

Two new *Nesticus* Thorell, 1869 (Araneae: Nesticidae) from caves in northwest Georgia, USA

Kirk S. Zigler¹ and Marc A. Milne²: ¹Department of Biology, University of the South, Sewanee, Tennessee, 37383, USA; ²Department of Biology, University of Indianapolis, Indianapolis, Indiana, 46227, USA. E-mail: milnem@uindy.edu

Abstract. We describe two new *Nesticus* Thorell, 1869 from Walker County, Georgia, USA. *Nesticus lula* sp. nov. is known from two caves on the eastern edge of Lookout Mountain and *N. cressleri* sp. nov. is known from three caves on Pigeon Mountain. Morphological and molecular evidence indicates the distinctiveness of both species when compared to other *Nesticus* from the southern Appalachians. *Nesticus lula* has reduced eyes and *N. cressleri* is eyeless. Both species are of conservation concern, as they are known from only a handful of sites spanning extremely limited ranges. This work contributes to our understanding of cave biodiversity in Georgia and of the *Nesticus* radiation in the southern Appalachians.

Keywords: Troglotic, subterranean, taxonomy, Araneoidea

<https://doi.org/10.1636/JoA-S-21-035>

ZooBank Registration: 536C1D2B-EBFD-4082-B7FE-C6E1A0BF08AC

The southern Appalachians are a hotspot for cave biodiversity with high levels of cave-obligate (troglotic) species richness and endemism (Culver et al. 2000; Niemiller & Zigler 2013; Christman et al. 2016). *Nesticus* Thorell, 1869 (Araneae: Nesticidae) spiders are a significant component of this diversity. A radiation of *Nesticus* in the southeastern United States comprises nearly thirty described species including ten troglotic species from Tennessee, Alabama, and Georgia (Gertsch 1984; Coyle & McGarity 1991; Hedin & Dellinger 2005). Troglotic nesticids typically have reduced eyes and pigmentation relative to surface species. Four of the troglotic species are single-cave endemics, and of particular conservation concern (Gertsch 1984; Hedin & Dellinger 2005). Other members of this radiation are troglites (or ‘eutroglites’ after Culver & Pipan 2019). Troglites are facultative cave inhabitants; they may complete their life cycle underground but can also be found in similar habitats outside of caves. Previous work on *Nesticus* from the southern Appalachians has focused on taxonomy (Gertsch 1984; Coyle & McGarity 1991; Hedin & Dellinger 2005), speciation and phylogenetics (Hedin 1997a, b), population genetics (Snowman et al. 2010; Balogh et al. 2020), and life history (Carver et al. 2016).

In the course of studying cave biodiversity in Georgia (Zigler et al. 2020), we encountered two undescribed cave-dwelling *Nesticus* species. We collected specimens from multiple populations of each species and describe both species here. In addition, we report a new locality for a troglotic *Nesticus* that was previously known from a single cave. This work contributes to our understanding of cave biodiversity in the southern Appalachians and of this remarkable *Nesticus* radiation.

METHODS

Sampling.—Specimens were collected by hand between 2013 and 2019 and preserved in 95% ethanol at -20°C. Samples were collected from five caves in Walker County, Georgia [Bee Rock Cave (Georgia Speleological Survey # GWK123), Lula Falls Cave (GWK617), Anderson Spring Cave (GWK46),

Pigeon Cave (GWK57), and Matthew Sink (GWK133)] and one cave in Marion County, Tennessee [Hugden Branch Cave (TMN127)]. Collections were permitted by the Georgia Department of Natural Resources (Permit #8934), the Tennessee Wildlife Resources Agency (Permit #1385), and various landowners.

Descriptions.—Character abbreviations: BL, body length (carapace length plus abdomen length measured in dorsal view); CL, carapace length (from posterior edge to front edge of clypeus, measured at midline); CW, maximum carapace width; LegIFL/LegIPL/LegITL/LegIML/LegITarL, total lengths of leg I segments; LegI total, sum of length of leg I segments; LegI/CW, LegI total divided by CW.

All measurements are in mm and were measured using a Leica M165C stereomicroscope with an attached DMC2900 camera and calibrated annotation tools within the Leica Application Suite X software (Leica Microsystems, LAS Suite X, Version 3.0.12.21488). Photographs were taken using the same stereomicroscope, camera, and software. Photographs were then enhanced in Photoshop (Adobe Photoshop Version 21.2.4) by removing unwanted background and increasing brightness to improve clarity. All illustrations were created by electronically tracing the images of previously described photographs using an XP-PEN Artist 13.3 Pro drawing tablet (XP-PEN Artist 13.3 Pro, B07VPHR6GD) and previously described Photoshop software. Shading and labeling were also completed using Photoshop. Epigyna were removed and cleared with clove oil prior to illustration. The left palp of male spiders was always used for illustration and photography.

We follow Hedin & Dellinger (2005) to describe anatomical structures. All specimens have been deposited in the American Museum of Natural History (AMNH).

DNA sequencing.—DNA extractions were carried out on tissues from two spiders per cave using the DNEasy Blood and Tissue Kit (Qiagen, Inc.) following the standard protocol. We amplified ~600 bp of the cytochrome *c* oxidase subunit I (*COI*) gene by polymerase chain reaction (PCR) using the primers 2776-SPIDER (5'-GGATAATCAGAATANCGNC-GAGG-3') and NESTL2 (5'-GGGGGGATCCAATTT-

TATTTCA-3'). The PCR conditions were 5 minutes at 95°C, followed by 35 cycles of 15 seconds at 95°C, 15 seconds at 50°C, and 1 minute at 72°C. The PCRs finished with 7 minutes at 72°C. PCR results were visualized on 1% agarose gels. Successful PCRs were purified by exonuclease and alkaline phosphatase treatment prior to sequencing.

DNA sequencing of purified PCR products was carried out at the DNA Analysis Facility on Science Hill at Yale University (New Haven, CT). Reactions were performed using BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Thermo Fisher Scientific, Inc.). Dye terminator removal was performed using Performa® DTR Ultra 96-Well Plates (Edge Biosystems). Capillary electrophoresis was performed on a 3730xl DNA Analyzer (Applied Biosystems, Thermo Fisher Scientific, Inc.) with a 96-capillary 50 cm array, using default instrument protocols. The sequences have been submitted to GenBank (GenBank Accession Nos. MZ604679-MZ604691).

Phylogenetic analyses.—We used MEGA X (Kumar et al. 2018) to trim and align the DNA sequences, and for phylogenetic analyses. We supplemented our COI sequences with previously published sequences from *Nesticus furtivus* Gertsch, 1984 (GenBank Accession #GQ421642), *N. georgia* Gertsch, 1984 (GQ421641), and *N. pecki* Hedin & Dellinger, 2005 (GQ421640), as well as an unpublished COI sequence for *N. barri* Gertsch, 1984 from Horseshull Cave (AJK631, Jackson County, Alabama). These additional sequences represent all cave-obligate *Nesticus* within 50 km of the new *Nesticus* populations. We used *Eidmannella pallida* (Emerton, 1875) (GQ421637) as an outgroup. Previous work (Hedin 1997b) indicates *Eidmannella* is an outgroup to the Appalachian *Nesticus* species. We used MEGA X to calculate p-distances between sequences, and to conduct phylogenetic analyses using parsimony and neighbor-joining methods.

