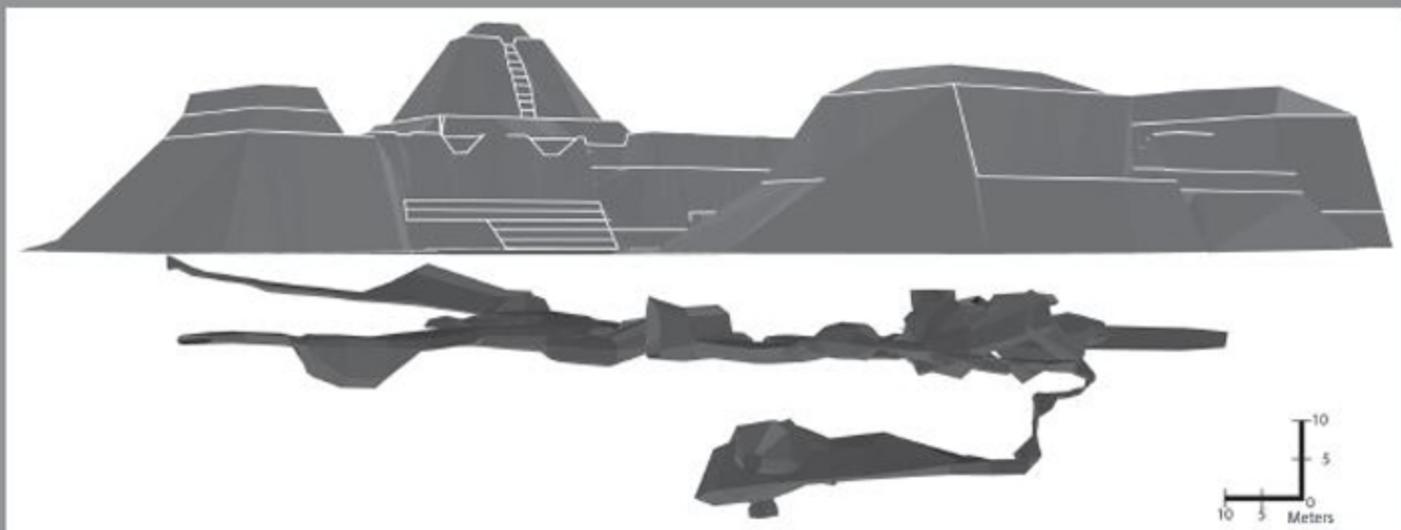


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Front cover: Graphic showing the cave passage profile under the remains of the Maya site of Xcoch. See Weaver et al. in this issue.



# CLUTCHES, NEST ATTENDANCE, AND HATCHING IN A ROCK-CAVE-DWELLING FROG, *ELEUTHERODACTYLUS (SYRROPHUS) ZEUS*, FROM CUBA

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EGLIS L. TORRES MARTÍNEZ<sup>3</sup>

**Abstract:** The Cuban archipelago hosts at least thirteen endemic species of the genus *Eleutherodactylus* that live in association with rocks, caves, or limestone landscapes. However, the reproductive biology of Cuban rock-cave dwelling frogs has received little attention. In this contribution, we offer the first report of oviposition, clutch size, nest site locations, and hatching in *Eleutherodactylus (Syrrophus) zeus*. Clutches of this species in different development stages were sighted in rock depressions and crevices inside of the Gran Caverna de Santo Tomás, located in Sierra de Quemados, Viñales, Pinar del Río province. Clutch sizes ranged from 48 to 74 eggs, and hatching success was 98% for one of them. Moreover, we document the first report of nest attendance by a species of the subgenus *Syrrophus*, the strategy of which may be biparental, with either male or female parental care. Our findings are important not only in understanding general life-history strategies, but also in understanding particular strategies for an endemic species whose habitat specialization is associated with an at-risk system.

## INTRODUCTION

The genus *Eleutherodactylus*, with more than 180 described species, is one of the largest genera of vertebrates. The species of this genus are the dominant terrestrial anuran component of most Caribbean-island forests, and most of these species lay terrestrial or arboreal eggs that undergo direct development into miniature froglets (Henderson and Powell, 2009). *Eleutherodactylus* species have experienced extensive island radiations with subsequent morphological, behavioral, and ecological diversification, colonizing a wide variety of microhabitats (Hedges et al., 2008). Three ecomorphs have been proposed for Caribbean species of *Eleutherodactylus*: aquatic or semiaquatic, rock-cave, and bromeliad associations (Hedges, 1989). At least thirteen Cuban endemic species of *Eleutherodactylus* (subgenus *Euhyas*: *E. blairhedgesi*, *E. etheridgei*, *E. guanahacabibes*, *E. bresslerae*, *E. greyi*, *E. klinikowskii*, *E. pezopetrus*, *E. michaelschmidi*, *E. pinar-ensis*, *E. thomasi*, *E. tonyi*; subgenus *Syrrophus*: *E. symingtoni* and *E. zeus*) are saxicolous, cavernicolous, or live in association with rocks, caves, or limestone landscapes (Díaz et al., 2007; Henderson and Powell, 2009). Most are known to occur in semidarkness at or near the entrance or in absolute darkness deeper in caves.

Both Cuban endemic species of subgenus *Syrrophus*, *E. symingtoni* and *E. zeus*, are large, have short vomerine denticerous processes, and share distinctive canthal tubercles (Schwartz, 1958; Hedges et al., 2008). They have become adapted to life in caves, rock crevices, and other sheltered sites in limestone landscapes associated with

forest habitats (Henderson and Powell, 2009). Unlike the first species, *Eleutherodactylus (Syrrophus) zeus* can be locally common in the Sierra del Rosario and the Sierra de Los Organos, in the Cordillera de Guaniguanico in Western Cuba. It has crepuscular to nocturnal activity, but active individuals have been sighted inside the caves during the day (Estrada et al., 1986). According to Alonso et al. (2007), adults of both sexes vocalize at night from the ground and rocks inside and outside caves. Males produce at least three different types of calls, including advertisement and distress calls (Díaz and Cádiz, 2008). Until now, the breeding activities, egg deposition sites, clutch size, and parental care were unknown. This contribution is the first account of the reproductive behavior and clutch characteristics in a Cuban giant frog, *Eleutherodactylus (Syrrophus) zeus*, for which we describe four clutches of eggs, nest sites, and presence of parental care from a karstic locality in western Cuba.

## MATERIALS AND METHODS

All the surveys were performed during the daytime. The observations were made in two areas inside of the Gran Caverna de Santo Tomás, located in Sierra de Quemados,

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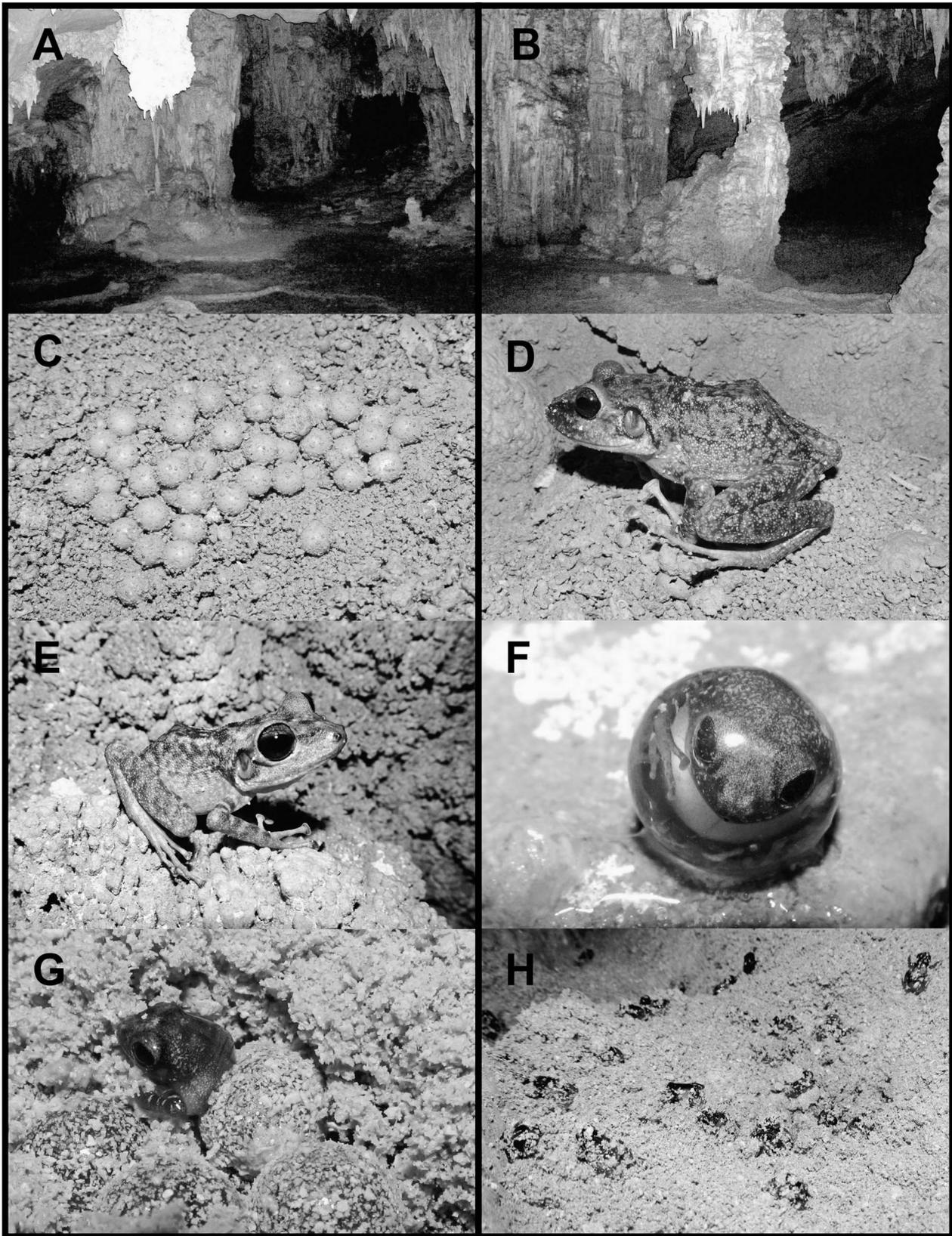


Figure 1. A. Las Perlas area in the Gran Caverna de Santo Tomás, Viñales, Pinar del Río province, Cuba. B. Incognita area, also in the Gran Caverna de Santo Tomás. C. First clutch sighted in Las Perlas, with eggs in three or four developmental stages. D. *Eleutherodactylus zeus* female attending eggs. E. *Eleutherodactylus zeus* male near the first clutch. F. Developing

Viñales municipality, Pinar del Río province, Cuba (83°50'37.024" W; 22°32'42.863" N). This is one of the larger cave systems in Cuba, with more than 46 km of explored galleries. It exhibits a great diversity of secondary karstic formations, some of them uncommon in Cuba (Luis, 2000). Using headlamps we inspected the roofs, walls, and floors in the entrance, semidark, and dark zones.

We measured the air temperature ( $\pm 0.5^\circ\text{C}$ ) and relative humidity ( $\pm 5\%$ ) using a thermohygrometer (HANNA Instruments). The animals were captured by hand and their snout-vent lengths were measured to the nearest 0.1 mm using calipers. After this procedure, all were released at the site of collection.

## RESULTS

We found two clutches in different stages of development in the Las Perlas area (Fig. 1A), Gran Caverna de Santo Tomás, Viñales, Pinar del Río, Cuba, at 17:20 on 1 May 2014. The following day at 15:25 we discovered a third nest in the Incógnita area (Fig. 1B), also inside the same system.

The first clutch was in total darkness, 20 to 30 m from the nearest entrance of the cave, in the soil of a rounded crevice. The clutch of forty-eight white-cream eggs, one measuring 6.5 mm in diameter, was among granules of secondary karstic formations approximately one millimeter in diameter (Fig. 1C). An undisturbed adult female *E. zeus* (93.4-mm snout-vent length) was sitting on the nest (Fig. 1D). A male conspecific (67.6-mm SVL; Fig. 1E) was sitting 1 m away. Air temperature measured near the site of oviposition was  $20.8^\circ\text{C}$ , and the relative humidity 88.3%.

We located the second clutch 5 m from the first nest, inside a 22-cm deep depression. Unlike the eggs of the first nest, eggs of this nest were covered by very moist soil. The clutch contained fifty-four large eggs, one measuring 9.5 mm in diameter, with completely pigmented and developed embryos that exhibited considerable mobility (Fig. 1F). Within a few minutes of discovery, fifth-four of the eggs (98%) hatched (Fig. 1G), and the froglets, one measuring 11.6-mm SVL, dispersed quickly along the ground to approximately 1 m from the nest (Fig. 1H).

The next day, we detected a third clutch in a very early stage of development in the Incógnita area in a dark zone approximately 120 m from the entrance. The nest was located on a mound 5 cm above the ground. All sixty-four creamy-brown eggs, one measuring 6.3 mm in diameter, were completely covered with substrate. In order to estimate the clutch size and to measure one of the eggs, we had to temporarily remove an adult individual thought

to be a male (67.7-mm SVL) that was positioned along the edge of the nest. Upon release at the site of capture, the frog emitted low, brief, and discontinuous vocalizations. When we inspected the site approximately one hour later, the male was at the same position and location.

In March 2010, one of us (LYG) had found a clutch of similar design and construction as those found in 2014, also deposited on a small mound 10 cm above the ground in complete darkness in Las Perlas area. The nest, also covered by soil, contained seventy-four yellow-cream spherical eggs in an early developmental stage, adhered together to form a semicircular group. Air temperature measured inside the cave was  $20.6^\circ\text{C}$  and relative humidity was 97.4%. The closest individual detected was an adult female *E. zeus* 1.1 m above the nest on the nearest wall.

## DISCUSSION

The intraspecific variation in eggs and clutch size has been studied in several species of anurans. Such variation is evident in comparisons among populations, among individual females in the same populations, and even within clutches of eggs produced by individual females (Wells, 2007). A positive correlation between clutch size and female body size has been reported for some species of *Eleutherodactylus* (Crump, 1974; Townsend and Stewart, 1994). The clutch-size differences among the four described nests of *E. zeus* could be a result of the variability in female size; females up to 127.4 mm in snout-vent length have been measured (García, 2012). However, in our largest clutch of seventy-four eggs we do not discard the possibility of communal nesting. This has been suggested for another Caribbean cave-dwelling frog, *E. cooki* from Puerto Rico (Rivero, 1978; Joglar et al., 1996; Burrowes, 2000).

Parental attendance of clutches affords a degree of protection from predation, parasitism, desiccation, and fungal attack (Taigen et al., 1984; Townsend et al., 1984; Duellman, 1989). Our contribution is the first report of attendance of eggs by frogs in the subgenus *Syrrophus*. Published information about two other species, *Eleutherodactylus (Syrrophus) marnockii*, also considered saxicolous and to some extent cavernicolous in nature, and *Eleutherodactylus (Syrrophus) cystignathoides*, does not include data about parental care in chirping frogs (Jameson, 1955; Hayes-Odum, 1990).

Parental care in the form of nest attendance has been documented in other Cuban *Eleutherodactylus* (*E. albipes*, *E. atkinsi*, *E. dimidiatus*, *E. guantanamera*, *E. intermedius*, *E. leberi*, *E. tetajulia*, and *E. varleyi*), but none of them is a rock-cave dwelling species. They nest on the ground, in leaf litter, or in elevated sites such as leaf axils of

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***Eleutherodactylus zeus* at stage 14-15. G. Neonate emerging from the egg in Las Perlas area. H. Neonatal *Eleutherodactylus zeus* after the simultaneous hatching of the same clutch. Photographs by Sergio L. Domínguez and Eduardo López.**

bromeliads and tree holes (Wells, 2007). Rodríguez and Alonso (2009) found a male *E. leberi*, an endemic Cuban frog of the subgenus *Eleutherodactylus*, vocalizing near a clutch of twenty-seven eggs in a small cavity in the rocky wall, which was considered evidence of territorial defense of oviposition sites. Our observations raise the possibility of biparental care in *Eleutherodactylus (Syrrophus) zeus*, in light of the presence of both sexes near nests. Similarly, both male and female parental care was found to occur in *E. alticola* from Jamaica and *E. johnstonei* from the Lesser Antilles (Townsend, 1996; Wells, 2007).

Many aspects of the natural history of *Eleutherodactylus (Syrrophus) zeus* are still unknown. Daily activity, abundance, demography, diet, predators, and particularly their reproductive biology have not been adequately studied (Alonso and Rodríguez, 2003). We do not believe this Cuban rock-cave frog fits well within the classic ecological classification of cavernicoles of Racovitza (1907). Instead, we prefer to classify them as subtroglaphiles, in accordance with the terminology suggested by Sket (2008). These are species that inhabit, perpetually or temporarily, a subterranean habitat, but are bound to the surface for some biological functions, such as feeding.

Caves and karst regions are fragile ecosystems that have unique scientific value. These environments provide habitats for secretive and rare endangered species, and they provide opportunities to study complex ecological relationships between cave- and surface-dwelling species. However, human activities are transforming and destroying the cave systems through intentional or accidental damage as a consequence of digging, exploration, and tourist visits and the associated disturbance that inevitably accompanies these activities. Consequently, these practices put at risk the survival and reproduction of troglomorphic and troglobitic faunas. Restricting access and indiscriminate use of karstic resources will enhance the likelihood of conserving poorly studied, at-risk endemic lineages such as *Eleutherodactylus (Syrrophus) zeus*.

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# UPDATE: LIVING RETICULATED FILAMENTS FROM HERBSTLABYRINTH-ADVENTHÖHLE CAVE SYSTEM, GERMANY

LESLIE A. MELIM<sup>1</sup>, DIANA E. NORTHUP<sup>2</sup>, MICHAEL N. SPILDE<sup>3</sup>, AND PENELOPE J. BOSTON<sup>4</sup>

**Abstract:** Previous reports of reticulated filaments, an unknown microbe, document that they are ubiquitous in subsurface environments, including limestone caves, lava tubes, and even granite tunnels. Although initial reports of fossil reticulated filaments described preserved organic matter, additional instances involve replacement by calcite, Mn-oxides, silica, or copper silicates. We report on living reticulated filaments found in the limestone Herbstlabyrinth-Adventhöhle Cave System, Hesse, Germany. Samples from soft pool-fingers, pool-bottom clays, and clay-coated rocks along the flow path of incoming water all contain living reticulated filaments associated with abundant biofilm. Most of the reticulated filaments are approximately 0.5  $\mu\text{m}$  in diameter, reach lengths between 150 and 200  $\mu\text{m}$ , and have irregular chambers with spines, a newly identified morphological variant. EDX of these filaments confirms an organic composition not replaced by minerals. They are the dominant visible microbial form in these biofilms, providing hope that they can be isolated and identified.

## INTRODUCTION

Reticulated filaments with enigmatic microbial filamentous morphologies were first reported by Melim et al. (2008) from five limestone caves and one lava cave. They described carbon-rich filaments with a diameter of about 0.5  $\mu\text{m}$  and lengths up to 75  $\mu\text{m}$  that have a cross-hatch or reticulated morphology. Additional examples have since been reported from a variety of subsurface environments. Jones (2009, 2010, 2011) reported on calcitized examples from cave pearls, terrestrial oncoids, and stalactites from the Cayman Islands. Northup et al. (2011) identified reticulated filaments from a copper-silicate stalactite in a Hawaiian lava cave. Miller et al. (2012) found Mn-mineralized reticulated filaments in a granite water tunnel near Oporto, Portugal. Miller et al. (2014) added to the list of lava-cave occurrences with an example from Easter Island, where the filaments are associated with siliceous deposits. This growing list of occurrences for reticulated filaments suggests they are a ubiquitous inhabitant of cave ecosystems, but we still do not know their affiliation.

One difficulty in identifying reticulated filaments is their typically sparse abundance. Although present in a large variety of settings, they are usually a small part of a larger community. Northup et al. (2011) and Miller et al. (2012) both did 16S rDNA clone libraries on samples containing reticulated filaments, but it is impossible to tell which results match up with the filaments. Miller et al. (2012) also cultured samples, but did not successfully culture reticulated filaments. We report here preliminary data from a limestone cave in Germany, where reticulated filaments dominate active pendant biofilm pool fingers and other pool environments. This occurrence confirms the microbial origin and documents samples not mineralized. In addition,

the great abundance of reticulated filaments in these samples provides hope they can finally be isolated and identified.

## FIELD LOCATION

The Herbstlabyrinth-Adventhöhle Cave System, Hesse, Germany, formed in the Upper Devonian Iberger Limestone, a reefal deposit built on an isolated seamount (Meyer and Plan, 2010). The limestone is overlain by a clay layer, likely a Mesozoic paleosol, then by Tertiary Westerwald volcanics (Richter et al. 2010, Mischel and Riechelmann, 2013). The volcanics are porous, and the clay layer acts as an aquitard that funnels water into the cave system (Al-Malabeh and Kempe, 2005). Cave pools in the system are either clay-free and coated in calcite spar or clay-rich and covered in clay instead (Fig. 1A). The clay-rich pools also contain pendant pool fingers composed of filamentous biofilm (Fig. 1B).

