

TAXONOMIC REVISION OF CAVE CRAYFISH IN THE GENUS *CAMBARUS*,  
SUBGENUS *AVITICAMBARUS* (DECAPODA: CAMBARIDAE) WITH  
DESCRIPTIONS OF TWO NEW SPECIES, *C. SPELEOCOOP*I AND  
*C. LACONENSIS*, ENDEMIC TO ALABAMA, U.S.A.

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A B S T R A C T

As historically recognized, three stygobitic species of the subgenus *Aviticambarus*, genus *Cambarus* inhabit Mississippian limestone caves along the southern edge of the Southern Appalachians and Highland Rim in southeastern Tennessee and Northern Alabama, U.S.A. These include *Cambarus hamulatus*, *C. jonesi*, and *C. veitchorum*. All stygobitic members of the genus *Procambarus* inhabit caves in Florida, Cuba, and Mexico with exception of *P. pecki* (in the monotypic subgenus *Remoticambarus*), which exists in only three caves with *C. jonesi* in Northwestern Alabama. It was hypothesized that *Procambarus pecki* was derived from a primitive *Procambarus* stock that gave rise to the genera *Cambarus* and *Orconectes* based on the morphological shapes of the gonopods. Excluding the unsampled rare *C. veitchorum*, here we present 16S rDNA phylogenetic evidence, contrary to former morphological-based inferences, for the recognition of five distinct *Aviticambarus* lineages including *P. pecki*. *Cambarus laconensis* is a new species restricted to one locality in Northern Alabama along the southern border of the Highland Rim. *Cambarus speleocoopi* is also a new species of subterranean crayfish restricted to Marshall County, Alabama. These two cryptic species, with distributions that do not overlap any other stygobitic species, were discovered during a previous phylogeographic survey of cave crayfishes in the Southern Appalachians. For cave crayfishes in particular, similar morphology owing to convergent evolution in replicate subterranean environments, obscures phylogenetic relationships and cryptic stygobitic lineages.

KEY WORDS: *Cambarus*, cave biotas, conservation, crayfish, genetics, phylogenetics

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INTRODUCTION

Within the freshwater crayfish genus *Cambarus*, there are four subgenera that contain stygobitic species: *Aviticambarus* (Hobbs, 1969) contains *C. hamulatus* (Cope, 1881), *C. jonesi* (Hobbs and Barr, 1960), and *C. veitchorum* (Cooper and Cooper, 1997a); *Erebicambarus* (Hobbs, 1969) contains *C. hubrichti* (Hobbs, 1952); *Jugicambarus* (Hobbs, 1969) includes *C. aculabrum* (Hobbs and Brown, 1987), *C. subterraneus* (Hobbs III, 1993), *C. setosus* (Faxon, 1889), *C. tartarus* (Hobbs and Cooper, 1972), *C. cryptodytes* (Hobbs, 1941), and *C. zophonastes* (Hobbs and Bedinger, 1964); and *Puncticambarus* (Hobbs, 1969) includes *C. nerterius* (Hobbs, 1964). With exception of *C. nerterius*, which retains body and eye pigment, all other stygobitic members exhibit reduced visual systems and loss of body pigment compared to surface-dwelling members of *Cambarus*.

In the southern Appalachians of the southeastern United States, the endemic subgenus *Aviticambarus* of the genus *Cambarus* inhabits caves at the southern edge of the mountain range extending westward across the southern edge of the Highland Rim (see Fig. 1 for geographic features relative to species distributions). All other stygobitic species within *Cambarus* inhabit caves around the Ozark Plateau, the Greenbrier region of West Virginia, and the Dougherty Plain of Southern Georgia and the Florida panhandle

(Hobbs and Barr, 1960). There are also five stygobitic crayfish species of the genus *Orconectes* (subgenus *Orconectes* Cope, 1872) and a member of the genus *Procambarus* (monotypic subgenus *Remoticambarus* Hobbs, 1972), which inhabit the Southern Appalachians (Hobbs et al., 1977; Cooper and Cooper, 1997a, b; Buhay and Crandall, 2008). Of these, two *Orconectes* species (*O. australis* and *O. sheltae*) along with *Procambarus pecki* (Hobbs, 1967) co-exist in some Northern Alabama caves with one *Aviticambarus* species, *Cambarus jonesi*. The Southern Appalachians are a global hotspot of stygobitic biodiversity (Peck, 1998; Culver et al., 2000) and the blind white cave crayfishes are the largest macro-invertebrates in subterranean communities.

As historically recorded (Hobbs et al., 1977), *Cambarus hamulatus* inhabits subterranean waters spanning the Sequatchie Valley and westward into the Jackson County Mountains of Alabama. The known distribution of *C. hamulatus* does not overlap with any other stygobitic species and includes 40 caves reported in Tennessee and Alabama (Buhay et al., 2007). *C. veitchorum* is endemic to White Spring Cave in Limestone County, Alabama. *C. veitchorum* is the rarest and smallest of the *Aviticambarus* assemblage, having not been seen during searches of White Spring Cave since 1968, and only seven individuals have

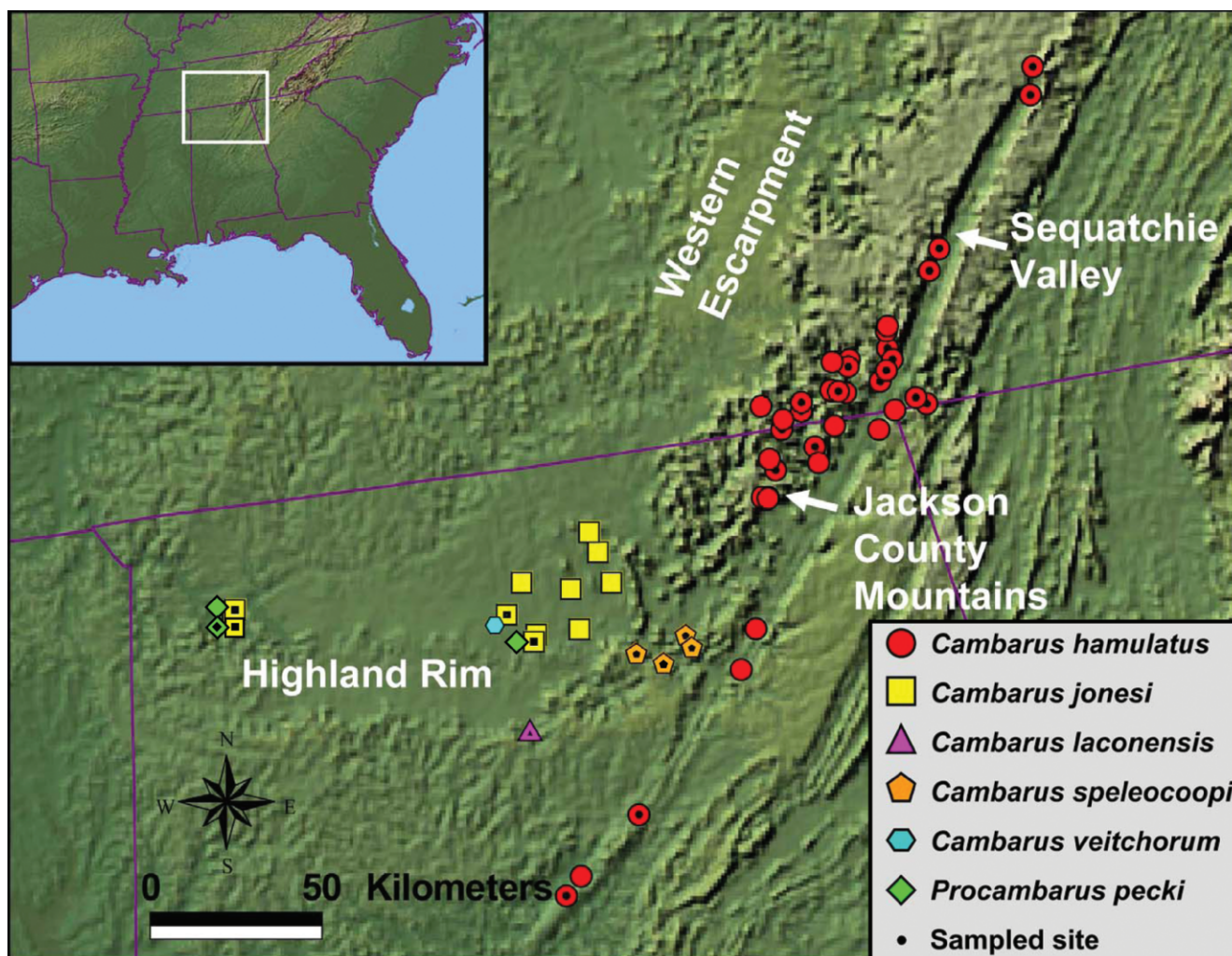


Fig. 1. Distributions of *Cambarus hamulatus* (red circles), *C. jonesi* (yellow squares), *C. speleocoopi* (orange pentagons), *C. laconensis* (purple triangle), *C. veitchorum* (blue hexagon), and *Procambarus pecki* (green diamonds) along the Cumberland Plateau and Highland Rim in the southeastern United States (adapted from Buhay et al., 2007).

ever been recorded (Cooper and Cooper, 1997a). *C. jonesi* occurs on both sides of the Tennessee River in Northern Alabama extending from Marshall County to Colbert County. In addition to co-existing with *C. veitchorum* at White Spring Cave, *C. jonesi* is also found in Madison County caves with *Orconectes australis* and *Orconectes sheltae* (Cooper and Cooper, 1997b), and in all three caves containing *Procambarus pecki* (Hobbs et al., 1977).

