Subterranean Biology 28: 1–13 (2018)
doi: 10.3897/subtbiol.28.29282
http://subtbiol.pensoft.net

SHORT COMMUNICATION

A peer-reviewed open-access journal
Subterranean Biology
Published by The International Society for Subterranean Biology

New occurrence records for stygobiontic invertebrates from the Edwards and Trinity aquifers in west-central Texas, USA

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Academic editor: *M. Niemiller* | Received 23 August 2018 | Accepted 30 September 2018 | Published 1 November 2018

http://zoobank.org/AC3F597C-05E8-42A8-A58B-A38B590FCC60

Citation: Nissen BD, Devitt TJ, Bendik NF, Gluesenkamp AG, Gibson R (2018) New occurrence records for stygobiontic invertebrates from the Edwards and Trinity aquifers in west-central Texas, USA. Subterranean Biology 28: 1–13. https://doi.org/10.3897/subtbiol.28.29282

Abstract

We report new occurrence records for stygobiontic invertebrates from the Edwards and Trinity aquifers in Blanco, Hays, and Travis counties of central Texas, USA. Our collection includes seven species from four families: *Caecidotea reddelli* (Steeves, 1968), Asellidae; *Crangonyx* nr. *pseudogracilis* Bousfield, 1958, *Stygobromus balconis* (Hubricht, 1943), *Stygobromus bifurcatus* (Holsinger, 1967), and *Stygobromus russelli* (Holsinger, 1967), Crangonyctidae; *Sphalloplana mohri* Hyman, 1938, Kenkiidae; and *Cirolanides* sp., Cirolanidae. Specimens of *Caecidotea reddelli* and *Crangonyx* nr. *pseudogracilis* are new records for Hays County and Travis county, respectively. Specimens of an undescribed species of *Cirolanides* were collected from a well in Hays County and from two localities in Travis County.

Keywords

karst, groundwater, stygofauna, Asellidae, Crangonyctidae, Kenkiidae, Cirolanidae

Introduction

The Edwards and Trinity aquifers in west-central Texas are some of the most biologically diverse aquifers in the world, home to at least 68 described species of endemic groundwater-obligate (stygobiontic) invertebrate species (Hershler and Longley 1986, Bowles and Arsuffi 1993, Hutchins 2018, Külköylüoğlu et al. 2017a, 2017b, 2017c, Camacho et al. 2018, Külköylüoğlu 2018, Külköylüoğlu and Gibson 2018). Of these species, 52 are in the Edwards (Balcones Fault Zone) Aquifer (hereafter, Edwards Aquifer), 18 are in the Trinity Aquifer, and 23 are in the Edwards-Trinity (Plateau) Aquifer. Seventeen stygobiontic invertebrates have been recorded in the Austin-Round Rock Metro Area (Table 1). The Edwards Aquifer is also the primary source of water for the city of San Antonio and other communities in central Texas (Gibson et al. 2008), which are currently experiencing rapid development and growth (Pendall et al. 2015). As new pumping wells are drilled for agricultural and municipal use, increased demands are placed on these aquifers, threatening regional groundwater biodiversity and ecosystem services. Documenting the stygobionts endemic to these aquifers is necessary to refine our knowledge of their distributions, thereby informing conservation and management of natural resources within the Edwards and Trinity aquifers. Furthermore, monitoring stygobiontic communities can prove useful in detecting changes in the water quality of these aquifers (Gibson et al. 2008), which many Central Texans rely on for drinking water, agriculture, and recreation.

Despite considerable recent research (Bowles and Arsuffi 1993, Gibson et al. 2008, Diaz and Alexander 2010, Hutchins et al. 2013), the distributions of stygobionts remain difficult to delineate due to the inaccessibility of their habitats, and low detection probabilities (Schneider and Culver 2004, Krejca and Weckerly 2007). Here, we

Table 1. Stygobiontic invertebrate fauna recorded in the Austin-Round Rock Metro area, Texas, USA.

Class	Order	Family	Species
Turbellaria	Kenkiidae	Kenkiidae	*Sphalloplana mohri* Hyman, 1938
Mollusca	Mesogastropoda	Hydrobiidae	*Phreatodrobia conica* Hershler & Longley, 1986
			Phreatodrobia nugax (Pilsbry & Ferriss, 1906)
			Phreatodrobia punctata Hershler & Longley, 1986
			Phreatodrobia rotunda Hershler & Longley, 1986
		Cochliopidae	*Stygopyrgus bartonensis* Hershler & Longley, 1986
Crustacea	Isopoda	Asellidae	*Caecidotea reddelli* (Steeves, 1968)
			Lirceolus bisetus (Steeves, 1968)
			Lirceolus hardeni (Lewis & Bowman, 1996)
		Cirolanidae	*Cirolanides texensis* Benedict, 1896
	Amphipoda	Bogidiellidae	*Artesia subterranea* Holsinger, 1980
		Crangonyctidae	*Crangonyx* nr. *pseudogracilis* Bousfield, 1958
			Stygobromus balconis (Hubrict, 1943)
			Stygobromus bifurcatus (Holsinger, 1967)
			Stygobromus flagellatus (Benedict, 1896)
			Stygobromus russelli (Holsinger, 1967)
		Sebidae	*Seborgia relicta* Holsinger, 1980

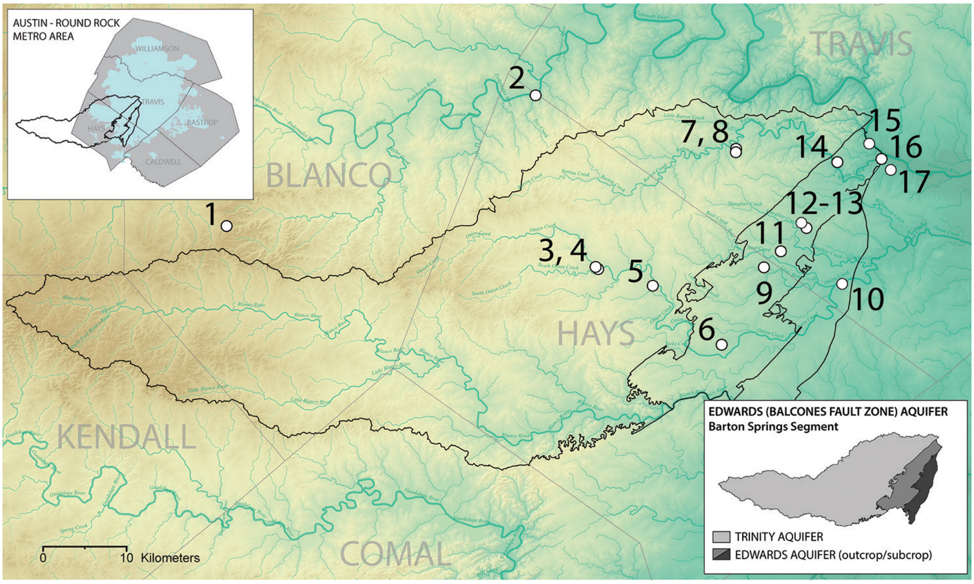

Figure 1. Sample Sites. Sampling map showing the extent of the Barton Springs Segment of the Edwards (Balcones Fault Zone) Aquifer and its hydrozones in Hays, Travis, and Blanco counties, Texas, USA. Sampling sites are numbered as follows: **1** Bamberger Ranch Spring **2** Red's Spring **3** Emerald Spring **4** Bello Spring **5** Ben McCulloch Spring **6** Sky Ranch Tract - State Well No. 5857507 **7** Sweetwater Spring 4 **8** Sweetwater Spring 1 **9** Hays County Ranch Tract - State Well No. 5849939 **10** Old San Antonio Spring **11** Ed's Crossing Tract - State Well No. 58499SH **12** Blowing Sink Cave **13** Blowing Sink Tract - State Well No. 5850411 **14** Barton Creek Greenbelt - State Well No. 5842820 **15** Cold Spring **16** Eliza Spring **17** Treadwell Spring. Boundaries of aquifer hydrozones courtesy of the Barton Springs Edwards Aquifer Conservation District. Wells are identified primarily by the Texas Water Development Board (TWDB) well-numbering system (Nordstrom and Quincy 1999).

present new occurrence records for seven species, including three new county records, from groundwater wells and springs in the Edwards and Trinity aquifers. Long-term monitoring of groundwater wells using bottle-traps allows a unique opportunity to sample a variety of locations over long periods of time with minimal effort (Hutchins and Orndorff 2009, Fenolio et al. 2017). In addition, we give a brief synopsis of known distributions of those species and relevant literature.

Methods

Sampling sites

Seventeen sites were sampled in and around the Barton Springs segment of the Edwards Aquifer and its catchment area in the Hill Country portion of the Trinity Aquifer in Blanco, Hays, and Travis counties (Figure 1). Most sampling took place in 2010–2011 and again in 2015–2018. We also report a handful of other specimens collected opportunistically over the past two decades (Table 2).

Table 2. Voucher specimens. Complete listing of all specimens collected. UTIC = University of Texas Insect Collection. Collector initials are as follows: TJD = Thomas J. Devitt; BDN = Bradley D. Nissen; MSS = Mark S. Sanders; NFB = Nathan F. Bendik; AGG = Andrew G. Gluesenkamp; RG = Randy Gibson; DAC = Dee Ann Chamberlain; PS = Peter Sprouse. N = Specimens collected. † = new county record. * = specimen accessioned at San Marcos US Fish and Wildlife Service Fish Hatchery.

Taxon	Sites	N	Date	Collectors	Catalog #
Caecidotea reddelli	Hays Co.: Roy Creek, Red's Spring†	4	16 Sep 2016	TJD	UTIC 92016
	Travis Co.: Zilker Park, Eliza Spring	1	1 Apr 1999	DAC	UTIC 93008
	Travis Co.: Barton Creek Habitat Preserve, Sweetwater Spring 4	4	10 Apr 2017	TJD, BDN	UTIC 92021
		3	17 Apr 2017	TJD, BDN	UTIC 92020
	Travis Co.: Barton Creek Habitat Preserve, Sweetwater Spring 1	3	17 Apr 2017	TJD, BDN	UTIC 92019
		1	1 May 2017	TJD, BDN	UTIC 92018
	Travis Co.: Old San Antonio District Park, Old San Antonio Spring	2	19 Jan 2018	TJD, BDN	UTIC 93014
Cirolanides nr. *texensis*	Travis Co.: Blowing Sink Cave†	1	14 Oct 2010	MSS	UTIC 91874
	Travis Co.: City of Austin WQPL, Blowing Sink Tract, State Well No. 5850411	1	4 Dec 2017	BDN	UTIC 210886
	Hays Co.: City of Austin WQPL, Hays County Ranch Tract, State Well No. 5849939†	2	1 Sep 2010	NFB, AGG	UTIC 91879
		3	12 Nov 2010	NFB, AGG	UTIC 91876
		1	3 Dec 2010	NFB, AGG	UTIC 91877
		3	14 Jan 2011	NFB, AGG	UTIC 91875
		2	27 Jan 2011	NFB, AGG	UTIC 91880
		2	21 Oct 2016	AGG, TJD, BDN	UTIC 92014
		1	15 Nov 2016	BDN	UTIC 92013
		3	6 Apr 2017	BDN	UTIC 210884
		2	20 Apr 2017	BDN	UTIC 210885
		1	5 Jan 2018	BDN	UTIC 210887
Crangonyx nr. *pseudogracilis*	Hays Co.: Old San Antonio Spring	2	31 Jan 2018	BDN	Cp31012018*
	Travis Co.: Treadwell Spring	3	21 June 2016	PS	UTIC 91369
Sphalloplana mohri	Travis Co.: Cold Spring	1	24 Feb 2011	RG	SM-Sm24022011*
Stygobromus balconis	Travis Co.: City of Austin WQPL, Ed's Crossing Tract, State Well No. 58499SH	1	6 Apr 2017	BDN	UTIC 92024
	Travis Co.: Barton Creek Habitat Preserve, Sweetwater Spring 4	4	10 Apr 2017	BDN	UTIC 92025
Stygobromus bifurcatus	Travis Co.: Zilker Park, Eliza Spring	1	29 Aug 2016	DAC	UTIC 92030
		1	5 Mar 2017	DAC	UTIC 93011
	Travis Co.: Barton Creek Habitat Preserve, Sweetwater Spring 4	8	17 Apr 2017	TJD, BDN	UTIC 92026
	Hays Co.: Onion Creek, Ben McCulloch Spring	1	31 Jan 2017	TJD	UTIC 92029
	Blanco Co.: Bamberger Ranch Spring	1	21 Jun 2017	TJD, BDN	UTIC 92028
Stygobromus russelli	Blanco Co.: Bamberger Ranch Spring	1	22 Mar 2018	TJD, BDN	UTIC 93016
	Travis Co.: Zilker Park, Eliza Spring	1	19 Nov 2015	DAC	UTIC 92033
	Travis Co.: Barton Creek Wilderness Park, Barton Creek Greenbelt tract, State Well No. 5842820	3	3 Dec 2010	NFB, AGG	UTIC 91888
		2	27 Jan 2011	NFB, AGG	UTIC 91882
		1	8 Mar 2011	NFB, AGG	UTIC 91883
	Travis Co.: City of Austin WQPL, Ed's Crossing Tract, State Well No. 58499SH	2	8 Mar 2011	NFB, AGG	UTIC 91886
		2	4 Dec 2017	BDN	UTIC 93012
		1	3 Jan 2018	BDN	UTIC 93013
	Travis Co.: City of Austin WQPL, Blowing Sink Tract, State Well No. 5850411	1	30 Mar 2018	BDN	UTIC 93017

Taxon	Sites	N	Date	Collectors	Catalog #
Stygobromus russelli	Hays Co.: Onion Creek, Ben McCulloch Spring	1	31 Jan 2017	TJD	UTIC 92029
		3	21 Mar 2017	TJD, BDN	UTIC 92031
		5	3 May 2017	TJD, BDN	UTIC 92039
	Hays Co.: City of Austin WQPL, Sky Ranch Tract, State Well No. 5857507	1	1 Sep 2010	NFB, AGG	UTIC 91889
		3	3 Dec 2010	NFB, AGG	UTIC 91885
		8	14 Jan 2011	NFB, AGG	UTIC 91887
		1	8 Mar 2011	NFB, AGG	UTIC 91884
	Hays Co.: Onion Creek, Bello Spring	2	18 Apr 2017	TJD, BDN	UTIC 92035
	Hays Co.: South Onion Creek, Emerald Spring	1	13 Jan 2017	TJD, BDN	UTIC 92040
		2	18 Apr 2017	TJD, BDN	UTIC 92032
	Hays Co.: Roy Creek, Red's Spring	2	23 Apr 2017	TJD, BDN	UTIC 92038
		1	25 Jun 2017	TJD, BDN	UTIC 92037

Trap designs

We sampled springs using cotton mophead "traps" lodged into spring outlets (modified per methods in Holsinger and Minckley 1971, Hershler and Longley 1986, Gibson et al. 2008, Huston et al. 2015). We separated the mopheads into individual strings, tied them into loose bunches, and securely wedged them into the spring outlets using rocks to keep them in place (Figure 2). The size of the mop bunches was determined by the size of the spring outlet, ideally filling a large portion of the outlet, to maximize the volume of water flowing through the trap. Where possible, we placed multiple clumps of mophead material into the spring outlets at various locations. We checked mops for invertebrates after approximately two weeks by removing them from the spring outlet, quickly placing them in a large handheld net and flushing water through the net to dislodge any invertebrates from mophead strands. We also searched through the strands by hand after flushing. Specimens were collected and stored in 99% ethanol.

