Final Report for 2012 Chopsticks for Salamanders Small Grant

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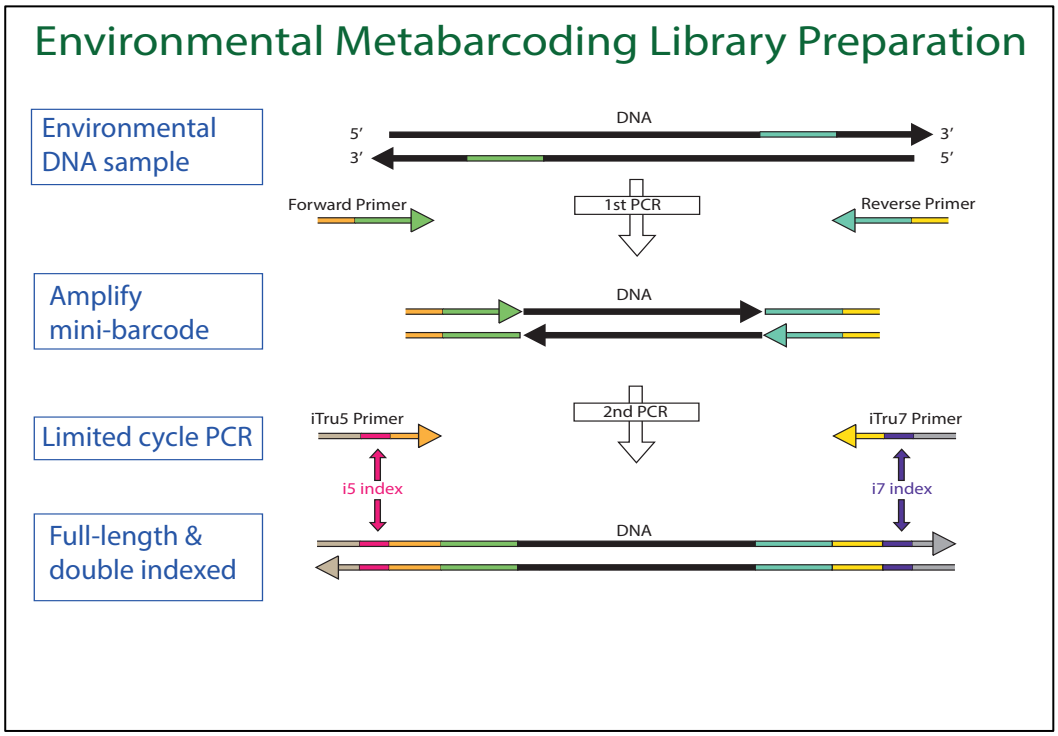
Objectives: We sought to design an environmental DNA (eDNA) assay capable of characterizing a diverse community of plethodontid salamanders in the Southern Appalachians. The use of eDNA to detect the presence of single species through a quantitative PCR assay has become a standard monitoring technique; however, even more promise is demonstrated by the use of universal primers and next-generation sequencing technologies to detect a suite of species.

Results and Report: Using publicly available genetic sequences on GenBank, we used the program ecoPrimers to design primers to amplify a short, highly-variable fragment of the mitochondrial 12S region. This region was chosen to minimize primer mismatches in target species and maximize variation in the amplicon; the end result is a region that is easily amplified from all plethodontids and capable of distinguishing between closely-related species (e.g. *Desmognathus quadramaculatus* and *D. folkertsi*). We designed a laboratory protocol for amplifying this mini-barcode, cleaning up the PCR product, adding unique tags for multiplexing with a second PCR, and pooling the samples for sequencing. A schematic of the technique can be seen below:

