Subterranean Biology 47: 1–27 (2023)
doi: 10.3897/subtbiol.47.113186
https://subtbiol.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
Subterranean Biology
Published by The International Society for Subterranean Biology

Two new phreatic snails (Mollusca, Caenogastropoda, Cochliopidae) from the Edwards and Edwards-Trinity aquifers, Texas

Kathryn E. Perez[1], Yamileth Guerrero[1], Roel Castañeda[1], Peter H. Diaz[2], Randy Gibson[3], Benjamin Schwartz[4,5], Benjamin T. Hutchins[4]

1 *School of Integrative Biological and Chemical Sciences, University of Texas Rio Grande Valley, Edinburg, TX, 78542, USA* **2** *Texas Fish and Wildlife Conservation Office, United States Fish and Wildlife Service, San Marcos, TX, USA* **3** *Aquatic Resources Center, United States Fish and Wildlife Service, San Marcos, TX, USA* **4** *Edwards Aquifer Research and Data Center, Texas State University, San Marcos, TX, 78666, USA* **5** *Department of Biology, Texas State University, San Marcos, TX, 78666, USA*

Corresponding author: Kathryn E. Perez (perezke@gmail.com)

Academic editor: Matthew L. Niemiller | Received 23 September 2023 | Accepted 8 November 2023 | Published 4 December 2023

https://zoobank.org/2DA26BD8-3066-4B88-8DD9-4EE8E9017E29

Citation: Perez KE, Guerrero Y, Castañeda R, Diaz PH, Gibson R, Schwartz B, Hutchins BT (2023) Two new phreatic snails (Mollusca, Caenogastropoda, Cochliopidae) from the Edwards and Edwards-Trinity aquifers, Texas. Subterranean Biology 47: 1–27. https://doi.org/10.3897/subtbiol.47.113186

Abstract
The Edwards and Edwards-Trinity Aquifers of Texas have diverse stygofauna, including fifteen species of snails found in phreatic and hyporheic habitats. These species have the hallmarks of adaptation to subterranean environments including extremely small body size and the loss of pigmentation and eyes. Here we use an integrative taxonomic approach, using shell, radula, and anatomical features as well as mitochondrial and nuclear DNA data, to circumscribe a new genus and two new cavesnail species from Central Texas. *Vitropyrgus lillianae* **gen. et sp. nov.** is described from Comal Springs (Comal County) and Fessenden Springs (Kerr County) and distinguished by a glassy, highly sculptured shell and distinctively simple, unornamented penial morphology. We also describe *Phreatodrobia bulla* **sp. nov.** from Hidden Springs (Bell County), and several other springs in Bell & Williamson Counties, Texas. This species has a smooth, unsculptured teleoconch, a reflected and flared lip, and deeply concave operculum.

Keywords
Cave snails, Edwards Trinity Aquifer System, groundwater, hyporheic, phreatic, spring

Introduction

The Edwards, Trinity, and Edwards-Trinity aquifers, constitute a large, interconnected karst aquifer systems in Texas and Northern Mexico and provide habitat for a diverse stygofauna (Longley 1981) including snails, crustaceans, worms, beetles, fish, and salamanders. These aquifers are the primary drinking water source for several large metropolitan areas (Maclay 1995) and support ranching and agriculture across a large portion of the Edwards Plateau. The Edwards (Balcones Fault Zone) Aquifer, a long, narrow region of faults, is a particularly biodiverse and productive subterranean ecosystem (Hutchins et al. 2016) which harbors a diversity of subterranean snails commonly called cave snails.

Cave or phreatic snails inhabit groundwater systems such as subterranean streams and aquifers (Hershler and Longley 1986b). Recent surveys have also found some species in hyporheic habitats (Alvear et al. 2020a; Hutchins et al. 2020). They are typically small (<3 mm), depigmented, and eyeless, with a suite of additional morphological adaptations attributed to the effects of miniaturization (Rysiewska et al. 2016; Osikowski et al. 2017; Falniowski 2018). These include loss or reduction of gills, lengthy coiled intestines, simplification of gonadal organs (Hershler and Holsinger 1990), and convergent evolution of characteristic shell shapes (Falniowski 2018). The phreatic snails of the Edwards and Edwards-Trinity Aquifers include 14 nominal species, with nine species in the genus *Phreatodrobia* Hershler & Longley, 1986. There are also several monotypic genera including: *Phreatoceras* (Hershler & Longley, 1986), *Stygopyrgus* Hershler & Longley, 1986, and *Texapyrgus* Thompson & Hershler, 1991. *Balconorbis* Hershler & Longley, 1986 has a single species endemic to Texas with an additional species in contiguous limestone regions of northern Mexico. Finally, the species referred to as *Tryonia diaboli* (Pilsbry & Ferriss, 1906) is a subterranean species, but requires taxonomic study to determine its generic assignment.

Phreatic snails of the Edwards and Edwards-Trinity Aquifers are all members of the Cochliopidae Tryon, 1866, a group of small freshwater and estuarine snails, comprising 260+ species found in the Nearctic, Neotropical, and Afrotropical regions of the world (Clark 2019). The family was, until recently, part of the Hydrobiidae Stimpson, 1865 which included 400+ genera and is undergoing splitting and taxonomic revision (Wilke et al. 2001; Wilke et al. 2013; Wilke and Delicado 2019). The most recent revisionary work (Hershler and Thompson 1992), divided the Cochliopidae into three subfamilies, Cochliopinae Tryon, 1966, Littoridininae Thiele, 1928, and Semisalsinae Giusti & Pezzoli, 1980 largely diagnosed by glandular features of the male reproductive anatomy. But the relationships among Cochliopidae remain unsettled with nearly twenty genera not assigned to a subfamily, including *Phreatodrobia* and the other subterranean genera from the Edwards and Edwards-Trinity. Surveys of this region encountered phreatic snail populations that were provisionally identified from gross shell morphology as nominal species (Alvear et al. 2020a; Alvear et al. 2020b; Gibson et al. 2021a, 2021b; Hutchins et al. 2021), however additional sampling and more extensive study has allowed these to be distinguished. In this study, we use mitochondrial and nuclear genetic data along with anatomical characteristics to examine relationships among the cave snails of central Texas and describe two new species from this diverse region.

Materials and methods

Groundwater was sampled using different methods as appropriate for the substrate or habitat (Fig. 1). Flowing springs and wells with outflow pipes were sampled by placing a 100 μm drift net into the spring opening, securing it with available cobble or by wedging it between rocks (Fig. 2). The net remained in place for 2–7 days. Well samples where there was no outflow pipe were sampled using a bottle trap baited with pistachios left in place for two weeks. Mophead samples were taken by placing the cotton head of a mop into the spring for ~30 days. Hyporheic samples were taken by Bou-Rouch sampling (Bou and Rouch 1967), where a stainless steel spike was hammered 30–50 cm into the hyporheic zone of a stream or gravel bed, allowing water to be pumped through a 100 μm mesh net. Bou-Rouch samples were further processed using a modified elutriation method (Lackey and May 1971) to remove sediments and retain organic materials, including animals. The sample is washed and agitated using faucet water with a small hose to flush lower-density organic materials (including snails and snail shells) out of a sorting tray and into a 100 μm filter. Inorganic sediments remain in the bottom of the tray. Sediments are repeatedly washed until all organic materials are removed. All samples were immediately preserved in 95% ethanol (refreshed at least once and stored in a refrigerator) until sorting under a dissecting microscope.

Types and paratypes are deposited in the Philadelphia Academy of Natural Sciences at Drexel University (**ANSP**). Additional reference materials include lots from the Texas Memorial Museum (**TMM**) and paravouchers deposited in the Texas State University Aquifer Biodiversity Collection (ABC). For some of the methods, such as DNA extraction, the animals are destroyed, paravouchers are intact individuals from the same population.

Photovouchers were created prior to DNA extraction since the procedure resulted in the dissolution of fragile shells (Suppl. materials 2–4). DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit, incubated at 65 °C for 24 hours. The same digestion was used to retain radula and opercula as well as DNA. PCR was conducted using the Platinum SuperFi DNA Polymerase Master Mix Kit (Thermo-Fisher). We followed the manufacturer protocol for PCR, conducting a temperature gradient between 48–54 °C for each species and proceeding with the optimal temperature, typically 51.7 °C. Primers included COIH2198 and COIL1490 (Folmer et al. 1994) for COI and LSU 1 & 3 (Wade et al. 2001) for the nuclear Large Ribosomal Subunit (LSU). Amplicons were purified using the PCR DNA Fragment Extraction Kit (IBI Scientific, Peosta) and quantified with a Qubit 3.0 Fluorometer and Qubit dsDNA HS Assay Kit (Invitrogen). DNA sequencing was conducted at Eton Biosciences, Inc.

Figure 1. Spring sites in Texas. Type locality of *Vitropyrgus lillianae* gen. et sp. nov. Comal Springs Upwelling #7, New Braunfels, Comal County **A** at normal water flow conditions, photo by Marcus Gary **B** at low water flow conditions, photo by Randy Gibson **C** drift nets in water flow at Fessenden Springs, Kerr County, photo by K.E. Perez **D** type locality of *Phreatodrobia bulla* sp. nov. Hidden Springs, Bell County, photo by P. Diaz **E** Robertson spring run hyporheic sampling site, Bell County, photo by K.E. Perez.

Following sequencing, Geneious 10.2.6 was used to assemble contigs, trim and visually inspect sequences, and align them using the MUSCLE algorithm. Model selection (Kalyaanamoorthy et al. 2017), Maximum likelihood analyses (Nguyen et al. 2015), and 10,000 ultrafast bootstrap replicates (Hoang et al. 2018) were conducted in IQTREE 1.6.12. For COI a 74 terminal alignment was assembled including all new sequences generated and members of all genera in the Cochliopidae available on Genbank. *Pomatiopsis lapidaria* (Say, 1817) was included as an outgroup. The COI alignment was partitioned to allow evaluation of 1st, 2nd, and 3rd codon positions separately. PartitionFinder and ModelFinder (via IQTREE) were used to assess whether partitions should be analyzed separately or merged and to determine the best fit model for each partition.

For the nuclear LSU gene an alignment with all available sequences (23) was generated using MUSCLE as implemented at https://www.ebi.ac.uk/Tools/msa/muscle/ and analyzed in IQ-TREE with 10,000 ultra-fast bootstrap replicates. Other Cochliopidae were not available for this locus, so *Pyrgulopsis* Call & Pilsbry, 1886

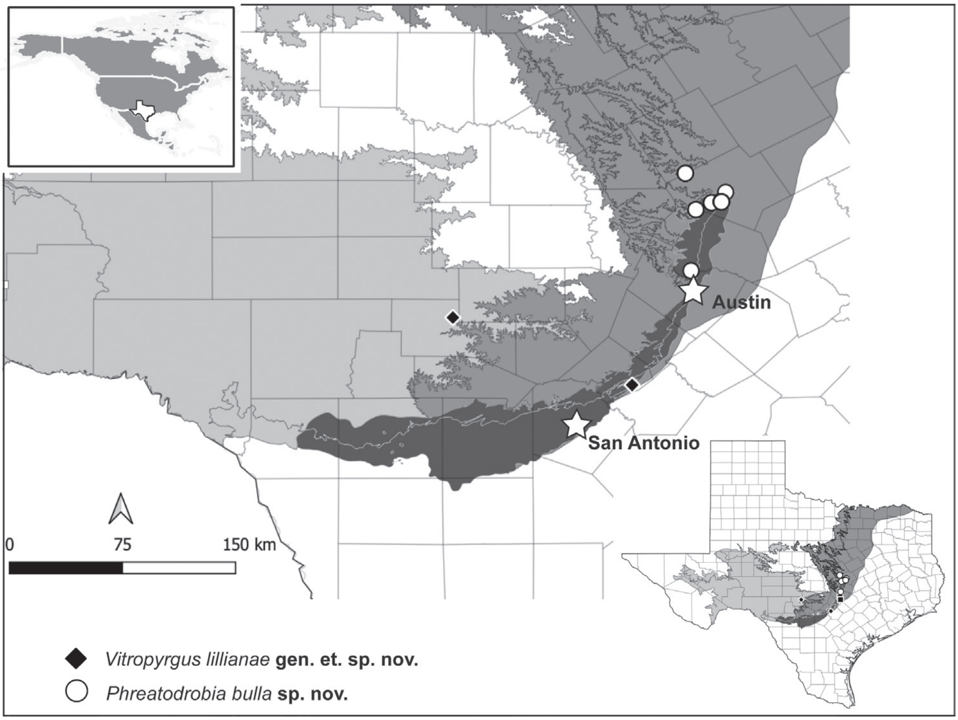

Figure 2. Map with known localities for *Vitropyrgus lillianae* gen et. sp. nov. and *Phreatodrobia bulla* sp. nov. The Edwards-Trinity Aquifer System is shown in gray shades with the lightest shade indicating the Edwards-Trinity, the medium gray indicating the Trinity, and the darkest portion indicating the Edwards portions. Major cities are indicated with a star and name label. Inset maps indicate the region of North America and state of Texas.

species were used as the outgroup. Model selection was conducted in IQ-TREE using the Bayesian Information Criterion (BIC). For the 74-terminal alignment for COI, the following models were used for 1st positions: TNe+I+G4, 2nd positions: TPM3u+F+I+G4, and 3rd positions: HKY+F+G4. For the LSU alignment (23 terminals) HKY+F+I+G4 was identified as the best fit model. Mega 11 was used to calculate P-distance (Tamura et al. 2013).

Anatomical terms follow recent works (Hershler and Longley 1986b; Hershler and Ponder 1998) on the Cochliopidae or Hydrobiidae. For anatomical examination, the calcareous portion of the shells were dissolved in 50% hydrochloric acid, then remaining proteinaceous material was removed by hand. Dissections were conducted in 70% ethanol and/or with the addition of Bouin's solution or 10% Eosin to allow visual contrast and hardening of tissues, resulting in a yellow color in anatomical photos. The unstained tissue of the species examined here are unpigmented and appear white after preservation in ethanol. Dissections and examination of shells and anatomical features were carried out using a Leica S9i, and the Leica LAS X software. Images were stacked using Helicon Focus. Shell measurements were taken using either Leica LAS X or AmScope calibrated with a S78-StageMicrometer 1mm/0.01 Div. Whorls were counted according to Burch (1989).

Scanning electron micrograph (SEM) images of *Vitropyrgus lillianae* gen. et sp. nov. were acquired using the methods of Perez et al., (2022) with a working distance of 10 mm and spot size between 48 and 52. Materials for SEM images of *Phreatodrobia bulla* sp. nov. were prepared with 75 angstroms of gold palladium alloy using a Quorum Sputter coater and imaged using a Zeiss EVO LS10, in high vacuum, at 20–100k magnification. The usual working distance was 4.5–5 mm, spot size was 242, and accelerating voltage was 10.94 kv.

Results

Phreatic snails were examined from 35 sites for this study (Suppl. material 1) and localities of the proposed new species are shown in Fig. 2.

An alignment of 74 COI sequences with 657 characters, 264 parsimony-informative, was analyzed using maximum likelihood. The ML tree (Fig. 3; best score = -7748.825) showed a close relationship between *Vitropyrgus lillianae* gen. et sp. nov. and a clade of epigean fresh and brackish water genera including *Onobops* F.G. Thompson, 1968 and *Heleobia* W. Stimpson, 1865. This relationship was not strongly supported with a bootstrap value of 36. *Phreatodrobia bulla* sp. nov. was sister to *Phreatoceras taylori* (Hershler & Longley, 1986) from the same region, but within the clade of *Phreatodrobia* species. P-distances calculated from COI were used to compare sequence divergence among genera, species, and populations in this group. COI was used because it is the DNA barcoding region, potentially allowing comparison with other phreatic Cochliopidae or Hydrobiidae. Intergeneric sequence divergence averaged 16.54% (range 5.19–25.37). Sequence divergence between *Vitropyrgus* gen. nov. and other genera in the clade (e.g., *Onobops* and *Heleobia*) averaged 16.0% (range 15.5–16.6). Interspecific sequence divergence averaged 10.7% (range 4.1–14.2) across all comparisons. Intraspecific sequence divergence averaged 2.5% (range 0–12.3) across all comparisons. Some species exhibited notably higher intraspecific divergence: 3.8% (range 3.8–5.7) for *Phreatodrobia nugax* and 12.2% (range 12.2–12.3) for *Balconorbis uvaldensis*. *Phreatodrobia bulla* sp. nov. was placed sister to *Phreatoceras* Hershler & Longley, 1986 (average sequence divergence between the species = 6%, range 6.2–5.7) with weak bootstrap support, and both species were embedded in the clade with other *Phreatodrobia* species. A small amount of sequence divergence exists between two subclades of *P. bulla*, with individuals from Tahuaya and Anderson springs more closely related to each other (average p-distance 0.9%, range 1.2–0.2) than to individuals from Solana Ranch springs (average p-distance 2.1%, range 2.3–1.9).

The LSU alignment had 23 sequences, 947 characters, with 224 parsimony-informative. A single ML tree with a score of -3416.312 was found (Fig. 4). Analysis

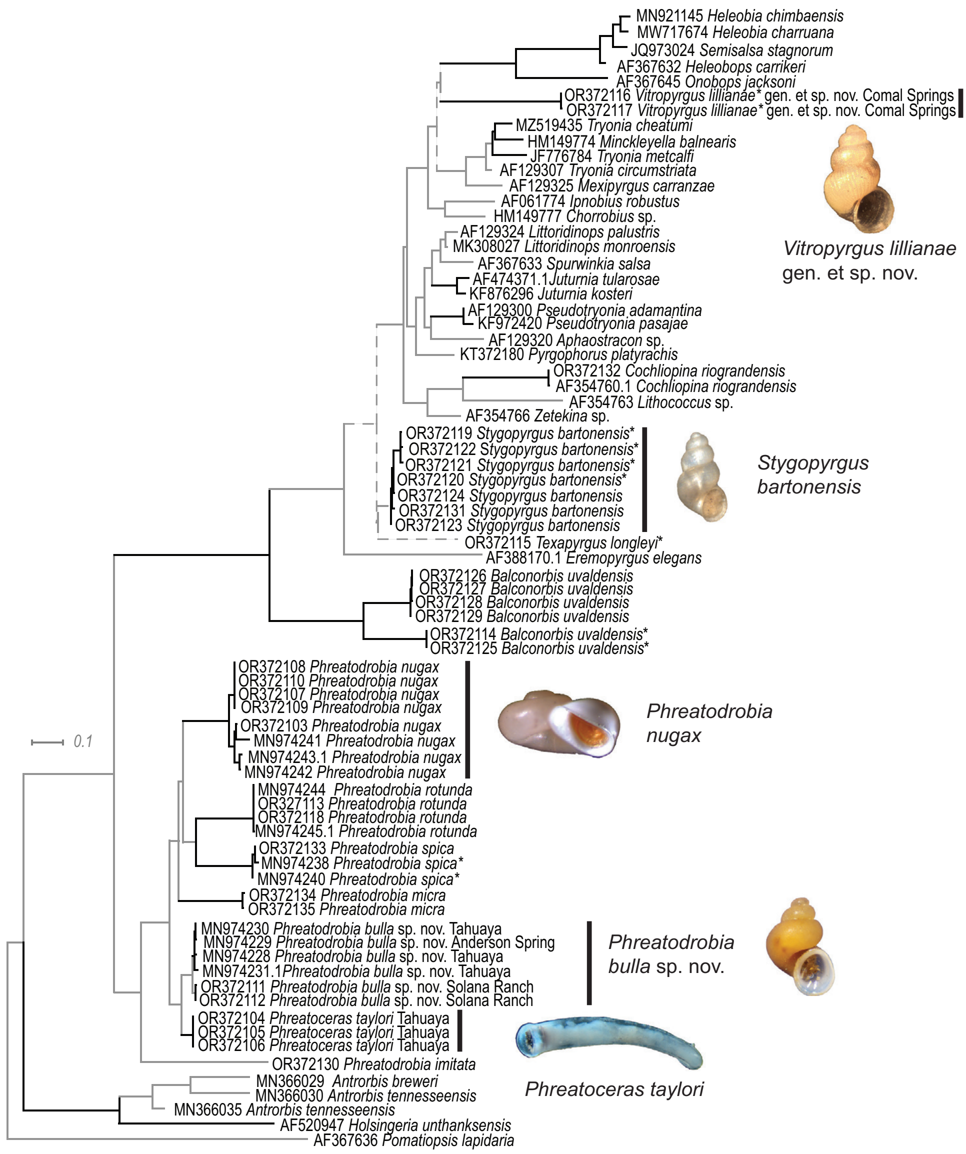

Figure 3. Highest likelihood trees resulting from maximum-likelihood analysis of COI alignment. Ultrafast bootstrap values >95% are shown with black branches, 50–94% with gray branches and <50 with dashed gray branches. Terminals are labeled with Genbank voucher numbers, species, and sampling locality for our focal taxa. Type localities are indicated with an *. The new species and select relatives are figured.

recovered each of the proposed new species as distinct monophyletic groups. Relationships are not entirely congruent with the COI tree, with the placement of *Tryonia* closer to *Stygopyrgus* than *Vitropyrgus*, however, there is relatively little support for these relationships in either gene tree.

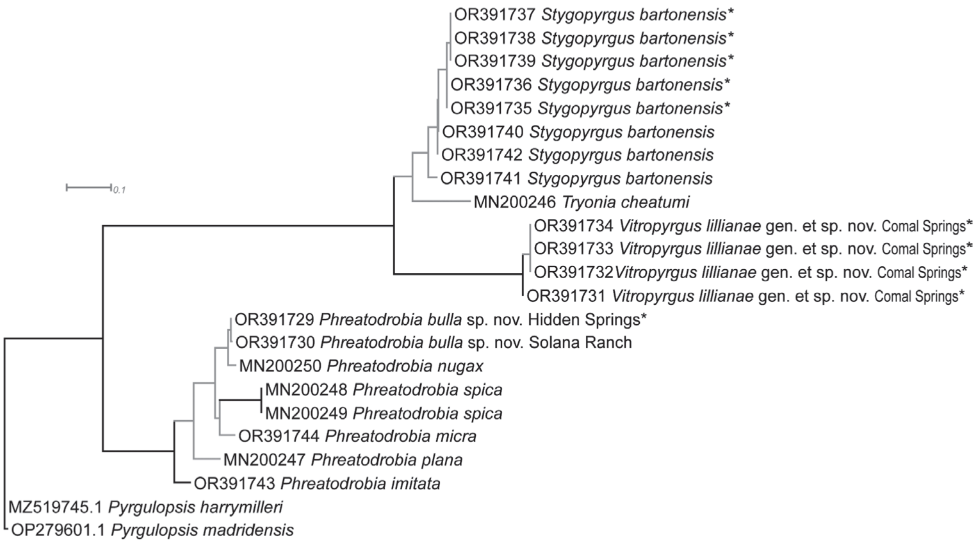

Figure 4. Highest likelihood trees resulting from maximum-likelihood analysis of LSU alignment. Ultrafast bootstrap values >95% are shown with black branches and 50 –94% with gray branches. Terminals are labeled with Genbank voucher numbers, species, and sampling locality for our focal taxa. Type localities are indicated with an *.

