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# A Taxonomic Investigation of the Black Ratsnake, Elaphe o. obsoleta (Say) [Reptilia, Squamata, Colubridae], in West Virginia using Morphometric Analyses

Thesis Submitted to The Graduate College of Marshall University

In partial fulfillment of the Requirements for the degree of Master of Science Biological Sciences

By

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Marshall University

May 2007

#### ABSTRACT

A Taxonomic Investigation of the Black Ratsnake, *Elaphe o. obsoleta* (Say) [Reptilia, Squamata, Colubridae], in West Virginia using Morphometric Analyses

### Adam M. Mann

A recent genetic study by Frank T. Burbrink (2000) determined that the common Black Ratsnake, Elaphe o. obsoleta, evolved from three separate evolutionary lineages and can no longer be classified under a single species name. The newly assigned species, which possess ranges that are separated into three regions of the eastern United States by geologic features such as rivers and mountains, are also said to possess distinct morphologic characteristics (Burbrink, 2001). This thesis study was initiated to mirror Burbrink's previous morphometric study and augment a previous lack of specimen data from West Virginia. Black Ratsnake museum specimens, collected from ranges of all three new species, were compared to West Virginia specimens and to Cornsnakes (a statistical outgroup). All specimens were measured for predetermined morphometric characters, including scale counts, scale measurements, and derived characters. Character data were subjected to multivariate statistical tests, including Canonical Discriminant Analysis (CDA), Principal Component Analysis, and Analysis of Variance (ANOVA). A dorsal pattern survey was also performed on Black Ratsnakes (using digital photography) to analyze trends in pattern retention of adult snakes. CDA, PCA, and ANOVA showed little to no significant (P>0.05) separation in Black Ratsnake specimens collected from different geographical areas. Individuals displayed much variation within and among groups. Cornsnake specimens were significantly different (P<0.0001) than Black Ratsnake specimens in all tests. The dorsal pattern survey showed no statistical difference in dorsal blotch retention among Black Ratsnakes of different areas; however, comparisons of mean values showed that one group of specimens displayed a more uniform and darker pigmentation than the other groups of specimens. West Virginia specimens were intermediate between the two pattern extremes.

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# **1.1 BACKGROUND**

Discrepancies in vertebrate taxonomic classification have increased in recent years, largely due to differences in opinions regarding the true definition of a species. Traditionally, separate species are defined as being morphologically distinct, as well as reproductively, geographically, and ecologically isolated. None of these characteristics alone give sufficient reason for species separation. Many species look similar; some can hybridize and produce viable offspring; many coexist in the same habitat; and some seem to fill the same ecological niche. Before the introduction of genetic analyses, morphologic characteristics were the strongest separators of taxa. Now, scientists are faced with new dilemmas as identical looking species are split or lumped into new groups based on evolutionary history. This problem can be seen in many herpetological taxa, such as hylid frogs, plethodontid salamanders, and (more recently) colubrid snakes.

Until recently, the taxonomy of the common ratsnake (*Elaphe obsoleta*) in the eastern United States had remained fairly consistent with five subspecies identified primarily on the bases of external pattern and coloration. Recent research studies performed by Burbrink et al. (2000) and Burbrink (2001) rejected the validity of the subspecies concept. Based on evidence obtained from morphologic and genetic comparisons, Burbrink et al. stated that the five traditional subspecies of *E. obsoleta* are not evolutionarily distinct, and should be replaced by three separate species that are isolated by geographical boundaries. These newly established species names are the Eastern Ratsnake (*Elaphe alleghaniensis*), Midland Ratsnake (*Elaphe spiloides*), and Western Ratsnake (*Elaphe obsoleta*) (Burbrink, 2001). Originally, only one of the five traditional subspecies of *E. obsoleta* was considered to be native to West Virginia. Specimens previously identified as the nominate *E. o. obsoleta*, or Black Ratsnake, must be re-evaluated as one of the three separate species based on evolutionary lineages in distinct geographical regions across the nation. The presence and distribution of two of the newly established species, which are considered allopatric due to the Appalachian Mountain chain, must be determined in West Virginia. One specimen from West Virginia, identified as *E. spiloides*, was collected from Wood County and documented for species determination (Burbrink et al. 2000). However, due to environmental factors such as moisture, topography, and natural barriers within the state, one specimen collected from a western border county cannot adequately determine statewide species distribution.

Although the new species described by Burbrink are published in reputable peer-reviewed journals and are currently recognized by the Society for the Study of Amphibians and Reptiles (SSAR) and Center for North American Herpetology (CNAH), it should be taken into consideration that much of the scientific and public community refuses to accept the taxonomic changes. Certain inconsistencies or problems in specimen selection and specific methods utilized by Burbrink, as well as insufficient evidence supporting differences in natural history and reproduction, have led others to initiate their own research into the classification of New World ratsnakes. This localized taxonomic study, in small part, will contribute to the overall understanding of this group of reptiles.

The objectives of this study on Black Ratsnake specimens were to:

- examine variations in ratsnake morphology within West Virginia using counts, measurements and digital photography;
- determine if morphometric characters confirmed significant by Burbrink (2001) can be used to separate specimens into distinct species;
- identity each specimen collected in West Virginia and surrounding states by employing statistical procedures to analyze morphometric data;
- verify or reject the validity of the traditional subspecies concept based on study data;
- 5) establish a statewide distribution map for the species complex based on identification of voucher specimens.

# **1.2 TAXONOMY**

#### **1.2.1** Nomenclature

Until very recently, the genus *Elaphe* Fitzinger was considered worldwide, encompassing dozens of species from the subtropic and tropic regions of Europe, Asia and North America. A recent study by Urs Utiger in the Russian Journal of Herpetology has determined that Old and New World ratsnakes are phylogenetically different, requiring a change in genus (Utiger et al, 2002). The proposed genus name for New World ratsnakes is *Pantherophis*, which is resurrected from a previous taxonomic synonym. Pending further evidence, the scientific community (including SSAR and CNAH) is not currently accepting this nomenclature change (Crother et al., 2003). For the purposes of this study, the long-established name of *Elaphe* will continue to be used.

Traditionally, seven species of *Elaphe* could be found in the New World, from southern Canada to Central America. These species were *E. bairdi*, *E. flavirufa*, *E. guttata*, *E. obsoleta*, *E. rosaliae*, *E. subocularis*, *E. triaspis* and *E. vulpina*. Only *E. obsoleta* and *E. guttata* were found along the eastern coast of the United States, including West Virginia.

Over the years, *Elaphe obsoleta*, the common ratsnake (a.k.a. Chicken Snake), has been divided into several geographic races or subspecies based on superficial, yet consistent, external morphological differences in adult color or pattern. Juveniles of all varieties are morphologically indistinct. Most recently, five subspecies were recognized, with intergrades or varieties often existing in peripheral areas of sympatry. The traditional taxonomic classification of *E. obsoleta* is listed in Table 1.

Using mitochondrial DNA evidence, Burbrink et al. (2000) demonstrated that recognition of *Elaphe obsoleta* and its subspecies is unwarranted and confusing. They suggested that *E. obsoleta* should be divided into three separate species, or clades, based on phylogenetic history in different areas of the eastern United States. Burbrink (2001) showed further evidence of species separation using statistical analyses of morphologic characters. In 2002, Collins and Taggart (of the Center for North American Herpetology) submitted the

proposals for species separation by Burbrink et al. 2000 and Burbrink 2001 for consideration to a snake systematist group composed of several scientists (including Burbrink), where the changes were made official (Collins and Taggart, 2002). In 2003, SSAR made the changes official in an update to their publication, Standard and Scientific Names of Amphibians and Reptiles of North America (Crother et al., 2003); however, the common names were slightly different from those originally proposed by Burbrink. Since both sources claim validity, no absolute consensus has been reached regarding standard names of these taxa, even among leading scientists. The newly accepted system of taxonomic classification for the common Ratsnake Complex is listed in Table 2.

Burbrink (2002) performed a follow-up genetic study involving Cornsnakes, and determined that there are three distinct species, including what was formerly known as *Elaphe guttata* spp. The nominate species (*E. guttata*), now called the Red Cornsnake, exists in the eastern portion of the former Cornsnake range, including West Virginia. For the purposes of this study, the traditional name of Cornsnake will continue be used.

Classification	Taxon	Common Name
Kingdom	Animalia	
Phylum	Chordata	
Subphylum	Vertebrata	
Superclass	Tetrapoda	
Class	Reptilia	
Subclass	Diapsida	
Order	Squamata	
Suborder	Serpentes	
Family	Colubridae	
Subfamily	Colubrinae	
Genus	Elaphe (Pantherophis)	
Species	obsoleta	
Subspecies	obsoleta (Say, 1853)	Black Ratsnake
Subspecies	lindheimeri (Baird and Girard, 1853)	Texas Ratsnake
Subspecies	quadrivittata (Holbrook, 1842)	Yellow Ratsnake
Subspecies	rossalleni (Neill, 1949)	Everglades Ratsnake
Subspecies	<i>spiloides</i> (Dumeril, Bibron and Dumeril, 1854)	Gray Ratsnake

Table 1. Traditional Classification of Elaphe obsoleta ssp.

Classification	Taxon	Common Name
Kingdom	Animalia	
Phylum	Chordata	
Subphylum	Vertebrata	
Superclass	Tetrapoda	
Class	Reptilia	
Subclass	Diapsida	
Order	Squamata	
Suborder	Serpentes	
Family	Colubridae	
Subfamily	Colubrinae	
Genus	Elaphe (Pantherophis)	
Species	obsoleta (Say, 1823)	Western Ratsnake
Species	alleghaniensis (Holbrook, 1836)	Eastern Ratsnake
Species	<i>spiloides</i> (Dumeril, Bibron and Dumeril, 1854)	Midland Ratsnake

Table 2. Newly-accepted Classification of *Elaphe* spp.

Since its first reported discovery in the early 1800's, Black Ratsnake nomenclature has undergone various taxonomic changes. Researchers have assigned many synonyms to this species and subspecies over the years (Table 3). Due to recent specific and generic changes or disputes by Burbrink (2000, 2001) and Utiger (2002), it is unclear which scientific name will prevail and which ones will ultimately become synonyms. Table 4 lists the several names assigned to the Black Ratsnake that are currently recognized by different members of the scientific community.

Type locality and holotype information is listed in Table 5. Information about the traditional classification of the Black Ratsnake is included, as well as the newly described species proposed by Burbrink. Each new species was assigned a new type specimen based on the earliest known holotypes of other *Elaphe obsoleta* subspecies that reside within the suspected ranges.

Scientific Name	Authority
Coluber obsoletus	Say (in James), 1823: 140
Scotophis alleghaniensis	Baird and Girard, 1853: 73
Scotophis confines	Baird and Girard, 1853: 76
Scotophis laetus	Baird and Girard, 1853: 77
Elaphis holbrookii	Dumeril, Bibron and Dumeril, 1854: 272
Elaphis alleghaniensis	Hallowell, 1856: 243
Scotophis obsoletus	Kennicott, 1860: 330
Elaphis alleghaniensis	Jan and Sordelli, 1867: 4
Coluber obsoletus obsoletus	Yarrow, 1882: 102
Elaphis obsoletus	Garman, 1883: 54
Elaphis obsoletus var. alleghaniensis	Garman, 1883: 54
Elaphis obsoletus var. obsoletus	Garman, 1892: 292
Elaphis obsoletus var. lindheimeri	Garman, 1892: 290
Pantherophis alleghaniensis	Garman, 1892: 108
Coluber confines	Cope, 1892: 632
Elaphe obsoletus	Dunn, 1915: 6
Callopeltis obsoletus	Medsger, 1919: 28
Elaphe obsoleta obsoleta	Stejneger and Barbour, 1923: 91
Elaphe obsoleta	Neill, 1947: 207

 Table 3. Synonyms of *Elaphe obsoleta obsoleta*, the Black Ratsnake, as Obtained from the Literature

Table 4. Current Unresolved Synonyms of the Black Ratsnake arising from Recent Taxonomic Dispute

Scientific Name	Source
Elaphe obsoleta obsoleta	Stejneger and Barbour, 1923
Pantherophis obsoleta obsoleta	Utiger et al., 2002
Elaphe obsoleta	Burbrink, 2001
Elaphe alleghaniensis	Burbrink, 2001
Elaphe spiloides	Burbrink, 2001
Pantherophis obsoleta	Utiger et al., 2002 / Burbrink, 2001
Pantherophis alleghaniensis	Utiger et al., 2002 / Burbrink, 2001
Pantherophis spiloides	Utiger et al., 2002 / Burbrink, 2001

 Table 5. Type Specimen and Locality Information

<i>Elaphe o. obsoleta</i> (Say, 1853) Black Ratsnake		
<u>Type Locality</u>		
"On the Missouri River from the vicinity of		
Isla au Vache (Cow Island) to Council		
Bluff". Cow Island is near Leavenworth,		
Leavenworth County, KA; over 100 miles		
downriver from Council Bluffs (Dowling,		
1951).		

Elaphe o. obsoleta	(Say, 1853)	) Black Ratsnake	)
--------------------	-------------	------------------	---

Elaphe obsoleta (Say, 1853) Western Ratsnake		
Type Specimen	Type Locality	
Identified originally as Coluber obsoletus.	"On the Missouri River from the vicinity of	
No type specimen is known at this time.	Isla au Vache (Cow Island) to Council	
There were three cotypes of this form,	Bluff". Cow Island is near Leavenworth,	
probably originally in the Peale Museum of	Leavenworth County, KA; over 100 miles	
Philadelphia, but none are known to still	downriver from Council Bluffs (Dowling,	
exist (Dowling, 1952).	1951).	

<i>Elaphe spiloides</i> (Dumeril, Bibron, a	and Dumeril, 1854) Midland Ratsnake
Type Specimen	<u>Type Locality</u>
Identified originally as <i>Elaphis spiloides</i> .	"La Nouvelle-Orleans" = New Orleans,
Holotype = MNHN 827	Louisiana.

Elaphe alleghaniensis (Ho	lbrook, 1936) Eastern Ratsnake
Type Specimen	Type Locality
Identified originally as Coluber	"Summit of the Blue Ridge Mountains in
alleghaniensis. Holotype = ANSP 16792	Virginia and Highlands of the Hudson"

#### 1.2.2 Karyotype

Various researchers have published chromosome numbers of snakes in the genus *Elaphe*. Only minor differences exist between New and Old World Elaphid species. The majority of species possess a diploid number of 2n=36. Becak and Becak (1969) and Trinco and Smith (1971) reported that a male individual of the nominate form of *E. obsoleta*, or Black Ratsnake, had only 35 chromosomes, while the subspecies *E. o. quadrivittata*, or Yellow Ratsnake, had 36 chromosomes. This work is often refuted, since differences in karyotype between two subspecies are highly unlikely, and an odd number of chromosomes is impossible (Schulz, 1996). The normal diploid number of 36 chromosomes (Baker et al., 1972). The five largest pairs are submetacentric; the sixth largest is acrocentric, and the remaining two largest are submetacentric and subtelocentric (Chang et. al., 1971; Baker et al., 1971).

#### **1.3 NATURAL HISTORY**

There have been no specific studies focusing on differences in morphology, status and natural history of the three newly described species of ratsnake proposed by Burbrink (2000). To avoid overlap of similar information, general and specific account information is presented only on the highly studied subspecies, *Elaphe o. obsoleta*, or Black Ratsnake.

#### 1.3.1 Morphology

New and Old World species of *Elaphe* are highly variable in all aspects of morphological features, such as: body size, external coloration and texture, numbers and sizes of scales (Table 6), skull and skeleton structure, organ and reproductive structure anatomy. They are traditionally characterized by a generally robust body form that is higher than wide in cross section, possessing longitudinal keels along the outer edges of the ventral scutes (Schulz, 1996). This feature gives the animals a "loaf of bread" appearance when viewed along the length of the body. Most species possess long tails, comprising from 1/5 to 1/3 the total body length. Except for relative size differences, little sexual dimorphism is present.

Ventrals	Subcaudals	Dorsal Scale Number	
234-264	81-105	27 (29)	
242-269	94-122	25-31 (32)	
197-245	47-84	25-29 (30-31)	
218-258	63-102	23-29	
222-238	63-90	25-27 (23, 29)	
218-238	72-88	25-27 (29)	
225-245	75-102	27-29 (25)	
220-235	70-95	25-27 (29)	
227-258	70-92	25-27 (29)	
276-288	31-35	31-35	
260-283	31-36	31-36	
243-282	83-126	31-39 (29-30)	
190-218	45-71	23-25 (27)	
	234-264 242-269 197-245 218-258 222-238 218-238 225-245 220-235 227-258 276-288 260-283 243-282	234-264       81-105         242-269       94-122         197-245       47-84         218-258       63-102         222-238       63-90         218-238       72-88         225-245       75-102         220-235       70-95         227-258       70-92         276-288       31-35         260-283       31-36         243-282       83-126	

Table 6. Variation in Scale Counts of New World Elaphe spp.

Numbers given in ranges. Rare numbers represented by parentheses. (Modified from Schultz, 1996)

The Black Ratsnake is one of the most common snakes found in West Virginia, and the entire eastern United States. It is one of the largest American non-venomous snakes in the family Colubridae. Individuals normally reach an adult size of 4 to 6 feet in length; however, specimens have been known to exceed 8 feet. The dorsal scales are weekly keeled, while the lateral scales are smooth. The anal plate is divided, consisting of two overlapping scales.

The bodies of adults are generally black on the dorsal side (Figure 1). Red, yellow, or white areas of skin often appear between the scales (Figure 2), showing evidence of the blotched pattern characteristic of juveniles (Conant and Collins, 1998). The ventral side is bright white in the throat and neck region, yielding to mottled black and white checkers along the midbelly. Posteriorly, the ventral scutes and subcaudals are uniformly black. Occasionally, individuals possess a light line running the length of the ventral surface of the tail.



Figure 1. Adult Black Ratsnake (showing solid black pattern)

Figure 2. Adult Black Ratsnake (showing blotched pattern)





Figure 3. Juvenile Black Ratsnake Juveniles (Figure 3) exhibit a strong pattern consisting of 28 to 40 dark brown or black dorsal blotches on a uniformly gray background (Mitchell, 1994). The venter is checkered black and white. There is also a distinct brown or black stripe on each side of the head, extending from the eye to the posterior jaw. Normally, the pattern begins to become obscure and darken in individuals over 2.5 feet, but can be retained much longer.

### 1.3.2 Habitat and Behavior

Black Ratsnakes are generally woodland dwellers, but are often found in a variety of habitats such as swamp borders, river floodplains, rocky hillsides, mountain ledges, and open fields (Green and Pauley, 1987). They are often found in more developed areas, residing in houses, yards, and farm buildings where they can obtain food.

Black Ratsnakes are powerful constrictors. They are semi-arboreal in nature and are often seen climbing trees to take shelter in hollowed cavities and to search for food. They feed almost exclusively on warm-blooded prey such as mice, rats, shrews, voles, squirrels, chipmunks, rabbits, and birds. They have been known to raid bird nests and devour the eggs. Juveniles have also been seen eating small amphibians and lizards. Black Ratsnakes are chiefly diurnal, but will often remain active at night during hot summer months.

The behavior of Black Ratsnakes is unpredictable. Some are quite docile; however, most are aggressive when cornered or captured. They often vibrate their tails in leaf litter, convincing some people that they are venomous rattlesnakes (potentially leading to their demise). In nature, this mimicry is often successful in warding off large predators.

