

Morphometrics and phylogeography of the cave-obligate land snail *Helicodiscus barri* (Gastropoda, Stylommatophora, Helicodiscidae)

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Abstract

Molecular studies have recently led to the detection of many cryptic species complexes within morphologically ambiguous species formerly undescribed by the scientific community. Organisms such as land snails are at a particularly higher risk of species misidentification and misinterpretation, in that gastropod systematics are based almost entirely on external shell morphology. Subterranean ecosystems are associated with especially high degrees of cryptic speciation, largely owing to the abiotic similarities of these systems. In this study, we attempt to diagnose the potential cryptic diversity in the troglobitic land snail *Helicodiscus barri*. Land snails are generally associated with having low vagility, and as such this species' broad, mosaic distribution indicates the misdiagnosis of this organism as a single species. We analyze both mitochondrial (16S, CO1) and nuclear (28S, H3) genetic data for 23 populations. Phylogeny for *H. barri* was reconstructed using both maximum-likelihood and Bayesian approaches to assess relationships among populations, and two species delimitation methods (mPTP and ABGD) were used to detect the presence of unique molecular operational taxonomic units (MOTUs). Species delimitation results revealed seven and sixteen MOTUs respectively, suggesting the presence of several cryptic lineages within *H. barri*. To assess how external shell morphology corresponds with patterns of genetic and environmental variation, two morphometric approaches were used incorporating 115 shells from 31 populations. Both morphometric approaches reveal a significant environmental influence on shell morphology, and one approach showed the significance of MOTU groups. We discuss the delimitation and morphometric results and additionally provide discussion on the taxonomic and conservation implications of this study.

Keywords

Helicodiscidae, subterranean ecology, morphometrics, MOTUs, cryptic species

Introduction

Caves provide a model system for studying the evolutionary processes and historical factors related to biogeography and speciation (Juan et al. 2010). Cave systems, characterized by geographic isolation and relatively simple biological communities, often are viewed as analogous to oceanic islands (Culver and Pipan 2009, Snowman et al. 2010). Strong selective pressures and the isolation of subterranean ecosystems can result in morphological stasis among otherwise genetically distinct species, largely due to the parallel or convergent evolution of these lineages (Lefébure et al. 2006, Finston et al. 2007, Niemiller et al. 2012). Further, many troglobites (i.e., terrestrial cave-obligates) exhibit broad, mosaic distribution patterns which, in conjunction with morphological stasis, often confound traditional approaches of delimitating species boundaries (Jochum et al. 2015). Consequently, troglobites are ideal models to address fundamental questions in ecology and evolution and provide a platform to approach a more modernized integration of taxonomic methods.

An increasing number of studies has examined population genetic and phylogeographic hypotheses of subterranean fauna (e.g., Moulds et al. 2007, Snowman et al. 2010, Weckstein et al. 2016), which have greatly increased our understanding of colonization history, speciation, dispersal, and biogeography of troglobitic taxa (Juan et al. 2010). Additional phylogeographic studies have uncovered considerable levels of cryptic diversity in subterranean species (Finston et al. 2007, Juan and Emerson 2010, Niemiller et al. 2012). Due to increasing advances in imaging technology, studies that incorporate morphometric analyses often complement such molecular findings (Jochum et al. 2015, Armbruster et al. 2016, Burrell et al. 2017, Inäbnit et al. 2019). The misidentification of species can hinder assessments of biodiversity and conservation of cryptic species. Therefore, an integrative taxonomic evaluation of troglobitic taxa is needed to fully assess species richness within these systems, and to better inform their respective evolutionary histories. Moreover, cryptic species complexes may be comprised of groups already at significant risk of extinction (Niemiller et al. 2013).

Land snails (Phylum Mollusca, Class Gastropoda) are a species-rich group, with over 24,000 currently recognized species and over 35,000 species thought to exist globally (Barker 2001, Lydeard et al. 2004). The land snail fauna of eastern North America is exceptionally diverse, with over 500 documented species (Hubricht 1985, Nekola 2014). However, this likely represents an underestimate of total species richness in this region. Larger species are often associated with mesic forest ecosystems with high levels of moisture, leaf litter, and calcium (Goodfriend 1986, Pearce and Örstan 2006). Yet, land snails utilize a variety of microhabitats often neglected in sampling efforts within these areas (Cameron and Pokryszko 2005). Further, land snails occur at high density in karst-rich landscapes, and subterranean habitats are particularly under-sampled within the region (Clements et al. 2008, Niemiller and Zigler 2013).

Nearly 75% of all land snails in eastern North America are considered terrestrial micromolluscs (< 5 mm) and comprise a significant portion of all land snail diversity (Nekola 2005, Liew et al. 2008). Many of these species tend to be particularly under-sampled and often require the collection of soil and leaf litter samples to discover them (Liew et al. 2008, Nekola and Coles 2010, Durkan et al. 2013). Regions hypothesized to have higher levels of snail biodiversity have had varying and potentially insufficient sampling effort, with many species remaining undescribed (Dourson 2007, Douglas et al. 2014, Dinkins and Dinkins 2018). Moreover, there is a paucity of studies examining intraspecific morphological variation in micromolluscs, obscuring accurate geographic ranges for these species (Nekola and Coles 2010). Thus, because most land snail species are delimited based on conchology (i.e., shell variation), a high incidence of misidentification of minute species occurs in many natural history collections (Hubricht 1985, Nekola and Coles 2010). The continued misidentification of species can have significant impacts on biodiversity assessments and conservation management (Bickford et al. 2007).

Strictly employing morphological data to delimit extant species in the genomic era is often met with criticism (Hermsen and Hendricks 2008, Duminil and Di Michele 2009, Carstens et al. 2013). An integrative taxonomy, i.e., a combination of morphological, ecological, and genetic data when considering phylogenetic relationships, is necessary to facilitate proper interpretations of biological patterns (Dayrat 2005, Weigand et al. 2012, Inäbnit et al. 2019). For gastropods, there are few discrete shell characters that can be used in phylogenetic hypotheses, and conchology is highly variable in response to environmental factors and other selective pressures (Goodfriend 1986, Smith and Hendricks 2013). However, morphometric analyses can contribute to species hypotheses when combined with genetic data (Hermsen and Hendricks 2008, Miller 2016, Inäbnit et al. 2019). Moreover, applying morphometric analyses can inform the causal mechanisms for shape variation between gastropod populations (Vergara et al. 2017).

Terrestrial micromolluscs of the genus *Helicodiscus* Morse, 1864 are found throughout the eastern United States (Hubricht 1985). This genus is known for its unique conchological sculpture, often exhibiting depigmented soft bodies and prominent spiraling striae on the shells of both surface and subterranean species. Many of these species are calciphiles, and two species – *H. barri* Hubricht, 1962 and *H. notius specus* Hubricht, 1962 – have even adopted a cave-obligate existence (Hubricht 1962). The distributions of these troglobites span both the Interior Low Plateau (ILP) and the Appalachians karst regions, covering multiple physiographic provinces within their ranges. The latter species is only known from six caves in Kentucky, Tennessee, and Virginia, whereas the former is known from 49 caves in Tennessee, Alabama, and Georgia. Two additional *Helicodiscus* species that were previously thought to be troglobitic – *H. hadenoecus* Hubricht, 1962 and *H. punctatellus* Morrison, 1942 – have been discovered at surface localities widely disjunct from their otherwise subterranean distribution (Coney et al. 1982; Hotopp et al. 2013). These distribution patterns suggest the potential for cryptic diversity among subterranean taxa within this genus. Morphological stasis is highly prevalent in troglo-

bites despite significant genetic divergence, and, therefore, the mosaic distributions of these snails warrant investigation (Juan and Emerson 2010, Weigand et al. 2012).

Here, we conduct the first study examining morphological variation and phylogeography of the cave-obligate land snail *Helicodiscus barri*. Recent cave bioinventory efforts within the ILP and Appalachians karst regions have yielded several additional specimens of this species for comparison across multiple physiographic provinces. The disjunct, mosaic distribution pattern of *H. barri* in conjunction with a lack of clear morphological variation is consistent with a high potential for cryptic diversity, as observed in other subterranean taxa (Snowman et al. 2010, Loria et al. 2011, Niemiller et al. 2012, Inäbnit et al. 2019). We examined museum accessions of *H. barri* while sampling caves within the states of Tennessee, Alabama, and Georgia for additional specimens. Using phylogeographic approaches, we (1) assessed patterns of genetic variation of *H. barri*; (2) employed two species delimitation methods (ABGD and mPTP) to infer the presence of cryptic lineages; and (3) tested if current species hypotheses based on conchology correspond with patterns of genetic variation. Further, we assessed morphological variation between *H. barri* populations using traditional morphometrics (TM) and landmark-based geometric morphometrics (GM).

Methods

Specimen collection

Shell specimens were collected from 31 populations of cave-dwelling *Helicodiscus barri* from the dark zone of caves within both the ILP and Appalachian karst regions in Tennessee and Alabama (115 total individual specimens collected). Each survey typically involved two to four researchers (maximum 12), with a search effort of two to 36 person-hours per cave visit. In total, 74 caves were visited from 13 March 2013 to 19 June 2018 by NSG, totaling ca. 300 person-hours. Snail specimens were preserved in 100% ethanol and identified using published keys and species descriptions (Pilsbry 1948, Hubricht 1962, Dourson 2010), as well as examination by taxonomic specialists (Dan and Judy Dourson). Specimens from ten additional populations were provided by the Field Museum of Natural History (FMNH), Florida Museum of Natural History (FLMNH), and the Auburn Museum of Natural History (AUM). In total, 154 shells were examined (see Table 1). The geographic distribution of populations utilized within this study can be found in Figure 1.

DNA extraction, amplification, and sequencing

Genomic DNA was obtained from soft tissue of each live specimen collected. The shells of smaller individuals were removed prior to DNA extraction. Tissue was removed from larger shells by breaking a small opening into the abapertural side of the

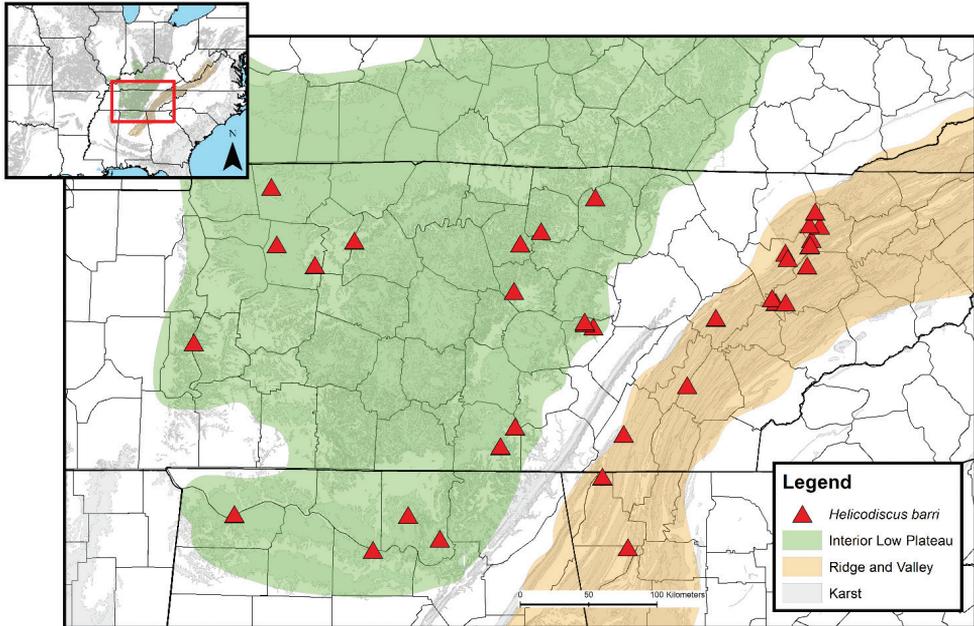


Figure 1. Geographic distribution of *Helicodiscus barri* from this study in relation to karst adapted from Weary and Doctor (2014). Triangles represent cave populations.

shell or the shell base, so that the shell was not completely destroyed and remained identifiable. Each DNA extraction was performed using the Qiagen® DNeasy Blood and Tissue kit following the manufacturer's protocol (Qiagen Sciences, Louisville, KY). Polymerase chain reaction (PCR) was used to amplify fragments of the mitochondrial (mt) 16S ribosomal RNA locus using the primer pair 16Sa/16Sb (Palumbi et al. 1991), mt cytochrome oxidase subunit 1 (COI) locus using the primer pair LCOI490/ HCO2198 (Folmer et al. 1994), nuclear 28S ribosomal RNA locus using the primer pair 28Sna1/28Sna2 (Kano et al. 2002), and nuclear histone 3 (H3) locus using the primer pair H3F/H3R (Colgan et al. 2000). PCR products were purified using ExoSAP-IT (Affymetrix) and sequenced in both directions with BigDye chemistry at Eurofins MWG Operon (Louisville, KY).

Genetic analyses

Forward and reverse sequences were assembled into contigs and edited in Sequencher v.5.1 (Gene Codes Corporation, Ann Arbor, MI). Alignments were modified by the manual trimming of the 3' and 5' primer ends. Ambiguous base calls and double peaks within heterozygotes were assessed visually with the chromatograms. Sequences were then aligned using MUSCLE under default parameters implemented in MEGA X v.10.0.5 (Kumar et al. 2018). All sequence data generated from this study was accessioned into

Table 1. *Helicodiscus barri* populations incorporated in this study, including 17 new populations. Cave names, Tennessee Cave Survey (TCS) cave number, county and state are provided, as well as information regarding which populations were considered in morphometric and genetic analyses.

Sample	Cave	TCS No.	County	State	References	n	Morphology	Genetic
NSG-DI3	Bowman Cave	TDI3	Dickson	TN	This study	2	X	X
NSG-KN112	Brents Cave	TKN112	Knox	TN	This study	3	X	X
AUM28348	Bull Run Cave	TDA4	Davidson	TN	Hubricht 1964	2	X	X
MLN 14-054.12; NSG-JK3	Carter Cave	TJK3	Jackson	TN	Gladstone et al. 2018	5	X	X
NSG-VB547	Cave Between the Caves	TVB547	Van Buren	TN	Lewis 2005	6	X	
NSG-RN5	Cave Creek Cave	TRN5	Roane	TN	This study	1		X
MLN 14-007	Christmas Cave	TDK72	DeKalb	TN	Gladstone et al. 2018	1	X	X
MLN 13-056	Clarksville Lake Cave	TMY11	Montgomery	TN	Gladstone et al. 2018	2	X	X
FMNH239117	Collier Cave	ALD100	Lauderdale	AL	Peck 1989	4	X	
FMNH239122; NSG-DI6	Columbia Caverns	TDI6	Dickson	TN	Hubricht 1962	7	X	X
NSG-KN50	Conner Creek Cave	TKN50	Knox	TN	This study	5	X	X
FMNH239121	Culbertson Cave	TUN22	Union	TN	Hubricht 1985	1	X	
NSG-AN5	Demarcus Cave	TAN5	Anderson	TN	This study	3		X
MLN 14-015.3	Dry Cave	TFR9	Franklin	TN	Gladstone et al. 2018	2		X
AUM27534-T2	Frazier Hollow Cave	DK11	DeKalb	TN	This study	1	X	X
NSG-DI27	East Fork Cave	TDI27	Dickson	TN	This study	2	X	
AUM28173	Hering Cave		Madison	AL	This study	1	X	X
NSG-FR14	Keith Cave	TFR14	Franklin	TN	Lewis 2005	10	X	X
UF 405128	Lady Finger Bluff Trail		Perry	TN	Gladstone et al. 2018	1	X	
WC13-165	Lovelady Cave	THM56	Hamilton	TN	This study	1		
NSG-MM10	McCorkle Cave	TMM10	McMinn	TN	This study	1		
NSG-VB9	McCoy Cave	TVB9	Van Buren	TN	This study	2	X	X
AUM27855	New Salem Cave Nr1	TSM10	Smith	TN	This study	2	X	X
MLN 15-007.9	Oaks Cave	TUN5	Union	TN	Gladstone et al. 2018	1	X	X
FMNH305126; NSG-AN12	Offut Cave	TAN12	Anderson	TN	Hubricht 1985	8	X	X
MLN 15-006.19; NSG-CM8	Panther Cave No. 1	TCM8	Campbell	TN	Gladstone et al. 2018	7	X	X
FMNH239120	Parkers Cave	GKH119	Chattooga	GA	Holsinger and Peck (1971)	2	X	
NSG-KN108	Pedigo Cave Nr. 2	TKN108	Knox	TN	This study	1		X

Sample	Cave	TCS No.	County	State	References	n	Morphology	Genetic
NSG-AN6	Robert Smith Cave	TAN6	Anderson	TN	This study	2		X
MLN 13-000	Rockhouse Cave	ALM312	Limestone	AL	Gladstone et al. 2018	1	X	
AUM27652	Rogers Hollow Cave	TUN23	Union	TN	This study	2	X	
FMNH239118	Shelta Cave	AMD4	Madison	AL	Peck 1989	3	X	
NSG-OV440; GC1	Slippery Slit Cave	TOV440	Overton	TN	Lewis 2005	3	X	X
KSZ15-313	Smartt Farm Cave	GWK124	Walker	GA	This study	1		
NSG-VB657	Swamp River Cave	TVB657	Van Buren	TN	This study	1	X	
MLN-16.0228	Weavers Cave	TAN22	Anderson	TN	Gladstone et al. 2018	2	X	X
NSG-KN80	Wilke Waller Cave	TKN80	Knox	TN	This study	1		

GenBank (see Suppl. material 1). PartitionFinder v.2.1.1 (Lanfear et al. 2012) was used to determine the best model of sequence evolution for each partition based on the Bayesian information criterion (BIC). A general time-reversible model of sequence evolution with corrections for a discrete gamma distribution and a proportion of invariant sites (GTR+ Γ +I) was chosen for 16S. The Hasegawa et al. (1985) model (HKY) with corrections for a discrete gamma distribution was chosen for the first and second codon positions of both CO1 and H3 as well as for 28S. A symmetrical model with corrections for a discrete gamma distribution (SYM+ Γ) was chosen for the third codon position of CO1 and H3 (Zharkikh 1994). Due to uneven coverage of genetic data across specimens, three unique *H. barri* datasets were assessed: CO1, *mtDNA* (CO1 + 16S), and *mtDNA* + *nDNA* (CO1 + 16S + 28S + H3). *Discus rotundatus* was used as an outgroup for all phylogenetic analyses. Summary statistics of the *H. barri* molecular dataset including haplotype and nucleotide diversity, number of segregating sites, haplotypes, and mutations were calculated in DnaSP v.6.12.01 (Librado and Rozas 2009). Uncorrected p-distances within and between cave populations were used as a metric of genetic divergence and calculated in MEGA X v.10.0.5 (Kumar et al. 2018). A haplotype network for all specimens for which genetic data were available was created in SplitsTree v.4.14.8 (Huson and Bryant 2005) using the NeighborNet network method with uncorrected p-distances.

Phylogenetic analyses and species delimitation

Phylogenetic trees were inferred utilizing both a Maximum-Likelihood (ML) and Bayesian-inference (BI) approach. ML analyses were conducted using RAxML v.8.0 (Stamatakis 2014) as implemented through the T-REX web server (Boc et al. 2012). A consensus tree was generated from the CO1, *mtDNA*, and *mtDNA* + *nDNA* datasets using rapid bootstraps for 100,000 replicates under a GTR+ Γ +I model of evolution.

The BI analyses were conducted in MrBayes v.3.2.6 (Ronquist et al. 2012) using a random start tree with three heated and one cold chain (default temperature of 0.1). This was run twice for 50,000,000 generations and sampled every 1,000 generations under the models of evolution determined by PartitionFinder. The first 25% of samples (12,500,000) were discarded as burn-in. Convergence of runs was assessed utilizing Tracer v. 1.4 (Rambaut and Drummond 2007).

The generation of molecular barcodes is often utilized in species delimitation in understudied groups (or in this case, those that are morphologically ambiguous; Pons et al. 2006; Rubinoff 2006; Weigand et al. 2012, 2014). As such, two species delimitation approaches were subsequently used in the identification of Molecular Operational Taxonomic Units (MOTUs; Floyd et al. 2002): 1) Automatic Barcode Gap Recovery (ABGD; Puillandre et al. 2012), and 2) Multi-rate Poisson Tree Processes (mPTP; Kapli et al. 2017). ABGD partitions samples into candidate species based on a statistically inferred barcode gap. The barcoding gap is defined as a notable disparity between pairwise genetic distances, presumably between intraspecific and interspecific distances. This process is applied recursively to newly obtained groupings of sequences, to assess the possibility of internal division. This method was employed on the CO1 dataset excluding the outgroup ($n = 24$) via the ABGD web server (<http://www.wabi.snv.jusieu.fr/public/abgd/abgdweb.html>) using the Kimura two-parameter (K2P; Kimura 1980) model with a standard X (relative gap width) = 1.5.

The initial development of PTP models assumed one exponential distribution for speciation events and one for all coalescent events (Zhang et al. 2013). In contrast, the mPTP approach fits speciation events for each candidate species to a unique exponential distribution, greatly improving the quality of results (Kapli et al. 2017). This method requires a rooted phylogenetic tree and partitions samples into candidate species based upon the number of substitutions under assumed Poisson processes. Intraspecific substitution rates should be notably smaller than interspecific rates. This method does not require an ultrametric tree, which is ideal given little reliable fossil data for Helicodiscidae and the variability of molecular clock rates in Stylommatophoran gastropods (Thomaz et al. 1996, Chiba 1999, Van Riel et al. 2005). A rooted tree was generated for the CO1 dataset using the methods previously outlined for RAxML under the models of evolution determined by PartitionFinder. Analysis was carried out on the mPTP webserver (<http://mptp.h-its.org>) for the maximum 100,000 MCMC generations, with 25% of samples (25,000) conservatively discarded as burn-in.

Morphometric analyses

Specimens were photographed using a Canon 6D digital SLR camera mounted on the Macropod PRO Micro Kit (Macroscopic Solutions, Tolland, CT). Each shell was photographed using a Canon MP-E 65mm f/2.8 1–5× macro lens in three views: ventral, dorsal and apertural. MacroMagnification settings were extracted from the images using ExifTool v.5.16.0.0. Images were imported to Adobe Photoshop CS5 Extended

v.12.1 and were subsequently scaled. Reproductive anatomy was not evaluated, given that most specimens were taken from museum collections with no soft tissue available. Two morphometrics methods were employed: geometric morphometrics (GM) and traditional morphometrics (TM).

GM techniques allow for the quantification and assessment of morphological variation. Biologically-meaningful landmarks (LMs) and semilandmarks (SLMs) of the *Helicodiscus* shells were digitized using tpsDig2 v. 2.32 (Rohlf 2015, available at <http://life.bio.sunysb.edu/morph/>). Nine homologous static LM were placed across each specimen. LM 1 is Type 1, characterizing the discrete juxtaposition of the homologous shell structure. LM 4 and LM 5 are Type 2, characterizing geometric maxima of curvature. All remaining LMs were Type 3, characterizing more than one region of each shell (Bookstein 1997). These nine LMs were combined with two manually traced curves of three equidistant SLMs anchored on LM 4–5 and LM 5–6 (see Figure 2A). Appending tps curves to SLM was achieved using tpsUtil v. 1.76 (Rohlf 2015). This results in a total of nine fixed and six semi-landmarks.

To eliminate variation due to orientation of the shell or size, a Procrustes superimposition was performed using the *geomorph* package v.2.0. in RStudio v 1.1.456 with R v. 3.5 (Adams and Otárola-Castillo 2013, Adams et al. 2014, RC Team 2014). These data were subjected to principal component analysis (PCA) to evaluate the distribution of populations in morphospace. Several alternative landmark schemes were tested but provided no notable differences in PCA results. The most conservative approach was employed to reduce the number of variables introduced into the downstream analyses. Correlation coefficients (PC loadings) of individual variables were assessed visually to determine which specific variables are significant to each PC as to interpret what shell characteristics account for variability of the dataset. To quantify error associated with landmark placement and shell placement during photography, a set of replicate images with digitized landmarks was used to calculate the disparity using the *morphol.disparity* function in *geomorph* (Adams et al. 2013, 2014).

Specimens were grouped by MOTUs to identify detectable differences in shell variation in concordance with genetic variation. Reduced datasets of those specimens with obtained genetic data were used for these groups. However, in the absence of genetic data, all specimens for which data are available were grouped by the physiographic province associated with the collection locality (i.e., Cumberland Plateau, Eastern Highland Rim, Valley and Ridge, and Western Highland Rim). These regions possess unique environmental characteristics (e.g., soil physiochemistry, rock type, vegetation; Fenneman 1917) and were utilized as broad-scale categories to test for the effect of environmental variation on conchology. Before assessing the significance of these groups in explaining morphological variation, the first and second PCs were subjected to a test of spatial autocorrelation (SAC) to prevent the increase of Type 1 errors introduced to the analyses (Perez et al. 2010). SAC was determined using the Moran's I statistic (Sokal and Oden 1978) and was found to be non-significant (PC 1=0.2184, PC 2=0.0832). These groups were subjected to Procrustes Analysis of Variance (ANOVA) following a randomized residual permutation procedure (RRPP) for 10,000 iterations.

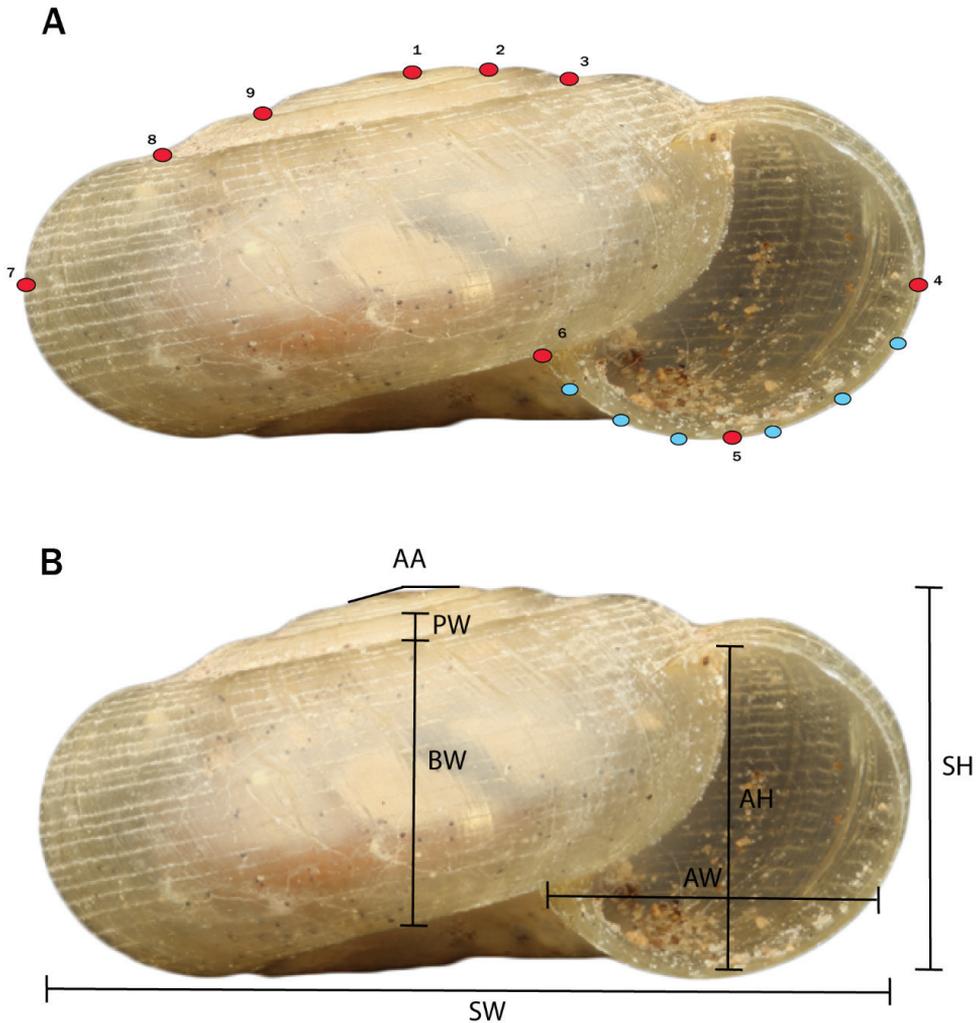


Figure 2. **A** Landmark scheme for geomorphometric analyses. Red circles represented landmarks (LM), blue circles represent semi-landmarks (SLM). **B** Shell measurements utilized for the traditional morphometric (TM) analyses.

Seven unique shell measurements from Burch (1962) were utilized in the TM approach (see Figure 2B): Shell width (SW), shell height (SH), aperture width (AW), aperture height (AH), body whorl height (BW), penultimate whorl height (PW), and angle of apex (AA). These shell characteristics are often utilized in morphometric analyses and are readily utilized in land snail species identification guides (Pearce and Örsan 2006, Dourson 2010). Scaled data were converted to a Euclidean distance matrix and subject to Permutational Multivariate Analysis of Variance (PERMANOVA). This test was performed in the *vegan* package in R for 10,000 permutations (Oksanen 2018). *P*-values extracted from pairwise comparisons were corrected using a Bonferroni test.

Results

Genetic analyses

Molecular sequence data were obtained from 32 specimens of 23 populations. Tissue samples were scarce, and the saturation of the land snail soft body with mucopolysaccharides inhibited the success of standard extraction procedures and subsequent sequencing. Thus, we were unable to obtain full genetic coverage (i.e., all four target genes sequenced) for all specimens. Summary statistics generated for the genetic data is presented in Table 2. The *mtDNA* dataset used for the gene tree estimation was unambiguously aligned (1316 base pairs; bp). A concatenated alignment of all specimens in which all four genes were amplified was also unambiguously aligned and assessed ($n = 16$; 3040 bp). The CO1 dataset was also assessed independently, as it was later utilized for the downstream species delimitation approaches. The CO1 dataset was unambiguously aligned ($n=24$; 704 bp). No shared haplotypes were observed between cave populations at CO1 (see Table 3), even in cases where caves were less than 15 m apart from one another (e.g., Demarcus Cave (AN5) and Robert Smith Cave (AN6) in Anderson County, Tennessee). The generated haplotype network strongly resembles MOTU delimitation results (see Figure 3), with the two most diverse MOTUs identified possessing five haplotypes each. Mean uncorrected p-distances between cave populations at CO1 was 16.14% (range 2.6–23.2%), indicating significant geographic isolation. For the concatenated genetic dataset, mean uncorrected p-distances between cave populations was 6% (range 1.3–10.3%). Due to the rarity of this species, there were only four instances of obtaining sequences of more than one individual per cave (Columbia Caverns (DI6), Keith Cave (FR14), Offut Cave (AN12), Panther Cave No. 1 (CM8)). Of these, two populations exhibited two haplotypes at CO1. Intrapopulation variation of these four populations was low, with a mean CO1 uncorrected p-distance of $1.48 \pm 0.3\%$.

Phylogenetic analyses

Both ML and BI approaches resulted in highly similar tree topologies for each unique concatenated genetic dataset (CO1, *mtDNA*, *mtDNA* + *nDNA*). The outstanding difference between the ML and BI phylograms generated from the *mtDNA* dataset was a resolution of polytomy from the ML approach in the Bowman Cave (DI3), Carter Cave (JK3), Keith Cave (FR14), McCoy Cave (VB9), and Slippery Slit Cave (OV440) clade. The *mtDNA* + *nDNA* phylograms also differed with the Hering Cave (AMD6) population representing a monotypic group in the ML approach, and grouping with the Brent's Cave (KN112), Columbia Caverns (DI6), and Weavers Cave (AN22) clade as it does in all other phylograms assessed. Additionally, there were several notable distinctions in the CO1 phylograms produced between BI and ML approaches (see Suppl. material 2). Despite these differences, only the representative phylogenies utiliz-

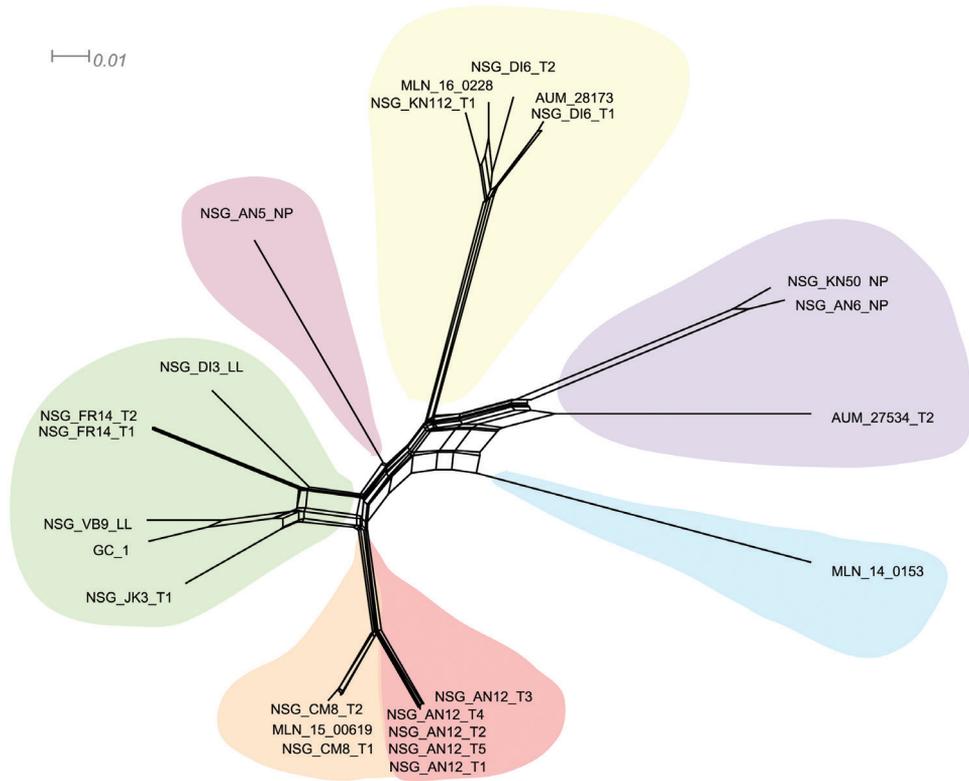


Figure 3. Haplotype network generated using the NeighborNet network method with uncorrected p-distances with the CO1 dataset. Species delimitation results are depicted using major color groups for the mPTP results, and subcolor groups for the ABGD results.

Table 2. Summary statistics generated for all four genes assessed (*mtDNA*: CO1, 16S; *nDNA*: 28S, H3).

	<i>n</i>	<i>bp</i>	<i>b</i>	<i>Hd</i>	<i>Π</i>	<i>S</i>	<i>Eta</i>
<i>mtDNA</i>							
CO1	24	668	18	0.957 ± 0.031	0.14346 ± 0.012	164	245
16S	28	605	20	0.968 ± 0.019	0.09019 ± 0.011	71	99
<i>nDNA</i>							
28S	29	1305	6	0.424 ± 0.111	0.00327 ± 0.001	20	20
H3	25	337	6	0.427 ± 0.122	0.00322 ± 0.001	7	7

n – number of sequences, *bp* – alignment size, *b* – number of haplotypes, *Hd* – haplotype diversity, *Π* – nucleotide diversity, *S* – number of polymorphic sites, *Eta* – number of mutations

ing the BI approach for the CO1, *mtDNA*, and *mtDNA* + *nDNA* datasets are shown (Figures 4, 5). All other trees are placed within Suppl. materials 2, 3.

Due to an inability to amplify all genes per specimen, some specimens are not represented in all phylogenies. However, among the representatives included in all three datasets, there is a consistent topology. The only differences between the *mtDNA* tree

Table 3. Species delimitation results from both ABGD and mPTP analyses. Haplotype diversity, specimen ID, state, karst region, and physiographic province also included.

mPTP	ABGD	Haplotype ID	Specimen ID	State	Karst	Physiographic
PG1	AG1	H1, H2	NSG-CM8-T1, NSG-CM8-T2, MLN-15-006.19	TN	APP	VR
PG2	AG2	H3	NSG-AN12-T1, NSG-AN12-T2, NSG-AN12-T3, NSG-AN12-T4, NSG-AN12-T5	TN	APP	VR
PG3	AG3, AG4, AG5, AG6, AG7	H4, H5, H6, H7, H8	NSG-JK3-T1, NSG-VB9-LL, GC1, NSG-DI3-LL, NSG-FR14-T1, NSG-FR14-T2	TN	ILP	CP, EHR, WHR
PG4	AG8	H9	NSG-AN5-NP	TN	APP	VR
PG5	AG9	H10	MLN-14-0153	TN	ILP	CP, EHR
PG6	AG10, AG11, AG12	H11, H12, H13	AUM27534-T2, NSG-KN50-NP, NSG-AN6-NP	TN	APP, ILP	VR, WHR
PG7	AG13, AG14, AG15, AG16	H14, H15, H16, H17, H18	MLN-16.0228, NSG-KN112-T1, NSG-DI6-T1, NSG-DI6-T2, AUM28173	AL, TN	APP, ILP	CP, VR, WHR

and the *mtDNA* + *nDNA* BI trees are 1.) the resolution of polytomy and varied topology in the Bowman Cave (DI3), Carter Cave (JK3), Keith Cave (FR14), McCoy Cave (VB9), Slippery Slit Cave (OV440) clade, and 2.) the relative placement of the Frazier Hollow Cave (DK11), Robert Smith Cave (AN6), and Conner's Creek Cave (KN50) clade. All other clades remain consistent. Bootstrap support for the ML approach were notably lower at deeper nodes in each phylogram, and the same occurred with posterior probabilities generated from the BI approach. Node posterior probabilities and confidence values increased overall after the addition of the *nDNA* data. Comparison of both *mtDNA* and *nDNA* phylograms show the existence of at least seven monophyletic clades across the Appalachians and ILP karst regions (Figure 5). The monotypic Dry Cave (FR9) and Demarcus Cave (AN5) samples seem to be considerably divergent from other groups. While the former is known from the southern extent of the Eastern Highland Rim, the latter monotypic clade is in immediate proximity to Robert Smith Cave (less than 15 m) yet both are significantly delineated in the CO1 phylogram and the subsequent delimitation approaches.

Species delimitation

The ABGD method generated two partition strategies. At prior intraspecific divergence (P) values between 0.0010 and 0.0215, sixteen MOTUs were recognized in initial and recursive partitions. Both partition schemes remained stable at these values until reaching congruency at $P = 0.0359$, grouping all populations together into a single MOTU. The barcode gap was discovered at 0.14–0.16 K2P distance. The PTP results generated seven MOTUs for both single and multi-coalescent rate models (see Suppl. material 4). Both delimitation approaches show highly similar MOTU designations, with most identified groups being known from individual caves (Figure 4).

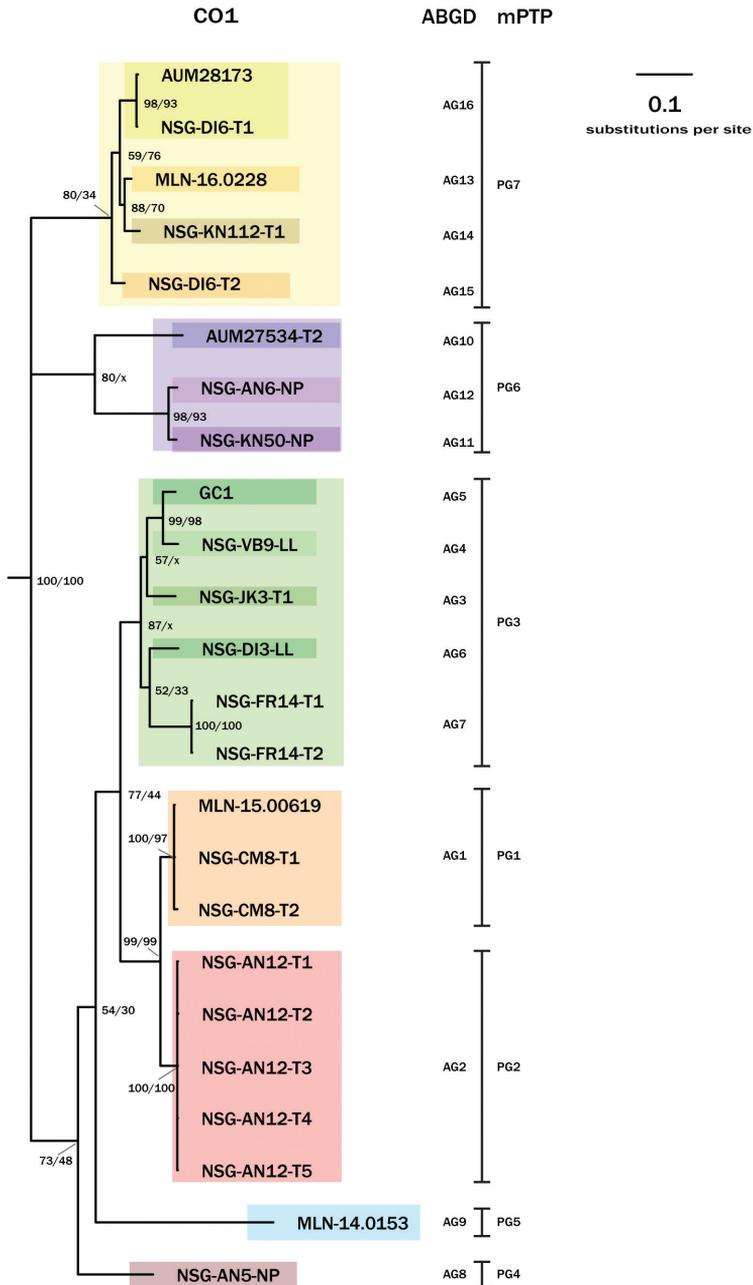


Figure 4. Phylogenetic tree of the CO1 dataset (808 bp) using the BI methodology. Posterior probabilities generated from the analysis are shown for each clade with the top numbers. Confidence values given from the bootstrapped ML method are shown for each clade with the bottom numbers. The 'x' symbols indicate varying topology between the BI and ML analyses. ML trees are reported in the Appendix for cross-reference. Species delimitation results are depicted using major color groups for the mPTP results, and subcolor groups for the ABGD results.

