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Two new species of burrowing crayfish in the genus *Lacunicambarus* (Decapoda: Cambaridae) from Alabama and Mississippi

MAEL G. GLON[1], SUSAN B. ADAMS[2], ZACHARY J. LOUGHMAN[3,4], GREG A. MYERS[3,5], CHRISTOPHER A. TAYLOR[6] & GUENTER A. SCHUSTER[7]
[1]*The Ohio State University Museum of Biological Diversity, Columbus, Ohio 43212.*
glon.1@osu.edu; https://orcid.org/0000-0003-4121-995X
[2]*USDA Forest Service, Southern Research Station, Center for Bottomland Hardwoods Research, Oxford, Mississippi 38655.*
susan.adams@usda.gov; https://orcid.org/0000-0002-8179-3748
[3]*West Liberty University Department of Biological Sciences, West Liberty, West Virginia 26074.*
[4] *zloughman@westliberty.edu; https://orcid.org/0000-0002-1486-4999*
[5] *gamyers@westliberty.edu; https://orcid.org/0000-0001-8860-0673*
[6]*Illinois Natural History Survey, Prairie Research Institute, Champaign, Illinois, 61820.*
cataylor@illinois.edu; https://orcid.org/0000-0002-8919-9030
[7]*224 Primrose Circle, Richmond, Kentucky 40475. Guenter.Schuster@eku.edu; https://orcid.org/0000-0003-0682-410X*

Abstract

While sampling for the Rusty Gravedigger, *Lacunicambarus miltus*, Taylor *et al.* (2011) found one or more potentially undescribed burrowing crayfish species in the genus *Lacunicambarus* inhabiting the area between the Pascagoula River and Mobile Bay in southern Alabama and Mississippi. Molecular analyses by Glon *et al.* (2018) confirmed that samples from this area were genetically distinct from other *Lacunicambarus* crayfishes. These findings prompted a dedicated sampling trip in January 2020. We used morphological and molecular analyses to investigate the specimens we collected and, based on our results, we describe two new crayfish species: the Lonesome Gravedigger, *L. mobilensis* **sp. nov.** and the Banded Mudbug, *L. freudensteini* **sp. nov.** *Lacunicambarus mobilensis* **sp. nov.** is sister to the Rusty Gravedigger, *L. miltus*, while *L. freudensteini* **sp. nov.** is sister to the Painted Devil Crayfish, *L. ludovicianus*. Both new species are currently known from a small number of sites in southern Alabama and Mississippi and may require conservation attention. In addition, we provide an updated key to *Lacunicambarus* crayfishes that includes these new species.

Key words: *miltus*, *ludovicianus*, *mobilensis, freudensteini*, painted, devil, crayfish, rusty, lonesome, gravedigger, banded, mudbug, taxonomy, systematics

Introduction

The genus *Lacunicambarus* (Hobbs, 1969) currently consists of nine primary burrowing crayfish species found east of the North American Great Divide. Among other distinguishing characteristics, *Lacunicambarus* crayfishes are generally large and have wide ranges. The one exception is the Rusty Gravedigger, *L. miltus* (Fitzpatrick, 1978), which in some ways is an outlier among its congeners. *Lacunicambarus miltus* is morphologically similar to other *Lacunicambarus* crayfishes and occupies comparable habitats. However, relative to its congeners, *L. miltus* is quite small, attaining a maximum size of approximately 31.00 mm carapace length (CL). For comparison, the Crawzilla Crawdad, *L. chimera* Glon & Thoma in Glon *et al.* (2019a), the largest member of the genus, exceeds 65.00 mm CL. In addition, *L. miltus* has a relatively narrow range. Indeed, upon its description and for several subsequent decades, *L. miltus* was known from fewer than five sites in Baldwin County, Alabama, and was considered for listing under the Federal Endangered Species Act (Fitzpatrick, 1978, 1991; Hartfield, 1991). Targeted sampling efforts by Taylor *et al.* (2011) increased the known localities of this species to 28 sites in southern Alabama and the Florida panhandle. Its conservation status was consequently deemed to be stable.

All known records of *L. miltus*, including the type locality (D'Olive Creek, Baldwin County, Alabama), are from east of Mobile Bay, Alabama. Mobile Bay is an inlet of the Gulf of Mexico that covers approximately 1,070

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km^2 and receives the Mobile and Tensaw rivers. In their search for additional populations of *L. miltus*, Taylor *et al.* (2011) also sampled west of Mobile Bay. Although they did not identify any *L. miltus* west of Mobile Bay, they found one or more possibly undescribed *Lacunicambarus* species (hereafter referred to as *L.* aff. *miltus*) in Mobile County, Alabama, and Jackson County, Mississippi. Voucher specimens collected at these sites were deposited into the Crustacean Collection of the Illinois Natural History Survey (INHS), and tissue samples from one of the undescribed species were sent to the primary author in 2017 for inclusion in a molecular phylogenetic analysis of *Lacunicambarus*. In the resulting phylogenies (Glon *et al.* 2018, 2019a), five representative specimens of *L.* aff. *miltus* from the west side of Mobile Bay form a maximally supported clade sister to a maximally supported clade consisting of 10 *L. miltus* specimens from the east side of Mobile Bay. Furthermore, the two clades are separated by long branches that are comparable in length to those leading to other *Lacunicambarus* species, indicating considerable genetic divergence between them.

Taken together, the observations of Taylor *et al.* (2011) and the molecular results from Glon *et al.* (2018, 2019a) sparked considerable interest in the *L.* aff. *miltus* populations on the west side of Mobile Bay among the authors. In January 2020, several of the authors sampled in the Mobile Bay area to collect additional specimens and delimit the range of *L.* aff. *miltus*. Although the purpose of our field sampling was to collect *L.* aff. *miltus* specimens, early in our sampling trip we encountered what we believed to be a second undescribed *Lacunicambarus* species and therefore expanded our search area to also collect specimens of this species. We then used an integrative taxonomic framework (sensu Dayrat, 2005) to determine whether these specimens simply represent disjunct populations of *L. miltus*, which would still be considered a favorable result as it would increase the known range of this species, or if these populations have diverged sufficiently from *L. miltus* to be considered a new species. Herein, we present evidence supporting the latter of these two hypotheses and describe two new species of *Lacunicambarus* crayfish, bringing the number of species in the genus to eleven.

Methods

Field sampling. We focused our sampling on an approximately 2,500 km^2 area delimited by Mobile Bay to the east, the Gulf of Mexico to the south, the Pascagoula River to the west, and U.S. Route 98 to the north (i.e., Mobile County, Alabama; Jackson and George counties, Mississippi). However, we also sampled east of Mobile Bay to collect additional *Lacunicambarus miltus* specimens, as well as west of the Pascagoula River and North of U.S. Route 98 to more clearly delimit the ranges of our two focal species. We collected crayfish from 58 sites during the period 5–15 January 2020 with a team of 3–5 people.

At all but one site, we collected specimens by excavating or pumping burrows by hand or with the aid of gardening tools or a clam pump. The exception was a single site in Mobile County, Alabama, where we collected *L. mobilensis* **sp. nov.** by hand while flipping rocks in a shallow creek. We primarily targeted roadside ditches and utility rights of way with aquatic vegetation indicative of a high water table, stream floodplains and boggy areas. At each site where we captured crayfish, we photographed each specimen then removed gill tissue using heat-sterilized forceps and immediately preserved it in individual vials of 100% ethanol for subsequent DNA extraction. We preserved the remainders of the specimens in jars of 70% ethanol and deposited them in the Ohio State University Museum of Biological Diversity crustacean collection (OSUMC; catalogue nos. 10862–10883). Lastly, we acquired preserved specimens from the INHS and OSUMC to fill in gaps in our sampling.

Molecular analyses. We followed the techniques outlined in Glon *et al.* (2018) to extract DNA and amplify and sequence sections of three mitochondrial DNA loci (12S, 16S, and CO1) from a representative subset of specimens of our focal species, as well as from several additional *Lacunicambarus* specimens collected by the primary author during the past two years. However, we created and used a new set of primers to better amplify 12S in cambarid crayfishes: 12ScrayfishF: AGGATTAGATACCCTAGTACAC; 12ScrayfishR: TTAAATGAAAGCGACGGG. We also amplified and sequenced a section of the nuclear locus H3 from 41 specimens across *Lacunicambarus* using the primers from Colgan *et al.* (1998) in order to determine if this gene could improve nodal support in our phylogenies. Unfortunately, this gene was uninformative, so although we included the 41 sequences that we generated in our alignments, we did not sequence this locus for additional specimens. To complete our dataset, we included sequences generated in Glon *et al.* (2018, 2019a), bringing the number of specimens included in the current analysis to 267 (i.e., 266 from across *Lacunicambarus* and 1 from the outgroup *Procambarus gracilis* (Bundy in Forbes, 1876)). We used the techniques and programs outlined in Glon *et al.* (2018) to assemble and align DNA sequences and infer phylogenies from our sequence data using maximum likelihood bootstrap in IQ-Tree (Nguyen *et al.*, 2015) and Bayesian inference in MrBayes (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), with the only exception being that we used IQ-Tree's ultrafast bootstrap (Minh *et al.*, 2013; Hoang *et al.*, 2018) rather than the regular bootstrap to establish nodal support. We uploaded all new sequences created during these analyses to GenBank (Supplementary Table 1).

Morphological analyses. In order to quantitatively differentiate *L. freudensteini* **sp. nov.** and *L. mobilensis* **sp. nov.** from each other and from *L. miltus* and *L. ludovicianus* (Faxon, 1884), we used digital calipers and a dissecting microscope to measure a standard set of morphological and meristic characters (see e.g., Fetzner & Taylor, 2018; Loughman & Williams, 2018; Supplemental File 1) from specimens of each species (*L. freudensteini* **sp. nov.**, n=27; *L. mobilensis* **sp. nov.**, n=25; *L. miltus*, n=26; *L. ludovicianus,* n=33). Where appropriate, to standardized continuous measurements to account for size variation among our specimens, we divided measurements by a reference body part such as carapace length or gonopod length (e.g., cephalon width/carapace length). We parsed our morphological and meristic data into two datasets, one consisting of Form I males only (n=48) and the other consisting of all specimens (n=111). We did not create specific datasets for Form II males because we collected few of them or for females because our preliminary examinations did not lead us to *a priori* hypotheses about female-specific morphometric features separating the taxa. For each dataset, we calculated means and standard deviations for each measurement and count and used these statistics, as well as our observations, to select measurements to compare across species with analysis of variance (ANOVA) followed by Tukey tests for pairwise differences. To minimize the risk of type I error, we used a Bonferroni correction to reduce our alpha value according to the number of tests that we ran (21 for Form I males, 16 for all specimens). To better understand and visualize the morphological differences between species, we ran a principal component analysis (PCA) on the characters that differed significantly in each dataset (specimens with missing data were omitted) and created scatterplots of the first two principal components from each PCA. We ran all analyses in R (R Core Team, 2017).

Results

Molecular analyses. The maximum likelihood tree generated by IQ-Tree had a log-likelihood value of -10460.790. The Bayesian analysis ran for 15,000,000 generations. The standard deviation of the split frequencies dropped below 0.01 during the analysis, and effective sample sizes for all parameters were well above 200, indicating convergence. Each of the two independent MrBayes runs generated 30,003 trees, 15,000 of which were discarded as burn in leaving 45,006 trees in the post-burnin posterior distribution. The Maximum Likelihood (ML) and Bayesian Inference (BI) trees recovered conflicting backbones within *Lacunicambarus*. However, both analyses found maximal support for all of the species-level clades within the genus. Because the focus of the study is on the species-level clades rather than the backbone of the phylogeny, we present here our ML phylogram annotated with both Bayesian posterior probabilities (PP_{BI}) and ML Bootstrap values (B_{ML}; Figure 1). We used asterisks to denote nodes in the ML tree that conflict with those in the BI phylogram.

Both analyses found maximal support (PP_{BI}=1, B_{ML}=100) for *L. mobilensis* **sp. nov.** and *L. freudensteini* **sp. nov.** species-level clades. *Lacunicambarus mobilensis* **sp. nov.** was found to be sister to *L. miltus* (PP_{BI}=1, B_{ML}=100), and *L. freudensteini* **sp. nov.** sister to *L. ludovicianus* (PP_{BI}=1, B_{ML}=100). The {*L. mobilensis* **sp. nov.**, *L. miltus*} and {*L. freudensteini* **sp. nov.**, *L. ludovicianus*} clades were not recovered as sister to each other in either of our phylograms despite morphological similarities among the four species; however, the relationship between the two species pairs should be revisited to obtain better support for the deeper nodes as additional data sources are used in subsequent studies.

Morphological analyses. Seventeen characters differed significantly across species for Form I males at a Bonferroni-adjusted alpha value of p=0.0024 and were included in the Form I PCA (Table 1). Fourteen characters differed significantly across species for all specimens at a Bonferroni-adjusted alpha value of p=0.0031 and were included in the all specimens PCA (Table 2).

The first five principal components of the Form I male PCA had eigenvalues greater than 1 and cumulatively explained 78.2% of the variation in the data. The first two principal components cumulatively explained 49.9% of the variation (PC1=32.5%, PC2=17.4%). Percent contributions of individual characters are shown in Table 1. On the PC1 and PC2 biplot, the four species form four, nonoverlapping clusters of points (Figure 2A). Key characters contributing to both PC1 and PC2 are indicated in Figure 2B.

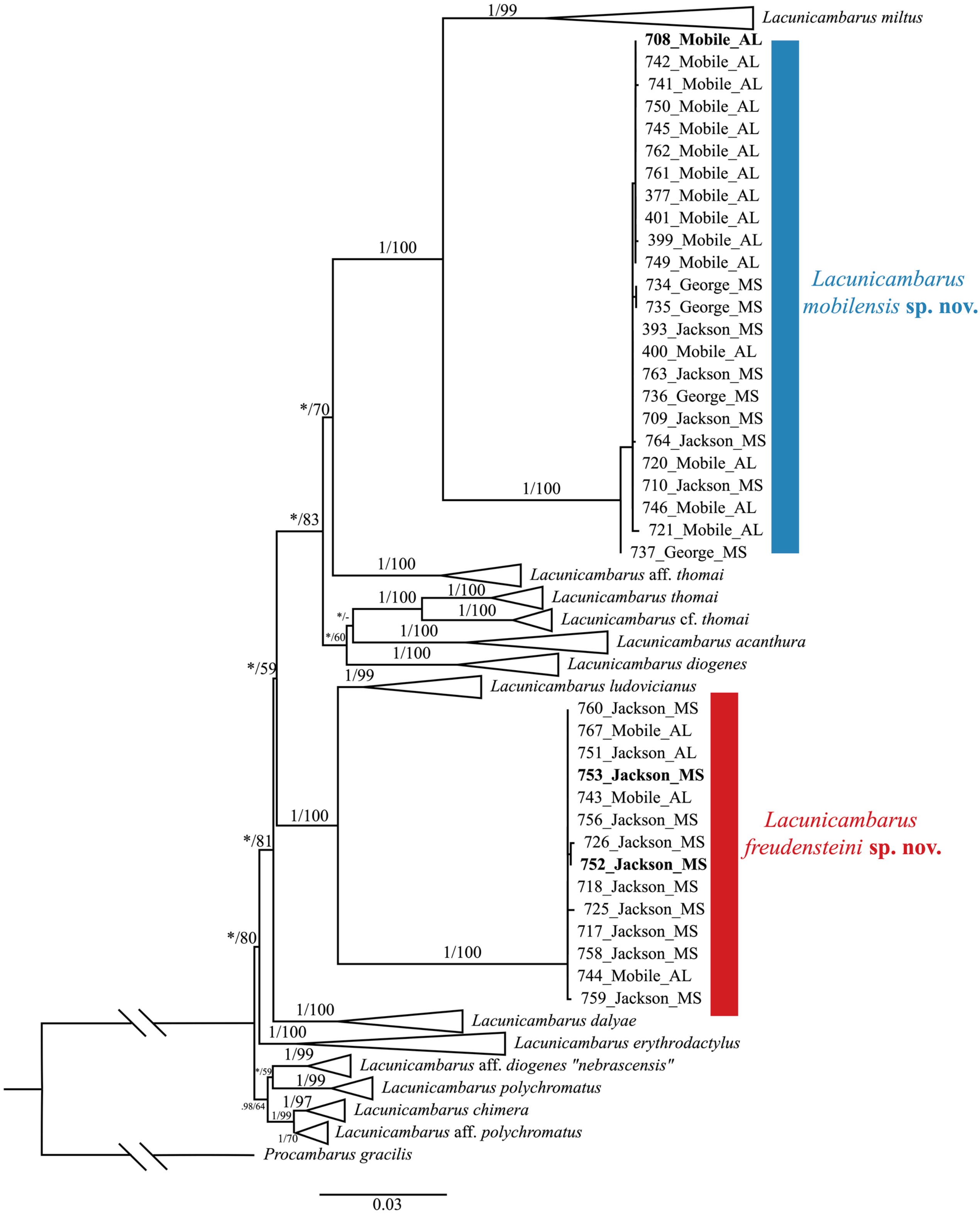

FIGURE 1. Phylogram showing maximum likelihood tree estimated from three mitochondrial loci (partial 12S, partial 16S, and partial CO1) and one nuclear locus (partial H3). Bayesian posterior probability values > 0.5 and maximum likelihood bootstrap values > 50 are given in that order. Dashes indicate lack of nodal support, and asterisks indicate nodes that conflict with Bayesian tree. Labels at tips of *L. mobilensis* **sp. nov.** and *L. freudensteini* **sp. nov.** clades correspond with specimen codes in Supplementary Table 1 and are followed by County and State of collection (AL, Alabama; MS, Mississippi). Bolded labels indicate specimens from each species' type locality. Other species-level clades are collapsed into triangles to improve legibility.

