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Is there anybody (new) out there? Seven new species of *Ligidium* (Isopoda, Oniscidea, Ligiidae) from the Southern Appalachians, eastern North America

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Abstract. In this study, we describe seven new species of *Ligidium* Brandt, 1833 from the Southern Appalachian Mountains based on morphological and molecular data: *Ligidium enotahensis* sp. nov., *L. nantahala* sp. nov., *L. protuberans* sp. nov., *L. gadalutsi* sp. nov., *L. pacolet* sp. nov., *L. whiteoak* sp. nov., and *L. schultzi* sp. nov. Our findings significantly enhance the known diversity of this genus in the Nearctic, particularly in eastern North America. Morphological diagnoses are primarily based on traits of the male pleopod 2 endopodite, while molecular data, including mitochondrial Cox1 and nuclear 28S and NaK genes, help delimit all newly described species. Together these genes reveal considerable genetic divergence among the new species and from previously described ones. Phylogenetic analyses revealed clear genetic distinctions among the new species, but did not resolve all relationships. Furthermore, we propose elevating three subspecies of *Ligidium elrodii* (Packard, 1873) to full species status based on consistent morphological differences, *Ligidium chatoogaensis* Schultz, 1970, *L. hancockensis* Schultz, 1970, and *L. scottensis* Schultz, 1970, thereby increasing the total number of *Ligidium* species to 68. This research underscores the high species diversity in the Southern Appalachians and the importance of integrating morphological and molecular approaches in taxonomy.

Keywords. Integrative taxonomy, Arthropoda, Crustacea, Nearctic Region, diversity.

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Introduction

The genus *Ligidium* Brandt, 1833 currently includes 58 species discontinuously distributed in Europe, Asia, and North America (Schmalfuss 2003; Wang *et al.* 2022). The first American species, *Ligidium gracile* (Dana, 1854), was described from California, although it was not assigned to this genus until much later (Richardson 1905). In eastern North America the first described species was *L. elrodii*

(Packard, 1873), although this name remained mostly ignored until it was placed in the genus *Ligidium* as a senior synonym of *L. longicaudatum* Stoller, 1902, almost one century after its description (Schultz 1970). These two species are considered the only widespread *Ligidium* in North America, the former in the west, the latter in the east. Also in the 19th century, the European species *L. hypnorum* (Cuvier, 1792) was reported from North America (San Francisco, CA, and Niagara, NY/ON; Stuxberg 1875); these records have never been confirmed and are most likely the result of misidentifications (Budde-Lund 1885).

During the 20th century, several new American taxa were described. From the Pacific Coast, Jackson (1923) described *L. latum* Jackson, 1923 from California, and named a variety of *L. gracile*, now considered a subspecies, *L. g. flavum* Jackson, 1923 from British Columbia. Also from California, Maloney (1930) and Mulaik & Mulaik (1942) described *L. kofoidi* Maloney, 1930 and *L. lapetum* Mulaik & Mulaik, 1942. In the eastern half of the continent, two species were described from the Gulf of Mexico area, *L. mucronatum* Mulaik & Mulaik, 1942, from Louisiana, and *L. floridanum* Schultz & Johnson, 1984, from Florida, as well as *L. blueridgensis* Schultz, 1964, from the Southern Appalachian Region.

Additionally, Schultz (1970) named four subspecies of *L. elrodii*: *L. e. chatoogaensi*s Schultz, 1970 from Georgia, *L. e. hancockensis* Schultz, 1970 from Tennessee, and *L. e. leensis* Schultz, 1970 and *L. e. scottensis* Schultz, 1970 from Virginia. These subspecies, based mainly in differences in the male pleopod 2 endopodite, together with the existence of other unnamed morphological forms (Schultz 1982), reflect the potential existence of poorly understood morphological variation and the need for further taxonomic research, particularly along the Appalachian Mountains.

The use of molecular data has indeed shown the existence of a much larger genetic diversity than previously thought in the Southern Appalachian *Ligidium* populations, including within several lineages that were initially assigned to *L. elrodii* or *L. blueridgensis* (Recuero & Caterino 2024a). The different genetic units identified represent very old lineages, probably the result of speciation events occurring since the Miocene (Recuero & Caterino 2024a), that present subtle but constant diagnostic morphological traits associated with the male pleopod 2 endopodite, the main structure used to delimit species in the genus morphologically (Wang *et al.* 2022).

Here, we describe seven new species of *Ligidium* from the southern Appalachian Mountains based on morphology and molecular data. We also propose a change of status of three subspecies of *L. elrodii*, considering them as full species, raising the total number of species of *Ligidium* to 68, 18 of which are found exclusively in the Nearctic Region.

Material and methods

The specimens analyzed for this study (see Type material or Material examined for each species in the Taxonomy section and Supp. file 1) were mostly collected as part of a larger project to study the diversity of arthropods living in leaf litter in the southern Appalachian Mountains (Caterino & Recuero 2024), for which numerous medium to high elevation sites were sampled. Most localities were visited twice, in spring and autumn, litter samples sifted and taken to the laboratory to be processed using Berlese-Tullgren funnels, and specimens collected directly into 100% ethanol and stored at -20°C. Maps showing localities and minimum convex polygons were generated with QGIS ver. 3.30 (https://qgis.org/).

Habitus images were generated by focus stacking 15–20 images taken with a Nikon EOS 6D camera, a Tamron AF 1.4× teleconverter and a Canon MP-E 65 mm macro lens, using a Visionary Digital Passport system; image stacking was done with Helicon Focus software ver. 8.1.1 (HeliconSoft, Ukraine). Morphological characters were observed using an Olympus SZX7 stereo microscope (up to

60 × magnification), and a Zeiss Axioskop 50 compound microscope (up to 400 × magnification) using temporary mounts prepared with glycerin. Drawings were made using a camera lucida and digitized with a Wacom Intuos Pro tablet.

Type specimens were deposited at the Invertebrate Zoology Collection (USNM, Smithsonian National Museum of Natural History) and at the Clemson University Arthropod Collection (CUAC) (see Material examined). All non-type material is deposited at CUAC.

Molecular data used to support the species here proposed, and for phylogenetic analysis, are those described in Recuero & Caterino (2024a) (Supp. file 1, Supp. file 2). Relationships between southern Appalachian species of *Ligidium* were explored with a multispecies coalescent approach using StarBeast3 (Douglas *et al.* 2022) implemented in BEAST ver. 2.7.6 (Bouckaert *et al.* 2014), allowing inference of a species tree from multilocus datasets. We defined seven partitions as suggested by PartitionFinder 2 (Lanfear *et al.* 2017): one for 28S and the rest for each codon position of Cox1 and NaK (but linking clock models and trees). Best-fitting substitution models were estimated with the package bModelTest (Bouckaert & Drummond 2017). We used a Yule tree prior and a species tree relaxed clock model, a Cox1 substitution rate with a mean value of 0.017 (± 0.007) (Recuero & Caterino 2024a), and the relative rates of the two nuclear markers estimated during the analyses, run for 100 million generations, sampling every 10 000. High ESSs (> 200) for all parameters were confirmed with Tracer ver. 1.7.2 (Rambaut *et al*. 2018) and TreeAnnotator ver. 1.10.4 was used to build maximum clade credibility trees considering a 25% burn-in. Molecular species delimitation results obtained in Recuero & Caterino (2024a) are summarized in the Supp. file 1. We used MEGA11 to calculate mean uncorrected pairwise genetic distances (p-distances) among and within species (Tamura *et al.* 2021). Intraspecific Cox1 diversity was represented with TCS haplotype networks (Clement *et al*. 2002) constructed in PopART (Leigh & Bryant 2015); broad geographic distribution for each haplotype was coded as traits including four main areas: SW (broadly the region south-west of the Little Tennessee River), SC (the region between the Little Tennessee River and the Asheville Depression), NE (the region north-east of the Asheville Depression in North Carolina) and WV (for the localities sampled in West Virginia).

Results

Phylogeny / Phylogenetic relationships

The phylogenetic relationships among the southern Appalachian species of *Ligidium* obtained with StarBeast3 analyses are identical to the analyses of concatenated alignments shown in Recuero & Caterino (2024a), although some nodes are not fully supported in the species tree (Fig. 1A). We found one clade including *Ligidium blueridgensis*, *L. pacolet* sp. nov. and *L. whiteoak* sp. nov.; the sister relationship between the latter two is suggested but not well supported in the species tree, but was fully supported when data was analyzed concatenating all gene alignments (Bayesian posterior probabilities = 1, Maximum likelihood ultrafast bootstrap = 96; Recuero & Caterino 2024a). This clade is sister to another one including *L. enotahensis* sp. nov., *L. schultzi* sp. nov. and *L. protuberans* sp. nov.; the relationships among these three species are not resolved, as observed in previous analyses (Recuero & Caterino 2024a). *Ligidium nantahala* sp. nov. is resolved as the sister to the previous two clades, and *L. elrodii* as the sister lineage to the previously mentioned seven species, with low support in the species tree but well supported in other analyses (Recuero & Caterino 2024a). Finally, *L. gadalutsi* sp. nov. appears as the sister species to the rest of the taxa included in this study.

Interspecific genetic p-distances are high (Supp. file 1), particularly for Cox1, with mean values ranging from 15.6% (*L. enotahensis* sp. nov./*L. schultzi* sp. nov.) to 26.9% (*L. protuberans* sp. nov./*L. gadalutsi* sp. nov.). Nuclear genes showed interspecific distances ranging from 0.3% (*L. whiteoak* sp. nov./*L. pacolet* sp. nov.) to 5.5% (*L. pacolet*/*L. gadalutsi*) for NaK, and 0.7% (*L. whiteoak*/*L. pacolet*) to 12% (*L. nantahala* sp. nov./*L. gadalutsi*) for 28S.

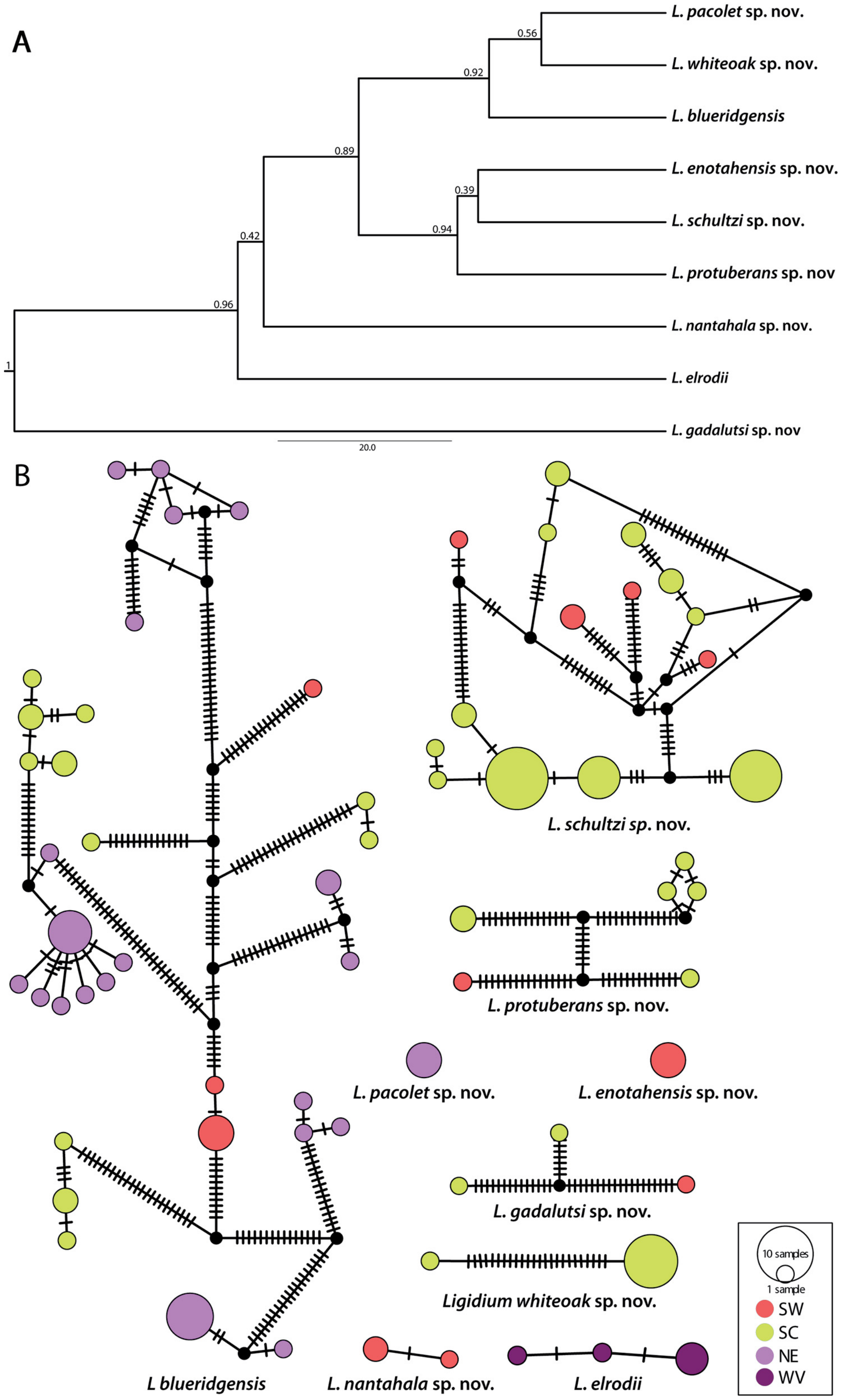

Fig. 1. A. Species tree based on the multispecies coalescent model implemented in StarBeast3; Support values on relevant branches are Bayesian posterior probabilities. **B**. Haplotype networks of Cox1 sequences; box shows a scale proportional to frequencies and color codes indicating broad geographical distribution (see Material and methods for region definition).

Cox1 haplotype networks (Fig. 1B), showed 34 haplotypes of *L. blueridgensis*, with six divergent groups distributed northeast of the Asheville Depression barrier and 5 to the west; among the latter, 2 of them are found southwest of the Little Tennessee River barrier. We found 15 Cox1 haplotypes within *L. schultzi* sp. nov., mostly found between the Asheville Depression and the Little Tennessee River, where they form 3 haplotype clusters, and only four divergent haplotypes southwest of the latter barrier.

Similarly, the 6 haplotypes of *L. protuberans* sp. nov. are mostly found between the Asheville Depression and the Little Tennessee River, with a single divergent haplotype found southwest of the latter barrier. *Ligidium gadalutsi* sp. nov. contains three divergent haplotypes, one southwest of the Little Tennessee River, two between the Asheville Depression and the Little Tennessee River. *Ligidium whiteoak* sp. nov. showed two divergent haplotypes; *L. nantahala* sp. nov. also has two, only weakly differentiated haplotypes; we found three haplotypes among the studied samples of *L. elrodii* and only one for *L. enotahensis* sp. nov. and *L. pacolet* sp. nov.

Taxonomy

Class Malacostraca Latreille, 1802
Superorder Peracarida Calman, 1904
Order Isopoda Latreille, 1816
Suborder Oniscidea Latreille, 1802
Family Ligiidae Leach, 1814

Genus ***Ligidium*** Brandt, 1833

Type species

Ligidium hypnorum (Cuvier, 1792).

Ligidium enotahensis sp. nov.
urn:lsid:zoobank.org:act:CF5E1EE6-9001-45DA-9DB2-5DE45CA56D20
Figs 2–3

Ligidium sp. 1 – Recuero & Caterino 2024: table 1.

Diagnosis

Ligidium enotahensis sp. nov. can be diagnosed morphologically based on the shape on the male pleopod 2 endopodite, showing a squarish tip with no excrescence, projection, or notch, and on the very broad male pleopod 1 endopodite projection. It also can be identified from all other Appalachian species based on molecular data, representing a distinct evolutionary lineage.

Etymology

From the Cherokee Enotah, a name of the mountain where the species is described, and the Latin suffix ‘*-ēnsis*’, forming an adjective meaning ‘from Enotah’.

Material examined

Holotype

USA – **Georgia** • ♂; Towns Co., Brasstown Bald; 34.8763° N, 83.8107° W; 1377 m a.s.l.; 17 Nov. 2020; M. Caterino and A. Haberski leg.; GenBank no: OR172584 (Cox1); USNM, CUAC000138051.

Other material examined

USA – **Georgia** • 3 imm.; same data as for holotype; CUAC000171312 to CUAC000171314.

Description

Body length of holotype 5.3 mm, body width (at pereonite 4) 2.6 mm. Color of holotype in ethanol (Fig. 2) dorsally brown with large whitish patches on dorsal surface, a dark brown longitudinal medial stripe running from pereonite 1 to telson, epimera dark brown with a large white patch; head, antennae and uropods dark brown, pereopods and pleopods lighter brown. Immatures much lighter and without longitudinal stripe. Antennule (Fig. 3G) three-segmented; first segment about 2 times as long as wide, with 3 strong spiniform setae on its distal border; second segment slender, about 4 times as long as wide, also bearing 3 strong spiniform setae on the distal border; third segment small, with round apex and a single seta. Antennae (Fig. 3F) with 5 segmented peduncle, relative size of antennomeres 5>4>3>2>1; antennal flagellum with 11 articles. Dactylus of pereopods with outer claw longer than inner one; no sexual dimorphism observed in pereopods 1 (Fig. 3H) and 7 (Fig. 3I). Male pleopod 1 endopodite (Fig. 3A) with broad, squarish projection bearing 3 strong, blunt setae, 1.4 times as long as endopodite. Pleopod 1 exopodite (Fig. 3B) with a flat caudal margin bearing 3 strong, blunt setae, 0.7 times as long as exopodite. Male pleopod 2 exopodite (Fig. 3C) 2 times as wide as long; inner and most part of frontal margin with dense, hairy setation, as in outer caudal corner. Male pleopod 2 endopodite (Fig. 3D–E) with squared tip showing a ventral oblique line of small, poorly defined denticles along the interior corner; inner margin with minute spiniform setae, no spines at the base. Telson (Fig. 2) with caudal margin obtusely produced. Uropod (Fig. 3J) with exopodite broken, endopodite about 1.5 times as long as basipodite; in the examined immatures endopodite 1.5–1.6 times as long as exopodite.

