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HOLOMORPHOLOGY AND SYSTEMATICS OF THE EASTERN NEARCTIC STONEFLY GENUS REMENUS RICKER (PLECOPTERA: PERLODIDAE)

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ABSTRACT

The holomorphology of the eastern Nearctic stonefly genus *Remenus* Ricker is reviewed using scanning electron microscopy, color photomicrographs and variation in the barcode region of the mitochondrial DNA Cytochrome Oxidase c Subunit I (COI) gene. Examination of all life stages has resulted in new comparative descriptions and a new key to adults. *Remenus daniellae* Verdone and Kondratieff, sp. n. is described from Great Smoky Mountains National Park, North Carolina and Tennessee, U.S.A. The new species is the fourth species to be included in the genus *Remenus* and is differentiated from *R. bilobatus* (Needham & Claassen, 1925), *R. duffieldi* Nelson & Kondratieff, 1995, and *R. kirchneri* Kondratieff & Nelson, 1995 by the short clavate epiproct with palmate hair-like spinulae.

Larvae can tentatively be separated into two groups based on basal cercal setal length: (1) variable length setae, *R. bilobatus* and *R. daniellae* sp. n., and (2) short setae, *R. duffieldi* and *R. kirchneri*. With the application of known distributions, larvae of *R. kirchneri* and *R. duffieldi* may be distinguished from *R. bilobatus* and *R. daniellae* sp. n. However, sympatry and range overlap precludes the use of this character in distinguishing *R. bilobatus* and *R. daniellae* sp. n. Ova of the four species apparently lack distinguishing characteristics and thus are not separable at this time. Distribution maps, biological notes, and a neighbor-joining tree based on COI DNA barcodes are also presented.

Keywords: Plecoptera, Perlodidae, *Remenus, Remenus daniellae* Verdone & Kondratieff, 2018, key to species, COI DNA sequences

INTRODUCTION

Remenus Ricker, 1952 (Fig. 1) is a small stonefly belonging to the family Perlodidae and is known only from the eastern Nearctic Region (Kondratieff & Nelson 1995). Ricker (1952) first proposed Remenus as a subgenus of Isogenus Newman, 1833

based on the well-developed lobes on sterna 7 and 8, the weakly sclerotized epiproct terminating a threadlike lash, and the larval lacinia which lacks accessory spines and hairs. Illies (1966) subsequently elevated *Remenus* to full generic status. Stark and Szczytko (1984) placed *Remenus* in

the tribe Diploperlini based on four apomorphies: 1) extreme reduction of setae on the larval lacinia; 2) presence of a lobe on the male sternum 7; 3) turtle shaped eggs; and 4) ventral position of the egg collar. Kondratieff and Nelson (1995) last reviewed the species of *Remenus* defining the genus based on two male characters: 1) tenth tergum cleft ½ of its length and producing short hemitergal lobes; 2) well developed lobes on sterna 7 and 8. Two additional species were described in the 1995 review, resulting in three species: *R. bilobatus* (Needham & Claassen, 1925), *R. duffieldi* Nelson & Kondratieff, 1995 and *R. kirchneri* Kondratieff & Nelson, 1995.



Fig. 1. *Remenus kirchneri*, spring fed stream, Blue Ridge Parkway, east of Mt. Olivet Church, Floyd County, Virginia.

Of the three described species, *R. bilobatus* is the most widely distributed, occurring from New York and Connecticut to Alabama in the Adirondack, Appalachian Plateau, Blue Ridge, New England and Piedmont Plateau physiographic provinces (Kondratieff & Nelson 1995). Two species are apparently restricted to the southern section of the Blue Ridge Physiographic Province. The southern Blue Ridge contains the highest peaks in the Appalachian Mountains, covers an approximate area of 40,000 km² and extends 616.4 km northeast

from northern Georgia to Roanoke, Virginia. *Remenus duffieldi* was known only from the Blue Ridge Physiographic Province in northern Georgia, while *R. kirchneri* was thought to be restricted to the Blue Ridge of southern Virginia.

In 2016, we began studying the population status and range of R. kirchneri for the U.S. Fish and Wildlife Service. Based on limited, available occurrence records we defined our study area to include southern Virginia, western North Carolina and eastern Tennessee. Specifically we focused our efforts in the Blue Ridge, and Ridge and Valley physiographic provinces. That year, collecting in Great Smoky Mountains National Park (GRSM), two males and two females of a distinctive undescribed species of Remenus were discovered in a small creek draining from the northeast boundary of the Park in the upper Tennessee River Basin. The epiproct of the two male specimens differed slightly in shape, which is not uncommon with Remenus, as epiproct is mostly membranous consequently, more variable in appearance depending on hemolymphatic pressure. Because of this morphological variability we did not describe the new taxon at that time. In 2017, we attempted to collect this new species again by sampling additional locations in the upper Tennessee River Basin in and around GRSM.

Over the two sampling seasons in 2016 and 2017, we collected 589 specimens of Remenus representing all life stages, affording us a unique opportunity to document the taxonomy of this genus using scanning electron microscopy (SEM) and high resolution color photography. Scanning electron microscopy has been widely used in the study of various stonefly structures (Stark and Kondratieff 2012), but SEM images are not presently available for species of Remenus. In this study, we describe a new species of Remenus, the larva of R. duffieldi and R. kirchneri, and the ova of R. kirchneri. We present for the first time, comparative SEM data for males, and high quality photomicrographs of males, females and larvae. In addition, we provide new diagnoses, biological notes, adult species keys and a neighbor-joining tree based on the barcode region of mitochondrial DNA Cytochrome Oxidase c Subunit I (COI) DNA.

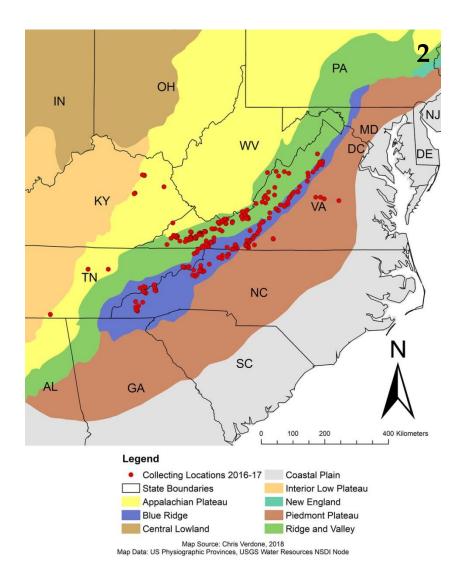


Fig. 2. Map of the eastern Nearctic physiographic provinces and 2016–17 collecting locations.

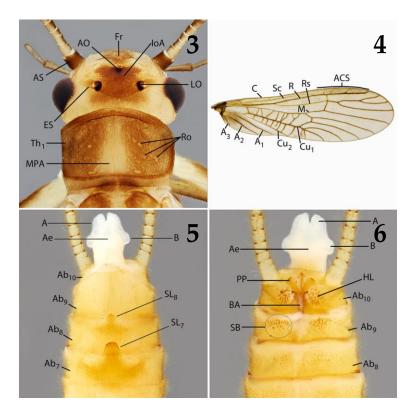
Distribution maps for each species compiled from examined material and published records are also presented.

METHODS AND MATERIALS Study Area

During 21 May–20 June 2016 and 3–30 May 2017, we conducted 300+ sampling events over an area of approximately 45,000 km² primarily within the Blue Ridge, and the Ridge and Valley physiographic provinces of North Carolina, Tennessee and Virginia (Fig. 2). The Blue Ridge Physiographic Province extends 885 km from northern Georgia to

southern Pennsylvania (Pride & Utgard 1985). Sampling in the Blue Ridge was conducted over a linear distance of 599 km from Shenandoah National Park, Virginia to south of Great Smoky Mountains National Park, North Carolina and Tennessee.

The Ridge and Valley Physiographic Province lies to the west of the Blue Ridge and is a belt of northeast-southwest trending ridges and valleys that extends for a distance of 1932 km from northeastern New York to central Alabama (Pride & Utgard 1985). Sampling in Ridge and Valley was conducted over a linear distance of 437 km from



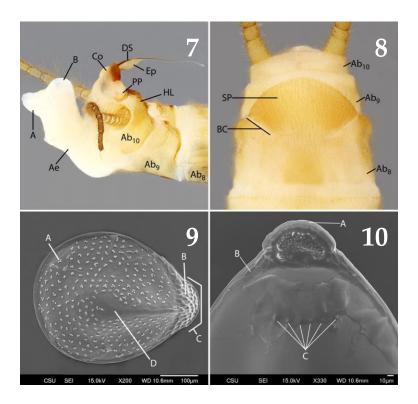
Figs. 3–6. *Remenus bilobatus*, adult male. 3. Head and pronotum, AO = anterior ocellus, AS = antennal scape, ES = epicranial suture, Fr = frons, IoA = interocellular area, LO = lateral ocelli, MPA = medial pale area, Ro = rugosities, Th₁ = pronotum. 4. Right forewing, A = anal vein, ACS, apical costal space, C = Costal vein, Cu = cubital vein, M = median vein, R = radial vein, Rs = radial sector vein, Sc = subcostal vein. 5. Male abdominal sterna 7–10, A = posterodorsal lobes, Ae = aedeagus, Ab = abdominal segment, B = lateral lobes, SL = sternal lobe. 6. Male abdominal terga 8–10, A = posterodorsal lobes, Ae = aedeagus, Ab = abdominal segment, B = lateral lobes, BA = basal anchor, HL = hemitergal lobe, PP = paragenital plate, SB = sensilla basiconica.

northcentral to southwestern Virginia. Sampling in both provinces was targeted at lower order (1^{st} – 3^{rd}) streams, but efforts were made to sample all lotic habitat types from small seeps to higher order (> 4^{th}) rivers.

Collecting

Research was conducted under the following permits: Blue Ridge Parkway, BLRI-2016-SCI-0010; Great Smoky Mountains, GRSM-2016-SCI-0023; Shenandoah National Park, SHEN-2016-SCI-0010; and Virginia Department of Game and Inland Fisheries, 056786. Adult stoneflies were collected using either a beating sheet or an aerial net. Larvae were collected using a 0.093 m² Surber sampler or an aerial net with the net pulled taut and disturbing the

stream substrate allowing contents to flow into the net. Larvae were either preserved in 80% ethanol or reared in aerated chambers in a cooler. Adult specimens were collected alive and kept in modified plastic tubes until fully sclerotized. Adult specimens were prepared under a dissecting microscope for identification. Using wide-tipped forceps, the abdomen of male specimens was gently squeezed until the epiproct was everted and recurved over the abdomen. While maintaining pressure on abdominal segments 7 and 8, specimens were submerged in near boiling water for 2–3 seconds to fix the epiproct in place. Specimens were then preserved in 80% ethanol.



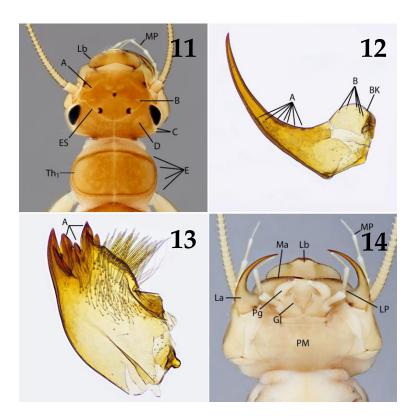
Figs. 7–10. *Remenus bilobatus* adult and ovum. 7. Male terminalia, lateral, A = posterodorsal lobes, Ae = aedeagus, Ab = abdominal segment, B = lateral lobes, Co = Cowl, DS = dorsal sclerite, Ep = epiproct, HL = hemitergal lobe, PP = paragenital plate. 8. Female abdominal sterna 8–10, Ab = abdominal segment, BC = basolateral crease, SP = subgenital plate. 9. Ovum, dorsal, A = globules, B = diamond-shaped follicle cell impressions, C = collar, D = dorsal keel. 10. Ovum, ventral, A = Collar, B = diamond-shaped follicle cell impressions, C = micropyles.

Coordinate data for new material were recorded directly using Topo Maps version 1.16 for iPhone. Additional material was examined from the C.P. Gillette Museum of Arthropod Diversity, Colorado State University, Fort Collins, Colorado (CSUIC), the Illinois Natural History Survey, Champaign, Illinois (INHS), and the United States National Museum of Natural History, Washington D.C. (NMNH). Coordinate data for legacy records were gathered using GEOLocate v. 3.22 (Rios & Bart 2010) and are indicated by "[]". Additional specimen records were provided by the following: Dr. Scott Grubbs, Western Kentucky University, Bowling Green, Kentucky (WKUC), and Luke Myers, Lake Champlain Research Institute, Plattsburgh State University of New York, Plattsburgh, New York (LCRI). Data for all species records may be downloaded from Remenus records.csv.

Plotting of coordinate data and measurements were accomplished using ArcMap, ArcGIS 10.4.1 (ESRI 2016). Watershed boundaries were obtained from USGS National Hydrography Dataset (U.S. Geological Survey 2016a), physiographic boundaries from USGS Water Resources NSDI Node (U.S. Geological Survey 2017), elevation data from USGS National Elevation Dataset (Gesch et al. 2002), and drainage data were acquired from USGS StreamStats program 4.1.1 (U.S. Geological Survey 2016b). Codens for National Parks are: Blue Ridge Parkway (BLRI), Great Smoky Mountains (GRSM), and Shenandoah (SHEN).

Photomicrographs

Color images of stoneflies were captured using a Canon EOS 5D digital camera with a Canon MP-E 65 mm 5X macro lens. Images are a compilation of serial photomicrographs taken at progressively



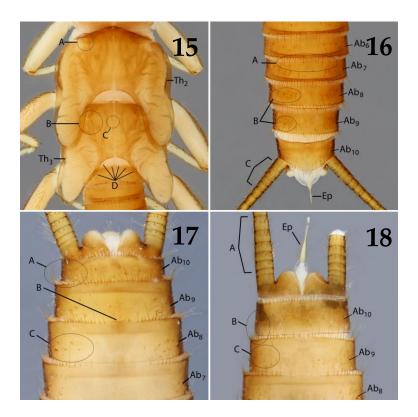
Figs. 11–14. *Remenus bilobatus*, larva, 11. Head and pronotum, A = pale M-shaped mark, B = pale enclosed anterolateral oval spot, C = postocular setae, D = large enclosed posterolateral oval spot, E = long erect marginal setae, ES = epicranial suture, Lb = labrum, Mp = maxillary palp, Th₁ = pronotum. 12. Left lacinia, dorsal, A = minute irregularly spaced setae, B = minute submarginal setae, BK = basal knob. 13. Left mandible, dorsal, A = Middle three teeth with proximal margins lightly serrated. 14. Head, ventral, Gl = glossa, La = lacinia, Lb = labrum, LP = labial palp, Ma = mandible, MP = maxillary palp, Pg = paraglossa, PM = prementum.

deeper focal planes using Stack Shot and controlled by Visionary Digital Passport software (Visionary Digital, Palmyra, Virginia). Composite images were assembled using Zerene Stacker version 1.04 (Zerene Systems LLC, Richland, WA). Measurements and image adjustments including background color correction, color levels and sharpening functions were achieved using Adobe Photoshop CS6 Extended.

