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Office

6001 Pulaski Pike NW
Huntsville, AL 35810 USA
Tel:256-852-1300
nss@caves.org

**Editor-in-Chief
Malcolm S. Field**

Washington, DC
703-347-8601
field.malcolm1@gmail.com

**Production Editor
Scott A. Engel**

Knoxville, TN
225-281-3914
saecaver@gmail.com

Journal Copy Editor

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Front cover: Olms. See Lucic in this issue.

AN UNDERWORLD TAILORED TO TOURISTS: A DRAGON, A PHOTO-MODEL, AND A BIOINDICATOR

Ivo Lučić

Abstract

The history of studies of a subterranean tailed amphibian, known as the olm or proteus (*Proteus anguinus*), is a colorful indicator of the changing view of the world beneath our feet. Throughout history, the underground has been presented as other worldly inhabited by life not of this world. At first, caves were the habitat of ghosts and spirits, and then as a symbol for hell with all its attributes. The olm, in light of this prominent worldview, is discussed here, in which its status changed from that of a mythical dragon, to a photo-model, to a biological indicator of environmental health. The mix of these roles, with which the modern notion of this animal is presented, is mostly generated by the experience of tour-guides in Postojna Cave in Slovenia. For a long time, Postojna was the only place that the wider public recognized as a home for proteus. This clearly shows the need to analyze the popular media constructions of environment.

INTRODUCTION

The first recognized cave-adapted animal, the olm, was described by Viennese zoologist J. N. Laurenti and named *Proteus anguinus* in 1768. Media interest in the olm has intensified in recent years. In mid-2016, it became one of the most frequent cave-related topics in the public media. This happened not only in local media from the Dinaric Karst region, where the olm is a local phenomenon, but included media from around the world, from Canada to Australia and Eastern Asia. Particular interest was raised by the hatching of olm in the Postojna Cave in Slovenia vivarium in early 2016 and reached its peak at the end of May and the beginning of June of that year. At the same time, images and videos of olm hatching were published for the first time. In this article, I try to provide answers to the question of how the media perceived the Postojna Cave olm in their coverage and why.

Dinaric Karst, which occupies half of the Adriatic Sea bed and areas to the east and north of the Adriatic Sea, is the largest karst area in Europe (Kranjc, 2003). It is the first area where karst was scientifically described and researched, and is the best known hot-spot of subterranean biodiversity in the world (Sket, 1999; Culver and Sket, 2000). Its most famous representative, the olm (Fig. 1), is one of the central figures in the history of karst research.

Subject and Method

In this article, I do not venture into empirical research on the frequency of media reports, nor do I deal with quantification of any aspect of that phenomenon. Rather, I focus on perceiving and defining key narratives or images of the olm that appeared and that are common to most of the articles and explain their background. First, I present the history of research and knowledge of the olm followed by the analysis of this knowledge based on cultural models or world-views. Then an explanation is provided for the media construction of reality (i.e., which of the scientific facts was valued and accepted as a standard form of this phenomenon by the communications community). In other words, on what did the media base its image of the olm? The fourteen most visible articles revealed by an Internet search, regardless from where in the world they originated, were taken into consideration. The most visible image presents the olm as a descendant of the dragon; the media titillated their readers with claims that actually “young dragons” have hatched in Postojna Cave. More than half of the articles contained the claim that the olm was a young dragon in their title (AFP, 2016; Anonymous, 2016; Fleur, 2016; Fox News, 2016; Gili, 2016; Hrala, 2016; Photopoulos, 2016; Tan, 2016; Vrdoljak, 2016; Webb, 2016; McKie, 2017) and this term appears in the articles several times (Figs. 2A, 2B). For instance, when explaining the mythological history of the olm, The Guardian (McKie, 2017) mentions the word “dragon” seven times and uses it as a synonym for the olm. Others have done a similar thing, with frequent use of the word “dragon.” I do not claim that the media wanted to literally present the olm as a dragon, which will be discussed later, but it was a feature that proved to be of extraordinary importance in its media presentation.

Unlike this dominant mythological image that originates without scientific knowledge, the other two images of the olm rely on scientific facts. One is based on the natural sciences: the olm is a living being, an aquatic salamander and a topic of biological research. When depicting the development of the knowledge about the olm in the past, the media use the historical science as well. This image remains factually credible however, since everything is happening in a tourist cave, with regard to business market conditions, it becomes a dimension of the tourist attraction itself.

Environmental science prevails in the third image of the olm, but it includes social and ethical aspects, as well. The olm is perceived as a part of an endangered environmental system. Hence, the survival of such a vulnerable spe-

¹Dubrava 256 d, 10000 Zagreb, Croatia, ivolucic@gmail.com



Figure 1. Olms.

TRILOBITES
In a Slovenian Cave, Hoping for a Batch of Baby 'Dragons'

A female olm, sometimes called a "baby dragon," pictured with one of its eggs at the Postojna Cave in Slovenia. izsak 3/10/16

By Nicholas St. Fleur
 Feb. 5, 2016

Creatures resembling dragons dwell deep within the caves of Slovenia. And now with the help of biologists, a new brood is set to hatch at an unusual aquarium, accessible by underground train.

Rare 'Baby Dragons' Recorded Hatching in Ancient Slovenian Cave
 By JAMIANE TAN Jan 3, 2016, 1:45 PM ET

POSTOJNA CAVE

The story is not over yet, as 22 more "dragons" want to break on through.

WATCH | Rare 'Baby Dragons' Hatch in Ancient Cave

Two "baby dragons" have been recorded hatching for the first time in a remote cave in Slovenia only accessible by an underground train.

No, these "baby dragons" aren't the fire-breathing kind you're probably thinking of. They're olms — an ancient species of blind salamanders that centuries ago were thought to be young versions of the mythological creature, according to biologist Saso Weltdt, who studies

Figure 2A. Media coverage of proteus hatching: New York Times, 2B ABC news (screenshots).

cies becomes imperative for the protection of nature in general: "Olms are a flagship species [in] need of protection. If there's too much pollution, they will disappear," warned Saso Weltdt, a biologist working at Postojna Cave (Fleur, 2016).

All three images are nicely visible in the article by local Al Jazeera Balcans (Vrdoljak, 2016). In it, the olm is referred to as: (a) small dragons, whose life (b) like that of the movie stars, is lived with the constant presence of a camera and (c) an animal that requires clean water to survive, otherwise it would become extinct. Other

articles on the olm, which appeared in the regional media (Šimičević, 2012; Vecernji list, 2017) unrelated to this campaign, contain similar ideas. Because the media often reach for arguments in historical reviews, I will present the history of proteus research first.

Living World of the Underground seen through the Eyes of Traditional Culture

For centuries, the olm was the only known inhabitant of the karst underground, but its home has been envisaged and presented in different ways during human history, depending on the cultural and historical circumstances. The subterranean fauna was not known in traditional culture in the sense of scientific knowledge, and the cave environment had strong mythological elements, mostly as a hellish place void of life.

Once upon a time, the most famous resident of the underworld in the northern part of the Dinaric Karst was Jure Grando. During his lifetime, he was an exemplary man from the town of Kringa. But after he died, Grando would rise from his grave and sow terrible fear all over Istria province. When he knocked at night on someone's door, the next day a dead person would be found there. Jure Grando was a prototype vampire. In 1672, local citizens finally disposed of him: his body was dug up during a special ceremony, but when the chief of the police cut off his head, Grando let out a scream and blood filled the grave. Peace finally returned to Kringa after Grando was decapitated. (Valvasor, 1969).

For modern educated people, this picture of the underground world must have been strange and weird; but for the traditional world that lives in various modifications up to now, caves are reserved for beings that have almost nothing in common with today's subterranean fauna. According to ethnological origins (Bajuk, 2012), the real master of the underworld is "kača" — locally called snake. For Old Slavs, snakes were sacred animals — the guardian of the front door of the house and the soul of ancestors (Bajuk, 2012). Some forms of remembrance of a holy snake are kept in numerous places. In Popovo Polje (Bosnia and Herzegovina, B&H), a belief existed until the mid 20th century that a snake cannot be killed because it always comes back to life (Ivanišević, 2001, 2019).

In South Slavic languages, the word snake originated from the term ground. Therefore, the snake was quite a normal subject for the underworld, but the same underworld was also the origin and dwelling place for dragons (Skok, 1971–1974). Some say dragons originate from the snake, but a special kind of snake. It can have seven heads, it may grow wings and become a winged serpent, a winged horse, or a winged ox. It lives in the clouds and in caves. There are many caves named after dragon (in Slavic languages zmaj is dragon) For example, an "immense large pit" Zmajevica on the hill of Klisura in Popovo Polje (B&H) (Mihajlović, 1890). There are numerous medieval tombstones, "stećci," with carved, stylized images of dragons. They bear witness that dragons were a significant part of the traditional lore.

Speleological features, especially springs, in old Slavic mythology usually meant source of life, and they were often places of worship. Women visiting Trbuhovica, the highest spring in Lubljanica Basin in Slovenia and Croatia, "did so to receive the gift of life." Today we would say to treat their infertility.

Moreover, it was believed that Trbuhovica spring literally brings babies. For a long period of time, the peasants of Babno Polje, placed on that basin, told their children that they were brought by the water (Pleterski and Šantek, 2012). Sometimes adults used to scare young children by telling them that Trbuhovica will also take them (Pleterski and Šantek, 2012). In a way, it was a continuation of the ancient belief that spring openings are the place through which you will reach another world. In essence, Trbuhovica was a two-way door between two worlds. Life comes through it into this world and finally leaves through it for another world or the afterlife. Trbuhovica is, in fact, the site of one of the three major deities of ancient Slavs: Mokoš or Baba, which means Mother Earth. Mokoš is the mistress of the house at the door (Katičić, 2011).

Why such a big difference between the underworld of Jure Grando and the old Slavs? This change was a result of the spreading of a new religion, Christianity. By accepting Christianity, Eve's serpent was equated with the devil or Satan (Bajuk, 2012), while early Christian iconography sometimes displays Jesus as a brass *snake* on a cross (Chevalier-Gheerbrant, 1994, after Bajuk, 2012). Therefore, women who drank water from Trbuhovica were now proclaimed as witches and were killed (Pleterski and Šantek, 2012). Caves became equated with hell, instead of giving new life.

Hence, everything that lived in the underground of the Dinaric Karst obtained an aura of evil, and the olm and snake were the only known physical members of that world at the time. The Christians had to relentlessly kill the dragon, in the manner of the legend of St. George and the Dragon, and a similar fate befell the human fish from Postojna Cave.

Scientific History of the Olm

In 1689, Johann Weikhard von Valvasor, the natural historian and pioneer of karst studies who acquainted us with Jure Grando, conveys statements of peasants from the Vrhnika area in Slovenia that they have seen a dragon's youngling in the Bela spring. Peasants explained its presence by the fact that the water in this spring appears twice a day, at midnight and around 9:00 a.m. When the water became high, the dragon becomes angry so he spits it out. A peasant had, he said, seen three young dragons in his life. A local mail carrier, who took one specimen home, described it to be around a span long (the width of a spread hand) and looking like a lizard (Valvasor, 1969).

Scientific knowledge of the olm began when peasants managed to catch living samples washed up at the Stična spring in Dolenjsko, Slovenia, and brought them alive to Ioannes Antonio Scopoli, who was a physician for the mercury mines in the nearby town of Idrija. Scopoli "received a hundred dragons" and was very thrilled with it. He put the samples into alcohol and sent them to numerous friends and colleagues, but he also made detailed studies of them including drawings and was preparing to publish an article about the discovery (Aljančič et al., 1993).

However, Viennese zoologist J. N. Laurenti, who received one of Scopoli's samples through a nature lover, Sigismund Hochewart, described the discovery of a new species named *Proteus anguinus* in 1768. Its name shows that, even in science, this little creature could hardly be removed from the mythological context. The first part of the name is from Proteus, Greek god of the sea, and the second part, *anguinus*, means related to or resembling a snake, an animal with extremely strong mythological tradition.

Literature indicates that Laurenti's description was faulty and superficial and that it left a number of unresolved questions (Aljančič et al., 1993). Some of them were resolved by Scopoli himself in his much more thorough description that was published four years later in 1772. In it, he classified proteus as an amphibian. However, the famous Carl von Linné, father of modern biological taxonomy, did not want to include it in his *Systema Naturae*, the Linnaean system of taxonomy. According to Linnaeus, as did Laurenti, the animal was in a larval stage. It truly is, but as such, it becomes reproductively mature and as such it lives its entire life in this state, as Scopoli claimed correctly, but without success.

The name human fish, which is now dominant in the region, was recorded for the first time some forty years after it was scientifically described. In an unsigned article published 1807, Slovenian biologist Žiga Zois says that peasants from the Stina surroundings call it human fish and white fish. He also wrote down the name little white snake. Hence, it seems that the name human fish is the original traditional name, fitting for mythological and tourist interpretation. Over time, there will be several more artificially-coined names that became widely used (e.g., German olm or Slovenian močeril (Aljančič, 1989)).

At the beginning of the 19th century, a new scientific interest for the olm arose, gaining global fame through several waves of research. Karl von Schreibers of the Viennese Natural History Museum — at the time when Slovenia was part of the Austro-Hungarian Empire — studied its anatomy in detail. In 1800, he held lectures in London and Paris that the experts mark as epochal. As a result of these lectures, the olm won over the entire learned world and became one of the animals of most interest on the planet (Aljančič et al., 1993). Specimens were sent to all parts of the world; Schreibers himself sent almost 300 of them.

This interest ignited the olm trade. From 1814, animals were sold at local inns in Postojna and sometimes outside of the cave. Guidebooks drew attention to their availability. Some were given to zoos, others were kept by amateur naturalists, some went to laboratories, and a few were even eaten. One was offered to Darwin for study (Shaw, 1999).

For the purpose of research, Zois started to breed olm in his apartment, followed by Schreibers. In 1806, Schreibers made a wax model of a nice specimen so the chroniclers attributed him the role of the “father of modern museum presentation of nature” (Aljančič et al., 1993). The research continued, and as early as 1850, curator of Viennese museum, L. Fitzinger, classified olm into seven species. However, the scientific community did not recognize this at the time. More recent research based on DNA methods confirmed that five populations of the olm differ enough genetically that they can be considered separate species (Gorički and Trontelj, 2006).

