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Morphometric Analysis and Comparisons of Electrophoretic Protein Profiles of the Scutellaria ovata Hill (Lamiaceae) Complex in West Virginia, Virginia and Ohio with Emphasis on Shale Barren Taxa

Eric W. Ewing
pedaleric@gmail.com

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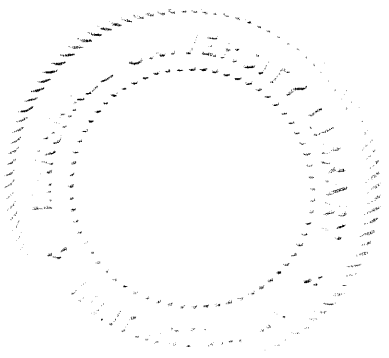
A Thesis Presented to the Faculty
of the Department of Biological Sciences

Marshall University

by

Eric W. Ewing

April 1996



THIS THESIS WAS ACCEPTED ON April 29, 1996
Month Day Year

as meeting the research requirements for the Master's Degree.

Research Advisor _____
Dan K. Evans, Ph.D.
Department of Biology

Committee Member _____
Donald C. Tarter, Ph.D.
Department of Biology

Committee Member _____
Jagan V. Valluri, Ph.D.
Department of Biology

_____
Leonard Deutsch, Ph.D.
Dean of the Graduate School

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When I began this project I was very confident of my abilities and I knew that I could carry out a good study and report it well in this thesis. I also knew that I wouldn't be able to do it alone. I was counting on help from professors, fellow graduate students, friends, family and my advisor. I wasn't disappointed! Numerous individuals provided assistance to me in some form during the time I was in graduate school. I would like to take this opportunity to mention these people and thank them for all that they have done for me.

First and foremost I want to thank P.J. Harmon and the West Virginia Natural Heritage Program for research grants awarded to Marshall University (Grant Nos. 94-0004-00 and 94-0004-01). These grants provided the support for this project and allowed us to complete the study without financial strain. The WVNHP also supplied plants for study and population data which were useful for completing field work.

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I would also like to thank my lovely wife, Karen, for her patience and understanding. She was very supportive even when school required me to be away from her for long periods of time. It takes a very special lady to put up with all that she has put up with in the last couple of years, but she has taken everything in stride without a single complaint. She didn't measure any plants or run any gels, but she did something much more important. She stood by me and gave me the confidence I needed when it seemed that I'd never get everything done. She always makes me feel like I can accomplish anything and as long as she's by my side, I know I can.

Finally, I want to express my sincere appreciation to my thesis advisor, Dr. Dan Evans. This is a man who truly loves what he does and conveys this enthusiasm to his students. His dedication to teaching and carrying out good science is incredible and I will always admire him for his work ethic. It seems that Dr. Evans teaches me something new every day and I certainly have enjoyed learning from him. To put it simply, taking on a project with him is the best decision I have made in my academic career. I hope he continues to teach and take on graduate students for a long time, so that other students may benefit from his knowledge and talent as I have.

Well, here it is! I've put a lot a sweat and tears into this work, but it's been worth it. Grad school has left me wiser, more confident and big time in debt. It's been great though and somehow I feel as if this won't be the last major project I'm involved in. Whatever and whenever that may be, I hope I have as much fun as I did working on this one. Thanks again to all the people who made this possible! Your efforts won't be forgotten!

Eric, April 1996

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Elements of the *Scutellaria ovata* complex in the eastern United States are reported to include *S. ovata* var. *pseudoarguta, S. ovata* var. *rugosa, S. ovata* var. *virginiana* and *S. ovata* ssp. *ovata.* All but *S. ovata* ssp. *ovata* occur in the shale barren habitat of eastern West Virginia and western Virginia. S. *ovata* var. *pseudoarguta* has been proposed as a threatened element in North America. A morphometric study of specimens collected in West Virginia, Virginia and Ohio was undertaken to determine which characters are most useful in separating taxa in the complex. Nineteen characters were assessed from 104 herbarium specimens and the data subjected to principal component analysis, canonical discriminant analysis and Duncan's model of analysis of variance. Specimens were grouped according to collection site in order to investigate intrapopulation and interpopulation variation. The results of this part of the study showed significant differences between western West Virginia and Ohio material and plants collected in eastern West Virginia and western Virginia. Variation between sites in West Virginia and Virginia was minimal. Plants were also grouped taxonomically according to variety or subspecies. The statistical procedures showed plant height, leaf length and width, petiole length, internode length, stem width and leaf cordateness to be the best characters for separating the subcomplex *S. ovata* var. *pseudoarguta* - *S. ovata* var. *rugosa* from *S. ovata* var. *virginiana* and *S. ovata* ssp. *ovata.* The latter taxa are separated by leaf width, floral bract length and width, first internode length, raceme length and raceme internode length. *S. ovata* var. *pseudoarguta* and *S. ovata* var. *rugosa* were shown to be very similar morphologically and separate only by leaf pubescence.

Studies incorporating electrophoretic protein profiles were also carried out in order to analyze biochemical differences among members of the *S. ovata* complex. Taxa included in the morphometric study were examined along with a single population of *Scutellaria elliptica* Muhl., which was used as an outlier species. One dimensional SDS-Polyacrylamide gel electrophoresis was used to separate soluble leaf proteins from 40 specimens representing four taxa. Intrapopulation and interpopulation variation was assessed as was variation within and between taxonomic groups. The results showed a high degree of homogeneity within the complex and the outlier element. Some differences were seen between taxa and populations, but variation was inconsistent and not significant enough to justify taxonomic separation based on protein profile data.

INTRODUCTION

Scutellaria L. is a genus of the Family Lamiaceae, which includes over 300 recognized species. Linnaeus (1735) first described *Scutellaria* and later listed 12 species of the genus in Species Plantarum (1753). *Scutellaria* is worldwide in distribution and a variety of distinct characters make it the most sharply defined genus of the Lamiaceae. These characters include a peg-like gynophore, dimidiate stamens, a curved embryo and a bilabiate calyx which closes at the mouth in fruit. These characters, although uncommon, are known in other mint genera. However, it is the combination of these that sets *Scutellaria* apart from the rest of the Lamiaceae (Paton, 1990). The most recognizable of the above characteristics is the cap-shaped projection (scutellum) that develops on the upper side of the mature calyx. This is responsible for the common name of the genus, Skullcap. Fernald (1970) and Gleason and Cronquist (1991) list 23 and 19 species of *Scutellaria,* respectively. Strausbaugh and Core (1977) recognize ten species of the genus from West Virginia. This study will concentrate on the morphology and biochemical characteristics of one of these: *Scutellaria ovata* Hill (Heart-Leaved Skullcap) and infraspecific taxa.

Scutellaria ovata is a pubescent herb with cordate-ovate, slender petioled, crenate-dentate leaves. The calyx is glandular and the corolla is blue with the lower side being lighter in color or white. This species, like

most members of the genus *Scutellaria,* is a perennial. It has fleshy, white underground tubers which provide nutritive and reproductive functions. However, Uttal (1966) reports that *S. ovata* behaves as an annual when transplanted from a Virginia shale barren to his home garden in the Piedmont region of Virginia. Wild populations at Millboro, Virginia were also examined by Uttal and their behavior was reported to be consistent with that of the transplants. Uttal also suggests that this annual habit, along with abundant nutlet production, are adaptations for survival in the rigorous conditions of the shale barrens. Since these tubers persist in all members of the species complex, appear to overwinter, and provide a means of vegetative reproduction, it seems logical to conclude that these plants are indeed perennials. More work needs to be done to determine if individual populations may have evolved the characteristics required to support a strictly annual habit.

There are four infraspecific taxa of *S. ovata* known to occur in West Virginia. Strausbaugh and Core (1977) list *S. ovata* var. *pseudoarguta, S. ovata* var. *rugosa, S. ovata* var. *virginiana* and *S. ovata* var. *versicolor. S. ovata* var. *versicolor* has been listed as a synonym for *S. ovata* ssp. *ovata,* so the latter taxon will be recognized in this study (Kartesz, 1994). Identification keys use plant height to separate taller *S. ovata* var. *virginiana* from *S. ovata* var. *pseudoarguta* and *S. ovata* var. *rugosa.* These two smaller taxa are distinguished from each other on the basis of leaf

pubescence, leaf shape and growth habit. The only character of these three that seems reliable is leaf pubescence. *S. ovata* var. *pseudoarguta* is nearly glabrous while *S. ovata* var. *rugosa* is hirsute. Scanning electron micrographs of leaf surfaces (Figures 1 and 2) show the difference in trichome density between these two taxa. However, this character, like the other key characters, is highly variable and often unhelpful in characterizing taxa. This makes identification below the species level difficult and raises many taxonomic questions. Heart-Leaved Skullcaps are also uncommon in West Virginia and have been reported to need special systematic attention within the state (Harmon *et al.,* 1995). One taxon within the complex, *S. ovata* var. *pseudoarguta,* has been listed as threatened in West Virginia (Ayensu and DeFilipps, 1978). This study will attempt to use morphology and protein profiles as a basis for solving some of the problems which make this species taxonomically difficult. The objectives of this study were:

1). To determine which morphological characters best separate members of the *Scutellaria ovata* complex.

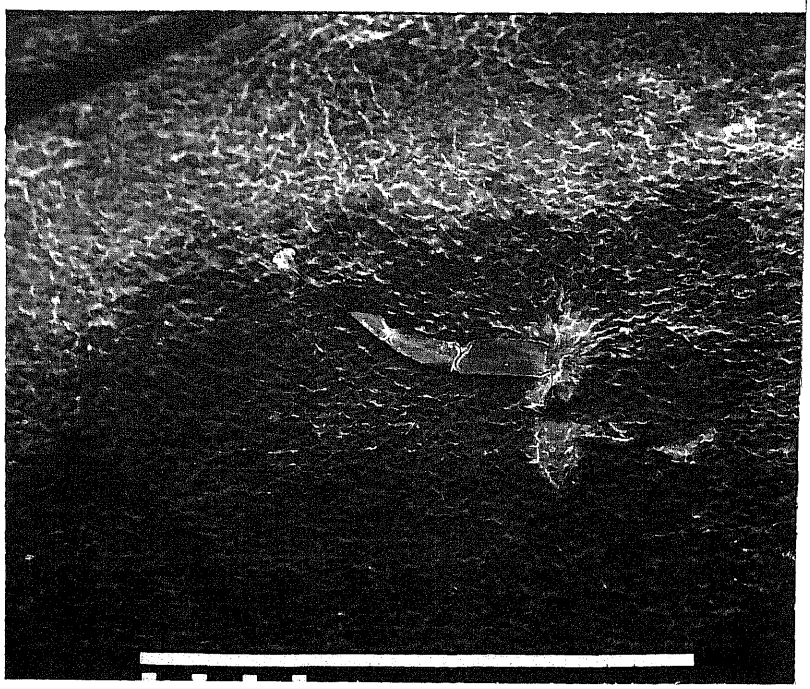
2). To determine the extent of morphological variability among taxa within the complex.

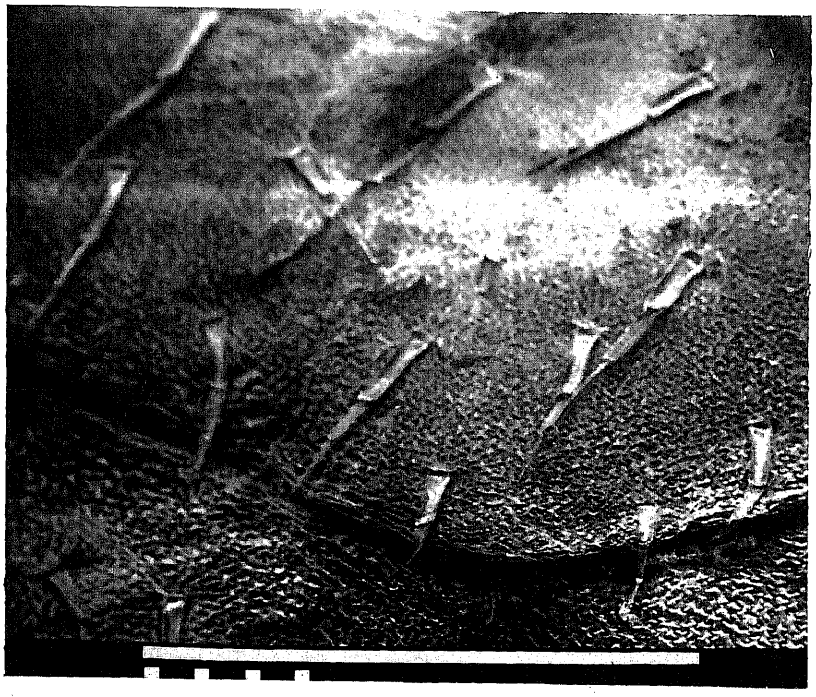
3). To determine the extent of morphological variability within and among populations of *Scutellaria ovata.*

4). To determine if protein profile patterns serve as a basis for showing relationships among taxa within the complex.

Figure 1. Scanning electron micrograph of *S. ovata* var. *pseudoarguta* leaf surface. 70X at 5 kV.

Figure 2. Scanning electron micrograph of *S. ovata* var. *rugosa* leaf surface. 70X at 5 kV.





5). To use protein profile patterns as a basis for showing relationships among populations of *Scutellaria ovata.*

6). To evaluate the taxonomic status of *Scutellaria ovata* in West Virginia based on morphological and biochemical data.

7). To determine if any of the taxa within the complex are to be considered rare and worthy of state tracking or federal listing.

Taxonomy

Classification of *Scutellaria ovata* according to Gleason and Cronquist (1991), Jones and Luchsinger (1986) and Sanders and Cantino (1984) is as follows:

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida (Dicotyledons)
Subclass: Asteridae
Order: Lamiales
Family: Lamiaceae
Subfamily: Scutellarioideae
Tribe: Scutellarieae
Subtribe: Scutellariinae
Genus: *Scutellaria*
Species: *S. ovata* Hill
Subspecies: *S. ovata* ssp. *ovata*
Varieties: *S. ovata* var. *pseudoarguta* (Epling) Core, *S. ovata* var. *rugosa* (Wood) Fernald, *S. ovata* var. *virginiana* (Epling) Core

The taxonomy of this species has seen many revisions and, as a result, synonyms exist for nearly all of the infraspecific taxa within the complex. Kartesz (1994) reports the following synonyms for the members of the *Scutellaria ovata* group:

Scutellaria ovata Hill
ssp. *bracteata* (Benth.) Epling
SY= *Scutellaria ovata* var. *bracteata* Benth.
ssp. *cuthbertii* (Alexander) Epling
SY= *Scutellaria cuthbertii* Alexander
ssp. *mexicana* Epling
ssp. *ovata*
SY= *Scutellaria cordifolia* Muhl.
SY= *Scutellaria ovata* ssp. *mississippiensis* (Mart.) Epling
SY= *Scutellaria ovata* ssp. *versicolor* (Nutt.) Epling
SY= *Scutellaria ovata* var. *calcarea* (Epling) Fern.
SY= *Scutellaria ovata* var. *versicolor* (Nutt.) Fern.
ssp. *pseudoarguta* Epling
SY= *Scutellaria ovata* var. *pseudoarguta* (Epling) Core
ssp. *rugosa* (Wood) Epling
SY= *Scutellaria ovata* var. *rugosa* (Wood) Fern.
ssp. *rupestris* Epling
ssp. *venosa* Epling
ssp. *virginiana* Epling
SY= *Scutellaria ovata* var. *virginiana* (Epling) Core

Many of the above synonyms for the taxa within the *S. ovata* complex result from the extreme variability of this species. Others have fluctuated between variety and subspecies status depending on whether they show morphological differences in local populations or discontinuities over a wide geographical area.

Literature Review

Scutellaria ovata was first described by Hill (1768) in Volume 1 of the Hortus Kewensis. The description and accompanying line drawing were based on a garden specimen which was apparently lost. Hill's description of the plant is as follows: "Caulis bepedalis, ramosus, subhirsutus. Folia ovata. Flores axillares, rubescentes. Bieennis. Ex America boreali. Julio florens."

The species was redescribed by Hill as *Scutellaria pilosa* in the thirteenth volume of the Vegetable System, dated 1773, but actually published in 1768. It was again called *S. ovata* in the second edition of Hortus Kewensis (Hill, 1768). The fact that Volume 12 of the Vegetable System is quoted in the first edition of Hortus Kewensis while Volume 13 is not, would seem to indicate the priority of *S. ovata* over *S. pilosa.*

After Hill described *S. ovata,* it was variously identified as *S. cordifolia* Muhl. or *S. versicolor* Nutt. Nuttall first collected *S. versicolor* in 1816 while working in the Ohio Valley (Stuckey, 1966). The name *S. ovata* was resurrected by Blake (1915) after his examination of some of Nuttall's *S. versicolor* specimens. Inspection of type material from the British Museum led Blake to conclude that *S. versicolor* Nutt. and *S. caroliniana* Walt. were both identical to the plant Hill described as *S. ovata.* Blake then proposed that the name *ovata* should have priority over *versicolor* as the earlier, valid specific epithet. Fernald (1942) however, states that *S. ovata* is probably the same as *S. versicolor* var. *bracteata,* but different from true *S. versicolor.* His conclusions were based upon Nuttall's description of *S. versicolor* and Hill's description of *S. ovata.* Fernald noted many discrepancies between the descriptions and proposed the name *S. ovata* var. *versicolor* for the plant that Blake declared equal to *S. versicolor.*

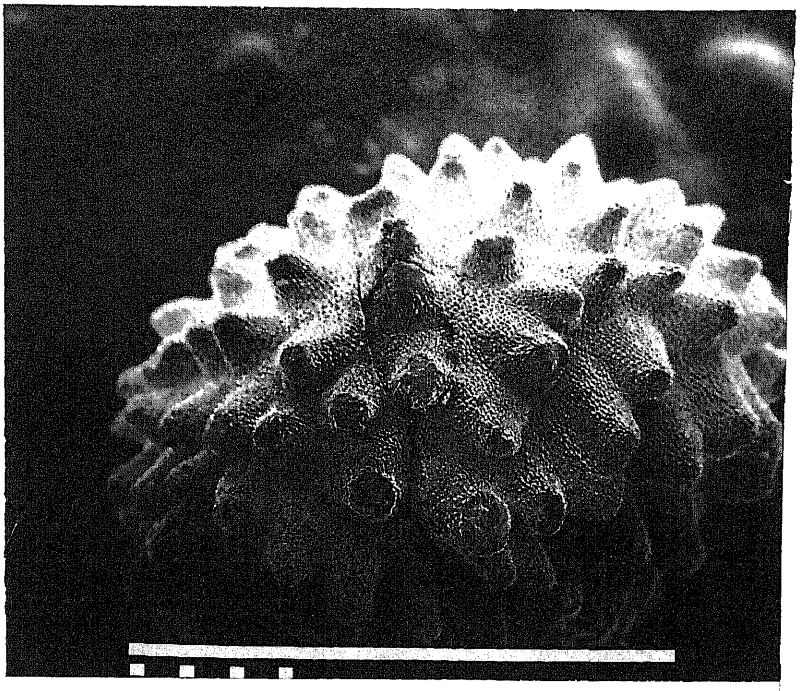
Penland (1924) completed one of the earliest taxonomic studies of the genus *Scutellaria.* Penland's work was unique in that the key to the species

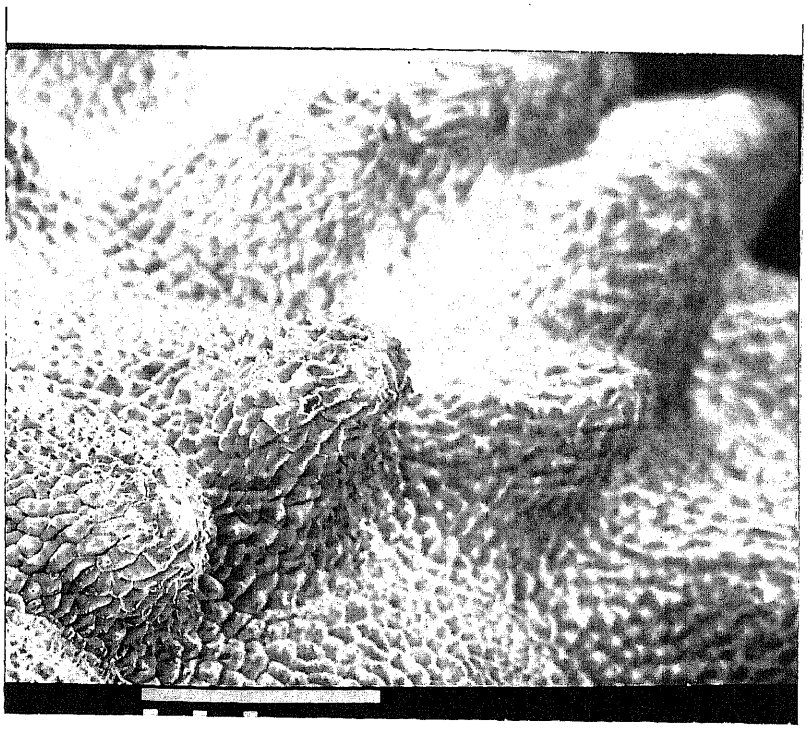
was based entirely on nutlet characteristics. This study does not mention *S. ovata,* but *S. versicolor* is included. The key describes the nutlets of *S. versicolor* as brown or black with short papillae that are broad at the base and sharply pointed. Scanning electron micrographs of *S. ovata* mericarps (Figures 3 and 4) show that Penland's description of *S. versicolor* nutlets also characterizes the nutlets of *S. ovata.* This may have been a case of synonomy between *S. ovata* and *S. versicolor.* Later studies have also utilized morphological characteristics of the nutlets to separate members of the genus *Scutellaria.* Lane (1983), in his study of the Great Plains *Scutellaria,* states that mericarp morphology is less variable than characters such as habit, plant size and pubescence, which makes it valuable as a taxonomic tool.

Leonard (1927) used a great number of morphological characters and produced a more thorough monograph of the North American species of *Scutellaria.* This study described *S. ovata* as a species that is highly variable for many of these characters, including leaf form and pubescence. The varieties *S. ovata* var. *bracteata* and *S. ovata* var *pilosior* were recognized by Leonard. Epling (1942) carried out what is considered to be the definitive work on the North American species of *Scutellaria.* This study placed *S. ovata* in the section Mixtae along with six other species and listed twelve subspecies within the complex. Characters such as leaf shape, growth pattern and pubescence were used by Epling to separate the subspecies,

Figure 3. Scanning electron micrograph of *S. ovata* var. *rugosa* nutlet. 70X at 5 kV.

Figure 4. Scanning electron micrograph of *S. ovata* var. *rugosa* nutlet. 300X at 5 kV.



