2005

The Natural History, Distribution, and Phenotypic Variation of Cave-dwelling Spring Salamanders, Gyrinophilus spp. Cope (Plethodontidae), in West Virginia

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Thesis submitted to
The Graduate College of
Marshall University

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

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May 2005

Abstract

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There are over 4000 documented caves in West Virginia, potentially providing refuge and habitat for a diversity of amphibians and reptiles. Spring Salamanders, *Gyrinophilus porphyriticus*, are among the most frequently encountered amphibians in caves. Surveys of 25 caves provided expanded distribution records and insight into ecology and diet of *G. porphyriticus*. Over 500 species locality records were compiled in a nearly comprehensive list. The *Gyrinophilus* population from General Davis Cave is of particular interest. In 1977, Besharse and Holsinger first described the West Virginia Spring Salamander, *G. subterraneus*; however, its taxonomic status is unclear. In order to document the degree of variability among cave-dwelling *Gyrinophilus* species, Principal Components Analysis was applied to measurements of external morphology. Eye diameter ($P \leq 0.05$) appears to be the primary morphological character separating *G. porphyriticus* from *G. subterraneus*. This investigation of cave-dwelling *Gyrinophilus* should broaden our understanding of amphibians in an often overlooked and threatened ecosystem.

Acknowledgements

A research assistantship from USGS and EPA, studying the potential use of salamanders in biomonitoring indices made it possible for me to attend Marshall University. The WVDNR Wildlife Diversity Program provided my primary thesis research grant. Additional funding came from the West Virginia Association for Cave Studies and the Marshall University Graduate College.

The fieldwork for this project required a lot of time, sweat, bruises, backaches, and long hours cold and wet underground. I am deeply grateful to all the adventurous Marshall grad students who enthusiastically crawled through the mud, over rocks, and through cave streams like the salamanders we were looking for. Roughly in the order of number of cave trips completed, the following friends hauled gear, caught salamanders, took photos, recorded data, and kept me company on long trips; Bill Sutton, Robert Makowsky, Lisa Smith, Keith Johnson, Seth Myers, Rob Fiorentino, Andy and Ellen Johnson, Zac Felix, Zac Loughman, Dr. Mark Watson, and Tiff Hutton. Jennifer Wykle of the West Virginia DNR has been an excellent friend of Dr. Pauley's herpetology lab. Her resilience and positive attitude through a 10 hour survey and later an all-nighter in General Davis Cave were remarkable.

The West Virginia Association for Cave Studies (WVACS) made this project possible by introducing me to the caving community and landowners, providing a warm place to sleep and space to work. Many thanks to Bill Balfour for sponsoring my WVACS membership and providing, maps, UTM coordinates, and advice from one of the true authorities on West Virginia caves. Harry Fair, Ed Swepston, Doug Boyer, Mike Corbett, Tony Buoy, Pat Bingham, Pam and Tom Malabad, Dave Cowan, and Bob Handley are all cavers who I greatly appreciate for helping out and being patient with my snail-like biologist pace. I am extremely grateful to Jeff Bray, Marianne Saugstad, and Ed Saugstad for not only showing me caves, but for being on-call, ready to call out a rescue if I did not return from a survey trip. Joseph Caldwell provided a map of Ludington Cave and Chuck Frostick donated the rope. Rick Samford, the resident at Ludington Cave, was kind enough to give me a lift through the pasture when the ground was soft and served as a check-in.

Input about PCA morphometrics from Dr. Michael E. Seidel and statistical advice from Dr. Laura Adkins were invaluable. Katie Schneider provided amphibian encounter records from her thesis inventories of the Buckeye Creek drainage. Dr. David C. Culver and Dr. Horton H. Hobbs III introduced me to the field of Biospeleology. I thoroughly enjoyed caving with them and appreciate all their advice. Addison Wynn and the Smithsonian Institution assisted me with specimens and insight into *Gyrinophilus* systematics.

I am very thankful for my friends and family who stuck by me as my laid-back life became hectic and busy. My parents, Vivian and Jerry, have always encouraged and supported my dreams. Mary Katherine Akers was my closest friend through the years we lived in Huntington. Deborah Merritt and Chris Barry are true friends who gave me a place to stay when I returned to Huntington from California and were always ready for adventure. Andy, Ellen, and Millhouse Johnson shared their home with me, great food, many laughs, and hundreds of running miles, training for a marathon.

Finally, I would like to thank my thesis committee. Dr. Tom Jones provided cave research gear, books, maps, and was very helpful with survey methods and analysis. I enjoyed learning from Dr. Dan Evans in the four classes I took from him at Marshall. Even though there were no living plants in my study ecosystem, he was interested in my project and very helpful. Lastly, I am eternally grateful to my mentor and friend, Dr. Tom Pauley, for the research opportunities he provided. I met him at a terrestrial salamander monitoring conference in Canada and two years later I moved to West Virginia to chase ‘manders as his student. I am honored to have had the opportunity to learn from the wise man of West Virginia natural history. His love of the natural wonders and creatures of this state has inspired countless student naturalists.

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Chapter I:
Introduction and Overview

Amphibians and reptiles have been studied extensively throughout West Virginia in a broad range of habitats including streams, rivers, spruce forests, roadside ditches, and vernal pools (T. K. Pauley pers. comm.). Caves may serve as critical habitat for many amphibian species, however with the exception of Longenecker's 2000 study of Cave Salamanders, *Eurycea lucifuga*, ecological research is largely lacking. Studying cave fauna can help elucidate and test population biology models and concepts. The relative simplicity of cave environments and cave communities better matches the assumptions inherent in many population models (Culver 1982).

Peck's (1998) list of cave fauna listed 1353 obligate cave species in the United States and Canada. According to Peck (1998), West Virginia ranks 7th in generic diversity with 32 documented genera of cave obligates. Counts of cave obligates are probably greatly underestimated due to the inaccessibility of small cracks and pockets of groundwater. Culver and Holsinger (1992) predicted there could be as many as 6000 cave obligates in the USA. This great diversity of cave life is vulnerable, however, to human land-use practices, particularly karst groundwater pollution (Peck 1998). The Greenbrier Valley is the largest karst region in West Virginia and is of particular concern. In 2001 the Karst Waters Institute named the Greenbrier Valley in their top 10 list of most endangered karst areas in the world (Tronvig and Belson 2001). Major impacts on the valley's caves include siltation, agricultural runoff, water contamination, and development. Doug Boyer (pers. comm.) of USDA reported witnessing a die off of spring salamanders in The Hole in 1992, following fertilizer application to adjacent fields. The protection and conservation of karst areas is critical for preserving this unique portion of the world's biodiversity. The goal of this research was to expand the base of knowledge that exists about amphibian and reptile cave-dwellers in West Virginia and hopefully assist in their conservation.

This project consisted of 4 independent studies of cave-dwelling amphibians and reptiles, with the major focus on Spring Salamanders, *Gyrinophilus* spp. The distribution of amphibians and reptiles in West Virginia is detailed in Chapter 2. This study is the result of cave inventories conducted throughout 2002 and 2003 and the compilation of species encounter records from literature and museum collections. Chapter 3 contains the results of a morphometric comparison of cave-dwelling *Gyrinophilus* spp. This analysis is of particular interest because of the systematic ambiguity and scarcity of the West Virginia Spring Salamander, *Gyrinophilus subterraneus*. Community ecology, diet, and feeding behavior of cave-dwelling *Gyrinophilus* spp. are examined in Chapter 4. The final study in this project involved monitoring 2 cave populations of *Gyrinophilus porphyriticus* using mark-recapture techniques. The purpose of population monitoring was to collect natural history data and estimate population sizes and densities. Unfortunately due to time constraints these results were not included in this thesis document, but will be reported in a future manuscript.

Chapter II:
The Distribution of Amphibians and Reptiles in West Virginia Caves

Introduction

Geographic Distribution of West Virginia Caves

There are approximately 4,150 known caves in West Virginia (B. Balfour pers. comm.), occurring in 16 counties. West Virginia's caves mainly occur in Paleozoic limestone strata of the Cambrian, Ordovician, Devonian, Silurian, and Mississippian ages. These cave-forming strata outcrop on valley floors and along the flanks of ridges in the eastern third of the state (Davies 1958). The karst regions of West Virginia occur primarily in the Ridge and Valley, portions of the Allegheny Mountains, and along the eastern edge of the Allegheny Plateau physiographic provinces.

In "The Invertebrate Cave Fauna of West Virginia", Holsinger et al. (1976) define 3 cave fauna regions based on species composition, geology, and drainage relationships (Figure 2-1). They defined these caves fauna regions as Upper Potomac Basin, Upper Monongahela Basin, and Upper Kanawha Basin. The Upper Potomac Basin in the eastern panhandle includes caves in Pendleton, Hardy, Grant, Mineral, Hampshire, Morgan, Berkeley, and Jefferson counties. This region is bordered by the Blue Ridge Mountains to the east and the Allegheny Front to the west. Most of this region is characterized by moderately rugged topography consisting of parallel ridges and valleys. The limestone strata of this region are exposed in narrow, isolated belts due to extensive faulting and folding. As a result, caves in the Potomac Basin are typically narrow with relatively low connectivity with other caves. Several hundred caves are known from this 3800 square mile region but broad karst corridors are mainly absent.

The Upper Monongahela Basin covers 2,000 square miles in the Allegheny Mountains and eastern edge of the Allegheny Plateau. This faunal region is drained by the Tygart and Cheat rivers and includes caves from Monongalia, Preston, Tucker, and Randolph counties. Most caves in this region occur in Mississippian limestone belts in Randolph and Tucker counties. This region is topographically rugged with caves limited to narrow strike belts with relatively low connectivity. Only about 10-15% of the Upper Monongahela Basin is floored with limestone and contains over 300 caves.

The third and most significant cave fauna region described by Holsinger et al. (1976) is the Upper Kanawha Basin, which includes caves in Pocahontas, Greenbrier, Monroe, and Mercer Counties. The cave-bearing portion of this region covers 2,200 square miles and is drained by the Elk, Greenbrier, and New Rivers. South of central Pocahontas County, the Greenbrier Valley forms an area of broad rolling karst with blind valleys and sinkholes. Roughly 60% of this region is floored with limestone, three-fourths of which is Mississippian-aged Greenbrier series. Cave interconnectivity is high in much of this area and many caves are quit extensive. The Greenbrier Valley contains the highest concentration of caves in the state with 1265 described. Most of the longest caves are located there as well, with some reaching over 45 miles long.

The Subterranean Environment

Caves typically have 3 environmental divisions based on light and temperature (Poulson and White 1969). The twilight zone is the area in cave entrances where sunlight can penetrate. The greatest fluctuations in air temperature occur here and caves that "suck" air in from the outside during the winter may be covered with ice in this area. The dark zone or inner cave is characterized by a complete absence of light and relatively stable air temperatures. In West Virginia caves, air temperature is typically around 11 ° C (52-54 ° F), year round. The third area is the transitional zone between the twilight and dark zones. This is the perpetually dark area adjacent to the twilight zone, which experiences wide temperature fluctuations (Reese 1934).

While a wide diversity of surface-dwelling, epigean, animals are able to inhabit or visit the twilight zones of caves, life in the dark zone is considerably more challenging. The absence of light and low nutritional resources throughout most of the cave make starvation the likely demise of animals accidentally swept-in or stumbled into the inner levels of caves (Poulson and White 1969; Culver 1982). The environment in caves is inhospitable to many organisms, however amphibians are the most well suited to be cave-dwellers of any terrestrial vertebrates. Amphibians generally have highly permeable skin prone to desiccation. As a result most amphibians have an aversion to bright sunlight and dry substrates, which they avoid by foraging nocturnally and following rains (Green and Pauley 1987). The cool, stable temperatures, damp substrates, high humidity, and low light environment of caves is an ideal habitat or refuge for many species. Members of the family Plethodontidae, the Lungless Salamanders, are particularly well adapted to be trogloxenes and it is possible to find any plethodontid from the surrounding area within the threshold of caves (Cliburn and Middleton 1983). All North American troglophilic and troglobitic salamanders are plethodontids, specifically from the taxonomic tribe Hemidactyliini (Ryan and Bruce 2000).

Amphibian and Reptile Cavernicoles

Cave-dwelling organisms, or cavernicoles, are divided into 4 principal classifications defined by thier degree of modification and dependency on caves for completing their life cycle (Barr 1960, 1968; Hamilton-Smith 1971; Barr and Holsinger 1985). Troglobites are cave obligates, so morphologically and physiologically modified that they are unable to complete their life cycle outside of caves. Troglophiles are facultative cavernicoles, frequently found both in and out of caves. They are able to complete their life cycle in caves, reproducing and feeding there, but do not exhibit modifications restricting them to caves. Trogloxenes are animals often found in caves but do not complete their entire life cycle there. Members of this class of cavernicoles may enter caves as a refuge or in search of food. Bats and cave crickets are good examples of trogloxenes. Many trogloxenes are considered to be "threshold trogloxenes" because they frequent the twilight zone of caves. The fourth classification of cavernicoles are of accidentals. They occur in caves as stray visitors, usually washed in or fallen down a pit (Barr 1960). These individuals lack the modifications necessary to complete their life cycles in caves but may be able to persist for limited periods of time. Additional categories such as parasites could also be found in caves (Hamilton-Smith 1971).

There are currently troglobitic salamanders described from 5 genera in North America, including *Eurycea*, *Gyrinophilus*, *Haideotriton*, *Typhlotriton*, and *Typhlomolge* (reclassification to *Eurycea* under review) (Brandon 1971). The West Virginia Spring Salamander, *Gyrinophilus subterraneus*, is the only troglobitic vertebrate species currently recognized in the state. The comparatively mild degree of cave modifications in this species has led some researchers to question whether it should be considered a troglophile, however there are currently no records of *G. subterraneus* from epigean habitats (T. G. Jones pers. comm.). While *G. subterraneus*, are extremely rare, only known to occur in one cave, *G. porphyriticus* are very widespread (Green and Pauley 1987). According to Green and Brant (1966), *G. porphyriticus* followed by *E. lucifuga* are the most commonly encounter salamander species in West Virginia Caves. Both these salamanders are troglophiles, able to feed, reproduce, and live out their entire life cycle either inside or outside of caves. *Gyrinophilus porphyriticus* occupy streams and adjacent muddy banks from the twilight zone to the deepest levels of caves. Cave Salamanders, *Eurycea lucifuga*, are typically found in crevices and along the walls in the twilight and adjacent dark areas near cave entrances (Hutchison 1958).

The Twilight areas at cave entrances are optimal habitat for a variety of trogloxene amphibians. Similar to *E. lucifuga*, other crevice-dwelling salamanders such as *E. longicauda*, *Plethodon glutinosus*, and *P. wehrlei* can be found along walls and crevices in cave entrances. These salamanders can feed on invertebrates within the cave or migrate out for nocturnal foraging in the forest leaf litter (Hutchison 1958; Peck 1974). Small terrestrial plethodontids such as *Plethodon cinereus* and *P.*

richmondi can also be found residing under leaves and logs in the twilight areas of West Virginia caves (Cooper 1961).

Stream salamanders often migrate along surface streams as their course flows into caves. Species that reside in interstitial spaces between stream gravel, under rocks, or in stream banks such as *G. porphyriticus*, may be preadapted for cave life. Aquatic larvae are adept at locating prey non-visually through mechanoreception and possibly chemoreception (Culver 1973). It is not uncommon to observe stream salamander species in upper levels of caves and as accidentals in the deeper dark areas after being washed in by high water. *Desmognathus ochrophaeus*, *D. fuscus*, *D. monticola*, *E. bislineata*, *E. cirrigera*, and *Pseudotriton ruber* have all been reported from West Virginia caves streams and may be either threshold trogloxenes or accidentals (Reese 1934; Cooper 1960; Green and Brant 1966; Garton et al. 1993).

Frogs and toads, are primarily visual predators, not well adapted for life in total darkness, however the cool, damp, low light environment of the twilight zone may be an attractive refuge during hot, dry summer months. Anurans may also take advantage of relatively mild, stable temperatures of caves during winter months. Rand (1950) observed Northern Leopard Frogs, *R. pipiens*, active in Indiana caves during winter when they are typically hibernating in mud at the bottom of ponds. Pickerel Frogs, *Rana palustris*, are trogloxenes inhabiting cave entrances and adjacent dark zones. They are the most frequently encountered anurans in West Virginia caves (Green and Brant 1966; Garton et al. 1993). Cave surveys from Alabama (Brown and Boschung 1954), Tennessee (Barr 1953), Mississippi (Brode 1958; Cliburn and Middleton 1983), and Missouri (Myers 1958) also reinforce the observation that *R. palustris* is the most cavernicoles frog within its range. Based on his observations of *R. palustris* in Mississippi, Brode (1958) speculated that they migrate out of caves at night and are abundant in the vicinity of the entrance. During periods of extremely warm weather they are inactive, occurring mostly in caves, or near by in crevices or under rocks in creek beds. During cool damp periods following rains *R. palustris* resume activity outside caves. Brode (1958) also observed *R. palustris* in caves during winter buried in the substrate, in crevices, and under rocks and postulated that a large portion of them hibernate in dark, damp recesses of caves rather than under water. In addition to *R. palustris*, other anurans including; *R. pipiens*, *R. clamitans*, *R. catesbeiana*, *R. sylvatica*, and *Bufo americanus* are occasional wash-ins to the deep reaches of West Virginia caves and may also be threshold trogloxenes to a lesser extent (Reese 1934; Cooper 1960; Garton et al. 1993).

Reptiles with thier thermoregulatory requirements seem to be unlikely cavernicoles, however box turtles and snakes are occasionally encountered in caves. It can usually be assumed that the majority of reptiles encountered in caves are there at no advantage to themselves. They occasionally fall down pits or are washed in by high water. Some reptiles may be true trogloxenes, entering caves to hunt or to use as a shelter or hibernaculum. *Elaphe guttata emoryi*, the Great Plains Rat Snake, has been reported as a regular inhabitant of caves in the Great Plains where it is an effective resident predator on bat colonies (Black 1974; McCoy 1975). One Rat Snake taken from an Oklahoma cave contained 2 adult *Tadarida* spp. bats. It is possible that records for Eastern Ratsnakes, *Elaphe alleghaniensis*, in West Virginia caves could also be individuals patrolling for bats or trogloxene Allegheny Woodrats, *Neotoma magister*. There are records for both West Virginia viper species, Northern Copperhead, *Agkistrodon contortrix* and Timber Rattlesnake, *Crotalus horridus* from caves. Vipers may be accidentals in caves, however they may also sometimes take residence. Black (1974) stated that Western Diamondback Rattlesnakes, *Crotalus atrox*, are so common in cave entrances in western Oklahoma that they may be considered trogloxenes.

It is theoretically possible to find any species which forages or migrates along the forest floor within a cave. From the perspective of a woodland salamander, a box turtle, or a toad, the damp leaf litter and woody debris in twilight zone of caves is essentially an extension of the forest floor. The same can be said for aquatic species following an epigean stream into a cave entrance. Many amphibians and a few reptiles are well suited to be threshold trogloxenes, but survival deep in the dark zone of caves requires specialized morphological and physiological adaptations (Brandon 1971; Barr and Holsinger 1985).

History of Cave Surveys in West Virginia

The first descriptions of cave-dwelling salamanders came from Johann Weichard von Valvasor in 1689 who described a "dragon" inhabiting subterranean lakes in present day Slovenia. It was believed that its sudden movements provoked floods and occasionally the "larvae" of these "dragons" would wash out. In 1789, J. N. Laurenti formally described the European cave salamander as *Proteus anguinus* (Camacho 1992). In the United States, the roots of biospeleology began to grow in the 19th century with Refinesque's 1822 description of the Cave Salamander, *Eurycea lucifuga* from caves near Lexington Kentucky. Also in Kentucky, at Mammoth Cave in 1842, De Kay first described the first North American cave obligate, the cave fish, *Amblyopsis spelaeus* (Barr 1960). In a career lasting from 1871 to 1905, A. S. Packard Jr. studied cave fish in at Mammoth Cave and became the central authority on North American biospeleology (Camacho 1992).

While studies of North American cave vertebrates began in Kentucky in the 19th century, West Virginia did not receive much attention until the mid 20th century. Reese conducted one of the earliest biospeleological surveys in the state for his 1934 paper "The Fauna of West Virginia Caves". He visited 43 caves in the eastern portion of the state and recorded 5 salamander species including *Desmognathus fuscus*, *D. ochrophaeus*, *Gyrinophilus porphyriticus*, *Plethodon cinereus*, and *Eurycea lucifuga* along with 2 frogs species, *Rana clamitans* and *R. sylvatica*. Fowler (1941 and 1944), added to this list the unusual records of *Hemidactylium scutatum* from the Sinks of Gandy and *Ambystoma jeffersonianum* from the base of Grape Vine Drop entrance to Lost World Caverns. In an attempt to bring together various cave records from the literature, Dearolf compiled the "Survey of North American Cave

Vertebrates" for the Pennsylvania Academy of Science in 1956. In addition to the species seen by Reese (1934), Dearolf (1956) reported records for the salamanders *E. longicauda*, *P. wehrlei*, and *P. glutinosus* and a frog species *Rana palustris*. According to Dearolf (1956), *G. porphyriticus* is more commonly seen than any other salamander in West Virginia caves. John E. Cooper published "Collective Notes on Cave-Associated Vertebrates" in 1960 and "Cave-Associated Salamanders of Virginia and West Virginia" in 1962. These compilations of species observed by cavers and researchers expanded the documentation of amphibians and reptiles in caves. Cooper (1962) added species encounter records *Notophthalmus viridescens*, *D. monticola*, and *P. richmondi*. In 1966, Green and Brant published "Salamanders Found in West Virginia Caves" as an account of salamanders collected from 47 caves combined with records from museum collections and literature. Their investigation added records for *Pseudotriton ruber* and *Eurycea bislineata* and contributed cave specimens to the West Virginia Biological Survey Collection at Marshall University. More resent surveys by Carey (1973), Storage (1977), Garton et al. (1993), Longenecker (2000), and Schneider (2003) have expended the lists of cave localities for amphibians and reptiles in the state. The objectives of this chapter are to report the findings of my 2002 and 2003 surveys of selected caves in Greenbrier and Monroe counties and to provide an updated comprehensive list of amphibian and reptile encounter records from literature, museum collections, and personal communications.

Methods

Cave Surveys 2002-2003

While searching for substantial *G. porphyriticus* populations in which to establish mark recapture studies, I conducted general herpetological surveys of 25 caves in Greenbrier and Monroe counties (Figure 2-2). Surveys consisted of thoroughly searching leaf litter and under rocks and logs on the cave floor. Crevices and wet seeps on the walls were inspected for climbing crevice-dwelling salamanders such as *Plethodon wehrlei* or *Eurycea lucifuga*. Cave stream pools and rimstone pools were visually searched and cobble lifted in stream riffles. Although these surveys were conducted thoroughly within the sampling area, only the smallest caves were completely examined. Since most amphibian species are found in the twilight area of caves near the entrance, it is assumed that a good examination of this area would produce the majority of species present. *Gyrinophilus porphyriticus,* however, are not limited to the upper levels of caves and can be found at considerable distances from the entrance. Probing into the deeper reaches of caves can also reveal accidentals washed in during periods of high water or tumbled down through vertical pits. The extent of each survey was limited by time, weather, stream height, and number of observers. For survey trips total survey time, relative humidity (RH), air temperature (AT), water temperature (WT), soil temperature (ST), water pH, and species observed were recorded. Amphibians and reptiles, encountered were identified, examined for abnormalities and reproductive status. Snout-vent length and total length were measured with dial calipers and recorded along with life stage and habitat information for each specimen. Some specimens and habitats were documented with digital photography.

Comprehensive List of Species Encounter Records

In addition to conducting new cave surveys, I compiled a comprehensive account of cave amphibian and reptile records through an extensive literature search and examination of museum collection records. Records from the Carnegie Museum of Natural History (CMNH), the US Museum of Natural History (USNM), and the West Virginia Biological Survey collection at Marshall University (WVBS) were examined for cave encounter records. Accuracy of identification of all specimens listed in published accounts and museum collections could not be verified. Some specimens were listed from localities well out of their range or given an obsolete taxonomic name. Wherever misidentifications were observed they were corrected. *Plethodon richmondi* records from

Greenbrier County, for example, were relisted as *P. hoffmani* in order to reflect current nomenclature. Locality information including cave name, county, USGS quad, and UTM coordinates were entered into an *MS Access* database. Cave coordinates were identified using Davies (1958), Garton and Garton (1976), Stevens (1988), Storrick (1972), Dasher and Balfour (1994), Ashbrook (1995), Medville and Medville (1995), and verified by Bill Balfour of the West Virginia Association for Caves Studies. Species encounter coordinates were then imported into *ArcMAP* GIS software to create distribution maps. These maps are intentionally vague and UTM coordinates were not listed in this account in an attempt to protect the locations of sensitive species.

Natural cave records were supplemented with additional species accounts from abandoned coal mines. Pauley (1993) conducted an inventory of the upland vertebrate fauna of the New River Gorge National River in which 80 species encounter records of amphibians and reptiles were reported from abandoned coal mines. Coal mines may serve as artificial caves, providing similar benefits to amphibians and reptiles such as shelter, moisture, and refuge from harsh climatic periods.

Results and Discussion

Cave surveys 2002-2003

Throughout 2002 and 2003, 25 caves were surveyed including 38 general searches and 25 monitoring visits for a total 63 cave visits (Table 2-1). Fifteen species, including 14 amphibians (9 Plethodontid salamanders, 4 Ranid frogs, and 1 Bufonid toad) and 1 reptile (*Terrapene carolina*) were observed within caves during this study. These results appear to support Green's (1942) assertion that *G. porphyriticus* are the most frequently encountered amphibians in West Virginia caves. They were found in over half of the 25 caves visited (Table 2-2), however this was biased by the fact that survey sites were chosen for their likelihood to contain *G. porphyriticus* habitat. *Eurycea lucifuga* was the second most frequently encountered salamander and was found at 9 caves, followed by *D. fuscus* (7 caves) and *D. ochrophaeus* (6 caves). *Rana palustris*, Pickerel Frogs, were found at 5 caves and were the most commonly encountered Anuran, followed by *Rana clamitans*, Green Frogs and *Bufo americanus*, American Toads. These cave surveys revealed 40 new species encounter locations, including 6 new locations for *D. fuscus* and *D. ochrophaeus,* 5 new locations for *G. porphyriticus* and *R. palustris* and 2 new sites for *T. carolina* (Table 2-2).

Surveys of General Davis Cave in Greenbrier County are of particular interest because of the rare endemic species found their. In August 2002 we counted 3 adult and 23 larval West Virginia Spring Salamanders, *Gyrinophilus subterraneus,* in the cave stream and along muddy banks. This survey also revealed 10 *G. porphyriticus* adults, 1 *E. lucifuga* larvae, 1 *E. longicauda* adult, and 1 *R. clamitans* subadult. In October of the following year, we counted 3 adult and 12 larval *G. subterraneus*, 7 *G. porphyriticus* adults, 1 *E. cirrigera* adult, 1 *D. fuscus* adult, and 1 *R. palustris* adult (Table 2-1).

Comprehensive Species Encounter Records of Amphibians in West Virginia Caves and Abandoned Coal Mines

A thorough review of literature and museum records uncovered over 500 amphibian and reptile accounts from caves. Forty-three species have been reported from 210 West Virginia caves (32 species) and 41 abandoned coal mines (27 species). Reports of amphibians in caves are far more numerous than reports of reptiles. Ninety seven percent of species encounter records were amphibians. Thirty amphibian species including 25 from natural caves and 20 from mines are documented compared to only 13 reptile species with 7 species reported from caves and 7 from mines (Tables 2-3 and 2-4).

The amphibian order Caudata, salamanders, make up the majority of West Virginia cavernicole records. There are 21 salamander species reported from 248 caves and 35 abandoned coal mines in the state. Nineteen salamander species reported from subterranean habitats are from the family Plethodontidae. Plethodontid salamanders may be exceptionally well suited for life in caves and accounted for 86% of the total species encounters. There are more cave records for *E. lucifuga* than any other species in West Virginia. They have been reported from 113 caves and 12 coal mines (Table 2-4). Their conspicuous orange pigmentation and tendency to occupy twilight areas of caves has probably facilitated observation of this species. *Gyrinophilus porphyriticus* is reported from 102 underground localities, including 100 caves and 2 coal mines. Other salamanders prevalent in subterranean habitats are *E. longicauda* (40 localities), *P. glutinosus* (32), *D. ochrophaeus* (27), *D. fuscus* (27), and *P. wehrlei* (24) (Tables 2-3 and 2-4).

Ten species of frogs and toads were observed in 26 caves and 8 abandoned coal mines. All 5 species of West Virginia ranid frogs have been encountered in caves. *Rana palustris* are the most widely encountered frogs in West Virginia caves and are known from 14 caves and 1 mine. *Rana clamitans* (12 localities), *B. americanus* (6), and *R. catesbeiana* (5) are also occasionally encountered underground (Tables 2-3 and 2-4).

Comprehensive Species Encounter Records for Reptiles in West Virginia Caves and Abandoned Coal Mines

Reptiles are far less frequently encountered underground in West Virginia than are amphibians and only represented 3 % of the total records. Thirteen species have been observed in 14 caves and 12 abandoned coal mines. Snakes are the most widely observed reptile in caves in the state. The most reported reptile, *Elaphe alleghaniensis*, Eastern Ratsnakes (Formerly *E. obsoleta*, Black Ratsnakes), is documented from 4 localities, followed by Eastern Box Turtles, *T. carolina* form 3 localities. Also there are 3 viper locality records, including 2 *Crotalus horridus*, Timber Rattlesnake from caves and 1 *Agkistrodon contortrix*, Northern Copperhead from a coal mine. No lizards have been reported from caves in West Virginia but 3 species were reported from 5 abandon coal mines in Fayette County.

Figure 2-3 shows general locations of West Virginia caves and abandon coal mines with amphibian and reptile records along with associated limestone areas and Holsinger et al. (1976) faunal regions. Cave records for *G. porphyriticus* are identified with green points. Sites with the greatest diversity of species records are Organ Cave (14 species), Buckeye Creek Cave (13), Rehoboth Church Cave (10), Ludington Cave (10), General Davis Cave (8), Higginbotham Cave #1 (8) and Norman Cave (8) (Table 2-7). These caves have been heavily visited by researchers which likely increased the chances of species encounters being reported. Rehoboth Church Cave has the highest number of anurans reported from any site. In 8 out of 12 visits in 2002 and 2003, anurans were encountered in Rehoboth Church Cave. The majority of frogs and toads encountered there were subadults, which my have dispersed downstream from a nearby stock pond.

Conclusions

This account of over 500 amphibian and reptile species encounter locations is the most comprehensive collection to date and should build on the works of Reese (1934), Cooper (1960 and 1962), Green and Brant (1966), Garton et al. (1993), and others. It is however, still a work in progress. There are still records at the U. S. Museum of Natural History which have not been examined, cavers and biospeleologists who have not been contacted, and countless new biological inventories to be conducted. This consolidation of species encounter records should be useful as a reference for managers and researches interested in the diversity and distribution of West Virginia's amphibians and reptiles in caves.

Chapter II

Tables and Figures

Table 2-1. Herpetological surveys of Caves in Greenbrier and Monroe Counties, West Virginia, completed in 2002 and 2003.

Higginbotham's Cave # 4, Several hundred meters of stream passage from entrance February 3, 2002				
Species observed: 1 adult *Gyrinophilus porphyriticus*				
Comments: Not thorough survey, sport trip. No environmental data.				
WT: --	pH: --	ST: --	AT: --	RH: --
Observers: M. Osbourn, Jeff Bray				

McClung's Cave, Entrance and first several hundred meters of dark zone. February 16, 2002				
Species observed: *Plethodon wehrlei:* 3 adult				
Comments: Scouting, not thorough survey. No environmental data.				
WT: --	pH: --	ST: --	AT: --	RH: --
Observers: M. Osbourn, Ed Swepston, Mike Corbett				

Buckeye Creek Cave, entrance and dry passage. February 17, 2002				
Species observed: none observed.				
Comments: no environmental data.				
WT: --	pH: --	ST: --	AT: --	RH: --
Observers: M. Osbourn, Mike Corbett				

Fuel's Fruit Cave, entrance to back of cave February 16, 2002				
Species observed: none seen.				
Comments:				
WT: --	pH: --	ST: --	AT: 10.6	RH: 50 %
Observers: M. Osbourn, Bob Handley				

US 219 Cave, twilight. March 24, 2002				
Species observed: none.				
Comments: Scouting, not thorough survey. No environmental data.				
WT: --	PH: --	ST: --	AT: --	RH: --
Observers: M. Osbourn				

Table 2-1. continued

US 219 Cave, entire cave.
March 29, 2002

Species observed:

- *Gyrinophilus porphyriticus*: 2 adult, 3 larvae
- *Eurycea lucifuga*: 11 adults, 5 larvae
- *Plethodon glutinosus*: 2 adult
- *Rana palustris*: 1 adult

Comments: *E. lucifuga* larvae found in pool at entrance. *E. lucifuga* adults found on vertical rock walls in dark zone. GYPO larvae in stream pools in dark zone.

WT: 11.0	pH: --	ST: --	AT: 10.7	RH: 82%

Observers: M. Osbourn, Zack Felix, Rob Fiorentino

McClung's Cave, Entrance to ~500 m along canyon stream.
March 30, 2002

Species observed:

- *Eurycea lucifuga*: 8 larvae
- *Plethodon wehrlei*: 18 adults
- *Rana palustris*: 2 adult

Comments: Thorough search of entrance and canyon stream. *P. wehrlei* in twilight and nearby dark zone. *R. palustris* 50 m in dark. *E. lucifuga* larvae in stream in canyon.

WT: 10.0	pH: --	ST: 10.0	AT: 12.3	RH: 83%

Observers: M. Osbourn, Zack Felix, Rob Fiorentino, Keith Johnson

Higginbotham's Cave # 2, entrance and rimstone pools.
March 30, 2002

Species observed: *Eurycea lucifuga*: 4 adults, 39 young larvae.

Comments: Most *E. lucifuga* larvae measured ~ 20 mm TL. Rimstone pools teeming with young larvae.

WT: 8.5	pH: --	ST: 11.0	AT: 9.7	RH: 98%

Observers: M. Osbourn, Keith Johnson

Higginbotham's Cave # 1, entire stream passage.
March 31, 2002

Species observed:

- *Eurycea lucifuga*: 4 adults, 2 larvae
- *Desmognathus ochrophaeus*: 1 adult
- *Gyrinophilus porphyriticus*: 4 adults, 1 larvae

Comments: Walked entire stream passage. *G. porphyriticus* found in leaf-litter in entrance, under stream rocks, and on mud banks. *E. lucifuga* adults on vertical walls. *E. lucifuga* larvae in small rimstone pool near rear of cave. *D. ochrophaeus* on stream bank in rear of cave, dark zone.

WT: 8.5	pH: --	ST: 9.0	AT: 9.3	RH: 88%

Table 2-1. continued

Observers: M. Osbourn, Keith Johnson

Union Cave, sump
April 14, 2002

Species observed:

- *Eurycea lucifuga*: 2 larvae
- *Gyrinophilus porphyriticus*: 15 adults and 13 larvae
- *Pseudotriton ruber*: 1 adult

Comments: Surveyed sump. Muddy banks and stream loaded with *Gyrinophilus*. *Pseudotriton ruber* emaciated. Had to depart before survey completed.

WT: 8.5	pH: --	ST: 10.0	AT: 11.0	RH: 91%

Observers: M. Osbourn, Keith Johnson. Guided to site by Jeff Bray and the Monroe Co. Cavers

Buckeye Creek Cave, Dry Passage and several hundred meters of stream passage.
May 16, 2002

Species observed:

- *Eurycea lucifuga*: 2 adult
- *Eurycea longicauda*: 1 adult
- *Gyrinophilus porphyriticus*: 1 adult

Comments:

WT: 10.0	pH: 7.9	ST: 9.5	AT: 14.0	RH: 78%

Observers: M. Osbourn

Spout Cave
June 9, 2002

Species observed: No amphibians

Comments: Accompanied David Culver, Horton H. Hobbs, and Katie Schneider on cave inventories of Buckeye Creek drainage.

WT: --	pH: --	ST: --	AT: --	RH: --

Observers: M. Osbourn, Katie Schneider

Upper Spout Cave
June 9, 2002

Species observed:

- *Gyrinophilus porphyriticus*: 1 adult
- *Desmognathus ochrophaeus*: 1 adult

Comments: Accompanied David Culver, Horton H. Hobbs, and Katie Schneider on cave inventories of Buckeye Creek drainage.

WT: --	pH: --	ST: --	AT: --	RH: --

Observers: M. Osbourn, David Culver, Horton H. Hobbs, and Katie Schneider

Table 2-1. continued

Ludington Cave, Ludington Entrance, down new drop to junction with thunderbolt passage. June 10, 2002				
Species observed: *Gyrinophilus porphyriticus*: >20 adults, 8 larvae				
Comments: First trip to salamander junction. There was not enough time to conduct a thorough count. Large number of adults seen on clay dunes and debris piles. Larvae seen in pools up stream. More adults in sump.				
WT: 9.0	pH: 7.6	ST: --	AT: 10.0	RH: 93%
Observers: M. Osbourn, Marianne Saugstad, Harry Fair.				

Goat Cave June 11, 2002				
Species observed: o *Eurycea lucifuga*: 6 adults o *Eurycea longicauda*: 1 adult o *Desmognathus ochrophaeus*: 1 sub adult				
Comments: Accompanied David Culver, Horton H. Hobbs, and Katie Schneider on cave inventories of Buckeye Creek drainage. Small cave entrance (FRO).				
WT: --	pH: --	ST: --	AT: --	RH: --
Observers: M. Osbourn, Jeff Hajenga				

Upper Buckeye June 11, 2002				
Species observed: o *Eurycea longicauda*: 1 adult o *Eurycea lucifuga*: 1 adult				
Comments: Accompanied David Culver, Horton H. Hobbs, and Katie Schneider on cave inventories of Buckeye Creek drainage. No environmental data. Banded Sculpin.				
WT: --	pH: --	ST: --	AT: --	RH: --
Observers: M. Osbourn, David Culver, Jeff Hajenga, and Katie Schneider				

Zimmerman's Pit June 11, 2002				
Species observed: *Eurycea lucifuga*: 1 adult, 1 subadult				
Comments: Accompanied David Culver, Horton H. Hobbs, and Katie Schneider on cave inventories of Buckeye Creek drainage.				
WT: --	pH: --	ST: --	AT: --	RH: --
Observers: M. Osbourn, David Culver, Jeff Hajenga, and Katie Schneider				

Table 2-1. continued

Upper Spout Cave
June 12, 2002

<u>Species observed</u>:

- *Eurycea lucifuga*: 2 adults
- *Desmognathus ochrophaeus*: 2 adults
- *Desmognathus fuscus:* 2 adults, 2 larvae

<u>Comments</u>: Accompanied David Culver, Horton H. Hobbs, and Katie Schneider on cave inventories of Buckeye Creek drainage.

WT: --	pH: --	ST: --	AT: --	RH: --

Observers: M. Osbourn and Katie Schneider

Buckeye Creek Cave, to sump
June 30, 2002

Species observed:

- *Gyrinophilus porphyriticus*: 6 adults
- *Desmognathus ochrophaeus*: 1 adult
- *Desmognathus fuscus:* 1 adult

Comments: *Gyrinophilus porphyriticus* found on mud banks, piles of organic matter, and in stream.

WT: 10.5	pH: 7.7	ST: 11.0	AT: 13.0	RH: 82%

Observers: M. Osbourn, Seth Meyers

Scott Hollow Cave, North-South passage
July 6, 2002

Species observed:

- *Gyrinophilus porphyriticus*: 1 larva
- *Eurycea lucifuga*: 11 larvae
- *Pseudotriton ruber*: 1 adult

Comments:

WT: 10	pH: 7.5	ST: 10.0	AT: 15.0	RH: 73%

Observers: M. Osbourn, Mike Door

Ludington Cave, Ludington entrance down "old drop" to sump.
July 7, 2002

Species observed:

- *Gyrinophilus porphyriticus*: 12 adults, 4 larvae
- *Eurycea lucifuga*: 1 larva
- *Bufo americanus*: 1 adult
- *Terrapene Carolina*: 1 adult

Table 2-1. continued

Comments: 3 GYPO larvae were in pool at base of drop with large *Cambarus* sp. Adult GYPO on floating woody debris and banks in sump. *Bufo* at sump, attracted to light. *T. carolina* along stream below 30 ft. drop. Appeared in good health except missing rear foot.				
WT:	pH:	ST:	AT:	RH:
Observers: M. Osbourn, Harry Fair.				

Lost World Caverns, base of Grapevine drop. July 8, 2002				
Species observed: none.				
Comments:				
WT: --	pH: --	ST: --	AT: --	RH: --
Observers: M. Osbourn				

Spencer Cave July 9, 2002				
Species observed: None seen.				
Comments: Buckeye Creek resurgence				
WT: 10.5	pH: 7.6	ST: 10.0	AT: 10.3	RH: 83%
Observers: M. Osbourn, Bob Handley				

Rehoboth Church Cave, entrance to breakdown pinch. July 10, 2002				
Species observed: o *Eurycea lucifuga*: 2 adults o *Rana palustris*: 1 adult o *Bufo americanus*: 1 subadult o *Rana clamitans*: 1 subadult o *Desmognathus ochrophaeus*: 1 adult				
Comments: Just after warm rain, water turbid and swift.				
WT: 16.5	pH: 7.6	ST: 10.5	AT: 12.0	RH: 96%
Observers: M. Osbourn				

Pilgrims Rest Church Cave #1, entrance to pinch. July 11, 2002				
Species observed: o *Gyrinophilus porphyriticus*: 1 larva o *Desmognathus ochrophaeus*: 1 adult				
Comments: Accompanied David Culver, Horton H. Hobbs, and Katie Schneider on cave inventories of Buckeye Creek drainage.				
WT: --	pH: --	ST: --	AT: --	RH: --

Table 2-1. continued

Observers: M. Osbourn, Katie Schneider, and Jeff Hajenga

Pilgrims Rest Church Cave #2
July 11, 2002

Species observed:

- *Gyrinophilus porphyriticus*: 3 larvae
- *Terrapene carolina*: plastron and bones

Comments: Accompanied David Culver, Horton H. Hobbs, and Katie Schneider on cave inventories of Buckeye Creek drainage. Very tight hillside entrance, had to remove my helmet to fit.

