



ISOPERLA ARCANA* AND *ISOPERLA BORISI* (PLECOPTERA: PERLODIDAE), TWO NEW STONEFLY SPECIES FROM NORTH CAROLINA, U.S.A. WITH NOTES ON THE DISTRIBUTION OF *ISOPERLA POWHATAN

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ABSTRACT

Two new perlodid stonefly species, *Isoperla arcana* Beaty, Holland, & Lenat, 2017 and *I. borisi* Beaty, Holland, & Lenat, 2017, are described from North Carolina, U.S.A. Associated images, scanning electron micrographs, and illustrations of reared adult males, females, larvae, and eggs are presented for each species. In addition, a map is included depicting the North Carolina distributions of each species. Adult males of the recently described *I. powhatan* Szczytko and Kondratieff, 2015 were also reared from larvae collected from a North Carolina stream. This represents a range extension for *I. powhatan*, previously known only from Pennsylvania and Virginia. Including *Isoperla borisi* sp. nov., *I. arcana* sp. nov. and the range extension of *I. powhatan*, thirty *Isoperla* species are now known from North Carolina.

Keywords: *Isoperla arcana* Beaty, Holland, & Lenat, 2017; *Isoperla borisi* Beaty, Holland, & Lenat, 2017; North Carolina; Slate Belt; eastern Nearctic; Southeast; North Carolina Biotic Index; *Fontinalis*

INTRODUCTION

Identification of adult eastern Nearctic species of *Isoperla* Banks, 1906 has historically relied on several disjunct regional keys authored by Frison (1935, 1942), Hitchcock (1974), Stark and Gaufin (1978), and Poulton & Stewart (1991). This has made species determinations of *Isoperla* difficult due to the non-overlapping geographical nature of the treatments. Identification of southeastern U.S.A. species of *Isoperla*, in both the adult and larval stage, have typically been attempted using

Hitchcock (1974), Stark and Gaufin (1978), and Unzicker and McCaskill (1982). Recently, Stark (2017) has published a new regional key for larvae known from the southeastern United States. However, there are still many species whose larvae are unknown, undescribed, or were described after the above treatments (see James 1974, Nelson 1976, Nelson & Kondratieff 1983, Grubbs & Szczytko 2010, Verdone & Kondratieff 2016).

A recent revision of the Isoperlinae by Szczytko & Kondratieff (2015a) provided the first

comprehensive treatment of all known *Isoperla* occurring in eastern North America. This extensively illustrated monograph and its companion photographic atlas, Szczytko & Kondratieff (2015b), recognizes 60 *Isoperla* species as occurring in eastern North America. Previously 41 eastern Nearctic species of *Isoperla* were known (Szczytko & Kondratieff 2015a). In addition to providing amended descriptions of the males, females, and eggs of all previously known species, Szczytko and Kondratieff (2015a) described 22 new species and proposed three new synonymies. They analyzed the taxonomic status of the 60 valid eastern North American *Isoperla* species based primarily on adult morphology and placed 53 species into 12 species groups, leaving seven species unassigned. However, as Szczytko and Kondratieff (2015a) noted, the phylogenetic relationships among many *Isoperla* species remain unclear as certain life stages, particularly the larvae, of numerous species are unknown or undescribed.

The *Isoperla* fauna of North Carolina presently includes 30 recognized species, 15 of which are newly described (Szczytko & Kondratieff 2015a, Verdone & Kondratieff 2017, DeWalt et al. 2017) and is easily the most species-rich stonefly genus in the state (Beaty 2015). Kondratieff et al. (1995) provided the first comprehensive list of *Isoperla* in North Carolina (Table 1), totaling only 14 species of *Isoperla* at that time. Four species recorded from North Carolina by Kondratieff et al. (1995), *Isoperla bilineata* (Say, 1823); *I. distincta* Nelson, 1976; *I. namata* Frison, 1942; and *I. similis* (Hagen, 1861), were not confirmed as valid North Carolina records by Szczytko & Kondratieff (2015a) likely caused by misidentification of larvae of cryptic species. In addition, Szczytko and Kondratieff (2015a) provide the first North Carolina published records of *I. montana* Banks, 1898, although these records may refer to either *I. kirchneri* Szczytko and Kondratieff, 2015 or *I. dewalti*, a species recently described from the southern Appalachians by Verdone and Kondratieff (2017).

During the course of rearing and associating larval stoneflies in North Carolina, the authors discovered two previously unknown species of

Isoperla not treated in Szczytko & Kondratieff (2015a). Numerous larvae of these species, herein named *I. arcana* sp. nov. and *I. borisi* sp. nov., have been collected over the last 30 years by North Carolina's Biological Assessment Branch of the Division of Water Resources during routine water quality bioassessments in headwater streams of the Appalachian Mountains and streams of the Carolina Slate Belt (Griffith et al. 2002), respectively. At least one more undescribed species of *Isoperla* is known to occur in North Carolina but requires additional material before a formal description can be completed.

Finally, numerous adult male specimens of the recently described species, *Isoperla powhatan* Szczytko & Kondratieff, 2015 were reared from a single North Carolina piedmont stream and represent a significant southern range extension for this species.