Scanning Electron Micrographs

Abdomens were cleaned using a fine tipped paint brush and sonicated in glass microvials filled with 80% ethanol for 30 seconds. Terminalia were serially dehydrated in ethanol in ten minutes intervals at concentrations of 80%, 90% and 95%. Further dehydration was accomplished using critical point

drying. Specimens were mounted on aluminum stubs using double stick copper tape and isopropanol graphite paint. Ova were either taken from an extruded egg mass or dissected from the female oviduct and cleaned in 80% ethanol using a fine tipped paint brush and sonicated for 30 seconds to remove tissue adhered to the ovum surface. Ova were air dried, then mounted on an aluminum stub using double stick copper tape. Abdomens and ova were sputter coated in 12 nm gold in preparation for scanning electron microscopy. Micrographs were taken using a JEOL JSM-6500F Field Emission Scanning Electron Microscope at the Central Instrument Facility, Imaging Laboratory, Colorado State University (http://cif.colostate.edu/imaginglaboratory/).



Figs. 15–18. Remenus bilobatus, larva. 15. Meso- Meta-nota, A = mesonotal anterolateral setae, B = metanotal anterolateral setae, C = mediolateral setae, D = marginal setae, $Th_2 = mesonotum$, $Th_3 = metanotum$. 16. Male abdominal terga 6–10, A = posterior setal fringe, Ab = abdominal segment, B = intercalary setae, C = basal cercal segments, Ep = male terminal process (developing epiproct). 17. Female sterna 7–10, E = abdominal segment, E = abdominal segmental setae, E = abdominal segmental se

DNA Barcodes

Tissue from 15 adult specimens representing each Remenus species was sent to the Canadian Center for DNA Barcoding for sequencing. Standard protocols were used to obtain the barcode region of the cytochrome oxidase I gene of the mitochondrial genome (deWaard et al. 2008). Sequences were aligned using MUSCLE (Edgar 2004) in Geneious version 11.0.5 (Kearse et al. 2012). Neighbor-joining analysis (Saitou & Nei 1987) was conducted in Geneious using the Tamura-Nei nucleotide substitution model (Tamura & Nei 1993) and pairwise deletion option. Nodal support was assessed using 1,000 bootstrap replicates (Felsenstein 1985). Sequences are available on the

Barcode of Life Database (BOLD; http://www.boldsystem.org; Ratnasingham & Hebert.

RESULTS

Taxonomic Characters

External male genitalia. The epiproct of *Remenus* is mostly membranous, variable in shape and may or may not possess a medial dorsal sclerite (Figs. 7, 23–24, 26–28, 45–46, 49–52, 67, 74–75, 77–80, 95–96, 98–100). The membranous portions are moderately to densely covered with minute hair-like setae (Figs. 27–28, 49–53, 77–79, 98–100). The epiproct is attached to a sclerotized basal anchor (Li & Murányi 2015). These structures are held within a membranous cowl and are movable (Figs. 7, 23–24,

26, 45–46, 49–50, 74–75, 95–96). The epiproct can be

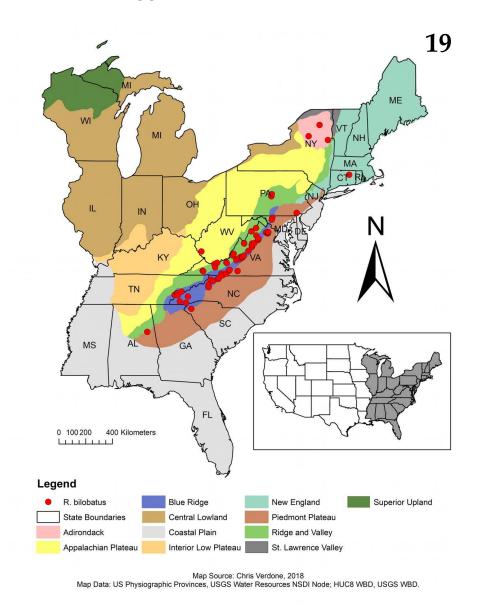


Fig. 19. Remenus bilobatus, distribution of examined material and published records.

everted such that it is recurved over the surface of the tenth tergum. The hemitergal lobes on the tenth tergum vary in length and sensilla density (Figs. 6–7, 25, 47–48, 76, 97). Hemitergal lobes typically possess two types of socketed sensilla, long hair-like trichoid sensilla and short conical sensilla basiconica (Figs. 25, 47–48, 76, 97). Terga 8 and 9 may or may not also possess patches of sensilla basiconica (Figs. 6–7, 23–24, 45–46, 74–75, 95–96).

Female genitalia. The subgenital plate is typically produced over sternum 9, or occasionally beyond (Figs. 8, 29–30, 54–56, 81, 97–99). The posterior and lateral margins are variable between and within species (Figs. 8, 29–30, 54–56, 81, 101–103). A variable glabrous crease is typically present at the basolateral margins of the subgenital plate and extends into tergum 8 to various degrees (Figs. 8, 29–30, 54–56, 81, 101–103).

Pigment patterns. Pronotal (Figs. 3, 22, 44, 72, 94) and abdominal (Figs. 6, 23, 45, 73, 95) pigment patterns can be useful in distinguishing adults of some species, but caution is suggested when using these pigment patterns after material is preserved. Larva. Remenus larvae are small, generally light to medium brown and possess few distinguishing characters. Basal cercal setae whorls (Stark 2017) tentatively separated the species into two groups: (1) Basal cercal segments with variable length setae (both short and long), R. bilobatus (Fig. 40) and R. daniellae sp. n. (Fig. 66); (2) Basal cercal segments with short setae, R. duffieldi (Fig. 90) and R. kirchneri (Fig. 113). Caution should be exercised in using this character as it is sometimes difficult to detect.

Keys to Remenus Species

Adult males

- 2' Epiproct dorsoventrally flattened; lacking a threadlike lash that greatly exceeds the epiproct apex (Figs. 74–75, 77–80); basal cowl covered in dense lightly pigmented spinulae (Figs. 74–75); tergum 9 lacking sensilla basiconica (Fig. 74); pronotum mostly pale (Fig. 72) *R. duffieldi*

Adult females

- 2 Subgenital plate broadly triangular (Fig. 29) or rounded (Figs. 30), extending ½ over sternum 9, or to the posterior margin of the sternum 9; pronotum mostly brown (Fig. 22) *R. bilobatus*
- 2' Subgenital plate broadly rounded, elongate, extending \% over of sternum 9 or slightly beyond the posterior margin of sternum 9 (Fig. 81); pronotum mostly pale (Fig. 72) ... R. duffieldi

Generic Description

Genus Remenus Ricker, 1952

(Figs. 1, 3–18)

Type species: *Perla bilobata* Needham & Claassen (1925: 95)

Isogenus (Remenus) Ricker (1952: 122)

Remenus: Illies (1966: 376) Remenus: Hitchcock (1974: 214)

Remenus: Stewart & Stark (1984: 406)

Remenus: Stewart & Stark (1988: 402)

Remenus: Kondratieff & Nelson (1995: 596)

Remenus: Stewart & Stark (2002: 437)

Remenus: Kondratieff (2004: 164)

Remenus: Stark (2017: 236)

Male. Macropterous; forewing length 9.1-11.0 mm (n = 40). Body length 7.9-10.6 mm (n = 40). General body color yellow-gold with light brown markings Fig. 1). Head as wide as, or wider than pronotum;



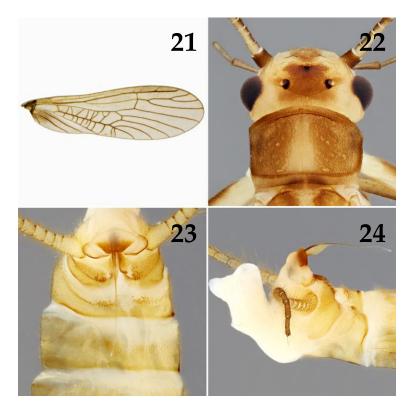
Fig. 20. Adult male habitus of *Remenus bilobatus*, Calfpasture River, Augusta County, Virginia.

Fig. 1). Head as wide as, or wider than pronotum; dorsum of head covered with sparse setae (Fig. 3); interocellular area enclosed with medium brown pigment (Fig. 3); pigment generally narrows towards the anterior ocellus and extends laterally from anterior ocellus to center of lateral ocelli; epicranial suture extending well beyond lateral ocelli (Fig. 3); frons and clypeus with medial triangular light brown patch (Fig. 3). Antennal scape medium brown, flagellum light brown, diffuse laterally on basal 4 segments (Fig. 3). Pronotum covered in regularly spaced setae, with medial pale area and glabrous rugosities mediolaterally (Fig. 3); anterior margin broadly rounded; lateral margins nearly straight, sometimes irregular; anterior and posterior margins light brown to brown (Fig. 3). Wings hyaline, covered in amber colored setae (Fig. 4); veins light brown; apical costal space with 2–3 crossveins (Fig. 4). Y-arms of mesosternum meeting posterior corners of furcal pits. Femora pale, light brown dorsally. Tibia pale, light brown dorsally. Tarsi light brown. Abdominal sterna pale to yellowgold; lobe on 7th sternum well developed, lightly pigmented, narrowly rounded, apex rounded (Fig. 5); lobe on 8^{th} sternum reduced, ~ $\frac{1}{3}$ the size of lobe on sternum 7, triangular, lightly pigmented, apex acutely rounded (Fig. 5). Abdominal terga usually yellow-gold, with variable color pattern, covered in regularly spaced with setae; terga 8 and 9 with variable patches of sensilla basiconica (Fig. 6). Hemitergal lobes variously produced with long trichoid sensilla and various densities of sensilla basiconica (Fig. 6). Cerci pale, setose (Figs. 5-6). Lateral stylets absent. Epiproct variable, small, mostly membranous; with or without mediodorsal sclerite (Fig. 7). Membranous portion covered in dense, minute hair-like spinulae. Paragenital plates flap-like, triangular or rounded (Figs. 6-7). Basal cowl clothed in dense variably pigmented spinulae (Fig. 7). Aedeagus entirely membranous with two lateral and two posterodorsal lobes (Figs. 5–7).

Female. Macropterous; forewing length 9.4–12.0 mm (n = 40). Body length 8.8–12.6 mm (n = 40). General body coloration similar to male. Abdominal terga and sterna generally uniformly pale yellow. Subgenital plate broadly rounded or broadly triangular, produced over ½ sternum 9, or beyond (Fig. 8); with regularly spaced setae; a variable glabrous basolateral crease is present at the base of the subgenital plate and extends anteriorly up to ½ into tergum 8 (Fig. 8).

Ovum. Length 434–460 µm; width 324–361 µm (n = 12). Turtle-shaped with a ventrally positioned collar (Fig. 9). Mature ovum with a membranous covering, adorned with regularly spaced globules in clusters of 2–5 (Fig. 9). Dorsum of chorion smooth with very faint diamond-shaped follicle cell impressions. Dorsal keel extending from collar center to ovum center (Fig. 9). Collar with prominent diamond-shaped follicle cell impressions dorsally and ventrally (Figs. 9–10). Ovum margin with a thickened raised edge. Micropyles positioned ventrally; arranged in a latitudinal row of 6 near the collar $\frac{1}{3}$ (Fig. 10).

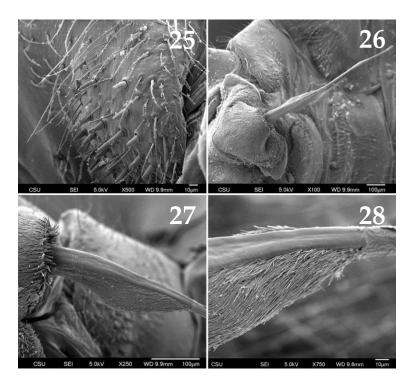
Larva. Body length 8.3–11.4 mm, (n = 19). Preserved



Figs. 21–24. *Remenus bilobatus*, adult male. 21. Right forewing, Crooked Creek, Carroll County, Virginia. 22. Head and pronotum, Calfpasture River, Augusta County, Virginia. 23–24. Blackrock Spring, SHEN, Albemarle County, Virginia. 23. Terminalia, dorsal, 24. Terminalia, lateral.

specimen yellow-brown. Head wider pronotum (Fig. 11); dorsum of head yellow- brown; frons with a faint, thin, pale M-shaped mark (Fig. 11); pale enclosed oval spots anterolateral of lateral ocelli, anterior of epicranial suture (Fig 11); large enclosed oval spots posterolateral of lateral ocelli, posterior of epicranial suture (Fig. 11); 2-4 postocular setae (Fig. 11); frontoclypeus unpigmented; labrum anterior margin with a setal fringe (Fig. 11); medial lobe of labrum with a dense fringe of golden setae. Lacinia unidentate (Fig. 12); lacinia sickle-shaped, tapering evenly from apical tooth and bearing stout, rounded, basal knob (Fig. 12); apical tooth with ~ 12 minute irregularly spaced setae extending halfway up the lacinia from the inner basal surface across the dorsal surface to the distal margin (Fig. 12). Basal knob with 5-6 submarginal minute setae (Fig. 12). Mandible with 5 teeth and without a deep cleft between the apical and subapical teeth (Fig. 13). Middle three teeth

with proximal margins lightly serrated; dorsum of mandibles with a dense patch of stout setae (Fig. 13); proximal margin with a dense brush of stout setae on apical 1/3 (Fig. 13). Maxillary palp slightly longer than lacinia (Fig. 14). Submental gills absent (Fig. 14). Pronotum yellow-brown; 10–16 long erect marginal setae on each side; lateral margins of pronotum brown (Fig. 11). Meso- and metanota yellow-brown; with 0-1 seta on either side of midline; 1–6 anterolateral setae (Fig. 15); wing pads with 1-7 marginal setae on proximal margin (Fig. 15). Y-arms of mesosternum reach the posterior corners of furcal pits. Femora pale yellow-brown with long stout setae over surface and without a dorsal fringe of silky setae. Tibia with short stout submarginal setae on dorsal and ventral surfaces and a dorsal fringe of silky setae. Tarsi with a short dorsal fringe of silky setae. Abdominal terga yellow-brown, with a posterior fringe of variable length setae (Fig. 16); longest setae ~ ⅓ length of tergum; terga 1–2



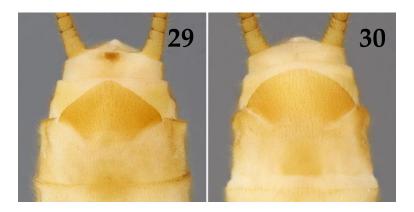
Figs. 25–28. *Remenus bilobatus*, adult male, Blackrock Spring, Albemarle County, Virginia. 25. Male hemitergal lobe. 26. Male terminalia, dorsal. 27. Male epiproct, dorsolateral. 28. Male epiproct, hair-like setae, dorsolateral.

without intercalary setae (Fig. 16); terga 4-10 with < 35 intercalary setae (Fig. 16). Abdominal sterna pale yellow-brown; sterna 1-2 without a posterior fringe or intercalary setae; sternum 3 occasionally with a single posterior seta laterally; sterna 4-8 with < 35 intercalary setae and a posterior fringe of variable length setae that is interrupted medially (Fig. 17); highest concentration of intercalary setae on abdominal segments 7-9 (Figs. 16-17); female 8th sternum with a thin darkened strip posteromedially (Fig. 17); posterior fringe complete on sterna 9–10 (Fig. 17). Mature male larvae with an elongate terminal process (developing epiproct) with a constriction at the basal 1/3, apical 2/3 thin and tapered (Fig. 18). Mature larvae with paired medial and lateral spots on the abdominal terga. Cerci with whorls of short or long setae, or a combination of both (Figs. 16, 18).

