eDNA Surveillance and Genomic Characterization of the Threatened Trispot Darter (*Etheostoma trisella*)

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[3] Georgia Department of Natural Resources

[4]Tennessee Aquarium Conservation Institute

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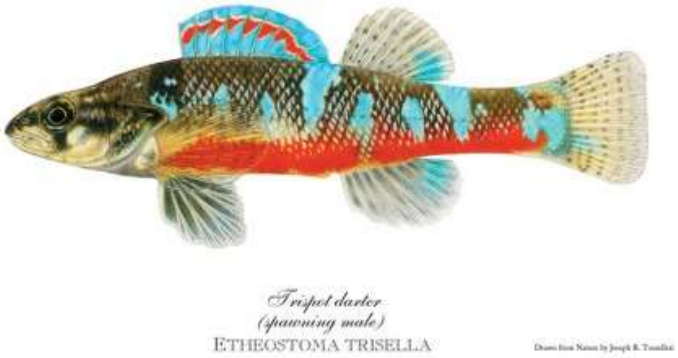
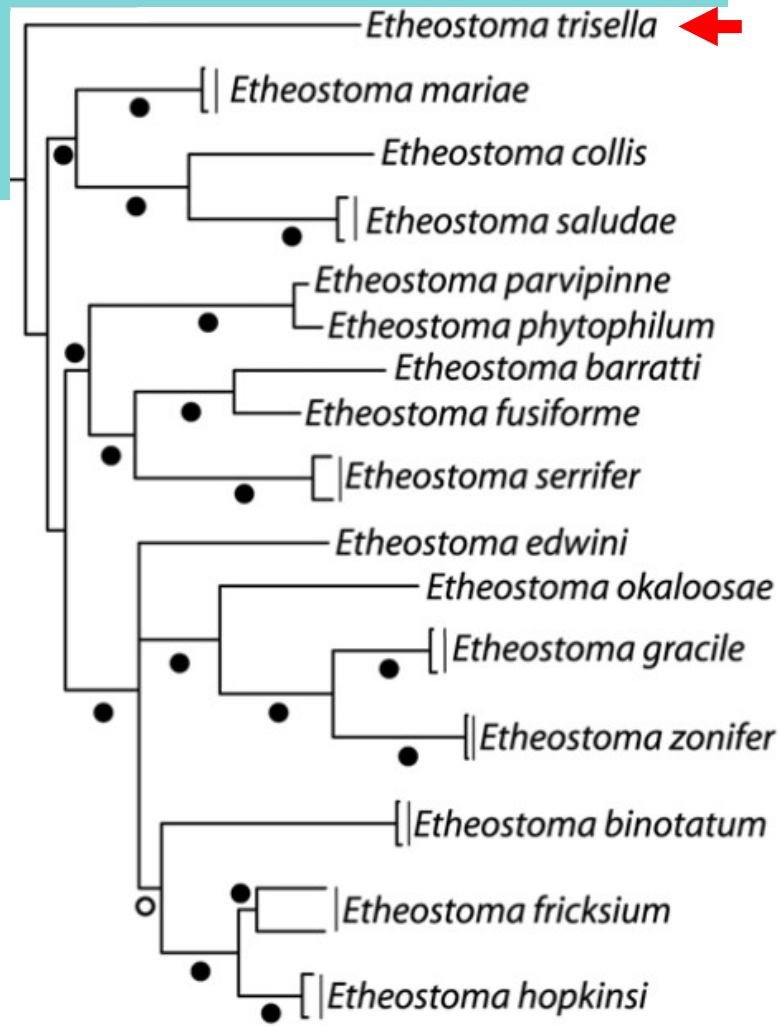
Background

- *Etheostoma trisella*
  - Trispot Darter
    - Order: Perciformes
    - Family: Percidae

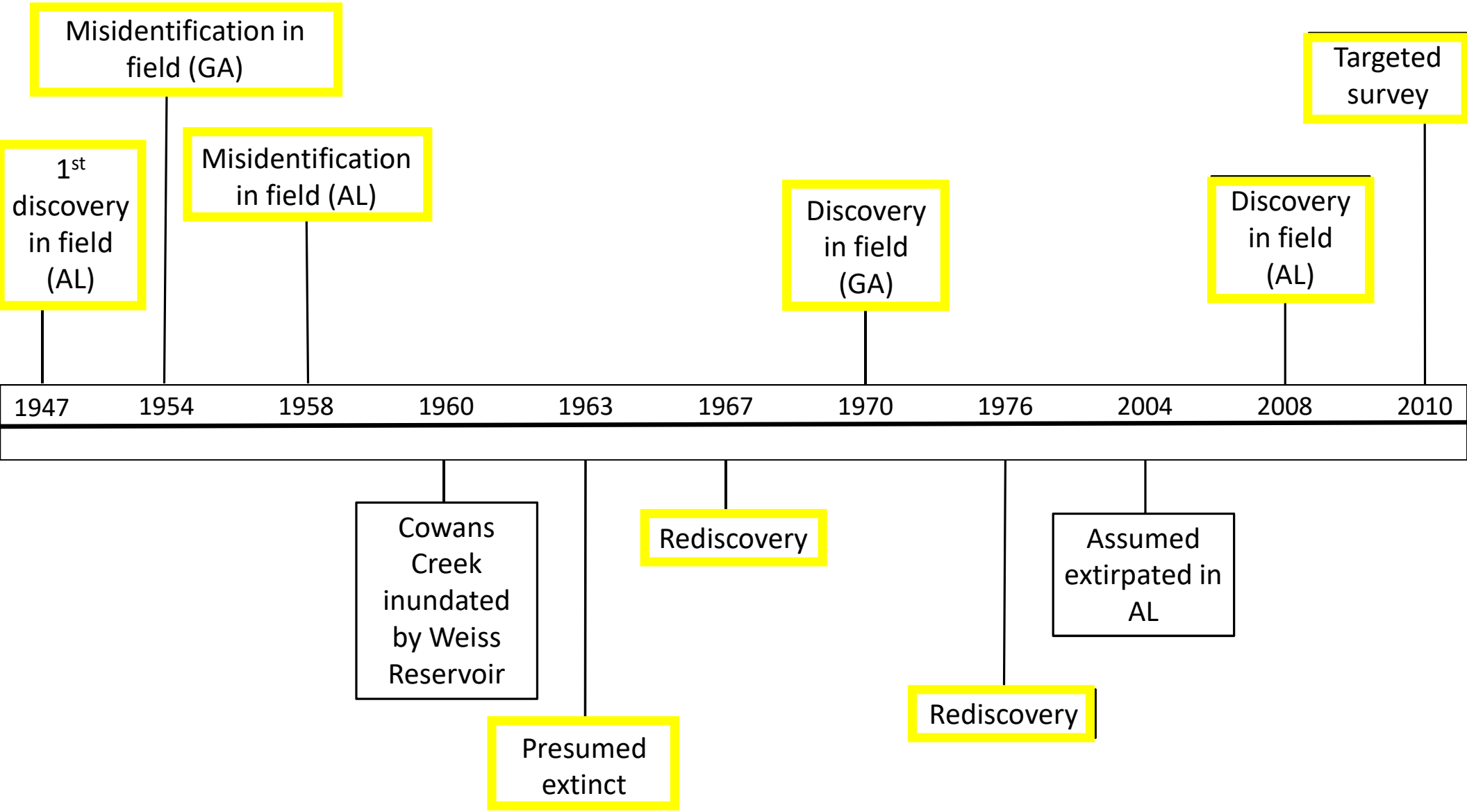




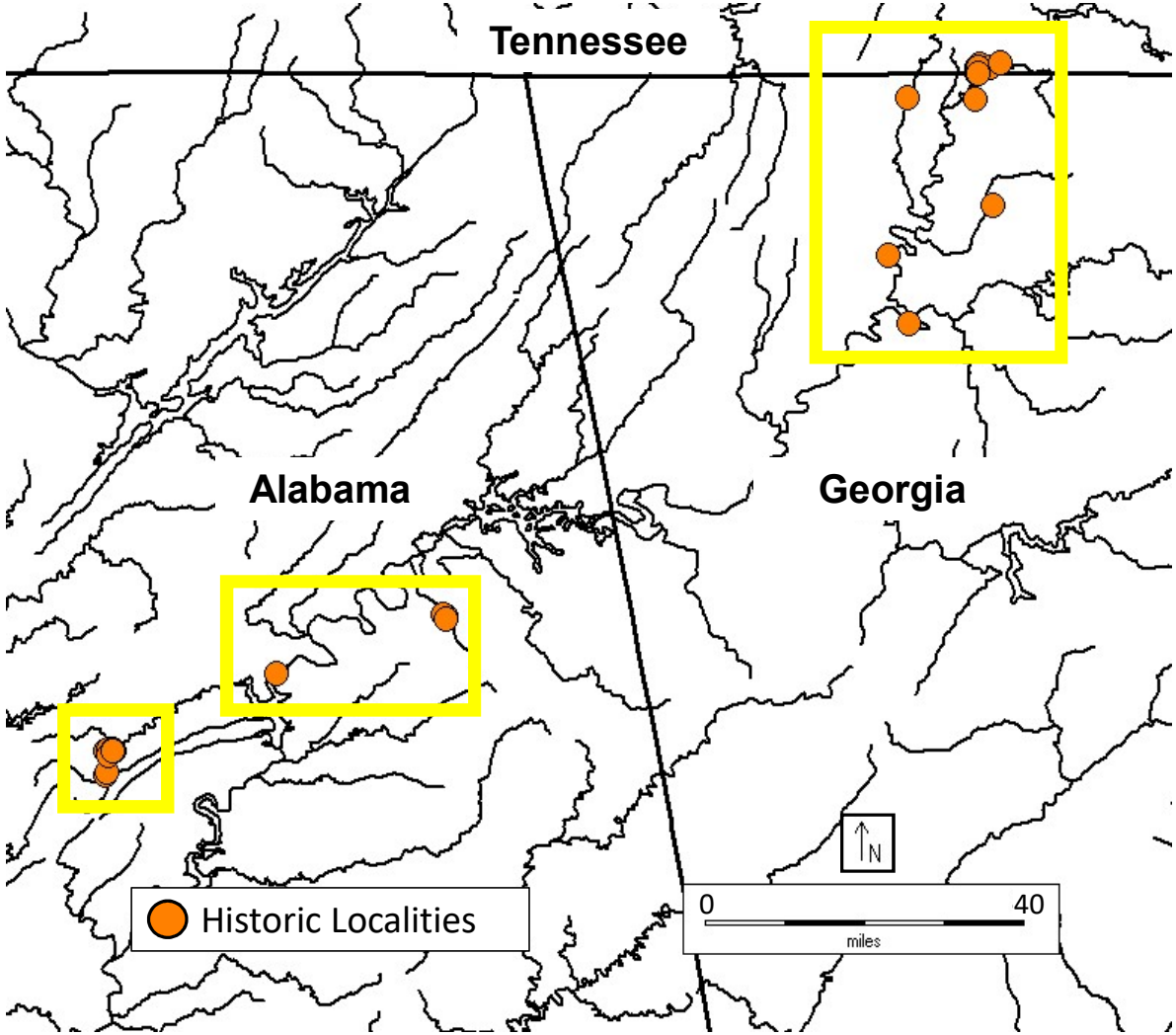
Near et al. 2011

Life History

- Non-breeding habitat
  - Mid-April to Mid-October
  - Slack water
    - Detritus, logs, sticks, beds of water-willow
- Breeding habitat
  - Late-November to Late-April
  - Rainfall lifts darters to spawning grounds
  - E.g., Seepage waters of pastures, flood plains