During our previous phylogeographic and phylogenetic study of *Aviticambarus* (Buhay et al., 2007), we concluded that a newly discovered disjunct population of cave crayfish actually represented a new *Aviticambarus* species which we labeled ‘*C. sp. nov 1*’ (herein described as *Cambarus laconensis*) using genetic sequence data from five genes (2686 base pairs from three mitochondrial: 12S, 16S, and COI; two nuclear: Histone H3 and GADPH). We also determined that a single population of *C. jonesi* along with some newly discovered neighboring populations all in Marshall County, Alabama actually represented a distinct cryptic evolutionary lineage (which we labeled ‘*C. sp. nov 2*’; herein described as *Cambarus speleocoopi*). These two

new species do not overlap in range with any other stygobitic crayfish species.

Following the Buhay et al. (2007) study, we collected a sample of *Procambarus pecki* from the type locale (McKinney Pit, Colbert County, Alabama) as part of an ongoing systematic revision for crayfishes (Sinclair et al., 2004). As currently recognized (Hobbs, 1972), the freshwater crayfish genus *Procambarus* includes stygobitic species in the subgenera *Austrocambarus*, *Leonticambarus*, *Lonnbergius*, and *Ortmannicus* all occurring in Mexico, Cuba, and Florida with exception of *P. pecki* in the monotypic subgenus *Remoticambarus* which occurs in three Northwestern Alabama caves along the southern Highland Rim (Fig. 1). Hobbs et al. (1977) hypothesized that *P. pecki* represents the “primitive stock” of the genus *Procambarus*, which was present “in and along the margin of the Cumberland Plateau during the Miocene.” It has also been hypothesized that the cave-adapted members of the genus *Orconectes* are descendants of this “primitive stock” of *Procambarus*, which included *P. pecki* (Hobbs and Barr, 1972). In fact, Hobbs (1967) claimed that *P. pecki* seemed

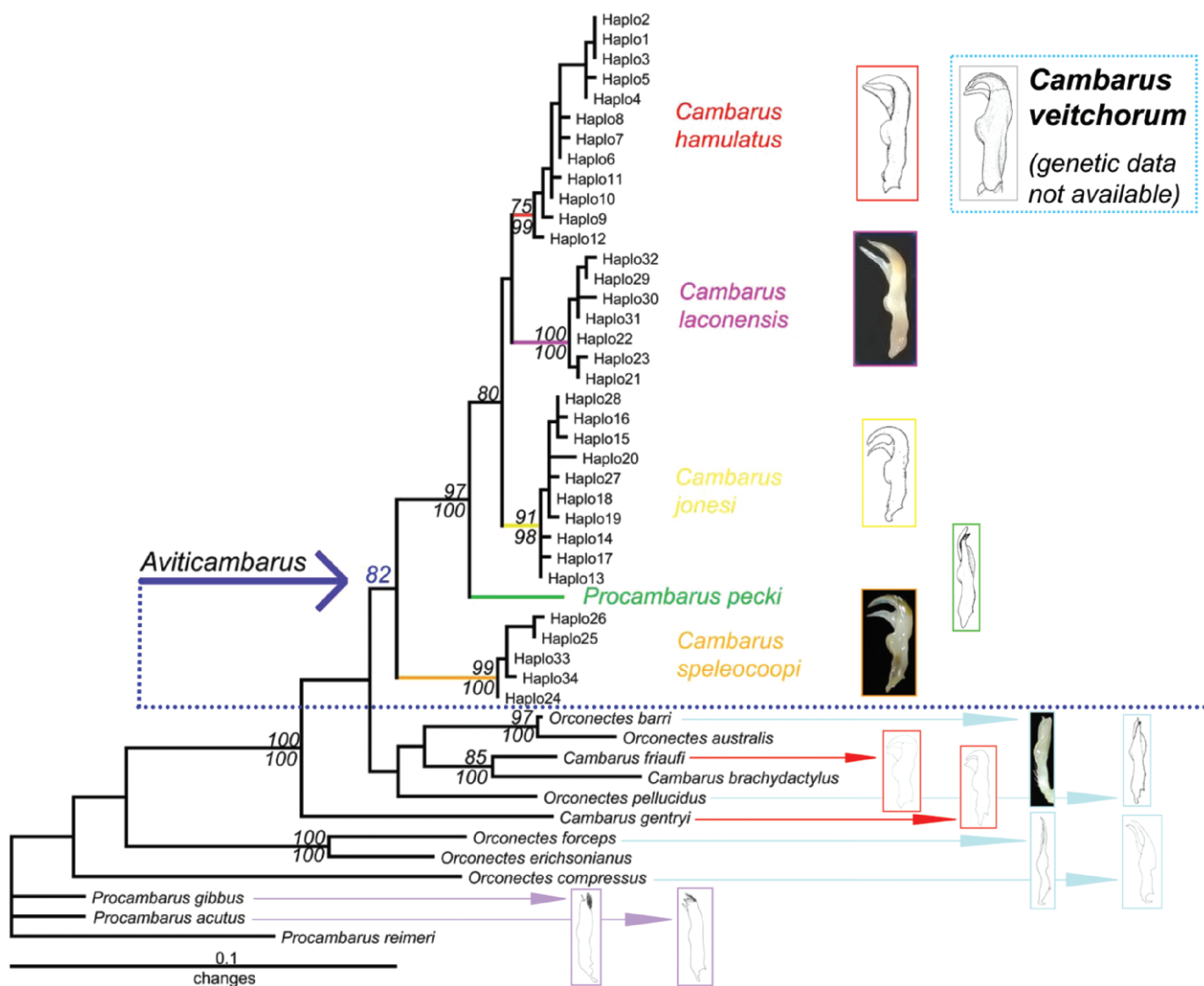


Fig. 2. Phylogenetic relationships using 35 unique sequences of the 16S mtDNA gene from *Aviticambarus* species and 12 out-group sequences. Analyses were performed with the maximum likelihood approach in PhyML (Guindon and Gascuel, 2003) and the Bayesian approach in MrBayes (Huelsenbeck and Ronquist, 2001). *Procambarus reimeri* was used to root the tree. ML topology shown. Numbers above branches indicate significant bootstrap support and numbers below branches indicate significant posterior probabilities (Hillis and Bull, 1993; Ronquist and Huelsenbeck, 2003). Lateral views of form I male pleopods are shown for shape comparisons across the genera *Procambarus*, *Cambarus* (including *Aviticambarus*), and *Orconectes*.

“equally closely related” to the troglitic members of the genus *Orconectes* and *Procambarus*, and there were good reasons “for assigning it to either of the two” genera. In contrast, though geographically overlapping, species of *Cambarus* in the subgenus *Aviticambarus* were suggested by Hobbs et al. (1977) to descend from stream-dwelling *Cambarus* ranging across the southern Cumberland Plateau.

As currently recognized, each crayfish genus is defined by distinctive differences among the shapes of their pleopods (Hobbs, 1976), but as several studies have shown, the hypothesized plesiomorphic qualities of the gonopods of obligate cave species are indeed confounding and do not accurately reflect evolutionary relationships (Fetzner, 1996; Crandall and Fitzpatrick, 1996; Sinclair et al., 2004; Buhay and Crandall, 2005; Buhay and Crandall, 2008). For example, the stygobitic members of the genus *Orconectes* are most closely related to burrowing members of the genus

*Cambarus* determined by genetic analyses, rather than their hypothesized morphologically similar ancestral relationships to surface stream-dwelling species of *Orconectes* (Fetzner, 1996; Crandall and Fitzpatrick, 1996; Sinclair et al., 2004; Buhay and Crandall, 2005; Buhay and Crandall, 2008). It is therefore imperative to employ molecular taxonomic approaches for phylogenetic study of cave-dwelling taxa not only to reveal cryptic species (Proudlove and Wood, 2003), but also to uncover incidents of convergent evolution (as in cave *Orconectes* species pleopods resembling “primitive” stream-dwelling *Orconectes* species pleopods rather than highly reduced *Cambarus* pleopods - see Fig. 2 for pleopod shapes). These same patterns of confounding morphological features, seemingly evolutionary reversals (Porter and Crandall, 2003), in systematic and phylogenetic studies of cave animals are becoming more apparent now that modern molecular studies test evolutionary relationships with neutral genetic charac-

ters rather than solely morphological characters influenced by the nature of inhabiting extreme subterranean biomes (salamander: Weins et al., 2003; amphipod: Lefebure et al., 2006; snail: Bichain et al., 2007; spider: Paquin and Hedin, 2004; catfish: Wilcox et al., 2004; cavefish: Protas et al., 2006; shrimp: Page et al., 2008).

