



A new species of *Neophemera* McDunnough, 1925 (Ephemeroptera: Neophemeridae) from North Carolina and Virginia

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Abstract

Neophemera eatoni **sp. nov.** is described based on nymphs, imagos, and eggs from North Carolina and Virginia, USA. Nymphs of the new species are associated only with hornleaf riverweed (*Podostemum ceratophyllum*) growing on the surfaces of cobbles and boulders in moderate to swift flows, at a depth of up to one meter. Life stages were associated by laboratory rearing. Nymphs of the new species are distinguished from other Nearctic *Neophemera* by the following combination of characters: all legs are short and stout, with overall lengths subequal to each other; tibial and tarsal lengths are subequal on all legs; tarsal claws are sharply curved; anterolateral projections on the pronotum and mesonotum are reduced; and anterosubmedian tubercles are absent from the pronotum. Male and female imagos exhibit unique abdominal maculations; the anterior protuberance of the mesonotum is obovate; and distinct leg banding patterns are absent. Male imagos have the fore tibia at least 2× the length of mid and hind tibiae, and female imagos have all legs subequal in length. Our new species may be most closely related to *N. youngi* based on imagos of the two species each having a small spine posteromedially on abdominal tergum II and having annulated terminal filaments. An updated key to North American species is provided.

Key words: mayflies, taxonomy, eggs, Nearctic, North America

Introduction

Neophemera McDunnough, 1925 (Ephemeroptera: Neophemeridae) was established for its type species, *N. bicolor* McDunnough, 1925, based on imagos from Québec, Canada. Shortly thereafter, Traver (1931) described *Oreianthus purpureus* Traver, 1931, a new genus and species from North Carolina, USA, based on the nymph, female subimago, and female imago stages. She considered the European species *Caenis maxima* Joly, 1870 to belong this new genus. About the same time, Lestage (1931) established the genus group *Leucorhoenanthus* Lestage, 1931 for *Rhoenanthus macedonicus* Ulmer, 1920 (a species later synonymized with *C. maxima* by Illies 1967). Traver (1935) subsequently established the subfamily Neophemerinae within Ephemeridae to include *Neophemera* and *Oreianthus* Traver, 1931. Burks (1953) synonymized *Oreianthus* under *Neophemera* and he was the first to recognize Neophemeridae at family rank. Demoulin (1961) established *Caenomera* Demoulin, 1961 as a subgenus of *Neophemera* for *Caenis maxima*, but he later recognized *Caenomera* as a junior synonym of *Leucorhoenanthus* (Demoulin 1962); at the time, he recognized three subgenera for *Neophemera*—*Leucorhoenanthus*, *Oreianthus*, and *Neophemera s.s.* However, Bae & McCafferty (1998) later did not recognize subgeneric divisions within the genus and formally placed *Leucorhoenanthus* and *Caenomera* as junior synonyms of *Neophemera*, in addition to recognizing Burks' (1953) synonymy of *Oreianthus*. Kluge (2004), however, accepted *Leucorhoenanthus* as a valid genus. Bauernfeind & Soldán (2012) recognized the synonymy of the two groups when they treated the European species of *Neophemera*. In this paper we follow this classification. Also worth mentioning here is that Bae & McCafferty (1998) established the genus *Ochernova* for the central Asian

species, *N. tshernovae* Kazlauskas, 1963, and Kluge (2004) and Bauernfeind & Soldán (2012) both concur that it is a valid monospecific genus. We note also that the date of publication of the species name, *C. maxima*, is questionable, but after examining a copy of Volume 4 of *Bulletin de la Société d'Histoire Naturelle de Toulouse*, we follow Bauernfeind & Soldán (2012) and use the earlier date of 1870 that is indicated on the publication.

Based on the prevailing classification, *Neophemera* contains six extant species from the Nearctic, Palearctic, and Oriental regions (Traver 1931; Burks 1953; Berner 1953, 1956; Bae & McCafferty 1998; Zhou & Zheng 2000; Schmude *et al.* 2012). These include: *N. bicolor* McDunnough, 1925 (= *Neophemera* sp. A Jacobus & McCafferty, 2002, per stage association by Schmude *et al.* 2012); *N. compressa* Berner, 1956; *N. purpurea* (Traver, 1931); and *N. youngi* Berner, 1953 (= *Oreianthus* sp. 1 Traver, 1937, by Berner's 1953 designation) from the eastern Nearctic region (Berner 1956; Bae & McCafferty 1998); *N. maxima* (Joly, 1870) from the western Palearctic region (Eaton 1883–1888; Kazlauskas 1959, 1963; Jazdzewska 1975; Bae & McCafferty 1998); and *N. projecta* Zhou & Zheng, 2000 from the eastern Palearctic and Oriental regions (Zhou & Zheng 2000). *Neophemera bicolor*, *N. compressa*, *N. purpurea*, and *N. youngi* are the only previously described extant species from North America (Bae & McCafferty 1998). One fossil species has been described for the genus (Sinitshenkova 1999), which also happens to be from North America. Berner (1956) provided a thorough review of the North American species known at the time, and he included identification keys for nymphs and imagoes.

McCafferty *et al.* (in press) include mention of a previously undescribed species designated as *Neophemera* sp. B in their identification key for mayfly nymphs of southeastern North America, based on information shared with them by biologists in the North Carolina Division of Water Resources (NCDWR). The objective of this paper is to name and describe this new species formally and to provide details about its biology.

Materials and methods

Distinctive but previously unknown *Neophemera* nymphs were collected by NCDWR biologists from the Dan River, North Carolina in 2014. Collections in 2015 by NCDWR biologists, and subsequent examination of material collected by D. R. Lenat in 2012, resulted in the identification of this morphotype from additional locations in the Mayo River in Rockingham County, North Carolina. Examination of nymphal specimens from the Gillette Museum of Arthropod Diversity, Colorado State University, Fort Collins, Colorado (CSUC) yielded additional nymphs of the same morphotype from both the New River, Grayson County, Virginia, USA (collected in 1973) and Sinking Creek, Craig County, Virginia (collected in 1974). Nymphs from the Dan River were laboratory reared in 2015, allowing us to associate life stages and confirm it as a new species, based on a morphological species concept.

The field methods that allowed for the laboratory rearing were as follows. In April and May 2015, *Neophemera* nymphs were collected by hand from the aquatic macrophyte, hornleaf riverweed (*Podostemum ceratophyllum* Michaux, 1803) (Malpighiales: Podostemaceae), growing on the surfaces of cobbles and boulders in the Dan and Mayo Rivers in Rockingham County, North Carolina. In cases where a nymph was difficult to collect due to its strong affinity for clinging to *P. ceratophyllum*, small shears were used to cut the plant away from the rock to avoid injuring the insect. Live nymphs, along with *P. ceratophyllum* were placed into containers with aerated water and returned to the laboratory, where they were reared in an artificial stream.

The rearing apparatus consists of a modified 55 L cooler connected to an Aqua Euro US Max-Chill® Aquarium Chiller (Model: MC-1/10 HP, Gardena, CA) to allow controlled water temperature adjustments and a plexiglass divider placed in the center of the cooler to provide circular flow. A water pump and activated charcoal filters promote clean water flow through the apparatus. A 14:10 hour light-dark cycle was maintained in the laboratory using an automated timer connected to compact florescent 34 watt bulbs mounted two meters above the rearing apparatus.

The nymphs and *P. ceratophyllum* were placed in 7.0 cm high by 7.5 cm wide modified cups with 3.5 cm by 4.5 cm mesh windows cut into the sides allowing water to flow through the cups. Floats for the cups were constructed using 3.75 cm thick insulation board appressed to the top of the cups, as this allowed shallow flow through the system containing nymphs and space above for subimago emergence. Small cone-shaped mesh tents with a central mesh ladder were attached to the tops of the cups to prevent insect escape and to allow a stable substrate for subimago landing and climbing. Following emergence, subimagos were transferred to a dry cup with

a moist condensed paper towel to reach the imago stage. Nymph and subimago exuviae, imagos, and eggs produced by female imagos were preserved in 95% ethanol.