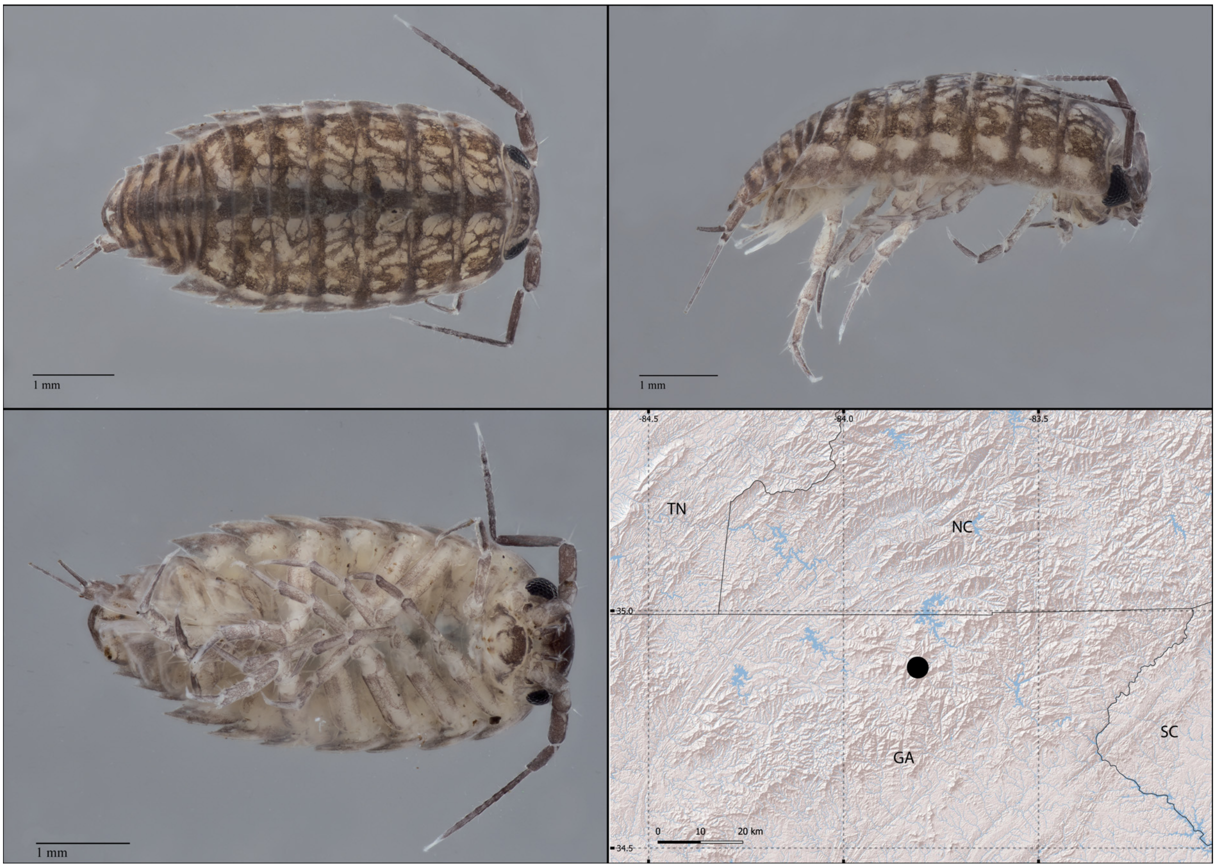

Fig. 2. General habitus of *Ligidium enotahensis* sp. nov., holotype, ♂ (USNM, CUAC000138051) in dorsal, lateral and ventral view, and map showing the type locality of the species.

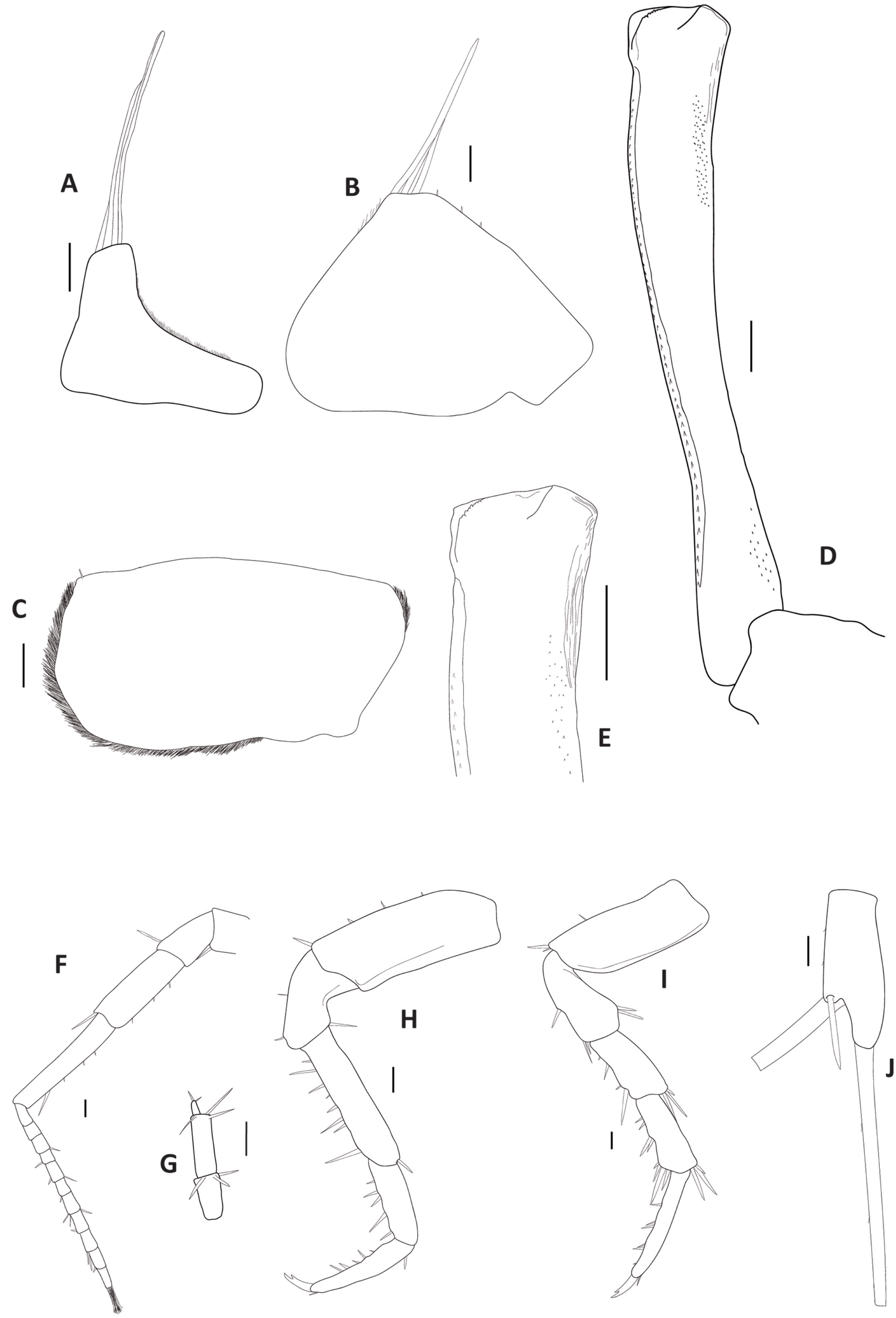

Fig. 3. *Ligidium enotahensis* sp. nov., holotype, ♂ (USNM, CUAC000138051). **A**. Pleopod 1 endopod. **B**. Pleopod 1 exopod. **C**. Pleopod 2 exopod. **D**. Pleopod 2 endopod. **E**. Pleopod 2 endopod, detail of tip. **F**. Antenna. **G**. Antennule. **H**. Pereopod 1. **I**. Pereopod 7. **J**. Uropod. Scale bars = 0.1 mm.

Distribution

The species is so far only known from its type locality, near the southern extreme of the southern Appalachian Mountains (Fig. 2). All specimens were collected in mixed forest leaf litter samples.

Remarks

Ligidium enotahensis sp. nov. corresponds with *Ligidium* sp. 1 in Recuero & Caterino (2024a). This new species is closely related to *L. protuberans* sp. nov. and *L. schultzi* sp. nov., but they can easily be identified based on the shape of male pleopod 2 endopodite, by lacking the conspicuous projection present in *L. protuberans*, or the marked notch typical of *L. schultzi*. *Ligidium enotahensis* also presents a much broader projection of male pleopod 1 endopodite. All three gene fragments analyzed are informative to identify this species, with high genetic p-distances for Cox1, and low in the conserved NaK and 28S fragments (Supp. file 1), forming a reciprocally monophyletic clade with respect to either of the other two in all cases.

Ligidium nantahala sp. nov.

urn:lsid:zoobank.org:act:A2CAA049-6BCB-4229-A06D-49F61B36B030

Figs 4–5

Ligidium sp. 2 – Recuero & Caterino 2024: table 1.

Diagnosis

Ligidium nantahala sp. nov. presents a male pleopod 2 endopodite with a robust excrescence occupying the inner part of the tip and projected outwards. The projection of male pleopod 1 endopodite presents a triangular profile. It also can be distinguished from all other Appalachian species based on molecular data, representing a distinct evolutionary lineage with no close relationships among the studied taxa.

Etymology

From the Cherokee Nantahala, the name of the forests where the species lives. A noun in apposition.

Material examined

Holotype

USA – **North Carolina** • ♂; Clay Co., Nantahala N.F., Chunky Gal Trail; 35.1471° N, 83.7144° W; 1274 m a.s.l.; 7 Jun. 2021; M. Caterino and E. Recuero leg.; GenBank no: OR169931 (Cox1); USNM, CUAC000180794.

Paratype

USA – **North Carolina** • 1 ♂; same data as for holotype; CUAC000180793.

Other material examined

USA – **North Carolina** • 1 imm. ♀; same data as for holotype; CUAC000171282.

Description

Body length of adult males 4.9–5.5 mm, width (at pereonite 4) 2.4–2.6 mm. Color in ethanol of adult males (Fig. 4) dorsally marbled brown and white, with a conspicuous dark brown longitudinal medial stripe running from pereonite 1 to pereonite 7 and a transverse dark brown band in the caudal margin of pereonites; epimera dark brown with a large white patch at the base; pleonites dark brown caudally, lighter in the central parts (showing in pleonites 4–5 in dorsal view); telson dark brown with a white anchor-shaped spot; head marbled brown and white; antennae with basal segments white and brown, distally brown with some white marking; uropod brown with a large white spot in the basipodite;

pereopods and pleopods white with brown marbling. Immature specimen with the same color pattern but lighter brown. Antennule (Fig. 5G) three-segmented; first segment about 1.6 times as long as wide, 3 spiniform setae on its distal border; second segment about 3 times as long as wide, also bearing 3 strong spiniform setae in the distal border; third segment small, with blunt apex and a single seta. Antennae (Fig. 5F) with 5 segmented peduncle, relative size of antennomeres 5>4>3>2>1; antennal flagellum with 10–11 articles. Dactylus of pereopods with outer claw longer than inner one; no sexual dimorphism observed in pereopods 1 (Fig. 5H) or 7 (Fig. 5I). Male pleopod 1 endopodite (Fig. 5A) with obtuse triangular projection, bearing 2 strong, blunt setae, 0.4–0.8 times as long as endopodite. Pleopod 1 exopodite (Fig. 5B) with rounded caudal margin bearing 2 strong, blunt setae, 0.4–0.6 times as long as exopodite. Male pleopod 2 exopodite (Fig. 5C) 1.6–1.7 times as wide as long; inner and frontal margins with limited pilose setation dense, present also along outer caudal corner; caudal margin markedly convex. Male pleopod 2 endopodite (Fig. 5D–E) with rounded tip and a soft, blunt, robust excrescence occupying most of inner part of tip and projecting outwards; inner margin with minute spiniform setae, no spines at the base. Telson (Fig. 4) with caudal margin obtusely produced, convex in immature female. Uropod (Fig. 5J) with exopodite and endopodite subequal in length when not broken, 1.5–1.7 times as long as basipodite.

Distribution

The species is so far only known from its type locality (Fig. 4). All specimens were collected in broadleaf forest leaf litter samples.

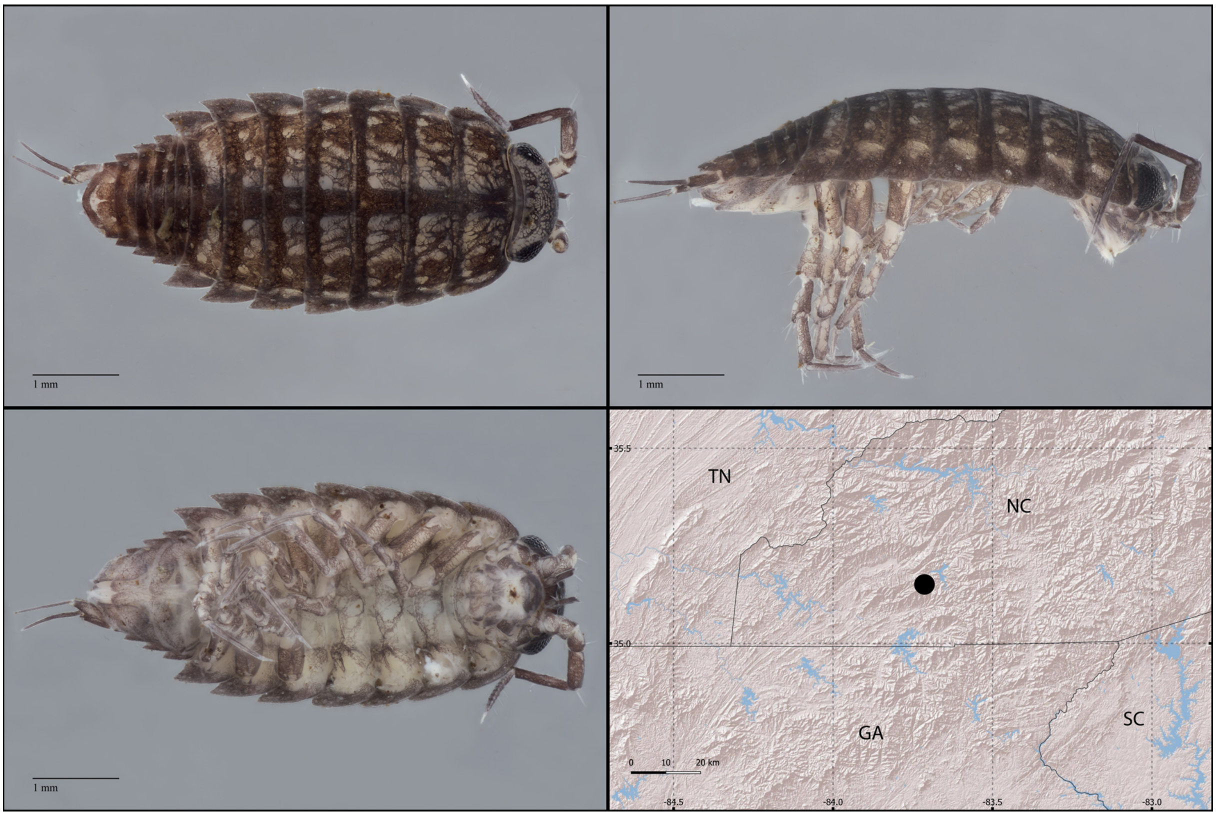

Fig. 4. General habitus of *Ligidium nantahala* sp. nov., holotype, ♂ (USNM, CUAC000180794) in dorsal, lateral and ventral view, and map showing the type locality of the species.