Five groundwater wells were sampled using a funnel trap fashioned from 1-L plastic water bottles with the top cut off and inverted into the bottle (Fenolio et al. 2017). We baited the traps using pistachio nuts, dried Mysis shrimp, Slim Jim (Conagra Brands) pieces, or catfish bait (Catalpa Worm: Little Stinker (Acme Tackle Company); Cricket: Berkley Gulp! Alive! (Berkley Fishing)). Traps were set between 0–10 m above the bottom of the well (Table 3). Wells were sampled every two weeks from September 2010

Table 3. Well Information. Data on sampled wells collected in the field and from the TWDB database. Tract names correspond to City of Austin WQPL tracts. The depth of trap in State Well No. 5857507 (Sky Ranch Tract) was not recorded. Water depth measurements were taken in the fall of 2017.

Tract Name/State Well No.	Depth to water	Depth of trap	Depth to bottom of well
Hays Co. Ranch – 58-49-939	17.2 m	27.7 m	29.3 m
Ed's Crossing – 58-49-9SH	42.6 m	44.2 m	44.2 m
Blowing Sink – 58-50-411	69.1 m	84.4 m	96.0 m
Barton Creek – 58-42-820	80.1 m	unknown	137 m
Sky Ranch – 58-57-507	48.8 m	unknown	306 m

Figure 2. Mophead in spring outlet at Cold Spring, Travis County, Texas, USA.

to December 2011 and then again 22 times between 21 October 2016 and 30 March 2018, for a total of over 1,000 days of trapping effort. Specimens are deposited in the Biodiversity Collections of the University of Texas at Austin and at the San Marcos Aquatic Resources Center, United States Fish and Wildlife Service (Table 2).

Results

New Occurrence Records

Caecidotea reddelli (Steeves, 1968) (Isopoda, Asellidae)

Site 1. TEXAS: Hays County: Roy Creek: Red's Spring (30.36324, -98.12315). Two specimens collected 21 October 2016 by TJD. Identified by RG. *New county record.*

Site 2. TEXAS: Travis County: Zilker Park, Eliza Spring (30.26425, -97.77006). One specimen collected 1 April 1999 by Dee Ann Chamberlain. Identified by RG.

Site 3. TEXAS: Travis County: Barton Creek Habitat Preserve, Sweetwater Tract Spring 1 (30.27535, -97.92709). One specimen collected 1 May 2017 by BDN and TJD. Identified by RG.

Site 4. TEXAS: Travis County: Barton Creek Habitat Preserve, Sweetwater Tract Spring 4 (30.27171, -97.92731). Three specimens collected 17 April 2017 by BDN and TJD. Identified by RG.

Site 5. TEXAS: Travis County: Old San Antonio District Park, Old San Antonio Spring (30.13217, -97.81750). Two specimens collected 19 January 2018 by BDN and TJD. Identified by RG.

Caecidotea reddelli is a stygobiontic isopod about 10 mm in length that occurs throughout central Texas in springs, caves, and wells in Bell, Burnet, Coryell, Dallas, Henderson, Hill, Limestone, Palo Pinto, Panola, San Augustine, Tarrant, Travis, and Williamson counties (Steeves 1968, Mitchell and Reddell 1971, Lewis and Bowman 1996, Lewis 2001, Hutchins 2018). *Caecidotea reddelli* is known from both the North Balcones Fault Zone and the adjacent part of the Gulf Coast Plain Province directly to the northeast in Dallas and Henderson counties (Lewis and Bowman 1996). Mitchell and Reddell (1971) showed an additional locality in Hays County (their fig. 30) but without further explanation, and there is no associated voucher specimen (Reddell, personal communication to TJD and BDN, 21 September 2017). Therefore, we present our specimen of *C. reddelli* collected from Red's Spring in Hays County as a new county record for this species.

Cirolanides nr. *texensis* (Isopoda, Cirolanidae).

Site 1. TEXAS: Hays County: City of Austin Water Quality Protection Lands (WQPL), Hays County Ranch Tract, State Well No. 5849939 (30.14722, -97.89691). First two specimens collected 1 September 2010 by NFB and AGG. Additional specimens are listed in Table 2. All specimens were identified by Benjamin F. Schwartz (Texas State University).

Site 2. TEXAS: Travis County: Blowing Sink Cave (30.189718, -97.851014). One specimen collected 14 October 2010 by Mark S. Sanders. Identified by Benjamin F. Schwartz. *New county record.*

Site 3. TEXAS: Travis County: City of Austin WQPL, Blowing Sink Tract, State Well No. 5850411 (30.18667, -97.84917). One specimen collected 4 December 2017 by BDN. Identified by Benjamin F. Schwartz.

These specimens are part of the *Cirolanides texensis* species complex, which needs revision (Ben Hutchins, Texas Parks and Wildlife, personal communication to BDN on 12 July 2018). These specimens represent a distinct lineage of *Cirolanides* related to *C. texensis* (Benedict, 1896) that warrants species-level designation, to be described elsewhere (Benjamin F. Schwartz, Texas State University, personal communication to BDN on 15 December 2017). All specimens were collected from a Hays County well and a cave and nearby well in Travis County.

Crangonyx nr. *pseudogracilis* (Bousfield, 1958) (Amphipoda, Crangonyctidae)

Site 1. TEXAS: Hays County: Old San Antonio District Park, Old San Antonio Spring (30.13217, -97.8175). Two specimens collected 31 January 2018 by BDN. Identified by RG.

Site 2. TEXAS: Travis County: Treadwell Spring (30.2549698, -97.7592774). Three specimens collected 21 June 2016 by Peter Sprouse. Identified by RG. *New county record.*

Crangonyx pseudogracilis is recorded in the east-central United States and southern Canada (Zhang and Holsinger 2003). Diaz and Alexander (2010) noted specimens of *Crangonyx* sp. collected in samples from the spring-fed San Marcos River, Hays County, Texas. Groundwater-adapted populations with reduced eyes and reduced pigmentation have been recorded in Comal and Kendall counties (Gibson et al. 2008). Specimens collected from Old San Antonio Spring and Treadwell Spring also show these stygobiontic adaptations.

Sphalloplana mohri (Hyman, 1938) (Triclada, Kenkiidae)

Site 1. TEXAS: Travis County: Cold Spring (30.27959, -97.78043). One specimen collected 24 February 2011 by RG. Identified by RG.

Sphalloplana mohri is a relatively large flatworm described by Hyman (1938) and recorded from the Edwards Plateau in Hays, Kendall, Mason, San Saba, Travis, and Uvalde counties (Kenk 1977, Hutchins 2018). This is the first record for Cold Spring.

Stygobromus balconis (Hubricht, 1943) (Amphipoda, Crangonyctidae)

Site 1. TEXAS: Travis County: City of Austin WQPL, Ed's Crossing Tract, State Well No. 58499SH (30.16472, -97.87889). One specimen collected 6 April 2017 by BDN. Identified by RG.

Site 2. TEXAS: Travis County: Barton Creek Habitat Preserve, Sweetwater Tract Spring 4 (30.27171, -97.92731). Four specimens collected 10 April 2017 by TJD and BDN. Identified by RG.

Stygobromus balconis is a relatively large species of *Stygobromus*. This species was originally described by Hubricht (1943) and later redescribed by Holsinger (1966, 1967) who subdivided the taxon into three species: *S. russelli*, *S. bifurcatus,* and *S. balconis*. *Stygobromus balconis* is known from very few localities in Hays, Travis and Kendall counties (Hutchins 2018).

Stygobromus bifurcatus (Holsinger, 1967) (Amphipoda, Crangonyctidae)

Site 1. TEXAS: Travis County: Zilker Park, Eliza Spring (30.26425, -97.77006). One specimen collected 29 August 2016 by Dee Ann Chamberlain. Identified by RG.

Site 2. TEXAS: Travis County: Barton Creek Habitat Preserve, Sweetwater Tract Spring 4 (30.27171, -97.92731). Eight specimens collected 17 April 2017 by TJD and BDN. Identified by RG.

Site 3. TEXAS: Hays County: Onion Creek, Ben McCulloch Spring (30.12732, -98.01709). One specimen collected 31 January 2017 by TJD. Identified by RG.

Site 4. TEXAS: Blanco County: Bamberger Ranch Spring (30.19185, -98.47723). One specimen collected 21 June 2017 by TJD and BDN. Identified by RG.

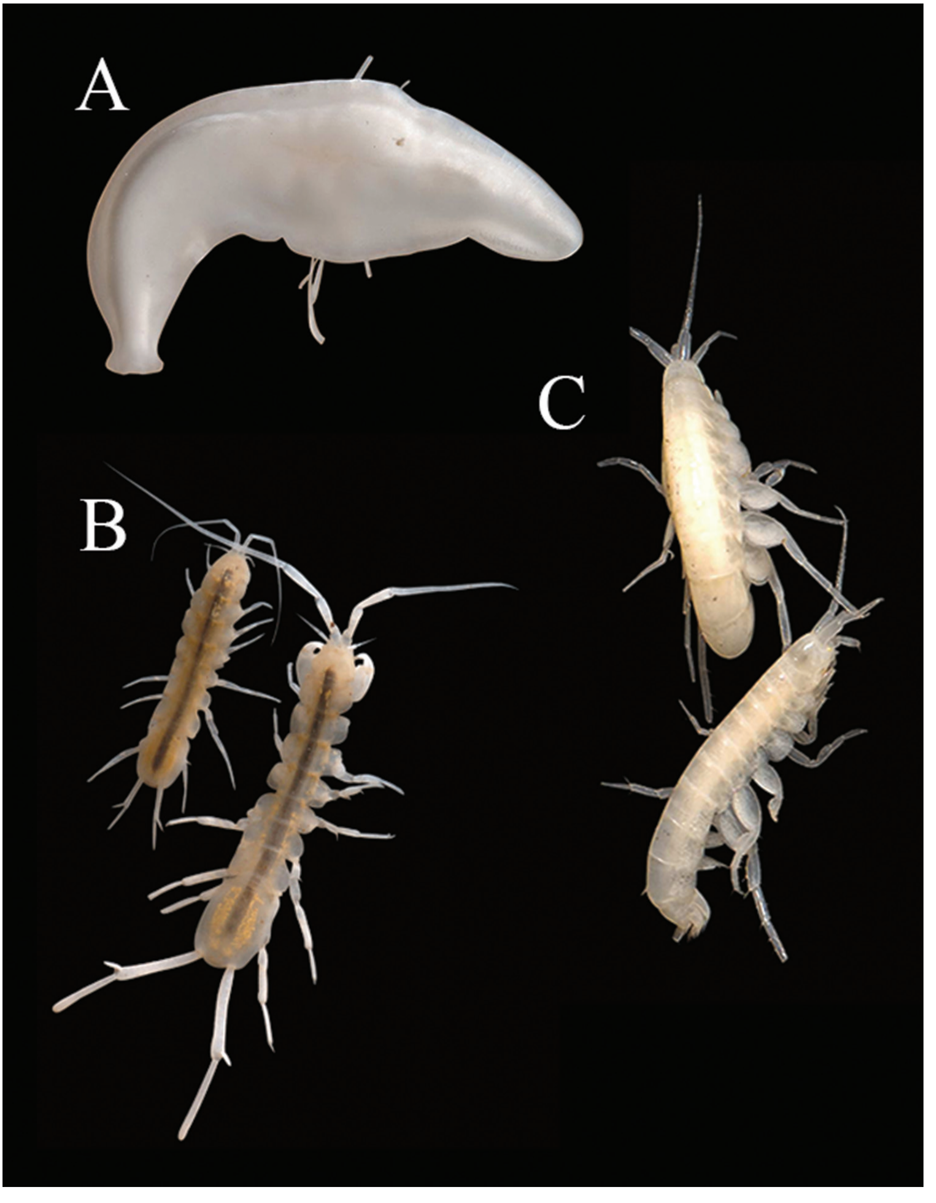

Figure 3. A *Sphalloplana mohri* Hyman, 1938 from Cold Spring, Travis Co., Texas, USA **B** *Caecidotea reddelli* (Steeves, 1968) from Rocket River Cave, Coryell Co., Texas, USA **C** *Stygobromus balconis* (Hubricht, 1943) from Autumn Woods Well, Hays Co., Texas, USA. All photographs by Dr. Jean K. Krejca, Zara Environmental LLC. Images not to scale.

Stygobromus bifurcatus (Holsinger, 1967) is rather widely distributed, often occurring syntopically with *S. russelli* (Mitchell & Reddell, 1971). It is currently known from Bell, Bexar, Blanco, Burnet, Comal, Coryell, Hays, Kendall, Lampasas, San Saba, Travis, and Williamson counties (Hutchins 2018).

Stygobromus russelli (Holsinger, 1967) (Amphipoda, Crangonyctidae)

Site 1. TEXAS: Travis County: Barton Creek Greenbelt, State Well No. 5842820 (30.26139, -97.816944). Three specimens first collected 3 December 2010 by AGG and NFB. Additional specimens listed in Table 2. All specimens identified by RG.

Site 2. TEXAS: Travis County: Zilker Park, Eliza Spring (30.26425, -97.77006). One specimen collected 19 November 2015 by Dee Ann Chamberlain. Identified by RG.

Site 3. TEXAS: Travis County: City of Austin WQPL, Blowing Sink Tract, State Well No. 5850411 (30.18667, -97.84917). One specimen collected 30 March 2018 by BDN. Identified by RG.

