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Cambarus (*Jugicambarus*) *magerae*, a new species of crayfish (Decapoda: Cambaridae) from Virginia

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Abstract.—A new species of crayfish, *Cambarus* (*Jugicambarus*) *magerae,* is described from southwestern Virginia within the Valley and Ridge physiographic province of the North American Appalachian Mountains. The species is one of the smallest and most geographically restricted in the subgenus and possibly the genus. Morphologically, it is similar to *Cambarus* (*J.*) *parvoculus* Hobbs & Shoup, 1947 and *Cambarus* (*J.*) *jezerinaci* Thoma, 2000. It can be distinguished from both by its proportionally wider areola. Its distribution appears to be restricted to a single gorge in the South Fork Powell River upstream of Cracker Neck, a village east-southeast of Big Stone Gap located at the base of Powell Mountain.

Keywords: *Cambarus*, crayfish, endangered species, *Jugicambarus*, Virginia

Cambarus (*Jugicambarus*) *parvoculus* Hobbs & Shoup, 1947 is considered to be widespread on the Cumberland Plateau in eastern Tennessee, southeastern Kentucky, southwest Virginia, and the northernmost area of the Georgia/Alabama state line (Hobbs 1989, Taylor & Schuster 2004). Thoma (2000) described *Cambarus* (*J.*) *jezerinaci*, splitting it from *C. parvoculus* and restricting the new species to the Powell River basin of southwest Virginia and northern Tennessee, thus eliminating *C. parvoculus* from the fauna of Virginia. Taylor & Schuster (2004) questioned the species-specific separation of *C. jezerinaci* from *C. parvoculus* and suggested a genetic study be conducted. A study was subsequently conducted by Thoma & Fetzner (2008; funded by a grant from the Virginia Department of Game and Inland Fisheries).

During the course of the investigation, RFT discovered a small, dark green crayfish inhabiting the South Fork Powell River. Initially the specimens were thought to be juveniles, but upon closer inspection all males were found to be first form. Subsequent collecting trips confirmed the small size of the species described herein and that it is confined to the narrow ravine of South Fork Powell River between the village of Cracker Neck and Big Cherry Reservoir, a stream reach of approximately 6 km.

Materials and Methods

Specimen and tissue collection.—All material was collected by hand or with a 4′ X 6′ minnow seine. All specimens were kept alive on ice until a single leg (or other muscle tissue) could be taken as a tissue sample for DNA analysis (which typically occurred 6–12 hours post capture) at

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which point the specimens were preserved in 70% ethanol. Sampled legs were cut into 2–3 mm pieces and placed directly into 1 mL of cell lysis buffer (10 mM Tris, 100 mM EDTA, 2% SDS, pH 8.0) that also contained 10 μL of Proteinase K (20 mg/mL stock). Samples were stored at ambient temperature until DNA extraction could be completed.

DNA extraction, amplification, and sequencing.—DNA was extracted using the high salt precipitation method described in detail by Fetzner & Crandall (2003). PCR amplifications of the mitochondrial DNA (mtDNA) cytochrome c oxidase subunit I gene (COI; EC 1.9.3.1) were conducted in a total volume of 25 μL. Each PCR reaction contained the following components: 1X PCR buffer, 3 mM magnesium chloride, 1.25 mM dNTP, 1 μM primer, 0.6 units of GoTaq Hotstart DNA polymerase (Promega), and 300 ng of sample DNA. PCR cycling conditions included an initial denaturation step of 2 min at 95°C followed by 50 cycles performed at 95°C for 30 sec, 50°C for 30 sec, and 72°C for 1.5 min. A final extension at 72°C for 10 min was conducted, followed by a final soak at 4°C until samples could be processed further (usually overnight). Primers used in the reaction were the standard set of Folmer et al. (1994) primers, but a universal primer sequence was added to the 5′ end of the Forward and Reverse COI primers (T7 and T3, respectively). These non-degenerate, non-homologous 5′ tails (in bold) were used to sequence all resulting PCR products. Primer sequences used were: HybLCO 5′-**TAATACGACTCACTATAGGG**GGTCAACAAATCATAAAGATATTGG-3′ and HybHCO 5′-**AATTAACCCTCACTAAAGGG**TAAACTTCAGGGTGACCAAAAATCA-3′. The PCR reactions were checked for amplification products in the correct size range (~700 bp) by electrophoresis through a 1% agarose gel (run at 140 volts for 20 min in TAE buffer). Viable PCR products were cleaned and purified using MultiScreen PCRμ96 plates (Millipore) in preparation for DNA sequencing.