## METHODS

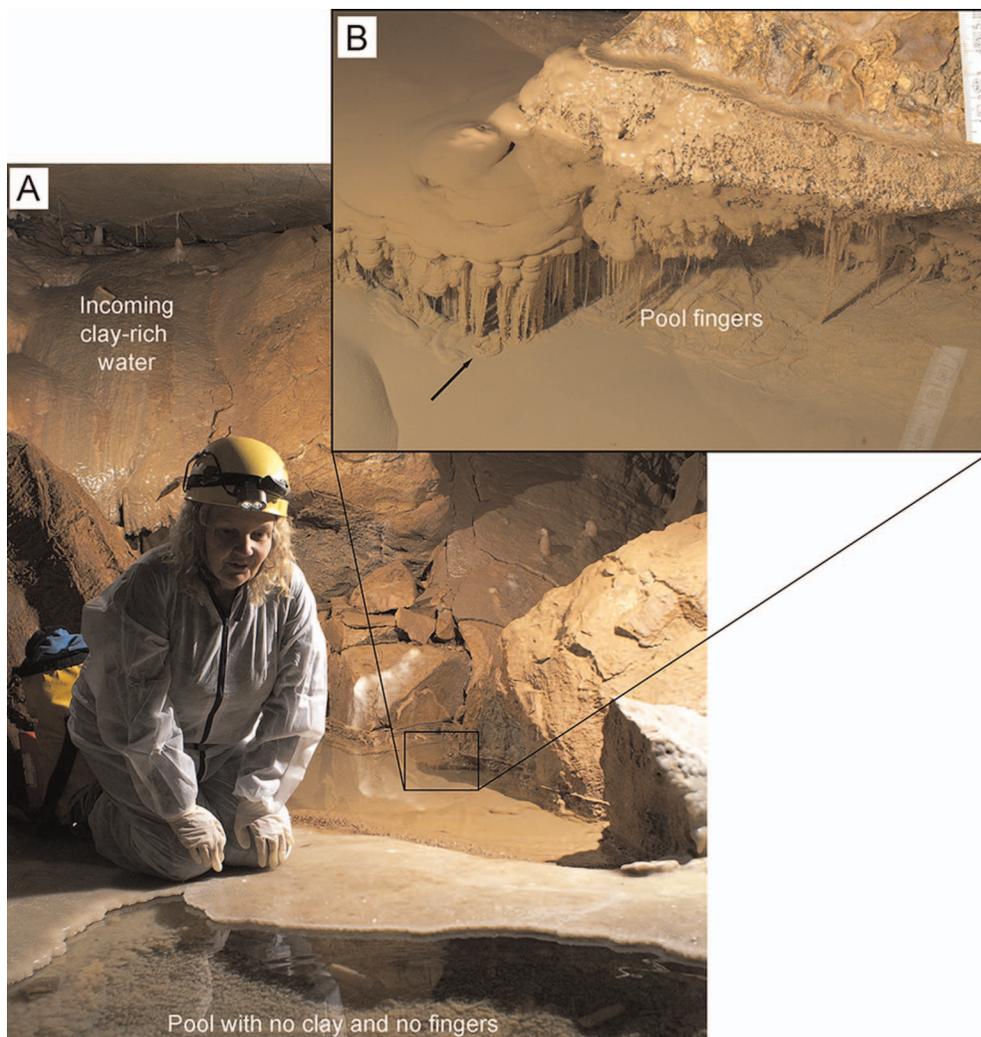
Two areas were sampled in 2011, the Rätselhalle in Herbstlabyrinth and an unnamed area in Adventhöhle; the Rätselhalle was resampled in 2014. All samples were collected using sterile techniques into sterile 50 ml vials. After overnight express shipping to the University of New Mexico, glutaraldehyde was added to kill and fix the samples. Several methods have been used in an attempt to separate out

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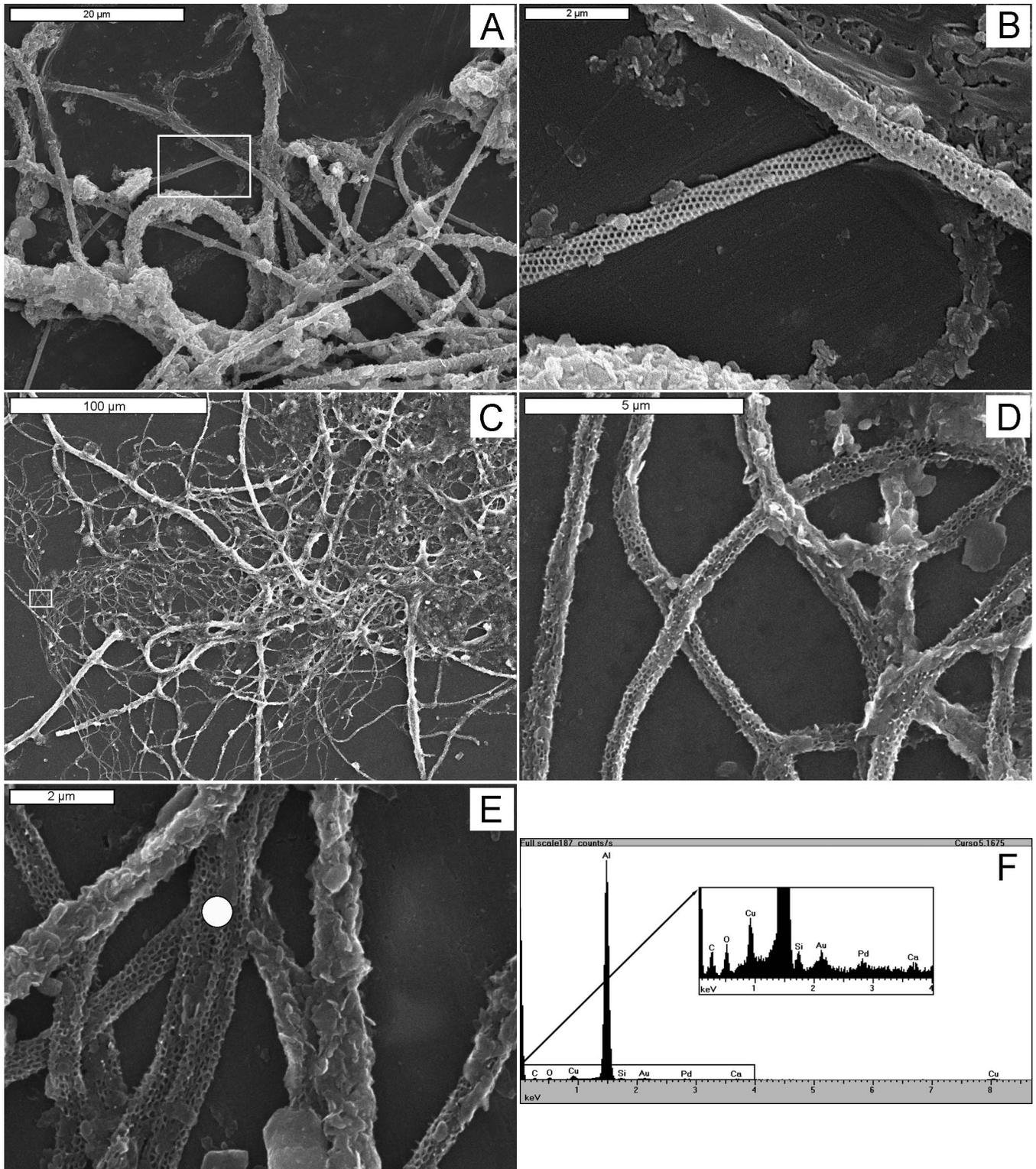


**Figure 1.** Cave pools in Rätshalle, Herbstlabyrinth Cave. Diana Northup for scale. 1A: Overview of pools. Pool to the rear has living pool fingers and is fed from above with clay-rich water. Pool to the front has a different, clay-free source and lacks pool fingers. 1B: Closer view of pool fingers with clay. Note coil of pendant clay-covered biofilm on pool bottom (arrow).

filaments from sediment. In 2011, a graded ethanol and water rinse followed by a hexamethyldisilazane rinse was used to clean and dehydrate filaments prior to electron microscopy. However, significant amounts of clay were still retained on the filaments, obscuring their examination. The 2014 samples were treated either with 10% Na-pyrophosphate and  $\text{Na}_2\text{CO}_3$  to disperse clays or with a simple deionized-water rinse. The best results, with the least residual clay, were obtained from the deionized-water rinse after a small amount of clay-rich sample was placed in 40 ml of deionized water, shaken by

hand to suspend, then centrifuged for 3 minutes at 300 rpm to settle the  $>5\text{-}\mu\text{m}$  fraction. The clay-rich liquid was decanted, and the process was repeated until the water was clear and a biofilm sludge remained in the vial. This sludge was smear-mounted on an aluminum sample stub and allowed to dry overnight before coating with approximately  $200\text{ \AA}$  of gold-palladium alloy prior to examination in a JEOL 5800LV scanning electron microscope at the University of New Mexico. The SEM is equipped with an Oxford Isis 300 energy-dispersive X-ray analyzer.

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**Figure 2.** SEM images of reticulated filaments from Rätshalle, Herbstlabyrinth Cave. 2A: Smear mount of biofilm, filaments, and clay treated with glutaraldehyde and rinsed with hexamethyldisilazane (from 2011 sample). Most filaments are coated in obscuring clay, but some are reticulated. Box is area in 2B. 2B: Detail from 2A of two reticulated filaments, one with regular chambers aligned in rows, one with irregular chambers partly obscured by clays. 2C: Smear mount of biofilm,



filaments, and clay treated with glutaraldehyde and rinsed in distilled water (from 2014 sample). The nature of the larger filaments, always coated with clay, is unknown. Smaller filaments are all reticulated. Box is area in 2D. 2D: Detail from 2C of reticulated filaments with irregular chambers and small spines. Clays coat some areas. 2E: Close view of reticulated filaments and unknown filaments coated with clays (2014 sample). White dot is location of EDX analysis in 2F. 2F: Energy-dispersive X-ray spectrum of reticulated filaments with minor clays, inset is same analysis with expanded scale. Sample mount is aluminum with minor copper, and samples are coated with gold-palladium. The rest of the spectrum includes oxygen, carbon, silicon and trace calcium.

## RETICULATED FILAMENTS

Reticulated filaments were found with biofilm in soft pool-fingers, pool-bottom clays, and clay-coated rocks along the incoming flow path (Fig. 2). Only one sample contains calcite crystals. Samples treated with hexamethyldisilazane are heavily coated in clays, obscuring most of the filaments (Fig. 2A). However, a few filaments are still clearly reticulated (Fig. 2B). The water-rinsed sample has much less residual clay, and two filament sizes are distinguishable, roughly 2- $\mu\text{m}$  diameter filaments completely coated in clays and roughly 0.5- $\mu\text{m}$  reticulated filaments (Fig. 2C, 2D, 2E). The most common reticulated filament, a new morphology not previously reported, has irregular chambers and small spines (Fig. 2D, 2E) and reaches lengths between 150 and 200  $\mu\text{m}$ . Unlike previous examples, reticulated filaments here are a dominant component in the filamentous biofilm (Fig. 2C).

To get an adequate X-ray energy spectrum, several intertwined filaments were analyzed (Fig. 2E, 2F). Minor clay was also present. Removing the influence of the substrate (Al, Cu), conductive coating (Au, Pd), and the clays (Si, O) leaves C, O, and lesser Ca as the apparent composition of the filaments (Fig. 2F). Typically calcite in a spectrum shows Ca much higher than C, but in this case, the filaments have higher C than Ca. This indicates that the filaments are composed of carbon (likely organic matter) and not replaced by calcite. Noting that these pool fingers were active in the cave prior to sampling and treatment, we conclude that the filaments must be microbial filaments, confirming the interpretation of Melim et al. (2008), Jones (2009, 2010, 2011), Northup et al. (2011), and Miller et al. (2012, 2014) that reticulated filaments are an unknown microbe. Ongoing research by our team is working to identify this microbe.

## CONCLUSIONS

Reticulated filaments are ubiquitous in cave environments, including limestone caves, lava caves, and even granite tunnels. In the limestone cave Herbstlabyrinth-Adventhöhle Cave System, Germany, living reticulated filaments are present in soft pool-fingers, pool-bottom clays, and clay-coated rocks along the incoming flow path. Most of the reticulated filaments are 0.5  $\mu\text{m}$  by 150 to 200  $\mu\text{m}$  and have irregular chambers with spines. Energy-dispersive X-ray analysis confirms carbon rather than mineral composition. This report of living reticulated filaments still composed of organic matter confirms their microbial nature. Their abundance in this cave provides hope that they can finally be isolated and identified, the subject of ongoing research.

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# HOLOCENE PALEONTOLOGY OF BAT CAVE, EDMONSON COUNTY, KENTUCKY

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**Abstract:** We report the excavation and analysis of a Chiroptera-dominated bonebed from Bat Cave, Edmonson County, Kentucky. Paleontological materials recovered in 1999 offered new insight into formation processes of the bonebed. Stratigraphic and geochronological information indicate a long, episodic history of the deposit spanning much of the Holocene. The vertebrate assemblage is dominated by *Myotis* spp. Although initially believed to represent a single, catastrophic kill event due to historic flooding, our results suggest that the Bat Cave bonebed was formed by a minimum of eleven accumulation events that took place between approximately 2200 and 10,800 cal BP.

## INTRODUCTION

Before Mammoth Cave was officially designated as a national park in 1936, three groups, the Mammoth Cave National Park Association, Kentucky National Park Commission, and the National Park Service (NPS), jointly operated the Mammoth Cave property; in 1941 the NPS became fully responsible (Goode, 1986). Bat Cave, presumably named for its large bat population, is located within the park in the limestone bluff above the Green River (Fig. 1). Since at least the 1930s, Bat Cave has drawn official attention, where an extensive deposit of bat bones has been of particular interest (Bailey, 1933; Hibbard, 1935a; Jegla, 1961; Keefer, 1969; MacGregor, 1991; Trevor-Deutsch, 1972).

In the 1930s, Claude Hibbard was concerned about management practices that were driving bats out of the park's caves. In July of 1934, Hibbard made a special trip to Bat Cave to assess the bat situation. He noted the virtual absence of bats, which he thought was due to local residents making fires at the base of formations, blasting them with dynamite in order to break off cave onyx for tourist items, or the Kentucky Park Commission's program to blast shut more than 25 cave entrances, including Bat Cave (Hibbard, 1935a). Hibbard did not mention that the absence of a large number of bats might have been due to the timing of his visit in July, a month when most species would be living outside the cave. Hibbard noted during his July 1934 trip that each of the two passages in Bat Cave "had been blasted shut but later worked out so that one could worm their way over the blasted material just below the ceiling though not enough room to allow a lantern to remain upright" (Hibbard, 1935a, p. 2). On June 19, 1935, Hibbard made another special trip to Bat Cave with geologist E. R. Pohl, about which he wrote,

the left avenue into the main portion of the cave had broken down due to the blast either early this spring or last winter. It is in this avenue that the bats hibernate. The question as to whether the bats were trapped by this breakdown cannot be answered until it is removed. The breakdown is small though forming a tight plug since the opening was just large enough to allow a medium sized man to slide through into the avenue. This should be opened by all means. (Hibbard, 1935a, p. 2)

In his diary entry for June 19, 1935, Hibbard (1935b) wrote, "Bat Cave was found closed at the main passage (I was through this passage a year ago)." Again, Hibbard's primary recommendation was "That all caves now closed due to blasting in the Park Area on our land shall be opened" (Hibbard, 1935a, p. 3). A report by E. R. Pohl (1935) dated July 1935 noted that an emergency request had been made to open Bat Cave, which had been closed "several years ago by the Kentucky National Park Commission." Documentary sources do not indicate whether this request was acted upon.

In 1960, Thomas Jegla became the first researcher to report the bone deposit in the A-Survey of Bat Cave. He described the deposit as measuring about 10-m long by 1.2-m wide by 0.6-m deep, with bones concentrated in the upper 8 cm (Jegla, 1961). Jegla collected a 45-cm<sup>3</sup> sample (exact dimensions unknown) from which he counted humeri and measured ten bat skulls. Based on these measurements, Jegla's study concluded that the skulls were consistent with those of *Myotis sodalis* and that the deposit was an accumulation of drowned bats.

In the mid-1990s Mammoth Cave Park Ecologist Rick Olson and Illinois State Museum researcher Rick Toomey proposed another examination of the bonebed as part of the Mammoth Cave Paleontological Inventory Project (Colburn, 2005). On May 20, 1999, Illinois State Museum researchers Toomey and Mona Colburn, accompanied by Olson, visited Bat Cave to excavate a small section of bones from the wall of the crawlway. It is unclear how effectively Hibbard's (1935a) number-one recommendation, opening the dynamited caves, was implemented at Bat Cave. We observed an 11-m long crawlway that had been dug through sediments in the passage of the A-Survey; it does not seem to be much larger than that described by Hibbard nearly 80 years ago (Fig. 2). Because the crawlway was dug through sediments containing a large,

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**Figure 1. Approach and entrance to Bat Cave.**

concentrated accumulation of bat bones, it provides an excellent profile of the bonebed (Fig. 3).

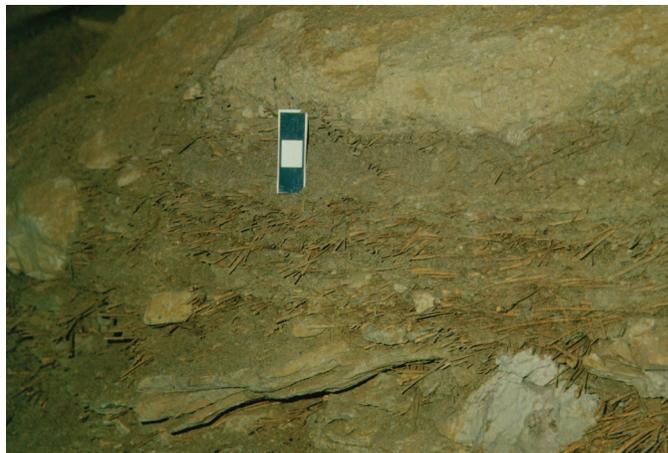
This study reports the results of paleontological work in Bat Cave, in particular the recent establishment of a geochronological framework for bonebed formation and the identification of bat taxa. Although the mechanism is still unclear, we also explore possible scenarios of bone accumulation.

#### METHODS

The entrance to Bat Cave lies 28 m above the normal pool of the Green River as ponded by Lock and Dam No. 6 (Fig. 4). Survey Station A9 has an elevation of 137.9 mamsl, 9.7 m above the modern river level. This station (Figs. 4 and 5), which is located on the ceiling of the passage, served as an excavation datum. The surface of the



**Figure 2. The excavated crawlway that cuts through the bone deposit in Bat Cave, looking north. The passage is approximately 1-m high.**

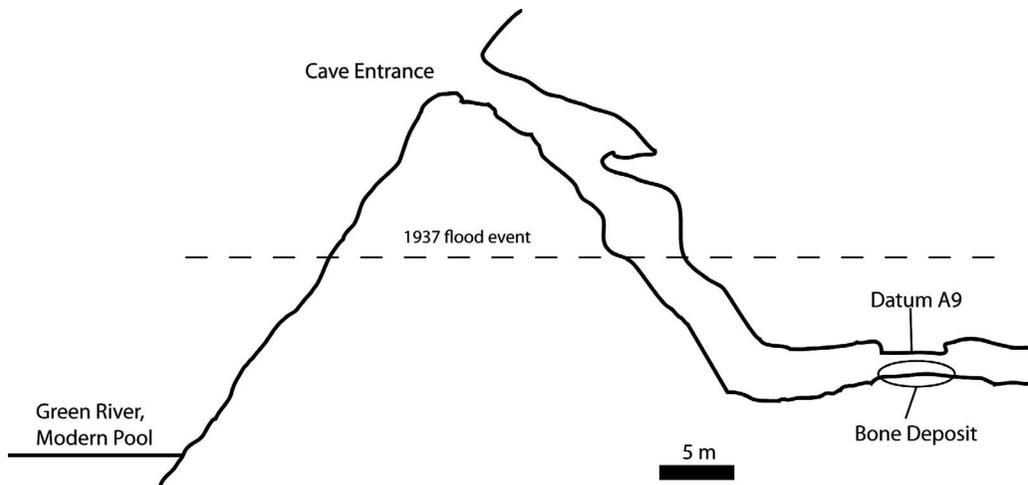


**Figure 3. Massive deposit of bat bone exposed in the wall of the crawlway in the Bat Cave. Scale bar in cm.**

excavation area was 50-cm below this datum, along the left edge of the crawl. A single unit (Unit 1) measuring 15-cm along the trail by 10-cm wide and 40-cm high was excavated in eleven levels, which ranged from 2.0-cm to 7.0-cm thick (Table 1). The deposit appears to have zones of almost sterile sediment separating some of the bones. These levels had to be teased out during excavation because of the intertwined nature of the bone deposits, thus the excavated levels reflect natural stratigraphy. After excavation, the small pit was lined with plastic and backfilled with rocks and sediment.

All samples were taken to the Illinois State Museum Research and Collection Center and dry-sifted through small mesh geological screens. Figure 6 shows an example of bone-deposit material from Level 4 that was  $>2.36$  mm; larger rocks have been removed from this sample. Screened material from Level 4 contains a large ratio of bone to rock. Bones selected for analysis included cranial elements and long bones that could be measured or could provide information for identification. To be consistent with Jegla's use of bat humeri to obtain an estimated number of individuals, distal humeri were also counted in the 1999 sample. All specimens selected for study were identified and measured by Blaine W. Schubert and one of us (Colburn) in 1999; Schubert screened and sorted samples and measured post-cranial elements of bat, and Colburn measured bat dentaries and skulls and identified non-bat mammals. Standard measurements on limb and skull elements follow Toomey (1993). The Illinois State Museum comparative osteology collections were referred to for making identifications.

Six *Myotis* species are known to live in the eastern US (Harvey et al., 2011). The size range for each taxon was assessed using the Museum's comparative samples and published size ranges for *Myotis* (e.g., Miller and Allen, 1928; van Zyll de Jong, 1984). Despite excellent comparative



**Figure 4.** Vertical section showing the elevation of the bonebed relative to the Green River and the cave entrance. Dashed line indicates the height of a 1937 flood event. Figure based on Jegla (1961).

specimens and references to body size, it is still difficult to distinguish between different species of *Myotis* on the basis of fragmentary or undiagnostic fossil material (Czaplewski et al., 2002; Jansky, 2013). In many cases, identification of the Bat Cave *Myotis* sample is limited to general size class (small, medium, or large *Myotis*) or only to genus level.

The skull is the most diagnostic element for identifying bat taxa. Dentaries, teeth, humeri, and element size are also useful for identifying bat genera and, potentially, species. In the case of bat dentaries and crania, the most useful characters include shape, overall size, skull measurements, tooth counts, and the presence or absence of a sagittal crest. Measurements were made to the nearest 0.1 mm using non-metallic 150 mm dial calipers. The following measurements were taken on bat skulls: greatest length of skull (GLS), condylobasal length (CBL), distance from front of maxilla (excluding incisor) to M3 (I-M3), maxillary breadth at the M3s (M3M3), least interorbital breadth (IOB), maximum breadth of the braincase (BB), occipital depth (OD), and total dentary length (TL). To distinguish *Myotis* species based on skulls, we relied on GLS, IOB, BB, cranial shape, and presence or absence of a sagittal crest.

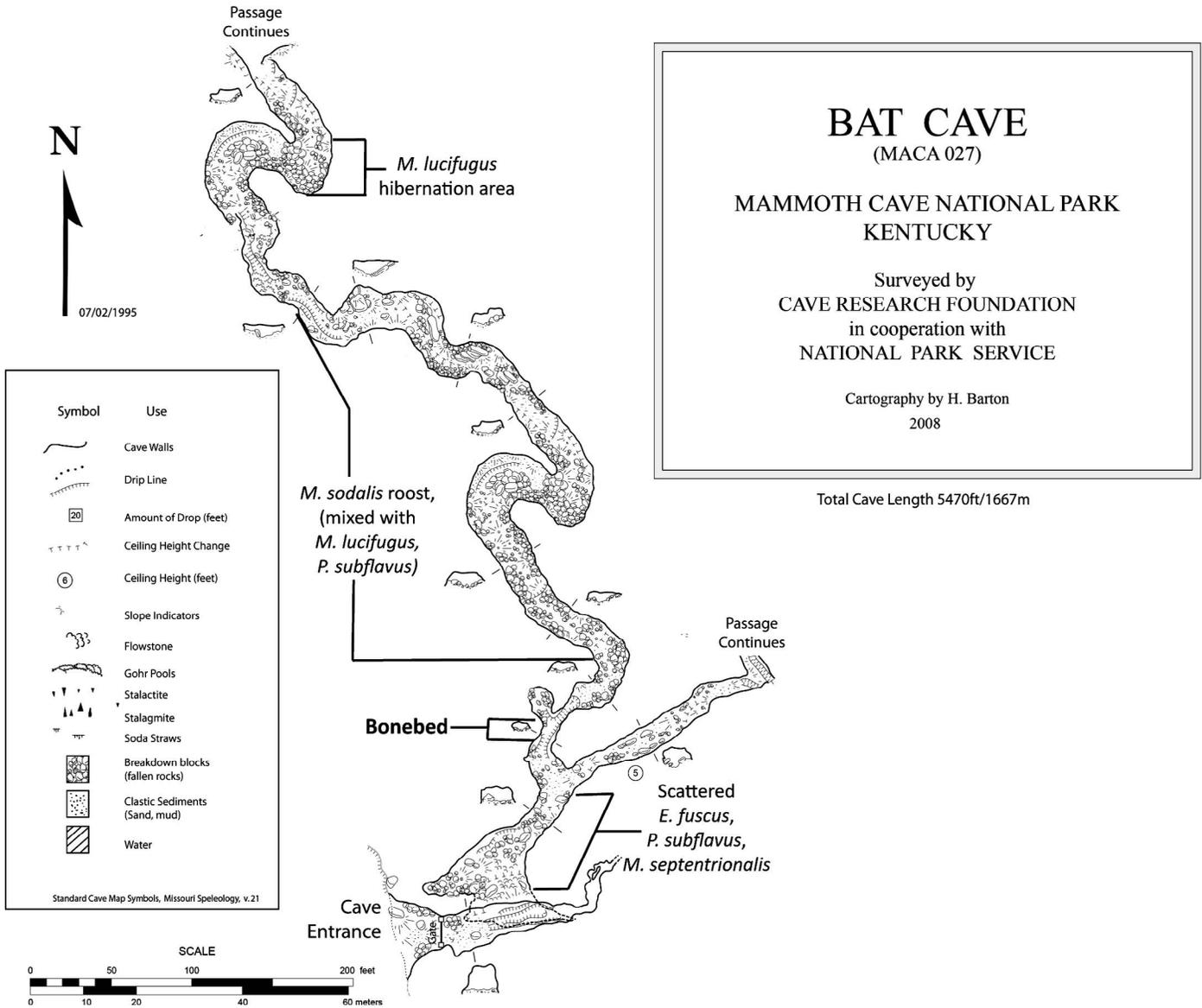
The eastern small-footed *Myotis* (*Myotis leibii*) is a small-sized bat, whereas the Indiana bat (*M. sodalis*), the little brown bat (*M. lucifugus*), the northern long-eared bat (*M. septentrionalis*), and the southeastern *Myotis* (*M. austroriparius*) are medium-sized *Myotis*, and the gray bat (*M. grisescens*) is large-sized. Compared to *M. lucifugus*, *M. sodalis* crania exhibit a pronounced sagittal crest and slightly narrower BB (*M. sodalis*: 6.4–7.2 mm; *M. lucifugus*: 7.0–7.6 mm) and IOB (*M. sodalis*: 3.3–4.3 mm; *M. lucifugus*: 3.6–4.4 mm) (Thomson 1982, p. 1). *M. leibii* dentaries average less than 10-mm in total length (TL) and are more gracile than those of other *Myotis*.

The majority of unassociated dentaries with 3-1-3-3 tooth counts were identified as *Myotis*. Because this dentition also occurs in *Corynorhinus* and *Lasionycteris*, there is a possibility that some of the dentaries identified as *Myotis* could represent one of those genera. However, only a single *Corynorhinus* cranium was identified, and no diagnostic elements of *Lasionycteris* are present, so it is unlikely that these taxa make up a significant percentage of the assemblage.

