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RESEARCH

Phylogenomics of endangered troglobiotic rove beetles (Coleoptera: Staphylinidae: Pselaphinae) from central Texas karst regions

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Abstract

The karst habitats of central Texas, USA, are home to an array of endemic subterranean-obligate (troglobiotic) invertebrates. This includes several species of rove beetles (Coleoptera: Staphylinidae: Pselaphinae). Here we developed a molecular dataset using sequence capture of Ultra-Conserved Elements (UCEs) from the Coleoptera-UCE-1.1 K v1 baits kit. These data were used to assess species relationships and patterns of diversification in this group, specifically among species within the genera *Batrisodes* Reitter 1882 and *Texamaurops* Barr and Steeves 1963; with a specific focus on the relationships of the federally listed as endangered *B. texanus* Chandler 1992 and *B.cryptotexanus* Chandler and Reddell 2001. Our final datasets consisted of 69 individuals (two genera, *Batrisodes* [five species] and *Texamaurops* [one species], from 34 localities), and a molecular dataset of 658,560 aligned base pairs across 672 UCE loci. Concatenated and species-tree phylogenetic analyses resolved all troglobiotic taxa as a monophyletic group. Within the Travis and Williamson County troglobionts, we recovered four well-supported clades that generally follow hypothesized geologic barriers to dispersal formalized as karst fauna regions (KFRs). A northward pattern of diversification was observed among these groups: (A) *Texamaurops reddelli* Barr and Steeves 1963 (Jollyville Plateau KFR); (B) *Batrisodes reyesi* Chandler 1997 (West Cedar Park and Post Oak Ridge KFRs); (C) *B. reyesi* (McNeil-Round Rock KFR); (D) *B. cryptotexanus*+*B. texanus* (Georgetown and North Williamson KFRs). The morphologically defined *Batrisodes texanus* and *B. cryptotexanus* were not reciprocally monophyletic, nor clustered into two unique groups in clustering analyses of single nucleotide polymorphisms (SNPs). Rather, we found support for five major subclades and five to seven genetic clusters. These results suggest that diversification and subsequent isolation of clades may have occurred with the progressive availability of karst habitats over time in the North Williamson and Georgetown KFRs resulting from the interactions of faulting, geologic structure, and drainage basin evolution. Comparison with recent U.S. Fish and Wildlife Service cave habitat resiliency assessments indicated that four genetic clusters occur within at least partially resilient habitat, whereas three are confined to caves with low or impaired resiliency. Integrating genetic results presented here along with results of other molecular studies of co-occurring troglobiotic invertebrates supports considering additional geological substructure within the North Williamson KFR in conservation efforts for these rare and unique lineages and systems.

Keywords Coleoptera: Staphylinidae: Pselaphinae · High-throughput sequencing · Ultra-conserved elements · Karst faunal regions · Phylogeography

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Introduction

The application of genomic data to biodiversity conservation has been facilitated by the advancement of low-cost high throughput sequencing, quicker and more efficient analytical tools, and the ability to conduct landscape genomic studies from small numbers of individuals per population (Chambers et al. 2023; Hohenlohe et al. 2021). Individual-based landscape genomics for conservation has benefited from this advancement given that large numbers of independent loci from small sample sizes can provide robust estimates of genetic differentiation, even if there are few localities (Chambers et al. 2023). This is optimal when working with organisms that are difficult to find owing to sampling challenges or short-range endemism. Genomic data have proven to be good tools for conservation efforts (e.g., Funk et al. 2012; Shafer et al. 2015), and these tools have seen increased use in the study of a variety of invertebrate species of conservation concern in the USA that have adapted to obligately inhabit subterranean ecosystems (troglobionts), including: arachnids (Derkarabetian et al. 2022; Hedin et al. 2018; Azevedo et al. 2024), beetles (Balogh et al. 2020; Boyd et al. 2020), and groundwater (stygobiotic) invertebrates (Hutchins 2018, and references therein).

Troglobiotic species in North America are most often associated with karst terrain, which form in porous and soluble bedrock such as limestone and gypsum. Karst landscapes are characterized by various subterranean habitats (e.g., caves, solution cavities, sinkholes, and springs) inhabited by numerous invertebrate groups that include troglobiotic species, many of which exhibit short-range endemism (Christman and Culver 2001; Niemiller and Zigler 2013; Culver and Pipan 2019). Accessing and studying karst habitats and their associated biodiversity is often challenging, and subterranean ecosystems are increasingly impacted by surface land-use changes or direct habitat modification and destruction (Niemiller et al. 2018; Mammola et al. 2019). For example, the karst habitats exposed along the Balcones Escarpment located in central Texas, which includes several rapidly growing metropolitan areas, are home to fifteen federally listed endangered troglobionts, referred to as 'karst invertebrates' (U.S. Fish and Wildlife Service [USFWS] 1988, 1993, 1994, 2011). These species are narrowly distributed within various karst fauna regions (KFRs), which are defined based on a combination of hypothesized environmental and biological barriers and restrictions that control the geographic distribution of karst invertebrates (Veni and Jones 2021; Veni et al. 2024). These KFRs serve as management and recovery units for federally listed karst invertebrate species (USFWS 1994, 2011).

There are several troglobiotic species of rove beetles (Coleoptera: Staphylinidae: Pselaphinae) within the genera *Batrisodes* and *Texamaurops* found within KFRs and surrounding karst habitats in Texas (Table 1a; Fig. 1) (Chandler 1992; Chandler and Reddell 2001; Chandler et al. 2009). In 1988, a species originally described from Travis and Williamson Counties, *Texamaurops reddelli* (Kretcshmarr Cave mold beetle), was federally listed as endangered given increased loss of karst habitats owing to ongoing development activities (USFWS 1988). Further sample collections of other troglobiotic *Batrisodes* (subgenus *Excavodes*) were later used to redescribe several populations of *T. reddelli* as a new species, *Batrisodes texanus* (Coffin Cave mold beetle, see Chandler 1992). This species was subsequently separately federally listed as endangered (USFWS 1993). Following this listing decision, taxonomic revisions of *Batrisodes* recognized an additional species, *B. cryptotexanus* (Dragonfly Cave mold beetle), which was known from caves previously considered occupied by *B. texanus* (Chandler and Reddell 2001; Chandler et al. 2009). The

Table 1 Rove beetles (*Batrisodes* and *Texamaurops*) included in this study, including their ecology and federal listing status (E = endangered, NL = not listed), Texas County and karst faunal regions sampled, number of caves and number of individuals sampled

Taxon	Taxonomic Authority	Ecology & Listing Status	County	Karst Faunal Region	Number of Caves	Number of Ind.
Batrisodes cryptotexanus	Chandler 1992; Chandler and Reddell 2001; split from *B. texanus*	Troglobiotic, E	Williamson	North Williamson	10	23
Batrosodes texanus	Chandler 1992; Chandler and Reddell 2001; split from *B. cryptotexanus*	Troglobiotic, E	Williamson	North Williamson	3	15
				Georgetown	4	5
Batrosodes reyesi	Chandler 1992	Troglobiotic, NL	Williamson	McNeil-Round Rock	1	4
			Travis	Post Oak Ridge	4	8
				West Cedar Park	1	1
Texamaurops reddelli	Barr and Steves 1963; Chandler 1992	Troglobiotic, E	Travis	Jollyville Plateau	6	11
*Batrosodes venyivi**	Chandler 1992	Troglobiotic, E	Bexar	Helotes	1	1
*Batrisodes uncicornis**	Casey 1897	Troglophilic, NL	Williamson	Georgetown	3	3
				McNeil-Round Rock	2	2

