Tennessee Journal of Herpetology, Vol. 2, pp. 16-19, 2019
ISSN 2576-9340

# NO ENVIRONMENTAL DNA DETECTION OF THE PATCH-NOSED SALAMANDER (*URSPELERPES BRUCEI*) IN NORTH CAROLINA

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***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).

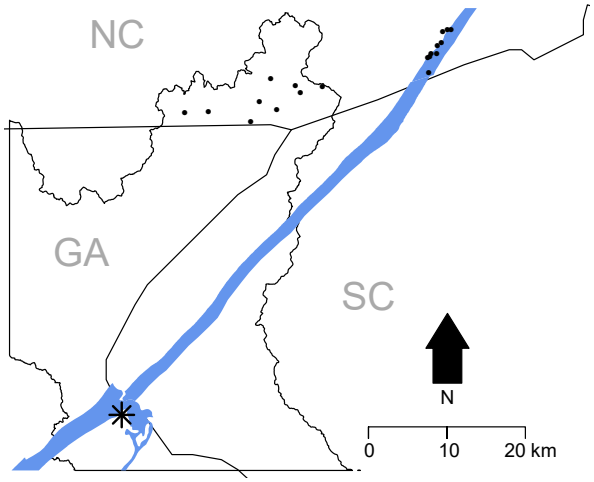


FIG. 1. Environmental DNA sampling localities. Black dots represent streams sampled in this study. The black star represents the approximate centroid of the known distribution of the patch-nosed salamander. The pink polygon represents the Tugaloo River drainage. The blue polygon represents geological 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.