Site 4. TEXAS: Hays County: South Onion Creek, Emerald Spring (30.14769, -98.07868). One specimen collected 13 January 2017 by TJD and BDN. Additional specimens listed in Table 2. Identified by RG.

Site 5. TEXAS: Travis County: City of Austin WQPL, Ed's Crossing Tract, State Well No. 58499SH (30.16472, -97.87889). One specimen collected 8 Mar 2011 by AGG and NFB. Additional specimens listed in Table 2. Identified by RG.

Site 6. TEXAS: Hays County: Onion Creek, Bello Spring (30.14537, -98.07599). Two specimens collected 18 April 2017 by TJD and BDN. Identified by RG.

Site 7. TEXAS: Hays County: Onion Creek, Ben McCulloch Spring (30.12732, -98.01709). One specimen first collected 31 January 2017 by TJD. Additional specimens listed in Table 2. All specimens were identified by RG.

Site 8. TEXAS: Hays County: City of Austin WQPL, Sky Ranch Tract, State Well No. 5857507 (30.06358, -97.94253). One specimen first collected 1 September 2010 by AGG and NFB. Additional specimens listed in Table 2. All specimens identified by RG.

Site 9. TEXAS: Hays County: Roy Creek, Red's Spring (30.36324, -98.12315). Two individuals first collected 23 April 2017 by TJD. Additional specimens are listed in Table 2. All specimens were identified by RG.

Site 10. TEXAS: Blanco County: Bamberger Ranch Spring (30.19185, -98.47723). One specimen collected 22 March 2018 by TJD and BDN. Identified by RG.

Stygobromus russelli is relatively common, morphologically variable, and widely distributed throughout the Edwards and adjacent Trinity aquifers (Hutchins et al. 2013). Its range covers most of the eastern half of the limestone area of central Texas, however most records are recorded from caves just west and northwest of Austin (Holsinger and Longley 1980). It is currently known from Bandera, Bell, Bexar, Burnet, Comal, Coryell, Hays, Kendall, Kerr, Mason, Medina, San Saba, Travis, and Williamson counties (Hutchins 2018).

Acknowledgments

We thank James Reddell (University of Texas) for cataloging all specimens, and valuable feedback; Benjamin Schwartz (Texas State University) and Ben Hutchins (Texas Parks and Wildlife Department) for specimen identification and helpful discussion; and Dee Ann Chamberlain (City of Austin), Mark Sanders (City of Austin), and Peter Sprouse (Zara Environmental, LLC) for specimen donations. Finally, we thank the many landowners and managers who provided access to lands under their care, including: Jared Holmes and the Bamberger Ranch; Reed and Holton Burns (Charro Ranch); Kevin Theusen (City of Austin); the owners of Camp Ben McCulloch; Brandon Crawford, John Karges, and The Nature Conservancy of Texas. The views presented herein are those of the authors and do not necessarily represent those of the U.S. Fish and Wildlife Service or the City of Austin.

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Subterranean Biology 28: 15–28 (2018)
doi: 10.3897/subtbiol.28.30700
http://subtbiol.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
Subterranean Biology
Published by The International Society for Subterranean Biology

Response of shallow subterranean freshwater amphipods to habitat drying

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Academic editor: *Oana Moldovan* | Received 19 October 2018 | Accepted 30 October 2018 | Published 16 November 2018

http://zoobank.org/359479FF-F292-49B8-8B39-754E7E01739D

Citation: Gilbert H, Keany J, Culver DC (2018) Response of shallow subterranean freshwater amphipods to habitat drying. Subterranean Biology 28: 15–28. https://doi.org/10.3897/subtbiol.28.30700

Abstract

The ability of three amphipods that occupy shallow subterranean habitats in the lower Potomac Basin of the US (hypotelminorheic), which often dry out seasonally, to withstand desiccation by burrowing in clay was investigated. Both *Crangonyx shoemakeri*, a wetland species, and *Stygobromus tenuis*, a subterranean species, burrowed in clay in the laboratory after surface water was removed. *Gammarus minus*, a spring species, did not. All three species exhibited behavioral changes as the habitat dried out.

Keywords

Adaptation to desiccation, burrowing, *Crangonyx shoemakeri*, *Gammarus minus*, Hypotelminorheic, shallow subterranean habitats, *Stygobromus*

Introduction

Freshwater habitats, such as wetlands and seasonal streams, typically first order streams, are subject to drying. Water level patterns, or hydroperiods, are an important way to categorize wetland habitats (Jackson et al. 2014, Batzer and Boix 2016). Permanent wetlands differ from seasonal wetlands (e.g., Scheffer 2004, Zimmer et al. 2016) in that their inhabitants are adapted to deal with a significant loss of water for months at a time (Wiggins et al. 1980). These adaptations include resting stages, migration, and burrowing into substrates that hold moisture. The fauna of wetlands varies, and among the most ubiquitous macro-fauna are Chironomidae and Dystiscidae (Batzer and Ruhí 2013).

In contrast with wetland habitats, caves and other subterranean habitats are typically among the most environmentally stable freshwater ecosystems (Poulson 1964, Culver and Pipan 2009). Cave streams dry up well after surface streams because they are fed by shallow subsurface aquifers in the epikarst (Williams 2008). Nevertheless, not all aquatic cave habitats are permanent. Many drip pools in caves, perched well above any streams, are subject to periodic drying either because of increased evaporation in cave passages, especially during winter summer months, or because of small changes in the flow path of water moving through epikarst. The epikarst itself, which is not accessible to direct observation or sampling, harbors both terrestrial and aquatic species (Culver and Pipan 2014). This outfall of animals suggests that habitats may switch between aquatic and terrestrial with water movement. Ginet (1960) and Mathieu and Turquin (1992) point out that the drying of pools is a typical part of the environment of some cave-dwelling *Niphargus* amphipods. The subterranean macro-fauna is less varied than that of wetlands, and is dominated by crustaceans, especially amphipods and isopods (Gibert and Culver 2009).

Burrowing to avoid desiccation is well known among wetland invertebrates, especially among species that have no resting stage and are unable to disperse, such as amphipods (Wiggins et al. 1980; DiSalvo and Haynes 2015). However, there are few reports of burrowing behavior of cave amphipods. Ginet (1960) reported burrowing in the subterranean amphipod *Niphargus virei*, an inhabitant of isolated pools in caves in France, and Mathieu and Turquin (1992) summarized his observations. Gounot (1960) suggested that burrowing was actually the result of ingestion of clay for its nutritional value, perhaps some micronutrient given the generally low level of organic carbon in clay. Holsinger and Dickson (1977) observed burrowing behavior in *Crangonyx antennatus*, a cave pool inhabitant in Virginia caves, in the laboratory, of both populations from permanent and temporary cave waters. The temporary cave waters in this case were epikarst drip pools.

While there is little discussion of wetlands in the cave and karst literature (see Pipan and Culver 2012, Sheehy Skeffington and Gormally 2007, Skeffington and Scott 2008), and even less discussion of caves and karst in the wetlands literature (Reynolds 2016), there is a unique habitat that bridges the two categories – the hypotelminorheic. First described by Meštrov (1962), it is in essence, a superficial subterranean drainage (Pipan et al. 2012) and a miniature wetland at its exit. This definition was later expanded by Culver and Pipan (2014) to include:

- A persistent wet spot that may dry up seasonally
- Underlain by clay or other impermeable layer
- Water exiting via seepage flow, typically from a slight depression
- Presence of blackened leaves
- A subterranean fauna, with species lacking or having reduced eyes and pigment

The hypotelminorheic is a subterranean habitat because it is aphotic except at its exit at a seep or seepage spring, where some light penetrates the leaf litter. We follow Williams' (2017) distinction between seeps and springs. He defines seeps as comprising groundwater that emerges over a more diffuse area [than springs], with a rate of flow that is generally insufficient to form an outflow spring. The exit point, or seep, is an isolated wetland, rich in organic carbon from decaying leaves, and is in turn habitat for amphipods. This, in many ways unremarkable habitat, is made remarkable by its fauna. Of the 56 species in the exclusively subterranean amphipod genus *Stygobromus* found in the eastern United States 12 (21%) are found primarily in hypotelminorheic habitats (Culver et al. 2010). In turn, amphipods and isopods are the dominant macroorganisms of all studied hypotelminorheic habitats (Pipan et al. 2012). Many of these species are both troglomorphic (reduced or absent eyes and pigment, and elaborated appendages) and stygobiotic (limited to subterranean habitats).

With increased monitoring and sampling of seeps in the mid-Atlantic region of the U.S. (e.g., Culver et al. 2012), it became apparent that most seeps have no flow during the summer months (May to October). Whether the subterranean hypotelminorheic habitat also dries out is not directly known, but if the exit to these small aquifers is dry, then the amount of free water in the aquifer is likely reduced. During these drying periods, water is likely retained in the clay layer. The two key properties of clay are both its high porosity (nearly as high as soil) and high specific retention (Heath 1983). Given the water retention of clay, it is plausible that the amphipod and isopod inhabitants of hypotelminorheic habitats burrow into clay as a refuge during dry periods.

In the course of sampling seeps in parklands of Washington, DC, specifically in National Capital Parks-East (Piscataway, Oxon Cove, Anacostia, Fort Dupont, Shepherd Parkway, and others), it was found that the macro-crustacean fauna was dominated by two species of amphipods – *Crangonyx shoemakeri* and a *Stygobromus* species in the *tenuis* group – and the isopod *Caecidotea kenki,* (Keany et al. 2018). The *Stygobromus* species lacks eyes and pigment, and *C. kenki* and *C. shoemakeri* have reduced eyes and pigment. The undescribed species of *Stygobromus* is likely limited to the Anacostia River drainage in Washington, DC, and adjoining parts of Maryland. *C. shoemakeri* is a common inhabitant of wetlands in National Capital Parks-East and elsewhere in its range from southcentral Maryland to southcentral Virginia (Zhang and Holsinger 2003). While not adapted to subterranean life in any obvious way, *C. shoemakeri* has likely adapted to the periodic drying of seasonal wetland habitats where it is found. Occasionally, a third amphipod is found in seeps of the DC metro area, especially those with direct connections to permanent surface streams – *Gammarus minus. G. minus* is primarily found in springs, and a distinct morphological variant – *Gammarus minus* var. *tenuipes* – is common in cave streams (but not cave pools, Culver et al. 1995). It is rarely, if ever, a permanent inhabitant of hypotelminorheic habitats. The isopod *Caecidotea kenki* is found in seeps and other small groundwater fed habitats. It is known from the lower Potomac drainage around Washington, DC, as well as two caves in Pennsylvania (Culver et al. 2012). It is not considered further in this study, as our study species were limited to amphipods.

Our goal in this study was to determine whether the ability to burrow into clay substrates is an important factor in determining the presence of *Stygobromus* species and *Crangonyx shoemakeri* in seeps and the general absence of *Gammarus minus*. In order to investigate this, we conducted laboratory experiments monitoring the behavior and success of the three amphipods when water was systematically removed from a clay-filled petri dish.

Methods

Species

Three species were studied – *Crangonyx shoemakeri* (Hubricht and Mackin), *Gammarus minus* (Say), and *Stygobromus tenuis potomacus* (Holsinger). *S. tenuis potomacus* was used rather than the undescribed species from the *tenuis* group because there were not sufficient specimens of the undescribed species available. The two species are very similar morphologically and ecologically. All three species were found in seeps in the parklands of the District of Columbia, although *G. minus* was only found in seepage springs, typically with a permanent outflow, and usually during the Spring when water levels were at their highest.

Specimens used in this study were taken from seeps and springs in C & O Canal National Historic Park under permit CHOH-2016-SCI-0023. These individuals were obtained using standard collecting procedures as described by Holsinger (1972). Early trials were completed using animals from Oxon Run in National Capital Parks-East under permit NACE-2016-SCI-0002. Unused animals were returned to their habitat.

Laboratory methods

The behavioral study was conducted in a Forma-lab Walk-in Environmental Room with a constant temperature of 5 °C. Glass pyrex dishes, with a volume of approximately 470 mL, (10 cm diameter and 4 cm depth) were lined with 30g of dry VWR™ clay, creating a clay layer approximately 2 cm deep. This clay was homogenized using a mortar and pestle and was sifted through a 125μm mesh sieve to ensure consistent grain size. Sixty mL of Deer Park™ spring water were added to each of the glass pyrex dishes, creating a water depth of approximately 2 cm.

The three species of amphipods – *S. tenuis potomacus*, *C. shoemakeri* and *G. minus* – were studied separately. Within one week from the date of specimen collection, five individuals, all adults, of the same species were added to each dish. Ovigerous females were not used. After a 48 hour period of acclimation to the pyrex dishes, the trials began. This acclimation period was chosen based on the 2–10 day amphipod acclimation period recommended by Environment Canada (1993) and Swartz et al. (1985).

Six dishes were designated for each species; three served as the control while the other three were used as experimental dishes that simulated drying conditions. Each trial lasted five weeks.

A supplemental experiment was completed to determine the amount of water loss (retention) of the clay over the course of eight weeks. The dry weight of the clay was compared to the weight of the clay after one to eight weeks of drying. Measurements began at the end of week 1, after all surface water was removed.

During the course of the drying experiment, behavioral observations were made for 60 second intervals three times a week. The following behaviors were recorded if one or more individuals was observed with this behavior during the 60 second interval:

1 Walking (at least one body length)
2 Swimming (at least one body length)
3 Burrowing (direct observation or buried in clay)
4 Resting (resting for more than 10 seconds)
5 Interaction (with other amphipods)
6 Other (amplexus,etc.)

The relative frequency of these behaviors is not a direct measure of the time spent at each behavior because a behavior was given the same weight whether one or five amphipods was displaying the behavior. This indirect measure was necessary because it was impossible to track each individual separately. All observations were made under red light.

Data analysis

Both survival and behavioral data were analyzed used log-likelihood tests (G-tests), comparing observed and expected values in each category (e.g., walking in control dishes). G, which is distributed as χ^2 was calculated as follows:

$$G = 2 \sum_i O_i \ln\left({}^{O_i}/_{E_i} \right)$$

where O_i is the observed number of cases in category *i* and E_i is the expected number of cases in category *i*.

Results

Gravimetric measurement of moisture levels of the clay at the start of each trial averaged at 65.5% and reduced to an average of 54.2% after the first week of drying.

Table 1. Results of experiments on effect of drying. All runs began with 15 individuals.