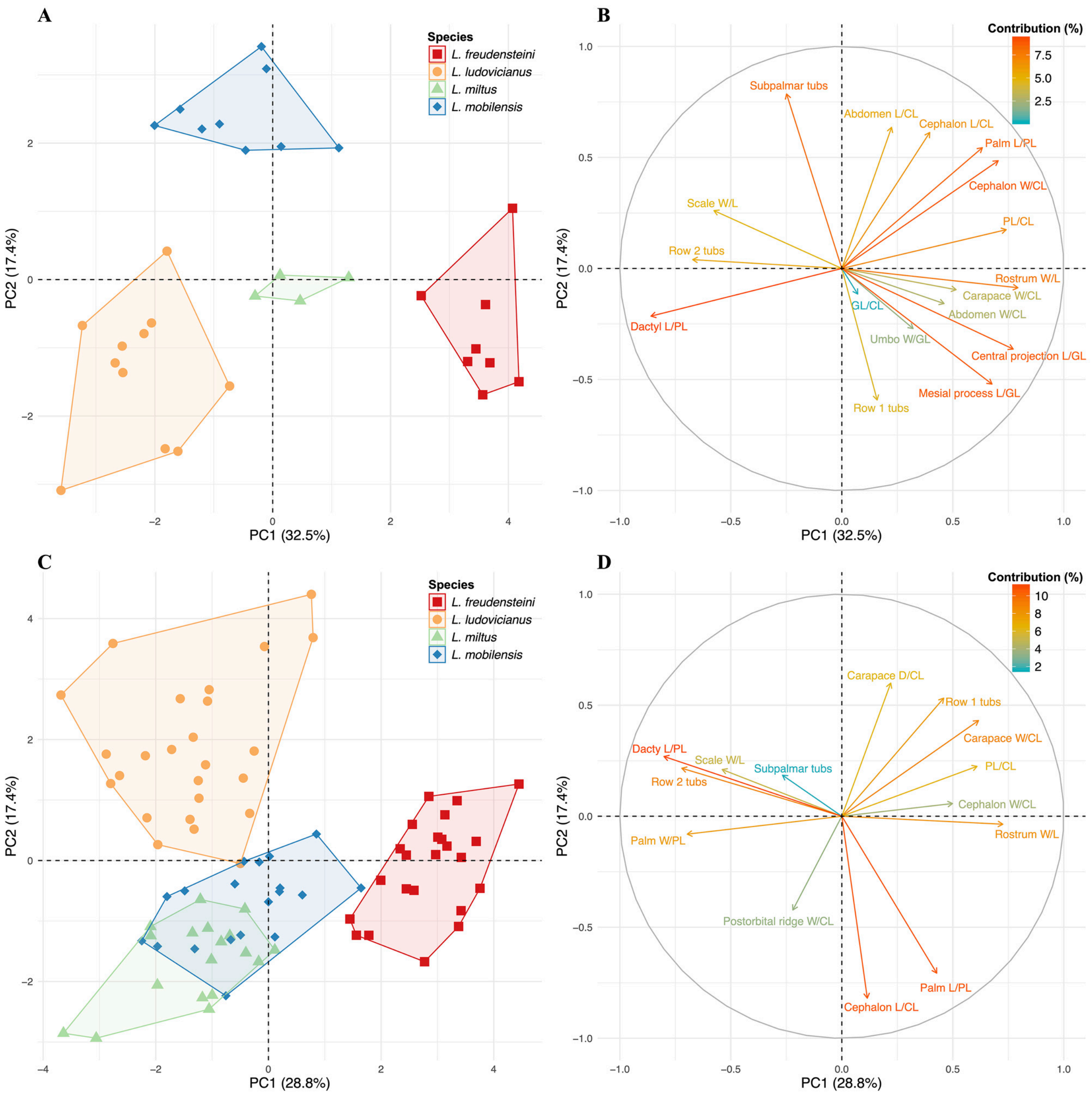

FIGURE 2. Biplots of first two principal components (i.e., PC1 and PC2) of morphometric and meristic data for Form I males (A & B) and all specimens (C & D). Percentages of variation in data explained by each principal component are indicated in axis labels. Panels A and C show specimen points, with shapes and colors corresponding to species in legend. Panels B and D show variables contributing to PC1 and PC2. Variables and arrows are color-coded and arrows are scaled according to their percent contribution to PC1 and PC2. Abbreviations: CL, carapace length; D, depth; GL, gonopod length; L, length; PL, propodus length; tubs, tubercles; W, width.

The first five principal components of the all specimens PCA had eigenvalues greater than 1 and cumulatively explained 72.1% of the variation in the data. The first two principal components cumulatively explained 46.2% of the variation in the data (PC1=28.8%, PC2=17.4%). Percent contributions of individual characters are shown in Table 2. On the PC1 and PC2 biplot, the four species form four clusters of points with the only meaningful overlap occurring between *L. mobilensis* **sp. nov.** and *L. miltus* (Figure 2C). Key characters contributing to PC1 and PC2 are indicated in Figure 2D.

TABLE 1. Mean ± standard deviation and percent contribution to PC1 and PC2 of morphometric measurements and meristic counts that are significantly different across species for Form I male dataset. Bolded letters denote results of pairwise Tukey tests. Abbreviations: L, length; W, width; CL, carapace length.

Character	*L. freudensteini* (n=11)	*L. mobilensis* (n=11)	*L. ludovicianus* (n=18)	*L. miltus* (n=8)	Contribution to PC1 (%)	Contribution to PC2 (%)
Cephalon L / CL	0.58 ± 0.01 **a**	0.58 ± 0.01 **a**	0.57 ± 0.01 **b**	0.58 ± 0.01 **a**	2.84	12.69
Carapace W / CL	0.48 ± 0.01 **a**	0.46 ± 0.01 **b**	0.46 ± 0.01 **b**	0.45 ± 0.01 **b**	4.80	0.31
Cephalon W / CL	0.39 ± 0.00 **a**	0.39 ± 0.01 **ab**	0.37 ± 0.01 **c**	0.38 ± 0.01 **b**	8.97	7.97
Rostrum W / Rostrum L	0.74 ± 0.04 **a**	0.67 ± 0.04 **b**	0.64 ± 0.03 **b**	0.66 ± 0.05 **b**	11.38	0.25
Scale W / Scale L	0.30 ± 0.02 **c**	0.34 ± 0.03 **a**	0.33 ± 0.02 **ab**	0.31 ± 0.01 **bc**	6.00	2.30
Abdominal L / CL	0.99 ± 0.03 **ab**	1.01 ± 0.02 **a**	0.97 ± 0.02 **b**	1.02 ± 0.02 **a**	0.91	13.66
Abdominal W / CL	0.37 ± 0.01 **a**	0.36 ± 0.01 **b**	0.36 ± 0.01 **b**	0.35 ± 0.01 **b**	3.84	0.85
Palm tubercles row 1	7.67 ± 0.50 **a**	6.20 ± 0.63 **b**	7.31 ± 1.11 **a**	6.75 ± 0.50 **ab**	0.46	11.87
Palm tubercles row 2	3.11 ± 0.60 **b**	4.50 ± 0.85 **a**	4.69 ± 0.85 **a**	4.75 ± 0.50 **a**	8.16	0.05
Subpalmar tubercles	1.56 ± 0.73 **b**	4.60 ± 1.26 **a**	2.00 ± 1.08 **b**	1.25 ± 0.50 **b**	1.12	20.89
Propodus L / CL	0.89 ± 0.04 **a**	0.86 ± 0.04 **ab**	0.81 ± 0.03 **c**	0.83 ± 0.01 **bc**	9.92	1.03
Palm L / Propodus L	0.35 ± 0.01 **a**	0.35 ± 0.01 **a**	0.32 ± 0.01 **b**	0.35 ± 0.01 **a**	7.24	10.02
Dacty L / Propodus L	0.61 ± 0.01 **c**	0.63 ± 0.02 **b**	0.66 ± 0.01 **a**	0.64 ± 0.01 **ab**	13.36	1.56
Gonopod L / CL	0.25 ± 0.01 **b**	0.24 ± 0.01 **b**	0.25 ± 0.01 **b**	0.26 ± 0.01 **a**	0.09	0.45
Umbo W / Gonopod L	0.28 ± 0.01 **b**	0.27 ± 0.01 **c**	0.27 ± 0.01 **bc**	0.29 ± 0.01 **a**	1.86	2.48
Central projection L / Gonopod L	0.32 ± 0.01 **a**	0.28 ± 0.01 **b**	0.28 ± 0.01 **b**	0.33 ± 0.01 **a**	10.78	4.46
Mesial process L / Gonopod L	0.35 ± 0.01 **a**	0.30 ± 0.01 **b**	0.31 ± 0.01 **b**	0.33 ± 0.02 **a**	8.27	9.17

Taxonomy

Family Cambaridae Hobbs, 1942a

Genus Lacunicambarus (Hobbs, 1969)

Lacunicambarus mobilensis Glon sp. nov.

Lacunicambarus aff. *miltus*.—Glon *et al.*, 2018:604. Glon *et al.*, 2019a:456.

Diagnosis. Eyes pigmented, not reduced. Rostrum narrow, curved ventrally in lateral view, margins moderately thickened to tip of acumen, lacking marginal spines or tubercles and median carina, shallowly excavated. Acumen poorly delimited at base from rostrum. Carapace subcylindrical, laterally compressed. Cervical spine absent; branchiostegal spine obsolete. Suborbital angle acute. Cervical groove uninterrupted. Postorbital ridges developed, ending cephalically in small tubercle. Areola obliterated, constituting in adults 39.99–43.14% ($\bar{x}$=41.52, σ=0.73, n=24) of entire length of carapace. Antennal scale 2.61–3.39 ($\bar{x}$=3.05, σ=0.19, n=26) times as long as wide, widest near midlength, antennal spine strongly developed. Cephalic lobe of epistome strongly truncated apically. Mesial margin of palm of chela with 3 rows of tubercles, mesialmost row normally consisting of 5–7 ($\bar{x}$=6.35, σ=0.57, n=23) tubercles, second row running parallel to first , with 3–6 ($\bar{x}$=4.65, σ=0.83, n=23) tubercles, third row running diagonally from mesial base of palm to mesialmost of two bulbous tubercles proximal to propodactyl articulation with 5–7 ($\bar{x}$=6.43, σ=0.59, n=23) small tubercles located in shallow dimples. Opposable margin of dactyl weakly concave at base. Ratio of dactyl length to palm length 1.62–1.96 ($\bar{x}$=1.80, σ=0.08, n=23). Dorsal longitudinal ridges of dactyl and propodus weakly developed. Dorsolateral impression at base of propodus weak. Ventral surface of chela with 3–7 ($\bar{x}$=4.70, σ=1.26, n=23) subpalmar tubercles running along arc from propodactyl articular condyle to midpoint of opposable margin of fixed finger. Mesial ramus of uropod with distomesial spine reaching and occasionally inconspicuously surpassing caudal margin. Gonopods of Form I males contiguous at base, with pronounced umbo near midlength of caudal surface; terminal elements consisting of 1) a moderately long central projection, thin and strongly arched in lateral aspect, bearing pronounced subapical notch, slightly shorter than mesial process, directed caudally at approximately 90^{o}, overreaching margin of umbo by noticeable amount, 2) a moderately long mesial process with conical base, tapering near midlength, tipped with protruding finger, directed caudally at approximately 90^{o} and overreaching margin of umbo by noticeable amount, 3) a weakly developed caudal knob protruding from caudolateral base of central projection. Hook on ischium of third pereiopod only. Female with annulus ventralis rhombus shaped, wider than long, deeply embedded in sternum, slightly moveable, with anterior half mildly pliable and posterior half sclerotized. Life colors include single longitudinal stripe running from cervical groove to telson.

TABLE 2. Mean ± standard deviation and percent contribution to PC1 and PC2 of morphometric measurements and meristic counts that are significantly different across species for all specimens dataset. Bolded letters denote results of pairwise Tukey tests. Abbreviations: L, length; W, width; D, depth; CL, carapace length.

Character	***L. freudensteini* (n = 27)**	***L. mobilensis* (n=25)**	***L. ludovicianus* (n=33)**	***L. miltus* (n=26)**	**Contribution to PC1 (%)**	**Contribution to PC2 (%)**
Cephalon L / CL	0.58 ± 0.01 **a**	0.58 ± 0.01 **a**	0.56 ± 0.01 **b**	0.58 ± 0.01 **a**	0.33	27.55
Carapace D / CL	0.46 ± 0.01 **ab**	0.46 ± 0.01 **b**	0.47 ± 0.01 **a**	0.45 ± 0.01 **c**	1.20	14.76
Carapace W / CL	0.48 ± 0.01 **a**	0.46 ± 0.01 **bc**	0.47 ± 0.01 **b**	0.45 ± 0.01 **c**	9.39	7.62
Cephalon W / CL	0.39 ± 0.01 **a**	0.39 ± 0.01 **a**	0.37 ± 0.01 **b**	0.37 ± 0.04 **b**	6.16	0.13
Postorbital W / CL	0.18 ± 0.01 **b**	0.18 ± 0.01 **b**	0.18 ± 0.01 **b**	0.19 ± 0.01 **a**	1.21	7.32
Rostrum W / Rostrum L	0.75 ± 0.04 **a**	0.67 ± 0.05 **b**	0.65 ± 0.04 **b**	0.65 ± 0.04 **b**	13.03	0.05
Scale W / Scale L	0.31 ± 0.02 **c**	0.33 ± 0.02 **ab**	0.33 ± 0.02 **a**	0.32 ± 0.01 **bc**	7.20	1.82
Palm tubercles row 1	7.78 ± 0.52 **a**	6.32 ± 0.57 **b**	7.38 ± 0.90 **a**	6.39 ± 0.61 **b**	5.22	11.61
Palm tubercles row 2	3.00 ± 0.67 **b**	4.68 ± 0.84 **a**	5.12 ± 0.99 **a**	4.61 ± 0.70 **a**	12.87	1.92
Subpalmar tubercles	1.65 ± 0.57 **b**	4.68 ± 1.29 **a**	2.85 ± 3.26 **b**	1.67 ± 0.91 **b**	1.77	1.40
Propodus L / CL	0.84 ± 0.06 **a**	0.89 ± 0.07 **ab**	0.78 ± 0.06 **b**	0.75 ± 0.05 **b**	9.19	2.07
Palm L / Propodus L	0.36 ± 0.01 **a**	0.35 ± 0.01 **a**	0.32 ± 0.01 **b**	0.35 ± 0.01 **a**	4.53	20.49
Palm W / Propodus L	0.45 ± 0.02 **b**	0.47 ± 0.02 **a**	0.48 ± 0.02 **a**	0.48 ± 0.02 **a**	12.01	0.27
Dacty L / Propodus L	0.60 ± 0.01 **c**	0.63 ± 0.02 **b**	0.65 ± 0.01 **a**	0.64 ± 0.02 **b**	15.90	2.98

FIGURE 3. Dorsal view of Form I holotypic male of *Lacunicambarus mobilensis* **sp. nov.** (catalogue no. OSUMC 10863).