Diagnosis. Regionally, *Remenus* is morphologically most similar to *Diploperla duplicata* (Banks, 1920). The two are often sympatric and share the following characters: (1) male tenth tergum partially cleft; (2)

lobe on 7th sternum well developed, narrowly rounded, and lobe on 8th sternum reduced, ~ ½ the size of lobe on 7th sternum; (3) female subgenital plate broadly rounded with a membranous basolateral crease; (4) interocellular area enclosed with medium brown pigment that generally narrows towards the anterior ocellus and extends laterally from anterior ocellus to center of lateral ocelli; (5) larvae and adults lack submental gills; (6) larvae are similarly concolorous; (7) larvae have a dorsal fringe of setae on the tibia and lack a fringe on the femora; and (8) ova are turtle shaped.

Adult males of *Remenus* are distinguished from *D. duplicata* by the presence of a well-developed epiproct and the lack of lateral stylets. Adult females of *Remenus* differ by the presence of distinct mesosternal Y-arms and < 3 cross veins in the apical costal space. Whereas *D. duplicata* has > 5 cross veins in the apical costal space. Larvae of *Remenus* are easily distinguished from *D. duplicata* by their unidentate lacinia and well-developed mesosternal Y-arms. Ova of *Remenus* are also easily separated by



Figs. 29–30. *Remenus bilobatus*, adult female subgenital plate variation. 29. Crooked Creek, Carroll County, Virginia. 30. Calfpasture River, Augusta County, Virginia.

their relatively smooth chorionic surface and the diamond-shaped follicle cell impression on the collar.

Species Accounts

Remenus bilobatus (Needham & Claassen, 1925)

(Figs. 3-18, 20-40)

Perla bilobata Needham & Claassen (1925: 95) Holotype 3: New York, Herkimer County, Old Forge (Cornell University Insect Collection). Figures: adult–fore- and hindwing, male stern 7–9, male terminalia (lateral), female subgenital plate.

Perla bilobata: Claassen (1931: 54). Figures: larvalabrum, mandibles, labium, maxilla, lacinia.

Diploperla bilobata: Frison (1942: 302). Figures: larvahabitus, female sterna, mandibles, labium, maxilla, lacinia.

Isogenus (Remenus) bilobatus: Ricker (1952: 122)

Remenus bilobatus: Illies (1966: 376)

Remenus bilobatus: Hitchcock (1974: 214). Figures: larva–habitus, female sterna (Frison 1942); adult–male terminalia (dorsal, lateral), female subgenital plate (incorrectly associated).

Remenus bilobatus: Surdick & Kim (1976: 9)

Remenus bilobatus: Lake (1980: 43)

Remenus bilobatus: Tarter & Kirchner (1980: 49)

Remenus bilobatus: Kondratieff & Voshell (1982: 761).

Figures: adult-head and pronotum, male terminalia (dorsal), female subgenital plate.

Remenus bilobatus: Stewart & Stark (1984: 406). Figures:

larva-head and pronotum, mandible, lacinia, foreleg, mesosternum, male and female sterna 7–10, cercus (basal, middle, apical).

Remenus: Stewart & Stark (1988: 402). Figures: larvamale habitus, and figures from Stewart & Stark (1984). Remenus bilobatus: Kondratieff & Nelson (1995: 596). Figures: adult–male terminalia (dorsal, lateral), female subgenital plate, ova (dorsal, ventral).

Remenus bilobatus: Grubbs (1997: 81)

Remenus bilobatus: Stewart & Stark (2002: 437) Figures: larva–figures from Stewart & Stark (1984, 1988)
Remenus bilobatus: Kondratieff (2004: 164). Figures: adult–figures from Kondratieff & Nelson (1995)
Remenus bilobatus: DeWalt & Heinold (2005: 40)
Remenus bilobatus: DeWalt et al. (2007: 142)
Remenus bilobatus: Parker et al. (2007: 171)

Remenus bilobatus: Grubbs (2011: 29)

Remenus bilobatus: Tarter & Nelson (2010: 162)

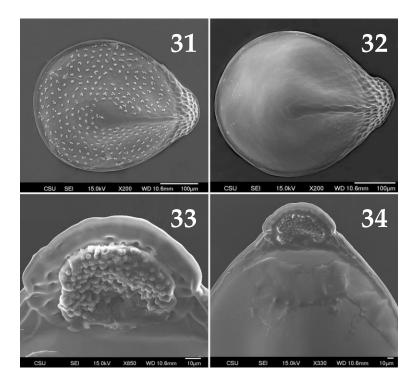
Remenus bilobatus: Li & Murányi (2015: 47). Figures:

adult–male epiproct, basal anchor. *Remenus bilobatus*: Tarter et al. (2015: 170)

Remenus bilobatus: Stark (2017: 236). Figures: larva-

cercus (basal, middle)

Material examined: Alabama, Cleburne Co., small stream above lake, Cheaha State Park, [N 33.47396, W 85.82077], 14 May 1988, B.C. Kondratieff, R.F. Kirchner, 3♂, 3♀ (CSUIC). Connecticut, Middlesex Co., brooklet, Rte 148, Killingworth [No GPS], 18 June 1965, S.W. Hitchcock, 4♂ (NMNH); nr. Madison, nr. Rte 80, [no GPS], 18 June 1965, S.W. Hitchcock, 1♂ (NMNH). Salem Co., Fraser Brook, Salem, Four Corners, [N 41.47291, W 72.26029], 15 June 1967, S. W. Hitchcock, 1♂ (NMNH). Tolland



Figs. 31–34. *Remenus bilobatus* ovum, Blackrock Spring, Albemarle County, Virginia. 31. Ovum with membranous covering, dorsal. 32. Ovum, bare, dorsal. 33. Collar, ventral. 34. Micropyles, ventral.

Co., Storrs, [N 41.80843, W 72.24952], 18 June 1954, J.A. Slater, $1 \circlearrowleft$, $1 \circlearrowleft$ (NMNH). Maryland, Frederick Co., Fishing Creek, Mountaindale, [N 39.51949, W 77.45114], 1 July 1958, P. Freytag, 3& (INHS). New York, Herkimer Co., Old Forge, [N 43.71007, W 74.97434], 18 July 1905, J.G. Needham, P.W. Claassen, $1 \circlearrowleft$, $1 \circlearrowleft$ (NMNH, paratypes) **North** Carolina, Alleghany Co., Brush Creek, Blue Ridge Parkway, BLRI, N 36.46067, W 80.99979, 26 May 2016, C. Verdone, B.C. Kondratieff, 1& (CSUIC); Stone Mountain Creek, Stone Mountain Rd., Stone Mountain State Park, N 36.39445, W 81.03341, B.C. Kondratieff, R.F. Kirchner, R.E. Zuellig, D.R. Lenat, 10\$\rightarrow\$, \$3\rightarrow\$ (CSUIC). **Ashe Co.**, tributary to Peak Creek, Peak Creek Rd., BLRI, N 36.38101, W 81.27936, 21 May 2017, [emerged 25 May 2017], C. Verdone, D. Fuller, 1♀ (CSUIC); Same data, [emerged 26 May 2017], 12 (CSUIC); Same data, [emerged 30 May 2017], 1♀ (CSUIC). Caldwell Co., Johns River, Old Johns River Rd., NE of Collettsville, N 35.94711, W 81.70472, 2 May 2005, B.C. Kondratieff, R.F. Kirchner, R.E. Zuellig, D.R. Lenat, 1L (CSUIC); Johns River, St. Johns River Rd. just upstream of

Franklin Branch NE of Collettsville, N 35.93361, W 81.69056, 17 May 2004, B.C. Kondratieff, R.F. Kirchner, R.E. Zuellig, D.R. Lenat, 1♂ (CSUIC); Thunderhole Creek, Rte 1366, N 36.07324, W 81.69975, 14 May 2017, C. Verdone, B.C. Kondratieff, 3♀, 1L (CSUIC). **Jackson Co.,** Balsam, [N 35.42667, W 83.08528], 24 April 1938, H.H. Ross, B.D. Burks, 6L (INHS). Macon Co., Big Creek, Lake Randall, [N 35.07861, W 83.20806], 20 June 1939, T. Howell, 1L (INHS); Jarrett Creek, Arrowwood Glade, [N 35.15444, W 83.58616], 25 May 1993, B.C. Kondratieff, R.F. Kirchner, 1♂ (CSUIC). Swain Co., Ekaneetlee Creek, 75 m upstream confluence Eagle Creek nr. Camp 89, N 35.49810, W 83.76640, 10-11 June 2003, B.D. Heinold, 1♂ (INHS); Skidder Branch, Straight Branch mi. 20, [N 35.59062, W 83.23494], 28 July 1982, B. Armitage, 1♀ (CSUIC); Twentymile Creek, Twentymile Creek Trail, Camp 93, GRSM, N 35.47300, W 83.85240, 1 July 2004, R.E. DeWalt, 1♀ (INHS). Transylvania Co., Pigeon Branch of South Fork Mills River, NFR 1206 off Rte 276, [N 35.35815, W 82.77810], 8 July 1981, B.C. Kondratieff, 1♂, 1♀ (CSUIC). Watauga Co., Clear

Branch Blue Ridge Parkway, BLRI, N 36.22954, W 81.54462, 21 May 2017, C. Verdone, D. Fuller, 1♀ (CSUIC). Wilkes Co., Betseys Rock Falls Creek, Walsh Rd. NW of Wilkesboro, N 36.28380, W 81.39729, 11 July 2008, B.C. Kondratieff, R.E. Zuellig, D.R. Lenat, 16 (CSUIC); Garden Creek, Stone Mountain Rd., N 36.38905, W 81.06922, 29 May 2006, B.C. Kondratieff, R.F. Kirchner, R.E. Zuellig, D.R. Lenat, 3♂, 4♀, 7L (CSUIC). **Pennsylvania, Chester** Co., Wet Lab at Stroud Research Center, London Grove, [N 39.86911, W 75.77245], 27 May 1980, D.H. Funk, 7♂ (INHS); Same location, 4 June 1980, D.H. Funk, 33, 39 (INHS). **Mifflin Co.**, Honey Creek, Reeds Gap, St. Park, New Lancaster Valley Rd., [N 40.72150, W 77.47426], 7 June 2013, B.C. Kondratieff, J. Sandberg, 2d (CSUIC); Little Poe Creek, Poe Valley, Little Poe Rd. 2 mi. E Poe Paddy State Park, [N 40.82977, W 77.44454], 8 June 2013, B.C. Kondratieff, J. Sandberg, 16 (CSUIC). South Carolina, Pickens Co., Wildcat Creek, 9 km SW Clemson, [N 34.75607, W 82.85767], 18 May 1976, P.H. Carlson, 16 (CSUIC); Wildcat Creek, Clemson Experimental Forest, [N 34.75607, W 82.85767], 5 May 1985, K.W. Stewart, B.C. Kondratieff, R.F. Kirchner, 1L (INHS). Tennessee, Blount Co., Abrams Creek, Cades Cove, Old Field ATBI Plot, [N 35.60470, W 83.77570], 9 May-3 June 2002, J. Burbank, 1♂ (CSUIC); Abrams Creek, Cades Cove, Sparks Lane, GRSM, N 35.60400, W 83.79390, 7 June 2001, B.D. Heinold, 1d (INHS); Abrams Creek, Cades Cove, GRSM, [N 35.60494, W 83.77653], 1-12 June 2003, C.R. Parker, 4♂ (CSUIC); Same location, 18–25 June 2003, C.R. Parker, ♂ (CSUIC); Cades Cove, GRSM, [N 35.60840, W 83.82700], 13 June 1940, T.H. Frison, 13, 1 exuvia (INHS); Cattail Branch, GRSM, N 35.51490, W 83.97630, 21-22 May 2003, R.E. DeWalt, B.D. Heinold 3L (INHS); Tabcat Creek, at first jeep trail crossing, GRSM, N 35.51956, W 83.97927, 21 May 2003, R.E. DeWalt, 4L (INHS). Sevier Co., Le Conte Creek, Gatlinburg, [N 35.70164, W 83.51361], 4 May 1939, T.H. Frison, 7L (INHS). Virginia, Albemarle Co., Blackrock Spring, Fire Rd. off Skyline Dr. (SHEN), N 38.20504, W 78.74916, 3 June 2016, C. Verdone, B.C. Kondratieff, S. Roble, 83, 29 (CSUIC); South Fork Moormans River, Fire Rd. of Skyline Dr. (SHEN), N 38.09850, W 78.77815, 3 June 2016, C. Verdone, B.C. Kondratieff, S. Roble, 1♀ (CSUIC). Alleghany Co.,