*included as outgroups

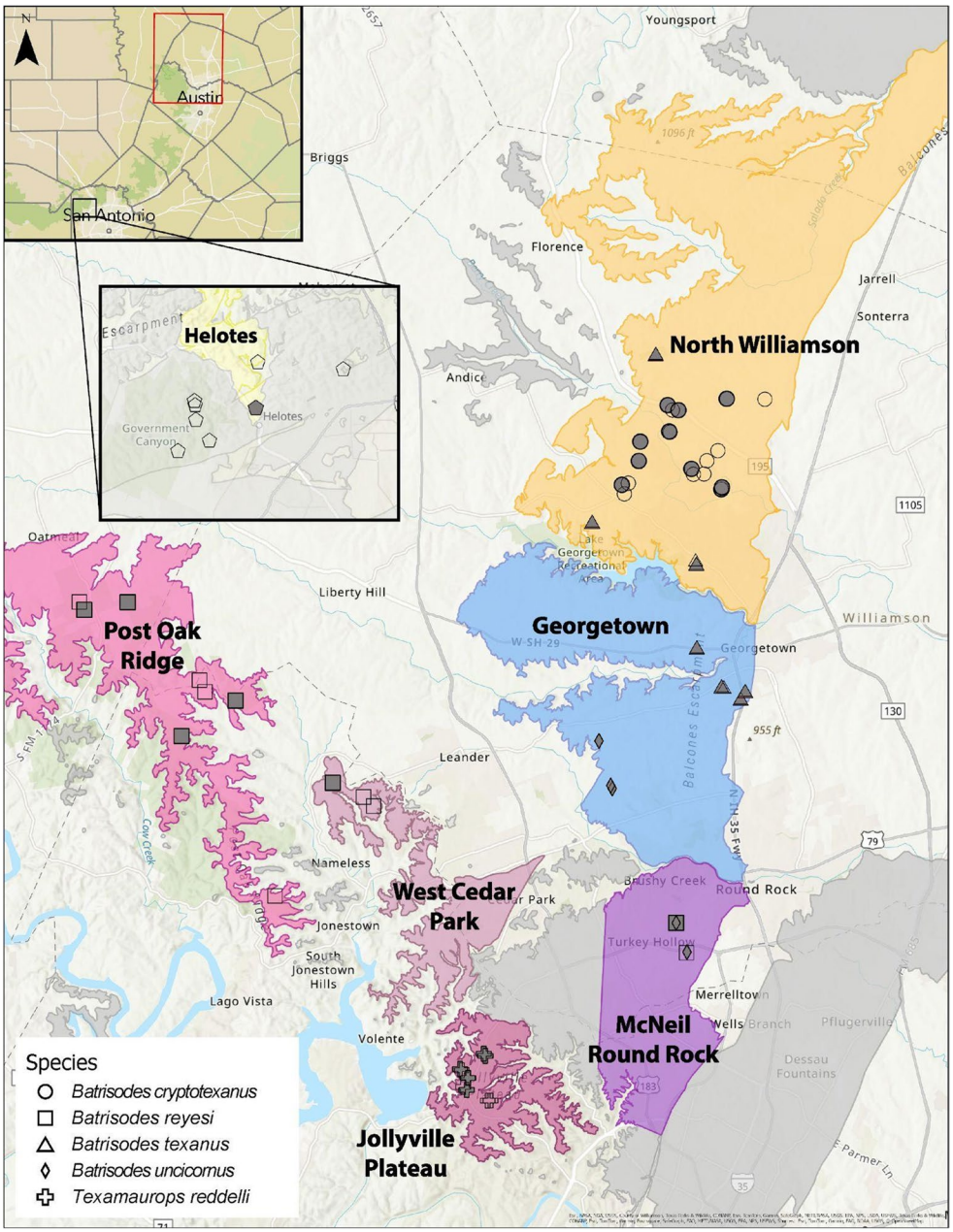


Fig. 1 Known geographic distributions of *Batrisodes* and *Texamaurops* species included in this study, excluding *B. uncicornis* which only includes sampled populations. Morphological species designations are shown with different shapes. Dark gray shapes denote sampled populations. Sampled karst fauna regions are shown in colored polygons and labeled. All other karst fauna regions are light gray. Major rivers, streams and reservoirs are overlaid in blue. (Map Sources: ESRI, TomTom, Garmin, FAO, NOAA, USGS, OpenStreetMap)

USFWS has not yet formally recognized *B. cryptotexanus* as a separate species and collectively considers populations of *B. cryptotexanus* and *B. texanus* as a single federally endangered *B. texanus* (USFWS 2018). These two taxa were hypothesized to form a monophyletic group with an additional species, *B.* (*Excavodes*) *reyesi* (Chandler et al. 2009). Despite substantial morphological and taxonomic work, prior to our study there were no published molecular data that could be used to assess the evolutionary relationships among these taxa.

Here we used targeted sequence capture of ultraconserved elements (UCEs) to develop a molecular phylogeny of several troglobiotic pselaphine beetles within the genera *Batrisodes* (*Excavodes*) and *Texamaurops*, emphasizing populations of species federally listed as endangered in the KFRs of Williamson and Travis Counties. UCEs have become widely used for phylogenomic analysis of different insect groups (Faircloth et al. 2012; Baca et al. 2017, 2021; Van Dam et al. 2017; Gustafson et al. 2020; Kobayashi et al. 2021; Bradford et al. 2022; Sota et al. 2022) and specifically other federally endangered karst invertebrates (Hedin et al. 2018; Derkarabetian et al. 2022; Azevedo et al. 2024).

This study had two major objectives. First, we assessed phylogenetic relationships among troglobiotic *Batrisodes* (*Excavodes*) and *Texamaurops* species and specifically the

relationships of several species (*Batrisodes texanus*, *B. cryptotexanus*, *B. reyesi*, and *Texamaurops reddelli*) which have geographic distributions across adjacent KFRs in this region. Second, the relationships among sampled locations were used to assess the currently defined boundaries between KFRs for conservation of diversity within this group, particularly within the range of *B. texanus* and *B. cryptotexanus*. Previous studies have noted that KFR boundaries may not align with currently understood species distributions, or phylogenetic structure within other karst invertebrates, and may require further adjustment (Van Kampen-Lewis et al. 2019; Derkarabetian et al. 2022; Azevedo et al. 2024). A better understanding of the relationships and distributions of federally listed karst invertebrates could assist in refining management units.

Methods

Sample collections

Samples were obtained from seven KFRs across Bexar, Burnet, Travis, and Williamson Counties, Texas: Georgetown, Helotes, Jollyville Plateau, McNeil-Round Rock, North Williamson, Post Oak Ridge, and West Cedar Park (Fig. 1). Although sampling troglobiotic species in Travis and Williamson Counties was prioritized, additional outgroup collections included the federally listed as endangered troglobiont *Batrisodes venyivi* Chandler 1992 (Helotes mold beetle) in Bexar County as well as the geographically widespread, facultatively subterranean (troglophilic) species *B.* (*Babnormodes*) *uncicornis* (Casey 1897). Beetles used in this study were collected by permitted biologists and provided by the USFWS or loaned from the University of Texas Insect Collection and the collections of DSC Chandler, University of New Hampshire (Table 1a, S1). We attempted to acquire at least two specimens from each sampling location for each sampled taxon. All beetles included in the sequencing analysis were reviewed and morphologically identified to species by DSC. Samples were either pinned and stored dry in museum conditions, or alternatively, were stored in 95% ethanol prior to extraction. Samples were shipped to the conservation genetics laboratory at the Western Ecological Research Center, San Diego Field Station for genetic analysis.

DNA extraction and quantification

In addition to being rare, troglobiotic rove beetles are extremely small (2–4 mm in length), and maximizing DNA yields while preserving the physical specimen integrity was of high priority. In preliminary tests of extraction methods, we found that soaking whole specimens in proteinase K (described in Santos et al. 2018) yielded much higher amounts of DNA than removal and crushing of a few legs. This method had the added benefit of being less destructive to the specimen, leaving the external features of the specimen intact for future morphological analyses. This method was applied to all samples. Whole bodies were soaked for up to 72 h in Proteinase K and lysis buffer at 37 °C, after which genomic DNA was extracted using the Qiagen Puregene Kit and following manufacturer's recommendations, with the exception that we eluted into 25 µl of ddH_2O. All extractions were quantified using a Qubit Fluorometer.

Library construction and sequencing

To develop our multi-locus dataset, we used targeted sequence capture of Ultra Conserved Elements (UCEs), previously developed for Coleoptera (see Faircloth 2017 for a full list of loci). These and other UCE probe kits have been shown to have high success when applied to specimens with lower quality or degraded DNA, such as dry museum specimens (without special preservation of DNA material) of bird skins (McCormack et al. 2016), formalin fixed snakes (Ruane and Austin 2017; Bernstein et al. 2022), and insects and spiders (Blaimer et al. 2016; Hedin et al. 2018). Thus, target capture was deemed to be a reasonable approach that could provide a robust, multilocus dataset, given the limitations of samples available to us for this study.

Seventy-three extracted samples with gDNA concentrations ranging from 1 ng/ul to 22 ng/ul were sent to Arbor Bioscience for sequencing and bait capture using the MyBaits Coleoptera 1.1 K version. Briefly, up to 500 ng of each DNA sample was sonicated and electrophoresed to verify sonication success. Libraries were prepared using the KAPA Hyper Prep Kit (Kapa Biosystems) followed by clean up with Ampure XP beads. Universal adapters were ligated onto libraries and libraries were amplified and indexed (Glenn et al. 2019). Final libraries were quantified and pooled. Target enrichment was performed on pooled libraries using the myBaits Coleoptera 1.1 K version 1 kit (Arbor Biosciences, Faircloth 2017) and following standard Illumina target enrichment protocols and sequencing on an Illumina HiSeq 4000 using 150 bp paired end reads. All raw sequencing reads, and ancillary data are deposited in the National Center for Biotechnology Information (NCBI) Sequencing Read Archive (SRA) under project ID PRJNA1228651.

