Observations of *Cambarus bartonii cavatus* (Decapoda: Cambaridae) and ectosymbiotic branchiobdellidans (Annelida: Clitellata) in Cruze Cave, Knox County, Tennessee, USA

Sarah W. Keenan[1], Matthew L. Niemiller[2] & Bronwyn W. Williams[3]

[1]Department of Earth and Planetary Sciences, University of Tennessee, 1412 Circle Drive, Knoxville, Tennessee 37996, USA
skeenan1@utk.edu (corresponding author)
[2]Department of Biology, University of Kentucky, 200 Thomas Hunt Morgan Building, Lexington, Kentucky 40506, USA
cavemander17@gmail.com
[3]Department of Zoology, Southern Illinois University, 1125 Lincoln Drive, Carbondale, Illinois 62901, USA
bwwilliams@siu.edu

Key Words: Decapoda, Cambaridae, *Cambarus bartonii cavatus*, ectosymbiosis, Annelida, Clitellata, Branchiobdellida, Cruze Cave, Knox County, Tennessee.

Branchiobdellidans, or crayfish worms, are obligate ectosymbionts of freshwater crustaceans, primarily crayfishes (Gelder 2010). Of the approximately 100 nominal branchiobdellidan species in North and Central America, nearly 20 have been reported from caves, eight of which are, to date, known only from caves (Holt 1973, 1984). Despite the apparent common occurrence of branchiobdellidans in North American karst habitats, current knowledge of diversity, host-affiliation, and distribution is limited. In this note, we report new findings of crayfish and associated branchiobdellidans in Cruze Cave, Knox County, Tennessee.

Despite containing over 9,500 caves, the biodiversity of cave fauna in Tennessee is largely understudied, leaving significant potential for the discovery of new species (Niemiller and Zigler 2013) and interspecific relationships. As part of an effort to fill gaps in our knowledge of Tennessee's subterranean fauna, a biological inventory survey was conducted on 13 May 2013 in Cruze Cave (TKN24). Cruze Cave is located within the Holston Formation, which is part of the Knox Group, and consists of Ordovician-aged carbonates that outcrop throughout East Tennessee (Barr 1961). The cave extends for approximately 300 m, forming a single main passage developed along the roughly southeast-dipping bedding and cross-cutting fracture planes. A dry to muddy upper passage diverges 50 m from the entrance, and connects with the main passage an additional 75 m downstream. A small stream enters the cave from the surface, and persists along the length of the cave passage. Anthropogenic (e.g., plastic lawn chair,

bottles, and other garbage) and terrigenous (e.g., tree branches) debris can be observed along the length of the cave passage, suggesting flow varies considerably during periods of intense rain transporting large debris into the cave system. Surfaces of exposed carbonates, particularly within the stream channel, are coated in a light brownish-orange biofilm, likely bacterial in origin with fine suspended sediment attached.

During the biological inventory of Cruze Cave, an ovigerous female Appalachian Brook Crayfish, *Cambarus bartonii cavatus* Hay, 1902 (**Figure 1**), was captured by hand in a disjunct pool ca. 100 m from the cave entrance. Upon further examination, numerous branchiobdellidans were observed on the undersurface of the female's abdomen and on the egg cases. Branchiobdellidan cocoons (**Figure 1**) were observed on exposed pleopods. The crayfish was transported live to the University of Tennessee, Knoxville, where it was transferred to formalin for storage and subsequent study. Crayfish egg cases were removed from the pleopods, and a subset (50 of 172) was photographed using the imaging software, ImageJ©. Branchiobdellidans were removed from the host and stored in separate containers for later identification.

Figure 1. Ventral view of egg cases attached to pleopods of a female Appalachian Brook Crayfish, *Cambarus bartonii cavatus*, from Cruze Cave (used with permission of M.E. Slay). Small, white branchiobdellidan cocoons are visible on exposed pleopods and uropods, in part highlighted by white circles.

