

THESIS

HOLOMORPHOLOGY AND SYSTEMATICS OF THE EASTERN NEARCTIC STONEFLY

GENUS *REMENUS* RICKER (PLECOPTERA: PERLODIDAE)

Submitted by

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ABSTRACT

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

The holomorphology of the eastern Nearctic stonefly genus *Remenus* Ricker is reviewed using scanning electron microscopy, color photomicrographs and COI DNA barcodes. 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 and Claassen, 1925), *R. duffieldi* Nelson and Kondratieff, 1995, and *R. kirchneri* Kondratieff and Nelson, 1995 by the short clavate epiproct with palmate hair-like spinulae. Nymphs 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, nymphs 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.

Three species, *R. daniellae* sp. n., *R. duffieldi* and *R. kirchneri* are apparently restricted to the Blue Ridge Physiographic Province in the southern Appalachian Mountains. Based on Nature Serve global conservation rankings these species are of high conservation concern. Maximum entropy modeling was used to identify potentially suitable habitats for these species. Model results were used to identify differences in the bioclimatic envelope inhabited by each species, and assess the current status of habitat protection. The models were able to successfully predict the potential distributions of *R. daniellae* sp. n., *R. duffieldi* and *R. kirchneri*. Model evaluation metrics were generally good, AUC = 0.83–0.90, and spatial predictions were plausible. Model results for *R. kirchneri* differed from both *R. daniellae* sp. n. and *R. duffieldi* in both environmental variable selection and percent contribution of these variables. Model response curves for *R. daniellae* sp. n. and *R. duffieldi* exhibited similar responses to environmental variables, which is not surprising as these species occupy similar habitats in relatively close geographic proximity. The amount of protected habitat varied between species. Based on model results, *R. kirchneri* had the least potentially suitable habitat in protected areas. Additional field surveys are needed to provide new occurrence records that can be used to create more accurate and refined distribution models.

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

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 nymphal 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 nymphal 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 $\frac{1}{3}$ 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 belonging to the genus *Remenus*: *R. bilobatus* (Needham and Claassen, 1925), *R. duffieldi* Nelson and Kondratieff, 1995 and *R. kirchneri* Kondratieff and Nelson, 1995.

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 (GSMNP), 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 GSMNP.

Over the two sampling seasons in 2016 and 2017, we collected 589 specimens of *Remenus* representing all life stages, which afforded us a unique opportunity to document the taxonomy of this enigmatic 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 data is not presently available for species of *Remenus*. In this study, we describe a new species of *Remenus*, the nymph of *R.*

duffieldi and *R. kirchneri*, and the ova of *R. kirchneri*. We present for the first time, comparative SEM data for males, high quality photomicrographs of males, females and nymphs. In addition, we provide new diagnoses, biological notes, adult species keys and a neighbor-joining tree based on COI DNA barcodes. Distribution maps for each species compiled from examined material and referenceable 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). Elevation in this province ranges from 64 m to 2035 m asl. Sampling in the Blue Ridge was conducted over a linear distance of 447 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). Elevation in this province ranges from 0 m to 1464 m asl. Sampling in Ridge and Valley was conducted over a linear distance of 551 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 (BioQuip, Rancho Dominguez, California, catalog # 2840C), or an aerial net (catalog # 7615NA). On warmer days, adults were most commonly collected in riparian trees by sweeping the canopy with an aerial net. Nymphs were collected using a 0.093 m² Surber sampler (Merritt et al. 2008) or an aerial net with the net pulled taut and disturbing the stream substrate allowing contents to flow into the net. Nymphs 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. 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 (WKU), and Luke Myers, Lake Champlain Research Institute, Plattsburgh State University of New York, Plattsburgh, New York (LCRI). Plotting of coordinate data and map measurements were accomplished using ArcMap, ArcGIS 10.4.1 (ESRI 201). 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 (GSMNP), 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

Male abdomens were removed using size 5s forceps with offset tips. 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 DNA barcoding protocols were used (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 2007).

RESULTS

Taxonomic Characters

External male genitalia. The epiproct of *Remenus* is small and generally 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, 47–53, 77–78, 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, 80, 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.

Nymph. *Remenus* nymphs 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, *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
- Epiproct without a medial dorsal sclerite (Figs. 45–46, 49–52, 95–96, 98–100)3
- 2 (1) Epiproct flattened laterally; terminating in a threadlike lash that greatly exceed 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)
..... *R. bilobatus*
- Epiproct dorsoventrally flattened; lacking a threadlike lash that greatly exceed 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 (1) 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.
- 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 Basolateral crease nearly straight or convex posteriorly (Figs. 29–30, 81); subgenital plate
broadly triangular (Fig. 29) or rounded (Figs. 30, 81); pronotum mostly brown (Fig. 22) or

- pale (Fig. 72).....2
- Basolateral crease 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 (1) Subgenital plate broadly triangular (Fig. 29) or rounded (Figs. 30), extending $\frac{1}{2}$ over sternum 9, or to the posterior margin of the sternum 9; pronotum mostly brown (Fig. 22)
..... *R. bilobatus*.
- Subgenital plate broadly rounded, elongate, extending $\frac{4}{5}$ over of sternum 9 or slightly beyond the posterior margin of sternum 9 (Fig. 81); pronotum mostly pale (Fig. 72).....
..... *R. duffieldi*
- 3 (1) Subgenital plate broadly rounded (Figs. 54–55), occasionally with a posteromedial emargination (Fig. 56); basolateral margins convergent (Figs. 54–56); found west of the French Broad River.....*R. daniellae* sp. n.
- Subgenital plate broadly rounded (Fig. 101), occasionally with a shallow posteromedial emargination (Fig. 102), or broadly triangular (Fig. 103); basolateral margins parallel; found east of the French Broad River *R. kirchneri*

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; 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 yellow-gold; lobe on 7th sternum well developed, lightly pigmented, narrowly rounded, apex rounded (Fig. 5); lobe on 8th sternum reduced, approximately $\frac{1}{3}$ 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 a 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 $\frac{1}{2}$ sternum nine, 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 $\frac{1}{3}$ 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).

Nymph. Body length 8.3–11.4 mm, ($n = 19$). Preserved 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); 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 approximately 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 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 $\frac{1}{3}$ (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 corner 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 $\sim \frac{2}{3}$ length of tergum; terga 1–2 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 nymphs with an elongate terminal process (developing epiproct) with a constriction at the basal $\frac{1}{3}$, apical $\frac{2}{3}$ thin and tapered (Fig. 18). Mature nymphs with paired medial and lateral spots on the abdominal terga. Cerci with a whorl of variable length setae (Figs. 16, 18).

Diagnosis. Regionally, *Remenus* is morphologically most similar to *Diploperla duplicata* (Needham & Claassen, 1925). 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, approximately $\frac{1}{3}$ 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) Nymphs and adults lack

submental gills; (6) Nymphs are similarly concolorous; (7) Nymphs 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. Nymphs 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 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 ♂: New York, Herkimer County, Old Forge (Cornell, CUIC). Figures: adult, fore- and hindwing, male stern 7–9, male terminalia (lateral), female subgenital plate.

Perla bilobata: Claassen (1931: 54). Figures: nymph–labrum, mandibles, labium, maxilla, lacinia.

Diploperla bilobata: Frison (1942: 302). Figures: nymph–habitus, ♀ sterna, mandibles, labium, maxilla, lacinia.

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

Remenus bilobatus: Illies (1966: 376)

Remenus bilobatus: Hitchcock (1974: 214). Figures: nymph–habitus, ♀ 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: nymph–head and pronotum, mandible,
lacinia, foreleg, mesosternum, male and female sterna 7–10, cerci (basal, middle, apical).

Remenus: Stewart & Stark (1988: 402). Figures: nymph–male 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: nymph–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: Tarter & Nelson (2010: 162)

Remenus bilobatus: Grubbs (2011: 29)

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: nymph–cerci (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♂ (USMN); nr. Madison, nr. Rte 80, [no GPS], 18 June 1965, S.W. Hitchcock, 1♂

(USMN). **Salem Co.**, Fraser Brook, Salem, Four Corners, [N 41.47291, W 72.26029], 15 June

1967, S.W. Hitchcock, 1♂ (USMN). **Tolland Co.**, Storrs, [N 41.80843, W 72.24952], 18 June

1954, J.A. Slater, 1♂, 1♀ (USMN). **New York, Herkimer Co.**, Old Forge, [N 43.71007, W

74.97434], 18 July 1905, J.G. Needham, P.W. Claassen, 1♂, 1♀ (USMN, 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♂, 3♀ (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], 1♀ (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, 1N (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♀, 1N (CSUIC). **Jackson Co.**, Balsam, [N 35.42667, W 83.08528], 24 April 1938, H.H. Ross, B.D. Burks, 6N (INHS). **Macon Co.**, Big Creek, Lake Randall, [N 35.07861, W 83.20806], 20 June 1939, T. Howell, 1N (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, GSMNP, 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, 1♂ (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♀, 7N (CSUIC). **Maryland, Frederick Co.**, Fishing Creek, Mountindale, [N 39.51949, W 77.45114], 1 July 1958, P. Freytag, 3♂ (INHS). **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, 3♂, 3♀ (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, 2♂ (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, 1♂ (CSUIC). **South Carolina, Pickens Co.**, Wildcat Creek, 9 km SW Clemson, [N 34.75607, W 82.85767], 18 May 1976, P.H. Carlson, 1♂ (CSUIC); Wildcat Creek, Clemson Experimental Forest, [N 34.75607, W 82.85767], 5 May 1985, K.W. Stewart, B.C. Kondratieff, R.F. Kirchner, 1N (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, Great Smoky Mountains National Park, N 35.60250, W 83.79390, 7 June 2001, B.D. Heinold, 1♂ (INHS); Abrams Creek, Cades Cove, GSMNP, [N 35.60494, W 83.77653], 1–12 June 2003, C.R. Parker, 4♂ (CSUIC); Same location, 18–25 June 2003, C.R. Parker, 1♂ (CSUIC); Cades Cove, GSMNP, [N 35.60840, W 83.82700], 13 June 1940, T.H. Frison, 1♂, 1N (INHS); Cattail Branch, GSMNP, N 35.51490, W 83.97630, 21 May 2003, R.E. DeWalt, 3N (INHS); Tabcat Creek, at first jeep trail crossing, GSMNP, N 35.51956, W 83.97927, 21 May 2003, R.E. DeWalt, 4N (INHS). **Sevier Co.**, Le Conte Creek, Gatlinburg, [N 35.70164, W 83.51361], 4 May 1939, T.H. Frison, 7N (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, 8♂, 2♀ (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, 2♂, 2♀ (CSUIC). **Amherst Co.**, Dancing Creek, Blue Ridge Parkway, Dancing Creek Overlook Parking, BLRI, N 37.63430, W 79.33258, 18 June 2016, C. Verdone, 1♂ (CSUIC). **Augusta Co.**, Calfpasture River, Jct. Hwy 250 and Rte 715, [N 38.27356, W 79.30068], 8 June 2017, B.C. Kondratieff, 5♂, 3♀ (CSUIC); Love bog Blue Ridge

Parkway, mile 15.61, BLRI, [N 37.88348, W 79.01054], 16-17 July 2007, C.R. Parker, 1♂ (CSUIC); Paine Run, Jct 661 and 614, [N 38.19880, W 78.79340], 19 July 1975, O.S., C.M Flint 1♂ (USMN); tributary to Braley Pond, NW Rte 715, [N 38.28963, W 79.30448], 9 June 2017, B.C. Kondratieff, 1♂ (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, 1♂, 1♀ (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, 1♂, 1♀ (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, 2♂ (CSUIC). **Fauquier Co.**, Bartons Brook, Jackson Hollow, [no GPS], 15 July 1974, R.W. Baumann, O. Flint, 1♀ (USMN); Little Bull Run, Bull Run Mtn., Shurburg Home,

Hopewell Rd., N 38.86665, W 77.703922, 1 June–10 July 2014, D.R. Smith, 7♂, 3♀ (USMN); tributary to Broad Run, Arlington Outdoor Lab, [N 38.80720, W 77.72170], 3–20 June 2016, D.R. Smith, 1♀ (USMN); 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♂, 7♀ (USMN). **Floyd Co.**, Dodd Creek, Blue Ridge Parkway, BLRI, N 36.87178, W 80.27901, 31 May 2016, C. Verdone, B.C. Kondratieff, 1♂, 1♀ (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, 1♂ (USMN). **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, 11♂, 6♀ (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, 6♂, 4♀ (CSUIC); tributary to Rapidan River, Rte 649 (SHEN), N 38.46519, W 78.36990, 2 June 2016, C. Verdone, B.C. Kondratieff, S. Roble, 4♂, 1♀ (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♂, 2♀ (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♂, 1♀ (CSUIC); Same location, 26 May–11 June 2012, D.R. Smith, 2♂, 2♀ (CSUIC); Same location, 26 May–11 June 2012, D.R. Smith, 12♂, 5♀ (USMN); 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♂ (USMN); 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♂ (USMN). **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, 1♂ (CSUIC). **Tazewell Co.**, Cove Creek, Rte 662, off Hwy 61, N 37.17837, 81.29900, 6 June 2016, C. Verdone, B.C. Kondratieff, 9♂, 9♀ (CSUIC); East Fork Cove Creek, Rte 662, [N 37.19464, W 81.30068], 12 June 1983, B.C. Kondratieff, 1♂ (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, 9♂, 9♀ (CSUIC); Detroit Cove, Rte 689, N 36.79664, W 82.05730, 4 May 2017, C. Verdone, B.C. Kondratieff, 1N (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, 2N (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, 1♂ (CSUIC); Same location, 18 June–1 July 2004, D.R. Smith 6♂, 2♀ (CSUIC); Same location, 12–15 July 2004, D.R. Smith, 4♂ (USMN). **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, 1♂ (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, 2N (INHS); Same location, 13 June 1975, R.F. Kirchner, 1♀, 7N (INHS).

Additional records. **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♂, 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, as for 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 $\frac{3}{4}$ terga 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 approximately 300–320 µm; width approximately 80–84 µm (n = 3); epiproct flattened laterally (Figs. 23, 26), with a ventral keel (Figs. 23, 26–27), 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 approximately 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 approximately $\frac{1}{2}$ over of 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 approximately $\frac{1}{3}$ length anteriorly into sternum 8 (Figs. 29–30)

Ovum. As for genus (Figs. 31–34). Length 439–440 μm ; width 348–361 μm (n = 3).

Nymph. (Fig. 35). Body length 9.0–10.4 mm, (n = 5). Head (Fig. 36), lacinia (Fig. 37), mandibles (Fig. 38), and pronotum (Fig. 36) as for genus. Mature male nymph with an elongate terminal process with a constriction at the basal $\frac{1}{3}$; apical $\frac{2}{3}$ thin and tapered (Fig. 39). Mature nymph with paired medial and lateral spots on the abdominal terga. Basal cercal segments with a whorl of variable length setae (Fig. 40).

Diagnosis. *Remenus bilobatus*, with its characteristic lash, in addition to several other characteristics, is easily separated from the other *Remenus* species. The lash is rarely complete however 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 notably, the sclerite does not greatly exceed the epiproct apex. Males of *R. bilobatus* are further differentiated in that 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.

Whereas in males of *R. duffieldi*, the epiproct is dorsoventrally flattened, tergum 9 is not divided, lacks sensilla basiconica, and the spinulae covering the basal cowl are pale.

As noted by Kondratieff & Nelson (1995), isolated females of *Remenus* are difficult to distinguish from one another without associated males. Minor differences in the shape of the subgenital plate and basolateral crease can be used to differentiate species, but should be done so with caution. 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 the in *R. duffieldi* it is pale with brown rugosities.

Mature nymphs of *R. bilobatus* can be separated from *R. kirchneri* and *R. duffieldi* by the presence of a long setae on the basal cercal segments. However, this character is shared by *R. daniellae* sp. n. which is sympatric in several locations.

Biological notes. No life history or biological studies have been done for this species. Anecdotal evidence suggests this predacious Perlodinae has a univoltine life cycle and inhabits leaf packs caught between cobbles 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* can occur in 1st order 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 can inhabit, providing it with greater habitat connectivity. Although this species is widely distributed, adults are seldom collected in large numbers. Of the 89 adult records presented only 13.5% (n = 12) were represented by more than 10 individuals, 40.4% (n = 36) 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 (GSMNP), 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 (GSMNP), 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, 1♂, 3♀, 3 exuvia (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♀ (CSUIC). **Swain Co.**, Collins Creek, Collins Creek Picnic Area, Hwy 441 (GSMNP), N 35.56752, W 83.09251, 14 May 2017, C. Verdone, B.C. Kondratieff, 1♂ (CSUIC); Gunna Creek, at confluence with Spence Cabin Branch, GSMNP, N 35.55120, W 83.73220, 3 June 2003, B.D. Heinold, C. Favret, 1♂ (INHS); Proctor Branch, Twentymile Creek Trail, GSMNP, 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, GSMNP, 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 (GSMNP), [N 35.68500, W 83.49888], 8 May–25 May 2010, C.R. Parker, 2♂ (CSUIC); Little Laurel Branch, Ramsey Cascade Trail (GSMNP), N 35.70270, W 83.35654, C. Verdone, B.C. Kondratieff, 4♂, 4♀ (CSUIC); tributary to Le Conte Creek, Twin Creeks Uplands Research Lab, GSMNP, N 35.68706, W 83.50096, 16 May 2017, C. Verdone, B.C. Kondratieff, 11♂, 9♀ (CSUIC).

Additional material. **North Carolina, Haywood Co.**, Ball Branch, Old Cataloochee Turnpike (GSMNP), N 35.71817, W 83.09251, 14 May 2017, C. Verdone, B.C. Kondratieff, 3N (CSUIC); Mt. Sterling Creek, Old Cataloochee Turnpike, GSMNP, N 35.70819, W 83.09658, 10 July 1983, B.C. Kondratieff, 2♀ (CSUIC); tributary to Hemphill Creek, 5 km WNW Jonathan, pump-house spring below Purchase Knob House, GSMNP, N 35.58220, W 83.07370, 31 May 2003, R.E. DeWalt, 2N (INHS). **Tennessee, Sevier Co.**, Le Conte Creek, Gatlinburg, [N 35.70164, W 83.51361], 14 June 1940, T.H. Frison, 6N (INHS).

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 “Smoky 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 as for 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. Tergum 9

with < 4 minute sensilla basiconica on either side of midline. 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 approximately 170–270 μm ; width approximately 82–89 μm (n = 3); epiproct clavate (club-shaped) and lightly sclerotized; covered in dense, thick palmate hair-like spinulae (Figs. 45–50); when produced forward rarely exceeding the anterior margin of the basal anchor; clavate in dorsal and lateral aspect, widest near the apical $\frac{1}{4}$ and typically bearing a short translucent tube at the apex (Figs. 51–52). 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. 54–55), occasionally with a posteromedial emargination (Fig. 56); lightly sclerotized, with regularly spaced setae, extending $\frac{1}{2}$ – $\frac{4}{5}$ over sternum 9; posterolateral margins convex; basolateral margins convergent posteriorly; basolateral crease typically curved, concave posteriorly, extending approximately $\frac{1}{4}$ length anteriorly into sternum 8 (Figs. 54–56).

Ovum. As for genus (Figs. 57–60). Length 434–435 μm ; width 324–355 μm (n = 3).

Nymph. (Fig. 61). Body length 8.3–10.8 mm, (n = 3) Head (Fig. 62), lacinia (Fig. 63), mandibles (Fig. 64), and pronotum (Fig. 62) as for genus. Mature male nymph with a short ovoid terminal process (developing epiproct) (Fig. 65). Basal cercal segments with a whorl of variable length setae (Fig. 66).

Diagnosis. Males of *R. daniellae* sp. n. are morphologically similar *R. kirchneri*, both of which lack a medial dorsal sclerite. However, *R. daniellae* sp. n. can be distinguished by details of the epiproct and terminalia. Males of *R. daniellae* sp. n. possess a clavate epiproct in dorsal and lateral aspect, which is widest towards the apical $\frac{1}{4}$ and bears a short translucent tube at the apex; whereas, the epiproct of *R. kirchneri* is dorsoventrally flattened, widest medially or near the basal $\frac{1}{4}$ 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* sp. n. 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* sp. n., the subgenital plate is typically convergent. Based on presently available records, the ranges of these two species do not overlap. *Remenus daniellae* sp. n. occurs west of the French Broad River, whereas *R. kirchneri* inhabits the region to the east. As such, morphology paired with geographic location should help inform identification of these two similar species.

Mature nymphs of *R. daniellae* sp. n. 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* sp. n. 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 Co. North Carolina based on two females

collected in 1985 from Mt. Sterling Creek in GSMNP. This location is 1.1 km from the type locality of *R. daniellae* sp. n. These specimens have been re-examined and have been re-determined as *R. daniellae* sp. n. 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 done for this species. *Remenus daniellae* is known only from ten locations in GSMNP 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 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 GSMNP, 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).

***Remenus duffieldi* Nelson & Kondratieff, 1995**

(Figs. 70–87)

Remenus duffieldi Kondratieff & Nelson (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: nymph–cerci (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♂ (CSUIC). **Macon Co.,** Nantahala River, [no GPS], 30 May 1939, Thelma Howell, 1N (INHS); Tellico Creek, Tellico Rd. [no GPS], 27 May 1993, B.P. Stark, R. Simmons, P. Kelly, 1♂ (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♂, 7♀ (CSUIC). **Swain Co.,** Collins Creek, Collins Creek Picnic Area, Hwy 441, GSMNP, N 35.56752, W 83.33663, 16 May 2017, C. Verdone, B.C. Kondratieff, 5♂, 2♀, 1N (CSUIC); Kanati Fork, Hwy 441 (GSMNP), 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. 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).

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, as for genus (Fig. 72). Pronotum pale, covered in regularly spaced setae, with light brown rugose area, 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 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 approximately 382–420 µm; width approximately 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 exceeds 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).

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 $\frac{4}{5}$ over sternum 9, or slightly beyond the posterior margin of sternum 9 (Fig. 81); basolateral crease nearly straight or convex posteriorly, extending approximately $\frac{1}{3}$ length anteriorly into sternum 8 (Fig. 81).

Ovum. As for genus (Figs. 82–84). Length 444–446 μm ; width 340–355 μm (n =3).

Nymph. (Fig. 85). Body length 9.1 mm, (n = 1). Head (Fig. 86), lacinia (Fig. 87), mandibles (Fig. 88), and pronotum (Fig. 86) as for genus. Mature male nymph with a short triangular terminal process (developing epiproct) (Fig. 89), Basal cercal segments with a whorl 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 nymphs of *R. duffieldi* are separable from the two other sympatric species, *R. bilobatus* and *R. daniellae* sp. n. 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.

Biological notes. No life history or biological studies have been done for this species. *Remenus duffieldi* is known from ten locations in Blue Ridge Physiographic Province of Georgia and North 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 GMSNP, Nantahala and Chattahoochee-Oconee National Forests.

***Remenus kirchneri* Kondratieff & Nelson, 1995**

(Figs. 1, 92–113)

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, 1♂ (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♀, 6N (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♂, 2♀ (CSUIC); tributary to Turkey Creek, Rte 620, N 36.61184, W 80.77082, 11 May 2017, 2♂, 6N (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♂, 1♀ (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, 5♂, 7♀ (CSUIC); Same location, 9 May 2017, C. Verdone, B.C. Kondratieff. 38N (CSUIC); Same data, [emerged 19 May 2017], 1♂, 1♀ (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♂, 1♀ (CSUIC); Spring tributary to Little River, Rte 706, N 36.98935, W 80.39589, 29 May 2016, C. Verdone, B.C. Kondratieff, 1♂, 2♀ (CSUIC); tributary to Dodd Creek, Blue Ridge Parkway, BLRI, N 36.86353, W 80.28702, 31 May 2016, C. Verdone, B.C. Kondratieff, 13♂, 10♀ (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, 8♂, 6♀ (CSUIC); tributary to Little River, Blue Ridge Parkway, N of Rte 642 "Poff", BLRI, N 37.01437, W 80.12343, 19 May 2017, C. Verdone, B.C. Kondratieff, S. Roble, 1♂, 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, 7♂, 4♀, 4N (CSUIC). **Franklin Co.**, Brogan Branch, Rte 680/793, N 36.91700, W 80.19504, 31 May 2016, C. Verdone, B.C. Kondratieff, 1♂ (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, 15N (CSUIC); Same location, 11 May 2017, C. Verdone, B.C. Kondratieff, 18N (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♂, 1♀ (CSUIC); same location, 26 May 2016, C. Verdone, B.C. Kondratieff, 1♂, 2♀ (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♂, 9♀ (CSUIC); Same location, 9 May 2017, C. Verdone, B.C. Kondratieff, 1N (CSUIC); small spring fed stream into [Talbot 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, 10N (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 as for 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 approximately 270–500 µm; width approximately 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), occasionally with a shallow posteromedial emargination (Fig. 102), or broadly triangular (Fig. 103); lightly sclerotized, with regularly spaced setae, extending $\frac{1}{2}$ – $\frac{4}{5}$ over sternum 9; posterolateral margins convex; basolateral margins typically parallel; basolateral crease typically curved, concave posteriorly, extending approximately $\frac{1}{4}$ length anteriorly into sternum 8 (Figs. 101–103).

Ovum. As for genus (Figs. 104–107). Length 438–460 μm ; width 348–353 μm .

Nymph. (Fig. 108). Body length 8.5–11.4 mm, (n = 10). Head (Fig. 109), lacinia (Fig. 110), mandibles (Fig. 111), and pronotum (Fig. 109) as for genus. Mature male nymph with a short terminal process (developing epiproct) with a constriction at the basal $\frac{1}{3}$; apical $\frac{2}{3}$ tapering to a narrowly rounded apex (Fig. 112). Basal cercal segments with a whorl of short setae (Fig. 113).