Data processing and quality control

To remove adapter contamination and low-quality bases from the untrimmed paired end sequencing reads we used the program illumiprocessor (Faircloth 2013), a wrapper for trimmomatic (Bolger et al. 2014). Following trimming and

cleaning we used the PHYLUCE v.1.6.8 (Faircloth 2016) pipeline to assemble contigs of the paired end sequences using SPAdes (Bankevich et al. 2012). To assess the quality control and performance of the assembly, we examined the length of the contigs from the assembly, number of contigs, and various other metrics (Table S2). Next the contigs were matched to the Coleoptera 1.1 K probe set and UCE loci that were enriched for each taxon were extracted. At this point we estimated individual statistics for UCE loci (Table S3). UCE loci were aligned using the mafft v7.526 (Katoh and Standley 2013) option in PHYLUCE and trimmed with default sequence edge trimming. Next internal trimming was performed using the program Clipping and Keeping Information Trimmer (ClipKIT), implementing the smart-gappy mode algorithm (Steenwyk et al. 2020). Finally, we constructed matrices of varying levels of completeness for further phylogenetic analyses, 50%, 75%, and 95% complete.

Extracting SNPs from UCE loci for population genetic analyses

Focusing on the relationships between and within *B. texanus* and *B. cryptotexanus* was an important objective of our study. Therefore, we developed a single nucleotide polymorphism (SNP) matrix using the samples of these two putative species, to assess population structure and genetic diversity patterns among collection sites within this group. Because including outgroup taxa and distantly related species in SNP discovery can result in large amounts of missing data in the target taxa, we started with a paired down UCE dataset that consisted of *B. texanus* and *B. cryptotexanus* samples only and created a new alignment in PHYLUCE, following the steps outlined above. We extracted all SNPs for each locus using the program SNP-sites (Page et al. 2016), and then randomly selected a single SNP per locus to retain. These "unlinked" SNPs were concatenated into a single phylip file for downstream analyses.

Phylogenomic analyses

We estimated phylogenetic trees from each of the 50%, 75%, and 95% complete matrices. We implemented a partitioned concatenated Maximum Likelihood (ML) analysis using IQ-TREE v.2.3.6 (Minh et al. 2020; Nguyen et al. 2015) implementing partitioned analysis for multigene alignments (Chernomor et al. 2016), which estimates models of molecular evolution for each partition (assessed using the Bayesian Information Criterion [BIC]; Schwarz 1978). We estimated nodal support for the ML analyses using 1000 pseudoreplicates via the ultrafast bootstrap (UFboot) approximation algorithm (Hoang et al. 2018; Minh et al. 2013).

In addition to a concatenated approach, we used a summary method of species tree estimation using ASTRAL-IV (Zhang and Mirarab 2022). This method has proven to perform well for handling incomplete lineage sorting (ILS) and is consistent with the multispecies coalescent model (MSC) (Mirarab and Warnow 2015). This method requires unrooted gene trees as input, and these were estimated using IQ-TREE with the same parameters as described above. The species tree was estimated using ASTRAL-IV v1.19.4.6 (Zhang and Mirarab 2022) by optimizing the objective function in ASTRAL (Zhang et al. 2018). We also estimated and computed branch lengths in substitutions-per-site using CASTLE-II (Tabatabaee et al. 2023). To estimate nodal support, we used local posterior probabilities (Sayyari and Mirarab 2016) instead of multilocus bootstrapping, which can be poor bootstrap support estimates for species trees from multilocus datasets (Mirarab et al. 2014). All nodes with UFboot≥95 and PP≥0.95 were considered well-supported (Huelsenbeck et al. 2001; Erixon et al. 2003; Huelsenbeck and Rannala 2004; Nguyen et al. 2015; Wilcox et al. 2002). Given the unknown phylogenetic relationships of *Batrisodes* and *Texamaurops* and the lack of extensive outgroup samples, we used a conservative approach of a midpoint root (Swofford 1996). All final trees were rooted using a midpoint root; this places the root of the tree at the midpoint between the longest path between any two taxa.

SNP-based analysis

To estimate population structure and estimating ancestry coefficients, we applied an R-based (R Core Team 2024) version of the program STRUCTURE (Pritchard et al. 2000; Frichot et al. 2014) implemented in the R package LEA (Frichot and François 2015; François 2016). While the algorithms for estimating population structure differ in LEA compared to STRUCTURE, the estimates of ancestry coefficients are similar to those of STRUCTURE and can perform better when inbreeding is present (Frichot et al. 2014). To estimate the optimal number of population clusters, we used cross-validation of an information theoretic measure, also known as the cross-entropy criterion (Alexander and Lange 2011; Frichot et al. 2014). We converted our SNP phylip file to a variant calling format (.vcf) using a python script phy2vcf.py. Next, the.vcf file was imported into R using the read.vcfR() function (Knaus and Grünwald 2017) and converted into a.geno file using the vcf2geno() function of the R package LEA v3.16.0 (Frichot and François 2015). A STRUCTURE-like analysis was implemented using sparse non-negative matrix factorization algorithms (sNMF: Frichot et al. 2014). Finally, we searched for population clusters (K) from 1 to 17, performing 10 repetitions each, and 50,000 iterations, with a sNMF regularization parameter set to 100. The optimal K was selected using cross-entropy validation when the points begin to plateau, and we selected the runs for the optimal Ks with the lowest cross entropy scores. For the optimal number of K we estimated the average nucleotide diversity (π) per

cluster using the program pixy (Korunes and Samuk 2021). We report and compare these with the estimated cave or cave complex resiliency estimated in USFWS (2025).

To investigate whether geographic distances were correlated with genetic distances (e.g., isolation by distance Slatkin 1993) we applied Mantel tests (Mantel 1967). Pairwise genetic distances among individuals were estimated from the random SNP alignment in R using ape v5.8.1 package (Paradis and Schliep 2018), while applying the JC69 (Jukes 1969) model of molecular evolution, and with pairwise deletion of missing sites. Geographic distances among all sampling locations were calculated in ArcGIS Pro v 3.4.0. Evolutionarily independent lineages without gene flow should not conform to isolation by distance (Hutchinson and Templeton 1999). To assess the effect of phylogeny on distance, a third indicator matrix was created for pairs of individuals within the same clade (assigned a value of 0) or in two different clades (assigned a value of 1), grouping individuals into the four major clades recovered in phylogenetic analyses (shown in Fig. 5). We tested for significant associations between distance matrices using Mantel tests and used partial Mantel test to understand the relationship of genetic distance with geographic distance while controlling for phylogeny. Tests were performed in IBD v 1.53 (Bohonak 2002).

Results

Sequencing and assembly

After final data processing the dataset contained 658,560 bp and 672 loci. A large percentage of these, (549 loci) were at least 50% complete across samples, the 75% complete matrix included 483 loci, and the 95% complete matrix included 294 loci (Fig. 2). Four samples (BACR33- Shaman