WT: 10.0	pH: 6.6	ST: 10.5	AT: 11.5	RH: 96 %

Observers: M. Osbourn and Katie Schneider

Ludington Cave, Entrance, down "new drop" to Salamander Junction.
July 12, 2002

Species observed: *Gyrinophilus porphyriticus*: 22 adults

Comments: Established Marked Recapture study. VIT needle to dull, none tagged. GYPO on debris pile, mud banks, and in stream.

WT: 9.0	pH: 6.4	ST: 9.0	AT: 10.0	RH: 98%

Observers: M. Osbourn, Harry Fair

Ludington Cave, Entrance, down "new drop" to Salamander Junction.
July 13, 2002

Species observed: *Gyrinophilus porphyriticus*: 20 adults (18 tagged with VIT), 1 larva

Comments: First tagging of mark-recapture study at Salamander Junction. All salamanders from study plot.

WT: 9.0	pH: 6.8	ST: 9.0	AT: 11.0	RH: 98%

Observers: M. Osbourn, Pat B., and Tony Buoy

Higginbotham Cave # 1, ~100 m of stream passage.
July 29, 2002

Species observed: *Gyrinophilus porphyriticus*: 1 larva

Comments: Not thorough search.

WT: 12.0	pH: --	ST: 9.5	AT: 15.0	RH: 70%

Observers: M. Osbourn, T. K. Pauley, and M. B. Watson

Higginbotham Cave # 2, entrance and rimstone pools.
July 29, 2002

Species observed:

- *Desmognathus ochrophaeus*: 1 adult
- *Eurycea lucifuga*: 1 adult

Table 2-1. continued

Comments: DEOC in twilight and gravid, EULU at rimstone pool.

WT: --	pH: --	ST: --	AT: --	RH: --

Observers: M. Osbourn, T. K. Pauley, and M. B. Watson

Norman Cave, entrance and stream passage.
July 30, 2002

Species observed:

- *Gyrinophilus porphyriticus*: 1 larva
- *Desmognathus fuscus*: 1 adult

Comments: In stream in inner cave.

WT: 11.0	pH: 7.6	ST: 10.0	AT: 11.5	RH: 86%

Observers: M. Osbourn, Seth Meyers

The Hole, Gibbs entrance to Bullwinkle passage
August 20, 2002

Species observed: *Gyrinophilus porphyriticus*: 9 adults, 7 larvae

Comments: Most salamanders seen in plunge pools

WT: 12.0	pH: 6.9	ST: 9.5	AT: 13.5	RH: --

Observers: M. Osbourn, Doug Boyer

General Davis Cave, main entrance along stream, sump to back of cave, pinch.
August 21, 2002

Species observed:

- *Gyrinophilus subterraneus*: 19 larvae, 3 adults
- *Gyrinophilus porphyriticus*: 10 adults
- *Eurycea lucifuga*: 1 larva

Comments: *E. lucifuga* larva in drip pool near entrance. *Gyrinophilus* spp. in stream and on mud banks. 1 GYPO gravid.

WT: 10.0	pH: 7.8	ST: 10.0	AT: 10.5	RH: 92%

Observers: M. Osbourn, Mark Watson, and Jennifer Wykle

General Davis Cave, lower entrance to sump.
August 22, 2002

Species observed:

- *Gyrinophilus subterraneus*: 6 large larvae
- *Eurycea longicauda*: 1 adult
- *Rana clamitans*: 1 subadult

Comments: 6 crayfish. *G. subterraneus*; 3 in pool before first sump, 2 in first sump, 1 in second sump.

WT: 10.0	pH: 7.9	ST: 9.5	AT: 11.5	RH: 89%

Observers: M. Osbourn

Table 2-1. continued

Ludington Cave, Entrance, down "new drop" to Salamander Junction.
August 24, 2002

Species observed: Study area

- *Gyrinophilus porphyriticus*: 4 adults (2 recaptures), 3 larvae
- *Eurycea lucifuga*: 1 larva

Outside study area: none

Comments: 2nd Mark-recapture trip.

WT: 9.5	pH: 7.6	ST: 9.5	AT: 10.0	RH: 93%

Observers: M. Osbourn

Ludington Cave, Entrance, down "new drop" to Salamander Junction.
September 8, 2002

Species observed: Study area

- *Gyrinophilus porphyriticus*: 5 adults (2 recaptures), 3 larvae
- *Eurycea lucifuga*: 1 larva

Outside study area: none

Comments: 3rd Mark-recapture trip.

WT: 9.5	pH: 7.6	ST: 9.0	AT: 10.9	RH: 88%

Observers: M. Osbourn, Andy Johnson, and Ellen Stone

Ludington Cave, Entrance, down "new drop" to Salamander Junction.
October 13, 2002

Species observed: Study area:

- *Gyrinophilus porphyriticus*: 2 adults (1 recapture), 3 larvae (3 recaptures)
- *Eurycea lucifuga*: 1 larva

Outside study area: none

Comments: 4th Mark-recapture trip. Surface stream flowing.

WT: 9.0	pH: 7.9	ST: 9.5	AT: 10.0	RH: 94%

Observers: M. Osbourn, Tiff Huntin

Ludington Cave, Entrance, down "new drop" to Salamander Junction.
November 2, 2002

Species observed: Study area: *Gyrinophilus porphyriticus*: 1 adult (recapture)

Comments: 5th Mark-recapture trip. High water, bank eroding in study area.

WT: 9.0	pH: 8.0	ST: 9.5	AT: 9.0	RH: 97%

Observers: M. Osbourn, Robert Makowsky

Table 2-1. continued

Rehoboth Church Cave, Entrance to Sand Passage junction.
November 3, 2002.

Species observed: Study area:

- *Gyrinophilus porphyriticus*: 13 adults(None tagged)
- *Rana palustris*: 1 adult
- *Rana clamitans*: 1 subadult

Outside study area:

- *Gyrinophilus porphyriticus*: 2 adults
- *Bufo americanus*: 1 subadult

Comments: Established Mark-recapture study. *Bufo* near entrance. *R. clamitans* deep in cave, sand passage junction. Fish species?

WT: 7.0	pH: 8.0	ST: 9.0	AT: 9.5	RH: 90%

Observers: M. Osbourn, Robert Makowsky

Ludington Cave, Entrance, down "new drop" to Salamander Junction.
November 23, 2002

Species observed: Study area:

- 0 in study area

Outside of study area:

- *Gyrinophilus porphyriticus*: 2 adults, 1 larva (0 recaptures)

Comments: 6th Mark-recapture trip. Stream flowing into cave.

WT: 5.5	pH: 8.3	ST: 7.5	AT: 8.0	RH: 91%

Observers: M. Osbourn, Bill Sutton, and Robert Makowsky

Rehoboth Church Cave, Entrance to Sand Passage junction.
November 24, 2002.

Species observed: Study area:

- *Gyrinophilus porphyriticus*: 11 adults
- *Rana clamitans*: 1 subadult

Outside study area:

- *Rana palustris*: 1 adult

Comments: 2nd Mark-recapture trip.

WT: 5.3	pH: 8.2	ST: 8.5	AT: 8.0	RH: 96%

Observers: M. Osbourn, Bill Sutton, and Robert Makowsky

Table 2-1. continued

Rehoboth Church Cave, Entrance to Sand Passage junction.
December 21, 2002.

Species observed: Study area:

- *Gyrinophilus porphyriticus*: 6 adults (2 recaptures), 9 hatchlings

Outside study area:

- *Gyrinophilus porphyriticus*: 1 adult

Comments: 3rd Mark-recapture trip.

WT: 4.0	pH: 8.1	ST: 6.0	AT: 6.5	RH: 96%

Observers: M. Osbourn, Andy Johnson

Ludington Cave, Entrance, down "new drop" to Salamander Junction.
December 22, 2002

Species observed: Study area:

- *Gyrinophilus porphyriticus*: 1 adult (1 recapture)

Outside study area:

- *Gyrinophilus porphyriticus*: 1 adult

Comments: 7th Mark-recapture trip. Stream flowing into cave. Water is up at study area and bank eroding.

WT: 4.5	pH: 7.9	ST: 6.0	AT: 6.5	RH: 98%

Observers: M. Osbourn

Rehoboth Church Cave, Entrance to Sand Passage junction.
January 19, 2003.

Species observed: Study site:

- *Gyrinophilus porphyriticus*: 7 adults (3 recaptures), 1 large larva, 9 hatchlings/ small larvae (recaptures)

Outside study area: none

Comments: 4th Mark-recapture trip. 2 large crayfish.

WT: --	pH: 8.7	ST: 4.5	AT: 6.0	RH: 92%

Observers: M. Osbourn, Robert Makowsky

Ludington Cave, Entrance, down "new drop" to Salamander Junction.
January 20, 2003

Species observed: Study area:

- *Gyrinophilus porphyriticus*: 12 adults (4 recaptures)

Outside study area: none

Comments: 8th Mark-recapture trip. Stream flowing into cave.

WT: 6.0	pH: 8.5	ST: 7.0	AT: 5.0	RH: 97%

Observers: M. Osbourn, Rob Fiorentino

Table 2-1. continued

Rehoboth Church Cave, Entrance to Sand Passage junction.
February 13, 2003.

Species observed: Study area:

- *Gyrinophilus porphyriticus*: 5 adults (3 recaptures) 1 larva, 8 hatchlings/small larvae(recaptures)

Comments: 5th Mark-recapture trip.

WT: --	pH: --	ST: 3.5	AT: 3.0	RH: 84%

Observers: M. Osbourn

Ludington Cave, Entrance, down "new drop" to Salamander Junction.
February 14, 2003

Species observed: Study area:

- *Gyrinophilus porphyriticus*: 26 adults (11 recaptures)

Outside of study area:

- *Gyrinophilus porphyriticus*: 2 adult, 2 larvae

Comments: 9th Mark-recapture trip. 1 ft. of snow. Stream frozen outside and 100 m in cave. Prolonged period of very cold, soil frozen.

WT: 4.0	pH: 7.0	ST: 7.0	AT: 7.5	RH: 98%

Observers: M. Osbourn

Ludington Cave, Entrance, down "new drop" to Salamander Junction.
March 22, 2003

Species observed: Study area:

- *Gyrinophilus porphyriticus*: 18 adults (10 recaptures), 1 larva
- *Desmognathus fuscus*: 1 adult, 1 larva

Outside of study area:

- *Desmognathus fuscus*: 1 larva

Comments: 10th Mark-recapture trip. After melt and flooded chamber to ceiling. Fresh layer of new mud.

WT: 7.0	pH: 7.9	ST: 5.0	AT: 5.5	RH: 98%

Observers: M. Osbourn

Rehoboth Church Cave, Entrance to Sand Passage junction.
March 23, 2003.

Species observed: Study area:

- *Gyrinophilus porphyriticus*: 16 adults (3 recaptures), 1 larva

Outside of study area:

- *Rana catesbeiana*: 1 subadult

Comments: 6th Mark-recapture trip.

WT: 7.0	pH: 8.1	ST: 6.0	AT: 7.0	RH: 98%

Table 2-1. continued

Observers: M. Osbourn

Rehoboth Church Cave, Entrance to Sand Passage junction.
April 19, 2003.

Species observed: Study area:
- *Gyrinophilus porphyriticus*: 8 adults (3 recaptures), 1 large larva
- *Rana catesbeiana*: 1 subadult

Outside of study area:
- *Gyrinophilus porphyriticus*: 1 adult, 1 larva

Comments: 7th Mark-recapture trip.

WT: 8.5	pH: 8.3	ST: 8.0	AT: 8.0	RH: 98%

Observers: M. Osbourn

Ludington Cave, Entrance, down "new drop" to Salamander Junction.
April 20, 2003

Species observed: Study area:
- *Gyrinophilus porphyriticus*: 18 adults (13 recaptures)
- *Desmognathus fuscus*: 1 adult

Outside of study area:
- *Gyrinophilus porphyriticus*: 1 adult (recapture), 1 larva.
- *Eurycea lucifuga*: 1 larva

Comments: 11th Mark-recapture trip. High water. Scoured out stream bed, deeper pools, higher bank, new debris layer. Water flowing into cave.

WT: 7.4	pH: 8.0	ST: 6.0	AT: 7.0	RH: 98%

Observers: M. Osbourn

Rehoboth Church Cave, Entrance to Sand Passage junction.
May 14, 2003.

Species observed: Study area:
- *Gyrinophilus porphyriticus*: 17 adults (9 recaptures)
- *Rana catesbeiana*: 1 adult
- *Eurycea lucifuga*: 1 larva

Outside of study area:
- *Gyrinophilus porphyriticus*: 3 adult.
- *Eurycea longicauda*: 1 adult

Comments: 8th Mark-recapture trip. Two very large crayfish. Low water, pooled.

WT: 10.0	pH: 7.9	ST: 9.0	AT: 11.6	RH: 94%

Observers: M. Osbourn

Table 2-1. continued

Ludington Cave, Entrance, down "new drop" to Salamander Junction.
May 15, 2003

Species observed: Study area:
- *Gyrinophilus porphyriticus*: 13 adults (7 recaptures) 1 large larva
- *Desmognathus fuscus*: 1 larva

Outside of study area:
- *Gyrinophilus porphyriticus*: 1 adult.
- *Eurycea lucifuga*: 1 larva
- *Desmognathus fuscus*: 1 adult, 1 larva

Comments: 12th Mark-recapture trip. Raining on and off out side. Creek running low outside but sinks before cave entrance. Creek low in cave.

WT: 8.0	pH: 7.9	ST: 9.0	AT: 7.5	RH: 98%

Observers: M. Osbourn

Rehoboth Church Cave, Entrance to Sand Passage junction.
June 10, 2003.

Species observed: Study area:
- *Gyrinophilus porphyriticus*: 13 adults (6 recaptures) 4 larvae
- *Rana catesbeiana*: 1 adult (recapture), 1 subadult

Outside of study area:
- *Gyrinophilus porphyriticus*: 4 adults, 1 larva.
- *Eurycea lucifuga*: 4 adults
- *Eurycea cirrigera*: 1 adult

Comments: 9th Mark-recapture trip. 1 gravid *Eurycea lucifuga* in twilight.

WT: 12.0	pH: 7.8	ST: 12.0	AT: 10.5	RH: 97%

Observers: M. Osbourn, Robert Makowsky

Ludington Cave, Entrance, down "new drop" to Salamander Junction.
June 11, 2003

Species observed: Study area:
- *Gyrinophilus porphyriticus*: 11 adults (3 recaptures), 1 larva

Outside of study area:
- *Gyrinophilus porphyriticus*: 1 adult

Comments: 13th Mark-recapture trip. Thunderstorm resulted in quickly rising water and an early exit.

WT: 10.0	pH: 7.9	ST: 11.2	AT: 11.7	RH: 94%

Observers: M. Osbourn, Robert Makowsky

Table 2-1. continued

Rehoboth Church Cave, Entrance to Sand Passage junction.
July 13, 2003.

Species observed: Study area:
- *Gyrinophilus porphyriticus*: 4 adults (3 recaptures), 1 larva

Outside of study area: none

Comments: 10th Mark-recapture trip.

WT: 13.0	pH: 7.8	ST: 14.2	AT: 13.4	RH: 98%

Observers: M. Osbourn, Lisa Smith, and Bill Sutton

Ludington Cave, Entrance, down "new drop" to Salamander Junction.
July 14, 2003

Species observed: Study area:
- *Gyrinophilus porphyriticus*: 14 adults

Outside of study area:
- *Eurycea lucifuga*: 1 larva
- *Eurycea cirrigera*: 1 larva
- *Rana palustris*: 1 adult carcass

Comments: 14th Mark-recapture trip.

WT: 12.0	pH: 7.1	ST: 12.1	AT: 12.3	RH: 98%

Observers: M. Osbourn, Lisa Smith, and Bill Sutton

General Davis Cave, main entrance along stream, sump to back of cave, pinch.
October 9, 2003

Species observed:
- *Gyrinophilus subterraneus*: 7 larvae, 3 adults
- *Gyrinophilus porphyriticus*: 7
- *Eurycea cirrigera*: 1 adult
- *Desmognathus fuscus*: 1 adult

Comments: *Gyrinophilus* spp. in stream and on mud banks. 1 gravid *G. subterraneus*.

WT: 11.8	pH: 7.9	ST: 11.6	AT: 11.6	RH: 98%

Observers: M. Osbourn, Bill Sutton, Lisa Smith, Zack Loughman, and Jennifer Wykle

General Davis Cave, lower entrance to sump.
October 10, 2003

Species observed:
- *Gyrinophilus subterraneus*: 5 larvae
- *Rana palustris*: 1 adult (dead)

Comments: *Gyrinophilus* spp. in stream and on mud banks. 3 crayfish.

WT: --	pH: --	ST: --	AT: --	RH: --

Table 2-1. continued

Observers: M. Osbourn, Bill Sutton, Lisa Smith, Zack Loughman, and Jennifer Wykle

Rehoboth Church Cave, Entrance to Sand Passage junction.
October 10, 2003.

Species observed: Study area:
- *Gyrinophilus porphyriticus*: 13 adults, 2 larva
- *Eurycea cirrigera*: 1 larvae
- *Rana catesbeiana*: 1 adult, 1 subadult

Outside of study area: none
- *Gyrinophilus porphyriticus*: 2 adults, 1 larva

Comments: 11th Mark-recapture trip. Collected stomach contents. Creek very low, but evidence of recent high water.

WT: 13.0	pH: 7.5	ST: 12.3	AT: 12.8	RH: 98%

Observers: M. Osbourn, Zack Loughman

Ludington Cave, Ludington entrance down "old drop" to sump.
November 4, 2003

Species observed:
- *Gyrinophilus porphyriticus*: 7 adults, 3 larvae
- *Eurycea lucifuga*: 1 larva
- *Pseudotriton ruber*: 1 larva
- *Desmognathus fuscus*: 1 adult
- *Bufo americanus*: 1 adult
- *Eurycea cirrigera*: 3 larvae

Comments: Collected stomach contents. Stream not flowing outside.

WT: 10.9	pH: 7.5	ST: 10.8	AT: 11.2	RH: 95%

Observers: M. Osbourn

Sinks-of-the-Run Cave, twilight zone only.
November, 2003

Species observed: none seen.

Comments: not full survey.

WT:--	pH:--	ST:--	AT:--	RH:--

Observers: M. Osbourn

Table 2-2. Tally of species encounter records from 2002 and 2003 cave surveys.

Taxa	**Species Encounters**	**New Localities**
Class Amphibia	**58**	**39**
Order Caudata	**47**	**28**
Family Plethodontidae	**47**	**28**
Desmognathus fuscus	7	6
Desmognathus ochrophaeus	6	6
Eurycea cirrigera	3	3
Eurycea longicauda	2	2
Eurycea lucifuga	9	2
Gyrinophilus porphyriticus	15	5
Plethodon glutinosus	1	1
Plethodon wehrlei	1	0
Pseudotriton ruber	3	3
Order Anura	**11**	**11**
Family Bufonidae	**2**	**2**
Bufo americanus	2	2
Family Ranidae	**9**	**9**
Rana catesbeiana	1	1
Rana clamitans	2	2
Rana palustris	5	5
Rana pipiens	1	1
Class Reptilia	**2**	**2**
Order Testudines	**2**	**2**
Family Emydidae	**2**	**2**
Terrapene carolina	2	2

Table 2-3. Amphibian encounter records for West Virginia caves and abandoned coal mines.

Species	Locality	County	USGS Quad	References
Order Caudata				
Family Ambystomatidae				
Ambystoma jeffersonianum	Lost World Caverns (Grapevine)	Greenbrier	Lewisburg	Cooper 1962
Ambystoma jeffersonianum	Organ Cave	Greenbrier	Ronceverte	WVBS 571
Ambystoma jeffersonianum	The Hole, 500 ft. from Pickens entrance	Greenbrier	Anthony	WVBS 3454
Ambystoma jeffersonianum	2.0 miles South of Union, 20 ft. from cave entrance	Monroe	Union	WVBS 3459
Ambystoma jeffersonianum	Seneca Caverns, near, Hell Hole Cave. Riverton	Pendleton	Onego	USNM 00109179
Ambystoma jeffersonianum	Hellhole	Pendleton	Onego	Fowler 1944, Cooper 1961, Carey 1973
Family Salamandridae				
Notophthalmus viridescens	At old mine air shaft, trail; Rush Run area	Fayette	Thurmond	Pauley 1993; WVBS 7840
Notophthalmus viridescens	Coal mine with 2 ft. of water, between Butcher Branch and Wolf Creek	Fayette	Fayetteville	Pauley 1993
Notophthalmus viridescens	Concho Mines; Mine portal	Fayette	Thurmond	Pauley 1993
Notophthalmus viridescens	Entrance to Kaymoor Mine #1	Fayette	Fayetteville	Pauley 1993
Notophthalmus viridescens	Head of Buffalo Creek;Old mine and small creek	Fayette	Thurmond	Pauley 1993
Notophthalmus viridescens	Mine portal (1.5 x 2m) on trail; Rush Run area	Fayette	Thurmond	Pauley 1993
Notophthalmus viridescens	Portal with tipple, very dry mine; Brooklyn area	Fayette	Thurmond	Pauley 1993
Notophthalmus viridescens	Lost World Caverns (Grapevine)	Greenbrier	Lewisburg	Carey 1973; Cooper 1962
Notophthalmus viridescens	Moose's Nose Pit	Greenbrier	Williamsburg	Garton et al. in Dasher and Balfour 1994
Notophthalmus viridescens	Organ Cave System	Greenbrier	Ronceverte	CM 14314, Hartline 1964
Notophthalmus viridescens	Our Pit	Greenbrier	Williamsburg	K. Schneider pers. comm.
Notophthalmus viridescens	Raceway Pit	Greenbrier	Williamsburg	Garton et al. in Dasher and Balfour 1995

Table 2-3. Continued.

Species	Locality	County	USGS Quad	References
Notophthalmus viridescens	Sinks of Gandy	Randolph	Spruce Knob	USNM 110949, 110950
Family Plethodontidae				
Aneides aeneus	Droop Mountain, Ice cave	Pocahontas	Droop	WVBS 48-50
Desmognathus fuscus	Head of Buffalo Creek; Old mine	Fayette	Thurmond	Pauley 1993
Desmognathus fuscus	Head of Buffalo Creek;Old mine and small creek	Fayette	Thurmond	Pauley 1993
Desmognathus fuscus	Old mine NW of Ames	Fayette	Fayetteville	Pauley 1993; WVBS 6335, 6577, 6579-6581
Desmognathus fuscus	Old mine; N. of Ames	Fayette	Fayetteville	Pauley 1993
Desmognathus fuscus	Biggers Cave	Greenbrier	Williamsburg	Rosevear in Ashbrook 1995
Desmognathus fuscus	Buckeye Creek Cave	Greenbrier	Williamsburg	M. Osbourn (pers. obsv. 2002); Cooper 1960, Carey 1973
Desmognathus fuscus	Cave Farm Cave	Greenbrier	Anthony	Cooper 1960-61, Carey 1973
Desmognathus fuscus	Field Station Pit	Greenbrier	Williamsburg	K. Schneider pers. comm.
Desmognathus fuscus	General Davis Cave	Greenbrier	Asbury	M. Osbourn (pers. obsv. 2003)
Desmognathus fuscus	Hannah Water Cave	Greenbrier	Asbury	K. Schneider pers. comm.
Desmognathus fuscus	Hillside Pit	Greenbrier	Williamsburg	K. Schneider pers. comm.
Desmognathus fuscus	Hinkle-Unus Cave	Greenbrier	Williamsburg	Carey 1973
Desmognathus fuscus	House Cave, Higganbotham Farm	Greenbrier	Williamsburg	WVBS 2223-2224; Carey 1973
Desmognathus fuscus	Ludington Cave	Greenbrier	Williamsburg	M. Osbourn (personal observation 2002 and 2003)
Desmognathus fuscus	McClung's Cave	Greenbrier	Williamsburg	Reese 1934, Carey 1973
Desmognathus fuscus	Norman Cave	Greenbrier	Droop	M. Osbourn (personal observation 2002)
Desmognathus fuscus	Organ Cave System	Greenbrier	Ronceverte	Reese 1934; Cadbury 1936 in Green and Brant 1966; Rutherford and Handley 1976
Desmognathus fuscus	Richlands Cave	Greenbrier	Lewisburg	Rosevear in Ashbrook 1995
Desmognathus fuscus	Turner's Pit, Renick, near	Greenbrier	Williamsburg	USNM 160504-160510
Desmognathus fuscus	Unus Cave	Greenbrier	Williamsburg	Reese 1934; WVBS 2456
Desmognathus fuscus	US Rt 219 Cave	Greenbrier	Williamsburg	WVBS 3402
Desmognathus fuscus	Wade's Cave	Greenbrier	Lewisburg	WVBS 3480, 3481
Desmognathus fuscus	Water Trough Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.

Table 2-3. Continued.

Species	Locality	County	USGS Quad	References
Desmognathus fuscus	Lower Beaver Hole Cave	Monongalia	Morgantown	Reese 1934, Carey 1973
Desmognathus fuscus	Sharps Cave	Pocahontas	Mingo	Storage 1977
Desmognathus fuscus	Waybrite Cave	Tucker	Parsons	Carey 1973
Desmognathus monticola	Abandoned mine, Between C & O Railroad and Backus Mountain	Fayette	Prince	Pauley 1993
Desmognathus monticola	Elverton mine	Fayette	Fayetteville	Pauley 1993; WVBS 7944
Desmognathus monticola	Head of Buffalo Creek; Old mine	Fayette	Thurmond	Pauley 1993;
Desmognathus monticola	Head of Buffalo Creek;Old mine and small creek	Fayette	Thurmond	Pauley 1993
Desmognathus monticola	Old mine NW of Ames	Fayette	Fayetteville	Pauley 1993; WVBS 6576
Desmognathus monticola	Old mine; N. of Ames	Fayette	Fayetteville	Pauley 1993
Desmognathus monticola	Buckeye Creek Cave	Greenbrier	Williamsburg	Grady Pers. Obsv. in Garton *et al.* 1993
Desmognathus monticola	Mystic Cave	Pendleton	Onego	Cooper 1960; Carey 1973; USNM 160511- 160513
Desmognathus monticola	Nelson Cave	Randolph	Horton	Garton et al. 1993
Desmognathus ochrophaeus	Abandoned mine, Between C & O Railroad and Backus Mountain	Fayette	Prince	Pauley 1993
Desmognathus ochrophaeus	Head of Buffalo Creek; Old mine	Fayette	Thurmond	Pauley 1993
Desmognathus ochrophaeus	Barber Pit #2	Greenbrier	Williamsburg	K. Schneider pers. comm.
Desmognathus ochrophaeus	Boothe Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.
Desmognathus ochrophaeus	Buckeye Creek Cave	Greenbrier	Williamsburg	M. Osbourn (personal observation 2002); K. Schneider pers. comm.
Desmognathus ochrophaeus	Field Station Pit	Greenbrier	Williamsburg	K. Schneider pers. comm.
Desmognathus ochrophaeus	Green Hollow FRO (Goat Cave)	Greenbrier	Asbury	K. Schneider pers. comm.
Desmognathus ochrophaeus	Hannah Water Cave	Greenbrier	Asbury	K. Schneider pers. comm.
Desmognathus ochrophaeus	Higginbotham Cave #1	Greenbrier	Williamsburg	M. Osbourn (personal observation 2002)
Desmognathus ochrophaeus	Higginbotham Cave #2	Greenbrier	Williamsburg	M. Osbourn (pers. obsv. 2002)
Desmognathus ochrophaeus	Ludington Cave	Greenbrier	Williamsburg	M. Osbourn (pers. obsv. 2003)
Desmognathus ochrophaeus	Our Pit	Greenbrier	Williamsburg	K. Schneider pers. comm.

Table 2-3. Continued.

Species	Locality	County	USGS Quad	References
Desmognathus ochrophaeus	Pilgrim Rest Church Cave #1	Greenbrier	Williamsburg	M. Osbourn (pers. obsv. 2002)
Desmognathus ochrophaeus	small unknown cave, 4 miles NW of Maxwelton	Greenbrier	__	WVBS 3263
Desmognathus ochrophaeus	Sunnyday Pit	Greenbrier	Williamsburg	K. Schneider pers. comm.
Desmognathus ochrophaeus	Turner's Pit, near Renick	Greenbrier	Williamsburg	USNM 160497-160502
Desmognathus ochrophaeus	Unnamed Cave	Greenbrier	__	Reese 1934, Carey 1973
Desmognathus ochrophaeus	Upper Spout	Greenbrier	Williamsburg	M. Osbourn (pers. obsv. 2002)
Desmognathus ochrophaeus	Water Trough Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.
Desmognathus ochrophaeus	Zimmerman's Pit	Greenbrier	Williamsburg	K. Schneider pers. comm.
Desmognathus ochrophaeus	Lower Beaver Hole Cave	Monongalia	Morgantown	Reese 1934, Carey 1973
Desmognathus ochrophaeus	Rehoboth Church	Monroe	Union	M. Osbourn (pers. obsv. 2002 and 2003)
Desmognathus ochrophaeus	Steeles Cave	Monroe	Alderson	H. H. Hobbs, pers. comm.
Desmognathus ochrophaeus	Crawford No. 1 Cave	Randolph	Pickens	Storage 1977
Desmognathus ochrophaeus	Devils Kitchen Cave	Randolph	Mingo	Storage 1977
Desmognathus ochrophaeus	FRO south of Falling Spring Cave	Randolph	Mingo	Storage 1977
Desmognathus quadramaculatus	"Wet Mine" on Kaymoor Trail; north of Butcher Branch	Fayette	Fayetteville	Pauley 1993; WVBS 7750
Desmognathus sp.	CB's Blowhole	Greenbrier	Williamsburg	K. Schneider pers. comm.
Desmognathus sp.	Spencer Waterfall	Greenbrier	Williamsburg	K. Schneider pers. comm.
Desmognathus **sp.**	Upper Spout Cave	Greenbrier	Williamsburg	H. H. Hobbs, pers. comm.
Desmognathus sp.	Deer (Upper Turner) Insurgence	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea bislineata / E. cirrigera	Old mine NW of Ames	Fayette	Fayetteville	Pauley 1993; WVBS 6578
Eurycea bislineata / E. cirrigera	Culverson Creek Cave	Greenbrier	Williamsburg	Carey 1973
Eurycea bislineata / E. cirrigera	Hern's Mill Cave #2	Greenbrier	Asbury	WVBS 3274, 3275
Eurycea bislineata / E. cirrigera	Hern's Mill Resurgence Cave	Greenbrier	Asbury	Carey 1973

Table 2-3. Continued.

Species	Locality	County	USGS Quad	References
Eurycea bislineata / E. cirrigera	Hinkle-Unus Cave	Greenbrier	Williamsburg	WVBS 3266, 3267
Eurycea bislineata / E. cirrigera	Organ Cave System	Greenbrier	Ronceverte	Rutherford and Handley 1976; WVBS 3479
Eurycea bislineata / E. cirrigera	Richlands Cave	Greenbrier	Lewisburg	Rosevear in Ashbrook 1995
Eurycea bislineata / E. cirrigera	Scott Hollow Cave	Monroe	__	Grady pers. obsrv. in Garton *et al.* 1993
Eurycea bislineata / E. cirrigera	Flower Pot Cave	Randolph	Whitmer	Newsom 1991 in Garton et al. 1993
Eurycea bislineata / E. cirrigera	Marshall Pit No. 2	Randolph	Mingo	Storage 1977
Eurycea cirrigera	Old mine; N. of Ames	Fayette	Fayetteville	Pauley 1993
Eurycea cirrigera	General Davis Cave	Greenbrier	Asbury	M. Osbourn (personal observation 2003)
Eurycea cirrigera	Ludington Cave	Greenbrier	Williamsburg	M. Osbourn (pers. obsv. 2003)
Eurycea cirrigera	Rehoboth Church	Monroe	Union	M. Osbourn (personal observation 2003)
Eurycea longicauda	Whitings Neck Cave	Berkeley	__	Carey 1973
Eurycea longicauda	"Wet Mine" on Kaymoor Trail; north of Butcher Branch	Fayette	Fayetteville	Pauley 1993; WVBS 7751
Eurycea longicauda	Dry mine and wet mine, between Butcher Branch and Wolf Creek	Fayette	Fayetteville	Pauley 1993
Eurycea longicauda	Elverton Mine	Fayette	Fayetteville	Pauley 1993; WVBS 7750
Eurycea longicauda	Mine portal air shaft with old building; W of Short Creek	Fayette	Fayetteville	Pauley 1993
Eurycea longicauda	Mine portal between Fayette and Big Bridge	Fayette	Fayetteville	Pauley 1993; WVBS 7456
Eurycea longicauda	Mine with fallen portal; N of Fayette	Fayette	Fayetteville	Pauley 1993
Eurycea longicauda	Old mine, Ames	Fayette	Fayetteville	Pauley 1993; WVBS 7799
Eurycea longicauda	Old mine; N. of Ames	Fayette	Fayetteville	Pauley 1993; WVBS 6417, 6418
Eurycea longicauda	Wet portal with big highwall and old railroad; N of Fayette	Fayette	Fayetteville	Pauley 1993

Table 2-3. Continued.

Species	Locality	County	USGS Quad	References
Eurycea longicauda	Small cave near Greenland Gap	Grant	__	WVBS, Carey 1973
Eurycea longicauda	Buckeye Creek Cave	Greenbrier	Williamsburg	Cooper 1960, Cooper 1961, Carey 1973
Eurycea longicauda	General Davis Cave	Greenbrier	Asbury	M. Osbourn (personal observation 2002)
Eurycea longicauda	Green Hollow FRO (Goat Cave)	Greenbrier	Asbury	K. Schneider pers. comm.
Eurycea longicauda	Hern's Mill No. 1 Cave	Greenbrier	Asbury	WVBS 3273, Carey 1973
Eurycea longicauda	Norman Cave	Greenbrier	Droop	Longenecker 2000.
Eurycea longicauda	Rapp's Cave	Greenbrier	Williamsburg	Cooper 1960, Cooper 1962, Carey 1973
Eurycea longicauda	Richlands Cave	Greenbrier	Lewisburg	Rosevear in Ashbrook 1995
Eurycea longicauda	Taylor No. 1 Cave	Greenbrier	Lewisburg	WVBS 3407
Eurycea longicauda	Turner's Cave	Greenbrier	Williamsburg	Hutchison 1956
Eurycea longicauda	Upper buckeye Creek Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea longicauda	US Rt 219 Cave	Greenbrier	Williamsburg	WVBS 3401
Eurycea longicauda	Wades Cave	Greenbrier	Lewisburg	WVBS 3473
Eurycea longicauda	Wake Robbin Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea longicauda	John Brown's Cave	Jefferson	Charles Town	Green and Brant 1966, Carey 1973
Eurycea longicauda	Greenville Saltpeter Cave	Monroe	Alderson	H. H. Hobbs, pers. comm.
Eurycea longicauda	Rehoboth Church	Monroe	Union	M. Osbourn (personal observation 2002 and 2003)
Eurycea longicauda	Flute Cave	Pendleton	Circleville	Carey 1973
Eurycea longicauda	McCoy's Mill Cave	Pendleton	Circleville	Springer 1992 in Garton *et al.* 1993
Eurycea longicauda	Thorn Mountain Cave	Pendleton	Circleville	Carey 1973
Eurycea longicauda	Durbin Cave No. 2	Pocahontas	Durbin	USFS records in Garton *et al.* 1993
Eurycea longicauda	Overholt's Blowing Cave	Pocahontas	Marlinton	Cooper 1960, Carey 1973
Eurycea longicauda	Wet Dream Cave	Pocahontas	__	Storage 1977
Eurycea longicauda	Big Run Cave	Randolph	Pickens	Storage 1977, Newsom 1991 in Garton et al. 1993
Eurycea longicauda	Bowden Cave	Randolph	Horton	Carey 1973
Eurycea longicauda	Marshall Cave	Randolph	Mingo	Storage 1977
Eurycea longicauda	Unknown cave 2 miles below Elkins	Randolph	__	Green 1937
Eurycea longicauda	Walt Allen Cave	Randolph	__	Storage 1977
Eurycea longicauda	Barger's Spring, Cave near, ca. 14 miles SE Hinton	Summers	__	USNM 27490, 33648, 33649; Carey 1973

Table 2-3. Continued.

Species	Locality	County	USGS Quad	References
Eurycea longicauda	Cave Hollow- Arbogast Cave	Tucker	Parsons	Carey 1973
Eurycea lucifuga	"Dry" mine on Kaymoor trail; beside a "wet" mine	Fayette	Fayetteville	Pauley 1993; WVBS 7940
Eurycea lucifuga	Air shaft east of Fayette	Fayette	Fayetteville	Pauley 1993; WVBS 7443
Eurycea lucifuga	Concho Mine (2nd mine); portal	Fayette	Thurmond	Pauley 1993; WVBS 8001
Eurycea lucifuga	Dry mine and wet mine, between Butcher Branch and Wolf Creek	Fayette	Fayetteville	Pauley 1993
Eurycea lucifuga	Elverton Mine	Fayette	Fayetteville	Pauley 1993
Eurycea lucifuga	Elverton mine	Fayette	Fayetteville	Pauley 1993; WVBS 7941, 7942, 7943
Eurycea lucifuga	Mine portal before creek and right-of-way; Road between Keeney Creek and Short Creek	Fayette	Fayetteville	Pauley 1993
Eurycea lucifuga	Mine portal; N of Fayette	Fayette	Fayetteville	Pauley 1993
Eurycea lucifuga	Old mine between Fayette and Big Bridge	Fayette	Fayetteville	Pauley 1993; WVBS 8023
Eurycea lucifuga	Old mine north of Ames	Fayette	Fayetteville	Pauley 1993; WVBS 6419
Eurycea lucifuga	Old mine, Ames	Fayette	Fayetteville	Pauley 1993; WVBS 7769
Eurycea lucifuga	Wet portal with big highwall and old railroad; N of Fayette	Fayette	Fayetteville	Pauley 1993
Eurycea lucifuga	Unnamed Cave	Grant	__	Carey 1973
Eurycea lucifuga	Alvon Cave	Greenbrier	Alvon	Green et al. 1967, CM 17147
Eurycea lucifuga	Apple Cave (Turner #3)	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Barber Pit #2	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Benjamen's Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Biggers Cave	Greenbrier	Williamsburg	Rosevear in Ashbrook 1995
Eurycea lucifuga	Bone-Norman Cave	Greenbrier	Droop	Garton *et al.* 1993; H. H. Hobbs, pers. comm.
Eurycea lucifuga	Boothe Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Bubble Cave	Greenbrier	Droop	H. H. Hobbs, pers. comm.
Eurycea lucifuga	Buckeye Creek Cave	Greenbrier	Williamsburg	USNM 160503; Cooper 1960 and 1961; Longenecker 2000; K. Schneider pers. comm.
Eurycea lucifuga	Buckeye Storage Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.

Table 2-3. Continued.

Species	Locality	County	USGS Quad	References
Eurycea lucifuga	Callisons Pond Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Cave above Second Creek near Dixon farm	Greenbrier	__	WVBS 4588
Eurycea lucifuga	Cave Farm Cave	Greenbrier	Anthony	Cooper 1960
Eurycea lucifuga	CB's Blowhole	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Crabapple	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Dead Tree Pit	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Fox Cave	Greenbrier	Droop	CM 14329-30, CM 14345, WVBS 572, Hutchison 1956; H. H. Hobbs, pers. comm.
Eurycea lucifuga	General Davis Cave	Greenbrier	Asbury	M. Osbourn (personal observation 2002); Garton *et al.* 1993
Eurycea lucifuga	Green Hollow FRO (Goat Cave)	Greenbrier	Asbury	K. Schneider pers. comm.
Eurycea lucifuga	Hannah Water Cave	Greenbrier	Asbury	K. Schneider pers. comm.
Eurycea lucifuga	Hern's Mill Cave #2	Greenbrier	Asbury	Green et al. 1967, WVBS 3455, Carey 1973
Eurycea lucifuga	Higginbotham Cave	Greenbrier	Williamsburg	WVBS 13427
Eurycea lucifuga	Higginbotham Cave #1	Greenbrier	Williamsburg	M. Osbourn (personal observation 2002); Green et al. 1967, CM 37585-86, WVBS 2260-61; H. H. Hobbs, pers. comm.
Eurycea lucifuga	Higginbotham Cave #2	Greenbrier	Williamsburg	M. Osbourn (personal observation 2002); Longenecker 2000. Green et al. 1967, CM 34619-20, CM 34599-602, WVBS 3272
Eurycea lucifuga	Hillside Pit	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Hit'N'Head Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	House Cave, Higginbotham Farm	Greenbrier	Williamsburg	Green et al. 1967, WVBS 2220, Carey 1973
Eurycea lucifuga	Jewell Cave	Greenbrier	Fort Spring	Green et al. 1967, WVBS 3260, Carey 1973
Eurycea lucifuga	Lizard Cave	Greenbrier	__	Reese 1934, Carey 1973
Eurycea lucifuga	Longanacre water cave	Greenbrier	Fort Spring	WVBS 3335, 4589, 4590
Eurycea lucifuga	Lost World Caverns (Grapevine)	Greenbrier	Lewisburg	Cooper 1962, Carey 1973, CM 43839-40
Eurycea lucifuga	Ludington Cave	Greenbrier	Williamsburg	M. Osbourn (personal observation 2002 and 2003)
Eurycea lucifuga	Ludington's Cave, 0.5 mile in	Greenbrier	Williamsburg	WVBS 3471
Eurycea lucifuga	Matts Black Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	MC Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.

Table 2-3. Continued.