A second trip to Cruze Cave occurred on 30 June 2013 for additional observation and collection of study material. Approximately 200 m of passage was surveyed for crayfish. When observed, capture of individuals was attempted by hand. Nine crayfish were caught and examined on-site, all with visible branchiobdellidans attached to the ventral abdomen. For most of the nine crayfish, branchiobdellidans were also observed in varying densities on the dorso-anterior rostral surface and the carapace. One male crayfish displayed noticeably higher branchiobdellidan abundance than the others, with >200 worms distributed across the dorso-anterior surface of the rostrum (**Figure 2**), the dorsal surface of the carapace, and ventral abdomen. Branchiobdellidan cocoons were observed on the ventral surface of the uropods and on the pleopods. The male crayfish was collected, transported live to the University of Tennessee, Knoxville, and stored at 4°C in habitat water. The live crayfish and previously preserved material were subsequently transported to Southern Illinois University for identification of the ectosymbionts. Preliminary species identification of branchiobdellidans was performed using live specimens and a wet-mount procedure under a compound microscope (Gelder 2010). Worms were then fixed in 95% ethanol, cleared with methyl salicylate, infiltrated with Canada balsam, and mounted on slides for final identification. Branchiobdellidans were identified to species using the keys and information in Gelder (2010), Hobbs et al. (1967) and Holt and Opell (1993).

Figure 2. Dorso-anterior view of *C. b. cavatus* with numerous branchiobdellidans attached to the rostrum (used with permission of D.R. Harmon).

The Appalachian Brook Crayfish is distributed across the Appalachian Highlands of eastern North America from Ohio and West Virginia to northern Georgia (Hobbs 1981, 1989). In Tennessee, *C. b. cavatus* is known from the Ridge and Valley Province (Williams et al. 2009). The species is common in surface streams, springs, and ditches, but is also found in streams of cave systems within the Appalachian karst region (Hobbs and Barr 1960; Hobbs 1994; Fong et al. 2012). As such, *C. b. cavatus* is primarily an inhabitant of surface waters, but considered a stygophile.

Crayfish in Cruze Cave were observed in disjunct pools along the entire length of the cave passage (~900 m). The observation of an ovigerous female *C. b. cavatus* suggests reproduction may be occurring in the cave, as interpreted for *Orconectes neglectus* Faxon, 1885 encountered in the January-Stansberry Cave, Oklahoma (Fenolio et al. 2013). Alternatively, the female may have mated in surface waters and moved into the cave during spring high water events that were particularly frequent during spring 2013. The cave stream is directly connected to the surface by a stream fed from surface sinks and runoff, and crayfish have been observed during every month of the year in Cruze Cave during previous surveys for *Gyrinophilus* salamanders (Miller and Niemiller 2008; Niemiller, unpublished data).

Three branchiobdellidan species were recovered from the two *C. b. cavatus* collected, including *Bdellodrilus illuminatus* (Moore, 1894) from the gill chambers, *Cambarincola holostomus* Hoffman, 1963 from regions around the rostrum (**Figure 2**), ventral cephalothorax, and ventral abdomen, and an undescribed species, also on the ventral abdomen. *Bdellodrilus illuminatus* and *C. holostomus* are known symbionts of *Cambarus bartonii sensu lato* (e.g., Holt 1973; Gelder and Williams 2011); however, only *B. illuminatus* has been previously reported on crayfish recovered from caves, including Cantwell Valley Cave (Tennessee), and Fuller's and Wade's caves (West Virginia) (Holt 1973).

Bdellodrilus illuminatus has a widespread distribution throughout the Appalachians from New Brunswick, Canada, to Georgia and Alabama (Gelder et al. 2002, 2009). Despite its large overall range, the species is locally rare, often found in low densities on few hosts, if at all, from a given site (Gelder and Williams 2011). All previous records of *B. illuminatus* in caves have been associated with *C. bartonii*; however, not all cave-caught *C. bartonii* have been reported to host *B. illuminatus* (Holt 1973). *Bdellodrilus illuminatus* is a gill chamber-dweller, and as a result is not readily apparent during superficial and/or external examinations of the crayfish host.