Diagnosis. Males of *R. kirchneri* are morphologically similar *R. daniellae* sp. n. Males possess a tongue-shaped epiproct in dorsal aspect, which is widest medially or near the basal $\frac{1}{4}$ and is dorsoventrally flattened; whereas, the epiproct of *R. daniellae* sp. n. is clavate, is widest towards the apical $\frac{1}{4}$ and narrows posteriorly both in dorsal and lateral aspect. Additionally, the epiproct of *R. daniellae* sp. n. 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* sp. n. 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* sp. n. Separation of these species may require associated males. Generally, the subgenital plate of *R. kirchneri* is parallel sided basally, whereas in *R. daniellae* sp. n., 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 nymphs 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.

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 ± 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 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. Other similarly sized perlodids are known to produce egg masses ranging in size from approximately 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 data supported the morphological species concepts presented above (Fig. 114). All four species, *R. bilobatus*, *R. daniellae* sp. n., *R. duffieldi*, and *R. kirchneri* were clearly delineated with bootstrap values supporting all major nodes.

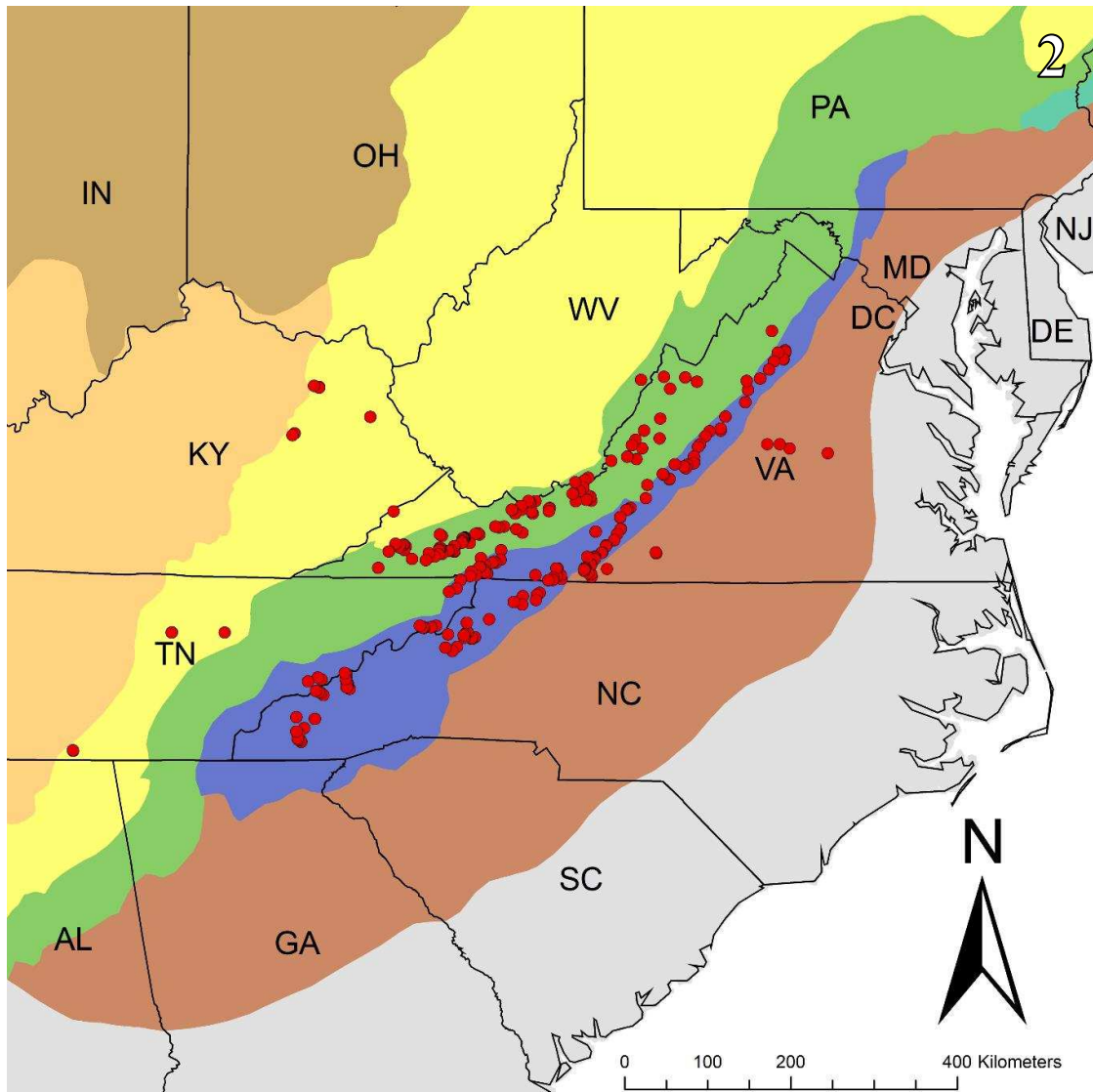
DISCUSSION

Four species of *Remenus* are now known, with species keys provided for adult males and females based on external genitalia. Nymphs 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, nymphs 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. 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. While this study has 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.

FIGURES AND CAPTIONS



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

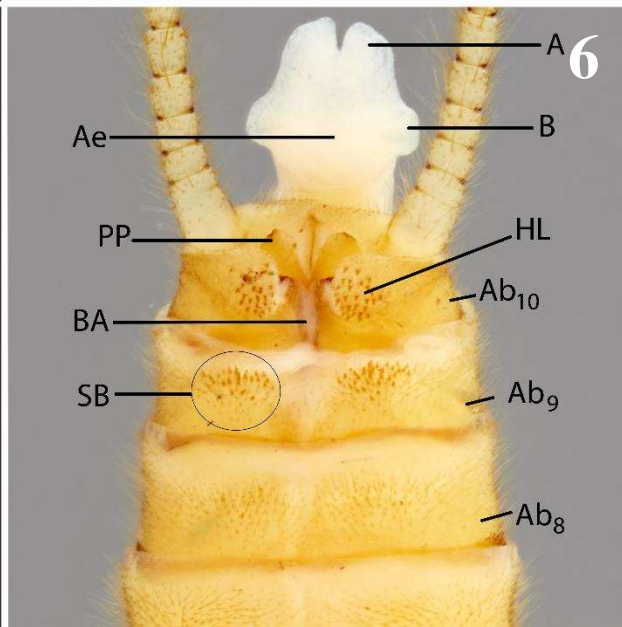
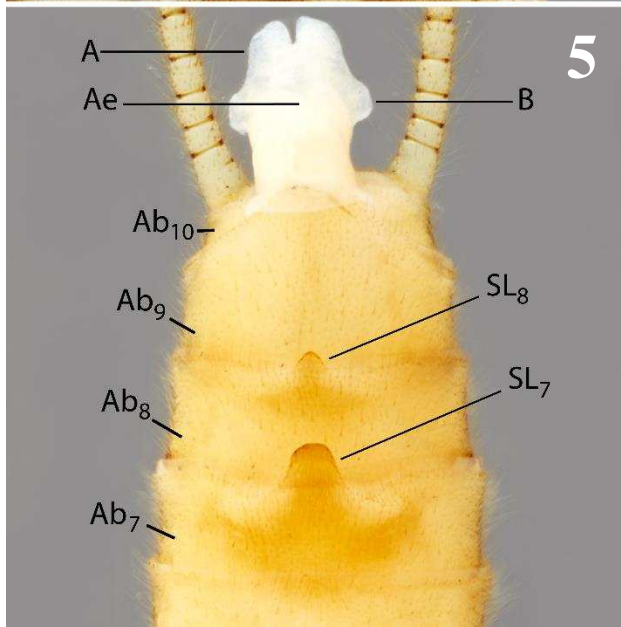
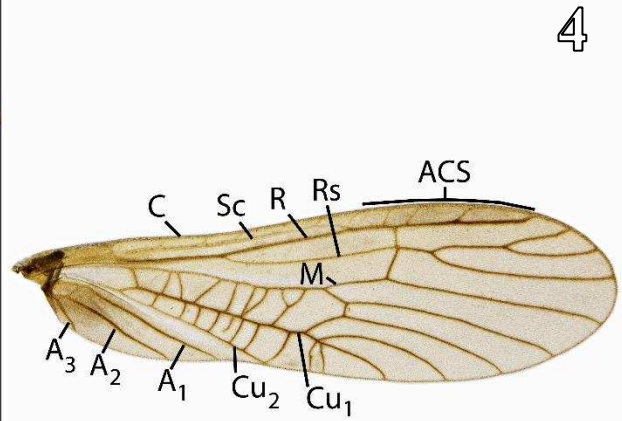
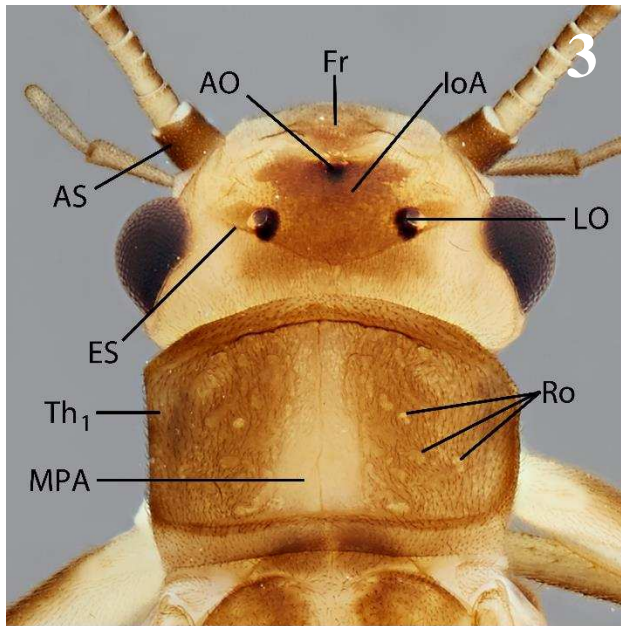


Legend

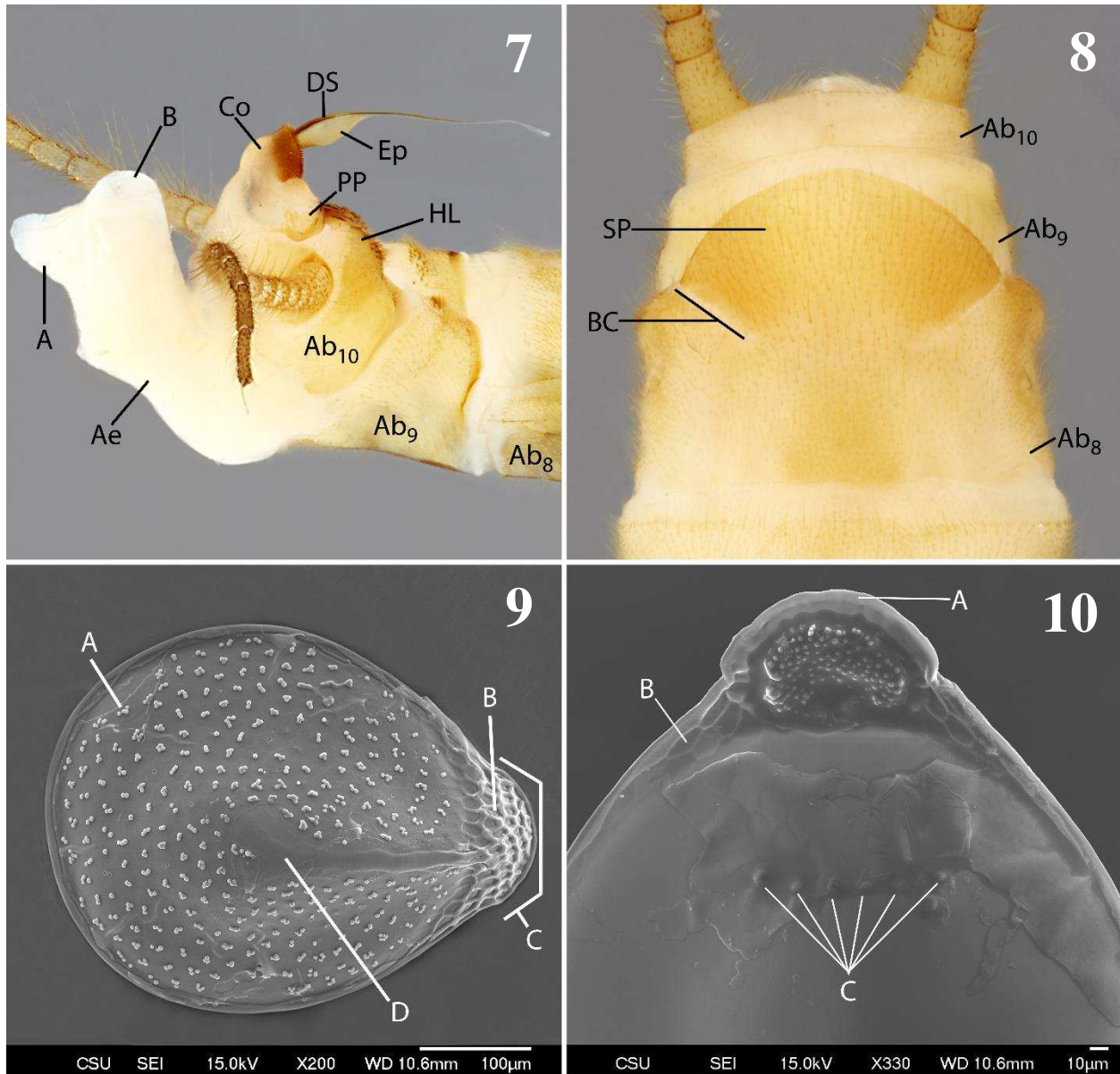
- Collecting Locations 2016-17
- State Boundaries
- Appalachian Plateau
- Blue Ridge
- Central Lowland
- Coastal Plain
- Interior Low Plateau
- New England
- Piedmont Plateau
- Ridge and Valley

Map Source: Chris Verdone, 2018
 Map Data: US Physiographic Provinces, USGS Water Resources NSDI Node

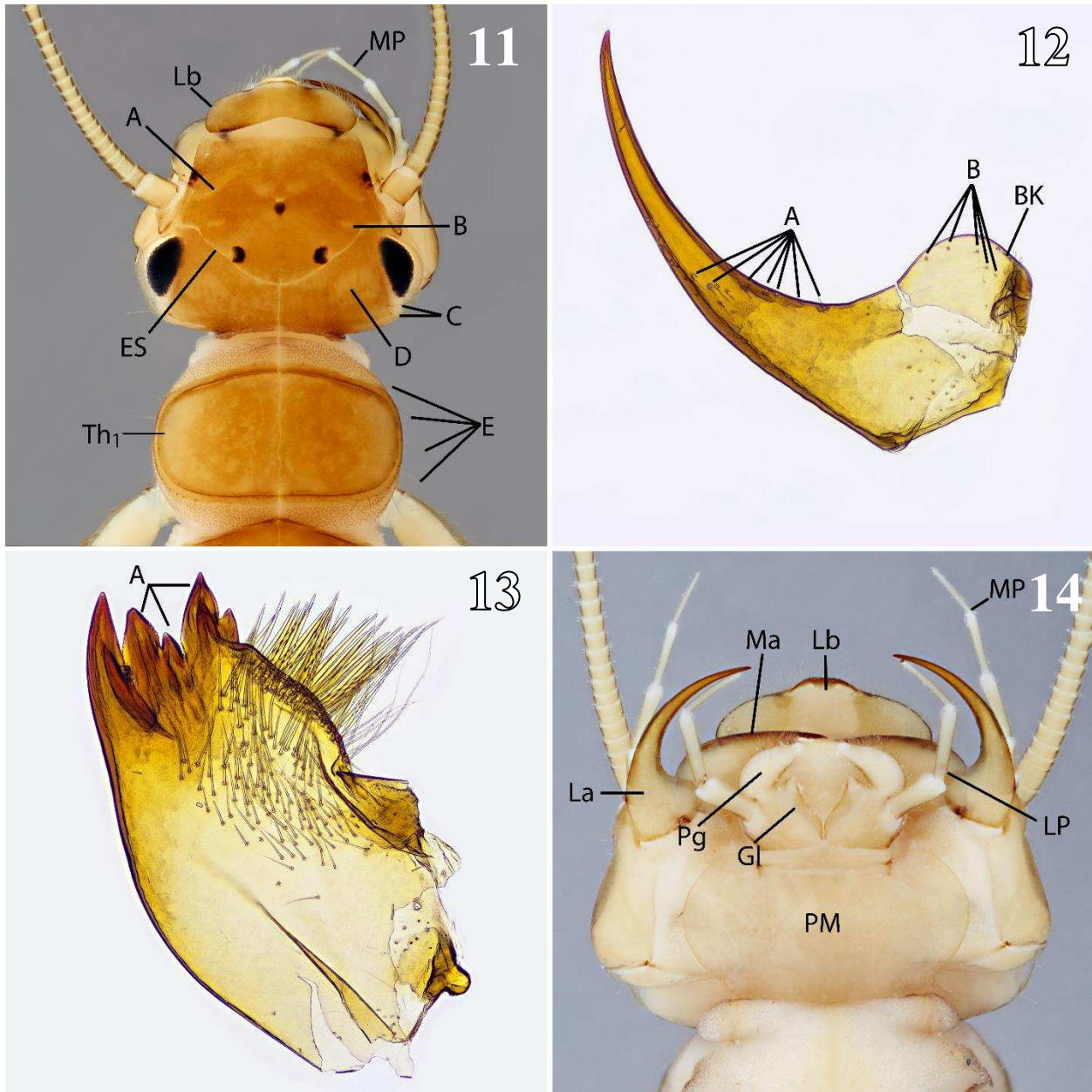
Figure 2. Map of the eastern Nearctic physiographic provinces and 2016–17 collecting locations.



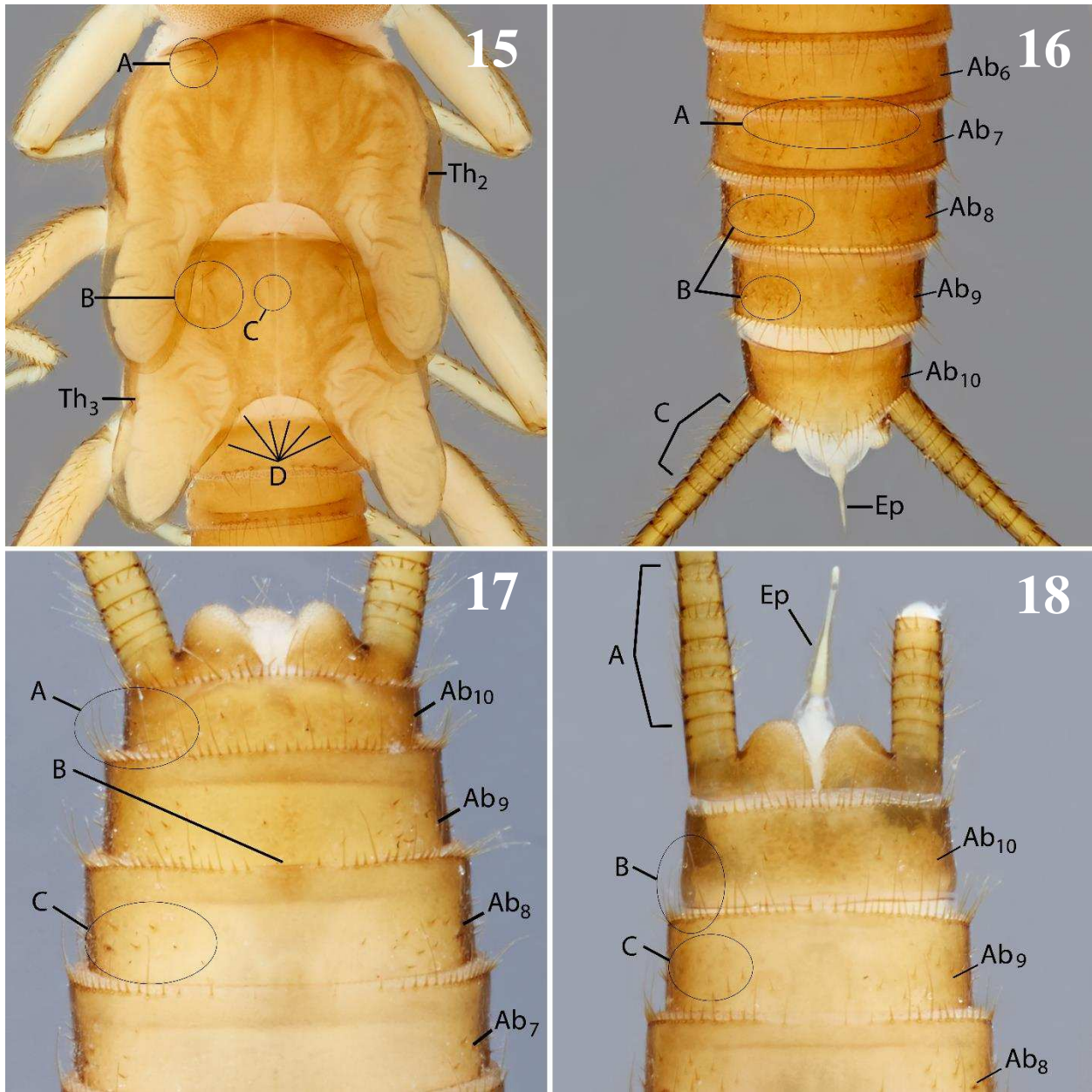
Figures 3–6. *Remenus bilobatus*, adult male. 3. Head and pronotum, AO = anterior ocellus, AS = antennal scape, ES = epicranial suture, Fr = frons, IoA = interocellular area, LO = lateral ocelli, MPA = medial pale area, Ro = rugosities, Th = pronotum. 4. Right forewing, A = anal vein, ACS, apical costal 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.



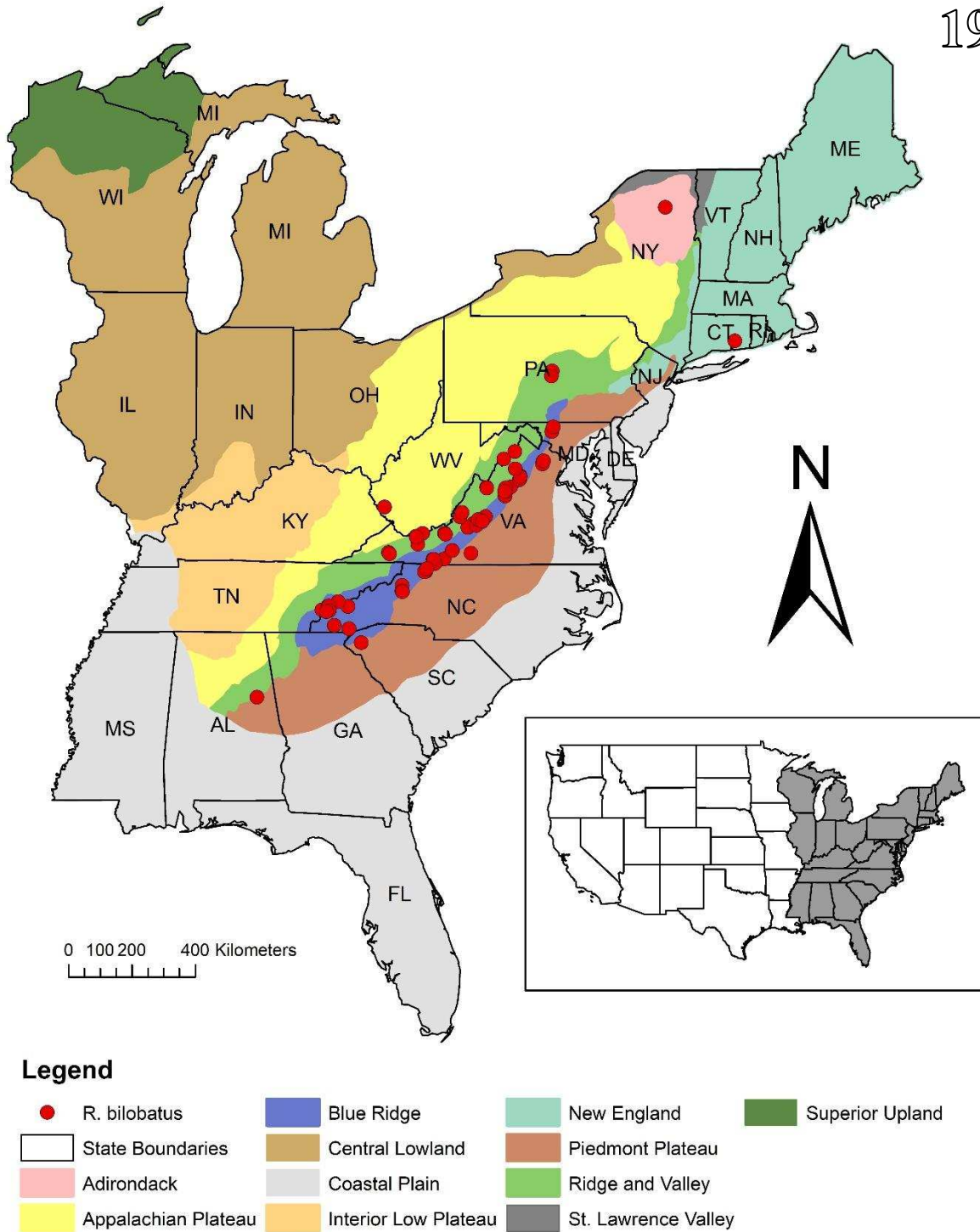
Figures 7–10. *Remenus bilobatus* adult and ovum. 7. Male terminalia, lateral, A = posterodorsal lobes, Ae = aedeagus, Ab = abdominal segment, B = lateral lobes, Co = Cowl, DS = dorsal sclerite, Ep = epiproct, HL = hemitergal lobe, PP = paragenital plate. 8. Female abdominal sterna 8–10, Ab = abdominal 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.



Figures 11–14. *Remenus bilobatus*, nymph, 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, Th = pronotum. 12. Left lacinia, dorsal, A = minute irregularly spaced setae, B = 5 submarginal minute 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.



