Salisa L. Lewis, Julian J. Lewis and William Orndorff. *Caecidotea burkensis*, new species, a unique subterranean isopod from Burke's Garden, with a synthesis of the biogeography and evolution of southwestern Virginia asellids. *Journal of Cave and Karst Studies*, v. 83, no. 2, p. 78-87. DOI:10.4311/2020LSC0126

CAECIDOTEA BURKENSIS, NEW SPECIES, A UNIQUE SUBTERRANEAN ISOPOD FROM BURKE'S GARDEN, WITH A SYNTHESIS OF THE BIOGEOGRAPHY AND EVOLUTION OF SOUTHWESTERN VIRGINIA ASELLIDS

Salisa L. Lewis[1], Julian J. Lewis[2,C] and William Orndorff[3]

Abstract

Caecidotea burkensis, a new species of subterranean asellid isopod, is described and illustrated from material collected from Lawson Cave, in Burke's Garden, Tazewell County, Virginia. The type-locality in Burke's Garden is located within the highest mountain basin in the southern Appalachians. Burke's Garden is a unique, geologically isolated area encompassing one of the headwater streams of the New River basin. Phylogenetically, the isopod is a member of the *forbesi* Group, a clade comprised primarily of epigean species. The complex mountain valleys and coves of southwestern Virginia are an area of intense speciation among asellids that have produced a bizarre array of cavernicolous species belonging to groups of otherwise epigean isopods. In addition to a few subterranean species of the *Caecidotea cannula* and *stygia* Groups, the *Lirceus hargeri* Group possesses over a dozen species endemic to caves and springs in the region, mostly only now in the process of being discovered and described. With so much species richness, syntopy of two, or even three, asellid species is commonplace in caves and springs in southwestern Virginia.

INTRODUCTION

Described as a mountain fortress of Nature by Hoffman (1996, 2012), Burke's Garden protects a unique habitat and considerable biodiversity. From the air, Burke's Garden looks like a crater. However, the crater is not the result of a volcano nor meteorite impact, but rather the breach of a broad sandstone anticlinal dome. This breach formed a broad circular valley in the underlying limestone that developed into a karst terrain of sinkholes, sinking streams, caves, and springs (Figure 1). Burke's Garden is the highest mountain basin in the southern Appalachians, with the floor at about 3,100 feet (almost 950 m) in elevation. The floor is encircled almost entirely by the northerly extension of Clinch Mountain. This circular mountain, formed of the remaining flanks of the anticline, attains elevations averaging above 4,000 feet, and peaks at 4,770 feet (1,454 m) on Bear Mountain on the west side. The single cleft in the mountain occurs at a water gap through which Burke's Garden Creek drains into Wolf Creek, eventually flowing to the New River.

Burke's Garden has long been known for the biodiversity of epigean fauna (Hoffman and Kleinpeter, 1948), although none of 29 obligate subterranean species occurring in the New River basin in Virginia are endemic. The majority are terrestrial. The carabid beetle (*Pseudanophthalmus hortulanus*) occurs in a cave in Burke's Garden and a single cave in Thompson Valley, the next valley to the southwest (Holsinger and Culver, 1988; Virginia Natural Heritage Program, Biotics, unpublished record). Other troglobites occurring in Burke's Garden are the Tazewell County endemic millipede *Pseudotremia tuberculata,* the New River endemic dipluran *Litocampa pucketti,* and the widespread spiders *Nesticus tennessensis* and *Porrhomma cavernicola.* The amphipod *Stygobromus mackini* is also documented in Burke's Garden and is the only widespread stygobiont in portions of the New River basin in Giles, Bland, and Tazewell counties, Virginia lying west of the New River (Holsinger et al, 2013*).*

Steeves (1969) reported the presence of an unusual asellid isopod inhabiting Lawson Cave in Burke's Garden. This isopod could not be assigned to any of the three recognized species groups of subterranean asellids known at the time (Steeves, 1963; 1964; 1966) and it was speculated to be the product of a relatively recent groundwater invasion. This isopod is the subject of this paper.

SYSTEMATICS

forbesi Group

The *forbesi* Group was erected by Lewis (2013) to receive *Caecidotea insula,* a troglomorphic species from a cave on South Bass Island in Lake Erie, Ohio. The significance of this assemblage was its dominance by epigean species (*C. forbesi*, *C. obtusa, C. attenuata* and *C. racovitzai*) that seemed to have little ecological or evolutionary inclination toward inhabiting groundwater. Lewis (2013) also noted the presence of the Burke's Garden asellid inhabiting Lawson Cave. Further examination indicates the Lawson Cave population is related to, but distinct from, *C. racovitzai* and is herein described as the second stygobiont species of the *forbesi* Group.

[1]Lewis and Associates, Cave, Karst and Groundwater Biological Consulting, LLC
[2]Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution
[3]Karst Program, Virginia Department of Conservation and Recreation
[C]Corresponding author: lewisbioconsult@gmail.com