Since its discovery, there has been uncertainty surrounding the chronology of the Bat Cave bonebed. Initially, Jegla (1961) suggested the bone accumulation took place in the recent, historic past, possibly during a 1937 flood event. In later correspondence, he allowed the possibility that the accumulation event could have predated European settlement. With these chronological questions in mind, we selected bat bones for  $^{14}\text{C}$  dating. Bat radii (*Myotis* spp.) were selected from excavation levels 2, 6, and 11 in order to assess the chronostratigraphy of the bonebed. Samples were submitted to Nancy Beavan, Rafter Radiocarbon lab, New Zealand, where collagen was isolated through standard acid-base-acid pretreatment. The carbon:nitrogen (C:N) ratio indicated preservation within the range of modern bone, and qualitative measures of collagen preservation were favorable. Radiocarbon results were calibrated in Calib 7.0html using the Intcal13 dataset. Two-sigma errors (95% probability) are reported.

## RESULTS

Within the 6,000 cm<sup>3</sup> of excavated material, a total of 3064 bat bones were diagnostic to family or better. The assemblage included five species of bat, four non-bat mammals, one salamander, and one fish (Table 2). Major bone-bearing units were levels 2 through 7, (53 through 73 cm below datum). To be consistent with Jegla, the



**Figure 5. Partial plan map of Bat Cave, Kentucky, showing the area of the bone deposit and the modern bat usage areas. Based on a map courtesy of the Cave Research Foundation.**

current study estimated the number of individual bats by counting distal humeri. Clearly, each specimen could not be identified to genus or species. Rather, the size and configuration of most humeri indicate medium-size *Myotis*; no humeri of large-size bat species were found. Several small humeri could be either tri-colored bat or very small *Myotis*, although no skull or dentary of a tri-colored bat was identified in the screened osteological material from the deposit. Thus, based on a tally of distal humeri (mostly *Myotis* spp.), an estimated 1322 individual bats are represented (Table 2).

A total of 141 skull fragments were identified to genus or better based on diagnostic portions (e.g., dentary, maxillary, rostrum). The majority of these specimens are lower jaws of *Myotis* spp., most being

**Table 1. Unit 1 excavation levels.**

Level	Elevation, cm (Below datum)	Thickness, cm
1	50–53	3
2	53–55.5	2.5
3	55.5–58.5	3
4	58.5–61	2.5
5	61–66	5
6	66–70	4
7	70–73	3
8	73–75	2
9	75–79	4
10	79–86	7
11	86–90	4



**Figure 6.** Material from Level 4 >2.36 mm, with rocks >12.5 mm removed before the photo was taken.

from individuals in the medium-size range. Six small dentaries were attributed to *M. leibii* due to their small size (TL <10 mm) and relatively gracile structure, but no cranial fragments of this species were encountered (Supplementary Table S1).

Two crania of *M. lucifugus* or *M. cf. lucifugus* were identified from Levels 5 and 6 and four crania of *M. sodalis* or *M. cf. sodalis* from Level 6. A *Corynorhinus* sp. skull was found in level 5. *Eptesicus fuscus* was identified from dentaries in Levels 4 and 9 and a canine in Level 10.

Collagen extracted for radiocarbon dating was well-preserved, and  $\delta^{13}\text{C}$  values were within the expected range of modern bats in the area (Table 3). These results indicate that the Bat Cave bonebed spans much of the Holocene (Table 3). A date of  $2250 \pm 25$  BP (NZA32583; 2160–2340 cal BP) was reported for Level 2. Bat bones from Level 6 were dated at  $3680 \pm 25$  BP (NZA32584; 3930–4090 cal BP). At the base of the unit, a date of  $9510 \pm 40$  BP (NZA32585; 10,610–10,880 cal BP) was acquired from Level 11. The upper half of the assemblage is late Holocene in age. The lower half of the assemblage is early and, possibly, middle Holocene in age.

In addition, the bonebed contained 11 specimens of non-chiropteran animals: two raccoon (*Procyon lotor*), one deer (*Odocoileus virginianus*), one mouse-sized rodent, one rat-sized rodent, two plethodontid salamander, two indeterminate mammal, one indeterminate fish, and a fragment that could not be identified beyond bird or mammal. The teeth of a juvenile raccoon (left p4, right dp3) and the unfused metacarpal diaphysis of a fetal deer offer seasonality information for Level 9.

**Table 2.** Number of identified specimens and minimum number of individuals, in parentheses, of taxa recovered from Bat Cave.

	1	2	3	4	5	6	7	8	9	10	11
<i>Myotis leibii</i>				1(1)	1(1)	1(1)		2(2)		2(1)	
<i>Myotis lucifugus</i>		1(1)				3(3)					
<i>Myotis sodalis</i>							1(1)				
<i>Corynorhinus</i> sp.					1(1)						
<i>Eptesicus fuscus</i>				1(1)					1(1)	1(1)	
Medium <i>Myotis</i>	2(1)	5(2)	8(3)	4(3)	9(5)	16(4)	8(5)	5(5)	7(4)	12(5)	16(8)
<i>Myotis</i> spp.		2(2)		1(1)	1(1)	1(1)	3(1)	1(1)		14(9)	10(2)
Vespertilionidae	57(26)	299(106)	380(165)	412(195)	414(183)	397(191)	304(138)	136(67)	173(88)	252(111)	99(52)
<i>Procyon lotor</i>									1(1)	1(1)	
<i>Odocoileus virginianus</i>									1(1)		
Medium Mammal						1				1(1)	
Small Rodent (mouse-sized)										1(1)	
Large Rodent (Rat-sized)										1(1)	
Bird or Mammal											1(1)
Plethodontid salamander										2(1)	
Fish											1(1)
Total NISP	59	307	388	419	426	419	316	144	183	287	127

## DISCUSSION

Table 3. AMS  $^{14}\text{C}$  dates on acid-base-acid purified bone collagen from Bat Cave.

Sample ID	Depth BD, cm	Lab. No.	$^{14}\text{C}$ age BP	Cal BP (2 sig.)	$\delta^{13}\text{C}$ (PDB)	Species	Material Dated
BC-2A	53–55.5	NZA32583	2250 ± 25	2160–2340	-20.8	<i>Myotis</i> sp.	purified bone gelatin; combined sample of 3 radii
BC-6A	66–70	NZA32584	3680 ± 25	3930–4090	-20.1	<i>Myotis</i> sp.	purified bone gelatin; combined sample of 3 radii
BC-11A	86–90	NZA32585	9510 ± 40	10610–10880	-20.0	<i>Myotis</i> sp.	purified bone gelatin; combined sample of 3 radii

Based on comparisons of the Bat Cave bat specimens with cranial and jaw elements of modern *Myotis* spp. from the Kentucky area, Jegla (1961) concluded that measurements on fossil crania were consistent with those of *Myotis sodalis*. MacGregor (1991) identified crania of *Eptesicus fuscus*, *Perimyotis* (= *Pipistrellus*) *subflavus*, and *Myotis* spp. from the deposit. The 1999 Illinois State Museum sample contained not only *M. sodalis* and *Eptesicus fuscus*, but also *M. leibii*, *M. cf. lucifugus*, hundreds of bones of indeterminate medium-sized *Myotis* spp., and *Corynorhinus* sp.

Consideration of the  $^{14}\text{C}$  chronology based on direct-dated bat remains clearly indicates that the Bat Cave bonebed predates historic flooding of the Green River and anthropogenic modification of the modern entrance by blasting. However, causes of death and the mechanisms of accumulation are still unclear. The high density of bat remains within the passage suggest either a series of catastrophic death events or the occasional concentration of bones from attritional deaths over time. The bonebed passage is not an ideal location for a large bat roost. The modern passage contains no concentrations of roosting bats, although there are hibernacula deeper within the cave (Fig. 5). Disease or flooding are possible causes of death in the Bat Cave fauna. Both may have occurred periodically through the Holocene.

The concentration of disarticulated bones within the bonebed passage suggests a mechanism of post-mortem accumulation is at work. Two taphonomic scenarios could be invoked to explain this concentration. The first scenario is periodic flooding within the cave due to an elevated water table that could have had the effect of redistributing and concentrating the remains of fauna that died upstream in the passage. The cave entrance is at a much higher elevation than the A9 section where the bonebed is located, and would have provided a barrier to high water, causing ponding and reduced flow in the area of the bonebed during draining of the cave. Alternatively, high floods from the Green River could have submerged the entrance, causing high-water conditions far back into the cave. As the floodwaters receded, ponding in low areas like the A9 section could have concentrated the bones of animals from nearby passages. These scenarios are not mutually exclusive.

Some non-bat remains within the bonebed offer clues to seasonality. A fetal deer metacarpal, identified on the basis of its small size, was recovered from Level 9 and suggests a winter death. This estimate is based on the rut taking place in November-December and parturition in May (Barbour and Davis, 1974, p. 287). Although young deer bones may be consumed or transported by carnivores or rodents, no gnawing is present on the surface of this specimen, so transport by woodrats or carnivores is unlikely. The presence of juvenile raccoon premolars may

support late winter to late spring as well. In Kentucky, raccoons mate from January to March and births can occur from early March through May (Barbour and Davis, 1974, p. 256). Because the bonebed is probably a secondary deposit, these seasonality indicators do not reflect the season-of-death for the bat fauna. Rather, they simply indicate that Bat Cave was open and accessible during the winter.

Jegla estimated that the Bat Cave deposit represented some 300,000 individual bats; the current study did not attempt to make an estimate for the entire bonebed. On his January 1960 trip, Jegla saw hibernating *Myotis sodalis* and recorded a temperature of 4°C in the passage just beyond the bonebed. Researchers before and after Jegla have reported on the various species found in Bat Cave. The United States National Museum has *Perimyotis subflavus* and *M. lucifugus* specimens collected by Vernon Bailey in 1929 from Bat Cave (Smithsonian Institution, 1996), where he wrote that the latter species was found in “small numbers... in late September” (Bailey, 1933, p. 453). In addition, Bailey commented that Bat Cave contains “large numbers” of *M. sodalis* (Bailey 1933, p. 457). In 1959 Hall captured *M. austroriparius* and *M. sodalis* (Smithsonian Institution, 1996). In the fall of 1969, Scott Keefer, a zoology student at Southern Illinois University, estimated that Bat Cave housed 200 to 250 *M. sodalis* and 300 *M. lucifugus*; in February of 1971 he estimated counts to be 200 to 300 and 250 to 300 for the two species and noted that *M. lucifugus* had shifted its hibernation location (Keefer, 1971). In November of 1972, B. Trevor-Deutsch of Carleton University, Ottawa, Canada, observed approximately 300 hibernating bats, primarily *Myotis*, in the first 1000 feet (~300 m) of the “large tubular part of this cave” and noted the bone deposit in the crawlway that led to the tubular passage (Trevor-Deutsch, 1972).

Endangered bat species in Kentucky are monitored by the Kentucky Department of Fish and Wildlife Resources, the Kentucky State Nature Preserves Commission, the U.S. Forest Service, and the U.S. Fish and Wildlife Service. A variety of bats have been recorded in Bat Cave since regular surveys were initiated by these agencies. Recent trips documented a hibernation roost of *Myotis sodalis* and a *M. lucifugus* hibernaculum in the passage beyond the bonebed (Fig. 5). Deeper in the cave, scattered *M. lucifugus* and *Perimyotis subflavus* occur, and a summer roost of *M. grisescens* has been recorded.

Bat counts are conducted every other winter. From 1985 to 1994, estimates of hibernating *M. lucifugus* remained consistent, in the range of 223 to 311. In recent years their numbers have been more varied, occasionally dropping below 100 individuals. *M. sodalis* declined from a high of 212 in 1982 to the current low, fewer than 50 individuals; *M. lucifugus* was not counted that year. In addition, small numbers of hibernating *P. subflavus*, *Eptesicus fuscus*, *Corynorhinus rafinesquii*, *M. septentrionalis*, and *M. grisescens* have been observed over the years,

and a bachelor colony of *M. grisescens* is present in summer (data on file, Mammoth Cave National Park). Because it is likely that taphonomic mechanisms have concentrated remains from periods of unknown duration, it is difficult to compare modern bat counts to estimates of individuals contained within the bonebed. Species composition of the identifiable cranial material found in the present study of the bonebed (though limited in numbers) suggests that the contributing population was a mixture of medium-sized *Myotis* (*M. lucifugus*, *M. sodalis*), *M. leibii*, *E. fuscus*, and *Corynorhinus* sp.

## CONCLUSIONS

Paleontological investigations of the Bat Cave bone deposit suggest multiple events that concentrated bat remains into a series of relatively discrete bonebeds over the last 10,800 years. Although sedimentation rates and accumulation frequencies were not uniform throughout this period, the earliest event at the base of the section occurred around 10,800 cal BP, while the most recent event occurred around 2200 cal BP. All bonebeds within the section are dominated by Chiroptera, especially *Myotis* spp. It is difficult to compare the paleoecological record of chiropteran osteological materials in Bat Cave to the modern record of population counts due to difficulty in identifying different species within the genus *Myotis* and uncertainty in the mode of accumulation. However, a number of taxa are identified based on diagnostic crania, including *M. lucifugus*, *M. sodalis*, *M. leibii*, *Eptesicus fuscus*, and *Corynorhinus* sp.

Supplemental Information, Table S1. Bat skull elements and measurements from Bat Cave, Unit 1. Abbreviations: n/m-not measured, med.=medium-sized, L=left; R=right; crest=sagittal crest. Greatest length of skull (GLS), condylobasal length (CBL), distance from front of maxilla (excluding incisor) to M3 (I-M3), maxillary breadth at the M3s (M3M3), least interorbital breadth (IOB), maximum breadth of the braincase (BB), occipital depth (OD), and total dentary length (TL).

## ACKNOWLEDGEMENTS

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# THE SUBTERRANEAN ASELLIDS OF INDIANA (ISOPODA), WITH THE DESCRIPTION OF *CAECIDOTEA* *DUNLAPORUM*, NEW SPECIES

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**Abstract:** *Caecidotea dunlaporum*, new species, is described from six localities peripheral to the Indiana karst areas. Endemic to Indiana, this new subterranean species occurs in saturated soil interstices. A second species inhabiting unconsolidated sediments, *C. beattyi*, is reported from Indiana for the first time. This brings to seven the number of obligate subterranean *Caecidotea* species known from the state. Two species, *C. stygia* and *C. rotunda*, are primarily found in caves, while *C. jordani*, *C. kendeighi*, and *C. teresae* are primarily inhabitants of non-cave hypogean habitats. The seven species are divided into the *stygia* and *hobbsi* species groups representing two phyletic lines. Conservation statuses of the subterranean isopod fauna include *C. teresae* extirpated from all known sites, *C. dunlaporum* and *C. jordani* critically imperiled, and *C. rotunda* imperiled.

## INTRODUCTION

The first report of an isopod from a subterranean habitat in Indiana was *Caecidotea microcephala*, described by Cope (1872) from material collected in Saltpeter Cave, near Wyandotte Cave, Crawford County. The name of the species was based on Cope's belief that the head was smaller than that of *C. stygia* described by Packard (1871) from Mammoth Cave, Kentucky. It is not surprising that Cope considered his species as different from *C. stygia*, considering that in the drawing accompanying his brief description he stitched together the body of an isopod with the egg sacs of a copepod. Fortunately, Packard (1873) synonymized *C. microcephala* with *C. stygia*.

Almost a century elapsed before another isopod species was described from groundwater in Indiana with the discovery of *Asellus* (= *Caecidotea*) *jordani* (Eberly 1966). Fleming (1973) believed this species was synonymous with *A. alabamensis*, but Lewis and Bowman (1981) examined the type-material and established the validity of *C. jordani*.

Lewis and Bowman (1981) also reported *Caecidotea kendeighi* (Steeves and Seidenberg 1971) from three collections made by Leslie Hubricht in 1942 from drain tiles in northern Indiana. Hubricht was among the first to recognize the significance of the outlets of field drainage systems, placed to allow cultivation of poorly drained ground, in sampling for groundwater crustaceans occurring in the saturated interstices of soil.

Lewis (1982a) described *Caecidotea teresae* from drain tile outlets on the campus of Indiana University Southeast in Floyd County. The last species discovered in the state was *C. rotunda*, described from caves in southeastern Indiana and adjacent Ohio (Bowman and Lewis, 1984).

Thus, five species of *Caecidotea* had been reported from subterranean habitats in Indiana. Herein a new species

is described from localities peripheral to the karst, and *C. beattyi* is reported from Indiana for the first time.

In the following species accounts all collections were made by Julian J. Lewis except where noted otherwise. Type-specimens and many of the other specimens have been deposited in the collection of the United States National Museum (USNM) of Natural History, Smithsonian Institution. Specimens of the new species described herein were assigned USNM catalog numbers. The following abbreviations have been employed: BONWR for Big Oaks National Wildlife Refuge, INAAP for Indiana Army Ammunition Plant, IUS for Indiana University Southeast. Cave locations are recorded in the Indiana Cave Survey database. Other locality data are reported in the English System, since sites were located as measured in the field by car odometer, with data in the specimen vials labelled accordingly; metric data are provided in parenthesis. Directions to a town or other landmark are abbreviated as capital letters, e.g., northwest for NW. The base map (Homoya, 1985) and nomenclature for natural regions of Indiana follows Homoya et al. (1985). Species rarity rankings incorporated are those standardized to conservation agencies in the United States (Master et al., 2000).

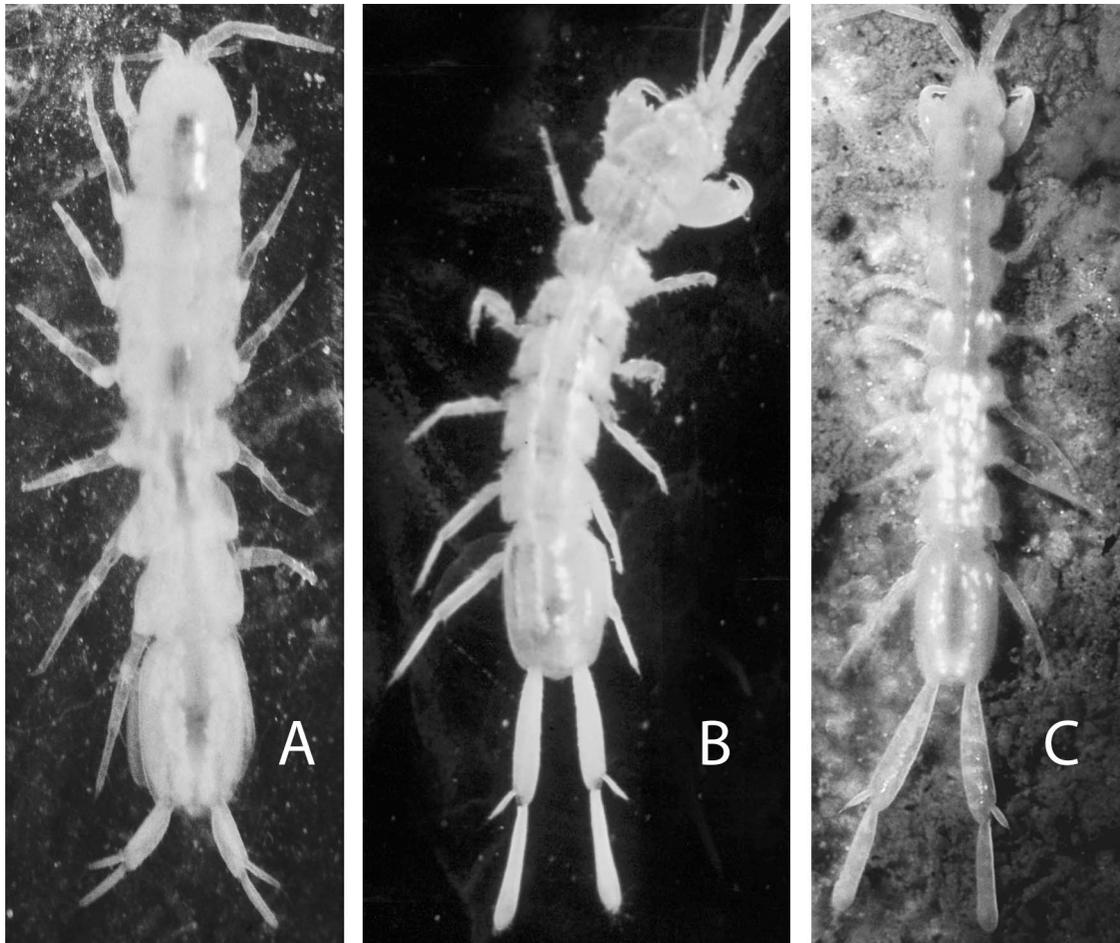
## FAMILY ASELLIDAE

*Caecidotea* Packard, 1871

*Caecidotea dunlaporum*, new species

Figures 1a, 2–4, 5a

**Material examined**—**INDIANA: Bartholomew Co.:** drain outlet on E side of Highway 31 approximately 1 mile (1.6 km) S intersection with Highway 7, 11 March 2012, 3 males (USNM 1251844); **Daviess Co.:** drain outlet near intersection of North Fork of Prairie Creek and CR N450E, 7.7 miles (12.4 km) NE Washington, 13 April 2014,

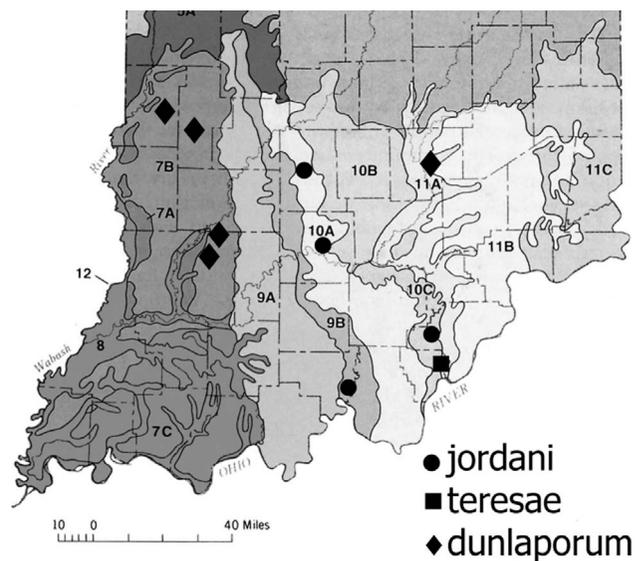


**Figure 1.** Dorsal view of representative Indiana *Caecidotea*: (a) *C. dunlaporum*, n. sp., drain outlet 0.8 mile east of Riley, Vigo County; (b) *C. teresae*, well, Floyd County; (c) *C. jordani*, drain outlet, Burns Hollow, Clark County.

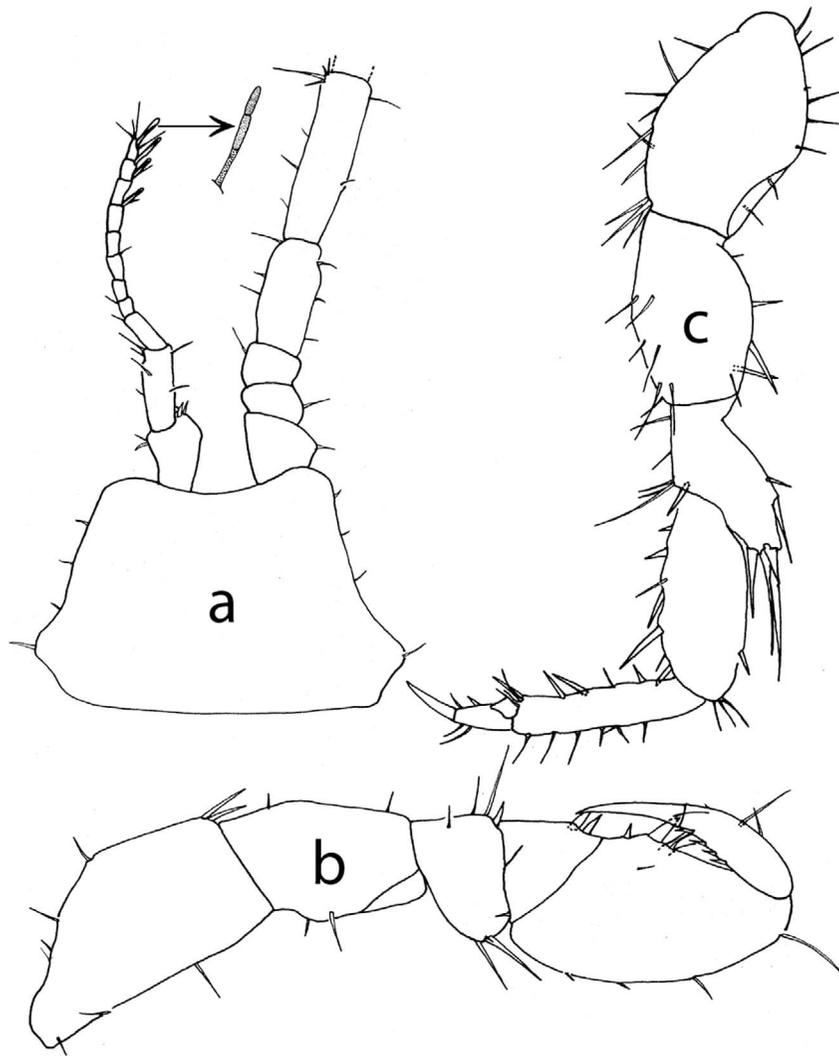
1 male fragment (USNM 1251846); drain outlet near intersection of North Fork of Prairie Creek and CR 775E, 8.7 miles (14 km) NE Washington, 13 April 2014, 1 female (USNM 1251847); drain tile outlet near intersection of Thomson Ditch (North Fork of Prairie Creek) and CR 750N, 9.8 miles (15.8 km) NE Washington, 13 April 2014, 3 males, 6 females (USNM 1251848); **Clay Co.:** drain outlet 2.5 miles (4 km) S of Cory, 8 May 2013, 1 male (USNM 1251845); **Vigo Co.,** drain outlet 0.8 mile (1.3 km) E of Riley on south side of Highway 4 (type-locality: N39°23'24" W87°16'57"), 16 April 2011, 1 male (USNM 1251842); 22 May 2011, 2 males, 1 ovigerous female (USNM 1251843).

