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Cambarus (*C.*) *hatfieldi*, a new species of crayfish (Decapoda:Cambaridae) from the Tug Fork River Basin of Kentucky, Virginia and West Virginia, USA

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Abstract

Cambarus (*Cambarus*) *hatfieldi* is a stream-dwelling crayfish that appears to be endemic to the Tug Fork River system of West Virginia, Virginia, and Kentucky. Within this region, it is prevalent in all major tributaries in the basin as well as the Tug Fork River's mainstem. The new species is morphologically most similar to *Cambarus sciotensis* and *Cambarus angularis.* It can be differentiated from *C. sciotensis* by its squamous, subtrinagular chelae compared to the elongate triangular chelae of *C. sciotensis*; its shorter palm length/palm depth ratio (1.9) compared to *C. sciotensis* (2.3); and a smaller areola length/total carapace length ratio (30.4% vs.36.5% respectively). *Cambarus hatfieldi* can be differentiated from *C. angularis* by its smaller areola length/total carapace length ratio (30.4% vs. 36.7% respectively); a smaller rostrum width/rostral length ratio (59.4% vs. 67.2% respectively); its rounded abdominal pleura as compared to the subtruncated pleura of *C. angularis*; the length of the central projection and mesial process of *C. hatfieldi* which both extend to the margin of the gonopod shaft or slightly beyond the margin compared to the central projection of *C. sciotensis* and *C. angularis* where both extend well beyond the margin of the gonopod shaft.

Key words: Crayfish, *Cambarus*, Kentucky, Virginia, West Virginia, Tug Fork River, Appalachian Mountains

Introduction

Cambarus sciotensis Rhoades, 1944, has one of the most disjunct ranges of any *Cambarus* species, with three geographically isolated populations (Jezerinac et al. 1995; Taylor and Shuster 2004) (Jezerinac *et al*. 1995; Taylor and Shuster 2004). The type population occurs in the Scioto River basin in Dublin Ohio, and shares morphological characters with populations in central and southern Ohio. *Cambarus sciotensis* also occurs upstream of Kanawha Falls in the New River system of West Virginia and Virginia; throughout the New River basin, *C. sciotensis* is the dominant large *Cambarus* species. *Cambarus sciotensis* is replaced in the Ohio River mainstem between the Scioto and eastern Kentucky populations and New River populations in the Big Sandy and Kanawha River system of West Virginia by *Cambarus robustus* Girard, 1852. The third population occurs in the Tug Fork River system of Kentucky, Virginia, and West Virginia.

The distribution of *C. sciotensis* in southwestern West Virginia and eastern Kentucky has long been inadequately documented (Jezerianc *et al*. 1995; Z. J. Loughman personal obs.). Jezerinac *et al*. (1995) documented *C. sciotensis* sporadically occurring throughout several watersheds in southwestern West Virginia outside of the New River basin. Recently, ZJL and SAW initiated a statewide survey of crayfishes in West Virginia, with special attention towards determining the ranges of *C. robustus* and *C. sciotensis*. Populations present outside of the New River in West Virginia's Guyandotte River basin of West Virginia, and the Big Sandy River basin of West Virginia and Kentucky excluding the Tug Fork River system of West Virginia and Kentucky were found to be an undescribed species since described as *Cambarus theepiensis* (Loughman *et al*. 2013).

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In 2009 independent crayfish surveys were performed in the Tug Fork River basin, a major tributary of the Big Sandy River, in West Virginia by ZJL and SAW, and in Kentucky by RFT. Both efforts collected what initially was considered *C. sciotensis*, and both noticed the distinct morphological differences from both Scioto and New River *C. sciotensis* populations. What was referred to as *C. sciotensis* in the Tug Fork appeared to have closer morphologic affinities with another species, *Cambarus angularis* Hobbs and Bouchard, 1994*;* which, prior to its description, was included in the *C. sciotensis* complex (Hobbs and Bouchard 1994). In addition to differing from both *C. sciotensis* and *C. angularis*, Tug Fork populations also were distinct from *C. theepiensis*, the Big Sandy River system's dominant tertiary burrowing *Cambarus* species. Subsequent meristic, morphologic and molecular analyses supported the conclusion of an undescribed species of crayfish in the Tug Fork River system in West Virginia, Virginia and Kentucky. This new species differs from both *C. sciotensis* and *C. angularis*, and is formally described herein as *Cambarus hatfieldi*.

Material and methods

All measurements were taken with digital calipers to the nearest 0.1 mm; specimen morphometrics follow Cooper 2006. Total carapace length is abbreviated TCL; postorbital carapace length is abbreviated PCL. DNA was extracted from crayfish pereiopods using DNeasy Blood & Tissue Kit (Qiagen, Inc.). PCR primer pairs for amplifying the mitochondrial cytochrome oxidase I (COI) subunit I gene were previously published in Folmer *et al*. (1994) modified with the addition of universal M13 forward and reverse primers on their 5'-ends: Forward primer, GTAAAACGACGGCCAGGGTCAACAAATCATAAAGATATTG, Reverse primer, CAGGAAACAGCTATGACTAAACTTCAGGGTGACCAAAAAATCA (M13 primer regions underlined). PCR reaction mixtures (from Fisher BioReagents exACTGene® PCR Kit, Fisher Scientific Inc.) contained 1X PCR buffer, 1.5 mM $MgCl_2$, 250 µM final concentration of each dNTP, a 0.3 µM final concentration of each primer, 0.3 U of Taq polymerase, and approximately 100 ng of template DNA in a final volume of 40 µl. PCR conditions were: 94°C for 3 min, followed by 40 cycles for 50 secs, 45°C for 1 min 10 secs, and 72°C for 1 min, and a final 10 min extension at 72°C. PCR products were purified using QIAquick PCR Purification Kit (Qiagen Inc.), and sequenced through SimpleSeq™ through Eurofins MWG Operon (http://www.operon.com).

The COI subunit I gene sequence of *Cambarus (cf.) bartonii cavatus* (GenBank Accession no. AY701190) was used as an outgroup in all phylogenetic analyses. Partial COI gene sequences were aligned using ClustalW (available on MEGA). Phylogenetic reconstruction was implemented using MEGA (Molecular Evolutionary Genetics Analysis) version 5.1 (http://www.megasoftware.net). Statistical support for all trees was obtained from 1,000 bootstrap replicates (only bootstrap values >50% are reported). Pairwise base comparisons of partial COI nucleotide sequences within and between taxa were determined using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/), with calculations for mean and standard error using Excel® ver. 12.3.2 (Microsoft® Corp.). Phylogenetic analyses based on Maximum Likelihood (ML, using Tamura-Nei model, gamma distributed rates among sites, nearest-neighbor-interchange for heuristic tree inference), Maximum Parsimony (MP, using subtree-pruning-regrafting for tree inference) and Neighbor Joining (NJ, using transitions and transversions substitution model based on the number of differences) were implemented on the alignment for partial COI nucleotide sequences, which consisted of 599 nucleotides for 17 sequences.

