The Tennessee Journal of Herpetology

Volume 2, 2019

Tennessee Herpetological Society

ISSN 2576-9340

The Tennessee Journal of Herpetology was founded to provide an avenue of conversation about amphibians and reptiles of Tennessee. Therefore, the Tennessee Journal publishes articles and notes about amphibians, reptiles, and the people who study them.

Find more information about the journal and how to submit at: tnherpsociety.wixsite.com/home

The Tennessee Herpetological Society is a not for profit organization dedicated to promoting the study and conservation of reptiles and amphibians worldwide, but particularly within Tennessee. We believe this can be accomplished through the education of the general public and the hard work of our members. Find out more about us at: www.tnherpsociety.org

Cover Photo by Matthew Niemiller

THE TENNESSEE JOURNAL OF HERPETOLOGY

CONTENTS 2019 VOLUME 2

Corrigendum: The Culturable Skin Microbiome of the Ocoee Salamander, *Desmognathus ocoee*. Daniel H. Hayes, Matthew Grisnik, Spencer A. Stone, Zachary W. Tarter, Kyle Ritter, Lucille M. Yanckello, Logan E. Oatman, Lydia E. Myers, Caroline C. Pellegrino, Carlos D. Camp, Donald M. Walker, and Jessica A. Wooten-Eagle

In the published version of this manuscript, the authors left a statement out of the acknowledgements section. Therefore, we wish to thank Aubree Hill for her thoughtful contributions. Her endeavors greatly strengthened the introduction of this manuscript.

Riparian Habitat of Streams used for Breeding by the Streamside Salamander (*Ambystoma barbouri*) in middle Tennessee

Daniel B. Estabrooks[a,b] and Brian T. Miller[a*]

[a]*Department of Biology, Middle Tennessee State University, Murfreesboro, TN, USA*
[b]*Department of Natural Sciences & Mathematics, Warner University, Lake Wales, FL, USA*
**Corresponding Author e-mail: brian.miller@mtsu.edu*

Abstract.— The Streamside Salamander (*Ambystoma barbouri*) is a stream-breeding ambystomatid that occurs in southeastern Indiana, southern Ohio, and central Kentucky, with disjunct populations forming the southern portion of the range in the Central Basin (CB) of Tennessee. Because of limited geographic distribution and association with low order, ephemeral streams that generally flow through hardwood forests, this species is under consideration by the US Fish and Wildlife Service for protection under the Endangered Species Act. The CB of Tennessee is a mosaic of habitat types with relatively small patches of forest interspersed amidst agricultural and residential lands, and many of the low-order streams have little, if any, riparian habitat that is forested. We characterized riparian habitat of 14 low-order streams in the CB that were used for breeding during the 2007–2008 and 2008–2009 seasons as forest, agriculture, or residential land. We calculated the percent coverage of these three habitat types in an area that extended 250 m and 500 m from the length of each section of stream in which we counted eggs. Riparian habitat was dominated by agricultural land (pastures and row crops), although at least a small amount of forest cover was found near most streams; thus, terrestrial stages of the Streamside Salamander likely inhabited agricultural land in the CB. Residential land was less prevalent in the vicinity of breeding sites than either agricultural land or forested land. Middle Tennessee, including the CB, is experiencing significant human population growth, a trend predicted to continue for at least the next two decades. The conversion of much of the agricultural and forested lands in the region into subdivisions potentially will negatively affect local populations of this species of conservation concern.

Key Words.— abundance, Central Basin, conservation, distribution, egg counts, population density, reproduction, terrestrial habitat.

The Streamside Salamander, *Ambystoma barbouri* Kraus and Petranka (1989), is a stream-breeding member of the Family Ambystomatidae (the Mole Salamanders) with a contiguous distribution extending from southeastern Indiana and southwestern Ohio into central Kentucky. Isolated populations occur in western and south-central Kentucky, southwestern West Virginia, and the Central Basin physiographic region (CB) of middle Tennessee (Scott et al. 1997; Petranka 1998; Niemiller et al. 2006; Niemiller et al. 2011; Anderson et al. 2014; Lockwood et al. 2016). The species is of conservation concern in most states it inhabits (NatureServe 2015. Available from http://explorer.natureserve.org [Accessed 22 February 2017]), and the IUCN lists the species as near threatened (Hammerson 2004). The Streamside Salamander is deemed in need of management by the Tennessee Wildlife Resources Agency (TWRA; Withers 2009), a state conservation listing used, in part, for species with either poorly understood distributions or unknown habitat needs, both types of information deemed vital for proper conservation and management (Withers 2009).

Streamside Salamanders breed in low-order streams from late fall through winter (Petranka 1984a). Many adults migrate to the streams during fall before the breeding season, and presumably live in burrows in the streambank while preparing for courtship (Petranka 1984a). Relatively little is known about terrestrial activities outside of the breeding season, but Petranka (1998) has found adults up to 400 m from streams, and he suggests that juveniles probably travel similar distances away from their natal stream. Regardless, the terrestrial stages are fossorial and inhabit burrows in the floor of hardwood forests (Petranka 1998).

Petranka (1998) indicates that terrestrial stages of the species require forested habitat adjacent to breeding streams. Although relatively little is known about population trends of the Streamside Salamander in middle Tennessee, Niemiller et al. (2009) suggest that populations are declining because of deforestation and residential development of the terrestrial landscape adjacent to streams used for breeding. Furthermore, because the Central Basin is a mosaic of habitat types, including small forest tracts interspersed among agricultural fields (pastures and cropland), cedar glades, and residential areas (urban and suburban areas) (Goodhue et al. 2000, Augustin et al. 2005), many of the low order streams flowing within the CB are not bordered by forested land. The objectives of our study are to characterize riparian habitat of streams used for breeding by the Streamside Salamander in the southern edge of its range and to compare number of females breeding, based on number of both egg masses/m and eggs/m of stream surveyed, among breeding sites with different types of riparian habitats.

METHODS AND MATERIALS

Streams surveyed—. To locate streams used for breeding by the Streamside Salamander, we searched for eggs, larvae, juveniles, and adults approximately 50 m upstream and downstream of road crossings of select low-order streams in southern Rutherford, northern Bedford, northeastern Marshall, eastern Maury, and southeastern Williamson counties from December 2007 through April 2008, and from December 2008 through April 2009 (Fig. 1). If we located eggs, we would continue to search the stream until we no longer encountered eggs for a distance of approximately 50 m. We selected streams based on site access and on

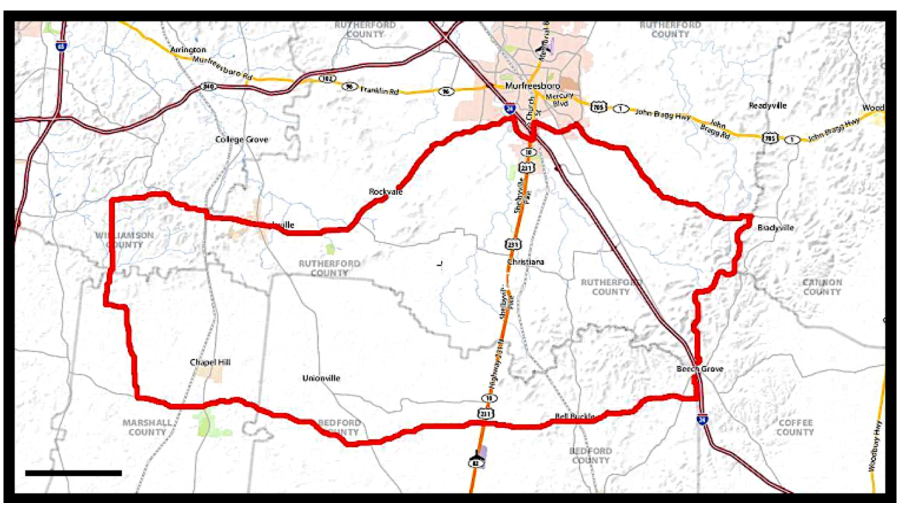

FIG. 1. The area bordered by the red polygon indicates the section of the Central Basin physiographic region in which we searched low-order streams for eggs, larvae, and adult Streamside Salamanders (*Ambystoma barbouri*) during either the 2007–2008 or the 2008–2009 breeding seasons.

similarity and proximity to known breeding sites reported by Niemiller et al. (2006). All streams that we searched were clear and ephemeral, and either became reduced to isolated pools or flowed underground during summer and fall.

Relative abundance of eggs and masses—. We counted eggs at six low-order streams during either the 2007–2008 or the 2008–2009 season. We lifted rocks in both pool and riffle habitats to locate eggs, larvae, and breeding adults, and rocks and other cover objects adjacent to streams to locate juveniles and adults. We carefully returned rocks and other objects to their original positions to limit habitat destruction. In these sections of streams, we lifted rocks suitable for egg deposition and checked the undersurface of the rock for the presence of eggs. We considered all eggs and embryos on the undersurface of a rock to form a single mass unless they were at distinctly different stages of development. When we found eggs and embryos at different stages of development on the undersurface of a rock, we regarded each group of similar staged embryos to represent a distinct mass. In addition to counting the number of masses, we counted the number of eggs within each mass. We counted eggs on site if the mass was relatively small; however, we photographed large masses, and those with either eggs or embryos tightly packed, with a digital camera. To accurately count eggs on digital photographs, we used the application Windows Paint (Microsoft Corporation, Redmond, Washington, USA) and placed a dot on each egg as it was counted.

