

SUPPLEMENTAL INFORMATION

THE DISTRIBUTION, ECOLOGY, LIFE HISTORY, AND CONSERVATION

STATUS OF THE BERRY CAVE SALAMANDER

(*GYRINOPHILUS GULOLINEATUS*)

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TABLE S1. Summary of surveys for Berry Cave Salamanders (*Gyrinophilus gulolineatus*) at historical sites in eastern Tennessee, USA between 2004 and 2019, including the current study.

County	Cave	TCS no.	Date	Salamanders observed
Knox	Aycock Spring Cave	KN172	9/17/2005	1
Knox	Aycock Spring Cave	KN172	7/10/2018	0
Knox	Christian Cave	KN49	9/17/2005	1
Knox	Fifth Entrance Cave	KN167	10/23/2004	0
Knox	Fifth Entrance Cave	KN167	11/8/2007	1
Knox	Fifth Entrance Cave	KN167	7/14/2018	0
Knox	Meads Quarry Cave	KN28	10/23/2004	11
Knox	Meads Quarry Cave	KN28	11/4/2006	11
Knox	Meads Quarry Cave	KN28	4/22/2007	14
Knox	Meads Quarry Cave	KN28	9/9/2007	24
Knox	Meads Quarry Cave	KN28	11/8/2007	5
Knox	Meads Quarry Cave	KN28	11/24/2007	6
Knox	Meads Quarry Cave	KN28	1/24/2008	7
Knox	Meads Quarry Cave	KN28	1/31/2008	18
Knox	Meads Quarry Cave	KN28	3/1/2008	10
Knox	Meads Quarry Cave	KN28	3/6/2008	4
Knox	Meads Quarry Cave	KN28	3/30/2008	16
Knox	Meads Quarry Cave	KN28	4/10/2008	11
Knox	Meads Quarry Cave	KN28	4/30/2008	17
Knox	Meads Quarry Cave	KN28	5/15/2008	7

Knox	Meads Quarry Cave	KN28	6/4/2008	24
Knox	Meads Quarry Cave	KN28	6/27/2008	8
Knox	Meads Quarry Cave	KN28	7/30/2008	15
Knox	Meads Quarry Cave	KN28	9/10/2008	17
Knox	Meads Quarry Cave	KN28	10/5/2013	5
Knox	Meads Quarry Cave	KN28	11/22/2017	10
Knox	Meads Quarry Cave	KN28	1/13/2018	5
Knox	Meads Quarry Cave	KN28	3/10/2018	8
Knox	Meads Quarry Cave	KN28	6/17/2018	2
Knox	Meads Quarry Cave	KN28	9/23/2018	9
Knox	Meads Quarry Cave	KN28	4/5/2019	1
Knox	Meads River Cave	KN151	10/23/2004	0
Knox	Meads River Cave	KN151	4/22/2007	0
Knox	Meads River Cave	KN151	11/8/2007	0
Knox	Meads River Cave	KN151	11/24/2007	1
Knox	Meads River Cave	KN151	12/2/2007	0
Knox	Meads River Cave	KN151	9/10/2008	0
Knox	Meads River Cave	KN151	2/17/2018	0
Knox	Meads River Cave	KN151	7/14/2018	0
Knox	Mudflats Cave	KN9	11/20/2004	5
Knox	Mudflats Cave	KN9	1/6/2005	3
Knox	Mudflats Cave	KN9	12/30/2005	5
Knox	Mudflats Cave	KN9	11/12/2006	2