Species	Start of Trial	Control-surviving at 4 weeks	Experiment-surviving at 4 weeks	Difference	Log-likelihood	p
Gammarus minus	7/20/2016	15	8	-7.00	5.15	<0.05
	7/20/2016	15	7	-8.00	4.44	<0.05
	8/9/2016	15	10	-5.00	3.45	<0.10
	1/8/2017	2	2	0.00	0.00	ns
	1/8/2017	1	2	1	0.27	ns
	Total	48	29	-19	7.61	<0.010
Crangonyx shoemaker	7/20/2016	13	12	-1	0.53	ns
	8/9/2016	15	15	0	0.00	ns
	1/8/2017	15	15	0	0.00	ns
	1/8/2017	15	15	0	0.00	ns
	1/8/2017	12	15	3	1.95	ns
	Total	70	72	2	0.25	ns
Stygobromus tenuis potomacus	7/20/2016	14	15	1	0.62	ns
	1/8/2017	15	15	0	0.00	ns
	1/8/2017	15	15	0	0.00	ns
	Total	44	45	1	0.61	ns

Moisture levels then reduced to 34.4% at week two, and remained relatively consistent from week two to week nine, at around 30% (Fig. 1).

Overall, there was a 25% increase in mortality for *G. minus* when subjected to drying (Table 1), which was a statistically significant difference between experiment and control (G=7.61, p<0.01). The difference was especially striking in the first three trials where no control animals died, and 44% of the amphipods died in the experimental treatment. In the last two trials, only a few amphipods survived (23%) in either experiment or control treatments. We cannot account for this difference between the first three and the last two experiments, but the high mortality suggests some water quality problem. However, no increased mortality was recorded for the other species. Both *C. shoemakeri* and *S. tenuis potomacus* showed the identical pattern of little or no mortality in either experiment or control (Table 1). For *C. shoemakeri* only 4% of experimental animals died, slightly fewer than the number of control animals that died. For *S. tenuis potomacus*, there was no mortality in the experimental treatments, and only one amphipod died in the control (Table 1). Thus, only *G. minus* had significant mortality associated with the drying experiment, and there were no differences in the survival of *C. shoemaker* and *S. tenuis potomacus*.

The three species showed differences in behavior, both between experiment and control, but also between species (Fig. 2, Table 2). In the controls, all three species showed frequent interaction (between 30% and 40% of the observational periods had one or more interactions) with other individuals, typically avoidance behavior (see Culver 1970). Locomotory behavior was observed about half the time, typically swimming in the case of *G. minus* and walking in the case of *C. shoemakeri*. Somewhat surprisingly, *S. tenuis potomacus* was equally likely to swim or walk, even

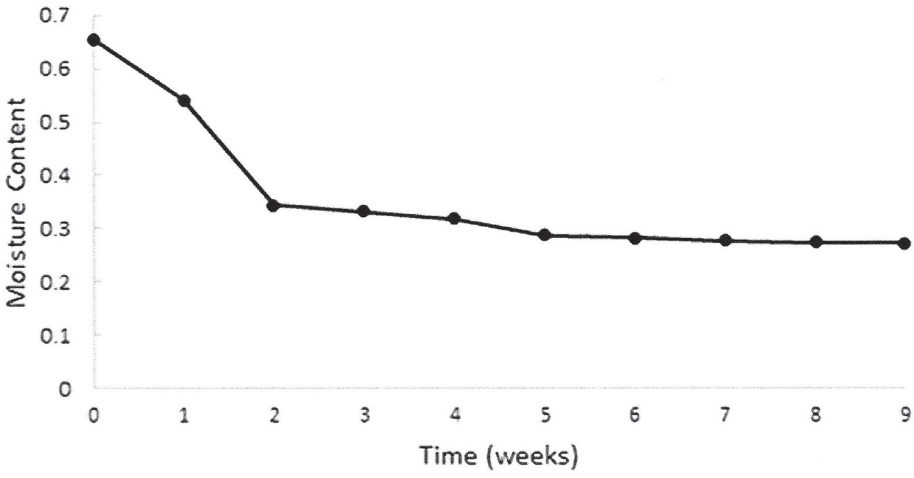

Figure 1. Average gravimetric soil moisture over the nine week trial.

though species in the genus are weak swimmer and usually walk (Culver 1970). Resting was uncommon in all three species in control containers, occurring less than 10% of the time (Fig. 2). *G. minus* were in amplexus 12 % of the time. This behavior was particularly frequent in the last two control trials. Overall, in the control containers, the three species had differences in behavior (Table 3) due to species specific behavioral patterns in locomotion (swimming vs. walking) and amplexus (only with *G. minus*).

When in the experimental containers, each species showed statistically significant differences in behavior relative to the control containers (Table 3). *G. minus* showed decreases in all categories except inactivity, which was observed 85% of the time (Fig. 2, Table 2). Typically, the *G. minus* animals were stretched out on the substrate and burrowing was only observed once. Amplexing was a particularly frequent behavior in the last two trials, perhaps due to seasonality of mating behavior.

For *C. shoemakeri,* resting was also the most common activity, occurring 77% of the time (Fig. 2). In comparison with resting *G. minus,* resting *C. shoemakeri* curled into 'C' shapes keeping their appendages tight to their bodies. Burrowing, and residence in burrows was the second most common activity, occurring 10.4% of the time. Not surprisingly, swimming almost completely disappeared (less than 1%) and walking was observed only 9.4% of the time, given that there was no open water (Fig. 2).

Overall, *S. tenuis potomacus* showed less behavioral change than the other two species, but the differences were statistically significant compared to its control group (Table 2). Resting was the most common behavior in the experimental conditions, occurring 42.1% of the time, but was still less frequent when compared to resting in the other two species. Walking and interaction remained common, although less so than in controls,

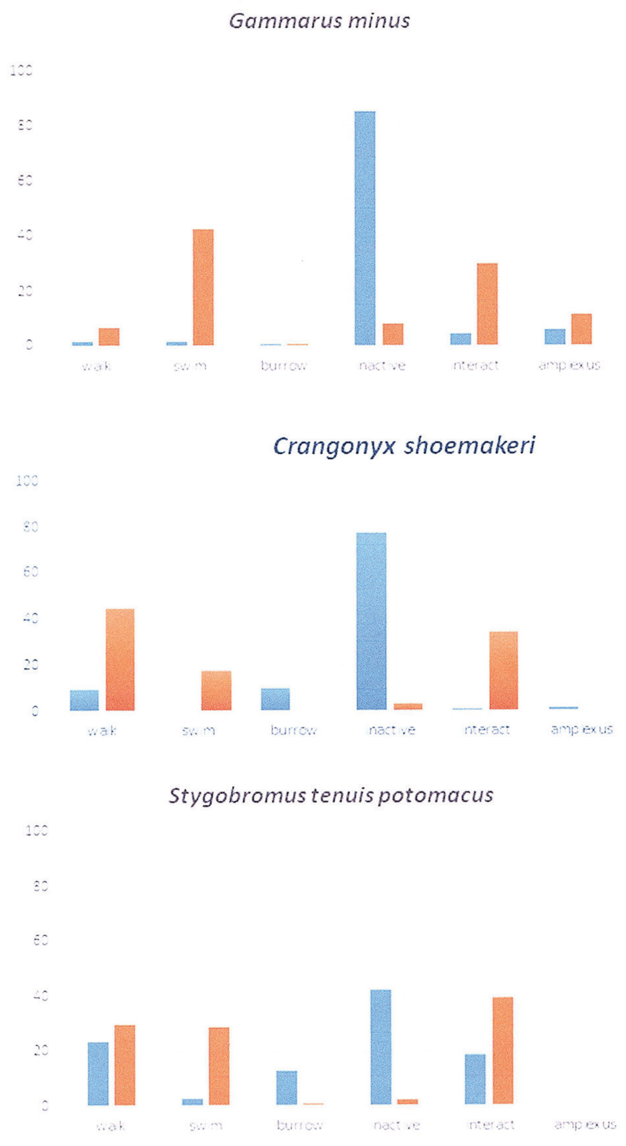

Figure 2. Relative frequency of different behaviors of the three species in control (red bars) and experimental (blue bars).

and swimming declined to less than 3% (Fig. 2, Table 2). Burrowing (and residence in burrows) was relatively frequent in *S. tenuis potomacus*, accounting for 12.9% of observations in the experimental group. The burrows of *S. tenuis potomacus* were deeper than those of *C. shoemakeri*, but they both had a similar C-shape when resting. *S. tenuis potomacus* leave circular depressions visible from the top of the soil where they have entered the clay. They exhibit the greatest amount of burrowing and travel the deepest into the clay; one *S. tenuis potomacus* hole was visible from the bottom of the dish.

Table 2. Number of observations of six different behaviors of *Crangonyx shoemakeri*, *Gammarus minus*, and *Stygobromus tenuis potomacus* in experimental and control containers. If at least one individual (and up to five) shows the behavior, this is counted as an observation. See methods for the observational protocols.

Category	*Gammarus*		*Crangonyx*		*Stygobromus*	
	Exp	Control	Exp	Control	Exp	Control
1- walk	3	20	19	138	40	55
2-swim	3	125	1	54	5	53
3-burrow	1	2	21	0	22	1
4-resting	161	24	156	10	72	4
5-interact	8	88	2	106	32	73
6-amplexus	11	34	3	0	0	0
Total	189	293	202	308	171	186

Table 3. Log-likelihood tests for differences between behaviors in experimental and control containers for *Crangonyx shoemakeri*, *Gammarus minus*, and *Stygobromus tenuis potomacus*, and among control behavior and among behavior in experimental containers. n_1, n_2, and n_3 are the total number of observations of a behavior, e.g., walking. The total number of separate observations was 180 for *G. minus* and *C. shoemakeri*; 108 for *S. tenuis potomacus*. For some observation periods more than one behavior was observed. See Figure 1.

Comparison	n_1	n_2	n_3	df	log - likelihood	p
G. minus-experiment vs. control[2]	188	291		4	147.6	<0.01
C. shoemakeri-experiment vs. control[1]	199	308		4	198,9	<0.01
S. t. potomacus-experiment vs. control[1]	171	186		4	70.4	<0.01
All control	308	291	186	10	112.1	<0.01
All experimental	199	188	171	10	70	<0.01

[1]Amplexus is excluded due to small sample size.
[2]Burrowing is excluded due to small sample size.

Discussion

Adaptation to the absence of open water

Crangonyx shoemakeri and *Stygobromus tenuis potomacus* have evolved mechanisms allowing for their success in habitats that periodically lack open water. Both of these species exhibited similar adaptations by burrowing into the substrate during experimental drying periods. Those individuals that failed to burrow, remaining exposed on the surface, curled in ‘C’ shapes and often times lay in shallow depressions. The length of time that burrowing in clay can provide a refuge from desiccation is unknown, beyond the four week duration of the experiment. We do know that moisture is retained in clay for longer periods (see Fig. 1).

Animals living in surface habitats that periodically dry, including many wetlands, have a large array of strategies to cope with desiccation (Wiggins et al. 1980). *Crangonyx* is a frequent inhabitant of wetlands (Boix and Baxter 2016), and Wiggins et al. (1980) report that *C. gracilis* uses crayfish burrows as refuges from desiccation. Wiggins et al. (1980) report that *C. rivularis* burrows into sediments and bottom vegetation during the absence of open water in summer and autumn. DiSalvo and Haynes (2015) describe an interesting strategy employed by *C. pseudogracilis* in temporary pools. During wet weather they actively enter existing small tubes in the soil, and are able to persist throughout the year in damp soil. Unfortunately, no phylogeny exists for *Crangonyx* and so we cannot look directly at evolutionary relationships of this behavior.

In addition to its widespread occurrence in wetlands, *Crangonyx* is also a frequent inhabitant of caves and some of these species occur in epikarst drip pools, a subterranean habitat that occasionally dries out (Pipan and Culver 2013). Holsinger and Dickson (1976) observed burrowing behavior in an epikarst drip pool population of *Crangonyx antennatus* in Molly Waggle Cave in Virginia.

The only other observations of burrowing behavior in subterranean habitats comes from the European amphipod genus *Niphargus*. Ginet (1960) observed *Niphargus* curling into a 'C' shape in a shallow burrow where individuals were at least partially visible from the surface (see also Mathieu and Turquin 1996). Interestingly, they reported that an air bubble was typically present on the dorsal side, presumably aiding in oxygen exchange. It remains unknown if either *Stygobromus tenuis potomacus* or *Crangonyx shoemaker* form such air bubbles, but none were directly observed. Gounot (1960) asserted that the clay supplies important nutrients from the presence of inert organic substances as well as microorganisms. She based this on experiments where survival was enhanced when *Niphargus virei* was provided a clay substrate compared to the control where no substrate was provided. Gounot showed that while the nutrients associated with clay were needed for immature *Niphargus*, they are not sufficient as the only source of nutrients for adults. To our knowledge, these preliminary experiments have never been followed up.

Conservation and protection

Clay substrates provide important resources for amphipod species from nutrition to surviving environmental stressors. Our findings indicate the importance of a clay layer for *Crangonyx shoemakeri* and *Stygobromus tenuis potomacus* amphipods inhabiting hypotelminorheic habitats in the Washington, DC region. To protect these species and the endemic and endangered species, *Stygobromus hayi*, it is essential to maintain healthy soil. Although these cryptic species may not be found during drying periods, it is apparent that the animals are still present. Due to the difficulty in accessing the microenvironments these animals create in the soil, biological surveys may report false negatives. The application of eDNA sampling holds promise for detecting these populations (Niemiller 2017).

By 2090, it is expected that the proportion of the global land surface in extreme drought will increase tenfold from current levels (Kundzewicz et al. 2008). Due to the extension of droughts and decreasing water levels, wetlands are at risk. While some of these wetlands are ephemeral and therefore not always wet, they constitute a large portion of the Earth's freshwaters and support freshwater biodiversity (Willliams 2006). As a result of wetland global significance to healthy ecological systems, climate-driven changes to wetland hydrology have been identified as a key global wetland conservation issue (Klein et al. 2005; Waterkeyn et al. 2008). Records of river and wetland hydroperiods already indicate pronged and more frequent dry periods (Strachan et al. 2014). These measurements are two components of a hydroregime. The characteristics of a particular hydroregime, particularly the hydroperiod, have a direct influence on aquatic community structure and its biodiversity (Brooks 2000; Williams 2006; Vanschoenwinkel et al. 2009).