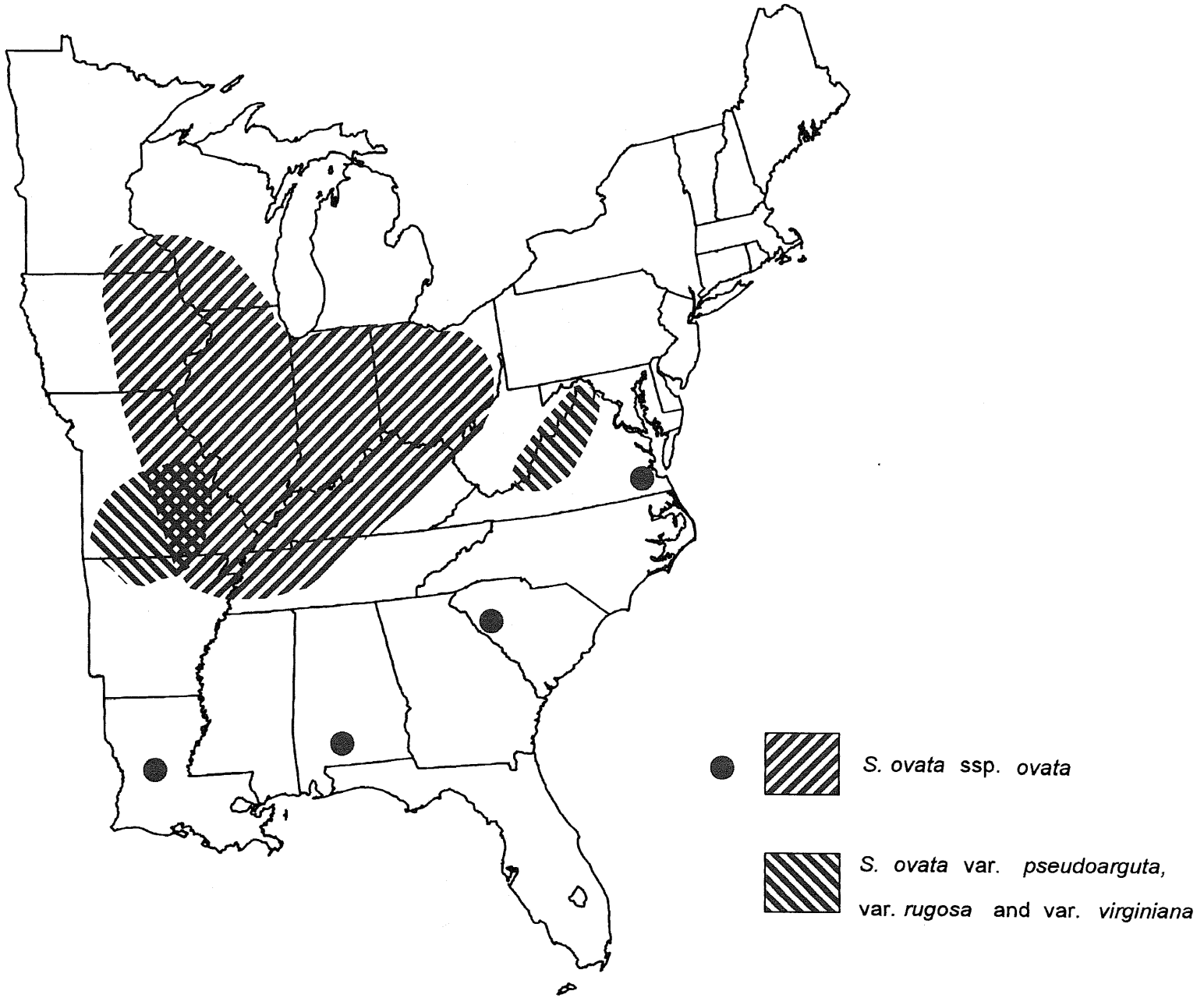


which he described as geographical variants. Four of these subspecies were reported from areas in or around West Virginia, Virginia and Ohio. *S. ovata* ssp. *pseudoarguta* and *S. ovata* ssp. *virginiana* were named by Epling, while *S. ovata* ssp. *rugosa* was declared equal to a plant named *S. rugosa* by Wood (1847) and *S. ovata* ssp. *versicolor* was based on Nuttall's *S. versicolor.* Later, these four subspecies were reduced to varieties and reported from several eastern West Virginia counties by Fernald (1945) and Core (1957). The most recent taxonomic revision, by Pittman (1987a,b), reduced Epling's original twelve subspecies to three subspecies including four varieties. Characters used for separation were floral morphology and pubescence, leaf dimensions and plant height. According to Pittman, the varieties of Heart-Leaved Skullcap found in West Virginia belong to a single taxon: *Scutellaria ovata* ssp. *rugosa* (Wood) Epling var. *rugosa.*

Geographical Distribution and Habitat

The North American distribution of the taxa reviewed in this study, shown in Figure 5, follows Pittman (1987b). There are two centers of distribution for the varieties recognized by Strausbaugh and Core (1977). These are the Ridge and Valley Province of the central Appalachians and the Ozark Plateau of Missouri and Arkansas. The disjunction between populations is most likely due to the lack of suitable habitat in the intervening areas. The distribution of *S. ovata* ssp. *ovata* is probably continuous throughout the eastern United States in areas where favorable conditions

Figure 5. Distribution of the *Scutellaria ovata* complex in the United States. (Distribution follows Pittman, 1987)


S. ovata ssp. ovata
S. ovata var. pseudoarguta,
var. rugosa and var. virginiana

exist. *S. ovata* distribution in West Virginia is primarily limited to the counties east of the Alleghenies, along the Virginia border. Virginia populations of the species are concentrated in the western counties although a few outlying populations can be found near the coastal plain (Harvill *et al.,* 1992). There are several populations of *S. ovata* var. *pseudoarguta, S. ovata* var. *rugosa* and *S. ovata* var. *virginiana* in these areas, but most are small and isolated (Figures 6, 7, 8). *S. ovata* ssp. *ovata* is very uncommon in West Virginia, with only one population on record. This single population is located in Wayne County, which borders southeastern Ohio (Figure 9). Dr. Phillip Cantino reports that the taxon is also uncommon in Ohio (personal communication, 1995). Literature reports of *S. ovata* ssp. *ovata* from Taylor and Mineral counties, West Virginia were listed in Strausbaugh and Core (1977) as *S. ovata* var. *versicolor,* which has since been listed as a synonym of *S. ovata* ssp. *ovata* (Kartesz, 1994). Since these specimens were not examined in this study, their identification as *S. ovata* ssp. *ovata* or *S. ovata* var. *virginiana* is still in question. Figure 10 shows the West Virginia distribution of all infraspecific taxa recognized in this study. The great amount of sympatry in the eastern part of the state contributes to the taxonomic problems within this complex.

The eastern West Virginia varieties of *S. ovata* are normally found on steep, dry slopes with a south or southwest aspect. This makes them well suited to a specific habitat known as shale barrens. The term shale barren

Figure 6. Distribution of *Scutellaria ovata* var. *pseudoarguta* in West Virginia.

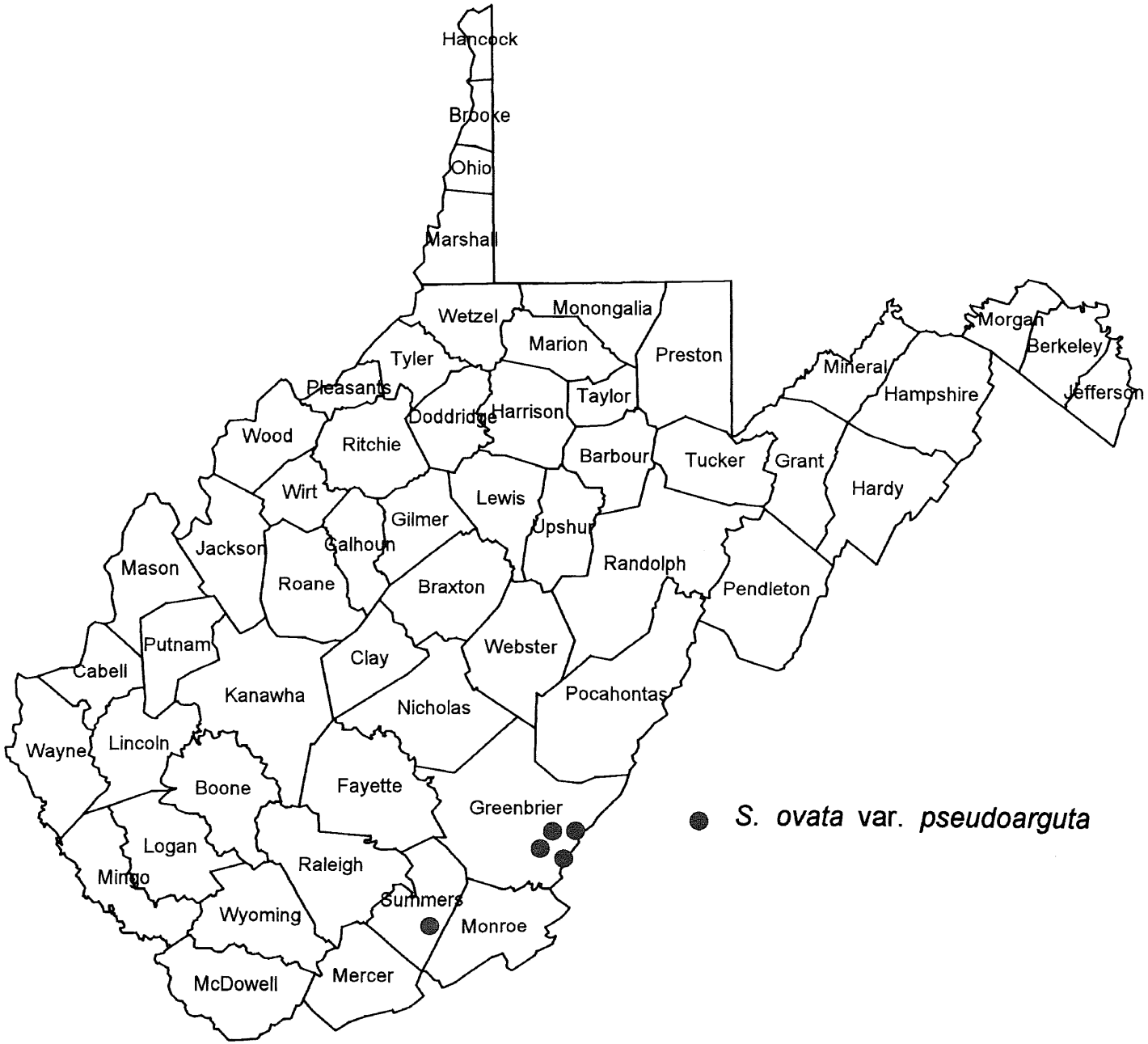
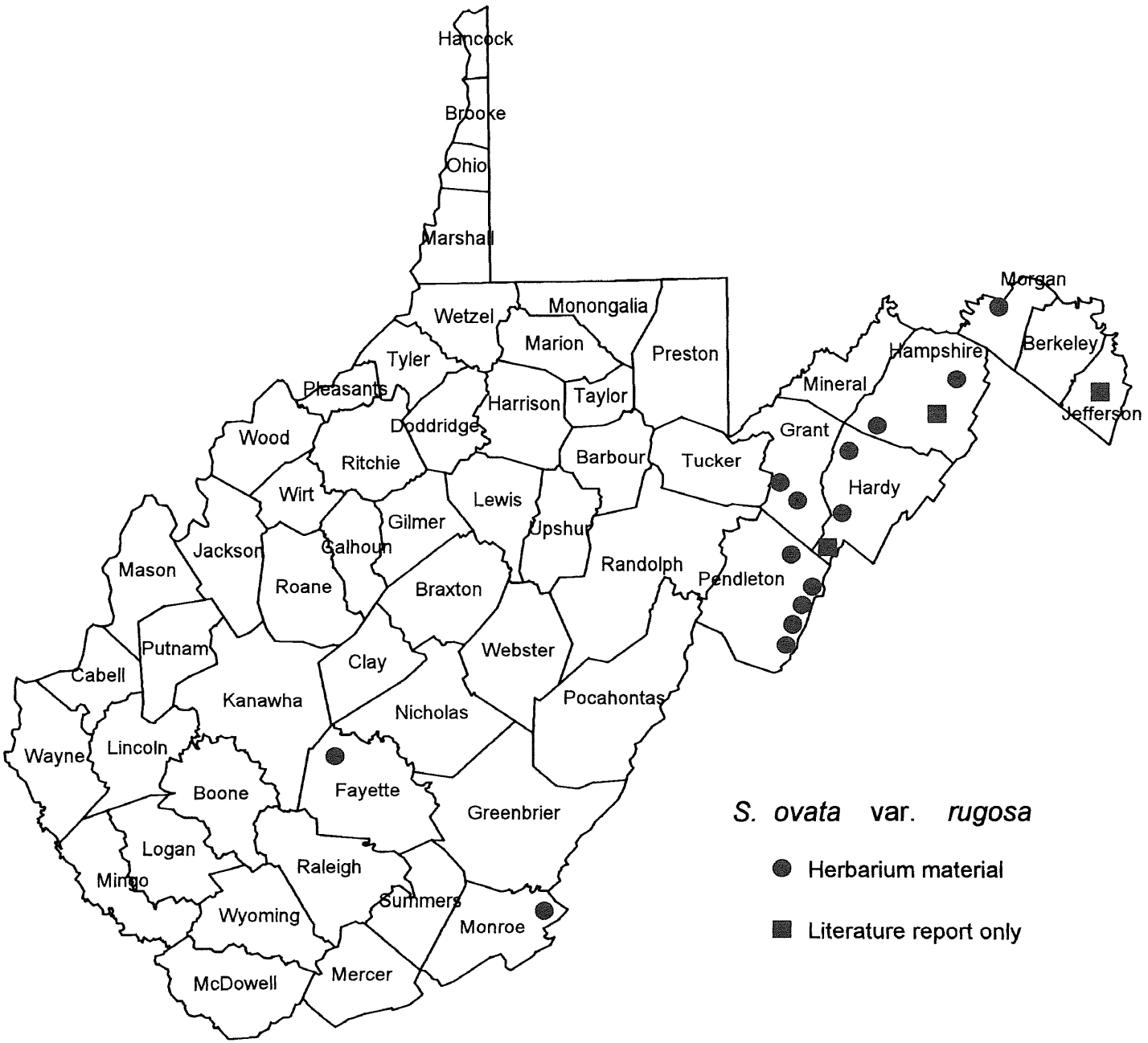

Hancock
Brooke
Ohio
Marshall
Wetzel
Monongalia
Marion
Preston
Tyler
Pleasants
Doddridge
Harrison
Taylor
Wood
Ritchie
Barbour
Tucker
Wirt
Lewis
Gilmer
Upshur
Jackson
Calhoun
Randolph
Mason
Roane
Braxton
Pendleton
Putnam
Clay
Webster
Cabell
Kanawha
Nicholas
Pocahontas
Wayne
Lincoln
Boone
Fayette
Greenbrier
Logan
Raleigh
Mingo
Summers
Monroe
Wyoming
McDowell
Mercer
Mineral
Morgan
Berkeley
Hampshire
Jefferson
Grant
Hardy
S. ovata var. pseudoarguta

Figure 7. Distribution of *Scutellaria ovata* var. *rugosa* in West Virginia.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Mingo
Summers
Wyoming
Monroe
McDowell
Mercer
Morgan
Berkeley
Hampshire
Mineral
Jefferson
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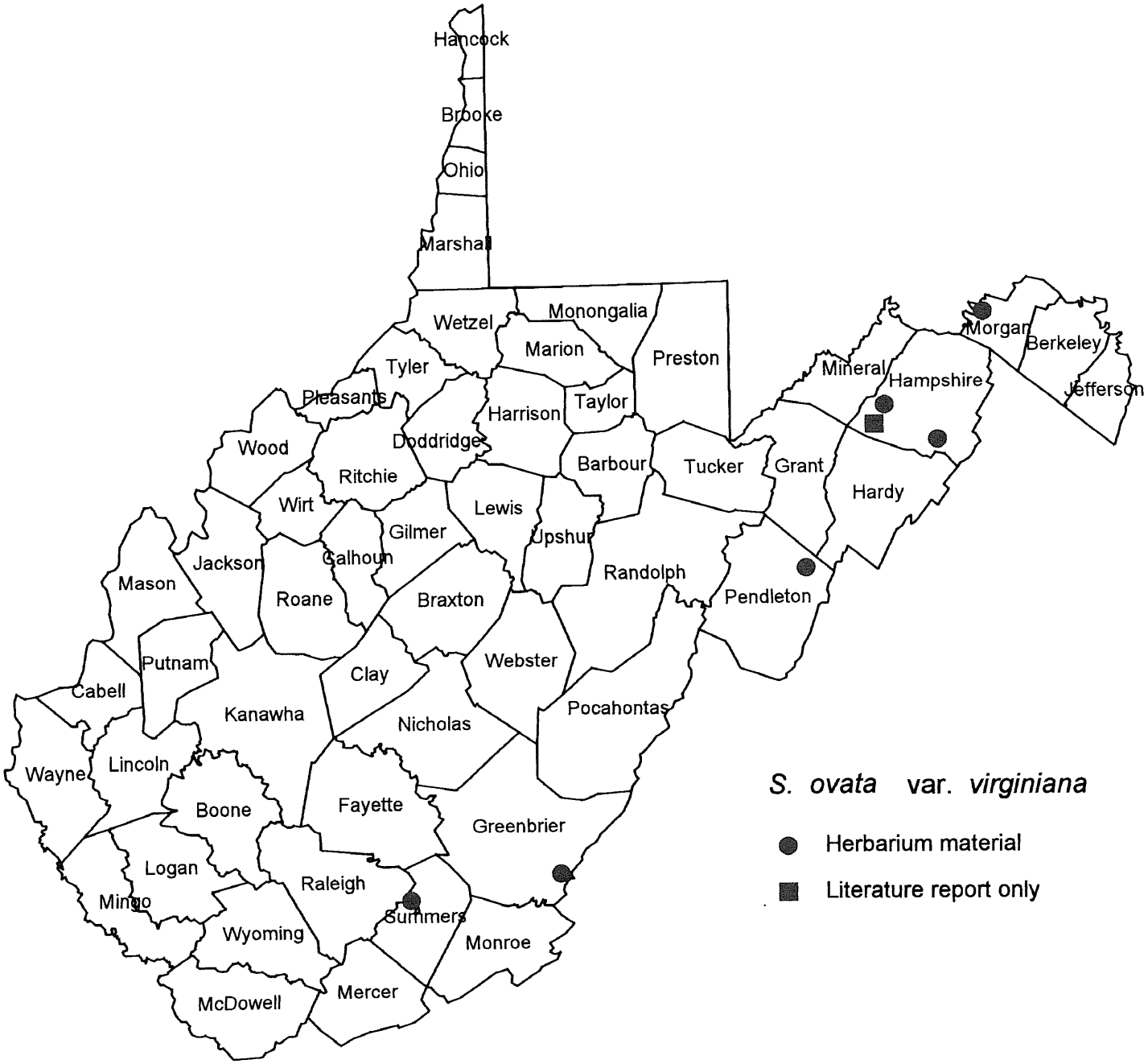
Hardy
S. ovata var. rugosa
Herbarium material
Literature report only

Figure 8. Distribution of *Scutellaria ovata* var. *virginiana* in West Virginia.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Mingo
Summers
Wyoming
Monroe
McDowell
Mercer
Morgan
Berkeley
Jefferson
Mineral
Hampshire
Grant
Hardy
S. ovata var. virginiana
Herbarium material
Literature report only

Figure 9. Distribution of *Scutellaria ovata* ssp. *ovata* in West Virginia.

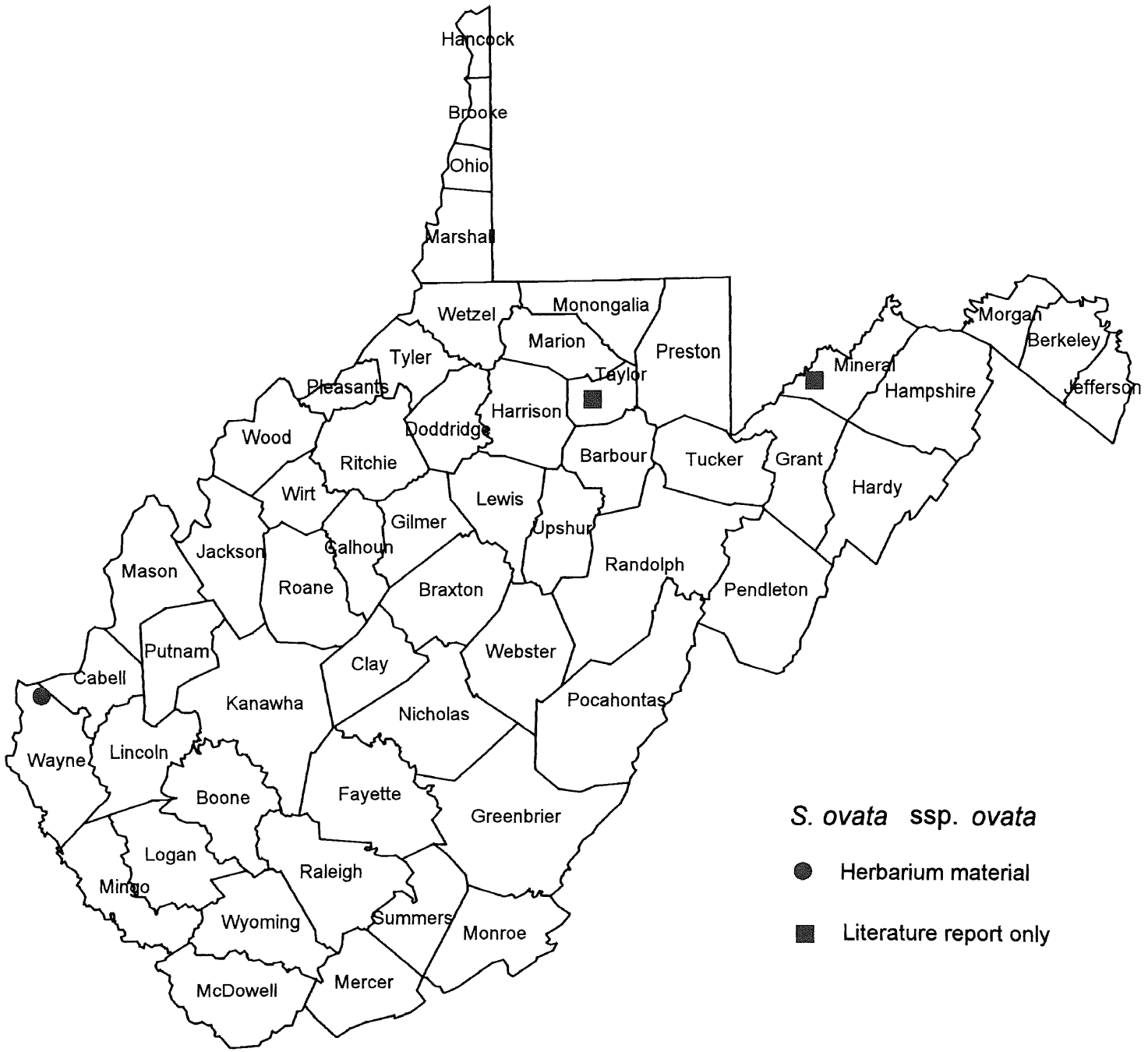
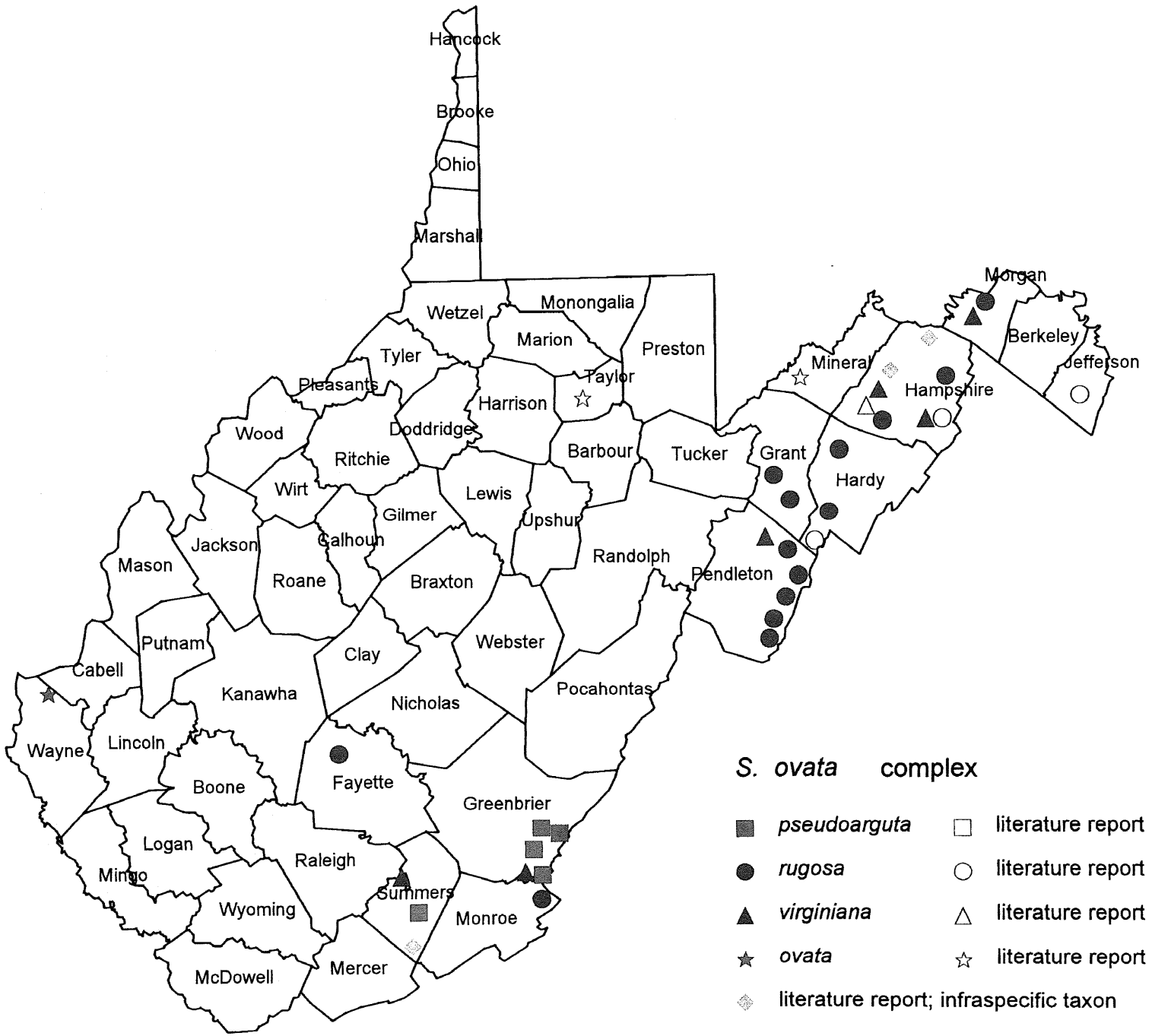

Hancock
Brooke
Ohio
Marshall
Wetzel
Monongalia
Marion
Preston
Tyler
Taylor
Pleasants
Harrison
Doddridge
Wood
Ritchie
Barbour
Tucker
Grant
Wirt
Lewis
Gilmer
Upshur
Jackson
Calhoun
Mason
Roane
Braxton
Randolph
Pendleton
Putnam
Webster
Clay
Cabell
Kanawha
Nicholas
Pocahontas
Wayne
Lincoln
Boone
Fayette
Greenbrier
Logan
Raleigh
Mingo
Wyoming
Summers
Monroe
McDowell
Mercer
Morgan
Berkeley
Mineral
Hampshire
Jefferson
Hardy
S. ovata ssp. ovata
Herbarium material
Literature report only

Figure 10. Distribution of the *Scutellaria ovata* complex in West Virginia.