1st discovery in field (AL)
Misidentification in field (GA)
Misidentification in field (AL)
Discovery in field (GA)
Discovery in field (AL)
Targeted survey
1947
1954
1958
1960
1963
1967
1970
1976
2004
2008
2010
Cowans Creek inundated by Weiss Reservoir
Presumed extinct
Rediscovery
Rediscovery
Assumed extirpated in AL

Previous Distribution



Background

- Reservoirs
  - Weiss Reservoir on Cowans Creek
  - Neely Henry Reservoir on the Coosa River
- Recently listed as a threatened species under the Endangered Species Act

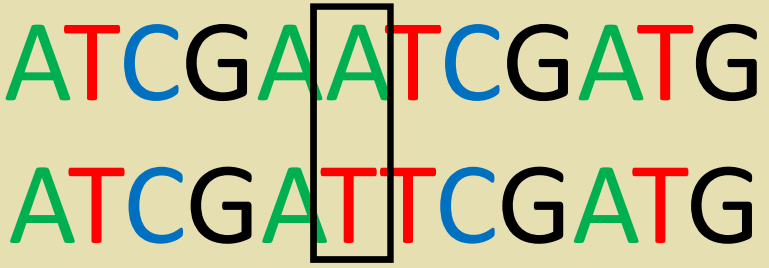
Objectives

1. Describe population genomic diversity in this newly protected species
2. Develop a minimally invasive protocol for occupancy monitoring using environmental DNA

Objective 1

Methods-Population Genomics

- DNA extracted from museum voucher specimens (n = 94)
  - Alabama, Georgia, and Tennessee
- Cytochrome oxidase I (COI)
  - 1 individual shows 0.5% divergence from other identical individuals (n = 8)
- DArTSeq
  - Reduced representation genome sequencing using enzyme digests
  - Product: 20,673 single nucleotide polymorphisms (SNPs)
  - Filtering:
    - Remove monomorphs: 20,669 SNPs
    - Allow 5.0% missing data: 10,497 SNPs
    - Remove secondaries: 9,732 SNPs



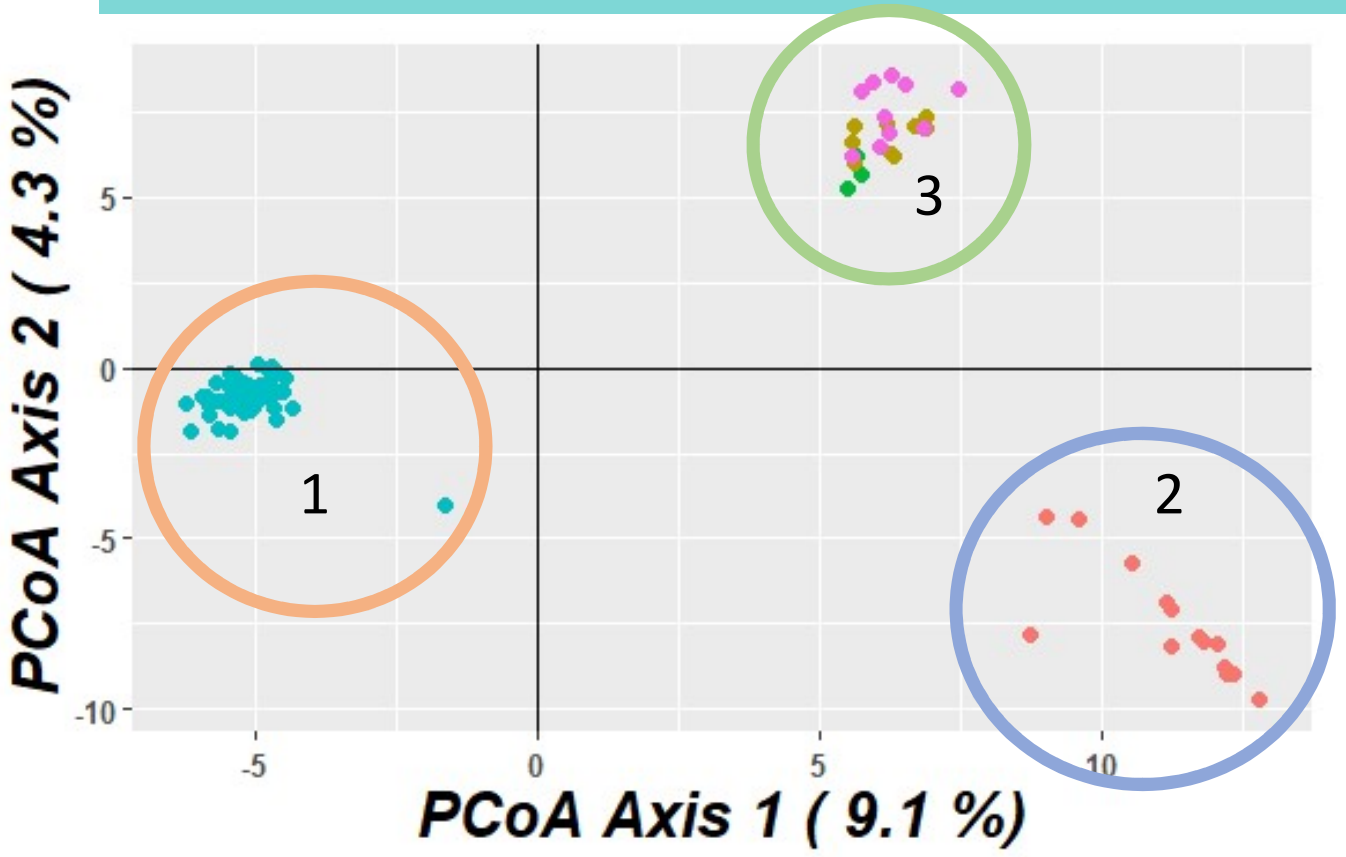
Objective 1

Methods-Population Genomics

- Principle Coordinate Analysis (PCoA)
  - dartR
- Bayesian Phylogeny
  - BEAST
- Ancestry matrix
  - fastSTRUCTURE in LEA
- Heterozygosity, AMOVA, F_{ST}
  - Arlequin
- Estimated population size
  - NeEstimator