TAXONOMY

Family Nesticidae Simon, 1894

Genus *Nesticus* Thorell, 1869

Nesticus lula sp. nov.

ZooBank LSID: 8EAB5E1A-B76F-41D0-84DE-D75A07618DE9
(Figs. 1A, 1C, 2, 3)

Type material.—*Holotype male*. U.S.A.: *Georgia*: Walker County, Lula Falls Cave (GWK617), near Lula Lake on Lookout Mountain, ~5 km S. of the town of Lookout Mountain, elevation ~400 m, 15 April 2014, K.S. Zigler, L.M. Carver, L. Lyles (KSZ13-169; AMNH male specimen ID# AMNH_IZC 00357042).

Paratypes. U.S.A.: *Georgia*: 1 ♀, same data as holotype except 3 August 2013, K.S. Zigler, L.M. Carver, A. Cressler (KSZ13-181; AMNH female specimen ID# AMNH_IZC 00357043); 1 ♀, Walker County, Bee Rock Cave (GWK123), near Flintstone, ~2 km SE of the town of Lookout Mountain, elevation ~200 m, 31 May 2015, K.S. Zigler, M. Abercrombie, T. Lichtefeld (KSZ15-388; AMNH female specimen ID# AMNH_IZC 00357044).

Etymology.—The specific name is a noun in apposition, after Lula Falls Cave on the Lula Lake Land Trust.

Diagnosis.—Slightly smaller than the geographically close *N. georgia*, males of this species can be distinguished by a thin and wide, anteriorly-pointed tegular apophysis. The shape of this tegular apophysis separates *N. lula* from the geographically close *N. furtivus* by being wider and not flattened against the median apophysis, and from *N. georgia* by being smaller, thinner, and not spatuliform. Male palps may also be distinguished from geographically close *Nesticus* by possessing a paracymbium with a ventral process with a pointed proximal portion and a concave, cup-like distal process (PVP and DVP in Figs. 2A, B). Specifically, in nearby *N. georgia*, the proximal portion of the paracymbium is flattened rather than pointed (cf. Gertsch 1984: fig. 156) and in *N. furtivus*, the proximal portion of the paracymbium is rounded and the distal ventral process is greatly reduced (cf. Hedin & Dellinger (2005): figs 15–16). Epigynum similar to *N. georgia*, but with thinner posteriolateral edges and epigynal pockets forming a much wider “V” shape on the anterior edge (Figs. 2C–D and 3E, G).

Description (male holotype; Figs. 1A, C, 2A, B, 3A–C; Table 1).—Appendages and carapace uniform light yellow in color. Abdomen light gray and mottled. AME reduced to small, pigmented spots with no lenses visible. Remaining eyes approximately the same size, rimmed with black pigmentation. Leg formula 1423. Leg I length over nine times carapace width. Paracymbium with proximally-pointed ventral process that flattens anteriorly and extends dorsally along the distal edge. Paracymbium possesses a concave, cup-like distal process that extends to a point (Figs. 2A and 3A). Dorsal process on paracymbium reduced to slight bump (Figs. 2B, 3B). Tegular apophysis thickened with a distal process pointing anteriorly. Median apophysis semi-translucent, rectangular, with distal, anterior edge pointed (Figs. 2A, 3A).

Description (female paratype ID# AMNH_IZC 00357043; Figs. 2C, D, 3D–G; Table 1).—Appendages and carapace color as in male. Abdomen gray with irregular, darker gray patches along midline. Eyes as in male. Leg formula 1423. Leg I tarsus length unknown but total leg I length estimated at ca. 12.6–12.7. This estimation makes leg I slightly more than nine times carapace width. Epigynum width over half the width of the abdomen. Median septum bluntly pointed posteriorly. Wing-shaped pockets lateral of median septum form a “V”-shape on anterior edge. Elongate spermathecae visible underneath bulging posteriolateral edges (Figs. 2C, D, 3E–G).

Distribution.—Known from two caves on the eastern side of northern Lookout Mountain in Walker County, Georgia. The two caves are ~4 km from one another.

Natural history.—The paratype female collected from Bee Rock Cave had an egg sac that was approximately 3.2 mm x 2.5 mm in size and contained 38 eggs.

Nesticus cressleri sp. nov.

ZooBank LSID: D4B36895-7CCE-4152-8FBF-337FCB384E6A
(Figs. 1B, 1D, 4, 5)

Type material.—*Holotype male*. U.S.A.: *Georgia*: Walker County, Anderson Spring Cave (GWK46), on Pigeon Mountain, ~13 km WSW of LaFayette, elevation ~350 m, 11 June 2014, K.S. Zigler, L.M. Carver, W.T. Coleman (KSZ13-159; AMNH male specimen ID# AMNH_IZC 00357045).

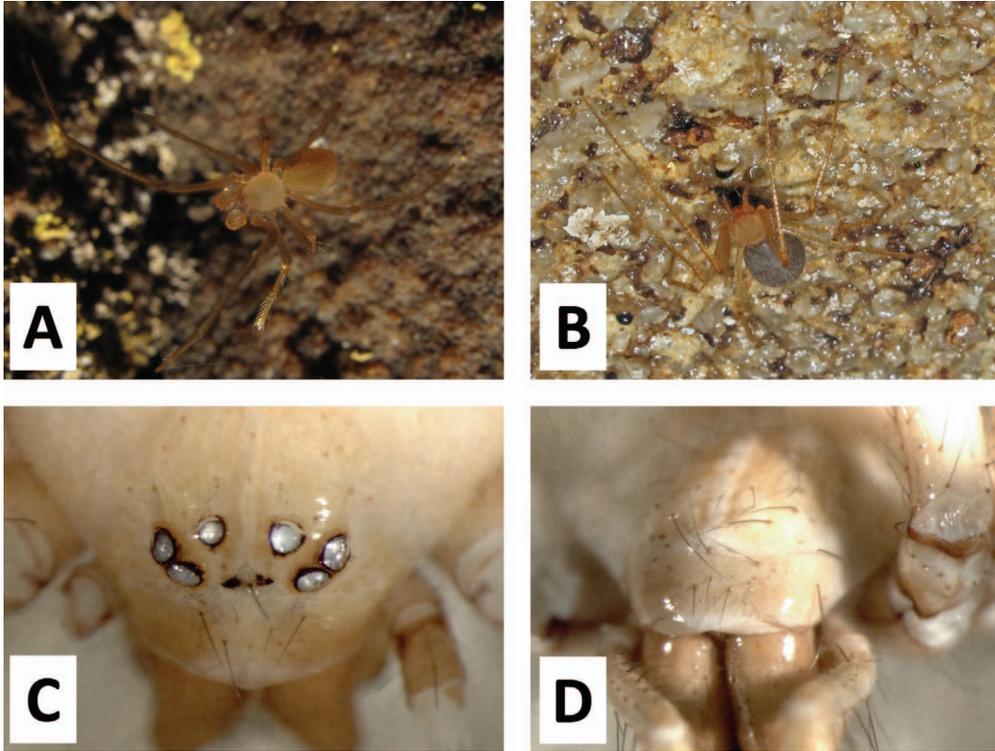


Figure 1.—Two new *Nesticus* species from caves in northwest Georgia: A, *Nesticus lula* sp. nov., penultimate male from Lula Falls Cave, Walker County; B, *N. cressleri* sp. nov., mature female from Pigeon Cave, Walker County. C, D, Frontal views of (C) *N. lula* sp. nov., male from Lula Falls Cave, and (D) *N. cressleri* sp. nov., female from Pigeon Cave. Images A & B by Alan Cressler.