Knox	Mudflats Cave	KN9	6/7/2007	5
Knox	Mudflats Cave	KN9	4/5/2014	1
Knox	Mudflats Cave	KN9	10/20/2014	1
Knox	Mudflats Cave	KN9	1/8/2015	1
Knox	Mudflats Cave	KN9	10/29/2017	0
Knox	Mudflats Cave	KN9	11/25/2017	0
Knox	Mudflats Cave	KN9	2/27/2018	0
Knox	Mudflats Cave	KN9	3/16/2018	2
Knox	Mudflats Cave	KN9	5/10/2018	0
Knox	Mudflats Cave	KN9	6/18/2018	0
Knox	Mudflats Cave	KN9	9/22/2018	1
Knox	The Lost Puddle	KN145	5/8/2012	3
Knox	The Lost Puddle	KN145	3/23/2018	4
Knox	The Lost Puddle	KN145	7/13/2018	2
McMinn	Small Cave	MM5	5/10/2014	1
Meigs	Blythe Ferry Cave	ME1	1/26/2018	0
Roane	Berry Cave	RN3	12/17/2004	1
Roane	Berry Cave	RN3	3/5/2005	4
Roane	Berry Cave	RN3	6/28/2014	3
Roane	Berry Cave	RN3	2/14/2016	2
Roane	Berry Cave	RN3	10/30/2017	9
Roane	Berry Cave	RN3	12/4/2017	10
Roane	Berry Cave	RN3	1/6/2018	6

Roane	Berry Cave	RN3	2/17/2018	3
Roane	Berry Cave	RN3	3/16/2018	3
Roane	Berry Cave	RN3	4/13/2018	6
Roane	Berry Cave	RN3	5/10/2018	5
Roane	Berry Cave	RN3	6/18/2018	3
Roane	Berry Cave	RN3	7/20/2018	4
Roane	Berry Cave	RN3	8/12/2018	5
Roane	Berry Cave	RN3	9/15/2018	19
Roane	Berry Cave	RN3	10/21/2018	5
Roane	Berry Cave	RN3	12/8/2018	9
Roane	Berry Cave	RN3	1/19/2019	0
Roane	Berry Cave	RN3	2/17/2019	0
Roane	Berry Cave	RN3	4/6/2019	1
Roane	Berry Cave	RN3	7/20/2019	2

TABLE S2. Summary of caves surveyed during the current study (2017–2019) and additional surveys associated with other projects (e.g., Niemiller et al. 2016b, 2017) between 2004 and 2017 in the Appalachian Valley and Ridge and adjacent Blue Ridge Mountains of eastern Tennessee, USA, including survey dates and Tennessee Cave Survey (TCS) number. Berry Cave Salamanders (*Gyrinophilus gulolineatus*) were not observed at these sites. Sites where related Spring Salamanders (*G. porphyriticus*, Gpor) were observed are indicated.

County	Cave	TCS no.	Date	Gpor
Anderson	Blowing Springs Cave	AN1	2016: 3 Jun 2018: 13 Apr	
Anderson	Offut Cave	AN12	2018: 18 May	
Anderson	Weaver Cave	AN22	2016: 22 Mar	
Anderson	Springhill Saltpeter Cave	AN3	2017: 28 Oct	
Anderson	Martin Cave	AN31	2016: 21 Feb	
Anderson	Rieders Lost Creek Cave	AN36	2016: 30 May	
Anderson	Wallace Cave	AN37	2015: 25 Oct	
Anderson	Rainy Knob Cave	AN42	2019: 10 May	
Anderson	Demarcus Cave	AN5	2018: 26 Jun	Y
Anderson	Robert Smith Cave	AN6	2018: 26 Jun	
Anderson	Carters Pit	AN8	2015: 19 Dec	
Blount	Tuckaleechee Caverns	BA11	2014: 20 Mar	
Campbell	Panther Cave No. 1	CM8	2015: 23 Mar 2018: 19 Jul	
Campbell	Panther Cave No. 2	CM9	2018: 19 Jul	