***Cambarus* (*Cambarus*) *hatfieldi n.* sp.**

Figures 1–5, Tables 1–4

Diagnosis. Body and eyes pigmented. Posterior dorsal region of rostrum concave and deflected anteriorly. Rostrum margins thickened, parallel to base of acumen. Acumen distinctly triangular with prominent dorsally deflected spiniform tubercle at terminus. Areola 2.7–4.5 ($\bar{x}$ = 3.3, *n* = 52, *SD* = 2.5) times as long as wide with 7–9 (mode = 7) punctations across narrowest point. Cervical spines absent. Mandibular, branchiostegal, and orbital regions of carapace with well-developed tubercles. Postorbital ridges short; spiniform, dorsally deflected tubercle present in juveniles and subadults; adult postorbital ridges terminating in either spiniform or truncated tubercles. Suborbital angle acutue. Antennal scale widest in middle, 0.9–2.6 ($\bar{x}$ = 1.9, *n* = 52, *SD* = 0.8) times as long as wide. Total carapace length (TCL) 1.6–2.2 ($\bar{x}$ = 1.9, n = 52, SD = 0.3) times longer than width. Form I and II males possessing hooks on ischium of third pereopods only; hook gently curved at apex, overarching basioischial joint in form I males, not reaching basioischial joint in form II males; hooks not opposed by tubercle on basis. Mesial surface of palm of chelae with single row of 5–9 ($\bar{x}$ = 7.7, n = 52, SD = 0.8) weakly developed tubercles; deep punctations found lateral to tubercles. Dorsal longitudinal ridge of dactyl consisting of several moderately-developed scattered tubercles. Dorsomedian ridge of fixed finger of propodus pronounced. Poorly defined lateral impression at the junction of the fixed finger portion of the propodus. Dactyl and fixed finger with sharp corneous tip. Form I male palm length 63.4–70.8 % ($\bar{x}$ = 67.5%, n = 12, SD = 2.3%) of palm width, form I male palm length 22.4–33.2% ($\bar{x}$ = 29.8%, n = 12, SD = 2.8%) of total propodus length; female dactyl length 52.6–62.3% ($\bar{x}$ = 58.4, n = 21, SD = 2.8%) of total propodus length.

First pleopod of form I male with short terminal elements. Central projection gently tapering distally; recurved > 90º to main shaft of gonopod, with distinct subapical notch. Mesial process directed 90° to shaft, bent cephalolaterally, inflated at its base, tapering to distinct caudal point at or slightly beyond terminance of central projection. Neither process of first pleopod projecting significantly beyond caudal margin of gonopod shaft. Annulus ventralis immovable; distinctly asymmetrical posteriorly; cephalic portion with median trough leading to strongly sculptured central fossa; exaggerated "S" bend in sinus terminating at caudal edge formed by two asymmetrical hardened ridges.

Description of holotypic male, form I. (Figs. 1 A–C, F–I, K–L, 2; Table 1). Body compressed dorsoventrally (Fig. 1A); carapace posterior to cervical groove wider than abdomen. Total carapace length 38.9 mm; postorbital carapace length 33.0 mm. Areola 3.8 times longer than wide, 7 punctations across narrowest part (Fig 1G); length of areola 36.2 % of TCL (42.7 % of postorbital carapace length (PCL). Rostrum weekly excavated; margins thickened, continuous to base of acumen, parallel at midpoint and flared at base; floor of rostrum with numerous punctations. Rostrum 1.8 times longer than wide. Acumen distinctly triangular, ending in dorsally oriented corneous tip (Fig. 1A). Postorbital ridges developed, short, terminating in weak dorsally oriented cephalic tubercles. Suborbital angle acute, lacking tubercle (Fig. 1A). Cervical spine/tubercles absent. Mandibular, branchiostegal, and orbital regions of carapace punctated with well-developed tubercles; highest tubercle density in hepatic region.

Abdomen supraequal in length to carapace, pleura rounded cephaloventrally, angled distoventrally. Lateral margin of terga angulate; lateral margin of second pleura deeply furrowed. Cephalic section of telson with 2 large spines in each caudolateral corner. Proximal podomere of uropod with distal spine on mesial lobe; mesial ramus of uropod with median ridge ending distally in distomedian spine not overreaching margin of ramus; laterodistal spine pronounced. Distal margin of proximal segment of lateral ramus of right uropod having 10 immovable, small spines and 1 lateral, large, movable spine.

Cephalomedian lobe of epistome subtriangular, zygoma moderately arched (Fig. 1K); cephalolateral margins thickened, forming sharp angle at junction with endostyle (Fig. 1K). Body of epistome possessing prominent cephalomedian fovea. Antennal scale broadest in middle (Fig. 1F); lateral margin thickened, terminating in large corneous spine; setiferous. Right antennal scale 5.5 mm long, 2.5 mm wide (Fig. 1F). Tip of right antenna reaching middle of telson when adpressed.

Mesial surface of right chela palm with single row of 9 tubercles (Fig. 1H), length 69.5 % of width; depth 8.6 mm, ventral surface with 0 subpalmar tubercles. Dorsal longitudinal ridge of dactyl developed, mesial margin possessing moderate equal-sized tubercles (Fig. 1F). Dorsomedian ridge of fixed finger of propodus pronounced. Poorly defined lateral impression at junction of fixed finger with the propodus. Dactyl and fixed finger of propodus with sharp, corneous tip.

Carpus with prominent dorsal furrow (Fig. 1H) and 6 weak dorsomesial tubercles; rest of surface with some setiferous punctations; mesial margin with large, procurved spine at midlength, subtended by reduced proximal spine. Distodorsal surface of merus with 8 spiniform tubercles; ventrolateral ridge with 3 small spines and large, corneous distal spine; ventrolateral margin of ischium with 2 small, spiniform tubercles. Carapace depth less than width. Hook on ischium of third pereopods only (Fig. 1L.); hook gently curved at apex, overarching basioischial joint, not opposed by tubercle on basis. Form I gonopod as described in diagnosis (Fig. 1C–D); tip reaching anterior margin of fourth caudomesial boss.

Description of allotypic female. (Fig. 1J, Table 1). Differing from holotype in following respects; carapace depth less than carapace width (15.8 and 20.2 mm, respectively); TCL 37.7 mm, PCL 32.0 mm. Areola 37.1% of

TCL (43.8% of PCL), 3.7 times as long as wide. Rostrum 1.3 times longer than wide. Abdomen length 39.9 mm; abdomen width 45% of abdomen length. Mesial surface of palm of chelae with single row of 8 weak tubercles. Palm length (8.6 mm) 66.1% of palm width (13.0 mm); depth of palm 8.0 mm. Antennal scale 5.9 mm long, 2.7 mm wide. All measurements and counts from right chela. Annulus ventralis as described in diagnosis (Fig. 1J); width of postannular sclerite 33.0% total width of annulus ventralis; first pleopods uniramous, reaching central region of annulus ventralis when abdomen flexed.

TABLE 1. Measurements (mm) of *Cambarus hatfieldi*, new species.