#### 1.3.3 Reproduction

Black Ratsnakes mate in late April, May or early June. One clutch of 4 to 25 eggs is laid in rotten logs, decaying leaf litter, sawdust piles, or fallen hollow trees during late June or July. The eggs are white and oblong, averaging less than 2 inches in diameter. Incubation takes approximately two months. Hatching occurs from late August into October, with young measuring 11 to 16 inches in length. Sexual maturity is reached after 4 years of age.

# **1.4 GEOGRAPHIC DISTRIBUTION**

# **1.4.1** Traditional Distribution

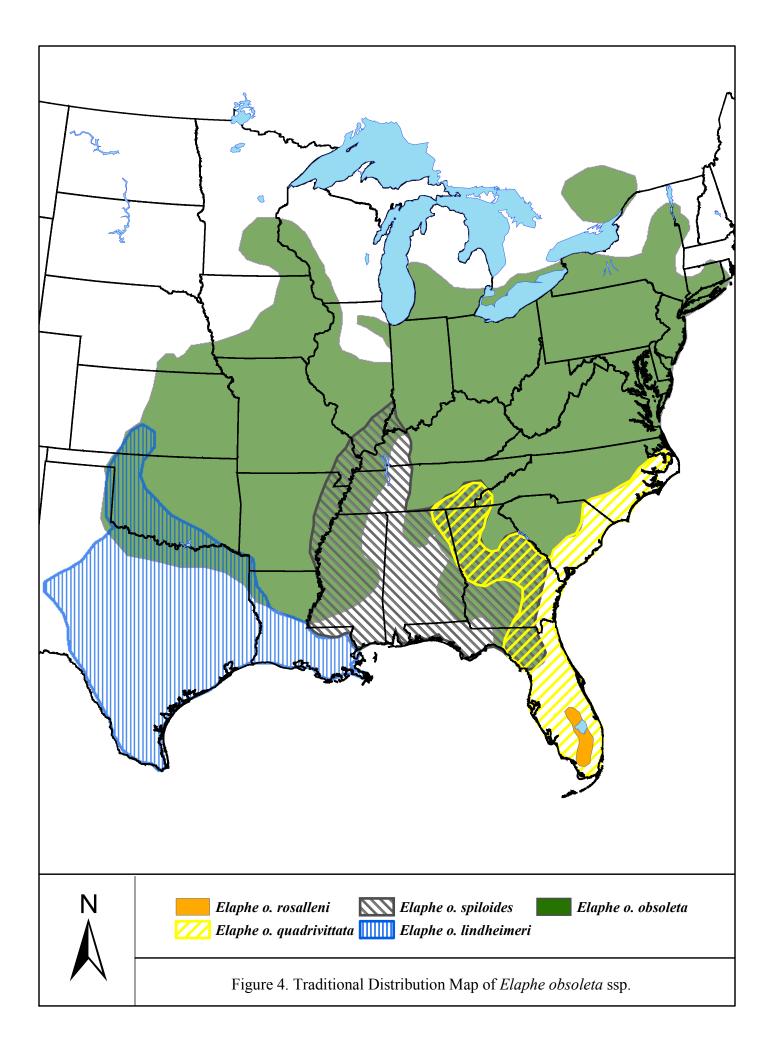
The range of the common ratsnake, *Elaphe obsoleta* ssp., encompasses the entire eastern half of the United States from the Great Lake region to the Gulf of Mexico. It can also be found in some parts of southern Canada. Due to inconsistencies in specimen data and problems in subspecific identification in different areas of the range, it is extremely difficult to precisely map individual subspecies. Most clinal areas of subspecies ranges possess individuals with overlapping characteristics, most likely due to hybridization. Figure 4 shows the range map for *Elaphe obsoleta* ssp. based on traditional classification.

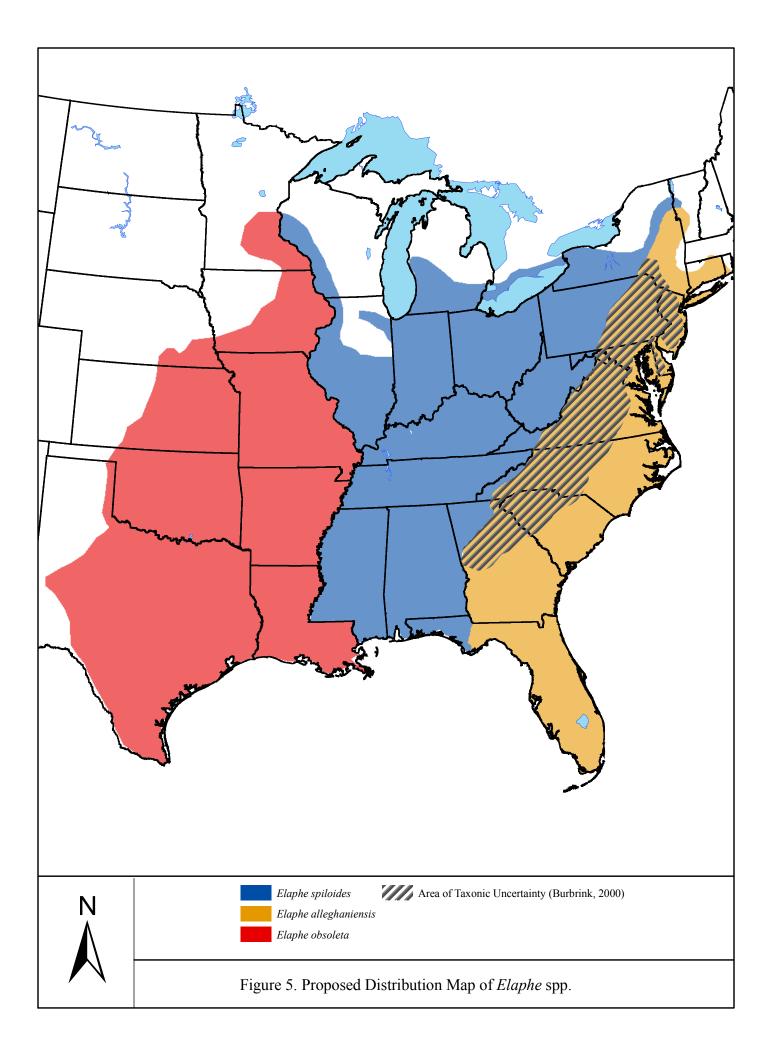
### 1.4.2 Newly Classified Distribution Pattern

The three new species proposed by Burbrink (2000, 2001) encompass the entire range of what was formerly divided between all *Elaphe obsoleta* subspecies. Burbrink determined that three evolutionary lineages actually exist in different areas of the eastern North America. Geographic boundaries such as major rivers and mountain ranges are said to divide the species into their respective ranges. Burbrink (2001) stated that these geographic boundaries isolated the *Elaphe* populations as they migrated northward from southern refuges following glacial retreat. The new geographic ranges of these species negate all previous subspecific ranges. The overall range map for *Elaphe obsoleta*, *Elaphe alleghaniensis*, and *Elaphe spiloides* is shown in Figure 5.

# 1.4.3 West Virginia Distribution

Within West Virginia, the Black Ratsnake is extremely common and prolific. It can be found in all types of habitats and has been observed from the lowest elevation in Harper's Ferry up to mountains as high as 3,760 feet (Green and Pauley, 1987). To date, voucher specimens have been collected from most (48 of 55) counties within the state.





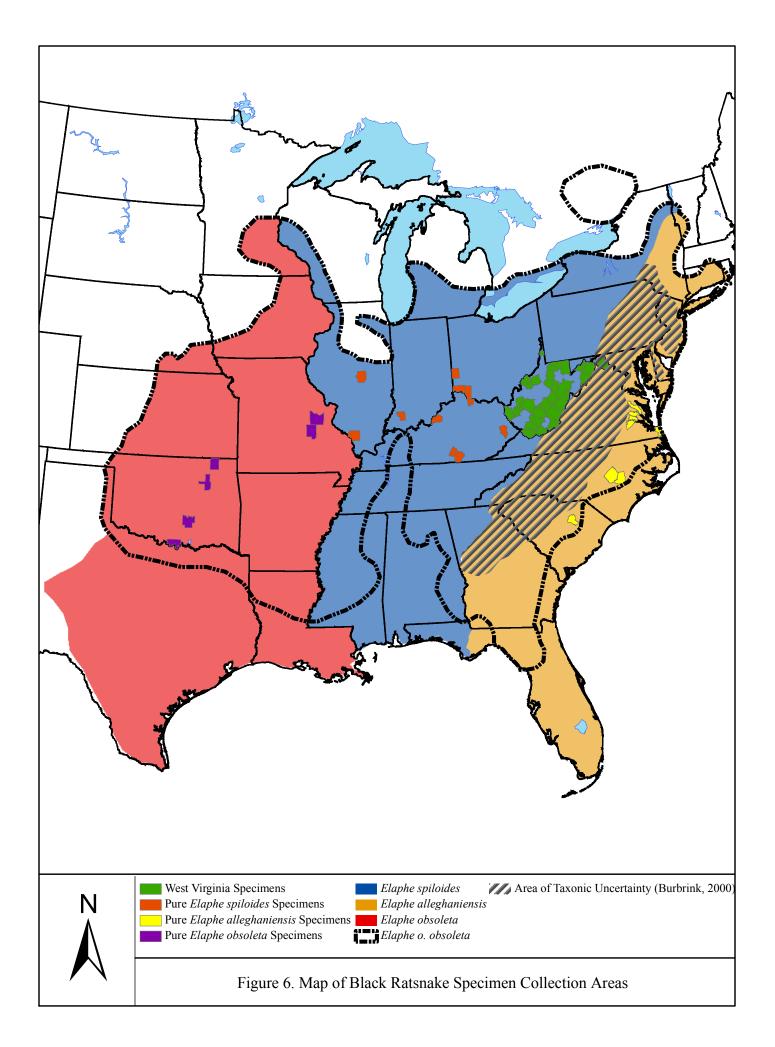
# **2.0 METHODS AND MATERIALS**

#### 2.1 SPECIMENS

One hundred and fourteen Black Ratsnake specimens were located and gathered from the Carnegie Museum of Natural History in Pittsburgh, Pennsylvania, and the Amphibian and Reptile Collection from the West Virginia Biological Survey at Marshall University in Huntington, West Virginia (Appendix A). Only adult specimens (greater than 200 mm in length) were used, in order to minimize the variation in growth rates among individual snakes before maturity. Due to the expense of large-scale trapping or surveying, difficulties in measuring small characteristics on live snakes without causing undue stress, and the overall availability of numerous museum specimens, only preserved individuals were analyzed. Special emphasis was placed on obtaining Black Ratsnake voucher specimens for each county in West Virginia where they have been previously captured.

#### 2.1.1 Newly Classified Populations

Three groups of specimens were selected to represent "pure" individuals of Burbrink's newly classified species. Fifty-four specimens were chosen based on their collection locations in counties within each species' represented range, and not from areas of taxonomic uncertainty (specified by Burbrink [2001]; Figure 5). Collection locations of all Black Ratsnake specimens used in this project can be found in Figure 6. Four specimens from western North Carolina were measured but excluded from certain statistical tests, for they were originally collected from the delineated area of taxonomic uncertainty within the Appalachian Mountains (Figure 6). Specimens (n=21) from coastal Virginia, North Carolina and South Carolina were chosen to represent pure *Elaphe alleghaniensis* populations (Figure 6). Specimens (n=17) from Illinois, Indiana, Kentucky, and Ohio were chosen to represent pure *Elaphe spiloides* populations (Figure 6). Specimens (n=12) from Kansas, Missouri, and Oklahoma were chosen to represent pure *Elaphe obsoleta* 



populations (Figure 6). Only specimens representing the typical Black Ratsnake subspecies morphology were used to represent these groups.

#### 2.1.2 West Virginia Population

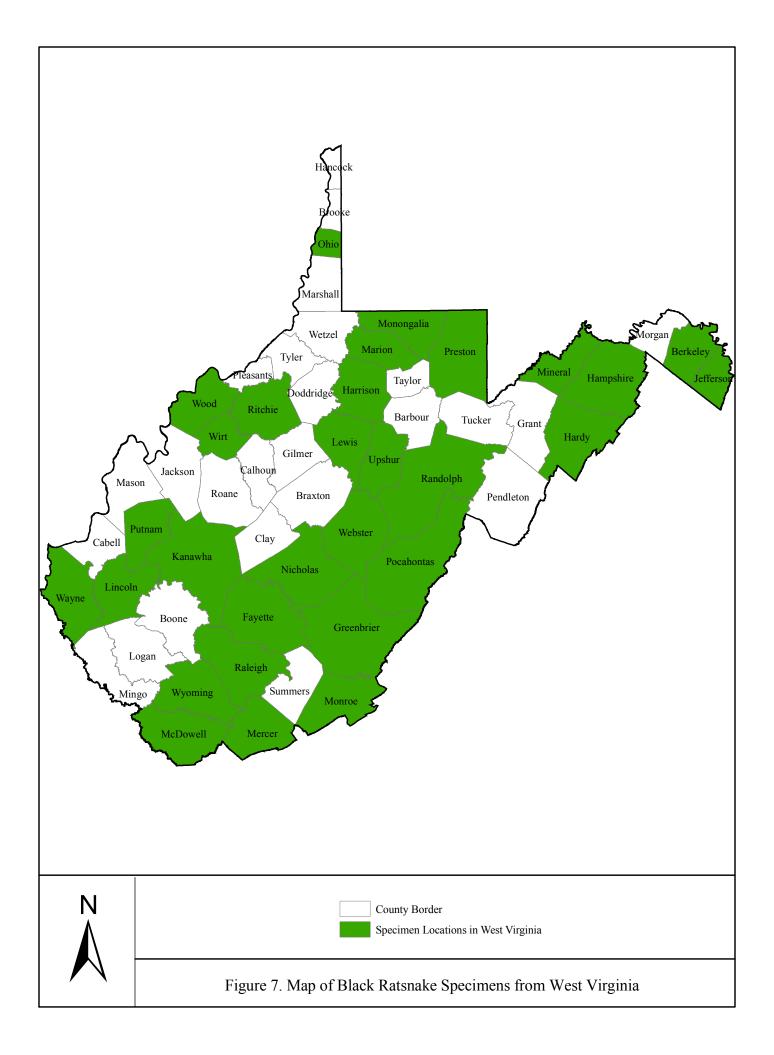
The main purpose of this study was to determine the taxonomy of West Virginia individuals; therefore, 60 Black Ratsnake specimens used were vouchers from West Virginia counties. Use of these specimens augments a lack of West Virginia data in previous experiments by Burbrink (2000, 2001). Burbrink intentionally avoided this area due to apparent taxonomic uncertainty within mountainous areas of the Appalachians. Specific county locations of West Virginia museum specimens used in this project can be found in Figure 7.

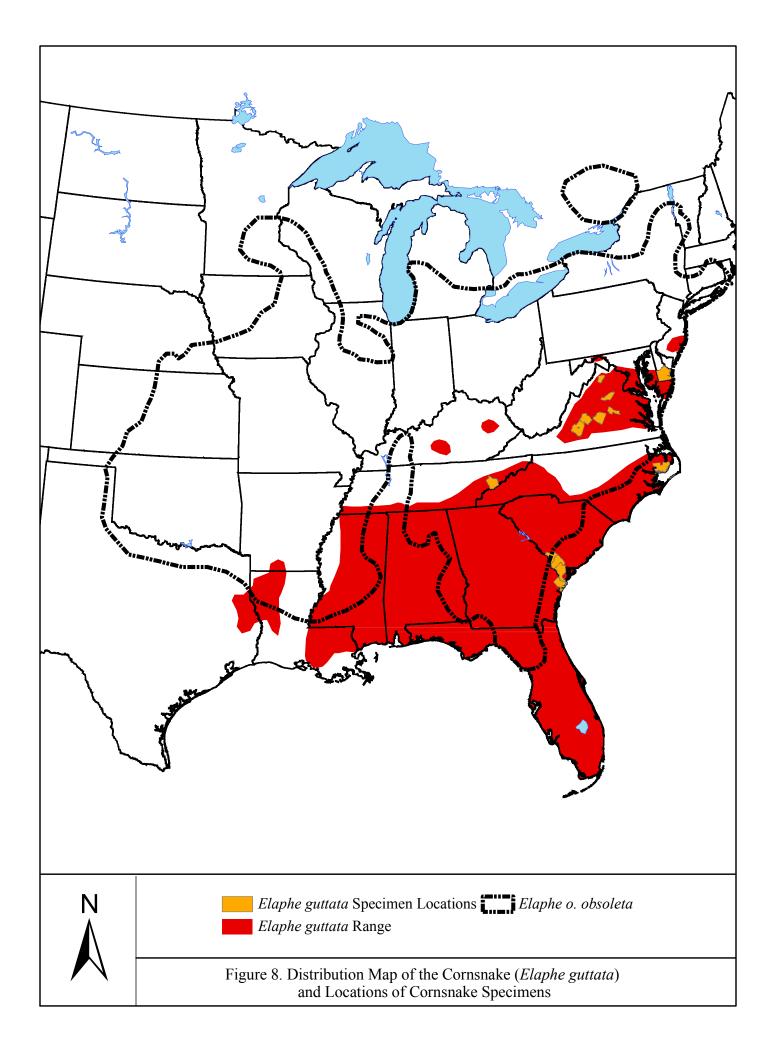
#### 2.1.3 Cornsnakes

Twenty Cornsnakes (*Elaphe g. guttata*) were also analyzed for this study, making a combined total for the study of 134 specimens. These Cornsnake specimens were added to serve as a statistical outgroup for morphologic character comparisons. The Cornsnake is a member of the same genus and is sympatric with the Black Ratsnake over the northern part of its range, including the eastern panhandle of West Virginia (Figure 8). Cornsnake specimens used in this study were originally collected in Delaware, Georgia, North Carolina, South Carolina, Tennessee, and Virginia. By examining this congener, the overall relationship between Black Ratsnakes over different areas can be better understood. Locations of Cornsnake specimens used in this project can be found in Figure 8.