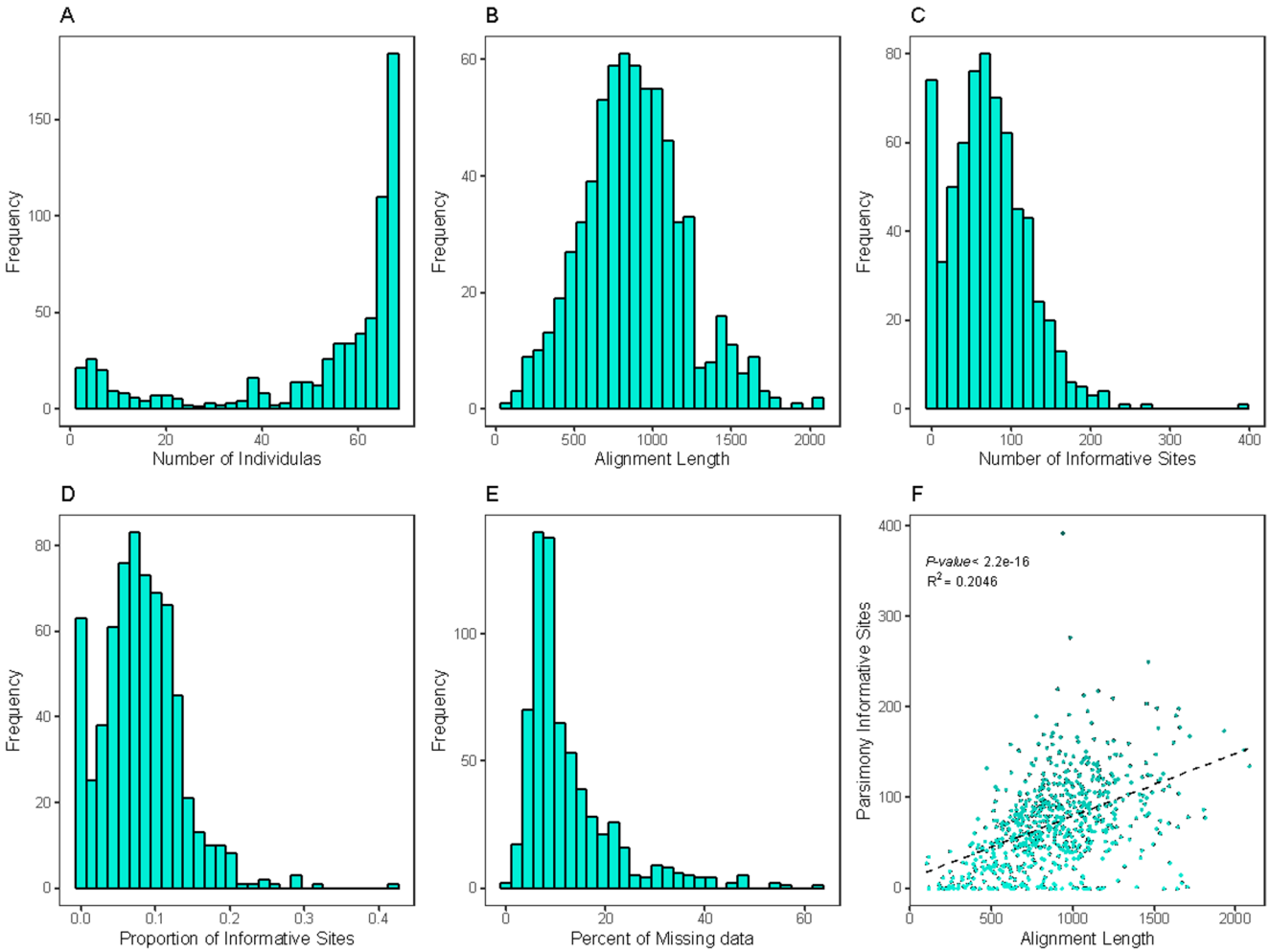


Fig. 2 Alignment frequency distributions for loci in *Batrisodes* and *Texamaurops* species examined in this study. (**A**) Number of individuals in each alignment. (**B**) Distribution of alignment length. (**C**) The number of informative sites per alignment. (**D**) The proportion of invariant sites. (**E**) Percent missing data for each alignment. (**F**) Scatter plot of informative sites vs. alignment length, p-value = 2.2e-16 and R-squared = 0.2046, the dashed line id is the result of the smoothing function and the shaded area in the confidence interval around the regression line estimated with the equation lm(informative sites ~ alignment length)

Cave; and BATE53, BATE76, BATE77- Cobb's Cavern) had poor assembly or UCE capture quality and were removed from further analysis. Individual sample statistics are available in Tables S2 and S3.

Phylogenetic trees and clade support

Concatenated analyses across the three completeness matrices recovered and highly supported the same relationships among major groups (Figs. 3, S1). Midpoint rooting placed the root with *B. uncicornis* as the first branching lineage in the trees. Additionally, the coalescent based analyses inferred the root using backward calculations of the tree as ((*B. uncicornus*+*B. venyivi*) (*Texamaurops reddelli*)), resulting in *Texamaurops* nested within *Batrisodes*. The southernmost troglobiont species *B. venyivi* within Bexar County was the first branching lineage to a clade containing the troglobiotic *Texamaurops* (Jollyville Plateau KFR*)* and *Batrisodes* species found in Burnet, Travis, and Williamson Counties. *Batrisodes reyesi* was recovered as paraphyletic (Clade B was found in adjacent Post Oak Ridge+West Cedar Park KFRs separate from Clade C McNeil-Round Rock KFR, Fig. 3). The *B. reyesi* population sampled from the McNeil-Round Rock KFR was sister to the clade comprising all *B. cryptotexanus* and *B. texanus* populations (Clade D, Fig. 3). Within the *B. cryptotexanus*+*texanus* clade, we recovered at least four subclades (Clades D1–D4, Fig. 3), although there were some differences in the branching order and support among analyses. In the 50% and 75% complete matrices, the clade containing all caves within the Georgetown KFR was recovered as sister to a clade containing Temples of Thor, Medicine Man and Ventilator Caves (Fig. 3). However, in the 95% complete matrix, the Georgetown KFR caves fell outside all other clades except Cobb's Cavern (Figure S1).

The species tree analysis supported the same major clades and branching order as recovered in concatenated analyses for all major clades (Fig. 4, Pp=1.0). Subclades within the *B. cryptotexanus*+*texanus* clade were less well supported (Clade D Fig. 4, Pp≤0.95), except that Cobb's Cavern was well supported as sister to all other clades (Clade D1, Fig. 4, Pp=1, 0.99). Branch lengths were also relatively short within the *B. cryptotexanus*+*texanus* clade. Finally, we note that in most cases across all phylogenetic analyses, where more than one individual was sampled from the same cave, these individuals clustered together (Figs. 3, 4 and 5). Exceptions occurred in cases where caves are geographically close together and likely connected, including Shamrock Cave and Inner Space Cavern (Clade D3–1 Fig. 5, Georgetown KFR), Powwow Cave and Shaman Cave (Clade D4–2, Fig. 5; North Williamson KFR), Rattlesnake Inn Cave and Corn Cobb Cave (Clade D4–1, Fig. 5; North Williamson KFR), and Persephone Caverns and Stovepipe Cave (*T. reddelli*, Figs. 3 and 4; Jollyville Plateau KFR).

SNP-based analyses

The SNP dataset contained 561 SNPs (1 SNP per locus) with 13.67% missing data and after removing 75 loci due to missing data, our final dataset contained 493 loci/SNPs. The sNMF analysis of the SNP dataset best supported seven clusters within the *B. cryptotexanus*+*texanus* clade using the cross-entropy criterion (Figure S2). However, clusters for five and six had relatively similar cross-entropy values as the best supported cross entropy score for seven (cross-entropy scores: K=7: 0.2394, K=5: 0.2395, K=6: 0.2412). The seven clusters largely mirrored the clades supported in the tree-based analyses (Fig. 6). Some co-ancestry or shared ancestral polymorphisms were apparent, particularly in Powwow and Shaman caves (Fig. 6). Mean pairwise genetic distances within clades was 0.121 (range 0.015–0.229) and the mean among clade genetic distance was 0.215 (range 0.121–0.340). We found significant genetic isolation by geographic distance across all samples within the *B. cryptotexanus*+*texanus* clade (Mantel $R=0.515$, $p\leq0.001$; Figure S3) and genetic distance was even more strongly correlated with the clade indicator matrix (Mantel $R=0.637$; $p\leq0.001$). Both factors were also significant in partial Mantel tests (genetic and geographic distance, controlled for clade $r=0.307$, $p\leq0.003$; genetics and clades, controlled for geographic distance $R=0.516$, $p\leq0.001$). Examining the plot, when comparisons were grouped within versus among clades, among clade genetic distances were high regardless of distance, while within clade distances strongly followed isolation by distance (Figure S2). This pattern supports stepping-stone colonization or gene flow within clades, but isolation among clades (Hutchinson and Templeton 1999). Nucleotide diversity (π) was highest in Cluster 3 and lowest in Cluster 6 (Table 2). Average nucleotide diversity within clusters and habitat resiliency were not correlated (Table 2). 10592_2025_1733_Article

Discussion

We utilized high resolution UCE data across the range of several troglobiotic rove beetles in karst regions of central Texas to examine phylogenetic relationships and assess whether currently understood KFR boundaries reflect diversity patterns within these species. The use of capture probes and UCE loci were highly informative in reconstructing the phylogeny of rove beetles in our study, joining other recent studies that have used UCEs to shed light on cave-dwelling invertebrates across central Texas karst regions (Derkarabetian et al. 2022; Azevedo et al. 2024).