Hays Creek, Rte 619, N 37.73125, W 80.00259, 10 June 2016, C. Verdone, B.C. Kondratieff, 23, 29(CSUIC). Amherst Co., Dancing Creek, Blue Ridge Parkway, Dancing Creek Overlook Parking, BLRI, N 37.63430, W 79.33258, 18 June 2016, C. Verdone, 16 (CSUIC). **Augusta Co.**, Calfpasture River, Jct. Hwy 250 and Rte 715, [N 38.27356, W 79.30068], 8 June 2017, B.C. Kondratieff, $5 \circlearrowleft$, $3 \circlearrowleft$ (CSUIC); Love bog Blue Ridge Parkway, mile 15.61, BLRI, [N 37.88348, W 79.01054], 16-17 July 2007, C.R. Parker, 16 (CSUIC); Paine Run, Jct 661 and 614, [N 38.19880, W 78.79340], 19 July 1975, O.S., C.M Flint 13 (NMNH); tributary to Braley Pond, NW Rte 715, [N 38.28963, W 79.30448], 9 June 2017, B.C. Kondratieff, 16 (CSUIC). **Bedford Co.,** Battery Creek, FR 951, N 37.55194, W 79.44059. 17 June 2016, C. Verdone, 2♂, 1♀ (CSUIC); Falling Rock Creek, Blue Ridge Parkway, BLRI, N 37.56403, W 79.40851, 27 May 2017, C. Verdone, D. Fuller, 2♂ (CSUIC); Hunting Creek, Rte 602, N 37.53511, W 79.42025, 17 June 2016, C. Verdone, 13, 19 (CSUIC); Little Stony Creek, Hwy 43, Peaks of Otter Picnic Area, BLRI, N 37.4454, W 79.59713, 17 June 2016, C. Verdone, 5♂, 6♀ (CSUIC). **Bland Co.,** Laurel Creek, Rte 613, under Hwy 77, N 37.25547, W 81.12081, 8 June 2016, C. Verdone, B.C. Kondratieff, 1♂ (CSUIC); Wolf Creek, Hwy 61, [No GPS], 10 June 1978, B.C. Kondratieff, 1♀ (CSUIC). **Botetourt Co.**, spring fed stream, Blue Ridge Parkway, BLRI, N 37.39235, W 79.83864, 31 May 2016, C. Verdone, B.C. Kondratieff, 3♂, 2♀ (CSUIC); Bearwallow Creek, Hwy 443, N of BLRI, Bearwallow Gap, N 37.4856, W 79.66866, 19 May 2017, C. Verdone, B.C. Kondratieff, 13, 14(CSUIC). Carroll Co., Big Reed Island Creek Rd. between Rte 640 & Rte 645, N 36.67665, W 80.51019, 30 May 2016, C. Verdone, B.C. Kondratieff, 1♂ (CSUIC); Crooked Creek, Rte 620, trout fishing trail, N 36.67296, W 80.80853, 29 May 2016, C. Verdone, B.C. Kondratieff, 20%, 27 (CSUIC); Stewarts Creek, Rte 896, N 36.58083, W 80.76437, 30 May 2016, C. Verdone, B.C. Kondratieff, 4♂ (CSUIC); tributary to Crooked Creek, Rte 620 nr. abandoned barn, N 36.67318, W 80.81921, 30 May 2016, C. Verdone, B.C. Kondratieff, 1° (CSUIC). Craig Co., seeps into Barbours Creek, Rte 617, Potts Jeep Trail, N 37.63398, W 80.05481, 10 June 2016, C. Verdone, B.C. Kondratieff, 26 (CSUIC). Fauquier Co., Bartons Brook, Jackson Hollow, [no GPS], 15 July 1974, R.W.

Baumann, O. Flint, 12 (NMNH); Little Bull Run, Bull Run Mtn., Shurburg Home, Hopewell Rd., N 38.86665, W 77.703922, 1 June-10 July 2014, D.R. Smith, 73, 3 (NMNH); tributary to Broad Run, Arlington Outdoor Lab, [N 38.80720, W 77.72170], 3–20 June 2016, D.R. Smith, 1♀ (NMNH); tributary to Piney Branch, Roland Farm, N 38.843, W 77.8261, 23 May-6 June 2013, D.R. Smith, 3° , 3° (CSUIC); Same location, 7–25 June 2013, D.R. Smith, $5 \circlearrowleft$, $7 \circlearrowleft$ (NMNH). Floyd Co., Dodd Creek, Blue Ridge Parkway, BLRI, N 36.87178, W 80.27901, 31 May 2016, C. Verdone, B.C. Kondratieff, $1 \circlearrowleft$, $1 \updownarrow$ (CSUIC). Franklin Co., Grassy Fork, Rte 619, N 36.81097, W 79.74516, 7 June 2016, C. Verdone, B.C. Kondratieff, 1 (CSUIC); **Frederick Co.,** Cold Spring, 800 m S FR 93 Gate, N 38.80720, W 77.72170, 20 June 2007, A.C. Chazal, 16 (NMNH). Greene Co., Fork Hollow, Ranger Station, nr. Simmons Gap (SHEN), N 38.3007, W 78.61979, 3 June 2016, C. Verdone, B.C. Kondratieff, S. Roble, 113, 69 (CSUIC). **Madison** Co., Cedar Run, Rte. 600, White Oak Canyon Trailhead ,SHEN, N 38.53907, W 78.34813, 2 June 2016, C. Verdone, B.C. Kondratieff, S. Roble, 6♂ (CSUIC); Rapidan River, Rte 649 (SHEN), N 38.46256, W 78.36535, 2 June 2016, C. Verdone, B.C. Kondratieff, S. Roble, 1♂ (CSUIC); Robinson River, Rte 600, N 38.52717, W 78.35015, 2 June 2016, C. Verdone, B.C. Kondratieff, S. Roble, 60° , 49° (CSUIC); tributary to Rapidan River, (SHEN), N 38.46519, W 78.36990, 2 June 2016, C. Verdone, B.C., Kondratieff, S. Roble, 40° , 19° (CSUIC). Montgomery Co., spring flowing into Craig Creek, 2.7 km off 460 on Rte 621, [No GPS], 17 June 1980, B.C. Kondratieff, 1♂ (CSUIC); spring tributary to Poverty Creek, Rte 708, N 37.26314, W 80.49781, 15 June 2016, C. Verdone, 8♂, 5♀ (CSUIC). Toms Creek, Rte 655, [N 37.238054, W 80.47339], 29 May 1978, B.C. Kondratieff, 1♂ 1 exuvia. Patrick Co., Little Rock Castle Creek, of milepost 165, Rt. 8, BLRI, [N 36.82747, W 80.32460], 18-19 May 2007, C.R. Parker, $3 \stackrel{?}{\circ}$, $2 \stackrel{?}{\circ}$ (CSUIC). **Prince William Co.**, Bull Run Mtn., Mountain House, N 38.82500, W 77.70500, 26 May−11 June 2012, D.R. Smith, 2♂, 1♀ (NMNH); Catharpin Creek., Jackson Hollow Campground, N 38.876667, W 77.68900, 11-24 June 2011, D.R. Smith, $5 \stackrel{\wedge}{\circ}$, $1 \stackrel{\wedge}{\circ}$ (CSUIC); Same location, 26 May–11 June 2012, D.R. Smith, 23, 29 (CSUIC); Same location, 26 May-11 June 2012, D.R. Smith,

123, 5 (NMNH); Same location, 12-27 June 2012, D.R. Smith, 2 ? 1 ? (CSUIC); Same location, 21 June– 10 July 2014, D.R. Smith, 2♂ (CSUIC); Same location, 20 May−2 June 2016, D.R. Smith, 3♂ (NMNH); tributary to North Fork Broad Run, Bull Run Mtn. Natural Area above rd., N 38.84700, W 77.70072, 21 May–5 June 2014, D.R. Smith, 1♂ (NMNH). Rockbridge Co., Back Run, Cave Mtn. Rec. Area, N 37.56891, W 79.54088, 17 June 2016, C. Verdone, 5♀ (CSUIC). Rockingham Co., Deep Run, Rte 708, N 38.27972, W 78.76374, 3 June 2016, C. Verdone, B.C. Kondratieff, S. Roble, 16 (CSUIC). Tazewell Co., Cove Creek, Rte 662, off Hwy 61, N 37.17837, 81.29900, 6 June 2016, C. Verdone, B.C. Kondratieff, 90° , 99° (CSUIC); East Fork Cove Creek, Rte 662, [N 37.19464, W 81.30068], 12 June 1983, B.C. Kondratieff, 16 (CSUIC). Washington Co., Detroit Cove, Jct. Brumley Gap Rd. & Rte 689, N 36.79664, W 82.05730, 24 May 2016, C. Verdone, B.C. Kondratieff, B. Evans, 93, 99 (CSUIC); Detroit Cove, Rte 689, N 36.79664, W 82.05730, 4 May 2017, C. Verdone, B.C. Kondratieff, 1L (CSUIC); Little Moccasin Creek, Rte 690 (below switchbacks), N 36.83005, W 82.08855, 13 June 2016, C. Verdone, 1♂, 1♀ (CSUIC). **Wythe Co.,** Gullion Fork, Rte 619, N 37.01228, W 81.25340, 27 May 2016, C. Verdone, B.C. Kondratieff, 2L (CSUIC). West Virginia, Hardy Co., Lower Cove Run, 3 mi. NE of Mathias, N 38.91938, W 78.81641, 10-24 July 2003, D.R. Smith, 13 (CSUIC); Same location, 18 June-1 July 2004, D.R. Smith 63, 29 (CSUIC); Same location, 12–15 July 2004, D.R. Smith, 4^{\wedge} (NMNH). **Mingo Co.**, Laurel Branch of Laurel Fork, 2 mi. S Dingess at CR-3/07, [N 37.85006, W 82.20055], 17 June 1975, R.F. Kirchner, 16, 1 exuvia (INHS); Laurel Fork of Pigeon Creek, 2 mi S Dingess at CR-3/07, [N 37.84891, W -82.20064], 8 May 1976, R.F. Kirchner, 2L (INHS); Same location, 13 June 1975, R.F. Kirchner, 1♀, 1 exuvia (INHS); Same location, 20 June 1975, R.F. Kirchner, 7N (INHS).

Additional records not examined. New York, Franklin Co., Dutton Brook, Rt. 3 nr. Saranac Lake, N 44.2495, W 74.23818, 4 August 2006, L. Myers, 3 (LCRI). Warren Co., seep to Lake George, Rt. 9 North, Basin Bay, N 43.52100, W 73.67410, 5 August 2009, L. Myers, 1 \circlearrowleft , 5 (LCRI).

Distribution. <u>USA</u> – AL, CT, DE, GA, KY, MD, NC, NY, PA, SC, TN, VA, WV. (Fig. 19).



Fig. 35. Larval habitus of *Remenus bilobatus*, Stone Mountain Creek, Alleghany County, North Carolina.

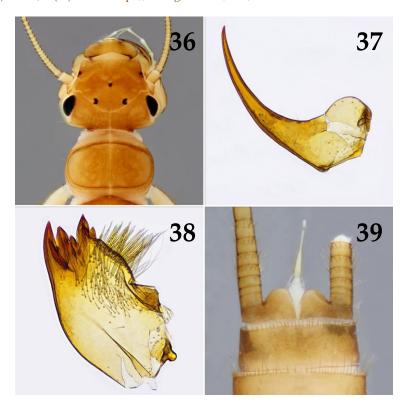
Male. (Fig 20). Macropterous; forewing length 10.0– 11.0 mm (n = 10) (Fig. 21). Body length 8.1–10.6 mm (n = 10). General body color yellow-gold with light brown markings. Dorsum of head typical of genus (Fig. 22). Pronotum light brown, covered in regularly spaced setae, with pale, glabrous rugosities mediolaterally (Fig. 22); medial pale area widest posteriorly (Fig. 22). Abdominal terga uniformly yellow-gold (Fig. 23). Tergum 8 with mediolateral patches of < 10 sensilla basiconica (Fig. 23). Tergum 9 with a medial glabrous division that anteriorly 3/4 tergum length mediolateral patches of ~ 20 sensilla basiconica (Fig. 23). Hemitergal lobes short, separated from 10th tergum, with long trichoid sensilla and > 20 sensilla basiconica on each lobe (Figs. 23-26). Epiproct length ~ 300–320 µm; width ~ 80–84 µm (n = 3); epiproct flattened laterally (Figs. 23, 26), with a ventral keel (Figs. 24, 27–28), and a mediodorsal sclerite that is broadest basally and greatly exceeds the epiproct apex terminating in a thread-like lash (Figs. 23, 26-27); complete lash length ~ 830 µm (n = 1); keel widest at mid-length (Fig. 26), covered in dense, hair-like spinulae (Figs. 28). Paragenital plates broadly triangular (Figs. 23–24, 26). Basal cowl clothed in dense golden-brown spinulae (Figs. 23–24).

Female. Macropterous; forewing length 9.8–12.0 mm (n = 10). Body length 8.8–11.1 mm (n = 10). Body coloration and morphology similar to male. Sternum 8 with variable subgenital plate extending $\sim \frac{1}{2}$ over sternum 9, or to the posterior margin of sternum 9; subgenital plate moderately sclerotized, broadly triangular (Fig. 29) or broadly rounded (Fig. 30); with regularly spaced setae; posterolateral margins convex; basolateral margins either parallel or convergent posteriorly; basolateral crease nearly straight or convex posteriorly, extending $\sim \frac{1}{3}$ length anteriorly into sternum 8 (Figs. 29–30).

Ovum. Shape typical of genus (Figs. 31–34). Length 439–440 μ m; width 348–361 μ m (n = 3).

Larva. (Fig. 35). Body length 9.0–10.4 mm, (n = 5). Head (Fig. 36), lacinia (Fig. 37), mandibles (Fig. 38), and pronotum (Fig. 36) typical of genus. Mature male larva with an elongate terminal process with a constriction at the basal ½; apical ½ thin and tapered (Fig. 39). Mature larva with paired medial and lateral spots on the abdominal terga. Basal cercal segments with whorls of both long and short setae (Fig. 40).

Diagnosis. *Remenus bilobatus*, with its characteristic epiproct lash is easily separated from the other *Remenus* species. However, the lash is rarely complete and may become broken as a result of collection, eversion, preservation, or possibly the lash is naturally broken during development or on mated individuals. Even when incomplete the lash generally still greatly exceeds the epiproct apex. The only other species of *Remenus* with a dorsal sclerite on the epiproct is *R. duffieldi*, but it does not greatly exceed the epiproct apex as in this species. Males of



Figs. 36–39. *Remenus bilobatus*, larva. 36. Head and pronotum, Stone Mountain Creek, Alleghany County, North Carolina. 37–38. Toms Creek, Montgomery County, Virginia. 37. Left lacinia, dorsal. 38. Left mandible, dorsal. 39. Male terminal process (developing epiproct), ventral, Gullion Fork, Wythe County, Virginia.

R. bilobatus are further differentiated in that the epiproct is laterally flattened, the 9th tergum has a medial glabrous division, both terga 8 and 9 have mediolateral patches of sensilla basiconica, and the basal cowl is clothed in dense golden-brown spinulae. Whereas in males of *R. duffieldi*, the epiproct is dorsoventrally flattened, tergum 9 is not divided and lacks sensilla basiconica, and the spinulae covering the basal cowl are pale.