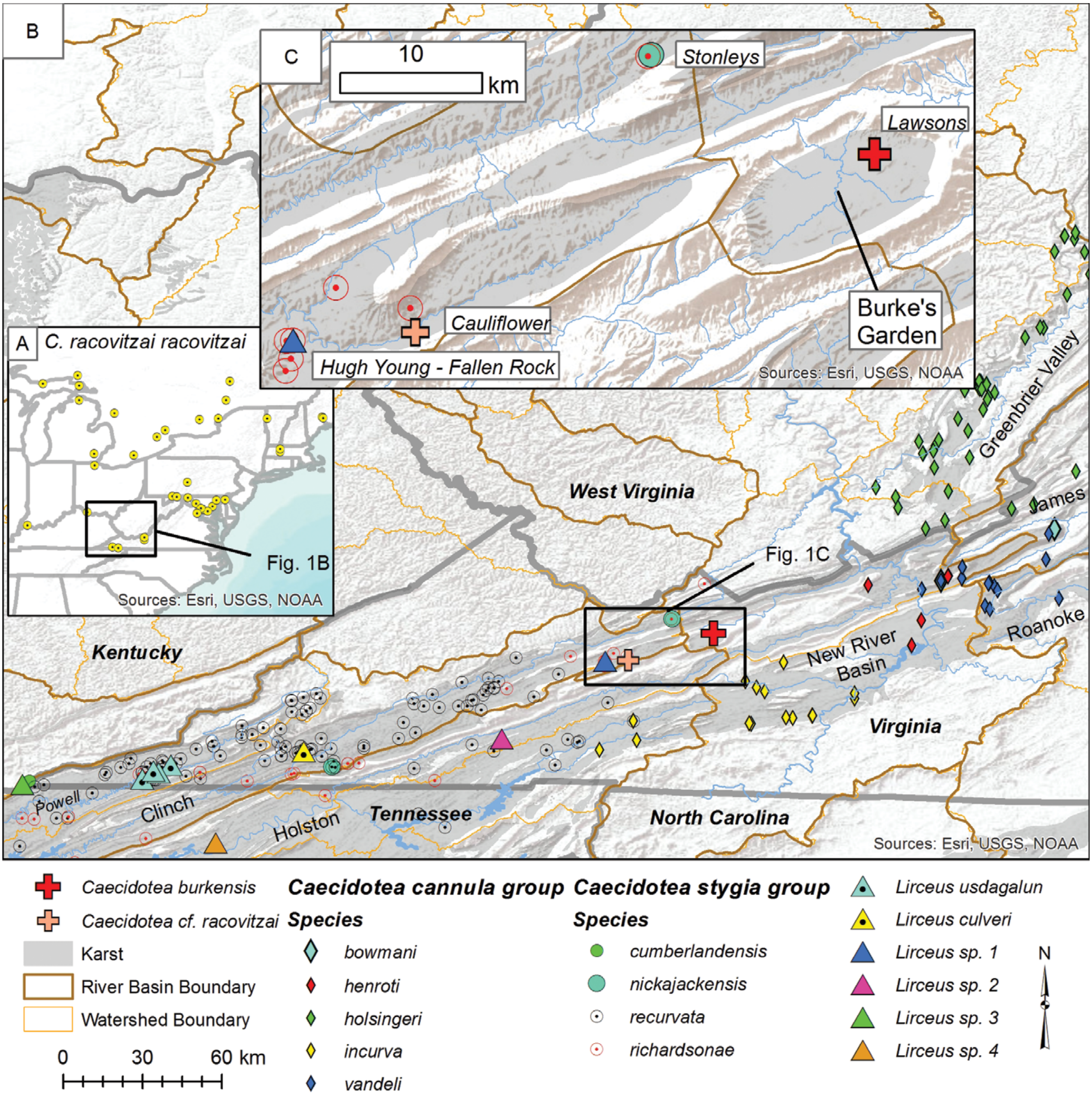

Figure 1. Asellid isopod range maps: (a) Complete range of *Caecidotea racovitzai racovitzai* in the eastern United States; (b) all subterranean asellids known from southwestern Virginia; (c) Burke's Garden and environs with known subterranean asellid populations.

Caecidotea burkensis, S. Lewis and J. Lewis, new species

A. sp. D. [*Asellus*] —Steeves, 1969: 60-61.

Caecidotea racovitzai racovitzai.—Fleming, 1972: 242; Holsinger and Culver, 1988: 32, 37.

Caecidotea racovitzai.—Lewis, 2013: 67.

Material examined—VIRGINIA: Tazewell County, Burke's Garden, Lawson Cave, cave stream, Salisa L. Lewis, Julian J. Lewis, 6 October 2019, 14♂ 12♀ (USNM 1622329); same locality, William D. Orndorff, Ellen Crowder, 7 July 2018, 18♂ 22♀ (USNM 1622330); Kenneth Dearolf, 3 July 1937, 3♂1♀ (USNM 108535); same locality, John R. Holsinger, Laurence E. Fleming, 12 April and 27 July, 1963, 5♂ 5♀ (USNM 230603); Lawson Insurgence Cave, mud-bottomed drip pools, William D. Orndorff, Ellen Crowder, 7 July 2018, 6♂ 7♀ (USNM 1622331).

Figure 2. *Caecidotea burkensis*, new species, paratype males, length approximately 8mm, from Lawson Cave, Tazewell County, Virginia.

The holotype is an 8.5mm ♂ from the collection of 6 October 2019 (USNM 1622328), all other specimens cited above are paratypes. The material examined has been deposited in the National Museum of Natural History, Smithsonian Institution, Washington, D.C. Of note, Fleming labelled specimens as holotype and allotype in the 1963 collection, but never published a description, thus, these specimens have no valid taxonomic status beyond that of paratypes established herein.

Diagnosis.—Within the *forbesi* Group, *C. burkensis* is morphologically most similar to *C. racovitzai racovitzai*. The two species are readily separated by the stygobiont morphology of *C. burkensis*, which possesses a more elongate body, >3X as long as wide, as compared to <3X in *C. r. racovitzai*, e.g., 2.7X in holotype (Williams, 1970), more elongate uropods, and eyes and pigmentation vestigial or absent. Pereopod 1 propodus is over 1.7X as long as wide and subovate in *C. burkensis*, 1.3X and subtriangular in *C. r. racovitzai*. Pleopod 1 protopod of *C. burkensis* has four retinaculae vs. two in *C. r. racovitzai*.