DNA sequencing methods also followed those outlined by Fetzner & Crandall (2003). Sequences obtained from the automated sequencer were initially corrected and aligned using the program Sequencher, version 5.0.1 (GeneCodes Corp., Inc.) and adjusted, as necessary, by eye.

Genetic data analysis.—After alignment in Sequencher, the COI barcode sequence data were checked for indels and also translated into the corresponding amino acids using Mesquite v1.7.6 (Maddison & Maddison 2011) to verify the presence of an open reading frame (i.e., no stop codons), and to avoid incorporating mtDNA nuclear pseudogenes (= numts) in the analysis (Song et al. 2008). The data were imported and analyzed using PAUP* v. 4.0b10 (Swofford 2003) via PaupUp v. 1.0.3.1 (Calendini & Martin 2005) in order to output distance matrices. For phylogenetic analyses of haplotype relationships, and for higher-level systematic relationships among related species in the genus *Cambarus*, models of DNA sequence evolution were tested for their fit to the data. Eighty-eight different models (11 substitution schemes) of DNA sequence evolution were tested using jMODELTEST v. 2.1.4 (Darriba et al. 2012). Both maximum likelihood (ML) and Bayesian inference (BI) optimality criteria were used to estimate phylogenies using raxmlGUI v. 1.3 (Silvestro & Michalak 2012), which includes RAxML v. 7.4.2 (Stamatakis 2006), and MrBayes v. 3.2.2 (Ronquist & Huelsenbeck 2003), respectively. For RAxML, the GTR+G model was selected for use over the only other alternative model (GTR+G+I), following the author's (JWF) suggestion that the GTR+G+I model may cause problems with the resulting model parameter optimization. The RAxML analyses used a combined ML topology search with the rapid bootstrap ($n = 1000$) setting, in order to determine nodal support.

Bayesian analyses were performed with MrBayes, using two independent runs with one cold chain and three hot chains. The program was run for 1×10^7 generations, with sampling every 1000 generations. Split frequencies below 0.01 were used to check for convergence, and the first 25% of trees were discarded as burn-in. The two independent runs were then combined after the deletion of burn-in and a majority rule consensus tree was created with nodal confidence for the trees assessed using node posterior probabilities.

Systematics

Cambarus (*Jugicambarus*) *magerae*, new species

Fig. 1, Table 1

Diagnosis.—Pigmented; eyes not reduced, size small (first form male [M-I] average carapace length = 17.8 mm, range 17.1–18.7 mm). Pre-acumen rostral margins slightly convergent, moderately thickened, without marginal spines or tubercles. Rostrum lacking median carina, shallowly excavated, angled near end at slightly less than 90° to poorly defined acumen. Carapace slightly dorsoventrally compressed in cross-view, without cervical spines or tubercles. Branchiostegal tubercles weakly developed. Suborbital angle acute. Postorbital ridges weak, not ending in distinct spines or tubercles. Areola open, length 1.7–3.5 times width ($\overline{X} = 2.7$), constituting, in adults, 30.8 to 35.4% ($\overline{X} = 33.6\%$) of entire carapace length, with room for 4–8 rows of punctuations in narrowest part. Antennal scale 2–2.6 times as long as wide ($\overline{X} = 2.34$), usually broadest at mid-point. Mesial palm of chelae usually with one row of 6–8 tubercles, occasional second row of 3–6 poorly developed tubercles, not cristiform. No tufts of elongate setae at base of propodus. Opposable margin of propodus with four enlarged tubercles on basal half, denticles extending on average 86.0% of distal length. Opposable margin of dactyl with four enlarged tubercles on basal half, denticles extending on average 54.3% of distal length. Palm length to dactyl length ratio averaging 1.6. Distinct dorsomedian longitudinal ridges on dactyl and opposable propodus. No dorsolateral impression at base of propodus. Carpus with one large tubercle on mesial margin, just distal of midpoint. First pleopods of form I male not contiguous at base, with convexity near mid-length of cephalic surface; terminal elements consisting of tapering, distally pointed central projection, with apical notch that extends beyond mesial process. Mesial process conically shaped at base and tapering to distal point. Both processes recurved greater than 90°. Mesial processes deflected laterally. Hooks on ischium of third pereopods only, not opposed by tubercle on coxa. Female with asymmetrical annulus ventralis formed by two hardened caudal parts, one curved in a C-shape, the other straight, rounded segment, forming flange projecting under C-shaped portion, creating fossa. Annulus ventralis sclerotized throughout. Postannular sclerite symmetrical bar shape, greatest height in center.