In general, the female subgenital plate of *R. bilobatus* is broadly triangular or broadly rounded, with basolateral margins either parallel or convergent posteriorly and a basolateral crease that is nearly straight or convex posteriorly. The subgenital plate can be quite similar to the other species, but the nearly straight or convex basolateral crease is consistently different compared to the concave crease seen in *R. daniellae* sp. n., and *R. kirchneri*. Females of *R. bilobatus* differ from *R.*

duffieldi by a generally shorter subgenital plate and in pronotal pigmentation. The pronotum of *R. bilobatus* is brown with pale rugosities, whereas in *R. duffieldi*, it is pale with brown rugosities. We still agree with Kondratieff & Nelson (1995) who asserted that females of *Remenus* should not routinely be identified in the absence of male specimens.

Mature larvae of *R. bilobatus* can be separated from *R. kirchneri* and *R. duffieldi* by the presence of long setae on the basal cercal segments. However, this character is shared by *R. daniellae* sp. n. which is sympatric in several locations. Rearing of larvae is still the best hope for species level identification. **Biological notes.** No life history or biological studies have been conducted on this species. Anecdotal evidence suggests that this predaceous perlodine has a univoltine life cycle and inhabits leaf packs in areas with swift current (Beaty 2015).



Fig. 40. Remenus bilobatus, larval cercus, dorsal, Gullion Fork, Wythe County, Virginia.

Based on the examined material, emergence begins in early May and continues into late July. In the northern part its range emergence can continue into early August. Remenus bilobatus inhabits springs to 6th order rivers (Kondratieff & Voshell 1982) with drainage areas ranging from 0.26-165.2 km² and has been recorded from six eastern Nearctic physiographic provinces (Adirondack, Appalachian Plateau, Blue Ridge, New England, Piedmont Plateau, and Ridge and Valley). The average elevation of occurrence localities is 503.4 m (SD \pm 219.1 m). The relatively large range of this species may be explained in part by the wide range of stream sizes it inhabits, providing it with greater habitat connectivity. Although this species is widely distributed, adults are seldom collected in large numbers. Of the 88 adult records presented only 13.6% (n = 12) were represented by more than 10 individuals, 43.2% (n = 38) were represented by a single specimen.

Remenus daniellae Verdone & Kondratieff, sp. n.

(Figs. 42–67)

Material examined: *Holotype* ♂: Tennessee, Sevier Co., tributary to Le Conte Creek, Twin Creeks Uplands Research Lab, GRSM, N 35.68706, W 83.50096, 16 May 2017, C. Verdone, B.C. Kondratieff (NMNH). Paratypes: North Carolina, Haywood Co., Ball Branch, Old Cataloochee Turnpike, GRSM, N 35.71817, W 83.09251, 25 May 2016, C. Verdone, B.C. Kondratieff, $2 \circlearrowleft$, $3 \circlearrowleft$ (CSUIC); Same location, 14 May 2017, [emerged 18 May 2017], C. Verdone, B.C. Kondratieff, $1 \stackrel{?}{\circ}$, $3 \stackrel{?}{\circ}$, 3 exuvia (CSUIC); Same location, 20 May 2017, C. Verdone, D. Fuller, 3^{\land} , 2^{\circlearrowleft} (CSUIC); Right Fork Cove Creek, Rte 284, [N 35.62104, W 83.05193], 23 May 1993, B.C. Kondratieff, R.F. Kirchner, $2 \circlearrowleft$, $1 \updownarrow$ (CSUIC). Swain Co., Collins Creek, Collins Creek Picnic Area, Hwy 441, GRSM, N 35.56752, W 83.09251, 14 May 2017, C. Verdone, B.C. Kondratieff, 1♂ (CSUIC); Gunna Creek, at confluence with Spence Cabin Branch,

GRSM, N 35.55120, W 83.73220, 3 June 2003, B.D. Heinold, C. Favret, $1 \circlearrowleft$ (INHS); Proctor Branch, Twentymile Creek Trail, GRSM, N 35.48558, W 83.83684, 5 June 2003, B.D. Heinold, $1 \circlearrowleft$ (INHS). **Tennessee, Blount Co.,** Anthony Creek, E Cades Cove Campground, Anthony Creek Trail at $3^{\rm rd}$ footbridge going upstream, GRSM, N 35.58680, W 83.75160, 26 May 2001, R.E. DeWalt, B.D. Heinold, $1 \circlearrowleft$, $2 \hookrightarrow$ (INHS). **Sevier Co.,** Greenbriar Cove, Smoky Mtns., [N 35.70704, W 83.38294], 15 June 1939, A.C. Cole, $1 \circlearrowleft$ (INHS); Le Conte Creek, Gatlinburg, [N

35.70164, W 83.51361], 14 June 1940, T.H. Frison, $1 \ \circlearrowleft$, $3 \ \circlearrowleft$ (INHS); Le Conte Creek, ATBI Plot, Twins Creek ,GRSM, [N 35.68500, W 83.49888], 8 May–25 May 2010, C.R. Parker, $2 \ \circlearrowleft$ (CSUIC); Little Laurel Branch, Ramsey Cascade Trail. GRSM, N 35.70270, W 83.35654, C. Verdone, B.C. Kondratieff, $4 \ \circlearrowleft$, $4 \ \circlearrowleft$ (CSUIC); tributary to Le Conte Creek, Twin Creeks Uplands Research Lab, GRSM, N 35.68706, W 83.50096, 16 May 2017, C. Verdone, B.C. Kondratieff, $11 \ \circlearrowleft$, $9 \ \circlearrowleft$ (CSUIC).

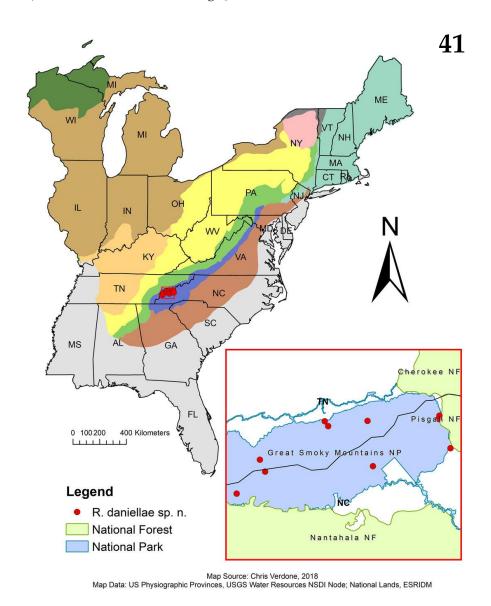


Fig. 41. Remenus daniellae sp. n. distribution of examined material.

Additional material not paratypes. North Carolina, Haywood Co., Ball Branch, Old Cataloochee Turnpike, GRSM, N 35.71817, W 83.09251, 14 May 2017, C. Verdone, B.C. Kondratieff, 3L (CSUIC); Mt. Sterling Creek, Old Cataloochee Turnpike, GRSM, N 35.70819, W 83.09658, 10 July 1983, B.C. Kondratieff, 2♀ (CSUIC); tributary to Hemphill Creek, 5 km WNW Jonathan, pumphouse spring below Purchase Knob House, GRSM, N 35.58220, W 83.07370, 31 May 2003, R.E. DeWalt, 2L (INHS). Tennessee, Sevier Co., Le Conte Creek, Gatlinburg, [N 35.70164, W 83.51361], 14 June 1940, T.H. Frison, 6L (INHS).

Distribution. <u>USA</u> – NC, TN (Fig. 41)

Etymology. The patronym honors the senior author's wife, Danielle M. Fuller, for her valued support and patience. The proposed common name is the "Danielle's Stripetail".



Fig. 42. Adult male habitus of *Remenus daniellae* sp. n., tributary to Le Conte Creek, Sevier County, Tennessee.

Male. (Fig. 42). Macropterous; forewing length 9.3–10.4 mm (n = 10) (Fig. 43). Body length, 8.0–9.8 mm

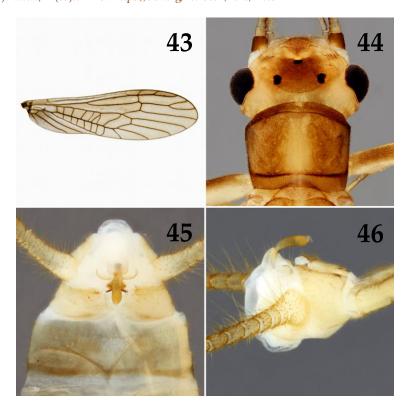
(n = 10). General body color yellow-gold with light brown markings. Dorsum of head typical of genus (Fig. 44). Pronotum light brown, covered in regularly spaced setae, with pale, glabrous rugosities mediolaterally (Fig. 44); medial pale area widest medially (Fig. 44). Abdominal terga with anterior margin darkened (Fig. 45); rarely darkened laterally. Hemitergal lobes short, not separated from 10th tergum, with long trichoid sensilla and 0-4 minute sensilla basiconica on each lobe (Figs. 45-48). Epiproct length ~ 170-270 μm; width ~ 82-89 μ m (n = 3); epiproct clavate (club-shaped) and lightly sclerotized (Figs. 45-46); covered in dense, thick palmate hair-like spinulae; when produced forward rarely exceeding the anterior margin of the basal anchor; clavate in dorsal and lateral aspects, widest near the apical ¼ and typically bearing a short translucent tube at the apex (Figs. 49-52). Base of epiproct with sparse palmate hair-like spinulae with between 4–10 seta arising from a common base (Fig. 53). Paragenital plates short, rounded, or triangular (Figs. 45, 49-50). Basal cowl covered in dense lightly pigmented spinulae (Figs. 45-46).

Female. Macropterous; forewing length 11.2–11.7 mm (n = 10). Body length, 9.2-10.2 mm (n = 10). General color and morphology similar to the male. Abdominal terga pale, without darkened anterior pigmentation. Subgenital plate broadly rounded (Fig. 54-56), occasionally with a posteromedial emargination (Fig. 56); lightly sclerotized, with regularly spaced setae, extending ½-4/5 over posterolateral sternum 9; margins convex; basolateral margins convergent posteriorly; basolateral crease typically curved, concave posteriorly, extending ~ 1/4 length anteriorly into sternum 8 (Figs. 54-56).

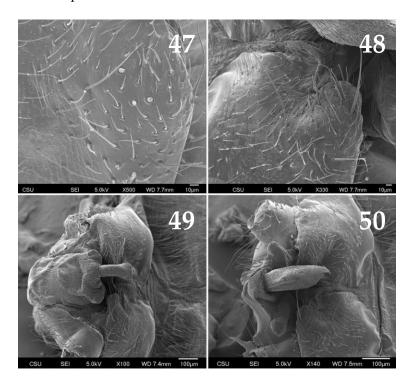
Ovum. Shape typical of genus (Figs. 57–60). Length $434-435 \mu m$; width $324-355 \mu m$ (n = 3).

Larva. (Fig. 61). Body length 8.3–10.8 mm, (n = 3). Head (Fig. 62), lacinia (Fig. 63), mandibles (Fig. 64), and pronotum (Fig. 62) typical of genus. Mature male larva with a short ovoid process on abdominal tergum 10 outlining the developing epiproct (Fig. 65). Basal cercal segments with whorls of both short and long setae (Fig. 66).

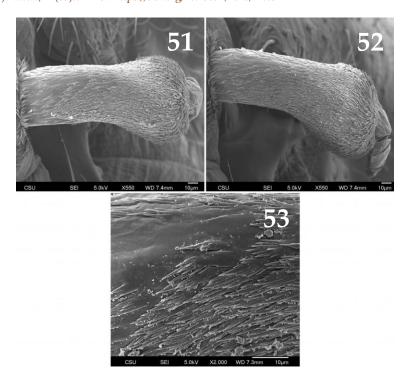
Diagnosis. Males of *R. daniellae* are morphologically similar *R. kirchneri*, both of which lack a medial



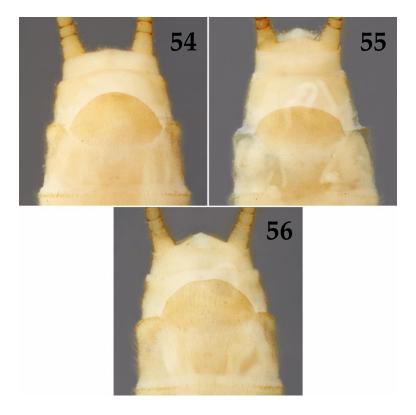
Figs. 43–46. *Remenus daniellae* sp. n., adult male, tributary to Le Conte Creek, Sevier County, Tennessee. 43. Right forewing. 44. Head and pronotum. 45. Terminalia, dorsal. 46. Terminalia, lateral.



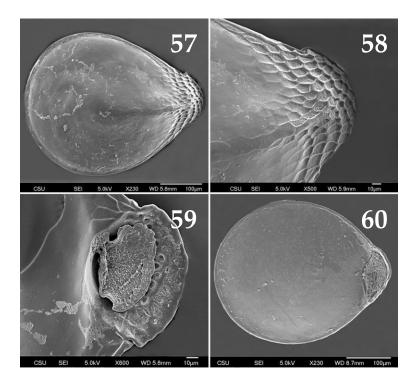
Figs. 47–50. *Remenus daniellae* sp. n. adult male. 47. Hemitergal lobe and sensilla basiconica, Ball Branch, Haywood County, North Carolina. 47–50. Tributary to Le Conte Creek, Sevier County, Tennessee. 48. Hemitergal lobe. 49–50. Epiproct variation, dorsal.



Figs. 51–53. *Remenus daniellae* sp. n., adult male, tributary to Le Conte Creek, Sevier County, Tennessee. 51. Epiproct, dorsal. 52. Epiproct, lateral. 53. Epiproct, palmate hair-like setae.



Figs. 54–56. *Remenus daniellae* sp. n., adult female subgenital plate variation. 54. Ball Branch, Haywood County, North Carolina. 55. Little Laurel Branch, Sevier County, Tennessee. 56. Tributary to Le Conte Creek, Sevier County, Tennessee.