Description—Light, dorsal magenta pigmentation visible under low magnification in large adults, most prominent in ovigerous females, most concentrated on anterior surface of head; under higher magnification (200X) pigment appears diffuse and in a somewhat reticular pattern. The isopods appear whitish to the naked eye (Figure 2). Eyes absent, vestigial as unformed magenta smudges of pigmentation, or up to 7-8 ocelli (Table 1), most individuals with pigmented ocelli possess 2-5 of irregular shape and size. Longest male 8.5 mm, female 6.5 mm, ovigerous females from 4.9-6.3 mm; body approximately 3.3X as long as wide. Margins of head, pereonites and pleonites moderately setose, post-mandibular lobes well produced. Pleotelson slightly longer than wide, caudomedial lobe moderately produced.

Antenna 1 of 9-10 articles, distal 3-4 articles each with single esthetes (Figure 3). Antenna 2, distal article of peduncle approximately 1.8X length of penultimate article, flagellum to 80 articles. Mouthparts per the diagnosis of the genus (Lewis, 2009b).

Pereopod 1, propodus of male approximately 1.75X as long as wide, palmar margin with two stout, elongate proximal spines, medial triangular process reaching to dactyl flexor margin, distal process absent; dactylus with row of stout

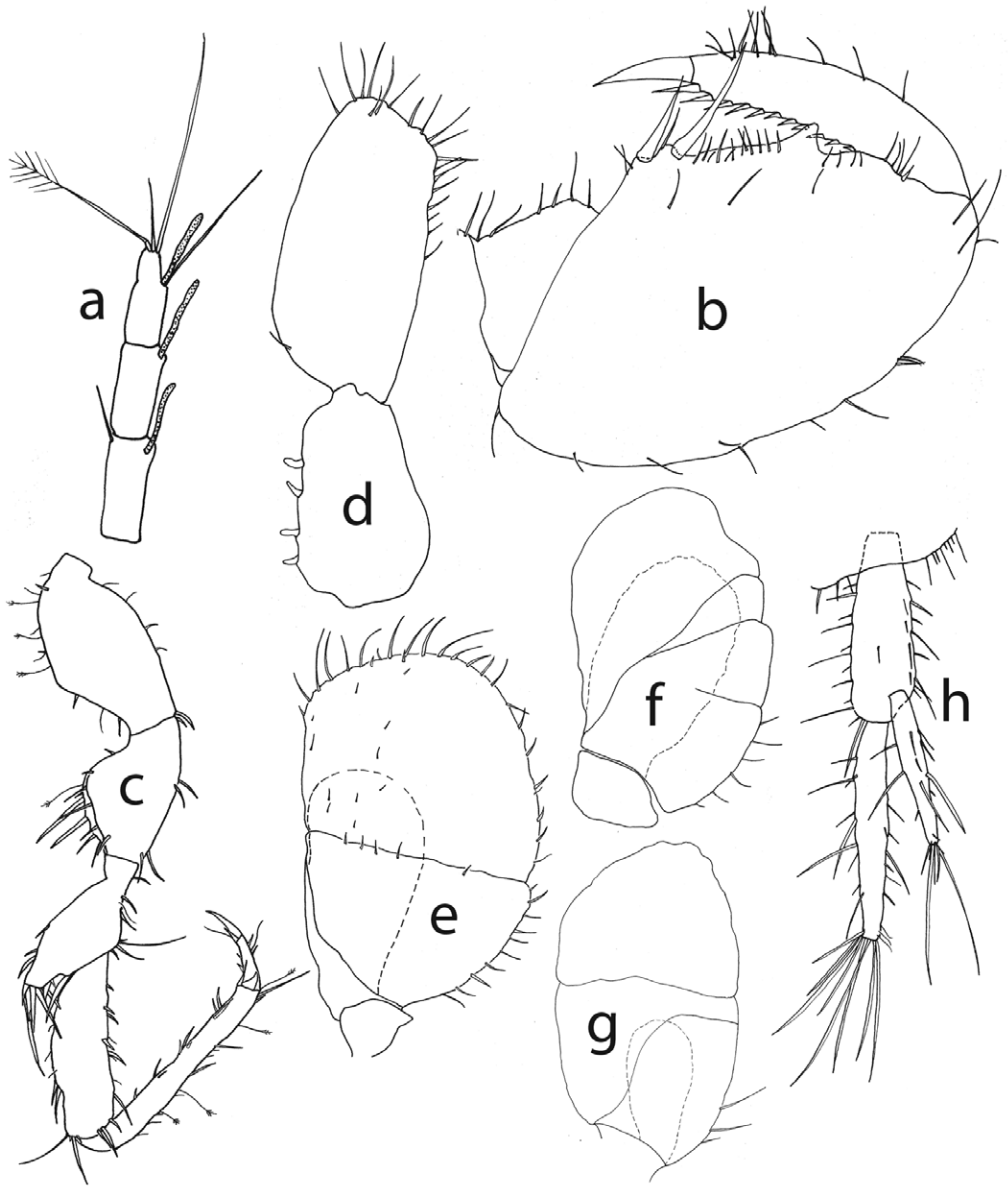

Figure 3. *Caecidotea burkensis,* new species, Lawson Cave, Tazewell County, Virginia, paratype male: (a) antenna 1 distal articles of flagellum; (b) gnathopod; (c) pereopod 4; (d) pleopod 1; (e) pleopod 3; (f) pleopod 4; (g) pleopod 5; (h) uropod.

spines along flexor margin, increasing in size distally (Figure 3). Pereopod 4 the shortest of the 6 walking legs (Table 2), carpus of male 2.8X as long as wide, carpus of female more slender, 3.1X as long as wide.

Pleopod 1 slightly longer than pleopod 2, protopod sub-rectangular, approximately 0.75X length of exopod, 4 retinaculae; exopod sub-rectangular, approximately 2.0X as long as wide, tapering slightly distally, mesial and lateral margins slightly convex, single proximomesial seta, distal half of lateral margin and distal margin with short to moderately long non-plumose setae, longest setae at apex (Figure 3).