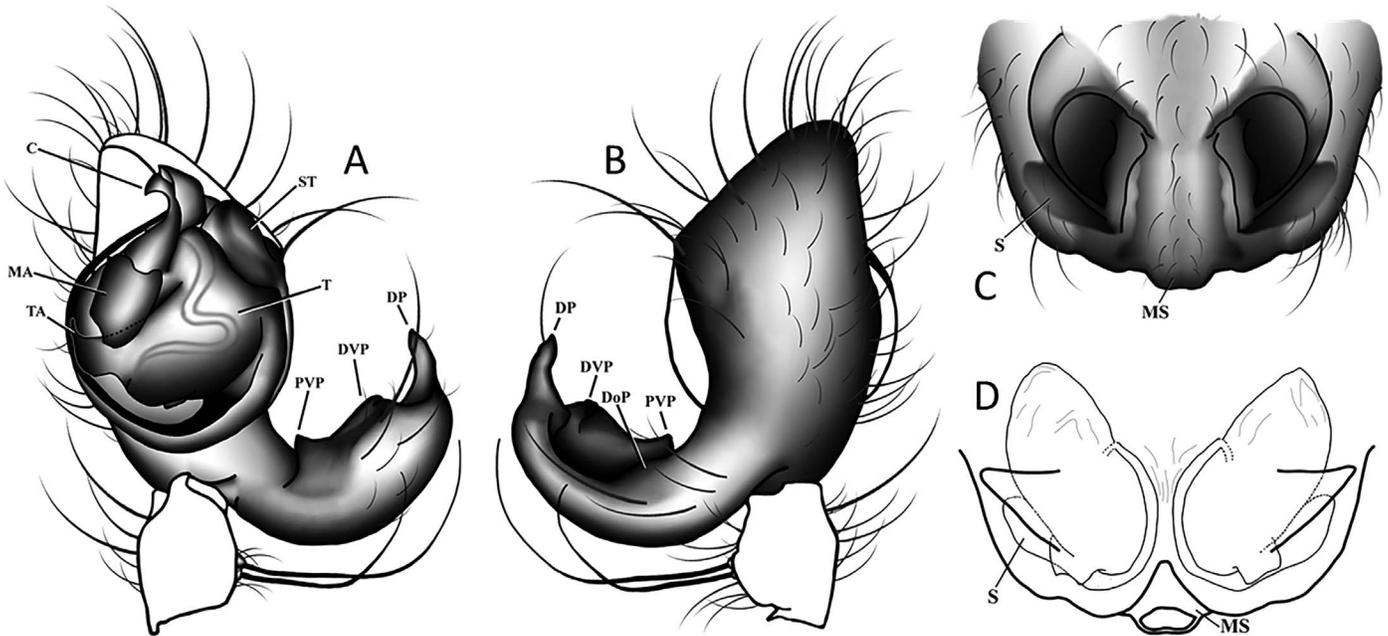


Figure 2.—Illustrations of *Nesticus lula* sp. nov.. A, Male left palp, ventral view; B, male left palp, dorsal view; C, epigynum, ventral view; D, epigynum, cleared dorsal view. Abbreviations: PVP, proximal ventral process of paracymbium; DVP, distal ventral process of paracymbium; DP, distal process of paracymbium; TA, tegular apophysis; MA, median apophysis; C, conductor; ST, subtegulum; T, tegulum; DoP, dorsal process of paracymbium; S, spermathecae; MS, median septum.

