

New and revised groundwater snails (Mollusca, Caenogastropoda, Cochliopidae) from karst and associated hyporheic habitats in western Texas and northern Mexico

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Abstract

The freshwater gastropod family Cochliopidae is a diverse component of the subterranean fauna in the karst and hyporheic habitats of western Texas and northern Mexico, with 11 genera and 27 described species found in the region. Building on previous phylogenetic studies of the family in the region we incorporate additional sampling, DNA, and morphological study for systematic revision of species found in the western Edwards-Trinity Aquifer System and associated karst and hyporheic habitats. We describe three new species, *Stygopyrgus variabilis* **sp. nov.**, *Stygopyrgus gracilis* **sp. nov.**, and *Phreatodrobia embossa* **sp. nov.** and revise the generic placement of two others, *Texapyrgus diaboli* **comb. nov.** and *Balconorbis coronae* **comb. nov.**

Keywords

Cave snails, dimorphic morphology, groundwater, phreatic, stygobionts

Introduction

Cochliopidae Tryon, 1866 are a group of minute to small freshwater and estuarine snails, comprising 250+ species in 48 genera found in the Nearctic, Neotropical, and Afrotropical realms (Clark 2019). The relationships of subfamilies and genera within the Cochliopidae remain unclear. A review focusing on detailed anatomical descriptions of the Cochliopidae distinguished the subfamily level groups Cochliopinae Tryon, 1966, Littoridininae Thiele, 1928, and Semisalsinae Giusti & Pezzoli, 1980 (Hershler and Thompson 1992), but several genera remain unassigned, limiting our understanding of relationships and evolution of traits in the family. More recent work has described new species (Czaja et al. 2017; Czaja et al. 2019; Alvear et al. 2020b; Perez et al. 2023) but there has not been a recent review of the relationships and classification of the family. Several recent descriptions have been based on shell materials only, due to the recent drying of spring habitats, precluding any information on anatomy or DNA (Czaja et al. 2021; Czaja et al. 2024). Prior assignment of species to Cochliopidae genera has been informed by combinations of shell shape, shell sculpture, penial morphology, female reproductive anatomy, and DNA (Hershler and Thompson 1992; Liu et al. 2001; Hershler et al. 2003). More recent work that included DNA data (Perez et al. 2023) has begun to clarify relationships among species, but the markers used were unable to adequately resolve relationships at the subfamily level.

A diverse radiation of cochliopids is known from the Edwards and Edwards-Trinity Aquifer Systems (ETAS), with 11 genera and 27 species of phreatic cochliopids described from the region (Table 1). The ETAS is a complex transboundary aquifer system extending across Texas, USA into the Mexican state of Coahuila (Sanchez et al. 2018; Sanchez and Rodriguez 2022). Hydrogeologically, discrete groundwater watersheds in the ETAS are contained in rocks of similar ages and depositional environments. These aquifers form a complex but geologically contiguous region of adjacent and overlapping aquifers. The phreatic snail fauna of the ETAS has been investigated in several waves of survey and taxonomic work, each emphasizing specific regions and incorporating new sampling techniques. The first works described shells collected from drift debris (Pilsbry and Ferris 1906) across the southern Texas portion of the ETAS. In the 1960's, numerous species were described (Taylor 1966) from Cuatro Ciénegas, which is renowned for endemic aquatic diversity.

Although not part of the ETAS, several taxa found in Cuatro Ciénegas in Mexico are also documented in the ETAS (Pilsbry and Ferriss 1906; Taylor 1966; Hershler and Longley 1986a; Czaja et al. 2017; Czaja et al. 2022). The bulk of our understanding of phreatic snail diversity comes from Hershler and others in the 1980s and 1990s (Hershler and Longley 1986b; Hershler and Longley 1987; Thompson and Hershler 1991) who drift-netted wells and spring heads to describe the snail fauna of the Eastern ETAS, particularly the Edwards Balcones Fault Zone in Texas. More recent work by Czaja et al. has focused on the Mexican extent of the ETAS and related fauna from karstic regions slightly further south in Coahuila, particularly in the Rio Sabinas Karstic Region, Coahuila (Boghici 2004; Czaja et al. 2017,

2019, 2021, 2022, 2024; Sanchez et al. 2018), at the southern edge of the ETAS. In this study we sampled hyporheic and spring sites across western Texas, including the western portion of the ETAS.

Table 1. List of the phreatic Cochliopidae of the Edwards-Trinity Aquifer System, Cuatro Ciénegas Basin, and Rio Sabinas Basin. Conservation status ranks as assigned by Hutchins (2018), Johnson et al. (2013), and Czaja et al. (2020) using Vulnerable for S3, Threatened for S2, and Endangered for S1.

Habitat	Region	Aquifer	Species	Authority	Conservation Rank Status
Hyporheic	Coahuila	Rio Sabinas	<i>Balconorbis sabinasensis</i>	Czaja, Cardoza-Martínez & Estrada-Rodríguez, 2019	Threatened
Phreatic, hyporheic	Texas	ETAS	<i>Balconorbis uvaldensis</i>	Hershler & Longley, 1986	Vulnerable
Phreatic	Coahuila	Cuatro Ciénegas	<i>Coahuilix hubbsi</i>	D.W. Taylor, 1966	Threatened/ Vulnerable
Phreatic	Coahuila	Cuatro Ciénegas	<i>Coahuilix landyei</i>	Hershler, 1985	Threatened/ Vulnerable
Hyporheic	Coahuila	Cuatro Ciénegas, Rio Sabinas	<i>Coahuilix parrasense</i>	Czaja, Estrada-Rodríguez, Romero-Méndez, Ávila-Rodríguez, Meza-Sanchez & Covich, 2017	unranked
Phreatic, surface, hyporheic	Texas & Coahuila	ETAS, Rio Sabinas, Cuatro Ciénegas	<i>Cochliopina riograndensis</i>	(Pilsbry & Ferris, 1906)	Threatened
Phreatic, surface	Coahuila	Cuatro Ciénegas	<i>Paludiscala caramba</i>	D.W. Taylor, 1966	Threatened/ Vulnerable
Phreatic, hyporheic	Texas & Coahuila	ETAS, Cuatro Ciénegas	<i>Phreatoceras taylori</i>	(Hershler & Longley, 1986)	Vulnerable
Phreatic, hyporheic	Texas	ETAS	<i>Phreatodrobia bulla</i>	Perez & Castañeda, 2023	unranked
Phreatic	Mexico	Rio Sabinas	<i>Phreatodrobia candeleensis</i>	Czaja, Becerra-López & Ávila Rodríguez, 2024	Endangered
Phreatic	Texas	ETAS	<i>Phreatodrobia conica</i>	Hershler & Longley, 1986	Threatened
Phreatic	Texas	ETAS	<i>Phreatodrobia coroneae</i>	Hershler 1987	Threatened
Phreatic	Texas	ETAS	<i>Phreatodrobia imitata</i>	Hershler & Longley, 1986	Endangered
Phreatic, hyporheic	Texas	ETAS	<i>Phreatodrobia micra</i>	(Pilsbry & Ferriss, 1906)	Threatened
Phreatic, hyporheic	Texas	ETAS	<i>Phreatodrobia nugax</i>	(Pilsbry & Ferriss, 1906)	Vulnerable
Phreatic	Texas	ETAS	<i>Phreatodrobia plana</i>	Hershler & Longley, 1986	Threatened
Phreatic, hyporheic	Texas	ETAS	<i>Phreatodrobia punctata</i>	Hershler & Longley, 1986	Endangered
Phreatic	Texas	ETAS	<i>Phreatodrobia rotunda</i>	Hershler & Longley, 1986	Threatened
Phreatic	Texas	ETAS	<i>Phreatodrobia spica</i>	Perez & Alvear, 2020	unranked
Phreatic	Coahuila	Rio Sabinas	<i>Phreatomascogos garciasaucedoi</i>	Czaja, Cardoza-Martínez & Estrada-Rodríguez, 2024	Endangered
hyporheic	Coahuila	Rio Sabinas	<i>Phreatomascogos gregoi</i>	Czaja & Estrada-Rodríguez, 2019	Threatened
Phreatic	Coahuila	Cuatro Ciénegas	<i>Phreatoviesca spinosa</i>	Czaja & Gladstone, 2021	Extinct or Endangered
Phreatic	Coahuila	Viesca	<i>Spinopyrgus luismaedae</i>	Czaja, Covich, Neubauer & Estrada-Rodríguez, 2022	Probably recently extinct.
Phreatic, hyporheic	Texas	ETAS	<i>Styopyrgus bartonensis</i>	Hershler & Longley, 1986	Endangered
Phreatic	Texas	ETAS	<i>Texaspyrgus longleyi</i>	Thompson & Hershler, 1991	Endangered
Phreatic & surface	Texas	ETAS	<i>Tryonia diaboli</i>	Pilsbry & Ferriss, 1906	Endangered
Phreatic	Texas	ETAS	<i>Vitropyrgus lillianae</i>	Perez & Guerrero, 2023	unranked

The groundwater cochliopids of the ETAS have the standard suite of soft tissue morphological features associated with adaptation to a subterranean environment, such as very small size, unpigmented bodies, a lack of eyes, and simplified or reduced body parts such as reduced or missing ctenidia (Hershler and Holsinger 1990). Some groups of snails, including phreatic snails are very similar morphologically, a pattern described as “morphostatic evolution” (Davis 1993). The phreatic snails of the ETAS, however, show a wide diversity of shell forms from depressed (flat) to tall, and tightly coiled, half coiled, or uncoiled (Alvear et al. 2020a), with diverse sculptural features such as ribs, keels, fine lines forming a crosshatch pattern, or rows of beads (Hershler and Longley 1986b). However, shell features may be variable or dimorphic in populations, including traditionally diagnostic features such as spines, keels, and shell form (Hershler and Longley 1987; Czaja et al. 2021). It is not currently possible to determine whether these features have evolved once or repeatedly in subterranean habitats due to the lack of a well resolved and supported phylogeny in the group. Nevertheless, snails with convergent morphological features are found in unrelated stygobiont groups around the world, suggesting at least the potential for repeated evolution of morphological characteristics within the ETAS Cochliopidae.

In this study, we build on previous molecular phylogenetic studies (Alvear et al. 2020; Perez et al. 2023) with newly generated mitochondrial and nuclear DNA data, and examination of shell shape, shell sculpture and soft anatomy, to clarify relationships and names of phreatic cochliopids in the Western ETAS of Texas and Mexico. We use new collections from this region to describe three new species, synonymize two species, and propose the reassignment of one species to a more appropriate genus.

Review of nominal taxa

As a first step in a revision of the taxonomy of groundwater snails of a region, putative new species must be distinguished from existing nominal species and relationships among species must be considered. Here we briefly introduce current taxa, some of which are described in more detail in the taxonomic remarks of relevant species. *Phreatodrobia*, with 11 species, is the most diverse of the ETAS genera. The genus is most diverse in the Eastern ETAS, with only two species extending to the West: *Phreatodrobia micra* (Pilsbry and Ferriss, 1906), the type species, has an east-west range of 600 km in the ETAS, supported by low genetic divergence (Alvear et al. 2020b) and *Phreatodrobia coronae* Hershler, 1987, restricted to the Western portion of the aquifer, in the Devils River and San Felipe Springs. In this study, we consider whether *P. coronae* should be reassigned to *Balconorbis* Hershler & Longley, 1986 based on DNA and morphology. The genus includes *Balconorbis sabinensis* Czaja, Cardoza-Martínez & Estrada-Rodríguez, 2019 from the Rio Sabinas drainage of Coahuila, and the type species *Balconorbis uwaldensis* Hershler & Longley, 1986 restricted to a narrow range in the Western ETAS of Texas.