A 6.2 mm dissected male from Vigo County is the holotype, the other specimens from that locality are paratypes. All material has been deposited in the collection of the United States National Museum of Natural History, Smithsonian Institution.

*Description of holotype*—Eyeless, unpigmented, 6.2-mm long, body elongate, approximately 5.2× as long as wide. Margins of head, pereonites, and pleotelson moderately



**Figure 2.** Localities of the three *Caecidotea* species endemic to Indiana.



**Figure 3.** *Caecidotea dunlaporum*, new species, Vigo County, Indiana, holotype male: (a) head, antenna 1, proximal articles antenna 2; (b) pereopod 1; (c) pereopod 4.

setose. Head with anterior margin concave, approximately 1.6× as wide as long, post-mandibular lobes moderately produced. Pleotelson approximately 1.5× as long as wide, caudomedial lobe not produced. Antenna 1 flagellum reaching mid-length of distal article of antenna 2 peduncle, with 8 articles, distal 4 with aesthetascs. Antenna 2 last article of peduncle approximately 1.6× length of preceding article, flagellum with 34 articles. Mouthparts per the diagnosis of the genus (Lewis 2009).

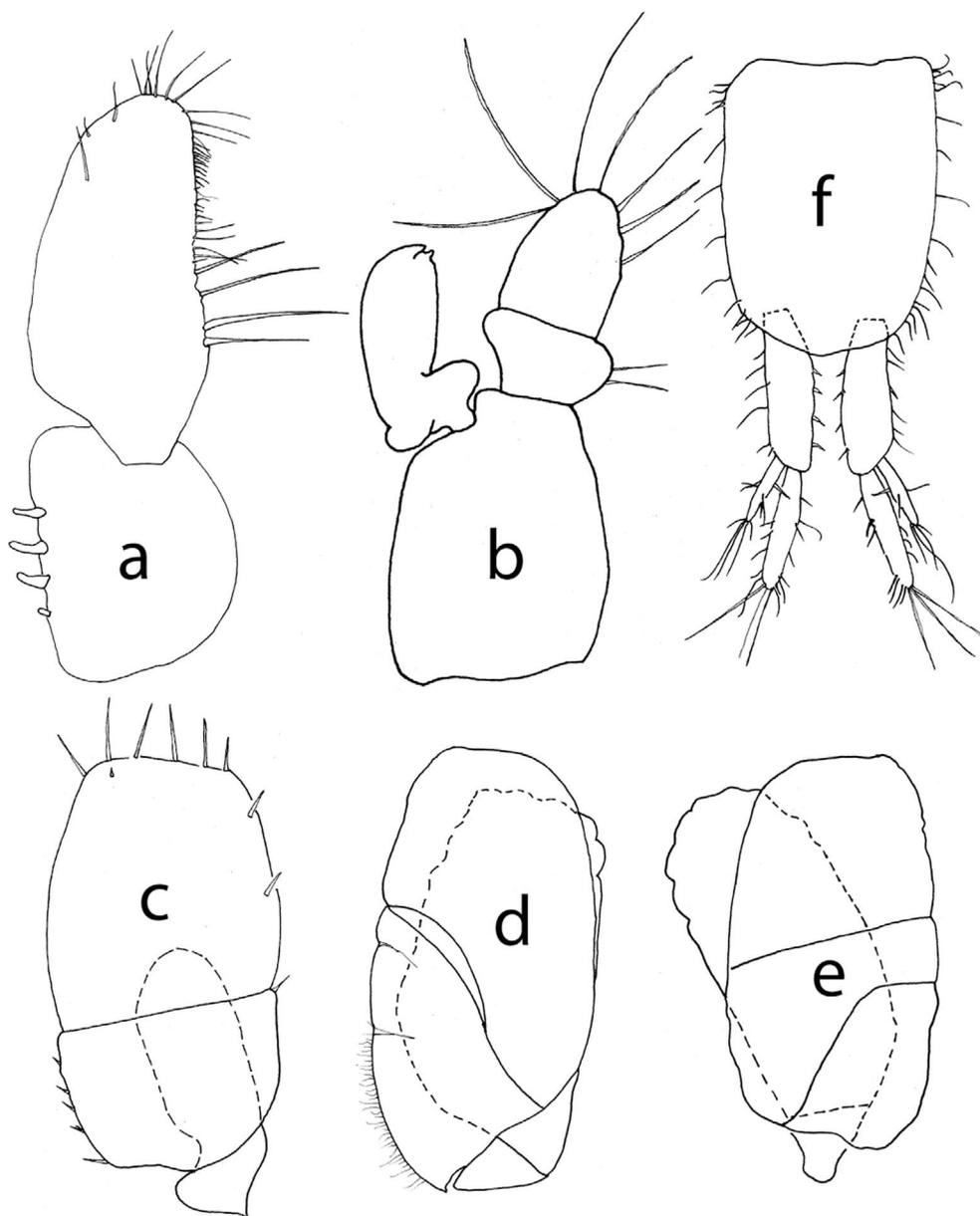
Pereopod 1 propodus subtriangular, approximately 1.7× as long as wide, palmar margin with stout proximo-medial spine. Dactylus extending beyond palmar margin of propodus approximately 1/3 length of carpus. Pereopod 4 as figure 3c.

Pleopod 1 slightly longer than pleopod 2, protopod approximately 0.7× length of exopod, 4 retinacula; exopod approximately 2.2× as long as wide, tapering slightly toward apex, elongate setae on lateral margin.

Pleopod 2, protopod subquadrate, about 1.4× as long as wide; exopod slightly longer than endopod, proximal article with 2 lateral plumose setae, distal article ovate, with 7 plumose setae along margins increasing in size at apex; endopod with broadly rounded basal apophysis, tip with 2 parallel processes extending distolaterally in anterior view: (1) cannula short, conical, (2) lateral process short, extending slightly beyond margin of endopod, apically blunt.

Pleopod 3 exopod, distance proximal to transverse suture about 0.65× length of distal part; 6 elongate plumose setae along distal margin. Pleopod 4 exopod with sigmoid false sutures, proximolateral margin with numerous setules. Pleopod 5 exopod without setation. Uropods slightly shorter than pleotelson, rami shorter than peduncle.

*Remarks*—Among some species of *Caecidotea*, one or more prominent spines along the palmar margin of the male gnathopod are replaced by processes in larger



**Figure 4.** *Caecidotea dunlaporum*, new species, Vigo County, Indiana, holotype male: (a) pleopod 1, (b) pleopod 2, (c) pleopod 3, (d) pleopod 4, (e) pleopod 5, (f) pleotelson and uropods.

specimens. The palmar margin of *C. dunlaporum* has such a large proximal spine, but in none of the material examined was it replaced by a process, including an 8.5 mm male from the Bartholomew Co. sample.

*Etymology*—This species is named in honor of Keith Dunlap and his sister Cynthia Rothrock in recognition of their stewardship of the land and a lifelong dedication to karst conservation. The suggested vernacular name is Dunlap's groundwater isopod.

*Habitat and range*—This species occurs in unconsolidated sediments of glacial origin. In the sites in Vigo, Daviess, and Clay counties, the field drainage systems from which the specimens were collected were placed in loess that is characteristically six feet or more in thickness, with

underlying till (McCarter, 1982; Montgomery, 1974). The drain outlet in Bartholomew County is in soil consisting of loamy glacial outwash (Brownfield, 1976).

A distance of 127 km is spanned by the collection sites. The sites in Vigo, Daviess, and Clay counties occur in the Southwest Lowlands Natural Region associated with the bottomlands of the Wabash River. The region was covered during the Illinoian glaciation and lies about 25 km south of the Wisconsin glacial boundary. The Bartholomew County site is just within this boundary in the Scottsburg Lowlands.

*Ecological notes*—The type-locality was visited on 8 May 2013 for the purpose of collecting additional material for genetic analysis. At that time the water flowing from the drain tile was 11 degrees C. As an indication of the

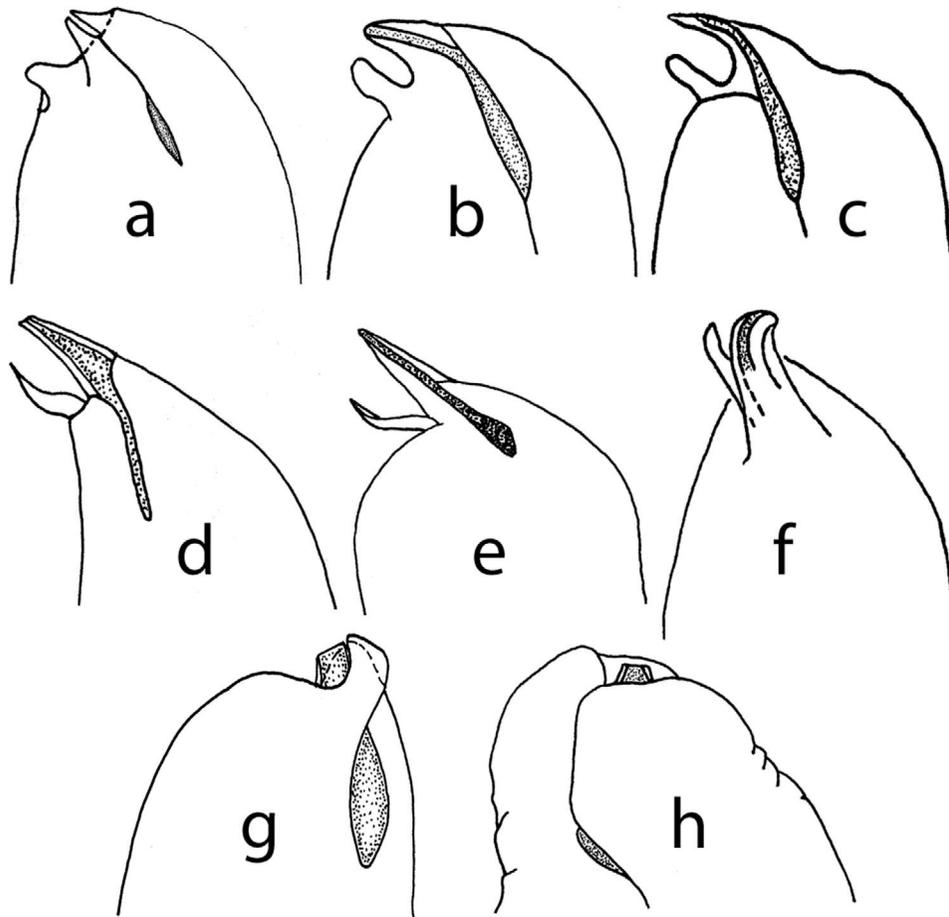


Figure 5. *Caecidotea* male pleopod 2 endopod tips: (a) *C. dunlaporum*, Vigo Co., Indiana; (b) *C. bicrenata*, Hidden River Cave, Hart Co., Kentucky; (c) *C. jordani*, seep, Burns Hollow, Clark Co., Indiana; (d) *C. beattyi*, pipe spring, Dixon Springs, Pope Co., Illinois; (e) *C. rotunda*, Three Raiders Monument Cave, Jefferson Co., Indiana; (f) *C. stygia*, Slider Cave, Jefferson Co., Indiana; (g) *C. teresae*, drain outlet, Indiana University Southeast, Floyd Co., Indiana; (h) *C. kendeighi*, drain outlet 1.4 miles W Nottingham, Wells Co., Indiana.

washout rate of the two macroscopic subterranean species occurring in the outlet, 97 amphipods *Baetruus mucronatus* were present, but only one *C. dunlaporum*. A plankton net placed over the drain outlet for 24 hours (8–9 May 2013) collected a similar number of *Baetruus* and one *Caecidotea*, plus numerous specimens of the groundwater ostracod *Pseudocandona jeanneli*.

The drain outlet in Bartholomew County where *C. dunlaporum* was collected on 11 March 2012 was revisited on 9 May 2013. Remarkably, *C. dunlaporum* was not found, but several dozen *C. kendeighi* were present on the rocks around the pipe opening. This is the only site in Indiana where two subterranean *Caecidotea* species have been reported.

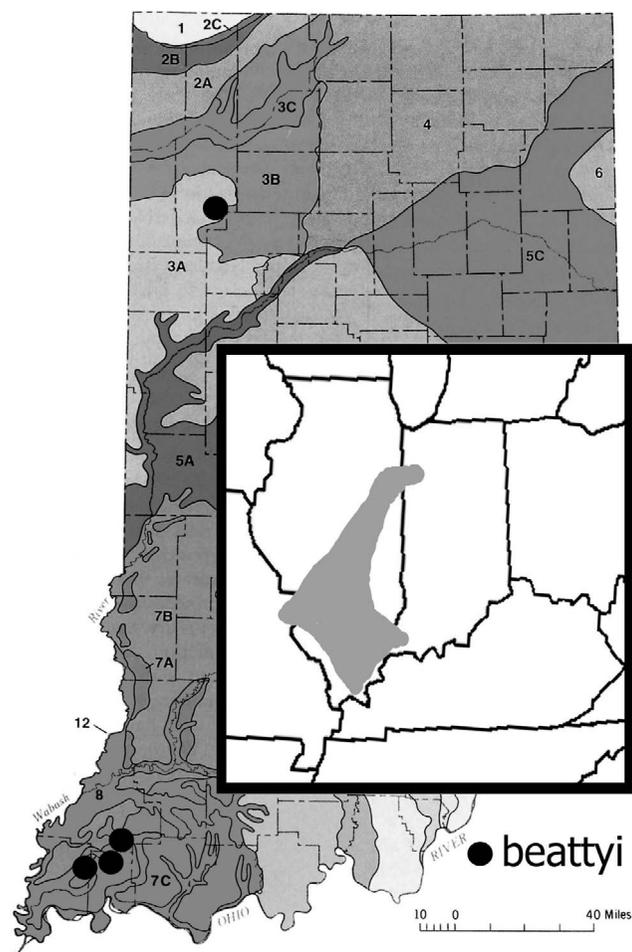
**Conservation status**—Coal strip-mining has caused wholesale destruction of potential habitat and fragmented the range. None of the known localities have any protection, three of which are clustered in the same vicinity. Suggested ranking is S1/G1, critically imperiled.

*Caecidotea beattyi* Lewis and Bowman  
Figures 5d, 6

**Localities**—INDIANA: **Gibson Co.:** drain outlet 2.6 miles (4.2 km) E Haubstadt; **Jasper Co.:** drain outlet 4 miles (6.4 km) SSE Rensselaer; **Posey Co.:** drain outlet 3.9 miles (6.3 km) SE Wadesville; drain outlet 3.5 miles SE Wadesville; **Vanderburgh Co.:** drain outlets 2.3 and 3.7 miles (3.7 and 5.9 km) N Darmstadt.

**Habitat and Range**—In Indiana, all collection sites for *Caecidotea beattyi* have been at the mouths of field-drain outlets where the isopods were emerging from the saturated soil interstices that they inhabit. Lewis and Bowman (1981) reported the isopods from ditches receiving flow from field drains, wells, seeps, and springs. *C. beattyi* has a predilection for occurring in groundwater near the headwaters of surface streams.

The range of *C. beattyi* encompasses the edge of the Ozark Plateau in eastern Missouri, the northern margin of the Gulf Coastal Plain in southern Illinois, and the till plains of the Central Lowland of Illinois. Previously unknown in Indiana,



**Figure 6. Known range and Indiana localities of *Caecidotea beattyi*.**

it was first discovered in 2011 in northwestern Indiana at a drain outlet in Jasper County in the Grand Prairie Natural Region. Shortly thereafter, *C. beattyi* was discovered at drain outlets in three counties in the southwestern corner of Indiana bounded by the bottomlands of the Ohio and Wabash rivers. Over much of its range *C. beattyi* is sympatric with *C. kendeighi*, but the two species are not known to co-occur.

*Vernacular name*—Beatty’s groundwater isopod.

*Conservation status*—*Caecidotea beattyi* is known from a total of 13 localities (six in Indiana) spanning 429 km. The relatively broad range for a subterranean species is deceptive, since this species is sporadic in occurrence, with a penchant for inhabiting groundwater in the headwater zones of streams. Significant portions of the range of *C. beattyi* are heavily impacted by surface coal mining and the use of agricultural pesticides. Suggested status is S2/G2-3, globally imperiled/vulnerable.

*Caecidotea jordani* (Eberly)

Figures 2, 5c

*Localities*—**INDIANA: Clark Co.:** Burns Hollow, 2.6 miles (4.2 km) ESE Borden; **Crawford Co.:** Blue River at Rothrock Mill, 3.4 miles (5.5 km) ENE Leavenworth;

**Lawrence Co.:** drip pool, Chase Cave, Buddha Karst Preserve; **Monroe Co.:** seep spring (type-locality) under Jordan Hall, Indiana University Bloomington (Eberly, 1966)

*Habitat and Range*—Three of the four known localities where *Caecidotea jordani* has been collected are in Indiana’s southcentral karst belt. The type-locality was a seep spring under the Biology Department building at Indiana University. The ground in the sub-basement was treated with termiticides that extirpated the population. In Crawford County, these isopods were found at a parafluvial seep on the bank of the Blue River, as well as a fragment retrieved from a Bou-Rouche sampling well placed a few meters from the seep. The pumpwell was placed one meter deep in a gravel bar that was dry during base-flow conditions on the river. In Chase Cave on one occasion *C. jordani* was found in a drip pool, where the isopods were entering the cave from the epikarst. Outside of the karst, in Clark County, a cluster of three sites in Burns Hollow have produced *C. jordani* from a pipe spring and two seeps.

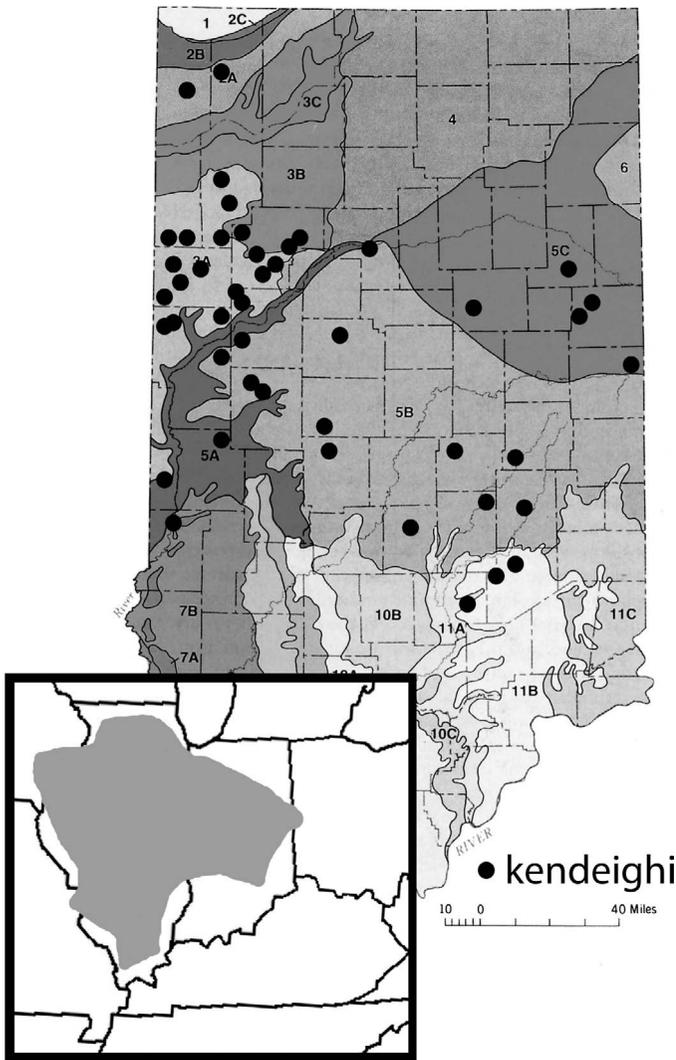
*Vernacular name*—Jordan’s groundwater isopod.

*Conservation status*—The range of this species spans 107 km, including the extirpated population in Monroe County. Within this range, the occurrence of *Caecidotea jordani* is frequently ephemeral, and its seemingly narrow habitat preferences remain poorly understood. Suggested status is S1/G1, critically imperiled.

*Caecidotea kendeighi* (Steeves and Seidenberg)

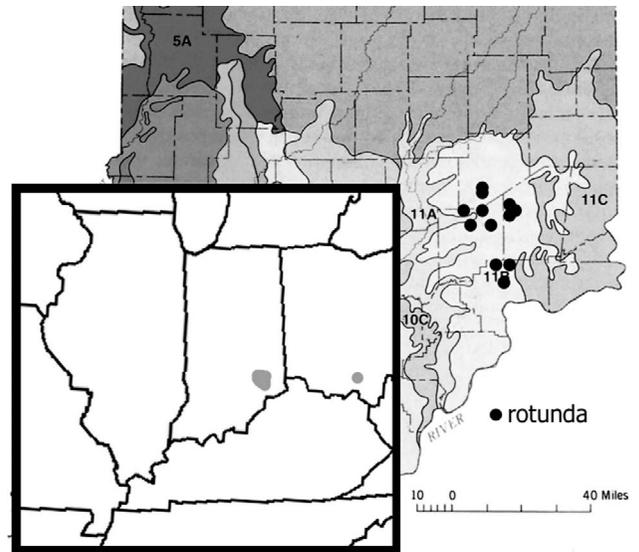
Figures 5h, 7

*Localities*—**INDIANA: Bartholomew Co.:** drain outlet 1.7 miles (2.7 km) N intersection Highways 31/46; **Benton Co.:** drain outlets 2.5 miles (3.9 km) NNE Ambia, 3 miles (4.8 km) N Boswell, 3 miles (4.8 km) WSW Fowler, 0.5 mile (0.8) S Fowler; **Boone Co.:** drain outlet 0.8 mile (1.3 km) SSW Max; **Cass Co.:** drain outlet 0.3 mile (0.5 km) W Poundstone Corner; **Clinton Co.:** drain outlet 0.5 mile (0.8 km) E Sedalia; **Decatur Co.:** drain outlets 1.5 miles (2.4 km) NNW Milford, 2 miles (3.2 km) W Greensburg; **Fountain Co.:** drain outlet at intersection Highways 28/341, 5 miles (8 km) E Attica; **Grant Co.:** drain outlet 5 miles (8 km) W Jonesboro (Koenemann and Holsinger, 2001); **Hancock Co.:** drain outlet 3 miles (4.8 km) NE Maxwell; **Hendricks Co.:** drain outlet 2.5 miles (3.9 km) NE Lizton; **Henry Co.:** drain outlets 3.2 miles (5.1 km) W Knightstown (Koenemann and Holsinger, 2001), 3.8 miles (6.1 km) W Knightstown (Lewis and Bowman, 1981); **Jasper Co.:** drain outlets 5 miles (8 km) NNE Rensselaer, 2.5 (3.9 km) miles SE Rensselaer, 4 miles (6.4 km) N Remington; **Jay Co.:** drain outlets 1 mile (1.6 km) SW Pennville, 1.3 miles (2 km) NE Pennville at head of Haines Creek; **Johnson Co.:** drain outlet 3 miles (4.8 km) NE Whiteland; **Lake Co.:** drain outlet 3.8 miles (6.1 km) SE Merrellville (Lewis and Bowman, 1981); **Montgomery Co.:** drain outlets 1.5 miles (2.4 km) N Manchester, 2 miles (3.2 km) NNW Manchester; **Newton Co.:** drain outlets 2 and 3 miles (3.2 and 4.8 km) SE Kentland, 4.5 miles (7.2 km) ESE Kentland; **Parke Co.:** drain outlet 4 miles (6.4 km) SW Russellville; **Porter**



**Figure 7. Known range and Indiana localities of *Caecidotea kendeighi*.**

**Co.:** drain outlet 0.5 mile (0.8 km) E Deep River (Lewis and Bowman, 1981); **Randolph Co.:** drain outlet 2 miles (3.2 km) WNW Union City; **Rush Co.:** drain outlet junction CR 350W/310S, 5.5 miles (8.9 km) SSW Rushville; **Shelby Co.:** drain outlet 2.5 miles (4 km) ENE Morristown; **Tippecanoe Co.:** drain outlets 1 mile (1.6 km) NE Otterbein, 1 mile (1.6 km) N Montmorenci, 5.8 miles (9.3 km) SSW Montmorenci; **Vermillion Co.:** drain outlet 2 miles (3.2 km) E St. Bernice at junction CR 200S/CR 100W; **Vigo Co.:** drain outlet on Wabash River floodplain 5.7 miles (9.2 km) N Terre Haute; well 9.5 miles (15.2 km) N Terre Haute; **Warren Co.:** drain outlets 0.2 mile (0.3 km) W intersection Highways 26/41, 2.8 miles (4.5 km) W intersection Highways 26/41, 3.5 miles (5.6 km) SSW Otterbein; **Wells Co.:** drain outlet 1.4 miles (2.3 km) W Nottingham; **White Co.:** drain outlets 3.3 miles (5.3 km) W Brookston, 3.5 miles (5.6 km) WSW Brookston, 6.5 miles (10.5 km) SSE Wolcott on Bell Ditch, 3.1 miles (5 km) ENE Wolcott, 4 miles (6.4 km)



**Figure 8. Known range and Indiana localities of *Caecidotea rotunda*.**

SW Reynolds, 4.2 miles (6.8 km) S Reynolds. **OHIO: Darke Co.:** drain outlets on Madison-Coletown Road, 6.3 miles W (10.1 km) and 7.0 (11.3 km) WSW Greenville.

