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CAVE SURVEYOR AS MICROBIOLOGIST

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INTRODUCTION TO CAVE MICROBIOLOGY: A REVIEW FOR THE NON-SPECIALIST

HAZEL A. BARTON

Department of Biological Sciences, Northern Kentucky University, Highland Heights, KY 41099

Within the past decade there has been an increasing interest in cave microbiota. Such interest has helped many speleologists both recognize and understand the importance of microbial species in caves, which has led to improvements in cave conservation practices to better conserve these unseen ecosystems. While much information on the metabolic properties and functions of such subterranean ecosystems has been published in the microbial ecology literature, is it sometimes unusable by the non-specialist due to technical "jargon" and unexplained background information. It is the aim of this review to provide such background information and to explain the current technologies available to study cave microbiota. In doing so, it is hoped that this material will make the microbiology literature more accessible to interested non-specialists, and open new areas of inquiry in the study of microbial-mineral interactions.

INTRODUCTION

The study of cave microbiology deals with the microscopic life that resides in caves. Microscopic life has historically been grouped into the Monera, one of the five kingdoms of life, and a pretty broad group that includes any microscopic organism that lacks a nucleus; also historically known as the prokaryotes (Whittaker, 1969). The protozoa, which are also microscopic and include such organisms as the amoeba, do contain a nucleus and are classed within the eukaryotes (Whittaker, 1969). While viruses are also of microscopic, they are not technically alive and, although fascinating, will not be discussed within the scope of this review. Animals, plants and fungi all constitute the forms of life that can be seen with the naked eye (macroscopic) and are nucleus-containing eukaryotes.

A BRIEF HISTORY OF MICROBIOLOGY

During the Dark Ages (c.400-700 AD) devastating diseases, such as the Black Death, ravaged humanity. At that time, there was only a vague inkling in medicine that these maladies were transmitted by an infectious agent. Rather, physicians believed in mystical spirits and lethal miasmas, which were responsible for such pestilence. Patients would attempt to ward off these evil spirits with charms and even flowers; the "pocket-full of posies" of the nursery rhyme. These attempts were somewhat beneficial, although not for immediately obvious reasons – breathing through containers of dried flowers effectively filtered out the bacteria that were responsible for the spread of pneumonic plague, perpetuating the myth of evil vapors.

The first awareness of a microscopic world occurred around 300 years ago, when Dutch linen merchant Antoni van Leeuwenhoek devised a rudimentary, hand-made microscope to look at linen fibers. Leeuwenhoek also used his microscope to look at other materials; in pond water he saw tardigrades (for slow-walker), small insect-like organisms he termed animalcules. Eventually, by refining his microscope lens, Leeuwenhoek went on to describe the first bacteria, which he saw in his own dental plaque. These bacteria were so small that it would take two thousand of them stacked end-to-end to traverse the head of a pin, which Leeuwenhoek accurately described in great detail.

Leeuwenhoek's animalcules were considered nothing more than a curiosity for almost 200 years, until the golden age of microbiology and the work of such luminaries as Pasteur and Koch. Louis Pasteur wanted to understand the then-accepted notion of spontaneous generation – that a vital force allowed life to arise spontaneously in food or wine. In attempting to define the existence of this vital force, in 1861 Pasteur successfully demonstrated that this "vital force" was comprised of microorganisms in the air. Pasteur subsequently went on to design ways of killing these organisms in food to prevent spoilage and developed the technique of pasteurization. Robert Koch took the principles of Pasteur and other investigators further, to show that the same microorganisms that could spoil food could also cause disease in humans. In 1876 Koch developed the techniques to identify the causative agent of anthrax. These techniques became an accepted scientific principle, termed Koch's postulates, that can be applied to any pathogenic microbe today, including as recently as in the case of severe acute respiratory syndrome (SARS) virus. Following the work of Pasteur, Koch and other eminent microbiologists, Leeuwenhoek's animalcules went from being simply a curiosity to the basis of the accepted science of microbiology.

With its founding in the medical sciences, the study of microorganisms has historically concentrated on the impact of microorganisms on humans, whether through disease or food spoilage. Nonetheless, in 1887 Sergei Winogradsky made a groundbreaking discovery; he identified microorganisms that did not obtain food through photosynthesis, but could use mineral energy to literally pull food out of thin air, a process termed chemolithotrophy (chemical energy – chemo, food from the environment – litho, to eat – trophy) (Winogradsky, 1887). Despite the identification of microorganisms that defied

Corresponding author address: Hazel A. Barton, Department of Biological Sciences, Northern Kentucky University, SC 204D, Nunn Drive, Highland Heights, KY 41099. Email: bartonh@nku.edu, Phone: 859-572-5303, Fax: 859-572-3956.

all the previously understood conventions of life (that it relies on plant-derived material), Winogradsky's discoveries were again considered a curiosity, and most microbiologists continued to concentrate on just a handful of microbes responsible for human disease. Of course, once again it was proved that an important microbial activity was overlooked: microbiologists have since determined that Winogradsky's chemical-eating organisms are among the most significant life forms on the planet (Madigan *et al.*, 2000).

Due to an ability to consume inorganic material for energy, it is not surprising that microorganisms interact directly with the geology of our planet. Their activities have helped shape the global environment in which we live, provided the oxygen that we breathe and even the oil that drives our economy (Gold, 1999; Madigan *et al.*, 2000). These microbes also turn over the essential nutrients, such as carbon (C), nitrogen (N), sulfur (S) and phosphorus (P), of our ecosystem. Indeed, without the activities of these microorganisms, all macroscopic life on the planet would cease, while these microbes would survive quite happily without us (Madigan *et al.*, 2000). Obviously, as the science of microbiology continues to evolve, our level of understanding continues to change. This has led to the rapid inception of extremophile research, which began in 1967 when Thomas Brock identified bacteria growing in the boiling hot springs of Yellowstone National Park (Brock, 1967). These hot conditions demonstrated the extreme conditions under which microorganisms can survive and the chemistries that can support their growth (Madigan *et al.*, 2000).

Extremophile research extended the physical and chemical limits of the environment in which microorganisms can survive, from the sterilizing temperatures (121° C) of volcanic vents miles below the surface of the Pacific Ocean, the battery-acid-like conditions of acid mine drainages, to surviving for millions of years within crystals of salt (Bond *et al.*, 2000; Jannasch *et al.*, 1992; Mormile *et al.*, 2003; Shivaji *et al.*, 2004; Thomas and Dieckmann, 2002). Today, almost every environment imaginable has been examined for the presence of microorganisms, including, of course, caves (Angert *et al.*, 1998; Barton and Luiszer, 2005; Barton *et al.*, 2004; Canaveras *et al.*, 2006; Canaveras *et al.*, 2001; Caumartin, 1963; Chelius and Moore, 2004; Cunningham *et al.*, 1995; Engel *et al.*, 2004; Groth and Saiz-Jimenez, 1999; Groth *et al.*, 1999; Holmes *et al.*, 2001; Hose *et al.*, 2000; Northup and Lavoie, 2001; Sarbu *et al.*, 1996)

STUDYING MICROORGANISMS IN EXTREME ENVIRONMENTS

In attempting to understand the difficulties in carrying out microbiology in cave environments, we must go back to the traditional methods developed by Koch and others in the 1880s (Koch, 1881). Traditional microbiology techniques rely on growing a microorganism in a Petri plate, and then identifying this organism based on the sum of its reactions to obtain food, respire and break down products (the sum of which is referred to as metabolism). This is why swabs are often taken when a

bacterial pathogen is thought to be responsible for an infection, which are used to grow the suspected organism and carry out metabolic tests for its identification. Unfortunately, there is a small problem when attempting to grow environmental microbes: the majority of microorganisms cannot be cultivated (or as microbiologists say 'cultured') in a Petri plate. This is a phenomenon that microbiologists have known about since the time of Winogradsky and is based on a simple observation: if you took a gram of soil, you would be able to see about 5,000 different bacterial species under the microscope, but you would be lucky if you could induce 50 different species, or 1% of those observed, to grow (Amann *et al.*, 1995; Amann *et al.*, 1996). This is even worse compared on a global scale: of the millions of species of bacteria thought to exist on Earth, we have only been able to cultivate approximately 16,000 (American Type Culture Collection, 2006).

The reason that so many microbes from the environment are non-culturable is still not completely understood (Bloomfield *et al.*, 1998; Bogosian *et al.*, 2000; Oliver, 1995; Whitesides and Oliver, 1997). One reason thought to be important comes from the founding of microbiology in medicine; historically all our cultivation strategies to grow microorganisms are based on the growth characteristics of a small number of microbes able to infect humans. As a result, the majority of microbial growth media (the contents of a Petri plate) are designed, to some extent, to be chemically similar to human tissues. Indeed, such media is often made from boiling down meat products with agar to make a nutrient gel. While these growth media work extremely well for human pathogens, for environmental microorganisms that grow using unusual chemical reactions, the 'food' in these Petri dishes is inappropriate. To grow these environmental microorganisms requires a certain amount of guesswork based on the environment from which they were isolated. It is like trying to decide which cave food to pack for someone else; everyone has their own preference and won't eat what they don't like (Amann *et al.*, 1995).

Such non-culturability is likely more pronounced in cave microorganisms. Without photosynthesis, caves are cut off from most energy that supports life on the surface, confining most nutrients to the entrance zone. As a result, cave microorganisms must turn to alternative sources of energy, such as those found in the atmosphere, or present in the very rock itself (Barton *et al.*, 2004; Chelius and Moore, 2004; Engel *et al.*, 2003; Northup *et al.*, 2003; Spilde *et al.*, 2005). In adapting to these extremely starved environments, microorganisms produce elaborate scavenging mechanisms to pull scarce nutrients into the cell (Koch, 1997). When these organisms are then exposed to the rich nutrients of a Petri plate, they cannot turn down these scavenging mechanisms and quickly gorge themselves to death (Koch, 1997, 2001). As a result, microorganisms from starved cave environments may have a hard time adapting to rapidly changing nutrient status, and simply die from osmotic stresses (Koch, 1997). Therefore, only a tiny minority of microorganisms from caves might grow on standard Petri plates by surviving the changing nutrient conditions,
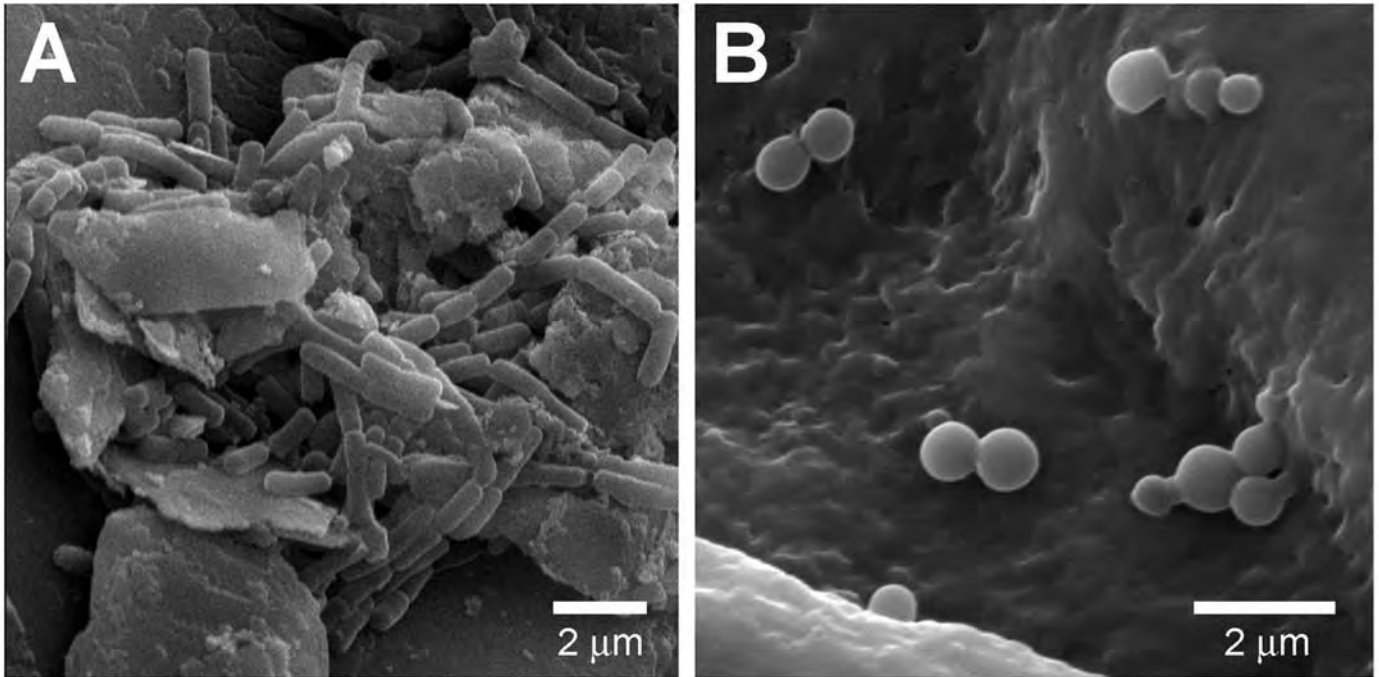
Figure 1. Scanning electron microscopy images of common themes in bacterial structure. The two most common structures are the A) bacilli (in chains, as streptococci) and B) cocci (in pairs, as diplococci).

including such species as *Pseudomonas* and *Bacillus* species (Pemberton *et al.*, 2005). Unfortunately these species are unable to tell us much about the unique microbial processes taking place in cave environments. In order to overcome these problems, cave microbiologists have therefore spent a great deal of time trying to developed improved techniques to grow cave microorganisms (Gonzalez *et al.*, 2006; Groth *et al.*, 1999; Laiz *et al.*, 1999). Many of these techniques are quite specialized, and it is unlikely that many microbiologists (such as those within diagnostic or hospital microbiology labs) have access to the materials necessary to cultivate rare cave species.

TO GROW OR NOT TO GROW

The non-culturability of microbial species, rather than simply being a curiosity, poses a significant road-block if you want to study microbes in natural cave environments. Fortunately, classical microbiology lost some of its dependence on cultivation in 1977, when Woese was attempting to understand the genetic differences between microbial species (Woese and Fox, 1977). Historically, bacteria had been identified by their growth characteristics and classified based on their shape and structure. This process of grouping life based on structure is called taxonomy, and is extremely useful in determining evolutionary relationships between plants, animals and insects, etc. (Whittaker, 1969). In bacteria, using structure to group species turns out to be pretty futile. Bacterial structures generally represent variations on a theme: either spheres (technically called cocci) or sausages (bacilli) (Figure 1). As a result, structural taxonomy tended to cluster the bac-

teria into simplified, but artificial groups. We therefore tended to believe that eukaryotic organisms were the most complex form of life on Earth, with their broad structural diversity; taxonomy identifies over 1,000,000 species of insects alone (May, 1988). Faced with this limitation, Woese decided on a different approach to resolve bacterial taxonomy. Rather than examining structure, he looked inside the cells at the DNA.

All of us inherit our genetic information from our parents, which creates a genetic blueprint of our family tree (blue-eyed children to blue-eyed parents, etc). By looking at the surnames in a traditional family tree, it is also quite easy to see who is related to whom, based on how the surnames change; the same surname indicates direct descendency, while a change in surname may indicate a marriage into the family. Similarly, Woese extracted the genetic surnames of a number of microorganisms to determine a family tree for microbial life (Woese and Fox, 1977). By examining this data, he discovered that bacteria are much more diverse than we previously suspected. This diversity, rather than being expressed through structure, was elaborated through phenomenal capabilities of physiology and metabolism (Woese, 1987). Woese also identified an entirely new kind of life using this technique, which was previously unknown to science. This new form of life looked just like the bacteria, but the internal genetic controls looked more like those found in eukaryotic organisms (such as plants and animals). These organisms were also primarily found in the harshest conditions; conditions similar to those that may have been found on early Earth. Woese therefore named this newly discovered form of life after its potentially ancient origins, calling them the Archaea (Woese and Fox, 1977). What
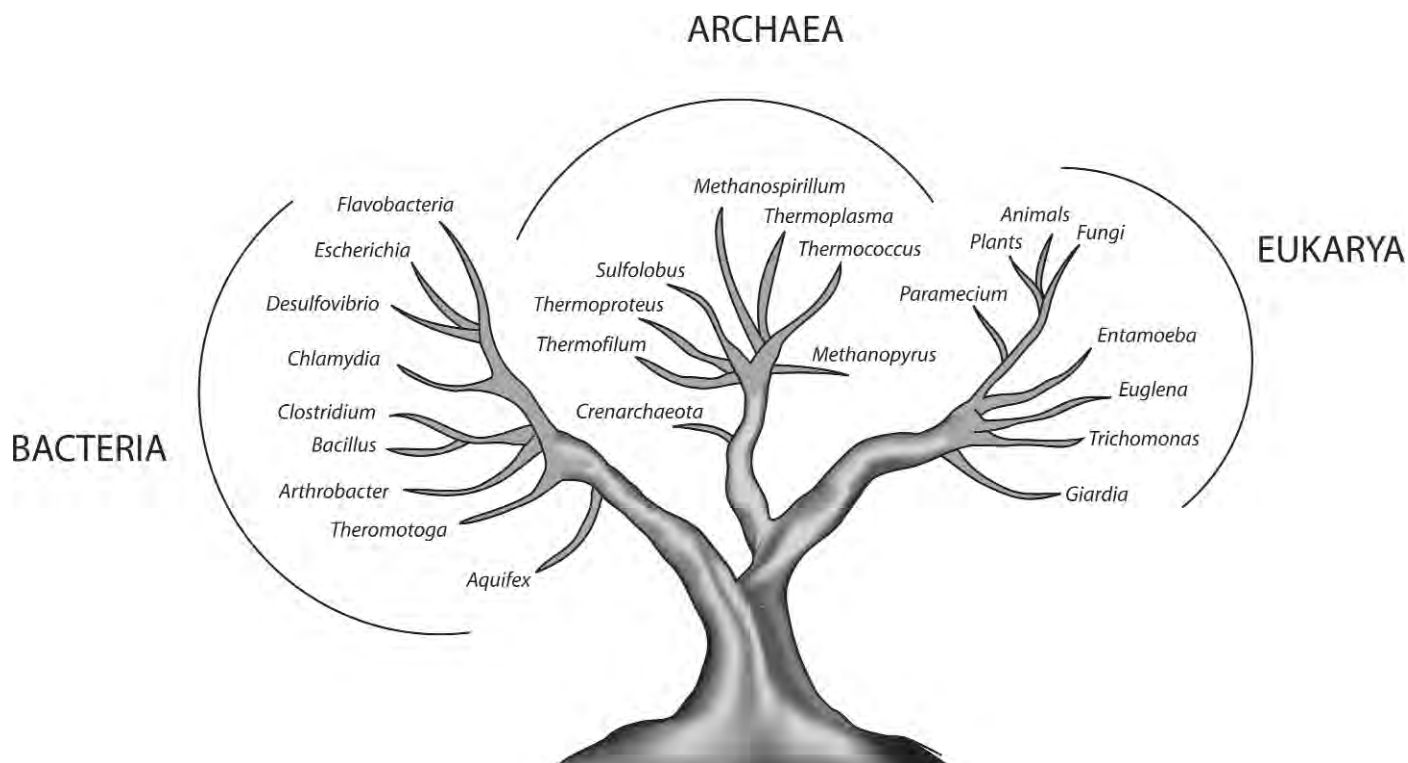
Figure 2. A universal tree-of-life based on the genetic comparisons of Woese and Fox (1977), with further refinement by Pace (1997). The base of the tree emerges from the 'root' or last common ancestor of life and splits into the three major domains of life; the Bacteria, Archaea and Eukarya. The branches represent the (simplified) major divisions (Kingdoms) of life within each domain.

emerged from this work was that, above and beyond the five-kingdom tree-of-life, there exist domains; the primary groups of life on Earth. These domains represent 1) the Bacteria (prokaryotes; lacking a nucleus), 2) the Archaea (also prokaryotic, but with structures that differentiate them from bacteria and a DNA structure more similar to a eukaryote), and 3) the Eukarya (eukaryotes; containing a nucleus) (Figure 2).

BEYOND THE GENETIC TREE

The work of Woese was groundbreaking and shifted our understanding of what is "life" on Earth. With further refinement by other investigators, it also showed a five-kingdom tree was an oversimplification, with a current estimate of almost 100 distinct kingdoms (divisions) of life on Earth (Hugenholtz *et al.*, 1998; Pace, 1997). The new tree also revealed that the most ancient, most complex and most abundant life on Earth was actually microscopic (Pace, 1997). This work excited a number of scientists, including Pace (a microbiologist and Indiana caver) who decided to look for Woese's genetic surnames in the environment without cultivation (Lane *et al.*, 1985; Stahl *et al.*, 1985). The technique uses the polymerase chain reaction, or PCR for short, and allows investigators to make millions of copies from a single piece of DNA. A similar technique is commonly used today to look for pieces of

genetic information in crime scene investigations, or to detect the presence of the HIV virus in a patient's blood. When Pace used the PCR reaction to look for bacterial genetic surnames within the environment, it altered our understanding of microbial ecology (Pace, 1997). Rather than generating a tree of lineage from a handful of organisms that could be grown in the laboratory, this new technique allowed entire microbial ecosystems to be examined at once without the limitations of cultivation. Soon, other investigators began using the same techniques to study environments, and the number of genetic surnames that were known to science rapidly went from 177 to the >120,000 that are available today (Maidak *et al.*, 2001). Such techniques have also improved our ability to estimate the true diversity of microorganisms (putting entomologists to shame) with an estimated worldwide population of 200,000,000 different species of bacteria alone (Curtis *et al.*, 2002; Dojka *et al.*, 2000; Torsvik *et al.*, 2002).

IDENTIFICATION WITHOUT CULTIVATION

Using genetic surnames to identify microorganisms (technically called molecular phylogenetics: molecular – using DNA molecules; phylogenetics – a reconstruction of the evolutionary history), it gave us tools to identify microorganisms within an environment without cultivation. Molecular phylo-
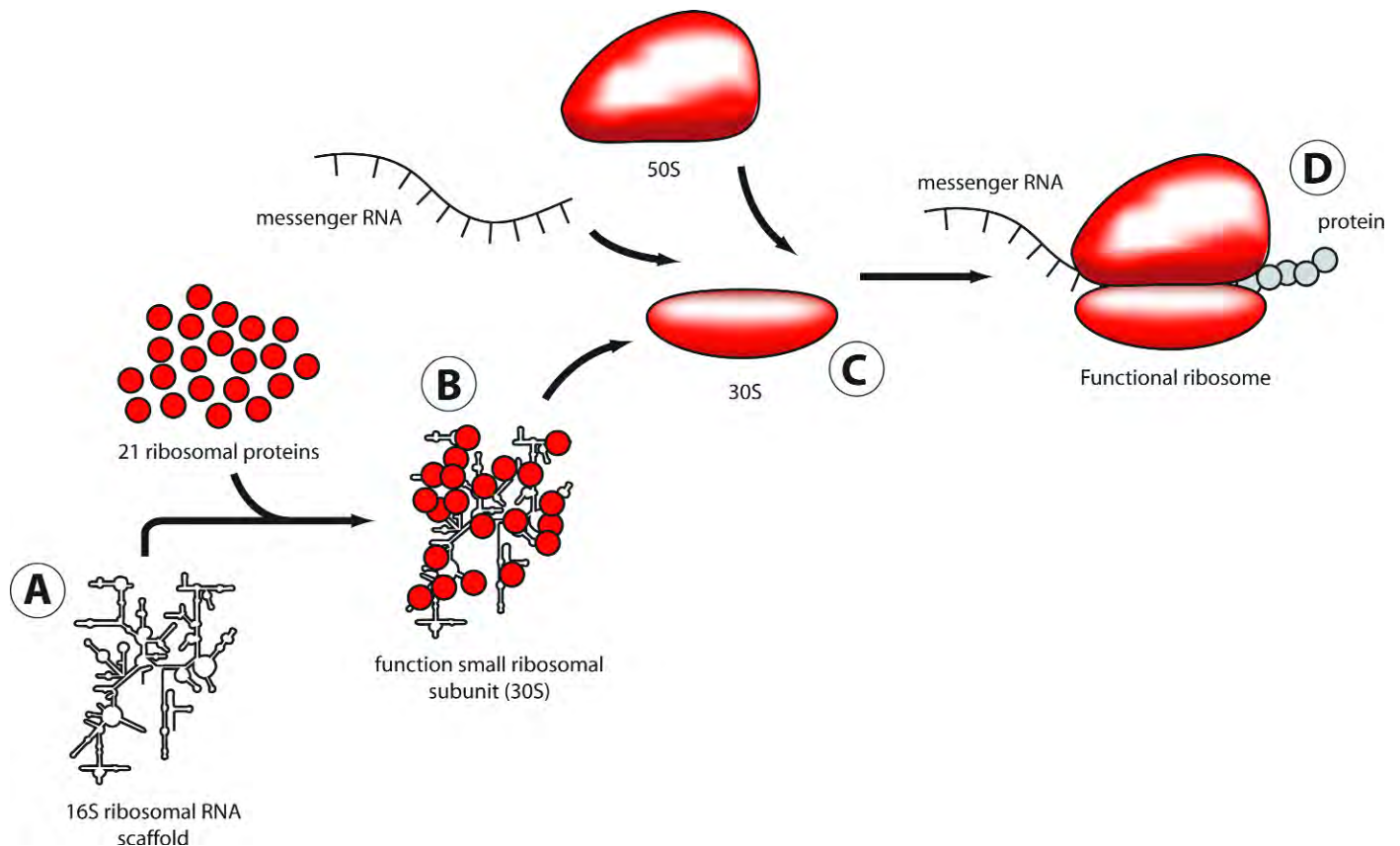
Figure 3. The function of 16S ribosomal RNA (rRNA) within assembly of the ribosome. The 16S rRNA is transcribed from genomic DNA, folding into a three-dimensional structure (A). This 'scaffold' allows the 21 ribosomal proteins to bind (B) and assemble into the 30S small subunit of the ribosome (C). In this functional structure, the 30S subunit assembles with the larger 50S subunit around protein encoding messenger RNA, allowing protein synthesis (D).

genetics have therefore allowed us to effectively examine microorganisms in extreme environments; conditions that would be almost impossible to replicate in the laboratory for growth using the approach of Koch. These genetic "surnames" are genes with a fairly long name: the 16S ribosomal RNA gene sequence, or 16S rRNA for short. This gene is critically important in the cell as it encodes a structural component of the ribosome, the cellular factory used to build proteins (Figure 3). The ribosome is a complicated structure, made from two functional domains, a large 50S and small 30S subunit. Each subunit is built from protein building blocks and due to its complexity, a piece of RNA acts as a scaffold upon which the ribosome can assemble, much like a scaffold can help the building of a house. The 30S subunit is assembled around the 16S rRNA. If the shape of the 16S rRNA was to change dramatically the ribosome would not be built correctly and the cell could no longer make proteins, leading to an almost instantaneous death. This need for an unaltered structure constrains the shape of the ribosomal RNA such that only tiny modifications can be made over time, without damage to protein synthesis – in biology this is known as an evolutionary constraint. Therefore, over the history of the microbes there have only been subtle changes in the structure of the rRNA molecules,

much like "Chinese Whispers" (where tiny mistakes in a sentence subtly change its context as it is whispered from person to person). These changes in the 16S rRNA allowed Woese to identify the primary lineages of life and trace descendency to predict the most ancient forms of life on Earth (Woese, 1987; Woese and Fox, 1977).