Riparian habitat—. We used aerial photographs available on Google Earth to determine the length of the section of stream surveyed, and we overlaid a grid onto aerial photographs of each site to estimate the proportion of the type of riparian habitat (forested, agricultural cropland, agricultural pasture, or residential) along the length of the section of the stream in which we found eggs or larvae. Because Streamside Salamanders have been reported to travel up to 400 m from a breeding stream (Petranka 1998), we characterized riparian habitat at distances up to 250 m and 500 m on each side of the surveyed sections. We revisited each site to verify our habitat characterization based on the aerial photographs.

Statistical analysis—. We used Microsoft Excel 2016 (Microsoft Corporation, Redmond, Washington, USA) to perform four single linear regression analyses to elucidate the relationships between egg density (eggs/m of stream length) and the following potential predictor variables: % forest cover within 250 m of stream, % forest cover within 500 m of stream, % field cover within 250 m of stream, and % field cover within 500 m of stream. For these analyses, we used only egg count data from 2009 for the Lynch Hill stream to ensure data independence, as our 2008 counts were conducted after many of the egg masses had hatched. Egg masses from other sites were only counted during one field season each. We also omitted egg count data from the unnamed tributary of the Middle Fork Stones River north of Christiana Hoovers Gap Road, as the salamanders did not appear to be breeding throughout most of the stream area searched, thus potentially skewing the egg density measurement.

RESULTS

Streams used for breeding—. We found eggs, embryos, or larvae of the Streamside Salamander in eight streams in the Stones River watershed of southern Rutherford County (Fig. 2a) and in four streams in the Duck River watershed of northern Bedford County (Fig. 2b). Furthermore, we found one juvenile in eastern Marshall County.

Egg counts—. We counted eggs only from those streams where we discovered salamanders breeding early during the season, before eggs had started hatching. Consequently, our egg

(a)

(b)

FIG. 2. Aerial photograph of a portion of (a) southern Rutherford County and (b) northern Bedford County, Tennessee. Note the mosaic landscape of agricultural fields, residential developments, and forest stands of different sizes. The white ellipses encompass sections of eight low-order streams where we found Streamside Salamanders (*Ambystoma barbouri*) breeding during either the 2007–2008 or 2008–2009 breeding seasons. The length of the colored path in each circle indicates the relative length of streams searched. White scale bar in lower left of photograph is 5 km.

counts were limited to six streams (five streams in southern Rutherford County and one of the northwest Bedford County). We counted 42,804 eggs in 528 egg masses in these six streams (Table 1). We found more than half (55%) of the egg masses and just under half (45%) of the eggs in two low-order tributaries to the Middle Fork of the Stones River (Table 1; Fig. 2a). However, we found a greater density of egg masses (masses/m) and of eggs (eggs/m) in the nearby first-order tributary to the Middle Fork of the Stones River near Lynch Hill Road (Table 1; Fig. 2a).

Egg die offs—. During the 2007–2008 breeding season, we found 38 egg masses comprising 2,550 eggs in Dry Creek (Table 1), with 17 masses and 1,668 eggs in the 690 m upstream and 21 masses and 882 eggs in the 420 m downstream of Cobb Road. However, nearly all embryos we found upstream of the road were dead (white and motionless); whereas, those we found downstream of the road were living. During the 2008–2009 breeding season, we found only a few egg masses and larvae

Table 1. Egg mass data for the Streamside Salamander (*Ambystoma barbouri*) at seven streams in the Central Basin, near the southern edge of the range of the species in southern Rutherford and northern Bedford counties, Tennessee, from December 2007 to May 2009. UNT = Un-named tributary.

Stream	Number of Egg Masses	Survey Length (m)	Min – Max eggs/mass	Total number of eggs	Eggs/m
UNT Middle Fork Stones River, north of Christiana Hoovers Gap Road	5	1090	25 – 205	446	0.4
UNT Middle Fork Stones River, south of Christiana Hoovers Gap Road	288	1730	1 – 439	19,371	11.0
Long Creek	64	620	2 – 345	6,064	9.8
Dry Creek	38	1110	2 – 376	2,550	2.3
Lynch Hill 2008	44	320	8 – 270	2,516	7.8
Lynch Hill 2009	65	320	6 – 910	10,249	32.0
Dolly Branch	24	230	6 – 276	1,608	7.0
Totals	528	5,420	1 – 910	42,804	7.9

upstream of Cobb Road, and we found only one larva and no eggs downstream from the road.

Riparian habitat—. The riparian habitat varied among streams used as breeding sites by Streamside Salamanders during the 2007–2008 and 2008–2009 seasons (Table 2). Although the riparian habitat of most streams included some forest cover, the extent of this coverage varied from 10% or less to nearly 75% (Table 2). Furthermore, the type of field coverage varied based on watershed. For example, the percent field coverage of the seven streams of the Stones River watershed varied from 25% to 89%, but all of the field coverage was either old field or pasture; none of the riparian habitat was cropland in the Stones River watershed. In contrast, the field coverage of the four streams in the Duck River watershed varied from 31% to 90%, but all of the field was tilled cropland (Table 2).

Statistical analysis—. Within 125 m of streams, we found no significant correlation between egg density and % forest (n = 5, r^2 = 0.281, P = 0.358) or % field cover (n = 5, r^2 = 0.298, P = 0.342). Within 250 m of streams, we found a significant positive correlation between egg density and % forest cover (n = 5, r^2 = 0.811, P = 0.037) and a significant negative correlation between egg density and % field cover (n = 5, r^2 = 0.803, P = 0.040).

Table 2. Riparian habitat at 12 low-order streams used by the Streamside Salamander (*Ambystoma barbouri*) in the southern Central Basin of middle Tennessee during the 2007– 2008 and 2008–2009 breeding seasons. UNT = Un-named tributary. Values presented indicate percent coverage of the habitat type in a polygon that extended 125 m on either side of the section of the stream searched.

Watershed	Sub-Watershed	Stream	Length of stream searched (m)	Area of riparian habitat analyzed (m^2)	Forest	Field (Pasture)	Field (Cropland)	Residential
Stones River	Middle Fork	UNT to Middle Fork Stones River, north of Christiana Hoovers Gap Road	1310	327,500	41	59	0	0
		UNT to Middle Fork Stones River, south of Christiana Hoovers Gap Road	2030	507,500	28	72	0	0
		UNT to Long Creek	620	155,000	30	69	0	1
		Dry Creek	1110	277,500	44	56	0	0
		Lynch Hill						
		UNT, downstream section	320	80,000	64	36	0	0
		Upstream and downstream sections	675	168,750	74	25	0	trace
		UNT of Lytle Creek	140	35,000	9	0	0	91
		Knox Branch of Hurricane Creek	100	25,000	13	84	0	3
	West Fork	UNT of Lytle Creek	100	25,000	0	86	0	16
Duck River	North Fork Creek	Dolly Branch of Alexander Creek	230	57,500	68	0	32	0
		UNT to Weakley Creek	50	12,500	9	0	91	0
		UNT to Weakley Creek	50	12,500	69	0	31	0
	Wilson Creek	Osteen Branch of Wilson Creek	100	25,000	10	0	90	0

Table 3. Riparian habitat at 12 low-order streams used by the Streamside Salamander (*Ambystoma barbouri*) in the southern Central Basin of middle Tennessee during the 2007– 2008 and 2008–2009 breeding seasons. UNT = Un-named tributary. Values presented indicate percent coverage of the habitat type in a polygon that extended 250 m on either side of the section of the stream searched.

Watershed	Sub-Watershed	Stream	Length of stream searched (m)	Area of riparian habitat analyzed (m^2)	Forest	Field (Pasture)	Field (Cropland)	Residential (includes industrial)
Stones River	Middle Fork	UNT to Middle Fork Stones River, north of Christiana Hoovers Gap Road	1310	327,500	48	52	0	0
		UNT to Middle Fork Stones River, south of Christiana Hoovers Gap Road	2030	507,500	33	67	0	0
		UNT to Long Creek	620	155,000	15	84	0	1
		Dry Creek	1110	277,500	20	78	0	2
		Lynch Hill						
		UNT, downstream section	320	80,000	51	49	0	0
		Upstream and downstream sections	675	168,750			0	
		UNT of Lytle Creek	140	35,000	3	73	0	24
		Knox Branch of Hurricane Creek	100	25,000	9	81	0	10
	West Fork	UNT of Lytle Creek	100	25,000	16	82	0	2
Duck River	North Fork Creek	Dolly Branch of Alexander Creek	230	57,500	21	0	79	0
		UNT to Weakley Creek	50	12,500	3	0	94	3
		UNT to Weakley Creek	50	12,500				

Discussion

Streamside Salamanders apparently use relatively few of the of the low-order streams available in the southern section of the CB of middle Tennessee as breeding sites (Niemiller et al. 2006, Anderson et al. 2014). Niemiller et al. (2006) found Streamside Salamanders breeding in only 5 of 40 low-order streams that they searched in this region. We searched 40 indicated by Niemiller et al. (2006) and extended our search area to include streams in southeastern Williamson County, northeastern Marshall County, and eastern Maury County, but found *A. barbouri* breeding in only seven additional streams. Thus, our results are in agreement with those of Anderson et al. (2014) who report that Streamside Salamanders breed in a small percentage of apparently suitable low-order streams in the CB of middle Tennessee.