Carter	Carter Saltpeter Cave	CR1	2014: 14 May	
Carter	Rockhouse Cave	CR3	2014: 14 May	
Claiborne	Obie Mill Cave	CB14	2019: 16 Mar	
Claiborne	Powell Mountain Cave	CB15	2019: 16 Mar	Y
Claiborne	Station Creek Cave	CB17	2019: 6 Jun	
Claiborne	Sour Kraut Cave	CB46	2015: 1 Jun	
Claiborne	Buis Saltpeter Cave	CB48	2015: 1 Jun	Y
Claiborne	Tom Balls Cave	CB51	2019: 6 Jun	
Claiborne	Kings Saltpeter Cave	CB52	2015: 30 May	Y
Claiborne	Coonsies Creek Cave	CB57	2016: 23 Mar	
Claiborne	Tiprell Spider Cave	CB78	2019: 6 Jun	
Claiborne	Fools Cave	CB90	2016: 23 Mar	
Grainger	Indian Cave	GA4	2014: 22 Feb; 29 Jun	Y
Hamblen	Soard Cave	HB3	2015: 29 Dec	
Hamblen	Miller Cave	HB5	2015: 29 Dec	
Hamilton	Pan Gap Cave	HM11	2019: 10 Jun	
Hamilton	Read Spring Cave	THM47	2019: 25 May	
Jefferson	Silo Pit Cave	JF71	2015: 3 Aug	
Jefferson	Tater Cave	JF8	2015: 3 Aug	
Knox	Campbell Cave	KN1	2014: 23 Dec	
			2018: 14 Jul; 26 Jul; 15	
Knox	Pedigo Cave	KN103	Dec	
			2019: 27 Jan	

Knox	Pedigo Cave No. 2	KN108	2018: 14 Jul	
Knox	Out and In Cave No. 1	KN111	2019: 13 Jan	
Knox	Brents Cave	KN112	2012: 8 May 2018: 23 Mar	
Knox	Heiskell Pit	KN12	2015: 19 Dec	
Knox	Burnett Cave	KN125	2008: 21 May	Y
Knox	Chriscroft Cave	KN127	2014: 20 Oct	
Knox	Carter Cave	KN14	2008: 21 May	Y
Knox	Ebenezer Rising Cave	KN150	2004: 20 Nov 2018: 22 Sep	
Knox	Watercress Cave	KN153	2019: 13 Jan	
Knox	Keller Bend Cave	KN16	2013: 16 May	
Knox	Steamboat Crawl	KN173	2007: 5 Apr	
Knox	Blowing Hole Cave	KN19	2013: 16 May 2015: 14 Nov	
Knox	Cherokee Caverns	KN22	2014: 5 Apr 2004: 31 Oct 2005: 6 Jan; 6 Mar; 31 Dec	
Knox	Cruze Cave	KN24	2006: 18 Jul; 10 Sep; 19 Nov 2008: 19 May; 7 Jul 2013: 13 May; 15 Jun	Y

			2014: 10 Apr; 11 May; 19 Jun; 14 Aug; 13 Oct 2018: 3 Jul	
Knox	Cherokee Bluff Cave	KN4	2015: 7 Mar	
Knox	Conner Creek Cave	KN50	2018: 10 Jul	
Knox	Kirkpatrick Cave	KN62	2014: 9 Feb; 6 Jul 2019: 25 Jun	Y
Knox	Wilke Waller Cave	KN80	2019: 10 Jul	
Knox	Thumping Cave	KN82	2019: 25 Jun	
Knox	Unreported Cave	KN90	2014: 5 Apr	
Loudon	Blankenship Cave	LN1	2014: 25 Jan	
Loudon	Benjos Cave	LN11	2014: 30 Aug	
Loudon	Ghost Cave	LN3	2014: 30 Aug	Y
Loudon	Melton Hill Spring Cave	LN4	2018: 6 Oct	
McMinn	McCorkle Cave	MM10	2018: 6 Jul	
McMinn	Too Small Cave	MM6	2014: 10 May	
Meigs	Sensabaugh Cave	ME3	2014: 31 Aug	Y
Monroe	The Lost Sea	MO1	2014: 9 Sep	
Monroe	Gay Cave	MO3	2013: 16 Nov	
Monroe	Morgan Cave	MO5	2013: 26 Oct	
Monroe	Nobletts Cave	MO6	2014: 26 Nov	Y
Monroe	Lick Creek Cave	MO8	2013: 16 Nov	