### 2.2 CHARACTER MEASUREMENTS

Burbrink (2001) distinguished certain meristic (countable) and mensural (measurable) characteristics that could be used to separate *Elaphe alleghaniensis*, *Elaphe spiloides*, and *Elaphe obsoleta*. Numerous measurements and counts were taken on each snake, including Cornsnake specimens (Table 7). Twenty-five characters were selected for statistical analysis in this study. These characters were composed of 3 meristic scale counts, 12 scale or body (mensural) measurements, and 10 derived scale measurements





Character	Figure		e # Description			
	MERISTIC CHARACTERS (COUNTS)					
Subcaudals	SC	11	Total number of subcaudals on one side of the tail, beginning with the first scale that contacts another subcaudal from the other side. The terminal spine is not included.			
Ventrals	V	11	Total number of ventral scales beginning with the first full-sized ventral on the neck and not including the anal plate.			
Dorsal Scale Row at Midbody	DSM	10	Total number of dorsal scales around the body starting and ending adjacent to the ventral scale.			
MENSURAL CHARACTERS (MEASUREMENTS)						
Head Length	HL	15	Measured from the rostral tip to the posterior-most point of the lower jaw.			
Cranial Width	CW	13	Measured across the width of the head at the posterior- most point of the lower jaws.			
Tail Length	TL	11	Measured from the posterior tip of the anal plate to the posterior terminus of the tail button.			
Parietal Length	PL	13	Measured from the anterior-most point at the suture with the frontal and supraocular scales to the posterior-most point of the scale itself.			
Parietal Width	PW	13	Measured from the median suture at the posterior-most point of the frontal scale to the point of contact with the left temporal and postocular scales.			
Frontal Width – Posterior	FWP	13	Measured from the point of contact with the left parietal- supraocular suture to the point of contact with the right parietal-supraocular suture.			
Internasal Width – Posterior	INWP	12	Measured from the median suture contact with the prefrontal scale to the dorsal-most suture contacting the posterior nasal scale.			
Eye Diameter	EYE	14	Measured at the widest point of the left eye between the preocular and postocular scales.			
Anterior Gular (Inframaxillar) Length	AG	15	Measured from the anterior most contact with the left infralabials to the posterior-most contact with the gular scales.			
Internasal Rostral Contact	INR	12	Measured across the width of the rostral scale at the left and right contacts with the internasal-nasal sutures.			
Rostral Width	RW	12	Measured across the width of the rostral scale at the left and right contacts with the nasal-supralabial sutures.			
Supralabial Length	LL	14	Total length of all supralabials on the left side of the maxilla, measured from the anterior-most contact with the rostral scale to the posterior-most contact with the dorsal body scales.			

Table 7. Morphometric Characters Obtained from Each Specimen

(Modified from Burbrink, 2001)

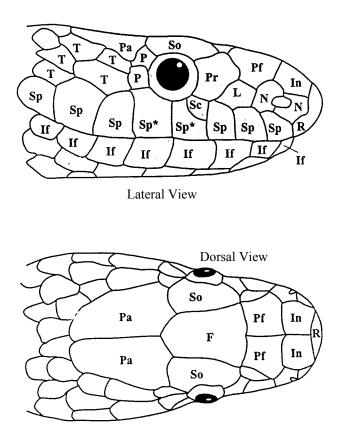
that were corrected for size variations of individual snakes (Table 8). Most derived characters were cited by Burbrink (2001) as significant in determining the relationship between *Elaphe alleghaniensis* and *Elaphe spiloides*, the two species likely present in West Virginia. Burbrink determined that these characters were significant, regardless of sex.

Derived Character	<b>Derivation Formula</b>
CWHL PLHL PWHL FWPHL INWPHL EYEHL AGHL INRHL RWHL	Ratios of scale measurements (mm) to Head Length (mm) in order to standardize for size differences Ex. CW (mm) / HL (mm) = CWHL (no units)
LLHL	

Table 8. Derived Characters used for Statistical Analyses

Figure 9, modified from Schultz (1996), illustrates the terminology of head and body scale features of ratsnakes. In many snake species, scale counts are essential in determining differences between closely related species. Conflicting scale numbers often signify different skeletal structure and musculature (e.g. different numbers of vertebrae); however, most ratsnakes exhibit a high degree of variability in scale numbers among individuals. Subcaudal, ventral, and dorsal scales were counted visually with the occasional assistance of a dissection microscope (Figures 10 and 11). Using PRO-MAX® digital calipers, measurements were taken of the head length and width, as well as several of its individual scales (Figures 12 to 15).

Derived measurements were those standardized for differences in snake body size by comparing the data to each individual's head length (Table 8).



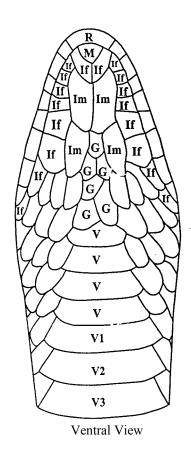


Figure 9. Terminology of Head and Body Scale Features of Ratsnakes - modified from Schultz (1996)

- $\mathbf{F} = Frontal.$
- $\mathbf{G} = \mathrm{Gular}.$
- If = Infralabial.
- In = Internasal.
- Im = Inframaxillar.
- $\mathbf{L} = \text{Loreal}.$
- M = Mental
- N = Nasal.
- $\mathbf{P}$  = Postocular.
- $\mathbf{Pa} = \mathbf{Parietal}.$
- $\mathbf{Pf} = \mathbf{Prefrontal}.$
- $\mathbf{Pr} = \mathbf{Preocular}.$
- $\mathbf{R}$  = Rostral.
- Sc = Subocular; other authors may refer to it as a second preocular or as presubocular.
- So = Supraocular.
- Sp = Supralabial.
- $\hat{Sp}^* = Supralabials$  in contact with the eye.
- $\mathbf{T}$  = Temporal.
- V = Ventral; V1-V3 are the first ventrals of equal size which, according to the method by DOWLING (1951c), are to be counted as the first plates. Those ventrals without numbers are reduced plates of unequal size which, in earlier times, were also counted. The reduced ventrals usually number only 2 to 3, rarely 4 to 5.

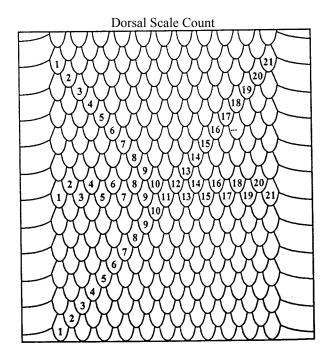




Figure 10. Morphometric Characters: Dorsal View of Body DSM = Dorsal Scale Row at Midbody

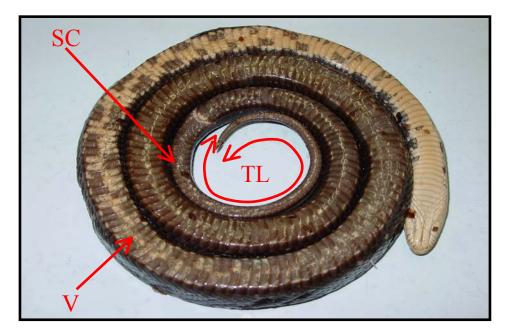


Figure 11. Morphometric Characters: Ventral View of BodySC = SubcaudalsV = VentralsTL = Tail length

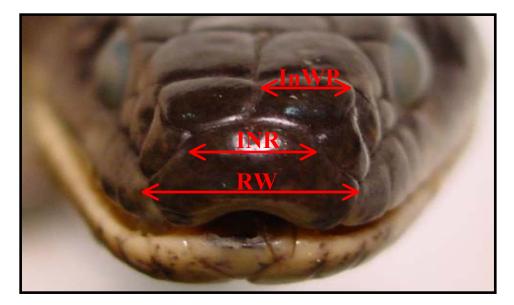


Figure 12. Morphometric Characters: Anterior View of Head

InWP = Internasal Width – Posterior INR = Internasal Rostral Contact RW = Rostral Width

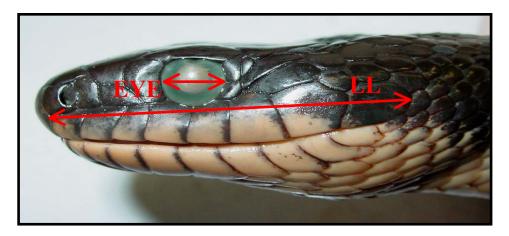


Figure 13. Morphometric Characters: Lateral View of Head EYE = Eye Diameter LL = Supralabial Length

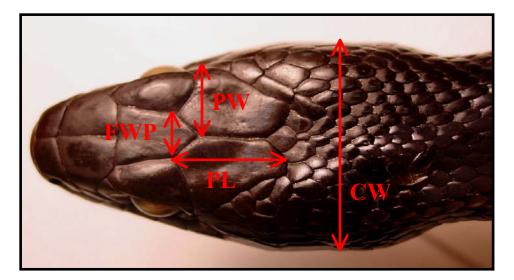


Figure 14. Morphometric Characters: Dorsal View of Head

FWP = Frontal Width - Posterior PW = Parietal Width PL = Parietal Length CW = Cranial Width

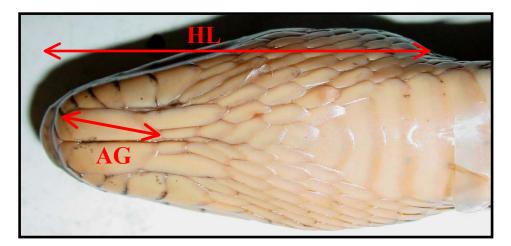


Figure 15. Morphometric Characters: Ventral View of Head

HL = Head Length AG = Anterior Gular Length

# 2.3 CHARACTER DATA ANALYSES

All character data were recorded on standardized data sheets and later entered into Microsoft Excel (Version 2000) spreadsheets. Completed data sheets are located in Appendix B.

Prior to statistical analyses, all specimens were divided into five categories or groups, each representing different taxa or geographically separated populations. These categories are:

- 1) Taxon A = Pure *Elaphe <u>alleghaniensis</u>* specimens (Eastern Ratsnakes)
- 2) Taxon O = Pure *Elaphe obsoleta* specimens (Western Ratsnakes)
- 3) Taxon S = Pure *Elaphe spiloides* specimens (Midland Ratsnakes)
- 4) Taxon  $W = \underline{W}$ est Virginia ratsnake specimens
- 5) Taxon G = *Elaphe* guttata (Cornsnake) specimens

Although 25 characters were measured, counted, or calculated for this study, not all were analyzed simultaneously. Multivariate statistical tests were divided into five separate classes or sets, each containing different combinations of characters:

- All Character Data = 24 characters. This set includes all meristic, mensural, and derived characters listed in Tables 7 and 8, excluding tail length (TL).
- Scale Count Data = 3 characters. This set includes only the meristic characters listed in Table 7.
- Raw Data = 11 characters. This set includes only the mensural characters listed in Table 7, excluding tail length (TL). Tail length was excluded due to potential for sexual dimorphic variation.
- Derived Data = 10 characters. This set includes only the derived characters listed in Table 8.
- 5) Scale Count and Derived Data = 13 characters. The set includes all meristic and derived characters, essentially a combination of Sets 1 and 3.

Although 134 specimens were measured, not all were used for each analysis. Seventeen Black Ratsnake specimens exhibited broken or regenerated tails; therefore, true subcaudal counts (SC) or tail lengths (TL) could not be determined. These individuals were not included in sets that included the SC character, because systematic error would be introduced into each analysis. The other data sets included these individuals, because every mensural character (with the exception of tail length) contained a data entry for each specimen. Certain data sets were repeated using a different combination of specimens. One data set was repeated to rule out potential error due to sexual dimorphism. The most effective set of characters was determined, and repeated without the presence of the outgroup in order to maximize separation between Black Ratsnake specimens.

Values for derived characters were calculated in Microsoft Excel using ratios of raw character measurements divided by head length (HD). Each multivariate character set was analyzed using SAS Version 9.1 for Microsoft Windows. Specific statistical analyses tests conducted were Canonical Discriminant Analysis (CDA) and Principle Component Analysis (PCA). Results of CDA and PCA were graphed using bivariate scatterplots of the first and second coordinate systems, which should show the greatest degrees of variation in each sample. On each graph, one point was plotted for each represented specimen in the data pool. Bivariate scatterplot graphs of CDA and PCA results were analyzed visually by observing relative placement and clustering of individuals compared to other individuals within the same or different sample groups or taxa. Bivariate scatterplot graphs of CDA results were also substantiated through interpretation of mathematical principles such as Eigenvalues, canonical coefficients, eigenvectors, and squared distances (D) between taxa.

All sets of multivariate statistical data were analyzed among each other to determine the precision and/or accuracy in separating assigned groups. Character means were also compared among groups. Using Microsoft Excel, column graphs for specific character means were constructed from the SAS data output. These graphs gave a visual representation of group means among taxa for each meristic, mensural, or derived character.

# 2.4 DORSAL PATTERN SURVEY

Black Ratsnakes receive their name for their tendency to fade from a juvenile pattern of dark dorsal blotches to a very uniform dark (or black) appearance. Individuals display this trait in varying degrees. Some adults seem to permanently retain an obvious juvenile pattern over the entire dorsal surface, while others become completely black. It is currently unknown whether genetic or environmental factors guide this process.

To study the variation in dorsal blotch pattern retention among or within assigned Black Ratsnake groups, all specimens were documented with a digital photograph at the time of data collection. Some photographed individuals were not included in this analysis due to deteriorated specimen conditions or poor photographs. Of 114 specimens, 100 were included in this study. Photographs were analyzed for dorsal pattern retention. Only Black Ratsnake specimens were used for this analysis, because Cornsnakes do not fade or lose their patterned appearance as adults.

A small graduated scale ranging from 1 to 4 was used to categorize each snake by color pattern. Figures 16 to 19 display examples of each color scale category. The color scale categories are as follows:

- **Category 1** = Individuals possess an obvious blotched pattern throughout almost the entire body. Dorsal blotches are distinct and countable on a lighter-colored background (Figure 16).
- **Category 2** = Individuals still retain the blotched pattern, but it is considerably faded or darkened. Many individual blotches can still be counted. Light skin color can often be seen between the scales (Figure 17).
- Category 3 = Individuals are almost entirely dark or black; however, a very slight pattern (or blotching) can still be discerned on some parts of the body (Figure 18).
- **Category 4** = Individuals are entirely black or uniformly dark in coloration, with no trace of pattern (Figure 19).





Figure 16. Color Scale Example Pictures - Category 1









Figure 17. Color Scale Example Pictures - Category 2









Figure 18. Color Scale Example Pictures - Category 3









Figure 19. Color Scale Example Pictures - Category 4





Snake specimen photographs were observed by 15 volunteer biologists, scientific professionals, or biology students, and ranked according to pattern category. These volunteers ranked the digital photographs using the pattern scales presented in Figures 16 to 19, and entered all values into Microsoft Excel. Mean values for each specimen were rounded to the nearest tenth. ANOVA was also performed to determine the effectiveness of the test. A correlation coefficient analysis was used to determine the precision of rankings among all observers. Maximum, minimum, and mean values for assigned groups were calculated and rounded to the nearest tenth. All values were analyzed statistically for trends in pattern retention based on separation into assigned taxa, and conclusions were drawn based on such variables as geographic location and topography.

# **3.0 RESULTS AND DISCUSSION**

Traditionally, even similar looking organisms could be distinguished from each other by one or two characters. For example, Snake X looks very similar to Snake Y; however, one or the other possesses keeled scales while the other does not, thereby distinguishing the two groups. With the ever-increasing use of genetic studies to separate or combine former species, taxonomy has changed drastically. Field identification of specimens has become more difficult as identical looking organisms are genetically determined to have different evolutionary lineages, and are therefore classified as different taxa.

# 3.1 CHARACTER DATA ANALYSES

## 3.1.1 Canonical Discriminant Analysis & Principal Component Analysis

Normally, univariate statistical techniques are not sufficient to account for subtle variations that exist between or among closely related groups of organisms, especially since variations also exist within these groups of organisms. Canonical Discriminant Analysis (CDA) and Principal Component Analysis (PCA) are statistical tests used to show variation in sample data that contain multiple variables, hence the phrase "multivariate analyses."

CDA examines differences in morphologic variables (characters) and identifies linear combinations of these variables, in order to provide maximum separation between userdefined groups or taxa. If presented with multiple groups of observations, each with different measured or counted characters, CDA indicates relative contributions of each character in discriminating among the assigned groups. This test inherently possesses user-defined bias, since the analysis is based on the initial placement into groups. Using group means for each character, the analysis will determine the relative weight of each character. Each canonical variable is a linear combination of characters and their relative contribution or weight toward separation among taxa. Most variation is usually accounted for on the first canonical variable, followed by the second, and so on. The number of canonical variables is generally equal to the number of defined groups, minus one; however, most variation is among groups is accounted for on the first and second canonical variables. These canonical variables are orthogonal, and can be plotted graphically on bivariate scatterplots to visually represent the data. All CDA graphs were plotted using the first and second canonical variables (Can 1 vs. Can 2).

PCA is also a multivariate technique that examines relationships among morphologic variables and identifies or detects linear combinations of these variables. Contradictory to CDA, it does so independently of user-defined groups. It is essentially a precursor to CDA, except that it does not compute group means of transformed variables. CDA actually involves PCA as a component of its test. Ultimately, PCA is a stronger test to show separation among groups, for it does not include user-defined bias. If groups show any separation, it is exclusively due to the represented data and not the assignment by groups. PCA transforms the data to a new coordinate system, where each principal component is a linear combination of the characters with coefficients based on relative importance in separating the data. Also similar to CDA, most variation in PCA is accounted for on the first and second principal components. These principal components are orthogonal, and can be plotted graphically on bivariate scatterplots to visually represent the data. All PCA graphs were plotted using the first and second principal components (Prin 1 vs. Prin 2).

## 3.1.1.1 All Character Data

The first multivariate statistical test included a combination of all meristic and mensural characters, as well as all derived characters that were standardized for size variations among specimens. It was assumed that use of more characters should provide greater insight into species separation. This data set included 113 specimens. Since scale count data were included, those specimens with missing SC values (due to short or injured tails) were excluded.

CDA showed distinct separation of the specimens into two the major species groupings, Cornsnakes and Black Ratsnakes (Figure 20). The majority of separation (D>10.7; P= <0.0001) between these groups can be seen on the first canonical variable (Can 1). Figure 21 shows that the means of these values are also separated mainly on the Can 1 axis. Some slight separation of the Black Ratsnake taxa can be seen on Can 2, with West Virginia specimens showing more relation to pure *Elaphe spiloides* specimens (D=1.24; P=0.139) than to the other two groups (P=0.063; P=0.073). Eigenvalues showed that approximately 94 percent of the variation was on the first and second canonical variables, with 86 percent on Can 1 alone (Table 9). One hundred percent of the variation was accounted for in four canonical variables. Standardized canonical coefficient values suggest that the characters PW, PL, InWP, RW, PLHL, PWHL, and InWPHL accounted for most of the variation on Can 1, while InWP, LL, EYE, HL, and InWPHL accounted for most of the variation on Can 2 (Table 10). These findings show heavy reliance on certain raw characters, which could be unduly influenced by individual specimen size.

PCA also showed some separation among taxa, especially between Cornsnakes and Black Ratsnakes (Figure 22). Separation was not as clearly defined as with CDA. Black Ratsnake specimens showed a high degree of within-group variation on both principal components; thereby showing no strong separation among taxa. Eigenvalues showed that approximately 63 percent of the variation could be explained on three principal components. Approximately 45 and 12 percent of the variation was shown on the first and second principal components, respectively (Table 9). Eigenvectors suggest that all raw characters accounted for most of the variation on Prin 1, while ventrals and derived characters accounted for most of the variation on Prin 2 (Table 10). This could also demonstrate the possibility for bias due to the inclusion of raw measurements.