Throughout most of their geographic range, terrestrial stages of the Streamside Salamander typically inhabit upland deciduous forests, with populations rarely found breeding in streams with riparian habitat lacking forests (Petranka 1998). Nonetheless, the CB is a mosaic of habitat types, with agricultural fields and residential land interspersed among remnant forest habitats (Goodhue et al. 2000, Augustin et al. 2005). Our data indicates that Streamside Salamanders breed in streams flowing through agricultural land (forest and cropland), but that more eggs are laid in streams with forest tracts within 500 m of the stream compared to those streams lacking forest tracts within this distance.

We cannot explain why reproduction failed at Dry Creek during the course of this study. Although used as a breeding site annually from 2001 through 2008 (Niemiller et al. 2006, this study, B. Miller pers. obs.), many eggs and embryos failed to develop through hatching upstream of Cobb Road, and we found very little evidence of reproduction during the 2008–2009 breeding season. Although we are uncertain of the cause of the die-off or if eggs have succumbed since our study ended, breeding inexplicably failed at Dry Creek for at least two consecutive years.

Terrestrial habitat—. The terrestrial habitat requirements of the Streamside Salamander are almost completely unknown, and what little is known is largely inferred from the habitat adjacent to streams used for breeding. Populations in Kentucky breed in streams that flow through large tracts of forest (James W. Petranka, pers. comm.). Perhaps in contrast to the landscape of central Kentucky inhabited by Streamside Salamanders, the CB is a mosaic of agricultural land (row crops, pasture, and old fields), residential development, commercial development, and relatively small forest remnants. The proportion of these land uses varies substantially among the smaller sub-watersheds within the Central Basin (Goodhue et al. 2000, Augustin et al. 2005). For example, the streams used as breeding sites in southern Rutherford County are tributaries of the Middle Fork and West Fork of the Stones River, and the sub-watersheds associated with these streams consist primarily of pasture land and forest (deciduous and mixed), with relatively little land devoted to row crops or development (Goodhue et al. 2000). Consequently, riparian habitat of low-order streams used for breeding by Streamside Salamanders is either forest or pasture in southern Rutherford County, but not cropland, which is what our data also indicates. However, encroachment of suburban development is occurring in this area, and residential coverage dominates at least one breeding site. Furthermore, the section of the stream where Regester and Miller (2000) found Streamside Salamanders breeding is now bordered by houses (without any forest buffer).

In contrast to the situation in southern Rutherford County, in northern Bedford County, Streamside Salamanders are restricted to the North Fork Creek sub-watershed of the Upper Duck River, where more land is devoted to pasture than deciduous forest, and more than

23% of the land is devoted to row crops (Augustin et al. 2005). Thus, the known breeding streams in Bedford County are more likely to bordered by agricultural fields than forests, and the agricultural fields are often cropland, rather than pasture land, which is substantiated by our data.

Eggs and egg masses—. The long duration of the breeding season of the Streamside Salamander in middle Tennessee, long length of streams used for breeding at some sites, and number of eggs found, prevented us from counting all eggs at each stream. For example, we did not conduct searches at previously searched stream sections after each rain event. Undoubtedly, additional eggs were laid in these sections throughout the breeding season. Consequently, our counts underestimate the number of eggs present at each stream. Nonetheless, our counts do reflect relative abundance of masses and eggs among sites. Furthermore, we found some sites late in the breeding season, after many eggs had hatched, and we did not attempt to count larvae. Consequently, in several streams we are unable to determine the number of eggs or number of masses deposited. Because of the relatively recent discovery of the Streamside Salamander in Tennessee (Scott et al. 1997), we lack data on demography and cannot comment on whether the populations in the CB are stable, declining, or increasing. Because of the relative ease in counting eggs, compared to either mark-recapture studies of adults migrating during the breeding season or counts of juveniles exiting streams after undergoing metamorphosis, egg count data (eggs/m of stream searched) can be used as a metric to evaluate trends in population dynamics (i.e., whether populations are stable, increasing, or decreasing). The use of egg-counts has been used for decades to assess trends in population dynamics of several species of amphibians that breed in ponds or pools (Cooke 1985, Crouch and Paton 2000, Grant et al. 2005, Paton and Harris 2009), and for a few species that breed in streams, including the Streamside Salamander (Kats and Sih 1992). Based on egg density, a few unnamed tributaries to the Middle Fork of the Stones River that cross Christiana Hoovers Gap Road and Lynch Hill Road are the most important breeding streams for the Streamside Salamander in middle Tennessee.

We discovered relatively late during the 2007−2008 breeding season that salamanders breed in the unnamed tributary draining the forests of Lynch Hill, and we found many larvae of various sizes in the stream channel at that time. Eggs were also present, but our count is a gross underestimate of their abundance. Nonetheless, the density estimate for this site during the 2007–2008 breeding season exceeds those that we obtained at most other sites that year. Furthermore, our count of 10,249 eggs from 65 masses during peak breeding of the 2008–2009 season yielded a density of 32 eggs/m of stream searched, which is nearly three times the density found at other sites. Because many masses of eggs were hatching, we stopped counting eggs after 320 m of stream length, but we did search an additional 675 m upstream of our study site where the riparian habitat was primarily forest. Based on the number of eggs we observed, egg density upstream of our survey section was at least equal to and potentially greater than in the section of stream in which we counted eggs. Furthermore, larvae were abundant; we found larvae near the stream source (near the summit of the hills serving as the Duck River/Stones River divide and the Rutherford County/Bedford County border). Similarly, the tributaries of the other unnamed tributaries, which also originate in the hills at the Bedford County/Rutherford County line, are important breeding streams as evidenced by the relatively high density of eggs, and include the longest known and essentially continuous breeding site for the species in Tennessee. The headwaters of these streams are adjacent to the headwaters of the streams that form the creek at Lynch Hill Road, and these tributaries share a large forested area associated with the hills at the Rutherford County/Bedford County boundary.

The forest that connects these two watersheds likely serves as a refugium for terrestrial stages.

Conservation—. Although populations of the Streamside Salamander persist along streams where forests have been converted to agricultural fields, the relatively greater density of eggs and egg masses in streams with associated with forest tracts within 500 m compared to those associated with indicates the importance of forested terrestrial habitat in the CB. Elimination of remaining forests for residential development poses a serious threat to the survival of the species in Tennessee. Terrestrial stages of the Streamside Salamander apparently are able to use agricultural land in the CB (pasture land in Rutherford County, crop land in Bedford County), but apparently, the species is not as tolerant of conversion of terrestrial habitat into residential use (Niemiller et al. 2006). Middleton and Murray (2009) project that the human population in Rutherford County will increase about 67% during the next 10 to 15 years (from 251,596 in 2010 to 420,465 in 2030). Much of this growth is projected to occur in the unincorporated areas of the county (Middleton and Murray 2009), which will result in additional destruction of terrestrial habitat. Unfortunately, destruction of some of the larger forested areas seems imminent. Water lines have been added near the unnamed tributaries on the south side of the Middle Fork of the Stones River, foreshadowing residential development. Furthermore, the land within the watersheds associated with those same unnamed tributaries and the small stream that drains Lynch Hill flow across property owned by several individuals, and a few landowners have indicated a desire to sell or develop their property.

The known breeding sites of the Streamside Salamander in southern Rutherford and northern Bedford counties, Tennessee occur entirely on private land, and, thus, there are few restrictions on how the land can be used. The Tennessee Division of Forestry (2003) requires a Streamside Management Zone (SMZ) adjacent to any permanent or ephemeral stream. This SMZ is essentially a forested buffer, for which there is no width requirement. There is, however, a recommended width, which varies from 7.6 to 44 m, depending on the slope from the cleared area to the streambed (Division of Forestry 2003). Rutherford County regulations require a 15-meter-wide conservation easement on each side of any natural waterway running through a subdivision (Rutherford County Planning & Engineering Department 2009), which is presumably similar in character to the state-defined SMZ. However, these regulations seem to be inconsistently enforced, as there is no apparent easement along at least one stream that flows through a subdivision. There is also no guarantee that a narrow strip of woodland adjacent to a stream is sufficient to continually support a population of the Streamside Salamander. Additional work is required to obtain more definite information on the terrestrial stages of the Streamside Salamander in Tennessee, including direct observations of individuals during the non-breeding phase of their life cycle and measurements of migration distances. These data would provide valuable information, which could be used to better understand and protect Tennessee populations of this species.

Acknowledgments.— Funding for this study was provided by the Tennessee Wildlife Resources Agency and the Department of Biology, Middle Tennessee State University. This research was conducted under Tennessee Wildlife Resources Agency Scientific Research Permits 1450, and an MTSU IACUC permit, both issued to BTM). We are grateful to assistance with fieldwork provided by Michael Anderson, Bethany Baker, Lacy Danikas, Erika Gray, Dani Millay, Joshua Miller, Will Monroe, Jerrod Shipman, and Emily Sumners.

Literature Cited

Anderson, M.A., J.R. Campbell, A.N. Carey, D.R. Dodge, R.A. Johnston, E.R. Mattison, R.J. Seddon, N.L. Singer, and B.T. Miller. 2014. Population survey of the Streamside Salamander in the Central Basin of Tennessee. Southeastern Naturalist 13:101-7.

Anderson, M.A. and B.T. Miller. 2011. Early iron deposition in teeth of the Streamside Salamander, *Ambystoma barbouri*. Journal of Herpetology 45:336-8.