MATERIALS AND METHODS

As part of a larger ecological study, unidentified *Isoperla* larvae were collected during the springs of 2012–2016 from various Appalachian mountain streams as well as both Collins Creek, a small North Carolina Slate Belt stream and Barnes Creek, a mid-order slate belt stream. Supplementary trips were made during late April and early May of 2015 and 2016 to collect additional adult *Isoperla* specimens.

Adult *Isoperla* were collected by beating sheet in riparian vegetation while pre-emergent *Isoperla* larvae were collected from in-stream habitat using dip nets and bucket sieves. Larvae were sorted streamside from moss and detritus using modified plastic pipettes and were placed in an aerated cooler containing ice for transport back to the laboratory. Concurrent with larval collection, approximately 50 liters of stream water, stream conditioned leaves, and gravel were collected for use in artificial flumes during rearing. At the laboratory, live pre-emergent *Isoperla* larvae were sorted out for rearing with the remaining larvae preserved in 80% ethanol for later examination. Along with a small leaf pack cleaned of resident competitors, up to 3 pre-emergent larvae were placed into a small plastic cup with screened sides



Fig. 1. Artificial stream used in rearing immature aquatic insects in the laboratory.

and enclosed with a tented screen top. Stream gravel was used to weigh down the leaf substrate and provide refuge. Chambers were subsequently floated on the water surface of a small artificial stream leaving the screened tented area of the rearing cup above the surface of the water for the emergent adult.

The artificial stream (Fig. 1) was constructed of a modified 55 liter cooler with a central Plexiglas™ divider and two internal pumps to provide circulation. Water temperature, controlled and maintained by an attached water chiller (Aqua Euro US Max Chill), was set to simulate field surface water temperatures. Room lighting was controlled by an integrated automatic timer to provide a natural diurnal cycle in an approximate 14:10 hour light: dark cycle. Successfully reared specimens were collected, along with the exuvia, and placed into separate containers in a cool, humid environment. Adults were allowed to feed for 24–48 hours on small pieces of apple used as a nectar substitute. Reared specimens were kept

alive for 48–72 hours after emergence to allow for complete hardening and darkening of the exoskeleton. Some males and females were placed together for mating in an attempt to yield mature ova.

Reared females were placed in 80% ethanol along with the associated exuvia while live males were prepared for aedeagal eversion. Eversion of the aedeagus was performed using a stereomicroscope to ensure complete and full extrusion. Upon successful eversion, specimens were immersed into near-boiling water to fix the aedeagus in place.

Preserved larvae and exuvia were examined at 20–40X and photographed using a Lumenera® Infinity 1–5C camera attached to a Leica® MZ6 stereomicroscope. The maxillae and legs from preserved larvae and exuvia were slide mounted using Hoyer's Solution as a clearing agent and mounting medium. The lacina and mandibles were examined using a phase contrast Nikon® Eclipse E 400 compound microscope and subsequently

photographed. Focus stacking software Zerene Stacker 1.04 was used to generate composite images of specimens and post-processing of the resulting stacked images was performed with Photoshop CS2. Line drawings were created in Photoshop CS2 and the open source software packages GIMP 2.82 and Inkscape 0.91. Terminology used in larval description follows that of Sandberg (2011).

Adult material and eggs were studied using a JEOL® JSM-6500F Field Emission Scanning Electron Microscope at Central Instrument Facility, Imaging Laboratory of Colorado State University. Specimens were critical point dried and sputter coated with 20 nm gold. In addition to SEM, the male aedeagus of one specimen was slide-mounted and the armature examined and photographed. Additional eggs were dissected from female specimens and slide mounted for surface microstructure analysis.

Archived historical aquatic macroinvertebrate samples up to 35 years old and collected by the North Carolina Division of Water Resources Biological Assessment Branch biologists were reexamined for *Isoperla* larvae similar to those collected and reared. Candidate specimens were examined using the same methods above. Additional new species records were used to generate distribution maps with ArcMap® 10.2 (NAD83 coordinate system). Watershed analysis, including drainage areas of the type localities, was performed using USGS StreamStats program 4.1.1 (U.S. Geological Survey 2016) and ecoregion information was provided by Griffith et al. (2002).

Specimens are deposited at the United States National Museum of Natural History, Washington, D.C. (NMNH), the C.P. Gillette Museum of Arthropod Diversity, Colorado State University, Fort Collins, Colorado, U.S.A. (CSUIC) and the North Carolina Division of Water Resources Biological Assessment Branch collections (NCDWR), Raleigh, North Carolina U.S.A.

RESULTS

Isoperla arcana sp. nov.