Pleopod 2, protopod quadrate, about 1.3X as long as wide, 2 setae present on mesial margin proximal to insertion of endopod; exopod about 1.1X length of endopod, proximal article with 3 lateral setae, distal article ovate, about 2.3X length of proximal article, with approximately 15 elongate plumose setae along margins (Figure 4). Endopod narrow, sides subparallel, small rounded basal apophysis, tip with three processes: (1) cannula conical, conspicuous, distally truncate, endopodial groove prominent adjacent to mesial process; (2) mesial process well-developed, obscuring base of cannula, curved mesiad, tapering to a point; (3) caudal process large, sclerotized, scallops on caudal surface, terminating in acute apex, small spine-like structures present along lateral margin.

Pleopods 3-5 per Figure 3; pleopod 4 exopod with sigmoid false suture, about 6 setae present along proximolateral margin; pleopod 5 with about 3 setae along proximolateral margin.

Uropod subequal in length to pleotelson, protopod about 0.8X length of endopod, exopod about 0.75X length of exopod (Figures 2, 3).

Etymology.—The name *burkensis* is a contraction of Burke + ensis, a Latinization referring to Burke's Garden, the site of the type-locality of the species. The suggested vernacular name is the Burke's Garden cave isopod.

Relationships.-- *Caecidotea burkensis* and *C. racovitzai* share many morphological similarities suggesting a close evolutionary relationship. At the time of the description of the species, Williams (1970) divided *Caecidotea racovitzai*

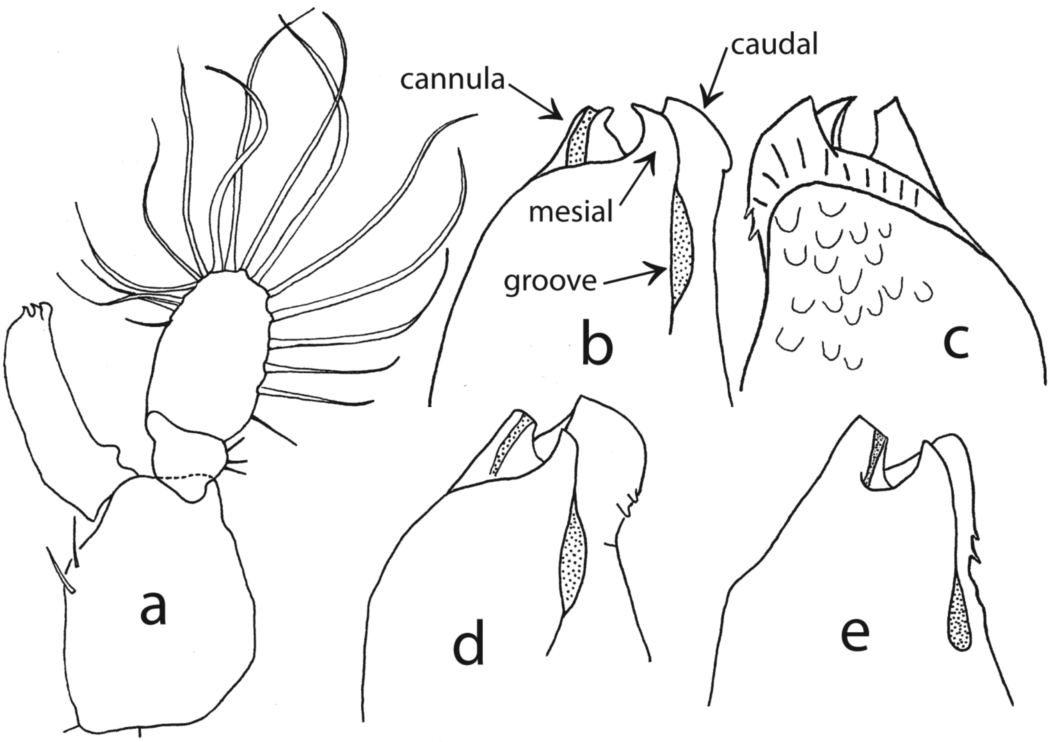

Figure 4. *Caecidotea burkensis,* new species, Lawson Cave, Tazewell County, Virginia, paratype male: (a) pleopod 2; (b) pleopod 2 endopodite tip, anterior; (c) same, posterior; *Caecidotea racovitzai racovitzai*, Cauliflower Cave, Tazewell County, Virginia: (d) pleopod 2 endopodite tip, anterior; Golf Course Spring, Smyth County, Virginia: (e) pleopod 2 endopodite tip, anterior.

into two subspecies: (1) *C. r. racovitzai*, occurring from Indiana east to Virginia, and through most of the Great Lakes, northeastern U.S. and adjacent Canada; and (2) *C. r. australis*, occurring in Florida and southern Georgia. To eliminate confusion if these subspecies are elevated to species at a later date, herein only *C. r. racovitzai* is considered. Lawson Cave is located near the southern edge of the range of this subspecies (Figure 1).

The key aspects of the morphology employed to characterize species of *Caecidotea* are structures of the gnathopod (first pereopod), first and second pleopods, and uropods. Examining first the propodus of the gnathopod, that of *C. racovitzai racovitzai* was described by Williams (1970) as being 1.3X as long as wide and subtriangular in shape, whereas in *C. burkensis* the gnathopod propodus is over 1.7X as long as wide and subovate. The palmar margin in both species has stout proximal spines and a triangular median process, but this process exceeds the width of the dactylus in *C. racovitzai,* whereas it is less than half that in *C. burkensis*. The appendages of *C. burkensis* are more slender and elongate than in *C. r. racovitzai,* as demonstrated by the index of length vs. width in the carpus of pereopod 4 (Table 2).