Holotypic male, Form I (catalogue no. OSUMC 10863; Figures 3, 4A–C, F–H, J–L; Table 3). Carapace bullet shaped in dorsal view (Figure 4H), width 100.90% of depth, wider than abdomen (12.25 and 9.69 mm, respectively; Figure 3); maximum width greater than depth at caudodorsal margin of cervical groove (12.25 and 12.14 mm, respectively). Areola obliterated; length 41.95% of total length of carapace (Figure 4H). Rostrum curved ventrally in lateral view, margins moderately thickened to acumen tip; acumen poorly delimited at base from rostrum, anterior tip upturned, not reaching ultimate podomere of antennular peduncle; dorsal surface of rostrum shallowly concave with minute punctations forming single row along rostral margins. Subrostral ridge moderate, evident in lateral aspect along entire length of rostrum, tapering distally. Postorbital ridges developed, grooved dorsolaterally, ending cephalically in small tubercle. Suborbital angle acute; branchiostegal spine obsolete (Figure 4A). Posterior margin of cervical groove lined by collar of 4 small tubercles. Branchiostegal region granulate. Anteroventral branchiostegal region with 11 small tubercles. Hepatic region with scattered granules and tubercles. Remainder of carapace with slight punctations dorsally and laterally. Abdomen longer than carapace (27.48 and 26.05 mm, respectively), 2.84 times as long as wide; pleura short, rounded caudoventrally. Cephalic section of telson with 2 spines in caudolateral corners, mesial spine slightly moveable. Proximal segment of lateral ramus of uropod with 16 spines on distal margin; mesial ramus of uropod with prominent median rib ending distally as strong distomesial spine just overreaching margin of ramus, laterodistal spine of ramus strong.

Anteromesial lobe of epistome (Figure 4G) bell shaped but truncated apically, with uniform ventrally raised margins, ventral surface shallowly concave; main body of epistome with shallow fovea; epistomal zygoma arched. Ventral surface of antennular peduncle's proximal podomere with small spine at midlength. Antennal peduncle without spines. Antennal scale 2.90 times as long as wide (Figure 4J), widest near midlength, lateral margin straight from basal area to widest distal point, ending in strongly developed antennal spine reaching past proximal margin of ultimate podomore of antennal peduncle. Ventral surface of entire third maxilliped densely studded with long, flexible setae; distolateral angle acute.

Length of right chela of cheliped (Figure 4K) 81.57% of carapace length; chela width 46.30% of chela length; palm length 34.21% of chela length; dactyl length 1.90 times palm length. Mesial margin of palm of chela with 3 rows of tubercles, mesialmost row with 7 tubercles, second row running parallel to first with 4 tubercles, third row running obliquely from mesial base of palm to mesialmost of two bulbous tubercles proximal to propodactyl articulation with 6 small tubercles located in shallow dimples; proximal dorsolateral half smooth, distolateral area punctate, punctations deep, large in vicinity of dorsolateral base of propodus; lateral margin of propodus not costate; ventromesial surface of palm with small punctations, 2 bulbous tubercles on articular rim opposite base of dactyl, lateralmost of which is spiniform; 5 subpalmar tubercles running along arc from propodactyl articular condyle to midpoint of opposable margin of fixed finger. Both fingers of chela with weakly developed dorsomesial longitudinal ridges. Opposable margin of propodus with row of 9 tubercles, decreasing in size from base except for third from base, which is much larger than adjacent tubercles, and ultimate tubercle; penultimate tubercle positioned ventrally relative to adjacent tubercles; single row of minute denticles extending distally from fifth tubercle. Opposable margin of dactyl with row of 8 tubercles decreasing in size from base except for fourth from base which is larger than adjacent tubercles; single row of minute denticles extends distally from third tubercle; mesial surface of dactyl studded with 15 tubercles proximally, not forming distinct rows, giving way to punctations distally. Dorsolateral impression at base of propodus weak.

Cheliped carpus with distinct dorsal furrow; mesial surface with row of 4 small tubercles; dorsolateral surface punctate; mesial surface with 10 scattered spiniform tubercles plus 1 large procurved spine near distal margin; ventral surface with spine on distal articular rim. Merus dorsodistal margin with 4 small tubercles, dorsal surface with 2 spiniform tubercles; ventrolateral margin with row of 3 spines increasing in size from base, ventromesial margin with row of 10 spines increasing in size from base. Basioischial segment with 2 small tubercles on ventral margin. Ischium of third pereiopod with simple hook extending proximally to basioischial articulation, not opposed by tubercles on basis. Coxa of fourth pereiopod with setiferous, caudomesial boss, ventral surface calcified; coxa of fifth pereiopod lacking boss, ventral surface membranous.

Gonopods contiguous at base, reaching past caudomesial boss of fourth pereiopod when abdomen flexed; terminal elements as described in diagnosis (Figure 4B–C, F).

Left gonopod and right antennal scale separated from specimen and placed in vial inside specimen jar. One gill has been extracted from left gill chamber of specimen, preserved in 100% ethanol, and frozen at OSUMC for future DNA extractions (MGG 768).

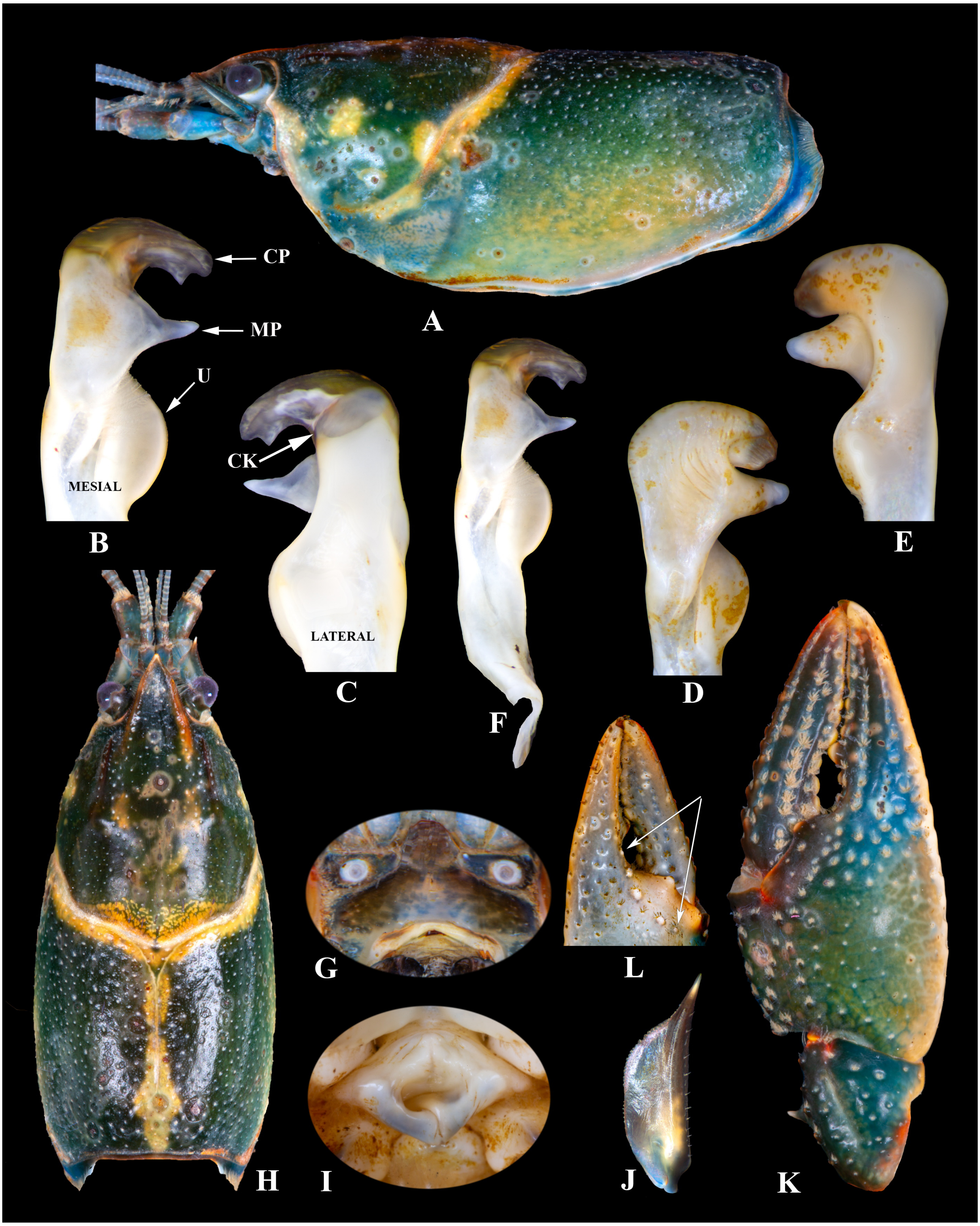

FIGURE 4. *Lacunicambarus mobilensis* **sp. nov.** (A) Lateral view of carapace; (B & F) mesial and (C) lateral views of Form I gonopod; (D) mesial and (E) lateral views of Form II gonopod; (G) epistome; (H) dorsal view of carapace; (I) annulus ventralis; (J) antennal scale; (K) dorsal view of right chela; (L) ventral view of right chela showing subpalmar tubercles. A–C, F–H, and J–L from holotype (catalogue no. OSUMC 10863); D–E from morphotype (catalogue no. OSUMC 10870); I from allotype (catalogue no. OSUMC 10868). Abbreviations: CP, central projection; MP, mesial process; CK, caudal knob; U, umbo.

Allotypic female (catalogue no. OSUMC 10868; Figure 4I; Table 3). Differs from holotype as follows: Carapace width 97.86% of depth. Maximum width of carapace less than depth at caudodorsal margin of cervical groove (11.01 and 11.25 mm, respectively). Posterior margin of cervical groove lined by collar of 5 small tubercles. Anteroventral branchiostegal region with 9 small tubercles. Abdomen 2.74 times as long as wide. Proximal segment of lateral ramus of uropod with 12 spines on distal margin. Antennal scale 3.09 times as long as wide. Right chela regenerated. Length of left chela 72.66% carapace length; chela width 48.02% of length; dactyl length 1.67 times palm length. Second row of tubercles on mesial margin of palm of chela with 5 tubercles, third row running diagonally from mesial base of palm to mesialmost of two bulbous tubercles proximal to propodactyl articulation with 7 small tubercles located in shallow dimples; 6 subpalmar tubercles running along arc from propodactyl articular condyle to midpoint of opposable margin of fixed finger. Opposable margin of propodus with row of 7 tubercles, decreasing in size from base except for third from base which is much larger than adjacent tubercles; single row of minute denticles extending distally from third tubercle. Opposable margin of dactyl with row of 9 tubercles decreasing in size from base except for fourth from base which is larger than adjacent tubercles; single row of minute denticles extends distally from fourth tubercle; mesial surface of dactyl studded with 16 tubercles proximally, not forming distinct rows, giving way to punctations distally. Cheliped carpus dorsomesial surface with row of 5 small tubercles; mesial surface with 7 scattered spiniform tubercles plus 1 large procurved spine near distal margin. Merus ventromesial margin with row of 9 spines increasing in size from base.

Annulus ventralis (Figure 4I) as in Diagnosis; 1.27 times wider than long, with bifurcated leathery ridge mesially located in anterior half; tongue extending from sclerotized lingual (left) wall into fossa of sclerotized supralingual (right) wall; supralingual wall more swollen caudomesially than lingual wall and curved on outer margin. Posterior margin of annulus ventralis reaching anterior margin of oblong, approximately symmetrical postannular sclerite lacking setae. First pleopods present, not reaching caudal edge of annulus ventralis when abdomen flexed. Specimen ovigerous, bearing 30 eggs.

Morphotypic male, Form II (catalogue no. OSUMC 10870; Figure 4D–E; Table 3). Differs from holotype as follows: Carapace width 105.69% of depth. Posterior margin of cervical groove with 1 small tubercle. Anteroventral branchiostegal region with 7 small tubercles. Abdomen 3 times as long as wide. Proximal segment of lateral ramus of uropod with 12 spines on distal margin. Antennal scale 3.32 times as long as wide. Length of right chela 75.76% of carapace length; chela width 47.22% of length; dactyl length 1.83 times palm length. First row of tubercles on mesial margin of palm of chela with 6 tubercles, third row running diagonally from mesial base of palm to mesialmost of two bulbous tubercles proximal to propodactyl articulation with 7 small tubercles located in shallow dimples, 3 subpalmar tubercles. Opposable margin of propodus with row of 7 tubercles; single row of minute denticles extending distally from third tubercle. Opposable margin of dactyl with row of 9 tubercles decreasing in size from base except for fourth from base which is larger than adjacent tubercles; single row of minute denticles extends distally from third tubercle; mesial surface of dactyl studded with 14 tubercles proximally, not forming distinct rows, giving way to punctations distally. Cheliped carpus dorsomesial surface with row of 7 small tubercles; mesial surface with 5 scattered spiniform tubercles plus 1 large procurved spine near distal margin. Merus distolateral margin with 3 small tubercles, dorsal surface with 3 spiniform tubercles; ventrolateral margin with 1 spine, ventromesial margin with row of 9 spines increasing in size from base. Basioischial segment of cheliped with 4 small tubercles on ventral margin. Ischium of third pereiopod with simple hook extending towards but not reaching basioischial articulation.

Form II gonopod central projection non-corneous, rounded, slightly shorter than mesial process, overreaching margin of umbo, mesial process subconical, overreaching margin of umbo (Figure 4D–E). Caudal knob not visible. Left gonopod separated from specimen, placed in glass vial inside specimen jar. One gill was extracted from left gill chamber of specimen, preserved in 100% ethanol, and frozen at OSUMC for future DNA extractions (MGG 772).

Disposition of Types. We deposited the holotype, allotype, and morphotype in the OSUMC (catalogue nos. 10863, 10868, and 10870). We also deposited Form I and female paratypes in the INHS (catalogue no. 16725) and the Center for Bottomland Hardwoods Research of the USDA Forest Service Southern Research Station (CBHR; catalogue no. 6972).

Type locality. We collected the holotype from a burrow in a roadside ditch on the west side of Rangeline Road in Mobile County, Alabama (30.5247° N, 88.1237° W). The closest flowing water to this ditch was the Deer River, which flowed directly into Mobile Bay.

TABLE 3. Measurements (mm) of holotype, morphotype, and allotype of *Lacunicambarus mobilensis* **sp. nov.** (Catalogue #: OSUMC 10863, 10868 & 10870).

Character	Holotype	Morphotype	Allotype
Carapace:			
Depth	12.14	11.24	11.25
Width	12.25	11.88	11.01
Length	26.05	26.16	24.36
Areola:			
Length	10.93	10.56	10.14
Rostrum:			
Width at eyes	2.87	2.65	2.77
Length			
Postorbital ridge:			
Width	4.90	4.77	4.47
Chela (right):			
Length of propodus	21.25	19.82	17.70
Length of palm	7.27	6.69	6.76
Width of palm	9.84	9.36	8.50
Length of dactyl	13.80	12.28	11.31
Abdomen:			
Length	27.48	26.77	26.21
Width	9.69	9.06	9.56
Gonopod:			
Length	6.69	6.20	NA
Width at umbo	1.69	1.56	NA
Annulus ventralis:			
Length	NA	NA	1.91
Width	NA	NA	2.42
Antennal scale:			
Length	3.86	4.12	3.77
Width	1.33	1.24	1.22

We collected the morphotype from a burrow in the bank of Little Cedar Creek, a tributary of the Pascagoula River in George County, Mississippi (30.8432° N, 88.5307° W). This site had few burrows, possibly because the creek banks were steep and sandy. We collected the allotype from a shallow burrow underneath a submerged a rock in Chickasaw Creek in Mobile County, Alabama (30.7822° N, 88.1191° W). This site had a high number of burrows both in the creek banks and among boulders in the creek bed. Collecting at this site was unusually easy because many of the crayfish were in shallow burrows that terminated directly underneath boulders.

Specimens examined. We examined 66 specimens (18 Form I males, 5 Form II males, 26 females, 17 juveniles; Table 4).

Coloration and color pattern. The background color of *L. mobilensis* **sp. nov.** varies both between and within sites. We regularly encountered specimens having a background color of olive green, brown, maroon, and navy blue. The background color is typically uniform on a specimen's carapace, abdomen, and dorsal surface of the chela (Figure 3).

The dorsal surface of the cheliped bears burgundy highlights at the base of the dactyl, the distal portions of the carpus and merus, and in some cases just proximal to the tips of the propodus and dactyl. The articulations between the merus and carpus and the carpus and propodus are highlighted in orange. The lateral margin and ventral surface of the chela diverge from the background coloration and are typically an apricot or cream color. The tubercles on the dorsal surface of the carpus, propodus and dactyl are of the same background color as the rest of the chela, but small, light-colored setae on their anterior surfaces make them conspicuous.