Cambarincola holostomus, known only from Virginia, Tennessee, and North Carolina (Hoffman 1963; Gelder and Williams 2011), has a more restricted distribution in the Appalachians than that of *B. illuminatus*. *Cambarincola holostomus* is primarily found on the external surface of the host, specifically on the ventral cephalothorax along the articulations of the walking legs and chelipeds (Gelder and Williams 2011). On the *C. b. cavatus* specimens from Cruze Cave, we observed *C. holostomus* on the dorsal and

ventral surfaces of the rostrum at high densities, suggesting a hierarchy in microhabitat selection and/or use. *Cambarincola holostomus* and the undescribed species were both observed on the ventral abdominal surface of the examined male crayfish, along with high numbers of cocoons arrayed along the pleopods. The undescribed species was identified as belonging to the genus *Cambarincola*, and unequivocally differed from all species descriptions in Holt and Opell (1993).

On the ovigerous female crayfish, egg cases (n = 172; 2.90 ± 0.12 mm in diameter) were each attached to the pleopods via a single, thin, filament (approximately 2 mm-length), together forming a densely packed mass on the ventral surface of the abdomen. The presence of branchiobdellidans on the host egg mass might suggest a parasitic interaction; however, no damage (e.g., scarring, tearing) was observed on examined egg cases. Instead, the egg cases likely prevented direct attachment to the ventral abdominal surface, thus displacing branchiobdellidans from large portions of their usual microhabitat. Indeed, branchiobdellidan cocoons were present on the pleopods as well as along the ventral abdominal surface on male and non-ovigerous female *C. b. cavatus* observed in Cruze Cave, whereas cocoons were only observed on exposed regions of the pleopods of the ovigerous female.

The caves of Tennessee are vastly understudied, leaving significant gaps in our knowledge of biodiversity, species distributions, potential endemism, biotic community structure, and the overall connectivity between biota in surface and cave systems. Our observations of ectosymbiotic associations between *Cambarus bartonii cavatus* and three species of branchiobdellidans—one of which, *Cambarincola holostomus,* has not previously been observed in a cave system, and another, an undescribed *Cambarincola* sp.—suggests that there is great potential for the discovery of novel species and ectosymbiotic associations in subterranean systems of Tennessee.

Acknowledgements

We wish to thank the speleobiologists involved in the biological inventory of Cruze Cave: M.L. Porter, M.E. Slay, A.S. Engel, S.A. Engel, S.J. Taylor, and D.W. Fong. D.R. Harmon is gratefully acknowledged for assisting with the second trip to Cruz Cave. Special thanks to H.H. Hobbs III for confirming the crayfish species identification. B.W.W. was supported by NSF-DEB 1036516 (Wormnet II: Assembling the Annelid Tree of Life).

Literature Cited

Barr, T.C. Jr. 1961. Caves of Tennessee. Bulletin 64. Tennessee Division of Geology, Nashville, Tennessee, USA. 567 pp.

Fenolio, D., Niemiller, M.L., Soares, D., Slay, M.E., Harris, A., & Harris, N. 2013. Subterranean reproduction of the Ringed Crayfish, *Orconectes neglectus* Faxon

1885 (Astacoidea: Cambaridae) within an Ozark Highlands cave in Oklahoma, USA. Speleobiology Notes 5: 43-46.

Fong, D.W., Porter, M.L., & Slay, M.E. 2012. Cave life of the Virginias: a field guide to commonly encountered species. Biology Section of National Speleological Society, Huntsville, Alabama, USA. 41 pp.