Two and a half centuries of olm research enabled exceptional insight into understanding its morphology, way of life, adaptation and functioning, even capturing its hatching on camera. It also brought about significant suffering of the olm. They were caught en masse and subjected to experiments, such as keeping them in captivity for ten-years without food to determine how long they could survive. The olm became a metaphor for knowledge of the secrets of the underground, as well as transformation of our knowledge over time. Numerous books, research papers, and popular publications promoted the scientific perception of the olm and its underground world. Proteus has been so well researched that there is no need to link it with mythological dragons.

Worldview Frame

To this day and despite the breadth of research, public perception of the Dinaric Karst and reference to the olm as a mythological creature continues, which has no scientific corroboration:

1. 1. Mythological image of the olm as a “dragon.” Truly, it is not as fearsome as its ancestor seems to be, it is more of a pet name. Its underground habitat is steeped in folklore and is dramatized as the origin of fear.
2. 2. An image of animal species that is in fact a “star” from the show business world. On the Postojna Cave web page, proteus is said to be a *star* amongst inhabitants of the cave. On millions of posters, flyers, and other media, for over a century, olm has had the starring role as a photo model for tourists, which attracts hordes of people. There is little discussion of other taxa — and there are over 80 species in Postojna Cave — apart from the star.
3. 3. More recently, the olm is often associated with clean water. In areas where the olm lives, the water is still environmentally sound, so an important characteristic of proteus is that it has become a bio-indicator of relative environmental health.

These three functions of the olm indicate that naming of this underground animal is more than a means of persuasion and shows the way we come to socially construct and know our natural world. That language is epistemic — it is how we come to know, and thus becomes central to the creation of our reality. Within this system of study, symbols and language are conceived of as perceptual lenses (Oravec and Clarke, 2004)

These three functions well illustrate the dominant worldviews with which people have observed the world through history. There are several classifications (e.g., Capra 1982; Merchant 1998; Geiger 2009), however, among them, the most suitable for our use is the division (1) traditional, pre-modern or organic, (2) modern, rational or mechanistic, and (3) postmodern, environmental or holistic.

The earliest worldview is traditional or organic. It provides a mythologized image of the world, and according to this view, the visible world is only the expression of spirits or deities and the entire world is connected into one organic entirety. In it, caves are doors to another world. Human fish was actually a dragon. Home snake is a spirit of our ancestors or an expression of evil.

The second worldview is rational, modern, or industrial. It is based on the perception of the world as understandable through the principles of science. Accordingly, knowledge must be explainable and verifiable. It gradually introduced geology, geophysics, hydrogeology, speleology, biology, etc. into the underworld, using conventional systems of description, calculation and reasoning. Max Weber claims that the task of science is the disenchantment of the world (Weber, 1946). Rational sciences largely expelled the many mythological beings from the underground. However, different worldviews influence the mix of different content under this perspective. They primarily depend on socially influenced circumstances, but also on numerous individual inclinations.

The third worldview is postmodern or environmental. It sees scientific data through the living community in relation to the non-living world. An essential component of that image of the world is the awareness of the negative effects of humans on ecosystems. The olm fits in this worldview as a bio-indicator. This worldview opened the door to the subterranean fauna as a whole. In the last few decades, subterranean fauna have become increasingly visible and acceptable in the Dinaric Karst, thanks to this worldview. Not only have hundreds of scientific papers been written about the subterranean fauna of the Dinaric Karst, but the popular media coverage has been received by a large, worldwide audience.

Media Construction of Environment

In the Dinaric Karst, cultural memory still connects the underground with mythological attributes. For instance, the snake still largely assumes its mythological aura. Each year, at the end of spring and beginning of summer, media in the Dinaric Karst area extensively reports on the danger of snakes. In the Dalmatia region, where snake bites are the most common, 5.2/100,000 inhabitants (Lukšić et al., 2006) are harmed annually, 0.4% of these bites are lethal, showing that there is not really a factual foundation for the danger of snake bites, because almost the same number of people die in traffic accidents each year in Croatia, of which Dalmatia is one of the five regions. This danger can resonate only in a public in which mythological memory of snakes still vibrates.

Science fosters the principle of physical evidence that excludes the impact of spiritual values, which in market conditions, gives more weight to the principle of utility. We often hear: Science must serve the economy. How could these small subterranean animals be useful in the dominant model of a market economy in which the external attractiveness is one of the most important characteristics of a good? Proteus has become a photogenic model in the tourist cave, enveloped in a mythological fog: "miracle of the underworld." It has become an important part of the tourist attraction, luring almost a million visitors to the cave in the most successful years.

On the other hand, our subconscious resists rationalization of life (Barešín, 2016), and taking into account the underworld, creates subversive phenomena such as dragons or vampires. The market economy relates them to contemporary myths for its commercial value. Many films were made and books written on Count Dracula. This is partly why many "consumers" of these products associate innocent bats with blood-thirsty vampires. Other underground species are scarcely known by the general public. It confirms what is well-known in environmental communication: the environmental issues are recognized by the public only when media and public forums present them as matters of public and political significance (Hansen, 2015). The media image of reality is a social construct.

Who made this construct and how? Here is an example. Primary definers in news coverage of environmental issues on British and Danish television (Hansen, 1991) are 23% authorities on the subject matter, 21% governmental representatives, 17% independent scientists, and 6% represent environmental groups.

To identify the environmental problems in the public arena as such, the media and public forums need to present them as issues of public and political importance. Therefore, environmental problems only become recognized, *per se*, through the process of public claims-making (Hansen, 2015), and the result depends on their balance of power.

With regard to the sources on which the articles on hatching of the olm relied, as well as the sources for images of proteus, it is obvious that they are largely based on the data obtained from the officials at Postojna Cave. Similarities can be found with the official web pages of the Cave, which are written with respect to the rules of a tourist guide (i.e., marketing of this tourist attraction).

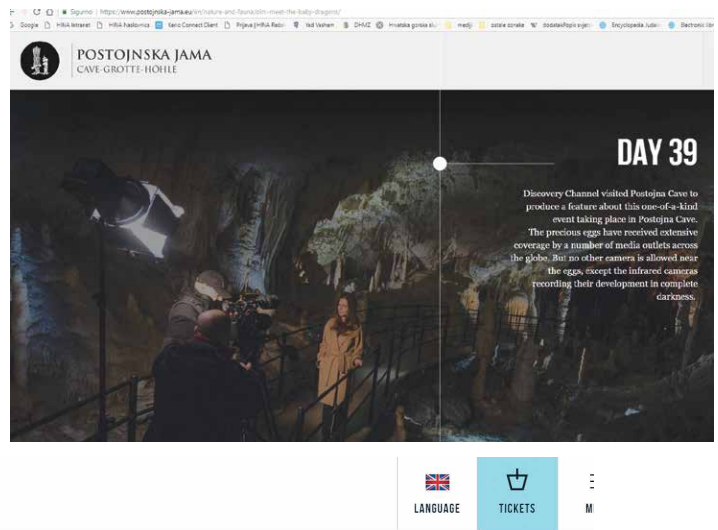
Olms have been intriguing people for centuries, the news about them reaching even the far corners of the region back in the day. This intrigue was mainly owing to people's vivid imagination, the mystery surrounding the species, and the polymath Johann Weikhard von Valvasor, who wrote about the dragon living the depths of the caves between Postojna and Vrhnika (Wonderland Stories, 2017; Postojnska-jama, 2017) (Figs. 3A, 3B).



OLM - MEET THE BABY DRAGONS

In January 2016, a fairy tale became reality: the news that the eggs of dragon's offspring had been laid spread around the world.

INTERESTING FACTS ABOUT OLMS | BLOG



POSTOJNSKA JAMA
CAVE-GROTTE-HÖHLE

VIVARIUM PROTEUS

The undisputed star among Postojna Cave's inhabitants is the olm, *Proteus anguinus*, however, the Karst underground world is inhabited by numerous other interesting inhabitants. According to explorers' estimates, Postojna Cave is home to more than 150 animal species. You can get to know some of the most interesting ones in more detail at Vivarium Proteus.



Figure 3A – 3D. Presentation of the proteus and proteus hatching by the management of the Postojna Cave (screenshots of the website).

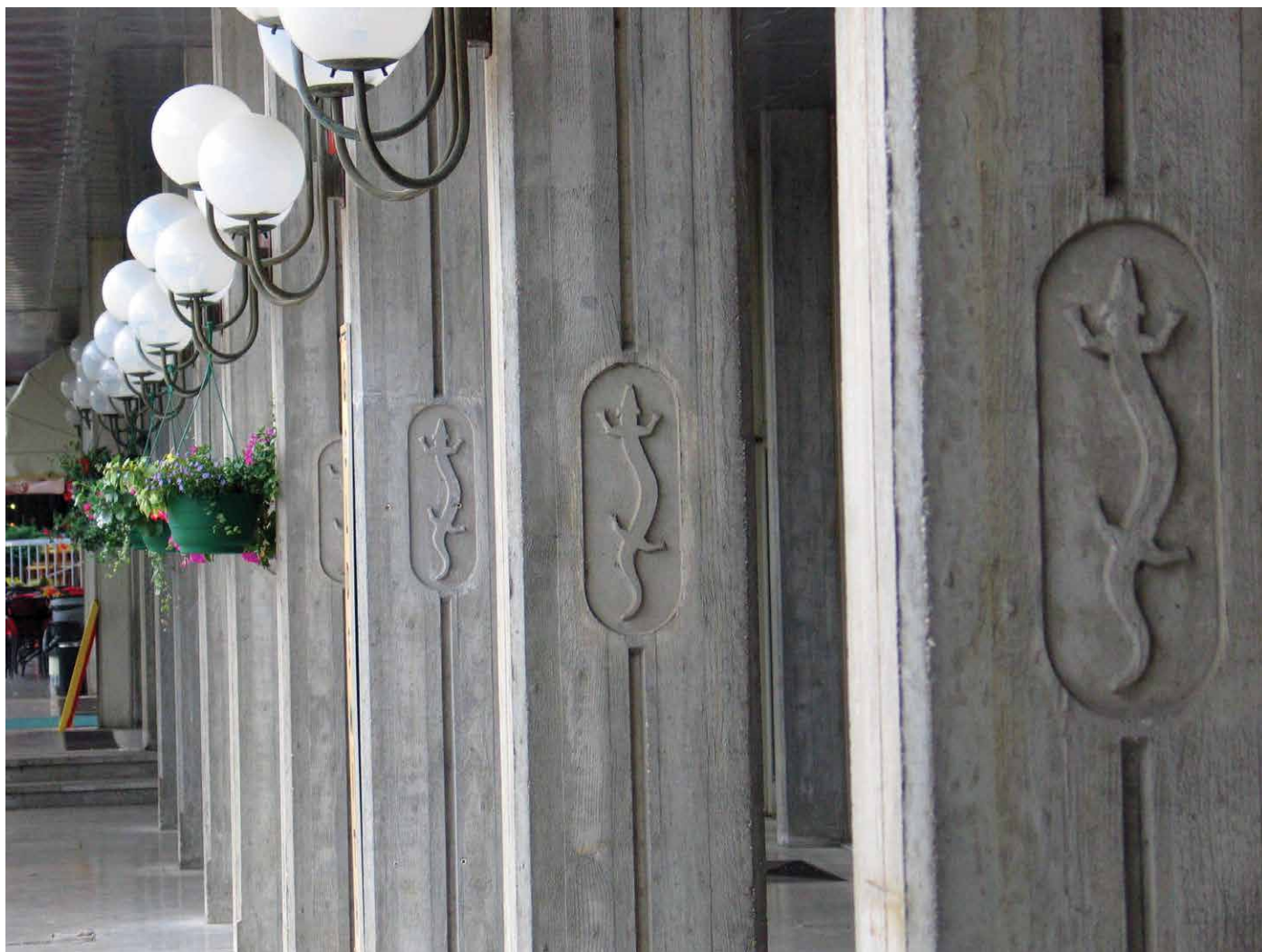


Figure 4. The figure of olm applied to the pillars in front of the Jama (Cave) Hotel in Postojna.

The precious eggs have received extensive coverage by media outlets across the globe. But the mystery remains hidden in the darkness, recorded only by means of an infrared camera (Fig. 3C). The undisputed star among Postojna Cave's inhabitants is the olm, *Proteus anguinus*; however, the karst underground world is inhabited by numerous other interesting inhabitants (Postojnska-jama, 2020) (Fig. 3D).

Actually, very few authors of the articles reviewed here looked for additional sources beyond the official Postojna Cave information. Thus, it is no wonder that some sentences in the articles are phrased as if they are part of Postojna Cave publicity material. It should be said, in this article I do not venture into a detailed analysis of cultural and historical aspects of the proteus's heritage, which is growing into elements of the national identity. I will only recall the various proteus prints on buildings (Fig. 4) and coins (Fig. 5). During the period in which a rational worldview gained strength, olm was mostly promoted in Postojna Cave, relying on scientific sources, on interest of a robust tourist industry, and on the appetite of the public. Tour guides in Postojna Cave played an extremely important role in interpretation of the underground, especially its living world, including the dominant elements of different worldviews. This tourist model of presentation is still dominant in the general public. For a long time, the olm has stopped being an epistemological issue and is now more predominantly a matter of media construction of the environment. A more careful interpretation of this phenomena requires more detailed research.

CONCLUSION

Historical analysis of the olm is a clear example of what usually happens in the natural sciences. It is traditionally believed that after a specific scientific discovery the knowledge reaches all the people in its integral form and that the public then creates a "real" image of the world based on that knowledge. Knowledge is an important element in the construction of the image of the world, but it is not the only image. This image is determined by different historical, political and cultural cir-



Figure 5. Proteus-coin.

cumstances, as well as the social interests of the people who constructed it. The example of s proteus shows that the tourist guides in the Postojna Cave were a significant factor in forming this image. They contributed to the fact that this image, besides the basic scientific facts, contains emphasized elements of a brand with a market value, i.e. as a tourist attraction. Further research is needed for more detailed answers that would cast more light into the connection between s proteus as a natural phenomenon and its public image. Thus, the results of this article should be considered in further research on caves and their public perception for purposes of their management and commercialization.