Stygopyrgus is a monotypic genus known only from the Eastern ETAS of Texas. *Stygopyrgus bartonensis* Hershler & Longley, 1986 is found in phreatic and hyporheic

habitats in Travis, Mason, Bell, Williamson Counties and has been considered for listing under the U.S. Endangered Species Act due to its relatively narrow range and intense human development in the region (USFWS 2009; Alvear et al. 2020a). In this study, we describe two additional species of *Stygopyrgus*, extending the range of the genus to include most of the ETAS.

Tryonia W. Stimpson, 1865 are epigeal snails that are found in thermal and mineral springs across the Western United States and Western Mexico. The type species is *Tryonia clathrata* W. Stimpson, 1865. Only a single species, *Tryonia diaboli* Pilsbry & Ferriss, 1906 was thought to occur in both subterranean and epigeal habitats (Diaz et al. 2020). In this paper we reassign *T. diaboli* to *Texapyrgus* Thompson & Hershler, 1991. *Texapyrgus* is monotypic, with *Texapyrgus longleyi* Thompson & Hershler, 1991 described from subterranean habitats along the Devils River: the same stream reach in which *Tryonia diaboli* occurs. Here we assess the validity of these sympatric species.

Material and methods

Specimens were collected from sites across the study region (Fig. 1) using several sampling methods. Phreatic habitats were sampled using 100-micron mesh drift nets over spring orifices or flowing wells and passively sampled for 1–14 days (Hershler and Longley 1986b). Alternatively, a small bottle trap (soda bottle with top portion cut off and inverted to form a funnel) baited with pistachios was used to collect some individuals from wells. Hyporheic habitats were sampled using a Bou-Rouch pump: a temporary well was installed to a depth of 30–50 cm then ~12 liters of water were pumped through a 100-micron mesh net (Bou and Rouch 1967). All samples were washed from nets and preserved with 95% alcohol. Following collection, samples had alcohol replaced once and were kept refrigerated until processing. Bou-Rouch samples were elutriated to remove excess sediment and debulk the sample. Sample processing included picking the snails from environmental detritus under 20X to 50X magnification. Snails with tissue were stored in 95% alcohol in a -20 °C freezer until DNA extraction or dissection. Water chemistry measurements were taken in the field using a Manta Sonde (Eureka Water Probes, Austin, TX). For most taxa, any individual with tissue present was photographed prior to digestion for DNA extraction. To evaluate the distinctiveness of *Tryonia diaboli* and *Texapyrgus longleyi*, individuals were selected that conformed to the descriptions of each species, including relatively smooth individuals representing *T. diaboli* and highly sculptured individuals representing *Te. longleyi*.

Specimens were photographed prior to DNA extraction or dissection (Suppl. materials 1–3) as both techniques destroy or damage the shell. DNA extractions were conducted using the DNeasy Kit (QIAGEN, Cat. No. 69506). PCR was conducted for cytochrome c oxidase subunit I (COI) using the Folmer COI (Folmer et al. 1994) or Liu COI primers (Liu et al. 2001) and for the nuclear large subunit ribosomal DNA with LSU 1 and LSU 3 primers (Wade et al. 2001) using the reaction conditions detailed in Perez (Perez et al. 2023). PCR products were purified using the Gel/PCR DNA

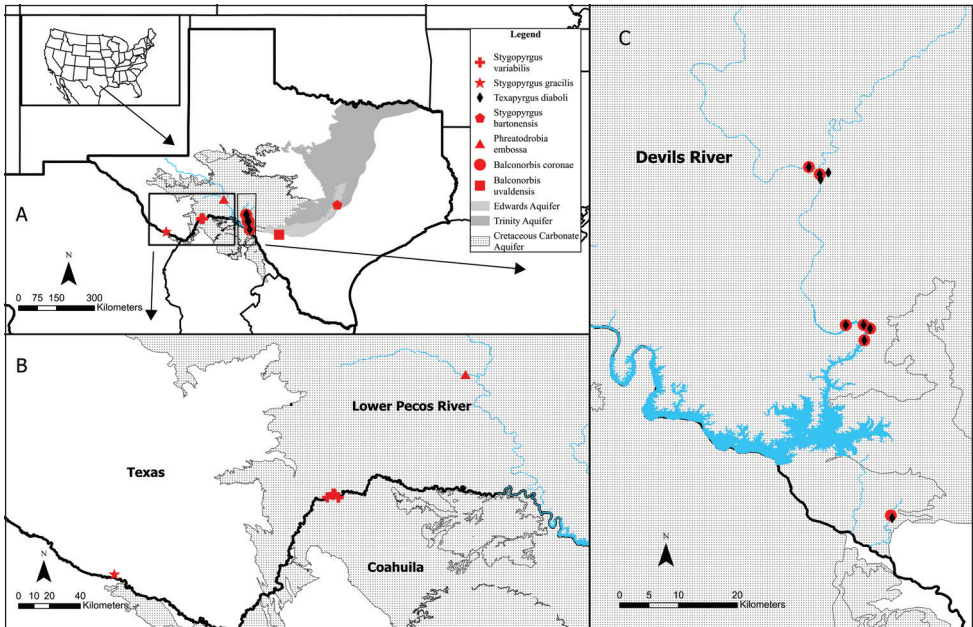


Figure 1. **A** subdivision of the ETAS in Texas and Northern Mexico with sites for new and related Cochliopidae **B** hyporheic and spring sites in western Texas along the Rio Grande, and Lower Pecos; and **C** hyporheic and spring sites in the Devils River. In the case of the Devils River sites, two symbols overlay each other (the circle and the diamond). Relevant karst formations are shown in shades of grey to black. Layer sources: Mexican State Map layer (<https://github.com/jschleuss/mexican-states>), Mexico Outline Map (<https://gisdata.mapog.com/mexico/Mexico%20Country%20Boundary>), Reservoir, Karst, and River layers from Texas Commission on Environmental Quality, Geographic Information System Hub: <https://gis-tceq.opendata.arcgis.com/maps/TCEQ::surface-water/about>.

Fragments Extraction Kit (IBI Scientific, Peosta), and then sent for Sanger Sequencing at Eton Biosciences using the PCR primers and the difficult template chemistry. Contigs were formed and sequences were edited and aligned in Geneious 10.2.6 (Kearse et al. 2012). Existing sequences of representatives of the Cochliopidae were included from GenBank along with new sequences generated during this study (Suppl. material 4). Although not the focus of this study, individuals of other phreatic Cochliopidae found in the region and the types of genera (e.g. *Phreatodrobia micra*, *Stygopyrgus bartonensis*, *Tryonia clathrata* W. Stimpson, 1865) were included to understand the best generic assignments for the new taxa. All new sequences were deposited in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) so they are available for future use (Suppl. material 4, COI: PP844662–PP844692; LSU: PP860047–PP860083).

Substitution saturation in COI was examined in the Data Analysis in Molecular Biology and Evolution software package (DAMBE7; Xia 2018). Each codon position was examined separately with 1st and 2nd positions showing no sign of saturation with transitions and transversions increasing in a linear fashion, but 3rd codon positions appear saturated with transversions outnumbering transitions at a TN84 genetic distance

of 0.5. The Xia (Xia et al. 2003; Xia and Lemey 2009) test of substitution saturation was conducted on all positions. The 1st and 2nd positions had Iss values less than Iss.c and significant p-values indicating little saturation. The 3rd codon did not have a significant p-value and had Iss values close to or greater than Iss.c, indicating substantial saturation. Therefore, we conducted replicate analyses of COI using just the 1st and 2nd positions and compared them to analyses with all three positions retained (analyses described below).

The COI sequence alignment did not require refinement, but LSU has large sections that are difficult to align. The LSU data was initially aligned using the CLUSTAL algorithm in Geneious followed by refinement using the MAFFT server online (Katoh et al. 2019). The default settings were used along with the E-INS-I iterative method and an attempt to align gappy regions (Katoh et al. 2002). To form a combined alignment, the COI and LSU alignments were concatenated by combining the same individual when possible or individuals from the same locality. The dataset was reduced to ~two individuals per species and individuals that were represented by only one gene were removed. There were two exceptions, *Phreatodrobia plana* and *Phreatodrobia embossa* sp. nov. were retained in the combined analysis to attempt to determine their placement.

Evolutionary model selection was conducted via the ModelFinder approach implemented in IQTree 2.3.6 (Kalyaanamoorthy et al. 2017) to test three partitions (COI: 1st, 2nd, and 3rd codons) and merge them until model fit does not increase. COI was partitioned into 1st, 2nd, and 3rd codon positions and models selected as follows: TNe+I+G4: 1st codon position, TPM3u+F+I+G4: 2nd codon position, HKY+F+G4: 3rd codon position. The best fit model for LSU was HKY+F+G4. For combined analyses, the dataset was divided into four partitions: the COI locus was split into 1st, 2nd, and 3rd codon positions, and the LSU region was treated as a separate partition. Partition-specific evolutionary models were selected for each, and their fit was evaluated. This resulted in analyses with four partitions retained: TNe+I+G4: 1st codon position, TPM3+F+R2: 2nd codon position, HKY+F+R2: 3rd codon position, HKY+F+G4: LSU. These parameters were then implemented with Bayesian Analyses. Pairwise distances in COI were calculated in MEGA11: Molecular Evolutionary Genetics Analysis version 11 (Tamura et al. 2021) as p-distances.

BEAUti (Drummond et al. 2012) was used to establish parameters for BEAST v1.10.4 (Suchard et al. 2018). These included the use of a Gamma Site Model, 4 Gamma categories, shape parameter of 0.1940 (Estimate), and the substitution models selected by PartitionFinder, an Optimized Relaxed Clock, Birth/Death Model, and other model parameters remained at the default values. Four independent Markov Chain Monte Carlo (MCMC) chains were run for 2,000,000 generations sampled every 1000 generations. Convergence and effective sample size were examined in Tracer 1.7.1 to ensure effective sample size values were > 200 (Rambaut et al. 2018). Log Combiner v1.10.4 (<https://beast.community/logcombiner>) was used to combine tree files. TreeAnnotator v1.10.4 (<https://beast.community/treeannotator>) was used to construct a Maximum Clade Credibility tree and discard 10% burnin trees. FigTree v1.10.4 (<http://tree.bio.ed.ac.uk/software/figtree/>) was used for tree visualization with posterior probabilities shown on each node.

Anatomical descriptions follow the nomenclature on hydrobioid morphology (Hershler and Ponder 1998). Whorl counts follow Burch (1989). For examination of soft anatomy, the shell was dissolved in 50% hydrochloric acid, followed by removal of the periostracum. Dissections were conducted in water with the addition of Bouin's solution for visual contrast and hardening tissues to allow separation. Dissections were conducted and measurements taken on a Leica S9i using the Leica LASX software. When needed, images were stacked with Helicon Focus. Very few specimens were available for some taxa, limiting the detail of some anatomical descriptions. For example, having only juveniles or males available limited our ability to describe the female reproductive anatomy in some species and the radula in others.

