Exploring Tiger Beetle Diversity Associated with Salt Domes across East Texas

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Summary

This report summarizes recent research conducted on tiger beetles of East Texas, around and within the Big Thicket National Preserve (BITH), Texas in 2022, made possible by funding from by the Big Thicket Association, and Rice University. The project explored the genetic structure and evolutionary relationships within and between populations of two salt-adapted tiger beetle species–*Eunota togata* and *Eunota circumpicta*, and culminated in the publication of two peer reviewed papers in *Nature Scientific Reports* and *Molecular Phylogenetics and Evolution*. Results revealed cryptic diversity among these beetles, with *E. togata* and *E. circumpicta* each representing at least three distinct species based on genetic analyses. Further, beyond the taxonomic group of focus, this work documented four additional tiger beetle species found within the Big Thicket National Preserve representing two additional genera.

Introduction

This project began in 2022 with the objective of investigating the genetic structure and evolutionary relationships within and between populations of two salt-adapted tiger beetle species–*Eunota togata* and *Eunota circumpicta* across East Texas. This work sampled genetic data from field collected specimens along with specimens from private and museum collections in order to assess the true diversity of *Eunota* species, in particular in the BITH region. Specifically, this research aimed to test the existing species hypotheses for these *Eunota* taxa, which was largely based on morphological characters, and identify connections between the evolutionary history of these groups and the unique geographic characteristics of East Texas. Our purpose here is to provide a final report of our results to date along with some key findings regarding our investigation into the genetic structure and evolutionary relationships of the *Eunota* tiger beetles in and around BITH.

Methods

In Summer 2022 counties in East Texas including the BITH region were investigated for the presence of our *Eunota* species of interest. We used data on the locations of known salt domes from historic geological surveys combined with visual surveys of Google Earth to identify specific salt domes within these counties to target for collection. Collection methods followed those used for previous studies including the use of bug nets and UV light trapping. Further, we

leveraged specimens collected previously by collaborators in this region in addition to local museum samples at Texas A&M, as well as national collections at the Smithsonian and the American Museum of Natural History, to look for historic samples of this region.

At Rice University, DNA was extracted from specimens using Qiagen DNeasy Blood and Tissue Kits and a non-destructive protocol in which the abdomen of the specimen is punctured 5-10 times before being soaked in lysis solution. Following DNA extraction, specimens were rinsed and kept in 70% ethanol for pinning and photography. One mitochondrial region, a 424 bp region of the cytochrome b gene (cytb), was amplified using primers developed by our research group and sent to the University of Arizona's Genetics Core, where paired end reads were produced via Sanger sequencing. Multilocus genetic data was produced using a restriction enzyme associated DNA sequencing (RAD-seq) approach. Pooled libraries including many specimens were prepared using a protocol used previously by our research group with other tiger beetle species. DNA sequencing of these libraries was conducted by the University of Texas Genomic Sequencing and Analysis Facility on the Illumina NovaSeq and NextSeq platforms.

A multitude of genetic analyses were used to investigate the evolutionary relationships between the *Eunota* specimens at different collection sites. These analyses included the inference of a mitochondrial genealogy, and, from multilocus data, the production of phylogenetic trees, principal component analyses, Bayesian clustering analyses, estimations of genetic distance, and measures of species delimitation. Further, morphological measurements of total length, elytra length, and fovea density were utilized to identify phenotypic differences between putative distinct species.

Results and Discussion

The results of this research highlight significant findings regarding the taxonomy, species delimitation, and conservation implications for *Eunota* tiger beetles in East Texas and beyond. For the *Eunota togata* species complex, genomic and mitochondrial DNA (mtDNA) analyses challenged traditional morphology-based taxonomic classifications. The results indicate that the four subspecies delineated by dorsal coloration and white markings often fail to align with genetic evidence. Instead, the genomic data reveal three distinct species within what was traditionally thought to be one species. This discrepancy underscores the limitations of morphological approaches, as convergent evolution driven by environmental pressures, such as adaptation to saline habitats, masks true genetic relationships. These findings advocate for an integrative taxonomic approach, blending molecular data with traditional methods to better inform conservation strategies.