Augustin, C., T. Wilder, J.R. Smith, A. Goodhue, J. Holland, R. Cochran, D. Duhl, R. McGahen, and S. Wang. 2005. *Upper Duck River Watershed of the Tennessee River Basin: Watershed Water Quality Management Plan*. Watershed Management Section, Division of Water Pollution Control, Tennessee Department of Environment & Conservation, Nashville.

Bi, K., and J.P. Bogart. 2010. Time and time again: unisexual salamanders (genus *Ambystoma*) are the oldest unisexual vertebrates. BMC Evolutionary Biology 10. PMID 20682056 DOI:10.1186/1471-2148-10-238.

Bogart, J.P., J. Bartoszek, D.W.A. Noble, and K. Bi. 2009. Sex in unisexual salamanders: discovery of a new sperm donor with ancient affinities. Heredity 103:483–93.

Cooke, A.S. 1985. The deposition and fate of spawn clumps of the Common Frog *Rana temporaria* at a site in Cambridgeshire, 1971–1983. 32:165–87.

Crouch, W.B., and P.W.C. Paton. 2000. Using egg-mass counts to monitor Wood Frog populations. Wildlife Society Bulletin 28:895–901.

Division of Forestry. 2003. Guide to Forestry Best Management Practices in Tennessee. Division of Forestry, Tennessee Department of Agriculture, Nashville, 51 pp.

Goodhue, A., J.E. Holland, J.R. Smith, R.E. Cochran, D. Duhl, S. Fathi, A. Vann, and S. Wang. 2002. *Stones River Watershed of the Cumberland River Basin: Water Quality Management Plan*. Watershed Management Section, Division of Water Pollution Control, Tennessee Department of Environment & Conservation, Nashville.

Grant, E.H.C., R.E. Jung, J.D. Nichols, and J.E. Hines. 2005. Double-observer approach to estimating egg mass abundance of pool-breeding amphibians. Wetlands Ecology and Management 13:305–20.

Holomuzki, J.R. 1991. Macrohabitat effects on egg deposition and larval growth, survival, and instream dispersal in *Ambystoma barbouri*. Copeia 1991:687–94.

Kats, L.B., and A. Sih. 1992. Oviposition site selection and avoidance of fish by Streamside Salamanders (*Ambystoma barbouri*). Copeia 1992:468–73.

Kraus, F., and J. W. Petranka 1989. A new sibling species of *Ambystoma* from the Ohio River Drainage. Copeia 1989:94–110.

Mattison, E. and B.T. Miller. 2011. *Ambystoma barbouri* (Streamside Salamander). Terrestrial breeding sites. Herpetological Review 42:578–579.

Middleton, E.J., and M.N. Murray. 2009. Population projections for the state of Tennessee, 2010-2030. Tennessee Advisory Commission on Intergovernmental Relations and The University of Tennessee Center for Business and Economic Research. 59 p.

Niemiller, M.L., B.M. Glorioso, C. Nicholas, J. Phillips, J. Rader, E. Reed, K.L. Sykes, J. Todd, G.R. Wyckoff, E.L. Young, and B.T. Miller. 2006. Status and distribution of the Streamside Salamander, *Ambystoma barbouri*, in middle Tennessee. American Midland Naturalist 156:394–9.

Niemiller, M.L., B.M. Glorioso, C. Nicholas, J. Phillips, J. Rader, E. Reed, K.L. Sykes, J. Todd, G.R. Wyckoff, E.L. Young, and B.T. Miller. 2009. Notes on the reproduction of the Streamside Salamander, *Ambystoma barbouri*, from Rutherford County, Tennessee. Southeastern Naturalist 8:37–44.

Niemiller, M.L., B.M. Glorioso, J. Spiess, R.G. Reynolds, and B.T. Miller. 2011. Herpetofauna of the Cedar Glades and associated habitats of the Inner Central Basin of middle Tennessee. Herpetological Conservation and Biology 6:135-49.

Paton. P.W.C., and R.N. Harris. 2009. Egg mass and nest counts. Pp. 143–163 in Dodd, C.K., Jr. (Ed), Amphibian Ecology and Conservation: *A Handbook of Techniques*. Oxford University Press, Inc. New York, New York, USA.

Petranka, J.W. 1982a. Courtship behavior of the Small-Mouthed Salamander (*Ambystoma texanum*) in central Kentucky. Herpetologica 38:333–6.

Petranka 1982b. Geographic variation in the mode of reproduction and larval characteristics of the Small-mouthed Salamander (*Ambystoma texanum*) in the east-central United States. Herpetologica 28:475–85.

Petranka, J.W. 1984a. Breeding migrations, breeding season, clutch size, and oviposition of stream-breeding *Ambystoma texanum*. Journal of Herpetology 18:106–12.

Petranka, J.W. 1984b. Incubation, larval growth, and embryonic and larval survivorship of Smallmouth Salamanders (*Ambystoma texanum*) in streams. Copei 1984:862–8.

Petranka, J.W. 1984c. Sources of interpopulational variation in growth responses of larval salamanders. Ecology 65:1957–65.

Petranka, J.W. 1998. Salamanders of the United States and Canada. First Edition. Smithsonian Institution Press, Washington, D.C., USA.

Regester, K. J. and B.T. Miller. 2000. *Ambystoma barbouri* (Streamside Salamander). Reproduction. Herpetological Review 31:232.

Rutherford County Planning & Engineering Department. 2009. Subdivision Regulations, Murfreesboro, Tennessee. 38 pp.

Scott, A. F., B. T. Miller, M. Brown, J. W. Petranka 1997. Geographic distribution. *Ambystoma barbouri* (Streamside Salamander). Herpetological Review 28:155.

Storfer, A. 1999. Gene flow and population subdivision in the Streamside Salamander, *Ambystoma barbouri*. Copeia 1999:174–81.

Withers, D.I. 2009. A Guide to Rare Animals of Tennessee. Division of Natural Heritage, Tennessee Department of Environment and Conservation, Nashville, Tennessee, USA.

No Environmental DNA Detection of the Patch-nosed Salamander (*Urspelerpes brucei*) in North Carolina

Todd W. Pierson[a*] & Elijah C. White[b]

[a]*Department of Ecology and Evolutionary Biology, University of Tennessee Knoxville, Knoxville, TN, USA*
[b]*Department of Biology, Western Carolina University, Cullowhee, NC, USA*
**Corresponding Author e-mail: tpierso1@vols.utk.edu*

Abstract.—The patch-nosed salamander (*Urspelerpes brucei*) is a geographically restricted plethodontid salamander known only from approximately 20 km^2 in the Tugaloo Mosaic of Georgia and South Carolina. All of the 17 documented localities are in first- and second-order streams in or near the Brevard Fault Zone and Tugaloo River. Here, we use environmental DNA surveys to test for the presence of the patch-nosed salamander in two regions of potential occupancy in North Carolina: 1) the Brevard Fault Zone in Gorges State Park; and 2) the Upper Chattooga River. We collected three 1L samples from each of 19 streams, but we failed to detect the patch-nosed salamander with any sample. Our results provide additional evidence that this species is likely restricted to the small region from which it is currently known.

Key Words.— amphibian, Appalachian, Brevard Fault, Chattooga River, eDNA, plethodontid

Environmental DNA (eDNA) surveys can be effective at locating previously undocumented populations of rare species, especially if those species are difficult to detect using traditional survey methods (e.g., Spear et al. 2015; de Souza et al. 2016). Furthermore, because eDNA surveys require less time in the field compared to traditional searching methods, they can allow researchers to thoroughly sample more locations while reducing environmental damage to sensitive sites.

One example of a rare species easily detected with eDNA is the patch-nosed salamander (*Urspelerpes brucei*). This recently discovered species is currently known from only 16 first- and second-order streams in an approximately 20 km^2 area in northeastern Georgia and a single first-order stream in South Carolina (Camp et al. 2009; Pierson et al. 2016; Camp et al. 2018). Many of the known localities are associated with the Tugaloo Mosaic, a small, unique region of diverse topography, soils, and plants (Garst and Sullvan 1993). Through the Tugaloo Mosaic runs the Brevard Fault Zone (BFZ); the heterogeneous geologic strata (e.g., carbonate rocks) within this regionally significant feature influence the distribution of local biota (Pruitt 1952; Graves and Monk 1985; Fig. 1). The late discovery of the patch-nosed salamander and its relatively cryptic nature suggest the possibility that biologists have thus far underestimated its actual distribution, including in regions noncontiguous with its known distribution, and emphasize the need for widespread surveys. Previous efforts have demonstrated that eDNA surveys can be more cost- and time-effective for discovering populations of the patch-nosed salamander than manual searches or leaf-litter bag surveys (Pierson et al. 2016).

Because many conservation decisions are structured by political boundaries, the documentation of rare species in new regions is important. Here, we used an established eDNA assay to survey for the patch-nosed salamander at what we deemed the sites most likely to be occupied in North Carolina. The known distribution of the patch-nosed salamander falls largely within the BFZ and within the Tugaloo River of the Savannah River drainage in Georgia and South Carolina, so we focused on these criteria in selecting sampling sites in North Carolina. The only place where the BFZ overlaps with the Savannah River drainage in North Carolina is near the Toxaway River in and around Gorges State Park. First, we sampled ten streams in this region. Second, because extensive amphibian surveys in the Upper Tallulah River in Georgia and North Carolina have produced no evidence of the patch-nosed salamander (e.g., Rothermel et al. 2013), we focused instead on the Tugaloo River's other major tributary—the Upper Chattooga River. We sampled nine more streams in this region. We refer to those two regions as BFZ and UCR, respectively, throughout the remainder of this manuscript (Table 1; Fig. 1).