In Australia, Sim et al. (2013) examined long-term data sets of temporal trends in the persistence and the stability of freshwater ecosystems and documented changes in wetland hydroperiods over the past 50 years. At one site, the hydroperiod was significantly correlated with invertebrate community richness. Ultimately, however, the study could not capture the true effects of long term drying. The magnitude of drying on these communities is yet to be fully recorded. As successive hydroperiods decrease without sufficient time and recharge, the flexibility of invertebrate survival strategies will be tested. Small wetlands that facilitate movement of species on a landscape scale may completely dry, leaving exposed the many species that rely on migrating to nearby permanent water after ephemeral habitats dry (Sim et al. 2013). This loss of water is cited as one of the most deleterious of environmental changes, changing the patch dynamics of species. As species geographic range is reduced, metapopulations will be altered (Robson et al. 2011). If surface water disappears completely, this would signify the end of the corresponding habitat niche, and only those inhabitants capable of surviving without even intermittent surface water could persist in the long term.

In order to further assess the impact these anthropogenic changes to the environment will have on amphipod species, it is necessary to learn more about thair physiological sensitivity. To better understand amphipod adaptations and estimate the impact of warming on their fitness, further studies on the burrowing behavior of *Crangonyx shoemakeri* and *Stygobromus tenuis potomacus* should be investigated.

Acknowledgements

This research was supported by Chesapeake Watershed Cooperative Ecosystem Studies Unit Cooperative Agreement P16AC01389 between Department of the Interior, National Park Service, National Capital Parks – East and American University, "A Survey of the Fauna of Seepage Springs in National Capital Parks-East (NACE)". All specimens were collected under CHOH-2016-SCI-0023 and NACE-2016-SCI-0002 permits. A reviewer offered many constructive comments.

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Subterranean Biology 28: 29–38 (2018)
doi: 10.3897/subtbiol.28.30506
http://subtbiol.pensoft.net

SHORT COMMUNICATION

A peer-reviewed open-access journal
Subterranean Biology
Published by The International Society for Subterranean Biology

A new maximum body size record for the Berry Cave Salamander (*Gyrinophilus gulolineatus*) and genus *Gyrinophilus* (Caudata, Plethodontidae) with a comment on body size in plethodontid salamanders

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Academic editor: *O. Moldovan* | Received 12 October 2018 | Accepted 23 October 2018 | Published 16 November 2018

http://zoobank.org/2BCA9CF1-52C7-47B6-BDFB-25DCF9EA487A

Citation: Gladstone NS, Carter ET, Niemiller KDK, Hayter LE, Niemiller ML (2018) A new maximum body size record for the Berry Cave Salamander (*Gyrinophilus gulolineatus*) and genus *Gyrinophilus* (Caudata, Plethodontidae) with a comment on body size in plethodontid salamanders. Subterranean Biology 28: 29–38. https://doi.org/10.3897/subtbiol.28.30506

Abstract

Lungless salamanders in the family Plethodontidae exhibit an impressive array of life history strategies and occur in a diversity of habitats, including caves. However, relationships between life history, habitat, and body size remain largely unresolved. During an ongoing study on the demography and life history of the paedomorphic, cave-obligate Berry Cave Salamander (*Gyrinophilus gulolineatus*, Brandon 1965), we discovered an exceptionally large individual from the type locality, Berry Cave, Roane County, Tennessee, USA. This salamander measured 145 mm in body length and represents not only the largest *G. gulolineatus* and *Gyrinophilus* ever reported, but also the largest plethodontid salamander in the United States. We discuss large body size in *G. gulolineatus* and compare body size in other large plethodontid salamanders in relation to life history and habitat.

Keywords

amphibian, habitat, life history, paedomorphosis, subterranean

Introduction

Body size in amphibians is driven by strong selective pressures, because it interacts with many aspects of life history (Whitford and Hutchison 1967, Blueweiss et al. 1978, Hairston and Hairston 1987, Stearns 1992). Although several ecological and evolutionary mechanisms can be responsible for body size variation in amphibians, overarching patterns are elusive (*e.g.*, Bernardo and Reagan-Wallin 2002, Adams and Church 2008, Slavenko and Meiri 2015). In response to Tilley and Bernardo (1993), Beachy (1995) argues that a primary influence on body size in amphibians is a delay in larval and juvenile period. In general, *K*-selected characteristics are correlated with increased longevity and a shift toward larger propagule size in stable environments. Prolonged developmental periods may promote neoteny (or prolonged maturation) and can be associated with reduced energy demand (McNamara and McNamara 1997). This suggests a possible correlation between increased body size and both paedomorphic and *K*-selected life history strategies. However, the relationship between amphibian body size and these life history strategies is largely unresolved (Yeh 2002, Wiens and Hoverman 2008).

While the reduction of body can be associated with paedomorphic traits (e.g., Alberch and Alberch 1981, Yeh 2002), Wiens and Hoverman (2008) concluded that obligately paedomorphic salamanders (Amphiumidae, Cryptobranchidae, Proteidae, Sirenidae) exhibit larger body sizes compared to those within clades that undergo metamorphosis. This pattern does not seem to translate to paedomorphic species within clades that possess metamorphic or direct-developing species (Wiens and Hoverman 2008). In fact, paedomorphic *Eurycea* (Plethodontidae) associated with springs and caves of the Edward's Plateau in Texas are characterized by reduced body size relative to their obligately metamorphic congeners, while both metamorphic and paedomorphic *Ambystoma* (Ambystomatidae) share similar body size (Ryan and Bruce 2000, AmphibiaWeb 2018).

Caves and other subterranean habitats are often viewed as extreme and inhospitable environments characterized by an absence of primary production and limited resources (Culver and Pipan 2009). Salamanders are one of only two vertebrate groups to have successfully colonized and obligately live in subterranean habitats. Fourteen species from two families (Plethodontidae and Proteidae) occur exclusively in caves, and most have evolved paedomorphosis (Goricki et al. 2012, in press, Niemiller et al. unpubl. data), which may be a response to limited food resources within terrestrial cave habitats (Brandon 1971, Wilbur and Collins 1973, Ryan and Bruce 2000). Few studies have examined the relationship between cave inhabitation and body size, and changes in body size may not necessarily be associated with shifts from surface to subterranean habitats (Romero 2009, Pipan and Culver 2017). However, many cave-obligate species (i.e., troglobites) exhibit *K*-selected life history traits such as reduced growth rate, delayed sexual maturity, and increased longevity (Brandon 1971, Culver and Pipan 2009, Hüppop 2012), and some troglobites and stygobites are larger than their surface congeners, such as in amblyopsid cavefishes (Poulson 1963, 1985, Niemiller and Poulson 2010).

The plethodontid genus *Gyrinophilus* Cope, 1869 includes four semi-aquatic to paedomorphic species endemic to the highlands of eastern North America. Three species are paedomorphic stygobionts found in caves of the Interior Low Plateau and Appalachians karst regions of Alabama, Tennessee, Georgia, and West Virginia in the United States (Niemiller et al. 2009, Goricki et al. 2012). Here, we report on a Berry Cave Salamander, *G. gulolineatus* Brandon, 1965, from the type locality in Roane Co., Tennessee that exceeds the current maximum body size record for the species and represents the largest *Gyrinophilus* and plethodontid salamander reported in the United States. *Gyrinophilus gulolineatus* is known from just ten localities in the Clinch and Tennessee River watersheds in the Appalachians karst region of eastern Tennessee (Figure 1). The largest *G. gulolineatus* previously reported measured 136 mm snout-vent length (SVL; tip of the snout to the posterior margin of the vent) from the type locality (Brandon 1965, 1966).

Methods

As part of an ongoing study on the demography and life history of *Gyrinophilus gulolineatus*, we captured a large *G. gulolineatus* at the type locality, Berry Cave (Tennessee Cave Survey no. TRN3), on 12 August 2018. Berry Cave is located 0.37 km west of the Tennessee River near Wright Bend in Roane County, Tennessee. The main entrance is in a large sink, with the passage from the entrance steeply sloping down to the main stream passage. The passage can be followed downstream to the northeast for ~160m along the stream until large debris and sediment buildup block further exploration. The stream is characterized by a series of riffles and shallow (<0.5 m) pools with primarily chert, cobble, and coarse gravel substrate and significant amounts of coarse woody debris, detritus, and fine mud and sediment in some areas. The salamander was observed and captured in the margin of a shallow (<0.5 m deep) pool located in a small passage upstream from the main entrance chamber. When first encountered, all but the salamander's head was out of the water, as it appeared to be moving partially over land to continue upstream.

The salamander was captured with a handheld dip net and immediately transferred to a clear plastic bag for processing. We massed to the nearest 0.5 g using a Pesola® spring scale and measured to the nearest 0.5 mm snout-vent length (SVL; tip of the snout to the posterior margin of the vent) and total length (TL; tip of the snout to the end of the tail) using a metric caliper. The salamander was measured four times by MLN, confirmed by NSG and ETC, and then photographed using an Olympus Tough TG-5 Camera. We also noted any physical abnormalities and the overall health of the salamander. Finally, we marked the salamander by injecting a 1.2 × 2.7 mm visible implant (VI) alpha tag (Northwest Marine Technology Inc., Shaw Island, WA) into the dermis of the tail. The salamander was released at its point of capture following processing.

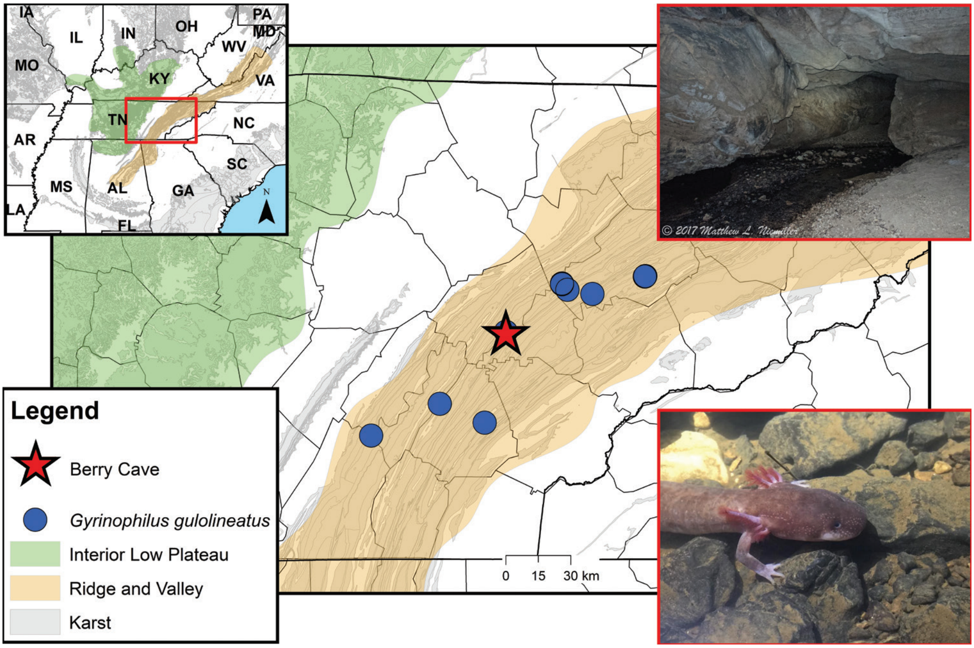

Figure 1. Geographic distribution of the Berry Cave Salamander (*Gyrinophilus gulolineatus*) in relation to karst adapted from Weary and Doctor (2014). Blue circles represent cave localities from which the species has been reported, and the red star represents the location of Berry Cave. The top right image shows the main stream passage near the entrance of Berry Cave that continues throughout the entirety of our sampling area. The bottom right image shows the large individual captured on 12 August 2018. Photo credits: Matthew L. Niemiller.

To provide a comparison of body size relations across other large-bodied plethodontids, we later compiled a list of maximum body sizes, modes of development, and habitat for several plethodontid salamanders by conducting a search of the primary literature and relevant field guides (see Table 1 and references therein).

Results

The *Gyrinophilus gulolineatus* observed and captured at Berry Cave on 12 August 2018 measured 145 mm SVL and 238 mm TL, with a mass of 35 g (Figure 2). Head width measured 22 mm. There was notable damage to the posterior end of the tail, and it is likely that this individual was >250 mm TL before tail tissue loss. Additionally, the two distal-most gill rachises on the right side of the head were notably smaller than those on the left side, while the most proximal right gill rachis was enlarged relative to that on the left side of the head.

A list of maximum body size and total length for several large plethodontid salamanders is reported in Table 1. Based on our literature review, *G. gulolineatus* is the largest plethodontid based on body size (SVL) in the United States, while

Table 1. Mode of development (DD = direct development, m = metamorphic; OP = obligately paedomorphic, FP = facultatively paedomorphic), habitat (AQC = aquatic cave, SAC = semi-aquatic cave, SAT = semiaquatic terrestrial, SUT = surface terrestrial), maximum body size (SVL) and total length (TL) of select plethodontid salamanders based on literature sources and the current study.

Size and life history characteristics of select plethodontid salamanders					
Species	**Mode of development**	**Habitat**	**SVL (mm)**	**TL (mm)**	**References**
Bolitoglossa dofleini	DD	SUT	130	205	Feder et al. (1982)
Desmognathus quadramaculatus	M	SAT	103	189	Bakkegard and Rhea (2012)
Gyrinophilus gulolineatus	OP	AQC	145	238	Brandon (1965, 1966), this study
Gyrinophilus palleucus	OP	AQC	113	186	Lazell and Brandon (1962), Dent and Kirby-Smith (1963), Niemiller et al. (unpubl. data)
Gyrinophilus porphyriticus	M	SAT/ SAC	134	221	Brandon (1966), Niemiller et al. (2010), Niemiller et al. (unpublished data)
Gyrinophilus subterraneus	FP	SAC	117	199	Niemiller et al. (2010)
Isthmura bellii	DD	SUT	146	327	Smith (1949), Feder et al. (1982), Raffaelli (2014)
Isthmura gigantea	DD	SUT	161	276	Taylor and Smith (1945)
Isthmura maxima	DD	SUT	128	244	Parra-Olea et al. (2005)
Phaeognathus hubrichti	DD	SUT	138	268	Schwaner and Mount (1970), Bakkegard and Guyer (2004), Graham et al. (2009)

Figure 2. Dorsal view of the *Gyrinophilus gulolineatus* captured at Berry Cave. Photo credit: Matthew L. Niemiller.

only *Phaeognathus hubrichti* attains a greater total length. Body size in *G. gulolineatus* rivals that observed in the direct-developing *Isthmura bellii* species complex endemic to Mexico.