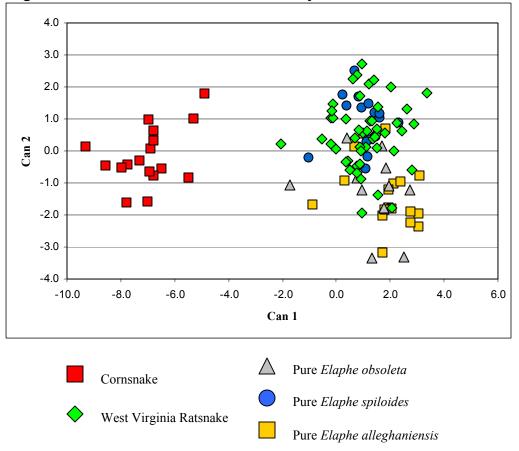
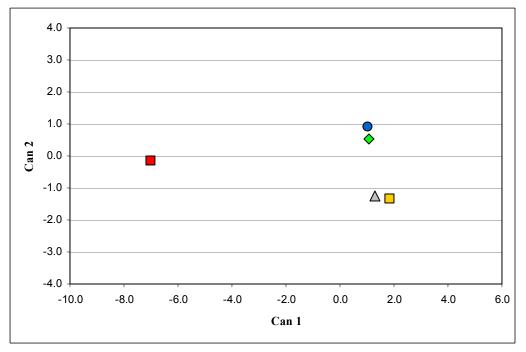


Figure 20. Canonical Discriminant Analysis of All Character Data

Figure 21. Canonical Discriminant Analysis of All Character Data Mean Values



Test		Eigenvalue	Difference	Proportion	Cumulative
	Can 1	9.1265	8.4035	0.8690	0.8690
CDA	Can 2	0.7230	0.3057	0.0688	0.9378
CDA	Can 3	0.4173	0.3057	0.0397	0.9776
	Can 4	0.2357		0.0224	1.0000
	Prin 1	10.7175	7.9386	0.4466	0.4466
PCA	Prin 2	2.7788	1.1585	0.1158	0.5623
	Prin 3	1.6202		0.0675	0.6299

Table 9. Eigenvalues for Canonical Discriminant and Principal Component Analyses of All Character Data

Table 10. Canonical Coefficients and Eigenvectors for All Character Data

	CI	CDA		CA
Characters	Canonical C	Coefficients	Eigenv	vectors
	Can 1	Can 2	Prin 1	Prin 2
SC	0.82741110	-1.31082887	0.202886	0.101452
V	0.58270012	0.09249970	0.149833	0.281107
DSM	-0.22182302	-0.03133028	-0.054032	-0.252572
HL	3.76749230	5.09442350	0.293662	-0.155222
CW	0.57871657	-2.55723951	0.275310	-0.048179
PL	11.74372619	3.47822881	0.279324	-0.124982
$\mathbf{PW}$	-12.31843987	-3.62466804	0.294461	-0.017125
FWP	-0.97127984	1.93598089	0.207360	-0.264264
InWP	-7.62270473	-7.31720731	0.277919	0.058056
EYE	3.83047679	6.36831185	0.285615	-0.024332
AG	1.41770658	-0.0972791	0.273139	-0.101494
InR	-0.19232534	-3.75013386	0.235435	0.051984
RW	-4.47974500	4.31212980	0.295476	-0.007095
LL	0.54465386	-6.40925359	0.289874	-0.138975
CWHL	-0.22901494	1.16975023	0.109703	0.177531
PLHL	-4.42421642	-1.52828062	-0.121106	0.116968
PWHL	4.62587655	1.31893150	0.051018	0.404943
FWHL	0.62654736	-1.08340006	-0.148831	-0.162032
InWPHL	4.71684791	4.45762217	0.133219	0.341569
EYEHL	-1.46889175	-2.08697552	-0.041190	0.376700
AGHL	-0.43731633	0.30889145	-0.021176	0.110019
InRHL	0.12598574	2.52727141	0.041376	0.241720
RWHL	1.58108998	-1.17238341	0.142376	0.348093
LLHL	-0.11879835	1.43220270	-0.100969	0.112663

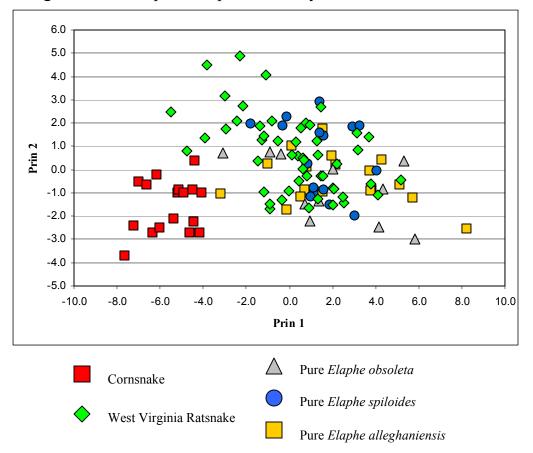


Figure 22. Principal Component Analysis of All Character Data

## 3.1.1.2 Scale Count Data

Snakes often possess different scale counts that can be used to separate between species. Scale counts show a relationship to musculo-skeletal structures, which should differ more among species than within them. Snakes with longer, slender bodies tend to have more ventral scales than those with shorter, thicker bodies. Snakes with longer tails also tend to have more subcaudals. These morphological differences sometimes signify differences in natural histories or niches of certain species. Unfortunately, all ratsnakes have a good degree of variability in scale counts even within species or subspecies, and between sexes. This test includes three meristic characters and 113 specimens. Specimens with missing SC values were excluded.

CDA showed distinct separation of the specimens into the two major species groupings, Cornsnakes and Black Ratsnakes (Figure 23). The majority of this separation was on the first canonical variable (D>20.9; P<0.0001), as also seen when viewing the mean canonical values for each taxa (Figure 24). A very slight separation of Black Ratsnake specimens is noticed on the Can 2 axis. Eigenvalues showed that 99.7 percent of the variation was on the first and second canonical variables, with approximately 95 percent on Can 1 (Table 11). Canonical coefficient values for both Can 1 and Can 2 (Table 12) suggest that the subcaudal count (SC) accounted for most of the variation and contributed most to separation of the taxa.

Test		Eigenvalue	Difference	Proportion	Cumulative
CDA for	Can 1	3.3986	3.2131	0.9451	0.9451
	Can 2	0.1856	0.1737	0.0516	0.9967
All Individuals	Can 3	0.0119		0.0033	1.0000
CDA for	Can 1	4.0747	3.8347	0.9343	0.9343
CDA for Males	Can 2	0.2400	0.1932	0.0550	0.9893
	Can 3	0.0468		0.0107	1.0000

Table 11. Eigenvalues for Canonical Discriminant and Principal Component Analyses of Scale Count Data

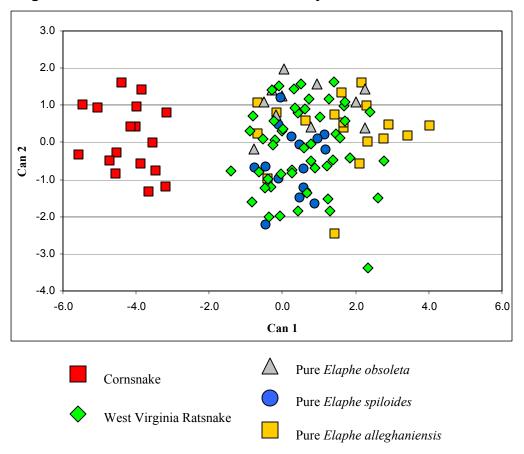
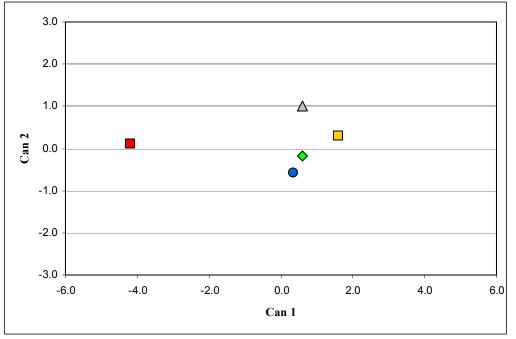


Figure 23. Canonical Discriminant Analysis of Scale Count Data

Figure 24. Canonical Discriminant Analysis of Scale Count Data Mean Values



		l Individuals	CDA for Males		
Characters	Canonical Coefficients		Canonical C	oefficients	
	Can 1	Can 1	Can 1	Prin 2	
SC	1.382107050	1.723079228	1.723079228	-0.635229	
V	0.846049349	0.413612725	0.413612725	0.730816	
DSM	-0.407979148	-0.436043718	-0.436043718	0.249784	

Table 12. Canonical Coefficients for Scale Count Data

Since subcaudal counts are heavily influenced by sexual dimorphism (i.e. male snakes generally have longer tails than female snakes), a separate test was performed using only male specimens. Although specimens were not chosen based on sex, some groups could have an unweighted portion of one sex or the other. This test would rule out any bias due sex of the snakes. Individuals were only identified as males if inverted hemipenes were observed. This test included 51 individuals. All five taxa were represented.

CDA showed distinct separation of the male specimens into the two major species groupings (Figures 25 and 26). The majority of separation between these groups was on Can 1 (D>21.5; P<0.0001). Some slight separation of Black Ratsnake specimens was seen on Can 2 (D<7.27). Eigenvalues showed that approximately 99 percent of the variation was on the first and second canonical variables, with 93 percent on Can 1 (Table 11). Again, canonical coefficient values for Can 1 and Can 2 (Table 12) show that SC accounted for the most variation and contributed most to separation of the taxa.

PCA showed separation of the taxa during both tests of Scale Count Data. Separations between major groupings were predominantly on Prin 1, with a great deal of variability on Prin 2 (Figures 27 and 28). Eigenvalues showed that approximately 84 and 90 percent of the separation was shown on the first two principal components for test of all individuals and males only, respectively (Table 13). Eigenvectors suggest that ventrals (V) accounted for the most variation, followed by subcaudals (SC; Table 14). Separation of specimens into groups did not appear to be dependent on sex of the sampled individuals.

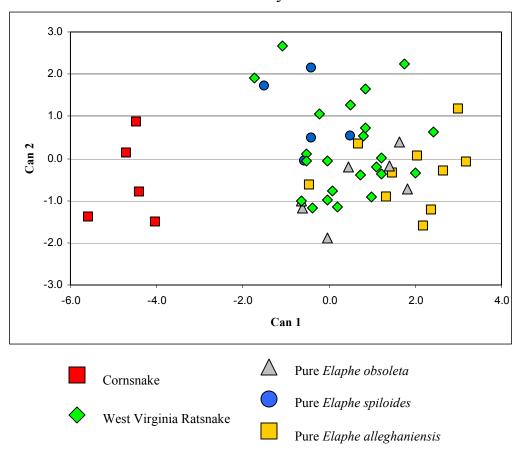
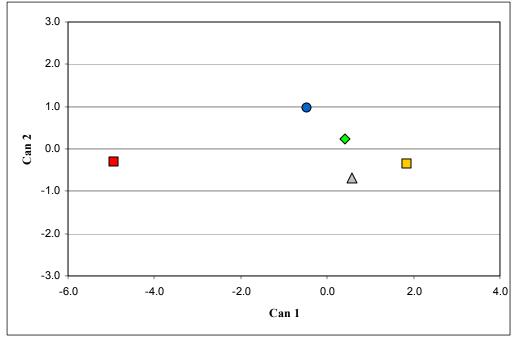


Figure 25. Canonical Discriminant Analysis of Scale Count Data for Males

Figure 26. Canonical Discriminant Analysis of Scale Count Data Mean Values for Males



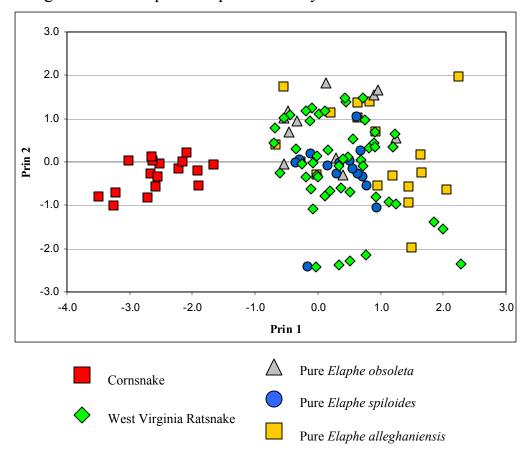
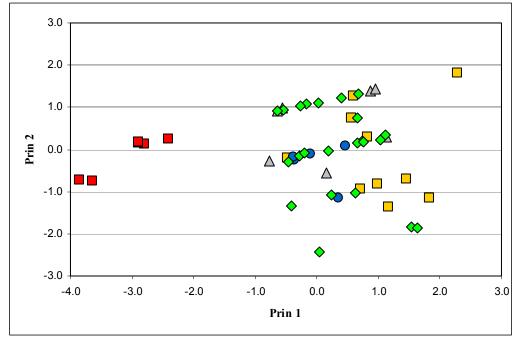


Figure 27. Principal Component Analysis of Scale Count Data

Figure 28. Principal Component Analysis of Scale Count Data for Males



Test		Eigenvalue	Difference	Proportion	Cumulative
DCA for	Prin 1	1.60877847	0.70218924	0.5363	0.5363
PCA for All Individuals	Prin 2	0.90658922	0.42195691	0.3022	0.8385
All individuals	Prin 3	0.48463231		0.1615	1.0000
DCA for	Prin 1	1.80654433	0.89292986	0.6022	0.6022
PCA for Males	Prin 2	0.91361446	0.63377325	0.3045	0.9067
	Prin 3	0.27984121		0.0933	1.0000

Table 13. Eigenvalues for Principal Component Analyses of Scale Count Data

Table 14. Eigenvectors for Scale Count Data

	PCA for Al	l Individuals	PCA for Males		
Characters	Eigenvectors		Eigenvectors		
	Prin 1	Prin 2	Prin 1	Prin 2	
SC	0.612786	-0.635229	0.663963	-0.696284	
V	0.673775	0.730816	0.677532	0.714463	
DSM	-0.412941	0.249784	-0.316390	0.946132	

# 3.1.1.3 Raw Data

To determine the influence by raw measurements of snake anatomy, CDA and PCA were performed on 11 mensural head characters. This data set included 130 specimens.

CDA showed separation of the specimens into the two major species groupings (Figures 29 and 30). The majority of separation between these groups was shown on Can 1 (D>14.7; P<0.0001). Little to no separation existed on Can 2 (D<2.91). Eigenvalues showed that approximately 92 percent of the variation was on the first and second canonical variables, with approximately 80 percent on Can 1 (Table 15). Canonical coefficient values suggest that characters PW, PL, and EYE accounted for most of the variation in Can 1, while HL and EYE accounted for most of the variation in Can 2 (Table 16).

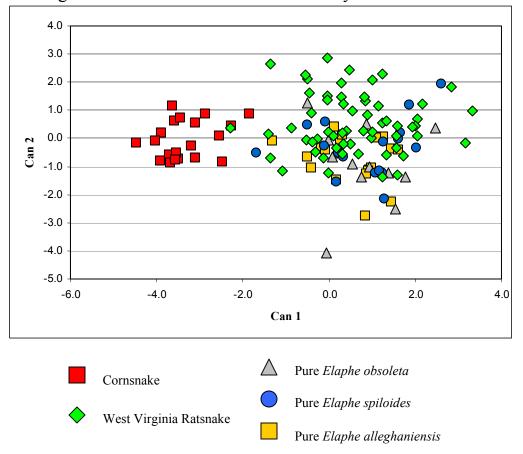
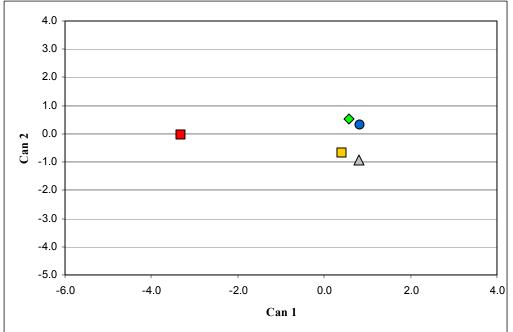


Figure 29. Canonical Discriminant Analysis of Raw Data





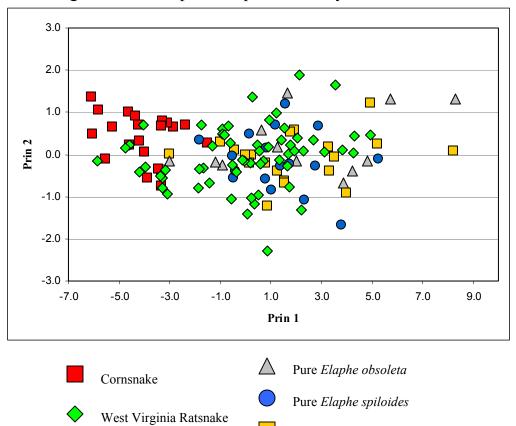
Test		Eigenvalue	Difference	Proportion	Cumulative
	Can 1	2.0987	1.8015	0.8022	0.8022
CDA	Can 2	0.2972	0.1480	0.1136	0.9158
CDA	Can 3	0.1492	0.0780	0.0570	0.9728
	Can 4	0.0712		0.0272	1.0000
	Prin 1	9.18606068	8.69916684	0.8351	0.8351
PCA	Prin 2	0.48689384	0.06810243	0.0443	0.8794
	Prin 3	0.41879140		0.0381	0.9174

Table 15. Eigenvalues for Canonical Discriminant and Principal Component Analyses of Raw Data

Table 16. Canonical Coefficients and Eigenvectors for Raw Data

	CI	DA	PC	CA	
Characters	Canonical (	Coefficients	Eigenvectors		
	Can 1	Can 2	Prin 1	Prin 2	
HL	-0.241401242	-3.342202891	0.324360	0.042815	
CW	-0.369700161	-0.664164894	0.298430	-0.136462	
PL	-1.094518744	-0.941116354	0.314345	0.102993	
PW	2.295111133	-0.046490212	0.318349	0.031231	
FWP	-0.765569975	-0.171797235	0.253470	0.811144	
InWP	0.516251834	0.618992094	0.293158	-0.149644	
EYE	1.064835137	1.125063091	0.306530	-0.107765	
AG	-0.102181203	-0.104308257	0.302732	-0.057504	
InR	-0.131787130	-0.049611071	0.256434	-0.503897	
RW	0.278554965	0.787527394	0.315674	-0.094160	
LL	-0.306428495	2.192449374	0.323059	0.099578	

PCA showed no clear separation of the specimens into major groupings of Cornsnakes and Black Ratsnakes (Figure 31); however, a slight gradient did exist. High variability could be seen, especially along Prin 1. Eigenvalues showed that approximately 92 percent of the variation could be explained on three principal components (Table 15). Approximately 84 percent of the variation was found on Prin 1; however, Eigenvectors suggested that no specific characters accounted for that variation (Table 16).



Pure Elaphe alleghaniensis

Figure 31. Principal Component Analysis of Raw Data

#### **3.1.1.4 Derived Data**

Much of the variation in Raw Data that is seen on Can 1 and Prin 1 can probably be attributed to differences in sizes of individual specimens. Adult snakes of many sizes were recorded from each group. Smaller individuals will undoubtedly have smaller raw data values, and larger would have the opposite. In general, Cornsnakes are smaller as adults than Black Ratsnakes; therefore, use of these characters could lead to systematic errors. To account for size variations in specimens, derived characters were calculated to standardize the measurements based on each snake's head length. Many of these derived characters were identified as significant in determining the new species of ratsnakes. This data set included 130 specimens.

CDA showed some separation of the specimens into the two major species groupings (Figures 32 and 33). Results were very similar to Raw Data in that almost all of the separation was shown on Can 1 (D>10.7; P<0.0001), with little to none on Can 2 (D<2.00). Eigenvalues were also similar to those for Raw Data (Table 17). Canonical coefficient values suggest that characters PWHL, FWPHL, and PLHL accounted for most of the variation in Can 1, while LLHL, EYEHL, and CWHL accounted for any variation seen in Can 2 (Table 18).