TABLE 4. Data (sorted by State and County) on examined specimens of *Lacunicambarus mobilensis* **sp. nov.** including catalogue number, State and County of collection, GPS coordinates in decimal degrees, collection date, number of specimens of each sex and Form, and whether sample included ovigerous females. Asterisk denotes type locality. Please note that some museum specimens were not associated with GPS coordinates so we estimated them based on provided locality information. Abbreviations: AL, Alabama; INHS, Illinois Natural History Survey; MS, Mississippi; OSUMC; Ohio State University Museum of Biological Diversity.

Field/catalogue #	State	County	Latitude (°N)	Longitude (°W)	Collection date	Form 1 males	Form 2 males	Females	Ovigerous?	Juveniles
INHS 10861	AL	Mobile	30.7829	88.1162	3/17/2007			2		
INHS 11139	AL	Mobile	30.8805	88.1981	4/21/2007			1		
INHS-10861	AL	Mobile	30.7829	88.1162	3/17/2007			2		
INHS-11142	AL	Mobile	30.7823	88.1157	3/12/2008	1	1	1		
INHS-15284	AL	Mobile	30.5241	88.1234	3/29/2016	1				
INHS-15289	AL	Mobile	30.8558	88.3341	3/28/2016			2		
OSUMC 10862*	AL	Mobile	30.5247	88.1237	1/8/2020	1		1		
OSUMC 10864	AL	Mobile	30.5927	88.2681	1/8/2020	1		1		3
OSUMC 10865	AL	Mobile	30.5432	88.1241	1/11/2020	1		2	yes	1
OSUMC 10866	AL	Mobile	30.5049	88.1697	1/11/2020	3		3	yes	2
OSUMC 10867	AL	Mobile	30.5016	88.1303	1/12/2020	3				1
OSUMC 10869	AL	Mobile	30.7822	88.1191	1/14/2020	2		4	yes	6
OSUMC 10871	MS	George	30.8432	88.5307	1/9/2020	1	2	1		
OSUMC 10872	MS	George	30.8743	88.4778	1/9/2020			3	yes	1
INHS-13987	MS	Jackson	30.6322	88.4482	4/3/2014	2	1			
OSUMC 10873	MS	Jackson	30.6326	88.4489	1/8/2020	1				
OSUMC 10874	MS	Jackson	30.6289	88.4348	1/14/2020			1		3
OSUMC 2638	MS	Jackson	30.7142	88.5132	3/22/1988	1	1	2		

The distal segments of the walking legs, from the merus to the dactyl, are usually light blue, but in some specimens can be cream or tan. The proximal segments of the walking legs, from the ischium to the coxa, as well as the ventral surface of the carapace and abdomen, are typically cream colored. The articulations between walking leg segments are highlighted in red or orange.

The tail fan is typically a similar color as a specimen's background coloration, although often a shade lighter. The margins of the entire tail fan, including the articulation between the telson and the sixth abdominal somite, are highlighted in conspicuous red or orange in some specimens.

This species bears a single conspicuous stripe running longitudinally from the posterior margin of the sixth abdominal somite to the anterior margin of the areola, where it forks and continues down the cervical groove. This stripe is typically a shade of orange and can be quite flamboyant. The rostral margins are highlighted in the same color, and these highlights sometimes extend posteriorly, forming a diamond shape that converges at a point just anterior to the cervical groove. In some specimens, small spots or dashes of the same color as the stripe are present on the abdominal pleurites, but we have never encountered a specimen where these spots or dashes connect to form complete stripes such as those on *L. ludovicianus*.

Size. The largest specimen of *L. mobilensis* **sp. nov.** that we examined was a Form I male from Mobile County, Alabama, measuring 32.45 mm CL. Form 1 males measured 24.31–32.45 mm CL ($\overline{x}$=28.70, σ=2.81, n=12), Form II males measured 24.32–26.16 mm CL ($\overline{x}$=25.24, σ=1.30, n=2) and females measured 24.36–31.38 mm CL ($\overline{x}$=26.47, σ=2.07, n=12). The smallest ovigerous female examined measured 24.36 mm CL.

Variation. Morphology varies minimally across the species' range; however, we saw some variation in color pattern. Specifically, we examined many specimens with pigment spots on the sides of the carapace, most frequently in the hepatic region of the cephalon. These spots are usually of the same color as the stripe but are bilaterally asymmetrical and appear random. Similarly, many specimens exhibit irregular and asymmetrical breaks in their stripes or in the highlighting of the cervical groove or cephalon. Taken together, these irregularities give the impression that the color pattern of this species is somehow unstable. Whether this variation is genetic or environmental in nature is difficult to determine but might be elucidated using transcriptomics. Lastly, the annulus ventralis of some specimens is a mirror image of what is described in the Diagnosis.

Range. *Lacunicambarus mobilensis* **sp. nov.** is currently known from an approximately 1,050 km^2 area spanning parts of Mobile County, Alabama and George and Jackson counties, Mississippi (see Figure 5). The farthest north that we found this species is approximately 16 km southeast of Lucedale, Mississippi. Additional sampling may locate this species further north, but our attempts have thus far only yielded *L. ludovicianus.* To the east, this species' range appears to be firmly bound by Mobile Bay and the Mobile-Tensaw River Delta. All sampling efforts east of Mobile Bay to date have failed to locate *L. mobilensis* **sp. nov.**, instead yielding *L. miltus, L. erythrodactylus* (Simon & Morris, 2014), and *L. dalyae* Glon, Williams & Loughman, 2019b. Similarly, to the west, *L. mobilensis* **sp. nov.**'s range appears to be firmly bound by the Pascagoula River. Our sampling west of the Pascagoula river has thus far only yielded *L. ludovicianus* and *L. erythrodactylus*. Interestingly, the species' range does not appear to extend south to the Gulf Coast. We found the species within 15 km of the coast, but all of our sampling efforts closer to the coast yielded only *L. freudensteini* **sp. nov.**

Conservation status. Based on our current information, we suggest that *L. mobilensis* **sp. nov.** be considered Vulnerable following the criteria of the American Fisheries Society (Taylor *et al.*, 2007), G3 (i.e., rare or uncommon but not imperiled) following the global conservation criteria of Master (1991), and Near Threatened following the criteria of the International Union for the Conservation of Nature (IUCN, 2019). *Lacunicambarus mobilensis* **sp. nov.** is currently known from 18 sites in an approximately 1,050 km^2 area, although future sampling will likely yield additional sites. Of concern to us is that *L. mobilensis* **sp. nov.** is quite rare even in seemingly appropriate habitats. At some sites where we managed to locate *L. mobilensis* **sp. nov.**, we only found very small colonies, consisting of fewer than five burrows, despite surveying large areas. To further refine the species' conservation status and suggest specific conservation actions, we recommend that future studies delve more deeply into possible threats to the species' persistence.

Life history notes. The life history of *L. mobilensis* **sp. nov.** remains largely unexplored. The bulk of our sampling was conducted in January and March. During these months, we collected an approximately equal number of adult males (Form I, n=18; Form II, n=5) and females (n=26). Most males were Form I, and almost all females were ovigerous or in glair. These observations, plus the fact that we never encountered male and female crayfish in the same burrow, suggest that mating was completed prior to our sampling. Ovigerous females carried 1–59 ($\overline{x}$=33.00, σ=21.15, n=6) with diameters ranging from 2.10–2.66 mm ($\bar{x}$=2.35, σ=0.18, n=6). We kept one ovigerous female alive following our January 2020 collecting trip, and the young hatched on 2 February 2020. We also collected a small number of juvenile specimens (n=17) whose size suggests that they were born in the preceding year. These juveniles were in their own burrows rather than sharing the burrows of adults. This information suggests that *L. mobilensis* **sp. nov.** follows the typical life history pattern of Cambarid crayfishes outlined in Hobbs III (2001), with one or two copulation events per year occurring in spring and/or autumn and females subsequently laying eggs in winter. We currently do not have any information about the lifespan of this species.

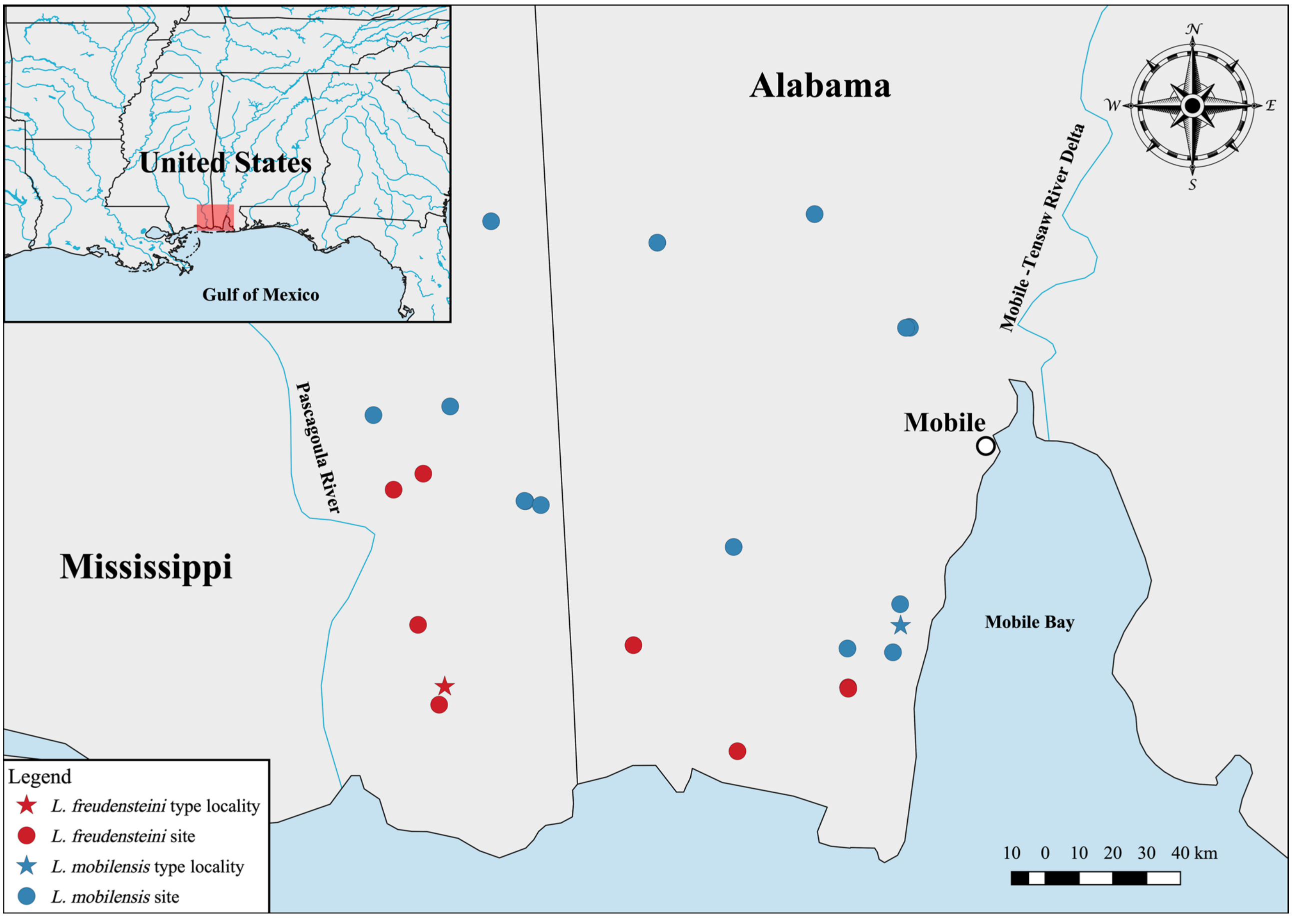

FIGURE 5. Range map of *Lacunicambarus mobilensis* **sp. nov.** and *L freudensteini* **sp. nov.** Colored dots indicate collection sites of *L. mobilensis* **sp. nov.** (blue) and *L. freudensteini* **sp. nov.** (red). Colored stars indicate type locality of *L. mobilensis* **sp. nov.** (blue) and *L. freudensteini* **sp. nov.** (red).

Ecological notes. *Lacunicambarus mobilensis* **sp. nov.** burrow morphology is similar to what is seen in other *Lacunicambarus* species and normally consists of 1–2 portals leading to a single resting chamber. On occasion tunnels bifurcate into multiple tunnels, most of which terminate in their own resting chamber. Resting chambers are not as pronounced as in other, larger-bodied *Lacunicambarus* species, and are normally only increased slightly in diameter relative to the burrow's tunnels. Given that the majority of burrows were excavated in January, this could be a seasonal aspect of the biology of *L. mobilensis* **sp. nov.**, and it is possible that burrow volume increases in warmer months. When present, chimneys were composed of homogeneous mud pellets arranged in a less organized fashion than those seen at the openings of burrows of sympatric *Creaserinus* species.

We encountered burrow colonies in both anthropogenic and natural habitats, including roadside ditches, residential yards with low-lying depressional wetlands, and bridge abutments. We also collected specimens from riparian forests adjacent to streams and natural wetlands. Canopies in these forests were composed of *Quercus*, *Nyssa*, *Liquidambar, Ilex*, *Magnolia*, and *Salix*. We frequently encountered burrows on stream floodplains that were excavated among tree roots and, in many cases, followed the subterranean path of the roots, making our excavations difficult. Burrows in the latter habitats were usually clustered in colonies compared to more sprawling distributions associated with anthropogenic habitats. Lastly, we also collected *L. mobilensis* **sp. nov.** from burrows in incised, channelized streambanks. These burrows were all simple in architecture and consisted of single downward shafts terminating in single resting chambers. Interestingly, most burrows of this nature lacked chimneys and maintained open portals.

Crayfish associates. We collected the following crayfish species at sites where we collected *L. mobilensis* **sp. nov.**: *Creaserinus burrisi* (Fitzpatrick, 1987) and *Procambarus* cf. *acutus* (Girard, 1852).

Relationships. *Lacunicambarus mobilensis* **sp. nov.** most closely resembles *L. ludovicianus* and *L. miltus*. It can be distinguished from *L. ludovicianus* based on the number of stripes on the abdomen (*L. mobilensis* **sp. nov.**: 1; *L. ludovicianus*: 3), the shape of the cephalic lobe of the epistome (*L. mobilensis* **sp. nov.**: truncated; *L. ludovicianus*: rounded or subtriangular), the number of tubercles in the first row of tubercles on the mesial margin of the chela palm (*L. mobilensis* **sp. nov.**: 5–7 [$\bar{x}$=6.32, σ=0.57, n=23]; *L. ludovicianus*: 6–10 [$\bar{x}$=7.38, σ=0.90, n=26]), and the chela palm length (*L. mobilensis* **sp. nov.** palm length/propodus length: 0.34–0.38 [$\bar{x}$=0.35, σ=0.01, n=23]; *L. ludovicianus* palm length/propodus length: 0.31–0.34 [$\bar{x}$=0.32, σ=0.01, n=25]). The maximum known size of *L. mobilensis* **sp. nov.** (32.45 mm CL) is also considerably less than that of *L. ludovicianus* (>50 mm CL).

Lacunicambarus mobilensis **sp. nov.** can be distinguished from *L. miltus* based on the number of subpalmar tubercles (*L. mobilensis* **sp. nov.**: 3–7 [$\bar{x}$=4.68, σ=1.29, n=22]; *L. miltus*: 1–4 [$\bar{x}$=1.67, σ=0.91, n=23]), and the length of the Form I male gonopod terminal elements (*L. mobilensis* **sp. nov.** central projection length/gonopod length: 0.26–0.29 [$\bar{x}$=0.28, σ=0.01, n=12], mesial process length/gonopod length: 0.27–0.31 [$\bar{x}$=0.29, σ=0.01, n=12]; *L. miltus* central projection length/gonopod length: 0.31–0.35 [$\bar{x}$=0.33, σ=0.01, n=8], mesial process length/gonopod length: 0.30–0.35 [$\bar{x}$=0.33, σ=0.02, n=8]).

Generally speaking, *L. mobilensis* **sp. nov.** can be differentiated from other *Lacunicambarus* crayfishes based its small size, the tuberculation pattern of the palm of its chela, its high number of subpalmar tubercles, its truncate cephalic lobe of the epistome, its single bright abdominal stripe, and its pronounced subapical notch.