The first pleopod of both species is similar in general shape and appearance. The protopod of *C. burkensis* has twice the number of retinaculae (four vs. two described in *C. r. racovitzai*). Both species also have in common a single seta near the proximomesial margin, and similar setation along the apical and lateral margins. Likewise, the second pleopod protopod of each species possesses two distomesial setae. Both possess an exopod proximal article with several short setae along the lateral margin, and the distal article is ovate with elongate plumose setae along all but part of the proximomesial margin. The second pleopod endopod has a rounded basal apophysis. The endopod tip of both species has three processes quite similar in appearance (Figure 4): (1) cannula conical, not exceeding caudal process in length; (2) mesial process acutely pointed, curved mesiad, about equal in length to cannula; (3) caudal process larger and slightly longer than the other processes, ending in distal apex. Williams (1970) illustrated a variety of pleopod 2 endopodite tips that suggests that differences in the relative size of the processes is variation between populations of *C. r. racovitzai*.

Lewis et al. (2020) indicated reliance on these conventional morphological characteristics to separate asellid species significantly underestimated diversity in the *Lirceus hargeri* Group. This is also the case with *C. burkensis* and *C. r. racovitzai*, particularly considering their morphologically similar pleopod 2 endopodite tips (Figure 4, b, d, e). Separating species in the *Lirceus hargeri* Group required using other morphological characteristics, including troglomorphisms such as the reduction of eyes and pigmentation, and elongation of the body and appendages. In the case of *C. burkensis*, as compared to *C. r. racovitzai*, the eyes are vestigial or absent, and the dorsal pigmentation is absent or greatly reduced. The body of *C. burkensis* is more elongate, as are the pereopods and uropods.

In addition to the morphological inference, molecular analysis shows divergence greater than 0.16 substitution per site in the COI gene, as measured by patristic distances, between *C. burkensis* and the populations tested of *C. racovitzai racovitza* and *C. r. australis* (Douady, C.J. and Malard, F., unpublished data). This strongly suggests three species are present (Lefébure et al., 2006).

Table 1. Variation in presence or absence of eye vestiges in *Caecidotea burkensis*.

Habitat	Gender	Ocelli absent, no pigmentation	Ocelli absent, magenta pigmentation	Ocelli present
Stream 7 July 2018	Males	6	8	4
	Females	11	8	3
Pools 7 July 2018	Males	0	5	1
	Females	1	1	5
Stream 6 October 2019	Males	12	1	1
	Females	10	1	1
	Total	40	24	15

Table 2. Comparison of approximate length of carpus (measured in micrometers) of pereopods 1-7 of *Caecidotea burkensis* and *Caecidotea racovitzai racovitzai*, and index of length versus width of pereopod 4 carpus in males and females. Measurement of the carpus is indicative of the total relative length of the legs, with pereopod 4 frequently being the shortest walking leg, modified in the male for grasping during copulation.

Carpus of ♂ pereopod: (length in micrometers)	1	2	3	4	5	6	7	Pereopod 4, Length/Width
Caecidotea burkensis (8.1 mm ♂; 6.5 mm ♀) Lawson Cave, Tazewell Co., Va.	50	70	80	60	88	103	123	♂ 2.8X ♀ 3.1X
Caecidotea r. racovitzai (11.8 mm ♂; 7.0 mm ♀) Golf Course Spring, Smyth Co., Va.	58	83	88	68	98	125	135	♂ 2.1X ♀ 2.5X

Table 3. Sites where syntopy of asellids occurs in southwestern Virginia.

Site	*C. recurvata*	*C. richardsonae*	*C. nickajackensis*	*L. usdagalun*	*L. culveri*	*L.* **undescribed spp.**
Flanary Bridge Springs (Lee Co.)	X			X		X
Olinger Cave (Lee Co.)	X	X				
Smiths Milk Cave (Lee Co.)	X	X				
Surgener Cave (Lee Co.)	X			X		
Thompson Cedar Cave (Lee Co.)	X			X		
Blair-Collins Cave (Scott Co.)	X	X				
Carters Pit (Scott Co.)	X				X	X
Coley No. 2 Cave (Scott Co.)	X	X				X
Lane Cave (Scott Co.)	X		X			X
McDavids Cave (Scott Co.)	X				X	
Fallen Rock Cave (Tazewell Co.)		X				X
Stonley Cave (Tazewell Co.)		X	X			

Distribution and ecology.— The location and a description of the type-locality, Lawson Cave, was presented by Holsinger (1975). The cave is entered via a karst window allowing egress to passages both upstream and downstream. The upstream segment is comprised of about 60 m of passage, truncated by a siphon prohibiting further exploration. Upstream from this siphon is another short section of passage termed here the Lawson Insurgence Cave, where the water from a surface stream sinks. Most of the enterable cave passage is on the downstream end of the karst window, extending for about 380 m before another siphon is encountered. The passage is relatively large, ranging from 7-12 m in width, and mostly 1-3 m in height. The entire length of the passage is occupied by the stream, with riparian mud-banks on either side. The stream is believed to resurge at Fish Spring, located about 1500 m to the west. On the visit to Lawson Cave in October 2019, the surface stream flowing across the floor of the karst window was searched, but no isopods were found. The search continued downstream, where *C. burkensis* was found in the dark zone of the cave, where the isopods occurred on sticks and logs washed in from the karst window.

The collection of 7 July 2018 contained three ovigerous females, measuring approximately 4.9, 6.0 and 6.3 mm in length. The 6 October 2019 collection had one ovigerous female approximately 5.8 mm in length. The brood pouch was not dissected, but under visual inspection appeared to contain 22 embryos.