Holotypic male, Form I.—Eyes pigmented, diameter 57% of rostrum width at base. Carapace subovate (Fig. 1L), dorsoventrally compressed (Fig. 1A). Abdomen narrower than cephalothorax; maximum width of carapace slightly greater than depth at caudodorsal margin of cervical groove. Areola wide (2.4 mm) with seven punctuations across narrowest part, length comprising 34% of total carapace length. Rostrum slightly recurved distally with slightly convergent, thickened margins and a 45° angle delimiting acumen, anterior tip upturned, not reaching to base of ultimate podomere of antennular peduncle; dorsal surface of rostrum flat with few punctuations mostly on basal half. Subrostral ridge moderately developed. Postorbital ridge moderately developed, grooved dorsolaterally and ending cephal-

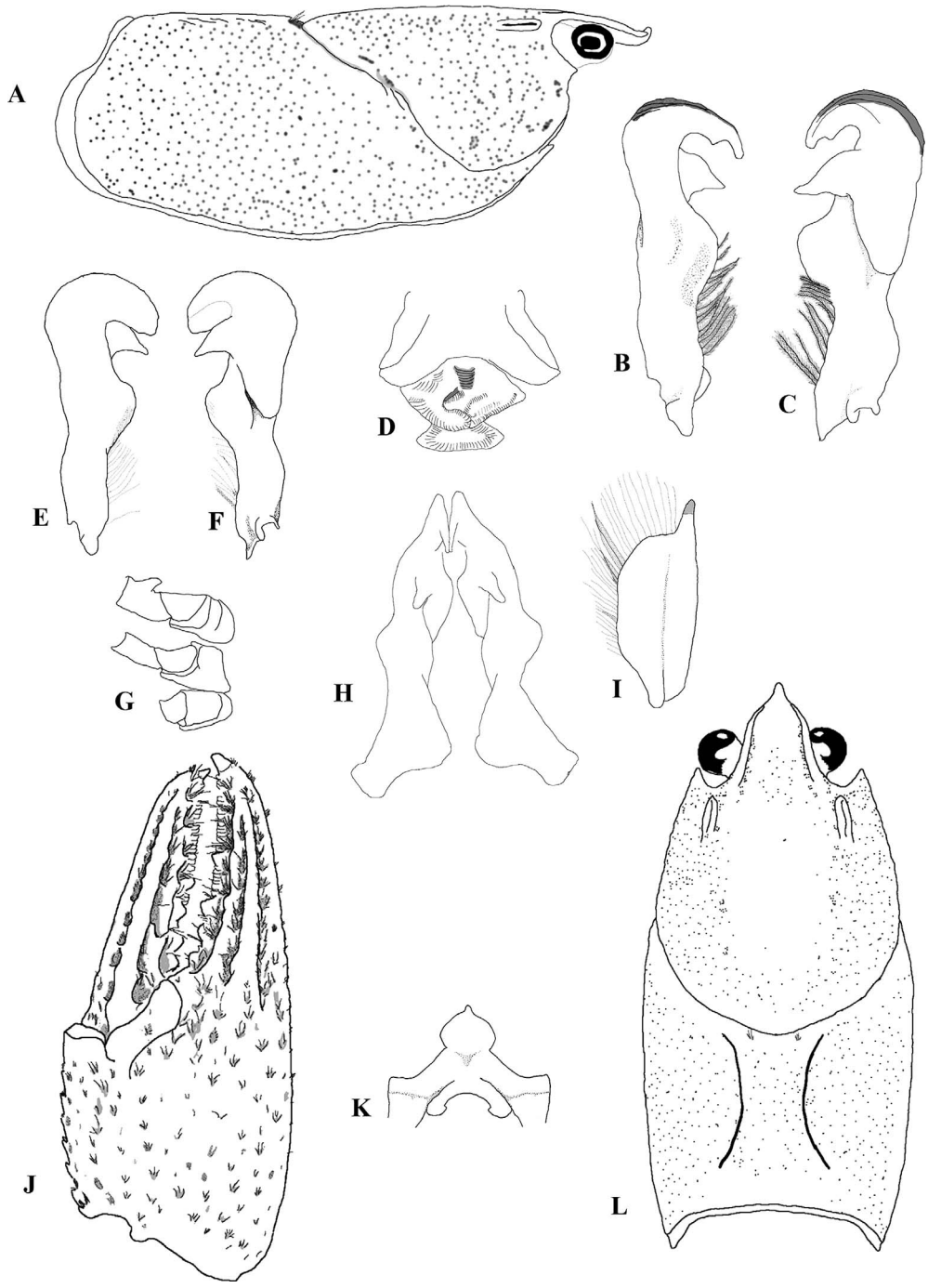

Fig. 1. *Cambarus* (*Jugicambarus*) *magerae*, all from holotype male, Form I (OSUMC 8928), except E and F from morphotype male, Form II (OSUMC 8929), and D from allotype female (OSUMC 8930). A, lateral aspect of carapace; B, C, lateral and mesial aspect of male I right gonopod (first pleopod); D, caudal aspect of annulus ventralis; E, F, lateral and mesial aspect of right gonopod male II; G, ventral aspect of right third, fourth, and fifth pereopods; H, ventral aspect of in situ gonopods; I, dorsal aspect of antennal scale; J, dorsal aspect of distal podomeres of right cheliped; K, epistome; L dorsal aspect of carapace.

Table 1.—Measurements (mm) of *Cambarus* (*J.*) *magerae*.

Character	Holotype	Allotype	Morphotype
Carapace			
Height	7.8	8.0	8.4
Width	9.3	9.0	9.6
Length	18.0	17.4	18.6
Areola			
Length	6.2	6.0	6.1
Width	2.4	2.3	3.6
Rostrum			
Width at base	3.0	3.5	3.1
Length	4.3	4.2	4.0
Eye			
Diameter	1.7	1.6	1.7
Chelae (right)			
Length lateral margin	13.4	11.4	12.7
Length mesial palm	4.9	4.0	4.5
Width of palm	5.9	5.1	5.5
Length of dactyl	7.9	6.8	7.5
Abdomen			
Length	20.7	20.1	18.9
Width	8.3	8.3	7.9
Gonopod			
Length	4.5	N/A	4.6
Antennal scale			
Length	3.3	3.4	3.7
Width	1.4	1.4	1.5

