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Description of a new stream-dwelling species of *Cambarus* (Decapoda: Cambaridae) from Virginia and Pennsylvania (USA) with notes on its distribution, ecology, and conservation status

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Abstract

A new species of crayfish, *Cambarus emegi* **sp. nov.**, is described from the upper James and Roanoke River basins in Virginia and the lower Delaware River basin in Pennsylvania, where it occurs in streams and small rivers. The species was formerly part of the *Cambarus acuminatus* species complex (*Cambarus* species C complex), a long-standing taxonomic enigma that is widely distributed across the Atlantic slope drainages of eastern North America. *Cambarus emegi* **sp. nov.** can be distinguished from *Cambarus acuminatus* due to the absence of a well-defined subpalmar tubercle, greater number of punctations across its areola, and differences in the mesial process of the form I male gonopod. Coloration and pattern in life differ between the two species. Additionally, the distributions of *C. emegi* **sp. nov.** and *C. acuminatus* are separated by hundreds of kilometers. The ranges of two other undescribed members of the *Cambarus* species C complex border that of *C. emegi* **sp. nov.** in Virginia. These species do not appear to overlap geographically; *C. emegi* **sp. nov.** is confined to the Ridge and Valley physiographic province, whereas the two undescribed species are found in the Blue Ridge and northern inner Piedmont regions. *Cambarus emegi* **sp. nov.** is morphologically similar but visually and genetically different from the undescribed species directly to the south in the upper Roanoke River system**.** *Cambarus emegi* **sp. nov.** is both visually and morphologically similar but genetically distinct from the undescribed species immediately downstream in the James River basin. *Cambarus emegi* **sp. nov.** is threatened by several potentially interacting factors including invasive crayfishes, narrow range, and urban and agricultural development. Alarmingly, the species is being rapidly replaced by invasive *Faxonius* species across a substantial part of its range. This paper represents an important step in providing the information needed to conserve and manage *Cambarus emegi* **sp. nov.**

Key words: *Cambarus* species C, *Cambarus acuminatus*, new species, Ridge and Valley, Northern Piedmont, Delaware, James, Roanoke, invasive crayfish

Introduction

The *Cambarus acuminatus* Faxon, 1884 complex, i.e., *Cambarus* species C (*C.* sp. C) [*sensu* Hobbs & Peters (1977)], is a poorly understood group of morphologically-similar crayfishes with a widespread distribution long assumed to extend from the Patapsco River drainage in Maryland southward to the Santee River basin in South Carolina (Hobbs 1972, 1974, 1989; see Williams *et al.* (2019) for a thorough discussion of *C. acuminatus* and the *C.* sp. C moniker). In 2000, an undescribed member of this complex was discovered in Valley Creek, a tributary to the Schuylkill River in southeastern Pennsylvania (Lieb *et al.* 2008), representing a substantial northward extension of the known range

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of the species complex. Subsequent surveys throughout eastern Pennsylvania revealed that the undescribed species has a very limited distribution in Pennsylvania, with reproducing populations restricted to six small drainages in the southeastern part of the state, all occurring in a rapidly urbanizing area within approximately 30 km of Philadelphia, one of North America's largest cities (Lieb *et al.* 2011a, b; Williams *et al.* 2020; unpublished data, D.A. Lieb). Additionally, in recent years, invasive crayfishes have been introduced to those drainages, resulting in substantial reductions or extirpation of *C*. sp. C from invaded sites, indicating that management intervention may be needed to protect extant populations in Pennsylvania (Lieb *et al.* 2011b). Unfortunately, uncertainty in its taxonomic status presents a substantial barrier to effective management.

In an attempt to resolve this taxonomic uncertainty, Fetzner & Crandall (2013) collected members of *C*. sp. C from 42 sites scattered across the range of the complex for genetic analysis. Their results suggested *C*. sp. C from southeastern Pennsylvania was similar genetically to individuals collected from the middle James and upper Rivanna Rivers in central Virginia; however, they could not definitively determine whether the species is native or introduced to Pennsylvania (Williams *et al.* 2020). As a result, Pennsylvania populations have remained in limbo with conservation and management intervention awaiting taxonomic clarification.

Williams *et al.* (2019) redescribed and circumscribed *Cambarus acuminatus*, a name that had previously been used interchangeably with *C*. sp. C and restricted its distribution to the Saluda River drainage in northwestern South Carolina from which it was described by Faxon in 1884. This action was significant as it effectively "freed up" the entirety of the *C*. sp. C complex for taxonomic investigation. In the same year, Perkins *et al.* (2019) described *Cambarus franklini* Perkins, Williams & Russ, 2019 from the upper Catawba River basin in western North Carolina, splitting it from the complex. Four years later, Perkins *et al.* (2023) described two narrow endemics (*Cambarus lapidosus* Perkins, Williams & Russ, 2023 and *Cambarus burchfielae* Perkins, Williams & Russ 2023) from the upper Yadkin River system in western North Carolina, not only removing them from the historic conceptual umbrella of *C*. sp. C but revising our understanding of the complex more generally. These three publications continued and expanded upon earlier efforts by John E. Cooper in North Carolina to solve the *C*. sp. C taxonomic riddle (Cooper 2001; Cooper & Cooper 2003, 2006; Cooper & Price 2010).

To date, members of the *C*. sp. C complex have been collected from at least one site in each HUC 10 (10-digit Hydrologic Unit Code) watershed within which it occurs, from southeastern Pennsylvania southward to the vicinity of Columbia, South Carolina. In total, over 1000 sites have been systematically sampled. Results of preliminary analyses of a multi-locus molecular dataset indicate the potential for dozens of taxa within the complex. We were also able to confirm that the member of the *C*. sp. C complex that occurs in Pennsylvania is an undescribed species. Additionally, populations that occur in Pennsylvania were found to be genetically affiliated with populations of the complex occurring in the upper James and upper Roanoke River drainages in Virginia, indicating that these disjunct populations belong to the same undescribed species. As in Pennsylvania, populations in Virginia are threatened by invasive crayfishes and are an immediate conservation concern. The objectives of this paper are to describe this species and provide data pertinent to its protection and management including information on its distribution, ecology, response to invasive crayfish introductions and habitat degradation, and conservation status.

Material and methods

Field methods and morphometrics.—Specimens were captured from running waters of all sizes (small headwater streams to large rivers) either by seine, dip net, snorkeling, electrofishing, or by hand when turning over rocks or by kick sampling gravel, cobbles, slab boulders, root masses, aquatic vegetation, woody debris, and leaf/detrital accumulations. A variety of habitats were sampled ranging from high velocity riffles and runs to moderate to low velocity eddies and pools; banks, main channel, and side channel areas were sampled. Standing waters were not surveyed for this study except for an impounded section of a small headwater stream in the Ridley Creek basin in Pennsylvania. At most sites, all habitats were sampled with the objective to collect all the epigean crayfish species present.

Crayfish were preserved in the field in 80–95% non-denatured EtOH. After removing gill tissue from individuals for DNA extraction (see below), we transferred the specimens into 70% EtOH for permanent storage in the North Carolina Museum of Natural Sciences (NCSM) Non-Molluscan Invertebrates (NMI) Collection.

All measurements were made with digital calipers to the nearest 0.1 mm, following those used by Loughman

et al. (2017). The following abbreviations are used below: TCL for total carapace length, and PCL for postorbital carapace length. All specimens used in this study, with the exception of five paratypes (see below in Taxonomy: Type material), are deposited in the NCSM NMI Collection.

DNA extraction, amplification, and sequencing.—We isolated total genomic DNA from gill tissue using a NucleoSpin 96 Tissue Core Kit (Machery-Nagel Inc., Allentown, PA, USA) following manufacturer protocols. We amplified a region of the mtDNA gene cytochrome *c* oxidase subunit I (COI) using the primer pairs LCO1490 and HCO2198 (Folmer *et al.* 1994) or mlCOIintF and jgHCO2198 (Leray *et al.* 2013), and the nuclear gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using the primer pair G3PCq157F and G3PCq981R (Buhay *et al.* 2007) Amplification was conducted in 10μL reactions consisting of 5μL Promega Taq Master Mix, 1.5 mM $MgCl_2$, 0.16 μM forward and reverse primers, and 25–100 ng template DNA. The thermal profile for polymerase chain reaction (PCR) amplification included an initial denaturation at 95°C for 2 minutes, followed by 35 cycles of 95°C for 30 sec, 30 sec at the annealing temperature (LCO1490/HC02198: 46°C; mlCOIintF/jgHCO2198: 42°C; G3PCq157F/G3PCq981RGAPDH: 58°C), and 72°C for 1 min, with a final extension of 72°C for 7 minutes. Amplified fragments were purified using Affymetrix ExoSAP-IT and subsequently sequenced and run on an ABI3130 DNA Analyzer (Thermo Fisher Scientific).