Caecidotea racovitzai racovitzai **(Williams, 1970)**

Material examined.—DISTRICT of COLUMBIA: Potomac River below Aqueduct Bridge, W.P. Hay, 15 March 1896, 24♂♀ (holotype USNM 122066, allotype USNM 122067, paratypes USNM 122068); OHIO: Lucas County: stream in old locks, Side Cut Metropark, Maumee, Salisa L. Lewis, Julian J. Lewis, 9 August 2017, 1♂. VIRGINIA: City of Bristol: spring, Old Abingdon Highway, Julian J. Lewis, Charles D.R. Stephen, 10 October 2019, 1♂1♀. Smyth County: I-81 Cave, 2.8 miles southwest Marion, Thomas E. Malabad, Katarina Kosič Ficco, 10 August 2018, 12♂♀; Golf Course Spring, 1.4 miles southwest Marion, Thomas E. Malabad, Katarina Kosič Ficco, 10 August 2018, 10♂♀; Tazewell County: Cauliflower Cave, Thomas E. Malabad, Katarina Kosič Ficco, 14 October 2018, 1♂. WASHINGTON: King County: Boeing Creek, Boeing Creek Park, Seattle, Julian J. Lewis, 8 June 2018, 15♂♀; Green Lake, Seattle, Julian J. Lewis, 6 June 2018, 10♂♀; East Fork Issaquah Creek, High Point, Julian J. Lewis, Victor M. Lewis, 10 June 2018, 10♂♀.

The specimens of the type series of *C. r. racovitzai* reside in the collection of the Smithsonian Institution. Under examination the isopods have the robust appearance typical of epigean *Caecidotea*, i.e., broad habitus, distinctly pigmented, prominent eyes, with stout appendages. The type-locality, the Potomac River on the Virginia side of the Aqueduct Bridge (Washington metro area) was visited in December 2019. No isopods were found, but many specimens collected from an area about 5 km northwest around the Chain Bridge are present in the Smithsonian collections and there is little doubt the species still exists in the area.

The isopod from Cauliflower Cave, Virginia is troglomorphic with vestigial eyes and pigmentation. It is tempting to identify this isopod as *C. burkensis* due to the morphological similarities and geographic proximity, with only 17 miles (27 km) separating Cauliflower and Lawson caves. However, Cauliflower Cave occurs in Thompson Valley, an adjacent mountain cove south of Burke's Garden, and moreover, is in a different river basin. The water in Cauliflower Cave drains to the Clinch River, part of the Tennessee River drainage, whereas Lawson Cave occurs in the New River basin. With only a single specimen too little is known at this time to further assess this intriguing isopod population.

As noted by Williams (1970), a population of *C. r. racovitzai* occurs in the vicinity of Seattle, Washington, where it was presumably introduced from the eastern U.S., perhaps transported with commercially available water lilies or some similar vector.

Caecidotea racovitzai australis **(Williams, 1970)**

Material examined.—FLORIDA: Jackson County: Russ Mill Creek at Union Road Bridge, 4.3 miles NNE Cottondale, Julian J. Lewis, Charles D.R. Stephen, 9 January 2018, 22♂♀; Leon County: Gopher Hole, Leon Sinks Geological Area, from sticks in entrance room pool, Julian J. Lewis, Charles D.R. Stephen, 10 January 2018, 13♂♀; Wakulla County: Wakulla River, Wakulla Springs State Park, Julian J. Lewis, Charles D.R. Stephen, 10 January 2018, 15 ♂♀.

The isopods from Gopher Hole (a cave) appear mildly depigmented and genetic sequencing also revealed divergence from local populations of *C. r. australis* greater than 0.16 substitution per site in the COI gene (Douady C.J. and Malard F., unpublished data).

Caecidotea* cf. *nickajackensis **Packard (1881)**

Material examined.—VIRGINIA: Scott County: Lane Cave, 7 miles southwest of Snowflake, William D. Orndorff, 12 October 2019, 18♂♀. Tazewell County, Stonley Cave, 5.2 miles northeast of Tazewell, Thomas E. Malabad, William D. Orndorff, 10 January 2019, 11♂♀.

Described by Packard (in Cope and Packard, 1881), this species was considered extinct after the type-locality, Nickajack Cave, was inundated by a TVA lake. Lewis (2009a) redescribed the species from a specimen collected prior to the creation of the lake. Coleman and Zigler (2015) discovered three new localities in southeastern Tennessee and adjacent northeastern Alabama. Despite the morphological similarities, we are dubious the Virginia populations are conspecific with those in the vicinity of the type-locality in southeastern Tennessee given the separation of over 320 km, but for the moment these are considered the first collections of *C. nickajackensis* in Virginia. The records are presented to allow inclusion in the discussion and Figure 1.

Biogeography and evolution of southwestern Virginia groundwater asellids

There are four species groups of stygobiont asellids occurring in caves in southwestern Virginia (Fig. 1). These are: (1) *Lirceus hargeri* Group (Lewis 2020 et. al), (2) *Caecidotea stygia* Group (Steeves, 1963; Lewis, 1988), (3) *Caecidotea cannula* Group (Steeves, 1966; Lewis 1980, 2009b), and (4) *Caecidotea forbesi* Group (Lewis, 2013). Collaborating with C. Douady, F. Malard and L. Konecny-Dupré (Université Lyon-1), work in progress on the molecular phylogeny of subterranean asellids suggests these species groups are monophyletic. Considering first the *Lirceus hargeri* Group (Lewis, et. al, 2020), the species of this group are nearly ubiquitous spring inhabitants in the upper Tennessee River basin. In the intermontane karst valleys of southwestern Virginia the *hargeri* Group comprises the most species-rich assemblage of asellids in North America. Six stygobiont species are troglomorphic to varying degrees, ranging from possessing vestigial eyes and pigmentation to complete absence (Holsinger and Bowman, 1973; Estes and Holsinger, 1976; Lewis and Lewis, in preparation). These subterranean species appear to be the results of localized groundwater invasions of related species, examples of which can be seen in the process in places like Speers Ferry or Lane caves (Scott County), or Young-Fugate Cave (Lee County).