ically in tubercle without spine or corneous portion. Suborbital angle acute; branchiostegal spine present. Cervical spine absent. Hepatic and branchiostegal regions with small tubercles. Remainder of carapace punctate dorsally and laterally. Abdomen slightly greater in length than carapace, pleura short, sub-truncate, rounded caudoventrally. Cephalic section of telson with two spines in caudolateral corners. Proximal podomere of uropod with weak distal spine on mesial lobe; mesial ramus of uropod with median rib ending distally as weak distomedian spine not overreaching margin of ramus; laterodistal spine of ramus also present.

Cephalomedian lobe of epistome (Fig. 1K) rounded with level margins, distal portion forming pointed projection, with small setae; main body with shallow fovea; epistomal zygoma strongly arched. Ventral surface of antennal peduncle's proximal podomere without spine at base of distal third. Antennal peduncle without spines; antennal scale (Fig. 1I) 2.4 times as long as wide, broadest at midpoint, mesial border rounded; distal spine strong, not reaching distal extremity of antennular peduncle. Ischium of third maxilliped with two rows of flexible setae; lateral row much longer than mesial, mesial margin with 18 teeth, numbers 1, 6, 9, and 11 from distal end larger than adjacent teeth.

Length of right chela (Fig. 1J) 75% that of carapace; width 44% of chela length; palm length 36% of chela length; dactyl length 1.6 times palm length. Dorsal surface of palm covered with punctations with setae in each, mesial margin with row of seven tubercles, no second row; ventral surface also punctate, punctations ornamented with setae; three low tubercles on articular rim opposite base of dactyl, one mesial and two lateral; lateral surface of chela smooth. Both fingers of chela with well-defined submedian dorsal ridges; opposable margin of fixed finger with row of four tubercles (third from base enlarged), gap between third and fourth tubercle, denticles extending distally from third tubercle over 66.7% of opposable propodus. Opposable margin of dactyl with row of four tubercles along proximal half, second and fourth enlarged; single row of minute denticles extending distally from tubercle three over 48.1% of dactyl length; mesial surface of dactyl lacking tubercles but having setiferous punctuations instead.