The need for thorough molecular phylogeographic examination of cave taxa is becoming increasingly more common and more important for uncovering cryptic species and determining close surface relatives (Porter, 2007). As in the case of cave shrimp (Page et al., 2008), the closest ancestors were found not to be the hypothesized similar morphological relatives from "further afield," but rather "localised surface species," which contradicted traditional systematic classifications. Furthermore, it is not appropriate to combine morphological analyses with genetic data in cases of possible convergence because it is not possible to tease out the confounding influence of the cave environment from the morphological patterns (Wiens et al., 2003). The combination of various types of data (morphological, genetic, geographic, behavioral, etc.) is often suggested for robust diagnoses of species (Sites and Marshall, 2003), but for cryptic and convergent species in cases like crayfish, molecular genetic data paired with geographic information can robustly determine evolutionary units, species' boundaries, and identity (Buhay and Crandall, 2005; Finlay et al., 2006; Buhay et al., 2007; Buhay and Crandall, 2008; Crandall et al., 2008).

As an extension to the previous study of *Aviticambarus* (Buhay et al., 2007), we formally revise the subgenus *Aviticambarus* to include the descriptions of two new species, *Cambarus laconensis* and *Cambarus speleocoopi*. Because of the disjunct nature of *Procambarus pecki* (both geographically and morphologically) from all other stygobitic species of *Procambarus* and its co-occurrence with *Cambarus jonesi*, we examine the phylogenetic position of the species with respect to the *Aviticambarus* assemblage. Finally, we provide a summary of morphological differences between stygobitic species co-occurring in Northern Alabama caves.

## MATERIALS AND METHODS

### Genetic Data Collection and Analyses

Tissue samples were obtained non-destructively by sampling a claw or leg, which can be regenerated in individual crayfish, that were then placed back into the cave stream at the point of capture. Some whole voucher specimens were taken for morphological examination and museum deposition. Tissues and voucher specimens were immediately placed in 95% ethanol and given unique identification numbers (JC numbers for claw, leg, and tail/gill tissues from preserved whole specimens and Monte L. Bean Museum Crustacean Collection at Brigham Young University (BYUC) collection numbers for whole specimens). After tail or gill tissue was removed from vouchers, the specimens were then placed in 70% ethanol for museum storage. Buhay et al. (2007) included sequence data from multiple genes from one individual of *Aviticambarus* representing every cave ( $n=27$ ) in addition to 16S data from every individual *Aviticambarus* ( $n=130$ ) for the phylogeographic analysis. We also previously analyzed each mtDNA gene separately (12S, 16S, and COI), the combined mtDNA genes (12S + 16S + COI), and the mtDNA genes with the nuclear genes dataset (12S + 16S + COI + Histone 3 + GADPH) for which we recovered similar topologies. Therefore, for this present study, we gathered data from only the most variable gene (16S) from new individuals sampled during trips to collect voucher specimens for the descriptions and the newly acquired *Procambarus pecki* individual from the type locality for investigation of the ancestral

hypothesis of cave species along the Cumberland Plateau. Sequence data were gathered, edited, and aligned by eye using BioEdit (Hall, 1999). For purposes of rooting the phylogenetic tree, we included three stygobitic members of *Orconectes*, three surface stream members of *Orconectes*, three surface stream and burrowing members of *Cambarus*, and three surface stream and burrow members of *Procambarus* (Table 1).

Phylogenetic analysis of the 16S dataset was run in MrBayes 3.04b (Ronquist and Huelsenbeck, 2003) and PhyML (Guindon and Gascuel, 2003; <http://atgc.lirmm.fr/phyml/>). Parameters were estimated in ModelTest (Posada and Crandall, 1998). PhyML was run with number of substitution types (nst) = 2 and model = HKY with 100 bootstrap replicates. The Bayesian analysis was run with nst = 2 and rates = invgamma for ten million generations over ten chains (nine heated, one cold) with 1/1000 trees sampled. Tracer (Rambaut and Drummond, 2007; <http://beast.bio.ed.ac.uk/Tracer>) was used to determine the burn-in, which was then discarded. Bootstrap support (BS) 70% and higher and Bayesian posterior probability (PP) 95% and higher are considered to be significant support for a clade (Felsenstein, 1985; Hillis and Bull, 1993; Huelsenbeck and Ronquist, 2001).

For purposes of delineating species' boundaries, we employed the lineage-based "Genealogical Concordance Species Concept," whereby a "genealogical species," is a group of organisms more closely related to each other ("exclusivity") than to organisms outside its group (Baum and Shaw, 1995).

### Morphological Data Collection and Analyses

Measurements (mm) of type specimens of the new species of *Aviticambarus* were taken using digital calipers (Fowler Sylvac, Model Number 54-100-444; Newton, Massachusetts, U.S.A.) to the nearest hundredth for the 1) carapace: height, width, total length, and postorbital length; 2) rostrum: length and width; 3) areola: length and width; 4) Pleon: length and width; 5) antennal scale: length and width; and 6) cheliped: length of lateral margin, length of mesial margin, width of palm, depth of palm, and length of dactyl.

In addition to the type specimens, we also took these same measurements for additional crayfish in the collection to provide an idea of the population variation in morphology. Freshwater crayfish are sexually dimorphic (Stein, 1976) and furthermore, the males have cyclically dimorphic modified first pleopods (gonopods) once mature. Thus, we measured individuals in the following three classes of crayfish, male form I (reproductively active), male form II (not reproductively active), and female. Juveniles were excluded from measurements. Digital photographs of type specimens were taken using a Nikon D70 with AF Micro Nikkor 60 mm lens.

## RESULTS

### Phylogenetic Evidence for Species Diagnosis

The phylogenetic relationships of the cave assemblage of *Aviticambarus* were previously determined using mitochondrial sequence data from the 16S, COI, and 12S genes and nuclear genes Histone H3 and GADPH totaling 2686 base pairs (Buhay et al., 2007), but we did not include *Procambarus pecki* in those analyses because the sample was captured on a collecting trip after publication (despite numerous previous attempts). With inclusion of *P. pecki* using just the most variable gene (mtDNA 16S), in this study, *Cambarus speleocoopi* is still resolved as the most basal member of *Aviticambarus* with 82% BS support at the node (Fig. 2). *Procambarus pecki* falls out as a sister to the rest of the species of *Aviticambarus* with 97% BS/100% PP support at the node. The relationships among *C. hamulatus*, *C. laconensis*, and *C. jonesi* were unresolved, but each species formed a well-supported monophyletic group and together this clade of three species forms a sister-group relationship to *P. pecki*.

Each species of *Aviticambarus* was significantly supported as monophyletic and each is designated as a separate species as determined by this study and supported by the previous phylogeographic analysis from Buhay et al.

Table 1. Specimens with mtDNA 16s sequence data added to Buhay et al. 2007 (which included GenBank accessions of Haplotypes 1 - 26).

Species	Specimen no.	State	County	Locality	Haplotype no.	GenBank no.
<i>Procambarus pecki</i>	JC3819	AL	Colbert	McKinney Pit (type)	n/a	EU433911
<i>Cambarus jonesi</i>	JC3818	AL	Colbert	McKinney Pit	19	DQ411752
<i>Cambarus jonesi</i>	JC3783, JC3784, JC3785, JC3788, JC3789, JC3793	AL	Limestone	White Spring Cave	13	DQ411746
<i>Cambarus jonesi</i>	JC3791, JC3794	AL	Limestone	White Spring Cave	18	DQ411751
<i>Cambarus jonesi</i>	JC3786, JC3790, JC3792	AL	Limestone	White Spring Cave	27	EU433903
<i>Cambarus jonesi</i>	JC3795	AL	Limestone	White Spring Cave	28	EU433904
<i>Cambarus laconensis</i>	JC3802, JC3803, JC3804, JC3806, JC3808, JC3810, JC3811, JC3814, JC3815, JC3816, JC3817	AL	Morgan	Lacon Exit Cave (type)	29	EU433905
<i>Cambarus laconensis</i>	JC3809	AL	Morgan	Lacon Exit Cave (type)	30	EU433906
<i>Cambarus laconensis</i>	JC3799, JC3800, JC3801, JC3812, JC3813	AL	Morgan	Lacon Exit Cave (type)	31	EU433907
<i>Cambarus laconensis</i>	JC3807	AL	Morgan	Lacon Exit Cave (type)	32	EU433908
<i>Cambarus speleocoopi</i>	JC3823, JC3825, JC3826, JC3827, JC3829, JC3830	AL	Marshall	Kellers Cave (type)	26	DQ411759
<i>Cambarus speleocoopi</i>	JC3820	AL	Marshall	Beech Spring Cave	33	EU433909
<i>Cambarus speleocoopi</i>	JC3821	AL	Marshall	Beech Spring Cave	34	EU433910
Outgroups						
<i>Cambarus brachydactylus</i>	JF2579	TN	Humphreys	Duck River, Blue Ck.	n/a	DQ411732
<i>Cambarus friaufi</i>	JF2543	KY	Monroe	Barren River, Salt Lick Ck.	n/a	DQ411733
<i>Cambarus gentryi</i>	JF2508	TN	Dickson	Piney River, Williams Br.	n/a	AY853664
<i>Orconectes australis</i>	JC2443	AL	Marshall	Hans Kennamer Cave	n/a	EU433912
<i>Orconectes barri</i>	JC2031	TN	Pickett	Kathryn Cave	n/a	EU433913
<i>Orconectes pellucidus</i>	JC1426	KY	Hart	Fisher Ridge Cave	n/a	EU433914
<i>Orconectes compressus</i>	JC35	TN	Hickman	Buffalo River, Cane Ck.	n/a	EU433917
<i>Orconectes erichsonianus</i>	JC527	AL	Lauderdale	Tennessee River, Cypress Ck.	n/a	EU433918
<i>Orconectes forceps</i>	JC311	TN	Claiborne	Powell River	n/a	EU433919
<i>Procambarus acutus</i>	NCSM173	NC	Randolph	Cape Fear River	n/a	EU433915
<i>Procambarus gibbus</i>	USNM147843	GA	Marion	Flint River, Muckalee Ck.	n/a	EU433916
<i>Procambarus reimeri</i>	KC2262	AR	Polk	Ouachita River, Ward Ck.	n/a	EF012342