For the *Eunota circumpicta* species complex, the results of this research reveal multiple cryptic species previously misclassified under subspecies categories. The study identifies clear genetic and geographic structuring, leading to the recognition of new species and subspecies within the group. For instance, the gulf coastal and more interior populations within *Eunota circumpicta* exhibit distinct genetic profiles, leading to taxonomic revisions and the description of a new species, *Eunota houstoniana*, from the Gulf region. This work emphasizes the need for comprehensive, multidisciplinary approaches to resolving taxonomic challenges.

Overall, these results reveal the hidden diversity of *Eunota* tiger beetles in East Texas within and around BITH and highlight the broader implications for biodiversity conservation. By refining species boundaries and recognizing cryptic diversity, these findings ensure that conservation efforts are accurately targeted, avoiding misallocation of resources. Additionally, the research provides a robust framework for addressing the challenges of integrating molecular and morphological data, paving the way for more reliable and effective conservation planning in the face of global biodiversity declines.

Outside of the *Eunota* genus, multiple additional species of tiger beetles representing at least two genera (*Cicindelidia* and *Tetrarcha*) were collected and successfully identified within BITH.

Future work could include further surveys of BITH utilizing additional collection methods such as pitfall traps. This could yield additional insights into the overall diversity of tiger beetles within the park and provide awareness of the multitude of unique BITH habitats which support a variety of species within this charismatic and elusive insect group.

The following presentations were made based on this work:

2023. Oral Presentation by coauthor Dan Duran. A genomic test of subspecies in the Eunota togata species group (Coleoptera: Cicindelidae): Morphology masks evolutionary relationships and taxonomy.

- November. National Harbor, MD. Entomology Meeting.

2022. Poster Presentation by undergraduate researcher Ethan Bull. Population Genomics and Morphology Support a New Species of Tiger Beetle Found on Isolated Saline Habitats in East Texas.

- August. Houston, TX. Gulf Coast Undergraduate Research Symposium.

The following publications were made based on this work:

Duran, D.P., Laroche, R.A., Roman, S.J., Godwin, W., Herrmann, D.P., Bull, E. and Egan, S.P. (2024). Species delimitation, discovery and conservation in a tiger beetle species complex despite discordant genetic data. Scientific Reports, 14(1), p.6617.

Laroche, R.A., Duran, D.P., Lee, C.T.A., Godwin, W., Roman, S.J., Herrmann, D.P. and Egan, S.P. (2023). A genomic test of subspecies in the Eunota togata species group (Coleoptera: Cicindelidae): Morphology masks evolutionary relationships and taxonomy. Molecular Phylogenetics and Evolution. https://doi.org/10.1016/j.ympev.2023.107937

These publications were featured in multiple media stories, including in the Houston Chronicle:

Johnson, O. (2024, April 1). New tiger beetle species detected in the Houston area by a Rice University Research Team. Houston Chronicle. https://www.houstonchronicle.com/news/houston-texas/trending/article/houston-new-tiger-beetle-19375050.php