Genetic data analysis.—We reconciled and aligned sequences by gene in Geneious Prime® 2020.2.4 using the auto algorithm of MAFFT v7.450 (Katoh *et al.* 2002; Katoh & Standley 2013) and default parameters (gap open penalty 1.53, offset value 0.123), adjusting for sequence direction. We then translated all sequences into amino acids to verify an open reading frame, in turn avoiding potential inclusion of pseudogenes into subsequent analyses. We concatenated the two genes in Geneious prior to phylogenetic reconstruction.

We used ModelFinder (Kalyaanamoorthy *et al.* 2017), implemented in IQTREE v2.1.3 (Minh *et al.* 2020; as "-m MFP+MERGE"), to find the best-fit partitioning strategy for our data using a heuristically optimized search and the Bayesian Information Criterion (Chernomor *et al.* 2016). The candidate pool of partition strategies ranged from a partition for each gene and codon position to a single partition for all data. The optimal partition-model scheme (COI and GAPDH first position combined as a single partition, TN+F+I; COI second codon position and GAPDH second and third codon positions combined as a single partition, HKY+F+I; COI third codon position alone, TN+F+G4) was automatically implemented in the subsequent maximum likelihood (ML) phylogenetic tree reconstruction (Nguyen *et al.* 2015), quantifying clade support with 1000 ultrafast phylogenetic bootstraps (Hoang *et al.* 2018). We visualized the resulting majority rule (>50%) consensus tree using FigTree v1.4.4. Pairwise p-distances among COI sequences were calculated in Geneious.

Results

We generated 96 and 54 new COI and GAPDH sequences including 34/21 for *C. emegi* **sp. nov.**, 11/7 for *Cambarus hobbsorum* Cooper, 2001, and 51/26 from eight additional taxa—currently subsumed within *C.* sp. C—in the Roanoke, James, and Lower Chesapeake basins. Maximum likelihood phylogenetic reconstruction placed *C. emegi* **sp. nov.** sister to an undescribed form of *C.* sp. C from the upper Roanoke Basin (bootstrap support, bs = 84; Fig. 1). This pair of taxa was in turn sister to a clade containing several distinct moderately-to-well-supported subclades from the James, Lower Chesapeake, and Roanoke Basins.

Average and minimum genetic distance among clades was moderate to low, ranging from 1.80% (J3–R2) to 5.22% (*Cambarus hobbsorum*-J2) and 1.52% (J3–R2) to 4.41% (*C. hobbsorum*-J2), respectively (Table 1). Maximum intraspecific and minimum interspecific genetic distances were non-overlapping. *Cambarus emegi* **sp. nov.** was most similar—by p-distance—to R2 (2.08%), a morphologically and geographically distinct taxon occurring in the Roanoke basin.

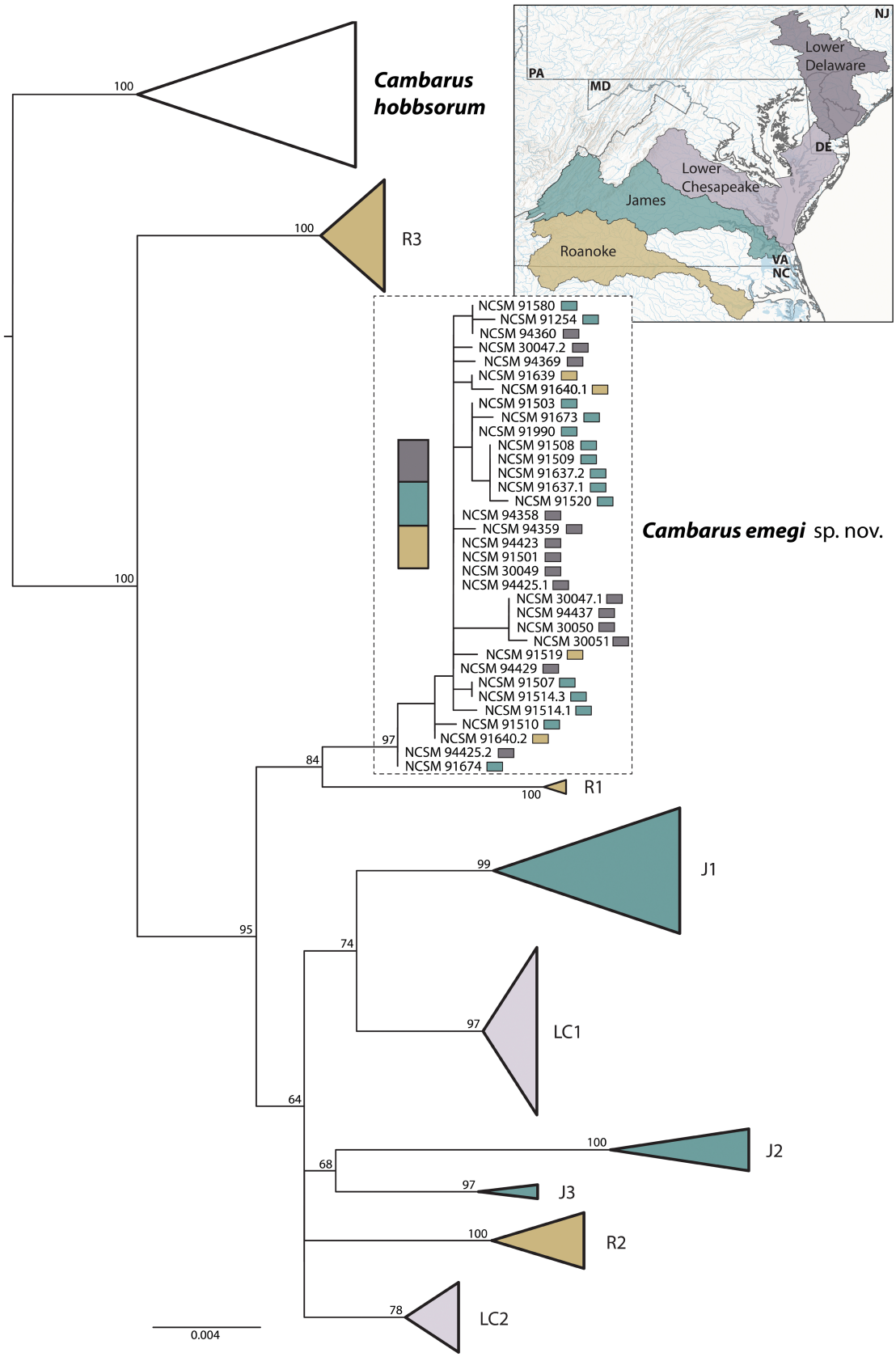


FIGURE 1. Maximum likelihood reconstruction (IQTree) of relationships among *Cambarus emegi* **sp. nov.** and closely related taxa based on concatenated COI and GAPDH sequences. Bootstrap values >50 are shown at associated nodes. Clades are color-coded to coincide with the river basin in which each taxon occurs, as shown in inset map. Clades representing currently undescribed taxa are labeled with identifiers corresponding to Table 1. Scale bar indicates estimated number of nucleotide substitutions per site.

TABLE 1. Uncorrected percent (%) pairwise genetic distances calculated from the COI sequence alignment calculated in Geneious. Minimum interspecific pairwise distances are in the upper half matrix; average interspecific pairwise distances are in the lower half matrix; and average and maximum intraspecific pairwise distances, along with sample size, are displayed on the diagonal.