Etymology. The species epithet *mobilensis* means "from Mobile." This species is primarily found in the vicinity of Mobile, Alabama and Mobile Bay. In addition, we suspect that Mobile Bay played an important role in dividing the range of the last common ancestor of *L. mobilensis* **sp. nov.** and *L. miltus*, leading to geographic isolation and hence speciation.

For a common name, we suggest "Lonesome Gravedigger" to recognize the close affinity between *L. mobilensis* **sp. nov.** and *L. miltus* (Rusty Gravedigger) and to recognize that this species remained undescribed for four decades after its sister species, *L. miltus*, was described.

Family Cambaridae Hobbs, 1942a

Genus *Lacunicambarus* (Hobbs, 1969)

Lacunicambarus freudensteini Glon sp. nov.

Diagnosis. Eyes pigmented, not reduced. Rostrum wide, directed ventrally in lateral view, margins moderately thickened to tip of acumen, lacking marginal spines or tubercles and median carina, shallowly excavated. Acumen distinctly delimited basally by 45° angles. Carapace subcylindrical, laterally compressed. Cervical spine absent; branchiostegal spine obsolete. Suborbital angle acute. Cervical groove uninterrupted. Postorbital ridges developed, ending cephalically in small tubercle. Areola obliterated, constituting in adults 39.74–44.49% ($\bar{x}$=42.12, σ=1.00, n=27) of entire length of carapace. Antennal scale 2.80 to 3.73 ($\bar{x}$=3.28, σ=0.21, n=28) times as long as wide, widest distal to midlength, antennal spine strongly developed. Cephalic lobe of epistome moderately truncated apically. Mesial margin of palm of chela with 3 rows of tubercles, mesialmost row normally consisting of 7–9 ($\bar{x}$=7.79, σ=0.51, n=28) spiniform tubercles, second row incomplete, running parallel to first, with 2–4 ($\bar{x}$=3.04, σ=0.69, n=28) tubercles located distally, third row running diagonally from mesial base of palm to mesialmost of two bulbous tubercles proximal to propodactyl articulation with 4–8 ($\bar{x}$=6.33, σ=0.87, n=28) small tubercles located in shallow dimples. Opposable margin of dactyl weakly concave at base. Ratio of dactyl length to palm length 1.52–1.82 ($\bar{x}$=1.68, σ=0.06, n=24). Dorsomesial longitudinal ridges of dactyl and propodus weakly developed. Dorsolateral impression at base of propodus weak. Ventral surface of chela with 1–3 ($\bar{x}$=1.63, σ=0.59, n=24) subpalmar tubercles. Mesial ramus of uropod with distomesial spine not reaching caudal margin. Gonopods of Form I males contiguous at base, with pronounced umbo near midlength of caudal surface; terminal elements consisting of 1) a long central projection, thin and strongly arched in lateral aspect, bearing pronounced subapical notch, slightly shorter than mesial process, directed caudally at approximately 90^o, overreaching margin of umbo by noticeable amount, 2) a long mesial process with conical base, tapering near midlength, tipped with protruding finger, directed caudally at approximately 90^o and overreaching margin of umbo by noticeable amount, 3) a weakly developed caudal knob protruding from caudolateral base of central projection. Hook on ischium of third pereiopod only. Female with annulus ventralis subcircular or subquadrangular, wider than long, deeply embedded in sternum, slightly moveable with anterior half mildly pliable and posterior half sclerotized. Life colors include orange latitudinal bands highlighting posterior margin of each abdominal somite and cervical groove, band enlarged on first abdominal somite, in some cases entire visible portion of somite highlighted. Life colors never include longitudinal stripe.

Holotypic male, Form I (catalogue no. OSUMC 10880; Figures 6, 7A–C, F–H, J–L; Table 5). Carapace bullet shaped in dorsal view (Figure 7H), width 103.89% of depth, wider than abdomen (12.54 and 9.78 mm, respectively; Figure 6); maximum width greater than depth at caudodorsal margin of cervical groove (12.54 and 12.07 mm, respectively). Areola obliterated; length 42.38% of total length of carapace (Figure 7H). Rostrum directed ventrally in lateral view, margins moderately thickened, asymmetrical likely due to life injury; acumen indistinctly delimited basally by 45° angles, anterior tip upturned, not reaching ultimate podomere of antennular peduncle; dorsal surface of rostrum shallowly concave with minute punctations forming single row along rostral margins. Subrostral ridge moderate, evident in lateral aspect along entire length of rostrum, tapering distally. Postorbital ridges developed, grooved dorsolaterally, ending cephalically in small tubercle. Suborbital angle acute; branchiostegal spine obsolete (Figure 7A). Posterior margin of cervical groove lined by collar of 6 small tubercles. Branchiostegal region granulate. Anteroventral branchiostegal region with 14 small tubercles. Hepatic region with scattered granules and tubercles. Remainder of carapace with slight punctations dorsally and laterally. Abdomen shorter than carapace (25.48 and 25.53 mm, respectively), 2.61 times as long as wide; pleura short, rounded caudoventrally. Cephalic section of telson with 2 spines in caudolateral corners, mesial spine slightly moveable. Proximal segment of lateral ramus of uropod with 18 spines on distal margin; mesial ramus of uropod with prominent median rib ending distally as strong distomesial spine not reaching margin of ramus, laterodistal spine of ramus strong.

Anteromesial lobe of epistome (Figure 7G) bell shaped but truncated apically, with uniform ventrally raised margins, ventral surface shallowly concave; main body of epistome with shallow fovea; epistomal zygoma weakly arched. Ventral surface of antennular peduncle's proximal podomere with small spine at midlength. Antennal peduncle without spines. Antennal scale 3.16 times as long as wide (Figure 7J), widest distal to midlength, lateral margin straight from basal area to widest distal point, ending in strongly developed antennal spine reaching past proximal margin of ultimate podomore of antennal peduncle. Ventral surface of entire third maxilliped densely studded with long, flexible setae; distolateral angle acute.

Length of right chela of cheliped (Figure 7K) 90.87% of carapace length; chela width 44.65% of length; palm length 36.12% of chela length; dactyl length 1.71 times palm length. Mesial margin of palm of chela with 3 rows of tubercles, mesialmost row with 8 spiniform tubercles, second row running parallel to first, with 4 tubercles located distally, third row running diagonally from mesial base of palm to mesialmost of two bulbous tubercles proximal to propodactyl articulation with 7 small tubercles located in shallow dimples; proximal dorsolateral half smooth, distolateral area punctate, punctations deep, largest in vicinity of dorsolateral base of propodus; lateral margin of propodus not costate; ventromesial surface of palm with small punctations, 2 bulbous tubercles on articular rim opposite base of dactyl, lateralmost of which is spiniform; 1 subpalmar tubercle. Both fingers of chela with weakly developed dorsomesial longitudinal ridges. Opposable margin of propodus with row of 7 tubercles, decreasing in size from base except for third from base, which is much larger than adjacent tubercles, and ultimate tubercle; penultimate tubercle positioned ventrally relative to adjacent tubercles; single row of minute denticles extending distally from fourth tubercle. Opposable margin of dactyl with row of 9 tubercles decreasing in size from base except for fifth from base which larger than adjacent tubercles; single row of minute denticles extends distally from fifth tubercle; mesial surface of dactyl studded with 15 tubercles proximally, not forming distinct rows, giving way to punctations distally. Dorsolateral impression at base of propodus weak.

Cheliped carpus with distinct dorsal furrow; dorsomesial surface with row of 6 small tubercles; dorsolateral surface punctate; mesial surface with 9 scattered spiniform tubercles plus 1 large procurved spine near distal margin; ventral surface with spine on distal articular rim. Merus dorsodistal margin with 1 small tubercle, dorsal surface with 3 spiniform tubercles; ventrolateral margin with row of 5 spines increasing in size from base, ventromesial margin with row of 8 spines increasing in size from base. Basioischial segment with 3 small tubercles on ventral margin. Ischium of third pereiopod with simple hook extending proximally to basioischial articulation, not opposed by tubercles on basis. Coxa of fourth pereiopod with setiferous, caudomesial boss, ventral surface calcified; coxa of fifth pereiopod lacking boss, ventral surface membranous.

Gonopods contiguous at base, reaching past caudomesial boss of fourth pereiopod when abdomen flexed; terminal elements as described in Diagnosis (Figure 7B–C, F).

Left gonopod and right antennal scale separated from specimen and placed in vial inside specimen jar. One gill has been extracted from left gill chamber of specimen, preserved in 100% ethanol and frozen at OSUMC for future DNA extractions (MGG 769).

Allotypic female (catalogue no. OSUMC 10880; Figure 7I; Table 5). Differs from holotype as follows: Carapace width 107.89% of depth. Posterior margin of cervical groove lined by collar of 5 small tubercles. Anteroventral branchiostegal region with 15 small tubercles. Abdomen longer than carapace (27.34 and 26.79 mm, respectively). Proximal segment of lateral ramus of uropod with 14 spines on distal margin. Antennal scale 3.32 times as long as wide. Right chela regenerated. Length of left chela 88.58% of carapace length; chela width 45.98% of length. Second row of tubercles on mesial margin of palm of chela with 3 tubercles, third row running diagonally from mesial base of palm to mesialmost of two bulbous tubercles proximal to propodactyl articulation with 6 small tubercles in shallow dimples. Two subpalmar tubercles. Opposable margin of dactyl with row of 10 tubercles decreasing in size from base except for fifth from base which larger than adjacent tubercles; mesial surface of dactyl studded with 16 tubercles proximally, not forming distinct rows, giving way to punctations distally. Cheliped carpus dorsomesial surface with row of 5 small tubercles; mesial surface with 6 scattered spiniform tubercles plus 1 large procurved spine near distal margin. Merus ventrolateral margin with row of 4 spines increasing in size from base, ventromesial margin with row of 6 spines increasing in size from base.

Annulus ventralis (Figure 7J) as in Diagnosis; 1.19 times wider than long, with bifurcated leathery ridge mesially located in anterior half; tongue extending from sclerotized lingual (left) wall into fossa of sclerotized supralingual (right) wall; supralingual wall more swollen caudomesially than lingual wall and curved on outer margin. Posterior margin of annulus ventralis reaching anterior margin of oblong, approximately symmetrical postannular sclerite lacking setae. First pleopods present, overreaching caudal edge of annulus ventralis when abdomen flexed.

Morphotypic male, Form II (catalogue no. OSUMC 10880; Figure 7D–E; Table 5). Differs from holotype as follows: Carapace width 106.13% of depth. Posterior margin of cervical groove lined by collar of 8 small tubercles. Anteroventral branchiostegal region with 9 small tubercles. Abdomen longer than carapace (22.01 and 21.79 mm, respectively), 2.69 times as long as wide. Proximal segment of lateral ramus of uropod with 19 spines on distal margin. Antennal scale 3.40 times as long as wide. Length of right chela 77.97% of carapace length. Mesial margin of palm of chela with 3 rows of tubercles, mesialmost row with 9 spiniform tubercles, running parallel to incomplete second row with 3 tubercles located distally, third row running diagonally from mesial base of palm to mesialmost of two bulbous tubercles proximal to propodactyl articulation with 6 small tubercles located in shallow dimples. Opposable margin of dactyl with row of 8 tubercles decreasing in size from base except for fourth from base which larger than adjacent tubercles; single row of minute denticles extends distally from fourth tubercle; mesial surface of dactyl studded with 17 tubercles proximally, not forming distinct rows, giving way to punctations distally. Cheliped carpus dorsomesial surface with row of 4 small tubercles; mesial surface with 6 scattered spiniform tubercles plus 1 large procurved spine near distal margin. Merus dorsal surface with 2 tubercles; ventromesial margin with row of 10 spines increasing in size from base. Basioischial segment of cheliped with 3 small tubercles on ventral margin. Ischium of third pereiopod with simple hook extending towards but not reaching basioischial articulation.

Form II gonopod central projection non-corneous, rounded, slightly shorter than mesial process, overreaching margin of umbo, mesial process short and blunt, just overreaching margin of umbo (Figure 7D–E). Caudal knob not visible. Left gonopod separated from specimen, placed in glass vial inside specimen jar. One gill has been extracted from left gill chamber of specimen, preserved in 100% ethanol and frozen at OSUMC for future DNA extractions (MGG 771).

Disposition of Types. We deposited the holotype, allotype, and morphotype in the OSUMC (catalogue no. 10880). We also deposited Form I and female paratypes in the INHS (catalogue nos. 16726–16727) and the CBHR (catalogue nos. 6970–6971).

FIGURE 6. Dorsal view of Form I holotypic male of *Lacunicambarus freudensteini* **sp. nov.** (catalogue no. OSUMC 10880).

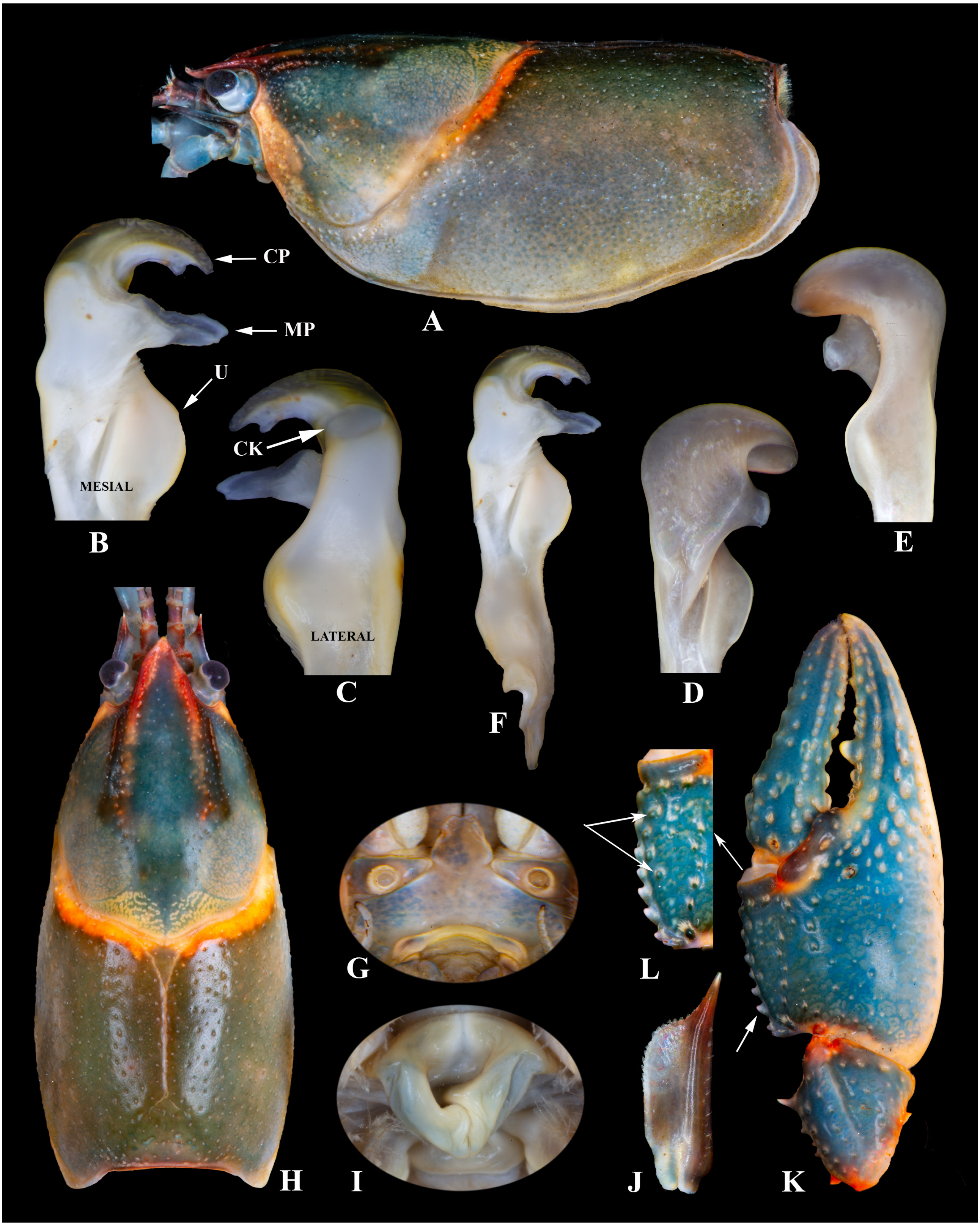

FIGURE 7. *Lacunicambarus freudensteini* **sp. nov.** (A) Lateral view of carapace; (B & F) mesial and (C) lateral views of Form I gonopod; (D) mesial and (E) lateral views of Form II gonopod; (G) epistome; (H) dorsal view of carapace; (I) annulus ventralis; (J) antennal scale; (K) dorsal view of right chela; (L) close up of chela showing tubercles on mesial margin of palm. A–C, F–H, and J–L from holotype (catalogue no. OSUMC 10880); D–E from morphotype (catalogue no. OSUMC 10880); I from allotype (catalogue no. OSUMC 10880). Abbreviations: CP, central projection; MP, mesial process; CK, caudal knob; U, umbo.