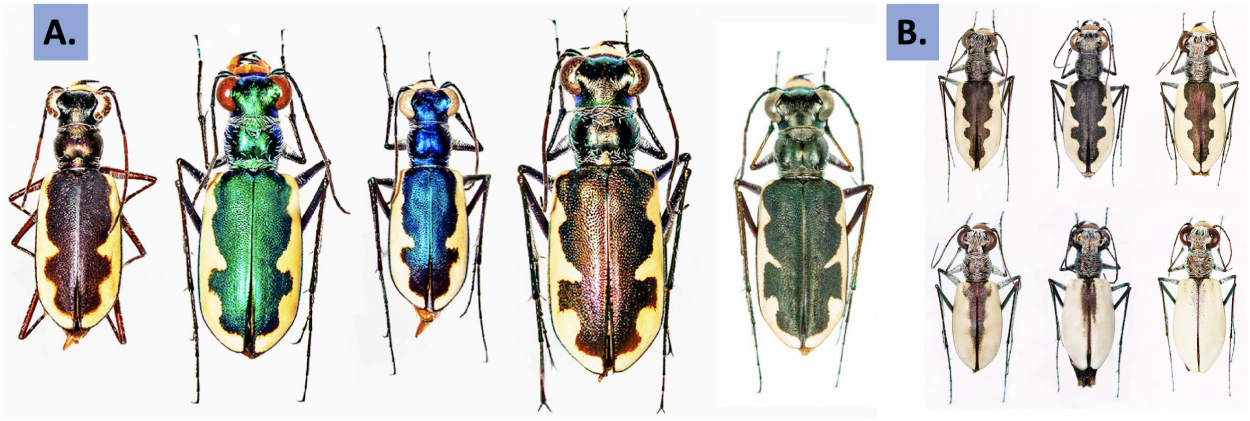
The following describes Laroche's journey as a Rice University graduate student:

2024. YouTube. Graduate student life at Rice: Robert Laroche combines science policy with adventure. https://www.youtube.com/watch?v=AJEwjmM7HQM