Carpus of cheliped with distinct dorsal furrow; dorsomesial surface with two tubercles; dorsolateral surface with setal punctuations; mesial surface with one large spiniform tubercle subtended by small tubercle proximally; ventral surface with small tubercle on distal articular rim. Merus with one premarginal tubercle dorsally, one distal, one ventrolateral, spiniform tubercle, and ventromesial row

of six, increasing in size distally. Ventral ridge of ischium without tubercles. Ischium of third pereopod (Fig. 1G) with simple hook extending proximally over basioischial articulation, not opposed by tubercles on basis. Coxa of fourth pereopod (Fig. 1G) with vertically disposed caudomesial boss; that of fifth pereopod lacking boss, its ventral membrane not setiferous.

First pleopods not contiguous at base (Fig. 1H), just reaching coxae of third pereopods; central projection (Fig. 1B, C) long, tapering, with subapical notch, angled greater than 90°, slightly longer than mesial process; mesial process conical at base and tapered apically, directed caudolaterally, bent greater than 90°, terminating in single point. Distal margin of proximal segment of lateral ramus of right uropod having nine small spines displayed distally, one larger, movable spine at lateral edge, median spine of mesial uropod ramus small, not overhanging distal margin.

Allotypic female.—Other than secondary sexual characters, differing from holotype in following respects: right chela 65% carapace length; dactyl of chela 1.7 times length of palm, palm of chela with two rows of six tubercles each, eye diameter 46% of rostrum width at base, denticles extending distally over 66.7% of opposable propodus, denticles extending distally over 48.1% of dactyl length.

Annulus ventralis (Fig. 1D) sclerotized throughout, moderately embedded in V-shaped sternum, asymmetrical in shape, formed by two hardened caudal parts: right curved in weak C-shape, left straighter, rounded, segment with a flange projecting under C-shaped portion and forming fossa. Postannular sclerite symmetrical bar shape, thickest height in center.

Morphotypic male, Form II.—Differing from holotype in following respects: palm of chela with two rows of tubercles (first row = 7, second = 3); areola length 33% of carapace length; areola 1.7 times as long as wide; antennal scale 2.5 times as long as wide; right chelae 68% of carapace length; palm length 60.5% of chela length; eye diameter 55% of rostral basal width; merus with row of eight tubercles dorsally, two ventrolateral spines and row of eight ventromesial spiniform tubercles; central projection of first pleopod (Fig. 1E, F) non-corneous and blunt, slightly longer than mesial process. Hook on ischium of third pereopod smaller. Distal margin of proximal segment of lateral ramus of right uropod having 12 tubercles displayed distally.

Type locality.—Holotype, morphotype, and allotype were collected from South Fork Powell River, at bridge crossing on Cracker Neck Road (SSR 616) adjacent to the Big Stone Gap Water Treatment Plant, 6.14 km SE of downtown Big Stone Gap, Wise County, Virginia (36.83606, –82.70552, WGS84). The stream here is high gradient in a narrow ravine with an abundance of boulders, some over ten meters in radius and most greater than three meters.

Disposition of types.—The holotype, allotype, and morphotype are in the collection of the Ohio State University Museum of Biological Diversity Crustacean Collection (OSUMC 8928, 8930, and 8929, respectively), Columbus, Ohio. Paratypes are housed at the National Museum of Natural History, Smithsonian Institution, Washington, D.C. (USNM 1202143, 1202144), Illinois Natural History Survey (INHS 14596), and Carnegie Museum of Natural History (CMNH Acc. No. 38364).

Range and specimens examined.—This species is found only in Wise Co., Virginia in the upper reaches of the South Fork Powell River between the community of Cracker Neck and Big Cherry Reservoir, a stream length of 7 km. Adjacent streams have been searched, but no other populations have yet been found. A total of 57 specimens from five collections from two locations were examined.

VIRGINIA: Wise Co.: OSUMC 6865 – South Fork Powell River upstream Cracker Neck at Virginia (VA) SSR 616 bridge (Cracker Neck Road) adjacent Big Stone Gap Water Treatment Plant, (36.836060, –82.705520, WGS84), R. F. Thoma (RFT) and M. Pucket, 2M-I, 2F, 1F juv., 1 Aug 2007; CMNH Acc. No. 38364 – South Fork Powell River upstream Cracker Neck at VA SSR 616 bridge (Cracker Neck Road) adjacent Big Stone Gap Water Treatment Plant, (36.836060, –82.705520, WGS84), RFT and J. W. Fetzner, Jr., 1M-I, 2F, 4 Oct 2007; OSUMC 6903 – South Fork Powell River 677 m upstream Big Stone Gap Water Treatment Plant at end of Cracker Neck Road (36.829640, –82.702420, WGS84), RFT and J. A. Thoma, 4M-I, 1F, 2M juv., 3F juv., 28 Sep 2007; OSUMC 7035 – South Fork Powell River upstream Cracker Neck at VA SSR 616 bridge (Cracker Neck Road) adjacent Big Stone Gap Water Treatment Plant, (36.847364, –82.711366, WGS84), RFT, 1M-I, 3F, 3M juv., 2F juv., 16 Oct 2008; OSUMC 8658 – South Fork Powell River at Big Stone Gap Water Treatment Plant upstream VA Rt. 616, 6.24 km SE of Big Stone Gap, (36.835930, –82.705420, WGS84), RFT and M. A. Luehrs, 1M-I, 1M-II, 2F, 2M juv., 8F juv., 15 Jul 2011.