While molecular phylogenetics may appear to be an interesting exercise in evolutionary biology, these techniques allow us to determine how microbes are related by how similar their 16S rRNA sequences are. To explain how this works, we need to return to the analogy of the genetic surname and the family tree. In determining ancestry, if a surname is the same then there is a high likelihood that two people are closely related; however, what about if the surnames are similar, but not the same? In the case of microbes, such as *Escherichia coli*, we know based on structure and biochemistry that it is closely related to *Salmonella entrica*. Due to their being close relatives, we can assume that *Salmonella* has many of the same metabolic activities and lives in the same environment as *E. coli*. This is indeed correct, in that they both reside in mammalian gastrointestinal tracts. In the same way, we can compare the 16S rRNA sequences from *E. coli* and *Salmonella* and see that they are 99% identical. Therefore, if we had no other
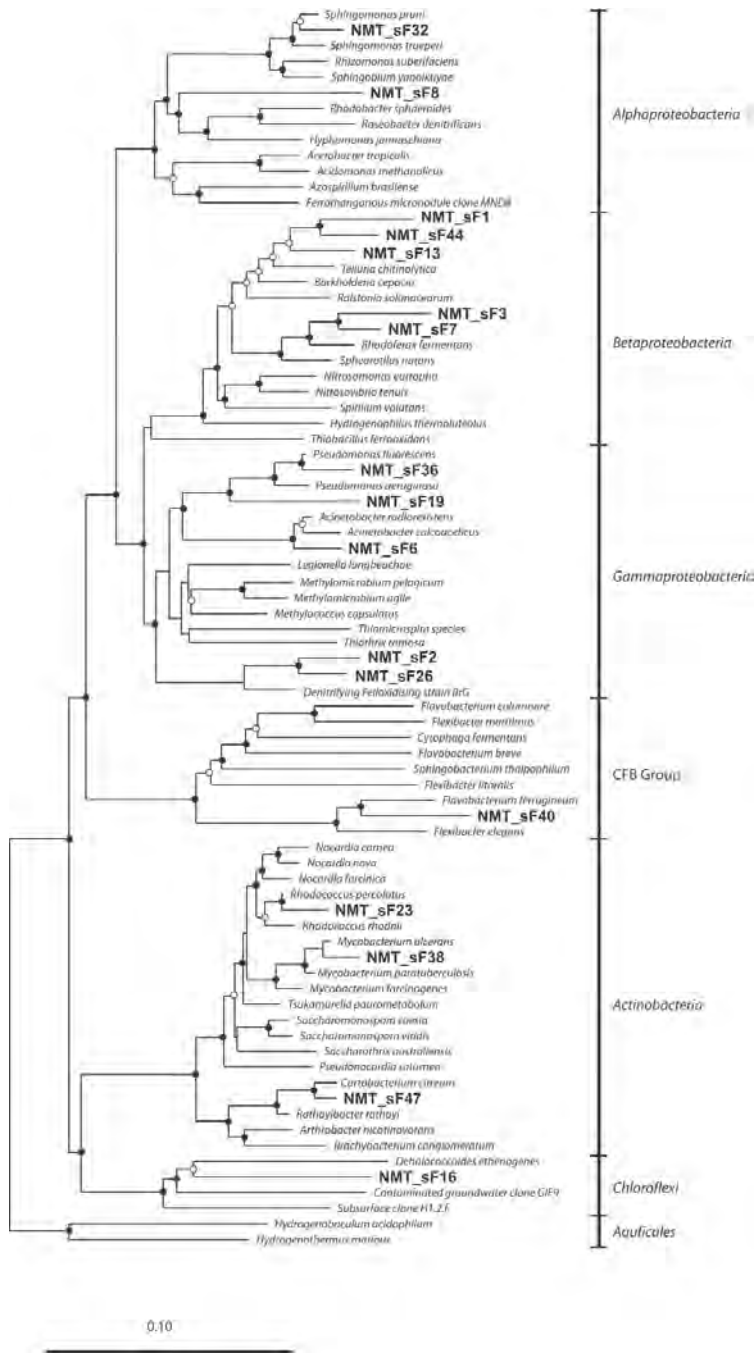
Figure 4. Dendogram of the 16S rRNA gene sequences identified within Carlsbad Cavern (NMT-sF). The bar indicates 10% divergence between the 16S rRNA sequences. Where the dendogram branches, those with branches supported by >70% of all alignments (bootstrap values >70% in neighbor-joining and a heuristic search) are indicated by closed circles. Marginal branch support (bootstrap values >50% but >70% in both analyses) are shown by an open circle.

information other than the 16S rRNA sequence, we can hazard a guess that *Salmonella* had a similar metabolic activity to *E. coli*. This is essentially the basis of the molecular phylogenetic approach, allowing analysis of microbial communities without cultivation. While the technique seems pretty straightforward, in practice it's actually quite a challenge due to the complexity of aligning thousands of DNA sequences to find the closest match (Maidak *et al.*, 2001; Maidak *et al.*, 2000; Nei *et al.*, 1998). Nonetheless, how closely species are related can be shown graphically as a dendrogram, which is a mathematical model of a family tree based on a 16S rRNA gene sequences (Figure 4). In scientific journals, molecular phylogenetic analyses of environments are often explained with dendrograms – with a scale at the bottom to indicate the relative distance between organisms. These dendrograms allow us to see how closely organisms are related to the cultivated representatives with known metabolic activity; the smaller the distance the more closely an organism is related. These dendograms also allow a visual representation of the structure of the community; much like a yellow pages allows us to determine what businesses drive the economy of a city, dendograms allow us to determine what activities drive the processes that support a microbial community within an environment. The tree in Figure 4 is from Carlsbad Caverns and suggests that there are a number of metabolic activities that are occurring to support growth in this extremely starved cave environment, such as fixing nitrogen gas from the atmosphere and obtaining energy from the rock itself (Barton *et al.*, 2005a). Molecular phylogenetic analyses can also give us information of what nutrients are available in an environment, providing clues as what to add to our Petri plates to improve the chances of growing those rare cave microbes.

BROADER IMPLICATIONS

As the original observations by Leeuwenhoek changed our understanding of the world around us, the recognition of microorganisms in geologic cave samples has altered our perception of cave ecosystems (Barton and Northup, 2006). The science of cave geomicrobiology has similarly mirrored the meteoric rise of microbiology as a science, with new insights suggesting that cave microorganisms may be involved in processes as varied as speleothem deposition to cavern enlargement (Canaveras *et al.*, 2006; Engel *et al.*, 2004). While the important role of cave microorganisms may be of interest to speleologists, the implications of this research go well beyond caves. For example, the work of Saiz-Jimenez and colleagues has led to the identification of microorganisms degrading the ancient, prehistoric paintings within Altamira Cave, Spain (Schabereiter-Gurtner *et al.*, 2002). Not only did this work contribute to understanding the
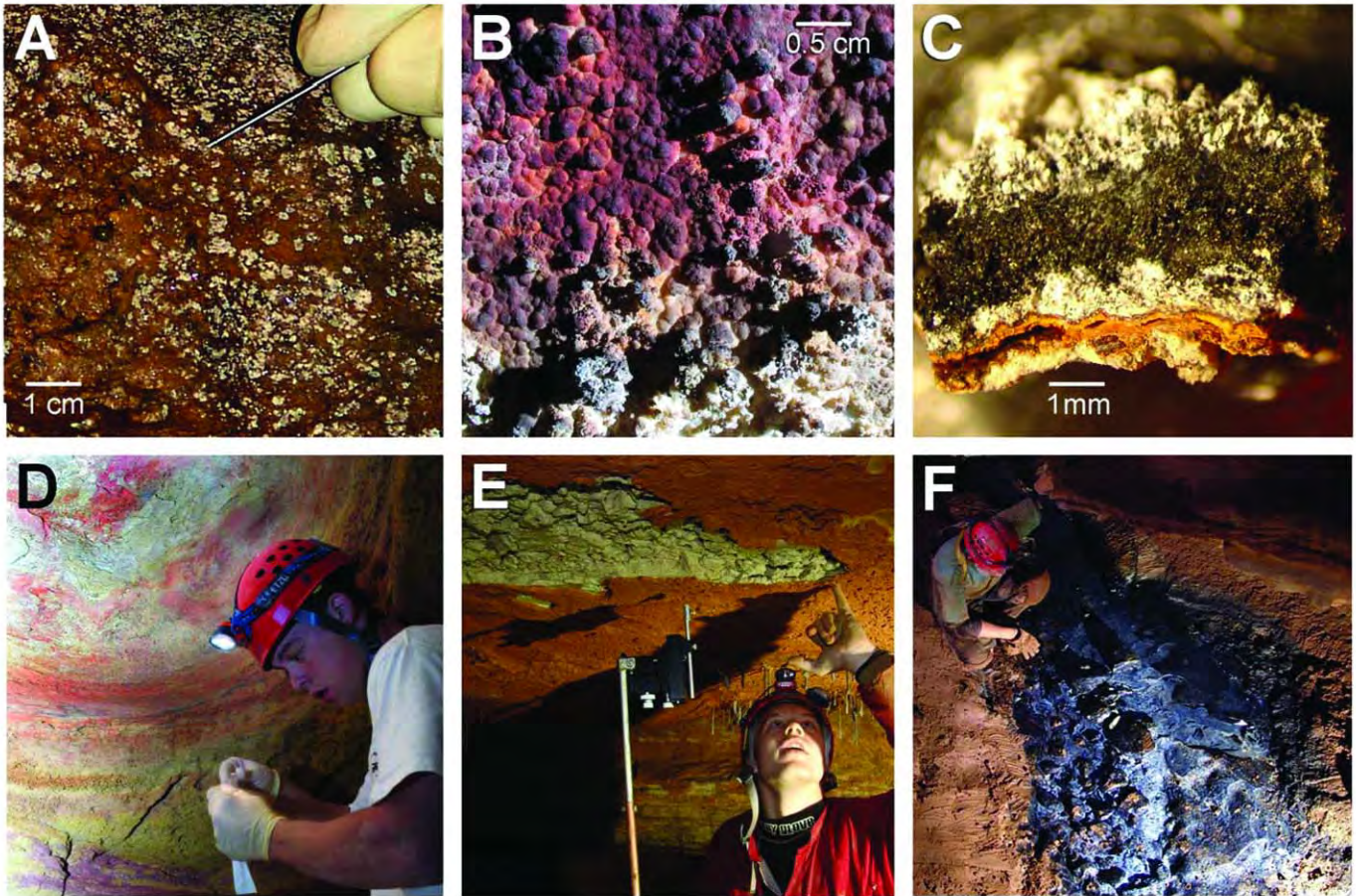
Figure 5. Microbiological activity in caves. A) Dots – microbial colonies on the surface of a rock being sampled using a hypodermic needle; B) Color – Microbial activity leading to the discoloration of a rock surface; C) Precipitation – Banded mineralization on a rock surface by microbial activity (the precipitate has peeled away revealing the deposit's banded formation); D) Corrosion residues – brightly-colored corrosion residue formed on a cave ceiling; E) Structural changes – using a microelectrode assembly to examine chemical gradients formed within a soft cave ceiling; F) Biofilms – a white biofilm coating in the streambed of a cave.

role of microorganisms in the degradation and subsequent conservation of these paintings, but it identified microbial species that could colonize carbonate surfaces, depositing calcite. Such work had significant implications in the preservation of ancient marble monuments and statues, where microorganisms could be used to deposit a veneer of calcite to protect ancient structures from continued erosion (Laiz *et al.*, 2003). Similarly, within Carlsbad Caverns we have identified a novel species of microorganism that can degrade complex aromatic compounds, such as benzothiazole and benezenesulfonic acid for growth, which are compounds involved in the manufacture of plastics and are dangerous environmental contaminants (Bennett and Barton, 2006). Such a capability could allow these species to be inoculated into contaminated environments, to rapidly degrade such pollutants and allow restoration of natural habitats in a process called bioremediation. Cave microorganisms, with their adaptation to extreme starvation, also have the potential to harbor other important biomolecules. Similar environments have yielded microbes with properties that allow

efficient ethanol production for fuel, enzymes for environmentally friendly paper processing and even the improved stonewashing of jeans. Cave microorganisms also have the potential to harbor unique antibiotics and cancer treatments (Onaga, 2001). Finally, one of the philosophical questions of humanity regards our place in the Universe: Are we alone? Is life on Earth unique? Cave microbiology can not only answer questions about the limits of life, but also help us to identify the geochemical signatures of life. Such signatures are capable of surviving geologic uplift, which allows them to be detected on the surface of planets, such as Mars (Boston *et al.*, 2001). While such ideas may seem an extraordinary application of cave geomicrobiology, NASA has recently undergone a dramatic refocus by gearing its activities to returning humans to the moon and exploration of Mars and world's beyond to find evidence of past life, activities in which cave geomicrobiology may play an important role (White House Press Release, 2004).

Cave Microbiology

While this extensive preamble has explained how difficult cave microbiology can be and the technical solutions possible, it does not preclude the average caver from being involved in cave microbiology; however, it does hopefully explain why running out and collecting "goo" in plastic bags is not going to advance the field. In addition to the traditional leave-no-trace conservation ethics of cave exploration, there are two significant roles that cavers can play in cave microbiology; 1) is the identification of new cave biota and ecosystems through continued exploration and survey, and 2) taking steps to limit human contamination and conserving the microbial habitats of caves.

Cave Surveyor as Microbiologist

It may be a stretch to suggest that by exploring and surveying a cave you are contributing to the field of cave geomicrobiology, but it remains almost impossible to predict the location of microbial activity without its active discovery. Microbial activity in caves range from the obvious slimy goop to the more subtle deposition of calcite or alteration of the rock surface. There are a number of features that can be identified within caves as evidence of microbial activity (Figure 5):

Dots on surfaces: The appearance of dots on surfaces may not necessarily be due to abiotic mineral deposition. Often, where conditions are appropriate, microorganisms can grow up as a colony large enough to be seen with the naked eye. These colonies are similar in shape to those that might be seen on a Petri plate and each represents the growth of millions of bacteria. Sometimes these colonies may be more apparent through the concurrent deposition of minerals (such as calcite), creating a contrast against the host bedrock. Such microbial colonies are particularly obvious in areas of seeping water.

Unusual coloration: When microorganisms grow on surfaces, they can alter the surface chemistry, leading to a subtle change in coloration of the rock. Classic examples of this are the black/red residues seen within the Lunch Room of Carlsbad Caverns. The exact mechanism of this coloration may vary from site to site with local chemistry and microbial activity.

Precipitates: Microorganisms use chemical gradients to generate energy, much as we use the chemical gradient between food and oxygen to generate energy for life. In using these gradients microorganisms may often change the surrounding conditions of their environment, leading to a change in the chemical properties of minerals. In the case of minerals such as iron and manganese oxides, this will lead to a change in the solubility of these minerals and lead to their precipitation. Such microbially-mediated precipitation often forms banded layers of minerals on surfaces.

Corrosion residues: A significant amount of work has been carried out in caves of the Guadalupe Mountains on the corrosion residues that form as a result of microbial interactions with the minerals of limestone and dolomite (Barton *et al.*,

2005a; Cunningham *et al.*, 1995; Northup *et al.*, 2003). While researchers are still trying to elucidate the exact mechanism of their formation, it appears that microbial metabolic activity is involved in dissolving the host rock, while energy-generating chemical gradients cause continued mineral transformation and precipitation. Whatever the mechanism, these soft and powdery corrosion residues are an exciting component of geomicrobial activity in cave environments and may provide useful insights to the mechanisms of energy generation in such extremely starved environments.

Structural changes: When microorganisms interact with the rock on which they live they can cause different chemical changes in the rock. These changes may be distinct from the bright color changes seen with corrosion residues, resulting in subtle structural changes, such as decreased density or softening of the host rock. In Grayson-Gunnar Cave in Kentucky, areas of the ceiling have been reduced to a wet, toothpaste-like consistency that allows unique microbial energy acquisition strategies.

Biofilms: One of the most obvious signs of microbiological activity in a cave environment is the presence of biofilms. These coatings are comprised of microbial communities, held together with gel-like polymers that produce a range of structures including: wads of snot-like goo, floating dumplings, slippery sub-aqueous coatings and hair-like tendrils. These structures tend to form a sticky polymer that clumps bacterial species together, with the environmental conditions and mechanical action of water dictating the structure the community will form. These biofilms often form where energy enters the cave environment and have proven important insights into the sources of energy supporting cave life (Angert *et al.*, 1998).

A rule of thumb as a caver is that if you see anything unusual that cannot be easily explained by geologic phenomena, it may be microbiological. There are a number of active caver/microbiology researchers in the United States who can help you determine its relative significance (Barton *et al.*, 2005b; Boston *et al.*, 2001; Chelius and Moore, 2004; Engel *et al.*, 2004; Northup *et al.*, 2003; Spear *et al.*, 2005). In addition to the identification of unique cave ecosystems, one of the most important tools of our research is a cave line-plot/survey and map. Such maps allow researchers to determine how far below the surface the microbial activity is found, its relative position to geologic faults and other factors that might bring energy in to the system for microbial growth.

Microbial conservation

While it can be difficult to remember to protect things that you cannot see, to minimize your impact on microbes, minimum impact caving techniques are appropriate. These include keeping to the established trail, removing all waste, avoid touching anything unnecessarily and generally minimizing your impact on the environment. While we often caution novices against touching formations, it is important to think similarly about placing limits on unnecessarily touching walls

(particularly where moisture is obvious), undisturbed sediments and corrosion residues. Some cave systems can withstand more human impact than others, and the same is true for microbial systems. If a cave floods, it is likely that the microbes are adapted to sudden influxes of material and energy, so a level of anxiety is not called for and less care need be taken. Other systems with perennially dry passages are more easily impacted. In some extreme instances, such as Lechuguilla Cave, flagged trails limit all travelers to the same area, to avoid walls and other surfaces unnecessarily.

In addition to conservation activities within the cave, also think about activities that you are carrying out in preparing for the cave. The biggest impact people can have on microbial ecosystems in caves is not washing caving gear and seeding one cave with another cave's microbes. I've heard of some cavers who will go down and smell their coveralls between caving trips, to get their cave fix from that earthy-smell of the dirt in their coveralls. That smell is a mixture of organic products being produced when microbial species, known as *Actinomycetes*, decompose organic material (Jachymova *et al.*, 2002; Scholler *et al.*, 2002). While you may get your kicks from smelling microbial excrement, it also means that if unwashed, those *Actinomycetes* will be traveling in to the next cave system with you.

In limiting your impact on such environments think about the potentially delicate nature of microbial ecosystems and, while I wouldn't recommend bathing in disinfectant (showers are counter productive as it dries skin out, increasing skin cell shedding) or wearing environmental suits, common sense should prevail. Think about your personal hygiene when entering a delicate cave system: Have you brushed your hair recently? Is long hair tied back so it won't be shedding in the cave? Think about the food you take with you into a cave. Avoid anything that generates crumbs and try to eat over a plastic bag, even when there is little chance of spilling, because one crumb is enough to feed a million microbes for many months. In addition to following leave-no-trace ethics to maintain the aesthetic beauty and visible ecosystems of the cave environment, similar care can go a long way to conserving microbial ecosystem health.

Conclusion

The science of geomicrobiology is still in its infancy, and as with other fields within microbiology, it is continuing to evolve. As it does, significant discoveries are updating our understanding of microbe-rock interactions and how microorganisms have helped shape our global environment (Balkwill *et al.*, 1997; Banfield and Nealson, 1997; Barghoorn and Schopf, 1966; Ben-Ari, 2002; Colwell *et al.*, 1997; Schopf, 1983). Similarly, cave microbiology is continually changing; from the preliminary description of cave microbiota at the turn of the last century to the sophisticated techniques employed today (Caumartin, 1963; Sarbu *et al.*, 1996). As cavers continue to explore caves in search of the unknown, they can help geomicrobiologists identify unique microbial ecosystems and help us to preserve this important resource.

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POTENTIAL EFFECTS OF RECURRENT LOW OXYGEN CONDITIONS ON THE ILLINOIS CAVE AMPHIPOD

S.V. Panno[1,*], K.C. Hackley[1], W.R. Kelly[2], H.H. Hwang[1], F.M. Wilhelm[3], S.J. Taylor[4], and B.J. Stiff[1]

The caves of Illinois' sinkhole plain are the sole habitat of the Illinois Cave amphipod (Gammarus acherondytes), a federally endangered species. The sinkhole plain is a hydrologically-connected sequence of karstified limestone that constitutes an extensive karst aquifer which serves as an important source of potable water for area residents. During this investigation, we examined the ground-water quality in caves within two ground-water basins: 1) Illinois Caverns, where the amphipod is now present after previously reported to have been extirpated from the lower reaches, and 2) Stemler Cave, where the amphipod is reported to have been extirpated. The chemical composition of cave streams in Illinois Caverns and Stemler Cave were compared to determine which parameters, if any, could have contributed to the loss of G. acherondytes from Stemler Cave. Stream water in Stemler Cave contained higher concentrations of organic carbon, potassium, silica, chloride, fluoride, sulfate, iron and manganese than Illinois Caverns. Perhaps most importantly, dissolved oxygen (DO) concentrations in Stemler Cave were, during periods of low flow, substantially lower than in Illinois Caverns. Based on land use, there are probably at least eight times more private septic systems in the Stemler Cave ground-water basin than in the Illinois Caverns ground-water basin. Low DO concentrations were likely the result of microbial breakdown of soil organic matter and wastewater treatment system effluent, and the oxidation of pyrite in bedrock. The near-hypoxic DO in Stemler Cave that occurred during low-flow conditions, and, we speculate, a limited range of G. acherondytes within the Stemler Cave ground-water basin due to a metabolic advantage of the stygophilic aquatic invertebrates over the stygobitic G. acherodytes, resulted in the apparent loss of G. acherondytes from Stemler Cave.

INTRODUCTION

GAMMARUS ACHERONDYTES IN ILLINOIS CAVES

The cave streams of Illinois' sinkhole plain comprise the only habitat of the Illinois Cave amphipod (*Gammarus acherondytes*) (Fig. 1), a federally endangered species (U.S. Fish and Wildlife Service, 2002). The sinkhole plain is a hydrologically connected sequence of karstified limestone that constitutes an extensive aquifer in southwestern Illinois (Fig. 2). Each cave within the sinkhole plain lies within or is the focal point of a ground-water basin (analogous to a watershed). Because of the rapid movement of ground water through conduits within the subsurface, ground water in karst aquifers undergoes little natural cleansing (White, 1988). Contaminants from row-crop agriculture, livestock waste, urban runoff, and wastewater treatment systems are easily transferred to the subsurface where they may have adverse effects on the quality of the aquatic environment and its aquatic biota (USFWS, 2002).

In this investigation, samples were collected from two reaches of the main cave stream in Illinois Caverns and the cave stream in Stemler Cave (Fig. 2). Based on a survey conducted along nine transects in Illinois Caverns in 2000, the upper reach of the stream in Illinois Caverns (upstream and just downstream of the main entrance) supports a viable/stable population of *G. acherondytes*, but the amphipod was absent in lower reaches of the cave stream (up to the "T-Junction;" a map of the cave may be found in Panno *et al.*, 2004). Its absence was assumed to be related to a poorer water quality in these reaches (Lewis, 2000). A "viable/stable population" is defined as density of *G. acherondytes* of approximately ten individuals per m^2 (Lewis, 2003; Venarsky 2005). In addition,

Lewis (2003) found a correlation between the increase in biofilms and filamentous microbial growth on cave stream gravels (these filaments bridged gaps between the gravels in the downstream parts of Illinois Caverns) and the decrease in population of *G. acherondytes* relative to the upstream areas where the amphipod was found. However, during our investigation, *G. acherondytes* was found in about equal densities in both the upstream and downstream portions of Illinois Caverns. Conversely, *G. acherondytes* is reported to have been completely extirpated from Stemler Cave (Fig. 2). *Gammarus acherondytes* was reported to be present in Stemler Cave based on an examination of samples collected in 1965 (Peck and Lewis, 1978), but more recent surveys (Peck and Lewis, 1978; Webb, 1995; Taylor and Webb, 2000; Lewis *et al.*, 2003) and our investigation failed to locate any individuals.

The objective of this investigation was to compare water-quality parameters between Stemler Cave and Illinois Caverns

[1] Illinois State Geological Survey, 121 Natural Resources Bldg., 615 E. Peabody Dr., Champaign, IL, 61821.
[2] Illinois State Water Survey, 2204 Griffith Dr., Champaign, IL, 61820.
[3] Dept. of Zoology and Center for Ecology, 1125 Lincoln Drive, Southern Illinois University, Carbondale, IL, 62901.
[4] Illinois Natural History Survey, 172 Natural Resources Bldg., 607 E. Peabody Dr., Champaign, IL, 61821.

Samuel V. Panno, panno@isgs.uiuc.edu
Keith C. Hackley, hackley@isgs.uiuc.edu
Walton R. Kelly, kelly@sws.uiuc.edu
Hue-Hwa Hwang, hwang@isgs.uiuc.edu
Frank M. Wilhelm, fwilhelm@zoology.siu.edu
Steven J. Taylor, sjtaylor@inhs.uiuc.edu
Barbara J. Stiff, stiff@isgs.uiuc.edu

Figure 1. Photograph of *G. acherondytes* collected from Illinois Caverns (photograph by Frank Wilhelm). Scale bar is 1 mm.

to determine if differences in water quality between the two cave streams could account for the presence of *G. acherondytes* in Illinois Caverns and its reported absence from Stemler Cave. Because the geology and hydrology of the two ground-water basins are similar (Panno *et al.* in press a, b), any differences in the chemical compositions of the two cave streams are most likely due to differences in land use between the ground-water basins. Specifically, changes in land use within the Stemler Cave ground-water basin between the 1960s and the 1990s may have resulted in changes in cave-water quality that could not be tolerated by *G. acherondytes*, resulting in its apparent extirpation.

GEOLOGY AND HYDROLOGY

The two caves lie within Illinois' sinkhole plain in the southwestern part of the state. Bedrock consists of calcite-rich, Mississippian-age limestone that either crops out or is covered by up to 15 m of glacial till and loess (Herzog *et al.*, 1994). The

bedrock surface is predominantly St. Louis Limestone, with relatively thin occurrences of the overlying Ste. Genevieve Limestone. Karst and cave formation are controlled by the lithology, poor primary porosity coupled with well-developed secondary porosity (*i.e.*, near vertical fractures and dilated bedding planes). The majority of caves in the study area, including Illinois Caverns and Stemler Cave, are branchwork type caves that formed along bedding planes within the St. Louis Limestone (Panno *et al.*, 2004).

Illinois Caverns is located near the center of the sinkhole plain and contains about 9.6 km of explored passage (Panno *et al.*, 2004). Stemler Cave is located 29 km to the north of Illinois Caverns and is shorter, with only about 1.8 km of explored passage. Both caves contain perennial streams, and the stream beds contain bedrock, cobble, gravel, sand and silt substrates.
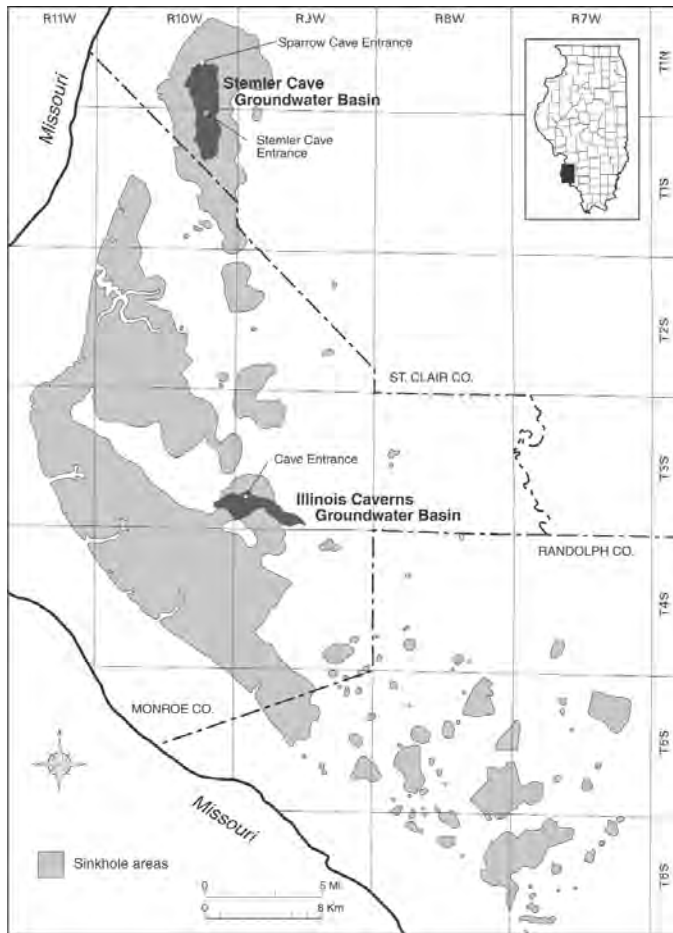
Figure 2. Map showing the locations of Illinois Caverns and Stemler Cave in southwestern Illinois' sinkhole plain (modified from Panno *et al*., 1997). The black shapes near the cave entrances represent the groundwater basins for each cave (basin boundaries modified from Aley *et al*. 2000).