	1	2	3	4	5	6	7	8	9	10
1. *C. emegi* **sp. nov.**	**0.38 | 0.91 | n=33**	1.67	1.98	2.13	2.72	1.98	1.67	2.08	3.04	3.04
2. *C.* species C R1	2.32	**0.15 | 0.15 | n=2**	2.74	2.89	3.19	2.58	2.74	2.58	3.50	3.04
3. *C.* species C J1	2.52	3.10	**0.53 | 1.37 | n=10**	2.28	3.50	2.74	2.28	2.89	3.95	4.26
4. *C.* species C LC1	2.41	3.12	2.61	**0.12 | 0.30 | n=13**	3.19	2.13	2.02	2.13	3.50	3.95
5. *C.* species C J2	3.71	3.76	4.20	3.84	**0.76 | 1.06 | n=4**	2.58	2.39	3.04	3.80	4.41
6. *C.* species C J3	2.41	2.81	3.11	2.43	3.13	**0.61 | 0.61 | n=2**	1.52	2.28	3.50	3.34
7. *C.* species C R2	2.08	2.90	2.60	2.36	3.49	1.80	**0.17 | 0.37 | n=6**	1.84	3.04	3.49
8. *C.* species C LC2	2.69	2.87	3.41	2.58	3.43	2.57	2.29	**0.76 | 1.06 | n=5**	2.58	3.65
9. *C.* species C R3	3.62	3.74	4.36	3.72	4.46	3.72	3.37	3.04	**0.36 | 0.76 | n=9**	2.74
10. *C. hobbsorum*	4.02	3.56	4.84	4.54	5.22	3.89	3.97	4.42	3.43	**0.91 | 1.67 | n=11**

Taxonomy

Family CAMBARIDAE Hobbs, 1942

Genus *Cambarus* Erichson, 1846

Cambarus emegi sp. nov.

(Figs. 2a–j, 3a–b, 4, 5, Table 2)

Type material.—The holotype, allotype, morphotype, and 7 paratypes are deposited in the North Carolina Museum of Natural Sciences Non-molluscan Invertebrates Collection with the following catalog numbers: holotype, NCSM 95987; allotype, NCSM 95988; morphotype, NCSM 91508; and paratypes, NCSM 91515, 91517, 91639. Additional paratypes are deposited in the Virginia Museum of Natural History's Recent Invertebrates Collection as VMNH 112523.1 and VMNH 112523.2 (both form I males) and VMNH112524.1, VMNH112524.2, and VMNH112524.3 (2 form II males and 1 female, respectively).

Other material examined.—We examined specimens of *C. emegi* **sp. nov.** from the following localities: VIRGINIA, ***Alleghany County*:** (1) NCSM 91510, Wilson Creek at Whisper Circle, north of Clifton Forge, 2 form I males (MI), D.A. Foltz II (DAF), 6 October 2019; ***Augusta County*:** (2) NCSM 91509, Little Calfpasture River off

state road (SR) 811 (Augusta Springs Road), east-northeast (ENE) of Craigsville, 1 MI, DAF, 5 October 2019; (3) NCSM 91637, Moffatts Creek off Virginia (VA) 252 (Middlebrook Road), south of Newport, 1 juvenile male (jM), 1 juvenile female (jF), DAF, 5 October 2019; (4) NCSM 91636, Moffatts Creek off VA 252 (Middlebrook Road), south of Newport, 2 jM, B.W. Williams (BWW) & D.A. Lieb (DAL), 9 October 2019; (5) West Liberty University Astacology Collection (WLU) 1381, Calfpasture River off SR 811 (Augusta Spring Road), ENE of Craigsville, 1 F, 1 form II male (MII), Z.J. Loughman (ZJL), K. Skalican (KS), R. Fagundo (RF), & M. Lucero (ML), 4 June 2012; ***Bath County*:** (6) NCSM 91674, Back Creek at SR 600 (Big Back Creek Road), southwest of Sunrise, 1 MII, 2 jM, 4 jF, D.R. Jones (DRJ), 20 May 2019; (7) NCSM 91638, Rowan Run at SR 687 (Richardson Gorge Road), west-southwest of Bacova, 1 MII, DRJ , 21 May 2019; (8) NCSM 95987, Holotype, Mill Creek at SR 629 (Douthat State Park Road), south of Bath Alum, 1 MI, DAF, 28 September 2022; (9) NCSM 95988, Allotype, Mill Creek at SR 629 (Douthat State Park Road), south of Bath Alum, 1 F, DAF, 28 September 2022; (10) NCSM 91271, Mill Creek at SR 629 (Douthat State Park Road), south of Bath Alum, 7 MI, 6 F, DAF, 28 September 2022; (11) NCSM 91294, Mill Creek at SR 629 (Douthat State Park Road), south of Bath Alum, 1 jF, DAF, 28 September 2022; (12) WLU 1383, Mill Creek bridge on VA 39/42 near Camp ACCOVAC, Hotchkiss, 3 F, 4 MII, ZJL, KS, RF, & ML, 4 June 2012; ***Botetourt County*:** (13) NCSM 91671, Little Catawba Creek at SR 779 (Catawba Road), southwest of Lone Star, 1 MI, 1 jM, DRJ, 3 May 2019; (14) NCSM 91676, Tinker Creek at SR 672 (Greenway Hollow Road), Glebe Mills, 3 F [1 ovigerous (ovig)], DRJ, 4 May 2019; (15) NCSM 91673, Beckner Branch at SR 606 (Blue Ridge Turnpike) west-northwest (WNW) of Spec, 3 MII, DRJ, 4 May 2019; (16) NCSM 91519, Buffalo Creek off United States (US) 11 (Lee Highway), southwest of Troutville, 1 F (ovig), 1 MII, DRJ, 4 May 2019; (17) WLU 1374, Back Creek at SR 600 (Big Back Creek Road), approximately 5 miles north of the intersection with VA 39, 1 F, 2 MII, ZJL, RF, & ML, 6 March 2012; ***Montgomery County*:** (18) NCSM 91675, South Fork Roanoke River off SR 637 (Alleghany Spring Road), behind Sowders Chapel Baptist Church, 1 MII, DRJ, 2 May 2019; (19) NCSM 91640, Elliott Creek off SR 639 (Mt. Pleasant Road), southwest of Alleghany Springs, 1 F, 2 jF, 2 jM, DRJ, 2 May 2019; (20) NCSM 91518, North Fork Roanoke River at SR 785 (Catawba Road), northeast of McDonalds Mill, 1 F (ovig), DRJ, 3 May 2019; (21) WLU 1517, North Fork Roanoke River at SR 785 (Catawba Road), northeast of McDonalds Mill, 1 j, WLU Summer 2012, 24 July 2012; (22) NCSM 91515, North Fork Roanoke River at SR 747 (Pepper Run Road), southwest of McDonalds Mill, 2 MI, DRJ, 3 May 2019; ***Roanoke County*:** (23) NCSM 91639, unnamed tributary (UNT) Roanoke River off SR 603 (Cove Hollow Road), east of Lafayette, 1 MII, DAF, 23 June 2019; (24) NCSM 91516, UNT Catawba Creek at SR 785 (Blacksburg Road), southwest of Catawba, 1 MI, 1 MII, 3 F (2 ovig), DRJ, 3 May 2019; ***Rockbridge County*:** (25) NCSM 91508, Morphotype, Calfpasture River off VA 39 (Maury River Road), at mouth of Mill Creek, Goshen, 1 MII, DAF, 5 October 2019; (26) NCSM 91503, Mill Creek off VA 39 (Virginia Avenue), WNW of Goshen, 2 F (2 ovig.), DRJ, 20 May 2019; (27) NCSM 91517, Mill Creek off VA 39 (Virginia Avenue), WNW of Goshen, 1 MI, 1 F (ovig.), 1 jF, DRJ, 20 May 2019; (28) NCSM 91672, Colliers Creek off VA 251 (Collierstown Road), southeast of Collierstown, 1 MII, 1 F, DRJ, 25 May 2019; (29) NCSM 91678, Sheep Creek at SR 716 (Timber Ridge Road), Crossroads Store, 4 MII, 4 F, DRJ, 25 May 2019; (30) NCSM 91507, Cedar Creek at SR 692 (Cedar Creek Road), next to I-81, Red Mills, 4 MII, 2 F, DAF, 23 June, 2019; (31) WLU 1420, Kerrs Creek at Kerrs Creek Baptist Church along SR 850 (W Midland Trail), 2 F, 1 MII, ZJL, KS, RF, & ML, 8 June 2012; (32) WLU 1393, Whistle Creek at SR 640 (Edgars Way), 6 F, 1 MI, 11 MII, 1 j, ZJL, KS, RF, & ML, 8 June 2012.