	Holotype	Allotype	Morphotype
Carapace			
Total carapace length	38.9	37.7	26.0
Postorbital length	33.0	32.0	21.8
Length cephalic section	24.7	18.0	16.8
Width	21.6	20.2	13.6
Depth	14.9	15.8	10.6
Length rostrum	8.6	8.2	5.0
Length acumen	2.6	2.8	1.6
Length areola	14.2	14.0	9.2
Width areola	3.7	3.8	1.8
Antennal scale			
Width	2.5	2.7	1.1
Abdomen			
Width	16.6	18.2	10.6
Cheliped (Right)			
Length mesial margin palm	10.7	9.4	6.2
Width palm	15.5	9.3	8.9
Depth palm	8.6	8.0	5.4
Length dactyl	20.4	18.1	10.3
Length carpus	11.0	10.4	7.9
Width carpus	7.3	9.2	6.0
Length dorsal margin merus	13.0	11.3	10.8
Depth merus	7.7	7.4	5.9
Gonopod length	7.1	N.A.	5.1

Description of morphotypic male, form II. (Fig. 1D–E, Table 1). Differing from holotype in the following respects: TCL 26.0 mm and PCL 21.8 mm. Areola length 35.3% of TCL (42.2% of PCL), 5.1 times longer than wide. Rostrum margins subparallel to base of acumen; rostrum 1.9 times as long as wide. Abdomen 14.0 mm long. Mesial row of tubercles on palm of chela with 8 tubercles. Palm length (6.2 mm) 69.7% of palm width (8.9 mm). All measurements and counts from right chela. Antennal scale 2.2 mm long, 0.9 mm wide. Gonopods 23.4% of TCL length. Central projection with complete apex rounded (Fig. 1D–E). Mesial process bluntly tapered, bulbous at base. Hook on ischium of third pereopod small, not reaching basioischial joint.

Size. Form I male (n = 12) TCL size range 19.0–40.1 mm (PCL 23.5–34.7 mm), mean TCL of 33.5 mm. Form II male (n = 19) mean TCL 32.3 mm, size range 18.4–36.6 mm (PCL 18.5–31.7 mm). Non-ovigerous female (n = 21) mean TCL 34.3 mm, size range 26.6–42.8 mm (PCL 21.9–36.5 mm). Ovigerous female (n = 4) TCL size range 26.1–39.5 mm, mean TCL of 32.8 mm. The largest specimen examined was a form I male with a TCL of 40.9 mm (PCL 34.7 mm).

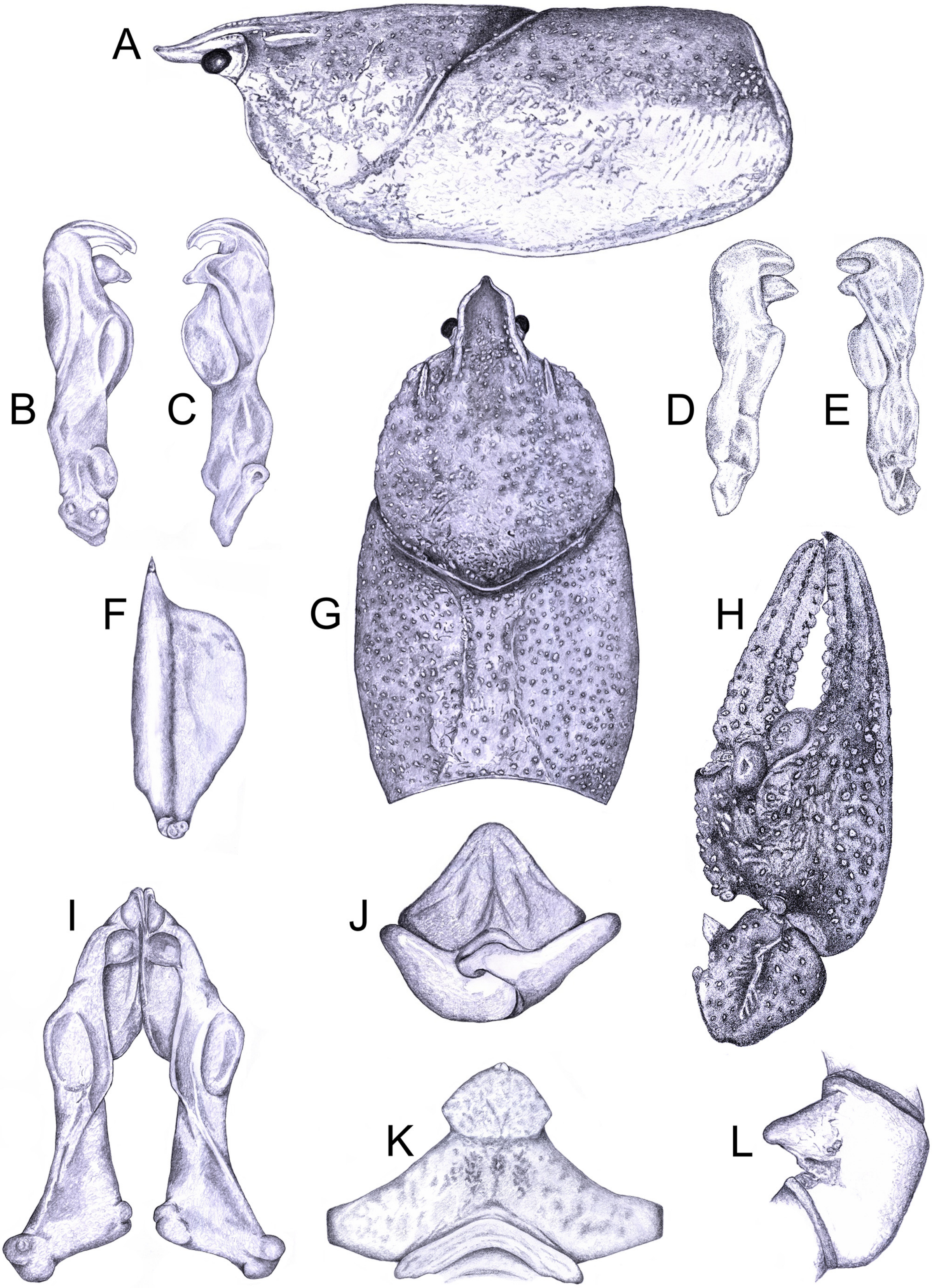

FIGURE 1. *Cambarus hatfieldi* **n. sp.**: A. lateral view of carapace; B. lateral and C. mesial view of first gonopod of form I male; D. lateral and E. mesial view of form II male gonopod; F. antennal scale; G. dorsal view of carapace; H. dorsal view of distal podomere of right cheliped of form I male; I. caudal view of in situ form I male gonopods; J. annulus ventralis; K. epistome; L. ischial hook; A–C, F–I, K–L from holotype; J from allotype; D–E from morphotype.

FIGURE 2. *Cambarus hatfieldi* **n. sp.**, holotype (USNM 1227861) in life.