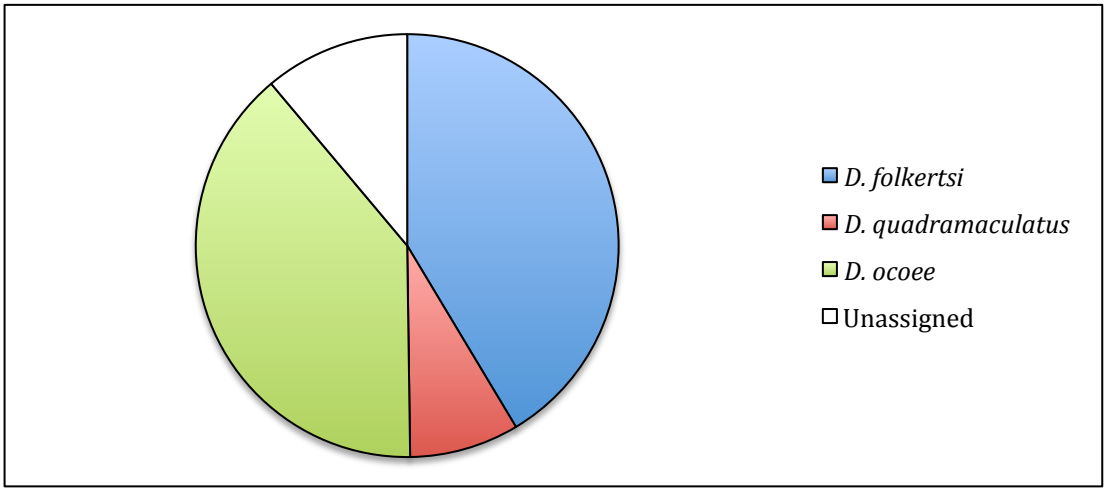


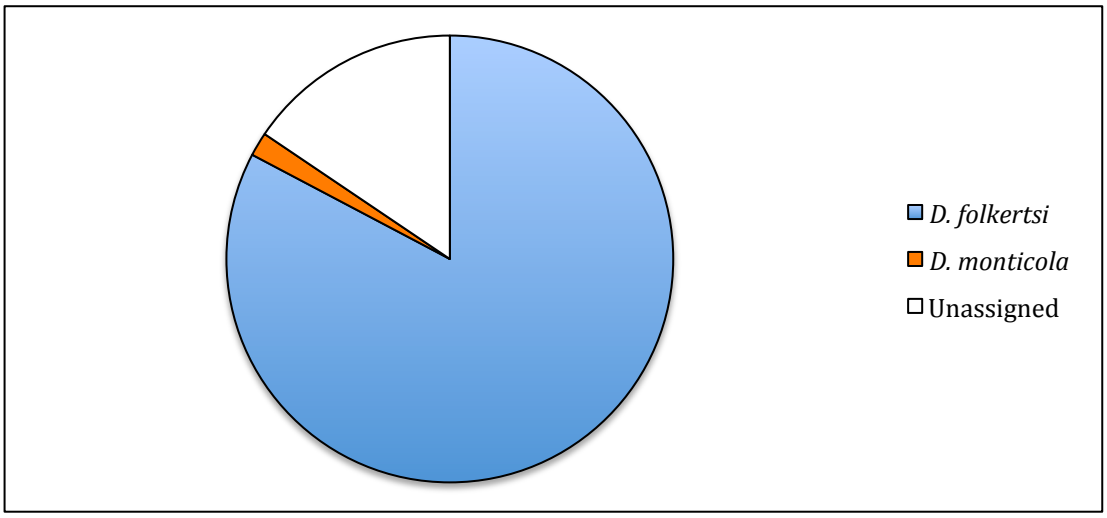
We visited three groups of localities (the upper Tugaloo Basin, the Wharton Conservation Center, and the Coweeta Basin) across the Southern Appalachians and collected a series of eDNA samples from headwater streams in each. Samples were filtered and extracted following the protocol of Goldberg *et al.* (2011).

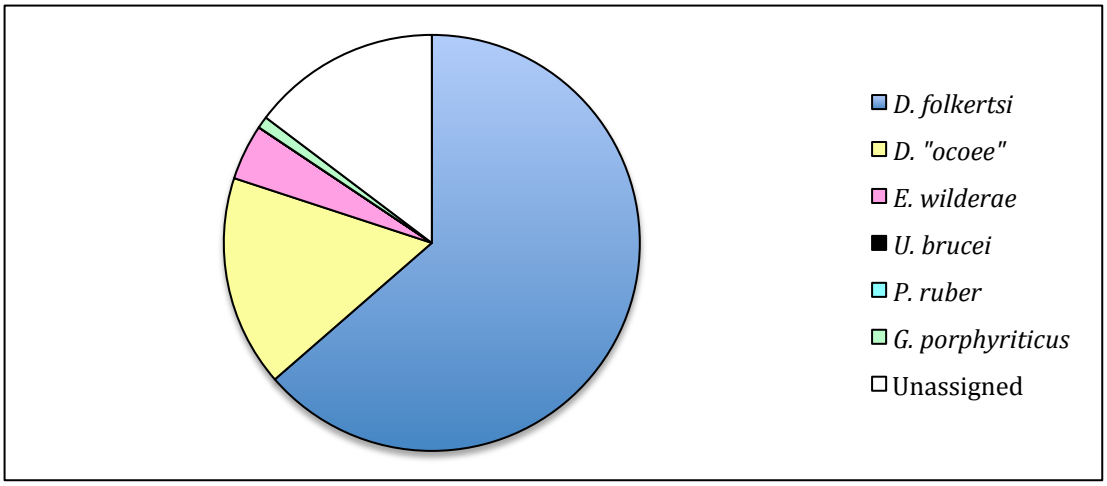
Additionally, we collected tissue samples from all aquatic and semi-aquatic plethodontids that inhabit the region. To build a reference library of sequences, we amplified our mini-barcode from DNA extracted from these tissues and sequenced the PCR product on an Illumina MiSeq.

To preliminarily test our assay on eDNA samples, we selected one sample each from three streams in the Upper Tugaloo Basin and amplified our mini-barcode. Samples were individually tagged, pooled, and sequenced for ~125,000 reads each on an Illumina MiSeq.

Of the ~125,000 reads recovered, approximately 27% originated from salamanders. Of these reads, almost 90% matched unambiguously with a sequence from our reference library. Between the three samples, we recovered DNA from all eight species that inhabit the streams: *Desmognathus quadramaculatus, D. folkertsi, D. monticola, D. ocoee* (two distinct haplotypes), *Eurycea wilderae, Urspelerpes brucei, Pseudotriton ruber,* and *Gyrinophilus porphyriticus*. The relative abundance of reads from each species in each sample can be visualized below.







The initial results demonstrated by our metabarcoding assay are incredibly promising, and we plan to further develop the assay. Future goals include:

1. Comparing estimates of relative abundance using eDNA and mark-recapture. These data have been collected from three sites at the Coweeta LTER and will soon be analyzed.
2. Examining seasonal and annual trends in eDNA results at a small series of sites.
3. Expanding our reference library to other regions of the United States.
4. Publishing our results.

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2 June 2014