Species	Locality	County	USGS Quad	References
Eurycea lucifuga	McClung's Cave	Greenbrier	Williamsburg	M. Osbourn (personal observation 2002); Reese 1934, Green et al. 1967, CM 28614, CM 23533-34, Gross 1987
Eurycea lucifuga	Moose's Nose Pit	Greenbrier	Williamsburg	Garton et al. in Dasher and Balfour 1994
Eurycea lucifuga	Mud Cave	Greenbrier	__	Reese 1934, Carey 1973
Eurycea lucifuga	Muddy Creek (Cave?) 2 mi. N Alderson	Greenbrier	Alderson	Reese 1934, Green et al. 1967, CM 12734
Eurycea lucifuga	Nellie's Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Norman Cave	Greenbrier	Droop	Longenecker 2000. Green et al. 1967, WVBS 3404, 3408
Eurycea lucifuga	One Little Room Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Organ Cave System	Greenbrier	Ronceverte	Carey 1973, Green et al. 1967; WVBS 3478; CM 14352, CM 14329-30, CM 41217
Eurycea lucifuga	Osborne Pit	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Our Pit	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Pignut Pit	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Pilgrim Rest Church Cave #1	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Point Pit	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Posthole Pit	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Raceway Pit	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Rapp's Cave	Greenbrier	Williamsburg	Cooper 1960, Cooper 1961, Carey 1973
Eurycea lucifuga	Richlands Cave	Greenbrier	Lewisburg	Rosevear in Ashbrook 1995
Eurycea lucifuga	Salamander Suicide Pit	Greenbrier	Williamsburg	M. Saugstad, pers. comm.
Eurycea lucifuga	Schoolhouse Cave	Greenbrier	__	Garton *et al.* 1993
Eurycea lucifuga	Short Stuff Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Spade's Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Spencer Waterfall	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Split Rock Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Sunbeam Cave	Greenbrier	__	H. H. Hobbs, pers. comm.
Eurycea lucifuga	Sunnyday Pit	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Teetering Rock Pit	Greenbrier	Williamsburg	K. Schneider pers. comm.

Table 2-3. Continued.

Species	Locality	County	USGS Quad	References
Eurycea lucifuga	The Hole	Greenbrier	Anthony	WVBS 3472, 3475
Eurycea lucifuga	Tin Cave	Greenbrier	__	H. H. Hobbs, pers. comm.
Eurycea lucifuga	Trillium Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Turner Pit #2	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Turner's Cave	Greenbrier	Williamsburg	Hutchison 1956, Carey 1973
Eurycea lucifuga	Unnamed cave near Renick	Greenbrier	Williamsburg	CM in Green et al. 1967
Eurycea lucifuga	Unnamed Caves	Greenbrier	__	Hutchison 1956, Carey 1973, CM 19421
Eurycea lucifuga	Upper buckeye Creek Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	US 219 Cave	Greenbrier	Williamsburg	M. Osbourn 2002 pers. obs.; Green et al. 1967, WVBS 3400, 3404; Carey 1973
Eurycea lucifuga	Wake Robbin Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Water Trough Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	Wild Dog Cave	Greenbrier	Williamsburg	Rosevear in Ashbrook 1995
Eurycea lucifuga	Zimmerman's Pit	Greenbrier	Williamsburg	K. Schneider pers. comm.
Eurycea lucifuga	John Brown's Cave	Jefferson	Charles Town	Carey 1973
Eurycea lucifuga	Honaker's Cave	Mercer	Narrows	Cooper 1961, Carey 1973
Eurycea lucifuga	1.5 miles NW of Sinks Grove Cave on Charles Scott Farm	Monroe	__	WVBS 3238, 3239
Eurycea lucifuga	2.0 miles South of Union, entrance to cave	Monroe	__	WVBS 3461
Eurycea lucifuga	Greenville Saltpeter Cave	Monroe	Alderson	H. H. Hobbs, pers. comm.
Eurycea lucifuga	Hay Bale Cave	Monroe	__	Garton *et al.* 1993
Eurycea lucifuga	Laurel Creek Cave	Monroe	Alderson	MCZ 27834, Cooper 1960, Blaney and Blaney 1978, Carey 1973
Eurycea lucifuga	McClung-Zenith Cave	Monroe	Alderson	H. H. Hobbs, pers. comm.
Eurycea lucifuga	Rehoboth Church	Monroe	Union	M. Osbourn (personal observation 2002 and 2003)
Eurycea lucifuga	Scott Cave	Monroe	__	Green et al. 1967, WVBS 3238-39, WV 3462, CM 3462, Carey 1973; H. H. Hobbs, pers. comm.
Eurycea lucifuga	Scott Hollow Cave	Monroe	__	M. Osbourn (pers. obs. 2002); H. H. Hobbs, pers. comm.

Table 2-3. Continued.

Species	Locality	County	USGS Quad	References
Eurycea lucifuga	Second Creek Cave	Monroe	__	Hutchison 1956, Carey 1973, Green et al. 1967, CM 15405-16
Eurycea lucifuga	Steele's Cave, 1 mi. N Salt Sulphur Springs	Monroe	Alderson	Hutchison 1956, Carey 1973, Green et al. 1967, CM 30100; H. H. Hobbs, pers. comm.
Eurycea lucifuga	Union (Caperton) Cave	Monroe	Alderson	M. Osbourn (pers. obs. 2002); Green et al. 1967; WVBS 3461; Schneider 2003
Eurycea lucifuga	Unnamed Cave 1.5 miles NW Sinks Grove	Monroe	__	WVBS
Eurycea lucifuga	Unnamed Cave on Second Creek	Monroe	__	CM, WVBS in Green et al. 1967; Carey 1973
Eurycea lucifuga	Flute Cave	Pendleton	Circleville	Carey 1973
Eurycea lucifuga	Thorn Mountain Cave	Pendleton	Circleville	Carey 1973
Eurycea lucifuga	Overholt's Blowing Cave	Pocahontas	Marlinton	Carey 1973
Eurycea lucifuga	Unnamed cave near Millpoint	Pocahontas	__	Hutchison 1956, Carey 1973, CM 5342
Eurycea lucifuga	Wet Dream Cave	Pocahontas	__	Storage 1977
Eurycea lucifuga	Big Run Cave	Randolph	Pickens	Storage 1977
Eurycea lucifuga	Bowden Cave	Randolph	Horton	Carey 1973
Eurycea lucifuga	Marshall Cave	Randolph	Mingo	Storage 1977
Eurycea lucifuga	Roadside Pit	Randolph	Mingo	Storage 1977
Eurycea lucifuga	Unnamed Cave	Randolph	__	Carey 1973
Eurycea lucifuga	Barger's Spring, near Hinton	Summers	__	Green et al. 1967, USNM 27491, USNM 33611, Carey 1973
Eurycea lucifuga	Cave Hollow- Arbogast Cave	Tucker	Parsons	Carey 1973
Eurycea sp.	Glady Cave	Randolph	Horton	Corbett 1969 in Garton *et al.* 1993
Gyrinophilus porphyriticus	Cheat Mountain Cave, Files Creek	?	__	USNM 33612
Gyrinophilus porphyriticus	Broken Nothing (My) Cave	__	__	Storage 1977
Gyrinophilus porphyriticus	Entrance to Kaymoor Mine #1	Fayette	Fayetteville	Pauley 1993
Gyrinophilus porphyriticus	Old mine; N. of Ames	Fayette	Fayetteville	Pauley 1993; WVBS 6333-6334
Gyrinophilus porphyriticus	Benedict Cave; 1 mile SE of Maxwelton	Greenbrier	Lewisburg	WVBS 3340-3343, 3348
Gyrinophilus porphyriticus	Biggers Cave	Greenbrier	Williamsburg	Rosevear in Ashbrook 1995
Gyrinophilus porphyriticus	Bransfords Cave	Greenbrier	Williamsburg	USNM 337470

Table 2-3. Continued.

Species	Locality	County	USGS Quad	References
Gyrinophilus porphyriticus	Buckeye Creek Cave	Greenbrier	Williamsburg	M. Osbourn (pers. obs. 2002); Carey 1973 in Garton *et al*. 1993; K. Schneider pers. comm.
Gyrinophilus porphyriticus	Cave Farm Cave	Greenbrier	Anthony	Cooper 1961, Carey 1973
Gyrinophilus porphyriticus	Coffman's Cave	Greenbrier	Williamsburg	Reese 1934; CM 6189, 6190, 29442-29445; USNM 319460
Gyrinophilus porphyriticus	Culverson Creek Cave	Greenbrier	Williamsburg	USNM 319461
Gyrinophilus porphyriticus	Fox Cave	Greenbrier	Droop	H. H. Hobbs, pers. comm.
Gyrinophilus porphyriticus	General Davis Cave	Greenbrier	Asbury	M. Osbourn (pers. obs. 2002 and 2003); USNM 525272-525278, 198542; WVBS 4515, 3261; H. H. Hobbs, pers. comm; Besharse and Holsinger 1977
Gyrinophilus porphyriticus	Hannah Water Cave	Greenbrier	Asbury	K. Schneider pers. comm.
Gyrinophilus porphyriticus	Higginbotham Cave	Greenbrier	Williamsburg	CM 30101, Carey 1973 in Garton *et al*. 1993
Gyrinophilus porphyriticus	Higginbotham Cave #1	Greenbrier	Williamsburg	M. Osbourn (personal observation 2002); J. Hajenga per. comm.
Gyrinophilus porphyriticus	Higginbotham Cave #2	Greenbrier	Williamsburg	M. Osbourn (personal observation 2002)
Gyrinophilus porphyriticus	Higginbotham Cave #4	Greenbrier	Williamsburg	M. Osbourn (personal observation 2002)
Gyrinophilus porphyriticus	Hinkle-Unus Cave	Greenbrier	Williamsburg	Carey 1973 in Garton *et al*. 1993
Gyrinophilus porphyriticus	Jewell Cave	Greenbrier	Fort Spring	WV 3261, 3340; CM 30099, WVBS, Carey 1973 in Garton *et al*. 1993
Gyrinophilus porphyriticus	Ludington Cave	Greenbrier	Williamsburg	M. Osbourn (personal observation 2002 and 2003); WVBS 3476
Gyrinophilus porphyriticus	Matts Black Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.
Gyrinophilus porphyriticus	McClung's Cave	Greenbrier	Williamsburg	WVBS 4241, 3348; Carey 1973; J. Hajenga per. comm.
Gyrinophilus porphyriticus	Norman Cave	Greenbrier	Droop	M. Osbourn (personal observation 2002)
Gyrinophilus porphyriticus	Organ Cave (Lipps Entrance)	Greenbrier	Ronceverte	H. H. Hobbs, pers. comm.
Gyrinophilus porphyriticus	Organ-Hedricks Cave System	Greenbrier	Ronceverte	USNM 319462, 319463; Cooper 1961, Carey 1973
Gyrinophilus porphyriticus	Piercy's Cave	Greenbrier	Asbury	J. Hajenga, pers. comm.
Gyrinophilus porphyriticus	Pilgrim Rest Church Cave #1	Greenbrier	Williamsburg	M. Osbourn (personal observation 2002); K. Schneider pers. comm.
Gyrinophilus porphyriticus	Pilgrim Rest Church Cave #2	Greenbrier	Williamsburg	M. Osbourn (pers. obs. 2002); K. Schneider pers. comm.: H. H. Hobbs, pers. comm.

Table 2-3. Continued.

Species	Locality	County	USGS Quad	References
Gyrinophilus porphyriticus	Rapp's Cave	Greenbrier	Williamsburg	Carey 1973 in Garton *et al.* 1993
Gyrinophilus porphyriticus	Richlands Cave	Greenbrier	Lewisburg	Rosevear in Ashbrook 1995
Gyrinophilus porphyriticus	Seep Cave #2	Greenbrier	Williamsburg	K. Schneider pers. comm.
Gyrinophilus porphyriticus	Sinks-of-the-Run Cave	Greenbrier	Asbury	Carey 1973 in Garton *et al.* 1993
Gyrinophilus porphyriticus	small cave 4 miles NW Maxwelton	Greenbrier	__	WVBS
Gyrinophilus porphyriticus	Taylor No. 1 Cave	Greenbrier	Lewisburg	WVBS 3406
Gyrinophilus porphyriticus	The Hole	Greenbrier	Anthony	M. Osbourn (personal observation 2002); WVBS 3477
Gyrinophilus porphyriticus	Tin Cave	Greenbrier	__	H. H. Hobbs, pers. comm.
Gyrinophilus porphyriticus	Unknown Cave	Greenbrier	__	WVBS 3262
Gyrinophilus porphyriticus	Unknown small cave, 4 miles NW of Maxwelton	Greenbrier	__	WVBS 3262
Gyrinophilus porphyriticus	Unnamed Cave	Greenbrier	__	Carey 1973 in Garton *et al.* 1993
Gyrinophilus porphyriticus	Unus Cave	Greenbrier	Williamsburg	WVBS 2455
Gyrinophilus porphyriticus	Upper buckeye Creek Cave	Greenbrier	Williamsburg	K. Schneider pers. comm.
Gyrinophilus porphyriticus	Upper Spout	Greenbrier	Williamsburg	M. Osbourn (pers. obs. 2002); H. H. Hobbs, pers. comm.
Gyrinophilus porphyriticus	US 219 Cave	Greenbrier	Williamsburg	M. Osbourn pers. obsv. 2002 and K. Schneider pers. comm.
Gyrinophilus porphyriticus	Wild Dog Cave	Greenbrier	Williamsburg	Rosevear in Ashbrook 1995
Gyrinophilus porphyriticus	Windy Mouth (Wind) Cave	Greenbrier	Alderson	J. Bray pers. comm.
Gyrinophilus porphyriticus	at mouth of Cave in Athens	Mercer	__	WVBS 4241
Gyrinophilus porphyriticus	Barret's Cave	Mercer	__	WVBS 4245
Gyrinophilus porphyriticus	Beacon Cave	Mercer	__	J. Hajenga, pers. comm.
Gyrinophilus porphyriticus	Beaver Pond Cave	Mercer	Bluefield	J. Hajenga, pers. comm.
Gyrinophilus porphyriticus	Big Spring Cave	Mercer	Bluefield	J. Hajenga, pers. comm.
Gyrinophilus porphyriticus	Argobrites Cave	Monroe	Alderson	USNM 319465-319467
Gyrinophilus porphyriticus	Crossroad Cave	Monroe	Alderson	J. Hajenga, pers. comm.
Gyrinophilus porphyriticus	Deales Hole	Monroe	__	A. Bird pers. comm.
Gyrinophilus porphyriticus	Fletcher Cave	Monroe	Ronceverte	USNM 319468; J. Hajenga, pers. comm.
Gyrinophilus porphyriticus	Greenville Saltpeter Cave	Monroe	Alderson	WVBS 4242; J. Hajenga, pers. comm.

Table 2-3. Continued.

Species	Locality	County	USGS Quad	References
Gyrinophilus porphyriticus	Hunt (Connell) Cave	Monroe	__	J. Hajenga, pers. comm.
Gyrinophilus porphyriticus	Indian Draft Cave	Monroe	Alderson	J. Hajenga, pers. comm.
Gyrinophilus porphyriticus	Laurel Creek Cave	Monroe	Alderson	Cooper 1960, Carey 1973; J. Hajenga, pers. comm.
Gyrinophilus porphyriticus	Miller Cave	Monroe	Ronceverte	J. Hajenga, pers. comm.
Gyrinophilus porphyriticus	Neel Insurgence	Monroe	Ronceverte	J. Hajenga, pers. comm.
Gyrinophilus porphyriticus	Rehoboth Church	Monroe	Union	M. Osbourn (personal observation 2002 and 2003)
Gyrinophilus porphyriticus	Scott Hollow Cave	Monroe	__	M. Osbourn (pers. obs. 2002); Garton et al. 1993; H. H. Hobbs, pers. comm.
Gyrinophilus porphyriticus	Steele's Cave	Monroe	Alderson	Reese 1934, Carey 1973
Gyrinophilus porphyriticus	Union Cave	Monroe	Alderson	M. Osbourn (personal observation 2002)
Gyrinophilus porphyriticus	Unknown Cave	Monroe	__	WVBS 3349
Gyrinophilus porphyriticus	Harman Waterfall Cave	Pendleton	__	Howard et al. 1982
Gyrinophilus porphyriticus	Keel Spring Cave	Pendleton	Onego	Carey 1973 in Garton *et al.* 1993
Gyrinophilus porphyriticus	Mystic Cave	Pendleton	Onego	Cooper 1960, Carey 1973
Gyrinophilus porphyriticus	Thorn Mountain Cave	Pendleton	Circleville	Carey 1973 in Garton *et al.* 1993
Gyrinophilus porphyriticus	Cass Cave	Pocahontas	Cass	Carey 1973
Gyrinophilus porphyriticus	Cave Creek Cave	Pocahontas	Hillsboro	CM 6192, 6193; Reese 1934
Gyrinophilus porphyriticus	Lobelia Saltpeter Cave	Pocahontas	Lobelia	J. Hajenga, pers. comm.
Gyrinophilus porphyriticus	Overholt's Blowing Cave	Pocahontas	Marlinton	USNM 319464, 319469-319475; Cooper 1960, Carey 1973 in Garton *et al.* 1993
Gyrinophilus porphyriticus	Raine's Cave	Pocahontas	__	WVBS 1185-1192, Carey 1973 in Garton *et al.* 1993
Gyrinophilus porphyriticus	Snedegar's Cave	Pocahontas	Droop	USNM 319476; Reese 1934; Carey 1973; J. Hajenga, pers. comm.
Gyrinophilus porphyriticus	Spice, Near, At Cochrane's Cave	Pocahontas	Droop	USNM 110506
Gyrinophilus porphyriticus	Bickle Hollow #1 Cave	Randolph	Horton	Garton et al. 1993
Gyrinophilus porphyriticus	Bowden Cave	Randolph	Horton	Carey 1973 in Garton *et al.* 1993; J. Hajenga, pers. comm.
Gyrinophilus porphyriticus	Bowden Cave #2	Randolph	Horton	WVBS, Carey 1973 in Garton *et al.* 1993
Gyrinophilus porphyriticus	cave 2 miles below Elkins	Randolph	__	Green 1937
Gyrinophilus porphyriticus	Crawford No. 1 Cave	Randolph	Pickens	Carey 1973 in Garton *et al.* 1993
Gyrinophilus porphyriticus	Flower Pot Cave	Randolph	Whitmer	Newsom 1991 in Garton et al. 1993
Gyrinophilus porphyriticus	Hermit's Cave	Randolph	__	USNM 110937, 110938

Table 2-3. Continued.

Species	Locality	County	USGS Quad	References
Gyrinophilus porphyriticus	Marshall Cave	Randolph	Mingo	Storage 1977 in Garton et al. 1993
Gyrinophilus porphyriticus	Mingo Cave No. 1	Randolph	Mingo	Reese 1934
Gyrinophilus porphyriticus	Simmons-Mingo Cave	Randolph	Mingo	Storage 1977
Gyrinophilus porphyriticus	Sinks No. 1	Randolph	Spruce Knob	CM 6191, Reese 1934
Gyrinophilus porphyriticus	Sinks of Gandy	Randolph	Spruce Knob	CM 9974, Carey 1973 in Garton *et al.* 1993; J. Hajenga, pers. comm.
Gyrinophilus porphyriticus	small cave near Aggregates	Randolph	__	Green 1941 in Green and Brant 1966
Gyrinophilus porphyriticus	Unnamed Caves	Randolph	__	Carey 1973 in Garton *et al.* 1993
Gyrinophilus porphyriticus	Cave Hollow- Arbogast Cave	Tucker	Parsons	Carey 1973
Gyrinophilus porphyriticus	Bear Heaven Cave	Tucker?	__	Howard et al. 1982
Gyrinophilus subterraneus	General Davis Cave	Greenbrier	Asbury	M. Osbourn (pers. obs. 2002 and 2003); USNM 198533-198541, 525271, 525279, 525280; WVBS 4516; Besharse and Holsinger 1977
Hemidactylium scutatum	Sinks of Gandy	Randolph	Spruce Knob	CM 20838, Carey 1973, Fowler 1941
Plethodon cinereus	Higginbotham Cave #1	Greenbrier	Williamsburg	Reese 1934, Carey 1973
Plethodon cinereus	Higginbotham Cave #2	Greenbrier	Williamsburg	Longenecker 2000.
Plethodon cinereus	Lost World Caverns (Grapevine)	Greenbrier	Lewisburg	Cooper 1962, Carey 1973
Plethodon cinereus	John Brown's Cave	Jefferson	Charles Town	CM 30089, Green and Brant 1966, Carey 1973
Plethodon cinereus	2.0 miles South of Union, just inside cave entrance	Monroe	Union	WVBS 3460
Plethodon cinereus	Steeles Cave	Monroe	__	H. H. Hobbs, pers. comm.
Plethodon cinereus or ***D. ochrophaeus***	Jone's Quarry Cave	Berkeley	__	CM 39015-39017
Plethodon glutinosus	Silar's Cave, Tomahawk	Berkeley	__	USNM 110939
Plethodon glutinosus	Whitings Neck Cave	Berkeley	__	Carey 1973
Plethodon glutinosus	Kaymoor Trail; at entrance to two coal mines	Fayette	Fayetteville	Pauley 1993; WVBS 7903-7905
Plethodon glutinosus	Mine portal between Fayette and Big Bridge	Fayette	Fayetteville	Pauley 1993; WVBS 7457

Table 2-3. Continued.

Species	Locality	County	USGS Quad	References
Plethodon glutinosus	Mine portal; Road between Keeney Creek and Short Creek	Fayette	Fayetteville	Pauley 1993
Plethodon glutinosus	Old mine shaft ~0.75 mi N of Big Bridge	Fayette	Fayetteville	Pauley 1993; WVBS 6689-6696
Plethodon glutinosus	Old mine shaft, Between big bridge (US 19) and Ames	Fayette	Fayetteville	Pauley 1993
Plethodon glutinosus	Old mine, Ames	Fayette	Fayetteville	Pauley 1993; WVBS 6522
Plethodon glutinosus	Old mine; N. of Ames	Fayette	Fayetteville	Pauley 1993
Plethodon glutinosus	Wet portal with big highwall and old railroad; N of Fayette	Fayette	Fayetteville	Pauley 1993
Plethodon glutinosus	Small cave near Greenland Gap	Grant	__	WVBS
Plethodon glutinosus	Unnamed Cave	Grant	__	Carey 1973
Plethodon glutinosus	Hern's Mill Cave #2	Greenbrier	Asbury	WVBS 3456
Plethodon glutinosus	Lost World Caverns (Grapevine)	Greenbrier	Lewisburg	CM 43841, 43842; Cooper 1962, Carey 1973
Plethodon glutinosus	Ludington's Cave, 0.5 mile in	Greenbrier	Williamsburg	WVBS 3474
Plethodon glutinosus	Norman Cave	Greenbrier	Droop	WVBS 3403
Plethodon glutinosus	Organ Cave System	Greenbrier	Ronceverte	WVBS 569; CM 14315-14319, 14325, 14326; Rutherford and Handley 1976
Plethodon glutinosus	Osborne Pit	Greenbrier	Williamsburg	K. Schneider pers. comm.
Plethodon glutinosus	US 219 Cave	Greenbrier	Williamsburg	M. Osbourn (personal observation 2002)
Plethodon glutinosus	John Brown's Cave	Jefferson	Charles Town	CM 30090-30092
Plethodon glutinosus	Wetzle's Cave	Ohio	Tiltonsville	CM 34059
Plethodon glutinosus	Hamilton Cave	Pendleton	Circleville	Grady in Garton et al. 1993
Plethodon glutinosus	Propst Cave	Pendleton	Circleville	Carey 1973
Plethodon glutinosus	Sinnott's Cave	Pendleton	Circleville	CM 30103
Plethodon glutinosus	Smoke Hole Cave	Pendleton	Onego	Reese 1934, Carey 1973
Plethodon glutinosus	Thorn Mountain Cave	Pendleton	Circleville	Carey 1973
Plethodon glutinosus	Big Run Cave #4	Randolph	Pickens	Storage 1977
Plethodon glutinosus	Bowden Cave	Randolph	Horton	Carey 1973
Plethodon glutinosus	Dumire Cave	Tucker	__	Carey 1973

Table 2-3. Continued.

Species	Locality	County	USGS Quad	References
Plethodon hoffmani	Buckeye Creek Cave	Greenbrier	Williamsburg	Carey 1973, Cooper 1962, Cooper 1965
Plethodon hoffmani	Higginbotham Cave	Greenbrier	Williamsburg	CM 37583, 37584
Plethodon hoffmani	Lost World Caverns (Grapevine)	Greenbrier	Lewisburg	CM 43843, Carey 1973
Plethodon hoffmani	Norman Cave	Greenbrier	Droop	WVBS 3405
Plethodon hoffmani	Rapp's Cave	Greenbrier	Williamsburg	Cooper 1962, Carey 1973
Plethodon hoffmani	Mystic Cave	Pendleton	Onego	CM 40783-40785
Plethodon kentucki	Mine portal (1.5 x 2m) on trail; Rush Run area	Fayette	Thurmond	Pauley 1993
Plethodon richmondi	Flower Pot Cave	Randolph	Whitmer	Newsom 1991
Plethodon richmondi	Marshall Cave	Randolph	Mingo	Storage 1977
Plethodon wehrlei	Mine portal air shaft with old building; W of Short Creek	Fayette	Fayetteville	Pauley 1993
Plethodon wehrlei	Mine portal; N of Fayette	Fayette	Fayetteville	Pauley 1993
Plethodon wehrlei	Mine with fallen portal; N of Fayette	Fayette	Fayetteville	Pauley 1993
Plethodon wehrlei	Old mine between Fayette and Big Bridge	Fayette	Fayetteville	Pauley 1993; WVBS 8024, 8025
Plethodon wehrlei	Wet portal with big highwall and old railroad; N of Fayette	Fayette	Fayetteville	Pauley 1993
Plethodon wehrlei	Arbuckle's Cave	Greenbrier	Lewisburg	WVBS 3626-3649, 3717, 3718, 4060-4066; CM 6195, 6196; Reese 1934; Netting 1936; Carey 1973; Gross 1982
Plethodon wehrlei	Culverson Creek Cave	Greenbrier	Williamsburg	Carey 1973
Plethodon wehrlei	Higginbotham Cave	Greenbrier	Williamsburg	CM 37587; WVBS 2262, 2263
Plethodon wehrlei	Higginbotham Cave #2	Greenbrier	Williamsburg	WVBS 4648
Plethodon wehrlei	Hillside Pit	Greenbrier	Williamsburg	K. Schneider pers. comm.
Plethodon wehrlei	Hinkle-Unus Cave	Greenbrier	Williamsburg	WVBS 3265
Plethodon wehrlei	McClung's Cave	Greenbrier	Williamsburg	M. Osbourn (pers. obs. 2002); CM 23535-23540, 28611-28613, 29446-29448, 34618; WVBS 3336-3339, 4416; Gross 1982

Table 2-3. Continued.

Species	Locality	County	USGS Quad	References
Plethodon wehrlei	Water Trough Cave	Greenbrier	Williamsburg	H. H. Hobbs, pers. comm.
Plethodon wehrlei	Flute Cave	Pendleton	Circleville	Carey 1973
Plethodon wehrlei	Keel Spring Cave	Pendleton	Onego	Carey 1973
Plethodon wehrlei	Seneca Caverns, 2 mi. NE Riverton	Pendleton	Onego	CM 34469-34471, 36575-36578, 52344, 52345
Plethodon wehrlei	Droop Mountain, Ice cave	Pocahontas	Droop	WVBS 46-47
Plethodon wehrlei	Linwood Water Cave	Pocahontas	Mingo	Storage 1977
Plethodon wehrlei	Stella's Cave	Pocahontas	__	H. H. Hobbs, pers. comm.
Plethodon wehrlei	Cornwell Cave	Preston	Bruceton	WVBS, Carey 1973
Plethodon wehrlei	Marshall Pit No. 2	Randolph	Mingo	Storage 1977
Plethodon wehrlei	Mingo Cave No. 2	Randolph	Mingo	WVBS
Plethodon wehrlei	Rosemont; NR. Mine	Taylor	__	WVBS 3599, 3602-3606
Pseudotriton montanus diastictus	Big Indian Cave, Hudnall	Kanawha	__	CM 19238
Pseudotriton ruber	Old mine NW of Ames	Fayette	Fayetteville	Pauley 1993; WVBS 6582-6585
Pseudotriton ruber	Buckeye Creek Cave	Greenbrier	Williamsburg	Carey 1973
Pseudotriton ruber	House Cave, Higganbotham farm	Greenbrier	Williamsburg	WVBS 2221, 2225
Pseudotriton ruber	Ludington Cave	Greenbrier	Williamsburg	M. Osbourn (personal observation 2003)
Pseudotriton ruber	Organ Cave System	Greenbrier	Ronceverte	CM 14320; WVBS 570
Pseudotriton ruber	Scott Hollow Cave	Monroe	__	M. Osbourn (personal observation 2002)
Pseudotriton ruber	Union Cave	Monroe	Union	M. Osbourn (pers. obs.)
Pseudotriton ruber	Overholt's Blowing Cave	Pocahontas	Marlinton	WVBS 3259; Carey 1973
Pseudotriton ruber	Big Run Cave	Randolph	Pickens	Storage 1977
Unidentified salamander	Coffman's Cave	Greenbrier	Williamsburg	CM 18178
Unidentified salamander	Field Station Pit	Greenbrier	Williamsburg	Garton et al. in Dasher and Balfour 1994
Unidentified salamander	Martha's Cave	Pocahontas	Marlinton	CM 18177

Table 2-3. Continued.

Species	Locality	County	USGS Quad	References
Order Anura				
Family Bufonidae				
Bufo americanus	Coal mine with 2 ft. of water, between Butcher Branch and Wolf Creek	Fayette	Fayetteville	Pauley 1993
Bufo americanus	Buckeye Creek Cave	Greenbrier	Williamsburg	Carey 1973, Cooper 1961
Bufo americanus	Ludington Cave	Greenbrier	Williamsburg	M. Osbourn (pers. obsv. 2002 and 2003)
Bufo americanus	Norman Cave	Greenbrier	Droop	Longenecker 2000.
Bufo americanus	Rehoboth Church	Monroe	Union	M. Osbourn (pers. obsv. 2002 and 2003)
***Bufo* spp.**	Baber Pit	Greenbrier	Williamsburg	H. H. Hobbs, pers. comm.
***Bufo* spp.**	Water Trough Cave	Greenbrier	Williamsburg	H. H. Hobbs, pers. comm.
Family Hylidae				
Hyla chrysoscelis	Mine portal; Red Ash area	Fayette	Thurmond	Pauley 1993
Pseudacris brachyphona	Entrance to Kaymoor Mine #1	Fayette	Fayetteville	Pauley 1993
Pseudacris crucifer	Head of Buffalo Creek;Old mine and small creek	Fayette	Thurmond	Pauley 1993
Pseudacris feriarum	Dryers Cave	Hardy	__	CM 36502-36507
Family Ranidae				
Rana catesbeiana	Buckeye Creek Cave	Greenbrier	Williamsburg	Grady in Garton et al. 1993
Rana catesbeiana	Organ Cave System	Greenbrier	Ronceverte	CM 29422; Grady in Garton et al. 1993
Rana catesbeiana	Greenville Saltpeter Cave	Monroe	Alderson	WVBS 4240
Rana catesbeiana	Rehoboth Church	Monroe	Union	M. Osbourn (personal observation 2003)
Rana catesbeiana	Entrance to unknown cave, Cave Mountain	Pendleton	__	CM 10106
Rana clamitans	Entrance to Kaymoor Mine #1	Fayette	Fayetteville	Pauley 1993; WVBS 6582-6585
Rana clamitans	Head of Buffalo Creek;Old mine and small creek	Fayette	Thurmond	Pauley 1993
Rana clamitans	Mine portal and road puddle; Road between Keeney Creek and Short Creek	Fayette	Fayetteville	Pauley 1993

Table 2-3. Continued.

Species	Locality	County	USGS Quad	References
Rana clamitans	Mine portal N. of Ames	Fayette	Fayetteville	Pauley 1993; WVBS 6336
Rana clamitans	Mine with running water; Rush Run area	Fayette	Thurmond	Pauley 1993
Rana clamitans	Buckeye Creek Cave	Greenbrier	Williamsburg	Carey 1973, Cooper 1960
Rana clamitans	General Davis Cave	Greenbrier	Asbury	M. Osbourn (personal observation 2002)
Rana clamitans	Hinkle-Unus Cave	Greenbrier	Williamsburg	WVBS 3264
Rana clamitans	Organ Cave System	Greenbrier	Ronceverte	CM 14322; Reese 1934, Carey 1973
Rana clamitans	Rehoboth Church	Monroe	Union	M. Osbourn (personal observation 2002 and 2003)
Rana clamitans	Overholt's Blowing Cave	Pocahontas	Marlinton	Cooper 1960, Carey 1973
Rana palustris	Whitings Neck Cave	Berkeley	__	Carey 1973
Rana palustris	Old mine; N. of Ames	Fayette	Fayetteville	Pauley 1993
Rana palustris	Biggers Cave	Greenbrier	Williamsburg	Rosevear in Ashbrook 1995
Rana palustris	Buckeye Creek Cave	Greenbrier	Williamsburg	Cooper 1960, Carey 1973
Rana palustris	General Davis Cave	Greenbrier	Asbury	M. Osbourn (pers. obsv. 2003)
Rana palustris	Higginbotham Cave	Greenbrier	Williamsburg	CM 37615
Rana palustris	House Cave, Higganbotham Farm	Greenbrier	Williamsburg	WVBS 2218-2219
Rana palustris	Ludington Cave	Greenbrier	Williamsburg	M. Osbourn (pers. obsv. 2003)
Rana palustris	McClung's Cave	Greenbrier	Williamsburg	M. Osbourn (pers. obsv. 2002)
Rana palustris	Richlands Cave	Greenbrier	Lewisburg	Rosevear in Ashbrook 1995
Rana palustris	Schoolhouse Cave	Greenbrier	__	Wilson 1946
Rana palustris	US 219 Cave	Greenbrier	Williamsburg	M. Osbourn (pers. obsv. 2002)
Rana palustris	Crossroads Cave	Monroe	Alderson	Cooper 1960, Carey 1973
Rana palustris	Rehoboth Church	Monroe	Union	M. Osbourn (pers. obsv. 2002 and 2003)
Rana pipiens	Organ Cave System	Greenbrier	Ronceverte	Rutherford and Handley 1976
Rana pipiens	Rehoboth Church Cave	Monroe	Union	WVBS 14818; M. Osbourn (pers. obsv. 2003)
***Rana* sp.**	McFerrin Breakdown Cave	Greenbrier	Williamsburg	Grady in Garton et al. 1993
***Rana* sp.**	Piercey's Cave	Greenbrier	Asbury	H. H. Hobbs, pers. comm.
Rana sylvatica	Organ Cave System	Greenbrier	Ronceverte	Reese 1934

Table 2-4. Classifications of amphibians and reptiles in West Virginia caves and abandon coal mines and their total occurrence records. Cavernicole classifications are; Accidental (AC), Trogloxene (TX), Troglophile (TP), and Troglobite (TB).

Taxa	Cavernicole Classification	Total Cave Localities	Total Mine Localities	Total Localities
Order Caudata				
Family Ambystomatidae				
Ambystoma jeffersonianum, Jefferson Salamander	AC	6	0	6
Family Salamandridae				
Notophthalmus viridescens, Red-spotted Newt	AC	6	7	13
Family Plethodontidae				
Aneides aeneus, Green Salamander	TX *sandstone	1 sandstone cave	0	1
Desmognathus fuscus, Northern Dusky Salamander	AC or TX	23	4	27
Desmognathus monticola, Seal Salamander	AC	3	6	9
Desmognathus ochrophaeus, Allegheny Mountain Dusky Salamander	AC or TX	24	3	27
Desmognathus quadramaculatus, Black-bellied Salamander	AC	0	1	1
***Desmognathus* sp.**	AC or TX	4	0	4
Eurycea bislineata / E. cirrigera, Two-lined Salamander Complex	AC or TX	12	2	14
Eurycea longicauda, Long-tailed Salamander	TX	31	9	40
Eurycea lucifuga, Cave Salamander	TP	113	12	125
***Eurycea* sp.**	AC or TX	0	1	1
Gyrinophilus porphyriticus, Spring Salamander	TP	100	2	102
Gyrinophilus subterraneus, West Virginia Spring Salamander	TB	1	0	1
Hemidactylium scutatum, Four-toed Salamander	AC	1	0	1
Plethodon cinereus, Eastern Red-backed Salamander	AC or TX	7	0	7
Plethodon glutinosus, Northern Slimy Salamander	TX	24	8	32

Table 2-4. Continued.

Taxa	Cavernicole Classification	Total Cave Localities	Total Mine Localities	Total Localities
_***Plethodon hoffmani***, Valley and Ridge Salamander	AC	6	0	6
Plethodon kentucki, Cumberland Mountain Salamander	TX	0	1	1
Plethodon richmondi, Southern Ravine Salamander	AC	2	0	2
Plethodon wehrlei, Wehrle's salamander	TX	19	5	24
Pseudotriton montanus diastictus, Midland Mud Salamander	AC	1 sandstone cave	0	1
Pseudotriton ruber, Northern Red Salamander	AC or TX	8	1	9
Order Anura			1	
Family Bufonidae				
Bufo americanus, American Toad	AC or TX	4	1	5
***Bufo* sp.**		2	0	2
Family Hylidae				
Hyla chrysoscelis, Cope's Gray Treefrog	AC	0	1	1
Pseudacris brachyphona, Mountain Chorus Frog	AC	0	1	1
Pseudacris crucifer, Northern Spring Peeper	AC	0	1	1
Pseudacris feriarum, Upland Chorus Frog	AC	1	0	1
Family Ranidae				
Rana catesbeiana, American Bullfrog	AC	4	1	5
Rana clamitans, Northern Green Frog	AC	7	5	12
Rana palustris, Pickerel Frog	TX	14	1	15
Rana pipiens, Northern Leopard Frog	AC	2	0	2
Rana sylvatica, Wood Frog	AC	1	0	1
***Rana* sp.**	AC	2	0	2

Table 2-5. Reptile encounter records for West Virginia caves and abandoned coal mines.

Species	Locality	County	USGS Quad	References
Order Squamata				
Suborder Sauria				
Family Iguanidae				
Sceloporus undulatus	Coal mine; SW of Lansing	Fayette	Fayetteville	Pauley 1993
Sceloporus undulatus	Old coal mine; McKendree Rd, S of Claremont	Fayette	Thurmond	Pauley 1993
Family Scincidae				
Eumeces fasciatus	Abandoned mine at Stone Cliff	Fayette	__	Pauley 1993; WVBS 5170
Eumeces fasciatus	Entrance to Kaymoor Mine #1	Fayette	Fayetteville	Pauley 1993
Eumeces laticeps	Mine portal; S of South Nuttall	Fayette	Fayetteville	Pauley 1993
Suborder Serpentes				
Family Colubridae				
Carphophis a. amoenus	Mine site with tipple; W of Short Creek	Fayette	Fayetteville	Pauley 1993
Carphophis a. amoenus	Old strip mine with two small portals, S. of Keeney Creek	Fayette	Fayetteville	Pauley 1993
Carphophis a. amoenus	Organ Cave	Greenbrier	Ronceverte	WVBS 568
Coluber constrictor	Head of Buffalo Creek;Old mine and small creek	Fayette	Thurmond	Pauley 1993
Diadophis punctatus	Elverton Mine; At portal	Fayette	Fayetteville	Pauley 1993; WVBS 7861
Elaphe alleghaniensis	River (Indian) Cave, near entrance	Berkeley	Williamsport	Cooper 1960
Elaphe alleghaniensis	Buckeye Creek Cave	Greenbrier	Williamsburg	Balfour in Garton et al. 1993
Elaphe alleghaniensis	Lost World Caverns (Grapevine)	Greenbrier	Lewisburg	Carey 1973
Elaphe alleghaniensis	Norman Cave	Greenbrier	Droop	Longenecker 2000.
Nerodia sipedon	Cave Farm Cave	Greenbrier	Anthony	T. Jones pers. Comm.
Nerodia sipedon	Organ Cave System	Greenbrier	Ronceverte	CM 14324
Thamnophis sirtalis	Higginbotham Cave	Greenbrier	Williamsburg	Balfour in Garton et al. 1993
Family Viperidae				
Agkistrodon contortrix mokasen	Coal mine air shaft; S. of Fayette; at coal mine air shaft	Fayette	Fayetteville	Pauley 1993

Table 2-5. Continued.

Species	Locality	County	USGS Quad	References
Crotalus horridus	Jone's Quarry Cave	Berkeley	__	CM 35929
Crotalus horridus	Sites Cave	Pendleton	Circleville	Grady in Garton et al. 1993
Order Testudines				
Family Chelydridae				
Chelydra serpentina	The Hole	Greenbrier	Anthony	D. Boyer pers. comm. 2002
Family Emydidae				
Terrapene carolina	Ludington Cave	Greenbrier	Williamsburg	M. Osbourn (pers. obsv. 2002)
Terrapene carolina	Pilgrim Rest Church Cave #2	Greenbrier	Williamsburg	M. Osbourn (pers. obsv. 2002)
Terrapene carolina	Steeles Cave	Monroe	Alderson	H. H. Hobbs, pers. comm.

Table 2-6. Classifications of reptiles in West Virginia caves and abandon coal mines and their total occurrence records. Cavernicole classifications are; Accidental (AC), Trogloxene (TX), Troglophile (TP), and Troglobite (TB).

Taxa	**Cavernicole Classification**	**Total Cave Localities**	**Total Mine Localities**	**Total Localities**
Order Squamata				
Suborder Sauria				
Family Scincidae				
Eumeces fasciatus, Common Five-lined Skink	AC	0	1	1
Eumeces laticeps, Broad-headed Skink	AC	0	1	1
Family Iguanidae				
Sceloporus undulatus, Eastern Fence Lizard	AC	0	2	2
Suborder Serpentes				
Family Viperidae				
Agkistrodon contortrix mokasen, Northern Copperhead	AC or TX	0	1	1
Crotalus horridus, Timber Rattlesnake	AC or TX	2	0	2
Family Colubridae				
Carphophis amoenus, Eastern Wormsnake	AC	1	2	3
Coluber constrictor, Northern Black Racer	AC	0	1	1
Diadophis punctatus, Northern Ring-necked Snake	AC	0	1	1
Elaphe alleghaniensis, Eastern Ratsnake	AC or TX	4	0	4
Nerodia sipedon, Common Watersnake	AC	2	0	2
Thamnophis sirtalis, Eastern Gartersnake	AC	1	0	1
Order Testudines				
Family Chelydridae				
Chelydra serpentina, Eastern Snapping Turtle	AC	1	0	1
Family Emydidae				
Terrapene carolina, Eastern Box Turtle	AC	3	0	3

Table 2-7. Diversity of amphibians and reptiles at 28 West Virginia caves with the greatest numbers of species encounter records.