LAND USE ANALYSIS

Land use in both the Illinois Caverns and Stemler Cave ground-water basins is dominated by agriculture (Table 1). The main difference between the two basins is the amount of urban land. The Stemler Cave Ground-Water Basin has a higher percentage of urban land (4.1%) than the Illinois Caverns basin (0.53%). Urbanization of the Stemler Cave basin has primarily occurred in the last two decades, and recently there has been a large increase in the number of permits to build single family houses in the basin (D. Newman, Illinois Nature Preserves Commission, personal communication, 2004). Municipal sewerage systems are not present in either basin, and all households use on-site wastewater-treatment systems. Many of these systems discharge within the drainage area of a sinkhole (Panno *et al*., 1997). An eight-fold increase in urban land use could mean a similar increase in the number of private septic systems in the Stemler Cave ground-water basin. In addition,

Table 1. The percent land use in the Illinois Caverns and Stemler Cave ground-water basins based on maps by Aley *et al.* (2000); land-use estimates were made using GIS techniques.

Land Use	Illinois Caverns (6.03 km²)	Stemler Cave (19.1 km²)
Row crop agriculture	62.8	57.7
Rural grasslands and misc.	17.0	17.5
Urban	0.53	4.05
Wooded	17.5	15.8
Waterways, wetlands, *etc.*	2.21	5.02

intensified construction activities in the region could have increased the sediment load of runoff; sedimentation associated with such development could have adverse affects on the cave biota (USFWS 2002).

METHODS

To investigate cave-water quality, 19 stream-water samples were collected from Illinois Caverns and seven from Stemler Cave. Nine of the samples in Illinois Caverns were collected about 50 m upstream from the main entrance, and 10 were collected from a location approximately 800 m downstream from the entrance, in a reach reported by Lewis (2000) to be devoid of *G. acherondytes*. Ground-water samples were collected monthly to bi-monthly between May 2003 and March 2004. Because of our findings, four months into the investigation we began sampling Stemler Cave where the Illinois Cave amphipod was reported to have been extirpated about 25 years earlier. Cave-stream samples from Stemler Cave were collected at its entrance (a sinkhole intersecting the downstream reach of the subsurface stream) monthly to bi-monthly between September 2003 and May 2004. Because of this adjustment in our sampling efforts, ground-water samples from April through August 2003 collected from the two caves were not time synchronous.

Ground-water samples were collected just before and soon after agrichemical application for five out of eight sampling events. Springtime applications of synthetic fertilizer (estimated to be 50% of the annual application) and herbicides generally occur between mid-April and mid-May; fall application (the other 50%) generally occurs in November (P. Kremmell, State Extension Agent, Southern Illinois University, Edwardsville, personal communication, 2000). Samples were collected during periods of both high and low stream discharges. In general, grab samples were collected, but nine of the samples from Illinois Caverns and 1 from Stemler Cave were collected using automatic samplers (ISCO®) in May, June, September, December 2003 and March 2004. These were composite samples that were mixtures of samples collected daily for 24 days in order to have integrated ground-water samples that included contaminants carried into the cave dur-

ing and following large rainfall events. Some of the water sampling events were coordinated with timed area searches and bait trapping of invertebrates at the same ground-water sampling locations in both caves. However, the tasks were separated by several days so that sediments suspended by invertebrate counts did not affect subsequent water sampling. Invertebrates were counted at Illinois Caverns on two occasions; one event was conducted during high-flow conditions (May 2003) and the other at low flow (September 2003). Invertebrates were counted in Stemler Cave on one occasion under high-flow conditions (May 2004). An inspection of Sparrow Creek Cave, the distal end of the Stemler Cave ground-water basin, was conducted in March 2004. Because of the relatively deep water in this part of the cave, a dip net and aquarium net were used to examine sediment whenever possible. When found, all adult amphipods were field identified with the aid of a dissecting microscope, counted, and released.

Temperature, pH, Eh, dissolved oxygen (DO) and specific conductance (SpC) were measured using standard field techniques and meters with temperature compensation. Stream discharge was measured using a stream velocity gage along measured cross sections of the cave streams in non-turbulent stream segments (*e.g.*, Panno *et al.*, 2003). All water samples were analyzed for major cations and anions; ammonium-nitrogen (NH_4-N); total Kjeldhal nitrogen (TKN); herbicides used on fields in the study area including atrazine, bentazon, chlorimuron, cyanazine, glyphosate, imazaquin, imazethapyr, metolachlor, sethoxydim and trifluralin (Panno *et al.*, 1996); total organic carbon (TOC); and caffeine. The detection limits for many of these analyses are available on Table 2; the detection limit for caffeine is 0.15 µg L^{-1}.

Samples for cations, anions and isotopic analyses were filtered through 0.45-µm high-capacity filters and stored in polyethylene bottles. Cation samples were acidified in the field

Table 2. Minimum, median, and maximum values of chemical and physical parameters measured in two locations in Illinois Caverns (n = 19) and Stemler Cave (n = 7). Results are reported in mg L^{-1} unless otherwise noted. Detection limits, where applicable, are reported as less than values.

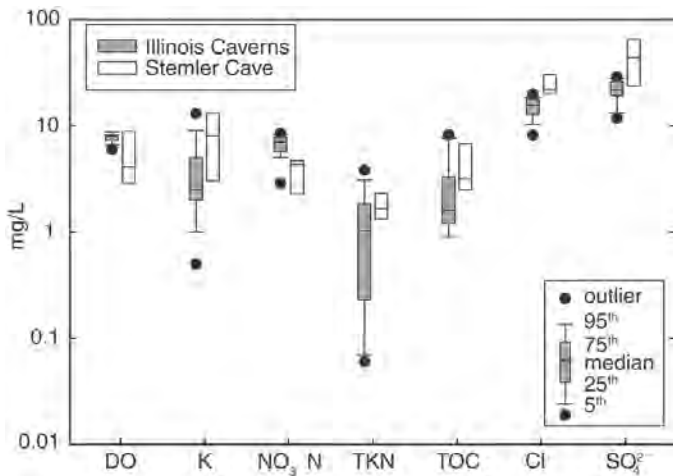| Parameters	Illinois Caverns			Stemler Cave		
	Minimum	Median	Maximum	Minimum	Median	Maximum
Stream discharge (L s^{-1})	1.8	19	57	18	93	>169
Temperature (°C)	13.6	13.8	14.6	10.7	13.5	14.3
pH (pH units)	7.0	7.8	8.4	7.2	7.6	7.8
DO (mg L^{-1})	6.0	7.9	8.8	2.8	4.0	9.8
O_2 Saturation (%)	58.8	74.5	85.4	24.4	39.6	92.5
SpC (µS cm^{-1})	250	537	622	184	620	753
Alkalinity ($CaCO_3$)	84	226	252	72	245	281
Na	5.60	22.0	33.5	2.06	22.6	29.8
K	<5	<5	13	3	8	14
Ca	36.6	78.7	103	22.1	95.0	105
Mg	5.58	10.3	14.7	4.00	15.5	18.5
Ba	0.07	0.08	0.09	0.05	0.08	0.87
B	<0.01	<0.01	0.04	<0.01	<0.02	0.06
SiO_2	14.6	17.2	21.6	5.91	19.3	20.4
SO_4	13.1	21.8	28.7	10.7	43.6	66.4
Cl	8.1	15.6	19.8	4.3	22	34.2
F	<0.04	0.1	0.3	<0.2	0.3	0.4
Fe	<0.01	<0.01	0.04	<0.01	0.01	0.05
Mn	<0.001	0.01	0.04	<0.001	0.03	0.07
Sr	0.11	0.17	0.20	0.07	0.21	0.23
NO_3-N	4.99	7.04	8.43	0.60	4.30	4.74
NH_4-N	0.02	0.04	0.12	<0.01	0.06	0.28
TKN	0.07	1.02	3.81	0.02	1.66	3.38
PO_4-P	<0.01	<0.01	0.30	<0.01	0.01	0.70
TOC	0.9	1.6	8.2	2.4	3.2	10.2
Atrazine (µg L^{-1})	<0.05	0.79	4.79	<0.05	<0.05	0.38
Metolachlor (µg L^{-1})	<0.02	0.12	1.54	<0.02	0.06	0.42
Trifuralin (µg L^{-1})	<0.01	0.02	0.05	<0.01	<0.01	0.01

Figure 3. Box and whisker plots comparing stream water quality in Illinois Caverns (n = 19) and Stemler Cave (n = 7). Whiskers are missing from the Stemler Cave data because there were an inadequate number of samples to calculate the 5th and 95th percentiles.
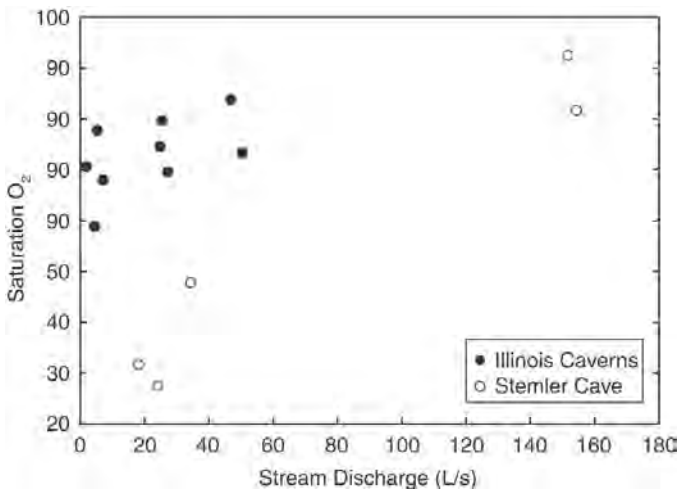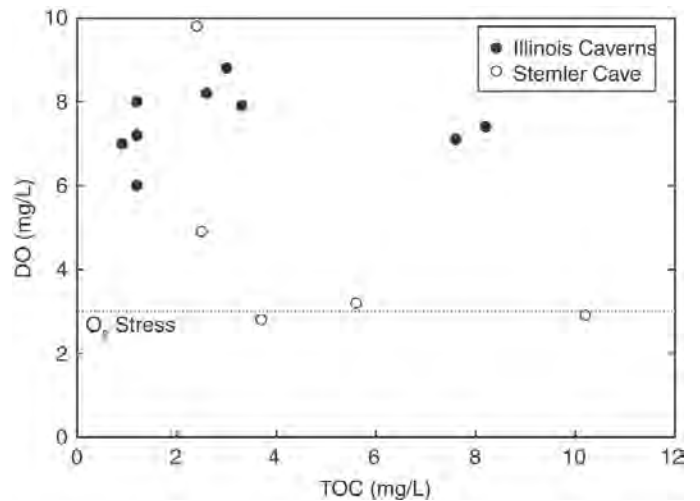

Figure 5. DO *vs* TOC concentrations of Illinois Caverns and Stemler Cave stream-water samples. Stemler Cave water exhibits a relationship with TOC not seen in Illinois Caverns water.

Caffeine was determined using high performance liquid chromatography. TOC was determined using a carbon analyzer.

Optical brighteners were measured during one sampling episode. The presence of optical brighteners in water is a strong indication of the presence of septic effluent (Alhajjar *et al.*, 1990). White cotton fabric devoid of optical brighteners was placed in both cave streams in February 2004 and retrieved the following week. Samples were analyzed with a spectrofluorophotometer using a technique described by Aley (1985).

<center>RESULTS AND DISCUSSION</center>

CHEMICAL COMPOSITION OF CAVE GROUND WATER

There were no statistically significant differences in the chemical composition of the cave-stream water in the two sample locations within Illinois Caverns, and the composition was within the normal range for karst ground water in this region (Panno *et al.*, 1996). Thus, we combined the results from both locations within Illinois Caverns when comparing them to Stemler Cave. The range and median of parameter concentrations from each sampling site were used as descriptors; mean values were not used because they are easily biased by extreme values.

There were differences in stream water quality between Stemler Cave and Illinois Caverns (Table 2; Fig. 3). The results of this investigation showed that Stemler Cave water generally had higher concentrations of TOC, potassium (K^+), chloride (Cl^-), sulfate (SO_4^{2-}) and manganese (Mn), and lower pH and NO_3-N concentrations than Illinois Caverns. Phosphate (PO_4-P) was detected in four of seven Stemler Cave samples but only two of sixteen Illinois Caverns samples. The differences were statistically significant for K^+, SO_4^{2-}, Cl^-, and NO_3-N, as

Figure 4. Percent O_2 saturation in cave streams as a function of stream discharge in Illinois Caverns and Stemler Cave. Stemler Cave exhibited a very low % O_2 saturation at low flow.

with ultra-pure nitric acid to a pH of < 2. All samples were transported to the laboratory in ice-filled coolers and kept refrigerated at approximately 4° C until analysis.

Cation concentrations were determined with an inductively coupled argon plasma spectrometer, and anions were determined by ion chromatography as described in Panno *et al.* (2001). TKN and NH_4-N were determined colorimetrically as described in Panno *et al.* (2006). Organic N was operationally defined as the difference between TKN and NH_4-N. Herbicides were determined with gas chromatography-mass spectrometry techniques using USEPA Method 508 (USEPA, 1988).
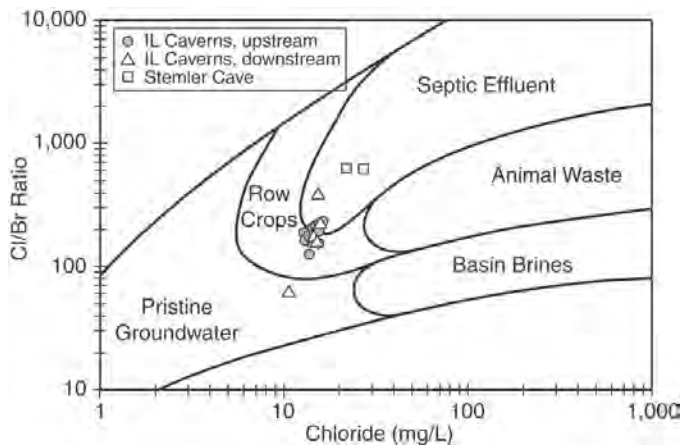
Figure 6. Cl/Br *vs* Cl⁻ concentrations (domains from Panno *et al.*, 2006) of Illinois Caverns and Stemler Cave stream-water samples revealed that groundwater in both caves is affected by both row crop agriculture and septic effluent. Data were from previous sampling efforts conducted by Panno et al. (ISGS, unpublished data).

determined using the rank sum test, but not for the other parameters. These results are consistent with those of Panno *et al.* (2001) who had time-synchronous, seasonally-collected samples from Illinois Caverns and the resurgence spring of Stemler Cave. In their study, Panno *et al.* (2001) also observed higher concentrations of iron (Fe), silica (SiO_2), and fluoride (F^-) in ground water from the Stemler Cave system. In this present investigation, stream water in Stemler Cave also had a wider range with both the lower and upper extremes of DO concentrations than that in Illinois Caverns stream water. Although based on limited data, DO concentrations in the cave streams appeared to be influenced by discharge; at high-flow rates, DO concentrations (converted to % saturation O_2, to take water temperature into account) were elevated, especially in Stemler Cave. At low flow rates, % O_2 saturation in Stemler Cave was much lower than in Illinois Caverns (Fig. 4).

The lower pH, DO, and NO_3-N, and elevated Fe, Mn and TOC concentrations in Stemler Cave waters suggest a more organic-rich soil-water component entering the Stemler Cave stream than that of Illinois Caverns (Panno *et al.*, 2001). Dissolution of soil organic matter (SOM), and human/animal waste can increase TOC concentrations; the water samples from Stemler Cave typically had a pale-yellow color (during low-flow conditions) suggesting the presence of elevated concentrations of organic acids. Reduction of SOM and human/animal waste consumes O_2 and generates CO_2, which lowers the pH due to formation of carbonic acid (H_2CO_3). Low-DO concentrations coincided with TOC concentrations greater than about 3 mg L^{-1} in Stemler Cave. However, DO in Illinois Caverns stream water didn't show this relationship with TOC (Fig. 5). Alkalinity, which is a proxy for dissolved CO_2 concentrations, was slightly higher in samples from

Stemler Cave than Illinois Caverns. Thus, if water recharging Stemler Cave remains in contact with soil longer than in the Illinois Caverns basin and receives more animal/human waste, it should be more reducing with a lower pH and higher organic carbon.

The stream-water chemistry in Stemler Cave appears to be influenced by two types of water: (1) soil water seeping into the ground water that feeds the cave, and (2) nutrient-enriched wastewater effluent or animal waste discharging and/or being washed into sinkholes directly connected to the karst aquifer. The water chemistry is also influenced by reaction with the limestone bedrock; the water is generally close to saturation with respect to calcite, and the likely source of elevated concentrations of SO_4^{2-} in Stemler Cave water (median concentration two times greater than Illinois Caverns) may be the oxidation of pyrite in the bedrock limestone (Panno *et al.*, 2001; Hackley *et al.*, in press). Elevated Fe concentrations and lower pH in Stemler Cave water, relative to Illinois Caverns, further supports this hypothesis. Oxidation of pyrite within the limestone could substantially contribute to the reduction of DO in ground water feeding Stemler Cave. The relatively elevated Cl^-, F^- and K^+ concentrations and the more frequent occurrence of PO_4-P above its detection limit in Stemler Cave cannot be explained by soil-water contributions or water-rock interactions alone, and are more likely the result of surface-borne contamination. Potential sources of Cl^-, F^-, K^-, and PO_4-P include human wastewater, animal waste, and fertilizer (KCl) (Panno *et al.*, 2002, 2006). Potassium and PO_4-P are generally immobile in the soil zone, being adsorbed by clay particles and organic compounds. Without a geologic or anthropogenic source, they are typically below detection or at low concentrations in subsurface waters. Chloride and K^+ were elevated in Stemler Cave throughout the year, except during a major recharge event when the Cl^- concentration was only 4.3 mg L^{-1}. This suggests a continuous source that generally bypasses the soil zone, where K^+ and PO_4-P would likely have been removed. The most likely source of Cl^-, F^-, K^+, and PO_4-P is effluent from on-site wastewater treatment systems, many of which typically discharge directly into sinkholes in this area (Panno *et al.*, 1997).

To test the hypothesis of the presence of septic effluent, we looked at caffeine, optical brighteners and Cl/Br ratios. Caffeine was detected in only one sample, in the downstream location of Illinois Caverns in September 2003. Optical brighteners were measured on only one occasion (March 2004) and were detected in Stemler Cave but not Illinois Caverns. These two compounds are indicators of human wastewater, and their detection is evidence of effluent from domestic wastewater treatment systems in both caves. Caffeine appears to be only episodically present at concentrations great enough to be detected by the technique used in this investigation. A new version of a Cl/Br *vs* Cl⁻ scatter diagram (developed by Panno *et al.*, 2006) including groundwater samples from both caves (only a few samples had Br⁻ concentrations above detection limits) provides further evidence of the presence of septic

effluent in Stemler Cave and, to a lesser extent, Illinois Caverns (Fig. 6). The relatively low Cl/Br ratio precludes road salt from being the source of Cl⁻ (Panno *et al.*, 2006). The infiltration of septic effluent into the shallow karst aquifer is also consistent with the lack of seasonal trends for Cl⁻ and K⁺, because effluent would be introduced into the hydrologic system year round. Elevated TOC and lower DO in Stemler Cave may also be due in part to a greater influx of septic effluent. Boron, another element associated with wastewater, was detected (> 0.01 mg L⁻¹) in about 5% of Illinois Caverns samples, whereas, it was detected in about 38% of the Stemler Cave samples. Livestock are present, but uncommon within the ground-water basins. Contamination of both Illinois Caverns and Stemler Cave by human/animal waste has been reported previously; enteric bacteria, sometimes at very high levels, are almost always detected when these cave streams have been tested (Panno *et al.*, 1996; Taylor *et al.*, 2000).

Herbicides have been shown to have adverse impacts on aquatic biota (U.S. Fish and Wildlife Service, 2002), but the fact that herbicides were detected in both Illinois Caverns and Stemler Cave, and at higher concentrations in Illinois Caverns, suggests that they alone are not responsible for the extirpation of *G. acherondytes* from Stemler Cave. The herbicides further suggest that there was a stronger agricultural influence within the Illinois Caverns ground-water basin.

CAVE BIOTA

Gammarus acherondytes was found in both upstream and downstream locations in Illinois Caverns in surveys undertaken in May 2003 and September 2003. Populations were roughly equal at both locations in spite of previous extirpation in the downstream reaches. *Gammarus acherondytes* was not detected in Stemler Cave in a survey during May 2004 or during reconnaissance trips in November 2003 and March 2004 to Sparrow Creek Cave, the resurgence of the Stemler Cave ground-water basin, located several km to the north (Fig. 2). The presence of biofilms and total absence of cave biota within Sparrow Creek Cave suggests the possible discharge of animal and/or human waste prior to the survey. No similar biofilms were observed in Illinois Caverns during or prior to this investigation. In July 2000, Lewis (2003) conducted a survey in Spider Cave, a small cave located about 1 km to the north of the entrance to Illinois Caverns. Within the cave system, there was a strong, putrid odor and evidence of sewage and/or animal waste. Lewis (2003) reported that a putrid, microbial mat covered all surfaces and was present in interstices within the cave; the microbial mat and odor were gone by the following September 2000. Subsequent work by F. Wilhelm at Spider Cave in September 2003 revealed no additional microbial mats or odor and *G. acherondytes* had returned. High levels of bacterial contamination associated with human and livestock waste have been shown to have impacted cave communities elsewhere in the eastern United States (Poulson, 1991; Simon and Buikema, 1997). In Virginia, *Caecidotea recurvata* (Steeves), an asellid isopod, had a high-

er tolerance for waters negatively influenced by septic effluent than did the gammarid amphipod *Stygobromus mackini* Hubricht. Elsewhere in Virginia, Culver *et al.* (1992) attributed the extirpation of the stygobitic crangonyctid amphipod *Crangonyx antennatus* Packard and the asellid isopods *C. recurvata* and *Lirceus usdagalun* Holsinger and Bowman to increased flux of organic pollutants in the karst system. These observations suggest that discharge or dumping of sewage and/or animal waste in the sinkhole plain may occur and could be responsible for the apparent extirpation of *G. acherondytes* from cave streams.

CONSEQUENCES TO CAVE BIOTA

Probably the most important difference that we observed in the chemical composition between the two cave streams is the periodically low DO concentrations in Stemler Cave. Differences in other chemical parameters do not appear to vary sufficiently to account for the apparent absence of *G. acherondytes* from Stemler Cave and its continued presence in Illinois Caverns. Low-DO concentrations are known to be deleterious to aquatic organisms; average DO concentrations less than 3 mg L⁻¹ are stressful for aquatic organisms, and concentrations less than 2 mg L⁻¹ are defined as hypoxic (SCDNR, 2004). The lowest measured DO concentration in Stemler Cave was 2.8 mg L⁻¹, and it is possible that even lower concentrations can occur. We conclude that of all the chemical data we examined, low DO is the most likely cause contributing to the apparent loss of *G. acherondytes* from Stemler Cave.

Even though we have a limited number of samples, the DO concentrations in Stemler Cave stream water were depressed (and possibly hypoxic) during periods of low flow. The loss of all biota at the resurgence of Stemler Cave (Sparrow Creek Cave) and observations of the formation of biofilm on rocks and walls of the cave suggest that the aquatic biota of Stemler Cave are subjected to periodic low oxygen stress. This likely resulted from periodic and some continuous influx of human and/or animal waste. Such a reduction of DO is often responsible for the decline of aquatic biota in waters receiving organic waste.

Taylor *et al.* (2003) suggested that cave facultative amphipods such as *Gammarus troglophilus* could out-compete (through numerical displacement) *G. acherondytes* because of metabolic advantages under heightened-food conditions in caves. Although the lower metabolic rate of *G. acherondytes* would have an advantage in a "food-poor hypogean environment" such as a near pristine cave, urbanization and the influx of abundant organic matter from anthropogenic sources (*e.g.*, septic effluent) would favor the proliferation of *G. troglophilus* (Wilhelm *et al.*, 2006). The influx of organic materials into the cave streams would increase in severity with greater anthropogenic surface activities (*e.g.*, Panno *et al.*, 1996, Simon and Buikema 1997). We speculate that because of the metabolic advantage of the stygophilic biota, the stygobitic *G. acheroudytes* may not have had as wide a range in the Stemler Cave ground-water basin as did the other aquatic invertebrates, thereby allowing the other aquatic invertebrates (but not *G.*

acherondytes) to recolonize Stemler Cave following periods of low DO in the cave stream.

The recent (since 2000) repopulation of downstream reaches in Illinois Caverns by *G. acherondytes* suggests either the quality of water recharging the cave has improved or that the distribution of *G. acherondytes* is sufficiently widespread in the cave to allow repopulation following low oxygen episodes in parts of the stream. This also suggests that the timing of population surveys (*i.e.*, amount of time following hypoxic episodes), is critical to accurately determining the abundance of invertebrate populations.

CONCLUSIONS

During this investigation, *G. acherondytes* was found in both upstream and downstream locations in Illinois Caverns, contrary to previous surveys when it was nearly absent from downstream reaches. This can be attributed to timing of surveys, improved water quality, or, more likely, periodic loss and repopulation of the amphipod in the downstream reaches of Illinois Caverns. Multiple surveys over the past 40 years support a conclusion that *G. acherondytes* has been extirpated from Stemler Cave; however, it is possible that the amphipod may exist in small tributaries within the ground-water basin and have the potential to repopulate the cave stream if the water quality improves. Stemler Cave stream-water samples had higher concentrations of TOC, K^+, Cl^-, F^-, SO_4^{2-}, SiO_2, Fe and Mn, and lower NO_3-N and pH than those from Illinois Caverns, and most importantly experienced substantially lower DO concentrations. The dominant influence on Illinois Caverns stream water appears to be agricultural. Whereas, Stemler Cave stream water appears to be influenced by two distinct sources: one with a large soil water component and the other with a human wastewater/animal waste component, the organic matter of which could contribute to low-DO concentrations. This characterization is further supported by the fact that the percentage of urban land use in the Stemler Cave ground-water basin is about eight times greater that in the ground-water basin of Illinois Caverns.