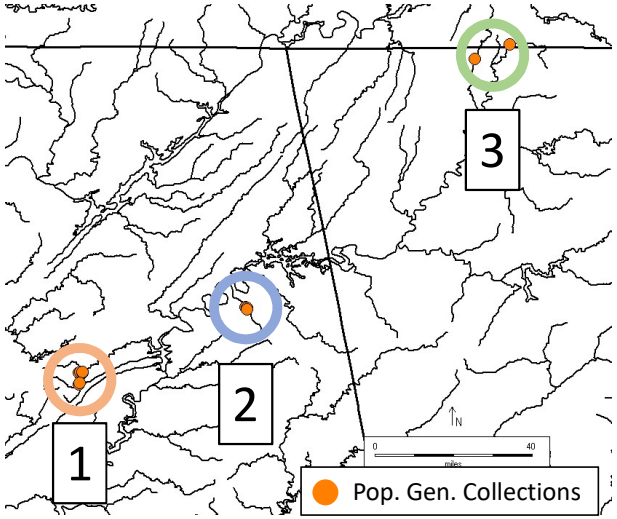
Objective 1

Results-Population Genomics



Three genetic populations

1. Little Canoe Creek
2. Ballplay Creek
3. Mill Creek, Coosawattee, and Coahulla Creek



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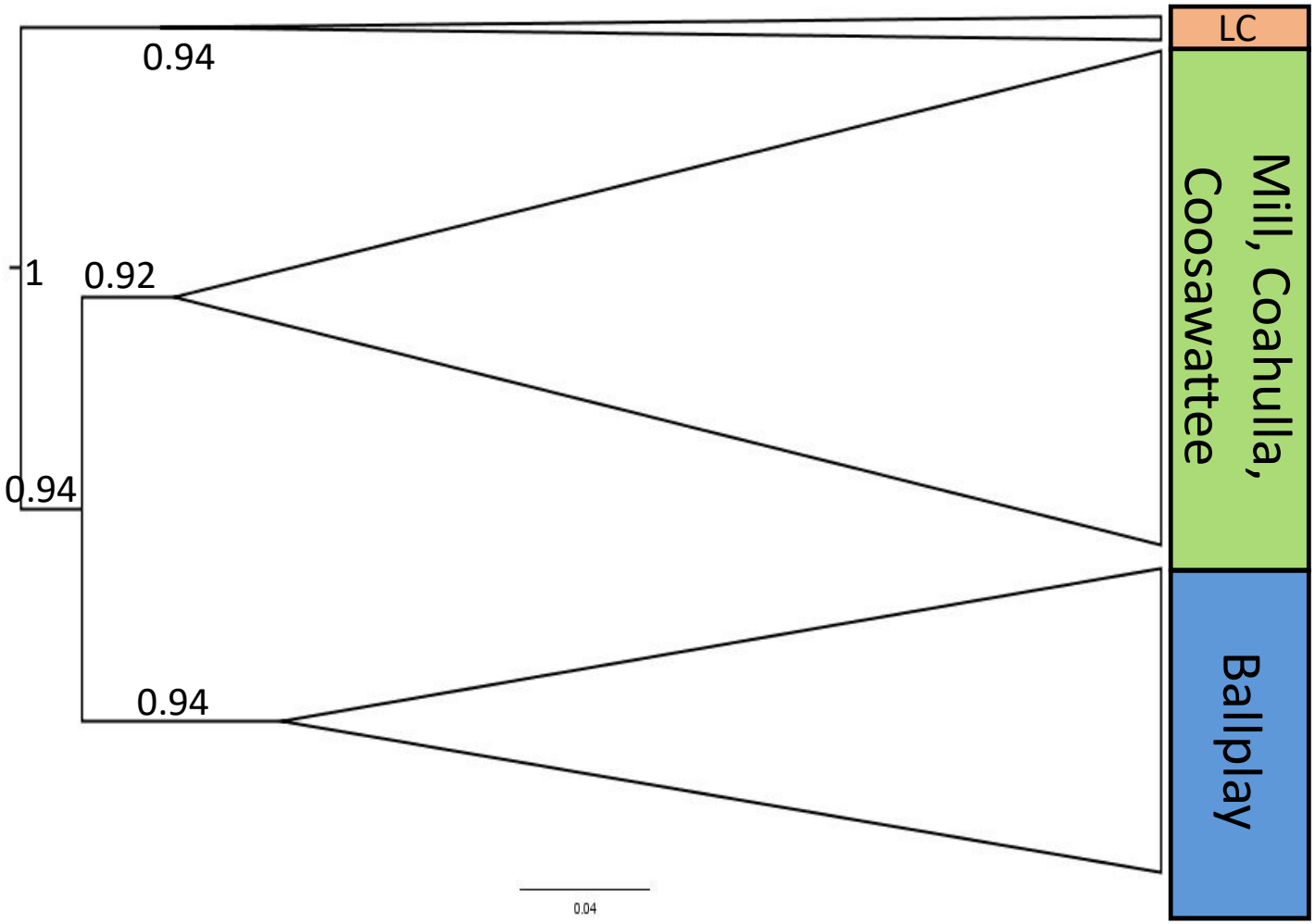
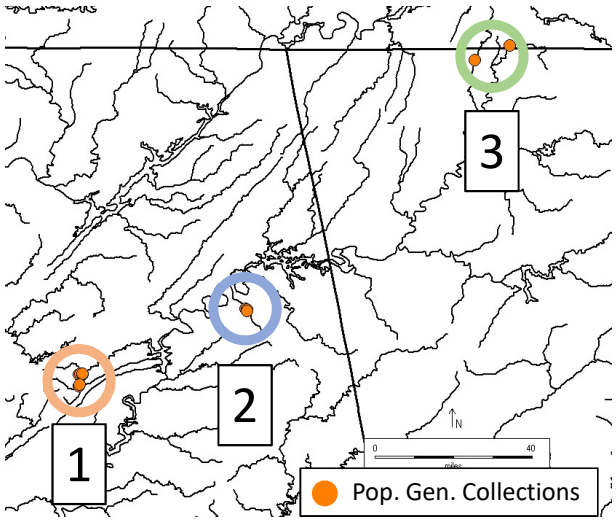


Bayesian Phylogeny

- BEAST



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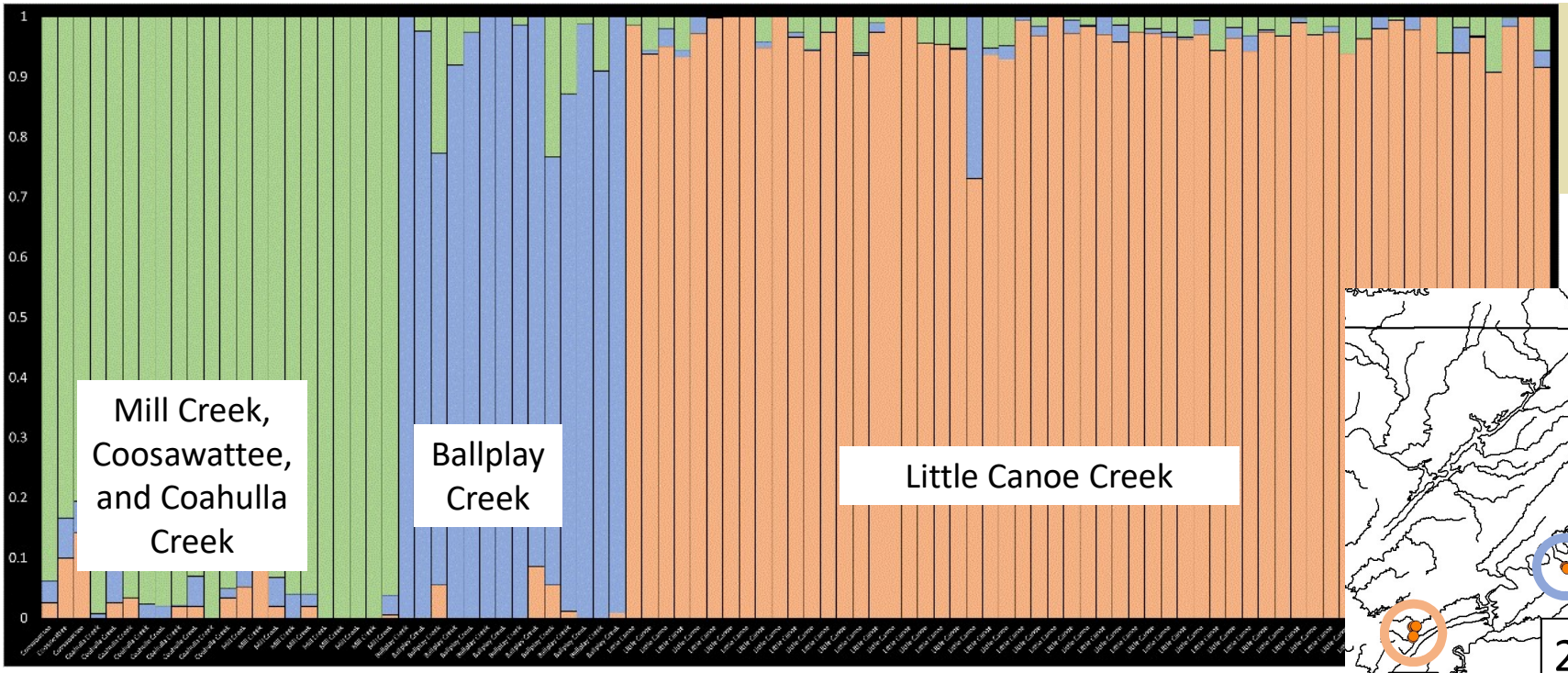
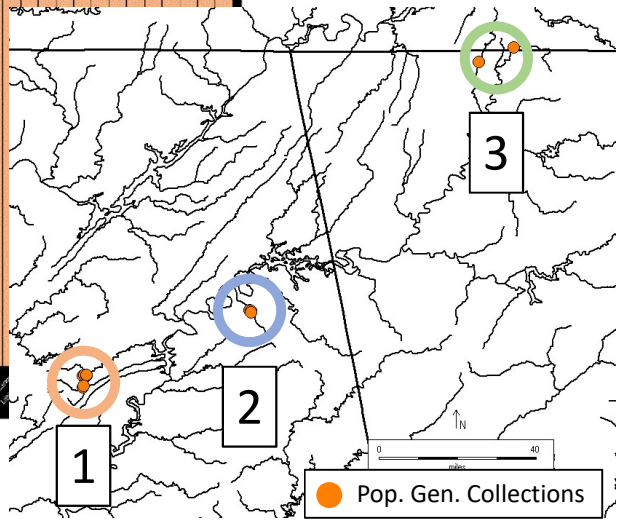
Results-Population Genomics



Ancestry Matrix
fastSTRUCTURE (LEA)
K = 3
CE = 0.47



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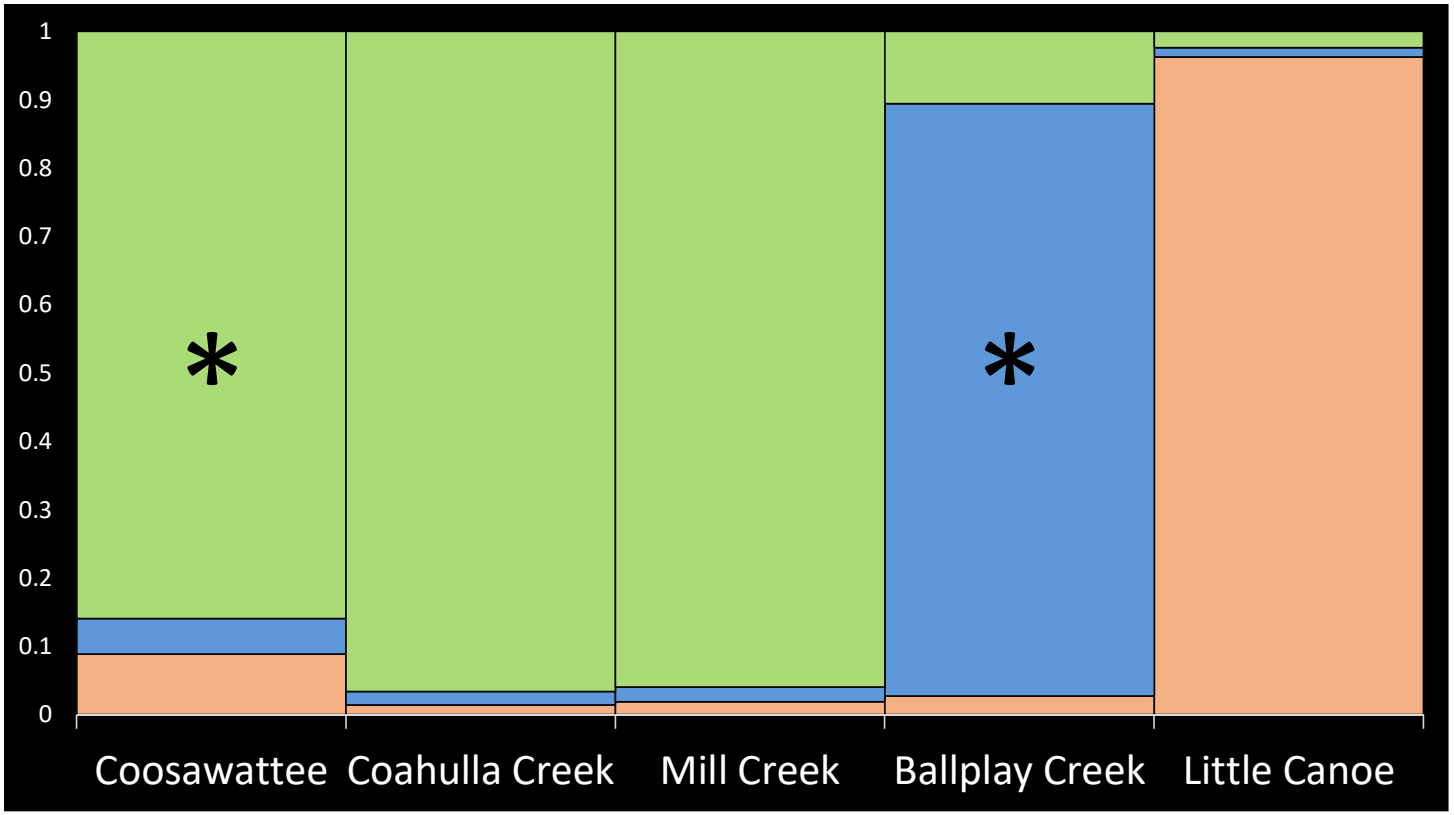
Results-Population Genomics