Shells and radula were prepared for scanning electron micrograph (SEM) imaging with 75 angstroms of gold palladium alloy using a Quorum Sputter Coater. Shell images were taken using a SEC Co. Tabletop SEM, SNE-4500 M Plus in high vacuum, at 10–500 um magnification. The working distance was 1 mm and accelerating voltage of 1 kv. Radula were imaged using a Zeiss EVO LS10 in high vacuum. The working distance was 4–5.5 mm and accelerating voltage of 15.38 kv.

Specimens examined for morphology and paravouchers were deposited in the Texas State University Aquifer Biodiversity Collection (ABC). Types deposited in the Academy of Natural Sciences of Philadelphia at Drexler University (ANSP). Other museum abbreviations used in the manuscript: USNM = United States National Museum, Smithsonian Institution; TMM = Texas Memorial Museum.

Results

Maximum Clade Credibility trees from the Bayesian analyses are presented in Figs 2, 3. There is resolution for most of the taxa we are considering, but weakness in the backbone of the tree. The analyses with just the first and second of COI also had this weak resolution in the backbone of the tree and lost resolution at the tips resulting in a tree with very little information. For that reason, we figure and discuss the COI tree with all three base positions included, acknowledging the problem of some saturation in the third position and taking this uncertainty into account by making conservative taxonomic revisions considering both DNA and morphological data. The lack of resolution at deeper nodes in this gene results in some uncertainty among our taxonomic targets, particularly uncertain placement for *Phreatodrobia coronae* and relationships within the *Stygopyrgus*+*Texapyrgus* clade.

In analyses excluding the third position of COI relationships (not figured) within the *Stygopyrgus*+*Texapyrgus* clade were varied with the position of the *Stygopyrgus* species differing within this overall clade. The Bayesian analysis of the COI alignment (Fig. 2, 8 terminals, 657 bp) found a clade comprising *Stygopyrgus bartonensis* and two new species belonging to *Stygopyrgus* (posterior probability of 0.32). Sister to this was a clade (posterior probability of 0.99) of specimens identified as *Tryonia diaboli* and *Texapyrgus longleyi* from Finegan, Blue Hole, Snake,

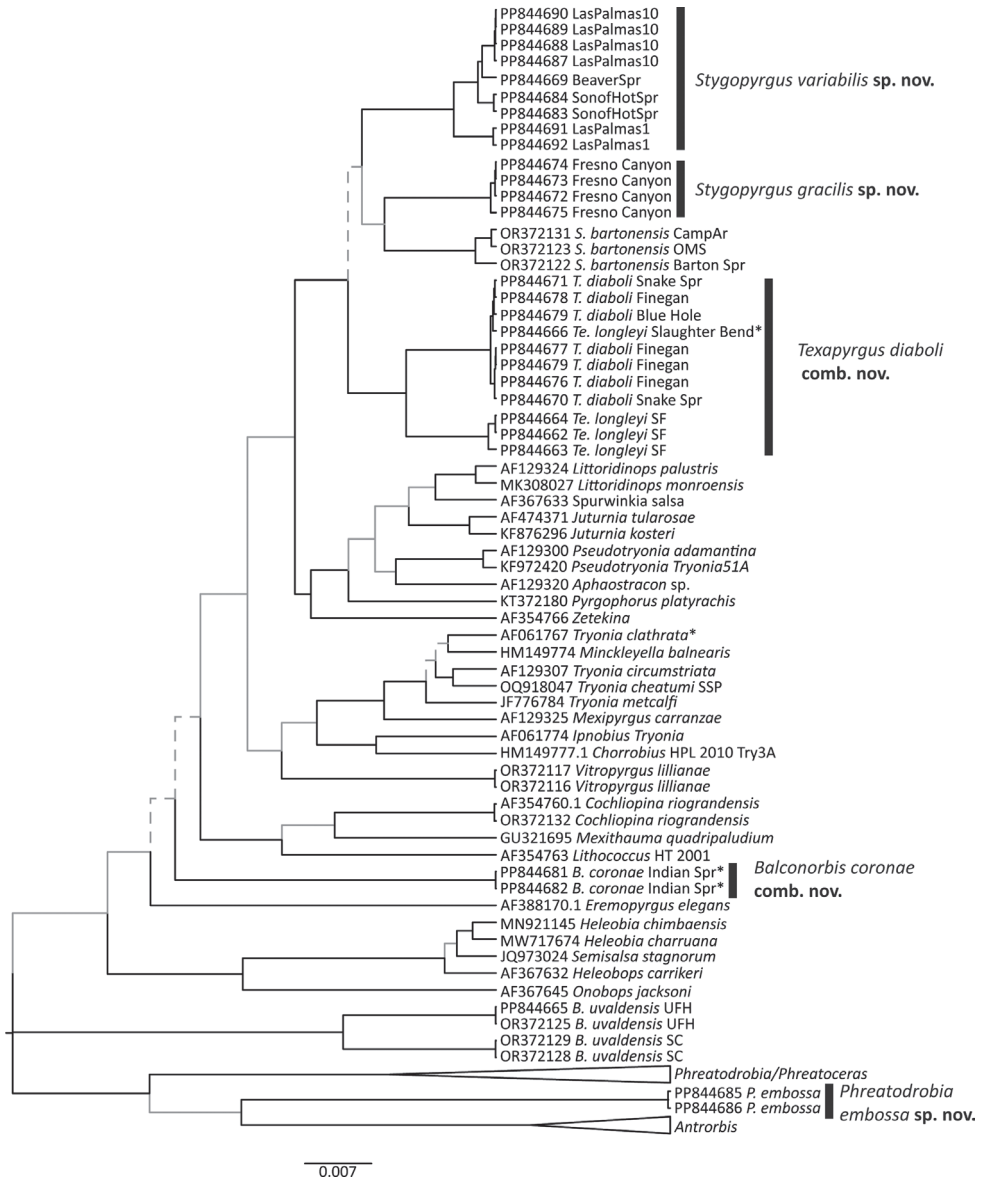


Figure 2. Maximum Clade Credibility tree resulting from Bayesian analysis of COI alignment. Posterior probability values < 0.50 indicated with dashed gray branches, 0.50–0.94 indicated with gray branches, and 1.0–0.95 are indicated with black branches. Terminals are labeled with a sample ID and sampling locality. Some nodes collapsed for non-target taxa for readability of the figure. Type localities are indicated with an *. Scale bars: substitutions per site.

and Slaughter Bend Springs (Devils River). Individuals with the morphology-based identification of each of these species formed a single clade: they are not reciprocally monophyletic. They formed a clade sister to individuals identified as *Texapyrgus*

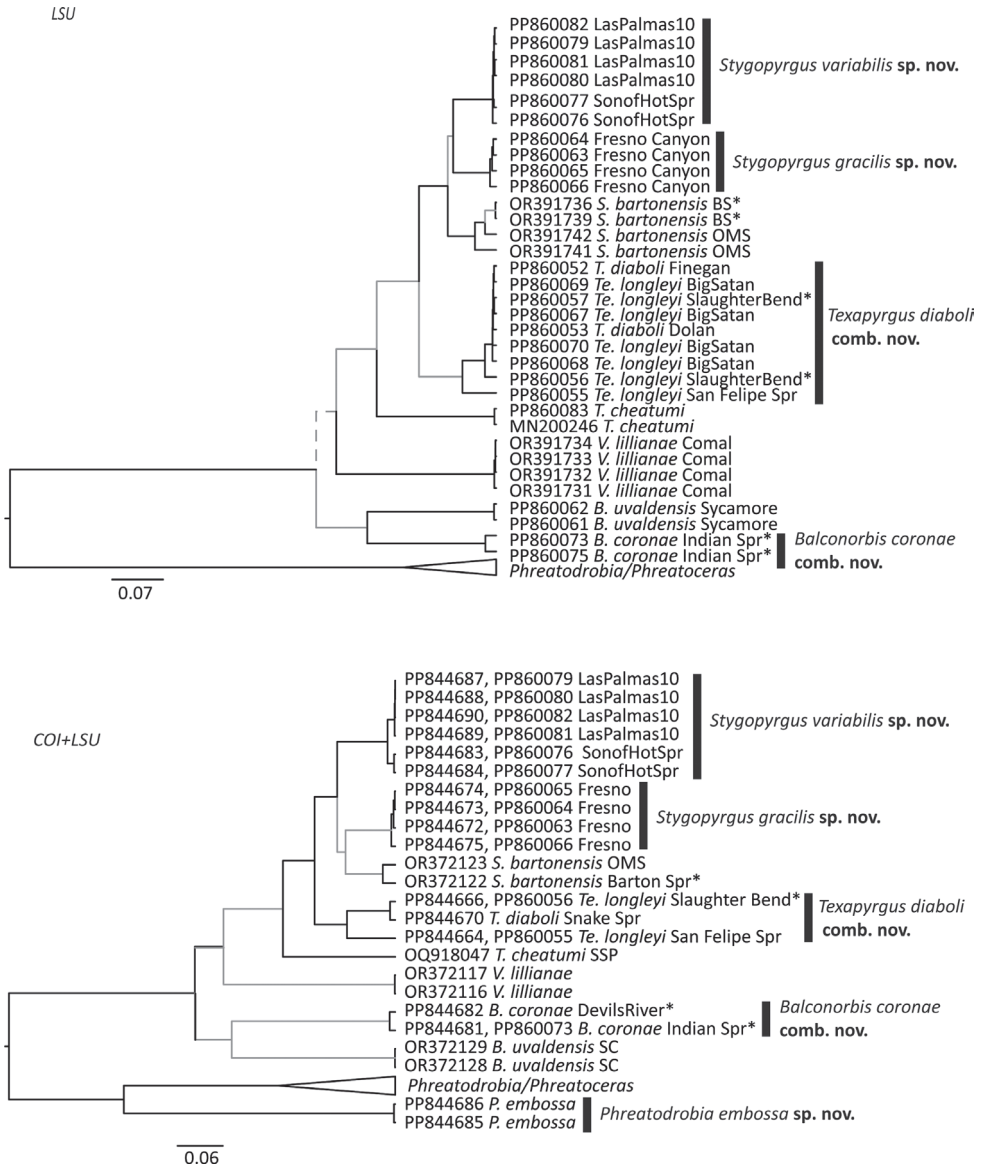


Figure 3. Maximum Clade Credibility tree resulting from Bayesian analysis of LSU and Combined alignment. Posterior probability values < 0.50 indicated with dashed gray branches, 0.50–0.94 indicated with gray branches, and 1.0–0.95 are indicated with black branches. Terminals are labeled with a sample ID and sampling locality. Type localities are indicated with an *. Scale bars: substitutions per site.

longleyi from San Felipe Springs. This “*Texapyrgus*” clade was not closely related to the type species *Tryonia clathrata* and other *Tryonia* species. There was weak support for relationships among additional genera of cochliopids. The COI analysis, with weak support, found *Phreatodrobia coronae* sister to a large clade of Littoridina. Finally, the new species from Caroline Springs was placed in a weakly supported clade

of *Phreatodrobia*, *Phreatoceras*, and *Antrorbis*. Analysis excluding third positions in COI placed the new species sister to *Phreatodrobia*.

The Bayesian analysis of the LSU alignment (Fig. 3, 55 terminals, 1,008 bp) supported the two new species and their placement within *Stygopyrgus*. This analysis found a weak sister relationship between *Balconorbis* and *P. coronae* (0.61 posterior probability). The Bayesian analysis of the combined alignment (Fig. 3, 37 terminals, 1,665 bp) is the best resolved of the analyses and found similar relationships among most taxa. This analysis found *P. coronae* sister to *Balconorbis* (posterior probability of 0.83) while the separate COI and LSU analyses found them in varying positions. The *Stygopyrgus*+*Texapyrgus* clade was supported as was the proposed new species as sister to *Phreatodrobia*.