Locality	County	Total	Amphibians	Salamanders	Anurans	Reptiles
Organ Cave	Greenbrier	14	12	8	4	2
Buckeye Creek Cave	Greenbrier	13	12	9	3	1
Rehoboth Church	Monroe	10	10	5	5	0
Ludington Cave	Greenbrier	10	9	7	2	1
General Davis Cave	Greenbrier	8	8	6	2	0
Higginbotham Cave #1	Greenbrier	8	7	6	1	1
Norman Cave	Greenbrier	8	7	6	1	1
Lost World Caverns (Grapevine)	Greenbrier	7	6	6	0	1
US 219 Cave	Greenbrier	6	6	5	1	0
Richlands Cave	Greenbrier	6	6	5	1	0
Hinkle-Unus Cave	Greenbrier	5	5	4	1	0
McClung's Cave	Greenbrier	5	5	4	1	0
Water Trough Cave	Greenbrier	5	5	4	1	0
Overholt's Blowing Cave	Pocahontas	5	5	4	1	0
Higginbotham Cave #2	Greenbrier	5	5	5	0	0
Steele's Cave	Monroe	5	4	4	0	1
Hannah Water Cave	Greenbrier	4	4	4	0	0
Rapp's Cave	Greenbrier	4	4	4	0	0
John Brown's Cave	Jefferson	4	4	4	0	0
Scott Hollow Cave	Monroe	4	4	4	0	0
Thorn Mountain Cave	Pendleton	4	4	4	0	0
Bowden Cave	Randolph	4	4	4	0	0
Marshall Cave	Randolph	4	4	4	0	0
Biggers Cave	Greenbrier	4	4	3	1	0
House Cave, Higginbotham Farm	Greenbrier	4	4	3	1	0
Greenville Saltpeter Cave	Monroe	4	4	3	1	0
Cave Farm Cave	Greenbrier	4	3	3	0	1
The Hole	Greenbrier	4	3	3	0	1

Figure 2-1. Karst regions of West Virginia and cave fauna regions as described by Holsinger et al. (1976).

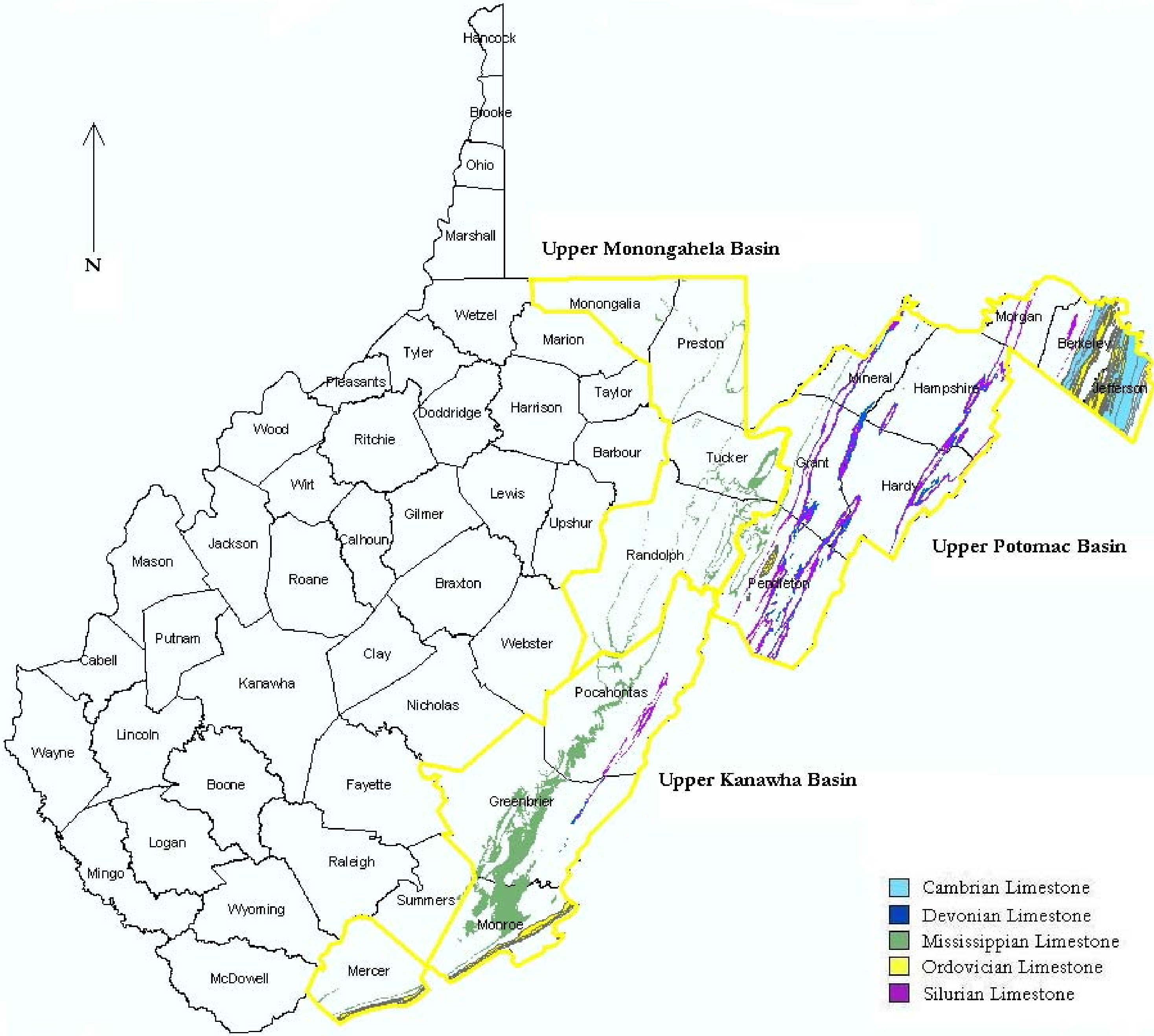
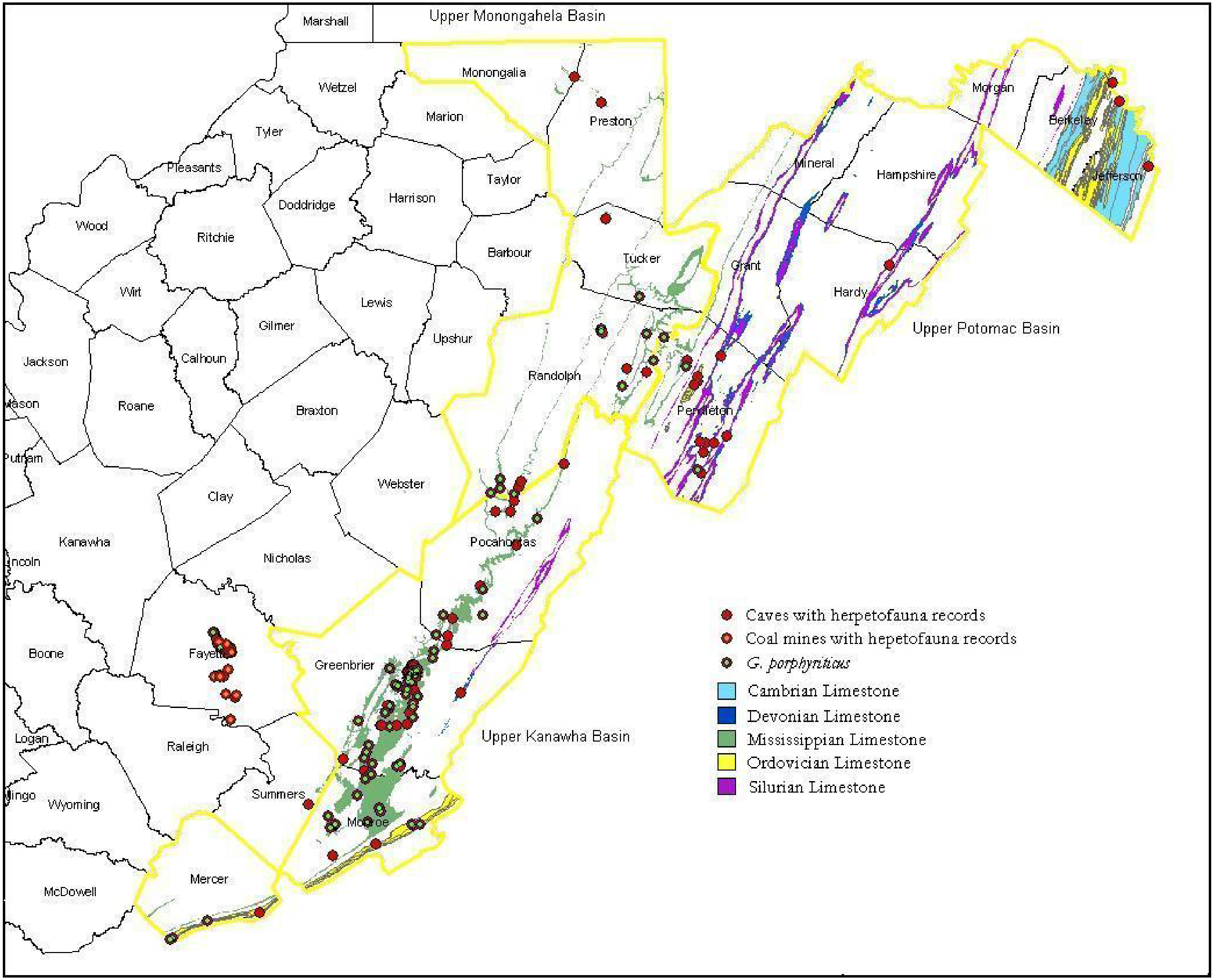

N
Upper Monongahela Basin
Upper Potomac Basin
Upper Kanawha Basin
Hancock
Brooke
Ohio
Marshall
Wetzel
Monongalia
Marion
Preston
Tyler
Pleasants
Doddridge
Harrison
Taylor
Wood
Ritchie
Barbour
Tucker
Wirt
Lewis
Gilmer
Upshur
Calhoun
Jackson
Mason
Roane
Braxton
Randolph
Putnam
Cabell
Clay
Webster
Kanawha
Pocahontas
Nicholas
Lincoln
Wayne
Boone
Fayette
Greenbrier
Logan
Mingo
Raleigh
Summers
Wyoming
Monroe
Mercer
McDowell
Morgan
Berkeley
Jefferson
Mineral
Hampshire
Grant
Hardy
Pendleton
Cambrian Limestone
Devonian Limestone
Mississippian Limestone
Ordovician Limestone
Silurian Limestone

Figure 2-2. Entrance to Ludington Cave, a typical stream cave inventoried in Greenbrier County, West Virginia.

Figure 2-3. Distribution of caves were amphibians and reptiles have been encountered.


Upper Monongahela Basin
Upper Potomac Basin
Upper Kanawha Basin
Marshall
Monongalia
Wetzel
Preston
Marion
Tyler
Pleasants
Taylor
Harrison
Doddridge
Wood
Ritchie
Barbour
Tucker
Wirt
Lewis
Gilmer
Upshur
Calhoun
Jackson
Randolph
Roane
Braxton
Putnam
Webster
Clay
Kanawha
Pocahontas
Nicholas
Boone
Greenbrier
Logan
Raleigh
Summers
Wyoming
Monroe
Mercer
McDowell
Mineral
Hampshire
Morgan
Berkeley
Jefferson
Grant
Hardy
Pendleton
Caves with herpetofauna records
Coal mines with hepetofauna records
G. porphyriticus
Cambrian Limestone
Devonian Limestone
Mississippian Limestone
Ordovician Limestone
Silurian Limestone

Chapter III:

Systematics and Phenotypic Variation in Cave-Dwelling *Gyrinophilus* spp.

Introduction

Salamanders of the genus *Gyrinophilus* are large semi-aquatic members of the Plethodontidae family of lungless salamanders. *Gyrinophilus* belong to the subfamily Plethodontinae and tribe Hemidactyliini. They are distinguished by a combination of characters including; a tongue which is free all around, continuous vomerine and parasphenoid teeth, and in transformed individuals a distinctive light line (canthus rostralis) extending from the anterior corner of the eye to the nasolabial groove (Brandon 1967; Green and Pauley 1987). Currently there are 4 species of *Gyrinophilus* recognized by the Society for the Study or Amphibians and Reptiles (SSAR) (Descriptions in Appendix 2). One species, *G. porphyriticus,* has a wide geographic range, while the other 3, *G. palleucus*, *G. gulolineatus*, and *G. subterraneus* are limited to isolated cave populations (Powell et al. 1998; Duellman and Sweet 1999; Crother et al. 2000) (Figure 3-1).

Descriptions of Gyrinophilus

Spring Salamanders, *Gyrinophilus porphyriticus*, are one of the largest plethodontids reaching a record total length of 232 mm (Conant and Collins 1991). Recently transformed individuals have dorsal background coloration of salmon red with dark reticulations which become clouded with age leading to a deep purplish brown appearance in old specimens (Green and Pauley 1987). Adult *G. porphyriticus* (Figures 3-2) do not exhibit sexual dimorphism in body proportions or size (Hulse et al. 2001). A knife-like keel extends along the distal third of the tail, reflecting their strongly aquatic tendencies (Green and Pauley 1987). Morphological proportions of larval *G. porphyriticus* (Figure 3-3) are similar to adults, however they have external gills, proportionately smaller eyes, larger heads, and a more laterally compressed tailfin (Hulse et al. 2001).

Gyrinophilus porphyriticus can be found from southern Quebec to northern Alabama (Conant and Collins 1998). They are rarely found far from water and are commonly associated with cool springs, swift mountain streams, and caves (Green and Pauley 1987). Cooper and Cooper (1968) suggested that in the limestone regions of Kentucky, Virginia, and West Virginia that *G. porphyriticus* are more common underground than above. In some caves they have become genetically isolated, resulting in speciation (Brandon 1965; Lazell and Brandon 1962; McCrady 1954).

Tennessee Cave Salamanders, *Gyrinophilus palleucus*, are found in isolated cave populations in middle Tennessee , northern Alabama, and northwest Georgia (Figure 3-1) (Brandon 1967; Petranka 1998). They have reduced eyes, broad heads, truncated snouts, long bright red gills, and laterally compressed tails (Figure 3-4). Their skin is very lightly pigmented and appears pinkish from blood capillaries (Petranka 1998). Lazell and Brandon (1962) found evidence that *G. palleucus* reproduce as neotenic, gilled adults. Application of metamorphic agents in laboratory tests readily induced transformation in some individuals (Blair 1961; Dent and Kirby-Smith 1963; Yeatman 1967) and a few metamorphosed individuals have been found in nature (Brandon et al. 1986; Yeatman and Miller 1985). Transformed *G. palleucus* found in nature have all been reported as extremely thin and emaciated. They differ from transformed *G. porphyriticus* in their pale skin pigmentation, gaunt appearance, narrower anterior skull, histologically reduced eyes (~1.00 mm), and retaining the larval characteristic of an undivided premaxillia (Brandon et al. 1986; Yeatman and Miller 1985).

Currently there are 3 subspecies of *G. palleucus* recognized, although studies in progress may result in taxonomic reordering. The holotype specimen is a *G. p. palleucus,* Pale Salamander, described by McCrady (1954), from Sinking Cove Cave on the southern Cumberland Plateau of Tennessee. The Big Mouth Cave Salamander, *G. p. necturoides* was described by Lazell and Brandon (1962) and differ from *G. p. palleucus* in having a darkly spotted dorsum in adults and uniformly darker juveniles (Brandon 1967). The maximal snout-vent length is probably around 100 mm for *G. p. palleucus* neotenes and at least 105 for *G. p. necturoides* (Dent and Kirby-Smith 1963; Lazell and Brandon 1962). These 2 subspecies intergrade in caves in the Tennessee Valley in northern Alabama (Brandon 1965).

Gyrinophilus palleucus gulolineatus, was described from Berry Cave in eastern Tennessee by Brandon in 1965. In 1986 Brandon et al. stated that due to the morphological differences of this group they should be considered as a separate species. In 1991 and 1997 Collins followed, recommending *G. p. gulolineatus* be reclassified from a subspecies to a full species, *G. gulolineatus*. This reclassification was recognized by the Society for the Study of Amphibians and Reptiles (SSAR) (Crother et al. 2000), and has been published in several texts (Powell et al., 1998 and Duellman and Sweet, 1999). Berry Cave Salamanders, *G. gulolineatus*, inhabit only a few caves in the Ridge and Valley physiographic province of eastern Tennessee. In addition to geographic distribution, they are distinguishable from *G. palleucus* by a distinct stripe on the throat, darker pigmentation, wider head, more spatulate snout, and possibly reaching greater adult size (Brandon 1965) (Figure 3-5). Brandon (1965) reported the largest *G. gulolineatus* neotenic adult to have a snout-vent length of 136 mm, considerably larger than *G. palleucus* specimens. In contrast to transformed *G. palleucus*, the premaxillary bone of the transformed *G. gulolineatus* reported by Simmons (1976) was divided similar to adult *G. porphyriticus*. Throughout their range *G. palleucus* is found in isolated cave populations, parapatric to surface dwelling *G. porphyriticus* (Cooper and Cooper 1968). In Mud Flats Cave and Meade's Quarry Cave in Knox County, Tennessee, *G. gulolineatus* and *G. porphyriticus* are sympatric and there is evidence that suggests hybridization between the two species (Simmons 1975; Brandon et al. 1986; A. Wynn, pers. comm.).

Gyrinophilus in West Virginia

In West Virginia, the genus *Gyrinophilus* is represented by Spring Salamanders, *G. porphyriticus* and West Virginia Spring Salamanders, *G. subterraneus. Gyrinophilus porphyriticus* range throughout West Virginia, up to an elevation of 1279 meters (Green and Pauley 1987). There are two subspecies of Spring Salamanders in West Virginia. Northern Spring Salamanders, *Gyrinophilus porphyriticus porphyriticus* occur in the northeastern portion of the state and Kentucky Spring Salamanders, *Gyrinophilus porphyriticus duryi,* found in a small corner of southwestern West Virginia.. *Gyrinophilus p. porphyriticus* are robust salamanders with prominently defined canthus rostralis, ranging in color from salmon with dark reticulations to deep purplish brown. *Gyrinophilus p. duryi* differ in being relatively smaller, having a dorsum of salmon pink to brownish pink color with dark spots, lacking clouded reticulations (Green and Pauley 1987; Conant and Collins 1998). Throughout the central region of the state, there is a broad band of intergradation between the two subspecies (Figure 3-6) (Green and Pauley 1987). Intergrades, *G. p. porphyriticus x duryi,* appear superficially to have a blend of characteristics of the two local subspecies. It is important to note, however, that the concept of a subspecies is not universally agreed upon and the results of genetic analysis currently underway (A. Wynn, pers. comm.) may lump or split these taxa. According to Green and Brant (1966), spring salamanders are the most widespread and abundant salamanders living in West Virginia caves and are considered to be troglophiles, able to complete their life cycle either inside or outside of caves.

In 1977, Besharse and Holsinger first described the West Virginia Spring Salamander, *Gyrinophilus subterraneus*, as a new troglobitic species. They are only known from their type locality, General Davis Cave in Greenbrier County, West Virginia (Figure 3-7) (Green and Pauley 1987). General Davis is a large stream passage cave prone to frequent flooding. *Gyrinophilus subterraneus* are often found within and along the muddy banks of the cave stream, where they are thought to feed on small invertebrates (Conant and Collins 1998). They coexists microsympatrically with *G.*

porphyriticus, but these morphologically similar species can be distinguished in several ways (Petranka 1998). Larvae of *G. subterraneus* (Figure 3-8) are large and robust relative to *G. porphyriticus*, with pale pink skin and dark reticulations (Figure 3-3). Large larvae usually have two or three irregular lateral rows of pale yellow spots, which are absent in *G. porphyriticus*. *Gyrinophilus subterraneus* larvae also have reduced eyes, wider heads, and more premaxillary and prevomerine teeth (Besharse and Holsinger 1977).

Metamorphosed *G. subterraneus* (Figure 3-9) appear gaunt and retain the pale reticulate pattern and reduced eyes of the larvae. They also exhibit an osteological characteristic which further distinguishes them from *G. porphyriticus*. Upon metamorphosis *G. porphyriticus* typically develop a suture between the anterior rami of the premaxillae (Brandon 1966). This suture was not present in 5 out of 5 transformed *G. subterraneus* examined by Besharse and Holsinger (1977). An undivided premaxillia is a characteristic also observed in transformed *G. palleucus* and is evidence of paedomorphosis (Blair 1961; Dent and Kirby-Smith 1963; Brandon 1966; Yeatman 1967)

Besharse and Holsinger (1977) speculated that metamorphic transformation from larva to adult in *G. subterraneus* occurs after a snout-vent length (SVL) of greater than 95 mm has been reached. By comparison, transformation in local *G. porphyriticus* populations is presumed to occur after they have reached an SVL of 55-70 mm (Besharse and Holsinger (1977). The largest larval *G. subterraneus* examined are sexually mature, indicating the possibility of neoteny, however it is uncertain if individuals reproduce as gilled adults (Petranka 1998). Besharse and Holsinger (1977) found counts of premaxillary and vomerine teeth to be intermediate between *G. porphyriticus* and *G. palleucus*. They suggested that *G. subterraneus* may be less cave specialized than *G. palleucus* and therefore represent a transitional form between it and *G. porphyriticus*.

Despite their morphological differences, the validity of *G. subterraneus* as a distinct species has been disputed. *Gyrinophilus porphyriticus* has been described as a phenotypically "plastic" species

(Howard et al. 1984). Blaney and Blaney (1978) argued that the *G. subterraneus* represents "only one extreme in a highly variable population of *G. porphyriticus*." They stated that the color patterns in cave populations vary from dark, typical of surface populations to extremely pale. Furthermore, Blaney and Blaney (1978) claimed that eyes ranged from normal to reduced and non-functional. The population described as *G. subterraneus*, they argued, also exhibits varying degrees of neoteny and appears to be transitional, with complete speciation having not yet occurred. According to Green and Pauley (1987), the evidence to demonstrate differences in the life history of *G. subterraneus* and *G. porphyriticus* has yet to be determined.

Life History and Troglobitic Speciation in Hemidactyliines

In addition to *Gyrinophilus*, Hemidactyliini encompasses the genera *Eurycea, Hemidactylium, Pseudotriton, Stereochilus, Typhlotriton, Typhlomolge,* and *Haideotriton.* Hemidactyliini is considered to be a morphologically conservative taxon lacking specialized morphological adaptations of the other 3 Plethodontid lineages (Collazo and Marks 1994; Ryan and Bruce 2000). All hemidactyliines have a larval stage with most exhibiting a complex life cycle, contrasting them with other members of Plethodontinae (Wilber 1980). A complex life cycle is considered to be ancestral in the family Plethodontidae and while members of the subfamily Desmognathinae also share this characteristic they are considered more evolutionarily derived (Schwenk and Wake 1993; Wake 1966). Based on morphology and life history, Hemidactyliini is believed to most closely reflect the ancestral developmental pattern of plethodontids and retain more ancestral traits than other lineages. *Gyrinophilus porphyriticus* is widely considered to be the most ancestral plethodontid because of their retention of many ancestral features and habitation of Appalachian mountain brooks believed by Dunn (1926) to be the location of origin of the family (Beachy and Bruce 1992; Bruce 1969; Collazo and Marks 1994; Wake 1966).

While the tribe Hemidactyliini is characterized as having conservative morphology, it exhibits great variations in life history. The larval period of *Hemidactylium scutatum*, for example, is only 1-2 months compared to 5 years in *G. porphyriticus* (Ryan and Bruce 2000). Also, the only examples of paedomorphosis within the family exist in Hemidactyliini (Chippindale 1995; Ryan and Bruce 2000; Wake 1966). Ryan and Bruce (2000) argued that generalized morphology and complex life cycle facilitates greater morphogenetic plasticity in hemidactyliines than any other plethodontid group, and has promoted adaptation to a broad range of habitats. According to Ryan and Bruce (2000) heterochronic changes in age of metamorphosis and maturation have lead to geographic and ecological expansion into areas uninhabitable to most plethodontids such as caves. With the exception of the European Cave Salamander, *Proteus anguinus*, all known troglobitic salamanders are hemidactyliines (Brandon 1971).

In cave-dwelling *Gyrinophilus*, neoteny or paedomorphosis via delayed metamorphosis appears to have evolved after cave colonization (Ryan and Bruce 2000). *Gyrinophilus porphyriticus* are facultative cave-dwellers, troglophiles, while *G. palleucus*, *G. gulolineatus*, and *G. subterraneus* are obligate cave-dwellers, troglobites (Bar 1968; Brandon 1971; Besharse and Holsinger 1977). Bruce (1979) argued that metamorphosis in *G. palleucus* was delayed as an adaptation to insufficient food in the terrestrial cave environment and to compensate for the energetically taxing morphological rearrangement and niche shift. A similar change in timing appears to have evolved independently in *G. subterraneus*. *Gyrinophilus subterraneus* exhibits a life history pattern intermediate between *G. porphyriticus* and *G. palleucus*, metamorphosing at an exceedingly large size and showing evidence in some specimens of reproductive maturity while still untransformed. Besharse and Holsinger (1977) reported male larvae with pigmented testes and female larvae with enlarged and convoluted oviducts indicating a trend toward paedomorphosis. The robust, larger appearance of *G. subterraneus* larvae compared to the gaunt, sometimes emaciated appearance of transformed individuals implies that

Bruce's (1979) insufficient terrestrial resources theory may have application to *G. subterraneus*. In addition to food availability, a prolonged or permanent larval stage may also be encouraged by the absence of predators in the cave environment (Ryan and Bruce 2000).

Adaptation to the cave environment has resulted in a striking convergence of morphology in troglobitic organisms (Christiansen 1992). Some of the same trends exemplified in cave invertebrates and cave fish are also evident in troglobitic salamanders (Brandon 1971; Culver et al. 1995). Brandon (1971) outlined the troglomorphic trends that occur in cave obligate salamanders. Troglobitic salamanders generally trend towards increasingly rigid paedomorphosis, greater tooth counts, reduced eyes, reduced number of trunk vertebrae, decreased body pigmentation, broadening of the head and flattening of the snout, and elongation and attenuation of limbs (Brandon 1971). In addition to morphological convergence there is evidence, though less documented, of physiological and ecological adaptive trends in cave organisms (Poulson 1964; Culver et al. 1995). *Gyrinophilus* exhibit a lesser degree of cave specialization than do other troglobitic salamanders such as the highly adapted *Haideotriton wallacei* (Brandon 1971).

Genetic Isolation

Paedomorphosis can facilitate speciation through diminished courtship success due to mechanical incompatibilities between transformed and untransformed perennibranchiate individuals (Ryan and Semlitsch 1998). Also, strictly aquatic salamanders are further isolated by there inability to disperse overland (Shaffer and Breden 1989). The result may be reduced gene flow from source populations and random genetic drift in isolated cave populations. Further genotypic diversification may occur as populations undergo adaptive pressures of the cave environment such as sparse nutritional resources and absence of light (Bruce 1979; Ryan and Bruce 2000). In *Gyrinophilus*, paedomorphosis accompanied by isolation in cave environments, may have lead to rapid macroevolutionary change (Ridley 1996; Ryan and Bruce 2000).

A useful technique for discerning the degree of genetic isolation of populations is electrophoresis. It is particularly useful when examining phenotypically "plastic" species such as *G. porphyriticus* (Howard et al. 1984). Electrophoresis separates proteins making it possible to identify the unique allozymes which mark a population. Howard et al. (1984) used electrophoresis to compare *G. subterraneus* to *G. porphyriticus* populations from General Davis Cave, adjacent Muddy Creek, inside and outside Harmon Waterfall Cave, and Bear Heaven Cave. Their results revealed 6 alleles unique to *G. subterraneus* not found in the *G. porphyriticus* examined. They concluded that *G. subterraneus* is a unique population, isolated from *G. porphyriticus* and probably a valid species. Unfortunately, due to marginal sample size, Howard et al. (1984) felt their results where not conclusive. Analysis of mitochondrial DNA using PCR (Polymerase Chain Reaction) is a very powerful tool for discerning genetic differences between groups and should provide more definitive results. Recent unpublished genetic analysis by Addison Wynn of the Smithsonian Institution, may clarify the status of the General Davis Cave *Gyrinophilus* population.

Objectives

The principal objectives of this study are to document the phenotypic variability among cave-dwelling *Gyrinophilus* species and determine which morphological characters are useful for separating groups. Specifically, this investigation should provide additional insight into the degree of morphological variation between *G. subterraneus* and *G. porphyriticus*. I am attempted to determine if external morphological differences described by Besharse and Holsinger (1977) and any additional ones, are sufficient to separate *G. subterraneus* and *G. porphyriticus* in multivariate statistical analysis. Due to the extreme rarity of these troglobitic species, collection of tissue from living specimens was not was not advisable for this study. Without genetic information a morphometric analysis can only establish the degree of phenotypic variability between these groups, as it is expressed in external morphology. While these methods cannot definitively answer the taxonomic challenge to *G. subterraneus* raised by Blaney and Blaney (1978), they can help to quantify and analyze their assertion that *G. subterraneus* is only one extreme variant in a highly variable population of *G. porphyriticus*.

It is possible that hybridization be occur between the microsympatric populations of *G. subterraneus* and *G. porphyriticus* at General Davis Cave. If this is the case then perhaps external morphology of sympatric *G. porphyriticus* may differ detectably from other cave populations. A comparison between General Davis *G. porphyriticus* and *G. porphyriticus* from other caves is necessary to address this question.

Finally, a comparison of gilled larval *G. subterraneus* and *G. porphyriticus*, and neotenic cave obligate *G. palleucus* and *G. gulolineatus* is needed to illustrate the variability that exists along the full gradient from troglobitic to troglophilic *Gyrinophilus* species. *Gyrinophilus subterraneus* appears in terms of life history and morphology to be more cave adapted than *G. porphyriticus*, but not to the extent of *G. palleucus* or *G. gulolineatus*. Results of this investigation may indicate that *G. subterraneus* is morphologically intermediate between the other troglobitic species and the less specialized and widespread *G. porphyriticus.* An additional objective is to compare *G. palleucus* and *G. gulolineatus* in order to demonstrate whether there is sufficient variability to support Collin's 1991 recommendation to elevate *G. palleucus gulolineatus* to species status.

Methods and Materials

Morphological Character Measurements

Twenty linear distance measurements of external morphological characters (Table 3-1) were recorded for each specimen. Linear distance measurements have been used to measure morphological variability within and among populations of similar groups (e.g., Adams and Beachy 2001; Seidel et al. 1999; Brandon 1966). Characters measured include snout vent length (SVL) from snout tip to posterior margin of cloaca, tail length (TL) from posterior margin of cloaca to tip of tail, gape width (GW) of widest span of the jaw, cranial length (CLV) measured ventrally from tip of snout to midpoint of gular fold, cranial length (CLL) measured laterally from tip of snout to fold posterior to articulation of jaw, cranial width (CW) at articulation of jaw, cranial width (CWG) at bulge anterior to gular fold in adults and at insertion of gill rami in larval and neotenic individuals, cranial depth (CD) vertical depth of head at articulation of jaw, eye to nostril (EN) length from anterior corner of eye to nostril, snout length (SL) from tip of snout to midpoint between eyes, interorbital distance (IOD) between anterior corners of eyes, internasal distance (IND) between nostrils, trunk width (TRW) posterior to axilla, trunk length (TRL) between axilla and groin, trunk height (TRH) vertical height of trunk posterior to axilla, tail width (TLW) posterior to insertion of posterior limbs, tail height (TLH) vertical height of tail posterior to insertion of posterior limbs, humerus length (HUL) from axilla to knee of left anterior limb, femur length (FL) from groin to knee of left posterior limb, and eye diameter (ED) measured from anterior to posterior corners of the left eyelid in adults and anterior to posterior margins of pigmented left eye in larval or neotenic individuals (Table 3-1 and Figure 3-10). Fowler Promax digital calipers were used for 19 measurements and for greater precision an ocular micrometer was used with a dissecting scope at 16x magnification to determined eye diameter. All measurements were recorded to the nearest 0.01 mm.

Study Specimens

For morphometric analysis, 106 specimens of cave dwelling *Gyrinophilus* spp. were measured, including 4 adult and 11 larval *G. subterraneus,* 33 adult and 24 larval *G. porphyriticus*, 1 transformed and 20 neotenic *G. palleucus palleucus*, and 6 neotenic *G. gulolineatus*. Measurements were taken of 81 specimens from the Smithsonian National Museum of Natural History (USNM), 22 from the West Virginia Biological Survey collection at Marshall University (WVBS), and from 3 specimens from the personal collection of Dr. Michael E. Seidel (MES) (Table 3-2 and Figure 3-11).

In an attempt to address to problem of low sample size, an additional 6 *G. subterraneus* and 17 *G. porphyriticus* adults from General Davis Cave were incorporated. These measurements were taken in the field on live specimens and limited to 5 morphological characters. Since these data were collected with different methods there results are treated separately from the analyses containing only museum specimens with 20 character measurements.

Statistical Analysis

For statistical analysis SAS software was used for analysis of variance (ANOVA), t-Test's (least significant difference), canonical discriminant analysis (CDA), and principal components analysis (PCA). Principal components analysis (PCA) is a powerful tool for distinguishing groups without bias toward an individual's taxonomic label. Due to the limited number of specimens available for rare taxa, it was impractical to choose only specimens from the same size class. Character measurements are usually highly correlated with body size, so any statistical differences between groups using unadjusted data could be the result of specimen body size differences and not other phenotypic variation (Adams and Beachy 2001). Canonical discriminant analysis results and principalcomponent 1 (PC 1) in PCA are heavily affected by body size (pers. comm. M. E. Seidel). To account for the influence of specimen body size 2 data sets were prepared for both larval and transformed individuals. For CDA, character measurements were converted to ratios with SVL. In PCA, the heavily size-influenced principal component 1 was ignored and only principal components 2 and 3 were analyzed. The risk with eliminating specimen size variation, however, is that average body size could possibly be a distinguishing characteristic between taxa, as suggested by Brandon (1965) and Besharse and Holsinger (1977). As a precaution, I have reported both size-adjusted ratios and unmodified results for characters measured. These analyses are based on 5 SAS statistical runs which are detailed in Table 3-3.

Skeletal Anatomy

Focus of this analysis is on external morphological features however a few observations were made of skeletal anatomy. A characteristic feature of *G. porphyriticus* is the appearance at metamorphosis of a suture between the anterior rami of the premaxillae (Grobman 1959; Martof and Rose 1962). Besharse and Holsinger (1977) reported that 5 of 5 metamorphosed *G. subterraneus* examined did not exhibit a suture. This apparent paedomorphic trait is similar to that found in transformed *G. palleucus* (Dent and Kirby-Smith 1963; Blair 1961). To test for the absence of a suture I was aided by Addison Wynn of the Smithsonian Institute. We used a digital X-ray machine at the U. S. Museum of Natural History to view skeletal anatomy of selected USNM specimens of *G. subterraneus* and *G. porphyriticus*.

Results

Metamorphosed *Gyrinophilus*

Multivariate Analysis

Principal Components Analysis of metamorphosed individuals revealed no separation between *G. porphyriticus* from General Davis Cave and those from other caves. *Gyrinophilus subterraneus,* however, exhibited clear separation from *G. porphyriticus* (Figure 3-12) (see Run 1 description in Table 3-3). Most separation between *G. subterraneus* and *G. porphyriticus* occurred along the PC 3 axis. Eye diameter is the most heavily weighted character on PC 3 with a coefficient value (eigenvector value) of 0.72 (Table 3-4). There were similar results, in the second PCA (Run 3 in Table 3), which was augmented with additional data collected from live specimens in the field. Again, there was strong overlap among the *G. porphyriticus* groups and clear separation with no overlap between *G. porphyriticus* and *G. subterraneus* (Figure 3-13). Most separation in this analysis occurred along the PC 2 axis. As in the first PCA, eye diameter is the dominant character loaded on the axis separating the taxa with a coefficient value (eigenvector value) of 0.93 (Table 3-5). Canonical discriminant analysis (Run 2 in Table 3-3) of metamorphosed specimens also revealed *G. subterraneus* clustering in a distinct group from *G. porphyriticus* (Figure 3-14).

Statistical Analysis of Morphological Characters

Characters useful for delineating groups where determined by comparing taxa character means using t-Test, corrected for error with ANOVA. Statistical Run 1 (see Table 3-3 for description) of metamorphosed *Gyrinophilus* found the characters listed in Table 3-7 to be significant ($P \leq 0.05$) for differentiating taxa. Correlation coefficients for nearly all character variables were highly correlated to SVL (Table 3-6). This correlation with specimen size is unadjusted for in Run 1. A more useful comparison of means may be the ratio values of Run 2 listed in Table 3-8. Results of comparing ratio values of the 20 characters analyzed revealed that 40% (8) are useful for separating *G. subterraneus* from *G. porphyriticus* from General Davis Cave ($GYPO_1$), 35% (7) are useful for separating *G. subterraneus* from *G. porphyriticus* from other cave populations ($GYPO_2$), and 20% (4) are useful for separating $GYPO_1$ from $GYPO_2$ (Table 3-8). Eye diameter, which was the most heavily weighted character for separating *G. porphyriticus* and *G. subterraneus* in PCA is also an important distinguishing character by t-Test (Figure 3-15). *Gyrinophilus subterraneus* have significantly ($P \leq 0.05$) smaller eye diameter than do *G. porphyriticus*, in this analysis (Table 3-9 and Figure 3-16).

Gilled Larval and Neotenic *Gyrinophilus*

Multivariate Analysis of Taxa

Analysis of 4 taxa of gilled larvae and neotenic *Gyrinophilus* species demonstrated distinct grouping in PCA and CDA. Principal components analysis (Run 4 in Table 3-3) revealed distinct separation between *G. gulolineatus, G. palleucus,* and *G. porphyriticus*. Most separation occurred along the PC 2 axis, which is heavily weighted by eye diameter (component loading/coefficient value = 0.75) followed by snout length and eye to nostril distance (Table 3-10). The greatest separation was between *G. gulolineatus* and *G. porphyriticus*. *Gyrinophilus subterraneus* plotted between *G. palleucus* and *G. porphyriticus* in PCA, strongly overlapping both (Figure 3-17). Canonical discriminant analysis (Run 5 in Table 3-3) also revealed separation between *G. gulolineatus, G. palleucus,* and *G. porphyriticus*. *Gyrinophilus subterraneus,* however, strongly overlapped *G. palleucus* and very slightly overlapped *G. porphyriticus* in CDA (Figure 3-18).

Statistical Analysis of Morphological Characters

Comparing taxa character means using t-Test, corrected for error with ANOVA, revealed characters useful for discerning groups. The characters listed in Table 3-11 are significant for differentiating gilled larval and neotenic *Gyrinophilus* taxa ($P \leq 0.05$). Much of this variation (Run 4 in Table 3-3) is probably related to specimen size, as with transformed individuals the correlation coefficients for nearly all character variables were highly correlated to SVL (Table 3-6). Consequently, a more useful comparison of means should be the ratio values of Run 5 in Table 3-12. Gular cranial width (CWG) appears to be a good character for separating all taxon pairs. Eye diameter (ED) is the best character for distinguishing *G. porphyriticus* from the troglobitic species, however it is not useful for separating troglobites from each other. *Gyrinophilus porphyriticus* in this analysis have a significantly ($P < 0.05$) greater eye diameter (Table 3-13 and Figure 3-19). This is also apparent in the polygonal plot of selected characters (Table 3-14 and Figure 3-20) and in the bivariate plot of ED verses SVL for *G. porphyriticus* and *G. subterraneus* (Figure 3-21).

Of the 6 taxa comparisons, *G. porphyriticus* and *G. subterraneus* had the fewest significantly different characters with 7 out of 20 (35%). The greatest number of significantly different characters was 13 out of 20 (65%) for the *G. gulolineatus* and *G. porphyriticus* comparison (Table 3-12). The highest degree of morphological separation and perhaps level of cave specialization appears to exist between *G. gulolineatus* and *G. porphyriticus.*

Cranial measurements are the majority of significant characters separating *G. gulolineatus* and *G. palleucus* from each other and the other taxa. *Gyrinophilus gulolineatus* had a significantly (t-Test, $P \leq 0.05$) wider head in all 3 measurements of cranial width (CW, CWG, GW) and a longer snout (SL) than *G. palleucus* (Table 3-12). This trend is illustrated in a bivariate plots of CW and SL plotted against SVL (Figures 3-22 and 3-23).

Ratios of tail length (TL), ventral cranial length (CLV), and trunk height (TRH) were not significantly different for any taxa comparisons. Tail length was not a useful character in either ratios (Run 5) or raw values (Run 4). This was to be expected since salamander tails are often in various stages of regeneration and are greatly influenced by the amount of injuries and antagonistic encounters.

Skeletal Anatomy

X-ray images of selected *Gyrinophilus* from General Davis Cave were very useful for revealing skeletal anatomy. As predicted, all larvae examined did not exhibit a suture between the anterior rami of the premaxillae (Grobman 1959; Martof and Rose 1962; Besharse and Holsinger 1977). Concurrent with the findings of Besharse and Holsinger (1977), metamorphosed *G. porphyriticus* exhibited a suture while the anterior rami *G. subterraneus* clearly remained fused (Figure 3-24).

Discussion

In PCA, the lack of separation between metamorphosed *G. porphyriticus* from General Davis Cave and those from other locations indicates that the 20 characters analyzed were not sufficiently different to separate these groups. If hybridization is occurring in General Davis Cave with *G. subterraneus*, it has not resulted in substantial phenotypic changes in sympatric *G. porphyriticus*. It is worth noting however, that General Davis *G. porphyriticus* had fewer significantly different characters separating them from *G. subterraneus* than did *G. porphyriticus* from other populations (Table 3-7). No larval *G. porphyriticus* from General Davis Cave where available for a comparison.

The clear separation of metamorphosed *G. subterraneus* from *G. porphyriticus* in PCA (Figures 3-12 and 3-13) and CDA (Figure 3-14) suggests morphological divergence between these groups and supports Besharse and Holsinger's (1977) assertion that they are distinct. When comparing gilled larval and neotenic *Gyrinophilus,* however, the clustering in PCA (Figure 3-17) and CDA (Figure 3-18) is much less delineated. *Gyrinophilus subterraneus* larvae greatly overlap *G. porphyriticus* larvae and *G. palleucus* neotenes. Of the 11 *G. subterraneus* larvae analyzed 3 plotted within the *G. porphyriticus* cluster, 2 plotted within the *G. palleucus* cluster, and 6 plotted independently of other groups. The 6 independent *G. subterraneus* larvae separated from the other groups along the PC 3 axis on which eye diameter (0.58), snout length (0.35), and humerus length (-0.31) are the most heavily weighted values. Interestingly, *G. subterraneus* larvae plotted in an intermediate position between *G. porphyriticus* and *G. palleucus*. Besharse and Holsinger (1977) stated that *G. subterraneus* may be less specialized than *G. palleucus* because tooth counts are intermediate between *G. porphyriticus* and *G. palleucus*. Evidence of delayed metamorphosis and possibly reproductively mature gilled *G. subterraneus* may indicate a degree of transition in life history as well. Furthermore, *G. subterraneus* had the fewer significantly different characters separating them from *G. porphyriticus* than did the 2 other troglobitic *Gyrinophilus* (Table 3-12) suggesting that *G. subterraneus* are less derived. The intermediate plotting in PCA of external morphological characters could be additional evidence of the intermediate degree of adaptation of *G. subterraneus* inferred by Besharse and Holsinger (1977) and Ryan and Bruce (2000).