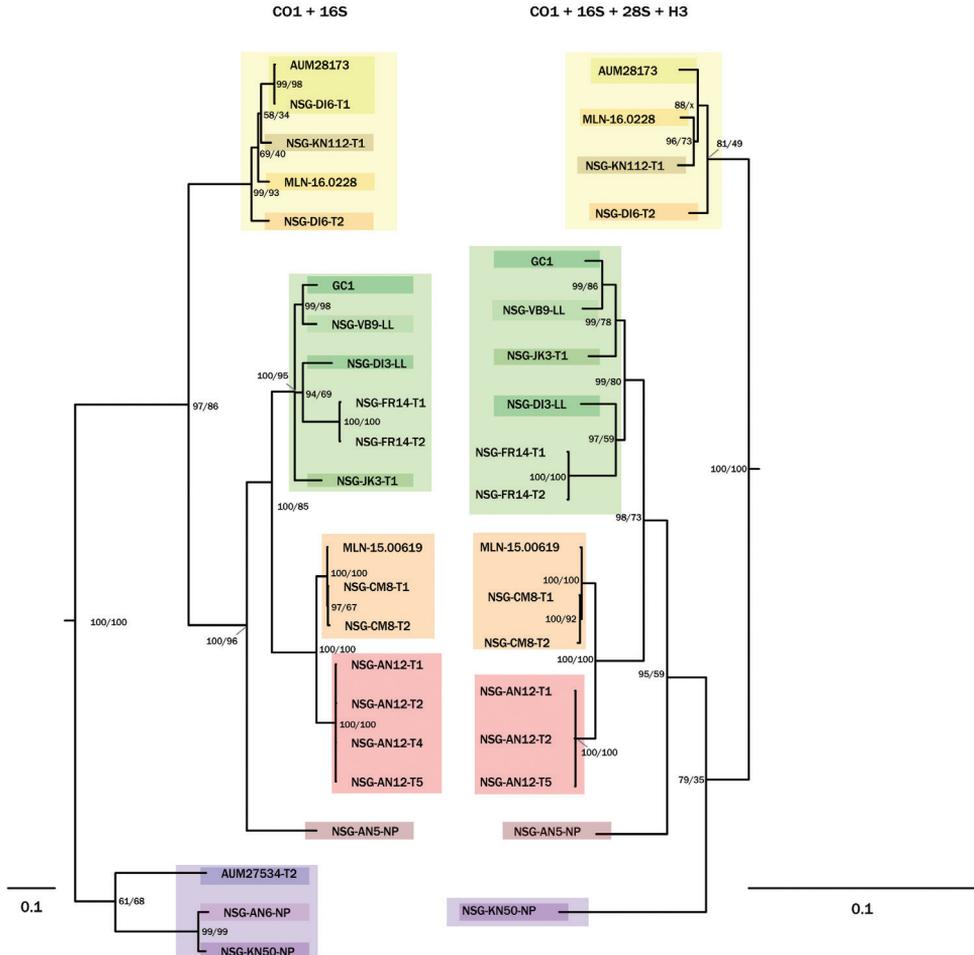


Figure 5. Phylogenetic trees of the concatenated *mtDNA* (CO1 + 16S; 1316 bp) and the full *mtDNA* + *nDNA* (CO1 + 16S + 28S + H3; 3040 bp) datasets. Posterior probabilities generated from the analyses are shown for each clade with the top numbers. Confidence values given from the bootstrapped ML method are shown for each clade with the bottom numbers. The 'x' symbols indicate varying topology between the BI and ML analyses. ML trees are reported in the Appendix for cross-reference. Species delimitation results are depicted using major color groups for the mPTP results, and subcolor groups for the ABGD results.

There were four cases of both delimitations methods producing the same results (PG1, PG2, PG4, PG5). Three groups of five, four, and three MOTUs generated by ABGD were consolidated into three MOTUs generated by mPTP (PG3, PG6, PG7), respectively. The consolidated PG3 MOTU group is largely clustered within the Eastern Highland Rim (AG3, AG4, AG5, AG7), with only one disjunct representative being found in a fragmented karst formation on the eastern extent of the Western Highland Rim (AG6). The PG6 and PG7 MOTU groups exhibit an irregular geographic struc-

ture, with both possessing representatives from both karst regions (Appalachians and ILP). Further, the ABGD results suggest two MOTU groups (AG15, AG16) within a single cave population at Columbia Caverns, with AG16 comprising this cave on the eastern extent of the Western Highland Rim and another in the southern extent of the Cumberland Plateau in the state of Alabama.

Morphometric analyses

In total, 65 specimens were incorporated into both the GM and TM datasets from 28 cave populations. The disparity test used to indicate possible error introduced from shell and landmark placement (2.28%) was negligible. PC plots for each grouping are displayed in Figure 6A–D. For the GM PCA, the first three principal components account for 69.52% of the total variance. PC 1 (31.24%) was interpreted as the curvature of the shell, with higher PC scores exhibiting a higher angle of apex and larger shell height. The X-coordinates of LM 3, LM 2, and LM 6 all had the highest PC loadings associated with PC 1 (0.418, 0.312, 0.243 respectively). PC 2 (25.18%) was interpreted as the size of the secondary body whorl in relation to the aperture, with higher PC scores exhibiting significantly wider secondary body whorls with an annular apertural structure. The Y-coordinate of LM 8 and the X-coordinates of LM 9 and LM 1 had the highest PC loadings associated with PC 2 (0.281, 0.189, 0.159 respectively). PC 3 (13.10%) was interpreted as the size of the aperture, with higher PC scores exhibiting larger apertures and higher shell width. The X-coordinates of LM 6 and SLM 15 and the Y-coordinate of LM 4 had the highest PC loadings associated with PC 3 (0.354, 0.333, 0.301 respectively). A smaller morphometric dataset ($n = 39$) was assessed for those individuals for which molecular data was available. Only the MOTU groups from the mPTP analysis were considered, as these were the larger groups. The first three principal components for this smaller dataset account for 74.90% of the total variance (PC 1 = 31.03%; PC 2 = 28.76%; PC 3 = 15.11%).

For the TM PCA, the first three principal components accounted for 79.36% of the total variance. PC 1 (66.03%) was interpreted as the overall size of the shell, with high PC scores exhibiting larger shell height and shell width (PC loadings = 0.4553, 0.4370 respectively). PC 2 (13.23%) was interpreted as the height of the shell, with higher PC scores exhibiting much larger penultimate whorls and shell height (PC loadings = 0.6336, 0.6011 respectively). PC 3 (9.85%) was interpreted as the curvature of the shell, with higher PC scores having higher angles of apex and smaller shell width (PC loadings = 0.6815). For the smaller mPTP dataset, the first three principal components accounted for 85.98% of the total variance. Procrustes ANOVA and PERMANOVA tested the influence of environmental variation (i.e., respective physiographic province) on external shell morphology, indicating significance for both morphometric approaches. MOTU groups did not significantly explain shell variation with the GM approach, but it was significant for the TM approach.

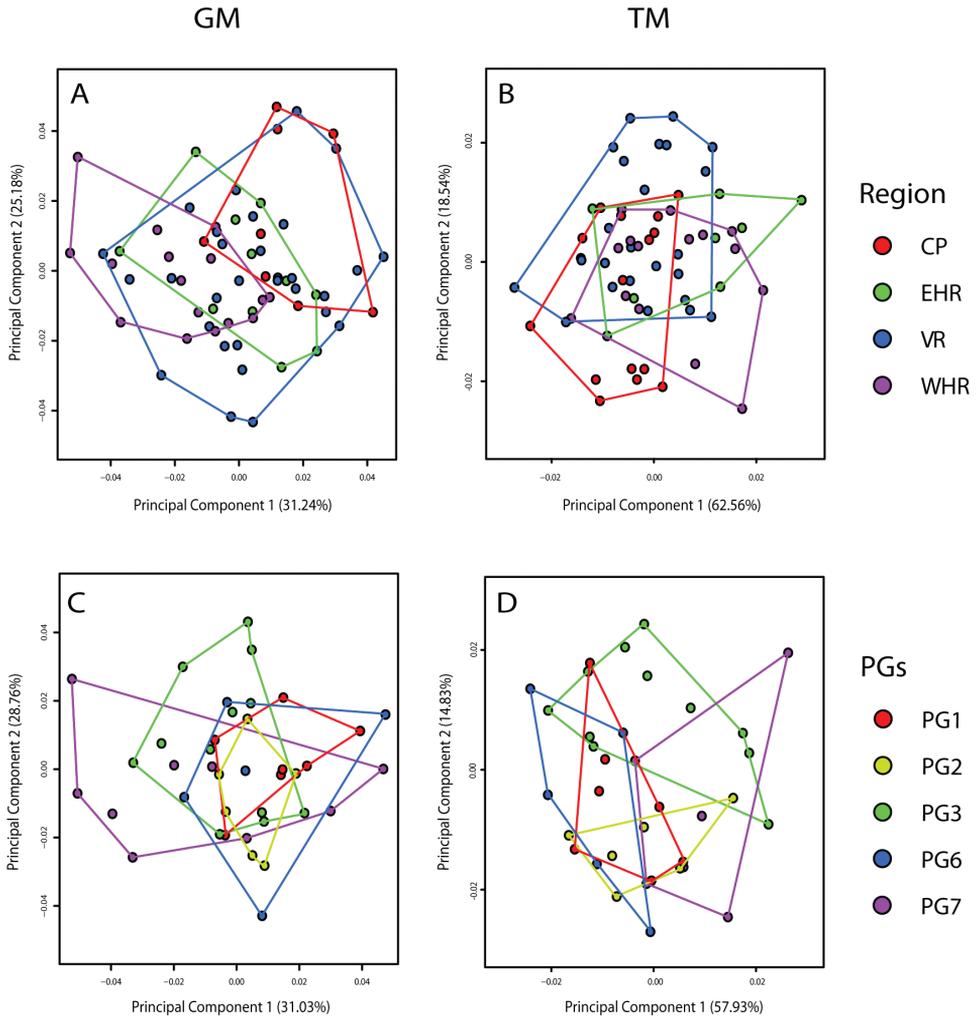


Figure 6. PCA results from both geometric morphometric (left) and traditional morphometric (right) analyses. **A, B** Total morphometric dataset (n=65) grouped by physiographic province. **C, D** Morphometric dataset with complimentary molecular data (n=39) grouped by MOTUs from the mPTP analysis.

Discussion

Many molecular studies of troglotic taxa have revealed previously unknown cryptic lineages in North America (Buhay and Crandall 2009, Snowman et al. 2010, Niemiller et al. 2012, Weckstein et al. 2016). Troglabites are hypothesized to have fewer opportunities for dispersal than obligately-subterranean aquatic species (i.e., stygobites), due to limited connectivity of terrestrial subterranean passages (Culver et al. 2009). This may promote isolation and short-range endemism in troglabites (Culver et al. 2009,

Niemiller and Zigler 2013). No phylogeographic study of troglobitic snails has been conducted in North America, and all other molecular studies of troglobites in the Appalachians and Interior Low Plateau have focused on organisms with comparatively higher vagility and dispersal potential (e.g., Buhay et al. 2007, Niemiller et al. 2008, Niemiller et al. 2012, Snowman et al. 2010, Loria et al. 2011). Using both a multilocus molecular and a morphometrics approach, we investigated genetic diversity within *H. barri* to identify potential cryptic populations within the species' range, and to further determine whether external shell morphology was a useful indicator of differing patterns of genetic variation.

Genetic diversity of *Helicodiscus barri*

Despite limited sampling success of this rare species, our study revealed high genetic diversity in *H. barri*. Haplotypic diversity is strongly dictated by individual caves, and there appears to be little to no dispersal between cave systems regardless of proximity. Mitochondrial genetic divergence among *H. barri* populations is significantly higher (16.14%) compared to other troglobitic invertebrate taxa studied in the region (e.g. 3.1% for *Nesticus* spiders (Snowman et al. 2010); 0.06% for *Tetracion* millipedes (Loria et al. 2011); 2.1% for *Ptomaphagus* beetles (Leray et al. 2019)), suggesting that the low vagility of land snails accentuates the isolation caused by subsurface habitat fragmentation. Rates of mitochondrial gene evolution for land snails vary considerably, with estimates of 1.6–12.9% per million years for ribosomal genes and 2.8–13% for CO1 (Thomaz et al. 1996, Chiba 1999, Van Riel et al. 2005). Further, land snails often exhibit high levels of intraspecific genetic divergence and population structure (Guiller et al. 1994, Davison et al. 2009, Perez et al. 2014). An estimated 1.6% divergence per million years has been a proposed standard for other gastropods (Liu and Hershler 2007, Murphy et al. 2012, Harris et al. 2013). With this conservative estimate, CO1 sequence divergence suggests the average timing of isolation between *H. barri* populations is 10.1 million years, and up to 14.5 million years. In this scenario, not only do these results indicate the evolutionary independence of these cave populations, they suggest that the subterranean colonization of this species predates Pleistocene glaciation. The Climatic Relict Hypothesis suggests that environmental stress (such as the often implicated Holsinger (1988) “Pleistocene-effect” model) drives the colonization of organisms into subterranean environments (Leys et al. 2003, Culver and Pipan 2009). Using this gastropod CO1 molecular clock, the Climate-relict hypothesis is not supported. Rather, a scenario in which a geographically widespread proto-troglobitic (i.e., troglophilic) species colonized different subterranean systems independent of obvious environmental stress is favored instead.

Mitochondrial divergence estimates of $\geq 10\%$ per million years are, however, often associated with terrestrial gastropods on island systems (Chiba 1996, Thacker and Hadfield 2000, Van Riel et al. 2005), which are highly comparable to cave systems due

to the isolation of subterranean environments and the discontinuity of these habitats across karst landscapes (Culver 1970, Snowman et al. 2010). In this latter scenario with a high rate of mitochondrial gene evolution (10% per million years), average timing of isolation is 1.6 million years. This would suggest a climatically-driven subterranean colonization during the mid-Pleistocene, failing to reject the Climatic Relict Hypothesis. Thus, it is difficult to differentiate between varying biogeographic scenarios without the application of a *Helicodiscus*-specific molecular clock model. However, the development of an accurate molecular clock is problematic due to a notable gap in fossil material for the genus in North America. There are several occurrences of fossil material in surface and cave habitats across central and eastern North America during the Pleistocene (Rinker 1949, Wetmore 1962, Slaughter 1966, Schultz and Cheatum 1970, Guilday et al. 1977, Dalquist and Stangl 1984, Eshelman and Hager 1984), and one fossil record in central North America in the upper Miocene (Liggert 1997). Moreover, dating based on biogeographic barrier formation is also problematic, as timing estimates of cave formation across the distribution of *H. barri* are highly variable. The formation of some caves in the eastern Appalachians and Cumberland Plateau have been estimated to occur in the late Pliocene to middle Pleistocene (Davies 1953, Anthony and Granger 2004, White 2009), while other caves along the Tennessee River Valley and the Highland Rim have been estimated to form in the late Mesozoic to the early Tertiary (Moneymaker 1948, Barr 1961). Therefore, assessing the timing of colonization is currently beyond the scope of this study.

Delimitation analyses revealed up to sixteen unique MOTUs within *H. barri*, largely organized by geographic and geological similarity (see Figure 7). Most MOTUs belong to similar rock groups, arranged largely in association with each respective physiographic province. There were two unique cases of MOTUs being distributed across both the Appalachians and ILP karst, each exhibiting irregular geographic structure. PG7 is distributed across four cave populations from the northeastern Valley and Ridge, the southernmost contact zone of the Cumberland Plateau and the Eastern Highland Rim, and the westernmost extent of the Western Highland Rim. PG6 is distributed across three cave populations in the eastern Central Basin and the northeastern Valley and Ridge. Further, the ABGD results reveal two distinct MOTUs (AG15, AG16) within a single cave population at Columbia Caverns (DI6). AG15 is comprised of a single individual from Columbia Caverns, whereas AG16 is comprised of one individual from Columbia Caverns and another from the Hering Cave population in northern Alabama. This pattern may be the product of multiple cave colonization events in Columbia Caverns, or perhaps this demonstrates a case of sympatric speciation because of niche partitioning (e.g., Cooper et al. 2002, Niemiller et al. 2008), as these individuals were found in two separate areas of this large cave system. However, due to a low sample size, the aforementioned limited fossil data, and the uncertainty in estimating biogeographic barrier formation, it is difficult to determine the evolutionary history of this species and the geologic context whereby these unique MOTU groups may have developed.

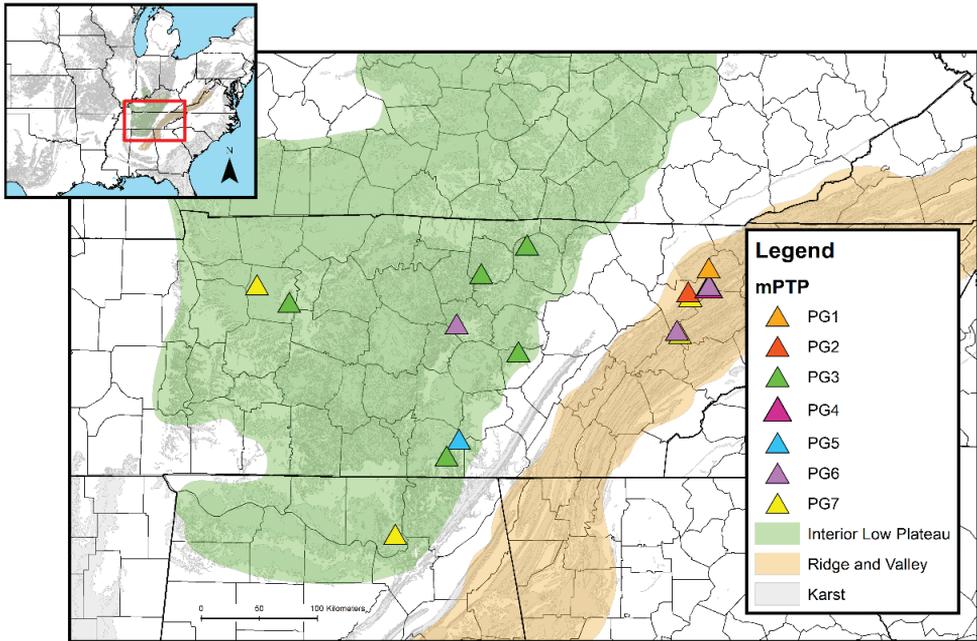


Figure 7. Geographic distribution of MOTUs generated from the mPTP delimitation method in relation to karst adapted from Weary and Doctor (2014). Triangles represent cave populations. The numbers associated with each unique color corresponds to the associated mPTP MOTUs found in Table 3.

Utility of shell morphometrics in species delimitation of cryptic terrestrial micromolluscs

There has been much debate regarding the use of gastropod shell morphology in phylogenetic analyses (Emberton 1995, Wagner 2001, Uit de Weerd et al. 2004, Smith and Hendricks 2013, Miller 2016). Shell variation, while informative at lower taxonomic resolutions (e.g., Smith and Hendricks 2013), may not be useful in accurate delimitation of cryptic lineages, owing to the high responsiveness of shell structure to environmental factors and commonality of local adaptations in land snails (Goodfriend 1986, Fiorentino et al. 2008, Stankowski 2011, Razkin et al. 2017). Moreover, though many subterranean taxa (including *Helicodiscus barri*) exhibit disjunct, fragmented distributions, the ecological similarity of subterranean environments can lead to the protraction of morphological distinguishability between distinct genetic lineages (Losos and Mahler 2010, Eme et al. 2018, Inäbnit et al. 2019). Terrestrial micromolluscs pose additional difficulty in morphological delimitation due to their small size and similarities in external shell morphology, and molecular approaches have been favored (e.g., Weigand et al. 2012). Recent study of troglobitic *Zospeum* snails show that external shell morphology shows high variability both within and between cave populations, further obscuring the taxonomic identity of these cryptic groups without molecular data and intensive study of internal shell structure and soft tissue histology (Jochum et al. 2015).

Table 4. Results from both TM and GM analyses. Asterisk (*) denotes significant p-values.

Group	Degrees of Freedom	Sums of Squares	R ²	F	P
Procrustes ANOVA					
MOTUs (mPTP)	4	0.00763	0.14037	1.388	0.1311
Physiographic Province	3	0.01291	0.13503	3.1742	2.00E-04*
Permutational MANOVA					
MOTUs (mPTP)	4	80.084	0.30107	3.6614	3.00E-04*
Physiographic Province	3	69.84	0.15589	3.7553	0.0021*

Results herein indicate geographic variation of shell morphology as shown by the distinction of physiographic province groups, although intensive study of habitat variation was not performed. Both GM and TM methods resulted in significant differences among physiographic provinces. These findings further suggest an environmental influence on overall external shell morphology, agreeing with previous studies (Goodfriend 1986, Fiorentino et al. 2008, Vergara et al. 2017). The smaller MOTU dataset, comparatively, exhibited large morphological overlap between MOTU groups. However, results were significant for distinction from the TM groupings (see Table 4). This significance is most likely the result of population-based similarity in shell size, rather than the respective MOTU, of which many consist of multiple populations. Further, the GM groups were not significantly different for the MOTU dataset. The small sample size is a potential drawback to the utilization of these morphometric approaches. Terrestrial micromolluscs are notoriously difficult to sample (Boag 1982, Durkan et al. 2013), and sampling in cave environments significantly increases this difficulty. Moreover, as many of these populations remain understudied, morphometric methods may negatively impact populations subject to high amounts of collection and disturbance. This said, application of molecular barcodes may be most useful in the identification of these terrestrial micromolluscs (Weigand et al. 2011, 2014).

Taxonomic and conservation implications

The discovery of cryptic evolutionary lineages within *H. barri* has significant conservation implications. Recent reassessment of the conservation status of *H. barri* listed this species as Vulnerable (G3) under NatureServe criteria and Least Concern (LC) under the IUCN Red List criteria (Gladstone et al. 2018). Though our study suggests that this species is more geographically wide-spread than previously known, the distribution of individual MOTUs is greatly reduced, sometimes being restricted to a single cave. However, this species' presence in both karst regions despite separation by a considerable amount of non-karst strata, and the discovery of a single specimen from surface habitat (see Table 1) suggests that it may not be limited to cave systems. Rather, like other *Helicodiscus* species, *H. barri* could be highly calciphilic, dwelling in rock talus piles or potentially interstitial habitats (Gladstone et al. 2018, Dr. Jeff Nekola, personal comm.). Few studies have investigated the significance of epikarst and

other subsurface habitats to troglobitic fauna in North America (Culver et al. 2012), and a more intensive sampling effort may be necessary to assess the importance of these habitats in facilitating the dispersal of such snail fauna.

This study offers an important first step in outlining the presence of cryptic lineages within *H. barri*. However, many aspects of this species' ecology and life history remain unknown, and the subsequent assessment of distinguishing ecology or habitat requirements for these cryptic groups is essential for their conservation and management. As with other recently discovered cryptic species, additional study of MOTU distribution, ecology, and conservation status are all necessary (Niemiiller et al. 2013, Schlesinger et al. 2018).

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References

- Adams DC, Otarola-Castillo E (2013) *geomorph*: an R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution* 4: 393–399. <https://doi.org/10.1111/2041-210X.12035>
- Adams DC, Otarola-Castillo E, Sherratt E (2014) *geomorph*: Software for geometric morphometric analyses. R package version 2.0. <http://cran.r-project.org/web/packages/geomorph/index.html>
- Anthony DM, Granger DE (2004) A Late Tertiary Origin for Multilevel Caves Along the Western Escarpment of the Cumberland Plateau, Tennessee and Kentucky, Established by Cosmogenic super (26) Al and super (10) Be. *Journal of Cave and Karst Studies* 66(2): 46–55.
- Armbruster JW, Niemiiller ML, Hart PB (2016) Morphological Evolution of the Cave-, Spring-, and Swampfishes of the Amblyopsidae (Percopsiformes). *Copeia* 104(3): 763–777. <https://doi.org/10.1643/CI-15-339>
- Barker GM (2001) Gastropods on land: phylogeny, diversity, and adaptive morphology. In: GM Barker (Ed.) *The Biology of Terrestrial Molluscs*. CABI Publishing, Wallingford, New Zealand: 1–146. <https://doi.org/10.1079/9780851993188.0001>
- Barr TC (1961) Caves of Tennessee. Bulletin 64. Tennessee Division of Geology, Nashville, TN.
- Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I (2007) Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution* 22(3): 148–155. <https://doi.org/10.1016/j.tree.2006.11.004>

- Boag DA (1982) Overcoming sampling bias in studies of terrestrial gastropods. *Canadian Journal of Zoology* 60: 1289–1292. <https://doi.org/10.1139/z82-173>
- Boc A, Diallo AB, Makarenkov V (2012) T-REX: a web server for inferring, validating and visualizing phylogenetic trees and networks. *Nucleic Acids Research* 40(1): 573–579. <https://doi.org/10.1093/nar/gks485>
- Bookstein FL (1997) *Morphometric tools for landmark data: geometry and biology*. Cambridge University Press, Cambridge, UK. <https://doi.org/10.2307/2534038>
- Buhay JE, Moni G, Mann N, Crandall KA (2007) Molecular taxonomy in the dark: evolutionary history, phylogeography, and diversity of cave crayfish in the subgenus *Aviticambarus*, genus *Cambarus*. *Molecular Phylogenetics and Evolution* 42(2): 435–448. <https://doi.org/10.1016/j.ympev.2006.07.014>
- Buhay JE, Crandall KA (2009) Taxonomic revision of cave crayfish in the genus *Cambarus* subgenus *Aviticambarus* (Decapoda: Cambaridae) with descriptions of two new species, *C. speleocoopi* and *C. laconensis*, endemic to Alabama, USA. *Journal of Crustacean Biology* 29: 121–134. <https://doi.org/10.1651/08-3089.1>
- Burch JB (1962) How to Know the Eastern Land Snails. In: William C (Ed.) *Brown Company Publishers, Dubuque, IA*: 214 pp.
- Burress PBH, Burress ED, Armbruster JW (2017) Body shape variation within the Southern Cavefish, *Typhlichthys subterraneus* (Percopsiformes: Amblyopsidae). *Zoomorphology*: 1–13. <https://doi.org/10.1007/s00435-017-0360-0>
- Cameron RAD, Pokryszko BM (2005) Estimating the species richness and composition of land mollusc communities: Problems, consequences and practical advice. *Journal of Conchology* 38(5): 529–548.
- Carstens BC, Pelletier TA, Reid NM, Satler JD (2013) How to fail at species delimitation. *Molecular Ecology* 22(17): 4369–4383. <https://doi.org/10.1111/mec.12413>
- Chiba S (1996) A 40,000-year record of discontinuous evolution of island snails. *Paleobiology* 22(2): 177–188. <https://doi.org/10.1017/S009483730001616X>
- Chiba S (1999) Accelerated evolution of land snails *Mandarina* in the oceanic Bonin islands: evidence from mitochondrial DNA sequences. *Evolution* 53: 460–471. <https://doi.org/10.1111/j.1558-5646.1999.tb03781.x>
- Clements R, Ng PKL, Lu X, Ambu S, Schilthuizen M, Bradshaw CJA (2008) Using biogeographical patterns of endemic land snails to improve conservation planning for limestone karsts. *Biological Conservation* 141(11): 2751–2764. <https://doi.org/10.1016/j.biocon.2008.08.011>
- Colgan DJ, Ponder WF, Egger PE (2000) Gastropod evolutionary rates and phylogenetic relationships assessed using partial 28S rDNA and histone H3 sequences. *Zoologica Scripta* 29(1): 29–63. <https://doi.org/10.1046/j.1463-6409.2000.00021.x>
- Coney CC, Tarpley WA, Bohannon R (1982) Ecological studies of land snails in the Hiawasse River Basin of Tennessee, U.S.A. *Malacological Review* 15: 69–106.
- Cooper SJB, Hinze S, Leys R, Watts CHS, Humphreys WF (2002) Islands under the desert: molecular systematics and evolutionary origins of stygobitic water beetles (Coleoptera: Dytiscidae) from central Western Australia. *Invertebrate Systematics* 16(4): 589–590. <https://doi.org/10.1071/IT01039>

- Culver DC (1970) Analysis of simple cave communities I. Caves as islands. *Evolution* 24(2): 463–474. <https://doi.org/10.1111/j.1558-5646.1970.tb01776.x>
- Culver DC, Pipan T (2009) *The biology of caves and other subterranean habitats*. Second edition. Oxford University Press, Oxford, UK.
- Culver DC, Pipan T, Schneider K (2009) Vicariance, dispersal and scale in the aquatic subterranean fauna of karst regions. *Freshwater Biology* 54(4): 918–929. <https://doi.org/10.1111/j.1365-2427.2007.01856.x>
- Culver DC, Brancelj A, Pipan T (2012) Epikarst communities. In: White WB, Culver DC (Eds) *Encyclopedia of caves*. Second edition. Elsevier, London, 288–295. <https://doi.org/10.1016/B978-0-12-383832-2.00039-6>
- Dalquist WW, Stangl FB (1984) Late Pleistocene and early Recent mammals from Fowlkes Cave, southern Culbertson County, Texas. *Carnegie Museum of Natural History Special Publication* 8: 432–455.
- Davies WE (1953) Geology of Pennsylvania caves. *National Speleological Society Bulletin* 15: 3–9.
- Davison A, Blackie RLE, Scothern GP (2009) DNA barcoding of stylommatophoran land snails: a test of existing sequences. *Molecular Ecology Resources* 9: 1092–1101. <https://doi.org/10.1111/j.1755-0998.2009.02559.x>
- Dayrat B (2005) Towards integrative taxonomy. *Biological Journal of the Linnean Society* 85(3): 407–415. <https://doi.org/10.1111/j.1095-8312.2005.00503.x>
- Dinkins BJ, Dinkins GR (2018) An Inventory of the Land Snails and Slugs (Gastropoda: Caenogastropoda and Pulmonata) of Knox County, Tennessee. *American Malacological Bulletin* 36(1): 1–22. <https://doi.org/10.4003/006.036.0101>
- Douglas DA, Dourson DC, Caldwell RS (2014) The Land Snails of White Oak Sinks, Great Smoky Mountain National Park, Tennessee. *Southeastern Naturalist* 13(1): 166–175. <https://doi.org/10.1656/058.013.0116>
- Dourson DC (2007) A selected land snail compilation of the Central Knobstone escarpment on Furnace Mountain in Powell County Kentucky. *Journal of the Kentucky Academy of Science* 68: 119–131. [https://doi.org/10.3101/1098-7096\(2007\)68\[119:ASLSCO\]2.0.CO;2](https://doi.org/10.3101/1098-7096(2007)68[119:ASLSCO]2.0.CO;2)
- Dourson DC (2010) *Kentucky's Land Snails and Their Ecological Communities*. Goatslug Publications, Bakersville, NC.
- Duminil J, Di Michele M (2009) Plant species delimitation: a comparison of morphological and molecular markers. *Plant Biosystems* 143(3): 528–542. <https://doi.org/10.1080/11263500902722964>
- Durkan TH, Yeung NW, Meyer WM, Hayes KA, Cowie RH (2013) Evaluating the efficacy of land snail survey techniques in Hawaii: implications for conservation throughout the Pacific. *Biodiversity and Conservation* 22(13–14): 3223–3232. <https://doi.org/10.1007/s10531-013-0580-7>
- Emberton KC (1995) When shells do not tell: 145 million years of evolution in North America's polygyrid land snails, with a revision and conservation priorities. *Malacologia* 37: 69–110.
- Eme D, Zagnajster M, Delić T, Fišer C, Flot JF, Konecny-Dupré L, Pálsson S, Stoch F, Zakšek V, Douady CJ, Malard F (2018) Do cryptic species matter in macroecology? Sequencing European groundwater crustaceans yields smaller ranges but does not challenge biodiversity determinants. *Ecography* 41(2): 424–436. <https://doi.org/10.1111/ecog.02683>

- Eshelman R, Hager M (1984) Two Irvingtonian (medial Pleistocene) vertebrate faunas from north-central Kansas. *Contributions in Quaternary Vertebrate Paleontology: a volume in memorial to John E. Guilday*, Special Publication of Carnegie Museum of Natural History 8: 384–404.
- Fenneman NM (1917) Physiographic subdivision of the United States. *Proceedings of the National Academy of Sciences of the United States of America* 3(1): 17–22. <https://doi.org/10.1073/pnas.3.1.17>
- Finston TL, Johnson MS, Humphreys WF, Eberhard SM, Halse SA (2007) Cryptic speciation in two widespread subterranean amphipod genera reflects historical drainage patterns in an ancient landscape. *Molecular Evolution* 16: 355–365. <https://doi.org/10.1111/j.1365-294X.2006.03123.x>
- Fiorentino V, Salomone N, Manganelli G, Giusti F (2008) Phylogeography and morphological variability in land snails: the Sicilian *Marmorana* (Pulmonata, Helicidae). *Biological Journal of the Linnean Society* 94: 809–823. <https://doi.org/10.1111/j.1095-8312.2008.01023.x>
- Floyd R, Abebe E, Papert A, Blaxter M (2002) Molecular barcodes for soil nematode identification. *Molecular Ecology* 11(4): 839–850. <https://doi.org/10.1046/j.1365-294X.2002.01485.x>
- Folmer O, Black M, Hoeh W, 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.
- Gladstone NS, Carter ET, McKinney ML, Niemiller ML (2018) Status and Distribution of the Cave-Obligate Land Snails in the Appalachians and Interior Low Plateau of the Eastern United States. *American Malacological Bulletin* 36(1): 62–78. <https://doi.org/10.4003/006.036.0107>
- Goodfriend GA (1986) Variation in land-snail shell form and size and its causes: a review. *Systematic Biology* 35(2): 204–223. <https://doi.org/10.1093/sysbio/35.2.204>
- Guilday JE, Parmalee PW, Hamilton HW (1977) The Clark's Cave bone deposit and the Pleistocene paleoecology of the central Appalachian Mountains of Virginia. *Bulletin of Carnegie Museum of Natural History* 2: 1–87.
- Guiller A, Madec L, Daguzan J (1994) Geographical patterns of genetic differentiation in the land snail *Helix aspersa* Müller (Gastropoda: Pulmonata). *Journal of Molluscan Studies* 60: 205–221. <https://doi.org/10.1093/mollus/60.3.205>
- Harris JD, Ferreira AF, De Frias Martins AM (2013) High levels of mitochondrial DNA diversity within oxychilid land snails (subgenus *Drouetia* Gude, 1911) from São Miguel island, Azores. *Journal of Molluscan Studies* 79(2): 177–182. <https://doi.org/10.1093/mollus/eyt009>
- Hasegawa M, Kishino H, Yano T (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* 22(2): 160–174. <https://doi.org/10.1007/BF02101694>
- Hermesen EJ, Hendricks JR (2008) W(h)ither fossils? Studying morphological character evolution in the age of molecular sequences. *Annals of the Missouri Botanical Garden* 95(1): 72–100. <https://doi.org/10.3417/2006206>
- Holsinger JR (1988) Troglobites: the evolution of cave-dwelling organisms. *American Scientist* 76(2): 146–153.

- Hotopp KP, Pearce T, Nekola JC, Slapcinsky J, Dourson DC, Winslow M, Kimber G, Watson B (2013) Land Snails and Slugs of the Mid-Atlantic and Northeastern United States. Carnegie Museum of Natural History, Pittsburgh, PA, USA.
- Hubricht L (1962) New species of *Helicodiscus* from the eastern United States. *The Nautilus* 75(3): 102–107. <https://doi.org/10.5962/bhl.part.25951>
- Hubricht L (1985) The distributions of the native land mollusks of the eastern United States. *Fieldiana n.S.* 24: 1–191. <https://doi.org/10.5962/bhl.title.3329>
- Huson DH, Bryant D (2005) Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* 23(2): 254–267. <https://doi.org/10.1093/molbev/msj030>
- Inäbnit T, Jochum A, Kampschulte M, Martels G, Ruthensteiner B, Slapnik R, Nesselhauf C, Neubert E (2019) An integrative taxonomic study reveals carychiid microsnailed of the troglitic genus *Zospeum* in the Eastern and Dinaric Alps (Gastropoda, Ellobioidea, Carychiinae). *Organisms Diversity & Evolution*: 1–43. <https://doi.org/10.1007/s13127-019-00400-8>
- Jochum A, Slapnik R, Klusmann-Kolb A, Pall-Gergely B, Kampschulte M, Martels G, Vrabec M, Nesselhauf C, Weigand AM (2015) Groping through the black box of variability: An integrative taxonomic and nomenclatural re-evaluation of *Zospeum isselianum* Pollonera, 1887 and allied species using new imaging technology (Nano-CT, SEM), conchological, historical and molecular data (Ellobioidea, Carychiidae). *Subterranean Biology* 16: 123–165. <https://doi.org/10.3897/subtbiol.16.5758>
- Juan C, Emerson BC (2010) Evolution underground: shedding light on the diversification of subterranean insects. *Journal of Biology* 9(3): 17. <https://doi.org/10.1186/jbiol227>
- Juan C, Guzik MT, Jaime D, Cooper SJB (2010) Evolution in caves: Darwin's 'wrecks of ancient life' in the molecular era. *Molecular Ecology* 19: 3865–3880. <https://doi.org/10.1111/j.1365-294X.2010.04759.x>
- Kano Y, Chiba S, Kase T (2002) Major adaptive radiation in neritopsine gastropods estimated from 28S rRNA sequences and fossil records. *Proceedings of the Royal Society of London B: Biological Sciences* 269(1508): 2457–2465. <https://doi.org/10.1098/rspb.2002.2178>
- Kapli P, Lutteropp S, Zhang J, Kobert K, Pavlidis P, Stamatakis A, Flouri T (2017) Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. *Bioinformatics* 33(11): 1630–1638. <https://doi.org/10.1093/bioinformatics/btx025>
- Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111–120. <https://doi.org/10.1007/BF01731581>
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution* 35(6): 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29(6): 1695–1701. <https://doi.org/10.1093/molbev/mss020>

- Lefébure T, Douady CJ, Gouy M, Trontelj P, Briolay J, Gibert J (2006) Phylogeography of a subterranean amphipod reveals cryptic diversity and dynamic evolution in extreme environments. *Molecular Ecology* 15(7): 1797–1806. <https://doi.org/10.1111/j.1365-294X.2006.02888.x>
- Leray VL, Caravas J, Friedrich M, Zigler KS (2019) Mitochondrial sequence data indicate “Vicariance by Erosion” as a mechanism of species diversification in North American Ptomaphagus (Coleoptera, Leiodidae, Cholevinae) cave beetles. *Subterranean Biology* 29: 35–57. <https://doi.org/10.3897/subtbiol.29.31377>
- Leys R, Watts CHS, Cooper SJB, Humphreys WF (2003) Evolution of subterranean diving beetles (Coleoptera: Dytiscidae Hydroporini, Bidessini) in the arid zone of Australia. *Evolution* 57(12): 2819–2834. <https://doi.org/10.1111/j.0014-3820.2003.tb01523.x>
- Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25(11): 1451–1452. <https://doi.org/10.1093/bioinformatics/btp187>
- Liew T, Clements R, Schilthuizen M (2008) Sampling micromolluscs in tropical forests: one size does not fit all. *Zoosymposia* 1: 271–280. <https://doi.org/10.11646/zoosymposia.1.1.16>
- Liggert GA (1997) The beckerdie local biota (early Hemphillian) and the first Tertiary occurrence of a crocodylian from Kansas. *Transactions of the Kansas Academy of Sciences* 100(3–4): 101–108. <https://doi.org/10.2307/3627997>
- Liu H, Hershler R (2007) A test of the vicariance hypothesis of western North American freshwater biogeography. *Journal of Biogeography* 34(3): 534–548. <https://doi.org/10.1111/j.1365-2699.2006.01611.x>
- Loria SE, Zigler KS, Lewis JL (2011) Molecular phylogeography of the troglobiotic millipede *Tetracion* Hoffman, 1956 (Diplopoda, Callipodida, Abacionidae). *International Journal of Myriapodology* 5: 35–48. <https://doi.org/10.3897/ijm.5.1891>
- Losos JB, Mahler LD (2010) Adaptive radiation: the interaction of ecological opportunity, adaptation, and speciation. In: Bell MA et al. (Eds) *Evolution since Darwin: the first 150 years*. Sinauer Associates, Sunderland, MA, 381–420.
- Lydeard C, Cowie RH, Ponder WF, Bogan AE, Bouchet P, Clark SA, Cummings KF, Frest TJ, Gargominy O, Herbert DG, Hershler R, Perez KE, Roth B, Seddon M, Strong EE, Thompson FG (2004) The global decline of nonmarine mollusks. *BioScience* 54: 321–330. [https://doi.org/10.1641/0006-3568\(2004\)054\[0321:TGDONM\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2004)054[0321:TGDONM]2.0.CO;2)
- McGuire JA, Linkem CW, Koo MS, Hutchison DW, Lappin KA, Orange DI, Lemos-Espinal J, Riddle BR, Jaeger JR (2007) Mitochondrial introgression and incomplete lineage sorting through space and time: phylogenetics of crotaphytid lizards. *Evolution: International Journal of Organic Evolution* 61(12): 2879–2897. <https://doi.org/10.1111/j.1558-5646.2007.00239.x>
- Miller JP (2016) Geometric morphometric analysis of the shell of *Cerion mumia* (Pulmonata: Cerionidae) and related species. *Folia Malacologica* 24(4): 239–250. <https://doi.org/10.12657/folmal.024.020>
- Moneymaker BC (1948) Some broad aspects of limestone solution in the Tennessee Valley. *Transactions of the American Geophysical Union* 29(1): 93–96. <https://doi.org/10.1029/TR029i001p00093>
- Moulds TA, Murphy N, Adams M, Reardon T, Harvey MS, Jennings J, Austin AD (2007) Phylogeography of cave pseudoscorpions in southern Australia. *Journal of Biogeography* 34: 951–962. <https://doi.org/10.1111/j.1365-2699.2006.01675.x>

- Morrison JPE (1942) Preliminary report on mollusks found in the shell mounds of the Pickwick landing basin in the Tennessee River Valley. *Bulletin of the Bureau of American Ethnology* 129: 337–392.
- Morse ES (1864) Observations on the terrestrial Pulmonifera of Maine, including a catalogue of all the species of terrestrial and fluviatile Mollusca known to inhabit the state. *Journal of the (Portland) Maine Society of Natural History* 1: 1–63.
- Murphy NP, Breed MF, Guzik MT, Cooper SJB, Austin AD (2012) Trapped in desert springs: phylogeography of Australian desert spring snails. *Journal of Biogeography* 39(9): 1573–1582. <https://doi.org/10.1111/j.1365-2699.2012.02725.x>
- Nekola JC (2005) Geographic variation in richness and shell size of eastern North American land snail communities. *Records of the Western Australian Museum Supplement* 68: 39–51. <https://doi.org/10.18195/issn.0313-122x.68.2005.039-051>
- Nekola JC (2014) Overview of the North American terrestrial gastropod fauna. *American Malacological Bulletin* 32(2): 225–235. <https://doi.org/10.4003/006.032.0203>
- Nekola JC, Coles BF (2010) Pupillid land snails of eastern North America. *American Malacological Bulletin* 28: 29–57. <https://doi.org/10.4003/006.028.0221>
- Niemiller ML, Fitzpatrick BM, Miller BT (2008) Recent divergence with gene flow in Tennessee cave salamanders (Plethodontidae: *Gyrinophilus*) inferred from gene genealogies. *Molecular Ecology* 17(9): 2258–2275. <https://doi.org/10.1111/j.1365-294X.2008.03750.x>
- Niemiller ML, Near TJ, Fitzpatrick BM (2012) Delimiting species using multilocus data: diagnosing cryptic diversity in the southern cavefish, *Typhlichthys subterraneus* (Teleostei: Amblyopsidae). *Evolution* 66(3): 846–866. <https://doi.org/10.1111/j.1558-5646.2011.01480.x>
- Niemiller ML, Zigler KS (2013) Patterns of cave biodiversity and endemism in the Appalachians and Interior Plateau of Tennessee, USA. *PLoS One* 8(5): e64177. <https://doi.org/10.1371/journal.pone.0064177>
- Niemiller ML, Fitzpatrick BM, Shah P, Schmitz L, Near TJ (2013) Evidence for repeated loss of selective constraint in rhodopsin of amblyopsid cavefishes (Teleostei: Amblyopsidae). *Evolution* 67: 732–748. <https://doi.org/10.1111/j.1558-5646.2012.01822.x>
- Palumbi S, Martin A, Romano S, McMillan WO, Stice L, Grabowski G (1991) *The Simple Fool's Guide to PCR*. Version 2.0. University of Hawaii, Honolulu.
- Pearce TA, Örstan A (2006) Terrestrial Gastropoda. In: Sturm CF, Pearce TA, Valdés A (Eds) *The Mollusks: A Guide to Their Study, Collection, and Preservation*. American Malacological Society, USA, 261–285.
- Perez KE, Defreitas N, Slapcinsky J, Minton RL, Anderson FE, Pearce TA (2014) Molecular phylogeny, evolution of shell shape, and DNA barcoding in Polygyridae (Gastropoda: Pulmonata), an endemic North American clade of land snails. *American Malacological Bulletin* 32(1): 1–31. <https://doi.org/10.4003/006.032.0103>
- Perez SI, Diniz-Filho JAF, Bernal V, Gonzalez PN (2010) Spatial regression techniques for inter-population data: studying the relationships between morphological and environmental variation. *Journal of Evolutionary Biology* 23(2): 237–248. <https://doi.org/10.1111/j.1420-9101.2009.01905.x>
- Pilsbry HA (1948) *Land Mollusca of North America (north of Mexico)*. Volume II, Part II. The Academy of Natural Sciences of Philadelphia. Philadelphia, PA.

- Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP (2006) Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology* 55(4): 595–609. <https://doi.org/10.1080/10635150600852011>
- Puillandre N, Lambert A, Brouillet S, Achaz G (2012) ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology* 21(8): 1864–1877. <https://doi.org/10.1111/j.1365-294X.2011.05239.x>
- Oksanen J, Blanchet GF, Friendly M, Kindt R, Legendre P, McGlenn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2018) *vegan*: Community Ecology Package. R package version 2.5-1. <https://CRAN.R-project.org/package=vegan>
- R Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>
- Rambaut A, Drummond AJ (2007) Tracer v. 1.4. <http://beast.bio.ed.ac.uk/Tracer>
- Razkin O, Gomez-Moliner BJ, Vardinoyannis K, Martinez-Orti A, Madeira MJ (2017) Species delimitation for cryptic species complexes: case study of *Pyramidula* (Gastropoda, Pulmonata). *Zoologica Scripta* 46: 55–72. <https://doi.org/10.1111/zsc.12192>
- Rinker GC (1949) Tremarctotherium from the Pleistocene of Meade County, Kansas. *Contributions from the Museum of Paleontology, University of Michigan* 7(6): 107–112.
- Rohlf FJ (2015) The tps series of software. *Hystrix, the Italian Journal of Mammalogy* 26: 1–4.
- Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61(3): 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Rubinoff D (2006) Utility of mitochondrial DNA barcodes in species conservation. *Conservation Biology* 20(4): 1026–1033. <https://doi.org/10.1111/j.1523-1739.2006.00372.x>
- Schlesinger MD, Feinberg JA, Nazdrowicz NH, Kleopfer JD, Beane JC, Bunnell JF, Burger J, Corey E, Gipe K, Jaycox JW, Kiviat E (2018) Follow-up ecological studies for cryptic species discoveries: Decrypting the leopard frogs of the eastern US. *PLoS One* 13(11): e0205805. <https://doi.org/10.1371/journal.pone.0205805>
- Schultz GE, Cheatum EP (1970) *Bison occidentalis* and associated invertebrates from the late Wisconsin of Randall County, Texas. *Journal of Paleontology* 44(5): 836–850.
- Slaughter BH (1966) The Moore Pit local fauna; Pleistocene of Texas. *Journal of Paleontology* 40(1): 78–91.
- Smith UE, Hendricks JR (2013) Geometric morphometric character suites as phylogenetic data: extracting phylogenetic signal from gastropod shells. *Systematic Biology* 62(3): 366–385. <https://doi.org/10.1093/sysbio/syt002>
- Snowman CV, Zigler KS, Hedin M (2010) Caves as islands: mitochondrial phylogeography of the cave-obligate spider species *Nesticus barri* (Araneae: Nesticidae). *Journal of Arachnology* 38: 49–56. <https://doi.org/10.1636/A09-057.1>
- Sokal RR, Oden NL (1978) Spatial autocorrelation in biology. 1. Methodology. *Biological Journal of the Linnean Society* 10: 199–228. <https://doi.org/10.1111/j.1095-8312.1978.tb00013.x>
- Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30(9): 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>

- Stankowski S (2011) Extreme, continuous variation in an island snail: local diversification and association of shell form with the current environment. *Biological Journal of the Linnean Society* 104: 756–769. <https://doi.org/10.1111/j.1095-8312.2011.01748.x>
- Thacker RW, Hadfield MG (2000) Mitochondrial phylogeny of extant Hawaiian tree snails (Achatinellinae). *Molecular Phylogenetics and Evolution* 16(2): 263–270. <https://doi.org/10.1006/mpev.2000.0793>
- Thomaz D, Guiller A, Clarke B (1996) Extreme divergence of mitochondrial DNA within species of pulmonated land snails. *Proceedings of the Royal Society of London Series B – Biological Sciences* 263: 363–368. <https://doi.org/10.1098/rspb.1996.0056>
- Uit de Weerd D, Piel WH, Gittenberger E (2004) Widespread polyphyly among Alopiinae land snail genera: when phylogeny mirrors biogeography more closely than morphology. *Molecular Phylogenetics and Evolution* 33: 533–548. <https://doi.org/10.1016/j.ympev.2004.07.010>
- Van Riel P, Jordaens K, Van Houtte N, Martins AMF, Verhagen R, Backeljau T (2005) Molecular systematics of the endemic Leptaxini (Gastropoda: Pulmonata) on the Azores islands. *Molecular Phylogenetics and Evolution* 37: 132–143. <https://doi.org/10.1016/j.ympev.2005.03.019>
- Vergara D, Fuentes JA, Stoy KS, Lively CM (2017) Evaluating shell variation across different populations of a freshwater snail. *Molluscan Research* 37(2): 120–132. <https://doi.org/10.1080/13235818.2016.1253446>
- Wagner PJ (2001) Gastropod phylogenetics: progress, problems, and implications. *Journal of Paleontology* 75: 1128–1140. <https://doi.org/10.1017/S0022336000017182>
- Weary DJ, Doctor DH (2014) Karst in the United States: A digital map compilation and database. USGS Open File Report 2014–1156. <https://doi.org/10.3133/ofr20141156>
- Weckstein JD, Johnson KP, Murdoch JD, Krejca JK, Tayika DM, Veni G, Reddell JR, Taylor SJ (2016) Comparative phylogeography of two codistributed subgenera of cave crickets (Orthoptera: Rhaphidophoridae: *Ceuthophilus* spp.). *Journal of Biogeography* 43: 1450–1463. <https://doi.org/10.1111/jbi.12734>
- Weigand AM, Jochum A, Pfenninger M, Steinke D, Klussmann-Kolb A (2011) A new approach to an old conundrum – DNA barcoding sheds new light on phenotypic plasticity and morphological stasis in microsnailes (Gastropoda, Pulmonata, Carychiidae). *Molecular Ecology Resources* 11(2): 255–265. <https://doi.org/10.1111/j.1755-0998.2010.02937.x>
- Weigand AM, Görze MC, Jochum A (2012) Outdated but established?! Conchologically driven species delineations in microgastropods (Carychiidae, *Carychium*). *Organisms Diversity & Evolution* 12 (4): 377–386. <https://doi.org/10.1007/s13127-011-0070-2>
- Weigand AM, Jochum A, Klussmann-Kolb A (2014) DNA barcoding cleans house through the Carychiidae (Eupulmonata, Ellobioidea). *American Malacological Bulletin* 32(2): 236–245. <https://doi.org/10.4003/006.032.0215>
- Wetmore A (1962) Notes on fossil and subfossil birds. *Smithsonian Miscellaneous Collections* 145 (2): 1–17.

- White WB (2009) The evolution of Appalachian fluviokarst: competition between stream erosion, cave development, surface denudation, and tectonic uplift. *Journal of Cave and Karst Studies* 71(3): 159–167. <https://doi.org/10.4311/jcks2008es0046>
- Zhang J, Kapli P, Pavlidis P, Stamatakis A (2013) A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* 29(22): 2869–2876. <https://doi.org/10.1093/bioinformatics/btt499>
- Zharkikh A (1994) Estimation of evolutionary distances between nucleotide sequences. *Journal of Molecular Evolution* 39(3): 315–329. <https://doi.org/10.1007/BF00160155>

Supplementary material 1

GenBank accession numbers for all sequence data

Authors: Nicholas S. Gladstone, Matthew L. Niemiller, Evelyn B. Pieper, Katherine E. Dooley, Michael L. McKinney

Data type: list

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Link: <https://doi.org/10.3897/subtbiol.30.35321.suppl1>

Supplementary material 2

Maximum-Likelihood (ML) CO1 Phylogram

Authors: Nicholas S. Gladstone, Matthew L. Niemiller, Evelyn B. Pieper, Katherine E. Dooley, Michael L. McKinney

Data type: multimedia

Explanation note: CO1 (704 bp) phylogram of *H. barri* generated from RAxML. Outgroup not shown due to long branch length.

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Link: <https://doi.org/10.3897/subtbiol.30.35321.suppl2>

Supplementary material 3

Maximum-Likelihood (ML) mtDNA + nDNA Phylogram

Authors: Nicholas S. Gladstone, Matthew L. Niemiller, Evelyn B. Pieper, Katherine E. Dooley, Michael L. McKinney

Data type: multimedia

Explanation note: mtDNA + nDNA (CO1 + 16S + 28S + H3; 3040 bp) phylogram generated of *H. barri* from RAxML. Outgroup not shown due to long branch length.

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Link: <https://doi.org/10.3897/subtbiol.30.35321.suppl3>

Supplementary material 4

ABGD Delimitation Results

Authors: Nicholas S. Gladstone, Matthew L. Niemiller, Evelyn B. Pieper, Katherine E. Dooley, Michael L. McKinney

Data type: multimedia

Explanation note: ABGD species delimitation results. A: Recursive and initial partitions under varying prior intraspecific divergences. B: Frequency histogram of K2P pairwise divergences.

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Link: <https://doi.org/10.3897/subtbiol.30.35321.suppl4>

First record of albinism for the doglike bat, *Peropteryx kappleri* Peters, 1867 (Chiroptera, Emballonuridae)

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Abstract

Albinism is a type of deficient in melanin production could be the result of genetic anomalies that are manifest as the absence of coloration of part or the entire body of an organism. This type of chromatic disorder can affect several vertebrate species, but is rarely found in nature. Among bats, more than 450 cases of total or partial loss of body pigmentation have been reported. Herein we provide the first report of albinism for the bat species *Peropteryx kappleri* (Chiroptera: Emballonuridae) with two such specimens being observed from iron formation caves in a conservation unit “Floresta Nacional de Carajás”, Amazon forest, northern of Brazil.

Keywords

Chiroptera, *Peropteryx*, hypopigmentation, Neotropics, Amazonia, cave

Deficiency in melanin production is a rare among vertebrates. Such a condition can cause changes in the coloration of specific body parts, such as skin, hair, feathers, scales or the eye, and can even reach extreme cases where there is complete absence of pigmentation throughout the entire body. Changes in body color caused by deficient melanin production can generate several conditions, which (Lucati and López-Baucells 2017) has classified into four types: true albinism, leucism, piebaldism and hypomelanism. All types of melanin production deficiency are hereditary and genetic (congenital) abnormalities. Such disorders have been observed in numerous species of wild mammals throughout the world, including marine (Fertl and Rosel 2002, Abreu et al. 2013), terrestrials (Lopucki and Mróz 2010, Abreu et al. 2013, Neves et al. 2013, Xu et al. 2013, Camargo et al. 2014, Nedyalkov et al. 2014, Sayyed et al. 2015, Kumar et al. 2016, Romero et al. 2018, Oliveira et al. 2019) and bats (Uieda 2000, Murariu and Chisamera 2006, McCardle 2012, López-Baucells et al. 2013, Ramasindrazana et al. 2014, Rengifo et al. 2014, Smirnov et al. 2014, Zalapa et al. 2016, Lucati and López-Baucells 2017).

Chromatic aberrations that lead to albinism in bats have been reported in 61 species of 10 families (Rhinolophidae, Hipposideridae, Rhinopomatidae, Emballonuridae, Nycteridae, Phyllostomidae, Mormoopidae, Molossidae, Miniopteridae, and Vespertilionidae), in a great number of countries worldwide, but they continue to be a rarely observed event (Uieda 2000, Rosa et al. 2017, Nascimento et al. 2018, Zortéa and Silva 2018). Although these aberrations interfere with the coloration of the animals, there have been, to date, no marked negative effects of this genetic inheritance that could compromise the survival of bats in the natural environment (Uieda 2001).

There have been 31 reports of the absence of melanin pigmentation for 17 species of bats in Brazil (Uieda 2000, Rosa et al. 2017, Nascimento et al. 2018, Zortéa and Silva 2018). Of these records, 20 were cases of albinism (t-inherited, hypopigmentary disorder characterized by a complete lack of melanin caused by the absence of the enzyme tyrosinase; it results in pale skin, white fur or feathers, and red eyes) and 11 case of piebaldism (total lack of melanin in part of the skin and/or hair follicles due to the absence of melanocytes in the affected part) (Lucati and López-Baucells 2017; Rosa et al. 2017; Uieda 2000; Zortéa and Silva 2018). This article reports the observations of two albino individuals of *Peropteryx kappleri* Peters, 1867, which increases the number of individual records of albinism in bats in Brazil to 33. Furthermore, it represents the first report of albinism for the genus *Peropteryx* and the third report in the world for the family Emballonuridae (Zortéa and Silva 2018) (Table 1).

The albino bats were observed in Floresta Nacional de Carajás (FLONA-Carajás), which is located completely within the Amazon Forest domain in the state of Pará, North Brazil. (Figure 1). The FLONA Carajás, created by Decree No. 2,486 of February 2, 1998, has more than 390,000 hectares and is a Conservation Unit for sustainable and diversified use of forest resources and scientific research. The main

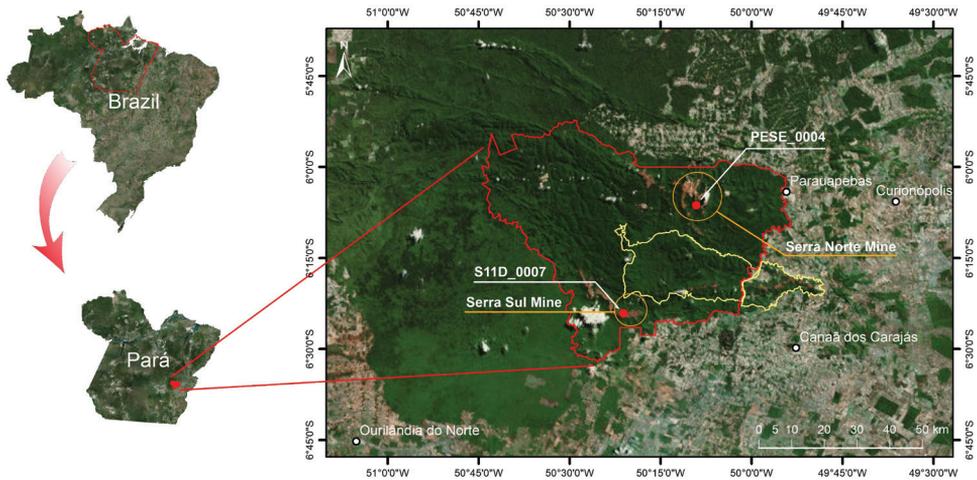


Figure 1. Location of the caves where the specimens of *Peropteryx kapleri* were observed in the municipalities of Parauapebas (cavern PESE-0004) and Canaã dos Carajás (cavern S11D-0007), in southeastern Pará, Brazil.

vegetation cover of the region is the Ombrophilous Forest, with local variations, the majority associated with changes in the relief. The forest areas are intercepted by rock outcrops of iron ore, which has a high economic interest. In these outcrops are concentrated the caves of the region, with mining being the main impact on this important habitat for some species of bats. One of the main purposes for creating FLONA-Carajás was to protect the environment and allow multiple uses of the forest, including the extraction of iron ore in a way that is minimally damaging to the environment, with emphasis on methods for sustainable exploitation of native forests (ICMBIO 2017). However, during the iron ore extraction some caves have been suppressed, this proceeds are allowed in this type of conservation unit area. And with the intention of keeping some forest areas and caves undisturbed, was established around of FLONA-Carajás the Carajás Ferruginous Fields National Park, created on June 5, 2017, with 80,000 hectares (Figure 1). In this new protected area are inserted hundreds of caves preserved as an environmental compensation and the iron ore extraction is commercial use of the forest is not allowed.

Species surveys at the site have been routine, but since 2008 faunal studies have intensified in the caves of the region, mainly in association with environmental licensing and monitoring. As a result, knowledge of the local chiropterofauna has increased and to date faunal surveys have been carried out in more than 1,000 caves in FLONA Carajás, in addition to several projects involving the survey of chiroptera in epigeal environments. To date, a total of 75 species of bats have been recorded in FLONA Carajás, of which 23 were recorded in caves, including those of the genus *Peropteryx* (Tavares et al. 2012).

Table 1. Records of albino bats in Brazil.

Species	Disorder	N° individuals	Location	Reference
<i>Artibeus cinereus</i>	Albinism	1♂	flyway	Oliveira and Aguiar 2008
<i>Artibeus concolor</i>	Piebaldism	1♂	flyway	Zortéa and Silva 2018
<i>Artibeus jamaicensis</i>	Albinism	1?	tree	Uieda 2000
<i>Artibeus planirostris</i>	Albinism	1	–	Uieda 2000
<i>Artibeus lituratus</i>	Piebaldism	1♂	flyway	Souza et al. 2013
<i>Carollia perspicillata</i>	Piebaldism	1♀	cave	Rocha et al. 2013
<i>Carollia perspicillata</i>	Albinism	1♀	flyway	Falcão 2014
<i>Carollia perspicillata</i>	Piebaldism	1♀	flyway	Zortéa and Silva 2018
<i>Carollia perspicillata</i>	Albinism	1?	tunnel	Rosa et al. 2017
<i>Dermanura cinerea</i>	Albinism	1?	–	Oliveira and Aguiar 2008
<i>Desmodus rotundus</i>	Albinism	2♂, 1♀, 1?	1 cave, 1 flyway, 1?	Uieda 2000, 2001
<i>Desmodus rotundus</i>	Albinism	1♂	cave	Uieda 2000, 2001
<i>Desmodus rotundus</i>	Albinism	1?	flyway	Moreira et al. 1992
<i>Eumops glaucinus</i>	Albinism	1♂	–	Sodré et al. 2004
<i>Gardnerycteris crenulatum</i>	Albinism	1	flyway	Zortéa and Silva 2018
<i>Molossus molossus</i>	Albinism	1	hollow tree	Veiga and Oliveira 1995
<i>Molossus molossus</i>	Albinism	1♀	hollow tree	Uieda 2000
<i>Molossus molossus</i>	Albinism	1♀	flyway	Nascimento et al. 2018
<i>Myotis lewis</i>	Albinism	1?	–	Miranda et al. 2012
<i>Nyctinomops laticaudatus</i>	Piebaldism	2♂, 3♀	building	Geiger and Pacheco 2006
<i>Peropteryx kappleri</i>	Albinism	1♂	cave	Present data
<i>Peropteryx kappleri</i>	Albinism	1 young	cave	Present data
<i>Phyllostomus discolor</i>	Piebaldism	1♀	flyway	Treitler et al. 2013
<i>Tadarida brasiliensis</i>	Piebaldism	1?	–	Zortéa and Silva 2018
<i>Tonatia saurophila</i>	Piebaldism	1?	flyway	Zortéa and Silva 2018

The albino bats were observed in 2016 and 2017 in two caves located in FLONA-Carajás; one in a cave in the municipality Parauapebas and the other in the municipality of Canaã dos Carajás, both in the state of Pará, Brazil (Figure 1). The first observation was made on 04 September 2016 and was of an adult male *P. kappleri* found in cave PESE-0004 (06°06'19.22"S; 50°09'5.96"W) (forearm measurement; 47,5 cm). The second observation was made on 23 October 2017 and was of a young *P. kappleri* found in cave S11D-0007 (06°24'06.42"S, 50°21'06.09"W) (no measurements were made).

The specimens were observed hanging on walls inside the caves and last than 5 metres from the entrance. The adult male was solitary, while the juvenile was close to other conspecific individuals with normal coloration. Both individuals had complete albinism, including reddish eyes, which are typical features of albinism (Figure 2). Aside from the obvious morphological differences of albino bats (i.e., coloration), no other differences were observed for the individuals during the brief period during which they were observed in the field (not more than 30 minutes), not even in their behavior. The observed individuals were not euthanized since the legal authorization of the research only permitted the capture of bats for purposes of identification, with the collection of specimens being allowed only for cases of taxonomic doubt (ABIO 639/2015 and ABIO 455/2014).

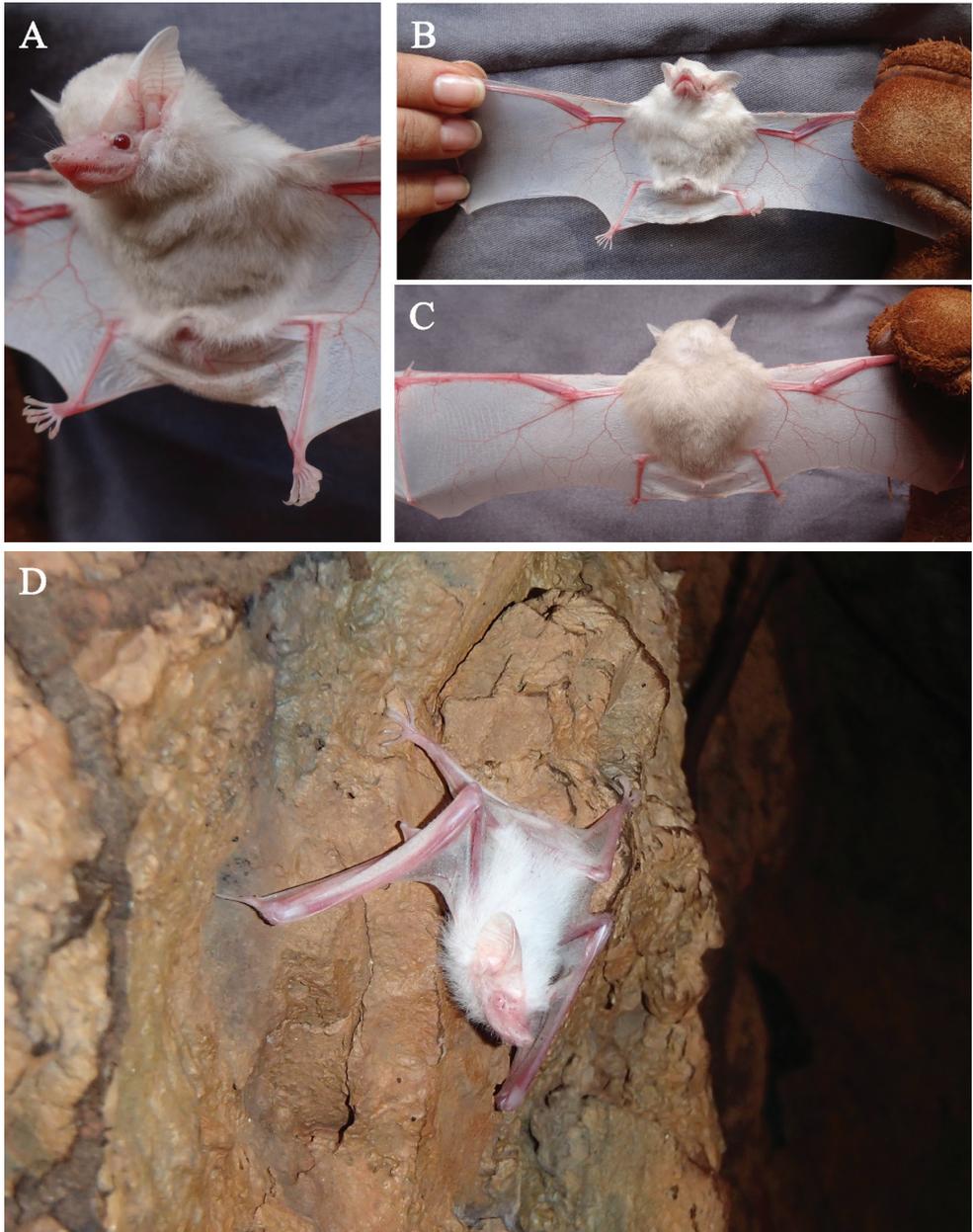


Figure 2. Albino specimens of *Peropteryx* observed in caves of Floresta Nacional de Carajás. **A–C** male specimen of *Peropteryx kappleri* **D** juvenile specimen of *Peropteryx kappleri*.

The adult specimens were captured and measurements were taken to confirm the condition, but the juvenile was simply observed during campaigns to monitor cave fauna.

Bats of the genus *Peropteryx* spp. are very common in the entrances to caves in the region of Carajás, and have been observed in more than 600 caves. Nonetheless,

despite this significant effort, there have been no other reports of albino specimens, which are testimony to the rarity of this condition in this group of animals, as demonstrated by Uieda (2000) and Lucati and López-Baucells (2017), highlighting the importance of these records.

The influence or not of albinism in bat individual fitness should be better evaluated, that's because lack of skin pigmentation could promote a major disadvantage to animals in the wild habitat, the most obvious being an animal's lack of crypsis with its surroundings, protects sun rays and lost of accuracy of eyesight (Uieda 2000, Sandoval-Castilho et al. 2006, Acevedo et al. 2009). However, bat animals have prevalent are nocturnal behaviour so the absence of pigment may not be interfere is significantly with individual fitness. Furthermore, bats seek for a daytime shelter in a variety of habitats, and caves could offer protection against sunlight, water loss, and visual hunting predators (Uieda 2001), promoting better chances to survive.

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References

- Acevedo J, Aguayo-Lobo A, Torres D (2009) Albino weddell seal at cape shirreff, Livingston island, Antarctica. *Polar Biology* 32(8): 1239–1243. <https://doi.org/10.1007/s00300-009-0680-8>
- Abreu MSL, Machado R, Barbieri F, Freitas NS, Oliveira LR (2013) Anomalous colour in Neotropical mammals: a review with new records for *Didelphis* sp (Didelphidae, Didelphimorphia) and *Arctocephalus australis* (Otariidae, Carnivora). *Brazilian Journal of Biology* 73(1): 185–194. <https://doi.org/10.1590/S1519-69842013000100020>
- Camargo I, Rios E, Cornejo-Latorre C, Álvarez-Castañeda, ST (2014) First Record of Leucism in the Genus *Peromyscus* (Mammalia: Rodentia). *Western North American Naturalist* 74(3): 366–368. <https://doi.org/10.3398/064.074.0301>
- Falcão FC (2014) First record of complete albinism in *Carollia perspicillata* (Chiroptera, Phyllostomidae). *Chiroptera Neotropical* 20(1): 1234– 2136.
- Fertl D, Rosel P (2002) Albinism. In: Perrin WF, Würsig B, Thewissen JGM (Eds) *Encyclopedia of Marine Mammals*, Academic Press, San Diego, 16–18.

- Geiger D, Pacheco SM (2006) Registro de albinismo parcial em *Nyctinomops laticaudatus* (E. Geoffroy, 1805) (Chiroptera: Molossidae) no sul do Brasil. *Chiroptera Neotropical* 12(1): 250–254.
- ICMBIO [Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis] (2017) Plano de pesquisa geossistemas ferruginosos da Floresta Nacional de Carajás: Temas prioritários para pesquisa e diretrizes para ampliação do conhecimento sobre os geossistemas ferruginosos da Floresta Nacional de Carajás e seu entorno. Brazil, 82 pp.
- Kumar K, Vaishnav V, Ojha AP, Parihar P, Barmera R, Parihar GR (2016) Occurrence of Albino *Gazella bennetti* in Viratra mata Oran (Sacred land) of Chohtan, Barmer (Thar Desert of Rajasthan). *India International Journal of Environmental & Agriculture Research* 2(11): 87–90.
- López-Baucells A, Mas M, Puig-Montserrat X, Flaquer C (2013) Hypopigmentation in vesperilionid bats: the first record of a leucistic soprano pipistrelle *Pipistrellus pygmaeus* Barbastella 6(1): 66–72. <https://doi.org/10.14709/BarbJ.6.1.2013.09>
- Lopucki R, Mróz I (2010) Cases of colouration anomalies in small mammals of Poland, and reasons for their incidence. *Annales Universitatis Mariae Curie-Sklodowska* 65(1): 67–76. <https://doi.org/10.2478/v10067-011-0006-4>
- Lucati F, López-Baucells A (2017) Chromatic disorders in bats: a review of pigmentation anomalies and the misuse of terms to describe them. *Mammal Review* 47: 112–123. <https://doi.org/10.1111/mam.12083>
- McCardle H (2012) Albinism in wild vertebrates. Texas State University, San Marcos 72pp.
- Miranda JMD, Kaku-Oliveira NY, Munster LC (2012) Primeiros dados de uma colônia reprodutiva de *Myotis levis* (I. Geoffroy, 1824) nos campos de Palmas, Paraná, Brasil (Vesperilionidae). *Chiroptera Neotropical* 16(2): 762–768.
- Moreira EC, Silva MCP, Veloso JG (1992) Albinismo em *Desmodus rotundus rotundus*, Chiroptera (E. Geoffroy, 1810). *Arquivo Brasileiro de Medicina Veterinária de Zootecnia* 44: 549–552.
- Murariu D, Chisamera G (2006) Partial albinism in noctule bat – *Nyctalus noctula* (Schreiber, 1774) (Mammalia: Chiroptera) from Romania. *Travaux du Muséum National d'Histoire Naturelle* 49: 353–357.
- Nascimento ACS, Dourado ACM, Trevelin LC, Bezerra AMR (2018) First record of total albinism in *Molossus molossus* (Chiroptera: Molossidae) from northeastern Brazil. *Boletim do Museu Paraense Emílio Goeldi. Ciências Naturais* 13: 273–277.
- Nedyalkov N, Koshev Y, Raykov I, Bardarov G (2014) Color variation of small mammals's (Mammalia: Rodentia and Insectivora) coats from Bulgaria. *North-western Journal of Zoology* 10(2): 1–4.
- Neves ACSA, Coutinho LC, Oliveira MB, Pessôa LM (2013) First report of partial albinism in genus *Thrichomys* (Rodentia: Echimyidae). *Papéis Avulsos de Zoologia* 54(9): 107–110. <https://doi.org/10.1590/0031-1049.2014.54.09>
- Oliveira HFM, Aguiar LMS (2008) A new case of complete albinism in a bat from Brazil. *Chiroptera Neotropical* 14: 421–423.
- Oliveira LS, Varjão LCG, Pereira LCM, Nicola-Pereira PA (2019) Primeiro registro de leucismo no cachorro-do-mato *Cerdocyon thous* (Linnaeus, 1766) (Carnivora: Canidae) no Brasil. *Biotemas* 32(1): 93–98. <https://doi.org/10.5007/2175-79252019v32n1p93>

- Ramasindrazana B, Wilkinson DA, Beral M, Dietrich, M (2014) An albino molossid bat from the southwestern Indian Ocean region Malagasy. *Nature* 8: 103–104.
- Rengifo EM, Linares V, Díaz F, Panafo J (2014) First record of albinism in the Black mastiff bat *Molossus rufus* E Geoffroy, 1805. *Chiroptera Neotropical* 20(2): 1288–1291.
- Rocha, PA, Feijó JA, Donato CR, Ferrari SF (2013) Leucism in Seba's short-tailed bat, *Carollia perspicillata* (Linnaeus, 1758), from a rock shelter in northeastern Brazil. *Chiroptera Neotropica* 19: 1151–1153.
- Romero V, Racines-Márquez CE, Britob J (2018) A short review and worldwide list of wild albino rodents with the first report of albinism in *Coendou rufescens* (Rodentia: Erethizontidae). *Mammalia* 82(5): 509–515. <https://doi.org/10.1515/mammalia-2017-0111>
- Rosa AR, Martorelli LFA, Almeida MF de, Aires CC (2017) Albinism in *Carollia perspicillata* (Chiroptera; Phyllostomidae), in the state of Rondônia, Brazil. A brief review of albinism in bats. *Revista Biotemas* 30: 71–77. <https://doi.org/10.5007/2175-7925.2017v30n3p71>
- Sandoval-Castilho J, Mariano-Melendez E, Villavicencio-Garayzar C (2006) New records of albinism in two elasmobranchs: the tiger shark, *Galeocerdo cuvier* and the giant electric ray, *Narcine entemedor*. *Cybium* 30(2): 191–192.
- Sayyed A, Mahabal A, Nale A (2015) A first record of albinism in Jungle Palm Squirrels *Funambulus tristriatus* (Rodentia: Sciuridea) from India. *ZOO's PRINT* 30(12): 18–19.
- Smirnov DG, Vekhnik VP, Kurmaeva NM, Baishev FZ (2014) The Detection of Partial Albinism at Three Species of Bats (Mammalia: Chiroptera) in European Part of Russia. *Open Journal of Animal Sciences* 4: 291–296. <https://doi.org/10.4236/ojas.2014.45037>
- Sodré MM, Uieda W, Baldim M (2004) First record of albinism in the bat *Eumops glaucinus* (Molossidae) from southeastern Brazil. *Chiroptera Neotropical* 10(1–2): 200–201.
- Tavares VC, Palmuti CFS, Gregorin R, Dornas TT (2012) Morcegos. In: Martins FD, Castilho A, Campos J, Hatano FM, Rolim SG (Eds) *Fauna da Floresta Nacional de Carajás: estudos sobre vertebrados terrestres*. Nitro Editora, São Paulo. 156–173.
- Treitler JT, López-Baucells A, Gomes FS, Tenaçol JF, Rocha R (2013) First record of a leucistic piebald *Phyllostomus discolor* (Chiroptera: Phyllostomidae). *Chiroptera Neotropical* 19: 1179–1181.
- Uieda W (2000) A review of complete albinism in bats with five new cases from Brazil. *Acta Chiropterologica* 1: 97–105.
- Uieda W (2001) Behavior of an albino vampire bat, *Desmodus rotundus* (E. Geoffroy) (Chiroptera, Phyllostomidae), in captivity. *Revista Brasileira de Zoologia* 18: 641–644. <https://doi.org/10.1590/S0101-81752001000200031>
- Xu X, Dong GX, Hu XS, Miao L, Zhang XL, Zhang DL, Yang HD, Zhang TY, Zou ZT, Zhang TT, Zhuang Y (2013) The genetic basis of white tigers. *Current Biology* 23: 1031–1035. <https://doi.org/10.1016/j.cub.2013.04.054>
- Zalapa RSS, Guerrero S, Romero-Almaraz ML, Sánchez-Hernández C (2016) Coloración atípica en murciélagos: frecuencia y fenotipos en Norte y Centroamérica e islas del Caribe y nuevos casos para México y Costa Rica. *Revista Mexicana de Biodiversidad* 87: 474–482. <https://doi.org/10.1016/j.rmb.2016.04.007>
- Zortéa M, Silva MC (2018) Albinism in the striped spear-nosed bat *Gardnerycteris crenulatum* (Chiroptera: Phyllostomidae) with an updated list of albino bats in the World. *Mammalia* 82: 78–84. <https://doi.org/10.1515/mammalia-2016-0080>

Two caves in western Honduras are important for bat conservation: first checklist of bats in Santa Bárbara

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Abstract

Caves are important reservoirs for species, including bats, but in Honduras there is little known information about these biodiverse ecosystems. We describe the importance of two caves in Ceguaca in western Honduras, based on the species richness of each cave. From December 2015 to May 2016, we used three mist-nets in seven journeys (74.27 mist-net/hours). We captured 139 bats belonging to 23 species of four families. We recorded 10 species in the crop of Quita Sueño, 10 in the cave of El Peñón, and 12 in the cave of Monte Grueso. In overall, 17% of the bats were captured in the crops of Quita Sueño, 62% in the cave of Monte Grueso, and 21% in the cave of El Peñón. About 9% of bat species of the total account for Honduras could be found in the cave of El Peñón, and 10% in the cave of Monte Grueso. If we consider the number of the species that have been recorded since 1979, between 20.9 and 49.2% of the expected species are still unrecorded, this suggests that despite low sampling effort there is a high diversity. However, the diversity of bats species using these caves embraced on the Tropical Dry Forest of Ceguaca is threatened by the fragmentation of the ecosystem due to the following reasons: intentional fires in the caves, extensive cattle raising, replacement of native plants with extensions of crops, and human-vampire conflicts (*Desmodus rotundus*).

Keywords

Ceguaca, Chiroptera, Mammalia, subterranean ecosystems, Tropical Dry Forest

Introduction

Caves are reservoirs for exceptional levels of endemic and threatened species, very high levels of genetic uniqueness, and they harbor unconventional taxa such as blind fishes, crustaceans, worms, and many other groups, including bats (Medellín et al. 2017). These ecosystems enclosed unique features for housing bats such as the complete or partial absence of light, constant temperature, and high air humidity (Deleva and Chaverri 2018).

Caves are also considered as a complex system with a variety of microclimates and roosting conditions for bats (Rodríguez-Durán 2009; Furey and Racey 2016). In other cases, bats are so abundant that they can significantly modify the caves by altering their microclimate conditions and providing important amounts of guano, which is an essential food source for trophic chains in most caves (Deleva and Chaverri 2018). In return, caves provide to bats a refuge from predators, inconstant weather, and a critical venue for social interactions, reproduction, hibernation, roosting and even alimentation (Furey and Racey 2016).

On the tropical regions of America, bat caves have been studied in Puerto Rico (Rodríguez-Durán 1998), West Indies (Rodríguez-Durán 2009), Mexico (Medellín et al. 2017), Brazil (Bichuette et al. 2018), and Costa Rica (Deleva and Chaverri 2018). Recently, in Honduras, there are records by Divoll and Buck (2013) of some bat species captured in caves: *Phyllostomus hastatus* and *Balantiopteryx io* captured in Río Masca, Piedra Cocha in Cortés (northern Honduras); and *Sturnira hondurensis* (referred as *S. ludovici*) in San Juancito, Parque Nacional La Tigra in Francisco Morazán (central Honduras). After those records, nothing is known about cave-dwelling bats in Honduras recently.

Chiroptera is the order with more mammalian species in Honduras, which is represented by approximately 114 species (Mora et al. 2018). Even though there is a high diversity of bats in Honduras, information of roosting sites, ecology, natural history, and systematic studies of each species is scarce (Turcios-Casco and Medina-Fitoria 2019). Herein, we describe the importance of two caves in Ceguaca, Santa Bárbara in western Honduras based on the number of species recorded and the historical records of Santa Bárbara. Finally, we provide the first checklist of bat species of Santa Bárbara with a discussion regarding the importance of this Tropical Dry Forest that embrace two caves for bat conservation.

Methods**Study area**

Three sites were studied in Ceguaca, Santa Bárbara in western Honduras (Figure 1). Site 1 in the locality of Quita Sueño (14°46'32"N, 88°12'00"W; 299 m), a crop of *Zea mays* (Poaceae) in the middle of a Tropical Dry Forest. One cave was studied in the locality of Monte Grueso (Figures 3A, 3B; 14°47'51"N, 88°12'19"W; 534 m) which

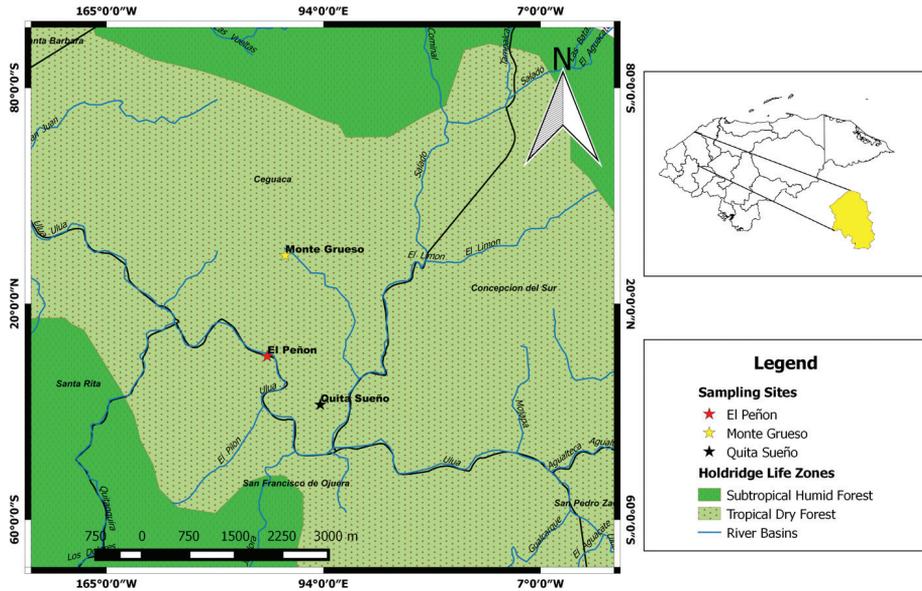


Figure 1. Study sites at Ceguaca, Santa Bárbara in western Honduras. Note that near the Tropical Dry Forest of Ceguaca there are Humid Subtropical Forest. Also, the cave of El Peñón is located near Río Ulúa, one of the longest rivers in Honduras. The life zones are based on Holdridge (1987). Map organized in QGIS software, version 2.18, Author: Diego Ordoñez.

has an entrance of approximately 5 m beneath the ground and a width of 10 m; the interior of the cave is divided into several branches, but the main branch is often used by bats for going in or out of the cave. The other cave was studied in the locality of El Peñón (Figure 4; $14^{\circ}46'57''\text{N}$, $88^{\circ}12'29''\text{W}$; 249 m), which is an agglomeration of 7 caves that are located riverside of the Río Ulúa; the entrance of the main cave has a height of approximately 3 m, and five meters of width, while the others have a height between 1 to 2 m, and a width of 2 to 3 m.

In general, 54.65% of the vegetation coverage of Ceguaca are crops and grasslands, and the other percentage include vegetation of Tropical Dry Forest and Secondary Deciduous Vegetation (ICF 2015). Ceguaca have a mid-annual precipitation of 1,900 mm, mid temperatures from 20–26 °C, and a mid-annual relative humidity from 78–79.4% (IH-CIT 2012; Suazo Oliva 2014). Based on Holdridge (1987), the sites studied in Ceguaca are embraced on a Tropical Dry Forest. Plants species include: *Aristolochia* (Aristolochiaceae), *Clusia* (Clusiaceae), *Crescentia* (Bignoniaceae), *Desmodium* and *Enterolobium*, (Fabaceae), *Ficus* (Moraceae), *Guazuma* (Malvaceae) *Ipomoea* (Convolvulaceae), *Mangifera* (Anacardiaceae), *Solanum* (Solanaceae); as well as crops of *Z. mays* (Poaceae) and cattle.

Sampling and bat identification

From December 2015 to May 2016 we made seven surveys, using three mist-nets (9 × 2.5 m and 14 × 2.5 m; mesh of 35 mm) that remained opened from 16:30 until

4:30 h and were checked every 20 minutes. In some occasions they remain opened for monitoring birds until 9:00, but the sampling effort was not taken in account. The positions of the mist-nets were selected according to Kunz and Kurta (1988), based on the vegetation, topography, and bodies of water.

We determined the sex of the bats according to Kunz et al. (1996), and the biological age according to Brunet-Rossinni and Wilkinson (2009). We took measurements with a vernier with spire Mitutoyo (505–675) to the closest 0.01 mm. Body mass was measured with a scale of 10 or 100 g. We followed Timm et al. (1999), Medellín et al. (2008), and Aguirre et al. (2009) for the taxonomical identification of the bats. Finally, we followed the taxonomical proposals of Velazco and Patterson (2013), Simmons (2005), and Baker et al. (2016).

