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Isoperla riverae, a new stonefly species from the southeast Nearctic, with notes on sympatric species including the larval description of *Isoperla lenati* Szczytko & Kondratieff, 2015 (Plecoptera: Perlodidae)

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Abstract

Isoperla riverae **sp. n.** is described from the southeastern USA. The new species is proposed based on details of the adult habitus, male aedeagus, vesicle, female subgenital plate, ovum chorion, and larval habitus. Supporting data includes color images, scanning electron micrographs, genetic analysis of DNA barcodes, and comparative morphology of cognate species. The larva of *Isoperla lenati* Szczytko & Kondratieff, 2015 is also described supported by color images.

Key words: Plecoptera, Perlodidae, *Isoperla*, new species, southeastern, Nearctic

Introduction

Isoperla Banks, 1906 is a diverse plecopteran genus with 193 valid species known worldwide (DeWalt *et al*. 2023). Undescribed species of *Isoperla* continue to be found throughout the Holarctic region with at least one new taxon being described each year since 2008 with the exception of 2022 (DeWalt *et al*. 2023). In the southeastern USA, *Isoperla* is particularly speciose with 51 valid taxa. They can be found in habitats ranging from high altitude seeps to large Coastal Plain rivers (Szczytko & Kondratieff 2015). Larvae are usually elaborately patterned, with certain habitus types representing multiple species. Adults are typically distinguishable by genitalic structures that require careful preparation. For these reasons, and apparently high adaptive radiation, *Isoperla* continues to be a fruitful source of undescribed species.

In May 2018, (CJV) ran a light trap at Drowning Creek on the county line between Moore and Richmond counties, North Carolina, USA, a favorite collecting spot of his mentor Dr. Boris Kondratieff, renowned plecopterologist, and Dave Lenat, former biologist with the North Carolina Division of Water Resources, Biological Assessment Branch, Raleigh, North Carolina, USA. That evening 63 specimens of an undescribed *Isoperla* were collected, only two of which were males. Attempts to collect additional specimens were met with limited success; however, in searching several museum collections, as well as the Biological Assessment Branch collection, we found an additional 1700+ specimens. This ultimately made the description of *Isoperla riverae* **sp. n.** possible.

The new species was historically identified as *Isoperla dicala* Frison, 1942 given the superficial similarity of the two species as both larvae and adults. Males of *I. riverae* **sp. n.** and *I. dicala* possess a distinctively long vesicle, which separates them from all other Nearctic congeners. Larvae of the new species also resemble several other sympatric *Isoperla* species including *Isoperla lenati* Szczytko & Kondratieff, 2015 (described below) and *Isoperla davisi* James, 1974. Herein, we provide a description of this new species supported by color images, scanning electron microscope (SEM) photomicrographs, and maximum likelihood (ML) and pairwise genetic distance analyses based on mitochondrial cytochrome *c* oxidase subunit I (COI) fragment data.

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Methods

Adult stoneflies were collected using beating sheets, aerial nets, UV light traps, and hand-picking from riparian vegetation and substrates. Larvae were collected using a D-frame sweep-net and kicknet and were immediately preserved in 80–95% ethanol or reared to the adult stage using the methods described by Beaty *et al*. (2017). Adults were held for a period of time in ventilated jars to allow for complete sclerotization. Male aedeagi were everted using wide tipped forceps; specimens were then fixed in position by briefly submerging them in near boiling water and preserved in 80–95% ethanol.

Additional specimens were examined from the Illinois Natural History Survey, Champaign, Illinois (INHS), the North Carolina Division of Water Resources Biological Assessment Branch Collections (NCDWR), Raleigh, North Carolina, and the Virginia Museum of Natural History, Martinsville, Virginia (VMNH). Dr. Bill Stark, Mississippi College, Clinton, Mississippi also provided specimens. Location coordinates for legacy specimen records were georeferenced from museum label information using GEOLocate v. 3.22 (Rios & Bart 2010) and are indicated by "[ ]". Location coordinates for newly collected material were recorded directly using Topo Maps version 1.16 for iPhone. Plotting of coordinate data and map measurements were accomplished using ArcMap, ArcGIS 10.8.1. Level III/IV eastern Nearctic ecoregion data were obtained from the US Environmental Protection Agency (USEPA 2019) and drainage data were acquired from USGS StreamStats program 4.1.1 (U.S. Geological Survey 2016).

Specimens were stack-photographed using a Nikon D2900 mounted on a Wild M5-A. Composite images were assembled using Zerene Stacker version 1.04 (Zerene Systems LLC, Richland, Washington) and subsequently adjusted using Adobe Photoshop version 21.0.3. Adult male terminalia were prepared for SEM via serial dehydration in 90%, 95% and 100% ethanol for 10 minutes each and subsequently critical point dried. Ova were taken from an extruded egg mass and cleaned in 80% ethanol using a fine tipped paint brush and sonicated for 30 seconds to remove tissue adhering to the ovum surface. Dehydrated terminalia and ova were attached to aluminum stubs with double-stick tape and sputter-coated with gold-palladium. Coated specimens were imaged using a variable pressure Hitachi S3200N at North Carolina State University, Raleigh, North Carolina.

DNA extraction, amplification, and sequencing. Total genomic DNA was isolated from full or partial legs using a NucleoSpin 96 Tissue Core Kit (Machery-Nagel Inc., Allentown, PA, USA) following manufacturer protocols. A region of COI was amplified using the primer pairs LCO1490 and HCO2198 (Folmer *et al*. 1994) and mlCOIintF and jgHCO2198 (Leray *et al*. 2013). We conducted amplification in 10μL reactions comprising 25–100 ng template DNA, 5μL Promega Taq Master Mix, 1.5 μM $MgCl_2$, and 0.16 μM forward and reverse primers. The thermal profile for polymerase chain reaction (PCR) amplification included an initial denaturation at 95°C for 2 minutes, followed by 35 cycles of 95°C for 30 seconds, 30 seconds at the annealing temperature (LCO1490/HC02198: 46°C; mlCOIintF/jgHCO2198: 42°C), and 72°C for 1 minute, with a final extension of 72°C for 7 minutes. Amplified fragments were purified using Affymetrix ExoSAP-IT and were subsequently sequenced and run on an ABI3130 DNA Analyzer (Thermo Fisher Scientific).

Genetic data analysis. Sequences were reconciled, compiled, and aligned in Geneious Prime® 2023.0.2 using the auto algorithm of MAFFT v7.450 (Katoh *et al*. 2002; Katoh & Standley 2013), and default parameters (gap open penalty 1.53, offset value 0.123), adjusting for sequence direction. We examined all sequences for the presence of indels and subsequently translated into amino acids to verify an open reading frame and avoid the inclusion of pseudogenes or NUMTs into analyses. All sequences, including duplicates, were retained in the alignment for downstream analysis. Uncorrected pairwise distance (p-distance) matrices were calculated in MEGA v11.0.8 accounting for transitions and transversions (d) and uniform rates among sites, treating missing data via pairwise deletion (Tamura *et al*. 2021).

We used ModelFinder (Kalyaanamoorthy *et al*. 2017), implemented in IQTREE v2.1.3 (Minh *et al*. 2020; as "-m MFP+MERGE"), to find the best-fit partitioning strategy for our data using a heuristically optimized search and the Bayesian Information Criterion (Chernomor *et al*. 2016). The candidate pool of partition strategies included a partition for each codon position as well as a single partition for all data. The optimal partition-model scheme (first, second, and third codon positions each independent as TNe+G4, F81+F, and TN+F+G4, respectively) was automatically implemented in the subsequent ML phylogenetic tree reconstruction (Nguyen *et al*. 2015), quantifying clade support with 1000 ultrafast phylogenetic bootstraps (Hoang *et al*. 2018). We visualized the resulting majority rule (>50%) consensus tree using FigTree v1.4.4 (Rambaut 2018). Average pairwise tree distances for COI were calculated from the ML tree using the Species Delimitation plugin in Geneious.

The holotype and paratypes of *I. riverae* **sp. n.** are deposited in the National Museum of Natural History, Washington, DC (NMNH). Additional paratypes are deposited at INHS and NCDWR, and additional specimens are deposited at VMNH. Morphology and terminology generally follow that of Sandberg (2011) and Szczytko & Kondratieff (2015).

Results

Isoperla lenati Szczytko & Kondratieff, 2015

(Figs. 1–9)
Little Stripetail
http//lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:468919

Isoperla lenati Szczytko & Kondratieff 2015:137. Holotype male (USNM), Naked Creek, Richmond Co., North Carolina, U.S.A.

Distribution. U.S.A.—FL, GA, NC, SC. (DeWalt *et al*. 2023; Fig. 15)

Material examined. U.S.A.—North Carolina: Hoke Co., Flat Creek, Manchester Rd., 35.18244, -79.17695, 13 March 2019, emerged 21 March 2019, C. Verdone, S. Beaty, L. Housley, 2F, 2E (NCDWR); same location, 13 March 2019, emerged 25 March 2019, C. Verdone, S. Beaty, L. Housley, 1F, 1E (NCDWR); same location, 13 March 2019, emerged 26 March 2019, C. Verdone, S. Beaty, L. Housley, 1M (NCDWR); same location, 13 March 2019, emerged 29 March 2019, C. Verdone, S. Beaty, L. Housley, 1M, 2F, 3E (NCDWR); same location, 13 March 2019, emerged 11 April 2019, C. Verdone, S. Beaty, L. Housley, 2F, 2E (NCDWR); same location, 13 March 2019, C. Verdone, S. Beaty, L. Housley, 1M, 2F, 2E (NCDWR); same data, 1F, 1E (NCDWR); same data, C. Verdone, S. Beaty, L. Housley, 10L (NCDWR); Rockfish Creek, Plank Rd., 35.05861, -79.27907, 23 April 1990, NCDWR, 1L (NCDWR). **Hoke/Scotland Co.,** Drowning Creek, Turnpike Rd., 34.97414, -79.37785, 19 May 2018, C. Verdone, 2F (NCDWR); Lumber River, NC 401, 34.90081, -79.34833, 28 March 2006, D. Lenat, 3L (NCDWR); same location, 25 March 2011, D. Lenat, 2L (NCDWR); same data, 2L (NCDWR); same location, 20 May 2018, C. Verdone, 1M (NCDWR); same location, 17 April 2019, C. Verdone, V. Holland, 2M, 5F (NCDWR); same data, 1M, 1F (NCDWR); same location, 9 May 2020, C. Verdone, V. Holland, 6M, 4F (NCDWR). **Moore Co.,** Little River, Morrison Bridge Rd., 35.19233, -79.18408, 17 April 2019, C. Verdone, V. Holland, 4M, 7F (NCDWR); same data, 1L (NCDWR); unnamed tributary to Drowning Creek, SR 1140, 35.23711, -79.66511, 4 May 2006, E. Fleek, S. Beaty, 3L (NCDWR). **Moore/Richmond Co.,** Drowning Creek, SR 1102, 35.08426, -79.58270, 28 March 2019, S. Beaty, C. Verdone, V. Holland, L. Housley, 1M, 1E (NCDWR); same data, 1M, 1E (NCDWR); same data, 2M (NCDWR); same location, 28 March 2019, emerged 14 April 2019, S. Beaty, C. Verdone, V. Holland, L. Housley, 1M, 1E (NCDWR); same location, 14 April 2019, C. Verdone, D. Fuller, S. Beaty, 2F (NCDWR); same data, 3M (NCDWR); same location, 2015, D. Lenat, 2L (NCDWR). **Richmond Co.,** Big Branch, SR 1487, 35.02833, -79.63833, 2 May 2006, E. Fleek, S. Beaty, 1F, 1L (NCDWR); Naked Creek, SR 1003, 35.08197, -79.58909, 17 April 2019, C. Verdone, V. Holland, 2F (NCDWR); same location, 28 April 2019, S. Beaty, C. Verdone, 3F (NCDWR); same data, 1F (NCDWR).

Isoperla davisi James, 1974

(Figs. 10–14)
Alabama Stripetail
http//lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:468717

Isoperla davisi James 1974:996. Holotype male (INHS), Saugahatchee Creek, Tallapoosa Co., Alabama, U.S.A.
Isoperla coushatta Szczytko & Stewart 1976:211. Holotype male (INHS), Saddler Creek, Anderson Co., Texas, U.S.A. Syn. Szczytko & Kondratieff, 2015:73.
Isoperla coushatta: Szczytko & Stewart 1977:358.
Isoperla coushatta: Poulton & Stewart 1991:47.
Isoperla davisi: Szczytko & Kondratieff 2015:73.

Distribution. USA: AL, AR, DE, FL, LA, MO, MS, NC, OK, SC, TX, VA. (DeWalt *et al*. 2023)

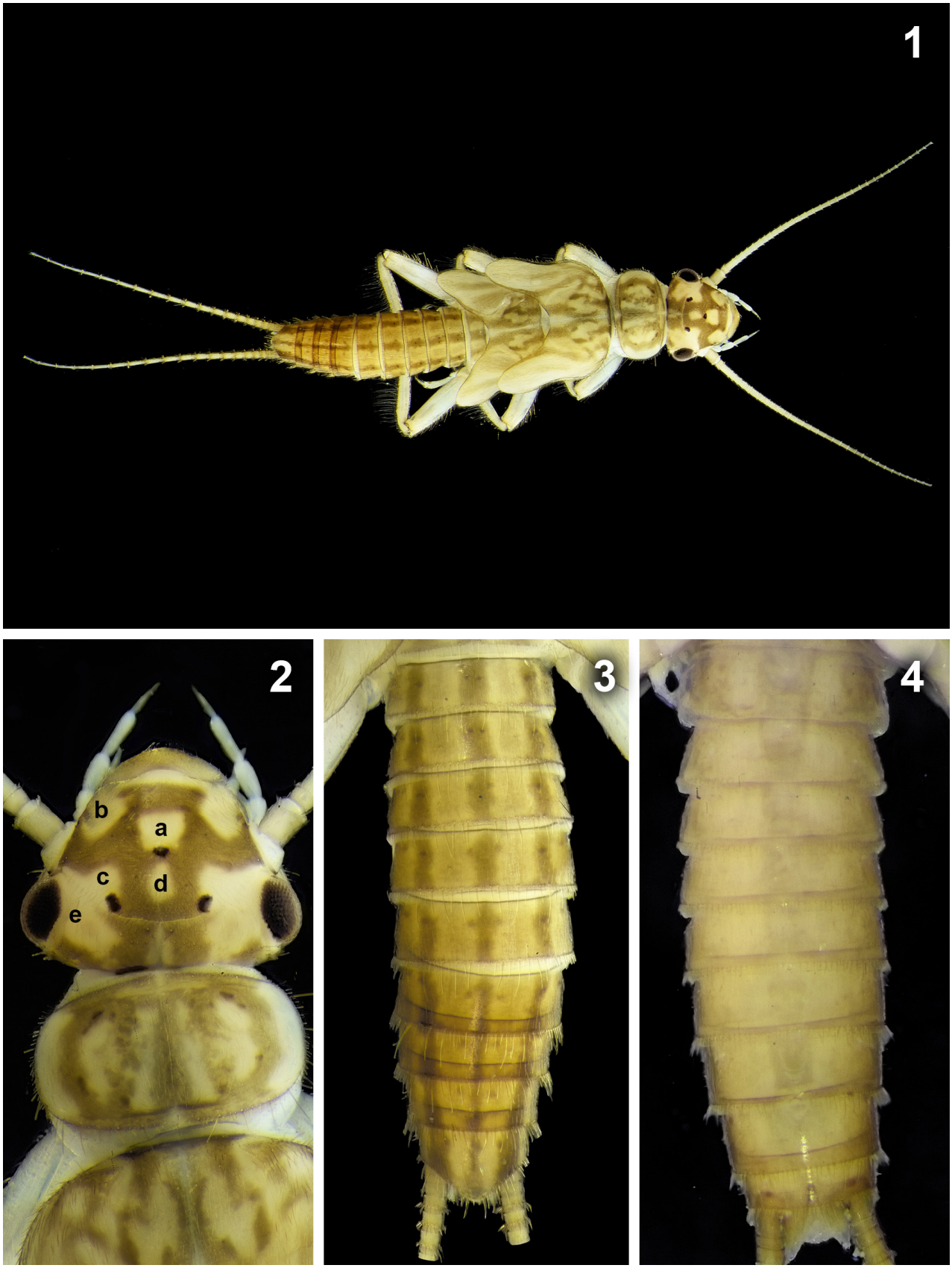
Material examined. U.S.A.—North Carolina: Alamance Co., Haw River, NC 87, 36.18250, -79.50944, 2 May 1985, T. MacPherson, 4L (NCDWR). **Bladen Co.,** Browns Creek, NC 87, 34.61339, -78.58489, 7 February 2008, S. Beaty, M. Walters, 6L (NCDWR). **Cleveland Co.,** Lick Branch, SR 2227, 35.17694, -81.50111, 17 March 1986, D. Penrose, 15L (NCDWR). **Edgecombe Co.**, Whiteoak Swamp, SR 1428, 36.00444, -77.62028, 3 May 1988, T. MacPherson, 1M, 13L (NCDWR); Whiteoak Swamp, NC 43, 36.00444, -77.62028, 5 February 2007, B. Crouch, B. Prusha, M. Walters, 9L (NCDWR). **Gaston Co.,** Beaverdam Creek, SR 1609, 35.40444, -81.24528, 5 May 2006, B. Prusha, M. Walters, 9L (NCDWR). **Guilford Co.,** Hickory Creek, SR 1132, 35.94027, -79.86888, 16 April 2003, K. Herring, B. Crouch, 10L (NCDWR). **Iredell Co.,** Patterson Creek, SR 1890, 35.92417, -80.82417, 4 April 2006, E. Fleek, T. MacPherson, 14L (NCDWR). **Moore Co.,** UT McLendons Creek, SR 1261, 35.34964, -79.47894, 28 March 2019, emerged 11 April 2019, S. Beaty, C. Verdone, V. Holland, L. Housley, 1M, 1E (NCDWR). **Moore/ Richmond Co.,** Drowning Creek, SR 1102, 35.08426, -79.58270, 28 March 2019, emerged 8 April 2019, S. Beaty, C. Verdone, V. Holland, L. Housley, 1M, 1E (NCDWR); same location, 28 March 2019, S. Beaty, C. Verdone, V. Holland, L. Housley, 1M, 1E (NCDWR); same location, 17 April 2020, S. Beaty, V. Holland, 2M, 2F (NCDWR); **Nash Co.,** Swift Creek, SR 1310, 36.11167, -77.92111, 24 April 2003, E. Fleek, T. MacPherson, 11L (NCDWR); **Richmond Co.,** Naked Creek, SR 1003, 35.08197, -79.58909, 12 March 2019, C. Verdone, V. Holland, S. Beaty, 1L (NCDWR); same location, 28 March 2019, emerged 4 April 2019, S. Beaty, C. Verdone, V. Holland, L. Housley, 2M, 2E (NCDWR); same location, 28 March 2019, S. Beaty, C. Verdone, V. Holland, L. Housley, 5L (NCDWR); **Stokes Co.,** Little Yadkin River, SR 1104, 36.29888, -80.41388, 6 May 1987, D. Penrose, 1L (NCDWR); **Wake Co.,** unnamed tributary to Middle Creek, Optimist Farm Rd., 35.65831, -78.73863, 23 April 2019, C. Verdone, D. Fuller, 2M (NCDWR); **Yadkin Co.,** North Deep Creek, SR 1510, 36.12583, -80.59222, 12 April 1993, D. Lenat, K. Herring, T. MacPherson, 11L (NCDWR); North Deep Creek, SR 1503, 36.17194, -80.67500, 12 April 1993, D. Lenat, K. Herring, T. MacPherson, 8L (NCDWR).