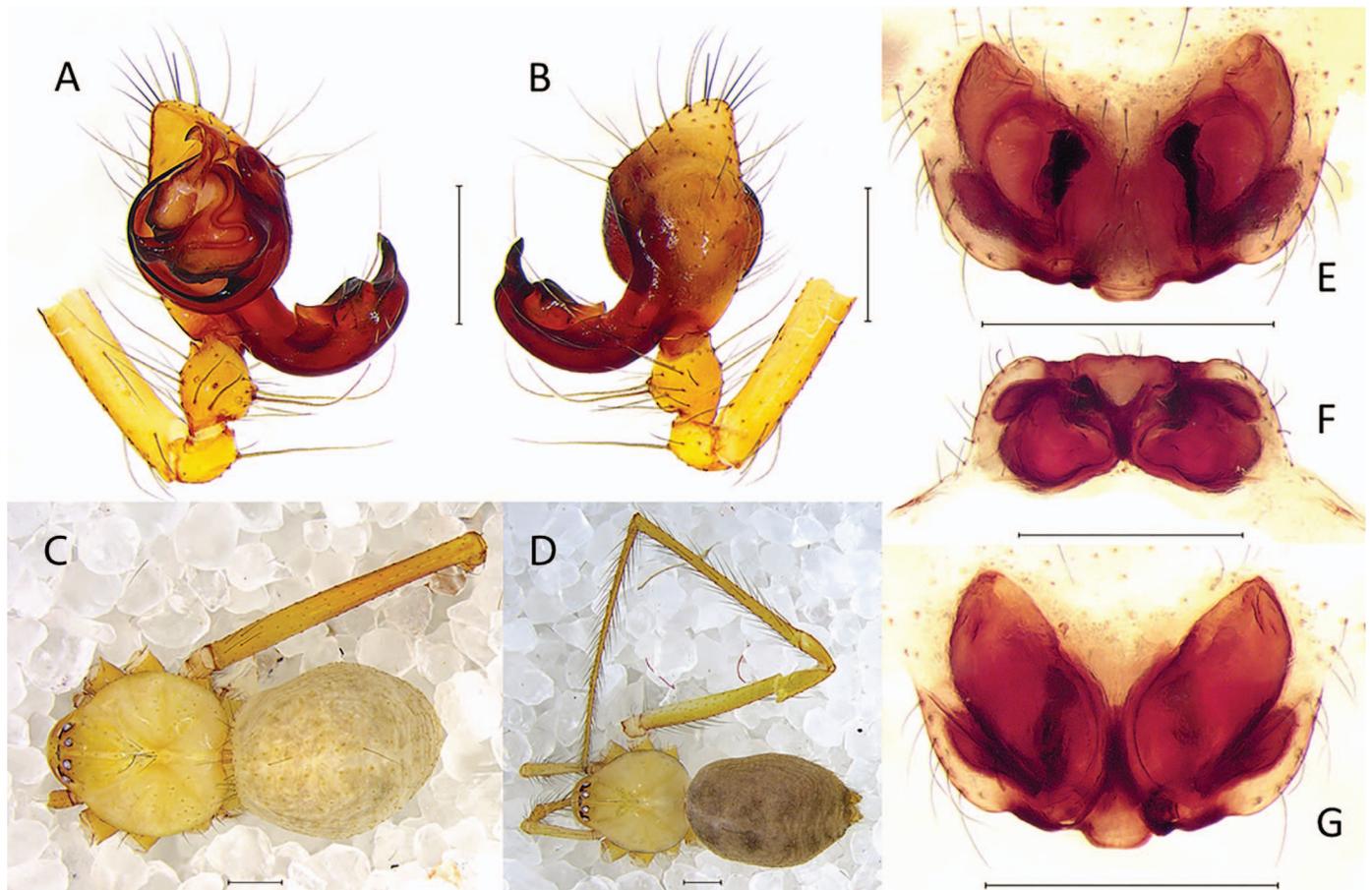


Figure 3.—Photographs of *Nesticus lula* sp. nov.. A, Male left palp, ventral view; B, male left palp, dorsal view; C, male habitus; D, female habitus; E, epigynum, ventral view; F, epigynum, posterior view; G, epigynum, cleared dorsal view. Scale bars represent 0.5mm.

Paratypes. U.S.A.: *Georgia*: 1 ♀, same data as holotype (KSZ13-159; AMNH female specimen ID# AMNH_ICZ 00357046); 1 ♀, 1 ♂, Walker County, Matthew Sink (GWK133), on Pigeon Mountain, ~6 km NW of LaFayette, elevation ~400 m, 7 October 2016, A. Cressler (KSZ19-255; AMNH female specimen ID# AMNH_ICZ 00357047, KSZ18-39; AMNH male specimen ID# AMNH_ICZ 00357048); 1 ♀, Walker County, Pigeon Cave (GWK57), on Pigeon Mountain, ~11 km W. of LaFayette, elevation ~350 m, 3 August 2013, L.M. Carver, A. Cressler, K.S. Zigler (KSZ13-184; AMNH female specimen ID# AMNH_ICZ 00357049).

Other material examined.—U.S.A.: *Georgia*: 1 juvenile, Walker County, Pigeon Cave (GWK57), 3 August 2013, K.S. Zigler, L.M. Carver, A. Cressler (not deposited); 4 juveniles, Walker County, Anderson Spring Cave (GWK46), 11 June 2014, K.S. Zigler, L.M. Carver, W.T. Coleman (not deposited).

Etymology.—The specific name is a patronym in honor of Alan Cressler—scientist, caver, naturalist, and photographer with a particular interest in *Nesticus* spiders.

Diagnosis.—Males and females can be easily distinguished from the geographically close *N. lula* sp. nov. and *N. georgia*

Table 1.—Specimen measurements (mm). Estimated values are marked by an asterisk. BL, body length; CL, carapace length; CW, maximum carapace width; LegIFL, length of leg I femur; LegIPL, length of leg I patella; LegITL, length of leg I tibia; LegIML, length of leg I metatarsus; LegITarL, length of leg I tarsus; LegI total, sum of length of leg I segments; LegI/CW, LegI total divided by CW. See Methods for more detail.

Specimen	BL	CL	CW	LegI FL	LegI PL	LegI TL	LegI ML	LegI TarL	LegI total	LegI/CW
<i>N. lula</i> holotype male (Lula Falls Cave)	3.57	1.65	1.53	4.33	0.77	4.57	4.27	—	~14.8*	~9.67*
<i>N. lula</i> paratype female (Lula Falls Cave)	3.67	1.62	1.37	3.81	0.73	3.8	3.49	—	~12.7*	~9.23*
<i>N. lula</i> paratype female (Bee Rock Cave)	3.48	1.28	1.26	—	—	—	—	—	—	—
<i>N. cressleri</i> holotype male (Anderson Spring Cave)	2.83	1.43	1.26	3.24	0.68	3.36	3.18	1.28	11.74	9.32
<i>N. cressleri</i> paratype male (Matthew Sink)	3.08	1.71	1.3	3.14	0.68	2.98	2.67	1.09	10.56	8.12
<i>N. cressleri</i> paratype female (Anderson Spring Cave)	4.81	1.65	1.38	3.68	0.73	3.63	3.55	1.41	13	9.42
<i>N. cressleri</i> paratype female (Matthew Sink)	3.62	1.63	1.39	3.43	0.71	3.35	3.36	1.37	12.22	8.79
<i>N. cressleri</i> paratype female (Pigeon Cave)	4.45	1.64	1.46	3.89	0.79	—	—	—	—	—