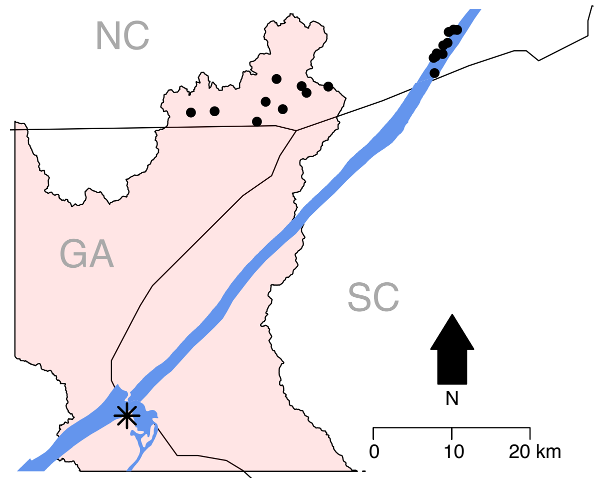

FIG. 1. Environmental DNA sampling localities. Black dots represent streams sampled in this study. The black star represents the approximate centroid of the known distribution of the patch-nosed salamander. The pink polygon represents the Tugaloo River drainage. The blue polygon represents geological strata associated with the Brevard Fault Zone. Watershed boundaries come from the Watershed Boundary Dataset (http://datagateway.nrcs.usda.gov), and we accessed the geological data (Dicken et al. 2005) from the USGS.

METHODS AND MATERIALS

We collected eDNA samples on 6 March 2015 (BFZ) and 1 June 2015 (UCR) following the methods described in Pierson et al. (2016). From each stream, we collected three 1L samples of water and one 1L negative control (i.e., distilled water poured into a collection bottle on-site). We stored these bottles on ice in a cooler, brought them back to the laboratory, and filtered them within 24 hours. We vacuum-filtered samples through 0.45 µm cellulose nitrate filters (Thermo Fisher Scientific, Waltham, MA, USA). We cut these filter papers in half, immediately putting one half into a digest and the other half in 95% EtOH for long-term storage. We extracted DNA from the filters using the modified Qiagen DNeasy Blood and Tissue Kit protocol (Valencia, CA, USA) described in Goldberg et al. (2011) and cleaned DNA extracts with a Zymo Inhibitor Removal Kit (Irvine, CA, USA). Following Pierson et al. (2016), we ran quantitative PCR (qPCR) assays in triplicate for all samples and negative controls on an ABI StepOnePlus (Foster City, CA, USA). This species-specific assay targets an 89-bp fragment of the mitochondrial cytochrome-b using primers and a hydrolysis probe. To test for PCR inhibition, we included an internal positive control (IPC) with all samples. We also included a no template control and a positive control (i.e., DNA extracted from patch-nosed salamander) with each plate. We evaluated the presence of patch-nosed salamander DNA using a manually-established amplification threshold near the beginning of exponential amplification of the IPC in the no template control. We conducted all DNA extractions and qPCRs in a laboratory dedicated to low-copy DNA at the University of Georgia's Department of Environmental Health Science.

RESULTS

We did not detect the presence of patch-nosed salamander DNA in any of our samples (Table 1). All negative controls were negative, and all internal positive controls were positive.

Table 1. Environmental DNA sampling localities. BFZ = Brevard Fault Zone, and UCR = Upper Chattooga River.

Region	Latitude	Longitude	eDNA
BFZ	35.10374°N	82.89491°W	Negative
BFZ	35.07407°N	82.91594°W	Negative
BFZ	35.07921°N	82.91161°W	Negative
BFZ	35.07544°N	82.91324°W	Negative
BFZ	35.08834°N	82.90250°W	Negative
BFZ	35.10643°N	82.88822°W	Negative
BFZ	35.09132°N	82.89654°W	Negative
BFZ	35.10612°N	82.88319°W	Negative
BFZ	35.07849°N	82.90340°W	Negative
BFZ	35.05661°N	82.91464°W	Negative
UCR	35.04111°N	83.06284°W	Negative
UCR	35.04168°N	83.10004°W	Negative
UCR	35.03402°N	83.09319°W	Negative
UCR	35.01525°N	83.12637°W	Negative
UCR	35.02372°N	83.15021°W	Negative
UCR	35.00090°N	83.16239°W	Negative
UCR	35.01286°N	83.22145°W	Negative
UCR	35.01138°N	83.25446°W	Negative
UCR	35.04987°N	83.13503°W	Negative

DISCUSSION

Because the patch-nosed salamander has a high detection probability using this eDNA assay, our results provide strong evidence for the absence of this species in all 19 surveyed streams and suggest its absence more broadly from the two regions surveyed. These results concur with the assertion that the patch-nosed salamander likely has a very small distribution, as suggested from other eDNA and traditional surveys for the species in Georgia and South Carolina (Pierson et al. 2016). This underscores the importance of headwater stream conservation within the small known distribution of the patch-nosed salamander, although additional surveys nearer to this region are necessary to conclusively determine the full distribution of the species.

Acknowledgements.— We thank the North Carolina Herpetological Society, the North Carolina Wildlife Resources Commission, and the Highlands-Cashiers Land Trust for funding this research. We especially thank N. Bayona-Vásquez, E. Corey, K. Pursel, and L. Williams for their help in gaining access to streams and collecting samples, and E. Lipp and T. Glenn for access to laboratory space at the University of Georgia. TWP's research was supported by a National Science Foundation Graduate Research Fellowship under Grant No. (DGE-1452154).

LITERATURE CITED

Camp, C.D., W.E. Peterman, J.R. Milanovich, T. Lamb, J.C. Maerz, and D.B. Wake. 2009. A new genus and species of lungless salamander (family Plethodontidae) from the Appalachian highlands of the south-eastern United States. Journal of Zoology 279:86–94.

Camp, C.D., T.L. Brock, T.W. Pierson, J.R. Milanovich, and J.C. Maerz. 2018. Life history and habitat of a rare salamander (*Urspelerpes brucei*). Herpetological Conservation and Biology 13:609-16.

de Souza, L.S., J.C. Godwin, M.A. Renshaw, and E. Larson. 2016. Environmental DNA (eDNA) detection probability is influenced by seasonal activity of organisms. PLoS One 11:e0165273.

Dicken, C.L., S.W. Nicholson, J.D., Horton, M.P. Foose, and J.A.L. Mueller. 2005. Preliminary integrated geologic map databases for the United States: Alabama, Florida, Georgia, Mississippi, North Carolina, and South Carolina. Open File Report No. 2005-1323.

Garst, J. and J. Sullivan. 1993. Tugaloo Mosaic: a unique area. Tipularia 8:18–26.

Goldberg, C.S., D.S. Pilliod, R.S. Arkle, and L.P. Waits. 2011. Molecular detection of vertebrates in stream water: a demonstration using Rocky Mountain tailed frogs and Idaho giant salamanders. *PLoS One* 6:e22746.

Graves, J.H. and C.D. Monk. 1982. Herb-soil relationships on a lower north slope over marble. Bulletin of the Torrey Botanical Club 109:500–507.

Pierson, T.W., A.M. McKee, S. F. Spear, J.C. Maerz, C.D. Camp, and T.C. Glenn. 2016. Detection of an enigmatic plethodontid salamander using environmental DNA. Copeia 2016:78–82.

Pruitt, R.G. 1952.The Brevard zone of northeasternmost Georgia. Ph.D. diss., Emory University, USA.

Rothermel, B.B., E.R. Travis, D.L. Miller, R.L. Hill, J.L. McGuire, and M.J. Yabsley. 2013. High occupancy of stream salamanders despite high *Ranavirus* prevalence in a Southern Appalachians watershed. EcoHealth 10:184–9.

Spear, S.F., J.D. Groves, L.A. Williams, and L.P. Waits. 2015. Using environmental DNA methods to improve detectability in a hellbender (*Cryptobranchus alleganiensis*) monitoring program. Biological Conservation 183:38–45.

Gross Morphology of Teeth on the Premaxillae of Streamside Salamanders (*Ambystoma barbouri*) and Small-mouthed Salamanders (*Ambystoma texanum*) from Middle Tennessee

Brian T. Miller[a*] and Joyce L. Miller[b]

[a]*Department of Biology, Middle Tennessee State University, Murfreesboro, Tennessee, USA*
[b]*MTSU Integrative Microscopy and Imaging Center, Middle Tennessee State University, Murfreesboro, Tennessee, USA*
**Corresponding Author e-mail: brian.miller@mtsu.edu*

Abstract.— Until 1989, the Streamside Salamander (*Ambystoma barbouri*) was considered conspecific with the Small-mouthed Salamander (*Ambystoma texanum*). Although they have distinct natural histories, particularly reproductive behaviors, individuals of these two species are nearly indistinguishable from each other. The similarity in appearance typically is not an issue because the two species are largely allopatric and geography can be used to determine which species is present. However, several narrow zones of contact (i.e. parapatry) have been reported from Kentucky, Indiana, and Ohio, and identifying an individual to species in these zones requires an examination of the dentition in postmetamorphic individuals. We used scanning electron microscopy to examine the gross morphology of teeth from adult Streamside Salamanders and adult Smallmouth Salamanders from middle Tennessee. Our observations of tooth morphology do not differ from those of these two sibling-species from other regions of their range. The lingual cusps of teeth on the upper jaw (premaxillae and maxillae) of Streamside Salamanders are short and rounded; whereas, cusps of these teeth in Small-mouthed Salamanders are long and narrow. Tooth morphology can be used to identify postmetamorphic individuals of each of these species from middle Tennessee.