Adult male, female, and ovum. Described in Szczytko & Kondratieff (2015).

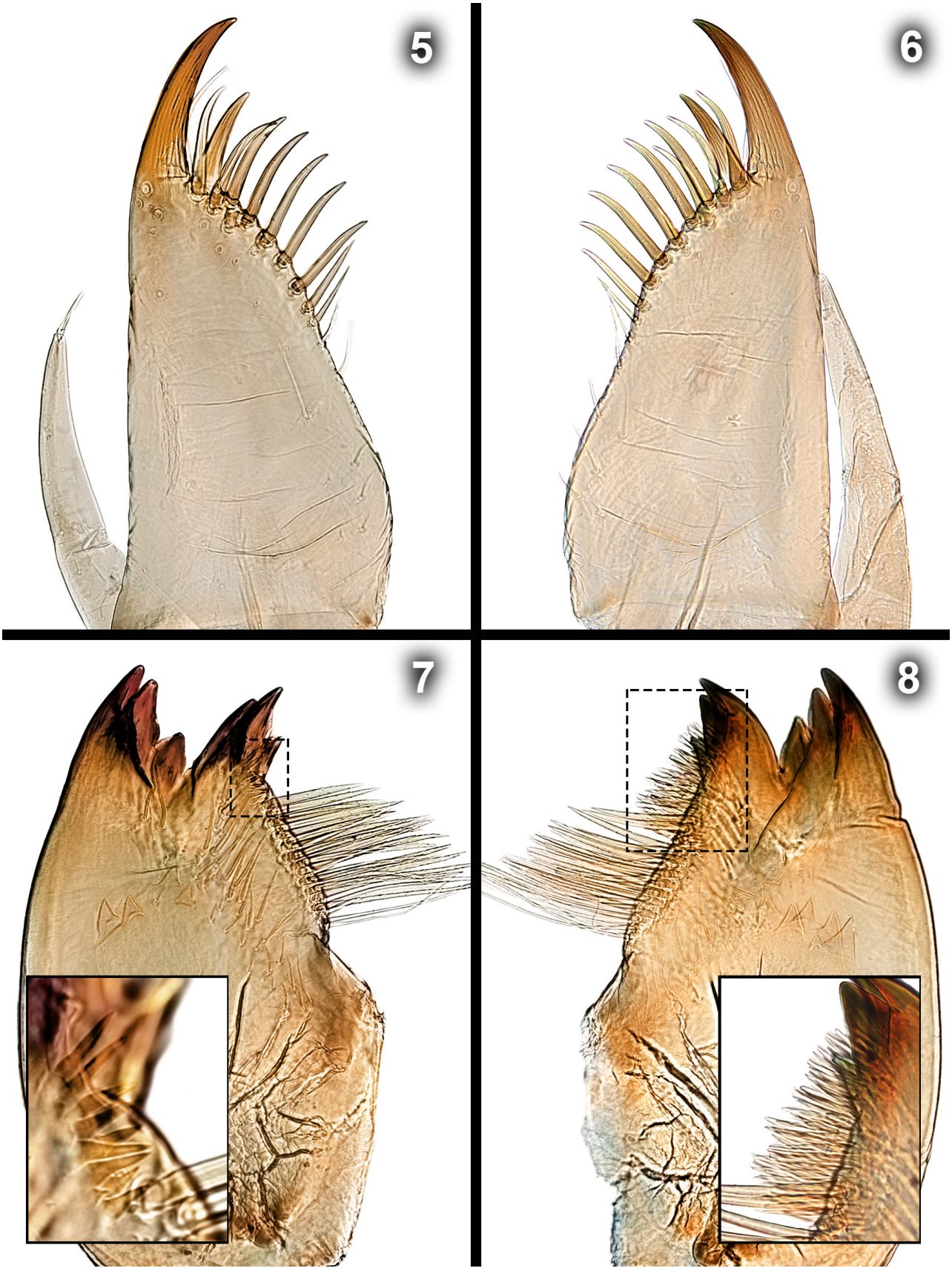
Mature larva (Figs. 1–4). Pre-emergent male larvae 4.9–5.5 mm (n = 6), female larvae 5.2–6.1 (n = 12). Body small and slender with contrasting body pattern (Fig. 1). Body usually with inconspicuous clear clothing hairs, some specimens with darker hairs.

Dorsum of head (Fig. 2). Ground color pale yellow with mottled brown mask and with a pale medial area anterior to median ocellus, a pair of large anterolateral pale spots, a pair of mediolateral pale spots, a small enclosed pale ocellar spot, and pale ocular areas; pale median area sub-trapezoidal and slightly wider anteriorly with anterior edge usually somewhat straight; fully enclosed interocellar spot triangular to subtriangular and reaching anterior ocellus, removed from lateral ocelli; frontoclypeus with additional semi-quadrate anterolateral pale areas; brown mask composed of an irregular median transverse stripe, lateral arms of stripe directed posteriorly from pale median area with an abrupt anterolateral turn to antennal base, similar to a bent arm; mask with posterior extensions to lateral ocelli; brown bar on anterior frontoclypeus with darker brown tentorial spots or dashes at vertices; area between eyes and lateral ocelli pale, mediolateral tentorial callosities brown, barely visible; occiput with irregular brown areas posterior to epicranial suture and on either side of the epicranial stem, stem overlaid with a triangular pale spot; an oblique brown stripe from the post-occipital margin reaching post ocular margin, spicules scattered along oblique stripe; labrum pale yellow with median area marginally darker, thinly edged with brown. Labrum yellow-brown, slightly darker basomedially. Antennal scape and pedicel yellow to yellow-brown, flagellum yellow with apical segments slightly darker; flagellum with 28–31 antennomeres (n=11); each segment with an apical circlet of small spines. Pale to brown clothing hairs covering head.

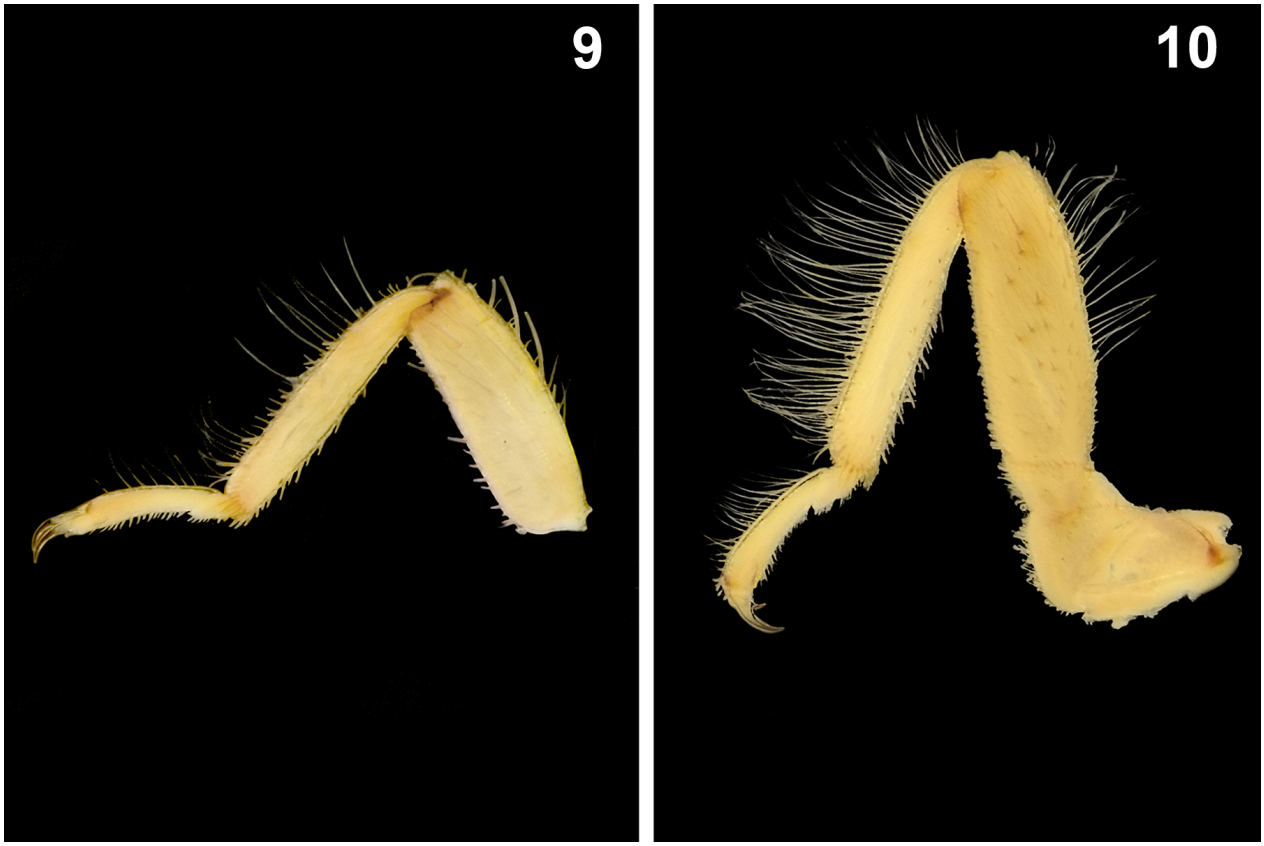
Maxilla (Figs. 5–6). Lacinia bidentate, receding evenly from base to subapical tooth; a long thin marginal seta between apical and subapical tooth present, and often a second shorter seta present at base of subapical tooth; 6–7 striated, stout, socketed marginal setae below subapical tooth, setae progressively thinner and shorter towards base; anterior marginal setae barely smaller than subapical tooth giving lacinia an overall comb-like appearance; 2–3 thin, unevenly-spaced setae just below last marginal seta on palm edge; a submarginal row of 3 closely set striated, stout, socketed setae below apical tooth, a fourth smaller seta in-line but slightly removed posteriorly; ventral face of palm with 3–4 setae, some bifurcate at tip, most set away from palm edge. Length of striated apical tooth of lacinia approximately 1/3 the palm length (0.38X) and 2/3 the basal palm width (0.64–0.72X). Subapical tooth about 2/3 (0.63–.68X) the length of the apical tooth. Galea about 0.9X lacinial palm length, with a sparse ventral row of thin setae and tipped with 1–3 apical long spines. Maxillary palp stout, about 1.5X (1.53–1.58X) length of lacinia; segments 1–4 successively longer, segment 5 subequal to segment 3; palp segments 1 and 2 with transverse row of spinous setae at apex; segment 3 with 1–2 spines on body of segment and 1–2 apical spines, segment 5 tipped with 1–2 sensillae.



FIGURES 1–4. *Isoperla lenati,* larva*,* Flat Creek, Hoke Co., North Carolina. 1. Habitus, dorsal. 2. Head and pronotum, dorsal, (a) pale medial area anterior to median ocellus, (b) anterolateral pale spots, (c) mediolateral pale spots, (d) enclosed pale ocellar spot, (e) pale ocular areas. 3. Abdomen, dorsal. 4. Abdomen, ventral.



FIGURES 5–8. *Isoperla lenati,* larva, Rockfish Creek, Hoke Co., North Carolina. 5. Right lacinia, ventral. 6. Left lacinia, ventral. 7. Right mandible, ventral; (inset) acanthae. 8. Left mandible, ventral; (inset) a dense brush of short spinous setae.



FIGURES 9–10. 9. *Isoperla lenati,* foreleg, dorsal, Flat Creek, Hoke Co., North Carolina. 10. *Isoperla davisi,* foreleg, dorsal, Sandy Creek, Randolph Co. North Carolina.

Mandibles (Figs. 7–8). Right mandible (Fig. 7) bicuspid, outer cusp with three teeth, ventral tooth largest, dorsal tooth smallest, tooth serrations indistinct or worn; ventral surface of mandible with an unorganized band of long spinous setae from base to outer ventral tooth, setae nearest ventral tooth thicker and with setules and serrations; inner cusp with three teeth, middle tooth longest and largest; a comb-like row of small sharply pointed acanthae at base of basal tooth, number and size of acanthae variable (Fig. 7 inset); molar ridge with a dense row of spine-like setae, setae longer and thinner towards base of mandible; dorsal surface of mandible also with band of unorganized serrated setae from inner cusp towards base of mandible, setae near cusp larger and with setules. Left mandible (Fig. 8) similar to right mandible except comb-like row of sharply pointed acanthae at base of inner cusp replaced by a dense brush of short spinous setae from basal tooth on inner cusp to molar ridge (Fig. 8 inset).

Thorax (Figs. 1–2). Pronotum (Figs. 1–2) about twice as wide as long, extensive brown patterning consisting of two brown "O" shaped markings with pale centers, one on each disk, and connected by a narrow anterior transverse band; a brown stripe along the medial suture, widest posteriorly, joins to an anterior transverse brown bar forming a "T"; brown pronotal markings encompass many small dark rugosities; a narrow pale stripe on either side of the brown median stripe; pronotal flange edged in brown on anterior and posterior margins, narrowly interrupted medially, widely pale laterally; about 1/3 of anterior pronotal furrow pale; pronotum edged with closely set row of stiff setae, with longer bristles interspersed, the longest on lateral and posterior corners and approximately 1/8 the width of pronotum. Mesonotal and metanotal discs with irregular brown longitudinal markings (Fig. 1); wing pads yellow-brown with one (mesonotum) or two (metanotum) faint longitudinal brown bands, inner stripe of metanotum widest posteriorly; lateral margins of nota fringed with a short, closely set row of stiff bristles, with intervening long bristles truncate, mesonotum additionally with a cluster of up to 10 longer stiff bristles on the anterolateral corners. Thoracic nota with sparse light brown to pale clothing hairs. Lateral aspect of thorax pale yellow with notal trochantins brown to dark brown at apex; procoxal process conspicuous, dark brown. Thoracic sterna pale, without distinctive markings; basisterna with short clear setae; furcal pits conspicuous, Y-stem arms inconspicuous. Entire sternum with pale to light brown clothing hairs.

FIGURES 11–14. *Isoperla davisi,* larva, Lumber River Hoke/Scotland Co., North Carolina. 11. Habitus, dorsal. 12. Head and pronotum, dorsal. 13. Abdomen, dorsal. 14. Abdomen, ventral.

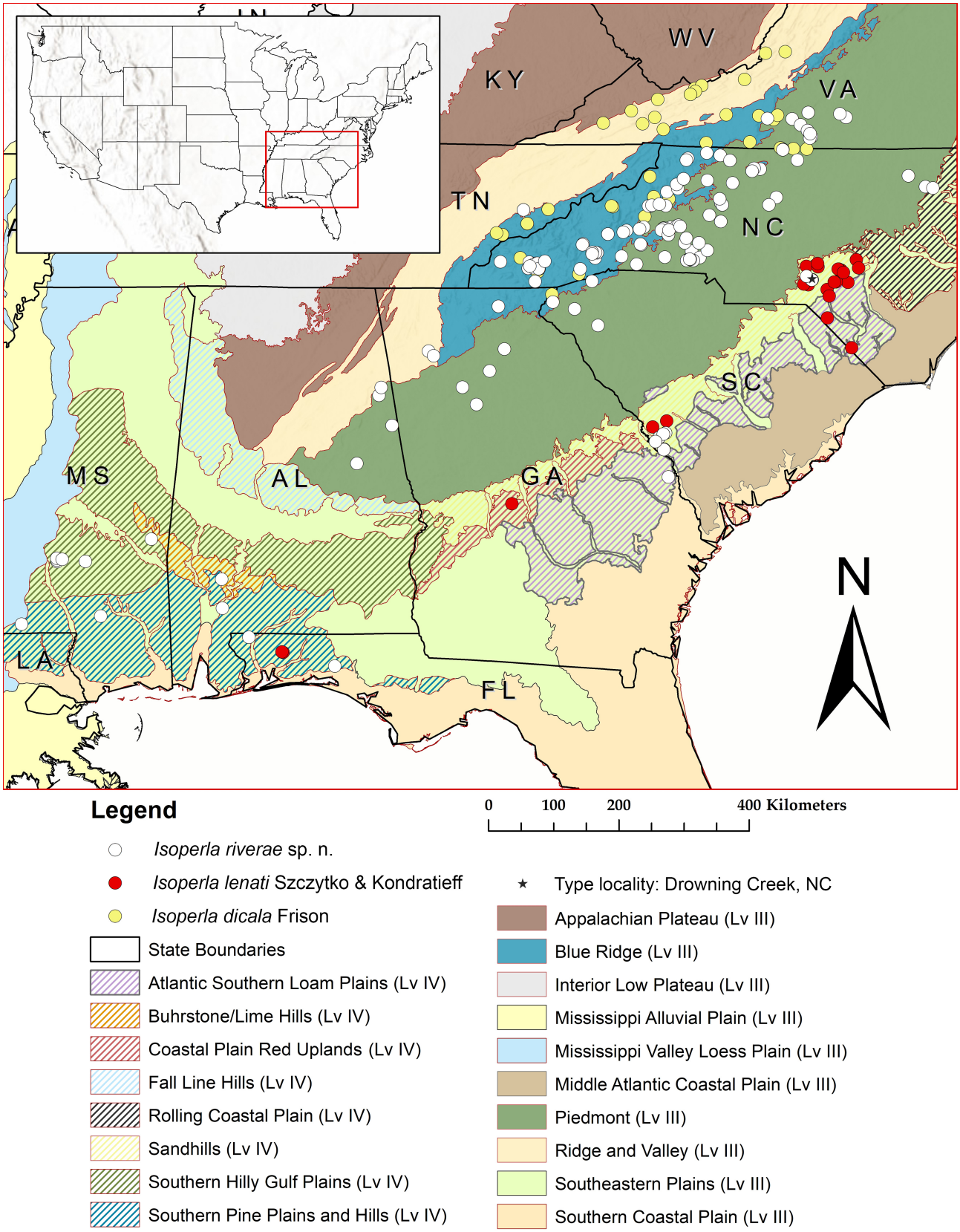


FIGURE 15. Distribution of *Isoperla riverae* **sp. n.** and examined material of *I. dicala* and *I. lenati.* Records of *I. lenati* from Szczytko & Kondratieff are also included.