Figures 15–18. *Remenus bilobatus*, nymph. 15. Meso- Meta-nota, A = mesonotal anterolateral setae, B = metanotal anterolateral setae, C = mediolateral seta, D = marginal setae, Th₂ = mesonota, Th₃ = metanota. 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, A = posterior setal fringe, Ab = abdominal segment, B = darkened posteromedially strip, C = intercalary setae. 18. Male sterna 8–10, A = Basal cercal segments, Ab = abdominal segment, B = intersegmental setae, C = intersegmental setae, Ep = Male terminal process (developing epiproct).



Map Source: Chris Verdone, 2018
 Map Data: US Physiographic Provinces, USGS Water Resources NSDI Node

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

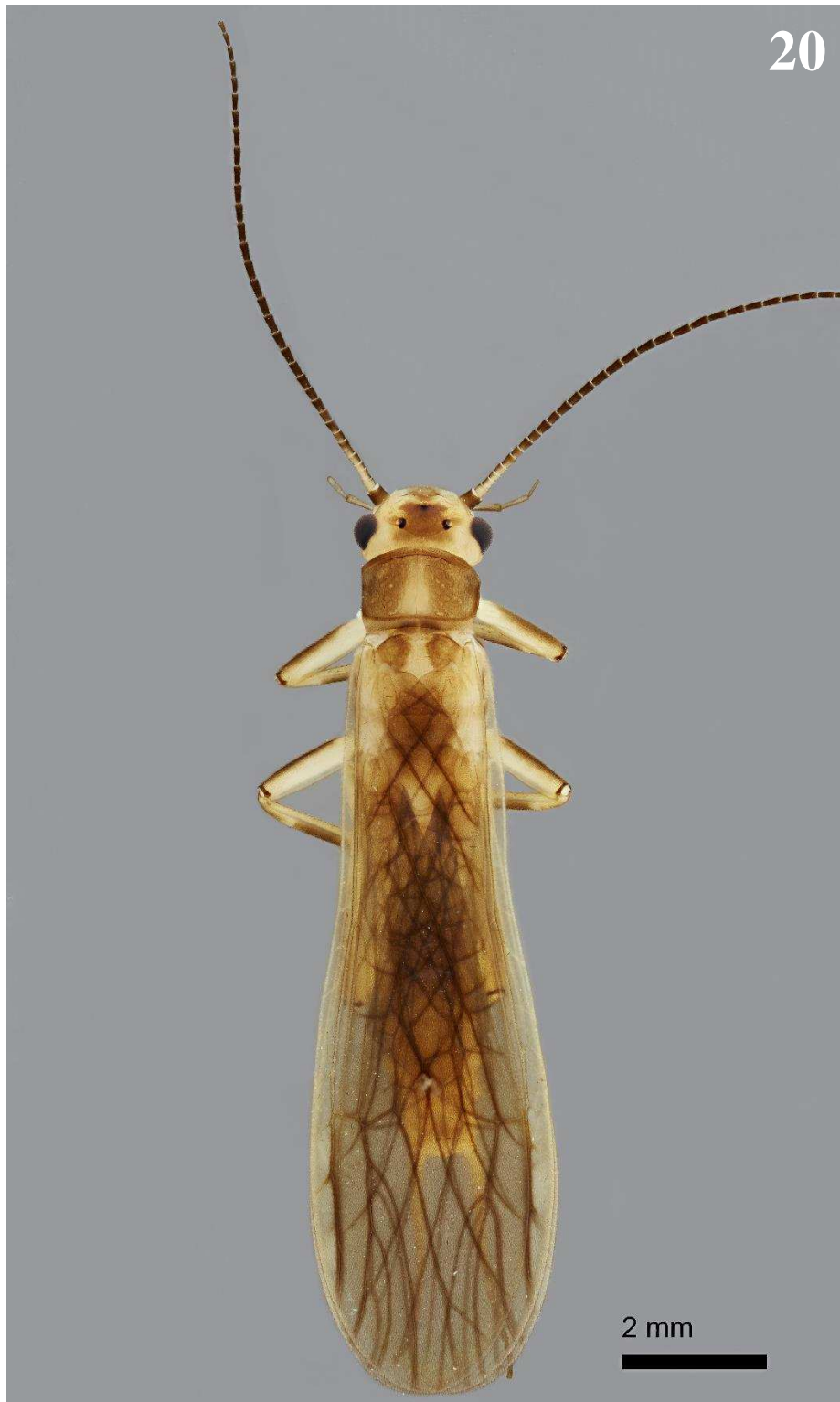
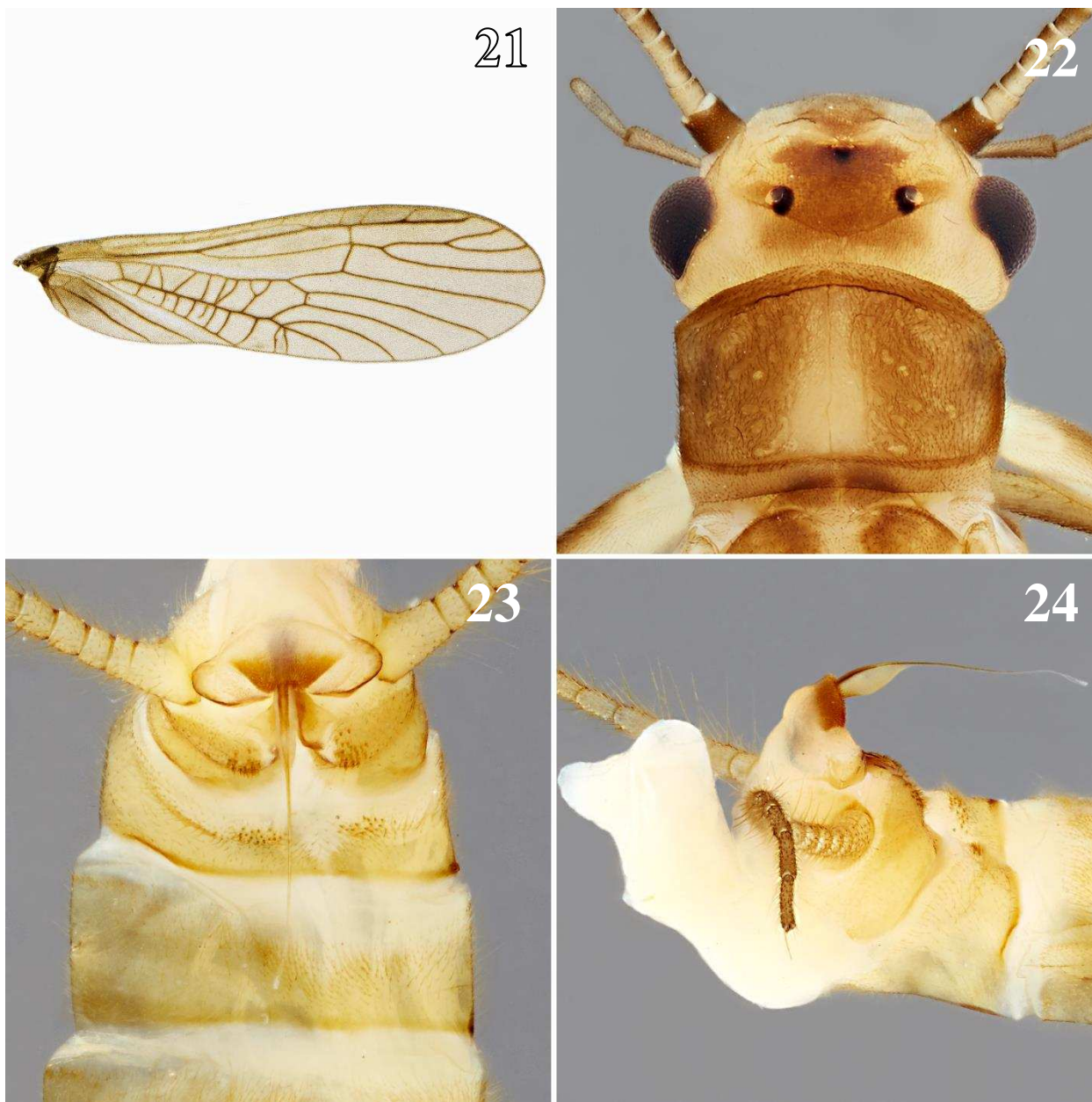
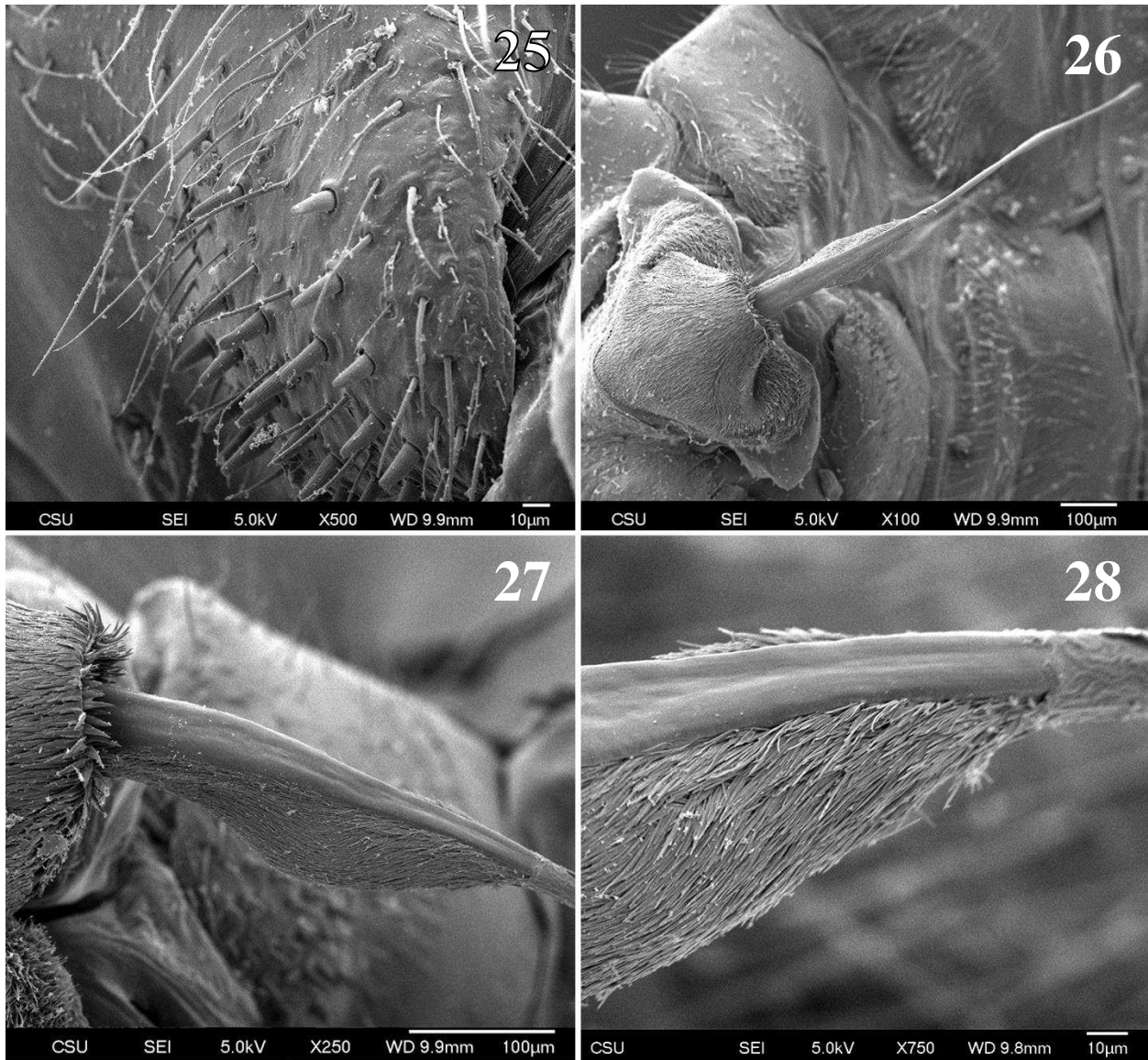


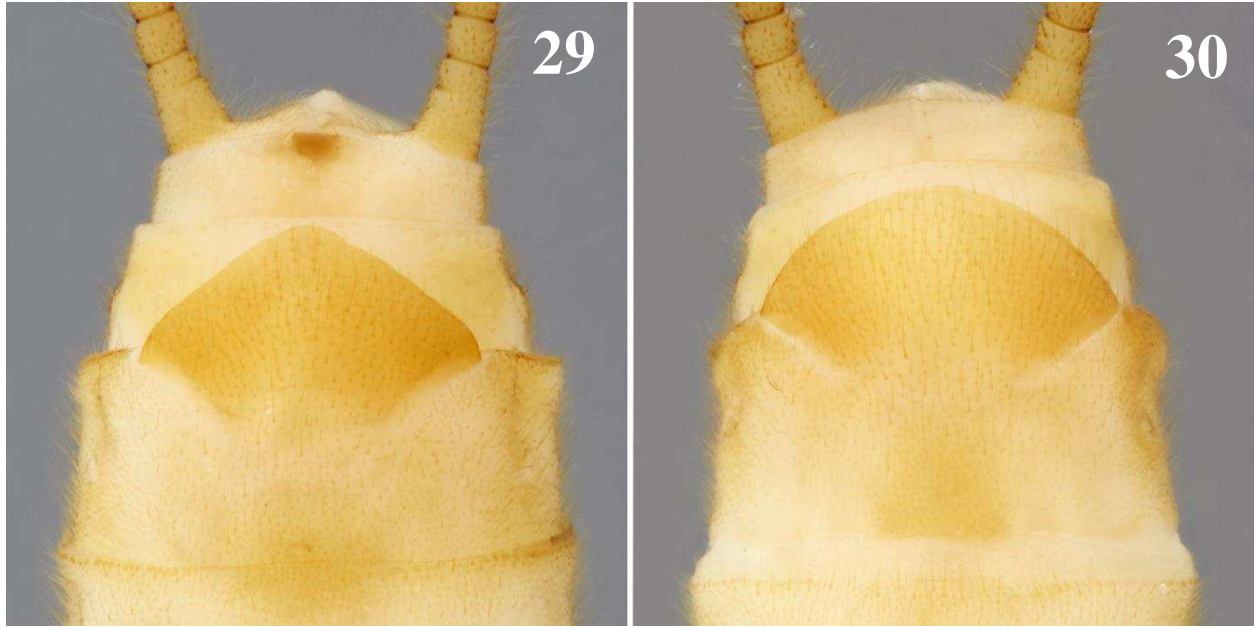
Figure 20. Adult male habitus of *Remenus bilobatus*, Calfpasture River, Augusta County, Virginia.



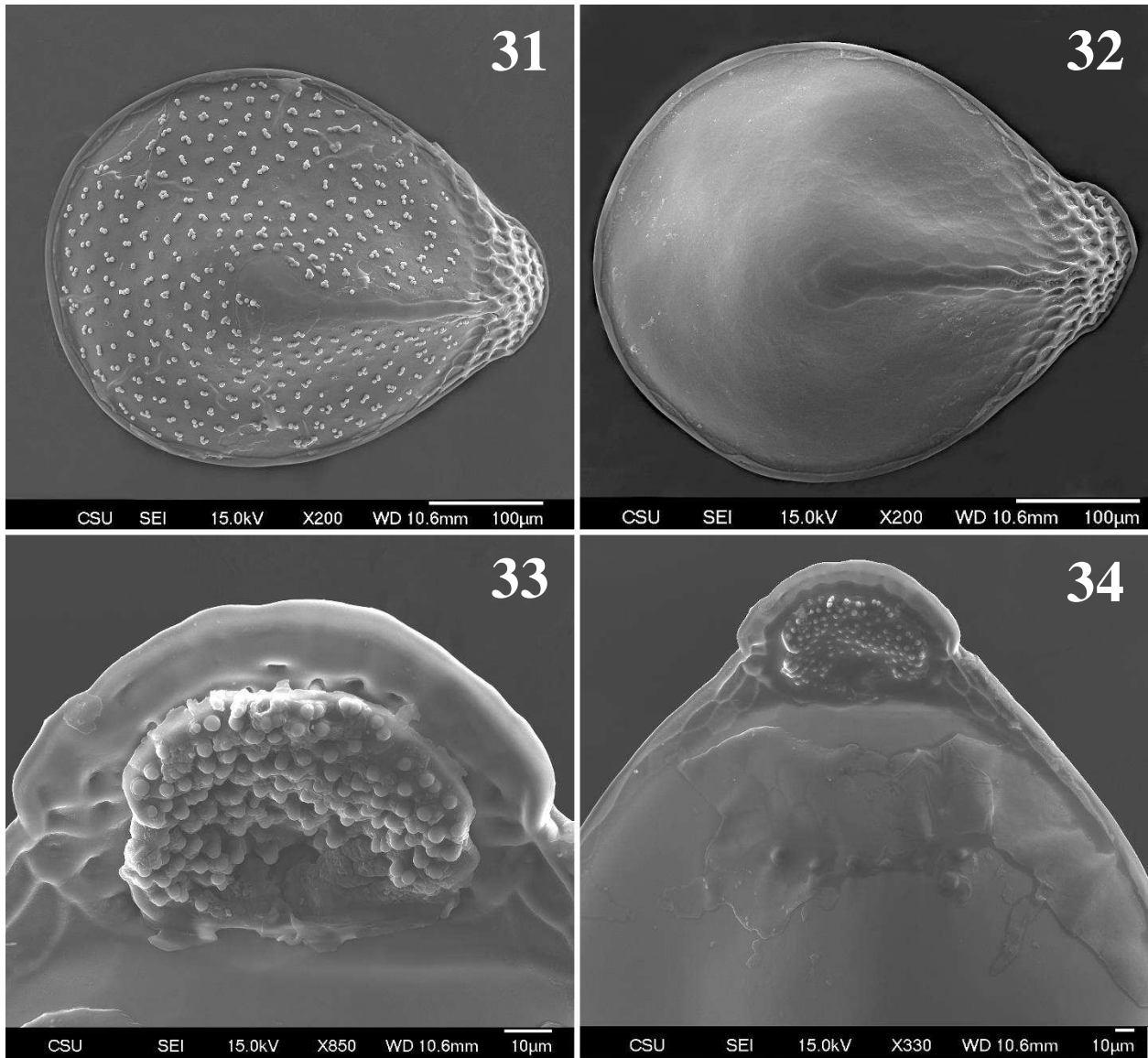
Figures 21–24. *Remenus bilobatus*, adult male. 21. Right forewing, Crooked Creek, Carroll County, Virginia. 22. Head and pronotum, Calfpasture River, Augusta County, Virginia. 23–24. Blackrock Spring, SHEN, Albemarle County, Virginia. 23. Terminalia, dorsal, 24. Terminalia, lateral.



Figures 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. Celsius



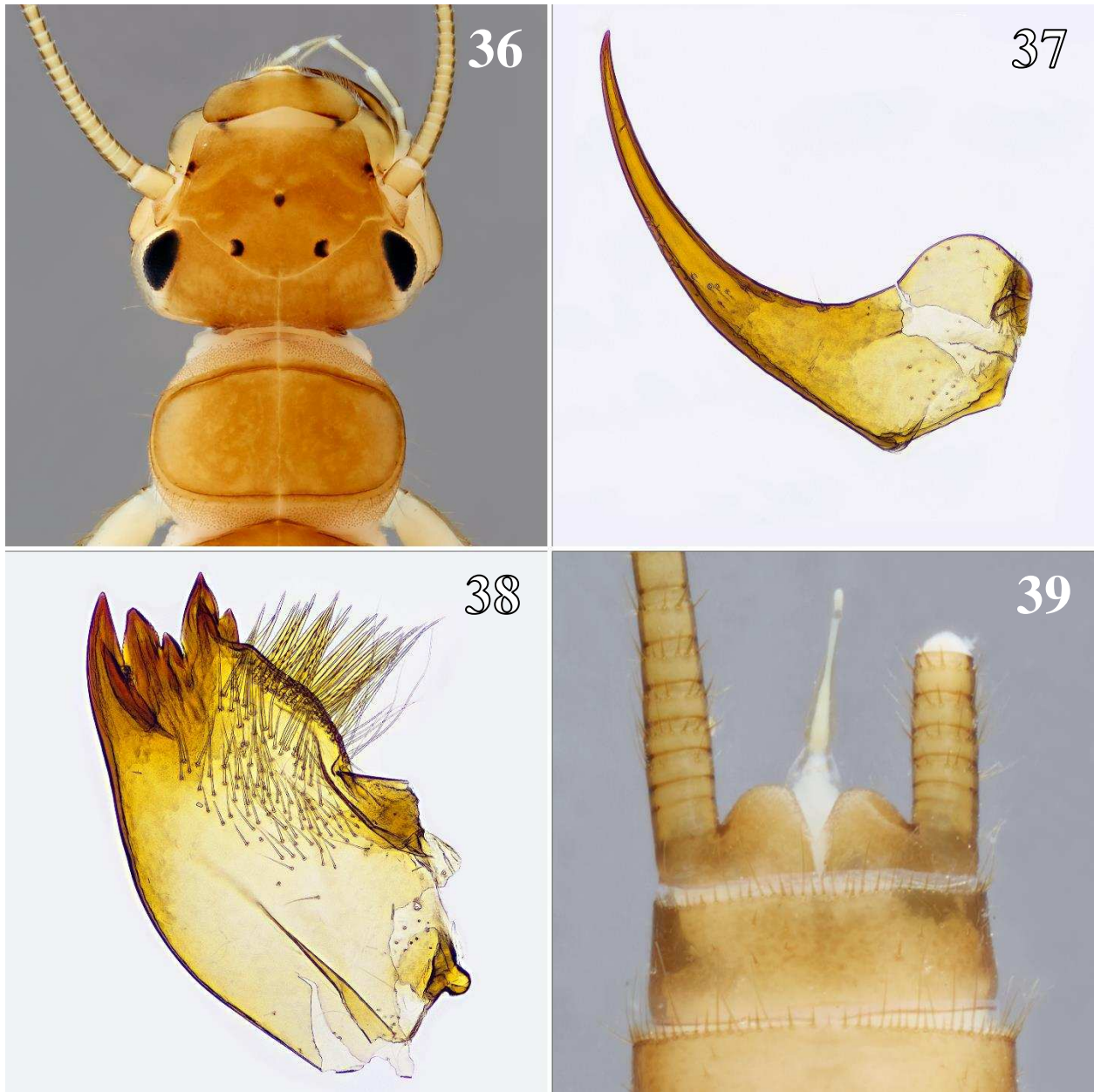
Figures 29–30. *Remenus bilobatus*, adult female subgenital plate variation. 29. Crooked Creek, Carroll County, Virginia. 30. Calfpasture River, Augusta County, Virginia.



Figures 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.



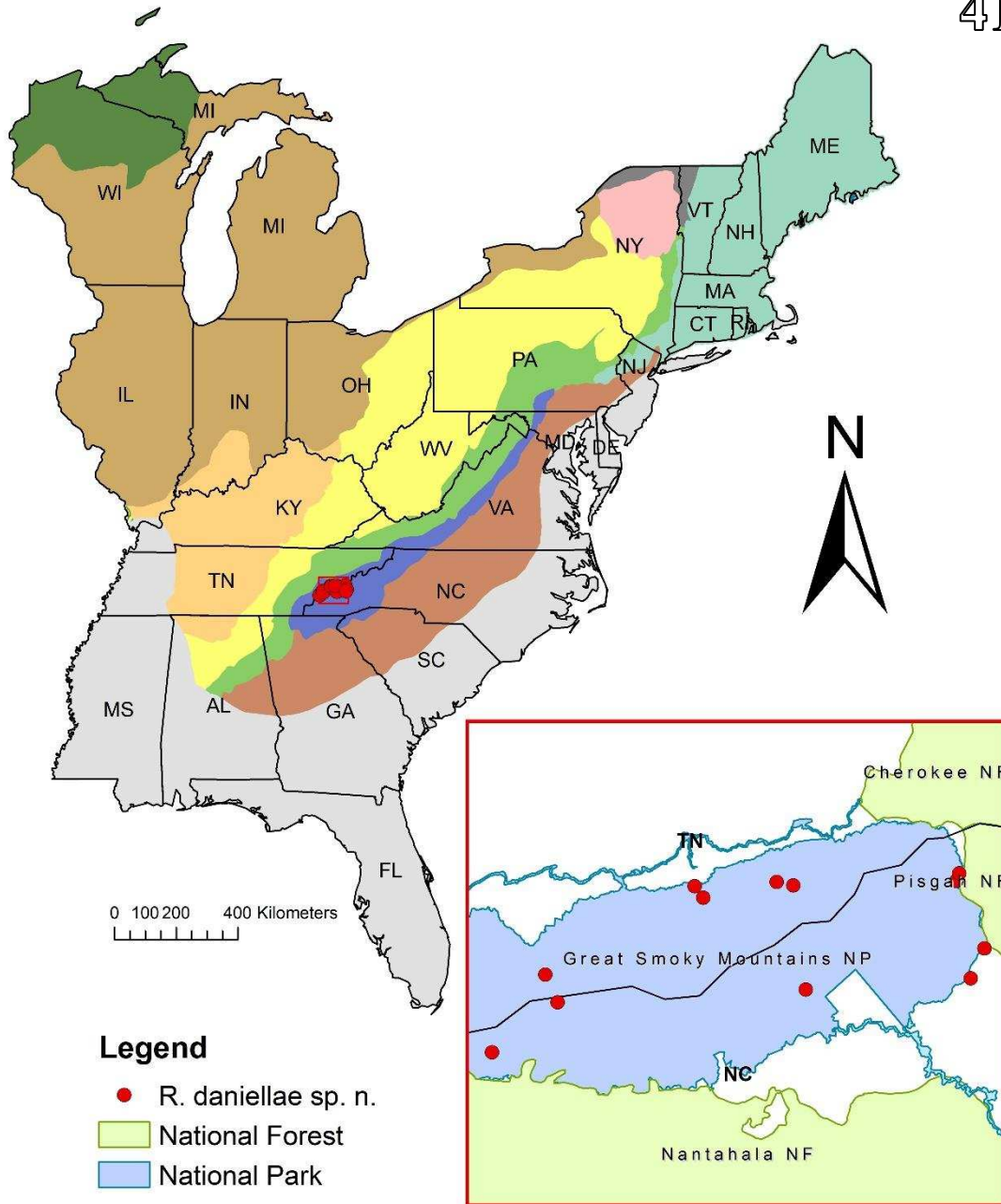
Figure 35. Nymphal habitus of *Remenus bilobatus*, Stone Mountain Creek, Alleghany County, North Carolina.