(2007). *Procambarus pecki*, therefore, must be placed within the genus *Cambarus* and subgenus *Aviticambarus*, and the monotypic subgenus *Remoticambarus* of the genus *Procambarus* must be dropped. The taxonomic status of *C. veitchorum* remains unknown until individuals are re-discovered in White Spring Cave, since attempts to acquire genetic data from preserved specimens proved futile. Genetic sequences acquired for this study were deposited into GenBank as accession numbers: EU433903-EU433919.

#### SYSTEMATICS

Cambaridae Hobbs, 1942

Cambarinae Hobbs, 1942

Genus *Cambarus* Erichson, 1846

Subgenus *Aviticambarus* Hobbs, 1969

***Cambarus (Aviticambarus) speleocoopi*, n. sp.**

Figs. 3, 4; Table 2

Diagnosis.—Albinistic; eyes reduced and without pigment; rostrum without marginal spines and with an acumen of moderate length, margins converging cephalically, its upper surface concave, without a median carina; postorbital ridges without cephalic spines; areola broad and long, 4.5 times longer than broad with five to six punctations across narrowest part, constituting approximately 43 percent of the carapace length; without lateral spines on each side of carapace; cephalolateral portion of carapace granulate; chela with many long conspicuous setae; hooks on ischiopodites of third pereopods in male. First pleopod of male

terminating in two strongly recurved parts with the apices of both terminals in first form male directed proximocaudally (Fig. 3a, d). Annual ventralis as figured (Fig. 4b).

Holotypic Male, form I.—Carapace subovate, slightly depressed (Fig. 4c). Pleon narrower than thorax (8.17 and 9.35 mm in widest parts, respectively). Width of carapace greater than depth in region of caudodorsal margin of cervical groove. Greatest width of carapace near midlength of areola.

Areola moderately broad (4.5 times longer than wide) with five to six punctations across narrowest part; cephalic section of carapace about 1.25 times longer than areola; length of areola about 43 percent of total length of carapace.

Rostrum with convergent margins, slightly thickened; acumen with corneous upturned tip; upper surface of rostrum concave with small setiferous punctations. Subrostral ridges weakly developed.

Postorbital ridges short and strongly depressed, grooved dorsolaterally, and without terminal spines. Suborbital angle lacking. Branchiostegal spine of moderate size and acute. Lateral surface of carapace without a spine along the cervical groove. Surface of carapace punctate except for the weakly granulate cephalolateral portions.

Pleon longer than carapace (22.05 and 21.03 mm). Cephalic section of telson with two spines in each caudolateral corner.

Epistome with a cephalo-median depression with raised cephalo-lateral borders but without median projection. Antennules with prominent spine on ventral surface of



Fig. 3. *Cambarus (Aviticambarus) speleocoopi*, new species: a, mesial view of first pleopod of holotypic form I male; b, mesial view of first pleopod of morphotypic form II male; c, lateral view of first pleopod of morphotypic form II male; d, lateral view of first pleopod of holotypic form I male.

basal segment. Antennae extend caudally beyond caudal margin of telson. Antennal scale with heavy lateral portion terminating in a long spine; lamellar portion comparatively short and broad.

One chela regenerated and comparatively shorter than second chela. Chela elongate, subovate in cross section with slightly inflated palm; entire hand with conspicuous long setae (Fig. 4a). Inner surface of palm with tubercles forming three irregular rows with a prominent tubercle on the lower surface at the base of the dactyl. Fingers not gaping. Upper surface of immovable finger with a submedian longitudinal ridge flanked laterally by deep setiferous punctations and mesially by shallower ones; lateral margin with row of deep punctations with each bearing several long setae; lower surface V-shaped in cross-section and with two or three irregular rows of long setae; opposable margin with row of 11 knob-like tubercles of which the fourth from the base is the largest; usual large tubercle lying below this row present at the base of distal third of finger; a single irregular row of denticles extends along distal half of finger. Upper and lower surfaces of dactyl similar to corresponding surfaces of immovable finger, but mesial margin with proximal tubercles. Opposable margin of dactyl with a row of 14 tubercles with largest four at base.

Carpus longer than broad with shallow longitudinal furrow above; entire surface of podomere with scattered setiferous punctations. Mesial surface with large spine and lower mesiodistal margin with similar spine.

Merus with scattered setiferous punctations on all surfaces. Upper surface with row of tubercles which broadens distally. Lower surface of merus with lateral row of 5 tubercles, distalmost the largest; mesial row of 11 tubercles with distalmost the largest. Ischium with a row of four tubercles along upper margin and three acute tubercles on lower margin.

Hooks on ischia of third pereiopods only; hooks strong and simple. Coxa of fourth pereiopod with prominent caudomesial protuberance.

First pleopod extends cephalically to coxa of third pereiopod when pleon is flexed. Tips terminating in two parts - strongly recurved, slender, and with the apices directed caudoproximally. Central projection corneous.

Allotypic Female.—Differs from the holotype as follows: Epistome shorter and with less developed cephalomedian depression; chela covered with many very long setae; opposable margin of immovable finger of chela with row of 9 tubercles with third from the base largest; dactyl with a row of 12 tubercles, fourth from base largest.

Annulus ventralis subovate, broader than long, and with an elevated, rounded caudal wall (Fig. 4b); a rounded hook-like elevation extends from caudodextral margin to the median line and forms the cephalic walls of a sinistral sinus; a tongue-like prominence extends cephalically from the caudal margin and passes below hook-like elevation.

Morphotypic Male, Form II.—Differs from holotype in possessing small terminal spines on postorbital ridges; opposable margin of dactyl with row of 18 tubercles, fourth from the base largest; opposable margin of immovable finger with 10 tubercles, third from base largest.

First pleopod extends to caudal margin of coxa of third pereiopods when pleon is flexed. Two terminal elements strongly recurved at an angle slightly greater than 90 degrees (Fig. 3b, c). Mesial process extends slightly beyond tip of the central projection, and neither element is corneous.

Morphological Variation.—Form I males showed an average carapace length of 17.78 mm across the three individuals measured. Females had an average carapace