TABLE 5. Measurements (mm) of holotype, morphotype, and allotype of *Lacunicambarus freudensteini* **sp. nov.** (Catalogue #: OSUMC 10880).

Character	Holotype	Morphotype	Allotype
Carapace:			
Depth	12.07	9.95	12.04
Width	12.54	10.56	12.99
Length	25.53	21.79	26.79
Areola:			
Length	10.82	9.16	11.26
Rostrum:			
Width at eyes	2.95	2.47	3.13
Length	3.77	3.36	4.00
Postorbital ridge:			
Width	4.51	3.91	4.69
Chela (right):			
Length of propodus	23.20	16.99	23.73
Length of palm	8.38	6.08	8.27
Width of palm	10.36	7.17	10.91
Length of dactyl	14.33	10.41	13.89
Abdomen:			
Length	25.48	22.01	27.34
Width	9.78	8.17	10.49
Gonopod:			
Length	6.66	5.31	NA
Width at umbo	1.77	1.31	NA
Annulus ventralis:			
Length	NA	NA	2.53
Width	NA	NA	3.01
Antennal scale:			
Length	4.14	3.60	4.31
Width	1.31	1.06	1.30

Type locality. We collected the holotype, allotype, and morphotype along an electric transmission line right of way south of Kings Road in Jackson County, Mississippi (30.4720° N, 88.5179° W). This site had a particularly high water table and yielded the highest number of specimens that we found in any site, possibly because the cleared vegetation made locating burrows easier. The closest running water to this site is Little Black Creek, a tributary of the Pascagoula River.

Specimens examined. We examined 42 specimens (15 Form I males, 1 Form II male, 20 females, 6 juveniles; Table 6).

Coloration and color pattern. The background color of *L. freudensteini* **sp. nov.** varies both between and within sites. Background colors that we encountered regularly include olive, green, brown, and blue (but see "Variations" below). The uniformity of this background color on any given specimen varies. For example, some specimens bear the same background color on their carapace, abdomen, and the dorsal surface of the chela. In others, the dorsal surface of the chela, cephalon, and/or abdomen is a vivid color, such as blue or green, whereas the branchiostegal region remains a dull color, such as brown or olive green (Figure 6).

The propodactyl, carpopropodal, and merocarpal articulations of the cheliped bear red-orange and burgundy highlights. The lateral margin and ventral surface of the chela diverges from the background coloration and is typically an apricot or cream color. The tubercles on the dorsal surface of the carpus, propodus and dactyl are of the same background color as the rest of the chela, but small, light-colored setae on their anterior surfaces that makes them conspicuous.

The background color of the walking legs can be cream, tan, or light blue. This color is typically lighter proximally and becomes gradually darker distally. The articulations between walking leg segments are highlighted in red or orange. The ventral surfaces of the carapace and abdomen are typically cream colored.

The tail fan is typically a similar color as a specimen's background color, although sometimes a shade lighter. The margins of the entire tail fan, including the articulation between the telson and the sixth abdominal somite, are highlighted in conspicuous red or orange.

Unlike many of its congeners, this species never bears a longitudinal stripe. The most prominent markings are orange latitudinal bands on the posterior margin of each abdominal somite. The band is enlarged on the first abdominal somite, and in some cases the entire visible portion of this somite is highlighted. The cervical groove is also highlighted in orange. The rostral margins are highlighted in orange, red-orange, or burgundy, and these highlights sometimes extend posteriorly on the dorsal surface of the cephalon, typically fading near the posterior margin of the postorbital ridge.

Size. The largest *L. freudensteini* **sp. nov.** that we examined was a Form I male from Mobile County, Alabama, that measured 30.90 mm CL. Form 1 males measured 21.88–30.92 mm CL ($\bar{x}$=25.00, σ=2.53, n=12) and females measured 21.32–28.40 mm CL ($\bar{x}$=24.90, σ=2.36, n=15). The only Form II male that we examined measured 21.79 mm CL. The smallest ovigerous female that we examined measured 23.30 mm CL.

Variation. Morphology varies minimally across the species' range. The most drastic variation that we saw was present in two specimens whose coloration differed dramatically from what is typical for this species. The first of these, collected from Mobile County, Alabama, had a powder blue background color that was particularly vibrant on the chelipeds, abdomen, and uropod, with the carapace being a dull blue (Figure 8A). This specimen still bore the usual highlights and markings of the species, albeit in uniform yellow orange. The second unusual specimen, collected in Jackson County, Mississippi, had a bright pink background color (Figure 8B). Like the blue specimen, this crayfish still had the characteristic markings of the species, but they were harder to see because they blended into the pink background color. These unusual specimens lend credence to Schuster (2020), who suggested that although color itself can vary, color pattern (in this case, the bands on the cervical groove and abdomen) typically remains consistent and is, therefore, more reliable than background color as a taxonomic character. Lastly, the annulus ventralis of some specimens is a mirror image of what is described in the Diagnosis.

Range. *Lacunicambarus freudensteini* **sp. nov.** is currently known from an approximately 475 km^2 area spanning parts of Mobile County, Alabama, and Jackson County, Mississippi (Figure 5). The species' range extends along the coast of the Gulf of Mexico, from Mobile Bay in the east to the Pascagoula River in the west, with some sites being 2 km from the Gulf of Mexico. In Mississippi the species' range extends north as far as Wade, Mississippi. Similar to *L. mobilensis* **sp. nov.**, we did not find this species east of Mobile Bay or west of the Pascagoula River. Interestingly, we never found *L. freudensteini* **sp. nov.** in sympatry with *L. mobilensis* **sp. nov.**, and their ranges do not overlap.

Conservation status. Based on our current information, we suggest that *L. freudensteini* **sp. nov.** be considered Vulnerable following the criteria of the American Fisheries Society presented in Taylor *et al.* (2007), G3 (i.e., rare or uncommon but not imperiled) following the global conservation criteria of Master (1991), and Vulnerable (B1ab[ii, iii]) following the criteria of the International Union for the Conservation of Nature (IUCN, 2019). *Lacunicambarus freudensteini* **sp. nov.** is currently known from 9 sites in an approximately 475 km^2 area, although future sampling will likely yield additional sites. Of concern to us is that *L. freudensteini* **sp. nov.** is quite rare, even in seemingly appropriate habitats. We failed to collect this species at seven sites within its range where other burrowing crayfish were abundant. At some sites where we managed to locate *L. freudensteini* **sp. nov.**, we only found a single specimen despite surveying large areas. Of additional concern is that several sites where we found this species were at low elevations and within a few km of the Gulf of Mexico. Numerous recent studies have projected that sea levels will rise by as much as 2 m by 2100 (e.g., Sweet *et al.*, 2017; Bamber *et al.*, 2019). According to NOAA's Sea Level Rise Viewer tool, such a dramatic rise in sea levels would inundate several *L. freudensteini* **sp. nov.** sites. In addition, given that *L. freudensteini* **sp. nov.** relies on ground water that it accesses by burrowing to survive dry periods, subsurface saltwater intrusion could harm this species before inundation occurs. To further refine the species' conservation status and suggest specific conservation actions, we recommend that future studies delve more deeply into possible threats to the species' persistence.

Life history notes. The life history of *L. freudensteini* **sp. nov.** remains largely unexplored. The bulk of our sampling was conducted in early to mid-January. During this time, we collected an approximately equal number of adult males (Form I, n=15; Form II, n=1) and females (n=19). Almost all male specimens were Form I, and almost all females were ovigerous or in glair. These observations, plus the fact that we never encountered male and female crayfish in the same burrow, suggest that mating had occurred prior to our collection efforts and had since ceased. Ovigerous females carried 4–34 ($\bar{x}$=17.67, σ=15.18, n=3) with diameters ranging from 2.20–2.35 mm ($\bar{x}$=2.25, σ=0.09, n=3). We also collected a small number of juvenile specimens (n=6) whose sizes suggests that they were born in the preceding year. These juveniles were in their own burrows rather than sharing the burrows of adults. Like for *L. mobilensis* **sp. nov.**, the available information suggests that *L. freudensteini* **sp. nov.** follows the typical life history pattern of Cambarid crayfishes outlined in Hobbs III (2001), with one or two reproductive events per year occurring in spring and/or autumn and females subsequently laying eggs in winter. We currently do not have any information about the lifespan of this species.

FIGURE 8. Dorsal view of blue morph (A) and pink morph (B) of *Lacunicambarus freudensteini* **sp. nov.**

Ecological notes. Burrow morphology and habitat association of *L. freudensteini* **sp. nov.** are similar to those discussed for *L. mobilensis* **sp. nov.**, with the exception of total burrow volume and habitat type. Whereas *L. mobilensis* **sp. nov.** excavates relatively simple, localized burrows, we found several *L. freudensteini* **sp. nov.** burrows that possessed ancillary tunnels with portals up to 4 m apart. *Lacunicambarus freudensteini* **sp. nov.** also differed from *L. mobilensis* **sp. nov.** in the frequency of open habitat utilization. Specifically, open, non-sloping grasslands with a shallow clay pan appear to provide optimal conditions for *L. freudensteini* **sp. nov.** Many populations were found in the vicinity of manmade rights of way (which served as either petroleum pipelines or electric power lines and were maintained by both mowing and controlled burns). It is possible that these rights of way imitate wet prairies that existed prior to the development of the Mobile Bay area, and the reliance of *L. freudensteini* **sp. nov.** on this particular habitat type is an area in need of future research.

Crayfish associates. We collected the following crayfish species at sites where we collected *L. freudensteini* **sp. nov.**: *Creaserinus danielae* (Hobbs, 1975), *Faxonella clypeata* (Hobbs, 1975), *Procambarus clarkii* (Girard, 1852), *Procambarus evermanni* (Faxon, 1890), and *Procambarus shermani* Hobbs, 1942b.

Relationships. *Lacunicambarus freudensteini* **sp. nov.** most closely resembles *L. diogenes* (Girard, 1852)*, L. chimera* and *L. ludovicianus*. It can be distinguished from the former two species based on the central projection of Form I male gonopods (*L. freudensteini* **sp. nov.**: thin and strongly arched in lateral aspect, bearing pronounced subapical notch; *L. diogenes* and *L. chimera*: squat and straight or moderately arched in lateral aspect, subapical notch lacking or inconspicuous). The maximum known size of *L. freudensteini* **sp. nov.** (30.90 mm CL) is also considerably less than that of *L. diogenes* (>47.00 mm CL) and *L. chimera* (>65 mm CL).

Lacunicambarus freudensteini **sp. nov.** can be distinguished from *L. ludovicianus* based on color pattern (*L. freudensteini* **sp. nov.**: abdominal bands; *L. ludovicianus*: abdominal stripes), the width of the rostrum (*L. freudensteini* sp. nov rostrum width/length: 0.69–0.83 [$\bar{x}$=0.75, σ=0.04, n=27]; *L. ludovicianus* rostrum width/length: 0.59–0.72 [$\bar{x}$=0.65, σ=0.04, n=33]) and the number of tubercles in the second row of tubercles on the mesial margin of the chela palm (*L. freudensteini* sp. nov: 2–4 [$\bar{x}$=3.00, σ=0.67, n=24]; *L. ludovicianus*: 4–7 [$\bar{x}$=5.11, σ=0.99, n=26]).

Generally speaking, *L. freudensteini* **sp. nov.** can be differentiated from other *Lacunicambarus* crayfishes based on its small size, the spiniform tubercles in the first row of tubercles on the mesial margin of the chela palm, the low number of tubercles in the second row of tubercles on the mesial margin of the chela palm, its wide rostrum, its abdominal bands, and its pronounced subapical notch.

Etymology. It is with great pleasure that we name this crayfish after Dr. John Freudenstein, a Professor in and Chair of the Department of Evolution, Ecology, and Organismal Biology at The Ohio State University and Director of the University's Museum of Biological Diversity's Herbarium. Dr. Freudenstein has had a distinguished career researching the systematics of the Orchidaceae and Ericaceae, as well as theory and methods of systematics. Dr. Freudenstein has provided tremendous support and guidance to the primary author's dissertation research despite specializing in an entirely different kingdom. In addition, Dr. Freudenstein has been a wonderful mentor and friend to the primary author, helping to make his dissertation research enjoyable. Lastly, there are many plants in the vicinity of *L. freudensteini* **sp. nov.** burrows that Dr. Freudenstein would probably find interesting.

We suggest the common name "Banded Mudbug" as a testament to this species' brightly colored lateral bands as well as the fact that it is a primary burrowing crayfish (which are sometimes colloquially referred to as mudbugs).

Updated key to *Lacunicambarus*

The following key is modified from Glon *et al.* (2019b) and is based on Form I male specimens with at least one original chela and which still exhibit life colors. This key may still be useful for preserved specimens lacking life coloration but the absence of this character may occasionally make identification more difficult.

1. Mesial margin of palm of chela with 2 parallel rows of tubercles: mesial first row complete, bearing 5–10 tubercles, lateral second row either incomplete, bearing 2–4 tubercles, or complete, bearing 5–8 tubercles; third row of tubercles running diagonally from mesial base of palm to mesialmost of two bulbous tubercles proximal to propodactyl articulation; additional scattered tubercles sometimes present between second and third rows **2**

\- Mesial margin of palm of chela studded with tubercles not forming distinct rows **7**

2(1). Form I male gonopod central projection thin and strongly arched in lateral aspect, bearing pronounced subapical notch (Figure 9A–D) **3**

\- Form I male gonopod central projection squat and straight or moderately arched in lateral aspect, subapical notch lacking or inconspicuous (Glon 2019a, Figure 12A, E) **6**

3(2). Life colors never include stripe on abdomen (Figure 10A); rostrum wide (rostrum width/length: 0.69–0.83 [$\bar{x}$=0.75, σ=0.04, n=12]); tubercles of first row of chela palm spiniform; tubercles on second row of mesial margin of chela palm: 2–4 ($\bar{x}$=3.2, σ=0.63, n=10). . . . . ***L. freudensteini* sp. nov.**

\- Life colors include 1–3 stripes on abdomen (Figure 10B–D), rostrum narrow (rostrum width/length: 0.59–0.74 [$\bar{x}$=0.65, σ=0.04, n=27]); tubercles on second row of mesial margin of chela palm: 3–6 ($\bar{x}$=4.63, σ=0.79, n=27) **4**

4(3). Life colors typically include three brightly colored dorsal stripes on abdomen (Figure 10C); cephalic lobe of epistome apically rounded or subtriangular; palm of chela relatively short (palm length/propodus length: 0.31–0.33 [$\bar{x}$=0.32, σ=0.01, n=12]); tubercles on first row of mesial margin of chela palm: 6–10 ($\bar{x}$=7.31, σ=1.11, n=13). . . . . ***L. ludovicianus* (Faxon, 1884)**

\- Life colors include only one brightly colored dorsal stripe on abdomen (Figure 10B, D); cephalic lobe of epistome apically truncated; palm of chela relatively long (palm length/propodus length: 0.33–0.37 [$\bar{x}$=0.35, σ=0.08, n=23]); tubercles on first row of mesial margin of chela palm: 5–8 ($\bar{x}$=6.87, σ=0.87, n=23) **5**

5(4). Subpalmar tubercles: 3–7 ($\bar{x}$=4.64, σ=1.21, n=11); gonopod terminal elements relatively short (central projection length/gonopod length: 0.26–0.29 [$\bar{x}$=0.28, σ=0.01, n=12]; mesial process length/gonopod length: 0.27–0.31 [$\bar{x}$=0.29, σ=0.01, n=12]) . . . . ***L. mobilensis* sp. nov.**

- Subpalmar tubercles: 1–2 ($\bar{x}$=1.25, σ=0.50, n=4); gonopod terminal elements relatively long (central projection length/gonopod length: 0.31–0.35 [$\bar{x}$=0.33, σ=0.01, n=8]; mesial process length/gonopod length: 0.30–0.35 [$\bar{x}$=0.33, σ=0.02, n=8]) . . . . ***L. miltus*** **(Fitzpatrick, 1978)**