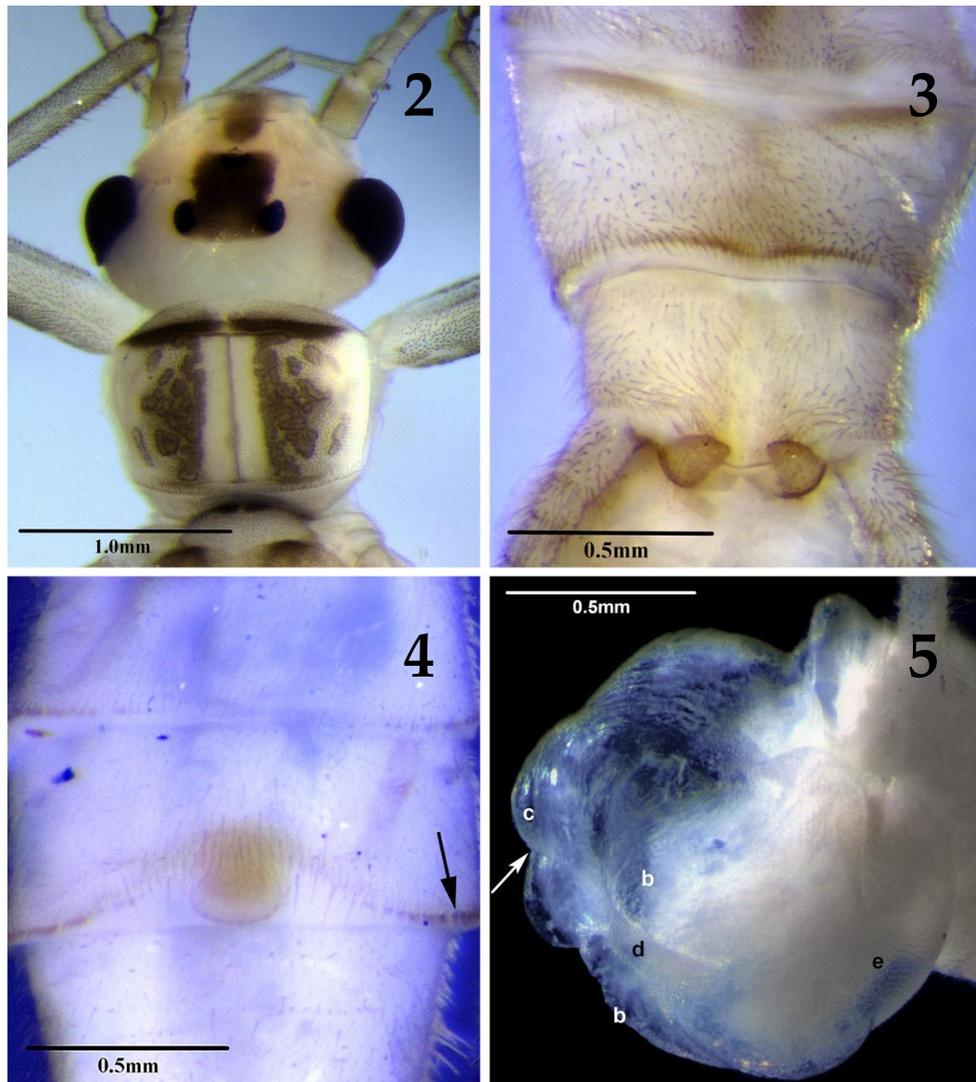
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Secret Stripetail

(Figs. 2–19)