Figures 36–39. *Remenus bilobatus*, nymph. 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.



Figure 40. *Remenus bilobatus*, nymphal cercus, dorsal, Gullion Fork, Wythe County, Virginia.



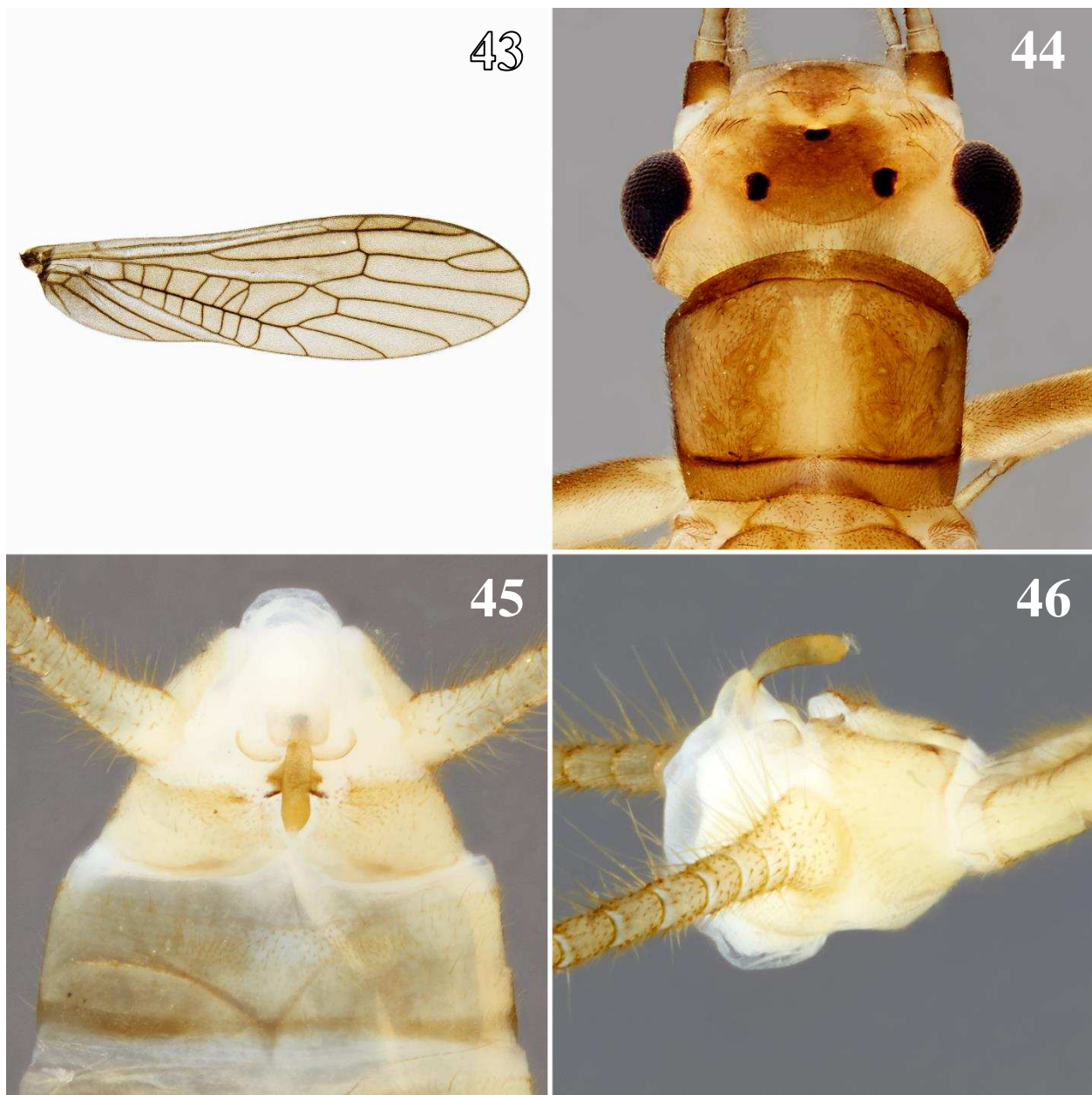
Map Source: Chris Verdone, 2018
 Map Data: US Physiographic Provinces, USGS Water Resources NSDI Node; National Lands, ESRIDM

Figure 41. *Remenus daniellae* sp. n. distribution of examined material.

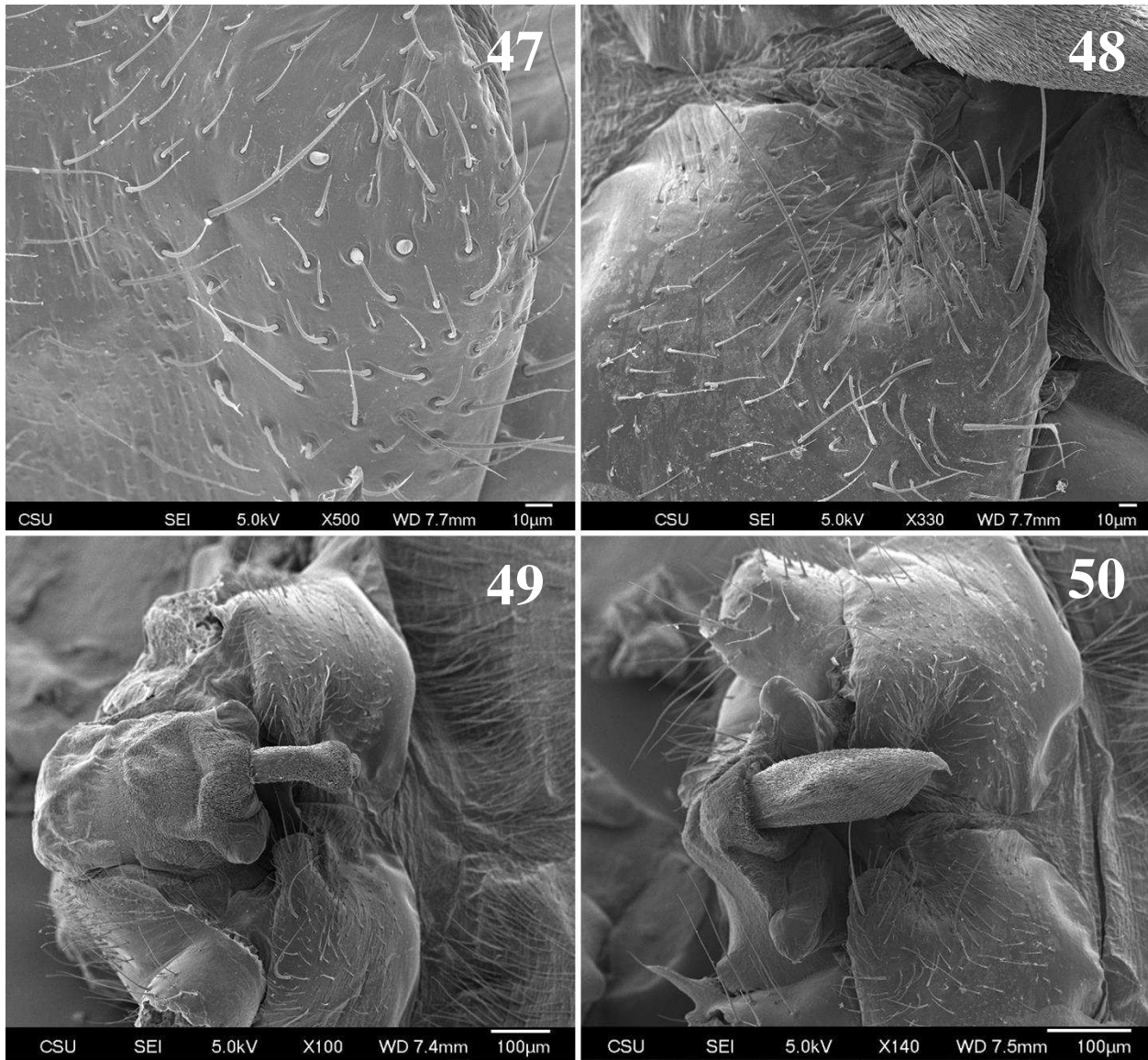
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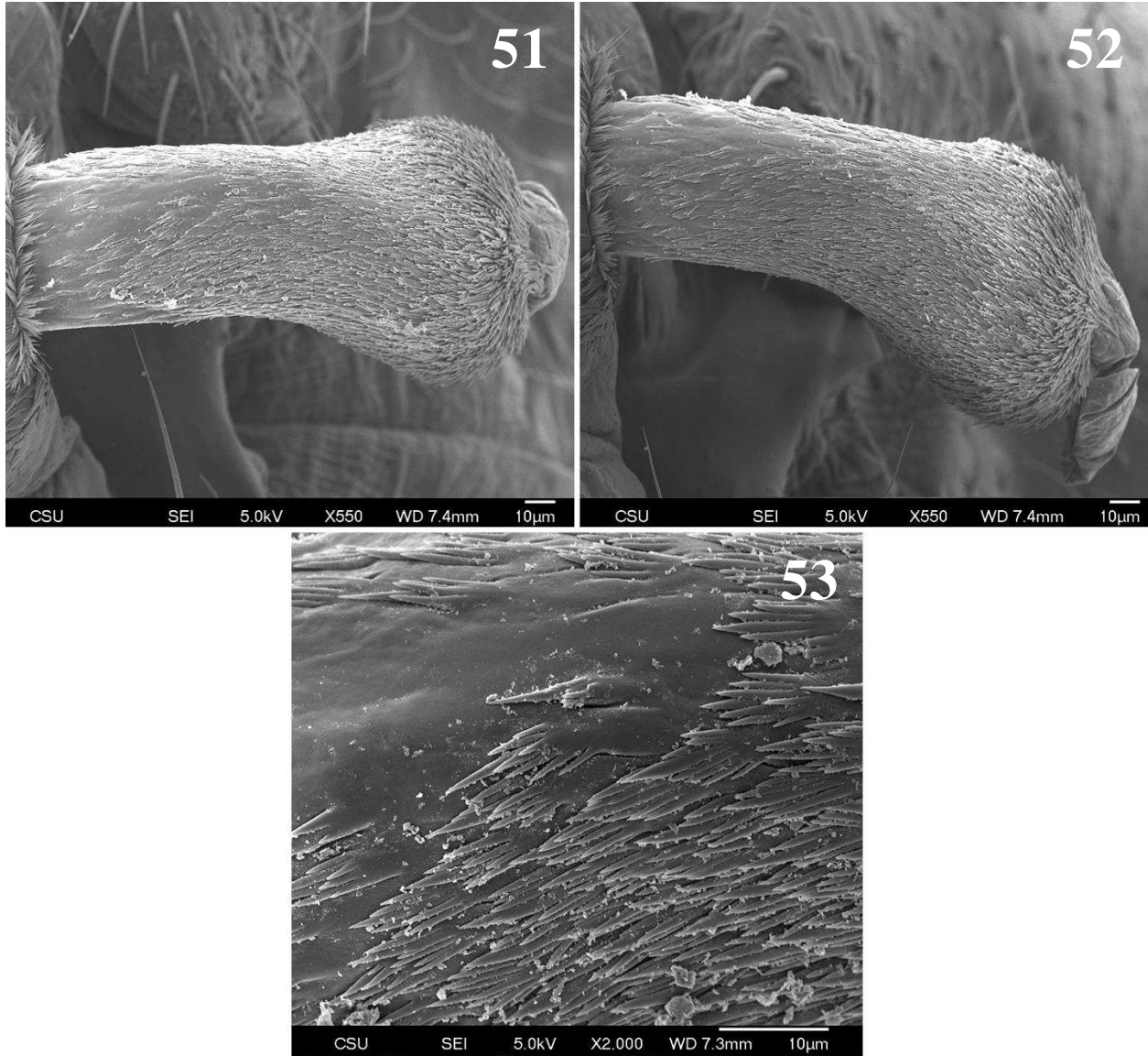
Figure 42. Adult male habitus of *Remenus daniellae* sp. n., tributary to Le Conte Creek, Sevier County, Tennessee.



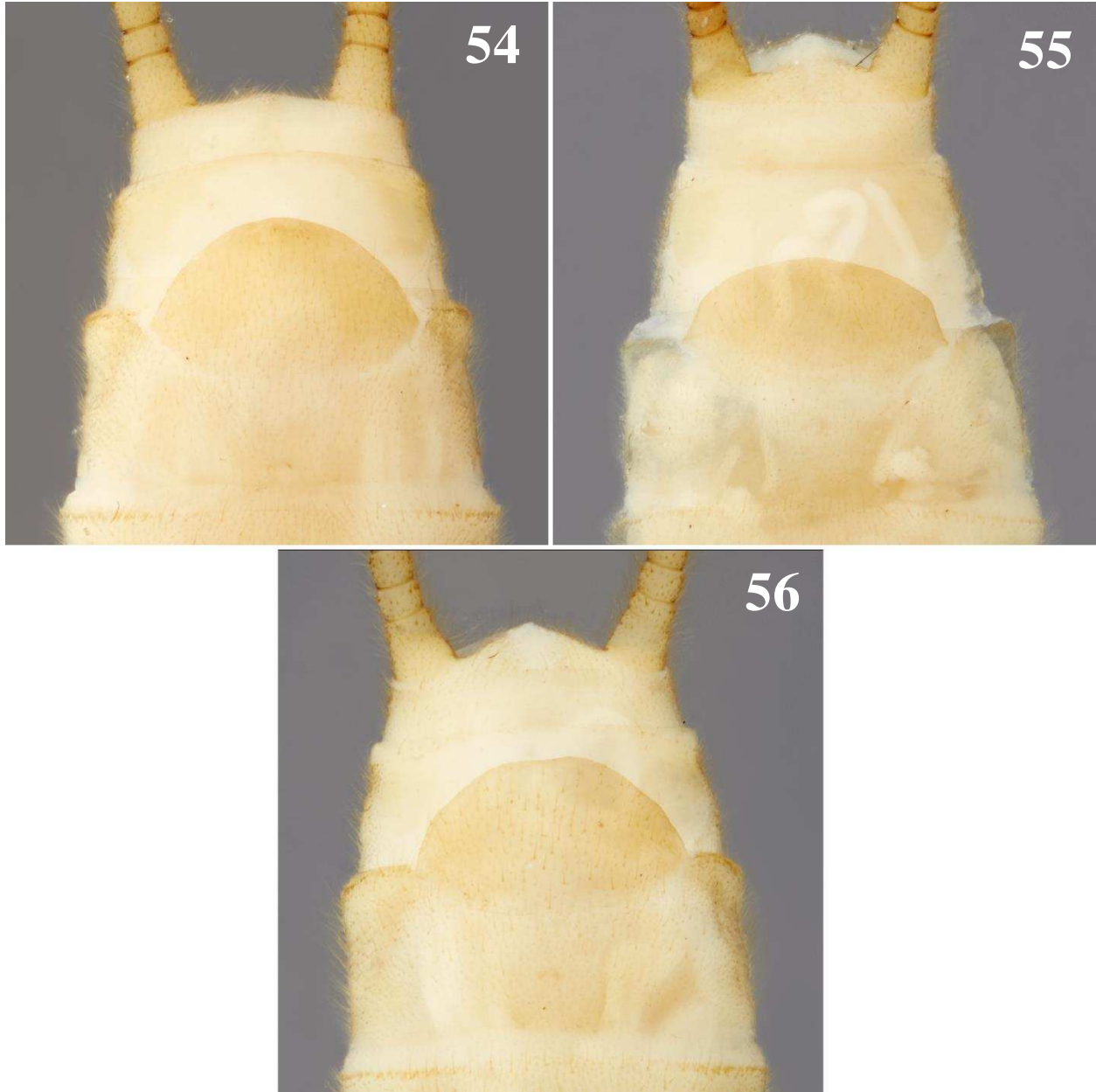
Figures 43–46. *Remenus daniellae* sp. n., adult male, tributary to Le Conte Creek, Sevier County, Tennessee. 43. Right forewing. 44. Head and pronotum. 45. Terminalia, dorsal. 46. Terminalia, lateral.



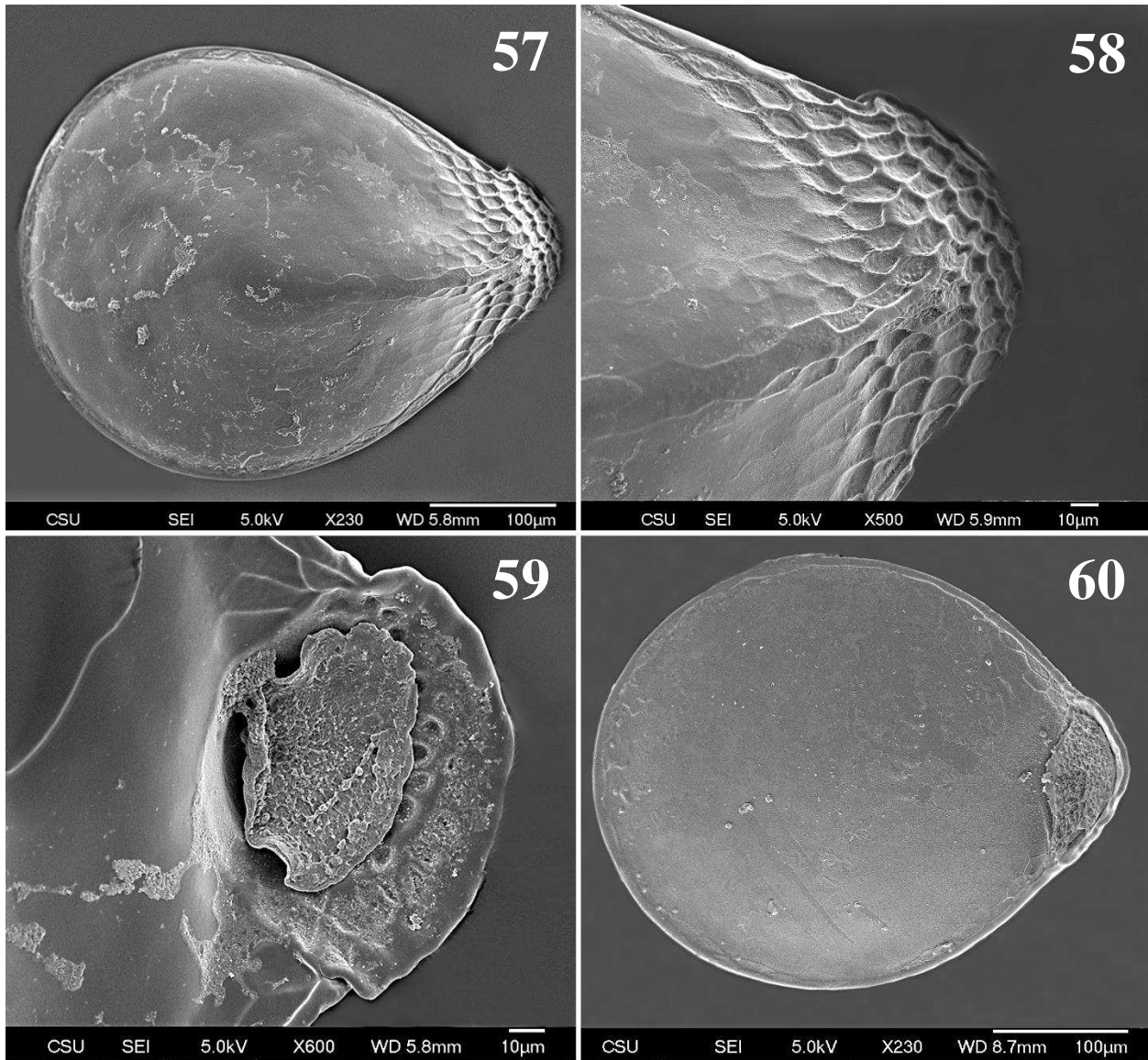
Figures 47–50. *Remenus daniellae* sp. n. adult male. 47. Hemitergal lobe and sensilla basiconica, Ball Branch, Haywood County, North Carolina. 47–50. Tributary to Le Conte Creek, Sevier County, Tennessee. 48. Hemitergal lobe. 49–50. Epiproct variation, dorsal.



Figures 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.



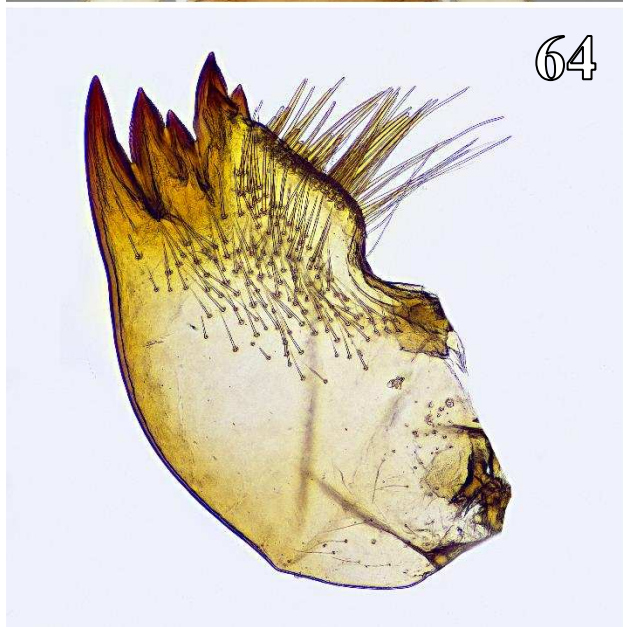
Figures 54–56. *Remenus daniellae* sp. n., adult female subgenital plate variation. 54. Ball Branch, Haywood County, North Carolina. 55. Little Laurel Branch, Sevier County, Tennessee. 56. Tributary to Le Conte Creek, Sevier County, Tennessee.



Figures 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.



Figure 61. Nymphal habitus *Remenus daniellae* sp. n., Ball Branch, Haywood County, North Carolina.



Figures 62–65. *Remenus daniellae* sp. n., nymph. 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.

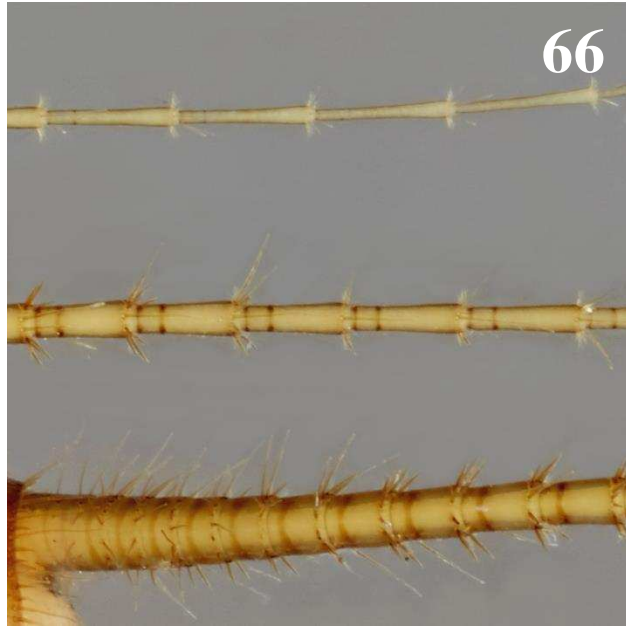


Figure 66. *Remenus daniellae* sp. n., nymphal cercus, dorsal, Ball Branch, Haywood County, North Carolina.