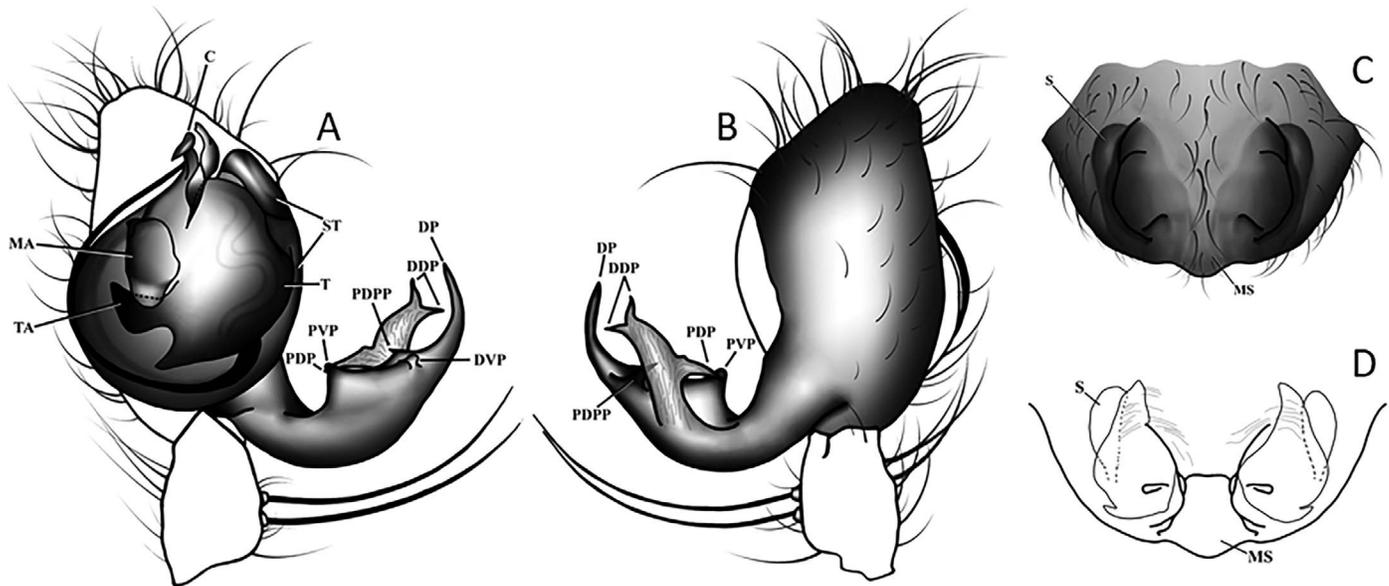


Figure 4.—Illustrations of *Nesticus cressleri* sp. nov.. A, Male left palp, ventral view; B, male left palp, dorsal view; C, epigynum, ventral view; D, epigynum, cleared dorsal view; PVP, proximal ventral process of paracymbium; DVP, distal ventral process of paracymbium; DP, distal process of paracymbium; DDP, distal dorsal processes of paracymbium; PDP, proximal dorsal process of paracymbium; PDPP, prolaterally-directed paradistal process of paracymbium; TA, tegular apophysis; MA, median apophysis; C, conductor; ST, subtegula; T, tegulum; S, spermathecae; MS, median septum.

by their absence of eyes. Males may be distinguished from the geographically close *N. furtivus* – and other *Nesticus* – by palp morphology, specifically *N. cressleri* sp. nov. males possess a fishtail-like dorsal process on the paracymbium which differs markedly from the elongated, thin dorsal process in *N. furtivus* (Figs. 4A, B, 5A, B; compare to Hedin & Dellinger (2005), figs. 15–16). Females may be differentiated from the geographically close *N. furtivus* – and other *Nesticus* – by the morphology of the epigynum, specifically the presence of elongated, lateral spermathecae that extend anteriorly parallel to the pockets lateral of the median septum (Figs. 4C, D, 5E–G; compare to Gertsch 1984: fig. 97).

Description (male holotype; Figs. 4A, B, 5A–C; Table 1).—Carapace with a pale, light yellow color with legs light yellow. Abdomen light yellow. Eyes absent; small spots of orange-brown pigment present. Leg formula 1423. Leg I length over nine times carapace width. Paracymbium with proximally-pointed, triangular translucent ventral process with distal portion ventrally bulged (PVP and DVP in Fig. 4A; Fig. 5A). Dorsal process on paracymbium fishtail-like, extending anteriorly and splitting into two extensions, one prolateral and the other retrolateral. The retrolaterally-pointed extension of the dorsal process splits again, with one extension pointing anteriorly and the other directed retrolaterally (DDP in Figs. 4A, B; Fig. 5B). Paracymbium possesses a pointed distal process and a black, sharply pointed, prolaterally-directed paradistal process (DP and PDPP in Fig. 4B; Fig. 5B). Posterior portion of tegulum with pointed prolaterally-directed process (Figs. 4A, 5A). Tegular apophysis thickened with a black, distal process pointing anteriorly (TA in Fig. 4A; 5A). Median apophysis semi-translucent, oval, with distal, anterior edge pointed ventrally and posterior portion over-

lapping prolaterally-directed process of posterior portion of tegulum (Figs. 4A, 5A).

Description (female paratype ID# AMNH_IJC 00357046; Figs. 1B, D, 4C, D, 5D–G; Table 1).—Appendages and carapace color as in male. Abdomen concolorous gray. Eyes as in male. Leg formula 1423. Leg I more than eight times carapace width. Epigynum width slightly less than half the width of the abdomen. Median septum bluntly pointed posteriorly. Oval pockets lateral of median septum heavily sclerotized on outer edge. Elongate spermathecae curve and extend anteriorly, parallel to outer edges of epigynal pockets (Figs. 4C, D, 5E–G).

Variation.—Male abdomen color varies from a light yellow to a dark gray between the holotype and paratype. Female abdomen color a darker gray to purple when gravid.

Distribution.—Known from three caves on Pigeon Mountain in northwest Georgia. The maximum straight-line distance between caves known to host this species is ~15 km.

Natural history.—The fourteen *Nesticus* observed on 3 August 2013 from Pigeon Cave included nine mature females, eight of which were carrying egg sacs (Carver et al. 2016).

Records.—Buhlmann (2001) first reported a “small, eyeless” *Nesticus* from Pigeon Cave and Anderson Spring Cave. Additional observations of eyeless *Nesticus* from other caves on Pigeon Mountain – Fingerhole Cave (GWK259), Mouldy Bat Pit (GWK257) (Zigler et al. 2020), and Vern’s Cave (GWK346) (A. Cressler, pers. comm) – likely correspond to *N. cressleri* sp. nov..

RESULTS

Molecular results.—We sequenced 580 bp of the *COI* gene from each spider. No indels or stop codons were observed in the sequence. Within each cave, the sequences from the two