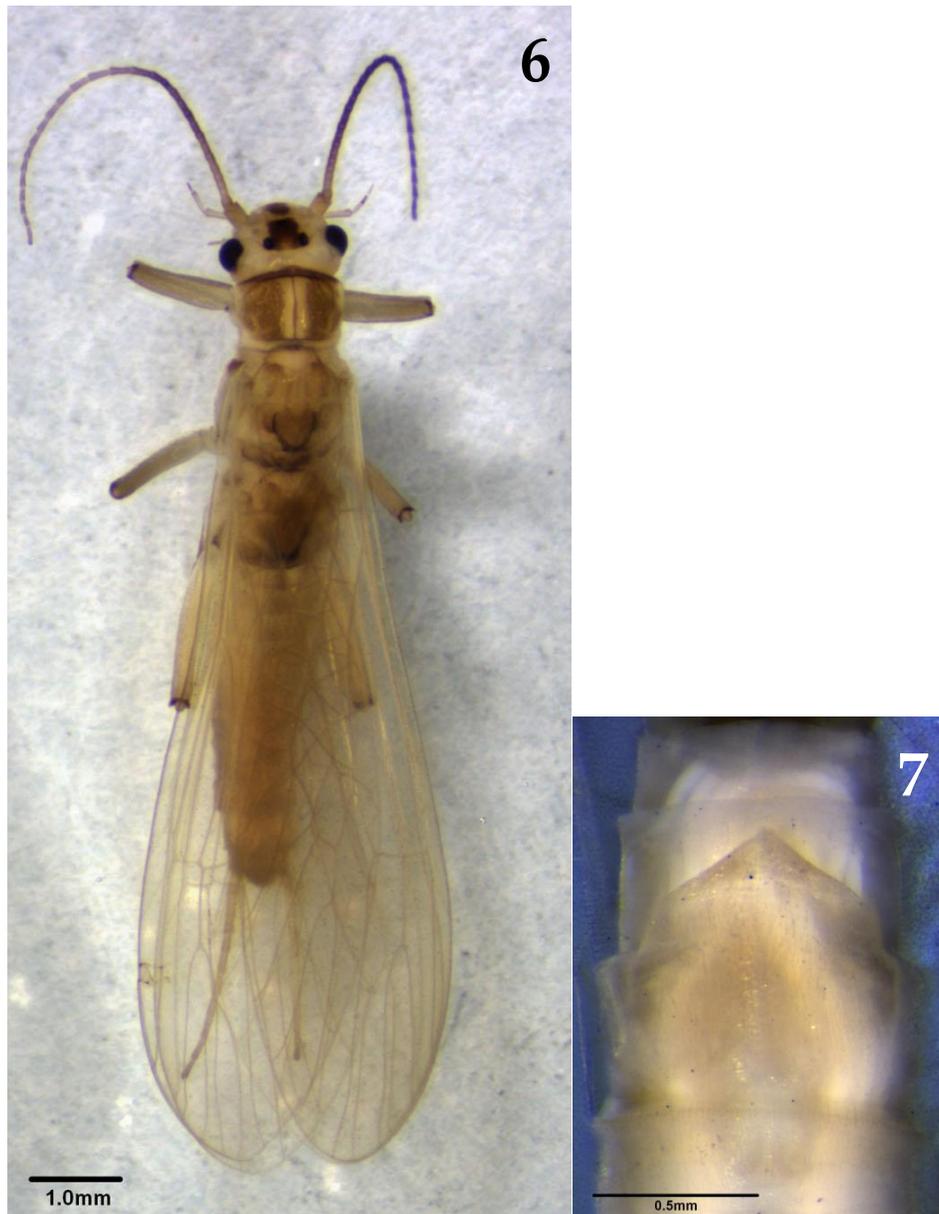
Material examined. USA – *Holotype* ♂ and larval exuvium, **NORTH CAROLINA, McDowell Co.**, Curtis Creek, Curtis Creek Rd, 35.71459, -82.19079, 28/IV/2016, S.R. Beaty, V.B. Holland, E.D. Fleek (NMNH). *Paratypes*: **Alleghany Co.**, unnamed tributary to Little River, SR 1418, 36.5348, -81.1065, 15/V/2013, S.R. Beaty, V.B. Holland, 6♀ (reared) and exuvia (NCDWR). **Avery Co.**, Elk Hollow Branch, off Roaring Creek Rd., 36.09947, -82.04547, 7/V/2014, V.B. Holland, E.D. Fleek, M.D. Walters, 1♂ (reared) and exuvium, 3♀ (reared) and exuvia, 1 larva (NCDWR); Little Wilson Creek, Edgemont Rd., 36.08316, -81.79131, 28/IV/2016, S.R. Beaty, V.B. Holland, E.D. Fleek, 1♂ (reared), 2♀ (reared) and exuvia (NCDWR); North Harper Creek, FSR 58, 36.00861, -81.85361, 9/V/2016, S.R. Beaty, V.B. Holland, E.D. Fleek, 1♂ (reared) and exuvium (NCDWR); Wilson Creek, US 221, 36.09694, -81.80777, 9/V/2016, S.R. Beaty, V.B. Holland, E.D. Fleek, 1♂ (reared) and exuvium (NCDWR). **Burke Co.**, Gingercake Creek, FSR 496, 35.92398, -81.87231, V.B. Holland, E.D. Fleek, M.D. Walters, 1♂ (reared) and exuvium (CSUIC). **Buncombe Co.**, Coles Cove Branch, off SR 2132, 35.73569, -82.49934, 8/V/2012, S.R. Beaty, M.D. Walters, D. Black, 2♀ (reared) and exuvia (NCDWR). **Haywood Co.**, unnamed tributary to Raccoon Creek, off Pippin Lane, coordinates unknown, 8/V/2012, S.R. Beaty, M.D. Walters, D. Black, 1♂ (reared) (NCDWR). **Macon Co.**, Robin Branch, Wayah Rd., 35.15498, -83.59143, 16/V/2014, S.R. Beaty, V.B. Holland, E.D. Fleek, 1♂ (reared) and exuvium (NCDWR). **Madison Co.**, Wolf Laurel Branch, off Oakridge Lane, 35.96783, -82.50488, 8/V/2012, S.R. Beaty, M.D. Walters, D. Black, 1♂ (reared) and exuvium, 1♀ (reared) and exuvium, 1 larva (NCDWR). **McDowell Co.**, Curtis Creek, Curtis Creek Rd, 35.71459, -82.19079, 28/IV/2016, S.R. Beaty, V.B.



Figs. 2–5. *Isoperla arcana*, adult male. 2. Head and pronotum. 3. Posterior abdominal terga and paraprocts. 4. Posterior abdominal sterna and vesicle, arrow indicates brush setae. 5. Aedeagus, three-quarter posterolateral view, white letters indicate lobes, black letters indicate spine patches; a. glabrous posteroventral lobe, b. paired posterolateral lobes, c. transverse posterodorsal lobe, supramedial invagination is indicated by the white arrow, d. quadrate median spine patch, e. basal stalk with short, sharp spinulae.

Holland, E.D. Fleek, 2♂ (reared) and exuvia, 1♀ (reared) and exuvium, 3 larvae (NMNH); 2♀ (reared) and exuvia (CSUIC); 7♂ (reared) and exuvia, 2♀ (reared) and exuvia, 2 larvae (NCDWR). **Transylvania Co.**, Cherryfield Creek, off Still Waters Lane, 35.18460, -82.81165, 12/V/2013, S.R. Beatty, T. Morman, M.D. Walters, 1♀ (reared) and

exuvium, 2 larvae (NCDWR). **Watauga Co.**, Boone Fork, Blue Ridge Parkway, 36.11904, -81.78168, 7/V/2014, V.B. Holland, E.D. Fleek, M.D. Walters, 1♂ (reared) and exuvium, 1♀ (reared) and exuvium (CSUIC); Buckeye Creek, near Smoketree Rd., 36.20194, -81.89583, 9/V/2016, V.B. Holland, E.D. Fleek, M.D. Walters, 1♀ (reared) and exuvium



Figs. 6-7. *Isoperla arcana*. 6. Adult female habitus. 7. Female posterior sterna and subgenital plate.