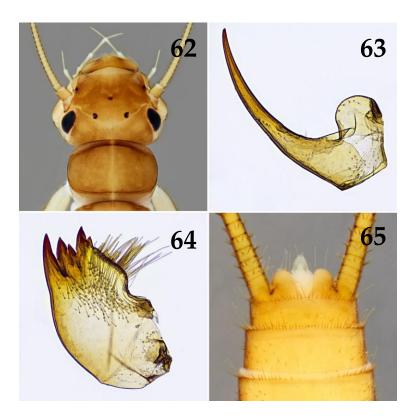
Figs. 57–60. *Remenus daniellae* sp. n., ovum, tributary to Le Conte Creek, Sevier County, Tennessee. 57. Ovum, dorsal. 58. Collar, dorsal. 59. Collar, ventral. 60. Ovum, ventral.

dorsal sclerite. However, *R. daniellae* can be distinguished by details of the epiproct and terminalia. Males of *R. daniellae* possess an epiproct that is clavate in dorsal and lateral aspects, which is widest towards the apical ¼ and bears a short translucent tube at the apex. Whereas the epiproct of *R. kirchneri* is dorsoventrally flattened, widest medially or near the basal ¼ and lacks a translucent tube at the apex. Additionally, the new species lacks sensilla basiconica on the 9th tergum and has 4 or fewer sensilla basiconica on each hemitergal lobe. *Remenus kirchneri* occasionally lacks sensilla basiconica on the 9th tergum, but consistently has 12–20 on each hemitergal lobe.

Females of *R. daniellae* are most similar to *R. kirchneri*. Separation of these spacies may require associated males. Generally, the subgenital plate of *R. kirchneri* is parallel sided basally, whereas in *R. daniellae*, the subgenital plate is typically convergent. Based on presently available records, the ranges of these two species do not overlap. *Remenus daniellae* occurs west of the French Broad River, whereas *R. kirchneri* inhabits the region to the



Fig. 61. Larval habitus *Remenus daniellae* sp. n., Ball Branch, Haywood County, North Carolina.



Figs. 62–65. *Remenus daniellae* sp. n., larva. Figs. 62–64. Ball Branch, Haywood County, North Carolina. 62. Head and pronotum. 63. Left lacinia, dorsal. 64. Left mandible, dorsal. 65. Male terminal process (developing epiproct), ventral, tributary to Hemphill Creek, Haywood County, North Carolina.

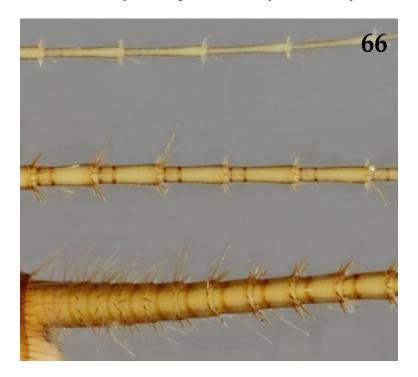


Fig. 66. Remenus daniellae sp. n., larval cercus, dorsal, Ball Branch, Haywood County, North Carolina.

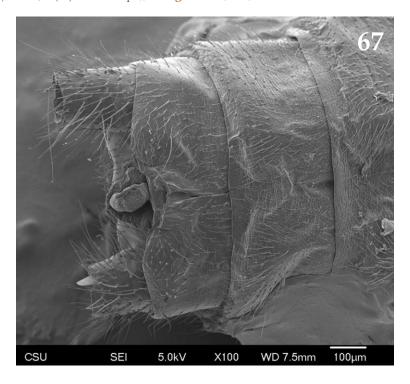


Fig. 67. Remenus daniellae sp. n., male epiproct variation, dorsal, Ball Branch, Haywood County, North Carolina.



Fig. 68. *Remenus daniellae* sp. n., type locality, tributary to Le Conte Creek, Great Smoky Mountains National Park, Sevier County, Tennessee.

east (Fig. 115). As such, morphology paired with geographic location should help inform identification of these two similar species.

Mature larvae of R. daniellae can be separated

from *R. kirchneri* and *R. duffieldi* by the presence of long setae on the basal cercal segments. However, this character is shared by *R. bilobatus* which is sympatric in some locations.

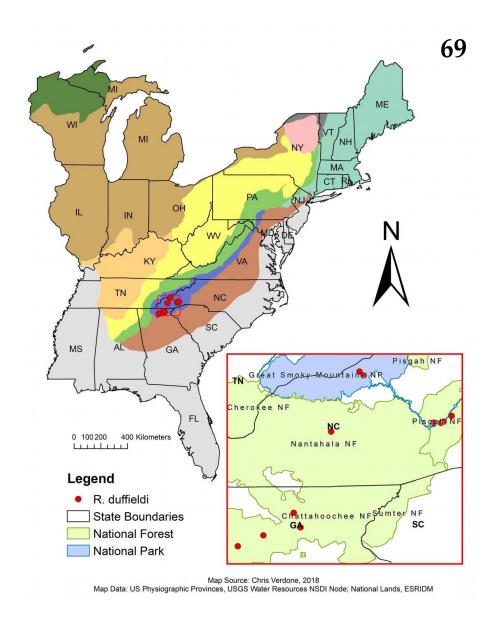


Fig. 69. Remenus duffieldi, distribution of examined material and published records.

Biological notes. Occasionally, the epiproct of *R. daniellae* is moderately to highly reduced, or appears flaccid and deflated, possibly due to insufficient hemolymphatic pressure (Fig. 67), an apparent aberrancy not observed in any other species of *Remenus*. Kondratieff and Nelson (1995) reported *R. bilobatus* from Haywood County, North Carolina based on two females collected in 1985 from Mt. Sterling Creek in GRSM. This location is 1.1 km from the type locality of *R. daniellae*. These specimens

have been re-examined and have been determined as *R. daniellae* based on the subgenital plate morphology, habitat similarity and proximity to the paratype locality at Ball Branch.

No life history or biological studies have been conducted on this species. *Remenus daniellae* is known only from 12 locations in GRSM in North Carolina and Tennessee. This species has been documented from 1st–3rd order streams with drainage areas ranging from 0.47–11.07 km². The



Fig. 70. Adult male habitus of *Remenus duffieldi*, Tellico Creek, Macon County, North Carolina.

average elevation of occurrence localities is 875.1 m (SD \pm 285.7 m). Based on the material examined, emergence occurs from mid-May to mid-July. Existing records are limited, but this species probably inhabits many other streams within GRSM, which possesses more than 3400 km of high quality stream habitats. The type locality (Fig. 68) of this new species is located in what was the heart of the Chimney Tops 2 fire that burned more than 46 km² in December 2016. The effects of this event on the aquatic macroinvertebrate community are unknown; however, stonefly species richness at the type locality six months later was relatively high. Other stoneflies collected with the holotype included Alloperla nanina Banks, 1911, A. usa Ricker, 1952, Amphinemura wui (Claassen, 1936), Isoperla dewalti Verdone & Kondratieff, 2017, Leuctra grandis Banks, 1906, L. sibleyi Claassen, 1923, Sweltsa lateralis (Banks, 1911), S. mediana (Banks, 1911), S. urticae (Ricker, 1952), Tallaperla anna (Needham & Smith, 1916), and T. laurie (Ricker, 1952).



Fig. 71. *Remenus duffieldi*, adult male, right forewing, Tellico Creek, Macon County, North Carolina.

Remenus duffieldi Nelson & Kondratieff, 1995 (Figs. 70–87)

http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org: TaxonName:607

Remenus duffieldi Nelson & Kondratieff (1995: 600). Holotype ♂: Georgia, Towns County, Soapstone Creek, Rt. 180, nr. jct. Rt. 180 & Owl Cr. Rd., Chattahoochee National Forest (NMNH). Figures: adult–male terminalia (dorsal, lateral), female subgenital plate, ova (dorsal, ventral).

Remenus duffieldi: Kondratieff (2004: 164). Figures: adult–figures from Kondratieff & Nelson (1995).

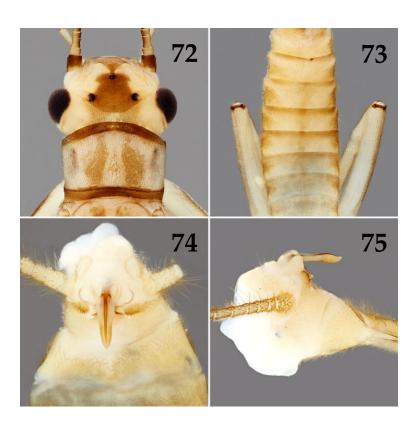
Remenus duffieldi: Stark (2017: 236). Figures: larvacercus (basal, middle).

Material examined: Georgia, Union Co., Rock Creek, Rte 69, 1.5 mi. W Hightower Gap, Chattahoochee National Forest, [N 34.67981, W 84.135914], 29 May 1990, R.M. Duffield, 2♂ (CSUIC, paratypes). White Co., Andrews Creek, Rte 17/75, Andrews Cove Campground, [N 34.77817, W 83.73738], 2 June 1994, C.H. Nelson, 1♂, 1♀ (CSUIC,

paratypes). North Carolina, Haywood Co., Yellowstone Prong of East Fork Pigeon River, Graveyard Fields, BLRI, [N 35.321966, W 82.845768, 17 June 2009, J. Robinson, $1 \circlearrowleft$ (CSUIC). Macon Co., Nantahala River, [no GPS], 30 May 1939, Thelma Howell, 1L (INHS); Tellico Creek, Tellico Rd. [no GPS], 27 May 1993, B.P. Stark, R. Simmons, P. Kelly, $1 \circlearrowleft$ (CSUIC); Tellico Creek, Tellico Rd. ~0.5 mi. W of Sugar Cove Rd., N 35.27688, W 83.54347, 15 May 2017, C. Verdone, B.C. Kondratieff, $7 \circlearrowleft$, $7 \circlearrowleft$ (CSUIC). Swain Co., Collins Creek, Collins Creek Picnic Area, Hwy 441, GRSM, N 35.56752, W 83.33663, 16 May 2017, C. Verdone, B.C. Kondratieff, $5 \circlearrowleft$, $2 \backsim$, 1L

(CSUIC); Kanati Fork, Hwy 441, GRSM, N 35.58738, W 83.36297, 16 May 2017, C. Verdone, B.C. Kondratieff, 2♂ (CSUIC). **Transylvania Co.**, Pigeon Branch South Fork Mills River, NFR 1206, off Rte 276, [N 35.35815, W 82.77810], 8 July 1981, B.C. Kondratieff, 1♂, 2♀ (CSUIC).

Additional records not examined. North Carolina, Haywood Co., Yellowstone Prong of East Fork Pigeon River, N 35.32814, W 82.82897, 26 May 2014, A.L. Sheldon 1♀ (WKUC); tributary to Flat Laurel Creek, TR 617, N 35.32303, W 82.89282, 22 July 2015, A.L. Sheldon, 1♂ (WKUC).



Figs. 72–75. *Remenus duffieldi*, adult male. 72. Head and pronotum, Collins Creek, Swain County, North Carolina. 73–75. Tellico Creek, Macon County, North Carolina. 73. Abdominal terga 2–9. 74. Terminalia, dorsal. 75. Terminalia, lateral.

Distribution. <u>USA</u> – GA, NC. (Fig. 69)

Male. (Fig. 70). Macropterous; forewing length 9.5–10.4 mm (n = 10) (Fig. 71). Body length, 7.9–9.8 mm (n = 10). General body color pale yellow-gold with light brown markings. Dorsum of head typical of genus (Fig. 72). Pronotum pale, covered in regularly

spaced setae, with light brown rugose areas, glabrous rugosities mediolaterally (Fig. 72); anterior and posterior margins brown (Fig. 72). Abdominal terga with a narrow mid-dorsal, dusky, interrupted brown stripe (Fig. 73); terga 1–4 pale yellow, terga 5–9 pale yellow to light brown; terga darkened

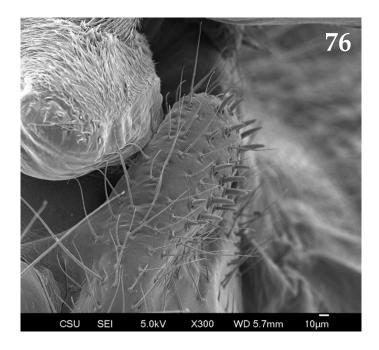
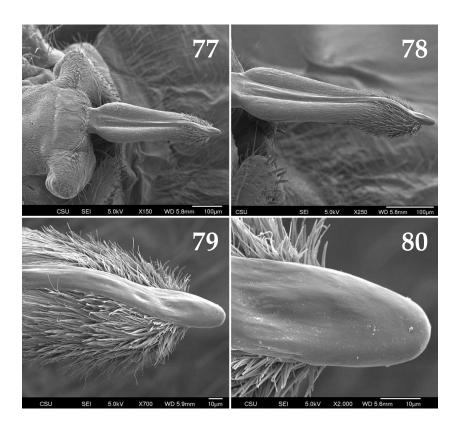


Fig. 76. Remenus duffieldi, adult male, hemitergal lobe, Tellico Creek, Macon County, North Carolina.



Figs. 77–80. *Remenus duffieldi*, adult male, Tellico Creek, Macon County, North Carolina. 77. Epiproct, dorsal. 78. Epiproct, dorsolateral. 79. Epiproct, splayed setae, dorsolateral. 80. Epiproct apex, dorsal.

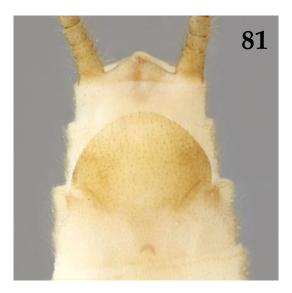
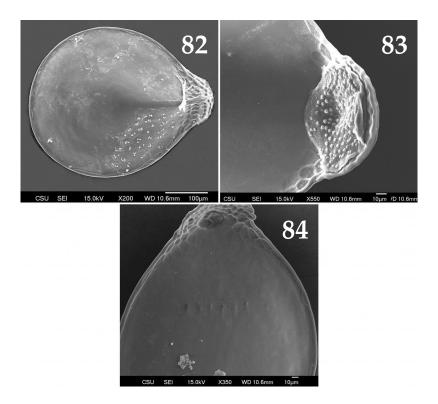


Fig. 81. Remenus duffieldi, adult female subgenital plate, Tellico Creek, Macon County, North Carolina.



Figs. 82–84. *Remenus duffieldi*, ovum, Tellico Creek, Macon County, North Carolina. 82. Ovum, dorsal. 83. Collar, ventral. 84. Micropyles, ventral.

laterally (Fig. 73); tergum 9 lacking sensilla basiconica (Fig. 74). Hemitergal lobes short, not separated from 10th tergum, with long trichoid

sensilla and 15–17 sensilla basiconica on each lobe (Figs. 74-77). Epiproct length ~ 382–420 μ m; width ~ 136–168 μ m (n = 3); epiproct flattened

dorsoventrally (Figs. 75, 78), clothed in appressed hair-like spinulae, and with a mediodorsal sclerite (Figs. 74, 77–78); epiproct widest basally; hair-like spinulae of distal ¼ splayed (Fig. 79). Mediodorsal sclerite narrow throughout its length (slightly wider at its base) and barely exceeding the epiproct apex (Figs. 77–80); sclerite with a dorsal hump near distal ¼ (Figs. 77–79); apex smoothly rounded and glabrous (Fig. 80). Paragenital plates short, rounded, or triangular (Figs. 74, 77). Basal cowl covered in dense lightly pigmented spinulae (Figs. 74–75).