Discussion

Plethodontid salamanders exhibit considerable variation in life history strategies and habitat that has resulted in an extraordinary range of growth rates and age at maturity (Tilley and Bernardo 1993, Beachy 1995, Beachy et al. 2017). Representative species with notable larger body sizes included in Table 1 represent four primary modes of development in salamanders, with paedomorphic and direct-developing species exhibiting larger body sizes relative to metamorphosing species. Larger species also are correlated with aquatic habitats, apart from the *Isthmura bellii* species complex, which inhabits Neotropical montane forests in southern North America.

Larger plethodontids are likely to occur in well-oxygenated, moist to fully aquatic habitats, which largely relax allometric constraints on gas exchange. This is particularly relevant to those species that exhibit paedomorphic life history strategies. Paedomorphic individuals may be able to grow unimpeded in their permanently aquatic state owing to indeterminate growth. Obligate paedomorphosis has evolved multiple times within Plethodontidae, with the subfamily Spelerpinae having the greatest richness of paedomorphic species (Chippendale 1995; Ryan and Bruce 2000; Bonnet et al. 2014). Additionally, neoteny has been predicted to be the primary causal mechanism of paedomorphosis in salamanders (Duellman and Trueb 1986, Ryan and Bruce 2000). Larger amphibian body sizes are further associated with longer juvenile periods, which significantly covary with age at maturation (e.g., *Desmognathus quadramaculatus* and *Gyrinophilus porphyriticus*, Bruce 1988, Beachy 1995, Beachy et al. 2017).

Many of the largest plethodontid salamanders are direct-developing (e.g., *Phaeognathus hubrichti* in the United States; *Isthmura bellii* in Mexico). Direct-developing species are generally characterized by having larger eggs and longer embryonic development relative to metamorphic or paedomorphic species, and this may related to attaining larger body sizes (Wake and Hanken 2004). There are, however, tradeoffs related to larger body size in these terrestrial plethodontids. The habitat must support gas exchange through adequate temperature and moisture gradients, and these taxa have evolved physiological mechanisms, such as waxy secretions, to reduce water loss. Second, terrestrial environments typically have lower food availability, and, accordingly, terrestrial salamanders often experience more extended periods of inactivity (Jaeger 1979, 1981, Scott et al. 2007). *Phaeognathus*, for instance, has rarely (if ever) been observed outside of burrows in densely forested ravines. Larger body size in such species is in accordance with the 'starvation hypothesis' that predicts that greater mass is positively correlated to seasonality and periods of low resource availability (Lundberg 1986), because larger individuals can persist through low-resource events by having greater energy stores and typically more efficient metabolism owing to positive allometry. The starvation hypothesis has received recent support in multiple amphibian taxa, where body size is positively related to extended inactivity (Valenzuela-Sánchez et al. 2015) and increased precipitation seasonality (Goldberg et al. 2018).

Cave environments are often characterized by low food resources and few natural predators, which likely shaped much of the evolution of many subterranean taxa (Gibert and Deharveng 2002). However, this archetype may not be representative of all subterranean systems, as many caves possess a high surface-environment connection with significant allochthonous organic input (i.e., higher influx of organic matter) driving both terrestrial and aquatic food webs. Cave obligate salamanders often exhibit reduced growth rates and low metabolic demand (e.g., Hervant et al. 2000), and they may also exhibit greater longevity owing to the slow pace of life and low predation pressure associated with subterranean environments (Brandon 1971, Culver and Pipan 2009, Voituron et al. 2011, Hüppop 2012). High resource environments may thus permit more rapid growth and sustain a larger overall body size. The exceptionally large *Gyrinophilus gulolineatus* reported here occurred within 10 m of the cave entrance in a high flow zone with an abundance of organic matter accumulated in the cave pool. Berry Cave is a diverse system relative to other caves in the Appalachian Valley and Ridge (Niemiller et al. 2016), likely due to the large influx of organic matter from the surface.There are a variety of invertebrate taxa that serve as prey for *G. gulolineatus* (e.g., isopods, amphipods, crayfish, flatworms, etc.).

While there has been much focus on life history evolution in salamanders, sampling biases may impact interpretations of the relationship between body size and mode of development. Paedomorphic species may be more difficult to capture, and they are often associated with extreme habitats such as underground springs and caves (Ryan and Bruce 2000, Bonnet et al. 2014). More thorough survey efforts and detailed life history observations within harsher or more isolated environments are necessary to better understand how paedomorphosis may relate to body size in amphibians.

Due to its subterranean existence and cryptic nature, many life history characteristics of *G. gulolineatus* have yet to be documented. Active survey efforts are continuing to assess the species' demography in Berry Cave, as well as to better understand the growth of this species. Further biological inventory within the Appalachian Valley and Ridge is underway with the intent to uncover additional localities. Future directions for research include additional life history characterization and study of the species' ecology.

Acknowledgements

Funding for this project was provided by the U.S. Fish & Wildlife Service (grant no. F17AC00939). All research was conducted under a TWRA scientific collection permit (nos. 1385 and 1605) and following an approved protocol by the University of Alabama in Huntsville Institutional Animal Care and Use Committee (protocol no. 2017.R005). We especially thank the Healy family for allowing access to Berry Cave.

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Subterranean Biology 28: 39–51 (2018)
doi: 10.3897/subtbiol.28.31801
http://subtbiol.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
Subterranean Biology
Published by The International Society for Subterranean Biology

An important site for conservation of bats in Brazil: Passa Três cave, São Domingos karst area, with an updated checklist for Distrito Federal (DF) and Goiás state

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Academic editor: *O. Moldovan* | Received 20 November 2018 | Accepted 4 December 2018 | Published 12 December 2018

http://zoobank.org/0CA319B0-98C8-4F65-942F-43FBBC570213

Citation: Bichuette ME, Gimenez EA, Arnone IS, Trajano E (2018) An important site for conservation of bats in Brazil: Passa Três cave, São Domingos karst area, with an updated checklist for Distrito Federal (DF) and Goiás state. Subterranean Biology 28: 39–51. https://doi.org/10.3897/subtbiol.28.31801

Abstract

A checklist of bats from Distrito Federal (DF) and Goiás state (GO) and, particularly a single cave (Passa Três cave), located in São Domingos karst area (GO), central Brazil, is presented. Data is based on literature and surveys carried out during 2,000 years. In total, 66 species were recorded, with 30 using caves as shelters. Passa Três cave harbors nine Phyllostomidae species; the most abundant species were *Platyrrhinus lineatus, Lonchorhina aurita*, *Desmodus rotundus* and *Carollia perspicillata*; and the less abundants *Trachops cirrhosus*, *Anoura caudifer* and *Glossophaga soricina*. Besides, the cave is shelter of two threatened bats - *Lonchophylla dekeyseri* (Endangered category) and *Lonchorhina aurita* (Vulnerable category), included at Brazilian List of Threatened Fauna, and of a rare species, *Lionycteris spurrelli*. Passa Três cave shows enough attributes to be considered as a SICOM (Sites of Importance for Conservation of Bats), which would ensure its protection.

Keywords

Mammalia, Chiroptera, Caves, Conservation, central Brazil

Introduction

The Order Chiroptera comprises about 22% of all extant mammals, with more than 1,300 described species (BCI 2018) and currently with 182 species known from Brazil (SBEQ 2018). This number is still increasing, with approximately two new bat species described per year in Brazil in the past two decades (Paglia et al. 2012, Nogueira et al. 2014). In the Cerrado, bats surpass rodents in diversity, with 103 species from all nine families that occur in Brazil (Aguiar and Zortéa 2008), representing more than 50% of all mammal species in this phytophysiognomy. The Cerrado is one of the richest tropical savannas, with a high endemism of plants and birds and a high diversity of vertebrates, being considered a "hotspot" for biodiversity conservation (Mittermeier et al. 1999, Myers et al. 2000). According to Reis et al. (2011), 9% of 195 mammals recorded in the Cerrado are endemic to it. Among bats, the nectarivorous *Lonchophylla bokermanni* Sazima, Vizotto & Taddei, 1978, was reported as endemic from this hotspot (Nogueira et al. 2014).

The main treats to Cerrado are land expansion for cattle raching, soybean plantations and other land uses that had reduced it to only 20% of the original area (Myers et al. 2000, Strassburg et al. 2017). This can directly affect bat assemblages, changing the habitat structure and reducing shelter and food availability.

Bats use many types of roosts, including caves, rocky crevices, foliage roosts, hollow trees and man-made structures such as the buildings, mines and tunnels (Kunz 1982, Pacheco et al. 2010). For several species, caves are the main roosts (Kunz 1982, Arita 1996, Trajano 1995). Bats are the typical trogloxenes, or organisms that regularly use subterranean habitats but that must return periodically to the surface (in their case, daily to feed) in order to complete their life cycle, and some are obligatory trogloxenes, that depend on caves as obligatory shelters at least during part of their life cycles (Trajano 2012; Trajano and Carvalho 2017). Therefore the conservation of caves and their surrounding are crucial for maintenance of bat populations (Trajano 1995, Arnone 2008, Rocha and Bichuette 2016).

The Red Latinoamericana y del Caribe para la Conservación de los Murciélagos -RELCOM is an international NGO that congregates Latin-American and Caribbean national programs for bat conservation in 22 countries. Among its actions, RELCOM certificates Areas and Sites of Importance for Conservation of Bats (AICOMs and SICOMs), recognized with basis on criteria of diversity, presence of endangered and rare species, maternity colonies, and importance as roosting and migration areas. AICOMs and SICOMs differ only in area size, the latter are smaller. So far, RELCOM certified 80 (64 AICOMs and 16 SICOMs) in 15 countries. However, only recently Brazil have recognized the first and so far the only Brazilian AICOM, the "Alto Ribeira e Alto Paranapanema AICOM", in southeast São Paulo state, SE Brazil (RELCOM 2017). Although many other Brazilian areas are known to fit the RELCOM criteria for Important Areas and Sites for Bat Conservation, no actions have been undertaken to certificate them.

We present herein a checklist of bat species from Distrito Federal and Goiás state, with data of a short-term study in Passa Três cave, São Domingos karst area, central

Brazil and part of a State Park (Terra Ronca State Park - PETeR). We discuss the criteria of proposition of SICOMs and the possibility of application to Passa Três cave.

Brazil has ca. of 17,000 recorded caves (CECAV 2018) and a potential to 100,000 according to Auler et al. (2001), and most of this heritage is under threat by mining and other huge economic purposes, such as hydro electrical projects. The São Domingos karst area, Goiás state, central Brazil, is characterized by the presence of huge cave systems crossed by allochthonous streams, with large amounts of organic matter available for cave organisms, supporting rich aquatic and terrestrial communities (e.g., Bichuette and Trajano 2003, Simões et al. 2013, Bichuette et al. 2015). It is the second most intensively surveyed Brazilian karst area after the Alto Ribeira, but so far no comprehensive study on the bat communities from these caves has been undertaken.

Methods

Study Area

Passa Três cave is located in the Parque Estadual Terra Ronca - PETeR, one of the largest protected areas in Goiás state (Figures 1, 2). The Cerrado phytophysionomy, which is the second largest in Brazil, after Amazonian rainforest, comprises about 2 million km^2 (IBGE 2004). There is still significant economic activity represented by cattle rising and agriculture threatening the original Cerrado. Therefore, although the cave is located inside a Conservation Unit, its protection is not warranted.

The Passa Três cave (GO 14) (Lat -13,60 and Long -46,39), São Domingos County, is a relatively small and confined cave when compared with the cave systems in São Domingos karst area. The cave entrance (sinkhole – Figure 2A), used by the bats for emergence, is about 2.5 m high and 4 m wide; after a flooded low conduit, the cave stream joins the São Vicente I Cave. There are ca. 1.5 km of vadose galleries potential for bat use, mostly the stream conduit, width and height varying from 2 to 8 m, with plenty of concavities and crevices in the ceiling and potential shelters. Stable spelaeoclimate due to spatial confinement and low noise in comparison with larger caves, make Passa Três cave a particularly good shelter.

According to Köeppen classification (1948), the climate is Aw type, with two well-defined seasons: dry (May-September) and wet (October-April), with annual precipitation between 600 and 2,000 mm (Lima and Silva 2005).

Sampling

Bats were sampled in three occasions at the Passa Três cave entrance; all in the dry season of 2000 (July 25, September 9 and 14), using one mist net (7× 3 m) at the beginning of the night (4 h total collecting efforts). Bats were euthanized through cervical dislodgment (one specimen or a couple per species) and identified using taxonomic

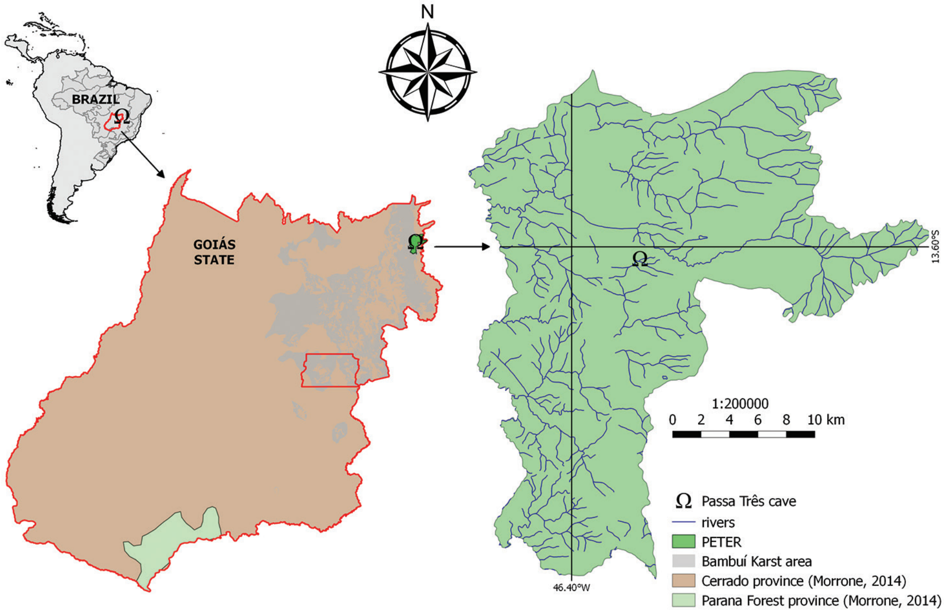

Figure 1. Study area, showing the limits of PETeR (Parque Estadual de Terra Ronca), northeastern state of Goiás, central Brazil. (Map organized in QGIS software, version 2.18, Author: Diego M. von Schimonsky).

keys (Vizotto and Taddei 1973, Gardner 2008). Vouchers of part of studied (collected) material are deposited in the Vertebrates Collection of Laboratório de Estudos Subterrâneos of Universidade Federal de São Carlos (LESV).