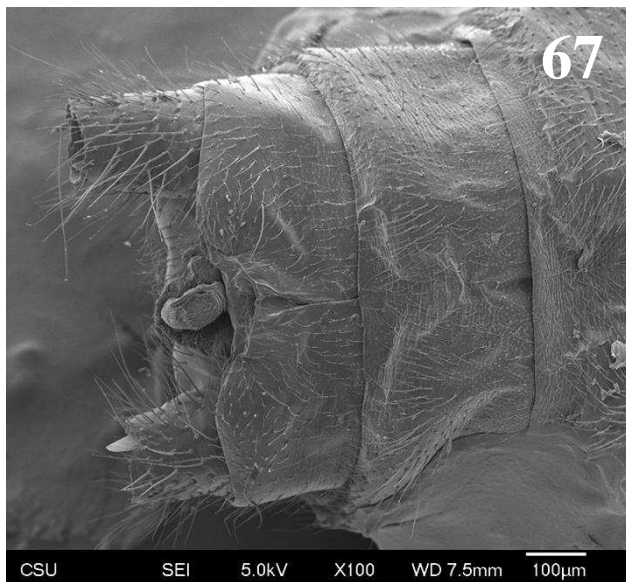
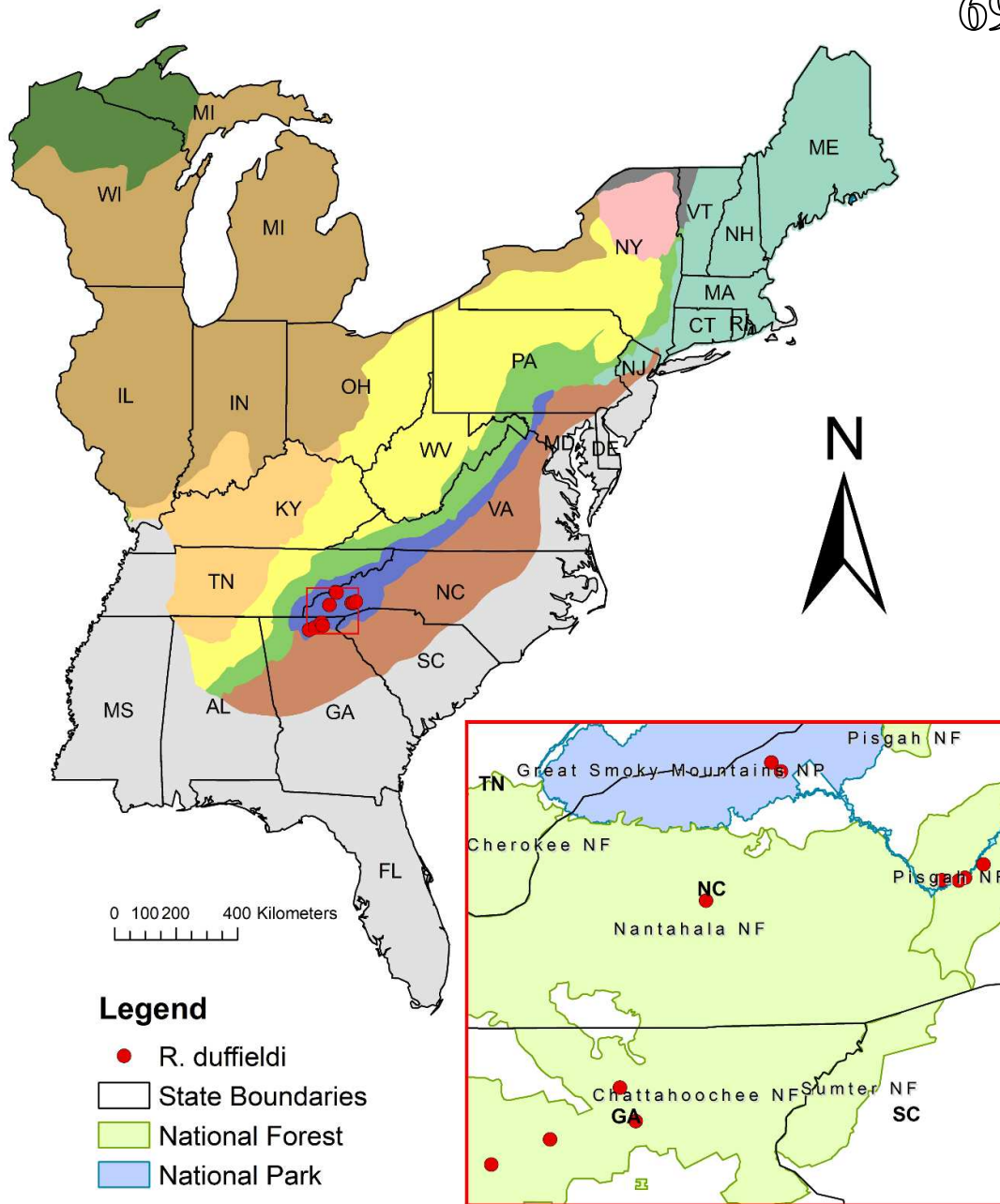


Figure 67. *Remenus daniellae* sp. n., male epiproct variation, dorsal, Ball Branch, Haywood County, North Carolina.



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



Map Source: Chris Verdone, 2018
 Map Data: US Physiographic Provinces, USGS Water Resources NSDI Node; National Lands, ESRIDM

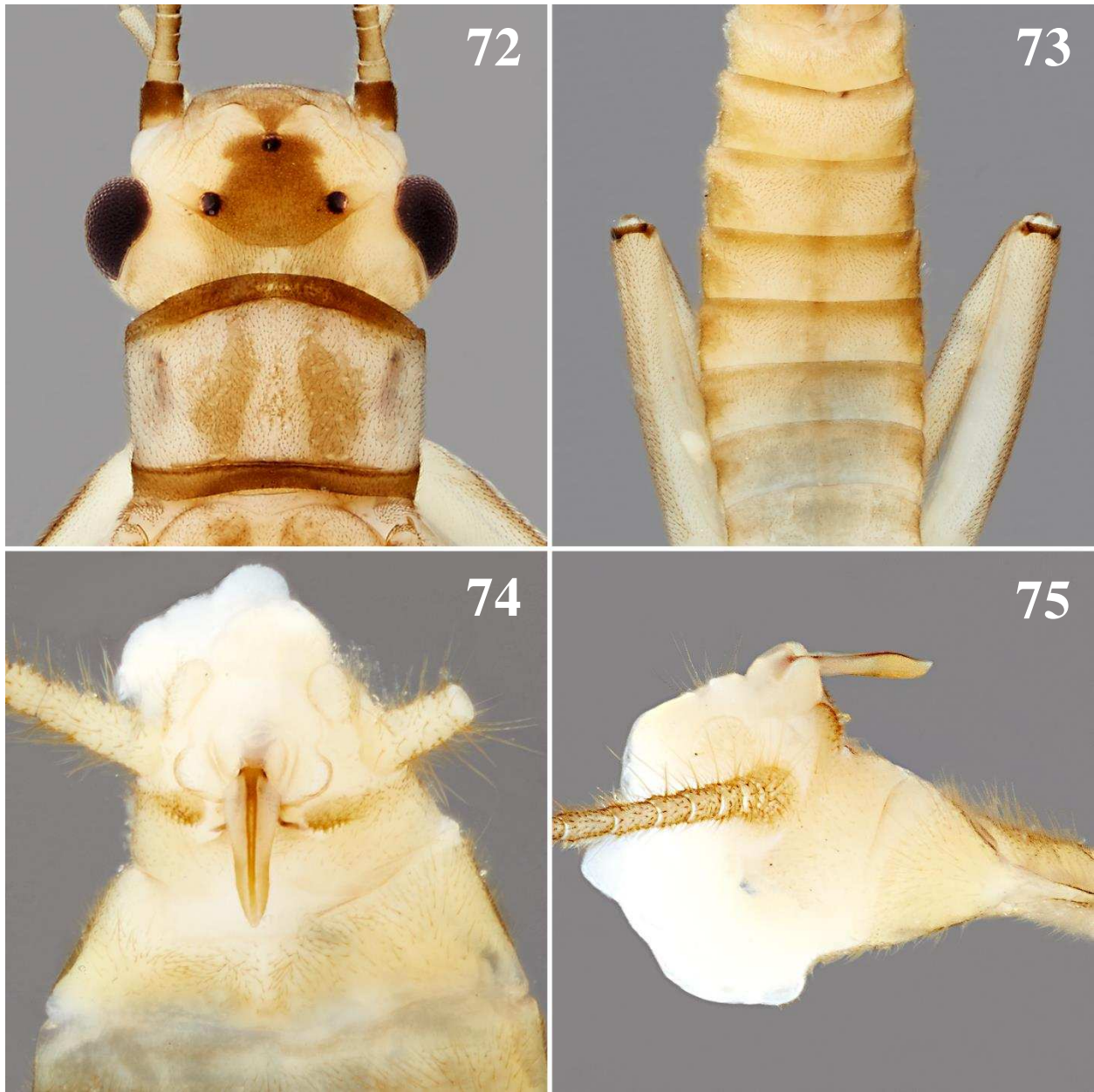
Figure 69. *Remenus duffieldi*, distribution of examined material and published records.



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



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



Figures 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.

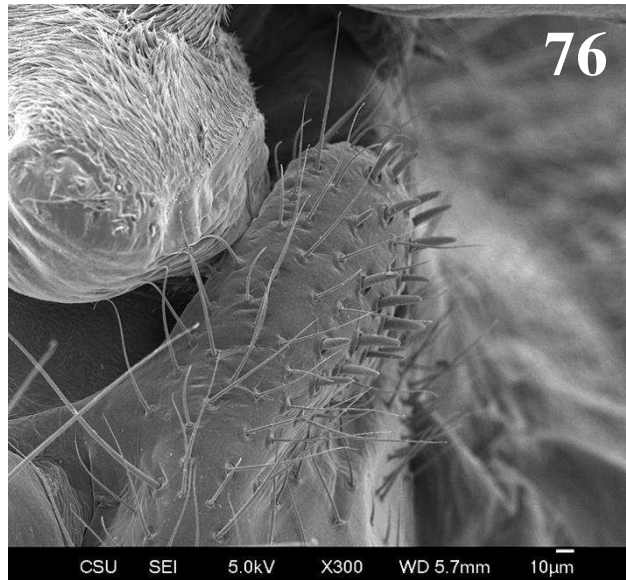
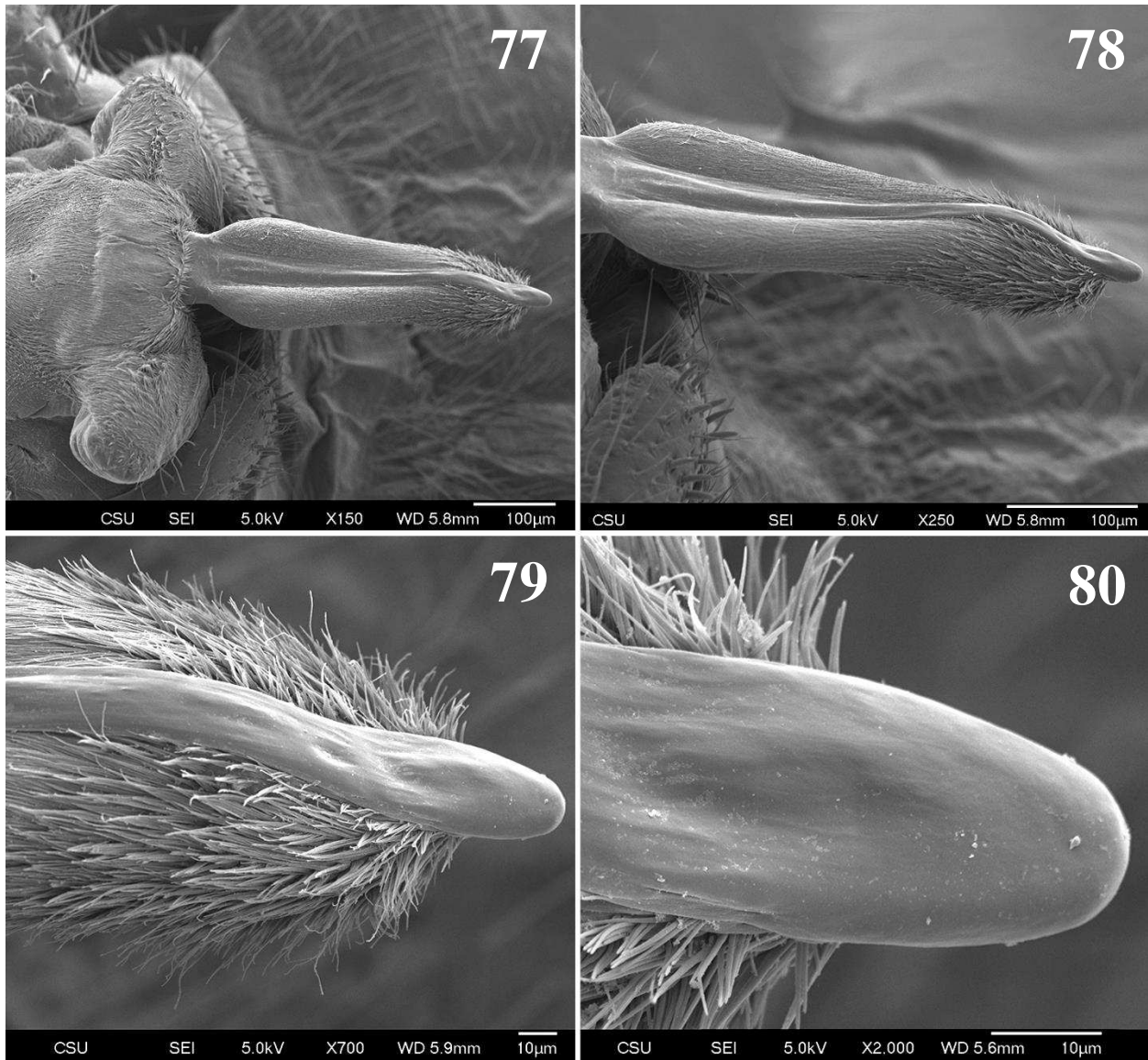


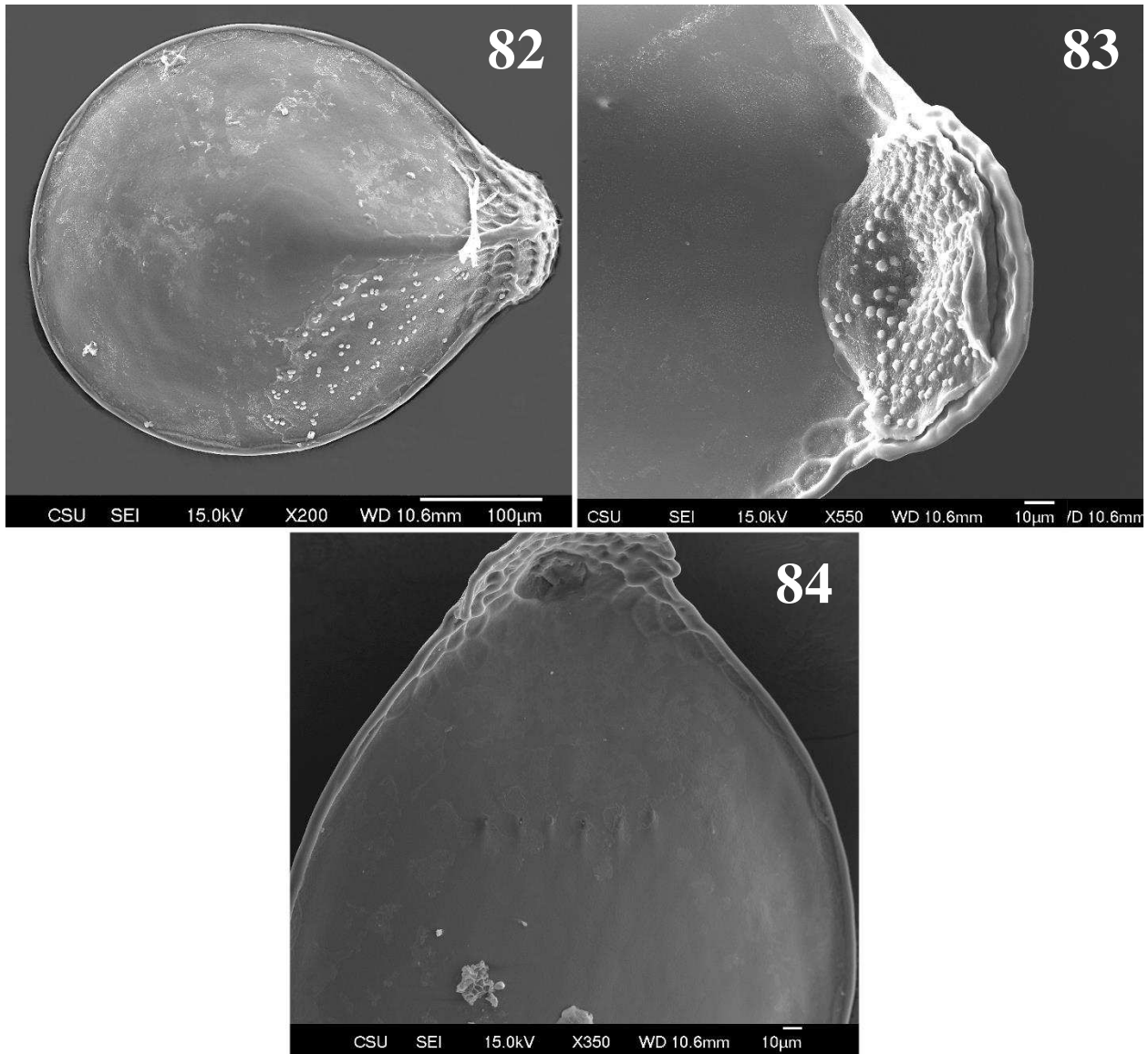
Figure 76. *Remenus duffieldi*, adult male, hemitergal lobe, Tellico Creek, Macon County, North Carolina.



Figures 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.



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



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

85



2 mm

Figure 85. Nymphal habitus of *Remenus duffieldi*, Nantahala River, Macon County, North Carolina.



Figures 86–89. *Remenus duffieldi*, nymph. 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.



Figure 90. *Remenus duffieldi*, nymphal cercus, dorsal, Collins Creek, Swain County, North Carolina.

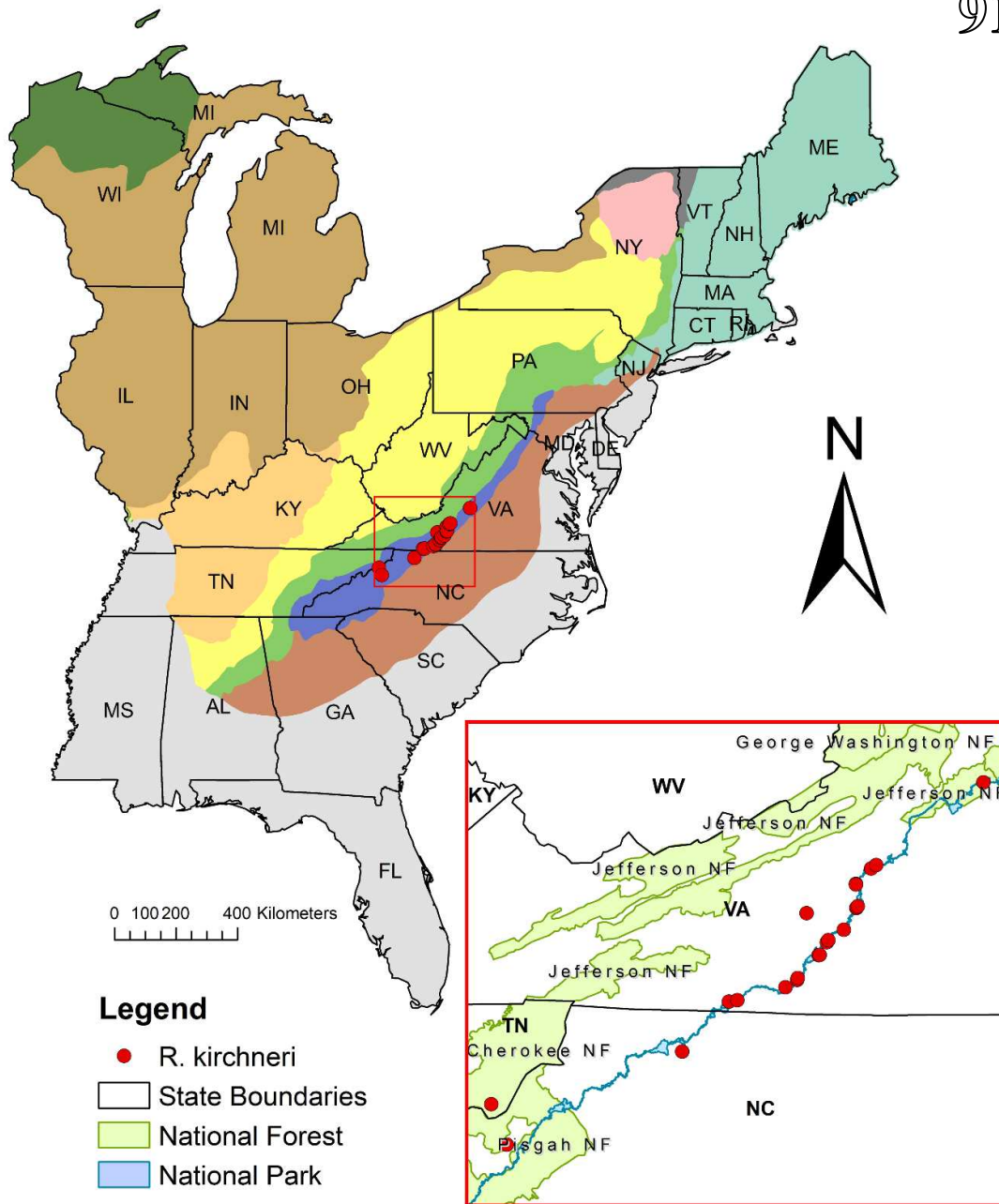
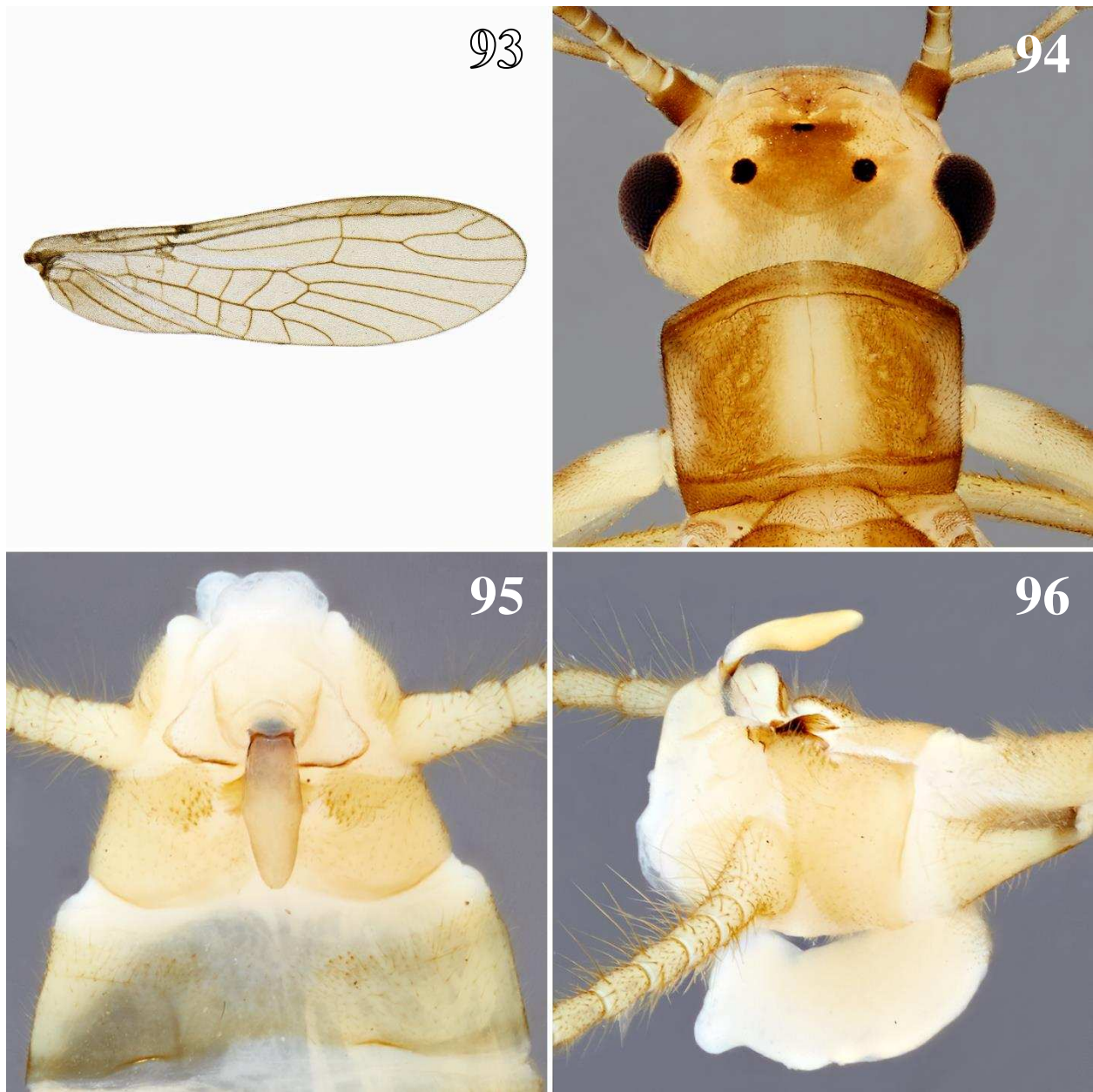


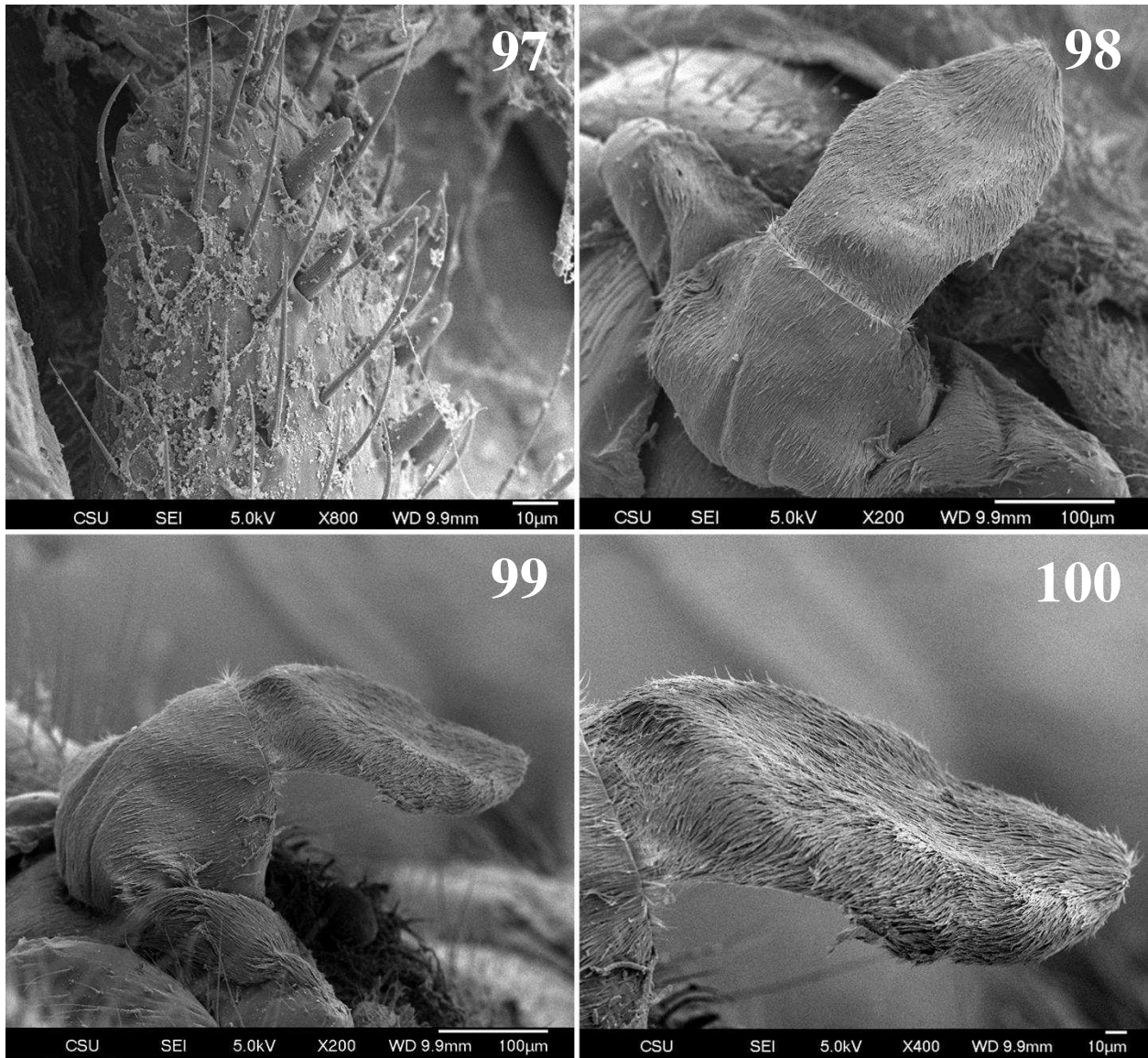
Figure 91. *Remenus kirchneri*, distribution of examined material and published records.