Systematics

Class Gastropoda Cuvier, 1795
Subclass Caenogastropoda Cox in Moore, 1960
Order Littorinimorpha Golikov & Starobogatov, 1975
Superfamily Truncatelloidea Gray, 1840
Family Cochliopidae Tryon, 1866

Genus *Vitropyrgus* Perez & Guerrero, 2023, gen. nov.
https://zoobank.org/4E6DE6B7-1891-4960-8211-AC921C8171D0
Figs 5, 6

Type species. *Vitropyrgus lillianae* gen. et sp. nov.

Diagnosis. Minute shell with spiral and collabral sculpture on teleoconch that extends to sutures. Embryonic whorl distinctively sculptured with wrinkles giving a malleated appearance. Aperture ovate to round, with slightly reflected lip near umbilicus. Umbilicus open. Animal eyeless and unpigmented. Penis attached behind right eye position, simple in glandular structure. The single known species of *Vitropyrgus* is a quarter of the size of related epigean taxa and is adapted to a subterranean environment (e.g., lacking pigmentation, eyes, and ctenidia). Simple penial morphology lacking the papillae or apocrine glands that define other members of Cochliopidae. Finally, the

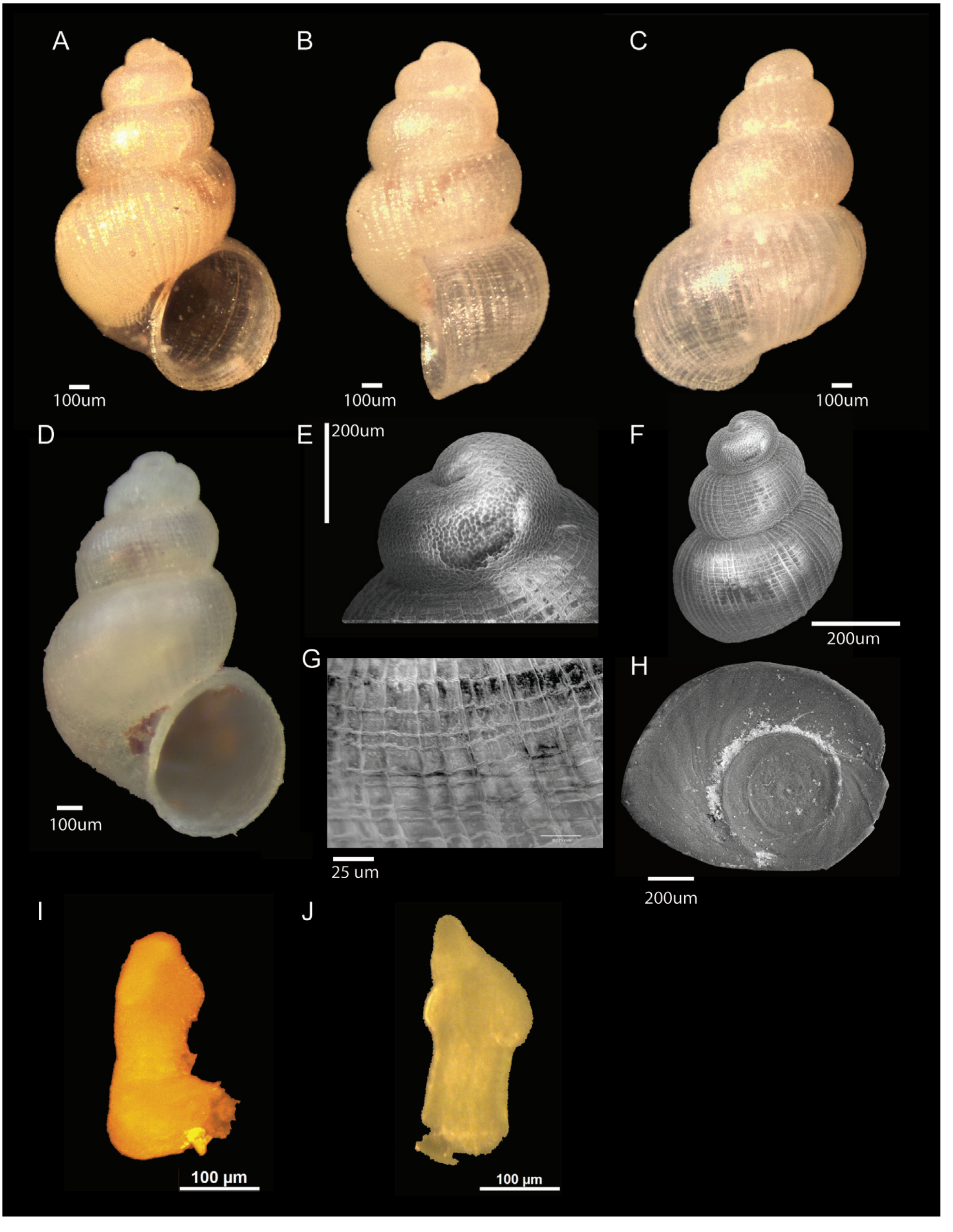

Figure 5. Shells and anatomical features of *Vitropyrgus lillianae* gen. et sp. nov. All localities in Texas **A–C** holotype, Comal Springs, Comal County, ANSP 494654 **D** shell frontal view of individual from Fessenden Springs, Kerr County, ABC 005622 **E** SEM of embryonic whorls to detail sculpture **F** SEM of rear shell and sculpture **G** SEM of teleoconch sculpture **H** SEM of operculum, outer view **I** penis, ventral view **J** penis, dorsal view. The yellow coloration in I and J is caused by immersion in Bouin's solution, the tissues are white, unpigmented in life.

shell has a distinctive clear and glassy appearance, lacking the tan color of *Tryonia* or *Stygopyrgus* Hershler & Longley, 1986 or the usual translucency of *Phreatodrobia*.

Taxonomic remarks. The most recent review of Cochliopidae (Hershler and Thompson 1992) divided the family into three subfamilies, Cochliopinae Tryon, 1966, Littoridininae Thiele, 1928, and Semisalsinae Giusti & Pezzoli, 1980 largely distinguished by glandular features of the male reproductive anatomy, including "Tribe" *Heleobia* Thompson, 1968 (Hershler and Thompson 1992; Liu et al. 2001) diagnosed by apocrine penial glands. Cochliopinae (e.g. *Cochliopina* W. Stimpson, 1865) is diagnosed by a simple, non-glandular penis with a long filament distinct from the wrinkled or folded base and Littoridininae (including *Stygopyrgus*, *Pyrgophorus*, *Mexipyrgus*, and *Tryonia*) is characterized by a long sperm duct and often with numerous glandular papillae. A subsequent molecular phylogenetic analysis broadly supported these groupings (Liu et al. 2001).

We do not attempt to place this genus among the subfamilies of Cochliopidae. The COI phylogeny has limited resolution at this level, we have limited sampling in LSU for placement among subfamilies. The COI tree places *Vitropyrgus* close (with no support) to a clade that included *Heleobia* (Semisalsinae) and *Onobops* (Littoridinae). Members of Semisalsinae are diagnosed by penial papillae or apocrine glands (Liu et al. 2001), which *Vitropyrgus* lacks. *Onobops* is one of several cochliopid genera that have a simple penis with no papillae or apocrine glands, resembling *Vitropyrgus*. *Onobops* are epigean, brackish water species from North America. The subfamily placement of this genus is best defined as uncertain along with many other genera in Cochliopidae.

Vitropyrgus is proposed as a new genus with the following rationale. First, it was found by COI and LSU phylogenies sister to epigean taxa. In the COI phylogeny, *Vitropyrgus* is most closely related to members of the *Heleobia* and *Onobops*. Divergence in COI between *Vitropyrgus* and other members of that clade averaged 16.0% with a range from 15.5–16.6. Intergeneric comparisons in our dataset averaged 16.54% with a range from 5.19–25.37. This places the level of divergence between *Vitropyrgus* and its closest known relatives within the range of intergeneric divergence and just under the average for the Texas genera. In other groups of subterranean hydrobioids the range of 14.5–16.7% has been used to justify genus level distinction (Delicado et al. 2019; Delicado and Gürlek 2021).

Vitropyrgus lillianae **Perez & Guerrero, 2023, sp. nov.**

https://zoobank.org/DB9F6F2C-749B-45C1-B496-8BB603C7BAF4
Figs 5, 6

Stygopyrgus bartonensis, Hutchins 2018, suppl. material 1: table S1.
Stygopyrgus bartonensis, Hutchins et al. 2021, suppl. material 1: table S2.

Type locality. USA, Texas. Comal County, New Braunfels, Comal Spring Upwelling #7, (29.7135, -98.1370).

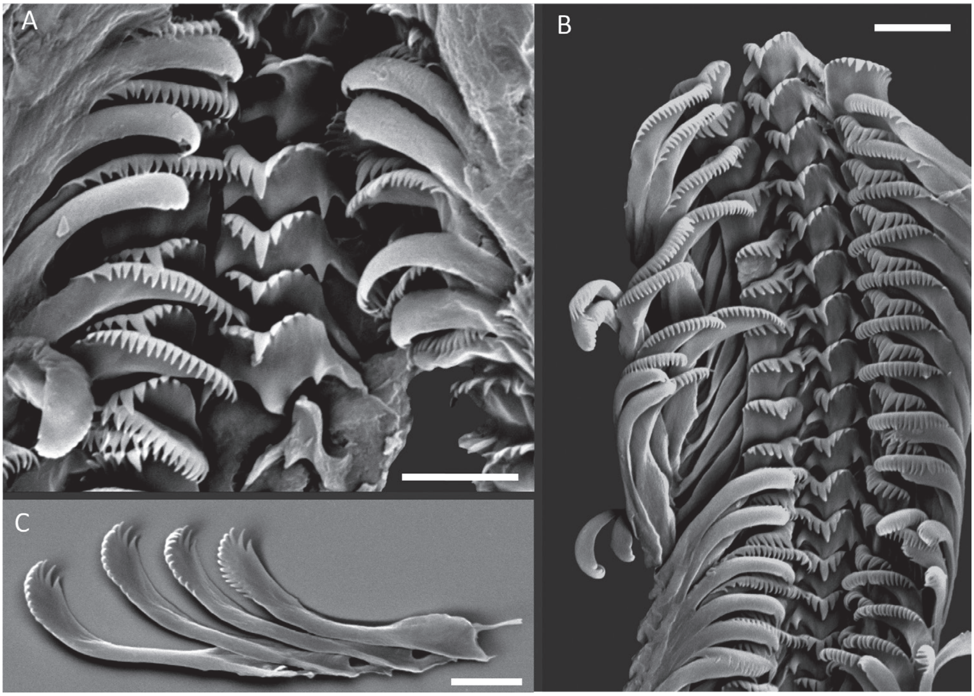

Figure 6. SEM of *Vitropyrgus lillianae* gen. et sp. nov. radula **A** view of central radula teeth **B** radula ribbon showing details of inner and outer marginals **C** outer marginal teeth.

Material examined. All sites are in Texas, USA. ***Holotype*** – Comal County, Landa Park, New Braunfels, Comal Spring Upwelling #7, (29.7135, -98.1370), drift net, collected by Randy Gibson, 2 May 2019 (ANSP 494654). ***Paratypes*** – Comal County, Landa Park, New Braunfels, Comal Spring Upwelling #7, (29.7135, -98.1370), drift net, collected by Randy Gibson, 1–5 June 2018 (ANSP 494656).

Additional material examined. – Kerr County, Fessenden Spring, near Heart of the Hills Fisheries Science Center (30.1670, -99.3427), drift net, collected by K.E Perez, D. Deshommes, N. Loveland, 4–6 November 2020 (ABC 005622).

Diagnosis. Minute shell with glassy appearance, with distinctive spiral and collabral sculpture on teleoconch that extends to sutures. *Vitropyrgus lillianae* differs from similar species in the region by shell shape, sculpture, or shell color. *Stygopyrgus bartonensis* has a taller, more columnar, and less heavily sculptured shell. The shell of *S. bartonensis* also has a pale brown tint in fresh shells that is not present in *V. lillianae*. The animals most easily confused with *V. lillianae* are very juvenile individuals of *Pyrgophorus spinosus* (Call & Pilsbry, 1886). While their sculpture can appear quite similar, juvenile *P. spinosus* are much larger, have a white base color and the aperture forms an oval, completely appressed to the body whorl. *Pyrgulopsis spinosus* shells have a more steeply tapering spire than *V. lillianae*. Dissection and comparison of penial anatomy will readily distinguish *V. lillianae* due to its simple structure with no papillae or apocrine glands.

Description. Shell very small, clear, glassy, heavily sculptured, ovate-conic with rounded whorl outlines (Fig. 5A–D). Average shell measurements for adults (n = 20): shell height = 0.737 mm (SD = 0.25), shell width = 0.470 mm (SD = 0.17), aperture height = 0.333 mm (SD = 0.11), aperture width = 0.268 mm (SD = 0.09), number of whorls = 4.5 (SD = 0.20).

First whorl of protoconch slightly elevated, separated from subsequent whorls (Fig. 5E, F). Protoconch surface heavily sculptured by wrinkles that form irregularly shaped shallow depressions or pits. Teleoconch sculpture includes finely spaced collabral ribs dissected by spiral lines (Fig. 5F, G). Ribs slightly more elevated, spaced 20–23 μm apart. Aperture ovate, slightly pulled away from body whorl, only lightly touching body whorl at parietal corner. Lip reflected on basal and umbilical portions in larger individuals. Outer lip straight, simple, slightly tilted forward (prosocline). Umbilicus open. Operculum clear, extremely thin, pliable, fragile (Fig. 5H). Shape elongate ellipsoidal, nucleus submarginal, spiral weakly convex. Growth lines not distinct or frilled.

Unpigmented body visible through shell. Snout nontapered, about as long as wide, with strong distal lobation. Foot short, anterior portion rounded, anterior edge indented, without lateral wings. Cephalic tentacles tapered, rounded, unpigmented, with no visible cilia, about 5 times as long as wide. Mantle tissue unpigmented. No visible eyes and no visible pigment at base of eyestalks. No ctenidium observed, osphradium rounded.

Intestine uncoiled, mostly filled with rounded fecal pellets, rectum exiting in pallial cavity, near mantle edge on right side of head. Esophagus entering stomach below, smaller posterior chamber with large digestive gland aperture and larger anterior chamber. Stomach speckled with dark flecks of pigment. Caecum not observed.

Penis large relative to body size tapering, attached well behind right eye, with an expanded, muscular base, narrow body segment, tapering to a distal tip (Figs 5I, J). Penis base with moderate folding along inner curvature. Distal portion tapered, inner and outer curves with aglandular curving lobes nearly opposite each other, giving a blunt, asymmetrical "arrowhead" shape to distal portion of penis. Neither apocrine glands or papillae present on examined individuals. Cilia not observed on distal penis.

Central radular tooth with indented dorsal edge (Fig. 6A); lateral cusps 4 on each side; central cusp ~1/3 longer than adjacent cusps, pointed but tapering at the end and at the base, wider in the middle, singular basal cusps pointed, with small buttress, paddle shaped, not needle-like, basal process triangular in shape; deep basal socket. Lateral tooth rectangular, narrowing slightly upon reaching the outer wing; outer wing tapering; central cusp longer than lateral cusps, 5–6 cusps outer and 5 cusps inner direction, decreasing in size distally. Inner marginal teeth with broad outer wing with basal notch, 17–19 cusps, mostly similar in length except two outermost cusps shorter, triangular, wide at base. Outer marginal teeth broad and curved at end, with 14–16 cusps. Cusps along inner edge longer; tooth face tapering to outer wing which then broadens again at base (Fig. 6B, C).

Etymology. We use the generic name "*Vitropyrgus*" reflecting the glassy appearance of the shell of this phreatic snail compared to related groups. The specific epithet "*lillianae*" is in honor of Dr. Lillian E. Perez, the first author's mother. We propose the common name "glass cavesnail".

Ecology. This new snail species is found among other phreatic snail fauna in Comal Springs including: *Phreatodrobia nugax* (Pilsbry & Ferriss, 1906), *Phreatodrobia plana* Hershler & Longley, 1986, *Phreatodrobia spica* K. E. Perez & Alvear, 2020, and *Phreatodrobia rotunda* Hershler & Longley, 1986. Other members of this unique subterranean fauna include the federally endangered amphipod *Stygobromus pecki* (Holsinger, 1967), the federally endangered dryopid beetle *Stygoparnus comalensis* Barr & Spangler, 1992, an undescribed stygobiontic salamander, and many other invertebrates (Hutchins et al. 2021). Federally endangered epigean fauna at Comal Springs include the riffle beetle, *Heterelmis comalensis* Bosse, Tuff, & Brown, 1988, fountain darter, *Etheostoma fonticola* (Jordan & Gilbert, 1886), and Comal Springs salamander, *Eurycea neotenes* Bishop & Wright, 1937.

Habitat. This species is known from two localities in the karstic Edwards and Edwards-Trinity Aquifers, separated by ~125 km. Comal Springs is the largest spring in Texas (mean annual discharge = 8.4 m^3/s, (USGS 2023)) and discharges water from the deep confined portion of the regional San Antonio segment of the Edwards Aquifer. The spring is a complex of openings discharging on and along a normal fault that divides the deep confined and recharge zones of the Edwards Aquifer, and the springs integrate a mix of species found in one or both aquifer zones.

Fessenden Spring on Johnson Creek is a smaller spring that is part of the large regional Edwards-Trinity Aquifer system. Fessenden Spring discharges from the base of the Edwards Limestone in the central Texas Hill Country and is one of many Edwards-Trinity springs that support baseflows in the headwater reaches of the Guadalupe River. Across much of the southeastern portion of this aquifer, springs discharge into streams and rivers in the contributing zone for the Edwards Aquifer. The Edwards-Trinity system is hydrologically connected to the Edwards Aquifer along the Balcones Fault zone through both groundwater and surface-water linkages. The Guadalupe River is the only river in the contributing zone to not consistently lose much or all its flow to the Edwards Aquifer as it crosses the aquifer recharge zone (Ockerman and Slattery 2008; Wehmeyer et al. 2013). Instead, it consistently gains discharge via Comal Spring, Hueco Spring, and several other springs discharging from both the Edwards and Edwards-Trinity aquifers.

In the boundary zone between the two aquifer systems, movement of organisms across blurry hydrologic boundaries between the aquifers is possible. Additionally, there is increasing evidence that the hyporheic zone along river corridors can provide important habitat and connectivity for a variety of Texas groundwater taxa, including snails (Hutchins et al. 2020; Sparks 2023). Because the Edwards Limestone is continuously exposed across the upper and middle Guadalupe River watershed between Fessenden and Comal Springs, it is likely that *Vitropyrgus lillianae* gen. et sp. nov. is more widespread than the localities reported here. More occurrences will likely be discovered once the species is characterized, and additional samples are collected across this watershed. However, given the prevalence of restricted range size in most (though not all) Texas groundwater snails (Alvear et al. 2020a), it is unlikely that the range for *Vitropyrgus lillianae* gen. et sp. nov. will be expanded considerably. With only two populations currently known, the species is classified as critically imperiled (G1S1) using NatureServe methodology and considering distribution data only.

Taxonomic remarks. The species superficially resembles *Stygopyrgus bartonensis* in overall shell form and sculpture and was initially identified as that species (e.g. Hutchins 2018, suppl. material 1: table S1, identification by R. Hershler, and Hutchins et al. 2021, suppl. material 1: table S2). Here we examine the relationship with both COI and LSU data of *V. lillianae* to several populations of *S. bartonensis*, including the type locality. In both analyses, while it is not certain which members of the Cochliopidae *V. lillianae* are closely related to, this species is not supported as closely related to *S. bartonensis.*

Class Gastropoda Cuvier, 1795
Subclass Caenogastropoda Cox in Moore, 1960
Order Littorinimorpha Golikov & Starobogatov, 1975
Superfamily Truncatelloidea Gray, 1840
Family Cochliopidae Tryon, 1866
Genus *Phreatodrobia* Hershler & Longley, 1986

Phreatodrobia bulla **Perez & Castañeda, sp. nov.**
https://zoobank.org/5393D9DA-DCC8-49B0-BC2C-4C45FC2E45F4
Figs 7, 8

Phreatodrobia cf *imitata* Perez et al., 2020, pp. 7.
Phreatodrobia conica Gibson et al., 2021b, pp. 33.

Type locality. USA, Texas, Bell County, Hidden Springs (30.9382, -97.6044).

Material examined. All sites are in Texas, USA. ***Holotype*** and ***Paratypes*** – Bell County, Hidden Springs, collected by Peter Diaz (30.9382, -97.6044), 27 October 2021 (ANSP 494658, 494660).

Additional material examined. – Bell County, Salado Springs Complex, Anderson Spring (30.9441, -97.5347); Stagecoach Inn Cave, Salado (30.9432, -97.5375), 1 May 2020, P. Diaz (ABC 005618); Copperhead Spring Cave, Ft. Cavazos (confidential location); Bent Oak Spring (30.8916, -97.7092), 17 August 2022 (ABC 005616); Gault Archaeological Site Spring (30.8916, -97.7095), 8 June 2019 (ABC 005615); Robertson Springs, Creek Springs (30.9445, -97.5413); Solana Ranch Spring (30.8997, -97.6390), 25 March 2020 (ABC 005620), P. Diaz; Spicewood Creek Pipe Spring (confidential location); Spring 23-398, Ft. Cavazos (confidential location); Camp Tahuaya, Tahuaya Spring Pool (31.0087, -97.5093). – Williamson County, PC Spring (30.4818, -97.7419), 23 March 2023 (ABC 005617).