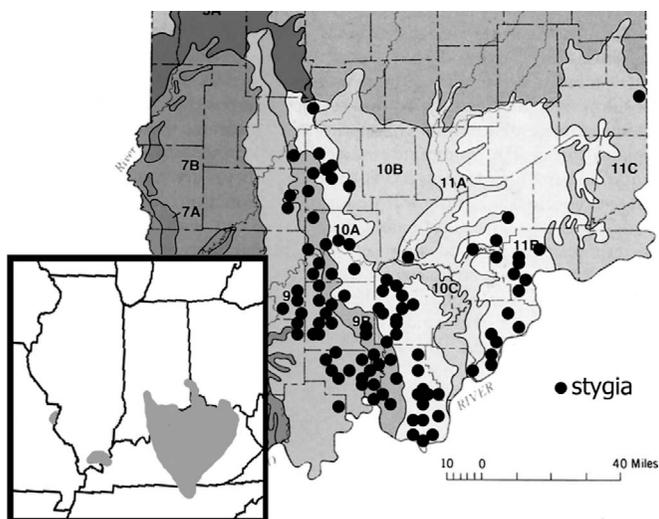
*Habitat and Range*—Lewis and Bowman (1981) discussed at some length the habitat of *Caecidotea kendeighi*. To summarize, the species is an inhabitant of saturated soil interstices, primarily found across glaciated plains from eastern Iowa and Missouri across most of Illinois into Indiana. Lewis and Bowman (1981) reported *C. kendeighi* from three counties in Indiana, to which are now added records from another 24 counties. The Ohio localities reported above are the first for the state.

*Vernacular name*—Kendeigh’s groundwater isopod.

*Conservation status*—The range of *Caecidotea kendeighi* spans almost 500 km. Although apparently secure, concerns for this species include use of agricultural pesticides through much of its range, septic contamination of groundwater, and habitat destruction by surface coal mining and other forms of human encroachment. Sampling suggests that this isopod (as well as *C. beattyi* and *C. dunlaporum*) is absent from many sites where it probably once occurred. In conducting sampling it was not unusual to visit 10 to 20 drain tiles to find one that was inhabited by isopods. In some cases, such as where sewage gray water was emerging from the outlet, the reason for the absence of isopods was obvious, but in other cases the lack of fauna remains a matter of speculation. Suggested status is S4/G4, apparently secure.

*Caecidotea rotunda* Bowman and Lewis  
 Figures 5e, 8

*Localities*—**INDIANA: Decatur Co.:** Faulty and Horse-thief caves; **Jefferson Co.:** BONWR: Charles Bear, Mary Spurgin, Grace Bear Sycamore, John and Daisy Smith, Three Raiders Monument, Edward Prenatt, Glen and Florence Shoots, Sadie, and Juanita Jines caves; **Jennings**



**Figure 9. Known range and Indiana localities of *Caecidotea stygia*.**

**Co.:** Asa and Sarah Edwards (BONWR), Meek, Muscattuck, and Cave Spring (Wyaloosing Creek) caves, spring below homesite on Middlefork Creek (BONWR); **Ripley Co.:** **BONWR:** Thomas and Effie Jessie, Lowell Cooper, Louis Neil, Timber Lodge Spring, and Bernice Chandler caves.

**Habitat and range**—Bowman and Lewis (1984) reported *Caecidotea rotunda* from Frost Cave, Pike County, Ohio (type-locality), and four caves in Decatur and Jennings counties in the southeastern Indiana karst. Hobbs and Hazelton (2011) added four localities in Adams and Highland counties, Ohio. Most of the newly discovered Indiana localities are in Big Oaks National Wildlife Refuge in Jefferson and Ripley counties. This species is an obligate inhabitant of cave streams.

**Vernacular name**—The Ohio population is called the Frost cave isopod in reference to the type-locality. The Indiana populations have been referred to as the Rotund cave isopod.

**Conservation status**—The range of *Caecidotea rotunda* spans 200 km, but Ohio localities are separated from the cluster of Indiana sites by the non-cavernous shale beds at the apex of the broad structural uplift known as the Cincinnati Arch. Thus, it is suspected that in actuality two cavernicolous species are present on opposite sides of the Cincinnati Arch. Research is currently being conducted (Lewis, Victor and Lewis, James, in progress) to ascertain if molecular genetic means can distinguish the two suspected species. As currently understood, *C. rotunda* is known from 19 sites. Suggested status is S2/G2 in Indiana and S1/G2 in Ohio, globally imperiled.

*Caecidotea stygia* Packard

Figures 5f, 9

**Localities**—**INDIANA:** **Clark Co.:** Ballistics Lab (INAAP), Cave Spring, CC Dryer (INAAP), Godecker Spring, Indian, Jenny Lind Run (INAAP), Moore Brothers Spring, Oak Tree (INAAP), Nine Penny Run Shelter, Peyton

Beechwood, Peyton Spring, RDX (INAAP), Pleasant Run #2, Poachers (INAAP), Sunset Village, Thomas Crews, and Twin caves, spring at Falls of the Ohio State Park; **Crawford Co.:** Archibald, Big Circle, Enlows Back Door, Firetail Spring, Hidden Spring, Horseshoe Bend, Hoton Canyon, Little Wildcat, Marengo, Megenity Peccary, Mesmore Spring, Robinson Ladder, Saltpeter, Siberts Well, Wildcat, Wyandotte, and Wymans caves; **Dubois Co.:** Hall Spring and Vowell caves; **Franklin Co.:** drain outlet, Springfield Road 1 mile (1.6 km) S Barth; **Greene Co.:** Ashcraft and Rays caves; **Harrison Co.:** Baker Hollow, Baseball Bat, Beech Tree Lane, Big Mouth Spring, Big Run, Binkley, Black Medusa, Bradford, Bunck, Bussabargers, Cadaver, Carter Byrnes, Devils Graveyard, Earl Williams, George Miles, Hiser Spring, Horsemans Hideaway, Jinglying Spring, Kings, Klinstiver Spring, Limekiln Hollow, Linds, Manhole, Munsons Spring, Otts Endless Agony, Pipe, Paul Wiseman, Pitmans, Seven Springs, Squire Boone, Stygian River, Tabler Spring, Wallier, and Zollmans caves; **Jackson Co.:** drain outlet 5.2 miles (8.4 km) S Vallonia at intersection SR 135 – CR 700S; **Jefferson Co.:** Boyd, Cave Spring, Cricket, Deputy Corner, Double, Gray (BONWR), Hardy, Indian, Mud, Slider, Waterfall, and Wilson caves; **Jennings Co.:** Biehle, Crosley Canyon, Crosley Crack, Garbage Hole, Horsethief, Lowry, Meek, and Skeeter Bluff caves; **Lawrence Co.:** Brick Pit, Buddha, Bug Ear, Burton Hollow, Connerly, Donnehue, Henshaw Bend, Salts, Shawnee System, Shiloh, and Sullivan caves; **Martin Co.:** Anderson Road, Bluff House, Gypsy Bill Allen, Redberry, Rose, and Silent Timber caves; **Monroe Co.:** Frog Pond Fault Pot, Harp Spring, Mayfields, Mays, Patton, and Wayne caves; **Orange Co.:** Apple, Beaver Attack, Black, Boiling Spring, Bond, Cleveland Spring, Critchfield, Concord Spring, Davids Spring, Diggers Delight, Dillon, Double Cola, Duggins Spring, Freeman Spring, Gallmeiers, Garlow Spring, Grease Gravy Road, Hudelson, Jerrys Jewel, Liars Bluff, Little Africa Pleasure Palace, Lost River System, Mount Horeb, Murray Spring, Nine Springs, Not Our Area, One Bat, Peacher, Piggy Pit, Pine Valley Spring, Roland Wetland, Shale Trail, Silent Brook, Smith Brothers, Snaggy Little, Splorin Hole, Spring Springs, Tucker Dam Quarry, Tucker Lake Spring, Wells, William Cleveland, and Wire Trap Spring caves; **Owen Co.:** Boone and Porters caves; **Perry Co.:** Ziegler Cave; **Scott Co.:** drain outlet 6 miles (9.6 km) NNE Austin on North Bogardus Road; **Washington Co.:** Baynes, Benign, Clicks, Charles, Dorsey, Endless, Fredericksburg, Greens Crawl, Karens Pit, May, McNeely Spring, Middle, Mill, Neven Spring, Saltpeter, Suicide, Trinkle, and Waterfall caves.

**Habitat and Range**—*Caecidotea stygia* is primarily an inhabitant of caves. Herein it is reported from drain outlets in unconsolidated deposits (i.e., saturated soil interstices) in Jackson and Scott counties in the Scottsburg Lowland that separate Indiana's southcentral and southeastern karst regions. *C. stygia* was also collected from another drain outlet in Franklin County, adjacent to southwestern Ohio where Bowman and Beckett (1978) reported the isopods from

seepage areas. This isopod is ubiquitous in caves of the Mitchell Karst Plain and Crawford Upland, composing the southcentral Indiana karst, and the southern half of the southeastern karst, where it is replaced by *C. rotunda* in northern Jefferson County. To the west *C. stygia* occurs in eastern Missouri and across the northwestern Interior Low Plateaus physiographic province in disjunct populations associated with the Shawnee Hills of southern Illinois (Lewis and Bowman 1981). The eastern edge of the range is the karst occurring along the edge of the Cumberland Escarpment in Kentucky and northern Tennessee (Lewis, 1982b).

*Vernacular name*—Stygian cave isopod.

*Conservation status*—This is one of the most widespread subterranean isopods in North America, with a range that spans more than 600 km. Suggested ranking is S5/G5, secure.

#### *Caecidotea teresae* Lewis

Figures 2, 5g

*Localities*—**INDIANA: Floyd Co.:** drain outlet below Life Sciences Building at IUS, drain outlet next to small pond by parking lot 5 at IUS, well on Grant Line Road adjacent to IUS campus, and drain outlet near I-265/Grant Line Road interchange.

*Habitat and Range*—*Caecidotea teresae* was an inhabitant of saturated soil interstices, and belonged to an assemblage of species that occurred across the Great Plains region in groundwater habitats (Lewis et al. 2006). The range of this species never exceeded a span of two kilometers and has been enveloped by the Louisville metropolitan area. The ground around the buildings above the type-locality on the campus of Indiana University Southeast, New Albany, was treated with termiticides, and the isopods have not been seen since. The area above the other drain outlet on the campus has been turned into an extensive asphalt parking lot. The well was on a farm that has now been bulldozed for on-going development, and the other drain outlet drains oily water from under an automotive repair shop. The species has been extirpated at all known localities. *C. teresae* was last collected, from the farm well, on 28 April 1995.

*Vernacular name*—IUS groundwater isopod.

*Conservation status*—All populations extirpated and all of the land within the narrow range of *Caecidotea teresae* has been heavily modified by human development. The species is possibly extinct (GX).

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# ESTIMATION OF GROUNDWATER MEAN RESIDENCE TIME IN UNCONFINED KARST AQUIFERS USING RECESSSION CURVES

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**Abstract:** A new parsimonious method is proposed to estimate the mean residence time of groundwater emerging at any specific time during recession periods from karst springs. The method is applicable to unconfined karstic aquifers with no-flow boundaries. The only required data are numerous consecutive spring hydrographs involving a wide range of discharge from high to low flow and the relevant precipitation hyetographs. First, a master recession curve is constructed using the matching-strip method. Then, discharge components corresponding to the individual hydrographs at any desired time are estimated by extrapolation of recession curves based on the master curve. Residence times are also taken from the time elapsed since the events' centroids. Finally, the mean residence time is calculated by a discharge-weighted average. The proposed method was evaluated for the Sheshpeer Spring in Iran. There are 259 sinkholes in the catchment area of the Sheshpeer unconfined aquifer, and all the boundaries are physically no-flow. The mean residence time calculated by the proposed method was about one year longer than that of uranine dye tracer. The tracer mean time is representative of flowing water between the injection and emergence points, but the mean time by the proposed method is representative of all active circulating water throughout the entire aquifer. The proposed method is more appropriate, in practice, especially in groundwater recharge and contaminant vulnerability assessments, than isotopic methods, in cases where a small portion of water with exceptionally high residence time increases the mean. Moreover, isotopic methods are more expensive and not capable of directly determining the residence-time distribution.

## INTRODUCTION

About 20 percent of the Earth's land surface is covered by carbonate-karst formations (White, 1988), and roughly 20 to 25 percent of the global population depends largely or entirely on karst groundwater (Ford and Williams, 2007). Carbonate karst comprises about 11 percent (~185,000 km<sup>2</sup>) of Iran's land surface (Raeisi and Kowsar, 1997) and is one of the major fresh-water resources of the country.

Groundwater residence time is defined as the elapsed time for a water molecule to travel from the recharge area to the discharge zone of the aquifer (Kazemi et al., 2006; Bethke and Johnson, 2008). Water emerging from a karst spring is composed of billions of water molecules from different precipitation events. Karst aquifer recharge water flows rapidly through conduits and fractures and very slowly through the small pores and fissures where most of the recharged water may be stored for a long period, which is especially the case in Iran. Consequently, water with a wide range of residence times may be observed in the spring water. The residence-time distribution represents the distribution of different times, which is calculated by mathematical age-transport modeling (Kazemi et al., 2006). According to the age-mass concept (Goode, 1996), groundwater mean

residence time (MRT) is the mass-weighted average residence time of all water emerging at a specific time. Knowledge of groundwater residence time is an invaluable tool for contaminant vulnerability assessment and evaluation of aquifer recharge rate during karst-water exploitation studies.

The MRTs of groundwater in karst aquifers have been estimated routinely by environmental and artificial tracers. Radioisotopes are the commonly used environmental tracer for this purpose. Considering the amount of a radioisotope in precipitation as input and in spring water as output, mathematical lumped-parameter (e.g. piston flow, dispersion, etc.) models have been used to estimate the MRT (e.g. Maloszewski, 1994).

Fluorescent dyes are the most practical and widely used artificial tracers in karst aquifers (Benischke et al., 2007). The mean transit time of dye can be approximated as the time difference between the injection and the centroid of the tracer-breakthrough curve (Benischke et al., 2007). For a sink-to-spring tracer test, the mean transit time represents the MRT for the part of the spring water that flows from the injection point during the conditions of the test.

Worthington (2007) reported that MRTs determined from environmental tracers are typically one hundred times longer than the mean transit times of artificial tracers in

karstic aquifers. He concluded that the artificial tracers gave velocities and residence times for conduit flow, while environmental tracers gave the average flow velocities and MRTs of groundwater as a whole, including not only rapid flow through the conduits, but also the slow flow through the matrix and fractures, as well as the soil cover and epikarst.

Methods of determining mean residence time using either environmental or artificial tracers are expensive, and the procedure is usually cumbersome; and therefore, innovative approaches of MRT estimation using readily available data are of interest. For example, Vitvar et al. (2002) developed a method for estimation of baseflow MRT from an expression of soil contact time, using mean values of subsurface hydraulic conductivity and storage coefficient derived by hydrograph-recession characteristics. Also, Lalbat et al. (2007) proposed a simple hydrochemical indicator based on the concentrations of some major ions for estimating groundwater MRT in carbonate aquifers.

In this study, a method is proposed to determine the MRT of groundwater emerging during recession periods from a karst aquifer with a special boundary condition. The method uses the age-mass concept (Goode, 1996) to develop a new MRT-estimation method. The concept has been already used in the MRT calculations for simple mixing of hypothetical water components with known residence times (e.g., Kazemi et al., 2006, pp. 11–12; Bethke and Johnson, 2008, p. 131–132) and for age-transport modeling (e.g., Castro and Goblet, 2005; Doyon and Molson, 2012). Groundwater components with known residence times were detected using hydrograph recession and precipitation hyetograph data.

Recession curves have been utilized for different aims in karst hydrogeology, such as determination of hydraulic parameters (Atkinson, 1977; Baedke and Krothe, 2001), aquifer dynamic volume (Ford and Williams, 2007) and groundwater budget parameters (Raeisi, 2008), as well as hydrograph decomposition (Mangin, 1975; Atkinson, 1977).

The average hydraulic behavior of an aquifer from high to sustained, low flows can be also assessed using the master recession curve, which is a combination of various recession curves. In surface-water hydrology, constructing the master recession curve is a routine step in base-flow studies. However, the master curve has been also constructed and used for karst springs (e.g., Angelini and Dragoni, 1997; Desmarais and Rojstaczer, 2002; Posavec et al., 2006, 2010; Doctor, 2008; Malík and Vojtková, 2012; Gregor and Malík, 2012).

There are three principal methods to construct a master recession curve, including matching-strip (Snyder, 1939), tabulating (Johnson and Dils, 1956) and correlation (Langbein, 1938). Toebes and Strang (1964), Nathan and McMahon (1990), and Sujono et al. (2004) reviewed the methods, highlighting their advantages and disadvantages, and providing some examples as well. In this study, the

matching-strip method was preferred, because of its simplicity, visibility of data irregularities, available automated computation tools, and wide usage in the karst-aquifer context. However, other methods of constructing the master recession curve can be applied as well, if one prefers them because of better fitting statistics.

In the matching-strip method, recession curves are superimposed and adjusted horizontally until the main parts overlap; the mean line through the overlapping parts is the master recession curve (Toebes and Strang, 1964). Formerly, this method has been carried out manually by plotting of individual recessions on tracing papers. However, automated techniques have been developed in recent years (e.g., Lamb and Beven, 1997; Rutledge, 1998; Posavec et al., 2006, 2010; Gregor and Malík, 2012) that significantly facilitate the procedure.

Different mathematical functions based on empirical relationships and exact or approximate analytical solutions have been used to describe karst springs' recession curves (see Dewandel et al., 2003; Fiorillo, 2014). Semi-log plots of recession curves, i.e.,  $\log[\text{discharge}]$  versus time, for many karst springs show two or more linear segments with decreasing recession coefficients (e.g., Milanovic, 1976; White, 1988; Baedke and Krothe, 2001; Ford and Williams, 2007), suggesting piecewise exponential functions for the fitting. In this study, the master recession curve is also defined by a piecewise exponential function, composed of  $m$  segments ending at times  $t_j$  with  $m$  recession coefficients  $\alpha_j = 1 \text{ to } m$  and base discharges  $Q_0, j = 1 \text{ to } m$ .

$$Q_t = \begin{cases} Q_{0(1)}e^{-\alpha_1 t}, & t \leq t_1, Q_t \geq Q_1 \\ Q_{0(2)}e^{-\alpha_2 t}, & t_1 < t \leq t_2, Q_1 > Q_t \geq Q_2 \\ \vdots & \vdots \\ Q_{0(m-1)}e^{-\alpha_{m-1} t}, & t_{m-2} < t \leq t_{m-1}, Q_{m-2} > Q_t \geq Q_{m-1} \\ Q_{0(m)}e^{-\alpha_m t}, & t > t_{m-1}, Q_t < Q_{m-1} \end{cases} \quad (1)$$

Equation (1) implies that the master recession curve is represented with  $m$  straight lines with slopes of  $-\alpha_j$  on a semi-log scale (see Figure 4b for a simple example). Segmentation of recession curves has been justified by changes in flow regimes (Milanovic, 1976; Baedke and Krothe, 2001), catchment area, and effective porosity of the declining saturated zone (Bonacci, 1993; Fiorillo, 2011).

## STUDY SITE

To examine the proposed method of mean residence time estimation, the Sheshpeer Spring (Fig. 1) 80 km northwest of Shiraz in the Zagros Mountain Range, south-central Iran, was considered. The Zagros orogenic



**Figure 1. The Sheshpeer Spring, Zagros Mountains, Iran.**

belt consists of three parallel northeast-southwest trending tectonic subdivisions, the Urumieh-Dokhtar Magmatic Assemblage, the Zagros Imbricate Zone, and the Zagros Fold-Thrust Belt (Alavi, 2007). Stratigraphy, lithology, and tectonic settings of the Zagros lithostratigraphic units are fairly well described in James and Wynd (1965), Falcon (1974), Stöcklin and Setudehnia (1971), and Alavi (1994, 2007). The Sheshpeer karst aquifer is situated at the border of the Zagros Fold-Thrust Belt and Imbricate Zone. Extensive hydrogeological researches have been carried out on the Sheshpeer aquifer (Pezeshkpour, 1991; Karami, 1993; Raeisi et al., 1993, 1999; Raeisi and Karami, 1996, 1997; Raeisi, 2008, 2010).

The Sheshpeer aquifer is composed of the calcareous Sarvak karstic formation (Albian-Turonian) in the northern flank of the Barm-Firooz and Gar anticlines and some portion of the southern flank of the Barm-Firooz anticline (Raeisi et al., 1993; Raeisi, 2008; Fig. 2). The anticlines are extended in the general direction of the Zagros Mountain Range and are connected by a saddle-shaped plunge. The exposed cores of the anticlines are mainly composed of the Sarvak Formation, underlain and overlain by impermeable shale and marl layers of the Kazhdumi (Albian-Cenomanian) and Pabdeh-Gurpi (Santonian-Oligocene) Formations, respectively. The most important tectonic feature is a major northwest-southwest trending thrust fault (Fig. 2). The northern flank of the anticlines has been brought up by tectonic forces, and the southern flank has been brecciated so that it's either completely removed or crops out as large rockslide blocks. Several normal and strike-slip faults are also present. The overall tectonic setting of the area has produced suitable conditions for extensive karstification (Raeisi and Karami, 1996, 1997; Raeisi, 2010).

The karst features of the study area are karrens, grikes, small caves, sinkholes, and springs. The most important karst feature is the presence of 259 sinkholes in the Gar and Barm-Firooz Mountains, lined up in a narrow zone on top of the northern flanks from the northern end of the catchment area to near the Sheshpeer Spring (Fig. 2). The sinkholes are primarily of the collapse type and coincide evidently with the direction of longitudinal faults. The biggest cave in the catchment area has a length of twenty meters and is located along a fracture (Raeisi, 2010). Out of twelve springs emerging from the Sarvak karstic formation in the Gar and Barm-Firooz anticlines, only Sheshpeer Spring, with a mean annual discharge of  $3247 \text{ L s}^{-1}$ , is located on the northern flanks (Fig. 2). Berghan Spring is the largest spring in the southern flanks (Fig. 2) and has a mean annual discharge of  $632 \text{ L s}^{-1}$ . The mean annual discharges for the other springs range from 1.41 to  $68.34 \text{ L s}^{-1}$  (Raeisi and Karami, 1996).