Across the COI alignment, pairwise distances on average among species were 3.8% (p-distance in COI) and among genera 17.2%. Intraspecific p-distance among *Stygopyrgus variabilis* was 3.2%, *Stygopyrgus gracilis* 0.4%, within the clade containing *T. longleyi* and *T. diaboli* 4.7% (within Devils River springs 0.6% and within San Felipe Springs 1.0%), within *Phreatodrobia embossa*, 0.3%, and within *P. coronae* 0.0%. The p-distance among *Texapyrgus* and *Stygopyrgus* was 10.5% and between *Phreatodrobia embossa* and *Phreatodrobia* was 17.5%. The p-distance among *Stygopyrgus gracilis* and *Stygopyrgus variabilis* was 11.1%.

Systematics

Class Gastropoda Cuvier, 1795

Subclass Caenogastropoda Cox in Moore 1960

Order Littorinimorpha Golikov & Starobogatov, 1975

Superfamily Truncatelloidea Gray, 1840

Family Cochliopidae Tryon, 1866

Stygopyrgus variabilis Perez & Saenz, 2025, sp. nov.

<https://zoobank.org/2514A89C-7FB7-4DE3-9AD1-74E83C66DCB1>

Figs 4A, B, 5A, E, 6A, 7A–C

Type locality. USA, Texas, Brewster County, Lower Canyons of the Rio Grande, hyporheic zone of Rio Grande below Las Palmas Spring #5, (29.76829, -102.57352, WGS84).

Material examined. All sites are in Texas, USA. **Holotype** and **Paratypes** – BREWSTER COUNTY • Las Palmas Spring #5, drift net sample, collected by B. Schwartz, K.E. Perez, R. Winton, B. Hutchins, A. Cottrell, A. Sovie, A. Cressler, and B. Tobin, (29.76829, -102.57352), 22 April 2022, (ANSP 506748, ANSP 506749, ABC-000901).

Additional material examined. – All sites are in Texas, USA. All sample collection through the joint effort of: B. Schwartz, K.E. Perez, R. Winton, B. Hutchins, A. Cottrell, A. Sovie, A. Cressler, and B. Tobin. BREWSTER COUNTY, Beaver Spring, drift net sample (29.77079, -102.57088) 22 April 2022 (ABC-000898);

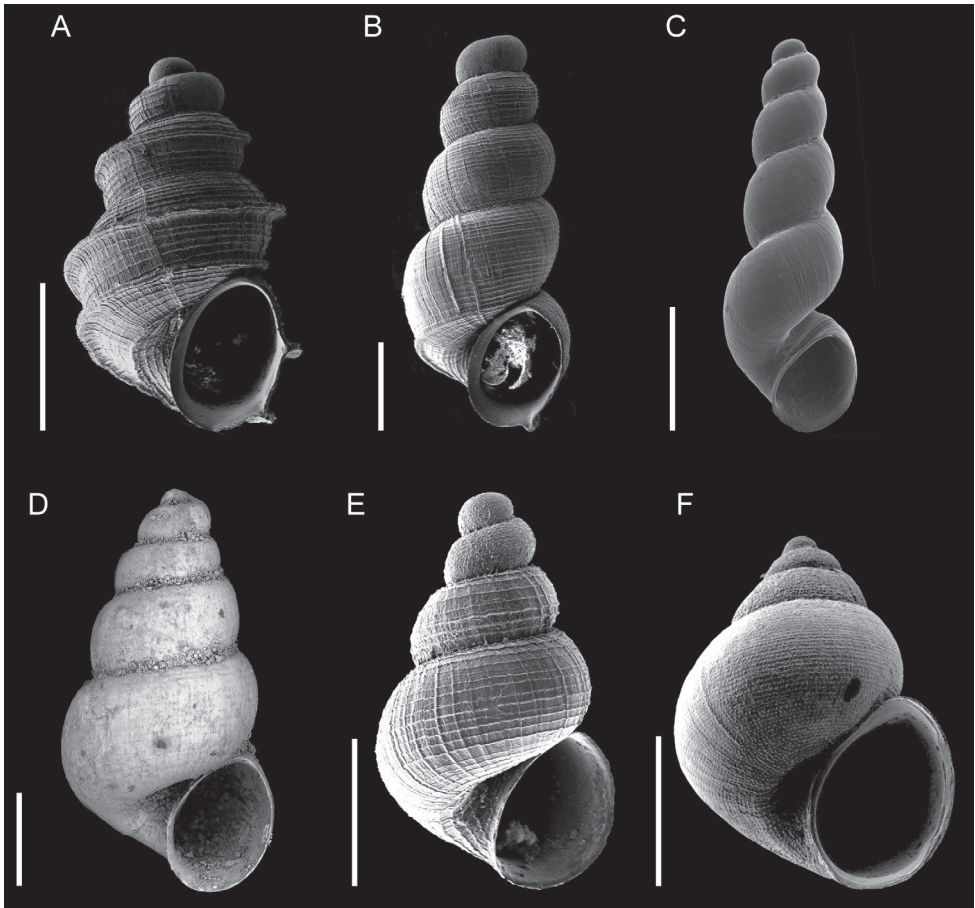


Figure 4. SEM of shells **A** shells of *Stygopyrgus variabilis* sp. nov., Las Palmas 5, Lower Canyons of the Rio Grande, Brewster Co., TX. ANSP 506748 **B** Las Palmas 1, Lower Canyons of the Rio Grande, Brewster Co., TX **C** shell of *Stygopyrgus gracilis* sp. nov., Fresno Creek, Fresno Canyon, Presidio County, TX. ANSP 506744 **D** *Texapyrigus diaboli*, comb. nov., lectotype of *Tryonia diaboli*, ANSP 91726, Drift of Devils River, Val Verde County, TX **E** Big Satan Canyon, Devils River, Val Verde County, TX **F** *Phreatodrobia embossa* sp. nov., Caroline Springs, Terrell County, TX. ANSP 506746. Scale bars: 500 μ m (**A–C, F**); 300 μ m (**D, E**).

- Las Palmas Spring #1, hyporheic sample (29.74829, -102.6075), 19 April 2022, (USNM);
- Son of Hot Springs, drift net sample (29.74846, -102.54075), 21 April 2022, (ABC-000900);
- Las Yeguas Spring, drift net sample (29.76233, -102.57348), 22 April 2022, (ABC-000903);
- Asa Jones Spring Complex, hyporheic sample (29.76833, -102.56351), 20 April 2022, (ABC-000902);
- Rio Grande below Las Palmas 5 and Las Yeguas Rapid, hyporheic sample (29.76916, -102.57192), 20 April 2022, (ABC-000904);
- Unnamed spring, downstream of Las Palmas 5, drift net sample (29.76884, -102.57198), 22 April 2022, (ABC-000905);
- Spigot Spring, drift net sample (29.76719, -102.56244), 23 April 2022, (ABC-000906);
- Las Palmas 10 Spring, drift net sample (29.74693, -102.54345), 23 April 2022, (ABC-000899).

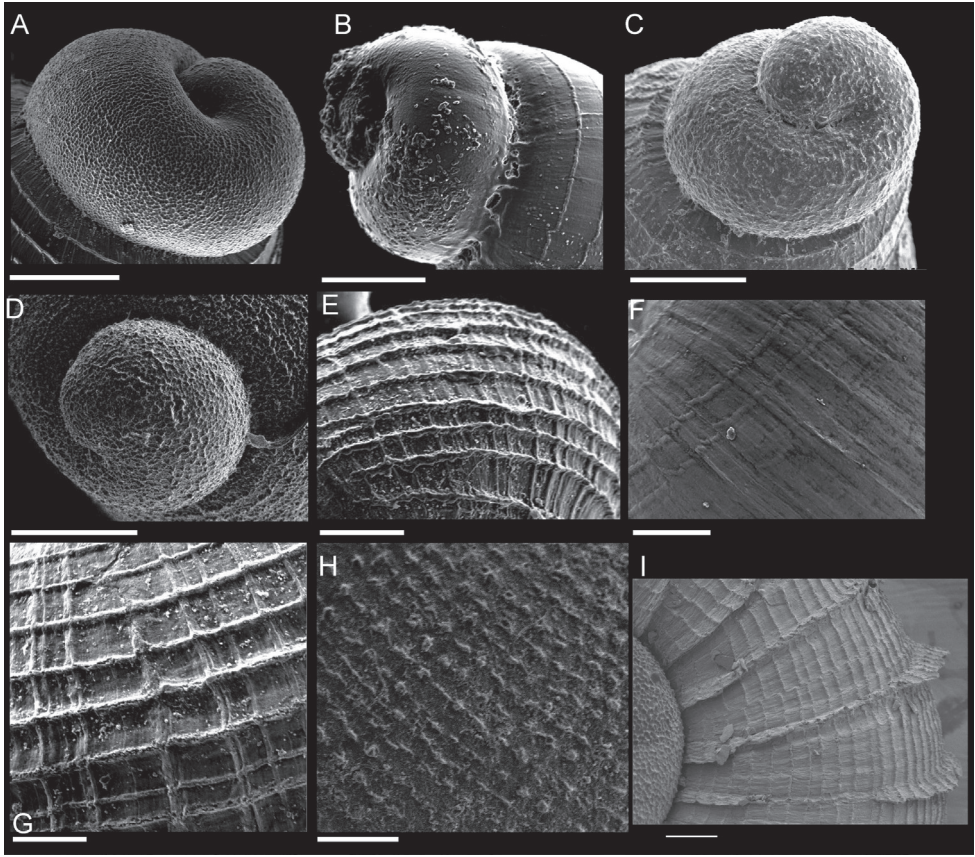


Figure 5. SEM of protoconch sculpture **A–D** & teleoconch sculpture **E–I** **A, E** *Stygopyrgus variabilis* sp. nov. Individual from Las Palmas 1, Lower Canyons of the Rio Grande, Brewster County, TX **B, F** *Stygopyrgus gracilis* sp. nov. Individual from Fresno Canyon, Presidio County, TX **C, G** *Texapyrgus diaboli* comb. nov. Individual from Big Satan Canyon, Devils River, Val Verde County, TX **D, H** *Phreatodrobia embossa* sp. nov. Individual from Caroline Springs, Terrell County, TX **I** *Balconorbis coronae* comb. nov. Individual from Indian Springs Canyon Springs, Val Verde County, TX. Scale bars: 100 μm (**A–E**); 50 μm (**E–I**).

Diagnosis. Minute shell, some individuals with prominent keeled appearance, with distinctive spiral and longitudinal lirae sculpture on teleoconch that extends to the sutures. *Stygopyrgus variabilis* differs from related species in the region by shell shape, and sculpture. Some populations (e.g. Son of Hot Springs, Rio Grande River, Brewster County) have a shorter, more ovate, and less heavily sculptured shell with fewer keeled individuals. A closely related species, *Stygopyrgus gracilis* sp. nov., has a turritiform shape with transverse growth lines and faint spiral raised lines that are not present in *Stygopyrgus variabilis*. *Stygopyrgus bartonensis* has similar sculpture that is finer, without keels, and with spiral and longitudinal striations rather than lirae. Penis of *Stygopyrgus variabilis* lacks the papillae or apocrine glands of other *Stygopyrgus* and *Texapyrgus* species. Cusps of central radula tooth more sharply pointed than other *Stygopyrgus* species.