The results of CDA for gilled larval and neotenic *Gyrinophilus* also plotted *G. subterraneus* between *G. porphyriticus* and *G. palleucus*. The strong overlap of *G. subterraneus* and *G. palleucus* indicates a degree of morphological parallelism. This similar morphology probably evolved independently in response to similar ecological demands of the cave environment. Genetically, *G. subterraneus* and *G. palleucus* are each more closely related to *G. porphyriticus* than to each other (Besharse and Holsinger 1977), but perhaps due to homoplasy they have evolved similar morphologies.

In all PCA runs, eye diameter was the most heavily weighted character on the axis separating *G. porphyriticus* from *G. subterraneus*, *G. palleucus,* and *G. gulolineatus*. Eye diameter is significantly (*P*

<0.05) greater in *G. porphyriticus* than in its troglobitic congenerics. This has been well documented in previous studies (McCrady 1954; Dent and Kirby-Smith 1962; Lazell and Brandon 1962; Brandon 1965; Brandon 1966; Brandon 1967; Brandon 1971; Besharse and Brandon 1973; Besharse and Holsinger 1977). Reduction in eye size, structure, and function is often a characteristic of troglobitic animals (Barr 1968; Brandon 1971; Besharse and Brandon 1973). When compared to their closest epigean relatives all troglobitic salamanders have some degree of eye size reduction, however, not necessarily a reduction in eye function. Stone (1964) found that in *Typhlotriton spelaeus* most larvae and some adults retained optomotor response. Besharse and Brandon (1973) investigated the optomotor response of *G. palleucus* to movement in the visual field. They found that while *G. palleucus* eyes are reduced compared to *G. porphyriticus* eyes (McCrady 1954; Brandon 1966), 21 of 23 individuals tested demonstrated optomotor response. Apparently *G. palleucus* structural reduction has not proceeded far enough to prevent visual function. Compared to other troglobitic species *G. palleucus* is in the early stages of regression, exhibiting size reduction and some structural reduction similar to that in *Typhlotriton spelaeus* and troglobitic amblyopsid fish (Besharse 1972; Besharse and Brandon 1973). This small degree of structural reduction is not near the extent of *Typhlomolge rathbuni, Haideotriton wallacei,* or *Proteus anguinus* in which visual function is impaired (Besharse and Brandon 1973).

Besharse and Holsinger (1977) determined that the eyes of *G. subterraneus* larvae and adults are smaller than *G. porphyriticus* and similar to *G. palleucus* in size (Besharse and Brandon 1973; Brandon 1966). In addition to size reduction, the *G. subterraneus* eyes they examined exhibited structural reduction sufficient to impair visual function. Of the 14 *G. subterraneus* observed for optomotor response only half responded to movement in the visual field. Those that did respond only showed weak and erratic reactions. Cave-dwelling *G. porphyriticus* tested by Besharse and Holsinger (1977) all exhibited strong and immediate optomotor responses.

Many of the characteristics distinguishing metamorphosed *G. subterraneus* from *G. porphyriticus* appear to be retained larval characteristics. Reduced eyes, pale coloration, reticulated patterning, and absence of the formation of a suture in the anterior rami of the premaxillae all are evidence of paedomorphosis. If the majority of phenotypic differences between these groups are the result of paedomorphosis, the greatest divergence in morphology would be expected to occur in post metamorphic individuals. This may explain why these PCA results revealed greater separation of *G. subterraneus* and *G. porphyriticus* in transformed individuals.

Gyrinophilus palleucus and *G. gulolineatus* clustered independently of each other with no overlap in both PCA (Figure 3-17) and CDA (Figure 3-18). *Gyrinophilus gulolineatus* is described by Brandon (1965) as having a wider head with a more "spatulate" snout than *G. palleucus* and these results support that characterization. *Gyrinophilus gulolineatus* had a significantly (t-Test, $P \leq 0.05$) wider head in all 3 cranial width characters (CW, CWG, GW) and a longer snout (SL) than all other taxa. Brandon (1965) also speculated that *G. gulolineatus* reach greater adult size. While my sample size for *G. gulolineatus* is too low to be definitive (n=6), this does appear to be the trend. The mean SVL for *G. gulolineatus* in this study is 86.45 mm compared to 64.50 mm for *G. palleucus*. The distinct black chin stripe described in Powell et al. (1998) as the primary diagnostic characteristic was not used in this statistical analysis, however it was observed in all *G. gulolineatus* specimens.

Conclusions

The principal objectives of documenting the phenotypic variability among cave-dwelling *Gyrinophilus* species and determining morphological characters useful for separating groups, have been accomplished. Without a thorough genetic analysis, taxonomic status of *G. subterraneus* cannot be fully supported or invalidated. When the results of genetic studies now in progress are published we should have a clearer picture of the status of *G. subterraneus* (pers. comm. Addison Wynn). This morphometric study provides an expanded account of the morphological differences between these taxa. Differences between metamorphosed *G. subterraneus* and *G. porphyriticus* are sufficient enough for clear separation in PCA and CDA. Eye diameter appears to be the principal morphological character separating *G. porphyriticus* from *G. subterraneus* and the other troglobitic congenerics. While the results for transformed *G. subterraneus* seem to confirm them as distinct from *G. porphyriticus*, *G. subterraneus* larvae do not separate out in PCA as clearly. *Gyrinophilus subterraneus* larvae plotted in an intermediate position between *G. porphyriticus* and *G. palleucus*, overlapping both. The position of *G. subterraneus* between *G. porphyriticus* and *G. palleucus* may reflect their degree of cave adaptation with *G. palleucus* being more specialized and *G. porphyriticus* being less specialized.

Comparison of transformed General Davis Cave *G. porphyriticus* and those from other cave populations revealed complete overlap in PCA, indicating no substantial difference in external morphology. If hybridization occurs at General Davis Cave then phenotypic changes to *G. porphyriticus* are too subtle for these tests to illuminate. *Gyrinophilus gulolineatus* never overlapped any other group in PCA or CDA. As described by Brandon (1965) in their original description *G. gulolineatus* specimens in this study had wider heads and longer snouts when compared to other *Gyrinophilus* species, including *G. palleucus*. The distinct separation of *G. gulolineatus* and *G. palleucus* seems to support Collin's 1991 recommendation to elevate *G. palleucus gulolineatus* to a species. *Gyrinophilus porphyriticus*, *G. gulolineatus*, and *G. palleucus* all plotted independently of each other with no overlap. This study supports these 3 taxa as distinct groups based on external morphology. For *G. subterraneus*, however, the picture is not as definitive. When dentition, skeletal anatomy, and genetics are considered more thoroughly the status of *G. subterraneus* should be solidified. Regardless of taxonomic label, *G. subterraneus* is clearly a unique population requiring careful management and continued study to insure its survival.

Chapter III:

Tables and Figures

Table 3-1. Description of characters measured for *Gyrinophilus spp.* All measurements to 0.01 mm.

Characters	Description
Eye Diameter (ED)	diameter of pigmented eye
Interorbital Distance (IOD)	distance between anterior corners of eyes
Internasal Distance (IND)	distance between nostrils
Eye to Nostril (EN)	length from anterior corner of eye to nostril
Snout Length (SL)	length from tip of snout to midpoint between eyes
Cranial Length, Ventral (CLV)	length from tip of snout to middle of gular fold
Cranial Length, Lateral (CLL)	length from tip of snout to fold posterior to articulation of jaw
Cranial Width (CW)	cranial width at articulation of jaw
Gape Width (GW)	width of widest span of the jaw
Cranial Width, Gular (CWG)	cranial width at gular fold
Cranial Depth (CD)	vertical depth of head at articulation of jaw
Snout Vent Length (SVL)	length from snout to posterior margin of cloaca
Trunk Height (TRH)	vertical height of trunk posterior to axilla
Trunk Width (TRW)	trunk width posterior to axilla
Trunk Length (TRL)	distance between axilla and groin
Tail Length (TL)	length from posterior margin of cloaca to tip of tail
Tail Width (TLW)	width of tail posterior to insertion of posterior limbs
Tail Height (TLH)	vertical height of tail posterior to insertion of posterior limbs
Humerus Length (HUL)*	distance from axilla to knee of anterior limb
Femur Length (FL)*	distance from groin to knee of posterior limb

* External measurement, not skeletal.

Table 3-2. Museum specimens measured for morphometric analysis.

Species	Life Stage	Meas. ID	Collection Number [1]	Locality
G. subterraneus	Adult	1	USNM 198533	General Davis Cave, Greenbrier Co., WV
G. subterraneus	Adult	2	USNM 198541	General Davis Cave, Greenbrier Co., WV
G. subterraneus	Adult	7	USNM 198539	General Davis Cave, Greenbrier Co., WV
G. subterraneus	Adult	12	USNM 525271	General Davis Cave, Greenbrier Co., WV
G. porphyriticus	Adult	82	WVBS 4515	General Davis Cave, Greenbrier Co., WV
G. subterraneus	Larva	3	USNM 198535	General Davis Cave, Greenbrier Co., WV
G. subterraneus	Larva	4	USNM 198536	General Davis Cave, Greenbrier Co., WV
G. subterraneus	Larva	5	USNM 198540	General Davis Cave, Greenbrier Co., WV
G. subterraneus	Larva	6	USNM 198534	General Davis Cave, Greenbrier Co., WV
G. subterraneus	Larva	8	USNM 198538	General Davis Cave, Greenbrier Co., WV
G. subterraneus	Larva	9	USNM 198537	General Davis Cave, Greenbrier Co., WV
G. subterraneus	Larva	18	USNM 525279	General Davis Cave, Greenbrier Co., WV
G. subterraneus	Larva	19	USNM 525280	General Davis Cave, Greenbrier Co., WV
G. subterraneus	Larva	83	WVBS 4516	General Davis Cave, Greenbrier Co., WV
G. subterraneus	Larva	84	MES	General Davis Cave, Greenbrier Co., WV
G. subterraneus	Larva	85	MES	General Davis Cave, Greenbrier Co., WV
G. porphyriticus	Adult	10	USNM 5252278	General Davis Cave, Greenbrier Co., WV
G. porphyriticus	Adult	11	USNM 525273	General Davis Cave, Greenbrier Co., WV
G. porphyriticus	Adult	13	USNM 525274	General Davis Cave, Greenbrier Co., WV
G. porphyriticus	Adult	14	USNM 525276	General Davis Cave, Greenbrier Co., WV
G. porphyriticus	Adult	15	USNM 525275	General Davis Cave, Greenbrier Co., WV
G. porphyriticus	Adult	16	USNM 525277	General Davis Cave, Greenbrier Co., WV
G. porphyriticus	Adult	17	USNM 525272	General Davis Cave, Greenbrier Co., WV
G. porphyriticus	Adult	52	USNM 524956	Pigeon Cave, Walker Co., GA
G. porphyriticus	Adult	53	USNM 319460	Coffman Cave, Greenbrier Co., WV
G. porphyriticus	Adult	54	USNM 319461	Culverson Creek Cave, Greenbrier Co., WV
G. porphyriticus	Adult	55	USNM 319462	Organ-Hedricks Cave Syst., Grnbr. Co., WV
G. porphyriticus	Adult	56	USNM 319463	Organ Cave, Greenbrier Co., WV
G. porphyriticus	Adult	58	USNM 319465	Argobrites Cave, Monroe Co., WV
G. porphyriticus	Adult	59	USNM 319466	Argobrites Cave, Monroe Co., WV
G. porphyriticus	Adult	60	USNM 319467	Argobrites Cave, Monroe Co., WV
G. porphyriticus	Adult	61	USNM 319468	Fletcher Cave, Monroe Co., WV
G. porphyriticus	Adult	62	USNM 319469	Overholt Blowing Cave, Pocahontas Co., WV
G. porphyriticus	Adult	63	USNM 319470	Overholt Blowing Cave, Pocahontas Co., WV
G. porphyriticus	Adult	64	USNM 319471	Overholt Blowing Cave, Pocahontas Co., WV
G. porphyriticus	Adult	68	USNM 319475	Snedegars Cave, Pocahontas Co., WV
G. porphyriticus	Adult	69	USNM 319476	Snedegars Cave, Pocahontas Co., WV
G. porphyriticus	Adult	70	USNM 337470	Bransford Cave, Greenbrier Co., WV
G. porphyriticus	Adult	71	USNM 198542	General Davis Cave, Greenbrier Co., WV
G. porphyriticus	Adult	80	USNM ?	Overholt Blowing Cave, Pocahontas Co., WV
G. porphyriticus	Adult	81	USNM 525243	Unthanks Cave, Lee Co., VA
G. porphyriticus	Adult	86	WVBS 3262	Unknown cave, Greenbrier Co., WV
G. porphyriticus	Adult	87	MES	General Davis Cave, Greenbrier Co., WV

Table 3-2. continued.

Species	Life Stage	Meas. ID	Collection Number [1]	Locality
G. porphyriticus	Adult	91	WVBS 3261	Jewell Cave, Greenbrier Co., WV
G. porphyriticus	Adult	95	WVBS 3340	Benedict Cave, Greenbrier Co., WV
G. porphyriticus	Adult	96	WVBS 3348	McClung's Cave, Greenbrier Co., WV
G. porphyriticus	Adult	98	WVBS 4241	The Hole, Greenbrier Co., WV
G. porphyriticus	Adult	99	WVBS 3349	Unknown cave, Monroe Co., WV
G. porphyriticus	Adult	100	WVBS 3406	Taylor Cave #1, Greenbrier Co., WV
G. porphyriticus	Adult	101	WVBS 2455	Unus Cave, Greenbrier Co., WV
G. porphyriticus	Adult	102	WVBS 3347	Bowden Cave #2, Randolph Co., WV
G. porphyriticus	Adult	103	WVBS 3344	Bowden Cave #2, Randolph Co., WV
G. porphyriticus	Adult	104	WVBS 3345	Bowden Cave #2, Randolph Co., WV
G. porphyriticus	Adult	105	WVBS 3346	Bowden Cave #2, Randolph Co., WV
G. porphyriticus	Adult	106	WVBS 4245	Barret's Cave Mercer Co., WV
G. porphyriticus	Larva	20	USNM 497685	Pigeon Cave, Walker Co., GA
G. porphyriticus	Larva	21	USNM 497686	Pigeon Cave, Walker Co., GA
G. porphyriticus	Larva	22	USNM 525228	McClure Cave, Lee Co., VA
G. porphyriticus	Larva	23	USNM 525223	McClure Cave, Lee Co., VA
G. porphyriticus	Larva	24	USNM 525229	McClure Cave, Lee Co., VA
G. porphyriticus	Larva	25	USNM 525227	McClure Cave, Lee Co., VA
G. porphyriticus	Larva	26	USNM 525225	McClure Cave, Lee Co., VA
G. porphyriticus	Larva	27	USNM 525226	McClure Cave, Lee Co., VA
G. porphyriticus	Larva	28	USNM 525231	McClure Cave, Lee Co., VA
G. porphyriticus	Larva	29	USNM 525232	McClure Cave, Lee Co., VA
G. porphyriticus	Larva	30	USNM 525224	McClure Cave, Lee Co., VA
G. porphyriticus	Larva	31	USNM 525233	McClure Cave, Lee Co., VA
G. porphyriticus	Larva	32	USNM 52530	McClure Cave, Lee Co., VA
G. porphyriticus	Larva	57	USNM 319464	Organ Cave, Greenbrier Co., WV
G. porphyriticus	Larva	65	USNM 319472	Overholt Blowing Cave, Pocahontas Co., WV
G. porphyriticus	Larva	66	USNM 319473	Overholt Blowing Cave, Pocahontas Co., WV
G. porphyriticus	Larva	67	USNM 319474	Overholt Blowing Cave, Pocahontas Co., WV
G. porphyriticus	Larva	88	WVBS 3343	Benedict Cave, Greenbrier Co., WV
G. porphyriticus	Larva	89	WVBS 3341	Benedict Cave, Greenbrier Co., WV
G. porphyriticus	Larva	90	WVBS 1192	Raine's Cave, Pocahontas Co., WV
G. porphyriticus	Larva	92	WVBS 1190	Raine's Cave, Pocahontas Co., WV
G. porphyriticus	Larva	93	WVBS 1191	Raine's Cave, Pocahontas Co., WV
G. porphyriticus	Larva	94	WVBS 1189	Raine's Cave, Pocahontas Co., WV
G. porphyriticus	Larva	97	WVBS 3477	The Hole, Greenbrier Co., WV
G. p. palleucus	Transformed Adult	48	USNM 317709	Custard Hollow Cave, Franklin Co., TN
G. p. palleucus	Neotenic Adult	33	USNM 545709	Sinking Cove Cave, Franklin Co., TN
G. p. palleucus	Neotenic Adult	34	USNM 545710	Sinking Cove Cave, Franklin Co., TN
G. p. palleucus	Neotenic Adult	35	USNM 337413	Custard Hollow Cave, Franklin Co., TN
G. p. palleucus	Neotenic Adult	36	USNM 337421	Custard Hollow Cave, Franklin Co., TN
G. p. palleucus	Neotenic Adult	37	USNM 337415	Custard Hollow Cave, Franklin Co., TN

Table 3-2. continued.

Species	Life Stage	Meas. ID	Collection Number [1]	Locality
G. p. palleucus	Neotenic Adult	38	USNM 337414	Custard Hollow Cave, Franklin Co., TN
G. p. palleucus	Neotenic Adult	39	USNM 337416	Custard Hollow Cave, Franklin Co., TN
G. p. palleucus	Neotenic Adult	40	USNM 337418	Custard Hollow Cave, Franklin Co., TN
G. p. palleucus	Neotenic Adult	41	USNM 337417	Custard Hollow Cave, Franklin Co., TN
G. p. palleucus	Neotenic Adult	42	USNM 337419	Custard Hollow Cave, Franklin Co., TN
G. p. palleucus	Neotenic Adult	43	USNM 337429	Custard Hollow Cave, Franklin Co., TN
G. p. palleucus	Neotenic Adult	44	USNM 337420	Custard Hollow Cave, Franklin Co., TN
G. p. palleucus	Neotenic Adult	45	USNM 337423	Custard Hollow Cave, Franklin Co., TN
G. p. palleucus	Neotenic Adult	46	USNM 337422	Custard Hollow Cave, Franklin Co., TN
G. p. palleucus	Neotenic Adult	47	USNM 317705	Custard Hollow Cave, Franklin Co., TN
G. p. palleucus	Neotenic Adult	49	USNM 317707	Custard Hollow Cave, Franklin Co., TN
G. p. palleucus	Neotenic Adult	50	USNM 317708	Custard Hollow Cave, Franklin Co., TN
G. p. palleucus	Neotenic Adult	51	USNM 317706	Custard Hollow Cave, Franklin Co., TN
G. p. palleucus	Neotenic Adult	72	USNM 139402	Custard Hollow Cave, Franklin Co., TN
G. p. palleucus	Neotenic Adult	73	USNM 139403	Sinking Cove Cave, Franklin Co., TN
G. gulolineatus	Neotenic Adult	74	USNM 317750	Berry Cave, Roane Co., TN
G. gulolineatus	Neotenic Adult	75	USNM 317751	Berry Cave, Roane Co., TN
G. gulolineatus	Neotenic Adult	76	USNM 317752	Berry Cave, Roane Co., TN
G. gulolineatus	Neotenic Adult	77	USNM 317753	Berry Cave, Roane Co., TN
G. gulolineatus	Neotenic Adult	78	USNM 317754	Berry Cave, Roane Co., TN
G. gulolineatus	Neotenic Adult	79	USNM 317755	Berry Cave, Roane Co., TN

[1] USNM: National Museum of Natural History, Washington, D.C.
WVBS: West Virginia Biological Survey Collection at Marshall University, Huntington, WV.
MES: Personal Collection of Michael E. Seidel, Marshall University, Huntington, WV.

Table 3-3. Description of statistical "runs" used.

Run	Life-stage	Data	Statistical Analyses	Preserved Specimens [1]	Live Specimens	Characters [2]
1	Metamorphosed	Unadjusted measurements	PCA, PC 3 / PC 2 ANOVA, t-Test	GYSU = 4 $GYPO_1$ = 10 $GYPO_2$ = 23	None	All 20
2	Metamorphosed	Ratios with SVL	CDA ANOVA, t-Test	GYSU = 4 $GYPO_1$ = 10 $GYPO_2$ = 23	None	All 20
3	Metamorphosed	Unadjusted measurements	PCA, PC 3 / PC 2 ANOVA, t-Test	GYSU = 4 $GYPO_1$ = 10 $GYPO_2$ = 23	GYSU = 6 $GYPO_1$ = 17 $GYPO_2$ = 23	5 : SVL, CLL, CW, TRW, ED
4	Gilled larvae and neotenic adults	Unadjusted measurements	PCA, PC 3 / PC 2 ANOVA, t-Test	GYSU = 11, GYPO = 24, GYPA = 20, GYGU = 6	None	All 20
5	Gilled larvae and neotenic adults	Ratios with SVL	CDA ANOVA, t-Test	GYSU = 11, GYPO = 24, GYPA = 20, GYGU = 6	None	All 20

[1] $GYPO_1$: *Gyrinophilus porphyriticus* from population microsympatric with *G. subterraneus* at General Davis Cave, Greenbrier County, WV.
$GYPO_2$: *Gyrinophilus porphyriticus* from various caves in West Virginia and southwestern Virginia.
GYSU: *Gyrinophilus subterraneus* from General Davis Cave, Greenbrier County, WV.
GYPA: *Gyrinophilus palleucus palleucus* from Custard Hollow Cave and Sinking Cove Cave in Franklin County, Tennessee.
GYGU: *Gyrinophilus gulolineatus* from Berry Cave in Roane County Tennessee.

[2] Character measurements are described in Table 1.

Table 3-4. Eigenvector values (coefficients) for principal components analysis of metamorphosed *Gyrinophilus* spp.

	PC 1	**PC 2**	**PC 3**
SVL	0.247107	-0.148735	-0.054465
TL	0.194983	0.00802	-0.095992
GW	0.254005	-0.006068	0.140937
CLV	0.237391	-0.235749	0.167743
CLL	0.247637	-0.139146	0.233457
CW	0.260968	0.020459	0.006807
CWG	0.237335	0.188631	-0.184471
CD	0.233501	0.205483	0.021643
EN	0.221725	-0.230781	0.104639
SL	0.228375	-0.169096	0.261276
IOD	0.249531	-0.086066	0.00523
IND	0.230299	-0.10232	0.052482
TRW	0.238134	0.176341	-0.1342
TRL	0.230381	-0.048124	-0.222041
TRH	0.146147	0.529969	0.099732
TLW	0.234039	0.228858	-0.186676
TLH	0.190778	0.440832	-0.175704
HUL	0.215958	-0.220172	-0.282384
FL	0.206947	-0.271994	-0.149773
ED	0.106627	0.203569	**0.723619**

Table 3-5. Eigenvector values for principal components analysis of metamorphosed *Gyrinophilus* spp. This includes specimens measured in the field in 2002 and 2003.

Characters	PC 1	PC 2	PC 3
SVL	0.473088	-0.235696	-0.286286
CLL	0.470165	0.017876	-0.670273
CW	0.487875	-0.169444	0.232867
TRW	0.467461	-0.226929	0.629245
ED	0.313993	0.929473	0.136375

Table 3-6. Total-sample correlation coefficients for variables verse SVL. Character variables are described in Table 1.

	Transformed		Gilled	
Variable	***r***	***P***	***r***	***P***
ED	-0.43450	0.7138	0.32083	0.0124
IOD	0.99997	0.0048	0.92106	<.0001
IND	0.97746	0.1354	0.83590	<.0001
EN	0.98635	0.1053	0.82340	<.0001
SL	0.79152	0.4186	0.76995	<.0001
CLV	0.90580	<.0001	0.94013	<.0001
CLL	0.90380	<.0001	0.92226	<.0001
CW	0.86498	<.0001	0.92905	<.0001
GW	0.83655	<.0001	0.93244	<.0001
CWG	0.75977	<.0001	0.93179	<.0001
CD	0.89147	0.2994	0.93608	<.0001
TRH	-0.42561	0.7201	0.92727	<.0001
TRW	0.80449	0.4049	0.95951	<.0001
TRL	0.99878	0.0314	0.98114	<.0001
TL	0.71451	<.0001	0.83745	<.0001
TLW	0.97324	0.1476	0.95641	<.0001
TLH	0.51135	0.6583	0.93982	<.0001
HUL	0.97366	0.1464	0.95713	<.0001
FL	0.99255	0.0778	0.92692	<.0001

Table 3-7. Significantly different characters ($P \leq 0.05$) for group comparisons of metamorphosed *Gyrinophilus* spp. (Run 1). Results were determined by t-Test (LSD) and corrected for error with ANOVA.

Group Comparison [1]	**Significantly Different Characters** [2]
GYSU- $GYPO_1$	TRH, HUL, ED
GYSU- $GYPO_2$	SVL, TRL, HUL, FL, ED
$GYPO_1$- $GYPO_2$	SVL, CWG, TRW, TRL, TRH, HUL

[1] $GYPO_1$: *Gyrinophilus porphyriticus* from population microsympatric with *G. subterraneus* at General Davis Cave, Greenbrier County, WV.
$GYPO_2$: *Gyrinophilus porphyriticus* from various caves in West Virginia and southwestern Virginia.
GYSU: *Gyrinophilus subterraneus* from General Davis Cave, Greenbrier County, WV.

[2] Character measurements are described in Table 1.

Table 3-8. Significantly different characters ($P \leq 0.05$) for group comparisons of metamorphosed *Gyrinophilus* spp. (Run 2). All characters are ratios with SVL to adjust for specimen size difference. Results were determined by t-Test (LSD) and corrected for error with ANOVA.

Group Comparison [1]	Significantly Different Characters [2]	Percentage of Significantly Different Characters
GYSU- $GYPO_1$	GW, CLL, CWG, TRW, TRH, TLH, ED	35%
GYSU- $GYPO_2$	GW, CLL, CW, SL, IND, TRH, TLH, ED	40%
$GYPO_1$- $GYPO_2$	CLV, CLL, SL, IND	20%

[1] $GYPO_1$: *Gyrinophilus porphyriticus* from population microsympatric with *G. subterraneus* at General Davis Cave, Greenbrier County, WV.
$GYPO_2$: *Gyrinophilus porphyriticus* from various caves in West Virginia and southwestern Virginia.
GYSU: *Gyrinophilus subterraneus* from General Davis Cave, Greenbrier County, WV.

[2] Character measurements are described in Table 1.

Table 3-9. Mean, standard error (SE), standard deviation (SD), maximum (MAX), and minimum (MIN) eye diameter for metamorphosed *Gyrinophilus*.

TAXON	MIN	-1 SD	-1 SE	MEAN	+1 SE	+1 SD	MAX
G. subterraneus	2.12	2.53	2.60	**2.68 ***	2.76	2.83	2.94
G. porphyriticus 1	3.24	3.58	3.66	**3.70**	3.74	3.82	4.25
G. porphyriticus 2	2.53	3.28	3.45	**3.49**	3.53	3.70	4.40

* *G. subterraneus* has significantly ($P \leq 0.05$)smaller ED than *G. porphyriticus*.

Table 3-10. Eigenvector values for principal components analysis of larval and neotenic *Gyrinophilus* spp.

Characters	**PC 1**	**PC 2**	**PC 3**
SVL	0.236139	0.137603	-0.058446
TL	0.203182	0.142935	-0.24884
GW	0.235485	-0.070688	0.10079
CLV	0.230352	0.167299	0.083545
CLL	0.234009	-0.069075	0.227292
CW	0.237197	-0.115202	0.107344
CWG	0.237359	-0.130222	0.031682
CD	0.231437	-0.025278	-0.101057
EN	0.217132	-0.22648	0.22848
SL	0.209473	-0.322415	0.352004
IOD	0.235407	-0.079092	0.075194
IND	0.221058	-0.218963	0.19656
TRW	0.237194	0.060316	-0.11686
TRL	0.228686	0.209097	-0.200006
TRH	0.227546	0.118415	-0.257739
TLW	0.231221	0.122021	-0.250436
TLH	0.225634	0.164855	-0.310956
HUL	0.236204	-0.031362	-0.039641
FL	0.233739	-0.036808	0.048587
ED	0.055421	**0.759459**	**0.582487**

Table 3-11. Significantly different characters ($P \leq 0.05$) for group comparisons of gilled larval and neotenic *Gyrinophilus* spp. (Run 4). Characters are unadjusted for specimen size difference. Results were determined by t-Test (LSD) and corrected for error with ANOVA

Taxa Comparison [1]	**Significantly Different Characters** [2]
GYSU - GYPO	SVL, TL, GW, CLV, CLL, CW, CWG, EN, SL, IOD, IND, TRW, TRL, TRH, TLW, TLH, HUL, FL, ED
GYPO - GYPA	TL, CD, EN, SL, IND, TRL, TLH, ED
GYPA - GYGU	SVL, GW, CLV, CLL, CW, CWG, CD, EN, SL, IOD, IND, TRW, TRH, TLW, TLH, HUL, FL
GYGU - GYSU	GW, CLL, CW, CWG, EN, SL, IOD, IND, TRH, FL
GYSU - GYPA	SVL, GW, CLV, CLL, CW, CWG, CD, IOD, TRW, TRL, TRH, TLW, TLH, HUL, FL
GYPO - GYGU	SVL, GW, CLV, CLL, CW, CWG, CD, EN, SL, IOD, IND, TRW, TRL, TRH, TRW, HUL, FL, ED

[1] GYPO: *Gyrinophilus porphyriticus* from various caves in West Virginia and southwestern Virginia.
GYSU: *Gyrinophilus subterraneus* from General Davis Cave, Greenbrier County, WV.
GYPA: *Gyrinophilus palleucus palleucus* from Custard Hollow Cave and Sinking Cove Cave in Franklin County, Tennessee.
GYGU: *Gyrinophilus gulolineatus* from Berry Cave in Roane County Tennessee.

[2] Character measurements are described in Table 1.

Table 3-12. Significantly different characters ($P \leq 0.05$) for taxa comparisons of gilled larval and neotenic *Gyrinophilus* spp. (Run 5). All characters are ratios with SVL to adjust for specimen size difference. Results were determined by t-Test (LSD) and corrected for error with ANOVA.

Taxa Comparison [1]	Significantly Different Characters [2]	Percentage of Significantly Different Characters
GYSU - GYPO	CWG, CD, TRW, TRL, TLW, HUL, ED	35%
GYPO - GYPA	GW, CLL, CW, CWG, EN, SL, IOD, IND, TRL, TLW, HUL, ED	60%
GYPA - GYGU	GW, CLL, CW, CWG, CD, SL, TRW, TLW, FL	45%
GYGU - GYSU	GW, CLL, CW, CWG, EN, SL, IOD, IND, TRL, FL	50%
GYSU - GYPA	CLL, CW, CWG, EN, SL, IOD, IND, TRW, TRL, TLW	50%
GYPO - GYGU	GW, CLL, CW, CWG, CD, EN, SL, IOD, IND, TRW, TRL, HUL, FL, ED	65%

[1] GYPO: *Gyrinophilus porphyriticus* from various caves in West Virginia and southwestern Virginia.
GYSU: *Gyrinophilus subterraneus* from General Davis Cave, Greenbrier County, WV.
GYPA: *Gyrinophilus palleucus palleucus* from Custard Hollow Cave and Sinking Cove Cave in Franklin County, Tennessee.
GYGU: *Gyrinophilus gulolineatus* from Berry Cave in Roane County Tennessee.

[2] Character measurements are described in Table 1.

Table 3-13. Mean, standard error (SE), standard deviation (SD), maximum (MAX), and minimum (MIN) eye diameter for larval or neotenic *Gyrinophilus*.

TAXON	MIN	-1 SD	-1 SE	MEAN	+1 SE	+1 SD	MAX
G. subterraneus	0.80	0.88	1.27	**1.29**	1.30	1.69	1.94
G. porphyriticus	1.18	1.43	1.81	**1.82***	1.82	2.20	2.47
G. palleucus	0.76	0.93	1.15	**1.15**	1.15	1.38	1.59
G. gulolineatus	1.00	1.14	1.41	**1.42**	1.44	1.70	1.76

* *G. porphyriticus* is significantly ($P \leq 0.05$) larger than the other taxa.

Table 3-14. Maximum, Minimum, and Mean values for characters used in polygonal analysis plot of *Gyrinophilus* spp.; a = actual values, r = relative values. See Table 1 for character abbreviations.

		ED		CLL		CWG		SL		IND	
		a	r	a	r	a	r	a	r	a	r
G. subterraneus	**MAX**	1.94	78.54	22.92	80.28	19.12	81.95	5.20	62.13	7.01	67.66
	MEAN	1.29	52.23	18.68	65.43	14.83	63.57	4.41	52.69	5.26	50.77
	MIN	0.80	32.39	11.19	39.19	7.04	30.18	2.70	32.26	2.49	24.03
G. porphyriticus	**MAX**	2.47	100.00	24.04	84.20	16.88	72.35	5.28	63.08	7.30	70.46
	MEAN	1.82	73.68	14.90	52.19	10.37	44.45	3.55	42.41	3.90	37.64
	MIN	1.18	47.77	10.02	35.10	6.33	27.13	2.33	27.84	2.27	21.91
G. palleucus	**MAX**	1.59	64.37	20.04	70.19	15.30	65.58	5.13	61.29	7.11	68.63
	MEAN	1.15	46.56	15.86	55.55	11.96	51.26	4.17	49.82	4.81	46.43
	MIN	0.76	30.77	11.70	40.98	8.10	34.72	3.14	37.51	3.37	32.53
G. gulolineatus	**MAX**	1.76	71.26	28.55	100.00	23.33	100.00	8.37	100.00	10.36	100.00
	MEAN	1.42	57.49	23.06	80.77	18.09	77.54	6.43	76.82	7.02	67.76
	MIN	1.00	40.49	19.42	68.02	13.30	57.01	5.16	61.65	5.20	50.19

		TRL		TLH		EN		IOD		TRW	
		a	r	a	r	a	r	a	r	a	r
G. subterraneus	**MAX**	67.17	100.00	19.10	100.00	4.93	73.36	7.78	73.81	17.12	100.00
	MEAN	49.30	73.40	10.72	56.13	4.04	60.12	5.96	56.55	11.76	68.69
	MIN	22.75	33.87	3.76	19.69	2.40	35.71	2.20	20.87	4.87	28.45
G. porphyriticus	**MAX**	56.57	84.22	14.05	73.56	5.24	77.98	7.54	71.54	14.67	85.69
	MEAN	35.85	53.37	7.39	38.69	3.06	45.54	4.48	42.50	8.28	48.36
	MIN	19.74	29.39	4.40	23.04	2.19	32.59	2.51	23.81	4.64	27.10
G. palleucus	**MAX**	50.52	75.21	10.95	57.33	4.76	70.83	6.94	65.84	11.41	66.65
	MEAN	34.23	50.96	7.11	37.23	3.60	53.57	4.99	47.34	8.17	47.72
	MIN	23.47	34.94	4.40	23.04	2.40	35.71	2.90	27.51	5.52	32.24
G. gulolineatus	**MAX**	62.18	92.57	15.25	79.84	6.72	100.00	10.54	100.00	15.59	91.06
	MEAN	45.60	67.89	10.59	55.45	5.22	77.68	7.35	69.73	12.85	75.06
	MIN	34.82	51.84	8.30	43.46	4.18	62.20	4.96	47.06	8.48	49.53

Figure 3-1. Distribution map of *Gyrinophilus*.

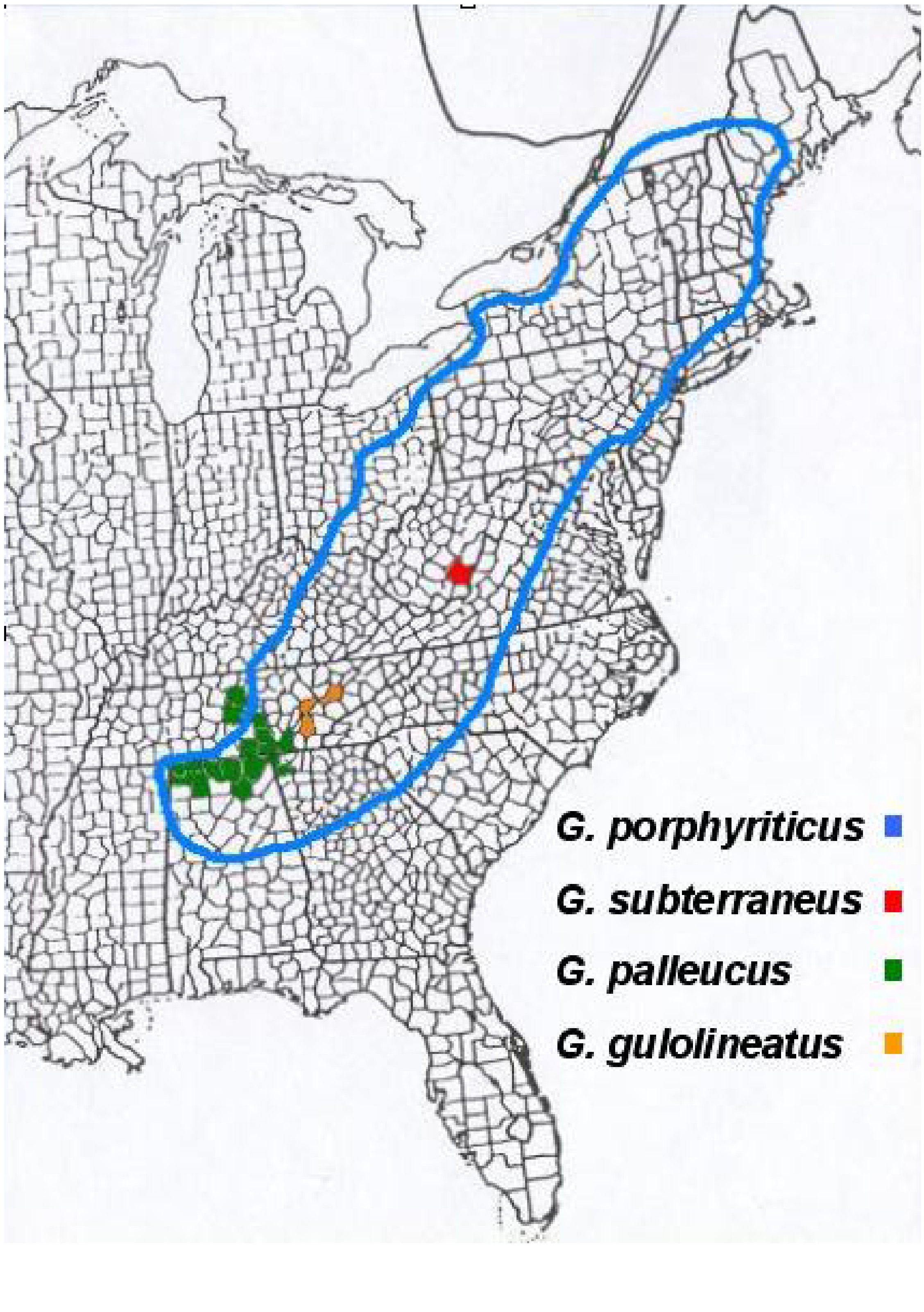

G. porphyriticus
G. subterraneus
G. palleucus
G. gulolineatus

Figure3- 2. Metamorphosed *Gyrinophilus porphyriticus* from General Davis Cave, Greenbrier county West Virginia.

(M. B. Watson)

(M. B. Watson)

Figure 3-3. Larval *Gyrinophilus porphyriticus* from Rehobeth Church Cave, Monroe County, West Virginia.


Carolina Biological Supply Company

(M. S. Osbourn)


Burlington, North Carolina
8
9
10

Figure 3-4. *Gyrinophilus palleucus necteroides.*

(© John R. McGregor)

Figure 3-5. *Gyrinophilus gulolineatus,* neotene above, transformed below.

(Tom Barr)

(© 2004 Henk Wallays)

Figure 3-6. Distribution of *Gyrinophilus* in West Virginia.