Hancock
Brooke
Ohio
Marshall
Wetzel
Monongalia
Marion
Preston
Tyler
Pleasants
Taylor
Harrison
Wood
Doddridge
Barbour
Tucker
Ritchie
Wirt
Lewis
Gilmer
Upshur
Randolph
Jackson
Calhoun
Roane
Braxton
Mason
Putnam
Webster
Clay
Cabell
Kanawha
Nicholas
Pocahontas
Wayne
Lincoln
Boone
Fayette
Greenbrier
Logan
Raleigh
Mingo
Summers
Monroe
Wyoming
Mercer
McDowell
Morgan
Berkeley
Jefferson
Mineral
Hampshire
Grant
Hardy
Pendleton
S. ovata complex
pseudoarguta
literature report
rugosa
literature report
virginiana
literature report
ovata
literature report
literature report; infraspecific taxon

was first used by Steele (1911) to describe land made up of "exposures of shale in different stages of disintegration." The mid-Appalachian shale barrens, which range from southcentral Pennsylvania to southwestern Virginia and adjacent West Virginia are characterized by 1) a generally southern exposure, 2) normally a steep slope (greater than 20^0) and 3) sparse vegetation growing on a mantle of thin weather-resistant rock flakes (Keener, 1970). These areas are primarily made up of shales that are derived from rock of the Upper Devonian age (Artz, 1948). However, shale barrens may also consist of Ordivician Martinsburg shales or Silurian strata (Artz, 1937; Morse, 1983).

The shale barrens support a scrubby growth of oak, pine and juniper with a sparse herbaceous cover of endemic or near endemic species. Several authors have studied shale barrens and hypothesized about the conditions that lead to the unique flora represented there (Allard and Leonard, 1946; Core 1940,1952; Henry, 1954; Keener, 1970,1983; Morse, 1993; Platt, 1951; Wherry, 1930). Wherry (1930) suggested that the habitat was a result of the sparsity of soil and the limited amount of available moisture and nutrient elements. Allard and Leonard (1946) maintained that the lack of true soil and the scarcity of available moisture and humus contributed to the formation of the highly selective habitat. Later work showed that the limiting factors were not lack of soil, moisture or soil nutrients. Platt (1951) reports that shale barren plants thrive because of high light intensity and insolation temperature,

which allows them to flourish when competitors cannot survive. This unique combination of light and temperature conditions, along with low levels of competition, have contributed to the development of eighteen endemic taxa (Keener, 1983). About half of these, including *Allium oxyphilum, Eriogonum allenii, Trifolium virginicum, Clematis albicoma* and *Oenothera argillicola,* have their type localities from shale barrens located in eastern West Virginia.

Historically, most shale barren research has concentrated on the distinctive flora inhabiting these communities. Only recently have entomologists begun to discover a diverse fauna of phytophagous insects on the barrens. The butterflies and skippers characteristic of shale barrens are well known (Clench and Opler, 1983; Opler and Krizek, 1984; Pague and Schweitzer, 1991) and some of Pennsylvania's rarest butterfly and moth species are found on shale barrens (Smith, 1989). Several other insect groups have also been studied, including grasshoppers (Gurney, 1941), psyllids (Wheeler, 1994) and plant bugs (Wheeler, 1995a). A.G. Wheeler (1995b) reports a diverse insect fauna from moss phlox (*Phlox subulata*) which is common on many shale barrens. This fauna includes two recently described insect species and at least four new species that remain undescribed. Joe Cavey, an entomologist with the United States Department of Agriculture, has discovered an interesting specimen of leaf beetle on a shale barren in Maryland. The beetle, which was found on a species of *Penstemon,* may be new to science (personal communication, 1995). As

more shale barren research is carried out, many new flora-fauna relationships may be discovered and described.

While common on many barrens, *Scutellaria ovata* is not an endemic species. It is found on other dry, rocky soils in habitats that are not described as shale barrens. However, these non-shale barren areas where the taxa examined in this study have been found, normally lie within the shale barren region and have many physical and biological features of shale barrens.

Cytology

Review of Indices to Plant Chromosome Numbers (Goldblatt, 1979-81; Goldblatt, 1982-83; Goldblatt and Johnson, 1986-87) provided the cytological data and citations shown in Table 1. Epling (1942) includes *S. ovata* in the section Mixtae along with *S. cardiophylla, S. saxatilis, S. Churchilliana* and *S. lateriflora.* Epling suggests that *S. ovata* and *S. saxatilis* are polyploids that may have had their origins from *S. lateriflora.* However, recent cytological data do not support this as base numbers of 8, 9, 10, 11, 13, 15 and 17 are recorded for the genus (Gill, 1980). This would suggest that *S. lateriflora* is a polyploid that has arisen from one of the more primitive forms.

Given the variation in chromosome number between members of Epling's Mixtae, Pittman (1987b) suggests that it is unlikely *S. cardiophylla, S. saxatilis* and *S. ovata* represent a polyploid or aneuploid series. They are only distantly related species that were grouped together based on overall morphological similarity. *S. ovata* seems to be more closely related to *S.*

TABLE 1. Chromosome numbers of related species of *Scutellaria*

TAXON	n	2n	CITATION
Scutellaria lateriflora	-	80	Love and Love, 1982
Scutellaria lateriflora	44	88	Gill and Morton, 1978
Scutellaria leonardi	10	-	Gill, 1980
Scutellaria parvula	10	-	Gill, 1980; Gill 1981
Scutellaria cardiophylla	24	-	Pittman, 1987b
Scutellaria saxatilis	15	-	Pittman, 1987b
Scutellaria Churchilliana	-	60	Gill and Morton, 1978
Scutellaria ovata ssp. *ovata*	10	-	Pittman, 1987b
Scutellaria ovata vars. *pseudoarguta, rugosa, virginiana*	10	-	Pittman, 1987b

leonardi and *S. parvula,* both of which belong to Epling's section Galericularia. These three species are similar in morphology, habitat and breeding system, as well as chromosome number.

MATERIALS AND METHODS

Morphometric Analysis

Scutellaria ovata specimens were collected from ten sites in eastern West Virginia (Appendix III) and deposited in the Marshall University Herbarium (MUHW) Huntington, West Virginia. These plants were examined along with specimens from herbaria at West Virginia University (WVA), Morgantown, West Virginia, Virginia Polytechnic Institute and State University (VPI), Blacksburg, Virginia and Ohio University (BHO), Athens, Ohio. Specimens from West Virginia Natural Heritage Program collections were also borrowed and included in the study. Appendix I contains a complete list of specimens examined. A total of nineteen morphological characters (including two ratios) (Table 2) were measured from specimens collected in West Virginia and adjacent Virginia and Ohio.

All measurements were quantitative and recorded in millimeters. Plant height was measured from the uppermost roots to the highest part of the plant. Leaf measurements were taken from the largest leaves on the plant, which are normally found at the second or third node. Leaf width was measured at a point one-fourth the length of the leaf blade from the apex. Floral bract measurements were taken from the youngest bracts near the top

TABLE 2. Characters measured for specimens of *S. ovata.*

ABBREVIATION	CHARACTER (mm)
PH	Greatest height of plant
LLG	Greatest length of largest leaf blade
LWD	Width of largest leaf blade at a point 1/4 the distance from the apex
FBLG	Greatest length of youngest floral bracts
FBWD	Greatest width of youngest floral bracts
P1LG	Petiole length at 1st node
P3LG	Petiole length at 3rd node
I1LG	Internode length between 1st and 2nd nodes
I3LG	Internode length between 3rd and 4th nodes
P/I1	Ratio of petiole length at 1st node to internode length between 1st and 2nd nodes.
P/I3	Ratio of petiole length at 3rd node to internode length between 3rd and 4th nodes.
SWD	Width of stem between 1st and 2nd nodes
CLG	Greatest length of corolla
CLLG	Greatest length of the corolla lower lip
CALG	Greatest length of the calyx in fruit
CRD	Amount of leaf cordateness
NTD	Greatest diameter of mature nutlet
RLG	Greatest length of raceme
RI	Raceme internode length

of the plant. Petiole lengths were measured from the first and third nodes as were the internodes they subtend. Ratios of petiole length to internode length were then computed. Measurements for stem width were taken from a point on the stem between the first and second nodes. Total corolla length and the length of the lower lip of the corolla were included, as were the lengths of the indeterminate racemes and raceme internodes. Lengths of mature calyces in fruit and diameters of mature nutlets were also recorded. Cordateness of leaves was measured by taking the distance from the margin of the leaf to the deepest part of the basal sinus. Where possible, three measurements were taken for each character and an average of these was recorded for a given collection. A dial caliper calibrated to .01mm was used for all measurements and a dissecting microscope was used to aid in measurements of small characters such as floral bracts and nutlets.

Data from all nineteen characters were subjected to quantitative analysis using a SAS computer program (SAS, 1982). Principal component analysis (PCA) was used to determine which morphological characters are best used to separate members of the complex, regardless of their species designation. Canonical discriminant analysis (CDA) was used to evaluate the significance of morphological characters based on assigned species groups (Gittins, 1985). CDA is useful when appointing more than one data group (Pielou, 1984). In this study, four matrices of log transformed character data were used, one for each taxon. Character data are often log transformed

when normal distributions are not represented. Maximum, mean and minimum values were determined for each character and standard deviation was calculated. Mean character measurements were also subjected to analysis of variance and Duncan's Multiple Range Test (Proc ANOVA; SAS, 1982). This procedure is used to assign letters to species groups based on the difference in their means. Means with different letter designations were considered to be significantly different for that particular character.

Specimens were grouped in two different ways before being analyzed statistically. Plants were grouped according to subspecies or variety in order to determine variability between taxa. Measurements were taken from 28 specimens of *S. ovata* var. *pseudoarguta,* 58 specimens of *S. ovata* var. *rugosa,* 11 specimens of *S. ovata* var. *virginiana,* and seven specimens of *S. ovata* ssp. *ovata* for a total of 104 herbarium collections. The same 104 collections were also grouped together based upon collection location in order to evaluate variability between populations and within populations. Table 3 shows the sites compared and the number of specimens measured from each. Sites A, B and C , all in Greenbrier County, West Virginia, supported populations of *S. ovata* var. *pseudoarguta. S. ovata* var. *rugosa* specimens were studied from sites D, E, F, G and J. Site N included specimens of *S. ovata* ssp. *ovata* from western West Virginia and southern Ohio. The remaining sites contained plants from more widespread distributions and thus included a mixture of *S. ovata* var. *pseudoarguta, S. ovata* var. *rugosa* and

TABLE 3. Sites for interpopulation and intrapopulation comparisons of *S. ovata* specimens.

LETTER DESIGNATION	SITE	NO. OF SPECIMENS MEASURED
A	Kate's Mt., Greenbrier Co., WV (shale barren)	10
B	Upper White's Draft, Greenbrier Co., WV (shale barren)	10
C	Blue Bend, Greenbrier Co., WV (shale barren)	8
D	Slaty Mt., Monroe Co. and Camp Lightfoot Rd., Summers Co., WV (shale barrens)	11
E	Smoke Hole Caverns, Grant Co., and Gauley Bridge, Fayette Co., WV (non-shale barren areas)	5
F	Brandywine, Pendleton Co., WV (shale barren)	7
G	Little Fork Rd. and Dam #10, Pendleton Co., WV (shale barrens)	7
H	Hardy, Hampshire, Morgan and Grant Cos., WV (shale barrens)	10
I	Pendleton Co., WV (shale barrens)	4
J	Bath Co., VA (shale barren)	10
K	Giles and Alleghany Cos., VA (shale barrens)	5
L	Rockbridge, Botetourt, Bedford Cos., VA (shale barrens)	7
M	Roanoke Co., VA (non-shale barren areas)	3
N	Athens, Adams, Pickaway and Ross Cos., Ohio and Wayne Co., WV (non-shale barren areas)	7

S. ovata var. *virginiana.* These sites were arranged in this manner in order to present a significant sample size and efforts were made to group geographically close sites together. Sites E and M were grouped separately because they are non-shale barren habitats.

Biochemical Analysis

Plants of the *Scutellaria ovata* complex were collected from ten sites in eastern West Virginia, including nine shale barren habitats and one non-shale barren site. Specimens of *S. ovata* ssp. *ovata* and *S. elliptica* Muhl., an outlier species, were collected from Wayne County, West Virginia (Table 4). Each collection was given a two number designation, the first representing the population and a second which specified the individual plant collected. The specimens included in the study are listed in Appendix II. Plants were carefully removed from the substrate and potted in on-site soil while in the field. Living material was transported to Marshall University in a cooler and allowed to acclimate in an environmental chamber which kept a constant temperature of 22°C (72°F) and a twelve hour photoperiod. Specimens were kept in the environmental chamber for at least two weeks before proteins were harvested.

Young leaves showing healthy characteristics were excised from plants and proteins were extracted from this tissue by grinding the leaves with a mortar and pestle in 0.5 mL of ice-cold buffer. The grinding buffer was composed of 50 mM Tris HCL, 20 mM KCL, and 10 mM $MgCl_2$ (pH 8.65).

TABLE 4. Collection sites for *S. ovata* specimens used in protein profile analysis.

NUMBER	SITE	TAXON
1	Upper Kate's Mt., Greenbrier Co., WV (shale barren)	*S. ovata* var. *pseudoarguta*
2	Lower Kate's Mt., Greenbrier Co., WV (shale barren)	*S. ovata* var. *pseudoarguta*
3	Little Fork, Pendleton Co., WV (shale barren)	*S. ovata* var. *rugosa*
4	Brandywine, Pendleton Co., WV (shale barren)	*S. ovata* var. *rugosa*
5	Blue Bend, Greenbrier Co., WV (shale barren)	*S. ovata* var. *pseudoarguta* & *S. ovata* var. *virginiana*
6	Slaty Mt., Monroe Co., WV (shale barren)	*S. ovata* var. *rugosa*
7	Smoke Hole Caverns, Grant Co., WV (xeric woodland)	*S. ovata* var. *rugosa*
8	Dam #10, Pendleton Co., WV (shale barren)	*S. ovata* var. *rugosa*
9	Greenbrier Mt., Greenbrier Co., WV (shale barren)	*S. ovata* var. *pseudoarguta*
10	Upper White's Draft, Greenbrier Co., WV (shale barren)	*S. ovata* var. *pseudoarguta*
11	Beech Fork, Wayne Co., WV (mixed hardwood forest)	*S. ovata* ssp. *ovata*
12	Beech Fork, Wayne Co., WV (mixed hardwood forest)	*Scutellaria elliptica*

The resulting slurry was centrifuged at 13,000 x G for 10 minutes in an International Biotechnologies, Inc. IMV-13 microcentrifuge. The supernatant obtained was transferred into fresh 1.5 ml microcentrifuge tubes and labeled. Protein samples were then stored at -70^{0}C in a Forma Scientific Bio-Freezer to minimize degradation.

Protein samples were assayed for total protein concentration by Bradford's Assay (Bradford, 1976). A 60 µl aliquot of sample was added to 2940 µL of Bradford's Reagent in 3mL silicon spectrophotometer cuvettes with a thickness of 1.00 cm. A plastic cuvette cap was placed over the open end of the cuvette and it was inverted once to mix. Samples were allowed to incubate at room temperature for five minutes. Following incubation, the solutions were analyzed for absorbance at 595 nm with a factor of 1.6244 and an integration time of two seconds on a Perkin-Elmer Lambda 4A UV/VIS Spectrophotometer with a slit of one.

Data obtained from Bradford's Assay presents the concentration of protein in the samples and is used to determine volumes of the supernatant required to load a constant amount of 5 µg of protein per well in SDS-PAGE gels. The gel system used was the Mini-Protean II by Bio-Rad Laboratories. Both 12 and 16 percent polyacrylamide separating gels of 0.75 mm thickness were prepared as described by Laemmli (1970). A 4 percent stacking gel placed on top of the separating gels contained ten wells, each with a capacity of 27 µL. The volume of sample necessary for 5 µg of protein was diluted 1:4

with sample buffer and loaded into each well. In order to provide a standard by which molecular weights of the sample proteins could be determined, 8 µL of either Low-Range (31-2.5 kD) or Mid-Range (97.4-14.4 kD) Molecular Weight Markers (Promega Corporation) were loaded into one well on each gel. To provide a dye front during the run, 0.2 mL of Bromophenol Blue was added to the upper buffer chamber immediately before the initiation of electrophoresis. The gels were run at an initial amperage of 120 mamps for an average of 40 minutes using 5X running buffer.

Proteins were fixed by allowing the gels to incubate overnight in 50 percent methanol with 0.1 percent formaldehyde. Gels were silver stained for nanogram sensitivity of the proteins present using the Accurate Chemical Corporation's Hi-Ho Silver Stain Kit based on the methodology of Wray et al. (1981). Gels were dried on filter paper with a Bio-Rad Slab Gel Drier. Gels were dried at 60°C for one hour under 30 psi pressure.

Each of the 28 gels run was scanned using a Hewlett Packard scanner with Adobe Photoshop software. The image obtained was then imported into the Sigma Scan (Jandel Scientific, v 1.20) program. Using Sigma Scan, the dye front was first measured as the distance from the top of the gel to the furthest point that the samples migrated. Molecular weight markers were identified and their distances migrated from the top of the gel were measured. Rf values for each marker were calculated by dividing the distance of the dye front by the distance migrated by the molecular weight standard. This data

was analyzed for regression/correlation and a least squares line was generated using the analysis software of Microsoft Excel v 5.0 (Figure 11). The Rf values were then calculated for each protein of interest in the samples and their molecular weights were estimated by the equation:

molecular weight = -slope(Rf) + y-intercept.

RESULTS AND DISCUSSION

Principal Component Analysis

The results of the principal component analysis (PCA) showed significant overlap of taxa within the *Scutellaria ovata* complex (Figure 12). The first principal component axis (PRIN1) accounted for 37 percent of the total variation between taxa, while the second principal component axis (PRIN2) accounted for an additional 14 percent. This suggests that the remaining 49 percent of the variation was associated with additional axes. Although considerable overlap did occur, *S. ovata* var. *pseudoarguta* and *S. ovata* var. *rugosa* was separated to a great degree from *S. ovata* var. *virginiana* and *S. ovata* ssp. *ovata* on PRIN1. There was no significant separation between *S. ovata* var. *pseudoarguta* and *S. ovata* var. *rugosa* or between *S. ovata* var. *virginiana* and *S. ovata* ssp. *ovata.*

Principal component correlations (Table 5) show those characters which were most significant for separating taxa using PCA. The larger the correlation value (plus or minus) the greater the character's value in separating groups. The taxa were first separated by leaf length (LLG), plant

Figure 11. Rf values as a function of molecular weight (kD) using mid-range molecular weight markers separated on a 12% SDS-Polyacrylamide gel.