The nymphal habitus illustration was completed using a M-series Leica® camera lucida attached to a Leica® M80 stereoscope. Photographs were completed using a Lumenera® Infinity 1-5C microscope camera attached to a Leica® Wild M3C stereoscope or Nikon® Eclipse E400 compound light microscope and Infinity Analyze Software Version 6.1. Outlines and illustrations using these photographs were finalized using Inkscape version 0.91 and Gimp version 2.8 open source software. Egg materials were studied using a JEOL JSM-6500F Field Emission Scanning Electron Microscope (FESEM), Central Instrument Facility, Imaging Laboratory (<http://cif.colostate.edu/imaging-laboratory/>), Colorado State University. Egg samples were critical point dried and coated with 20 nm of gold prior to SEM study. Images were captured in tagged image file format (TIFF).

Specimens are deposited with the following institutional collections: Gillette Museum of Arthropod Diversity, Colorado State University, Fort Collins, Colorado, USA [CSUC]; National Museum of Natural History, Smithsonian Institution, Washington, District of Columbia, USA [NMNH]; Water Sciences Section, North Carolina Division of Water Resources, Raleigh, North Carolina, USA [NCDWR]; Purdue University Entomological Research Collection, West Lafayette, Indiana, USA [PERC].

Results

Neoephemera eatoni, new species

(Figs. 1–10, 14, 18, 20–25, 29, & 31–34)

=*Neoephemera* sp. B, McCafferty *et al.* (in press)

Description. *Mature Nymph* (preserved in 95% ethanol). Dimensions: Body length 8.1–11.8 mm; cerci and median filament ca. 0.7–0.8× body length. Body color: speckled brown and cream.

Head: Speckled brown with coronal and epicranial sutures pale. Dorsal diameter of compound eye 0.5 mm; one pale spot on left and right side of epicranial suture at base of each antenna (Fig. 1). Antenna light brown at base, white medially to translucent at apex and length 0.8× width of head capsule; base of scape and head capsule forming triangular shape. Labrum (Fig. 2) with broadly rounded anteromedial notch with six short plumose setae along margin of notch and constricted arched row of 60 or more finely ciliated setae located dorsally, 100 µm from apicomedial margin; various sizes of plumose setae scattered posterior to arched labral setal row. Mandibles heavily mottled and sclerotized with stout bifurcate and plumose setae on outer margins (Figs. 3–4). Outer and inner incisors cleft on both mandibles with four and two denticles, respectively; protheca bifid with sharp subapical spines; molars with reduced apical and subapical dissections. Hypopharynx (Fig. 8) with lingua apically truncate and covered with fine setae; superlinguae with two basal rows of sharp setae and covered apically with long hispid setae. Each galealacinia of maxillae with five distinct denticles (Fig. 5) and hispid setae at base and along remaining lacinial lateral margin. Segment I of maxillary palp slightly broader than segments II and III, with lengths of segments I–III=0.3 mm+0.2 mm+0.3 mm (Fig. 5); labial palp (LP) segments I–III each with length of 0.3 mm; LP segment I slightly concave basally abutting basal segment of paraglossa and with dense plumose setae across ventral surface (Fig. 6). Paraglossa two segmented with dense hispid spines across ventral surface; glossa with similar setae ventrally (Fig. 7).

Thorax: Speckled yellowish brown to piceous; pronotal anteromedial dorsal tubercles absent, but with ridge extending diagonally from anteromedial submargin to anterior corners; pronotum and mesonotum with reduced anterolateral projections (Figs 1 & 14); hindwing pads small and covered by forewing pads in dorsal view. All legs subequal in length to each other; tibiae and tarsi lengths subequal on each leg (Fig. 1) with adentiate claws sharply curved, darkening to piceous at approximately 90° from vertex to apex (Figs 1 & 9); length ratio range of foreleg femur: tibia: tarsus: claw = 1.3–1.5: 1.0–1.1: 1.0: 0.4–0.5; midleg femur: tibia: tarsus: claw = 1.3–1.4: 1–1.1: 1.0: 0.5; hindleg femur: tibia: tarsus: claw = 1.4–1.7: 1.0–1.2: 1.0–1.1: 0.5. Legs pale to brown, with distinct black spots present ventrally, at coxal-femoral junctions. Femora (Fig. 9) broader than tibiae and tarsi, with distinctive short bifurcate plumose setae in broad longitudinal row along medial dorsolateral surface (Fig. 10); submarginal dorsal surface with row of short plumose setae with few, scattered longer setae and ventral margin