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GLACIER CAVES: A GLOBALLY THREATENED SUBTERRANEAN BIOME

Francis G. Howarth¹

Abstract

Caves and cave-like voids are common features within and beneath glaciers. The physical environment is harsh and extreme, and often considered barren and devoid of life. However, accumulating evidence indicates that these caves may support a diverse invertebrate fauna with species endemic to each region. As glaciers continue to disappear at an alarming rate due to global warming, they take their largely unknown fauna with them. Thus, glacier caves may harbor one of the most endangered ecosystems globally, and yet their biodiversity is among the least studied or known. Faunal surveys and ecological studies are urgently needed before all examples are lost.

INTRODUCTION

Glacier caves are voids within and beneath glaciers that are formed mostly by surface meltwater sinking into the glacier through crevasses, moulins, and fissures (Piccini and Mecchia, 2013; Smart, 2003; Kováč, 2018; Gulley and Fountain, 2019). Glacier caves can be enlarged by geothermal melting (Kiver and Mumma, 1971; Giggenbach, 1976), as well as by pressure and friction at the contact between the ice and bedrock. These caves are created by natural phenomena during the life of the glacier and are common features in glaciers. They are best developed in montane glaciers in comparison to polar glaciers, largely because of the steeper gradient, greater flow rate, and seasonally warmer temperatures (Smart, 2003). The cave structure is dynamic; for example, changing shape and course as the glacier flows downslope; enlarging during warm periods; and collapsing and deforming under pressure. However, the voids within sediments at the base of glaciers may remain open and contain liquid water during most of the life of the glacier (Hodson et al., 2015) especially in montane glaciers at low and mid latitudes (Hotaling et al., 2017b). A meltwater stream often flows along the contact between the glacier and bedrock. This meltwater exits glaciers through springs and seeps that feed surface streams. Glacier caves are distinct from 'ice caves' (the latter more accurately referred to as 'freezing caverns'), which are conventional caves in rock, such as limestone and basalt, that trap cold air and contain permanent ice (Kováč, 2018; Perşoiu and Lauritzen, 2018). The known fauna of freezing caverns was reviewed by Iepure (2018).

Glacier caves can be considered a subgroup of the larger subterranean biome, as well as a subgroup of the glacial biome (Anesio et al., 2017). The latter is a subgroup of the aeolian biome (Papp, 1978; Swan, 1992). A biome is a grouping of ecosystems that share similar features of their physical environment and characteristics of the resident community of organisms. The physical environment within glacier caves is extreme. Temperatures remain near or below freezing, and the polished ice walls often appear clean and devoid of organic material. There are few food resources visible that could support life. Liquid water necessary for cellular function would be unavailable except during periods when meltwater is present. Shallow glacier caves receive subdued sunlight filtered through the translucent ice, but deeper caves remain completely dark (Hotaling et al., 2017b). Subglacial sediments are normally anoxic unless refreshed by incoming aerated surface water (Hodson et al., 2015). In addition, survey of subglacial habitats can be extremely difficult and dangerous (Hodson et al., 2015; Gulley and Fountain, 2019; Templeton and Jahn, 2019).

The barren appearance is deceiving. Organic material can be relatively abundant. The surface of glaciers traps wind-borne organisms and organic detritus that become encased in the ice when covered by new snow fall (Mani, 1968; Papp, 1978; Edwards, 1987; Lockwood et al., 1991; Cartaya and McGregor, 2014; Vanlooy and Vandenberg, 2019). The organic material is preserved often for millennia within the ice until meltwater releases it and washes it into caves or it is released at the margin of receding glaciers (Mani, 1968). The interface between the ice and bedrock under montane glaciers contains relatively abundant nutrient resources that support distinct and diverse microbial communities (Bhatia et al., 2006; Hodson et al., 2015; Hotaling et al., 2017b).

Meltwater on the glacial surface support diverse assemblages of microbiota on nearly all glaciers studied worldwide (Takeuchi 2011; Hotaling et al., 2017b). The main source of nutrients supporting these surface communities is transported by wind (Mani, 1968; Swan, 1992). Additional nutrients are contributed by autotrophic algae growing on the snow, but these organisms also require aeolian-derived nutrients for growth (Swan, 1992). These communities contribute significant organic material to meltwater that sinks into the glacier through crevasses and moulins (Hodson et al., 2015; Anesio et al., 2017; Hotaling et al., 2017b). The invertebrates are represented by five phyla: Rotifera, Annelida, Tardigrada, Nematoda, and Arthropoda (Zawierucha et al., 2015). Surprisingly, rotifers live on and in the snow and ice of glaciers in Iceland (Shain et al., 2016). A few macroscopic organisms exploit organic resources on the glacial surface, e.g., the obligate glacial ice worm, *Mesenchytraeus solifugus* on glaciers of northwestern North America (Hotaling et

¹Distinguished Research Associate, B.P. Bishop Museum, Honolulu, Hawaii
Corresponding Address: 4033 SW Tunnelwood Street, Portland, OR, 97221, USA; fghcavebug@gmail.com

al., 2019) and numerous arthropods such as Arachnida, Collembola and Diptera in the Himalayas (Swan, 1992) and Plecoptera in Patagonia (Takeuchi 2011).

Meltwater exiting the downslope edge of glaciers feeds surface streams and springs. These glacially-fed surface waterways support a diverse fauna of cold-adapted aquatic invertebrates (Hotaling et al., 2017a) such as the Himalayas (Hamerlik and Jacobsen, 2011), New Zealand (Milner et al., 2001; Winterbourn et al., 2008), N. America (Giersch et al., 2016), S. America (Jacobsen et al., 2010), and Europe (Brown et al., 2007; Scotti et al., 2019). Most of the nutrients supporting this community is supplied by organic material exiting the glacier, at least close to the glacier forefront (Hotaling et al., 2017a). Although faunal surveys confirm the existence of a diverse fauna in glacially-fed streams, the true diversity remains unknown. In fact, most glaciers and associated surface water ways have yet to be surveyed, and as glaciers disappear through climate change, many species will be lost without having been described or recognized (Brown et al., 2007; Muhlfeld et al., 2011; Jacobsen et al., 2012; Giersch et al., 2015; Hotaling et al., 2017a; b; Jordan et al., 2016; Maurer et al., 2019; Zawierucha and Shain, 2019). For example, 125 of the estimated 150 glaciers have disappeared from Glacier National Park in the last 175 years, and by 2050, all of the iconic glaciers in the park will be gone (Hall and Fagre, 2003).

FAUNA OF GLACIAL CAVES

In contrast to these surface meltwater habitats, the harsh environment beneath glaciers has long been considered inhospitable and devoid of life. However, that view is changing with the discovery of microbial communities in subglacial lakes and within the sediments between the glacier and bedrock (Bhatia et al., 2006; Mikucki et al., 2009; Hotaling, 2017b). The macroinvertebrate fauna of subglacial habitats remains poorly known. I know of no overview of the fauna of any glacier cave, but at least a few subglacial arthropods are known. Two species of subterranean amphipods have been described from subglacial aquatic habitats in Iceland. Their endemism on the isolated oceanic island indicates that they survived in refugia (possibly maintained by geothermal heating) beneath glaciers (Kornobis et al., 2010). At least six species of cave-adapted amphipods in North America are thought to have persisted in subglacial refugia (Taylor and Niemiller, 2016), and in northern Europe several subterranean species of *Niphargus* amphipods most likely survived multiple glaciation events (McInerney et al., 2014). In addition to these aquatic species, a few terrestrial cave species survived glaciation including an anomalous cave-adapted campodeid dipluran that is known from the edge of the last glacial maximum on Vancouver Island, Canada (Sendra and Wagnell, 2019), and several species of collembola evidently survived glaciation in the British Isles (Faria, 2019). The habitat of these subglacial animals includes the medium-sized voids in the gravel between the glacier and bedrock, analogous to the “mesocaverns” in Howarth (1983). Liquid water may be available in this habitat by heating from a geothermal source, melting point reduction from pressure, and friction from the moving glacier. In August 1988, I visited Paradise Ice Cave in Paradise Glacier, Mt Rainier National Park (Fig. 1) and noted that in places the walls seemed alive with insects resting or running on the ice. I did not have a collecting permit, and as far as I know, the dozen or more species in four orders that I saw are still unknown. Observed were mayflies (Ephemeroptera), stoneflies (Plecoptera), caddisflies (Trichoptera), and flies and midges (Diptera). None of the species observed showed morphological characters that might indicate adaptation to subterranean habitats, and all may also occur in nearby melt water streams. However, because of their isolation within the glacier, most if not all species were successfully living in the cave. Due to glacial retreat from warming, Paradise Ice Cave is no longer accessible. Comprehensive surveys of aquatic insects were conducted by Kubo and colleagues (2012), and for stoneflies by Kondratieff and Lechleitner (2002) occurring in meltwater streams on Mt. Rainier; however, neither study apparently included glacial caves in their surveys. There is undoubtedly significant overlap in the aquatic arthropod fauna of meltwater streams and the fauna within glacier caves, but there may be significant differences based on degree of isolation, food resources, age and history of the glacier, the source of fauna, and differences in the physical environment. Even the meltwater stream fauna that successfully colonized glacier caves might have highly evolved additional physiological traits that allow them to survive in the harsh environment, such as being frozen for prolonged periods as well as being subjected to periodic anoxic conditions. For example, Bhatia et al. (2006) found that the bacterial communities beneath John Evans Glacier in Nunavut, Canada, were highly distinct from the composition of bacterial communities in adjacent supraglacial meltwater and ice-free proglacial sediments indicating that the bacterial community beneath the glacier were adapted to subglacial conditions.

The subglacial microbial communities along with aeolian debris and surface animals falling into subglacial voids would provide resources needed by cavernicoles. Animals that can exploit these resources and reproduce in subglacial voids would, over time, become adapted to life underground, possibly by an adaptive shift (Howarth et al., 2019). Potential candidates for colonizing aquatic subglacial habitats include the invertebrates inhabiting the glacial surface and glacial fed streams noted above. A potential source for terrestrial animals that could colonize glacial caves is the community of scavengers and predators living in talus slopes adjacent to snow fields. These animals characteristically venture onto the ice during favorable weather to feed on moribund aeolian waifs trapped on the ice. They retreat into the

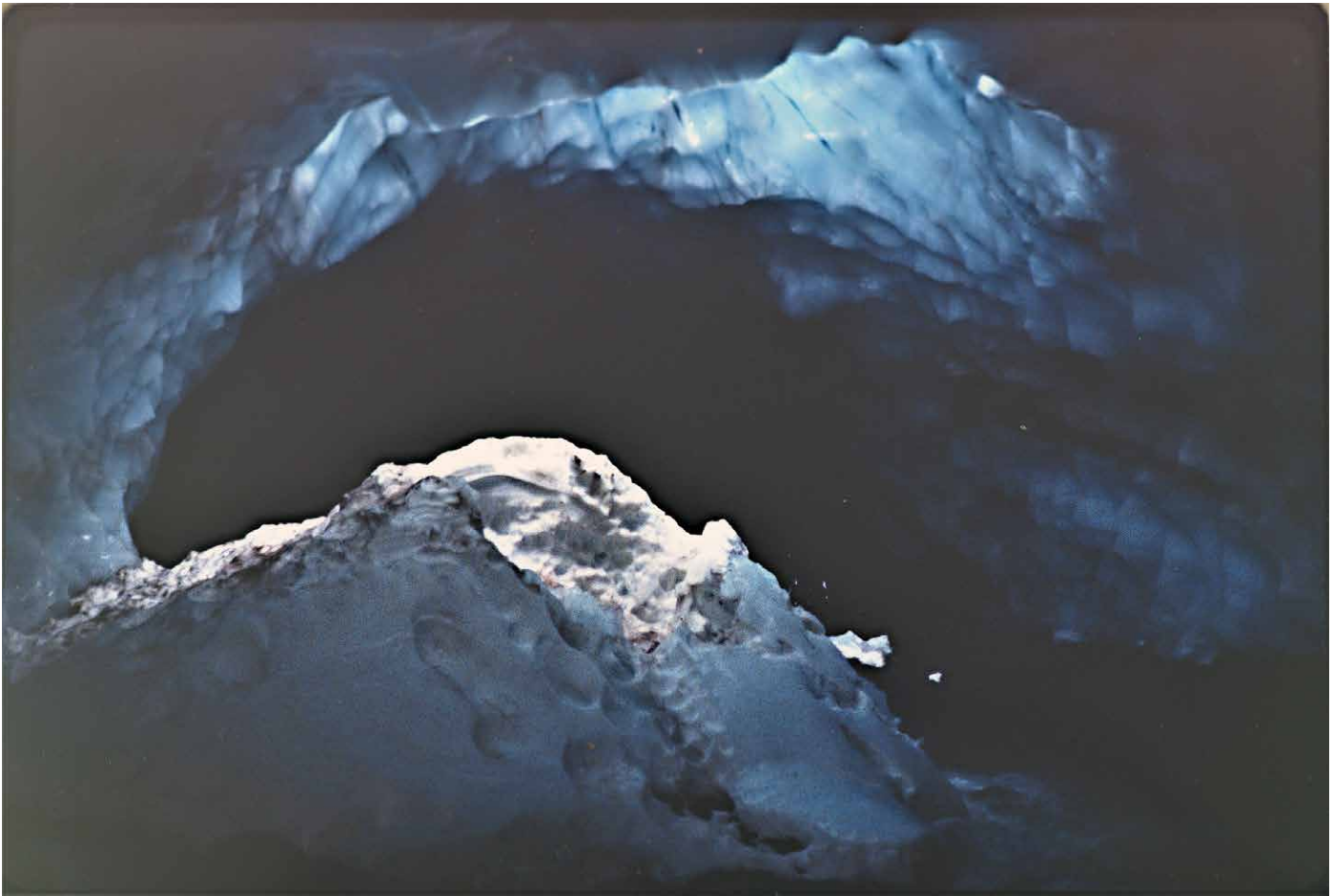


Figure 1. View downslope in Paradise Ice Cave, Mt. Rainier National Park, Washington, U.S.A. showing snow cone below skylight.

talus to escape unfavorable conditions (Edwards, 1987). These communities are dominated by cryophilic arthropods including spiders, harvestmen, springtails, grylloblattids, and staphylinid and carabid beetles (Mani, 1968; Edwards, 1987; Wipfler et al., 1914). Many of these cryophilic talus-inhabiting arthropods have close relatives adapted to live in conventional caves including freezing caverns (Iepure, 2018). Without biological surveys, one cannot assume that these caves do not harbor unique animals (Ficetola et al., 2018). If these cave faunas were to go extinct before being studied, the assumption that nothing could possibly live there would remain true, and biologists could claim that they never existed. Our understanding of the fauna of glacial caves is currently analogous to the assumption pre-1970 that young lava tubes on oceanic islands were barren of life (Howarth, 1972). A diverse fauna including species highly specialized to live only underground in caves and similar habitats is now known from most temperate and tropical regions that have been adequately surveyed (Howarth, 1983; Howarth and Moldovan, 2018). The animals living in glacial caves provide unique research opportunities in evolutionary ecology, behavior and physiology. For example, how do the animals survive in such a harsh environment? Specifically, how long can glacier cave arthropods survive freezing? Meltwater streams maintain annual freezing and thawing cycles, and the surface stream fauna is known to survive in such regimes. The same temperature regime may not be true in glacier caves as slight prolonged cooling of the climate or collapse of passages may isolate the animals in frozen crypts for decades or centuries. How do animals disperse within or below the glacier? Which populations are permanent residents, and which are meta populations? The latter require periodic recolonization from surface habitats. Understanding the environment and faunal adaptations may help explain how the recently discovered anomalous campodeid survived beneath the glacier (Sendra and Wagnell 2019).