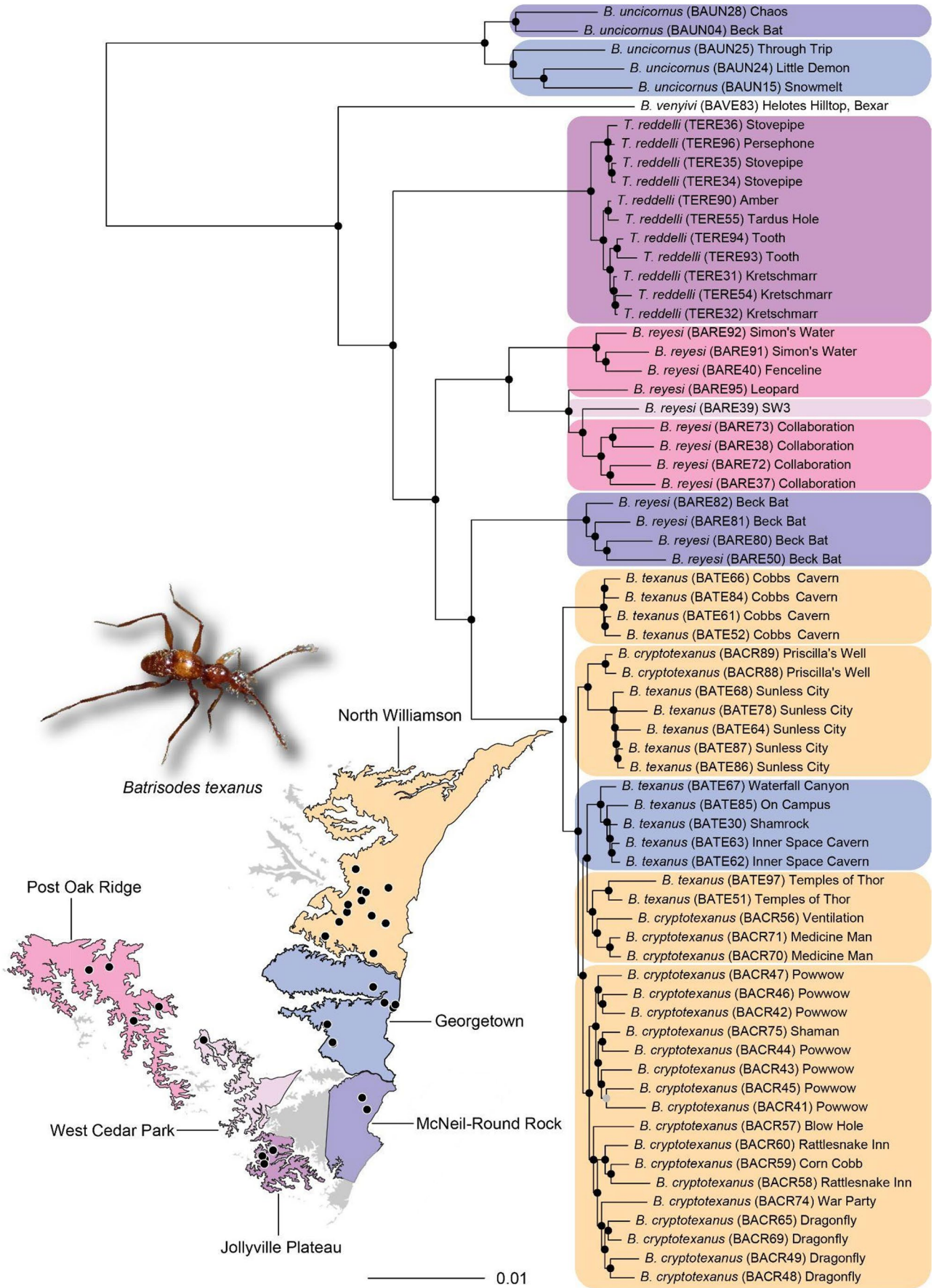

B. uncicornus (BAUN28) Chaos
B. uncicornus (BAUN04) Beck Bat
B. uncicornus (BAUN25) Through Trip
B. uncicornus (BAUN24) Little Demon
B. uncicornus (BAUN15) Snowmelt
B. venyivi (BAVE83) Helotes Hilltop, Bexar
T. reddelli (TERE36) Stovepipe
T. reddelli (TERE96) Persephone
T. reddelli (TERE35) Stovepipe
T. reddelli (TERE34) Stovepipe
T. reddelli (TERE90) Amber
T. reddelli (TERE55) Tardus Hole
T. reddelli (TERE94) Tooth
T. reddelli (TERE93) Tooth
T. reddelli (TERE31) Kretschmarr
T. reddelli (TERE54) Kretschmarr
T. reddelli (TERE32) Kretschmarr
B. reyesi (BARE92) Simon's Water
B. reyesi (BARE91) Simon's Water
B. reyesi (BARE40) Fenceline
B. reyesi (BARE95) Leopard
B. reyesi (BARE39) SW3
B. reyesi (BARE73) Collaboration
B. reyesi (BARE38) Collaboration
B. reyesi (BARE72) Collaboration
B. reyesi (BARE37) Collaboration
B. reyesi (BARE82) Beck Bat
B. reyesi (BARE81) Beck Bat
B. reyesi (BARE80) Beck Bat
B. reyesi (BARE50) Beck Bat
B. texanus (BATE66) Cobbs Cavern
B. texanus (BATE84) Cobbs Cavern
B. texanus (BATE61) Cobbs Cavern
B. texanus (BATE52) Cobbs Cavern
B. cryptotexanus (BACR89) Priscilla's Well
B. cryptotexanus (BACR88) Priscilla's Well
B. texanus (BATE68) Sunless City
B. texanus (BATE78) Sunless City
B. texanus (BATE64) Sunless City
B. texanus (BATE87) Sunless City
B. texanus (BATE86) Sunless City
B. texanus (BATE67) Waterfall Canyon
B. texanus (BATE85) On Campus
B. texanus (BATE30) Shamrock
B. texanus (BATE63) Inner Space Cavern
B. texanus (BATE62) Inner Space Cavern
B. texanus (BATE97) Temples of Thor
B. texanus (BATE51) Temples of Thor
B. cryptotexanus (BACR56) Ventilation
B. cryptotexanus (BACR71) Medicine Man
B. cryptotexanus (BACR70) Medicine Man
B. cryptotexanus (BACR47) Powwow
B. cryptotexanus (BACR46) Powwow
B. cryptotexanus (BACR42) Powwow
B. cryptotexanus (BACR75) Shaman
B. cryptotexanus (BACR44) Powwow
B. cryptotexanus (BACR43) Powwow
B. cryptotexanus (BACR45) Powwow
B. cryptotexanus (BACR41) Powwow
B. cryptotexanus (BACR57) Blow Hole
B. cryptotexanus (BACR60) Rattlesnake Inn
B. cryptotexanus (BACR59) Corn Cobb
B. cryptotexanus (BACR58) Rattlesnake Inn
B. cryptotexanus (BACR74) War Party
B. cryptotexanus (BACR65) Dragonfly
B. cryptotexanus (BACR69) Dragonfly
B. cryptotexanus (BACR49) Dragonfly
B. cryptotexanus (BACR48) Dragonfly
Batrisodes texanus
North Williamson
Post Oak Ridge
Georgetown
West Cedar Park
McNeil-Round Rock
Jollyville Plateau
0.01

Fig. 3 Majority rule consensus tree of *Batrisodes* and *Texamaurops* from maximum likelihood analysis of the concatenated matrix with 75% completeness performed in IQ-TREE. Colors correspond to karst fauna regions in Fig. 1. Black and grey dots represent UFboot node support≥95% and <95%, respectively. Photograph of *B. texanus* by Colin Strickland

We found that all troglobiotic *Batrisodes* (*Excavodes*) species included in this study form a clade. At the broad scale across the four major species groups there are two patterns to note with potential taxonomic implications. First, *B. reyesi* is paraphyletic across disjunct karst regions (Clade B in adjacent Post Oak Ridge and West Cedar Park KFRs separate from clade C in McNeil – Round Rock KFR; Figs. 3 and 4). There may be few inhabitable karst features between these occupied karst regions. This taxon could represent multiple putative species pending further morphological evaluation. The second is the placement of *Texamaurops* (Clade A, Jollyville Plateau KFR) within *Batrisodes*. Sharing similar morphologies, Chandler (1992) described *T. reddelli* as a likely derived lineage of other troglobiotic *Batrisodes* that is only distinguished by a lack of the metatibial pencil of setae. However, recently described *Batrisodes* species discovered outside of central Texas exhibit further variability or absence of metatibial setae (Ferro and Carlton 2014; Jiang and Yin 2017), suggesting that this character may not warrant separation of *T. reddelli* from other *Batrisodes*. Further, a more comprehensive cladistic analysis of 96 *Batrisodes* taxa and outgroups based on 81 morphological characters placed *T. reddelli* within the *Batrisodes*, sister to most *Batrisodes* (*Excavodes*) including those in our study, apart from *B. venyivi* which was nested within the *Batrisodes* (*Babnormodes*) clade (Vasquez-Velez 2020). Similarly, our findings do not support the concept of *Texamaurops* as an independent genus.