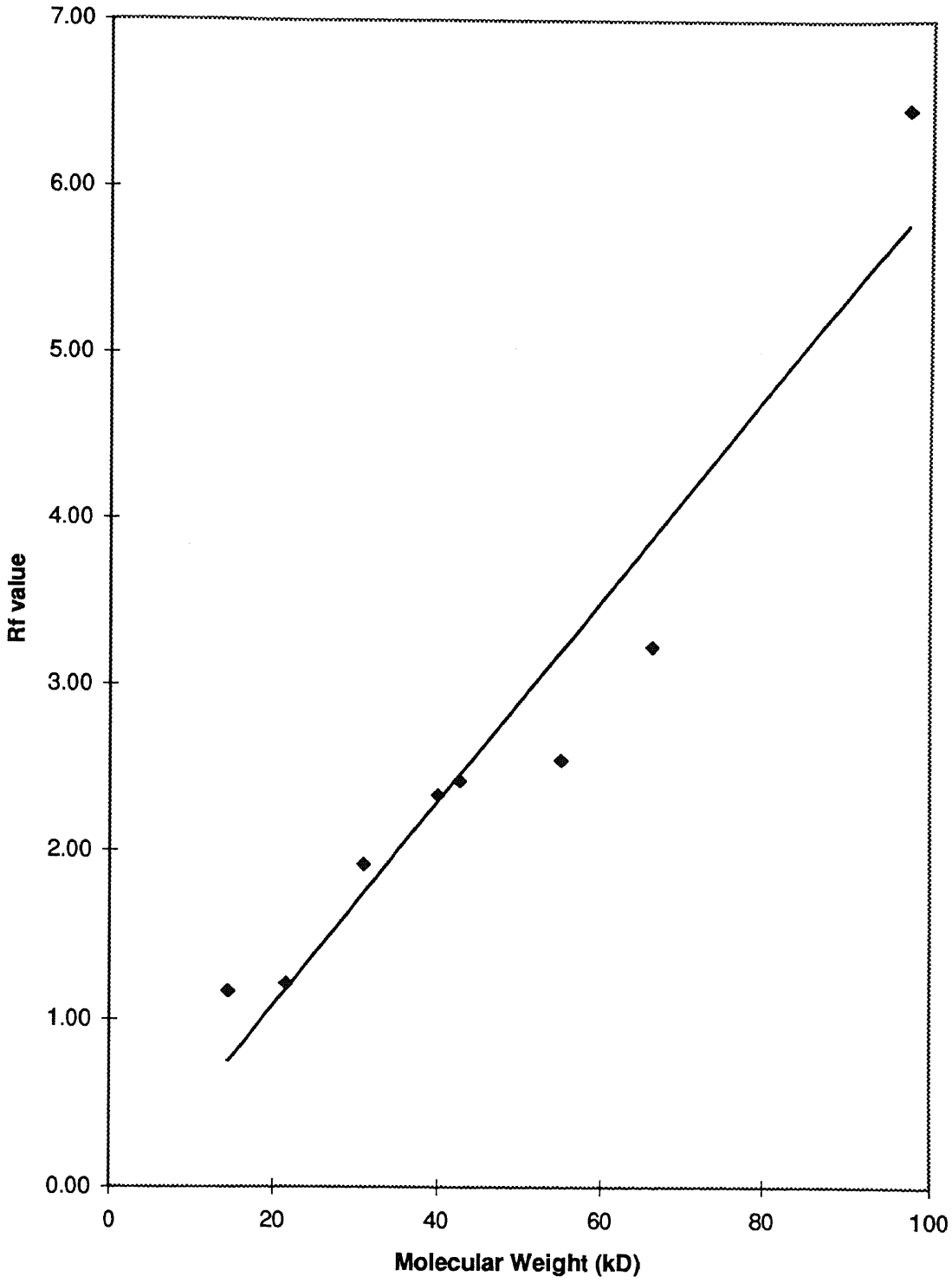

7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Rf value
0
20
40
60
80
100
Molecular Weight (kD)

Figure 12. Principal component analysis ordination of 104 specimens of *Scutellaria ovata*. Mean values for each taxon are represented by a closed symbol.

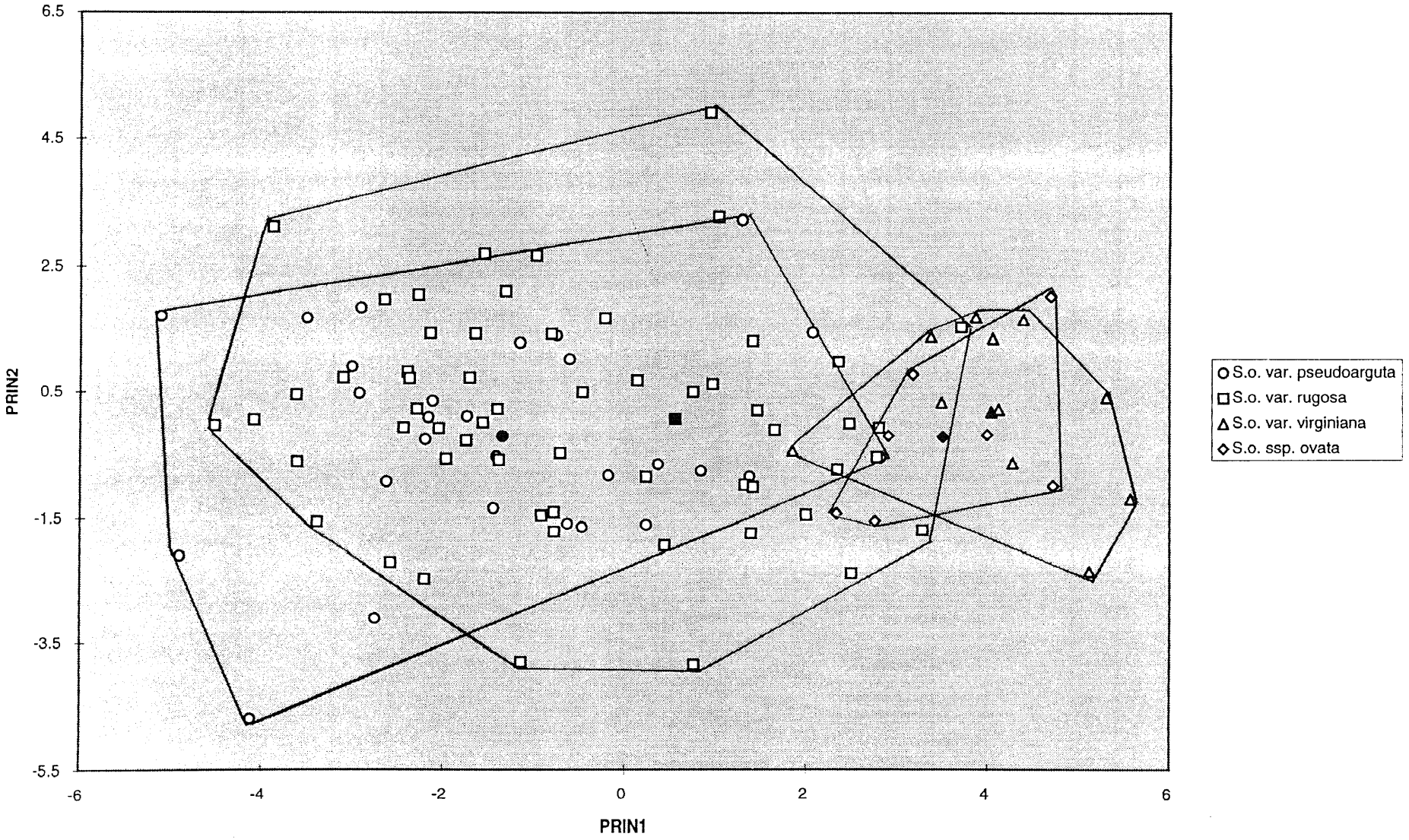

6.5
4.5
2.5
0.5
-1.5
-3.5
-5.5
PRIN2
-6
-4
-2
0
2
4
6
PRIN1
S.o. var. pseudoarguta
S.o. var. rugosa
S.o. var. virginiana
S.o. ssp. ovata

TABLE 5. Total sample correlation between ln-transformed character measurements and first two principal component variables (PRIN1 and PRIN2). See TABLE 2 for character abbreviations.

CHARACTER	PRIN1	PRIN2
PH	0.338264	0.065043
LLG	0.346825	0.023710
LWD	0.327059	0.032738
FBLG	-0.084478	0.459367
FBWD	-0.052398	0.448271
P1LG	0.322657	-0.152973
P3LG	0.310101	0.045163
I1LG	0.312725	-0.064388
I3LG	0.232968	0.274378
P/I1	-0.124147	-0.088911
P/I3	0.069268	-0.299595
SWD	0.319996	-0.023053
CLG	-0.134691	0.136170
CLLG	-0.111249	0.177521
CALG	0.056045	0.304922
CRD	0.302437	0.009019
NTD	-0.103427	-0.147185
RLG	0.193539	0.137860
RI	0.014457	0.441954

height (PH) and leaf width (LWD) on PRIN1. Petiole lengths (P1LG, P3LG), internode lengths (I1LG, I3LG), stem width (SWD) and cordateness (CRD) accounted for most of the remaining separation on PRIN1. The taxa were separated on PRIN2 first by floral bract length (FBLG) and floral bract width (FBWD), then by raceme internode length (RI) and calyx length (CALG).

Canonical Discriminant Analysis

The results of the canonical discriminant analysis (CDA) showed three distinct groups within the *S. ovata* complex (Figure 13). Canonical axis 1 (CAN1) accounted for 76 percent of the separation and Canonical axis 2 (CAN2) accounted for an additional 17 percent. Only 7 percent of the total variation was unaccounted for on the first two axes. *S. ovata* var. *pseudoarguta* and *S. ovata* var. *rugosa* showed significant overlap with little to no separation. *S. ovata* var. *virginiana* was separated from these two taxa on CAN2 and *S. ovata* ssp. *ovata* separated from the other three taxa on CAN1.

Canonical correlations (Table 6) showed first internode length (I1LG), leaf length (LLG) and leaf width (LWD) as the most significant characters for separation on CAN1. First petiole length (P1LG), stem width (SWD), cordateness (CRD) and plant height (PH) were also important for CAN1 separation. Characters important for separation along CAN2 included third internode length (I3LG), plant height (PH), third petiole length (P3LG), leaf length (LLG) and stem width (SWD). As in PCA, the larger the correlation

Figure 13. Canonical discriminant analysis ordination of 104 specimens of *Scutellaria ovata*. Mean values for each taxon are represented by a closed symbol.

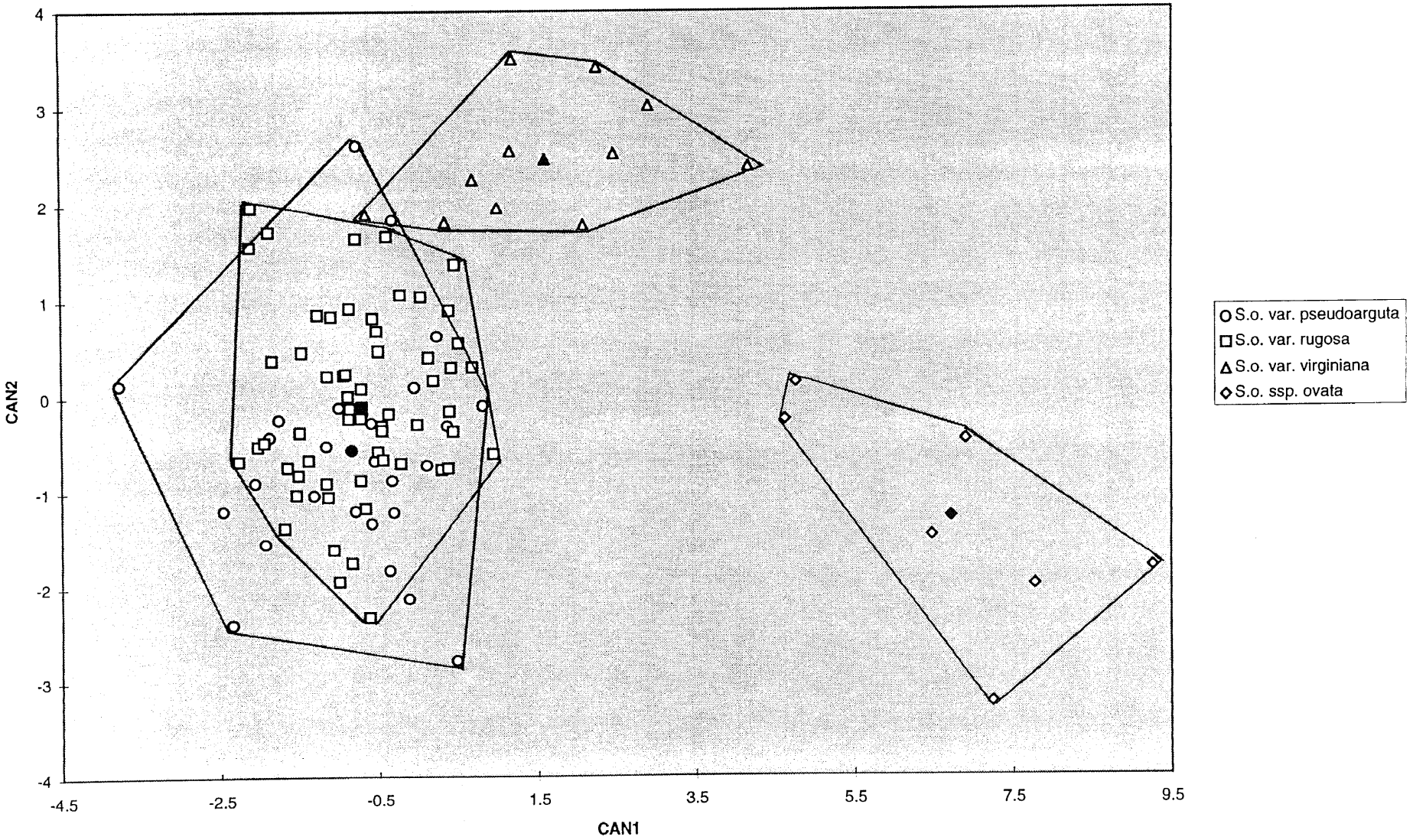

4
3
2
1
0
-1
-2
-3
-4
CAN2
-4.5
-2.5
-0.5
1.5
3.5
5.5
7.5
9.5
CAN1
S.o. var. pseudoarguta
S.o. var. rugosa
S.o. var. virginiana
S.o. ssp. ovata

TABLE 6. Total sample correlation between ln-transformed character measurements and first two canonical discriminant variables (CAN1 and CAN2). See TABLE 2 for character abbreviations.

CHARACTER	CAN1	CAN2
PH	0.533799	0.580079
LLG	0.746745	0.531033
LWD	0.702986	0.390938
FBLG	0.118568	-0.308231
FBWD	0.155295	-0.375315
P1LG	0.589720	0.444032
P3LG	0.339354	0.547484
I1LG	0.756961	0.365681
I3LG	-0.002051	0.648488
P/I1	-0.479285	-0.112079
P/I3	0.461385	-0.254909
SWD	0.567957	0.504481
CLG	0.127384	-0.329212
CLLG	0.118961	-0.154725
CALG	0.208958	0.078174
CRD	0.564315	0.468846
NTD	-0.119232	-0.189159
RLG	0.058298	0.439199
RI	-0.280301	0.412199

value (plus or minus) the greater the character's value in separating taxa.

Duncan's Multiple Range Test

The results of Duncan's multiple range test showed the means of three characters to be significantly different for three of the four taxa, while thirteen characters were sufficient for separating the four taxa into two groups of two taxa each. A total of three characters showed no significant mean differences for any of the taxa (Table 7). *S. ovata* var. *virginiana* and *S. ovata* ssp. *ovata* had significantly larger means than *S. ovata* var. *pseudoarguta* and *S. ovata* var. *rugosa* for seven characters. These characters, shown in bold face in Table 7, included plant height (PH), leaf length (LLG), leaf width (LWD), first petiole length (P1LG), first internode length (I1LG), stem width (SWD), and cordateness (CRD). A total of eleven character means were significantly different between *S. ovata* var. *virginiana* and *S. ovata* ssp. *ovata.* Of these characters, leaf width (LWD), floral bract length (FBLG) and width (FBWD), first internode length (I1LG), third petiole/internode ratio (P/I3) and corolla length (CLG) were larger for *S. ovata* ssp. *ovata* while third petiole length (P3LG), third internode length (I3LG), first petiole/internode ratio (P/I1), raceme length (RLG) and raceme internode length (RI) were larger for *S. ovata* var. *virginiana.* There were no characters of the nineteen measured that showed significant mean differences between *S. ovata* var. *pseudoarguta* and *S. ovata* var. *rugosa.* Floral bract width (FBWD), first petiole/internode ratio (P/I1) and corolla length (CLG) showed slight, but not

Table 7. Mean values ± 1 SD for morphological characters measured in the *Scutellaria ovata* complex. Values with different superscripts for any character are significantly different ($p \leq 0.05$).

Character	*S. o. pseudoarguta*	*S. o. rugosa*	*S. o. virginiana*	*S. ovata ovata*
PH	$154.68^{B} \pm 65.83$	$196.64^{B} \pm 69.30$	$358.32^{A} \pm 54.49$	$329.28^{A} \pm 89.96$
LLG	$29.39^{B} \pm 9.07$	$31.46^{B} \pm 9.20$	$69.63^{A} \pm 17.68$	$76.07^{A} \pm 10.95$
LWD	$14.34^{C} \pm 4.62$	$15.56^{C} \pm 6.67$	$29.41^{B} \pm 6.73$	$36.39^{A} \pm 3.44$
FBLG	$3.25^{AB} \pm 0.856$	$3.17^{AB} \pm 0.707$	$2.82^{B} \pm 0.328$	$3.61^{A} \pm 0.375$
FBWD	$2.13^{AB} \pm 0.618$	$2.07^{B} \pm 0.457$	$1.79^{B} \pm 0.321$	$2.48^{A} \pm 0.203$
P1LG	$15.02^{B} \pm 6.67$	$16.04^{B} \pm 7.23$	$34.17^{A} \pm 13.13$	$34.63^{A} \pm 7.69$
P3LG	$17.10^{B} \pm 6.96$	$17.38^{B} \pm 10.47$	$36.35^{A} \pm 9.43$	$23.95^{B} \pm 9.35$
I1LG	$15.62^{C} \pm 10.09$	$18.46^{C} \pm 8.69$	$47.13^{B} \pm 18.30$	$80.83^{A} \pm 31.32$
I3LG	$13.57^{B} \pm 8.23$	$17.35^{B} \pm 11.06$	$31.91^{A} \pm 14.50$	$14.73^{B} \pm 18.15$
P/I1	$1.12^{A} \pm 0.409$	$0.934^{AB} \pm 0.359$	$0.762^{B} \pm 0.223$	$0.460^{C} \pm 0.121$
P/I3	$1.43^{B} \pm 0.598$	$1.11^{B} \pm 0.498$	$1.27^{B} \pm 0.457$	$2.59^{A} \pm 1.09$
SWD	$1.13^{B} \pm 0.376$	$1.36^{B} \pm 0.329$	$1.95^{A} \pm 0.282$	$1.99^{A} \pm 0.256$
CLG	$15.06^{AB} \pm 1.73$	$14.42^{B} \pm 2.00$	$13.76^{B} \pm 2.04$	$15.92^{A} \pm 1.03$
CLLG	$3.71^{A} \pm 0.827$	$3.93^{A} \pm 1.03$	$3.62^{A} \pm 0.951$	$4.37^{A} \pm 0.889$
CALG	$5.00^{A} \pm 0.935$	$4.94^{A} \pm 0.688$	$5.24^{A} \pm 0.289$	$5.50^{A} \pm 0.915$
CRD	$0.968^{B} \pm 0.819$	$1.09^{B} \pm 1.10$	$3.66^{A} \pm 1.19$	$3.45^{A} \pm 0.805$
NTD	$1.21^{A} \pm 0.289$	$1.19^{A} \pm 0.292$	$1.05^{A} \pm 0.246$	$1.11^{A} \pm 0.204$
RLG	$55.52^{B} \pm 27.07$	$63.96^{B} \pm 37.03$	$95.27^{A} \pm 42.02$	$56.57^{B} \pm 29.88$
RI	$10.28^{A} \pm 3.91$	$10.90^{A} \pm 4.65$	$13.23^{A} \pm 4.33$	$5.73^{B} \pm 1.63$

significant, differences.

Population Range Diagrams

In order to further show differences between character means of taxa, population range diagrams were constructed for six of the most important characters used for separating members of the complex. These diagrams show ranges, means, standard deviations and standard errors of characters for each taxon. The range and mean are represented by the vertical and horizontal lines, respectively. The open box shows the standard deviation and the closed box is the standard error. Diagrams for plant height (PH) (Figure 14) and stem width (SWD) (Figure 15) show separation of *S. ovata* var. *virginiana* and *S. ovata* ssp. *ovata* from *S. ovata* var. *pseudoarguta* and *S. ovata* var. *rugosa.* However, plant height and stem width are not useful characters for separating *S. ovata* var. *virginiana* from *S. ovata* ssp. *ovata. S. ovata* ssp. *ovata* separated from the other three taxa by having the largest values for leaf width (LWD) (Figure 16) and first internode length (I1LG) (Figure 17), while *S. ovata* var. *virginiana* had the longest racemes (RLG) (Figure 18) and raceme internodes (RI) (Figure 19). The raceme internodes of *S. ovata* ssp. *ovata* were significantly shorter than those of the other three taxa.

Scatter Diagrams

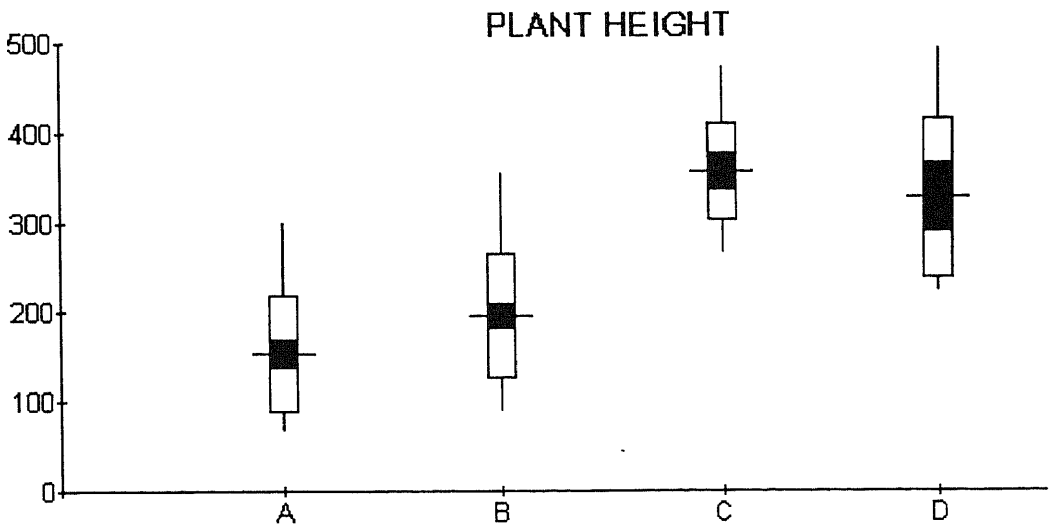
Two dimensional scatter diagrams were plotted in order to show the relationship between taxa using two significant characters. Figure 20 shows

Figure 14. Population range diagram of **plant height** for specimens of the *Scutellaria ovata* complex.

A- *S. ovata* var. *pseudoarguta*
B- *S. ovata* var. *rugosa*
C- *S. ovata* var. *virginiana*
D- *S. ovata* ssp. *ovata*

Figure 15. Population range diagram of **stem width** for specimens of the *Scutellaria ovata* complex.