As with the Raw Data test, PCA showed no clear separation of the specimens using the derived data characters (Figure 34). Most values were highly variable and spread out along the entire range of Prin 1. Some slight separation could be seen with Cornsnakes in Prin 2; however, much overlap existed. Eigenvalues showed that only approximately 51 percent of the variation could be explained on three principal components (Table 17). Only approximately 20 percent of the variation was on Prin 1. Eigenvectors suggested again that no particular characters clearly accounted for variation in either Prin 1 or Prin 2 (Table 18).

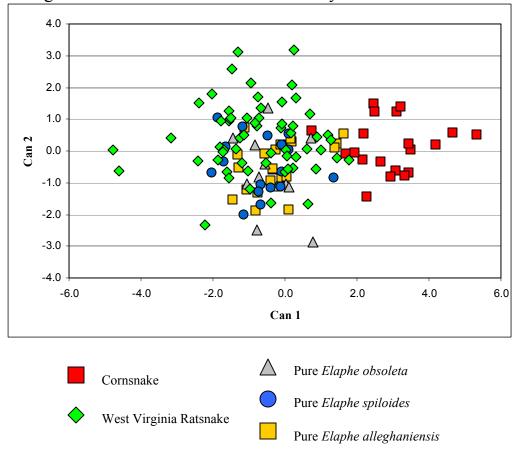
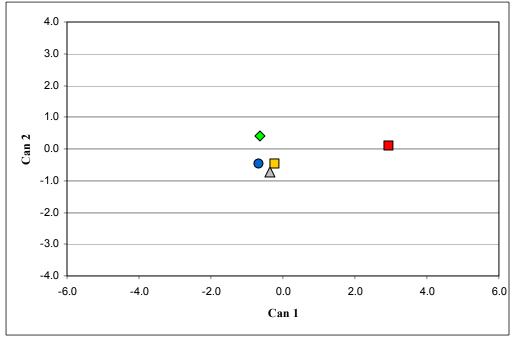


Figure 32. Canonical Discriminant Analysis of Derived Data

Figure 33. Canonical Discriminant Analysis of Derived Data Mean Values



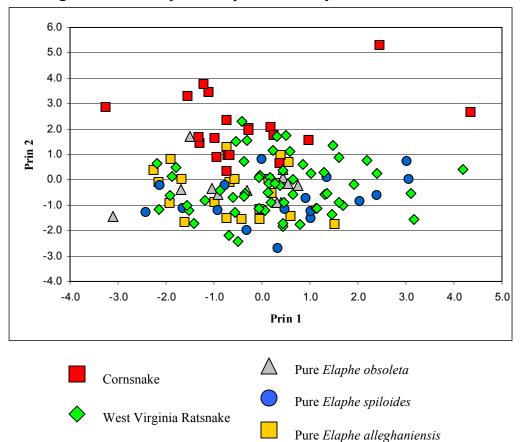


Figure 34. Principal Component Analysis of Derived Data

Test		Eigenvalue	Difference	Proportion	Cumulative
	Can 1	1.6672	1.4711	0.8183	0.8183
CDA	Can 2	0.1960	0.0865	0.0962	0.9145
CDA	Can 3	0.1096	0.0450	0.0538	0.9683
	Can 4	0.0646		0.0317	1.0000
	Prin 1	1.96195930	0.12762772	0.1962	0.1962
PCA	Prin 2	1.83433158	0.56511832	0.1834	0.3796
	Prin 3	1.26921326		0.1269	0.5086

Table 17. Eigenvalues for Canonical Discriminant and Principal Component Analyses of Derived Data

Table 18. Canonical Coefficients and Eigenvectors for Derived Data

	CI	CDA		CA	
Characters	Canonical (	Coefficients	Eigenvectors		
	Can 1	Can 2	Prin 1	Prin 2	
CWHL	0.0805360613	-0.4418751831	0.268137	-0.30172	
PLHL	0.6728751021	-0.1363222583	0.362654	0.400163	
PWHL	-0.9004970529	-0.0464715644	0.508066	-0.126675	
FWHL	0.7719874647	0.0027597242	0.142984	0.521712	
InWPHL	0.0923805090	0.0596763702	0.114186	0.251959	
EYEHL	-0.1534544944	0.6748264751	0.303454	-0.106086	
AGHL	0.0852038222	-0.0314583868	0.252992	0.098221	
InRHL	0.1196802840	0.0481494891	0.263319	-0.238683	
RWHL	-0.3843102323	0.0978768283	0.406497	-0.407100	
LLHL	0.1003600964	0.7041004225	0.338090	0.391792	

## 3.1.1.5 Counts and Derived Data

A combination of both Scale Count Data and Derived Data were determined to have the highest potential to separate taxa of Cornsnakes and Black Ratsnakes, and to possibly separate Black Ratsnakes into their newly assigned species. This assumption was based on the traditional value of scale counts in separating snake taxa, as well as its ability to distinguish between major groups using CDA and PCA. Raw Data were excluded due to the potential for error from size variations in individual specimens. Scale counts are independent of size, and derived characters are standardized for size using each individual's head length. All sexes of specimens were used, since sex was not determined to significantly affect results of the Scale Count Data test. To assess the validity of these arguments, this test included 13 characters and 113 specimens.

CDA showed distinct separation of the specimens into the two major species groupings (Figures 35 and 36). As was the case with most of the other tests, the majority of separation between groups was on Can 1 (D>32.0; P<0.0001). Means of these values are also mainly separated on the Can 1 axis (Figure 36). A smaller separation in Black Ratsnake taxa can be seen on Can 2, where West Virginia specimens have a close association with pure *Elaphe spiloides* specimens (D=1.32; P=0.363), and pure *E. alleghaniensis* and *E. obsoleta* specimens also have a close association (D=2.91; P=0.193). Eigenvalues showed that approximately 95 percent of the variation was on the first two canonical variables, with approximately 86 percent accounted for on Can 1 (Table 19). Canonical coefficient values suggest that the meristic characters SC and V and derived characters PWHL and FWPHL accounted for most of the variation on Can 1, while SC, RWHL, and EYEHL accounted for most of the variation on Can 2 (Table 20). Therefore, subcaudal count is an important character in separating the studied taxa.

PCA also showed good separation among the two major species groups; however, Black Ratsnake specimens showed a high degree of within- and between-group variation, thus were not as clearly separated (Figure 37). Like the other tests, separation was not as clearly defined as with CDA. Eigenvalues showed that approximately 48 percent of the variation could be explained on three principal components (Table 19).

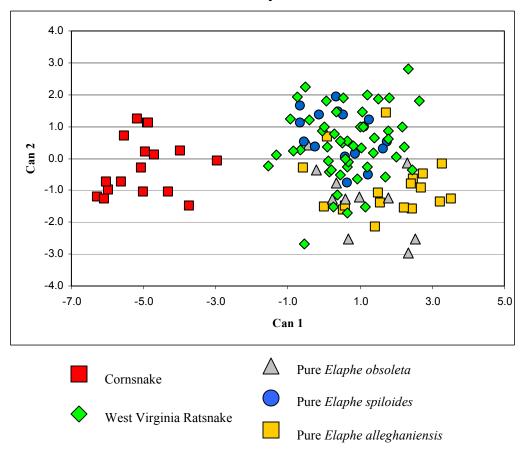
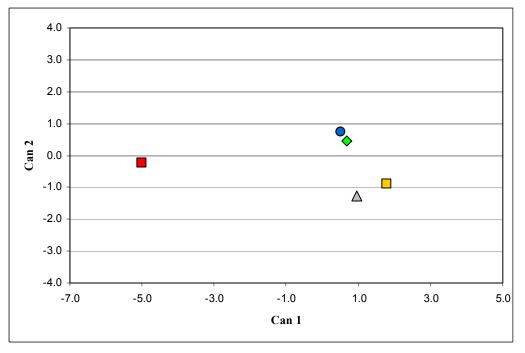


Figure 35. Canonical Discriminant Analysis of Scale Count and Derived Data

Figure 36. Canonical Discriminant Analysis of Scale Count and Derived Data Mean Values



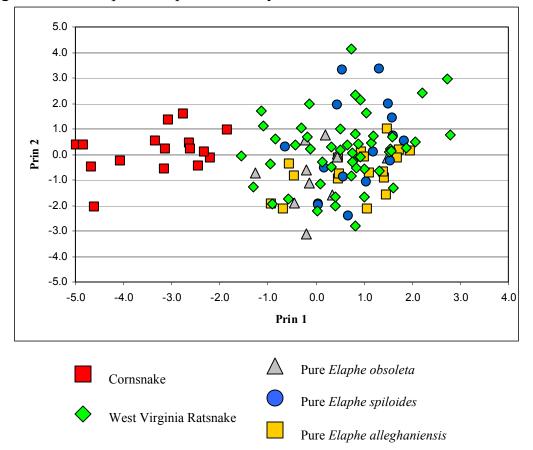


Figure 37. Principal Component Analysis of Scale Count and Derived Data

Test		Eigenvalue	Difference	Proportion	Cumulative
	Can 1	4.8173	4.3373	0.8640	0.8640
CDA	Can 2	0.4800	0.3323	0.0861	0.9501
CDA	Can 3	0.1478	0.0171	0.0265	0.9766
	Can 4	0.1306		0.0234	1.0000
	Prin 1	2.98905493	1.02611557	0.2299	0.2299
PCA	Prin 2	1.96293935	0.63512541	0.1510	0.3809
	Prin 3	1.32781395		0.1021	0.4831

Table 19. Eigenvalues for Canonical Discriminant and Principal Component Analyses of Scale Count and Derived Data

Table 20. Canonical Coefficients and Eigenvectors for Scale Count and Derived Data

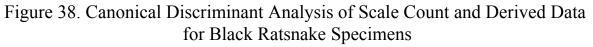
	CI	DA	PCA		
Characters	Canonical (	Coefficients	Eigenvectors		
	Can 1	Can 2	Prin 1	Prin 2	
SC	1.337014550	-0.955009870	0.391257	-0.225915	
V	0.591384675	0.389637959	0.448015	0.000251	
DSM	-0.380570667	-0.220321272	-0.239298	0.122624	
CWHL	0.116801426	-0.158071196	0.233000	0.383426	
PLHL	-0.339467714	-0.166693832	-0.191490	-0.044403	
PWHL	0.487934702	0.117284718	0.286058	-0.274213	
FWHL	-0.485181951	0.142931489	-0.362787	-0.275167	
InWPHL	0.078557402	-0.156753986	-0.170885	0.316650	
EYEHL	-0.052002614	0.591885898	0.182619	-0.344996	
AGHL	0.007164709	-0.141325766	-0.014325	0.530234	
InRHL	0.035456138	-0.133247369	0.177754	0.176465	
RWHL	-0.122661279	0.681431625	0.415307	0.126723	
LLHL	-0.149883967	0.027430528	-0.133054	-0.293927	

Only 23 percent of this variation was shown on Prin 1. This differs significantly with the All Data test, perhaps due to variation in raw data measurements caused by individual snake sizes. Eigenvectors suggest that meristic characters SC and V and derived characters RWHL and FWPHL accounted for most of the variation on Prin 1, while PLHL, PWHL, and LLHL accounted for most of the variation on Prin 2 (Table 20).

## 3.1.1.6 Black Ratsnake Specimens Only

The overall purpose of this study was to validate or refute the separation of Black Ratsnake specimens into distinct groups, and to draw a connection to the taxon or taxa that most closely relate to individuals from West Virginia. For the final multivariate statistical data set, the Cornsnake outgroup was removed to allow comparison strictly between specimens with Black Ratsnake morphology. By removing the outgroup, which always showed the most separation among all groups, more subtle variations could be expressed graphically and statistically among the four Black Ratsnake groups. This data set contained 96 specimens.

CDA showed slight (but noticeable) separation of the specimens into loosely clustered groups (Figure 38). West Virginia specimens appeared to have a closer association with pure *Elaphe spiloides* specimens on the Can 1 axis (D=1.36; P=0.36). Pure E. alleghaniensis specimens had a closer association with pure E. obsoleta specimens on the Can 1 axis and a slight separation on the Can 2 axis (D=2.88; P=0.22). This can be seen more easily when comparing the mean values of all four taxa (Figure 39). Eigenvalues show that approximately 85 percent of the variation was on the first two canonical variables, with approximately 65 and 20 percent on Can 1 and Can 2, respectively (Table Canonical coefficient values suggest that characters SC, RWHL, and EYEHL 21) accounted for the most variation on Can 1 (Table 22), helping to separate West Virginia and pure E. spiloides specimens from the other two groups. These characters corresponded to those that account for the most variation in Can 2 when the Cornsnake was included in the analysis (Table 20; Section 3.1.1.5). Characters V, DSM, and PWHL accounted for most of the variation in Can 2 in this analysis (Table 22), helping to separate pure E. alleghaniensis specimens from pure E. obsoleta specimens. Two of these characters, V and PWHL, accounted for a good portion of the variation in Can 1 when the Cornsnake was also included (Table 20; Section 3.1.1.5). In this analysis, subcaudal count was the overall strongest character.



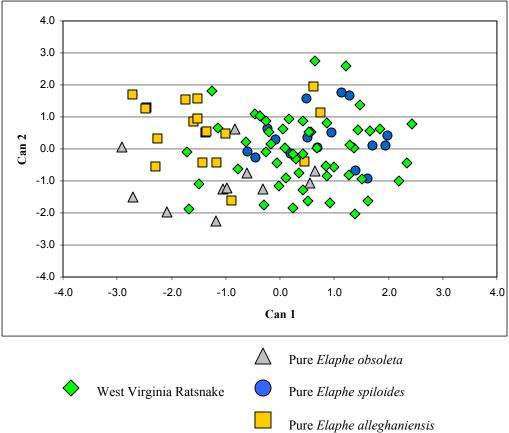
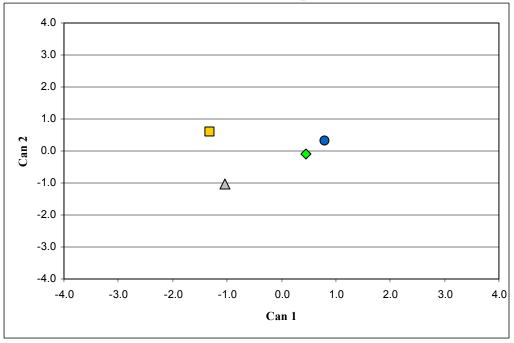


Figure 39. Canonical Discriminant Analysis of Scale Count and Derived Data Mean Values for Black Ratsnake Specimens



Test		Eigenvalue	Difference	Proportion	Cumulative
CDA	Can 1	0.6906	0.4759	0.6464	0.6464
	Can 2	0.2147	0.0518	0.2010	0.8474
	Can 3	0.1630		0.1526	1.0000
РСА	Prin 1	2.50143670	0.82802052	0.1924	0.1924
	Prin 2	1.67341618	0.31091562	0.1287	0.3211
	Prin 3	1.36250057		0.1048	0.4260

 Table 21. Eigenvalues for Canonical Discriminant and Principal Component Analyses of Counts and Derived Data for Black Ratsnakes Only

Table 22. Canonical Coefficients and Eigenvectors for Counts and Derived Data for Black Ratsnakes Only

	CI	DA	PCA Eigenvectors	
Characters	Canonical (	Coefficients		
	Can 1	Can 2	Prin 1	Prin 2
SC	-1.057206126	0.140746861	-0.414354	-0.263773
V	0.093295745	0.532093914	0.103105	0.367002
DSM	0.061206969	-0.547523508	-0.204419	-0.104317
CWHL	-0.225041959	0.304436409	0.177032	0.243755
PLHL	-0.062337366	0.045586676	0.401848	-0.234748
PWHL	-0.020473555	-0.522928047	0.494975	-0.162129
FWPHL	0.366842958	0.041506304	0.203401	-0.454023
InWPHL	0.044311235	-0.228395657	0.312492	0.095645
EYEHL	0.459967083	0.201626395	0.304985	-0.167323
AGHL	-0.229906720	0.301231704	0.188429	0.347465
InRHL	-0.155714129	-0.021680575	0.163607	0.328439
RWHL	0.572008374	0.255405103	0.277959	0.360142
LLHL	0.063639346	-0.188807318	0.343811	-0.206043

Although general trends can be derived from this CDA, applying it to each specimen does not show any correlation. Logic would dictate that specimens that overlap with pure *E. alleghaniensis* should theoretically be those gathered from eastern-most counties; however, this was not always the case. Five specimens possessed Can 1 values less than -1.0 (comparable to the mean Can 1 value of *E. alleghaniensis*). Those specimens were from Monongalia, Kanawha, Berkeley, Hardy, and Mineral counties. Three of these counties **are** in the Eastern Panhandle; however, other specimens from these counties do not possess similar values. Therefore, classification of Black Ratsnake specimens from West

Virginia into newly-defined ratsnake taxa is not statistically or graphically feasible using CDA of specific morphometric characters.

PCA showed no clear separation of the specimens into distinct clusters; however, some varied clustering existed along the Prin 1 axis (Figure 40). West Virginia and pure *Elaphe spiloides* specimens were highly variable and spread out along the entire range of Prin 1, but pure *E. alleghaniensis* and *E. obsoleta* specimens tended to cluster in only half of the value field. Eigenvalues showed that only approximately 43 percent of the variation could be explained on three principal components (Table 21). Eigenvectors suggested that no particular characters clearly accounted for the variations in either Prin 1 or Prin 2 (Table 22).

Results of these multivariate statistical analyses show no clear way to separate Black Ratsnake individuals based on geographic location, despite the use of scale count characters (often used in species separation) or characters defined as significant by Burbrink (2001) in separating his newly-defined taxa.

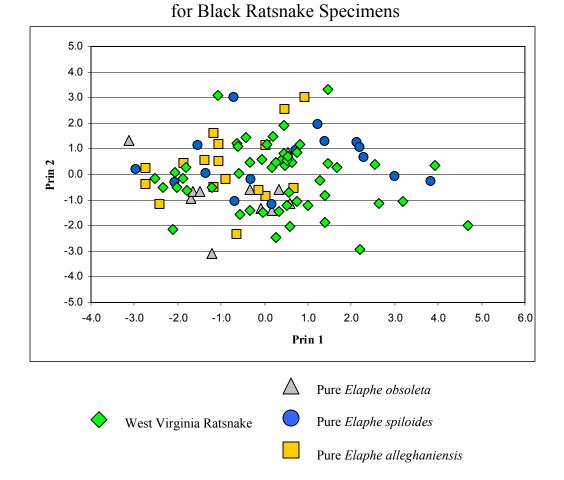


Figure 40. Principal Component Analysis of Scale Count & Derived Data

## 3.1.2 Character Comparisons Among Taxa

Column graphs were created to show the relationships of character means among groups or taxa. Characters were separated into their respective types: scale counts (meristic), raw data (mensural), and derived characters, and plotted on separate graphs. Using simple comparisons of mean values for each character, relative closeness of each taxon could be determined. In theory, those taxa with similar means should have similar morphology. Two types of column graphs were created for each type of character. The first graph displayed the mean values for each character; however, due to the relative difference in scale among characters, some graphs had a large range on the y-axis, showing less variation among each taxon. Therefore, a second graph was created for each set of characters. These graphs made a standardized percentage scale on the y-axis, allowing easier comparisons among taxa. This was accomplished by determining the mean for each character, then dividing each taxon value into that mean, yielding a relative percentage.