The low DO in Stemler Cave apparently occurs only during low-flow conditions, causing the cave stream aquatic invertebrates to periodically (seasonally?) experience low-oxygen conditions and increased stress. The presence of optical brightener, wastewater microbial taxa, and the results of Cl/Br *vs* Cl^- scatter diagrams of Illinois Caverns and Stemler Cave water suggest that effluent from wastewater-treatment systems is also entering their ground-water basins, most likely through discharge into sinkholes. This situation would make organics (a heightened-food condition) much more abundant within the caves. Based on land-use data, the ground-water basin of Stemler Cave could contain at least eight times more private septic systems that that of Illinois Caverns. Dissolved oxygen is probably lost not only from degradation of human waste, but also from degradation of SOM, as well as pyrite oxidation. We speculate that because of metabolic differences

between *G. acherondytes* and other aquatic invertebrates, *G. acherondytes* may not have had as wide a range in the Stemler Cave ground-water basin as the other aquatic invertebrates. This situation would allow them, but not *G. acherondytes*, to recolonize the downstream reaches of the cave following periods of low DO. The fact that cave facultative amphipods have a metabolic advantage over *G. acherondytes* under heightened-food conditions probably led to its displacement and apparent extirpation from Stemler Cave.

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ARCHITECTURE OF AIR-FILLED CAVES WITHIN THE KARST OF THE BROOKSVILLE RIDGE, WEST-CENTRAL FLORIDA

LEE J. FLOREA

Department of Geology, University of South Florida, Tampa, FL 33620, lflorea@chuma1.cas.usf.edu

Air-filled caves surveyed in the Brooksville Ridge of west-central Florida provide insight into the organization of karstic permeability within the unconfined portions of the Upper Floridan Aquifer. The morphology of the passages that compose these caves in geologically young, high-permeability limestones is strikingly different from caves found in ancient carbonates far from the influence of the coast. Cave passages in west-central Florida are laterally extensive and tiered. Principal horizons of cave development occur between +3 m and +5 m, +12 m and +15 m, and +20 m and +22 m above modern sea level. The primary guide of cave passage orientations within these cave levels is widespread fractures oriented approximately NE-SW and NW-SE. Cave passages of human dimensions form at the intersection of the laterally extensive cavities and fractures and often acquire a characteristic plus-sign shape. The walls of cave passages in west-central Florida are porous and complex, with small-scale solution features such as pockets and tafoni structures extending into the host bedrock. Additionally, these cave passages often end in blind pockets, ever-narrowing fissures, sediment fills, and collapses. The passages do not appear to represent an integrated system of conduits between aquifer inputs and outputs.

INTRODUCTION

Cavers and karst scientists have long appreciated and recorded information concerning the morphology of passages in caves. These data about caves are important to understanding the flow of water in karst aquifers, which cover approximately 15% of the land surface and provide water to approximately one-fifth of the world's population (Ford and Williams, 1989). For example, compilations of cave maps reveal patterns in both the organization of passages in a cave and the shape of individual passage cross-sections that are a direct consequence of hydrogeological conditions within karst aquifers (*e.g.*, Palmer, 2000; White 1988). To date, these observations are drawn primarily from experiences in caves formed far from the influence of the coast and within ancient carbonate rocks that are remarkably different from carbonate rocks that are geologically recent or are forming today. This paper presents a case study of the morphology of caves within the coastal karst aquifers of west-central Florida.

GEOLOGIC FRAMEWORK OF THE BROOKSVILLE RIDGE AND THE UPPER FLORIDAN AQUIFER

The Tertiary limestones that compose the highly productive Upper Floridan Aquifer are intensely karstified in regions that experience active groundwater circulation (*e.g.*, Lane, 1986; Stringfield and LeGrand, 1966), particularly in the portion of west-central Florida where the Upper Floridan Aquifer is semi-confined to unconfined. This region, characterized by 33 springs with average discharge greater than 2.8 m³ s⁻¹ (*e.g.*, Scott *et al.*, 2004; Roseneau *et al.*, 1977; Meinzer, 1927), stretches from the panhandle near Tallahassee in the north to Tampa in peninsular Florida (Fig. 1A) and encompasses several physiographic provinces including the Brooksville Ridge (White, 1970).

The Brooksville Ridge, a linear, positive-relief topographic feature extending from northern Citrus County, through Hernando County, and into southern Pasco County (White, 1970), is bounded by coastal lowlands to the west and south and wetlands of the Withlacoochee River to the east and north. The ridge system is a consequence of a localized geologic high termed the Ocala Platform by Scott (1988), who attributed this topographic feature to a westward tilt of thickened Eocene strata. Elevations in the Brooksville Ridge range from five to more than 75 m above sea level (Fig. 1B). The topography is rolling with internal drainage (Fig. 2). Upland mesic-hardwood hammocks separate sinkhole lowlands that are mostly occupied by wetlands or lakes. The Withlacoochee State Forest manages more than 525 km² (157,000 acres) in the region, including the 100-km² (30,000 acre) Citrus Tract that includes much of the study area. Pasture land and lime-rock quarries compose the remaining land uses. The city of Brooksville lies in the heart of the Brooksville Ridge (Fig. 1A).

Upper-Eocene and Oligocene carbonates (42–33 Mya) compose the Upper Floridan Aquifer, which is semi-confined to unconfined in the Brooksville Ridge. The strata of the Upper Floridan Aquifer thicken to the south along a regional dip that averages less than half of one degree (Scott *et al.*, 2001; Miller *et al.*, 1986). Miocene-age sands and clays of the Hawthorn Group thicken to more than 150 m in northern and southern Florida where the Upper Floridan Aquifer is confined (Scott, 1988). The Hawthorn Group is thin to missing in the center of the Brooksville Ridge in northern Hernando and southern Citrus Counties (Fig. 3).

The Suwannee Limestone, a pale-orange, partially recrystallized limestone that is extensively quarried in northern Hernando County, is more than 30 m thick to the south. In the up-dip sections of the northern Brooksville Ridge of Citrus County, the Suwannee Limestone is thin to nonexistent as a
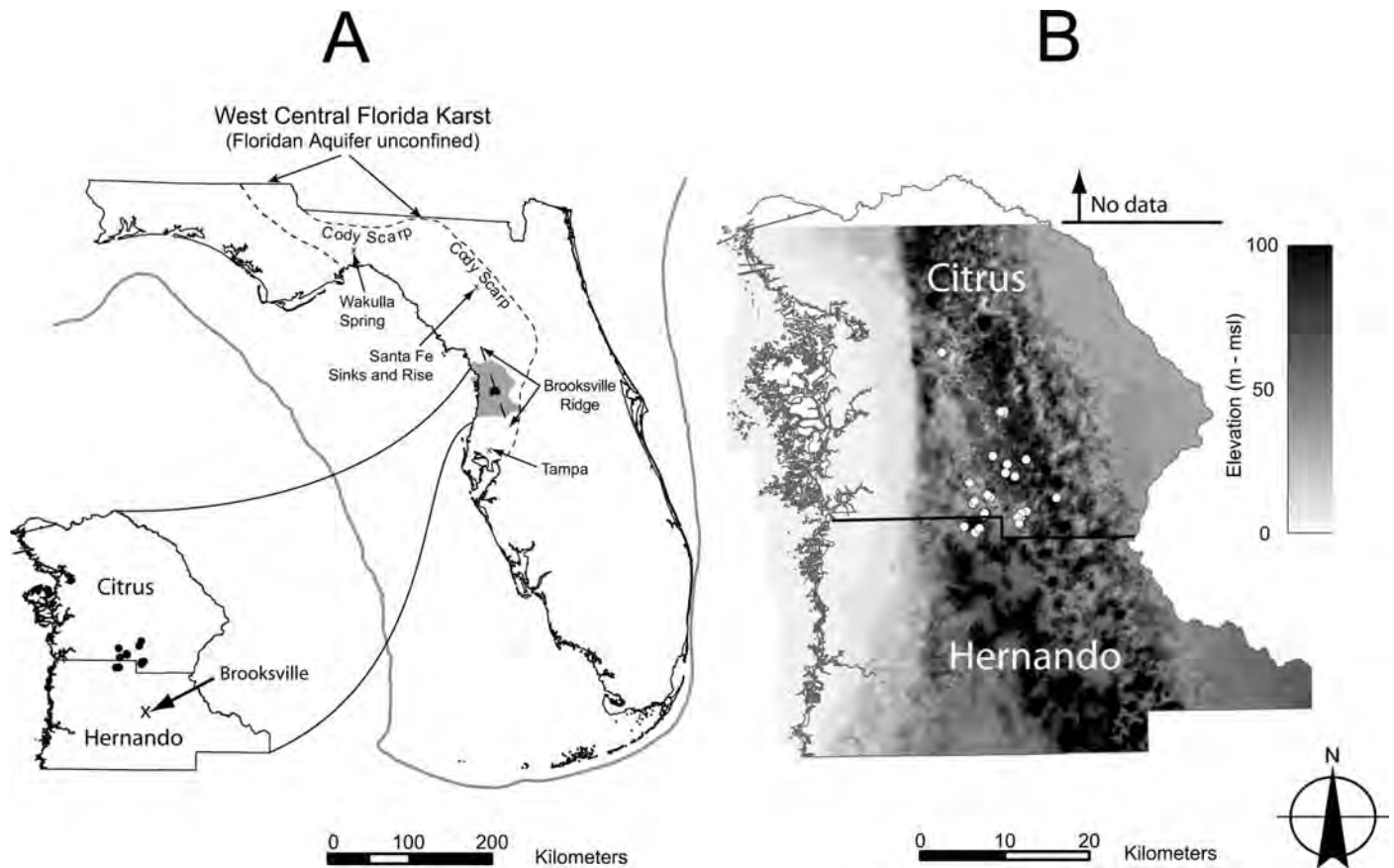
Figure 1. Data locations and topographic elevations. A) The grey line surrounding Florida is the –120 m bathymetric contour on the continental shelf. Inset is included for Citrus and Hernando Counties. Air-filled caves surveyed in this study are indicated by black dots. An "x" indicates the location of the city of Brooksville. B) Elevations for the Brooksville Ridge in Citrus and Hernando Counties are generated using GIS topographic data. Known air-filled caves in the Brooksville Ridge are indicated by white circles.

result of post-Oligocene exposure and erosion (Yon and Hendry, 1972). As a result, the Suwannee Limestone is thickest beneath the topographic highs and missing in many topographic lows (Yon *et al.*, 1989). Paleokarst filled with Miocene-age siliciclastics pierces the Suwannee Limestone throughout the Brooksville Ridge (Yon and Hendry, 1972). These paleokarst sinkholes indicate a period of intense karstification during the end-Oligocene exposure.

An irregular exposure surface with chert lenses, clay-rich marls, and a transition to non-recrystallized limestone marks the boundary between the Oligocene carbonates and the Ocala Limestone of late Eocene age. The Ocala Limestone is cream to white, soft, friable, and very porous in the Brooksville Ridge. It ranges in thickness from 30 m north of the study area to more than 120 m south of the Brooksville Ridge (Miller, 1986). Petrographic investigations of the Ocala Limestone by Loizeaux (1995) demonstrate three 3rd-order cycles of deposition. Shallow-water, high-energy facies, such as cross-bedded, low-mud grainstones and mixed-skeletal packstones, dominate all three cycles of the Ocala Limestone in the Brooksville Ridge.

Figure 2. Gentle rolling topography of the Brooksville Ridge near the city of Brooksville. An upland mesic-hardwood hammock is visible in the background. The foreground is a sinkhole lowland (photo by author).
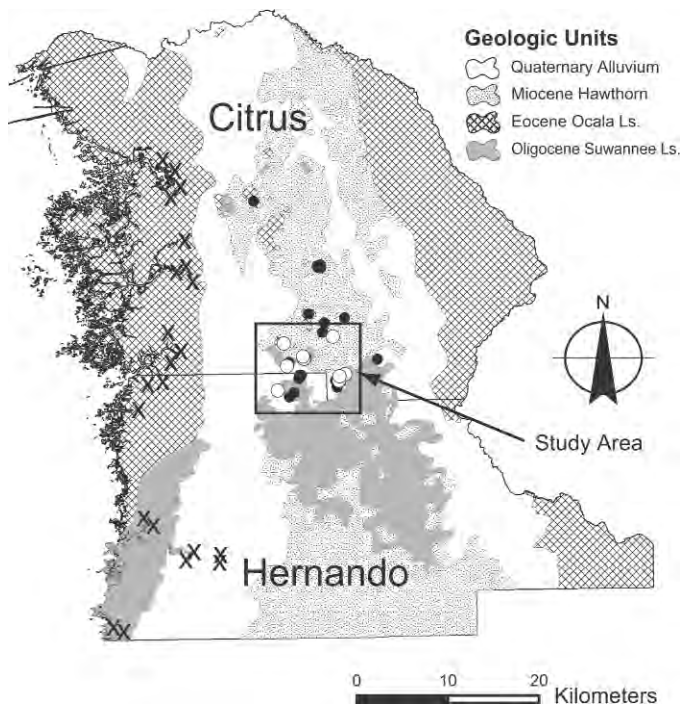
Figure 3. Geologic map of Citrus and Hernando Counties. Geologic units generally dip and thicken to the south. The Miocene Hawthorn Group is thin to non-existent in northern Hernando and southern Citrus Counties. The Oligocene Suwannee Limestone occupies only the topographic highs in the study area. Air-filled caves surveyed in this study are indicated by white circles. Additional air-filled caves known in the region are indicated by black dots. Springs are indicated with a black "X."

The geologically young carbonates of the Upper Floridan Aquifer retain much of their original porosity and permeability, which is highly heterogeneous and facies-dependent (Budd and Vacher, 2004). Measurements during this study from cave and core samples from the Brooksville Ridge indicate that the matrix permeability of the Ocala Limestone averages $10^{-12.7}$ m^2, which compares to an estimated value of $10^{-17.7}$ m^2 for the much older Paleozoic limestones of the Mammoth Cave region of Kentucky (Worthington *et al.*, 2000).

KARST OF THE BROOKSVILLE RIDGE

Historically, exploration of air-filled caves in Florida has been concentrated in portions of the panhandle near Florida Caverns State Park (Lane, 1986) and along the Cody Scarp in north-central Florida (*e.g.*, issues of the Florida Speleologist, published by the Florida Speleological Society). In west-central Florida, the emphasis of karst research has surrounded the first-magnitude springs concentrated near the Gulf of Mexico (Meinzer, 1927) (Fig. 3). These large springs, such as Weeki-Wachee, Crystal River, Chassahowitzka, and Homosassa, discharge several hundred million gallons of water per day (Scott

et al., 2004). The known underwater caves near these springs, such as Eagle's Nest, Twin-Dees, and Diepolder, are famous in the popular press for their large passages, great depths (in excess of 100 m), and technical diving challenges.

Less is known about the caves within the watersheds of the large springs along the coast in west-central Florida. These watersheds cover hundreds of square kilometers and include portions of the coastal lowlands and the Brooksville Ridge.

In the coastal lowlands, most caves are currently underwater because the depth to the water table is less than 15 m. Thick Quaternary sediments mantle karst features, subduing their surface expression (Tihansky, 1999). In contrast, the depth to the water table exceeds 45 m in the uplands of the Brooksville Ridge, and Quaternary sediments are thin to non-existent. Air-filled caves in the Brooksville Ridge have been known for decades; *e.g.*, the Dames Cave complex of southern Citrus County (Brinkmann and Reeder, 1994). However, there has been only limited exploration or scientific documentation of these caves until this study. The restricted number of natural, human-sized cave entrances contributes to the lack of exploration.

Beginning in 2001, local cave explorers located several previously unknown caves of significant size in the uplands of the Brooksville Ridge (*e.g.*, Turner, 2003). These newly-found caves are the focus of this study. Many of the discoveries were fortuitous; for example, otherwise hidden passages were revealed after structural collapses of cave roofs below abandoned lime-rock quarries. Such air-filled caves provide insight into the architecture of cave-scale porosity in the Upper Floridan Aquifer and greatly expand our perception of karst features in west-central Florida.

DATA COLLECTION

The data for this study are largely from surveys of seven caves within a study area in northern Hernando and southern Citrus Counties in west-central Florida (Fig. 3, Table 1). Maps of additional air-filled caves in the Brooksville Ridge were acquired from the archives of the Florida Cave Survey. The seven surveyed cave sites are in the central portion of the Brooksville Ridge where Miocene siliciclastics are thin and the Suwannee Limestone occupies only the upland hammocks. The Withlacoochee State Forest manages five of the seven sites; private landowners own the other two.

At each of the seven caves, I established elevation control using established data where available or by using an Ashtech Z-Extreme RTK (real-time kinematic) GPS base station and rover unit operated by the Coastal Research Group at the University of South Florida. I used a NOAA-HARN benchmark for our base station. The elevation of each in-cave survey station above mean sea level is based upon these control points. Subsequent survey from the control points, using a fiberglass tape and a hand-held compass and clinometer, is accurate to one-degree per station; this error propagates through the survey. In most of the surveyed caves, the number

Table 1. Caves surveyed in this study.

Cave Name	County	Length (m)	$n_{(sta)}$[a]	$n_{(az)}$[b]
Big Mouth Cave	Citrus	96	13	14
Blowing Hole Cave	Citrus	257	50	54
BRC Cave	Hernando	1,033	276	281
Football Cave	Citrus	142	29	31
Legend Cave	Citrus	44	12	12
Morris cave	Citrus	92	12	13
Werner Cave	Citrus	561	105	115
Totals		2,225	497	520

[a] Number of survey stations.
[b] Number of azimuth readings.

of azimuth readings exceeds the number of survey stations (Table 1), because some stations were located at passage junctions where multiple azimuth readings were required to accommodate splay shots or loop surveys.

I generated detailed maps of each cave in Adobe Illustrator and ESRI ArcGIS software using a combination of detailed sketches and the cave survey data. These maps were used to assess the overall cave morphology in plan and profile view, including height-width ratios of the passages, length-weighted rose diagrams of passage orientations, and a histogram of all the survey-station elevations.

RESULTS AND ANALYSIS

The data include more than 2.2 km of new cave survey (Table 1). Small-scale maps of the caves are presented in plan view in Figure 4. Of the caves surveyed, BRC Cave is by far the longest with more than a kilometer of mapped passage
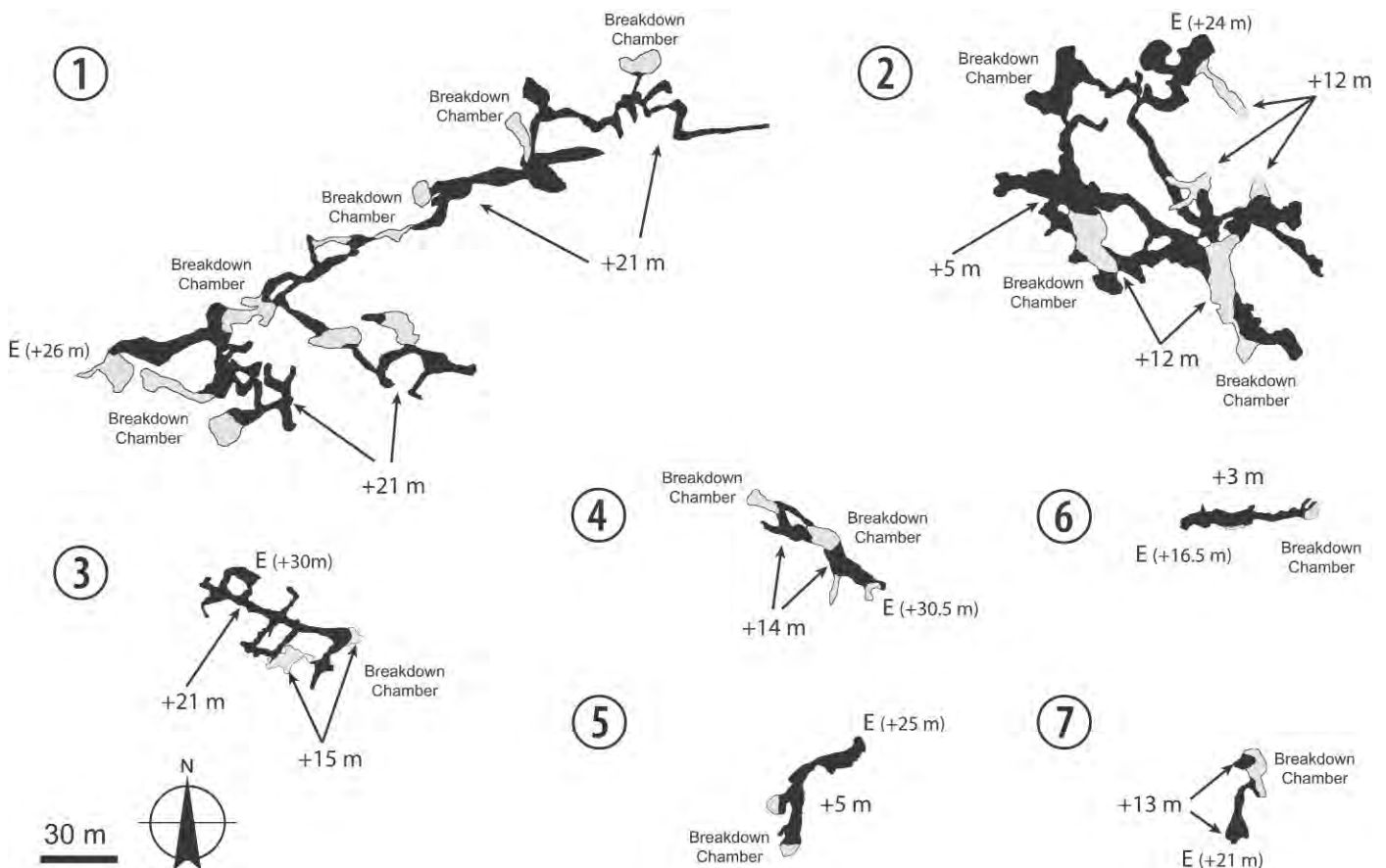

Figure 4. Index maps from air-filled caves surveyed during this study. 1 – BRC Cave, 2 – Werner Cave, 3 – Blowing Hole Cave, 4 – Football Cave, 5 – Big Mouth Cave, 6 – Morris Cave, 7 – Legend Cave. The cave passages occur on distinct levels. For instance, Werner Cave, Big Mouth Cave, and Morris Caves contain passages near the present-day water table between +3 m and +5 m. Werner Cave, Blowing Hole Cave, Football Cave, and Legend Cave all have passages between +12 m and +15 m. BRC Cave and Blowing Hole Cave both have extensive passages at +21 m. The entrances to every cave surveyed are above the level of passage development. Only Blowing Hole Cave and Football Cave have natural entrances that are fractures enlarged by dissolution that are several meters deep. All of the caves surveyed contain collapse features.
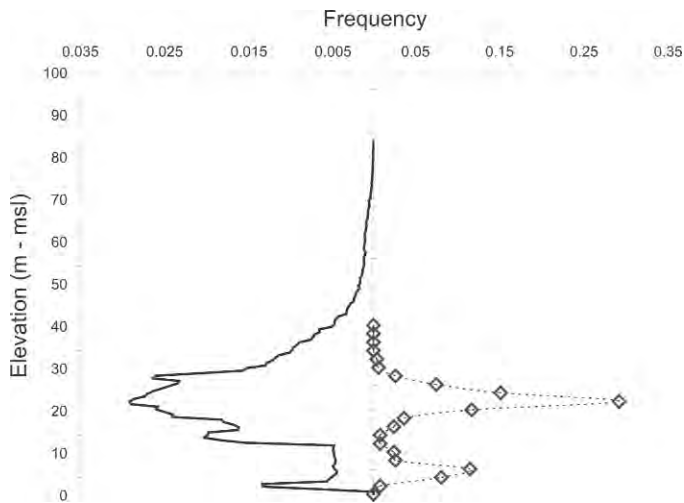
Figure 5. Frequency of data of land elevations in Citrus-Hernando Counties (left) compared to elevations of cave-survey stations in this study (right). Modes in the cave-survey data correspond with modes in the elevation data set from Citrus and Hernando Counties and with known marine terraces.
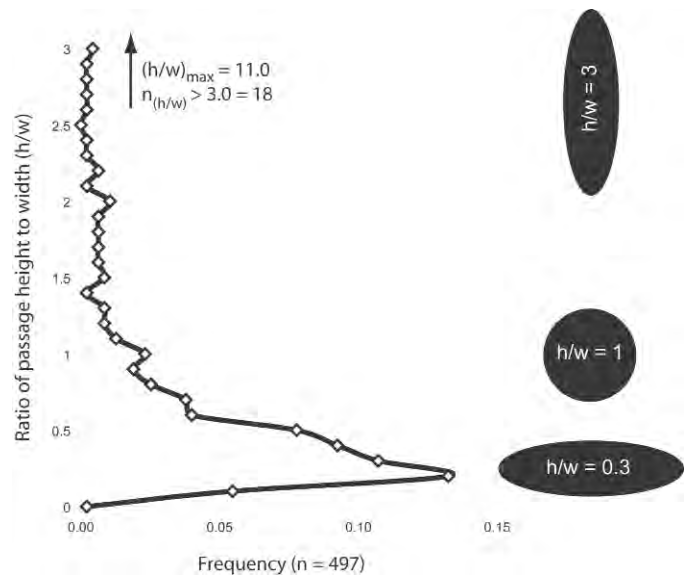

Figure 6. Frequency of passage height-width ratios at all survey stations in this study. Almost 15% of measured passages are more than four-times wider than they are tall, and 47% of measured passages are more than twice as wide as they are tall.

(Table 1); Werner Cave, (561 m, Table 1), together with Blowing Hole Cave (257 m, Table 1), round out the longest three caves in the study.

The entrances to all seven caves surveyed in this study, as well the entrances to other air-filled caves in the Brooksville Ridge, are at a higher elevation than the level of passages in the cave (Fig. 4). Football Cave and Blowing Hole Cave have natural entrances that are fractures enlarged by dissolution that are several meters deep. The entrance to Legend Cave is a small hole in a rock choke at the edge of a small lime-rock quarry. Werner, Big Mouth, and Morris Caves did not have natural entrances. Rather, a quarry operation intersected structural collapses within the cave.

Figure 5 collects elevation data for all caves surveyed in this study and compares the data to a frequency plot of elevations for Citrus and Hernando Counties from Figure 1B. Figure 6 presents a frequency plot of passage dimensions. Figure 7 presents the length-weighted rose diagrams of passage orientations and compares this data to a similar dataset from 14 caves in Marion County 40-50 km to the north and east of the study area.

Upon first inspection, all of the caves within the study area are strikingly similar in their appearance. For instance, natural solution walls, ceilings, and floors of all caves of the study area, as well as many caves throughout west-central Florida, contain cuspate, pocket-like, or even tafoni features (Fig. 8). The passages in the caves of Figure 4 terminate in blind pockets, ever-narrowing fissures, sediment fills, and collapses. Development of cave passages along fractures is visible from cave maps in plan view (Fig. 4), and individual caves demonstrate a preferred orientation of passages (BRC, Werner, and

Figure 7. Length-weighted rose diagrams for the orientation of all segments of cave survey obtained during this study and from 14 caves in Marion County to the north and east of the study area. The data from this study reveal a regional WNW-ESE (100°-120°) and NNE-SSW (20°-40°) pattern of passages similar to the data from Marion County. Both are related to regional fracture sets. Individual caves have a preferred orientation to cave passages.

Blowing Hole Caves, Fig. 7). The cumulative length-weighted rose diagram of passage directions reveals a WNW-ESE (100°-120°) and NNE-SSW (20°-40°) pattern of passages (Fig. 7).

Figure 8. Spongework-like features present in the walls of an exposed cavity in the Haile Quarry near Gainesville, Florida. Height of cavity is approximately 40 cm (Figure 12 of LaFrenz *et al.*, 2003).

Observations from quarry highwalls in the study area and throughout west-central Florida reveal laterally extensive cavities (Fig. 9). These laterally extensive cavities occur at particular elevations throughout the study area (Figs. 4 and 5). The elevations of cave survey stations cluster between +3 m and +5 m and between +20 m and +22 m (Fig. 5) above mean sea level. The individual cave maps reveal a third, less-pervasive level of passages between +12 m and +15 m (Fig. 4) which is not visible in Figure 5 because it is masked by the scatter in the survey data for the higher-elevation peak.