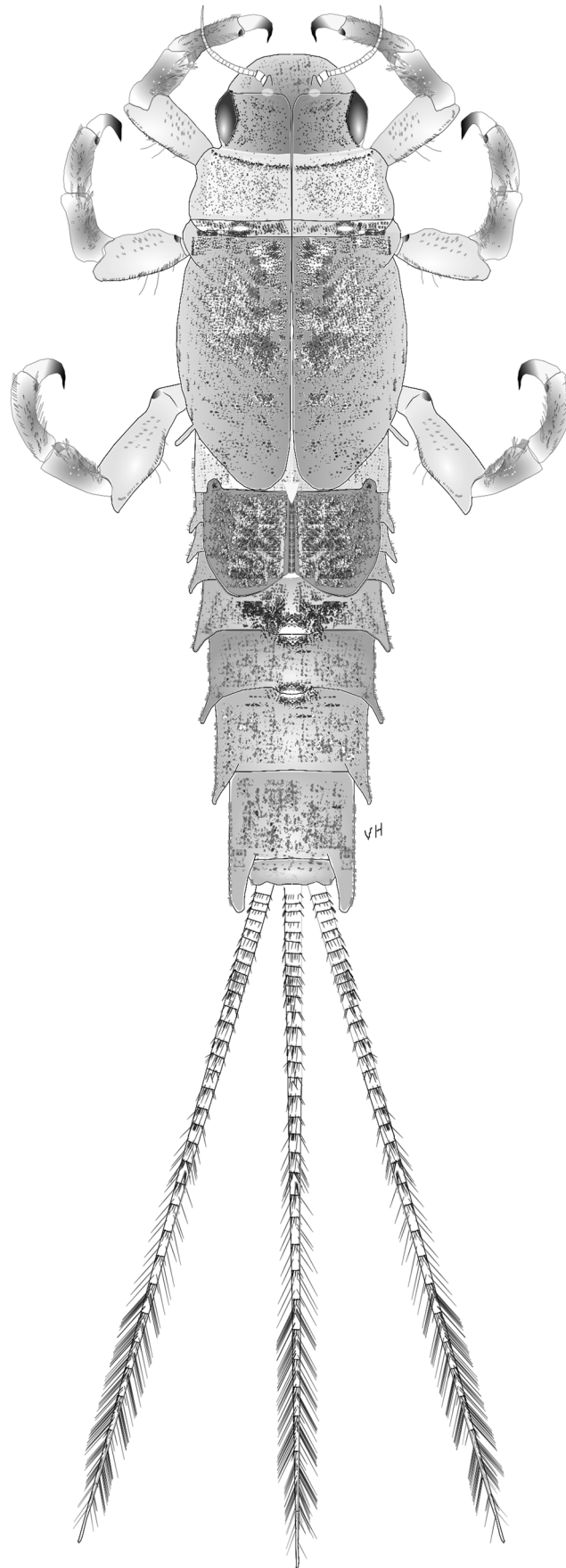
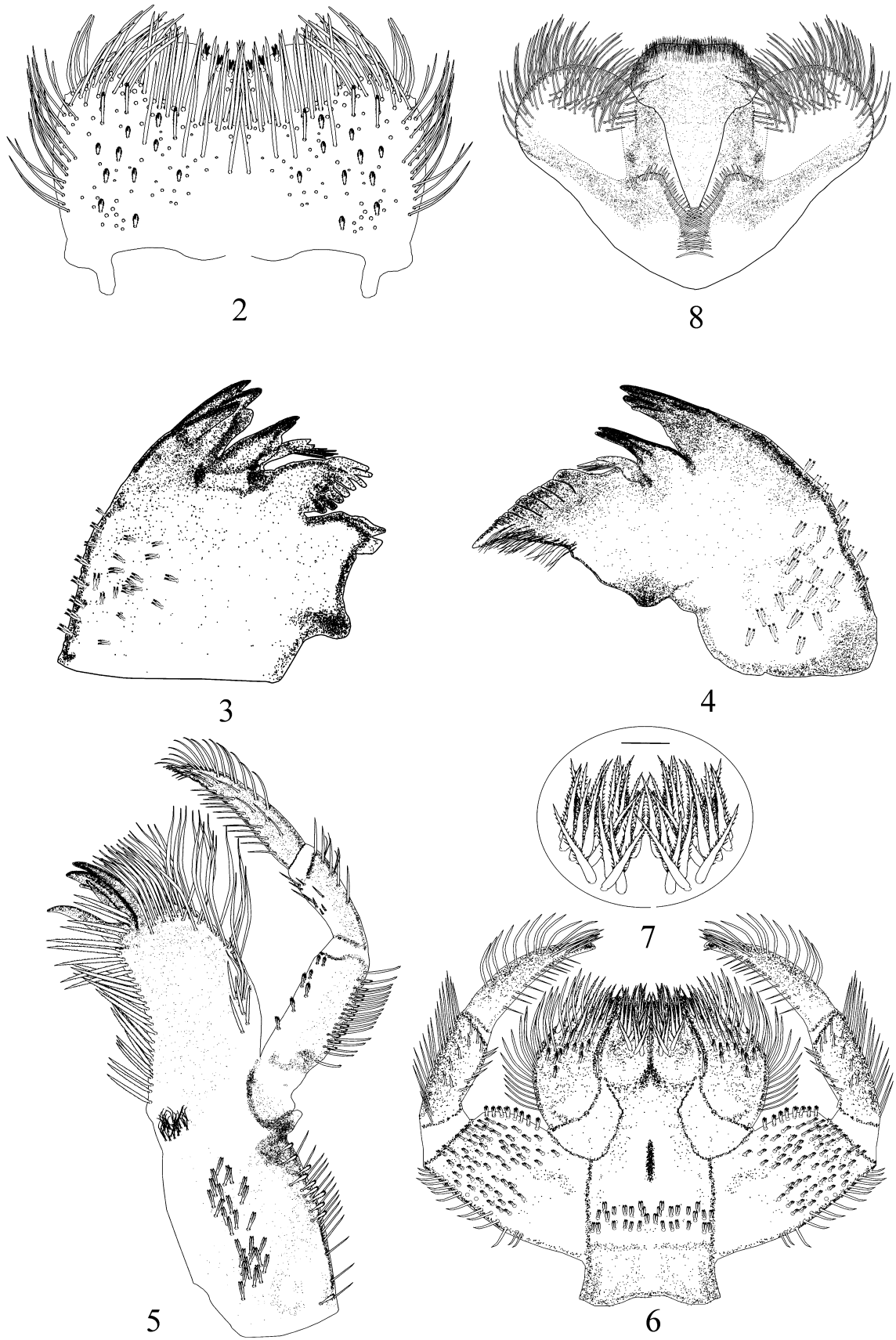
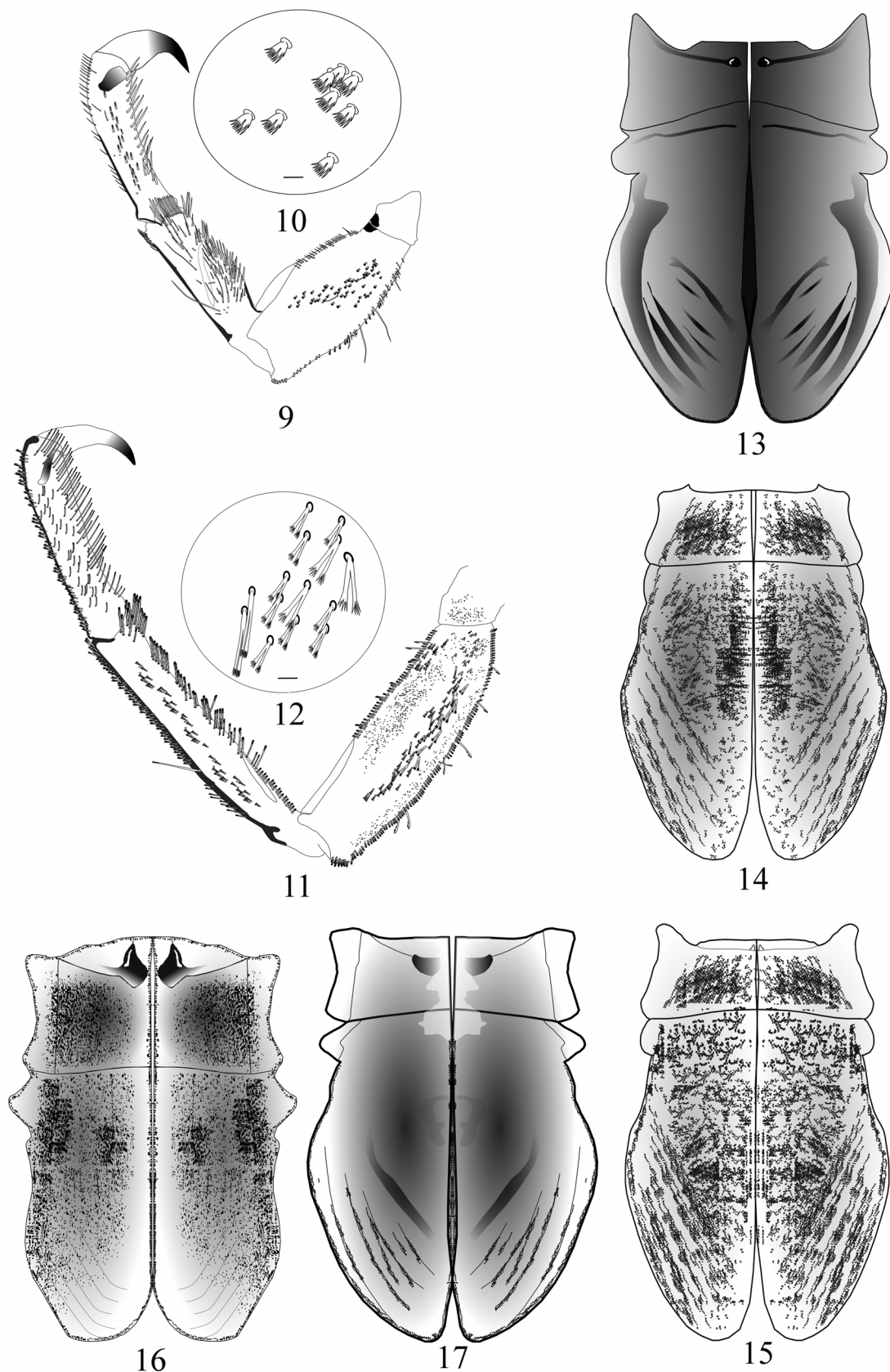