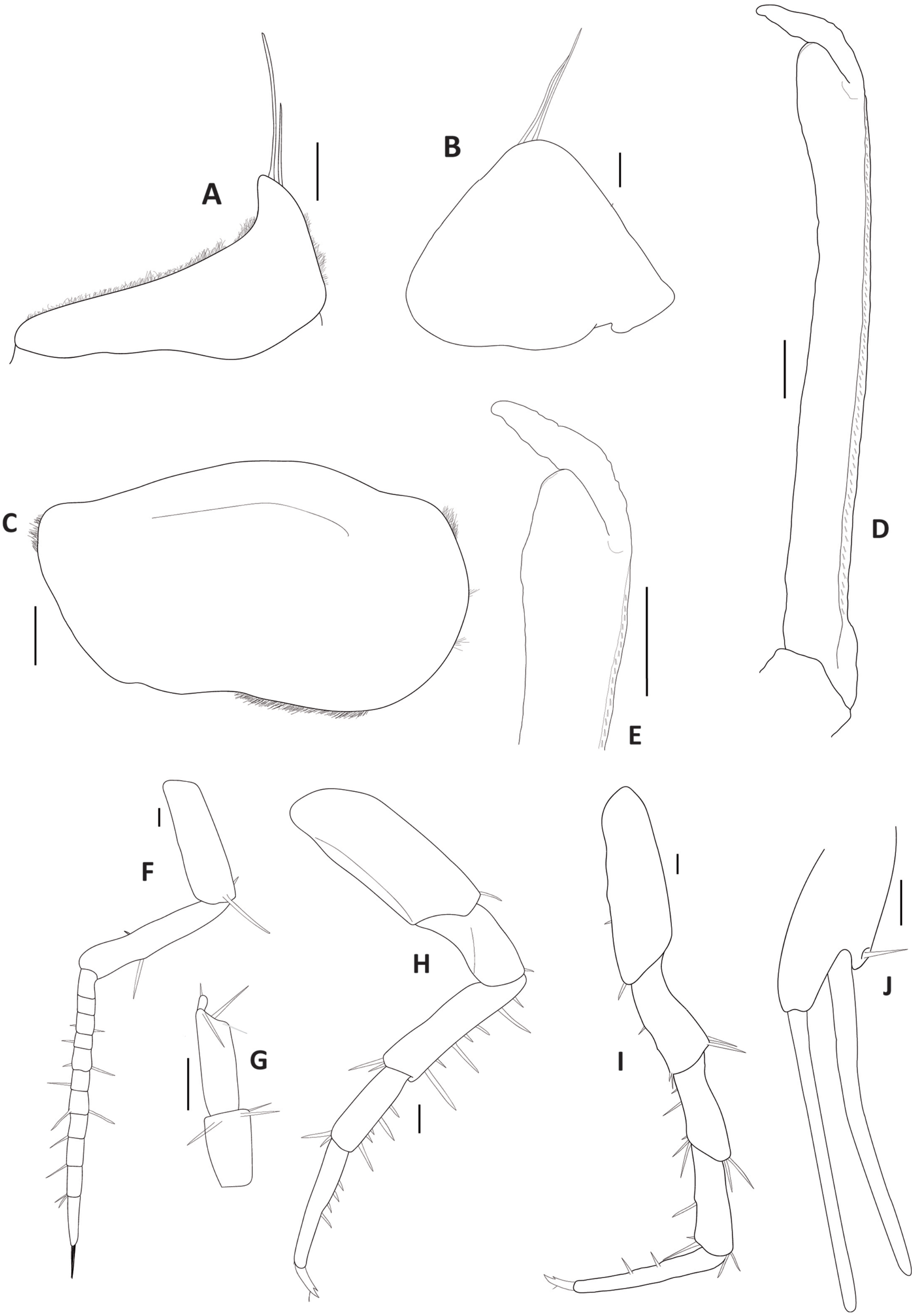

Fig. 5. *Ligidium nantahala* sp. nov. **A–C, F–J**. Holotype, ♂ (USNM, CUAC000180794). **D–E**. Paratype, ♂ **(**CUAC000180793). **A**. Pleopod 1 endopod. **B**. Pleopod 1 exopod. **C**. Pleopod 2 exopod. **D**. Pleopod 2 endopod. **E**. Pleopod 2 endopod, detail of tip. **F**. Antenna. **G**. Antennule. **H**. Pereopod 1. **I**. Pereopod 7. **J**. Uropod. Scale bars = 0.1 mm.

Remarks

Ligidium nantahala sp. nov. corresponds with *Ligidium* sp. 2 in Recuero & Caterino (2024a). It is not closely related to any of the other species studied here (Fig. 1A). Morphologically, the presence of a projecting excrescence at the tip of male pleopod 2 endopodite resembles that observed in *L. blueridgensis*, *L. pacolet* sp. nov. and *L. whiteoak* sp. nov. However, the disposition of the excrescence is different in all four species. In *L. nantahala* it covers the whole inner part of the tip ventrally, and projects outwards. In *L. blueridgensis* the excrescence is more slender, and projects from the inner tip corner rising near the margin dorsally, and projecting caudally and inwards. *Ligidium pacolet* has also a more slender excrescence rising medially or near the inner corner from the end of the tip, projecting caudally or inwards. In *L. whiteoak*, the excrescence is broad, almost as much as the endopodite stem, rising dorsally and medially from the caudal margin of the tip and projecting caudally and slightly outwards. *Ligidium nantahala* differs also in the triangular shape of male pleopod 1 endopodite projection, squarish in the other mentioned species. All three gene fragments analyzed are informative to identify this species, with high genetic p-distances for Cox1 and even for the conserved NaK and 28S fragments (Supp. file 1), forming a monophyletic clade in all cases.

Ligidium protuberans sp. nov.

urn:lsid:zoobank.org:act:65FAB813-B5FB-4865-9F36-925FC2C1DCA2

Figs 6–7

Ligidium sp. 3 – Recuero & Caterino 2024: table 1.

Diagnosis

This new species is characterized by a male pleopod 2 endopodite distally broadened, with rounded tip presenting a short, squarish projection on its distal margin. It also can be identified from all other Appalachian species based on molecular data, representing a distinct evolutionary lineage.

Etymology

From the latin '*protubero*', meaning 'protuberance', referring to the characteristic projection in the male pleopod 2 endopodite.

Material examined

Holotype

USA – **North Carolina** • ♂; Swain Co., Great Smoky Mountains N.P., Payne Creek at Lakeshore Trail; 35.4855° N, 83.8028° W; 553 m a.s.l.; 12 Apr. 2022; M. Caterino, E. Recuero, A. Haberski and P. Wooden leg.; GenBank no: OR169915 (Cox1); USNM, CUAC000171349.

Paratypes

USA – **South Carolina** • 1 ♂; Pickens Co., Chimney Top Gap; 35.0644° N, 82.7953° W; 781 m a.s.l.; 23 Mar. 2023; C.W. Harden leg.; CUAC000177090 • 1 ♀; same data as for preceding; CUAC000177089 • 1 ovi ♀; same data as for preceding; CUAC000177088.

Other material examined

USA – **North Carolina** • 2 ♀♀; Macon Co., Nantahala N.F., Van Hook Glade Campground; 35.0783° N, 83.245° W; 1006 m a.s.l.: 21 Apr. 2022; S. Payne leg.; CUAC000171316, CUAC000171317 • 1 ♀; Clay Co., Nantahala N.F., Tusquitee Bald; 35.1467° N, 83.7146° W; 1262 m a.s.l.; 1 Sep. 2020; M. Caterino and F. Etzler leg.; CUAC000138058.

Description

Body length of males 6.3–6.5 mm, of females 6.8–8.6 mm; width (at pereonite 4) of males 2.8–3, of females 3.3–4.6 mm. Color in ethanol (Fig. 6) dorsally marbled brown and white, with a conspicuous dark brown longitudinal medial stripe running from pereonite 1 to pereonite 7; caudal margin of pereonites with no defined dark brown bands; epimera brown with large white spots in the outer surface an at the base; pleonites brown to dark brown with conspicuous white spots; telson with two large white spots laterally, and a white anchor-shaped spot caudally; head marbled brown and white, white above the eyes; antennae brown with white spots in basal segments and a white setae bundle in the flagellum; uropod brown with iner part of basipodite white; pereopods brown and white, pleopods mostly white. Antennule (Fig. 7G) three-segmented; first segment about 1.4 times as long as wide, 3 spiniform setae on its distal border; second segment about 2.5 times as long as wide, also bearing 3 strong spiniform setae on the distal border; third segment with blunt apex and a single seta. Antennae (Fig. 7F) with 5 segmented peduncle, relative size of antennomeres 5>4>3>2>1; antennal flagellum with 11–13 articles. Dactylus of pereopods with outer claw longer than inner one; no sexual dimorphism observed in pereopods 1 (Fig. 7H) and 7 (Fig. 7I). Male pleopod 1 endopodite (Fig. 7A) with obtuse triangular projection, bearing 2–3 strong, blunt setae, up to 1.5 times as long as endopodite; caudal and inner margins with pilose setae. Pleopod 1 exopodite (Fig. 7B) with broadly rounded caudal margin bearing 3–4 strong, blunt setae, 0.7–0.9 times as long as exopodite. Male pleopod 2 exopodite (Fig. 7C) 2 times as wide as long; inner and most part of frontal margin with dense, hairy setation, as in outer caudal corner; caudal margin with 5–6 short but strong setae. Male pleopod 2 endopodite (Fig. 7D–E) distally broadened, with rounded tip presenting a row of small denticles in the inner corner and a short, squarish terminal projection in the

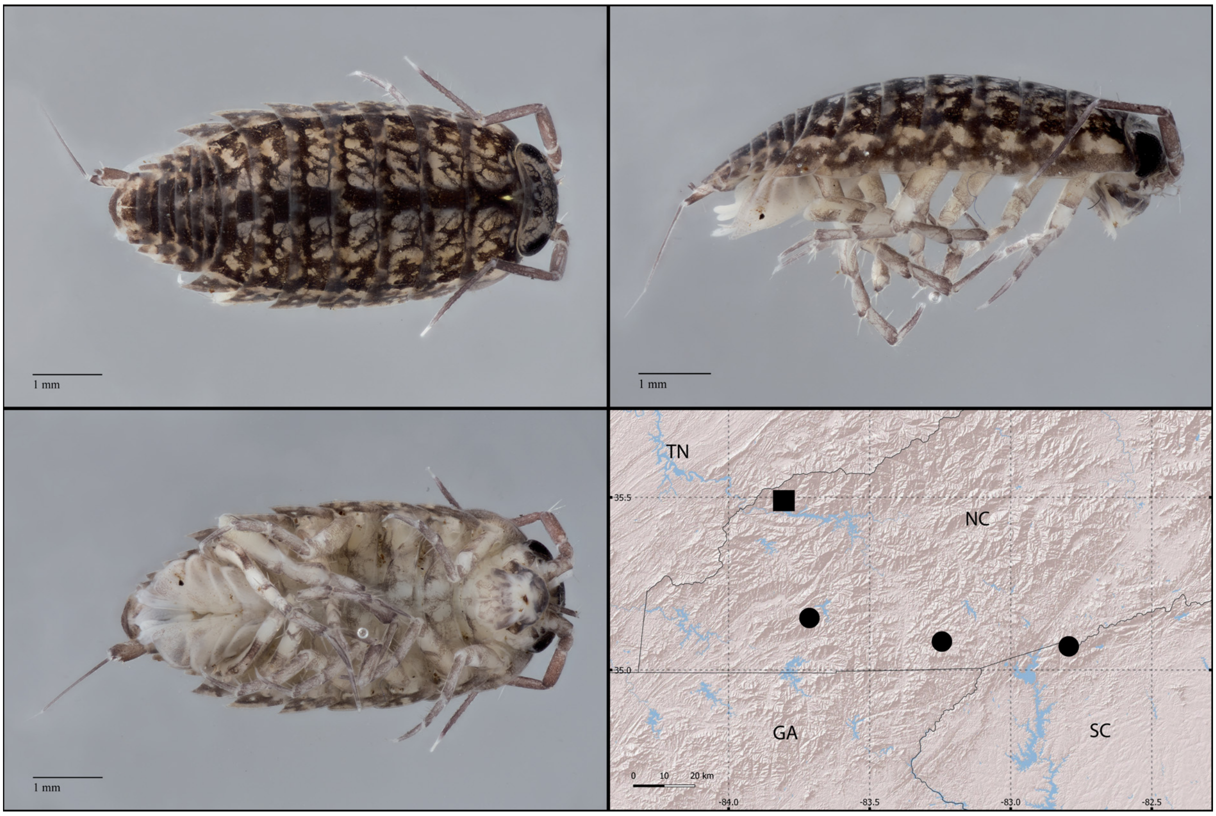

Fig. 6. General habitus of *Ligidium protuberans* sp. nov., holotype, ♂ (USNM, CUAC000171349) in dorsal, lateral and ventral view, and map showing the known localities of the species (type locality indicated with a square).

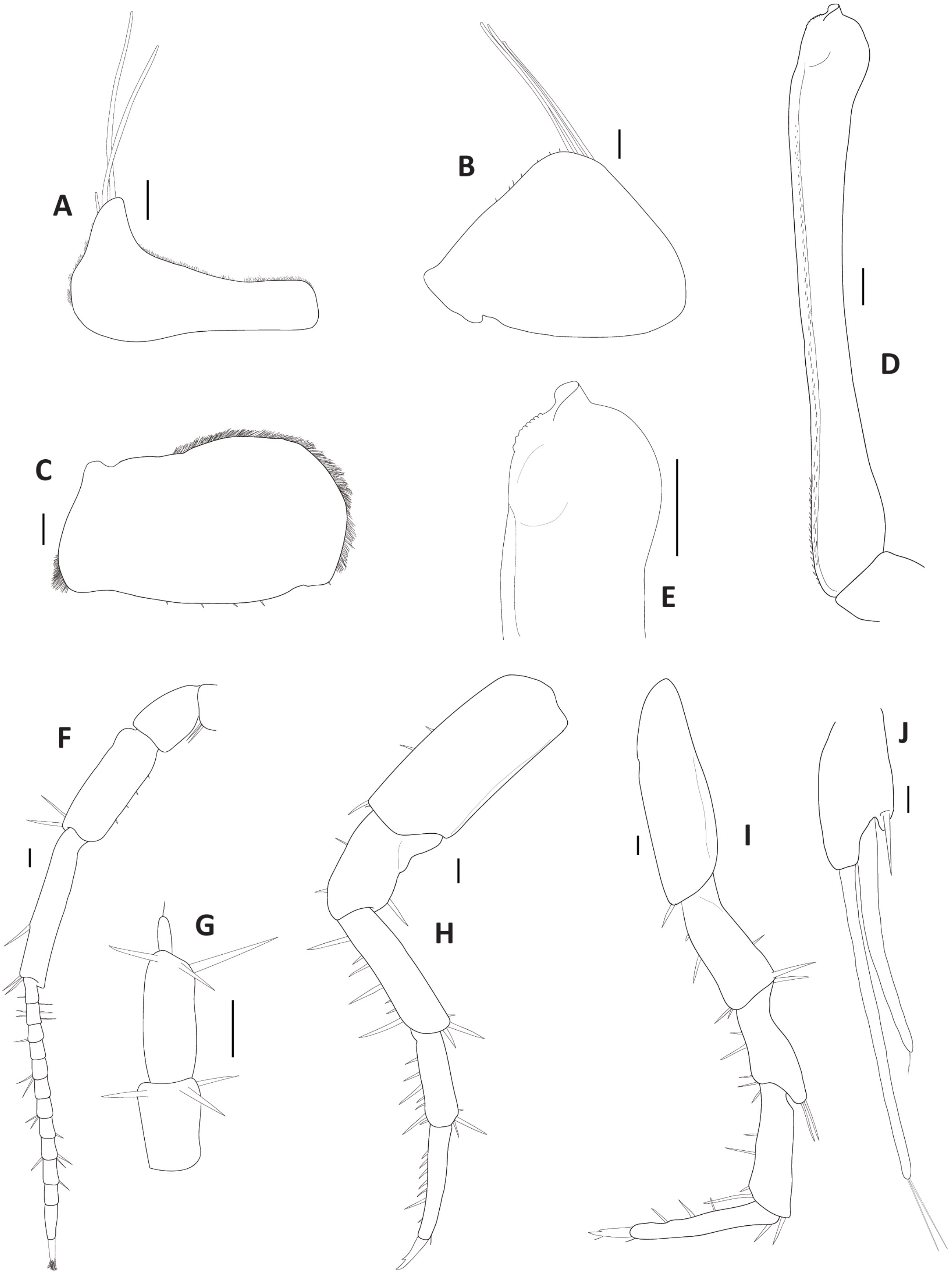

Fig. 7. *Ligidium protuberans* sp. nov. **A–I**. Holotype, ♂ (USNM, CUAC000171349). **J.** Female from Van Hook Glade Campground, NC (CUAC000171316). **A**. Pleopod 1 endopod. **B**. Pleopod 1 exopod. **C**. Pleopod 2 exopod. **D**. Pleopod 2 endopod. **E**. Pleopod 2 endopod, detail of tip. **F**. Antenna. **G**. Antennule. **H**. Pereopod 1. **I**. Pereopod 7. **J**. Uropod. Scale bars = 0.1 mm.

distal margin; inner margin with minute spiniform setae, larger and more conspicuous at base. Telson (Fig. 6) with caudal margin obtusely produced. Uropod (Fig. 7J) with endopodite 1.3–1.4 times as long as exopodite when not broken, and about 2 times as long as basipodite.

Distribution

The species has been found scattered in the southern Blue Ridge Mountains (Fig. 6), at low to mid-altitudes. All studied specimens have been collected in leaf litter from hardwood forests.

Remarks

Ligidium protuberans sp. nov. corresponds with *Ligidium* sp. 3 in Recuero & Caterino (2024a). This species is closely related to *L. enotahensis* sp. nov. and *L. schultzi* sp. nov. (Fig. 1A), but it can be easily diagnosed from them by the conspicuous short projection present in the male pleopod 2 endopodite. All three gene fragments analyzed are informative to identify this species, with high interspecific p-distances for Cox1, and low to medium in the conserved NaK and 28S fragments (Supp. file 1), forming a reciprocally monophyletic clade in all cases.

Ligidium gadalutsi sp. nov.

urn:lsid:zoobank.org:act:3EAEAB27-5C45-4183-9B5D-70FB794D8094

Figs 8–9

Ligidium sp. 4 – Recuero & Caterino 2024: table 1.

Diagnosis

This new species differs from all other Appalachian species in its male pleopod 2 endopodite having an acuminate tip, strongly projected posteriorly and outwards. It has a distinctive male pleopod 1 endopodite, showing a poorly defined, broadly triangular projection, and a male pleopod 1 exopodite with its caudal margin flattened. It also differs from all other Appalachian species based on molecular data, representing a distinct evolutionary lineage with no close relationships among the studied taxa.

Etymology

A noun in apposition, ‘*gadalutsi*’ is the Cherokee word that originated the name Cataloochee, and consequently the origin of the name of the type locality, Big Cataloochee Mountain.

Material examined

Holotype

USA – **North Carolina** • ♂; Haywood Co., Great Smoky Mountains N.P., Big Cataloochee Mt; 35.6675° N, 83.1805° W; 1703 m a.s.l.; 14 Jul. 2020; M. Caterino and F. Etzler leg.; GenBank no: PP737148 (Cox1); USNM, CUAC000138053.

Other material examined

USA – **North Carolina** • 1 ♀; Clay Co., Nantahala N.F., Chunky Gal Trail; 35.1471° N, 83.7144° W; 1274 m a.s.l.; 6 Jul. 2021; M. Caterino and E. Recuero leg.; CUAC000171281. – **Tennessee** • 1 ♀; Sevier Co., Smoky Mountains N.P., Mount LeConte (Alum Cave Trail); 35.6382° N, 83.4387° W; 1317 m a.s.l.; 28 Sep. 2021; M. Caterino and E. Recuero leg.; CUAC000171324.