The following specimens were sacrificed according to the guidelines of the use of mammals in wildlife research (Rabinowitz et al. 2000; Kingston 2016; Sikes et al. 2016), and deposited in the Zoological Collection in the Escuela Agrícola Panamericana (EAP): *Diphylla ecaudata* (CZB–2019–1; CZB–2019–7), *Glossophaga soricina* (CZB–2019–2), *Desmodus rotundus* (CZB–2019–8), *Sturnira parvidens* (CZB–2019–13), *Micronycteris schmidtorum* (CZB–2019–14), *Carollia subrufa* (CZB–2019–15), and *Dermanura phaeotis* (CZB–2019–15).

Species richness and sampling effort

We estimated species richness based on our sampling effort and the abundance of each species using the software EstimateSMac 910 with 100 randomizations to eliminate the specific order of the data (Colwell and Coddington 1994; Colwell 2013). We made accumulation curves using Chao 1, Chao 2, and ICE as species richness estimators, and the sampling effort was calculated by the time each mist-net remained open during each survey (Moreno 2001; Rex et al. 2008).

Results

Species richness and sampling effort

We accumulated 74.27 mist-net/hours in seven journeys and captured 139 bats (1.87 individuals per mist-net/hour) belonging to 23 species (0.31 species per mist-net/hour) of four families (0.05 families per mist-net/hour). The sampling effort was distributed as the following: 39% in the crops of Quita Sueño, 39% in the cave of Monte Grueso, and 22% in the cave of El Peñón. Based on Chao 1, Chao 2 and ICE (Table 2), we recorded between 41.71–64.95% of the expected species in Ceguaca, Santa Bárbara (Figure 2), 10 species in the crops of Quita Sueño, 10 in the cave of El Peñón, and 12 in the cave of Monte Grueso. We captured 17% of the individuals in the crop of Quita Sueño, 62% in the cave of Monte Grueso, and 21% in the cave of El Peñón.

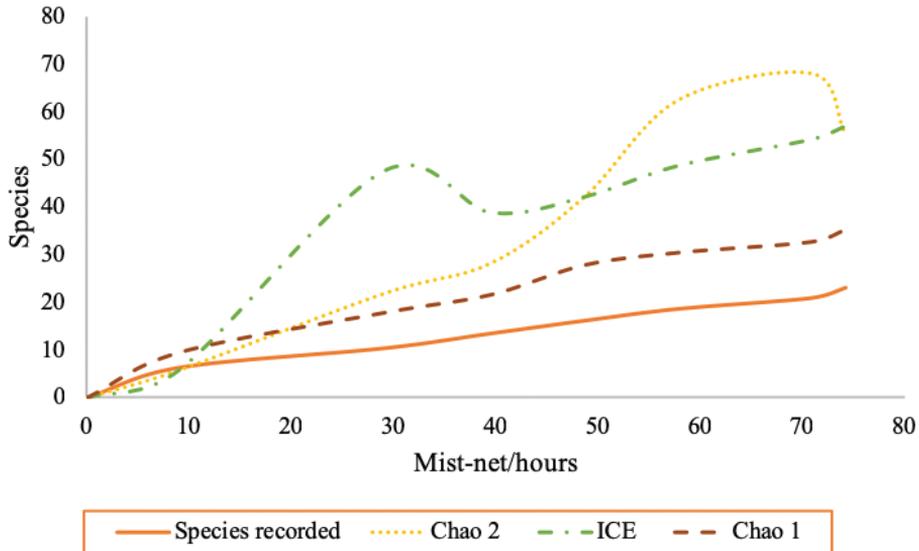


Figure 2. Species richness of bats in Ceguaca, Santa Bárbara based on the abundance of individuals captured during 2015 and 2016. Based on the estimator indexes, between 35.05 and 58.29% of bat species are still unrecorded. None of these accumulation curves reached an asymptote, which reflects that more sampling effort is needed in the area.

In general, the most common family was Phyllostomidae with 83.5% of the captures, followed by Emballonuridae (13.7%), Vespertilionidae (1.4%), and Mormoopidae (1.4%). The most common species was *Glossophaga soricina* with 28.8% of the captures, followed by *Artibeus jamaicensis* and *D. rotundus* with 27.3% of the captures and *Peropteryx macrotis* (12.2% of the captures). The most uncommon species with one capture each were: *Carollia subrufa*, *Chiroderma villosum*, *C. salvini*, *Dermanura phaeotis*, *D. tolteca*, *D. watsoni*, *Micronycteris microtis*, *M. schmidtorum*, *Myotis albes-cens*, *M. nigricans*, and *Sturnira parvidens* (Table 1).

Discussion

Caves in Honduras

Hernández (2015) mentioned that only two caves have approval for tourism in Honduras: Taulabé, Comayagua (central Honduras) and Talgua, Olancho (eastern Honduras), but unfortunately are altered by footpaths and lightings. In Honduras, there is a cave declared as a SICOM (abbreviation in Spanish for Site with Importance for the Conservation of Bats) by the RELCOM (abbreviation in Spanish for Latin American Network for the Conservation of Bats), and PCMH (abbreviation in Spanish for Program for the Conservation of Bats in Honduras) known as Hato Viejo, a cave in which



Figure 3. **A** the main entrance of the cave of Monte Grueso is a climb of approximately 5 meters drop. The inside of the cave is divided into tree branches. The photo was taken by Hefer Ávila **B** main branch of the cave, which is often used by the bats whenever they entered or exited the cave. Notice the guano in the floor of the cave of hematophagous bats (*D. ecaudata* and *D. rotundus*). The photo was taken by Manfredo Turcios Padgett.



Figure 4. Caves of El Peñon are located riverside of Río Ulúa. This agglomeration of 7 caves is located riverside of the Río Ulúa. During the surveys we found an owl (Strigidae) coming out of the cave when the activity of the bats (18:00) started, and also, we found remains of the food belonging to a presumably *Chironectes minimus* (Didelphidae), that use rocks of these caves to eat fishes. The photos were taken by Hefer Ávila.

is estimated that 35,000 bats inhabit on it, and still is the biggest aggregation of bats in a natural refuge for Honduras.

Conservation of bat caves

Most of the species recorded on this study are typically recorded on intervened habitats, with the exception of species of Phyllostominae which are considered bioindicators of

Table 1. Checklist of bat species in Santa Bárbara including historical records. Abbreviations are defined as: 1 = Davis (1970); 2 = Dolan and Carter (1979); 3 = Davis (1984); 4 = McCarthy et al. (1993); 5 = Mora et al. (2018); EP = Cave of El Peñón; MG = Cave in Monte Grueso; QS = crops in Quita Sueño. The abundance presented on this table is based on the individuals captured during this study.

No.	Family	Subfamily	Species, author and year	Site in which was captured and/or historical records	Abundance
1	Emballonuridae		<i>Balantiopteryx plicata</i> Peters, 1867	4	0
2			<i>Peropteryx macrotis</i> (Wagner, 1843)	EP	17
3			<i>Rhynchonycteris naso</i> (Wied-Neuwied, 1820)	EP (captured in the upstream of Río Ulúa)	2
4	Mormoopidae		<i>Preronotus personatus</i> (Wagner, 1843)	EP	2
5	Phyllostomidae	Micronycterinae	<i>Micronycteris microtis</i> Miller, 1898	MG	1
6			<i>Micronycteris schmidtorum</i> Sanborn, 1935	QS, 4	1
7		Lonchorhininae	<i>Lonchorhina aurita</i> Tomes, 1863	4, 5	0
8		Phyllostominae	<i>Phyllostomus hastatus</i> (Pallas, 1767)	4	0
9		Desmodontinae	<i>Desmodus rotundus</i> (É. Geoffroy, 1810)	EP, MG, QS	19
10			<i>Diphylla ecaudata</i> Spix, 1823	MG	5
11		Glossophaginae	<i>Glossophaga leachii</i> Gray, 1844	MG	2
12			<i>Glossophaga soricina</i> (Pallas, 1766)	MG, QS	40
13		Carollinae	<i>Carollia castanea</i> H. Allen, 1890	MG	3
14			<i>Carollia perspicillata</i> (Linnaeus, 1758)	MG	4
15			<i>Carollia sowelli</i> Baker, Solari & Hoffmann, 2002	EP, MG, QS	6
16			<i>Carollia subrufa</i> (Hahn, 1905)	EP	1
17		Stenodermatinae	<i>Artibeus jamaicensis</i> Leach, 1821	MG, QS, 1	19
18			<i>Artibeus lituratus</i> (Olfers, 1818)	MG, QS, 3	8
19			<i>Chiroderma salvini</i> Dobson, 1878	MG	1
20			<i>Chiroderma villosum</i> Peters, 1860	QS	1
21			<i>Dermanura phaeotis</i> (Miller, 1902)	QS	1
22			<i>Dermanura toteca</i> Saussure, 1860	QS	1
23			<i>Dermanura watsoni</i> (Thomas, 1901)	MG	2
24			<i>Sturnira parvidens</i> (Goldman 1917)	QS	1
25	Natalidae		<i>Natalus stramineus</i> Gray, 1838	4	0
26	Vespertilionidae	Myotinae	<i>Myotis albescens</i> (É. Geoffroy, 1806)	EP	1
27			<i>Myotis nigricans</i> (Schinz, 1821)	EP	1
28	Molossidae		<i>Molossus sinaloae</i> J.A. Allen, 1906	2	0
Total					139

well-conserved areas in the sites in which they forage and live, maintaining themselves fragile to the changes of their habitats (Emmons and Feer 1997). In accordance with Vela-Vargas and Pérez-Torres (2012), we identified that the Tropical Dry Forest in Ceguaca is undergoing deforestation, as well as in Dry Forests of Colombia, due to two main reasons: (1) fragmentation of the forest for crops of *Zea mays*, and (2) extensive livestock around remnants of Tropical Dry Forests.

If we considered the number of species reported by Mora et al. (2018), 9% of bat species of the total account for Honduras could be found in the cave of El Peñón, and 11% in the cave of Monte Grueso. We must consider that some of the species re-

Table 2. Accumulation of individuals, mist-net hours, species recorded and expected based on statistical estimators (Chao 2, ICE and Chao 1). Sample sites 1, 2 and 5 were in Quita Sueño; 3 and 4 were in Monte Grueso; 6 and 7 were in El Peñón.

Samples sites	Individuals	Mist-net/hours	Species recorded	Chao 2	ICE	Chao 1
1	19.86	9.03	6.29	5.81	5.81	9.52
2	39.71	29.03	10.33	21.82	47.62	17.85
3	59.57	39.54	13.43	28.22	38.89	21.67
4	79.43	48.715	16.11	42.16	42.22	27.92
5	99.29	57.89	18.57	62.48	48.77	30.53
6	119.14	70.97	20.86	68.02	54.33	32.81
7	139	74.27	23	55.14	57.13	35.41

corded only in the crops of Quita Sueño, could be also living in the caves of El Peñón and Monte Grueso (e.g. *Micronycteris schmidtorum*). Interestingly, if we considered the number of the species that have been recorded since 1979, between the 20.9 and 49.2% of the species expected are still unrecorded. Besides the diversity of bats that can be encountered in those caves and based on the comments of people of the community in Ceguaca, another importance of the cave of Monte Grueso is its use by pregnant females of White-tailed deer (*Odocoileus virginianus*, national mammal of Honduras) as a refuge. Remarkably, we recorded individuals of *C. perspicillata* and *G. soricina* returning at 4:00 h to the caves of Monte Grueso, as well as a record of *C. perspicillata* captured at 9:00 h returning from the crops of *Z. mays* to the Tropical Dry Forests in Quita Sueño (these records could reveal different activity patterns from the usual). Also, people from the community have encountered crystalized teeth of mammals, fragments of bones, obsidian, and artifacts such as vessels of clay with printings on them. These caves could not only represent an importance for the conservation of bats but also for knowing more about an unstudied culture of the area.

Based on Mora et al. (2018), *D. ecaudata* (Figure 7) is a species that is considered threatened in Honduras, specifically due to the human-vampire conflicts, and these caves could represent one of their principal refuges for conservation. Moreover, after the additional records of *M. schmidtorum* by McCarthy et al. (1993), there is one more record of this species in northwestern Honduras, in Cusuco National Park in Cortés by Estrada-Villegas et al. (2007). The record of this species might represent the first one after eight years. Additionally, we encountered pregnant females of *Pteronotus personatus* (May; Figure 5), *Peropteryx macrotis* (May), *Artibeus jamaicensis* (December), *Desmodus rotundus* (December), *Carollia subrufa* (May), and females in lactation period of *Myotis albescens* (May) and *Sturnira parvidens* (December). Reproductive males of *Artibeus lituratus* (December), *Desmodus rotundus* (January), *Glossophaga leachii* (January), and *G. soricina* (January), were also recorded. Importantly, these caves could represent their primary roosting site and nursery when they have newborns. Destroying and not protecting those caves could determine the decrease of the populations of bats specialized in seed dispersal, pollination (Figure 6) as well as controlling pest species (e.g. insects of crops) in Ceguaca.



Figure 5. Pregnant female of *Pteronotus personatus* in the Cave of El Peñón during the survey of May 8, 2016. The photo was taken by Hefer Ávila.



Figure 6. An adult female of *Glossophaga soricina* returning to the cave of Monte Grueso after the pollination of certain species of plants. During these surveys, we found trees with opened flowers of *Crescentia alata* (Bignoniaceae). The photo was taken by Hefer Ávila.



Figure 7. Adult female of *Diphylla ecaudata* captured in the cave of Monte Grueso when leaving the cave for searching food. The shelters of this unstudied species in Honduras in Monte Grueso could represent an important site for their conservation. The photo was taken by Hefer Ávila.

Importance of bat caves in Ceguaca

We encountered 0.30 species per mist-nets/hours on those located in the Tropical Dry Forest in Ceguaca. This result is comparably higher to other studies in different Dry Forests of America, for example: Chávez and Ceballos (2001) reported 15 species in a Dry Forest in Jalisco, México with a sampling effort of 410 mist-net/hours (0.04 species per mist-net/hours); Medina et al. (2004) reported 24 species in a Dry Forest in Rivas, Nicaragua, with a sampling effort of 5376 mist-net/hours (0.005 species per mist-net/hours); Vela-Vargas and Pérez-Torres (2012) reported 20 species in a Tropical Dry Forest in El Refugio, Colombia, with a sampling effort of 4673 mist-net/hours (0.004 species per mist-net/hours); and Ramírez Fráncel et al. (2015) recorded 10 species with a sampling effort of 7776 mist-net/hours (0.001 species per mist-net/hours) in Tolima, Colombia. Although, there is a low sampling effort in our study, the richness represented is higher in comparison to other studies with a higher sampling effort. However, the asymptote of the accumulation curves was not reached, and more effort is needed to support the high diversity estimated.

The Dry Forests of Honduras are one of the most threatened ecosystems due to the continuous fragmentation processes of habitats that are related to the use of soil in crops, cattle, and other agricultural systems. We conclude that the fragmentation of ecosystems

in Ceguaca, is due to the following reasons: extensive cattle raising, replacement of native plants with extensions of grasslands for crops, and human-vampire conflicts (*D. rotundus*) which lead to intentional fires in the caves caused by the demonized concept of bats in the area, for example, the caves of El Peñón have been set on fire at least three times in the last five years. This study represents the first preliminary inventory of those caves on that Tropical Dry Forest. Thus, we strongly recommend the continuity of this study for a longer period of time, including wet and dry seasons, with a greater sampling effort. Furthermore, other methodologies to study bats must be included: harp nets, pulley nets, and vocalization recording. Finally, with this baseline, we hope to encourage the PCMH to declare these diverse caves as AICOMS (abbreviation in Spanish for Areas with Importance for the Conservation of Bats) for their conservation.

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References

- Aguirre L, Vargas A, Solari S (2009) Clave de campo para la identificación de los murciélagos de Bolivia. Centro de Estudios en Biología Teórica y Aplicada (BIOTA), Cochabamba, 38 pp.
- Baker RJ, Solari S, Cirranello A, Simmons NB (2016) Higher level classification of phyllostomid bats with a summary of DNA synapomorphies. *Acta Chiropterologica* 18: 1–18. <https://doi.org/10.3161/15081109ACC2016.18.1.001>
- Bichuette ME, do Amaral Gimenez E, Arnone IS, Trajano E (2018) An important site for conservation of bats in Brazil: Passa Três cave, Sao Domingos karst area, with an updated checklist for Distrito Federal (DF) and Goiás state. *Subterranean Biology* 28: 39–51. <https://doi.org/10.3897/subtbiol.28.31801>
- Brunet-Rossinni A, Wilkinson G (2009) Methods for age estimation and the study of senescence in bats. In: Kunz TH, Parsons S (Eds) *Ecological and behavioral methods for the study of bats*. The John Hopkins University Press, Baltimore: 315–325.
- Chávez C, Ceballos G (2001) Diversidad y abundancia de murciélagos en selvas secas de estacionalidad contrastante en el oeste de México. *Revista Mexicana de Mastozoología* 5: 27–44.

- Colwell RK (2013) EstimatesS (version 9): statistical estimation of species richness and shared species from sample. <http://www.purl.oclc.org/estimates> [Accessed at January 2018]
- Colwell RK, Coddington JA (1994) Estimating terrestrial biodiversity through extrapolation. *Philosophical Transactions: Biological Sciences* 345(1311): 101–118. <https://doi.org/10.1098/rstb.1994.0091>
- Davis WB (1970) The large fruit bats (genus *Artibeus*) of Middle America, with a review of the *Artibeus jamaicensis* complex. *Journal of Mammalogy* 51(1): 105–122. <https://doi.org/10.2307/1378537>
- Davis WB (1984) Review of the large fruit-eating bats of the *Artibeus* “*lituratus*” complex (Chiroptera: Phyllostomidae) in Middle America. *Occasional Papers The Museum Texas Tech University* 93: 1–16. <https://doi.org/10.5962/bhl.title.156552>
- Deleva S, Chaverri G (2018) Diversity and conservation of cave-dwelling bats in the Brunca Region of Costa Rica. *Diversity* 10(2): 1–15. <https://doi.org/10.3390/d10020043>
- Dolan PG, Carter DC (1979) Distributional notes and records for Middle American Chiroptera. *Journal Mammalogy* 60(3): 644–649. <https://doi.org/10.2307/1380115>
- Divoll TJ, Buck DG (2013) Noteworthy field observations of cave roosting bats in Honduras. *Mastozoología neotropical* 20(1): 149–151.
- Emmons L, Feer F (1997) Neotropical rainforest mammals, a field guide. The University of Chicago Press, Chicago, 368 pp.
- Estrada-Villegas SL, Allen M, García M, Hoffman M, Munroe ML (2007) Bat assemblage composition and diversity of the Cusuco National Park, Honduras. *Operation Wallacea, San Pedro Sula*, 6 pp.
- Furey NM, Racey PA (2016) Conservation ecology of cave bats. In: Voigt C, Kingston T (Eds) *Bats in the anthropocene: conservation of bats in a changing world*. Springer, Cham: 463–500. https://doi.org/10.1007/978-3-319-25220-9_15
- Hernández DJ (2015) Programa para la conservación de los murciélagos de Honduras (PCMH). In: Rodríguez Herrera B, Sánchez R (Eds) *Estrategia centroamericana para la conservación de los murciélagos*. Sistema Editorial y de Difusión de la Investigación, San José, 41–55.
- Holdridge L (1987) *Ecología basada en zonas de vida* (Trad. H. Jiménez Saa). Instituto Interamericano de Cooperación para la Agricultura (IICA), San José, 216 pp.
- ICF (2015) *Atlas municipal forestal y cobertura de la tierra*. Municipio de Ceguaca, Santa Bárbara. Comayagüela, MDC, 43 pp.
- IHCIT (2012) *Atlas climático y de gestión de riesgo de Honduras*. Cooperación Suiza en América Central, Tegucigalpa. MDC, 148 pp.
- Kingston T (2016) Bats. In: Larsen L (Ed.) *Core standardized methods for rapid biological field assessment*. Conservation International, Virginia, 59–82.
- Kunz TH, Kurta A (1988) Capture methods and holding devices. In: Kunz TH (Ed.) *Ecological and behavioral methods for the study of bats*. Smithsonian Institution Press, Washington DC, 1–28.
- Kunz T, Wemmer C, Hayssen V (1996) Sex, age, and reproductive condition of mammals. In: Wilson DE, Nichols J, Rudrin R, Cole R, Foster M (Eds) *Measuring and monitoring biological diversity*. Smithsonian Institution Press, Washington DC, 279–290.

- McCarthy TJ, Davis WB, Hill JE, Jones Jr. JK, Cruz GA (1993) Bat (Mammalia: Chiroptera) records, early collectors, and faunal lists for northern Central America. *Annals Carnegie Museum* 62: 191–228.
- Medellín R, Arita H, Sánchez O (2008) Identificación de los murciélagos de México, clave de campo. Ed. 2. Universidad Nacional Autónoma de México (UNAM), Distrito Federal, 83 pp.
- Medellín RA, Wiederholt R, Lopez-Hoffman L (2017) Conservation relevance of bat caves for biodiversity and ecosystem services. *Biological Conservation* 211: 45–50. <https://doi.org/10.1016/j.biocon.2017.01.012>
- Medina A, Harvey C, Sánchez D, Vilchez S, Hernández B (2004) Diversidad y composición de Chiropteros en un paisaje fragmentado de bosque seco en Rivas, Nicaragua. *Revista Encuentro* 68: 24–43. <https://doi.org/10.5377/encuentro.v0i68.4255>
- Mora JM, López LI, Espinal M, Marineros L, Ruedas L (2018) Diversidad y Conservación de los murciélagos de Honduras. Master Print S. de R.L., Tegucigalpa, 300 pp.
- Moreno CE (2001) Métodos para medir biodiversidad. M&T-Manuales y Tesis SEA, Zaragoza, 84 pp.
- Rabinowitz A, Hart J, White L (2000) Information from dead animals and their curation. In: White LJ, Edwards A (Eds) *Conservation research in the African rain forests: a technical handbook*. Wildlife Conservation Society, New York, 185–195.
- Ramírez Fráncel LA, Rivas Pava M del P, Reinos Flórez G (2015) Murciélagos insectívoros de dos fragmentos de bosque seco tropical, Tolima-Colombia. *Revista de la Asociación Colombiana de Ciencias Biológicas* 27: 32–41.
- Rex K, Kelm DH, Wiesner K, Kunz TH, Voigt CC (2008) Species richness and structure of three Neotropical bat assemblages. *Biological Journal of the Linnean Society* 94(3): 617–629. <https://doi.org/10.1111/j.1095-8312.2008.01014.x>
- Rodríguez-Durán A (1998) Nonrandom aggregations and distribution of cave-dwelling bats in Puerto Rico. *Journal of Mammalogy* 79: 141–146. <https://doi.org/10.2307/1382848>
- Rodríguez-Durán A (2009) Bat assemblages in the West Indies: the role of caves. In: Fleming TH, Racey PA (Eds) *Island bats: evolution, ecology and conservation*. University of Chicago Press, Chicago, 279–290.
- Sikes RS, Animal Care and Use Committee of the American Society of Mammalogists (2016) Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *Journal of Mammalogy*. 97: 663–688. <https://doi.org/10.1093/jmammal/gyw078>
- Simmons N (2005) Order Chiroptera. In: Wilson DE, Reeder DM (Eds) *Mammal species of the world: a taxonomic and geographic reference* (3rd edn). The Johns Hopkins University Press, Baltimore, 312–529.
- Suazo Oliva EA (2014) Propuesta para la implementación de un manual de procedimientos administrativos de la municipalidad de Ceguaca, departamento de Santa Bárbara. Tesis MSc, Universidad Nacional Autónoma de Honduras, Tegucigalpa, M.D.C., 113 pp.
- Timm RM, LaVal RK, Rodríguez-H B (1999) Clave de campo para los murciélagos de Costa Rica. *Brenesia* 52: 1–32.

- Turcios-Casco MA, Medina-Fitoria A (2019) Occurrence of *Hylonycteris underwoodi* (Chiroptera, Phyllostomidae) and *Thyroptera tricolor* (Chiroptera, Thyropteridae) in Honduras. *Studies on Neotropical Fauna and Environment* 54(1): 69–72. <https://doi.org/10.1080/01650521.2018.1544205>
- Vela-Vargas M, Pérez-Torres J (2012) Murciélagos asociados a remanentes de bosque seco tropical en un sistema de ganadería extensiva (Colombia). *Chiroptera Neotropical* 18(1): 1089–1100.
- Velazco PM, Patterson BD (2013) Diversification of the yellow-shouldered bats, genus *Sturnira* (Chiroptera, Phyllostomidae), in the new world tropics. *Molecular Phylogenetics and Evolution* 68(3): 683–698. <https://doi.org/10.1016/j.ympev.2013.04.016>

Cave millipede diversity with the description of six new species from Guangxi, China

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Abstract

We synthesized the current knowledge of cave-dwelling millipede diversity from Guangxi Zhuang Autonomous Region (Guangxi), South China Karst, China and described six new millipede species from four caves from the Guilin area, northeastern Guangxi. Fifty-two cave-dwelling millipedes are known for the region consisting of 38 troglobionts and 14 troglaphiles. Of the troglobionts, 24 are presently considered single-cave endemics. New species described here include *Hyleoglomeris rukouqu* **sp. nov.** and *Hyleoglomeris xuxiakei* **sp. nov.** (Family Glomeridae), *Hylomus yuani* **sp. nov.** (Family Paradoxosomatidae), *Eutrichodesmus jianjia* **sp. nov.** (Family Haplodesmidae), *Trichopeltis liangfengdong* **sp. nov.** (Family Cryptodesmidae), and *Glyphiulus maocun* **sp. nov.** (Family Cambalopsidae). Our work also resulted in range expansions of *Pacidesmus trifidus* Golovatch & Geoffroy, 2014, *Blingulus sinicus* Zhang & Li, 1981 and *Glyphiulus melanoporus* Mauriès & Nguyen Duy-Jacquemin, 1997. As with many hypogean animals in Southeast Asia, intensive human activities threaten the persistence of both cave habitats and species. We provide both assessments on the newly described species' distributions and recommendations for future research and conservation efforts.

Keywords

Single cave endemic, disturbance relict, cave conservation

Introduction

Southeast Asia is considered the most biologically diverse for troglomorphic animals among the well-sampled tropical regions of the globe (Clements et al. 2006). Yet despite research conducted over the past three decades, few areas in Southeast Asia have been sufficiently investigated and knowledge of cave biological diversity and ecological processes is limited (Deharveng and Bedos 2000). The expansive South China Karst represents one of these regions (Clarke 2006, Chen et al. 2001). Among the four administrative units in China where this formation occurs, Guangxi is considered the most taxonomically well-studied region. At least 100 troglomorphic (subterranean-adapted) arthropods have been identified from Guangxi (Wynne, unpublished data). Most have been identified as short-range endemic species (Tian 2011, Deharveng et al. 2008) with more than half (or 58 species) considered single cave endemics (Gao et al. 2018).

Common in cave-arthropod communities, millipedes represent a dominant and widespread group in southern China (Golovatch 2015, Liu et al. 2017a). Approximately 150 cave-dwelling millipedes have been described from China (Golovatch 2015, Liu unpublished data) with 52 species known from Guangxi. Of these, 38 species are troglobionts with 24 identified as single-cave endemics (Table 1). Fig. 1 summarizes millipede diversity by family with Cambalopsidae (14 species), Glomeridae (11) and Paradoxosomatidae (10) being among the most speciose groups.

In the paper, we describe six new species (representing five families, Glomeridae, Paradoxosomatidae, Haplodesmidae, Cryptodesmidae and Cambalopsidae), as well as discuss range expansions of three additional cave-dwelling species. We have also summarized the cave-dwelling millipedes known to Guangxi, their known distributions, and provide recommendations to guide future research and management efforts.

Material and methods

Study area

Located in southwest China, Guangxi encompasses 236,700 km². Once an ancient shallow sea during the middle Cambrian to Late Triassic periods, this region is now largely characterized by a massive karst (limestone) stratum over 10,000 m thick (Cao et al. 2007) with steep-sided mountains called *fenglin* or “tower karst” protruding skyward. As a result of the subtropical climate and rock stratum, Guangxi supports at least 564 known caves (Yuanhai Zhang, pers. comm. 2019).

We sampled four caves in the northeastern most extent of Guangxi within a 30 km radius of the city of Guilin, China (Fig. 2). Caves were selected based upon two criteria – sufficient length to support deep zone conditions, and the availability of a current cave map. Cave deep zones are defined as completely dark region with relatively stable temperature, low to no airflow, and a near water saturated atmosphere with a

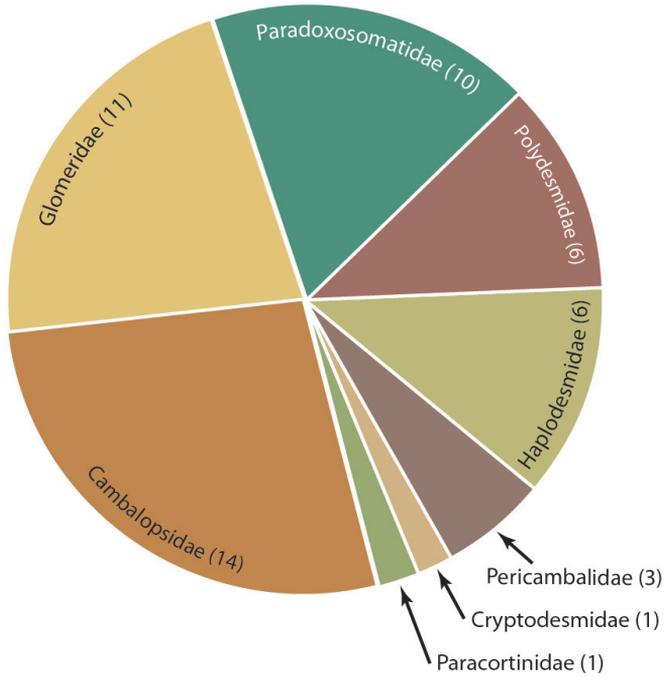


Figure 1. Species richness (total number of species provided in parentheses) for the eight known families of subterranean-dwelling millipedes from Guangxi, China.

negligible evaporation rate (Howarth 1980, 1982). While we recognize other factors may contribute to the occurrence of cave deep zones (e.g., mazy and/or constricted passageways, small or partially rock-fall obstructed entrances, and cave structure in general), we used this criterion because logistics prevented us from selecting study sites based upon site visit evaluations. This work represents the first effort to sample these caves for subterranean-adapted arthropods.

All caves occurred at low elevations within tower karst formations. While extensive agriculture, as well as rural village and suburban habitation characterized the surrounding lowlands, vegetation on the tower karst represented a marginally disturbed combination of native and introduced plant and tree species.

Literature review and synthesis

Based on our knowledge of the South China Karst (SCK) cave-dwelling millipede literature, we summarized the total number of caves sampled and cave-dwelling millipedes detected for Guangxi. For selected species occurring within two or more caves, we determined maximum distances between caves and, in some cases, average distances across all caves using ArcGIS 10.6.1.

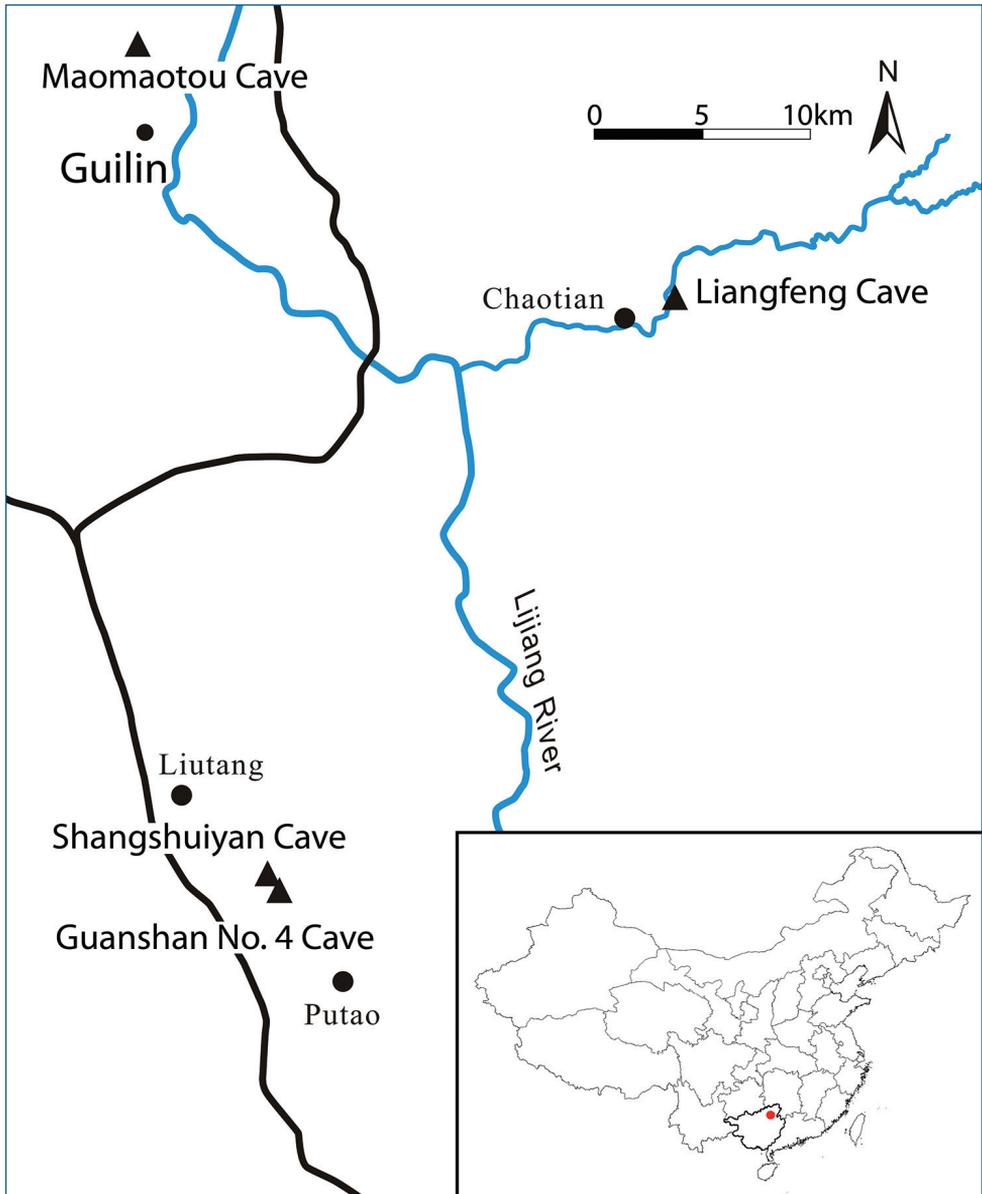


Figure 2. Study area with general locations of the four study caves (black triangles). Locator map depicts the Guilin area (red dot), Guangxi (bold black outline), China.

Field sampling

We hand collected cave-dwelling arthropods at four caves from 15 to 18 November 2016. Approximately eight hours (2 observers at 4 hours per observer) was spent conducting direct intuitive searches within estimated deep zones of each cave. We examined bat guano, dead insects, mud banks and floors, flood detritus, or vegetation

brought in by humans and deposited within the cave. Given the four caves varied in size and the diversity of arthropods encountered, the areal extent sampled varied. We also sampled the vegetation within the entrance of Liangfeng Cave applying a direct intuitive search approach. For all four caves, we also opportunistically collected arthropods as encountered while transiting from entrance to estimated deep zones. Sampling methods were applied *sensu stricto* Wynne et al. (2019).

Analysis and preparation

All specimens examined in this study were collected by the second author in the Guilin area, Guangxi, southern China and preserved in 95% ethanol. Holotypes and paratypes are deposited in the Zoological Collection of the South China Agricultural University, Guangzhou, Guangdong Province, China (SCAU). Detailed examination of characters and dissections were performed using a Leica S8 APO stereo microscope. Line drawings were prepared with a ZEISS Axioskop40 microscope with a camera lucida attached. Photographs were taken using a Keyence VHX-5000 digital microscope, and further edited using Adobe Photoshop CS5.

Cave locations

We recognize standard practice for new species description is to provide sample locality information including geographical data to facilitate future collecting, interpretation and research. Because caves often represent sensitive resources, we provided general geographical information and offset the latitude and longitude coordinates by ~1 km. This level of detail is sufficient for future comparative studies, while protecting the precise location of our study caves.

Terminology

Cave ecosystems typically consist of four zonal environments (Howarth 1980, 1983): (1) *entrance zone* – combination of surface and cave environmental conditions; (2) *twilight zone* – both diminished light conditions and influence of surface environment; (3) *transition zone* – aphotic, yet barometric and diurnal shifts are observed at a significantly diminished rate approaching near stable climatic conditions; and, (4) *deep zone* – complete darkness, high environmental stability, constant temperature, and near water-saturated atmosphere with low to no airflow (typically occurs in the deepest portion of the cave). While there are four primary cave specific functional groups generally recognized, the specimens discussed are *troglophilic* (subterranean-adapted) organisms known as *troglobionts* (Sket 2008). These animals are obligate cave dwellers that require the stable environmental conditions of the deep zone to complete their life cycle and exhibit morphological characteristics indicative of cave adaptation. We also

Table 1. Fifty-two known cave-dwelling millipede species from Guangxi, South China Karst, China. ‘Order: Family: Species’, ‘Functional Group’ (TB=presumed troglobiont, TP=troglophile), number of caves (# Caves) to suggest a level of potential endemism), and main Reference(s) are provided. As troglophiles are expected to have regional distributions, the number of caves where TPs were detected is not included.

Order: Family: Species	Functional group	# Caves	Reference(s)
Glomerida: Glomeridae			
<i>Hyleoglomeris baxian</i> Liu & Tian, 2015	TB	1	Liu and Tian 2015a
<i>Hyleoglomeris curtisulcata</i> Golovatch, Liu & Geoffroy, 2012	TB	1	Golovatch et al. 2012a
<i>Hyleoglomeris grandis</i> Liu & Tian, 2015	TB	1	Liu and Tian 2015a
<i>Hyleoglomeris heshang</i> Golovatch, Liu & Geoffroy, 2012	TB	1	Golovatch et al. 2012a
<i>Hyleoglomeris kunnan</i> Golovatch, Liu & Geoffroy, 2012	TB	1	Golovatch et al. 2012a
<i>Hyleoglomeris lii</i> Golovatch, Liu & Geoffroy, 2012	TP	–	Golovatch et al. 2012a
<i>Hyleoglomeris mashanorum</i> Golovatch, Liu & Geoffroy, 2012	TB	1	Golovatch et al. 2012a
<i>Hyleoglomeris mulunensis</i> Golovatch, Liu & Geoffroy, 2012	TB	1	Golovatch et al. 2012a
<i>Hyleoglomeris rukouqu</i> sp. nov.	TP	–	This study
<i>Hyleoglomeris xueju</i> Golovatch, Liu & Geoffroy, 2012	TB	1	Golovatch et al. 2012a
<i>Hyleoglomeris xuxiakei</i> sp. nov.	TB	1	This study
Polydesmida: Cryptodesmidae			
<i>Trichopeltis liangfengdong</i> sp. nov.	TB	1	This study
Haplodesmidae			
<i>Eutrichodesmus distinctus</i> Golovatch, Geoffroy, Mauriès & VandenSpiegel, 2009	TB	1	Golovatch et al. 2009a
<i>Eutrichodesmus jianjia</i> sp. nov.	TB	1	This study
<i>Eutrichodesmus latus</i> Golovatch, Geoffroy, Mauriès & VandenSpiegel, 2009	TB	4	Golovatch et al. 2009b
<i>Eutrichodesmus lipsae</i> Golovatch, Geoffroy, Mauriès & VandenSpiegel, 2015	TB	1	Golovatch et al. 2015
<i>Eutrichodesmus planatus</i> Liu & Tian, 2013	TB	1	Liu and Tian 2013
<i>Eutrichodesmus similis</i> Golovatch, Geoffroy, Mauriès & VandenSpiegel, 2009	TB	2	Golovatch et al. 2009b
Paradoxosomatidae			
<i>Piccola golovatchi</i> Liu & Tian, 2015	TB	1	Liu and Tian 2015b
<i>Hylomus longispinus</i> (Loksa, 1960)	TB	1	Loksa 1960, Golovatch et al. 2010a, Srisonchai et al. 2018
<i>Hylomus lui</i> (Golovatch, Li, Liu & Geoffroy, 2012)	TB	1	Golovatch et al. 2012b, Srisonchai et al. 2018
<i>Hylomus nodulosus</i> (Liu, Golovatch & Tian, 2014)	TP	–	Liu et al. 2014, Srisonchai et al. 2018
<i>Hylomus phasmoides</i> (Liu, Golovatch & Tian, 2016)	TB	1	Liu et al. 2016, Srisonchai et al. 2018
<i>Hylomus scolopendroides</i> Golovatch, Geoffroy & Mauriès, 2010	TB	4	Golovatch et al. 2010a, Liu et al. 2014, Srisonchai et al. 2018
<i>Hylomus scutigeroideus</i> Golovatch, Geoffroy & Mauriès, 2010	TB	6	Golovatch et al. 2010a, Liu et al. 2014, Srisonchai et al. 2018
<i>Hylomus spinissimus</i> (Golovatch, Li, Liu & Geoffroy, 2012)	TB	1	Golovatch et al. 2012b, Srisonchai et al. 2018
<i>Hylomus variabilis</i> (Liu, Golovatch & Tian, 2016)	TP	–	Liu et al. 2016, Srisonchai et al. 2018
<i>Hylomus yuani</i> sp. nov.	TB	1	This study
Polydesmidae			
<i>Epanerchodus orientalis</i> Attems, 1901	TP	–	Golovatch et al. 2012c
<i>Pacidesmus armatus</i> Golovatch, Geoffroy & Mauriès, 2010	TB	3	Golovatch et al. 2010b
<i>Pacidesmus bedosae</i> Golovatch, Geoffroy & Mauriès, 2010	TB	3	Golovatch et al. 2010b
<i>Pacidesmus bifidus</i> Golovatch & Geoffroy, 2014	TB	1	Golovatch and Geoffroy 2014
<i>Pacidesmus tiani</i> Golovatch, Geoffroy & Mauriès, 2010	TB	2	Golovatch et al. 2010b
<i>Pacidesmus trifidus</i> Golovatch & Geoffroy, 2014	TB	4	Golovatch and Geoffroy 2014, This study

Order: Family: Species	Functional group	# Caves	Reference(s)
Callipodida: Paracortinidae			
<i>Paracortina yinae</i> Liu & Tian, 2015	TP	–	Liu and Tian 2015c
Spirostreptida: Pericambalidae			
<i>Bilingulus sinicus</i> Zhang & Li, 1981	TP	–	Zhang and Li 1981, This study
<i>Parabilingulus aramulus</i> Zhang & Li, 1981	TP	–	Zhang and Li 1981
<i>Parabilingulus simplicius</i> Mauriès & Nguyen Duy-Jacquemin, 1997	TP	–	Mauriès and Nguyen Duy-Jacquemin 1997
Cambalopsidae			
<i>Hypocambala polytricha</i> Golovatch, Geoffroy, Mauriès & VandenSpiegel, 2011	TB	1	Golovatch et al. 2011a
<i>Glyphiulus acutus</i> Golovatch, Geoffroy, Mauriès & VandenSpiegel, 2011	TB?	2	Golovatch et al. 2011b
<i>Glyphiulus calceus</i> Jiang, Guo, Chen & Xie, 2018	TP	–	Jiang et al. 2018
<i>Glyphiulus difficilis</i> Golovatch, Geoffroy, Mauriès & VandenSpiegel, 2011	TB	2	Golovatch et al. 2011b
<i>Glyphiulus echinoides</i> Golovatch, Geoffroy, Mauriès & VandenSpiegel, 2011	TP	–	Golovatch et al. 2011c
<i>Glyphiulus foetidus</i> Jiang, Guo, Chen & Xie, 2018	TP	–	Jiang et al. 2018
<i>Glyphiulus impletus</i> Jiang, Guo, Chen & Xie, 2018	TP	–	Jiang et al. 2018
<i>Glyphiulus maocun</i> sp. nov.	TB	1	This study
<i>Glyphiulus melanoporus</i> Mauriès & Nguyen Duy-Jacquemin, 1997	TP	–	Mauriès and Nguyen Duy-Jacquemin 1997, This study
<i>Glyphiulus mulunensis</i> Golovatch, Geoffroy, Mauriès & VandenSpiegel, 2011	TB	2	Golovatch et al. 2011b
<i>Glyphiulus paramulunensis</i> Golovatch, Geoffroy, Mauriès & VandenSpiegel, 2011	TB	2	Golovatch et al. 2011b
<i>Glyphiulus proximus</i> Golovatch, Geoffroy, Mauriès & VandenSpiegel, 2011	TB	2	Golovatch et al. 2011b
<i>Glyphiulus speobius</i> Golovatch, Geoffroy, Mauriès & VandenSpiegel, 2011	TB	2	Golovatch et al. 2011b
<i>Glyphiulus tiani</i> Golovatch, Geoffroy, Mauriès & VandenSpiegel, 2011	TB	1	Golovatch et al. 2011b

reference *troglophiles* (or *troglophilous* organisms) – non-troglobiontic animals that occur facultatively within caves and complete their life cycles there, but also exist in similar cave-like habitats on the surface (Barr 1967, Howarth 1983, Sket 2008). Terms used for taxonomic descriptions follow Minelli (2015).