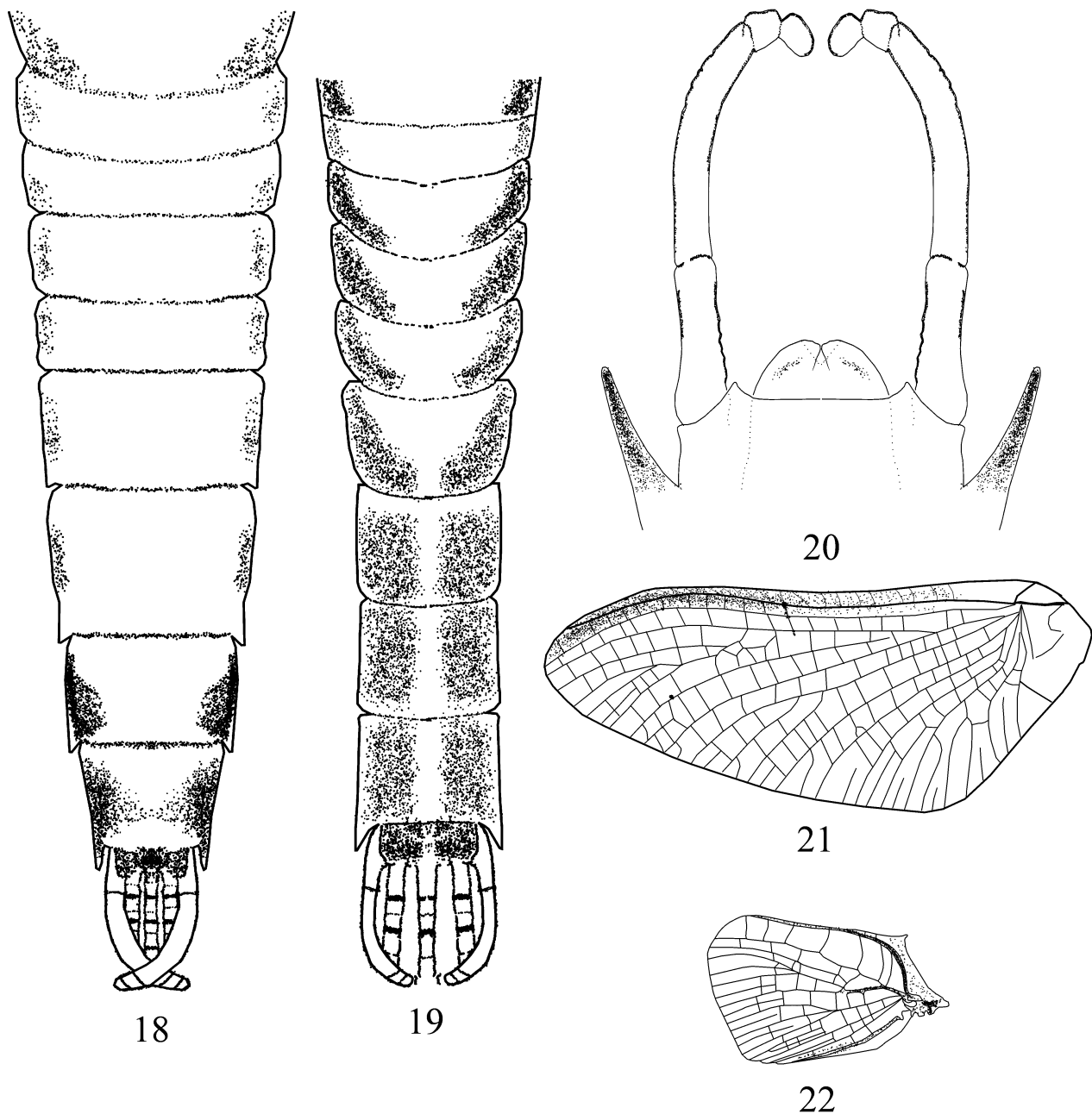
FIGURE 1. *Neophemera eatoni* sp. nov. Dorsal habitus of nymph.



FIGURES 2–8. *Neophemera eatoni* sp. nov., nymph: 2, Labrum, dorsal face; 3–4, mandibles; 3, right mandible, dorsal; 4, left mandible, dorsal; 5, right maxilla; 6, labium, ventral view; 7, setae on glossa, scale bar = 36 μ m; 8, hypopharynx.



FIGURES 9–17. *Neoephemera* species, nymphs: 9, *N. eatoni* sp. nov. hindleg, dorsolateral view; 10, short bifurcate plumose setae on dorsolateral surface of *N. eatoni* sp. nov. femora, scale bar = 40 μ m; 11, *N. youngi* hindleg, dorsolateral view; 12, bifurcate plumose setae on dorsolateral surface of *N. youngi* femora, scale bar = 40 μ m; 13–17, *Neoephemera* nymphs thoracic nota, dorsal view; 13, *N. purpurea*; 14, *N. eatoni* sp. nov.; 15, *N. youngi*; 16, *N. compressa*; 17, *N. bicolor*.

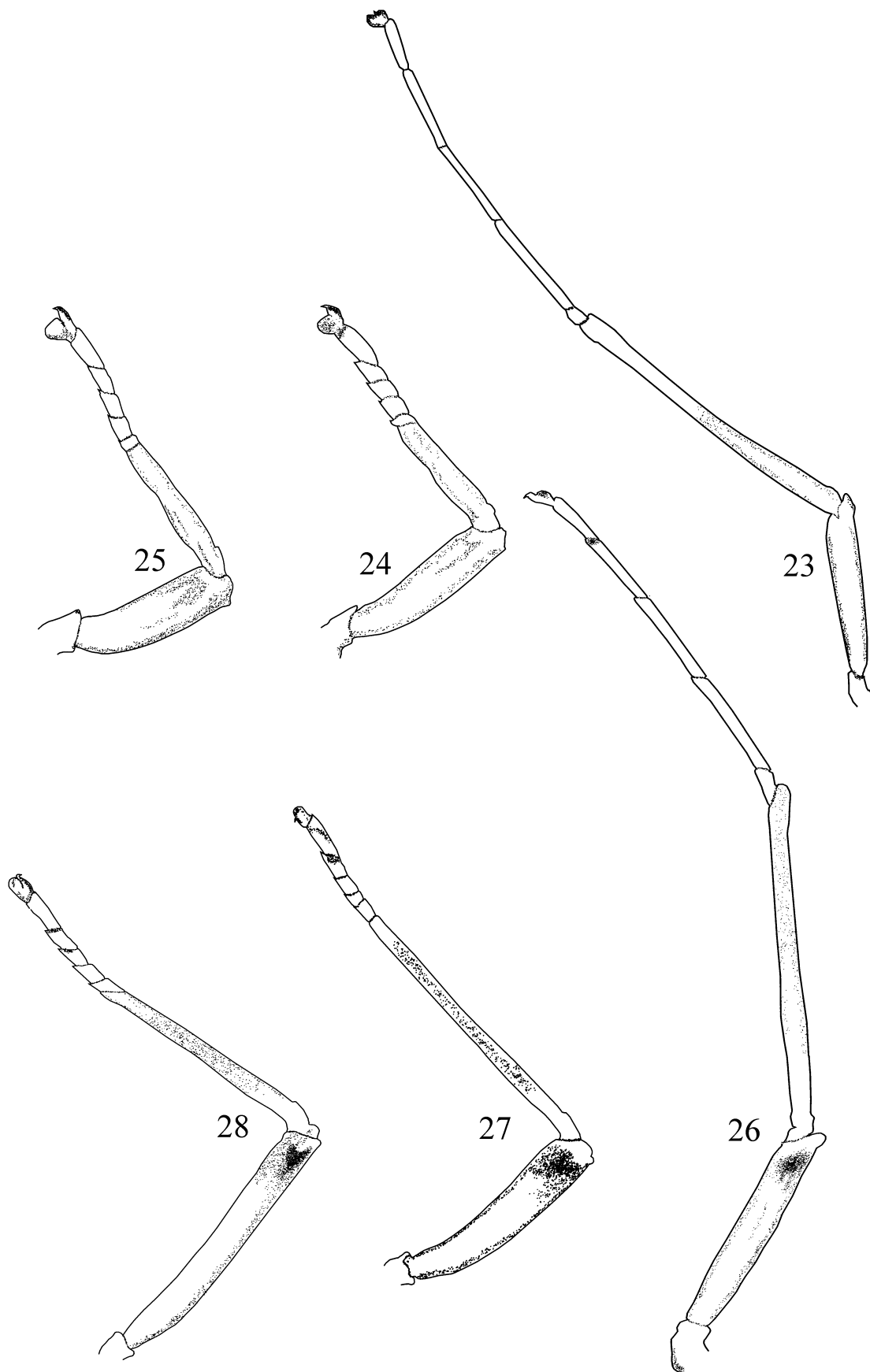


FIGURES 18–22. *Neoephemera eatoni* sp. nov. and *N. youngi*, male imago: 18, *N. eatoni* sp. nov. dorsal habitus; 19, *N. youngi* dorsal habitus; 20–22, *N. eatoni* sp. nov.: 20, genitalia; 21, forewing; 22, hindwing.