Monroe	Alans Hiaway Cave	MO9	2013: 16 Nov	
Rhea	Dayton Quarry Cave	RH1	2017: 14 Jul	
Rhea	Grassy Creek Cave	RH2	2014: 22 Dec	
Rhea	Starve Rock Cave	RH7	2016: 26 Mar	
Rhea	Clear Creek Cave	RH8	2016: 26 Mar	
Rhea	Piney River Cave	RH9	2016: 26 Mar	
Roane	Big Cave	RN13	2005: 5 Mar	
Roane	Chimney Cave	RN14	2005: 5 Mar	
Roane	Marble Bluff Cave	RN19	2018: 27 Feb	
			2007: 7 Jun	
			2014: 28 Jun	
Roane	Cave Creek Cave	RN5	2018: 3 May; 3 Jun; 3 Jul; 15 Dec 2019: 3 Feb 2005: 30 Dec	Y
Roane	Eblen Cave	RN6	2013: 15 May 2019: 3 Feb; 24 Mar	Y
Sevier	Two County Cave	SV36	2014: 5 Jul	
Sullivan	Bristol Caverns	SL1	2017: 17 Oct	Y
Union	Big Cave	UN10	2015: 22 Mar	Y
Union	Rogers Hollow Cave	UN23	2015: 22 Mar	
Union	Mossy Spring Cave	UN25	2015: 22 Mar	

Union	Big Coon Caverns	UN30	2018: 19 Jul	
Union	Little Coon Cave	UN36	2018: 19 Jul	
Union	Ellison Hollow Cave	UN46	2015: 22 Mar	
Union	Oaks Cave	UN5	2015: 23 Mar	Y
Union	Wright Cave	UN9	2015: 21 Mar	

TABLE S3. Summary of average parameter estimates and AICc for best model distributions comparing abundance over time (salamanders observed ~ days) at Berry, Mudflats, and Meads Quarry Cave. Days represents number of days since 01 January 1983 (before first survey in dataset). Conditional (c.m.) and zero-inflation (zi.m) model parameters of hurdle models are included. Models in bold indicate top fitting distributions (i.e., $\Delta AICc < 2$). Significance: *** - $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

Model distribution	Berry Cave	Mudflats Cave	Meads Quarry Cave
Gaussian	intercept: 5.09*	intercept: 5.53***	intercept: 28.1***
	days: -2.0e-5	days: -3.57e-4***	days: -0.0018**
	df: 3	df: 3	df: 3
	AICc: 153.2	AICc: 76.4	AICc: 161.9
Poisson	intercept: 1.63***	intercept: 1.80***	intercept: 4.29***
	days: -4.04e-6	days: -1.29e-4***	days: -1.95e-4***
	df: 2	df: 2	df: 2
	AICc: 172.1	AICc: 75.4	AICc: 171.9
Zero-inflated Poisson	intercept: 1.60***	intercept: 1.80***	intercept: 4.29***
	days: 6.54e-6	days: -1.29e-4***	days: -1.95e-4***
	zi.m. intercept: -	zi.m. intercept: -	zi.m. intercept: -21.78
	2.50**	21.03	df: 3
	df: 3	df: 3	AICc: 174.5
	AICc: 168.0	AICc: 78.1	