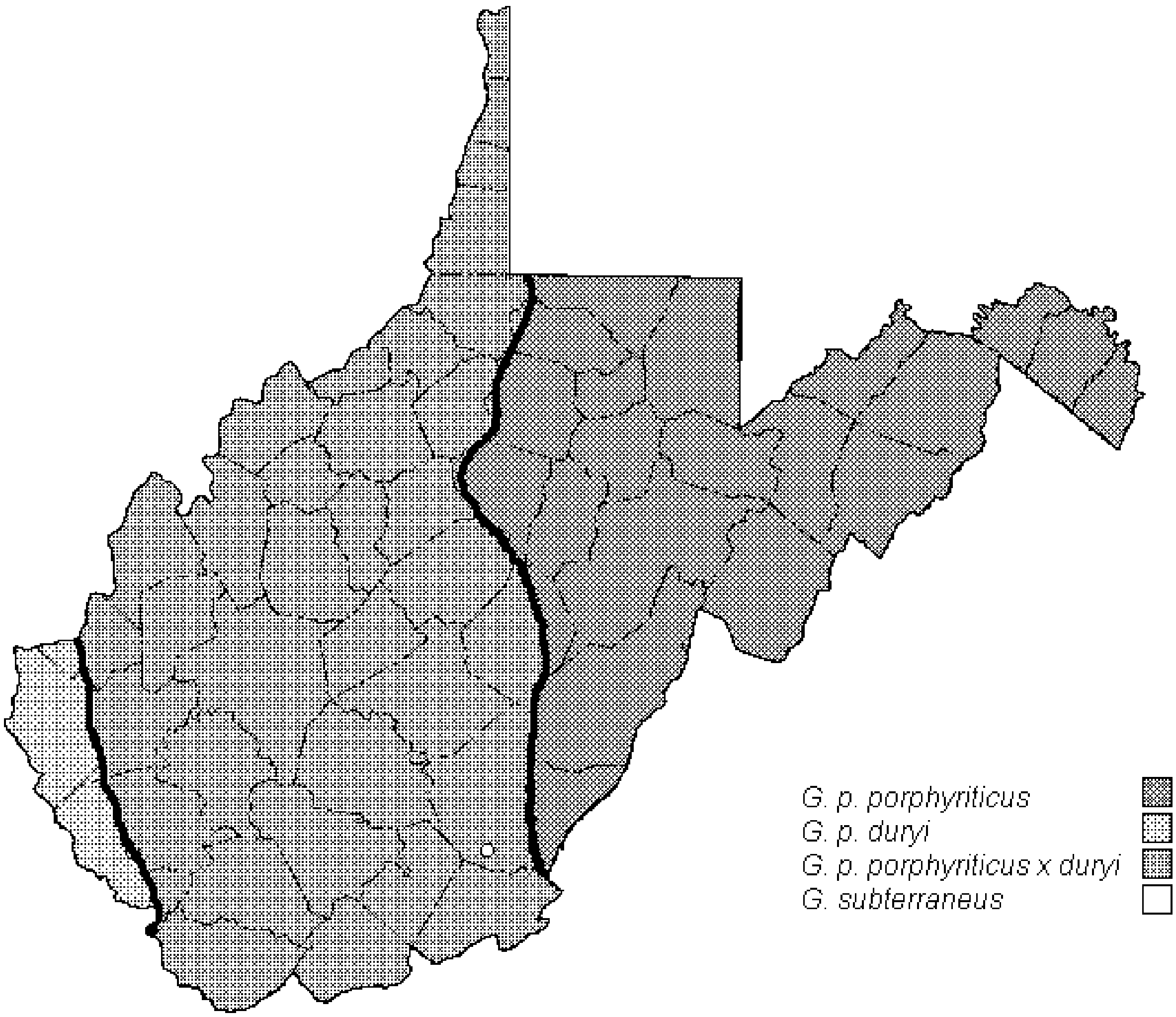

G. p. porphyriticus
G. p. duryi
G. p. porphyriticus x duryi
G. subterraneus

Figure 3-7. Michael Osbourn at entrance to General Davis Cave.

(M. B. Watson)

Figure 3-8. Larval *Gyrinophilus subterraneus* from stream in General Davis Cave, Greenbrier County West Virginia.

(M. B. Watson)

(M. B. Watson)

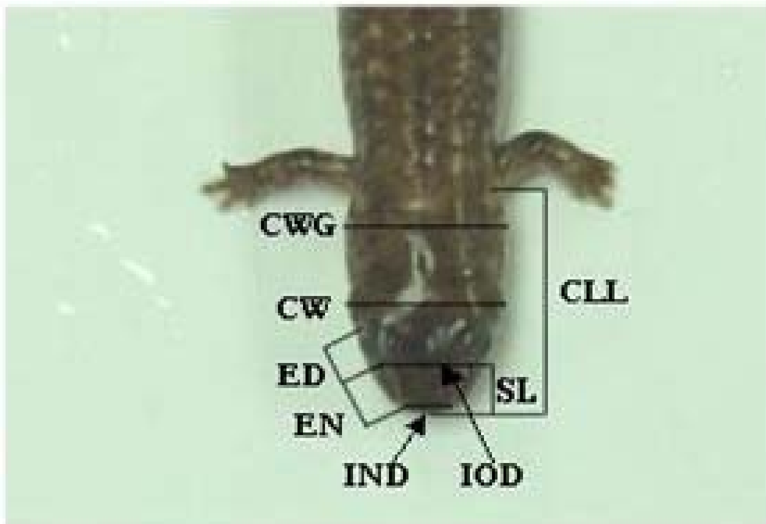
Figure 3-9. Metamorphosed *Gyrinophilus subterraneus* from General Davis Cave, Greenbrier County West Virginia.


(M. B. Watson)

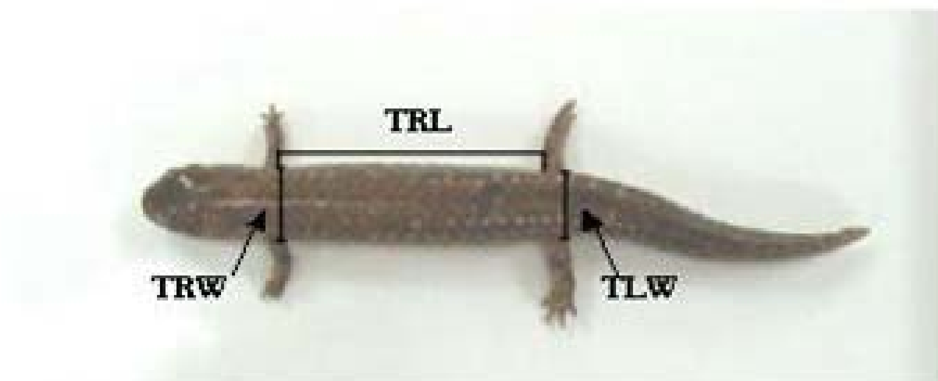

(M. B. Watson)

Figure 3-10. Linear distance measurements of external morphological characters of *Gyrinophilus*, showing cranial (a), dorsal (b), ventral (c), and lateral (d) views.

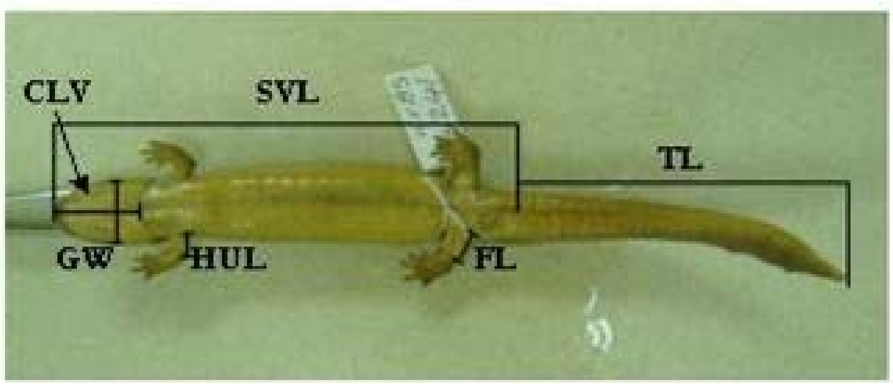
a.


CWG
CW
CLL
ED
SL
EN
IND
IOD

b.


TRL
TRW
TLW

c.


CLV
SVL
TL
GW
HUL
FL

d.

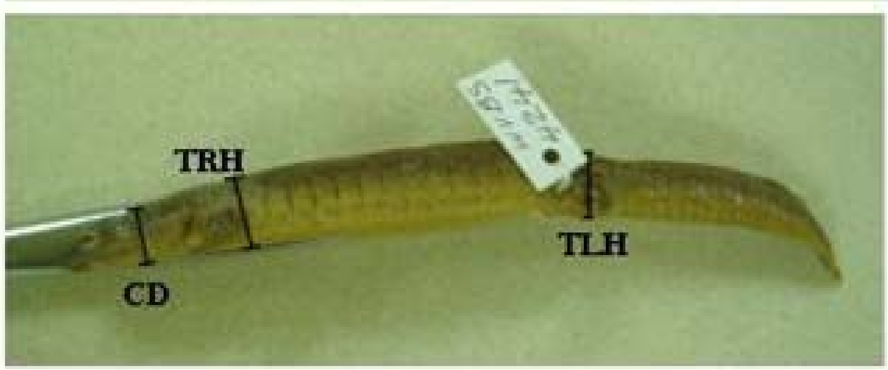

TRH
TLH
CD

Figure 3-11. Distribution of specimens used in morphometric analysis.

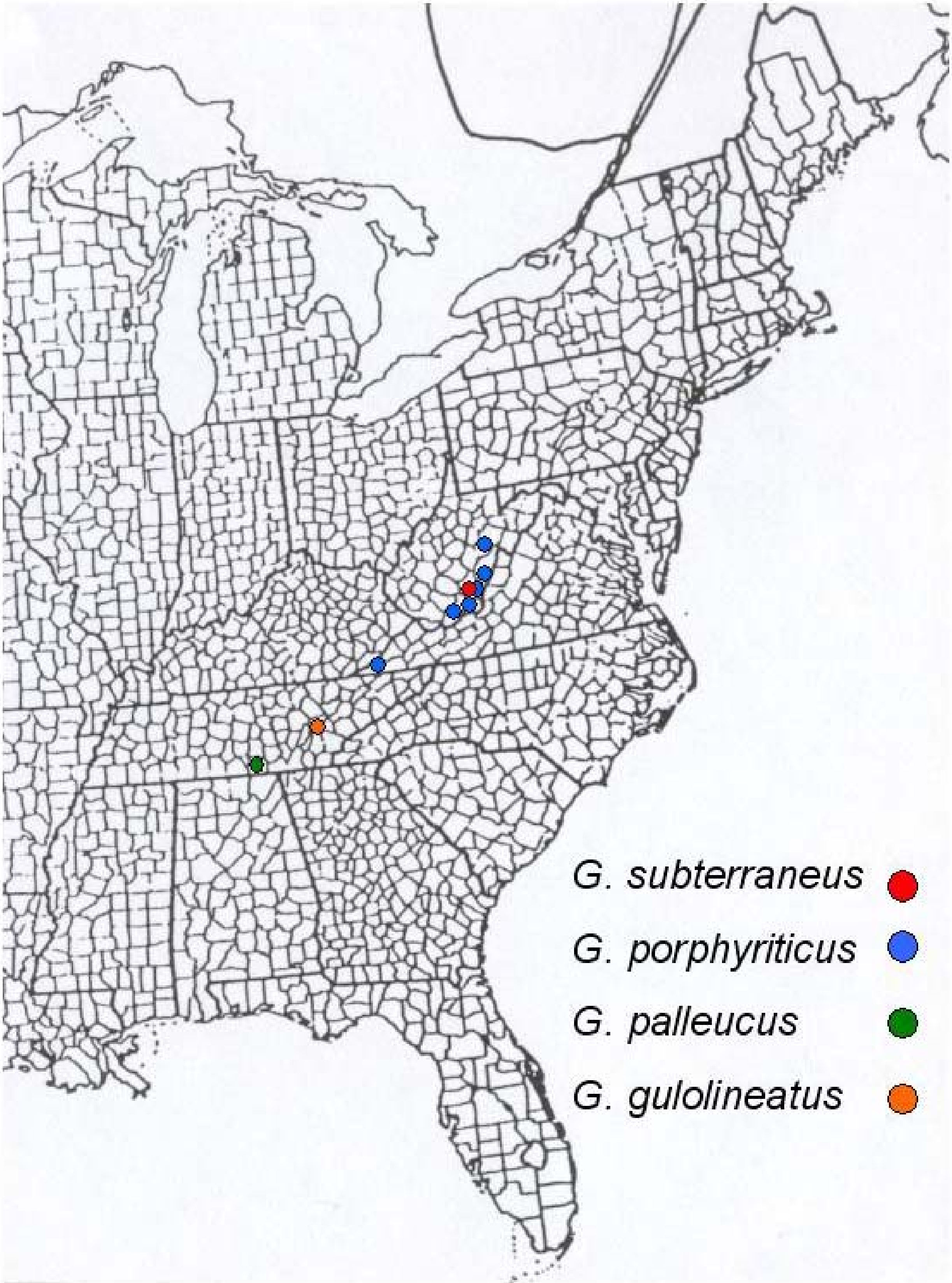
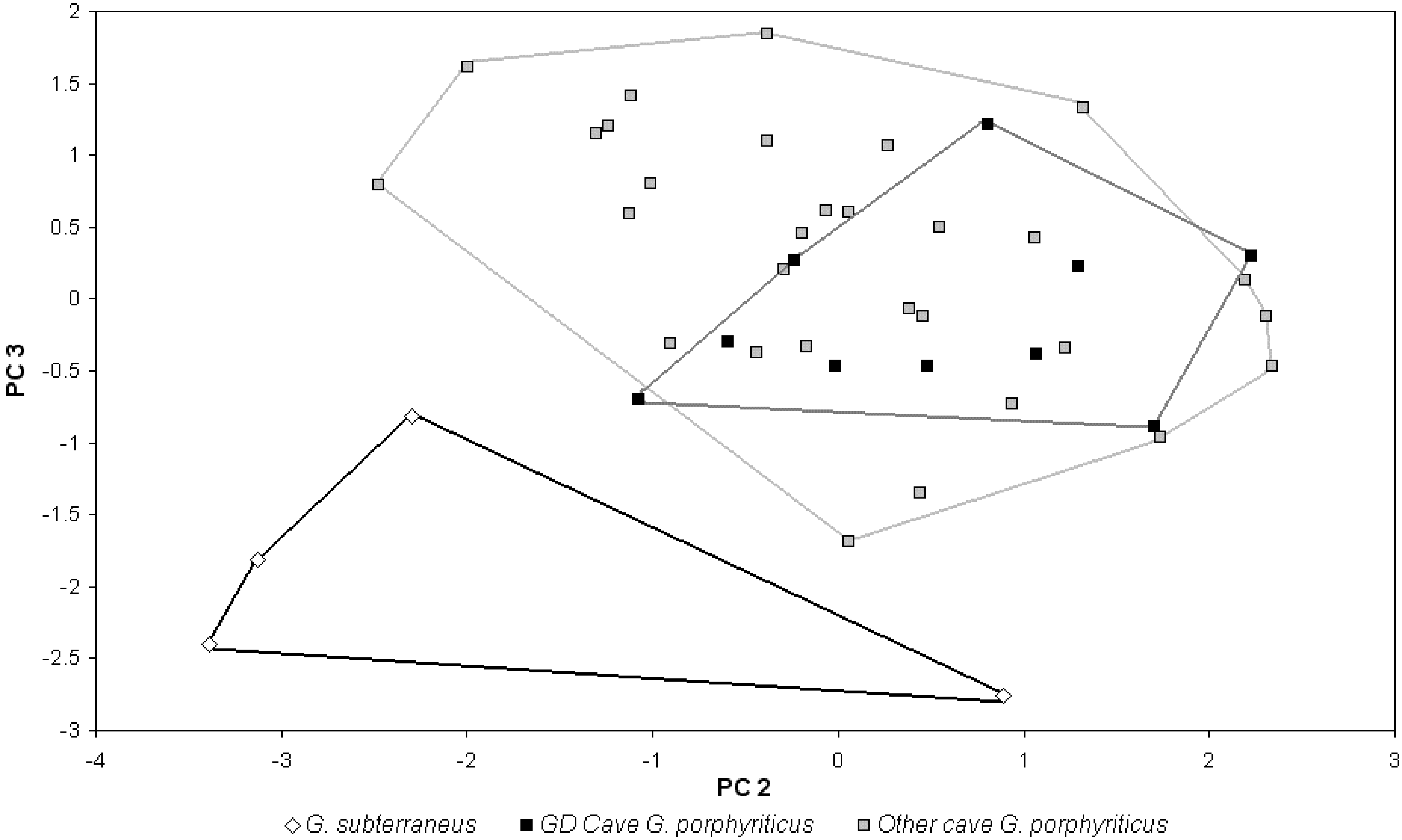

G. subterraneus
G. porphyriticus
G. palleucus
G. gulolineatus

Figure 3-12. PCA plot of metamorphosed *Gyrinophilus*.



PC 3
PC 2
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-4
-3
-2
-1
0
1
2
3
◇ G. subterraneus ■ GD Cave G. porphyriticus ■ Other cave G. porphyriticus

Figure 3-13. PCA plot of metamorphosed *Gyrinophilus* supplemented with live specimens measured in the field.

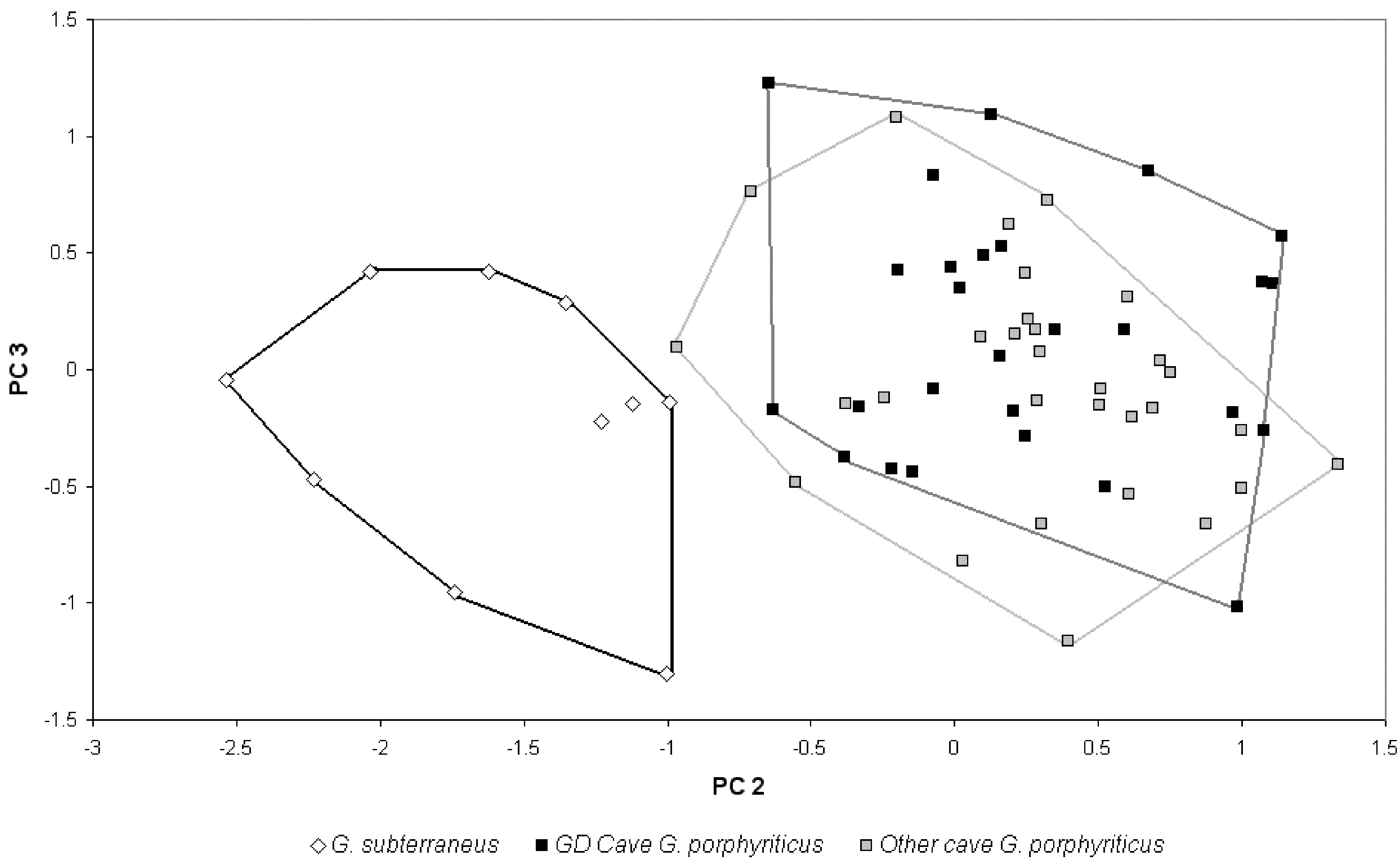
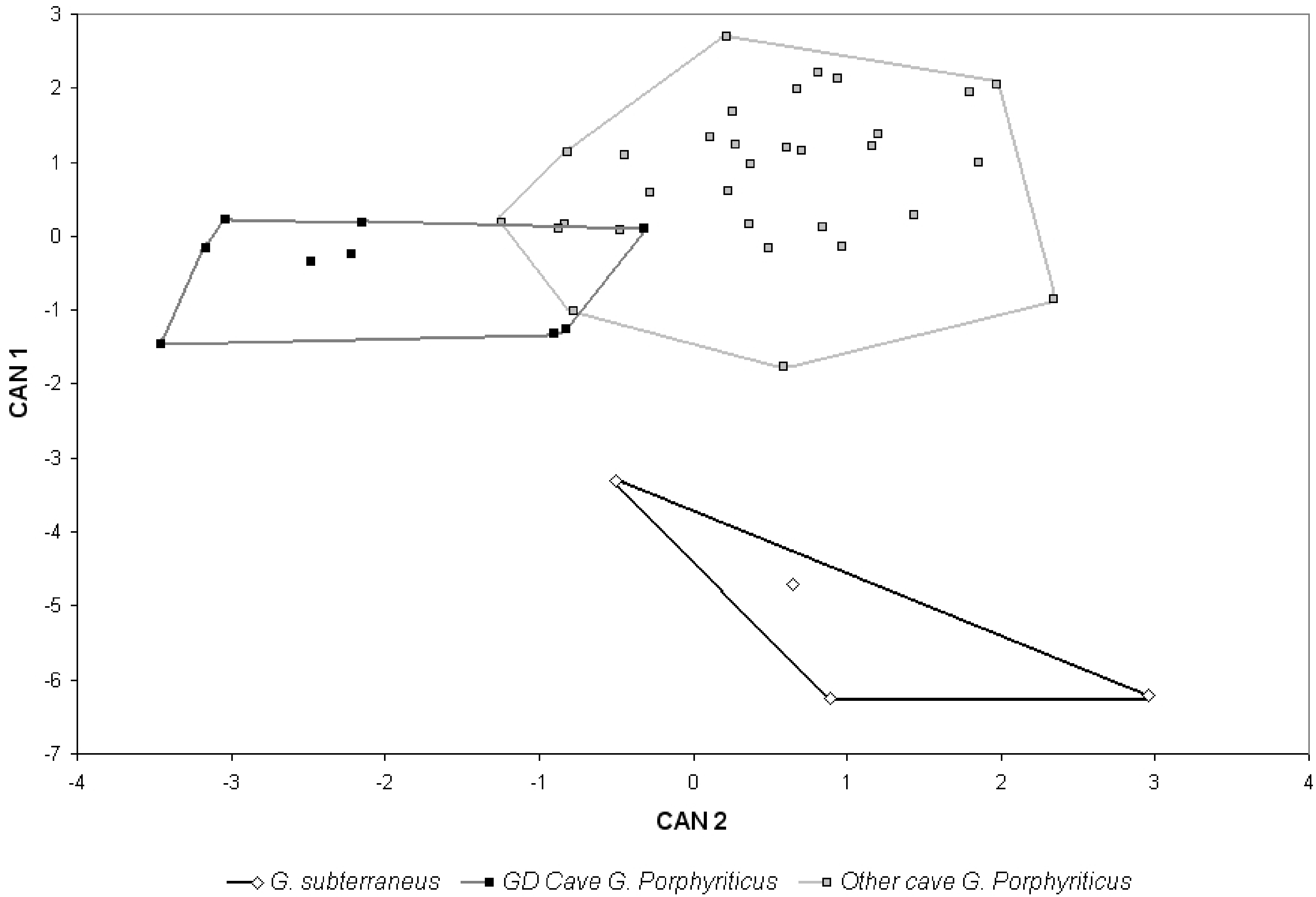

PC 3
PC 2
1.5
1
0.5
0
-0.5
-1
-1.5
-3
-2.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
◇ G. subterraneus ■ GD Cave G. porphyriticus ■ Other cave G. porphyriticus

Figure 3-14. CDA plot of metamorphosed *Gyrinophilus*.



3
2
1
0
-1
-2
-3
-4
-5
-6
-7
CAN 1
-4
-3
-2
-1
0
1
2
3
4
CAN 2
G. subterraneus
GD Cave G. Porphyriticus
Other cave G. Porphyriticus

Figure 3-15. Comparison of eye diameter of metamorphosed *Gyrinophilus porphyriticus* (above) and *G. subterraneus* (below).


(W. B. Sutton)


(W. B. Sutton)

Figure 3-16. Population range diagram of eye diameter for metamorphosed *Gyrinophilus subterraneus, G. porphyriticus* from General Davis Cave (GYPO1), and *G. porphyriticus* from other caves (GYPO2). *G. subterraneus* eye diameters are significantly smaller ($P \leq 0.05$).

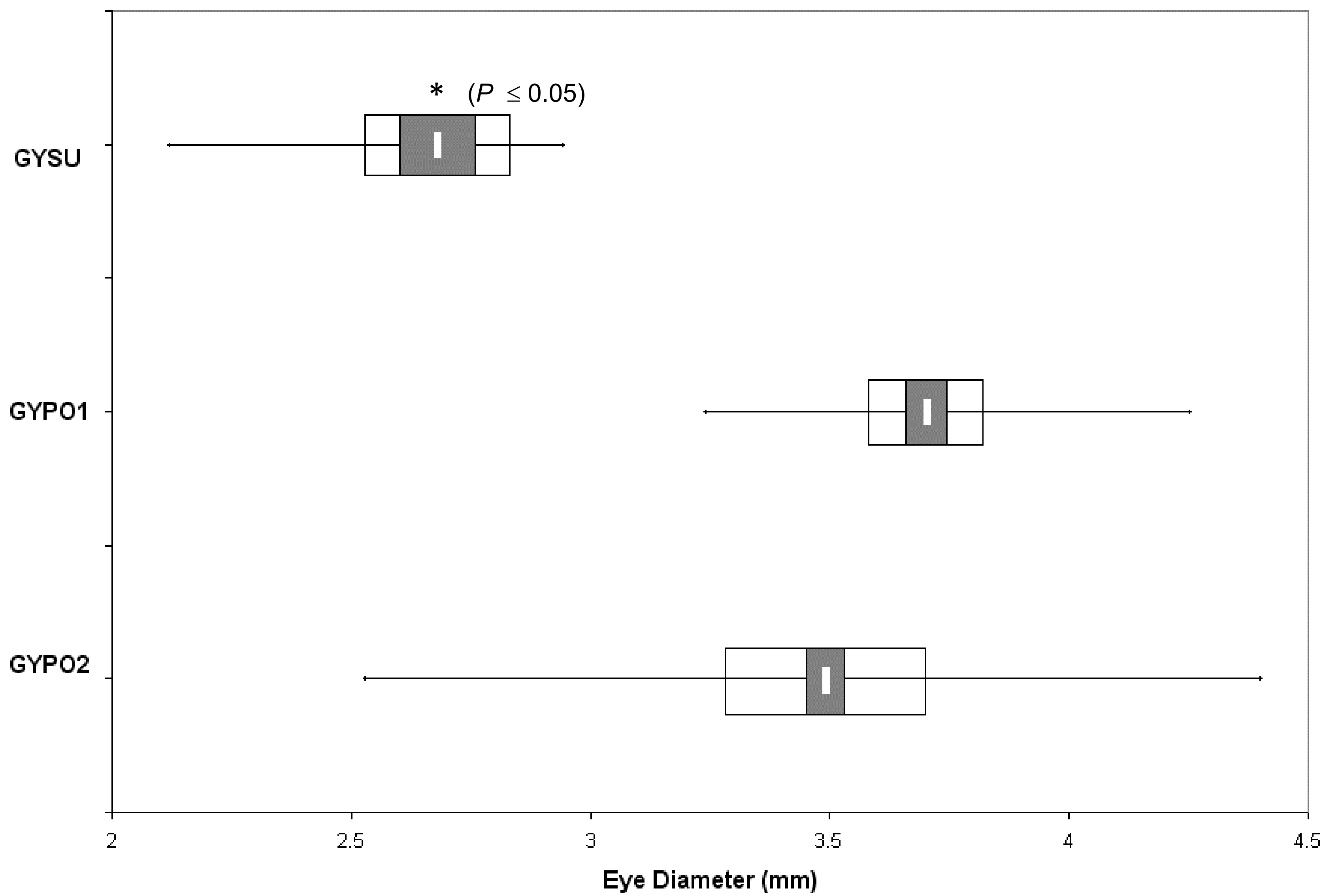
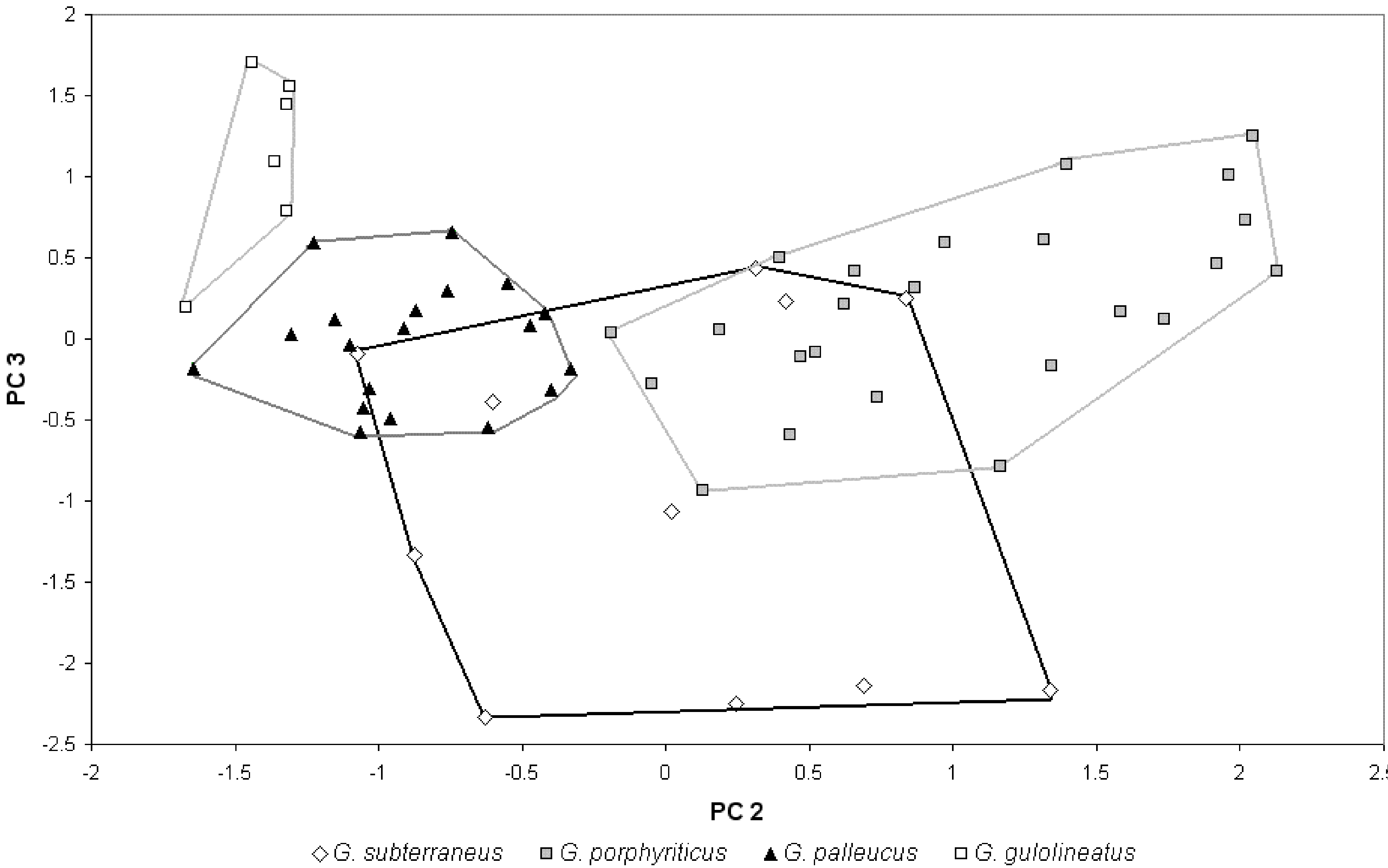
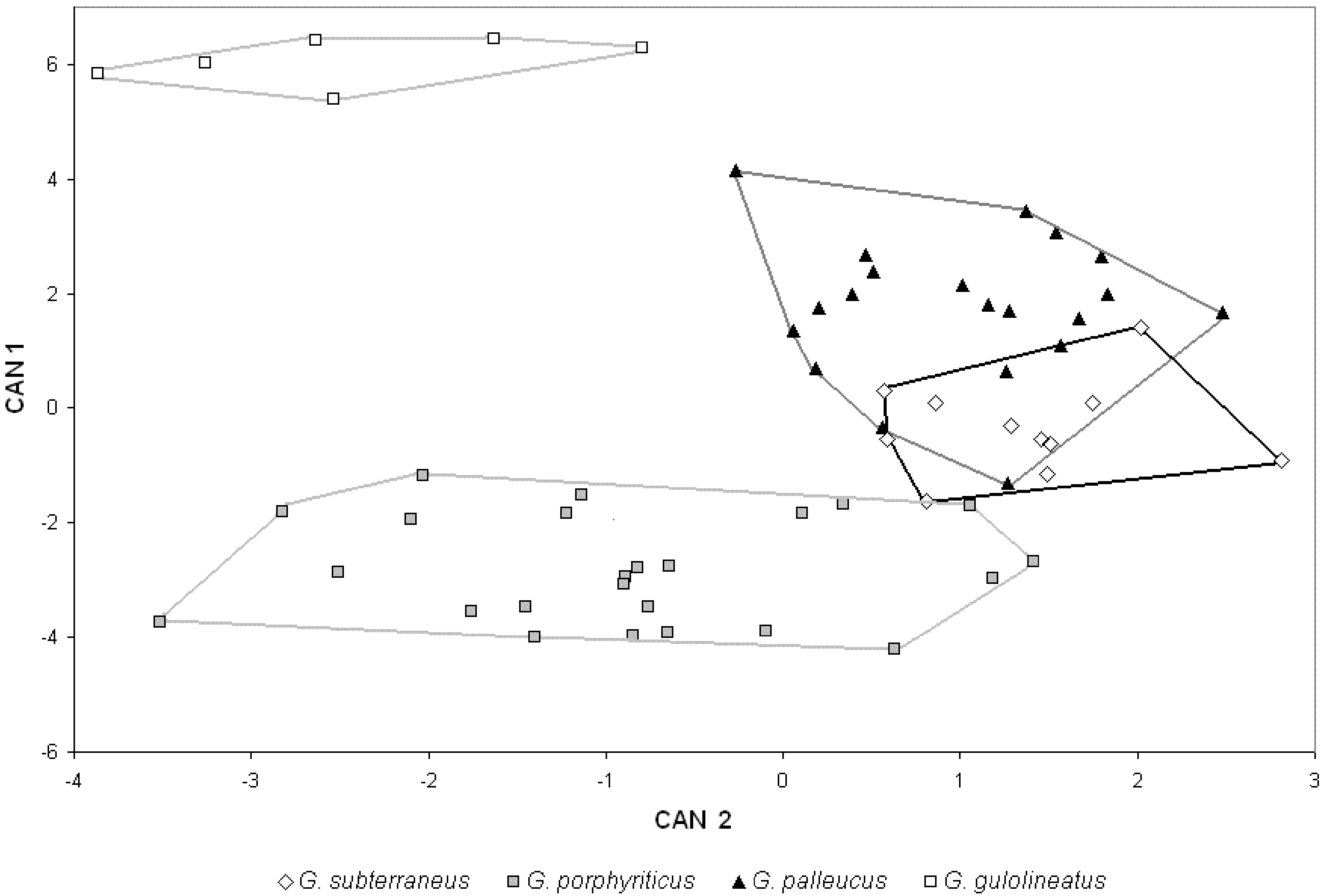

* (P ≤ 0.05)
GYSU
GYPO1
GYPO2
2
2.5
3
3.5
4
4.5
Eye Diameter (mm)

Figure 3-17. PCA plot of gilled larval and neotenic *Gyrinophilus*.



PC 3
PC 2
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
2.5
G. subterraneus
G. porphyriticus
G. palleucus
G. gulolineatus

Figure 3-18. CDA plot of gilled larval and neotenic *Gyrinophilus*.



6
4
2
0
-2
-4
-6
CAN 1
-4
-3
-2
-1
0
1
2
3
CAN 2
◇ G. subterraneus ■ G. porphyriticus ▲ G. palleucus □ G. gulolineatus

Figure 3-19. Population range diagram of eye diameter for larval *Gyrinophilus subterraneus* (GYSU), *G. porphyriticus* (GYPO), *G. palleucus* (GYPO), and *G. gulolineatus* (GYGU). *G. porphyriticus* eye diameters are significantly larger ($P \leq 0.05$).

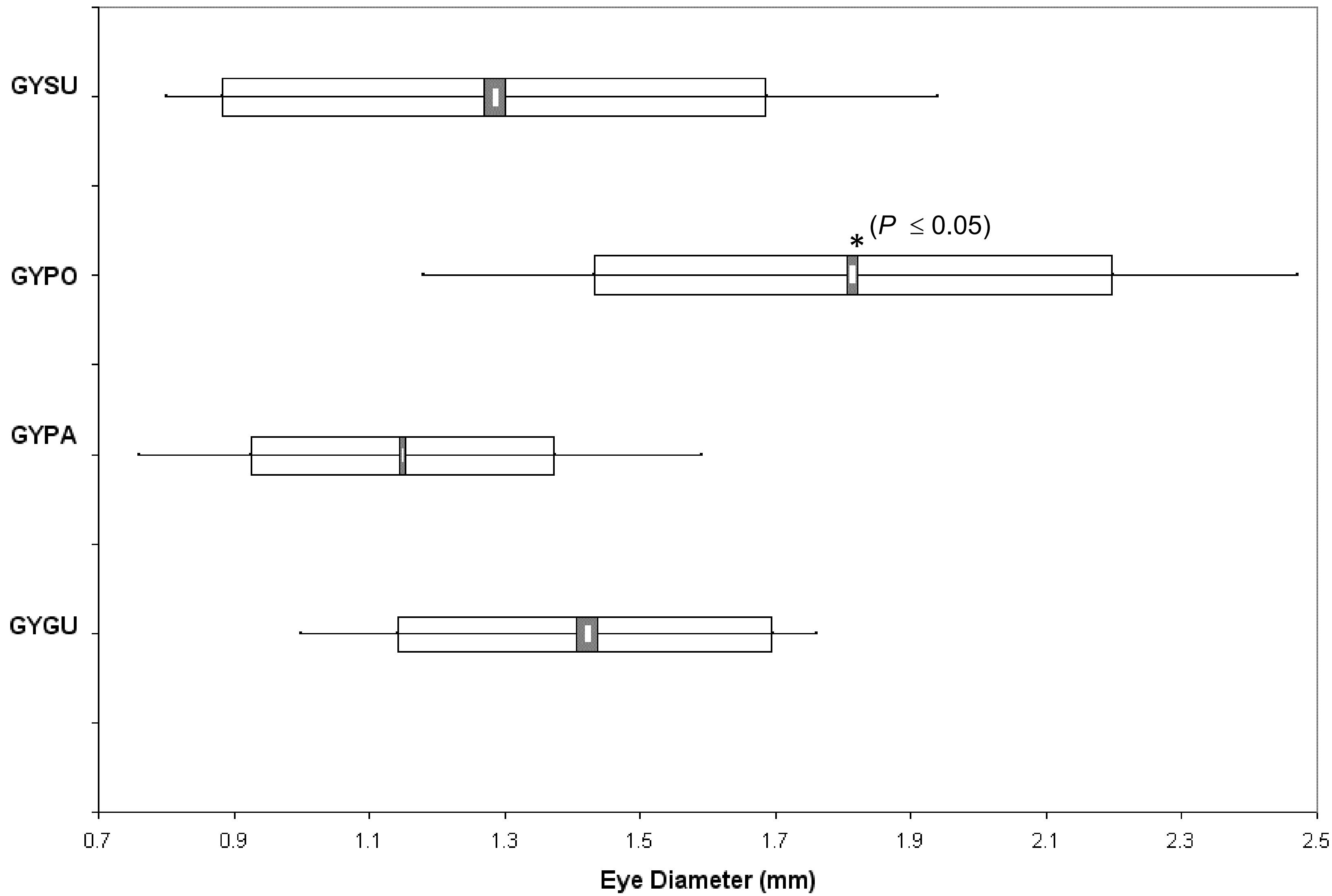
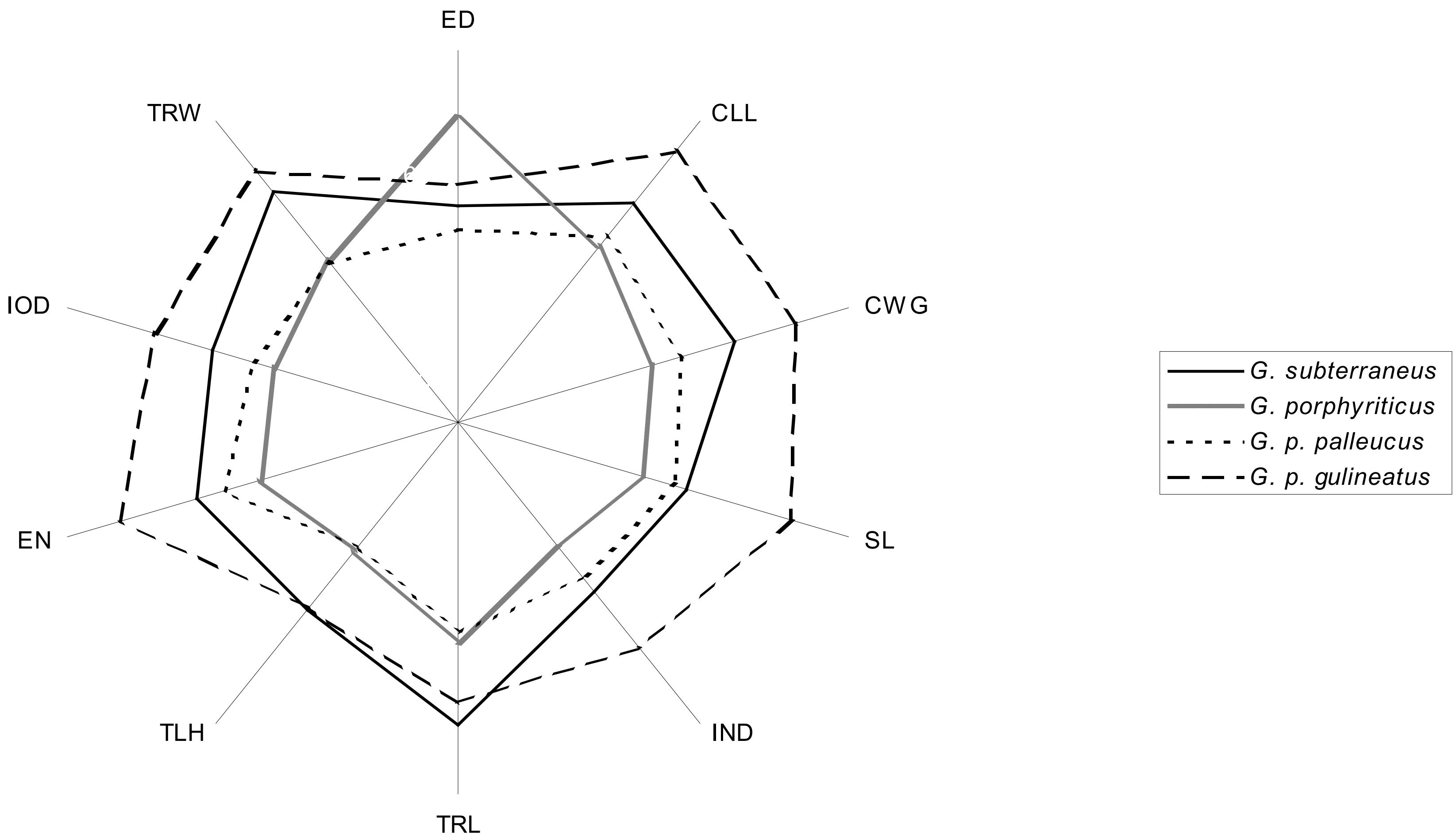
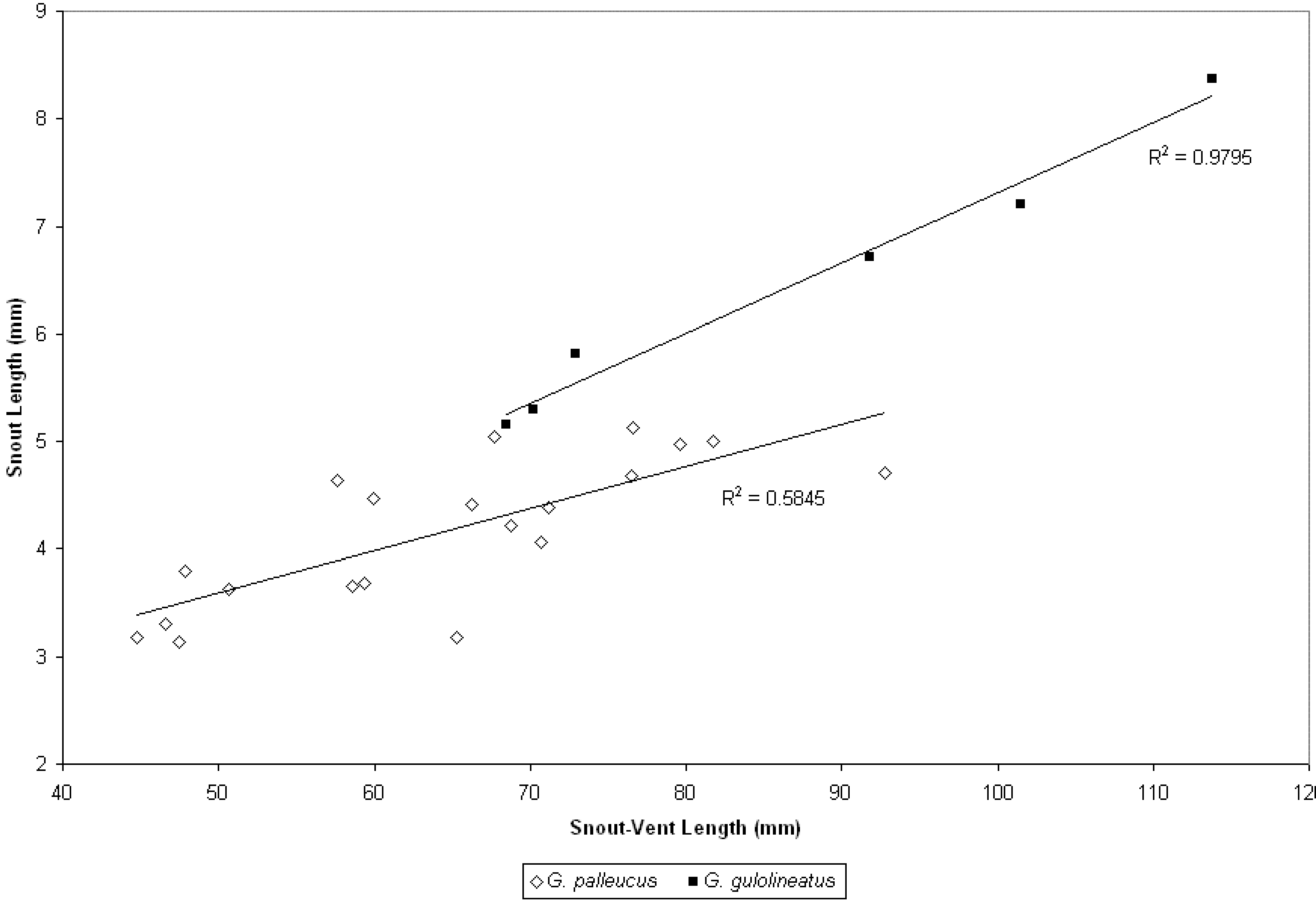

GYSU
GYPO
GYPA
GYGU
* (P ≤ 0.05)
0.7
0.9
1.1
1.3
1.5
1.7
1.9
2.1
2.3
2.5
Eye Diameter (mm)

Figure 3-20. Polygonal plot of relative values for gilled larval and neotenic *Gyrinophilus* (see Table 14 for character values.)