PENNSYLVANIA, ***Chester County*:** (1) NCSM 24749, Valley Creek downstream of Knox Covered Bridge (Yellow Springs Road), southeast of Phoenixville, Valley Forge National Historical Park (VFNHP), Chester–Montgomery County line, 5 MI, DAL, R.F. Carline (RFC), V.M. Mengel (VMM), A. Smith (AS), & P. Mooney (PM), 22 April 2003; (2) NCSM 24750, Valley Creek downstream of Knox Covered Bridge (Yellow Springs Road), southeast of Phoenixville, VFNHP, Chester–Montgomery County line, 1 MI, DAL, RFC, VMM, AS, & PM, 22 April 2003; (3) NCSM 24751, Valley Creek downstream of Wilson Road Bridge, southeast of Phoenixville, VFNHP, 8 MI, DAL, RFC, VMM, AS, & PM, 22 April 2003; (4) NCSM 24752, Valley Creek off Route 252 (Valley Creek Road) approximately halfway between Knox Covered Bridge and Route 23 (Valley Forge Road) Bridge, southeast of Phoenixville, VFNHP, 6 MI, DAL, RFC, VMM, AS, & PM, 22 April 2003; (5) NCSM 24753, Valley Creek off Route 252 (Valley Creek Road) approximately halfway between Knox Covered Bridge and Route 23 (Valley Forge Road) Bridge, southeast of Phoenixville, VFNHP, 1 MI, DAL, RFC, VMM, AS, & PM, 22 April 2003; (6) NCSM 26548, Pickering Creek at PA 29 (State Road), east of Charlestown, 1 F, 2 jF, 3 jM, DAL & J. Harper (JH), 10 May 2006; (7) NCSM 26549, Little Valley Creek at Mill Road Park, northwest of Howellville, 1 jM, unknown

collector, 18 October 1985; (8) NCSM 26550, Welch Run, east-southeast (ESE) of Phoenixville, VFNHP, 1 F, PM & JH, 31 March 2005; (9) NCSM 26553, Crum Creek off Boot Road downstream of Bartram's Covered Bridge (Goshen Road), west of Newtown Square, Chester–Delaware County line, 2 MI,1 F, 1 jF, 2 jM, DAL, 2 November 2008; (10) NCSM 30047, Valley Creek at Le Boutillier Road, north-northeast (NNE) of Paoli, 8 MI, 2 MII, 2 F, 8 jF, 3 jM, DAL, BWW, E.M. Delekta (EMD), & ZJL, 31 May 2018; (11) NCSM 30048, Crum Creek off Boot Road downstream of Bartram's Covered Bridge (Goshen Road), west of Newtown Square, Chester–Delaware County line, 1 F (ovig), DAL, BWW, EMD, & ZJL, 31 May 2018; (12) NCSM 30049, UNT Pine Creek at Worthington Road, east of Lionville, 1 MI, 2 MII, 5 F, 3 jM, DAL, BWW, EMD, & ZJL, 31 May 2018; (13) 20220718-01-WLU, East Branch Brandywine Creek downstream from R.G. Struble Trail, upstream from Route 282 (Wallace Avenue), north of Downingtown, 2 F, J. Krochmal (JK), A. Shanor (AS), & K. Deihl (KD), 18 July 2022; (14) 20220718-02-WLU, UNT to East Branch Brandywine Creek off of Dowlin Forge Road upstream of Route 282 (Creek Road), across from Whittaker Trail Head Memorial Park, north of Downingtown, 3 MII, 2j, JK, AS, & KD, 18 July 2022; (15) 20220718-03-WLU, East Branch Brandywine Creek at Brandywine Creek Campground, north of Lyndell, 1 MII, 2 F, JK, AS, & KD, 18 July 2022; (16) 20220804-01-WLU, Little Valley Creek at Vanguard 50 Morehall Road, downstream from Down East Park, north-northwest of Malvern, 1 MII, 3 F, DAL, J. Uplinger (JU), E.C. Lieb (ECL), KD, & AS, 4 August 2022; (17) 20220713-01-WLU, UNT to Valley Creek at Whitford Country Club golf course, north of Business Route 30 (W Lincoln Highway), west of Exton, 1 MI, 1 MII, 1 F, DAL, ECL, JU, S. Shaw (SS), & JK, 13 July 2022; (18) 20220716-02-WLU, East Branch Brandywine Creek beneath Route 322 (Brandywine Avenue/Downingtown Pike) Bridge, south-southeast of Downingtown, 1 MI, JK, H. Wells (HW), M. Ranson (MR), S. McGovern (SM), & MF, 16 July 2022; (19) 20220713-01-WLU, UNT to Pickering Creek upstream of intersection of Saint Mathews Road and Route 401 (Conestoga Road) across from Saint Matthew's Church, ESE of Ludwigs Corner, 5 MII, 7 F, DAL, ECL, JU, SS, & JK, 13 July 2022; ***Delaware County*****:** (20) NCSM 26552, Darby Creek off Darby Creek Road downstream of Marple Road, NNE of Brookthorpe Hills, 2 MI, 1 F, 1 jF, 2 jM, DAL, 2 November 2008; (21) NCSM 30050, Darby Creek off Darby Creek Road downstream of Marple Road, NNE of Brookthorpe Hills, 4 MII, 4 F, DAL, BWW, EMD, & ZJL, 31 May 2018; (22) NCSM 30051, Darby Creek off Darby Creek Road downstream of Marple Road, NNE of Brookthorpe Hills, 1 F (ovig), DAL, BWW, EMD, & ZJL, 31 May 2018; (23) 20220711-01-WLU, Little Darby Creek at The Willows Park, upstream of Willows Road, South of Wayne, 2 MII, 2 F, DAL, ECL, JU, SS, & JK, 11 July 2022; (24) 20220712-01-WLU, UNT to Ridley Creek off Tanglewood Drive, northwest of Hershey Mill, south-southwest of Frazer, 3 MII, 4 F, DAL, JU, ECL, SS, & JK, 12 July 2022; (25) 20220713-01-WLU, Darby Creek off Darby Creek Road downstream of Marple Road, NNE of Brookthorpe Hills, 2 F, HW, MR, SM, & MF, 13 July 2022; ***Montgomery County*****:** (26) NCSM 26551, Schuylkill River at US 422 (Pottstown Expressway), WNW of King of Prussia, 1 jF, DAL, JH, & N. Bhattarai, 9 May 2006.

Diagnosis.—Body and eyes pigmented. Rostrum weakly to moderately excavated and ventrally deflected anteriorly; rostral margins subparallel, pronounced and entire through acumen, and more thickened posteriorly than anteriorly; acumen acuminate with prominent dorsally deflected spiniform tubercle at terminus. Areola 2.2–3.6 ($\bar{x}$=2.7, n=42, s=0.4) times as long as wide, with 6–10 ($\bar{x}$=8.5, n=42, s= 0.7) punctations across narrowest point. Lateral portion of branchiostegal region of carapace heavily punctate; hepatic regions also punctate with well-developed tubercles. Strong cervical spines present; branchiostegal spine prominent; postorbital ridge terminating anteriorly in spiniform tubercle or spine. Weak to acute suborbital angle typically present, rarely absent; suborbital angle terminating anteriorly in spiniform tubercle or spine. Antennal basipodite spines prominent; ischiopodite spines present but not prominent.

Cheliped palm punctate, mesial surface with 2 rows of tubercles; first row consisting of 6–9 tubercles, second row 5–8. Subpalmar tubercles usually absent, when present represented by a singular rudimentary tubercle. Dorsomedian ridge of pollex pronounced. Lateral impression apparent at base of pollex. Dactyl possessing strong dorsal longitudinal ridges with pronounced tubercles. Pollex possessing enlarged tooth or denticle at midlength. Dactyl possessing enlarged tooth or denticle near junction with palm in some individuals, particularly large adults. Dactyl and pollex each with sharp corneous subterminal tip. Merus ventrolateral margin with 3–5 enlarged spines; ventral interior (mesial) margin with 5–9 smaller spines, occasionally with 10. Merus with 1–3 dorsal spines, prominent in juveniles, rounded in large adults though occasionally well defined. Carpus with well-developed enlarged lateral spine; ventral tubercles with spiniform apex, occasionally terminating in a spine.