Human-scale passages within these cavities often occur where they intersect fractures enlarged by solution. Each cave presented in Figure 4 is a group of these human-scale cavities.

Passages formed along fractures in the caves of the Brooksville Ridge often develop "fissure" morphologies. In contrast, passages not associated with fractures acquire a "tabular" morphology. The cave-survey data demonstrate the latter

to be more common; 47% of the surveyed stations are more than twice as wide as they are tall (Fig. 6). Commonly, passages combine fissure and tabular morphologies into a signature "plus-sign" cross-section.

DISCUSSION

Caves in the young, high-permeability, coastal karst aquifers of west-central Florida differ substantially from those of the traditional, textbook perspective (*e.g.*, White, 1988; Ford and Williams, 1989) of caves in ancient, low-permeability limestones of inland karst regions. The differences in cave morphology were anticipated by Palmer (2000) and briefly examined using examples of caves from the panhandle and north-central Florida by Palmer (2002).

The common conception of caves within the ancient limestones of the mid-continent is that water generally enters at discrete sites, travels through conduits, and discharges at springs. Caves in these settings have predictable geometries. According to Palmer (2003, p. 2):

> Within karst aquifers, most of the dissolution porosity consists of conduits, usually arranged in dendritic patterns in which tributaries join each other to produce fewer but larger conduits in the downstream direction.

In such caves, the porosity tends to form "continuous conduits rather than isolated voids" Palmer (2003, p. 2).

The current perception of karst aquifers in the young carbonates of Florida is similar to this sinking-stream, spring model. For example, when speaking about the evolution of karst landscapes in Florida, Lane (1986, p. 14) states:

> Continuing dissolution…will divert more of the surface water into the underground drainage. Eventually, all of the surface drainage may be diverted underground, leaving dry stream channels that flow only during floods, or disappearing streams that flow down swallow holes…and reappear at distant points to flow as springs or resurgent streams.
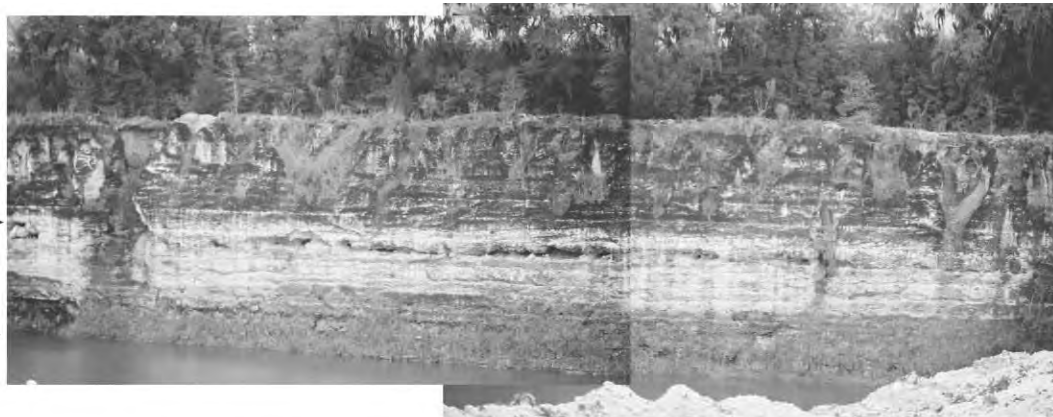

Figure 9. Photo of highwall at Haile Quarry near Gainesville in north-central Florida. The highwall is approximately 14 m tall, and the land surface is approximately 27.5 m above mean sea level. Note the laterally continuous cavernous zone 7 m below the top of the highwall at +20.5 m (Figures 5a and 9 of LaFrenz *et al.*, 2003).

Figure 10. Breakdown chamber in Werner Cave. Such collapse features are common in the caves of the study area. Main level of passages is approximately 3 m below the top of the breakdown (photo by Tom Turner).

Figure 11. Plus-sign passage in Roosevelt Cave in Marion County, Florida. Note that the vertical extension of the passage visually correlates to a fracture. Also note the laterally continuous horizon of passage approximately 1 m above the water table (photo by Sean Roberts).

Certainly there are many examples of underground river caves in Florida that follow this model. In fact, most major surface streams that cross the Cody Scarp in the Florida panhandle and north-central Florida sink into the Upper Floridan Aquifer (Upchurch, 2002). The water from several of these sinking streams travels through conduits and returns to the surface as major springs (Scott, *et al.*, 2004). Well-studied examples include the Santa Fe River Sinks and Rise (Martin and Dean, 2001) and the Wakulla-Leon Sinks Cave System (Loper *et al.*, 2005; Lane, 1986).

On the other hand, the Cody Scarp is just one physiographic feature in an otherwise large karst region, and the underground river caves associated with the Cody Scarp account for only a small fraction of the nearly 1,500 known caves in the current Florida Cave Survey database. The Brooksville Ridge is not related to the Cody Scarp and it contains many caves that are not of the underground river type. What do the caves in the Brooksville Ridge look like? How do they differ from the caves of the mid-continent, and what do these caves reveal about the hydrogeology of the Upper Floridan Aquifer in west-central Florida?

To answer these questions, I will inspect the cave architecture documented from my cave-survey data from four viewpoints: passage cross-section, directionality, horizontality, and connectivity.

PASSAGE CROSS-SECTION

Many passages in the caves of the Brooksville Ridge and throughout west-central Florida are wider than they are tall (Fig. 6). These low, wide cavities can be laterally extensive (Fig. 9). Interspersed in the tabular voids created by the later-

ally extensive cavities are pillars of rock that have not dissolved (Fig. 4). As in an underground coal mine, these pillars hold the ceiling intact. Structural collapse of the ceiling is common between these rock pillars, predominantly where rock pillars are widely spaced or where ceiling blocks are bounded by fractures. These collapses are a mixed blessing to exploration, because, while they often create large rooms in the otherwise low, wide cave (Fig. 10), they also impede progress by blocking access (Fig. 4) to cave beyond the breakdown.

Tall, narrow passages in the caves of the Brooksville Ridge and throughout west-central Florida are always associated with fractures. Human-scale passages commonly occur where fractures and the laterally extensive cavities intersect, producing a characteristic plus-sign passage morphology (Fig. 11).

Walls of the cave passages in this study have complex, small-scale solution features (Fig. 8). These cuspate, pocket-like, or tafoni structures are an indication of water-filled conditions during at least part of the cave-forming period. However, these features are not flow indicators as are scallops in caves within ancient carbonates of the mid-continent. Rather, they closely resemble spongework features found in the caves of young carbonate islands such as in the Bahamas (Mylroie *et al.*, 1995) and caves of hypogenic settings such as in the Guadalupe Mountains of New Mexico (Hill, 1987).

PASSAGE DIRECTIONALITY

Caves in west-central Florida, regardless of cross-section, exhibit a preferred orientation of passages along fractures in the aquifer (Figs. 4 and 7). The datasets from the Brooksville Ridge and from Marion County are similar; both generally reveal a regional NW-SE and NE-SW pattern of passages.
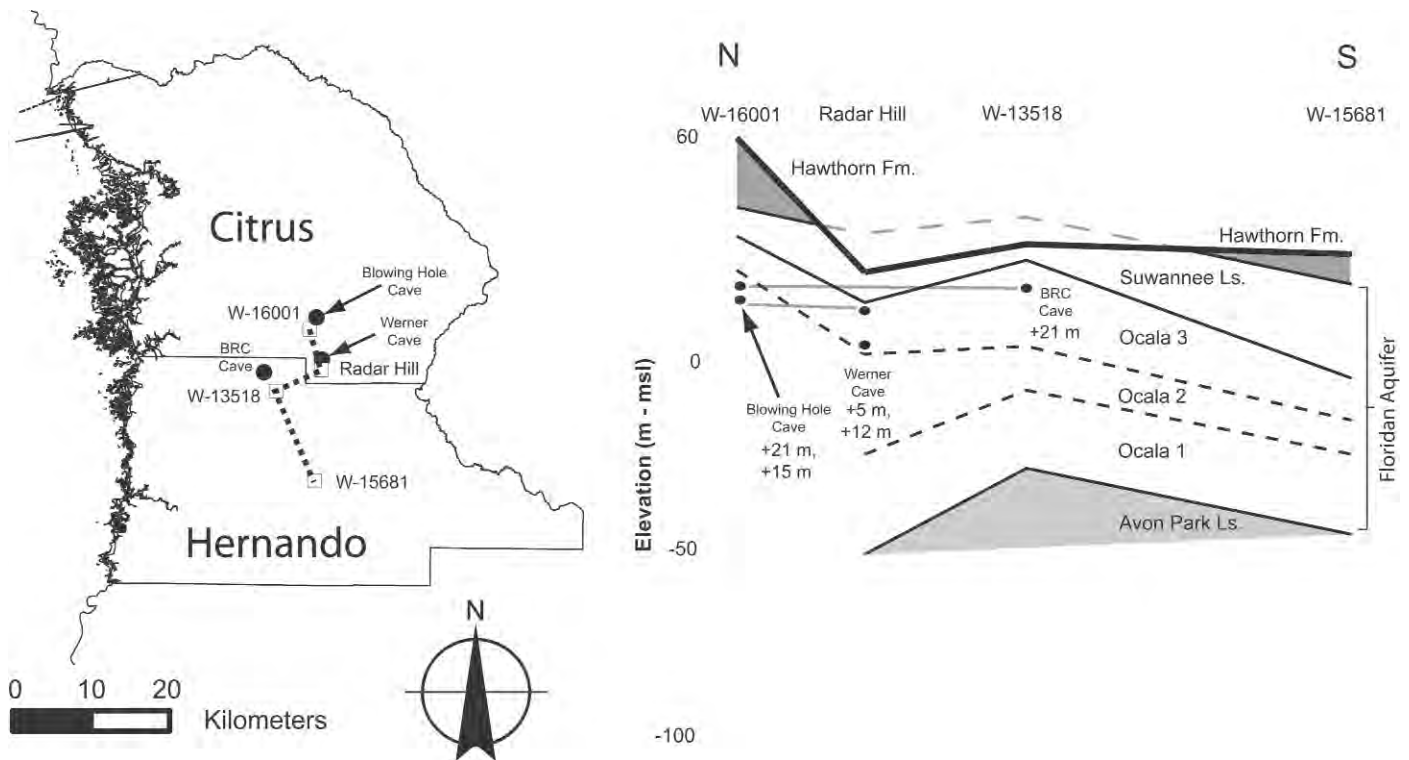
Figure 12. North-south cross-section through the study area in the Brooksville Ridge. Dashed line on the map at left indicates the location of the cross-section. White squares are the wells used for lithologic identification. Black dots are the caves from this study near the line of cross-section. Note that the levels within these caves do not occur in the same geologic units throughout the study area.

Vernon (1951), who looked at topographic and physiographic features (such as linear segments of the Withlacoochee River), and Littlefield *et al.* (1984), in a detailed study of sinkhole alignments in west-central Florida, identified a large number of photo-linear features attributed to fractures that follow this NW-SE and NE-SW pattern. The widespread nature of this pattern is a manifestation of a pervasive cause of the fractures that is not yet identified.

Individually, the rose diagrams of passage orientations vary amongst the caves in the study area and in the caves in Marion County (Fig. 7). However, these data do not provide credible evidence that explains the reason for the variation. For instance, it is unclear whether the passages surveyed in a particular cave are a representative subset of all passages in the vicinity of that cave. What is clear is that the passages are some measure of the anisotropy of the aquifer at the time the cave formed.

PASSAGE HORIZONTALITY

Cave passages in west-central Florida are not only laterally expansive, they occur at particular elevations much like the levels of cave passages within ancient limestones, such as at Mammoth Cave (Palmer, 1987). At Mammoth Cave, cave levels formed near the water table as the elevation of the Green

River experienced staged base-level lowering during glacial-interglacial cycles (Granger *et al.*, 2001). In Florida, the origin of cave levels may also result from changing positions of the water table, but one must also consider the role of lithology and, more specifically, variations in matrix permeability.

This second option, variations in matrix permeability, is often ignored in the study of caves in ancient limestones. However, the matrix permeability of the young carbonates that comprise the Upper Floridan Aquifer may be more than 10^5 times more permeable than the ancient limestones of the mid-continent. Additionally, matrix permeability in the Upper Floridan Aquifer is facies-dependent and spans three orders of magnitude (Budd and Vacher, 2004). Such variations would provide preferred horizons of ground-water flow (Vacher *et al.*, 2006).

If the cave levels in Florida are related to lithologic units with high matrix permeability, the elevations of these cave levels would change in accordance with the geologic structure. However, the widespread levels of cavities do not follow the geologic structure; the cave levels are at the same elevation even though the lithologic units dip to the south (Fig. 12). Therefore, lithologic variability does not exert the first-order influence on the locus of cave development.

There is, however, some correspondence between the cave levels in the study area and modes in the histogram of topographic data for Citrus and Hernando Counties (Fig. 5). The

modes in the topographic data manifest the classic marine terraces identified in Florida by Cooke (1945) and later Healy (1975) including the Silver Bluff (+2.4 m), Talbot (+12.8 m), Penholoway (+21.3 m), and Wicomico (+30.5 m) terraces. These marine terraces are directly related to previous elevations of sea level.

In this near-coastal setting, the position of sea level has a direct influence on the position of the water table. Since the elevations of cave levels in the survey data generally correspond to the elevation of marine terraces, it appears that the development of air-filled caves in west-central Florida may be related to positions of the water table, and thus sea level, when they were higher than present.

PASSAGE CONNECTIVITY

Of the seven caves in the Brooksville Ridge surveyed during this study, none contain continuous conduits that connect sites of recharge to points of discharge within the Upper Floridan Aquifer. Neither do passages in the surveyed caves comprise a dendritic network of conduits with tributary passages. Only one cave, BRC Cave, receives occasional water from a sinking stream and contains natural indicators of localized directional flow such as sediment ripples and pebble imbrication. Three other caves, Big Mouth, Morris, and Werner, receive recharge from artificial sinking streams created during quarry reclamation. Discharge for the water that enters all seven caves rises some 15–20 km to the west at the large springs along the coast.

Connections between the caves and the surface are limited in the karst of west-central Florida. Many caves in the Brooksville Ridge, including four of the caves in this study (BRC, Big Mouth, Morris, and Werner), had no known human-scale entrance prior to lime-rock mining. In fact, most air-filled caves that are known in the karst of west-central Florida were discovered by human alteration of the land, in particular lime-rock quarries that excavate to the level of the cave passages. The subdued topography of Florida contributes to the lack of entrances by restricting the natural intersection of the land surface with the horizontal cave passages. The implication is that there are many more caves in west-central Florida than are currently known. The burgeoning sinkhole insurance industry in Florida is a manifestation of this fact.

Surveyed passages within the air-filled caves of west-central Florida do not extend long distances. Tabular passages pinch into low cavities. Fissure-type passages thin into increasingly-narrowing fractures. Quaternary-age siliciclastic sediments and structural collapse features are pervasive, and further segment the caves. The connections between human-scale passages at the same level, therefore, are small, and additional exploration requires excavation by dedicated cavers (Turner, 2003). Vertical exploration in the caves is achieved where structural collapse features or solution-enlarged fractures connect multiple levels (Fig. 4).

POSSIBLE HYDROLOGIC IMPLICATIONS

Data from the air-filled caves in the Brooksville Ridge of west-central Florida contradict the notion of an integrated network of conduits above the modern water table. If the observations from this study are representative of conditions below the present water table, then connectivity between input and output points within the Upper Floridan Aquifer may be limited.

It also appears that caves in west-central Florida do not follow the sinking stream-spring model so widely accepted by karst scientists who study the ancient limestones of the mid-continent. Rather, water in the karst aquifers of west-central Florida may travel through a maze of passages, fractures, sediment fills, and rock matrix at several horizons.

Available data support this conjecture of multi-level discontinuous mazes. For instance, maps of underwater caves reveal passages throughout west-central Florida that occur at specific depths up to 120 m below the water table (Florea and Vacher, in review). Furthermore, Quaternary-age siliciclastic sediments infiltrate these underwater caves, and these sediments are commonly recovered from cavities encountered during well construction (*e.g.*, Hill and DeWitt, 2004).

Disjunct or occluded underwater passages in the Upper Floridan Aquifer would impede ground-water flow, resulting in higher elevations of the water table and steep hydrologic gradients. These are both observed within the karst of west-central Florida. As one example, a regional, finite-difference ground-water model that includes the northern portions of the Brooksville Ridge, developed for the Southwest Florida Water Management District by GeoTrans (1988), concluded that model calibration to known elevations of the water table is possible only if fractures or solution features are not regionally extensive or hydraulically connected. If the opposite case were true (*i.e.*, if solution features were regionally extensive or hydraulically connected), the gradient of the water table would reduce to near-zero and the elevation of the water table would equilibrate near sea level. The coastal, carbonate aquifers in the Yucatań Riviera of Mexico, with more than 400 km of mapped underwater cave and water-table gradients of less than 0.00001 (Worthington *et al.*, 2000), illustrates this possibility. This hydrogeologic contrast between the great peninsulas of Florida and Yucatań, and its relation in part to the presence of infiltrating clastics in the case of Florida, was pointed out more than 30 years ago by Back and Hanshaw (1970).

CONCLUDING REMARKS

This study of air-filled caves in the Brooksville Ridge of west-central Florida offers an improved understanding of cave-scale porosity in the Upper Floridan Aquifer. How does the architecture of these caves compare with that of other cave systems? It is instructive to review summaries from two contrasting geologic settings, the caves of ancient low-permeability limestones of the mid-continent (Palmer, 2003) and the

caves of small islands composed of Pleistocene limestone (Mylroie *et al.*, 1995).

The first example, the caves of the mid-continent, is important because it is the paradigm view of near-surface caves. Palmer (2003, p. 2) uses the following description for such caves:

> Most accessible caves are surrounded by rock in which the vast majority of openings have hardly enlarged at all. The conduits are not surrounded by porous zones, with walls like a sponge, where progressively smaller openings extend indefinitely into the cave wall. The conduits are quite discrete.

Cave passages in the young carbonates of west-central Florida do not fit this description. Tabular passages are laterally extensive, and fissure-type passages thin into increasingly-narrowing fractures; both extend beyond the limits of human exploration. The walls of the passages are porous and complex, with small-scale solution features such as pockets and tafoni structures extending into the host bedrock, which itself has high permeability. Cave passages in the Brooksville Ridge are not discrete conduits, and they do not connect together into a dendritic-style drainage system as described by Palmer (1991). Ground water in the Upper Floridan Aquifer may readily exchange between the cave and the rock matrix (Martin and Dean, 2001).

The second example, from the young carbonate islands, is important because it is the paradigm for caves in young limestone. These flank margin caves, which form by mixing at the water table and at the freshwater-saltwater interface, are summarized as follows by Mylroie and Carew (1995, p. 252-253):

> Typically these caves are dominated by large globular chambers that are broad in the horizontal plane but vertically restricted…At the rear of the chamber there is usually a series of smaller chambers that change into tubular passages…Commonly there are many cross-connections between adjacent chambers and passages that give the caves a maze-like character. The passages…end abruptly. The chamber and passage walls are often etched into a variety of dissolution pockets and tubes…Flow markings, such as ablation scallops, are absent.

Many of the features found in the caves of the Brooksville Ridge are remarkably similar to this description. Laterally extensive cavities contain bedrock pillars and cuspate dissolution features, and the passages often terminate in blind pockets. Flow indicators are generally not present. However, there are distinct differences between caves of west-central Florida and caves on young, carbonate islands. Whereas flank margin caves, for example, are composed of amorphous voids and rudimentary, spongework mazes (Palmer, 1991), the caves in west-central Florida contain passages with a sense of directionality imposed by fractures in the rock matrix. The result is maps that resemble network maze caves in plan view, such as those in the Black Hills of South Dakota (Palmer, 1991).

In conclusion, caves in west-central Florida do not fit existing models of cave architecture. They represent a style of cavern development important within coastal karst aquifers composed of young carbonates.

These west-central-Florida caves that lie above the water table demonstrate the extreme heterogeneity of permeability within the unconfined Upper Floridan Aquifer that lies below. This study offers the following insights to the architecture of cave-scale porosity in this critical-use aquifer: 1) cave-scale porosity is widespread but often composed of isolated or partially connected passages; 2) cave passages are generally restricted to specific elevations within the aquifer framework, and 3) the direction of cave passages in these levels occurs along a NE-SW and NW-SE system of fractures.

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AGE CONSTRAINTS ON CAVE DEVELOPMENT AND LANDSCAPE EVOLUTION IN THE BIGHORN BASIN OF WYOMING, USA

GREG M. STOCK*
Department of Geological Sciences, University of Michigan, Ann Arbor, MI 48109-1005, USA, gstock@umich.edu, (734) 615-4076
CATHERINE A. RIIHIMAKI
Department of Geology, Bryn Mawr College, Bryn Mawr, PA 19010, USA, criihima@brynmawr.edu, (610) 526-7971
ROBERT S. ANDERSON
Department of Geological Sciences and Institute of Arctic and Alpine Research, University of Colorado, Boulder, CO 80309, USA, andersrs@colorado.edu, (303) 735-8169

Cosmogenic $^{26}Al/^{10}Be$ burial dating and tephrochronology of cave deposits provide minimum estimates for the timing of cave development in the Bighorn Basin of Wyoming. Spence Cave is a linear phreatic passage formed along the fold axis of the Sheep Mountain anticline and subsequently truncated by 119 m of Bighorn River incision. A fine-grained eolian (windblown) sand deposit just inside the entrance yields a $^{26}Al/^{10}Be$ burial age of 0.31 ± 0.19 million years (Ma). This represents a minimum age for the development of Spence Cave, and provides a maximum incision rate for the Bighorn River of 0.38 ± 0.19 mm/yr. Horsethief Cave is a complex phreatic cave system located 43 km north of Spence Cave on a plateau surface ~340 m above the Bighorn River. Electron microprobe analyses of white, fine-grained sediment in the Powder Mountain section of Horsethief Cave confirm that this deposit is Lava Creek B fallout ash, erupted from the Yellowstone Plateau volcanic field ca. 0.64 Ma. Assuming this as a minimum age for the development of Horsethief Cave, extrapolation of the cave profile gradient westward to the Bighorn River gorge suggests a maximum incision rate of 0.35 ± 0.19 mm/yr. Incision rates from both caves match well, and are broadly similar to other estimates of regional incision, suggesting that they record lowering of the Bighorn Basin during the late Pleistocene. However, we caution that deposition of both the Spence Cave sand and the Horsethief Cave volcanic ash may postdate the actual timing of cave development. Thus, these ages place upper limits on landscape evolution rates in the Bighorn Basin.

INTRODUCTION

Caves offer important geomorphic markers that can be used to determine rates of landscape evolution, including rates of canyon cutting (here termed river incision). Cave passages that originally formed in shallow phreatic conditions (Palmer, 1991; Ford and Williams, 1989; White, 1988), but which are now perched high above modern water-table levels can be used to reconstruct the history of base level lowering (Palmer, 1987). Because base-level lowering is usually dictated by the rate that the local river incised into bedrock, dated caves can provide a robust record of this process (e.g., Ford *et al.*, 1981; Atkinson and Rowe, 1992; Sasowsky *et al.*, 1995; Granger *et al.*, 1997, 2001; Stock *et al.*, 2004; Anthony and Granger, 2004).

Determining cave ages is difficult because caves are voids that usually cannot be directly dated. Although there are some exceptional cases where dated deposits derive from speleogenesis, such as clays resulting from sulfuric acid dissolution (Polyak *et al.*, 1998), more often only limiting estimates of cave age can be determined by dating either speleothems or sediments deposited within cave passages after the passages formed. U/Th dating of calcite speleothems or paleomagnetic or cosmogenic $^{26}Al/^{10}Be$ burial dating of clastic sediment are the primary methods of constraining cave ages. Sediment and

speleothems are sometimes deposited during the waning stages of speleogenesis, but can also be deposited much later; speleothems are particularly prone to this effect. Dating coarse clastic sediment that has clearly washed into caves most directly ties the cave age to the former position of the local base-level river. Thus, coarse fluvial sediment is usually the better material for dating cave development (Stock *et al.*, 2005). Once an age has been determined, river incision rates can then be determined by dividing the height of the dated cave passage above the modern river by the age of the deposit. Because even in the best of circumstances dated deposits represent minimum estimates of cave age, rates of river incision calculated from these ages must be considered maximum rates (e.g., Ford *et al.*, 1981; Atkinson and Rowe, 1992; Stock *et al.*, 2005). Using dated deposits of uncertain relation to base level tends to reduce the estimated age of the cave, thereby increasing the estimated incision rate.

We investigated several caves in the northeast Bighorn Basin and adjacent Bighorn Mountains of Wyoming as part of a larger study of the geomorphic history of the this region (Anderson *et al.*, 2006; Riihimaki *et al.*, in press; Riihimaki, 2003). Although most of the caves we investigated did not con-

* Present address: Yosemite National Park, 5083 Foresta Road, P.O. Box 700, El Portal, CA 95318, USA, (209) 379-3263, greg_stock@nps.gov
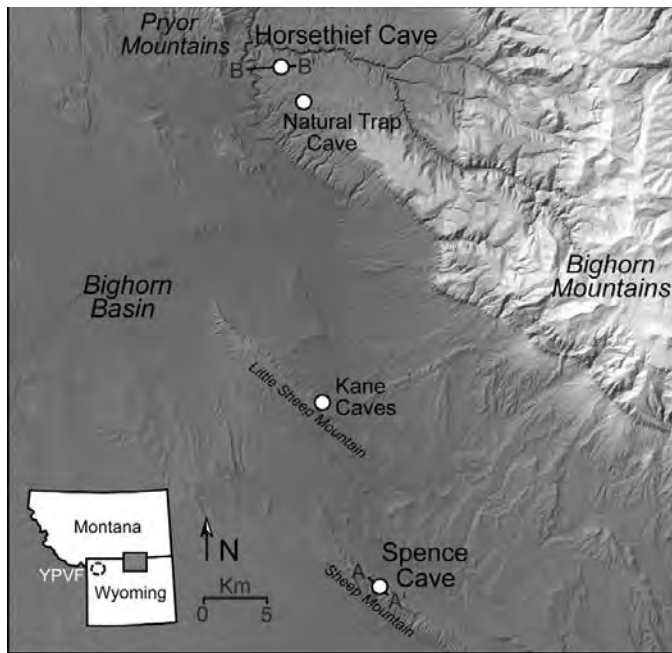
Figure 1. Map of the northeastern Bighorn Basin, Wyoming, showing locations of Spence, Kane, Horsethief, and Natural Trap caves. Cross-sections A-A′ and B-B′ are shown in Figures 2 and 4, respectively. YPVF: Yellowstone Plateau volcanic field, source of the ca. 0.64 Ma Lava Creek B ash.

tain datable fluvial sediments, we were able to obtain ages from two of the larger caves in the region, Spence Cave and Horsethief Cave (Fig. 1). In both cases, the setting and morphology of these caves suggest that they record former positions of the Bighorn River. However, they do not contain coarse clastic sediments washed in when the caves were at (or very near) river level. Rather, they contain datable sediments (windblown sand and volcanic ash, respectively) that likely entered the caves after they formed. As such, the dated sediments provide minimum estimates of the timing of cave development in the region. Combining these age data with the positions of these caves in the modern landscape yields maximum incision rates of the Bighorn River.