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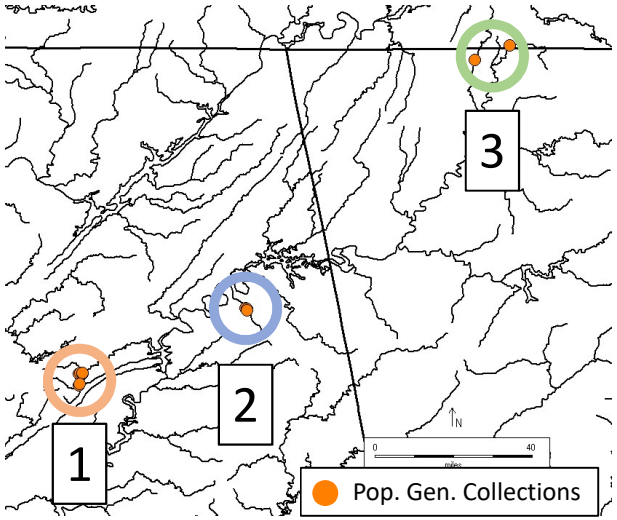

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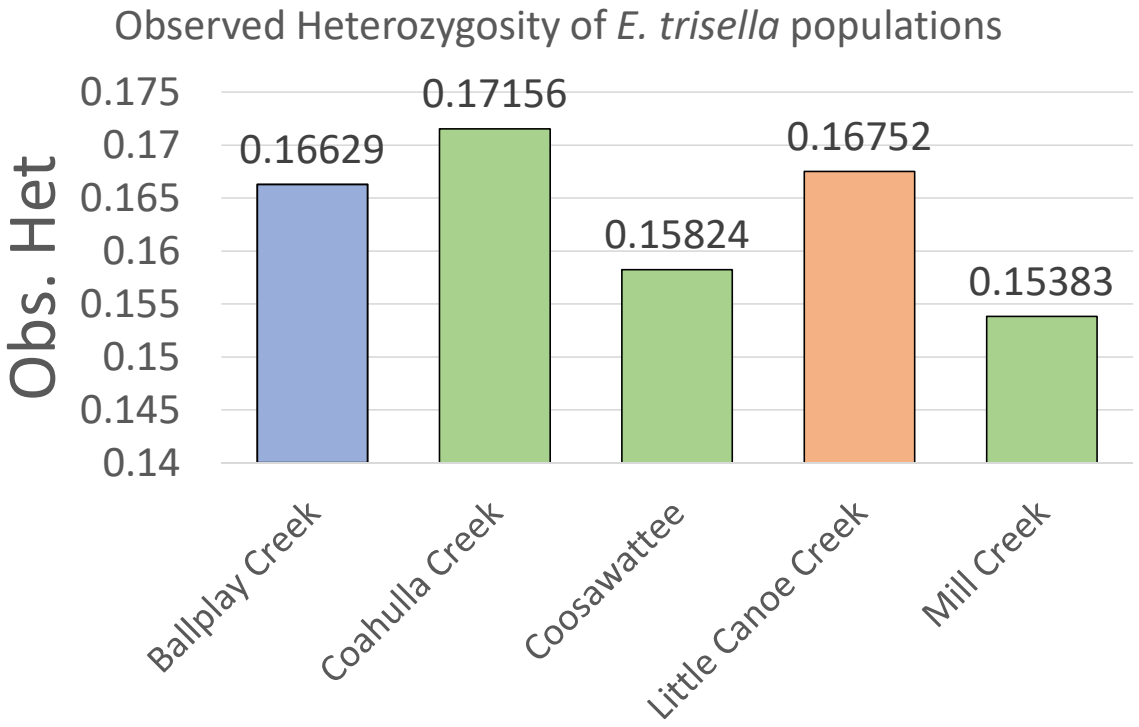
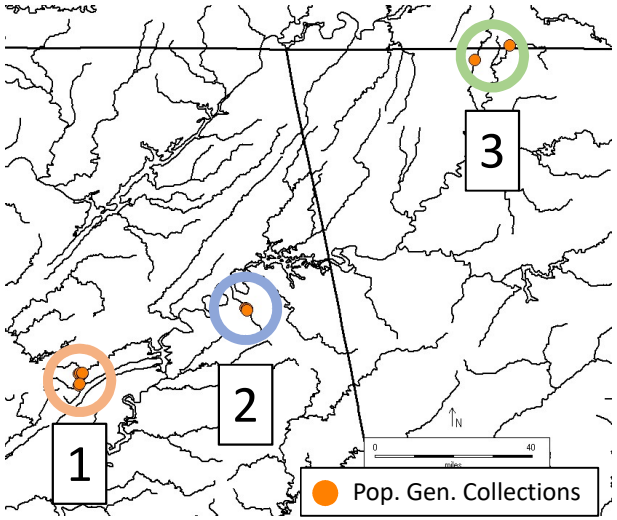
Results-Population Genomics





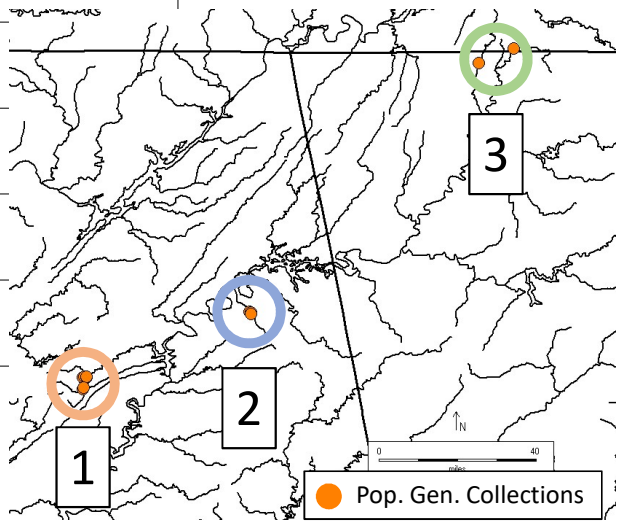
Objective 1

Results-Population Genomics

Population pairwise FSTs and AMOVA

	Ballplay Creek	Coahulla Creek	Coosawattee	Little Canoe	Mill Creek
Ballplay Creek	0				
Coahulla Creek	0.11669	0			
Coosawattee	0.12925	0.05985	0		
Little Canoe	0.14149	0.10074	0.10634	0	
Mill Creek	0.12193	0.05273	0.06617	0.10304	

FST: 0.11529

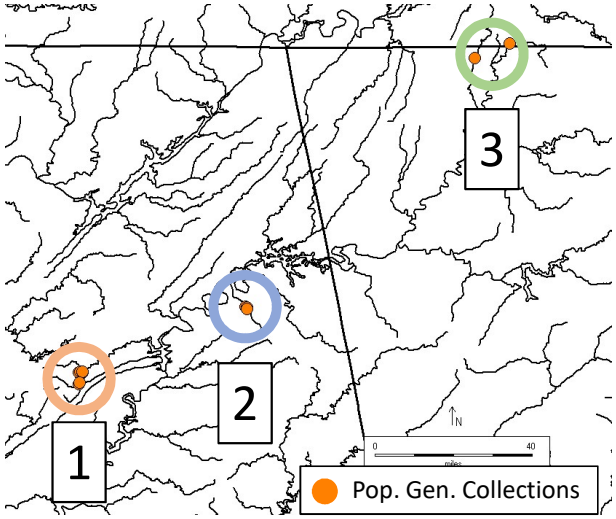


Objective 1

Results-Population Genomics

Effective Population Size Estimates (N_e)

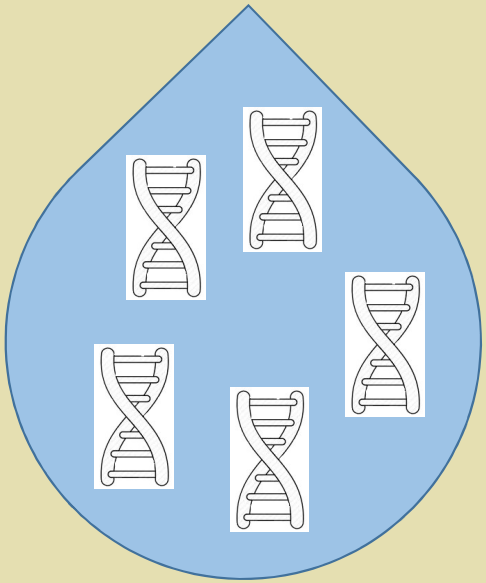
	Estimated Ne	n
Little Canoe	232.5	57
Coahulla Creek	∞	9
Mill Creek	∞	10
Ballplay Creek	33.1	14
Coosawattee	∞	3