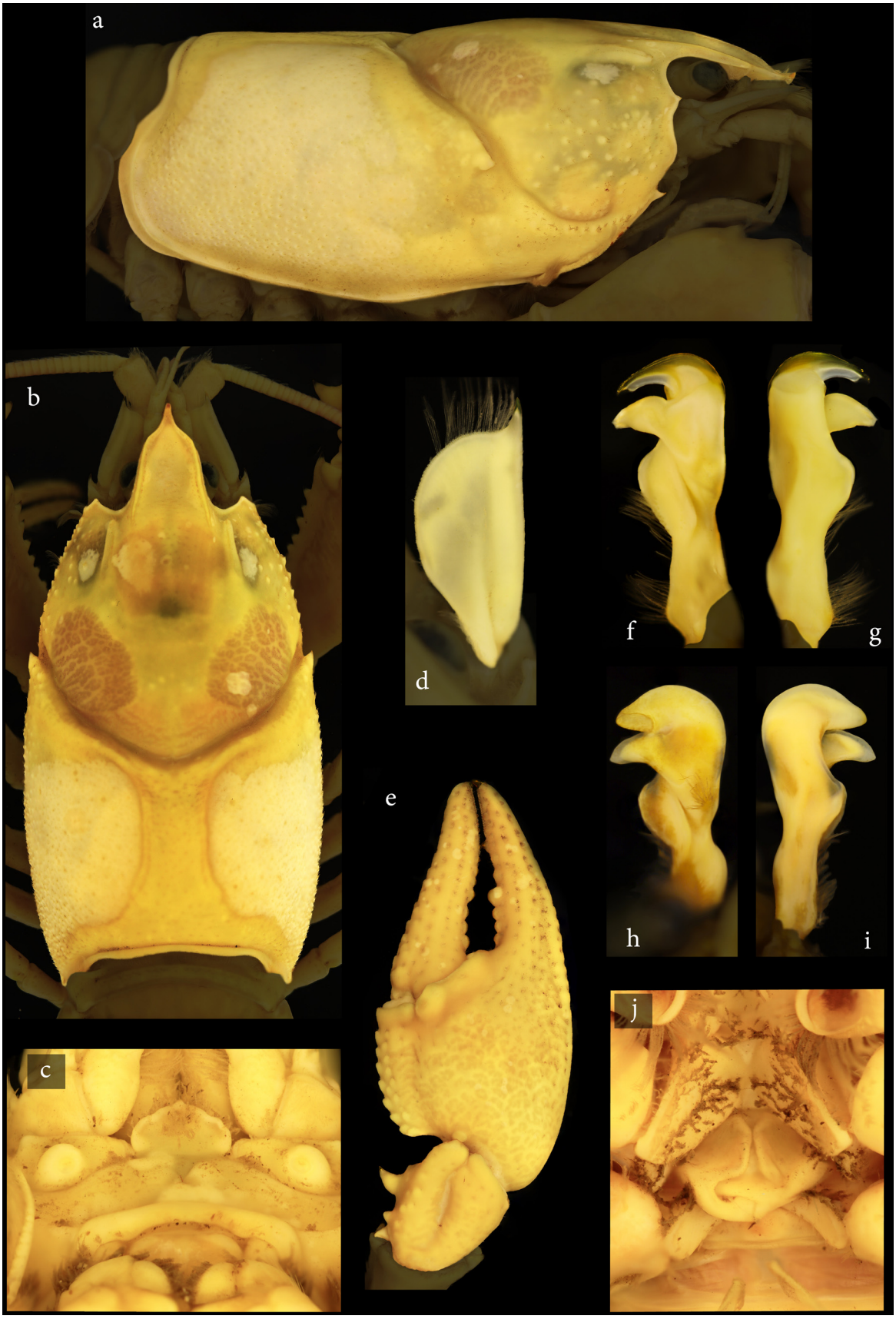


FIGURE 2. *Cambarus emegi* **sp. nov.:** (a–g) holotype, NCSM 95987; (h–i) morphotype, NCSM 91508; (j) allotype, NCSM 95988. (a) carapace, right lateral view; (b) carapace, dorsal view; (c) epistome; (d) right antennal scale, dorsal view; (e) right chela and carpus, dorsal view; (f) form I gonopod, mesial view; (g) form I gonopod, lateral view; (h) form II gonopod, mesial view; (i) form II gonopod, lateral view; (j) annulus ventralis.

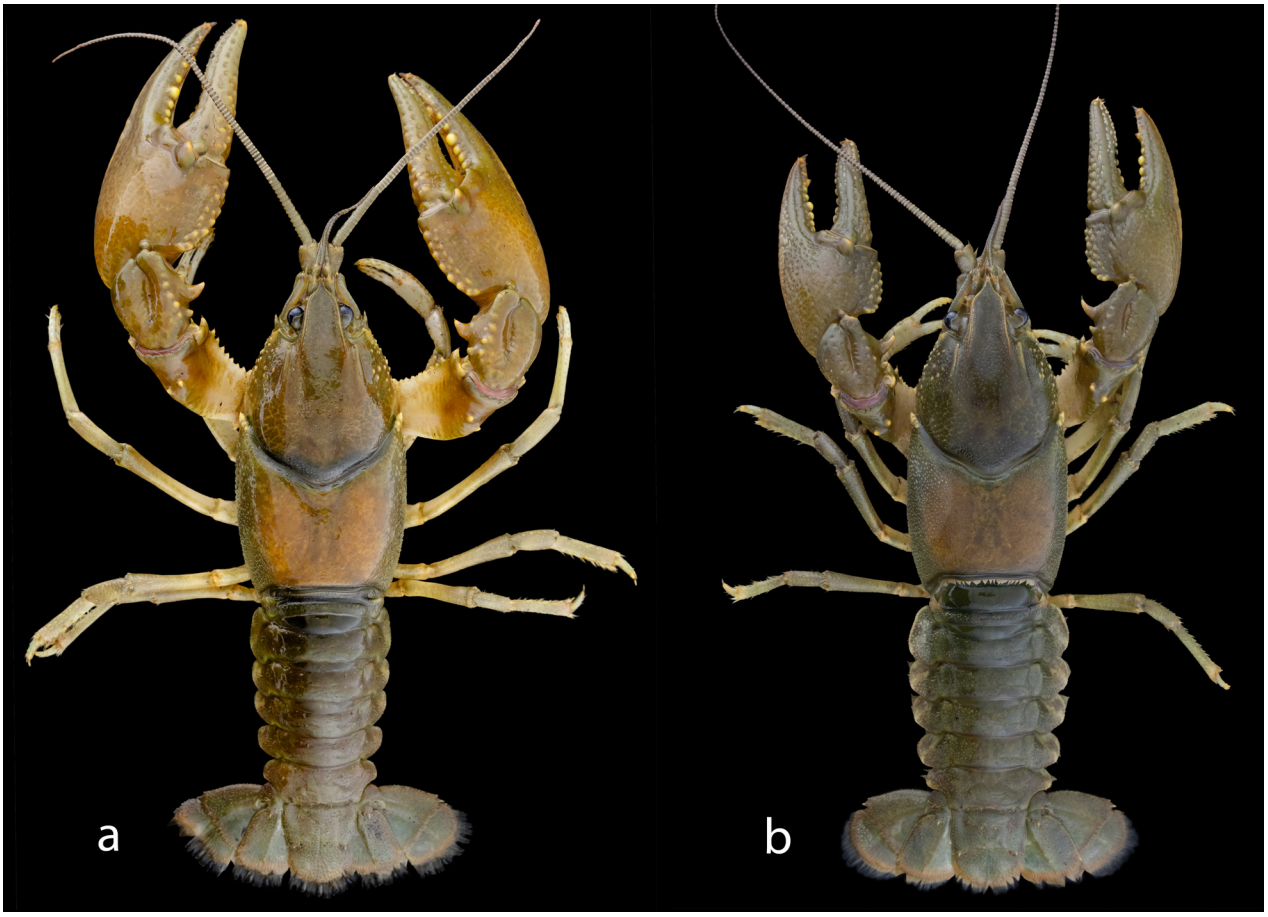


FIGURE 3. *Cambarus emegi* **sp. nov.**, color in life: (a) holotype, NCSM 95987; (b) allotype, NCSM 95988.



FIGURE 4. Life color and color pattern of juvenile *C. emegi* **sp. nov.**



FIGURE 5. Life color and color pattern of adult *C. emegi* **sp. nov.**: (a) dorsal view; (b) oblique lateral view (photo credit Zackary A. Graham).

Form I and II males possessing hook on ischium of third pereopod only; hook gently curved at apex, overarching basioischial joint in form I males, not reaching basioischial joint in form II males. Hook not opposed by tubercle on third pereopod boss.

Gonopod of form I male with 2 long terminal elements, lacking caudal knob; central projection sickle-shaped and not tapering distally, recurved > 90° to main shaft of gonopod, possessing subapical notch. Mesial process directed approximately 90° to shaft, bent distolaterally, inflated proximally with distinct hump, tapering to a point distinctly caudal to and slightly distal to terminus of central projection, projecting beyond margin of main shaft. Annulus ventralis immovable, distinctly asymmetrical caudally; cephalic portion with median trough leading to strongly sculptured central fossa; exaggerated "S" bend in sinus terminating at caudal edge.