Another major focus of our sampling was to shed light on the relationships between *B. cryptotexanus* and *B. texanus* to help inform the conservation management of this species group. A monophyletic subclade including all *B. cryptotexanus* and *B. texanus* was consistently recovered in our analyses, with strong regional genetic structuring (Clade D Fig. 5). However, we did not recover *Batrisodes texanus* and *B. cryptotexanus* as monophyletic groups. Instead, multiple clades of *B. texanus* and *B. cryptotexanus* are interspersed on the tree (Fig. 5).

Genetic diversity of *B. cryptotexanus* and *B. texanus* across KFRs

Separate species designation was conferred to *B. cryptotexanus* from populations originally described as *B. texanus* based on observed morphological differences in the size and shape of abdominal tergite five and sternite six (Candler and Reddell 2001; Chandler et al. 2009; see Figure S4 for examples of morphological differences). Based on limited sampling at the time of description, *B. cryptotexanus* was described as "definitely known only from caves north of the North Fork San Gabriel River, which has cut through the cavernous limestone and thus serves as a barrier separating this species from *B. texanus"* (Chandler and Reddell 2001, pp. 116). The recognition of *B. cryptotexanus* as a new species contributed to the designation of the North Fork San Gabriel River as a barrier that could restrict troglobiont dispersal through down-cutting and removal of cavernous bedrock; it also divides the Georgetown and North Williamson KFRs (Veni and Jones 2021). However, subsequent sampling efforts led to new populations with the morphology of *B. texanus* being discovered on either side of both the North Fork and South Fork San Gabriel Rivers. Populations found at Waterfall Canyon Cave (between the forks) and Sunless City Cave, and Cobbs Cavern (north of both forks) in the North Williamson KFR (Chandler et al. 2009; USFWS 2018; current study) led to further ambiguity in the species ranges and overlap, and the hypothesis that the river was a fundamental boundary leading to the diversification of the two species (Veni 1992; Veni and Jones 2021).

Rather than supporting *B. texanus* and *B. cryptotexanus* as separate monophyletic groups, we find genetic divergence among geographically clustered subclades inferred by both phylogenetic and sNMF analyses. Further, the progression of clades appears to generally align with the temporal geologic progression of available karst habitat as well as drainage basin evolution, supporting considering overarching hydrogeologic processes related to drainage formation and underlying temporal geologic processes in the evolution of this group. The most distinctive group appears to be the Cobbs Cavern population (Clade D1, Fig. 5). Cobbs Cavern is one of the largest caves in central Texas with more than 1,000 m of passage. Cobbs Cavern recharges Cobbs Spring and both features occupy an upland outcrop of karstic Edwards Limestone that is isolated from other karst habitats by two factors. First, the karst rock has been completely eroded away to the north and west. Second, although Cobbs Cavern sits next to karst rock to the southeast, a northeast-to-southeast trending fault about 600 m east of the cave has shifted the Edwards Limestone so that it is no longer connected across the fault. The fault likely blocks habitat connectivity, especially for terrestrial species, since conduits crossing the fault plane may be below the aquifer base level (Collins 1997; White 2011 Figs. 2 and 4). This isolation may contribute to the unique genetic diversity observed in the Cobbs Cavern population.

The North and South Forks of the San Gabriel River, separating the North Williamson and Georgetown KFRs are the largest and most deeply incised streams crossing the northern

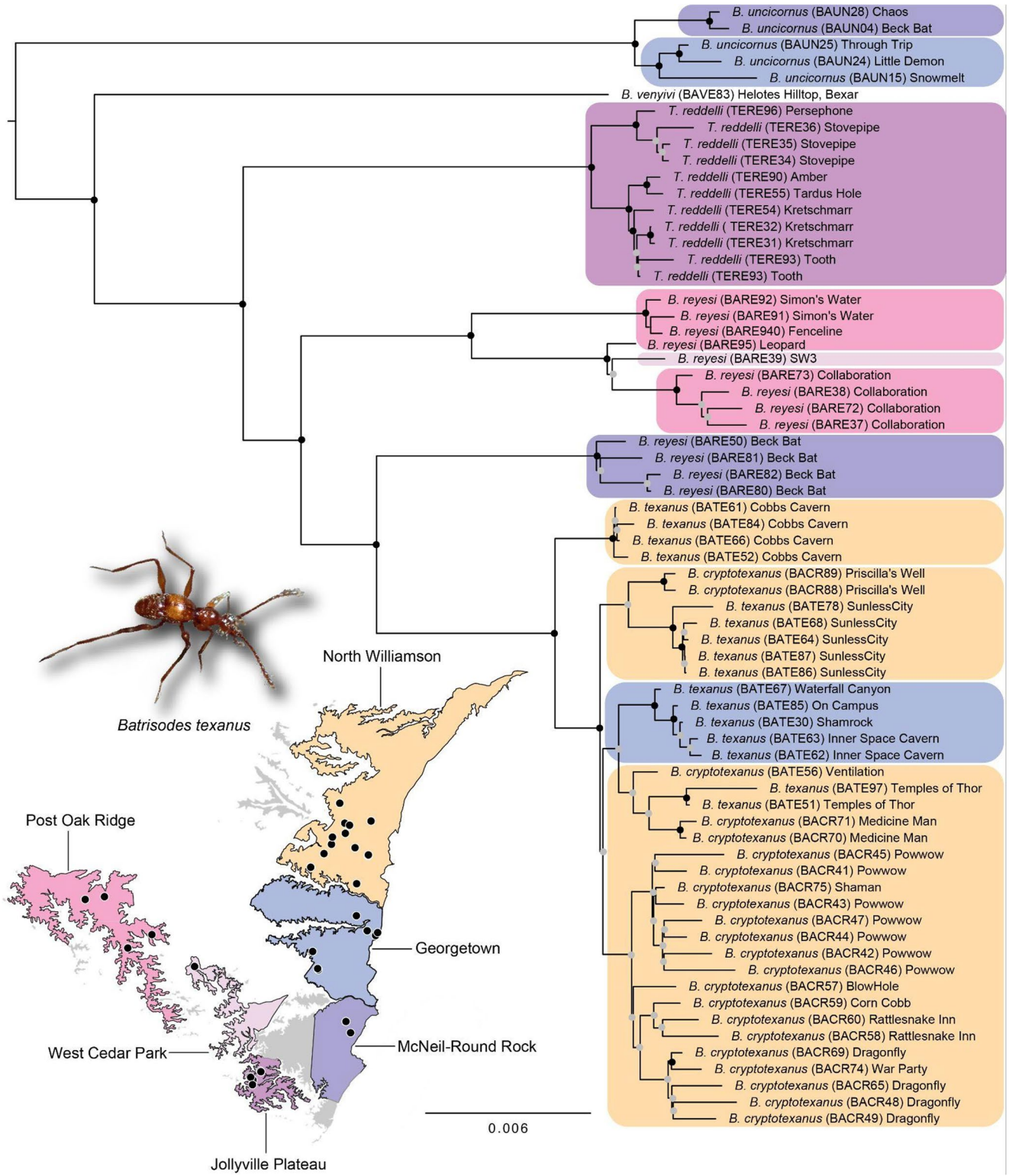


Fig. 4 Species tree of *Batrisodes* and *Texamaurops* species estimated using ASTRAL-IV. Local posterior probabilities ≥ 0.95 are shown as black circles and ≤ 0.94 shown as grey circles supporting each node are shown. Colors correspond to karst fauna regions in Fig. 1. Photograph of *B. texanus* by Colin Strickland