Objective 2

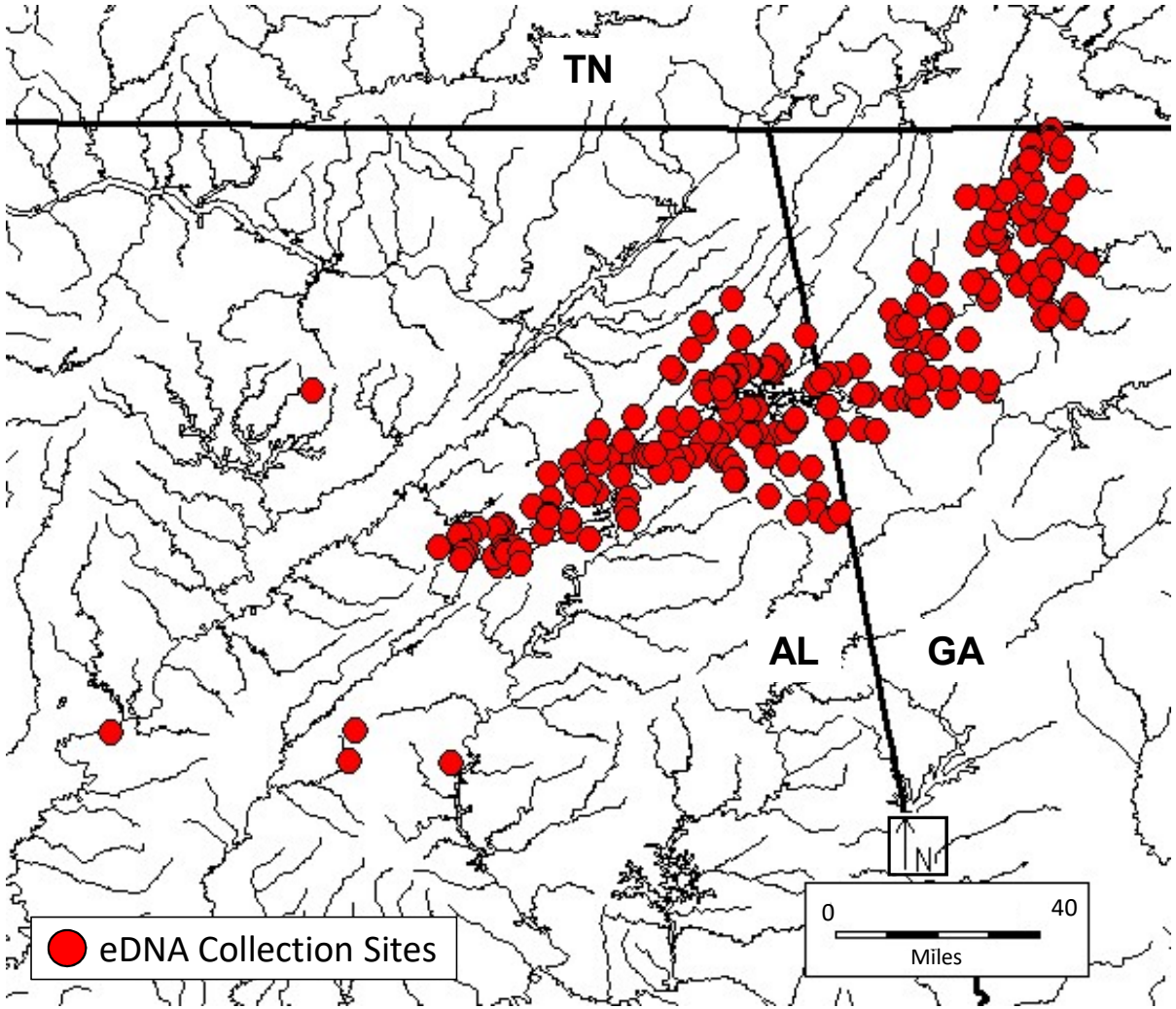
Methods-Surveillance

- Environmental DNA (eDNA)
  - Genetic material extracted from environmental samples
- Water collections
  - Geological Survey of Alabama
  - Georgia Department of Natural Resources
    - January 22- March 18, 2019
    - 136 collection sites
    - 17 watersheds
      - Experimental: 16
      - Negative control: 1



Objective 2

2019 Water Collections

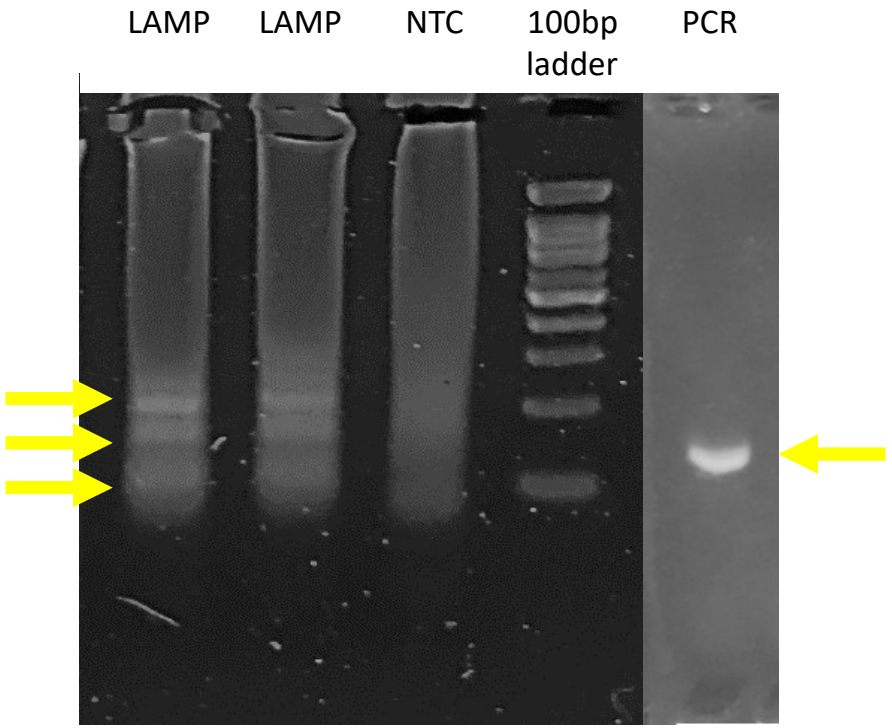


n = 136

Objective 2

Methods-Surveillance

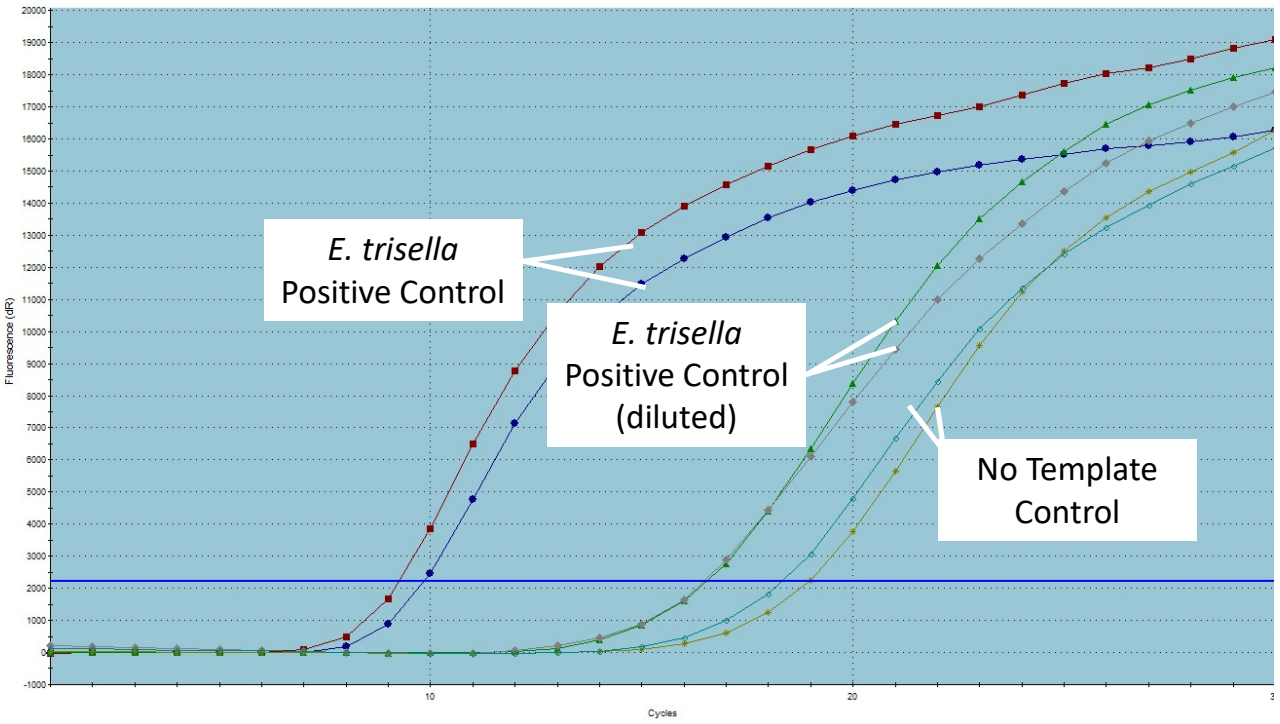
- Water → DNA
  - Water filtration
  - Modified Qiagen DNeasy extraction using the QIAshredder
- LAMP (loop-mediated isothermal amplification)
  - Primers designed specifically for trispot darter