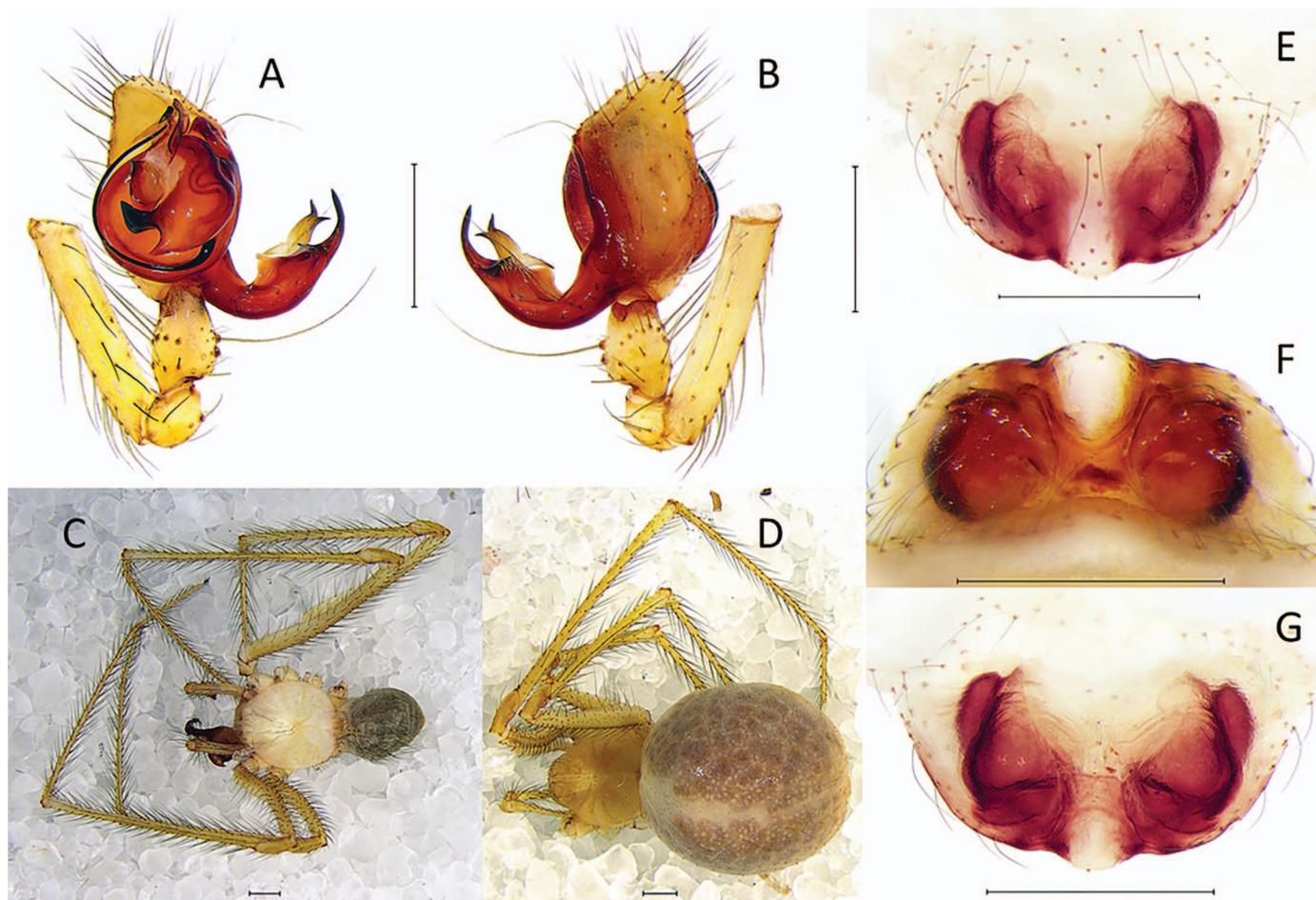


Figure 5.—Photographs of *Nesticus cressleri* sp. nov.. A, Male left palp, ventral view; B, male left palp, dorsal view; C, male habitus; D, female habitus; E, epigynum, ventral view; F, epigynum, posterior view; G, epigynum, cleared dorsal view. Scale bars represent 0.5mm.

individuals were identical (that is, we observed no intra-population genetic diversity at this locus). *COI* p-distances between populations within species ranged from 0.000 to 0.031 (Table 2). *COI* p-distances between *Nesticus* species ranged from 0.084 to 0.126 (Table 2). *COI* p-distances between *Nesticus* populations and the outgroup *Eidmannella pallida* ranged from 0.160 to 0.178 (Table 2).

Phylogenetic analysis of the *COI* sequences grouped the presumed members of *N. cressleri* (from Anderson Spring Cave, Pigeon Cave, Matthew Sink), *N. lula* (Lula Falls Cave, Bee Rock Cave), and *N. furtivus* (Hugden Branch Cave, and the previously known population from Raccoon Mountain Caverns) with 100% bootstrap support (Fig. 6). Relationships between species were not resolved (Fig. 6). Neighbor-joining

Table 2.—Genetic distances between cytochrome *c* oxidase subunit I sequences. Pairwise p-distances (the proportion of nucleotide differences per site) are shown, calculated from 580 bp of sequence. Intraspecific distances are indicated for *Nesticus lula* sp. nov. (**bold**), *N. cressleri* sp. nov. (**bold italics**), and *N. furtivus* (**bold underline**).

	Bee Rock Cave	Lula Falls Cave	Anderson Spring Cave	Pigeon Cave	Matthew Sink	Hugden Branch Cave	<i>N. furtivus</i>	<i>N. barri</i>	<i>N. georgia</i>	<i>N. pecki</i>
Lula Falls Cave	0.031									
Anderson Spring Cave	0.103	0.109								
Pigeon Cave	0.103	0.109	0.000							
Matthew Sink	0.110	0.112	0.026	0.026						
Hugden Branch Cave	0.084	0.093	0.103	0.103	0.107					
<i>N. furtivus</i>	0.088	0.097	0.107	0.107	0.107	0.007				
<i>N. barri</i>	0.096	0.094	0.106	0.106	0.108	0.089	0.092			
<i>N. georgia</i>	0.088	0.092	0.121	0.121	0.123	0.099	0.099	0.103		
<i>N. pecki</i>	0.117	0.121	0.117	0.117	0.121	0.126	0.124	0.110	0.125	
<i>Eidmannella pallida</i>	0.164	0.166	0.160	0.160	0.160	0.164	0.164	0.146	0.162	0.178