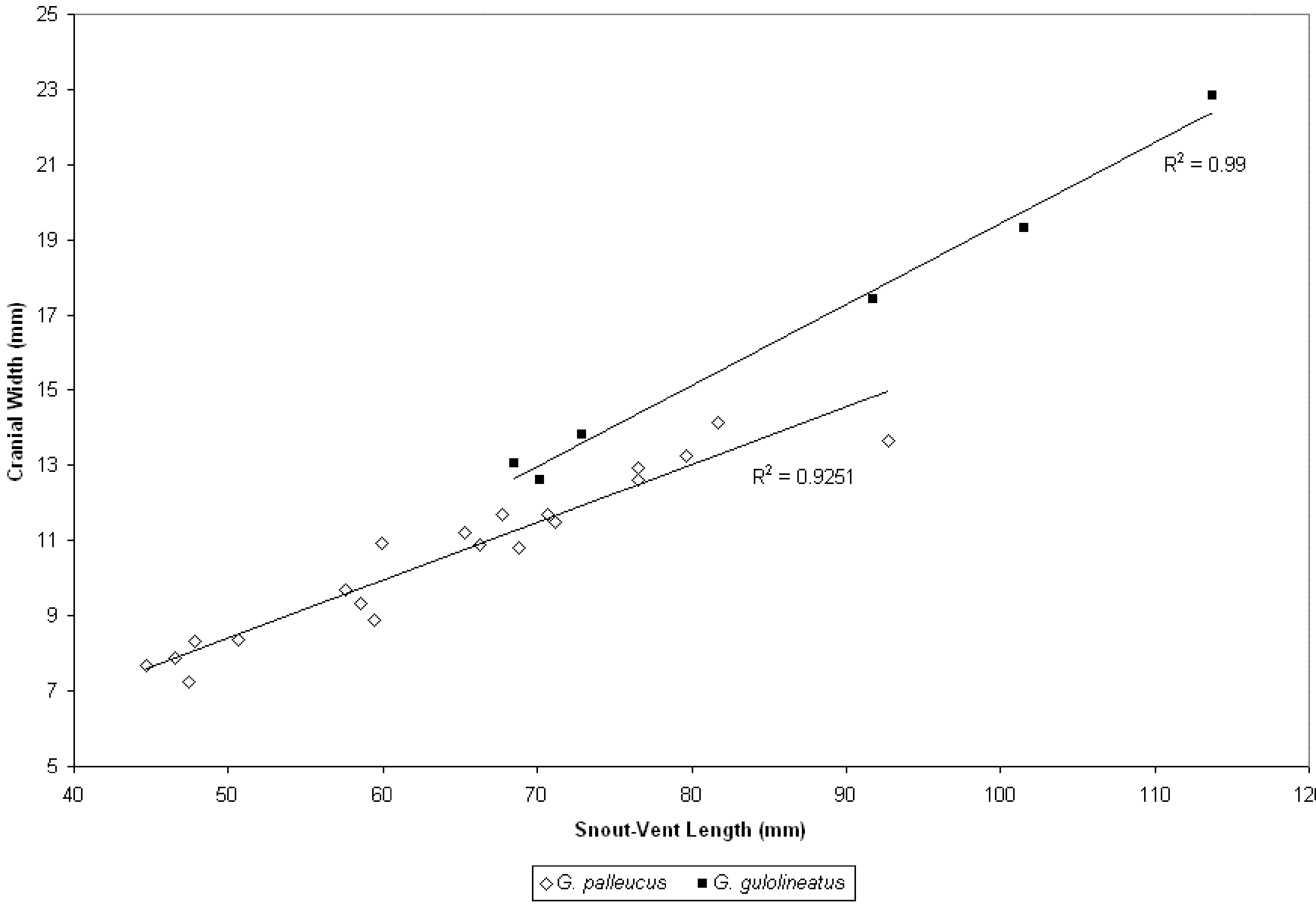
ED
CLL
CWG
SL
IND
TRL
TLH
EN
IOD
TRW
G. subterraneus
G. porphyriticus
G. p. palleucus
G. p. gulineatus

Figure 3-21. Bivariate plot of eye diameter verses snout-vent length for *Gyrinophilus*.



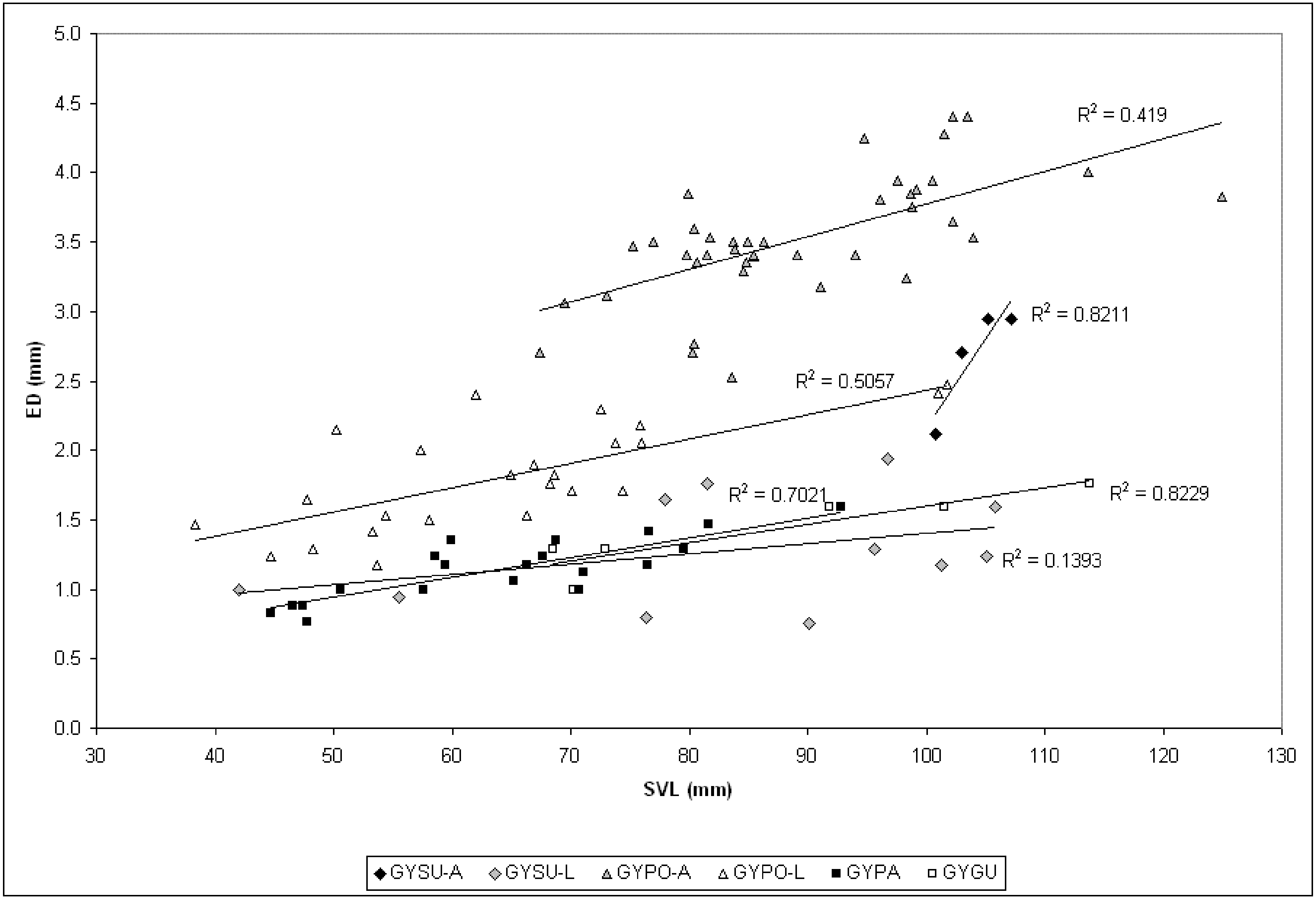
9
8
7
6
5
4
3
2
40
50
60
70
80
90
100
110
120
Snout Length (mm)
Snout-Vent Length (mm)
R² = 0.9795
R² = 0.5845
G. palleucus
G. gulolineatus

Figure 3-22. Bivariate plot of cranial width verses snouth-vent length for *Gyrinophilus palleucus* and *G. gulolineatus.*



25
23
21
19
17
15
13
11
9
7
5
Cranial Width (mm)
40
50
60
70
80
90
100
110
120
Snout-Vent Length (mm)
R2 = 0.99
R2 = 0.9251
G. palleucus
G. gulolineatus

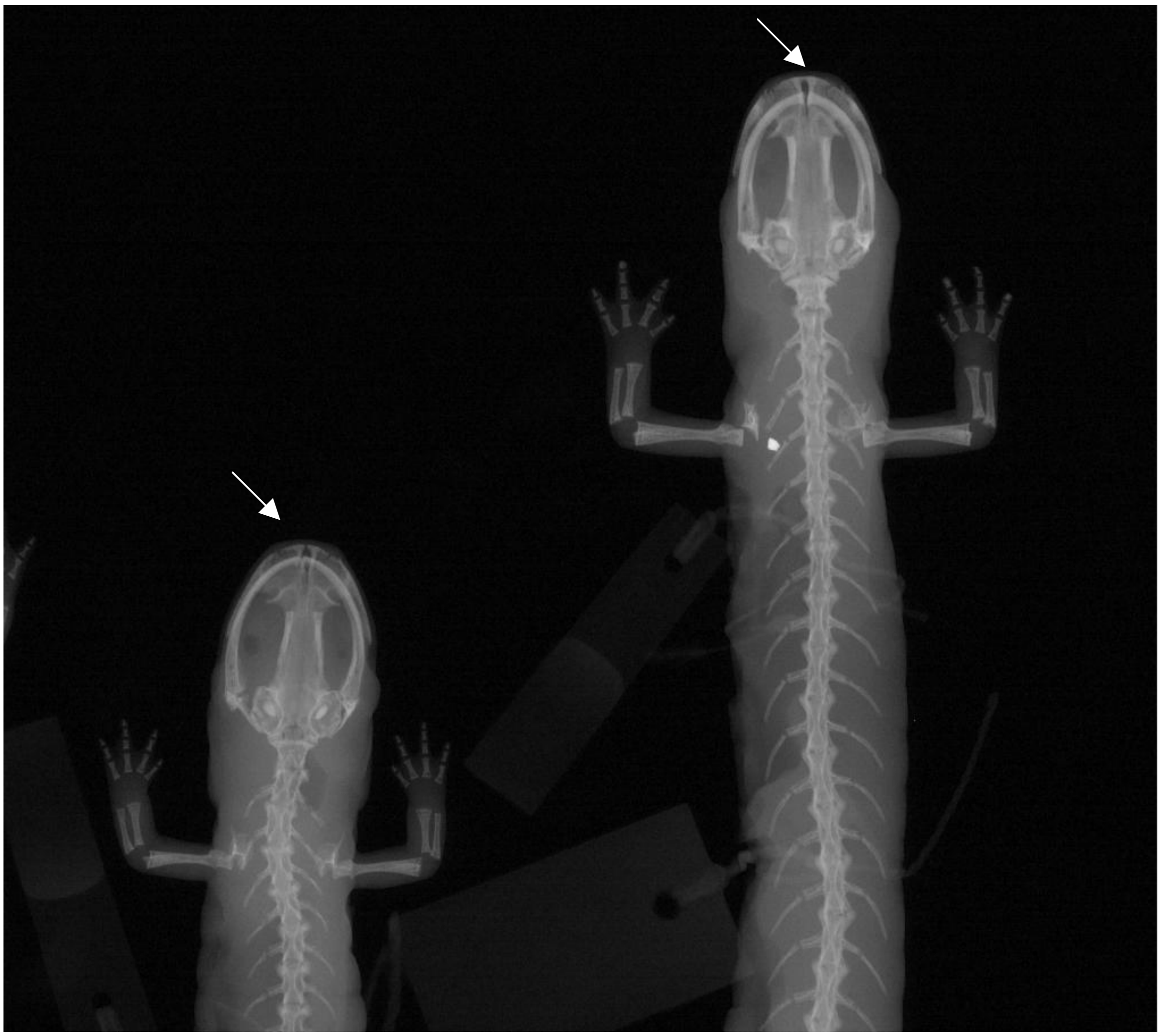
Figure 3-23. Bivariate plot of eye diameter verses snouth-vent length for *Gyrinophilus spp.*



5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ED (mm)
30
40
50
60
70
80
90
100
110
120
130
SVL (mm)
R2 = 0.419
R2 = 0.8211
R2 = 0.5057
R2 = 0.7021
R2 = 0.8229
R2 = 0.1393
GYSU-A
GYSU-L
GYPO-A
GYPO-L
GYPA
GYGU

Figure 3-24. X-ray images of transformed *G. porphyriticus* (left) and *G. subterraneus* (right) demonstrating the undivided premaxilla of *G. subterraneus* .

(A. Wynn, USNM)



G. porphyriticus ***G. subterraneus***

Chapter IV:

Community Ecology, Diet, and Feeding Behavior in Cave-dwelling *Gyrinophilus* spp.

Introduction

The feeding habits of Gyrinophilus porphyriticus in surface habitats

Gyrinophilus porphyriticus ssp. inhabit a variety of habitats including cool mountain headwater streams, seepages, springs, and caves throughout a large geographic range from northern Alabama to southern Quebec (Green and Pauley 1987). This wide range of geographic variation results in a diversity of ecological contexts. Spring Salamanders in the Cowee Mountains of western North Carolina, for instance, interact with a high diversity and abundance of species compared to Spring Salamanders in Adirondack Mountains of New York (Bruce 1972 and 1979; Bishop 1941). There have been diet analysis studies on *Gyrinophilus* populations in New York, New Hampshire, Pennsylvania, West Virginia, Virginia, North Carolina, Tennessee, and Alabama. These studies have demonstrated regional, ecological, and life-stage variation in the diet of Spring Salamanders.

Examinations of stomach contents in the northeastern states revealed a heavy dominance of invertebrates, particularly insects, in the diet of *Gyrinophilus p. porphyriticus.* Studies in New York by Hamilton (1932) and Bishop (1941), in New Hampshire by Burton (1976), and in Pennsylvania by Surface (1913) found the following food items within the dissected digestive tracts of Northern Spring Salamanders; ephemeropterans, tricopterans, plecopterans, coleopterans, dipterans, annelids, araneids, diplopods, gastropods, hymenopterans, hemipterans, chilopods, hydracarinans, homopterans, collembolas, lepidopterans, and amphibian species.

Gyrinophilus spp. are notorious for feeding on other salamanders (Petranka 1998). There are many observations of salamanders being consumed in captivity and in the wild. *Desmognathus fuscus*, *D. ochrophaeus*, *D. wrighti*, *Eurycea bislineata*, *E. cirrigera*, *E. wilderae*, *Pseudotriton ruber*, *Plethodon oconaluftee*, *Plethodon jordani*, *Plethodon serratus*, *Hemidactylium scutatum*, *Notophthalmus v. viridescens*, other *G. porphyriticus*, and a young Wood Frog, *Rana sylvatica*, have all been reported in the literature as Spring Salamander prey items (Bishop 1941; Pope and Noble 1921; Wright and Haber 1922; Bruce 1972, 1979; Hueey and Stupka 1967; Surface 1913; Hamilton 1932; Burton 1976; Secki and Queral-Regil 1997). Both adult and larval *G. porphyriticus* feed on adults and larvae of other species (Bruce 1979). In studies in the northeastern U.S., salamanders only accounted for a small proportion of the overall diet (Surface 1913; Bishop 1941; Burton 1976). Salamanders appear to be a much greater component of the diets of Blue Ridge Spring Salamander adults, *G. p. danielsi*, in the southern Appalachians. Bruce (1972) found that in the mountains of western North Carolina 47 % of identifiable stomach contents were salamanders. In the southern Appalachians, there seems to be a shift at metamorphosis from a generalized diet of aquatic invertebrates and an occasional larval salamander, to a specialized diet focused on salamanders (Bruce 1979).

Differences in feeding patterns between northern and southern populations could represent a genetic predisposition of two different subspecies (or perhaps species) (pers. comm. Addison Wynn) or more likely is the response of an opportunistic feeder to the most abundant prey of the appropriate size. Salamanders are typically generalist feeders and according to Hairston (1949) "will eat almost anything that falls within the proper size range." The community structure in the southern Appalachians is characterized by higher densities of salamanders competing for invertebrate prey. In this context, it would be advantageous for metamorphosed *G. p. danielsi* to shift to feeding upon other salamanders (Bruce 1979).

West Virginia Gyrinophilus porphyriticus ssp. Diet in Surface Habitats

Diet studies in West Virginia have also documented predation on salamanders by *Gyrinophilus porphyriticus* (Green 1941; Lindley 1999). Green (1941) observed captured *G. p. porphyriticus* regurgitate earthworms, snails, and the salamanders; *Plethodon richmondi*, *Eurycea bislineata*, and *Desmognathus fuscus*. Lindley (1999) reported finding a *E. bislineata* larva in the stomach of a *G. porphyriticus* larva and a *Desmognathus ochrophaeus* juvenile inside a *G. porphyriticus* adult. Aggressive behavior and attempts at predation on other salamanders have also been observed in captive animals (pers. comm. T. K. Pauley). On one occasion a *Desmognathus monticola* was mistakenly placed in a bag with a *G. porphyriticus* and was quickly seized by the mouth of the *G. porphyriticus* (pers. comm. W. B. Sutton). The degree to which salamanders are an important food source for West Virginia *Gyrinophilus* is uncertain. It is possible to deduce from Lindley's (1999) findings that 11% of adults sampled (n=9) and 3% of larvae sampled (n=36) consumed salamanders. These percentages appear to be more in line with studies of northeastern populations were 10% (Hamilton 1932) and 3% (Bishop 1941) of specimens contained salamanders, as opposed to a study in North Carolina where 47% of adults and 18% of larvae contained salamander remains (Bruce 1979).

Lindley's 1999 study at the Westvaco Wildlife and Ecosystem Research Forest in Randolph County, West Virginia, revealed a heavy proportion of invertebrates in *G. p. porphyriticus* diet and supports the notion of them as opportunistic generalist feeders. The stomach contents in this study varied seasonally as prey abundance varied. In spring (March- June), when Plecoptera nymphs were common they were taken by both adults and larvae. Plecopterans constituted 54.5% of the total larval prey. Crayfish (*Cambarus* sp.) were only found in stomach contents during summer (June-September 1). Autumn was characterized by a flush of Lepodopteran larvae, equaling 73% of the prey volume of *Gyrinophilus* larvae (Lindley 1999).

Community Ecology and Trophic Relationships in Surface Populations

Within surface stream salamander communities, Spring Salamanders usually occupy the highest trophic position (Resertarits 1991). Their large size, up to a record total length of 232 mm (Conant and Collins 1991), and propensity to feed on other salamanders contribute to their status as top predators. The presence of *G. porphyriticus* greatly influences the growth, survival, activity, and microhabitat use of other salamanders. *Eurycea cirrigera* and *E. wilderae* exhibit decreased survival rates and lowered activity levels (Gustafson 1994; Beachy 1994), *Pseudotriton* spp. have reduced growth rates (Gustafson 1993), and small *Desmognathus ochrophaeus* may alter there microhabitat use (Formanowicz and Brodie 1993) when *G. porphyriticus* are present. Gustafson (1993) also found that large *G. porphyriticus* larvae reduced the growth rate of small *G. porphyriticus* larvae through interference competition or threat of predation. *Gyrinophilus porphyriticus* was also found to be a more effective and efficient predator of salamanders than another large stream Plethodontid, such as *Desmognathus quadramaculatus* (Formanowicz and Brodie 1993; Beachy 1994).

Spring Salamanders may sit atop the salamander trophic pyramid, but they too can be prey for larger predators. Bright coloration associated with noxious skin secretions and anti-predatory posturing may help protect them from shrews (Brodie et al. 1979). Common Water Snakes, *Nerodia sipedon* and Eastern Garter Snakes, *Thamnophis sirtalis* have been known to occasionally eat *G. porphyriticus* (Uhler et al. 1939). They are probably preyed upon by a variety of terrestrial predators such as raccoons and some birds. In the aquatic community, the presence of predatory fish greatly influences *G. porphyriticus* growth and distribution. Resertarits (1991 and 1995) determined that *G. porphyriticus* individuals from streams not containing Brook Trout were larger and healthier than individuals from streams containing Brook Trout. In the presence of trout, *G. porphyriticus* tend to be more concentrated in shallow areas (Resertarits 1991 and 1995). *Gyrinophilus porphyriticus* also metamorphose more quickly. Lindley (1999) found in West Virginia streams containing trout, *G.*

porphyriticus larvae started to transform at 56 mm SVL, while an individual from a troutless stream had an SVL of 64.8 mm with no sign of metamorphosing.

Salamander Feeding Behavior in Subterranean Environments

Cave adapted animals exhibit morphological and physiological modifications that increase their ability to survive in a subterranean environment. The scarcity of food in caves may be the driving selective force for cave-adapted organisms. In caves, selection favors increased feeding and metabolic efficiency (Poulson 1964 and Mitchell 1969 in Peck 1973). There is evidence for increased feeding and metabolic efficiency in Amblyopsid fish species as they approach greater morphological specialization (Poulson 1963). Poulson (1963 in Culver 1985) found that the more cave-adapted species of troglobitic fish were able to locate scarce prey faster than less troglomorphic ones. There also appears to be an increase in metabolic efficiency in *Gyrinophilus* spp. By counting the number of erythrocyte surges per minute through gill and foot capillaries, Cooper and Cooper (1968) determined the heartbeat rate for *Gyrinophilus palleucus* and *Gyrinophilus porphyriticus*. The *G. palleucus* individual showed an average heartbeat rate 27-28% lower than two cave-dwelling *G. porphyriticus* larvae of comparable size. This suggests that like fish, salamanders exhibit an increased metabolic efficiency with cave specialization.

The ability to locate and capture prey effectively in total darkness is critical for cave predators. *Gyrinophilus porphyriticus* are the least morphologically and physiologically modified salamanders found deep within caves, yet they can be found in large numbers in Kentucky, Virginia, and West Virginia caves (Cooper and Cooper 1968). Feeding behavior and community ecology of *G. porphyriticus* larvae from caves in southwest Virginia were analyzed by Culver (1973 and 1975). Culver collected 7 *G. porphyriticus* larvae from 3 caves in Lee County Virginia, and studied their behavior when presented with amphipods and isopods in darkroom aquaria. None of the larvae responded to dead prey, however, when live prey was added to the water they would assume a predatory posture. *Gyrinophilus porphyriticus* larvae responded to aquatic crustacean prey by raising up on their legs, remaining motionless until the amphipod or isopod came within 2-4 cm of its snout and ate it with a rapidly sucking motion, sometimes lunging at the prey (Culver 1973). Pylka and Warren (1958) described a similar posture and sucking technique in the troglobitic salamander *Haideotriton wallacei*. By rising up on there legs, aquatic cave-dwelling salamanders maximize the surface area available for detecting the water movements of prey.

Mechanoreception appears to be the primary method of prey detection for *G. porphyriticus* larvae, even when observed in a brightly lighted room (Culver 1973). Foraging in stream bank burrows and streambed interstitial spaces has probably preadapted spring salamanders for feeding in the absence of light. *Gyrinophilus porphyriticus* larvae may use some chemoreception for detecting prey as well. Culver (1973) found that after a larva had eaten an isopod or amphipod, a feeding response could be initiated by merely moving a pair of forceps slowly through the water. Prior to feeding, larvae would not respond to the forceps, implying the need for a chemical cue along with movement. Cooper and Cooper (1968) observed *G. porphyriticus* larvae react immediately to mechanical stimulation of the water and would even bite bare forceps, however this might have been the result of conditioning of the captive individuals to feeding by the observers (Brandon 1971). *Gyrinophilus palleucus* also appears to be sensitive to mechanical stimulation of the water (Cooper and Cooper 1968).

By counting food boluses in the digestive tracts of Georgia Blind Salamanders, *Haideotriton wallacei*, Peck (1973) determined 67% of feeding attempts were successful. Laboratory observations of feeding activities of *G. porphyriticus* larvae, revealed that when they did not lunge at prey their success rate was comparable to *Haideotriton* at 69% (Culver 1973). Feeding success was greatly decreased when larvae lunged at prey. Lunging occurred in ~46% of feeding attempts and resulted in a 40% success rate for isopod prey and 0% for amphipod prey. This behavior may be an evolutionary carry-over from life in epigean habitats where prey could be visually located and is maladaptive for feeding in total darkness (Culver 1973).

The Diets of Cave-dwelling Salamanders

Published accounts of the cave diets of *Haideotriton wallacei* (Lee 1969; Peck 1973), *Typhlotriton spelaeus* (Smith 1948; Brandon 1970), *Eurycea lucifuga* (Smith 1948; Hutchison 1958; Peck 1974), *Eurycea longicauda* (Hutchison 1958), *Plethodon glutinosus* (Peck 1974), *Gyrinophilus palleucus* (Brandon 1967; Cooper and Cooper 1968; Simmons 1975), and *Gyrinophilus porphyriticus* (Cooper and Cooper 1968; Culver 1973, 1975, and 1982) indicate that cave-dwelling salamanders are generalized predators. The more terrestrial troglophilic salamanders *Eurycea lucifuga*, *E. longicauda*, and *Plethodon glutinosus* were found to eat a variety of prey from the twilight and near surface portions of caves. Dipterans, mainly Heleomyzid flies, were their most common food accompanied by isopods, coleopterans, collembolas, araneaens, lepidopterans, orthopterans, and others (Smith 1948; Hutchison 1958; Peck 1974). In Missouri caves, the troglobitic Grotto Salamander, *Typhlotriton spelaeus*, subsists on isopods, dipterans, coleopterans, gastrapods, and orthopterans (Smith 1948). In caves studied by Smith (1948) isopods accounted for 96% of the food bulk of aquatic larvae, however in another cave studied by Brandon (1970) there appeared to be a complete lack of aquatic crustaceans. Brandon (1970) found one Missouri *Typhlotriton spelaeus* population to be dependent on the seasonal influx of organic material and invertebrates, such as dipteran larvae, from outside the cave. *Haideotriton wallacei* are neotenic, blind, aquatic, troglobitic salamanders from isolated populations in southern Georgia and northern Florida. They are one of the most highly cave-specialized plethodontid salamanders and also appear to be generalist feeders subsisting on troglobitic amphipods, isopods, ostracods, copepods, and other invertebrates common in their aquatic cave communities (Lee 1969; Peck 1973). In food poor environments such as caves, it is likely that natural selection favors individuals that eat a broad range of food (Culver 1985).

Gyrinophilus Diet in Caves

The diets of *Gyrinophilus* in caves appears to be as generalized as in surface populations, however there is usually a much lower diversity of prey available and little competition with other salamander species. *Gyrinophilus palleucus* ssp., Tennessee Cave Salamanders, are found in isolated populations in central Tennessee, northern Alabama, and northwest Georgia (Brandon 1967). In northern Alabama the ranges of *G. palleucus* and *G. porphyriticus* slightly overlap, however they do not occur within the same caves. *Gyrinophilus palleucus* is a troglobitic, neotenic, salamander that largely resembles *G. porphyriticus* larvae with reduced eyes (Brandon 1971). Cooper and Cooper (1968) examined populations of both species in northern Alabama and found differences in the species composition and community structure of the caves they inhabit. Caves with *G. porphyriticus* contained a greater diversity and abundance of terrestrial fauna such as millipedes and crickets. Caves inhabited by *G. palleucus palleucus* had many more troglobitic species such as the crayfish *Orconectes pellucidus autralis* and the isopod *Asellus alabamensis* (Cooper and Cooper 1968). Brandon (1971) found the aquatic invertebrate fauna of a cave inhabited by *G. palleucus nectoriodes*, in Tennessee, to be neither diverse nor abundant and many stomachs he examined were empty. Analysis of *G. palleucus* ssp. stomach contents from various studies have revealed oligochates, amphipods, isopods, crayfish, cladocerans, coleopterans, epheminopterans, plecopterans, dipterans, tricopterans, thrips, and salamanders including *Eurycea lucifuga* and conspecifics as prey (Lazell and Brandon 1962; Brandon 1967; Simmons 1975). *Gyrinophilus palleucus* is probably better adapted for survival in low prey density cave environments than *G. porphyriticus* through a lower metabolism (Cooper and Cooper 1968), neoteny (Bruce 1979), and perhaps feeding efficiency and prey detection. The lack of microsympatry in Alabama caves may be the result of the more cave-adapted *G. palleucus palleucus* out competing *G. porphyriticus* in certain cave environments (Cooper and Cooper 1968). There are however a few records of microsympatry with *G. porphyriticus* in some populations of *Gyrinophilus gulolineatus* in Tennessee (Simmons 1975) and *Gyrinophilus subterraneus* in West Virginia (Besharse and Holsinger 1977). In General Davis Cave, *G. porphyriticus* adults are found alongside *G. subterraneus*. Larval *G. porphyriticus*, however, have not been reported from the cave stream, while large *G. subterraneus* larvae are found frequently (Besharse and Holsinger 1977).

Prior to this study there was little investigation of the diets of cave-dwelling *Gyrinophilus* in West Virginia. Culver's (1973, 1975 and 1982) observations and experiments with cave-dwelling *Gyrinophilus porphyriticus* larvae in southwest Virginia documented predatory responses to isopod and amphipod species. In McClure's Cave their diet consists almost entirely of the amphipod *Crangonyx antennatus* and the isopod *Caecidotea recurvata* (Culver 1973). According to Culver, larvae in the Powell Valley of southwest Virginia and northeastern Tennessee are paler and thinner than larvae in other areas. Also, *G. porphyriticus* adults in Powell Valley are found primarily in the stream in contrast to adults in West Virginia, which are generally found out of water (Culver 1973). He further observed that in West Virginia, *G. porphyriticus* larvae were more sluggish, plumper, and feed on earthworms that are in or near the stream. Brandon's (1971) idea that feeding history can have a large effect on feeding behavior, may explain some of the differences in behavior and habitat use of the Virginia and West Virginia populations observed.

In an earlier diet study of northern Alabama caves, *G. porphyriticus* regurgitated a troglobitic crustacean and reacted "voraciously" to enchytraeid worms in captivity (Cooper and Cooper 1968). The only reference to *G. subterraneus* diet reported in the literature states that they are found along the cave stream, feeding on small invertebrates (Conant and Collins 1991). The diet of *G. subterraneus* is likely to be composed of a broad assortment of local invertebrates and an occasional hapless salamander of the right size.

Community Ecology and Trophic Relationships in Cave Populations

The most obvious discrepancy separating cave from surface food webs is the lack of primary production through photosynthesis. The result is a relative scarcity of food in many caves and a dependency on organic material transported in from the surface (Moore and Sullivan 1978). Food enters caves through three main ways; organic material carried in directly by streams or vertical shafts, dissolved organic material and microorganisms in water percolating through limestone, and finally as feces, eggs, and carcasses of trogloxenes which forage outside caves (Culver 1982). Plant detritus is a very important food source for cave organisms. As floodwaters recede, layers of fine leaf particles, twigs, and mud are deposited throughout the terrestrial environment. These nutrient rich areas are often accompanied by higher densities of cave fauna such as oligochaete worms (Culver 1982). Cave stream ecosystems are characterized by relatively low trophic complexity compared to other freshwater habitats (Culver *et al.* 1995). Culver (1970) found that the 6-8 species in Organ cave streams were typical of the Greenbrier Valley. In many caves, there may be only a few links that connect detritus, detritivore, and top predator.

Salamanders, along with fish and crayfish, are the largest predators in cave streams. *Gyrinophilus porphyriticus* is the often the top predator in West Virginia caves and in Virginia caves where it has a major impact on the microdistribution of amphipods and isopods (Culver 1975). Laboratory experiments by Culver (1973) revealed that *G. porphyriticus* larvae did not discriminate between the isopod, *Caecidotea recurvatus*, and the amphipod, *Crangonyx antennatus*, however the faster swimming ability of the amphipod resulted it being eaten less often. Observations in McClure's Cave and Sweet Potato Cave in the Powell Valley of southwest Virginia, illustrated the effects of *G.*

porphyriticus larvae predation on cave stream communities in contrasting habitats. Culver (1975 and 1982) found densities of *Caecidotea recurvatus* to decrease and *Crangonyx antennatus* to increase in the immediate vicinity of *G. porphyriticus* larvae. In areas where *G. porphyriticus* larvae were absent, *Crangonyx antennatus* densities were lower, indicating that *G. porphyriticus* predation helps stabilize this community. In contrast, in Sweet Potato Cave *C. recurvatus* was completely absent from areas near *G. porphyriticus* larvae and *C. antennatus* densities were reduced. The disparity between these two cave communities is the result of differences in their physical environments. Sweet Potato Cave is characterized by a series of mud bottom rimstone pools. This environment provides little refugia for isopods and amphipods. *Caecidotea recurvatus* is extremely vulnerable to predation in still water and no cover, while *C. antennatus* can persist by swimming away and burrowing in the mud. The physical habitat of McClure's Cave consists of gravel bottom riffles and pools. *Gyrinophilus porphyriticus* larvae were only found in pools, where the still water facilitates mechanoreception. Isopods and amphipods used riffles as refugia from *G. porphyriticus* larvae, leading to the establishment of equilibrium (Culver 1975 and 1982). As top predators *G. porphyriticus* are important in shaping community structure in caves.

Objectives

Culver's (1973 and 1975) studies of *G. porphyriticus* larvae in Virginia caves laid the foundation for our knowledge of Spring Salamander feeding behavior and trophic relationships in cave communities. My objective for this study was to document the composition of *Gyrinophilus* stomach contents in selected West Virginia Caves. I also wanted to look for differences in the diets of different cave populations, life stages, and seasons in which data were collected.

Materials and Methods

Field Methods

Monitoring Sites

In July of 2002, salamander population monitoring was initiated at Ludington Cave in Greenbrier County near the town of Maxwelton (Figure 2-2). In November 2002 a second monitoring site was established at Rehoboth Church Cave in Monroe County near Union, West Virginia. Both monitoring sites are characterized by surface streams flowing into the mouth of the cave from cow pastors. Monitoring trips were spaced approximately three weeks apart, however some trips were postponed due to weather hazards. Monitoring was concluded in July 2003, for a total of 14 surveys of Ludington Cave and 10 surveys of Rehoboth Church Cave. Additional data was collected on for these populations during supplemental diet analysis trips in October 2003.

The Ludington study site is reached in 45 minutes to 1 ½ hours of crawling through the zigzagging "polar passage", a ~35 foot repel, and following the stream passage until you rejoin the original stream (Figure 4-1). The study area was nicknamed "Salamander Junction" by Marianne Saugstad of the West Virginia Association for Cave Studies, while she was involved with mapping the cave. According to Marianne, they counted as many as 80 large spring salamanders in this area on one extremely hot dry summer day. The mud banks along the stream are structured like a layer-cake of sticks, organic debris, gravel, and mud (Figure 4-2). The stream consists of riffles and pools with small and medium sized cobble. The presence of debris lodged in the ceiling and sticking to the walls is evidence that water completely fills the stream corridor during the spring floods. The sampling area consists of a 20 m length of stream and the adjacent mud banks and dunes, totaling 100 m^2.

The second monitoring site lies below the oldest church west of the Allegheny Mountains. The Rehoboth Church Cave study area is reached in about 30-45 minutes of scrambling over boulders, crawling through break-down, and climbing around plunge-pools (Figure 4-3). The study area is located at the junction of the main stream passage and the Sand Passage. This area is comprised of large boulders and high, steep mud banks. Organic debris is prevalent in the layers of mud throughout the banks and clinging to the walls and ceiling. Like Ludington, this cave floods to the ceiling during high water events and it is not uncommon to see still green duckweed plastered to the ceiling. The stream consists of riffles, pools, and undercut bedrock with small and medium sized cobble.

The two cave populations were monitored for one year using mark-recapture techniques described by Donnelly and Guyer in Heyer et al. 1994. This mark-recapture data will be useful for estimating population sizes, however I have saved this analysis for future a manuscript. Only community ecology observations and diet analysis information will be detailed in this chapter.

Salamander Sampling

Sampling, consisted of flipping cover-objects, scanning the mud banks and streambed, and carefully examining the bank-cut profile, cracks, and tunnels. When salamanders were located they were captured using an aquarium net or by hand. A numbered piece of trail tape was used to mark the capture point of each salamander. The number on the tape corresponded with the bag containing the captured salamander, ensuring release at the point of capture. The location of each capture point was measured in meters and degrees from a designated wire flag, using a meter tape and compass. Additional capture point information collected includes; substrate (mud, sand, gravel, bedrock, organic debris), habitat (mud bank, bank-cut, stream, drip pool, seep), water depth, and cover object size.

Captured salamanders were examined for abnormalities such as parasites, regenerating body-parts, scars, deformities, prominence of lateral line sensory organs, and unusual pigmentation (Figure

4-4). Life stage and evidence of reproductive morphology such as maturing follicles were also recorded. Spring Salamanders are notoriously slippery and squirmy, so it was necessary to anesthetize them to decrease stress upon them and facilitate examination and stomach flushing. A 1:2000 solution of 0.125 g tricaine methanesulfonate (Finquil, MS-222) per 250 ml water was used to temporally anesthetize salamanders (Cooper 2003). This dosage is recommended by the Canadian Council on Animal Care (CCAC) (1993) and buffered with sodium bicarbonate as recommended. The solution proved to be very effective on *G. porphyriticus*, with anesthesia taking place in under 3 to 5 minutes. Once an individual was anesthetized it was promptly removed from the bath for examination, then placed in fresh water. Recovery time was 5 to 10 minutes with no observed deleterious effects.