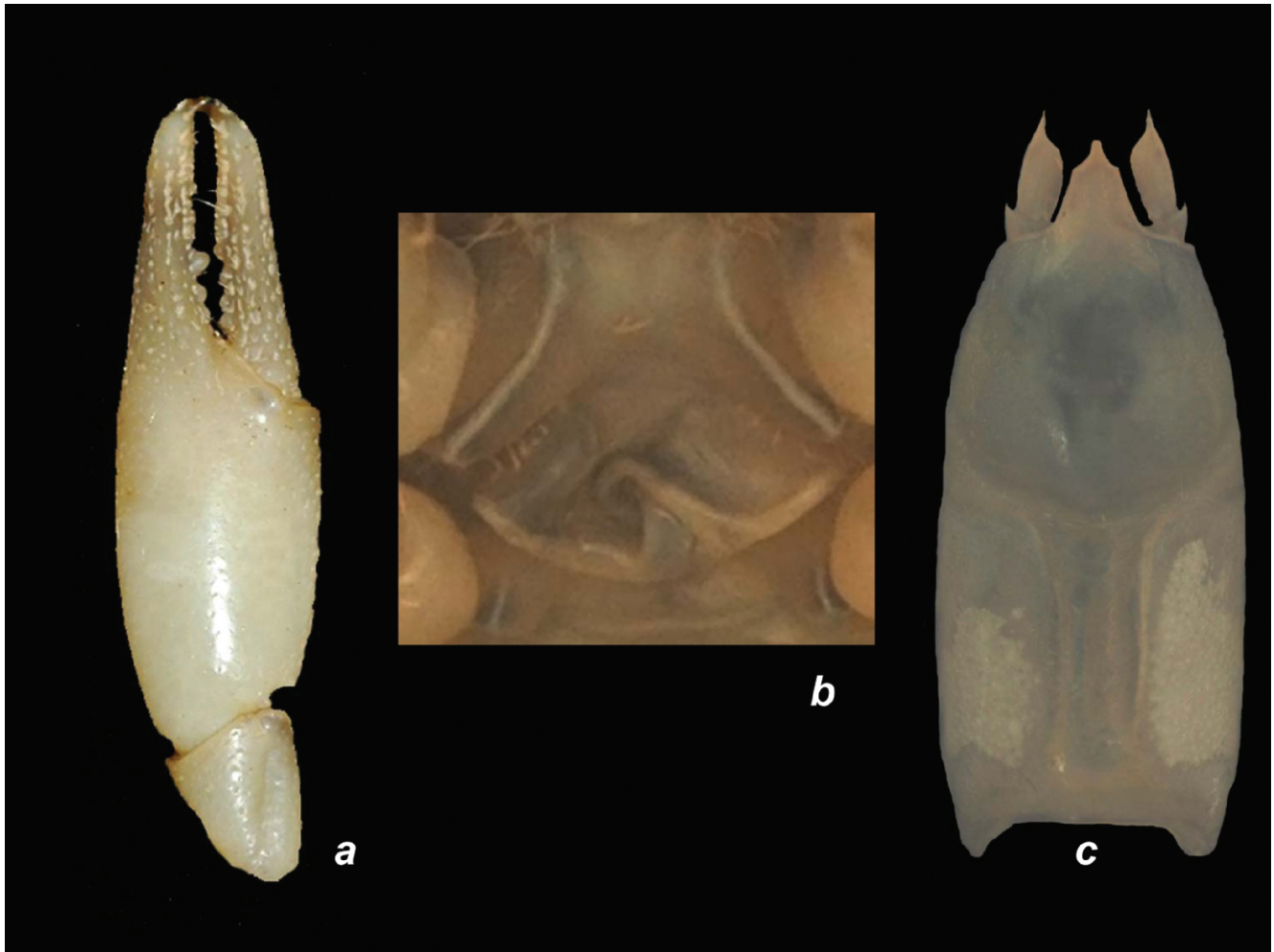


Fig. 4. *Cambarus (Aviticambarus) speleocoopi*, new species: a, dorsal view of chela of holotypic form I male; b, annulus ventralis and portion of sternum of allotypic female; c, dorsal view of carapace of holotypic form I male.

length of 17.54 mm ( $n = 7$ ) and form II males 15.34 mm ( $n = 9$ ). As is often the case with cave species, the measurements across carapace, rostrum, areola, pleon, and antennal scale were relatively similar across individuals (both within sexual class and among sexual class). The only exception to this trend is in the cheliped which is longer in the form I males compared to the females and form II males ( $P = 0.027$  Kruskal-Wallis with  $\chi^2$  approximation and 2 degrees of freedom).

**Type Locality.**—Kellers Cave (Alabama Cave Survey, ACS#326, Marshall County) is situated just below the road with an entrance approximately 1.2 m high and 2 m wide. There is a 30 m crawlway leading to a very tight flowstone choke in the stream passage. Cave continues <1 m high for another 50 m in a 1m wide streambed.

**Common Name.**—Sweet Home Alabama Cave Crayfish.

**Disposition of Types.**—The holotypic form I male (JC3839, USNM 1109966), morphotypic form II male (JC3840, USNM 1109969), and allotypic female (JC3841, USNM 1109967) all collected from Kellers Cave are deposited at

the United States National Museum as. One female paratype (JC2287), one form II male paratype (JC2288), and one juvenile paratype (JC2289) are deposited in the Monte L. Bean Museum at Brigham Young University as BYUC06-69. Two form II males (JC3822, JC3823), two females (JC3826, JC3827), and five juveniles (JC3824, JC3825, JC3828-JC3830) are also deposited as paratypes at the USNM as USNM 1109968. The type series was collected May 5, 2006 by J.E.B., T. Mann, M. Niemiller, and A. Toon.

**Range.**—*Cambarus speleocoopi* is endemic to Marshall County, Alabama, northwest and downstream of Guntersville Dam along both sides of the Tennessee River. It is restricted on the western side of its range to the area around the town of Mt. Olive which is located on the southwest side of the main reach of the Tennessee River and on the eastern side of its range to the area around the town of Cushion along the Paint Rock River on the north side of the Tennessee River.

**Non-type Material Examined.**—Type locality: June 26, 1967 (1 form II male, 2 female, 2 juveniles: NCSM24649, North Carolina State Museum of Natural Science) collected

Table 2. Measurements (mm) of *Cambarus speleocoopi*, new species.

Structure	Holotype JC3839 Form I Male	Allotype JC3841 Female	Morphotype JC3840 Form II Male
<b>Carapace</b>			
Height	6.64	6.56	5.63
Width	9.35	8.71	7.90
Total length	21.03	19.43	16.68
Postorbital length	17.98	17.05	14.69
<b>Rostrum</b>			
Width at base	2.55	2.56	2.22
Acumen length	0.79	0.81	0.84
<b>Areola</b>			
Length	9.19	8.68	7.35
Width	2.03	1.74	1.90
<b>Abdomen</b>			
Length	22.05	20.64	19.31
Width	8.17	8.03	6.83
<b>Antennal scale</b>			
Length	3.23	3.02	2.97
Width	1.72	1.74	1.45
<b>Cheliped</b>			
Length lateral margin chela	20.10	16.92	12.55
Length mesial margin palm	8.30	7.54	5.06
Width palm	6.33	5.47	4.00
Depth palm	4.34	3.76	2.82
Length dactyl	9.47	7.55	6.45

by S. B. Peck and A. Fiske; July 10, 1967 (1 form I male, 3 form II males, 1 female: NCSM24663) collected by S.B. Peck and A. Fiske; July 8, 2005 (1 form II male: JC2288, 1 female: JC2287, 4 tissue samples: JC2289, JC2440-JC2442) collected by J.E.B, T. Mann, D. Mann, G. Moni, and K. Toepke; Beech Spring Cave (ACS# 347), Marshall County, Alabama: July 1969 (1 female: NCSM24648) collected by R. Graham and J. Wilson; May 6, 2006 (1 form I male JC3820 and 1 juvenile JC3821 = BYUC06-72) collected by J.E.B., T. Mann, M. Niemiller, and A. Toon. Cherry Hollow Cave (ACS# 1710), Marshall County, Alabama: July 10, 2005 (1 juvenile female JC2412) collected by J.E.B, T. Mann, and G. Moni. Porches Spring Cave (ACS# 693), Marshall County, Alabama: July 8, 2005 (1 juvenile female JC2227) collected by J.E.B, J. Douglas, T. Mann, and G. Moni.

**Etymology.**—Named in honor of John E. Cooper, Curator of Crustaceans at the North Carolina State Museum of Natural Sciences. Coop along with his buddies Dick Graham and Bill Torode tore up the hills of northern Alabama eons ago in search of anything white and moving in the most horrible of dark places. His long time love affair with Northern Alabama turned up two new cave crayfish species and a cavefish to say the least. It has been an honor to crawl, slide, and squeeze into those same mud holes as Coop and while he always suspected a species complex was eluding him, he didn't suspect that a redneck woman from the coal region of Pennsylvania going to a Mormon school out west would practically lay on it decades later. From 50813 to 4803 with love.

**Crayfish Associates.**—The facultative cave crayfish species *Cambarus tenebrosus* (Hay, 1902a) and *C. striatus* (Hay,

1902b) are also found in subterranean habitats containing *C. speleocoopi*.

***Cambarus (Aviticambarus) laconensis*, n. sp.**

Figs. 5, 6; Table 3

**Diagnosis.**—Albinistic; eyes reduced and without pigment; rostrum without marginal spines and with an acumen of moderate length, margins converging cephalically, its upper surface concave, without a median carina; postorbital ridges without cephalic spines; areola broad and long, 4.3 times longer than broad with four punctations across narrowest part, and constituting approximately 40 percent of carapace length; with an acute lateral spine on each side of carapace; cephalolateral portion of carapace granulate; chela with scattered long setae; hooks on ischiopodites of third pereopods in male. First pleopod of male terminating in two recurved parts with apices of both terminals in first form male directed proximocaudally (Fig. 5a, d). Annual ventralis as figured (Fig. 6b).

**Holotypic Male, Form I.**—Carapace subovate, slightly depressed (Fig. 6c). Pleon narrower than thorax (7.26 and 8.47 mm in widest parts, respectively). Width of carapace greater than depth in region of caudodorsal margin of cervical groove. Greatest width of carapace near mid-length of areola.

Areola moderately broad (4.3 times longer than wide) with four punctations across narrowest part; cephalic section of carapace about 1.4 times longer than areola; length of areola about 40 percent of total length of carapace.

Rostrum with convergent margins with are slightly thickened; acumen with corneous upturned tip; upper surface of rostrum concave with small setiferous punctations. Subrostral ridges weakly developed.