Comparisons of mean values for scale count data among groups are shown in Figures 41 and 42. On average, Cornsnakes had noticeably fewer subcaudals and ventrals, while no apparent differences were present among means of Black Ratsnake groups. Subcaudal counts were also considered for male specimens only, to determine if sexual dimorphism affects mean values for each taxon. Subcaudal counts were similar for both data sets. Greater differences were seen when comparing percentages of mean values, especially between the Cornsnake and all other Black Ratsnake groups. In general, Cornsnakes are shorter snakes with shorter tails, and are known to possess fewer ventrals and subcaudals. Differences in dorsal scale numbers were insignificant among all groups; counts only varied by two or three scales at most. All members of the *Elaphe* genus have inherent variability in dorsal scale rows within species and even within each individual.

Comparisons of mean values for raw data among groups are shown in Figures 43 and 44. As expected, Cornsnakes had significantly smaller measurements for all characters, predominantly because adults of this species to not attain the same length or girth as Black Ratsnakes. Black Ratsnake groups showed consistent trends among all characters.

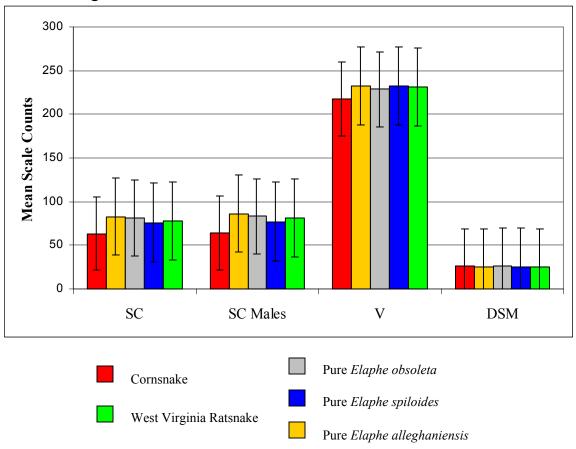
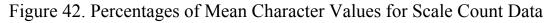
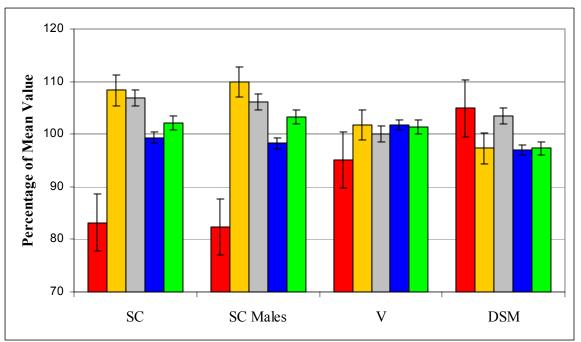


Figure 41. Mean Character Values for Scale Count Data





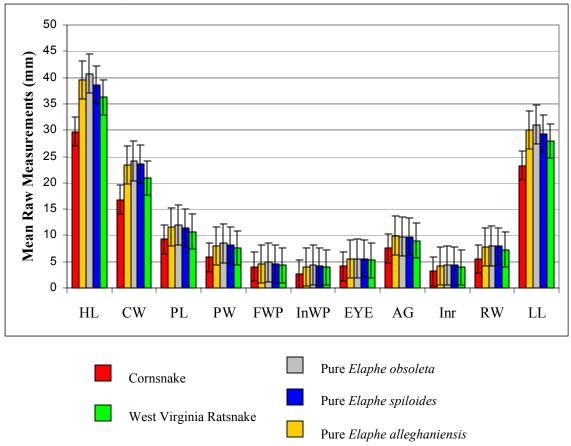
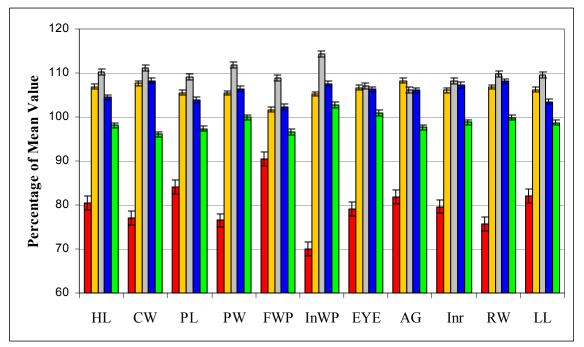


Figure 43. Mean Character Values for Raw Data

Figure 44. Percentages of Mean Character Values for Raw Data

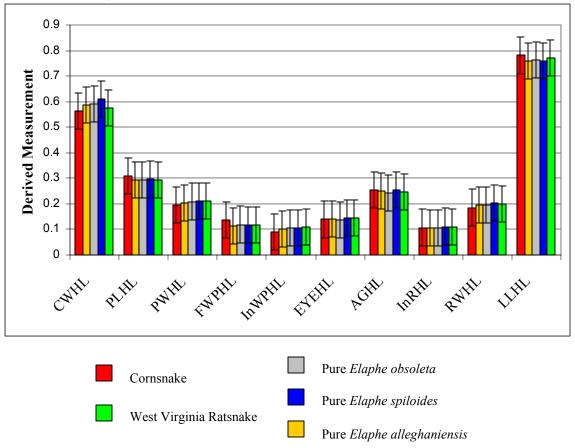


In general, pure *E. alleghaniensis* specimens appeared larger than other Black Ratsnake groups. Mean values of West Virginia specimens were smaller than the other three groups. Overall, the Black Ratsnake groups had similar values that varied based primarily on sizes of individual snakes used during data collection. Due to the small sample sizes in this study, no assumptions can be made regarding differences in raw specimen data among Black Ratsnake groups from different areas.

Comparisons of mean values for derived data among ratsnake groups are shown in Figures 45 and 46. Derived data were calculated by standardizing each raw measurement by the head length. Little difference among groups was seen in mean values of these derived characters. Standard error bars overlapped any potential difference in values. When standardized again using percentages of mean values, some separation occurred. Most variation existed in Cornsnake character values, which were drastically different from other groups on several characters. West Virginia specimen values varied slightly, but were always correlated with other Black Ratsnake groups. They showed no greater correlation to any particular other group of newly-described ratsnake species. Therefore, no conclusions could be drawn about West Virginia specimens by analyzing mean values for derived character data.

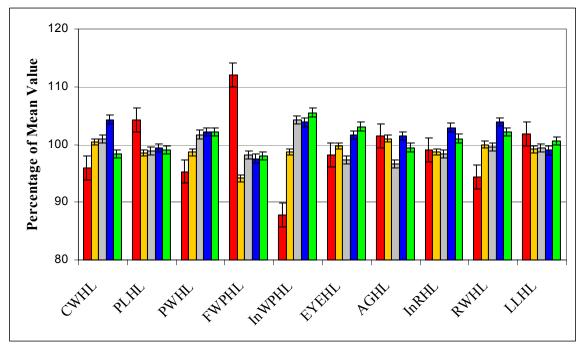
#### 3.2 DORSAL PATTERN SURVEY

Like most other subspecific individuals of the traditional species *Elaphe obsoleta*, Black Ratsnakes gradually change their dorsal and ventral coloration as they mature from juveniles to adults. Some subspecies loose their blotches completely in exchange for longitudinal stripes running the length of the bodies. Black Ratsnakes, which loose their blotches and fade darker as they mature, are most widely known to the general public as pure dark black individuals, which is how the name component "black" was originally derived. They are also often mistaken for Black Racers (*Coluber constrictor*) or Black Kingsnakes (*Lampropeltis getula niger*) and collectively grouped into the term of "Black Snakes". Unfortunately, their blotched juvenile pattern (which aids in cryptic coloration and aposematic defenses from predators) often causes humans to mistake them for other blotched snakes, including rattlesnakes.



#### Figure 45. Mean Character Values for Derived Data

Figure 46. Percentages of Mean Character Values for Derived Data



Black Ratsnakes are known to display a great deal of variation in the retention or loss of this dorsal pattern; however, no known studies have attempted to investigate the extent of this variability. As an added component to the morphometric study, digital photographs were taken of every specimen and ranked for dorsal blotch pattern retention using a pre-established scale.

Fifteen observers ranked the specimens based on pattern retention. Observers were from different backgrounds or professions; however, only two were trained herpetologists. Photographs were named based on specimen number. To prevent user bias, snake pictures were arranged in order from highest to lowest specimen number, and not based on assigned groups or taxa.

All observer results were compiled into an Excel spreadsheet and analyzed. Although the pattern scale had only four categories, and each specimen could be assigned to only one of those categories, the range of values varied greatly between observers (F=6.58, P<0.001). This demonstrates the inherent subjectivity of the test. A correlation analysis was performed for all data to analyze the similarity between individual observations. In this particular study, correlation was used to assess the degree of comparability between observers. Most observer correlations ranked between 60 and 80 percent (i.e., the scores were 60 to 80 percent similar to each other). The two herpetologist observers had the highest correlation value, approximately 85 percent. These two observers were used as standards for comparison to other observers. A direct relationship could be seen among all observers based on biological experience and correlation values. Those with more vertebrate field biology experience showed a higher degree of similarity to the herpetologists' assessments. Based on this analysis, two observers were eliminated from remaining analyses, for their correlation values ranked less than 50 percent when compared to all other observers.

Within the Excel spreadsheet, specimens were sorted into their preassigned taxa. Single Factor Analysis of Variance (ANOVA) was performed for each taxa to test the hypothesis that the pattern data were significant in separating specimens into predetermined taxa. The

P value for every taxon was <0.001, signifying that members within each group were significantly different; therefore, the groups could not be separated numerically using pattern rankings. Within the ANOVA analysis, a variance value was also determined for each specimen. Those specimens with variances over a value of 0.75 were examined for potential bias and discarded from further analyses. Six specimens were discarded, including one from Taxon A and five from Taxon W. In all six cases, observed specimens were deteriorated in some manner, predominantly due to scale sloughing (Figure 47).

Mean specimen values were created by averaging all 13 observer entries. Maximum, minimum, and mean values were then calculated for each taxon based on mean specimen values (Table 23). Despite the lack of statistical validation, these values yielded interesting results. Pure *Elaphe alleghaniensis* specimens were much darker (retaining less pattern) than all other defined groups, with a mean value of 3.5. *E. obsoleta* and *E. spiloides* specimens displayed much more pattern, with mean values of 2.2 and 1.9, respectively. West Virginia specimens, which are effectively located on the peripheral ranges of *E. alleghaniensis* and *E. spiloides*, displayed a mean value of 3.0, showing a potential gradient of dark and blotched individuals. It is also interesting to note that ranges for maximum and minimum values of *E. alleghaniensis* and *E. spiloides* overlap very little.

Taxon	Number of	Specimen	Specimen	Taxon	Herpetologist
1 8 2011	Specimens	Min	Max	Mean	Taxon Mean
Pure E. alleghaniensis	22	2.1	3.9	3.5	3.6
Pure E. spiloides	11	1.0	2.7	1.9	1.8
Pure E. obsoleta	7	1.0	3.8	2.2	2.1
West Virginia	54	1.2	4.0	3.0	3.0

Table 23. Minimum, Maximum, and Mean Values by Taxon for the Dorsal Pattern Survey

Figure 47. Specimens Excluded from Analysis for the Dorsal Pattern Survey



WVBS # 2934



WVBS # 3983



WVBS # 4310



CM # 6075



WVBS # 10542



CM # 73669

As previously mentioned, the two herpetologist observers were used as standards for comparison to all other observers. The overall mean values for each taxon were compared to the combined means of both herpetologist observers (Table 23). The mean values were 90% similar to herpetologists' observations for all four taxa, showing that, despite observer bias and subjectivity, this methodology showed some degree of objectivity when analyzed as a whole. It is assumed that the addition of more observers, especially herpetologist observers, could only improve the results of the study.

As a personal observation, the author has noticed that Black Ratsnakes from different areas of West Virginia seem to retain patterns differently. Many individuals from the Ohio River Valley tend to retain heavy blotching, even as large adults. On the other hand, individuals from mountainous areas or other higher elevation sites seem to turn dark more quickly (at a younger age) and retain very little dorsal blotching. Theoretically, this "blacker" color could prove advantageous for individuals in colder climates, allowing more efficient thermoregulation on colder days by providing faster solar absorption due to darker pigmentations.

Preliminary data for this portion of the study yielded interesting results; however, results were not completely conclusive. Further studies should be conducted on a wider scale. For this study, digital photographs were mainly taken to supplement the morphometric data; therefore, only a limited number of specimens were actually photographed. In the future, overall sample size should be increased, as should geographic sample size. More preserved specimens could be photographed, whether or not morphometric data are gathered. In addition, photographs of live specimens could also be incorporated. Higher numbers of observers should decrease the amount of subjectivity even further. In order to increase consistency among the increased amount of observers, a higher percentage of vertebrate biologists (especially herpetologists) should be polled. Data could be entered into ArcGIS and represented graphically at each location by unique data value categories.

#### 4.0 CONCLUSIONS

The purpose of this project was to utilize morphometric character analyses to determine morphologic variation, species status, and distribution of ratsnakes found within the state of West Virginia. By employing such methods as Canonical Discriminant Analysis, Principal Component Analysis, and column graphs, group separation trends (or lack thereof) can be detected.

The first objective of this study was to examine variations in morphology using different morphometric characters. It was found that a great deal of variation does indeed exist within all groups, especially with the West Virginia specimens. Not only was there variation in certain static characteristics (such as scales), but color pattern and relative size differences could also be seen. Within-group variation makes it extremely difficult to assign group-wide identifiable traits.

The second objective was to determine if any of the morphologic characters (designated as instrumental by Burbrink (2001) in separating *E. alleghaniensis* and *E. spiloides*) were significant in this study. Twenty-four characters were analyzed; however, none appeared to show a strong ability to separate assigned Black Ratsnake groups. Certain scale count characters were effective in showing separation of the two major groupings, Black Ratsnakes and Cornsnakes; however, due to high variability among groups and individuals within groups, no characters were reliable in separating the Black Ratsnakes.

The third objective was to assign a species status to each West Virginia specimen examined during the study. This objective could not be fulfilled, since there was no clear way to separate Black Ratsnake specimens into different species. At the current time, most specimens (among all Black Ratsnake groups) continued to appear as one highly variable group, supporting the traditional subspecies concept. CDA and PCA did show a slight similarity between West Virginia and pure *Elaphe spiloides* specimens when compared to the other two groups. This trend was logical, though, given the geographic proximity of

the groups. Unfortunately, species determination was not predictable based on geographic areas or counties; therefore, individual West Virginia specimens could not be officially placed within any group.

The fourth objective was to expand on the morphometric work initiated by Frank Burbrink (2000, 2001) by including West Virginia specimens in the data set. Although the sample sizes of grouped specimens used in this study were far less than those used by Burbrink, little evidence existed to support the claim that individuals found in one area of the country were significantly different than those found in another area. Burbrink's main supportive conclusions relied on Canonical Discriminant Analysis, which inherently gives bias to the results by attempting to show as much variation as possible within user-defined groups. Principal Component Analysis is a more powerful tool for showing true variation in data sets. In this study, both tests were conducted on the same data. CDA showed good separation of some taxa (especially the Cornsnake outgroup), and very slight separation among Black Ratsnake groups. PCA showed little to no separation among Black Ratsnakes. Genetic studies by Burbrink (2000) provided evidence that Black Ratsnakes in different areas of the country evolved from numerous lineages, thereby causing their separation into multiple species. Results of this limited study cannot refute the genetic evidence; however, they do raise questions as to the ability of separating specimens into newly designated taxa based strictly on morphology. Unfortunately, morphology is usually the only factor available to field biologists or museum curators when making species determinations.

The last objective of the study was to create a statewide distribution map for the *Elaphe* genus in West Virginia. Since West Virginia Black Ratsnake specimens were unable to be separated into distinct groups, a composite distribution map and dichotomous key could not be established. The data would suggest that all Black Ratsnake individuals found within the state are indeed a part of one highly variable group. This is more consistent with traditional taxonomic nomenclature. Should separation into one of Burbrink's (2000) newly classified species become necessary, results of this study and overall geographic location data support the designation of all West Virginia specimens as *E. spiloides*.

#### **5.0 LITERATURE CITED**

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## **APPENDIX A**

**Museum Specimen Information** 

Specimen #	Museum	State	County	Collector	Year
S 7174	СМ	NC	Cherokee	Beck, J.A.	1903
13276	СМ	VA	New Kent	Richmond, N.D.	1938
18571	СМ	VA	New Kent	Richmond, N.D.	1939
18572	СМ	VA	New Kent	Richmond, N.D.	1939
18574	СМ	VA	New Kent	Richmond, N.D.	1939
18853	СМ	VA	New Kent	Richmond, N.D.	1940
18854	СМ	VA	New Kent	Richmond, N.D.	1940
19577	СМ	VA	Prince George	Llewillyn, L.	1940
19578	СМ	VA	Prince George	Llewillyn, L.	1940
22887	CM	VA	Virginia Beach	Werler, J.E.	1944
22889	СМ	VA	Virginia Beach	Werler, J.E.	1944
23209	СМ	VA	Virginia Beach	Werler, J.E.	1943
32711	СМ	VA	New Kent	Richardson, G.	1952
34540	CM	VA	New Kent	Richmond, N.D.	1956
36739	СМ	VA	Charles City	Wood, J.T.	1951
37443	СМ	NC	Buncombe	Parkes, K.C. and E. S.	1960
39614	CM	NC	Graham	Morrison, F.	1947
39615	СМ	NC	Macon	Morrison, F.	1947
73491	CM	NC	Johnston	Freed, P.S.	1979
73559	CM	NC	Johnston	Freed, P.S.	1979
73669	CM	NC	Wayne	Freed, P.S.	1979
73704	CM	SC	Lee	Peters, E.L. and Thomson, D.C.	1979
113669	CM	VA	Virginia Beach	Young, D.A.	1985
114043	CM	VA	King and Queen	Young, D.A.	1985
114066	СМ	VA	Virginia Beach	Young, D.A. and Lovette, R.	1983

Taxon A – Pure *Elaphe alleghaniensis* Specimens

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	Specimen #	Museum	State	County	Collector	Year
	S 5128	СМ	OH	Hamilton	Rexroat, E.A	1929
	S 5133	СМ	OH	Hamilton	Rexroat, E.A	1929
	S 9762	СМ	IN	Pike	Swanson, D.C. and P. L.	1936
	17029	СМ	KY	Floyd	Adams, W.	1939
	17030	СМ	KY	Floyd	Adams, W.	1939
	17031	СМ	KY	Floyd	Adams, W.	1939
	17262	СМ	KY	Jefferson	Unglaub, A.	1939
	20684	СМ	KY	Russell	Morrison, F.D.	1941
	23954	СМ	OH	Clermont	Goodpaster, W.	1945
	23956	СМ	OH	Clermont	Goodpaster, W.	1945
	25898	СМ	IN	Unknown	Unknown	Unknown
	56915	СМ	OH	Preble	Ashton, R.E	1969
	58712	СМ	KY	Bracken	Collins, J.T.	1960
	66518	СМ	IL	Macon	Williams, K.	1963
	58711	СМ	KY	Wayne	Hirshfeld, C.	1960
	114334	СМ	IL	Jackson	Busack, S.D. and Rielly, S.	1986
	147754	СМ	KY	Jefferson	Taylor, G.	1994