Description

Body length of holotype 4.9 mm, width (at 4 pereonite) 2.4 mm; body length of females 5.5–7 mm, width 2.5–3.6 mm. Color in ethanol (Fig. 8) of holotype dorsally marbled brown and white, with a conspicuous dark brown longitudinal medial stripe running from pereonite 1 to pereonite 7 and a transversal dark brown band in the caudal margin of pereonites; epimera dark brown with a large white patch at the base; pleonites dark brown with lateral white spots; telson dark brown with a white anchor-shaped spot; head and antennae marbled brown and white, distal segments of antennae darker brown with white setae bundle in flagellum; uropod brown with a white spot in the inner part of basipodite; pereopods and pleopods white with brown marbling. Among females one resembles the holotype in coloration, the other is dorsally almost black with large white spots from head to telson. Antennule (Fig. 9G) three-segmented; first segment about 1.5–1.7 times as long as wide, 2–3 spiniform setae in its distal border; second segment about 2.3–2.5 times as long as wide, bearing 3 strong spiniform setae in the distal border; third segment with blunt apex and 2 setae. Antennae (Fig. 9F) with 5 segmented peduncle, relative size of antennomeres 5>4>3>2>1; antennal flagellum with 11 articles. Dactylus of pereopods with outer claw longer than inner one, inner one reduced in pereopod 1; no sexual dimorphism observed in pereopods 1 (Fig. 9H) and 7 (Fig. 9I). Male pleopod 1 endopodite (Fig. 9A) with short, blunt projection, bearing a single strong, blunt seta, 0.8 times as long as endopodite; caudal and inner margins with pilose setae. Pleopod 1 exopodite (Fig. 9B) with flat caudal margin bearing 4 strong, blunt setae, up to 0.8 times as long as exopodite. Male pleopod 2 exopodite (Fig. 9C) 1.9 times as wide as long; inner and half of the frontal margin with dense, hairy setation, as in outer caudal corner; caudal margin evenly convex, with 2 short, strong setae in the outer corner. Male pleopod 2 endopodite (Fig. 9D–E) with a broad and acuminate tip, projected posteriorly-laterally, with small setae in the interior margin; inner margin of the peduncle with minute spiniform setae, no spines at base. Telson (Fig. 8) with caudal margin slightly concave in the sides and obtusely produced in the middle. Uropod (Fig. 9J) with endopodite 1.3–1.4 times as long as exopodite when not broken, and about 2 times as long as basipodite.

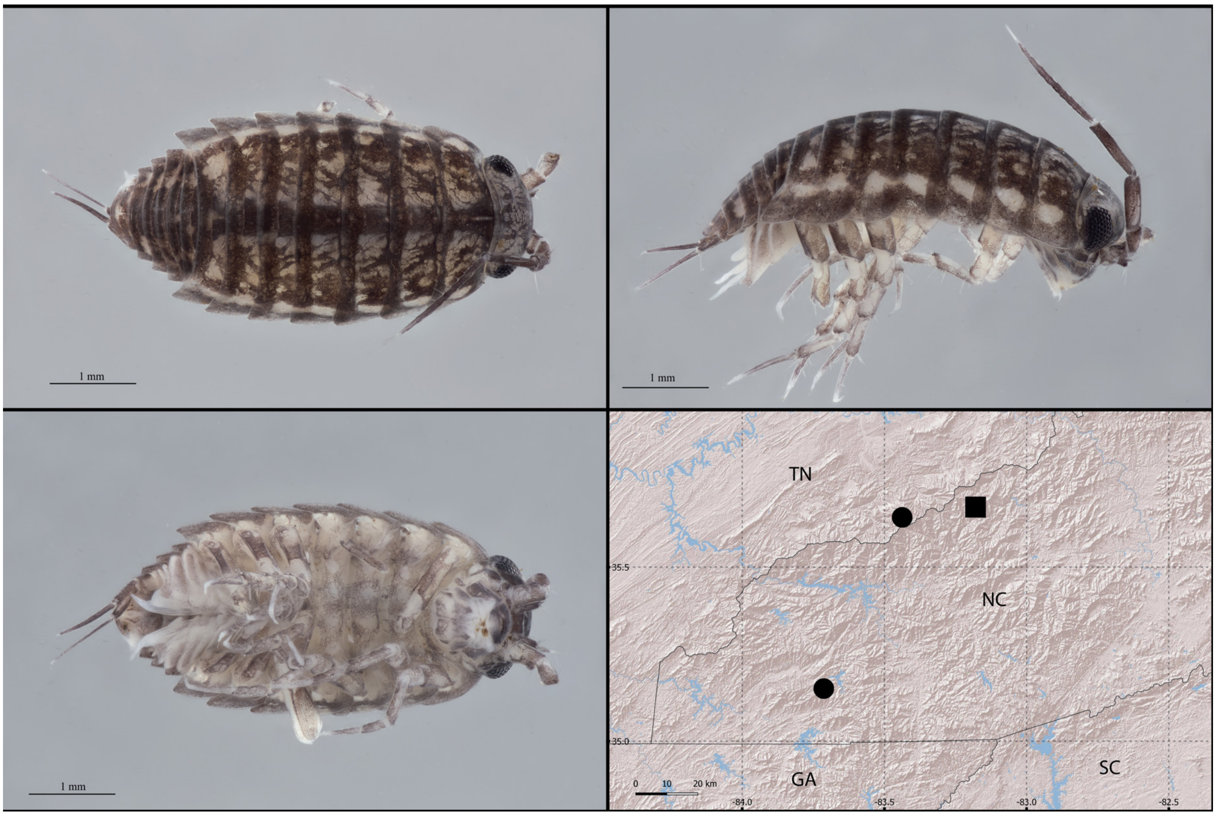

Fig. 8. General habitus of *Ligidium gadalutsi* sp. nov., holotype, ♂ (USNM, CUAC000138053) in dorsal, lateral and ventral view, and map showing the known localities of the species (type locality indicated with a square).

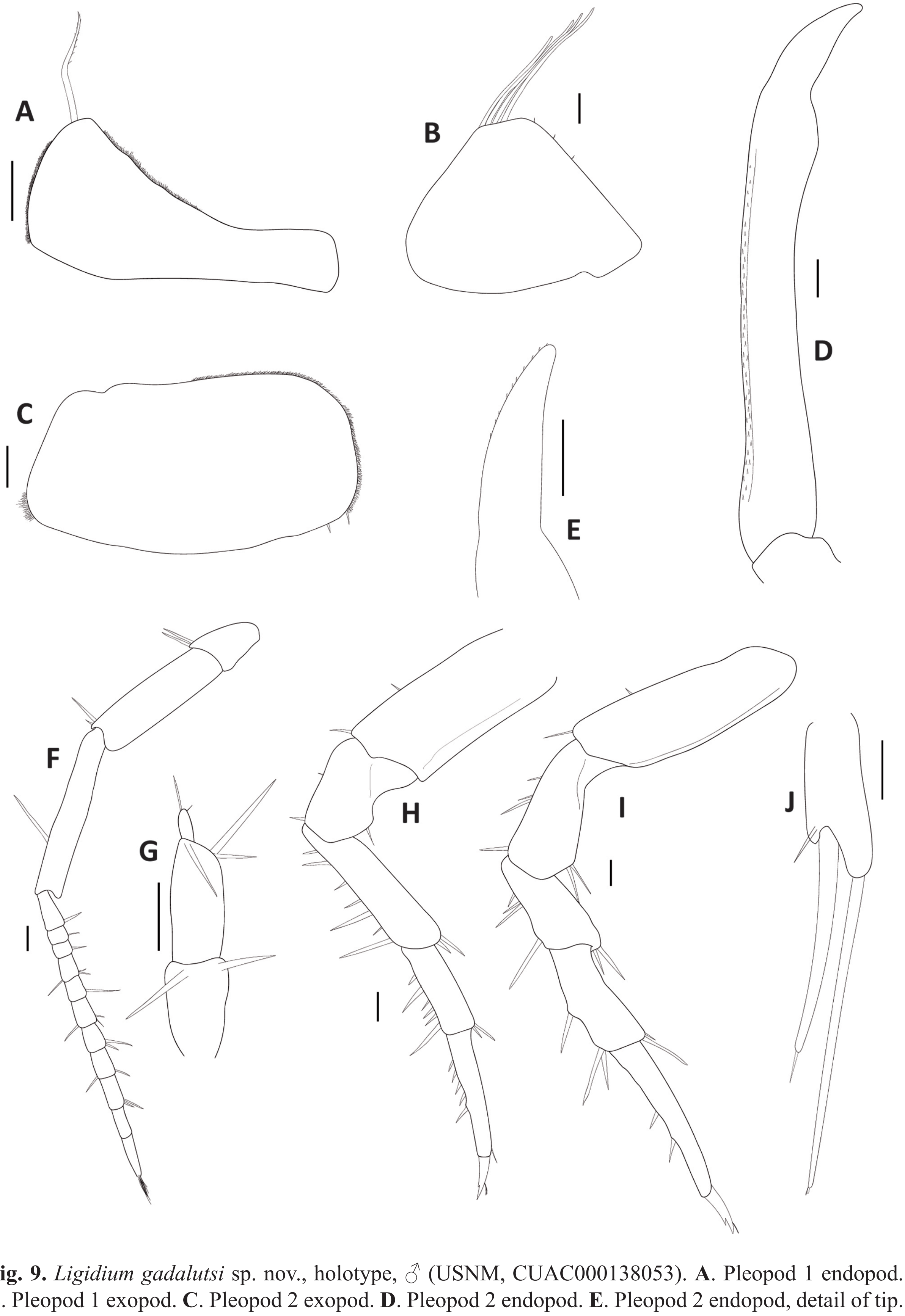

Fig. 9. *Ligidium gadalutsi* sp. nov., holotype, ♂ (USNM, CUAC000138053). **A**. Pleopod 1 endopod. **B**. Pleopod 1 exopod. **C**. Pleopod 2 exopod. **D**. Pleopod 2 endopod. **E**. Pleopod 2 endopod, detail of tip. **F**. Antenna. **G**. Antennule. **H**. Pereopod 1. **I**. Pereopod 7. **J**. Uropod. Scale bars = 0.1 mm.

Distribution

The species has been found in two localities in the Great Smoky Mountains and one at the Nantahala National Forest further south, at mid- to high elevations (Fig. 8). Studied specimens have been collected in leaf litter from conifer and mixed forests.

Remarks

Ligidium gadalutsi sp. nov. corresponds with *Ligidium* sp. 4 in Recuero & Caterino (2024a). It is not closely related to any of the studied Appalachian species (Fig. 1A). The shape of the male pleopod 2 endopodite somewhat resembles that of *L. mucronatum*, which could indicate a shared ancestry. However, in *L. mucronatum* the tip is shorter and blunt, while much longer and acuminate in *L. gadalutsi*. This new species shows the highest genetic p-distances compared to all other studied species (Supp. file 1).

Ligidium pacolet sp. nov.

urn:lsid:zoobank.org:act:6A3E7CC8-1EF9-4451-889E-81878B6B5250
Figs 10–11

Ligidium sp. 5 – Recuero & Caterino 2024: table 1 (in part).

Diagnosis

This new species is characterized by its male pleopod 2 endopodite presenting a soft, long, slender excrescence rising ventrally in the distal margin of the tip, close to its inner corner, and projected caudally or inwards. It also can be identified from all other Appalachian species based on molecular data, representing a distinct evolutionary lineage.

Etymology

A noun in apposition, in reference to the Pacolet River area where the species has been found.

Material examined

Holotype

USA – **North Carolina** • ♂; Polk Co., North Pacolet River; 35.2221° N, 82.3059° W; 384 m a.s.l.; 15 Feb. 2022; M. Caterino, E. Recuero, C. Harden and P. Wooden leg.; GenBank no: OR169911 (Cox1); USNM, CUAC000171345.

Paratypes

USA – **North Carolina** • 2 ♂♂; same data as for holotype; CUAC000171341, CUAC000171342 • 1 ♀; same data as for holotype; CUAC000171343.

Description

Body length of males 5.5–6.1 mm, of female 7.9 mm; width (at pereonite 4) of males 2.6–2.9, of female 3.6 mm. Color in ethanol (Fig. 10) dorsally dark brown with large off-white spots, with a conspicuous dark brown longitudinal medial stripe running from pereonite 1 to pereonite 7, frequently broken with light spots in the caudal margin of pereonites, which carry dark brown transverse bands; epimera dark brown with large white spots at the base and sometimes smaller white spots in the external margin; pleonites 1–4 with two pairs of off-white spots, pereonite 5 (and rarely 4) with a single pair; telson with two large white spots laterally, merging caudally with the white anchor-shaped spot; head marbled brown, with white areas above the eyes; antennae dark brown with large white spots in basal segments and a white setae bundle in the flagellum; uropod brown with inner part of basipodite white; pereopods brown and white, pleopods mostly white. Antennule (Fig. 11G) three-segmented; first segment about 1.7–1.8 times as long as wide, 3 spiniform setae in its distal border; second segment about 2.5–2.6 times as long as wide, also bearing 3 strong spiniform setae in the distal border; third segment with rounded apex and a single seta. Antennae (Fig. 11F) with 5 segmented peduncle, relative size of antennomeres 5>4>3>2=1; antennal flagellum with 11–12 articles. Dactylus of pereopods with outer claw longer than inner one; no sexual dimorphism observed in pereopods 1 (Fig. 11H) and 7 (Fig. 11I). Male pleopod 1 endopodite (Fig. 11A) with narrow projection truncated distally, bearing 3–4 strong, blunt setae, up to 1.3 times as long as endopodite; caudal and inner margins with pilose setae. Pleopod 1 exopodite (Fig. 11B) with broadly rounded caudal margin bearing 3–5 strong, blunt setae, 0.8–0.9 times as long as exopodite; external margin with 2–3 short setae, a few short piliform setae in the frontal external corner and in the inner margin. Male pleopod 2 exopodite (Fig. 11C) 2.1–2.3 times as wide as long; inner and most part of frontal margin with dense, hairy setation; caudal margin convex. Male pleopod 2 endopodite (Fig. 11D–E) with a broad tip, bearing a soft, long, slender excrescence rising ventrally at its distal margin near the inner corner; inner margin of stem with minute spiniform setae, denser at the base and absent well before the tip. Telson (Fig. 10) with caudal margin obtusely produced, with a sinuate profile. Uropod (Fig. 11J) with endopodite 1.3–1.4 times as long as exopodite when not broken, and about 2 times as long as basipodite.

Distribution

The species is known only from its type locality, by the North Pacolet River near Saluda, North Carolina, along the boundary between the Blue Ridge Mountains and the Piedmont Region (Fig. 10). All studied specimens have been collected in moist leaf litter from deciduous forest.

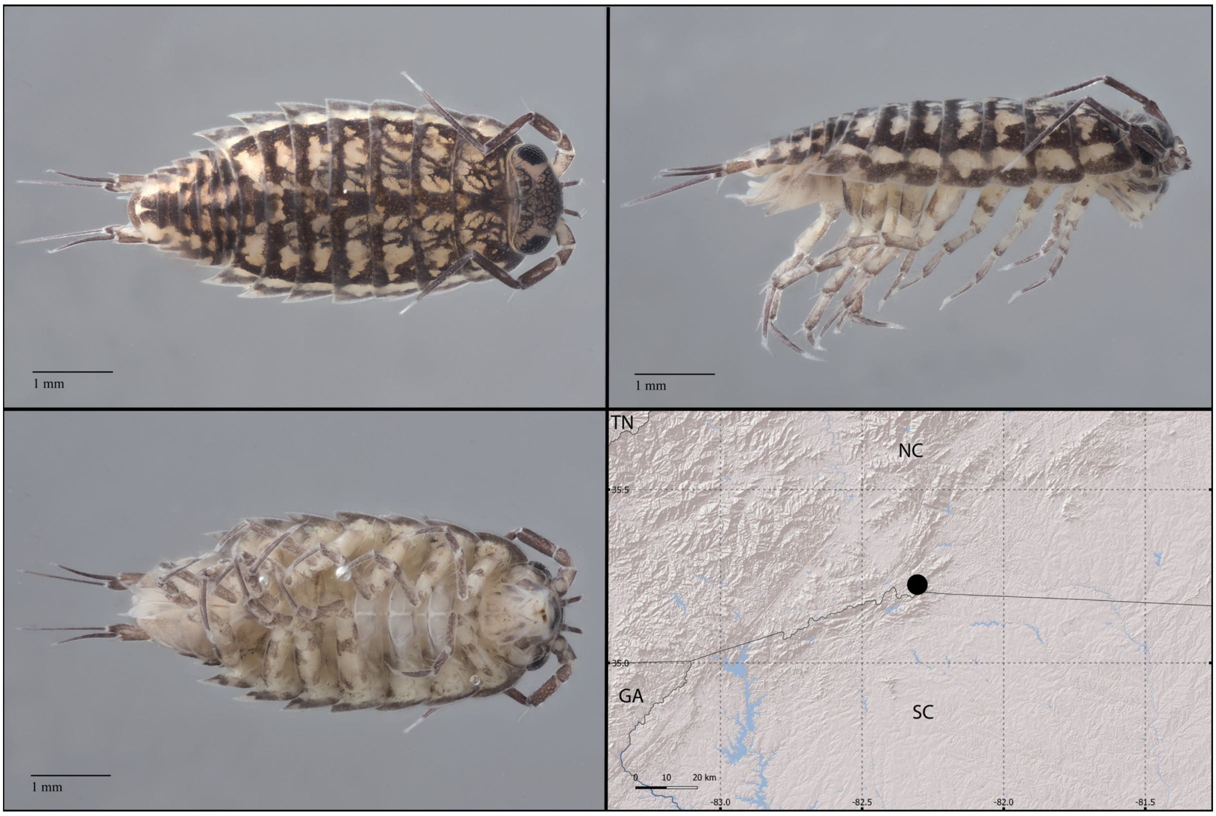

Fig. 10. General habitus of *Ligidium pacolet* sp. nov., holotype, ♂ (USNM, CUAC000171345) in dorsal, lateral and ventral view, and map showing the type locality of the species.