(NCDWR). **Wilkes Co.**, Garden Creek, SR 1379, 36.39027, -81.07111, 8/V/2014, V.B. Holland, E.D. Fleek, M.D. Walters, 1♂ (reared) and exuvium (NCDWR).

Additional material: **NORTH CAROLINA, Avery Co.**, Elk Hollow Branch, off Roaring Creek Rd., 36.09947, -82.04547, 7/V/2014, V.B. Holland, E.D. Fleek, M.D. Walters, 11 larvae (NCDWR); Gragg

Prong, FSR 261, 36.03091, -81.80307, 6/V/2014, V.B. Holland, E.D. Fleek, M.D. Walters, 2♀ (reared) and exuvia, 1 larva (NCDWR); Wilson Creek, Blue Ridge Parkway, 36.10084, -81.80778, 28/IV/2016; US 221, 9/V/2016, S.R. Beaty, V.B. Holland, E.D. Fleek, 3 larvae (NCDWR). **Buncombe Co.**, North Fork Swannanoa River, upstream Burnett Reservoir, 35.70414, -82.32984, 6/V/2014, V.B. Holland, E.D.

Fleek, M.D. Walters, 3 larvae (NCDWR). **Caldwell Co.**, Thorp Creek, NC 90, 35.9953, -81.7604, 12/IV/2012, S.R. Beaty, V.B. Holland, E.D. Fleek, 3 larvae (NCDWR). **Clay Co.**, Fires Creek, FSR C, 35.14027, -83.75638, 19/IV/1988, L.E. Eaton, D. Penrose, C. Smith, 1 larva (NCDWR). **Jackson Co.**, small seep, off Cold Mountain Rd., coordinates unknown, 1/III/2006, D.R. Lenat, 3 larvae (NCDWR). **Macon Co.**, North Fork Coweeta Creek, SR 1114, 35.06525, -83.40253, 17/V/2011, V.B. Holland, T. Morman, M.D. Walters, 3 larvae (NCDWR). **Madison Co.**, Big Creek, Big Creek Rd., 36.02388, -82.65333, 5/IV/2015, S.R. Beaty, V.B. Holland, E.D. Fleek, 1 larva (NCDWR). **McDowell Co.**, Cow Creek, SR 1443, 35.79666, -82.12166, 20/IV/2005, W.B. Crouch, E.D. Fleek, M.D. Walters, 7 larvae (NCDWR); Reedy Branch, NC 80, 35.73277, -82.01779, 21/IV/2005, W.B. Crouch, B. Prusha, M.D. Walters, 4 larvae (NCDWR); unnamed tributary to Peppers Creek, Peppers Creek Rd., 35.87586, -82.01779, S.R. Beaty, E.D. Fleek, B.C Kondratieff, C.J. Verdone, 1♀ (reared) and exuvium (NCDWR). **Transylvania Co.**, Cove Branch, FSR 225, 35.30310, -82.82783, V.B. Holland, E.D. Fleek, M.D. Walters, 10 larvae (NCDWR).

Distribution. USA – NC.

Adult Male – Macropterous. Forewing length 9.0–10.0 mm (n=12), body length 7.5–8.5 (n=14). General pattern on head dark and bell or hat-shaped with a brown U-shaped to triangular spot on anterior edge of head. General body color pale yellow in life with some brown markings.