SUGGESTED COLLECTION METHODS

Biological surveys of caves can be challenging both physically and logistically (Wynne et al., 2019). Fortunately, improved equipment and training have made exploring caves and conducting research safer and more productive. Furthermore, protocols for biological surveys have evolved and become more standardized (Hunt and Millar, 2001; Culver and Sket, 2002; Wynne et al. 2019). These methods can be utilized or modified for surveying glacial caves; however,

because of the additional risks inherent in exploring glacial caves, it seems prudent that the initial reconnaissance surveys employ passive collecting techniques that can be deployed remotely or quickly in accessible locations. For example, for terrestrial habitats, baited pitfall traps, including mesocavern traps (López and Oromí, 2010) could be lowered by rope into crevasses that reach the substrate. For aquatic habitats, plankton nets and baited shrimp traps could be lowered into waterways. Where possible, traps can be placed in and along the margins of springs exiting glaciers. This latter strategy should include surveys of the volume and composition of organic material released from the glacier. In addition, biologists might collaborate with glaciologists and utilize the holes drilled into glaciers. The results from these reconnaissance surveys will indicate which accessible caves would be suitable for more intensive surveys.

CONCLUSIONS

The fauna and microbiome of caves occurring in and beneath glacial ice are poorly known, and their ecology little understood. The few studies done to date indicate that a diverse fauna exists, which includes narrowly endemic species. Research in glacial caves is difficult due to the harsh environment and dynamic nature of the caves. Nevertheless, biodiversity and ecological studies are valuable and potentially improve our understanding of how organisms adapt to harsh environments. Glaciers and their associated habitats are disappearing at alarming rates. Glacial caves harbor one of the most endangered ecosystems on earth. Without successful mitigation to reverse global warming and limit the loss of montane glaciers, most glaciers, along with their associated fauna, will disappear in the next few decades.

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IDENTIFICATION OF FUNGI FROM SOIL AND SEDIMENT IN JEFRIZ CAVE; THE FIRST SURVEY IN A CAVE FROM IRAN

Azadeh Habibi^{1, c} and Banafsheh Safaiefarahani²

Abstract

The aim of this study was to characterize the mycobiota in soil and sediment samples of Jefriz cave in Kerman, Iran. During 2018–2019, the culturable mycobiota from several sites within the Jefriz cave, resulted in 82 fungal isolates. Morphological characteristics of the isolates, as well as molecular sequence data, were used for species identifications. The fungi were identified as species of *Fusarium*, *Fusicolla*, *Geomyces* (*Pseudogymnoascus*), *Humicola*, *Chalastospora*, *Penicillium*, *Aspergillus*, *Epiciccum*, *Podospora* and *Mucor*. The most prevalent was *Aspergillus spelunceus*, followed by *Geomyces pannorum* and *Humicola grisea*. The majority of these species have been reported as cave residents in previous studies of cave environments. Our data showed that the fungal community composition varied between the samples from the entrance and less visited sites deeper in the cave. This study is the first cave mycological investigation in Iran, and one of the identified species is reported for the first time from a cave.

INTRODUCTION

Caves are considered as extreme environments characterized by darkness, low temperatures, high humidity, and limited organic matter (Barton and Northup, 2007). Microbial communities in caves are uniquely adapted to oligotrophic conditions (Jurado et al., 2009). Fungi are an important group of cave microflora that play critical roles as decomposers, mutualists, and pathogens (Shapiro and Pringle, 2010) and they interact with cave fauna. Some fungal species are involved in processes of disintegration and formation of cave speleothems by providing nucleation sites for precipitation of calcium carbonate (Barton and Northup, 2007), while some other species play roles in biodeterioration of cave structures via physical penetration, metabolite secretion, and pigmentation (Cañiveras et al., 2001; Wang et al., 2011). These facts make the caves microorganisms interesting to microbiologists.

A number of studies have explored the fungal community occurring in caves soil and sediments and have proved that caves harbor a high diversity of fungi (Vanderwolf et al., 2013). In July 2001, an outbreak of *Fusarium solani* in the prehistoric painted Lascaux Cave in France was discovered and biocide treatments were applied (Dupont et al., 2007; Bastian et al., 2009b). *Pseudogymnoascus destructans*, the causal agent of white-nose syndrome emerged in North America and spread in 2006, drawing more attention to fungal cave communities (Meteyer et al., 2009). Vanderwolf et al. (2013) published a list of species and genera of fungi, slime molds, and yeasts reported from caves worldwide. The fungal community composition is affected by factors such as sampling site, isolation method, time of year (Wang et al., 2011), human activity, and presence of nutrient sources such as bat guano and droppings (Nováková, 2009; Shapiro and Pringle, 2010).

No data have been reported on metabolic properties and the mechanisms of adaptation of the fungal flora in caves in Iran. Jefriz cave, located in Baft, Kerman province, Iran (Fig. 1), is an attractive limestone cave with unique speleothems. This cave is important since there are reports of bat species residing in the cave (personal communication with local people). In this study, we aimed to investigate fungal flora of Jefriz cave based on culture-dependent methods to determine the diversity of fungi and thereby enhance our understanding of the evolution of adaptations to the extreme ecosystems in caves.

MATERIALS AND METHODS

Sampling Site

Jefriz is a limestone cave located 3 km east of Jefriz village, Baft, Kerman province, Iran (Fig. 1). This cave has a vertical, eight-meter deep entrance located on a hillside. A gate has been installed over the entrance (Fig. 2). Exploration into the cave is by rappelling through a shaft that requires the use of specialized equipment such as a rope or cable ladder. The cave length is about 332 m (personal communications from the explorers). The cave is linear with few side passages, but there are two main tunnels. After entry, there is a big chamber with passageways covered with stalactites and stalagmites. The main passage in some parts is only accessible through narrow tunnels that requires the explorer to crawl to pass. The cave floor is covered with wet debris and tiny ponds are present. Jefriz cave is not scientifically

¹Assistant professor, Department of Biodiversity, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran

²Assistant Professor, Plant Protection Research Department, Fars Agricultural and Natural Resources Research and Education Center, AREEO, Shiraz, Iran

^cCorresponding author: a.habibi@kgut.ac.ir

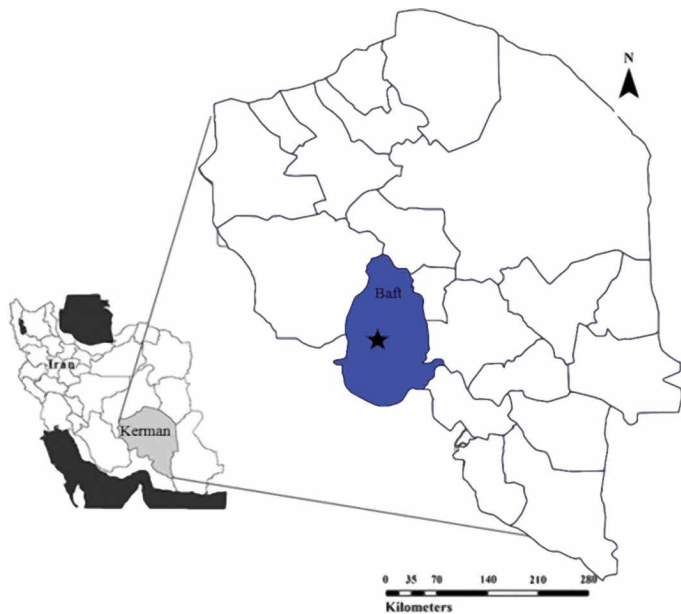


Figure 1. Location of the study area in Iran.



Figure 2. Entrance to Jefriz cave.

explored and a map has not been published. A gate at the entrance (Figure 2b) of the cave was installed by local people to prevent entering of unprofessional cavers, to preserve the cave from damages produced by some careless visitors, and to protect the untrained visitors because the exploration needs training and special equipment.

Sample Collection and Isolation of Culturable Fungi

Soil and sediment samples were collected using a sterilized spatula from 0-4 cm of the cave floor, placed in sterile bags, transferred to the laboratory, and stored at 4 °C. Four visits were carried out for sampling and four samples were taken during each visit. Sample collections took place in all four seasons, on January 11, 2018, and May 14, 2019, August 19, 2019, and October 22, 2019. For each trip, one sample was collected from sites close to the entrance corridor in the first large chamber, and three samples were collected randomly throughout the cave from deeper sites. Isolations were carried out by direct plating and plate dilution methods (Garrett, 1981). Soil samples (1 g) were suspended in nine mL sterile distilled water, vortexed vigorously for 1 min and allowed to stand

for 3 min. Two hundred μL of 10^{-2} to 10^{-4} -fold diluted suspensions was inoculated onto potato dextrose agar (PDA; Merck, Germany) and dichloran rose bengal chloramphenicol (DRBC) agar (peptone 5 g, KH_2PO_4 1 g, MgSO_4 0.5 g, glucose 10 g, dichloran solution (0.2% (w/v) in ethanol, Rose Bengal 0.025 g, chloramphenicol 0.1 g, agar 15 g, and distilled water to 1 L; King et al., 1979). All ingredients of culture media were obtained from Merck, Germany. Chloramphenicol (Sigma–Aldrich, Germany) was added to eliminate bacteria. Cultures were incubated at 25 °C for four weeks in darkness. Plates were examined for fungal growth every three days. Colonies that appeared on cultures were transferred to new

plates for further examination. All the isolates were deposited in the KGUT Fungal Culture Collection at Kerman Graduate University of Advanced Technology, Kerman, Iran.

Morphological Studies

The morphological identification of fungal isolates was based on the macroscopic and microscopic characteristics of the isolates, such as colony morphology, conidia, and conidiophores according to reliable identification guides (Domsch et al., 1980; Barnett and Hunter, 1998; Hanlin et al., 1998; Leslie and Summerell, 2006; Seifert et al., 2011). All cultures were done in triplicate. Media used were Malt Extract Agar (MEA: malt 20 g/L, glucose 20 g/L, peptone 1 g/L, agar 16 g/L), Czapek Yeast autolysate Agar (CYA: sucrose 30 g/L, powdered yeast extract 5 g/L, K_2HPO_4 1 g/L, NaNO_3 2 g/L, KCl 0.5 g/L, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g/L, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01 g/L, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01 g/L, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.005 g/L, agar 20 g/L) and Czapek–Dox agar (CZ: sucrose 30 g/L, K_2HPO_4 1 g/L, NaNO_3 2 g/L, KCl 0.5 g/L, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g/L, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01 g/L, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01 g/L, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.005 g/L, agar 20 g/L) (Hubka et al., 2016) to observe colony characteristics. All ingredients of culture media were obtained from Merck, Germany. Lactophenol, lactophenol cotton blue, and 50% lactic acid (Merck, Germany) solutions were used to prepare microscope slides. The measurements and microphotographs of fungal features were taken using Dino-eye microscope camera USB lens (The Microscope Store, LLC., USA) from agar plates and slides. Colony diameter was assessed on plates after 7–28 days at 25 °C. The mean diameter was obtained from three replicates.

Molecular Examination Of Isolates

Genomic DNA of fungal isolates was extracted using a CTAB extraction procedure (Doyle and Doyle, 1987; Zhang et al., 2010) from mycelia grown on PDA for 7–15 days at 25 °C. The DNA concentrations were estimated by a NanoDrop spectrophotometer (NanoDrop Technologies, USA). ITS-rDNA regions were amplified using two primers, ITS1 and ITS4 (White et al. 1990). A ~700 bp portion of the *TEF1-α* gene (Translation Elongation Factor 1α) was amplified from *Fusarium* isolates using the primers, EF1 (5'-ATG GG TAA GGA RGA CAA GAC-3') and EF2 (5' GGA RGT ACC AGT SAT CAT G-3') (O'Donnell et al., 1998; Geiser et al., 2004; Edel-Hermann et al., 2012). Primers were synthesized by Macrogen, Inc. (South Korea). Twenty-five μL polymerase chain reaction (PCR) contained 1X reaction buffer, 2.5 mM MgCl₂, 200 mM dNTPs, 0.4 mM of each primer, 20 ng of DNA, and 1 unit of Taq polymerase (Ampliqon, Denmark). A Biometra TAdvanced Thermal Cycler (Biometra, Göttingen, Germany) were used to perform PCRs. The cycling conditions for ITS1 and ITS 4 consisted of 95 °C for 5 min, followed by 30 cycles of 95 °C for 45 s, 60 °C for 30 s, and 72 °C for 1 min, and then 5 min at 72 °C. The annealing temperature of 54 °C was used for *TEF1-α*. The sequencing was carried out by Macrogen (Macrogen Inc., South Korea). The sequences were edited by Geneious (Biomatters Inc., USA) when needed and compared to the sequences in the NCBI (GenBank) database using BLAST (Basic Local Alignment Search Tool) (Altschul et al., 1990) to find the most likely taxonomic designation of each isolate. All nucleotide sequences obtained in this study were deposited in GenBank and accession numbers were obtained as MN643060 to MN643071.