SETTING

The Bighorn Basin is a large structural basin located in north-central Wyoming (Fig. 1). The northeast part of the basin, where the study caves are located, is flanked on the east by the Bighorn Mountains and on the north by the Pryor Mountains (Fig. 1). The Bighorn River flows northward through the basin, meandering across basin fill for much of its length, but is occasionally restricted to deep gorges incised into uplifted structures such as the Sheep Mountain and Little Sheep Mountain anticlines and the northern Bighorn Mountains (Fig. 1). The numerous caves in this region have developed primarily in the Madison Limestone of Mississippian age (360 to 325 million years ago [Ma]), but are
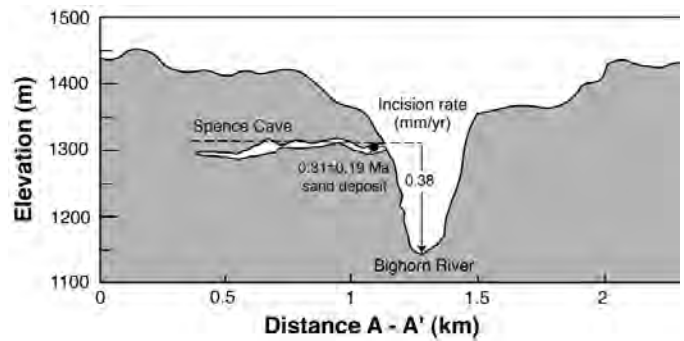

Figure 2. Topographic cross section along A-A′ (see Fig. 1) showing position of Spence Cave in relation to Bighorn River where it incises through Sheep Mountain anticline. Note vertical exaggeration. Dashed line indicates approximate water table position during cave development. Minimum cave age of 0.31 ± 0.19 Ma, based on burial age of sand deposit just inside cave entrance, suggests a maximum Bighorn River incision rate of 0.38 ± 0.19 mm/yr.

also present in the Bighorn Dolomite of Ordovician age (500 to 440 Ma). The caves discussed in this paper have formed within the Madison Limestone (Hill *et al.*, 1976).

CAVE DESCRIPTIONS, ANALYTICAL METHODS, AND RESULTS

SPENCE CAVE

Spence Cave is located at an elevation of 1312 m, 119 ± 2 m above the Bighorn River (Fig. 2), in the folded core of the Sheep Mountain anticline (Fig. 3). The cave consists of a single phreatic passage 728 m long, with a short constriction and 12 m drop approximately halfway through. Egemeier (1981) interpreted Spence Cave to have been dissolved by sulfuric acid upwelling along joints parallel to the core of the anticline and mixing with a shallow paleo-water table surface graded to

Figure 3. Photograph taken from Spence Cave entrance, looking across gorge cut by Bighorn River through curving strata of Sheep Mountain anticline.

Figure 4. Photograph of sand deposit just inside entrance to Spence Cave. Fine grain size, high degree of sorting, uniform lithology, and position near the entrance suggest an eolian (windblown) origin. Cosmogenic ^{26}Al/^{10}Be burial dating of the sand indicates that it was emplaced ca. 0.31 ± 0.19 Ma.

a former level of the Bighorn River. This model of cave development is analogous to ongoing sulfuric acid dissolution in lower Kane Cave (Engel *et al.*, 2004; Egemeier, 1981), located 15 km north of Spence Cave in the smaller Little Sheep Mountain anticline (Fig. 1). Lower Kane Cave is situated within a few meters of the present Bighorn River and consists of a single horizontal passage extending ~330 m into the canyon wall, parallel to the folded core of the anticline. Situated 32 m above Lower Kane Cave, 329-m-long Upper Kane Cave likely represents an earlier phase of cave development that occurred when the Bighorn River was at that level. Although we were not able to find sedimentary deposits suitable for dating in either Upper or Lower Kane Caves, they do present useful analogies for the development of Spence Cave.

The arched entrance to Spence Cave is ~5 m wide and 3 m high. The floor of the entrance area consists of an extensive deposit of well-sorted, very fine, quartz-rich sand (Fig. 4). The deposit extends ~15 m into the cave before sloping steeply

down at the angle of repose into the large main passage. The deposit has faint bedding 1 mm to 1 cm thick. We observed no finer-grained sediments further inside the cave, as might be expected if the deposit were fluvially emplaced as a prograding delta into a water-filled passage, nor were there ripples or channels indicative of deposition by subaerially flowing water. Therefore, we interpret this deposit as eolian (windblown) in origin. Although this interpretation does not directly tie the deposit to a cave position near river level, we infer that this sand was in fact deposited when the cave entrance was very near the Bighorn River. This is because concentrations of eolian sand decrease rapidly with height above the ground surface (Zheng *et al.*, 2004; Anderson, 1986); even high winds usually do not mobilize sand grains of this size more than a few meters into the air. In addition, we did not identify any modern source for quartz sand close to the entrance. Thus, we consider dry sand along the banks of the Bighorn River to be the most likely source for the sand in Spence Cave, and argue that this sand entered the cave when it was within a few vertical meters of river level (*i.e.*, prior to the 119 m of incision). Constant replenishment of riverside sand banks would have provided the large source needed to produce the considerable volume of the Spence Cave deposit.

We dated sand from the top of the Spence Cave deposit using cosmogenic burial dating (*e.g.*, Granger *et al.*, 1997, 2001; Granger and Muzikar, 2001; Anthony and Granger, 2004; Stock *et al.*, 2004, 2005). This dating method exploits the fact the quartz-rich sediment at or near the Earth's surface accumulates the rare cosmogenic isotopes aluminum-26 (^{26}Al) and beryllium-10 (^{10}Be) by cosmic ray bombardment, but no longer accumulates these isotopes once the sediment enters a cave and is shielded from further bombardment. Because these isotopes decay radioactively at rates that differ by about a factor of two, the ratio of ^{26}Al to ^{10}Be in the sediment changes through time, and may be used to assess the duration of burial within the cave. We collected ~500 g of sand, purified the quartz in it by chemical dissolution (Kohl and Nishiizumi, 1992), and isolated ^{26}Al and ^{10}Be using methods described in Stock *et al.* (2005).

Table 1. Cosmogenic nuclide concentrations and burial age for Spence Cave.

Cave	Mass quartz (g)	P_{10}[a] (atm g^{-1} yr^{-1})	^{26}Al (10^5 atm g^{-1})	^{10}Be (10^5 atm g^{-1})	^{26}Al/^{10}Be	Burial age[b] (Ma)
Spence	80.08	15.0	3.754 ± 0.035	0.726 ± 0.002	5.17 ± 0.05	0.31 ± 0.19 (0.20)

[a] Local ^{10}Be production rate at Spence Cave site, scaled for altitude and latitude and assuming sea-level high latitude production rate of 5.1 atm g^{-1} yr^{-1} (Stone, 2000). Multiply by 6.1 to get local ^{26}Al production rate.

[b] Uncertainties represent one standard error measurement uncertainty. Systematic uncertainties in nuclide production rates, production rate ratio (Stone, 2000), and radioactive decay constants are added in quadrature and shown as total uncertainty in parentheses.
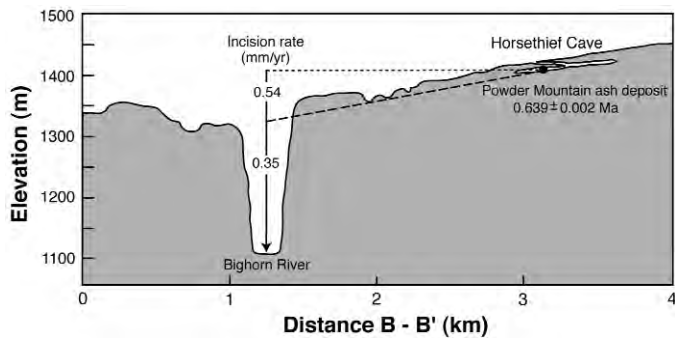
Figure 5. Topographic cross section along B-B′ (see Fig. 1) showing position of Horsethief Cave in relation to Bighorn River. Note vertical exaggeration. Assuming Bighorn River was at the same elevation as Horsethief Cave (dotted line) when Powder Mountain Passage formed, dividing this height above modern river level by the age of the Lava Creek B ash (0.639 ± 0.002 Ma) yields a maximum incision rate of 0.54 ± 0.01 mm/yr. Extrapolation of cave gradient (dashed line) suggests a river position 220 m above modern at the time Horsethief Cave formed, yielding an incision rate of 0.35 ± 0.19 mm/yr (see text).

The ^{26}Al/^{10}Be ratio of the Spence Cave sample suggests that the top of the sand deposit was emplaced ca. 0.31 ± 0.19 Ma (Table 1). This age is close to the practical lower limit of the technique (Granger *et al.*, 1997; Granger and Muzikar, 2001), and is thus subject to relatively large uncertainty resulting both from analytical uncertainty and uncertainty in the decay constants of ^{26}Al and ^{10}Be (Norris *et al.*, 1983; Middleton *et al.*, 1993). The 0.31 ± 0.19 Ma burial age of the sand represents a minimum age for the development of Spence Cave; this is especially true because we were not able to sample the base of the deposit. This minimum age, combined with the height of the cave above the modern Bighorn River, provides a maximum incision rate of 0.38 ± 0.19 mm/yr for the Bighorn River at this location (Fig. 2). The uncertainty on the incision rate is large because of the large analytical uncertainty on the burial age.

HORSETHIEF CAVE

Horsethief Cave is located on a broad plateau surface southeast of the Bighorn River Gorge at an elevation of 1428 m (Fig. 5). Horsethief Cave is one of the longest caves in the Rocky Mountains, comprising roughly one half of the Bighorn-Horsethief Cave system. The combined length of the two caves is 23.5 km, though a natural connection between them is presently sealed (Hill *et al.*, 1976).

Horsethief Cave is an example of a network maze, with secondary spongework maze development (Palmer, 1991; Sutherland, 1976). The cave appears to have formed entirely under phreatic conditions. Although there is likely some structural control on the cave is longitudinal profile (Fig. 5), including a system of joints and a semi-impermeable layer strati-

Figure 6. Photograph of Powder Mountain Passage in Horsethief Cave. White cone-shaped deposit illuminated at far end of room is Lava Creek B fallout ash erupted from Yellowstone Plateau volcanic field 0.639 ± 0.002 Ma.
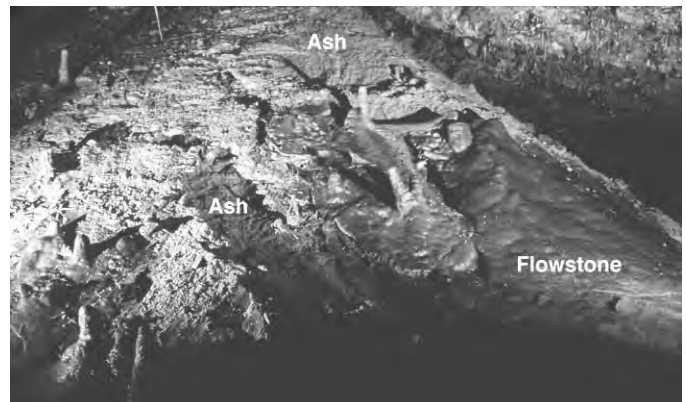

Figure 7. Close up view of Powder Mountain ash deposit. Width of view is ~2 m. Note several-cm-thick flowstone and stalagmite deposits on top of pristine white ash.

graphically below the cave (Sutherland, 1976), the fundamental control on ground-water flow through Horsethief Cave appears to have been the position of the Bighorn River. This is supported by the fact that the cave gradient trends almost due west, which represents the steepest hydraulic gradient to the Bighorn River, whereas the bedding dips southwest (Sutherland, 1976). As Horsethief Cave formed, ground-water moving through low-gradient phreatic passages likely eventually emerged as springs along the banks of the entrenched Bighorn River (e.g., Palmer, 1987). Thus, age constraints on the development of Horsethief Cave provide rates of incision for the Bighorn River due west of the cave.

The single entrance to Horsethief Cave is in a large doline, but there is evidence for additional former entrances within the cave. Some of the most striking depositional features within Horsethief Cave are the so-called "Buddhas," large conical mounds of various sedimentary materials. Most of the Buddha formations are composed of siliciclastic sediment that has been

interpreted either as paleofill following an earlier period of cave development (e.g., the "Red Buddha"), or as surficial material entering the cave through now-closed fissure or doline entrances (Sutherland, 1976). In the Powder Mountain section of the cave (Fig. 6), there is a large conical deposit of white powdery material positioned below a narrow fissure extending upward into the ceiling (Fig. 7). We agree with Sutherland (1976) that this fissure was likely a former entrance, similar to the modern entrances of adjacent Bighorn Cave, and was only open for a relatively short period of time. Sutherland (1976) examined the Powder Mountain deposit and other similar deposits from the Powder Mountain area and suggested a tentative correlation with one of the Pearlette family of volcanic ashes. As subsequently shown by Izett (1981) and Izett and Wilcox (1982), the Pearlette ashes were erupted from the Yellowstone Plateau volcanic field in northwestern Wyoming and eastern Idaho (Fig. 1) and include two very widespread ash beds, the 0.64 Ma Lava Creek B ash bed and the 2.06 Ma Huckleberry Ridge ash bed.

The Powder Mountain deposit is a prime candidate for tephrochronology (chemical correlation with well-dated volcanic ashes and tuffs) because it is nearly pristine (*i.e.*, contains very few non-glass grains) and has been shielded from weathering and erosion. A sample (082201-ca) of the Powder Mountain deposit was processed and analyzed at the University of Utah tephrochronology laboratory. Analyses were performed on a Cameca SX-50 electron microprobe using methods described in Perkins *et al.* (1995) and under analytical conditions described by Nash (1992). Twenty-five glass shards were analyzed for 13 major and minor elements including oxygen. Of these 25 analyses, one shard with a Ti concentration well outside the range observed in the other 24 shards was eliminated as an outlier prior to calculating the average concentrations shown in Table 2.

Comparison of sample 082201-ca glass shard analyses with those in an extensive database of late Cenozoic tephra of the western U.S. indicates that sample 082201-ca is most likely the Lava Creek B ash bed. In particular, the glass shards of 082201-ca show the distinctive bimodal composition commonly observed in the Lava Creek B ash bed (Williams, 1994) with a dominant low Fe mode and a secondary high Fe mode (Table 2). It is worth noting that there is considerable compositional overlap between the averages of analyses for the Lava Creek B ash bed and the older Huckleberry Ridge ash bed (Table 2). However, glass shards of the Huckleberry Ridge ash bed generally display a range of compositions from lower to higher Fe rather than the discrete lower and higher Fe modes typical of Lava Creek B. Furthermore, for a given Fe concentration the concentration of Ca is lower in the glass shards of Lava Creek B ash bed relative to those of the Huckleberry Ridge ash bed. Finally, we note that other Yellowstone Plateau source ash beds, such as the 1.26 Ma Mesa Falls ash bed, the 0.64 Ma Lava Creek A ash bed, the late Pleistocene Hebgen Narrows ash bed and the 0.11 Ma Natural Trap Cave (Fig. 1) ash bed are distinctly different than either the Lava Creek B or

Table 2. Composition of glass shards from Powder Mountain, Horsethief Cave (082201-ca), and Lava Creek B and Huckleberry Ridge ash beds — Electron microprobe analyses[a].

Sample[b]	n[c]	SiO_2[d]	TiO_2	Al_2O_3	Fe_2O_3[e]	MnO	MgO	CaO	BaO	Na_2O	K_2O	Cl	F	sum	H_2O[f]	−O
082201-ca	24	75.0	0.098	11.6	1.57	0.029	0.018	0.519	0.008	3.14	4.88	0.156	0.20	97.2	5.1	0.03
082201-ca-I	17	75.0	0.086	11.6	1.45	0.028	0.017	0.497	0.006	3.16	4.83	0.171	0.24	97.1	5.3	0.04
082201-ca-II	7	74.9	0.126	11.5	1.84	0.032	0.020	0.572	0.011	3.09	5.00	0.119	0.12	97.3	4.8	0.03
LCBavg	25	73.5	0.112	11.6	1.57	0.035	0.020	0.515	0.009	3.15	4.95	0.139	0.18	95.8	4.5	0.03
LCBmax	...	75.0	0.120	11.9	1.64	0.043	0.023	0.560	0.013	3.32	5.11	0.159	0.26	...	...	...
LCBmin	...	72.0	0.098	11.5	1.50	0.030	0.015	0.470	0.006	3.07	4.78	0.120	0.07	...	...	...
HBRavg	22	74.1	0.102	11.8	1.64	0.036	0.018	0.561	0.013	3.34	4.04	0.139	0.19	96.0	4.7	0.03
HBRmax	...	75.1	0.107	11.9	1.70	0.039	0.025	0.583	0.022	3.73	5.25	0.150	0.24	...	...	...
HBRmin	...	73.4	0.092	11.7	1.59	0.032	0.015	0.532	0.008	3.03	1.51	0.126	0.11	...	...	...

[a] Analyses of Powder Mountain sample (082201-ca) by Michael E. Perkins, others by Steven W. Williams, Barbara P. Nash, and Michael E. Perkins, University of Utah.
[b] LCB = Lava Creek B ash bed with results from analyses for 8 samples. HBR = Huckleberry Ridge ash bed with results from analyses of 6 samples.
[c] Number or average number of analyzed glass shards.
[d] All oxides and elements in wt%.
[e] Total Fe reported as Fe_2O_3.
[f] H_2O content calculated from difference between measured and stoichiometric oxygen content assuming all Fe as Fe_2O_3.

Table 3. Bighorn River incision rates based on cave sediment ages.

Cave	Height Above Bighorn River (m)	Adjusted Height[a] (m)	Age (Ma)	Incision Rate (mm yr⁻¹)	Adjusted Incision Rate[a] (mm yr⁻¹)
Spence	119 ± 2	119 ± 2	0.31 ± 0.20	0.38 ± 0.19	0.38 ± 0.19
Horsethief	343 ± 5	221 ± 60	0.639 ± 0.002	0.54 ± 0.01	0.35 ± 0.19

[a] Adjusted heights and incision rates account for paleo-hydraulic gradient indicated by dip of cave passages.

Huckleberry Ridge ash beds. In particular, they have measurably different average glass shard compositions and show tight unimodal compositions rather than either the distinctive bimodal composition of the Lava Creek B glass shards or the range of compositions of the Huckleberry Ridge glass shards.

The Lava Creek B ash is preserved across much of the western and central United States because of its substantial volume, broad initial dispersal, and the aggrading depositional environments into which the ash fell (Izett and Wilcox, 1982; Dethier, 2001). Based on the chemical correlation, the large volume of material in the Powder Mountain deposit, and the occurrence of Lava Creek B ash elsewhere in the Bighorn Basin (Izett and Wilcox, 1982; Reheis et al., 1991; Dethier, 2001), we conclude that the voluminous deposit in the Powder Mountain Passage is the Lava Creek B ash.

Precisely how old is this Lava Creek B deposit? ⁴⁰Ar/³⁹Ar dating by Lanphere et al. (2002) placed the age of member B of the Lava Creek Tuff at 0.639 ± 0.002 Ma and found excellent agreement between ⁴⁰Ar/³⁹Ar ages from members A and B of the Lava Creek tuff and those of the Lava Creek fallout ash. Given the pristine appearance of the Powder Mountain deposit (i.e., nearly pure ash with little to no terrigenous sediment; Fig. 7), we infer that this ash entered the cave rapidly, soon after falling on the surface. That the ash fell directly into the entrance rather than being washed in is supported by (1) the steeply sloped, conical shape of Powder Mountain (Fig. 7), (2) the powdery nature of the deposit, (3) a lack of fluvial sedimentary structures, (4) the angularity of glass shards, and (5) the nearly 100% glass composition of the material. Sutherland (1976) made similar observations in the Powder Mountain area. Therefore, we argue that the 0.639 ± 0.002 Ma eruption age of the Lava Creek B member closely marks the timing of deposition within the cave. Although development of this level of Horsethief Cave could have occurred before deposition of the ash, the eruption age provides a minimum estimate of the age of the Powder Mountain passage.

Although the development of Horsethief Cave is clearly linked to former levels of the Bighorn River, the position of Horsethief Cave in the landscape creates large uncertainty in our estimates of river incision rates. Unlike Spence Cave, which is situated directly above the modern Bighorn River and is therefore a relatively unambiguous marker of the former river level, Horsethief Cave is set back ~1.75 km from the modern river (Fig. 5). As a result, the position of the Bighorn

River when the Powder Mountain passage formed is harder to define. We have made two estimates of the incision rate of the Bighorn River west of Horsethief Cave. The first is determined simply by dividing the present height of the Powder Mountain passage above the modern river taken at a point due west of the cave (343 ± 5 m) by the age of the passage (here taken to be 0.639 ± 0.002 Ma). This most simple calculation yields an incision rate of 0.54 ± 0.01 mm yr⁻¹. The uncertainty in the rate is low in this case, because it is based only on how well the height of the Powder Mountain passage above the modern river is known (± 1.3%) and on the analytical precision of the ⁴⁰Ar/³⁹Ar age (± 0.3%). However, a more accurate, though less precise, method of determining the incision rate is to account for the paleo-hydraulic gradient by extrapolating the cave profile down the hydraulic gradient to the river. In most cases this will estimate a river position lower than the more simple calculation above, resulting in a slower rate of incision. We used the profile of Horsethief Cave to extrapolate west, down gradient to the Bighorn River canyon (Fig. 5). This exercise suggests that the spring outlet for the Horsethief Cave system was located ~220 m above the modern river level, rather than the 343 m used in the prior calculation, and yields a maximum incision rate of 0.35 ± 0.19 mm yr⁻¹. In this case, the uncertainty on the rate is large because of the considerable uncertainty in the reconstructed river position, which we estimate to be ~50%.

DISCUSSION

The incision rates we calculate based on dated sediment deposits in Spence and Horsethief caves are nearly identical (0.38 ± 0.19 and 0.35 ± 0.19 mm yr⁻¹, respectively; Table 3), suggesting that they represent accurate estimates of river incision. They are also broadly similar to other estimates of incision in the region. For example, Reheis et al. (1991) used the presence of the Lava Creek B ash in river terraces to calculate incision rates of 0.16 mm yr⁻¹ for the upper Bighorn River in Montana and Wyoming. Regionally, the depth of incision since Lava Creek B ash deposition reveals that rates of > 0.15 mm yr⁻¹ are typical of Rocky Mountain rivers; these rates can be as high as 0.3 mm/yr (Dethier, 2001). Reiners et al. (2002) found erosion rates of ~0.15-0.4 mm yr⁻¹ in the nearby Powder River Basin.

These independent incision rate estimates are within the uncertainty of our calculated incision rates from the caves. However, our best incision rate estimates are somewhat faster (0.3–0.4 vs. 0.15–0.2 mm yr^{-1}). If this discrepancy is real, and not a product of our uncertainty, then there are several possible explanations. The discrepancy may relate to geologic processes, such as isostatic uplift in response to sediment removal, that increase river incision rates preferentially in certain parts of the basin (Riihimaki, 2003). However, the discrepancy may also result from bias resulting from the various methods used to estimate incision rates. For example, incision estimates based on river terraces (*e.g.*, Reheis *et al.*, 1991) could be erroneously fast because incision below the terraces may have commenced well after deposition of the Lava Creek B ash. As mentioned at the beginning of this paper, caves are also prone to a similar bias, which can be further exacerbated depending on what type of material is dated. Unless the dated deposits can be shown to relate directly to cave development, then they represent minimum estimates of cave ages, and may underestimate the actual cave ages considerably. For example, in order to yield an incision rate of 0.15 mm yr^{-1}, the age of Spence Cave should be ~0.8 Ma, nearly 0.5 Ma older than the sand burial age we determined. Although this is outside the range of burial age uncertainty, we stress that while this age may accurately date deposition of the sand, it may not accurately date development of the cave. The same is true for the Horsethief Cave ash deposit; the Lava Creek B ash could have fallen into the Powder Mountain room well after the passage formed. The 0.11 Ma ash in Natural Trap Cave (Fig. 1; Gilbert, 1984) presents a useful example of this problem; if the age of this ash, which erupted nearly 0.5 Ma after the Lava Creek B ash, were combined with the height of Natural Trap Cave above the Bighorn River to calculate an incision rate, the resulting rate would be 3.63 mm yr^{-1}. This rate is clearly much too fast, and simply results from performing the calculation using dated material that considerably underestimates the true age of the cave. In the case of Spence and Horsethief caves, the nature of the deposits and the consistent incision rates they provide increase our confidence that the incision rates we have calculated are accurate. The important distinction between the age of a cave deposit and the actual age of the cave it resides in, noted decades ago (*e.g.*, Ford *et al.*, 1981; Atkinson and Rowe, 1992) and quantified more recently (Stock *et al.*, 2005), might explain why our calculated incision rates are higher than other regional estimates, independent of geological processes. In any case, the dated sediment deposits in Spence and Horsethief caves set important constraints on river incision rates in the Bighorn Basin since 0.31 and 0.64 Ma, respectively, and further highlight the importance of cave studies in geomorphic and tectonic research.

CONCLUSIONS

A cosmogenic ^{26}Al/^{10}Be burial date for fine (eolian) sand within the entrance area of Spence Cave yields a burial age of 0.31 ± 0.19 million years. Tephrochronology suggests that a fine white sediment deposit in the Powder Mountain area of Horsethief Cave is the Lava Creek B fallout ash, erupted from the Yellowstone Plateau volcanic field ca. 0.64 million years ago. These two dates provide minimum estimates for the timing of cave development in the Bighorn Basin, and yield maximum incision rates for the Bighorn River of 0.38 ± 0.19 mm yr^{-1} and 0.35 ± 0.19 mm yr^{-1}, respectively. These rates are broadly similar to independent estimates of river incision in the region. An apparent two-fold discrepancy between the rates based on the dated cave deposits and other independent estimates may be due to geologic processes, but may also result from the possibility that the dated deposits postdate the actual time of cave development. Investigation of other caves (*e.g.*, Tongue River Cave, Cliff Dwellers Cave, Spirit Mountain Caverns) may further clarify rates of landscape evolution in the region.

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A REVISION OF THE GENUS *TYPHLOGASTRURA* IN NORTH AMERICAN CAVES WITH DESCRIPTION OF FIVE NEW SPECIES

KENNETH CHRISTIANSEN AND HANGHANG WANG*
Grinnell College, Grinnell IA 50112

The peculiar troglobitic, polyphyletic nature of Typhlogastrura *and the small amount of troglomorphism found in the genus is discussed. New features of chaetotaxy are systematized and used . The Nearctic members are distinguished, keyed out and five new species*—asymmetrica, fousheensis, helleri, steinmanni *and* unica—*are described. Species are shown to occur in 5 widely separated regions ranging from Colorado to Pennsylvania.*

INTRODUCTION

Typhlogastrura is, in our view, an artificial genus consisting of all members of the *denticulata* branch of *Ceratophysella*, which have successfully invaded caves and become troglomorphic. They have a number of unusual features: A) they are the result of many widely separated invasions and most of these paraphyletic groups occur in only one cave or a few closely connected caves[1], B) there appear to be no troglophilic or edaphic species (with one possible exception[2]) and C) in the Nearctic region, there has been no evolution in any of these forms into more troglomorphic species within the cave systems they have invaded. The parallel genus from the *armata* branch of *Ceratophysella* (*Bonetogastrura*) has both cave and troglophilic species as well as one surface species *(B. variabilis*) which is the only one found in the Northern North America. Both genera are characterized by a reduction in eyes and the maintenance of a fully developed furcula. This is in contrast to the genus *Schaefferia* which includes reduced eye *Ceratophysella* that are largely edaphic and have reduced furculas, which we have shown is uniquely an edaphic phenomenon (Christiansen, 1985). Only one species of *Schaefferia* has been found in Nearctic caves (*S. hubbardi* Thibaud 1995). While this species has only been found in a cave, a number of species have been found in Palearctic caves. The majority of these are troglophiles. This study is limited to *Typhlogastrura*. We briefly discuss *T. alabamensis* and *christianseni*, redescribe *T. valentini* and describe five new species: *asymmetrica, fousheensis, helleri, steinmanni* and *unica*.