Objective 2

Methods-Surveillance

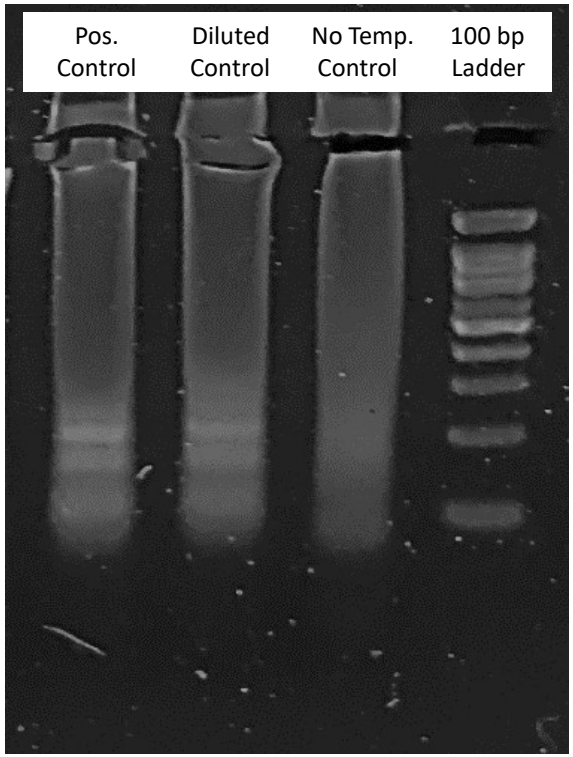
- Stratagene Mx3000P
- Triplicate
- Positive Controls
- Negative Controls



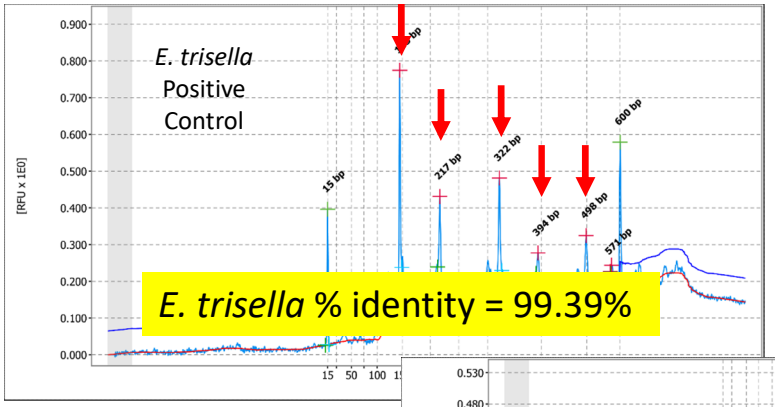
Objective 2

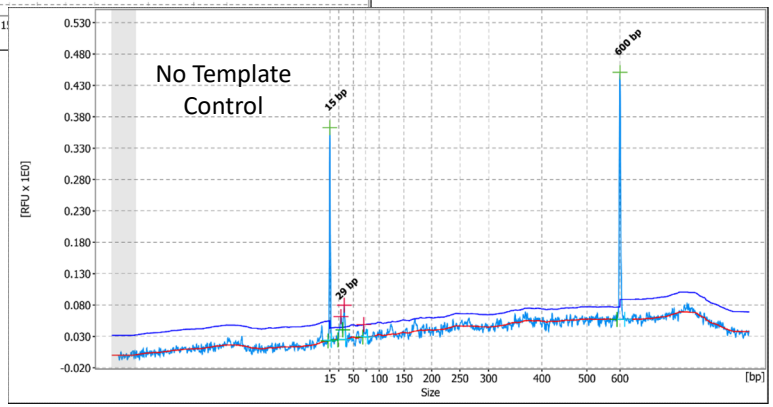
Methods-Surveillance

Gel electrophoresis



QIAxcel (Quantitative electrophoresis)



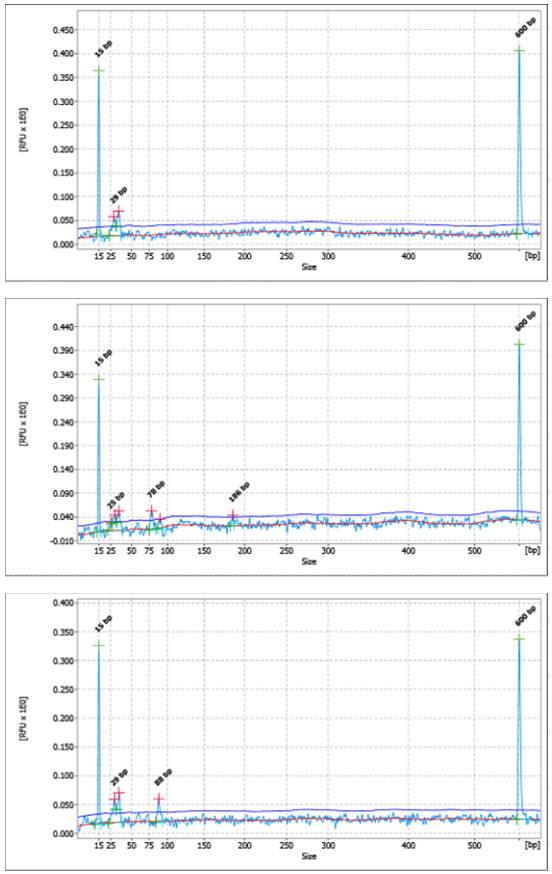


Objective 2

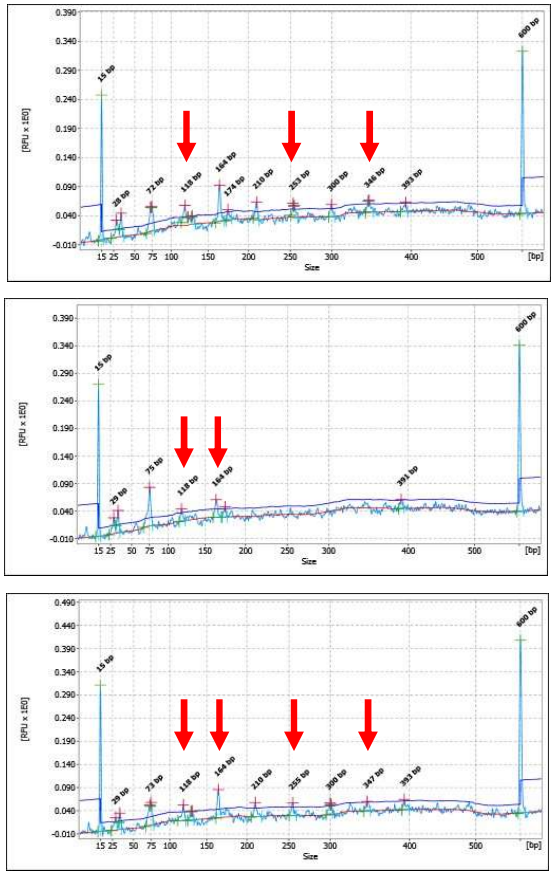
Methods-Surveillance

- QIAxcel
- Standards for positive status
  - Match at least 3 out 5 segments recovered from positive control
  - 116, 182, 254, 330, and 422bp
  - Majority of replicates must be positive

Negative eDNA



Positive eDNA



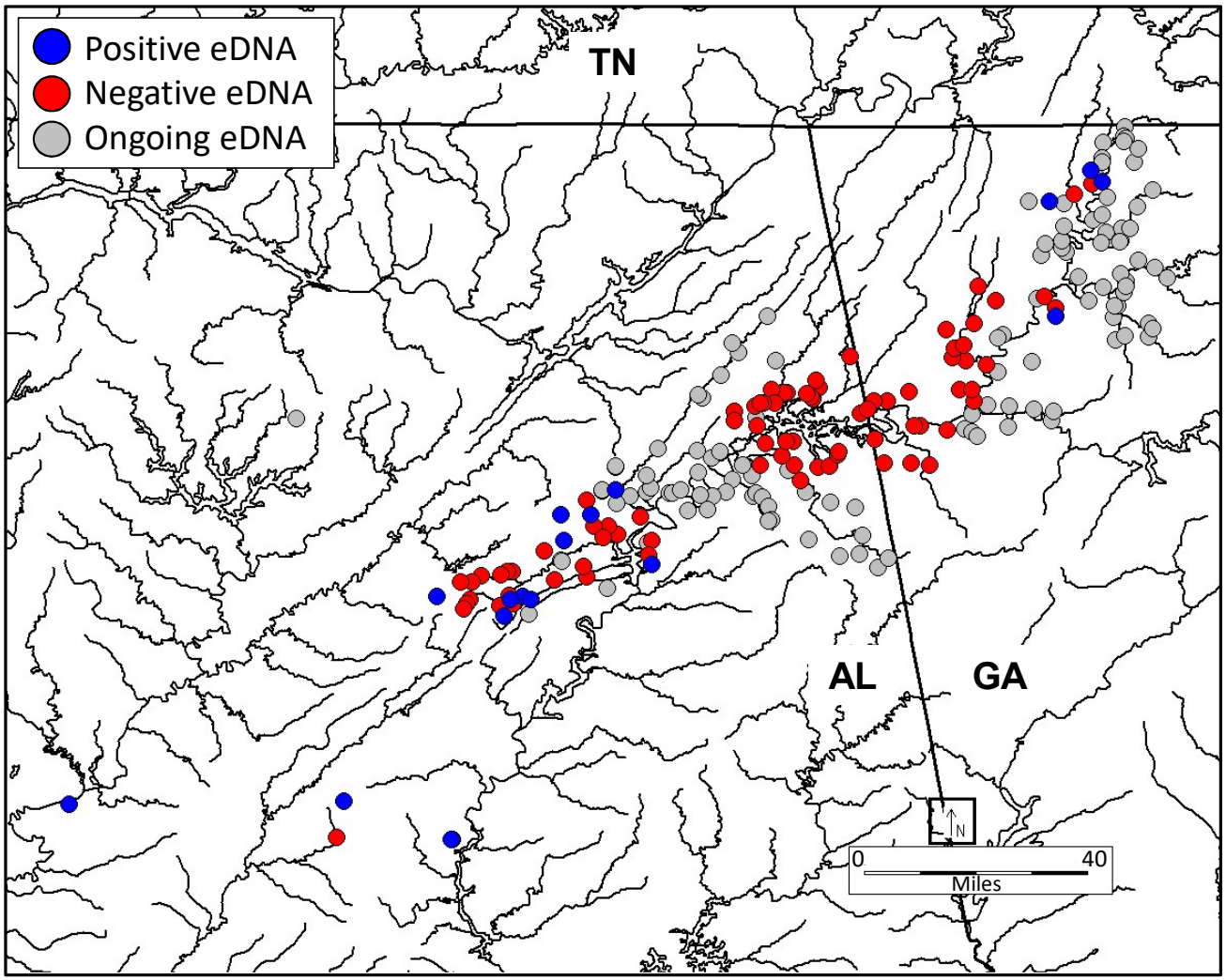
Objective 2

Results-Surveillance

- eDNA extracted from all ~250 water samples
- 155 water samples screened for *E. trisella* presence using LAMP
  - 22 positive sites
  - 93 negative sites