Results

Since ~1960, at least 49 caves have been sampled for cave-dwelling millipedes. Of these, nearly half (24 caves) supported single-cave endemic troglobionts, nine caves supported at least two troglobitic millipede species, and three caves (present study; Table 2) contained three troglobionts. For species occurring in two or more caves, maximum distance between caves ranged from 11.22 km for *Glyphiulus speobius* to 137.27 km for *Hylomus scutigeroideus* (Table 3).

A total of nine cave-dwelling millipede species were collected from four caves near Guilin (Table 2). Four millipede species were detected in Liangfeng Cave, the other three caves contained three species each. Of the six undescribed species, four are troglo-

Table 2. Nine cave-dwelling millipedes including both troglotic and potentially relict species from four caves near Guilin, Guangxi, South China Karst, China.

Species	Maomaotou cave	Guanshan No. 4 cave	Shangshuiyan cave	Liangfeng cave
<i>Hyleoglomeris rukouqu</i> sp. nov.	–	–	×	–
<i>Hyleoglomeris xuxiakei</i> sp. nov.	–	×	–	–
<i>Hylomus yuani</i> sp. nov.	–	–	–	×
<i>Eutrichodesmus jianjia</i> sp. nov.	–	×	–	–
<i>Trichopeltis liangfengdong</i> sp. nov.	–	–	–	×
<i>Pacidesmus trifidus</i>	×	×	×	–
<i>Blingulus sinicus</i>	×	–	×	×
<i>Glyphiulus melanoporus</i>	×	–	–	–
<i>Glyphiulus maocun</i> sp. nov.	–	–	–	×

Table 3. Fourteen troglomorphic millipede species detected in more than one cave, Guangxi, South China Karst, China. ‘Order: Family: Species’, number of caves (# Caves) to suggest a level of potential endemism), and maximum distance between caves in kilometers (Max Dist.) are provided. *Indicates coordinate data for all caves was unavailable for that species. ‘?’ denotes maximum distance was indeterminate due to lack of cave coordinate data. ‘Landscape features’ potentially affecting distribution of caves separated by more than 10 km.

Order: Family: Species	# Caves	Max dist. (km)	Landscape features
Polydesmida: Haplodesmidae			
* <i>Eutrichodesmus latus</i>	4	14.32	Maximum distance between two caves provided, the third cave occurs in between the two.
* <i>Eutrichodesmus similis</i>	2	?	
Paradoxosomatida			
<i>Hylomus scolopendroides</i>	4	81.27	Three caves clustered within 21.24 km; one cave separated by Diaojiang, Hongshui and Jincheng Rivers and lowland areas
<i>Hylomus scutigeroideus</i>	6	137.6	One cave separated from others by Hongshui River; others may occur in same formation
Polydesmidae			
* <i>Pacidesmus armatus</i>	3	12.4	Two of three caves separated by lowland areas
* <i>Pacidesmus bedosae</i>	3	2.67	
<i>Pacidesmus tiani</i>	2	1.88	
<i>Pacidesmus trifidus</i>	4	59.7	Separated by lowland areas
Spirostreptida: Cambalopsidae			
<i>Glyphiulus acutus</i>	2	8.85	
<i>Glyphiulus difficilis</i>	2	9.32	
<i>Glyphiulus mulunensis</i>	2	7.77	
<i>Glyphiulus paramulunensis</i>	2	6.67	
<i>Glyphiulus proximus</i>	2	2.67	
<i>Glyphiulus speobius</i>	2	11.22	Separated by lowland areas

morphic and may represent single cave and/or short-range endemics. One undescribed epigeal species, *Hyleoglomeris rukouqu* sp. nov., was identified within cave entrance vegetation, and may represent a ‘disturbance relict’ species (*sensu stricto* Wynne et al. 2014). *Glyphiulus maocun* sp. nov., a troglophile, was detected in one cave, but likely has a more regional distribution. For known troglophiles, *Blingulus sinicus* Zhang & Li, 1981 were more widely distributed occurring in three caves each, while *Glyphiulus melanoporus* Mauriès & Nguyen Duy-Jacquemin, 1997 was detected in one cave.

Class Diplopoda**Order Glomerida Leach, 1814****Family Glomeridae Leach, 1815****Genus *Hyleoglomeris* Verhoeff, 1910**

Type species. *Hyleoglomeris multilineata* Verhoeff, 1910.

Remarks. The genus can be characterized by the telopods, which with frontomesal trichosteles on the prefemur and femur, and the caudomesal femoral process forming a distinct angle to femur proper (Golovatch et al. 2006, Liu and Tian 2015a). It encompasses over 90 species distributed in Eurasia from Japan and Sulawesi to the Balkans (Enghoff et al. 2015). At present, 30 species of this genus occur in China, 25 are subterranean-adapted.

***Hyleoglomeris rukouqu* sp. nov.**

<http://zoobank.org/EFA33915-ECD8-4B25-B874-28D0354B907C>

Figs 3A, 5, 6

Type material. Holotype male (SCAU), China, Guangxi Zhuang Autonomous Region, Yangshou County, Shangshuiyan Cave [24°57'43.6"N, 110°20'37.21"E], 191 m elevation (el.), cave entrance, ferns and other vegetation (refer to habitat section below), direct intuitive search, 17 November 2016, J.J. Wynne leg. Paratype, 1 female (SCAU), same data as holotype.

Etymology. The species name, *rukouqu*, is used as a noun in apposition from the Mandarin phrase, rùkǒu qū (入口区). When translated to English it means “entrance zone” or “entrance area” to denote the area in the cave where this species was collected.

Diagnosis. Adult male of *H. rukouqu* sp. nov. is distinct from other *Hyleoglomeris* species based on the following combination of characters: (1) peculiar color pattern (Fig. 5); (2) telopods with a large, rectangular, central syncoxital lobe (Fig. 6D). This new species is similar to *H. lii* (a troglophile from a cave in Guangxi), but is distinguished by (1) 2+2 dark brown spots on the thoracic shield (Fig. 5B, C) *vs.* 1+1 light grey-yellow spots in *H. lii*; (2) sycoxital lobe of telopods being high, large and rectangle-shaped (Fig. 6D) *vs.* low, linguiform, apically evidently concave in *H. lii*.

Description. Based on type specimens. Length *ca.* 8.0 mm (holotype), 11.0 mm (paratype), width 5.0 mm (holotype), 7.0 mm (paratype). **Coloration:** pattern vivid (Fig. 3A). With the exception of the brown dorsal spots, this species had similar coloration as that of the limestone rock and sediment within the entrance. General coloration in alcohol (Fig. 5) light yellowish with contrastingly dark spots, latter absent on rings 3 and 11. Head only brownish caudal margin, antennae and ommatidia dark-brownish. Collum mostly marbled dark-brown except frontal margin part. Thoracic shield with 2+2 dark brown spots, but lateral ones smaller than middle ones. Terga 4 with 1+1 brownish spots, smaller than the above. Terga 5–7 and pygidium with 1+1 large, oblong, transverse, paramedian, marbled, dark spots. Terga 8–10, these paramedian spots increasingly separated

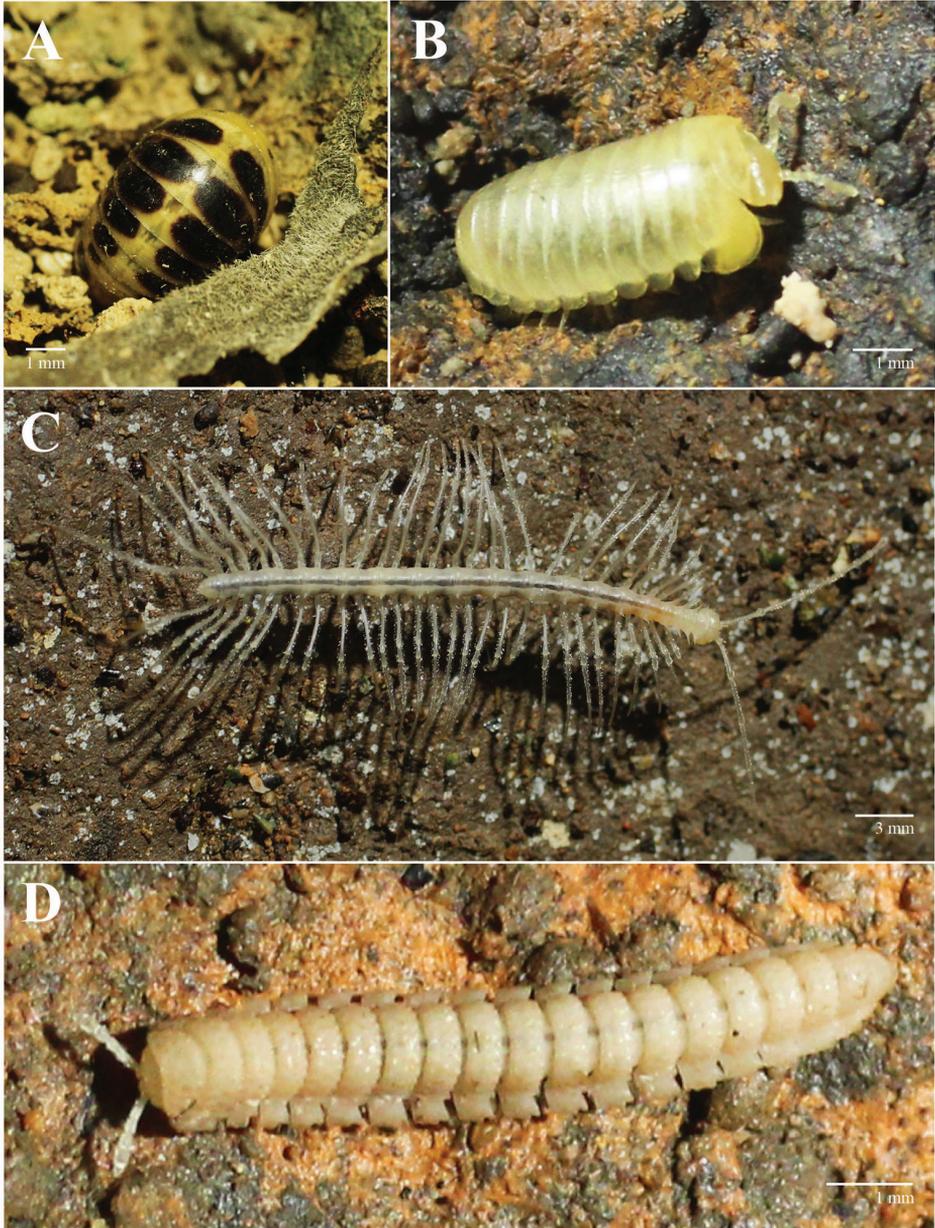


Figure 3. **A** *Hyleoglomeris rukouqu* sp. nov. from Shangshuiyan Cave **B** *Hyleoglomeris xuxiakei* sp. nov. from Guanshan No. 4 Cave **C** *Hylomus yuani* sp. nov. from Liangfeng Cave **D** *Eutrichodesmus jianjia* sp. nov. from Guanshan No. 4 Cave.

into 2+2, but lateral ones smaller than middle ones. **Head:** Ommatidium at least 7+1, lenses rather convex. Tömösváry's organ transverse-oval, parallel to the body, only slightly wider than long. Antennae with four apical cones, antennomere 6 *ca* 2.5 times as long as

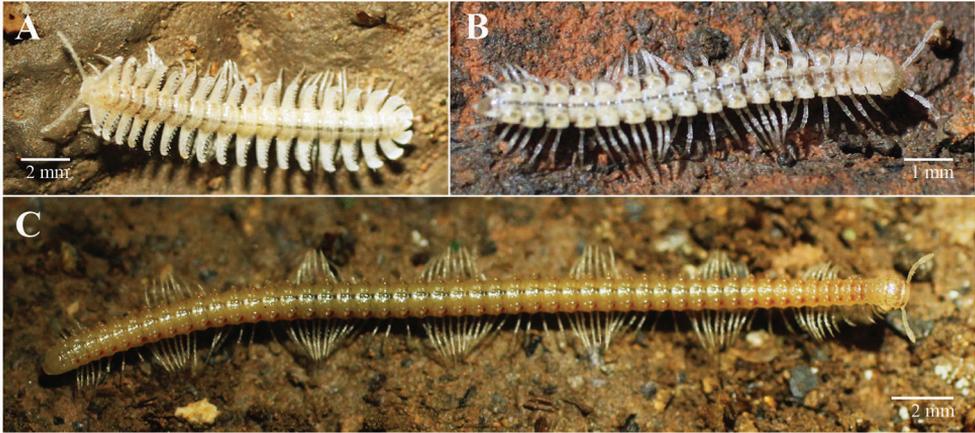


Figure 4. **A** *Trichopeltis liangfengdong* sp. nov. from Liangfeng Cave **B** *Pacidesmus trifidus* Golovatch & Geoffroy, 2014 from Maomaotou Cave **C** *Glyphiulus maocun* sp. nov. from Liangfeng Cave.

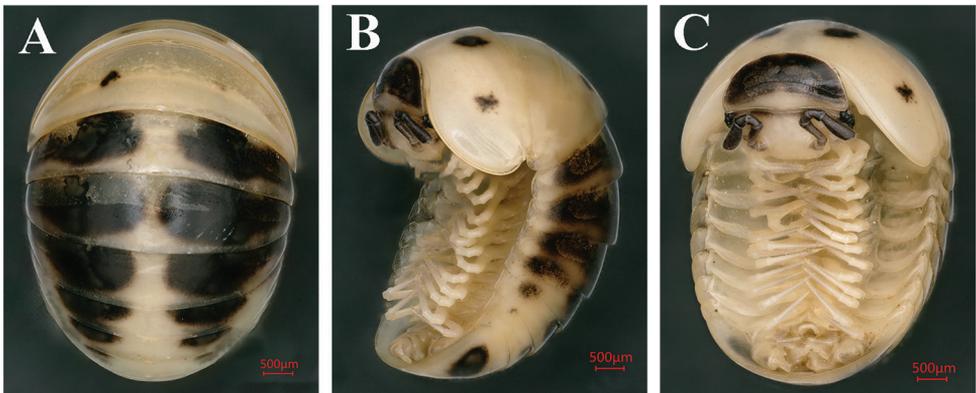


Figure 5. *Hyleoglomeris rukouqu* sp. nov., holotype. **A–C** Habitus, dorsal, lateral and ventral views, respectively.

wide. **Exoskeleton:** Collum with two transverse striae (Fig. 5C). Thoracic shield (Fig. 5B) with a narrow hyposchism, the latter not reaching behind caudal tergal margin; 7 transverse striae: 3 starting below, 1 level to, 3 above schism; 5 striae (never the first and last from below) crossing the dorsum. Following terga 3–11 with two striae above lateral edge (Fig. 5B). Pygidium (last tergite) of both sexes slightly concave medially at caudal margin. ♂ leg 17 with a low, rounded, outer coxal lobe; telopodite 4-segmented (Fig. 6A). ♂ leg 18 with a subtriangular syncoxital notch; telopodite 4-segmented (Fig. 6B). **Telopods:** (Fig. 6C–E) with a large, subrectangular, central syncoxital lobe flanked by high setose horns, each of the latter with a small lobe on top. Prefemur micropapillate laterally, with a long, digitiform, frontomesal trichostele. Femur with a smaller, digitiform, frontomesal trichostele. Caudomedial femoral process prominent, apically with an evident lobe. Tibia with a frontomesal seta. Caudomesal tibial process evident, recurved; an indistinct, papillate tubercle at base on caudal face. Tarsus strongly sigmoid, narrowly rounded apically.

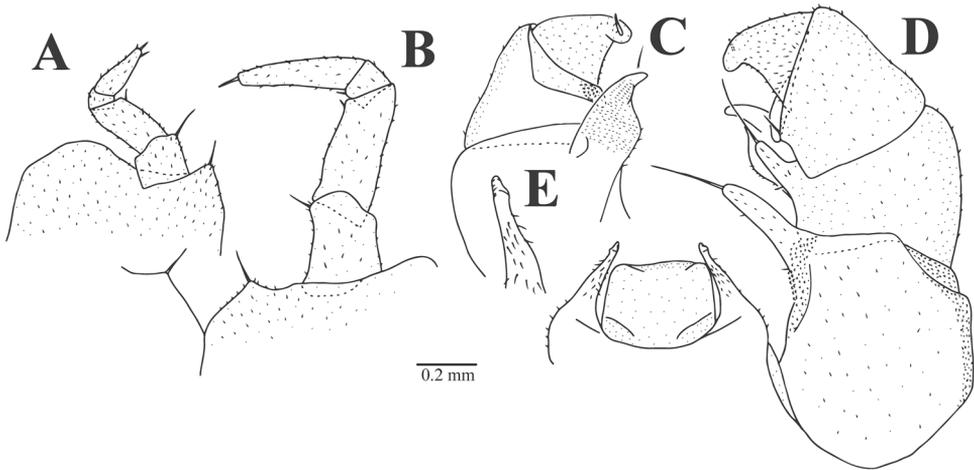


Figure 6. *Hyleoglomeris rukouqu* sp. nov., holotype. **A** leg 17, anterior view **B** leg 18; anterior view **C** right half of telopods, posterior view **D** right half of telopods, anterior views **E** tip of syncoxital horn, anterior view.

Habitat. Specimens were collected within a vegetation association that may be limited to the cave entrance zone and similar geographic features (e.g., sinkholes and fissures in rock). At least three plant species occurred within the entrance including Gesneriaceae sp., *Adiantum* sp., and one other fern species, which cannot be identified without examining the sorii (A. Monro, pers. comm. 2018).

Notes. Based on the vivid color pattern and well-developed ommatidia, as well as the location where it was detected within the cave, this animal represents an epigeal species and may be functioning as an obligate troglophile (*sensu* Peck 1970). While it is likely *H. rukouqu* sp. nov. either occurred or still occurs in similar habitats on the surface, the importance of relict plant species restricted to cave entrances has been discussed for southern China (Monro et al. 2018). Additionally, several arthropod species globally are restricted to cave entrances in Polynesia (Mockford and Wynne 2013, Wynne et al. 2014, Bernard et al. 2015, Taiti and Wynne 2015) and North America (Benedict 1979, Wynne and Shear 2016) due to either extensive surface disturbance and glacial interglacial cycles, respectively. Thus, it is possible this species is a ‘disturbance relict’ restricted to the entrance of Shangshuiyan Cave and potentially other area cave entrances with similar vegetation.

***Hyleoglomeris xuxiakei* sp. nov.**

<http://zoobank.org/D3CA0855-76FD-4683-8A7C-3F0505DC3D8E>

Figs 3B, 7, 8

Type material. Holotype male (SCAU), China, Guangxi Zhuang Autonomous Region, Yangshuo County, Guanshan No. 4 Cave [24°56'58.34"N, 110°20'53.52"E],

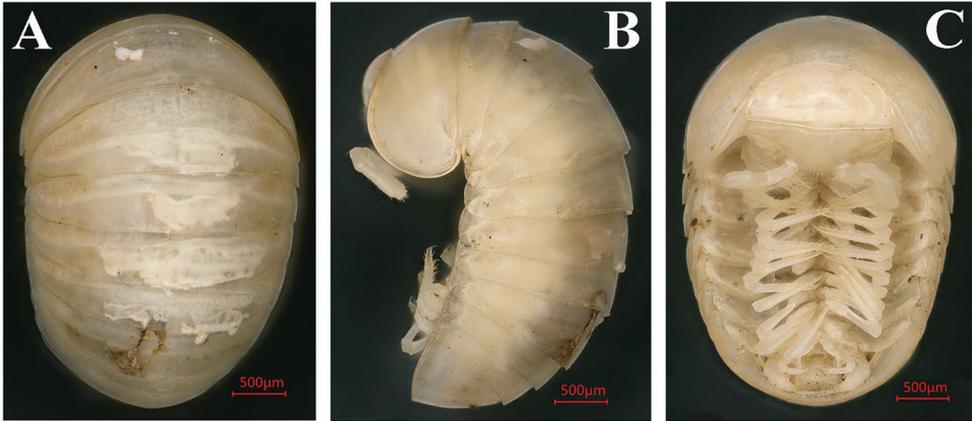


Figure 7. *Hyleoglomeris xuxiakei* sp. nov., holotype. **A–C** Habitus, dorsal, lateral and ventral views, respectively.

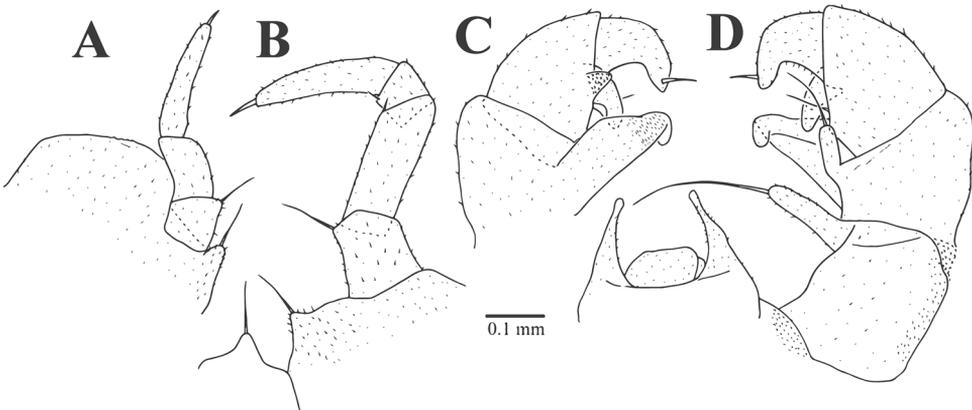


Figure 8. *Hyleoglomeris xuxiakei* sp. nov., holotype. **A** Leg 17, anterior view **B** leg 18; anterior view **C** right half of telopods, posterior view **D** right half of telopods, anterior views.

186 m el., deep zone, direct intuitive search, 16 November 2016, J.J. Wynne leg. Paratype, 1 male (SCAU), same data as holotype. 3 females (SCAU), same data as holotype, but deep zone, slightly muddy flat area.

Etymology. The species name, *xuxiakei*, was Latinized using a combination of the surname and forename of Xu Xiake (徐霞客). Xu was a traveler, explorer, and the first speleologist of China, who studied caves and karst geology during the decline of the Ming Dynasty. He conducted a four-year expedition (1636–1640) across southern China where he examined over 300 caves (including more than 100 from the Guilin area; Sweeting 1995, Ravbar 2016). In his book, *Xu Xiake's Travels* (first published in 1642), he described underground streams and ponds, provided sketch maps of caves, as well as proposed terminology for karst features; many of his speleological terms are still used today (Ravbar 2016).

Diagnosis. Adult male of *H. xuxiakei* sp. nov. is distinct from other *Hyleoglomeris* species based on the following combination of characters: (1) nearly pallid color (Fig. 7); (2) ♂ leg 17 with 3-segmented telopodite (Fig. 8A); (3) telopods with a low, oval-shaped, central syncoxital lobe; (4) horns of syncoxital lobe without any structure on top (Fig. 8D). This new species is clearly distinguished by the depigmented body (Fig. 3B) *vs.* vivid color pattern in *H. rukouqu* sp. nov. (Fig. 3A).

Description. Based on type specimens. Lengths of body *ca* 3.8–4.5 mm, width 2.5–3.0 mm in both sexes. **Coloration:** entirely pallid (Figs 3B, 7). **Head:** Ommatidium at least 5(6) + 1, translucent, barely visible (Fig. 7C). Tömösváry's organ transverse-oval, parallel to the body, only slightly wider than long. Antennae with four apical cones, antennomere 6 *ca* 2.0 (♂) or 1.8 (♀) times as long as wide. **Exoskeleton:** Collum with two transverse striae (Fig. 7C). Thoracic shield with a narrow hyposchism, the latter reaching behind caudal tergal margin; 8–9 transverse striae: 4(5) starting below, one level to, 3(4) above schism; 5 striae (never the first and last from below) crossing the dorsum. Following terga 3–11 with two striae above lateral edge. Pygidium of both sexes regularly rounded at caudal margin. ♂ leg 17 with a low, subrounded, outer coxal lobe; telopodite 3-segmented (Fig. 8A). ♂ leg 18 with an arch-shaped syncoxital notch; telopodite 4-segmented (Fig. 8B). **Telopods:** (Fig. 8C–D) with a rather low, oval-shaped, transverse, central syncoxital lobe flanked by high setose horns, each of the latter without any structure on top. Prefemur micropapillate laterally, with a well-developed frontomesal trichostele. Femur with a smaller frontomesal trichostele. Caudomesal femoral process prominent, apically with an evident lobe strongly curved to frontad. Tibia with a frontomesal seta. Caudomesal tibial process evident, recurved; a distinct, papillate tubercle at base on caudal face. Tarsus strongly sigmoid, narrowly rounded apically.

Habitat. This species was collected from a chamber within the estimated cave deep zone, approximately 50 m from the cave entrance. Cave sediment was compact mud with a small amount of rock breakdown from the ceiling.

Notes. Based on a depigmented habitus and translucent ommatidia, we consider this species is a troglobiont.

Order Polydesmida Leach, 1815

Family Paradoxosomatidae Daday, 1889

Genus *Hylomus* Cook & Loomis, 1924

Type species. *Hylomus draco* Cook & Loomis, 1924.

Remarks. The genus is often referred to as “dragon millipedes” because of the paraterga is antler-like, wing-shaped or spiniform. It can be characterized by the collum and metaterga often with granulate, tuberculate and/or speculate; male femora (5, 6, 7 and/or 9) often humped ventrally; and gonopods mostly suberect, some subfalcated, a shortened solenomere mostly sheathed by a usually condensed, rather simple solenophore

(Liu et al. 2014, 2016). This genus encompasses 33 species distributed from China, Laos, Vietnam and Thailand (Srisonchai et al. 2018). At present, 19 species are known in China and 13 are subterranean-adapted (Liu et al. 2016; Srisonchai et al. 2018).

***Hylomus yuani* sp. nov.**

<http://zoobank.org/89255A98-84A8-4D57-ADF4-F7B4F0BDBE48>

Figs 3C, 9–12

Material examined. Holotype male (SCAU), China, Guangxi Zhuang Autonomous Region, Lingchuan County, Liangfeng Cave [25°12'34.86"N, 110°31'56.8"E], 184 m el., deep zone, direct intuitive search, 18 November 2016, J.J. Wynne leg. Paratypes, 1 male, 3 females (SCAU), same data as holotype.

Etymology. This species is named for Dr. Yuan Daoxian (袁道先), a preeminent and globally recognized Chinese hydrologist. He has made numerous significant contributions in advancing both the science and management of karst and karst waters in southern China since the 1960s.

Diagnosis. Adult males of *H. yuani* sp. nov. are distinct from other *Hylomus* species based on the following combination of characters: (1) paraterga long and spiniform only on collum and rings 2–6, short and coniform thereafter (Figs 9, 10A, B); (2) collum with 8+8 frontal, 3+3 in the middle, 3+3 caudal, setigerous spinules; (3) ♂ femur 6 strongly inflated in distal 1/4 (Fig. 10D); (4) gonopod with a flagelliform solenomere and a strongly condensed solenophore (Figs 9–12). This new species is similar to *H. lui* (a troglobiont from a cave in Guilin), but is distinguished by (1) paraterga long and spiniform on collum and rings 2–6 (Fig. 9) *vs.* on collum and rings 2–5 in *H. lui*; (2) collum and following metaterga with three transverse rows of spinules (Fig. 10A–B) *vs.* two transverse rows of spinules in *H. lui*.

Description. Based on type specimens. Lengths of body *ca* 27–28 (♂), 30–35 mm (♀); width of mid-body pro- and metazonae 1.5–1.6 and 2.0 (♂), 2.0–2.2 and 2.5–2.6 mm (♀). **Coloration:** light brownish to nearly pallid (Figs 3C, 9–10). Antennomere 7 dark brown. **Body:** with 20 rings. In width, head < ring 2 < collum < 4–5 < 3 = 6–7 < 8–9 < 10–13; thereafter body gradually tapered posteriorly towards telson. **Head:** densely setose, but more sparsely on vertex, epicranial suture conspicuous (Fig. 10A). Antennae long and slender, reaching past ring 8 (♂) or 7 (♀) when extended posteriorly. **Exoskeleton:** Collum with 8+8 evident setigerous spinules arranged in a row at front margin, behind it with 3+3 in the middle and 3+3 similarly spinules at posterior margin. Metaterga 2–4 each with 5+5, 3+3 and 5+5(6) similarly setigerous spinules arranged in three transverse rows (Fig. 9A). Following metaterga with same sculpture, but 7–9+7–9 setigerous spinules at posterior margin, 2–4 spinules of them extended to the lateral side (Figs 9C, 10B). Paraterga clearly spiniform, but well-developed only in paraterga 1–6, directed more dorsad than laterad (Fig. 9A). Following paraterga increasingly stout and short, paraterga 7–14 directed dorsolaterad, thereafter directed clearly caudad (Figs 9C, 10A–B). Tegument shining, prozonae delicately microalveolate, metaterga

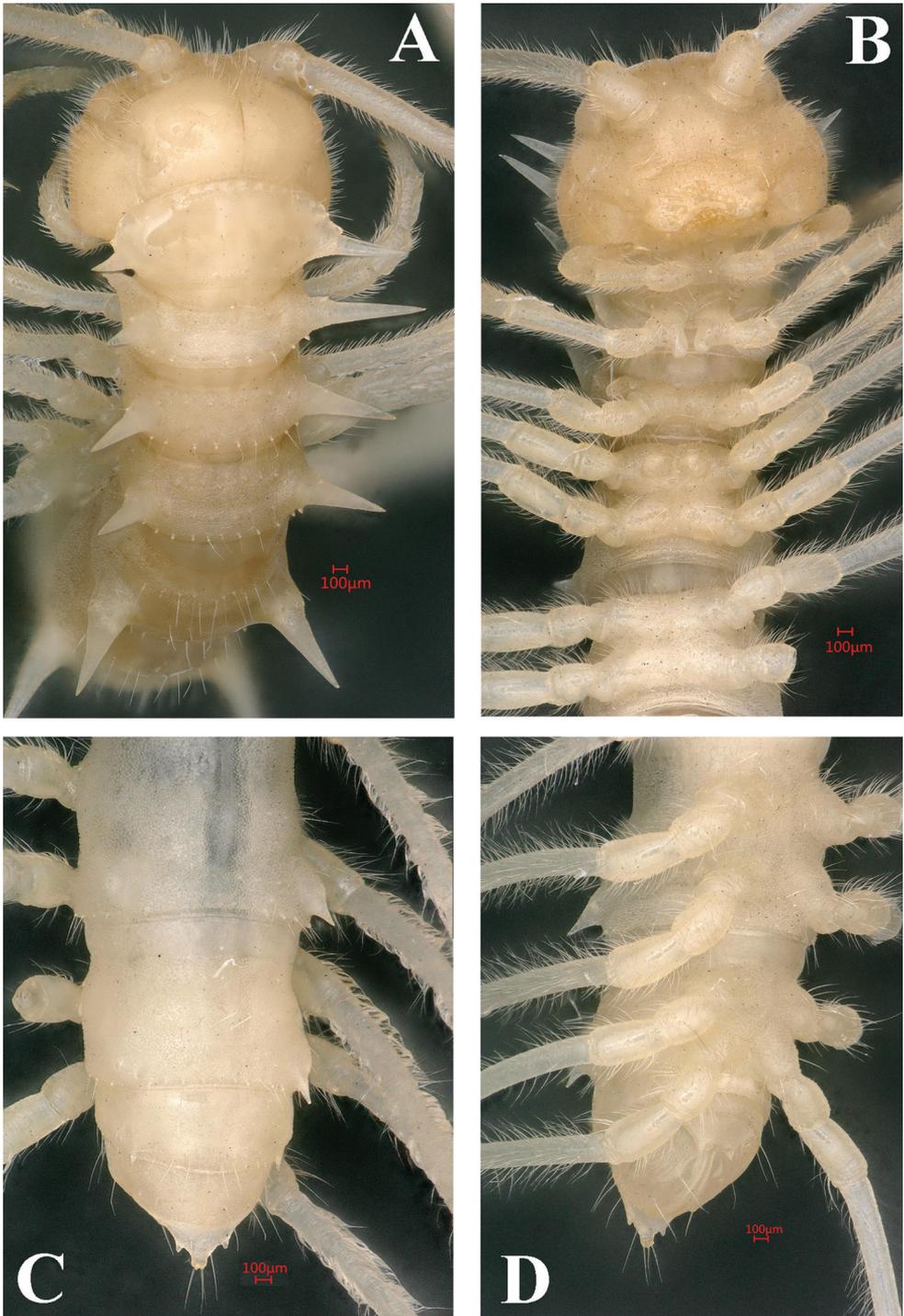


Figure 9. *Hylomus yuani* sp. nov., ♂ paratype. **A** Anterior part of body, dorsal view **B** anterior part of body, ventral view **C** posterior part of body, dorsal view **D** posterior part of body, ventral view.

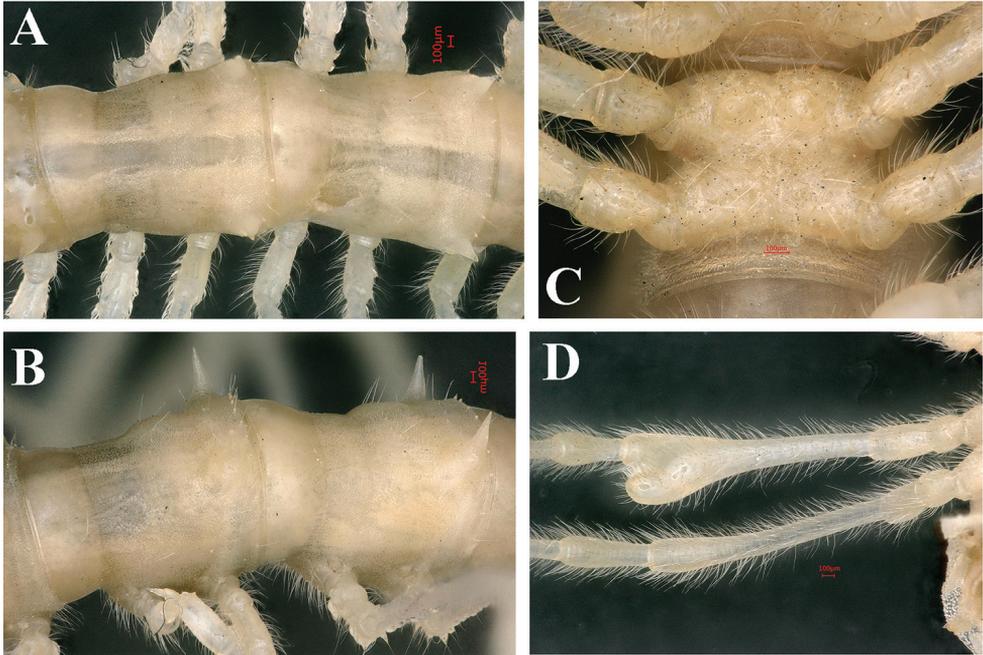


Figure 10. *Hylomus yuani* sp. nov., ♂ paratype. **A** Mid-body rings, dorsal view **B** mid-body rings, lateral views **C** sternite V, ventral view **D** femora 6 and 7, subventral view.

and surface below paraterga finely microgranulate. Constriction between pro- and metazonae broad and shallow (Fig. 10A–B). Pore formula normal; transverse sulcus usually very vague, but traceable in rings 5–19 (Fig. 10A). Ozopores inconspicuous, located near the base on lateral side of pore-bearing paraterga (Fig. 10B). Pleurosternal carinae evident only on rings 2 and 3 in both sexes, absent thereafter. Epiproct simple, lateral pre-apical papillae distinct (Fig. 9C). Hypoproct subtrapeziform, caudal margin emarginate, setigerous cones at caudal edge large, widely separated (Fig. 9D). Sterna sparsely setose, cross-shaped impressions weak. A paramedian pair of short, rounded, independent tubercles between ♂ coxae 4 (Figs 9B, 10C). Legs long and slender, *ca* 5.0 (♂) or 3.5 (♀) times as long as mid-body ring height. ♂ femur 6 strongly inflated ventrally in distal 1/4 (Fig. 10D). **Gonopods:** (Figs 11, 12) Coxite short, subcylindrical, densely setose distodorsally, about 1/3 as long as telopodite. Prefemur densely setose and about half as long as acropodite. Femorite elongate, slightly curved, with seminal groove running entirely on mesal face. Solenophore (**sph**) strongly condensed; solenomere (**sl**) flagelliform, evidently separated at base from solenophore.

Habitat. This species was collected within the estimated cave deep zone, approximately 20 m from the cave entrance. Cave sediment was compact mud and the passageway was swaddled by a slightly visible fog. This species and cave crickets (family Rhaphidophoridae) were among the most abundant species within this portion of cave deep zone.

Notes. Based on the slender elongate antennae and legs, a depigmented cuticle, the species is considered a troglolobiont.



Figure 11. *Hylomus yuani* sp. nov., ♂ paratype. **A** Right gonopod, lateral view **B** right gonopod, mesal views.

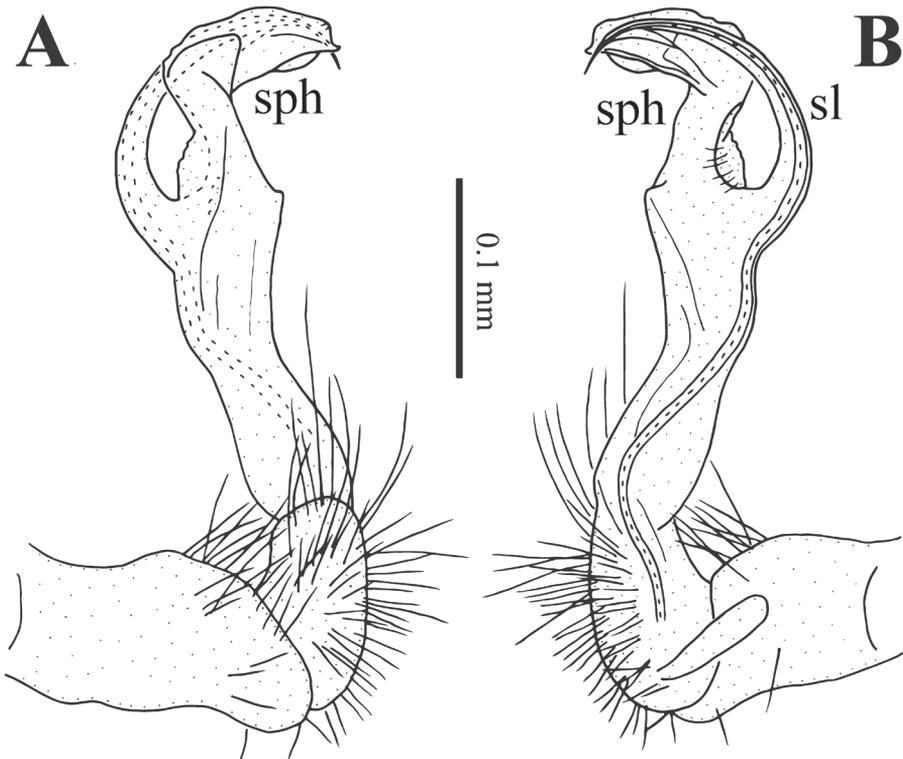


Figure 12. *Hylomus yuani* sp. nov., ♂ paratype. **A** Right gonopod, lateral view **B** right gonopod, mesal views. Designations: sl = solenomere; sph = splenophore.

Family Haplodesmidae Cook, 1895**Genus *Eutrichodesmus* Silvestri, 1910**

Type species. *Eutrichodesmus demangei* Silvestri, 1910.

Remarks. The genus can be characterized by the body often capable of volvation, with or without mid-dorsal projections; paraterga 2 strongly enlarged; and gonopod femorite with a more or less distinct process or outgrowth laterally (Golovatch et al. 2009a, b). It encompasses 53 species distributed from south Japan, southern China, and Southeast Asia to Vanuatu, Melanesia (Enghoff et al. 2015, Liu et al. 2017b). At present, 23 species have been known in continental China, 20 are subterranean-adapted.

***Eutrichodesmus jianjia* sp. nov.**

<http://zoobank.org/403323E4-C776-4DC8-8795-9C53C3B64E02>

Figs 3D, 13, 14

Type material. Holotype male (SCAU), China, Guangxi Zhuang Autonomous Region, Yangshuo County, Guanshan No. 4 Cave [24°56'58.34"N, 110°20'53.52"E], 186 m el., deep zone, direct intuitive search, 16 November 2016, J. J. Wynne leg. Paratypes, 4 females, 1 juv. (SCAU), same data as holotype; 1 male (SCAU), same data as holotype, but collected on mud bank.

Etymology. The species name, *jianjia* (肩甲), is used as a noun in apposition. This phrase was used to denote the well-developed paraterga, which covers the millipede's legs much like the pauldrons of body armor cover the shoulder of the warrior.

Diagnosis. Adult males of *E. jianjia* sp. nov. is distinct from other *Eutrichodesmus* species based on the following combination of characters: (1) collum with five, and metaterga 2–19 with three transverse rows of round microvillose tubercles (Fig. 13). (2) gonopod acropodite with a dorsolateral tooth (t) at midway and apical with a large tube-shaped lobe (l) (Fig. 14). This new species is clearly distinguished from *E. lipsae* (a troglobiont from a cave in Guilin) by (1) metaterga without mid-dorsal projections *vs.* most metaterga with mid-dorsal projections; (2) gonopod acropodite with a triangular, ventral process (p) at about basal 1/3 (Fig. 14) *vs.* without this process in *E. lipsae*.