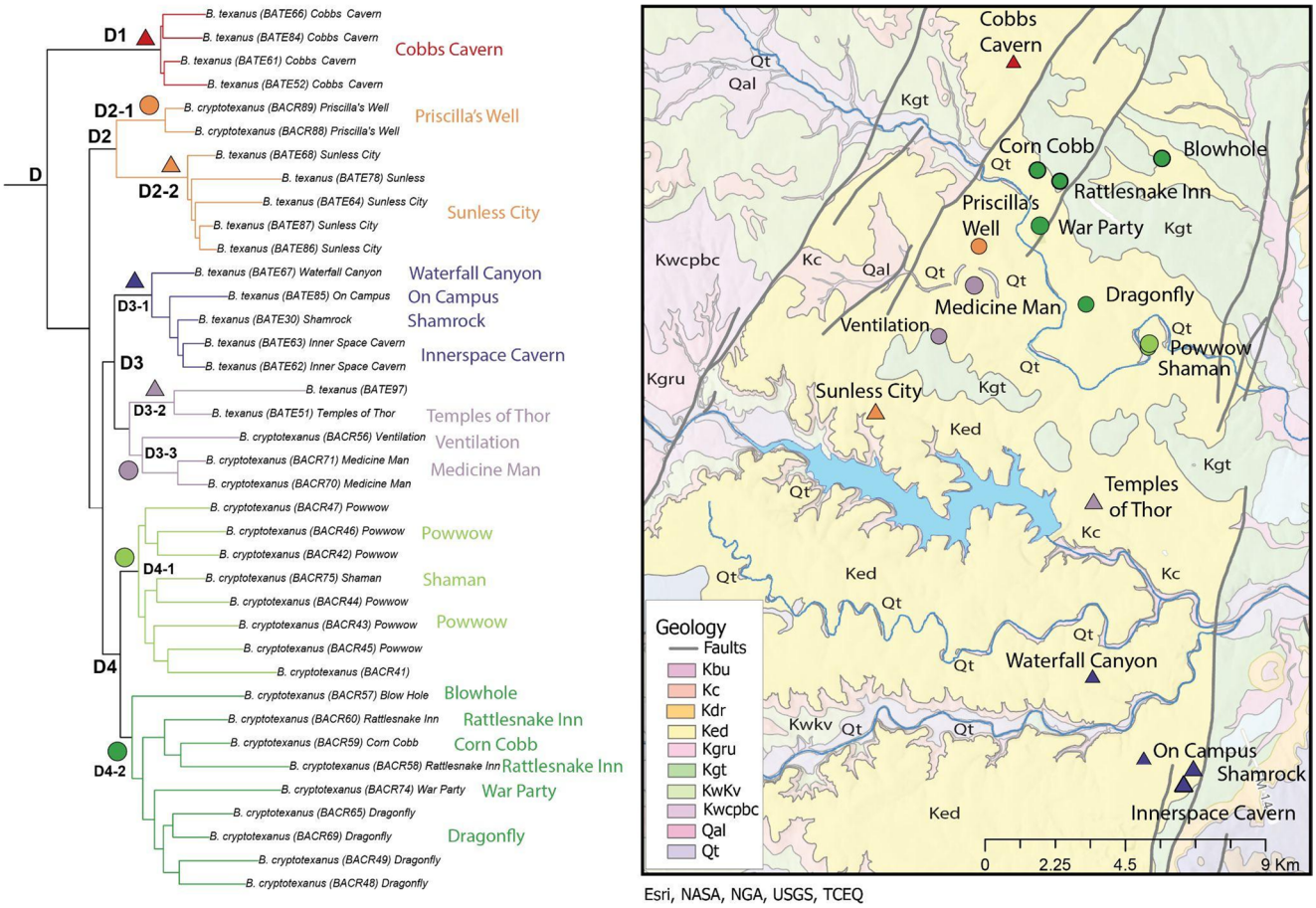


Fig. 5 Close-up of the *B. cryptotexanus* + *texanus* clade from the concatenated tree from the 75% complete data matrix and corresponding map including faults (mapped at 24 K) and rock formations from the USGS, Geologic Database of Texas. Subclades are colored along with their corresponding collection locations. Triangles are locations where individuals were morphologically identified as *B. texanus*, circles are locations where individuals were morphologically identified as *B. cryptotexanus*. Geological formation codes are as follows: Kbu- Buda Formation, Upper Cretaceous, Kc- Comanchean rocks (Lower Cretaceous), Kdr- Del Rio Clay, Upper Cretaceous, Ked- Edwards Limestone (Lower Cretaceous), Kgru- upper Glen Rose limestone (Lower Cretaceous), Kgt- Georgetown Limestone (Lower Cretaceous), Kwkv and Kwcpbc- Cretaceous, Qal- Alluvium (Quaternary), Qt- Terrace deposits (Quaternary). (Map sources: Esri, NASA, NGA, USGS and TCEQ)

segment of the Edwards (Balcones Fault Zone) Aquifer (Senger et al. 1990; Veni 1992). They have downcut through the Edwards Limestone creating almost complete separations in the karst rock, which splits populations - one group (D3–1) in the Georgetown KFR and others (D3–2 and D3–3) in the Williamson KFR. In contrast, the Berry Creek drainage basin is far less incised and is therefore understood as a relatively immature karst terrain, with caves that were exposed more recently. Subclade D4 (Fig. 5) appears restricted to the Berry Creek drainage basin which has been more recently down-cut into the karstic Edwards Limestone formation. Further clustering analyses suggest some mixed assignment in clade D4, consistent with retained ancestral polymorphism and more recent geographic isolation. Therefore, in summary, we find that the genetic patterns observed in the *B. cryptotexanus* + *texanus* clade could be explained by progressive availability of karst habitat as the Edwards became exposed (White et al. 2009).

The apparent mismatch between genetic relationships and morphological taxonomy could indicate that there is a continuum of morphological variability across populations, although there is no evidence of intermediates in examined specimens to date, including all specimens sequenced here (DSC unpubl. data). Another possibility is that there has been some morphological convergence across multiple independent caves or cave complexes. More generally throughout the genus *Batrisodes*, it has been noted that cave-adapted species have reduced character differences and evidence of morphological convergence across major lineages (Vasquez-Velez 2020). In this group, abdominal morphology, including characters that differentiate *B. texanus* and *B. cryptotexanus*, may play an important role in allowing proper attachment during copulation. It is plausible that morphological changes in structures that confer a mating advantage could be driven rapidly via sexual selection in isolated cave populations (Vasquez-Velez

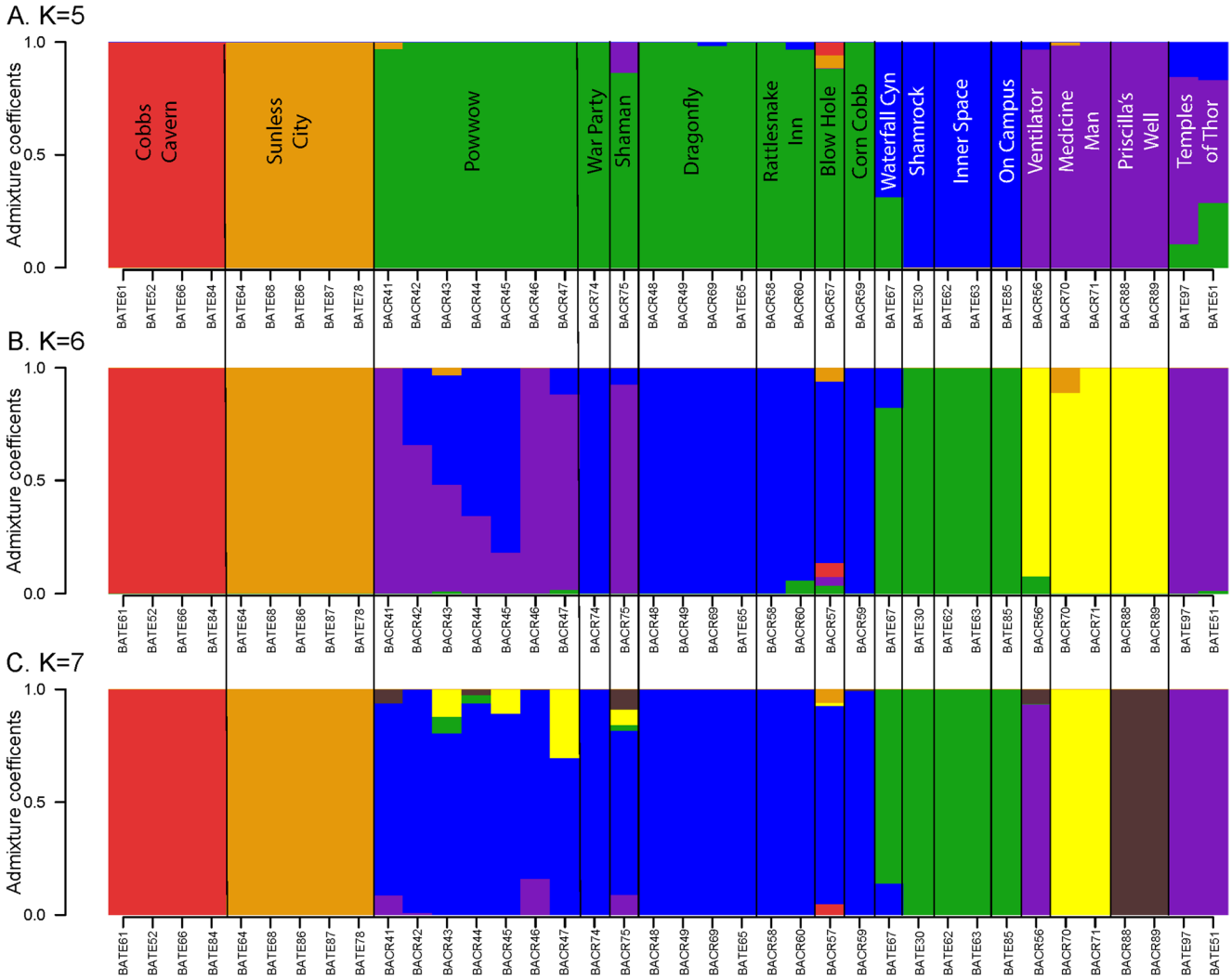


Fig. 6 *Batrisodes texanus/cryptotexanus* individual co-ancestry proportions based on 493 SNPs. Best supported assignment for (**A**) K = 5, (**B**) K = 6, and (**C**) K = 7