Diagnosis. Shell translucent, conical, with nearly smooth teleoconch, dome-shaped protoconch with wrinkles. Aperture round to slightly ovate, usually separated

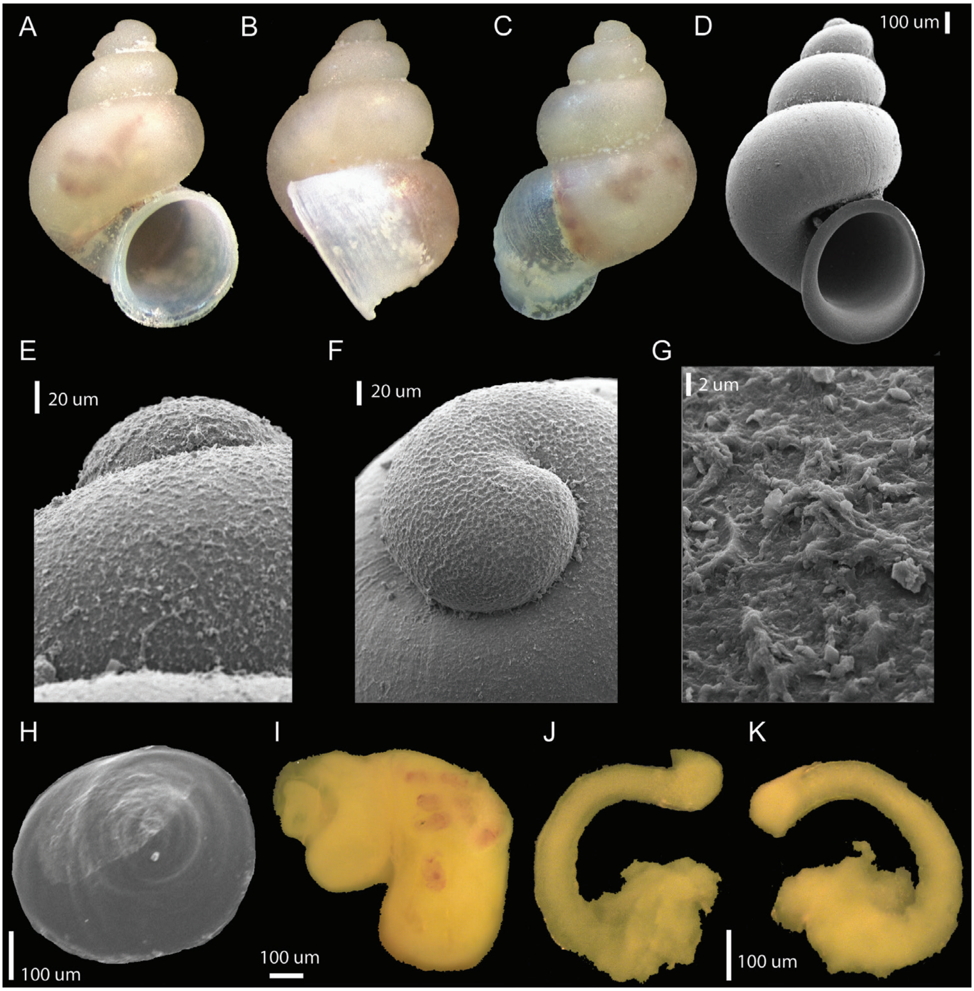

Figure 7. Shells and anatomical features of *Phreatodrobia bulla* sp. nov. All sites are in Texas **A–C** holotype, Hidden Springs, Bell County, ANSP 494658 **D** SEM of individual from Hidden Springs, Bell County ANSP 494660 **E–G** SEM of embryonic shell sculpture **H** SEM of operculum, inner surface **I** dorsal view of body **J** ventral view of penis **K** dorsal view of penis. Scale bars: 100 µm (**A–E**); 100 µm (**J, K**).

from body whorl in adults. Mantle tissue white, unpigmented. Sharply pointed median cusp of central radular teeth with small basal cusp. Penis long, equal width most of the length, tapering sharply near tip, loosely to tightly coiled, length 2–3 times length of snout.

Description. Shell translucent, usually pale tan, conical with 3.5–4.5 well rounded whorls (Fig. 7A–D). Shell height ranges from 1.1–2.39 mm. Average shell measurements (n = 14 adult individuals): height = 1.86 mm (SD = 0.5), width = 1.22 mm (SD = 0.3), aperture height = 0.78 mm (SD = 0.2), aperture width = 0.77 mm (SD = 0.2), number of whorls = 4.0 (SD = 0.5). Sutures deeply impressed giving whorls a very rounded aspect. Body whorl wider than others, which taper steeply to a dome-shaped embryonic whorl. Spire with distinctive "bubble" or dome shape. Dome-like embryonic whorl sculptured with irregular granules and wrinkles (Fig. 7E–G), teleoconch smooth, without visible sculpture, except under high-magnification. Regular growth lines visible on recent shells. In most individuals, aperture fully detached from previous whorl (appressed only at top of aperture in some smaller individuals). Aperture ovate, with simple, prosocline, reflected lip that flares at base. Umbilicus present.

Operculum round to broadly ovate, concave, amber in color, deeply concave, with narrow band of thinner material on outer margin, tapering to a point but without nuclear peg (Fig. 7H). Opercular growth lines vague, simple. Nucleus slightly eccentric, central, paucispiral. Muscle attachment scar distinct and thickened toward edges, with undifferentiated edges. Attachment region callus thin.

Body visible through translucent shell. No eyes present. Ctenidium composed of triangular filaments, approximately as broad as high, stretching from posterior portion of pallial cavity nearly to mantle edge. Osphradium oval shaped, elongate, positioned opposite posterior portion of ctenidium, occupying ~25% of pallial cavity. Pallial portion of intestine with loops in posterior portion of pallial cavity similar to *P. imitata*. Fecal pellets in the coiled intestine usually clearly visible through the shell, bright orange, oval-elongate in shape (Fig. 7I). Rectum ends just before mantle edge.

Snout narrow, longer than wide, deeply lobate distally, with folds along sides. Tentacles tapered, with scattered granules or pigmentation at base, length equal to snout. No eye visible. Foot rounded anteriorly, with lateral wings. Penis base well behind right tentacle, slightly wider and with deeper folds near base, tapering quickly to a consistent length until sharply tapering at tip. Slight folds continue until midway along penis length. Penis long, loosely to tightly coiled, curved and 2–3 times longer than snout (Fig. 7J).

Central radular tooth with deeply indented dorsal edge; central cusp longer than adjacent cusps; lateral cusps 5–6 on each side, evenly decreasing in width towards tip, sharply pointed; basal cusps small, triangular; basal socket deep, v-shaped. Lateral tooth rectangular, with a longer central cusp and 4 (inner) – 7 (outer) cusps on either side. Some laterals with wide deposit down mid-line. Base of lateral tooth with triangular, well excavated ventral process, tapering to wing. Inner marginal teeth with ~25–30 cusps, similar in length, decreasing slightly in outermost cusps. Tooth surface tapering towards outer wing with narrow neck before flaring smoothly towards the base. Outer marginal teeth rounded, spoon-shaped, wide at top, smoothly curving, with 12–20 small cusps, tapering slightly to short neck.

Etymology. We use the specific epithet "*bulla*" from the latin for "bubble", referring to the rounded appearance of each whorl, particularly the rounded spire. We propose the common name "Brown's cave snail" in honor of Mr. Tim Brown, a Bell County native and former Commissioner who has worked extensively to promote conservation of archaeological and groundwater resources in the region.

Ecology. This new species is part of a diverse aquifer community. Relative abundance varies across the range of the species. At Creek Springs (part of the Robertson Springs Complex), as many as 200 snails can be captured over a couple of days of drift

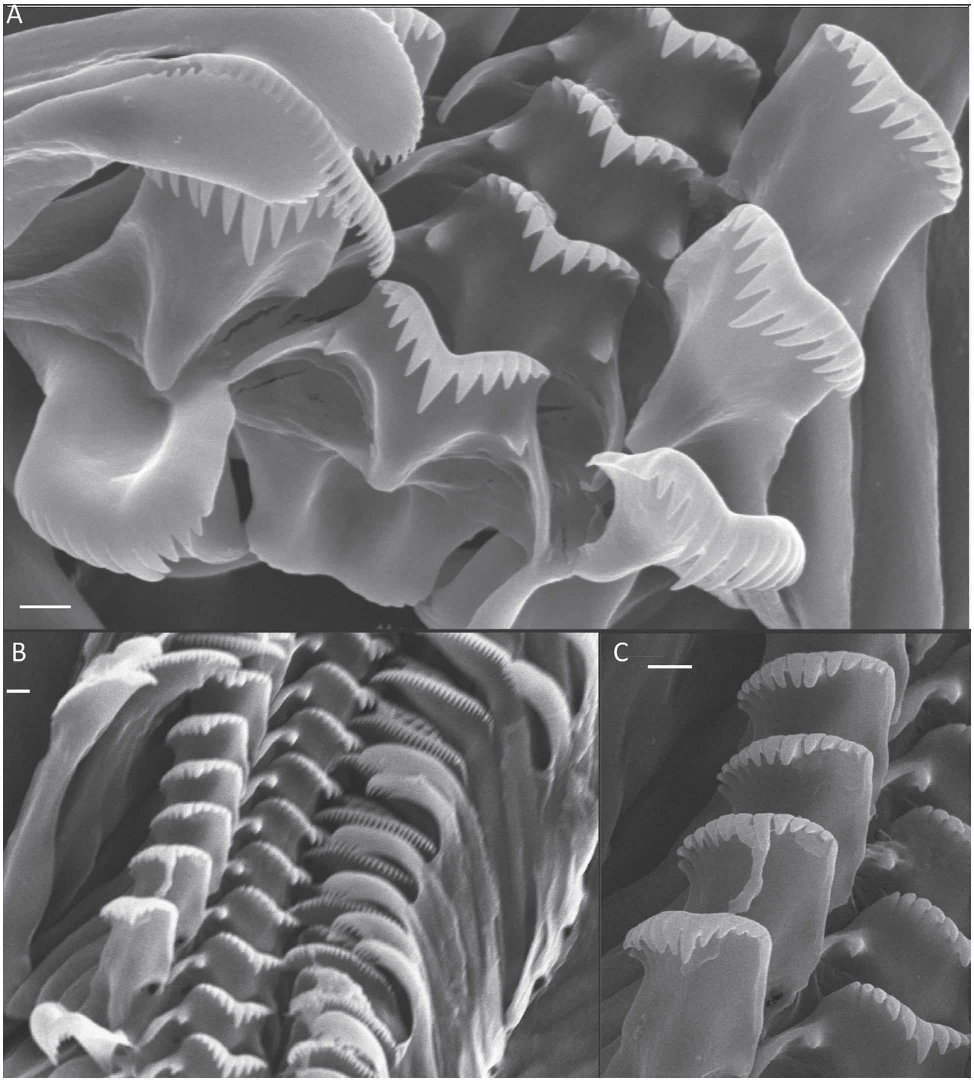

Figure 8. SEMs of radula of *Phreatodrobia bulla* sp. nov. Hidden Springs, Bell County **A** central and marginal radular teeth **B** portion of radular ribbon **C** lateral teeth. Scale bars: 2 μm.

net collection, whereas at PC Springs, similar sampling effort yields only one or a few specimens. *Phreatodrobia bulla* sp. nov. is often collected with other phreatic snails: *P. nugax*, *P. micra* (Pilsbry & Ferriss, 1906), and *Phreatoceras taylori* (Diaz and Warren 2018). Depending on site, *P. bulla* sp. nov. may also occur with several crustaceans *Lirceolus* sp., *Stygobromus bakeri* Gibson et al. 2021, *Parabogidiella americana* Holsinger, 1980, and undescribed Bathynellacea and Microcerberidae. They also occur with the federally threatened Salado salamander (*E. chisholmensis* Chippindale, Price, Wiens & Hillis, 2000) and Jollyville salamander (*E. tonkawae* Chippindale, Price, Wiens & Hillis, 2000).

Habitat. All known localities for *Phreatodrobia bulla* sp. nov. are springs or hyporheic samples taken near springs discharging from the northern segment of the karstic Edwards Aquifer (north of the Colorado River). The northern segment lies adjacent to, but is disconnected from, the Barton Springs segment of the Edwards Aquifer, with the Colorado River a topographic low that forms the boundary between the two segments. Faults, erosion, and other geologic and geomorphic factors in the northern segment have resulted in groundwater basins that are relatively smaller and more dissected than in the Barton Springs and San Antonio segments to the south (Jones 2003). More and smaller springs in the region, combined with relatively intensive sampling at many of those springs, are likely factors contributing to the higher number of known occurrences for this species, and it is likely that additional sampling in Bell, Williamson, and northern Travis Counties will result in more localities, particularly in the 45km gap between PC Spring (the southernmost location) and Kings Garden Spring. In particular, very little hyporheic sampling has been performed along streams and rivers in the region and this has been a productive method for sampling groundwater snails in other parts of Texas. Nevertheless, for the same reasons discussed for *V. lillianae* gen. et. sp. nov., it is unlikely that additional work will substantially expand the known range of *P. bulla* sp. nov.

Currently, *P. bulla* sp. nov., is known from 12 sites across a range of approximately 680 km^2. Occurrence at multiples sites provides some security against catastrophic events (redundancy, sensu Shaffer and Stein 2000). Nevertheless, Bell and Williamson Counties are among the fastest growing counties in Texas, resulting in substantial pressure on groundwater resources. Eleven of the 12 locations occur within the Clearwater Underground Conservation District, which is tasked with developing and implementing a groundwater management plan for the Edwards and Trinity aquifers in Bell County (Clearwater Underground Water Conservation District 2020). The desired future condition adopted by the conservation district, which provides a basis for some permitting and regulation of groundwater extraction, is preservation of a minimum acceptable springflow of 1.66 cfs at the Salado Springs complex (which includes Anderson and Creek springs) during hydraulic conditions equal to the 1950s drought of record. That is approximately 10% of average flows during the 1980s (Brune 1995). Currently, several spring orifices in the region go dry during drought periods (Diaz et al. 2015), illustrating that groundwater availability is the central conservation concern for *P. bulla* sp. nov. The sites where this species has been encountered are restricted to springs and spring-run hyporheic habitats, with sampling of wells or caverns in Bell and Williamson counties needed to determine its' full extent. Without quantifying the severity and scope of threats, *P. bulla* sp. nov. is ranked as imperiled (G2S2) using NatureServe methodology.

Taxonomic remarks. Intraspecific and interspecific sequence divergence averaged 2.45% and 10.73%, respectively, in our dataset of Texas phreatic snails. *Phreatodrobia bulla* has an average sequence divergence of 10.34% with the other members of *Phreatodrobia* and *Phreatoceras*, and 6% divergence with its sister *Phreatoceras taylori*. Interspecific variability in COI has been examined in several groups of subterranean hydrobioid gastropods inhabiting karstic environments. In *Belgrandiella* A. J. Wagner, 1928 "species" (Hydrobiidae) COI divergence ranged from 5.2–9.9% (Jaszczyńska et al. 2022). An analysis of *Bythinella* Moquin-Tandon, 1856 (Bythinellidae Locard, 1893) from a karstic region of France, which included epigean and cave species, found that maximum species-level divergence was 1.5% (Bichain et al. 2008). In *Kerkia* Radoman, 1978 (West Balkans), a group of snails that resembles *Phreatodrobia* in habitat and morphology, interspecific genetic divergence ranged from 4.2%–14.7% (Hofman et al. 2022) and similar values were found in *Balkanica* Georgiev, 2011 and related lineages (Hydrobiidae, 7.8%–11.8%) in Bulgaria (Osikowski et al. 2017). Thus, gene flow seems to vary by group and may be relatively low within some taxa or high, possibly facilitated by movement through routes such as the hyporheic or phreatic rhizosphere (Haase et al. 2021). While there is not a molecular ruler denoting species-level distinction among subterranean species, *Phreatodrobia bulla* has sequence divergence comparable to other species of *Phreatodrobia* and greater than average species level divergence relative to most subterranean gastropods.

Lacking circumscription, *Phreatodrobia bulla* has been previously identified in recent literature (Alvear et al. 2020a; Gibson et al. 2021b) as *P. conica* or *P.* cf *imitata* as it resembles these species in some aspects of shell morphology, the basis for those identifications. When we consider internal anatomy or DNA, these species are diagnosably different from *P. bulla*. The shell of *Phreatodrobia conica* is described (Hershler and Longley 1986b) as having a simple aperture and a varix (ridge behind the aperture marking previous aperture position) near the end of the body whorl. It also has a distinctive teleoconch sculpture with numerous ridges. Its internal anatomy is distinguished by the lack of a ctenidium and a square-shaped central radular tooth. *Phreatodrobia bulla* in contrast has a flared and reflected aperture in adults with no sign of a varix in any material examined. The teleoconch sculpture is smooth without ridges and with a few collabral growth lines near the aperture, however, these are not elevated as described in *P. conica*. Finally, *P. bulla* has a robust ctenidium and the usual V-shaped central radular tooth both in contrast to what is described for *P. conica.* Access to the type locality of *P. conica* has not been possible during this study, preventing collection of tissues for DNA data collection. Even in the absence of DNA data, however, the anatomical distinctions between these species are sufficient to describe *P. bulla* as distinct from *P. conica*.

Phreatodrobia imitata and *P. bulla* share the same general shell shape and highly flared aperture (Fig. 9). However, the shells are readily distinguished. *Phreatodrobia imitata* has a translucent or clear shell which is heavily sculptured shell with collabral costae (ribs) and spiral lines (running opposite the ribs) while the teleoconch of *P. bulla* is unsculptured. Even though these sculptural features appear to consistently distinguish *P. imitata* and *P. bulla*, sculptural characters alone are insufficient to distinguish these species as ribs are polymorphic among *Phreatodrobia* and other freshwater snails. There are more pronounced differences found in the radula and DNA. The central radular tooth of *P. imitata* has a very narrow central cusp, 6–7 cusps on either side, and it lacks a basal cusp. The central radular tooth of *P. bulla* has a wider central cusp, ~ 5 cusps on

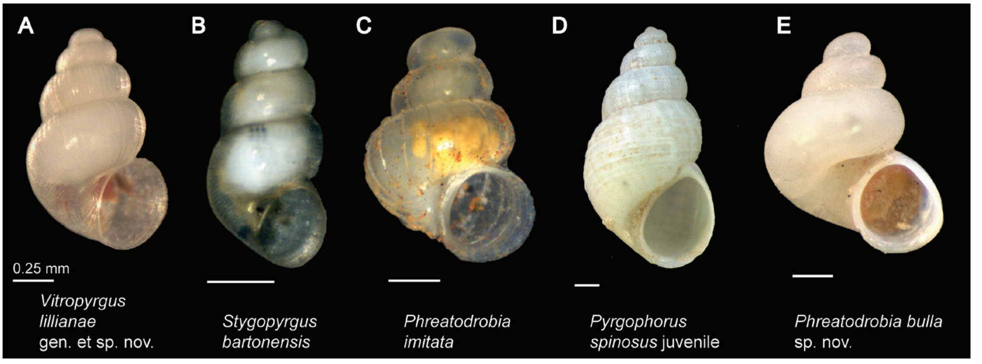

Figure 9. Shells of the species described here compared to shells of similar species in the region. All localities are in Texas **A** *Vitropyrgus lillianae* gen. et sp. nov. Comal Springs, Comal County **B** *Stygopyrgus bartonensis*, Barton Springs, Travis County **C** *Phreatodrobia imitata*, Verstræten Well, Bexar County **D** *Pyrgophorus spinosus* juvenile, San Marcos River, near Martindale, Guadalupe/Caldwell County Line **E** *Phreatodrobia bulla* sp. nov. PC Spring, Williamson County.

either side, and it possesses a distinct basal cusp. We obtained *P. imitata* individuals for DNA analysis from Aldridge Well near the type locality (Verstræten Well), and both COI and LSU phylogenies have strong support for placement of *P. imitata* as the sister lineage to other species of *Phreatodrobia*, not part of the *P. bulla* clade.

Previous classification efforts have not determined the placement of *Phreatodrobia* and *Phreatoceras* within a subfamily of Cochliopidae (Hershler and Thompson 1992; Liu et al. 2001). They are both found in the Edwards and Edwards-Trinity Aquifers and share features such as a minute, colorless, translucent shell and pitted protoconch microsculpture (Hershler and Longley 1986b; Hershler and Longley 1986a). *Phreatoceras* is diagnosed primarily by its unique, uncoiled, horn-like shell (see Fig. 3), and some features that are shared with various *Phreatodrobia* species such as a smooth teleoconch, loss of ctenidium, long central cusp of the central radular tooth. In the COI phylogeny, *P. bulla* is found sister to *Phreatoceras taylori* from the same springs in Bell County, Texas, but with weak support, and both are embedded within a clade of *Phreatodrobia* species. When nine anatomical characteristics of the two genera are compared (Table 1), *P. bulla* shares one distinctive characteristic with *Phreatoceras*, four characteristics with both genera, and five characteristics with *Phreatodrobia*. As this proposed new species shares more morphological characteristics with *Phreatodrobia*, is found by the DNA data within a clade of *Phreatodrobia* and does not have the distinctive trumpet shaped shell of *Phreatoceras*, we place it in *Phreatodrobia*.

We propose two potential explanations for the sister relationship of *P. bulla* and *Phreatoceras taylori*. In this study, we have not sampled the type locality of *Phreatoceras*, so it is possible that snails with a trumpet-shaped shell that we sampled in Bell County are not *Phreatoceras taylori sensu stricto*, described from Real County, Texas, ~250 km distant. Alternatively, *Phreatoceras* may be better considered a morphologically divergent member of *Phreatodrobia* rather than a separate genus. We have observed other species of *Phreatodrobia*, such as *P. nugax*, with a loosely coiled or partially uncoiled

Table 1. Comparison of morphological features of *Phreatoceras* and *Phreatodrobia* with *P. bulla* sp. nov.

Character	*Phreatoceras*	*Phreatodrobia* spp.	*P. bulla* sp. nov.
Shell shape	horn-like	planispiral, trochoid, conical	conical
Protoconch sculpture	pitted/wrinkled microsculpture	pitted microsculpture	wrinkled
Teleoconch sculpture	smooth	variable	regular collabral growth lines
Aperture	simple	flared	flared
Operculum	near circular, concentric, multispiral, large ventral central process.	Round to oval, non-concentric, nucleus subcentral, sometimes with central knob	Oval, non-concentric, nucleus subcentral with ventral mound
Ctenidium	absent	absent, nearly absent, present	present
Radula - central tooth	central cusp long, basal tooth long at origin of lateral angle	central cusp long or not, basal cusp present or not at origin of lateral angle	central cusp long, basal tooth long at origin of lateral angle
Penial morphology	tight coil	coil or uncoiled	coiled

shell in some individuals, lending some observational support to this possibility. Examination and sequencing of *Phreatoceras taylori* from the type locality, is needed to resolve its relationship to *Phreatodrobia*.