Description. Based on type specimens. Lengths of adult body *ca* 6.0–7.0 mm, widths of mid-body pro- and metazonae 0.6–1.0 and 1.2–1.3 mm in both sexes. **Coloration:** generally light-yellowish to pallid (Fig. 3D). **Body:** subcylindrical, conglobation complete, adults with 20 rings (Fig. 10A). **Head:** frons densely setose, finely microgranulate on vertex, with a paramedian pair of rounded knobs above antennal sockets. Epicranial suture conspicuous. Antennae short, slightly clavate. **Exoskeleton:** Collum subtrapeziform, slightly broader than head, not covering the latter from above, with five transverse rows of round microvillose tubercles (Fig. 13B). Metaterga 2–19 each with three transverse mixostictic rows of similar tubercles extending onto paraterga, about 6–8+6–8 per row. The middle row of tubercles slightly larger than others. Pre-anal ring short, with four transverse rows of small tubercles (Fig. 13D, E). Paraterga with evident shoulders anteriorly, strongly declivous, broad and usually triobate laterally, evidently

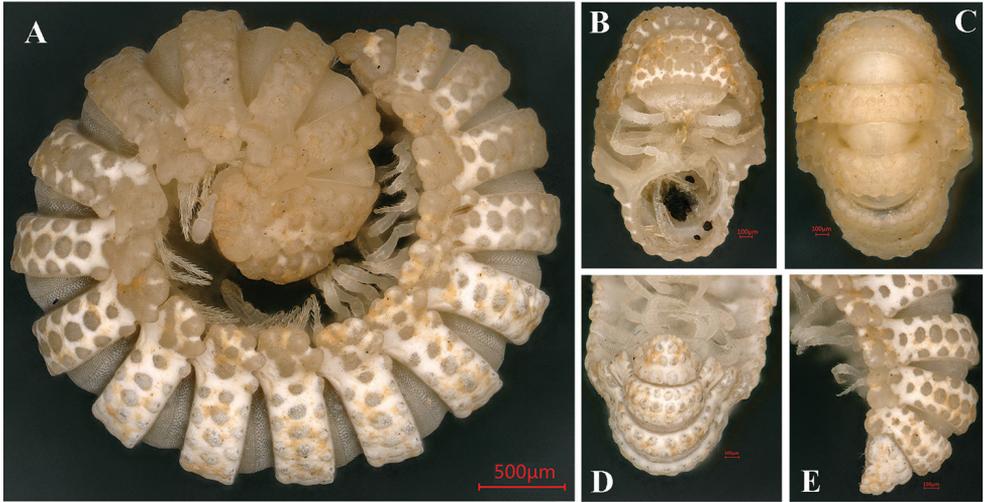


Figure 13. *Eutrichodesmus jianjia* sp. nov., ♂ paratype. **A** Habitus, lateral view **B** anterior part of body, ventral view **C** anterior part of body, dorsal view **D** posterior part of body, ventral view **E** posterior part of body, lateral view.

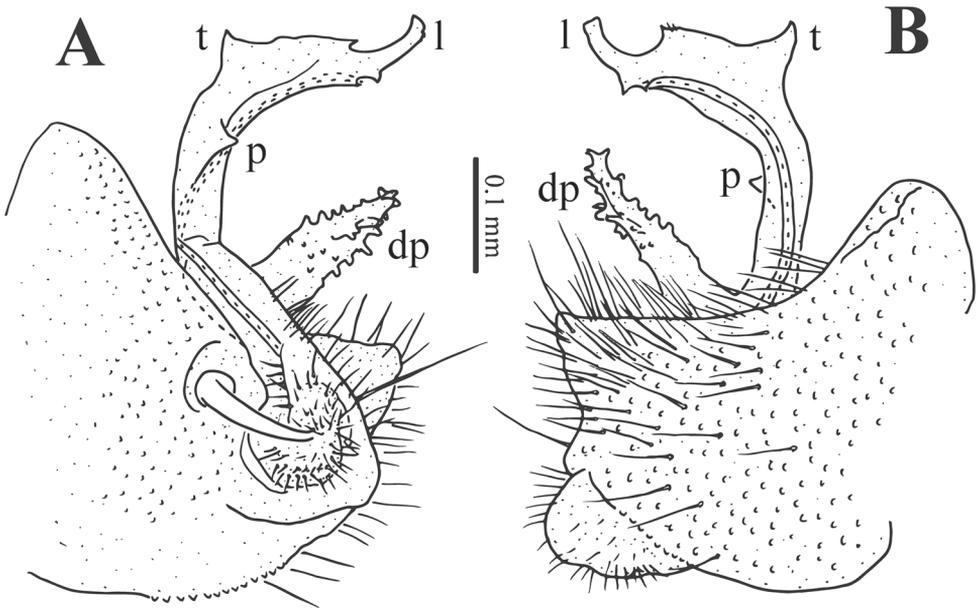


Figure 14. *Eutrichodesmus jianjia* sp. nov., ♂ paratype. **A** Left gonopod, lateral view **B** left gonopod, mesal view. Designations: dp = distofemoral process; l = lobe; p = process; t = tooth.

extending down below level of venter; caudolaterally at base with two distinct lobulations (Fig. 13A). Paraterga 2 strongly enlarged. Tergal setae and ozopores invisible. Ozopores hardly visible. Prozonae finely alveolate; constriction between pro- and metazoan

narrow and shallow. Epiproct apically with four spinnerets. Hypoproct subtrapeziform, with two long setae. Sterna narrow, but much broader between ♂ coxae 6–7 and 9. Gonopod aperture suboval. Legs long and slender, but hardly reaching tips of paraterga. **Gonopods:** (Fig. 14) Coxite large, abundantly setose and micropapillate ventrolaterally. Telopodite subfalcate, distinctly curved ventrad, setose in its basal part, with a prominent, denticulate, lateral, distofemoral process (**dp**) at midway. Acropodite with a triangular, ventral process (**p**) at about basal one-third and a dorsolateral tooth (**t**) at midway; apical with a large tube-shaped lobe (**l**). Seminal groove long, terminating without hair.

Habitat. Specimens were collected in the same general location as *H. xuxiakei* sp. nov. specimens. Thus, refer to the *H. xuxiakei* sp. nov. habitat description.

Notes. Based on the long slender legs and a depigmented cuticle, we consider this species a troglobiont.

Family Cryptodesmidae Karsch, 1880

Genus *Trichopeltis* Pocock, 1894

Type species. *Cryptodesmus bicolor* Pocock, 1894.

Remarks. The genus can be characterized by the metaterga distinctly several transverse rows of tuberculations, usually setose, and gonopod usually foliate, with a tripartite or deeply notched telopodite. It encompasses 12 species distributed from the Himalayas of India, through Bangladesh, Myanmar to southern China, Laos, Vietnam, Cambodia and Indonesia (Likhitrakarn et al. 2017). At present, 5 species have been known in China, 4 are subterranean-adapted.

Trichopeltis liangfengdong sp. nov.

<http://zoobank.org/7E972E70-ADDA-4CA1-924F-3016EF5E3FA7>

Figs 4A, 15–17

Type material. Holotype male (SCAU), China, Guangxi Zhuang Autonomous Region, Lingchuan County, Liangfeng Cave [25°12'34.86"N, 110°31'56.8"E], 184 m el., deep zone, steam bank, direct intuitive search, 18 November 2016, J.J. Wynne leg. Paratypes, 4 females (SCAU), same data as holotype.

Etymology. This species name, *liangfengdong*, is used as a noun in apposition and is the name of the type locality.

Diagnosis. Adult male of *T. liangfengdong* sp. nov. is distinct from other *Trichopeltis* species based on the following combination of characters: (1) metaterga 2–15 with four transverse rows of small, setigerous tubercles (Fig. 15B); (2) gonopod coxae without seta and acropodite with several small subapical lobules (los) (Fig. 17). This new species is similar to *T. reflexus* (a troglobiont from a cave in Hunan), but is distinguished by (1) metaterga 2–15 with four transverse rows of setigerous tubercles *vs.* two transverse rows in *T. reflexus*; (2) tip of acropodite bifid (Fig. 17) *vs.* distorted in *T. reflexus*.

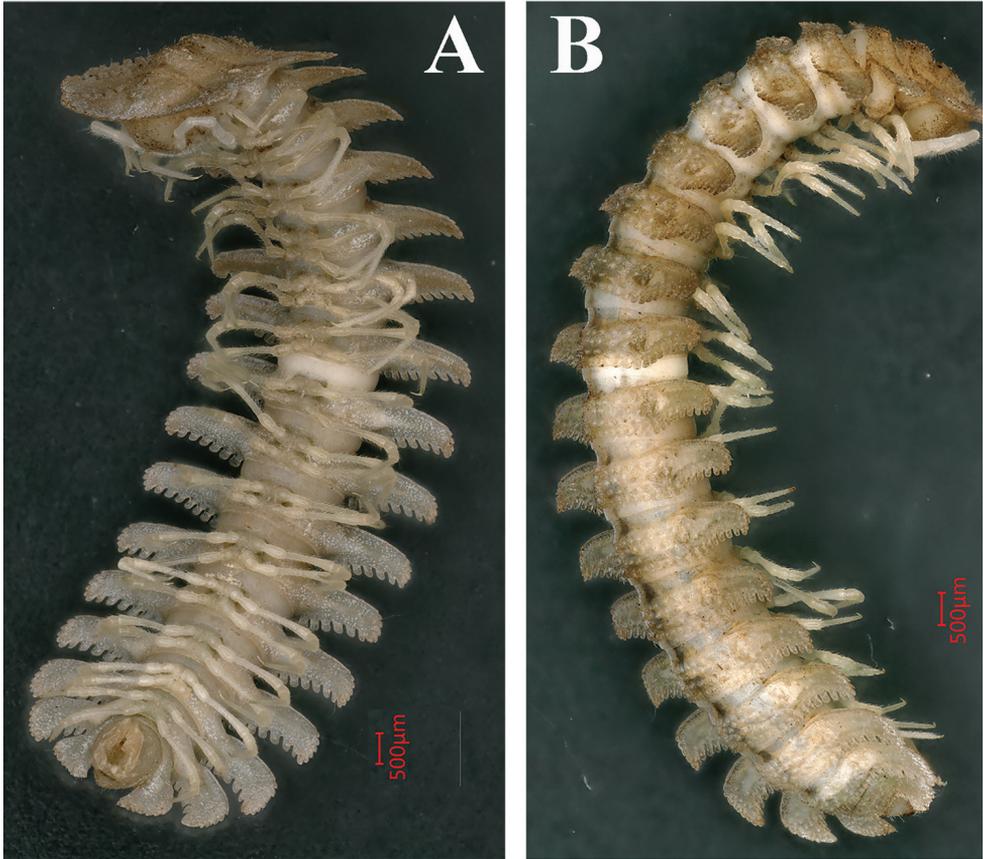


Figure 15. *Trichopeltis liangfengdong* sp. nov. holotype. **A** Habitus, ventral view **B** habitus, sublateral view.

Description. Based on type specimens. Length of both sexes *ca* 14.0–15.0 mm, widths of mid-body pro- and metazonae 1.5–2.0 and 4.8–5.0 mm. **Coloration:** generally pallid (Fig. 4B). **Body:** with 20 rings (Fig. 15). In width, collum < ring 2 < 3 = 4 < 5 < 6 < 7–16; thereafter body gradually tapered posteriorly towards telson. **Head:** densely pilose and microgranulate, epicranial suture present (Fig. 16A). Antennae long, reaching past ring 3 when extended posteriorly; in length, antennomere 6 = 5 > 4 = 3 > 2 > 7 > 1. **Exoskeleton:** Collum fan-shaped (Fig. 15A), covering the head from above, dorsal surface with irregular several small, round, setigerous tubercles. Marginal lobules on collum: 13+13 small, setigerous, rounded anteriorly, 7+7 relative larger, laterally and 5+5 very small caudally. Mid-dorsal regions on rings 2–15 with four more or less regular, transverse rows of similarly small, setigerous tubercles, 4–5 + 4–5 per row (Figs 15B, 16B). Several tubercles extending onto paraterga. Following metaterga with five rows of smaller tubercles, 6–7 + 6–7 per row (Fig. 15B). Paraterga strongly developed, clearly upturned dorsally above the dorsum only on the collum, other paraterga flat. Each with 5–6 small, dentiform, lateral and 7–8 much larger, squarish caudolateral lobules, all evident, setigerous and microvillose (Figs 15, 16A, B). Cau-

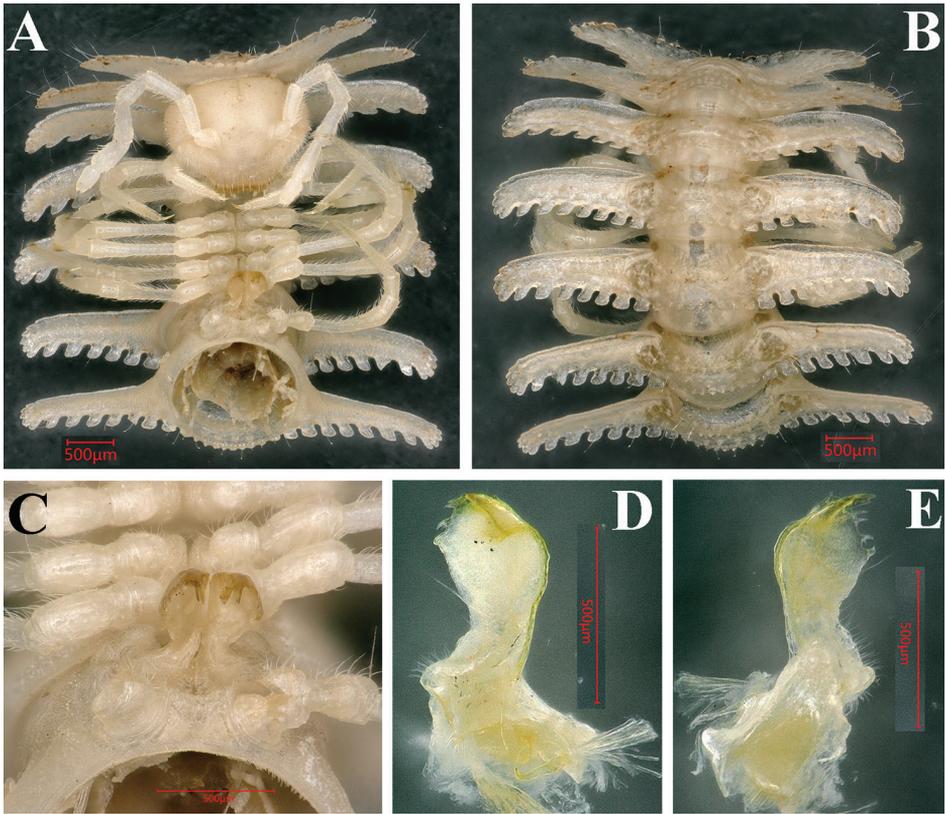


Figure 16. *Trichopeltis liangfengdong* sp. nov., holotype. **A** anterior part of body, ventral view **B** anterior part of body, dorsal view **C** sterna 6 and 7, ventral view **D** right gonopod, mesal view **E** right gonopod, lateral view.

dolateral lobules on paraterga mostly oblong, relatively large, and well separated from one another (Fig. 16A). Caudolateral corner of paraterga projecting behind rear tergal margin on rings 15–19 (Fig. 15). Integument clearly microgranulate throughout, prozonae finely alveolated. Limbus regularly crenulated. Stricture between pro- and metazonae broad, shallow and finely microgranulated. Tergal setae simple and short (Fig. 16A, B). Ozopores invisible, pore formula untraceable. Epiproct short, with four spinnerets apically. Hypoproct subtrapeziform, 1+1 caudal setigerous papillae clearly separated. Pleurosternal carinae present on rings 2 and 3 in both sexes. Sterna modestly setose, cross-shaped impressions moderate, broadened between ♂ coxae 9 (Fig. 16C). Legs long and slender, unmodified, produced beyond paratergal lateral margin (Fig. 15), about 1.8 times as long as mid-body ring height in both sexes. **Gonopods:** (Figs 16D, E, 17) Coxite short and squarish, without seta. Prefemur densely setose and a particularly long setae; nearly half the length of telopodite. Femorite strongly flattened, pie-shaped, with a small lobe (l) ventrally. Acropodite folded, with several small subapical lobules (los), tip bifid. Seminal groove terminating with a hairy pulvillus, forming no distinct solenomere.

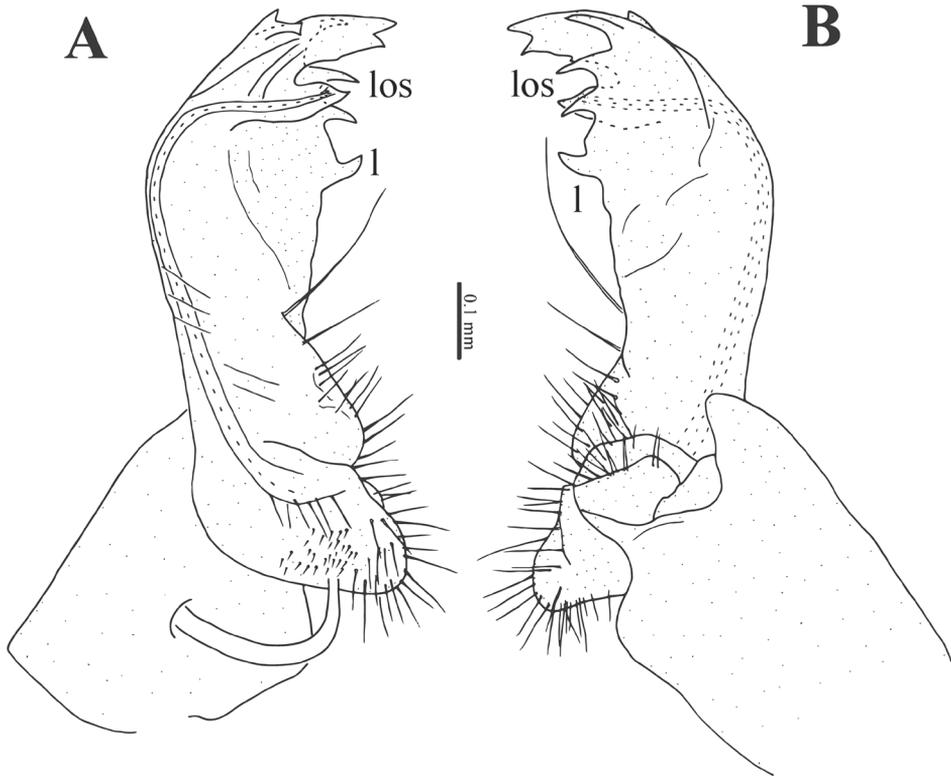


Figure 17. *Trichopeltis liangfengdong* sp. nov., holotype. **A** Left gonopod, mesal view **B** left gonopod, lateral view. Designations: l = lobe; los = lobules.

Habitat. Specimens were collected in the same general location as *H. yuani* sp. nov. specimens. Thus, refer to the *H. yuani* sp. nov. habitat description.

Notes. Based on the long slender antennae and legs and a depigmented cuticle, the species is considered a troglobiont.

Family Polydesmidae Leach, 1815
Genus *Pacidesmus* Golovatch, 1991

Type species. *Pacidesmus shelleyi* Golovatch, 1991

Remarks. The genus can be characterized by metaterga often with three transverse rows of 3+3 sculpture, and gonopod structure showing no prominent clivus to recurve laterad of the seminal groove (Golovatch and Geoffroy 2006). It encompasses 9 species, only the type species from a mountain in Chiangmai, Thailand, all other 8 species have been known from caves in China.

***Pacidesmus trifidus* Golovatch & Geoffroy, 2014**

Fig. 4B

Material examined. 6 males, 3 females (SCAU), China, Guangxi Zhuang Autonomous Region, Yangshuo County, Guanshan No. 4 Cave [24°56'58.34"N, 110°20'53.52"E], 186 m el., deep zone, direct intuitive search, 16 November 2016, J.J. Wynne leg. 1 male, 1 juv. (SCAU), China, Guangxi Zhuang Autonomous Region, Xiufeng District, Maomaotou Cave [25°18'46.12"N, 110°16'12.64"E], 225 m el., deep zone, direct intuitive search, 15 November 2016, J. J. Wynne leg. 2 males, 1 female, 4 juv. (SCAU), China, Guangxi Zhuang Autonomous Region, Yangshou County, Shangshuiyan Cave [24°57'43.6"N, 110°20'37.21"E], 191 m el., deep zone, direct intuitive search, 17 November 2016, J. J. Wynne leg.

Notes. This troglobiont was first found in Skeleton Cave [25°17'13"N, 110°13'26"E], el. 186 m (Golovatch and Geoffroy 2014). We later confirmed *P. trifidus* within three caves with the furthest cave (Guanshan # 4) approximately ~13 km to the south of type locality. The three caves where we detected *P. trifidus* are separated by a distance of ~41 km. The northernmost cave, Maomaotou Cave occurs 39.6 km north from Guanshan No. 4 Cave and Shangshuiyan Cave, which are co-located occurring within 1 km of each other. As it is currently morphologically described as one species, it now has a regional distribution.

Habitat. This species was collected from the estimated deep zones of the three caves. For Maomaotou cave, we collected it within bamboo detritus at mid-cave. In Shangshuiyan Cave, specimens were collected within decomposing detritus and along muddy cave sediment deposits within a sinuous passageway. For Guanshan No. 4 Cave, refer to habitat descriptions of *H. xuxiakei* sp. nov.

Order Spirostreptida Brandt, 1833**Family Pericambalidae Silvestri, 1909****Genus *Bilingulus* Zhang & Li, 1981**

Type species. *Bilingulus sinicus* Zhang & Li, 1981.

Remarks. The genus can be characterized by the lingular lamellae divided into two parts. ♂ legs 1 with a syncoxite carry a pair of long, band-like coxal process; femur with a finger-shaped process at the end protruding posteriorly, tarsus without claw at the end. Anterior gonopod with a very broad coxa; telopodite of posterior gonopod slender, devoid of branch (Zhang and Li 1981).

***Bilingulus sinicus* Zhang & Li, 1981**

Material examined. 3 males, 3 females, 5 juv. (SCAU), China, Guangxi Zhuang Autonomous Region, Yangshou County, Shangshuiyan Cave [24°57'43.6"N, 110°20'37.21"E], 191 m el., deep zone, cartography station #19, direct intuitive

search, 17 November 2016, J.J. Wynne leg. 4 females (SCAU), China, Guangxi Zhuang Autonomous Region, Xiufeng District, Maomaotou Cave [25°18'46.12"N, 110°16'12.64"E], 225 m el., deep zone, direct intuitive search, 15 November 2016, J.J. Wynne leg. 1 juv. (SCAU), China, Guangxi Zhuang Autonomous Region, Lingchuan County, Liangfeng Cave [25°12'34.86"N, 110°31'56.8"E], 184 m el., deep zone, direct intuitive search, 18 November 16, J.J. Wynne leg.

Notes. This species lacked characters suggestive of troglomorphy. It had a pigmented cuticle and ommatidia were present. Thus, *B. sinicus* is considered a troglophile. It was first described by Zhang and Li (1981) from a cave in Guilin; no cave name or coordinate data were provided. While we detected this species in three different Guilin caves, we're unable to determine a distance from the type locality. The caves where we detected *P. trifidus* is separated by an average distance of 34.1 km. Maomaotou Cave occurs to the north-northwest and is 45 km from Liangfeng Cave and 40.4 km to the north of Shangshuiyan Cave. Based upon its epigeal characteristics and that this species is regionally distributed on the landscape, we consider it a troglophile.

Family Cambalopsidae Cook, 1895

Genus *Glyphiulus* Gervais, 1847

Type species. *lulus* (recte: *Julus*) *granulatus* Gervais, 1847.

Remarks. The genus has been divided into two groups, *granulatus*-group and *javanicus*-group. Both groups shared the same gonopods characters as the anterior gonopods usually with a plate-like coxosternum, 1-segmented telopodite. Posterior gonopods highly compressed, showing a plumose, subflagelliform, distal process (Golovatch et al. 2007). It encompasses over 60 species distributed in Southeast Asia and southern China. At present, 42 species have been known in China, 34 are subterranean-adapted. The below two species belong to *granulatus*-group because ♂ legs 1 strongly reduced, sternum devoid of median structures.

Glyphiulus melanoporus Mauriès & Nguyen Duy-Jacquemin, 1997

Material examined. 1 male, 1 female, 1 juv. (SCAU), China, Guangxi Zhuang Autonomous Region, Xiufeng District, Maomaotou Cave, [25°18'46.12"N, 110°16'12.64"E], 225 m el., deep zone, direct intuitive search, 15 November 2016, J. J. Wynne leg.

Notes. *G. melanoporus* is considered a troglophile. The type locality for this species is Skeleton Cave [25°17'13"N, 110°13'26"E] (Mauriès and Nguyen Duy-Jacquemin 1997). We collected this species from Maomaotou Cave, which represents a 21.1 km range expansion to the south of the type locality.

Habitat. For our specimens, we collected them within the deep zone of Maomaotou Cave. Sediment where specimens were collected was compacted and wet.

***Glyphiulus maocun* sp. nov.**

<http://zoobank.org/5CDE1D3A-71A2-48EA-B4BB-F9FDD20D860D>

Figs 4C, 18–20

Type material. Holotype male, China, Guangxi Zhuang Autonomous Region, Lingchuan County, Maocun Village, Liangfeng Cave [25°12'34.86"N, 110°31'56.8"E], 184 m el., deep zone, direct intuitive search, 18 November 2016, J.J. Wynne. Paratypes, 4 females, 4 juv. (SCAU), same data as holotype.

Etymology. The species name, *maocun*, is used as a noun in apposition and is the name of the village nearest to the type locality.

Diagnosis. This new species is similar to *G. melanoporus* (from caves in Guilin) in showing carinotaxy formula of collum I–IV+5c+6a+pc+ma. However, *G. maocun* sp. nov. is distinct from *G. melanoporus* by (1) carinotaxy formula of metaterga 2/2+I/

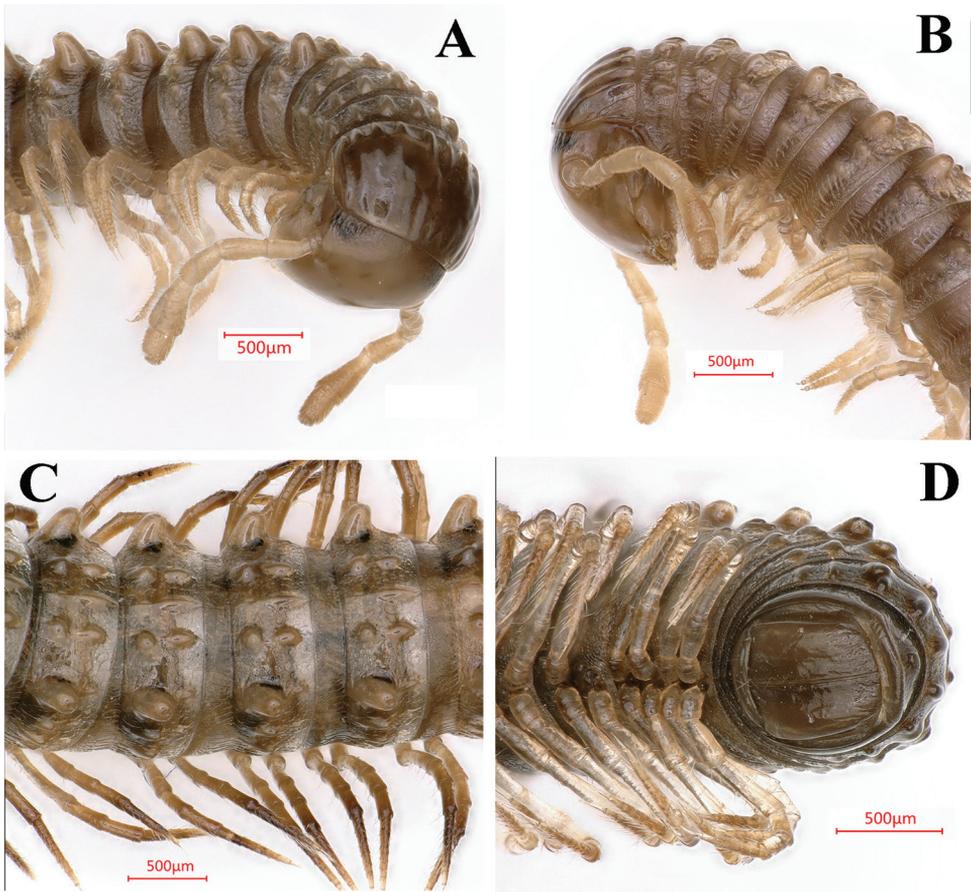


Figure 18. *Glyphiulus maocun* sp. nov., holotype. **A, B** Anterior part of body, sublateral views **C** mid-body rings, dorsal view **D** posterior part of body, ventral view.

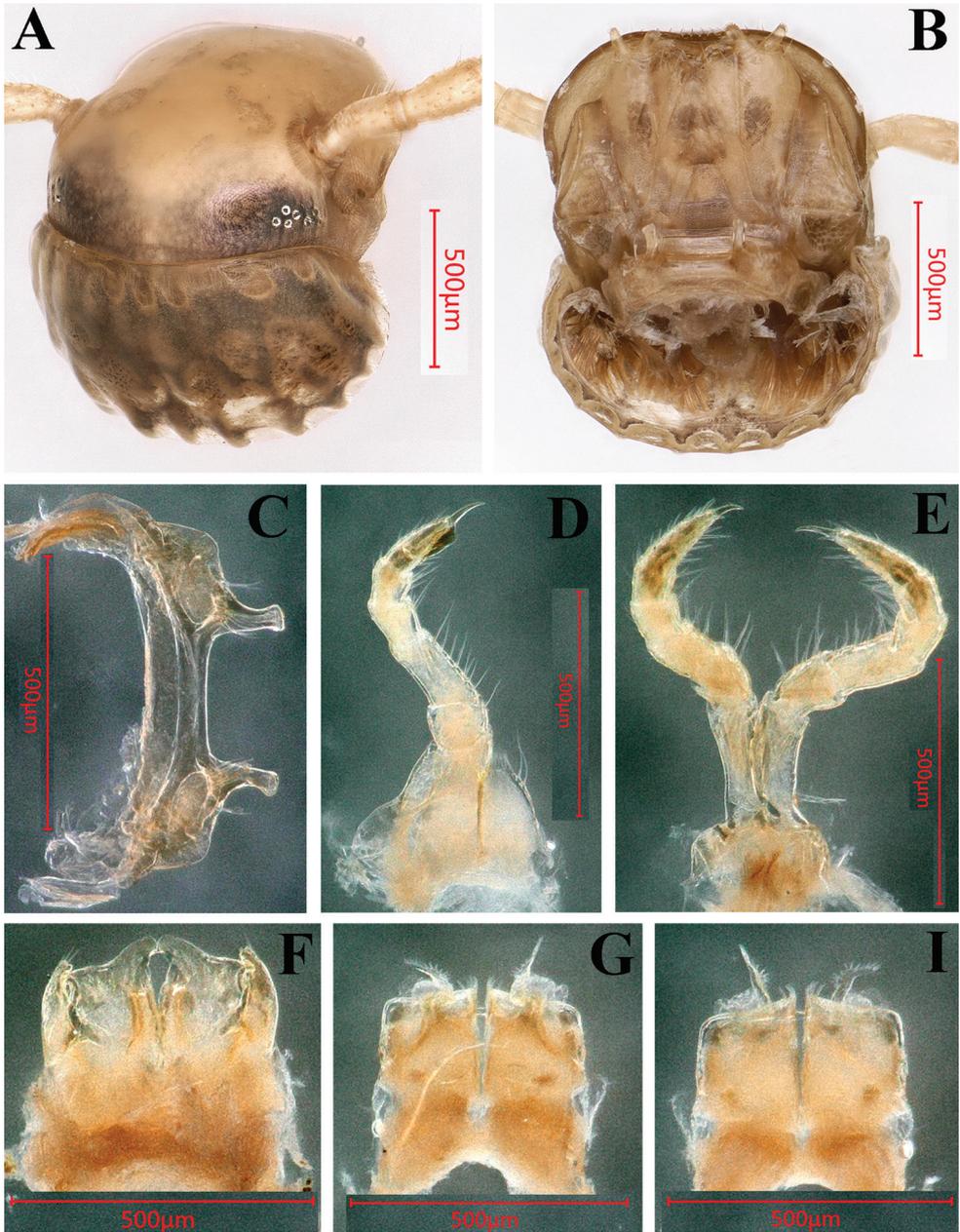


Figure 19. *Glyphiulus maocun* sp. nov., holotype. **A** Head and collum, dorsal view **B** head, collum, and legs 1, ventral view **C** legs 1, anterior view **D** leg 2, caudal view **E** legs 3, frontal view **F** anterior gonopods, caudal view **G** posterior gonopods, anterior view **H** posterior gonopods, caudal view.

i+3/3; (2) anterior gonopod with a conspicuous, high, curved downward process on coxosternum (Figs 19F, 20A); (3) posterior gonopod tip branch plumose flagellum (Figs 19G–I, 20B, C).

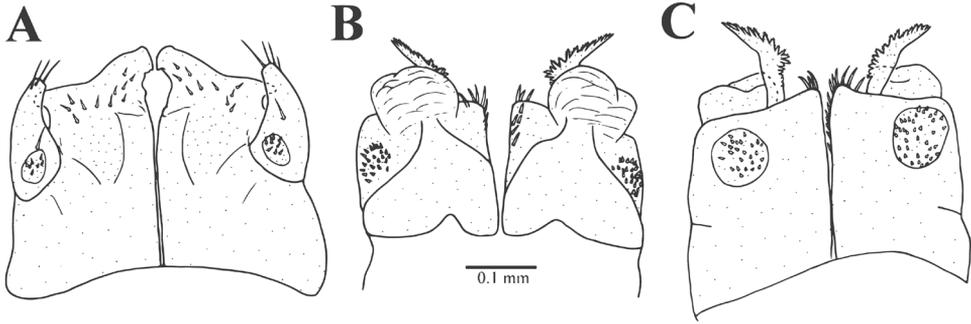


Figure 20. *Glyphiulus maocun* sp. nov., holotype. **A** Anterior gonopods, caudal view **B** posterior gonopods, anterior view **C** posterior gonopods, caudal view.

Description. Based on the type specimens. Lengths of both sexes *ca* 26–32 mm, mid-body rings round in cross-section, their widths and heights similar, 1.2–1.8 mm. **Coloration:** yellow-brown to brownish in alcohol. Ommatidia blackish (Fig. 18A). **Body:** with 46–54 podous rings + 2–1 apodous one(s) + telson. **Head:** clypeus with 4 teeth anteromedially. Each eye patch with about 8–12 ommatidium arranged in three irregular vertical rows (Figs 18A, 19A). Antennae long, antennomere 7 with four short apical cones (Fig. 18A, B). Gnathochilarium usually, with a separate promentum (Fig. 19B). **Exoskeleton:** Carinotaxy formula of collum I–IV+5c+6a+pc+ma, anterior margin crests lower (Fig. 19A). Subsequent metaterga strongly crested, carinotaxy formula 2/2+I/i+3/3. Ozoporiferous tubercles round (Fig. 18). Prozonae delicately alveolate; strictures and metazonae fine longitudinal striations. Rings 2 and 3 with long pleural flaps. Epiproct simple, with a short, low, rounded tubercle medially. Paraprocts rather regularly convex. Hypoproct broadly emarginated (Fig. 18D). ♂ legs 1 very strongly reduced, represented only by a sternum devoid of any median or paramedian structures but carrying 1+1 strongly separated prongs both curved anteriorly and bearing several strong setae (Fig. 18B, C). ♂ legs 2 with large coxae; penes oblong, each with two strong setae distolaterally (Fig. 19D). ♂ legs 3 with slender and elongate coxae (Fig. 19E). Legs slender, about 1.2 times as long as mid-body ring height. Claw simple, without any spine at base. **Anterior gonopods:** (Figs 19F, 20A) with a conspicuous, high, lobe-shaped, curved downward process on coxosternum, which is much higher than telopodite; telopodite slightly coiled, 1-segmented, lateral in position, with 2 or 3 strong apical setae. **Posterior gonopods:** (Figs 19G–I, 20B, C) laterally with a low, lateral lobe carrying a dozen of long setae, tip branch very slender and high, plumose flagellum.

Habitat. Specimens were collected within or at proximity to flood detritus.

Notes. Specimens exhibited no characteristics suggestive of cave adaptation. It had a pigmented cuticle and well-developed blackish ommatidia. We consider this species to be troglophilic within Liangfeng Cave. Subsequently, this species may have a larger, more regional distribution.

Discussion

Our work increased the number of subterranean-adapted millipedes from 34 to 38 species. With 564 known caves in Guangxi (Yuanhai Zhang, pers. comm. 2019), but a fraction (8.7% or 49 caves) of these caves have been examined for troglomorphic millipedes. The identification of six new species of millipedes from four caves in the Guilin area underscores the potential for many additional discoveries. For millipede-specific surveys, direct intuitive searches of selected cave deep zones (*sensu* Wynne et al. 2019) has been identified as the best technique; Mesibov et al. (1995) reported that four weeks of hand collecting during the fall was more efficient than an intensive pitfall trapping effort involving trapping seven days per month for over one year.

Conversely, while advancing our knowledge of regional troglomorphic millipede diversity stands to contribute significantly to the conservation value of the SCK, a more comprehensive understanding (i.e., for all cave-dwelling taxa) of regional diversity, as well as advancing procedures to assessing the vulnerability of cave systems to anthropogenic impact should be considered. Wynne et al. (2018, 2019) provides a systematic framework and guidance for sampling cave-dwelling arthropods using a repeatable framework, which can be modified for application in the SCK. Using such an approach, these data may be then be examined by applying principles similar to the cave vulnerability assessment developed by Tanalgo et al. (2018). Through such an effort, systematic techniques may be used to assemble robust and comparable landscape scale data, and each cave subsequently be evaluated for its vulnerability due to human activities.

Moreover, for conservation biologists and resource managers to best evaluate the importance of SCK cave biological diversity, we will require a more robust understanding of the distributional ranges of troglomorphic species (as well as other subterranean-restricted taxa). Specifically, while nearly 40 percent of troglomorphic millipedes were identified as single cave endemics, 14 troglobionts (including one whose range was expanded from this work) occurred within two or more caves. One species, *P. trifidus*, was confirmed within three caves in the Guilin region (two caves from this work, plus the type locality); the maximum distance between caves for this species was 59.7 km. Three additional subterranean-adapted species occurred in multiple caves with maximum distances ranging from 81.27 and 137.6 km (Table 3). As many troglomorphic arthropods are identified as short-range endemic species, occurring in a single cave or geological formation (Reddell 1994, Culver et al. 2000, Christman et al. 2005, Deharveng et al. 2008, Tian 2011, Harvey and Wynne 2014, Gao et al. 2018, Nitzu et al. 2018) and that rivers and valleys/ lowland areas often result in vicariance (Barr 1985, Faillie et al. 2015, Katz et al. 2018), the genetic relatedness of at least these three species should be further examined using genetic techniques. While these species may be morphological similar, we suggest they may be genetically distinct – potentially representing different subspecies or lineages.

We also reported a possible ‘disturbance relict’, *H. rukouqu* sp. nov., discovered within a cave entrance vegetation community. As similar cave entrance vegetation

communities have been identified as either supporting distinct relict plant communities and/or plant species in southern China (Monro et al. 2018), Easter Island, Chile (Wynne et al. 2014), and west-central New Mexico, USA (Lindsey 1951, Northup and Welbourn 1997, Wynne 2013), their importance in supporting cave-restricted arthropod populations demonstrated (Northup and Welbourn 1997, Wynne 2013, Wynne et al. 2014, Wynne and Shear 2016), and widespread land cover conversion of both lowlands and uplands has occurred in China since 1958, this finding warrants both additional research into this species distribution, as well as a larger scale examination of other potential 'disturbance relict' arthropod species within cave entrance vegetation communities of the SCK.

Although caves are often considered distinct from the surface environment, cave ecosystems are inextricably linked to surface processes. Caves require an allochthonous energy supply, which may include flood detritus, guano deposition from bats, birds and crickets, and dissolved organic materials that percolate from the surface. Thus, when humans adversely change the surface environment, the cave ecosystem may change as well. Deforestation (Trajano 2000, Ferreira and Horta 2001, Clements et al. 2006, Stone and Howarth 2007), intensive agriculture and water diversion (van Beynen and Townsend 2005, Stone and Howarth 2007, Harley et al. 2011), livestock grazing (Stone and Howarth 2007), alien species introductions (Elliott 1992, Reeves 1999, Taylor et al. 2003, Howarth et al. 2007, Price 2016), heavy metals and agrochemicals (Whitten 2009), and global climate change (Chevaldonné and Lejeune 2003, Mamola et al. 2018) have negatively affected both cave organisms and ecological processes.

Despite the impressive biological diversity found in China, there are no government regulations, nor is any government agency responsible for managing and protecting cave resources. Development projects typically progress in caverniferous regions and tourist caves are developed without consideration for subterranean resources and the rich biodiversity they often support (Whitten 2009). While we recognize environmental conditions are improving and environmental regulations have strengthened in China, Whitten (2009) and Cao et al. (2007) identified most of the aforementioned impacts continue to expand and severely stress both epigeal and hypogean ecosystems in southern extent of the country. Thus, we recommend monitoring environmental conditions of the surface and subsurface of caves identified as supporting rare endemic species populations and/or sensitive ecosystems.

This paper revealed that at least 38 troglomorphic millipede species occur in Guangxi. Other examples of SCK's high cave diversity includes at least 19 species of troglomorphic pseudoscorpions (Gao et al. 2018), 29 vascular plant species believed restricted to cave entrances (Monro et al. 2018), and the richest globally diversity of cavefishes with at least 154 species and nearly half of these species considered single cave endemics (Zhao et al. in press). As this work continues, we anticipate the number of subterranean-adapted millipedes, as well as other arthropod species will increase. Through ours and other efforts, we believe the SCK will ultimately emerge as a global hotspot for cave biological diversity.