Color. Carapace ground color orange-brown to pink-brown; posterior and anterior margins of carapace dark. Hepatic and antennal region of carapace punctuated with yellow, white, or cream tubercles. Postorbital ridge reddish to orange-brown. Rostrum margins and acumen distinctly orange to red-orange. Cephalic section of carapace immediately anterior to and including cervical groove black; mandibular abductor scars ranging from black to brown. Lateral margin of antennal scale gray-blue to gray; body of antennal scale gray to blue-gray. Antennal flagellum and antennules blue-gray, with olivaceous hue; dorsal surface of lamellae green-brown to blue-brown; ventral surface tan to olivaceous. Dorsal surface of chelae green, olive green to brown-orange with green highlights, olivaceous to orange mottling; mesial surface of dactyl tubercles amber, orange or red-brown. Denticles on opposable surfaces of fingers yellow, white, or tan. Ventral surface of chelae orange or orange-brown. Dorsal surface of carpus brown, olivaceous or green-brown; occasionally orange; region adjacent to and including furrow olivaceous orange to green; carpus spine orange. Merus orange-brown, green-brown, or olivaceous brown. Podomeres of pereopods light blue, blue-green, or blue-gray; joints of pereopod podomeres white. Dorsal and dorsolateral surface of abdomen olivaceous, green-brown or brown; tergal margins brown, reddish brown or crimson red. Uropods green-brown, with olivaceous tint; margins gray to brown. Ventral surface of abdomen and carapace tan. Dorsal ridge of form I gonopod central projection amber; body of central projection, gonopod, and mesial process tan. Form II gonopod and all associated processes cream. Cephalic portion of annulus ventralis pink to pink-cream; ridge of fossa pink; caudal region of annulus ventralis ranges from pink to cream colored.

Type locality. Mate Creek at CR 6 crossing in Red Jacket, Mingo County, West Virginia (37.64807° N, -82.13524° W). The holotype, allotype, and morphotype were all collected mid channel in a riffle underneath large slab boulders in 0.75 m of water. Mate Creek ranged between 9.0–15.0 m wide, and 0.3–1.0 m deep when the type-series was collected. Stream substrate was composed primarily of gravel, cobbles, boulders and slabs. Anthropogenic impacts included siltation, channelization, and bank erosion. Coal fines were also prevalent in situations with sands and gravels. *Cambarus hatfieldi* also was associated with coarse woody debris snags and leaf packs. The holotype, morphotype, and allotype were collected on 20 Mar 2013 by K. R. Loughman, C. Z. Loughman, and ZJL.

Disposition of types. The holotype, allotype, and morphotype are deposited in the National Museum of Natural History (USNM), Smithsonian Institution, Washington, D. C. (catalogue numbers USNM 122861 122862, 122863, respectively). Paratypes are deposited in the following museums and collections: Carnegie Museum of Natural History, Pittsburgh, PA (CMNH 3829.1, 3829.2), and West Liberty University Astacology Collection, West Liberty, WV (WLU 2000).

Range and specimens examined. *Cambarus hatfieldi* appears to be endemic to the Tug Fork River system and its associated tributaries in Kentucky, Virginia and West Virginia. Kentucky's population occurs in Pike and Martin counties, with substantial populations occurring in Blackberry and Pond Creeks (Fig. 3). Virginia populations are confined to the Dry Fork watershed in Tazewell Co. West Virginia's populations are limited to McDowell, Mingo and Wayne counties, with stable populations occurring in Dry Fork, Mate, Panther, and Horse Creeks. All three states share the Tug Fork mainstem population. *Cambarus hatfieldi* appears to be replaced by *Cambarus theepiensis* in lower reaches of the Tug Fork River where the river loses gradient and gains sandy substrates.

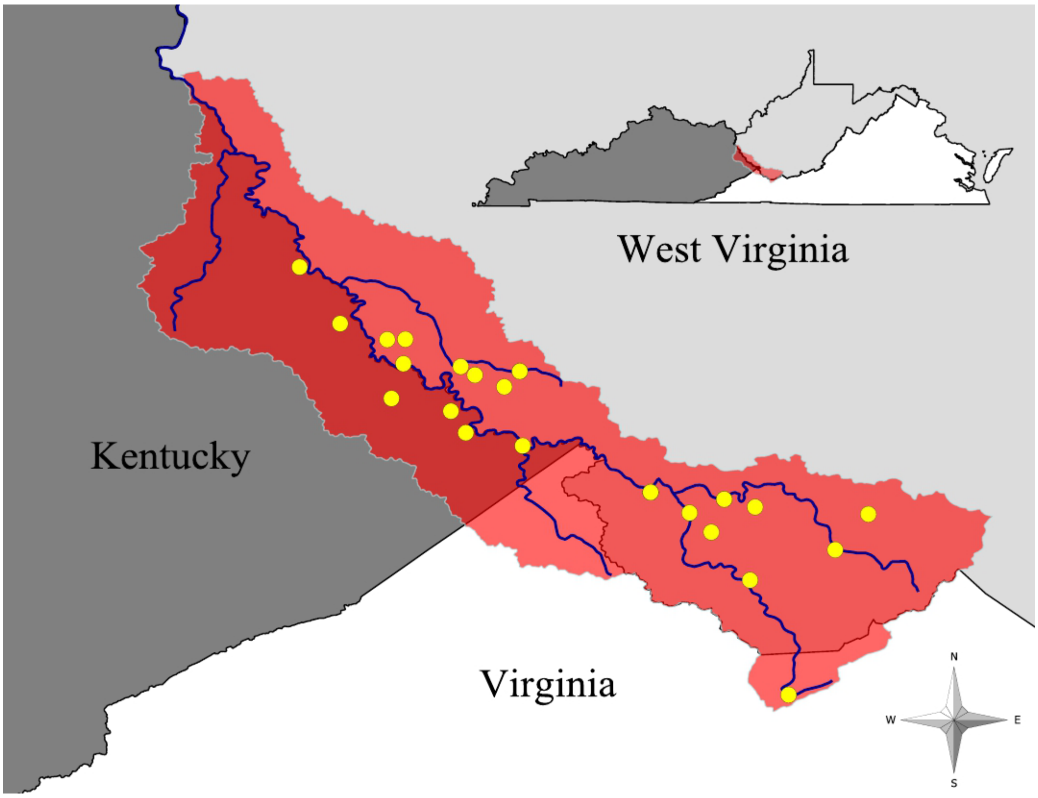

FIGURE 3. Known range of *Cambarus hatfieldi* **n. sp.** in Kentucky, Virginia, and West Virginia. Tug Fork River basin is highlighted in red; yellow circles represent *C. hatfieldi* collection locales.

All West Virginia collections are housed in the West Liberty University Astacology Collection, denoted with the prefix WLU and were collected in the summer of 2009 unless otherwise noted. West Liberty University field crew members for 2009 included ZJL, SAW, Nicole L. Garrison, David A. Foltz, Evan I. Hewitt, and Mathew I. McKinney. All Kentucky and Virginia specimens examined were collected by RFT and are housed in the Ohio State University Museum of Biological Diversity Crustacean Collection. Abbreviations are defined as follows: CR = county road; I = interstate; KY = Kentucky state highway; mi = miles; RD = road; US = U. S. route; VA = Virginia state highway; WV = West Virginia state highway; Rd = road; F = female; OF = ovigerous female; IM = Form I male; IIM = form II male; JV = juvenile.