Acknowledgements

We thank UTRGV School of Integrative Biological and Chemical Sciences and the College of Sciences. Funding for DNA data collection provided by U.S. Army Corps of Engineers (Project number W912HZ2120033). Sampling supported by several agencies: City of Austin (grant # NA190000181); Clearwater Underground Water Conservation District; and Texas Parks and Wildlife Department (U.S. Fish and Wildlife Service, TPWD Interagency Cooperation Contract #532109). We thank many students for DNA data collection: Dominique Alvear, Vanessa Saenz, Natalia Salazar-Lozano, Mariana Gonzalez Tiscareño, Evan Guerrero, Manuel Spor Leal, Taylor Villanueva, Christina Ortega, Megan Solis, Jonathan Dominguez, and Mercedes Serrano. We thank the following for assistance with collections for this study: Didier Deshommes, Peter Sprouse, Jessica Gordon, Zara Environmental, Chase Corrington, Ashley Casarez, Weston Nowlin, Victor Castillo, III, Nate Bendik, Nick Loveland, Jack Johnson, Thomas Devitt, Valentin Cantu, Sarah Donelson, and Patricia Duncan. The Texas State University Analysis Research Service Center facilitated use of a scanning electron microscope, and Joyce Anderson provided technical guidance for SEM imaging. Tom Eubanks and Satinderpal Kaur at UTRGV provided SEM assistance. Martin Haase provided advice on imaging and handling of minute subterranean snails. We thank the curators and collections staff at the Texas Memorial Museum, United States National Museum, Texas State University Aquifer Biodiversity Collection, and Academy of Natural Sciences of Philadelphia, especially James Reddell and Paul Callomon. The views presented herein are those of the authors and do not necessarily reflect those of the U.S. Fish and Wildlife Service.

References

Alvear DA, Diaz PH, Gibson JR, Hutchins B, Schwartz BF, Perez KE (2020a) Expanding the known ranges of the endemic, phreatic snails (Mollusca, Gastropoda) of Texas, USA. Freshwater Mollusk Biology and Conservation 23: 1–17. https://doi.org/10.31931/fmbc.v22i2.2020.1-17

Alvear DA, Diaz PH, Gibson JR, Perez KE (2020b) An unusually sculptured new species of *Phreatodrobia* Hershler & Longley (Mollusca; Caenogastropoda; Cochliopidae) from central Texas. Zootaxa 4810: 143–152. https://doi.org/10.11646/zootaxa.4810.1.8

Bichain J-M, Gaubert P, Samadi S, Boisselier M-C (2008) A gleam in the dark: Phylogenetic species delimitation in the confusing spring-snail genus *Bythinella* Moquin-Tandon, 1856 (Gastropoda: Rissooidea: Amnicolidae). Molecular Phylogenetics and Evolution 45: 927–941. https://doi.org/10.1016/j.ympev.2007.07.018

Bou C, Rouch R (1967) Un nouveau champ de recherches sur la faune aquatique souterraine. Comptes rendus de l'Académie des Sciences 265: 369–370.

Brune G (1995) Salado Springs. Handbook of Texas Online. Texas State Historical Association.accessed September 14, 2023. https://www.tshaonline.org/handbook/entries/salado-springs

Burch JB (1989) North American Freshwater Snails: Introduction, systematics, nomenclature, identification, morphology, habitats, distribution. Walkerana 2: 1–80. https://molluskconservation.org/publications/walkerana/Vol2/walkerana%20vol2%20no6%201-80.pdf

Clark SA (2019) Cochliopidae Tryon, 1866. In: Lydeard C, Cummings KS (Eds) Freshwater Mollusks of the World: A Distribution Atlas. Johns Hopkins Press, 256–258.

Clearwater Underground Water Conservation District (2020) District Groundwater Management Plan. Belton, 34 pp.

Delicado D, Arconada B, Aguado A, Ramos MA (2019) Multilocus phylogeny, species delimitation and biogeography of Iberian valvatiform springsnails (Caenogastropoda: Hydrobiidae), with the description of a new genus. Zoological Journal of the Linnean Society 186: 892–914. https://doi.org/10.1093/zoolinnean/zly093

Delicado D, Gürlek M (2021) Taxonomic transfer of two species of hydrobiid snails from western Anatolia (Caenogastropoda, Truncatelloidea) to two new genera, based on molecular and morphological evidence. Archiv für Molluskenkunde 150: 119–131. https://doi.org/10.1127/arch.moll/150/119-131.

Diaz P, Warren JB (2018) Salado salamander monitoring final report 2018. San Marcos, Texas, 20 pp.

Diaz PH, Montagne M, Gibson R, Najvar P, Nice CC, Warren JB (2015) Salado salamander monitoring final report 2015. San Marcos, Texas, 20 pp.

Falniowski A (2018) Species distinction and speciation in hydrobioid gastropods (Mollusca: Caenogastropoda: Truncatelloidea). Archives of Zoological Studies 1: 1–6. https://doi.org/10.24966/AZS-7779/100003

Folmer OM, Black W, Hoeh R, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

Gibson R, Hutchins BT, Krejca JK, Diaz PH, Sprouse PS (2021a) Corrigenda: *Stygobromus bakeri*, a new species of groundwater amphipod (Amphipoda, Crangonyctidae) associated with the Trinity and Edwards aquifers of central Texas, USA. Subterranean Biology 38: 19–45. https://doi.org/10.3897/subtbiol.38.61787

Gibson R, Hutchins BT, Krejca JK, Diaz PH, Sprouse PS (2021b) *Stygobromus bakeri*, a new species of groundwater amphipod (Amphipoda, Crangonyctidae) associated with the Trinity and Edwards aquifers of central Texas, USA. Subterranean Biology 38: 19–45. https://doi.org/10.3897/subtbiol.38.61787

Haase M, Grego J, Erőss ZP, Farkas R, Fehér Z (2021) On the origin and diversification of the stygobiotic freshwater snail genus *Hauffenia* (Caenogastropoda: Hydrobiidae) with special focus on the northern species and the description of two new species. European Journal of Taxonomy 775: 143–184. https://doi.org/10.5852/ejt.2021.775.1555.

Hershler R, Holsinger JR (1990) Zoogeography of North American hydrobiid cavesnails. Stygologia 5: 5–16.

Hershler R, Longley G (1986a) *Hadoceras taylori*, a new genus and species of phreatic Hydrobiidae (Gastropoda: Rissoacea) from south-central texas. Proceedings of the Biological Society of Washington 99: 121–136. https://repository.si.edu/handle/10088/11314

Hershler R, Longley G (1986b) Phreatic hydrobiids (Gastropoda: Prosobranchia) from the Edwards (Balcones Fault Zone) Aquifer region, south-central Texas. Malacologia 27: 127–172. https://archive.org/stream/malacologia271986inst#page/126/mode/2up

Hershler R, Ponder WF (1998) A review of morphological characters of hydrobioid snails. Smithsonian Contributions to Zoology 600: 1–55. https://doi.org/10.5479/si.00810282.600

Hershler R, Thompson FG (1992) A review of the aquatic gastropod subfamily Cochliopinae (Prosobranchia: Hydrobiidae). Malacological Review Supplement 5: 1–140.

Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS (2018) UFBoot2: Improving the ultrafast bootstrap approximation. Molecular Biology and Evolution 35: 518–522. https://doi.org/10.1093/molbev/msx281

Hofman S, Grego J, Beran L, Jaszczyńska A, Osikowski A, Falniowski A (2022) *Kerkia* Radoman, 1978 (Caenogastropoda: Hydrobiidae): endemism, apparently morphostatic evolution and cryptic speciation. Molluscan Research 42: 295–319. https://doi.org/10.1080/13235818.2022.2129943

Hutchins BT (2018) The conservation status of Texas groundwater invertebrates. Biodiversity and Conservation 27: 475–501. https://doi.org/10.1007/s10531-017-1447-0

Hutchins BT, Engel AS, Nowlin WH, Schwartz BF (2016) Chemolithoautotrophy supports macroinvertebrate food webs and affects diversity and stability in groundwater communities. Ecology 97: 1530–1542. https://doi.org/10.1890/15-1129.1

Hutchins BT, Gibson JR, Diaz PH, Schwartz BF (2021) Stygobiont diversity in the San Marcos artesian well and Edwards Aquifer Groundwater ecosystem, Texas, USA. Diversity 13: 234. https://doi.org/10.3390/d13060234

Hutchins BT, Swink AP, Diaz PH, Schwartz BF (2020) Environmental influences on invertebrate diversity and community composition in the hyporheic zone ecotone in Texas, USA: contrasts between co-occurring epigean taxa and stygobionts. Hydrobiologia 847: 3967–3982. https://doi.org/10.1007/s10750-020-04379-8

Jaszczyńska A, Falniowski A, Prevorčnik S, Osikowski A, Hofman S (2022) Isolation and endemism in the subterranean aquatic snails of the genus *Belgrandiella* A. J. Wagner, 1928 (Caenogastropoda: Truncatelloidea: Hydrobiidae). Hydrobiologia 850: 4089–4113. https://doi.org/10.1007/s10750-022-05106-1

Jones IC (2003) Groundwater availability modeling: northern segment of the Edwards Aquifer, Texas. Texas Water Development Board Report 358, 75 pp.

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14: 587–589. https://doi.org/10.1038/nmeth.4285

Lackey RT, May BE (1971) Use of sugar flotation and dye to sort benthic samples. Transactions of the American Fisheries Society 100: 794–797. https://doi.org/10.1577/1548-8659(1971)100%3C794:UOSFAD%3E2.0.CO;2

Liu H-P, Hershler R, Thompson FG (2001) Phylogenetic relationships of the Cochliopinae (Rissooidea: Hydrobiidae): an enigmatic group of aquatic gastropods. Molecular Phylogenetics and Evolution 21: 17–25. https://doi.org/10.1006/mpev.2001.0988

Longley G (1981) The Edwards Aquifer: Earth's most diverse groundwater ecosystem? International Journal Speleology 11: 123–128. https://doi.org/10.5038/1827-806X.11.1.12

Maclay RW (1995) Geology and hydrology of the Edwards Aquifer in the San Antonio area, Texas. Water-Resources Investigations Report, Austin, TX, 64 pp. https://doi.org/10.3133/wri954186

Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Molecular Biology and Evolution 32: 268–274. https://doi.org/10.1093/molbev/msu300

Ockerman DJ, Slattery RN (2008) Streamflow conditions in the Guadalupe River Basin, south-central Texas, water years 1987–2006–An assessment of streamflow gains and losses and relative contribution of major springs to streamflow. U.S. Geological Survey Scientific Investigations Report 2008–5165, 22 pp. https://doi.org/10.3133/sir20085165

Osikowski A, Hofman S, Georgiev D, Rysiewska A, Falniowski A (2017) Unique, ancient stygobiont clade of Hydrobiidae (Truncatelloidea) in Bulgaria: the origin of cave fauna. Folia Biologica 65: 79–93. https://doi.org/10.3409/fb65_2.79

Perez KE, Solis M, Hutchins BT, Schwartz B (2022) New species of *Pyrgulopsis* Call & Pilsbry, 1886 (Mollusca: Caenogastropoda: Hydrobiidae) from two Texas Trans-Pecos springs. Zootaxa 5213: 064–074. https://doi.org/10.11646/zootaxa.5213.1.4

Rysiewska A, Georgiev D, Osikowski A, Hofman S, Falniowski A (2016) *Pontobelgrandiella* Radoman, 1973 (Caenogastropoda: Hydrobiidae): a recent invader of subterranean waters. Journal of Conchology 42: 193–203.

Shaffer ML, Stein BA (2000) Safeguarding Our Precious Heritage. In: Precious heritage: the status of biodiversity in the United States. Oxford University Press, New York. https://doi.org/10.1093/oso/9780195125191.001.0001

Sparks K (2023) Distribution and occurrence of stygobionts in hyporheic systems downstream of karst regions (Unpublished thesis). M.S., San Marcos, Texas: Texas State University, 54 pp.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30(12): 2725–2729. https://doi.org/10.1093/molbev/mst197

USGS (2023) Current Conditions for the Nation. https://waterdata.usgs.gov/nwis/uv?site_no=08169000&legacy=1 [accessed August 14, 2023]

Wade CM, Mordan PB, Clarke B (2001) A phylogeny of the land snails (Gastropoda: Pulmonata). Proceedings of the Royal Society of London, B 268: 413–422. https://doi.org/10.1098/rspb.2000.1372

Wehmeyer LL, Winters KE, Ockerman DJ (2013) A preliminary assessment of streamflow gains and losses for selected stream reaches in the lower Guadalupe River Basin, Texas, 2010–12. US Geological Survey Scientific Investigations Report 2013–5209, 30 pp. https://doi.org/10.3133/sir20135209

Wilke T, Davis GM, Andrzej F, Folco G, Marco B, Magdalena S (2001) Molecular systematics of Hydrobiidae (Mollusca: Gastropoda: Rissooidea): testing monophyly and phylogenetic relationships. Proceedings of the Academy of Natural Sciences of Philadelphia 151: 1–21. https://doi.org/10.1635/0097-3157(2001)151[0001:MSOHMG]2.0.CO;2

Wilke T, Delicado D (2019) Hydrobiidae Stimpson, 1865. In: Lydeard C, Cummings KS (Eds) Freshwater Mollusks of the World: A Distribution Atlas. Johns Hopkins Press, 111–117.

Wilke T, Haase M, Hershler R, Liu H-P, Misof B, Ponder W (2013) Pushing short DNA fragments to the limit: phylogenetic relationships of 'hydrobioid'gastropods (Caenogastropoda: Rissooidea). Molecular Phylogenetics and Evolution 66: 715–736. https://doi.org/10.1016/j.ympev.2012.10.025

Supplementary material 1

List of specimens, including their sampling localities and voucher information

Authors: Kathryn E. Perez

Data type: xlsx

Explanation note: *denotes type localities. Alphanumeric identifiers correspond to Genbank accession numbers. USNM = United States National Museum, Smithsonian Institution; ANSP = Academy of Natural Sciences of Philadelphia at Drexler University, TMM = Texas Memorial Museum, ABC = Texas State University Aquifer Biodiversity Collection.

Link: https://doi.org/10.3897/subtbiol.47.113186.suppl1

Supplementary material 2

Photo-vouchers of individuals sampled for DNA or radula, *Vitropyrgus lillianae* gen. et sp. nov. and *Stygopyrgus bartonensis*

Authors: Kathryn E. Perez

Data type: tif

Explanation note: Shells are destroyed during both procedures. Photo vouchers are supplied here and paravouchers deposited in museum collections. All localities are in Texas. *Vitropyrgus lillianae* gen. et sp. nov., Comal Springs, Comal County, TX **A** OR372116 **B** OR372117, OR391734 **C** OR391731 **D** OR391732 **E** OR391733, Paravouchers (paratypes) = ANSP 494656; *Stygopyrgus bartonensis*, Parthenia Springs in Barton Springs Pool, Travis County, TX **F** OR391735 **G** OR372120 **H** OR372121, OR391737 **I** OR391738 **J** OR372122, OR391739 Paravouchers = ABC 003350; Old Mill Springs, Travis County, TX **K** OR391740 **L** OR391741; **M** OR372123, OR391742 **N** OR372124, Paravouchers = ABC 003357; ABC 003358**;** Camp Aransas Springs, Travis County, TX **O** OR372131, Paravouchers = ABC 005621. Scale bar: 0.5 mm.

Link: https://doi.org/10.3897/subtbiol.47.113186.suppl2

Supplementary material 3

Photo-vouchers of individuals sampled for DNA or radula, *Phreatodrobia bulla* sp. nov.

Authors: Kathryn E. Perez

Data type: tif

Explanation note: Shells are destroyed during both procedures. Photo vouchers are supplied here and paravouchers deposited in museum collections. All localities are in Texas. *Phreatodrobia bulla* sp. nov. Hidden Springs, Bell County, TX **A–N** Hidden Springs, Bell County, TX, Paravouchers = USNM 157128, radula specimens. Solana Ranch, Bell County, TX, Paravouchers = ABC 005620 **O** OR372111 **P** OR372112, OR391730 **Q** OR391729 **R** Anderson Spring, Bell County, TX MN974229. Paravouchers = USNM 1571284. Locality information in Suppl. material 1. Scale bar: 1 mm.

Link: https://doi.org/10.3897/subtbiol.47.113186.suppl3

Supplementary material 4

Photo-vouchers of individuals sampled for DNA

Authors: Kathryn E. Perez

Data type: tif

Explanation note: All localities are in Texas. *Balconorbis uvaldensis*, Uvalde Fish Hatchery, Uvalde County **A** OR372125; Sycamore Creek at HWY 277, Uvalde County **B** OR372126 **C** OR372127 **D** OR372128 **E** OR372129; *Cochliopina riograndensis*, Lake Amistad National Recreation Area, small spring 25 m W of Indian Springs, Val Verde County **F** OR372132; *Phreatodrobia imitata*, Aldridge Well, Bexar County **G** OR372130; *Phreatodrobia micra*, San Marcos River at Scull Road Crossing, Hays County **H** OR372134 **I** OR372135, OR391744; *Phreatodrobia nugax*, Little Hunt Spring, Travis County **J** OR372107 **K** OR372108 **L** OR372109 **M** OR372110, Paravouchers = TMM 12388; *Phreatodrobia rotunda*, Comal Springs, Comal County **N** OR372113 **O** OR372118; *Phreatodrobia spica*, Garden Ridge Well, Comal County **P** OR372133; *Texapyrgus longleyi*, Lake Amistad National Recreation Area, small spring 25 m W of Indian Springs, Val Verde County **Q** OR372115. Locality information in Suppl. material 1. Scale bar: 0.5 mm.

Link: https://doi.org/10.3897/subtbiol.47.113186.suppl4

Subterranean Biology 47: 29–62 (2023)
doi: 10.3897/subtbiol.47.113219
https://subtbiol.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
Subterranean Biology
Published by The International Society for Subterranean Biology

Cave-dwelling fauna of Costa Rica: current state of knowledge and future research perspectives

Stanimira Deleva[1,2], Andres Ulloa[3,4], Hernani F. M. Oliveira[5,6], Nikolay Simov[2], Ferdinando Didonna[7], Gloriana Chaverri[1,8]

1 *Sede del Sur, Universidad de Costa Rica, 4000 Alamedas, Golfito 60701, San Jose, Costa Rica* **2** *National Museum of Natural History - Bulgarian Academy of Sciences, 1 Tsar Osvoboditel str., Sofia 1000, Bulgaria* **3** *Escuela Centroamericana de Geología, Universidad de Costa Rica, San Jose, Costa Rica* **4** *Centro de Investigación en Ciencias Geológicas, Universidad de Costa Rica, San Jose, Costa Rica* **5** *Departamento de Zoologia, Universidade de Brasilia, Campus Universitário Darcy Ribeiro, Brasilia, Brazil* **6** *Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Brazil* **7** *Grupo Espeleologico Anthros, San Jose, Costa Rica* **8** *Smithsonian Tropical Research Institute, Apartado 0843-03092 Balboa, Ancón, Panama*

Corresponding author: Stanimira Deleva (stanimira.deleva@gmail.com)

Academic editor: Fabio Stoch | Received 24 September 2023 | Accepted 23 November 2023 | Published 8 December 2023

https://zoobank.org/84402EAD-ECA8-45CF-9DC7-4A6F8375436A

Citation: Deleva S, Ulloa A, Oliveira HFM, Simov N, Didonna F, Chaverri G (2023) Cave-dwelling fauna of Costa Rica: current state of knowledge and future research perspectives. Subterranean Biology 47: 29–62. https://doi.org/10.3897/subtbiol.47.113219

Abstract

This study focused on the cave fauna of Costa Rica, which has remained relatively understudied despite the presence of more than 435 recorded natural caves and artificial subterranean sites. We collected and reviewed all available literature data on cave fauna in Costa Rica and created the first comprehensive review of the existing information. In addition, we report new records from field surveys conducted between 2015 and 2018. This study reported approximately 123 animal species, whereas the remaining records (n = 82) represented taxa that could not be identified at the species level. Data were collected from 127 locations throughout the country, with new cave fauna records from 41 sites. Notably, we reported the first occurrence of the true bug *Amnestus subferrugineus* (Westwood 1837) within Costa Rican caves, which represents an addition to the country's faunal inventory. As this study highlights the knowledge gaps in the subterranean fauna, it will serve as an important stepping stone for future research and conservation efforts related to caves in Costa Rica.

Keywords

cave fauna, caves, Central America, inventory, karst

Introduction

Caves are important habitats and roosts for a large number of animal taxa, such as bats and other highly specialized organisms, adapted to specific conditions, which usually consist of the absence of light, high humidity, and almost constant temperature (Romero 2009). Because of their isolation from the surface and other subterranean systems across evolutionary time, caves can provide refuge for numerous endemic species in a confined area, making them intriguing subjects for research (Culver and Pipan 2009). Tropical regions are fascinating for biospeleological research because of their high biodiversity and the presence of large underground systems (Deharveng and Bedos 2012). Despite the recent rapid progress in the study of cave-dwelling organisms in tropical regions such as Brazil (Campos-Filho et al. 2023), numerous areas and taxonomic groups remain underexplored (Niemiller et al. 2018 and Wynne et al. 2021). With its diverse and relatively understudied cave-dwelling fauna, Central America has enormous potential for speleological discoveries (Day and Koenig 2002; Taylor et al. 2011; Pacheco et al. 2020). Expanding research efforts in these areas is essential to better understand the unique biological communities in caves and their ecological roles.

Costa Rica is a small country located in the Neotropical region. It is a natural bridge between North and South America, and has been estimated to hold at least 5% of the world's biodiversity (Avalos 2019). Despite the existing knowledge, this country has great potential for taxonomic investigation and discovery. The country has a limestone surface area of approximately 430 km² with numerous karst landscapes and more than 435 described caves (Ulloa et al. 2011; Grupo Espeleológico Anthros 2023). Although the carbonated platforms in Costa Rica cover less than 1% of the country's area, several karstic systems exceed a kilometer in length (Ulloa 2009a). In addition to limestone caves, Costa Rica has several volcanic caves with a total length of 2.2 km (Ulloa and Alvarado, personal communication). The dimensions of the largest cave systems in Costa Rica are modest compared with those in other parts of Central and South America. However, Costa Rican caves are undoubtedly captivating research objects that have been attracting numerous expeditions since the first speleological explorations in 1943 (Goicoechea 2015).

The earliest records of cave fauna in Costa Rica were from 1965 to 1969, with studies on some cave-dwelling bat species (Armstrong 1969). Long-term research on Seba's short-tailed bats (*Carollia perspicillata*) was conducted in the late 1970s and the early 1980s in Santa Rosa National Park (Heithaus and Fleming 1978; Fleming and Heithaus 1986). Costa Rican caves grabbed the attention of the US National Speleological Society (NSS) and different European speleological groups, and several expeditions were conducted in the country, providing a tremendous scientific contribution (Hempel 1989; Peacock and Hempel 1993). Several dedicated biospeleological studies have been conducted in the country, with one describing a new species of stygobiont (Hobbs 1991). The existing biospeleological data are summarized in the book "Introduction to Speleology" (Alpizar et al. 2006). Cave-dwelling bats have been the topic of recent studies (Cubero and Artavia 2016; Deleva and Chaverri 2018; Mitchell et al. 2018). Unfortunately, all previous efforts have covered only a portion of caves in the country. Many caves and taxonomic groups remain unknown.