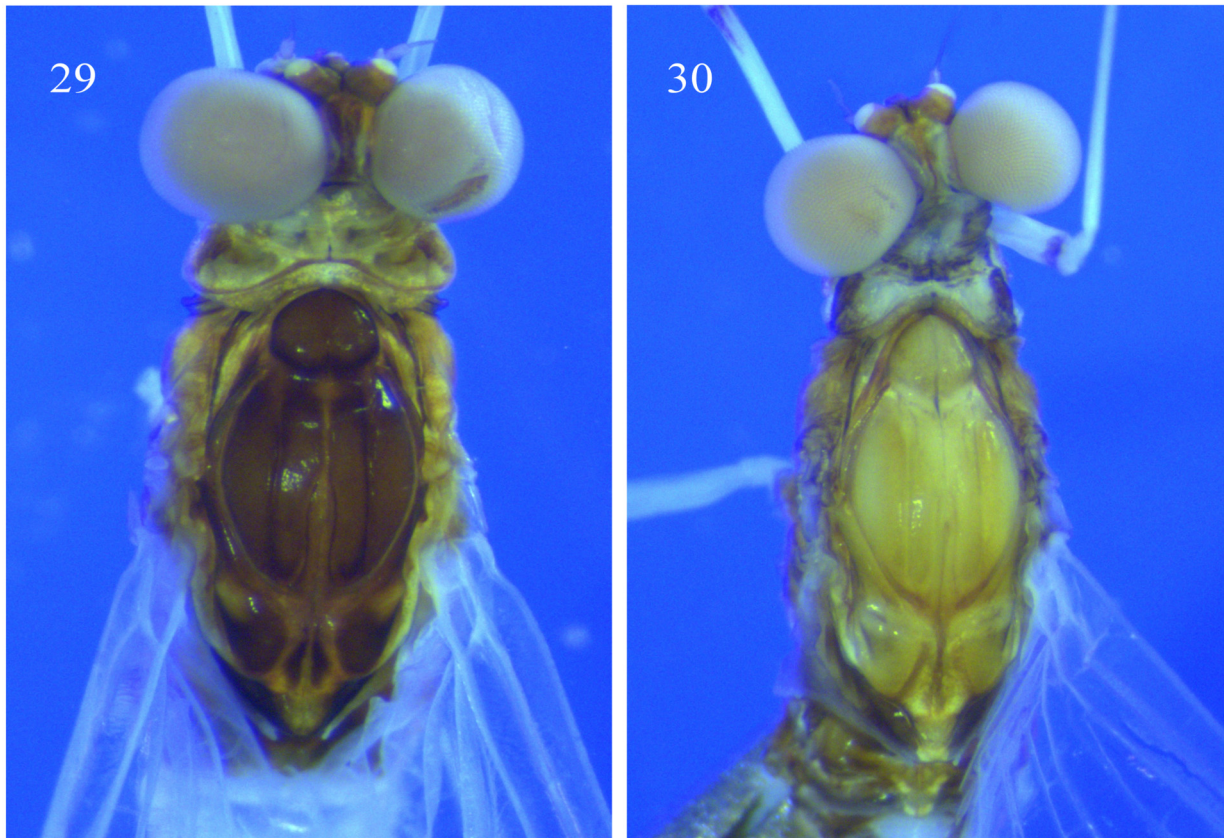
with full row of setae; tibiae and tarsi with scattered, apically fringed setae; dorsolateral surfaces of tarsi with rows of bifurcate, apically fringed setae. Thoracic sterna speckled yellow to brown, with blunt medial protuberances between fore and midcoxae.

Abdomen: Speckled yellowish brown to dark brown (Fig. 1); terga I & II with distinct dorsomedial projections; terga with scattered bifurcate plumose setae on surfaces, with setae densely distributed along margins; well-developed apically pale posterolateral projections present on terga III–IX, with largest projection on tergum IX; terga VI–VII each with large, pale posteromedial spot (Fig. 1). Terga VI–VIII with rudimentary posteromedial dorsal projections. Sternal coloration similar to terga, with distinct pale spots anteromedially on segments II–VIII. Terminal filament subequal in length to cerci, with whorls of spines at junction of each segment; intercalary setae longer, increasing in density from mid-length to apex.

Male imago (preserved in 95% ethanol). Dimensions: Body length 10.0–10.5 mm; terminal filament and cerci lengths 10.1–10.5 mm.



FIGURES 23–28. *Neophemera eatoni* sp. nov. and *N. youngi*, male imago: 23–25, *N. eatoni* sp. nov.: 23, foreleg; 24, midleg; 25, hindleg; 26–28, *N. youngi*: 26, foreleg; 27, midleg; 28, hindleg.



FIGURES 29–30. *Neophemera eatoni* sp. nov. and *N. youngi*, dorsal view of male imagos: 29, *N. eatoni* sp. nov. anterodal protuberance on the mesothorax, 30, *N. youngi* anterodal protuberance on the mesothorax.

Head: Compound eyes large, dorsal diameters 1.0 mm; separated by 0.25× width of one compound eye; yellow to light brown with purple outer margins. Vertex of head dark brown, with yellow paralleling eye margins. Ocelli large and yellow, surrounded by dark brown band at base, meeting anterolateral margins of compound eyes (Fig. 29); antenna pale to transparent, but with scape and pedicel white.

Thorax: Yellow to dark brown. Pronotum yellow and light brown medially; shaded to dark brown along posterior and lateral margins; emarginate posterior margin. Mesonotum lustrous dark brown with obovate anterodal protuberance (Fig. 29); scutum dark brown; median notal suture and sutural ommatium light brown; anterior parapsidal suture dark brown and posterior parapsidal suture yellow; scutellum yellow with two large dark brown ovate lateral spots and two smaller median triangular spots. Sterna yellow to dark brown; episternum dark brown; basisternum yellow to light brown; furcisternum dark brown with yellow median invagination. Metathorax dark brown laterally and yellow to light brown on remaining surface. Legs white, all femora with indistinct band at apex (Figs. 23–25); length ratios of foreleg femur: tibia and tarsal segment (TS) I: TS II: TS III: TS IV: TS V: claw = 1.0: 1.73 and 1.0: 8.0: 6.5: 6.5: 3.0: 1.5; length ratio of midleg femur: tibia: tarsus = 1.0: 0.76: 0.62; and length ratios of hindleg femur: tibia: tarsus = 1.0: 0.86: 0.57; all inner claws obtuse, and all outer claws pointed. Forewing length 9.2 mm and width 3.8 mm; hindwing length 2.5 mm and width 1.9 mm. Forewing hyaline with all longitudinal veins and crossveins pale; area between costal veins and border clouded, but with no distinctive markings on remainder of wing; single crossvein connecting A_1 to hind margin (Fig. 21); A_1 variable with one or two veinlets basally between CuP and A_1 ; hindwing with acute costal projection basally (Fig. 22).

Abdomen: Segments VI–IX with bluntly pointed posterolateral projections directed ventrally. Terga I–VII translucent to white; terga VIII–X brown laterally; tergum X nearly all brown (Fig. 18). Small median spine on posteromedial margin of tergum II. Sterna I–VII translucent to white with distinct anteromedial purple spots; sterna VIII–IX white; sternum X mostly brown. Genitalia pale (Fig. 20); penes furcate with median ventral apical v-shaped notch, not separated beyond subgenital plate; forceps segments I–IV lengths ratio = 1.0: 2.0: 0.3: 0.3. Cerci and terminal filament annulated on alternating segments.

Female imago (preserved in 95% ethanol). Dimensions: Body length 9.2–11.0 mm; terminal filament and cerci lengths 10.0–10.5 mm.

Head: Brown; dorsal compound eye diameter 0.4 mm; compound eyes separated by distance of 1.0 mm; ocelli and antennae same as male.