RESULTS

Fungal Isolates



Figure 3. Colony morphology of fungal species from Jefriz cave incubated on Potato Dextrose Agar (PDA) a, *Fusarium solani*. b, *Fusicolla septimanifiniscentiae*. c, *Pseudogymnoascus pannorum*. d, *Humicola grisea*. e, *Chalastospora gossypii*. f, *Penicillium brevicompactum*. g, *Penicillium expansum*. h, *Aspergillus spelunceus*. i, *Epiciccum nigrum*.

air resulting in biased isolation toward common airborne fungi from outside of the cave.

Sampling was carried out in four visits, one in each season of the year. In each visit, samples were collected from the sites close to the entrance corridor and from sites located deep in the cave. Comparisons showed that the composition of fungal species was different among sampling sites. *Penicillium expansum* and *Aspergillus spelunceus* were the most abundant species detected from samples close to the entrance of the cave in the first large room while the most abundant species in remote and out of reach parts were *Pseudogymnoascus pannorum* and *Humicola grisea*. In addition, there was no observed difference in the composition of fungal species isolated in different seasons of the year.

In our current investigation, 16 samples of soil and sediments from Jefriz cave were collected in 4 visits and a total of 82 fungal isolates were isolated. The morphological and molecular investigations led to the identification of *Fusarium solani*, *Fusicolla septimanifiniscentiae*, *Pseudogymnoascus (Geomyces) pannorum*, *Humicola grisea*, *Chalastospora gossypii* (formerly *Alternaria malorum*), *Penicillium expansum*, *P. brevicompactum*, *Aspergillus spelunceus*, *Epiciccum nigrum*, *Podospora* sp., *Ochroconis* sp. and *Mucor* spp. (Figs. 3 and 4). Fifteen isolates were mycelia sterilia and were not identified to species level. The most prevalent genera were *Aspergillus spelunceus* (21%), *Pseudogymnoascus pannorum* (13%) and *Humicola grisea* (10%) (Table 1). Species diversity in this cave were not attempted to estimate due to the method of isolation that was limited to culture-dependent methods. These

methods give an estimate of the fungi present but is biased toward isolation of spore-forming species. Fungal species in cave air were not attempted because the air, especially in the entrance corridor, is in contact with outside

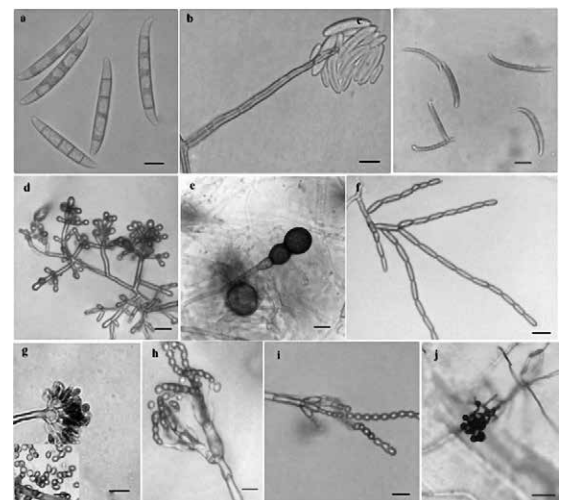


Figure 4. a-b, *Fusarium solani*: Macroconidia (a), false head and microconidia (b). c, *Fusicolla septimanifiniscentiae*: Macroconidia. d, *Pseudogymnoascus pannorum*: conidiophores and conidia. e, *Humicola grisea*: Aleurioconidia. f, *Chalastospora gossypii*: Elipsoid conidia. g, *Aspergillus spelunceus*: Conidial head. h, *Penicillium brevicompactum*: Conidia and conidiophore. i, *Penicillium expansum*: Conidia and conidiophore. j, *Epicocccum nigrum*: Conidia. Scale bars = 10 μm, j = 50 μm.

Table 1. Fungal species isolated from Jefriz cave. The relative frequency is calculated by dividing the frequency of each species by the total number of all isolates.

Species	Relative frequency	Isolate used for sequencing	Accession numbers obtained from GenBank
<i>Pseudogymnoascus pannorum</i>	13	J2	MN643060
<i>Humicola grisea</i>	10	J4	MN643061
<i>Chalastospora gossypii</i>	5	J10	MN643062
<i>Penicillium brevicompactum</i>	5	J11	MN643063
<i>Penicillium expansum</i>	7	J17	MN643064
<i>Aspergillus spelunceus</i>	21	J14	MN643065
<i>Epicoccum nigrum</i>	4	J20	MN643066
<i>Podospora</i> sp.	2	J12	MN643067
<i>Ochroconis</i> sp.	1	J21	MN643068
<i>Fusicolla septimanifiniscentiae</i>	2	J0	MN643069
<i>Fusicolla septimanifiniscentiae</i>	...	J0	MN643070
<i>Fusarium solani</i>	2	J22	MN643071
<i>Mucor</i> spp.	7
<i>Mycelia sterilia</i>	20

DISCUSSION

Our goal was to provide preliminary data on fungal communities of caves to study biological interactions in subterranean and extreme environments. The present study is the first one studying the mycobiota of a cave in Iran. We were able to successfully identify eleven fungal genera from soil and sediment samples of Jefriz cave on the basis of macroscopic, microscopic, and molecular analyses.

Literature suggests that most of these species are commonly associated with cave habitats around the world (Cunningham et al., 1995; Dupont et al., 2007; Jurado et al., 2009; Bastian et al., 2010; Docampo et al., 2010; Wang et al., 2011; Jacobs et al., 2017). Some of the isolated species such as *Fusarium* spp. are common soil inhabitants (Leslie and Summerell, 2006) and it is possible that they have been carried in by the water streams that run from outside through the walls of Jefriz cave. A similar scenario was suggested for *F. solani* entering Lascaux Cave by Dupont et al. (2007). Human visitors, small animals, and arthropods are other vectors that may have contributed to the fungal flora composition of Jefriz cave. The relationship between fungi and arthropods in caves is reported in several studies. Greif and Currah (2007) and Dromph (2003) isolated several fungal species from collembolans. *Isaria farinosa* (syn: *Paecilomyces farinosus*) is reported as the one of frequent parasites occurring on insects in underground environments in the Czech Republic by Kubátová and Dvořák (2005), and also as a parasite of *Stenophylax* (syn: *Micropterna*) *fissus* in Spanish caves by Jurado et al. (2008). Arthropod roles as host and vectors of fungal species (Bastian et al., 2009a) in Jefriz cave remain to be studied in further research by entomologists.

Water films resulting from condensation cover Jefriz walls and speleothems. These wet surfaces are a suitable niche for fungal growth (Barton and Jurado, 2007). *Lecanicillium psalliotae*, *L. araneorum*, *Engyodontium album* and *Torrubiella* spp. in biofilm samples of Roman catacombs are reported (Saarela et al., 2004; Jurado et al., 2008). Another factor influencing the cave mycobiota is availability of potential food sources. Droppings and guano especially of bats may serve as possible source of nutrients and energy input for many fungal species as well (Zhang et al., 2017).

The predominant species in Jefriz cave were *Aspergillus spelunceus* (21%), *Pseudogymnoascus pannorum* (13%), and *Humicola grisea* (10%). *Aspergillus spelunceus* (sect. *Nidulantes*) have been frequently isolated from caves (Vanderwolf et al., 2013; Hubka et al., 2016). Species related to *A. nidulans* may have medical importance (Hubka et al., 2016); the prevalence of this species should receive more attention from cavers. *Aspergillus* spp. are known to be allergenic, and causal agents of aspergillosis and mycotoxin producers (IARC 2002; Perrone et al., 2007). Mycotoxins are associated with immune deficiency and cancer (Shephard, 2008). *Pseudogymnoascus pannorum* was one of the dominant species in Jefriz cave. This species expresses keratinolytic enzymes and can cause skin infections and nail onychomycosis (Gianni et al., 2003; Zelenková; 2006; Reynolds and Barton, 2014) and may be dangerous to humans. *P. pannorum* tolerates low temperatures and higher salinity than seawater (Poole and Price, 1971; Leushkin et al., 2015). Characteristics such as tolerating low temperatures justifies its high prevalence in extreme environments such as caves. This species is reported from caves, arctic, and low temperature soils worldwide (Out et al., 2016). It should be noted that its close relative, *P. destructans*, is one of the commonly reported fungi from caves all over the world

(Martinkova et al., 2010; Turner et al., 2011) causing white nose syndrome in bats (Shelley et al., 2013). Another commonly-occurring species isolated from the cave samples was *Humicola grisea*. This species has high potential in starch saccharification (Maheshwari et al. 2000), which makes it a promising species in the process of producing bioethanol by microbial fermentation (Pervez et al., 2014). Several studies have reported bio-deterioration of structures in caves associated with fungal species extracellular metabolites. Gargani (1968) studied the microbial floras of damaged wall paintings in Florence after a flood and associated the damage with the growth of microorganisms. The deteriorations are the result of extracellular metabolites secreted while hyphae are penetrating inside the wall painting and cave speleothems (Strzelczyk, 1981; Garg et al., 1995).

Fusicolla septimanifiniscentiae is reported here from a cave for the first time. *Fusarium solani* was one of the especially interesting microorganisms that was identified. An aggressive isolate of this species was discovered in 2001 threatening the Paleolithic paintings of Lascaux cave, France, and resulted in multiple applications of biocides (Dupont et al., 2007). Arthropods have symbiotic relationships with *Fusarium* spp. (Morales-Ramos et al., 2000; Sharma and Marques, 2018) and are suggested as possible vectors of some species (Jurado et al., 2008). The pathogenicity of *Fusarium verticillioides* on grasshoppers (Pelizza et al., 2011) and *F. keratoplasticum* and *F. proliferatum* on *Tribolium* species (Chehri, 2017) have been reported.

Penicillium expansum, followed by *Aspergillus spelunceus*, prevailed in samples collected near the cave entrance, while *Humicola grisea* was the most abundant species in the samples collected in remote parts of the cave. Air exchange with the outside environment and the activities of cave explorers are possibly impacting on the cave airborne fungal community at the entrance. The number of visitors is not officially reported, however, the number is recently reduced because of the door implemented by locals. The impact of human activity on fungal diversity in caves is documented by Shapiro and Pringle, (2010). Their investigation showed complex associations between levels of human disturbance and the diversity of fungal species in four caves in Kentucky and Tennessee. They showed that the fungal diversity was higher in sites with moderate levels of disturbance than highly visited sites.

CONCLUSIONS

This survey was conducted to determine the major components of the mycoflora present in Jefriz cave to understand the complex biological interactions in subterranean habitats. In conclusion, 12 fungal species associated with Jefriz cave soil and sediments were identified in this study. Our data showed that the biological and climatic condition of this cave is selective. The fungal species were composed of both cosmopolitan species and common cave residents. Fungal species that can be harmful to the human health were found in Jefriz cave that evidenced the need to further investigate the hygienic safety of this place for cavers, although no illnesses have been reported. Work on unculturable species is needed to provide a thorough perspective of the fungal community in this cave. Furthermore, studies on metabolic activity and bioactive compounds of fungi, such as *Humicola grisea*, help our understanding of the biological interactions in a subterranean environment. We suggest studying the possible agents contributing to the dispersion of spores (*i.e.* arthropod vectors) in order to better take preventive actions, if needed, about the preservation of the cave and the safety of the explorers.

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CAECIDOTEA BURKENSIS, NEW SPECIES, A UNIQUE SUBTERRANEAN ISOPOD FROM BURKE'S GARDEN, WITH A SYNTHESIS OF THE BIOGEOGRAPHY AND EVOLUTION OF SOUTHWESTERN VIRGINIA ASELLIDS

Salisa L. Lewis¹, Julian J. Lewis^{2,C} and William Orndorff³

Abstract

Caecidotea burkensis, a new species of subterranean asellid isopod, is described and illustrated from material collected from Lawson Cave, in Burke's Garden, Tazewell County, Virginia. The type-locality in Burke's Garden is located within the highest mountain basin in the southern Appalachians. Burke's Garden is a unique, geologically isolated area encompassing one of the headwater streams of the New River basin. Phylogenetically, the isopod is a member of the *forbesi* Group, a clade comprised primarily of epigeal species. The complex mountain valleys and coves of southwestern Virginia are an area of intense speciation among asellids that have produced a bizarre array of cavernicolous species belonging to groups of otherwise epigeal isopods. In addition to a few subterranean species of the *Caecidotea cannula* and *stygia* Groups, the *Lirceus hargerii* Group possesses over a dozen species endemic to caves and springs in the region, mostly only now in the process of being discovered and described. With so much species richness, syn-tyopy of two, or even three, asellid species is commonplace in caves and springs in southwestern Virginia.

INTRODUCTION

Described as a mountain fortress of Nature by Hoffman (1996, 2012), Burke's Garden protects a unique habitat and considerable biodiversity. From the air, Burke's Garden looks like a crater. However, the crater is not the result of a volcano nor meteorite impact, but rather the breach of a broad sandstone anticlinal dome. This breach formed a broad circular valley in the underlying limestone that developed into a karst terrain of sinkholes, sinking streams, caves, and springs (Figure 1). Burke's Garden is the highest mountain basin in the southern Appalachians, with the floor at about 3,100 feet (almost 950 m) in elevation. The floor is encircled almost entirely by the northerly extension of Clinch Mountain. This circular mountain, formed of the remaining flanks of the anticline, attains elevations averaging above 4,000 feet, and peaks at 4,770 feet (1,454 m) on Bear Mountain on the west side. The single cleft in the mountain occurs at a water gap through which Burke's Garden Creek drains into Wolf Creek, eventually flowing to the New River.