Zero-inflated hurdle	intercept: 1.59***	intercept: 1.72	intercept: 4.29
Poisson	days: 7.46e-6	days: -9.7e-5	days: -1.96e-4
	zi.m. intercept: 106.8	zi.m. intercept: -	zi.m. intercept: -0.437
	zi.m. days: 0.008	35.69	zi.m. days: -0.0023
	df: 4	zi.m. days: 0.0028	df: 4
	AICc: 166.4	df: 4	AICc: 177.3
		AICc: 73.8	
Negative binomial	intercept: 1.52***	intercept: 1.81***	intercept: 4.26***
	days: 5.77e-6e-4***	days: -1.30e-4***	days: -1.92e-4**
	k: 2.83	k: 0.09	k: 1.75
	df: 3	df: 3	df: 3
	AICc: 139.2	AICc: 78.1	AICc: 157.3
Zero-inflated	intercept: 1.52	intercept: 1.80	intercept: 4.29
negative binomial	days: 5.77e-6	days: -1.29e-4	days: -1.92e-4
	k: 2.83	k: 0.09	k: 1.75
	zi.m. intercept: -20.1	zi.m. intercept: -20.9	zi.m. intercept: -21.8
	df: 4	df: 4	df: 4
	AICc: 142.1	AICc: 81.2	AICc: 160.3
Zero-inflated hurdle	intercept: 0.760	intercept: 1.72	intercept: 4.39
negative binomial	days: 6.85e-5	days: -9.74e-5	days: -2.06e-4
	zi.m. intercept: -	zi.m. intercept: -35.7	zi.m. intercept: 0.003
	106.8	zi.m. days: 0.003	zi.m. days: -0.001
	zi.m. days: 0.008	k: 2.43e-6	k: 1.87

	k: 3.44	df: 5	df: 5
	df: 5	AICc: na	AICc: 162.8
	AICc: 139.8		
Negative binomial	intercept: 1.63***	intercept: 1.80***	intercept: 4.35***
with NB2	days: -3.80e-6	days: -1.29e-4***	days: -2.02e-4***
parameterization	k: 1.72	k: 6.56e7	k: 6.16
	df: 3	df: 3	df: 3
	AICc: 139.2	AICc: 78.1	AICc: 157.5
Zero-inflated	intercept: 1.63***	intercept: 1.80	intercept: 4.35
negative binomial	days: -3.80e-6	days: -1.29e-4	days: -2.02e-4
with NB2	zi.m. intercept: -20.4	zi.m. intercept: -16.5	zi.m. intercept: -22.1
parameterization	k: 1.72	k: 3.22e7	k: 6.16
	df: 4	df: 4	df: 4
	AICc: 142.1	AICc: na	AICc: 160.3
Zero-inflated hurdle	intercept: 1.44**	intercept: 1.72***	intercept: 4.39***
negative binomial	days: 7.89e-6	days: -9.74e-5	days: -2.07e-4
with NB2	zi.m. intercept: -	zi.m. intercept: -35.7	zi.m. intercept: -0.362
parameterization	106.8	zi.m. days: 0.003	zi.m. days: -0.0024
	zi.m. days: 0.008	k: 3.91e6	k: 5.89
	k: 1.38	df: 5	df: 5
	df: 5	AICc: na	AICc: 163.2
	AICc: 140.6		

TABLE S4. Threats, existing and recommended conservation and management actions for Berry Cave Salamander (*Gyrinophilus gulolineatus*) sites in east Tennessee, USA.

Location	Last observed	Last surveyed	Threats/impacts	Contribution to assess species viability	Severity (expert opinion)	Actions in place	Specific recommended actions
Aycock Spring Cave (TKN172)	2005	2018	<ul style="list-style-type: none"> Habitat degradation and contamination associated with urbanization (residential) 	Low	High to medium	None	<ul style="list-style-type: none"> Water quality monitoring Delineate recharge basin
Christian Cave (TKN49)	2005	2005	<ul style="list-style-type: none"> Habitat degradation and contamination associated with urbanization (residential) 	Low	High to medium	Gated	<ul style="list-style-type: none"> Water quality monitoring Delineate recharge basin
Fifth Entrance Cave (TKN167)	2007	2018	<ul style="list-style-type: none"> Habitat degradation and contamination associated with urbanization (residential and commercial) 	Very high	Very high to high	Gated	<ul style="list-style-type: none"> Remove lime deposits in recharge zone
Meads Quarry Cave (TKN28)	2019	2019	<ul style="list-style-type: none"> Habitat loss and degradation and changes in hydrology associated with past mining operations Possible competition/hybridization with <i>G. porphyriticus</i> 			Managed by Ijams Nature Center	<ul style="list-style-type: none"> Water quality monitoring Increased regulation of cave visitation Increase natural buffers around infiltration and recharge zone
Meads River Cave (TKN151)	2007	2018	<ul style="list-style-type: none"> Human visitation 				<ul style="list-style-type: none"> Assess levels and risk of hybridization with <i>G. porphyriticus</i>