This study aimed to provide an overview of the current state of knowledge regarding the cave-dwelling fauna of Costa Rica. Given that previous efforts have provided valuable, albeit scattered, information, we sought to systematize the existing data on the cave fauna of Costa Rica and add original preliminary research from our field expeditions. We hope that this study will provide insights for new studies and conservation efforts in Costa Rican cave-dwelling animals.

Methods

Literature review

We searched for literature sources that mentioned cave-dwelling fauna in Costa Rica, including but not limited to peer-reviewed articles, expedition reports, conference papers, short notes, and dissertations. We conducted searches using Google Scholar, Web of Science, and ResearchGate. We searched separately using each of the keywords "cave," "underground," "subterranean," "cave fauna," "speleology," "troglobiont", "troglobite," stygobiont," "stygobite" in combination with the keyword "Costa Rica," using the Boolean operator "and." We examined the references in the articles obtained during the search for additional relevant sources. We searched for studies published in Spanish by translating the keywords and performing the search with the same word combinations. Furthermore, we checked the expedition reports of the Anthros Speleological group (Grupo Espeleológico Anthros 2023) and the archives of the University of Costa Rica's library (UCR 2023). The last search was performed in October 2022.

Field research

In addition to the literature review, we also included preliminary data from observations of cave-dwelling animals during speleological expeditions between December 2015 and August 2018. The research sites included natural caves, artificial tunnels, and abandoned mines. We used direct observations inside the roosts where the specimens were documented with photographs. Field guides were used to identify animals at the species level (Henderson 2011). A small number of invertebrates was collected and preserved in 96% ethanol. The collected material was distributed for further identification among specialists in the different taxonomic groups. Bats were divided into two categories, following the assessment of Sagot and Chaverri (2015): 1) cave-dependent – only known to roost in caves or cave-like structures, and 2) not cave-dependent – roosting in caves as well as in other types of roosts. We created a dataset for each record of cave-dwelling fauna, which included the following attributes: location, site type, protected area name (if applicable), conservation status of the species, and citation (if applicable).

Spatial data

We used GIS software (ArcGIS Desktop 10.8.1) to create the maps. The locations of the sites were obtained from the database of the Anthros Speleological Group (Grupo Espeleológico Anthros 2023). We included information on the origin of each subterranean site: karst, volcanic, marine, artificial or unknown. We used publicly available geospatial data from the National Geographical Institute to determine whether the sites were located in protected areas (SNIT 2023). Because of the sensitive species inhabiting the subterranean sites, we did not disclose the exact coordinates of each cave, as unregulated visitation may further affect these sensitive resources. Therefore, we plotted site locations at a low resolution following the best practices for generalizing sensitive species occurrence data (Chapman 2022).

Conservation status

We determined the management status of all species identified by cross-checking each species with the IUCN Red List (IUCN 2023b), appendices of the Convention on the Conservation of Migratory Species of Wild Animals (CMS Convention 2023), the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 2023), and the Costa Rican Law for the protection of wildlife (Ley de Conservación de Vida Silvestre, MINAE 2017).

Results

We identified a total of 62 sources reporting organisms in Costa Rican caves. Data from the published sources contained 773 records, with 123 unique organisms identified at the species level. The articles and reports were written in Spanish (33), English (25), French (4), and Italian (1). Of these, one-third (23) were peer-reviewed, and the earliest study was published in 1965. The majority of the studies (43) were published after 2003. Our study included 186 observations (both vertebrates and invertebrates) from 42 sites, four of which had no prior biospeleological records. The combined literature data and field research provided information for 127 locations, accounting for 30% of the 435 described subterranean sites in Costa Rica.

Conservation status of Costa Rican caves and cave-dwelling fauna

Regarding the level of protection, most sites (91) were located outside of protected areas. The categories of the protected areas were national park (25), national wildlife refuge (4), forestry reserve (2), and protected zone (2) (Fig. 1). The subterranean sites across the country's specific administrative regions, also known as "Conservation Areas", were distributed as follows: Osa (63), Central Region (16), Tempisque (16), Guanacaste (7), Huetar Norte (6), La Amistad Pacifico (6), Pacifico Central (4), Arenal Tempisque (3), La Amistad Caribe (2). Most sites were concentrated in the two

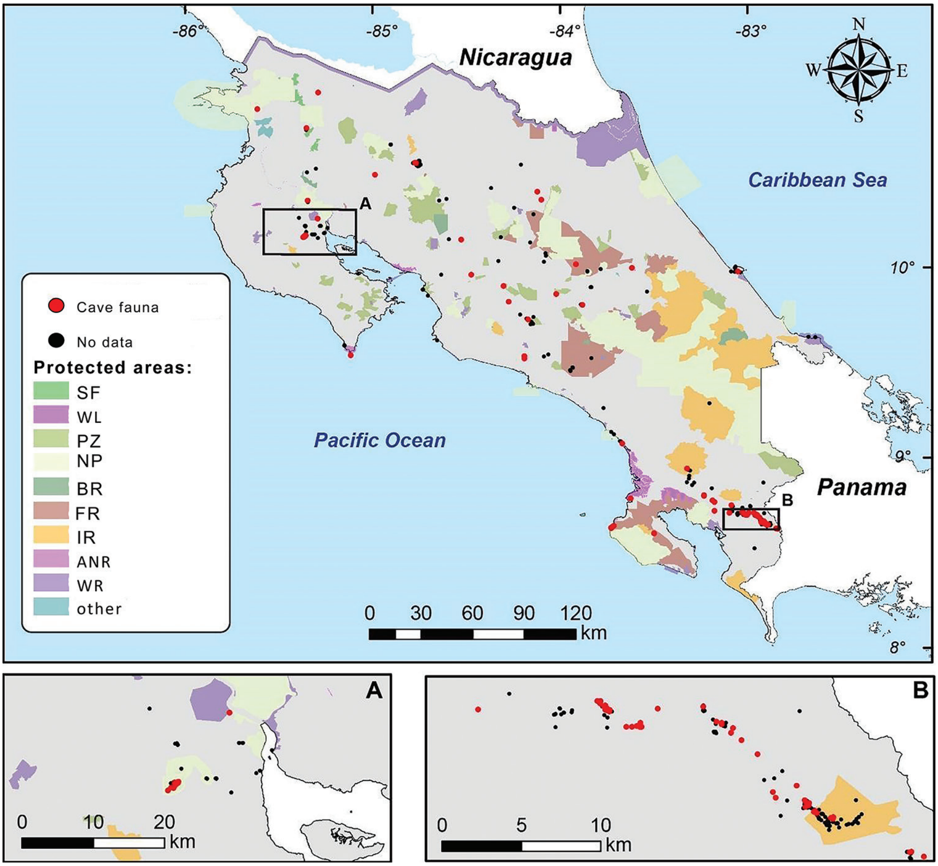

Figure 1. Costa Rica's subterranean sites: sites with records of cave fauna (red) vs. sites without records of cave fauna (black). The state-protected areas are presented in different colors: state farm (SF), wetland (WL), protected zone (PZ), national park (NP), biological reserve (BR), forest reserve (FR), indigenous reserve (IR), absolute natural reserve (ANR), and wildlife refuge (WR). The maps inserted at the bottom represent two karstic areas in Costa Rica with the most significant clusters of caves **A** Barra Honda karst area and **B** Zona Sur karst area.

largest karst areas in the country: Barra Honda, which is located in the Tempisque Conservation Area (Fig. 1A), and the Zona Sur Karst Area in the Osa Conservation Area (Fig. 1B). Fifteen of the 16 sites in Barra Honda were located within the borders of the Barra Honda National Park; only five of the 63 sites in Zona Sur had some level of state protection. Data on the of six of the sites were unavailable.

The distribution of global IUCN Red List conservation status among the species of cave-dwelling fauna was: "Least Concern" (75), "Not Evaluated" (43), "Data Deficient" (3), "Vulnerable" (2). Five species were included in the appendices of the CITES Convention. The Costa Rican Law for Wildlife Protection included nine species listed as "Reduced or Threatened population" and one species listed as "Endangered." No species were included in the appendices of the CMS Convention. The conservation status of all species is presented inTables 1–3 and Suppl. material 1.

Faunistic diversity in Costa Rican caves

Approximately 123 animal species were recorded in Costa Rican caves, along with 82 other records that could not be identified at the species level. Bats (Chiroptera) were the most studied vertebrate group with 36 species, followed by 26 species of other mammals (e.g., oppossums, rodents, or cats), 11 reptiles (Reptilia), and eight amphibians (Amphibia). Additionally, seven species of cave-dwelling fish (Actinopterygii) were identified. Only three species of birds (Aves) were observed in caves. Studies on invertebrate diversity were predominantly represented by insects (Insecta) with 81 reported taxa, followed by 43 arachnids (Arachnida), 11 springtails and bristletails (Entognatha), eight crustaceans (Malacostraca), and several other unique taxa, including snails, millipedes, clitellate worms, mollusks, centipedes, copepods, various worms, bristle worms, garden centipedes, and flatworms, each represented by one or a few species (Fig. 2).

Invertebrates

Mollusca

Snails and slugs (Gastropoda) were reported from 11 sites, and shellfish (Bivalvia) from one marine cave. Snails from the subfamily Subulininae have been observed in Costa Rican caves. The only species of snail identified at the species level was the miniature Awlsnail (*Subulina octona* (Bruguière, 1789)), which was observed in an abandoned gold mine in the Osa Peninsula.

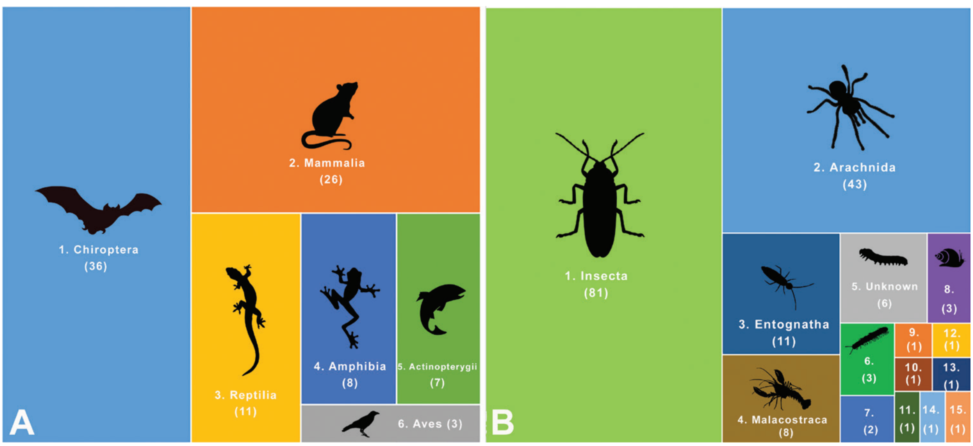

Figure 2. Number of animal taxa reported from subterranean sites in Costa Rica **A** vertebrates: 1. Chiroptera, 2. Mammalia (excluding bats) 3. Reptilia 4. Amphibia 5. Actinopterygii 6. Aves **B** invertebrates: 1. Insecta 2. Arachnida 3. Entognatha 4. Malacostraca 5. Unknown 6. Diplopoda 7. Clitellata 8. Gastropoda 9. Bivalvia 10. Chilopoda 11. Thecostraca 12. Oligochaeta 13. Polychaeta 14. Symphyla 15. Turbellaria.

Crustacea

Subterranean crustaceans included *Macrobrachium carcinus* (Linnaeus, 1758) (Fig. 3A), *Potamocarcinus magnus* (Rathbun, 1896), and *Ptychophallus montanus* (Rathbun, 1898). The troglophilic freshwater crab *Pseudothelphusa puntarenas* (Hobbs, 1991) from the Emus cave is currently the only known cave-dwelling species described from Costa Rica (Fig. 1B). Although it does not have any external troglomorphic modifications, the crab was observed only underground. The other crustaceans observed belonged to the classes Copepoda and Malacostraca (Bathynellacea and Isopoda) (Fig. 3C). A single observation of goose barnacles (*Pollicipes elegans* (Lesson, 1831)) (Fig. 3D) was reported from a marine cave.

Arachnida

A minimum of 16 mites and ticks (Acari) were identified (Table 1). The reported species of mites belong to the superfamily Hydrachnellae and the families Arrenuridae, Limnesiidae, Omartacaridae, and Torrenticolidae of the order Trombidiformes. Other identified mites belonged to the families Ascidae, Dinychidae, Macronyssidae, Spinturnicidae, Uropodidae (order Mesostigmata), and Torrenticolidae (order Trombidiformes). Several studies mentioned ticks and mites only at the order or family levels.

Figure 3. Crustaceans from Costa Rican caves **A** bigclaw river shrimp (*Macrobrachium carcinus*) **B** freshwater crab from Emus cave (*Pseudothelphusa puntarenas*) **C** woodlice (Armadillidae) **D** goose barnacles (*Polliceps elegans*).

Figure 4. Arachnids in Costa Rican caves **A** wandering spider (*Ancylometes bogotensis*) with an egg sack **B** tarantula (Theraphosidae) **C** tailless whip scorpions (Phrynidae).

Spiders (Araneae) were often observed in caves, but we could not find relevant peer-reviewed publications on their diversity. We report observations of spiders in the families Attidae, Ctenidae, Dysderidae, Lycosidae, Theraphosidae, Theridiidae, Theridiosomatidae, and Trechaleidae (Fig. 4). Harvestmen (Opiliones) consisted of,two identified species, *Pachylicus hispidus* Goodnight & Goodnight, 1983 from the family Phalangodidae and *Panopiliops inops* Goodnight & Goodnight, 1983 from the family Zalmoxidae. Both species were reported from the Damas cave. The remaining records (10) only mention the order without providing further details.

False scorpions (Pseudoscorpiones) were reported from five Costa Rican caves, but none of the reviewed studies provided species-level information. True scorpions (Scorpiones) were reported from three caves, and short-tailed whip scorpions (Schizomida) were observed in five caves in the Puntarenas region.

Records from 27 caves and subterranean sites mentioned Amblypygi, and there is a cave named after this animal (the Amblipigio cave). Cave-dwelling Amblypygi were reported to belong to the family Phrynidae, with one record of Tarantulidae. The second family was not mentioned in other sources and may refer to *Phrynus parvulus* (Pocock, 1902) of the family Phrynidae, previously known as *Tarantula marginemaculatus*. All Amblypygi in Costa Rica belong to the family Phrynidae, within the genera *Paraphrynus* (Moreno, 1940) and *Phrynus* (Harvey 2019). The identified species of cave-dwelling whip scorpions was *Paraphrynus laevifrons* (Pocock, 1894).

Myriapoda

Millipedes (Diplopoda) and centipedes (Chilopoda) were observed in at least 19 caves, but there were almost no data on their taxonomy, except for one record mentioning the family Polyxenidae. Garden centipedes from the class Symphyla were recorded from two caves.

Insecta

The reported species of cockroaches (Blattodea) were *Blaberus giganteus* (Linnaeus, 1758) and *Megaloblatta blaberoides* (Walker, 1871). A noteworthy refuge for cockroaches was the Hediondo cave, which harbors a large number of cockroaches from the *Blaberus* genus. Beetles (Coleoptera) from the families Alleculidae (Tenebrionidae), Bostrichidae, Carabidae, Cerambycidae, Curculionidae, Cleridae, Passalidae, Scarabaeidae, Scolytidae, Staphylinidae, and Tenebrionidae were observed inside Costa Rican caves and near their entrances. The Lamiinae subfamily and Clytini tribe of the Cerambycidae family, *Temnocheila* sp. (Trogossitidae), *Pyrophorus* sp. (Elateridae), and *Zophobas atratus* (Blanchard, 1845) (Tenebrionidae) were the only beetles classified at a lower taxonomic level. Cave crickets were observed on cave walls, but the available records only refer to them by their common names.

Bugs (Auchenorrhyncha) belonging to the families Fulgoridae and Cicadellidae were documented in Barra Honda National Park. True bugs (Heteroptera) from the families Reduviidae, Pentatomidae, Lygaeidae, Coreidae, Corixidae, and Cydnidae were reported to inhabit caves. Based on the specimens we collected during our field trips, we present the first record of the true bug *Amnestus subferrugineus* (Westwood 1837) (Heteroptera: Cydnidae) for the fauna of Costa Rica (Fig. 5). This is the first record of the genus *Amnestus* Dallas, 1851 in Costa Rican caves.

A few earwigs (Dermaptera) were mentioned in expedition reports from the Puntarenas province. Flies (Diptera), including but not limited to the families Streblidae, Tabanidae, Tachinidae, and Heleomyzidae, were reported in caves. Parasitic wingless flies *Strebla wiedemanni* Kolenati, 1856 and *Trichobius parasiticus* Gervais, 1844 were collected from vampire bats in various parts of the country. A single record of Ephemeroptera was reported from the Corredores cave. Ants (Formicidae) were observed in at least nine caves. Other Hymenoptera included the families Eumenidae, Ichneumoni-

Figure 5. Morphological characteristics of the true bug (*Amnestus subferrugineus*) found in Costa Rican caves.

dae, Mutillidae, Pompilidae, Sphecidae, Tenthredinidae, and the wasp *Polistes instabilis* de Saussure, 1853 (Vespidae). All of the observed insect orders are listed in Table 1.

Table 1. Classes of invertebrates in Costa Rican caves. The first column represents the taxon. The second column (CS) presents the conservation status of the species: 1. IUCN Red List - "Least Concern" (LC), "Not Evaluated" (NE), "Data Deficient" (DD), "Vulnerable" (VU), 2. Included in the CITES convention: CITES, 3. Included in the annexes of the Costa Rican Biodiversity law (LEY) – "Vulnerable" (VU), "Reduced or threatened population" (TR). The third column (N) represents the number of individual sites where the taxon was present. The last column presents the sources of information regarding the taxa.

Taxon	CS	N	References
TURBELLARIA			
Turbellaria indet.		1	(Peacock and Hempel 1993)
POLYCHAETA			
Phyllodocida			
Nereididae			
Lycastopsis sp.		1	(Peacock and Hempel 1993)
OLIGOCHAETA			
Haplotaxida		1	(Peacock and Hempel 1993)
CLITELLATA			
Clitellata indet.		1	(Graening 2004)
Hirudinea			
Hirudinea indet.		1	(Lips and Lips 2008)
BIVALVIA			
Bivalvia indet.		1	ND[†]
GASTROPODA			
Heterobranchia			
Stylommatophora			
Achatinidae			
Subulininae		8	ND
Subulina octona (Bruguière, 1789)		1	ND
Gastropoda indet.		10	(Lips and Lips 2008; Palacios 1994; Peacock and Hempel 1993), ND
THECOSTRACA			
Pollicipedidae			
Pollicipes elegans (Lesson, 1831)		1	ND
COPEPODA			
Copepoda indet.		1	(Peacock and Hempel 1993)
MALACOSTRACA			
Bathynellacea		PS[§]	(Peacock and Hempel 1993)
Decapoda			
Palaemonidae			
Macrobrachium carcinus (Linnaeus, 1758)	IUCN-LC	5	(Hobbs 1994; Peacock and Hempel 1993), ND
Pseudothelphusidae			
Potamocarcinus magnus (Rathbun, 1896)	IUCN-LC	PS[§]	(Hobbs 1994)
Pseudothelphusa puntarenas Hobbs 1991[‡]	IUCN-DD	1	(Hobbs 1991; Hobbs 1994; Peacock and Hempel 1993), ND
Pseudothelphusa sp.		1	(Gonzalez 2012)
Ptychophallus montanus (Rathbun, 1898)		1	(Hobbs 1994; Peacock and Hempel 1993)
Pseudothelphusidae indet.		7	(Hobbs 1994; Lips and Lips 2008; Peacock and Hempel 1993b; Quesada 2016), ND

Taxon	CS	N	References
Isopoda			
Oniscidea		11	(Graening 2004; Hempel 1989; Lips and Lips 2008; Palacios 1994; Peacock and Hempel 1993; Strinati et al. 1987), ND
Armadillidae		1	ND
ARACHNIDA			
Opiliones			
Phalangodidae		4	(Peacock and Hempel 1993)
Zalmoxidae			
Pachylicus hispidus Goodnight & Goodnight, 1983		1	(Goodnight and Goodnight 1983; Juberthie and Strinati 1994)
Panopiliops inops Goodnight & Goodnight, 1983		1	(Goodnight and Goodnight 1983; Juberthie and Strinati 1994)
Indet.		9	(Graening 2004; Hempel 1989; Lips and Lips 2008; Peacock and Hempel 1993), ND
Acari			
Acariformes			
Pyemotidae		1	(Palacios 1994)
Ixodida		1	(Hempel 1989)
Mesostigmata			
Ascidae		1	(Palacios 1994)
Dinychidae			
Urodiaspis sp.		1	(Palacios 1994)
Macronyssidae			
Radfordiella desmodi Radovsky, 1967		1	(Rojas et al. 2008)
Spinturnicidae			
Periglischrus herrerai Machado-Allison, 1965		1	(Rojas et al. 2008)
Uropodidae			
Neodiscopoma sp.		1	(Palacios 1994)
Indet.		1	(Palacios 1994)
Oribatida			
Carabodidae		1	(Palacios 1994)
Galunmidae		1	(Lips and Lips 2008)
Indet.		1	(Lips and Lips 2008)
Trombidiformes			
Arrenuridae			
Arrenurus golfitensis Cook, 1980		1	(Juberthie and Strinati 1994)
Arrenurus plevamus Cook, 1980		1	(Juberthie and Strinati 1994)
Arrenurus zukovus Cook, 1980		1	(Juberthie and Strinati 1994)
Hydrachnellae			
Protolimnesia mesoamericana Cook, 1980		1	(Juberthie and Strinati 1994)
Limnesiidae			
Neomamersa costarriquensis Cook, 1980		PS[§]	(Juberthie and Strinati 1994)
Neomamersa decussa Cook, 1980		PS[§]	(Juberthie and Strinati 1994)
Psammolimnesia costarriquena Cook, 1980		PS[§]	(Juberthie and Strinati 1994)
Omartacaridae			
Omartacarus motasi Cook, 1980		NC[§]	(Juberthie and Strinati 1994)
Rhagidiidae		1	(Palacios 1994)
Torrenticolidae			
Torrenticola amala Cook, 1980		1	(Juberthie and Strinati 1994)
Frontipodopsis mesoamericana Cook, 1980		1	(Juberthie and Strinati 1994)
Frontipodopsis staheli Walter, 1919		1	(Juberthie and Strinati 1994)
Maharashtracarus neotropicus Cook, 1980		1	(Juberthie and Strinati 1994)