*Typhlogastrur*a was first described as a subgenus of *Hypogastrura* by Bonet (1930) and later placed by many as a subgenus of *Schaefferia,* but since Stach 1949 the majority of workers have treated it as a separate genus. Presently there are 14 described species in *Typhlogastrura*, but no Nearctic species were described until Thibaud described *alabamensis* and *christianseni* from Alabama caves in 1975 (Thibaud,

1975). Thibaud redescribed these two species in detail in his revision of the genus in 1980 (Thibaud, 1980). He described a third species, *T. valentini*, from a cave in Virginia in 1996. We herein redescribe *T. valentini*, give some supplementary details about *T. christianseni* and *alabamensis,* and describe five new species from Pennsylvania, Colorado and Missouri. Palacios-Vargas and Thibaud (1986) and Palacios-Vargas (1997) have described two species from Mexico which we include in the key and diagnostic table.

With the single exception of *Typhlogastrura valentini* from Virginia, Nearctic species of this genus have been found only in one or two closely connected caves. The striking picture of the distribution of these species bespeaks a single, separate adaptation to cave life in each species which was physiological, and only after some time allowing sufficient change to occur to make survival outside caves improbable.

The species are normally associated with guano or scat although they are also found on rotted wood. They are often found on pool surfaces, but these are almost certainly trapped there accidentally.

SPECIES CHARACTERISTICS

Thibaud's excellent works on this genus, including his large works of 1970 and 1980, distinguished species using the number of sensory setae and apical bulbs on the fourth antennal segment, the structure of the PAO (Post Antennal Organ), the number of eyes (Fig. 1), structure of the mucro and the thoracic chaetotaxy. In our study we had the good fortune of being able to study many populations of one species, *T. valentini*, to

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[1] The one exception to this is *T. korenevski* which occurs in two caves 120 km apart; however the conspecific nature of these two remains in doubt. When Babenko first saw them he thought of them as two different species but later changed his mind.

[2] Jose Palacios-Vargas reports (pers. comm.) having seen an edaphic eyeless member of the genus, but the specimens have been lost and not described in detail, and no detail of their capture or of their location is available other than they were from Chiapas.
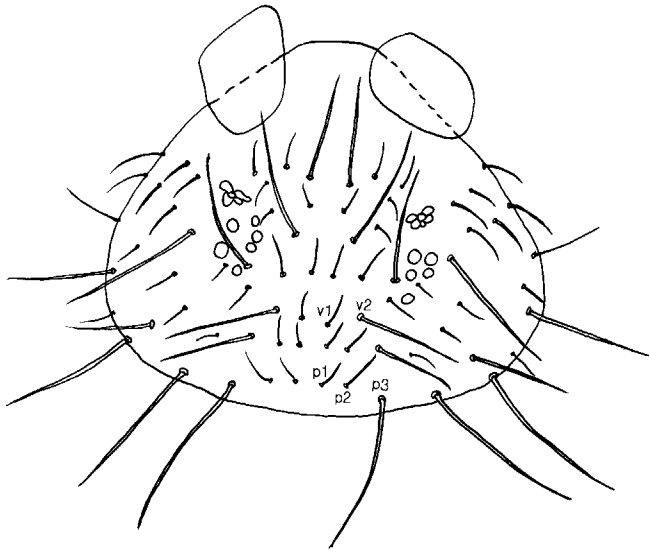
* CHRISTAK@GRINNELL.EDU, WANGHANG@GRINNELL.EDU

Figure 1. Generalized head with chaetotaxy.

obtain a good picture of the intraspecific variability of features. This demonstrated to us that the chaetotaxy of the second thoracic segment and abdominal segments 3–5 (Fig. 2) was extremely intraspecifically constant, but interspecifically varying, and thus furnished an excellent tool for taxonomic separation.

We found that the chaetotaxy shows a number of patterns which we defined in Table 1. In the second thoracic segment the useful character was the triangle formed by P1–P3, with A being the length of the line between setae 1 & 3 and B line from P2 perpendicular to this line, with Ratio = A/B (Fig. 2; Table 2). In abdominal segments 3–5 it is the presence or absence of setae and their relative length which are of importance as well as their positions (Fig. 2; Table 2). Except for two pairs, *fousheensis – veracruzana* and *steinmanni – asymmetrica*, all species are easily separable by their chaetotaxy alone (Table 2). We also found Yosii A measurement (number of tubercles between the P1 setae on abdominal segment 5) to be useful. A key to the Nearctic & Mesoamerican species is on the next page.
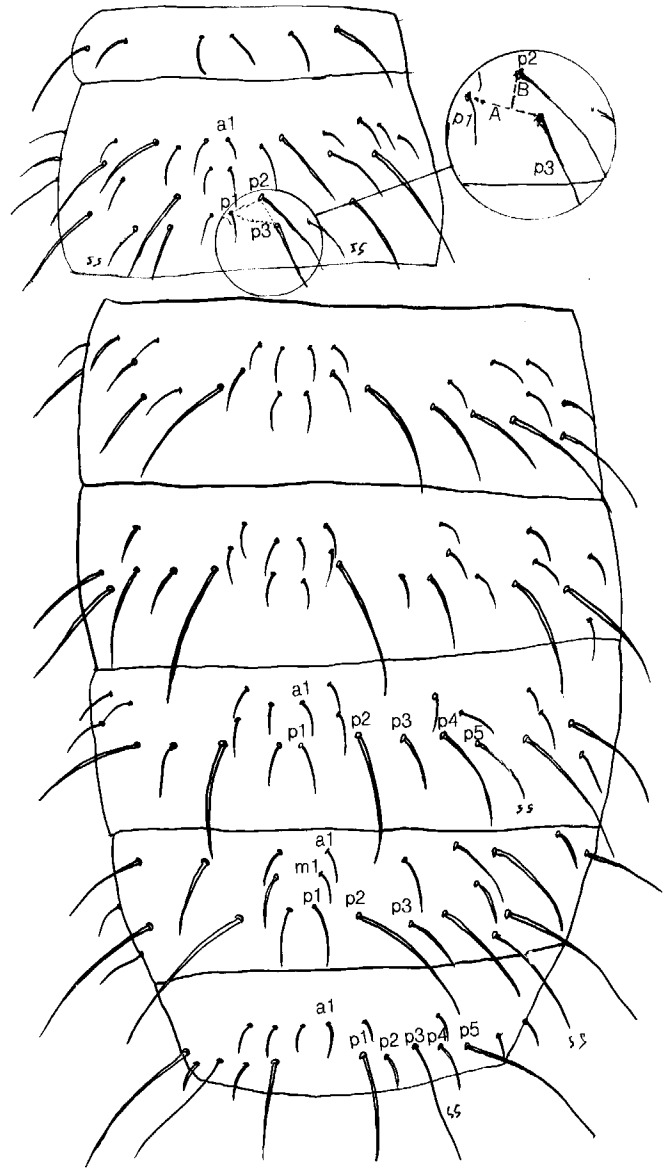

Figure 2. Generalized dorsal chaetotaxy first–second thoracic and first–fifth abdominal segments.

1) Eye 0 or 1 + 1..2
1') Eyes at least 3 + 3...3

2) Fourth antennal segment with seven blunt, thick setae
....*T. elsarzolae*
2') Fourth antennal segment with ten blunt, thick setae
....*T. veracruzana*

3) Fifth abdominal segment with a clear median swelling
(Fig. 3)...*T. unica*
3') Fifth abdominal segment without a clear median
swelling...4

4) Fifth abdominal segment with three–four setae between
the P1 setae level (Fig. 4A)..............................*T. alabamensis*
4') Fifth abdominal segment with two setae between the P1
setae level (Fig.4B)..5

5) Apical antennal bulb large and double (Fig. 5A)
....*T. steinmanni*
5') Apical antennal bulb small and single, sometimes apically
indented (Fig. 5B)...6

6) Fifth abdominal segment with setae P1–P5 all present
(Fig. 2)...7
6') Fifth abdominal segment with seta P2 and/or P4 absent
....8

7) Eyes 5–6 per side.....................................*T. helleri*
7') Eyes 4 per side.................................*T. fousheensis*

8) B/A ratio on second thoracic segment less than 1.8
(Fig. 6A)..*T. asymmetrica*
8') B/A ratio on second thoracic segment greater than 3.0
(Fig. 6B)..9

9) Fifth abdominal segment with both setae P2 and P4
(Fig. 2) absent......................................*T. christianseni*
9') Fifth abdominal segment with seta P4 present
....*T. valentini*

Figure 6 (right) Second thoracic segment with B -horizontal unbroken line) / A -vertical unbroken line) ratio less that 1.8 (A) and more than 3.0 (B).
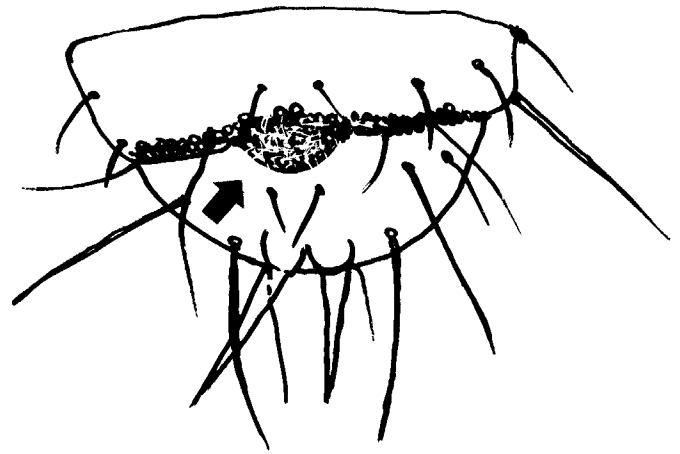

Figure 3. Fifth and sixth abdominal segments illustrating the hump in the center of the fifth segment.
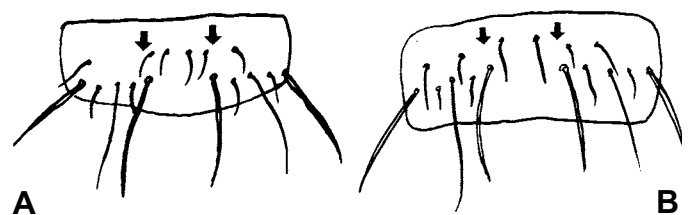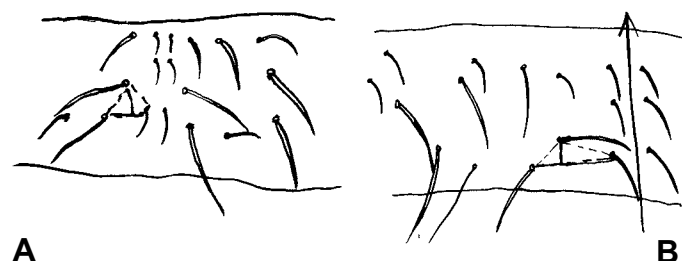

Figure 4. Fifth abdominal segment with A (left) 3-4 setae between level of P1 setae and B (right) two setae between the P1 setae.

Figure 5. Apex of antenna A (left) with two large retractile bulbs and B (right) a single small bulb.

Table 1. Body Chaetotaxy Types[a]

Abdominal Segment 5	Description	Figure
Type 1	P3 long, P4 short, P2 absent, 2 A setae between level p1 setae	Fig. 14 G
Type 2	P3 long, P2 & 4 absent, 2 A setae between level p1 setae	Fig. 9 E
Type 3	P3 long, P2 & 4 short , 3-4 A setae between level p1 setae	Fig. 7 C
Type 4	P3 short, P4 absent, 2 A setae between level p1 setae (only *elsarzolae*)	
Type 5	P3 long, P2 & 4 short, 2 A setae between level p1 setae	Fig. 10 H

Abdominal Segment 4		
Type 1	P3 present , setae P1, m1, & A1' in a nearly straight line	Fig. 13 F
Type 2	P3 absent, inner setae with A1 strongly angled from M1 and P1	Fig. 7 C
Type 3	P3 absent , setae P1, m1, & A1' in a nearly straight line	Fig. 12 E
Type 4	P3 present inner setae with A1 strongly angled from M1 and P1 (only *elsarzolae*)	

Abdominal Segment 3		
Type 1	6 setae between P2 setae, either P3 or P4 missing	Fig. 9 D
Type 2	8 setae between P2 setae, P2 & P4 present	Fig. 7 C
Type 3	6 setae between P2 setae, P2 & P4 present	
Type 4	8 setae between P2 setae, P2 or P4 missing (only *elsarzolae*)	

Thoracic Segment 2		
Ratio = A/B	Length line from A) P1–P3 and B) line from p2 perpendicular to line A	

[a]Thibaud (1970) in his study of *Typhlogastrura balazuci* showed that the chaetotaxy was fully developed after the third instar.

Table 2. Species characteristics Nearctic *Typhlogastrura*

Species	Eyes/ side	Segment Type			Thor.2 A/B[a]	Ant. 4 blunt thick setae	PAO lobes	Anal spine/ inner unguis	Region
		Abd.5	Abd.4	Abd.3					
steinmanni	6	2	3	1	1.2–1.8	6	(4) 5 (6)	1.3	Colorado Eagle Co.
alabamensis	4-6	3	2	2	1.4–2.4	7,8 (9)	(3) 4 (5)	1.1–2.2	Alabama Blount Jackson Co.s
asymmetrica	6 (5,7)	(2) 1	3	1	1.45–1.7	6	4 (3,5)	1.1	Colorado Custer Co.
christianseni	3-4	2	3	1	3.2–4.0	7	4	0.85–0.9	Alabama Morgan Co.
fousheensis	4	5	1	2	2.0–3.1	7	4	1.33	Arkansas Independence Co.
helleri	5-6	5	(1) 4	2	1.25–1.58	(8) 7	5	1.4	Pennsylvania Huntingdon Co.
unica	5	5	1	(3) 1	1.0–1.4	5-6	7 (5,8)	2.0	Colorado Gunnison Co.
valentini	3 (4,5)	1[b]	3	1	3.2–4.2	6-7	4 (5)	0.69–0.85	Virginia Scott, Giles, Wythe, Washington, Russell Co.
elsarzolae	0	4	4	4	1.7	7	5-6	1.1	Mexico Nuevo Leon
veracruzana	(1)-0	5	1	2	1.9–2.5	10	5-6	.8	Mexico Veracruz

( ) = rare conditions.

[a] From a triangle drawn from seta 1-3 with seta 2 as the apex. A = line drawn from seta 1 to seta 3, and B = perpendicular line drawn from this line to seta 2.

[b]A single specimen has seta P2 present, but P4 absent.

Table 3. Measurements (in mm) of individuals of new species.

Locality no. (type localities)	Total Length	Hind inner unguis	Hind unguiculus	Anal horns	Dens	Mucro	Seta P1 on Abd. 4	Seta P2 on Abd. 4	Lateral sensilla antenna 1	Ocellus B	PAO	Sensilla P3 on Abd. 5
						asymmetrica						
9676 sl.1 #2	1.9	0.082	0.04	0.106	0.07	0.028	0.052	0.142	0.018	0.018	0.028	0.05
9676 sl.1 #1	1.08	0.052	0.016	0.07	0.042	0.018	0.022	0.07	0.016	0.012	0.02	0.04
9676 sl.2 #1	1.3	0.066	0.02	0.078	0.054	0.02	0.022	0.102	0.014	0.01	0.02	0.066
9676 sl.2 #2	1.38	0.06	0.022	0.08	0.056	0.018	0.026	0.092	?	0.01	0.02	0.054
9675	?	0.06	0.022	0.108	?	?	0.044	0.12	0.014	0.012	0.028	0.054
9661	1.84	0.084	0.034	0.09	0.06	0.024	0.04	0.122	0.016	0.016	0.032	0.076
						fousheensis						
3890 spec. 1	1.5	0.066	0.018	0.08	0.058	0.02	0.048	0.1	0.02	0.01	0.03	0.106
3890 spec. 2	1.54	0.068	0.02	0.071	0.06	0.022	0.04	0.11	0.02	0.012	0.034	0.1
9782 spec. 1	1.7	0.058	0.02	0.056	0.066	0.024	0.044	0.11	0.022	0.014	0.028	0.124
9782 spec. 5	1.4	0.056	0.02	0.06	0.052	0.022	0.04	0.1	0.02	0.012	0.02	0.098
9782 spec. 2	1.4	0.044	0.018	0.05	0.052	0.018	0.04	0.108	0.018	0.016	0.028	0.088
9782 spec. 3	1.04	0.032	0.012	0.024	0.03	0.022	0.03	0.1	0.02	0.01	0.022	0.092
						helleri						
9276 sl.1 #1 (holotype)	1.4	0.06	0.038	0.082	0.068	0.028	0.04	0.16	0.024	0.012	0.036	0.11?
9276 sl.1 #2	2	0.084	0.05	0.1	0.088	0.026	0.048	?	0.03	0.012	0.04	?
9276 sl.2 #1 (allotype)	2.08	0.086	?	0.1	0.072	0.032	0.056	0.208	0.03	0.014	0.036	0.164
9276 sl.2 #2	1.64	0.07	0.026	0.08	0.07	0.022	0.054	0.196	0.026	0.014	0.038	0.134
						steinmanni						
9677 sl.1 #2	2.26	0.084	0.02	0.09	0.08	0.032	0.044	0.144	0.02	0.02	0.034	0.084
9677 sl.1 #1	1.98	0.09	0.032	0.08	0.074	0.028	0.04	0.12	0.02	0.022	0.03	0.096
9677 sl.2	2.1	0.086	0.03	0.1	0.09	0.032	0.04	0.166	0.02	0.02	0.03	0.082
9677 sl.4 #2	1.86	0.08	0.024	0.1	0.09	0.03	0.048	0.152	0.024	0.022	0.032	0.082
9677 sl.4 #1	2.12	0.078	0.024	0.104	0.11	0.034	0.038	0.126	0.022	...	...	0.09
						unica						
9737 sl.2 #1	0.96	0.044	0.022	0.052	0.042	0.016	0.028	0.088	0.02	0.012	0.03	0.06
9737 sl.2 #2	1.08	0.042	0.02	0.048	0.04	0.016	0.036	0.09	0.018	0.008	0.026	0.074
9737 sl.1 #1	1.44	0.05	0.024	0.07	0.04	0.016	0.03	0.09	0.02	0.01	0.03	0.078
9737 sl.1 #2	1.2	0.056	0.024	0.08	0.06	0.02	0.058	0.1	?	0.008	0.028	0.09

SPECIES DESCRIPTIONS AND COMMENTS

All measurement of the new species herein described are given in Table 3.

Typhlogastrura alabamensis Thibaud 1975 (Fig. 7A–D)
This species is unique among the Nearctic *Typhlogastrura* in having 3–4 A setae between the levels of the P1 setae on abdominal segment 5 (Fig. 7C). It also has the most sharply angled A1 and M1 setae on abdominal segment 4 (Fig. 7C). The apical organ of the fourth antennal segment consists of several elements, similar to those of *T. christianseni* and *T. valentini*. (Fig. 7A). All of the 11 verifiable specimens, including four type specimens, are from Swaim Cave in Morgan Co., Alabama. Thibaud also recorded a specimen from a salamander stomach in Bryant Cave in Blount Co., but this record is very questionable (for fuller description see Thibaud 1980).

Typhlogastrura asymmetrica new species (Fig. 8A–J)
Description: Color slightly tan in life, white in alcohol. Apical bulb of fourth antennal segment when expanded small and weakly trilobed, but when withdrawn appearing as a single structure, sometimes with an apical indentation. Longest setae ~0.7 as long as maximum width of segment. Dorsal surface with six blunt setae, which are not clearly distinguished
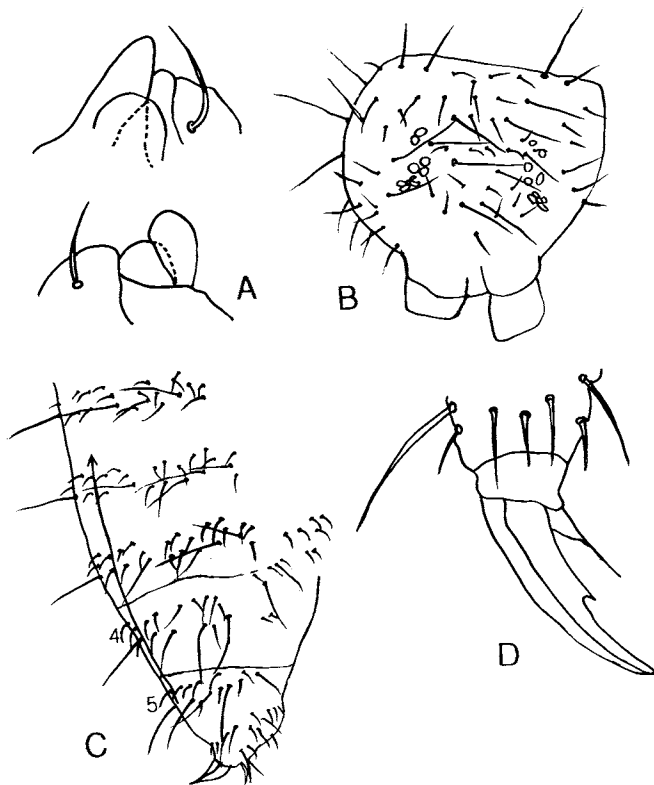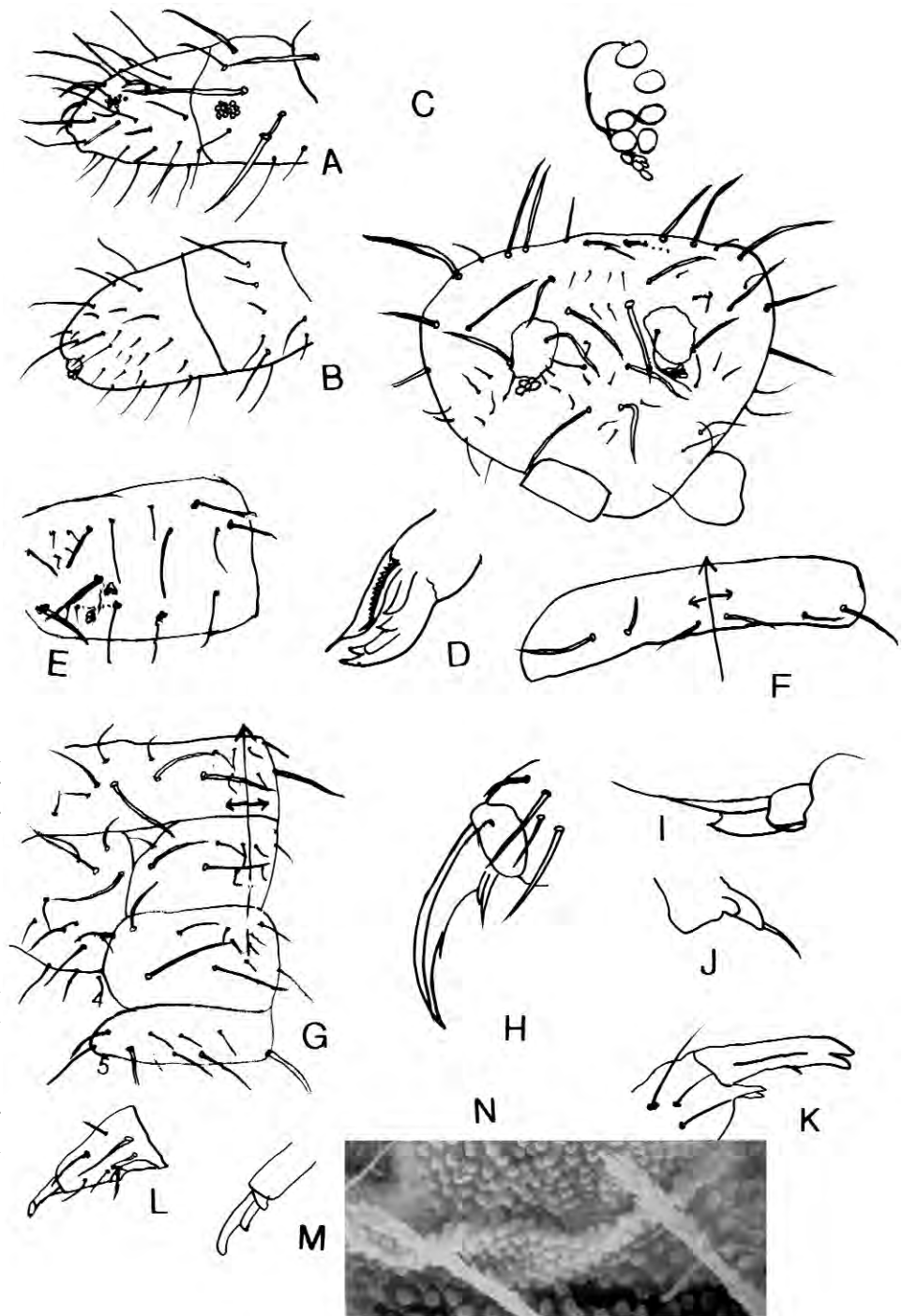

Figure 7. *Typhlogastrura alabamensis* Thibaud. All figures of specimens from type locality. A, apical organs of fourth antennal segment; B, dorsum of head; C, dorsal chaetotaxy abdominal segments 1–6; D, hind foot complex.

from normal setae. Ventral surface without "file" but with 9–10 short, acuminate, straight or weakly curved setae. Apical organ of third antennal segment with two very short curved blunt rods and lateral sensillae not clearly differentiated from other setae. Second antennal segment with 4–5 cylindrical, apically blunt or pointed, ciliate setae. Large specimens with 1–2 such also on third segment. First antennal segment with five dorsal and one (or possibly two) ventral setae. Three or four of the dorsal setae are cylindrical and similar to those on second segment with the remaining setae acuminate. Eyes usually 6 + 6 and moderately to weakly pigmented. PAO with 4–6 lobes and ~2 times nearest eye. Maxillae with 2 large lamellae, reaching apex of teeth and without ciliations or teeth along edge. Other lamellae unclear. Six clear labral papillae. Area verticalis, with 2 + 2 setae, outer setae 4–5 times as long as the inner, thick, cylindrical, coarsely ciliate and truncate. On the posterior margin P3 and P4 are similar and ~2–3 times as long as P1 & P2 which are sparsely ciliate and acuminate. First thoracic segment with all setae thick and ciliate. Seta 2 is 0.51–0.71 seta 3. Setae 1 usually asymmetrical with one 1.5–1.7 times the other. One of these setae is usually ~seta 2. Remaining thoracic and abdominal chaetotaxy as shown in Table 1 and Fig. 8E–G. Large setae all cylindrical, coarsely ciliate (Fig. 8N), and apically blunt or conical. Small setae vary from slender, acuminate and smooth to cylindrical and coarsely ciliate. Sensory setae slender, smooth and clearly differentiated from normal setae. Cuticle coarsely granulate, Yosii A measure 14–21, average 18.5. Tenaculum with four teeth and no setae. Dens with seven slender, straight, dorsal setae. Mucro with lateral basal flap-like tooth absent or reduced. Tibiotarsi with 16–16–17 setae. Unguis with strong inner tooth. Unguiculus varying from having a simple basal lamella to having a distinct tooth-like structure on the lamellar apex.

Holotype: Colorado, Custer Co., 1 adult female, White Marble Halls Cave, August 11, 2000, at 3,566 m., 3.33° C, on moist mud and scat. Locality 9676. Paratypes 1 immature female and 1 subadult male same locality, 1 adult female Burns Cave, August 11, 2000, altitude 3,429 m, on mud and scat; also same locality 9675 & 9661 David Steinmann coll., Biogeographic zone 8.

Derivatio nominis: Named for the large degree of asymmetry found in the species.