Legs (Fig. 9). Pale yellow to yellow-brown overall. Femoral-tibial ratio 0.9 to 1.0 for each leg (n = 12). Posterior face of femora with a sparse and uneven medial row of stout golden spines; anterior face of femora with golden spines scattered along length, with a row of short spines along posterior edge of sclerite; dorsal edge of femora with a cluster of bristles in apical half, longest bristles about half the width of corresponding femur, many bristles truncate; femora with silky setae very sparse to absent (Fig. 9). Tibiae with two longitudinal dorsal rows of short spines and a sparse dorsal row of long silky setae along length, setae twice as long as width of tibia; tibiae also with two ventral rows of spines. Tarsi pale yellow-brown with a comb-like ventral row of stiff setae and a sparse dorsal row of silky setae. Two claws darker apically, moderately long and gradually curved.

Abdomen (Figs. 1, 3–4). Terga yellow-brown with three dark brown longitudinal stripes, two lateral and one median, often bordered by obscure pale lines (Figs. 1, 3); anterior half of each segment with a transverse row of eight small, variably dark dots, obscure in some specimens; posterior edges of each tergal sclerite bearing a transverse row of golden spines interspersed with long truncate to slightly clavate bristles, longest bristles about ¾ length of respective segment; terga also with a few curved erect setae on body of sclerite. Abdominal sterna (Fig. 4) yellow-brown, darker than thoracic sterna; posterior edges of sterna bearing a transverse row of short golden spines, successively longer on distal segments; first and last dot of dorsal transverse row visible in ventral aspect on segments 9 and 10. Clothing hairs clear to light brown. Paraprocts triangular, pointed, 1.3–1.5X as long as wide. Cerci yellow-brown; each cercal segment with an apical circlet of spines, and bearing one long ventral spine about 2/3 length of respective segment and one dorsal spine about ½ length of segment; distal third of cerci with a sparse setal fringe, bearing 1–3 fine dorsal setae.

Diagnosis. The larval habitus of *I. lenati* is defined by the following combination of characters: small body size (4.9–6.1 mm, n = 12) ; head pattern with a pale medial area anterior to median ocellus with a straight anterior margin, a pair of large anterolateral pale areas, a small enclosed pale ocellar spot, a pair of mediolateral pale spots that are confluent with the lateral ocelli and pale ocular areas; each pronotal disc with a brown "O" marking; femora with a sparse to absent fringe of silky setae and with long truncate bristles in apical half; three dark longitudinal abdominal stripes bordered by pale lines. Additionally, the overall habitus of this species appears quite bristly.

Taxonomic notes. The larva of *I. lenati* is superficially most similar to *I. davisi* (Figs. 10–14), with which it is sometimes sympatric. Both taxa have striped abdomens (Figs. 3, 13) and the same configuration of markings on the head and pronotum (Figs. 2, 12). The diagnostic characters listed above differ from *I. davisi* in the following: pale medial area anterior to median ocellus is pointed anteriorly in *I. davisi* (Fig. 11–12), femora of *I. davisi* have long silky setae (Fig. 10), and the abdominal stripes have more pronounced dark submedial stripes (Fig. 13).

Isoperla riverae sp. n. Verdone, Beaty, Holland & Williams
(Figs. 16–39)

Isoperla dicala: Stark & Gaufin, 1978:401
Isoperla dicala: Szczytko & Kondratieff, 2015:88 (in part)

Etymology. The new species is named for the senior author's daughter. May she enjoy the splendor of the natural world and value all its inhabitants. The proposed common name is "River's Stripetail".

Distribution. U.S.A.—AL, FL, GA, NC, MS, SC, TN, VA (Fig. 15)

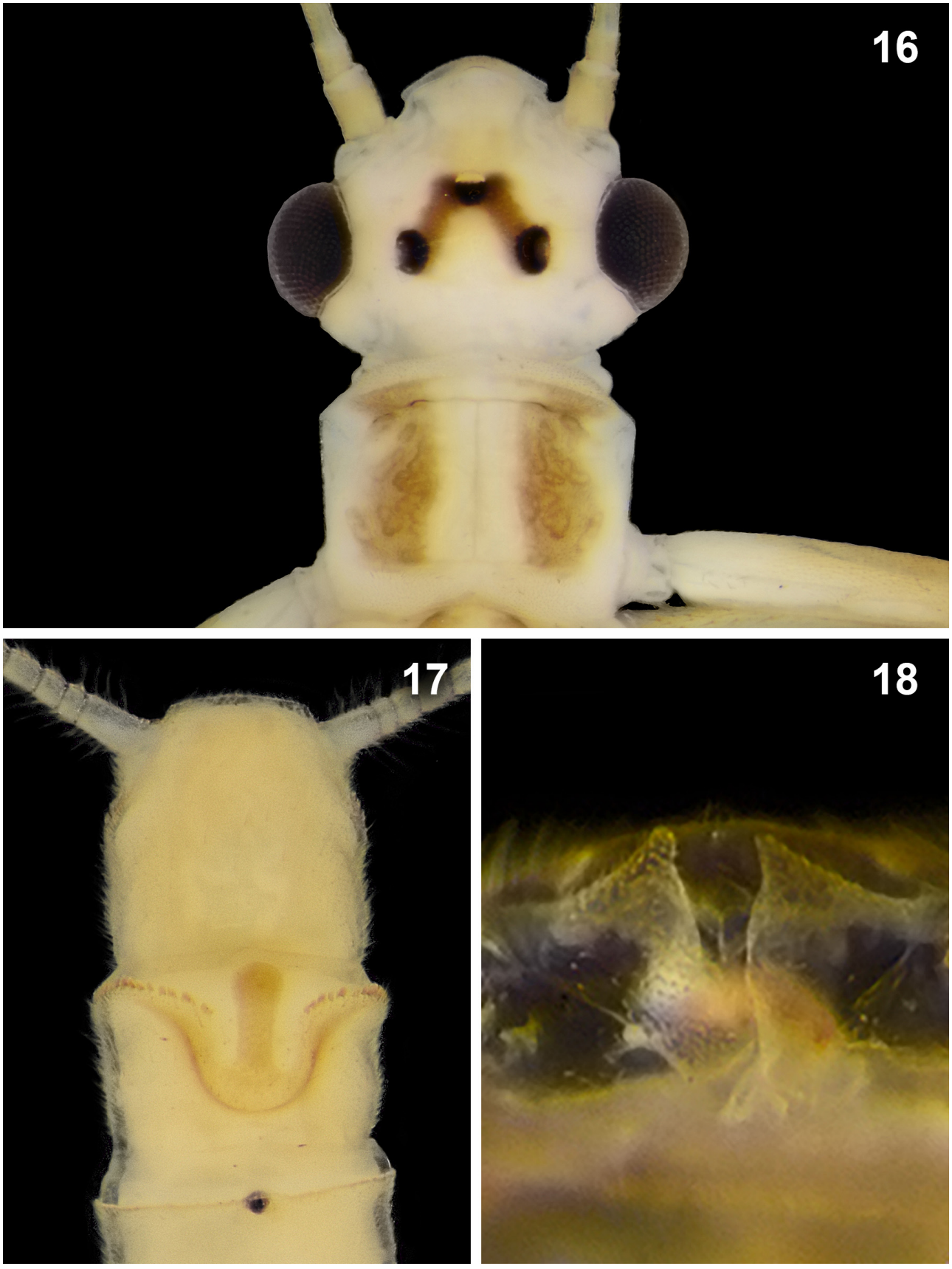
Material examined. *Holotype* M: **U.S.A.—North Carolina: Moore/Richmond Co.**, Drowning Creek, SR 1102, 35.08426, -79.58270, 28 April 2019, emerged 6 May 2019, C. Verdone, S. Beaty (NMNH). *Paratypes*: **Georgia: DeKalb Co.,** Snapfinger Creek, 5 mi SE of Panthersville, [33.66552, -84.21116], 4 June 1945, P.W. Fattig, 9M (INHS, Insect Collection 875867). **Gwinnett Co.,** Ivy Creek, 5 mi NS Buford, [34.04788, -84.03133], 11 June 1945, P.W. Fattig, 2M (INHS, Insect Collection 875931). **North Carolina: Caldwell Co.,** Johns River, Old Johns River Rd., [35.93359, -81.69053], 17 May 2004, B. Kondratieff, D. Lenat, R.F. Kirchner, R. Zuellig, 1M (NCDWR); same location, 20 May 2019, C. Verdone, S. Beaty, V. Holland, 1M (NCDWR). **Henderson Co.,** French Broad River, Hwy 64, [35.32853, -82.57524], 23 May 1990, B. Kondratieff, R.F. Kirchner, 5M, 3F (NMNH). **Moore Co.,** Drowning Creek, SR 1004, 35.06575, -79.54998, 28 April 2022, emerged 4 May 2022, C. Verdone, S. Beaty, V. Holland, 1M (NCDWR). **Moore/Richmond Co.,** Drowning Creek, SR 1102, 35.08426, -79.5827, 19 May 2018, C. Verdone, 2M, 61F (NCDWR); same location, 28 April 2019, emerged 6 May 2019, C. Verdone, S. Beaty, 1M, 1E (NCDWR); same location, 28 April 2019, emerged 6 May 2019, C. Verdone, S. Beaty, 2M (NCDWR);

same location, 28 April 2019, emerged 13 May 2019, C. Verdone, S. Beaty, 1M, 1E (NCDWR). same location, 28 April 2019, emerged 13 May 2019, C. Verdone, S. Beaty, 1F (NCDWR). **Richmond Co.,** Naked Creek, SR 1003, 35.08197, -79.58909, 27 April 2012, emerged 30 April 2012, D. Lenat, 1F,1E (NCDWR); same location, 27 April 2012, emerged 5 May 2012, D. Lenat, 2F, 2E (NCDWR); same location, 28 April 2019, C. Verdone. S. Beaty, 1F, 1E, 3L (NCDWR); same location, 30 April 2019, C. Verdone, S. Beaty, 1F, 1E (NCDWR); same location, 9 May 2020, C. Verdone, V. Holland, 1M, 1F (NCDWR). **Rockingham Co.,** Mayo River, SR 1358, 36.53483, -79.99086, 11 May 2018, C. Verdone, S. Beaty, V. Holland, 1M (NCDWR); same location, 15 May 2018, C. Verdone, S. Beaty, V. Holland, L. Housley, 2M (NCDWR); same location, 21 May 2019, V. Holland, 1M (NCDWR). **South Carolina: Aiken Co.,** Upper Three Runs, Savannah River Site Rd. C, [33.28518, -81.69533], 5 May 1984, B. Kondratieff, 29M, 8F (NCDWR). **Virginia: Franklin Co.,** Big Chestnut Creek, Rte 718 E, [36.87630, -79.86254], 21 May 2002, B. Kondratieff, R.F. Kirchner, 2M (NMNH).

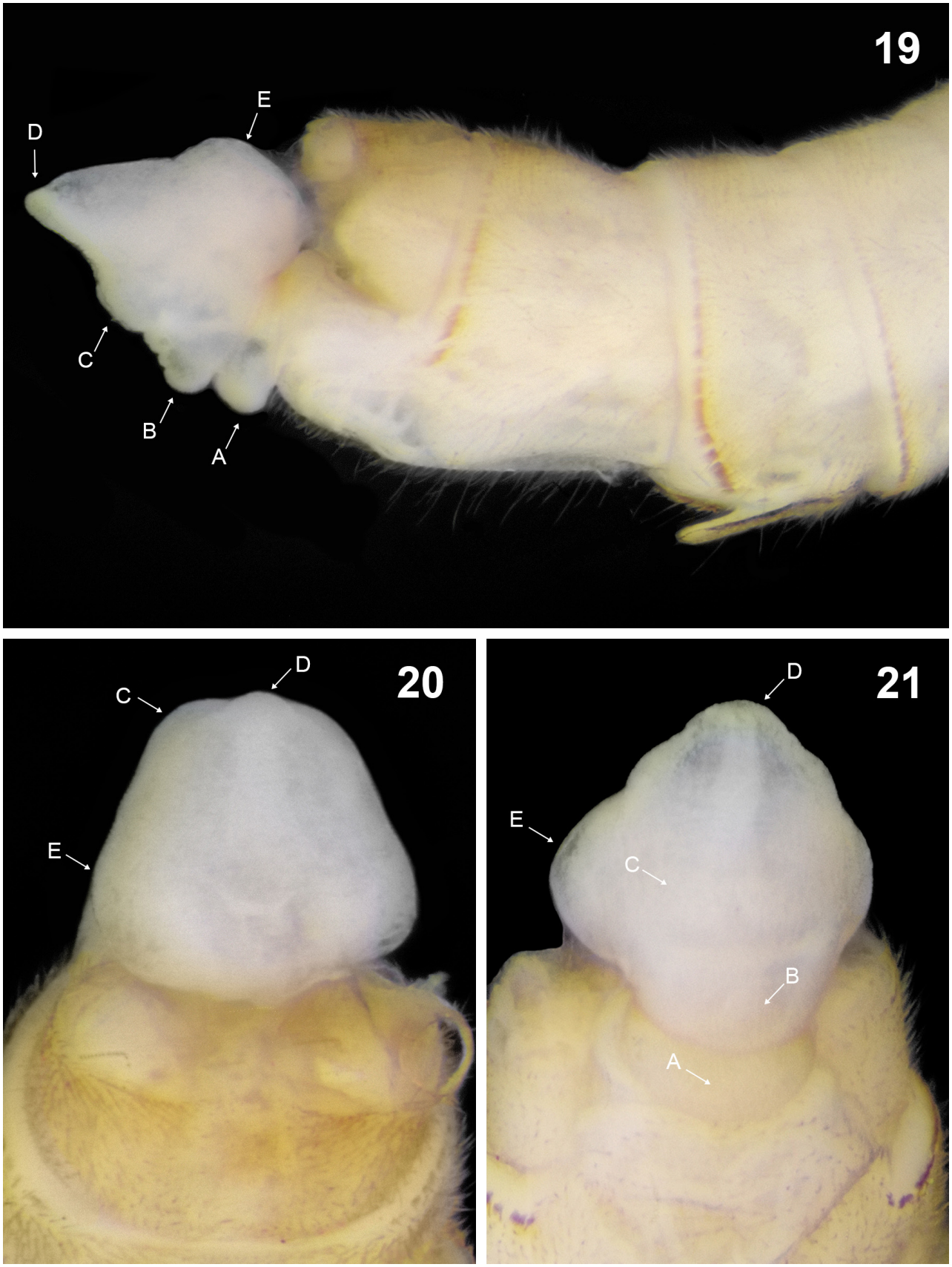
Additional material examined. U.S.A.—Alabama: Clarke Co., Fishers Creek, nr Whatley, [31.63982, -87.71937], 8 May 1986, S. Harris, P. O'Neil, 44M, 11F (NCDWR). **Cleburne Co.,** Shoal Creek, Shoal Creek Campground, [33.77071, -85.55572], 25 May 1984, S. Harris, P. Lago, 4M, 2F (NCDWR); South Fork Terrapin Creek, Hwy 55, [33.86017, -85.52440], 25 May 1984, S. Harris, P. Lago, 3M, 1F (NCDWR)**. Monroe Co.,** Little River, Hwy 59, [31.29888, -87.71048], 11 May 1982, S. Harris, 1M (NCDWR). **Randolph Co.,** Cutnose Creek, Hwy 48, [33.42541, -85.37436], 20 May 1984, S. Harris, P. Lago, 1M, 2F (NCDWR). **Tallapoosa Co.,** Josie Leg Creek, off Hwy 22, [32.98634, -85.85926], 19 June 1983, S. Harris, P. Lago, 1M (NCDWR). **Florida: Escambia Co.,** Canoe Creek, above Bratt Rd., [30.95664, -87.34758], 24 April 2009, D. Ray, 1M (NCDWR). **Walton Co.,** Alaqua Creek, Base Rd. 201, Eglin Air Force Base, [30.61667, -86.16444], 17 April 2006, R.W. Flowers, M.L. Pescador, A.K. Rasmussen, B.A. Richard, 10M (NCDWR). **Georgia: Bartow Co.,** Clear Creek, 6 mi N Cartersville, [34.27560, -84.87250], 3 June 1939, P.W. Fattig, 1M (INHS, Insect collection 875885); Pettit Creek, 2 mi N Cartersville, [34.22127, -84.80251], 3 June 1939, P.W. Fattig, 2F (INHS, Insect Collection 875855). **Fulton Co.,** Nancy Creek, 8 mi N Atlanta, [33.86331, -84.40475], 26 June 1945, P.W. Fattig, 3F (INHS, Insect Collection 875853). **Hall Co.,** no waterbody data, Gainesville, [34.29778, -83.82417], 9 May 1944, T.H. Frison, H.H. Ross, 1F (INHS, Insect Collection 875862). **Screven Co.,** Brier Creek, 7 mi NE Sylvania, [32.83172, -81.56612], D.C. Scott, 1M (INHS, Insect Collection 875895). **Union Co.,** Wolf Creek, 3.5 mi N Neel[s] Gap, [34.78515, -83.90730], 30 May 1943, P.W. Fattig, 3M (INHS, Insect Collection 875891); same location, 8 June 1945, P.W. Fattig, 5M (INHS, Insect Collection 875875). **Mississippi: Clarke Co.,** Moore Mill Creek, Clarkco State Park, [32.10818, -88.69334], 26 May 1990, Tanner, Parham, 1F (NCDWR). **Lamar Co.,** Sandy Run, Okahola Rd., [31.20737, -89.38775], 13 May 1992, B. Stark, M. Hicks, 2F (NCDWR). **Pike Co.,** Tangipahoa River, 3 mi Magnolia, [31.11275, -90.47943], 25 March 1977, B. Stark, 2M, 1F (NCDWR). **Simpson Co.,** Mill Creek, Bushtowns Shiver Rd., [31.84672, -89.98869], 12 May 1992, B. Stark, E. Stark, C. Bishop, 1M, 5F (NCDWR); Mill Creek, Hwy 472, [31.86365, -89.99195], 3 May 1990, B. Stark, 2M, 5F (NCDWR); Strong River, 3 mi W of Pinola, Hwy 28, [31.88622, -89.99349], 22 June 1988, S. Szczytko, 1M (NCDWR); Westville Creek, Jake Barnes Rd. blw. Hale Branch jct., [31.87234, -89.91891], 13 April 1991, B. Stark, 6M, 3F (NCDWR). **Smith Co.,** Clear Creek, 3 mi SW Mize [31.84949, -89.59955], 24 April 1991, B. Stark, D. Tanner, 12F (NCDWR). **North Carolina: Alleghany Co.,** Glade Creek, SR 1422, nr mouth, 36.50000, -81.03639, 20 June 2008, S. Beaty 15L (NCDWR). **Ashe Co.,** Cranberry Creek, SR 1609, 36.4028, -81.29833, 13 May 1998, D. Lenat, 1L (NCDWR); Peak Creek, Off SR 1599, 350M upstream Ore Knob Branch, 36.42083, -81.31944, 15 April 1991, D. Penrose, 1L (NCDWR); Cranberry Creek, SR 1609, 36.44028, -81.29833, 13 May 1998. D. Lenat, 1L (NCDWR). **Buncombe Co.,** South Hominy Cr, Davis Creek Rd., 35.47831, -82.75003, 2 June 2010, V. Holland, 1L (NCDWR); South Hominy Creek, Dale Pen Rd., 35.48817, -82.74259, 1 June 2010, V. Holland, 1L (NCDWR); [Swannanoa River], Swannanoa, 35.59789, -82.39984], 19 June 1938, T.H. Frison, T.H. Frison Jr., 2M (INHS, Insect Collection 875897); same data, 2M (INHS, Insect Collection 875851). **Burke Co.,** Black Fox Cr, SR 1917, 35.66417, -81.63278. 19 April 1989, T. McPherson, 15L (NCDWR); Henry Fork, NC 18, 35.67361, -81.53611, 20 April 1988, D. Lenat, 25L (NCDWR); Henry Fork, SR 1803, 35.68944, -81.45028, 1 May 2006, B. Crouch, 15L (NCDWR); Henry Fork SR 1854, 35.69556, -81.42722, 1 May 2006, B. Crouch, 16L (NCDWR); Henry Fork, SR 1916, 35.66167, -81.60361, 2 May 2006, B. Crouch, 10L (NCDWR); Henry Fork, SR 1922, 35.66167, -81.63694, 19 April 1988, T. McPherson, 15L (NCDWR); same location, 2 May 2006, T. Mormon, 11L (NCDWR); Ivy Creek, SR 1919, 35.64111, -81.66167, 19 April 1988, D. Lenat, 1L (NCDWR); Jacob Fork, SR 1924, 35.59056, -81.56722, 2 May 2006, B. Crouch, 6L (NCDWR); Little Fork, FSR 228, 35.91056, -81.81833, 19 May 2015, M. Walters, 1L (NCDWR); Rock Creek, SR 1915, 35.64167, -81.55750, 19 April 1988, D. Lenat, 2L (NCDWR); Steels Creek, at FR 228, [35.912264, -81.838327] 25 June 1988, S. Dunkle,