Taxon S – Pure *Elaphe spiloides* Specimens

Taxon O – Pure Elaphe obsoleta Specimens

Specimen #	Museum	State	County	Collector	Year
S 7157	СМ	MO	Crawford	Beck, J.A	Unknown
54782	CM	KA	Douglas	Montanucci, R.R.	1971
55432	CM	KA	Douglas	Edwards, S.R.	1971
58572	CM	KA	Douglas	Montanucci, R.R.	1970
58611	CM	KA	Douglas	Collins, J.T.	1971
58741	CM	KA	Douglas	Pisani, P. & G.R.	1971
61916	CM	OK	Tulsa	McCoy, C.J	1976
66520	CM	MO	Franklin	Baley, J.	1966
66521	CM	MO	Franklin	Baley, J.	1966
88632	CM	OK	Nowata	Wood, D.S.	1982
91163	CM	OK	Love	Wood, D.S.	1982
93682	СМ	OK	Pontotoc	Wood, D.S.	1983

Specimen #	Museum	State	County	Collector	Year
168	WVBS	WV	Kanawha		
544	WVBS	WV	Putman		
1498	WVBS	WV	Pocahontas		
2405	WVBS	WV	Harrison		
2686	WVBS	WV	Wood		
2934	WVBS	WV	Wetzl		
3172	WVBS	WV	Ritchie		
3182	WVBS	WV	Wirt		
3835	WVBS	WV	Berkeley		
3836	WVBS	WV	Preston		
3979	WVBS	WV	Nicholas		
3983	WVBS	WV	Raleigh		
4310	WVBS	WV	Monroe		
4311	WVBS	WV	Mercer		
S 5329	CM	WV	Pocahontas	Netting, M.G.	1931
S 5585	CM	WV	Pocahontas	Netting, M.G.	1931
S 5588	CM	WV	Pocahontas	Netting, M.G.	1931
S 6075	CM	WV	Ohio	Netting, M.G.	1932
S 7511	СМ	WV	Berkeley	Poland, L.	1934
S 7764	CM	WV	Mineral	Llewillyn, L.	1934
S 9096	СМ	WV	Monongalia	McClintock, J.	1936
S 9097	CM	WV	Monongalia	McClintock, J.	1936
S 9098	СМ	WV	Monongalia	McClintock, J.	1936
S 9432	CM	WV	Monongalia	Richmond, N.D.	1936
S 9602	СМ	WV	Preston	Netting, M.G. and Llewellyn, L.	1937
11976	CM	WV	Pocahontas	Goin, C.J.	1936
11999	СМ	WV	Nicholas	Goin, C.J.	1936
12038	CM	WV	Webster	Goin, C.J.	1936
13037	СМ	WV	Monongalia	Netting, M.G.	1933
13876	СМ	WV	Mineral	Llewillyn, L.	1938
14053	CM	WV	Mineral	Llewillyn, L.	1938
14085	СМ	WV	Marion	Boggess, G.S	1938
14217	СМ	WV	Upshur	Richmond, N.D.	1938
14453	CM	WV	Raleigh	Richmond, N.D.	1938
15389	CM	WV	Lewis	Green, N.B.	1938
15608	CM	WV	Randolph	Green, N.B.	1936
15658	CM	WV	Randolph	Green, N.B.	1937
15728	CM	WV	Randolph	Green, N.B.	1938
15927	CM	WV	Wyoming	Richmond, N.D.	1938
15970	CM	WV	McDowell	Richmond, N.D.	1938

Taxon W – West Virginia Specimens

Specimen #	Museum	State	County	Collector	Year
17570	CM	WV	Lincoln	Richmond, N.D.	1939
19474	CM	WV	Wayne	Green, N.B.	1940
24068	CM	WV	Hardy	Wilson, L.W.	1945
24069	CM	WV	Hardy	Wilson, L.W.	1945
27325	CM	WV	Lewis	Netting, M.G.	1946
30049	CM	WV	Jefferson	Netting, M.G.	1951
30050	CM	WV	Jefferson	Netting, M.G.	1951
30087	CM	WV	Berkeley	Scott, F.E.	1949
35269	CM	WV	Greenbriar	Schwartz, F.J.	1955
117328	СМ	WV	Raleigh	Buhlmann, K.A.	1985

Taxon G – Cornsnake Specimens

Specimen #	Museum	State	County	Collector	Year
15093	СМ	NC	Hyde	Clanton, W.	1937
26706	СМ	VA	Warren	Ulmer, F.A.	1946
27750	CM	GA	Chatham	McCauley, R.H.	1946
32488	CM	VA	Orange	Richmond, N.D.	1953
35385	CM	TN	Sevier	Wood, J.T.	1946
124668	CM	VA	Goochland	Hart, D.	Unknown
91975	CM	SC	Allendale	Romano, A.W.	1966
91994	CM	SC	Hampton	Romano, A.W.	1965
108988	CM	DE	Sussex	Arndt, R.G. & Lindsay, B.	1962
114053	CM	VA	Buckingham	Young, D.A. & Norris, J.A.	1984
116847	CM	GA	Chatham	De Marco, V.	1977
116848	CM	SC	Jasper	O'Connell, A.	1977
116916	CM	DE	Sussex	Brown, E.	1978
124667	CM	VA	Goochland	Hart, D.	Unknown
124671	CM	VA	Goochland	Hart, D.	1981
124673	CM	VA	Bedford	Miller, M. & A.	1983
136864	CM	NC	Tyrell	Unknown	1956
136865	CM	NC	Tyrell	Unknown	1956
146381	CM	VA	Amherst	Sullivan, M.C.	1986
146485	СМ	VA	Bedford	Unknown	1988

## **APPENDIX B**

**Completed Data Sheets** 

pM.	a Cán dong	>										•								,						
	*****	State	County			SC	_					1	HLV	SL	cw	TL	PL	PW	FWP	PrFWP	InWP	EYE	AG	InR	RW	LL
CM		w	Mon-				a27			207		35.92	-	23.50			10.10	6.98	4,56	3.29	3.86	4.79	8.31	4.69	7.23	26.50
CM				M			223			723		34,15				21.3	10.70	7.65	4,55	2.86	3.67	5.33	7,70	3.76	6.66	26.82
CM	30049	WV				79			27	126	19		and an at a sure of the second se	22.50		22,3	11.79	8.25	4,29	3.25	3.97	5.45	8.68	3.44	7.38	29.14
CM	5585	WV	Poca-			Salley	336	- Varia		23				24,44		16,0	12.10	7.65	3.92	3.84	4.57	5,29	2,26	3.19	7.75	30.07
CM	1	₩√	Bal-		A		233				19				23.96		10,43	7.79	3.52	2.73	3.76	5.3)	10.78	4.12	6.97	26.43
CM	·· · · · ·	WV	Nich-			73					118		34.24				11.03	8.03	3,90		3,55	5.07	9,53	3,36	7.56	29.26
CM		WV	Lewis	M	<u> </u>		238						29.98			<u>al.5</u>	10,11	6.77	3,72		3,53	4.88		3.92	1	26.05
CM			Upshur						~~~~~~				32.37			18.3	9.58	7.17		3.93	3.76	5.04	8.03		1	26.24
CM			Poca		A	87	233		~~~~~	·					25.04		12,71	1	5.62	4.32	4,09	6.24	11.61	5.341	10.00	33.80
CM	9602	WV	Prestor	15	J	74	231						26.45				9,00			3.03	3,64	4.70	7.48		6.52	
CM	14085				·		~~~~						33.91			124.0	11.46	7.95		+	4.59	5,62	+	3.99	7,92	29.86
<u>CM</u>	15927	NV	Wyomina	F	A	75	333	ĺλ			18	37.30	1				11.22	7.65	3.89	3.99	3,88	5,68	8,76	4,55	7.22	28.04
CM				-+	Ą	70	229	11	aĵ	21	17	37.12		23.87	20.21	19.0	10.96	7,42	4.02	3.70	3,82	5.66		6.85	7.06	27.95
CM	17570	W√	Lincoln	M	A	82	233	11	25	605	17	34.37			19,48	21,8	10,65	6.78	4.02	3.74	3.84	5,06	9.04	4.19	7.01	a6.00
CM	24068	WV	Hardy	M	A	85.	232	1	24	126	, ]	35.28	32,54	22,60	21,14	24	9,80	6.87	3.84	3.72	7,02	5.01	9.27	4.30	7,48	27.81
CM	35269	W	Giken-		A	75	954	12	25	125		36,62	30.42	23,81	19.28	21,1	10,53	6.78		3.95	3,60	5.18	9.39	3.7	7.11	28.04
СМ	15970	WV	McD-		A	75	234	12	25	125	·	36.45	30.15	24.28	21,93	20.5	10.78	7.42	4.28	4,02	4,13	5.20	9.67	3.90	726	28,09
CM	14453	WJ	Raleigh		A	72	232	1	27	1 24		29.61	24.4	20,13	18.74	17	8,99	6.63	3.46	3.54	3.45	4.60	8.28	2.99	5.77	22.83
CM	15728	WV	Rand-		4	75	229	13	27	126		30,94	25.64	19.66	17.35	17	9,25	6.74	3,48	3.30	3.21	4.44	6:71	3,80	6.35	24.41
CM	9098	WV	Mon-	M	A	72 8	331	12		25		37,72	33.03	24.77	22.91	22	11,09	7.53	4,61	4.16	3.55	5.60		4.23	7.52	28.43
(M	14053	WV	Mineral	F	A	79	232	.11		25	5	39,26	31.87	25.09	21,98	22.5	12,17	8.15	4.31	4.16	41.08	5.24	11,20	4.32	7,47	31.28
СМ	30050	W٧	Jeff-	M	A	85	234	1		27		41,78	35.84	26,91	22.28	26	11.16	8.31	4.78	3.71	4.30	5.98	9,72	3,81	8.55	31.36
CM	24069	WV	Hardy			85	232	13		a1	and the second se	410,9	28,46	26.413	23,01	25,2	11.55	7,80	4.66	3.84	41.01	5.83	9.37	4.36	8.00	31.0.6
СМ	30087	WV	Berk-	M	A	81	વેર્ટ્ડ	12		a	1	39.07	32,05	24.75	21.02	23.8	12,08	7.99	4.88	4.31	4.07	5.16	10,07	4,62	7,17	30.59
См	15658	W	Rand.		A	76	237	11	and a subset	23		37.87	33.22	24.49	21.12	23.8	11.13	7.91	4.81	3.17	3.41	5.34	9,98	2.96	6.71	36.68
(M	1000		Mon-	M		78 :		11	Alternativa	25	anticester.	39,05	f-		:27.38		11,74	8.11	4.71	4,04	4,42	5,83	9.13	3.21	7.27	30,17
PM	27325	WV	Lewis		A	80	231	13	Ancourses	87		37.30	33-82	23,90	22.93	20,8	11,01	7.71	4.22	4.22	3.99	5.65	9,27	4.67	7.81	29.01
СМ	7764	WV	Mineral	f	A	70	225			al	auconceller.	34,8	31.17	22,2-	20.58	19.3	10,83	7.50	4.35	3.67	3.77	5.07	8,67	3.35	6.07	27.49
CM	5588	W	Poca-	M	A	71	330	12		21	and the second sec	39,20	33.50	24.35	21.79	21.7	11,88	8.11	4,76	3.76	3.98	5.98	9,45	3,09	7,63	30,14
CM	13037	WN	Mon-	M	A	81	225	11	Paramontation	25		40.63	35.12	26,62	21.27	24.8	11,32	7.19	4.29	4.04	3.58	6.11	9.81	3.90	8,21	30.79
	15608									24		36,51	33,60	23,39	24,24	22.0	11.23	7.79	3.78	4.42	4.09	5.58	9.29			28.61
	7511									27		40,66	33,54	25.98	23,04	20.8	11,57	8,27	4.26	4.36	4.27	5,63	8.86	4.76	8,28	30,50
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Museum Specimen # State County Sex Age SC V IFL DSN		HLV	SŁ	cw	TL	PL	PW	FWP	PrFWP	InWP	EYÉ	AG	InR	RW	LL
CM 11976 WU Poca- MA 78 23211	25 40,0	30,59	25.35	22.39	22.7	11,20	8,49	4.27	4.27	5.40	5.79	9.06	5.22	8.12	29,67
CM 12038 WV Webster F. A 71 23411				21,901				4.75	4.16	4.39	5.37	9.25	4,13	7,76	27.96
CM 6968 WV Preston f J @7 2411 (1	27 29.30	22.84	20.02	15.66	1.2	8.63	6.52	3,90		3.63	1				22.56
CM 9096 WV Mon- MA 8322711	27 41,44	33.74	25.66	25.58 2	25,5	12.11	8.49	4.38	3.95	4.47	5.67	10,12	3.50	8.21	32,12
CM 7134 WV Berk- FA 5224211				24.70 1											33.84*
CM 39615 NC Macon F A 72 23512				21.59											
CM 39614 NC Braham FA 70 24111				20.381											
	24 36.4	1 31.42	23,65	20.34 2	13.2	10,30	7.39	4.27	4.09	4.36	4,88	9.58	4,47	7.48	26.65
CM 7349 NC John - MA 89 233 11	22 35.77	30,84	23.47	21.05 2	22.8	10,67	7.57	4.39	4.03	3,41	5,35	\$.63	3.87	7.18	26.88 3
		32,57	26,58	22.211	8.7	12.39	7.95	5.03	4.57	3.90	5,85	10,20	4,28	7.94	30,03
				20.79 3											29,19
CM 7174 WC Cher- FA 75 235 2	25 37.5-	34.62	24,46	20.42 =	21.0	11.50	7,84	4.72	4.18	4.62	5.47	9,24	4.34	7.21	28.88
		28.91			9.3	10,07	6.86	3.56	3,33	2.61	5.04	7.94	2-83	6.47	24.38
CM 18854 VA New Kent M A 79 224 11	25 50, 6	39,41	31.37	36.45 3	7.3	13,52	9.72	5.99	5.27	5.27	6.73	13,79	5.81	16,02	38.25
CM 13669 VA V Beach M A 90 246 11	27 44.54	41.28	29.77	22,11 3	30.0	13.61	9.52	5.83	4.49	5,02	6.17	11.31	4.70	9,22	34.39
CM 114043 VA Kingaveen MA 85230 11	22 43.3	37.76	27.91	26.89 6	5.9	12.30	8.79	4,78	4.42	4.27	6.11	10.32	4.98	8.16	31.37
CM 18571 VA New Kent M A 88 227 11				29.36 2											
CM 19577 VA Prince Geo M A 86 23011	26 42,69	30.93	25.50	28.26 2	18,2	12,66	8,55	4.98	4.39	4,07	6.02	9,75	4,92	8.85	32.65
CM 18572 VA NewKent M A 84 228 11	23 39.76	, 30,09	25.64	25.406	24.0	11,08	7,96	4,19	3.47	4,15	5.77	9,67	4.27	7.88	30.53
CM 23209 VAV Beach A 74 238 12	al 37.95	30,05	24.76	21.38 2	a1.5	11,23	7.80	3.91	7,30	4.18	5.36	9.72	4.75	7:92	28.44
CM 18853 VA New Kent M A 88 23212	23 37.80	32,20	24.27	24.47:	22.2	11.22	7.80	4.09	3.46	3,70	5.69	9.45	3,20	6.99	27.72
CM 114066 VAN Beach A722311	25 42.15	36.54	27.36	27.28 6	2,3	12.34	8.85	4.73	4.13	4.27	5.65	11.90	5.58	9,30	32.24
CM 32711 VA New Kent A 86 236 2	24 38.4	31.68	24.35	20.24 6	22.0	10.86	7.55	4,40	3.84	3,13	5.34	9.18	4.58	7.54	28.53
CM 34540 VA New Kent A 80 233 11	27 32,50	31.39	20,84	17.33	19,5	9.21	6,23	3.51	2.77	3,05	4.44	7.91			25.33
CM 22889 VAV Beach A 78 223 11				21.82 2			7.61	4.15	4.29	3.86	5.2.8	8-86	3.57	6.99	29,15
CM 18574 VA New Kent A \$323711				25.480											28.90
CM 36739 VA Charles- MA 83 235 11	27 41.52	34.12	27.29	25.46 2	24.4	12.28	872	484	4.45	5,35	5.58	11.25	4.00	8.30	32,07
CM 22887 VA V Beach M A 89 228 11	25 39.72	31.61	24,45	21.07	25.2	12,14	7,99	5,04	4,49	4.32	5.85	9.68	4,45	7.11	30,47
CM 66518 11 Macon A 74 239	25 38.94	131,28	25,44	20,34 2	21.3	10.91	7.75	4.65	4,25	4,44	5,15	9,95	4,13	7.47	28,95
CM 114334 1L Jackson f A 73 234	25 39,87	33,66	25,98	24.632	1.6	11.50	8.30	4.09	4,66	5,39	5.73	9.23	4.94	8.36	31.93
CM 25898 IN Unk. 78231	as 39,24	32,33	26,44	22,932	4.3	11.77	8.39	4,72	4.73	3.88	5.77	9.49	3,44	7.45	28.80
CM 19578 VA Prive Geo 63335	a5 35.98	28.04	23.84	18.31											
(M 59762 IN Rike 72230	25 42.78	35.54	27.75	26,20 2	14.7	12.79	8.25	4.41	3.85	3.89	6.03	10.83	6.26	9.62	31.53

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	Specimen #	State	County	Sex	Age	SC	V	IFL	DSN	DSM	DSC		HLV	SL	CW		PL	PW	FWP 3,44	PrFWP	InWP	EYE	AG	InR	RW	
Civi	13210	V/V	New Yer		4	82	<u>~35</u>			d 3	<u></u>	35,10	27.33	22.80	20,75	19.2	10,49	6,93	3,44	3.3/	3.77	4.91	9,16	3.17	6.31	ab.61
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Museum	Specimen #	State	County	Sex	Age	sc		DSM	HL	cw	TL	PL	PW	FWP	InWP	EYE	AG	InR	RW	LL
CM	66520	Mo	Franklin	F	A	73	232	27	45.77	27.20	232	13.12	8.84	4.30	5.02	5.91	10.52	4.42	8.61	33. 49
CM	88632	OK	Nowata	M	A	85	235	25	43.67	25.51	264	12.34	9.05	5.29	5.36	6.37	11.59	523	8.85	33.29
CM	55432	KA	Douglas	Μ	A	64)	229	27	50.22	30.01	204	14.57	10.59	6,67	5.51	6,41	11.02	5.74	11.63	38.92
CM	91163	ōΚ	Love	Μ	A	88	234	27	46.71	31.29	272	13,49	9.70	6.22	4,62	6.00	11.24	5.09	8.79	34.63
CM	93682	oK	Pontotoc	Μ	A	80	225	27	39.75	24.49	214	11.54	8.27	4.38	3.94	5,49	9.76	3.53	7.75	30.21
CM	17031	KΥ		Μ	A	81	ટેકર	25	39.32	24.21	233	11.55	8.50		4.86	•		4.77	8.60	30.81
См	58611	KA	Douglas	M	A	83	223	27	43.40	27,28	261	13.01	8.97					5.06	8,24	33.17
	10 M	KA	Douglas	M	J	79	221	25	31.41	16.89	165	9.27	6.52	3.79			8.16			24.29
CM	0			F	A	1				24.92		12.86	8.55	4,36			9.48		8.07	30.42
CM	66521	MO			i					19.35		12,43	8.54	5.24	3.88	5.87	9.49	3.69	7,31	31.07
см										21.25		1	7,64	,				4,03	6.45	27.56
CM			~~~	1					38.46		205	11.06	7.76	4.83	······································					28.49
CM	61916	OK	Tolsa	1		ļ.				20.09	1	9.88		4.18			8.16			26.72
CM	23954	OН	Clermont	1							1	11.53		5.10		1	1			31.54
CM	58712	KY	Backen								1	11.35		4,15			9.31		1	28.76
CM	58711	KΥ	5 C	1		1				23.17	1	1		4.14	1		9.92			28.43
CM	S 5133	ØН	Hamilton	1							1	10.34	6.91	4.20	3.18	4,67	8.92	3.11	6.42	24.72
CM		OH			1							10.34		4.9	4.07	5.21	7.84	3.87	8.09	26.51
CM	17029	ΚY	Fload							23,99		10.24		4.19	3.55	5.15	8.86		6.95	27.23
CM			1							1		12.25		4.50	1				7,67	28.01
CM														4.59	3.67	5.66	10.07	4,40	7,68	28.35
CM	55128									19.20										26.97
CM						$\sim$				30,74			9.05				12.23			34.78
CM		5	Russell	1						a1.37		•				1	16.12	5		
CM			Jefferson								253				1					30.15