Remarks: The PAO is compacted and difficult to analyze. All specimens show some degree of asymmetry. Three specimens have seta P4 on only one side of the fifth abdominal segment while two have it on both sides and one on neither. Three specimens have the setae 1 on the first thoracic segment very different in length and many other features show bilateral asymmetry. This asymmetry is far greater than any other species we have seen. In addition the unguiculus shows unusual states. In some legs the inner margin, which appears to be thickened, is entire; however on most legs it does not reach the shaft of the unguiculus resulting in a tooth-like projection (Fig. 8I). In collection 9675 from Burns Cave this reaches an extreme with the unguiculus shortened and the unguis showing

Figure 8. *Typhlogastrura asymmetrica* **n. sp. Figures A-C, E, I & L holotype. Others paratypes. Microchatae serrations not shown. A, dorsum of antennal segments 3–4; B, venter of same; C, cephalic chaetotaxy with enlargement of eyepatch and PAO; D, maxilla; E, chaetotaxy second thoracic segment; F, chaetotaxy first abdominal segment; G, chaetotaxy abdominal segments 2–5; H, fore foot complex of holotype; I, unguiculus of holotype; J, unguiculus of paratype; K, abnormal unguis and unguiculus from Burns Cave; L, dens and mucro, dorsal view; M, mucro lateral view; N, electron micrograph of base of antenna showing serrations seen on most macrochaetae.**

a separation between the outer unguis margin and the inner margin (Fig. 8K). White Marble Halls and Burns Cave are on the same mountain and are both much colder and higher (1,350 m and 1,213 m) than any other U.S. *Typhlogastrura* caves. It is possible that the unusual features of this species are an ecomorphic response to these conditions.

This species is similar to *T. steinmanni* but differs in seta P4 normally on the fifth abdominal and chaetotaxy of third thoracic segment, They also differ in the chatotaxy of thorcic segment 1 where seta 1 is much shorter than 3 whereas they are similar in length in *steinmani*. The much smaller and unlobed apical antennal bulb and the structure of the macrochaetae, particularly on the antennae, are the most striking differences.

Typhlogastrura christianseni
Thibaud 1975 (Fig. 9A–F)

This species has two unusual features. First, the mucro shows no basal unilateral lamella or flap-like tooth (Fig. 9B); second, the apical antennal bulb shows no sac-like exsertile bulb but rather two or three triangular or conical projections (Fig. 9A). In the latter it is somewhat similar to *T. valentini* but differs in that the latter is exsertile. They differ in fifth abdominal segment chaetotaxy. It is probable they both evolved from the same *Ceratophysella* ancestor (for a fuller description see Thibaud 1980).

Typhlogastrura fousheensis new species (Fig. 10A–I)

Description: Color light brown to white with dark eyespots. Fourth antennal segment apical bulb simple without apical indentation (Fig. 10A). Longest setae about as long as maximum width of segment. Dorsal surface with seven blunt setae moderately distinguished from normal setae. Ventral surface without clear file, with 13–15 short, straight acuminate setae. Apical organ of third antennal segment with two very short blunt curved pegs and lateral sensillae slightly differentiated from normal setae. All antennal setae smooth, acuminate,

Figure 9. *Typhlogastrura christianseni* Thibaud. All figures of type specimens. A, apex of antenna; B, mucrones, lateral view; C, chaetotaxy second thoracic segment, left side; D, chaetotaxy third abdominal segment, left side; E, chaetotaxy abdominal segments 4–6; F, hind foot complex.

straight or weakly curved and smooth. First antennal segment with seven setae. Eyes 4 + 4, PAO with 4–5 lobes. Maxillae similar to *Ceratophysella pecki*. Labral papillae weakly developed. Area verticalis, with 2 + 2 setae with outer setae 3.5–6 times as long as inner. On the posterior margin seta 1 is ~2 times seta 2 and 0.4–0.5 times seta 3. First thoracic segment with all setae acuminate and smooth. Seta 1 only a little longer than seta 2 and 0.4–0.6 as long as seta 3. All pairs subequal in length. Remaining thoracic and abdominal chaetotaxy as shown in Table 1 and Fig. 10E–H. All large setae acuminate and smooth or very finely granulate. Sensory setae clearly differentiated from normal setae. Ventral tube with 4 + 4 setae. Cuticle finely granulate on head and appendages but coarsely granulate on the body dorsum particularly on abdominal segments 4–6. Yosii A measure 11–14. Tenaculum with four teeth and no setae. Dens with seven straight acuminate dorsal setae. Mucro with a very small flaplike lateral tooth, visible only from the side. Unguis with one clear inner tooth.

Holotype: Adult female USA, Arkansas, Independence Co., Foushee Cave. March 18, 2005, Locality 9782, slide 1 specimen 1. Norman & Jean Youngsteadt colls. Paratypes 1 subadult female and 4 adult females same slide and five specimens in alcohol plus 1 adult female and 1 subadult same locality May 1978 with same collector and site data. Biogeographic zone 7B.

Derivatio nominis: Named after the type locality, Foushee Cave.

Remarks: Larger specimens are sometimes pale brown but the smaller specimens are white. The former all have full guts whereas guts of the latter are sometimes empty. This species is one of the few having thoracic segment 2 B/A ratio greater than 2.0. (Fig. 10E). It, along with *alabamensis*, has a range of values for this bridging *christianseni* and *valentini* with ratios greater than 3.1 and the other species all with ratios below 1.8; however the two ranges barely overlap (Table 1). This also is one of the few species having well developed pigment and the only species we have seen with a projecting slender ciliate or weakly serrate lamella 1 of the maxilla (Fig. 10C).

All specimens were collected from bat guano. We were very fortunate that the Youngsteadts were able and willing to go back to the same cave after 27 years and collect more specimens since the first collection contained only one adult specimen.

Typhlogastrura helleri new species (Fig. 11A–H)

Description: Color tan with black eye spots. Fourth antennal segment apical bulb simple without apical indentation.

Figure 10. *Typhlogastrura fousheensis* **n. sp. All figures of type specimens. A, fourth antennal segment, dorsal view; B, eyepatch and PAO; C, maxilla; D, cephalic chaetotaxy; E, chaetotaxy second thoracic segment; F, chaetotaxy third abdominal segment; G, chaetotaxy fourth abdominal segment; H, chaetotaxy fifth abdominal segment; I, fore foot complex, dentes and mucrones.**

Longest setae about as long as maximum width of segment. Dorsal surface with seven blunt setae poorly distinguished from normal setae. Ventral surface without clear file with 23–25 short, acuminate, mostly straight setae. Apical organ of third antennal segment with two short straight blunt rods and lateral sensillae slightly differentiated from normal setae. All antennal setae smooth, acuminate, straight or weakly curved and smooth. First antennal segment not seen clearly. Eyes usually 5 + 5, PAO with 4–5 lobes and 2.5–3.2 times nearest eye. Maxillae not clearly seen but appear similar to that of *T. unica*. Labral papillae weakly developed. Area verticalis, with 2 + 2 setae with outer setae ~4 times as long as inner. On the posterior margin seta 1 is 1.2–0.15 times seta 2 and 0.2–0.42 as long as P3. First thoracic segment with all setae acuminate and smooth. Seta 2 is 0.36–0.34 seta 3 and 0.54–0.42 setae. All pairs subequal in length. Remaining thoracic and abdominal chaetotaxy as shown in Table 1 and Fig. 11E–F. All large setae acuminate and smooth or very finely granulate. Sensory setae weakly differentiated from normal setae except at their thinner apices. Cuticle mostly finely granulate except on the dorsum of abdominal segments 4–6. Yosii A measure 12–20. Tenaculum with four teeth and no setae. Dens with seven straight acuminate dorsal setae. Mucro without clear flap-like lateral tooth. Tibiotarsi with 16–16–17 setae. Unguis with one clear inner tooth.

Holotype: Adult male USA, Pennsylvania, Huntingdon Co., Heller Cave, January 1997, on pool surface, Locality 9276. Keith Christenson coll. Allotype adult female, and 2 paratypes, subadult females, all with same collection data. Biogeographic zone 7A.

Derivatio nominis: Named after the type locality, Heller cave, and in honor of the owners, the Heller family.

Remarks: This is by far the most Northeastern Nearctic species of the genus. It is somewhat similar to *T. alabamensis* but differs in mucronal structure, apical antennal bulb, and chaetotaxy (Table 1). The specimens display a considerable degree of asymmetry. It is possible that one or two specimens have only four ocelli on one side as the outlines of the ocelli are often obscured by the eye pigment.

Typhlogastrura steinmanni new species (Fig. 12A–G)
Description: Color white except for black ocelli. Fourth antennal segment apical bulb when expanded large and clearly double (Fig. 12A) but when withdrawn appearing as a single structure. Longest setae about as long as maximum width

Figure 11. *Typhlogastrura helleri* n. sp. All figures of type specimens. A, fourth antennal segment; B, ocelli and PAO right side; C, cephalic chaetotaxy; D, chaetotaxy first thoracic segment; E, chaetotaxy right half of second thoracic segment; F, chaetotaxy abdominal segments 3–5; G, hind foot complex; H, mucrones and dens.

of segment. Dorsal surface with six blunt setae with only apical two clearly distinguished from normal setae. Ventral surface without file but with eight short, acuminate setae. Apical organ of third antennal segment with two very short curved blunt rods and lateral sensillae not clearly differentiated from other setae. First antennal segment with seven setae. 6 + 6 usually well-pigmented eyes. PAO with 4–6 lobes and ~1.5 times nearest eye. Maxillae not seen clearly but not having any lamellae surpassing the apical maxillary tooth. Labral papillae 4 and weakly developed. Area verticalis, with 2 + 2 setae, outer setae 4–5 times the inner, thick, weakly tapered and rugose or finely ciliate and blunt. On the posterior margin P3 are similar and ~4 times P1 & P2 which are smooth and acuminate. First thoracic segment with all setae thick and rugose or sparsely ciliate. Seta 1 is 1.1–1.5 seta 2, which is 0.59–0.74 seta 3, which is 1.7–2.6 seta 1. Thoracic and abdominal chaetotaxy as shown in Table 1 and Fig. 12C–E. Large setae varying from acuminate to almost cylindrical and blunt. Sensory setae with long filamentous apices and clearly differentiated from normal setae. All large setae are rugose or very sparsely ciliate. Cuticle granulations moderate, Yosii A measure 23–28. Tenaculum with four teeth and no setae. Dens with 7–8 slender, straight, dorsal setae. Mucro with lateral basal flap-like tooth absent or reduced. Tibiotarsi with 16–16–17 setae. Unguis with strong inner tooth and sometimes with a minute outer tooth.

Holotype: Adult male USA, Colorado, Eagle Co., Fulford Cave. August 22, 2000, altitude 3,139 m, temp. 1.67° C, on rotted wood and pool surfaces. Allotype: same data Locality 9677. Paratypes: same data as holotype, three adult or subadult females and one immature specimen. Biogeographic zone 8. Found only in type locality.

Derivatio nominis: Named in honor of David Steinmann who discovered most of the new species described in this work.

Remarks Most of the specimens have seven dorsal dental setae but at least two specimens have eight on one or both sides. The allotype and all but one paratype are females, apparently adult with a well-developed genital plate but poorly developed genital opening. The mucro is quite varied in structure but never has the typical flap-like lateral tooth typical of *Ceratophysella*. While this species resembles *T. asymmetrica* in some respects, it differs stringly in having the antennal apical bulb large and double versus small and single as well as many other features (see remarks under *asymmetrica*).

***Typhlogastrura unica* new species (Fig. 13A–H)**

Description: Color white. Fourth antennal segment apical bulb when expanded small and clearly trilobed (Fig. 13A), but when withdrawn appearing as a single structure sometimes with apical indentations visible. Longest setae ~0.9 as long as maximum width of segment. Dorsal surface with seven blunt setae. Ventral surface with weakly developed file but with ~20
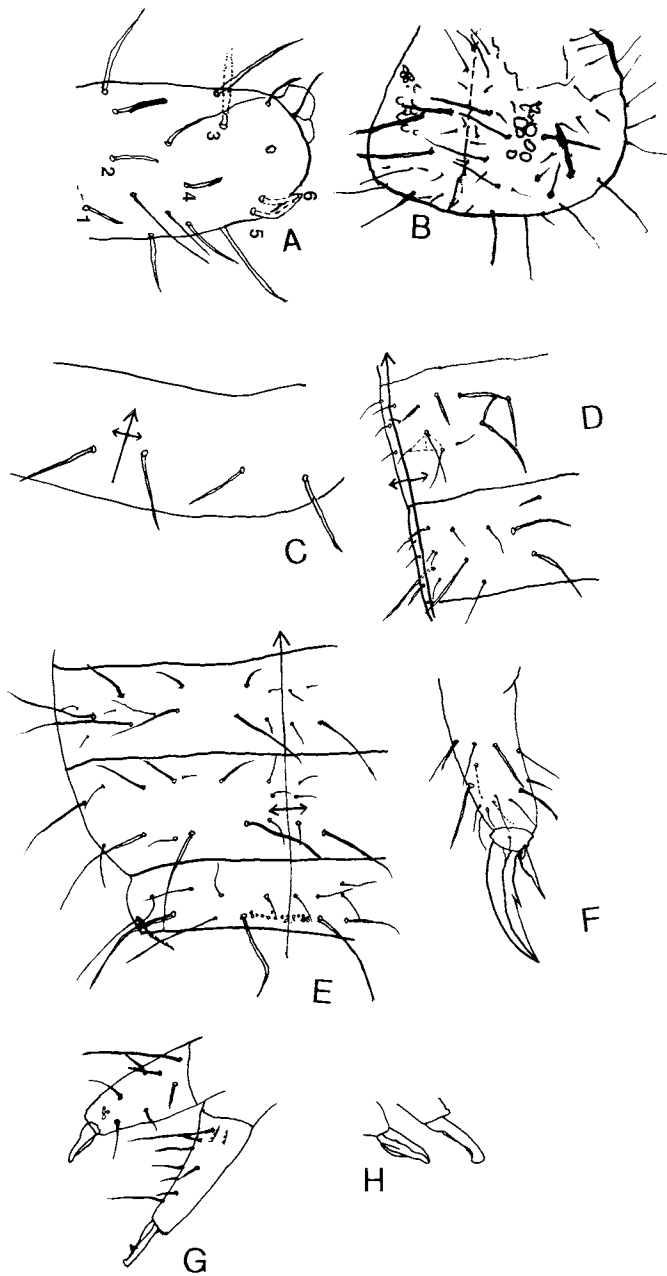
Figure 12. *Typhlogastrura steinmanni* **n. sp. Figures A–C holotype, others paratypes. A, fourth antennal segment, dorsal view; B, dorsum of head; C, chaetotaxy first thoracic segment; D, chaetotaxy right half of thoracic segments 2–3; E, chaetotaxy right half of abdominal segments 3–5; F, hind tibiotarsus and foot complex; G, dentes and mucrones.**

short, acuminate, straight or weakly curved setae. Apical organ of third antennal segment with two very short curved blunt rods and lateral two slightly basally enlarged, acuminate sensillae. Large setae of antennal segments 1–3 acuminate, heavy and rugose. First antennal segment with five dorsal and two ventral setae. Four labral papillae. Maxilla of *denticulata* type

with lamella 1 clearly surpassing the apex of the teeth but without visible inner serrations. Eyes 5 + 5 and moderately pigmented. PAO usually with seven lobes. Area verticalis, with 2 + 2 setae, outer setae 3.5–5 times the inner. On the posterior margin, seta P1 is 2.5–3 times P2 and 0.52–0.80 times P3. First thoracic segment with seta 2 ~½ as long as seta 3 and 0.55–0.64 as long as seta 1. Remaining thoracic and abdominal chaetotaxy as shown in Table 1 and Fig. 13D–F. Small setae all acuminate and varying from smooth to ciliate. Large setae all acuminate, straight to slightly curved and sparsely ciliate. Sensory setae slender, smooth and clearly differentiated from normal setae. A well-developed hump occurs between the P1 setae on abdomen 5. Integument moderately granulate but coarsely granulate on the posterior part of abdomen 5 and abdomen 6. Yosii A measure 14–17. Tenaculum with 4–5 teeth and no setae. Dens with 6–7 slender, straight, dorsal setae. Mucro with a small lateral basal flap-like tooth. Tibiotarsi with 16–16–17 setae. Unguis with strong inner tooth.

Holotype: Adult male USA, Colorado, Gunnison Co., Hunters Camp Cave. ?- August 11, 2000, altitude 3050 m, 12.2 m from entrance, on scat; Locality 9737, David Steinmann coll. Allotype adult female, same data as holotype. Paratypes 1 adult and 1 subadult females, same data. Known only from type locality.

Derivatio nominis: Latin unica = only, in view of the fact that it is the only member of the genus having a fifth abdominal hump.

Remarks: This is the most striking member of the genus from the northern Nearctic. The combination of fifth abdominal segment hump, many-lobed PAO, and trilobed apical bulb make it easy to identify. Both the tenacular teeth and dental chaetotaxy are variable.

Typhlogastrura valentini Thibaud 1996 (Fig. 14A–I)
Ref.: Revue Franc. Ent. (N.S.) 18:11-12

Redescription: Color pale brown to white. Eyespots usually black. Fourth antennal segment apical bulb when unexpanded appearing like a broad peg or pair of pegs more or less conjoined. When fully expanded it is single, with a larger or smaller apical indentation. Intermediate stages of expansion show a variety of forms. Longest setae about as long as maximum width of segment. Dorsal surface with 6–7 blunt setae. Ventral surface without clear file but with 16–22 short, acuminate or slightly truncate setae. Apical organ of third antennal segment with two very short blunt pegs and lateral sensillae not clearly differentiated from other setae. First antennal segment with seven setae. 3 + 3 (rarely 2, 4, 5 or 6) usually well-pigmented eyes. PAO with four lobes (one specimen with five) ~1.4–2.5 times nearest eye. Maxillae similar to those of *Ceratophysella pecki*. Labral papillae weakly developed but apparently with six lobes. Area verticalis, with 2 + 2 setae, outer setae 2.2–2.7, all setae acuminate and smooth. On the posterior margin setae P1 & P2 are subequal and seta 3 is 2.0 to 2.3 times these. All are smooth and acuminate. First thoracic segment with setae 1 & 3 usually thick and straight but seta 2 curved and slender.

Figure 13. *Typhlogastrura unica* n. sp. All figures of paratypes. A, fourth antennal segment, dorsal view; B, dorsum of head; C, first thoracic segment; D, left side of second thoracic segment; E, left side of third abdominal segment; F, left side of abdominal segments 4–5; G, dentes and mucrones; H, foot complex.

Seta 1 is 1.5–2.25 seta 2, and 0.48–0.84 seta 3. Thoracic and abdominal chaetotaxy as shown in Table 1 and Fig. 14D–G. Large setae varying from acuminate to almost cylindrical and blunt. Sensory setae with long filamentous apices and clearly differentiated from normal setae. All large setae are rugose or very sparsely ciliate. Cuticle granulations moderate, heavier on the last three abdominal segments, Yosii A measure 23–28. Tenaculum with four teeth and no setae. Dens with 7–8 slender, straight, dorsal setae. Mucro with lateral basal flap-like tooth absent but with a clear basal expansion. Tibiotarsi with 16–16–17 setae. Unguis with strong inner tooth and sometimes with a minute outer tooth.

Holotype: USA, Virginia, Scott Co., Grigsby Cave, August 8, 1995, and Herons Echo Hall Cave August 9, 1996. D. Hubbard coll. on rotted wood and pool surfaces; 14 paratypes same localities.

Also collected from many other Virginia caves: Scott County: six additional caves; Wythe County: nine caves; Russell County: four caves; Pulaski County: two caves; and Washington, Smyth and Giles County: one cave each.

Remarks: The apical bulb of the fourth antennal segment is very confusing. At first it appears that there are several different types of organ, but one sample shows that this is due to different levels of expansion of the exsertile bulb. In the unexpanded condition it is similar to the apical organ of *T. christianseni*. *T. valentini* is quite similar to *christianseni*, appearing to differ primarily in the non- exsertile nature of the apical bulb in the latter and the fifth abdominal chaetotaxy and the shape of the mucro. Thibaud in his original description says there are nine cylindrical setae on the fourth antennal segment but we were never able to see more than seven.

This species has the most remarkable distribution of any species of the genus. It occurs over the western third of the State of Virginia. This is in striking contrast to almost all other species of the genus, which occur in one or two close-by caves, or at most three such. It is almost impossible that these Virginia caves are presently connected by cave passages, although it is possible that there are connections through the MSS, which has not been studied in the state. The chaetotaxy is remarkably constant throughout its range. There is some variation in eye number and relative sizes of organs, but these occur largely in isolated specimens in the two counties with the greatest number of collections. As far as we can see there are several possible explanations:

1) The present distribution reflects geological changes in the cave systems and the various caves were at one time linked; 2) there actually are MSS linkages between the various cave systems; or 3) this represents a series of invasions of

Figure 14. *Typhlogastrura valentini* Thibaud. A, fourth antennal segment, dorsal view (Giles County); B, dorsum of head (Wythe County); C, maxilla (Wythe County); D, chaetotaxy first thoracic segment (Washington County); E, chaetotaxy second thoracic segment (Wythe County); F, right side of third abdominal segment (Wythe County); G, chaetotaxy abdominal segments 4–5 (Wythe County); H, dentes and mucrones (Wythe County); I, foot complex (Wythe County).

caves followed by parallel morpho-speciation. The latter hypothesis could be readily tested by genetic analysis.

DISCUSSION

The three new species from Colorado come from three cave regions, which are both topographically and geologically separated. Each also comes from a very high-altitude cold cave or caves (3,048 m or over). It would appear to be ideal for a series of glacial relict species; however, while it is possible that *T. steinmanni* and *T. asymmetrica* arose from a common ancestor, *T. unica* clearly arose from a different group. We have seen no clear candidates for putative ancestors to either group from arctic species of *Ceratophysella.*

There are a number of mysterious features about this polyphyletic genus. First, all described species are exclusively or almost exclusively troglobitic, yet most show only slight troglomorphy, usually limited to eye and pigment reduction. The exceptions to this are two European species: *T. mendizabili* from Spain and *T. morozovi* from Georgia. Both are eyeless and have the troglomorphic feature of elongate ungues and the putatively troglomorphic hypertrophy of the PAO. It is possible but not certain that loss of the mucronal tooth is also a troglomorphic feature. This is supported by the fact that the two Mexican species, which show the greatest troglomorphy in the American species, show the most reduced mucronal structure. The other frequent Collembola troglomorphic features — elongation of antennae, hypertrophy of third antennal segment sense organ, elongation of furcula, and elongation of unguis or other appendages, gracilization, increase of size (Christiansen, 2004) — are absent. That such increase in troglomorphy is possible in the Hypogastruridae is clearly shown by the highly troglomorphic genus *Ongulogastrura* (Thibaud & Massoud, 1983); however this genus is virtually impossible to associate with any species of *Typhlogastrura.*

Second, we have the rarity of their occurrence. We have only one species from the very heavily collected Missouri–Arkansas region, two each from the very thoroughly collected Virginia and Alabama–Georgia regions, and none from the heartland of cave Collembola collections — the Southeast of the U.S., nor from the also heavily collected Iowa, Indiana and Illinois regions, nor the also heavily collected California region. In contrast, three species are known from poorly collected Colorado and one from poorly collected Pennsylvania caves. An explanation for this may be that there are no troglophile species, which allow invasions of caves over wider regions. Another possible explanation is that the cave invasions were not successful where there were pre-existing troglomorphic Collembola.

This brings up the third mystery: why no troglophiles? The simplest explanation would be that the physiological and reproductive and/or behavioral changes required to succeed in the caves prevents survival outside them. If so, this raises another question: why does the analogous genus *Bonetogastrura*, which differs only in the ancestral group of

species of *Ceratophysella,* have both edaphic and troglophilic forms? If the undescribed edaphic species should turn out to be a member of *Typhlogastrura,* this would make even more mysterious the absence of troglophilic forms.

Fourth: why is it that the species, after invading the caves, show no evidence of evolving into progressively more troglomorphic species as do most cavernicole lineages (Christiansen and Culver, 1987; Christiansen, op. cit.)? All these puzzles furnish an ample source of future interesting scientific investigations.

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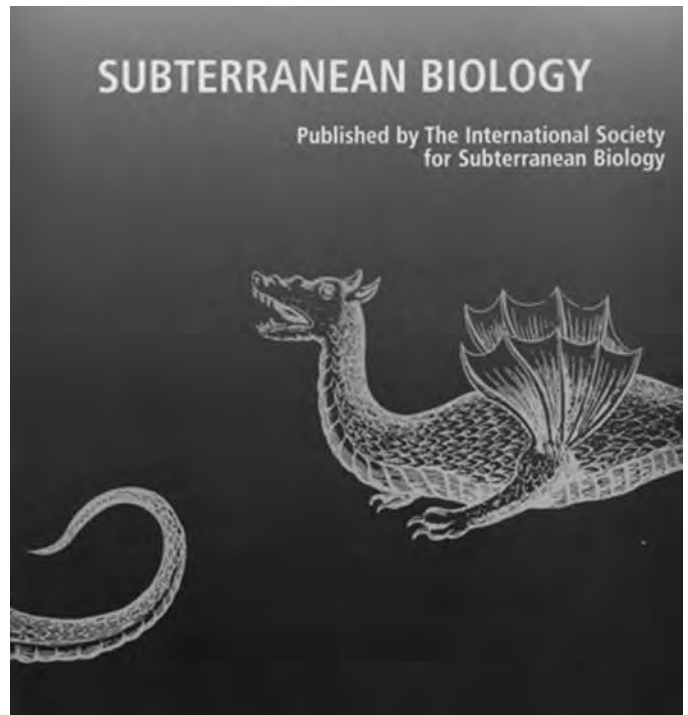
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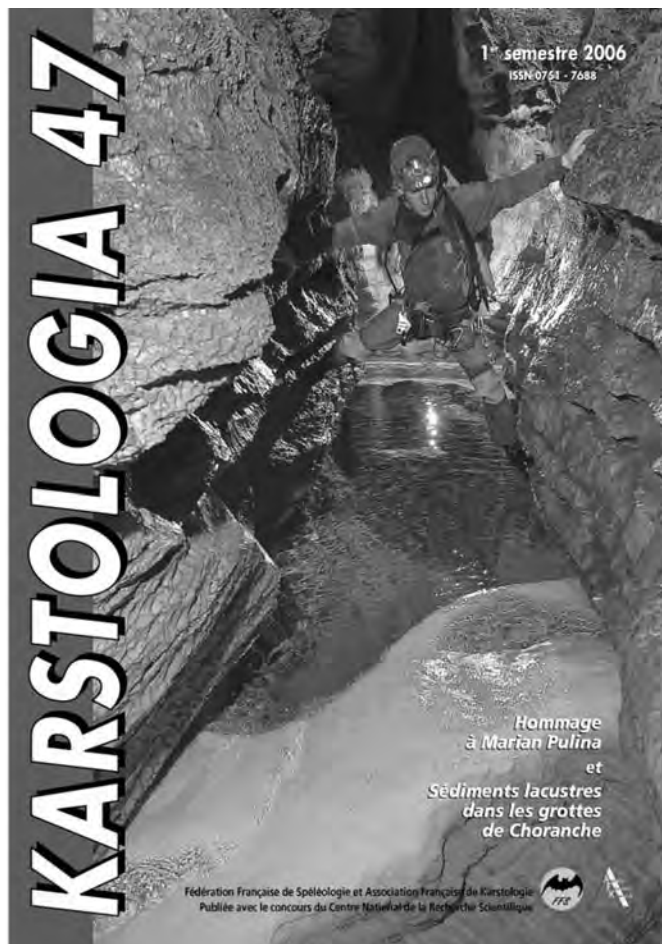
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