TABLE 2. Morphological measurements (mm) of the primary type specimens of *Cambarus emegi* **sp. nov.** (NCSM 95987, holotype; NCSM 95988, allotype; NCSM 91508, morphotype). Measurements taken from the right chela and right antennal scale.

	Holotype	Allotype	Morphotype
Carapace:			
Total length	40.2	41.5	46.8
Postorbital length	32.6	33.5	38.5
Width	21.9	21.8	25.3
Depth	16.8	18.4	21.2
Areola:			
Width	5.3	5.0	4.2
Length	13.1	13.8	16.3
Rostrum:			
Width	5.1	6.0	5.4
Length	8.9	11.4	11.8
Cheliped:			
Length of mesial palm margin	12.7	8.4	15.9
Palm width	18.8	9.9	19.4
Length of lateral margin	39.6	32.2	51.0
Dactyl length	23.5	18.8	31.7
Abdomen:			
Width	18.4	22.4	19.7
Length	41.8	45.5	47.8
Antennal scale:			
Width	3.0	3.4	4.2
Length	7.3	7.5	8.2

Size.—Form I male (*n*=21) TCL ranges in size from 30.0–41.7 mm (23.4–33.3 mm PCL), TCL $\bar{x}$ = 35.1 mm. Form II male (*n*=11) TCL $\bar{x}$ = 34.3 mm, ranging from 28.2–46.8 mm (PCL 22.2–38.5 mm). Female (*n*=10) TCL $\bar{x}$ = 37.3 mm, ranging from 30.4–43.8 mm (PCL 24.2–35.4 mm). The largest specimen examined was a form II male with a TCL of 46.8 mm (PCL 38.5 mm).

Male, form I, holotype (Figs. 2a–g, Table 2).—Body (Figs. 2a, b) slightly compressed dorsoventrally; thoracic section of carapace slightly wider than abdomen and cephalon. Carapace height less than width (16.8 and 21.9 mm, respectively). Total carapace length 40.2 mm; PCL 32.6 mm. Areola 2.5 times longer than wide, with 8 punctations across narrowest part; length of areola 32.6% of TCL (40.2% of PCL). Rostrum narrow, lanceolate, and moderately excavated equally along length with acuminate acumen; margins moderately thickened and converging caudal to acumen. Floor of rostrum with numerous punctations. Rostrum 1.8 times longer than wide, distinctly lanceolate, ending in dorsally turned corneous tip and prominent acumen (Fig. 2a). Postorbital ridges moderate in length, terminating in spinose dorsally directed tubercles. Suborbital angle acute (Fig. 2a). Prominent, large cervical spine present. Orbital region weakly tuberculate; mandibular, branchiostegal, and antennal regions of carapace heavily tuberculate; greatest tubercle density in hepatic region. Hepatic tubercles prominent and well developed. Prominent, large branchiostegal spine present (Figs. 2a, b).

Abdomen slightly longer than carapace; pleura sharply rounded anteroventrally, angled caudoventrally. Abdomen 41.8 mm long. Anterior section of telson with 2 large spines in each caudolateral corner. Uropod with 2 distolateral spines on mesial lobe; median spine of mesial lobe originating from ventral surface; mesial ramus of uropod with median ridge ending distally in distomedian spine not overreaching margin of ramus. Distal margin of proximal segment of lateral ramus of right uropod bearing 15 immovable, small spines.

Epistome anteromedian lobe subtriangular, forming weak angle at junction with endostyle; anterolateral margins moderately thickened (Fig. 2c); main body possessing weak anteromedian fovea.

Antennal basipodite spine well-developed; weakly defined antennal ischiopodite tubercle present (Figs. 2a, b). Antennal scale broadest distally; lateral margin thickened, terminating in large corneous spine; setiferous mesial margin (Fig. 2d). Right antennal scale 2.4 times longer than wide. Tip of left antennal flagellum reaching middle of telson when adpressed posteriorly.

Right cheliped with mesial surface of palm with 2 well-defined tubercle rows; 8 tubercles in mesial-most first row, 7 in second row (Fig. 2e). Palm length (12.7 mm) 67.6% of palm width (18.8 mm); depth of palm 10.4 mm. Well-defined subpalmar tubercles absent. Dorsal longitudinal ridge of dactyl well developed and possessing 10 prominent disorganized tubercles; dactyl terminating in large corneous spine. Dorsomedian ridge of pollex strongly developed with prominent lateral impression at junction of pollex and palm. Single large prominent tubercle (tooth) on pollex at mid-length. Pollex with sharp corneous tip. Carpus with prominent dorsal furrow (Fig. 2e), surface covered with punctations; ventral mesial margin with large, procurved spine at about mid-length and 2 smaller proximal spines. Merus with 2 spiniform tubercles on dorsal surface; 3 large spines on ventrolateral surface, and 9 spines on ventral mesial margin. Hook on ischium of third pereopod only; gently curved at apex, overarching basioischial joint, not opposed by tubercle on base. Form I gonopod as described in Diagnosis (Figs. 2f, g).

Female, allotype (Fig. 2j, Table 2).—Differing from holotype as follows (only taxonomically relevant characters listed): right cheliped palm length 84.8% of palm width; other proportions similar to holotype (Table 2). Mesial surface of palm of right chelae with 7 tubercles in first row and 6 tubercles in second row. Suborbital angle weakly acute, nearly absent. Annulus ventralis as described in diagnosis (Fig. 2j); width of postannular sclerite approximately half total width of annulus ventralis. First pleopods uniramous, reaching central region of annulus ventralis when abdomen flexed.

Male, form II, morphotype (Figs. 2h, i, Table 2).—Differing from holotype as follows (only taxonomically relevant characters listed): carapace with areola 3.9 times longer than wide, rostrum 2.2 times longer than wide; right palm length 82% of palm width; right antennal scale 2 times longer than wide; other proportions similar to holotype (Table 2). Suborbital angle weakly acute. Gonopod with central projection curved 90° to shaft, tapering to a rounded terminus (Figs. 2h, i); mesial process tapered, bulbous, directed caudolaterally. Hook on ischium of third pereopod small, not reaching basioischial joint.

Color.—Carapace ground color orange-brown to olivaceous (Figs. 3, 5); thoracic section of carapace olivaceous to distinctly orange-brown dorsally; thoracic section of carapace frequently mottled laterally in juveniles (Fig. 4); posterior margin of carapace olivaceous to brown lacking saddle. Hepatic and antennal region of carapace with beige to yellow tubercles (Fig. 5). Postorbital ridge olivaceous to brown terminating in beige spine or tubercle. Rostrum margins and acumen are orange, orange-brown, olivaceous or tan. Cephalic section of carapace immediately anterior to cervical groove olivaceous to brown; cervical groove same color as previous; mandibular abductor scars mottled, ranging from light-brown, brown, to brown-green. Lateral margin of antennal scale olivaceous to brown; body of antennal scale same as previous. Antennal flagellum and antennules green-brown, with olivaceous hue; dorsal surface of lamellae tan to brown; ventral surface light-green to olivaceous. Dorsal surface of chelae generally olivaceous or green-brown to brown, orange-brown, or tan, with beige tubercles. Denticles on opposable surfaces of fingers yellow, white, or tan. Ventral surface of chelae cream or tan. Dorsal surface of carpus olivaceous or green-brown; region adjacent to, and including, furrow orange-brown to olive-brown to olive; carpus spine yellow to olivaceous. Merus olive-brown or olive. Articles of pereopods olivaceous, light brown, or green-brown; joints of pereopod articles pink or beige. Dorsal and dorsolateral surface of abdomen olivaceous, brown, or green-brown; tergal margins beige to pink; abdomen lacking dorsal stripe. Uropods same colors as abdomen. Ventral surface of abdomen and carapace cream. 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. Anterior 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.—Mill Creek at SR 629 (Douthat State Park Road), south of Bath Alum, Bath County, Virginia. Mill Creek at this locality ranges from 3 to 8 meters wide, and 0.3 to 1.0 meters deep. The stream runs through a mixed mesophytic forest and is heavily forested on both banks with only a single road and bridge serving as anthropogenic influences. Riffles, runs, glides, and pools are present throughout the site and the stream's gradient can best be described as moderate. The stream bottom consists primarily of bedrock with an overlay of cobbles and slab boulders. Gravel, sand, and occasional silt pockets were present in glides, and at the heads of riffles. Some riffles exhibit exposed bedrock jutting upwards perpendicular to flow. Occasional large, slab boulders are scattered

throughout the stream. Individuals of *Cambarus emegi* **sp. nov.** were taken primarily from under slab rocks mid channel in runs and glides.