6(2). Spines on ventrolateral row of merus: 1–3 ($\bar{x}$ =1.67, σ=0.59, n=18); terminal elements relatively long (central projection/gonopod length: 0.28–0.32 [$\bar{x}$ =0.29, σ=0.01, n=18]; mesial process/gonopod length: 0.28–0.33 [$\bar{x}$ =0.31, σ=0.01, n=18]); stripes on dorsal side of abdomen never present . . . . ***L. diogenes*** **(Girard, 1852)**

- Spines on ventrolateral row of merus: 4–8 ($\bar{x}$ =5.82, σ=1.40, n=11); terminal elements relatively short (central projection/gonopod length: 0.20–0.24 [$\bar{x}$ =0.22, σ=0.01, n=11]; mesial process/gonopod length: 0.23–0.28 [$\bar{x}$ =0.26, σ=0.02, n=11]); single stripe on dorsal side of abdomen usually present (often faded in large adults). . . . . ***L. chimera*** **Glon and Thoma in Glon et al. (2019a)**

7(1). Median spine on mesial ramus of uropod conspicuously overreaching caudal margin of ramus . . ***L. acanthura*** **(Hobbs, 1981)**

- Median spine on mesial ramus of uropod not overreaching caudal margin of ramus **8**

8(7). Rostrum straight in lateral view. Life colors olive to brown, occasionally with red or orange highlights on rostral margins and tip of chela, but body typically monochromatic and not adorned with dorsal stripe . . . . ***L. thomai*** **(Jezerinac, 1993)**

- Rostrum moderately to strongly deflected in lateral view. Life colors vary but body typically polychromatic and adorned with dorsal stripe **9**

9(8). Central projection and mesial process of Form I male gonopod long, both distinctly overreaching umbo; central projection approximately equal in length to mesial process (central projection/mesial process: 0.88–1.03 [$\bar{x}$=0.96, σ=0.04, n=28]; Glon 2019b, Figure 6) . . . . ***L. dalyae*** **Glon, Williams & Loughman, 2019**

- Mesial process of Form I male gonopod overreaching umbo, but central projection short and not overreaching umbo; central projection conspicuously shorter than mesial process (central projection/mesial process: 0.76–0.89 [$\bar{x}$=0.81, σ=0.04, n=23]; Glon 2019b, Figure 6) . . . . ***L. polychromatus*** **(Thoma *et al.*, 2005)**

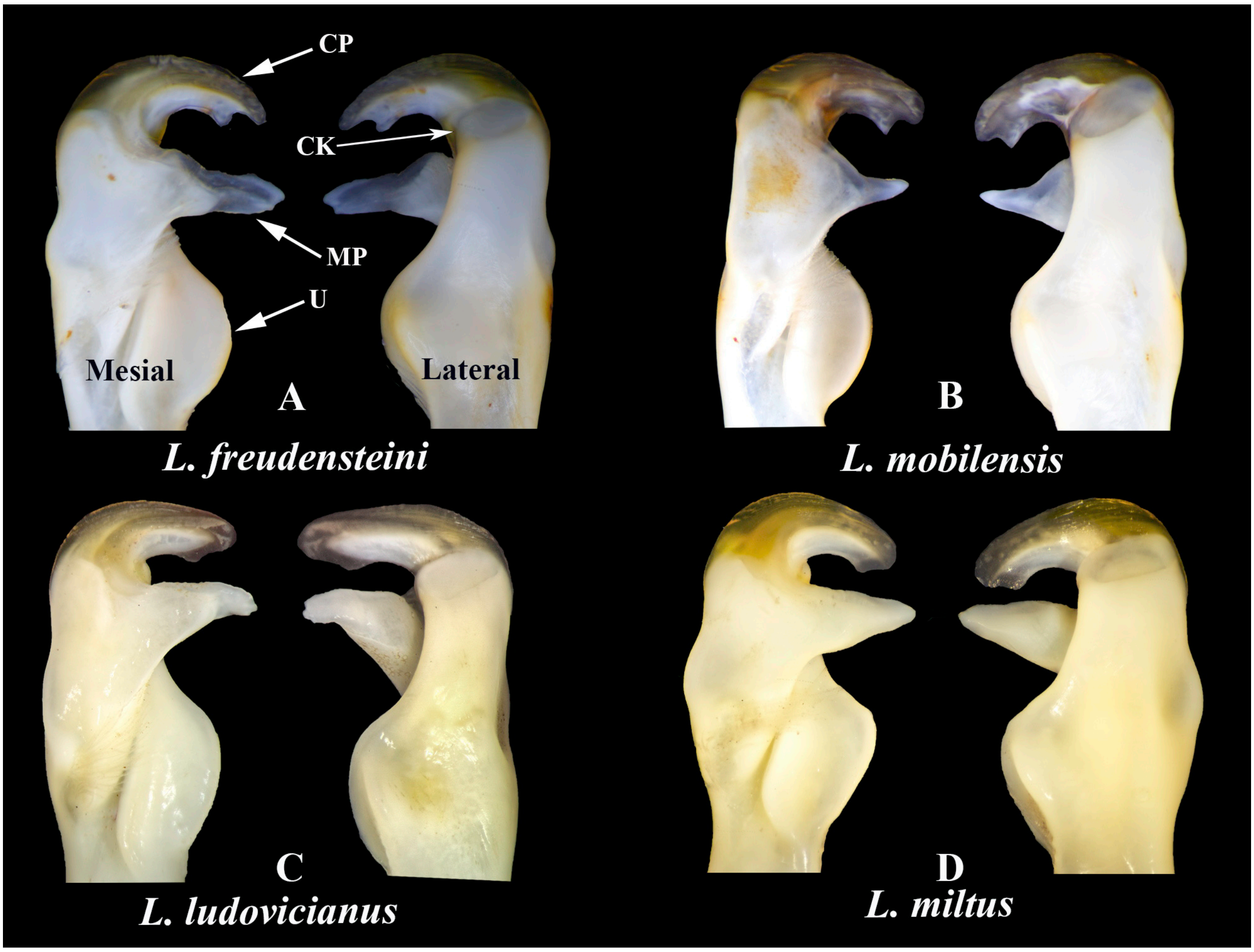

FIGURE 9. Mesial and lateral view of Form I gonopods of *Lacunicambarus freudensteini* **sp. nov.** (A), *L. mobilensis* **sp. nov.** (B), *L. ludovicianus* (C) and *L. miltus* (D). Abbreviations: CP, central projection; MP, mesial process; CK, caudal knob; U, umbo.

Table 6. Data (sorted by State and County) on examined specimens of *Lacunicambarus freudensteini* **sp. nov.** including catalogue number, State and County of collection, GPS coordinates in decimal degrees, collection date, number of specimens of each sex and Form, and whether sample included ovigerous females. Asterisk denotes type locality. Please note that some museum specimens were not associated with GPS coordinates so we estimated them based on provided locality information. Abbreviations: AL, Alabama; INHS, Illinois Natural History Survey; MS, Mississippi; OSUMC; Ohio State University Museum of Biological Diversity.

Catalogue #	State	County	Latitude (°N)	Longitude (°W)	Collection date	Form 1 males	Form 2 males	Females	Ovigerous?	Juveniles
INHS 14610	AL	Mobile	30.4161	88.2648	28-03-2006			1		
OSUMC 10875	AL	Mobile	30.4713	88.1691	8-01-2020	1				
OSUMC 10876	AL	Mobile	30.4702	88.1690	11-01-2020			4		1
OSUMC 10877	AL	Mobile	30.5078	88.3547	12-01-2020	1				
OSUMC 10878	MS	Jackson	30.6422	88.5621	8-01-2020			2		2
OSUMC 10879	MS	Jackson	30.4563	88.5227	9-01-2020	1		4	yes	1
OSUMC 10881*	MS	Jackson	30.4720	88.5179	13-01-2020	8	1	3	yes	1
OSUMC 10882	MS	Jackson	30.5254	88.5410	13-01-2020	2		5	yes	
OSUMC 10883	MS	Jackson	30.6561	88.5364	13-01-2020	2		1		1

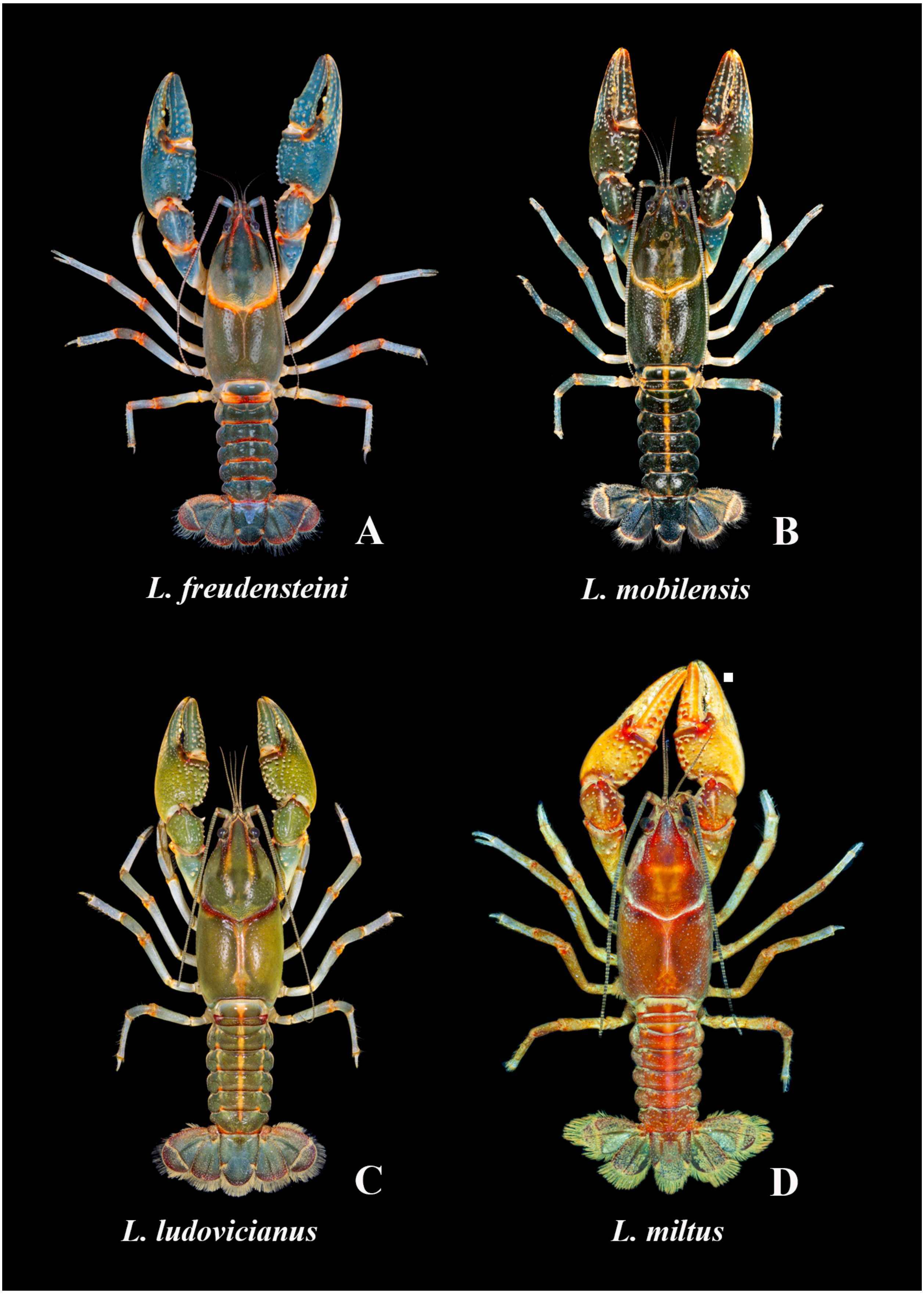

FIGURE 10. Dorsal views of *Lacunicambarus freudensteini* **sp. nov.** (A), *L. mobilensis* **sp. nov.** (B), *L. ludovicianus* (C) and *L. miltus* (D).

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https://doi.org/10.2988/0006-324X(2005)118[326:CTPDCA]2.0.CO;2

SUPPLEMENTAL FILE 1. We used the following measurements to collect data for our morphometric analyses. We used digital calipers and a dissecting scope to obtain all measurements, although an ocular micrometer can also be used. As a general rule, we recommend taking measurements at the widest/deepest point on a particular structure. We typically measure all structures, including the gonopods and antennal scales, without dissecting them from the specimen.

A. Carapace depth (hook calipers ventrally between coxa of pereiopods)
B. Gonopod length
C. Central projection length
D. Mesial process length
E. Umbo width
F. Carapace length
G. Rostrum length (hook calipers on tip of rostrum and measure diagonally to posteriormost point behind eye)
H. Rostrum width (hook calipers around rostrum and slide calipers to posteriormost point of rostral margins)
I. Cephalon length
J. Cephalon width
K. Areola length
L. Carapace width
M. Annulus ventralis width
N. Annulus ventralis length
O. Antennal scale width
P. Antennal scale length
Q. Dactyl length
R. Palm width
S. Palm length
T. Propodus length
U. Abdomen width
V. Abdomen length

Not shown: palm depth (position chela between calipers and measure at deepest point of palm).


A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T


U
V

SUPPLEMENTAL TABLE 1. Genbank accession numbers for new sequences of partial H3, 12S, 16S and CO1 generated since Glon *et al.* (2019a) used in the present manuscript's analyses, along with sample number in MGG's database, species name, and State and County of collection.

Sample #	Species	State	County	H3	12S	16S	CO1
cm164	*Lacunicambarus miltus*	Alabama	Baldwin	MT108744	NA	NA	NA
1	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Ohio	Auglaize	MT108747	NA	NA	NA
26	*Lacunicambarus diogenes*	North Carolina	Wake	MT108741	NA	NA	NA
31	*Lacunicambarus* aff. *thomai*	Tennessee	Cocke	MT108778	NA	NA	NA
101	*Lacunicambarus chimera*	Kentucky	Union	MT108762	NA	NA	NA
102	*Lacunicambarus dalyae*	Tennessee	Humphreys	MT108761	NA	NA	NA
113	*Lacunicambarus ludovicianus*	Tennessee	Carroll	MT108760	NA	NA	NA
132	*Lacunicambarus acanthura*	Georgia	Murray	MT108759	NA	NA	NA
134	*Lacunicambarus acanthura*	Tennessee	Bradley	MT108764	NA	NA	NA
141	*Lacunicambarus* aff. *thomai*	Tennessee	Bradley	MT108777	NA	NA	NA
153	*Lacunicambarus chimera*	Indiana	Pike	MT108768	NA	NA	NA
166	*Lacunicambarus polychromatus*	Indiana	Pike	MT108749	NA	NA	NA
169	*Lacunicambarus thomai*	Ohio	Perry	MT108779	NA	NA	NA
182	*Lacunicambarus thomai*	Kentucky	Boyd	MT108754	NA	NA	NA
204	*Lacunicambarus thomai*	West Virginia	Cabell	MT108752	NA	NA	NA
226	*Lacunicambarus ludovicianus*	Louisiana	Lafourche	MT108776	NA	NA	NA
231	*Lacunicambarus diogenes*	Pennsylvania	Bucks	MT108743	NA	NA	NA
337	*Lacunicambarus thomai*	Pennsylvania	Beaver	MT108751	NA	NA	NA
370	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Iowa	Lee	MT108758	NA	NA	NA
373	*Lacunicambarus miltus*	Florida	Washington	MT108745	NA	NA	NA
377	*Lacunicambarus mobilensis*	Alabama	Mobile	MT108757	NA	NA	NA
393	*Lacunicambarus mobilensis*	Mississippi	Jackson	MT108775	NA	NA	NA
396	*Lacunicambarus* cf. *polychromatus*	Illinois	Woodford	MT108767	NA	NA	NA
399	*Lacunicambarus mobilensis*	Alabama	Mobile	MT108753	NA	NA	NA
400	*Lacunicambarus mobilensis*	Alabama	Mobile	MT108772	NA	NA	NA
401	*Lacunicambarus mobilensis*	Alabama	Mobile	MT108756	NA	NA	NA

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SUPPLEMENTAL TABLE 1. (Continued)