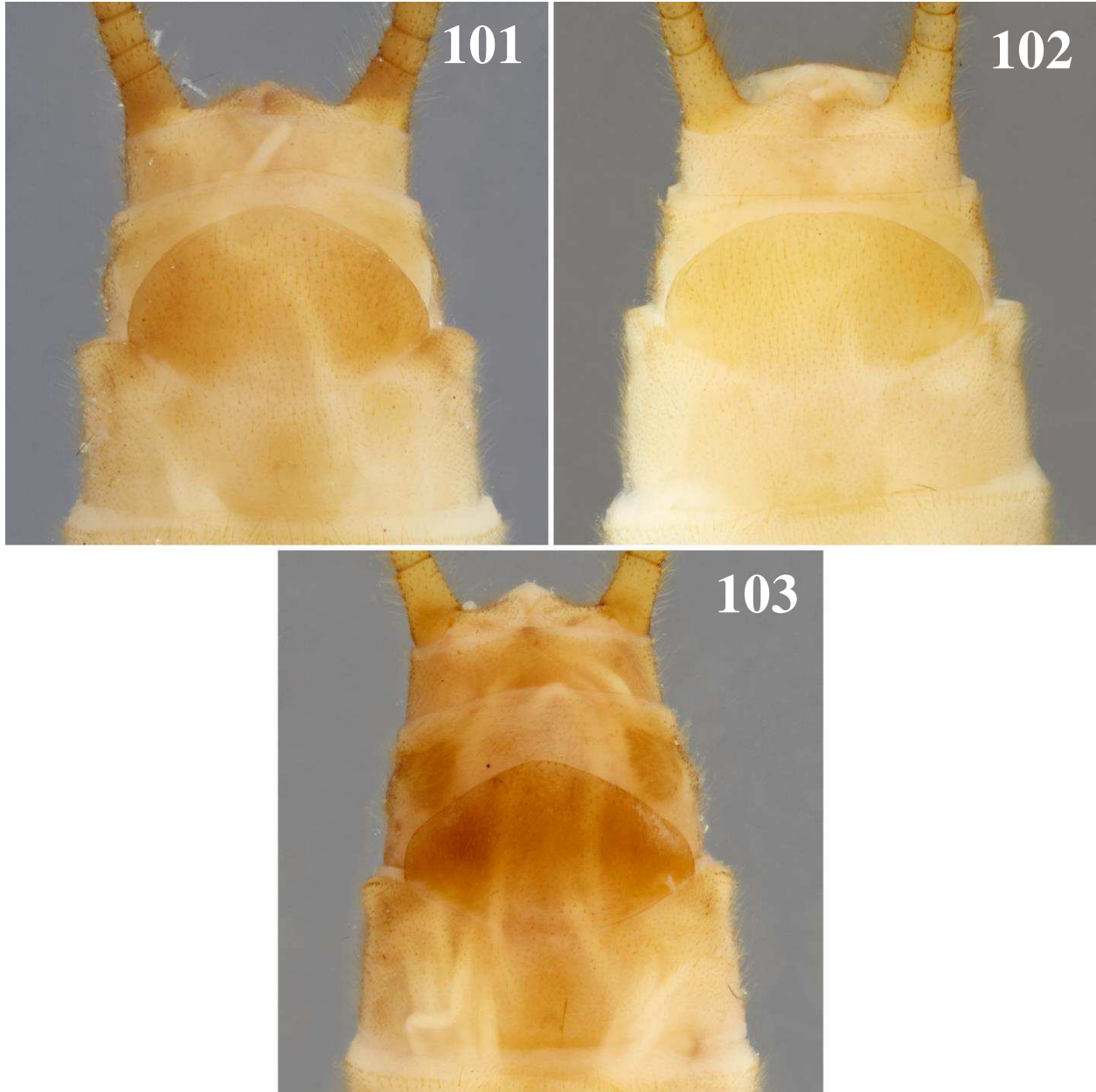
Figure 92. Adult male habitus of *Remenus kirchneri*, tributary to Dodd Creek, Floyd County, Virginia.



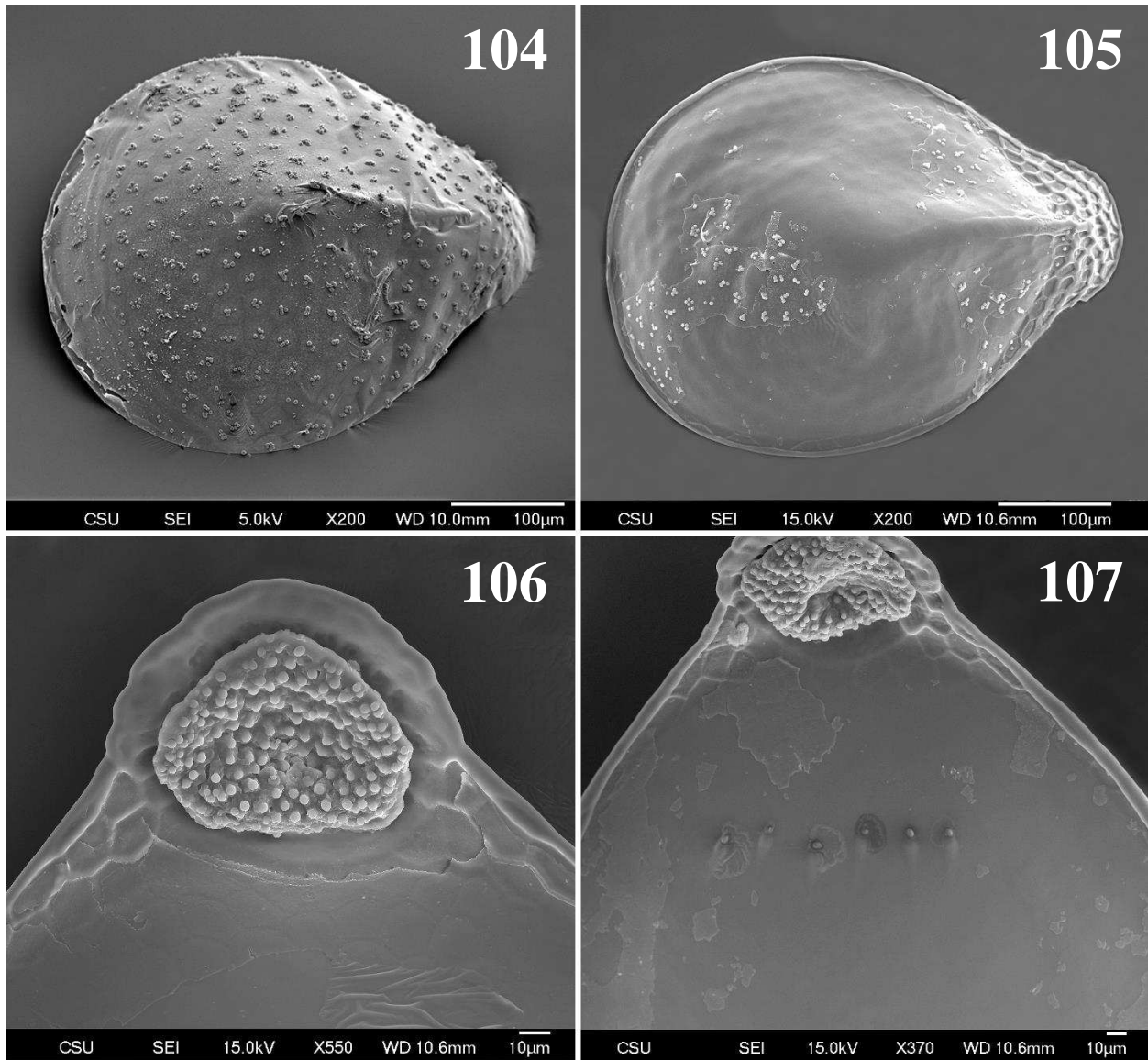
Figures 93–96. *Remenus kirchneri*, adult male. 93. Right forewing, spring fed stream, Floyd County, Virginia. 94. Head and pronotum, tributary to Dodd Creek, Floyd County, Virginia. 95–96. Tributary to Rock Castle Creek, Patrick County, Virginia. 95. Terminalia, dorsal. 96. Terminalia, lateral.



Figures 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.



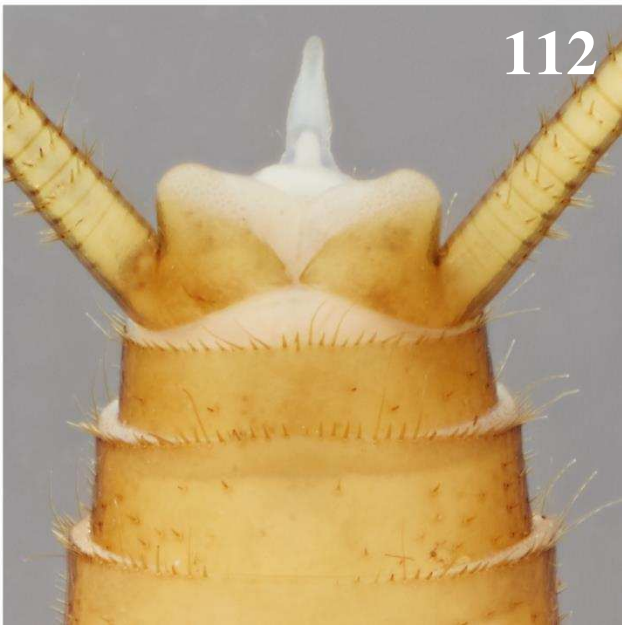
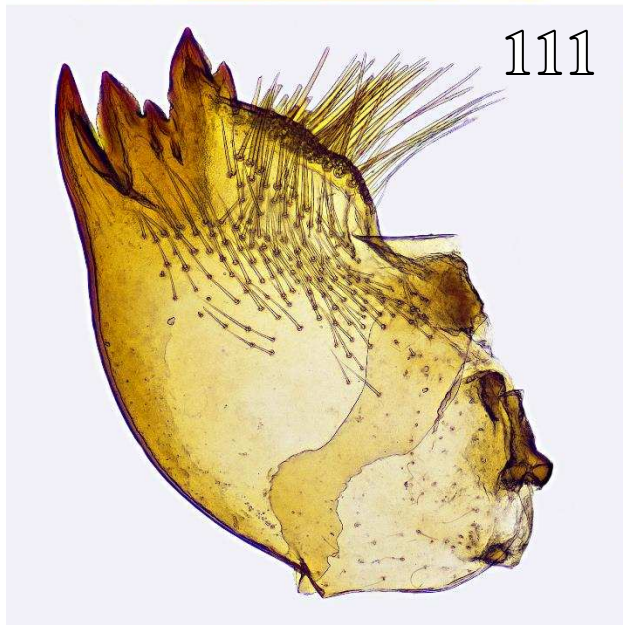
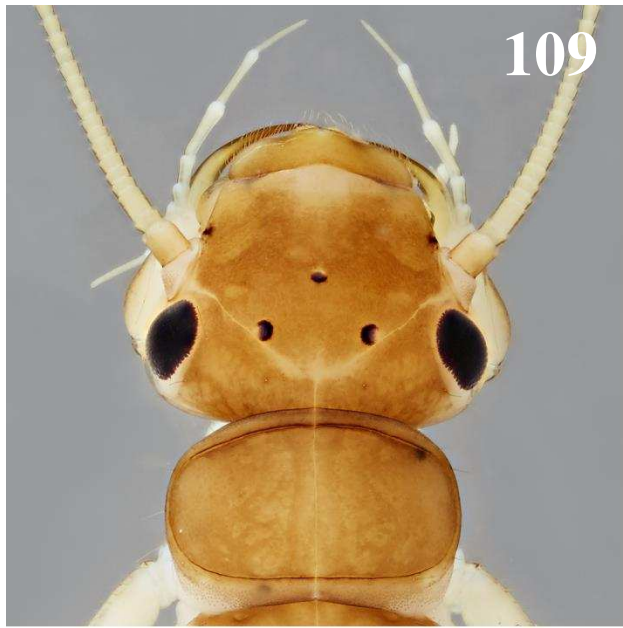
Figures 101–103. *Remenus kirchneri*, adult female, subgenital plate variation. 101. Tributary to Rock Castle Creek, Patrick County, Virginia. 102. Tributary to Lick Fork, Floyd County, Virginia. 103. Tributary to Dodd Creek, Floyd County, Virginia.



Figures 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.



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



Figures 109–112. *Remenus kirchneri*, nymph. 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.

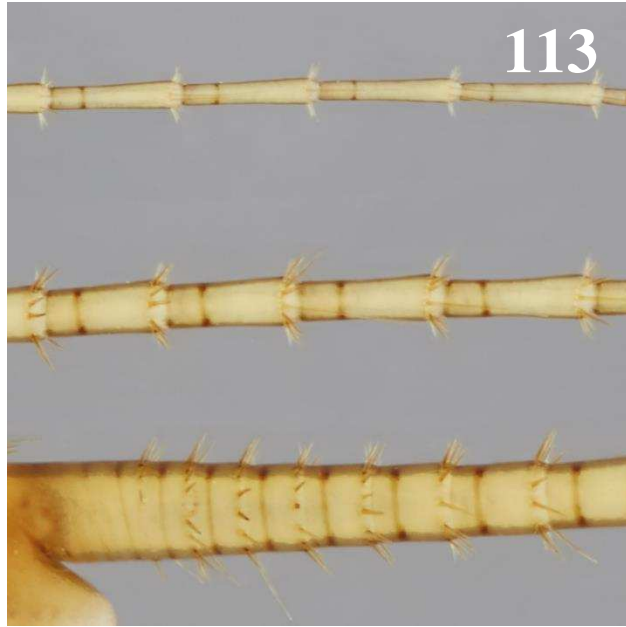


Figure 113. *Remenus kirchneri*, nymphal cercus, dorsal, tributary to Rock Castle Creek, Patrick County, Virginia.

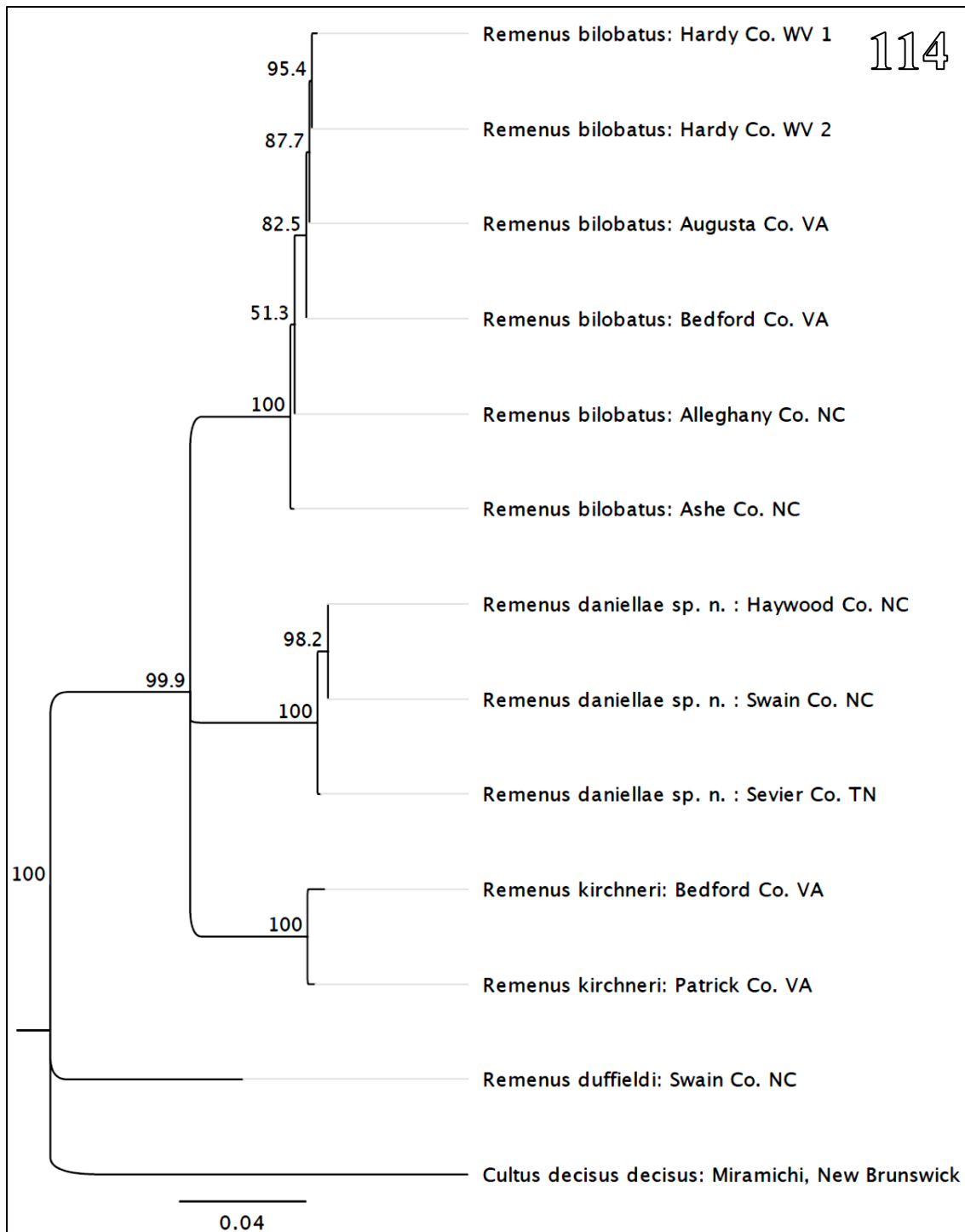


Figure 114. Neighbor-joining tree of four *Remenus* species based on a 658 bp fragment of COI estimated using the Tamura-Nei parameter model with pairwise deletion option. Nodal support values are bootstrap percentages from 1,000 replicates. Scale indicates divergence.

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PART TWO: PREDICTING POTENTIALLY SUITABLE HABITAT OF *REMENUS* RICKER
IN THE SOUTHERN APPALACHIANS USING MAXIMUM ENTROPY MODELING

INTRODUCTION

The present day distribution of *Remenus* Ricker (Plecoptera: Perlodidae) is limited to the mid-elevation regions of the Appalachian Mountains. The four species can be found in similar habitats, but are rarely sympatric. Three species, *R. daniellae* sp. n., *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* sp. n. and *R. duffieldi* are restricted to the mountains south 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).

Remenus duffieldi, and *R. kirchneri* are currently considered rare and highly endemic and have been assigned a Nature Serve global conservation ranking of G2 (imperiled—at high risk of extinction due to very restricted range, very few populations (often 20 or fewer), steep declines, or other factors) (NatureServe 2016). The newly described *R. daniellae* sp. n. also meets the G2 criteria. The more widespread *R. bilobatus*, on the other hand, has been evaluated as G4 (Apparently Secure—uncommon but not rare; some cause for long-term concern due to declines or other factors) (NatureServe 2016). Little is known regarding the biology or population statuses of the species of *Remenus*. Effective conservation of these rare species will rely on identifying populations suitable for ecological study to improve our understanding of their autecology and population trends.

Species distribution models (SDMs) under various titles including bioclimatic envelope, ecological niche, and habitat suitability models, are popular tools for predicting species ranges and identifying the environmental variables which determine species distributions (Elith & Leathwick 2009). Here, I specifically focus on the theory of bioclimatic envelopes, which describes the relationship between a species observed distribution and climate variables (Luoto et al. 2005). The theory of bioclimatic envelopes is derived from Hutchinson's (1957) niche concept, in which species distributions are limited by biotic interactions, such as competition and predation, and abiotic gradients (e.g. elevation, temperature and precipitation) (Whitaker 1956). Temperature and precipitation patterns are important environmental variables that affect the growth and life histories of stoneflies (Ward & Stanford 1982). Resulting adaptation to specific climatic conditions can lead to localized distributions (Hynes 1976). Recently, SDMs have been developed for two other rare and endemic stoneflies: *Arsapnia arapahoe* (Nelson & Kondratieff, 1988), and *Lednia tumana* (Ricker, 1952) (Young et al. 2016, Muhlfeld et al. 2011). Both studies used maximum entropy method (Maxent) (Phillips & Dundik 2008) to model the potential geographic distribution of the target species for the purpose of identifying additional populations.

Remenus daniellae sp. n., *R. duffieldi*, *R. kirchneri* are known to inhabit similar physical habitats along a latitudinal gradient in the Appalachian Mountains, but the distributions of these species are ill-defined. Additionally, little is known regarding the environmental conditions present at known occurrence locations. The objectives of this study are to: (1) use location data for *Remenus* species collected in 2016 and 2017, museum records, and climate data to develop distribution models using ArcGIS and Maxent to predict potentially suitable habitats for each southern Appalachian endemic (*R. daniellae* sp. n., *R. duffieldi*, *R. kirchneri*); (2) describe the bioclimatic envelope inhabited by each species and identify potential differences; (3) assess

current status of habitat protection based on model results. The results of this study will help guide future surveys and inform conservation priorities.

METHODS AND MATERIALS

Study Area

The study area was restricted to the Blue Ridge Physiographic Province, which trends northeast from northern Georgia to southern Pennsylvania covering an area of approximately 45,000 km² (Pride & Utgard 1985). The Blue Ridge contains the highest peaks in the Appalachian Mountains including Mount Mitchell (2037 m) and Clingmans Dome (2025 m). The Appalachian Mountains are one of the oldest mountain ranges in the world and have a complex history. The Alleghenian orogeny, the primary mountain building event, is believed to have taken place between 300–250 Mya (Hatcher 1978), which aligns closely to the earliest known Plecopteran fossil records (Béthoux et al. 2011). More recently, stonefly distributions have been correlated to physiographic regions (Ross & Ricker 1971, Kondratieff 1987, Grubbs 1997); however, surface water drainages to which stoneflies are directly tied do not perfectly conform to the physiographic boundary. As such, a hydrologically delineated boundary was created by intersecting sub-watershed boundaries (HUC-12) (U.S. Geological Survey 2016a) with the Blue Ridge Physiographic Province (U.S. Geological Survey 2017).

Occurrence Data

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 all records were manually adjusted to

improve position accuracy using GEOLocate v. 3.22 (Rios & Bart 2010). Additional specimen records were taken from published literature or provided by Dr. Scott Grubbs, Western Kentucky University, Bowling Green, Kentucky (WKU). Occurrence locations were restricted to records represented by adult specimens, which provide the most reliable characters for species identification. The total number of locations used for each model were as follows: *R. daniellae* sp. n. (10), *R. duffieldi* (11), and *R. kirchneri* (22) (Table 1).

Topographical Data

Flowlines and watershed boundaries and 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), national land cover classifications from the National Land Cover Database (NLCD 2011) (Homer et al. 2015), and protected areas from USGS Protected Areas Database (U.S. Geological Survey 2016b). Elevation data was downloaded from USGS National Elevation Dataset (Gesch et al. 2002). Data included 1 arc-second ($\sim 30 \text{ m}^2$) digital elevation model (DEM) grids.

Environmental Data

Climate data were downloaded from Worldclim.org (Fick & Hijmans 2017). Worldclim provides 19 bioclimatic variables in addition to average monthly climate data for minimum, mean, and maximum temperature and for precipitation from 1970-2000 in 30 arc-seconds ($\sim 1 \text{ km}^2$) resolution. Bioclimatic predictors capture information about annual conditions, seasonal mean climate conditions and intra-year seasonality that relate to species physiology (O'Donnel & Ignizio 2012).

Data Preparation

All environmental variables considered for the model (Table 2) were processed using ArcGIS ArcMap 10.4.1 (ESRI 2016). All variables were projected to projected coordinate system (PCS) NAD 1983 Zone 17N and were subsequently clipped to the HUC-12 sub-watershed boundaries, using the extract by mask tool (Spatial Analyst: Extraction). Output coordinate systems (Output Coordinates) were set to geographic coordinate system North America 1983. Extent (Processing Extent) was set to the DEM file which was the first layer to be processed. Snap raster was also set to the DEM file. Cell size (Raster Analysis) was set to 29.3639 m as specified by the DEM file. All variables were subsequently converted to ASCII file type using the raster to ASCII tool (Conversion Tools: From Raster).