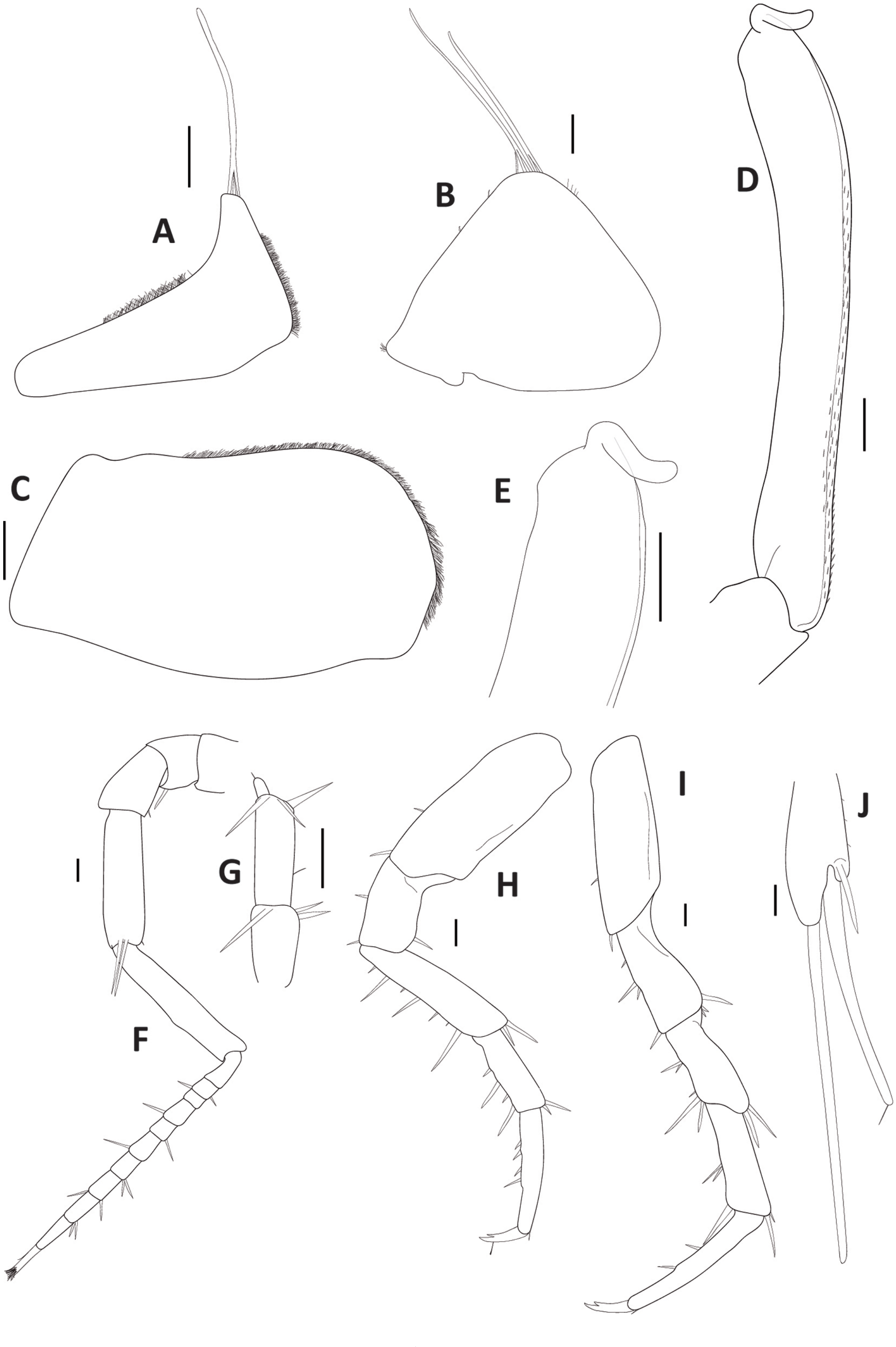

Fig. 11. *Ligidium pacolet* sp. nov., holotype, ♂ (USNM, CUAC000171345). **A**. Pleopod 1 endopod. **B**. Pleopod 1 exopod. **C**. Pleopod 2 exopod. **D**. Pleopod 2 endopod. **E**. Pleopod 2 endopod, detail of tip. **F**. Antenna. **G**. Antennule. **H**. Pereopod 1. **I**. Pereopod 7. **J**. Uropod. Scale bars = 0.1 mm.

Remarks

Ligidium pacolet sp. nov. corresponds with one of the two lineages named as *Ligidium* sp. 5 in Recuero & Caterino (2024a). It was clustered together with *L. whiteoak* sp. nov. in that paper given their morphological and genetic affinities, although species delimitation methods recognized the two species using multilocus data for species delimitation (BPP), or even three with Cox1 single-locus species delimitation methods (ASAP, mPTP) (Supp. file 1). It belongs to a clade including *L. blueridgensis* and *L. whiteoak*, being genetically closer to the latter (Fig. 1A). They are also morphologically close, all presenting an excrescence projecting from the male pleopod 2 endopodite; in *L. pacolet* the excrescence is slender and rises medially or near the inner corner from the margin of the tip, projecting caudally or inwards. In *L. blueridgensis* the excrescence is also slender, projecting from the inner tip corner rising from the dorsal surface near the margin, and projecting caudally and inwards. *Ligidium whiteoak* presents a broader excrescence, almost as wide as the endopodite stem, rising dorsally and medially from the caudal margin of the tip and projecting caudally and slightly outwards. The stem of male pleopod 2 endopodite in *L. pacolet* sp. nov. is slightly broadened in the distal third, while in *L. blueridgensis* and *L. whiteoak* its width is constant. The projection of male pleopod 1 endopodite is narrower in *L. pacolet* than in the other two species. All three gene fragments analyzed are informative to identify this species, with high genetic p-distances for Cox1, moderate to high for NaK and 28S fragments (Supp. file 1), forming a reciprocally monophyletic clade in all cases.

Ligidium whiteoak sp. nov.

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Figs 12–13

Ligidium sp. 5 – Recuero & Caterino 2024: table 1 (in part).

Diagnosis

Ligidium whiteoak sp. nov. is diagnosed from other Appalachian species by the presence in the male pleopod 2 endopodite of a broad excrescence, almost as broad as the endopodite stem, rising dorsally and medially from the caudal margin of the tip and projecting caudally and slightly outwards. It also can be identified from all other Appalachian species based on molecular data, representing a distinct evolutionary lineage.

Etymology

A noun in apposition, in reference to the species type locality.

Material examined

Holotype

USA – **Tennessee** • ♂; Blount Co., Great Smoky Mountains N.P., Whiteoak Sink area; 35.6362° N, 83.7412° W; 536 m a.s.l.; 27 Oct. 2021; M. Caterino, A. Haberski and P. Wooden leg.; GenBank no: OR169835 (Cox1); USNM, CUAC000171267.

Paratypes

USA – **Tennessee** • 3 ♂♂; same data as for holotype; CUAC000171268, CUAC000180800, CUAC000180801 • 5 ♀♀ ; same data as for holotype; CUAC000171269 to CUAC000171273.

Description

Body length of males 4.9–5.5 mm, of females 4.9–5 mm; width (at pereonite 4) of males 2.3–2.5, of females 2.4–2.6 mm. Color in ethanol (Fig. 12) dorsally brown to dark brown with off-white and greenish spots, a conspicuous dark brown longitudinal medial stripe running from pereonite 1 to pereonite 7, more or less broken with light spots in the caudal margin of pereonites; not all pereonites with darker transverse bands; epimera brown and off-white, with large white spots at the base; pleonites with a pair of lateral off-white spots, medially brown sometimes with off-white spots; telson with two white spots laterally, and a white anchor-shaped spot; head marbled brown; antennae dark brown with large white spots in basal segments and a white setae bundle in the flagellum; uropod brown, dorsally with inner part of basipodite white; pereopods brown and white, pleopod exopodites brown. Antennule (Fig. 13G) three-segmented; first segment about 1.7–1.8 times as long as wide, 3 spiniform setae in its distal border; second segment about 2.6–2.7 times as long as wide, also bearing 3 strong spiniform setae in the distal border; third segment with rounded apex and up to 3 setae. Antennae (Fig. 13F) with 5 segmented peduncle, relative size of antennomeres 5>4>3>2>1; antennal flagellum with 10–11 articles. Dactylus of pereopods with outer claw longer than inner one; no sexual dimorphism observed in pereopods 1 (Fig. 13H) and 7 (Fig. 13I). Pereopod 7 ischium broadened medially. Male pleopod 1 endopodite (Fig. 13A) with narrow projection truncated distally, bearing 2–3 strong, blunt setae, about as long as endopodite; most part of caudal and inner margins with pilose setae. Pleopod 1 exopodite (Fig. 13B) with rounded caudal margin bearing 2–3 strong, blunt setae, 0.6–0.7 times as long as exopodite; external margin with 3–4 short setae. Male pleopod 2 exopodite (Fig. 13C) 1.8–2 times as wide as long; inner and most part of frontal margin with dense, hairy setation, as in outer caudal corner; caudal margin convex, with 3–4 short, but strong setae. Male pleopod 2 endopodite (Fig. 13D–E) with narrow, rounded tip, bearing a soft, long excrescence rising medially, almost as broad as the stem; inner margin with minute spiniform setae. Telson (Fig. 12) with caudal margin obtusely produced, subtriangular. Uropod (Fig. 13J) with endopodite 1.4–1.5 times as long as exopodite when not broken, and about 2 times as long as basipodite.

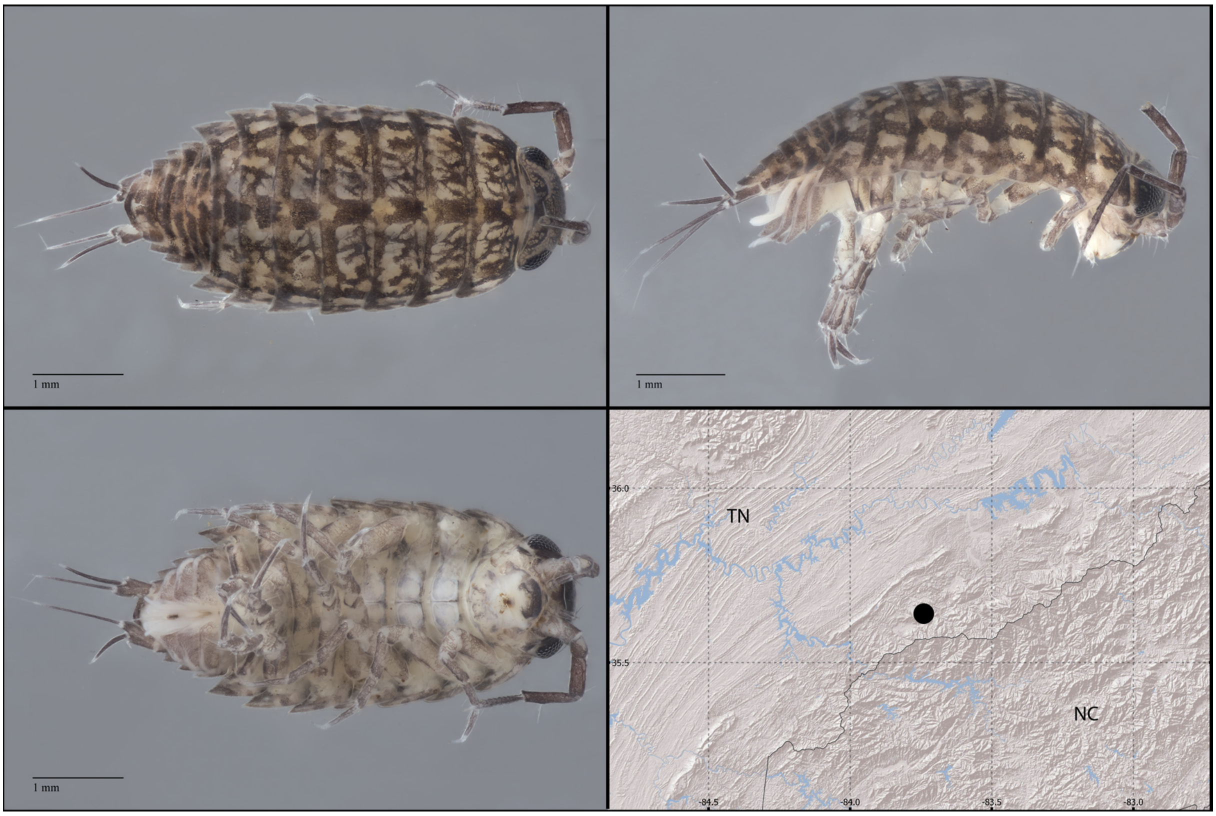

Fig. 12. General habitus of *Ligidium whiteoak* sp. nov., paratype, ♂ (CUAC000171268) in dorsal, lateral and ventral view, and map showing the type locality of the species.

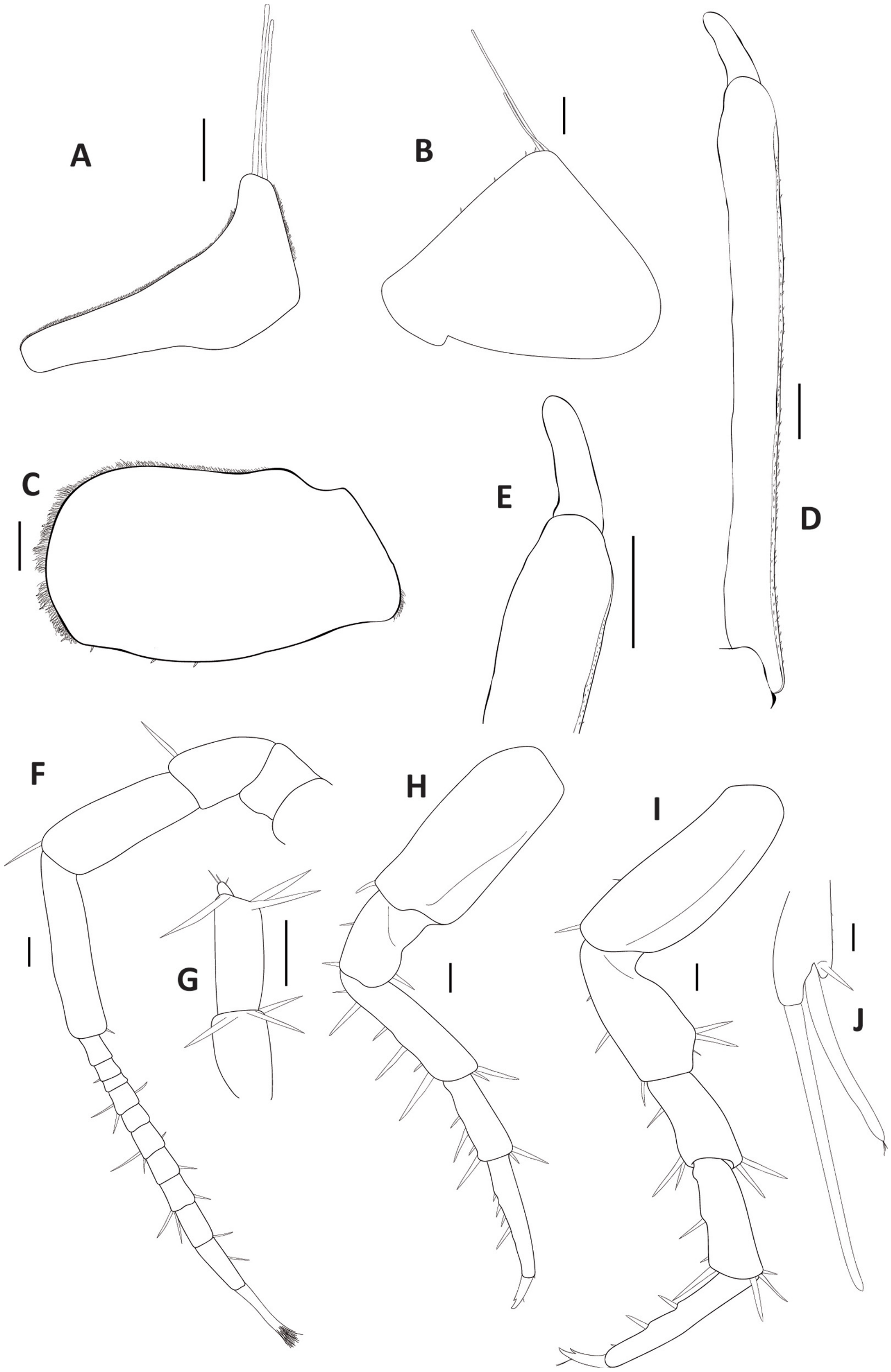

Fig. 13. *Ligidium whiteoak* sp. nov., holotype, ♂ (USNM, CUAC000171267). **A**. Pleopod 1 endopod. **B**. Pleopod 1 exopod. **C**. Pleopod 2 exopod. **D**. Pleopod 2 endopod. **E**. Pleopod 2 endopod, detail of tip. **F**. Antenna. **G**. Antennule. **H**. Pereopod 1. **I**. Pereopod 7. **J**. Uropod. Scale bars = 0.1 mm.

Distribution

The species is known only from its type locality, at low elevations near the western edge of the Great Smoky Mountains (Fig. 12). All studied specimens have been collected in moist leaf litter from deciduous forest.