1M (NCDWR); Upper Creek, FSR 982 upstream of Timbered Br, 35.92000, -81.79306, 13 June 1994, D. Penrose, 1L (NCDWR). **Caldwell Co.,** Abingdon Creek, SR 1315, 35.91344, -81.60104, 18 April 2011, T. Mormon, 1L (NCDWR); Yadkin River, off SR 1372 downstream of Jackson Camp Creek, 36.06895, -81.58553. 23 May 2011, D. Black, E. Fleek, S. Beaty, 2L (NCDWR). **Catawba Co.,** Henry Fork, SR 1124, 35.68417, -81.40278. 1 May 2006, B. Crouch, 16L (NCDWR). **Cleveland Co.,** Brushy Creek, SR 1323, 35.33972, -81.61580, 16 May 1995, N. Guthrie, 1L (NCDWR). **Davidson Co.,** Abbott Creek, SR 1735, 36.00833, -80.0775, 10 May 2006, D. Black, S. Beaty, B. Prusha, 4L (NCDWR); Brushy Fork. SR 1810, 35.92444, -80.18028, 14 May 2003, T. Mormon, 9L (NCDWR). **Franklin Co.,** Sandy Creek, US 401, 36.24806, -78.25833, 3 May 1988, T. McPherson, 3L (NCDWR). **Gaston Co.,** Beaverdam Creek, SR 1609, 35.40444, -81.24528, 5 May 2006, B. Crouch, B. Prusha, M. Walters, 15L (NCDWR); Hoyle Creek, SR 1836, 35.33472, -81.13389, 4 May 2006, B. Crouch, 2L (NCDWR); Long Creek, SR 1405, 35.30194, -81.31333, 4 April 1994, N. Guthrie, 1L (NCDWR); same location, 18 April 1995, D. Penrose, 1L (NCDWR); Long Creek, NC 275, 35.30222, -81.22278, 5 April 1991, L. Eaton, 1L (NCDWR); same location, 1 April 1992, D. Lenat, 1L (NCDWR); same location, 19 April 1995, D. Penrose, 1L (NCDWR); Long Creek, NC 274, 35.30778, -81.30333, 4 April 1994, D. Penrose, 1L (NCDWR); same location, 18 April 1995, D. Penrose, 2L (NCDWR); Long Creek, SR 1448, 35.31222, -81.26472, 4 April 1994, K. Herring, 1L (NCDWR), Long Creek, SR 1446, 35.31639, -81.27194, 5 April 1994, D. Penrose, 2L, (NCDWR); UT Long Creek, SR 1446, 35.31750, -81.27194, 5 April 1994, N. Guthrie, 1L (NCDWR). **Graham Co.,** Little Snowbird Creek, off SR 1115, 35.28697, -83.87841, 4 June 2014, M. Walters, 1L (NCDWR). **Henderson Co.,** Mills River, SR 1353, 35.38778, -82.54611, 2 May 2011, T. Mormon, 2L (NCDWR); same location, 23 April 2012, E. Fleek, 1L (NCDWR); Mills River, NC 191/280, 35.39139, -82.56806, 2 June 2009, T. Mormon, E. Fleek, M. Walters, 4L (NCDWR); North Fork Mills River, SR 1343, 35.39425, -82.61503, 3 May 2011, T. Mormon, 1L (NCDWR); South Fork Mills River, SR 1340, 35.37569, -82.6144, 1 June 2009, T. Mormon, E. Fleek, M. Walters, 1L (NCDWR). **Iredell Co.,** Olin Creek, SR 1891, 35.94472, -80.84444, 9 May 2006, D. Black, B. Prusha, S. Beaty, 3L (NCDWR); Patterson Creek, SR 1890, 35.92417, -80.82417, 4 April 2006, E. Fleek, 2L (NCDWR); Rocky Creek, SR 1854, 35.99139, -80.85944, 5 June 2003, D. Lenat, 4L (NCDWR); Third Creek, SR 2318, 35.73889, -80.84444, 21 June 1989, D. Lenat, 1L (NCDWR); Third Creek, SR 1521, 35.80917, -81.00222, 8 May 2006, S. Beaty, D. Black, 1L (NCDWR). **Jackson Co.,** [Savannah Creek] Fort Tatham Campground, [US 441], [35.29354, -83.26746], 8 July 1981, B.C. Kondratieff, 1F (INHS, Insect Collection 875913). **Lincoln Co.,** Howards Creek, SR 1200, 35.49833, -81.34056, 3 May 2006, B. Crouch, 2L (NCDWR); Killian Creek, SR 1511, 35.41583, -81.02889, 8 June 1994, D. Lenat, 2L (NCDWR); Leepers Creek, SR 1354, 35.49861, -81.14619, 8 May 2006, B. Prusha, 4L (NCDWR); Pott Creek, SR 1217, 35.55194, -81.31889, 3 May 2006, T. Mormon, 1L (NCDWR). **Macon Co.,** Burningtown Creek, Tellico Rd, 35.28277, -83.48240, 6 May 2008, T. Mormon, 1L (NCDWR); Caler Fork, off Ruby Mine Rd, 35.26860, -83.38656, 7 May 2008, R. Thorpe, 1L (NCDWR); Coon Creek, US23/441, 35.23033, -83.34921, 9 May 2008, T. Mormon, 1L (NCDWR); Cowee Creek, Leatherman Gap Rd, 35.27046, -83.39047, 8 May 2008, R. Thorpe, 3L (NCDWR); Cowee Creek, NC 28, 35.2625, -83.40944, 23 May 2007, D. Black, 1L (NCDWR); Downes Branch, SR 1392, 35.21263, -83.49833, 28 April 2011, V. Holland, 1L (NCDWR); Iotla Branch, SR 1387, 35.22425, -83.42923, 7 May 2008, R. Thorpe, 3L (NCDWR); Iotla Creek, SR 1385, 35.21887, -83.42293, 7 May 2008, C. Tyndall, 2L (NCDWR); Iotla Creek, off SR 1378, 35.23473, -83.39824, 24 May 2007, D. Black, 2L (NCDWR); same location, 7 May 2008, T. Mormon, 1L (NCDWR); Mica City Creek, SR 1346, 35.2696, -83.37045, 25 April 2011, V. Holland, 3L (NCDWR); North Fork Coweeta Creek, SR 1114, 35.06525, -83.40253, 17 May 2011, V. Holland, 2L (NCDWR); Rabbit Creek, off SR 1504, 35.20690, -83.34836, 8 May 2008, R. Thorpe, 12L (NCDWR); Rabbit Creek, SR 1504, 35.20837, -83.35209, 8 May 2008, T. Mormon, 3L (NCDWR); Younce Creek, Younce Creek Rd., 35.23775, -83.47404, 26 April 2011, V. Holland, E. Fleek, S. Beaty, 1L (NCDWR). **McDowell Co.,** Catawba River, SR 1273, 35.61944, -82.18833, 18 April 1985, NCDWR, 1L (NCDWR); Catawba River, SR 1234, 35.63694, -82.14389, 18 April 1985, NCDWR, 1L (NCDWR); North Muddy Creek, SR 1760, 35.67528, -81.90639, 17 April 1985, NCDWR, 16L (NCDWR); Youngs Fork, SR 1819, 35.65389, -81.96306, 17 April 1985, NCDWR, 1L (NCDWR). **Nash Co.,** Sandy Creek, SR 1405, 36.12694, -78.02472, 21 April 2003, E. Fleek, 3L (NCDWR); Swift Creek, SR 1310, 36.11167, -77.92111, 24 April 2003, E. Fleek, 2L (NCDWR). **Polk Co.,** Rotten Creek, off Jeep Trail 35.34709, -82.21117, 16 April 2014, V. Holland, 1L (NCDWR); Whiteoak Creek, SR 1 352, 35.26055, -82.00388, 15 May 1995, N. Guthrie, L. Eaton, K. Herring, 1L (NCDWR). **Richmond Co.,** Naked Creek, SR 1003, 35.08194, -79.59028, 6 April 1990, L. Eaton, 2L (NCDWR); Naked Creek, SR 1003, 35.08197, -79.58909, 18 April 2013, D. Lenat, 1L (NCDWR); Rocky Ford Branch, SR 1424, 35.12417, -79.66250, 9 May 1990, D. Lenat, 8L (NCDWR). **Rockingham Co.,** Falls Creek, Mayo River State Park, 36.539178, -79.988084, 1 May 2012, D. Lenat, 1L (NCDWR); Rock House Creek, SR

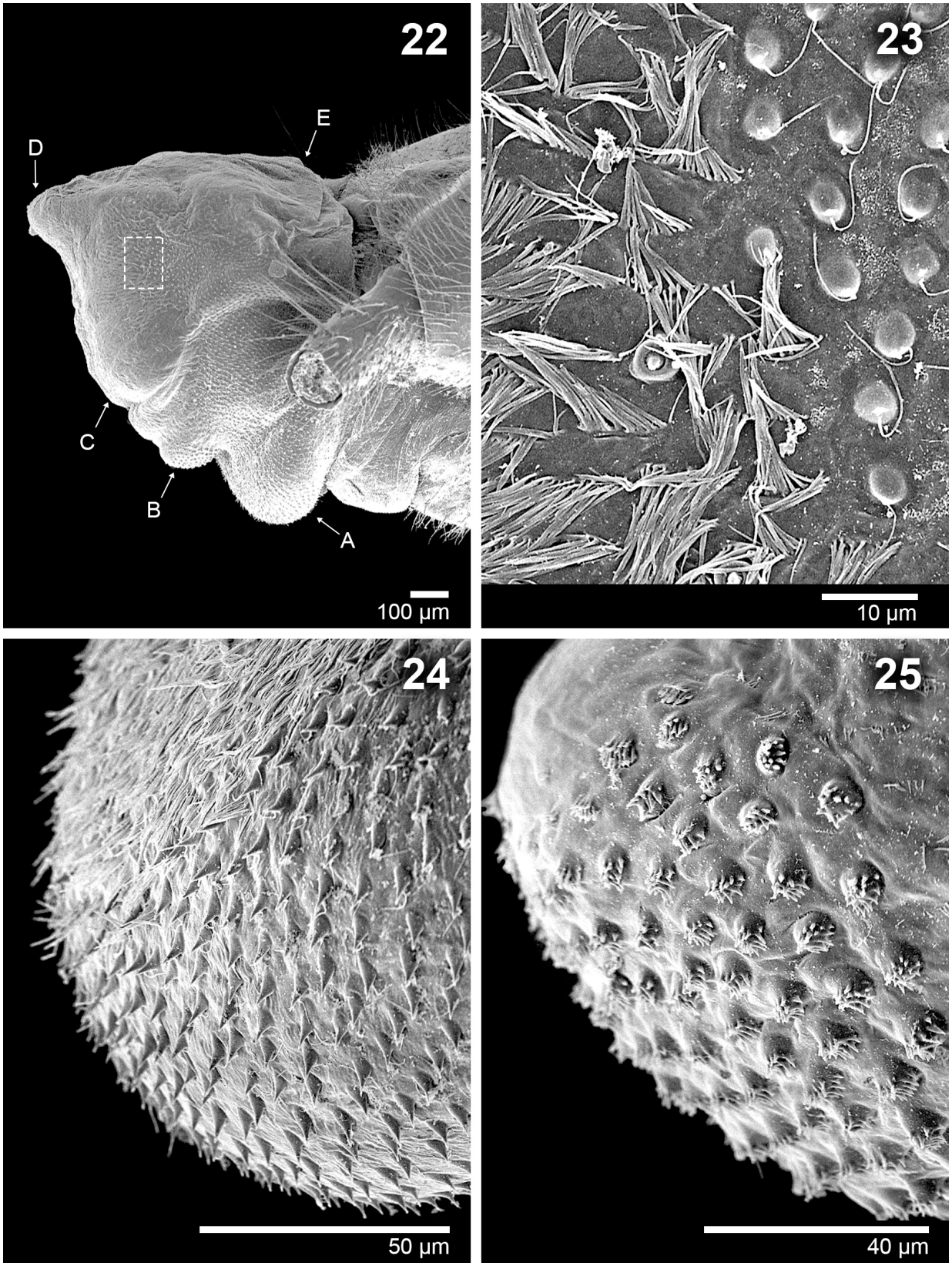
2127, 36.42083, -79.79055, 12 April 2001, L. Eaton, 1L (NCDWR). **Rutherford Co.,** Second Broad River, above Union Mills, 35.47917, -81.94417, 19 May 1999, K. Herring, 4L (NCDWR); Second Broad River, below Union Mills, 35.47667, -81.94250, 19 May 1999, K. Herring, 1L (NCDWR) Second Broad River, SR 1504, 35.48401, -81.94921, 18 May 2010, D. Black, S. Beaty, E. Fleek, 1L (NCDWR). **Stokes Co.,** Brushy Fork, SR 1998, 36.32306, -80.30278, 18 May 2004, N. Flint, 1L (NCDWR); East Prong Little Yadkin River, SR 1224, 36.33126, -80.38586, 17 May 1994, N. Guthrie, 4L (NCDWR); Little Yadkin River, SR 1104, 36.29889, -80.41389, 18 May 1994, D. Penrose, 1L (NCDWR); same location 31 May 1989, D. Penrose, 1L (NCDWR); Little Yadkin River, SR 1104, 36.29899, -80.41388, 6 May 1987, D. Penrose, 2L (NCDWR); same location, 31 May 1989, D. Penrose, 1L (NCDWR). **Surry Co**., Endicott Creek, Raven Knob Rd., 36.47274, -80.85152, 5 May 2010, V. Holland, M. Walters, 2L (NCDWR); Fisher River, nr SR 1346, 36.41806, -80.71752, 4 May 2010, V. Holland, M. Walters, 4L (NCDWR). **Transylvania Co.,** Catheys Creek, SR 1338, 35.20750, -82.78056, 21 May 1987, D. Penrose, 1L (NCDWR). **Wilkes Co.,** Elk Creek, SR 1175, 36.10639, -81.43808, 7 June 2006, M. Walters, 1L (NCDWR); Left Prong Stony Fork, off SR 1155, 36.15065, -81.45392, 20 April 2012, S. Beaty, 1L (NCDWR); North Prong Lewis Fork, nr SR 1300, 36.21309, -81. 35806, 7 May 2010, V. Holland, M. Walters, 2L (NCDWR); Yadkin River, nr Abtco Rd., 36.19694, -81.02917, 7 June 1993, D. Penrose, 1L (NCDWR). **Yadkin Co.,** North Deep Creek, NC 601, 36.16861, -80.68111, 12 April 1993, D. Lenat, 6L (NCDWR). **South Carolina: Aiken Co.,** Tinker Creek, Savannah River Site, SRP B-1, [33.33769, -81.60466], 1 June 1977, Herlong, Prichard, 3F (NCDWR); same data, 40M, 7F (INHS, Insect Collection 875859); same data, 4M, 119F (INHS, Insect Collection 875917); same data, 136F (INHS, Insect Collection 875919); same data 1M, 62F (INHS, Insect Collection 875902); same data, 1M, 165F (INHS, Insect Collection 875845); same data, 1M, 100F (INHS, Insect Collection 875868); same location, 28 June 1977, Herlong, Prichard, 5F (NCDWR); Upper Three Runs, Savannah River Site Rd. C, [33.28518, -81.69533], 5 May 1984, B.C. Kondratieff, 29M, 8F (NCDWR); same location, 27 May 1984, B. Kondratieff, 1M, 27F (NCDWR); same location, 4 June 1984, B. Kondratieff, 14M, 26F (NCDWR); same location, 17 April 1985, B. Kondratieff, 2M, 1E (NCDWR); Upper Three Runs, Savannah River Site Rd. F, [33.23926, -81.74427], 4 May 1977, Herlong, Prichard, 4M, 1F (INHS, Insect Collection 875918); same location, 17 May 1977, Herlong, Prichard, 12M, 5F (INHS, Insect Collection 875916); same location, 1 June 1977, Herlong, Prichard, 3M, 159F (INHS, Insect Collection 875847); same data, 215F (INHS, Insect Collection 875861). **Anderson Co.,** Rock Creek, N of S-4-670, [34.56723, -82.53546], 3–4 June 1980, J. Weaver, 1M (NCDWR). **Barnwell Co.,** Meyers Branch, 600 m upstream from Savannah River Site Rd. A, [33.15049, -81.62530], 3 June 1983, B. Kondratieff, 5F (NCDWR); same location, 3 June 1983, B. Kondratieff, 4F (NCDWR); Steel Creek, Savannah River Site Rd. A, [33.14589, -81.62874], 3 June 1982, B. Kondratieff, 2M, 1F, 2E, (NCDWR); same data, 1M, 4F (NCDWR). **Oconee Co.,** Chauga River, 4 mi S. of Mountain Rest, [34.83317, -83.15801], 10 June 1969, J. Morse, 1M, 2F (INHS, Insect Collection 875857). **Pickens Co.,** Wildcat Creek, 9 km NW Clemson, [34.75589, -82.85776], 25 May 1980, J.S. Weaver III, 1F (INHS, Insect Collection 875870); South Saluda River, 4 mi N Pumpkintown, [35.06719, -82.61793], 27 June 1969, H. Douglas, Fox & Fox, 2M, 1F (INHS, Insect Collection 875858). **Spartanburg Co.,** South Saluda River, 5 mi W Cleveland, [35.06759, -82.61827], 2 June 1969, J. Morse, G. Townes, 2M (INHS, Insect Collection 875850); same location, 2 July 1969, H. Douglass, G. Townes, 1F (INHS, Inset Collection 875899). **Tennessee: Blount Co.,** Abrams Creek, 100 m upstream Abrams Falls Trailhead, Great Smoky Mountains National Park, 35.59210, -83.85200, 8 June 2001, B.D. Heinold, 5F (INHS, Insect Collection 3383); same location, 6 July 2001, B.D. Heinold, R.E. DeWalt, S.L. Johnson, 1F (INHS, Insect Collection 3457). **Virginia: Franklin Co.,** Big Chestnut Creek, Rte 718, [36.87630. W 79.86254], 21 May 2002, B.C. Kondratieff, R.F. Kirchner, 2M (VMNH); Grassy Fork, 2 mi S of Snow Creek, [36.81097, -79.74516], 2 June 2000, R.L. Hoffman, 3M, 8F (VMNH); Pigg River, 0.3 km upstream of Rte 890 bridge SW Penhook, [36.94638, -79.64051, 26 June 2005, R.L. Hoffman, 1M, 7F (VMNH); Shooting Creek, Rte 860, 1.4 mi W jct. VA 40, [36.88060, -80.20147], 13 July 1991, R.L. Hoffman, 1F (VMNH). **Halifax Co.,** no waterbody data, Berry Hill (Delorme27? D5-6) at light in logged floodplain, [no coordinates], 8 June 1993, R.L. Hoffman, C. Hampton, 1F (VMNH); no waterbody data, swamp 2.5 mi E of Riceville, [no coordinates], 21 May 2000, (VMNH) survey, 1M (VMNH). **Pittsylvania Co.,** Banister River, below Mt. Airy Mill, Rte 683, ca 1 mi SW Mt. Airy, [36.93180, -79.20300], 25 June 1989, R.L. Hoffman, 1M (VMNH); Crooked Run, Rte 841, 6 mi SW Callands, [36.74825, -79.65121] 9 June 2000, R.L. Hoffman 3M, 5F (VMNH); Sandy River, Rte 845 bridge 4.5 mi N Brosville, [36.67935, -79.59389], 24 June 1993, R.L. Hoffman, 1F (VMNH); South Prong Sandy River, NW of Brosville, [36.66365, -79.61798], 19 June 1990, R.L. Hoffman, 1F (VMNH); [Bawley Branch], jct. 939 & 614 3 mi SE of Sandy River, [36.71343, -79.61181], 2 June 1998, R.L. Hoffman, 1M, 2F (VMNH).