A- *S. ovata* var. *pseudoarguta*
B- *S. ovata* var. *rugosa*
C- *S. ovata* var. *virginiana*
D- *S. ovata* ssp. *ovata*


PLANT HEIGHT
500
400
300
200
100
0
A
B
C
D

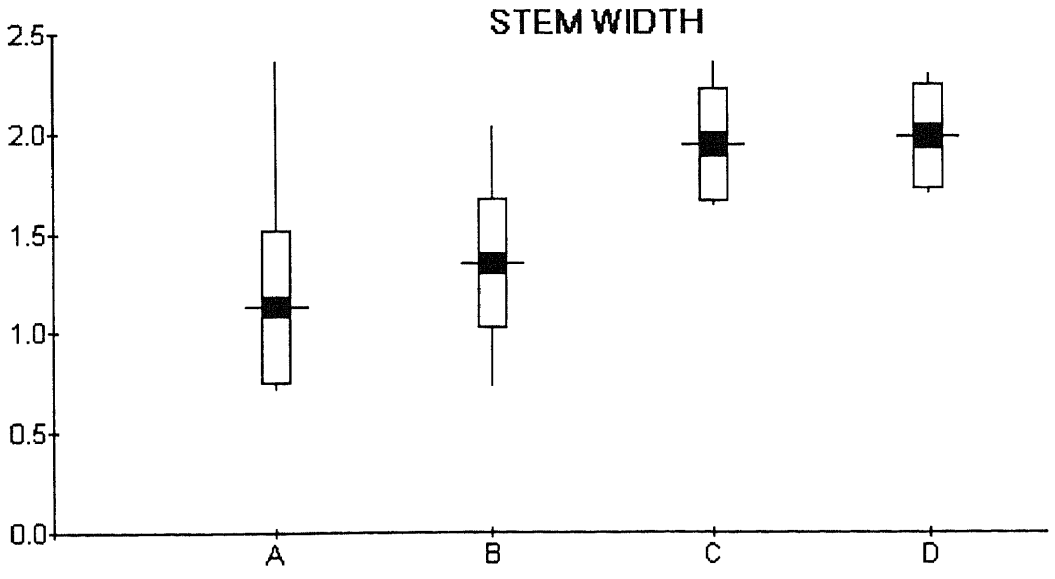
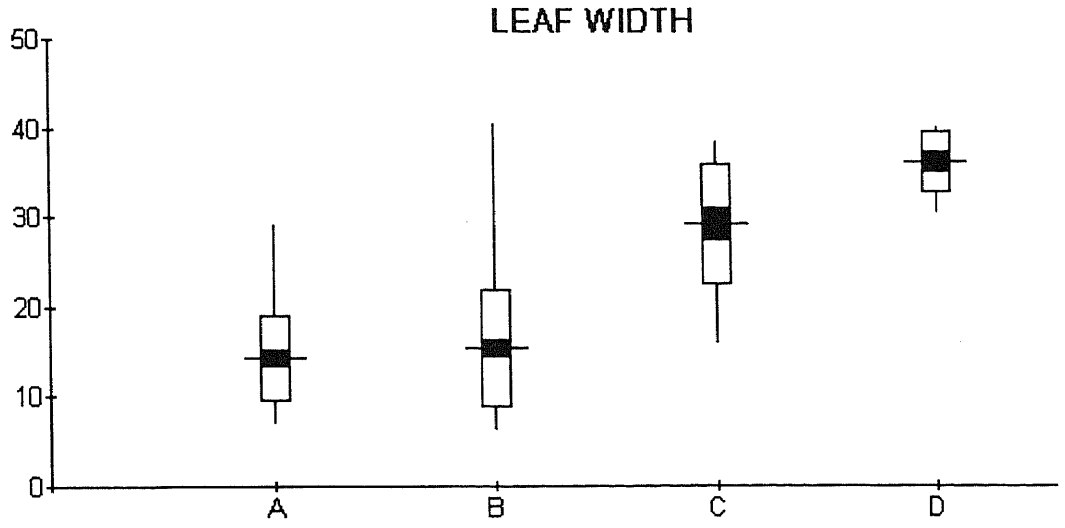

STEM WIDTH
2.5
2.0
1.5
1.0
0.5
0.0
A
B
C
D

Figure 16. Population range diagram of **leaf width** for specimens of the *Scutellaria ovata* complex.

A- *S. ovata* var. *pseudoarguta*
B- *S. ovata* var. *rugosa*
C- *S. ovata* var. *virginiana*
D- *S. ovata* ssp. *ovata*

Figure 17. Population range diagram of **1st internode length** for specimens of the *Scutellaria ovata* complex.

A- *S. ovata* var. *pseudoarguta*
B- *S. ovata* var. *rugosa*
C- *S. ovata* var. *virginiana*
D- *S. ovata* ssp. *ovata*


LEAF WIDTH
50
40
30
20
10
0
A
B
C
D

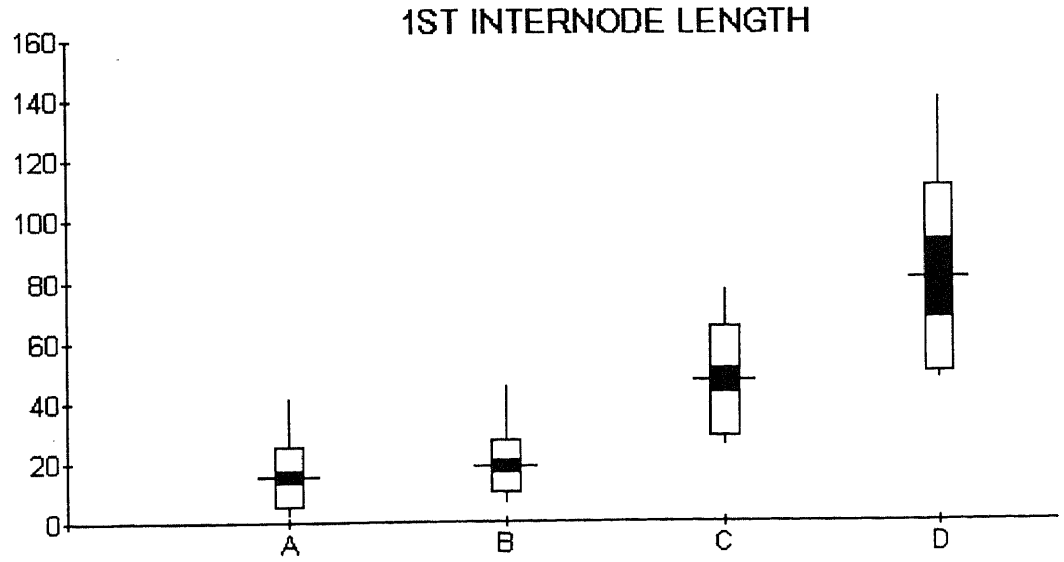
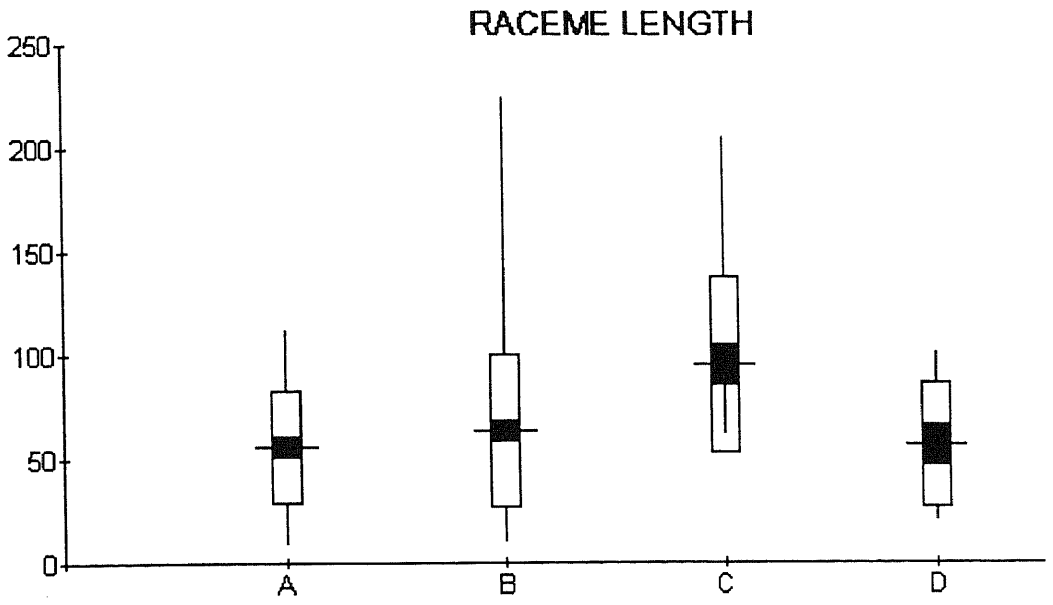

1ST INTERNODE LENGTH
160
140
120
100
80
60
40
20
0
A
B
C
D

Figure 18. Population range diagram of **raceme length** for specimens of the *Scutellaria ovata* complex.

A- *S. ovata* var. *pseudoarguta*
B- *S. ovata* var. *rugosa*
C- *S. ovata* var. *virginiana*
D- *S. ovata* ssp. *ovata*

Figure 19. Population range diagram of **raceme internode length** for specimens of the *Scutellaria ovata* complex.

A- *S. ovata* var. *pseudoarguta*
B- *S. ovata* var. *rugosa*
C- *S. ovata* var. *virginiana*
D- *S. ovata* ssp. *ovata*


RACEME LENGTH
250
200
150
100
50
0
A
B
C
D

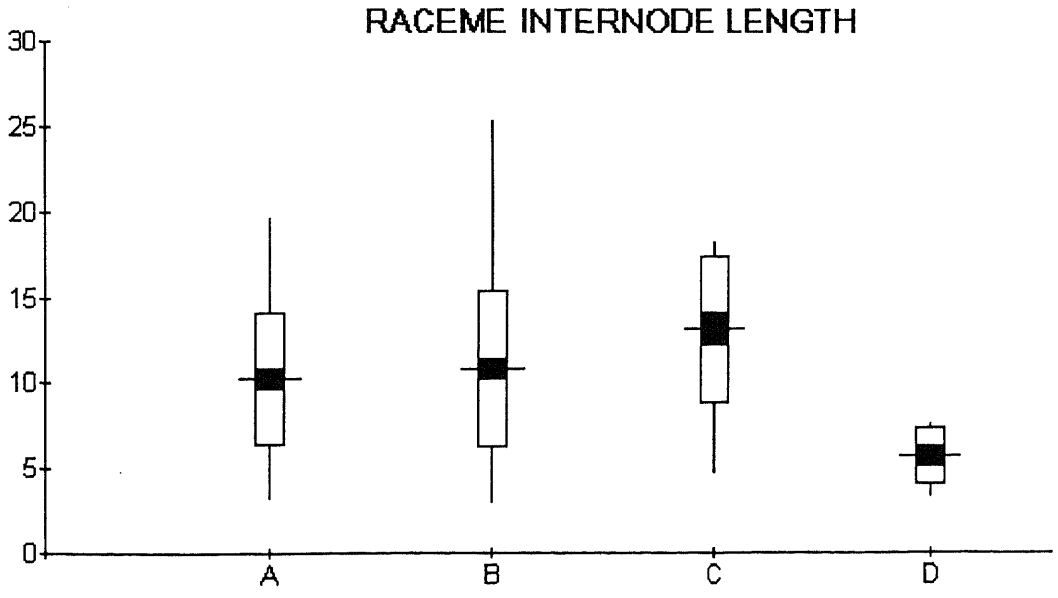
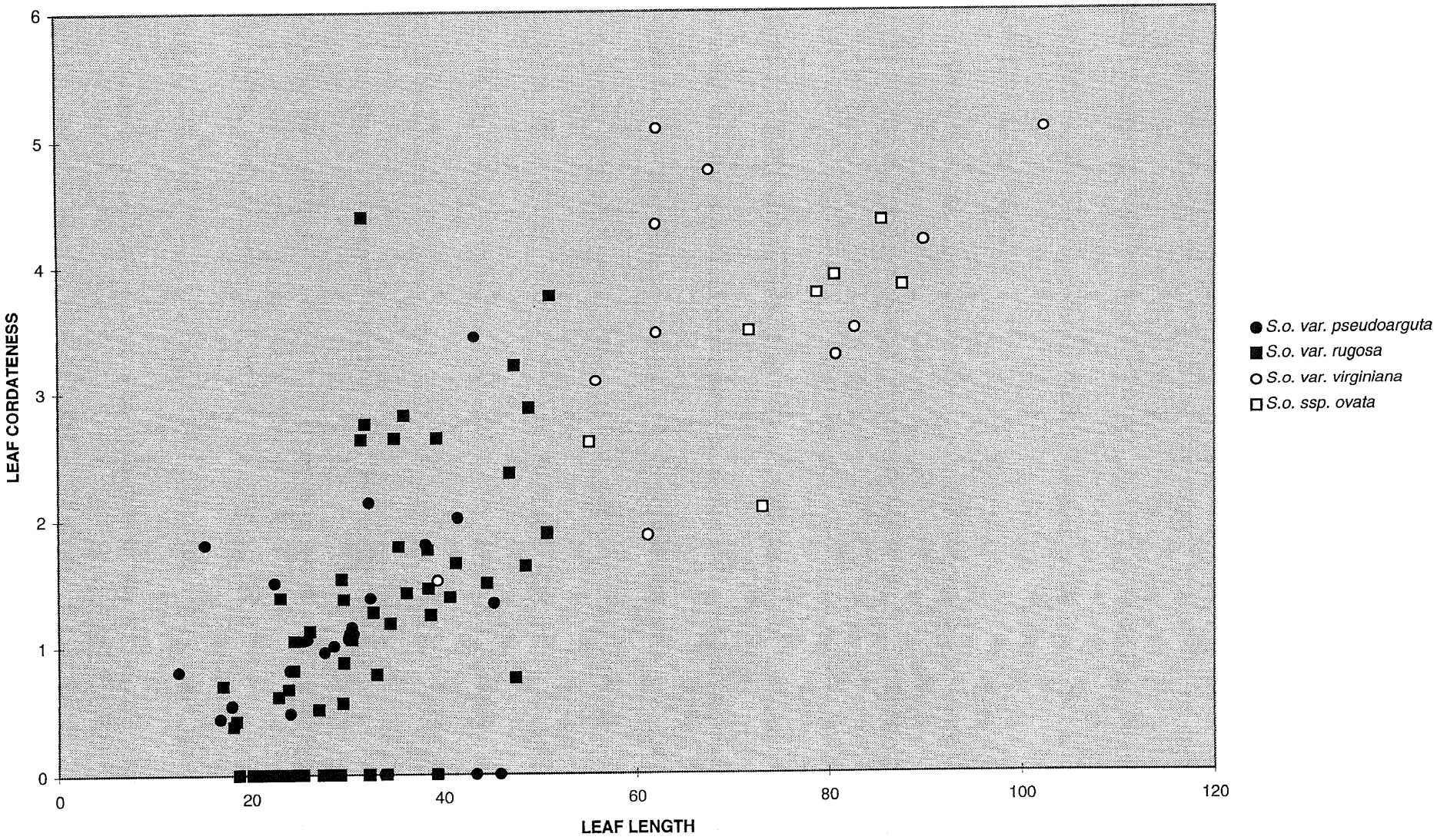

RACEME INTERNODE LENGTH
30
25
20
15
10
5
0
A
B
C
D

Figure 20. Scatter diagram comparing **leaf length** and **leaf cordateness** for members of the *Scutellaria ovata* complex.

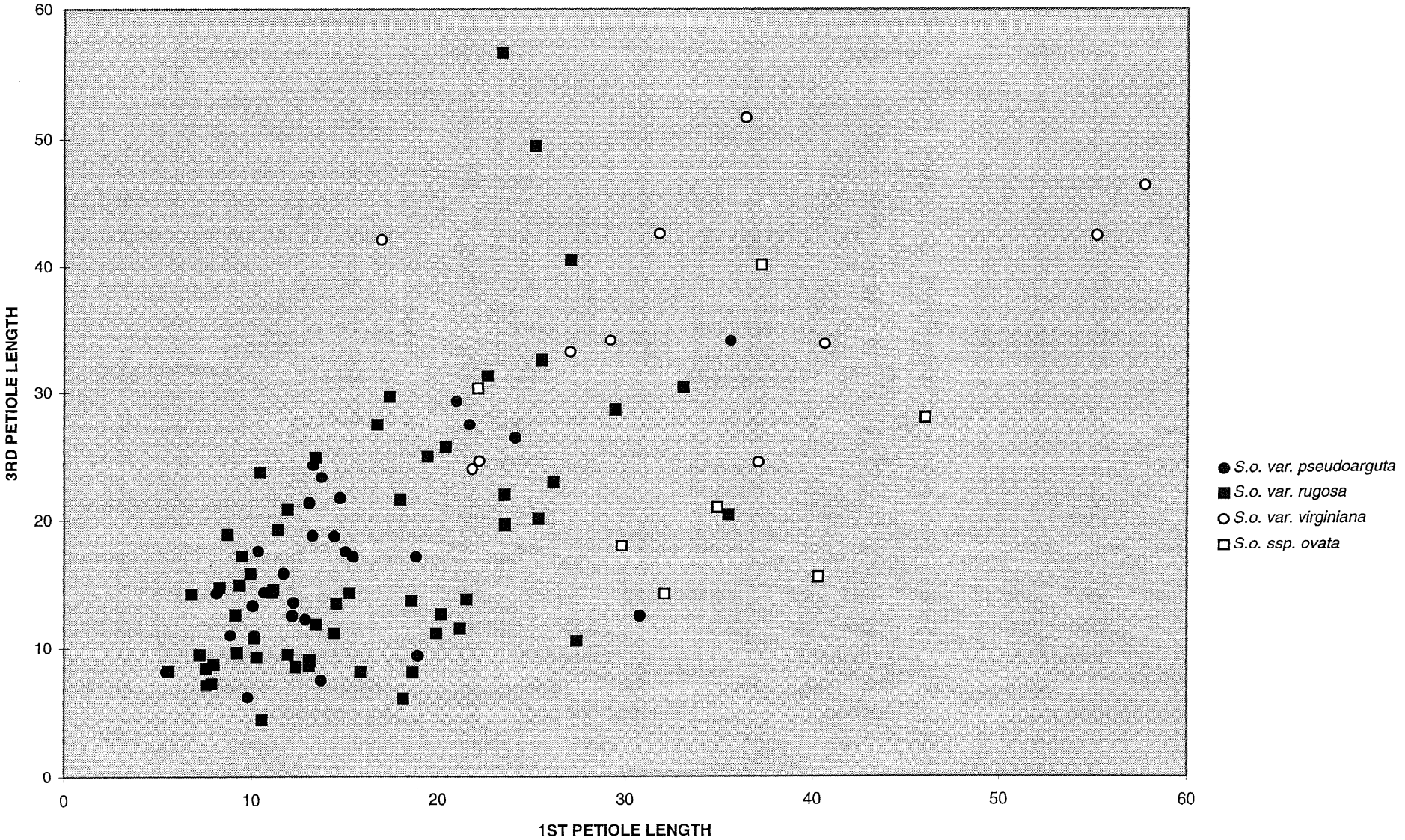

LEAF CORDATENESS
LEAF LENGTH
0
1
2
3
4
5
6
0
20
40
60
80
100
120
S.o. var. pseudoarguta
S.o. var. rugosa
S.o. var. virginiana
S.o. ssp. ovata

that *S. ovata* var. *virginiana* and *S. ovata* ssp. *ovata* have longer, more cordate leaves than the other two taxa. The tendency of these two taxa to cluster together in the upper right corner, away from the specimens of *S. ovata* var. *pseudoarguta* and *S. ovata* var. *rugosa* supports this notion. A similar situation is seen in Figure 21 which shows a general trend toward longer petioles for *S. ovata* var. *virginiana* and *S. ovata* ssp. *ovata.*

Population Comparisons

When *S. ovata* specimens were grouped according to collection site, little variation was seen between or within populations. Principal component analysis showed significant overlap between populations with no pattern of separation. PRIN1 and PRIN2 accounted for 50 percent of the total separation and the strongest characters were the same as those proven to be significant in the comparison of taxa. Canonical discriminant analysis showed separation of western West Virginia and southern Ohio specimens of *S. ovata* ssp. *ovata* along CAN1. The strongest characters used for separating this group are the same as those that can be used to separate *S. ovata* ssp. *ovata* from other taxa. CAN1 and CAN2 accounted for only 57 percent of the total separation and showed plant height (PH), leaf length (LLG) and width (LWD), petiole lengths(P1LG & P3LG), internode lengths (I1LG & I3LG), stem width (SWD) and cordateness (CRD) to be the most important characters. Sites K, L and M (Table 3) had large mean values for many characters and showed a slight separation that was comparable to that of *S. ovata* ssp.

Figure 21. Scatter diagram comparing **1st petiole length** and **3rd petiole length** for members of the *Scutellaria ovata* complex.


60
50
40
30
20
10
0
3RD PETIOLE LENGTH
0
10
20
30
40
50
60
1ST PETIOLE LENGTH
S.o. var. pseudoarguta
S.o. var. rugosa
S.o. var. virginiana
S.o. ssp. ovata

ovata. This is most likely due to the fact that these groups included more specimens of *S. ovata* var. *virginiana* than the smaller taxa, *S. ovata* var. *pseudoarguta* and *S. ovata* var. *rugosa.*

Biochemical Analysis

An assessment of differences in protein patterns in the four *S. ovata* taxa and the outlier species, *S. elliptica,* has yielded information on cytosol proteins. *S. ovata* var. *pseudoarguta, S. ovata* var. *virginiana, S. ovata* var. *rugosa* and *S. ovata* ssp. *ovata* are compared in Figure 22. A 70 kD protein is common to all of the taxa with the exception of *S. ovata* var. *virginiana* (lanes C and D). The more common situation is for proteins to be homogeneous and conserved among the variants. This is evident in the 50, 44, 39, 37, 36, 35 and 33 kD proteins indicated on the gel.

The same taxa are compared in Figure 23 using different populations. In this gel, proteins corresponding with 54, 48, 46, 38, 37, 36 and 30 kD are observed in all of the variants. A 68 kD protein is observed in all of the taxa except *S. ovata* var. *virginiana.* This protein is strongly suspected to be the same as the 70 kD protein observed in Figure 22. The absence of a 32 kD protein is unique to *S. ovata* var. *rugosa.*

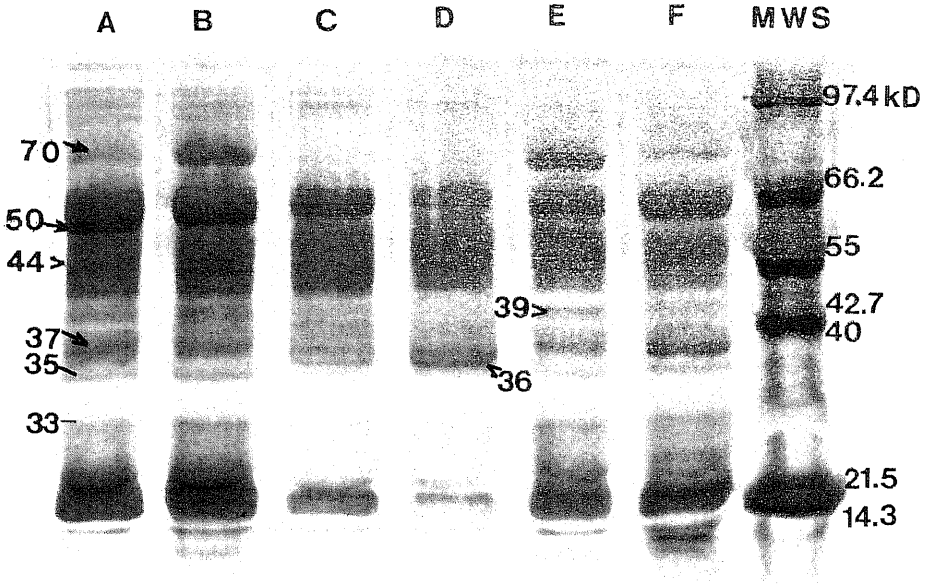
Conserved expression of 50, 47, 42, 39, 31 and 21 kD proteins among *S. ovata* var. *rugosa, S. ovata* ssp. *ovata* and *S. ovata* var. *virginiana* is observed in Figure 24. *S. ovata* var. *virginiana* is marked by the absence of a 76 kD band. However, one of the *S. ovata* var. *rugosa* specimens also

Figure 22. Gel showing variation in protein profiles among *S. ovata* var. *pseudoarguta, S. ovata* var. *rugosa, S. ovata* var. *virginiana* and *S. ovata* ssp. *ovata* specimens. See Table 4 for site descriptions.