A total of 256 specimens were examined from the following 24 localities. KENTUCKY: *Martin Co:* (1.) OSUM 7423, Knox Creek upstream of confluence with Tug Fork and intersection of Woodman Creek Road & unnamed road, 1.2 mi. NW of Woodman, 18 Sep 2009, 1 F. *Pike Co*: (2.) OSUM 7423, Knox Creek upstream of confluence with Tug Fork River and intersection of Woodman Creek Road & unnamed road 1.17 mi. NW of Woodman, 17 Sep 2009, 1 F. (3.) OSUM 7428, Peter Creek at Freeburn adjacent to KY 194 1.2 mi. W of Vulcan, 17 Sep 2009, 1 JV. (4.) OSUM 7431, Blackberry Creek adjacent KY Rt. 1056 2.26 mi. S of Matewan, 17 Sep 2009, 1 F, 2 IIM. (5.) OSUM 7423, Pond Creek at Sherondale adjacent US. 119 3.9 mi N of Pinsonfork, 17 Sep 2009, 6 F, 2 IIM, 9 JV. (6.) OSUM 7441, Big Creek adjacent to KY 468 at confluence of Lick Branch 1.29 mi. SW of Nolan, 18 Sep 2009, 3 JV. VIRGINIA: *Tazewll Co*.: (7.) OSUM 8737, Dry Fork of Tug Fork at VA Rt. 636 bridge; 1.42 Km NNW of Rourkes Gap, 10.53 Km NW of Tazewell, 28 Jul 2011, 2 F, 3 IIM, 23 JV. WEST VIRGINIA: *McDowell Co*: (8.) WLU 2, Crane Creek adjacent to CR 5/02, 2.0 mi SE of Avondale, 11 Jun 2009, 10 F, 5 IIM. (9.) WLU 3, Elk Horn Creek 0.1mi NW of intersection of Coe Street and E Main Street in Kimball, 13 Jun 2009, 2 F, 2 II M. (10.) WLU 7, Horse Creek adjacent to CR 1–4, 0.5 mi from Johnny Cake Road 3.4 mi SE of Panther, 10 Jun 2009, 2 OF. WLU 26, 10 Jul 2009, 22 F, 24 IIM, 16 JV. (11.) WLU 9, Dry Fork adjacent to SR 80, 2.7 mi SE of Iaeger, 1.0 mi SE of Apple Grove, 10 Jun 2009, 4 F, 2 IIM, 1 JV. (12.) WLU 10, Barrenshe Creek adjacent to CR 83–20/CR 83 intersection in Yukon, 10 Jun 2009, 1 OF, 2 F, 1 IM, 3 IIM, 2 JV. (13.) WLU 12, Tug Fork River parallel to SR 103, 0.6 mi NW of Gary, 10 Jun 2009, 3 F, 1 IIM. (14.) WLU 13, Clear Fork parallel to CR 2, 1.1 mi W of CR 2-1 in Coalwood, 10 Jun 2009, 2 F, 8 IIM, 15 JV. (15.) WLU 23, Tug Fork at Clear Fork confluence in Roderfield, 13 Jun 2009, 9 F, 5 IIM. (16.) WLU 25, Spice Creek adjacent to US 52, 0.3 mi SE of Erin, 13 Jun 2009, 6 F, 10 IIM. (17.) *Mingo Co*: (17.) WLU 3, Left Fork of Gilbert Creek adjacent to CR 13 & CR 13-4 junction, 2.4 mi SE of Baisden, 11 Jun 2009, 1 F, 4 IIM. (18.) WLU 14, Pigeon Creek parallel to US 52 in Musick, 11 Jun 2009, 1 OF, 7 F, 4 IIM. (19.) WLU 16, Pigeon Creek at SR 65/ US 52 intersection 2.8 mi W of Varney, 11 Jun 2009, 1 F. (20.) TYPE SERIES (USNM 12861 holotype, USNM 122862 allotype, USNM 12863 morphotype; CMNH 3829.1,3829.2 paratypes, WLU 2000 paratypes, Mate Creek at CR 6 crossing in Red Jacket, 20 Apr 2013, 12 F, 9 IM, 3 IIM, 11 JV. (21.) WLU 18, Mate Creek parallel to CR 6, 0.1 mi NW of Mark's Branch Rd in Newtown, 11 Jun 2009. 2 F. (22.) Sycamore Creek parallel to US 52, 0.2 mi N of SR 49 in Williamson, 11 Jun 2009, 4 F, 3 IIM. (23.) WLU 20, Buffalo Creek adjacent to CR 14, at intersection with Mullberry Street in Chattaroy, 11 Jun 2009, 2 F, 1 IM, 5 IIM, 2 JV. *Wayne Co*: (24.) WLU 22, Mill Creek at intersection of CR 36-3/CR 34-3, 2.75 mi W of Radnor, 11 Jun 2009, 2 F, 2 IIM.

Conservation status. It is recommended *Cambarus hatfieldi* be listed as vulnerable (V) according to the American Fisheries Society criteria (Taylor *et al*. 2007), and assigned a G3 ranking according to the global conservation criteria (Masters 1991) for conservation listing as a consequence of its limited range. *Cambarus hatfieldi* should be listed as near threatened (NT) using the International Union for the Conservation of Nature (IUCN 2001) criteria due to its narrow distribution. All of the range of *C. hatfieldi* occurs in portions of West Virginia, Virginia, and Kentucky that currently undergo extensive amounts of surface mining. The majority of streams harboring *C. hatfieldi* experience elevated siltation rates and high conductivity due to mine drainage issues (Pond *et al*. 2008). At present, the impact of environmental change on *C. hatfieldi* populations remains unknown.

Habitat and life history notes. *Cambarus hatfieldi* occurs in small to large, moderate to high gradient streams with substrates composed of cobbles, boulders and slabs. Slab boulders are the preferred refuge of *C. hatfieldi*, though when absent course woody debris snags, leaf packs, and rootwads are readily used as shelter. As stream gradient decreases and sandy substrates pervade, *C. hatfieldi* numbers decline (ZJL, personal observation). In situations with these habitat parameters in lower reaches of the Tug Fork River, *C. hatfieldi* is replaced by *C. theepiensis.*

Cambarus hatfieldi male reproductive form state is seasonal. Nine percent of males collected in June and July were first form, compared to 89% collected in March and April. These results suggest the majority of males molt from form II to form I in late summer/early fall, with an additional molting event occurring in May/Jun back to form II. Females collected from Mate Creek, Mingo Co. West Virginia in March and April 2013 exhibited active glair glands. Three ovigerous females were collected from Horse Creek, McDowell Co. West Virginia and a single female with stage 1 instars and unhatched eggs was collected on 11 Jul 2009 from Barenske Creek, McDowell Co. West Virginia (Table 4). Hatched eggs were still attached to the Barenske Creek females pleopods, indicating hatching had occurred very close to the time of capture (ZJL, personal observation). All ovigerous females were taken from embedded boulders in slack water environments with sandy substrates (ZJL, personal observation). Young of the year were collected in September 2011 from the same environs ovigerous females were collected from in Horse Creek during the preceding years.

Crayfish associates. *Cambarus (Cambarus.) hatfieldi* has been collected with *Cambarus (C.) bartonii cavatus* Hay, 1902, *Cambarus* (*Jugicambarus*.) *dubius* Faxon, 1884, *Cambarus* (*Puncticambarus*.) *veteranus* Faxon, 1914, and *Orconectes (Procericambarus) cristavarius* Taylor, 2000.