Fig. 85. Larval habitus of *Remenus duffieldi*, Nantahala River, Macon County, North Carolina.

Female. Macropterous; forewing length 10.5–11.1 mm (n = 10). Body length, 8.9–10.4 mm (n = 10). General color and morphology similar to the male. Abdominal pigment pattern usually less developed. Medial dusky stripe is faint and lateral margins are not distinctly darkened. Subgenital plate broadly rounded, elongate, lightly sclerotized, with regularly spaced setae, extending ½ over sternum 9, or slightly beyond the posterior margin of sternum

9 (Fig. 81); basolateral crease nearly straight or convex posteriorly, extending $\sim \frac{1}{3}$ length anteriorly into sternum 8 (Fig. 81).

Ovum. Shape typical of genus (Figs. 82–84). Length 444–446 μ m; width 340–355 μ m (n =3).

Larva. (Fig. 85). Body length 9.1 mm, (n = 1). Head (Fig. 86), lacinia (Fig. 87), mandibles (Fig. 88), and pronotum (Fig. 86) typical of genus. Mature male larva with a short triangular terminal process (developing epiproct) (Fig. 89), Basal cercal segments with whorls of short setae (Fig. 90).

Diagnosis. Remenus duffieldi is distinguished from all other Remenus species in coloration and genitalic structure. The pale pronotum and grayish wings are unlike any other species in the genus. The epiproct is most similar to R. bilobatus in that it has a dorsal sclerite, but in contrast, it does not greatly exceed the epiproct apex. Additionally, R. duffieldi is differentiated in that the epiproct is dorsoventrally flattened, tergum 9 is not divided, lacks sensilla basiconica, and the spinulae covering the basal cowl are pale. Whereas in males of R. bilobatus, the epiproct is flattened laterally, the 9th tergum has a medial glabrous division, both terga 8 and 9 have mediolateral patches of sensilla basiconica and the basal cowl is clothed in dense golden-brown spinulae.

Females are distinguished by the distinctive pronotal coloration in addition to differences in the subgenital plate. In *R. duffieldi*, the subgenital plate is broadly rounded and elongate, generally, as long as, or longer than tergum 9. Whereas in *R. bilobatus*, the subgenital plate is either broadly triangular or broadly rounded and typically does not exceed the posterior margin of tergum 9.

Mature larvae of *R. duffieldi* are separable from the two other sympatric species, *R. bilobatus* and *R. daniellae* by the lack of long setae on the basal cercal segments. *Remenus kirchneri* also lacks long setae on the basal cercal segments, but based on presently available records, the ranges of these two species do not overlap with *R. duffieldi* occurring west of the French Broad River and *R. kirchneri* occurring to the east (Fig. 115).

Biological notes. No life history or biological studies have been conducted on this species. *Remenus duffieldi* is known from 11 locations in Blue Ridge Physiographic Province of Georgia and North



Figs. 86–89. *Remenus duffieldi*, larva. Head and pronotum, Nantahala River, Macon County, North Carolina. Figs. 87–88. Collins Creek, Swain, County North Carolina. 87. Left lacinia, dorsal. 88. Left mandible, dorsal. 89. Male terminal process (developing epiproct), dorsal, Nantahala River, Macon County, North Carolina.

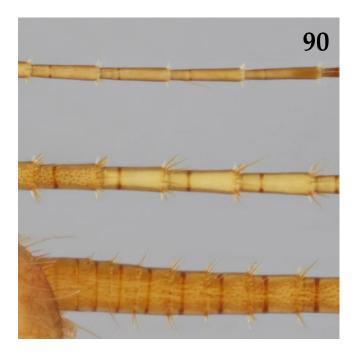


Fig. 90. Remenus duffieldi, larval cercus, dorsal, Collins Creek, Swain County, North Carolina.

Carolina. This species has been documented from 1^{st} – 3^{rd} order streams with drainage areas ranging from 0.28–9.72 km². The average elevation of occurrence localities is 987.1 m (SD ± 346.1 m). Based on examined material, emergence occurs from mid-

May to late July. Existing records are limited, but this species probably inhabits many other streams within GRSM, Nantahala and Chattahoochee-Oconee National Forests.

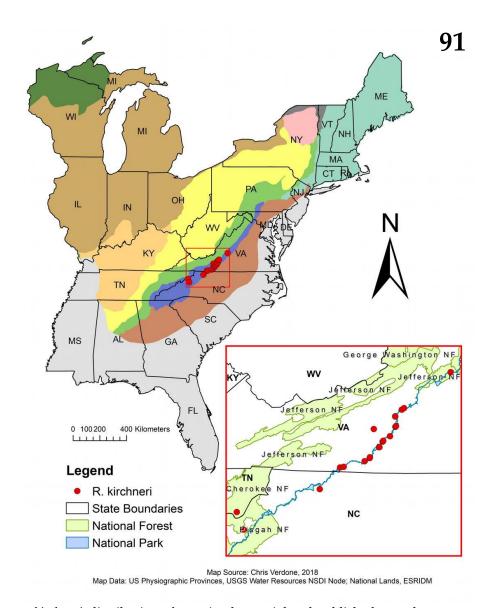


Fig. 91. Remenus kirchneri, distribution of examined material and published records.

Remenus kirchneri Kondratieff & Nelson, 1995 (Figs. 1, 92–113)

 $\frac{http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:}{TaxonName:606}$

Remenus kirchneri Kondratieff & Nelson (1995: 600). Holotype ♂: Virginia, Patrick County, Little Rock Castle Creek, Rock Castle Gorge National Recreation Area (NMNH). Figures: adult–male terminalia (dorsal, lateral), female subgenital plate.

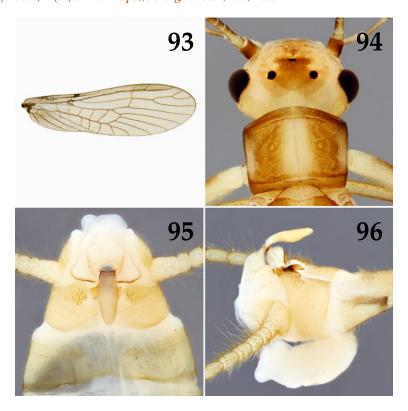
Remenus kirchneri: Kondratieff (2004: 165). Figures: adult–figures from Kondratieff & Nelson (1995).

Material examined: North Carolina, Avery Co., Jones Creek, Old Hanging Rock Rd., N 35.982213, W 82.01676, 22 June 2013, C. Verdone, B.C. Kondratieff, 1♂ (CSUIC). **Wilkes Co.**, Garden Creek, Stone Mountain Rd., N 36.38905, W 81.06922, 29 May 2008, B.C. Kondratieff, R.F. Kirchner, R.E. Zuellig, D.R. Lenat, 2♂ (CSUIC). **Tennessee**, **Carter Co.**, Roan Mountain State Park, [N 36.19623, W 82.07040], 2 June 2000, J. Enshinger, 1d (CSUIC). Virginia, Bedford Co., Battery Creek FR 951, N 37.55194, W 79.44059, 17 June 2016, C. Verdone, 2♂, 3♀ (CSUIC). Carroll Co., North Fork Stewarts Creek, Blue Ridge Parkway, BLRI, N 36.60630, W 80.81663, 11 May 2017, C. Verdone, B.C. Kondratieff, 11° , 6° , 6L(CSUIC); Same location, 25 May 2017, C. Verdone, 3, 1 (CSUIC); Pine Creek, nr. Jct. Co. Rd. 633 & Co. Rd. 640, N 36.66773, W 80.51043, 30 May 2016, C. Verdone, B.C. Kondratieff, $4 \circlearrowleft$, $2 \updownarrow$ (CSUIC); tributary to Turkey Creek, Rte 620, N 36.61184, W 80.77082, 11 May 2017, 2\$\frac{1}{2}\$, 6L (CSUIC). Floyd Co., Dodd Creek, Blue Ridge Parkway, BLRI, N 36.87178, W 80.27901, 31 May 2016, C. Verdone, B.C. Kondratieff, 1♂ (CSUIC); small spring fed stream, 6 miles east of Floyd Rte 221, [no GPS] 28 June 1981, B.C. Kondratieff, $1 \circlearrowleft$, $1 \circlearrowleft$ (CSUIC, paratype); spring fed stream, Blue Ridge Parkway, E. of Mt. Olivet Church, BLRI, N 37.11577, W 80.12949, 31 May 2016, C. Verdone, B.C. Kondratieff, 14° , 19° (CSUIC); Same location, 11 June 2016, C. Verdone, 53, 7(CSUIC); Same location, 9 May 2017, C. Verdone, B.C. Kondratieff. 38L (CSUIC); Same data, [emerged 19 May 2017], $1 \circlearrowleft$, $1 \circlearrowleft$ (CSUIC); Same data, [emerged 24 May 2017], 1♀ (CSUIC); spring fed stream, Blue Ridge Parkway (spring house S of Rte 642), BLRI, N 37.01133, W 80.12679, 31 May 2016, C. Verdone, B.C. Kondratieff, $1 \circlearrowleft$, $1 \circlearrowleft$ (CSUIC); Spring tributary to Little River, Rte 706, N 36.98935, W 80.39589, 29 May 2016, C. Verdone, B.C. Kondratieff, $1 \circlearrowleft$, $2 \circlearrowleft$ (CSUIC); tributary to Dodd Creek, Blue Ridge Parkway, BLRI, N 36.86353, W 80.28702, 31 May 2016, C. Verdone, B.C. Kondratieff, $13 \circlearrowleft$, $10 \updownarrow$ (CSUIC); tributary to Dodd Creek, Blue Ridge Parkway, BLRI, N 36.8717, W 80.27943, 31 May 2016, C. Verdone, B.C. Kondratieff, 2♂ (CSUIC); tributary to Little River, BLRI, N 37.01919, W 80.11913, 18-19 July 2007, C.R. Parker, 83, 64 (CSUIC); tributary to Little River, Blue Ridge Parkway, N of Rte 642 "Poff", BLRI, N 37.01437, W 80.12343, 19 May 2017,

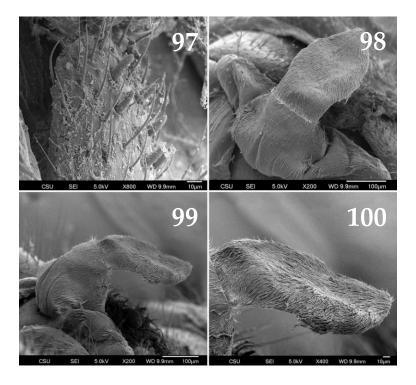


Fig. 92. Adult male habitus of *Remenus kirchneri*, tributary to Dodd Creek, Floyd County, Virginia.

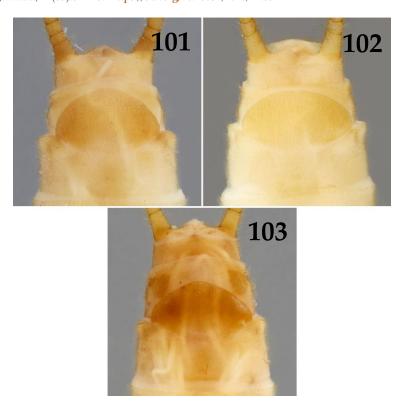
C. Verdone, B.C. Kondratieff, S. Roble, 13, 3(CSUIC); tributary to Lick Fork, Blue Ridge Parkway, 0.9 mi. S of Rte 602, BLRI, N 37.11251, W 80.12924, 19 May 2017, C. Verdone, B.C. Kondratieff, S. Roble, 73, 44, 4L (CSUIC). Franklin Co., Brogan Branch, Rte 680/793, N 36.91700, W 80.19504, 31 May 2016, C. Verdone, B.C. Kondratieff, 18 (CSUIC). Patrick Co., Haunted Branch, Blue Ridge Parkway ~1 mi. S of Rte 634, BLRI, N 36.70024 W 80.44730, 9 May 2017, C. Verdone, B.C. Kondratieff, 15L (CSUIC); Same location, 11 May 2017, C. Verdone, B.C. Kondratieff, 18L (CSUIC); Same location, 19 May 2017, C. Verdone, B.C. Kondratieff, S. Roble, 2♂, 1♀ (CSUIC); Little Rock Castle Creek, Rocky Knob Rec. Area, Rte 605, BLRI, N 36.80783, W 80.33112, 24 May 1994, B.C. Kondratieff, R.F. Kirchner, $1 \circlearrowleft$, $1 \updownarrow$ (CSUIC); same location, 26 May 2016, C. Verdone, B.C. Kondratieff, 13, 29 (CSUIC); Mayberry Creek, Blue Ridge Parkway, ~ 0.25 mi. S of Rte 634, BLRI, N 36.70842, W 80.44395, 26 May 2016, C. Verdone, B.C. Kondratieff, $6 \circlearrowleft$, $9 \circlearrowleft$ (CSUIC);



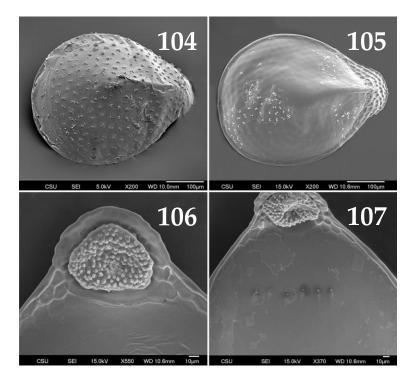
Figs. 93–96. *Remenus kirchneri*, adult male. 93. Right forewing, spring fed stream, Floyd County, Virginia. 94. Head and pronotum, tributary to Dodd Creek, Floyd County, Virginia. 95–96. Tributary to Rock Castle Creek, Patrick County, Virginia. 95. Terminalia, dorsal. 96. Terminalia, lateral.



Figs. 97–100. *Remenus kirchneri*, adult male, tributary to Dodd Creek, Floyd County, Virginia. 97. Hemitergal lobe. 98. Epiproct dorsal. 99. Epiproct dorsolateral. 100. Epiproct, hair-like setae, dorsolateral.



Figs. 101–103. *Remenus kirchneri*, adult female, subgenital plate variation. 101. Tributary to Rock Castle Creek, Patrick County, Virginia. 102. Tributary to Lick Fork, Floyd County, Virginia. 103. Tributary to Dodd Creek, Floyd County, Virginia.



Figs. 104–107. *Remenus kirchneri*, ovum, spring fed stream, Floyd County, Virginia. 104. Ovum with membranous covering, dorsal. 105. Ovum, bare, dorsal. 106. Collar, ventral. 107. Micropyles, ventral.

