Successful amplification of Alabama Cave Shrimp eDNA from a spring water sample demonstrates the potential utility of this approach for the detection and monitoring of this and other endangered groundwater species.

We plan to screen additional water samples from spring, caves, and wells throughout the range of the Alabama Cave Shrimp to potentially identify new localities of this species.

We also plan to develop qPCR assays for other groundwater species of conservation concern, such as the Alabama Cavefish and Manitou Cavesnail.

We designed a qPCR assay that amplifies a 133-bp fragment of the 16S locus for the Alabama Cave Shrimp. In silico tests showed that no non-target species were likely to be amplified.

The assay successfully amplified the positive control (a synthetic gene sequence of cave shrimp 16S rRNA) with a lowest limit of detection of $1 \times 10^{-6}$ ng/µl.

We detected Alabama Cave Shrimp DNA at 1 of the 20 sites sampled thus far, with amplification in a total of 3 of 12 qPCR replicates for one site (Cave Spring, Madison Co., AL).

Cave organisms represent one of the most understudied and threatened biodiversity on the planet. These species are characterized by their unique traits that enable them to survive underground, including elongated appendages, loss of eyes and pigment, and reduced metabolisms. It is imperative to monitor these species in order to mitigate further species loss and conserve existing cave biodiversity.

A contemporary method that shows incredible promise for monitoring and detecting species is eDNA (environmental DNA). Compared with traditional approaches, eDNA can yield faster, more reliable, and cost-effective results, especially for species that are difficult to study using traditional approaches.

In this study, we assess the efficacy of eDNA for the detection and monitoring in subterranean habitats of the Alabama Cave Shrimp (*Palaemonias alabamae*), a federally endangered species found in the Huntsville area.

Introduction

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**Methods**

- We compiled a list of potential subterranean habitats to collect water samples in the Huntsville area, including caves, springs, and wells
- 2-L of water was collected from 20 sites to date
- Water samples were vacuum-filtered through 0.45-µm cellulose nitrate filters
- eDNA was extracted from filters using Qiagen Dneasy® kits following a modified protocol (4)
- We designed a species-specific qPCR primer-probe assay using IDT’s PrimerQuest® tool based on published mitochondrial 16S rRNA sequences
- The assay was tested in silico using NCBI’s Primer-BLAST against the *nr* database
- eDNA water samples were screened using the primer-probe assay via qPCR
- Five PCR replicates for each sample were screened and both positive and negative controls were included

**Results**

- We designed a qPCR assay that amplifies a 133-bp fragment of the 16S locus for the Alabama Cave Shrimp
- In silico tests showed that no non-target species were likely to be amplified
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**Discussion**

- Successful amplification of Alabama Cave Shrimp eDNA from a spring water sample demonstrates the potential utility of this approach for the detection and monitoring of this and other endangered groundwater species.
- We plan to screen additional water samples from spring, caves, and wells throughout the range of the Alabama Cave Shrimp to potentially identify new localities of this species.
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