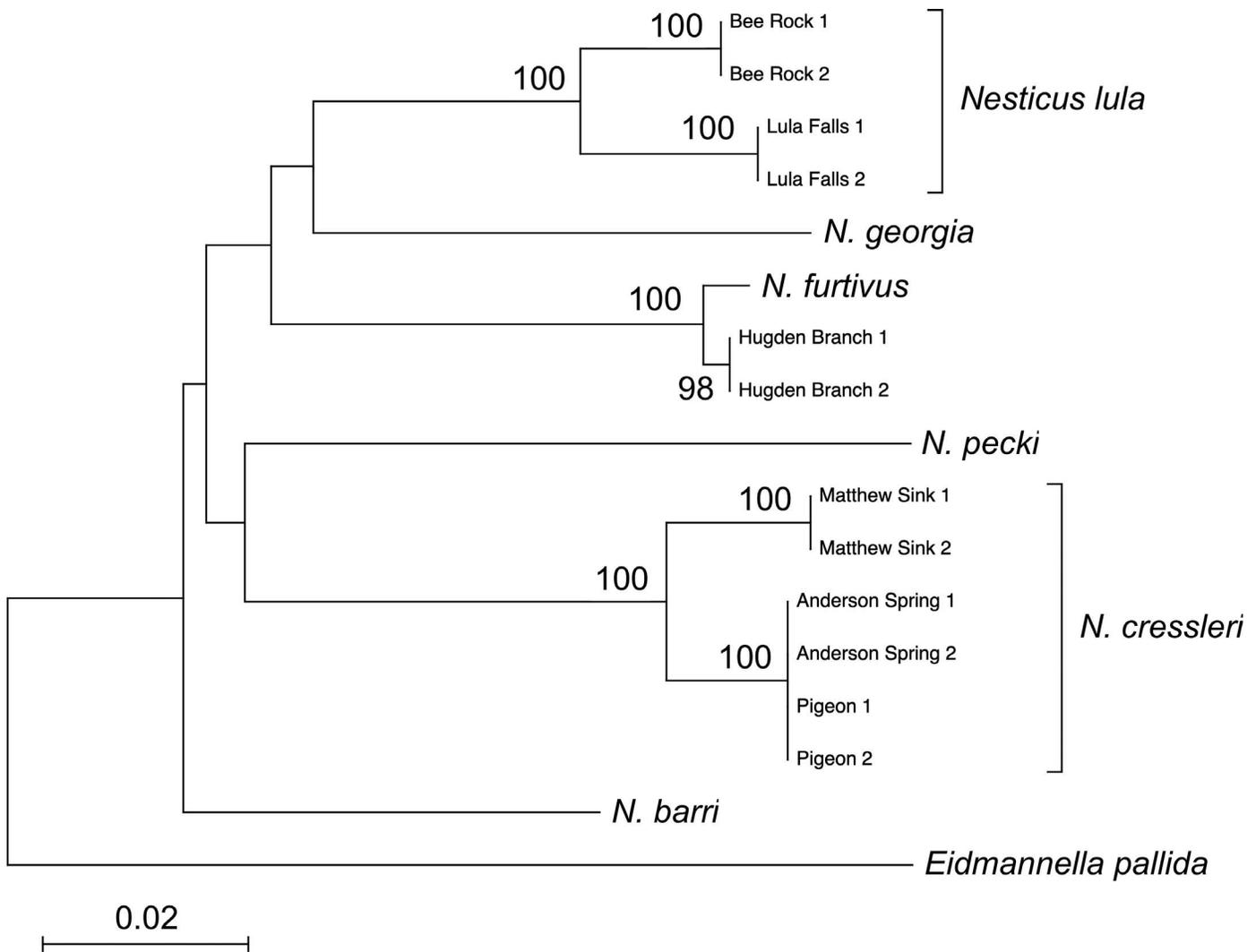


Figure 6.—Neighbor-joining phylogenetic tree showing relationships between *Nesticus* species and populations based on p-distances derived from cytochrome *c* oxidase subunit I sequences. Bootstrap values >90% (from 500 replicates) are indicated on the branches.

(Fig. 6) and parsimony (not shown) analyses produced nearly identical results in terms of topology and branch support.

DISCUSSION

Nesticus cave spiders often have highly restricted ranges bounded by geologic features that define the distribution of available habitat (Gertsch 1984; Hedin & Dellinger 2005). We observed this pattern with the species and populations described here. The previously known *N. georgia* is restricted to several caves in Lookout Valley, west of Lookout Mountain in (Fig. 7). *Nesticus furtivus*, previously known only from Raccoon Mountain Caverns on Raccoon Mountain, is now reported from Hugden Branch Cave, a second site on Raccoon Mountain. The two *N. lula* sites are on the northeastern edge of Lookout Mountain less than five km apart, and the three *N. cressleri* sites are on Pigeon Mountain less than 15 km apart (Fig. 7).

The molecular results correlate with the geographic patterns. In every case, individuals from the same cave shared

the same *COI* haplotype. Caves in close proximity support populations from the same species (Fig. 7). For all three species examined here, genetic distances between populations (i.e., between caves) are less than 0.04 (Table 2), which is consistent with the between-cave genetic distances observed in the trogllobiont *N. barri*, using the same molecular marker (Snowman et al. 2010).

Although we have only a handful of records for *N. lula* and *N. cressleri*, it appears both species are trogllobiotic. These spiders are known only from caves, and both exhibit morphological changes consistent with subterranean life, as *N. lula* has reduced eyes and *N. cressleri* is eyeless (Fig. 1). *Nesticus* spiders are not the only trogllobionts from northwest Georgia that exhibit extremely limited ranges (Niemiller & Zigler 2013; Zigler et al. 2020). *Nesticus cressleri* joins a handful of other trogllobiotic species known only from Pigeon Mountain: the isopod *Caecidotea cyrtorhynchus* Fleming & Steeves, 1972, the amphipod *Stygobromus minutus* Holsinger, 1978, and the leptonetid spider *Appaleptoneta fiskei* (Gertsch, 1974) (Zigler et al. 2020). The cave-associated Pigeon

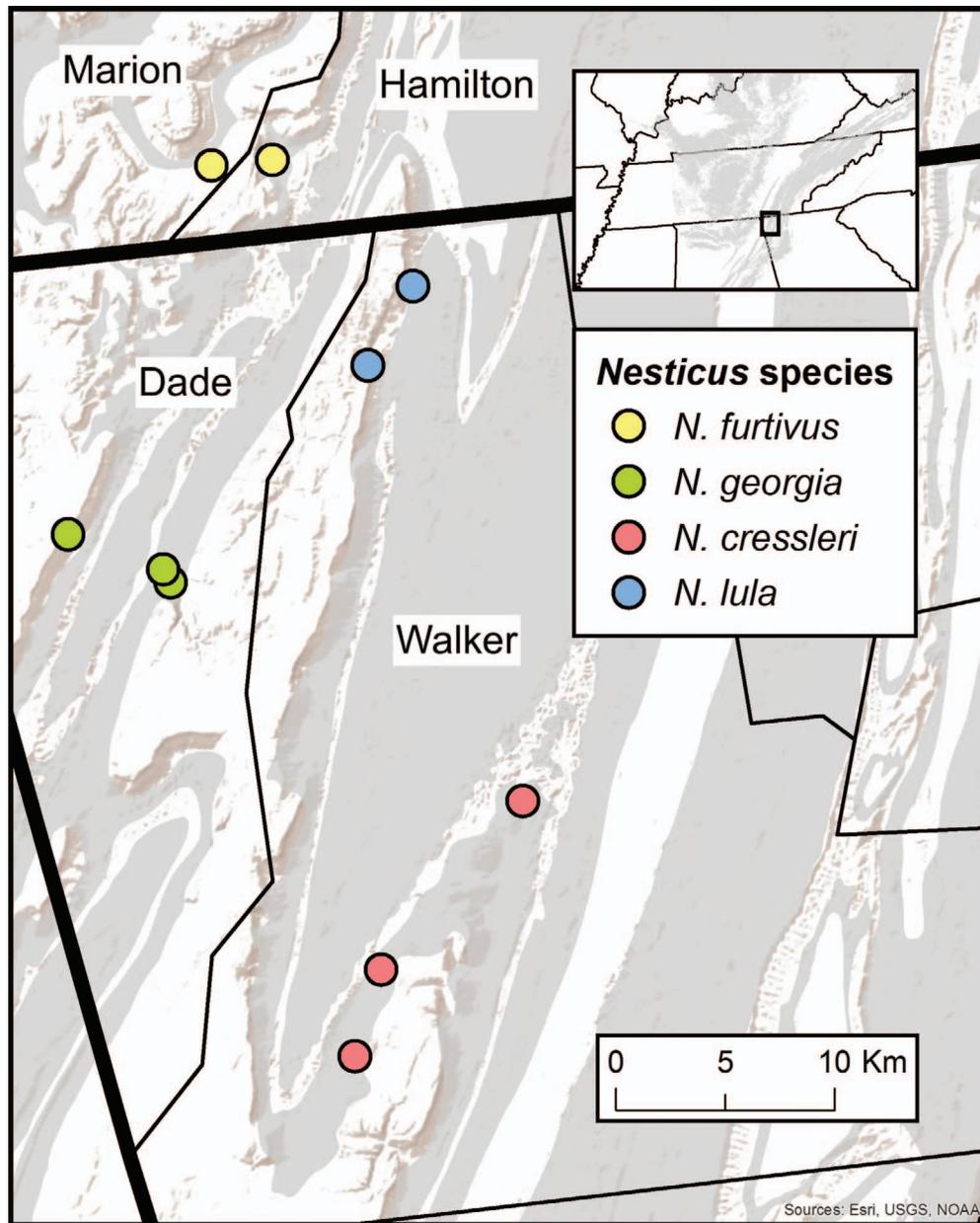


Figure 7.—All known cave populations of *Nesticus* in northwest Georgia and adjacent southeast Tennessee. County boundaries are indicated by thin black lines, and counties are labeled. State boundaries are indicated by thick black lines. Karst is indicated by grey shading on the map and its inset (Weary & Doctor 2014). Higher elevations typically correspond to sandstone (non-karst) strata.