Examined material (part): *Anoura caudifer* (LESV 0365 and 0366), *Platyrrhinus lineatus* (LESV 0355 and 0358), *Glossophaga soricina* (LESV 0356 and 0362), *cirrhosus* (LESV 0357, 0367 and 0391), *Lonchorhina aurita* (LESV 0358, 0360), *Lionycteris spurrelli* (LESV 0359 and 0381), *Carollia perspicillata* (LESV 0361), *Lonchophylla dekeyseri* (LESV 0363 and 0364).

Literature review

Secondary data were gathered from literature: Bredt et al. (1999), Esbérard et al. (2001), Esbérard et al. (2005), Zortéa and Tomaz (2006), Reis et al. (2007), Silva et al. (2009), Zortéa and Alho (2008), Silva et al. (2009), Bezerra and Marinho-Filho (2010), Zortéa et al. (2010), Chaves et al. (2012), Reis et al. (2013) and Guimarães (2014).

Following Garbino and Tejedor (2012) and Nogueira et al. (2014), we treat the *Natalus* species recorded in Brazil as *Natalus macrourus* (Gervais, 1856), replacing the

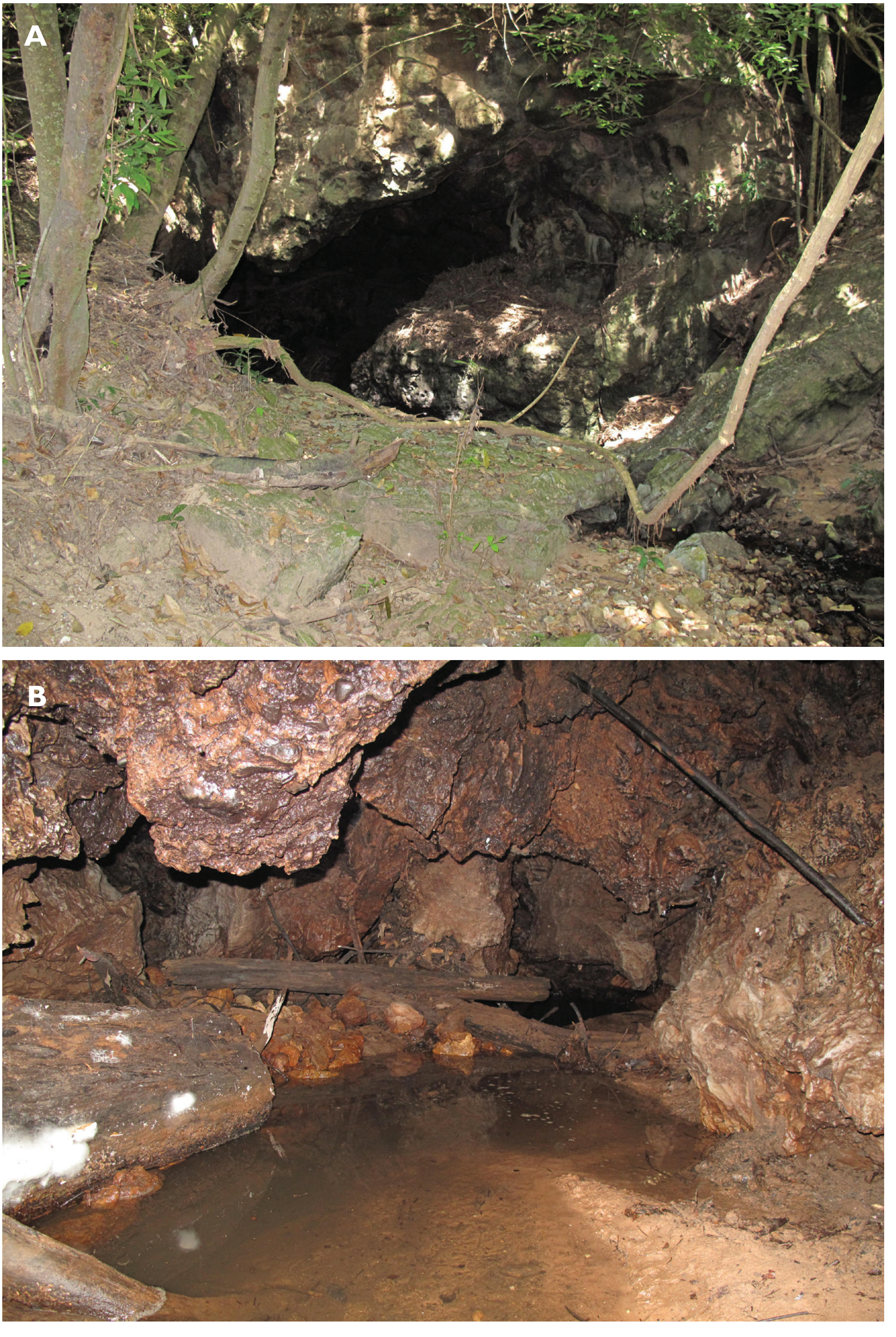

Figure 2. Passa Três cave, São Domingos karst area, Goiás state, central Brazil. **A** cave entrance **B** cave stream conduit close to entrance with short ceiling passages. Photos: M. E. Bichuette.

names *N. stramineus* (Gray, 1838) and *N. espiritosantensis* Ruschi, 1951; *A. planirostris* (Spix, 1823) instead of *Artibeus jamaicensis* (Leach, 1821); *Dermanura cinerea* Gervais, 1856 instead of *Artibeus cinereus* (Gervais, 1856); and *Cynamops planirostris* (Peters, 1866) instead of *Molossops planirostris* (Peters, 1865). The list does not include *Platyrrhinus helleri* (Peters, 1866), which after revision, seems not to occur in Brazil (Velazco et al. 2010, Nogueira et al. 2014).

Results

An updated list of bats for Distrito Federal and Goiás state is shown in Table 1, with 66 species belonging to eight families, including 41 phyllostomids. Among these, 30 species were recorded in caves previously (22 belonging to Phyllostomidae family- see literature cited in Table 1). Two of them are threatened and included in the Brazilian List of Threat-

Figure 3. *Lonchophylla dekeyseri*, Endangered species at Brazilian List of Threatened Fauna. Photo: Roberto Leonam Morim Novaes.

Figure 4. *Lonchorhina aurita*, Vulnerable species at Brazilian List of Threatened Fauna. Photo: Roberto Leonam Morim Novaes.

ened Fauna (MMA 2016): *Lonchophylla dekeyseri* (Figure 3), Endangered (EN), and *Lonchorhina aurita* (Figure 4), Vulnerable (VU) and the rare species, *Lionycteris spurrelli*.

We recorded nine species in Passa Três cave (in parenthesis, the number of specimens captured in July/September, both nights combined): *Anoura caudifer* (0/2), *Carollia perspicillata* (0/2), *Desmodus rotundus* (6/8), *Glossophaga soricina* (2/3), *Lionycteris spurrelli* (1/0), *Lonchophylla dekeyseri* (0/4), *Lonchorhina aurita* (1/1), *Platyrrhinus lineatus* (11/4) and *Trachops cirrhosus* (1/2). Pregnant females of *P. lineatus* and *Lonchorhina aurita* were captured in July; in September we recorded pregnant females of *P. lineatus* and *T. cirrhosus*. Moreover, Passa Três cave is a shelter of two threatened species, *Lonchophylla dekeyseri* (EN) and *Lonchorhina aurita* (VU).

Table 1. Checklist of bats recorded in Distrito Federal and Goiás state. Source: 1 - Bredt et al. (1999), 2 - Esbérard et al. (2001), 3 - Esbérard et al. (2005), 4 - Zortéa and Tomaz (2006), 5 - Reis et al. (2007), 6 - Silva et al. (2009), 7 - Bezerra and Marinho-Filho 2010, 8 - Zortéa et al. (2010), 9 - Chaves et al. (2012), 10 - Reis et al. (2013), 11 - LESV, 12 - Zortéa and Alho (2008), 13 - Guimarães (2014), **14 – Present study**. * Species recorded in caves.

Family/Subfamily/Species	Data Source
Family Emballonuridae	
Subfamily Emballonurinae	
Centronycteris maximiliani (Fisher, 1829)	10
Peropteryx macrotis (Wagner, 1843)*	1, 3, 5, 6, 10
Rhynchonycteris naso (Wied-Neuwied, 1820)	10
Saccopteryx bilineata (Temminck, 1838)	10
Saccopteryx leptura (Schreber, 1774)	10
Family Furipteridae	
Furipterus horrens (Cuvier, 1828)*	1, 2, 3, 10, 13
Family Mormoopidae	
Pteronotus gymnonotus Natterer, 1843*	1, 3, 9, 10, 13
Pteronotus parnellii (Gray, 1843)*	1, 2, 3, 9, 10, 12, 13
Family Natalidae	
Natalus macrourus (Gervais, 1856)*	2, 3, 6, 10, 13
Family Noctilionidae	
Noctilio leporinus (Linnaeus, 1758)	7, 10
Family Molossidae	
Subfamily Molossinae	
Cynomops abrasus (Temminck, 1826)	10
Cynomops planirostris (Peters, 1866)	8
Eumops maurus (Thomas, 1901)	10
Molossops temminckii (Burmeister, 1854)	7, 8, 10, 12
Molossus molossus (Pallas, 1766)	10
Neoplatymops mattogrossensis (Vieira, 1942)	10
Family Phyllostomidae	
Subfamily Carolliinae	
Carollia brevicauda (Schinz, 1821)	10
Carollia perspicillata (Linnaeus, 1758)*	1, 2, 3, 6, 7, 8, 9, 10, 11, 12, **14**
Subfamily Desmodontinae	
Desmodus rotundus (É. Geoffroy Saint-Hilaire, 1810)*	1, 2, 3, 6, 7, 8, 9, 10, 12, **14**
Diaemus youngii (Jentink, 1893)	10
Diphylla ecaudata Spix, 1823*	1, 2, 3, 7, 9
Subfamily Glossophaginae	
Anoura caudifer (É. Geoffroy Saint-Hilaire, 1818)*	1, 2, 3, 7, 8, 11, 12, 13, **14**
Anoura geoffroyi Gray, 1838*	1, 2, 3, 8, 10, 12
Choeroniscus minor (Peters, 1868)*	3, 10
Glossophaga soricina (Pallas, 1766)*	1, 2, 3, 6, 7, 8, 10, 11, 12, **14**
Subfamily "Glyphonycterinae"	
Glyphonycteris behnii (Peters, 1865)	10, 12
Subfamily Lonchophyllinae	
Lonchophylla mordax Thomas, 1903	5

Family/Subfamily/Species	**Data Source**
Lonchophylla dekeyseri Taddei, Vizotto & Sazima, 1983*	1, 2, 3, 4, 5, 6, 7, 10, 11, **14**
Lionycteris spurrelli Thomas, 1913	2, 5, 10, 11, **14**
Subfamily Lonchorhininae	
Lonchorhina aurita Thomas, 1863*	1, 2, 3, 5, 6, 9, 10 11, 13, **14**
Subfamily Micronycteridae	
Micronycteris megalotis (Gray, 1842)*	1, 3, 6, 8, 9, 10
Micronycteris minuta (Gervais, 1856)*	1, 2, 3, 5, 7, 10, 12
Subfamily Phyllostominae	
Chrotopterus auritus (Peters, 1856)*	1, 2, 3, 5, 6, 7, 10, 12
Lophostoma brasiliensi Peters, 1866	10, 12
Lophostoma carrikeri (J.A. Allen, 1910)	5, 10
Macrophyllum macrophyllum (Schinz, 1821)	5, 10
Mimon bennettii (Gray, 1838)*	1, 2, 3, 5, 6, 7, 8, 10, 12
Mimon crenulatum (É. Geoffroy Saint-Hilaire, 1803)	8, 12
Tonatia bidens (Spix, 1823)	5, 7
Trachops cirrhosus (Spix, 1823)*	1, 2, 3, 5, 11, **14**
Subfamily Stenodermatinae	
Artibeus concolor Peters, 1865	4, 5, 10
Artibeus lituratus (Olfers, 1818)*	3, 5, 10, 12
Artibeus obscurus (Schinz, 1821)	5, 7
Artibeus planirostris Spix, 1823*	2, 3, 8, 9, 10, 12
Chiroderma villosum Peters, 1860	10
Chiroderma vizottoi (Taddei & Lim, 2010)	5
Dermanura cinerea Gervais, 1856	5, 8, 10, 12
Mesophylla macconnelli Thomas, 1901	4, 8, 10, 12
Phylloderma stenops (Peters, 1865)*	1, 2, 3, 5, 10
Phyllostomus discolor (Wagner, 1843)	5, 8, 10, 12
Phyllostomus hastatus (Pallas, 1767)*	1, 3, 5, 8, 9, 10, 12
Platyrrhinus incarium (Thomas, 1912)	5, 10
Platyrrhinus lineatus (É. Geoffroy Saint-Hilaire, 1810)*	1, 3, 5, 7, 10, 11, 12, **14**
Sturnira lilium (É. Geoffroy Saint-Hilaire, 1810)*	3, 5, 7, 8, 10, 12
Uroderma bilobatum Peters, 1866	5, 10
Uroderma magnirostrum Davis, 1968	5, 7
Vampyressa pusilla (Wagner, 1843)*	3, 5, 10
Family Vespertilionidae	
Subfamily Vespertilioninae	
Eptesicus diminutus (Osgood 1915)	12
Eptesicus furinalis (d'Orbigny & Gervais, 1847)	7, 8
Eptesicus andinus J.A. Allen, 1914	5, 10
Eptesicus brasiliensis (Demarest, 1819)*	1, 5, 10
Histiotus laephotis Thomas, 1916	5
Histiotus velatus (I. Geoffroy, 1824)	5
Lasiurus blossevillii ([Lesson, 1826])	5, 10
Lasiurus ega (Gervais, 1856)	10, 12
Myotis nigricans (Schinz, 1821)*	1, 2, 3, 5, 8, 10

Discussion

Majority of records for Distrito Federal (DF) and Goiás state (GO) are Phyllostomids, as observed by other authors (e.g. Bredt et al. 1999, Bezerra and Marinho-Filho 2010, Carrijo et al. 2011). In a regional scale, more than 40% of the species recorded in DF and GO use caves, which represents about 16% of Brazilian bats (30 of 179), a high percentage compared to other temperate regions.