Burke's Garden has long been known for the biodiversity of epigeal fauna (Hoffman and Kleinpeter, 1948), although none of 29 obligate subterranean species occurring in the New River basin in Virginia are endemic. The majority are terrestrial. The carabid beetle (*Pseudanophthalmus hortulanus*) occurs in a cave in Burke's Garden and a single cave in Thompson Valley, the next valley to the southwest (Holsinger and Culver, 1988; Virginia Natural Heritage Program, Biotics, unpublished record). Other troglobites occurring in Burke's Garden are the Tazewell County endemic millipede *Pseudotremia tuberculata*, the New River endemic dipluran *Litocampa pucketti*, and the widespread spiders *Nesticus tennesseensis* and *Porrhomma cavernicola*. The amphipod *Stygobromus mackini* is also documented in Burke's Garden and is the only widespread stygobiont in portions of the New River basin in Giles, Bland, and Tazewell counties, Virginia lying west of the New River (Holsinger et al, 2013).

Steeves (1969) reported the presence of an unusual asellid isopod inhabiting Lawson Cave in Burke's Garden. This isopod could not be assigned to any of the three recognized species groups of subterranean asellids known at the time (Steeves, 1963; 1964; 1966) and it was speculated to be the product of a relatively recent groundwater invasion. This isopod is the subject of this paper.

SYSTEMATICS

forbesi Group

The *forbesi* Group was erected by Lewis (2013) to receive *Caecidotea insula*, a troglomorphic species from a cave on South Bass Island in Lake Erie, Ohio. The significance of this assemblage was its dominance by epigeal species (*C. forbesi*, *C. obtusa*, *C. attenuata* and *C. racovitzae*) that seemed to have little ecological or evolutionary inclination toward inhabiting groundwater. Lewis (2013) also noted the presence of the Burke's Garden asellid inhabiting Lawson Cave. Further examination indicates the Lawson Cave population is related to, but distinct from, *C. racovitzae* and is herein described as the second stygobiont species of the *forbesi* Group.

¹Lewis and Associates, Cave, Karst and Groundwater Biological Consulting, LLC

²Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution

³Karst Program, Virginia Department of Conservation and Recreation

^CCorresponding author: lewisbioconsult@gmail.com

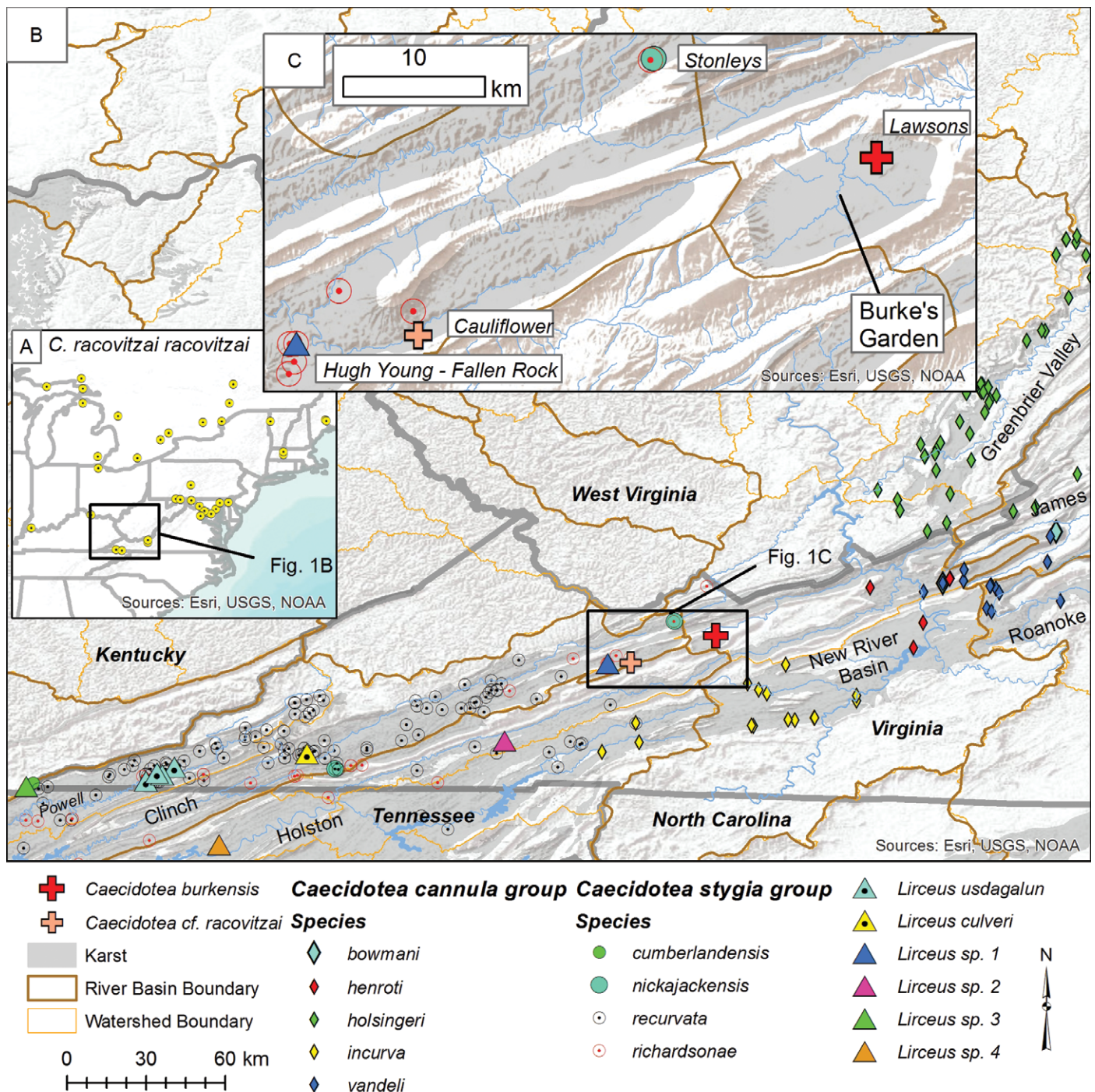


Figure 1. Asellid isopod range maps: (a) Complete range of *Caecidotea racovitzai racovitzai* in the eastern United States; (b) all subterranean asellids known from southwestern Virginia; (c) Burke's Garden and environs with known subterranean asellid populations.

Caecidotea burkensis, S. Lewis and J. Lewis, new species

A. sp. D. [*Asellus*]—Steeves, 1969: 60-61.

Caecidotea racovitzai racovitzai.—Fleming, 1972: 242; Holsinger and Culver, 1988: 32, 37.

Caecidotea racovitzai.—Lewis, 2013: 67.

Material examined—VIRGINIA: Tazewell County, Burke's Garden, Lawson Cave, cave stream, Salisa L. Lewis, Julian J. Lewis, 6 October 2019, 14♂ 12♀ (USNM 1622329); same locality, William D. Orndorff, Ellen Crowder, 7 July 2018, 18♂ 22♀ (USNM 1622330); Kenneth Dearolf, 3 July 1937, 3♂ 1♀ (USNM 108535); same locality, John R. Holsinger, Laurence E. Fleming, 12 April and 27 July, 1963, 5♂ 5♀ (USNM 230603); Lawson Insurgence Cave, mud-bottomed drip pools, William D. Orndorff, Ellen Crowder, 7 July 2018, 6♂ 7♀ (USNM 1622331).



Figure 2. *Caecidotea burkensis*, new species, paratype males, length approximately 8mm, from Lawson Cave, Tazewell County, Virginia.

The holotype is an 8.5mm ♂ from the collection of 6 October 2019 (USNM 1622328), all other specimens cited above are paratypes. The material examined has been deposited in the National Museum of Natural History, Smithsonian Institution, Washington, D.C. Of note, Fleming labelled specimens as holotype and allotype in the 1963 collection, but never published a description, thus, these specimens have no valid taxonomic status beyond that of paratypes established herein.

Diagnosis.—Within the *forbesi* Group, *C. burkensis* is morphologically most similar to *C. racovitzai racovitzai*. The two species are readily separated by the stygobiont morphology of *C. burkensis*, which possesses a more elongate body, >3X as long as wide, as compared to <3X in *C. r. racovitzai*, e.g., 2.7X in holotype (Williams, 1970), more elongate uropods, and eyes and pigmentation vestigial or absent. Pereopod 1 propodus is over 1.7X as long as wide and subovate in *C. burkensis*, 1.3X and subtriangular in *C. r. racovitzai*. Pleopod 1 protopod of *C. burkensis* has four retinaculæ vs. two in *C. r. racovitzai*.

Description—Light, dorsal magenta pigmentation visible under low magnification in large adults, most prominent in ovigerous females, most concentrated on anterior surface of head; under higher magnification (200X) pigment appears diffuse and in a somewhat reticular pattern. The isopods appear whitish to the naked eye (Figure 2). Eyes absent, vestigial as unformed magenta smudges of pigmentation, or up to 7-8 ocelli (Table 1), most individuals with pigmented ocelli possess 2-5 of irregular shape and size. Longest male 8.5 mm, female 6.5 mm, ovigerous females from 4.9-6.3 mm; body approximately 3.3X as long as wide. Margins of head, pereonites and pleonites moderately setose, post-mandibular lobes well produced. Pleotelson slightly longer than wide, caudomedial lobe moderately produced.

Antenna 1 of 9-10 articles, distal 3-4 articles each with single esthetes (Figure 3). Antenna 2, distal article of peduncle approximately 1.8X length of penultimate article, flagellum to 80 articles. Mouthparts per the diagnosis of the genus (Lewis, 2009b).

Pereopod 1, propodus of male approximately 1.75X as long as wide, palmar margin with two stout, elongate proximal spines, medial triangular process reaching to dactyl flexor margin, distal process absent; dactylus with row of stout

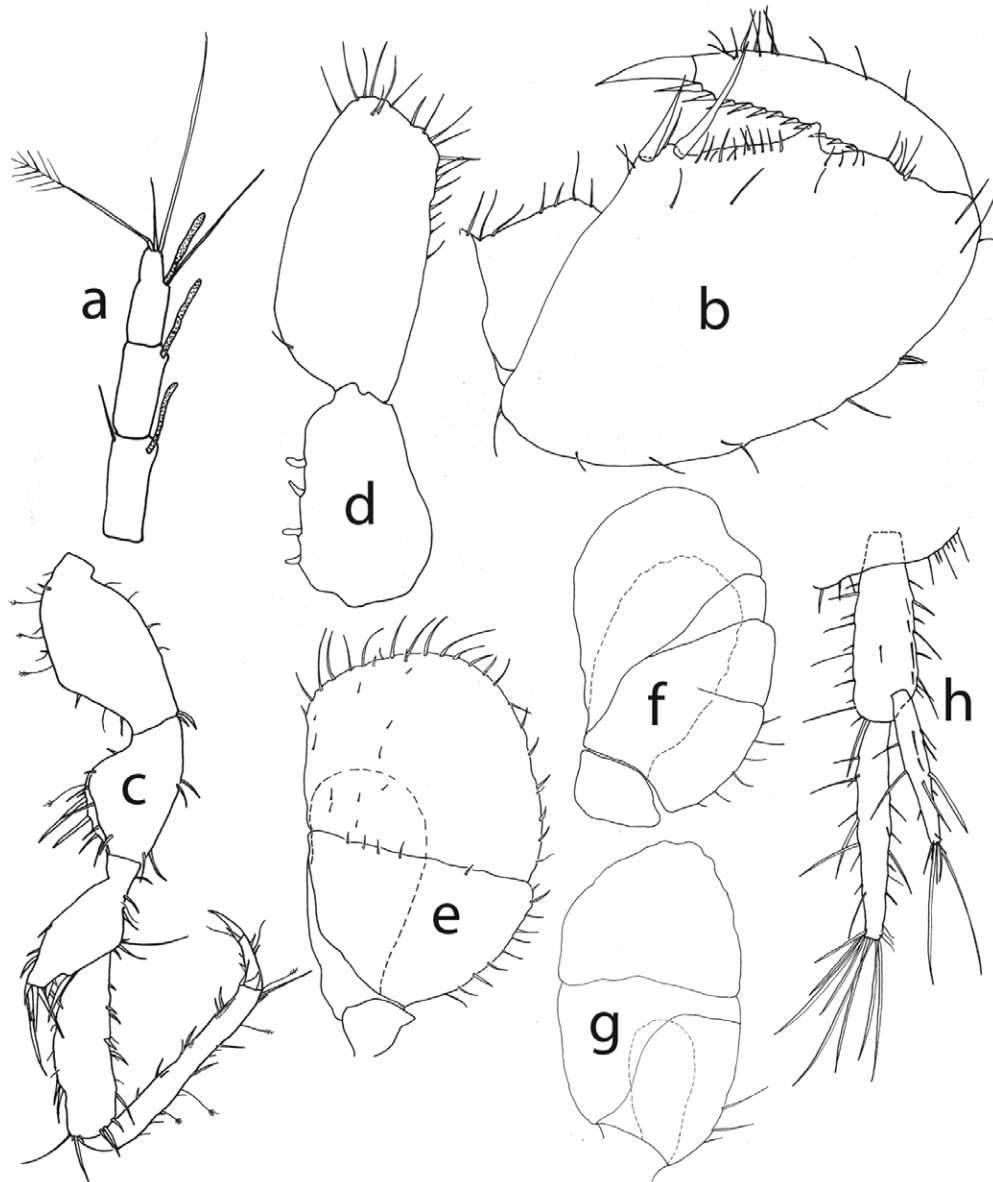


Figure 3. *Caecidotea burkensis*, new species, Lawson Cave, Tazewell County, Virginia, paratype male: (a) antenna 1 distal articles of flagellum; (b) gnathopod; (c) pereopod 4; (d) pleopod 1; (e) pleopod 3; (f) pleopod 4; (g) pleopod 5; (h) uropod.

article, with approximately 15 elongate plumose setae along margins (Figure 4). Endopod narrow, sides subparallel, small rounded basal apophysis, tip with three processes: (1) cannula conical, conspicuous, distally truncate, endopodial groove prominent adjacent to mesial process; (2) mesial process well-developed, obscuring base of cannula, curved mesiad, tapering to a point; (3) caudal process large, sclerotized, scallops on caudal surface, terminating in acute apex, small spine-like structures present along lateral margin.

Pleopods 3-5 per Figure 3; pleopod 4 exopod with sigmoid false suture, about 6 setae present along proximolateral margin; pleopod 5 with about 3 setae along proximolateral margin.

Uropod subequal in length to pleotelson, protopod about 0.8X length of endopod, exopod about 0.75X length of exopod (Figures 2, 3).