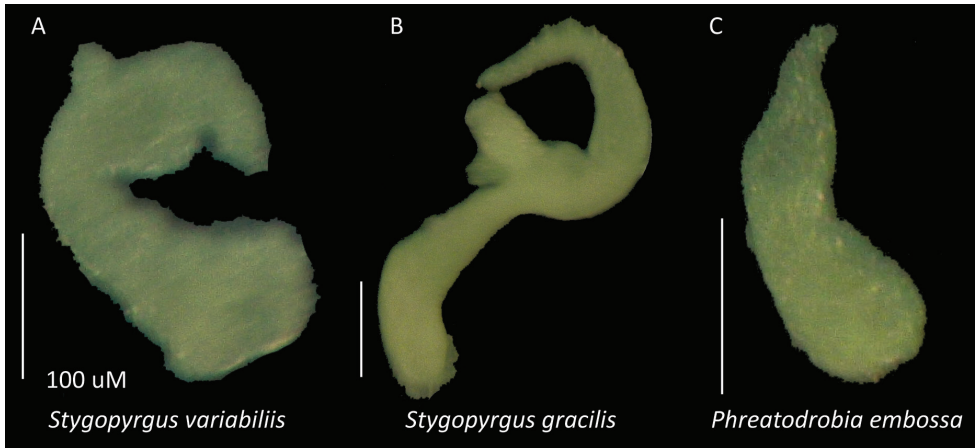


Figure 6. Dorsal view of the penis of the three new species **A** *Stygopyrgus variabilis* **B** *Stygopyrgus gracilis* **C** *Phreatodrobia embossa*. Yellow color is due to Bouin's solution for dissection, preserved tissues are white. Scale bars: 100 μ m.

Description. Shell minute, translucent to opaque, heavily sculptured, elongate ovate-conic with 1–3 prominent keel outlines per whorl on some individuals, some individuals with only a single weak keel most prominent on the body whorl or none (Fig. 4A, B, Suppl. material 1: fig. 1J–R). First whorl of protoconch is elevated, separated from subsequent whorls (Fig. 5A). Protoconch surface heavily sculptured by punctum that form irregularly shaped pits or wrinkles. Teleoconch sculpture includes irregularly spaced raised lateral lirae and more elevated longitudinal lirae dissected by prominent spiral keels. 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, umbilicus open but partially obscured by reflected lip. Keels (when present) extend to the edge of the outer apertural lip. Operculum ovate, extremely thin, translucent, light brown with darker brown region at nucleus, submarginal nucleus, distinct oval muscle attachment, slightly raised on inner surface. Average shell measurements for adults ($n = 20$) from Las Palmas Springs 1 & 5 and Beaver Springs: shell height = 1.35 mm (SD = 0.08), shell width = 0.68 mm (SD = 0.04), aperture height = 0.45 mm (SD = 0.03), aperture width = 0.40 mm (SD = 0.03), number of whorls = 4.75 (SD = 0.20).

Body unpigmented. Snout nontapered, slight distal lobation. Foot short, rounded, without lateral wings. Cephalic tentacle tapered, rounded, unpigmented, no visible cilia. Mantle tissue unpigmented. No visible eyes or pigmented patches at base of eyestalks. Ctenidium with about 12–15 triangular lobes. Rectum ends near edge of mantle on right side of head, intestine straight and uncoiled with elongate oval fecal pellets. Esophagus enters stomach below, stomach speckled with scattered dark flecks of pigment. Penis large, tapering, attached behind right eye, with expanded base and irregularly ridged along the proximal 2/3, narrows before the “arrowhead” like terminal portion. The tip

tapers with two rounded lobes near the distal end. No apocrine glands or papillae observed. In preserved specimen, penis has distinct curve forming a “fish-hook” shape.

Central radular tooth trapezoidal with rounded dorsal edge (Fig. 7A) deeply curved; lateral margin thickened, lateral cusps 4–5 on each side; central cusp about 1/3 longer than adjacent cusps but similar in shape with an elongate oval shape, tapering at the end and at the base, one pair of basal cusps pointed, with small buttress, broadly conical, not needle-like, basal tongue v-shaped, medium deep basal socket. Face of lateral tooth rectangular, tooth curved, narrowing upon reaching the outer wing; outer wing tapering; central cusp slightly longer than lateral cusps, 5–6 cusps outer and 4–5 cusps inner direction, decreasing in size distally (Fig. 7B). Inner marginal teeth with broad outer wing, 23–24 cusps visible, longer in length toward the central cusps, outermost cusps shorter, pointed, wide at base, cutting edge extends less than 25% of the length of the tooth, lateral wing present. Outer marginal teeth broad and slightly curved at end, with 9–11 cusps. Cusps along inner edge longer; tooth face tapering to outer wing (Fig. 7B, C).

Taxonomic remarks. Both mitochondrial and nuclear phylogenies place this new species close to *Stygopyrgus bartonensis* with 11.1% p-distance and with 10.1% p-distance from *Stygopyrgus gracilis*. However, the branch support is only moderate for placement in *Stygopyrgus*. In addition, these species share a minute, elongately conic shell outline, pitted or malleated protoconch sculpture, and teleoconch sculpture including regular, raised spiral lines and collabral growth lines. These sculptural features are larger and more pronounced in *Stygopyrgus variabilis*. *Stygopyrgus variabilis* share aspects of penial morphology with *S. bartonensis* with a relatively slender penis tapering with a muscular base but lacks papillae. This combination of DNA and morphological features seems to situate this species squarely in the genus *Stygopyrgus*, but sufficiently merit species status. Beaver Spring specimens were 100% keeled individuals, with both male and female individuals keeled. Other populations such as the type locality, Rio Grande at Las Palmas Spring 1, ~5% are unkeeled, 20% have one keel on the lower whorls only.

Etymology. The name *variabilis* was chosen to reflect the highly variable nature of shell sculpture of this species from heavily keeled to nearly smooth.

Ecology and habitat. The Lower Canyons Reach of the Rio Grande Wild and Scenic River is a remote section of the Rio Grande/Rio Bravo del Norte that forms the international border between the US and Mexico. This section of the Rio Grande is downstream from Big Bend National Park in a region of deep canyons through Chihuahuan Desert habitat, accessible only by multiple-day canoe excursion. Flow in the river includes some minor input from upstream, depending on the season, but is mostly maintained by groundwater input from numerous karst springs (some are thermal) discharging from the Edwards-Trinity Aquifer System (Smith Trevizo 2004; Brauch 2012). The Las Palmas spring group discharges from the Glen Rose and Del Carmen Formations (Brauch 2012) between river miles 739.6–735.8. *Stygopyrgus variabilis* was found in springs or hyporheic samples with water chemistry values ranging from: 25.79–32.26 °C, pH from 7.22–8.78, conductivity from 471.7–986.6 µS/cm, and dissolved oxygen from 3.7–10.8 mg/L.

The springs and hyporheic zone of the Lower Canyons of the Rio Grande are a region of high invertebrate diversity, including both spring-associated and ground-

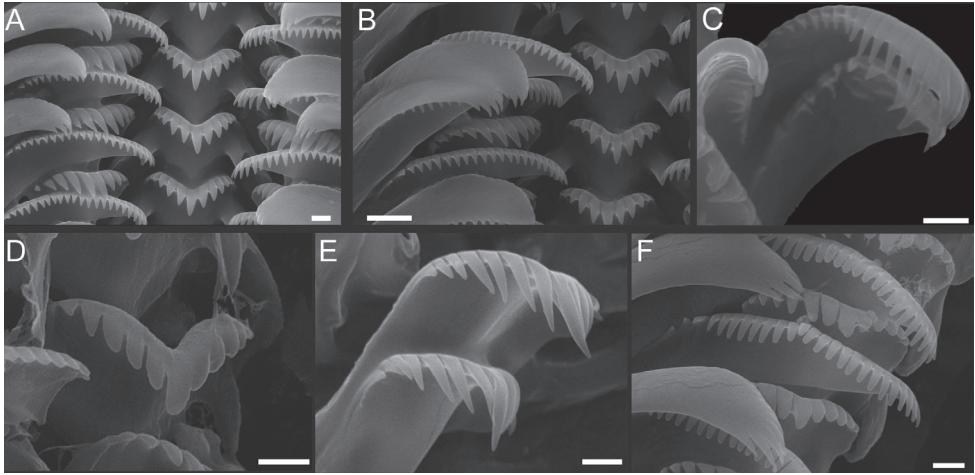


Figure 7. SEM of radula (**A–F**) of new *Stygopyrgus* species **A–C** *Stygopyrgus variabilis*, Las Palmas 1, Lower Canyons of the Rio Grande, Brewster County, TX **D–F** *Stygopyrgus gracilis*, Fresno Canyon, Presidio County, TX. Scale bar radula: 1 μ m.

water-associated (stygobiotic) taxa. Species found with *Stygopyrgus variabilis* included nematodes and freshwater annelida, crustaceans including ostracods, cyclopoid and harpacticoid copepods, *Texanobathynella* sp. (Parabathynellidae), Ingolfiellida, the amphipods *Paraholsingerius* sp., *Paramexiweckelia* sp., *Parabogidiella* sp., *Simplexia* sp., and *Seborgia* sp., the isopods *Speocirolana hardeni*, *Texicerberus amistad*, *Texicerberus schotteae*, *Thermosphaeroma subequalum*, and *Tethysbaena texana* (Thermosbaenacea). Apart from *Thermosphaeroma subequalum* (associated with thermal freshwater springs) and the ostracods and copepods of unknown ecology, the crustaceans present are considered stygobionts. Other molluscs present included Physidae and Sphaeriidae.

Class Gastropoda Cuvier, 1795

Subclass Caenogastropoda Cox in Moore 1960

Order Littorinimorpha Golikov & Starobogatov, 1975

Superfamily Truncatelloidea Gray, 1840

Family Cochliopidae Tryon, 1866

Stygopyrgus gracilis Perez, Saenz & Gonzalez, 2025, sp. nov.

<https://zoobank.org/AAC648E3-12FA-4136-B799-BD5E2829EF66>

Figs 4C, 5B, F, 6B, 7B

Diagnosis. Shell minute, elongate, turritiform, nearly smooth on both protoconch and telcoconch with few fine spiral lines and longitudinal growth lines. Penis slender with single, blunt, elongate papillae on inner curve near mid-length and sharply tapering distal end.

Type locality. USA, Texas, Presidio County, Big Bend Ranch State Park, Fresno Creek, hyporheic zone (29.74829, -102.6075).

Material examined. *Holotype* and *Paratypes* – USA, Texas, PRESIDIO COUNTY • Big Bend Ranch State Park, Fresno Creek, hyporheic sample, collected by K.E. Perez, A. Cottrell, L. Pustka, (29.30064, -103.84596), 5 October 2021 (ANSP 506744, ANSP 506745).

Additional material examined. – USA, Texas, PRESIDIO COUNTY • Big Bend Ranch State Park, Fresno Creek, hyporheic sample, collected by K.E. Perez, B. Schwartz, B. Hutchins (29.30064, -103.84596), 10 December 2021 (ABC-000919).