All boundaries of the Sheshpeer aquifer are physically no-flow, and all recharged water only emerges via the Sheshpeer Spring (Pezeshkpour, 1991). Groundwater balance indicated a catchment area of about  $81 \text{ km}^2$  for the Sheshpeer Spring (Raeisi et al., 1993), which is in accordance with the area bounded by geological formations. The aquifer boundaries were drawn based on the following observations (Raeisi, 2008): (1) The northern flank of the Gar and Barm-Firooz anticlines have been brought up by tectonic stresses, such that the aquifers of the northern and southern flanks have been disconnected by the underlying impermeable Khazdumi Formation that crops out in some parts of the anticline core (Fig. 2). Tracer tests have confirmed this hydrogeological disconnection. (2) The northwest and northeast sides of the catchment area are bounded by the impermeable Pabdeh-Gurpi Formations. Groundwater flow through the Sarvak Formation under these formations is not possible, because karstification usually does not occur under an 800-m thick layer of the Pabdeh-Gurpi Formations, there are no outcrops of the Sarvak Formation in adjacent parallel anticlines, and tracer tests confirmed the hydrogeological disconnection with the springs of the adjacent anticlines. (3) Sinkholes are only located in the catchment area of the Sheshpeer Spring, and the dye tracer injected in a sinkhole 18 km away (Fig. 2) only emerged from this spring, as described below.

In order to characterize the function of the Gar and Barm-Firooz karst systems, a tracer test was performed by Raeisi et al. (1999) during the recession period of the year 1992. Sodium fluorescein (uranine) dye was injected into a sinkhole in the Barm-Firooz Mountain, 18 km away from the Sheshpeer Spring (Fig. 2). The inflow of snowmelt to the sinkhole was about  $150 \text{ L s}^{-1}$  at the time of injection. The dye was detected only in the Sheshpeer Spring, and most of it was recovered before the recession was interrupted (Fig. 3). The first appearance, peak concentration, and centroid times of the tracer breakthrough curve

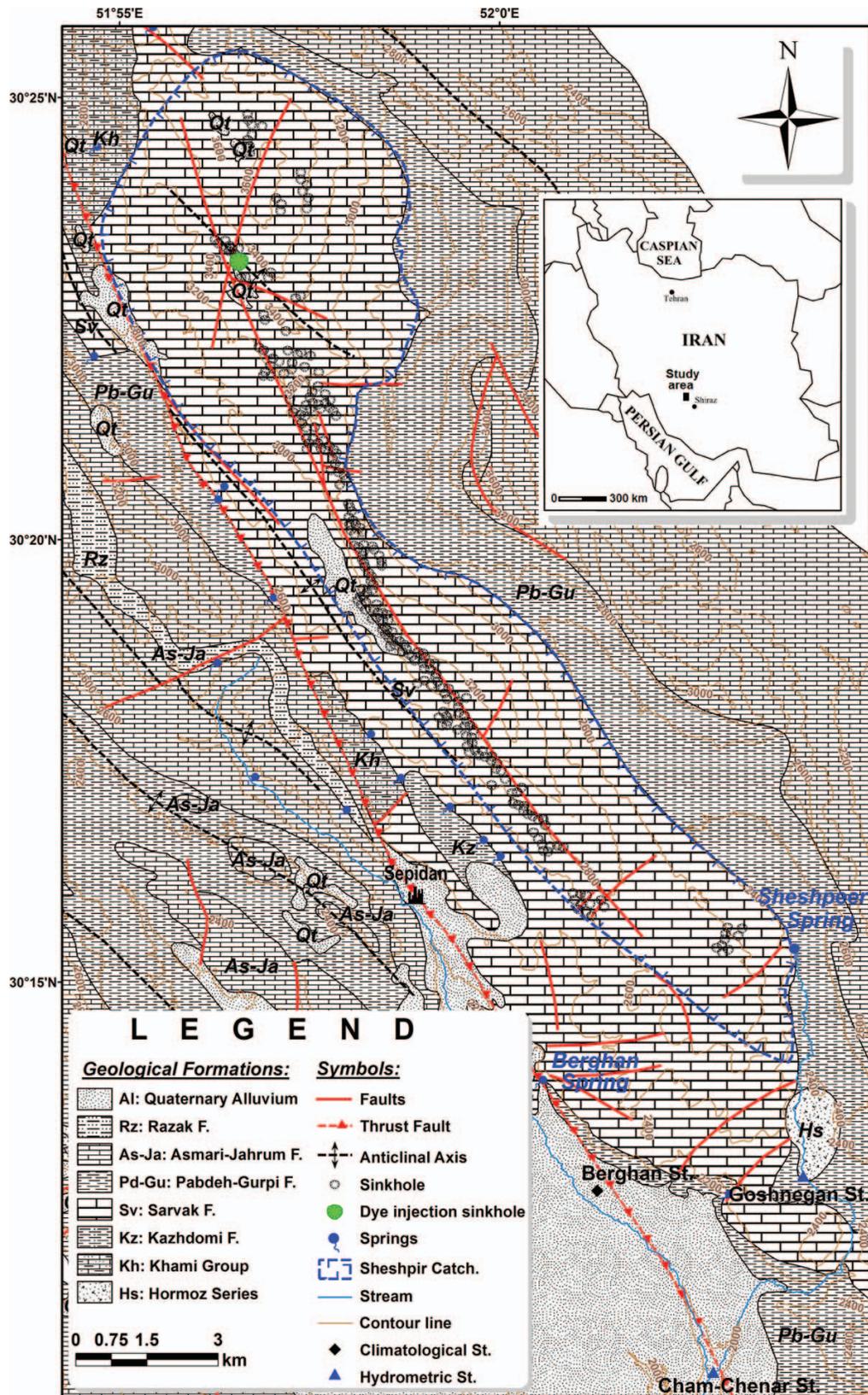
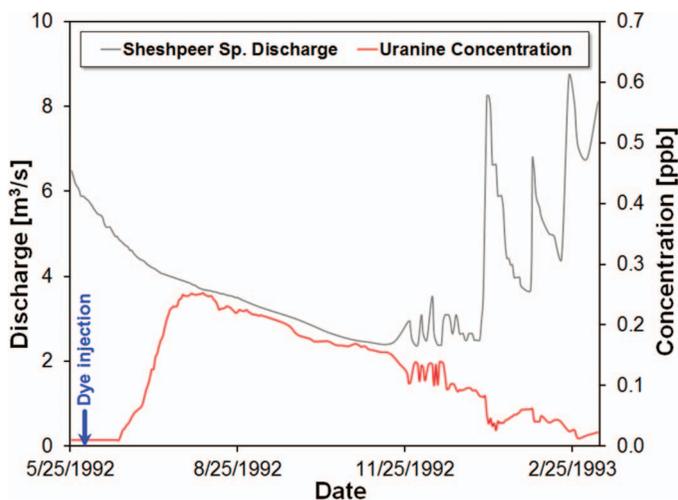


Figure 2. Hydrogeological map of the study area. The recharge area of Sheshpeer Spring is outlined in blue. Besides gauging information from Sheshpeer Spring, stage data from the Goshnegan and Cham-Chenar stations (shown as blue triangles) and precipitation data from Berghan station (shown as a black rhombus) were used in the calculations.



**Figure 3.** Tracer breakthrough curve for a dye trace during the 1992 recession period, plotted along with the spring discharge.

were estimated to be about 19, 64, and 115 days, respectively. Accordingly, the maximum, dominant, and mean linear flow velocities were calculated to be about 40, 12 and  $7 \text{ m h}^{-1}$ , respectively; which are within the range of conduit flow, as is expected from a sink-to-spring tracer test.

## METHODS

The proposed method estimates the mean residence time of groundwater leaving via springs or underground outflows to neighboring aquifers during recession period of an unconfined karst aquifer with no-flow boundaries. The no-flow boundaries can consist of impermeable layers or groundwater divides.

Recharge water might be stored for a long period within the aquifer, gradually discharging through the spring, hence the measured hydrograph is regarded here as a composite hydrograph that is a combination of several consecutive individual hydrographs caused by numerous previous precipitation events. Figure 4a shows a hypothetical spring composite hydrograph that is decomposed to its individuals by graphical extrapolation of recession curves.

At any specific time ( $T$ ) during the recessions, the measured discharge is the summation of discharge components ( $\Delta Q_{(i)}$ ) that are the contributions of the individual extrapolated hydrographs to time  $T$ . The discharge component corresponding to the fifth precipitation event at time  $T$ ,  $\Delta Q_{(5)}$ , is indicated in Figure 4a. The residence time for each discharge component at the time,  $tR_{(i)}$ , is defined as the time elapsed since the centroid of precipitation event  $i$ . The residence times for the different discharge components at time  $T$  are indicated in Figure 4a.

Once the discharge components and residence times corresponded to the all individual extrapolated hydro-

graphs are calculated for time  $T$ , the mean residence time ( $\bar{t}_R$ ) at the time is calculated as the discharge-weighted average residence times,

$$\bar{t}_R = \frac{1}{Q_T} \sum_{i=1}^n (t_{R(i)} \times \Delta Q_{(i)}) \quad (2)$$

where  $Q_T$  is the measured total spring discharge at time  $T$  on the composite hydrograph, equal to the sum of the discharge components, and  $i$  from 1 to  $n$  is the number of a preceding precipitation event, from oldest to more recent.

Equation 2 implies that the estimated MRT at time  $T$  is controlled by the total spring discharge  $Q_T$ , the residence times, and the discharge components. The total spring discharge is directly measured; high accuracy in discharge measurement can be achieved using high-tech devices. Residence times need the precipitations' centroids to be determined and can be estimated fairly well using high-frequency (e.g. daily) precipitation data. Therefore, the most uncertain parameters in the MRT calculation are the discharge components, which are based on the recession-curve extrapolations. Discharge components are estimated by graphical extrapolation of recessions in Fig. 4a. However, this method is based on subjective judgment and does not consider the characteristics of the aquifer. The recessional behavior depends on the lithology, porosity, degree of karstification, and type of flow regime, as well as the spatial and temporal characteristics of the precipitation.

A master recession curve is considered as the most probable recession scenario under a given situation (Nathan and McMahon, 1990), and it is constructed using all prior recorded recessions, from high to low flows. Therefore, it is an index of the particular aquifer's recessional behavior and is used here to extrapolate the recession curves, decompose the composite hydrograph, and estimate the discharge components.

In this study, the matching-strip method was applied to construct a segmented exponential master recession curve. Figure 4b presents a typical curve composed of three exponential segments with decreasing recession coefficients.

Because we are only interested in the recessions, the rising limbs are ignored in assembling the composite curves in Figure 4c. Since the figure is a semi-log plot, even the measured parts of the individual recession curves are approximated as segmented straight lines. To extrapolate from the last measured discharge on each recession curve, such as  $Q_{e(5)}$  in Figure 4, the portion of the master curve below that value is shifted and appended, extending to time  $T$ . The extrapolated straight line segments are shown dashed in Figure 4c.

After extrapolation of all the recession curves to time  $T$ , the discharge due to each individual event is the difference between successive curves. The hatched area on Figure 4c

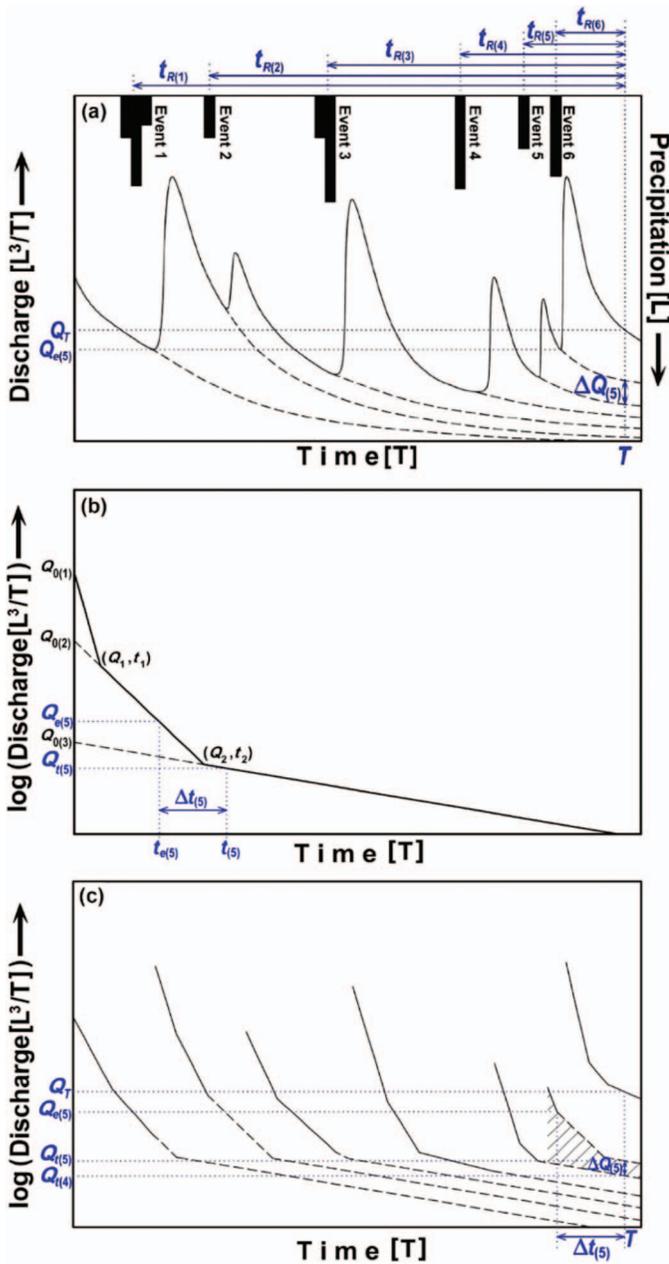


Figure 4. Diagrams showing the principle of the method, using hypothetical discharge curves and precipitation events. At time  $T$  during the recession following rainfall event 6, the total flow  $Q_T$  is decomposed into components due to several recent events, such as  $\Delta Q_{(5)}$  due to event 5, by extrapolating the recession curves of the various events using a master recession curve approximated by a series of exponential decline segments. Part (a) shows the assumed data and the precipitation events on a linear scale. Extrapolated recession curves are graphically appended. Part (b) shows segments of the master recession curve, which become straight lines on the semi-log plot; the notation is that of Equation 1.  $Q_{e(5)}$  is the flow at the end of the recession following event 5 at master-recession-curve equivalent time  $t_{e(5)}$ , and  $Q_{t(5)}$  is the inferred flow of the extrapolated event 5 recession at time  $T$ ,

represents the volume of discharged water due to the fifth precipitation event, since the hydrograph peak. At time  $T$ , the discharge component from the fifth precipitation event,  $\Delta Q_{(5)}$ , is shown in Figure 4c as the difference between the extrapolated discharges  $Q_{t(5)}$  and  $Q_{t(4)}$ .

The residence time corresponding to each discharge component at time  $T$ , i.e.  $t_{R(i)}$ , is calculated as the time difference between the relevant precipitation centroid and time  $T$ , as previously described. Finally, the mean residence time at time  $T$  is estimated using Equation 2.

## RESULTS AND DISCUSSION

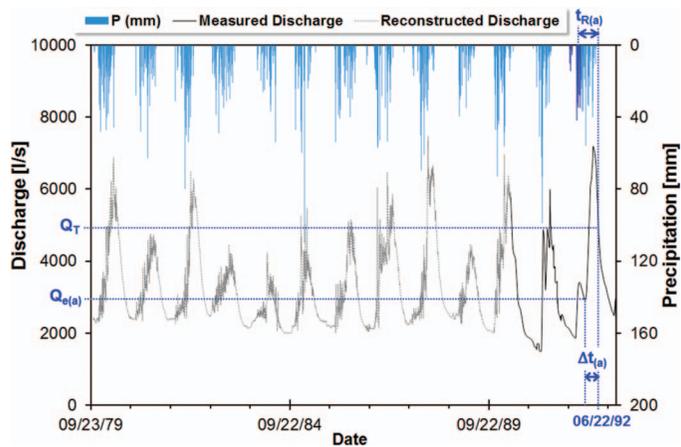
The hydrograph data of the Sheshpeer Spring were only measured for three hydrological years from 1990 to 1992 and contain five hydrograph recessions. These hydrograph data are not enough to apply the proposed method. Therefore, a multiple time series regression model (Phillips and Durlauf, 1986) was applied to reconstruct spring discharge from 1979 to 1989, using daily data of two hydrometric stations and one meteorological station, for use in addition to the measured Sheshpeer Spring hydrographs (Fig. 5).

Daily precipitation at the Berghan meteorological station (Fig. 2) is available for almost 51 years. The Goshnegan and Cham-Chenar hydrometric stations (Fig. 2), with 30 and 20 years of daily discharge data, are located about five and ten kilometers downstream of the Sheshpeer Spring, respectively. Goshnegan is the best hydrometric station to monitor the Sheshpeer Spring discharge other than a gage at the spring itself. Flood water from a small area of Pabdeh-Gurpi formations reaches the station during high flows, but there is no inflow during dry seasons. Discharge at the Goshnegan station was measured daily from 1979 to 1986, but the station was ungauged from 1987 to 1992.

The multiple time series regression model (Phillips and Durlauf, 1986) was used to find the relationship between the measured seven years of daily discharge data from Goshnegan Station (1979 to 1986) with the daily discharge data from the Cham-Chenar Station and the daily precipitation at the Berghan station. The adjusted  $R^2$  was 0.98, which is very close to 1, showing the accuracy available for reconstructing the missing six years (1987 to 1992) of daily discharge at the Goshnegan Station using that regression model.

←

which is  $\Delta t_{(5)}$  after the end of the event 5 recession (not be confused with  $t_{R(5)}$  in part (a), which is the time between the centroid of event 5 and  $T$ ). Part (c) is like part (a) except that the extrapolations are based on the master recession curve, and the recession parts of the hypothetical discharge curves, together with their extrapolations, are only shown. Notice that the plot is on semi-log scale.

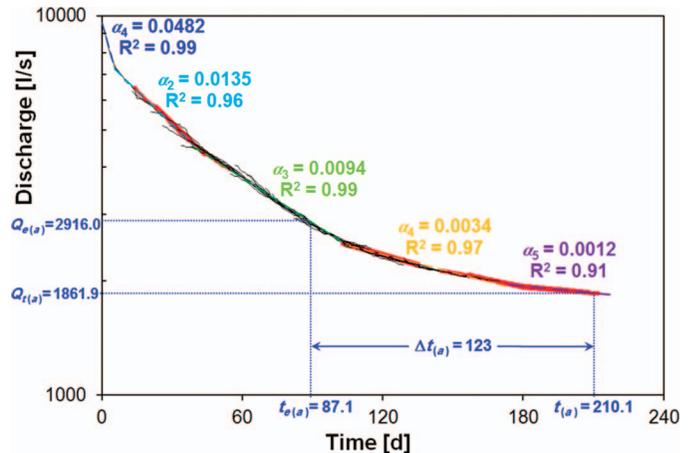


**Figure 5.** Measured discharge at Sheshpeer Spring for 1990–1992 (solid line) and discharge inferred from other data for 1979–1989 (dotted line). Notations are the same as those in Figure 4, with (a) replacing (5) for the first event of the year 1992. June 22, 1992, is the date for which the calculations are described in detail in the text. Also shown is the rainfall record for the period, on an inverted scale.

Daily Sheshpeer Spring discharge from 1979 through 1989 were subsequently reconstructed by the same procedure, using the daily precipitation of Berghan station and discharge at the Cham-Chenar and Goshnegan stations and Sheshpeer Spring. The adjusted  $R^2$  was 0.89, which is again close to 1. The reconstructed data for Sheshpeer Spring are shown in Figure 5, along with the later measured data.

Master recession curve (Fig. 6 and Table 1) was manually constructed using Hydro-Office RC 4.0 Module (Gregor and Malik, 2012), using both measured and reconstructed Sheshpeer discharge data from Figure 5. The curve was fitted by five exponential segments, shown as straight line segments on the semi-log plot in Figure 6, with recession coefficients, time ranges, and discharge ranges for each segment given in Table 1. A good match between the measured discharges for 1990–1992 and the master recession curve indicates the accuracy of data reconstruction.

Table 2 presents the recession coefficients, discharge ranges, and durations of the exponential segment of the



**Figure 6.** The master recession curve for Sheshpeer Spring on a semi-log scale. The master curve shown by the thin black line has been broken into five segments that are straight lines on that scale. The recession coefficients for each segment are indicated. Dotted lines show how  $Q_{e(a)}$ , the final measured discharge for the event (a), and the time between that measurement and  $\Delta t_{e(a)}$ , the time for which calculations are being made, here 123 days, are used to infer from the master curve  $Q_{r(a)}$ , the flow due to event (a) at the later time.

measured recession curves for the years 1990 to 1992 (Pezeshkpour, 1991; Karami, 1993; Raeisi et al., 1993), compared to the corresponding data from the constructed master recession curve. The curve's recession coefficients  $\alpha_1$  to  $\alpha_5$  were 0.0482, 0.0135, 0.0094, 0.0034 and 0.0012  $\text{d}^{-1}$ , respectively. Those corresponding to the lower discharge ranges (i.e.,  $\alpha_2$ ,  $\alpha_3$ ,  $\alpha_4$ , and especially  $\alpha_5$ ), which have significant effects on the mean residence time estimation, are within the range of recession coefficients of the measured recession curves.

As an example of the proposed method, the mean residence time is calculated for a specific time, June 22, 1992, during the second recession of the year 1992 (Fig. 5). Fig. 7 presents the measured recession curves of the Sheshpeer Spring and their extrapolations based on the master recession curve in Figure 6. Detailed calculations of discharge component for only the first recession of year 1992, using the subscript  $a$ , and the corresponding residence time are presented here.

**Table 1.** Sheshpeer spring master recession curve shown as five exponential straight-line segments on the semi-log plot in Figure 6, with time ranges, and discharge ranges for each segment with respect to  $Q_t$ .

Recession Segments	Time Ranges, d	Discharge Ranges, $\text{L s}^{-1}$
$9469.5 e^{-0.0482t}$	$t \leq 5.87$	$Q_t \geq 7137.2$
$7725.2 e^{-0.0135t}$	$5.87 < t \leq 38.26$	$7132.2 > Q_t \geq 4612.3$
$6607.8 e^{-0.0094t}$	$38.26 < t \leq 105.24$	$4612.3 > Q_t \geq 2457.9$
$3529.7 e^{-0.0034t}$	$105.24 < t \leq 172.31$	$2457.9 > Q_t \geq 1951.6$
$2419.0 e^{-0.0012t}$	$t > 172.31$	$Q_t < 1951.6$



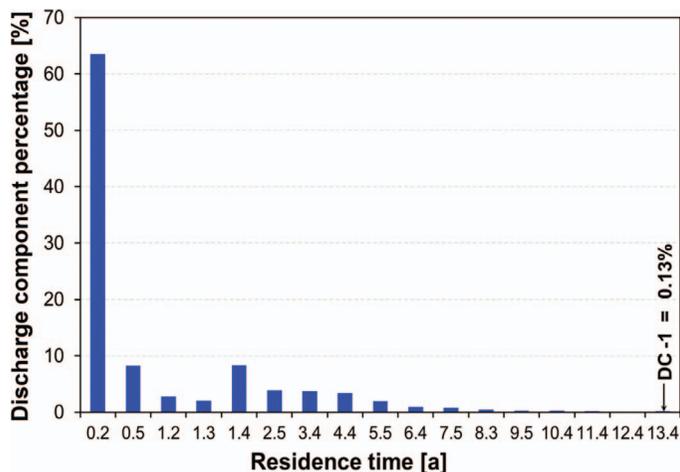


Figure 8. Residence-time distribution on June 22, 1992, based on the decomposing of total discharge on June 22, 1992, into discharge components by the method described in the text and shown in Figure 7. The percentage contribution of each prior recharge event is plotted. Note that the horizontal axis of residence-time (years) is not linear, but simply contains a point for each  $t_{R(i)}$ , the time since the contributing event, as labeled. DC-1, nominally for 1979, includes all water that old or older.

calculating the mean. As the first alternative, the starting time of  $t_{R(1)}$  was considered as the precipitation centroid of 1979, and other mean residence times for that component were assumed to be 100, 500, 1000, or 10000 years. The assumption of a long residence time is reasonable due to the small amount of water that might take a long time to join the main karst conduit because it has been retained in very small pores and fissures of the karst aquifer. Assuming the first alternative the mean residence time of the discharge on June 22, 1992, is estimated to be about 1.1 years using Equation 2. The means for all alternatives are presented in Table 3. The MRT would not change significantly even if the mean age of the oldest component  $t_{R(1)}$  was taken to be 100 years. However, if it was increased

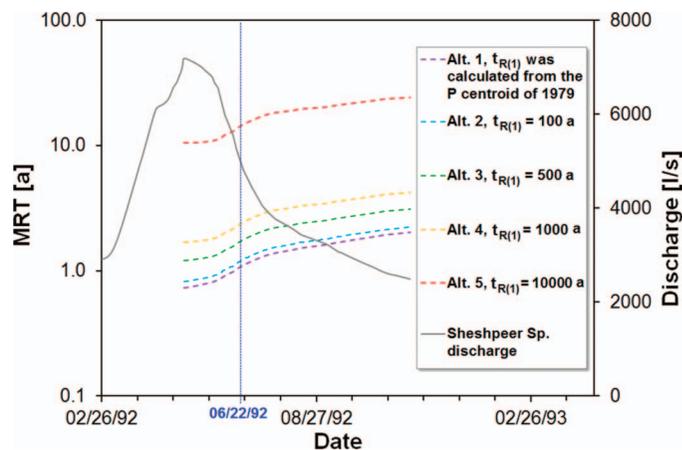


Figure 9. The colored curves show the mean residence time computed for the outflow from Sheshpeer Spring at different times during the second recession of 1992 for different assumptions about the mean age in years of the earliest discharge component. The vertical blue line is the date of the computation discussed in most detail, and its intersections with the colored curves show the data in the left half of Table 3. The vertical extents of the colored curves give the data for the right half of Table 3. Note that the residence-time data are plotted on a logarithmic scale. The total discharge curve for the spring is shown for comparison.

to considerably more than 100 years, the MRT would significantly increase.