Considering the Passa Três cave, all species are Phyllostomidae; the most abundant (e.g., *Platyrrhinus lineatus*, *Lonchorhina aurita*, *Desmodus rotundus* and *Carollia perspicillata*) follow partially the pattern observed in some limestone and sandstone caves in Brazil, when *Desmodus rotundus* and *Carollia perspicillata* are the most abundant. In some cases, *Phyllostomus hastatus*, has shown a high abundance (e.g.,Tocantins, in central Brazil, and Bahia, in northeastern Brazil; M.E. Bichuette, pers. obs.). However, *P. hastatus* has not been registered in Passa Três cave. The less abundant species (e.g., *Trachops cirrhosus*, *Anoura caudifer* and *Glossophaga soricina*) follow the pattern observed in some karst areas in Brazil (Trajano and Gimenez 1998, Arnone 2008).

Protection of roosting sites is an essential component of any strategy for the conservation of bats, with caves being the main roots for several bat species (Arita 1996, Kunz 1982). On the other hand, bat guano is an important food source for many subterranean organisms, especially for species restricted to subterranean habitats (troglobites), totally dependent on the resources present in these habitats and prone to rapid extinction following any ecological disequilibrium (e.g. alterations in the energy input), even very localized (Trajano 1995). Therefore, protecting bats is a fundamental part of any program or action for conservation of subterranean systems.

Passa Três cave follows the criteria of Site of Importance for Conservation of Bats (SICOM) according to the RELCOM. However, the proposition must be sent to RELCOM coordination for appreciation and validation. The following attributes were observed: presence of two threatened species – *Lonchophylla dekeyseri* and *Lonchorhina aurita*; presence of a rare species – *Lionycteris spurrelli*; a high diversity of bats compared to other caves in Brazil; reproducing colonies of at least three among these species, including the vulnerable *L. aurita*. Its protection is a priority since the Parque Estadual de Terra Ronca – PETeR has no Management Plan and is impacted by pastures, agriculture and poorly controlled visitation. In addition, the cave is the type-locality of two troglobitic (restricted to subterranean habitats) fishes, both included in the Brazilian Red List of Threatened species, the catfishes *Ancistrus cryptophthalmus* Reis, 1987 and *Ituglanis passensis* Fernández and Bichuette 2002 (Teleostei: Siluriformes) (Bichuette and Trajano 2003). Considering its peculiarity and attributes, it is urgent the inclusion of Passa Três in conservation programs, and implementation of monitoring programs.

Acknowledgements

We are grateful to Hertz F. Santos, Dirk Möller, Yvone Meyer-Lucht, Jens Poschadel, and Ramiro H. dos Santos for assistance in fieldwork at São Domingos karst area; to

Roberto Leonam Morim Novaes for permission use of photographs of Figures 3 and 4; to Regina Schultz and Arnor V. Mello, Park officials during the study, for the infrastructure and support to carry out this work; to Diego M. von Schimonsky for map confection; to the Secretaria de Meio Ambiente, Recursos Hídricos, Infraestrutura, Cidades e Assuntos Metropolitanos (SECIMA) and Instituto Chico Mendes de Conservação da Biodiversidade (ICMBIO) for the collection permits; to the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, #98/13858-1) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, #303715/2011-1) for the financial support to the senior author. We also thank Adarene Motta and Susi Missel Pacheco for suggestions.

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Subterranean Biology 28: 53–66 (2018)
doi: 10.3897/subtbiol.28.32093
http://subtbiol.pensoft.net

IN MEMORIAM

A peer-reviewed open-access journal
Subterranean Biology
Published by The International Society for Subterranean Biology

In Memoriam – John R. Holsinger

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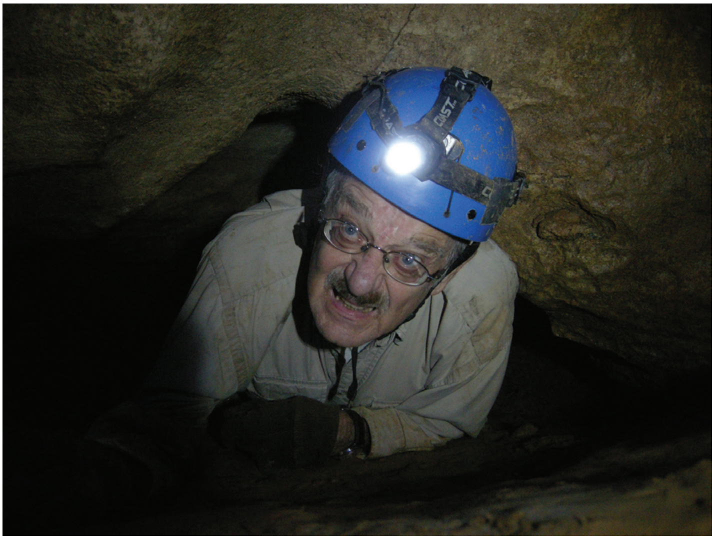
Received 1 December 2018 | Accepted 2 December 2018 | Published 12 December 2018

http://zoobank.org/3E89770E-C6C7-4941-9132-5C037A3C5F5E

Citation: Culver DC (2018) In Memoriam – John R. Holsinger. Subterranean Biology 28: 53–66. https://doi.org/10.3897/subtbiol.28.32093



John R. Holsinger, a prominent biospeleologist, both on the national and international scene, died on November 10, 2018. John's caving career spanned nearly 70 years, and his career as a biospeleologist and amphipod systematist spanned more than 50 years. Overall, John wrote more than 135 scientific papers and monographs (listed below). He has left a lasting and significant impact in several areas, not only amphipod systematics, but also subterranean biodiversity, biogeography, and conservation.

A native of Virginia's Shenandoah Valley, John began going into caves in the 1950's when he was a student at Virginia Polytechnic Institute and State University (now Virginia Tech). After his graduation from VPI in 1955 and a stint in the Army signal corps in Hawaii, he taught high school biology in Fairfax County, Virginia, and started the Biological Survey of Virginia Caves, an NSS supported project. During the late 1950's and early 1960's John was an active vertical caver. Together with his longtime friend and colleague John Cooper, they not only made the first biological collections in many vertical caves in the Virginias, but also helped lead the exploration and survey of these same caves. His speleological career began as an avid caver.

John Holsinger's interest in cave organisms was crystallized by his research for a Master of Science thesis, awarded in 1963 from Madison College (now James Madison University.) Much of his thesis was published in the *NSS Bulletin* in the form of a checklist of the obligate cave-dwelling organisms found in Virginia caves (1). He developed an interest in the overall diversity of cave life, which continued throughout his scientific career. It is reflected in papers and articles not only about amphipods (see below) but also on bats (2), salamanders (35,124), isopods (9,10,20,24,44,52,56,86) and mites (4,5). He pioneered the idea that overall biological surveys of cave faunas were important, and co-authored annotated lists for Georgia (17), Pennsylvania (31), east Tennessee (67), West Virginia (32), and two monograph length updates of the Virginia cave fauna (67, 133), first in 1988 and again in 2013. And he encouraged others to produce lists as well. This seemingly simple lists, often underappreciated, made it possible, in later years, for John and others to make generalizations and maps of subterranean biodiversity hotspots.

After extensive caving in the Virginias during the late 1950's and early 1960's, and with growing interest in subterranean biodiversity, John returned to graduate school at the University of Kentucky, where he obtained his Ph.D. in 1967 under the direction of Thomas C. Barr, a prominent biospeleologist. John's dissertation was on the taxonomy of a group of subterranean amphipods in the genus *Stygonectes* (7,8) which he eventually classified in the genus *Stygobromus*. The genus now has about 140 described species, the vast majority of which were described by John (12,28,38,40,60,65,96,121,123,127,134, 135). John described hundreds of species, not only in the genus *Stygobromus*, but in all of the other 8 amphipod genera that occur in North American caves, including the speciose genus *Crangonyx* (98, 103). John documented that *Stygobromus*, all of which are eyeless, occurred in a variety of subterranean habitats, not just caves. These included deep interstitial habitats, the hyporheic of streams, epikarst, and the hypotelminorheic. While European biospeleologists were well acquainted with non-cave subterranean habitats, Americans were not, and John brought this extra dimension to North American studies. As the years went on, his interest in subterranean amphipods became global, and he described dozens of new species from throughout the world. These included species of *Crangonyx* and *Stygobromus* from outside North America (42,123,130), and the cave and interstitial species in the families Bogidiellidae (89,90,91,108,111,113) and Hadziidae (27,53,57,61, 74,78,79,87,97,99,100,102,105). His enthusiasm for the morphology of cave amphipods was boundless. Anyone who was fortunate enough to hear him give a presentation on subterranean amphipods was treated to an enthusiastic and interesting talk, even if John had already described many similar species. In lusiess skilled hands, his talks would have become soporific.

His studies of subterranean amphipods on a global basis led John in two new directions. One was a study of the biogeography of subterranean amphipods and the likely scenarios of colonization and dispersal (55,66,70,75,88,104,129) and he introduced the two-step model of colonization of freshwater subterranean habitats from marine habitats (92). John always remained flexible with respect to the various schools of biogeography and systematics, using and testing components of each (76). His work on biogeography of subterranean amphipods is among the most cited of his publications, with several papers being cited more than 100 times (55,66).

The second direction his studies of subterranean amphipods on a global level was a strong commitment to international cooperation and collaboration. He started attending meeting outside the U.S. in the 1970's at a time well before globalization and well before a time when international cooperation was the norm. This is all the more remarkable for someone who grew up in a small town in the southern United States. The first international meeting John attended was "International Colloquium on *Gammarus* and *Niphargus*" in Schlitz, Germany in 1975. It was at this meeting that he forged friendships and collaborations with a multitude of biospeleogists from throughout the world, including Magniez (France), Ruffo (Italy), Siderov (Russia), Skalski (Poland) Sket (Slovenia), and Straškraba (Czech Republic). In 1978, together with Arthur Buikema, he organized an "International Symposium on Groundwater Ecology" in Blacksburg, Virginia, and provided Americans with their first opportunity to meet their international colleagues. John was a mainstay of the International Society for Subterranean Biology, attending most of the biennial meetings up to the meeting in Košice, Slovakia in 2012. He always treated his colleagues, not just with dignity and respect, but with enthusiasm and genuine appreciation of their work

Although John never thought of himself as an isopod taxonomist, he authored or co-authored eight papers on isopods and described several new species (9,10,20,24,44,52,56,86). He also made significant contributions to our understanding of the ecology and natural history of subterranean organisms, especially with respect to amphipods (6,14,33,44,49,80,122).

John was a lifelong teacher. A high school biology and earth science teacher in the early days, he went on to be a professor at East Tennessee State University and Old Dominion University, where he spent most of his professorial career. He mentored a number of graduate students both at the Masters and PhD levels, including Ph.D. students Jill Yager, discoverer of the Remipedes, a previously unknown class of Crustacea; Lynn Ferguson, expert on cave adapted diplurans of North America; Jun Zhang, expert on *Crangonyx* amphipods; Stefan Koenneman, expert on Bogidiellid and Crangonyctid amphipods; Tom Sawicki, expert on *Hadzioidea* amphipods; and Julian Lewis, the leading expert on freshwater isopods of North America. John enlightened hundreds of Old Dominion University students over the decades through his unique course on Cave Biology, leading weekend long field trips to caves in Virginia and West Virginia to see some of the fascinating places and creatures covered in his lectures. He was a teacher to almost everyone he met, and had a singular ability to impart knowledge without condescension or arrogance. All of us learned a lot from him.

I am not sure if John had one favorite cave, but a good candidate would be Unthanks Cave in Lee County, an 12 km long cave with an abundant and diverse fauna. John was instrumental in persuading The Nature Conservancy to purchase the land above the entrance. The Virginia state Natural Heritage Program and The Nature Conservancy continue working together to protect all of the land overlying or draining to the cave. John Holsinger's conservation efforts extended far beyond protecting a handful of his favorite caves. John was part of a group of pioneers who saw a role for the state in the recognition and protection of cave and karst resources. Starting with the Commission on Virginia Caves and culminating with the Virginia Cave Board, John served thirty years as a governor appointed citizen volunteer working to protect the state's karst. Notable achievements of these boards included the passage of the Virginia Cave Protection Act in 1979, regulation of scientific studies to limit impacts to caves, recognition of the paleontological, archaeological, and associated cultural significance of caves, and direct actions to conserve and restore threatened and degraded cave systems. John's efforts led to the 1982 listing of the Madison Cave isopod (*Antrolana lira*) as threatened under the endangered species act as part of a successful attempt to prevent discharge of chlorinated water to a sinkhole overlying the species' type locality. In the 1980s John led the Cave Board campaign to restore and protect Lee County's Thompson Cedar Cave, which he discovered had been contaminated by leachate flowing from massive sawdust piles adjacent to and overlying the cave. The impact was so severe that almost all life in the cave had been extinguished, resulting in the Endangered Species listing in 1992 of the Lee County Cave isopod (*Lirceus usdagalun)*. The protection and resources afforded these two species have resulted in increased knowledge and long term protections not only of these animals, but the caves and groundwater in which they live. Early on, John recognized the threats human activity posed to caves and cave fauna (6) and actively sought their protection, even when he was a very lonely voice calling for protection. It is not an overstatement that his actions have brought species like *Lirceus usdagalun* back from the brink of extinction.

John still somehow managed to make time for Linda, his wife of four decades, and their extended family, who together with us mourn his passing and celebrate his life. Linda was his mainstay and devoted caregiver in his later years. In honor of John's work toward our understanding of caves, cave biology, conservation and taxonomy, the Virginia Natural Heritage Program established the John R. Holsinger Cave Conservation Fund. Those wishing to contribute should contact the Virginia Department of Conservation and Recreation Division of Natural Heritage at 804-786-7951. Checks can be sent c/o John R. Holsinger Cave Conservation Fund, 600 East Main Street, 16th floor, Richmond, Virginia, 23219.

In all his activities, John Holsinger was incredibly generous in sharing both time and credit. He always welcomed new people to caving and cave biology. He could also be irascible, and it is fair to say he did not suffer fools gladly. Setting an example which we all would do well to follow, John took the time to carefully document and publish nearly everything he did, leaving a lasting contribution to both the caving and broader scientific communities. In a real sense, he devoted his life to speleology, which will be much diminished by his passing.

I wish to thank Chris Hobson and Wil Orndorff of the Virginia Department of Conservation and Recreation for help in tracking down details of John's remarkable life.

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