Variation. Morphological variation was homeoplasic across *Cambarus hatfieldi's* range, with little variation observed between similar age cohorts occurring in different streams. Ontogenic morphologic variation does occur in the following respects. Juvenile rostrums and chelae are noticeably elongate compared to adults. With age, both structures become both broader and deeper in form. Mesial margin tubercles on chelae are reduced in juveniles and become steadily more pronounced as adulthood is reached. Tubercles on the post-orbital ridge and acumen are always spinose in juveniles, compared to adults where they can either be spinose or truncated. Coloration also differs between juveniles and adults, with juvenile coloration dominated by various shades of brown. With maturity, blues, greens, and grays begin to dominate the walking legs, cephalothorax, and abdomen as described in the color section of the manuscript.

Relationships and comparisons. *Cambarus hatfieldi* is placed in the subgenus *Cambarus* based on the presence of a subapical notch in the form I gonopod and the lack of a well-developed mesial second tubercle row on the palm (Hobbs 1969). Among described members of the subgenus, *C. hatfieldi* is most similar to *C. sciotensis* and *C. angularis* in overall body size and shape and thickening of the rostral margins.

Meristic percentages/ratios that distinguish *C. hatfieldi* from *C. sciotensis* include areola length/carapace length, propodus length/areola length, two chelae ratios and size of the central projection and mesial process in comparison to gonopod shaft in form I-males. Areola length on average represents 30.4 % (n = 52; SE ± 3.9%) of the carapace length compared to 36.5% (n = 30; SE ± 0.1%) in *C. sciotensis*. *Cambarus hatfieldi* areola length is 2.3 (n = 52; SE ± 0.2) times the propodus length compared to 2.8 (n = 30; SE ± 0.2) times in *C. sciotensis*. *Cambarus hatfieldi's* palm length/dactyl length ratio is smaller ($\bar{x}$ = 1.9; n = 52; SE ± 0.3) than *C. sciotensis's* ($\bar{x}$ = 2.3; n = 48; SE = 2.1–2.6); palm depth to palm length ratio is also smaller in *C. hatfieldi* ($\bar{x}$ = 1.4; n = 52; SE ± 0.1) compared to *C. sciotensis* ($\bar{x}$ = 1.8; n = 30; SE ± 0.3).

The length of *C. hatfieldi* central projection and mesial process both extend to the margin of the shaft of the gonopod and not beyond compared to *C. sciotensis* with the central projection extending beyond the margin of the gonopod shaft (Fig. 4). *Cambarus hatfieldi* can be distinguished from Scioto River *C. sciotensis* easily by the absence of a second row of mesial tubercles on the chelae palm, both New and Scioto River *C. sciotensis* also have noticeably elongate chelae compared to *C. hatfieldi's* squamous, truncated chelae. Finally, *C. hatfieldi's* average total body length is smaller ($\bar{x}$ = 67.6 mm; n = 52; SE ± 7.3 mm) as an adult compared to *C. sciotensis* ($\bar{x}$ = 80.5 mm; n = 30; SE ± 6.5 mm).

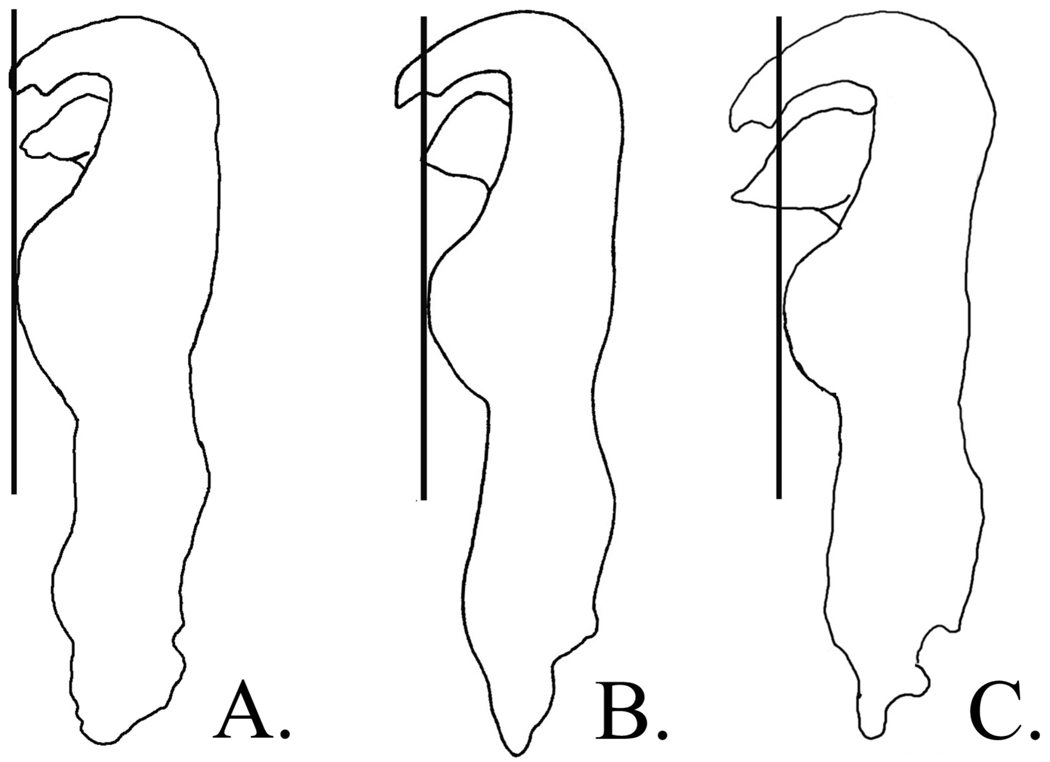

FIGURE 4. Form I gonopod's of (A.) *Cambarus hatfieldi*, (B.) *Cambarus sciotensis*, and (C.) *Cambarus angularis*.

Cambarus angularis and *C. hatfieldi* share in common short, subrectriangular chelae with swollen palms and a single row of mesial tubercles on the chelae. *Cambarus hatfieldi* can be differentiated from *C. angularis* by the rostrum width/length ratio, areola length/carapace length ratio, shape of the cephalothorax, and length of the central projection and mesial process in comparison to gonopod shaft in Form I-males (Fig. 4). *Cambarus hatfieldi* rostrum width constitutes a smaller percentage of the rostrum length ($\bar{x}$ = 59.4%; n = 52; SE ± 5.2%) compared to *C. angularis* ($\bar{x}$ = 67.2%; n = 22; SE ± 8.3%), and a smaller areola length/carapace length ratio ($\bar{x}$ = 30.4%; n = 52; SE ± 2.1%) compared to *C. angularis* ($\bar{x}$ = 36.7%; n = 22; SE ± 1.7%).

Cambarus hatfieldi's abdominal pleura are rounded ventrally compared to *C. angularis's* subtruncate pleura. Dorsally *C. angularis* cephalon is anteriorly swollen compared to *C. hatfieldi* cephalon, which begins to taper anteriorly at the junction of the cephalon with the cervical groove. The length of *C. hatfieldi* central projection and mesial process both extend to the caudal margin of the shaft of the gonopod but not beyond when compared to *C. angularis* where both extending beyond the caudal margin of the gonopod shaft (Fig. 4). Finally, like *C. sciotensis*, *C. angularis'* total body length on average is larger ($\bar{x}$ = 84.1 mm; n = 22; 9.8 mm) than *C. hatfieldi* ($\bar{x}$ = 67.6 mm; n = 52; SE ± 7.3 mm).