Key Words.— Central Basin, Eastern Highland Rim, labial cusps, lingual cusps, sibling species, teeth, tooth morphology

The Streamside Salamander (*Ambystoma barbouri*) and the Small-mouthed Salamander (*Ambystoma texanum*) are a pair of cryptic species that inhabit middle Tennessee (Fig. 1; Redmond and Scott 1996, Scott et al. 1997). No external feature can be used to reliably identify salamanders of either of these two species (Krause and Petranka 1989). However, the dentition is different between the two species, and tooth structure has been used to identify species of individuals collected in zones of contact (Kraus and Petranka 1989) and in newly discovered populations (Scott et al. 1997).

Little is known about the variation in tooth structure of either Streamside Salamanders or Small-mouthed Salamanders. Kraus and Petranka (1989) describe the dentition of both species, but they did not examine specimens of either species from middle Tennessee. Their descriptions of the dentition of the Small-mouthed Salamander are based on specimens they obtained throughout the range of the species, and they describe distinct variation in morphology of the teeth from western and eastern populations. Beneski and Larsen (1989) describe the morphology of the teeth of the Small-mouthed Salamander, but their publication precedes the recognition of the Streamside Salamander as a distinct species, and they do not indicate the collection locality of the

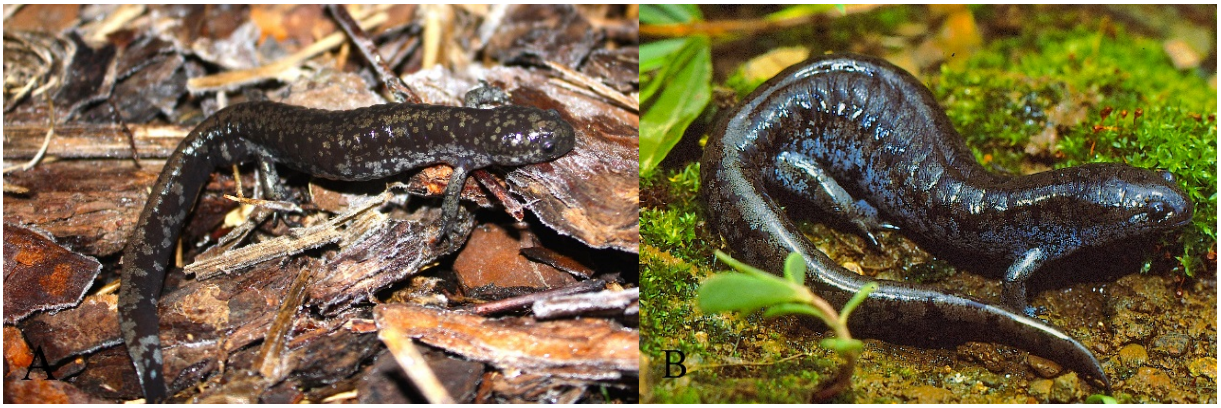

FIG 1. (A) Streamside Salamander (*Ambystoma barbouri*) from Wilson County, Tennessee. (B) Small-mouth Salamander (*Ambystoma texanum*) from Coffee County, Tennessee. (Photographs by Brian T. Miller).

specimens they use in their study. Gregory et al. (2016) describe tooth morphology of both species, but also do not indicate collection locality of their specimens. Thus, variation in morphology of the teeth occurs among populations of Small-mouthed Salamanders, but too little information exists to determine if similar variation occurs among populations of Streamside Salamanders.

The Streamside Salamander is known from fewer than fifty sites in middle Tennessee, all restricted to the Central Basin Physiographic region (Niemiller et al. 2006; Anderson et al. 2015, Lockwood et al. 2016). In contrast, the Small-mouthed Salamander is widespread in west Tennessee, but has a more limited distribution in middle Tennessee, where it is known primarily from a few locations in the Barrens of the Western Highland Rim physiographic region (Redmond and Scott 1996) and even populations from the Barrens of the Eastern Highland Rim (Miller et al. 2005). No aspect of the dentition has been described for either of these two species in middle Tennessee. The objective of this study is to use scanning electron microscopy to describe and document the morphology of teeth on the premaxillary bone of Streamside Salamanders and Small-mouthed Salamanders from middle Tennessee.

METHODS AND MATERIALS

We collected sexually mature Streamside Salamanders during January 1997 as they migrated to a small, ephemeral pond in the vicinity of Sinking Pond in Arnold Air Force Base in northern Coffee County, Tennessee. We also collected sexually mature salamanders as road kill in southern Rutherford County, Tennessee during breeding migrations of winter and spring of 2002. To prepare for SEM, we fixed specimens in 10% buffered formalin and preserved in 70% ETOH. We macerated three heads of preserved specimens of each species in a 4% KOH solution. We washed resulting skulls or disarticulated bones with distilled water for at least 24 h to remove the KOH. We then dehydrated the bones via a graded series of ethanol rinses (70%–95%–100%). Following dehydration, we air dried bones, mounted them on aluminum stubs, and sputter-coated specimens of each stub with about 30 nm of gold in a Hummer 6.2 sputter coater (Anatech USA, Union City, California, USA). We utilized a Hitachi S-3400N scanning electron microscope (Hitachi High-Technologies Corporation, Tokyo, Japan) to examine preparations at 20 KV.

RESULTS

Gross morphology of teeth on the premaxillary bone was similar in these two species (Figs. 2, 3). In each species, two rows of functional teeth were attached to the premaxillae, and the teeth on this jaw bone were pedicellate and bicuspid (Figs. 2, 3). The labial cusp was spade-like in each species; whereas, the lingual cusp was either short, blunt, and rounded (Streamside Salamander), or long, tapered and pointed (Small-mouthed Salamander; Fig. 3). Furthermore, in each species the outer surfaces of the cusps (labial and lingual) had an intricate network of ridges (Fig. 3).

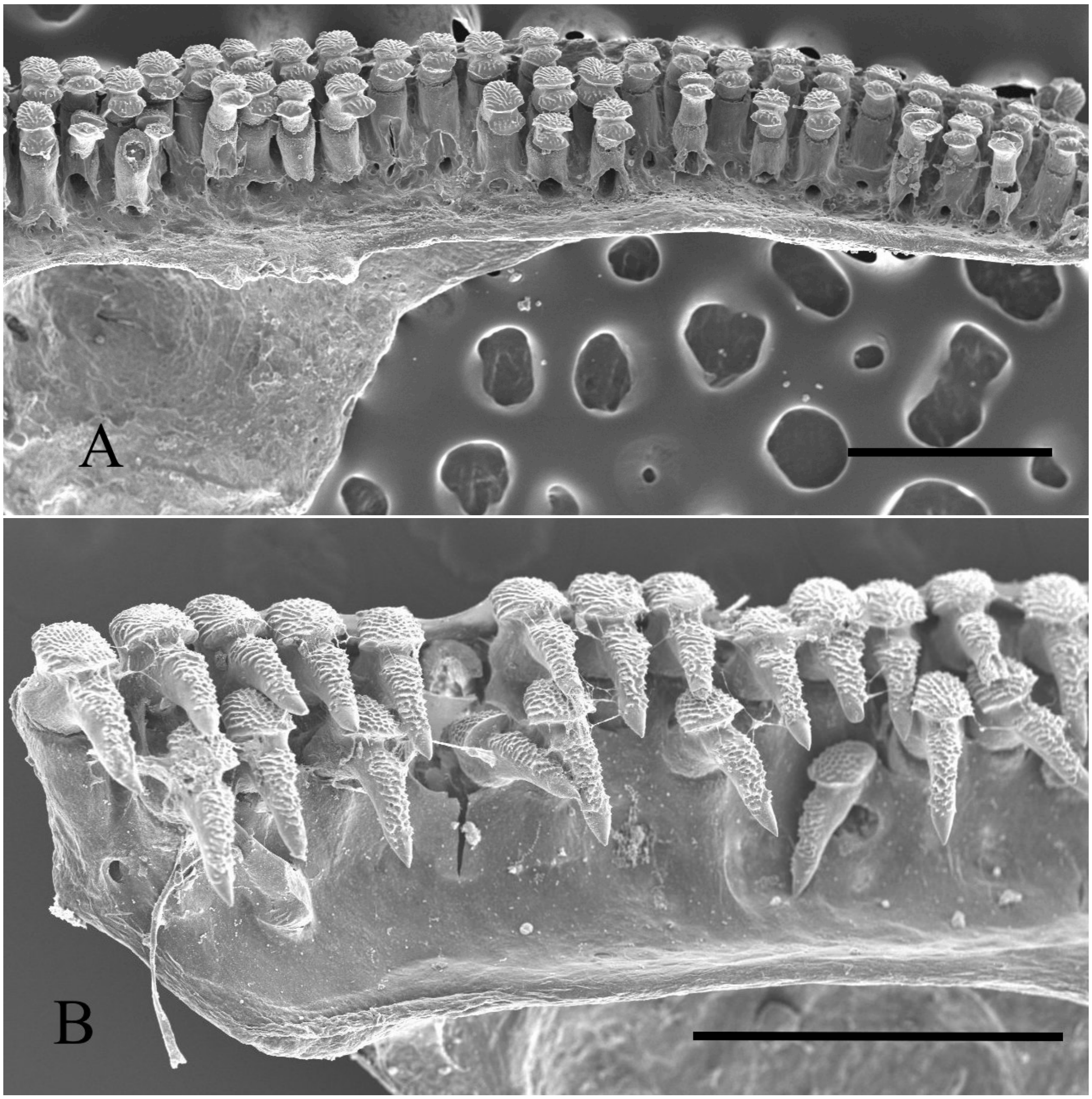

FIG. 2. (A) Teeth on the premaxilla of a Streamside Salamander (*Ambystoma barbouri*) from Rutherford County, Tennessee. (B) Teeth on the premaxilla of a Small-mouthed Salamander (*Ambystoma texanum)*, from Coffee County, Tennessee. The scale bar in the lower right of each photograph is 500 μ. (Photographs by Joyce L. Miller)