Etymology.—The name *burkensis* is a contraction of Burke + ensis, a Latinization referring to Burke's Garden, the site of the type-locality of the species. The suggested vernacular name is the Burke's Garden cave isopod.

Relationships.—*Caecidotea burkensis* and *C. racovitzai* share many morphological similarities suggesting a close evolutionary relationship. At the time of the description of the species, Williams (1970) divided *Caecidotea racovitzai*

spines along flexor margin, increasing in size distally (Figure 3). Pereopod 4 the shortest of the 6 walking legs (Table 2), carpus of male 2.8X as long as wide, carpus of female more slender, 3.1X as long as wide.

Pleopod 1 slightly longer than pleopod 2, protopod sub-rectangular, approximately 0.75X length of exopod, 4 retinaculae; exopod sub-rectangular, approximately 2.0X as long as wide, tapering slightly distally, mesial and lateral margins slightly convex, single proximomesial seta, distal half of lateral margin and distal margin with short to moderately long non-plumose setae, longest setae at apex (Figure 3).

Pleopod 2, protopod quadrate, about 1.3X as long as wide, 2 setae present on mesial margin proximal to insertion of endopod; exopod about 1.1X length of endopod, proximal article with 3 lateral setae, distal article ovate, about 2.3X length of proximal arti-

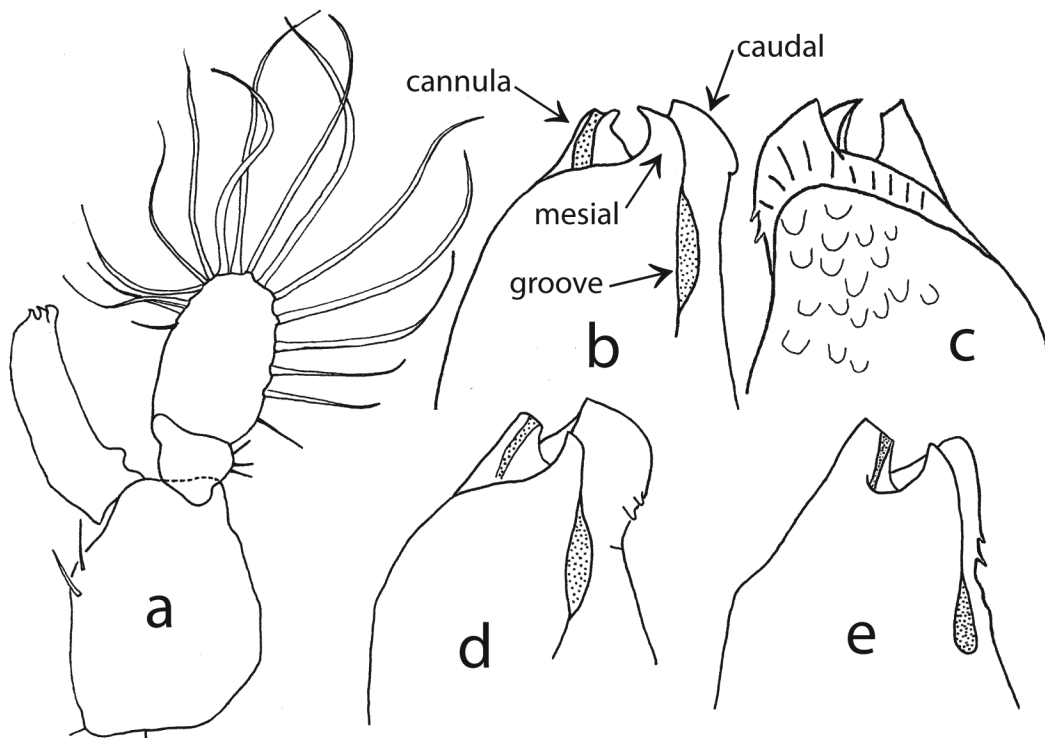


Figure 4. *Caecidotea burkensis*, new species, Lawson Cave, Tazewell County, Virginia, paratype male: (a) pleopod 2; (b) pleopod 2 endopodite tip, anterior; (c) same, posterior; *Caecidotea racovitzai racovitzai*, Cauliflower Cave, Tazewell County, Virginia: (d) pleopod 2 endopodite tip, anterior; Golf Course Spring, Smyth County, Virginia: (e) pleopod 2 endopodite tip, anterior.

vitzei racovitzai was described by Williams (1970) as being 1.3X as long as wide and subtriangular in shape, whereas in *C. burkensis* the gnathopod propodus is over 1.7X as long as wide and subovate. The palmar margin in both species has stout proximal spines and a triangular median process, but this process exceeds the width of the dactylus in *C. racovitzai*, whereas it is less than half that in *C. burkensis*. The appendages of *C. burkensis* are more slender and elongate than in *C. r. racovitzai*, as demonstrated by the index of length vs. width in the carpus of pereopod 4 (Table 2).

The first pleopod of both species is similar in general shape and appearance. The protopod of *C. burkensis* has twice the number of retinaculæ (four vs. two described in *C. r. racovitzai*). Both species also have in common a single seta near the proximomesial margin, and similar setation along the apical and lateral margins. Likewise, the second pleopod protopod of each species possesses two distomesial setae. Both possess an exopod proximal article with several short setae along the lateral margin, and the distal article is ovate with elongate plumose setae along all but part of the proximomesial margin. The second pleopod endopod has a rounded basal apophysis. The endopod tip of both species has three processes quite similar in appearance (Figure 4): (1) cannula conical, not exceeding caudal process in length; (2) mesial process acutely pointed, curved mesiad, about equal in length to cannula; (3) caudal process larger and slightly longer than the other processes, ending in distal apex. Williams (1970) illustrated a variety of pleopod 2 endopodite tips that suggests that differences in the relative size of the processes is variation between populations of *C. r. racovitzai*.

Lewis et al. (2020) indicated reliance on these conventional morphological characteristics to separate asellid species significantly underestimated diversity in the *Lirceus hargerii* Group. This is also the case with *C. burkensis* and *C. r. racovitzai*, particularly considering their morphologically similar pleopod 2 endopodite tips (Figure 4, b, d, e). Separating species in the *Lirceus hargerii* Group required using other morphological characteristics, including troglomorphisms such as the reduction of eyes and pigmentation, and elongation of the body and appendages. In the case of *C. burkensis*, as compared to *C. r. racovitzai*, the eyes are vestigial or absent, and the dorsal pigmentation is absent or greatly reduced. The body of *C. burkensis* is more elongate, as are the pereopods and uropods.

In addition to the morphological inference, molecular analysis shows divergence greater than 0.16 substitution per site in the COI gene, as measured by patristic distances, between *C. burkensis* and the populations tested of *C. racovitzai racovitzai* and *C. r. australis* (Douady, C.J. and Malard, F., unpublished data). This strongly suggests three species are present (Lefébure et al., 2006).

into two subspecies: (1) *C. r. racovitzai*, occurring from Indiana east to Virginia, and through most of the Great Lakes, north-eastern U.S. and adjacent Canada; and (2) *C. r. australis*, occurring in Florida and southern Georgia. To eliminate confusion if these subspecies are elevated to species at a later date, herein only *C. r. racovitzai* is considered. Lawson Cave is located near the southern edge of the range of this subspecies (Figure 1).

The key aspects of the morphology employed to characterize species of *Caecidotea* are structures of the gnathopod (first pereopod), first and second pleopods, and uropods. Examining first the propodus of the gnathopod, that of *C. raco-*

Table 1. Variation in presence or absence of eye vestiges in *Caecidotea burkensis*.

Habitat	Gender	Ocelli absent, no pigmentation	Ocelli absent, magenta pigmentation	Ocelli present
Stream 7 July 2018	Males	6	8	4
	Females	11	8	3
Pools 7 July 2018	Males	0	5	1
	Females	1	1	5
Stream 6 October 2019	Males	12	1	1
	Females	10	1	1
	Total	40	24	15

Table 2. Comparison of approximate length of carpus (measured in micrometers) of pereopods 1-7 of *Caecidotea burkensis* and *Caecidotea racovitzai racovitzai*, and index of length versus width of pereopod 4 carpus in males and females. Measurement of the carpus is indicative of the total relative length of the legs, with pereopod 4 frequently being the shortest walking leg, modified in the male for grasping during copulation.

Carpus of ♂ pereopod: (length in micrometers)	1	2	3	4	5	6	7	Pereopod 4, Length/Width
<i>Caecidotea burkensis</i> (8.1 mm ♂; 6.5 mm ♀) Lawson Cave, Tazewell Co., Va.	50	70	80	60	88	103	123	♂ 2.8X
								♀ 3.1X
<i>Caecidotea r. racovitzai</i> (11.8 mm ♂; 7.0 mm ♀) Golf Course Spring, Smyth Co., Va.	58	83	88	68	98	125	135	♂ 2.1X
								♀ 2.5X

Table 3. Sites where syntopy of asellids occurs in southwestern Virginia.

Site	<i>C. recurvata</i>	<i>C. richardsonae</i>	<i>C. nickajackensis</i>	<i>L. usdagalun</i>	<i>L. culveri</i>	<i>L. undescribed spp.</i>
Flanary Bridge Springs (Lee Co.)	X			X		X
Olinger Cave (Lee Co.)	X	X				
Smiths Milk Cave (Lee Co.)	X	X				
Surgener Cave (Lee Co.)	X			X		
Thompson Cedar Cave (Lee Co.)	X			X		
Blair-Collins Cave (Scott Co.)	X	X				
Carters Pit (Scott Co.)	X				X	X
Coley No. 2 Cave (Scott Co.)	X	X				X
Lane Cave (Scott Co.)	X		X			X
McDavids Cave (Scott Co.)	X				X	
Fallen Rock Cave (Tazewell Co.)		X				X
Stonley Cave (Tazewell Co.)		X	X			

Distribution and ecology.— The location and a description of the type-locality, Lawson Cave, was presented by Holsinger (1975). The cave is entered via a karst window allowing egress to passages both upstream and downstream. The upstream segment is comprised of about 60 m of passage, truncated by a siphon prohibiting further exploration. Upstream from this siphon is another short section of passage termed here the Lawson Insurgence Cave, where the water from a surface stream sinks. Most of the enterable cave passage is on the downstream end of the karst window,

extending for about 380 m before another siphon is encountered. The passage is relatively large, ranging from 7-12 m in width, and mostly 1-3 m in height. The entire length of the passage is occupied by the stream, with riparian mud-banks on either side. The stream is believed to resurge at Fish Spring, located about 1500 m to the west. On the visit to Lawson Cave in October 2019, the surface stream flowing across the floor of the karst window was searched, but no isopods were found. The search continued downstream, where *C. burkensis* was found in the dark zone of the cave, where the isopods occurred on sticks and logs washed in from the karst window.

The collection of 7 July 2018 contained three ovigerous females, measuring approximately 4.9, 6.0 and 6.3 mm in length. The 6 October 2019 collection had one ovigerous female approximately 5.8 mm in length. The brood pouch was not dissected, but under visual inspection appeared to contain 22 embryos.

***Caecidotea racovitzai racovitzai* (Williams, 1970)**

Material examined.—DISTRICT of COLUMBIA: Potomac River below Aqueduct Bridge, W.P. Hay, 15 March 1896, 24♂♀ (holotype USNM 122066, allotype USNM 122067, paratypes USNM 122068); OHIO: Lucas County: stream in old locks, Side Cut Metropark, Maumee, Salisa L. Lewis, Julian J. Lewis, 9 August 2017, 1♂. VIRGINIA: City of Bristol: spring, Old Abingdon Highway, Julian J. Lewis, Charles D.R. Stephen, 10 October 2019, 1♂1♀. Smyth County: I-81 Cave, 2.8 miles southwest Marion, Thomas E. Malabad, Katarina Kosič Ficco, 10 August 2018, 12♂♀; Golf Course Spring, 1.4 miles southwest Marion, Thomas E. Malabad, Katarina Kosič Ficco, 10 August 2018, 10♂♀; Tazewell County: Cauliflower Cave, Thomas E. Malabad, Katarina Kosič Ficco, 14 October 2018, 1♂. WASHINGTON: King County: Boeing Creek, Boeing Creek Park, Seattle, Julian J. Lewis, 8 June 2018, 15♂♀; Green Lake, Seattle, Julian J. Lewis, 6 June 2018, 10♂♀; East Fork Issaquah Creek, High Point, Julian J. Lewis, Victor M. Lewis, 10 June 2018, 10♂♀.

The specimens of the type series of *C. r. racovitzai* reside in the collection of the Smithsonian Institution. Under examination the isopods have the robust appearance typical of epigeal *Caecidotea*, i.e., broad habitus, distinctly pigmented, prominent eyes, with stout appendages. The type-locality, the Potomac River on the Virginia side of the Aqueduct Bridge (Washington metro area) was visited in December 2019. No isopods were found, but many specimens collected from an area about 5 km northwest around the Chain Bridge are present in the Smithsonian collections and there is little doubt the species still exists in the area.

The isopod from Cauliflower Cave, Virginia is troglomorphic with vestigial eyes and pigmentation. It is tempting to identify this isopod as *C. burkensis* due to the morphological similarities and geographic proximity, with only 17 miles (27 km) separating Cauliflower and Lawson caves. However, Cauliflower Cave occurs in Thompson Valley, an adjacent mountain cove south of Burke's Garden, and moreover, is in a different river basin. The water in Cauliflower Cave drains to the Clinch River, part of the Tennessee River drainage, whereas Lawson Cave occurs in the New River basin. With only a single specimen too little is known at this time to further assess this intriguing isopod population.

As noted by Williams (1970), a population of *C. r. racovitzai* occurs in the vicinity of Seattle, Washington, where it was presumably introduced from the eastern U.S., perhaps transported with commercially available water lilies or some similar vector.

***Caecidotea racovitzai australis* (Williams, 1970)**

Material examined.—FLORIDA: Jackson County: Russ Mill Creek at Union Road Bridge, 4.3 miles NNE Cottdale, Julian J. Lewis, Charles D.R. Stephen, 9 January 2018, 22♂♀; Leon County: Gopher Hole, Leon Sinks Geological Area, from sticks in entrance room pool, Julian J. Lewis, Charles D.R. Stephen, 10 January 2018, 13♂♀; Wakulla County: Wakulla River, Wakulla Springs State Park, Julian J. Lewis, Charles D.R. Stephen, 10 January 2018, 15 ♂♀.