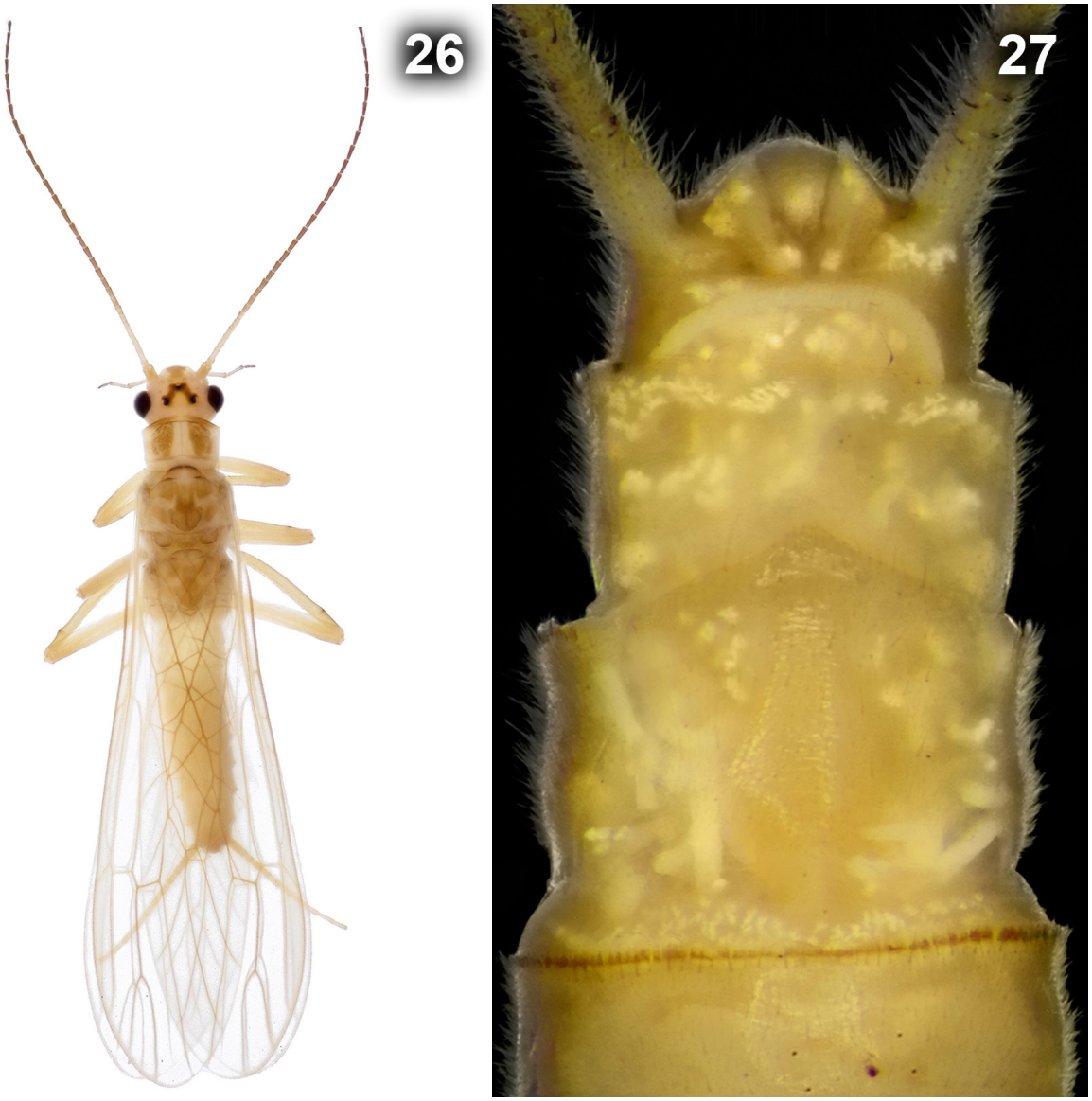
FIGURES 16–18. *Isoperla riverae* **sp. n.**, adult male. 16. Head and pronotum, dorsal, Drowning Creek, Moore/Richmond Co., North Carolina. 17–18. Grassy Fork, Franklin Co. Virginia. 17. Vesicle, ventral. 18. Paraprocts, caudal.



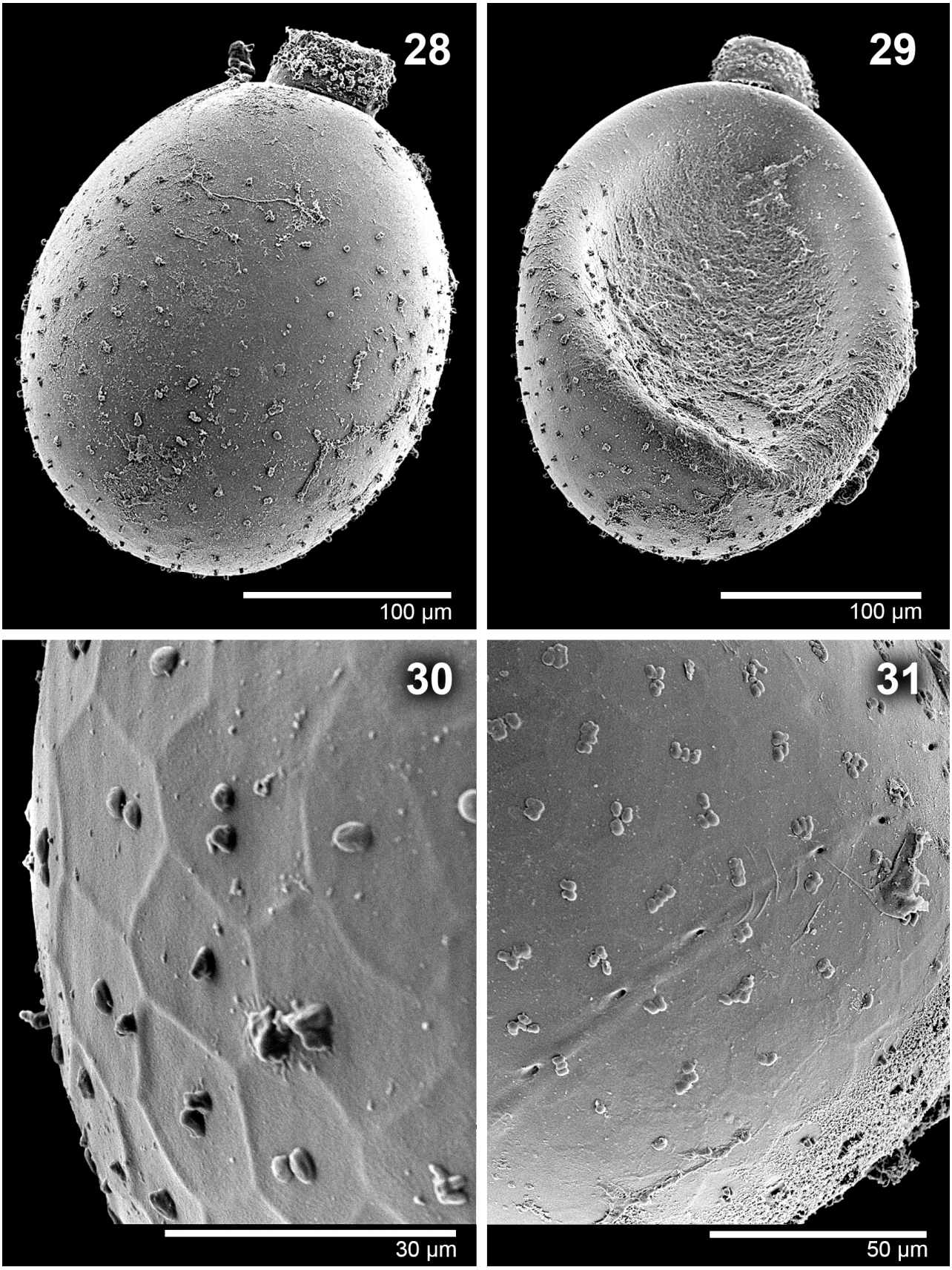
FIGURES 19–21. *Isoperla riverae* **sp. n.**, adult male terminalia, (A) posteroventral lobe, (B) posterior lobe, (C) pair of small caudal lobes, (D) conical apical lobe, (E) paired hemispherical anterior lobes, Mayo River, Rockingham Co., North Carolina. 19. Lateral, 20. Dorsal, 21. Ventral.



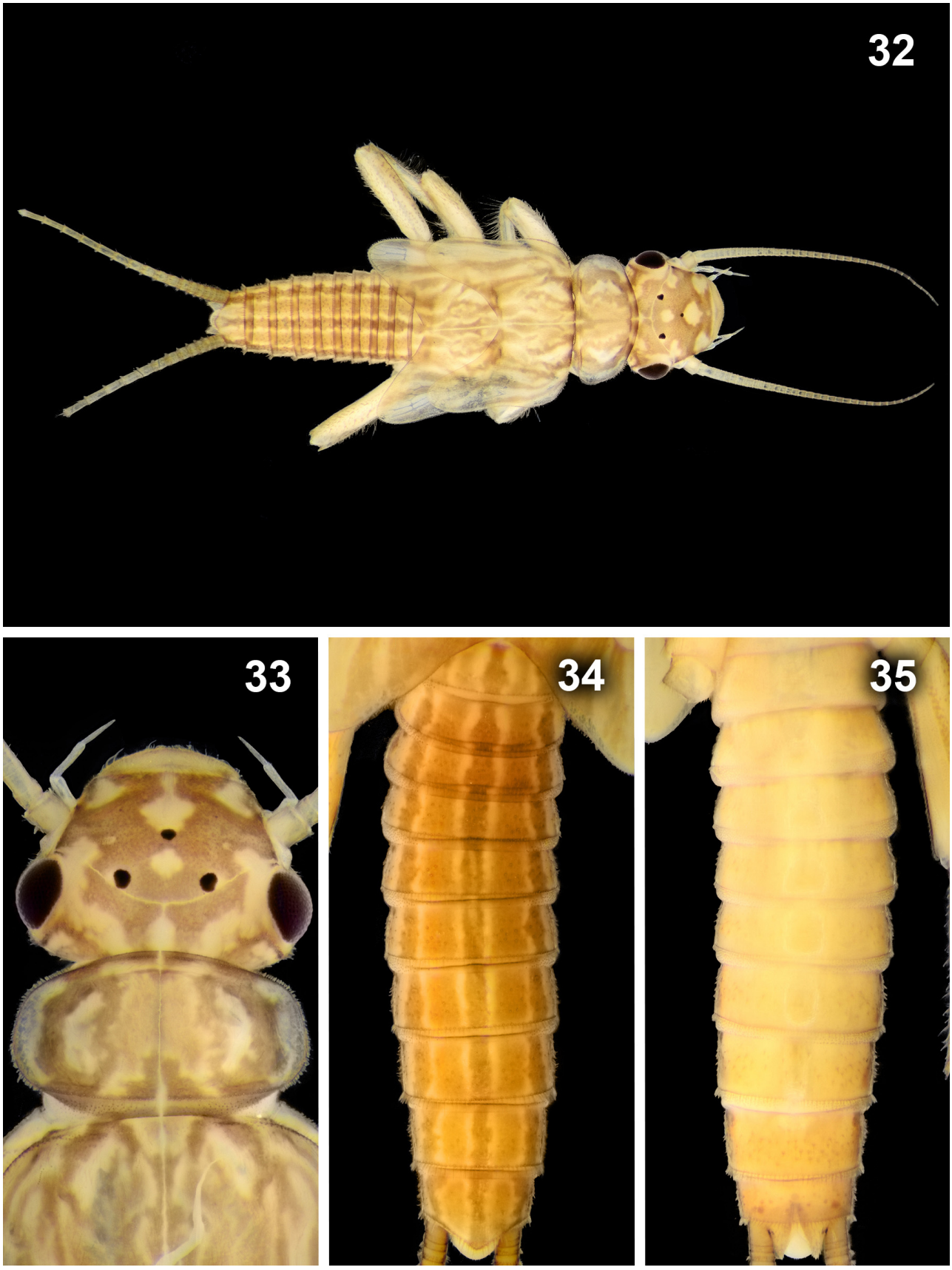
FIGURES 22–25. *Isoperla riverae* **sp. n.**, adult male, aedeagus, (A) posteroventral lobe, (B) posterior lobe, (C) pair of small caudal lobes, (D) conical apical lobe, (E) paired hemispherical anterior lobes, Drowning Creek, Moore/Richmond Co., North Carolina. 22. Lateral. 23. Details of fig. 22 inset, hair-like spinulae, sensilla basiconica and small rounded knobs with apical filaments. 24. Sharp spinulae and hair-like spinulae of posteroventral lobe (A) 25. Small mounds with sharp spinulae on posterior lobe (B).



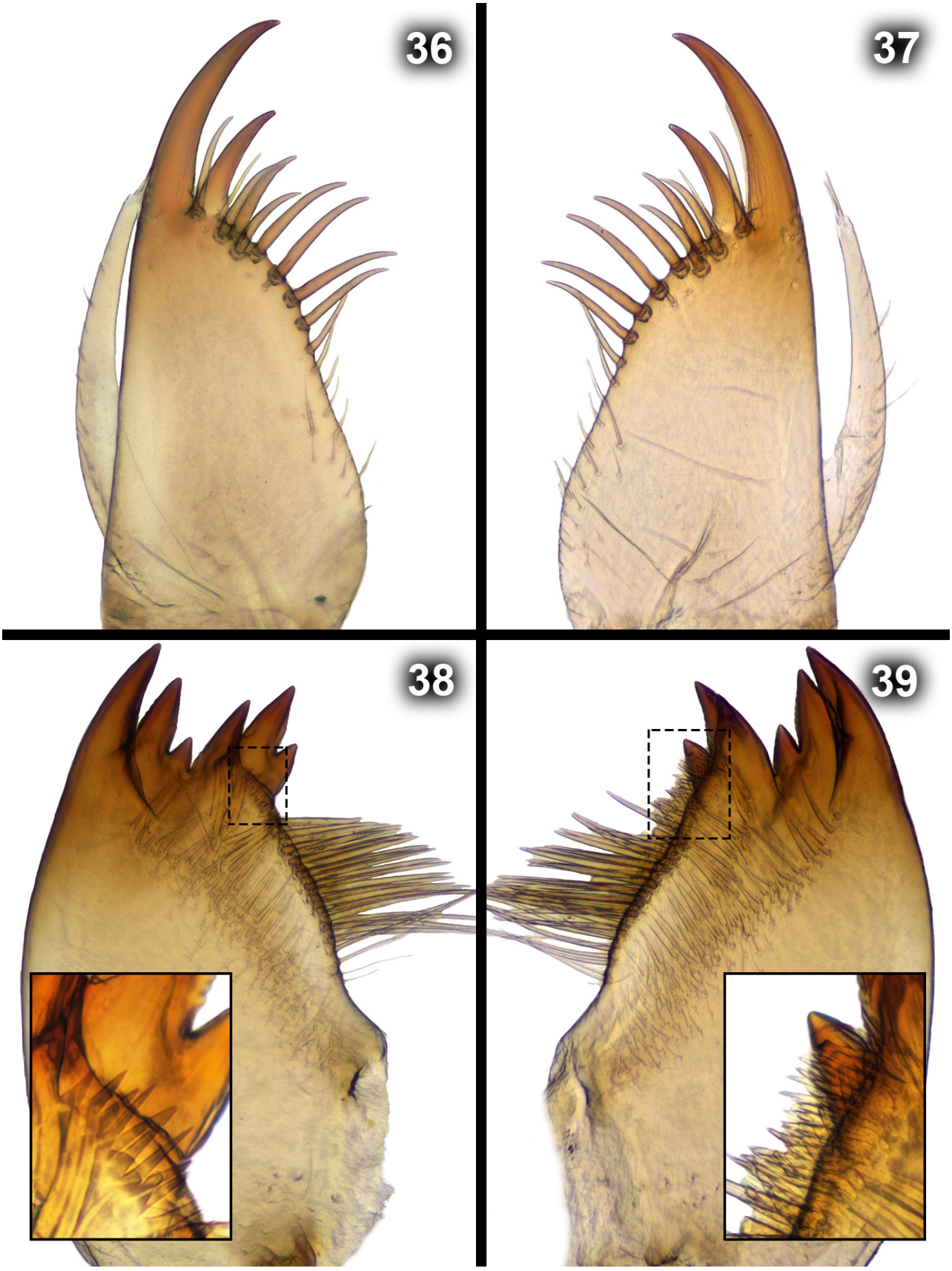
FIGURES 26–27. *Isoperla riverae* **sp. n.**, adult female, Drowning Creek, Moore/Richmond Co., North Carolina. 26. Habitus, dorsal. 27. Subgenital plate, ventral.