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References

- Barr Jr TC (1967) Observations on the ecology of caves. *American Naturalist* 101: 475–491. <https://doi.org/10.1086/282512>
- Barr Jr TC (1985) Pattern and process in speciation of trechine beetles in eastern North America (Coleoptera: Carabidae: Trechinae). *Phylogeny and zoogeography of beetles and ants*, Junk, Dordrecht [1985 Sep 30]: 350–407.
- Benedict EM (1979) A new species of *Apochthonius* Chamberlin from Oregon (Pseudoscorpionida, Chthoniidae). *Journal of Arachnology* 7: 79–83.
- Bernard EC, Soto-Adames FN, Wynne JJ (2015) Collembola of Rapa Nui (Easter Island) with descriptions of five endemic cave-restricted species. *Zootaxa* 3949: 239–267. <https://doi.org/10.11646/zootaxa.3949.2.6>
- Cao J, Yang D, Zhang C, Jiang Z (2007) Karst ecosystem of Guangxi Zhuang Autonomous Region Constrained by Geological Setting: Relationship between carbonate rock exposure and vegetation coverage. *International Conference on Karst Hydrogeology and Ecosystems, USA*: 211–218.
- Chen Z, Decu V, Juberthie C, Ueno SI (2001) China. In: Juberthie C, Decu V (Eds) *Encyclopaedia Biospeologica, Part III*, Société Internationale de Biospéologie, Moulis, France, 1763–1781.
- Chevaldonné P, Lejeune C (2003) Regional warming-induced species shift in northwest Mediterranean marine caves. *Ecology Letters* 6: 371–379. <https://doi.org/10.1046/j.1461-0248.2003.00439.x>
- Christman MC, Culver DC, Madden MK, White D (2005) Patterns of endemism of the eastern North American cave fauna. *Journal of Biogeography* 32: 1442–1452. <https://doi.org/10.1111/j.1365-2699.2005.01263.x>
- Clarke AK (2006) Guangxi 2005 cave fauna report (China Caves Project). *YRC Bulletin*, Autumn 2006.
- Clements R, Sodhi NS, Schilthuizen M, Ng PK (2006) Limestone karsts of Southeast Asia: imperiled arks of biodiversity. *Bioscience* 56: 733–742. [https://doi.org/10.1641/0006-3568\(2006\)56\[733:LKOSAI\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2006)56[733:LKOSAI]2.0.CO;2)

- Culver DC, Master LL, Christman MC, Hobbs HH, III (2000) Obligate cave fauna of the 48 contiguous United States. *Conservation Biology* 14: 386–401. <https://doi.org/10.1046/j.1523-1739.2000.99026.x>
- Deharveng L, Bedos A (2000) The cave fauna of Southeast Asia: origin, evolution and ecology. In: Wilkens H, Culver DC, Humphreys WF (Eds) *Ecosystems of the World 30: Subterranean Ecosystems*, Elsevier, Amsterdam, 603–632.
- Deharveng L, Bréhier F, Bedos A, Tian MY, Li YB, Zhang F, Qin WG, Tan XF (2008) Mulun and surrounding karsts (Guangxi) host the richest cave fauna of China. *Subterranean Biology* 6: 75–79.
- Elliott WR (1992). Fire ants invade Texas caves. *American Caves Winter* 5: 13.
- Enghoff H, Golovatch SI, Short M, Stoev P, Wesener T (2015) Diplopoda – taxonomic overview. In: Minelli A (Ed) *Treatise on Zoology-Anatomy, Taxonomy, Biology. The Myriapoda* 2: 365–457.
- Faille A, Tänzler R, Toussaint EF (2015) On the way to speciation: shedding light on the karstic phylogeography of the microendemic cave beetle *Aphaenops cerberus* in the Pyrenees. *Journal of Heredity* 106: 692–699. <https://doi.org/10.1093/jhered/esv078>
- Ferreira RL, Horta LCS (2001) Natural and human impacts on invertebrate communities in Brazilian caves. *Revista Brasileira de Biologia* 61: 7–17. <https://doi.org/10.1590/S0034-71082001000100003>
- Gao Z, Wynne JJ, Zhang F (2018) Two new species of cave-adapted pseudoscorpions (Pseudoscorpiones, Neobisiidae, Chthoniidae) from Guangxi, China. *Journal of Arachnology* 46: 345–354. <https://doi.org/10.1636/JoA-S-17-063.1>
- Golovatch SI, Geoffroy JJ (2006) Review of the Southeast Asian millipede genus *Pacidesmus* Golovatch, with the description of a new troglobitic species from southern China (Diplopoda: Polydesmida: Polydesmidae). *Zootaxa* 1325: 363–368. <https://doi.org/10.11646/zootaxa.1325.1.24>
- Golovatch SI, Geoffroy JJ, Mauriès JP (2006) Review of the millipede genus *Hyleoglomeris* Verhoeff, 1910 (Diplopoda, Glomerida, Glomeridae), with descriptions of new species from caves in southeast Asia. *Zoosystema* 28: 887–915.
- Golovatch SI, Geoffroy JJ, Mauriès JP, VandenSpiegel D (2007) Review of the millipede genus *Glyphiulus* Gervais, 1847, with descriptions of new species from Aoutheast Asia (Diplopoda, Spirostreptida, Cambalopsidae). Part 1: the *granulatus*-group. *Zoosystema* 29: 7–49.
- Golovatch SI, Geoffroy JJ, Mauriès JP, VandenSpiegel D (2009a) Review of the millipede genus *Eutrichodesmus* Silvestri, 1910 (Diplopoda, Polydesmida, Haplodesmidae), with descriptions of new species. *ZooKeys* 12: 1–46. <https://doi.org/10.3897/zookeys.12.167>
- Golovatch, SI, Geoffroy, JJ, Mauriès JP, VandenSpiegel D (2009b) Review of the millipede family Haplodesmidae, with descriptions of some new or poorly-known species (Diplopoda, Polydesmida). *ZooKeys* 7: 1–53. <https://doi.org/10.3897/zookeys.7.117>
- Golovatch SI, Geoffroy JJ, Mauriès JP (2010a) Two new species of the millipede genus *Desmoxytes* Chamberlin, 1923 (Diplopoda: Polydesmida: Paradoxosomatidae) from caves in southern China. *Arthropoda Selecta* 19: 57–61. <https://doi.org/10.15298/arthscl.19.2.01>
- Golovatch SI, Geoffroy JJ, Mauriès JP (2010b) Review of the millipede genus *Pacidesmus* Golovatch, 1991, with descriptions of three new species from caves in southern China (Diplopoda: Polydesmida: Polydesmidae). *Tropical Natural History* 10: 159–169.

- Golovatch SI, Geoffroy JJ, Mauriès JP, VandenSpiegel D (2011a) Two new species of the millipede genus *Hypocambala* Silvestri, 1895 from China and Vietnam (Diplopoda: Spirostreptida: Cambalopsidae). *Arthropoda Selecta* 20: 167–174. <https://doi.org/10.15298/arthsel.20.3.03>
- Golovatch SI, Geoffroy JJ, Mauriès JP (2011b) New species of the millipede genus *Glyphiulus* Gervais, 1847 from the *granulatus*-group (Diplopoda, Spirostreptida, Cambalopsidae). *Arthropoda Selecta* 20: 65–114. <https://doi.org/10.15298/arthsel.20.2.01>
- Golovatch SI, Geoffroy JJ, Mauriès JP, VandenSpiegel D (2011c) New species of the millipede genus *Glyphiulus* Gervais, 1847 from the *javanicus*-group (Diplopoda: Spirostreptida: Cambalopsidae). *Arthropoda Selecta* 20: 149–165. <https://doi.org/10.15298/arthsel.20.3.02>
- Golovatch SI, Liu WX, Geoffroy JJ (2012a) Review of the millipede genus *Hyleoglomeris* Verhoeff, 1910 in China, with descriptions of new species (Diplopoda, Glomerida, Glomeridae). *Zootaxa* 3358: 1–27. <https://doi.org/10.11646/zootaxa.3358.1.1>
- Golovatch SI, Li Y, Liu W, Geoffroy JJ (2012b) Three new cavernicolous species of dragon millipedes, genus *Desmoxytes* Chamberlin, 1923, from southern China, with notes on a formal congener from the Philippines (Diplopoda, Polydesmida, Paradoxosomatidae). *ZooKeys* 185: 1–18. <https://doi.org/10.3897/zookeys.185.3082>
- Golovatch SI, Li Y, Liu W, Geoffroy JJ (2012c) One new and two little-known species of the millipede family Polydesmidae from southern China (Diplopoda: Polydesmida). *Arthropoda Selecta* 21: 131–136. <https://doi.org/10.15298/arthsel.21.2.02>
- Golovatch SI, Geoffroy JJ (2014) On some new or poorly-known species of the millipede family Polydesmidae from southern China (Diplopoda: Polydesmida). *Russian Entomological Journal* 23: 91–105. <https://doi.org/10.15298/rusentj.23.2.01>
- Golovatch SI (2015) Cave Diplopoda of southern China with reference to millipede diversity in Southeast Asia. *ZooKeys* 510: 79–94. <https://doi.org/10.3897/zookeys.510.8640>
- Golovatch SI, Geoffroy JJ, Mauriès JP, VandenSpiegel D (2015) Review of the millipede genus *Eutrichodesmus* Silvestri, 1910, in China, with descriptions of new cavernicolous species (Diplopoda, Polydesmida, Haplodesmidae). *Zookeys* 505: 1–34. <https://doi.org/10.3897/zookeys.505.9862>
- Harley GL, Polk JS, North LA, Reeder PP (2011) Application of a cave inventory system to stimulate development of management strategies: The case of west-central Florida, USA. *Journal of Environmental Management* 92: 2547–2557. <https://doi.org/10.1016/j.jenvman.2011.05.020>
- Harvey MS, Wynne JJ (2014) Troglomorphic pseudoscorpions (Arachnida: Pseudoscorpiones) of northern Arizona, with descriptions of two new short-range endemic species. *Journal of Arachnology* 42: 205–219. <https://doi.org/10.1636/K14-34.1>
- Howarth FG (1980) The Zoogeography of Specialized Cave Animals: A Bioclimatic Model. *Evolution* 34: 394–406. <https://doi.org/10.1111/j.1558-5646.1980.tb04827.x>
- Howarth FG (1982) Bioclimatic and geological factors governing the evolution and distribution of Hawaiian cave insects. *Entomologia Generalis* 8: 17–26.
- Howarth FG (1983) Ecology of cave arthropods. *Annual Review of Entomology* 28: 365–389. <https://doi.org/10.1146/annurev.en.28.010183.002053>

- Howarth FG, James SA, McDowell W, Preston DJ, Imada CT (2007). Identification of roots in lava tube caves using molecular techniques: Implications for conservation of cave arthropod faunas. *Journal of Insect Conservation* 11: 251–261. <https://doi.org/10.1007/s10841-006-9040-y>
- Jiang X, Guo X, Chen H, Xie Z (2018) Four new species of the *Glyphiulus javanicus* group from southern China (Diplopoda, Spirostreptida, Cambalopsidae). *ZooKeys* 741: 155–179. <https://doi.org/10.3897/zookeys.741.23223>
- Katz AD, Taylor SJ, Davis MA (2018) At the confluence of vicariance and dispersal: Phylogeography of cavernicolous springtails (Collembola: Arrhopalitidae, Tomoceridae) codistributed across a geologically complex karst landscape in Illinois and Missouri. *Ecology and Evolution* 8: 10306–10325. <https://doi.org/10.1002/ece3.4507>
- Lindsey AA (1951) Vegetation and habitats in a southwestern volcanic area. *Ecological Monographs* 21: 227–253. <https://doi.org/10.2307/1943559>
- Likhitrakarn N, Golovatch SI, Srisonchai R, Panha S (2017) A new species of *Trichopeltis* Pocock, 1894 from southern China, with a checklist and a distribution map of *Trichopeltis* species (Diplopoda, Polydesmida, Cryptodesmidae). *ZooKeys* 725: 123–137. <https://doi.org/10.3897/zookeys.725.22014>
- Liu WX, Tian MY (2013) Four new cavernicolous species of the millipede genus *Eutrichodesmus* Silvestri, 1910 from southern China (Diplopoda: Polydesmida: Haplodesmidae). *Zootaxa* 3734: 281–291. <https://doi.org/10.11646/zootaxa.3734.2.11>
- Liu WX, Golovatch SI, Tian MY (2014) A review of the dragon millipede genus *Desmoxytes* Chamberlin, 1923 in China, with descriptions of four new species (Diplopoda: Polydesmida: Paradoxosomatidae). *ZooKeys* 448: 9–26. <https://doi.org/10.3897/zookeys.448.8081>
- Liu WX, Tian MY (2015a) A checklist of millipede genus *Hyleoglomeris* Verhoeff, 1910 in mainland China, with descriptions of seven new species (Diplopoda, Glomerida, Glomeridae). *Zootaxa* 4032: 103–116. <https://doi.org/10.11646/zootaxa.4032.1.5>
- Liu WX, Tian MY (2015b) Occurrence of the millipede genus *Piccola* Attems, 1953 in China (Diplopoda: Polydesmida: Paradoxosomatidae). *Zootaxa* 3904: 403–408. <https://doi.org/10.11646/zootaxa.3904.3.5>
- Liu W X, Tian MY (2015c) Two new cave-dwelling species of the millipede genus *Paracortina* Wang & Zhang, 1993 from southern China (Diplopoda, Callipodida, Paracortinidae). *ZooKeys* 517: 123–140. <https://doi.org/10.3897/zookeys.517.9949>
- Liu WX, Golovatch SI, Tian MY (2016) Six new species of dragon millipedes, genus *Desmoxytes* Chamberlin, 1923, mostly from caves in China (Diplopoda, Polydesmida, Paradoxosomatidae). *ZooKeys* 557: 1–24. <https://doi.org/10.3897/zookeys.557.7825>
- Liu WX, Golovatch SI, Wesener T, Tian MY (2017a) Convergent Evolution of Unique Morphological Adaptations to a Subterranean Environment in Cave Millipedes (Diplopoda). *PLoS One* 12: e0170717. <https://doi.org/10.1371/journal.pone.0170717>
- Liu WX, Golovatch SI, Wesener T (2017b) Four new species of the millipede genus *Eutrichodesmus* Silvestri, 1910 from Laos, including two with reduced ozopores (Diplopoda, Polydesmida, Haplodesmidae). *ZooKeys* 660: 43–65. <https://doi.org/10.3897/zookeys.660.11780>
- Loksa I (1960) Einige neue Diplopoden- und Chilopodenaren aus chinesischen Höhlen. *Acta Zoologica Academiae Scientiarum Hungaricae* 6: 135–148.

- Mauriès JP, Nguyen Duy-Jacquemin M (1997) Nouveaux craspedosomides et glyphiulides cavernicoles de Chine (Diplopoda). *Mémoires de Biospéologie* 24: 49–62.
- Mammola S, Goodacre SL, Isaia M (2018) Climate change may drive cave spiders to extinction. *Ecography* 41: 233–243. <https://doi.org/10.1111/ecog.02902>
- Mesibov R, Taylor RJ, Brereton RN (1995) Relative efficiency of pitfall trapping and hand-collecting from plots for sampling of millipedes. *Biodiversity and Conservation* 4: 429–439. <https://doi.org/10.1007/BF00058426>
- Minelli A (2015) *Treatise on Zoology-Anatomy, Taxonomy, Biology. The Myriapoda 2*. Brill., 482 pp. <https://doi.org/10.1163/9789004188273>
- Mockford EL, Wynne JJ (2013) Genus *Cyptophania* Banks (Psocodea: Lepidopsocidae): Unique features, augmented description of the genotype, and descriptions of three new species. *Zootaxa* 3702: 437–449. <https://doi.org/10.11646/zootaxa.3702.5.3>
- Monro AK, Bystriakova N, Fu L, Wen F, Wei Y, (2018) Discovery of a diverse cave flora in China. *PloS One* 13: e0190801. <https://doi.org/10.1371/journal.pone.0190801>
- Nitzu E, Vlaicu M, Giurginca A, Meleg IN, Popa I, Nae A, Baba Ş (2018) Assessing preservation priorities of caves and karst areas using the frequency of endemic cave-dwelling species. *International Journal of Speleology* 47: 43–52. <https://doi.org/10.5038/1827-806X.47.1.2147>
- Northup DE, Welbourn WC (1997) Life in the twilight zone – Lava tube ecology, natural history of El Malpais National Monument. *New Mexico Bureau of Mines and Mineral Resources Bulletin* 156: 69–82.
- Peck SB (1970) The terrestrial arthropod fauna of Florida caves. *Florida Entomologist* 53: 203–207. <https://doi.org/10.2307/3493189>
- Price L (2016) An introduction to some cave fauna of Malaysia and Thailand. *Acta Carsologica* 33: 311–317. <https://doi.org/10.2307/3493189>
- Ravbar N (2016) The earliest Chinese karstologist Xu Xiake. *Acta Carsologica* 32: 243–254. <https://doi.org/10.3986/ac.v32i1.376>
- Reddell JR (1994) The cave fauna of Texas with special reference to the western Edwards Plateau. In: Elliott WR, Veni G (Eds) *The caves and karst of Texas*. National Speleological Society, Huntsville, 31–50.
- Reeves WK (1999) Exotic species of North American Caves. In: Henderson K (Ed.) *Proceedings of the 1999 National Cave and Karst management symposium, Chattanooga, Tennessee: National Cave & Karst Management Symposia*, 164–166.
- Sket B (2008) Can we agree on an ecological classification of subterranean animals? *Journal of Natural History* 42: 1549–1563. <https://doi.org/10.1080/00222930801995762>
- Srisonchai R, Enghoff H, Likhitrakarn N, Panha S (2018) A revision of dragon millipedes I: genus *Hylomus* Chamberlin, 1923, with the description of eight new species (Diplopoda, Polydesmida, Paradoxosomatidae). *ZooKeys* 761: 1–177. <https://doi.org/10.3897/zookeys.761.24214>
- Stone FD, Howarth FG (2007) Hawaiian cave biology: status of conservation and management. In: *Proceedings of the 2005 National Cave and Karst Management Symposium; Albany, New York [October 31–November 4, 2005]*, 21–26.
- Sweeting MM (1995) *Karst in China. Its geomorphology and environment*. Springer Verlag, New York, 265 pp. <https://doi.org/10.1007/978-3-642-79520-6>

- Taiti S, Wynne JJ (2015) The terrestrial Isopoda (Crustacea, Oniscidea) of Rapa Nui (Easter Island), with descriptions of two new species. *ZooKeys* 515: 27–49. <https://doi.org/10.3897/zookeys.515.9477>
- Tanalgo KC, Tabora JA, Hughes AC (2018) Bat cave vulnerability index (BCVI): A holistic rapid assessment tool to identify priorities for effective cave conservation in the tropics. *Ecological Indicators* 89: 852–860. <https://doi.org/10.1016/j.ecolind.2017.11.064>
- Taylor SJ, Krejca J, Smith JE, Block VR, Hutto F (2003) Investigation of the potential for red imported fire ant (*Solenopsis invicta*) impacts on rare karst invertebrates at Fort Hood, Texas: A field study. In: Illinois Bexar County Karst Invertebrates Draft Recovery Plan Natural History Survey. Center for Biodiversity Technical Report, vol. 28, 1–153.
- Tian MY (2011) A new subgenus and two new species of the troglobitic genus *Dongodytes* Deuve from Guangxi, China (Coleoptera, Carabidae). *Subterranean Biology* 8: 57–64. <https://doi.org/10.3897/subtbiol.8.1232>
- Trajano E (2000) Cave faunas in the Atlantic tropical rain forest: Composition, ecology and conservation. *Biotropica* 32: 882–893. <https://doi.org/10.1111/j.1744-7429.2000.tb00626.x>
- van Beynen P, Townsend K (2005) A disturbance index for karst environments. *Environmental Management* 36: 101–116. <https://doi.org/10.1007/s00267-004-0265-9>
- Whitten T (2009) Applying ecology for cave management in China and neighboring countries. *Journal of Applied Ecology* 46: 520–523. <https://doi.org/10.1111/j.1365-2664.2009.01630.x>
- Wynne JJ (2013) Inventory, conservation and management of lava tube caves at El Malpais National Monument, New Mexico. *Park Science* 30: 45–55.
- Wynne JJ, Shear WA (2016) A new millipede (*Austrotyla awishashola*, n. sp., Diplopoda, Chordeumatida, Conotylidae) from New Mexico, USA, and the importance of cave moss gardens as refugial habitats. *Zootaxa* 4084: 285–292. <https://doi.org/10.11646/zootaxa.4084.2.8>
- Wynne JJ, Bernard EC, Howarth FG, Sommer S, Soto-Adames FN, Taiti S, Mockford EL, Horrocks M, Pakarati L, Pakarati-Hotus V (2014) Disturbance relicts in a rapidly changing World: The Rapa Nui (Easter Island) factor. *BioScience* 64: 711–718. <https://doi.org/10.1093/biosci/biu090>
- Wynne JJ, Sommer S, Howarth FG, Dickson BG, Voyles KD (2018) Capturing arthropod diversity in complex cave systems. *Diversity and Distributions* 24: 1478–1491. <https://doi.org/10.1111/ddi.12772>
- Wynne JJ, Howarth FG, Sommer S, Dickson BG (2019) Fifty years of cave arthropod sampling: techniques and best practices. *International Journal of Speleology* 48: 33–48. <https://doi.org/10.5038/1827-806X.48.1.2231>
- Zhang CZ, Li ZY (1981) Bilinguidae family nov. of Diplopoda in karst landform of south China. *Acta Zootaxonomica Sinica* 6: 373–377. [In Chinese]
- Zhao Y, Gluesenkamp A, Fenolio D, Soares D, Niemiller M, Bichuette M-E, Chakrabarty P (in press) The species diversity and conservation of cavefishes in China. In: Wynne JJ (Ed.) *Cave Ecology: Drivers of Diversity and Speciation*. NOVA Science Publishers, NY.

Sinkhole and brackish water nereidid polychaetes: Revision of *Stenoninereis* Wesenberg-Lund, 1958 (Annelida)

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Abstract

Stenoninereis species are nereidids with a small body (ca. 35 chaetigers), dorsal cirri with conspicuous cirrophores and cirrostyles, bare pharynx, and lack of neuropodial ventral ligules throughout the body. Currently, there are two valid species, *S. martini* Wesenberg-Lund, 1958 from the Lesser Antilles and *S. tecolultensis* de León-González & Solís-Weiss, 1997 from Eastern Mexico. *Nicon lackeyi* Hartman, 1958 has been regarded as a junior synonym of *S. martini*. The examination of type and topotype specimens indicated that *N. lackeyi* is a distinct species and both species are redescribed, *S. martini* is restricted to the Caribbean islands, and *N. lackeyi* is reinstated and transferred to *Stenoninereis*. Further, Puerto Rican specimens differ from these known species and a new species, *S. elisae* is also described, featured mainly by large cirrophores and short cirrostyles in anterior and middle chaetigers. Further discussions about their morphology and phylogenetic affinities and a key to identify all known *Stenoninereis* species are also included.

Keywords

Notopodial dorsal ligules, morphology, ecology, blood vessels, innervation, cirrophores

Introduction

The nereidid genus *Stenoninereis* was established by Wesenberg-Lund (1958) for a new species, *S. martini*, discovered in a sinkhole from Sint Maarten, the Caribbean Sea, featured by having a small body of about 35 chaetigers, and large dorsal cirri with

distinct cirrophores and cirrostyles, pharynx without papillae or paragnaths, and a progressive reduction and disappearing of notopodial dorsal ligules in the posterior end (Wesenberg-Lund 1958). Wesenberg-Lund (1958) argued that the set of features presence of antennae, lacking pharyngeal ornamentation, biramous parapodia but lacking hypertrophied dorsal ligules, separate the new species from genera with similar morphology such as *Micronereis* Claparède, 1863 or *Leptonereis* Kinberg, 1865. Later works have found morphological affinities among *Stenoninereis* and other plesiomorphic genera such as *Namalycastis* Hartman, 1959, *Namanereis* Chamberlin, 1919, and *Profundilycastis* Hartmann-Schröder, 1977 (Fitzhugh 1987; Santos et al. 2005), which have smooth pharynx and parapodia with few or no ligules.

At the same year, Hartman (1958) described *Nicon lackeyi* from a spring in Sarasota, Florida. She concluded it should belong to *Nicon* Kinberg, 1865 because it had a bare pharynx, biramous parapodia, notochaetae spinigers, and neurochaetae as spinigers and falcigers. Hartman (1958) also included a list and a key to species of all then known *Nicon*, but relevant features such as having dorsal cirri with distinct cirrophores and cirrostyles, the disappearing of notopodial dorsal ligules toward posterior end of body, and the lack of neuropodial ventral ligules throughout body, were disregarded as diagnostic at generic level. Pettibone (1971) studied the type material of *N. lackeyi* and *S. martini* and regarded them as synonyms because of their high resemblance. Consequently, *S. martini* has been reported along the Gulf of Mexico, the Caribbean Sea and the Eastern coasts of United States of America, especially in ecological studies, and even used as a bioindicator of hypoxic or anoxic conditions. Only another species has been described after *S. martini*, *S. tecolutlensis* de León-González & Solís-Weiss, 1997. This was found in oysters shells attached to mangrove roots from the Estero de Larios, Veracruz, Mexico (de León-González and Solís-Weiss 1997).

After a prospective examination of available material of *Stenoninereis* species in the National Museum of Natural History, Smithsonian Institution (USNM) from several localities, some doubts arose about if *Nicon lackeyi* is a synonym of *Stenoninereis martini*, and if some records of *S. martini* really correspond with it. With the aim to clarify the status of these species, all type, topotypes and additional specimens available of the involved species were revised. As a result of the revision, the type species is redescribed, *N. lackeyi* is reinstated and transferred to *Stenoninereis*, and a new species is proposed. Further, a key to identify all known species is also given.

Material and methods

Type and non-type specimens are deposited in the Museum of Natural History of Los Angeles County, California, United States of America (LACM-AHF); and the National Museum of Natural History, Smithsonian Institution, Washington D.C., United States of America (USNM). The holotype of *S. tecolutlensis* (USNM 174870) was examined for comparison.

All specimens were whole-mounted and observed under stereomicroscope and compound microscopes for observing parapodial and chaetal modifications along the body. Some parapodia were removed and mounted on semi-permanent slides with ethanol-glycerol, and examined under the compound microscope. Parapodia from first or two chaetiger were revised and sometimes dissected, and three to four additional parapodia were removed throughout the body. Due to the delicate tissues of the anterior end of specimens, dissection of the pharynx was avoided. The photographs were made with a digital camera with adaptor for both microscopes. In some cases, a set of photographs were made and combined manually to improve composition. Some contrast and brightness adjustments were performed in photographs to improve the visibility of structures. For describing parapodia, Bakken and Wilson's (2005) terminology was followed, and terms 'achaetous ring' and 'anterior cirri' were used instead of 'peristomium' and 'tentacular cirri', respectively, as suggested by Santos et al. (2005).

Results

Systematics

Order Phyllodocida Dales, 1962

Family Nereididae de Blainville, 1818

Genus *Stenoninereis* Wesenberg-Lund, 1958

Stenoninereis Wesenberg-Lund 1958: 12.

Type species. *Stenoninereis martini* Wesenberg-Lund, 1958, by monotypy.

Diagnosis (modified from León-González and Solís-Weiss 1997, additions are highlighted in boldface). Prostomium with anterior margin cleft. Antennae and eyes present. Four pairs of **anterior cirri**. Pharynx bare. First two chaetigers with neuroacicular ligules and ventral cirri only. **Anterior chaetigers with dorsal cirrophores and notopodial dorsal ligules ciliated.** Dorsal cirri with distinct cirrophores and cirrostyles throughout body. Notopodial dorsal ligules present in anterior and middle chaetigers, disappearing in posterior-most ones; neuropodial ventral ligules absent throughout body. **Notochaetae sesquigomph spinigers; neurochaetae sesquigomph spinigers and falcigers and heterogomph falcigers in supra-acicular fascicles, heterogomph spinigers and falcigers in sub-acicular fascicles.** Pygidium with two plate-like lobes.

Remarks. Main morphological features of *Stenoninereis* species are depicted in Figure 1. The species *S. tecolutlensis* was described as having two notopodial fascicles, the superior one bearing homogomph spinigers and the inferior one with sesquigomph spinigers. However, after the examination of the holotype (USNM 174870), there are no notopodial homogomph spinigers, likely being that indication an artifact due to the position of the shaft, and therefore this feature is deleted from the emended diagnosis.

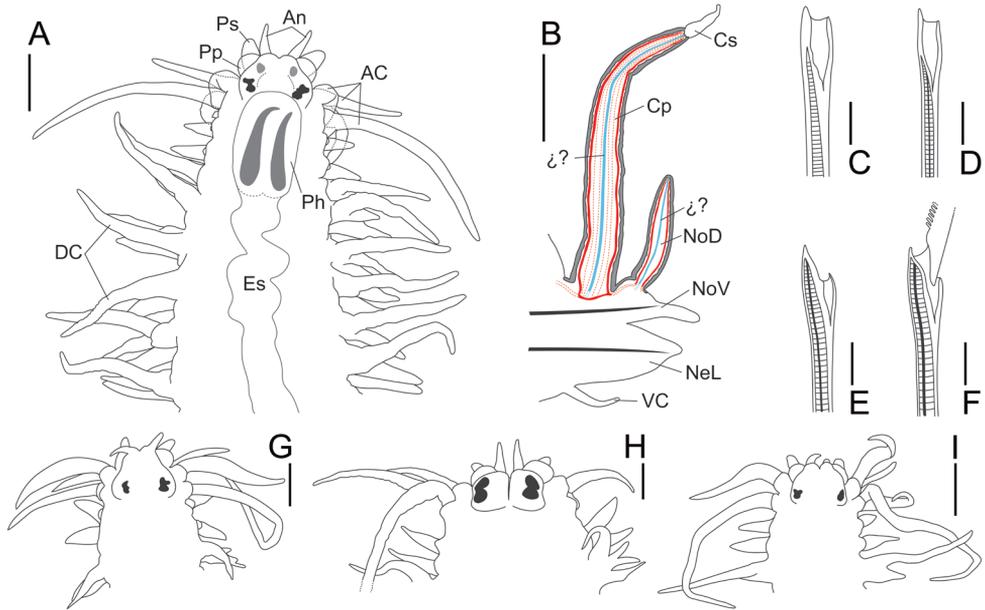


Figure 1. Morphology of *Stenoninereis* species **A** non-type of *S. lackeyi* comb. n. (USNM 53273) **B, I** syntypes of *S. elisae* sp. nov. (USNM 55366) **C–F** non-type of *S. elisae* sp. nov. (USNM 55360) **G** non-type of *S. martini* Wesenberg-Lund 1958 (USNM 61623) **H** holotype of *S. teocolutlensis* de León-González & Solís-Weiss, 1997 (USNM 174870) **A** anterior end, dorsal view **B** chaetiger 6, left parapodium (solid and dashed red lines: vessels; solid and dashed light blue lines: unknown structures, likely nerves) **C** shaft of notopodial sesquigomph spinigers, chaetiger 20 **D** shaft of neuropodial supra-acicular sesquigomph spiniger, same chaetiger **E** shaft of neuropodial sub-acicular heterogomph spiniger, chaetiger 20 **F** Shaft of neuropodial sub-acicular heterogomph falciger, chaetiger 20 **G–I** anterior ends, dorsal view. Abbreviations: AC, anterior cirri; An, antennae; Cp, cirrophore; Cs, cirrostyle; DC, dorsal cirrus; Es, esophagus; NeL, neuroacicular ligule; NoD, notopodial dorsal ligule; NoV, notopodial ventral ligule; Ph, pharynx; Pp, palpophore; Ps, palpostyle; VC, ventral cirrus; $\zeta?$, unknown structures, likely nerves by their position. Scale bars: 0.2 mm (**A, G–I**); 0.1 mm (**B**); 5 μ m (**C–F**).

Stenoninereis martini Wesenberg-Lund, 1958, restricted

Figures 1G, 2–3

Stenoninereis martini Wesenberg-Lund 1958: 9–12, figs 2, 3, 4a–c. Pettibone 1971: 39–41, figs 23a–n (partim).

Material examined. Syntypes. CARIBBEAN SEA, NETHERLANDS ANTILLES • 2; Sint Maarten, Devil’s Hole; 26 Jul. 1955; P.W. Hummelinck leg.; 20x5x1.5 m, water almost clear and slightly greenish brown, 10900 mg Cl/I; USNM 29726.

Additional material. CARIBBEAN SEA, PUERTO RICO • 21; Laguna Joyuda, off Inlet canal; 18°7’30”N, 67°10’0.12”W; 9 Oct. 1979; R. Castro leg.; mud and shells, host gastropods; USNM 61623.

Description. Two syntypes (USNM 29726) in poor condition, dissections previously performed (Figs 2D–E). One syntype posteriorly incomplete, some anterior pa-

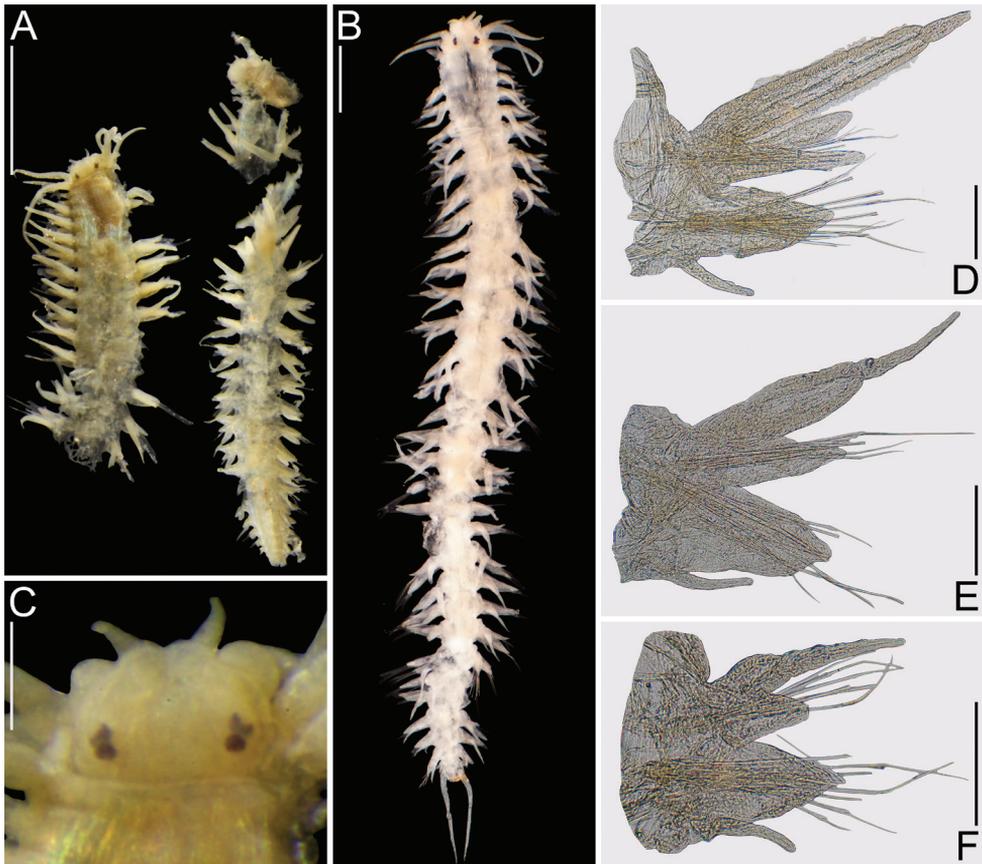


Figure 2. *Stenoninereis martini* Wesenberg-Lund, 1958 **A, C–F** syntypes (USNM 29726) **B** non-type (USNM 61623) **A** whole specimens, dorsal view **B** whole specimen, dorsal view **C** close-up of prostomium, dorsal view **D** chaetiger 6, left parapodium, anterior view (dorsal cirrostyle incomplete) **E** chaetiger 13, left parapodium, anterior view **F** chaetiger 18, left parapodium, anterior view. Scale bars: 0.5 mm (**A–B**); 0.1 mm (**C–F**).

rapodia previously dissected, 2.4 mm long, 0.4 mm wide at chaetiger 10, 16 chaetigers (Fig. 2A). The other syntype in two portions, anterior end very damaged, 3.5 mm long, 0.3 mm wide, 21 chaetigers, some posterior chaetigers previously dissected (Fig. 2A). Non-type material (USNM 61623) complete, 5 mm long, 0.3 mm wide, 32 chaetigers (Fig. 2B). All specimens pale, no pigmentation remaining.

Prostomium wider than long, anterior margin shallowly cleft (Figs 1G, 2C); antennae subulate, half as long as prostomium; eyes black, subequal, anterior eyes reniform, twice larger than posterior rounded eyes, anterior and posterior pairs slightly overlapped (Figs 1G, 2B–C). Achaetous ring half as long as first chaetiger; four pairs of anterior cirri, longest one reaching chaetiger 8 (Figs 1G, 2A–B).

Pharynx dissected; jaws light brown, translucent, 13 teeth (Fig. 3J). Pharynx surface bare.

All chaetigers having both noto- and neuroaciculae; dorsal cirri cirrophores and notopodial dorsal ligules of anterior chaetigers ciliated. In first two chaetigers (Fig. 3A), no-

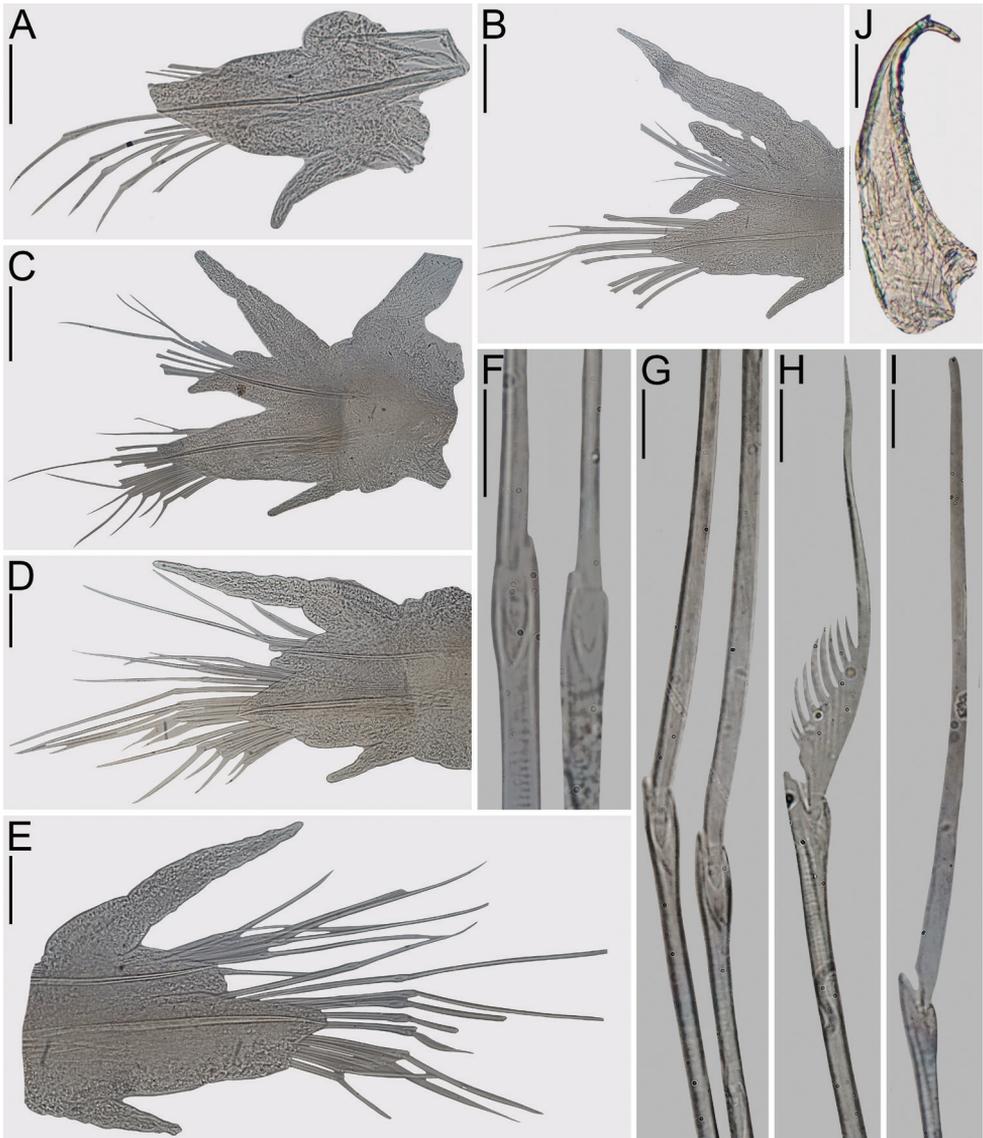


Figure 3. *Stenonineris martini* Wesenberg-Lund, 1958 **A–J** non-type specimens (USNM 61623) **A** chaetiger 2, right parapodium, anterior view **B** chaetiger 9, right parapodium, anterior view **C** chaetiger 21, right parapodium, anterior view **D** chaetiger 27, right parapodium, anterior view **E** chaetiger 28, left parapodium, anterior view **F** notopodial sesquigomph spinigers, chaetiger 28 **G** supra-acicular sesquigomph spinigers, chaetiger 28 **H** sub-acicular heterogomph spiniger, chaetiger 28 **I** sub-acicular heterogomph spiniger, chaetiger 28 **J** left jaw, dorsal view. Scale bars: 50 μ m (**A, D**); 0.1 mm (**B–C**); 10 μ m (**F–I**); 50 μ m (**J**).

topodia small, rounded lobe with notoaculum. Neuroacicular ligule subconical, twice longer than ventral cirrus. Ventral cirrus subulate, cirrophore and cirrostyle indistinct.

In anterior chaetigers (Figs 2D, 3B), dorsal cirrus as long as chaetiger width, excluding parapodia; cirrophore 2–3 times longer than cirrostyle, 1.6–2.0 times longer

than notopodial dorsal ligule, twice longer than notopodial ventral ligule. Notopodial dorsal ligule digitiform, basally attached to, and slightly shorter than, notopodial ventral ligule; notopodial ventral ligule subconical to digitiform, 2.8–3.0 times longer than wide, 1.0–1.3 times longer than neuroacicular ligule. Neuroacicular ligule subconical, 1.8–2.0 times longer than wide, 2.0–2.5 times longer than ventral cirrus. Ventral cirrus subulate, cirrophore and cirrostyle indistinct.

In middle chaetigers (Figs 2E, 3C), dorsal cirrus shorter than wide of chaetiger excluding parapodia; cirrophore 1.5 times longer than cirrostyle, 1.5 times longer than notopodial ventral ligule. Notopodial dorsal ligule absent in dissected syntype, digitiform in non-type specimen, 0.4 times as long as notopodial ventral ligule; notopodial ventral ligule subconical, 1.8–2.0 times longer than wide, as long as neuroacicular ligule. Neuroacicular ligule subconical, 1.4–1.8 times longer than wide, 1.7 times longer than ventral cirrus. Ventral cirrus subulate, cirrophore and cirrostyle indistinct.

In posterior chaetigers (Figs 2F, 3D–E), dorsal cirrus shorter than chaetiger width, excluding parapodia; cirrophore 0.6 times as long as cirrostyle, half as long as notopodial ventral ligule. Notopodial dorsal ligule absent; notopodial ventral ligule subconical, 1.3–1.4 times longer than wide, half as long as neuroacicular ligule. Neuroacicular ligule subconical, 1.4–1.5 times longer than wide, 1.7 times longer than ventral cirrus. Ventral cirrus subulate, cirrophore and cirrostyle indistinct.

Notochaetae sesquigomph spinigers. Neurochaetae sesquigomph spinigers in supra-acicular fascicles, heterogomph spiniger and falcigers in sub-acicular fascicles.

Notopodial and neuropodial supra-acicular sesquigomph spinigers with blade smooth (Figs 3F–G). Neuropodial heterogomph spinigers with blades basally serrate, coarse teeth, larger teeth longer than blade width, 2/3 of the blade edentate and subulate (Fig. 3H). Neuropodial heterogomph falcigers with very long blades (Fig. 3I), increasing their length from upper to lower positions in the same fascicle; falcigers with blades smooth (Fig. 3I).

Pygidium with two anal plate-like lobes; anal cirri missing in types (Fig. 2A), as long as last five chaetigers in a non-type specimen (Fig. 2B).

Remarks. The syntypes revised are damaged, but some parapodia in good conditions were dissected and examined. There are a few differences between the current description and the original one. The syntypes include about 25 specimens of a wide range of size, including juveniles having the larval eyes (“In young specimens there are 6 eyes arranged in two triangles of three each...”) (Wesenberg-Lund 1958). Wesenberg-Lund (1958) noted that notopodial dorsal ligules disappear from chaetiger 26 in the specimen dissected, but there is no indication about the number of chaetigers of such specimen, likely being the largest one 6 mm long, and 34 chaetigers. In the specimens examined, the notopodial dorsal ligules disappear from anterior chaetigers (about chaetiger 12). Remaining parapodial features in the current redescription match the original description, and the redescription by Pettibone (1971). Pettibone (1971) described the notopodia of the first two chaetigers as having “slender notoaciculum with short, conical, basal cirrophore of dorsal cirrus; style short (sometimes missing)”, and such dorsal cirrus was depicted in the anterior end and the second chaetiger draw-

ings (Pettibone 1971, Figs 23a and g, respectively). However, there are no dorsal cirri in first two chaetigers of the specimens of this and all other known species.