Conservation status.—*Cambarus* (*J.*) *magerae* is narrowly distributed and currently found only in a 7 km reach of South Fork Powell River between the community of Cracker Neck and Big Cherry Reservoir. It is best classified as Endangered following Taylor et al. (2007) and Endangered using IUCN criteria (IUCN 2001).

Color notes.—All specimens observed were a dark brownish-green to dark green that can appear to be almost black. An orange band runs along the lateral margin of the chelae. Tubercles on the opposable margins of the chelae fingers are cream-yellow. The tips of chelae fingers can be orange. Lighter colored individuals show mottling created by light and dark patches of the overall body coloration.

Variation.—No geographic variation has been identified in this species.

Size.—The largest individual observed was a female, with a total carapace length of 22.95 mm. The five largest individuals were female. Mature form I males averaged 17.83 mm ($n = 18$, 17.09–18.73), form II males 15.73 mm ($n = 6$, 13.85–18.64), and females 19.11 mm ($n = 22$, 15.83–22.95) carapace length.

Life history.—Collections were made in July, August, September, and October. First form males and females were observed in all months sampled, mature second form males were observed in July. No ovigerous females or young-of-year were observed. The food habits of *C.* (*J.*) *magerae* are unknown.

Crayfish associates.—*Cambarus* (*Jugicambarus*) *magerae* was collected with three other undescribed species. The species appears to be related to *C.* (*Cambarus*) *bartonii* Fabricius, 1798, *C.* (*Puncticambarus*) *robustus* Girard, 1852, and *C.* (*J.*) *dubius* Faxon, 1884.

Relationships.—This species is tentatively assigned to the subgenus *Jugicambarus*. It is speculated as derived from an ancestral population that led to the evolution of *Cambarus* (*J.*) *parvoculus* Hobbs & Shoup, 1947. It is hypothesized the South Fork Powell River population became isolated from the ancestral stock as the Appalachian Mountains eroded into their current state leaving a wide, low gradient, South Fork Powell River mainstem between them and other stocks inhabiting the eastern flanks of Stone Mountain to the west. Neither *C.* (*J.*) *parvoculus* nor *C.* (*J.*) *jezerinaci* are tolerant of warmwater streams, both preferring cold, spring feed, small tributaries.

Comparisons.—Within the genus, *C.* (*J.*) *magerae* is most likely to key out under *C.* (*J.*) *parvoculus* or *C.* (*J.*) *jezerinaci*. Both species have strongly recurved central

Table 2.—Summary of locality data for specimens examined in this study. Abbreviations: #Haplo, number of haplotypes detected at that sampling locality; *n*, number of individuals sampled. Note that some haplotypes were found at more than one locality.

Species	*n*	State	County	Tributary	Latitude	Longitude	#Haplo
C. (*J.*) *magerae*	3	Virginia	Wise	South Fork Powell River	36.83606	−82.70552	1
C. (*J.*) *jezerinaci*	12	Virginia	Lee	tributary to Dry Branch	36.65444	−83.49277	3
	2	Kentucky	Bell	tributary to Clear Creek	36.74288	−83.70293	2
	4	Kentucky	Bell	Centers Branch Creek	36.73006	−83.79775	2
C. (*J.*) *parvoculus*	4	Tennessee	Fentress	Rocky Branch	36.19530	−85.06862	4
	5	Tennessee	Fentress	Big Branch	36.17685	−85.01018	2
	10	Tennessee	Cumberland	Fox Creek	36.06560	−84.93774	2
	1	Tennessee	Cumberland	Little Laurel Creek	35.84066	−85.12354	1
	1	Tennessee	Overton	Little Hurricane Creek	36.15369	−85.13872	1
	3	Tennessee	Morgan	Roadside Ditch	36.01253	−84.52116	3
	5	Tennessee	Roane	tributary to Emory River	35.92930	−84.55756	3
C. (*C.*) *b. cavatus*	1	Kentucky	Bell	tributary Clear Creek	36.74288	−83.70293	1
C. (*J.*) *distans*	10	Kentucky	McCreary	tributary Cumberland River	36.83672	−84.34557	1
C. (*V.*) *pristinus*	2	Tennessee	Cumberland	White Oak Creek	35.96954	−85.20007	1
C. (*J.*) *unestami*	8	Georgia	Dade	Daniel Creek	34.81615	−85.49111	1
C. (*H.*) *longulus*	3	Virginia	Patrick	Dan River	36.59678	−80.44852	2