The elevation layer was created by combining 129 DEM grids by using the mosaic to new raster tool (Data Management Tools: Raster: Raster Dataset). Pixel type was set to 32 bit integer. The DEM was projected to PCS NAD 1983 Zone 17 using bilinear sampling technique. The elevation mosaic was then processed as described above.

Maxent Analysis

I used maximum entropy modeling, Maxent software (Phillips & Dundik 2008) to develop suitable habitat predictions for three species of *Remenus*. Maxent software estimates a target probability distribution by finding the probability distribution of maximum entropy which is constrained by incomplete information about the target distribution (Phillips et al. 2006). Information about the distribution are typically environmental variables such as climate, elevation, land cover, or stream order. An advantage of Maxent is that it uses presence only data which is important in modeling rare and cryptic species as true absences are often difficult or

impossible to verify. Another advantage of Maxent is that it performs well with small sample sizes (Hernandez et al. 2006, Pearson et al. 2007, Kumar et al. 2009, Young et al. 2016).

Model development and execution was performed using the Software for Assisted Habitat Modeling (SAHM) (Morissette et al. 2013) within the Vistrails application (Freire et al. 2006). The SAHM software uses a workflow interface that can efficiently run multiple species distribution models and iterations. The SAHM software also stores pre- and post-modeling processes to document analyses and results for reproducibility and comparison of model results and evaluations (Morissette et al. 2013). Default settings for Maxent were used except replicate type, which was changed to cross-validation. Output from Maxent included a logistic probability map, and a Multivariate Environmental Similarity Surface (MESS). The logistic probability map shows potential habitat as probability between 0 and 1. The MESS map identifies novel regions outside the environmental range of the model training data. Model predictions in novel areas should be interpreted with more caution than those areas within the defined environmental ranges (Elith et al. 2010).

Variable selection was conducted using the SAHM covariate correlation and selection module which compares each pair of variables using the maximum of the Kendall, Pearson, and Spearman correlation coefficients. To avoid multicollinearity, one of each pair of highly correlated variables ($|r| > 0.7$) were removed. Variables were retained based on the percentage of deviance explained, ecological relevance and expert opinion. Additionally, direct variables were given preference over indirect variables. Indirect variables such as elevation are often used as surrogates for direct variables, such as temperature or precipitation which can have direct physiological impacts on organisms (Austin 2007, Elith & Leathwick 2009). The selection process retained up to four variables for each Maxent model.

As noted above, Maxent uses presence only data. Presence data is often biased by accessibility, such as proximity to roads and ownership (e.g. publicly owned land is typically more accessible). If the bias is sufficiently large, presence only SDMs effectively estimate the sampling distribution as opposed to estimating the species distribution (Phillips & Dundik 2009). To reduce the effect of sampling bias I employed a method reviewed by Phillips (2008) and Phillips et al. (2009) and generated a random sample of 10,000 background points drawn from the HUC-12 sub-watersheds that were sampled in 2016 and 2017. This technique creates background data with a selection bias similar to the presence data, which improves prediction accuracy when the model is transferred to the rest of the study area. Use of a “bias file” as described above, has been shown to reduce omission and commission errors (false negatives and false positives) compared to non-corrected models (Kramer-Schadt et al. 2013).

Model Evaluation

The Maxent models were developed using 5-fold (*R. daniellae* sp. n. and *R. duffieldi*) or 10-fold (*R. kirchneri*) cross-validation, which trains the models 5 times by randomly selecting 80% of the occurrence data, then testing the model on the remaining 20%, or 10 times, which trains on 90% and tests on 10%. The final models are the average of the 5(10)-fold cross-validation results. The models were evaluated using four commonly used metrics of predictive accuracy: (1) area under the receiver-operator curve (AUC), (2) sensitivity and specificity, (3) True Skill Statistic (TSS), and (4) percent correctly classified (PCC). These metrics are independent of prevalence (fraction of the study area occupied by the species), which is known to affect other evaluation metrics such as Cohen’s kappa (Allouche et al. 2006).

The AUC, which ranges between 0 and 1, is considered to be threshold independent because it is derived from all possible thresholds, classifying the scores into confusion matrices

and obtaining sensitivity and specificity for each matrix, then plotting sensitivity against the proportion of false positives ($1 - \text{specificity}$) (Allouche et al. 2006). In other words, it is the probability that a randomly selected presence point will rank higher than a randomly selected background (pseudo-absence) point, where values of 0.5 are considered no better than random (Merow et al. 2013). Sensitivity is the ability of the model to correctly predict presences, whereas specificity is the ability to correctly predict absences. The TSS is a normalized measure of accuracy that accounts for both omission and commission errors and the success rate of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values < 0 are no better than random. The PCC considers both sensitivity and specificity expressed as a percentage (West et al 2016).

Predictor variables were evaluated based on percent contribution and response curves produced in SAHM. Variable percent contribution is determined during each iteration of the training algorithm and the increase in regularized gain is added to the contribution of the corresponding variable. Model response curves illustrate the relationship between each predictor included in the model and the fitted values, while holding the other predictors constant at their means (Talbert & Talbert 2012). In general, response curves should be smooth showing a positive/negative trend or central optimum. Response curves with multiple maxima or minima are indicative of over-fit models.

Spatial Analysis

To identify specific streams segments that contained potentially suitable habitat based on model results, I generated a drainage network from the DEM mosaic depicting Strahler Stream Order (Strahler 1957). I selected streams with orders 1–3 and intersected them with the National Hydrography Dataset flowlines. Additionally, I overlaid National Land Cover Database (NLCD

2011) land cover data onto the binary model results and erased areas classified as Developed (low, medium, high intensity) (NLCD 22–24), Pasture/Hay (NLCD 81), and Cultivated Crops (NLCD 82). Then I overlaid the NHD flowline data onto the binary model results and selected by location stream segments that intersected the predicted suitable habitats.

Modified binary outputs and selected streams were subsequently overlaid onto protected areas with Gap Analysis Project (GAP) codes 1–3, which corresponds to lands managed for biodiversity or multiple uses. The union of binary outputs or selected streams and protected lands (GAP code = 1–3) was used to calculate the land area and length of predicted suitable stream habitats within protected areas.

RESULTS

Model Evaluation

The Maxent models performed well based on the four evaluation metrics (Table 3). The AUC ranged between 0.85 to 0.93 (Training), and 0.83 to 0.90 (Test), models with an AUC value > 0.75 are generally considered potentially useful (Elith 2000). Sensitivity (Training: 0.86 to 1.00) and (Test: 0.70 to 0.87) and Specificity, (Training: 0.75 to 0.87–0.75) and (Test: 0.75 to 0.87) were similar, indicating that the models were balanced in identifying true positives (presences) and true negatives (absences). The TSS were (Training: 0.61 to 0.61) and (Test 0.50 to 0.70), which indicates the models scored relatively well for accuracy. The PCC were (Training: 75.43% to 83.99%) and (Test 75.12 % to 83.67%), which indicates relatively good model performance.

The most predictive variables in the final models varied between species (Table 4). Maxent models for *R. daniellae* sp. n. and *R. duffieldi* both responded similarly to Mean

Temperature of the Driest Quarter, which contributed 91.06% and 85.07% to the respective models. Precipitation Seasonality (5.36%), which is the measure of variation in monthly precipitation totals over the course of the year, was the second most predictive variable in the *R. daniellae* sp. n. model, followed by Isothermality (2.92%), i.e. day to night temperature oscillations relative to the summer to winter annual oscillations (O'Donnel & Ignizio 2012). Temperature Seasonality (temperature variation over a given year (or averaged years) based on the standard deviation of monthly temperature averages) was the second most predictive in the *R. duffieldi* model (14.93%). Conversely, Temperature Seasonality, contributed a negligible amount to the *R. daniellae* sp. n. model (0.67%). Neither Precipitation Seasonality nor Isothermality contributed positively to the *R. duffieldi* model. The highest contributing variable in the *R. kirchneri* model was Precipitation of the Coldest Quarter (54.74%), followed by Temperature Annual Range (31.61), and Precipitation Seasonality (13.65%).

The model response curves for both *R. daniellae* sp. n. (Fig. 116) and *R. duffieldi* (Fig. 117) exhibited higher predicted habitat suitability in response to higher Mean Temperature in the Driest Quarter, with the highest suitability in areas with mean temperatures approaching 20°C. Response curves for *R. daniellae* sp. n. showed marginal decreases in habitat suitability as values for Isothermality and Temperature Seasonality increased. Conversely, predicted habitat suitability increased in response to higher values of Precipitation Seasonality. Response curves for *R. duffieldi* exhibited no response to values of Precipitation Seasonality or Isothermality. Similar to the *R. daniellae* sp. n. model, the *R. duffieldi* model also showed a marginal decrease in predicted habitat suitability in response to increased values of Temperature Seasonality. Model response curves for the *R. kirchneri* model (Fig. 118) showed sharp decreases in predicted habitat suitability in areas receiving more than 200 mm of precipitation during the coldest

quarter. Additionally, predicted habitat suitability was lower in areas where annual temperature range exceeds 33° C. Further, the response curves showed decreased habitat suitability as values of Precipitation Seasonality increased beyond 11%.

Spatial Results

The maximum training sensitivity + specificity/2 was used as a threshold (Table 5) to convert the continuous model results (Fig. 119) into a binary map (Fig. 120) depicting potentially suitable and unsuitable habitat. This threshold has been shown to perform well across different training data sets and with presence only models (Liu et al. 2013, Young et al. 2016). The spatial model prediction for *R. daniellae* sp. n. covered 22,734.50 km² (Table 5) in the extreme southwest corner of North Carolina and included areas in northern Georgia, southeastern Tennessee and northwestern South Carolina. The northeastern boundary of the predicted area extended to the Asheville Basin, with a small area of predicted suitable habitat crossing the Basin west of the Tennessee border. Much of the predicted area in northern Georgia was included in the MESS output and should be interpreted with caution.

Model result for *R. duffieldi* were very similar to *R. daniellae* sp. n. covering 21,037.45 km² (Table 6) over much of the same area. Predicted suitable habitat for *R. duffieldi* differed in that suitable habitat did not extend as far north, stopping near the northern boundary of Great Smoky Mountains National Park. Additionally, predicted suitable habitat extended farther east across the Asheville Basin into South Carolina. Notably, predictions in northern Georgia were not included in the MESS output as they were for the *R. daniellae* sp. n. model.

Model results for *R. kirchneri* were largely restricted to Virginia, with only a few small areas extending into North Carolina and Tennessee. Predictions in the latter two states did not include known occurrence localities. Predicted suitable habitat covered 4,794.58 km² (Table 6)

and was generally more fragmented than the model predictions for *R. daniellae* sp. n. and *R. duffieldi*. Predicted suitable habitat covered much of the Iron Mountains and the higher elevation areas extending northeast from Lamsburg to Roanoke, Virginia. The model also predicted areas northeast of the Roanoke and James rivers with narrow bands of habitat extending as far north as Shenandoah National Park.

Spatial Analysis

National Land Cover Database (NLCD 2011) land cover data classified as Developed (low, medium, high intensity) (NLCD 22–24), Pasture/Hay (NLCD 81), and Cultivated Crops (NLCD 82) were erased from the binary model predictions (Fig. 121), which reduced prediction areas by 7.73% to 14.46% (Table 6). National Hydrography Dataset flowlines, which included only streams of orders 1–3 were subsequently intersected with the modified predicted area. The resulting selection included 51,722.95 km of streams that may be suitable habitat for *R. daniellae* sp. n., 47,611.41 km for *R. duffieldi* and 13,477.67 km for *R. kirchneri* (Table 7).

To calculate land area and length of potentially suitable stream habitats in protected areas the modified binary output and selected streams were overlaid onto protected lands managed for biodiversity or multiple uses. The resulting union included approximately 57% of predicted suitable habitat for all species being within protected areas (Table 8). The proportion of potentially suitable streams in protected areas ranged between 32.53% and 49.35% (Table 8). The majority of predicted stream habitats are on lands managed by the United States Forest Service (USFS), which are typically managed for multiple uses. In total, 80.01% of *R. daniellae* sp. n., 84.21% of *R. duffieldi*, and 89.53% of *R. kirchneri* predicted stream habitats reside on lands managed by the USFS.

DISCUSSION

The Maxent models presented here and other SDMs previously developed for stoneflies have relied on terrestrial environmental variables due to the lack of available hydrological and biological variables that directly influence distributions, such as substrate type, water chemistry, food availability, competition, and dispersal ability (Hynes 1976). The objectives of this study were to describe the bioclimatic envelopes of *R. daniellae* sp. n., *R. duffieldi* and *R. kirchneri* and use those variables to predict the potential distributions of these species, which was achieved using maximum entropy modeling. Model response curves for *R. daniellae* sp. n. and *R. duffieldi* exhibited similar responses to environmental variables, which is not surprising as these species occupy similar habitats in relatively close geographic proximity. Additionally, these species are sympatric in at least one location. Model results for *R. kirchneri* differed from *R. daniellae* sp. n. and *R. duffieldi* in both environmental variable selection and percent contribution of these variables. The differences in predictive bioclimatic variables between model results are indicative of the unique environmental conditions present at occurrence localities and may explain, in part, observed species distributions.

Interestingly, predicted suitable habitat for *R. daniellae* sp. n. was largely contained to the region west of the Asheville Basin, indicating climatic variation between regions east and west of the French Broad River. The Asheville Basin had been postulated as a biogeographic barrier separating the new species and the closely related *R. kirchneri*. These results are consistent with those reported for two other aquatic species, *Desmognathus wrighti* (King, 1936) and *D. organi* Crespi, Browne & Rissler, 2010, (Caudata: Plethodontidae), which are similarly distributed in both elevation and geographic space (Crespi et al. 2010).

While all three species are of conservation concern, conservation priority should be given to *R. kirchneri* based on the spatial results. Potentially suitable habitat, whether it be land area or stream kilometers were lowest overall for this species. Additionally, *R. kirchneri* has the smallest area of potentially suitable habitat in protected areas with only 2,361.24 km² (57.57% of binary output - (NLCD 22–24, 81–82)) on lands managed for biodiversity or multiple uses (Table 8). Further, only 4,384.74 km (32.53% of binary output - (NLCD 22–24, 81–82)) of potentially suitable stream reaches are within protected areas and this unfortunately does not imply that the headwaters are protected. Only two occurrence localities have their headwaters entirely within protected areas, tributary to Lick Fork, Floyd County, Virginia, and Battery Creek, Bedford County, Virginia, and the latter are on lands managed for multiple use.

Little is known regarding the autecology of *R. daniellae* sp. n., *R. duffieldi*, and *R. kirchneri*. As such, the tolerance of these species to disturbances remains undefined. Stoneflies are generally considered one of the most environmentally sensitive inhabitants of freshwater ecosystems (Baumann 1979, Masters et al. 2000). Like other sensitive aquatic organisms, stoneflies exhibit a range of responses to environmental stress (Rosenberg & Resh 1993). Their distribution and abundance can also be influenced by habitat modifications that affect current velocity and substrate type. Effective conservation of these rare species will require a better understanding of their autecology and population trends. The results presented in this study mark the beginning of an iterative procedure for generating new biogeographical data outlined by Pearson et al. (2006), whereby predictive models are developed to guide future field surveys and in turn, field surveys provide new occurrence records that can be used to create more accurate and refined distribution models.

FIGURES AND TABLES

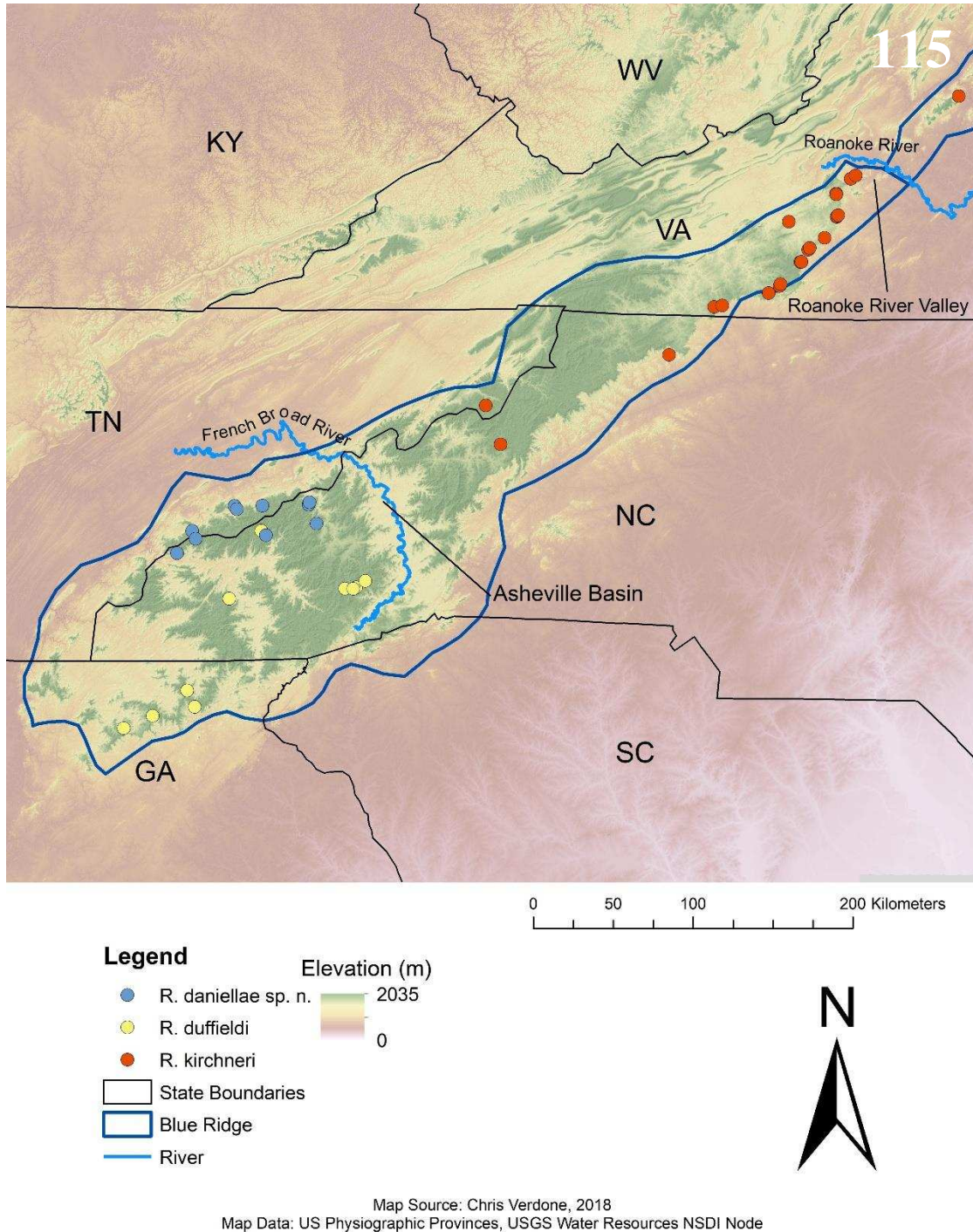


Figure 115. Distribution of *Remenus daniellae* sp. n., *R. duffieldi*, and *R. kirchneri* in the Blue Ridge Physiographic Province.

Table 1. Location data for occurrence records used in *Remenus* Maxent models.

Species	State	County	Habitat	Latitude	Longitude
<i>R. daniellae</i> sp. n.	NC	Haywood	Ball Branch	35.71838	-83.09217
	NC	Haywood	Mt. Sterling Creek	35.70849	-83.09657
	NC	Haywood	Right Fork Cove Creek	35.62111	-83.05192
	NC	Swain	Collins Creek	35.56768	-83.33660
	NC	Swain	Gunna Creek	35.55112	-83.73204
	NC	Swain	Proctor Branch	35.48625	-83.83651
	TN	Blount	Anthony Creek	35.58697	-83.75153
	TN	Sevier	Le Conte Creek	35.70160	-83.51354
	TN	Sevier	Little Laurel Branch	35.70270	-83.35662
	TN	Sevier	tributary to Le Conte Creek	35.68656	-83.49996
<i>Remenus duffieldi</i>	GA	Towns	Soapstone Creek	34.85396	-83.78073
	GA	Union	Rock Creek	34.68003	-84.13579
	GA	Union	Slaughter Creek	34.73692	-83.97383
	GA	White	Andrews Creek	34.77807	-83.73734
	NC	Haywood	tributary to Flat Laurel Creek	35.32321	-82.89283
	NC	Haywood	Yellowstone Prong of East Fork Pigeon River	35.32827	-82.82930
	NC	Haywood	Yellowstone Prong of East Fork Pigeon River	35.32199	-82.84743
	NC	Macon	Tellico Creek	35.27686	-83.54347
	NC	Swain	Collins Creek	35.56768	-83.33660
	NC	Swain	Kanati Fork	35.58744	-83.36295
	NC	Transylvania	Pigeon Branch South Fork Mills River	35.35808	-82.77833
<i>Remenus kirchneri</i>	NC	Avery	Jones Creek	35.98205	-82.01669
	NC	Wilkes	Garden Creek	36.38944	-81.06953
	TN	Carter	Bearwallow Branch	36.15934	-82.10077
	VA	Bedford	Battery Creek	37.55152	-79.44081
	VA	Carroll	North Fork Stewarts Creek	36.60662	-80.81706
	VA	Carroll	Pine Creek	36.66780	-80.51047
	VA	Carroll	tributary to Turkey Creek	36.61176	-80.77106
	VA	Floyd	Dodd Creek	36.87099	-80.27918
	VA	Floyd	spring fed stream	37.11633	-80.12965
	VA	Floyd	spring fed stream	37.01126	-80.12707
	VA	Floyd	spring tributary to Little River	36.98934	-80.39582
	VA	Floyd	tributary to Dodd Creek	36.86324	-80.28718
	VA	Floyd	tributary to Dodd Creek	36.87170	-80.27943
	VA	Floyd	tributary to Little River	37.01936	-80.11926
	VA	Floyd	tributary to Little River	37.01486	-80.12351
	VA	Floyd	tributary to Lick Fork	37.11290	-80.12930
	VA	Franklin	Brogan Branch	36.91694	-80.19498
	VA	Patrick	Haunted Branch	36.70027	-80.44728
VA	Patrick	Little Rock Castle Creek	36.80786	-80.33115	

VA	Patrick	Mayberry Creek	36.70846	-80.44408
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Table 1 continues on next page

Table 1 continued

Species	State	County	Habitat	Latitude	Longitude
<i>Remenus kirchneri</i>	VA	Patrick	tributary to Rock Castle Creek	36.80884	-80.32540
	VA	Roanoke	tributary to Back Creek	37.18174	-80.04740
	VA	Roanoke	tributary to Back Creek	37.19634	-80.02027

Table 2. Environmental variables and sources considered for *Remenus* Maxent models.

Environmental Variable	Source
BIO 1 - Annual Mean Temperature 1970-2000 (°C)	Worldclim
BIO 2 - Mean Diurnal Range Mean of monthly (max temp-min temp) 1970-2000 (°C)	Worldclim
BIO 3 - Isothermality (BIO2/BIO7) (* 100) 1970-2000 (%)	Worldclim
BIO 4 - Temperature Seasonality (standard deviation *100) 1970-2000 (°C*100)	Worldclim
BIO 5 - Max Temperature of Warmest Month 1970-2000 (°C)	Worldclim
BIO 6 - Min Temperature of Coldest Month 1970-2000 (°C)	Worldclim
BIO 7 - Temperature Annual Range (BIO5-BIO6) 1970-2000 (°C)	Worldclim
BIO 8 - Mean Temperature of Wettest Quarter 1970-2000 (°C)	Worldclim
BIO 9 - Mean Temperature of Driest Quarter 1970-2000 (°C)	Worldclim
BIO 10 - Mean Temperature of Warmest Quarter 1970-2000 (°C)	Worldclim
BIO 11 - Mean Temperature of Coldest Quarter 1970-2000 (°C)	Worldclim
BIO 12 - Annual Precipitation 1970-2000 (mm)	Worldclim
BIO 13 - Precipitation of Wettest Month 1970-2000 (mm)	Worldclim
BIO 14 - Precipitation of Driest Month 1970-2000 (mm)	Worldclim
BIO 15 - Precipitation Seasonality 1970-2000 (Coefficient of Variation) (%)	Worldclim
BIO 16 - Precipitation of Wettest Quarter 1970-2000 (mm)	Worldclim
BIO 17 - Precipitation of Driest Quarter 1970-2000 (mm)	Worldclim
BIO 18 - Precipitation of Warmest Quarter 1970-2000 (mm)	Worldclim
BIO 19 - Precipitation of Coldest Quarter 1970-2000 (mm)	Worldclim
Elevation (m)	USGS NED

Table 3. Evaluation metrics for *Remenus* Maxent models.

Evaluation Metric	<i>R. daniellae</i> sp. n.		<i>R. duffieldi</i>		<i>R. kirchneri</i>	
	Training	Test	Training	Test	Training	Test
AUC	0.91	0.88	0.93	0.90	0.85	0.83
Sensitivity	1.00	0.70	1.00	0.87	0.86	0.75
Specificity	0.84	0.84	0.83	0.83	0.75	0.75
True Skill Statistic	0.84	0.54	0.83	0.70	0.61	0.50
Percent Correctly Classified	83.99	83.67	82.57	83.25	75.43	75.12

Table 4. Environmental variable percent contribution to *Remenus* Maxent models.