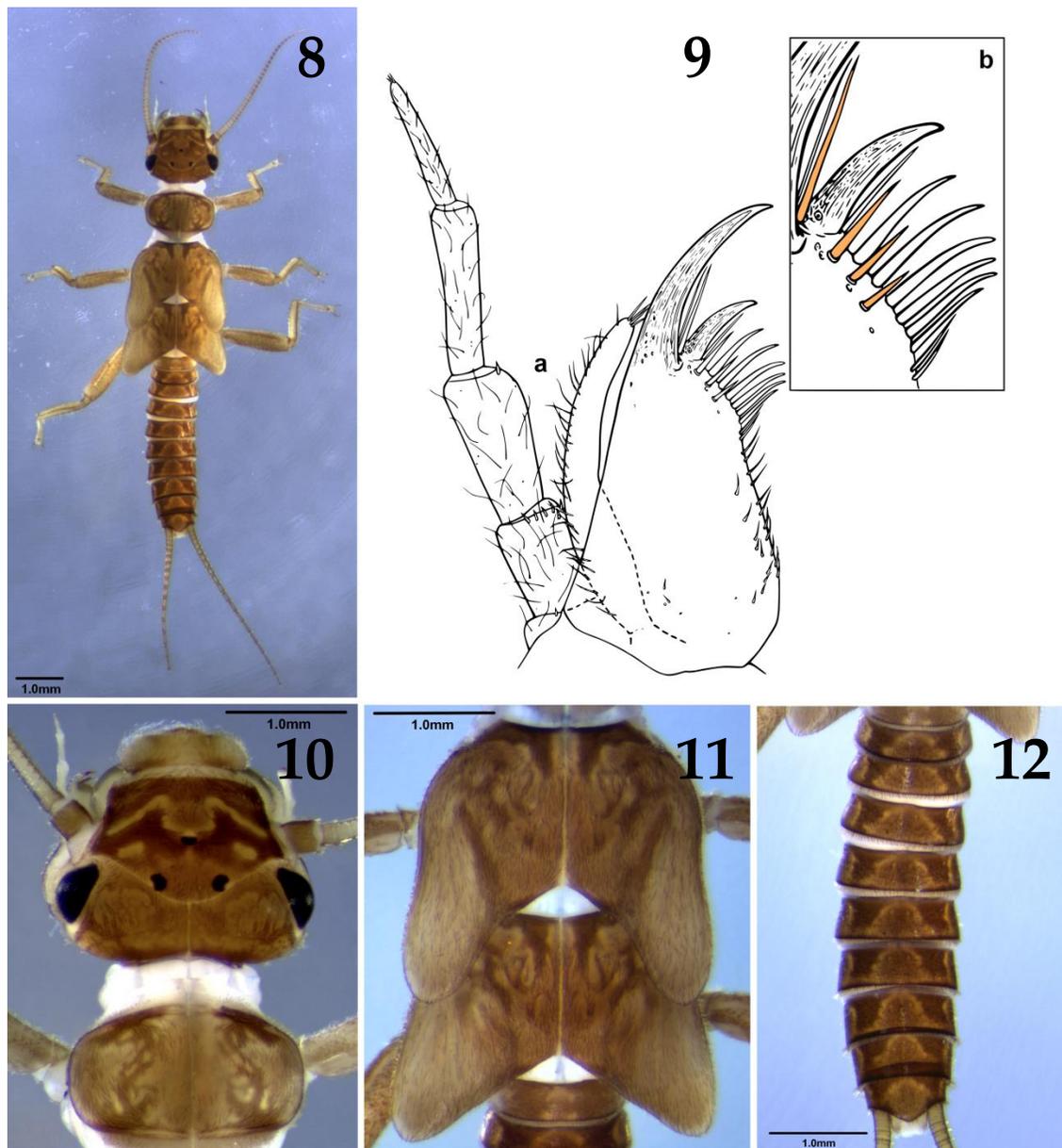
Head: Palpi pale yellow-brown. Labrum with stiff, short setae. Dorsum of head (Fig. 2) mostly pale cream with variable dark brown quadrate pattern connecting median ocellus with lateral ocelli and covering interocellar area; interocellar area may be paler posteriorly between the lateral ocelli on some individuals thus appearing as simple dark bands extending from the median ocellus to each lateral ocellus; usually with a U-shaped to triangular brown area on frons separated from median ocellus by pale yellow-brown line; frontoclypeus pale anterolaterally and anterior to eyes; occiput completely pale white to pale yellow; tentorial callosities indistinct; short, dark setae

restricted to interocellar area, some setae visible just anterior to lateral ocelli. Antennal scape and pedicel pale yellow-brown tending darker towards apex of each segment; flagellum brown with basal few segments lighter than remainder, antennal length about $\frac{3}{4}$ length of body with 33–36 segments.

Thorax: Pronotum with wide, pale median stripe (Fig. 2); pronotal margin anterior to furrow dark brown, sometimes narrowly interrupted medially; posterior edge of pronotum barely margined with brown, if at all, and delineated only by a row of thick, dark, short setae along edge; lateral pronotal margins pale white; mid-dorsal pronotal suture with thin brown line; pronotal disks yellow-brown with most rugosities brown, edged with darker brown, some yellow-brown; rugosities coalesced medially, more distant laterally, raised; short, dark setae anterior and posterior to pronotal sclerites, pronotal disks with short dark setae restricted to rugosities and areas in-between, setae absent from medial pale stripe and lateral edges of disks. Meso- and metanota yellow-brown with brown scutal humps (Fig. 6), pale yellow-brown stripe medially, anterior areas of nota pale; nota covered entirely with short, dark setae. Wings smoky hyaline with light brown veins. Meso- and metabasisterna mostly pale yellow; furcal sternites pale white.

Legs: Pale overall. Anterior and posterior faces of femora pale white, narrowly yellow-brown dorsally, dark brown at extreme apical margin; tibiae pale to yellow-brown with thin dark brown band near basal fourth and slightly darker apically; tarsi brown, segment 3 darker apically; claws and arolia darkened dorsally; leg vestiture dense with short dark setae giving legs a darker overall appearance; tibiae with a subdorsal and ventral row of larger spines.