Four species of the *stygia* Group occur in southwestern Virginia: *Caecidotea richardsonae, C. recurvata, C. cumberlandensis* and *C. nickajackensis* s. latu. All species of the *stygia* Group are obligate inhabitants of caves or other groundwater habitats, with only one midwestern species (*C. beattyi*) retaining vestigial pigmentation in evidence of an ancestral epigean derivation. The four Virginia species occur in caves located along the Powell, Clinch and Holston rivers (headwater streams of the Tennessee River). Except for one isolated case involving a stream capture, there are no records of any of these species extending northward into the drainage of the New River.

Looking next at the *cannula* Group, all species are stygobionts restricted to the Appalachians. In southwestern Virginia, the *cannula* Group is represented in the Tennessee River drainage by *C. incurva* (*C. nortoni* occurs in adjacent northeastern Tennessee). *Caecidotea incurva* also occurs to the north in the New River basin, along with *C. henroti* and *C. vandeli* (plus *C. holsingeri* in West Virginia). *Caecidotea incurva* is restricted to caves west of the course of the modern New River, while *C. vandeli* is restricted to caves east of the channel. *Caecidotea henroti* is only found in caves within a few kilometers of the river, but on either side.

The last group to be considered is the *forbesi* Group, with only two described subterranean species, *C. burkensis* in Virginia and *C. insula* in a cave on South Bass Island in Lake Erie, Ohio. Both species were derived from obvious epigean ancestors in geographic areas so isolated no other asellids presented any competition. In Virginia, the dearth of aquatic cave isopods extends beyond Burke's Garden to an entire sub-basin of the New River, where there exists only one record of *C. henroti*. The sub-basin encompasses the drainages of Wolf, Cove and Laurel creeks, on the west side of the present-day river channel and north of the Pulaski Fault. Numerous caves occur in this area with abundant, well-sampled and seemingly suitable aquatic habitat, making the lack of stygobiontic asellids puzzling.

Syntopy

The species richness of asellids in southwestern Virginia groundwater habitats has led to discovery of several instances of syntopy, with as many as three asellid species in the same site (Table 3). Estes (1978) demonstrated niche partitioning between *Lirceus usdagalun* and *Caecidotea recurvata* in Surgener Cave (Lee County) based on water velocity and depth ratios. Stygobiont *Lirceus* species exist in high energy streams where the isopods grasp on bare limestone substrates in waterfalls where *Caecidotea* is more likely to wash out during high flows as demonstrated in flume studies (Culver, 1973). A third species, *C. richardsonae*, occurs in Smiths Milk Cave, an upstream subterranean tributary to the Surgener-Gallohan System.

Springs, as ecotones connecting subterranean and epigean habitats, are excellent sites for syntopy to occur. For example, at Flanary Bridge Springs in Lee County, three asellids occur. In the modest spring orifice *Lirceus usdagalun* and *Caecidotea recurvata* can be found on the same piece of stone, along with a site-endemic undescribed epigean species of *Lirceus*.

SUMMARY AND CONCLUSIONS

Caecidotea burkensis is the second troglomorphic species of the *forbesi* Group to be described. Belonging to an assemblage of primarily epigean species, *C. burkensis* retains significant plasticity in the presence of vestigial eyes and pigmentation. Two other troglomorphic populations of the *forbesi* Group in Florida caves remain undescribed. *Caecidotea burkensis* is one of over a dozen recognized stygobiont asellids inhabiting southwestern Virginia and the fringes of adjacent states. The presence of *C. burkensis* in Burke's Garden occurs immediately to the north of the termination of the ranges of the *Caecidotea stygia* and *Lirceus hargeri* groups in the upper reaches of the Tennessee River tributaries. The *Caecidotea cannula* Group is also locally absent. High biodiversity attracts attention from those striving for conservation or endeavoring to understand the intricacies of species interactions, and the asellid fauna of southwestern Virginia does not disappoint. In a region with the highest asellid species richness in North America, of equal interest are isolated caves where only single species occur. Examples are *Lirceus culveri* in McDavids Cave (Rye Cove, Scott County) or *C. burkensis* in Lawson Cave (Burke's Garden, Tazewell County). Southwestern Virginia is replete with opportunities for future work.

ACKNOWLEDGEMENTS

The description of this species was funded by the Cave Conservancy of the Virginias, administered through the Virginia Department of Conservation and Recreation. Much of the material was examined and the initial illustrations prepared at the Department of Invertebrate Zoology, Smithsonian Museum Support Center, Smithsonian Institution. Research at the Smithsonian Institution was funded by the U.S. Fish and Wildlife Service, facilitated by Rose Agbalog. Dr. Karen Osborn hosted our visit to the Smithsonian Institution, which at the Smithsonian Museum Support Center was kindly facilitated by Collection Manager William Moser, and Museum Specialists Karen Reed and Geoff Keel.

Our heartfelt thanks are extended to the owner of Lawson Cave, Mr. John Moss, for allowing us access to Lawson Cave. For their assistance in the field we thank Ms. Ellen Crowder, Dr. Katarina Kosič Ficco, Dr. Victor M. Lewis, Mr. Thomas E. Malabad and Dr. Charles D.R. Stephen. Finally, we thank Dr. Florian Malard, Prof. Christophe Douady and Ms. Lara Konecny-Dupré of the Université Lyon-1 (Villeurbanne, France) for sharing molecular phylogenetic inferences gleaned from our collections of North American asellids. Details are deferred until further analysis has been completed and will then be published.

The Virginia Natural Heritage Karst Program wishes to acknowledge those who provided data for the preparation of the range map: Cave Conservancy of the Virginias, Tennessee Cave Survey, Virginia Speleological Survey, West Virginia Speleological Survey, and Dr. Matt Niemiller.

REFERENCES

Coleman, W. T., and K. S. Zigler, 2015, The rediscovery of *Caecidotea nickajackensis* in Tennessee and Alabama: Speleobiology Notes, v. 7, p. 10-13.