The MRTs were also calculated in the same way for different dates, other than June 22, during the second recession period of the year 1992 (Fig. 9). The MRTs increased as the recession proceeded, due to a decrease in the most recent component and the increased residence times. Table 3 shows the minimum, average, and maximum MRTs over the range of end-point dates during the recession for all alternative values of the oldest age. The average MRTs in the table are the mean age of all groundwater emerging from the spring during the entire recession.

Table 3. The approximate calculated mean residence times for June 22, 1992, and throughout the duration of the recession, for various assumed mean yearly ages for the oldest discharge component.

Alternative	Assumed $t_{R(1)}$ , a	MRT June 22, 1992, a	MRT during the second recession period of 1992, a		
			Minimum	Average	Maximum
1	13.4 <sup>a</sup>	1.1	0.7	1.4	2.0
2	100	1.2	0.8	1.5	2.2
3	500	1.7	1.2	2.1	3.1
4	1000	2.4	1.7	2.9	4.2
5	10000	14.1	10.5	17.4	24.2

<sup>a</sup> Calculated from the precipitation centroid of 1979, and was equal to 4906.1 days (ca. 13.4 years), for June 22, 1992

Recalculation of MRTs for different dates during the recession period also confirms that the MRT would significantly increase if the oldest water was assumed to be considerably older than 100 years. The percentage contribution of the oldest discharge component during the recession period was also calculated over that range of end dates, and its minimum, average and maximum were 0.10, 0.16 and 0.22 percent, respectively. Therefore, it can be concluded that the proposed method estimates, at least, the residence times of 99.78 percent of the total spring discharge. The precision of the MRT would be improved if the number of hydrographs increased, therefore, reducing the amount of discharge of unknown age. The portion of spring-water with uncertain residence times in our study is a very small amount and it doesn't have a significant impact for most practical applications such as groundwater exploitation studies.

Since a mean age of more than 100 years is very unlikely for the oldest discharge component, the mean residence time of Sheshpeer Spring discharge during the second recession period of the year 1992 most likely ranged from 0.7 to 2.2 years (see Table 3).

The mean transit time of uranine tracer, which is approximated as the time difference between the injection and the centroid of the breakthrough curve, was calculated to be about 112 days for the Sheshpeer Spring during the 1992 recession (Fig. 3). The average of the MRTs calculated by our method from the dye injection date to the end of recession is estimated to be about 1.4 or 1.5 years. The dye's mean transit time of 112 days is smaller than our calculated value because the dye was directly injected to the conduit system via a sinkhole with inflow rate of  $150 \text{ L s}^{-1}$ , and it is representative for a special groundwater flow path between the injection point and Sheshpeer Spring, whereas our calculation applies to the entire aquifer, including water paths through the aquifer matrix and conduits, as well as the soil, epikarst, and vadose zones.

A qualitative comparison highlights some superiorities of the proposed method over the conventional isotopic methods: (a) The proposed method is very parsimonious, such that the only required data are the spring hydrograph and the relevant precipitation hyetograph, which have been frequently recorded for many karst springs even in developing countries. (b) The mean residence time and the residence-time distribution at any specific time during a hydrograph recession can be determined. (c) The proposed method directly estimates the residence-time distribution of spring-water; previously the only method to determine this was mathematical modeling based on the solution of age-transport equations (Kazemi et al., 2006). (d) In some cases, a very small portion of water with exceptionally high residence time can dramatically increase the mean obtained by isotopic methods. Those methods are correct in principle, but the estimated MRT is misleading, as it is not representative of the bulk of the emerging water.

Therefore, the proposed method is more practical for all cases in which the residence time of a small portion of the water is unimportant, such as groundwater-recharge estimation and contaminant-vulnerability assessment.

## CONCLUSIONS

A new method is proposed to estimate the mean residence time of water emerging from karst springs during recession periods. The method is based on a discharge-weighted average formula (Eq. 2), which needs the size of various discharge components and their residence times relative to the target time. The discharge components can be estimated by decomposition of the measured composite hydrograph into the individual hydrographs from preceding precipitation events using a master recession curve, and the residence time can be taken as the time elapsed since the event's centroid. The proposed method of MRT estimation was successfully applied to the Sheshpeer Spring. The number of available previous hydrographs was limited for this case study. The greater the number of recession hydrographs over a range from high to low flows available, the greater the reliability of the result.

The proposed method is capable of estimating the MRT for unconfined karstic aquifers with no-flow boundaries. This hydrogeological setting is typical for the Zagros region anticlines, where carbonate karstic formations are enclosed by thick, impermeable non-karstic formations. The method is most practical in regions with relatively long gaps between precipitation events, so that the individual hydrographs corresponding to each event are easily identifiable. This situation is frequently observed in arid or semi-arid karst regions, like most parts of Iran. The method is not applicable if old water emerges from the spring due to piston flow after the beginning of recessions, and is applicable after the time of minimum electrical conductivity and maximum turbidity following the hydrograph peak.

The proposed method estimates the MRT for average flow paths in the catchment area of the karst spring, whereas the costly dye-tracer test can estimate the MRT only between the injection and the emergence points. The proposed method has some superiority over the conventional isotopic methods, too, since it's cheaper, able to determine mean residence times and residence-time distributions from existing hydrologic data and practically more appropriate in karst-water exploitation studies.

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# PRELIMINARY INVESTIGATION OF A RITUAL CAVE SITE IN THE PUUC REGION OF YUCATÁN, MEXICO: ACTUN XCOCH

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**Abstract:** Within the Lowland Maya site of Xcoch is a deep cave first described by John Lloyd Stephens in 1843. Evidence indicates the Maya settled the area during the Middle Preclassic (800 BC) and continued to maintain occupation at the site until the Terminal Classic. The cave's central location within the Xcoch site and its location at the base of a pyramid indicate that the cave played an important role in replicating Maya cosmology. Initial exploration of the cave in 2006 by Michael Smyth revealed examples of the oldest ceramic known in the Yucatán-Yotolin Patterned Burnished. Mapping of the cave in 2009 and 2010 revealed a cave that had been heavily used for ritual purposes. Throughout its reaches are broken ceramic vessels, and the lowest chamber, which contains a pool of water, contains piles of ceramic meters deep. As few caves in this region contain freshwater and the land above is void of natural surface water, it is assumed that the focus of interest was the water in the cave and that Chaac, the Maya rain god, was the principal deity for which the offerings were made. An obsidian blade found in a passage suggests bloodletting, and human bones indicate the cave was used for funerary purposes or for human sacrifice. It is evident that this cave was an important religious site for the Maya, and future excavations will produce a better understanding of the site.

## INTRODUCTION

Early investigations of a deep cave in the Lowland Maya site of Xcoch were first described by John Lloyd Stephens in 1843. Stephens' wrote: "The water was in a deep, stony basin running under a shelf of overhanging rock, with a pole laid across on one side, over which the Indians leaned to dip it up . . . [T]he sight of it was more welcome to us than gold or rubies" (Stephens, 1843). Caves have been a significant component of Maya worldview and their assessment of the sacred landscape for millennia. The rain deity, Chaac, was believed to reside within caves, where wind was formed that carried water to the sky. While caves were places connected with supernatural and often malevolent forces, they were also places that the Maya considered as wombs of the earth, connected to the primordial water that was the source of creation (Halperin et al, 2003). This duality is best described as reflecting breaks in the quadrilateral fabric of the world through which destructive forces and essential elements could enter (Bassie-Sweet, 2008). The Maya connected with caves and their cosmic denizens by integrating caves into town and city plans (Ashmore and Brady, 1999; Bassie-Sweet, 1991; Brady and Stone, 1986). Rituals were often performed both near and within caves. In 1841, John Lloyd Stephens and Frederick Catherwood were the first outsiders to visit one such cave, filled with the remains of past ritual activities, while visiting the ancient Maya site of Xcoch. This article reports on the first systematic exploration and mapping of Xcoch Cave, undertaken in 2009 and 2010.

The site of Xcoch is located just outside of the town of Santa Elena in Yucatán, Mexico, and approximately 10 km east of the famous ruins of Uxmal (Fig. 1). Xcoch lies in the heart of the Puuc Hills, a region famous for its high density of ancient Maya sites, most of which date to the Late and Terminal Classic periods (ca. AD 600–900). It is located south of a line of fault-block ridges known as the Sierrita de Ticul, and the permanent freshwater table in the Puuc lies tens of meters below the ground surface and is accessible through only a small handful of deep caves, including Xcoch (Dunning, 1992). Excavation of the site began in 2006 under the direction of Michael Smyth and has continued since then. Xcoch includes a 10 ha acropolis, primarily displaying megalithic-style architecture dating to the Middle and Late Preclassic periods (ca. 800 BC–AD 150). The largest pyramid, referred to as the Great Pyramid, is 30 meters high and exhibits Late Classic construction (ca. AD 600–800) overlying massive, earlier Preclassic phases. Actun Xcoch is located near the base of another pyramid, dubbed the Cave Pyramid. This edifice had four entrances and stairways indicating that it once may have been a radial pyramid, an architectural device used to align the site with the Maya model of the cosmos (Dunning, 2011). The Great Pyramid and an associated plaza are connected to other parts of the site by two

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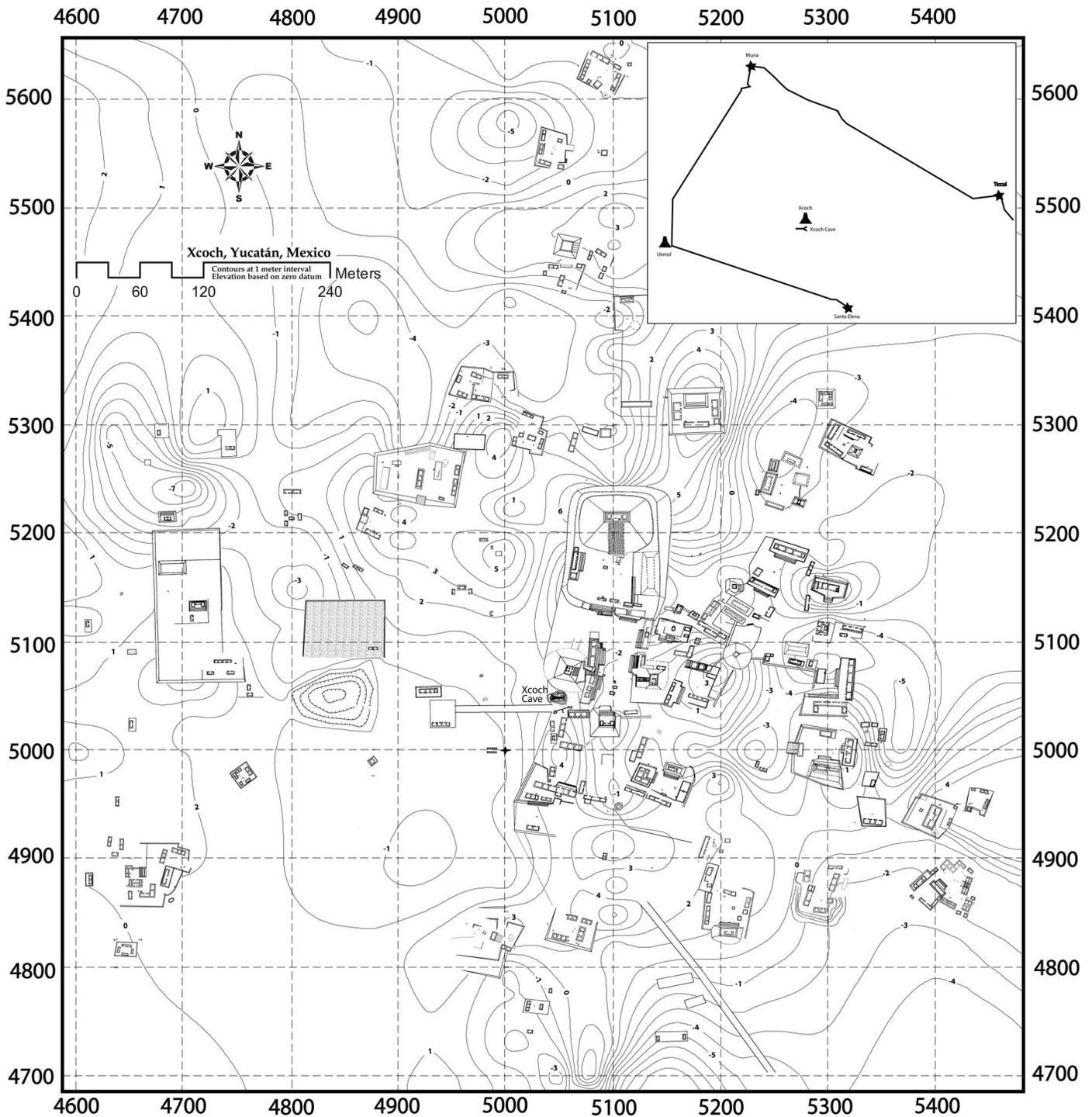
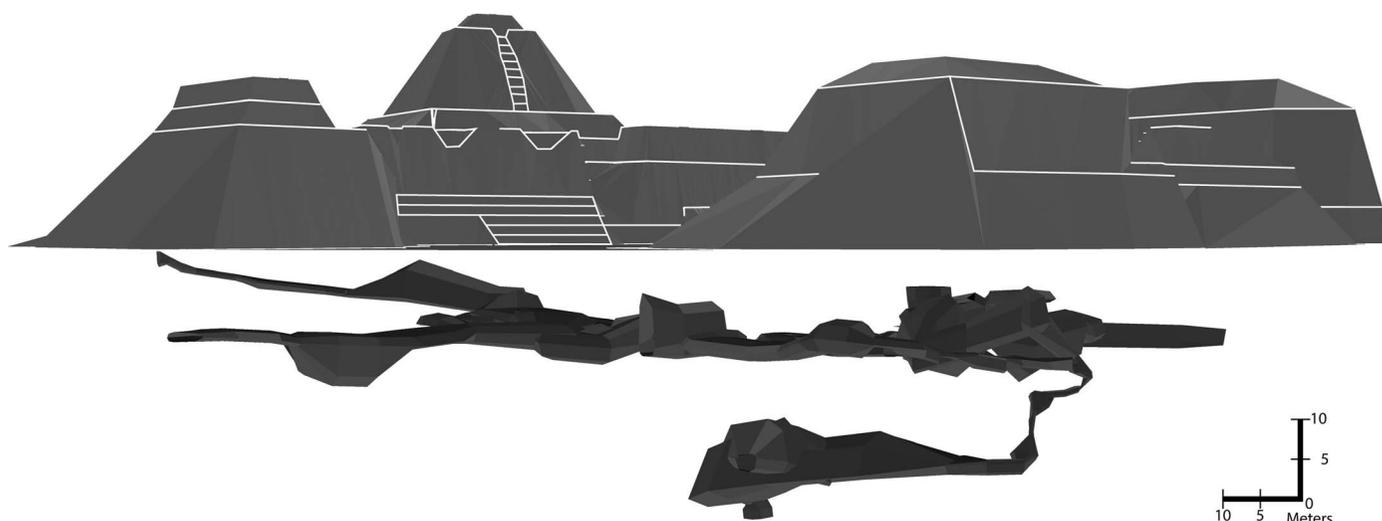


Figure 1. Map, with topography and identified structures, of the Xcoch archaeological site (Smyth et al, 2010).

causeways, one of which runs due west from near the cave entrance to a nearby ancient reservoir. The central plaza is approximately 100 meters by 50 meters in size. An overlay of the maps of central Xcoch and the underlying cave system (Fig. 2) indicates that the main plaza and associated monumental architecture were developed around and above the cave. A series of structures around the plaza form what appears to be an “E-Group Complex,” a

Preclassic solar observatory also used to cosmically align urban space (Smyth, 2008). Most of the central architecture shows evidence of construction in early styles (e.g., Early Puuc, Proto-Puuc, and Megalithic) that likely span the period between 800 BC and AD 600. Very few buildings, mainly those on the outskirts of the acropolis, resemble the later AD 700–900 Classic Puuc style of architecture for which the region is best known. Xcoch shows evidence of a



**Figure 2. Profile of Actun Xcoch underlying monumental structures on the surface above.**

significant population as early as 800 BC during the Middle Preclassic. While the site is still in the early stages of excavation, it seems evident that Xcoch was a major early center, but played a relatively minor role in the Late Classic/Terminal Classic florescence of the Puuc region (Smyth and Ortigón Zapata, 2008).

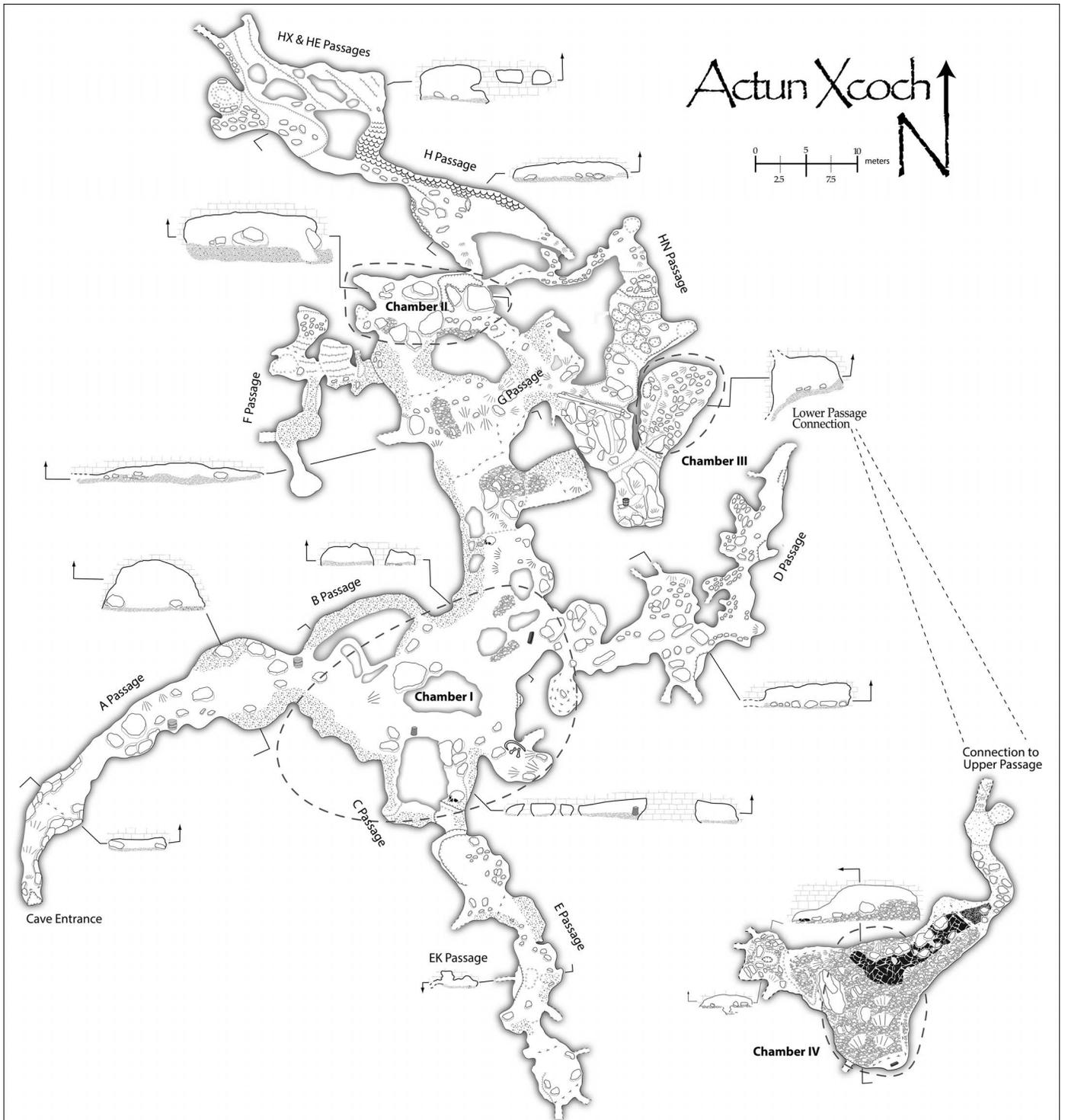
The first published account of Actun Xcoch is from John Lloyd Stephens (1843) in his book *Incidents of Travel in the Yucatán*. Stephens' account of the cave expresses both disappointment, because it did not contain an underground cathedral as he had been lead to believe by the residents of the area, and excitement because of what the cave did contain. The next report of any exploration of the cave is from a group of entomologists in 1971, who indicated that the cave entrance required reopening for them to gain access. They were unable to reach the water pool in the deep recesses of the cave because the passage to it had been intentionally sealed. The cave was entered again in 2006 by Michael Smyth during the course of mapping and excavating the Xcoch acropolis (Smyth and Ortigón Zapata, 2008). The survey of the cave began in 2009 and continued in 2010. At the onset of the survey, very minimal excavation work had occurred within the cave; but some ceramics had been collected, including a rare, specialized ware known as Chac Polychrome and the Middle Preclassic diagnostic ware Yotolin Patterned Burnished (Smyth and Ortigón Zapata, 2008).

#### MODERN EXPLORATION AND MAPPING

After two field seasons in the cave, its total surveyed length is currently 1,286 m with a surface length of 131 m. The depth of the cave is 34.9 m. There are two significant upper rooms, Chambers I and II, in the cave and two lower rooms, Chambers III and IV (Fig. 3). The cave entrance is situated at the bottom of a large sinkhole, where

excavation in 2010 revealed a staircase that had descended from the top of the depression to the entrance of the cave. The entrance is a small pit 2.3 m deep in which there is a carved rock at the bottom on the left-hand side. The rock is a spool element common in the decorated facades of Puuc architecture, and it is unclear whether this stone simply fell into the cave entrance or was deliberately placed. The pit immediately becomes a crawl space less than 1 m in height, where a strong breeze blows through the cave passage, and then proceeds to a stoop-walk between 1 and 1.5 m in height. Stephens aptly describes this breeze as capable of taking a person's breath away, and, indeed, the breeze blows dirt directly into one's face when crawling into the entrance. The breeze seems to diminish after several meters of crawling. Nearby is another carved rock that marks the location of what may be a blocked passage. No efforts have been made yet to remove the debris for further exploration.

Eventually the cave becomes large enough to accommodate upright walking, and the ceiling has multiple cupolas. There is a side lead to the left, the B Passage, marked by a round stone; it loops back into the main passage. The main passage then begins to open up into a relatively complex room, referred to as Chamber I. It would have been an ideal meeting location in the cave, as it is large enough to comfortably hold twenty or more people. Several significant passages branch from this room. The C Passage is a small passage that loops into the much longer E Passage. Multiple sets of human remains are identifiable at this location, primarily in the E Passage. From there, the E Passage continues in a southerly direction and eventually surpasses the entrance as the southern-most point in the cave. A major extension discovered during the second year of survey substantially increased the length of this passage. The connection of the A Passage with the E and C Passages is marked by an inverted conical stone altar (Fig. 4). On the surface such altars are typically found upright in the



**Figure 3. Map of Actun Xcoch based on the 2009 survey; 2010 extensions not shown or reflected in the statistics.**

center of courtyards and are thought to represent world trees or cosmic centering points (Kowalski and Dunning, 1999). The emblematic Maya world tree is the Ceiba (*Ceiba pentandra*), the branches of which created 13 celestial tiers and the roots of which formed 9 subterranean layers. The inverted cave altar may represent the roots of the world tree.