projections in M-I specimens. The areola width to length ratio of *C.* (*J.*) *parvoculus* ($\bar{X} = 5.7$) and *C.* (*J.*) *jezerinaci* ($\bar{X} = 4.5$) is proportionally greater than in *C. magerae* ($\bar{X} = 2.7$). Correspondingly, the number of punctations across the narrowest portion of the areola is greater in *C.* (*J.*) *magerae* (6–7 vs. 2 or 3). Coloration and size at maturity also differ in *C.* (*J.*) *magerae* with it ($\bar{X} = 17.8$ mm) being much smaller than *C.* (*J.*) *parvoculus* ($\bar{X} = 27.9$ mm) and *C.* (*J.*) *jezerinaci* ($\bar{X} = 21.5$ mm) and dominated by green pigmentation.

Etymology.—It is the senior author's honor to name this species after his mother Bernice Maud (Mager) Thoma. Without her selfless help and encouragement I would never have graduated from college. Both my mother and father supported me in my collegiate efforts but one "thomai" seems about enough. "Big Stone Crayfish" is recommended as the common name for this species in recognition of the proximity of the city of Big Stone Gap, their ownership of all the land on which the species is found, and the predominance of very large boulders in its preferred habitat.

Phylogenetic Results

Seventy-four specimens were sequenced for the COI gene in this study and included: 3 *C.* (*J.*) *magerae* from the type locality; 18 *C.* (*J.*) *jezerinaci* from 3 sites, including the species' type locality; 29 *C.* (*J.*) *parvoculus* from 7 sites (1 specimen from near the type locality); 10 *C.* (*J.*) *distans* Rhoades, 1944 from the type locality; 8 *C.* (*J.*) *unestami* Hobbs & Hall, 1969 from the type locality; 1 *C.* (*C.*) *bartonii cavatus* Hay, 1902; 3 *C.* (*Hiaticambarus*) *longulus* Girard, 1852; and 2 *C.* (*Veticambarus*) *pristinus* Hobbs, 1965 from the type locality (see Table 2). A total of 23 unique haplotypes were recovered from the 74 analyzed sequences. Unique haplotype sequences generated as part of this study are deposited in GENBANK under accession numbers (KM099317–KM099339). These unique haplotypes were divided among the species as follows: *C.* (*J.*) *magerae* ($n = 1$), *C.* (*J.*) *jezerinaci* ($n = 6$), *C.* (*J.*) *parvoculus* ($n = 10$), *C.* (*C.*) *b. cavatus* ($n = 1$), *C.* (*J.*) *distans* ($n = 1$), *C.* (*V.*) *pristinus* ($n = 1$), *C.* (*J.*) *unestami* ($n = 1$), and *C.* (*H.*) *longulus* ($n = 2$).