On the other hand, the holotype of *S. tecolutlensis* (USNM 174870) was examined for comparison, and differences are evident between it and *S. martini*. In *S. tecolutlensis*, the prostomial margin is deeply cleft and a median groove is present, reaching the anterior pair of eyes, whereas in *S. martini* the cleft is shallow, and there is no median groove. In *S. tecolutlensis*, the dorsal cirrophores are 1.5 times longer than cirrostyles and as long as notopodial dorsal ligules in anterior chaetigers, whereas in *S. martini* cirrophores are 2–3 times longer than cirrostyles and twice longer than notopodial dorsal ligules. Further, in *S. tecolutlensis* the cirrophores are as long as notopodial dorsal ligules in middle chaetigers and as long as them in posterior chaetigers, whereas in *S. martini* cirrophores are twice longer than notopodial dorsal ligules in middle chaetigers and half as long as them in posterior chaetigers. Both species are similar in other respects. The differences with *S. elisae* sp. nov. and *S. lackeyi* comb. n. are discussed in the remarks for these species.

The record of *S. martini* for the Gulf of Mexico by de León-González and Solís-Weiss (1997) is also different. The authors described specimens with 'trilobate' notopodia', i.e., with notopodial prechaetal lobes, a feature absent in all known species; also, the notopodial dorsal ligules are present throughout the body in specimens from the Gulf of Mexico, whereas in *S. martini* and other known species the notopodial dorsal ligules disappear in posterior chaetigers. Further, the cirrophores are several times longer than cirrostyles along the body in the specimens from the Gulf of Mexico (4 in anterior, 3.3 in middle, and 2.3 in posterior chaetigers), and shorter than cirrostyles in posterior chaetigers (0.5) in the syntypes of *S. martini*; this development of the dorsal cirri resembles the one found in *S. elisae* sp. nov. Furthermore, the cirrostyle/notopodial ventral ligule ratios in the specimens of *S. martini* from the Gulf of Mexico are almost the same along the body (1.4–1.5), whereas in the syntypes of *S. martini* the ratio decreases toward posterior chaetigers. Finally, the neuropodial sub-acicular spinigers in specimens from the Gulf of Mexico have 2/3 of the blade dentate, whereas in the syntypes of *S. martini* only 1/3 is dentate. These differences make doubtful the conspecificity of the specimens from the Gulf of Mexico with specimens from the Caribbean Sea, so a further study is needed to clarify their status.

***Stenoninereis lackeyi* (Hartman, 1958), reinst. comb. n.**

Figures 1A, 4, 5

Nicon lackeyi Hartman 1958: 263–265, figs 1–5.

Stenoninereis martini Pettibone 1971: 39–41, figs 24a–c (*partim, non* Wesenberg-Lund, 1958)

Material examined. Paratypes. GULF OF MEXICO, UNITED STATES • 8, paratypes of *Nicon lackeyi*; Florida, Sarasota County, Warm Mineral Springs; 27°02'43"N,

82°17'35"W; J. Lackey leg.; no date, 86 °F, 17000 ppm dissolved solids, 7.2±0.2 pH; LACM-AHF 806.

Additional material. GULF OF MEXICO, UNITED STATES • 60; Florida, Tampa Bay; 1963; J. L. Taylor leg.; USNM 45699 • 16; Halstead Bayou, Jackson County, Mississippi; Sep. 1975; R. Herd leg.; USNM 53273.

NORTHWESTERN ATLANTIC OCEAN, UNITED STATES • 6; North Carolina, Beaufort, Town Marsh; L. Cammen leg.; USNM 55619 • 7; North Carolina, Bogue Sound, Tar Landing Bay; Oct. 1976; R. T. Kneib leg.; USNM 55618.

Description. Paratypes (LACM-AHF 806) complete, some parapodia previously dissected; one complete paratype selected for description, 4 mm long, 0.5 mm wide at chaetiger 10, 31 chaetigers (Fig. 4A). Specimens from Florida (USNM 45699) most complete, some filled with large oocytes, two per segment (Fig. 5A); one paratype used for variation, complete, 4.8 mm long, 0.4 mm wide, 33 chaetigers (Fig. 5A). All specimens pale.

Prostomium wider than long, anterior margin shallowly cleft (Fig. 4B); antennae subulate, half as long as prostomium; eyes black, anterior eyes reniform, slightly larger than posterior rounded ones, anterior and posterior eyes slightly overlapped (Fig. 4B). Achaetous ring as long as first chaetiger; four pairs of anterior cirri, anterodorsal pair lanceolate, remaining ones subulate, longest one reaching chaetiger 7, (Figs 4A–B).

Pharynx previously removed in paratype. In non-type material, jaws light brown, 10 teeth restricted to half cutting edge (Fig. 5B). Pharynx bare.

All chaetigers with noto- and neuroaciculae; dorsal cirri cirrophores and notopodial dorsal ligules of anterior chaetigers ciliate. In first two chaetigers (Fig. 5C), notopodium consists in a small, rounded lobe bearing notoaciculum. Neuroacicular ligule subconical, 2.5 times longer than ventral cirrus. Ventral cirrus subulate, cirrophore and cirrostyle indistinct.

In anterior chaetigers (Figs 4H, 5D–E), dorsal cirrus shorter than chaetiger width, excluding parapodia; cirrophore 1.5–2.0 times longer than cirrostyle, 1.3 times longer than notopodial dorsal ligule, 1.0–1.2 times longer than notopodial ventral ligule. Notopodial dorsal ligule digitiform, basally attached to, and half as long as, notopodial ventral ligule; notopodial ventral ligule subconical, 3.0–3.5 times longer than wide, 1.0–1.2 times longer than neuroacicular ligule. Neuroacicular ligule subconical, 2.3–2.5 times longer than wide, 2.8–3.0 times longer than ventral cirrus. Ventral cirrus subulate, cirrophore and cirrostyle indistinct.

In middle chaetigers (Figs 4I, 5F), dorsal cirrus shorter than chaetiger width, excluding parapodia; cirrophore 1.2–1.4 times longer than cirrostyle, 4 times longer than notopodial dorsal ligule, 1.0–1.2 times longer than notopodial ventral ligule. Notopodial dorsal ligule digitiform, basally attached to, and 0.2 times as long as, notopodial ventral ligule; notopodial ventral ligule subconical, 2.2–2.4 times longer than wide, as long as notopodial ventral ligule. Neuroacicular ligule subconical, 2.5–2.7 times longer than ventral cirrus. Ventral cirrus subulate, cirrophore and cirrostyle indistinct.

In posterior chaetigers (Figs 4J, 5G), dorsal cirrus shorter than wide of chaetigers excluding parapodia; cirrophore 0.6 times as long as cirrostyle, 0.7 times as long as

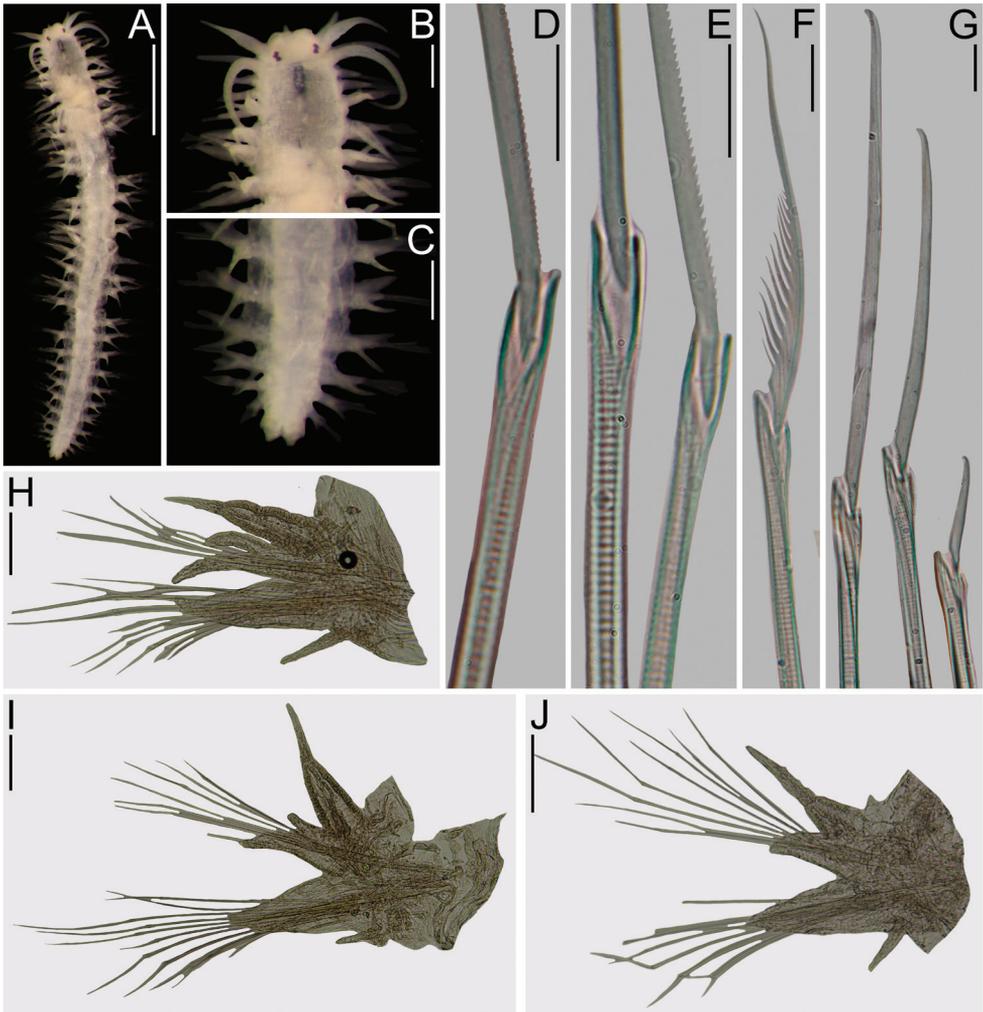


Figure 4. *Stenoninereis lackeyi* (Hartman, 1958) comb. n. **A–J** paratype (AHF-POLY-806) **A** whole specimen, dorsal view **B** anterior end, dorsal view **C** posterior end, dorsal view **D** notopodial sesquigomph spiniger, chaetiger 27 **E** supra-acicular sesquigomph spinigers, chaetiger 27 **F** sub-acicular heterogomph spiniger, chaetiger 27 **G** sub-acicular heterogomph falcigers (uppermost one at the left), chaetiger 27 **H** chaetiger 7, right parapodium, anterior view **I** chaetiger 19, right parapodium, anterior view **J** chaetiger 26, right parapodium, anterior view. Scale bars: 1 mm (**A**); 0.25 mm (**B–C**); 10 μ m (**D–G**) 0.1 mm (**H–J**).

notopodial ventral ligule. Notopodial dorsal ligule absent; notopodial ventral ligule subconical, 1.5–2.0 times longer than wide, 0.7–1.0 times as long as neuroacicular ligule. Neuroacicular ligule subconical, 2.0–2.1 times longer than wide, 2.5 times longer than ventral cirrus. Ventral cirrus subulate, cirrophore and cirrostyle indistinct.

Notochaetae sesquigomph spinigers. Neurochaetae sesquigomph spinigers in supra-acicular fascicles, heterogomph spinigers and falcigers in sub-acicular fascicles.

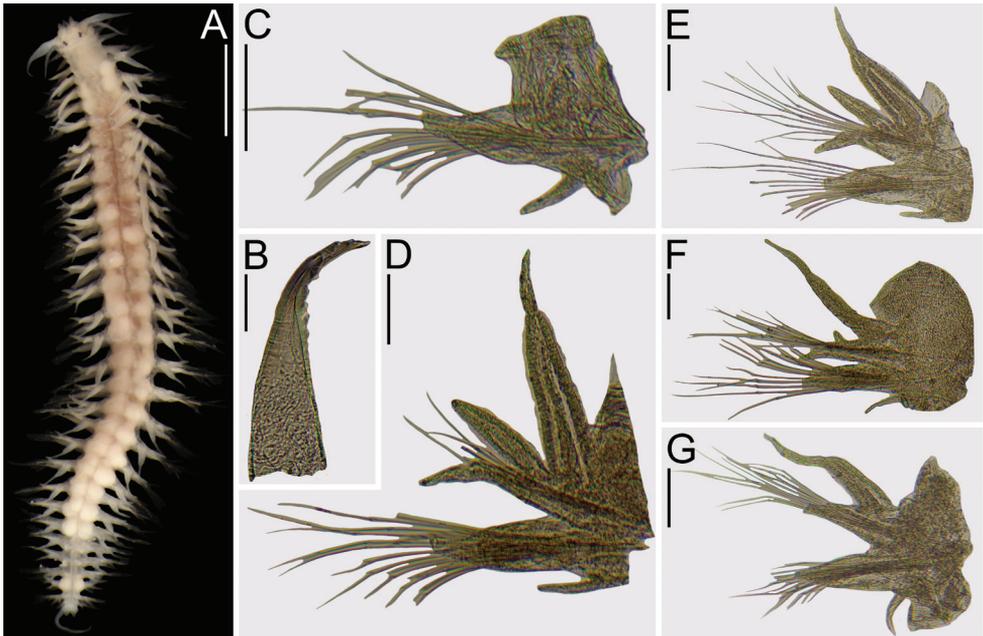


Figure 5. *Stenoninereis lackeyi* (Hartman, 1958) comb. n. **A–F** non-type specimen (USNM 45699) **A** whole specimen, dorsal view **B** left jaw, dorsal view **C** Chaetiger 2, right parapodium, anterior view **D** Chaetiger 6, right parapodium, anterior view **E** Chaetiger 17, right parapodium, anterior view **F** Chaetiger 23, right parapodium, anterior view **G** Chaetiger 29, right parapodium, anterior view. Scale bars: 1 mm (**A**); 50 μ m (**B**); 0.1 mm (**C–G**).

Notopodial (Fig. 4D) and neuropodial (Fig. 5E) supra-acicular sesquigomph spinigers pectinate, very minute teeth, progressively disappearing distally. Neuropodial heterogomph spinigers (Fig. 4F) serrate, coarse teeth, longer teeth are longer than the wide of the blade, half of 1/3 of the blade edentate and subulate. Neuropodial heterogomph falcigers (Fig. 4G) with very long blades, blades increasing their length toward posterior chaetigers, blades with minute teeth throughout, often inconspicuous; blades increasing their length from upper to lower positions in the same fascicle (Fig. 4G).

Pygidium with two plate-like lobes; anal cirri subulate, as long as last four chaetigers (Figs 4C, 5A).

Remarks. Hartman (1958) described *Nicon lackeyi* with several specimens but the exact number of them was not stated; the specimens were supposedly deposited in a single lot (USNM 29627). Pettibone (1971) examined the lot and found the holotype and an additional lot with catalog number USNM 29628 containing two paratypes, but no further comments about the supposed splitting of the type material were added. These lots were not found during three research visits at the USNM (2015, 2016, and 2018). Some specimens, however, are deposited in the Los Angeles Museum; they were examined by Hartman, with identical field data as those recorded for the supposed

type specimens. LACM material was labeled as paratypes by Kristian Fauchald according to the collection records, but no further comments were found. Because the specimens belonged to the original set of specimens, and because the specimens previously regarded as holotype and remaining type specimens are currently missing, the species was redescribed with available specimens in the LACM.

After a comparison between type material of *N. lackeyi* and *S. martini* led Pettibone (1971) to regard them as synonyms. However, there are relevant differences to separate them as two distinct species of the same genus. The main distinctive feature is the slender aspect of both notopodial ventral ligules and neuroacicular ligules and mainly observed in middle and posterior chaetigers, as seen when comparing the length/wide ratios in these ligules along the body. In anterior chaetigers, both species have similar ratios in notopodial ventral ligules, but *Stenoninereis lackeyi* comb. n. has larger ratios in the middle (2.2–2.4) and posterior (1.5–2.0) chaetigers than *Stenoninereis martini* (1.8–2.0 and 1.3–1.4, respectively). Likewise, in neuroacicular ligules, *S. lackeyi* comb. n. has larger ratios in anterior (2.3–2.5), middle (2.5–2.7), and posterior (2.0–2.1) chaetigers than *S. martini* (1.8–2.0, 1.4–1.8, and 1.4–1.5, respectively). Further, in anterior chaetigers, dorsal cirrophores in *S. lackeyi* comb. n. are 1.5–2.0 times longer than their respective cirrostyles, whereas in *S. martini* they are 2–3 times longer; in middle and posterior chaetigers, both species have similar ratios.

Stenoninereis lackeyi comb. n. is easily recognized from *S. tecolutlensis*, the other Gulf of Mexico species. In *S. lackeyi* comb. n., the prostomium is shallowly incised and the eyes are minute, whereas in *S. tecolutlensis* the prostomium is deeply incised and the eyes occupy a larger prostomial surface (Fig. 1H). In addition, in *S. lackeyi* comb. n. the neuroacicular lobes are blunt, whereas in *S. tecolutlensis* they are acuminate.

***Stenoninereis elisae* sp. nov.**

<http://zoobank.org/C5EF40A3-DCE5-45DC-B804-A6A9D8A16585>

Figures 1B–F, I; 6

Type material. Syntypes. CARIBBEAN SEA, PUERTO RICO • 5; Rio Grande, Espiritu Santo River, 50 m below Castanon confluent; Apr. 1977; W.R. Bhajan leg.; USNM 55366.

Additional material. CARIBBEAN SEA, PUERTO RICO • 3; Rio Grande, Espiritu Santo River, 50 m above Castanon confluent; W.R. Bhajan leg.; USNM 55360.

Type locality. Espiritu Santo River, Rio Grande, Puerto Rico.

Description. Five syntypes (USNM 55366) complete, in good condition. Specimens dissected complete, 62 mm long, 0.4 m wide at chaetiger 10, 27 chaetigers. All specimens pale (Figs 6A–B).

Prostomium wider than long, anterior margin shallowly cleft (Figs 1I, 6C); antennae subulate, half as long as prostomium; eyes black, anterior and posterior eyes

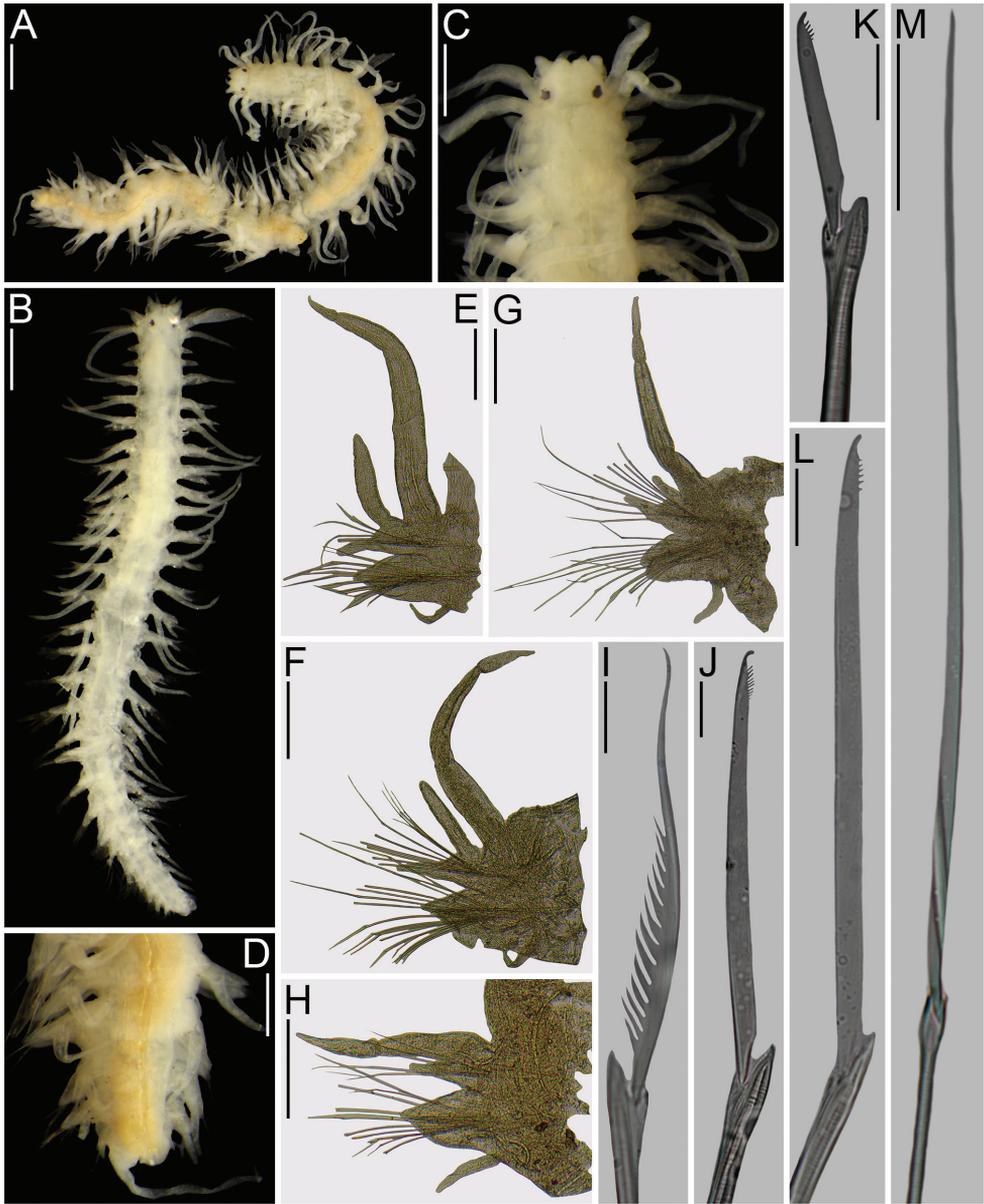


Figure 6. *Stenoninereis elisae* sp. nov. **A–M** Syntypes (USNM 55366) **A** whole specimen, dorsal view **B** whole specimens, dorsal view **C** anterior end, dorsal view **D** Posterior end, dorsal view **E** chaetiger 6, right parapodium, anterior view **F** chaetiger 16, right parapodium, anterior view **G** chaetiger 18, right parapodium, anterior view **H** chaetiger 24, right parapodium, anterior view **I** subacicular heterogomph spinigers, chaetiger 18 **J–L** subacicular heterogomph falcigers, chaetiger 18 **M** notopodial homogomph spiniger, chaetiger 49. Scale bars: 0.5 mm (**A–B**); 0.25 mm (**C**); 0.2 mm (**E–H**); 10 μ m (**I–L**); 30 μ m (**M**).

rounded, anterior eyes smaller than posterior ones, strongly overlapped, forming a pyriform spot (Figs 1I, 6A–C). Achaetous ring as long as first chaetiger; four pairs of anterior cirri, longest one reaching chaetiger 6, anterodorsal pair lanceolate, remaining ones subulate (Figs 1I, 6A–C).

Pharynx dissected; jaws brown, translucent, 8 teeth. Pharynx surface bare.

All chaetigers with noto- and neuroaciculae; dorsal cirri cirrophores and notopodial dorsal ligules of anterior chaetigers with ciliate surface. In first two chaetigers, notopodium with a small, rounded lobe bearing notoaciculum. Neuroacicular ligule subconical, twice longer than ventral cirrus. Ventral cirrus subulate, cirrophore and cirrostyle indistinct.

In anterior chaetigers (Figs 6E), dorsal cirrus longer than chaetiger width, excluding parapodia; cirrophore 5.5–6.0 times longer than cirrostyle, 2.6 times longer than notopodial dorsal ligule, 3 times longer than notopodial ventral ligule. Notopodial dorsal ligule digitiform, basally attached to, and 1.2 times longer than, notopodial ventral ligule; notopodial ventral ligule subconical, 2.3 times longer than wide, 1.3 times longer than neuroacicular ligule. Neuroacicular ligule subconical, 1.5 times longer than wide, 2.2 times longer than ventral cirrus. Ventral cirrus subulate, cirrophore and cirrostyle indistinct.

In middle chaetigers (Figs 6F–G), dorsal cirrus longer than chaetiger width, excluding parapodia; cirrophore 2.5–3 times longer than cirrostyle, twice longer than notopodial dorsal ligule and becoming shorter toward posterior chaetigers, twice longer than notopodial ventral ligule. Notopodial dorsal ligule digitiform, as long as notopodial ventral ligule, rapidly decreasing in size toward posterior chaetigers and disappearing from chaetiger 23–25; notopodial ventral ligule subconical, twice longer than wide, 1.6 times longer than neuroacicular ligule. Neuroacicular ligule subconical, 1.2–1.4 times longer than wide, twice longer than ventral cirrus. Ventral cirrus subulate, cirrophore and cirrostyle indistinct.

In posterior chaetigers (Fig. 6H), dorsal cirrus as long as chaetiger width, excluding parapodia; cirrophore 1.4 times longer than cirrostyle, 1.5 times longer than notopodial ventral ligule. Notopodial dorsal ligule absent; notopodial ventral ligule subconical, twice longer than wide, as long as neuroacicular ligule. Neuroacicular ligule subconical, 1.3 times longer than wide, 1.3 times longer than ventral cirrus. Ventral cirrus subulate, cirrophore and cirrostyle indistinct.

Notochaetae sesquigomph spinigers. Neurochaetae sesquigomph spinigers in supra-acicular fascicles, heterogomph spinigers and falcigers in sub-acicular fascicles.

Notopodial (Figs 1C, 6M) and neuropodial (Fig. 1D) supra-acicular sesquigomph spinigers pectinate, very minute teeth, disappearing toward distal end. Neuropodial heterogomph spinigers (Figs 1E, 6I) serrate, coarse teeth, larger teeth longer than blade width, 1/3 of blade edentate, subulate. Neuropodial heterogomph falcigers (Fig. 1F) with very long blades, blades increasing their length toward posterior chaetigers, blades with basal and medial teeth minute, often inconspicuous (Figs 6J–L), rarely some small teeth basally and distally (Fig. 1E); supra-acicular blades shorter than sub-acicular ones, blades of sub-acicular falcigers increasing their length from upper to lower positions in the same fascicle (Figs 6J–L).

Pygidium with two anal plate-like lobes; anal cirri as long as last five chaetigers (Fig. 6D).

Etymology. The name is after the late Dr. Elise Wesenberg-Lund, in recognition of her many works in polychaete taxonomy, especially freshwater ones, and by proposing the genus *Stenoninereis*. The name is a noun in apposition and was formed by the elision of the last vowel for euphony.

Remarks. Due to the morphological variability observed in specimens belonging to *Stenoninereis*, the designation of syntypes was preferred (ICZN 1999, Art. 72.3). *Stenoninereis elisae* sp. nov. is easily recognized by the enlarged dorsal cirri in anterior and middle chaetigers, being two or three times longer than the width of their respective segments, and by the size and proximity of the eyes resulting into a pyriform shape. The cirrophore/cirrostyle lengths ratio is also distinctive for this species because the cirrophore is longer than cirrostyle throughout the body, whereas in *S. lackeyi* comb. n., *S. martini*, and *S. tecolutlensis*, cirrostyles are longer than cirrophores in posterior chaetigers. Also, the cirrophore/cirrostyle lengths ratios in *S. elisae* sp. nov. are greater than in the other species, especially in anterior (5.5–6.0) and middle (2.5–3.0) chaetigers. Finally, the blades of the neuropodial heterogomph falcigers are denticulated, whereas in the remaining species they are smooth.

The notopodial dorsal ligules exhibit a high variation in their length as shown in the cirrophore/ notopodial dorsal and notopodial ventral ligules/notopodial dorsal ligules ratios in anterior (2.3–7.1 and 1.3–3.4, respectively) and middle (1.3–3.8 in both ratios) chaetigers. This variability in the size of the notopodial dorsal ligules was also observed in specimens of *S. lackeyi* comb. n., and *S. martini*, but the ratios are less variable.

Ecological notes

Stenoninereis species are known by living in unusual habitats and organically enriched or polluted environments. They live in inland waters such as sinkholes, marshes, mineral springs with tidal influences and freshwater inputs, often with high fluctuations in salinity and dissolved oxygen (McBee and Brehm 1979; Wenner and Beatty 1988). Also, *Stenoninereis* species have been recorded in bays associated with mangroves, reaching high population densities in some places (Ibáñez 1997; McBee and Brehm 1979; Wenner and Beatty 1988; Williams et al. 1976); or in tidal creeks (Walton et al. 2013).

Williams et al. (1976) reported *S. martini* from Texas coasts and found some environmental differences regarding those previously reported as shown in Table 1. Hartmann-Schröder (1977) reported this species from Cuba in a sinkhole pond having brackish water with the proliferation of marine algae. Gillet (1986) reported several specimens of *S. martini* from Guadeloupe, in warm waters with low dissolved oxygen and found in places organically enriched with abundant mangrove leaves. Table 1 summarizes the environmental features where *Stenoninereis* species have been recorded; most of these records were under *S. martini*. The most remarkable ecological characteristics are the findings in warm waters, and in areas with low oxygen levels, or deprived of it, with high contents of hydrogen sulfide and solids. *Stenoninereis martini* has been

Table 1. Records and ecological notes of *Stenomimeris* species. * = records in need of a new evaluation.

Species	Locality	Habitat	Inland distance	Temperature	pH	Salinity	Dissolved oxygen	Dissolved Hydrogen sulfide	Additional features	References
<i>S. martini</i>	Devil's Hole, Sint Maarten, Netherlands	Sinkhole pond	150 m	–	8	10.9–13.8 ppt	–	–	Limestone area with tidal movements, <i>Batophora</i> and <i>Apicennia</i>	Wesenberg-Lund 1958
	Cenote Aerolito, Quintana Roo, Mexico*	Sinkhole	–	25–28 °C	7.25–7.42	15.8–20.41 ppt	–	–	Soft bottoms, in mangrove roots	Frontana-Urbe and Solis-Weiss 2011
<i>S. lackeyi</i>	Warm Mineral Springs, Florida, USA	Spring	18000 m	30 °C	7.2 ±0.2	17 ppt	0 ppt	0.162 ppm	No direct connection with the Gulf of Mexico	Hartman 1958
	Tampa Bay, Florida, USA	Bay	–	28.5 °C	7.9	24.2 ppt	–	–	Fine sand, at 0.3 m depth	Taylor 1971
	Cedar Bayou, Texas, USA (as <i>S. martini</i>)	Bay	0	30°C	8.6	17.2 ppt	3.5 ppm	–	Polluted with domestic and industrial waters	Williams et al. 1976

reported in association with *Sigambra bassi* (Gillet 1986) in Guadeloupe, and with and *Streblospio benedicti* (Ibáñez 1997) in water with high organic enrichment in Cuba.

The presence of *Stenoninereis* species in low oxygen environments could be possible after the high vascularization shown in the dorsal cirri and notopodial dorsal ligules. Also, it is remarkable the presence of long-bladed chaetae (especially falcigers) because they can be as long as the ligules, and the enlarged dorsal cirri, which are unusual in most nereidids. These elongations could be related to a cryptic life and be regarded as troglomorphic features developed in these aphotic environments, including the almost transparent body wall, linked to living in habitats with low or no light. Moreover, the presence of *Stenoninereis* species in karstic inland environments very likely corresponds to a secondary invasion from the ocean, likely through subterranean connections, because specimens belonging to the same species found in the coasts are almost identical to inland specimens. Therefore, elongation of chaetal blades and dorsal cirri are features developed beyond subterranean environments, but aphotic ones, as already suggested for namanereidins (Conde-Vela 2017). The secondary invasion is also possible because of the high endurance to drastic changes of temperature, salinity, and oxygen availability shown by these species.

Discussion

The strongly reduced notopodia lacking dorsal cirri and dorsal ligules in the first two chaetigers is a distinctive feature of species belonging to *Stenoninereis* and *Micronereis* Claparède, 1863. They also have small body with few chaetigers, parapodial surfaces ciliated, and the absence of several parapodial structures such as the neuropodial ventral ligules. In fact, the parapodia of *Micronereis* species consist of two main acicular ligules with their respective cirri, resembling the posterior parapodia of *Stenoninereis* species. The main differences between these two genera are the presence of notopodial dorsal ligules, and that ciliated surfaces are restricted to dorsal cirrophores and notopodial dorsal ligules in *Stenoninereis*, whereas in *Micronereis* both parapodial lobes have cilia in ventral and dorsal surfaces, and notopodial dorsal ligules are absent throughout body.

The clear distinction between cirrophores and cirrostyles is also remarkable in *Stenoninereis* species, where the gymnonereidins *sensu stricto* (Santos et al. 2005) such as *Ceratocephale* Malmgren, 1865, *Gymnonereis* Horst, 1919, and *Tambalagamia* Pillai, 1961, have very similar development (pers. obs.). The cirrophores in gymnonereidins and in *Stenoninereis* species are strongly vascularized, with two main vessels running longitudinally the structure and a thick superficial tissue. Moreover, gymnonereidins and *Stenoninereis* species share the presence of cilia in cirrophores. Sometimes, the cilia are missing perhaps as a consequence of fixation, but when visible, the cilia in gymnonereidins are present in anterior and middle parapodia and even form tufts of cilia in the dorsal surface that cross each segment (Banse 1977). *Stenoninereis* species and gymnonereidins are similar in other respects such as the cleft anterior prostomial margin, and the progressive reduction of cirrophores toward posterior chaetigers.

Gymnonereidins show some relevant differences such as the presence of papillae in the pharynx oral ring, presence of neuropodial ventral ligules, double ventral cirri throughout the body, and remarkable chaetal bundles with abundant homogomph spinigers in anterior chaetigers. These features are not present in *Stenoninereis* species.

The notopodial structures are worth further comments. The vascularization of the cirrophores in *Ceratocephale*, *Gymnonereis*, and *Stenoninereis* consists in at least two thick vessels running close to the cirrophore wall, and also some slender vessels running parallel to the main ones (Fig. 1B). The inner structure of the notopodial dorsal ligules is almost identical to the one seen in cirrophores, and in both cases, a second structure running along the ligule is sometimes visible (Fig. 1B). The linking among cirrophore and notopodial dorsal cirri vessels and nerves are not clear because of the high transparency of the tissues. By its central position, the second structure shown by the cirrophores and the notopodial dorsal ligules in *Stenoninereis* could be the parapodial and notopodial dorsal ligule nerves, respectively, as observed in other nereidids (e.g. Winchell et al. 2010), but further studies are needed to elucidate the blood vessels and nerves patterning in *Stenoninereis*. A second particular feature is that the length of notopodial dorsal ligules is the most variable feature in parapodia, which could mean that this structure is muscular and contractile. Because of this, the cirrophores/notopodial dorsal ligules ratio are not used as diagnostic for these species.

Another relevant feature is the absence of neuropodial ventral ligules, which is shared with *Lycastonereis* Rao, 1981 and *Tylorrhynchus* Grube, 1866 (Conde-Vela 2019; Pettibone 1971). *Tylorrhynchus* species also lack notopodial dorsal ligules throughout the body, and both *Lycastonereis* and *Stenoninereis* species lack such ligules toward posterior chaetigers, such that the posterior parapodia are similar to those present in the posterior end of the body. Main differences and similarities among these genera are: *Lycastonereis* and *Tylorrhynchus* species have notopodial structures in chaetigers 1 and 2, papillae in pharynx, and neuroacicular ligules distally bilobated (i.e. superior and inferior lobes) in anterior chaetigers, but *Stenoninereis* species lack such features; *Stenoninereis* and *Tylorrhynchus* species have dorsal cirri with distinct cirrophores and cirrostyles, whereas in *Lycastonereis* species articulations are inconspicuous.

The phylogenetic affinities among *Ceratocephale*, *Gymnonereis*, and *Stenoninereis* was addressed by Fitzhugh (1987), and he concluded that *Stenoninereis* could be regarded as part of Gymnonereidinae by having bare pharynx and both noto- and neuroacicular in independent lobes (i.e. biramous parapodia). In the same analysis, namanereidins occupied the most basal positions in the cladogram because the character 'uniramous parapodia' was regarded as plesiomorphic, followed by *Tylorrhynchus* and *Stenoninereis* as the most basal genera with biramous parapodia and no directly related with gymnonereidins. The study by Glasby (1991) had a similar conclusion, where *Stenoninereis* was the most basal taxon with biramous parapodia, but in this case, gymnonereidins taxa were excluded. In the phylogenetic analysis by Santos et al. (2005), *Stenoninereis* appeared as the most plesiomorphic nereidid, even basal to namanereidins, whereas gymnonereidins are the most derived taxa and the sister group of the clade containing *Kinberginereis* Pettibone, 1971 and *Paraleonnates*

Khlebovich & Wu, 1962, both genera with several parapodial processes. Futures studies should be focused on clarifying the blood vessel and innervation patterning in *Stenoninereis* and allied genera, as a means to reach a better understanding of their phylogenetic affinities.

Key to species of *Stenoninereis* Wesenberg-Lund, 1958

- 1 Anterior prostomial margin deeply incised, dorsal prostomial groove reaching posterior pair of eyes; eyes large, covering half prostomial surface ***S. tecolutlensis* de León-González & Solís-Weiss, 1997 (Tecolutla, Mexico, Gulf of Mexico)**
- Anterior prostomial margin shallowly incised, dorsal prostomial groove prostomium often inconspicuous, or not reaching anterior eyes **2**
- 2 Anterior chaetigers with dorsal cirrophores as long as notopodial ventral ligules ***S. lackeyi* (Hartman, 1958) comb. n. (Florida, USA, Gulf of Mexico)**
- Anterior chaetigers with dorsal cirrophores at least twice longer (>2) than notopodial ventral ligules **3**
- 3 In anterior chaetigers, cirrophores of dorsal cirri are 2–3 times longer than cirrostyles ***S. martini* Wesenberg-Lund, 1958 (Sint Maarten, Netherlands, Caribbean Sea)**
- In anterior chaetigers, cirrophores of dorsal cirri are 5.5–6.0 times longer than cirrostyles ***S. elisae* sp. nov. (Rio Grande, Puerto Rico, Caribbean Sea)**

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References

- Bakken T, Wilson RS (2005) Phylogeny of nereidids (Polychaeta, Nereididae) with paragnaths. *Zoologica Scripta* 34: 507–547. <https://doi.org/10.1111/j.1463-6409.2005.00200.x>
- Banse K (1977) Gymnonereidinae new subfamily: the Nereididae (Polychaeta) with bifid parapodial neurocirri. *Journal of Natural History* 11: 609–628. <https://doi.org/10.1080/00222937700770541>

- Conde-Vela VM (2017) The troglomorphic adaptations of Namanereidinae (Annelida, Nereididae) revisited, including a redescription of *Namanereis cavernicola* (Solís-Weiss & Espinasa, 1991), and a new Caribbean species of *Namanereis* Chamberlin, 1919. *Subterranean Biology* 23: 19–28. <https://doi.org/10.3897/subtbiol.23.13701>
- Conde-Vela VM (2019) Re-evaluation of the morphology of the monotypic genera *Lycastonereis* Rao, 1981 and *Ganganereis* Misra, 1999 (Annelida, Phyllodocida, Nereididae). *Zootaxa* 4567: 450–460. <https://doi.org/10.11646/zootaxa.4567.3.2>
- de León-González JÁ, Solís-Weiss V (1997) A new species of *Stenoninereis* (Polychaeta: Nereididae) from the Gulf of Mexico. *Proceedings of the Biological Society of Washington* 110: 198–202.
- Fitzhugh K (1987) Phylogenetic relationships within the Nereididae (Polychaeta): implications at the subfamily level. *Bulletin of the Biological Society of Washington* 7: 174–183.
- Frontana-Uribe SC, Solís-Weiss V (2011) First records of polychaetous annelids from Cenote Aerolito (sinkhole and anchialine cave) in Cozumel Island, Mexico. *Journal of Cave and Karst Studies* 73: 1–10. <https://doi.org/10.4311/jcks2009lsc0107>
- Gillet P (1986) Contribution à l'étude des Annélides Polychètes des lagunes de la Manche-à-Eau et de Belle-Plaine (Guadeloupe). Description d'un nouveau Capitellidae: *Scyphoproctus guadalupensis* n. sp. *Bulletin du Muséum National d'Histoire Naturelle, Paris*, 4 sér 8: 803–817.
- Glasby CJ (1991) Phylogenetic relationships in the nereididae (Annelida: Polychaeta), chiefly in the subfamily Gymnonereidinae, and the monophyly of the Namanereidinae. *Bulletin of Marine Science* 48: 559–573.
- Hartman O (1958) A new nereid worm from Warm Mineral Springs, Fla., with a review of the genus *Nicon* Kinberg. *Journal of the Washington Academy of Sciences* 48: 263–266.
- Hartmann-Schröder G (1977) Die Polychaeten der Kubanisch-Rumänischen Biospeologischen Expedition nach Kuba 1973. *Résultats des expéditions biospéologiques cubano-roumaines à Cuba* 2: 51–63.
- Ibáñez DR (1997) Poliquetos bentónicos de la Bahía de la Habana, Cuba. *Revista de Biología Tropical* 44: 341–359.
- McBee JT, Brehm WT (1979) Macrobenthos of Simmons Bayou and an adjoining residential canal. *Gulf Research Reports* 6: 211–216. <https://doi.org/10.18785/grr.0603.01>
- Pettibone MH (1971) Revision of some species referred to *Leptonereis*, *Nicon*, and *Laonereis* (Polychaeta: Nereididae). *Smithsonian Contributions to Zoology* 104: 1–53. <https://doi.org/10.5479/si.00810282.104>
- Santos CSG, Pleijel F, Lana PDC, Rouse GW (2005) Phylogenetic relationships within Nereididae (Annelida: Polychaeta). *Invertebrate Systematics* 19: 557–576. <https://doi.org/10.1071/IS05001>
- Taylor JL (1971) Polychaetous annelids and benthic environments in Tampa Bay Florida. PhD Thesis. University of Florida. <https://doi.org/10.5962/bhl.title.37164>
- Walton AS, Nelson JL, Nappi CJ, Duffey RM, Rasnake EC (2013) Description of the benthic macroinvertebrate communities of four tidal creeks along the Eastern shore of Charlotte Harbor. *Florida Scientist* 76: 121–137.

- Wenner EL, Beatty HR (1988) Macrobenthic communities form wetland impoundments and adjacent open marsh habitats in South Carolina. *Coastal and Estuarine Research* 11: 29–44. <https://doi.org/10.2307/1351715>
- Wesenberg-Lund E (1958) Lesser Antillean polychaetes, chiefly from brackish water with a survey and a bibliography of fresh and brackish–water polychaetes. *Studies on the Fauna of Curaçao and other Caribbean Islands* 8: 1–41.
- Williams GE, Poff MJ, McBee JT (1976) Western Gulf of Mexico records of *Stenoninereis martini* Wesenberg-Lund 1958 (Polychaeta, Nereidae) with contributions to its habitat ecology. *Contributions in Marine Science* 20: 83–85.
- Winchell CJ, Valencia JE, Jacobs DK (2010) Confocal analysis of nervous system architecture in direct-developing juveniles of *Neanthes arenaceodentata* (Annelida, Nereididae). *Frontiers in Zoology* 7: 1–19. <https://doi.org/10.1186/1742-9994-7-17>