Remarks

Ligidium whiteoak sp. nov. corresponds with one of the two lineages referred to as *Ligidium* sp. 5 in Recuero & Caterino (2024a). See remarks for *Ligidium pacolet* sp. nov.

Ligidium schultzi sp. nov.

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Figs 14–15

Ligidium sp. 6 – Recuero & Caterino 2024: table 1.

non *Ligidium elrodii* – Schultz 1982: 14.

Diagnosis

This new species is characterized by the distally broadened male pleopod 2 endopodite, which presents on the caudal margin as a marked notch. Male pleopod 2 exopodite has its frontal margin concave. It

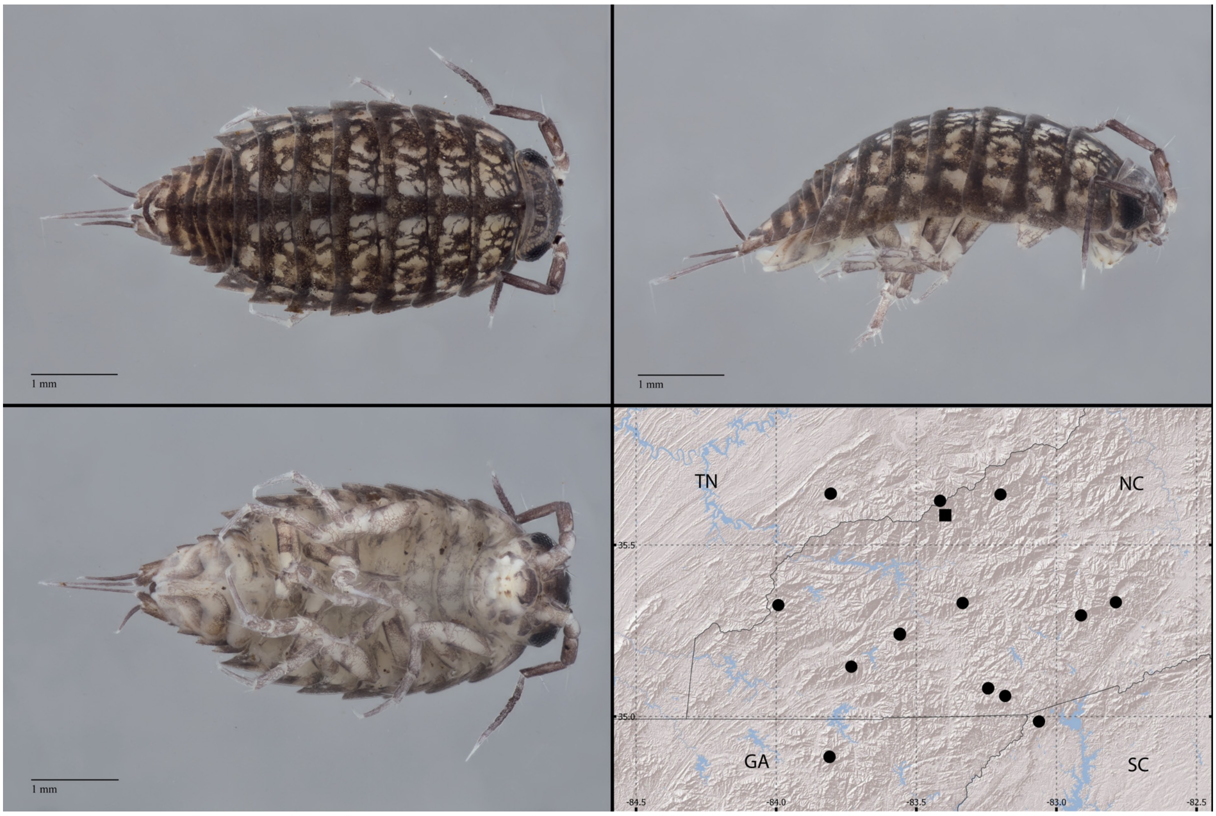

Fig. 14. General habitus of *Ligidium schultzi* sp. nov., male from Brasstown Bald, GA (CUAC000171279) in dorsal, lateral and ventral view, and map showing the known localities of the species (type locality indicated with a square).

also can be distinguished from all other Appalachian species based on molecular data, representing a distinct evolutionary lineage.

Etymology

The species is named after George A. Schultz, for his important contributions to our knowledge of the genus *Ligidium* in North America, and for being the first to illustrate this species from North Carolina.

Material examined

Holotype

USA – **North Carolina** • ♂; Swain Co., Great Smoky Mountains N.P., off Highway 441, Thomas Divide Trail; 35.5824° N, 83.3979° W; 1405 m a.s.l.; 12 Mar. 2020; M. Caterino and F. Etzler leg.; GenBank no: OR169857 (Cox1); USNM, CUAC000171290.

Paratypes

USA – **North Carolina** • 1 ♂; same data as for holotype; CUAC000171291 • 4 ♀♀; same data as for holotype; CUAC000171292 to CUAC000171295.

Other material examined

USA – **Georgia** • 2 ♂♂; Towns Co., Brasstown Bald; 34.8782° N, 83.8108° W; 1347 m a.s.l.; 17 Nov. 2020; Caterino and F. Etzler leg.; CUAC000171279, CUAC000180791 • 1 ♀; same data as for preceding; CUAC000138052 – **North Carolina** • 1 ♂; Graham Co., Nantahala N.F., Huckleberry Knob; 35.3210° N, 83.9934° W; 1674 m a.s.l.; 4 May 2020; M. Caterino and F. Etzler leg.; CUAC000138050 • 1 ♀, Haywood Co., Balsam Mountain Trail; 35.6425° N, 83.2007° W; 1565 m a.s.l.; 5 Nov. 2020; M. Caterino and F. Etzler leg.; CUAC000138054 • 3 ♂♂; Macon Co., Highlands Biological Station, Coker Rhododendron Trail; 35.0558° N, 83.1840° W; 1191 m a.s.l.; 20 Jun. 2022; P. Marek and L. Kairy leg.; CUAC000171315, CUAC000171319, CUAC000171320 • 1 ♂; Macon Co., Nantahala N.F., Copper Ridge Bald; 35.3270° N, 83.3359° W; 1535 m a.s.l.; 15 Sep. 2020; M. Caterino and F. Etzler leg.; CUAC000171277 • 2 ♀♀; same data as for preceding; CUAC000171278, CUAC000138046 • 3 ♀♀; Macon Co., Nantahala N.F., Cowee Bald; 35.3270° N, 83.3359° W; 1503 m a.s.l.; 15 Sep. 2020; F. Etzler, A. Haberski and P. Wooden leg.; CUAC000138047, CUAC000171283, CUAC000171284 • 5 imm.; same data as for preceding; CUAC000171285 to CUAC000171289 • 2 ♂♂; Macon Co., Nantahala N.F., Van Hook Glade Campground; 35.0783° N, 83.245° W; 1018 m a.s.l.; 21 Jun. 2022; CUAC000171321, CUAC000171322 • 1 ♀; same data as for preceding; CUAC000171318 • 2 ♀♀; Transylvania Co., Pisgah Forest; 35.3291° N, 82.789° W; 910 m a.s.l.; 23 Dec. 2021; E. Recuero and P.C. Rodríguez-Flores leg.; CUAC000171302, CUAC000171303 • 1 ♀; Transylvania Co., Pisgah National Forest, Hwy 215; 35.2910° N, 82.9133° W; 1561 m a.s.l.; 8 May 2018; M. Caterino, R. Kucuk and L. Cushman leg.; CUAC000171304 • 2 ♂♂; same data as for preceding; CUAC000171305, CUAC000171306 • 1 ovi. ♀; Clay Co., Nantahala N.F., Tusquitee Bald; 35.1415° N, 83.7273° W; 1582 m a.s.l.; 6 Jul. 2021; M. Caterino and E. Recuero leg.; CUAC000165724. – **South Carolina** • 1 ♀; Oconee Co., Sumter N.F., Walhalla Hatchery; 34.9853° N, 83.0731° W; 765 m a.s.l.; 21 Jan. 2023; C.W. Harden leg.; CUAC000177091 • 1 ♂; same data as for preceding; CUAC000177092 – **Tennessee** • 1 ♂; Blount Co., Great Smoky Mountains N.P., Rich Mountain Gap; 35.645° N, 83.81° W; 600 m a.s.l.; 27 Oct. 2021; M. Caterino, A. Haberski and P. Wooden leg.; CUAC000171264 • 2 ♀♀; same data as for preceding; CUAC000171265, CUAC000171266 • 3 ♂♂; Sevier Co., Great Smoky Mountains N.P., off Highway 441; 35.6240° N, 83.4163° W; 1394 m a.s.l.; 12 Mar. 2020; M. Caterino and F. Etzler leg.; CUAC000138081, CUAC000171297, CUAC000171298 • 2 ♀♀; same data as for preceding; CUAC000171300, CUAC000171301 • 1 imm.; same data as for preceding; CUAC000171296.

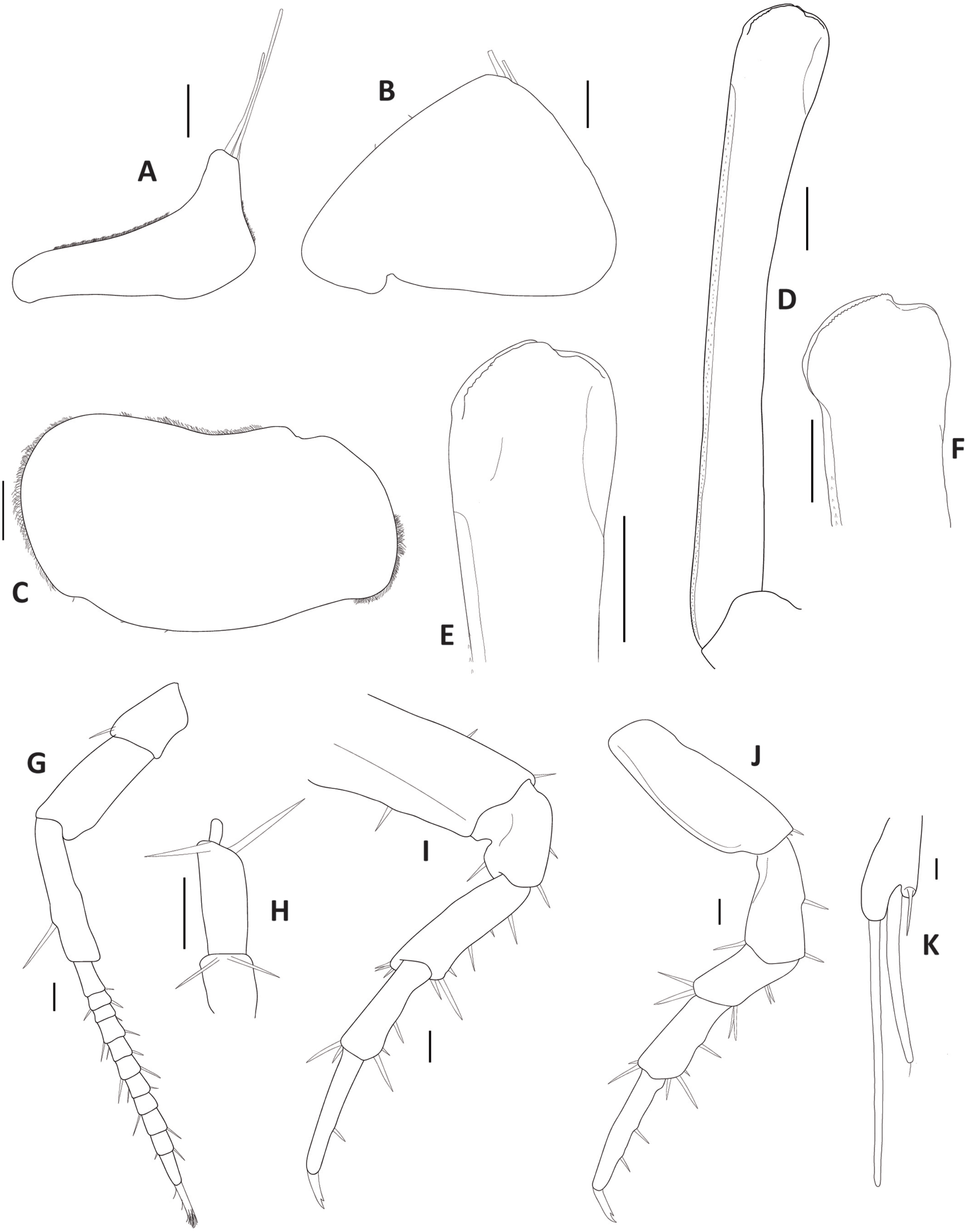

Fig. 15. *Ligidium schultzi* sp. nov. **A–E, G–J**. Holotype, ♂ (USNM, CUAC000171290). **F**. Male from Cooper Ridge Bald, NC (CUAC000171277). **K**. Female from the Great Smoky Mountains N.P., TN (CUAC000171266). **A**. Pleopod 1 endopod. **B**. Pleopod 1 exopod. **C**. Pleopod 2 exopod. **D**. Pleopod 2 endopod. **E–F**. Pleopod 2 endopod, detail of tip. **G**. Antenna. **H**. Antennule. **I**. Pereopod 1. **J**. Pereopod 7. **K**. Uropod. Scale bars = 0.1 mm.

Description

Body length of males 4.6–5.7 mm, of females 4.7–5.7 mm; width (at pereonite 4) of males 2.5–2.8, of females 2.6–2.9 mm. Color in ethanol (Fig. 14) dorsally brown with large off-white, a dark brown longitudinal medial stripe running from pereonite 1 to pereonite 7, frequently broadening caudally; pereonites with darker transverse bands in the caudal margin, frequently with clearer spots; epimera brown marbled with off-white, with large white spots at the base; pleonites brown with a pair of more or less defined off-white spots, paramedian in pleonites 1 and 2, more lateral in pleonites 3–5; telson with two white spots laterally, and a white anchor-shaped spot; head marbled brown, sometimes with white area above eyes; antennae brown, sometimes with large white spots in basal segments, and a white setae bundle in the flagellum; uropod brown, dorsally with inner part of basipodite white; pereopods brown and white, pleopod exopodites white, often with inner and frontal parts brown. Antennule (Fig. 15G) three-segmented; first segment about 1.7–1.8 times as long as wide, 2–3 spiniform setae in its distal border; second segment about 2.5–2.6 times as long as wide, bearing 2–3 strong spiniform setae in the distal border; third segment with blunt apex and 1 or no setae. Antennae (Fig. 15F) with 5 segmented peduncle, relative size of antennomeres 5>4>3=2>1; antennal flagellum with 10–12 articles. Dactylus of pereopods with outer claw longer than inner one; no sexual dimorphism observed in pereopods 1 (Fig. 15H) and 7 (Fig. 15I). Male pleopod 1 endopodite (Fig. 15A) with narrow projection obliquely truncated distally, bearing 2 strong, blunt setae, up to 1.2 times as long as endopodite; part of caudal and inner margins with pilose setae. Pleopod 1 exopodite (Fig. 15B) with broadly rounded caudal margin bearing 2–3 strong, blunt setae, up to 0.5–0.7 times as long as exopodite; external margin with 2–3 very short but strong setae. Male pleopod 2 exopodite (Fig. 15C) 1.8–1.9 times as wide as long; inner, outer and part of frontal margin with dense, hairy setation; caudal margin strongly convex, with 2–4 very short, but strong setae; frontal margin concave. Male pleopod 2 endopodite (Fig. 15D–E) with unevenly rounded tip, presenting a marked notch or indentation; inner margin with minute spiniform setae denser near the base. Telson (Fig. 14) with caudal margin obtusely produced. Uropod (Fig. 15J) with long endopodite, 1.6–1.8 times as long as exopodite when not broken, and about 2.1–2.2 times as long as basipodite.

Distribution

The species is widely distributed in the southern part of the Blue Ridge Mountains, with all known records found west of the Asheville Depression (Fig. 14). Studied specimens have been collected in moist leaf litter from conifer, deciduous, and mixed forest, as well as under dead logs and stones, usually in damp places near streams. From 600 m to 1674 m a.s.l., most frequently above 1000 m.

Remarks

Ligidium schultzi sp. nov. corresponds with *Ligidium* sp. 6 in Recuero & Caterino (2024a). The shape of male pleopod 2 endopodite of this species was first illustrated by Schultz (1982), as a morphological variant of what he identified as *L. elrodii*, and indicated the need for further taxonomic study to determine the specific status. The new species is closely related to *L. enotahensis* sp. nov. and *L. protuberans* sp. nov. (Fig. 1A; see Remarks for those species). All three gene fragments analyzed are informative to identify this species, with high genetic p-distances among the mentioned taxa for Cox1, moderate for 28S and low for NaK (Supp. file 1), forming reciprocally monophyletic clades in all cases.

Ligidium blueridgensis Schultz, 1964

Figs 16–17

Ligidium blueridgensis Schultz, 1964: 90, pl. 1 figs 1–11, pl. 2 figs 12–14.