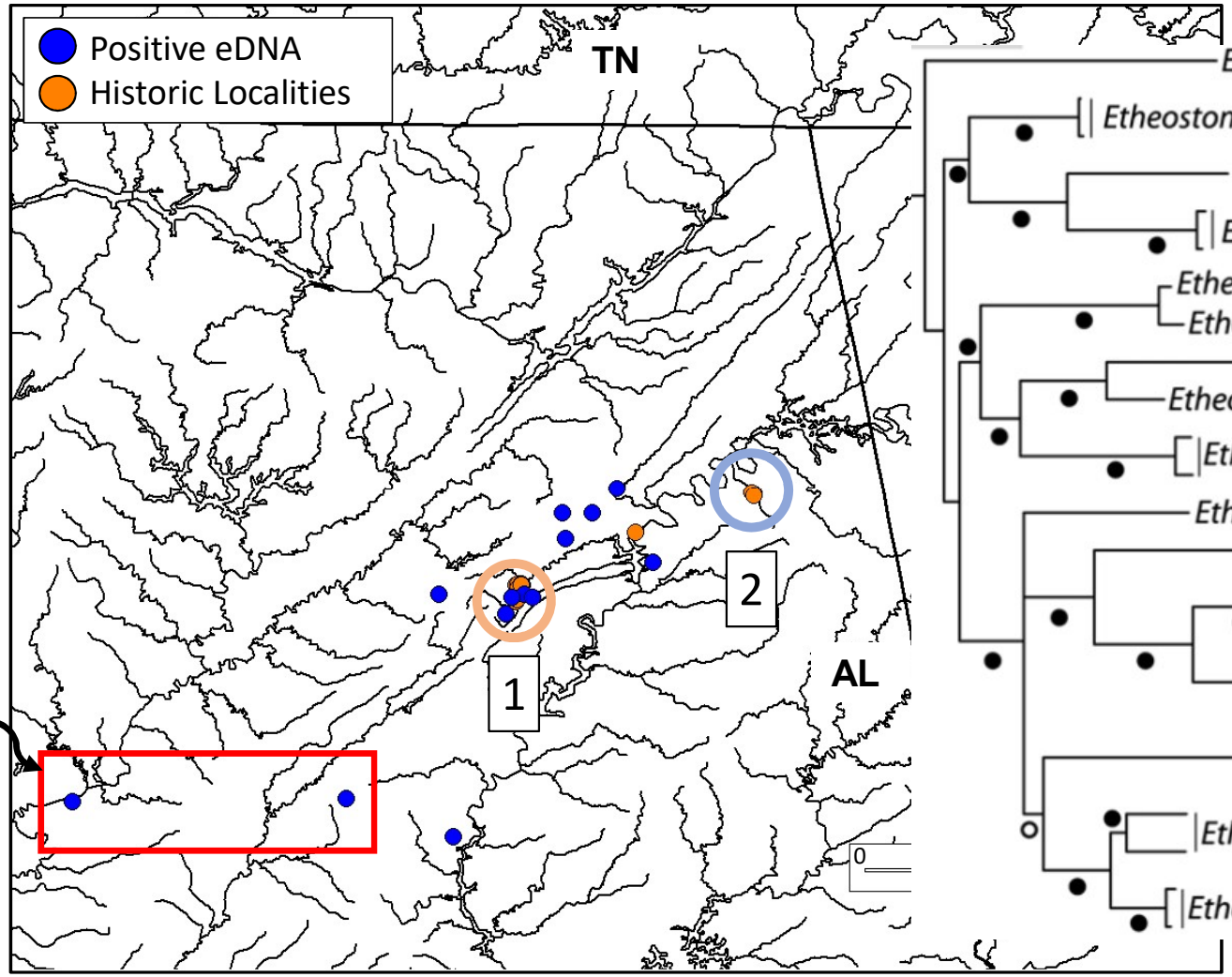
Objective 2

E. trisella Status by Site



Objective 2

Comparison of *E. trisella* Positive and Historic Sites

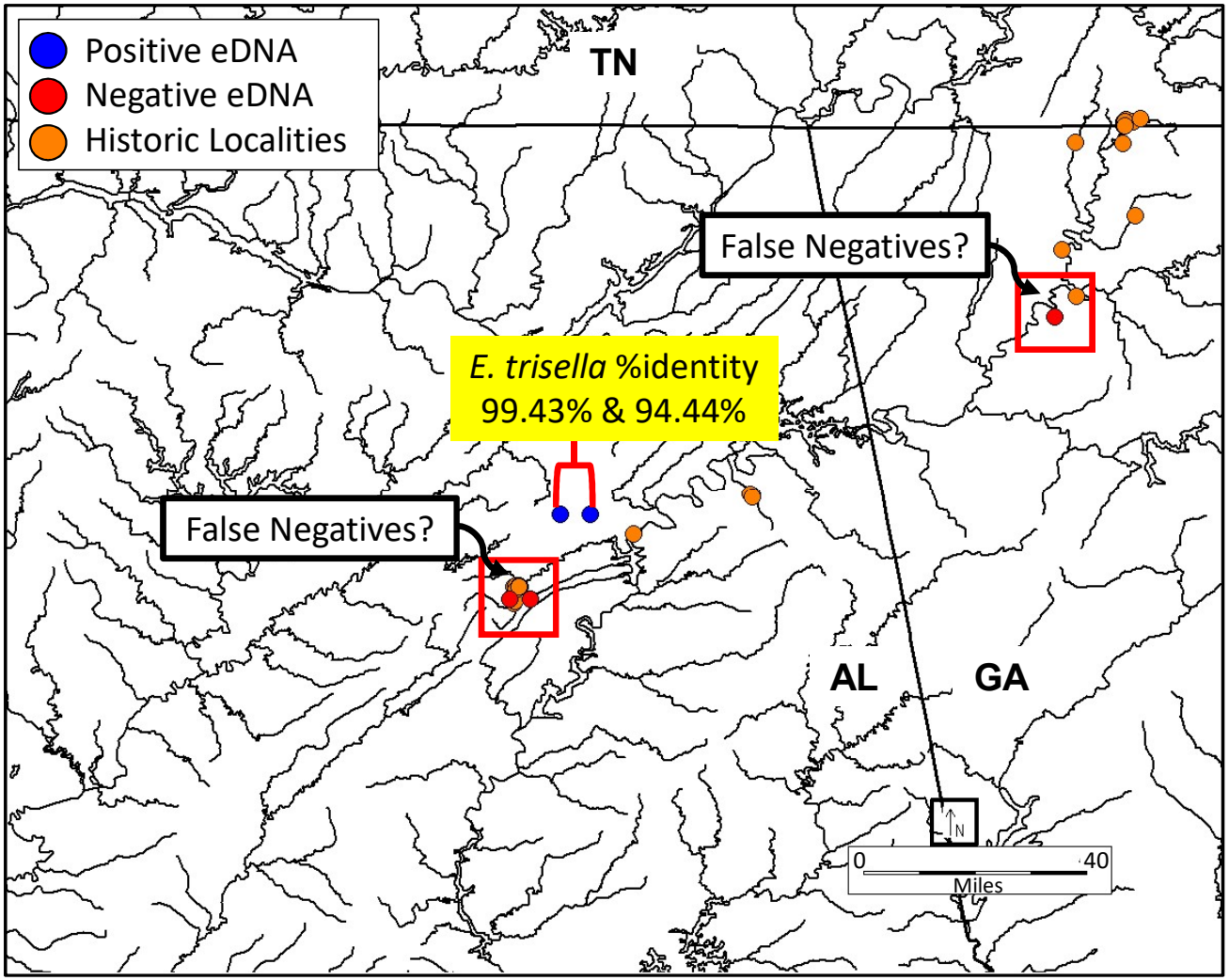


False Positives?