Mudflats Cave (TKN9)	2019	2018	<ul style="list-style-type: none"> • Habitat degradation and contamination associated with urbanization (residential & commercial) • Habitat loss/degradation and changes in hydrology associated with impoundments • Possible competition/hybridization with <i>G. porphyriticus</i> • Human visitation 	High	Very high to high	None	<ul style="list-style-type: none"> • Water quality monitoring • Delineate recharge basin • Assess levels and risk of hybridization with <i>G. porphyriticus</i>
The Lost Puddle (TKN145)	2018	2018	<ul style="list-style-type: none"> • Habitat degradation and contamination associated with urbanization (residential) 	High	Medium to low	None	<ul style="list-style-type: none"> • Water quality monitoring
Oostanaula Creek south of Athens	1953	1953	<ul style="list-style-type: none"> • Unknown 	Very low	Na	None	<ul style="list-style-type: none"> • Determine aquatic/karst connectivity
Small Cave (TMM5)	2014	2014	<ul style="list-style-type: none"> • Habitat degradation and contamination associated with urbanization (residential) • Possible competition/hybridization with <i>G. porphyriticus</i> • Human visitation 	Low	Medium to low	None	<ul style="list-style-type: none"> • Water quality monitoring • Delineate recharge basin
Blythe Ferry Cave (TME1)	1975	2018	<ul style="list-style-type: none"> • Habitat loss/degradation and changes in hydrology associated with impoundments • Human visitation 	Very low	High	Gated Owned and managed by TVA	<ul style="list-style-type: none"> • Water quality monitoring • Increased regulation of human visitation • Delineate recharge basin
Berry Cave (TRN3)	2019	2019	<ul style="list-style-type: none"> • Habitat degradation and contamination associated with 	Very high	High to medium	Conservation easement	<ul style="list-style-type: none"> • Water quality monitoring • Increase natural buffers around infiltration and recharge zone

urbanization (residential) and
agriculture (pasture/cattle)

General recommended actions for all sites

- Map hydrologic and karst connectivity
- Delineate surface recharge zones
- Identify and mitigate contaminant sources
- Limit cave visitation without compromising facultative cave fauna
- Develop captive breeding programs (accredited)
- Monitor human-inaccessible habitats
- Leverage noninvasive survey methods (e.g., eDNA)

TABLE S5. Potential threats facing Berry Cave Salamanders (*Gyrinophilus gulolineatus*).

Threat impacts are negligible (N), low (L), medium (M), high (H), and very high (VH) based on the scope, severity, and known timing of each threat.

Threat	Threat impact
Residential & commercial development	H
Housing & urban areas	H
Commercial & industrial areas	L
Tourism & recreation areas	L
Agriculture & aquaculture	L
Mining & quarrying	L
Transportation & service corridors	L
Roads & railroads	L
Biological resource use	L
Hunting & collecting animals	L
Human intrusions & disturbance	L
Recreational activities	L
Natural system modifications	M
Dams & water management/use	M
Invasive & other problematic species, genes, & diseases	L
Introduced genetic material	L
Contamination and pollution	M-H
Domestic & urban wastewater (i.e., sewage)	M-H
Agricultural & forestry effluents	L
Climate change & severe weather	L?
Droughts	L?