Gelder, S.R., Gagnon, N.L., & Nelson, K. 2002. Taxonomic considerations and distribution of the Branchiobdellida (Annelida: Clitellata) on the North American continent. Northeastern Naturalist 9: 451–468.

Gelder, S.R., McCurry, L., & McAlpine, D.F. 2009. Distribution and first records of Branchiobdellida (Annelida: Clitellata) from crayfishes (Crustacea: Decapoda) in the Maritime Provinces of Canada. Northeastern Naturalist 16: 45–52.

Gelder, S.R., & Williams, B.W. 2011. First distributional study of Branchiobdellida (Annelida: Clitellata) in the Great Smoky Mountains National Park, North Carolina and Tennessee, USA, with a redescription of *Cambarincola holostomus* Hoffman, 1963. Southeastern Naturalist 10: 211–220.

Gelder, S.R. 2010. Branchiobdellida, section III. Pp. 402–410. In Govedich, F.R., Bain, B.A., Moser, W.E., Gelder, S.R., Davies, R.W., & Brinkhurst, R.O. 2010. Annelida (Clitellata): Oligochaeta, Branchiobdellida, Hirudinida and Acanthobdellida. In Thorp, J.K., & Covich, F., eds. Ecology and Classification of North American Freshwater Invertebrates, 3rd Edition. Academic Press, Elsevier, San Diego, California, U.S.A.

Hobbs, H. H., Jr. 1981. The crayfishes of Georgia. Smithsonian Contributions to Zoology 318: 1–549.

Hobbs, H.H., Jr. 1989. An illustrated checklist of the American crayfishes (Decapoda: Astacidae, Cambaridae, and Parastacidae). Smithsonian Contributions to Zoology 480: 1–236.

Hobbs, H.H. III 1994. Biogeography of subterranean decapods in North and Central America and the Caribbean region (Caridae, Astacidea, Brachyura). Hydrobiologica 287: 95–104.

Hobbs, H.H., Jr., & Barr, T.C., Jr. 1960. The origins and affinities of the troglobitic crayfishes of North America (Decapoda, Astacidae), I, the genus *Cambarus*. American Midland Naturalist 64: 12–33.

Hobbs, H.H., Jr., Holt, P.C., & Walton, M. 1967. The crayfishes and their epizootic ostracod and branchiobdellid associates of the Mountain Lake, Virginia, region. Proceedings of the United States National Museum 123: 1-84.

Hoffman, R.L. 1963. A revision of the North American annelid worms of the genus *Cambarincola* (Oligochaeta: Branchiobdellidae). Proceedings of the United States National Museum 114: 271–371.

Holt, P.C. 1973. Branchiobdellids (Annelida: Clitellata) from some eastern North American caves, with descriptions of new species of the genus *Cambarincola*. International Journal of Speleology 5: 219–256.

Holt, P.C. 1984. On some branchiobdellids (Annelida: Clitellata) from Mexico with the description of new species of the genera *Cambarincola* and *Oedipodrilus*. Proceedings of the Biological Society of Washington 97: 35–42.

Holt, P.C., & Opell, B.D. 1993. A checklist of and illustrated key to the genera and species of the Central and North American Cambarincolidae (Clitellata: Branchiobdellida). Proceedings of the Biological Society of Washington 106: 251–295.

Miller, B.T., & Niemiller, M.L. 2008. Distribution and relative abundance of Tennessee cave salamanders (*Gyrinophilus palleucus* and *Gyrinophilus gulolineatus*) with an emphasis on Tennessee populations. Herpetological Conservation and Biology 3: 1–20.

Niemiller, M.L., & Zigler, K.S. 2013. Patterns of cave biodiversity and endemism in the Appalachians and Interior Plateau of Tennessee, USA. PLoS One 8: e64177.

Williams, C.E., Bivens, R.D., & Carter, B.D. 2009. Annotated list of the crayfishes of Tennessee. Tennessee Wildlife Resources Agency, Nashville, Tennessee, USA.