Material examined

USA – **Georgia** • 1 ♀; Rabun Co., Chattahoochee N.F., Rabun Cliffs; 34.9713° N, 83.2978° W; 1244 m a.s.l.; 11 May 2021; M. Caterino and A. Haberski leg.; CUAC000138049 • 2 ♂♂; same data as for preceding; CUAC000180796, CUAC000180797 • 1 ♀; Rabun Co., Chattahoochee N.F., Rabun Cliffs; 34.9709° N, 83.3004° W; 1308 m a.s.l.; 25 Nov. 2019; M. Caterino leg.; CUAC000138078 • 1 ♂; same data as for preceding; CUAC000171280. – **North Carolina** • 1 ♀; Avery Co., Grandfather Mountain; 36.0893° N, 81.8388° W; 1387 m a.s.l.; 21 Apr. 2022; M. Caterino, E. Recuero and A. Haberski leg.; CUAC000171348 • 2 ♂♂; same data as for preceding; CUAC000171346, CUAC000171347 • 1 ♀; Avery Co., Grandfather Mountain, Bridge Trail; 36.0948° N, 81.8311° W; 1554 m a.s.l.; 21 Apr. 2022; M. Caterino, E. Recuero and A. Haberski leg.; CUAC000171339 • 1 ♂; same data as for preceding; CUAC000171340 • 1 ♀; Avery Co., Grandfather Mountain, Grandfather Trail; 36.0978° N, 81.8293° W; 1637 m a.s.l.; 21 Apr. 2022; M. Caterino, E. Recuero and A. Haberski leg.; CUAC000171334 • 3 ♂♂; Caldwell Co., Grandfather Mountain, Boone Scout Trail; 36.1164° N, 81.7844° W; 1259 m a.s.l.; 6 Oct. 2020; M. Caterino, F. Etzler and A. Haberski leg.; CUAC000171307, CUAC000171308, CUAC000138059 • 1 ♀; same data as for preceding; CUAC000171309 • 1 ♂; Graham Co., Nantahala N.F., Huckleberry Knob; 35.3210° N, 83.9934° W; 1674 m a.s.l.; 4 May 2020; M. Caterino and F. Etzler leg.; CUAC000180792 • 1 ♀; Haywood Co., Black Balsam Knob; 35.3289° N, 82.8745° W; 1839 m a.s.l.; 20 Oct. 2020; M. Caterino and F. Etzler leg.; CUAC000138045 • 1 ♂; Madison Co.: Pisgah N.F.: Camp Creek Bald; 36.0220° N, 82.7167° W; 1445 m a.s.l.; 1 Mar. 2022; M. Caterino, E. Recuero and P. Wooden leg.; CUAC000171352 • 5 ♀♀; same data as for preceding; CUAC000171353 to CUAC000171357 1 ♂; Polk Co., North Pacolet River; 35.2221° N, 82.3059° W; 384 m a.s.l.; 15 Feb. 2022; E. Recuero, M. Caterino, C. Harden and P. Wooden leg.; CUAC000171344 • 1 ♂; Polk Co., Tryon, Melrose Falls; 35.2199° N, 82.2987° W; 407 m a.s.l.; 10 Aug. 2021; E. Recuero, M. Caterino, A. Haberski and P. Wooden leg.; CUAC000171335 • 2 ♀♀; same data as for preceding; CUAC000171336, CUAC000171337 2 ♂♂; Rutherford Co., Chimney Rock; 35.4372° N, 82.2506° W; 383 m. a.s.l.; 3 Apr.

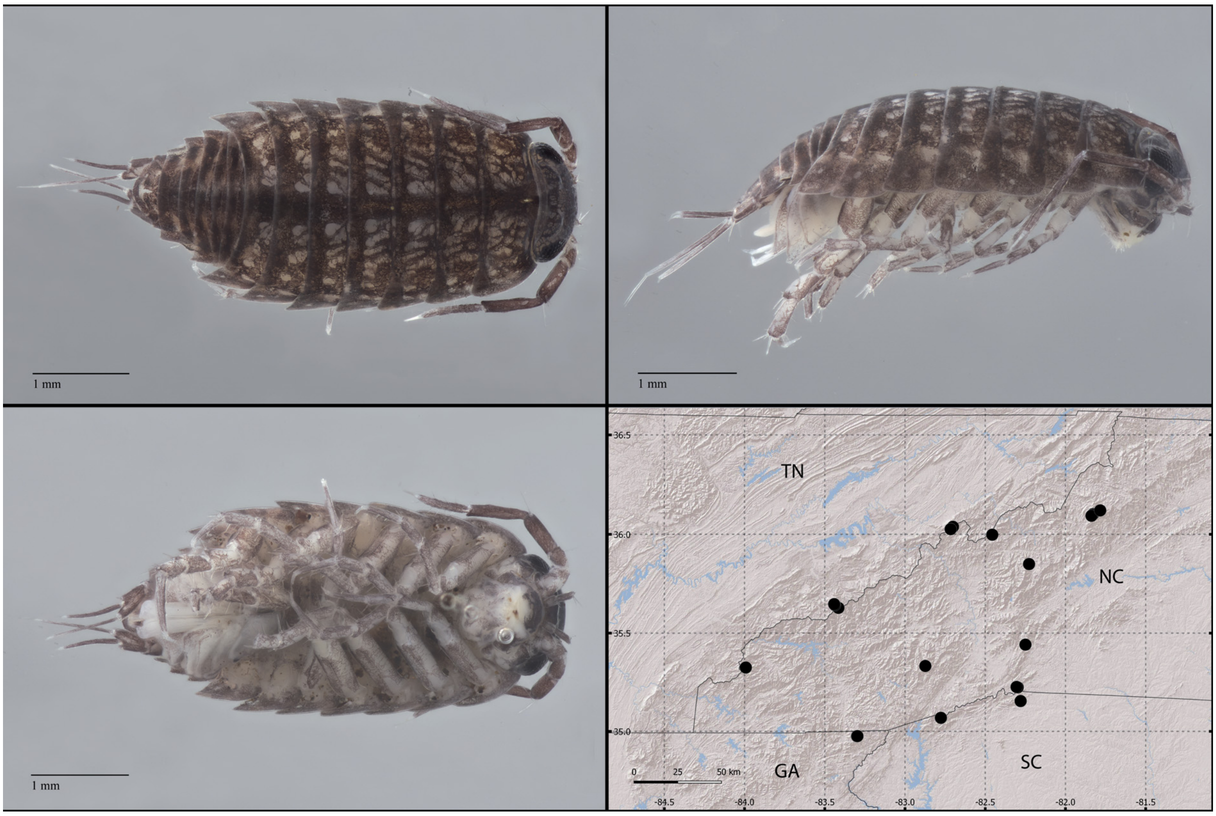

Fig. 16. General habitus of *Ligidium blueridgensis* Schultz, 1964, male from the Great Smoky Mountains (CUAC000171299) in dorsal, lateral and ventral view, and map showing the studied localities of the species.

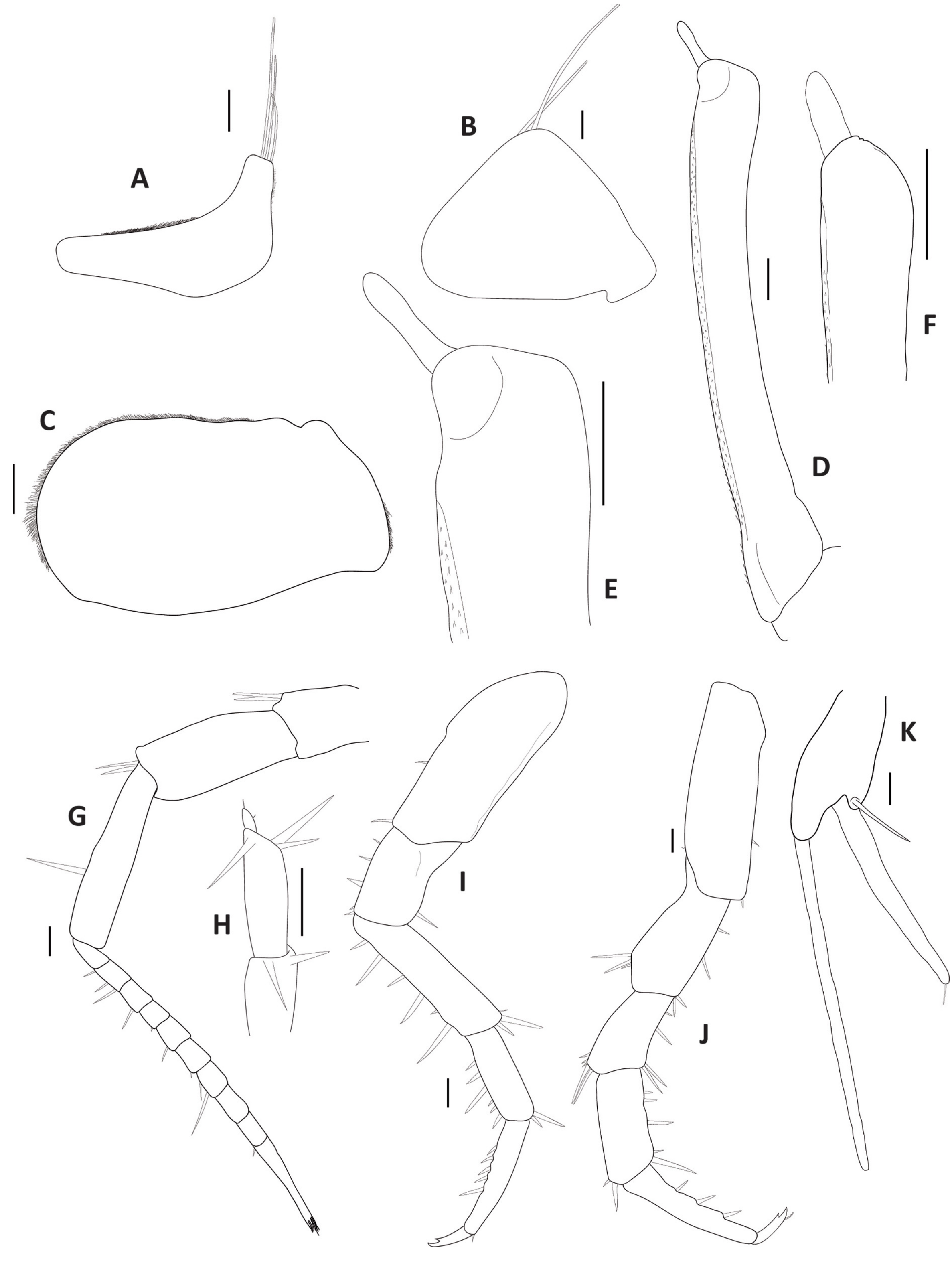

Fig. 17. *Ligidium blueridgensis* Schultz, 1964. **A–E, G–J**. Male from Rabun Cliffs, GA (CUAC000180797) **F**. Male from Woody Ridge Trail, NC (CUAC000180795). **K**. Male from Rabun Cliffs, GA (CUAC000180796). **A**. Pleopod 1 endopod. **B**. Pleopod 1 exopod. **C**. Pleopod 2 exopod. **D**. Pleopod 2 endopod. **E–F**. Pleopod 2 endopod, detail of tip. **G**. Antenna. **H**. Antennule. **I**. Pereopod 1. **J**. Pereopod 7. **K**. Uropod. Scale bars = 0.1 mm.

2023; M. Caterino, E. Recuero and C.W. Harden leg.; CUAC0001169835, CUAC0001169838 • 3 ♂♂; Yancey Co., Pisgah N.F., Woody Ridge Trail; 35.8455° N, 82.2279° W; 1259 m a.s.l.; 19 Oct. 2021; M. Caterino, E. Recuero and A. Haberski leg.; CUAC000180795, CUAC000171326, CUAC000171327 • 6 ♀♀; same data as for preceding; CUAC000171328 to CUAC000171333. – **South Carolina** • 1 ♀; Greenville Co., Chestnut Ridge H.P., South Pacolet River; 35.1503° N, 82.2804° W; 344 m a.s.l.; 15 Feb. 2022; E. Recuero leg.; CUAC000171350 • 2 ♂♂; Pickens Co., Sassafras Mountain; 35.0647° N, 82.7774° W; 1029 m a.s.l.; 11 Jun. 2020; F.E. Etzler leg.; CUAC000180798, CUAC000180799 • 1 ♀; same data as for preceding; CUAC000138055. – **Tennessee** • 1 ♂; Greene Co., Cherokee N.F., Firescald Knob; 36.0337° N, 82.7024° W; 1353 m a.s.l; 1 Mar. 2022; M. Caterino, E. Recuero and P. Wooden leg.; CUAC000171351 • 1 ♀; Sevier Co., Great Smoky Mountains N.P., Mount LeConte; 35.6427° N, 83.4426° W; 1581 m a.s.l.; 28 Sep. 2021; M. Caterino, E. Recuero, A. Haberski and P. Wooden leg.; CUAC000171276 • 1 ♂; same data as for preceding; CUAC000171338 • 1 ♀; Sevier Co., Smoky Mountains N.P., Mount LeConte, Alum Cave; 35.6382° N, 83.4387° W; 1317 m a.s.l.; 28 Sep. 2021; M. Caterino, E. Recuero, A. Haberski and P. Wooden leg.; CUAC000171325 • 1 ♂; same data as for preceding; CUAC000171323 • 1 ♀; Sevier Co., Great Smoky Mountains N.P., off Highway 441; 35.6237° N, 83.4163° W; 1394 m a.s.l.; 12 Mar. 2020; M. Caterino and F. Etzler leg.; CUAC000171275 • 1 ♂; same data as for preceding; CUAC000171299 • 2 imm.; Unicoi Co., Cherokee N.F., Big Bald; 35.9938° N, 82.4573° W; 1596 m a.s.l.; 5 Aug. 2020; M. Caterino, A. Haberski and P. Wooden leg.; CUAC000171310, CUAC000171311.

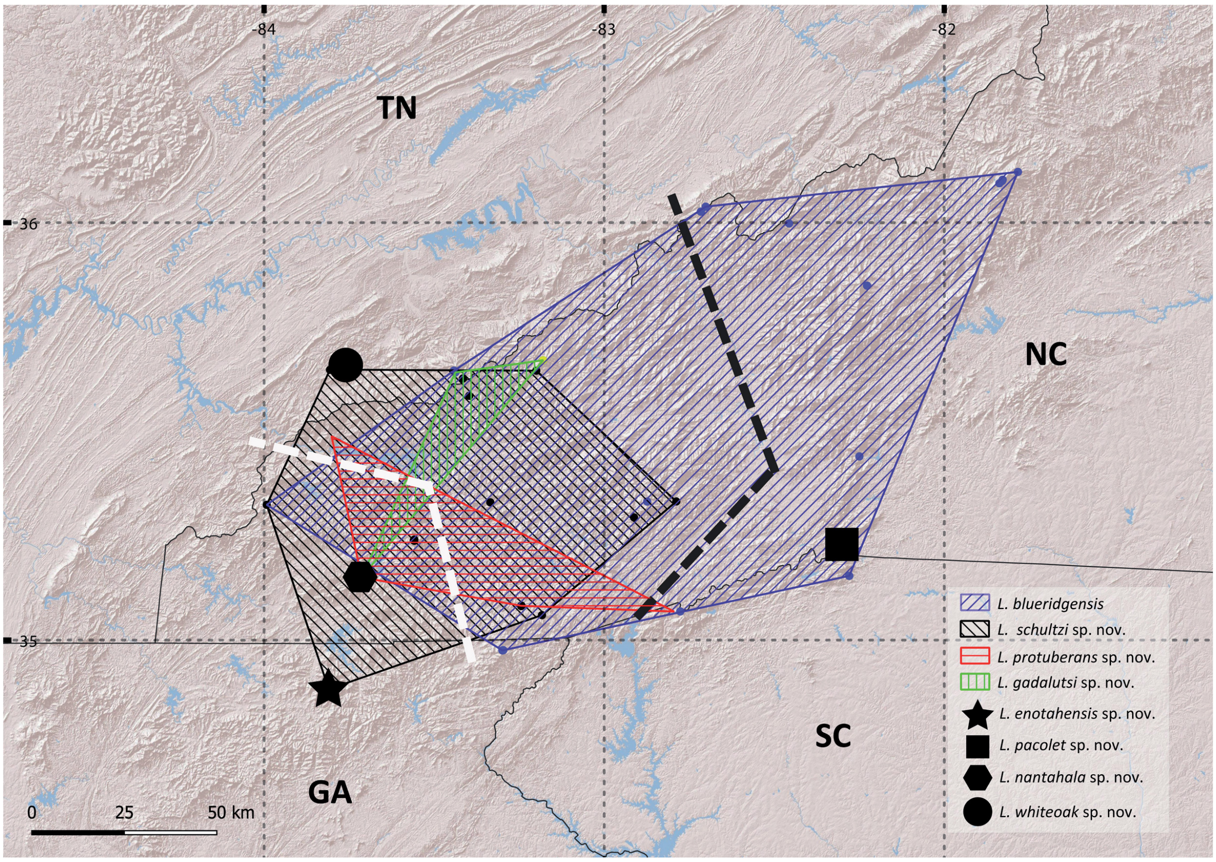

Fig. 18. Minimum convex polygons showing extent of occurrence of species of *Ligidium* Brandt, 1833 with several known localities, and type localities for species known from a single locality (see figure legend). Dotted black line indicates the location of the Asheville Depression Barrier. Dotted white line indicates the location of the Little Tennessee River Barrier.

Remarks

This is the most widely distributed *Ligidium* species in the southern Appalachian Mountains (Fig. 16), presenting also a broad altitudinal range, from 344 to 1674 m a.s.l. Although the species' distribution spans important biogeographical barriers, such as the Asheville Depression, it includes several deep mitochondrial lineages that seem to be geographically restricted to particular areas delimited by that barrier and one formed by the Little Tennessee River (Figs 1, 18). This pattern is not common among other litter arthropods in this area (Caterino & Recuero 2023; Recuero & Caterino 2024b, 2024c). This suggests an old presence and diversification within these mountains. See Remarks under *Ligidium pacolet* sp. nov. and *L. whiteoak* sp. nov. and Fig. 17 for diagnostic information.

Ligidium elrodii (Packard, 1873)
Figs 19–20

Euphiloscia elrodii Packard, 1873: 97.
Ligidium longicaudatum Stoller, 1902: 208, fig. 1.