FIGURES 28–31. *Isoperla riverae* **sp. n.**, ovum, Drowning Creek, Moore/Richmond Co., North Carolina. 28. Convex aspect. 29. Concave aspect. 30. Reticulation of egg chorion and globules. 31. Eclosion line and micropyles.



FIGURES 32–35. *Isoperla riverae* **sp. n.**, larva, Naked Creek, Richmond Co., North Carolina. 32. Habitus, dorsal. 33. Head and pronotum, dorsal. 34. Abdomen, dorsal. 35. Abdomen, ventral.



FIGURES 36–39. *Isoperla riverae* **sp. n.**, larva. 36–37. Naked Creek, Richmond Co., North Carolina. 36. Right lacinia, ventral. 37. Left lacinia, ventral. 38–39. Rocky Ford Branch, Richmond Co., North Carolina. 38. Right mandible, ventral; (inset) acanthae. 39. Left mandible, ventral; (inset) dense brush of short spinous setae.

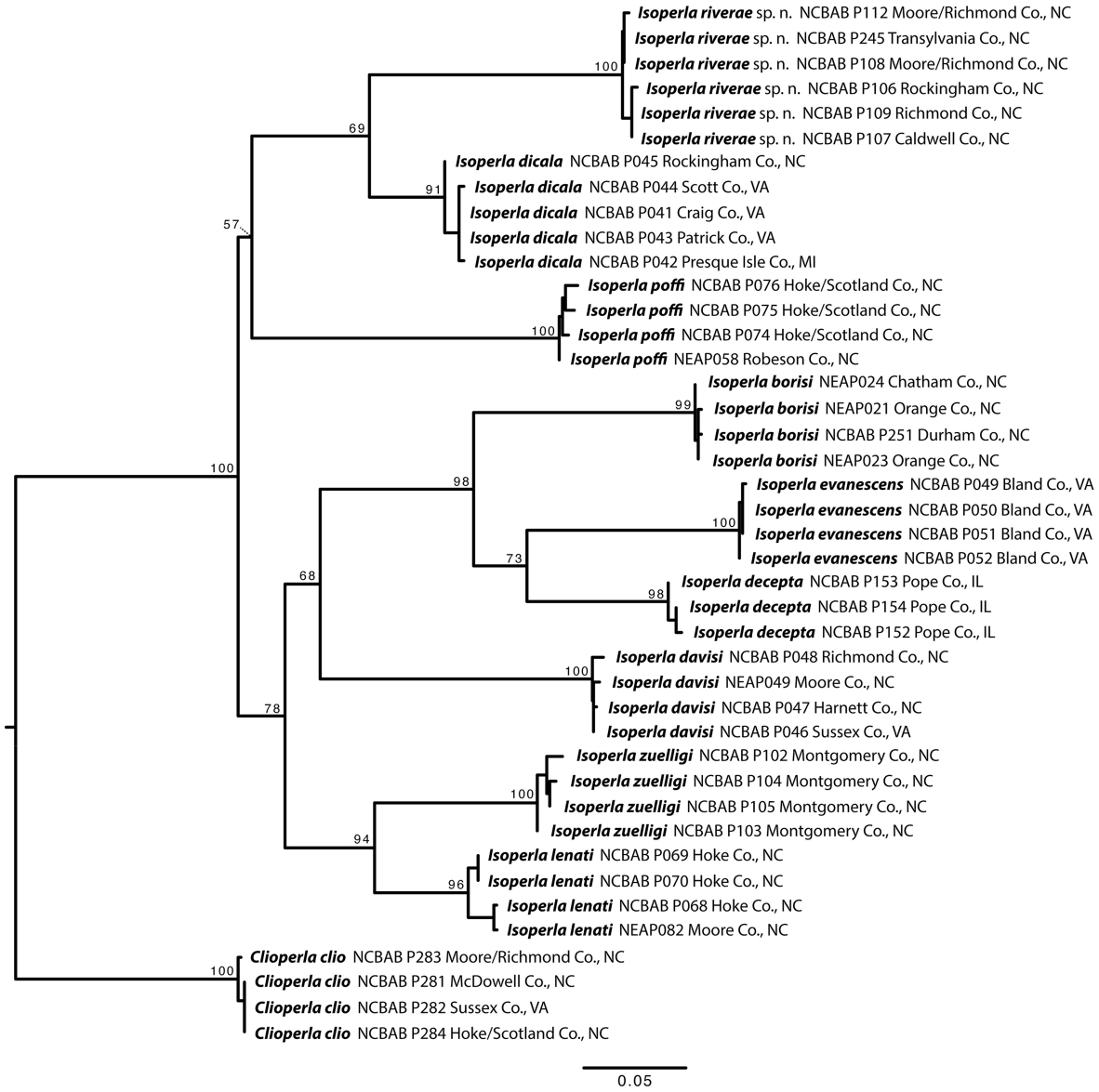


FIGURE 40. Phylogenetic representation of the IQTREE-estimated maximum likelihood tree of cytochrome c oxidase I (COI). Bootstrap values of 50 or greater are shown at associated nodes; bootstrap values are not provided for short internodes within species clades. Tips are labeled with species name, sample number, and county and state. The scale bar represents estimated number of nucleotide substitutions per site.

Isoperla dicala Frison, 1942

(Figs. 41–48)

Sable Stripetail

http//lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:468699

Isoperla dicala Frison 1942:321. Holotype male (INHS), Free Soil, Great Sable River, Mason Co., Michigan, U.S.A.
Isoperla dicala: Hitchcock 1974:197.
Isoperla dicala: Poulton & Stewart 1991:48.
Isoperla dicala: Szczytko & Kondratieff 2015:86.

Distribution. Canada—MB, NB, NS, ON, PQ. U.S.A.—AL*, AR, CT, GA, FL* IA, IN, KY, MA, MD, ME, MI, MN, MO, MS*, NC, NY, OH, PA, SC, TN, VA, WI, WV (DeWalt *et al*. 2023). (records with * are considered dubious based on the present study)

Material examined. U.S.A.—Michigan: Presque Isle Co., Ocqueoc River, Ocqueoc Falls Hwy parking area, 45.39724, -84.01754, 11 June 2019, S. Beaty, A. Beaty, 1M, 2F (NCDWR). **Missouri: Shannon Co.,** Round Springs, Round Springs State Park, [37.28270, -91.40768], 22 June 1974, J. Morse, 1F (NCDWR). **Texas Co.,** Current River, Montauk State Park, [37.45059, -91.68704], 4 June 1976, B. Stark, J. Lackey, 5M, 9F (INHS, Insect Collection 875889)**. North Carolina: Alleghany Co.**, Brush Creek, SR 1444, 36.49416, -81.00472, 3 April 2006, T. MacPherson, 2L (NCDWR). **Ashe Co.,** South Fork New River, US 221, 36.47380, -81.33694, 14 May 1990, D. Penrose, 1L, (NCDWR)**. Buncombe Co.,** Flat Creek, SR 1741, 35.72833, -82.61528, 24 April 1986, D. Penrose, 5L, (NCDWR); [Swannanoa River], Swannanoa, [35.59789, -82.39984], 19 June 1938, T.H. Frison, T.H. Frison Jr., 1M (INHS, Insect Collection 875897). **Burke Co.,** Linville River, NC 126, 35.79472, -81.89027, 10 April 1990, L. Eaton, D. Penrose, K. Lynch, 1L, (NCDWR)**. Caldwell Co.,** Johns River, SR 1356 (Old Johns River Rd.), 35.93416, -81.69138, 6 April 2019, C. Verdone, S. Beaty, V. Holland, 1F (NCDWR)**. Caldwell Co.,** Thorps Creek, Mortimer Campground, 35.99054, -81.76096, 13 April 2012, S. Beaty, V. Holland, E. Fleek, 1L, (NCDWR); Wilson Creek, NC 90, 36.01381, -81.76700, 13 April 2012, S. Beaty, V. Holland, E. Fleek 1L (NCDWR); Yadkin River, near SR 1516, 35.99166, -81.55805, 6 June 2006, M. Walters 1L, (NCDWR); Yadkin River, off SR 1372, 36.06895, -81.58553, 23 May 2011, D. Black, S. Beaty, E. Fleek 1L (NCDWR)**. Henderson Co.,** North Fork Mills River, SR 1343, 35.39425, -82.61503, 3 May 2011, T. Mormon, M. Walters, D. Black 1L, (NCDWR); South Fork Mills River, SR 1340, 35.37569, -82.61440, 1 June 2009, T. MacPherson, M. Walters, E. Fleek 2L, (NCDWR); same location, 35.37569, -82.61440, 3 May 2011, T. Mormon, M. Walters, D. Black, 4L, (NCDWR); same location, 25 June 2002, T. Mormon, 1L, (NCDWR). **Macon Co.,** Cullasaja River, off Fox Ridge Rd, 35.17172, -83.36720, 28 May 2019, V. Holland, S. Beaty, C. Verdone, 1L (NCDWR). **Rockingham Co.,** Mayo River, Anglin Mill Rd., 36.53530, -79.99070, 11 May 2018, C. Verdone, 2M, 1F (NCDWR); same location, 22 April 2020, S. Beaty, V. Holland, C. Verdone, 1F, 1E (NCDWR); same location, 2 May (no year given), D. Lenat, 3L (NCDWR). **Surry Co.,** Fisher River, near SR 1346, 36.41803, -80.71752, 4 May 2010, V. Holland, M. Walters, T. Mormon, 2L (NCDWR). **Swain Co.,** Nantahala River, Hwy 19, 35.33184, -83.60671, 27 May 1994, S.W. Szczytko, J.B. Sandberg, B. Stark, 1M (INHS, Insect Collection 875908). **Transylvania Co.,** French Broad River, SR 1129, 35.14861, -82.79972, 9 April 2019, C. Verdone, S. Beaty, V. Holland, E. Fleek, 10L (NCDWR). **Watauga Co.,** Cove Creek, off SR 1121, 36.23944, -81.82138, 15 May 2007, E. Fleek, T. MacPherson, S. Beaty 2L (NCDWR). **Yancey Co.,** Cane River, off SR 1114, 35.90437, -82.33250, 7 May 2008, D. Black, M. Walters, B. Crouch, 5L (NCDWR); Cane River, US 19E, 35.91222, -82.34944, 14 May 2013, D. Lenat, 1L (NCDWR). **South Carolina: Oconee Co.,** Chattooga River, Hwy 28 at Georgia State Line, [34.91958, -83.16882], 24 May 1993, B. Kondratieff, R. Kirchner, 1M (NCDWR). **Pickens Co.,** South Saluda River, 4 mi N Pumpkintown, [35.06719, -82.61793], 22 June 1969, H. Douglass, 1F (INHS, Insect Collection 875884). **Tennessee: Blount Co.,** Abrams Creek, Abrams Creek Campground, Great Smoky Mountains National Park, 35.61030, -83.93270, 10 June 2001, B.D. Heinold, 2F (INHS, Insect Collection 3491). **Sevier Co.,** Little Pigeon River, Gatlinburg, [35.71417, -83.51028], 12 June 1935, H.H. Ross, 1F (INHS, Plecoptera 2957); Gatlinburg, [35.71426, -83.51016], 11 June 1938, T.H. Frison, T.H. Frison Jr., 1F (INHS, Insect Collection 875854); same location, 14 June 1940, [T.H.] Frison, [C.O. Mohr, A.W. Hawkins] 2F, (INHS, Plecoptera 2956)**;** Sevierville, 35.86806, -83.56194, 11 June 1938 T.H. Frison, T.H. Frison Jr., 5F (INHS, Insect Collection 875863); same data**,** 3F (INHS, Insect Collection 875856)**;** same data, 1M (INHS, Insect Collection 875892). **Virginia: Bland Co.,** Clear Fork, Hwy 61, 0.5 mi W of Clear Fork, 37.20992, -81.20296, 6 June 2016, C. Verdone, B. Kondratieff, 1F (NCDWR)**;** Wolf Creek, Grapefield Rd., Stephen Levitt Property, 37.14702, -81.26314, 4 June 2016, C. Verdone, B. Kondratieff, 3F (NCDWR)**;** same location, 6 June 2016, C. Verdone, B. Kondratieff, 2F (NCDWR); **s**ame location, 8 June 2016, C. Verdone, B. Kondratieff, 2F (NCDWR); same location, 23 May 2017, C. Verdone, 3F, 2F (NCDWR); same location, 27 May 2017, C. Verdone, D. Fuller, 1F (NCDWR); Wolf Creek, Grapefield Rd., Wolf Creek Picnic Area, 37.18026, -81.19496, 8 June 2016, C. Verdone, B. Kondratieff, 1F (NCDWR); same location, 7 May 2017, C. Verdone, B. Kondratieff, S. Roble, 1M, 2F (NCDWR); Wolf Creek, Hwy 61, War Memorial, 37.24324, -81.10800, 27 May 2016, C. Verdone, B. Kondratieff, 4F (NCDWR); same location, 8 June 2016, C. Verdone, B. Kondratieff, 1F (NCDWR). **Botetourt Co.,** Craig Creek, Craig Creek Rec Area (2 mi SE of Oriskany), 37.61345, -79.95624, 9 June 2016, C. Verdone, B. Kondratieff, 1F (NCDWR). **Craig Co.,** Potts Creek, Rte 18, 37.59814, -80.22697, 4 May 2019, C. Verdone, D. Fuller, 1M (NCDWR). **Floyd Co.,** Floyd, [36.91124, -80.32005], no date, no collector data, 3F (VMNH). **Franklin Co.,** Otter Creek, Philpott Reservoir, end of Rte 789, 2.4 mi SE of Endicott, [36.86837, -80.11660], 25 August 2000, A.C. Chazal, 2F (VMNH); Pigg River, 0.3 km upstream of Rte 890 bridge SW Pennook, [36.94638, -79.64052], 26 June 2005, R.L. Hoffman, 1M, 2F (VMNH); UT Nicholas Creek, VA

781, 2.5 mi W Ferrum, 36.91615, -80.06237, 6 July 2000, R.L. Hoffman, 1F (VMNH). **Giles Co.,** Sinking Creek, at VA 700 Bridge, 37.31149, -80.51610, 3 July 1992, R.L. Hoffman, 3F (VMNH). **Grayson Co.,** New River, Hwy 58 Boat Access, 36.61340, -81.04464, 29 May 2016, C. Verdone, B. Kondratieff, 1F (NCDWR), same location, 11 May 2017, C. Verdone, B. Kondratieff, 1F (NCDWR). **Henry Co.,** South Mayo River, at VA 695, ca 5 mi SSW of Spencer, [36.55577, -80.02128], 6 June 1990, R.L. Hoffman, 2F (VMNH); Toeclout Branch, at VA 637, 3 mi SE of Ridgeway, [36.54852, -79.84263], 12 May 1990, R.L. Hoffman, 1M, 2F (VMNH). **Patrick Co.,** Dan River, Rte 648, 36.62230, -80.44497, 11 May 2017, C. Verdone, B. Kondratieff, 2M (NCDWR). **Pittsylvania Co.,** Crooked Run, VA 841 6 mi SW Callands, [36.74825, -79.65121], 9 June 2000, R.L. Hoffman, 1F (VMNH); Dan River, VA 860 bridge, ca 1 mi SE of Cascade, [36.54417, -79.66183], 31 May 1989, R.L. Hoffman, 1M (VMNH); Sandy River, Rte 845 bridge 4.5 mi N Brosville, [36.67935, -79.59389], 7 June 1993, R.L. Hoffman, 1F (VMNH). **Prince William Co./ Fairfax Co.,** Bull Run, Manassas National Battle Field, [38.82435, -77.50375], 30 July 1997, C.S. Hobson, 1F (VMNH). **Pulaski Co.,** Little Reed Island Creek, Rte 607 at Boone Furnace, [36.92150, -80.76976], 10 June 1990, R.L. Hoffman, 1F (VMNH). **Russell Co.,** Clinch River, Rte 798 SE of Rte 645 nr Nash Ford, 36.96549, -82.07749, 24 May 2017, C. Verdone, 3M, 4F (NCDWR). **Scott Co.,** Clinch River, Rte 65, Dungannon Boat Access, 36.83108, -82.46252, 6 May 2017, C. Verdone, B. Kondratieff, S. Roble, 3M, 6F (NCDWR). **Smyth Co.,** North Fork Holston River, Carl Slate Memorial Boat Landing, 36.89335, -81.75539, 5 May 2017, C. Verdone, B. Kondratieff, S. Roble, 5M, 5F (NCDWR); South Fork Holston River, jct. Riverside Rd./ Chestnut Ridge Rd., 36.76200, -81.63585, 8 May 2017, C. Verdone, B. Kondratieff, 8M, 3F (NCDWR). **Tazewell Co.,** spring pond on Smith Ridge, 1 mi N of Pounding Mill, [37.09095, -81.71416], 4 June 1999, Rodney Cordle, 1M, 2F (VMNH). **Washington Co.,** Wolf Creek, St. Rte 80 nr Conflu w/ North Fork Holston River, 36.82404, -81.92167, 4 May 2017, C. Verdone, B. Kondratieff, 1M (NCDWR).

Adult male. Macropterous; forewing length 7.5–9.3 mm (n = 16), body length 6.9–8.9 (n = 16). Head pale yellow with brown bands connecting lateral ocelli to anterior ocellus. General body color pale yellow in life with few brown markings.

Dorsum of Head (Fig. 16). Palpi pale yellow-brown. Labrum with stiff, short setae. Dorsum of head pale yellow with brown bands connecting median ocellus with lateral ocelli; interocellar area pale, open posteriorly; frons generally pale yellow. Antennal scape and pedicel yellow; flagellum brown with basal 7–8 segments pale, antennal length about ½ length of body with 32 segments.

Thorax. Pronotum with a wide, pale median stripe which extends to anterior margin (Fig. 16); rugose area laterad of median stripe pale brown; pigment extends anteriorly to pronotal margin; rugose area with short stout brown setae interspersed; posterior edge of pronotum pale; lateral pronotal margins pale. Mesonota and metanota yellow with yellow-brown scutal humps; nota with sparse, short, fine pale setae. Wings hyaline with light brown veins (Fig. 26). Mesobasisterna and metabasisterna pale yellow.