The isopods from Gopher Hole (a cave) appear mildly depigmented and genetic sequencing also revealed divergence from local populations of *C. r. australis* greater than 0.16 substitution per site in the COI gene (Douady C.J. and Malard F., unpublished data).

***Caecidotea cf. nickajackensis* Packard (1881)**

Material examined.—VIRGINIA: Scott County: Lane Cave, 7 miles southwest of Snowflake, William D. Orndorff, 12 October 2019, 18♂♀. Tazewell County, Stonley Cave, 5.2 miles northeast of Tazewell, Thomas E. Malabad, William D. Orndorff, 10 January 2019, 11♂♀.

Described by Packard (in Cope and Packard, 1881), this species was considered extinct after the type-locality, Nickajack Cave, was inundated by a TVA lake. Lewis (2009a) redescribed the species from a specimen collected prior to the creation of the lake. Coleman and Zigler (2015) discovered three new localities in southeastern Tennessee and adjacent northeastern Alabama. Despite the morphological similarities, we are dubious the Virginia populations are conspecific with those in the vicinity of the type-locality in southeastern Tennessee given the separation of over 320 km, but for the moment these are considered the first collections of *C. nickajackensis* in Virginia. The records are presented to allow inclusion in the discussion and Figure 1.

Biogeography and evolution of southwestern Virginia groundwater asellids

There are four species groups of stygobiont asellids occurring in caves in southwestern Virginia (Fig. 1). These are: (1) *Lirceus harger* Group (Lewis 2020 et. al), (2) *Caecidotea stygia* Group (Steeves, 1963; Lewis, 1988), (3) *Caecidotea cannula* Group (Steeves, 1966; Lewis 1980, 2009b), and (4) *Caecidotea forbesi* Group (Lewis, 2013). Collaborating with C. Douady, F. Malard and L. Konecny-Dupré (Université Lyon-1), work in progress on the molecular phylogeny of subterranean asellids suggests these species groups are monophyletic. Considering first the *Lirceus harger* Group (Lewis, et. al, 2020), the species of this group are nearly ubiquitous spring inhabitants in the upper Tennessee River basin. In the intermontane karst valleys of southwestern Virginia the *harger* Group comprises the most species-rich assemblage of asellids in North America. Six stygobiont species are troglomorphic to varying degrees, ranging from possessing vestigial eyes and pigmentation to complete absence (Holsinger and Bowman, 1973; Estes and Holsinger, 1976; Lewis and Lewis, in preparation). These subterranean species appear to be the results of localized groundwater invasions of related species, examples of which can be seen in the process in places like Speers Ferry or Lane caves (Scott County), or Young-Fugate Cave (Lee County).

Four species of the *stygia* Group occur in southwestern Virginia: *Caecidotea richardsonae*, *C. recurvata*, *C. Cumberlandensis* and *C. nickajackensis* s. latu. All species of the *stygia* Group are obligate inhabitants of caves or other groundwater habitats, with only one midwestern species (*C. beattyi*) retaining vestigial pigmentation in evidence of an ancestral epigeal derivation. The four Virginia species occur in caves located along the Powell, Clinch and Holston rivers (headwater streams of the Tennessee River). Except for one isolated case involving a stream capture, there are no records of any of these species extending northward into the drainage of the New River.

Looking next at the *cannula* Group, all species are stygobionts restricted to the Appalachians. In southwestern Virginia, the *cannula* Group is represented in the Tennessee River drainage by *C. incurva* (*C. nortoni* occurs in adjacent northeastern Tennessee). *Caecidotea incurva* also occurs to the north in the New River basin, along with *C. henroti* and *C. vandeli* (plus *C. holsingeri* in West Virginia). *Caecidotea incurva* is restricted to caves west of the course of the modern New River, while *C. vandeli* is restricted to caves east of the channel. *Caecidotea henroti* is only found in caves within a few kilometers of the river, but on either side.

The last group to be considered is the *forbesi* Group, with only two described subterranean species, *C. burkensis* in Virginia and *C. insula* in a cave on South Bass Island in Lake Erie, Ohio. Both species were derived from obvious epigeal ancestors in geographic areas so isolated no other asellids presented any competition. In Virginia, the dearth of aquatic cave isopods extends beyond Burke's Garden to an entire sub-basin of the New River, where there exists only one record of *C. henroti*. The sub-basin encompasses the drainages of Wolf, Cove and Laurel creeks, on the west side of the present-day river channel and north of the Pulaski Fault. Numerous caves occur in this area with abundant, well-sampled and seemingly suitable aquatic habitat, making the lack of stygobiontic asellids puzzling.

Syntopy

The species richness of asellids in southwestern Virginia groundwater habitats has led to discovery of several instances of syntopy, with as many as three asellid species in the same site (Table 3). Estes (1978) demonstrated niche partitioning between *Lirceus usdagalun* and *Caecidotea recurvata* in Surgener Cave (Lee County) based on water velocity and depth ratios. Stygobiont *Lirceus* species exist in high energy streams where the isopods grasp on bare limestone substrates in waterfalls where *Caecidotea* is more likely to wash out during high flows as demonstrated in flume studies (Culver, 1973). A third species, *C. richardsonae*, occurs in Smiths Milk Cave, an upstream subterranean tributary to the Surgener-Gallohan System.

Springs, as ecotones connecting subterranean and epigeal habitats, are excellent sites for syntopy to occur. For example, at Flanary Bridge Springs in Lee County, three asellids occur. In the modest spring orifice *Lirceus usdagalun* and *Caecidotea recurvata* can be found on the same piece of stone, along with a site-endemic undescribed epigeal species of *Lirceus*.

SUMMARY AND CONCLUSIONS

Caecidotea burkensis is the second troglomorphic species of the *forbesi* Group to be described. Belonging to an assemblage of primarily epigeal species, *C. burkensis* retains significant plasticity in the presence of vestigial eyes and pigmentation. Two other troglomorphic populations of the *forbesi* Group in Florida caves remain undescribed. *Caecidotea burkensis* is one of over a dozen recognized stygobiont asellids inhabiting southwestern Virginia and the fringes of adjacent states. The presence of *C. burkensis* in Burke's Garden occurs immediately to the north of the termination of the ranges of the *Caecidotea stygia* and *Lirceus harger* groups in the upper reaches of the Tennessee River tributaries. The *Caecidotea cannula* Group is also locally absent. High biodiversity attracts attention from those striving for conservation or endeavoring to understand the intricacies of species interactions, and the asellid fauna of southwestern Virginia does not disappoint. In a region with the highest asellid species richness in North America, of equal interest

are isolated caves where only single species occur. Examples are *Lirceus culveri* in McDavids Cave (Rye Cove, Scott County) or *C. burkensis* in Lawson Cave (Burke's Garden, Tazewell County). Southwestern Virginia is replete with opportunities for future work.

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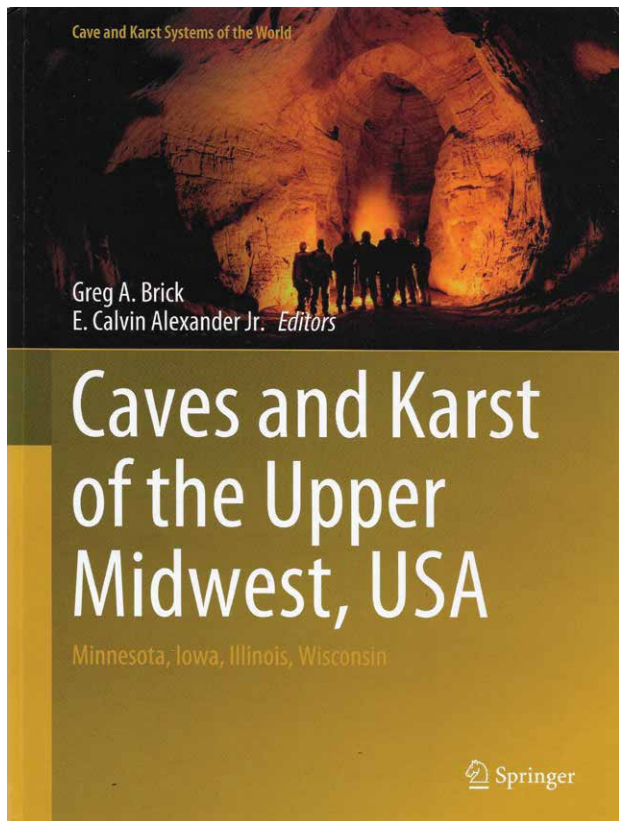
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Caves and Karst of the Upper Midwest, USA: Minnesota, Iowa, Illinois, Wisconsin

Greg A. Brick and E. Calvin Alexander Jr., editors, 2021. Springer Nature Switzerland AG, Cave and Karst Systems of the World series; 314 p., 8.3 × 11 inches; ISBN 978-3-030-54632-8 (hardcover), ISBN 978-3-030-54633-5 (e-book). Hardcover \$137.99 retail, USA. Kindle version \$119.99 from Amazon.

A recent addition to Springer's extensive series on *Cave and Karst Systems of the World*, this is the first comprehensive book that covers the karst of the Upper Midwest, including the caves and karst of Minnesota, Iowa, Illinois and Wisconsin. This is a low-relief region, most of which has undergone serious glaciation, and it receives relatively little attention in the karst literature. However, it contains hundreds of significant caves, including several that are open to visitors. Some have been the focus of significant historical and scientific studies. The well-known geologist J Harlen Bretz (no period after the "J") lived in the area and in 1961 co-authored a book on Illinois caves, published by the Illinois Geological Survey, which contained some innovative hypotheses about their origin. David Morehouse, who made many studies of Iowa caves in the 1960s, was one of the earliest to document cave origin by sulfuric acid derived from the oxidation of local sulfide minerals.

The major caves of these four states are described and interpreted in this book, along with their human histories, some of

which are quite odd. The longest in the area is Iowa's Coldwater Cave, with a surveyed length of 17 miles. Much of its length has been mapped beyond upstream sumps. Mystery Cave, Minnesota, contains at least 12.8 miles of maze-like passages between meanders in the Root River. Much of the cave requires climbing through slippery, muddy fissures in cold, wet conditions. However, easily accessible dry sections are open to tours as part of the Forestville/Mystery Cave State Park. Fogelpole Cave, in Illinois, is a significant biological refuge containing at least 12 miles of passages.

The first chapter covers the regional geology with a map and geologic column for all four states that identify the Paleozoic bedrock stratigraphy which host the karst. Significant aspects of each stratigraphic system are described. Some unique paleokarst features are introduced, as well as Mississippi Valley Type lead and zinc ore deposits that are associated with the carbonates and considered an integral part of the karst. Carbonate and evaporite karst are clearly distinguished, as are the relation of the karst to patterns of glaciation. A general summary of Pleistocene glacial history is provided. Caves and karst in each state are covered in detail, along with their history of exploration, interpretation of potential hypogenic processes, and the impact of glaciation on karst.

Chapter 2 deals with the long, complex, and at times rather unusual history of cave exploration and the development of commercial tours. Some of the smallest caves have had the most unusual histories.

Chapters 3–6 describe the karst and caves of the four states in the area, each presented by appropriate specialists, including both editors and other well-recognized speleologists (Michael Lace, Raymond Anderson, Patricia Kambesis, Samuel Panno, Donald Luhman, Joseph Devera, and Michael Day).

Chapter 7 concerns lead-zinc ores and their relation to karst (James Dockal). Because the Mississippi Valley Type ore deposits are so important economically and historically for the area, there is a chapter dealing with the fissures created by the oxidation of sulfide-related ore minerals to produce sulfuric acid. Some of the resulting crevices are several kilometers long. A detailed discussion of the formation of the ore and then its oxidation to form the fissures is given.

Chapter 8 considers "paradigmatic studies in Midwestern cave science" by co-editor Greg Brick. Several basic concepts are examined (e.g., *karst* and *speleogenesis*) that are ordinarily taken for granted. This chapter shows how these concepts have changed with time.

Chapter 9 examines the cave fauna of the region, with a contribution by Stewart Peck, a speleobiologist who has been active since the 1950s, and Julian Lewis, and John Whitaker. This chapter covers invertebrates, such as amphipods, spiders, and crickets; as well as vertebrates such as bats, packrats, fish and salamanders. Their distribution and origins are discussed, including their fate during the Pleistocene.

Chapter 10, by Kevin Erb and Benjamin Maas, considers the interaction between agriculture and karst, including the impact of karst on this important agricultural area – and vice versa. Case studies are given that relate to the susceptibility of the karst to contamination by fecal waste and fertilizer, and how to limit their effects.

Chapter 11, by Jeffrey Dorale, describes paleoclimate studies based on caves of the upper Midwest. Many important paleoclimate studies have been made in Midwestern caves - dating speleothems, especially stalagmites and then determining their carbon isotopes, which data can be used to interpret past climate conditions. Early paleoclimate studies in the U.S. included analyses of speleothems from Iowa's Coldwater Cave. Methods of studying speleothems are described, along with significant findings. A reminder is made that speleothems are fragile and non-replaceable, and collections should be very conservative and kept to a minimum.

The book is well illustrated with a map or photo on almost every page. Most images and maps are in color. The printing (at least in the review copy) is on non-glossy paper, so that color images lack sharpness, although they still convey the appropriate information. The many diverse topics in the book are well organized. All authors are specialists in their topics, and their work is carefully written. It covers an important, but often overlooked, karst area, and introduces thought-provoking topics that apply to all karst areas. It will be of special interest to those who are concerned about the overall character of karst in the United States. Knowledgeable authors and depth of coverage make it a strong addition to any speleological library.

Reviewed by Margaret V. Palmer, NSS 23685HM, 619 Winney Hill Rd., Oneonta, NY 13820, January 1, 2021.

GUIDE TO AUTHORS

The *Journal of Cave and Karst Studies* is a multidisciplinary journal devoted to cave and karst research. The *Journal* is seeking original, unpublished manuscripts concerning the scientific study of caves or other karst features. Authors do not need to be members of the National Speleological Society, but preference is given to manuscripts of importance to North American speleology.

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