Material examined

USA – **West Virginia** • 1 ♂; Pocahontas Co., Pocahontas Campground; 38.1026° N, 79.9666° W; 756 m a.s.l.; 13 Oct. 2022; M.. Caterino and E. Recuero leg.; CUAC000174439 • 1 ♀; Randolph Co.,

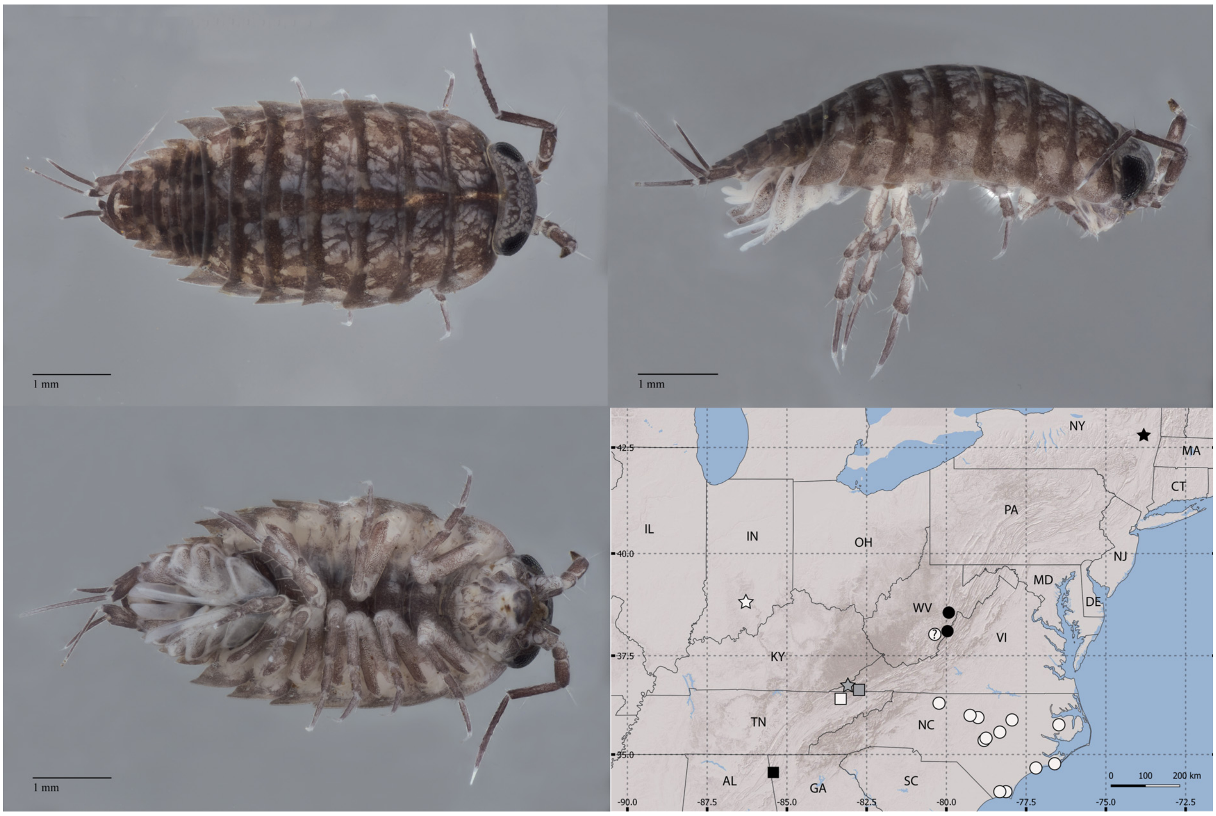

Fig. 19. General habitus of *Ligidium elrodii* (Packard, 1873), male from Monongahela N.F., WV (CUAC000174442) in dorsal, lateral and ventral view, and map showing known localities of *L. elrodii* (black circle: studied populations, white circles: published records, white star: type locality of *L. elrodii*, black star: type locality of *L. longicaudatum* Stoller, 1902), *L. e. leensis* Schultz, 1970 (grey star), *L. chatoogaensis* Schultz, 1970 (black square), *L. hancockensis* Schultz, 1970 (white square) and *L. scottensis* Schultz, 1970 (grey square).

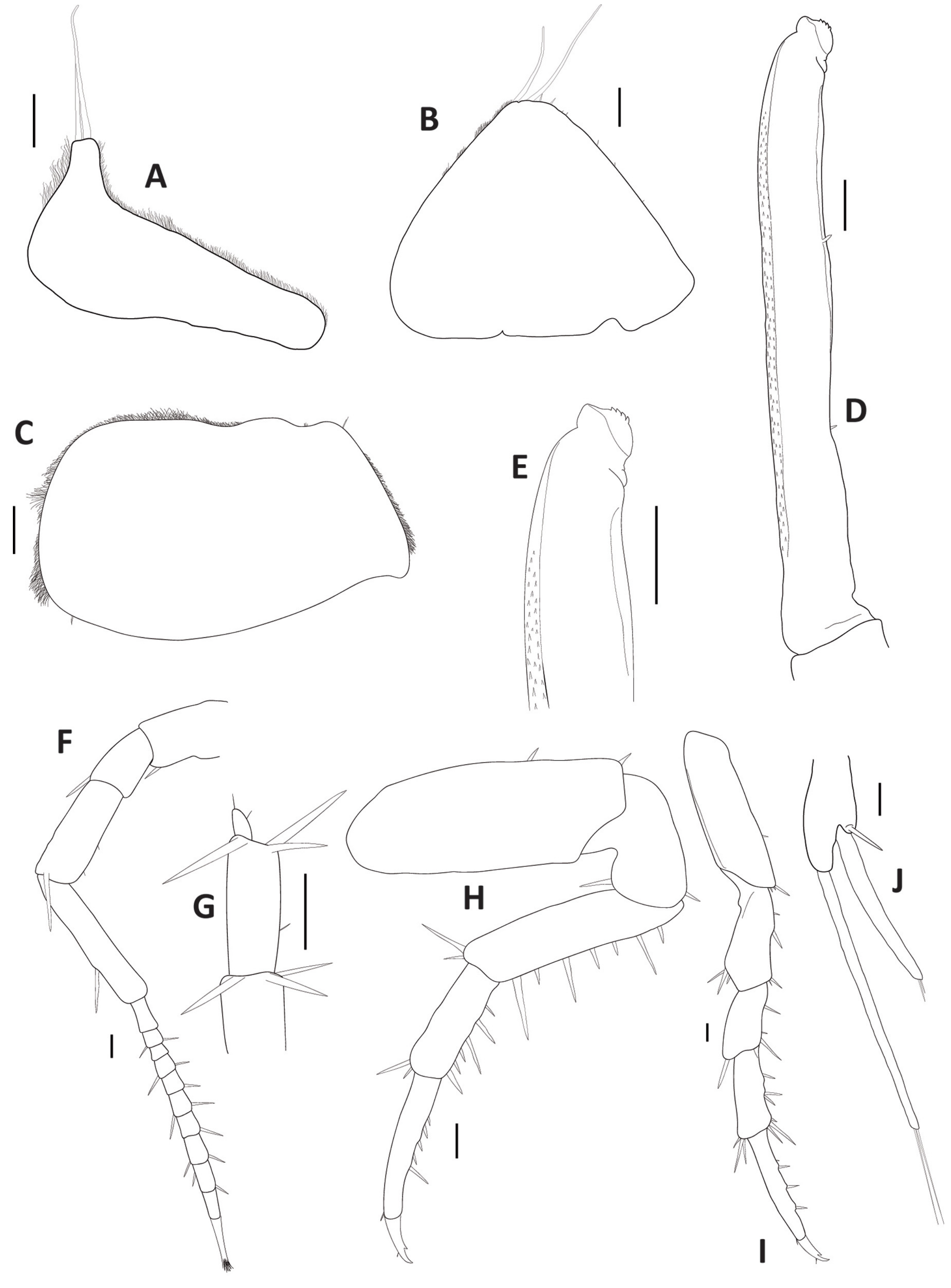

Fig. 20. *Ligidium elrodii* (Packard, 1873). **A–I**. Male from Monongahela N.F., WV (CUAC000174442). **J**. Male from Monongahela N.F., WV (CUAC000174443). **A**. Pleopod 1 endopod. **B**. Pleopod 1 exopod. **C**. Pleopod 2 exopod. **D**. Pleopod 2 endopod. **E**. Pleopod 2 endopod, detail of tip. **F**. Antenna. **G**. Antennule. **H**. Pereopod 1. **I**. Pereopod 7. **J**. Uropod. Scale bars = 0.1 mm.

Monongahela N.F.; 38.5595° N, 79.9262° W; 1135 m a.s.l.; 12 Oct. 2022; M. Caterino and E. Recuero leg.; CUAC000174441 • 2 ♂♂; same data as for preceding; CUAC000174440, CUAC000174442 • 1 imm.; same data as for preceding; CUAC000174443.

Remarks

The studied specimens from West Virginia have been collected in leaf litter from deciduous and conifer forests at mid-elevations (Fig. 18). They differ slightly from the ones illustrated from North Carolina (Schultz 1970, 1982). Particularly, males from North Carolina present ten scale-like spines on the distal margin of the tip of male pleopod 2 endopodite, while those from West Virginia present four (Fig. 19D–E). Also, the illustration of a male pleopod 2 exopodite from North Carolina shows a large spiniform or plumose seta on the inner corner of the caudal margin, not present in the samples from West Virginia (Fig. 19C). These differences could indicate different species, but we refrain from proposing any more new names for this complex, until the true status of *Ligidium elrodii* and *L. longicaudatum* may be clarified. This species is not closely related to any of the other taxa included in our analyses (Fig. 1A).

Four subspecies of *Ligidium elrodii* have been described from caves in the states of Virginia, Tennessee and Georgia, based on differences in the male pleopod 2 endopodite (Schultz 1970). One of them, *L. e. leensis*, from Bowling Cave, Lee Co., Virginia (see map in Fig. 18), is relatively similar to our specimens and to those illustrated from North Carolina, differing in a more markedly squarish process on the inner margin of male pleopod 2 endopodite, with only two scale-like spines on its distal margin. Indeed, it could represent a different species, but until *L. elrodii* is revised we prefer to maintain its status. The other three have more pronounced differences in this particular structure, which are indicative of specific status. We propose to raise them to full species.

Ligidium chatoogaensis Schultz, 1970

Ligidium elrodii chatoogaensis Schultz, 1970: 43, figs 29–33.

Remarks

In the form of male pleopod 2 endopodite this species resembles *Ligidium elrodii*, but having a constricted tip, and only five scale-like spines on the distal margin of the tip.

The species is so far known only from its type locality, Blowing Spring Cave, 2.5 miles NE Cloudland, Chattooga Co., Georgia (see map in Fig. 18).

Ligidium hancockensis Schultz, 1970

Ligidium elrodii hancockensis Schultz, 1970: 41, figs 24–28.

Remarks

In the form of male pleopod 2 endopodite this species resembles *Ligidium nantahala* sp. nov., but its tip has a much shorter process and a squared section.

The species is so far known only from its type locality, Cantwell Valley Cave, Hancock Co., Tennessee (see map in Fig. 18).

Ligidium scottensis Schultz, 1970

Ligidium elrodii scottensis Schultz, 1970: 41, figs 20–23.

Remarks

In the form of male pleopod 2 endopodite this species resembles *L. gadalutsi* sp. nov., but the tip is shorter and blunt, not acuminate. They also differ in the shape of male pleopod 1 endopodite.

The species is so far known only from its type locality, Coley Cave #2, in Virginia, Scott Co. (see map in Fig. 18).

Discussion

The species described here, together with the change of status of three subspecies of *Ligidium elrodii*, raises the total number of species of *Ligidium* to 68, including 18 exclusive to North America north of Mexico, with four in the west and 14 in the east and southeast. Up to 18 species have been described since 2000, the seven new species in this paper and 11 from China (Nunomura & Xie 2000; Nunomura 2002; Li 2015, 2017; Wang *et al.* 2022).

Classical taxonomy has relied on the identification of fixed morphological differences among studied specimens to delimit species. In certain groups, the most commonly used characters are linked to specific structures, such as genitalia in many insect groups, gonopods in several millipede orders, or modified male pleopods in terrestrial isopods (Vandel 1960; Tuxen 1970; Koch 2015), and in some cases they are practically the only reliable source of diagnostic characters. Interestingly enough, these structures are associated with reproductive behavior, and observed differences are often interpreted as the result, and sometimes the cause of reproductive isolation (Masly 2012; Yassin 2016). However, in most cases the interpretation of such morphological variability has been relegated to the taxonomist's subjective evaluation of whether it represents normal intraspecific variation, or is enough to separate species. The integration of further sources of information, like ecological or distributional data, to complement morphological evidence in taxonomic decisions has been a major help for many taxonomists throughout history, in what is called today integrative taxonomy (Valdecasas *et al.* 2007). Currently, the advancement of molecular techniques has allowed the incorporation of genetic information as a major source of taxonomic information, and phylogenetic and species delimitation analyses allow a better understanding of morphological variation and speciation (Padial *et al.* 2010; Sánchez-Vialas *et al.* 2020), while filling DNA barcoding databases should facilitate species identification even without taxonomic expertise (Recuero *et al.* 2023). Such has been the case for many species of *Ligidium* all over its distribution range.

Of the 18 species of *Ligidium* described since 2000, 13 have been informed by molecular evidence (Li 2017; Wang *et al.* 2022; this paper). Several other potentially new species have been identified based on DNA sequencing, although not resulting in formal taxonomic revisions yet (Klossa-Kilia *et al.* 2006; Yoshino & Kubota 2022; Harigai *et al.* 2023). In most cases, these newly described species showed a high morphological conservatism, with only subtle differences in male pleopod shapes. In the eastern USA, such morphological variability had been identified, but it was considered to represent intraspecific variability with moderate taxonomic significance (Schultz 1970, 1982), resulting in the description of four subspecies of a widespread *Ligidium elrodii* and the mention of another morphological variant within that species. The use of molecular data resulted in the discovery of deep, reciprocally monophyletic lineages (Recuero & Caterino 2024a). This indicates that in this region, subtle morphological differentiation is indicative of species-level taxa. Even if our sampling covers only a fraction of the distribution of *Ligidium* in Eastern North America, we have found several new morphotypes, and we anticipate that further exploration will result in the discovery of yet undescribed species. More taxonomic work is needed, including a revision of the status of *L. elrodii*.

It is important to note that the observed genetic divergences in the commonly used Cox1 barcoding fragment are very high even at the intraspecific level. This seems to be a relatively common pattern among terrestrial isopods (e.g., Zimmermann *et al.* 2015; Recuero *et al.* 2021; Raupach *et al.* 2022), and interpretation of such results must be made with caution and always together with other sources of evidence, such as nuclear genes and morphology, to avoid overestimation of specific diversity (Recuero & Caterino 2024a). However, a finer scale study of such diversity could discover further speciation events among those mtDNA lineages.

Schultz (1970) redefined the concept of *Ligidium elrodii* based on male specimens from the piedmont and the coastal plain of North Carolina (see map in Fig. 18), after revising the available female syntypes of *Euphiloscia elrodii* and, not surprisingly, finding no useful diagnostic characters to differentiate them from specimens assigned to *Ligidium longicaudatum*. The high diversity found in the Appalachian Mountains, despite the marked conservatism of general morphology, and the large distance, around 1500 km, between the type localities of *Ligidium elrodii* (a cave near Orleans, Orange Co., Indiana) and *L. longicaudatum* (Schenectady, Schenectady Co., New York) (see map in Fig. 18), could indicate that indeed both names could belong to different species. Detailed examination of topotypic males, ideally including molecular data, will resolve this issue, as well as the identity of populations from the coastal plain of North Carolina.

Another point that may have impaired the taxonomy of *Ligidium* in the Southern Appalachians is the curious distribution patterns observed for several of the species (Recuero & Caterino 2024a) (Fig. 18). It was surprising to find that in several localities two or even three species could coexist. Given the small morphological differences, finding several of the described morphotypes together could suggest such variation to be intraspecific, refraining previous taxonomic decision regarding species delimitation (e.g., Schultz 1982). That could have been the case of *L. blueridgensis* and *L. pacolet* sp. nov., both found in sympatry by the North Pacolet River in North Carolina, or *L. protuberans* sp. nov. and *L. schultzi* sp. nov., with several cases of sympatry. Indeed, the absence of molecular data would probably lead us to be more taxonomically conservative when considering several of the species here described.

The most widespread species in the Southern Appalachians is *L. blueridgensis*, and the only one that spans both sides of the main biogeographic barrier in these mountains, the Asheville Depression (Fig. 18). It includes several deep mtDNA lineages that show distinct geographic structure (Fig. 1), although it seems to have been able to expand across this and other barriers (i.e., the Little Tennessee River) several times during its evolutionary history. Similar patterns, but restricted to the area west of the Asheville Depression, and with more limited mtDNA diversity, are found in *L. schultzi* sp. nov., the second most widespread species, and *L. protuberans* and *L. gadalutsi*. Although more data is needed, we have not observed obvious differences in the ecological preferences of these species, as they have been collected in very similar habitats. Current distributions could be the result of range contractions and expansions following Pleistocene glacial cycles (Hewitt 2011), associated with possible differences in their dispersal capabilities. Following this hypothesis, we would expect species like *L. blueridgensis* and *L. schultzi* to be more prone to disperse, outcompeting other species in expanding their ranges following climate changes. This would have allowed them to occupy larger areas faster than other species, and eventually to enter into secondary contact with more restricted species, which would remain near glacial microrefugial areas as expansion into other favorable areas would be restricted by the presence of widespread species. However, it is likely that we are still far from knowing the extent of the geographic distribution of most of the species of *Ligidium* living in this region, and even possibility of the presence of further taxa cannot be disregarded, so more studies are needed before we start to fully understand the complex evolutionary history of this group in the southern Appalachian Mountains and, more broadly, in North America.

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Supplementary files

Supp. file 1. Studied specimens of *Ligidium* Brandt, 1833, including results of species delimitation analyses and GenBank accession numbers for analyzed sequences.
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Supp. file 2. Alignments, in FASTA format, of the analyzed sequences of *Ligidium* Brandt, 1833.
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