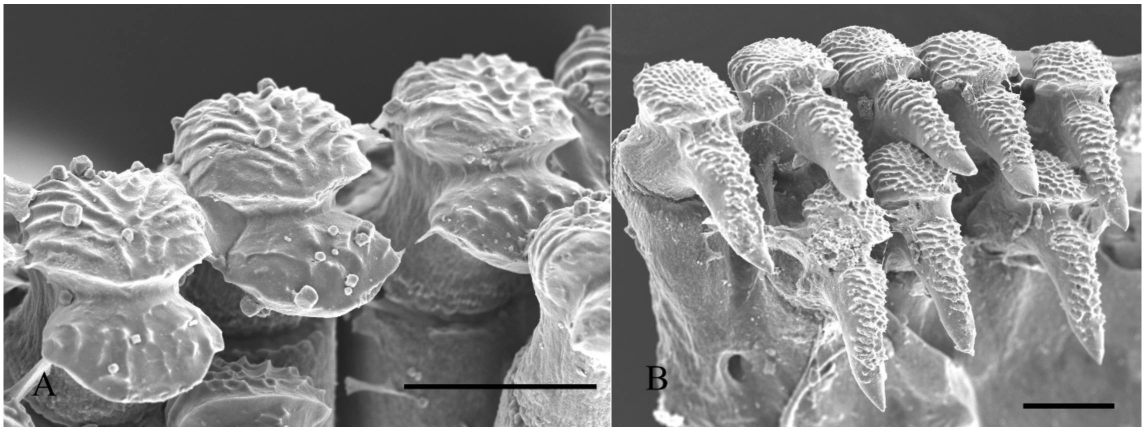

FIG. 3. (A) Teeth on the premaxilla of a Streamside Salamander (*Ambystoma barbouri*) from Rutherford County, Tennessee. (B) Teeth on the premaxilla of a Small-mouthed Salamander (*Ambystoma texanum*) and from Coffee County, Tennessee. The scale bar in the lower right of each photograph is 100 μ. (Photographs by Joyce L. Miller).

DISCUSSION

Teeth of the premaxillary bone of Streamside Salamanders and Small-mouthed Salamanders from middle Tennessee are similar to the few descriptions provided for these species from other regions (Beneski and Larsen 1989; Kraus and Petranka 1989; Gregory et al. 2016). At least in central Kentucky, the lingual cusps of teeth on the upper jaw of Streamside Salamanders are short and rounded; whereas, these cusps are long and narrow in eastern populations of the Small-mouthed Salamander (Kraus and Petranka 1989). The presence of multiple rows of functional teeth on the jaws of these two species and a few other ambystomatids has long been noted and is unusual (Beneski and Larsen 1989; Kraus and Petranka 1989); however, the functional significance, if any, is unknown. Furthermore, the functional significance of the peculiar ridges on the outer surfaces of the cusp is unknown (Beneski and Larsen 1989; Gregory et al. 2016).

Although the Streamside Salamander has been known to occur in Tennessee for approximately 20 years (Scott et al. 1997), relatively little information has been published on either the ecology or morphology of the species in the state. Regester and Miller (2000), Niemiller et al. (2009), and Mattison and Miller (2011) report on aspects of reproduction in the species, and Anderson and Miller (2011) report on iron deposition in the teeth of larvae. Most other published information is concerned with distribution of the species in the Central Basin, and most of these reports are based on discovery of either egg masses or adults (Niemiller et al. 2006; Niemiller et al. 2011; Anderson et al. 2014; Lockwood et al. 2016). Certainly, the typical reproductive behavior of Streamside Salamanders laying eggs on the undersurface of rock differs from that of the Smallmouth Salamander, which will usually lay eggs on in small clusters attached to vegetation in ponds (Kraus and Petranka 1989; Petranka 1998). However, Small-mouth Salamanders occasionally breed in ditches and streams, and Streamside Salamanders also occasionally breed in ponds (Petranka 1998). Thus, location of breeding site and manner of egg deposition is not necessarily a definitive means of identifying species. We suggest that the teeth of adult salamanders also be examined to ensure proper identification of this cryptic pair of sibling species.

Acknowledgements.— We collected animals under Tennessee Wildlife Resources Agency Scientific Research Permit 1450 and MTSU

Institutional Animal Care and Use Protocol Number 97-004, both issued to BTM.

LITERATURE CITED

Anderson, M.A. and B.T. Miller. 2011. Early iron deposition in teeth of the Streamside Salamander, *Ambystoma barbouri*. Journal of Herpetology 45:336–8.

Beneski, J.T., J.H. Larsen, Jr. 1989. Interspecific, ontogenetic, and life history variation in the tooth morphology of mole salamanders (Amphibia, Urodela, and Ambystomatidae). Journal of Morphology 199:53–69.

Gregory, A.L., B.R. Sears, J.A. Wooten, C.D. Camp, A. Falk, K. O'Quin, and T.K. Pauley. 2016. Evolution of dentition in salamanders: relative roles of phylogeny and diet. Biological Journal of the Linnean Society 119:960–97

Kraus, F., and J. W. Petranka 1989. A new sibling species of *Ambystoma* from the Ohio River Drainage. Copeia 1989:94–110.

Mattison, E. and B.T. Miller. 2011. *Ambystoma barbouri* (Streamside Salamander). Terrestrial breeding sites. Herpetological Review 42:578–9.

Miller, B.T., J.W. Lamb, and J.L. Miller. 2005. The herpetofauna of Arnold Air Force Base in the Barrens of Tennessee. Southeastern Naturalist 4:51–62.

Niemiller, M.L., B.M. Glorioso, C. Nicholas, J. Phillips, J. Rader, E. Reed, K.L. Sykes, J. Todd, G.R. Wyckoff, E.L. Young, and B.T. Miller. 2006. Status and distribution of the Streamside Salamander, *Ambystoma barbouri*, in middle Tennessee. American Midland Naturalist 156:394–9.

Niemiller, M.L., B.M. Glorioso, C. Nicholas, J. Phillips, J. Rader, E. Reed, K.L. Sykes, J. Todd, G.R. Wyckoff, E.L. Young, and B.T. Miller. 2009. Notes on the reproduction of the Streamside Salamander, *Ambystoma barbouri*, from Rutherford County, Tennessee. Southeastern Naturalist 8:37–44.

Niemiller, M.L., B.M. Glorioso, J. Spiess, R.G. Reynolds, and B.T. Miller. 2011. Herpetofauna of the Cedar Glades and associated habitats of the Inner Central Basin of middle Tennessee. Herpetological Conservation and Biology 6:135–49.

Petranka, J.W. 1998. Salamanders of the United States and Canada. First Edition. Smithsonian Institution Press, Washington, D.C., USA.

Redmond, W.H. and A.F. Scott. 1996. Atlas of Amphibians in Tennessee. Miscellaneous Publication No. 12, The Center for Field Biology, Austin Peay State University, Clarksville, Tennessee. 94 pp. Hard copy and internet versions [http://apbrwww5.apsu.edu/amatlas] accessed 22 March 2017. Latest Update January 2017)

Scott, A. F., B. T. Miller, M. Brown, J. W. Petranka 1997. Geographic distribution. *Ambystoma barbouri* (Streamside Salamander). Herpetological Review 28:155.

Withers, D.I. 2009. A Guide to Rare Animals of Tennessee. Division of Natural Heritage, Tennessee Department of Environment and Conservation, Nashville, Tennessee, US.

.

CARPHOPHIS AMOENUS **(Common Wormsnake). HATCHING.** At 0932 h on 19 September 2017, one of us (DAM) overturned a trailside rock in in a mesic hardwood forest in Knox County, Tennessee (36.104444°N, 83.763056°W, WGS 84, 360 m elev.) to reveal a recently-hatched *Carphophis amoenus* nest containing at least five eggshells and two hatchlings, but no visible adult female. The rock was approximately 20 x 25 cm in size and was loosely embedded into the ground beneath it. To the best of our knowledge, this is the first documentation of the location of a nest and the timing of hatching in Tennessee (Niemiller et al. 2013. The Reptiles of Tennessee. University of Tennessee Press, Knoxville, Tennessee. 366 pp.). The location of the nest, size of the clutch, and timing of hatching are similar to what has reported in neighboring states (e.g., North Carolina, summarized in Palmer and Braswell 1995. Reptiles of North Carolina. University of North Carolina Press, Chapel Hill, North Carolina. 412 pp.; Virginia, summarized in Ernst and Ernst 2003. Snakes of the United States and Canada. Smithsonian Books, Washington, D.C. 668 pp.).

