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 ⅓ 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, while 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 the epiproct is mostly membranous and 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.
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...


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

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/imaging-laboratory/).


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 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**

1 Epiproct with a medial dorsal sclerite (Figs. 23–24, 26–28, 74–75, 77–80) ........................................ 2

1’ Epiproct without a medial dorsal sclerite (Figs. 45–46, 49–52, 95–96, 98–100) ................................. 3

2 Epiproct flattened laterally; terminating in a threadlike lash that greatly exceeds the epiproct apex (Figs. 23–24, 26–28); Basal cowl clothed in dense golden-brown spinulae (Figs. 23–24); tergum 9 with mediolateral patches of ~20 sensilla basiconica (Fig. 23); pronotum mostly brown (Fig. 22) ..................... *R. bilobatus*

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*

3 Epiproct widest toward apex in dorsal and lateral view, clavate; bearing a short translucent tube on apex (Figs. 45–46, 49–52); hemitergal lobes with <4 sensilla basiconica (Figs. 45–50) .............................................. *R. daniellae* sp. n.

3’ Epiproct dorsoventrally flattened, widest basally or at mid-length in dorsal view, tongue-shaped (Figs. 95–96, 98–100); hemitergal lobes with 12–20 sensilla basiconica (Figs. 95–97) .............................................. *R. kirchneri*

**Adult females**

1 Basalateral crease of subgenital plate nearly straight or convex posteriorly (Figs. 29–30, 81); subgenital plate broadly triangular (Fig. 29), or rounded (Figs. 30, 81) .......................... 2

1’ Basalateral crease of subgenital plate concave posteriorly (Figs. 54–56, 101–103); subgenital plate broadly rounded (Fig. 54–55, 101), occasionally with a shallow posteromedial emargination (Fig. 56, 102), or broadly triangular (Fig. 103) .................................................. 3

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 ¾ over sternum 9 or slightly beyond the posterior margin of sternum 9 (Fig. 81); pronotum mostly pale (Fig. 72) ... *R. duffieldi*

3 Subgenital plate broadly rounded (Figs. 54–56), occasionally with a posteromedial emargination (Fig. 56); basolateral margins convergent (Figs. 54–56); found west of the French Broad River (Fig. 115) .......................... *R. daniellae* sp. n.

3’ Subgenital plate broadly rounded (Fig. 101–102), occasionally with a shallow posteromedial emargination (Fig. 102), or broadly triangular (Fig. 103); basolateral margins parallel; found east of the French Broad River (Fig. 115) .............................................. *R. kirchneri*

**Generic Description**

**Genus Remenus Ricker, 1952**

(Figs. 1, 3–18)

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

Isogenous (*Remenus*) Ricker (1952: 122)

*Remenus*: Illies (1966: 376)


*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;
specimen yellow-brown. Head wider than 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); frons and clypeus 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 1/2 (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 ~ 1/2 length of tergum; terga 1–2
without intercalary setae (Fig. 16); terga 4-10 with < 35 intercalary setae (Fig. 16). Abdominal sternae 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 ½, apical ¾ 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)
http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:608

Perla bilobata Needham & Claassen (1925: 95) Holotype ♀: 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.


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

Remenus bilobatus: Illies (1966: 376)


Remenus bilobatus: Surdick & Kim (1976: 9)

Remenus bilobatus: Lake (1980: 43)

Remenus bilobatus: Tarter & Kirchner (1980: 49)


Remenus bilobatus: Grubbs (1997: 81)


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)


Illiesia – http://illiesia.speciesfile.org

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Additional records not examined. New York, Franklin Co., Dutton Brook, Rte. 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♂, 5♀ (LCRI).

Distribution. USA – AL, CT, DE, GA, KY, MD, NC, NY, PA, SC, TN, VA, WV. (Fig. 19).
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 extends anteriorly ¼ tergum length and 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 (Fig. 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 ~ ½ 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 ~ ½ length anteriorly into sternum 8 (Figs. 29–30).