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Museum	Specimen #	State	County	Sex	Age	sc	v	DSM	HL	cw	TL	PL	PW	FWP	InWP	EYE	AG	InR	RW	LL_
CM	S 9474	WV	Marion	F	ĨM	72	233	<i>a5</i>	28:52	15.60	150	9.07	6.09	3.77	a.93	3.87	7.15	3.02	5,48	22.26
CM	59579	Nν	Randolph	F	M	74	239	25	26.39	14.07	120	7.78	6,10	3.30	2.72	3.87	6.62	2.65	5.65	20,61
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September 2003

Cornsnakes

6381 4673 5706 4671 4667 6485 488 8647	VA VA VA VA	Bedford Warren Goochland Goochland Bedford	M M F M F	A A A	58 63 59 58	206 232	26 27	ні 34/59 31.3.5 30.07	17.64		рь 10,89	рw 6.32	FWP	InWP 3,06	<b>EYE</b>	AG 8 8 4	1nR 371	<b>к</b> 6,29	
4673 5706 4671 4667 6485 488 8647	VA VA VA VA	Bedford Warren Goochland Goochland Bedford	M F M F	A A A	63 59 58	206 232	26 27	31.35	17.64			6.32	4.27	3.06	4.99	886	371	6.29	210-
5706 4671 4667 6485 488 8647	VA VA VA VA	Warren Goochland Goochland Bedford	F M F	A A	59 58	232	27			175			1.3 👾	19 9 T		01-1		· · · · · · · · · · · · · · · · · · ·	10,0,00
4671 4667 6485 488 8647	VA VA VA	Goochland Goochland Bedford	M F	A	58			30.07			9.51	6.01	4.41	2.82	4.26	1		1	23.88
4667 6485 488 3647	VA VA VA	Goochland Badford	F			23	1 1	10.01	16-10	148	9.22								22.72
6485 488 3647	VA VA	Bedford	ł	A		0.10	26	26.78	15.37	133	1	1				1	1	1	20.74
488 3647	VA		-	+	64	228	26	28,95	13.68	165	1			1					22.51
488 3647	VA		1	A	60	217	26	25.97	15.63	138				1		1			19.53
		Drange		1			1 1	29.46						1			1	1	23,29
	VA	Goochland																	21.93
6916		Sussex																	24.94
		5-ssex			1														24.95
1			F					29.63			1								23.95
093	NC	Hyde	F	A	69	218	27	27.56	15.12	160									21.20
6865	NC																		22,08
975	SC																		
											1								
		*							1										
6847	GA	1			· · · · · · · · · · · · · · · · · · ·														
385	TN	Sevier	M	A	69	aiz	27	31.31	19.07	174	9.86	5.88	3.40						
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September 2003

Cornsnakes

Museum	Specimen #	State	County	Sex	Age	sc	v	DSM	HL	cw	TL	PL	PW	FWP	InWP	EYE	AG	InR	RW	LL
WVBS	10539	WV	Fayette	F	A	74	স্প	20	36.48	2273	185	10.55	7,70	3.93	4,12	5.01	.9,43	4.67	7,78	28.01
WBS	10542	WV	Geenbeige	M1	A	80	225	27	43,11	23.93	260	12.97	8,93	5.45	4.94	6.79	10.15	4.95	8.34	33.48
WVBS	12099	WV	Hampshire	?m	YA	84	232	27	29.10	15,18	185	8,07	5,75	3.84	2.92	4.55	6.60	3.94	5.87	23.38
NVBS	4311	wV	Mercer	?	A	83	225	25	31.61	19.75	209	9,80	7.70	3.72	3.67	5,00	7.62	3.89	6,710	23.97
WVBS	383,6		Preston	M	A	83	224	23		al.58	251	11.64	8.56	5.92	4.76	5,06	9,39	4.01	8,00	31.26
WVBS	3983	WV	Raleigh	F	A	77	237	26	37.79	21.86	217	11,42	7.81	4.26	3.43	5.55	9.58	4.15	7.55	28,65
WBS	4310	WV	Monroe	F?	A	86	230	27	40,05	24,03	235	12.06	8.76	4.90	4.42	5.82	10.12	4,64	9.19	30.65
WVBS	3835	WV	Berkeley	m?	A	81)	a24	27	41.10	22.00	261	12.43	8.78	6.18	4.56	5.45	10,42	4.86	9.03	32,45
NVBS	544	WV	Potnaw	M	A	<u>(3</u>	233	23	32.72	16.35	177	9,99	7.04	3.91	3.84	5.22	8.17	3.67	6.58	25.30
NV BS	2934	wν	Wetzl	?	YA	78	225	25	27.24	17.39	153	8.77	6.26	3,84	3.28	4,60	6.97	3.51	5.61	21.44
WVB5	168	WV	Kanawha	M	YA	81	237	21	29,49	18.23	174	9.15	6.65	343	3.49	4.55	7.35	3.03	6.68	23.70
WVBS	3182	WN	Wirt	F?		81	238		30.58	17,44	186	8.33	5.99	3.78	3.21	4.84	8.73	3.31	6.54	23,50
WVBS	3172	wV	Ritchie	?	A	67)	230	23	35.35	19.68	158	10.49	7.66	4.38	4.07	5,24	9.18	4.12	7.12	27,91
WVBS	3979	WV	Nicholas	M			234		40.53	24.27	240	11,04	8,83	5.16	4.66	6.09	9.97	4.83	8.98	31.90
WVBS	1498	WV	Porchantas	?			233		35,40	19.60	192	10,68	רה.ד	4.13	3.68	5,26	8,36	4,22	7.51	27.72
WVBS	2405	WV	Harrison	F?	A	60	241	27	38.30	24.38	162	11.25	8.73	4.98	4.80	5.57	9.25	4.61	7.52	29.32
WVBS	10540	W	Greenbrier	M?	YA	84	232	25	33,16	15.77	206	9.37	7,19	4.16	3.64	5.22	8,45	4.16	6.55	25,83
WVBS	10530	WV	Y	Μ		White the second s			36.70	18.23	220	10.50	7.40	4.58	3.74	5.57	8.63	3.47	6.49	27,43
WVBS	2686	WV	Wood	MR,	YA	48	230	25	27.21	14.35	106	8.12	6,08	3.78	2.85	4.66	6.83	2.85		21.15
WVBS	1611	WV	Cabell	F.	YA	(57)	234	25	28,97	15.18	150	8,45	6.62	3.57	3.03	4.79	6,98		~ :	23,33
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January 2005

 $X_{ij} = \sum_{i=1}^{n} \sum_{j=1}^{n} \sum_{j=1}^{n} \sum_{i=1}^{n} \sum_{j=1}^{n} \sum_{j=1}^{n} \sum_{i=1}^{n} \sum_{i=1}^{n} \sum_{i=1}^{n} \sum_{j=1}$ 

## **APPENDIX C**

Curriculum Vitae

# Adam M. Mann

### **Curriculum Vitae**

EDUCATION	Marshall University, Huntington, WV Master of Science in Biology Concentration: Herpetology Summa Cum Laude, May 2007 Thomas More College, Crestview Hills, KY Bachelor of Arts in Biology Cum Laude, 1997
KNOWLEDGE/ SKILLS/ ABILITIES	<ul> <li>Handling and caring for a variety of captive herpetofauna</li> <li>Mark/recapture techniques on reptiles and amphibians</li> <li>Frog call surveying</li> <li>Knowledge of physiology, taxonomy, and ecology of extant vertebrate species, specializing in reptiles, amphibians, bats, birds, and bony freshwater fishes</li> <li>Knowledge of physiology, taxonomy, and ecology of native vascular plants</li> <li>Curation of reptile and amphibian museum specimens</li> <li>Field identification of numerous vertebrate taxa (by sight and call)</li> <li>Field identification of trees and other woody vegetation</li> <li>Public speaking to children and young adults regarding biological topics and wildlife</li> <li>Trapping: harp (for bats), funnel, pitfall, live mammal, snap, minnow</li> <li>Netting: hoop, seine, gill, fyke, mist (for bats/birds)</li> <li>Radio-telemetry of endangered bats (including transmitter attachment, foraging telemetry, triangulation, location of bat roosts) and timber rattlesnakes (triangulation, location of bat roosts) and timber rattlesnakes (triangulation, location of den sites)</li> <li>Boat and backpack electro-fishing</li> <li>Mapping, field orientation, and GPS navigation</li> <li>Technical operation and maintenance of audio and visual equipment</li> <li>Project management, including: formulation of study plans, supervision of biologists, maintenance of project budgets, keeping client relations, coordination with agencies, writing of associated technical reports and large regulatory documents</li> <li>Supervision of multiple field crews</li> <li>Writing and editing of detailed technical reports and large regulatory documents</li> <li>Routine maintenance of vehicles and field equipment</li> <li>Computer skills: ArcGIS mapping and analysis, MS Windows, MS Office, MS FrontPage, Adobe Acrobat, Adobe Photoshop, SAS, LOAS, Biotas</li> <li>SCUBA diving (PADI-certified) for freshwater mussels</li> <li>Operation of ATV's, 4-wheel drive vehicles, motorcycles, powerboats and other small watercraft</li></ul>

#### EXPERIENCE <u>Scientist / Group Manager</u>

Environmental Solutions & Innovations, Inc. August 2003 – Present

- Manage multiple projects simultaneously
- Consult on regulatory issues, usually pertaining to endangered species
- Supervise, hire, and schedule all technical staff
- Perform project-related field work
- Write technical reports and regulatory documents
- Project Experience to Date:
  - Project Manager KDFWR Vertebrate Inventory: 2007
  - o Project Manager Fort Drum Indiana Bat Survey and Radio-telemetry Study: 2007
  - o Biologist Texas Eastern Transmission Time II Expansion: 2007
  - Project Manager Wright-Patterson Air Force Base: 2006
  - Field Supervisor Eagle Ridge Townhouses: 2006
  - Project Manager Camp Dawson Indiana Bat Survey: 2006
  - Project Manager Algonquin Ramapo Pipeline Expansion: 2006
  - Project Manager Tuxedo Reserve Project: 2006
  - o Biologist Kentucky SR 163 Endangered Bat Survey: 2006
  - Biologist Interstate 66 Endangered Bat Survey: 2006.
  - Biologist-Big Sandy Pipeline Portal Survey: 2006.
  - Biologist American Electric Power 765kv Transmission Line: 2003-2006
  - Project Manager Millennium Pipeline: 2005-2006
  - Project Manager Columbia Gas Pipeline A-5 Replacement Pipeline: 2005-2006
  - Biologist Lewisburg Mine Winter Hibernaculum Survey: 2006
  - Project Manager Interstate 69 Section 2 Environmental Studies: 2004-2006
  - Biologist Indiana Bat Habitat Conservation Plan: 2003-2006
  - Project Manager Naval Support Activity Crane Bat Inventory: 2005
  - Biologist Interstate 69 Section 1 Environmental Studies: 2005
  - Biologist Route 33 Nelsonville Bypass: 2003-2005
  - Biologist US Route 24 Improvement: 2004-2005
  - Biologist Spring Staging Study: 2005
  - Biologist Indiana Bat Winter Hibernacula Surveys: 2005
  - o Field Supervisor Pennsylvania DEP Abandoned Mine Surveys: 2004
  - o Biologist German Ridge Restoration EIS: 2003-2005
  - Project Manager Delaware County Indiana Bat Survey: 2005
  - Project Manager Michigan City Municipal Airport Indiana Bat Survey: 2005
  - Project Manager Rainelle Power Plant Endangered Species Survey: 2004
  - Biologist Licking River Mussel Survey: 2003
  - Biologist Lewis Creek Surface Mine Biological Surveys: 2003
  - Biologist Summit Engineering Summer Mist Net Survey: 2003

#### **Volunteer Herpetologist**

Ohio Frog and Toad Calling Survey (sponsored by Ohio DNR) - (March 2004-present)

- Currently volunteering in statewide program to inventory anurans calling at different times of year
- Established permanent routes to be monitored on yearly basis
- Visit sites monthly during early and late spring, identify species, and record calling data.

### EXPERIENCE <u>Herpetology Research Assistant</u>

(continued)

Marshall University Department of Biological Sciences, under Dr. Thomas K. Pauley August 2001 – May 2003

- Long-term Gypsy Moth Study Project Coordinator (Funded by US Department of Agriculture – Forest Service)
  - Act as liaison between project administrators and students
  - Organize data, literature, and report information
  - Assist in the writing process of reports and papers
- Teaching Assistant
  - Teach and moderate Herpetology and Ornithology laboratory sessions
  - Chaperone students on trips to the field
  - Prepare supplemental learning material for students
  - Administer and grade examinations
- Marshall Herpetology Web-Site Webmaster (http://www.marshall.edu/herp)
  - Continually modify and update site with new information from various sources
  - Respond to correspondence relating to web site information
  - Write new information and material for web site
- Chief Animal Caretaker
  - Maintain health and well-being of all live animals located at school
  - Educate students, children, and visitors using live animal displays
  - Travel when necessary to give live animal presentations
- Mud River Study Co-coordinator (Funded by US Army Corps of Engineers)
   Direct efforts to survey multiple study sites for reptiles, birds, and amphibians
- Organize data obtained in the field
- Assist in preparation of written reports
- Field Crew Member
  - Work on cooperative projects located throughout the state
  - Use various reptile and amphibian collection techniques
  - Perform duties in field on herpetology research projects such as:
  - Stream Salamander Survey (funded by US Geologic Survey and the EPA)
  - Mudpuppy and Hellbender inventories (funded by WV Division of Natural Resources)
  - Mud River Inventory of Birds and Herps (funded by US Army Corps of Engineers)
  - Long-term Gypsy Moth Non-target Salamander Study (funded by USDA)
  - West Virginia Herp Atlas (funded by WV Division of Natural Resources)
  - Gauley River Inventory (funded by National Park Service)

### Aquatics Lab Research Assistant

Marshall University Department of Integrated Science and Technology under Dr. Thomas G. Jones

July 2002 – August 2003

- Conducted controlled collections of freshwater fish on large rivers and small streams
- Towed and piloted boats during day and night in order to conduct surveys
- Implemented mark-recapture techniques on freshwater fishes
- Operated and maintained backpack and boat-mounted electro-shocking units
- Sampled benthic organisms and examined water chemistry

### EXPERIENCE

(continued)

#### 4-H Camp Instructor, Barboursville 4-H Camp

June 2002

- Taught one-week course on local small stream ecology to 20 grade-school students
- Lead expeditions into the field to collect and study native aquatic organisms •

### Animal Caretaker, Thomas More College Biology Department

May 1993 – August 2001

- Possessed responsibility for the welfare of numerous species of reptiles, amphibians, fishes, and arthropods
- Gained valuable teaching experience to a variety of age groups concerning wildlife and • nature

#### Museum Volunteer, Cincinnati Museum of Natural History

Sept 1996-Jan 1999; Sept 1993-June 1994

- Identified vertebrate species and curate the herpetological specimen collection •
- Assisted with research opportunities •

**Biology Research Assistant,** Thomas More College Ohio River Biology Field Station Summers 1994 - 1997

- Collected and analyzed research data on large river and small stream systems •
- Achieve extensive knowledge of fish and other aquatic organisms

#### Audio-Visual Technician / Account Representative, MAC Productions, Inc.

January 1998 - August 2001

- Provided customer service concerning equipment rental and set up
- -Transported, maintained, and set up a variety of electronic and computer equipment •
- Named Character First Award Winner for January 2001 •

#### Convention Services Houseman, Drawbridge Estate

May 1993 - December 1998

- Used teamwork necessary to complete banquet room set-up assignments efficiently
- Named Employee-of-the-Month, April 1998
- Mann, A., M.R. Obermeyer, and J.W. Ferner. 2000. Geographic Distribution. Opheodrys **PUBLICATIONS** aestivus. Herpetological Review 31(2): 114.
  - Lorentz, C.N., J.R Hageman, D. Espenscheid, S. Galbraith, C. Gieske, T. King, A. Mann, K. McCafferty, K. McPhillips, M. Obermever, D. Phirman, J.D. Schaeffer, B. Stamm, R. Tewes, and J. Thomas. An Investigation of the Fish Populations, QHEI, Zebra Mussels, Limnology, Thermal Plume, and Screen Impingement at the Walter C. Beckjord Power Plant, New Richmond, Ohio, Summer 1997. Bulletin #28. Thomas More College Ohio River Biology Field Station. California, Kentucky.
  - McCafferty, K. and A. Mann. 1996. Geographic Distribution: Apalone mutica (Smooth Softshell Turtle). Herpetological Review 27(1): p. 31.

PUBLISHED ABSTRACTS	Mann, Adam M. and Thomas K. Pauley. Marshall University Department of Biological Sciences – <u>Status and distribution of the Black Ratsnake (<i>Elaphe</i>) complex in West <u>Virginia using morphometric techniques</u>. Southeastern Biology Vol. 50, No. 2, April 2003.</u>
	Tackett, Fred, Eric Emory, Melissa Mann, Adam Mann and Thomas Jones. Marshall University, Huntington, West Virginia and ORSANCO, Cincinnati, Ohio – <u>Fish</u> <u>community structure of the Kanawha River</u> . Southeastern Biology Vol. 50, No. 2, April 2003.
	Pauley, Thomas K., Melissa Obermeyer, Seth Myers, and Adam Mann. Department of Biological Sciences, Huntington, West Virginia – <u>Influence of UV-b radiation, dissolved</u> <u>aluminum and pH on amphibians in high elevation fens in West Virginia</u> . Southeastern Biology Vol. 49, No. 2, April 2002.
PROFESSIONAL AFFILIATIONS	Partners in Amphibian and Reptile Conservation (PARC) Greater Cincinnati Herpetological Society Northeast Bat Working Group (NEBWG)
GRADUATE COURSEWORK	Herpetology Conservation Biology Seminar I & II Ornithology Economic Botany Spatial Analysis for the Environment Independent Study (Fish Sampling Kanawha/Ohio River) Taxonomy of Vascular Plants II Aquatic Diversity
TRAINING	Natural Rivers Mechanisms, Morphology & Management Course: 2003 Rosgen Level 1: Applied Fluvial Geomorphology Course: 2004 Rosgen Level 2: River Morphology & Applications Course: 2005 USFWS – Interagency Consultation for Endangered Species: 2006 Ohio Department of Transportation – Ecological Training: 2006