The D Passage branches to the east and reaches the eastern-most point of the cave. Human bones in the D Passage are marked off by wooden torches in a way that appears to frame their location. When or by whom these bones were marked remains unclear. While broken pottery and used torches are evident in the smaller passageway, Chamber I is the point where torch fragments become



**Figure 4.** The inverted cone altar at the entrance to Passage E.

conspicuously visible. Radiocarbon dates through Chamber I range from Middle Preclassic through Colonial (Smyth and Ortégón Zapata, 2010). An area with a relatively high concentration of pottery is found in a small alcove, and some human bones as well as a *pila*, a carved stone water basin, are also located in the vicinity. This location is almost directly above the deeper subterranean water pool. During a heavy rainstorm, it was observed that water from the entrance ran directly to the *pila*. Pollen analysis of sediment samples taken in this area identified insect-pollinated plants, including *Passiflora*, Solanaceae, *Annona*, *Bauhinia*, *Ceiba* (two species), *Pachira*, Sapindaceae, and Tiliaceae. These pollens were likely introduced into the sediments through bat feces, as they are normally not associated with wind-borne, surface-water assemblages in the Maya Lowlands. *Gossypium* (cotton), *Manihot* (manioc; probably both wild and domesticated forms), and *Zea mays* (maize) of lower quantities were present in the sediment samples as well. Typically, grains from these cultigens are too heavy to travel far from their source, and this suggests that these were brought in as a result of human activity, possibly in dedicatory offerings such as flowers or honey.

Beyond Chamber I, the ceiling drops and there is a small crawl space before another smaller room opens up. A diminutive passage, the F Passage, breaks off to the right and may have once reconnected with the A Passage. A wooden beehive that appeared to have been ceremonially broken was found in this passage. After the crawl, the A Passage begins to expand into Chamber II. This large room appears to be the most geologically complicated in the cave

and contains unusual ceiling features resembling boxwork. One large boulder, approximately 3 m long and 2 m high, has a peculiar hole 20 cm in diameter at its tip, with some of the rock around the hole appearing chiseled. A small passage that quickly ends in breakdown debris was found by climbing to the bottom of the boulder. A significant amount of pottery and a large number of human infant bones were identified at the base of the boulder. A polished iron-ore mirror was also found in this area, and a charcoal sample taken from on top of the mirror indicated a Late Classic date of AD 700 (Smyth and Ortégón Zapata, 2010).

Above a drop in the floor is a small traverse that connects the A Passage with the H Passage. It is evident that the floor of this traverse was built up as a trail. The H Passage is composed of a combination of several interconnected passages, beginning as a large walking passage with a relatively steep incline. Evidence suggests that a significant amount of water ran through this passage in the distant past, with several winding holes in the ceiling that appear to have rapidly fed surface water at one time. The wall in this area is often flowstone with associated travertine pools that are now dried up and heavily weathered. Below this zone is a rounded-out hole that may have been modified to isolate some of the flowing water, with an obsidian blade found in proximity. Small rock cairns of unknown origin and age were also located in this passage. The passage splits into a northwestern passages to the left, the HX and HE Passages, and a southeasterly or southerly passage to the right, the HN Passage. Based on overlays of the site, this area rests

underneath the megalithic staircase of the Great Pyramid. There is a climb that reaches an additional 10 or more meters up at this location, but it was not attempted because of the instability of the rocks in the area. It has the potential to be another entrance to the surface or to a feature within the pyramid. In the second year of field work, further investigation revealed a significant extension of the HX passage. A small alcove at the end of this passage has heavily calcified walls and the only remaining speleothems in the cave. Unfortunately, none of the speleothems were of sufficient quality to assist in paleoclimate studies of the site. Several stone tools and markings on the walls suggested that the ancient Maya extracted crystallized crust and speleothems, which were commonly used in rain-related rituals, from this location (Brady et al., 1997; Brady and Rissolo, 2006; Moyes, 2002).

Past Chamber II is a junction of several passages. It is the pivot for the continuation of the A Passage, and the HN passage connects here, as well as the G Passage. This is another location that figured prominently in Stephens' (1843: v. 1, 215) description of his descent into the cave: "We . . . came out upon a ledge of rock, which ran up on the right to a great height, while on the left was a deep, yawning chasm. A few rude logs were laid along the edge of this chasm, which, with a pole for a railing, served as a bridge, and, with the torchlight thrown into the abyss below, made a wild crossing place." The drop here is approximately 5.5 m before it is choked up with break down. A small opening in the breakdown reveals a further drop that reaches into a room below, Chamber III. An old decaying log bridge is still present and is used to prevent slipping into the chasm rather than as a true bridge across the chasm. The A Passage goes around a bend on the right, and then proceeds to wind downward into a room through a small trail that maneuvers through a large breakdown pile. A wooden barrel, probably a Colonial Era artifact, was noted at the top of this climb-down. At the bottom of this route is a large room filled with breakdown, Chamber III. A large number of bats are present in this room, and the rocks are coated in a layer of guano. The breakdown pile contains significant amounts of broken pottery, and Chamber III contains some very small but active stalactites and flowstone. A marked drop in the oxygen level at this point is apparent, and breathing becomes increasingly difficult. A test of air quality indicated oxygen levels as low as 12%. From this point to the end of the survey at the water pool, the oxygen levels remain low and any movement results in labored breathing and profuse sweating.

A steep drop in the floor of Chamber III is the continuation of the A Passage, a location directly below the chasm. The remains of an old wooden ladder are scattered near a hole in the floor, apparently once used to assist with the difficult climb down necessary to reach the lower chambers of the cave. The climb leads into a further downward-sloping crawl. Another climb-down after this descent reaches the lower passage. This lower passage

begins as a crawl and is filled on each side with many broken pottery sherds. The floor of the passage changes from black, fine sediment and guano to primarily charcoal, and a hollow sound is heard when traveling across the charcoal. Whether this indicates the floor has been built up or it is a result of the acoustics is unclear. The passage proceeds into a large room, Chamber IV. At the entrance of Chamber IV, where one transitions from a hands-and-knees crawl to full standing, there are two deep deposits of charcoal, dubbed fire pits, to the left and right of the trail. These are the likely origin of the charcoal used to develop the trail. Two radiocarbon dates obtained from one of the fire pits indicated Postclassic usage (Smyth and Ortigón Zapata, 2010). Directly past these two pits one can follow a small path up a slope, with the entire slope composed of sediment and broken pottery (Fig. 5). At the end of the trail is a small alcove that has a broken *pila* or *metate* (corn-grinding basin) set near its center (Fig. 6), and it is possible that this was used to hold *vuhuy ha*, "virgin water" taken from the water source. Its location at the center of the alcove at the top of the hill of broken pottery indicates that it was the primary focus for whatever ceremonial activity occurred in the chamber for countless generations. Many ancient Maya rituals involved the breaking or killing of objects in order to make the essence of the material available to deities or ancestors (Brady et al., 1997; Brady and Colas, 2005). The mountain of broken artifacts attests to thousands of such rituals.

Returning to the main trail, the survey continues towards the water source, a small pool of water trapped in a drop in the floor. The entire pool is encircled with layers of broken pottery. It is the lowest point in the cave and is located 32.6 m below the entrance and 16.259 m above sea level. The bottom of the pool is littered with the skeletal remains of a significant number of bats. The rather anticlimactic pool (Fig. 7) is 10 to 15 cm in depth and has a visible extent of about a square meter before dipping under a stone shelf. However, in a dry land—within the confines of an oxygen-starved chamber of a dry cave—a pool of cool water containing translucent invertebrates swimming over a layer of broken ceramics becomes a mesmerizing sight. The cave passage continues through breakdown past the water source. This area has not been mapped or extensively explored, but it does not appear that any human activity has occurred in this area. This marked the end of the survey of the primary passage.

Further exploration of the E Passage in 2010 resulted in a significant amount of new cave passage to survey. The total passage surveyed there was 397.8 m. The passage is very distinct from the rest of the cave. In particular, it is much larger in comparison with the majority of the rest of the cave, with the air quality better, the temperature much cooler, and significant air movement present. While it was evident that the Maya had used this passage, it did not show the same kind or intensity of use that is present in the rest of the cave. No evidence of heavy traffic or even



**Figure 5. The slope of sediment and broken pottery in Chamber IV.**

footprints was encountered during the survey. A small number of spent torches were found, as well as vines used to wrap torches. A sample from one large torch appears to be a dicot, possibly *Vitex gaumeri* (2011 personal communication, David Lentz). Radiocarbon dates derived from the torch indicate the remains are from the Colonial Era (Smyth and Ortégón Zapata, 2010). Small amounts of

broken ceramic were also located in the passage. The most prevalent indicator of past usage of the passage was large cairns used to mark off the maze of side passages. An unusual aspect of these cairns is that they do not resemble the few others found elsewhere in the cave. Charcoal was noted in various areas of the passage. Toward the terminus of the main passage in the E and EK areas, it was noted



**Figure 6. Broken *pila* or *metate* in Chamber IV.**



**Figure 7. Pool of water at the lowest level of the cave.**

that the floor was coated with a layer of charcoal. The E Passage was also interesting because its terminus appears to be several meters higher than the known entrance to the cave. This strongly suggests that the E Passage once had an entrance to the surface that has now been buried. This assessment is further reinforced by the presence of ground-termite nests in wall and ceiling cracks, as well as some leaves and roots present in the passages. Using the coordinates drawn from the cave survey, a surface examination of the area above the E Passage was conducted. A surface feature was identified in an ancient plaza situated above the location of the EK and E junction. This feature is a significant depression that appears to be ringed with carved stones. Several workers were dispatched in an attempt to locate an entrance to the E Passage by digging into the bottom of the surface feature. While a physical connection was not made with the surface, voice and tapping communication was established, indicating a very close proximity from the workers digging from the surface to the surveyors in the cave.

#### DISCUSSION

Stephens' original description included the following passage: "As a mere cave, this [place] was extraordinary; but as a well or watering-place for an ancient city, it was past belief, except for the proofs under our own eyes (Stephens, 1843)." Stephens was absolutely correct that Actun Xcoch is an extraordinary place. However, his assessment of the cave and its pool as the sole water source

for an ancient city was erroneous. Recent mapping and excavations have revealed that water was supplied to the ancient city of Xcoch by an elaborate system of rain-fed reservoirs and household cisterns (Dunning et al., 2012). Nevertheless, there is compelling evidence that the cave was the symbolic heart of this water system and of the ancient population that depended on it. The cave has been used ritualistically from the time of the Preclassic Maya (ca. 800 BC) through Classic Maya times and into the Colonial Era. Ritual activity at Xcoch appears to have even occurred when the center above it experienced periods of abandonment. Based on the spatial arrangement of some of the earliest structures at the site, it is posited that the cave was a key rationale for original settlement at Xcoch. Because the cave is one of only three caves in the area known to contain a permanent water source, there is no doubt that it was seen as a very special place to the Maya. The suffocating, low-oxygen experience of reaching the water, if present during the time of the ancient Maya, may have only reinforced the supernatural nature of this cave. Certain caves are known to have functioned as places to communicate with the rain deity in bringing forth rain (Moyes, 2006; Prufer, 2002; Brady, 1989); Xcoch appears to have been such a cave. A radial pyramid close to the cave entrance and abundant deposits of broken pottery suggest that ritual activity also occurred outside of the feature (Smyth et al, 2012). Of special note is the causeway that runs from the cave entrance to a nearby reservoir, suggesting that virgin water may have been brought forth from the home of the rain god and carried to the reservoir, replicating the passage of trade-

wind-borne rain clouds, to begin filling the reservoir and initiate the onset of seasonal rains.

It is evident that other rituals occurred in Xcoch cave that may not have been as closely associated with water. Obsidian blades indicate at least some bloodletting occurred in the cave. At present, it is unclear whether the human bones found within the cave represent human-sacrifice rituals within the cave or important burials. Both practices are known from other caves in the Maya Lowlands, probably reflecting the belief that caves were entrances to the Maya underworld and places to communicate with a variety of deities as well as venerated ancestors (Vogt, 1993; Thompson, 1970; Redfield, 1941; León-Portilla, 1988). One can imagine that the ritually broken beehive might provide an interesting story, if it were ever retold. The E passage may be the most perplexing. While it was obviously known to the Maya, it has very little evidence of ritual activity, despite the fact that the passage is much more comfortable than any other spot in the cave.

In short, while our survey work has greatly expanded our knowledge concerning Actun Xcoch, much remains unknown. Test pits during the next field season should help to increase the stratigraphic knowledge of the samples. Future research, including systematic excavations, will begin to shed more light on this sacred place of the ancient Maya.

#### ACKNOWLEDGEMENTS

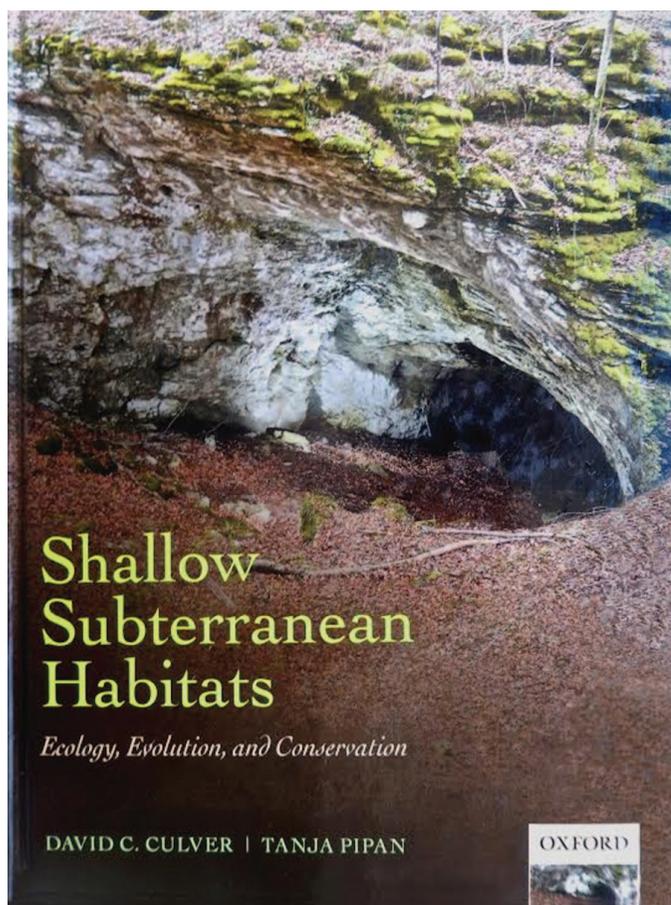
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## BOOK REVIEW



### Shallow Subterranean Habitats: Ecology, Evolution, and Conservation

David C. Culver and Tanja Pipan, 2014. Oxford University Press, UK, 258 p., 10 × 7.5 inches, ISBN 978-0-19-964617-3, hardbound, \$99.95.

#### OVERVIEW

As the authors point out in their preface, this book is unlike their 2009 introductory textbook *The Biology of Caves and Other Subterranean Habitats*. It extends their studies to many other shallow subterranean habitats. Their first book emphasized caves, but they later expanded their scope to include many other subterranean habitats with spaces of various sizes. In particular, they extend European workers' hypothesis that interstitial spaces among gravels in the beds of surface streams are the "staging areas" for colonization of deeper spaces, including caves. The shallow subterranean habitats discussed are seepage springs and other hypotelminorheic habitat of the soil-rock epikarst interface, intermediate-sized terrestrial shallow habitats in

calcareous aquifers, interstitial habitats along rivers and streams, and soil and lava tubes. Like caves, these habitats have many eyeless and de-pigmented species.

Their approach is heavily quantitative, with 76 graphs and 65 tables, but not nearly as theoretical, in terms of testing of ecological and evolutionary models, as Culver's incredible 1982 book *Cave Life*. They do report cases of rigorous multivariate analyses that associate past and ongoing competition with morphological divergence in size and shape among closely related species, as a result of past and ongoing competition, and they approach the problem of the impossibility of direct sampling of shallow subterranean habitats by substituting statistical approaches to estimate species diversity at increasing spatial and temporal scales. A surprising example of copepods collected from ceiling drips in caves is that 12% of all species ever collected from drips over time, 20% between drips with very different chemistry only a meter apart, and 68% between different caves.

#### CHAPTER-BY-CHAPTER REVIEW

Culver and Pipan's book is divided into two parts. After the first chapter, which summarizes the whole book, the next seven chapters provide "a detailed description of shallow subterranean habitats," and the next six chapters are "an exploration of the biological consequences of the existence of these habitats." There follows a short chapter on conservation. Finally a four-page "Epilog and Prospects" discusses what unites and divides shallow subterranean habitats and whether they are "staging areas" for colonization of deeper habitats and, finally, attacks the efficacy of troglomorphy as a unifying theme.

To me, the most interesting shallow subterranean habitat is the epikarst, since Pipan has made this her research focus. The authors show that certain copepod species are epikarst specialists, since they are only found in ceiling drips and drip pools in caves. Their tantalizing results beg, to me, for simple behavioral experiments, as done by Culver in the 1970s to use the diversity of biotic interactions, such as competition, predation, cannibalism, and indirect mutualism, to explain patterns of co-occurrence among species of Appalachian cave-stream isopods and amphipods. For example, it would be easy to put different species of copepods in a bowl alone with a food particle and to watch them, or to make a shallow subterranean analogue of artificial streams to see what aspects of size and leg morphology explain differences in washout rate. These behavioral studies could be complemented with measures of activity and metabolic rate. And raising species in the lab could give data on growth rate, fecundity, and egg size. Sadly, such data are virtually lacking for any species in any such habitat.

Some of the later chapters in the book are the most interesting and controversial. Chapter 9, “The role of light in shallow subterranean habitats,” is merely descriptive. Chapter 10, “Environmental fluctuations and stresses in shallow subterranean habitats,” is more analytical. Culver and Pipan point out a seeming contradiction: such habitats provide a refuge from unfavorable surface conditions (climate adversity colonization) or an opportunity to exploit new or better resources (habitat shift colonization), or else they are an extreme environment with many environmental barriers, especially absence of cyclic changes that entrain circadian rhythms and rest homeostasis on the surface.

Chapter 13, “Colonization and dispersal in shallow subterranean habitats” is an area of special expertise of the authors, and I have written nothing but “great” and “wonderful” in the margins. My only slight quibble is in their use of the term *exaptation*. They define it correctly in their glossary, but with no examples that fit the definition. To be fair, they do say that both *exaptation* and *preadaptation* “remain rather elusive concepts.” I believe that the fact that some soil and litter taxa do not occupy caves does not imply that the adaptations of many surface taxa to dark and cryptic habitats were not necessary for successful colonization of caves and some shallow subterranean habitats.

I also have nothing but praise for the authors’ perspectives and choices of a few excellent papers to review in Chapter 14, “Phylogeny in shallow subterranean habitats.”

Chapter 15, “Conservation and protection of shallow subterranean habitats” is mostly standard fare, but well done. They go beyond the usual by discussing types of rarity and a landscape approach to protection on different spatial scales. They show the importance of both these issues in connection with the various types of habitat of the first half of the book.

In their final chapter, “Epilog and Prospects,” the authors start with a discussion of what separates and what unites different types of shallow subterranean habitat and then consider which, if any, are “staging areas” for colonization of deeper subterranean habitats and caves. There is a brief bit on the confusing proliferation of terms, with a plea to focus on the selective factors in each type of habitat. They end with a very brief “What about troglomorphy?”

#### KUDOS AND CRITICISMS

The authors do their usual excellent job of discussing the areas of their particular expertise in biogeography and species diversity at different spatial scales and in patterns of morphological variation among species. They discuss pitfalls and limitations of some approaches, especially multivariate statistics, and they are clear where more and

better data are needed. Their care in discussing alternatives and the data of others is shown in just one chapter by the phrases *incomplete sampling* (four times), *correlation* (three times), *possibly* (twice), *conflated distance and chemistry* (twice), *may or may not, perhaps*, and *many possibilities*. On the other hand they do not hedge when discussing in detail the few very best research studies in several areas.

They do an especially nice job of picking critical research studies and discussing both the data and the implications. In addition to their own studies on copepods in the epikarst, I especially liked the following:

1. Gers’s (1992) special traps that sample living fauna from the surface to 2-m deep in the epikarst.
2. A number of studies of calcrete aquifers in Australia and the amazing radiation of Dytiscid beetles.
3. Canonical variate analyses of size, shape, and other morphology in relation to habitat metrics. These include their own on copepods, another on *Niphargus* amphipods, and another on cholevine beetles. Each unmasks unexpected relationships that suggest experimental studies.
4. Rouch’s (1988) many-year and all-season studies of the entire fauna in a 75 m<sup>2</sup> stream sample with its surface and shallow subterranean microhabitats.
5. As reviewed by Peck and Finston (1993) for Hawaii, Galapagos, and Canaries there are many sister species and on this basis researchers have championed habitat shift colonization with parapatric speciation.
6. Pioneering work by Howarth et al. (1980) in pointing out high tropical Hawaiian troglobite diversity, as opposed to some views that tropical caves have few troglobites.
7. In discussing organic carbon and nutrients in shallow subterranean habitats, the emphasis on Huppopp’s comprehensive review (2012) that cave fish of several species show a number of metabolic economies that are best explained by restricted food input into caves. In aquatic shallow subterranean habitats Simon and colleagues show a change in the quality of organic carbon with depth in the epikarst and into caves with increasing carbon limitation.

The authors provide a less unbiased review, and less complete citation, of examples that do and do not support their favorite hypotheses. “Our first book was in a way a consensus view... and we did not emphasize the controversies of the field.” “This book is different, and we have put forward our views, even when it is perhaps a minority view.” Since their book is intended for students and advanced researchers, this is excusable. But I would have liked them to address other views and give evidence to support their favorite hypotheses. I agree that absence of light is the only universal agent of selection with universal

reduction in eyes and pigment. But they miss a pattern that there are increasing degrees of troglomorphy, as taxa have increasing costs of doing business (Poulson, 2012) such as finding food in the face of increasing scarcity and dealing with circulation and respiration costs of increasing size. In some cases, they have missed important papers and not given due credit to earlier researchers. The most important example is the quasi-annual timing of flooding and its relation to circannual cycles of reproductive readiness. In other cases, they do not make sufficiently critical analyses where data are incomplete or even lacking. The most important example is the poorly conceived and supported hypotheses about the supposed stress of high humidity and the importance of “bad air” with high CO<sub>2</sub> and low O<sub>2</sub> in the habitat of troglobites. I would have liked to see an update of their table (12.1), which reproduces verbatim Christiansen’s (1962) list of troglomorphic features that does not distinguish reducing and increasing traits, has many traits found only in a few taxa, and is incomplete on behavioral, physiological, and life history. They selectively, and sometimes incorrectly, cite studies that support their thesis that natural selection for reducing traits is ubiquitous and that neutral mutations are rare. Examples are some new papers on *Astyanax* cavefish and both old and new papers on amblyopsid cavefish. They give too much emphasis and uncritical support to a study of an evolutionarily new troglobitic beetle that supposedly uses remnant eyes and has vision genes that are supposedly functional. A main critique centers on a few pages at the end of Chapter 12 subtitled “A new look at troglomorphy.” They laud early studies and a landmark review on convergent evolution, but then say that “cracks have appeared in the façade of convergent evolution of troglomorphy.” In my view, they have been selective in citing support for this statement and in two cases are incorrect about facts.

There are several additions that I suggest would have improved their book. At the top of my list are:

1. A detailed look at whether different shallow subterranean habitats are source populations, with sustaining reproduction, or sinks.
2. Comparative drawings of surface forms, troglophile generalists, and troglobites, including epikarst specialists.
3. Inclusion of discussion of elaborated troglomorphies of physiology, behavior, and life history.

4. Simple lab experiments, since most of the species in their habitats cannot be watched in the field. Good models are Culver’s own studies of amphipods in artificial streams and Christiansen’s clever experiments on Collembola that showed how behavioral deployment of their tiny foot complex allows them to essentially walk on water.
5. Discussion of leaf litter as a staging area for colonization of some small-volume subterranean habitats. What traits are really pre-adaptations?

#### SUMMARY

This book is the second coauthored by Culver and Pipan, and the fourth that Culver has authored or coauthored. Like those, this book is well-written and very professional. Some indicators of its rigor are the 76 graphs, 65 tables, 40 drawings and diagrams, 24 photos, 16 maps, 550 literature citations, and an 8-page glossary. This book will best serve professionals and advanced students with some background in biospeleology.

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