Description. Shell minute, transparent, thin, nearly smooth, turritiform, lightly sculptured. Spire finely tapering. Sutures deeply incised, growing deeper towards the body whorl, until aperture is separated from body whorl. Fine transverse lines along occasional whorls of teleoconch. Fine collabral growth lines at regular intervals on last two whorls. Sutures contain irregular indentations increasing in quantity approaching apex. Body whorl and aperture separated from previous whorl in adults. First whorl of protoconch elevated. Aperture ovate, lip thin, slightly reflected at base of aperture in adults, and not thickened. Aperture tilted forward. Umbilicus a wide slit due to separation of aperture from body whorl. Average shell measurements for adults ($n = 8$): shell height = 1.32 mm (SD = 0.14), shell width = 0.52 mm (SD = 0.06), aperture height = 0.38 mm (SD = 0.04), aperture width = 0.30 mm (SD = 0.03), number of whorls = 5.34 (SD = 0.55).

Body unpigmented, snout rounded, slightly tapered, with rounded distal lobes. Tentacles short, squat, tapered with no eyes or eye patches visible. Foot short, anterior portion rounded, without lateral wings. Ctenidium, when present, a series of 10+ triangular lobes found through the pallial roof, not present in some individuals. Intestine curves in “s” shape through pallial cavity, with rectum ending near edge of mantle. Intestine lined with short oval fecal pellets. Operculum extremely thin, lightly pigmented amber, nucleus submarginal, edge rounded, distinct muscle attachment scar. No apparent thickened or raised portion on inner surface. Penis very long, thin, and tapering, hooked at the distal end in preserved specimens. Proximal half of the penis length with shallow folds, one papilla present about 2/3 of way towards distal end. Female reproductive anatomy not described due to a lack of female specimens.

Central radula tooth trapezoidal. Central cusp of central radular tooth oval with rounded edge (Fig. 7D); lateral cusps 5–6 on each side; central cusp about 1/3 longer than adjacent cusps with elongate oval shape, lateral cusps become less rounded and more pointed distally, tapering at the end. Single pair of basal cusps conical, pointed, singular basal cusps pointed, with small buttress. Basal tongue broadly v-shaped. Face of lateral tooth rectangular, narrowing upon reaching the outer wing; outer wing tapering; central cusp slightly longer than lateral cusps, 6–7 cusps outer and 5 cusps inner direction, decreasing in size distally. Inner marginal teeth with broad outer wing, no basal notch, 22–23 cusps visible, similar in length, inner cusps slightly longer, middle cusps slightly wider at base, last 2–3 cusps shorter than the rest. Outer marginal teeth broad and curved at end, cusps 9–11. Middle cusps longer, fingerlike (Fig. 7E, F).

Taxonomic remarks. Both mitochondrial and nuclear gene phylogenies place this new species near *Stygopyrgus bartonensis* (9.6% p-distance) and with 10.1% p-distance from *Stygopyrgus variabilis*. However, the branch support is only moderate for placement in *Stygopyrgus*. In addition, these species share a minute shell, elongately conic

shell outline, and teleoconch sculpture including raised spiral lines and collabral growth lines. These sculptural features are greatly reduced in *Stygopyrgus gracilis*. *Stygopyrgus gracilis* share aspects of penial morphology with *S. bartonensis* with a slender penis tapering but with a single instead of two papillae and a more elongate and sharply tapering distal end. This combination of DNA and morphological features seems to situate this species squarely in the genus *Stygopyrgus* but is sufficiently distinctive to merit species status.

Etymology. The name “*gracilis*” was chosen to reflect the elongate, slender form of the shell.

Ecology and habitat. Fresno Creek watershed contains a mostly dry gravelly desert stream channel with intermittent reaches of permanent water, flowing southward through Fresno Canyon on the west side of the Solitario in Big Bend Ranch State Park (Rush 1960). Fresno Creek joins the Rio Grande/Rio Bravo ~2.3 km below the type locality. Upstream reaches of Fresno Creek receive small amounts of water from springs in the Chorro Canyon tributary, where water emerges from volcanic rock layers. The eastern side of the basin contains extensive, karstified limestones of the Solitario and Terlingua monocline. Small springs along Fresno Creek itself, and groundwater underflow discharging into the extensive gravels forming the creek bed, also contribute to flows. *Stygopyrgus gracilis* was found in hyporheic samples collected from gravel deposits in a short perennial reach where a bedrock exposure in the streambed forces subsurface flows in upstream gravels to reach the surface. Water chemistry values averaging 24.89 °C, pH 7.95, conductivity of 1071 µS/cm, and dissolved oxygen of 5.37 mg/L.

Fresno Creek fauna included nematodes, freshwater mites, freshwater Annelida, and crustaceans (ostracods, cyclopoid and harpacticoid copepods). Insects included Dytiscidae and Dryopidae.

Class Gastropoda Cuvier, 1795

Subclass Caenogastropoda Cox in Moore 1960

Order Littorinimorpha Golikov & Starobogatov, 1975

Superfamily Truncatelloidea Gray, 1840

Family Cochliopidae Tryon, 1866

Subfamily Littorinidae Thiele, 1928

***Texapyrgus diaboli* (Pilsbry & Ferris, 1906), comb. nov.**

Figs 4D, E, 5C, G

Paludestrina diaboli Pilsbry & Ferris, 1906, pp. 170, fig. 36.

Texapyrgus longleyi Thompson & Hershler, 1991, pp. 680–682.

Tryonia diaboli, Diaz et al. 2020, pp. 18–24.

Types. Lectotype ANSP 91726.

Type locality. Spring on east side of Devil’s [sic] River in canyon just downflow from Slaughter bend (Indian Springs Canyon Springs), Amistad National Recreation Area, 32 km N of Del Rio, Val Verde County, TX, 29.6582, -100.91765.

Additional material examined.—All sites are in Texas, USA. VAL VERDE COUNTY • Finegan 105, Devils River (29.9048, -101.0111), P. Diaz (ABC-000882); • Dolan Drift #383 (29.8858, -100.9933), 1 August 2016, P. Diaz (USNM 1571310); • San Felipe Creek. SW3-HZ1, (29.36887, -100.88388), 6 December 2020, B. Schwartz (ABC-000879); • Lake Amistad National Recreation Area, Slaughter Bend, small spring 25 m W of Indian Springs (29.66385, -100.9275), 12/6/2020, K.E. Perez, C. Ortega, R. Chastain (ABC-000878); • Lake Amistad National Recreation Area: Indian Springs Canyon Springs (29.6582, -100.91765), K.E. Perez, C. Ortega, R. Chastain (no specimens remaining); • Snake Spring (29.896319, -100.981214), B. Schwartz (ABC-000883); • Blue Hole, Finegan Springs, Devils River (29.8938, -100.99456), B. Schwartz (USNM 1571309); • Indian Springs (29.66383, -100.9275), 6 December 2020, Big Satan Canyon (29.66349, -100.95492), 8 December 2020, B. Schwartz (ABC-000876); • Big Satan Canyon Spring (29.6635, -100.95452), 8 December 2020, B. Schwartz (ABC-000880); • Boiling Trough Spring (29.6402, -100.92647), J. Gordon, M. Turner, R. Hoffman (ABC-000917).

Description. Slender, elongate shell comprised of 4 ½ - 5 ½ convex whorls. Sutures deeply impressed. Average size of 1.6 mm in height, and 0.82 mm in diameter. Protoconch strongly wrinkled followed by variable teleoconch sculpture with some individuals smooth and others heavily striated. Aperture ovate, measuring an average of 0.50 mm in height, and 0.40 mm in width. Umbilicus small. Peristome thin, and slightly reflected in adults (Thompson and Hershler 1991).

Taxonomic remarks. *Tryonia diaboli* (originally *Paludestrina diaboli*) was described from drift debris of the Devils River, “about four miles from its mouth”, and from “the Rio San Felipe near Del Rio”, now called San Felipe Springs. The habitat was unknown, as the types were dry, bleached shells deposited by flowing water. This region has a rich freshwater fauna of both surface and aquifer snails; however, it was uncertain from which habitat this species originated. *Tryonia diaboli* was described from shell anatomy, not internal morphology. The shell is described as small, 1.3 mm length, 0.62 mm diameter, very slender, and turrite (tower shaped), with 4.5 very convex whorls with deep sutures and a smooth surface (Pilsbry and Ferriss 1906). The description and illustration (Pilsbry and Ferriss 1906, pp. 170, fig. 36) depict a smooth shell. However, scanning electron microscope images of a lectotype (Fig. 4D, Academy of Natural Sciences of Philadelphia, ANSP 91726), which appears to be the shell illustrated in the original description, show a crosshatched pattern of longitudinal and spiral sculpture, similar to the sculpture of *Texapyrgus longleyi*.

Texapyrgus longleyi Thompson & Hershler, 1991 was collected from a spring on the Devils River just downstream from Slaughter Bend (USNM 860551). The species was collected using a net over a spring opening. That habitat and the small size and unpigmented body indicated that the species is subterranean. The shell of *Tē. longleyi* was 1.4–1.8 mm, thin and translucent with prominent spiral lines, crossed by strong longitudinal striae. The shell was described as having deep sutures, small umbilicus, aperture lip complete and touching body whorl at the top. The name includes the word “*pyrgus*” indicating the turrite or tower shape of the shell. The description of *Tē. longleyi* is more extensive but closely resembles in many features the description and type of *T. diaboli*.

Due to the obscurity of the original description, it appears likely that Thompson and Hershler were unaware of *T. diaboli*. They do not mention *T. diaboli*, also from the Devils River, in their description of *Te. longleyi*. The original description, and a mention in a list of the type specimens in the Academy of Natural Sciences of Philadelphia are the only mention of *T. diaboli* in the scientific literature for more than 100 years. To further support our supposition that Hershler was unaware of *T. diaboli*, in a review of all species of *Tryonia* of North America (Hershler 2001), Hershler does not mention *T. diaboli*. The species was not mentioned again in the scientific literature until it was included in a list of North American freshwater snails (Johnson et al. 2013) and was reported in both above and below ground habitats in the Devils River (Diaz et al. 2020). Since the “rediscovery” of this species name, the generic assignment of *T. diaboli* has not been assessed. Due to the close geographical proximity of their collection localities and close similarities of shell features, we questioned whether *T. diaboli* and *Te. longleyi* are distinct species. We conducted DNA sequencing of individuals with both “morphological forms” i.e. smooth vs highly sculptured. In addition, new sequences were included in an alignment with *Tryonia* and species from across the Cochliopidae to test the hypothesis that *T. diaboli* is a member of *Tryonia* or, if not, to determine the best generic placement.

We found that snails closely resembling the type material and descriptions of *T. diaboli* from springs of the Devils River are not closely related to members of *Tryonia* (Figs 2, 3), meriting reassignment to a different genus. Further we find that snails resembling the type material of *T. diaboli* and *Te. longleyi* from springs of the Devils River and San Felipe springs form a monophyletic group. The snails identified as each of these species, based on shell morphology, are not reciprocally monophyletic (Figs. 2, 3). There is some divergence (9.8%) between the populations found in springs near the Devils River and those sampled from San Felipe Springs. This could be an artifact of sequencing a limited number of individuals and the geographical gap in samples between these populations. There are some morphological differences to support further distinguishing those populations. We have considered whether populations in the Devils River and San Felipe drainages should be considered as distinct species, and while the data do not preclude the possibility, we take the more conservative approach of preserving these populations as a single species, acknowledging that additional evidence may call for further revision in the future. The average intraspecific divergence in COI in this clade is 4.6%, within the Devils River springs is 0.6% and within the San Felipe Springs 1.0%.