Near et al. 2011

Objective 2

LAMP Validation via PCR



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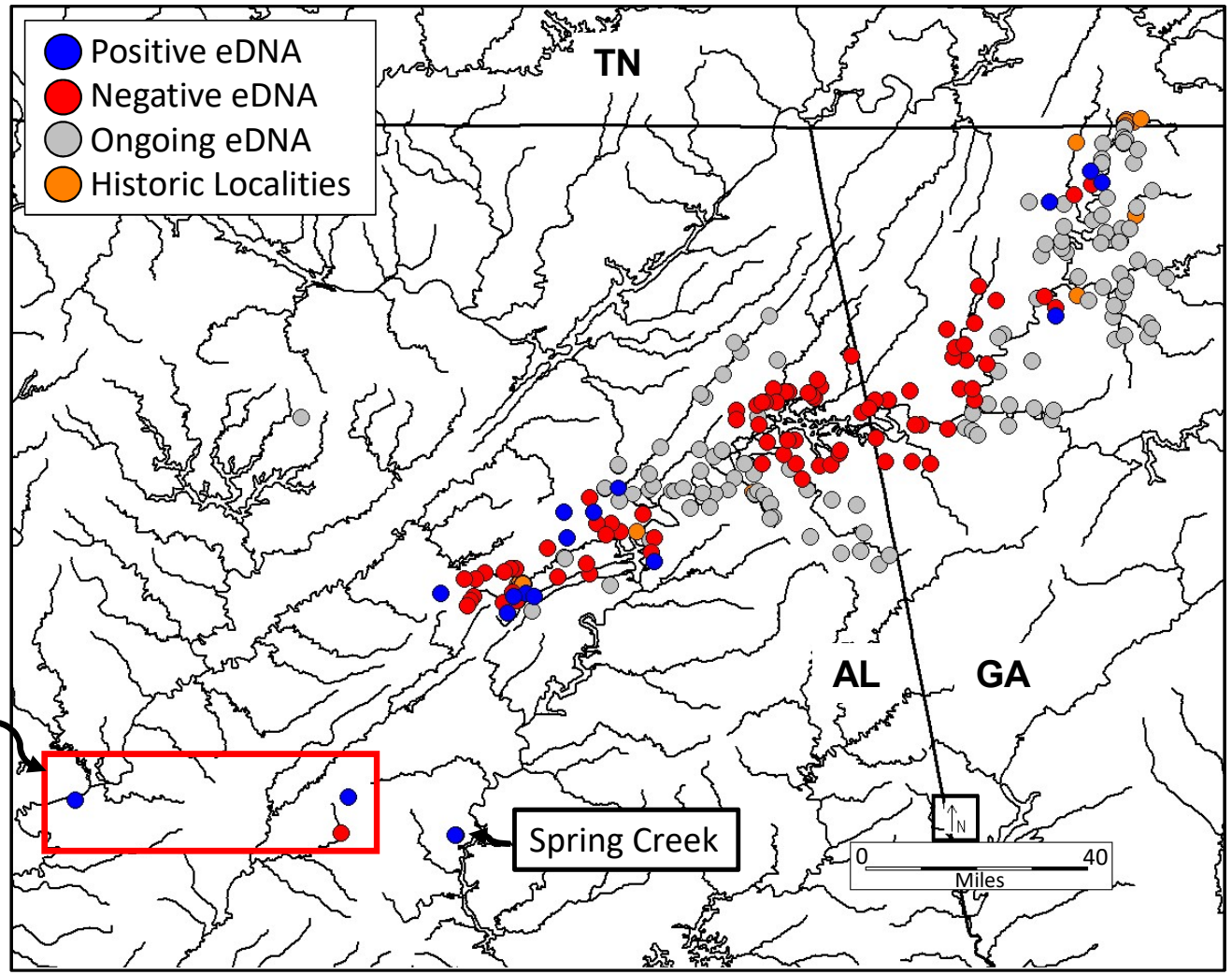
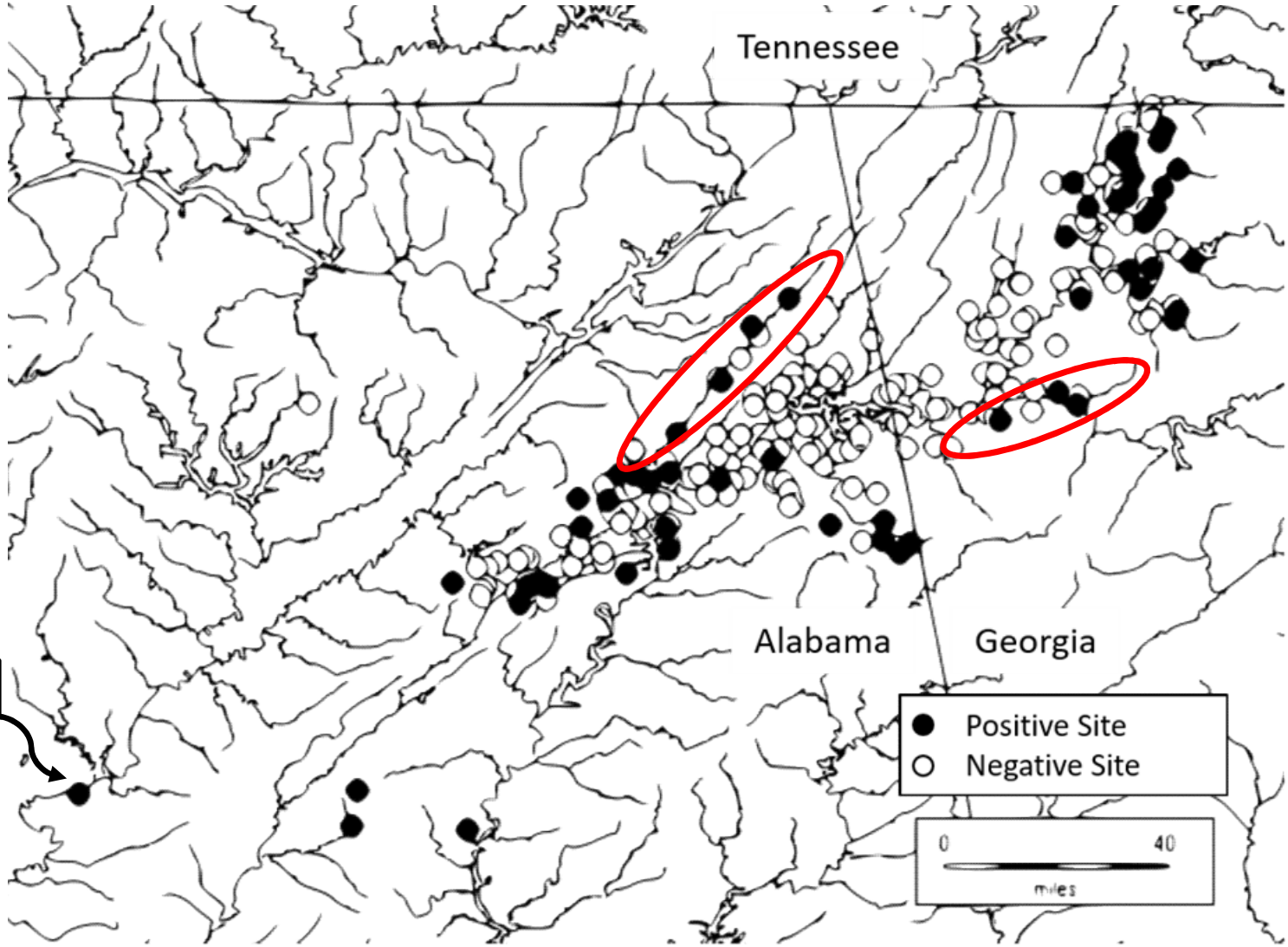


E. artesiae
E. coosae
E. ditrema
E. jordani
E. rupestre
E. stigmaeum
E. swaini

Objective 2

Comparison of *E. trisella* Status and Historic Sites



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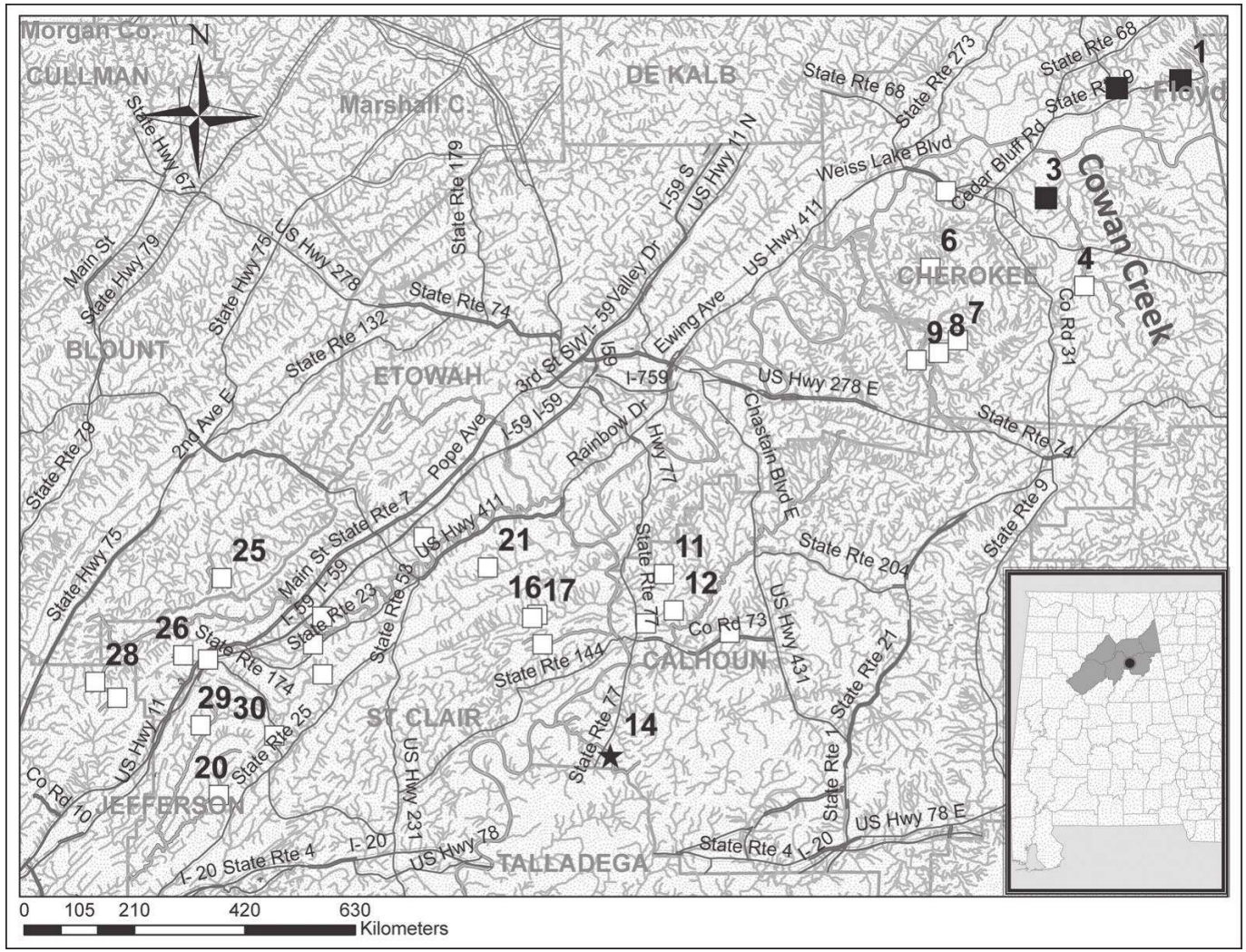

Discussion

1. Population genomics
   - Three evolutionarily significant units corresponding to geographically distinct populations
   - Gene flow follows stepping stone model
     - ↑ admixture at center of expected range (Ballplay & Coosawattee)
   - Ballplay and Little Canoe are the most genetically isolated and have low Ne
2. Surveillance
   - LAMP is a robust protocol for surveying imperiled freshwater fish
   - Results generally correspond with field observations and provide new opportunities for sampling
   - Cross-amplification is still possible

Next steps

- Capture and release trispot at eDNA positive sites during 2019-2020 spawning
- Confirm eDNA positive sites with additional water collections
- Collect water from suspected sites at different times during spawning

Acknowledgements

- Kellie Johnston

  LAQE (Sandel Lab)
- Dominique Dawson
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