Cope, E. D., and A. S. Packard, 1881, The fauna of Nickajack Cave: American Naturalist, v. 15, p. 877-882. https://doi.org/10.1086/272948

Culver, D. C., 1973, Competition in spatially heterogeneous systems: an analysis of simple cave communities: Ecology, v. 54, p. 102-110. https://doi.org/10.2307/1934378

Estes, J. A., 1978, The comparative ecology of two populations of the troglobitic isopod crustacean *Lirceus usdagalun* (Asellidae): [M.S. Thesis], Department of Biological Sciences, Old Dominion University, Norfolk, Virginia., 85 p.

Estes, J. A., and J. R. Holsinger, 1976, A second troglobitic species of the genus *Lirceus* (Isopoda, Asellidae) from southwestern Virginia: Proceedings of the Biological Society of Washington, v. 89, no. 42, p. 481-490.

Fleming, L. E., 1972, The evolution of the eastern North American isopods of the genus *Asellus* (Crustacea: Asellidae) Part I: International Journal of Speleology, v. 4, p. 221-256. https://doi.org/10.5038/1827-806X.4.3.1

Hoffman, R. L., 1996, A garden of biotic delights: Virginia Explorer, v. 12, no. 2, p. 7-11.

Hoffman, R. L., 2012, A garden of biotic delights: Bannisteria, v. 40, p. 87-89.

Hoffman, R. L., and H. I. Kleinpeter, 1948, Amphibians from Burke's Garden, Virginia: American Midland Naturalist, v.39, no. 3, p. 602-607. https://doi.org/10.2307/2421526

Holsinger, J. R., 1975, Descriptions of Virginia Caves: Virginia Department of Mineral Resources, Bulletin 85, 450 p.

Holsinger, J. R., and T. E. Bowman, 1973, A new troglobitic isopod of the genus *Lirceus* (Asellidae) from southwestern Virginia, with notes on its ecology and additional cave records for the genus in the Appalachians*:* International Journal of Speleology, v. 5, p.261-271. https://doi.org/10.5038/1827-806X.5.3.5

Holsinger, J. R., and D. C. Culver, 1988, The invertebrate cave fauna of Virginia and a part of eastern Tennessee: Zoogeography and ecology: Brimleyana, v. 14, p. 1-162.

Holsinger, J. R., Culver, D. C., Hubbard, D. A., Jr., Orndorff, W. D., and C. S. Hobson, 2013, The invertebrate cave fauna of Virginia: Banisteria, v. 42, p. 9-56.

Lefébure T., Douady C.J., Gouy M., and J. Gibert, 2006, Relationship between morphological taxonomy and molecular divergence within Crustacea: proposal of a molecular threshold to help species delimitation: Molecular Phylogenetics and Evolution, v. 40, p. 435-447.

Lewis, J. J., 1980, A comparison of *Pseudobaicalasellus* and *Caecidotea*, with a description of *Caecidotea bowmani*, new species (Crustacea: Isopoda: Asellidae): Proceedings of the Biological Society of Washington, v. 93, no. 2, p. 314-326.

Lewis, J. J., 1988, The systematics, zoogeography and life history of the troglobitic isopods of the Interior Plateaus of the eastern United States: [Ph.D. dissertation], Department of Biology, University of Louisville, Louisville, Kentucky, 281 p.

Lewis, J. J., 2009a, On the identity of *Caecidotea nickajackensis* (Crustacea: Isopoda: Asellidae): Proceedings of the Biological Society of Washington, v. 122, no. 2, p. 215–224. https://doi.org/10.2988/08-47.1

Lewis, J. J., 2009b, Three new species of subterranean asellid from Virginia (Crustacea: Isopoda: Asellidae), in Roble, S. M. and J. C. Mitchell, eds, A lifetime of contributions to Myriapodology and the natural history of Virginia: A festschrift in honor of Richard L. Hoffman's 80th birthday: Virginia Museum of Natural History Special Publication 16, p. 251-265.

Lewis, J. J., 2013, *Caecidotea insula,* a new species of subterranean asellids from Lake Erie's South Bass Island, Ohio (Crustacea: Isopoda: Asellidae): Journal of Cave and Karst Studies, v. 75, no. 1, p. 64-67. https://doi.org/10.4311/2011LSC0218

Lewis, J. J., Lewis, S. L., Orndorff, W D., Malard, F., Douady, C., and L. Konecny-Dupré, Endangered species management in an era of ever-increasing biodiversity: A case study of the molecular phylogenetics of *Lirceus hargeri*: 2019 National Cave and Karst Management Symposium Proceedings, Bristol, Virginia, National Speleological Society, Huntsville, Alabama, USA, p. 48-54.

Steeves, H. R., III, 1963, The troglobitic asellids of the United States: The *Stygius* Group:American Midland Naturalist, v. 69, no. 2, p. 470-481. https://doi.org/10.2307/2422923

Steeves, H. R., III, 1964, The troglobitic asellids of the United States: The *Hobbsi* Group:American Midland Naturalist, v. 71, no. 2, p. 445-451. https://doi.org/10.2307/2423303

Steeves, H. R., III, 1966, The troglobitic asellids of the United States: The *Hobbsi, Stygius* and *Cannulus* groups: American Midland Naturalist, v. 75, no. 2, p. 392-403.https://doi.org/10.2307/2423400

Steeves, H. R., III, 1969, The origins and affinities of the troglobitic asellids of the southern Appalachians, in Holt, P.C., The distributional history of the biota of the southern Appalachians, Part I, Invertebrates: Virginia Polytechnic and Institute and StateUniversity, Research Division Monograph 1, p. 51-65.

Williams, W. D., 1970, A revision of North American epigean species of *Asellus*: Smithsonian Contributions to Zoology, v. 49, p. 1-79. https://doi.org/10.5479/si.00810282.49