From these lines of evidence (monophyly in both nuclear and mitochondrial sequence data and similar morphologies), we conclude that *T. diaboli* and *Te. longleyi* are synonymous and cannot remain assigned to *Tryonia*. The original genus *Paludestrina* d’Orbigny, 1840 is unavailable (ICZN 2008). That being the case, it would be most appropriate to refer to this species as *Texapyrgus diaboli* (Pilsbry & Ferris, 1906) with *Texapyrgus longleyi* a junior synonym.

Ecology and habitat. The springs along the Devils River where we collected *Texapyrgus diaboli*, are all from the karstic Edwards-Trinity Aquifer (Georgetown Limestone) and form most of the flow for the Devils River (Toll et al. 2017; Texas Water Development Board 2018). The Devils River, a groundwater-dependent river, is fed by numerous springs and drains a large portion of the southwestern Edwards Plateau to

the Rio Grande River. One population of *Texapyrgus diaboli* was found in San Felipe Springs, which are among the largest in Texas with an average discharge of 3473 L/s (Hutchison 2021). The springs discharge from Cretaceous Salmon Peak Limestone (Barnes et al. 1992). *Texapyrgus diaboli* was found in drift net and hyporheic samples with water chemistry values ranging from 15.06–23.28 °C, pH 6.49–7.4, conductivity of 366.3–608.8 µS/cm, and dissolved oxygen of 4.33–8.47 mg/L.

The springs and hyporheic zone of the Devils River and San Felipe Springs watershed support high invertebrate diversity, especially stygobiotic taxa. The groundwater obligate taxa that have been documented at the Devils River Springs of Blue Hole and Finegan Springs are: *Artesia subterranea* Holsinger, 1980, *Bicornucandona fineganensis* Küllköyliüoğlu, Gibson, Diaz & Colin, 2011, *Cirolanides texensis* Benedict, 1896, *Hobbsinella edwardensis* Camacho, Hutchins, Schwartz, Dorda, Casado & Rey, 2017, *Ingolfiella* sp., *Lirceolus bisetus* (Steeves, 1968), *Parabogidiella americana* Holsinger, 1980, *Paraholsingerius smaragdinus* (Holsinger, 1992), *Paramexiweckelia ruffoi* Holsinger, 1996, *Phreatodrobia coronae* Hershler, 1987, *Phreatodrobia spica* Perez & Alvear, 2020, *Seborgia hershleri* Holsinger, 1982, *Speocirolana hardeni* Bowman, 1992, *Stygobromus hadenoecus* (Holsinger, 1966), *Texanobathynella* sp., *Texapyrgus longleyi* Thompson & Hershler, 1991, Microcerberidae sp. (probably *Texicerberus schotteae* Hutchins & Schwartz, 2021, but unconfirmed), and *Typhloelms finegan* Barr, 2015. Other molluscs present included Physidae, Sphaeridae, Planorbidae, and *Cochliopina riograndensis*.

The groundwater obligate taxa that have been documented at San Felipe Springs are: *Artesia subterranea* Holsinger, 1980, *Balconorbis uvaldensis* Hershler & Longley, 1986, *Cirolanides texensis* Benedict, 1896, *Lirceolus* sp., *Mexistenasellus coahuila* Cole & Minckley, 1972, *Parabogidiella americana* Holsinger, 1980, *Paramexiweckelia ruffoi* Holsinger, 1996, *Phreatodrobia coronae* Hershler, 1987, *Psychopomporus felipi* Jean, Telles & Miller, 2012, *Seborgia hershleri* Holsinger, 1982, *Speocirolana hardeni* Bowman, 1992, *Stygobromus* sp., *Tethysbaena texana* (Maguire, 1965), and *Typhloelms sanfelipe* Barr, 2015.

Class Gastropoda Cuvier, 1795

Subclass Caenogastropoda Cox in Moore 1960

Order Littorinimorpha Golikov & Starobogatov, 1975

Superfamily Truncatelloidea Gray, 1840

Family Cochliopidae Tryon, 1866

***Balconorbis coronae* (Hershler, 1987), comb. nov.**

Figs 4F, 5I

Phreatodrobia coronae Hershler, 1987, pp. 133–139.

Phreatodrobia coronae Alvear et al., 2020, pp. 7, fig. 6.

Types. Holotype USNM 859219.

Type locality. USA, Texas, Val Verde County, unnamed spring (now named Indian Springs Canyon Springs) on E side of Devils River in canyon (named Indian Springs Canyon) just downflow from Slaughter Bend, Devils River.

Material examined. All sites are in Texas, USA. VAL VERDE COUNTY • Lake Amistad National Recreation Area, Indian Springs Canyon Springs, Devils River, drift net sample, collected by K.E. Perez, C. Ortega, R. Chastain (29.6582, -100.91765), 8 December 2020, (ABC-000886).

Additional material examined. – VAL VERDE COUNTY • Indian Springs (29.66383, -100.9275), 6 December 2020, K.E. Perez, C. Ortega, R. Chastain, (ABC-000881); • San Felipe Creek, drift net sample, (29.36887, -100.88388), 6 December 2020, B. Schwartz (USNM 1571292); • Blue Hole, Finegan Springs, Devils River (29.8938, -100.99456), B. Schwartz (USNM 1571290); • Finegan Springs, Devils River, (29.900708, -100.998476), (USNM 1571291); • Snake Spring (29.896319, -100.981214), B. Schwartz (ABC-000915); • Boiling Trough Spring (29.6402, -100.92647), J. Gordon, M. Turner, R. Hoffman (ABC-000917); • Finegan 105, (29.9048, -101.0111381), Devils River, Pete Diaz, (ABC-000922); • Blue Hole, (29.893872, -100.99453), 19 March 2013, Randy Gibson, (ABC-000923).

Description. Shell minute, transparent and colorless. Near-planispiral to depressed-trochoid. Deeply impressed sutures. First whorl of protoconch uncoiled to a horn-like apex, sculptured with wrinkled pits. Teleoconch sculpture with strong raised spiral lines near protoconch, and later with both raised spiral and longitudinal lines. On some specimens, collabral ribs (costae) are strongly defined while absent or very faint in others. If present, ribs start on the penultimate whorl and increase in size towards the body aperture. Widely open umbilicus, aperture circular to ovate depending on overall shell shape (planispiral to trochoid). Peristome flared all around and tilted adapically.

Taxonomic remarks. *Phreatodrobia coronae* was assigned to *Phreatodrobia* based on a similar shell outline to *P. micra* (Pilsbry & Ferriss, 1906) and *P. nugax* (Pilsbry & Ferriss, 1906) (depressed to sub-trochoid), however, both mitochondrial and nuclear data consistently place *P. coronae* outside *Phreatodrobia*, with strong support. *Phreatodrobia coronae* displays dimorphism in several features, an aspect not common among *Phreatodrobia*, and has a distinctive, uncoiled protoconch, also not found among other *Phreatodrobia* species. We have considered whether this species should be reassigned to *Balconorbis* based on the DNA sequence relationship. This relationship is weak, and different genes and analysis parameters affect the placement of both *Balconorbis uvaldensis* and *Phreatodrobia coronae*. These two genes are insufficient to fully resolve the backbone of the tree of the Cochliopidae. The average divergence in COI between *Balconorbis uvaldensis* and *P. coronae* was 18.5%, slightly above the average divergence among genera overall (17.2%) and the 3rd position of the COI locus was saturated at this level, making the relationship in the LSU phylogeny more reliable. The LSU phylogeny places *P. coronae* with *Balconorbis*, but this is far from certain.

In the ETAS and nearby aquifers, there are members of the cochliopid fauna with depressed or subtrochoid shells that are also known only from shell or shell+anatomical descriptions and not represented in our phylogeny (e.g., *Coahuilix* and *Phreatomascogos*). We compare some key features of these species in Table 2.

Table 2. Comparison of morphological features of *B. coronae* and other similar genera from the region.

Morphological features	<i>B. coronae</i>	<i>Balconorbis</i>	<i>Coahuilix</i>	<i>Phreatomascogos</i>
Size	Minute	Minute	Minute	Minute
Shape	Near-planispiral to low-trochoid	Planispiral	planispiral	Valvatiform to low trochoid to low conical
Protoconch sculpture	Wrinkles, pitted	Wrinkles, pitted and/or spiral lines	Punctate, coarsely wrinkled	Smooth
Protoconch position	Raised, uncoiled	Slightly depressed	Slightly depressed to moderately protruding	Slightly depressed
Teleoconch sculpture	Spiral lines, longitudinal lines, lamelliform costae	Spiral lines	Smooth	Longitudinal keel
Operculum	Nucleus raised into a peg	Raised nucleus	No peg	Campanulate
Ctenidium	Absent	Absent	Absent or nearly absent	unknown
Penial shape	Coiling on right side of neck, filament 1/3 length, tapering distally.	Slender, uncoiled, filament 1/3 of length, tapering distally	Filament 1/3 of length, tapering distally	Unknown
Penial glands	No apocrine gland	Single apocrine gland	Single apocrine gland	Unknown
Vas deferens	Does not undulate in penis	Does not undulate in penis	Undescribed	Unknown
Female reproductive traits	Sperm pouch not observed, but description of female reproductive tract limited due to lack of material	Absence of sperm pouches	Sperm storage site in posterior albumen gland	Unknown

Coahuilix is diagnosed by raised riblets on the teleoconch, a flared aperture, and a flat, incised spire. In contrast, *B. coronae* lack all these features. *Phreatomascogos* has prominent keels, an unraised spire, a campanulate operculum, and a relatively closed umbilicus. In contrast, *B. coronae* is unkeeled, has a raised spire, a round operculum, and an umbilicus that is the width of several whorls.

Although our evidence is inconclusive about whether *P. coronae* is a member of *Balconorbis* or a new genus, it seems more conservative to move the species to *Balconorbis* rather than erecting a new genus at this time. Lacking strong support in the phylogeny and DNA sequences of *Balconorbis sabinasensis* and other Mexican taxa, the affinity of this species is not fully resolved. It is clear though that it is not a member of *Phreatodrobia*. We propose the new combination *Balconorbis coronae* comb. nov. with the understanding that the inclusion of more resolved DNA data and more thorough sampling of the region, especially in Mexico, will likely shed additional light on these relationships in the future.

Ecology and habitat. Habitat and associated fauna for *Balconorbis coronae* localities is similar to *Texapyrgus diaboli*, as they are found in many of the same springs in the Devils River watershed. *Balconorbis coronae* was collected from spring drift net samples with water chemistry values ranging from 15.06–23.28 °C, pH 6.49–7.4, conductivity of 366.3–608.8 µS/cm, and dissolved oxygen of 4.33–8.47 mg/L.

Class Gastropoda Cuvier, 1795

Subclass Caenogastropoda Cox in Moore 1960

Order Littorinimorpha Golikov & Starobogatov, 1975

Superfamily Truncatelloidea Gray, 1840

Family Cochliopidae Tryon, 1866

***Phreatodrobia embossa* Perez, 2025, sp. nov.**

<https://zoobank.org/43F12F11-A5DF-451A-AE3F-1F89563FBA9F>

Figs 5 D, H, 6C, 7C

Diagnosis. Shell minute, glassy, clear, ovately conic. Elevated protoconch and teleoconch with regular rows of spirally arranged nodules. Broadly ovate aperture. Penis simple, tapering, with little muscular ridging.