Abdomen: Overall pale white to yellow-brown with a longitudinal brown median dorsal stripe. Terga 2–9 pale yellow to yellow-brown, each tergum with a medial brown patch, contiguous with successive segments and thus appearing as a longitudinal brown stripe, becoming indistinct to absent on posterior segments; brown medial patch on anterior segments subtriangular, wider posteriorly; each tergum with a dense patch of long



Figs. 8–12. *Isoperla arcana*, larva 8. Dorsal habitus. 9. Right maxilla, ventral, a. lacinia, galea, and maxillary palp, b. marginal and submarginal (colored) setae. 10. Head and pronotal pattern. 11. Meso- and metanotal pattern. 12. Abdominal pattern.

silky clear setae laterally and shorter darker setae along posterior half to two-thirds on each segment; tergum 9 and 10 yellow-brown, paler laterally; 9 with a medially interrupted, transverse brown line at anterior edge of segment and with a setose, brown posteromedial protuberance; setae and

spinule patches on 10 absent from shallow medial furrow (Fig. 3). Paraproct length short, subtriangular in dorsal view, apices bluntly pointed and with outer surface lightly sclerotized and light brown; dorsum of paraprocts slightly rugose and with setae; paraprocts curved upwards

and medially, barely recurved over the apex of tergum 10. Anterior sterna pale white but gradually changing to pale yellow-brown at abdominal apex; sterna 2–5 mostly glabrous, setae become more numerous on each successive posterior segment. Sternum 8 with posterior edge slightly darkened and with a row of brown brush-like setae, with a pale brown vesicle about 1.4X wider than long; posterior edge of vesicle darker, evenly rounded to subtruncate, extending to or barely over base of segment 9 (Figs. 4, 13), the distal third of vesicle glabrous but with marginal hairs. Cerci pale yellow gradually darkening to pale yellow brown, last segment darkened distally, cercal length $\frac{1}{2}$ – $\frac{2}{3}$ as long as abdomen, with 11–14 segments, each segment with one long, colorless ventral seta near posterior margin, basal 4–6 segments with dorsal and ventral tufts of long stiff setae, setae becoming shorter and less numerous on each successive segment.

Aedeagus: Basal stalk encircled with a moderately dense band of short, sharp spinulae (Figs. 5e, 15); a rectangular to quadrate median patch of golden spinules with apical finger-like projections (Figs. 5d, 16, 17) above a glabrous posteroventral lobe (Figs. 5a, 14a), between paired posterolateral lobes (Figs. 5b, 14b), and below a transverse posterodorsal lobe which is invaginated supramedi ally (Figs. 5c, 14c); a large, mostly glabrous terminal dorsal lobe; a few sensillae basiconica scattered at base of the paired posterolateral lobes (Fig. 18).

Adult Female — Macropterous. Forewing length 10.0–12.0 mm (n=18), body length 8.0–10.0 (n=18). General head pattern and body color similar to that of the male, although the dorsal abdominal stripe is less conspicuous and may be absent in lighter individuals (Fig. 6).

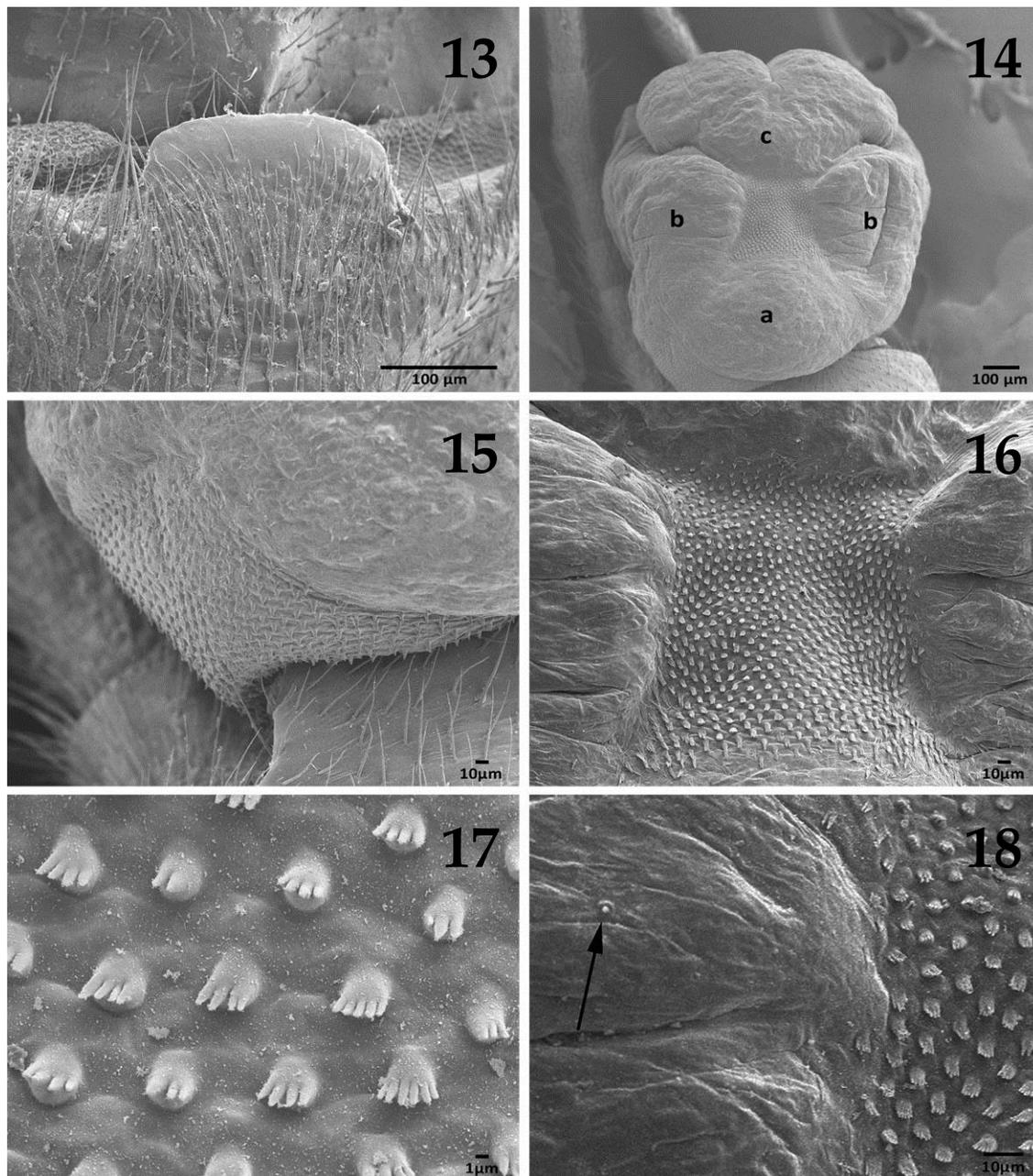
Subgenital plate: generally acutely triangular, originating from the distal third of sternum 8 from near pleural folds and produced posteriorly approximately $\frac{3}{4}$ the length of sternum 9; plate basally broad, about $\frac{3}{4}$ the width of segment 8 on most specimens, apex pointed (Fig. 7), plate with long and short setae on body; coloration of plate similar to remainder of segment 8.