Trees resulting from ML, MP and NJ analyses were identical in their placement of taxa within clades. The phylogenetic tree based on ML analysis is shown in Fig. 5. *Cambarus sciotensis* sequences form two groups, New River Basin, WV (Glade Creek, Meadow River, Cherry River, Bluestone River, Gauley River, and Whitewater Branch) and Scioto River, OH, clustered separately and formed a large, well-supported clade. Both groups (i.e., New River Basin and Scioto River) are phylogenetically related (Fig. 5). *Cambarus hatfieldi* sequences (from Browns Creek and Mate Creek, WV) are more distantly related to *C. sciotensis* and are placed in a well-supported cluster outside of the above mentioned clade consisting of *C. sciotensis* taxa.

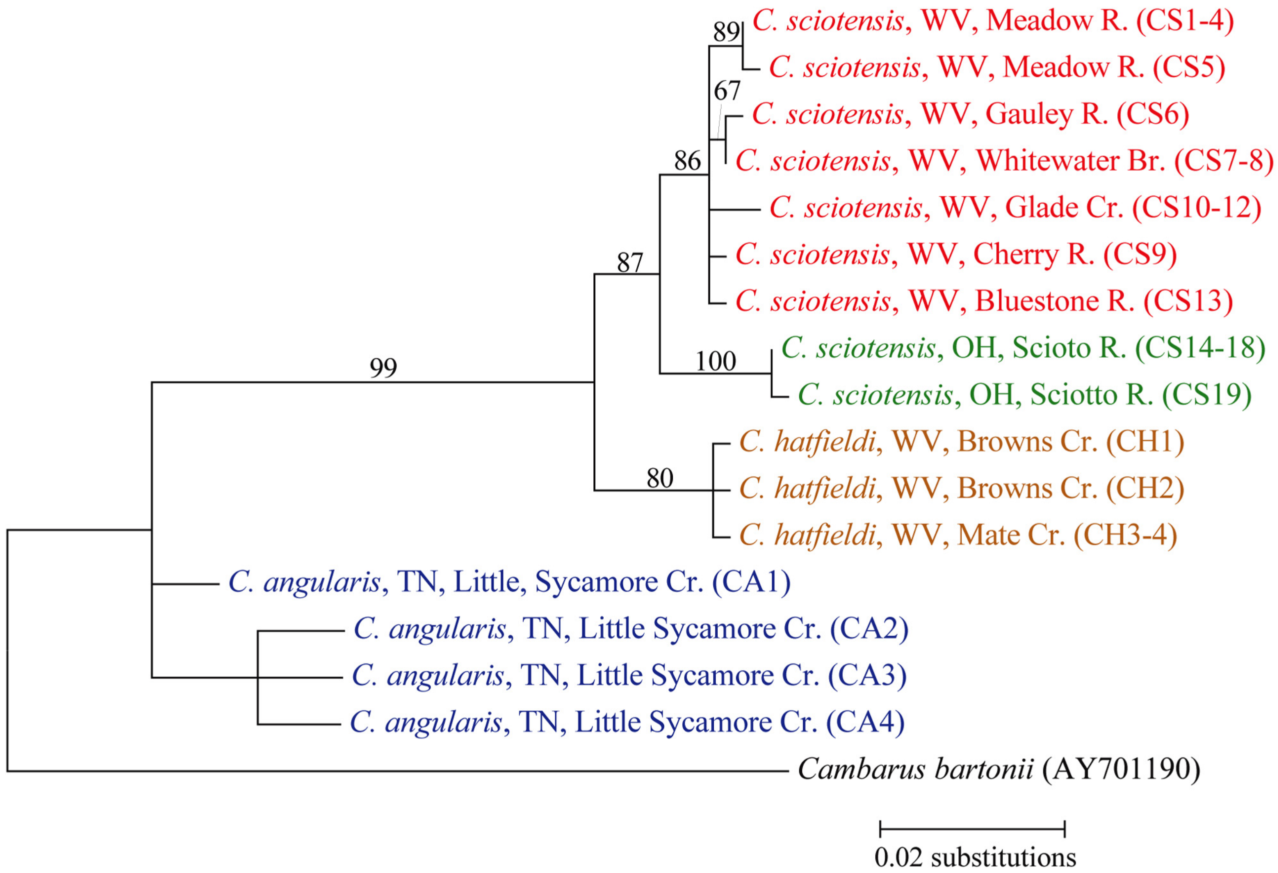

FIGURE 5. Maximum Likelihood tree of partial COI sequences from analysis of 17 unique sequences (599 bp) retrieved in this study. The species and locations of *Cambarus* sp. are listed in Table 3. The COI gene of *C. bartonii* was used as the outgroup. Accession numbers of sequences are indicated in parentheses. Bootstrap values from 1,000 replicates are indicated at the nodes of branches (if >50). The scale bar represents the number of nucleotide changes.

Pairwise nucleotide sequence divergence rates within the (i) *C. sciotensis* clade consisting of sequences from New River Basin, WV, (ii) *C. sciotensis* cluster from Sciotto River, OH, (iii) *C. hatfieldi* clade (from Browns Creek, Mate Creek, WV), and (iv) *C. angularis* from Sycamore Creek, TN, were 0.002–0.008, 0.002, 0.002–0.005 and 0.022–0.037, respectively. In contrast, *C. sciotensis* from New River Basin, WV, and *C. sciotensis* from Scioto River, OH, diverged on average, 0.026 (approx. 3%) and 0.029 (approx. 3%), respectively, from *C. hatfieldi* (from Browns Creek, Mate Creek), WV (Table 2). It is our hypothesis that divergence results indicate that *C. hatfieldi* is an incipient species, and likely diverged from Teays River *C. sciotensis* populations sometime in the Pleistocene epoch. GenBank accession numbers, specimen locations are provided in Table 3.

TABLE 2. Proportion of uncorrected base substitutions (from sequence comparisons of 599 bp) between all taxa (as average values) from the identified clusters (above diagonal) and associated standard errors (below diagonal). Analyses were performed using ClustalW2. New River sites include Glade Creek, Meadow River, Cherry River, Bluestone River, Gauley River, Whitewater Branch; Tug Fork sites include Browns Creek and Mate Creek. Both the Scioto River and Sycamore Creek represent type locations for their representative species.

	C. sciotensis New River Basin, WV	*C. sciotensis* Sciotto River, OH	*C. hatfieldii* Tug Fork River, WV	*C.angularis* Sycamore Cr, TN.
C. sciotensis New River Basin, WV		0.019	0.026	0.057
C. sciotensis Sciotto River, OH	0.001		0.029	0.061
C. hatfieldii Tug Fork River, WV	0.000	0.000		0.055
C.angularis Sycamore Cr, TN.	0.001	0.002	0.001	

Three additional *Cambarus* species occur in the Tug Fork River system that can easily be differentiated from *C. hatfieldi*. *Cambarus theepiensis* replaces *C. hatfieldi* in the Big Sandy River system, but is syntopic with *C. hatfieldi* in lower reaches of the Tug Fork River (ZJL personal observation). *Cambarus bartonii cavatus* occurs throughout the headwaters of the Tug Fork River system, and is the dominant species in ephemeral streams throughout the watershed. Both *C. theepiensis* and *C. b. cavatus* possess two rows of tubercles on the mesial surface of the chelae's palm, and 1–2 subpalmer tubercles on the ventral surface of the chelae. *Cambarus hatfieldi* possesses a single row of adpressed tubercles on the mesial surface of the palm and lacks subpalmer tubercles. *Cambarus veteranus* is syntopic with *C. hatfieldi* in mid to headwaters reaches of Tug Fork, though noticeably rarer than the latter (ZJL and RFT, personal observation). *Cambarus veteranus* rostrum is lanceolate compared to *C. hatfieldi's* broad rostrum. In addition to rostrum morphology, *C. veteranus* possess strong cervical spines; *C. hatfieldi* lacks both cervical spines and cervical tubercles.