Mountain Salamander (*Plethodon petraeus* Wynn et al., 1988) is also limited to Pigeon Mountain (Camp & Jensen 2007, 2021). Interestingly, the distribution of leptonetids in northwest Georgia partially mirrors the distribution of *Nesticus* from the region. As mentioned earlier, like *N. cressleri*, *A. fiskei* is known only from Pigeon Mountain (Ledford et al. 2011). In addition, like *N. georgia*, the leptonetid *Ozarkia georgia* (Gertsch, 1974) is known from only a handful of caves in Lookout Valley in Dade County, Georgia (Ledford et al. 2011). Thus, leptonetids provide another example of cave-limited spiders known from one or a few sites in northwest Georgia.

Previous authors have highlighted conservation concerns related to the cave-obligate *Nesticus* of the southern Appalachians (Hedin & Dellinger 2005; Carver et al. 2016). As observed with *N. lula* and *N. cressleri*, many species are known from one or a handful of caves within an extremely limited geographic area. The combination of such extreme endemism, limited potential for dispersal, small population sizes, and a lack of knowledge about the species' ecology contribute to concerns about the security of these species.

Our recent review of cave biodiversity in Georgia (Zigler et al. 2020) highlighted more than two dozen undescribed cave-associated taxa. If all of the reported, but undescribed, cave-obligate taxa were described, it would increase the known

cave-obligate biodiversity in the state by ~25%. Describing *N. lula* and *N. cressleri* is a step toward a better understanding of cave biodiversity in Georgia, increasing the number of known cave-obligate species from 51 to 53, raising the number of known cave spiders from six to eight, and emphasizing the importance of Pigeon Mountain as a hotspot for endemic cave biodiversity. In addition, our discovery of a second population of *N. furtivus* indicates a slightly reduced threat of habitat loss for a species that was previously known from a single site. With the aim of protecting known populations, uncovering new populations of known species, and discovering new species, we encourage further study of cave-dwelling *Nesticus* from the region.

ACKNOWLEDGMENTS

We thank M. Abercrombie, L. Carver, W. Coleman, A. Cressler, P. Heald, M. Keller, and T. Lichtefeld for their assistance in the field and the lab. We thank the Lula Lake Land Trust and the Tennessee River Gorge Trust for permitting us to access caves on their properties. This work was supported by the University of the South and the University of Indianapolis. We also thank two anonymous reviewers for their help in improving the manuscript.

LITERATURE CITED

- Balogh A, Ngo L, Zigler KS, Dixon G. 2020. Population genomics in two cave-obligate invertebrates confirms extremely limited dispersal between caves. *Scientific Reports* 10:17554.
- Buhlmann KA. 2001. A biological inventory of eight caves in northwestern Georgia with conservation implications. *Journal of Cave and Karst Studies* 63:91–98.
- Camp CD, Jensen JB. 2007. Use of twilight zones of caves by plethodontid salamanders. *Copeia* 2007:594–604.
- Camp CD, Jensen JB. 2021. Long-term observations of salamander abundance in twilight zones of caves in Georgia, USA. *Herpetological Conservation and Biology* 16:63–71.
- Carver LM, Perlaky P, Cressler A, Zigler KS. 2016. Reproductive seasonality in *Nesticus* (Araneae: Nesticidae) cave spiders. *PLoS ONE* 11:e0156751.
- Christman MC, Doctor DH, Niemiller ML, Weary DJ, Young JA, Zigler KS, et al. 2016. Predicting the occurrence of cave-inhabiting fauna based on features of the earth surface environment. *PLoS ONE* 11:e0160408.
- Coyle FA, McGarity AC. 1991. Two new species of *Nesticus* spiders from the southern Appalachians (Araneae, Nesticidae). *Journal of Arachnology* 19:161–168.
- Culver DC, Pipan T. 2019. *The Biology of Caves and Other Subterranean Habitats*. Oxford University Press, Oxford, United Kingdom.
- Culver DC, Master LL, Christman MC, Hobbs III HH. 2000. Obligate cave fauna of the 48 contiguous United States. *Conservation Biology* 14:386–401.
- Gertsch WJ. 1984. The spider family Nesticidae (Araneae) in North America, Central America, and the West Indies. *Texas Memorial Museum Bulletin* 31:1–91.
- Hedin MC. 1997a. Molecular phylogenetics at the population/species interface in cave spiders of the Southern Appalachians (Araneae: Nesticidae: *Nesticus*). *Molecular Biology and Evolution* 14:309–324.
- Hedin MC. 1997b. Speciation history in a diverse clade of habitat-specialized spiders (Araneae: Nesticidae: *Nesticus*): inferences from geographic-based sampling. *Evolution* 51:1929–1945.
- Hedin M, Dellinger B. 2005. Descriptions of a new species and previously unknown males of *Nesticus* (Araneae: Nesticidae) from caves in Eastern North America, with comments on species rarity. *Zootaxa* 904:1–19.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35:1547–1549.
- Ledford J, Paquin P, Cokendolpher J, Campbell J, Griswold C. 2011. Systematics of the spider genus *Neoleptoneta* Brignoli, 1972 (Araneae: Leptonetidae) with a discussion of the morphology and relationships for the North American Leptonetidae. *Invertebrate Systematics* 25:334–388.
- Niemiller ML, Zigler KS. 2013. Patterns of cave biodiversity and endemism in the Appalachians and Interior Plateau of Tennessee, USA. *PLoS One* 8:e64177.
- Snowman CV, Zigler KS, Hedin M. 2010. Caves as islands: mitochondrial phylogeography of the cave-obligate spider species *Nesticus barri* (Araneae: Nesticidae). *Journal of Arachnology* 38:49–56.
- Weary DJ, Doctor DH. 2014. Karst in the United States: a digital map compilation and database. US Department of the Interior, US Geological Survey.
- Zigler KS, Niemiller ML, Stephen CD, Ayala BN, Milne MA, Gladstone NS, et al. 2020. Biodiversity from caves and other subterranean habitats of Georgia, USA. *Journal of Cave and Karst Studies* 82:125–167.

Manuscript received 11 June 2021, revised 24 February 2022, accepted 7 March 2022.