Environmental Variable	% Contribution		
	<i>R. daniellae</i> sp. n.	<i>R. duffieldi</i>	<i>R. kirchneri</i>
Isothermality (BIO2/BIO7)*(100)	2.92	0.00	–
Temperature Seasonality (standard deviation *100)	0.67	14.93	–
Temperature Annual Range (mean of monthly (max temp – min temp))	–	–	31.61
Mean Temperature of Driest Quarter	91.06	85.07	–
Precipitation Seasonality (Coefficient of Variation)	5.36	0.00	13.65
Precipitation of the Coldest Quarter	–	–	54.74

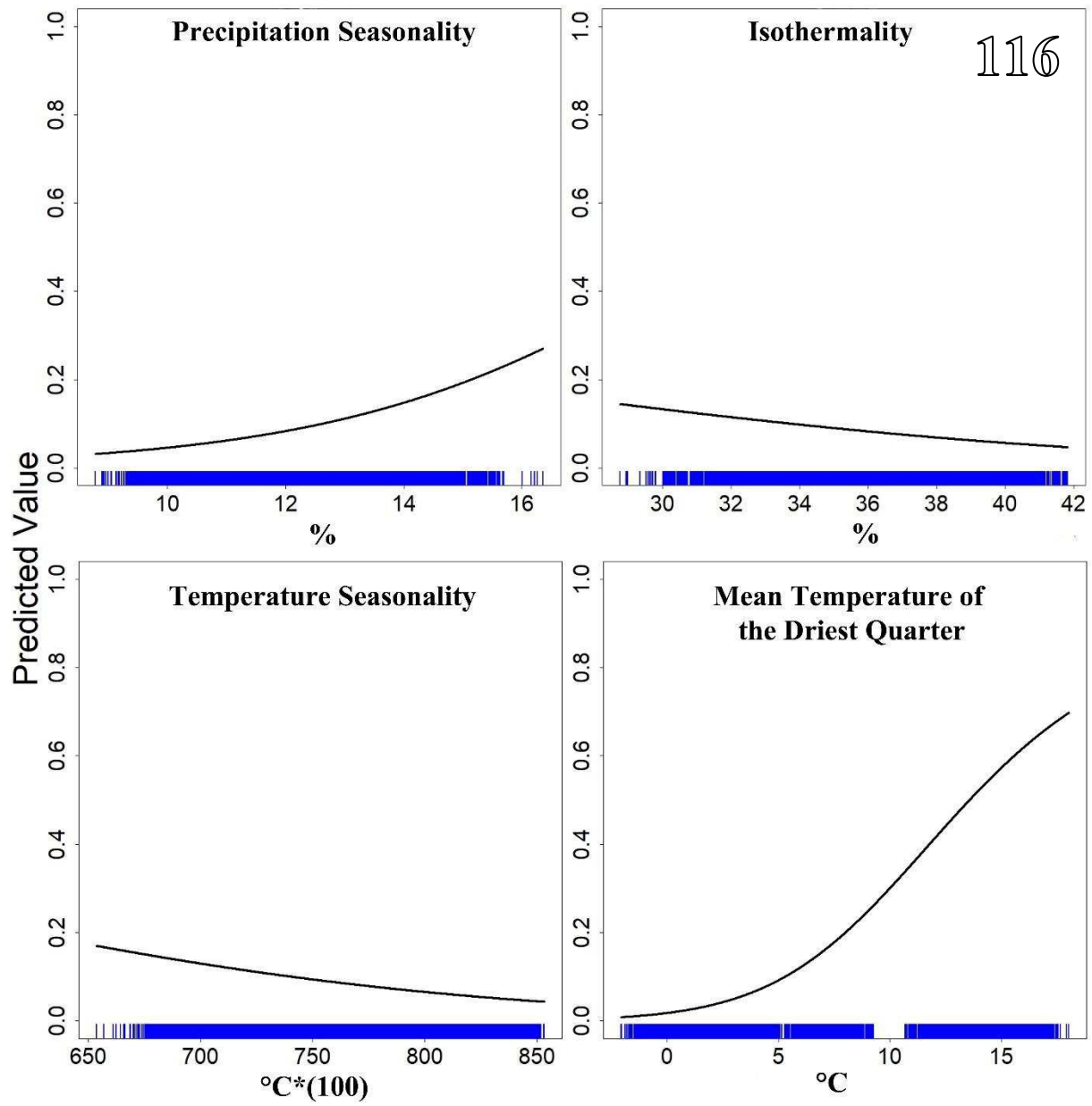


Figure 116. *Remenus daniellae* sp. n. Maxent model environmental variable response curves.

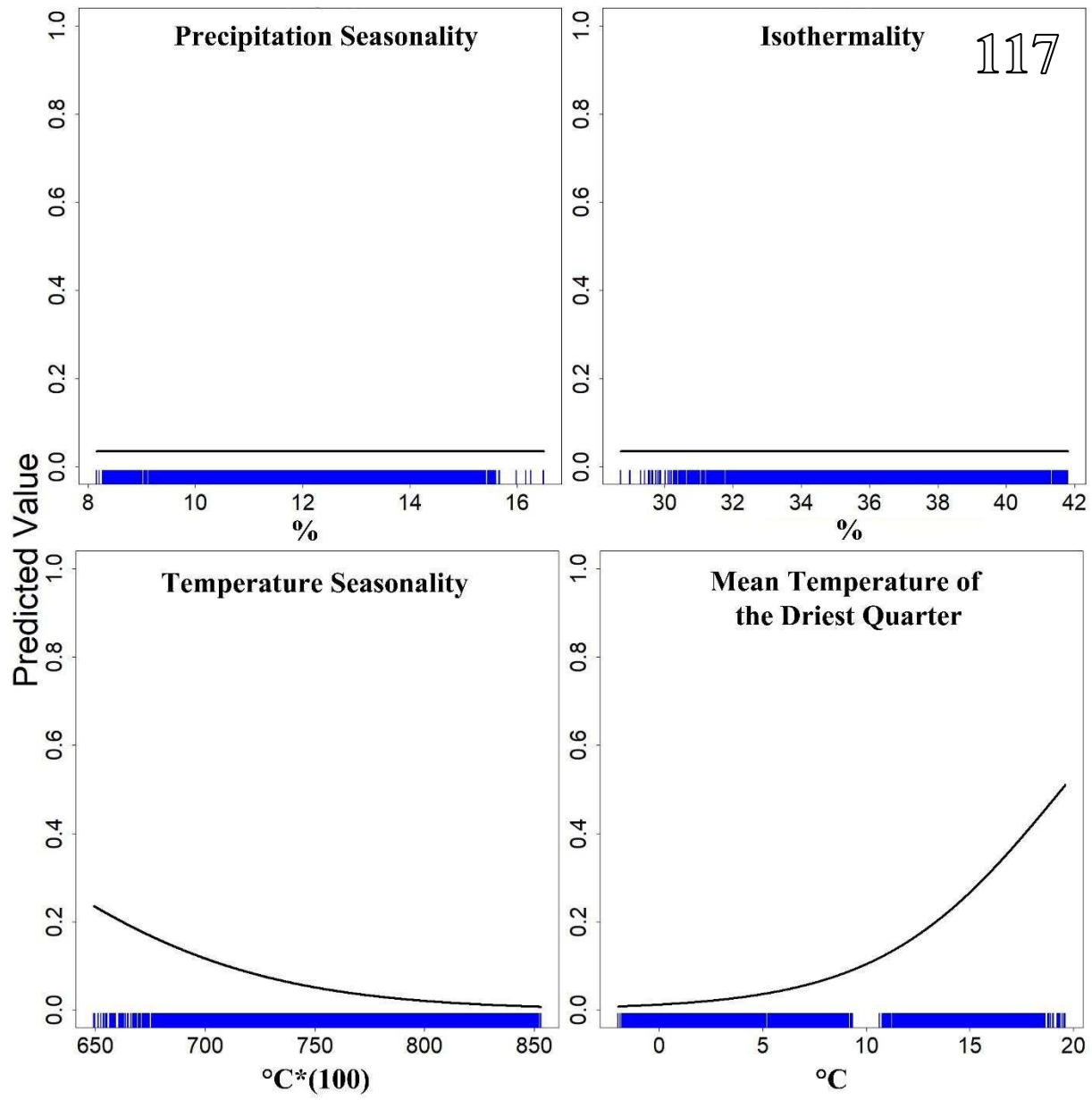


Figure 117. *Remenus duffieldi* Maxent model environmental variable response curves.

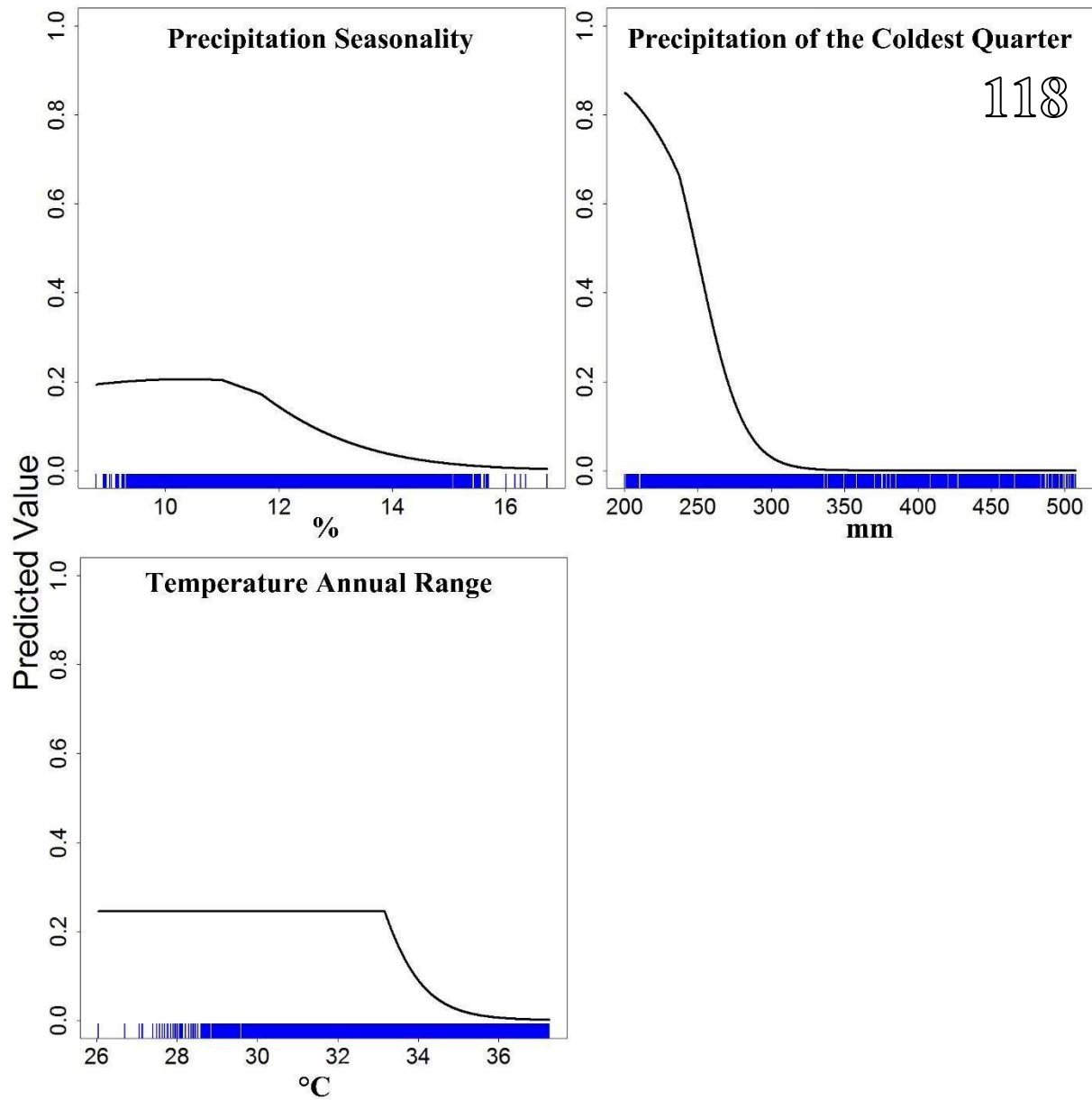


Figure 118. *Remenus kirchneri* Maxent model environmental variable response curves.

Table 5. Threshold values used to convert the continuous model results into binary using the maximum of sensitivity (proportion of true presences correctly classified) plus specificity (proportion of true absences correctly classified) divided by 2.

Threshold	<i>R. daniellae</i> sp. n.	<i>R. duffieldi</i>	<i>R. kirchneri</i>
Maximum Sensitivity + Specificity/2	0.416	0.342	0.357

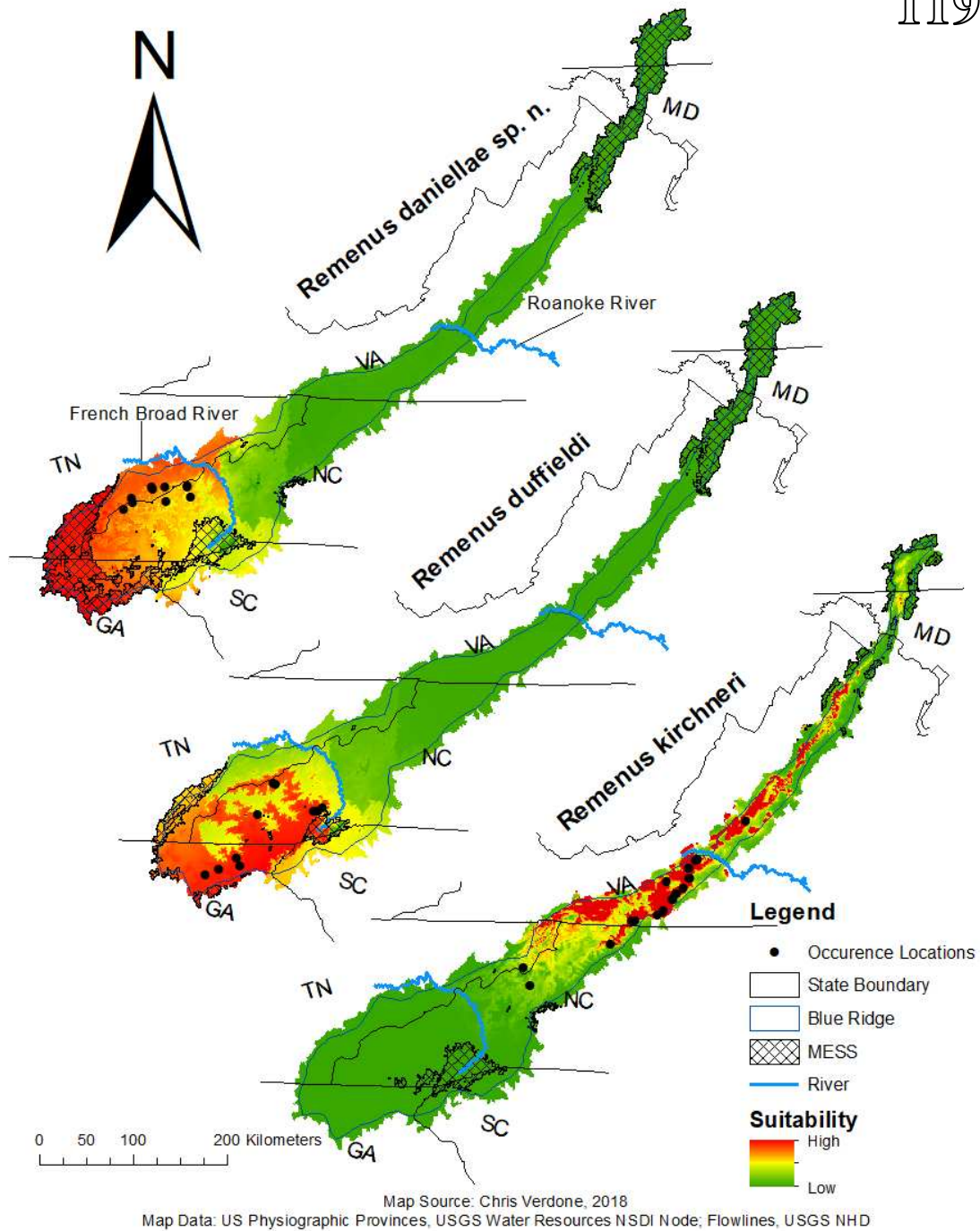
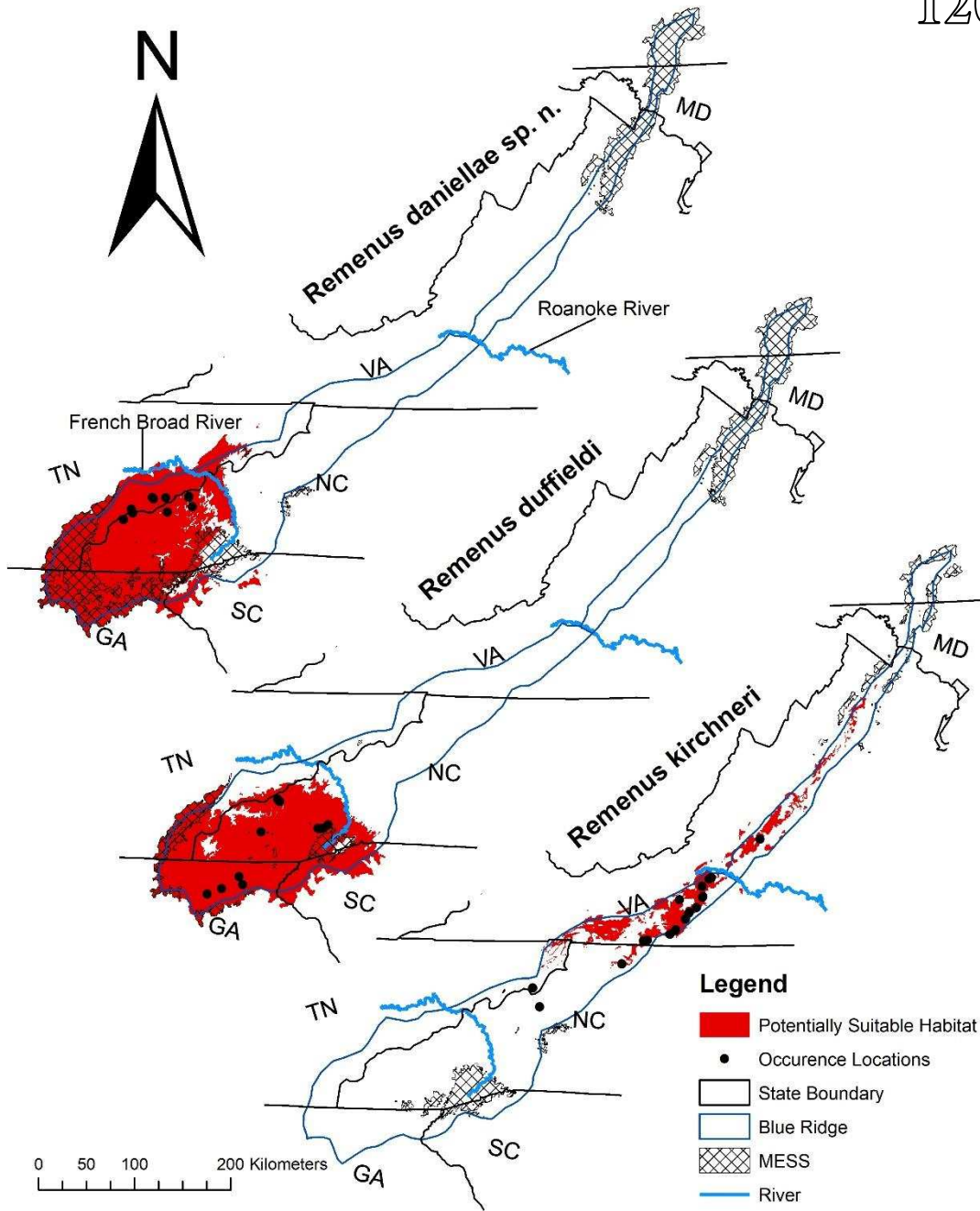


Figure 119. Maxent continuous probability predictions for potentially suitable habitat for *Remenus daniellae* sp. n., *R. duffieldi*, and *R. kirchneri* in the Blue Ridge Physiographic Province.



Map Source: Chris Verdone, 2018
 Map Data: US Physiographic Provinces, USGS Water Resources NSDI Node; Flowlines, USGS NHD

Figure 120. Maxent binary predictions for potentially suitable habitat for *Remenus daniellae* sp. n., *R. duffieldi*, and *R. kirchneri* in the Blue Ridge Physiographic Province.

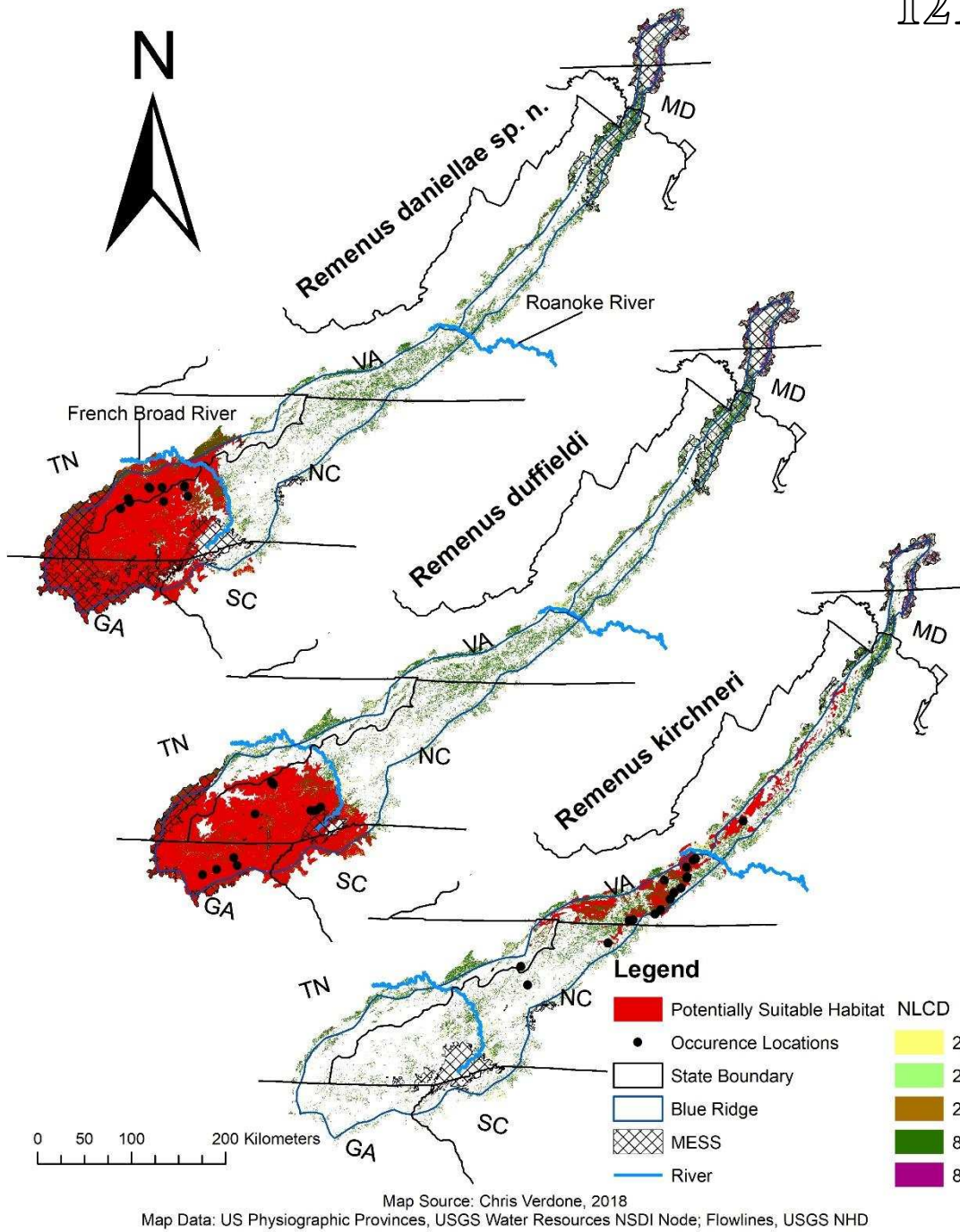


Figure 121. Maxent binary spatial predictions for potentially suitable habitat for *Remenus daniellae* sp. n., *R. duffieldi*, and *R. kirchneri* in the Blue Ridge Physiographic Province and National Land Cover classifications, Developed: low intensity (NCLD 22), medium intensity (NCLD 23), high intensity (NCLD 24); Pasture/Hay (NLCD 81); and Cultivated Crops (NCLD 82).

Table 6. Predicted suitable habitat land area based on binary output minus National Land Cover classifications, Developed: low intensity (NCLD 22), medium intensity (NCLD 23), high intensity (NCLD 24); Pasture/Hay (NCLD 81); and Cultivated Crops (NCLD 82).

Model Results	Predicted Habitat Area (km²)		
	<i>R. daniellae</i> sp. n.	<i>R. duffieldi</i>	<i>R. kirchneri</i>
Binary output	22734.50	21037.45	4794.58
Binary output - (NCLD 22–24, 81–82)	20485.52	19410.48	4101.29

Table 7. Predicted suitable stream habitat (stream order 1–3) length based on binary output minus National Land Cover classifications, Developed: low intensity (NCLD 22), medium intensity (NCLD 23), high intensity (NCLD 24); Pasture/Hay (NCLD 81); and Cultivated Crops (NCLD 82).

Model Results	Predicted Stream Habitat (km)		
	<i>R. daniellae</i> sp. n.	<i>R. duffieldi</i>	<i>R. kirchneri</i>
Binary output - (NCLD 22–24, 81–82)	51722.95	47611.41	13477.67

Table 8. Proportion of predicted suitable habitat within lands managed for biodiversity or multiple uses (subject to mining or logging).

Binary output - (NCLD 22–24, 81–82)	<i>R. daniellae</i> sp. n.	<i>R. duffieldi</i>	<i>R. kirchneri</i>
Predicted suitable habitat in protected lands (km ²)	11671.56	11098.01	2361.24
Percent of predicted suitable habitat in protected lands	56.97	57.17	57.57
Streams in protected areas (km)	24547.51	23495.62	4384.74
Percent of predicted suitable streams in protected areas	47.46	49.35	32.53

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