Thorax: Yellow to brown; pronotum brown with posterior margin indented; mesonotum yellow along scutal sutures and sutural ommatium, brown otherwise as in male; anteronotal protuberance same as male; sternum same as male but lighter brown. Foreleg length ratios of femur: tibia: tarsus = 1.0: 0.92: 0.85; midleg length ratios of femur: tibia: tarsus = 1.0: 0.85: 0.69; hindleg femur: tibia: tarsus length ratios = 1.0: 0.8: 0.57. All legs pale, with indistinct apical maculations on femora. Forewing length 10.2 mm and width 3.9 mm; hindwing length 2.7 mm and width 2.0 mm; wings with longitudinal veins and crossveins same as male (Figs. 21–22). Metathorax light brown.

Abdomen: Mostly brown speckled with light yellow spots dorsally; terga I–III light brown; terga IV–X with dark brown pigmentation increasing posteriorly; distinct longitudinal or lateral maculations absent. Sterna yellow with distinct purple spots posteromedially on segments I–VII and anterolaterally on segments II–VIII. Cerci and terminal filament annulated on alternating segments.

Egg. Length 180–190 μm ; width 110–115 μm . Oval, rugose and without polar caps (Figs. 31–32); surface densely covered with fine threads, forming fibrous adhesive layer with numerous minute bumps, parallel ridges, and minute mushroom-shaped fibers (MF) (Fig. 34), together terminating in blunt finger-like projections (FP) scattered over surface of egg (Figs. 31–34). One or two tageniform micropyles (TM) along equatorial region, with sperm guides 30–36 μm long and 17 μm wide.

Material examined. Type material. Holotype: ♂ imago (reared), associated exuviae, North Carolina, Rockingham Co., Dan River, NC 700, 36.4985, -79.6815, 16/V/2015, V.B. Holland [NMNH]. Paratypes: One ♂ imago (reared), associated exuviae, ♀ imago (reared), associated exuviae, same data as holotype [PERC]; one nymph (final instar), North Carolina, Dan River, off SR 1779, 36.48488, -79.7172, 9/IV/2015, V.B. Holland, M.D. Walters, L.E. Eaton [CSUC]; one nymph (final instar), same data [NCDWR]; one nymph (final instar), North Carolina, Mayo River, SR 1358, 36.53552, -79.9906, 1/V/2012, D.R. Lenat [NCDWR].

Additional material. Two nymphs, Virginia, Craig Co., Sinking Creek, 15/V/1974, J. Wright [CSUC]; two nymphs, Virginia, Grayson Co., New River, 6/VI/1973, J. Wright [CSUC]; nine nymphs, North Carolina, Rockingham Co., Dan River, off SR 1779, 36.48488, -79.7172, 28/X/2014, V.B. Holland, E.D. Fleek, M.D. Walters, L.E. Eaton, R.W. Smith, M.J. Stillwell [NCDWR]; seven nymphs, ♀ imago (reared), North Carolina, Dan River, off SR 1779, 36.48488, -79.7172, 9/IV/2015, V.B. Holland, M.D. Walters, L.E. Eaton [NCDWR]; three nymphs, ♂ imago (reared), ♀ imago (reared), ♂ subimago (reared), ♀ subimago (reared), North Carolina, Dan River, off SR 1779, 36.48488, -79.7172, 7/V/2015, V.B. Holland [NCDWR]; one nymph, ♂ imago (reared), two ♀ imagos (reared), ♂ subimago (reared), Rockingham Co., North Carolina, Dan River, NC 700, 36.4985, -79.6815, 16/V/2015, V.B. Holland [NCDWR]; one nymph, Rockingham Co., North Carolina, Mayo River, NC 770, 36.47083, -79.9514, 30/III/1989, L.E. Eaton, D.R. Lenat, M. Wicker [NCDWR]; three nymphs, Rockingham Co., North Carolina, Mayo River, SR 1358, 36.53552, -79.9906, 18/V/2015, S.R. Beaty, V.B. Holland, M.J. Stillwell [NCDWR].

Etymology. We are honored to name this species after Larry E. Eaton (North Carolina Division of Water Resources) in recognition of his long-term contributions to the study of aquatic macroinvertebrates as indicators of water pollution. He was one of the first biologists to observe and collect this species in its unique habitat.

Diagnosis. *Neoephemera eatoni* nymphs have been collected together with *N. youngi*. *Neoephemera eatoni* nymphs and imagos will key to *N. youngi* in Berner (1956) and Bae & McCafferty (1998) with the exception of imagos lacking distinct bands apically on femora and the absence of purplish-gray banding on tarsal segments II–IV presented in couplet 2 of Berner (1956). However, *N. eatoni* can be distinguished easily from all described North American *Neoephemera* species by the following combination of characters. In the **nymph**: (1) short robust legs; lengths of forelegs, midlegs, and hindlegs are subequal to each other and tibial to tarsal length ratios are subequal on all legs (Figs 1 & 9); (2) tarsal claws sharply curved (Fig. 9); (3) anterosubmedian tubercles absent on the pronotum; (4) rudimentary pronotal anterolateral projections and broadly rounded reduced mesonotal anterolateral expansions (Figs 1 & 14).

In addition to the above characters, **nymphal setae** characters separating *N. eatoni* from *N. youngi* include: (1) shorter bifurcate plumose setae (Figs 9–12) in a broad longitudinal row along medial dorsolateral surface on all femora (Figs 9–10); (2) denser plumose setae on the first segment of the labial palps (Fig. 6); (3) a constricted

arched row of 60 or more hispid setae situated dorsally 100 μm distal to the apicomedial labral notch margin (Fig. 2). In *N. youngi*, this arched row of setae is less constricted and situated 50 μm distal to the apicomedial labral notch margin.

In the **imago**, this species shares the presence of annulated caudal filaments with *N. youngi*; however, it is easily distinguished by the following characters: (1) unique dorsal maculation in males (Fig. 18) and females; (2) males with fore tibia at least 2 \times the length of mid and hind tibiae (Figs 23–25), female leg lengths are subequal to each other; (3) absence of distinct leg banding; (4) obovate anterotal protuberance on the mesothorax (Fig. 29).

In the **eggs**, this species is separated from *N. youngi* by the presence of apically blunt finger-like projections rather than mushroom-shaped projections (Figs 31–36).

Discussion

Systematics. With the description of *N. eatoni*, there are currently five extant *Neoephemera* species known in North America and seven extant species known worldwide. Both nymphs, imagos, and eggs are sufficiently distinct from congeners to recognize this new species. Preliminary DNA barcoding data also support the species as a new and distinct phylogenetic taxon (data for “*Neoephemera* sp. NC vbh” available from <http://www.boldsystems.org>). It may be a sibling species of *N. youngi* based on the following shared characters that are not found in other North American *Neoephemera* imagos (Berner 1953, Bae & McCafferty 1998): (1) the presence of a small spine posteromedially on abdominal terga II and (2) annulated terminal filaments. However, the short robust legs, sharply curved claws, and reduced anterolateral pronotal and mesonotal projections in the nymphs and dorsal maculations in imagos are unique among North American *Neoephemera*. In addition to the aforementioned characters, nymphal chaetal differences, tibial and leg length ratios in nymphs and imagos, and the anterotal protuberance differences easily separate *N. youngi* and *N. eatoni* imagos. A more comprehensive, worldwide review of all *Neoephemera* species is needed, however, to confirm this sibling species hypothesis.