A - *S. ovata* var. *pseudoarguta* from Site 1.
B - *S. ovata* var. *pseudoarguta* from Site 5.
C - *S. ovata* var. *virginiana* from Site 5.
D - *S. ovata* var. *virginiana* from Site 5.
E - *S. ovata* var. *rugosa* from Site 4.
F - *S. ovata* ssp. *ovata* from Site 11.
MWS - Molecular Weight Standards

Figure 23. Gel showing variation in protein profiles among *S. ovata* var. *rugosa, S. ovata* var. *pseudoarguta, S. ovata* var. *virginiana* and *S. ovata* ssp. *ovata* specimens. See Table 4 for site descriptions.

A - *S. ovata* var. *rugosa* from Site 7.
B - *S. ovata* var. *rugosa* from Site 8.
C - *S. ovata* var. *pseudoarguta* from Site 9.
D - *S. ovata* var. *pseudoarguta* from Site 10.
E - *S. ovata* ssp. *ovata* from Site 11.
F - *S. ovata* var. *virginiana* from Site 5.
G - *S. ovata* var. *virginiana* from Site 5.
MWS - Molecular Weight Standards


A
B
C
D
E
F
MWS
97.4 kD
66.2
55
42.7
40
21.5
14.3
70
50
44
39
37
35
36
33

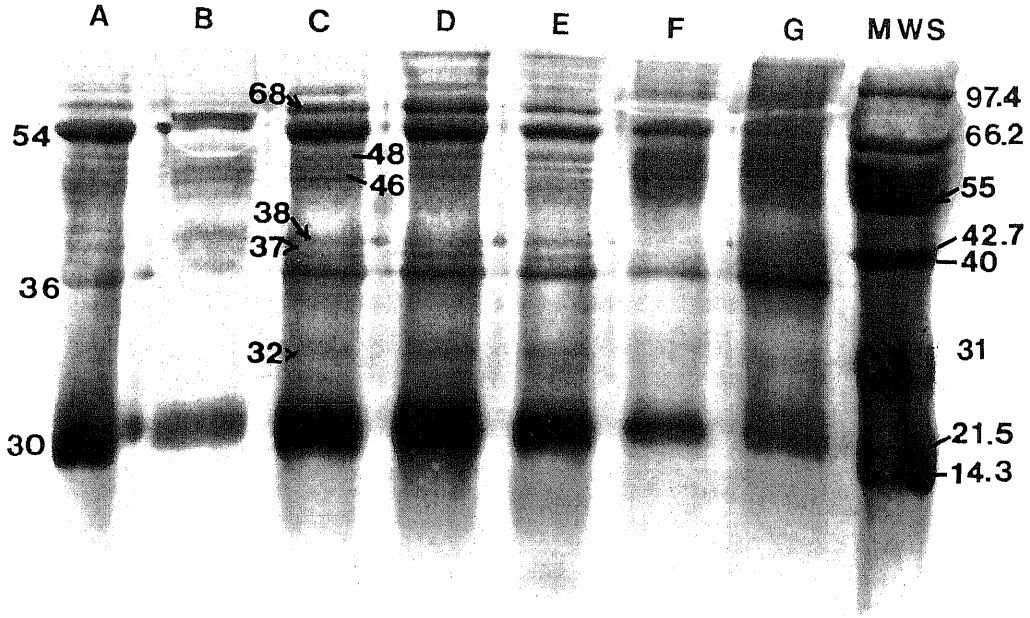
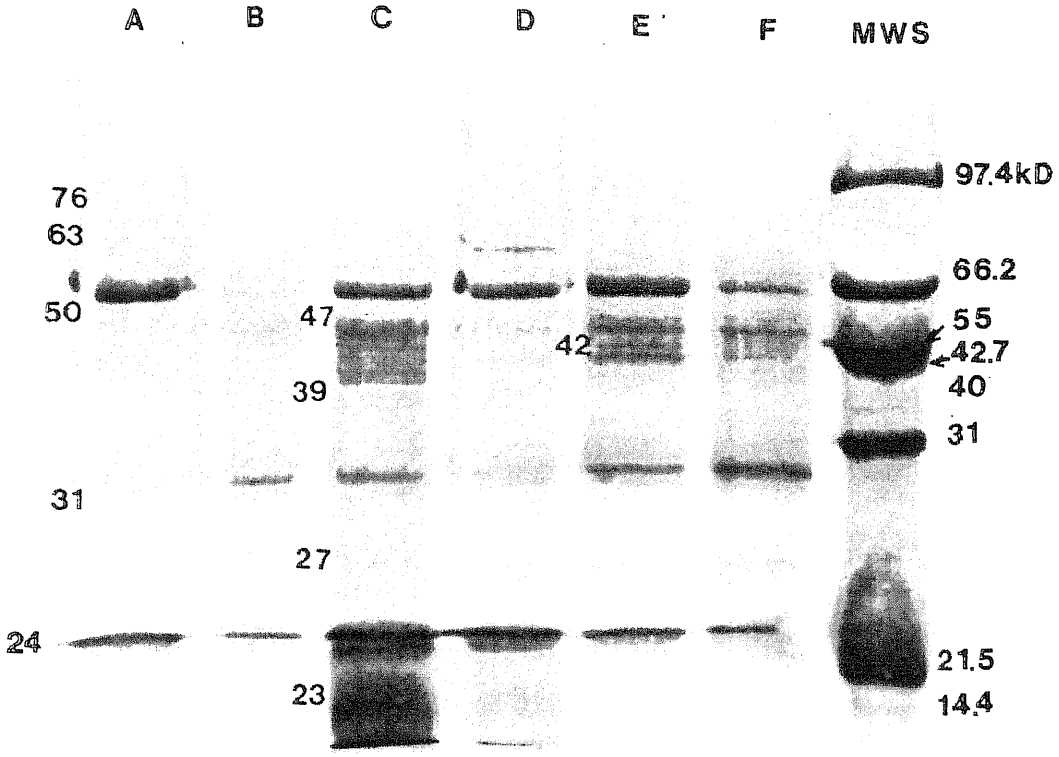

A
B
C
D
E
F
G
MWS
97.4
66.2
55
42.7
40
31
21.5
14.3
68
54
48
46
38
37
36
32
30

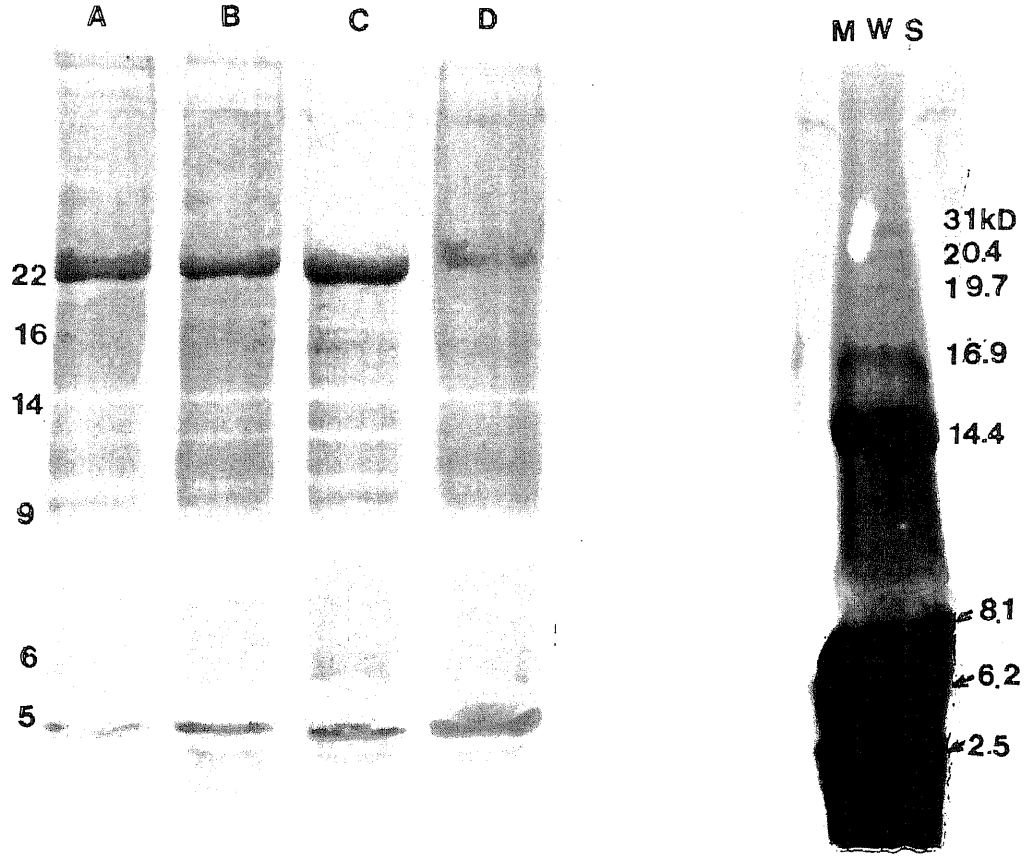
Figure 24. Gel showing variation in protein profiles among *S. ovata* var. *rugosa, S. ovata* ssp. *ovata* and *S. ovata* var. *virginiana* specimens. See Table 4 for site descriptions.

A - *S. ovata* var. *rugosa* from Site 4.
B - *S. ovata* var. *rugosa* from Site 6.
C - *S. ovata* ssp. *ovata* from Site 11.
D - *S. ovata* ssp. *ovata* from Site 11.
E - *S. ovata* var. *virginiana* from Site 5.
F - *S. ovata* var. *virginiana* from Site 5.
MWS - Molecular Weight Standards

Figure 25. Gel showing variation in protein profiles among *S. ovata* specimens from three habitats with an outlier species, *Scutellaria elliptica.* See Table 4 for site descriptions.

A - *S. ovata* var. *rugosa* from Site 4.
B - *S. ovata* ssp. *ovata* from Site 11.
C - *S. ovata* var. *rugosa* from Site 7.
D - *Scutellaria elliptica* from Site 12.
MWS - Molecular Weight Standards


A
B
C
D
E
F
MWS
76
63
50
47
42
39
31
27
24
23
97.4kD
66.2
55
42.7
40
31
21.5
14.4


A
B
C
D
M W S
22
16
14
9
6
5
31kD
20.4
19.7
16.9
14.4
8.1
6.2
2.5

lacks this protein (Lane B, Figure 24). One of the predominant features on this gel is the appearance of a 23 kD band that is unique to *S. ovata* ssp. *ovata.*

The gel represented by Figure 25 was run with 16.5 percent acrylamide and low molecular weight standards to identify lower molecular weight proteins. A high degree of homogeneity is observed in 22, 16, 14, 9, 6 and 5 kD proteins among *S. ovata* ssp. *ovata, S. ovata* var. *rugosa* and the outlier, *S. elliptica.* This reveals similarity between the *S. ovata* complex and an element outside of the complex, but of the same genus.

In order to assess intrapopulation and intrataxonomic variation in protein patterns, Figures 26 and 27 were analyzed. Figure 26 shows variation within and between two populations of *S. ovata* var. *rugosa.* An 84 kD protein is observed in the Dam #10 population but not in the Smoke Hole Caverns population. A 37 kD protein is also observed in the Smoke Hole Caverns population and in one individual of the Dam #10 population. This protein is not found in the other Dam #10 individual and is representative of variations within and among populations of the same taxon.

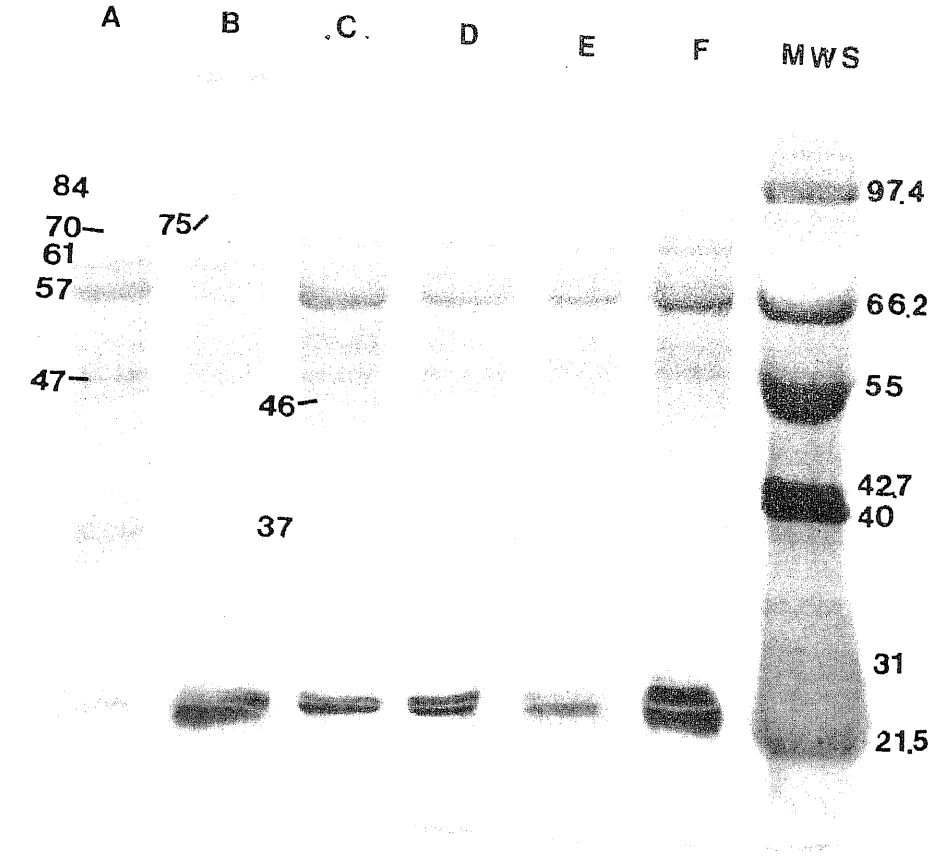
The same variation in lower molecular weight protein patterns is observed in Figure 27. Within five different populations of *S. ovata* var. *pseudoarguta,* four relatively intense protein bands are limited to a single population or are present in only a few. The 31 kD protein that appears in the Upper Kate's Mountain and Lower Kate's Mountain populations is absent in

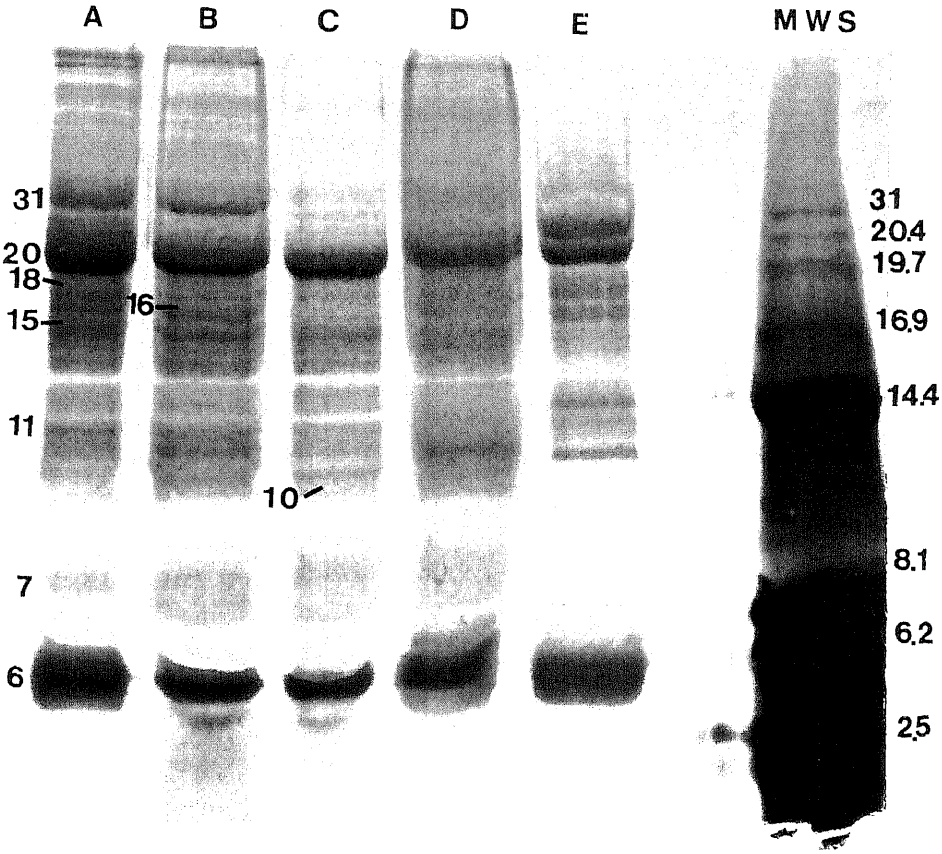
Figure 26. Gel showing interpopulation and intrapopulation variation in protein profiles of *S. ovata* var. *rugosa* specimens.

A, C, D, E - Specimens from Smoke Hole Caverns, Grant County, WV.
B, F- Specimens from Dam #10 shale barren, Pendleton County, WV.
MWS - Molecular Weight Standards

Figure 27. Gel showing interpopulation variation in protein profiles of *S. ovata* var. *pseudoarguta* specimens.

A - Upper Kate's Mt., Greenbrier Co., WV
B - Lower Kate's Mt., Greenbrier Co., WV
C - Blue Bend, Greenbrier Co., WV
D - Greenbrier Mt., Greenbrier Co., WV
E - Upper White's Draft., Greenbrier Co., WV
MWS - Molecular Weight Standards


A
B
C
D
E
F
MWS
84
70
61
57
47
75
46
37
97.4
66.2
55
42.7
40
31
21.5


A
B
C
D
E
MWS
31
20
18
16
15
11
10
7
6
31
20.4
19.7
16.9
14.4
8.1
6.2
2.5

the other populations sampled. Proteins of 7, 10 and 15 kD are observed in all but the Upper White's Draft population.

These intrapopulation and intrataxonomic variations are not sufficient to distinguish taxa based on protein patterns alone. As examples of the complexity of the problem, Figures 28 and 29 show protein patterns for various populations of *S. ovata* taxa. In Figure 28, where proteins from *S. ovata* var. *pseudoarguta* and *S. ovata* var. *rugosa* are separated, no differences are seen between the variants. Further, no apparent differences were noted between shale barren and non-shale barren populations of *S. ovata* var. *rugosa.* Figure 29 shows some variation within populations. A single specimen of *S. ovata* var. *pseudoarguta* from Upper White's Draft has 38 and 35 kD proteins that are not found in any of the other specimens, including another specimen from the same population. A 76 kD protein found in one of the of *S. ovata* var. *rugosa* is not found in any of the other samples.

The protein bands identified in this study are summarized in Tables 8 and 9. The tables show proteins of 30 molecular weights correlated with taxa and populations in which they were found. Those bands which show the most variation are marked with a bold **X**.

SUMMARY AND CONCLUSIONS

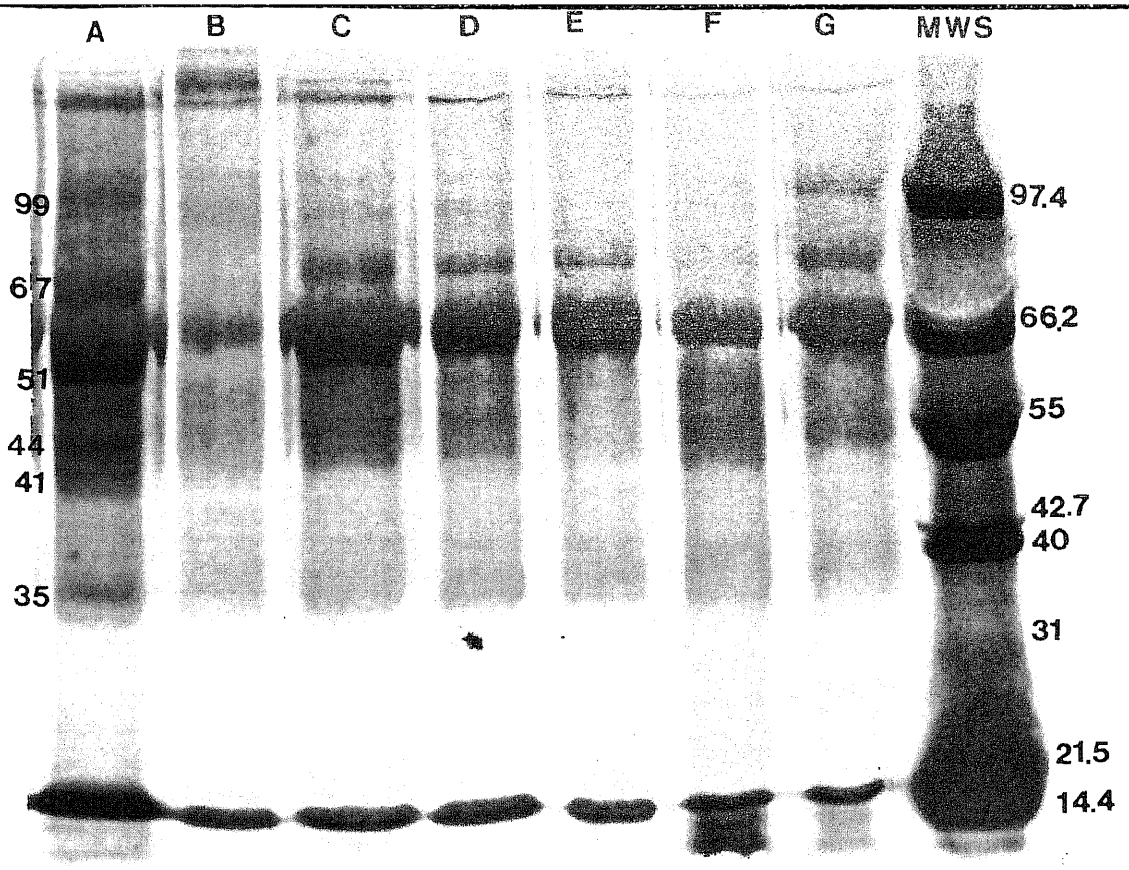
Scutellaria ovata is a highly variable species that has presented many taxonomic problems in the past. Three varieties of the species are known to occur sympatrically in West Virginia and Virginia, while occupying a restricted

Figure 28. Gel showing variation in protein profiles between *S. ovata* var. *pseudoarguta* and *S. ovata* var. *rugosa* specimens. See Table 4 for site descriptions.

A - *S. ovata* var. *pseudoarguta* from Site 2.
B - *S. ovata* var. *rugosa* from Site 4.
C - *S. ovata* var. *pseudoarguta* froom Site 5.
D - *S. ovata* var. *rugosa* from Site 6.
E - *S. ovata* var. *rugosa* from Site 6.
F - *S. ovata* var. *rugosa* from Site 6.
G - *S. ovata* var. *rugosa* from Site 7.
MWS - Molecular Weight Standards

Figure 29. Gel showing variation in protein profiles among *S. ovata* var. *rugosa, S. ovata* var. *pseudoarguta* and *S. ovata* ssp. *ovata* specimens. See Table 4 for site descriptions.