Ovum. Shape typical of genus (Figs. 31–34). Length 439–440 μm; width 348–361 μm (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 ¼; apical ¾ 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...
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).
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 ± 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♂, 3♀ (CSUIC); Same location, 14 May 2017, [emerged 18 May 2017], C. Verdone, B.C. Kondratieff, 2♂, 3♀ (CSUIC); Same location, 20 May 2017, C. Verdone, D. Fuller, 3♂, 2♀ (CSUIC); Right Fork Cove Creek, Rte 284, [N 35.62104, W 83.05193], 23 May 1993, B.C. Kondratieff, R.F. Kirchner, 2♂, 1♀ (CSUI).  
*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♂ (INHS); Proctor Branch, Twentymile Creek Trail, GRSM, N 35.48558, W 83.83684, 5 June 2003, B.D. Heinold, 1♂ (INHS).

**Tennessee, Blount Co.,** Anthony Creek, E Cades Cove Campground, Anthony Creek Trail at 3rd footbridge going upstream, GRSM, N 35.58680, W 83.75160, 26 May 2001, R.E. DeWalt, B.D. Heinold, 1♂, 2♀ (INHS).

**Sevier Co.,** Greenbriar Cove, Smoky Mtns., [N 35.70704, W 83.38294], 15 June 1939, A.C. Cole, 1♂ (INHS); Le Conte Creek, Gatlinburg, [N 35.70164, W 83.51361], 14 June 1940, T.H. Frison, 1♂, 3♀ (INHS); Le Conte Creek, ATBI Plot, Twins Creek, GRSM, [N 35.68500, W 83.49888], 8 May–25 May 2010, C.R. Parker, 2♂ (CSUIC); Little Laurel Branch, Ramsey Cascade Trail. GRSM, N 35.70270, W 83.35654, C. Verdone, B.C. Kondratieff, 4♂, 4♀ (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♂, 9♀ (CSUIC).

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

Distribution. USA – 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”.

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 μm (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 ½–⅔ over sternum 9; posterolateral margins convex; basolateral margins convergent posteriorly; basolateral crease typically curved, concave posteriorly, extending ~ ¼ length anteriorly into sternum 8 (Figs. 54–56).

Ovum. Shape typical of genus (Figs. 57–60). Length 434–435 μm; width 324–355 μm (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. 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.
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 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. *Remenus daniellae* occurs west of the French Broad River, whereas *R. kirchneri* inhabits the region to the west of the French Broad River.


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.
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.
average elevation of occurrence localities is 875.1 m (SD ± 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 km² 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).

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

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


**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,

**Distribution. USA** – 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.

Figs. 72–75. *Remenus dufffieldi*, 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.

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

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 μm; width ~ 136–168 μm (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 ~ ½ length anteriorly into sternum 8 (Fig. 81).

**Ovum.** Shape typical of genus (Figs. 82–84). Length 444–446 μm; width 340–355 μm (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

Fig. 90. *Remenus duffieldi*, larval cercus, dorsal, Collins Creek, Swain County, North Carolina.
Carolina. This species has been documented from 1st–3rd 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)

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.

Same location, 9 May 2017, C. Verdone, B.C. Kondratieff, 1♂ (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, 1♂ (CSUIC), paratype); spring fed tributaries of [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, 2♂, 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. USA – 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 μm; width ~ 20–59 μm (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 ½–⅔ 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 μm; width 348–353 μm.

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 ⅔; apical ⅓ tapering to a narrowly rounded apex (Fig. 112). Basal cercal segments with whorls of short setae (Fig. 113).

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 1<sup>st</sup>–3<sup>rd</sup> order streams with drainage areas ranging in size from 0.09–8.75 km<sup>2</sup>. The average elevation of occurrence localities is 717.8 m (SD ± 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/m<sup>2</sup> (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.

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.

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