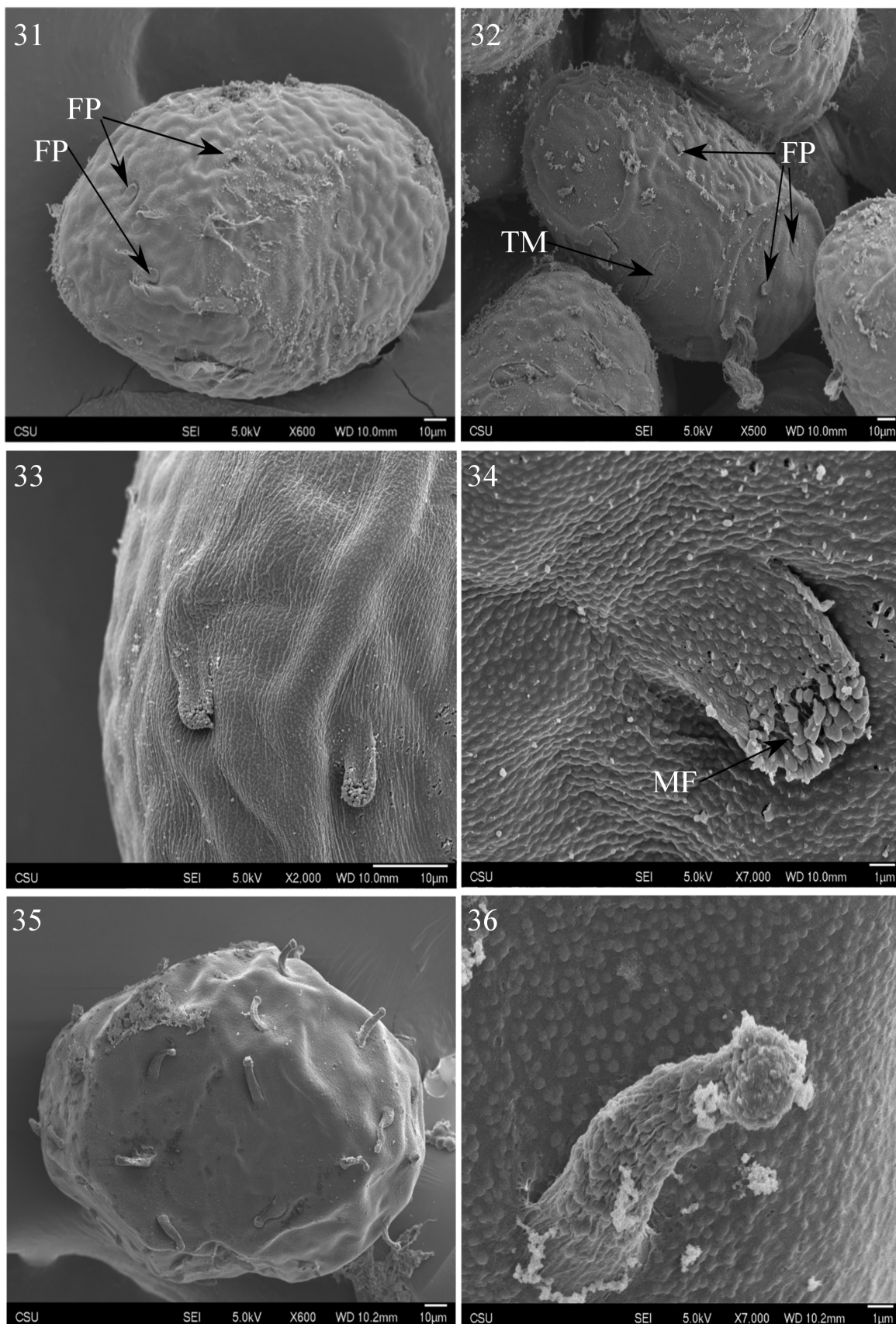
Ecology. *Neoephemera eatoni* has only been collected from hornleaf riverweed (*P. ceratophyllum*) growing on the surfaces of cobbles and boulders in moderate to swift flows, at a depth of up to one meter. These data suggest that the nymphs are specifically associated with this plant species. Other mayfly nymphs associated with *P. ceratophyllum* at the study sites were the Ephemerellidae species *Drunella tuberculata* (Morgan, 1911), *D. walkeri* (Eaton, 1884), *Teloganopsis deficiens* (Morgan, 1911), and *Tsalia bernerii* (Allen & Edmunds, 1958). The stocky morphology of the new species is consistent generally with other mayfly species that demonstrate at least partial affinity with this habitat (Jacobus 2013).

In the Mayo River, *N. youngi* nymphs were collected with dip nets approximately 30 meters away from *P. ceratophyllum* habitat and were clearly associated with riparian zone root mats, demonstrating potential habitat partitioning within the genus. This root mat habitat preference of *N. youngi* has been well documented (Berner & Pescador 1988; Bae & McCafferty 1998).

All nymphs in this study were collected from large rivers with drainage areas > 518 km². The Mayo and Dan Rivers are known for their outstanding water quality and exceedingly high aquatic macroinvertebrate richness (NCDWR, unpublished). Approximately 290 separate benthic macroinvertebrate taxa and 140 unique Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa have been recorded from the Mayo River in North Carolina since 1986. In addition, a new *Isoperla* Banks, 1906 stonefly species (Plecoptera: Perlodidae) was discovered in this river during the progression of this study, and we anticipate that it will be described in a subsequent publication. Similar to the Mayo River, approximately 320 benthic macroinvertebrate species and 125 EPT taxa have been recorded from North Carolina Dan River locations since 1984. Conservation of these unique and species-rich rivers in the transitional zones between the Appalachian Mountains and Piedmont region should be a priority, given that current and future increases in development pressures likely will impact negatively both water quality and the fauna supported by these waters.

Geographic distribution. We have examined specimens from north-central North Carolina and the mountains of Virginia.

Laboratory observations of behavior. Nymphal emergence in artificial streams occurred with water temperatures ranging from 22–26 °C. Subimago to imago molting times ranged from 12–16 hours at temperatures ranging from 18–24 °C.



FIGURES 31–36. *Neophemera eatoni* sp. nov. and *Neophemera youngi*, eggs: 31–32, typical outline of the egg (TM represents tageniform micropyle), scale bar = 10 μ m; 33–34, finger-like projections (FP) and minute mushroom-shaped fibers (MF), scale bars = 10 μ m and 1 μ m, respectively; 35, typical outline of *Neophemera youngi* egg, scale bar 10 μ m; 36, closeup shape of *N. youngi* FP.

Eggs. Few studies have been conducted analyzing egg morphology of the genus *Neoephemera* with Scanning Electron Microscopy (SEM) techniques. Bae & McCafferty (1998) report a general description of Neoephemeridae eggs and provide figures of *N. purpurea* and *N. youngi* eggs. Klonowska-Olejniki & Jazdzewska (2003) provide SEM photography of *N. maxima* eggs with a description of the finger-like projections found on the chorionic surfaces. Bauernfeind & Soldán (2012: fig. 368.1) also showed *N. youngi*. The finger-like projections observed on *N. eatoni* eggs (Figs. 31–34) are a unique shape compared to the projections reported for *N. youngi* (Figs. 35–36, herein; Bae & McCafferty 1998: figs. 31 & 33). Finger-like projections in *N. eatoni* are apically blunt, distinctly different than the mushroom-shaped projections in *N. youngi*. Further SEM studies are needed to decipher the morphological differences between various *Neoephemera* species eggs, especially with regard to unique attachment structures, and could have important phylogenetic, taxonomic and biological implications.

Notable morphological variability. All *N. eatoni* nymphs examined are copiously speckled with cream to dark brown spots, but the density of speckles varies among nymphs. Later instars are speckled darker brown than earlier instars. Based on materials examined, size of final instars appears sexually dimorphic with male nymph lengths 8.1–10.0 mm, and female lengths usually 10.0 mm or greater. The darkness of the brown dorsolateral maculation in male imagos varies on abdominal segments II–VII (Fig. 18); however, in reared specimens of *N. eatoni* that we examined, these maculations do not converge inward along the posterior border of the terga to near the median line, creating a striped appearance (Fig. 19) as in the original description of *N. youngi* (Berner 1953).

Wing venation was examined extensively, and venation patterns were similar to *N. bicolor*, *N. compressa*, and *N. youngi*. In all *N. eatoni* specimens examined, a single crossvein connecting A_1 to the hind margin of the forewing (Fig. 21) was present, however veinlets of A_1 were variable with one or two veinlets basally between CuP and A_1 and intercalaries between CuA and A_1 varied in lengths.

