

**Applicant:** Elizabeth K. Timpe, Univ. of Connecticut, Dept. of Ecology and Evolutionary Biology  
**Title:** Uncovering Genetic Diversity of a Salamander Species Restricted to a Critically Threatened Habitat  
**Salamanders Species:** Brownback Salamander (*Eurycea aquatica*: Spelerpinae: Plethodontidae)

**Project Overview:** The springs of the southern Appalachian Mountains are home to numerous endemic species; many which are federally or state protected (e.g., Watercress Darter, Spring Pygmy Sunfish). Spring-dwelling fauna are found in narrow and restricted distributions, and are often subjected to anthropogenic disturbances, such as agriculture, urbanization, aquifer draining, and contamination. Due to these threats on vulnerable spring habitats across the Southern Appalachians, many genetically divergent, endemic lineages could be extirpated before they are even recognized. This may be the case for the Brownback Salamander (*Eurycea aquatica*; Fig. 1), a strictly spring-dwelling, lungless salamander that is found in the Ridge and Valley (RV) and Appalachian Plateau (AP) Physiographic Provinces of Alabama and Georgia [1]. Lungless salamanders (family Plethodontidae) possess widespread morphological conservatism, and in many cases species boundaries have only recently been determined by molecular techniques, as with *E. aquatica*. Since its formal description in 1963 [1], it has faced a tumultuous taxonomic history, and recently was confirmed as a distinct evolutionary lineage using sequence data [2,3]. Molecular analysis of populations of *E. aquatica* from springs of Alabama and Georgia reveal not just one but three distinct lineages within this taxon [3]. Furthermore, preliminary analysis of samples collected from 10 different localities in southern and eastern Tennessee reveals additional (>2) genetically distinct *E. aquatica*-type (undescribed) animals [4]. This warrants the need to extensively sample limestone springs in the RV and AP of Tennessee and adjacent states to uncover additional cryptic lineages/species. Given the large degree of genetic diversity within *E. aquatica*, their preference to inhabit a highly restricted and threatened habitat, and the recognized decline in abundance/populations of other cohabitating spring endemics, state agencies and researchers will benefit from a complete phylogeny of *E. aquatica* as a means of identifying genetically divergent populations that may necessitate additional conservation protection.



**Fig. 1: Male Brownback Salamander collected from St. Clair Co., Alabama**

**Budget Overview:** Awarded funds for this project would finance both field collection and molecular analysis. Three hundred dollars for field collection (e.g., transportation, camping fees) would allow more extensive sampling of springs in central and eastern Tennessee and adjacent states. I have already sufficiently collected tissues in Alabama and Georgia and published my findings in *Molecular Phylogenetics and Evolution* [3]. The molecular analysis portion of this research project requires \$700 for the reagents and fees required to perform DNA extraction, amplification, and sequencing of at least two genetic (one mitochondrial, one nuclear) markers.

**Timeline of Work and Dissemination of Results:** Field collection will take place late Fall 2010 to early Spring 2011, when animals are found in the largest numbers. Molecular techniques will be conducted during Spring 2011 in the Jockusch Lab at the University of Connecticut where all necessary resources and equipment for analyses are available. Sequence collection will be completed by Summer 2011. All voucher specimens and duplicate tissues of *E. aquatica* will be deposited at the American Museum of Natural History. Results of this study will be published in a peer-reviewed journal (e.g., *Molecular Phylogenetics and Evolution*, *Copeia*) and made available to interested nonprofit organizations and federal/state agencies. Additionally, this research will be presented at the 2011 Joint Meeting of Ichthyologists and Herpetologists and to other local audiences.

[1] Rose, F.L., Bush, F.M., 1963. A new species of *Eurycea* (Amphibia: Caudata) from the southeastern United States. *Tulane Stud. Zool.* 10, 121-128.

[2] Kozak, K.H., Blaine, R.A., Larson, A., 2006b. Gene lineages and eastern North American palaeodrainage basins: phylogeography and speciation in salamanders of the *Eurycea bislineata* species complex. *Mol. Ecol.* 15, 191-207.

[3] Timpe, E.K., Graham, S.P., Bonett, R.M. 2009. Phylogeography of the brownback salamander reveals patterns of local endemism in Southern Appalachian springs. *Molecular Phylogenetics and Evolution.* 52, 368-376.

[4] Timpe, E.K. and Graham, S.P. unpublished findings.