Same location, 9 May 2017, C. Verdone, B.C. Kondratieff, 1L (CSUIC); small spring fed stream into [Talbott Reservoir], [no GPS], 2 August 1982, B.C. Kondratieff, 1° (CSUIC, paratype); spring fed tributaries of [Rock Castle Creek] Rte 605, [N 36.80874, W 80.3255], 10 May 1983, B.C. Kondratieff, 1♂ (CSUIC, paratype); tributary to Rock Castle Creek, Rte 605 at Cemetery, N 36.80874, W 80.3255, 26 May 2016, C. Verdone, B.C. Kondratieff, 8♂, 7♀ (CSUIC); Same location, 9 May 2017, C. Verdone, B.C. Kondratieff, 10L (CSUIC); Same location, 19 May 2017, C. Verdone, B.C. Kondratieff, S. Roble, 23, 3 (CSUIC). **Roanoke Co.**, tributary to Back Creek, Blue Ridge Parkway, BLRI, N 37.18181, W 80.04742, 31 May 2016, C. Verdone, B.C. Kondratieff, 7♂, 12♀ (CSUIC); tributary to Back Creek; Blue Ridge Parkway, 0.5 mi. E of Rte 688 overpass, BLRI, N 37.19653, W 80.02029, 11 June 2016, C. Verdone, 2♂ (CSUIC).

Distribution. <u>USA</u> – NC, TN, VA (Fig. 91)

Male. (Fig. 92). Macropterous; forewing length, 9.1– 10.5 mm (n = 10) (Fig. 93). Body length, 8.2–10.2 mm (n = 10). General body color yellow-gold with light brown markings. Dorsum of head typical of genus (Fig. 94). Pronotum light brown, covered in regularly spaced setae, with pale, glabrous rugosities mediolaterally (Fig. 94); medial pale area slightly wider posteriorly (Fig. 94). Abdominal terga yellow-gold with darkened anterior margins; rarely with darkened lateral margins. Tergum 9 with or without mediolateral patches of 3-5 sensilla basiconica (Fig. 95). Hemitergal lobes short, not separated from 10th tergum, with long trichoid sensilla and 12-20 sensilla basiconica on each lobe (Figs. 95–97). Epiproct length ~ 270–500 μ m; width ~ $20-59 \mu m$ (n = 3); epiproct dorsoventrally flattened, tongue-shaped and lightly sclerotized (Figs. 95-96, 98-100); covered in dense, thick hair-like spinulae (Fig. 100); when produced forward, exceeding the anterior margin of the basal sclerite. Paragenital plates triangular (Figs. 95, 98). Basal cowl covered in dense lightly pigmented spinulae (Figs. 95-96).

Female. Macropterous; forewing length 10.0-11.9 mm (n = 10). Body length, 9.2-12.6 mm (n = 10). General color and morphology similar to the male. Abdominal terga pale, without darkened anterior or lateral margins. Subgenital plate broadly rounded (Fig. 101-102), occasionally with a shallow

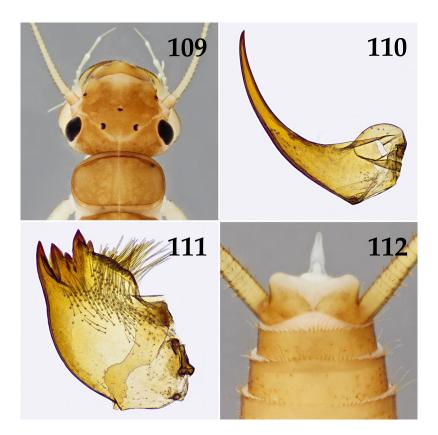
posteromedial emargination (Fig. 102), or broadly triangular (Fig. 103); lightly sclerotized, with regularly spaced setae, extending ½-½ over sternum 9; posterolateral margins convex; basolateral margins typically parallel; basolateral crease typically curved, concave posteriorly, extending ~ ¼ length anteriorly into sternum 8 (Figs. 101–103).

Ovum. Shape typical of genus (Figs. 104–107). Length $438-460 \mu m$; width $348-353 \mu m$.



Figure 108. Larval habitus of *Remenus kirchneri*, tributary to Rock Castle Creek, Patrick County, Virginia.

Larva. (Fig. 108). Body length 8.5–11.4 mm, (n = 10). Head (Fig. 109), lacinia (Fig. 110), mandibles (Fig. 111), and pronotum (Fig. 109) typical of genus. Mature male larva with a short terminal process (developing epiproct) with a constriction at the basal ½; apical ½ tapering to a narrowly rounded apex (Fig. 112). Basal cercal segments with whorls of short setae (Fig. 113).



Figs. 109–112. *Remenus kirchneri*, larva. 109. Head and pronotum, tributary to Rock Castle Creek, Patrick County, Virginia. 110–112. Spring fed stream, Floyd County, Virginia. 110. Left lacinia, dorsal. 111. Left mandible, dorsal. 112. Male terminal process (developing epiproct), ventral.

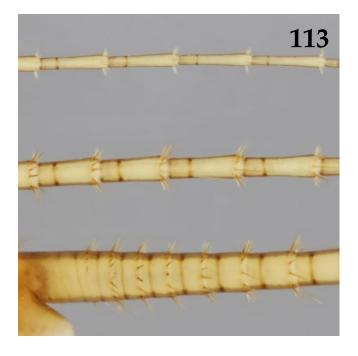


Fig. 113. Remenus kirchneri, larval cercus, dorsal, tributary to Rock Castle Creek, Patrick County, Virginia.

Diagnosis. Males of *R. kirchneri* are morphologically similar *R. daniellae*. Males possess a tongue-shaped epiproct in dorsal aspect, which is widest medially or near the basal ¼ and is dorsoventrally flattened. Whereas the epiproct of *R. daniellae* is clavate, is widest towards the apical ¼ and narrows posteriorly both in dorsal and lateral aspects. Additionally, the epiproct of *R. daniellae* typically possesses a short translucent tube at the apex, which *R. kirchneri* lacks. Furthermore, *R. kirchneri* generally has sensilla basiconica on tergum 9 and consistently has 12–20 sensilla basiconica on each hemitergal lobe. In contrast, *R. daniellae* generally lacks sensilla basiconica on tergum 9 and has < 4 minute sensilla basiconica on each hemitergal lobe.

Females of *R. kirchneri* are most similar to *R. daniellae*. Separation of these species may require associated males. Generally, the subgenital plate of *R. kirchneri* is parallel sided basally, whereas in *R. daniellae*, the subgenital plate is typically convergent. Based on presently available records, the ranges of these two species do not overlap, as previously mentioned. As such, morphology paired with geographic location should help inform identification of these two similar species.

Mature larvae of *R. kirchneri* are separable from the other sympatric species, *R. bilobatus*, by the lack of long setae on the basal cercal segments. *Remenus duffieldi* also lacks long setae on the basal cercal segments, but it does not occur east of the French Broad River (Fig. 115).

Biological notes. Prior to this study *R. kirchneri* was known from six locations in Virginia, one in North Carolina and one in Tennessee. Despite considerable effort, we were unable to find this species in either year in North Carolina or Tennessee. However, R. kirchneri proved to be a frequent inhabitant of headwater streams along the Blue Ridge Parkway in southern Virginia. This species is now known from 25 locations ranging from Bedford County, Virginia to Carter County, Tennessee. Remenus kirchneri occurs in the Blue Ridge Physiographic Province in 1st-3rd order streams with drainage areas ranging in size from 0.09–8.75 km². The average elevation of occurrence localities is 717.8 m (SD \pm 163.5 m). Based on the material examined, emergence begins in mid-May and continues until early August.

This species can achieve moderate benthic densities ranging from 11–161/m² (n = 12) despite apparently low fecundity. Females collected from a spring fed stream, along the Blue Ridge Parkway near Mt. Olivet Church, were kept alive in ventilated plastic jars with moistened paper towels (and no food) so that they might mate and produce eggs. In total, only three females produced eggs, each consisting of a single clutch of 59-61 ova. However, ova dissected from another female numbered 134. The ova were not fully formed and may have numbered more if fully developed. Possibly R. kirchneri produces several clutches of ova, or may need to feed to do so. Other similarly sized perlodids are known to produce egg masses ranging in size from ~ 90 to 1000 ova (Peckarsky & Cowan 1991, Tierno De Figueroa et al. 2000, Sandberg & Stewart 2001, Tierno De Figueroa & López-Rodríguez 2005).

DNA Barcodes

The neighbor-joining tree constructed from COI DNA barcode sequences supported the morphological species concepts presented above (Fig. 114). All four species, *R. bilobatus*, *R. daniellae*, *R. duffieldi*, and *R. kirchneri* were clearly delineated and supported by high bootstrap values for species level nodes.

DISCUSSION

Four species of Remenus are now known, with species keys provided for adult males and females based on external genitalia. Larvae can tentatively be separated into two groups based on basal cercal setal length (1) variable length setae (both short and long), R. bilobatus and R. daniellae, and (2) short setae, R. duffieldi and R. kirchneri. With the application of known distributions, larvae of R. kirchneri and R. duffieldi may be distinguished from R. bilobatus and R. daniellae. However, sympatry and range overlap precludes the use of this character in distinguishing R. bilobatus and R. daniellae. Caution should be exercised in using the basal cercal setae character as it is sometimes difficult to detect. Ova of the four species apparently lack distinguishing characteristics and thus are not separable at this time.

The present day distribution of *Remenus* Ricker (Plecoptera: Perlodidae) is limited to the mid-

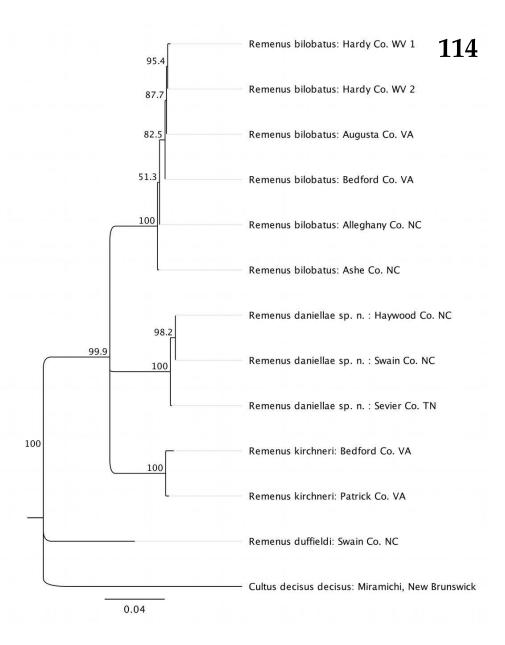


Fig. 114. Neighbor-joining tree of four *Remenus* species based on a 658 bp fragment of COI estimated using the Tamura-Nei parameter model with pairwise deletion option. Nodal support values are bootstrap percentages from 1,000 replicates. Scale indicates divergence.

elevation regions of the Appalachian Mountains. The four species can be found in similar habitats, but are rarely sympatric. Three species, *R. daniellae*, *R. duffieldi* and *R. kirchneri* are apparently restricted to the Blue Ridge Physiographic Province of the southern Appalachians. The ranges of the three southern species appear to be limited by low

elevation valleys that bisect the Blue Ridge near Asheville, North Carolina and Roanoke, Virginia. *Remenus daniellae* and *R. duffieldi* are restricted to the mountains west of the Asheville Basin, while *R. kirchneri* occurs primarily in the mountains between the Asheville Basin and the Roanoke River Valley to the northeast (Fig. 115). While this study has

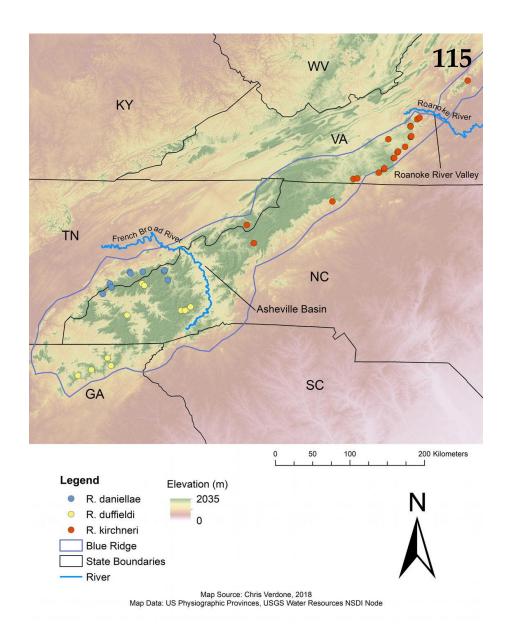


Fig. 115. Distribution of *Remenus daniellae*, *R. duffieldi*, and *R. kirchneri* in the Blue Ridge Physiographic Province.

clarified the taxonomy of these species, much remains to be discovered about their biology and behavior. Additional sampling is needed to elucidate further the distribution of these species in addition to identifying robust populations suitable for ecological study.

ACKNOWLEDGMENTS

Thanks to Steven R. Beaty (North Carolina Department of Environmental Quality, Division of Water Resources, Raleigh, North Carolina) for providing an early review of the manuscript. Drs. R.E. DeWalt (Illinois Natural History Survey, Champaign, Illinois), S.A. Grubbs (Western

Kentucky University, Bowling Green, Kentucky), and O.S. Flint (Smithsonian Institution, Washington, DC) are thanked for their assistance, support, and for loaning material. Dr. Steven M. Roble (Staff Zoologist, Virginia Department of Conservation & Recreation, Richmond, Virginia) is thanked for his assistance in the field and quality checking location data. Dr. Franz Lichtner (Colorado State University, Fort Collins, Colorado) is thanked for his help analyzing and interpreting DNA sequences. Kim Vanderpool and Dr. Patrick McCurdy (Colorado State University) are thanked for their help with scanning electron microscopy. Our gratitude to the employees of the Blue Ridge Parkway, Great Smoky Mountains, and Shenandoah National Parks and Cherokee, Jefferson, Washington, Nantahala, and Pisgah National Forests and the Clinch Mountain Wildlife Management Area. Access to these outstanding habitats made this study possible. We especially thank the U.S. Fish and Wildlife Service (grant number 5306259) for funding this research and the work they do to protect all wildlife.

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Submitted 15 February 2018, Accepted 10 April 2018, Published 30 May 2018

Hosted and published at the University of Illinois, Illinois Natural History Survey, Champaign, Illinois, U.S.A.

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Zoologisch-Botanische Datenbank/Zoological-Botanical Database

Digitale Literatur/Digital Literature

Zeitschrift/Journal: Illiesia

Jahr/Year: 2018

Band/Volume: 14

Autor(en)/Author(s): Verdone Chris J., Kondratieff Boris C.

Artikel/Article: Holomorphology and systematics of the eastern Nearctic stonefly genus Remenus

Ricker (Plecoptera: Perlodidae). 81-125