Postorbital ridges short and strongly depressed, grooved dorsolaterally, and with acute terminal spines. Suborbital angle lacking. Branchiostegal spine of moderate size and acute. Lateral surface of carapace with an acute spine along the cervical groove. Surface of carapace punctate except for the weakly granulate cephalolateral portions.

Pleon longer than carapace (21.33 and 19.88 mm). Cephalic section of telson with two spines in each caudolateral corner.

Epistome with a cephalomedian depression with raised cephalolateral borders but without a median projection. Antennules with a prominent spine on ventral surface of basal segment. Antennae extend caudally beyond caudal margin of the telson. Antennal scale with heavy lateral portion terminating in long spine; lamellar portion comparatively short and broad.

Chela elongate, subovate in cross section with slightly inflated palm; entire hand covered with long scattered setae (Fig. 6a). Inner surface of palm with tubercles forming three rows with a prominent tubercle on lower surface at base of dactyl. Fingers not gaping. Upper surface of immovable finger with a submedian longitudinal ridge flanked laterally by deep setiferous punctations and mesially by shallower ones; lateral margin with row of deep punctations with each bearing several long setae; lower surface V-shaped in cross-section and with three rows of long setae; opposable margin



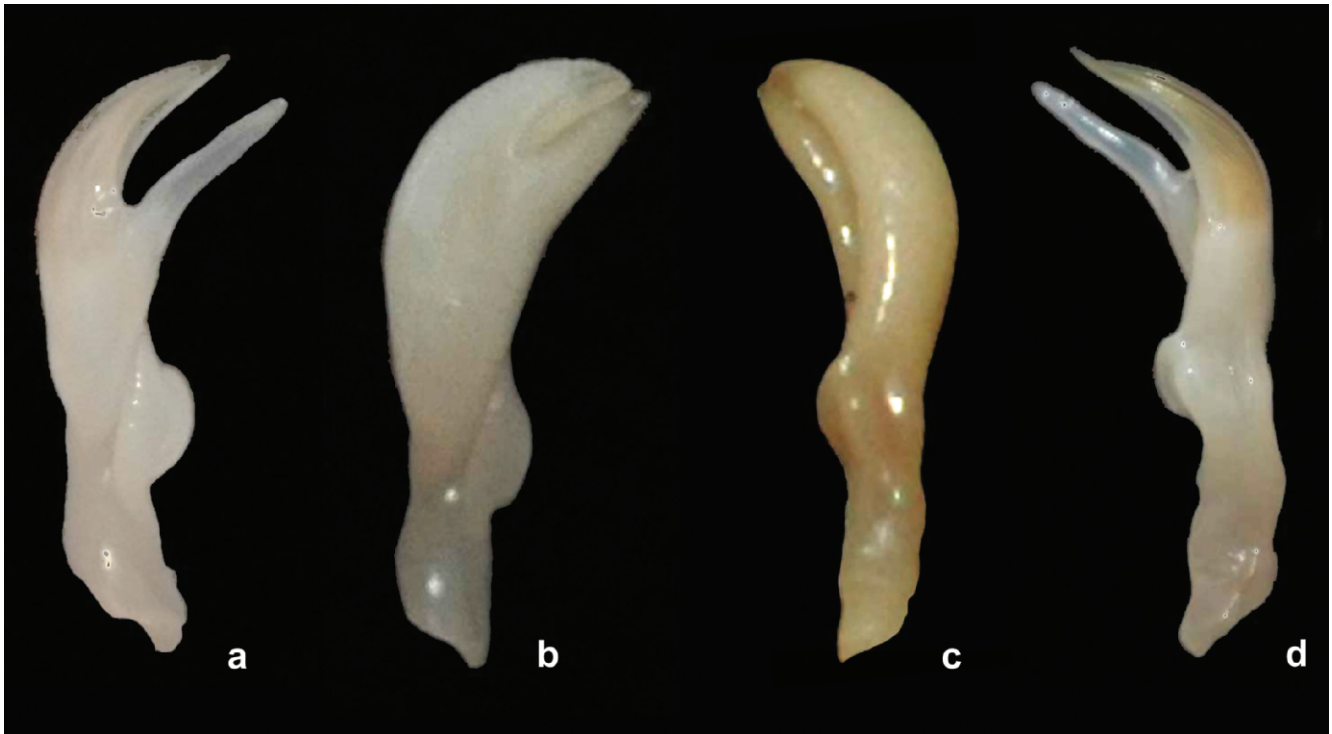


Fig. 5. *Cambarus (Aviticambarus) laconensis*, new species: a, mesial view of first pleopod of holotypic form I male; b, mesial view of first pleopod of morphotypic form II male; c, lateral view of first pleopod of morphotypic form II male; d, lateral view of first pleopod of holotypic form I male.

with row of 9 tubercles of which sixth and seventh from base are largest; without large tubercle at base of distal third of finger; single irregular row of denticles extends along distal half of finger. Upper and lower surfaces of dactyl similar to corresponding surfaces of immovable finger, but mesial margin with proximal tubercles. Opposable margin of dactyl with row of 6 tubercles at base.

Carpus longer than broad with shallow longitudinal furrow above; entire surface of podomere with scattered setiferous punctations. Mesial surface with large spine and lower mesiodistal margin with similar large spine, surrounded by several moderately sized spines.

Merus with scattered setiferous punctations on all surfaces. Upper surface with a row of tubercles which broadens distally. Lower surface of merus with scattered row of 9 tubercles, distalmost largest; mesial row of 13 tubercles with distalmost largest.

Hooks on ischia of third pereopods only; hooks strong and simple. Coxa of fourth pereopod with prominent caudomesial protuberance.

First pleopod extends cephalically to coxa of third pereopod when Pleon is flexed. Tips terminating in two parts. Corneous central projection moderately recurved and directed caudally at 45 degrees to shaft of appendage. Mesial process extends slightly farther caudally than central projection.

Allotypic Female.—Differs from holotype as follows: Epistome shorter and with less developed cephalomedian depression; opposable margin of immovable finger of chela with row of 9 tubercles with seventh from base largest; dactyl with a row of 12 tubercles, fourth from base largest.

Annulus ventralis subovate, broader than long, and with an elevated, rounded caudal wall with narrow median longitudinal furrow in cephalic half ending in central depression; tongue extending caudosinistrally across caudal side of depression, disappearing beneath caudosinistral wall of U-shaped sinus (Fig. 6b).

Morphotypic Male, Form II.—Differs from the holotype in lacking a lateral spine on each side of the carapace; opposable margin of dactyl with a row of 8 tubercles, the fourth from the base largest; opposable margin of immovable finger with 7 tubercles, third from base largest; hooks lacking on ischia.

First pleopod extends to caudal margin of coxa of third pereopods when pleon is flexed. Two terminal elements recurved at an angle approximately 45 degrees to the shaft of the appendage (Fig. 5b, c). Mesial process extends slightly beyond tip of the central projection, and neither element is corneous.

Morphological Variation.—The average carapace length for form I males was 18.95 mm ( $n = 4$ ), for form II males 19.28 mm ( $n = 5$ ), and for females 19.83 mm ( $n = 8$ ). There was no significant difference among sex class in any of the morphologies measured, including the cheliped.

Type Locality.—Lacon Exit Cave (ACS #3343, Morgan County) is located on the onramp to Interstate 65 in the blasted rock wall with an out-flowing spring. The entrance is 1 m high leading to a small room with a standing pool of water. The cave continues past a tight wedge for another 150 m in the stream bed.

