

Transcriptome assembly, annotation, and comparative analysis for characterizing adaptive potential of the Streamside Salamander (Ambystoma barbouri) Miranda Gaupp, Joshua Hall, Carla Hurt Department of Biology, Tennessee Technological University

Introduction

- Habitat loss and climate change are some of the greatest threats to amphibian biodiversity
- The persistence of species in the face of climate change depends on underlying genetic variation that allows populations to adapt
- The transcriptome is the complete set of mRNA molecules expressed within a cell
- Transcriptome studies characterize protein-coding genes to detect genes and pathways involved in responses to environmental conditions and understand the molecular mechanism of specific physiological processes
- Through transcriptome sequencing, adaptively significant gene regions can be identified and population-level gene expression can be compared. This information can then be used to predict population responses to future environmental conditions





Fig. 1. Streamside Salamander Fig. 2. *A. barbouri* distribution and collection sites

Study species: *Ambystoma barbouri* (Fig. 1)

- Near threatened with declining populations
- Currently under review for updated status
- **Sample sites:** Represent the latitudinal expanse of the species' distribution (Fig. 2)

Overarching Goals

- 1. Create conservation genomic resources for downstream application
- 2. Quantify variation temperature-mediated gene expression to understand population level responses to environmental conditions











Split clutches into treatment groups; allow eggs to develop





Bioinformatic Pipeline

	2000
Determine genes identified within all analysis methods	
entify gene sets showing significant differences in expression between treatments; Ger Set Enrichment Analysis	ne
Identify genes correlated with temperature; Weighted gene co-expression network analysis	ANAL
Identify differentially expressed genes between treatments; DESeq2	
Estimate gene expression levels; RSEM	
Align samples to the created reference transcriptome; Bowtie	
Assign gene ontology terms, enzyme codes, and KEGG pathways; Blast2GO	ANNO
Identify likely coding sequences; TransDecoder	
Assess transcriptome completeness; BUSCO	
Obtain transcriptome assembly statistics; TransRate	
Remove redundant transcripts and produce unigenes; CD-HIT	MBLY
Identify and remove sequences from sources of contamination; Kraken2	ASSE
De novo transcriptome assembly; Trinity	
Quality filter, trim, and correct random sequencing errors; FastP, Rcorrector	

State	County	Site	10°C	20°C	Total
Ohio	Preble	Hueston Woods	6	6	12
Kentucky	Scott	J. Whitfield	6	6	12
	Boone	Beaver Road	6	6	12
Tennessee	Wilson	Fair Grounds	9	9	18
	Wilson	Williams Farm	6	6	12
	Rutherford	Leconte Court	7	7	14





Library Prep

Sequence

Results

Species A. barbouri average Plethodon cinereus Salamandra salamandra Hyla sarda Bombina pachypus Table 2. Assembly statistics a individual assemblies and pu

A. barbouri de novo referenc Average of individual assemble Minimum of individual asser

Maximum of individual asse Table 3. Busco scores to assess the completeness of transcriptome assemblies

Discussion and Next Steps

- - bioinformatic protocol
 - annotation and analysis

Management applications

- variation

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Total transcripts	Mean length	N50	GC content (%)		
162358	810	1740	0.46		
117812	1084	2345			
1146571		1529	44.70		
1295741		914	44.00		
896992	616	1082	39.87		
generated by TransRate for the averaged A. barbouri ublished assemblies of other amphibian species					
Complete (%) Fragmented (%) Missing (%)					
93	8	2	4 2		

		Taginenieu (70)	WIISSING (70)
e	93.8	2	4.2
blies	67.4	7.4	25.2
mblies	43.2	4.4	13.3
mblies	82.2	12	45.4

• Assemblies possess quality similar to those of related species in published literature (Table 2) • Suggests successful wet lab, sequencing, and • Provides confidence in moving forward with • Due to the individual assembly average Busco completeness scores, data from individuals in the same clutch-treatment group should be merged to provide more complete transcriptome assemblies

• Monitor adaptation to climate change and assess ability of populations to acclimate to future conditions • Guide temperature-related land management actions • Prioritize populations with valuable adaptive genetic

• Provide insight into population response to future climatic conditions and assess long-term viability of populations