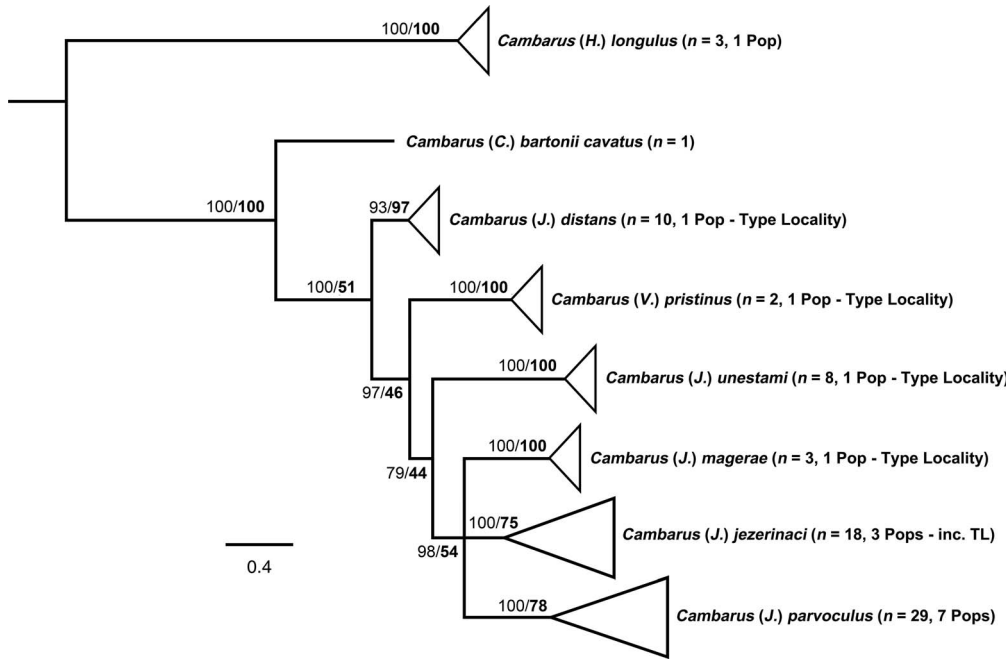

Fig. 2. Bayesian phylogenetic tree depicting relationships among *Cambarus* (*J.*) *magerae* and other closely related species and selected outgroup taxa. Numbers at nodes indicate both posterior probabilities and bootstrap values (in bold), with the latter generated by RAxML, using the GTR+G model and 1000 pseudoreplicate dataset.

The Bayesian phylogenetic tree (Fig. 2) suggests that *C.* (*J.*) *magerae* is most closely related to *C.* (*J.*) *jezerinaci* and *C.* (*J.*) *parvoculus*. However, the relationships among these three taxa were unresolved in the tree due to a short branch length leading to these taxa, which resulted in a lack of branch support at that node of the phylogeny. However, the branches leading to each of these three species were highly supported in both analyses, as were many of the other nodes in the tree.

The specimens of *C.* (*J.*) *magerae* differed from all other species (except *C.* (*J.*) *distans*) by an uncorrected sequence divergence (*p*-distance) of at least 5%

Table 3.—Average genetic distances among species using sequences from the COI gene, uncorrected *p*-distances below diagonal, HKY+G distances above diagonal. For the columns, values in parentheses are the number of sampled individuals and the number of sampled populations (= localities), respectively. **Values** along the diagonal are average *p*-distance estimates within species.

	Magerae (3, 1)	*Jezerinaci* (18, 3)	*Parvoculus* (29, 7)	*Distans* (10, 1)	*Unestami* (8, 1)	*bartonii cavatus* (1, 1)	*Pristinus* (2, 1)	*Longulus* (3, 1)
C. (*J.*) *magerae*	**0.000**	0.0730	0.0806	0.0602	0.0729	0.1187	0.0806	0.3145
C. (*J.*) *jezerinaci*	0.050	**0.021**	0.0729	0.0761	0.0956	0.1361	0.0871	0.3501
C. (*J.*) *parvoculus*	0.054	0.049	**0.009**	0.0786	0.1029	0.1211	0.0997	0.2739
C. (*J.*) *distans*	0.044	0.050	0.052	**0.000**	0.0648	0.0771	0.0503	0.3091
C. (*J.*) *unestami*	0.050	0.057	0.061	0.044	**0.000**	0.1535	0.0945	0.3210
C. (*C.*) *b. cavatus*	0.072	0.075	0.072	0.052	0.080	**N/A**	0.1265	0.2937
C. (*V.*) *pristinus*	0.055	0.055	0.062	0.038	0.058	0.073	**0.000**	0.3143
C. (*H.*) *longulus*	0.120	0.123	0.110	0.115	0.118	0.114	0.118	**0.002**

(range 4.40–12.0%) (see Table 3, lower diagonal). The best model selected by jMODELTEST using the BI criterion was the HKY+G model with the following settings: base = (0.2555, 0.1323, 0.2103), nst = 2, tratio = 4.2716, rates = gamma, shape = 0.1470, ncat = 6, pinvar = 0. These corrected distances ranged from a low of 6.0% to a high of 31.2% for comparisons between *C.* (*J.*) *magerae* and the other species included in the study (Table 3, upper diagonal). These values are typical of divergences seen among other described species of *Cambarus* (JWF, pers. obs.) and are consistent with the recognition of *C.* (*J.*) *magerae* as a new species.

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