A - *S. ovata* var. *rugosa* from Site 7.
B - *S. ovata* var. *rugosa* from Site 8.
C - *S. ovata* var. *rugosa* from Site 8.
D - *S. ovata* var. *pseudoarguta* from Site 10.
E - *S. ovata* var. *pseudoarguta* from Site 10.
F - *S. ovata* ssp. *ovata* from Site 11.
G - *S. ovata* ssp. *ovata* from Site 11.
MWS - Molecular Weight Standards


A
B
C
D
E
F
G
MWS
99
67
51
44
41
35
97.4
66.2
55
42.7
40
31
21.5
14.4

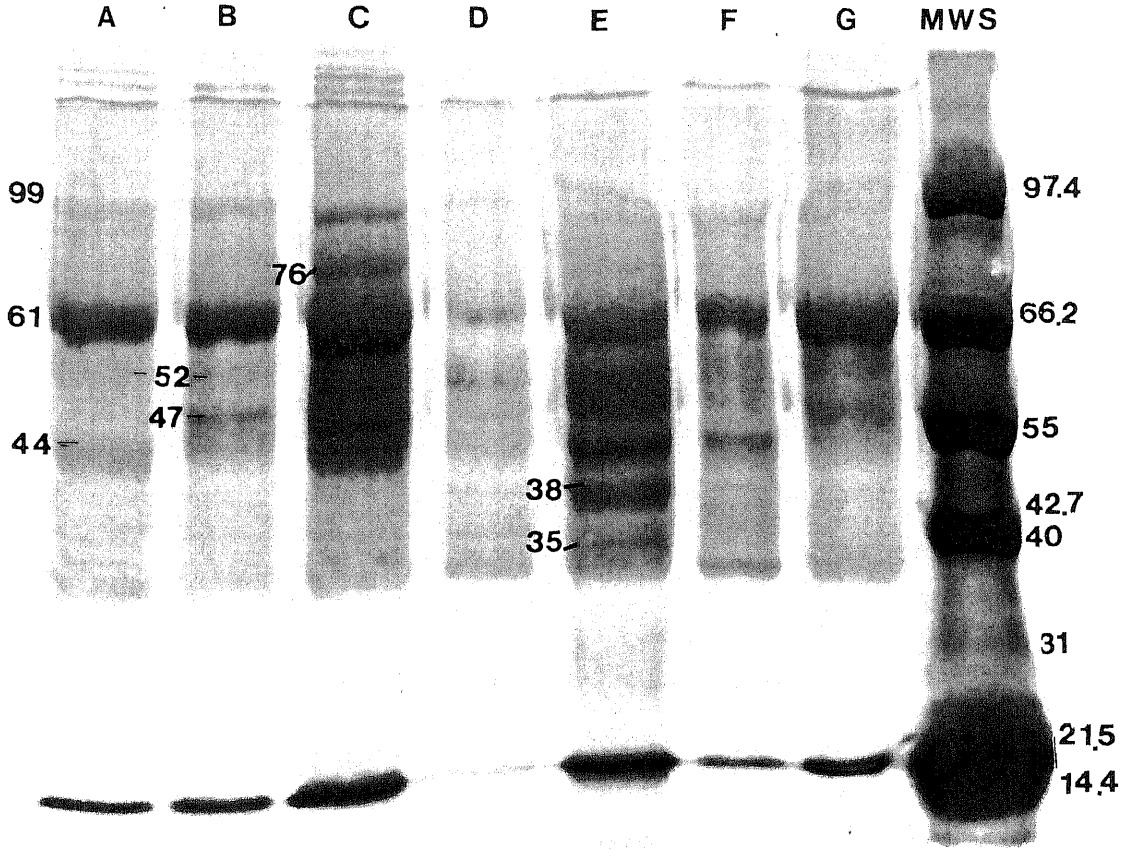

A
B
C
D
E
F
G
MWS
99
61
44
52
47
76
38
35
97.4
66.2
55
42.7
40
31
21.5
14.4

Table 8. Proteins resulting from gel electrophoresis in 12 populations of *S. ovata* representing 4 infraspecific taxa.

Taxa & Site Numbers	Protein Bands (6 - 35 kD)														
S. o. var. *pseudoarguta*	6	7	9	10	14	15	16	21	22	23	30	31	32	33	35
Site 1	X	**X**		**X**		**X**						X		X	X
Site 2	X	**X**		**X**		**X**						X			
Site 5	X	**X**		**X**		**X**								X	X
Site 9	X	**X**		**X**		**X**					X		**X**		
Site 10	X										X		**X**		X
S. o. var. *rugosa*															
Site 4	X		X		X		X	X	X			X		X	X
Site 6								X				X			
Site 7	X		X		X		X		X		X				
Site 8											X				
S. o. var. *virginiana*															
Site 5								X			X	X	**X**	X	X
S. o. ssp. *ovata*															
Site 11	X		X		X		X	X	X	**X**	X	X	**X**	X	X
S. elliptica															
Site 12	X		X		X		X		X						

Table 9. Proteins resulting from gel electrophoresis in 12 populations of *S. ovata* representing 4 infraspecific taxa.

Taxa & Site Numbers	Protein Bands (36 - 84 kD)														
S. o. var. *pseudoarguta*	36	37	38	39	42	44	46	47	48	50	54	68	70	76	84
Site 1	X	X		X		X				X			**X**		
Site 2															
Site 5	X	X		X		X				X			**X**		
Site 9	X	X	X				X		X		X	**X**			
Site 10	X	X	X				X	X	X		X	**X**			
S. o. var. *rugosa*															
Site 4	X	X		X	X	X		X		X			**X**	X	
Site 6				X	X			X		X					
Site 7	X	X	X				X	X	X		X	**X**	**X**		
Site 8	X	X	X				X	X	X		X	**X**	**X**	X	**X**
S. o. var. *virginiana*															
Site 5	X	X	X	X	X	X	X	X	X	X	X				
S. o. ssp. *ovata*															
Site 11	X	X	X	X	X	X	X	X	X	X	X	**X**	**X**	X	
S. elliptica															
Site 12															

geographical and ecological range. These distribution characteristics add to the problems of identification below the species level. However, the results of analysis by PCA, CDA and Duncan's multiple range test presented in this study, show several morphological characters that can be used to separate members of the complex into three groups.

The least amount of morphological variation shown in this study was between *S. ovata* var. *pseudoarguta* and *S. ovata* var. *rugosa.* These taxa were not significantly different for any of the nineteen characters measured, suggesting that they are morphologically identical. Strausbaugh and Core (1977) use growth habit, leaf shape and leaf pubescence to separate these two taxa. These characters are highly variable and not reliable for separation. Presence or absence of leaf pubescence is the best of these characters, but intermediate forms exist making variety determination difficult and inconsistent. These two taxa are scattered in small, isolated populations throughout the shale barren region. This has likely resulted in interbreeding, intergression and selection which may be responsible for the slight population differences, including leaf shape and pubescence. Yet, these morphological differences are apparently not significant enough for these plants to be separated into varieties.

The data for *S. ovata* ssp. *ovata* were interesting in that some of the character means were comparable to *S. ovata* var. *virginiana,* while others were more like those of *S. ovata* var. *pseudoarguta* and *S. ovata* var.

rugosa. For example, the first internode length for *S. ovata* ssp. *ovata* was almost twice as long as that for *S. ovata* var. *virginiana* and over four times those for *S. ovata* var. *pseudoarguta* and *S. ovata* var. *rugosa.* However, the third internode length was less than half that of *S. ovata* var. *virginiana* and not significantly different from *S. ovata* var. *pseudoarguta* or *S. ovata* var. *rugosa.* The same situation existed for the petiole lengths as *S. ovata* var. *virginiana* and *S. ovata* ssp. *ovata* had nearly identical lengths for petioles at the first node and significantly different lengths for petioles at the third node.

In addition to being separated by the above mentioned characters, *S. ovata* ssp. *ovata* has wider leaves, longer and wider floral bracts and shorter, more crowded racemes than the other taxa. This taxon also occupies a different geographical range than the others. Specimens examined in this study were collected in extreme western West Virginia and southern Ohio, while collections of the three varieties came from eastern West Virginia and western Virginia. It may be that *S. ovata* var. *pseudoarguta, S. ovata* var. *rugosa* and *S. ovata* var. *virginiana* are primarily restricted to the Ridge and Valley province of West Virginia while *S. ovata* ssp. *ovata* persists at the western end of the state in the Allegheny Plateau. More field work is required to determine if these taxa are indeed separated by the Alleghenies or if zones of sympatry occur in the central portions of West Virginia.

The results of the biochemical studies revealed a great deal of

homology of proteins among the four elements of the *S. ovata* complex and the outlier species. This suggests that all taxa within the complex are closely related biochemically. Similar protein patterns were shared among shale barren and non-shale barren populations of a single taxon and of different taxa. However, protein patterns among different populations of the same taxon show considerable variation. As a result, at this level of investigation, we cannot conclude that any protein pattern observed in the complex is specific to a particular taxon.

With few exceptions, similarity in protein patterns among taxa in the *Scutellaria ovata* complex support the similarity in morphology reported earlier in this study. In one exception, *S. ovata* ssp. *ovata* shares a common protein pattern with others in the complex, but is quite distinct in morphology. In a second exception, *S. elliptica,* a species outside of the complex and morphologically distinct, has a protein pattern quite similar to *S. ovata* taxa.

Thus, evidence suggests two possible conclusions. One, that protein patterns derived from electrophoretic analysis are not sufficiently sensitive to separate closely related taxa in the complex. Or, two, that there are indeed insignificant biochemical differences among taxa in the *S. ovata* complex.

Isozyme analysis provides a widely used, more specific method for comparing biochemical data from closely related taxa. The method is based on the fact that isozymes are a phenotypic trait which represent an unambiguous phenotype (Rattazzi *et al.,* 1983). Thus, this method allows the

investigator to come closer to the level of the gene in the taxonomic problem. Also, isozyme gels have less protein bands, making them easier to interpret. Future work involving isozyme analysis may prove useful in defining molecular differences among *Scutellaria ovata* elements.

In conclusion, the following statements can be made as a result of the morphometric and biochemical studies that were carried out:

1). Morphological studies revealed three distinct taxonomic groups within the *S. ovata* complex. These taxa are: *S. ovata* var. *pseudoarguta-S. ovata* var. *rugosa* subcomplex, *S. ovata* var. *virginiana* and *S. ovata* ssp. *ovata.*

2). The following morphological characters were found to be the most significant for separating taxa within the *S. ovata* complex: Plant height, leaf length and width, floral bract length and width, petiole lengths, internode lengths, stem width, cordateness, raceme length and raceme internode length.

3). *S. ovata* var. *pseudoarguta* and *S. ovata* var. *rugosa* were found to be morphologically inseparable.

4). *S. ovata* var. *rugosa* is a small plant that is usually less than 30 cm tall, while *S. ovata* var. *virginiana* is a tall variety with large leaves, long petioles, long internodes and long racemes. *S. ovata* ssp. *ovata* has wider leaves, longer and wider floral bracts, a longer first internode and shorter raceme internodes than the other taxa.

5). Little morphological variation exists between or within populations of *S.*

ovata. However, *S. ovata* ssp. *ovata* from western West Virginia and southern Ohio can be separated from eastern West Virginia and western Virginia members of the complex.

6). Gel electrophoresis data suggests that all taxa within the *S. ovata* complex are very similar biochemically and cannot be separated based on protein profiles alone.

7). Protein patterns show some variation when comparing different populations of the same taxon. However, this variation is not consistent.

8). No biochemical differences were seen between shale barren and non-shale barren populations of *S. ovata.*

9). *S. ovata* var. *rugosa* is the most common taxon within the complex with seventeen populations known in eastern West Virginia. *S. ovata* var. *pseudoarguta* is a less common element and is known from only five sites. *S. ovata* var. *virginiana* is also uncommon with records from seven sites in West Virginia. *S. ovata* ssp. *ovata* is the rarest of all the taxa within the state. A single site in Wayne County supports the only known population of these plants in West Virginia.

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Appendix I

CITATION OF SPECIMENS EXAMINED IN MORPHOMETRIC ANALYSIS

Scutellaria ovata Hill var. *pseudoarguta* (Epling) Core

WEST VIRGINIA

Greenbrier County: Upper White's Draft, shale barren: *Evans 3840-1, 3840-2, 3840-3, 3840-4, 3840-5, 3840-6* (MUHW), *Bush 254* (WVA), *Clarkson 2985* (WVA), *Grafton s.n. 9-6-1981* (WVA). *Wieboldt 4766* (VPI). Kate's Mountain, shale barren: *Ewing 2-1, 2-2, 2-3, 2-4, 2-5, 1-1, 1-2* (MUHW), *W.V.U. Botanical Expedition 7-21-1928* (WVA), *Core s.n. 7-19-1932* HOLOTYPE (WVA). Blue Bend, shale barren: *Ewing 5-2, 5-3, 5-5, 5-7, 5-8, 5-10, 5-11* (MUHW), *Grafton s.n. 9-6-1981* (WVA).

Summers County: Camp Lightfoot Road, moist shale barren: *Grafton s.n. 6-22-1978* (WVA).

VIRGINIA

Alleghany County: Mad Ann Ridge, shale barren: *Ludwig 2315* (MUHW).

Scutellaria ovata Hill var. *rugosa* (Wood) Fernald

WEST VIRGINIA

Monroe County: Slaty Mountain, shale barren: *Ewing 6-1, 6-2, 6-5, 6-7, 6-8, 6-9, 6-10* (MUHW), *Jessee s.n. 8-4-1994* (WV Natural Heritage Program Collection).

Pendleton County: Brandywine, shale barren: *Ewing 4-2, 4-4, 4-5, 4-6, 4-7, 4-8* (MUHW), *Core 3663* (WVA). Dam #10 East of Oak Flat, shale barren: *Ewing 8-3, 8-4, 8-5, 8-6, 8-7* (MUHW). Western edge of Little Fork Road, shale barren: *Ewing 3-1, 3-3* (MUHW). Ugly Mountain, shale barren: *Stevens 1303* (WVA). Smoke Hole Cave, dry rocky soil: *Grafton s.n. 8-22-1976* (WVA). South Fork of South Branch of Potomac, shale barren: *Taylor s.n. 8-15-1978* (WVA).

Grant County: Hillside East of Smoke Hole Caverns, xeric: calcareous woodland, *Ewing 7-3, 7-4, 7-5, 7-6* (MUHW). North Mill Creek: *W.V.U. Botanical Expedition 7-6-1926* (WVA).

Hampshire County: Sector, wooded shale barren: *Grafton s.n. 6-5-1982* (WVA). North River, shale barren: *Jessee s.n. 8-10-1994* (WV Natural Heritage Program Collection).

Morgan County: Great Cacapon: *Davis 2915* (WVA).

Fayette County: Gauley Bridge, thin woods between sandstone ledges: *Wieboldt 5261* (WVA).

Hardy County: Durgon, shale barren: *Frye s.n. 6-25-1941* (WVA), *Jessee s.n. 8-4-1994* (WV Natural Heritage Program Collection). High Knob Mountain northeast of Old Fields: *Wratchford 902* (WVA).

VIRGINIA

Bath County: Southwest of Millboro, shale barren: *Wieboldt 4350-A* (WVA) *Sargent s.n. 8-5-1950* (WVA), *Wieboldt 4811, 4350-A, 4158* (VPI), *Uttal s.n.*

7-27-1960, 3012 (VPI). Short Mountain, rocky shale barren: *Ludwig 2325* (MUHW). Shenandoah Mountain, shale ridge bald: *Ludwig 2362, 2365* (MUHW).

Giles County: Glenlyn, rocky hillside: *Core 2987* (WVA).

Alleghany County: Longdale Furnace, shale barren: *Wieboldt 4392,4822* (VPI).

Rockbridge County: Natural Bridge: *Uttal 56643* (VPI).

Botetourt County: Oriskany, shale cliff: *Wieboldt 3404* (VPI). Appalachian Trail in woods below Blue Ridge Parkway: *Freer 12380* (VPI). Patterson Mountain, shale barren: *Wieboldt 4382* (VPI).

Roanoke County: Dixie Caverns, dolomitic bluffs: *Uttal 6549* (VPI).

Scutellaria ovata Hill var. *virginiana* (Epling) Core

WEST VIRGINIA

Greenbrier County: Kate's Mountain, shale barren: *Grafton s.n. 8-20-1979* 2 specimens (WVA).

Pendleton County: Smoke Hole Cave, dry rocky soil: *Bartgis 1032* (WVA).

Morgan County: Cacapon State Park: *Duppstadt s.n. 6-22-1977* (WVA).

Hampshire County: Romney: *Strausbaugh s.n. 6-15-1955* (WVA).

VIRGINIA

Bedford County: Peaks of Otter, Flat Top Mountain, wooded mountain

slopes on rocks: *Pittman 131* (VPI), *Wieboldt 4382* (VPI), *Palmer 161*, *Freer and Ramsey* 4336 (VPI).

Roanoke County: Poor Mountain, dry mixed hardwoods: *Wieboldt 6727* (VPI). Fort Lewis Mountain, dry mixed hardwoods: *Wieboldt 7303* (VPI).

Giles County: Glenlyn: *Phillips s.n. 7-21-1968* (WVA).

Scutellaria ovata Hill ssp. *ovata*

WEST VIRGINIA

Wayne County: Beech Fork Lake and dam area, west facing slope of Beech-Maple forest: *Frisch 12* (MUHW).

OHIO

Athens County: Crumley's Run, mixed woodland: *Hall 1403* (BHO). Stroud's Run: *Cantino 1232, 1276* (BHO).

Adams County: Donaldson Run: *Cusick 20842* (BHO).

Pickaway County: Darby Creek: *Bartley 20010* (BHO).

Ross County: Mt. Logan: *Hall 1210* (BHO).

Appendix II

CITATION OF SPECIMENS EXAMINED IN BIOCHEMICAL STUDIES

Scutellaria ovata Hill var. *pseudoarguta* (Epling) Core

WEST VIRGINIA

Greenbrier County: Upper White's Draft, shale barren: *Ewing 10-1, 10-2, 10-3* (MUHW). Kate's Mountain, shale barren: *Ewing 1-8, 2-6, 2-7, 2-8, 2-9* (MUHW).

Blue Bend, shale barren: *Ewing 5-6, 5-12, 5-13, 5-14, 5-15, 5-16* (MUHW).

Greenbrier Mountain, shale barren: *Ewing 9-1* (MUHW).

Scutellaria ovata Hill var. *rugosa* (Wood) Fernald

WEST VIRGINIA

Monroe County: Slaty Mountain, shale barren: *Ewing 6-4, 6-11, 6-12, 6-13, 6-14, 6-15* (MUHW).

Grant County: Hillside East of Smoke Hole Caverns, xeric calcareous woodland: *Ewing 7-7, 7-8, 7-9, 7-10* (MUHW).

Pendleton County: Brandywine, shale barren: *Ewing 4-10, 4-11, 4-12, 4-13, 4-14* (MUHW). Dam #10 East of Oak Flat, shale barren: *Ewing 8-1, 8-2, 8-9, 8-10, 8-11, 8-12* (MUHW).

Scutellaria ovata Hill var. *virginiana* (Epling) Core

WEST VIRGINIA

Greenbrier County: Blue Bend, shale barren: *Ewing 5-17, 5-18* (MUHW).

Scutellaria ovata Hill ssp. *ovata*

WEST VIRGINIA

Wayne County: Beech Fork Lake Dam area, North facing slope of moist woodland: *Ewing 11-1, 11-2* (MUHW).

Appendix III

COLLECTION SITES FOR *Scutellaria ovata* IN WEST VIRGINIA

Site 1- Kate's Mountain, Greenbrier County, WV.

30 July 1994. This site was on a southwest facing shale barren near the top of the mountain. A population of 150+ plants was concentrated on the disturbed area near the road. Other species present included *Allium oxyphilum, Clematis albicoma* and *Convolvulus purshianus.* Five plants were potted for live material and two were collected for pressing.

21 June 1995. Collected one plant for live material. This population seemed to be a little smaller than the previous year.

Site 2- Kate's Mountain, Greenbrier County, WV.

30 July 1994. This site is located about half-way up the mountain and appears to be the largest barren on Kate's Mountain. This area was characterized by several endemics, including *Allium oxyphilum, Clematis albicoma, Convolvulus purshianus* and *Trifolium virginicum.* The *Scutellaria ovata* population was represented by at least 500 plants. Four specimens were collected for pressing and one for live material.

21 June 1995. Collected four plants for live material. The population seemed stable and very similar to the previous year.

Site 3- Little Fork Shale Barren, Pendleton County, WV.

4 August 1994. This site, which is on land owned by the United States Navy, is a south facing shale barren with good cover and a variety of shale barren

endemics. The population of *Scutellaria ovata* was thinly distributed throughout the barren in clusters of 10-20 plants, with the largest groups being found on large rock outcrops. Most of the plants were immature and insect damaged. A total of four plants were collected; two for pressing and two for potting. This site was not visited in the summer of 1995 because of limited access to the land.

Site 4- Brandywine Shale Barren, Pendleton County, WV.

5 August 1994. Steep south facing barren with good cover and many shale barren endemics including *Clematis albicoma, Oenothera argillicola, Trifolium virginicum* and *Convolvulus purshianus. Scutellaria ovata* populations were distributed throughout the slope in many groups of 15-50 plants. There were also a few clusters of 70-100 plants. Collections were made from these larger groups. Plants were healthy with only slight insect damage and many specimens were mature. Six plants were collected for pressing and three were collected for live material.

24 June 1995. Collected five live plants for biochemical studies. A good population of *S. ovata* existed, but most plants were immature. The flowering period may start later in the summer in this area of West Virginia.

Site 5- Blue Bend Shale Barren, Greenbrier County, WV.

19 August 1994. This shale barren, which was characterized by a diverse group of shale barren endemics and other common barren species, supported a very large and healthy population of *Scutellaria ovata* with

hundreds of mature specimens. On a south facing ridge line with good cover, three plants were collected for pressing and two were collected for potting. Just west of this ridge line on a steep, shaly southwest facing slope, two plants were collected for live material and four were collected for pressing.

22 June 1995. Collected five plants for live material. The population of *S. ovata* was again excellent with many mature specimens. Some very large specimens were found. These were probably *S. ovata* var. *virginiana,* however most plants were smaller.

18 August 1995. Collected two large plants for biochemical studies. These specimens have been identified as *S. ovata* var. *virginiana.*

Site 6- Slaty Mountain Shale Barren, Monroe County, WV.

20 August 1994. This site consisted of many south and southwest facing slopes of very loose shale. Many shale barren endemics were found both above and below the road that cuts through the area. The most common species present were *Convolvulus purshianus, Oenothera argillicola, Eriogonum alleni* and *Antennaria virginica.* A very healthy population of *Scutellaria ovata* was found scattered along the roadside for .50 to .75 miles. Hundreds of mature plants were found near the disturbed area of the road. Seven specimens were collected for pressing and three were collected for potting.

22 June 1995. Collected five plants for live material. An excellent population of *S. ovata* was present with several large groups spread along the road.

Site 7- Smoke Hole Caverns, Grant County, WV.

3 September 1994. This site is on a dry, south-facing slope that can best be described as a xeric, calcareous woodland. Flora was not typical of a shale barren as no endemics were found. Previous reports listed a population of about 50 specimens of *Scutellaria ovata.* The current population consists of at least 300-400 plants. Many clusters of 20-50 plants were found scattered throughout the slope, with the largest groups being found associated with large limestone outcroppings. A total of six plants were collected; four for pressing and two for potting. This is the only non-shale barren site where populations of *Scutellaria ovata* were found.

24 June 1995. Collected four live plants for biochemical studies. The *S. ovata* population was very good, but nearly all of the plants were immature.

Site 8- Dam #10 Shale Barren, Pendleton County, WV.

3 September 1994. This site is located on a steep southwest facing slope with typical shale barren flora. *Opuntia compressa* and *Clematis albicoma* were prevalent along with a healthy population of *Scutellaria ovata* which consisted of hundreds of plants scattered in small groups throughout the slope. The largest concentration was found in the mid-slope area. Five plants were collected for pressing and three were potted for live material.

24 June 1995. Collected four plants for live material. *The S. ovata* population was similar to last year with a few flowering specimens.

Site 9- Greenbrier Mountain Shale Barren, Greenbrier County, WV.