Submitted by: **DANIEL A. MALAGON** (e-mail: dmalagon@vols.utk.edu) and **TODD W. PIERSON** (e-mail: tpierso1@vols.utk.edu), University of Tennessee, Knoxville, TN, 37996.

FIG. 1. A recently-hatched *Carphophis amoenus* nest as uncovered. Photograph by Daniel A. Malagon

EURYCEA CIRRIGERA (Southern Two-lined Salamander). **CLIMBING ABILITY**. McEntire (2016, Copeia 104:124–131) suggests that arboreality or climbing behavior is either overlooked or under reported for many species of plethodontid salamanders. She indicates that arboreal behavior has been reported in at least 35% of species of plethodontid salamanders inhabiting the temperate forest regions of North America (McEntire 2016, *op cit*.), which includes many species that are typically considered to be terrestrial. For example, arboreal behavior has been reported in four species of *Eurycea*, including the Northern Two-lined Salamander (*E. bislineata*; LeGros 2013, Canadian Field-Naturalist 127:67–69), Longtail Salamander (*E. longicauda*; Anderson and Martino, 1966 American Midland Naturalist 75:257–279; Nazdrowicz 2015 Ph.D. Dissertation, University of Delaware, Newark, Delaware, USA 129 p.), Blue Ridge Two-lined Salamander (*E. wilderae;* McEntire *op cit*.), and Southern Two-lined Salamander (*E. cirrigera*; Miloski, 2010. M.S. thesis, Marshall University, Marshall, West Virginia, USA, 106 p.). However, the importance of arboreality has been demonstrated only for the Northern Two-lined Salamander (LeGros *op cit.)*, with data on the arboreality of other three species limited to only one or two observations. For example, McEntire (op cit.) indicates that the Southern Two-lined Salamander is facultatively arboreal because of a single observation of one individual found under a burlap bag tied about 1.5 m up the trunk of a tree in West Virginia (Miloski op cit.). Here, we provide additional information on climbing behavior by a Southern Two-lined Salamander in middle Tennessee.

On 27 September 2017 at approximately 0330 h, we found a male Southern Two-lined Salamander perched on top of a narrow (1.0 cm dia), cylindrical metal-rod used to support a glass hummingbird feeder (Fig. 1). To reach its perch, the salamander had to climb 185 cm up, 45 cm along and down the narrow metal rod. Although humidity was high (approaching 100%) during the early morning, no precipitation was recorded that day; consequently, the metal rod was dry when the salamander was discovered (Fig. 2). We do not know when the salamander climbed the rod, but it remained perched in position until 0500 hours, when we either inadvertently startled the salamander, or it was stimulated to seek cover because of impending daybreak. Regardless, from the time of discovery the salamander remained perched on the rod for at least 1.5 hours.

Although many plethodontids are known to climb vegetation (McEntire *op cit*.), presumably to forage (but see McEntire *op cit*. for discussion of this topic), most reports of arboreality are of salamanders in low vegetation during or immediately after rain storms. However, our observation indicates that Southern Two-lined Salamanders are capable of climbing to heights of nearly 2 m, taking circuitous routes to reach their destination, and climbing even during rainless nights. During night surveys for plethodontid salamanders, we seldom search for Southern Two-lined Salamanders in vegetation, and never look for them at eye-level; consequently, we are uncertain how important arboreality is to individuals of this species. However, we agree with McEntire (*op cit*.) who suggests that arboreality in temperate species of plethodontid salamanders might be more common than currently recognized.

Submitted by **BRIAN T. MILLER** (e-mail: brian.miller@mtsu.edu) Department of Biology, Middle Tennessee State University, Murfreesboro, Tennessee, USA 37132, and **JOYCE L. MILLER** (e-mail: joyce.miller@mtsu.edu) MTSU Integrative Microscopy and Imaging Center, Middle Tennessee State University, Murfreesboro, Tennessee, USA, 37132.

FIG. 1. A male Southern Two-lined Salamander (*Eurycea cirrigera*) perched 1.8 m above the ground on a metal pole used to support a hummingbird feeder. We discovered the salamander at 0300 h on 27 September 2017 in southern Cannon County, Tennessee, USA (Photographs by Joyce L. Miller).

TANTILLA CORONATA (Southeastern Crowned Snake). USA: TENNESSEE: McNairy Co.: Mt. Peter (35.289797°N, 88.534467°W; datum WGS84). 19 April 2018. Brian P. Butterfield, Wayne Baker, Leah Campbell, Sarahy Montoya, and Abigail Tatum. Verified by A. Floyd Scott. David H. Snyder Museum of Zoology, Austin Peay State University (APSU 19869, color photo). We searched VertNet and found four specimens collected from 1.5 miles east of Selmer in 1954 (Herp-17081 – Herp-17084. Chicago Academy of Sciences. CHAS Herpetology. http://ipt.vertnet.org:8080/ipt/resource.do?r=chas_herps[accessed on 2018-04-25]). However, our record is the first vouchered record to be published as well as the first with specific locality data for McNairy County (Redmond and Scott 2008. Atlas of Reptiles in Tennessee. The Center for Field Biology, Austin Peay State University, Clarksville, Tennessee. Internet version, available at http://apsu.edu/repatlas/ [updated 17 March 2018]; accessed 19 April 2018). Two individuals were found under debris remaining from a removed mobile home located in a rural upland oak-hickory forest. Tennessee Wildlife Resource Agency permit #1419.

Submitted by **Brian P. Butterfield** (e-mail: bbutterfield@fhu.edu), **Wayne Baker**, **Leah Campbell**, **Sarahy Montoya**, and **Abigail Tatum**, Freed-Hardeman University, 158 E. Main Street, Henderson, Tennessee 38334, USA.

List of 2017 hatchlings by Zoo Knoxville Herpetology Department

Summarized by Stephen Nelson

56 reptiles were hatched representing 14 species (8 of which are assessed as Critically Endangered, 5 are Endangered, and 1 Vulnerable by the International Union Conservation of Nature (IUCN) Red List of Threatened Species™ (* Pancake Tortoises and Home's Hingeback Tortoises are currently listed as Vulnerable by the Red List but have been recommended to be upgraded by the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group as Critically Endangered).

Table 1. Summary of reptile hatchlings in 2017 at Zoo Knoxville. VU = Vulnerable, EN = Endangered, CR = Critically endangered.

Scientific Name	Common Name	Status	# Hatchlings
Pyxis arachnoides brygooi	Northern Spider Tortoise	CR	5
Pyxis arachnoides arachnoides	Common Spider Tortoise	CR	3
Pyxis arachnoides oblonga	Southern Spider Tortoise	CR	1
Phelsuma klemmeri	Neon Day Gecko	EN	4
Astrochelys radiata	Radiated Tortoise	CR	7
Shinisaurus crocodilurus	Chinese Crocodile Lizard	EN	17
Malacochersus tornieri	Pancake Tortoise	CR*	1
Kinixys homeana	Home's Hingeback Tortoise	CR*	3
Terrapene carolina carolina	Eastern Box Turtle	VU	1
Clemmys guttata	Spotted Turtle	EN	3
Glyptemys muhlenbergii	Bog Turtles	CR	3
Geoemyda spengleri	Black-breasted Leaf Turtle	EN	2
Leucocephalon yuwonoi	Sulawesi Forest Turtle	CR	1
Pituophis ruthveni	Louisiana Pine Snake	EN	5

24rd Annual Meeting of the Tennessee Herpetological Society

Lichterman Nature Center, Memphis, TN
September 27-28, 2018
Business Meeting Notes
Recorded by Stephanie Chance

Award Recipients:
Congratulations to Shawn Snyder (Tennessee State University) & Emma Zeitler (University of the South) for being awarded the 2018 Chad Lewis Memorial Grant for their work on habitat modeling of Western pygmy rattlesnakes and wastewater effects on amphibians respectively. Todd Pierson (University of Tennessee) was the inaugural recipient of the Niemiller Travel Award for his work on the phylogeography and ecology of the *Eurycea* spp. in the Eastern U.S.

Conservation Committee:
Nothing new to report from the committee.

Chad Lewis Memorial Grant Committee:
Committee will make the announcement about award competition earlier in the year. This committee also evaluated the Niemiller Travel Award applicants as well. THS matched the Niemiller donation for 2018 and would like to continue to do so.

Website Committee:
The Website Committee was charged with migrating the current THS website to a more user-friendly and easier to administer website that could either be free with ads or ad-free for a smaller fee than the current website. Species accounts are being transferred to TN Watchable Wildlife or could be duplicated on the THS website with the assistance of student volunteers.

Publication/Newsletter Committee:
We continue to request new submissions. The Tennessee Journal of Herpetology may be found at:
https://www.tnherpsociety.org/tennessee-journal-of-herpetology

Treasurer's Report:
Members approved last year's report. Balance in the checking account is $14,793 and the CD balance is $12,489. The society approved motions to transfer CD to maintain $2,489 in a bank account and invest $10,000 with Larissa Barton at Northwestern Mutual Funds.

New Business:
New Elections:
President: Donny Walker
Secretary: Dustin Thames
West TN Representative: Lee Barton
Sergeant at Arms: Josh Ennen

2019 Annual Meeting of the Tennessee Herpetological Society
Gray Fossil Site & Museum
Gray, TN
We hope to see you there!