Sample #	Species	State	County	H3	12S	16S	CO1
402	*Lacunicambarus erythrodactylus*	Alabama	Fayette	MT108746	NA	NA	NA
427	*Lacunicambarus diogenes*	Washington D.C.	2nd Ward	MT108740	NA	NA	NA
438	*Lacunicambarus* cf. *polychromatus*	Kentucky	Muhlenberg	MT108774	NA	NA	NA
446	*Lacunicambarus* cf. *polychromatus*	Kentucky	Crittenden	MT108755	NA	NA	NA
469	*Lacunicambarus* cf. *polychromatus*	Illinois	Massac	MT108763	NA	NA	NA
476	*Lacunicambarus* cf. *polychromatus*	Illinois	Massac	MT108750	NA	NA	NA
504	*Lacunicambarus erythrodactylus*	Illinois	Fayette	MT108765	NA	NA	NA
509	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Illinois	Champaign	NA	NA	NA	NA
511	*Lacunicambarus diogenes*	South Carolina	Florence	NA	NA	NA	NA
513	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Wisconsin	La Crosse	NA	NA	NA	NA
514	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Wisconsin	La Crosse	MT108742	NA	NA	NA
517	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Illinois	DuPage	NA	NA	NA	NA
518	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Illinois	DuPage	NA	NA	NA	NA
522	*Lacunicambarus erythrodactylus*	Alabama	Montgomery	NA	NA	NA	NA
523	*Lacunicambarus ludovicianus*	Alabama	Perry	NA	NA	NA	NA
524	*Lacunicambarus dalyae*	Alabama	Perry	NA	NA	NA	NA
525	*Lacunicambarus polychromatus*	Alabama	Colbert	NA	NA	NA	NA
528	*Lacunicambarus chimera*	Illinois	White	MT108773	NA	NA	NA
529	*Lacunicambarus chimera*	Illinois	White	NA	NA	NA	NA
531	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Indiana	Decatur	NA	NA	NA	NA
532	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Indiana	Blackford	NA	NA	NA	NA
533	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Indiana	Madison	NA	NA	NA	NA
534	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Indiana	Howard	NA	NA	NA	NA
536	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Indiana	Hendricks	NA	NA	NA	NA
541	*Lacunicambarus chimera*	Indiana	Dubois	NA	NA	NA	NA

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SUPPLEMENTAL TABLE 1. (Continued)

Sample #	Species	State	County	H3	12S	16S	CO1
548	*Lacunicambarus erythrodactylus*	Mississippi	Lafayette	MT108766	NA	NA	NA
551	*Lacunicambarus dalyae*	Mississippi	Lafayette	MT108771	NA	NA	NA
552	*Lacunicambarus ludovicianus*	Mississippi	Lafayette	MT108770	NA	NA	NA
554	*Lacunicambarus erythrodactylus*	Mississippi	Grenada	NA	NA	NA	NA
558	*Lacunicambarus ludovicianus*	Mississippi	George	NA	NA	NA	NA
559	*Lacunicambarus erythrodactylus*	Mississippi	Webster	NA	NA	NA	NA
560	*Lacunicambarus erythrodactylus*	Mississippi	Tallahatchie	NA	NA	NA	NA
561	*Lacunicambarus ludovicianus*	Mississippi	Chickasaw	NA	NA	NA	NA
562	*Lacunicambarus ludovicianus*	Mississippi	Chickasaw	NA	NA	NA	NA
563	*Lacunicambarus erythrodactylus*	Mississippi	Tallahatchie	NA	NA	NA	NA
564	*Lacunicambarus ludovicianus*	Mississippi	Monroe	NA	NA	NA	NA
565	*Lacunicambarus erythrodactylus*	Mississippi	Leake	NA	NA	NA	NA
566	*Lacunicambarus erythrodactylus*	Mississippi	Choctaw	NA	NA	NA	NA
567	*Lacunicambarus erythrodactylus*	Mississippi	Holmes	NA	NA	NA	NA
568	*Lacunicambarus erythrodactylus*	Mississippi	Leake	NA	NA	NA	NA
569	*Lacunicambarus erythrodactylus*	Mississippi	Webster	NA	NA	NA	NA
570	*Lacunicambarus erythrodactylus*	Mississippi	Carroll	NA	NA	NA	NA
571	*Lacunicambarus erythrodactylus*	Mississippi	Carroll	NA	NA	NA	NA
573	*Lacunicambarus erythrodactylus*	Alabama	Montgomery	NA	NA	NA	NA
574	*Lacunicambarus dalyae*	Georgia	Bibb	MT108748	NA	NA	NA
575	*Lacunicambarus dalyae*	Alabama	Russell	NA	NA	NA	NA
577	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Michigan	Kalamazoo	NA	NA	NA	NA
578	*Lacunicambarus polychromatus*	Michigan	Oakland	MT108769	NA	NA	NA
579	*Lacunicambarus polychromatus*	Michigan	Ingham	NA	NA	NA	NA
581	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Michigan	Kalamazoo	NA	NA	NA	NA
582	*Lacunicambarus polychromatus*	Michigan	Monroe	NA	NA	NA	NA

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SUPPLEMENTAL TABLE 1. (Continued)

Sample #	Species	State	County	H3	12S	16S	CO1
584	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Michigan	Mackinac	NA	NA	NA	NA
585	*Lacunicambarus polychromatus*	Michigan	Monroe	NA	NA	NA	NA
586	*Lacunicambarus polychromatus*	Michigan	Monroe	NA	NA	NA	NA
588	*Lacunicambarus polychromatus*	Michigan	Washtenaw	NA	NA	NA	NA
590	*Lacunicambarus polychromatus*	Michigan	Wayne	NA	NA	NA	NA
591	*Lacunicambarus polychromatus*	Michigan	Wayne	NA	NA	NA	NA
592	*Lacunicambarus polychromatus*	Michigan	Calhoun	NA	NA	NA	NA
594	*Lacunicambarus erythrodactylus*	Florida	Jackson	NA	NA	NA	NA
595	*Lacunicambarus dalyae*	Florida	Jackson	NA	NA	NA	NA
596	*Lacunicambarus miltus*	Florida	Washington	NA	NA	NA	NA
597	*Lacunicambarus miltus*	Florida	Washington	NA	NA	NA	NA
598	*Lacunicambarus erythrodactylus*	Alabama	Baldwin	NA	NA	NA	NA
600	*Lacunicambarus dalyae*	Alabama	Clarke	NA	NA	NA	NA
601	*Lacunicambarus miltus*	Alabama	Baldwin	NA	NA	NA	NA
602	*Lacunicambarus miltus*	Alabama	Baldwin	NA	NA	NA	NA
605	*Lacunicambarus erythrodactylus*	Mississippi	Panola	NA	NA	NA	NA
618	*Lacunicambarus erythrodactylus*	Mississippi	Yalobusha	NA	NA	NA	NA
619	*Lacunicambarus erythrodactylus*	Mississippi	Yalobusha	NA	NA	NA	NA
626	*Lacunicambarus erythrodactylus*	Mississippi	Tishomingo	NA	NA	NA	NA
631	*Procambarus gracilis*	Illinois	Champaign	MT108739	NA	NA	NA
632	*Lacunicambarus erythrodactylus*	Tennessee	McNairy	NA	MT183072	MT182988	MT183524
639	*Lacunicambarus acanthura*	Georgia	Floyd	NA	MT183073	MT182989	MT183525
640	*Lacunicambarus acanthura*	Georgia	Bartow	NA	MT183074	MT182990	MT183526
641	*Lacunicambarus miltus*	Alabama	Baldwin	NA	MT183075	MT182991	MT183527
642	*Lacunicambarus acanthura*	Alabama	Chilton	NA	MT183076	MT182992	MT183528
643	*Lacunicambarus erythrodactylus*	Alabama	Montgomery	NA	MT183077	MT182993	MT183529
644	*Lacunicambarus miltus*	Florida	Santa Rosa	NA	MT183078	MT182994	MT183530
645	*Lacunicambarus miltus*	Florida	Santa Rosa	NA	MT183079	MT182995	MT183531

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SUPPLEMENTAL TABLE 1. (Continued)

Sample #	Species	State	County	H3	12S	16S	CO1
646	*Lacunicambarus miltus*	Florida	Okaloosa	NA	NA	MT182996	MT183532
647	*Lacunicambarus erythrodactylus*	Florida	Santa Rosa	NA	MT183080	MT182997	MT183533
648	*Lacunicambarus dalyae*	Florida	Holmes	NA	MT183081	MT182998	MT183534
649	*Lacunicambarus miltus*	Florida	Santa Rosa	NA	MT183082	MT182999	MT183535
650	*Lacunicambarus miltus*	Florida	Santa Rosa	NA	MT183083	MT183000	MT183536
651	*Lacunicambarus erythrodactylus*	Florida	Jackson	NA	MT183084	MT183001	MT183537
652	*Lacunicambarus dalyae*	Florida	Jackson	NA	MT183085	MT183002	MT183538
653	*Lacunicambarus dalyae*	Florida	Walton	NA	MT183086	MT183003	MT183539
655	*Lacunicambarus* cf. *thomai*	Tennessee	Scott	NA	MT183087	MT183004	MT183540
656	*Lacunicambarus dalyae*	Tennessee	Humphreys	NA	MT183088	MT183005	MT183541
657	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Arkansas	Lawrence	NA	MT183089	MT183006	MT183542
658	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Arkansas	Lawrence	NA	MT183090	MT183007	MT183543
660	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Missouri	Pemiscot	NA	MT183091	MT183008	MT183544
664	*Lacunicambarus dalyae*	Tennessee	Humphreys	NA	MT183092	MT183009	MT183545
665	*Lacunicambarus dalyae*	Tennessee	Humphreys	NA	MT183093	MT183010	MT183546
669	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Nebraska	Knox	NA	MT183094	MT183011	MT183547
670	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Nebraska	Knox	NA	MT183095	MT183012	MT183548
671	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Nebraska	Keya Paha	NA	MT183096	MT183013	MT183549
672	*Lacunicambarus* aff. *diogenes "nebrascensis"*	South Dakota	Tripp	NA	MT183097	MT183014	MT183550
673	*Lacunicambarus* aff. *diogenes "nebrascensis"*	South Dakota	Tripp	NA	MT183098	MT183015	MT183551
674	*Lacunicambarus* aff. *diogenes "nebrascensis"*	South Dakota	Tripp	NA	MT183099	MT183016	MT183552
675	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Nebraska	Cherry	NA	MT183100	MT183017	MT183553
677	*Lacunicambarus* aff. *diogenes "nebrascensis"*	Nebraska	Cherry	NA	MT183101	MT183018	MT183554
678	*Lacunicambarus* aff. *diogenes "nebrascensis"*	South Dakota	Todd	NA	NA	MT183019	MT183555
680	*Lacunicambarus* aff. *diogenes "nebrascensis"*	South Dakota	Todd	NA	NA	MT183020	MT183556
682	*Lacunicambarus erythrodactylus*	Louisiana	West Feliciana Parish	NA	MT183102	MT183021	MT183557
685	*Lacunicambarus erythrodactylus*	Florida	Liberty	NA	MT183103	MT183022	MT183558

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SUPPLEMENTAL TABLE 1. (Continued)

Sample #	Species	State	County	H3	12S	16S	CO1
686	*Lacunicambarus erythrodactylus*	Florida	Calhoun	NA	MT183104	MT183023	MT183559
687	*Lacunicambarus dalyae*	Florida	Calhoun	NA	MT183105	MT183024	MT183560
688	*Lacunicambarus erythrodactylus*	Florida	Santa Rosa	NA	MT183106	MT183025	MT183561
689	*Lacunicambarus aff. diogenes "nebrascensis"*	South Dakota	Todd	NA	NA	MT183026	MT183562
692	*Lacunicambarus miltus*	Alabama	Escambia	NA	MT183107	MT183027	MT183563
693	*Lacunicambarus miltus*	Alabama	Escambia	NA	MT183108	MT183028	MT183564
694	*Lacunicambarus miltus*	Alabama	Baldwin	NA	MT183109	NA	MT183565
695	*Lacunicambarus miltus*	Alabama	Baldwin	NA	MT183110	MT183029	MT183566
696	*Lacunicambarus miltus*	Alabama	Baldwin	NA	MT183111	NA	MT183567
697	*Lacunicambarus miltus*	Alabama	Baldwin	NA	MT183112	NA	MT183568
698	*Lacunicambarus miltus*	Alabama	Baldwin	NA	MT183113	MT183030	MT183569
699	*Lacunicambarus miltus*	Alabama	Baldwin	NA	MT183114	MT183031	MT183570
701	*Lacunicambarus miltus*	Alabama	Baldwin	NA	MT183115	MT183032	MT183571
702	*Lacunicambarus miltus*	Alabama	Baldwin	NA	MT183116	MT183033	MT183572
708	*Lacunicambarus mobilensis*	Alabama	Mobile	NA	MT183117	MT183034	MT183573
709	*Lacunicambarus mobilensis*	Mississippi	Jackson	NA	MT183118	MT183035	MT183574
710	*Lacunicambarus mobilensis*	Mississippi	Jackson	NA	MT183119	MT183036	MT183575
717	*Lacunicambarus freudensteini*	Mississippi	Jackson	NA	MT183120	MT183037	MT183576
718	*Lacunicambarus freudensteini*	Mississippi	Jackson	NA	MT183121	MT183038	MT183577
720	*Lacunicambarus mobilensis*	Alabama	Mobile	NA	MT183122	MT183039	MT183578
721	*Lacunicambarus mobilensis*	Alabama	Mobile	NA	MT183123	MT183040	MT183579
725	*Lacunicambarus freudensteini*	Mississippi	Jackson	NA	MT183124	MT183041	MT183580
726	*Lacunicambarus freudensteini*	Mississippi	Jackson	NA	MT183125	MT183042	MT183581
730	*Lacunicambarus erythrodactylus*	Mississippi	Jackson	NA	MT183126	MT183043	MT183582
734	*Lacunicambarus mobilensis*	Mississippi	George	NA	MT183127	MT183044	MT183583
735	*Lacunicambarus mobilensis*	Mississippi	George	NA	MT183128	MT183045	MT183584

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SUPPLEMENTAL TABLE 1. (Continued)

Sample #	Species	State	County	H3	12S	16S	CO1
736	*Lacunicambarus mobilensis*	Mississippi	George	NA	MT183129	MT183046	MT183585
737	*Lacunicambarus mobilensis*	Mississippi	George	NA	MT183130	MT183047	MT183586
738	*Lacunicambarus ludovicianus*	Mississippi	Perry	NA	MT183131	MT183048	MT183587
739	*Lacunicambarus ludovicianus*	Mississippi	Perry	NA	MT183132	MT183049	MT183588
741	*Lacunicambarus mobilensis*	Alabama	Mobile	NA	MT183133	MT183050	MT183589
742	*Lacunicambarus mobilensis*	Alabama	Mobile	NA	MT183134	MT183051	MT183590
743	*Lacunicambarus freudensteini*	Alabama	Mobile	NA	MT183135	MT183052	MT183591
744	*Lacunicambarus freudensteini*	Alabama	Mobile	NA	MT183136	MT183053	MT183592
745	*Lacunicambarus mobilensis*	Alabama	Mobile	NA	MT183137	MT183054	MT183593
746	*Lacunicambarus mobilensis*	Alabama	Mobile	NA	MT183138	MT183055	MT183594
749	*Lacunicambarus mobilensis*	Alabama	Mobile	NA	MT183139	MT183056	MT183595
750	*Lacunicambarus mobilensis*	Alabama	Mobile	NA	MT183140	MT183057	MT183596
751	*Lacunicambarus freudensteini*	Alabama	Mobile	NA	MT183141	MT183058	MT183597
752	*Lacunicambarus freudensteini*	Mississippi	Jackson	NA	MT183142	MT183059	MT183598
753	*Lacunicambarus freudensteini*	Mississippi	Jackson	NA	MT183143	MT183060	MT183599
756	*Lacunicambarus freudensteini*	Mississippi	Jackson	NA	MT183144	MT183061	MT183600
758	*Lacunicambarus freudensteini*	Mississippi	Jackson	NA	MT183145	MT183062	MT183601
759	*Lacunicambarus freudensteini*	Mississippi	Jackson	NA	MT183146	MT183063	MT183602
760	*Lacunicambarus freudensteini*	Mississippi	Jackson	NA	MT183147	MT183064	MT183603
761	*Lacunicambarus mobilensis*	Alabama	Mobile	NA	MT183148	MT183065	MT183604
762	*Lacunicambarus mobilensis*	Alabama	Mobile	NA	MT183149	MT183066	MT183605
763	*Lacunicambarus mobilensis*	Mississippi	Jackson	NA	MT183150	MT183067	MT183606
764	*Lacunicambarus mobilensis*	Mississippi	Jackson	NA	MT183151	MT183068	MT183607
765	*Lacunicambarus erythrodactylus*	Mississippi	Jackson	NA	MT183152	MT183069	MT183608
767	*Lacunicambarus freudensteini*	Alabama	Mobile	NA	MT183153	MT183070	MT183609