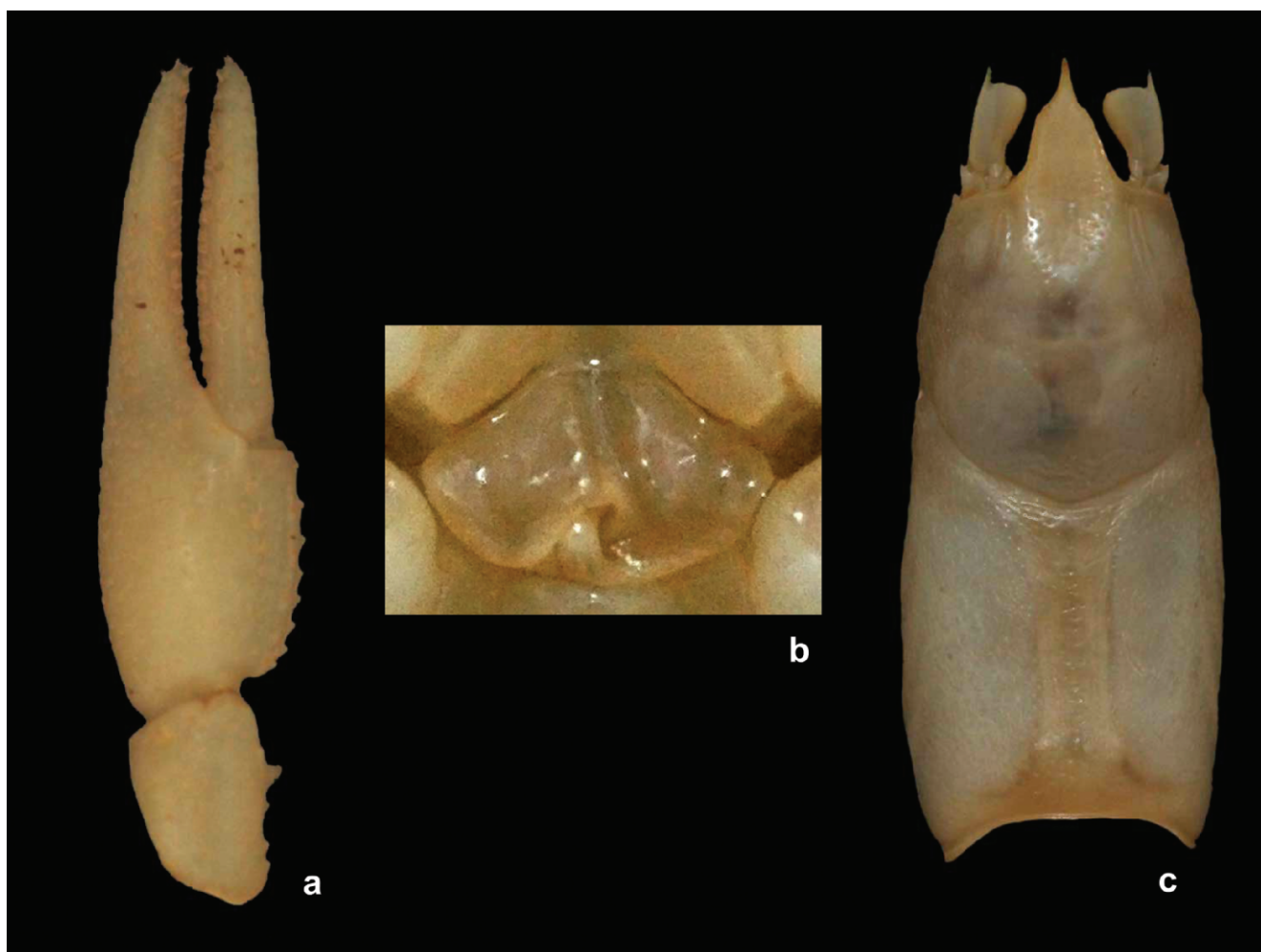


Fig. 6. *Cambarus (Aviticambarus) laconensis*, new species: a, dorsal view of chela of holotypic form I male; b, annulus ventralis and portion of sternum of allotypic female; c, dorsal view of carapace of holotypic form I male.

**Common Name.**—Lacon Exit Cave Crayfish.

**Disposition of Types.**—The holotypic form I male (JC3836, USNM 1109970), morphotypic form II male (JC3837, USNM 1109973), and allotypic female (JC3838, USNM 1109971) all collected from Lacon Exit Cave on May 8, 2006 by J.E.B., T. Mann, M. Niemiller, and A. Toon are deposited at the United States National Museum. A paratype series is deposited at the Monte L. Bean Museum including a form I male (JC3800), form II male (JC3803) and female (JC3808) as BYUC06-71 and a form II male (JC2540) collected on October 27, 2005 by J.E.B., T. Mann, and G. Moni, as BYUC06-70. Additional paratypes are deposited at the USNM as 1109972, which includes 2 form I males (JC3801, JC3802), 3 form II males (JC3804-JC3806), 6 females (JC3809-3814), and 4 juveniles (JC3807, JC3815-3817). Tissues samples from the gills were removed from all voucher specimens and are housed at BYU. Tissue from four claws (JC2436-JC2439) without voucher specimens collected on October 27, 2005 by J.E.B., T. Mann, and G. Moni are also housed at BYU.

**Range.**—*Cambarus laconensis* is endemic to Morgan County, Alabama, south of the Tennessee River along

Interstate 65. It is currently restricted to Lacon Exit Cave, but may occur in one or two other stream caves nearby as part of the same population.

**Etymology.**—Named for the geographic location of the type locality, the Lacon Exit off Interstate 65 in northern Alabama. Will Drake, an undergraduate student from Alabama attending Brigham Young University, was mapping the cave in 2005 and contacted J.E.B. about the presence of white crayfish after reading about the Crandall Lab genetic research online. One month later on a whim after not seeing blind crayfish anywhere for days, Thany Mann, Gerald Moni, and J.E.B. drove to Lacon Exit and turned up a new disjunct locale of cave crayfish and surprisingly a new species in the blasted rockwall of the highway!

**Crayfish Associates.**—*Cambarus tenebrosus* is also found in Lacon Exit Cave with *C. laconensis*.

#### DISCUSSION

Phylogenies determined using solely morphological similarities have been shown in some cases to be erroneous when comparing supposedly closely-related surface and

cave species (Wiens et al., 2003; Wilcox et al., 2004; Bonett and Chippindale, 2004). While the placement of species in polyphyletic genera hinders direction for conservation efforts for groups of subterranean taxa, even worse is the failure to diagnose and recognize species that are cryptic owing to habitat and/or morphological constraints (Hedin, 1997; Kiefer et al., 2002; Buhay and Crandall, 2005; Lefebure et al., 2006; Bichain et al., 2007; Buhay and Crandall, 2008). We have previously shown that cave-adapted members of the freshwater crayfish genus *Orconectes* are most closely related to surface-dwelling members of the genus *Cambarus* rather than surface-dwelling members of *Orconectes* (Crandall and Fitzpatrick, 1996; Sinclair et al., 2003; Buhay and Crandall, 2005; Buhay and Crandall, 2008). The morphological differences in the shapes of pleopods of cave and surface stream *Orconectes* with the genus *Cambarus* are striking (Fig. 2), but there is little difference between pleopod shape of cave and surface-dwelling species of *Cambarus*. A morphological feature cannot be determined to be ancestral, reduced, or regressed simply because subterranean taxa are thought to be primitive – the term “troglomorphy” (Christiansen, 1961) refers to selected adaptations for the cave environment without regard for “direction,” whether they are increased length of antennae and limbs or decreased chela and eye size. These “troglomorphic” changes are driven by small population sizes, isolation, and mutation (Porter and Crandall, 2003) and may reach “extreme” stages (such as the reduction of eye structures in *Aviticambarus*) or may not (such as the retention of visual pigments and eye structures in *Cambarus nerterius*).

For this current study of the cave crayfish of *Aviticambarus*, we found erroneous taxonomic placement of *Procambarus pecki*. Interestingly, Hobbs (1972) named the monotypic subgenus *Remoticambarus* to “encompass the disjunct *Procambarus pecki*” for which he considered “distant with other members of the genus.” Moreover, Hobbs et al. (1977) speculated that *P. pecki* could have been assigned to the cave assemblage of *Orconectes* because of its geographical occurrence near the margin of the Cumberland Plateau. Yet, it has never been speculated in crayfish publications that *P. pecki* could possibly be closely related to species of *Aviticambarus* with the argument of a modified *Cambarus* pleopod and geographic proximity in the Southern Appalachians. Clearly, our results using a molecular taxonomy approach demonstrate that *P. pecki* is actually a member of the genus *Cambarus*, subgenus *Aviticambarus* (Fig. 2), and we therefore recognized this species as *Cambarus pecki* to more accurately reflect the underlying evolutionary relationships in the taxonomic binomial.

The closest ancestors to the cave-dwelling *Aviticambarus* are indeed surface-dwelling members of *Cambarus* and similarly, we hypothesize that further surface sampling around the Southern Appalachians will help elucidate which species of *Cambarus* is the closest ancestor. Based on our preliminary analyses of most members of the genus *Cambarus*, it appears that three surface species (*C. friauffi*, *C. gentryi*, and *C. brachydactylus*) played an important role not just in the speciation of *Aviticambarus*, but also

Table 3. Measurements (mm) of *Cambarus laconensis*, new species.

Structure	Holotype JC3836 form I male	Allotype JC3838 female	Morphotype JC3837 form II male
Carapace			
Height	5.95	7.61	5.91
Width	8.47	9.84	8.28
Total length	19.88	23.64	19.93
Postorbital length	17.09	20.10	16.58
Rostrum			
Width at base	2.36	3.24	2.33
Acumen length	1.14	1.18	1.10
Areola			
Length	8.13	10.66	8.56
Width	1.88	2.01	1.79
Abdomen			
Length	21.33	24.68	21.31
Width	7.26	8.66	7.13
Antennal scale			
Length	2.82	3.55	3.11
Width	1.39	1.67	1.51
Cheliped			
Length lateral margin chela	15.05	16.46	16.5
Length mesial margin palm	5.21	5.77	6.14
Width palm	4.55	5.68	5.14
Depth palm	3.02	3.79	3.35
Length dactyl	8.84	9.00	8.82

as the possible ancestral lineages to the obligate cave-dwelling species of *Orconectes* along the Cumberland Plateau (Fig. 2).