Geographic range.—*Cambarus emegi* **sp. nov.** exhibits a disjunct distribution, occurring in the upper James and Roanoke River basins in Virginia and the lower Delaware River basin in Pennsylvania (Fig. 6). In Pennsylvania, *C. emegi* **sp. nov.** has a very limited distribution in the southeastern part of the state, with reproducing populations restricted to six adjacent small to medium-sized drainages clustered closely together across the landscape (Fig. 6.2). The headwaters of adjacent drainages are typically < 2 km apart and some stream segments are separated by < 500 m.

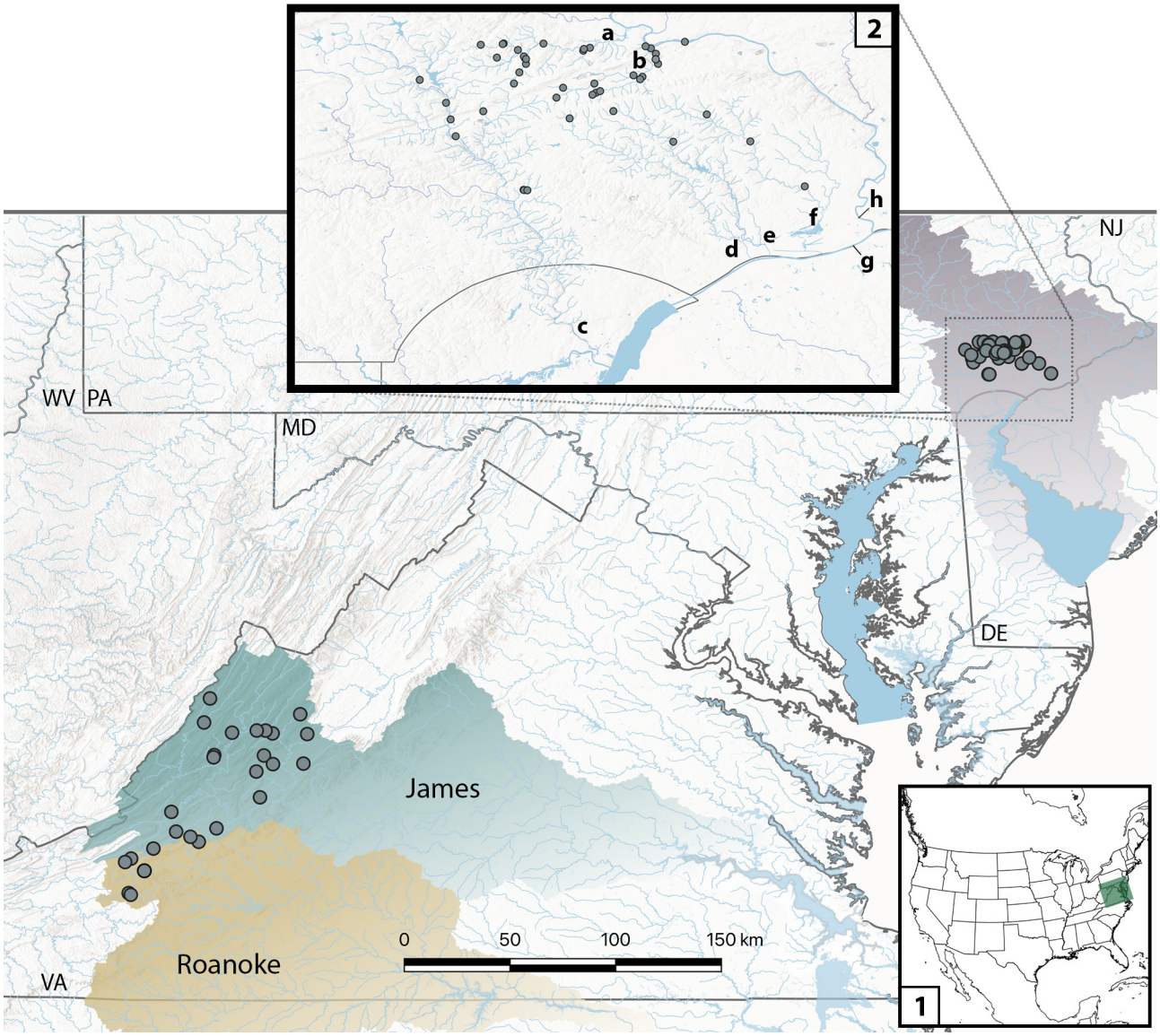


FIGURE 6. Terrain map showing *C. emegi* **sp. nov.** collection sites in Virginia and Pennsylvania. Inset 1 provides broader geographic context of the study area in the Mid-Atlantic U.S.A. Inset 2 magnifies part of southeastern Pennsylvania to show the six drainages in which *C. emegi* **sp. nov.** occurs or once occurred and bordering rivers: (a) Pickering Creek; (b) Valley Creek; (c) Brandywine Creek; (d) Ridley Creek; (e) Crum Creek; (f) Darby Creek; (g) Delaware River; and (h) Schuylkill River.

In Pennsylvania, most of the range of *C. emegi* **sp. nov.** is within the Piedmont; however, it has also been collected from the Coastal Plain physiographic province (Lieb *et al.* 2011a). The species occurs, or once occurred, across entire drainage networks (headwater streams to large creeks and small rivers) in some systems (Pickering, Valley, East Branch Brandywine), whereas in other systems the distribution of *C. emegi* **sp. nov.** appears more restricted/fragmented (Darby, Crum, Ridley), possibly due to a combination of invasive crayfishes, poor habitat and water quality typical of urbanized watersheds, and incomplete sampling. Invasive crayfish impacts have been

particularly pronounced in the Pickering and Crum Creek drainages, resulting in the disappearance of *C. emegi* **sp. nov.** from large portions of those drainages. *Cambarus emegi* **sp. nov.** has not been collected from large rivers in Pennsylvania (e.g., Schuylkill River, Delaware River), except for one juvenile collected from the Schuylkill River in 2006 (Lieb *et al.* 2011a, Fig. 6.2).

In Virginia, *C. emegi* **sp. nov.** is restricted within the James River and Roanoke River basins to streams located within the Ridge and Valley (Fig. 6). Within these drainages, *C. emegi* **sp. nov.** inhabits both large and small streams; however, it does not occur in high elevation headwater streams which typically contain only *Cambarus bartonii* (Fabricius, 1798). Despite historical occurrences in the largest streams in the region (James and Roanoke Rivers), *C. emegi* **sp. nov.** is currently extirpated or occurs in extremely low numbers due to high densities of invasive crayfish at these sites.

Crayfish associates.—In the upper James River system in Virginia, *C. emegi* **sp. nov.** co-occurs with *Cambarus bartonii*, *Cambarus longulus* Girard, 1852, *Faxonius cristavarius* (Taylor, 2000), *Faxonius obscurus* (Hagen, 1870), *Faxonius ozarkae* (Williams, 1952), and *Faxonius virilis* (Hagen, 1870). The latter four species are non-native. In the upper Roanoke River system in Virginia, *C. emegi* **sp. nov.** co-occurs with *C. bartonii*, *C. longulus*, *F. ozarkae*, and *F. virilis*, where the latter two species are non-native. In the lower Delaware River drainage of Pennsylvania, *C. emegi* **sp. nov.** co-occurs with *C. bartonii*, *Faxonius limosus* (Rafinesque, 1817), *Faxonius rusticus* (Girard, 1852), *F. virilis*, *Procambarus clarkii* (Girard, 1852), and *Procambarus acutus* (Girard, 1852)/*zonangulus* Hobbs & Hobbs, 1990 (indistinguishable in Pennsylvania without genetic information, D.A. Lieb & B.W. Williams, unpublished data), where the latter four species are non-native. In Pennsylvania, coexistence with *F. virilis* is often transient with *F. virilis* typically replacing *C. emegi* **sp. nov.** at invaded sites.