Legs. Anterior and posterior faces of femora pale yellow. Tibiae yellow with a thin transverse dark brown band near proximal ¼. Tarsi light brown, segment 3 darker apically. Leg vestiture dense with short dark setae giving legs a darker overall appearance; tibiae with widely spaced subdorsal and ventral rows of larger spines.

Abdomen. Terga pale yellow and clothed with short, fine golden setae. Sterna pale yellow. Sterna 6–9 with a posterior fringe of short, brush-like, brown setae. Sternum 8 with a deeply recessed, pale brown vesicle (Fig. 17) 2.1–3.0X (n = 14) longer than wide when measured from apex to anterior sclerite / maximum subapical width; apex slightly expanded, evenly rounded, extending to base of segment 9. Paraprocts triangular, minimally sclerotized, pale yellow; apices narrow and acutely rounded; not recurved over tergum 10 (Fig. 18). Cerci pale yellow; cercal length ½ as long as abdomen, with 12 segments, each segment with one long, light brown ventral seta near posterior margin, basal 4–6 segments with dorsal and ventral tufts of long stiff setae, setae becoming shorter and less numerous on each successive segment.

Aedeagus (Figs. 19–25). Male appendage with a short, broad basal stalk, posteroventral lobe, posterior lobe, pair of small caudal lobes, conical apical lobe and paired hemispherical anterior lobes (Figs. 19, 22). Paired caudal lobes (Figs. 19–22), small, often difficult to discern. Conical apical lobe (Figs. 19, 22) often depressed apically, flap-like, appearing triangular in dorsal and ventral aspects (Figs. 20–21); apex broadly rounded. Anterior hemispherical lobes membranous (Figs. 19–22).

Aedeagal armature (Figs. 22–25). Basal stalk encircled with short stout spinulae (Fig. 22); posteroventral lobe with sharp spinulae which transitions to dense hair-like spinulae dorsally (Fig. 24). Mediolateral areas of aedeagus also covered in sharp spinulae (Fig. 22). Posterior lobe with small mounds each adorned with a cluster of small

sharp spinulae (Fig. 25). Area dorsolateral to paired caudal lobes with linear brushes of hair-like spinulae and sensilla basiconica which transition to small, rounded knobs with apical filaments anteriorly (Fig. 23).

Adult female (Fig. 26). Macropterous; forewing length 8.1–10.1 mm (n = 11). Body length 7.9–10.3 (n = 11). General head pattern and body color similar to that of the male. Posterior margin of tergum 7 and posterolateral margins of terga 8–9 bearing brush-like setae.

Subgenital plate (Fig. 27). Pale yellow, broadly triangular and produced posteriorly approximately ⅛–¼ the length of sternum 9; plate basally broad, about ¾ the width of segment 8 on most specimens, apex obtuse; plate with scattered short golden setae and a few longer setae interspersed.

Ovum (Figs. 28–31). Length 268.7–277.4 μm (n = 2); width 200.3–202.2μm (n = 2). Color pale yellow. General shape oblong; cross section concave; chorion covered with subtle reticulate ridges; collar well-developed (Figs. 28–29). Follicle cell impressions hexagonal or pentagonal, weakly developed and with clusters of globules at their center (Figs. 30–31). Eclosion line weakly developed, micropyles positioned on eclosion line, spaced regularly around egg circumference (Figs. 31). Collar height 28.7–34.3 μm, width 56.2–56.9 μm (n = 2); width either subequal or slightly narrowed from base to apex; densely covered in globules (Figs. 28–29).

Mature larva (Figs. 32–35). Pre-emergent larvae 6.7–9.2 mm (n = 17). Body with lightly contrasting body pattern.

Dorsum of head (Fig. 33). Mottled brown with pale medial area anterior to median ocellus, a pair of large anterolateral pale spots, a pair of mediolateral pale spots, a small enclosed pale ocellar spot, and pale ocular areas. Anterior frontoclypeus with a dark bar that is obliterated medially by medial extension of pale median area. Pale medial area often shaped like a spinning top; sometimes with a short anteromedial extension and with small anterolateral projections, resembling a duck foot overall. Anterolateral pale spots variable and irregularly shaped, usually distinctly separated from pale medial area. Pale medial spot and anterolateral spot bordered by a dark M-line on posterior margins; pale ocellar spot small, usually round, fully enclosed and centered equidistant from ocelli; mediolateral pale spots subquadrate, extending anteromedially from epicranial suture but not reaching lateral ocelli; closed anterior to suture, confluent with pale ocular area. Pale ocular spots extend posteromedially towards posterior occipital margin; pale area divided by a thin angulate line of brown pigment and spicules. Epicranial stem with a pale oval spot covering posterior 2/3. Brown areas of occipital area with clusters of spicules. Clothing hairs on head lightly colored. Antennae yellow; apical segments slightly darker; scape and pedicel yellow to yellow-brown; flagellum with 30–39 antennomeres (n=13); each segment with an apical circlet of small spines.

Maxilla (Figs. 36–37). Lacinia bidentate; receding evenly from base to subapical tooth; a thin, marginal seta ¾ subapical tooth length, proximal to subapical tooth; 5–6 (rarely 7) striated, stout marginal setae below subapical tooth, last few setae progressively smaller; 8–10 thin, unevenly-spaced setae along palm edge and curving dorsad; a submarginal row of 3–4 striated stout setae below apical tooth; ventral face of palm basally with 5–7 setae. Length of striated apical tooth of lacinia between 0.5–0.6X palm length and 0.6–0.7X the palm width. Subapical tooth 0.5–0.6X the length of the apical tooth. Galea 1.0–1.1X the lacinial palm length, with a ventral row of less than 30 setae and tipped with 2–4 apical spinous setae. Maxillary palp setose, 1.4–1.5X length of lacinia; segments 1–4 successively longer, segment 4 slightly longer than 5; segments 1 and 2 with transverse row of apical spinous setae; segment 3 sometimes with one apical spine, segment 5 tipped with 1–2 sensillae.

Mandibles (Figs. 38–39). Right mandible (Fig. 38) bicuspid, outer cusp with three teeth, ventral tooth largest and serrated basally, dorsal tooth smallest; a band of unorganized, long spinous setae on ventral surface from base of outer ventral tooth to base of mandible; inner cusp with three teeth, middle tooth longest and largest; a few sharply pointed acanthae from basal tooth on inner cusp to molar ridge (Fig. 38 inset); molar ridge with a dense row of spine-like setae, setae longer and thinner towards base of mandible; dorsal surface of mandible with a band of unorganized setae from inner cusp towards base of mandible. Left mandible (Fig. 39) similar to right except acanthae at base of inner cusp replaced by a dense brush of short spinous setae (Fig. 39 inset).

Thoracic nota (Figs 32–33). Pronotum (Figs. 32–33) brown with contrasting pattern. Medial stripe yellow-brown, wider both anteriorly and posteriorly; medially interrupted on both anterior and posterior margins by brown triangular markings. Anterior pronotal flange with alternating stripes of pale and dark pigment; lateral edges pale, with sublateral brown stripes. Pronotal disk with an irregular pale crescent enclosed by brown markings. Posterior pronotal flange similar to anterior flange. Pronotum rimmed with short, closely set, stiff setae, intervening long setae mostly absent except at pronotal corners. Mesonota and metanota with irregular brown markings; wing pads yellow-brown with three sublateral longitudinal brown stripes (Fig. 32). Lateral margins of nota fringed with a short, closely set row of stiff setae. All thoracic nota with sparse, short, pale to brown clothing hairs. Thoracic sterna pale,

without distinctive markings; furcal pits and Y-stem slightly darker in most specimens. Clothing hairs on sternum pale, sparse.

Legs: Femora yellow to yellow-brown; ventral face mostly pale with a few stout, golden spines; anterior face yellow with an obscure submedial yellow-brown longitudinal band and regularly spaced stout golden setae; dorsally with a sparse fringe of silky setae. Tibiae pale yellow-brown with a broad ventral row of stout setae; anterior face with two longitudinal rows of stout setae separated by a row of long silky setae, setae twice as long as width of tibia. Tarsi pale yellow-brown with a ventral row of stiff setae and a sparse dorsal row of silky setae. Two moderately long claws darker apically, gradually curved.

Abdomen (Figs. 32, 34–35). Terga (Figs. 32, 34) with three dark brown longitudinal stripes bordered by pale lines, with intervening stripes wider and paler; in lateral aspect, anterior half of each segment with a pair of small, variably dark dots sometimes overlaid by a coalescing stripe on distal segments. Terga with numerous spicules with dark origins; posterior edges of abdominal segments bearing a transverse row of stout spines, longer spines interspersed among 2–3 shorter spines; Sterna (Fig. 35) pale and with spicules with dark origins typically restricted to segments 7–10; spicules may be present on anterior segments, but rarely have dark origins; posterior edges of sterna bearing a transverse row of long clear spines; female larvae with row incomplete medially on tergum 8. Cerci yellow-brown with 18–22 segments (n = 13), each with an apical circlet of short spines; cerci with sparse dorsal fringe of silky setae on distal half.

Diagnosis. *Isoperla riverae* **sp. n.** is defined in the adult male stage by having a vesicle that is 2.1–3.0X as long as wide, dark bars connecting the anterior ocellus to the lateral ocelli, and an aedeagus that lacks an elongate posteromedian spine. Females are defined also by dark bars connecting the anterior ocellus to the lateral ocelli, in addition to having a broadly triangular subgenital plate that is produced posteriorly approximately 1/8–1/4 the length of sternum 9. Larvae are defined by the following combination of characters: the head pattern having a pale medial area anterior to median ocellus shaped like a spinning top or duck foot, a pair of large anterolateral pale spots, a small enclosed pale ocellar spot, and lateral ocelli that are separated from the mediolateral and ocular pale areas; three dark longitudinal abdominal stripes bordered by pale lines and sternal spicules with dark origins typically restricted to segments 7–10.

Genetic data results. We generated a total of 42 new COI sequences (Table 1) for this study, including six for *I. riverae* **sp. n.**, five for *I. dicala*, four for each *Isoperla borisi* Beaty, Holland & Lenat, 2017, *I. davisi, Isoperla evanescens* Verdone & Kondratieff, 2016, *I. lenati*, *Isoperla poffi* Szczytko & Kondratieff, 2015, *Isoperla zuelligi* Szczytko & Kondratieff, 2015, and *Clioperla clio* (Newman, 1839) (the outgroup taxon), and three for *Isoperla decepta* Frison, 1935. Genetic distance within sampled *I. riverae* **sp. n.** was low (avg. p-dist = 0.67%; Table 2), and similar to that observed within each species clade (avg. p-dist 0.15%–1.27%; Table 2). Genetic distance was high between *I. riverae* **sp. n.** and *I. dicala* (avg. p-dist = 13.66%; Table 2) and among all included *Isoperla* species (avg. p-dist 11.40%–18.83%; Table 2). Maximum intraspecific distance for *I. riverae* **sp. n.** was 1.58%, and 1.27% for *I. dicala.* The minimum interspecific distance between the two taxa was 13.02%.

Maximum likelihood phylogenetic reconstruction placed *I. riverae* **sp. n.** as sister to *I. dicala* in a distinct moderately well-supported clade (Table 2, Fig. 40); average pairwise tree distance = 17.8% (Table 2), bootstrap support, bs = 69%; Fig. 40). These two taxa are in turn weakly supported (bs = 57%; Fig. 40) as sister to *I. poffi*. All species clades were recovered with high nodal support (bs = 100% for; *I. davisi, I. evanescens, I. poffi, I. riverae* **sp. n.**; bs = 99% for *I. borisi*; bs = 98% for *I. decepta*; bs = 96% for *I. lenati*; bs = 91% for *I. dicala*; Fig. 40).

Taxonomic notes. Similar to other eastern Nearctic *Isoperla* described since 2015 (*I. evanescens*, *Isoperla dewalti* Verdone & Kondratieff, 2017; and *I. borisi*), *I. riverae* **sp. n.** had been lumped with previously described species; *I. dicala* in the adult stage (Stark & Gaufin 1978; Stark & Harris 1986; Szczytko & Kondratieff 2015) and *I. dicala* and *I. davisi* in the larval stage (unpublished NCDEQ data). Although several vials containing the new species had been flagged by both Drs. Szczytko and Kondratieff, many specimens of the new species were identified as *I. dicala* (Figs. 41–48) based on similarities of the vesicle (Figs. 17, 43) and subgenital plate (Figs. 27, 44) under the assumption that the head pattern was variable (B. Kondratieff *personal comm.*). Males of the new species and *I. dicala* both possess a long vesicle (*I. dicala*: 1.7–2.9X L/W, n = 10; *I. riverae* **sp. n.**: 2.1–3.0X L/W, n = 14) and females have a broadly triangular subgenital plate. Adult specimens of *I. dicala* were examined from throughout its range and while a disjunctive population in Missouri had darker pigmentation around the anterior ocellus only, all other specimens completely lacked darkly colored bars (Fig. 41). As such, the absence of dark bars connecting the anterior ocellus to the lateral ocelli consistently allows for the separation of *I. dicala* and the new species if the distinctive aedeagus (Fig. 42) is not everted.

TABLE 1. Data for specimens included in the phylogenetic analysis.

Accession #	GeneBank #	Taxon	Stage	Sex	State	County	Waterbody	Date	Lat.	Long.	Barcoded by
NCBAB P281	OQ644685	*Clioperla clio*	A	M	NC	McDowell	Wilson Creek	18-Jan-19	35.97978	-81.76504	NCMNS
NCBAB P282	OQ644684	*Clioperla clio*	A	F	VA	Sussex	Nottoway River	4-Apr-19	36.85893	-77.18948	NCMNS
NCBAB P283	OQ644687	*Clioperla clio*	A	M	NC	Moore/Richmond	Drowning Creek	28-Mar-19	35.08426	-79.58270	NCMNS
NCBAB P284	OQ644686	*Clioperla clio*	A	M	NC	Hoke/Scotland	Lumber River	17-Apr-19	34.90092	-79.34909	NCMNS
NCBAB P251	OQ644669	*Isoperla borisi*	A	M	NC	Durham	UT Flat River	1-Apr-22	36.13470	-78.84134	NCMNS
NEAP021	-	*Isoperla borisi*	A	M	NC	Orange	Collins Creek	23-Apr-15	35.93114	-79.20568	CCDB
NEAP023	-	*Isoperla borisi*	A	F	NC	Orange	Collins Creek	9-May-15	35.93114	-79.20568	NCMNS
NEAP024	-	*Isoperla borisi*	A	F	NC	Chatham	Georges Creek	18-Apr-15	35.60179	-79.25818	CCDB
NCBAB P046	OQ644660	*Isoperla davisi*	A	M	VA	Sussex	Nottoway River	4-Apr-19	36.85893	-77.18948	NCMNS
NCBAB P047	OQ644659	*Isoperla davisi*	A	M	NC	Harnett	Parkers Creek	31-Mar-19	35.53940	-78.91912	NCMNS
NCBAB P048	OQ644661	*Isoperla davisi*	A	M	NC	Richmond	Naked Creek	28-Apr-19	35.08197	-79.58909	NCMNS
NEAP049	-	*Isoperla davisi*	L	-	NC	Moore	Little Crane Creek	24-Apr-14	35.32534	-79.25102	CCDB
NCBAB P152	OQ644664	*Isoperla decepta*	A	M	IL	Pope	Gibbons Creek	18-May-19	37.58479	-88.44215	NCMNS
NCBAB P153	OQ644662	*Isoperla decepta*	A	F	IL	Pope	Gibbons Creek	18-May-19	37.58479	-88.44215	NCMNS
NCBAB P154	OQ644663	*Isoperla decepta*	A	M	IL	Pope	Eagle Creek	18-May-19	37.59974	-88.42854	NCMNS
NCBAB P041	OQ644681	*Isoperla dicala*	A	M	VA	Craig	Potts Creek	4-May-19	37.59814	-80.22697	NCMNS
NCBAB P042	OQ644680	*Isoperla dicala*	A	M	MI	Presque Isle	Ocqueoc River	11-Jun-19	45.39724	-84.01754	NCMNS
NCBAB P043	OQ644682	*Isoperla dicala*	A	M	VA	Patrick	Dan River	11-May-17	36.22300	-80.44497	NCMNS
NCBAB P044	OQ644683	*Isoperla dicala*	A	M	VA	Scott	Clinch River	6-May-17	36.83108	-82.46252	NCMNS
NCBAB P045	OQ644679	*Isoperla dicala*	A	M	NC	Rockingham	Mayo River	11-May-18	36.53483	-79.99086	NCMNS
NCBAB P049	OQ644668	*Isoperla evanescens*	L	-	VA	Bland	Wolf Creek	3-May-19	37.18026	-81.19496	NCMNS
NCBAB P050	OQ644665	*Isoperla evanescens*	L	-	VA	Bland	Wolf Creek	3-May-19	37.18026	-81.19496	NCMNS
NCBAB P051	OQ644666	*Isoperla evanescens*	A	M	VA	Bland	Wolf Creek	3-May-19	37.18026	-81.19496	NCMNS
NCBAB P052	OQ644667	*Isoperla evanescens*	A	M	VA	Bland	Wolf Creek	3-May-19	37.18026	-81.19496	NCMNS
NCBAB P068	OQ644658	*Isoperla lenati*	A	M	NC	Moore	Little River	17-Apr-19	35.19233	-79.18441	NCMNS
NCBAB P069	OQ644656	*Isoperla lenati*	L	-	NC	Hoke	Flat Creek	13-Mar-19	35.18244	-79.17695	NCMNS
NCBAB P070	OQ644657	*Isoperla lenati*	L	-	NC	Hoke	Flat Creek	13-Mar-19	35.18244	-79.17695	NCMNS
NEAP082	-	*Isoperla lenati*	A	M	NC	Moore	Little River	1-May-03	35.26877	-79.50821	CCDB