Taxon	CS	N	References
Acari indet.		14	(Graening 2004; Hempel 1989; Lips and Lips 2008; Peacock and Hempel 1993; Strinati et al. 1987), ND
Pseudoscorpiones		5	(Lips and Lips 2008; Palacios 1994; Peacock and Hempel 1993; Strinati et al. 1987), ND
Scorpiones		3	(Hempel 1989; Peacock and Hempel 1993; Strinati et al. 1987)
Araneae			
Attidae		1	ND
Ctenidae			
Ctenus sp.		1	ND
Ancylometes bogotensis (Keyserling, 1877)		1	ND
Dysderidae		1	(Alpizar et al. 2006)
Lycosidae		1	ND
Segestriidae			
Ariadna isthmica Beatty, 1970		1	(Alpizar et al. 2006)
Theraphosidae		2	(Alpizar et al. 2006), ND
Sericopelma upala Valerio, 1980	LEY-RTP	1	(Alpizar et al. 2006)
Theridiidae		1	(Alpizar et al. 2006)
Theridiosomatidae		1	(Alpizar et al. 2006)
Trechaleidae			
Trechalea sp.		1	ND
Araneae indet.		20	(Graening 2004; Hapka et al. 1992; Hempel 1989; Lips and Lips 2008; Quesada and Deleva 2016; Palacios 1994; Peacock and Hempel 1993; Strinati et al. 1987)
Amblypygi			
Phrynidae			
Paraphrynus laevifrons (Pocock, 1894)		1	(Mullinex 1975; Juberthie and Strinati 1994; Alpizar et al. 2006)
Paraphrynus viridiceps (Pocock, 1894)		7	(Peacock and Hempel 1993)
Paraphrynus sp.		7	ND
Phrynidae indet.		20	(Graening 2004; Debeljak 1988; Hapka et al. 1992; Hempel 1989; Lips and Lips 2008; Quesada 2018; Peacock and Hempel 1993; Strinati et al. 1987), ND
Schizomida		5	(Lips and Lips 2008; Juberthie and Strinati 1994; Strinati et al. 1987)
SYMPHYLA			
Symphyla indet.		2	(Lips and Lips 2008; Strinati et al. 1987)
CHILOPODA			
Chilopoda indet.		3	(Lips and Lips 2008; Strinati et al. 1987), ND
DIPLOPODA			
Polyxenidae		1	(Palacios 1994)
Diplopoda indet.		17	(Graening 2004; Hempel 1989; Lips and Lips 2008; Palacios 1994; Peacock and Hempel 1993; Strinati et al. 1987), ND
ENTOGNATHA			
Collembola			
Neelipleona			
Neelidae			
Megalothorax cf. minimus Willem, 1900		1	(Palacios 1994)
Megalothorax sp.		1	(Palacios 1994)
Entomobryomorpha			
Paronellidae			
Cyphodeus sp.		1	(Juberthie and Strinati 1994)
Trogolaphysa sp.		1	(Palacios 1994)

Taxon	CS	N	References
Isotomidae			
Folsomides sp.		1	(Palacios 1994)
Folsomina onychiurina Denis, 1931		1	(Palacios 1994)
Isotomurus minor UN		1	(Palacios 1994)
Isotomiella cf. minor (Schäffer, 1896)		1	(Palacios 1994)
Collembola indet.		1	(Hapka et al. 1992; Quesada and Deleva 2016; Lips and Lips 2008; Peacock and Hempel 1993; Strinati et al. 1987), ND
Diplura			
Japygidae		1	(Strinati et al. 1987)
Diplura indet.		4	(Graening 2004; Hempel 1989; Lips and Lips 2008), ND
INSECTA			
Archaeognatha			
Meinertellidae			
Grasiella sp.		1	(Juberthie and Strinati 1994)
Zygentoma			
Nicoletiidae			
Nicoletia cf. phytophile Gervais, 1844		1	(Juberthie and Strinati 1994)
Zygentoma (reported as Thysanura)		1	(Strinati et al. 1987)
Ephemeroptera			
Heptageniidae		1	ND
Odonata		2	(Hempel 1989; Peacock and Hempel 1993)
Orthoptera			
Acrididae		1	(Hempel 1989)
Gryllacrididae		1	(Hempel 1989)
Gryllidae		1	(Hempel 1989)
Phalangopsidae		11	(Ulloa and Quesada 2010), ND
Tettigoniidae		1	(Hempel 1989)
Orthoptera indet.		12	(Quesada and Deleva 2016; Goicoechea 2010; Graening 2004; Hapka et al. 1992; Lips and Lips 2008; Peacock and Hempel 1993; Strinati et al. 1987)
Neuroptera			
Chrysopidae		1	(Hempel 1989)
Myrmeleontidae		2	(Graening 2004; Hempel 1989)
Dermaptera		7	(Lips and Lips 2008; Peacock and Hempel 1993)
Mantodea			
Mantidae		1	(Hempel 1989)
Blattodea			
Blaberidae			
Blaberus giganteus (Linnaeus, 1758)		3	(Graening 2004), ND
Blaberus sp.		1	(Ulloa and Quesada 2010)
Ectobiidae			
Megaloblatta blaberoides (Walker, 1871) (as *M. rufipes* Dohrn, 1887)		1	(Palacios 1994)
Blattodea indet.		16	(Hapka et al. 1992; Hempel 1989; Lips and Lips 2008; Peacock and Hempel 1993), ND
Isoptera		1	(Hempel 1989)
Hemiptera			
Coreidae		1	(Hempel 1989)
Cicadellidae		1	(Hempel 1989)
Corixidae		1	(Hempel 1989)
Cydnidae			
Amnestus subferrugineus (Westwood, 1837)		1	ND

Taxon	CS	N	References
Fulgoridae		1	(Hempel 1989)
Lygaelidae		1	(Hempel 1989)
Pentatomidae		1	(Hapka et al. 1992; Hempel 1989)
Reduviidae			
Triatoma sp.		1	(Hempel 1989)
Hemiptera indet.		9	(Graening 2004; Hapka et al. 1992; Lips and Lips 2008; Peacock and Hempel 1993)
Hymenoptera			
Eumenidae		1	(Hempel 1989)
Formicidae		10	(Graening 2004; Hapka et al. 1992; Hempel 1989; Peacock and Hempel 1993), ND
Ichneumonidae		1	(Hempel 1989)
Mutillidae		1	(Graening 2004; Hempel 1989)
Pompilidae		1	(Hempel 1989)
Sphecidae		1	(Graening 2004)
Tenthredinidae		1	(Hempel 1989)
Vespidae		1	(Hempel 1989)
Polistes instabilis de Saussure, 1853		1	(Graening 2004)
Hymenoptera indet.		4	(Hempel 1989; Lips and Lips 2008; Peacock and Hempel 1993)
Coleoptera			
Alleculidae		1	(Hempel 1989)
Bostrichidae		1	(Hempel 1989)
Carabidae		2	(Graening 2004)
Cerambycidae		2	(Hempel 1989)
Cleridae		1	(Hempel 1989)
Curculionidae		1	(Hempel 1989)
Elateridae			
Pyrophorus sp.		1	(Hempel 1989)
Passalidae		1	(Hempel 1989)
Scarabaeidae		1	(Graening 2004)
Scolytidae		1	(Hempel 1989)
Staphylinidae		1	ND
Tenebrionidae		1	(Graening 2004)
Zophobas atratus (Fabricius, 1775)		2	(Tschinkel 1984; Juberthie and Strinati 1994)
Trogossitidae			
Temnoscheila (as *Temnochila*) sp.		1	(Hempel 1989)
Coleoptera indet.		12	(Hapka et al. 1992; Lips and Lips 2008; Palacios 1994; Peacock and Hempel 1993)
Trichoptera		1	(Hempel 1989)
Lepidoptera			
Nymphalidae (as Brassolidae)		1	(Hempel 1989)
Hesperiidae		1	(Hempel 1989)
Lycaenidae		1	(Hempel 1989)
Noctuidae		1	(Hempel 1989)
Nymphalidae		1	(Hempel 1989)
Tineidae		1	(Lips and Lips 2008)
Lepidoptera indet.		3	(Hapka et al. 1992; Lips and Lips 2008; Peacock and Hempel 1993, (Strinati et al. 1987)
Diptera			
Heleomyzidae		1	(Graening 2004)
Streblidae			
Exastinion clovisi (Pessõa & Guimarães, 1937)		1	(Mitchell et al. 2018)

Taxon	CS	N	References
Megistopoda aranea (Coquillett, 1899)		1	(Mitchell et al. 2018)
Strebla wiedemanni Kolenati, 1863		1	(Rojas et al. 2008)
Trichobius lionycteridis Wenzel, 1966		1	(Mitchell et al. 2018)
Trichobius pallidus (Curran, 1934)		3	(Mitchell et al. 2018)
Trichobius parasiticus Gervais, 1844		1	(Rojas et al. 2008)
Indet.		1	(Hempel 1989)
Tabanidae		1	(Hempel 1989)
Tachinidae		1	(Hempel 1989)
Insecta indet.		15	(Debeljak 1988; Goicoechea 2010a; Lips and Lips 2008), ND
Unknown arthropods		4	(Lips and Lips 2008)
Unknown invertebrates		5	(Lips and Lips 2008)

† - ND - new data: original contribution to this paper, ‡ - stygobiont, § - some sources report a region instead of a single site: PS – Puntarenas, NC – Nicoya, | - species is most likely misidentified.

Entognatha

Springtails (Collembola) have also been observed in Costa Rican caves, but there have only been a few mentions of lower taxa. *Megalothorax minimus* Willem, 1900, *Isotomiella minor* (Schaeffer, 1896), *Folsomina onychiurina* (Denis, 1931), *Folsomides* sp., *Isotomurus* sp. *Trogolaphysa* sp. Bristletails (Diplura) were found in caves, but no specific information regarding their taxonomy was available.

Other invertebrates

Reports exist regarding worms belonging to Turbellaria, Oligochaeta, and Clitellata. The term "worm" was also used as a general morphological descriptor for invertebrates observed within caves.

Vertebrates

Actinopterygii

Two species of Costa Rican fish (Actinopterygii) display adaptations to cave life. These species are the three-barbed catfish from the *Rhamdia* genus and the characid Mexican tetra (*Psalidodon fasciatus* (De Filippi, 1853)). Pale-colored individuals of the catfish species *Rhamdia guatemalensis* (Günther, 1864) were observed in the Corredores and Bananal cave systems as well as in other adjacent caves (Fig. 6). Furthermore, pale-colored individuals of the same genus have been reported in an artificial tunnel near Arenal volcano. The Mexican tetra, also known as the blind cave fish, was studied in a karstic spring in Guanacaste. Livebearing fishes from the *Brachyrhaphis* genus, rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)), Nile tilapia (*Oreochromis niloticus* (Linnaeus, 1758)), and various unidentified characids (Characidae), cyprinids (Cyprinidae), and catfish (Heptapteridae) were reported from caves and springs (Table 2, Suppl. material 1).

Figure 6. Pale-colored catfish (*Rhamdia guatemalensis*) in Corredores cave.

Amphibia

Frogs and toads (Anura) were observed both at the entrances and deep inside the caves (Fig. 7). We found records of at least eight species belonging to five frog and toad families (Table 2). These included poison dart frogs, *Dendrobates auratus* (Girard, 1855), *Oophaga granulifera* (Taylor, 1958), thin-toed frogs (*Leptodactylus savagei* Heyer, 2005), grass frogs (*Lithobates forreri* (Boulenger, 1883)), *L. warszewitschii* (Schmidt, 1857)), toads (*Rhinella horribilis* (Wiegmann, 1833)), and *Incilius aucoinae* (O'Neill & Mendelson, 2004). Cane toads (*Rhinella horribilis*) were observed at the bottom vertical shafts on several occasions. There were two observations of tadpoles from the Carma and Corredores caves. The cave "Pozo Sapo Gordo" ("Fat Toad Abyss") received its name because of the presence of a large cane toad.

Reptilia

The South American snapping turtle (*Chelydra acutirostris* (Peters, 1862)) and the white-lipped mud turtle (*Kinosternon leucostomum* (Duméril, Bibron & Duméril, 1851)) were observed on multiple occasions deep inside a flooded artificial tunnel (Fig. 8A, B). The fer-de-lance (*Bothrops asper* (Garman, 1883)) was observed both at the entrances and in narrow passages inside the caves (Fig. 8C). The caves "Serpiente Dormida," "Pozo del Chispero," "Terciopelo," and "Pozo Oropel" were named after encounters between snakes and cave explorers. The aquatic prawn snake (*Hydromorphus concolor* (Peters, 1859)) was observed in an artificial tunnel near Arenal volcano

Figure 7. Frogs and toads found in Costa Rican caves **A** forrers grass frog (*Lithobates forreri*) **B** green and black poison dart frog (*Dendrobates auratus*) **C, E** Fitzinger's Robber Frog (*Craugastor fitzingeri*) **D** rainforest toad (*Incilius aucoinae*) **F** thin-toed frog (*Leptodactylus savagei*) **G** cane toad (*Rhinella horribilis*).

Figure 8. Reptiles living in caves **A** South American snapping turtle (*Chelydra acutirostris*) **B** white-lipped mud turtle (*Kinosternon leucostomum*) **C** fer-de-lance - (*Bothrops asper*) **D** Costa Rican tropical night lizard (*Lepidophyma reticulatum*) **E** prawn snake (*Hydromorphus concolor*).

(Fig. 8E). Additionally, there were sightings of a boa (Boaidae) and an unknown species of snake, solely identified based on visual characteristics. The night lizard (*Lepidophyma reticulatum* (Taylor, 1955)) was observed in at least three caves (Fig. 8D). Reports also mentioned the presence of geckos (Geckota) within a cave.

Birds

Information regarding birds residing in and around caves was limited. However, there were a few noteworthy observations. The entrance of an artificial tunnel near Rio Terraba served as a nesting site for a black vulture (*Coragyps atratus* (Bechstein, 1793)). Additionally, sightings near cave entrances included a great tinamou (*Tinamus major* (Gmelin, 1789)) and a wood rail (*Aramides cajaneus* Müller, 1776).

Mammalia

Non-volant mammals in the subterranean ecosystem predominantly comprise of small predators and rodents. Various tracks attributed to carnivorous mammals such as cats and mustelids have been observed in different caves. In Palo Verde National Park, the "Tigre cave" presumably served as a roosting site for a large cat, possibly a jaguar or puma. An ocelot (*Leopardus pardalis* (Linnaeus, 1758)) was sighted in an artificial tunnel, and bones of kinkajou (*Potos flavus* (Schreber, 1774)) were discovered in Trampa vertical cave. Opossums (Didelphidae) of at least four species were observed within the caves. Caves in Barra Honda yielded bones from various mammals, including peccary (*Dicotyles tajacu* (Linnaeus, 1758)), cottontail rabbit (*Sylvilagus* sp.), white-tailed deer (*Odocoileus virginianus* (Zimmermann, 1780)), armadillo (*Dasypus novemcinctus* (Linnaeus, 1758)), porcupine (*Coendou mexicanus*), and several species of rodents (Rodentia). It remains unclear whether these mammals entered the caves or whether their carcasses were brought in by predators.

Table 2. Classes of vertebrates in Costa Rican caves. The first column represents the taxon. The second column (CS) presents the conservation status of the species: 1. IUCN Red List - "Least Concern" (LC), "Not Evaluated" (NE), "Data Deficient" DD), "Vulnerable" VU), 2. Included in the CITES convention: CITES, 3. Included in the annexes of the Costa Rican Biodiversity law (LEY) – "Vulnerable" (VU), and "Reduced or threatened population" (TR). The third column (N) represents the number of individual sites where the taxon was present. The last column presents the sources of information regarding the taxa.

Taxon	CS	N	Reference(s)
ACTINOPTERYGII			
Cypriniformes			
Cyprinidae		1	(Peacock and Hempel 1993)
Characiformes			
Characidae			
Psalidodon fasciatus (De Filippi, 1853) (as *Astyanax fasciatus*)§	IUCN-LC	1	(Romero 1985)
Characidae indet.		1	ND

Taxon	CS	N	Reference(s)
Siluriformes			
Heptapteridae			
Rhamdia guatemalensis (Günther, 1864)[§]	IUCN-LC	5	(Debeljak 1988; Juberthie and Strinati 1994; Grupo Espeleológico Anthros 2023), ND
Rhamdia nicaraguensis (Günther, 1864)	IUCN-LC	1	(Gonzalez 2012)
Rhamdia sp.		4	(Strinati et al. 1987), ND
Heptapteridae indet.		3	(Quesada and Deleva 2016; Peacock and Hempel 1993)
Salmoniformes			
Salmonidae			
Oncorhynchus mykiss (Walbaum, 1792)		1	(González 2010)
Cichliformes			
Cichlidae			
Oreochromis niloticus (Linnaeus, 1758)	IUCN-LC	1	(Gonzalez 2012)
Cyprinodontiformes			
Poeciliidae			
Brachyrhaphis rhabdophora (Regan, 1908)	IUCN-VU	1	(Romero 1985)
Brachyrhapis olomina (Meek, 1914)	IUCN-DD	1	(Gonzalez 2012)
Actinopterygii indet.[‡]		1	(Woodman 1988)
AMPHIBIA			
Anura			
Craugastoridae			
Craugastor fitzingeri (Schmidt, 1857)	IUCN-LC	2	(Quesada 2018), ND
Bufonidae			
Rhinella horribilis (Wiegmann, 1833)	IUCN-LC	5	(Gonzalez 2012; Graening 2004), ND
Incilius aucoinae (O'Neill & Mendelson, 2004)	IUCN-LC	1	ND
Bufonidae indet.		2	(Hapka et al. 1992), ND
Dendrobatidae			
Dendrobates auratus (Girard, 1855)	IUCN-LC, CITES-II, LEY-RTP	1	(Quesada 2018), ND
Oophaga granulifera (Taylor, 1958)	IUCN-VU, CITES-II, LEY-RTP	1	ND
Leptodactylidae			
Leptodactylus savagei Heyer, 2005	IUCN-LC	2	(Quesada and Deleva 2016), ND
Ranidae			
Lithobates warszewitschii (Schmidt, 1857)	IUCN-LC	1	(Ulloa and Quesada 2010)
Lithobates forreri (Boulenger, 1883)	IUCN-LC	1	ND
Ranidae indet.		1	(Lips and Lips 2008; Peacock and Hempel 1993; Strinati et al. 1987), ND
Anura indet.		1	(Quesada 2009b)
Amphibia indet.[‡]		8	(Woodman 1988)
REPTILIA			
Testudines			
Chelydridae			
Chelydra acutirostris Peters, 1862	CITES-II	1	(Gonzalez 2012), ND
Kinosternidae			
Kinosternon leucostomum (Duméril, Bibron & Duméril, 1851)	CITES-II	1	(Gonzalez 2012), ND
Squamata			
Boaidae		2	(Lyon et al. 2004), Vicente-Santos 2019)

Taxon	CS	N	Reference(s)
Colubridae			
Hydromorphus concolor Peters, 1859	IUCN-LC	1	ND
Viperidae			
Bothrops asper (Garman, 1883)	IUCN-LC	2	(Quesada 2009a), ND
Bothriechis schlegelii (Berthold, 1846)	IUCN-LC	1	(Hapka et al. 1992)
Serpentes indet.		2	(Hapka et al. 1992)
Xantusiidae			
Lepidophyma reticulatum Taylor, 1955	IUCN-LC	2	(Ulloa 2009b), ND
Gekkota		1	(Graening 2004)
Reptilia indet.‡		1	(Goicoechea 2010; Graening 2004; Hapka et al. 1992; Woodman 1988)
AVES			
Tinamiformes			
Tinamidae			
Tinamus major (Gmelin, 1789)	IUCN-LC	1	ND
Gruiformes			
Rallidae			
Aramides cajaneus (Müller, 1776)	IUCN-LC	1	(Gonzalez 2012)
Cathartiformes			
Cathartidae			
Coragyps atratus (Bechstein, 1793)	IUCN-LC	1	(Quesada 2018), ND
MAMMALIA			
Cingulata			
Dasypodidae			
Dasypus novemcinctus Linnaeus, 1758‡	IUCN-LC	2	(Hempel 1989; Woodman 1988)
Didelphimorphia			
Didelphidae			
Caluromys derbianus (Waterhouse, 1841)	IUCN-LC	1	(Trescott and Vicente-Santos 2019)
Didelphis marsupialis Linnaeus, 1758	IUCN-LC	1	(Hempel 1989)
Didelphis sp.‡		1	(Woodman 1988)
Marmosa mexicana Merriam, 1897	IUCN-LC	1	(Hempel 1989)
Philander opossum (Linnaeus, 1758)	IUCN-LC	1	(Gonzalez 2012)
Didelphidae indet.		2	ND
Lagomorpha			
Leporidae			
Sylvilagus sp.		2	(Hempel 1989)
Rodentia			
Cricetidae			
Oryzomys sp.		1	(Hempel 1989)
Ototylomys phyllotis Merriam, 1901‡	IUCN-LC	1	(Hempel 1989; Woodman 1988)
Peromyscus stirtoni Dickey, 1928‡	IUCN-LC	1	(Woodman 1988)
Peromyscus sp. ‡		1	(Woodman 1988)
Reithrodontomys sp. ‡		1	(Woodman 1988)
Sigmodon hispidus Say & Ord, 1825‡	IUCN-LC	3	(Hempel 1989; Woodman 1988)
Cuniculidae			
Cuniculus paca (Linnaeus, 1766)	IUCN-LC, LEY-RTP	3	(Hempel 1989; Woodman 1988)
Dasyproctidae			
Dasyprocta punctata (Gray, 1842)‡	IUCN-LC	4	(Hempel 1989; Lips and Lips 2008; Woodman 1988)
Erethizontidae			
Coendou mexicanus (Kerr, 1792)‡	IUCN-LC	2	(Hempel 1989; Woodman 1988)
Geomyidae			
Orthogeomys sp.		1	(Hempel 1989)

Taxon	CS	N	Reference(s)
Heteromyidae			
Liomys salvini (Thomas, 1893)‡	IUCN-LC, LEY-RTP	2	(Hempel 1989; Woodman 1988)
Carnivora			
Procyonidae			
Potos flavus (Schreber, 1774)‡	IUCN-LC	1	(Hempel 1989)
Felidae			
Leopardus pardalis (Linnaeus, 1758)	IUCN-LC, CITES-I, LEY-VU	1	(Gonzalez 2012)
Felidae indet.		2	(Graening 2004; Hapka et al. 1992)
Carnivora indet.		1	ND
Perissodactyla			
Equidae			
Equus ferus caballus Linnaeus, 1758‡		1	ND
Artiodactyla			
Cervidae			
Odocoileus virginianus (Zimmermann, 1780)‡	IUCN-LC	1	(Hempel 1989)
Tayassuidae			
Dicotyles tajacu (Linnaeus, 1758)‡	IUCN-LC	2	(Hempel 1989; Woodman 1988)

† - ND - new data: original contribution to this paper, ‡ - bones, § - observed individuals with morphological adaptations toward cave life.