Distribution of *C. hatfieldi, C. theepiensis*, and *C. sciotensis* in Kentucky and West Virginia. In light of *C. hatfieldi's* description herein, and the recent description of *C. theepiensis*, the distribution of *C. sciotensis* in both Kentucky and West Virginia comes into question. *Cambarus sciotensis* previously was recorded in the Big and Little Sandy River drainages of Kentucky, and hadn't formerly been documented in the Tug Fork drainage in the state (Taylor and Schuster 2004). Currently, populations previously recognized as *C. sciotensis* in the Big and Little Sandy River watersheds of Kentucky are synonymized under *C. theepiensis* (Loughman *et al.* 2013). Records for *C. sciotensis* do exist for Kentucky in Tygart Creek, a direct tributary to the Ohio River in the vicinity of the Scioto River confluence with the Ohio River. At present Tygart Creek and streams in its associated watershed possess the only populations of *C. sciotensis* known to occur in Kentucky. Thoma (2010) was the first to document *C. hatfieldi* in Kentucky, and reported the species as *C. angularis.*

In West Virginia, *C. sciotensis* is distributed in the greater New River system upstream of Kanawha Falls, Kanawha County as well as the Kanawha River mainstem and tributaries immediately downstream of Kanawha Falls. *Cambarus robustus* replaces *C. sciotensis* downstream of Kanawha Falls, and is the dominant tertiary burrowing *Cambarus* throughout the Coal, Upper, and Lower Kanawha drainages in West Virginia. What previously was documented as *C. sciotensis* in the Guyandotte and Twelvepole systems of West Virginia (Jezerinac *et al*. 1993; Loughman *et al*. 2009) is synonymized with *C. theepiensis* (Loughman *et al*. 2013).

TABLE 3. Genbank accession numbers and locations for *Cambarus sciotensis*, *Cambarus hatfieldi,* and *Cambarus angularis* sequences used in this study.

Specimen#	GenBank Accession #	Species	Location	Coordinates
CS1	KF437297	*C. sciotensis*	Meadow River, WV	37.446123 N 81.120730 W
CS2	KF437298	*C. sciotensis*	Meadow River, WV	37.446123 N 81.120730 W
CS3	KF437299	*C. sciotensis*	Meadow River, WV	37.446123 N 81.120730 W
CS4	KF437300	*C. sciotensis*	Meadow River, WV	37.446123 N 81.120730 W
CS5	KF437301	*C. sciotensis*	Meadow River, WV	37.446123 N 81.120730 W
CS6	KF437302	*C. sciotensis*	Gauley River, WV	38.290968 N 80.640797 W
CS7	KF437303	*C. sciotensis*	Whitewater Branch, WV	38.274921 N 80.9324328 W
CS8	KF437304	*C. sciotensis*	Whitewater Branch, WV	38.274921 N 80.9324328 W
CS9	KF437305	*C. sciotensis*	Cherry River, WV	38.254229 N 80.510992 W
CS10	KF437306	*C. sciotensis*	Glade Creek, WV	37.704200 N81.052423 W
CS11	KF437307	*C. sciotensis*	Glade Creek, WV	37.704200 N81.052423 W
CS12	KF437308	*C. sciotensis*	Glade Creek, WV	37.704200 N81.052423 W
CS13	KF437309	*C. sciotensis*	Bluestone River, WV	37.446120 N81.120730 W
CS14	KF437310	*C. sciotensis*	Scioto River, OH	40.141670 N 83.11968 W
CS15	KF437311	*C. sciotensis*	Scioto River, OH	40.141670 N 83.11968 W
CS16	KF437312	*C. sciotensis*	Scioto River, OH	40.141670 N 83.11968 W
CS17	KF437313	*C. sciotensis*	Scioto River, OH	40.141670 N 83.11968 W
CS18	KF437314	*C. sciotensis*	Scioto River, OH	40.141670 N 83.11968 W
CS19	KF437315	*C. sciotensis*	Scioto River, OH	40.141670 N 83.11968 W
CA1	KF437316	*C.angularis*	Little Sycamore Creek, TN	36.442270 N 83.51006 W
CA2	KF437317	*C.angularis*	Little Sycamore Creek, TN	36.442270 N 83.51006 W
CA3	KF437318	*C.angularis*	Little Sycamore Creek, TN	36.442270 N 83.51006 W
CA4	KF437319	*C.angularis*	Little Sycamore Creek, TN	36.442270 N 83.51006 W
CH1	KF437320	*C.hatfieldii*	Browns Creek, WV	37.443050 N 81.56886 W
CH2	KF437321	*C.hatfieldii*	Browns Creek, WV	37.443050 N 81.56886 W
CH3	KF437322	*C.hatfieldii*	Mate Creek, WV	37.648070 N 82.13524 W
CH4	KF437323	*C.hatfieldii*	Mate Creek, WV	37.648070 N 82.13524 W

TABLE 4. Pleopodal egg diameters and instar condition for *Cambarus hatfieldi,* **n. sp.** ED denotes average egg diameter in mm; asterisk denotes incomplete complement of eggs or instars.

Stream	Date	Female TCL	Condition of eggs/instars
Horse Creek, McDowell Co. WV	10 July 2009	26.1	13 attached eggs*; ED = 1.9
Horse Creek, McDowell Co. WV	10 July 2009	33.3	33 attached eggs; ED = 1.6
Horse Creek, McDowell Co. WV	10 July 2009	39.5	36 attached eggs; ED = 1.8
Barenske Creek, McDowell Co. WV	11July 2009	32.1	64 attached instars; 3 attached eggs* ED = 1.5

Resultant of the description of C. *hatfieldi* and *C. theepiensis*, the distribution of *C. sciotensis,* as it is currently is understood, is disjunct with populations in the Scioto River basin of Central Ohio as well as direct Ohio River tributaries in Kentucky near the Ohio /Scioto River confluence and in the New River system of West Virginia and Virginia. Both populations were likely connected via the ancient Teays River, which is evident given the low COI divergence values exhibited between both populations (Table 2). That being said, Scioto and New river populations of *C. sciotensis* differ morphologically and are readily identifiable from each other using morphological characters (Z. J Loughman and R. F. Thoma personal observation). Given morphologic and zoogeographic differences, further taxonomic investigation is warranted between the Scioto and New River populations of *C. sciotensis*.

Etymology. Latinized form of Hatfield in honor of the Hatfield and McCoy feud which occurred in the Tug Fork River Valley of Kentucky and West Virginia in the 1860s–1870s.

Common name. Tug Valley Crayfish.

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