Cryptic species contained within subterranean taxa and species' complexes are becoming increasingly more common with discovery through the use of molecular methods (Bickford et al., 2007; Finston et al., 2007; Page et al., 2008; Buhay and Crandall, 2008). In our study, the presence of two cryptic species of *Aviticambarus*, *Cambarus speleocoopi* and *C. laconensis*, suggests that the number of subterranean taxa are probably underestimated in biodiversity surveys using strictly morphologically based diagnoses. In fact, we found no statistical difference between the morphology of our cryptic species, *C. speleocoopi* and *C. laconensis* even though they are clearly distinct evolutionary lineages based on both nuclear and mitochondrial genetic data (Fig. 2). The presence of cryptic species is particularly troublesome because subterranean fauna are considered to be highly imperiled across the globe and in need of immediate conservation attention (Danielopol et al., 2003). It would be disastrous to lose species before they are discovered, described, and hopefully protected.

Convergent morphological characters are inadequate and misleading in resolving species' boundaries and taxonomic position for cave crayfishes of *Aviticambarus* that all exhibit troglomorphy, including loss of body and eye pigment, extension of antennae and limbs, and reduced eye structures (Fig. 7). Thorough geographic sampling coupled with high resolution genetic data were employed to accurately depict phylogenetic relationships and diagnose independent evolutionary lineages of these subterranean taxa. When paired together, geography and genetic data are adequate and diagnosable characters for the identification of species of

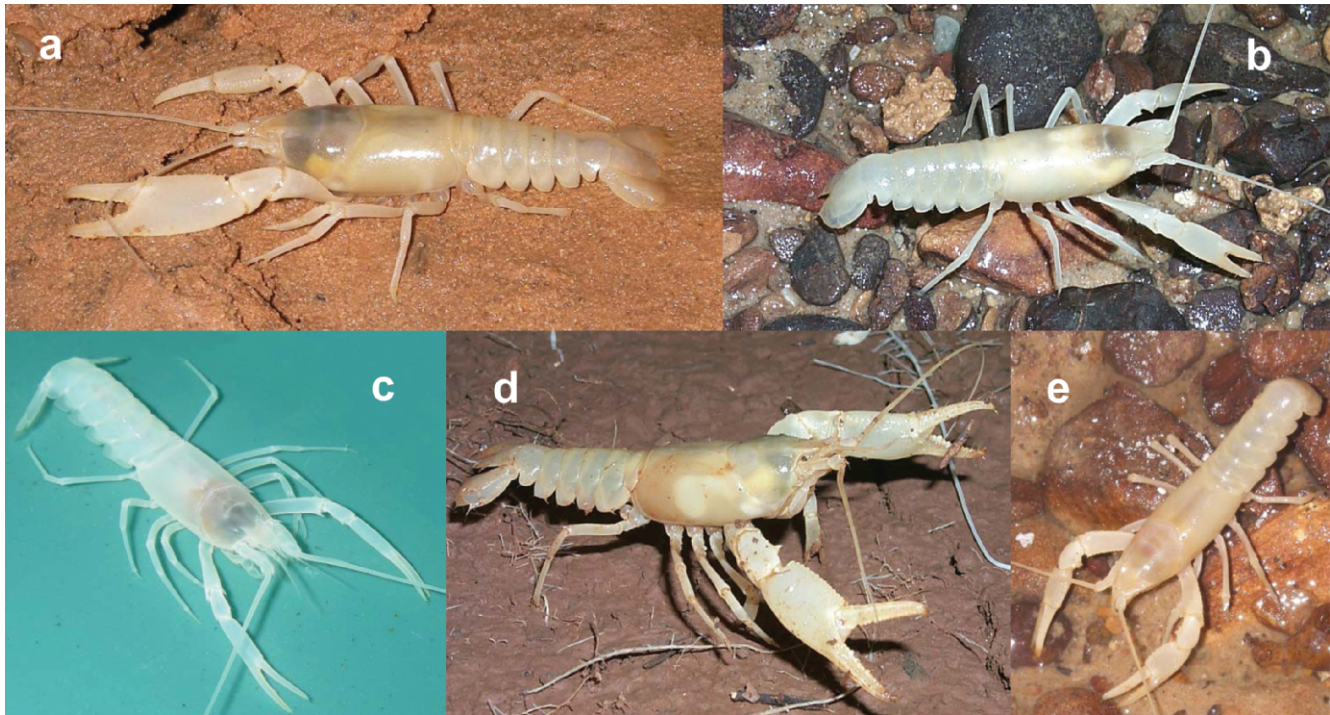


Fig. 7. Photographs of each *Aviticambarus* species, excluding *Cambarus veitchorum*: a, *C. speleocoopi*; b, *C. hamulatus*; c, *C. pecki*; d, *C. jonesi*; e, *C. laconensis*.

*Aviticambarus* in a “molecular taxonomy” framework (Buhay et al., 2007). Each lineage is a distinct evolutionary unit and meets the criteria of a “genealogical species” whereby members of a species are more closely related to each other than to members of other species (Baum and Shaw, 1995).

In the case of *Aviticambarus*, the two new species, *Cambarus speleocoopi* and *C. laconensis*, along with *C. hamulatus*, do not co-inhabit caves with other stygobitic species. Therefore, by knowing geographic location (cave name and county in Alabama), the identity of these species can be determined. Also, we previously provided a listing of all known locations for *Aviticambarus* (Buhay et al., 2007). For cave crayfish species that co-occur with *Cambarus jonesi*, we summarize (below) the morphological differences used to determine the identities of individuals at only

the particular locations with more than one stygobitic species. *Cambarus jonesi* co-occurs with *C. pecki*, *C. veitchorum*, *O. australis*, and *O. sheltae*, and morphological characters of form I males can be used to identify each of these species:

In Madison County.—1) *Orconectes sheltae*: smallest species with maximum total carapace length of 19.7 mm; narrow elongate chela with long palm and subvertical orientation, and not covered with conspicuous setae; gonopod terminating in two elements; twisted corneous central projection and a noncorneous mesial process; currently only known from Shelta Cave (Fig. 8a). 2) *Cambarus jonesi*: chela covered with long conspicuous setae; gonopod terminating in two strongly recurved elements that are directed caudoproximally; corneous

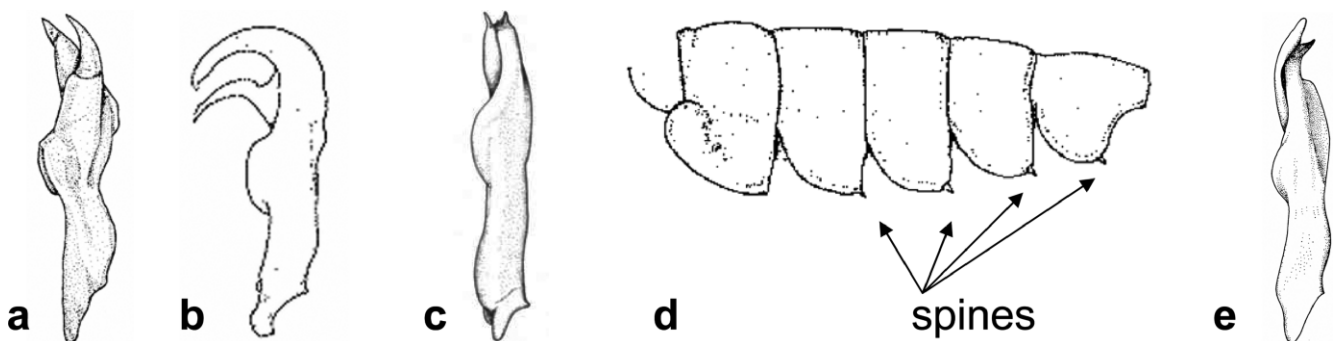


Fig. 8. Morphological differences between co-occurring cave species in Northern Alabama: a, *Orconectes sheltae* lateral view of form I male pleopod; b, *Cambarus jonesi* lateral view of form I male pleopod; c, *Orconectes australis* lateral view of form I male pleopod; d, *Cambarus veitchorum* spines along outer margin of tail; e, *Cambarus pecki* lateral view of form I male pleopod.

central projection and tapering noncorneous mesial process (Fig. 8b). 3) *Orconectes australis*: chela not conspicuously covered with setae; gonopod terminating in two acute elements; corneous basally flattened central projection and a noncorneous mesial process (Fig. 8c).

At White Spring Cave, Limestone County.—1) *Cambarus veitchorum*: smallest species with maximum carapace length of 16.7 mm; chela not conspicuously covered with setae; second through fifth pleonal pleura with spine on the caudal ventral angle (Fig. 8d). 2) *Cambarus jonesi*: chela covered with conspicuous setae; lacking pleonal spines.

At McKinney Pit, Colbert County; Cave Spring Cave, Morgan County; Key Cave, Lauderdale County.—1) *Cambarus pecki*: narrow elongate chela not conspicuously covered with setae; gonopod terminating in two acute elements; central projection slightly bent and mesial process tapered and directed distolateral (Fig. 8e). 2) *Cambarus jonesi*: chela covered with conspicuous setae; gonopod terminating in two strongly recurved elements that are directed caudoproximally (Fig. 8b).

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