Type locality. USA, Texas, Terrell County, Caroline Springs hyporheic zone (30.46622, -101.79429).

Material examined. *Holotype* and *Paratypes* – TERRELL COUNTY • Caroline Springs near Sheffield, 2nd pool of raceway, hyporheic sample, collected by K.E. Perez, H. Glover, P. Sprouse (30.46622, -101.79429), 7 December 2020 (ANSP 506746, ANSP 506747).

Description. Shell minute, clear, glassy, with regular rows of sculpture, ovately to globosely conic with rounded whorl outlines and impressed sutures (Figs. 5 D&H). First whorl of protoconch elevated, separated from the whorls that follow. Protoconch sculpture is a uniform network of raised wrinkles giving an irregular malleated appearance. Teleoconch sculpture is distinctive and includes regularly spaced (~10 µm apart), spirally arranged rows of raised nodules (Fig. 5H) and transverse growth lines. Nodules resemble irregularly sized knots on a string. Aperture broadly ovate, usually appressed to body whorl at upper parietal corner. Outer margin of aperture straight, slightly thickened and reflected, stronger at apex and base. Outer lip straight, simple, slightly prosocline. Umbilicus deep and open. Average shell measurements for adults (n = 8): shell height = 1.07 mm (SD = 0.09), shell width = 0.80 mm (SD = 0.08), aperture height = 0.53 mm (SD = 0.06), aperture width = 0.44 mm (SD = 0.04), number of whorls = 4.75 (SD = 0.38). Operculum clear, thin, pliable. Shape ellipsoidal, nucleus submarginal, strongly convex. Growth lines not distinct or frilled. Muscle attachment scar oval, nuclear area with raised thickening inside.

Tissue unpigmented, tentacles longer than snout, unpigmented, no visible eyes, snout short, deeply lobate, foot short with no lateral wings. Ctenidium across pallial roof, ~10 elongate, low-triangular lobes, filling 50% of pallial roof. Osphradium large, ovate, near posterior end of the ctenidium. Intestine winds in U shape through pallial cavity, filled with small round fecal pellets, rectum ends near edge of the mantle. Esophagus muscular, enters stomach below, smaller anterior chamber and larger posterior chamber. Penis small, attached above right tentacle, lightly furrowed along narrow, muscular base, narrowing to a short neck about half the width of the base before widening to a lobe on the distal 1/3, followed by a tapering tip. Female reproductive anatomy and radula not described due to a lack of material.

Taxonomic remarks. *Phreatodrobia embossa* is placed sister to *Phreatodrobia* in the phylogenetic analyses of both genes with strong support, and in a clade with *Phreatodrobia* and *Antrorbis* with weak support in the COI-only analysis. We were not able to obtain LSU sequence data for *Phreatodrobia embossa*. In COI, *Phreatodrobia embossa* has an average p-distance of 17.5% from *Phreatodrobia*. The sculpture is distinctive

among the Cochliopidae, and the genetic distance is close to what we would expect for genera in the family (which averaged 17.2%). *Phreatodrobia embossa* shares aspects of penial morphology with other *Phreatodrobia* and *Phreatoceras* species with a simple, slender penis tapering at the distal end, but with less muscular ribbing and no coiling. We chose to include *Phreatodrobia embossa* in *Phreatodrobia* to reflect the relatively close phylogenetic relationship with members of that genus, though we acknowledge that further understanding of the relationships among Cochliopidae might result in reassignment in the future.

Etymology. The name “embossa” refers to the teleoconch sculpture pattern similar to embossed designs on leather or paper.

Ecology and habitat. Caroline Springs (previously T5 springs), located at The Nature Conservancy’s Independence Creek Preserve, has a discharge of 189–315 L/s (TNC website), emanating from the early Cretaceous Edwards Limestone (Barnes et al. 1992; Brune 2002; Brown 2003). The spring run at Caroline springs is heavily modified and impounded. There are several elongate pools forming a raceway immediately downstream from the large main spring pool. *Phreatodrobia embossa* was found in hyporheic samples taken from gravels in the 2nd pool of the raceway. This pool is the site discussed and figured as the type locality of *Tryonia oasiensis* Hershler, Liu, Landye, 2011. Water chemistry values were as follows: temperature = 15.5 °C, pH = 9.52, conductivity = 863.6 µS/cm, and dissolved oxygen = 8.26 mg/L. Species found in the sample with *Phreatodrobia embossa* included Turbellaria and freshwater Annelida. Other molluscs included Physidae, *Cochliopina riograndensis* and *Ferrissia* sp. Other hyporheic samples taken at the same site included ostracods, *Gammarus seidelii* Cannizzaro, Walters, Berg, 2017, *Hyaella* sp., *Seborgia hershleri* Holsinger, 1992, and *Lirceolus* sp. We did not encounter *Phreatodrobia embossa* from numerous drift net samples which were taken at the springs.

Discussion

Our understanding of the phreatic snail fauna of the ETAS is steadily growing, especially with renewed investigation since 2020. However, the fauna has historically been unevenly examined, with sampling and effort biases, different management priorities, and a greater abundance of sampling sites all contributing to more research in central Texas karst systems relative to other parts of the ETAS. Additionally, sampling that has traditionally relied on drift nets has resulted in an ecological bias toward a subset of habitat types (i.e., springs and flowing artesian wells). In this study, we contribute to a greater understanding of the diversity across a much larger region by building on surveys in the western portion of the ETAS in Texas. We used examination of DNA, shell, and anatomical features to build this understanding and support our taxonomic proposals. Importantly, this work takes advantage of collections from both karst aquifer and hyporheic zone sites, but our knowledge of the western portion, where greater aridity and geologic dissection has resulted in smaller, more fragmented groundwater-

dependent habitats, still lags behind that of the eastern portion. The Mexican portion of the ETAS is known even less well, though recent work continues to shed light on that important region (Czaja et al. 2022). This work has shown that regional groundwater snail diversity does not stop at the boundaries of the ETAS: adjacent regions, particularly other sites with karstic groundwater habitats, may also contain important elements of the fauna, as evidenced by the discovery of *S. gracilis*.

There is still much to be done in all portions of the ETAS and surrounding karst formations, but bridging the knowledge gap for the groundwater-obligate snail fauna of the region will require integrating our understanding across international, geologic, and ecologic boundaries. Currently, high species richness and diversity hotspots of Cochliopidae (e.g., Salado Springs (n = 5), Comal Springs (n = 9), San Marcos Springs (n = 6), San Marcos Artesian Well (n = 4), and Barton Springs (n = 4)) appear to occur near the freshwater-saline water interface of the eastern Edwards Aquifer (Balcones Fault Zone). However, analogous Edwards Aquifer Springs in Coahuila are largely uninvestigated. Furthermore, taxa such as *Phreatodrobia embossa* illustrate that divergent lineages may occur far from centers of biodiversity. Leveraging the large environmental, geological, and biodiversity gradients across the Mexican and US ETAS and adjacent regions affords great opportunity for exploring the evolutionary history of repeated invasion and subterranean adaptation in cochliopid snails. Specifically, our understanding is currently limited by a lack of information on anatomy and DNA of the Mexican fauna as well as DNA markers that will allow resolution of the relationships among genera and subfamilies in Cochliopidae.

While we are building a better understanding of the fauna of the Western ETAS, we have attempted to approach taxonomic revisions conservatively. For example, *Texapyrgus diaboli* populations in the Devils River compared to San Felipe Springs are relatively deeply divergent (9.8%), at a level usually associated with greater than species-level distinction. However, they are relatively similar morphologically, and we do not have specimens from the Sycamore Creek and other nearby drainages where this (or related) species also likely occur. At this time, it seems reasonable to point out that there might be additional diversity in that group, but more exploration is needed. Based on our best available evidence, we have additionally placed two species in genera where they are not a perfect fit, rather than describing new monotypic genera. In the future, *Phreatodrobia embossa* and *Balconorbis coronae* may need to be placed into new (or different) genera, but it seemed like unnecessary taxonomic exuberance to do so with the data we have available.

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Supplementary material I

Photo-vouchers of individuals sampled for DNA S1

Authors: Kathryn E. Perez, Vanessa Saenz, Yamileth Guerrero, Lisa Gonzalez, Evan Guerrero, Pete Diaz, Benjamin T. Hutchins, Benjamin F. Schwartz

Data type: tif

Explanation note: Photo vouchers are supplied here and paravouchers deposited in museum collections. All localities are in Texas. Locality information in Supplemental Table 1. *Balconorbis uvaldensis* **A** PP844665 **B** PP860061 **C** PP860062. *Phreatodrobia embossa* **D** PP844685 **E** PP844686. *Stygopyrgus gracilis* **F** PP844672, PP860063 **G** PP844673, PP860064 **H** PP844674, PP860065 **I** PP844675, PP860066. *Stygopyrgus variabilis* **J** PP844669 **K** PP844683, PP860076 **L** PP844684, PP860077 **M** PP844687, PP860079 **N** PP844688, PP860080 **O** PP844689, PP860081 **P** PP844690, PP860082 **Q** PP844691 **R** PP844692. *Texapyrgus diaboli* **S** PP844662 **T** PP844663. Scale bar = 500 μ m.

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Link: <https://doi.org/10.3897/subtbiol.50.138174.suppl1>

Supplementary material 2

Photo-vouchers of individuals sampled for DNA S2

Authors: Kathryn E. Perez, Vanessa Saenz, Yamileth Guerrero, Lisa Gonzalez, Evan Guerrero, Pete Diaz, Benjamin T. Hutchins, Benjamin F. Schwartz

Data type: tif

Explanation note: Photo vouchers are supplied here and paravouchers deposited in museum collections. All localities are in Texas. Locality information in Supplemental Table 1. *Texapyrgus diaboli* **A** PP844664, PP860055 **B** PP844666, PP860056 **C** PP860057 **D** PP860067 **E** PP860068 **F** PP860069 **G** PP860070 **H** PP844670 **I** PP844671 **J** PP844676 **K** PP844677 **L** PP844678 **M** PP844679 **N** PP860047. *Phreatodrobia coronae* **O** PP844681, PP860073 **P** PP844682 **Q** PP860075. *Phreatodrobia micra* **R** PP860054. *Phreatodrobia plana* **S** PP860071 **T** PP860072. Scale bar = 500 μm .

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Link: <https://doi.org/10.3897/subtbiol.50.138174.suppl2>

Supplementary material 3

Photo-vouchers of individuals sampled for DNA S3

Authors: Kathryn E. Perez, Vanessa Saenz, Yamileth Guerrero, Lisa Gonzalez, Evan Guerrero, Pete Diaz, Benjamin T. Hutchins, Benjamin F. Schwartz

Data type: tif

Explanation note: Photo vouchers are supplied here and paravouchers deposited in museum collections. All localities are in Texas. Locality information in Supplemental Table 1. *Phreatodrobia punctata* **A** PP860060 **B** PP844667 **C** PP844668. *Phreatodrobia rotunda* **D** PP860058 **E** PP860059 **F** 4560 **G** PP860074 **H** PP860078 **I** PP860083. Scale bar = 500 μm .

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Link: <https://doi.org/10.3897/subtbiol.50.138174.suppl3>

Supplementary material 4

List of specimens used in our study, including their sampling localities and voucher information

Authors: Kathryn E. Perez, Vanessa Saenz, Yamileth Guerrero, Lisa Gonzalez, Evan Guerrero, Pete Diaz, Benjamin T. Hutchins, Benjamin F. Schwartz

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.

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