Chiroptera

Bats were documented at least in 97 subterranean sites throughout the country (Table 3). Thirty-six bat species from the families Emballonuridae, Mormoopidae, Natalidae, Noctilionidae, Phyllostomidae, and Vespertilionidae have been reported inside caves or at their entrances (Fig. 9). The most frequently observed species was Seba's short-tailed bat (*Carollia perspicillata* (Linnaeus, 1758)), found in 44 locations, followed by the common vampire bat (*Desmodus rotundus* (Geoffroy, 1810)) (34 locations), greater dog-like bat (*Peropteryx kappleri* (Peters, 1867)) (22 locations), greater sac-winged bat (*Saccopteryx bilineata* (Temminck, 1838)) (17 locations), Pallas's long-tongued bat (*Glossophaga soricina* (Pallas, 1766)) (12 locations), and Tomes' sword-nosed bat (*Lonchorhina aurita* Tomes, 1863 (10 locations)). Parnell's mustached bat was reported as either *Pteronotus parnellii* or *P. mesoamericanus* at 17 locations. The funnel-eared bat, found in 10 locations, was identified as *Natalus mexicanus* in some sources and either *Natalus stramineus* or *Natalus lanatus* in others. However, these scientific names are currently accepted as synonyms, suggesting that they likely represent the same species (Solari 2019). Regarding the importance of caves as bat roosts, eight species (*Glossophaga leachii*, *Lonchophylla robusta*, *Lonchorhina aurita*, *Natalus mexicanus*, *Pteronotus davyi*, *Pteronotus gymnonotus*, *Pteronotus mesoamericanus*, and *Pteronotus personatus*) were considered cave-dependant. Among the caves with the highest reported bat species richness were La Trampa (13 species), Corredores (11 species), Gabinarraca (8 species), Emus (8 species), Damas (7 species) Pozo Hediondo (6 species), and an artificial tunnel near Arenal volcano (6 species) (Suppl. material 1). The global conservation status of all the observed cave-dwelling bats was Least Concern. The Costa Rican Law for the protection of wildlife included four species (*Anoura cultrata*, *Chrotopterus auritus*, *Lonchophylla concava* and *Trinycteris nicefori*) in the category "Reduced or Threatened population" (Table 3).

Table 3. Bats (Chiroptera) in Costa Rican caves. The first column represents the taxon. The second column (CD) represents the species dependence on caves. The third column (CS) shows the species conservation status, according to the IUCN Red List (IUCN) and the Costa Rican Biodiversity law (LEY). The abbreviations are "Least concern" (LC) and "reduced or threatened population" (RTP). The fourth column (№) represents the number of individual sites where the taxon was present. The last column presents the sources of information regarding the taxa.

Taxon	CD	CS	№	References
Emballonuridae				
Balantiopteryx plicata (Peters, 1867)		IUCN-LC	2	(Timm and McClearn 2007; Graening 2004)
Peropteryx kappleri (Peters, 1867)		IUCN-LC	22	(Deleva and Chaverri 2018; Goicoechea and Quesada 2019; Lips and Lips 2008; Quesada 2009b), ND†
Peropteryx macrotis (Wagner, 1843)		IUCN-LC	2	(Deleva and Chaverri 2018)
Peropteryx sp.		IUCN-LC	1	(Lips and Lips 2008),
Saccopteryx bilineata (Temminck, 1838)		IUCN-LC	17	(Deleva and Chaverri 2018; Goicoechea and Quesada 2019; Gonzalez 2012; Quesada 2018; Quesada and Deleva 2016; Timm and McClearn 2007)
Mormoopidae				
Pteronotus davyi (Gray, 1838)	+	IUCN-LC	4	(Cubero and Artavia 2016; Flemming 2003; Hempel 1989)
Pteronotus gymnonotus (Natterer, 1843)	+	IUCN-LC	5	(Cubero and Artavia 2016; Deleva and Chaverri 2018), ND
Pteronotus parnellii (= *mesoamericanus*) (Gray, 1843)	+	IUCN-LC	17	(Cubero and Artavia 2016; Deleva and Chaverri 2018; Gonzalez 2012; Heithaus and Fleming 1978; Hempel 1989; Mitchell et al. 2018; Quesada 2018; Quesada and Deleva 2016; Trescott and Vicente-Santos 2019), ND
Pteronotus personatus (Wagner, 1843)	+	IUCN-LC	3	(Cubero and Artavia 2016), Deleva and Chaverri 2018; Lips and Lips 2008)
Pteronotus sp.			2	(Hempel 1989; Ulloa and Quesada 2010), ND
Natalidae				
Natalus mexicanus (= *lanatus* or *stramineus*) (Wagner, 1843)	+	IUCN-LC	10	(Cubero and Artavia 2016; Deleva and Chaverri 2018; Hempel 1989; Flemming 2003; Rodríguez-Herrera et al. 2011; Trescott and Vicente-Santos 2019), ND
Noctilionidae				
Noctilio leporinus (Linnaeus, 1758)		IUCN-LC	1	(Romero 1985)
Phyllostomidae				
Anoura cultrata Handley, 1960		IUCN-LC, LEY-RTP	1	(Mitchell et al. 2018)
Anoura sp.			2	(Deleva and Chaverri 2018)
Artibeus jamaicensis Leach, 1821		IUCN-LC	10	(Cubero and Artavia 2016; Deleva and Chaverri 2018; Goicoechea and Quesada 2019; Hempel 1989; Mitchell et al. 2018; Quesada 2018), ND
Artibeus lituratus (Olfers, 1818)		IUCN-LC	1	(Cubero and Artavia 2016)
Artibeus sp.			3	(Hempel 1989)
Carollia perspicillata (Linnaeus, 1758)		IUCN-LC	44	(Cubero and Artavia 2016; Deleva and Chaverri 2018; Fleming and Heithaus 1986; Flemming 2003; Gonzalez 2012; Goicoechea and Quesada 2019; Heithaus and Fleming 1978; Hempel 1989; Lips and Lips 2008; Mitchell et al. 2018; Ulloa 2009b; Quesada and Deleva 2016; Quesada 2018; Trescott and Vicente-Santos 2019; Villalobos-Chaves et al. 2016), ND
Carollia sowelli Baker, Solari & Hoffmann, 2002		IUCN-LC	2	(Deleva and Chaverri 2018; Villalobos-Chaves et al. 2016)
Carollia subrufa (Hahn, 1905)		IUCN-LC	1	(Heithaus and Fleming 1978)
Chrotopterus auritus Peters, 1856	+	IUCN-LC, LEY-RTP	2	(Deleva and Chaverri 2018; Graening 2004)

Taxon	CD	CS	№	References
Dermanura phaeotis Miller, 1902		IUCN-LC	1	(Cubero and Artavia 2016)
Desmodus rotundus (Geoffroy, 1810)		IUCN-LC	34	(Cubero and Artavia 2016; Deleva and Chaverri 2018; Gonzalez 2012; Goicoechea and Quesada 2019; Graening 2004; Hapka et al. 1992; Heithaus and Fleming 1978; Hempel 1989; Lips and Lips 2008; Peacock and Hempel 1993; Quesada 2013; Quesada 2015; Quesada and Deleva 2016; Trescott and Vicente-Santos 2019; Timm and McClearn 2007; Ulloa 2009b; Villalobos-Chaves et al. 2016; Vicente-Santos 2019), ND
Diphylla ecaudata Spix, 1823		IUCN-LC	4	(Cubero and Artavia 2016; Hempel 1989; Trescott and Vicente-Santos 2019), ND
Glossophaga commissarisi Gardner, 1962		IUCN-LC	1	(Cubero and Artavia 2016)
Glossophaga leachii Gray, 1844	+	IUCN-LC	1	(Cubero and Artavia 2016)
Glossophaga soricina (Pallas, 1766)		IUCN-LC	12	(Cubero and Artavia 2016; Deleva and Chaverri 2018; Gonzalez 2012; Flemming 2003; Hempel 1989; Lips and Lips 2008), ND
Lampronycteris brachyotis (Dobson, 1879)		IUCN-LC	3	(Cubero and Artavia 2016)
Lonchophylla concava Goldman, 1914		IUCN-LC, LEY-RTP	3	(Deleva and Chaverri 2018)
Lonchophylla robusta Miller, 1912	+	IUCN-LC	7	(Armstrong 1969; Deleva and Chaverri 2018; Goicoechea and Quesada 2019; Lips and Lips 2008; Trescott and Vicente-Santos 2019), ND
Lonchorhina aurita Tomes, 1863	+	IUCN-LC	10	(Deleva and Chaverri 2018; Mitchell et al. 2018; Nelson 1965; Trescott and Deleva 2016; Trescott and Vicente-Santos 2019; Villalobos-Chaves et al. 2016; Vicente-Santos 2019), ND
Micronycteris megalotis (Gray, 1842)		IUCN-LC	2	(Hempel 1989; Peacock and Hempel 1993)
Micronycteris microtis (Miller, 1898)		IUCN-LC	1	(Villalobos-Chaves et al. 2016)
Micronycteris schmidtorum Sanborn, 1935		IUCN-LC	1	(Woodman 1988)
Phyllostomus discolor Wagner, 1843		IUCN-LC	1	(Deleva and Chaverri 2018)
Phyllostomus hastatus (Pallas, 1767)		IUCN-LC	6	(Deleva and Chaverri 2018; Goicoechea and Quesada 2019; Gonzalez 2012; Hempel 1989; Quesada and Deleva 2016; Timm and McClearn 2007), ND
Phyllostomus sp.			1	(Peacock and Hempel 1993)
Tonatia saurophila Koopman & Williams, 1951		IUCN-LC	1	(Trescott and Vicente-Santos 2019)
Trachops cirrhosus (Spix, 1823)		IUCN-LC	5	(Deleva and Chaverri 2018; Trescott and Vicente-Santos 2019; Vicente-Santos 2019), ND
Trinycteris nicefori Sanborn, 1949		IUCN-LC, LEY-RTP	2	(Vásquez and Artavia 2017)
Phyllostomidae indet.			10	(Aguilar 2010; Brizuela et al. 2015; Goicoechea 2019; Lips and Lips 2008; Madrigal 2010; Quesada and Alfaro 2005; Quesada 2015; Quesada and Deleva 2016), ND
Vespertilionidae				
Rhogessa bickhami Baird, Marchán-Rivadeneira, Pérez & Baker, 2012		IUCN-LC	1	(Cubero and Artavia 2016)
Chiroptera indet.			16	(Carvajal 2014; Hapka et al. 1992; Goicoechea 2018; Hempel 1989; Lips and Lips 2008; Peacock and Hempel 1993; Quesada 2010; Strinati et al. 1987; Trescott 2012; Ulloa 2012; Woodman 1988)

† - ND - new data: original contribution to this paper,

Figure 9. Cave-dwelling bats in Costa Rica **A** Mexican greater funnel-eared bat (*Natalus mexicanus*) **B** a group of common vampire bats (*Desmodus rotundus*) with pups **C** sword-nosed bats (*Lonchorhina aurita*) **D** Parnell's mustached bats (*Pteronotus parnellii*) **E** Jamaican fruit bat (*Artibeus jamaicensis*) **F** greater sac-winged bat (*Saccopteryx bilineata*) **G** Goldman's nectar bat (*Lonchophylla concava*) **H** a group of Seba's short-tailed bats (*Carollia perspicillata*) with an albino pup **I** hairy-legged vampire bat (*Diphylla ecaudata*) with a pup **J** orange nectar bat (*Lonchophylla robusta*) **K** fringe-lipped bat (*Trachops cirrhosus*) **L** greater spear-nosed bat (*Phyllostomus hastatus*).

Discussion

Our literature review and field observations of cave-dwelling fauna in Costa Rica yielded a database of 959 records encompassing 123 species, with the remainder mentioning higher taxonomic levels. Some literature records are expedition reports that introduce the possibility of misidentification, particularly in cases involving closely related species. However, the information gathered undoubtedly represents a valuable depiction of the current state of knowledge regarding cave-dwelling fauna in Costa Rica. There are a few noteworthy records of possible "troglobites," which are typical cave-dwelling organisms morphologically adapted to subterranean life. Notably, a freshwater crab from Southern Costa Rica, *Pseudothelphusa puntarenas*, has been described as a cave dweller (Hobbs, 1994). Furthermore, various specimens of springtails (*Trogolaphysa* sp.) and mites (Rhagidiidae) from Barra Honda (Palacios 1994) exhibit morphological adaptations that are indicative of cave life. Similarly, a single harvestman species from Southern Costa Rica (Goodnight and Goodnight 1983) displayed morphological changes that were attributed to cave adaptation.

Although only long-term studies could confirm their exact categorization, it is likely that most vertebrates in Costa Rican subterranean ecosystems fall under the category of troglophiles, referring to species that find suitable living conditions within caves but still rely on surface access for activities, such as feeding or reproduction. During our field observations, we frequently encountered cave-dwelling cane toads (*Rhinella horribilis*) thriving on abundant invertebrate prey as well as numerous frog species located near the entrances. A thin-toed frog (*Leptodactylus savagei*) was also noted in the caves. However, these species cannot be considered as true cave dwellers if they are unable to reproduce underground. Nonetheless, live tadpoles have been documented in subterranean lakes in Southern Costa Rica (Peacock and Hempel 1993), suggesting the potential for the long-term survival of cave-dwelling populations of amphibians. We confirm the recent commentary of Sperandei et al. (2023) that neotropical frogs should not be considered accidentals in caves and that more attention should be given to their monitoring in subterranean habitats. Reptiles such as night lizards (*Lepidophyma reticulatum*) and turtles (*Chelydra acutirostris*) inhabit deep subterranean passages. Additionally, two distinct fish species, the three-barbed catfish *Rhamdia guatemalensis* and the Mexican tetra *Psalidodon fasciatus*, exhibited signs of adaptation to subterranean life, such as pale coloration and reduced eye size (Romero 1985; Juberthie and Strinati 1994). Their biology, adaptations, and taxonomy have yet to be studied in detail.

Our study highlights that the number of taxa recorded in Costa Rican caves is relatively low compared to the country's enormous potential as a biodiversity hotspot (Avalos 2019). We argue that this is due to the lack of detailed research and low sampling effort rather than the true scarcity of biodiversity. It is also important to note that some subterranean sites in Costa Rica have not been studied and most have limited biospeleological records. For comparison purposes, the cave fauna of Venezuela, which has received considerable research attention, includes over 350 identified invertebrate species, 46 of which are classified as troglobites (Galán and Herrera 2006). A detailed review of Central American subterranean aquatic fauna revealed rich biodiversity in a relatively small geographic area (Reddell 1981; Mejía-Ortíz et al. 2021). Recent studies on a limited number of caves in Belize (Wynne and Pleytez 2005; Taylor et al. 2011) have resulted in the discovery of at least 80 unique taxa with possible new species for science. Several studies have focused on the diversity and ecology of cave invertebrates in the Guatemala (Pacheco et al. 2020; Pacheco et al. 2021). These studies are encouraging and could hint at the results expected in the Costa Rican caves if we apply a more systematic approach to biospeleological research.

Costa Rican caves and artificial subterranean sites are crucial habitats for bats, as most of them (72%, n=97) were occupied by these mammals. The existing literature shows that at least 52 bat species that occur in Costa Rica dwell in caves across their geographic ranges (Sagot and Chaverri 2015; Oliveira et al. 2018; IUCN 2023a). For example, studies conducted in Brazil have identified 81 species that inhabit caves (Oliveira et al. 2018; Barros and Bernard 2023). For Costa Rica, the studies we found focused primarily on quantifying the diversity of cave-dwelling bats, yet there is limited information on the abundance and the seasonal dynamics of populations (Peacock and Hempel 1993; Gonzalez 2012; Cubero and Artavia 2016; Villalobos-Chaves et al. 2016; Deleva and Chaverri 2018). Since some species of bats that rely on caves as their roosting sites are highly specialized and may be more vulnerable to disturbance, it is crucial to identify and prioritize the conservation of important underground bat roosts in the country (Sagot and Chaverri 2015; Tanalgo et al. 2022). Long-term monitoring of cave-dwelling bats should be a high priority for local authorities, as it would provide a valuable contribution to research and conservation efforts in the country and decision-making for sustainable activities within caves, most notably tourism (Deleva and Chaverri 2018). Other research questions worth pursuing in future studies on Costa Rican bats are related to their ecology and behavior. Special attention must be paid to the importance of artificial subterranean sites such as roosts, as they can provide excellent conditions for bats and other animals (Gonzalez 2012; Deleva and Chaverri 2018). However, these are often overlooked in monitoring and conservation measures (Weigand et al. 2022; Deleva et al. 2023).

The relatively low number of species discovered in caves suggests the need to expand research on the subterranean fauna of Costa Rica. For example, there are considerable gaps in fundamental knowledge about whole taxonomic groups, such as Amphipoda, Schizomida, Gastropoda, and Diplura, and there are no studies on the ecology or behavior of cave organisms. A promising research topic would be to study in detail the adaptations of pale catfish toward cave life (Perdices et al. 2002) and more studies on the stygofauna. With the use of more advanced methods, such as environmental DNA (Saccò et al. 2022), acoustic monitoring, different trapping techniques, and citizen science, we believe that there is enormous potential for discovering new species and gaining a better understanding of the ecology and diversity of Costa Rica's subterranean ecosystems. In particular, we suggest that future studies focus on long-term investigations of cave invertebrate communities and compare them with other habitats in the country (Smith et al. 2023). Future studies should also include soil-dwelling organisms, particularly the Mesovoid Shallow Substratum (MSS). The study of MSS is very promising, as this habitat is an integral part of the subterranean environment (Gers 1998) and has been proven to bring valuable discoveries in other parts of the world (Langourov et al. 2014; Mammola et al. 2016; Ortuño et al. 2023). Apart from undoubtedly critical taxonomic studies, some fundamental questions in subterranean biology worth pursuing are related to the ecology of cave organisms and their adaptation to the environment. Particularly interesting topics could be related to the ecosystem services of subterranean communities and their functional diversity (Mammola et al. 2020).

The Barra Honda National Park is an excellent example of successful cave and karst conservation in Costa Rica. This national park was created primarily to protect the unique karstic landscape (Goicoechea 2015), and the subterranean sites within its borders were well preserved. However, most of the subterranean sites in the country are located outside protected areas, indicating severe challenges to their conservation. The Zona Sur karst area, which consists of an extensive karst surface with the most significant number of caves in the country (Ulloa 2011), lacks state protection. In addition, most cave-dwelling species lack legal protection. For example, all bat species in our database are stated as Least Concern in the global IUCN red list because of their wide distribution. However, they may be locally rare, particularly because cave-dependent species are vulnerable to disturbances in their roosts. Some of the country's most crucial subterranean bat roosts, Corredores, Gabinarraca, Damas, and Emus, lack any state of protection. Cave ecosystems are particularly vulnerable to anthropogenic threats such as pollution, disturbance due to tourist activities, and climate change (Mammola et al. 2019). With the rapid development of tourism and speleological activities (Ulloa and Goicoechea 2013), it is essential to preserve and protect the subterranean habitats and unique species assemblages that inhabit these sites. Understanding the unique adaptations and survival strategies of subterranean organisms will provide crucial data for developing effective conservation strategies to preserve fragile ecosystems.

Conclusions

Although the Costa Rican subterranean fauna has been the subject of a limited number of studies, our review and research have shown the current state of knowledge on the biodiversity of one-third of the known subterranean sites in the country. However, compared with cave-dwelling fauna from cave systems in other countries in the region, such as Belize, Guatemala, and Venezuela, we can infer that it is likely that a large number of Costa Rican subterranean organisms are yet to be described and reported within Costa Rican caves. Finally, with the current work, we hope to inspire and encourage future studies to focus on the exploration and documentation of new species in the underground habitats of the country.

Acknowledgments

We dedicate this article to the memory of Carlos Goicoechea, whose remarkable contributions to speleology inspired an entire generation of cave researchers in Costa Rica. We extend our gratitude to Gustavo Quesada for providing us with valuable information and access to the national speleological database of Costa Rica. We thank Jerzy Lis and Ivaylo Dedov for their help in identifying the collected material from Cydnidae and Gastropoda. We would also like to thank Angel Ivanov for his unwavering support during all stages of this study. We also thank Andrea Hidalgo, who assisted us with collecting literature data, and Angel Chonin, who created part of the figures used in this article. Finally, we express our deep appreciation to all the cavers of the Anthros Speleological Group who contributed their time and expertise to the fieldwork for this research. Our research was conducted under research permits issued by the National System of Conservation Areas (SINAC) (№ ACG-054-2018, M-P-SINAC-PNI-ACAT-024-2018, R-SINAC-ACG-PI-028-2018, ACT-OR-DR-095-18, and NV-ACOSA-040-18).

This article is part of a doctoral project at the University of Costa Rica (B69533). The fieldwork was partially funded by The Rufford Foundation (grant № 16923-1), National Geographic Society (grant № EC-194R-18), Alvaro Ugalde Scholarship of Osa Conservation, Idea Wild, and the University of Costa Rica. This article was created within the National Science Program "Young Scientists and Postdoctoral Fellows" of the Ministry of Education and Science of Bulgaria. The laboratory work of S. Deleva and N. Simov was supported by the Bulgarian National Science Fund, project КП-06-Н51/9 "Caves as a reservoir for novel and reoccurring zoonoses - ecological monitoring and metagenomic analysis in real-time."

References

Aguilar R (2010) Exploración del "pozo perdido", bautizado "Pozo La Ceiba". Espeleo Informe Costa Rica 5(6): 24–27.

Alpizar R, Bermúdez J, Didonna F, Goicoechea C, Guevara R, Oses J, Quesada G (2006) Introducción a la espeleología en Costa Rica. 7th edn. Asociación Anthros C.R. Grotto. Grupo Espeleológico Anthros, San José, Costa Rica, 176 pp.