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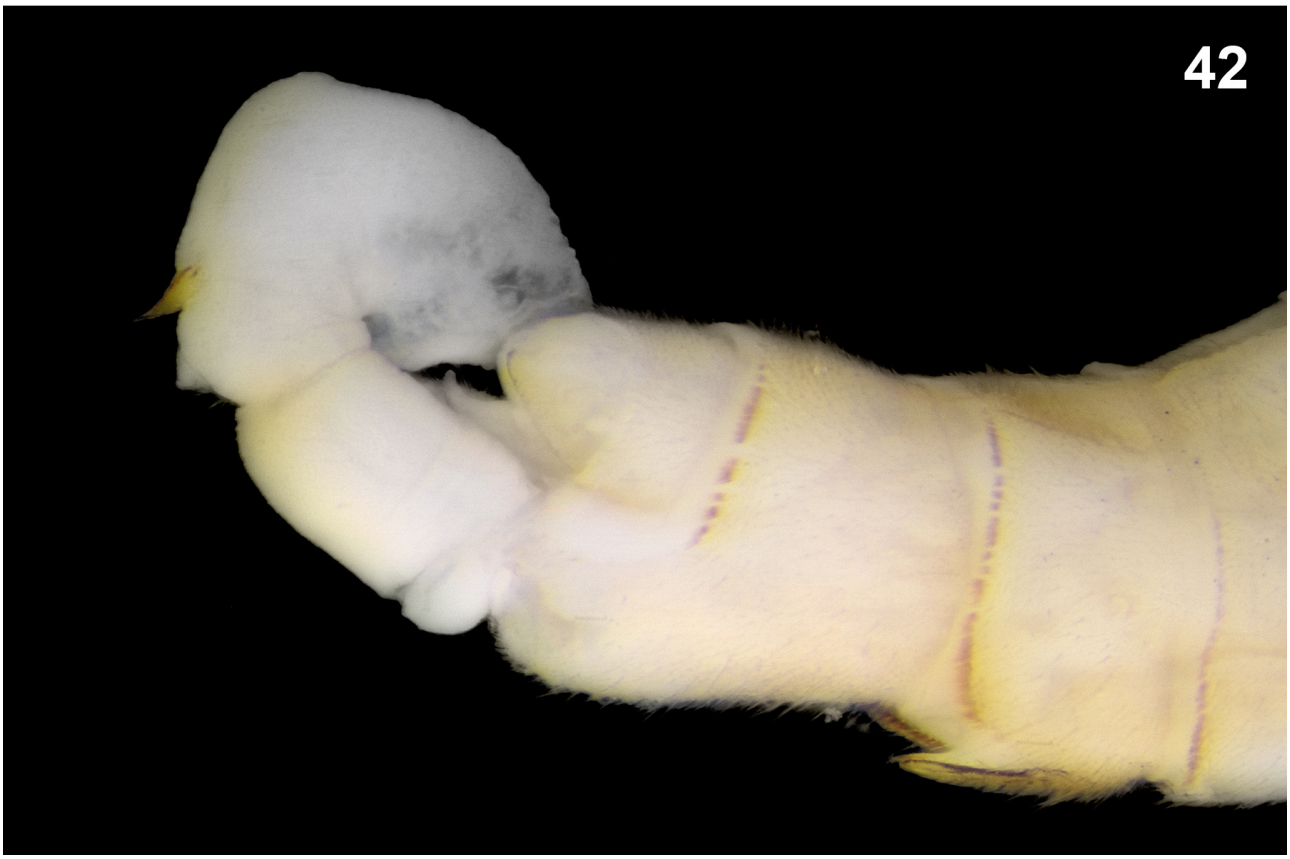
TABLE 1. (Continued)

Accession #	GeneBank #	Taxon	Stage	Sex	State	County	Waterbody	Date	Lat.	Long.	Barcoded by
NCBAB P074	OQ644670	*Isoperla poffi*	A	M	NC	Hoke/Scotland	Lumber River	20-Feb-19	34.90092	-79.34909	NCMNS
NCBAB P075	OQ644671	*Isoperla poffi*	A	F	NC	Hoke/Scotland	Lumber River	20-Feb-19	34.90092	-79.34909	NCMNS
NCBAB P076	OQ644672	*Isoperla poffi*	L	-	NC	Hoke/Scotland	Lumber River	21-Feb-19	34.90092	-79.34909	NCMNS
NEAP058	-	*Isoperla poffi*	L	-	NC	Robeson	Gum Swamp	22-Feb-11	34.71740	-79.27092	CCDB
NCBAB P106	OQ644675	*Isoperla riverae* **sp. n.**	A	M	NC	Rockingham	Mayo River	21-May-19	36.53483	-79.99086	NCMNS
NCBAB P107	OQ644673	*Isoperla riverae* **sp. n.**	A	M	NC	Caldwell	Johns River	6-Apr-19	35.93359	-81.69053	NCMNS
NCBAB P108	OQ644678	*Isoperla riverae* **sp. n.**	A	M	NC	Moore/Richmond	Drowning Creek	28-Apr-19	35.08426	-79.58270	NCMNS
NCBAB P109	OQ644674	*Isoperla riverae* **sp. n.**	L	-	NC	Richmond	Naked Creek	28-Apr-19	35.08197	-79.58909	NCMNS
NCBAB P112	OQ644676	*Isoperla riverae* **sp. n.**	A	F	NC	Moore/Richmond	Drowning Creek	19-May-18	35.08426	-79.58270	NCMNS
NCBAB P245	OQ644677	*Isoperla riverae* **sp. n.**	L	-	NC	Transylvania	Little River	28-Apr-21	35.14821	-82.65801	NCMNS
NCBAB P102	OQ644650	*Isoperla zuelligi*	A	M	NC	Montgomery	Barnes Creek	12-Mar-19	35.43864	-79.99918	NCMNS
NCBAB P103	OQ644651	*Isoperla zuelligi*	L	-	NC	Montgomery	Barnes Creek	12-Mar-19	35.43864	-79.99918	NCMNS
NCBAB P104	OQ644652	*Isoperla zuelligi*	A	M	NC	Montogomery	Little River	10-May-18	35.38679	-79.83131	NCMNS
NCBAB P105	OQ644653	*Isoperla zuelligi*	A	F	NC	Montogomery	Little River	10-May-18	35.38679	-79.83131	NCMNS



FIGURES 41–42. *Isoperla dicala,* adult male, Potts Creek, Craig Co., Virginia. 41. Head and pronotum, dorsal. 42. Terminalia, lateral.

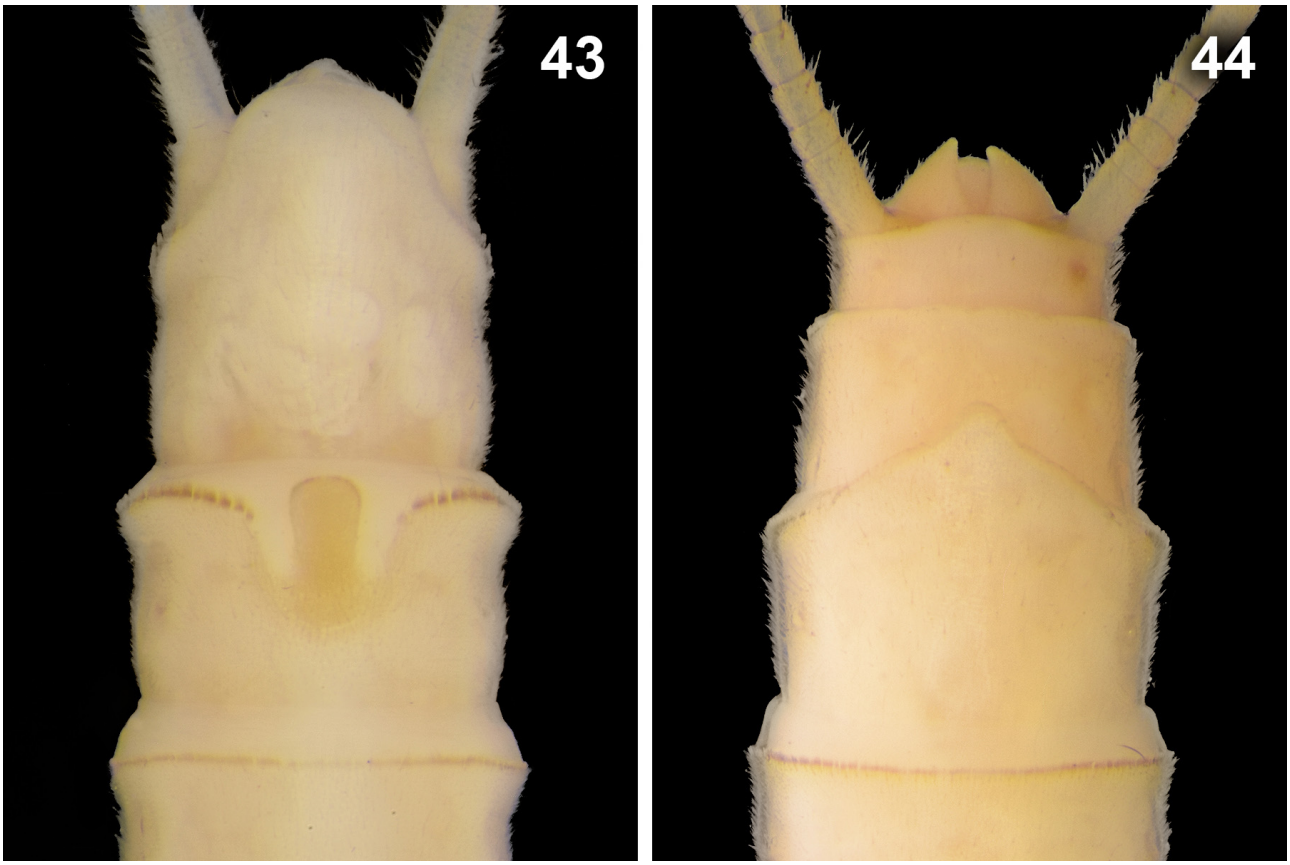
TABLE 2. Average percent (%) pairwise tree distances, calculated from the COI-based ML tree using the Species Delimitation in Geneious Prime 2023.0.2, shown in white. Uncorrected average percent (%) pairwise genetic distances, calculated from the COI data using MEGA v11.0.8, shown in gray. Average percent (%) uncorrected intraspecific pairwise distances, along with sample size, are shown in green.

Species	*Clioperla clio*	*Isoperla riverae* **n. sp.**	*Isoperla poffi*	*Isoperla borisi*	*Isoperla evanescens*	*Isoperla decepta*	*Isoperla dicala*	*Isoperla davisi*	*Isoperla zuelligi*	*Isoperla lenati*
Clioperla clio	**0.23; n=4**	35.40	30.70	34.90	36.40	33.10	28.40	32.30	29.70	27.00
Isoperla riverae **n. sp.**	16.31	**0.67; n=6**	32.50	35.60	37.00	33.70	17.80	32.90	30.30	27.60
Isoperla poffi	15.00	16.16	**0.87; n=4**	32.10	33.50	30.20	25.50	29.40	26.80	24.10
Isoperla borisi	15.77	16.01	13.47	**0.23; n= 4**	29.90	26.70	28.50	25.90	23.20	20.50
Isoperla evanescens	16.19	16.16	13.61	11.40	**0.15; n=4**	14.40	29.90	27.80	28.00	25.30
Isoperla decepta	18.01	17.09	15.32	11.89	10.87	**0.57; n=3**	26.70	24.50	24.70	22.00
Isoperla dicala	17.63	13.66	18.83	18.60	18.33	16.42	**0.63; n=5**	25.90	23.20	20.50
Isoperla davisi	15.12	16.58	13.93	12.61	14.82	13.99	17.35	**0.53; n=4**	23.90	21.20
Isoperla zuelligi	14.63	14.96	13.54	13.34	13.53	14.22	16.33	12.99	**0.86; n=4**	12.50
Isoperla lenati	13.87	15.23	13.41	12.80	12.99	15.09	13.54	11.97	8.74	**1.27; n=4**

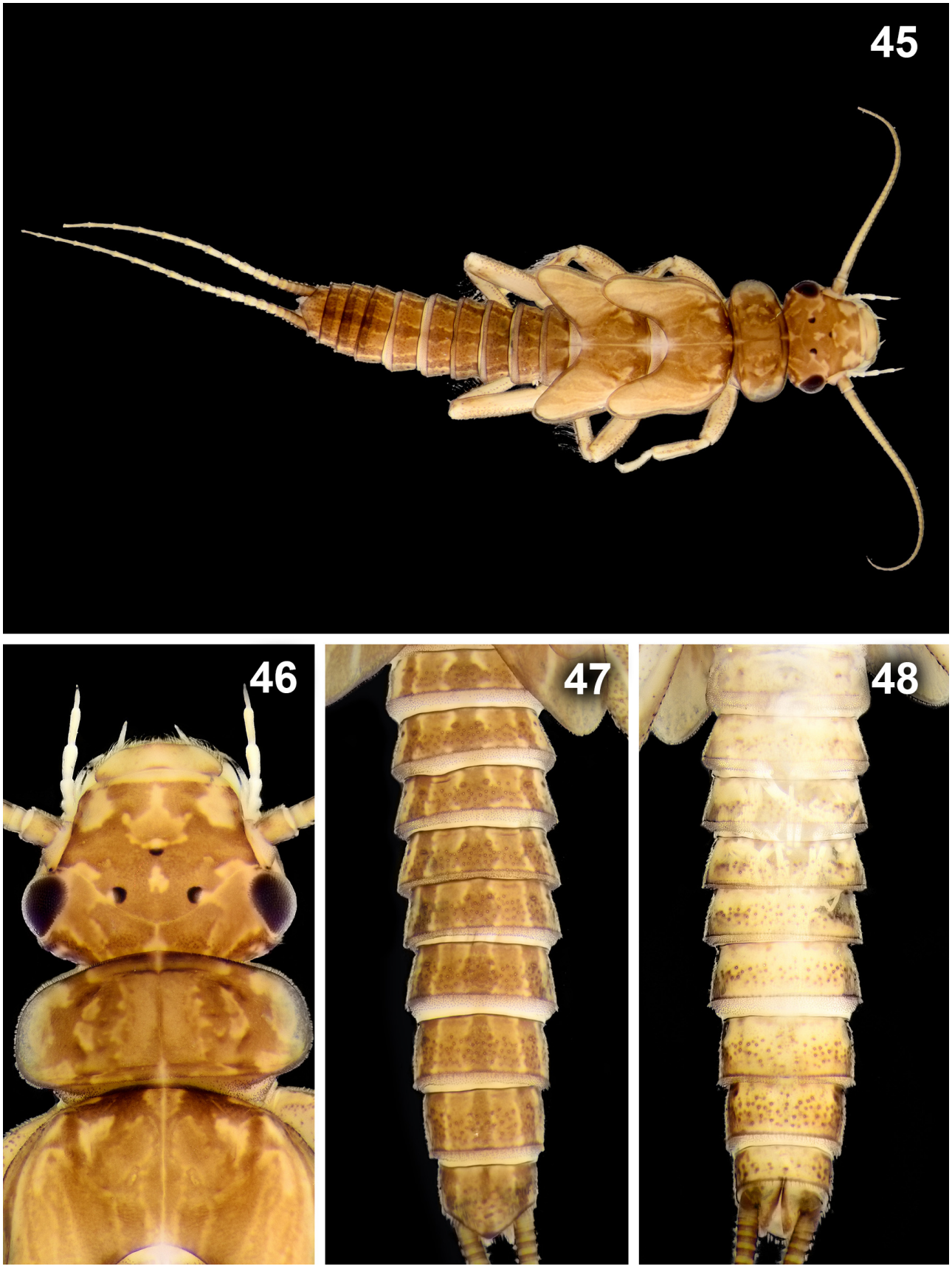
Larvae of the new species have been collected in North Carolina Biological Assessment Branch benthic samples dating back to 1985. However, much like the adult, the uniqueness of larval characteristics was considered to be intraspecific variation of either *I. davisi* or *I. dicala.* As a result of ongoing efforts to rear and associate *Isoperla* life stages, we found that the larva of *I. lenati* is superficially similar to the new species as well. A confounding factor that likely contributed to the past misidentification of *I. riverae* **sp. n.** is that it is sympatric with all the species mentioned above.

These four species have similar maculations, but with close examination of the head pattern, can be readily and confidently separated. The most reliable character state that distinguishes *I. riverae* **sp. n.** from the other three species is that the lateral ocelli are separated from the mediolateral and ocular pale areas. On both *I. davisi* (Fig. 12) and *I. lenati* (Fig. 2) the mediolateral pale spots are broadly confluent with the lateral ocelli. On *I. dicala* (Fig. 46) this is less obvious because the pale ocular areas are somewhat obfuscated, but they are connected to the lateral ocelli via thin isthmi. When distinguishing the new species from *I. dicala* it is also helpful to look at spicules with dark origins on the abdomen (Figs. 47–48). Most importantly, these spicules are present on all abdominal sterna on *I. dicala* (Fig. 48), whereas on *I. riverae* **sp. n.** (Fig. 35)*,* they are only present on sterna 7–10. There are additional characters that distinguish the larvae of the new species from other congeners and will be detailed in a comprehensive review of North Carolina *Isoperla* (S. Beaty *et al*. in progress); however, the characters mentioned above are sufficiently diagnostic in most cases.

Type locality (Fig. 49). Located in south-central North Carolina, Drowning Creek begins in, and flows through, the Sand Hills level IV Ecoregion (Griffith *et al*. 2002) and is the primary tributary of the Lumber River which is, in turn, a major tributary of the Little Pee Dee River. The collection site is 91 m ASL and has a drainage area of 214 km^2. Drowning Creek is typically clear and tannic and is complemented by substrates consisting of gravel, white sand, woody debris and coarse particulate organic matter. Water quality at the type locality has not been assessed using North Carolina Biotic Index (NCBI) protocols (Lenat 1993; NCDEQ 2015), but a long-term monitoring site approximately 4.5 km downstream has rated "Excellent" for 10 consecutive samples dating back to 1985. Although it has not been sampled in the spring during peak species richness, EPT richness often exceeds 30 taxa in the summer months.



FIGURES 43–44. *Isoperla dicala,* adults. 43. Male vesicle, ventral, South Fork Holston River, Smyth Co., Virginia. 44. Female subgenital plate, ventral, Clinch River, Tazewell Co., Virginia.



FIGURES 45–48. *Isoperla dicala,* larva. 45–46. Cullasaja River, Macon Co., North Carolina. 45. Habitus, dorsal. 46. Head and pronotum, dorsal. 47–48 French Broad River, Transylvania Co., North Carolina. 47. Abdomen, dorsal. 48. Abdomen, ventral.

FIGURE 49. Type locality: Drowning Creek, SR 1102, Moore/Richmond Co., North Carolina, 20 May 2018.

Biological notes. *Isoperla riverae* **sp. n.** is widespread in the southeast Nearctic, occurring in four EPA level III Ecoregions (Blue Ridge, Piedmont, Southeastern Plains, Ridge & Valley) across eight states. The North Carolina Division of Water Resources Biological Assessment Branch has 90 verified larval records of *I. riverae* **sp. n.** dating back to 1985. Of these, 61 are from the Blue Ridge, 25 are from the Piedmont, and only four are from the Southeastern Plains. The new species is known to occur in small streams (stream width = 2 m; drainage area = 2.8 km^2) to large rivers (> 40 m; 1572 km^2). The North Carolina Biotic Index pollution tolerance value calculated for *I. riverae* **sp. n.** is 2.3, indicating relatively low pollution tolerance. Additionally, 84% of the streams assessed by NCDWR in which the new species was detected have rated either "Excellent" or "Good".

Larvae can be found in riffle habitats and leaf packs in swift current. Adults typically emerge in mid-April through mid-May. Adult females have been collected as late as mid-July. Like most other *Isoperla,* a univoltine-slow life cycle is presumed for this species.

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