2020). Alternatively, given relatively short branch lengths across subclades, the mismatch could also reflect incomplete lineage sorting or recent gene flow between previously isolated lineages (e.g., Hart et al. 2024). Further morphological and genitalic examination utilizing greater sample sizes could be a useful step prior to any formal taxonomic revision.

Conservation status of *B. cryptotexanus* + *texanus*

Currently this lineage is treated as a single endangered species (*B. texanus* inclusive of *B. cryptotexanus*) by the U.S. Fish and Wildlife Service (USFWS 2025). The most recent status review (USFWS 2025) evaluated the habitat resiliency of occupied single caves or cave complexes based on surface habitat features including the surrounding area of naturally vegetation, distance of cave entrance to the nearest edge, and the status of cave cricket foraging areas surrounding each cave, as cave crickets are thought to be the most prevalent source of nutrient input into these cave systems (Taylor et al. 2007). Although we did not find a relationship between nucleotide diversity and current resiliency status within clusters (Table 2), efforts to protect the unique genetic variation represented in each genetic cluster or clade could help maximize representation and resiliency across the species complex (e.g., Forester and Lama 2023). At the finest (and most conservative) scale of genetic delineation, three of the seven genetic clusters have habitat resiliency that is low (Sunless City Cave) or impaired (Medicine Man Cave and the whole of genetic cluster K6/Clade D3–1 restricted to the Georgetown KFR). Future efforts to protect and enhance cave habitat could be prioritized within the clusters to protect the unique genetic variation that they include. At the broadest clade level, all clades contain at least some caves with high resilience habitat.

Table 2 Current resiliency of *Batrisodes texanus* and *B. cryptotexanus* for cave clusters and single caves based on USFWS (2025). See Fig. 5 for clades and Fig. 6C for clusters. KFR NW = North Williamson, GT = Georgetown N = number of individuals and average π = average nucleotide diversity per cluster

Clade	Cluster (K = 7)	KFR	Cave	*N*	Average π	Cave Cluster or Individual Cave (USFWS 2025)	Resiliency (USFWS 2025)
D1	**K1**	**NW**	**Cobbs Cavern**	**4**	**0.0206**	**Cobbs Cavern**	**High**
D2	**K2**	**NW**	**Sunless City**	**5**	**0.0338**	**Sunless City Cave**	**Low**
D2	**K3**	**NW**	**Priscilla's Well**	**2**	**0.1102**	**Priscilla's Well Cave**	**High**
D3	**K4**	**NW**	**Medicine Man**	**2**	**0.0493**	**Medicine Man Cave**	**Impaired**
D3	K5	NW	Ventilation	1		Ventilation & Viper Cave Cluster	Impaired
D3	K5	NW	Temples of Thor	2		Godwin Ranch Preserve	High
	K5	**NW**	**Total**	**3**	**0.0848**		**High, Impaired**
D3	K6	GT	Waterfall Canyon	1		Waterfall Canyon Cave	Impaired
D3	K6	GT	On Campus	1		On & Off Campus Cave Cluster	Impaired
D3	K6	GT	Shamrock	1		Inner Space Cave Cluster	Impaired
D3	K6	GT	Interspace Cavern	2		Inner Space Cave Cluster	Impaired
	K6	**GT**	**Total**	**5**	**0.0165**		**Impaired**
D4	K7	NW	Powwow	7		Shaman Preserve	High
D4	K7	NW	Shaman	1		Shaman Preserve	High
D4	K7	NW	War Party	1		Karankawa Preserve	High
D4	K7	NW	Dragonfly	4		Dragonfly & Deliverance No. 2 Cave Cluster	Impaired
D4	K7	NW	Rattlesnake Inn	2		SH-195 Cluster	Impaired
D4	K7	NW	Corn Cobb	1		SH-195 Cluster	Impaired
D4	K7	NW	Blow Hole	1		Blowhole	High
	K7	**NW**	**Total**	**17**	**0.0190**		**High, Impaired**

Comparisons with other karst invertebrates

Similar phylogenetic patterns have been observed in other karst invertebrates occurring in these karst regions (Ledford et al. 2012; Gómez et al. 2016; Derkarabetian et al. 2022). Ledford (2023) recovered a Cobbs Cavern endemic spider, *Tayshaneta anopica*, further supporting the isolation and distinctiveness of the Cobbs Cavern system. In addition, a recent conservation genomic study of troglobiotic *Texella* harvesters (Opiliones: Phalangodidae) showed some clade relationships (specifically in *Texella reyesi*) that mirror those presented here (Derkarabetian et al. 2022). Individuals from Sunless City and Pricilla's Well Caves (subclade D2 Fig. 5) shared a close relationship in both studies, for example, despite being geographically separated by other cave systems (Ventilator and Medicine Man Caves) that, in our study, form a subclade with the Georgetown KFR *Batrisodes cryptotexanus* + *texanus* group (subclade D3 Fig. 5). Genetic similarities between distant caves that are greater than those between more proximal caves could be explained by the progressive availability of karst habitats over time (White et al. 2009). While all *Texella reyesi* populations sampled from the Georgetown and North Williamson KFRs formed a clade, Derkarabetian et al. (2022) consistently recovered divergence between populations north of the North Fork San Gabriel River and those populations that occur south of the river, which may mirror patterns in *Batrisodoes cryptotexanus* + *texanus* clade D3. However, *Tayshaneta* spiders (Araneae: Leptonetidae) showed significant admixture between populations in the Georgetown and North Williamson KFRs, though this may be attributed to potential surface-dispersal capacities of these species (Ledford 2023). While we find some concordance across species, remaining differences highlight that further genomic studies of additional karst invertebrate species groups could better elucidate the biogeographic significance of the North Fork San Gabriel River and other features in this region.

Conservation of karst invertebrate diversity

Together, previous karst invertebrate genomic studies and the results presented here support recognizing subregional diversity within the North Williamson KFR, particularly in relation to drainage basins and fault blocks. The spatial distribution and genetic relationships of the morphologically defined *B. cryptotexanus* + *texanus* are highly interspersed, but geographic structuring of clades provides a more nuanced picture of diversification in this group that may have conservation implications. Cobbs Cavern, for example, stands out as an isolated and potentially older karst complex within the North Williamson KFR. In addition, the phylogenetic relationships among the caves appear

to reflect the geologic history of the region and support that both relay ramps of block-faulted limestone and drainage basin evolution have played an important part in diversification of these beetles (White et al.2009). Recognizing these processes in developing regional conservation units within the karst habitats of central Texas could improve representation and conservation of genetic diversity within this unique group of invertebrates.

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Author contributions PLW: methodology, formal analysis, visualization, data curation, writing – original draft, review and editing. DSC: resources, investigation, writing – review and editing. NG: visualization, writing – original draft, review and editing. AM: methodology, investigation, writing – review and editing. JGS: methodology, investigation, writing – review and editing. KW: resources, writing – original draft, review and editing. JW: Project administration, funding acquisition, resources, writing- review and editing. AGV: conceptualization; funding acquisition, project administration, supervision, methodology, data analysis, visualization, writing – original draft, review and editing.

Data availability Demultiplexed sequences are available on NCBI SRA (Bioproject PRJNA1228651). All alignments and tree files are available from DRYAD https://doi.org/10.5061/dryad.djh9w0wcg.

Declarations

Competing interests The authors declare no competing interests.

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