Armstrong DM (1969) Noteworthy records of bats from Costa Rica. Journal of Mammalogy 50: 808. https://doi.org/10.2307/1378261

Avalos G (2019) Still Searching the Rich Coast: Biodiversity of Costa Rica, Numbers, Processes, Patterns, and Challenges. In: Pullaiah T (Ed.) Global Biodiversity Selected Countries in the Americas and Australia. Apple Academic Press, 1689–1699. https://doi.org/10.1201/9780429433634-4

Barros JS, Bernard E (2023) Species richness, occurrence and rarity of bats in Brazilian caves. Austral Ecology 00: 1–27. https://doi.org/10.1111/aec.13453

Brizuela D, Aguilar LR, Quesada G (2015) Exploraciones en los Altos del Río Coto, Zona Sur (Costa Rica). Espeleo Informe Costa Rica 8(17): 24–30.

Campos-Filho IS, Sfenthourakis S, Gallo JS, Gallão JE, Torres DF, Chagas Jr A, Horta L, Carpio-Díaz YM, López-Orozco CM, Borja-Arrieta R, Araujo PB, Taiti S, Bichuette ME (2023) Shedding light into Brazilian subterranean isopods (Isopoda, Oniscidea): Expanding distribution data and describing new taxa. Zoosystema 45(19): 531–599. https://doi.org/10.5252/zoosystema2023v45a19

Carvajal V (2014) Exploración del Pozo del Vecino, Damas de Quepos. Espeleo Informe Costa Rica 8 (14): 35–44.

Chapman AD (2022) Current Best Practices for Generalizing Sensitive Species Occurrence. GBIF Secretariat, Copenhagen, 1–50. https://doi.org/10.15468/doc-5jp4-5g10

CITES (2023) Convention on International Trade in Endangered Species of Wild Fauna and Flora. Appendices I, II and III, valid from 4 May 2023. Geneva, Switzerland. https://cites.org

CMS (2020) Convention on the Conservation of Migratory Species of Wild Animals, Appendices I and II. Valid from 22 May 2020. Bonn, Germany. https://cms.int

Cubero O, Artavia E (2016) Bats living in caves, Barra Honda National Park. Projects Abroad.

Culver DC, Pipan T (2009) Oxford University press The Biology of Caves and Other Subterranean Habitats. Oxford University Press, Oxford, UK.

Day M, Koenig S (2002) Cave monitoring priorities in Central America and the Caribbean. Acta Carsologica 31: 123–134. https://doi.org/10.3986/ac.v31i1.408

Debeljak GC (1988) Ricerche e Scoperte Speleologiche Costa Rica ´88. Trieste.

Deharveng L, Bedos A (2012) Diversity patterns in the tropics. In: Encyclopedia of Caves. 2nd edn. Elsevier Inc., 238–250. https://doi.org/10.1016/B978-0-12-383832-2.00032-3

Deleva S, Chaverri G (2018) Diversity and conservation of cave-dwelling bats in the Brunca region of Costa Rica. Diversity 10: 1–15. https://doi.org/10.3390/d10020043

Fleming TH, Heithaus ER (1986) Seasonal foraging behavior of the frugivorous bat *Carollia perspicillata*. Journal of Mammalogy 67: 660–671. https://doi.org/10.2307/1381127

Flemming TH (2003) A Bat Man in the Tropics: chasing El Duende. University of California Press, Berkeley and Los Angeles, California, 333 pp.

Galán C, Herrera F (2006) Fauna cavernícola de Venezuela: una revisión. Boletín de la Sociedad Venezolana de Espeleología 40: 39–57.

Gers C (1998) Diversity of energy fluxes and interactions between arthropod communities: From soil to cave. Acta Oecologica 19: 205–213. https://doi.org/10.1016/S1146-609X(98)80025-8

Goicoechea C (2010a) Avance de las investigaciones en el sector Quebrada Seca / Caverna Bananal / Río Corredor, Puntarenas, Costa Rica. San José, Costa Rica.

Goicoechea C (2010b) Karst y Calcáreo por explorar: Sector Valle Central. In: Guía Kárstica de Costa Rica. Grupo Espeleológico Anthros, San José, Costa Rica, 17 pp.

Goicoechea C (2015) Proceso histórico de la Espeleología en Costa Rica. Ediciones Anthros / GEA, San Jose.

Goicoechea C (2018) Exploración Caverna La Higuera. Espeleo Informe Costa Rica 23: 12–14.

Goicoechea C (2019) La Legendaria 'Cueva del Cerro Corral de Piedra' en Nicoya, Guanacaste. Espeleo Informe Costa Rica 13 (25): 27–28.

Goicoechea C, Quesada G (2019) Damas, caverna de. Detalle cueva. Registro Kárstico Nacional (RKN). https://anthros.org/es/anthros/registro-karstico-nacional/ [January 16, 2023]

Gonzalez X (2012) Murciélagos en refugios artificiales: Diversidad de murciélagos y caracterización de factores ambientales en un túnel, Guanacaste, Costa Rica.

González X (2010) Inspección al "Resumidero" del río San Gerardo. Espeleo Informe Costa Rica 5 (7): 49–54.

Goodnight CJ, Goodnight ML (1983) Opiliones of the family Phalangodidae found in Costa Rica. Journal of Arachnology 11: 201–242.

Graening G (2004) Use of the Costa Rican Cave, Cueva del Tigré, as a Classroom for cave Ecology and Conservation by the Organization for Tropical Studies. NSS News: 168–171.

Grupo Espeleológico Anthros (2023) Registro Kárstico Nacional (RKN). https://anthros.org/es/anthros/registro-karstico-nacional/ [January 16, 2023]

Hapka R, Brasey J, Goicoechea C, Jeannin P-Y, Jeannin V (1992) Explorations dans les karsts tropicaux du Costa Rica. Cavernes 1–1992: 1–27.

Heithaus ER, Fleming TH (1978) Foraging Movements of a Frugivorous Bat, Carollia perspicillata (Phyllostomatidae). Ecological society of America, Ecological Monographs 48: 127–143. https://doi.org/10.2307/2937296

Hempel J (1989) Report of the 1982 Expedition to Barra Honda National Park. Bulletin of the National Speleological Society, 1–23.

Henderson CL (2011) 48 Mammals, amphibians, and reptiles of Costa Rica: a field guide. University of Texas Press, 214 pp.

Hobbs III H (1991) A new pseudothelphusid crab from a cave in southern Costa Rica (Decapoda: Brachyura). Proceedings of the Biological Society of Washington 104: 295–298.

Hobbs III HH (1994) Biogeography of subterranean decapods in North and Central America and the Caribbean region (Caridea, Astacidae, Brachyura). Hydrobiologia 287: 95–104. https://doi.org/10.1007/BF00006899

Howarth FG (2023) Why the delay in recognizing terrestrial obligate cave species in the tropics? International Journal of Speleology 52: 22–43. https://doi.org/10.5038/1827-806X.52.1.2446

IUCN (2023a) Bats in Costa Rica. The IUCN Red List of Threatened Species. Version 2023-4. https://www.iucnredlist.org/

IUCN (2023b) The IUCN Red List of Threatened Species. Version 2023-4. https://www.iucnredlist.org/

Juberthie C, Strinati P (1994) Encyclopedie Biospeologique. Tome I, Cap. IV. Historique de la Biospelogie. 1.7. Costa Rica. CNRS-Fabbro, Saint-Girons, France.

Langourov M, Lazarov S, Stoev P, Guéorguiev B, Deltchev C, Petrov B, Andreev S, Simov N, Bekchiev R, Antonova V, Ljubomirov T, Dedov I, Georgiev D (2014) New and interesting records of the MSS and cave fauna of Vitosha Mt., Bulgaria. In: Balkan Speleological Conference "Sofia 2014".

Lips B, Lips J (2008) Spéléologie au Costa Rica 2008. Echo des Vulcains 66: 1–38.

Lyon GM, Bravo AV, Espino A, Lindsley MD, Gutierrez RE, Rodriguez I, Corella ANA, Carrillo F, Mcneil MM, Warnock DW, Hajjeh RA (2004) Histoplasmosis associated with exploring a bat-inhabited cave in Costa Rica, 1998 – 1999. American Journal of Tropical Medicine and Hygiene 70: 438–442. https://doi.org/10.4269/ajtmh.2004.70.438

Madrigal H (2010) Exploración Caverna del Piquete. Espeleo Informe Costa Rica 5(6): 28.

Mammola S, Cardoso P, Culver DC, Deharveng L, Ferreira RL, Fišer C, Galassi DMP, Griebler C, Halse S, Humphreys WF, Isaia M, Malard F, Martinez A, Moldovan OT, Niemiller ML, Pavlek M, Reboleira ASPS, Souza-Silva M, Teeling EC, Wynne JJ, Zagmajster M (2019) Scientists' warning on the conservation of subterranean ecosystems. BioScience 69: 641–650. https://doi.org/10.1093/biosci/biz064

Mammola S, Amorim IR, Bichuette ME, Borges PAV, Cheeptham N, Cooper SJB, Culver DC, Deharveng L, Eme D, Ferreira RL, Fišer C, Fišer Ž, Fong DW, Griebler C, Jeffery WR, Jugovic J, Kowalko JE, Lilley TM, Malard F, Manenti R, Martínez A, Meierhofer MB, Niemiller ML, Northup DE, Pellegrini TG, Pipan T, Protas M, Reboleira ASPS, Venarsky MP, Wynne JJ, Zagmajster M, Cardoso P (2020) Fundamental research questions in subterranean biology. Biological Reviews 95: 1855–1872. https://doi.org/10.1111/brv.12642

Mammola S, Giachino PM, Piano E, Jones A, Barberis M, Badino G, Isaia M (2016) Ecology and sampling techniques of an understudied subterranean habitat: the Milieu Souterrain Superficiel (MSS). Die Naturwissenschaften 103: 88. https://doi.org/10.1007/s00114-016-1413-9

Mejía-Ortíz L, Sprouse P, Tejeda-Mazariegos J, Valladarez J, Frausto-Martínez O, Collantes-Chavez-Costa A, Ruíz-Cancino G, Yáñez G (2021) Tropical Subterranean Ecosystems in Mexico, Guatemala and Belize: A Review of Aquatic Biodiversity and Their Ecological Aspects. In: Natural History and Ecology of Mexico and Central America, 210 pp. https://doi.org/10.5772/intechopen.97694

MINAE (2017) Ley de Conservación de Vida Silvestre. La Gaceta Diario Oficial 239: 28–40. https://www.sinac.go.cr/ES/visasilves/Enlaces%20Inters/05.%20Lista%20especies%20en%20peligro%20de%20extinci%C3%B3n%20y%20poblaciones%20reducidas.pdf

Mitchell M, Vicente-Santos A, Rodriguez-Herrera B, Corrales-Aguilar E, Gillespie T (2018) Genetic diversity of *Bartonella* spp. in Cave-Dwelling Bats and bat flies, Costa Rica, 2018. Emerging Infectious Diseases 28: 2–3. https://doi.org/10.3201/eid2802.211686

Mullinex C (1975) Revision of *Paraphrynus*, Moreno (Amblypigida: Phrynidae) for North America and the Antilles. Occasional papers California Academy of sciences 116: 1–80.

Nelson CE (1965) *Lonchorhina aurita* and other bats from Costa Rica. Texas Journal of Science 17: 303–306.

Niemiller ML, Taylor SJ, Bichuette ME (2018) Conservation of Cave Fauna, with an Emphasis on Europe and the Americas. In: Moldovan O, Kováč Ľ, Halse S (Eds) Cave Ecology. Springer International Publishing, 451–478. https://doi.org/10.1007/978-3-319-98852-8_22

Oliveira HFM, Oprea M, Dias RI (2018) Distributional patterns and ecological determinants of bat occurrence inside caves: A broad scale meta-analysis. Diversity 10(3): 49. https://doi.org/10.3390/d10030049

Ortuño VM, Jiménez-Valverde A, Baquero E, Jordana R, Ledesma E, Pérez-Suárez G, Sendra A, Barranco P, Tinaut A, Herrero-Borgoñón JJ (2023) Fauna del Medio Subteráneo Superficial (MSS) en el parque nacional de la Sierra de Guadarrama (España).

Pacheco GSM, Silva MS, Cano E, Ferreira RL (2020) The role of microhabitats in structuring cave invertebrate communities in Guatemala. International Journal of Speleology 49: 161–169. https://doi.org/10.5038/1827-806X.49.2.2333

Pacheco GSM, de Oliveira MPA, Cano E, Souza Silva M, Ferreira RL (2021) Tourism effects on the subterranean fauna in a Central American cave. Insect Conservation and Diversity 14: 294–306. https://doi.org/10.1111/icad.12451

Palacios J (1994) Nuevos datos faunísticos para cuevas de Barra Honda, Costa Rica. Mundos Subterráneos México, 23–29.

Peacock N, Hempel J (1993) Studies in the Rio Corredor basin. The NSS Bulletin, Journal of Caves and Karst Studies 55: 134.

Perdices A, Bermingham E, Montilla A, Doadrio I (2002) Evolutionary history of the genus *Rhamdia* (Teleostei: Pimelodidae) in Central America. Molecular Phylogenetics and Evolution 25: 172–189. https://doi.org/10.1016/S1055-7903(02)00224-5

Quesada G (2009a) Exploración Pozo del Chispero. Espeleo Informe Costa Rica 3(5): 37.

Quesada G (2009b) Exploraciones en los Altos del Río Esquinas. Espeleo Informe Costa Rica 3(4): 32–35.

Quesada G (2010) Exploración Cueva de Los Murciélagos. Espeleo Informe 5(6): 29–34.

Quesada G (2013) Descubrimiento y exploración del Sistema La Trampa - Baquiano, Barra Honda de Nicoya. Espeleo Informe Costa Rica 7: 22–30.

Quesada G (2015) Reporte de Exploración de Caverna en Montserrat, Coronado. Espeleo Informe Costa Rica, 5 pp.

Quesada G (2016) Nuevas exploraciones en la Caverna CARMA. Espeleo Informe Costa Rica 9(19): 20–30.

Quesada G (2018) Exploración espeleológica en el Río Térraba, Zona Sur. Espeleo Informe Costa Rica 11(22): 14–35.

Quesada G, Alfaro J (2005) Exploración en el Parque Nacional Piedras Blancas, Golfito. Expedition report. Anthros.org

Quesada G, Deleva S (2016) Exploración en el Parque Nacional Piedras Blancas, Golfito. Espeleo Informe 8(16): 9–19.

Reddell JR (1981) A review of the cavernicole fauna of Mexico, Guatemala and Belize. The Bulletin of the Texas Memorial Museum, 1–327.

Rodríguez-Herrera B, Sánchez R, Pineda W (2011) First record of *Natalus lanatus* (Chiroptera: Natalidae) in Costa Rica, and current distribution of Natalus in the country. Ecotropica 17: 113–117.

Rojas A, Jiménez A, Vargas M, Zumbado M, Herrero MV (2008) Ectoparasites of the common vampire bat (*Desmodus rotundus*) in Costa Rica: Parasitism rates and biogeographic trends. Mastozoología neotropical 15: 181–187.

Romero A (1985) Cave Colonization by Fish: Role of Bat Predation. American Midland Naturalist 113: 7. https://doi.org/10.2307/2425342

Romero A (2009) Cave Biology - Life in darkness. Cambridge University Press, Cambridge, 1–319. https://doi.org/10.1017/CBO9780511596841

Saccò M, Guzik MT, van der Heyde M, Nevill P, Cooper SJB, Austin AD, Coates PJ, Allentoft ME, White NE (2022) eDNA in subterranean ecosystems: Applications, technical aspects, and future prospects. Science of the Total Environment 820: 153223. https://doi.org/10.1016/j.scitotenv.2022.153223

Sagot M, Chaverri G (2015) Effects of roost specialization on extinction risk in bats. Conservation Biology 29: 1666–1673. https://doi.org/10.1111/cobi.12546

SNIT (2023) Sistema Nacional De Información Territorial - Visores Temáticos. https://www.snitcr.go.cr/

Smith MA, Smith MA, Warne C, Pare K, Dolson S, Loewen E, Jones K, Mcphee M, Stitt L, Janke L, Smith RM, Coatsworth H, Loureiro AMMC, Solis A, Viquez CF, Rodriguez J, Fernandez-triana J, Sharkey MJ, Whitfield J, Masís A, Chavarría MM, Blanco R, Chavarria F, Phillips- E, Fernández R, Garcia D, Pereira G, Ramirez H, Pereira M, Hallwachs W, Janzen DH (2023) Communities of Small Terrestrial Arthropods Change Rapidly Along a Costa Rican Elevation Gradient. In: Myster R (Ed.) Neotropical Gradients and Their Analysis. Springer Cham, 255–307. https://doi.org/10.1007/978-3-031-22848-3

Solari S (2019) *Natalus mexicanus* (Mexican Greater Funnel-eared Bat). The IUCN Red List of Threatened Species 2019 8235. https://doi.org/10.2305/IUCN.UK.2019-2.RLTS.T123984355A22011975.en

Sperandei VF, Alvarenga DA, Araújo MS, Pereira CC (2023) Are caves true habitats for anurans or more a favorable rocky environment? A discussion of habitat occupation by frogs in Neotropical caves. Neotropical Biology and Conservation 18: 131–137. https://doi.org/10.3897/neotropical.18.e100778

Strinati P, Goicoechea C, Cortés G (1987) Quelques grottes du Costa Rica et leur faune. In: Actes du 8° Congrès National De Spéléologie. SSS, Geneve, Suisse, 23–32.

Tanalgo KC, Oliveira HFM, Hughes AC (2022) Mapping global conservation priorities and habitat vulnerabilities for cave-dwelling bats in a changing world. Science of the Total Environment 843: 156909. https://doi.org/10.1016/j.scitotenv.2022.156909

Taylor SJ, Slay ME, Jacoby J, Hoese GB, Krejca JK, Slay CM, Bond JE (2011) 2011 Belize Biospeleology Expedition. Illinois Natural History Survey Technical Report 43: 51.

Timm RM, McClearn D (2007) The bat fauna of Costa Rica's Reserva Natural Absoluta Cabo Blanco and its implication for bat conservation. In: Kelt DA, Lessa EP, Salazar-Bravo J, Patton JL (Eds) The Quintessential Naturalist: Honorinbg the life and legacy of Oliver P. Pearson. University of California Press, Berkeley and Los Angeles, California, 303–352. https://doi.org/10.1525/california/9780520098596.003.0012

Trescott S (2012) The expansion of Mennonites Cavern. Espeleo Informe Costa Rica 6(11): 56–59.

Trescott S, Vicente-Santos A (2019) Caverna Los Araya (CR387): Plano y resumen de Indagaciones. Espeleo Informe Costa Rica 13(24): 66–70.

Tschinkel W (1984) *Zophobas atratus* (Fab.) and *Z. rugipes* Kirsch (Coleoptera: Tenebrionidae) Are Same Species. The Coleopterists Bulletin 38: 325–333.

UCR (2023) Sistema de Bibliotecas, Documentacion e Informacion. https://sibdi.ucr.ac.cr/

Ulloa A (2009a) Caves of Costa Rica (Central America) and their geologic origin. In: 15th International Congress of Speleology, 1930–1936.

Ulloa A (2009b) Resultados de las exploraciones realizadas por espeleólogos franceses (AKL) y miembros del GEA en la Zona Sur de Costa Rica, Agosto 2008. Espeleo Informe Costa Rica 3: 20–27.

Ulloa A (2012) Informe de exploración, caverna “Huecos” (Bonilla Abajo, Peralta, Cartago). Espeleo Informe Costa Rica 10: 21–23.

Ulloa A, Quesada G (2010) Informe de exploración de la cueva “Torre 14”. Espeleo Informe Costa Rica, 1–15.

Ulloa A, Goicoechea C (2013) Geotourism Potential of Underground Sites in Costa Rica. Pesquisas em Turismo e Paisagens Cársticas 6: 43–56.

Ulloa A, Aguilar T, Goicoechea C, Ramírez R (2011) Descripción, clasificación y aspectos geológicos de las zonas kársticas de Costa Rica description, classification and geological aspects of the Karstic regions of Costa Rica. Revista Geológica de América Central 45: 53–74. https://doi.org/10.15517/rgac.v0i45.1910

Vásquez O, Artavia E (2017) First record of the bat *Trinycteris nicefori* (Chiroptera: Phyllostomidae) in the Nicoya Peninsula, Guanacaste, Costa Rica. Cuadernos de Investigación UNED 9: 35–37. https://doi.org/10.22458/urj.v9i1.1675

Vicente-Santos A (2019) Informe de cuevas – Cueva Bijagual. Espeleo Informe Costa Rica n.d.

Villalobos-Chaves D, Murillo JV, Valerio ER, Keeley BW (2016) Understory bat roosts, availability and occupation patterns in a Neotropical rainforest of Costa Rica 64: 1333–1343. https://doi.org/10.15517/rbt.v64i3.21093

Woodman N (1988) Subfossil remains of *Peromyscus stirtoni* (Mammalia: Rodentia) from Costa Rica. Revista Biología Tropical 36: 247–253.

Wynne JJ, Pleytez W (2005) Sensitive ecological areas and species inventory of Actun Chapat Cave, Vaca Plateau, Belize. Journal of Cave and Karst Studies 67: 148–157.

Wynne JJ, Howarth FG, Mammola S, Ferreira RL, Cardoso P, Lorenzo T Di, Galassi DMP, Medellin RA, Miller BW, Sánchez-Fernández D, Bichuette ME, Biswas J, BlackEagle CW, Boonyanusith C, Amorim IR, Borges PAV, Boston PJ, Cal RN, Cheeptham N, Deharveng L, Eme D, Faille A, Fenolio D, Fišer C, Fišer Ž, 'Ohukani'ōhi'a Gon SM, Goudarzi F, Griebler C, Halse S, Hoch H, Kale E, Katz AD, Kováč Ľ, Lilley TM, Manchi S, Manenti R, Martínez A, Meierhofer MB, Miller AZ, Moldovan OT, Niemiller ML, Peck SB, Pellegrini TG, Pipan T, Phillips-Lander CM, Poot C, Racey PA, Sendra A, Shear WA, Silva MS, Taiti S, Tian M, Venarsky MP, Pakarati SY, Zagmajster M, Zhao Y (2021) A conservation roadmap for the subterranean biome. Conservation Letters 14(5): e12834. https://doi.org/10.1111/conl.12834

Supplementary material 1

Dataset of cave-dwelling organisms occurring in Costa Rica

Authors: Stanimira Deleva, Andres Ulloa, Hernani F. M. Oliveira, Nikolay Simov, Ferdinando Didonna, Gloriana Chaverri

Data type: xlsx

Explanation note: The dataset includes information on taxonomic diversity, location, protected areas, conservation status, and references.

Link: https://doi.org/10.3897/subtbiol.47.113219.suppl1