Key to final instar nymphs of North American *Neoephemera* species

(modified from Bae & McCafferty 1998 and Berner 1956)

- 1 All legs with lengths subequal to each other (Fig. 1); tarsal claws sharply curved (Fig. 9); anterolateral projections rudimentary and anterosubmedian tubercles absent on pronotum (Figs 1 & 14) USA: North Carolina, Virginia *N. eatoni*
- Legs increase in length from pronotum to metanotum; tarsal claws gradually curved (Fig. 11); pronotum and mesonotum with moderate or well developed anterolateral projections (Figs 13 & 15–17) 2
- 2(1) Distinct, well developed pronotal anterolateral projections present (Fig. 13). Body 14–17 mm, eastern Nearctic USA: Georgia, North Carolina, South Carolina, Tennessee, Virginia, West Virginia *N. purpurea*
- Moderately developed anterolateral projections present on pronotum and mesonotum (Figs 15–17). Body 8–11 mm 3
- 3(2) Small distinct anterosubmedian tubercles present on pronotum (Fig. 15). Rounded anterolateral projections present on mesonotum. Rudimentary rounded projections present posteromedially on abdominal terga VI–VIII, eastern Nearctic USA; Alabama, Florida, Georgia, North and South Carolina. *N. youngi*
- Pronotum with large, distinct anterosubmedian tubercles (Figs 16–17). Mesonotum with distinct pointed anterolateral expansions; distinct tubercles present posteromedially on abdominal terga VI–VIII 4
- 4(3) Anterosubmedian pronotal tubercles approximate and apically parallel to convergent (Fig. 16) USA: Florida and Georgia *N. compressa*
- Anterosubmedian pronotal tubercles well separated and apically divergent (Fig. 17) Canada: Quebec; USA: Indiana, Michigan (Bae & McCafferty 1998), Wisconsin (Schmude *et al.* 2012), and Virginia (CSU, see Additional Materials Examined) *N. bicolor*

Key to imagos of North American *Neoephemera* species

(modified from Bae & McCafferty, 1998)

- 1 Forewing longitudinal veins purplish black. Body length > 13 mm. *N. purpurea*
- Forewing longitudinal veins pale. Body length 7–10.5 mm 2
- 2(1) Caudal filaments annulated on alternating segments 3
- Caudal filaments not annulated. 4
- 3(2) Male dorsal maculations as in Fig. 18; male foretibia at least 2× as long as mid- and hind- tibia and all female leg lengths subequal (Figs 23–25); legs white with apical banding on femora faint or absent; obovate anterotal protuberance on the mesothorax (Fig. 29) *N. eatoni*

- Male dorsal maculations as in Fig. 19; male fore tibia < 2× the length of mid and hind tibia (Figs 26–28); females with hind legs longer than mid legs; legs smoky with distinct purplish black apical banding on femora; anteronotal protuberance a curvilinear triangle (Fig. 30) *N. youngi*
- 4(2) Forefemora colored with purplish grey markings. Tarsi annulated. *N. compressa*
- Forefemora pale. Tarsal annulation absent. *N. bicolor*

Additional material examined

Neoephemera bicolor.—One nymph, ♀ imago (reared), Wisconsin, Ashland Co., White River, Hwy 13, 23/VI/2009, K.L. Schmude, University of Wisconsin-Superior; Virginia, Carroll Co., New River, RT 721, junction RT 606, 5/VI/1981, B.C. Kondratieff, exuvia, det. V.B. Holland & S.R. Beaty [CSUC].

Neoephemera compressa.—Five nymphs, Florida, Okaloosa Co., Yellow River, Below Griffith Ferry Stretch, 30.76956, -86.6216, 10/II/2015, R. Abad, M. King, Florida Department of Environmental Protection.

Neoephemera purpurea.—One ♂ imago, Virginia, Floyd Co., Little River, RT 615, 21/V/1979, B.C. Kondratieff, [CSUC]; five nymphs, North Carolina, Polk Co., North Pacolet River, near SR 1179, 35.22408, -82.2707, 29/VI/2015, M.D. Walters, E.D. Fleek, V.B. Holland, W.M. Turner [NCDWR]; one nymph, North Carolina, Swain Co., Deep Creek, GSMNP, 35.47295, -83.43, 30/V/2003, R.E. DeWalt [CSUC]; Two ♂ imagos, two ♀ imagos, North Carolina, Wilkes Co., Middle Fork Reddings River, Old NC 16, 36.26189, -81.3007, 29/V/2006, B.C. Kondratieff, R.F. Kirchner, R.E. Zuellig, D.R. Lenat [CSUC]; one nymph, North Carolina, Wilkes Co., North Prong Lewis Fork, SR 1300, 36.21309, -81.3581, 7/V/2010, V.B. Holland, M.D. Walters, T.L. Morman [NCDWR].

Neoephemera youngi.—Twelve nymphs, North Carolina, Duplin Co., Northeast Cape Fear River, NC 41, 34.82764, -77.8324, 26/VIII/1989, L.E. Eaton, S.R. Mitchell [NCDWR]; one nymph, North Carolina, Franklin Co., Sandy Creek, NC 561, 36.16472, -78.1617, 23/IV/2003, E.D. Fleek, T.F. McPherson, W.B. Crouch [NCDWR]; three nymphs, North Carolina, Henderson Co., Mills River, SR 1353, 35.38778, -82.5461, 23/IV/2012, T.L. Morman, V.B. Holland, E.D. Fleek [NCDWR]; one nymph, North Carolina, Henderson Co., South Fork Mills River, SR 1338, 35.38851, -82.6036, 25/IV/2012, V.B. Holland, T.L. Morman, E.D. Fleek [NCDWR]; one nymph, North Carolina, Nash Co., Swift Creek, SR 1310, 36.11167, -77.9211, 5/III/1996, D.R. Lenat, L.E. Eaton, D.R. Penrose [NCDWR]; one nymph, North Carolina, Nash Co., Swift Creek, SR 1310, 36.11167, -77.9211, 24/IV/2003, E.D. Fleek, T.F. McPherson, W.B. Crouch [NCDWR]; four nymphs, North Carolina, Richmond Co., Naked Creek, SR 1003, 35.08194, -79.5903, 9/V/1990, D.R. Lenat [NCDWR]; one ♂ imago (reared), two ♀ subimagos (reared), North Carolina, Rockingham Co., Mayo River, SR 1358, 36.53552, -79.9906, 11/V/2015, V.B. Holland, S.R. Beaty, M.J. Stillwell [NCDWR]; five nymphs, ♀ imago (reared), North Carolina, Rockingham Co., Mayo River, SR 1358, 36.53552, -79.9906, 16/V/2015, V.B. Holland [NCDWR]; one nymph, North Carolina, Rockingham Co., Mayo River, SR 1358, 36.53552, -79.9906, 21/V/2015, V.B. Holland [NCDWR]; five nymphs, North Carolina, Rutherford Co., Second Broad River, Above Union Mills, 35.47917, -81.9442, 19/V/1995, K. Herring, T.F. McPherson, D.R. Lenat [NCDWR]; one nymph, North Carolina, Stokes Co., Crooked Creek, off SR 1626, 36.53566, -80.0767, 1/XI/2011, V.B. Holland, S.R. Beaty, M.D. Walters, M.E. Simonson [NCDWR]; one ♂ imago, Georgia, Bibb Co., Ekeconnee Creek, 11 miles South of Macon, Georgia, 5/V/1939, P.W. Fattio, Illinois Natural History Survey; one nymph North Carolina, Harnett/Cumberland Co., Little River, Elliot Bridge Road northeast of Fayetteville, 35.23198, -78.87857, 4/V/2005, B.C. Kondratieff, R.F. Kirchner, R.E. Zuellig, D.R. Lenat [CSUC]; one ♂ imago, Florida, Gadsden Co., Rocky Comfort Creek, on dirt road at bridge, 6 miles South State Hwy. 268, 2/IV/1970, J. Jones, det. W.L. Peters [PERC].

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