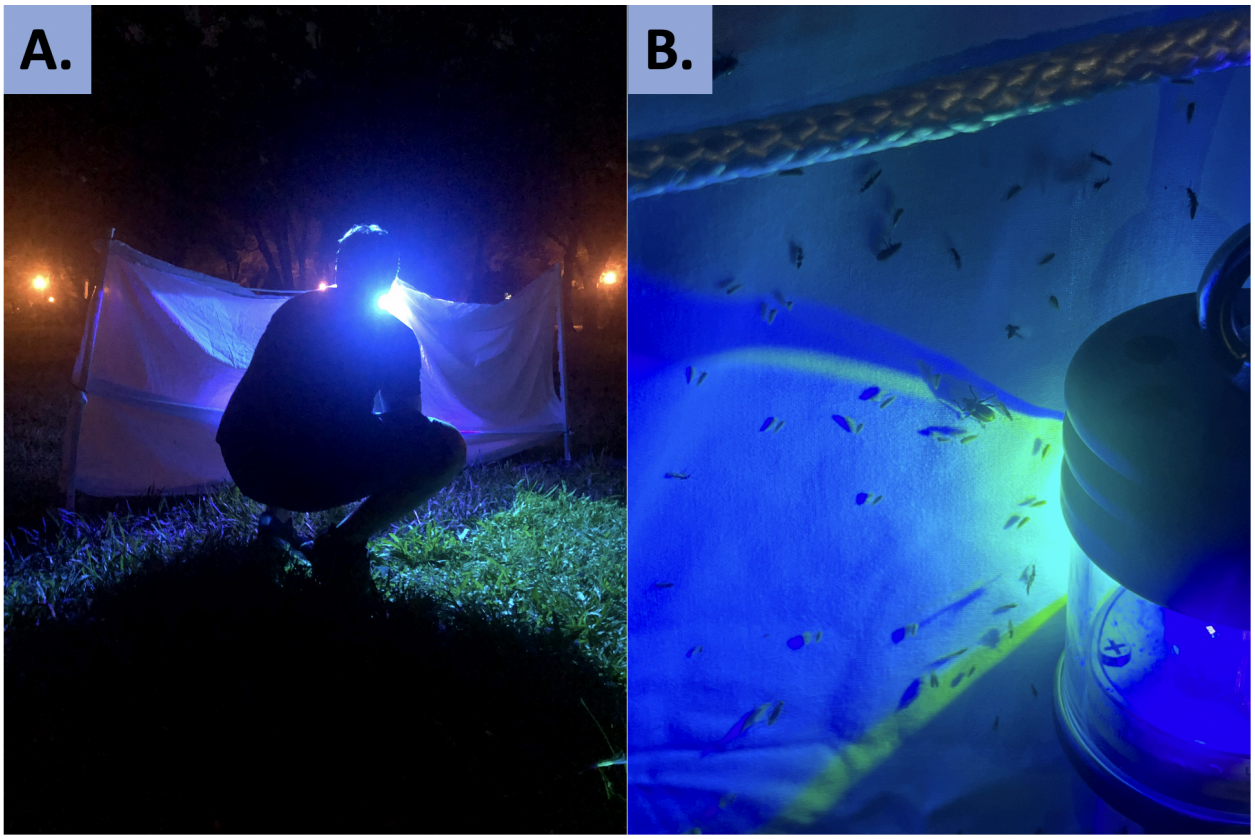
Species List Chart

Genus	Species	Collector	Collection Date	County	State	Park Unit
Cicindelidia	*punctulata*	Laroche, Robert	6/19/22	Hardin	TX	LR
Cicindelidia	*punctulata*	Laroche, Robert	6/19/22	Hardin	TX	LR
Cicindelidia	*punctulata*	Laroche, Robert	6/19/22	Hardin	TX	LR
Cicindelidia	*ocelatta*	Laroche, Robert	5/5/22	Hardin	TX	LR
Cicindelidia	*ocelatta*	Laroche, Robert	6/23/22	Hardin	TX	LR
Cicindelidia	*ocelatta*	Laroche, Robert	6/23/22	Hardin	TX	LR
Tetrarcha	*viriginica*	Bull, Ethan	6/23/22	Hardin	TX	LR
Tetrarcha	*viriginica*	Laroche, Robert	6/23/22	Hardin	TX	LR
Tetrarcha	*carolina*	Bull, Ethan	6/23/22	Hardin	TX	LR
Tetrarcha	*carolina*	Laroche, Robert	6/23/22	Hardin	TX	LR
Tetrarcha	*carolina*	Laroche, Robert	6/23/22	Hardin	TX	LR
Eunota	*togata*	Egan, Scott	2022	Hardin	TX	LR

Research Photos:

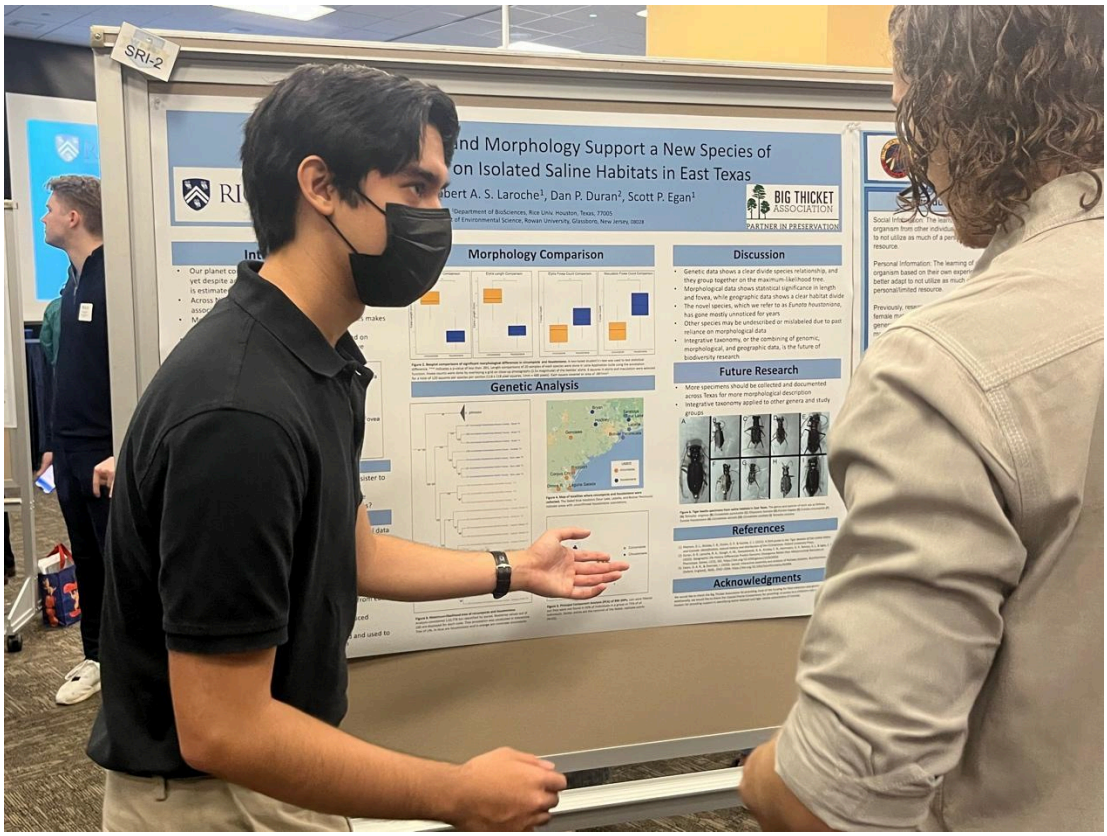


Photographs of some of the tiger beetle specimens from the studies published in (A) *Molecular Phylogenetics and Evolution* and (B) *Scientific Reports*.

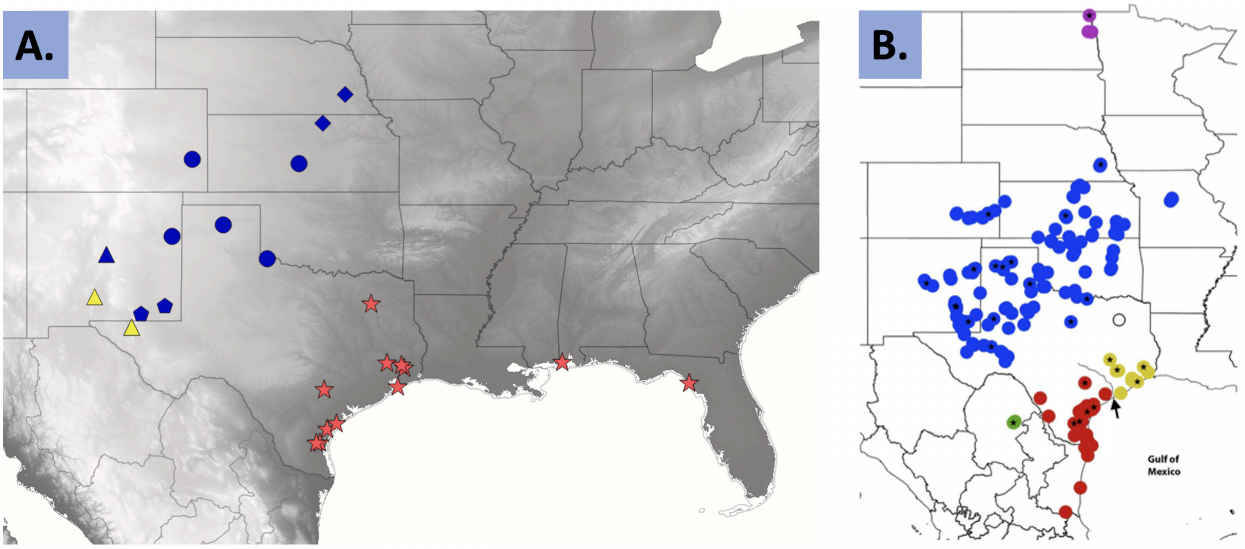


UV light traps (A) from a distance and (B) up close, used to attract insects and search for tiger beetle species of interest

Graduate student researcher Robert Laroche posing with a bug net on a tiger beetle collection field trip



Undergraduate researcher Ethan Bull presenting his analyses on the morphological differences between tiger beetles in East Texas at the Gulf Coast undergraduate Research Symposium



Maps of tiger beetle localities for the studies published in (A) *Molecular Phylogenetics and Evolution* and (B) *Scientific Reports*.

We greatly appreciate the patience and guidance of Mary Catherine Johnston over the course of this project and its review by members of the Big Thicket Association Science Committee.