Habitat.—Throughout its range, *C. emegi* **sp. nov.** occurs in all stream habitats; however, it occurs most frequently in slower moving sections such as pools, glides, slower moving runs, and lateral areas along banks. It is rarely found in high velocity riffles and seems to avoid areas of high flow and scour. In areas with high anthropogenic influence (e.g., man-made stream channels, heavily mowed or altered streambanks), *C. emegi* **sp. nov.** occurs in reduced numbers or is not present. It is primarily found under boulders and large cobbles with large adults typically occurring under the largest available substrate while juveniles can be found under smaller cobbles. Juveniles are often abundant in shallow lateral areas along stream banks (e.g., Lieb *et al.* 2008). While apparently preferring boulders and cobbles, *C. emegi* **sp. nov.** will utilize other more atypical substrates and has been collected under concrete and asphalt slabs, large logs, plywood sheets, and discarded metal signs. In the fall, in some streams, *C. emegi* **sp. nov.** of all sizes may be found in large leaf packs that build at the head of riffles. While *C. emegi* **sp. nov.** have been observed burrowing down into the stream channel, particularly in drought and low water conditions, they do not appear to exhibit any true burrowing behavior and have not been collected from burrows in stream banks.

Conservation status.—*Cambarus emegi* **sp. nov.** is restricted to the upper reaches of the James and Roanoke River drainages in Virginia and a relatively small part of the lower Delaware River drainage in Pennsylvania (Fig. 6). Across its range, *C. emegi* **sp. nov.** is patchily distributed and threatened by a number of invasive crayfishes, which have displaced the species from parts of its former range. In some areas, extant populations are located in rapidly urbanizing landscapes in the vicinity of large cities (Lieb *et al.* 2011a). Although anecdotal, the senior author has observed marked declines in habitat and water quality in streams following residential development or riparian habitat destruction that coincided with substantial reductions in *C. emegi* **sp. nov.** abundance and has found the species to be uncommon or absent in highly urbanized stream reaches with poor habitat and/or water quality (Williams *et al.* 2020). This suggests that urbanization and associated development pose a serious threat to *C. emegi* **sp. nov.** populations in some areas. *Cambarus emegi* **sp. nov.** is also frequently found in agricultural settings where abundance anecdotally appears to be lower than in forested streams with intact riparian corridors.

Given that invasive crayfish tend to thrive in altered habitats (Lindqvist & Huner 1999; Wang *et al.* 2021), the combined impacts of urbanization/agricultural development and invasive crayfish may be greater than the sum of their individual effects. Because of these threats, conservation and management activities that minimize the impacts of urban/agricultural development and reduce the likelihood of non-native crayfish introductions are warranted in drainages with populations of *C. emegi* **sp. nov.**

Relationships and comparisons.—*Cambarus emegi* **sp. nov.** is a member of the *C.* sp. C complex, based on several morphometric characters, including two rows of tubercles located along the mesial margin of the palm of the chelae, an acuminate rostrum, and large cervical spines. These characters occur throughout the *C.* sp. C complex, making it difficult to delimit individual taxa morphologically. Nonetheless, there are distinct morphological differences between *C. acuminatus* and *C. emegi* **sp. nov.** First, *C. acuminatus* possesses a well-defined subpalmar

tubercle, whereas *C. emegi* **sp. nov.** typically lacks a subpalmar tubercle, and if present, it is only rudimentary. Second, *C. acuminatus* has fewer punctations across its areola (3–5) than *C. emegi* **sp. nov.** (6–10). Third, the mesial process of the form I male gonopod in *C. acuminatus* forms a blunted terminus, while the form I male gonopod of *C. emegi* **sp. nov.** forms a tapered point. Fourth, *C. emegi* **sp. nov.** typically has a weak to acute suborbital angle, whereas *C. acuminatus* usually lacks a suborbital angle, although occasionally an acute angle is present. Additionally, *C. acuminatus* and *C. emegi* **sp. nov.** are geographically distant: *C. acuminatus* is confined to the Saluda River drainage in South Carolina (Williams *et al.* 2019), while *C. emegi* **sp. nov.** is found in the upper James and Roanoke Rivers in Virginia and the lower Delaware drainage in Pennsylvania. Lastly, coloration and color pattern in life differ, with *C. acuminatus* consisting primarily of a grounded flat orangish brown with highlights of cream, tan, and brown, whereas *C. emegi* **sp. nov.** consists of an olivaceous green with highlights of yellow, red, orange and brown, though specimens from Pennsylvania are often orangish brown but lack the cream-colored highlights.

Cambarus emegi **sp. nov.** is genetically and morphologically most similar to other undescribed members of the *C.* sp. C complex that occur in Virginia. *Cambarus emegi* **sp. nov.** is the only member of the complex occurring in—and in turn restricted to—the Ridge and Valley physiographic province. The species differs from *C.* sp. C R1 (Fig. 1) genetically and in its distribution (i.e., Ridge and Valley versus Blue Ridge/inner Piedmont) and coloration/color pattern in life. *Cambarus emegi* **sp. nov.** in Virginia has an olivaceous green to orange-brown base coloration highlighted by yellows, reds, oranges, and browns, while *C.* sp. C R1 consists of a base brown-hay coloration highlighted by brown and cream. The most visually striking feature of *C.* sp. C R1 is the bright white to cream on the tips of the dactyl and propodus of each chelae, while the tips of the propodus and dactyl of *C. emegi* **sp. nov.** are brown. *Cambarus* sp. C J1 occurs in the James River basin immediately downstream of *C. emegi* **sp. nov.** and is superficially similar in color and morphology. Yet, the two taxa differ genetically, and their distributions do not appear to overlap.

Etymology.—Derived from the Turkish work "emeği" meaning "labor of" or "hard work of", which seems appropriate given this project required many years and the collection of thousands of specimens from South Carolina to Pennsylvania. Additionally, the name honors Emily (Em) and Megan (Meg) Lieb, the senior author's two daughters, who happily accompanied him on many crayfish sampling trips as children and later helped him collect *C. emegi* **sp. nov.** from sites in its Pennsylvania range.

Common name.—We propose Enigma Crayfish as the common name.

Discussion

More crayfish species are found in the United States than anywhere else in the world (Taylor *et al.* 2019). Approximately a quarter to a half of those species are of conservation concern, threatened by one or more often interacting factors including invasive crayfish, narrow range, urban and agricultural development, habitat destruction, and pollution (Taylor *et al.* 1996, 2007, 2019; Richman *et al.* 2015). *Cambarus emegi* **sp. nov.** follows this pattern and is negatively impacted by many of those factors simultaneously across much of its range. As populations disappear and its already narrow range further contracts, it is clear that efforts to conserve and manage the species are needed. Unfortunately, such efforts, which fall under the jurisdiction of state and federal natural resource agencies, were not possible because the species had not been formally described. In this paper, we correct that deficiency and provide a suite of characters and geographical information that separate *C. emegi* from *Cambarus acuminatus* (as redescribed by Williams *et al.* 2019) and other undescribed members of the *Cambarus* sp. C complex that occupy drainages bordering the range of *C. emegi*. This in turn will allow biologists to identify and monitor extant populations.

In addition to monitoring populations, to effectively conserve and manage species and mitigate human impacts, natural resource managers must know exactly where species occur and do not occur. *Cambarus emegi* **sp. nov.** is often found in drainage networks that are anthropogenically disturbed to varying degrees, creating a mosaic of suitable and unsuitable stream reaches. As such, its distribution is fragmented across a substantial part of its range, and it is not uncommon to find the species in isolated stream segments where habitat and water quality have been reasonably maintained and invasive crayfish are absent, uncommon, or recent arrivals. Because of this and the fact that areas where the species occurs are riddled with complex, dendritic stream networks, the effort required to find new populations of *C. emegi* **sp. nov.** or new sections of stream where the species occurs will be substantial; however, to protect the species from additional extirpations those efforts are needed.

The *Cambarus* sp. C species complex has long been a taxonomic enigma in need of attention. *Cambarus*

emegi **sp. nov.** is no exception, with over 20 years having passed since it was discovered in the lower reaches of Valley Creek in southeastern Pennsylvania (Lieb *et al.* 2008), puzzling crayfish biologists. Questions regarding the taxonomy, distribution, and conservation status of the species have swirled since its discovery, preventing those charged with its protection and management from making any meaningful progress. However, this paper represents an important step in providing the information needed to finally conserve and manage the species. More broadly, this paper continues the quest to bring clarity to the *C.* species C complex.

Author contributions

DAL has collected and monitored *C. emegi* **sp. nov.** in Pennsylvania for over two decades. BWW and ZJL assisted with sampling in Pennsylvania; DAF, BWW, ZJL, and DAL collected the species in Virginia. ZJL and DAF made specimen measurements. BWW conducted the molecular genetic lab work and analyses, specimen photography, and designed and created the composite figures. All authors wrote and edited the manuscript.

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