Gyrinophilus porphyriticus were measured using dial calipers. The following measurements were taken for each adult; total length (TL), snout vent length (SVL), cranial width (CW), cranial length (CL), trunk width (TW), and snout length (SL). Occasionally a rough measurement of eye diameter was taken (ED). These measurements are described in detail in Table 3-1. Larval *G. porphyriticus* and other salamander species were typically only measured for TL and SVL.

Environmental data were collected with each monitoring survey. Soil temperature at 3 cm (ST) taken with 2 Reotemp dial thermometers and later a Taylor Professional digital pocket thermometer, ambient air temperature (AT) was taken with 2 Enviro-Safe armored thermometers and a digital thermometer, relative humidity (RH) was measured with 2 Digital Max/Min Thermohygrometers, water temperature (WT) taken with 2 Enviro-Safe armored thermometers, and water pH measured with an Oakton Instruments pHTestr 2 with ATC. Stream depth and width was measured at 2 designated locations at each study site. The high water mark was determined by pouring white sand in a perpendicular line from the stream edge up the bank to the wall or ceiling. Upon return visits the lower portions of this line were washed away to reveal the highest water level since the last visit. After each survey the sand line was replenished. Additional observational data such as stream turbidity and external weather were also recorded. These environmental data will be analyzed along with mark-recapture data as a supplemental manuscript.

Diet Analysis

Diet information was collected in the field through stomach flushing anesthetized individuals. A 10cc or 20cc syringe fitted with an 18 gauge rubber tube was inserted into the mouth of the salamander and carefully guided down the esophagus to the stomach. As water was flushed into the stomach of the salamander it regurgitated its contents into a container. After flushing, anesthetized salamanders were placed in bags of fresh water until they became active again. Upon their revival, salamanders were released and showed no signs of injury. Stomach contents were placed in 70 % ethanol and taken back to the lab for analysis with a microscope. Life stage, total length, snout-vent length, and habitat were recorded for each flushed salamander. These methods for capturing stomach contents were proven to be effective in earlier amphibian diet studies at Marshall University conducted by Raimondo (1999), Lindley (1999), and Longenecker (2000). Forty-nine salamander stomach contents were analyzed for this study including; 17 metamorphosed *G. porphyriticus* and 6 larval *G. porphyriticus* from Rehoboth Church Cave and 20 metamorphosed *G. porphyriticus*, 4 larval *G. porphyriticus*, 1 adult *Desmognathus fuscus*, and 1 larval *Pseudotriton ruber* from Ludington Cave. Stomach contents were collected during summer (n= 23), autumn (n= 21), and winter (n= 3).

In addition to the live specimens flushed, the digestive tracts of 3 *G. subterraneus* larvae and two metamorphosed *G. porphyriticus* preserved specimens from General Davis Cave were removed and contents analyzed. These specimens are from the personal collection of Michael E. Seidel and the West Virginia Biological Survey collection at Marshall University (WVBS 4516 and WVBS 4515).

Stomach contents were identified according to Borror et al. (1989), Merritt and Cummings (1996), and with assistance from Dr. Thomas G. Jones of Marshall University. Feeding behavior and ecological interactions were observed during cave inventory and monitoring trips. This information was not systematically gathered and therefore is strictly observational.

Statistical Analysis

Individual prey items were difficult to discern due to their varying degrees of digestion. Since it was not possible to record accurate numerical counts of individual prey items, stomach contents were recorded categorically as presence or absence of prey taxonomic orders. Statistical analysis was completed using SPSS version 11.5 software. Logistic regressions were used to test for statistical significance ($P < 0.05$) of location, life stage, and season as predictors of stomach contents. These categorical predictor variables were incorporated into logistic modeling by converting them to dummy variables as recommended by Quinn and Keough (2002). The presence of a taxonomic order of prey was represented as a "1" when present and a "0" when absent for this analysis.

Results

Logistic regression analysis for the occurrence of annelids did not reveal a statistically significant difference in terms of life stage, location, or season. There was, however, a statistically significant difference ($P<0.05$) between life stages in terms of the presence of prey items other than annelids. These data indicate that metamorphosed *G. porphyriticus* are less likely than larvae to contain prey items other than annelids.

Although differences between locations (Tables 4-1 and 4-2) and seasons (Table 4-3) were not statistically significant, observable differences can be described. Twenty-three of the 37 (62.2%) metamorphosed *G. porphyriticus* (Table 4-1) and 8 of the 10 (80%) larvae (Table 4-2) contained food items. Annelids were the only prey items found in both Rehoboth adults and larvae, while the Ludington salamanders contained 6 taxonomic orders of prey and 3 shed skins (Table 4-4). Of the 13 stomachs containing food in Ludington metamorphosed *G. porphyriticus,* 12 (92.3%) contained earthworms (Annelida), 1 contained a mite (Acarina), and 1 contained a beetle larva (Coleoptera) (Table 4-1). Ludington larvae contained food in 3 out of 4 stomachs of which there were 2 (50%) amphipods, 1 annelid, 1 isopod, and 1 snail (Gastropoda) (Table 4-2). Every stomach in this analysis contained debris in the form of sand, gravel, mud, leaf particles, or other organic debris. The *Desmognathus fuscus* adult and *Pseudotriton ruber* larva flushed from Ludington cave were mostly empty with traces of indistinguishable debris.

All 5 specimens from General Davis Cave contained food items (Table 4-5). As a result of small sample sizes no statistical analysis was possible for this population and all results are merely descriptive. A mass of annelid remains was found in 1 *G. porphyriticus* adult, and tiny ant (Hymenoptera) particles were found in the other. Of the 3 larval *Gyrinophilus subterraneus* specimens, 2 (66.7%) contained annelids, and 1 contained a mite (Acarina). Similar to the other study caves, all individuals in General Davis Cave contained sand, gravel, mud, leaf particles, or other organic debris.

Discussion

I choose to monitor populations in Ludington and Rehoboth Church caves because of the relatively large *Gyrinophilus* populations. These communities are rich in detritus and invertebrates appear to be abundant. Earthworms and their castings are visible on the mud banks and amphipods and isopods are under most rocks in the streams.

These data indicate a statistically significant difference ($P<0.05$) between metamorphosed and larval *G. porphyriticus* in terms of presence of non-annelid prey items. The higher occurrence of prey items other than annelids in larvae may suggest a higher diversity of prey available in the aquatic environment. An inventory of potential prey species in these study caves would be useful for making comparisons between terrestrial and aquatic habitats.

Analysis of all stomach contents from Ludington and Rehoboth revealed 62.2 % of metamorphosed *G. porphyriticus* (n=37) (Table 1), and 80% of larval *G. porphyriticus* (n=10) (Table 2) contained discernable food items. This relatively high percentage may attest to the relative adeptness of *G. porphyriticus* as predators in total darkness. These results are also comparable to the feeding efficiencies of *G. porphyriticus* larvae calculated by Culver (1973) of 69% and the 67% determined for *Haideotriton wallacei* by Peck (1973). In General Davis Cave 100% of specimens contained some food remains. The low sample size (n=5), however, make these results less definitive than those from Ludington and Rehoboth Church Caves.

All individuals sampled from the 3 caves contained debris such as sand, small gravel, mud, and leaf particles. Compared to this study, Lindley's (1999) diet analysis of *G. porphyriticus* in West Virginia surface streams, produced much lower percentages of stomachs containing debris. Only 11.1% of metamorphosed *G. porphyriticus* and 5.6 % larval *G. porphyriticus* in Lindley's study contained debris. The much higher occurrence of ingested debris in cave populations could be the result of a higher likelihood of missed feeding attempts. In total darkness, missed feeding attempts may produce a mouth full of substrate. Ingested debris could also be mud and detritus from the digestive tracks of consumed earthworms. Annelids accounted for 95.7% of prey items of metamorphosed *G. porphyriticus* in this study and 0 % of the prey in Lindley's (1999) epigean study. Annelids were found in 60 % of the total stomachs of both metamorphosed and larval *G. porphyriticus* in this study. Other studies revealed annelids in the diets of surface-dwelling *G. porphyriticus* but never were they as prevalent as in these cave populations. For example, earthworms were found in 15% of stomachs in New York (Davis 1932 in Bishop 1941) and 14.3% in North

Carolina (Bruce 1979). Brandon (1967) found annelids in only one (12.5%) of the troglobitic *G. palleucus necturoides* he examined. Cooper and Cooper (1968) noted that the species assemblages of *G. palleucus* caves contrasted with *G. porphyriticus* caves in northern Alabama. A greater reliance on aquatic cave fauna due to a lower abundance of annelids in certain cave communities, may have contributed to *G. palleucus* speciation.

Comparable to my cave *G. porphyriticus* results, Brandon (1967) found "dirt" in 75% of *G. palleucus necturoides*. Other cave-dwelling salamanders have also been reported to contain debris in their stomachs. Studies of *Eurycea lucifuga*, *Eurycea longicauda*, *Typhlotriton spelaeus*, and *Plethodon glutinosus* found grains of sand and plant debris in their digestive tracts (Smith 1948; Hutchison 1958; Peck 1974). Both Lee (1969) and Peck (1973) found silt and debris in the digestive tracts of 87.5% of *Haideotriton wallacei* examined. This silt and debris was probably sucked in inadvertently while feeding or attempting to feed. Lee (1969) suggested that silt may be ingested intentionally as a source of nutrition. Vandel and Bouillon (1959) found that captive young European cave salamanders, *Proteus anguinus*, thrived on a diet of clayey mud and its microfauna. *Proteus anguinus* fed exclusively on clay and silt for one year grew 22-60 mm, possibly due to organic nutrients and micro-crustaceans contained within (Vandel and Bouillon 1959; Culver 1985). It is unlikely that *Haideotriton* or *Gyrinophilus* ingest mud and debris intentionally, since this would be a very unusual behavior for an exclusively predatory group such as Plethodontid salamanders. Clayey mud however, could represent a major source of nutrition in cave environments. Culver (1985) speculated that natural selection should favor dietary shifts to alternate food sources when they are much more predictable and calorically rewarding.

Three *G. porphyriticus* adults from Ludington Cave contained shed skins. Salamanders periodically shed their skins as they grow and eat the old slough as a source of nutrients (Stebbins and Cohen 1995). Nutrients contained in a shed skin could be particularly essential in a cave, where food can be scarce. A *G. porphyriticus* study in New York revealed 9.4 % of stomachs contained shed skin (Hamilton 1932 in Bishop 1941). Diet studies of *Eurycea lucifuga* and *E. longicauda* also discovered shed skins within stomach contents (Smith 1948; Hutchison 1958).

Conclusions

The predominance of annelids in the diets of the *G. porphyriticus* in this study may appear to suggest an earthworm specialization. I, however, do not support this interpretation. Research on a wide array of Plethodontid salamanders describes broad generalist diets limited only to live prey small enough to fit within the salamander's mouth. Earthworms are probably the most abundant and easily captured food source in these ecological communities. In addition, soft-bodied organisms such as earthworms require less energy to digest and may therefore be more calorically rewarding than organisms with chitinous exoskeletons (Jaeger and Barnard 1981). The occurrence of the high densities of earthworms associated with thick mud and detritus banks is probably essential for supporting large densities of spring salamanders. In areas of caves without concentrations of mud, debris, and earthworms, observed salamander densities were always low. In West Virginia, cave-dwelling *G. porphyriticus* are most likely opportunistic generalist predators, which thrive in areas of high concentrations of mud, detritus, and earthworms.

Chapter IV:

Tables and Figures

Table 4-1. Adult *Gyrinophilus porphyriticus* stomach contents from Ludington and Rehoboth Church Caves, West Virginia.

	Ludington (n= 20)			Rehoboth Church (n= 17)			Combined Total (n= 37)		
	Frequency Occurrence	% Occurrence	% Stomachs w/ Food	Frequency Occurrence	% Occurrence	% Stomachs w/ Food	Frequency Occurrence	% Occurrence	% Stomachs w/ Food
Annelida	12	60.0	92.3	10	58.8	100.0	22	59.5	95.7
Acarina (mite)	1	5.0	7.7	0	0	0	1	2.7	4.4
Coleoptera (beetle larva)	1	5.0	7.7	0	0	0	1	2.7	4.4
Shed Skin	3	15.0	23.1	0	0	0	3	8.1	13.1
Debris: Mud, Sand, Plant	20	100.0	100.0	17	100.0	100.0	37	100.0	100.0
Stomachs w/ Food	13	65.0	100.0	10	58.8	100.0	23	62.2	100.0
No Food Items	7	35.0	0.0	7	41.2	0	14	37.8	0

Table 4-2. Larval *Gyrinophilus porphyriticus* stomach contents from Ludington and Rehoboth Church Caves, West Virginia.

	Ludington (n= 4)			Rehoboth Church (n= 6)			Combined Total (n= 10)		
	Frequency Occurrence	% Occurrence	% Stomachs w/ Food	Frequency Occurrence	% Occurrence	% Stomachs w/ Food	Frequency Occurrence	% Occurrence	% Stomachs w/ Food
Annelida	1	25.0	33.3	5	83.3	100.0	6	60.0	75.0
Amphipoda	2	50.0	66.7	0	0	0	3	30.0	37.5
Gastropoda (snail)	1	25.0	33.3	0	0	0	1	10.0	12.5
Isopoda	1	25.0	33.3	0	0	0	1	10.0	12.5
Debris: Mud, Sand, Plant	4	100.0	100.0	6	100.0	100.0	10	100.0	100.0
Stomachs w/ Food	3	75.0	100.0	5	83.3	100.0	8	80.0	100.0
No Food Items	1	25.0	0	1	16.7	0	2	20.0	0

Table 4-3. Seasonal comparison of *Gyrinophilus porphyriticus* adult and larval stomach contents from Ludington and Rehoboth Church Caves combined.

	Summer (n= 23)			Autumn (n= 21)			Winter (n= 3)		
	Frequency Occurrence	% Occurrence	% Stomachs w/ Food	Frequency Occurrence	% Occurrence	% Stomachs w/ Food	Frequency Occurrence	% Occurrence	% Stomachs w/ Food
Annelida	15	65.2	93.8	10	47.6	83.3	3	100.0	100.0
Amphipoda	2	8.7	12.5	2	9.5	16.7	0	0	0
Isopoda	0	0	0	1	4.8	8.3	0	0	0
Acarina (mite)	1	4.4	6.3	0	0	0	0	0	0
Gastropoda (snail)	0	0	0	1	4.8	8.3	0	0	0
Coleoptera (beetle larva)	0	0	0	1	4.8	8.3	0	0	0
Cast Skin	1	4.4	6.3	2	9.5	16.7	0	0	0
Debris: Mud, Sand, Plant	23	100.0	100.0	21	100.0	100.0	3	100.0	100.0
Stomachs w/ Food	16	69.6	100.0	12	57.1	100.0	3	100.0	100.0
No Food Items	7	30.4	0	9	42.9	0	0	0	0

Table 4-4. Diversity of taxonomic orders of prey found in metamorphosed *Gyrinophilus porphyriticus*, *larval G. porphyriticus,* and larval *G. subterraneus* stomach contents from Ludington Cave, Rehoboth Church Cave, and General Davis Cave, West Virginia.

Location	Species and Life Stage	Life Stage Prey Order Diversity	Location Prey Order Diversity
Ludington Cave	*G. porphyriticus,* metamorphosed	3	6
	G. porphyriticus, larval	4	
Rehoboth Church Cave	*G. porphyriticus*, metamorphosed	1	1
	G. porphyriticus, larval	1	
General Davis Cave	*G. porphyriticus*, metamorphosed	2	3
	G. subterraneus, larval	2	

Table 4-5. Metamorphosed *Gyrinophilus porphyriticus* and larval *Gyrinophilus subterraneus* stomach contents from General Davis Cave, West Virginia.

	Metamorphosed *G. porphyriticus* (n= 2)			Larval *G. subterraneus* (n= 3)			Combined Total (n=10)		
	Frequency Occurrence	% Occurrence	% Stomachs w/ Food	Frequency Occurrence	% Occurrence	% Stomachs w/ Food	Frequency Occurrence	% Occurrence	% Stomachs w/ Food
Annelida	2	100.0	100.0	2	66.7	66.7	4	80.0	80.0
Acarina (mite)	0	0	0	1	33.3	33.3	1	20.0	20.0
Hymenoptera (ant)	1	50.0	50.0	0	0	0	1	20.0	20.0
Debris: Mud, Sand, Plant	2	100.0	100.0	3	100.0	100.0	5	100.0	100.0
Stomachs w/ Food	2	100.0	100.0	3	100.0	100.0	5	100.0	100.0
No Food Items	0	0	0	0	0	0	0	0	0

Figure 4-1. Michael Osbourn repelling down "New Drop" at the end of the Polar passage in Ludington Cave

(W. B. Sutton)

Figure 4-2. Typical stream bank habitat associated with *Gyrinophilus* populations.
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(W. B. Sutton)

Figure 4-3. Formations in Rehoboth Church Cave.


(W. B. Sutton)

Figure 4-4. Lisa Smith and Michael Osbourn processing salamanders for diet analysis in Rehoboth Church Cave (above). *Gyrinophilus porphyriticus* before processing (below).

(W. B. Sutton)

(W. B. Sutton)

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Appendix I:

***Gyrinophilus* Taxonomy and Nomenclature**

SPRING SALAMANDER

***Gyrinophilus porphyriticus* ssp. (Green)**

Class **Amphibia**
Order **Caudata**
Family **Plethodontidae**
Tribe **Hemidactyliini**
Genus ***Gyrinophilus* Cope, 1869**
Species ***porphyriticus* (Green)**

Synonyms:

Salamandra porphyritica Green, 1827

- Green, J. 1827. An account of some new species of salamanders. Contrib. Maclurian Lyceum, 1(1):3-8.
- Holotype not extant.
- Neotype- same location. Brandon, R. A. 1966. Systematics of the salamander genus *Gyrinophilus*. Illinois Biol. Monograghs., 35:1-86.

Spelerpes? porphyritica Gray, 1850

- Gray, J. E. 1850. Catalogue of the specimens of amphibia in the collection of the British Museum, part 11. Batrachia Gradientia, ect. [British Museum], London. 72 pp.

Gyrinophilus porphyriticus Cope, 1869

- Cope, E. D. 1869. A review of the species of the Plethodontidae and Desmognathidae. Proc. Acad. Nat. Sci. Philadelphia, 21: 93-118.
- Transfer of genus

Geotriton porphyritica Garman, 1884

- Garman, S. 1884. The North American reptiles and batrachians. Bull. Essex Inst., 16:1-46.

Pseudotriton porphyriticus Organ, 1961

- Organ, J. A. 1961. The eggs and young of the spring salamander, *Pseudotriton porphyriticus*. Herpetological, 17(1): 53-56.

Salamandra salmonea Storer, 1838

- Holbrook, J. E. 1838. North American herpetology. Vol. 3, 122 pp. J. Dobson and Son, Philadelphia.

Pseudotriton salmoneus Baird, 1850
Spelerpes salmonea Gray 1850
*Ambystoma salmon*eum: Durmeril, Bibron, & Dumeril, 1854
Spelerpes salmoneus: Cope, 1866

Description of Genus: (Cope 1869 in Brandon, 1966):

- Tongue free all around
- Premaxillae usually separate

- Fontanel between unfused nasal processes of premaxilla
- Septomaxilla present
- Prefrontals present
- Prevomerine and paravomerine teeth continuous
- Occipital condyles not stalked
- Canthus rostralis present

Type-locality: "French Creek, near Meadville, Crawford County, Pa."

Diagnosis: "*G. porphyriticus* differs from *G. palleucus* in that it naturally metamorphoses from an aquatic larva dwelling in springs or small streams into a semiterrestrial adult which lacks gills, has a canthus rostralis, develops eyelids, loses the caudal fin, and undergoes the cranial changes already discussed (pp. 8-11). *G. palleucus* is neotenic.

Eyes of larval *G. porphyriticus* are larger than those of *G. palleucus*, tooth counts are lower, and the head is not so broad, flat, and spatulate" (p. 30 Brandon, 1966).

Holotype: not extant. See *G. p. porphyriticus* neotype.

West Virginia Subspecies

***Gyrinophilus porphyriticus porphyriticus* (Green), Northern Spring Salamander**

Synonyms:

- *Salamandra porphyritica* Green, 1827
- *Gyrinophilus porphyriticus porphyriticus* Stejneger & Barbour, 1933
- *Gyrinophilus porphyriticus inagnoscus* Mittleman, 1942

Neotype: "MCZ 35778, male, collected 15 April 1962, by J.D. Lazell, Jr., and L.M. Lazell in a small spring-fed stream (flowing directly into French Creek) at Liberty and Linden streets, Meadville, Crawford Co., Pa." (pp. 32-33 Brandon, 1966).

Description of neotype: "Male, 18 trunk vertebrae (17 costal grooves); measurements taken one day after fixation in 10 per cent formalin – head width just behind eyes, 17.7 mm; snout to gular fold mid-ventrally, 21 mm; snout-anterior margin of vent, 105 mm; snout-posterior margin of vent, 113 mm; posterior margin of vent to tip of tail, 67 mm; distance from axilla to groin, 64 mm; canthus rostralis interrupted near anterior end of snout by dark pigment, continuous in nasolabial groove region; lower eyelid clear, upper darkly pigmented; dorsum and sides above upper limb insertion covered by brown and black mottlings – black pigment especially pronounced on dorsum of head and forelimbs, and a pair of patches on the anterior third of trunk; entire ventral surface flesh colored and heavily covered with fine dark flecks (color notes were taken from the specimen in life)." (pp. 32-33 Brandon, 1966).

***Gyrinophilus porphyriticus duryi* (Weller), Kentucky Spring Salamander**

Synonyms:

- *Triturus lutesens* Rafinesque, 1832
- *Gyrinophilus lutesens* Mittleman, 1942
- *Pseudotriton duryi* Weller, 1930
- *Gyrinophilus duryi* Weller, 1931
- *Gyrinophilus porphyriticus duryi* Stejneger & Barbour, 1933
- *Gyrinophilus danielsi duryi*, King, 1939

Lectotype: "Female, USNM 84300 (designated by Walker and Weller, 1932), collected 6 April 1930, by R. Dury and W.H. Weller" (p.42 Brandon, 1966).

Type-locality: Cascade Caverns, near Grayson, Carter Co., Ky.

Paralectotypes: "Six topotypes mentioned by Weller (1930), and identified by Cincinnati Society of Natural History numbers 499 a-c, e-g. Two of these specimens are known to be in other collections now (MCZ 17540; CM 10937). The other four have not been located. They are no longer in the CSNH collection (now a part of the University of Cincinnati collection)" (p.42 Brandon, 1966)..

Diagnosis: *G. p. duryi* (Fig. 1B) was well distinguished from *p. porphyriticus* and *p. danielsi* by Walker and Weller (1932, p. 82): ". . .*duryi* differs from *porphyriticus* in the presence of distinct black spots and in the lighter color of the upper parts; from *danielsi* in the lateral concentration of t he spots, the absence of a black line along the canthus, and, when large specimens are compared, in the narrower head." Additionally, *duryi* differs from *dunni* by not being profusely flecked dorsally and in lacking the black line along the canthus, and from *palleucus* by undergoing metamorphosis." (p. 42 in Brandon, 1966).

<u>Southern Subspecies (Not within West Virginia):</u>

***Gyrinophilus porphyriticus dunni* Mittleman & Jopson**
***Gyrinophilus porphyriticus danielsi* (Blatchley)**

WEST VIRGINIA SPRING SALAMANDER

***Gyrinophilus subterraneus* Besharse & Holsinger, 1977**

Class **Amphibia**
Order **Caudata**
Family **Plethodontidae**
Tribe **Hemidactyliini**
Genus ***Gyrinophilus* Cope, 1869**
Species ***subterraneus* Besharse & Holsinger, 1977**

Besharse, J. C. and J. R. Holsinger, 1977. *Gyrinophilus subterraneus,* a new troglobitic salamander from southern West Virginia. Copeia. (4)

Synonyms: *Gyrinophilus porphyriticus ssp.* (Green)

Type-locality: General Davis Cave, Greenbrier County, West Virginia.

Diagnosis: "A cave dwelling *Gyrinophilus* apparently closely related to *G. porphyriticus* but differing greatly from it as follows: larva attaining greater maximum body size [112 mm svl (= distance in mm from snout to posterior angle of vent) compared to 80 mm svl in *G. porphyriticus*, having smaller eyes (ratio of eye diameter to svl of 0.015-0.031 compared to 0.026-0.031 in *G. porphyriticus*), greater head width behind the eyes (ration of head width to head length of 0.80-1.03 compared to 0.80-0.90 in *G. porphyriticus*), and two to three irregular rows of pale spots laterally: transformed adult having smaller, reduced eyes (ration of eye diameter to svl of 0.023-0.031 compared to 0.032-0.036 in *G. porphyriticus*), paler color and an indistinct canthus rostralis" (Besharse and Holsinger, 1977).

Description of holotype: (Holotype, USNM 198533) "Mature, postmetamorphic female with oviducts enlarged (0.5 mm diameter), convoluted and medially placed and oocytes small (less than 0.5 mm). 159 mm total length: 101 mm svl: weight 10.1 g (wet). Length of head from tip of snout to gular fold 19 mm; greatest width of head posterior to eyes 13.1 mm; snout width at anterior margin of eyes 9.8 mm; length of snout from anterior margin of eyes 5.6 mm. Eye easily visible, diameter (right) 3 mm. Length of forelimbs 15 mm: length of hind limbs 18.5 mm. Toes of forelimb, in order of increasing length, 1-4-2-3; toes of hind limb, in order of increasing length, 1-5-2-3-4. Costal grooves between axilla and groin 17, 7 between appressed limbs; trunk vertebrae 18. In life the holotype had a pale gray ground color, with dark lateral and dorsal reticulations. The venter was flesh-colored with a few widely scattered, dark spots on the lower jaw. After 16 months in ethyl alcohol the ground color had become lighter, making the dark reticulations more distinct" (Besharse and Holsinger 1977).
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Description of larva: (paratype, USNM 198535) "Immature male 177 mm total length; 107 mm svl; weight 12.6 g (wet). Length of head from tip of snout to gular fold 19.2 mm; greatest width of head posterior to eyes 18.5 mm; snout width at anterior margin of eyes 13.2 mm; length of snout from anterior margin of eyes 4.9 mm. Diameter of right eye 2.4 mm; eye sunken, cornea barely visible from surface (diameter of cornea 1 mm). Length of forelimbs 17 mm; length of hind limbs 17 mm. Toes of forelimb in order of increasing length, 1-4-2-3; toes of hind limb, in order of increasing length, 1-5-2-4-3. Costal grooves between axilla and groin 17, 7 between appressed limbs; trunk vertebrae 18. The ground color after 9 months preservation in ethyl alcohol was light gray, being somewhat darker on the head and dorsum than on the tail and sides. Sides of body with 2 or 3 irregular rows of light, flesh-colored spots extending from the forelimbs posteriorly onto the tail. Dorsum with very fine, light gray reticulations. Venter flesh-colored, without reticulations" (Besharse and Holsinger 1977).

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TENNESSEE CAVE SALAMANDER

Gyrinophilus palleucus ssp. McCrady, 1954

Class **Amphibia**
Order **Caudata**
Family **Plethodontidae**
Tribe **Hemidactyliini**
Genus ***Gyrinophilus* Cope, 1869**
Species ***palleucus* McCrady, 1954**

McCrady, E. 1954. A new species of *Gyrinophilus* (Plethodontidae) from Tennessee caves. Copeia, 1954:200-206.

Synonyms:
Pseudotriton palleucus Blair, 1961

Type-locality: "Sinking Cove Cave, Franklin Co., Tenn., 5 miles west of Sherwood, at 900-feet elevation." (pp. 62 & 66 in Brandon, 1966).

Diagnosis: " This rather stout-bodied, neotenic species is similar in body form to larva *G. porphyriticus* (Fig. 18). It differs from the latter by having smaller eyes (see key to species), an increased number of premaxillary, prevomerine, and ptergyoid teeth (averages of 25, 32, 20 and 16, 26, 14 in *palleucus* and *porphyriticus*, respectively), a wider head, and a distinctly spatulate snout.

The reported difference from *porphyriticus* in number of costal grooves (McCrady, 1954) resulted from different methods used in counting them (Lazell and Brandon, 1962). Actually the range in number of costal grooves and trunk vertebrae is the same in *palleucus* as in *porphyriticus* (17-19 costal grooves, 18-20 trunk vertebrae)" (pp. 62 & 66 in Brandon, 1966).

Holotype: CNHM 72585, female, collected by E. McCrady, January 1944.

Subspecies:
Gyrinophilus palleucus palleucus McCrady, 1954
***Gyrinophilus palleucus necturoides* Lazell and Brandon, 1962**

BERRY CAVE SALAMANDER

***Gyrinophilus gulolineatus* Brandon, 1965**

Class **Amphibia**

Order **Caudata**

Family **Plethodontidae**

Tribe **Hemidactyliini**

Genus ***Gyrinophilus* Cope, 1869**

Species ***gulolineatus* Brandon, 1965**

Synonyms:

Gyrinophilus palleucus gulolineatus Brandon, 1965

- Brandon, R. A. 1965. A new race of neotenic salamander Gyrinophilus palleucus. Copeia (3).

Gyrinophilus gulolineatus Brandon, 1965

- Collins, J. T. 1991. Viewpoint: A new taxonomic arrangement for some North American amphibians and reptiles. Herpetological Review 22(2), pp. 42-43.

Diagnosis: "A neotenic, cave-dwelling *Gyrinophilus* similar to *G. palleucus*, but differing from them as follows: From *G. p. palleucus* by being more heavily pigmented and having generally fewer trunk vertebrae (18 in 80% of *G. p. gulolineatus,* in 52% in *G. p. palleucus*); from *G. p. necturoides* in vertebral number (no specimen of *G. p. necturoides* has 18); from both *G. p. palleucus* and *G. p. necturoides* by having a distinctive dark stripe on the anterior half of the throat, by having a wider head and more spatulate snout, and perhaps by reaching a greater adult size" (p.347 in Brandon, 1965).

Type-Locality: Berry Cave, Roane Co., Tennessee.

Holotype: CNHM 14327, female. Collected by R. Brandon and J. E. Huheey, 10 July 1963.

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Osbourn, Michael. S. and Thomas. K. Pauley. 2004. The natural history of cave-dwelling spring salamanders, *Gyrinophilus* spp. Cope (Plethodontidae), in West Virginia. Southeastern Biology Vol 51 (2) In Press.

Osbourn, Michael. S. and Thomas. K. Pauley. 2004. Phenotypic variation among cave-dwelling spring salamanders, *Gyrinophilus* spp. Cope (Plethodontidae), in West Virginia, Virginia, and Tennessee Southeastern Biology Vol 51 (2) In Press.

Phu, L. D., M. S. Osbourn, J. E. Bailey, and T. K. Pauley. 2004 The use of streamside salamanders as indicators of headwater stream health in West Virginia. Southeastern Biology Vol 51 (2) In Press.

Osbourn, M. S., L. D. Phu, J. E. Bailey, and T. K. Pauley. 2003. Streamside salamanders as indicators to health of headwater streams in West Virginia. Southeastern Biology Vol. 50 (2): 132.

Johnson, K. J., M. S. Osbourn, and T. K. Pauley. 2002. Seasonal activity patterns of *Plethodon cinereus* and *P. hoffmani* in West Virginia. Southeastern Biology Vol. 49 (2).

ORAL PRESENTATIONS:

Osbourn, Michael. S. and Thomas. K. Pauley. 2004. The natural history of cave-dwelling spring salamanders, *Gyrinophilus* spp. Cope (Plethodontidae), in West Virginia. The 65th Annual Association of Southeastern Biologists Meetings, Memphis TN.

Osbourn, Michael. S. and Thomas. K. Pauley. 2004. Phenotypic variation among cave-dwelling spring salamanders, *Gyrinophilus* spp. Cope (Plethodontidae), in West Virginia, Virginia, and Tennessee. The 65th Annual Association of Southeastern Biologists Meetings, Memphis TN.

Osbourn, M. S., L. D. Phu, J. E. Bailey, M. B. Watson, and T. K. Pauley. 2003. Stream Salamanders as Potential Indicators of Headwater Stream Quality in West Virginia. Mid Atlantic Water Pollution Biology Workshop, sponsored by EPA.

TECHNICAL REPORTS:

Fellers, Gary M. and Michael S. Osbourn. 2004. Inventory of California Red-legged Frogs, *Rana draytonii*, within the wilderness areas of Point Reyes National Seashore. Prepared for the National Park Service, Point Reyes National Seashore, Division of Natural Resources.

Osbourn, M. S., L. D. Phu, J. E. Bailey, M. B. Watson, and T. K. Pauley. 2003. Stream Salamanders as Potential Indicators of Headwater Stream Quality in West Virginia. Prepared for U. S. EPA, U. S. Geological Survey, and WV Division of Environmental Protection.

Osbourn, Michael S. 2001. Winter 2000-2001 Waterbird Survey of Golden Gate National Recreation Area. Prepared for the National Park Service, GGNRA Division of Natural Resources.

RESEARCH GRANTS:

2002 West Virginia Division of Natural Resources Wildlife Diversity Program Grant $5138.00
2002 Marshall University Research Foundation Summer Research Grant $500.00
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2003 Marshall University Research Foundation Grant $ 200.00

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Graduate Research Assistant, August 2001- May 2003
Marshall University, Huntington, WV

Stream Salamander Biomonitoring Study. Funded by USGS/BRD and EPA.

- Coordinated and planned fieldwork as lead research assistant.
- Sampled stream salamanders through out the various eco-regions of WV.
- Collected stream quality data.
- Communicated with partnering agencies.
- Constructed and maintained *MS Access* databases.
- Co-authored and a technical report about stream salamander's potential use in biomonitoring indices in WV, for use by government agencies.

Long Term Monitoring of the Effects of Gypsy Moth Pesticide Applications on Salamander Populations in Monongahela National Forest. Funded by USDA.

- Sampled stream salamanders along transects and with litter bags.
- Sampled terrestrial salamanders in quadrats along transects and night visual encounter surveys.

West Virginia Atlas of Amphibians and Reptiles. Funded by WVDNR.

- Trapped aquatic turtles with hoop and basking traps.
- Collected, measured, and photographed county record amphibians and reptiles.
- Transferred encounter records from topographic maps to GIS using *Arcview*

Amphibian and Reptile Inventory of Gauley River National Recreation Area. Funded by the National Park Service.

- Surveyed for salamanders, anurans, lizards, turtles, and snakes with visual encounter searches and evening call surveys.
- Measured captured amphibians and reptiles and recorded locations with a GPS.

West Virginia Biological Survey Herpetology Collection, Marshall University.

- Preserved and cataloged voucher specimens.

Additional Research Assisted

- Inventory of Cave Invertebrates in Buckeye Creek Drainage, West Virginia
- *Rana pipiens* population study, Green Bottom Wildlife Management Area, WV
- Vernal Pool Monitoring, Beach Fork State Park, WV
- Timber Rattlesnake (*Crotalus horridus*) Radio telemetry Randolph Co., WV

Thesis Research, February 2002 - Spring 2004
Marshall University, Huntington, WV

Phenotypic Variation in Cave-dwelling Spring Salamanders, *Gyrinophilus* spp.

- Collected measurements of morphological characters for *Gyrinophilus* spp. specimens from the U. S. Natural History Museum and WV Biological Survey Collection.
- Used *SAS* statistical software for Principal Components Analysis (PCA), Canonical Discriminate Analysis (CDA), ANOVAs, and t-Tests to reveal variation between groups.

Cave-dwelling *Gyrinophilus* spp. Population Monitoring

- Established mark-recapture study of two cave populations.
- Marked salamanders with visible implant tags (VITs) and visible acrylic elastimer.
- Collected environmental data, i.e. pH, Temp., RH.
- Plotted capture location to calculate home range
- Estimated population size with *Program MARK*.
- Flushed stomachs of live specimens.
- Applied logistic regression analysis of stomach content data using *SPSS* statistical software.

Distribution of Amphibians in West Virginia Caves

- Inventoried Caves throughout Greenbrier and Monroe Counties, West Virginia.
- Compiled historical records of amphibians from WV caves
- Used *Arcview* GIS software to create distribution maps

PROFESSIONAL EXPERIENCE:

Biological Science Technician, GS-0404-07 May 17- September 30, 2004
National Park Service, Point Reyes National Seashore, Point Reyes Station, CA

- Surveyed for federally listed amphibian species.
- Worked independently to inventory ponds within the wilderness areas of Pt. Reyes NS for California Red-legged Frogs, *Rana draytonii*.
- Worked as part of a team to survey longterm monitoring sites of *Rana boylii* and *Rana muscosa* in the Sierra Nevada foothills.
- Conducted dip-net surveys, funnel trap surveys, and night eye shine surveys.
- Navigated cross-country through thick brush, using map, compass, and GPS.
- Weighed, measured, and inspected amphibians for abnormalities.
- Recorded amphibian and habitat data with PDA for upload into database.
- Documented sites with digital photography.
- Prepared a technical report for use by Park Service management staff.

Biological Field Technician, May- August 2001
Penn State University, State College PA

- Conducted stream salamander surveys throughout PA., MD., WV., and VA.
- Collected stream quality data.
- Surveyed for Bog Turtles in Eastern PA.
- Surveyed for Massasauga rattlesnakes (*Sistrurus c. catenatus*) in Western PA.

Aquatic Ecology Intern, National Park Service. December-March 2000
Golden Gate National Recreation Area, Sausalito CA

- Designed and implemented surveys of waterfowl and California Red-legged frogs (*Rana aurora draytonii)*.
- Monitored intertidal organisms.
- Collected stream quality data
- Wrote technical report for use by park resource management staff.

Biological Technician, (Part time) December- February 2000
USGS-Biological Resources Division, Point Reyes, CA

- Conducted an inventory of mammals, reptiles, and amphibians for John Muir and Eugene O'Neill National Historic Sites.

Park Ranger, National Park Service. Oct.-Dec. 2000 and Jun. 1998 – Feb. 1999

Muir Woods National Monument, Mill Valley, CA

- Designed and implemented interpretive walks and talks on redwood ecology and the history of Muir Woods.
- Participated in various resource management projects and ecology research, including; salamanders, small mammals, Coho salmon, Red-Legged Frogs, and native plant restoration.
- Hiked the trails providing nature interpretation and resource protection.
- Provided minor first aid and participated in search and rescue.

Biological Technician, USGS-Biological Resources Division. May-October 2000

Tahoe National Forest, Yosemite National Park, and other parks and forests throughout the Sierra Nevada Mountains, CA.

- Surveyed amphibians in Parks and Forest throughout the Sierra Nevadas and southern Cascade Mountains.
- Navigated cross-country, using map, compass, and GPS.
- Weighed, measured, and inspected amphibians for abnormalities.
- Recorded habitat data i.e.; turbidity, substrate, temperature, and vegetation.
- Documented sites with 35mm photography.

Biological Technician, National Park Service Volunteer, February-April 2000

Big South Fork National River and Recreation Area, TN/KY

- Initiated an amphibian and reptile inventory of the park.
- Conducted backcountry cliff-line surveys for endangered plants and archeological sites.
- Organized Threatened and Endangered Species Data for input into GIS.
- Prepared herbarium specimens.
- Used GPS to plot the locations of historic fields on topographic maps.

Park Ranger, National Park Service. May-September 1999

Olympic National Park, Port Angeles, WA

- Designed and conducted interpretive programs.
- Provided information and assistance to visitors.
- Operated visitor center.
- Roved Hurricane Ridge, providing interpretation and resource protection.

Farm Naturalist, April-June1998

Emandal: A Farm on A River, Willits, CA

- Instructed school groups in organic farming and sustainability.
- Led nature walks.
- Responsible for care of farm animals.

SCA Resource Assistant, **National Park Service Intern** January - March1998

Glacier National Park, West Glacier, Montana

- Led naturalist snowshoe hikes.
- Educated groups on the winter ecology of Glacier National Park.
- Operated the Visitor Center, providing information, book sales, and backcountry permits to visitors.

Instructor/Naturalist, August 1996 - November 1997
Blue Ridge Outdoor Education Center, Toccoa, GA

- Instructed classes on Forest Ecology and Aquatic Ecology.
- Tested water quality through dissolved oxygen tests, turbidity, temperature, pH, and sampling aquatic invertebrates.
- Facilitated High Ropes, Low Ropes, and Rappelling.
- Guided white water rafts on the Chattooga River.
- Responsible for care of captive animals.
- Led wilderness backpacking camps.

Whitewater Raft Guide, June 1997 - November 1997
Wildwater LTD., Long Creek, SC

- Guided rafts on day-long trips down the Chattooga River
- Negotiated class III - V rapids .
- Performed rescues and first aid.
- Educated guests about the history and ecology of the Chattooga National Wild and Scenic River.

Volunteer Botanist/ Horticulturalist, June - August 1996
Maquipucuna Biological Reserve, Ecuador

- Collected, identified, and created gardens of rainforest orchids, epiphytes, and tree ferns.
- Surveyed the reserve's pre-Columbian Indian archeological sites.
- Aided in capturing and relocating a spectacled bear.

Volunteer Horticulturalist, February – June 1996
Atlanta Botanical Garden, Atlanta GA

- Cared for poison-dart frogs
- Designed and maintained exhibits of rainforest plants
- Assisted with propagation and routine care of tropical plants

RELEVANT COURSEWORK :

Highlands Biological Station, Highlands N. C., University of North Carolina, C. H.

- *The Biology of Plethodontid Salamanders* , Instructor: Steven Tilley. Spring, 2002
- *Conservation Biology of Amphibians*, Instructor: Raymond D. Semlitsch. Spring, 2003

Marshall University: *Herpetology, Ornithology, Wetland Ecology, Biomonitoring (Aquatic Biology), Plant Taxonomy, Advanced Plant Taxonomy, Economic Botany, Biostatistics.*

Tambopata Biological Preserve, Peru: *Tropical Ecology Field School*. Spring, 1999

Emory University: *Ecology, Vertebrate Population Biology, Tropical Ecology, Coastal Biology.*

SOCIETY MEMBERSHIPS:

American Society of Ichthyologists and Herpetologists
Association of Southeastern Biologists
Partners in Amphibian and Reptile Conservation
National Speleological Society
West Virginia Association for Cave Studies