24 September 1994. This is a large shale barren complex that consists of many south and southwest facing slopes. Several shale barren endemics including *Allium oxyphilum, Eriogonum alleni* and *Clematis albicoma* were found. The largest concentrations of these were located at the higher elevations. Small clusters of *Scutellaria ovata* were found scattered throughout the mountain with the largest groups being from mid to upper-slope. One small group, which was found at the bottom of the slope, consisted mainly of immature plants. Because of the thin distribution of the species, only four plants were collected. One was collected for live material and three were collected for pressing. A few dried fruits were also collected from two mature plants for an attempt at future seed propagation. This site was not visited in the summer of 1995 because of overall inaccessibility and small *S. ovata* populations.

Site 10- Upper White's Draft Shale Barren, Greenbrier County, WV.

30 July 1994. Steep south facing slope with typical shale barren flora. The population of *Scutellaria ovata* consisted of hundreds of plants distributed in the mid-slope area of the barren. Six plants were collected for pressing and two were collected for potting.

22 June 1995. Collected three plants for live material. The *S. ovata* population consisted of hundreds of plants distributed throughout the barren. The highest concentrations were in the mid-slope area.

Site 11- Beech Fork Lake and Dam Area, Wayne County, WV.

28 June 1995. A population of about 100 *S. ovata* ssp. *ovata* specimens was found at the bottom of a north facing slope in a 5m X 2m area. Associated species included *Aesculus octandra, Fagus grandifolia, Acer saccharum, Lindera benzoin, Polystichum acrostichoides, Podophyllum peltatum* and *Asarum canadense.* This is the only known population of this taxon in the state. One plant was collected for biochemical studies and one plant was pressed for a herbarium voucher specimen.

Other sites visited:

-Little Cacapon Shale Barren B, Hampshire County, WV.

2 September 1994. Extensive search turned up only three specimens of *Scutellaria ovata* so no collections were made.

-Little Cacapon Shale Barren C, Hampshire County, WV.

2 September 1994. *Scutellaria ovata* population was found to be 20-30 plants for the entire barren. No specimens were collected because of the small number of plants.

Appendix IV. Raw data for selected character measurements of *S. ovata.*

OBS	PLTHGT	LEAFLG	LEAFWD	FLBRLG	FLBRWD	PETLG1	PETLG3	INTLG1	INTLG3	PET/INT1
1	293.74	47.44	21.44	5.37	3.07	10.47	23.81	19.49	33.67	0.537
2	181.12	29.25	10.82	4.07	2.63	14.54	13.58	10.86	13.39	1.34
3	130.12	21.52	9.26	3.33	1.76	10.55	4.4	6.62	5.26	1.59
4	157.78	29.51	15.35	4.29	2.75	13.47	11.96	16.7	12.39	0.807
5	103.85	20.14	9.18	3.56	2.49	12.37	8.56	12.93	8.55	0.957
6	213.23	27.03	14.25	3.75	2.54	10.14	10.81	7.5	23.35	1.35
7	258.45	38.68	17.98	4.39	2.7	18.59	13.84	23.92	28.21	0.777
8	91.4	28.59	14.7	3.76	2.66	9.94	15.85	11.18	16.02	0.889
9	147.48	32.24	14.24	4.02	3.01	6.78	14.25	11.86	14.52	0.571
10	114.2	24.73	12.2	2.67	2.12	11.18	14.63	20.5	17.12	0.545
11	161.64	25.4	11.68	3.26	2.5	9.5	17.19	10.9	20.51	0.872
12	97.02	18.73	9.43	5.1	3.02	7.56	8.46	14.2	10.04	0.532
13	218.63	33.07	18.7	3.82	2.37	26.13	22.99	25.86	10.85	1.01
14	143.31	34.49	18.07	3.81	2.44	11.44	19.35	11.04	14.36	1.04
15	161.76	41.29	17.85	4.29	2.95	9.37	14.99	8.08	12.46	1.16
16	183.44	36.21	36.33	3.28	1.75	8.3	14.79	8.68	13.5	0.956
17	118.79	24.09	11.06	3.64	2.16	10.07	13.35	10.78	9.77	0.934
18	108.58	17.97	10.62	3.48	1.76	8.88	11.03	12.07	13.92	0.736
19	119.58	23.02	11.2	3.97	2.73	10.68	14.41	9.1	11.18	1.17
20	105.51	25.49	11.96	3.28	2.39	15.46	17.21	11.16	15.52	1.39
21	70.37	22.9	10.64	2.12	0.98	12.25	13.61	9.91	6.59	1.24
22	94.68	24.66	11.72	1.96	1.4	10.37	17.64	6.75	9.84	1.54
23	203.64	38.18	14.86	4.32	2.6	24.12	26.52	31.32	41.39	0.77
24	135.24	17.04	8.67	2.87	2.16	7.23	9.49	9.58	10.55	0.755
25	219.7	32.3	9.95	3.38	1.86	18.64	8.16	19.12	5.37	0.975
26	149.6	27.46	11.86	3.48	1.85	15.84	8.2	15.44	11.32	1.03
27	110.56	22.97	9.98	3.47	2.12	7.98	8.76	13.48	6.83	0.592
28	97.37	18.44	6.72	3.54	1.91	5.58	8.23	9.59	10.09	0.582
29	216.18	23.64	9.44	3.53	2.46	7.86	7.2	16.68	5.79	0.471
30	127.27	23.87	12.51	3.47	2.35	11.11	14.39	10.03	11.29	1.11
31	113.06	27.66	16.14	2.2	1.58	15.05	17.65	10.8	10.28	1.39

OBS	PLTHGT	LEAFLG	LEAFWD	FLBRLG	FLBRWD	PETLG1	PETLG3	INTLG1	INTLG3	PET/INT1
32	85.28	15.21	10.49	3.67	2.73	5.45	8.18	2.41	8.14	2.26
33	89.18	22.47	12.74	5.16	2.66	12.89	12.32	9.92	15.75	1.3
34	120.06	16.74	8.24	3.58	2.76	10.16	11.03	10.86	12.06	0.936
35	123.26	30.52	15.74	4.09	2.91	14.76	21.83	22.75	15.51	0.649
36	201.05	30.66	11.8	3.68	2.77	14.45	18.89	20.12	18.68	0.718
37	114	25.86	12.19	3.79	2.16	11.73	15.9	7.88	10.04	1.43
38	111.21	24.02	11.43	3.13	2.17	12.19	12.59	10.56	6.39	1.15
39	160.88	43.38	22.84	5.45	4.08	21.67	27.55	9.84	9.59	2.2
40	179.73	45.88	29.08	2.75	1.83	13.32	24.4	13.85	9.6	0.962
41	119.64	28.64	14.75	2.83	1.82	8.15	14.3	6.53	9.25	1.25
42	201.75	32.46	11.94	2.56	1.75	13.28	18.93	14.94	10.51	0.889
43	132.07	30.14	14.09	2.84	1.96	18.84	17.23	18.07	8.44	1.04
44	163.91	32.3	16.65	2.58	1.69	13.77	23.43	12.63	9.54	1.09
45	235.43	39.32	16.26	2.29	1.52	21.17	11.62	31.75	20.16	0.667
46	196.94	34.09	15.68	2.82	1.73	18.14	6.14	23.44	9.75	0.774
47	149.04	23.33	9.68	3.12	1.84	13.09	9.09	18.53	12.88	0.706
48	282.72	34.17	20.95	2.44	1.81	18.93	9.48	37.34	14.23	0.507
49	79.85	12.44	7.18	2.64	1.82	9.8	6.16	6.79	5.71	1.44
50	320.5	39.44	16.22	2.61	1.48	22.19	24.69	26.12	21.97	0.849
51	414.48	61.19	24.35	2.83	2.2	29.18	34.16	30.94	25.32	0.943
52	331.1	62.12	27.14	2.75	2.35	16.96	42.15	43.01	36.04	0.394
53	192.26	24.41	10.46	2.92	1.98	9.14	12.64	20.83	11.95	0.439
54	170.51	22.54	9.52	2.25	1.76	14.45	11.26	9.33	6.1	1.55
55	197.5	35.37	15.07	2.13	1.36	11.94	20.88	10.64	22.38	1.12
56	353.64	62.03	28.19	3.08	1.78	40.62	33.92	48.73	56.47	0.834
57	300.55	29.44	12.71	2.74	1.75	20.4	25.8	31	25.48	0.658
58	160.94	48.87	24.08	2.79	2.22	25.35	20.18	18.35	14.22	1.38
59	107.53	26.12	13.98	2.55	1.52	20.17	12.77	20.07	5.15	1
60	287.42	43.23	18.91	2.46	1.4	35.59	34.15	40.87	27.96	0.871
61	360.9	67.54	27.88	3.07	2.16	31.77	42.57	26.1	62.03	1.22
62	143.3	22.91	11.98	3.17	1.72	10.27	9.31	6.43	4.78	1.59

OBS	PLTHGT	LEAFLG	LEAFWD	FLBRLG	FLBRWD	PETLG1	PETLG3	INTLG1	INTLG3	PET/INT1
63	190.52	34.96	20.34	3.89	2.02	16.74	27.58	15.24	38.3	1.09
64	206.38	30.48	20.17	3.43	1.79	8.73	18.97	16.6	23.25	0.526
65	142.02	24.48	14.65	3.53	2.52	7.59	7.13	9.17	8.91	0.828
66	249.84	29.66	12.68	2.82	1.52	35.48	20.52	18.79	20.44	1.89
67	215.37	31.65	34.54	2.82	1.77	23.37	56.74	45.02	24.5	0.519
68	239.86	32.73	12.88	2.94	2.21	21.52	13.89	14.28	14.95	1.51
69	354.82	40.67	19.67	2.19	1.46	33.04	30.42	20.09	12.3	1.64
70	166.41	22.83	10.91	3.05	1.86	13.07	8.63	17.83	13.16	0.733
71	296.97	31.9	14.54	3.15	2.24	19.9	11.29	25.86	16.5	0.769
72	275.66	31.48	13.62	2.29	1.42	17.99	21.72	15.28	10.97	1.18
73	306.14	46.87	21.18	2.48	1.8	22.62	31.35	28.02	27.36	0.807
74	246.38	47.38	20.1	2.37	2.04	29.43	28.68	16.41	24.34	1.79
75	237.3	44.52	19.26	2.9	1.91	27.03	40.48	34.48	37.1	0.784
76	214.43	38.38	20.46	2.37	2.04	23.55	22.03	37.02	17.2	0.636
77	115.24	20.2	6.52	2.75	1.55	9.23	9.65	16.65	9.8	0.554
78	322.62	51.04	40.54	2.8	2.96	25.16	49.47	25.02	41.9	1.01
79	473.2	102.33	35.09	2.02	1.46	37.07	24.63	61.03	18.93	0.607
80	242.23	50.74	23.07	3.05	2.13	25.51	32.61	21.56	36.77	1.18
81	150.14	23.05	11.01	2.73	2.15	15.26	14.39	15.91	12.34	0.959
82	232.8	41.5	17.2	2.89	1.98	30.77	12.6	36.67	15.33	0.839
83	342.55	38.45	18.17	3.22	1.82	13.43	25.01	10.73	26.41	1.25
84	305.49	35.92	14.93	2.15	1.58	23.56	19.75	39.28	34.68	0.599
85	190.68	39.34	18.66	1.9	1.38	27.38	10.61	36.14	8.19	0.758
86	329.66	48.51	19.12	2.69	1.73	17.4	29.76	22.95	62.81	0.758
87	347.32	80.78	36.11	2.89	1.8	36.37	51.73	52.9	32.91	0.688
88	267.03	55.82	22.7	2.99	1.83	27.04	33.21	54.52	29.18	0.496
89	366.4	82.75	34.13	3.28	1.68	21.82	24.07	26.58	23.68	0.821
90	389.36	89.9	38.68	2.72	1.39	55.16	42.4	76.74	22.42	0.719
91	317.61	62.08	33.06	2.77	1.6	57.71	46.34	71.72	22	0.805
92	104.88	29.62	14.52	3.22	1.72	19.43	25.09	23.2	11.18	0.838
93	296.35	87.69	38.47	3.65	2.33	46.03	28.05	84.2	9.82	0.547

OBS	PLTHGT	LEAFLG	LEAFWD	FLBRLG	FLBRWD	PETLG1	PETLG3	INTLG1	INTLG3	PET/INT1
94	294.53	73.1	34.12	3.43	2.17	40.29	15.63	92.94	5.15	0.434
95	224.49	55.1	30.69	3.5	2.71	29.79	18.08	83.52	5.06	0.357
96	369.99	71.73	34.32	3.8	2.73	34.88	21.08	58.79	11.18	0.593
97	493	78.77	37.5	3.23	2.55	22.12	30.36	49.3	55.44	0.449
98	257.12	80.63	39.44	3.32	2.39	32.06	14.32	56	5.63	0.573
99	369.5	85.49	40.18	4.34	2.47	37.21	40.14	141.03	10.84	0.264
100	188.58	18.13	8.52	2.86	1.88	13.13	9.04	21.75	12.05	0.604
101	203.85	20.98	12.48	2.88	1.93	11.96	9.49	18.78	12.67	0.637
102	298.37	45.25	17.8	3.52	2.04	20.97	29.36	26.02	33.92	0.806
103	235.1	33.88	14.35	2.53	1.83	13.11	21.41	12.77	13.38	1.03
104	178.68	30.3	14.96	3.4	1.82	13.73	7.53	14.54	7.43	0.944

OBS	PET/INT3	STEMWD	CORLG	CORLPLG	CALLG	CORDATE	NUTDIAM	RACLG	RACINTLG	SPECNO
1	0.707	1.38	14.85	5.68	5.55	0.76	0.73	116.96	23.05	B
2	1.01	1.5	14.8	3.57	5.12	0	1.22	49.2	14.62	B
3	0.837	1.12	15.95	4.69	5.18	0	1.74	59.58	15.85	B
4	0.965	1.16	13.62	3.8	5.61	0.56	1.52	54.48	19.89	B
5	1	1.03	15.18	4.33	5.6	0	1.62	21.14	10.74	B
6	0.463	1.41	15.94	4.09	5.29	0.51	1.48	89.39	22.98	B
7	0.491	1.86	14.31	3.82	5.24	1.26	1.02	81.75	25.29	B
8	0.989	1.16	19.48	4.36	5.78	0	1.4	31.9	14.38	B
9	0.981	1.01	15.21	4.68	5.1	0	1.27	43.7	11.15	B
10	0.855	1.21	15.78	3.89	5.61	0	1.39	43.45	15.52	B
11	0.838	1.07	15.93	4.11	5.64	0	1.54	40.7	13.03	B
12	0.843	0.97	17.07	6.73	5.52	0	0.75	33.52	11.62	B
13	2.12	1	16.54	3.89	5.42	0.79	1.48	93.68	13.32	B
14	1.37	1.08	16.43	3.89	5.17	1.19	0.55	55.35	10.23	B
15	1.2	1.21	14.92	5.9	5.37	1.66	0.69	82.48	12.68	B
16	1.09	0.92	16	2.72	5.35	1.43	1.18	72.73	12.66	B
17	1.37	0.96	13.77	2.72	5.08	0.48	0.91	51.88	12.4	A
18	0.792	0.91	18.14	4.74	4.35	0.54	0.63	43.72	8.23	A
19	1.29	0.93	16.43	4.63	4.15	0	0.52	29.78	7.92	A
20	1.11	1.14	13.8	4.12	5.44	1.05	1.41	43.67	7.79	A
21	2.07	0.94	18.24	5.77	3.25	0	1.55	9.26	3.24	A
22	1.79	1.01	15.05	3.3	2.61	0	0.98	37.35	11.52	A
23	0.641	1.57	15.38	4.27	5.88	1.82	0.86	51.21	19.65	A
24	0.899	1.05	15.84	4.84	5.93	0.7	1.45	57.49	11.19	B
25	1.52	1.41	16.57	6.23	4.82	0	1.08	30.17	8.39	B
26	0.724	1.32	15.32	5.17	5	0	1.18	26.53	6.41	B
27	1.28	1.13	17.05	6	3.47	0	1.44	37.38	7.32	B
28	0.816	0.78	15.08	5.46	4.45	0.42	1.52	21.87	8.26	B
29	1.24	1.29	14.42	4.63	5.87	0	1.4	63.67	9.96	B
30	1.27	1.11	11.15	3.38	4.75	0.67	1.14	50.75	7.99	B
31	1.72	0.93	15.35	3.83	6.59	0.96	1.36	46.81	8.5	A

OBS	PET/INT3	STEMWD	CORLG	CORLPG	CALLG	CORDATE	NUTDIAM	RACLG	RACINTLG	SPECNO
32	1.01	0.74	17.7	5.32	5.82	1.81	1.28	34.4	13.59	A
33	0.782	0.86	15.34	4.01	5.67	1.51	1.41	24.47	9.88	A
34	0.915	0.8	17.01	3.97	5.94	0.44	1.37	35.18	14.05	A
35	1.41	1.05	14.86	3.09	4.43	1.16	1.28	39.82	17.41	A
36	1.01	0.82	11.82	2.77	6.03	1.11	1.51	42.41	9.99	A
37	1.58	0.89	15.35	3.83	5.57	1.06	0.75	52.67	5.57	A
38	1.97	0.9	16.33	3.22	5.14	0.82	1.48	44.08	8.31	A
39	2.87	1.18	15.28	3.69	4.09	0	1.58	50.62	11.49	A
40	2.54	1.21	15.25	2.9	6.09	0	1.16	109.88	9.56	A
41	1.55	0.91	15.62	3.27	5.31	1.01	1.25	55.76	13.76	A
42	1.8	1.26	14.33	3.27	5.63	1.39	1.57	111.44	10.78	A
43	2.04	1.12	14.42	2.33	4.82	1.07	0.9	38.49	7.75	A
44	2.46	1.07	15.35	3.83	3.88	2.14	1.53	75.76	12.22	A
45	0.576	1.57	12.93	3.77	3.87	0	1.16	53.84	5.12	B
46	0.629	1.31	13.57	2.38	4.19	0	1.52	58.6	8.51	B
47	0.706	1.07	14.05	3.44	5.07	0	1.33	55.7	10.31	B
48	0.666	2.36	15.83	4.22	4.93	0	1.24	74.97	5.99	A
49	1.08	0.72	15.84	4.23	4.04	0.81	1.14	29.9	4.74	A
50	1.12	1.68	15.36	3.27	5.13	1.53	1.38	123.19	11.86	C
51	1.35	2.33	12.63	2.23	5.42	1.88	1.47	204.59	12.45	C
52	1.17	1.7	11.92	4.91	5.41	5.09	1.17	112.85	18.23	C
53	1.06	1.36	15.53	5.23	4.93	0.82	1.07	36.73	9.69	B
54	1.85	1.33	15.45	4.91	4.83	0	1.04	89.54	3.04	B
55	0.933	1.31	11.96	4.36	4.61	1.8	0.9	59.5	12.56	B
56	0.6	1.66	13.22	4.36	5.17	4.33	1.02	63.74	17.28	C
57	1.01	1.47	13.51	2.74	5.12	1.54	1.34	152.44	11.35	B
58	1.42	1.86	16.01	4.1	4.25	2.88	0.56	70	13.67	B
59	2.48	1.23	11.3	3.88	2.99	1.13	1.17	39.3	6.2	B
60	1.22	1.12	12.24	3.59	5.46	3.45	1.18	90.64	14.2	A
61	0.686	2.36	11.32	4.36	5.84	4.76	1.1	75.1	14.82	C
62	1.95	1.43	14.34	2.83	5.16	0	1.26	69.18	3.76	B

OBS	PET/INT3	STEMWD	CORLG	CORLPG	CALLG	CORDATE	NUTDIAM	RACLG	RACINTLG	SPECNO
63	0.72	1.84	13.22	3.88	4.96	2.65	1.02	25.9	6.79	B
64	0.816	1.02	14.84	4.88	5.01	1.06	0.86	80.82	15.47	B
65	0.8	0.74	13.18	2.04	4.26	1.05	0.79	45.77	15.3	B
66	1	1.73	11.92	2.28	5.43	1.38	1.06	107.82	10.34	B
67	2.32	1.76	10.86	3.06	4.97	4.4	1.18	28.84	6.17	B
68	0.929	1.5	18.48	3.98	3.29	1.28	1.26	46.42	10.71	B
69	2.47	2.04	11.33	3.59	5.64	1.4	1.4	104.16	7.81	B
70	0.656	1.05	9.85	2.71	5.02	0.61	1.37	40.83	11.14	B
71	0.684	1.38	15.39	3.39	3.98	2.76	0.95	118.38	10.76	B
72	1.98	1.64	14.29	2.88	5.84	2.64	0.89	137.9	13.61	B
73	1.15	1.95	13.73	3.31	4.35	2.37	1.14	96.75	10.58	B
74	1.18	1.86	13.73	3.31	4.88	3.22	1.24	59.06	4.9	B
75	1.09	1.88	13.36	2.35	5.08	1.51	1.22	78.04	10.79	B
76	1.28	1.22	13.42	3.24	4.93	1.77	1.61	84.82	3.9	B
77	0.985	1.31	16.64	3.71	5.02	0	1.49	10.85	6.76	B
78	1.18	1.59	13.42	3.24	5.4	3.77	0.87	85.71	11.88	B
79	1.3	1.92	12.29	2.8	5.09	5.09	1.2	79.47	4.78	C
80	0.887	1.93	11.48	2.63	4.1	1.9	1.29	39.78	7.34	B
81	1.17	1.38	10.48	3.24	4.87	1.39	1.4	47.67	6.06	B
82	0.822	1.47	10.96	3.25	3.94	2.02	1.19	56.15	11.76	A
83	0.947	1.51	12.63	3.31	5.64	1.47	1.28	223.62	17.73	B
84	0.569	1.9	15.06	3.75	4.83	2.83	0.59	78.93	10.52	B
85	1.29	1.34	11.36	3.31	2.99	2.65	1.5	37.5	8.09	B
86	0.474	1.99	16.36	4.3	4.33	1.64	0.7	62.22	10.73	B
87	1.57	2.21	12.55	3.36	5.13	3.29	0.75	102.74	14.78	C
88	1.14	1.65	12.82	2.7	4.68	3.09	0.74	62.89	15.35	C
89	1.02	2.02	16.32	4.37	5.39	3.51	1.02	93.75	18.16	C
90	1.89	2.2	15.38	2.7	5.1	4.2	0.81	63.19	9.52	C
91	2.11	1.76	17.56	4.73	5.31	3.47	0.86	66.49	8.27	C
92	2.24	1.09	13.73	3.31	4.78	0.88	1.03	17.63	6.86	B
93	2.86	1.9	15.92	4.37	6.82	3.85	1.28	49.96	5.68	D

OBS	PET/INT3	STEMWD	CORLG	CORLPG	CALLG	CORDATE	NUTDIAM	RACLG	RACINTLG	SPECNO
94	3.04	2.17	16.36	5.45	5.79	2.09	1.19	24.7	3.36	D
95	3.57	1.76	14.52	3.31	6.05	2.61	0.76	86.12	7.53	D
96	1.89	1.82	16.54	5.44	5.36	3.49	1.03	66.59	6.54	D
97	0.548	2.3	15.37	3.95	5.62	3.79	1.02	100.73	7.56	D
98	2.54	1.71	17.61	4.71	3.88	3.93	1.39	47.14	5.46	D
99	3.7	2.3	15.13	3.36	5.01	4.36	1.11	20.72	3.99	D
100	0.75	0.98	15.45	4.91	5.14	0.38	1.32	27.25	9.99	B
101	0.749	1.33	15.94	3.9	5.68	0	1.52	80.1	7.44	B
102	0.866	2.01	14.76	3.97	5.12	1.35	1.38	102.11	15.14	A
103	1.6	1.6	12.92	2.1	5.15	0	1.18	105.82	5.7	A
104	1.01	1.09	14.4	3.51	5.58	1.11	1.4	66.2	6.68	A