Ovum — Unknown.

Mature Larva — Pre-emergent larvae 7.0–10.0 mm (n=19). Body moderately slender with contrasting body pattern (Fig. 8). Clothing hairs present, dorsally dark, ventrally brown to clear.

Maxilla: Lacinia bidentate (Fig. 9); receding evenly from base to subapical tooth; a thin, long marginal seta present between apical and subapical teeth; 5–7 striated stout marginal setae below subapical tooth, last few setae progressively smaller; first marginal seta below subapical tooth somewhat isolated, removed from remaining marginal setae (Fig. 9a); 8–14 thin, unevenly-spaced setae along palm edge and curving dorsad; a widely-set submarginal row of 3–5 striated stout setae from apical tooth (Fig. 9b); some specimens may have an occasional extra thin submarginal seta interrupting the submarginal row (not illustrated); uneven row of 5–10 thin setae on ventral surface near palm edge and approaching base of lacinia, setae near base short; dorsal setae few to absent. Length of striated apical tooth of lacinia between 0.5–0.6X palm length and 0.7–0.9X the palm width. Subapical tooth 0.5–0.6X the length of the apical tooth. Galea 1.0–1.1X the lacinial palm length, with a ventral row of more than 30 setae and tipped with 2–4 apical spinous setae. Maxillary palp setose, 1.3–1.6X length of lacinia; segments 1–3 successively longer, segments 3 and 4 subequal and slightly longer than 5; segments 1 and 2 with transverse row of apical spinous setae, segment 3 sometimes with 1 apical spine, segment 5 tipped with 1–2 sensillae.

Mandible: Left mandible bicuspid; outer cusp with 3 teeth, ventral tooth largest and serrated basally, dorsal tooth smallest; a band of unorganized, long spinous setae on ventral surface from base of teeth to base of mandible; inner cusp with 3 teeth, middle tooth longest and largest; a brush of dense short spinous setae basal to cusp near molar ridge; a series of spine-like setae along molar ridge, setae longer and thinner towards base of mandible; a band of unorganized and serrated setae on dorsal surface from base of inner cusp towards base of mandible, setae shorter and thinner towards base. Right mandible similar to left mandible except dense brush of setae at base of inner cusp replaced by a short patch of denticle-



Figs. 13–18. *Isoperla arcana*. Scanning electron micrographs. 13. Male vesicle on sternum eight. 14. Posterior view of male aedeagus, a. glabrous posteroventral lobe, b. paired posterolateral lobes, c. transverse posterodorsal lobe. 15. Spinulae on basal aedeagal stalk. 16. Subquadrate patch of golden spinules between posterolateral lobes. 17. Detail of golden spinules with apical finger-like projections. 18. Sensillum basiconica on posterolateral lobe.

like acanthae.

Dorsum of head: head brown with distinctive pale M-pattern (Fig. 10), pattern sometimes

medially obfuscated; a pair of transversely oriented pale dashes anterior to M-pattern, sometimes obscure; a medial dark brown transverse band

posterior to M-pattern encompassing median ocellus, usually darker than remainder of head and appearing somewhat like a mask, mask may be difficult to see in old preserved specimens or younger instars; ocellar spot obscure to absent, if present then small and faint; a pair of obliquely oriented pale mediolateral spots always present; occiput with brown areas along epicranial suture, pale with brown reticulated pattern behind enclosed posteriorly by a heavily spiculate curved dark bar from epicranial stem to eye; labrum pale, darker basally, anterior margin with long setae, medial bulge clothed with a short, brown pubescence. Entire head with dark clothing hairs. Antennae yellow-brown, darker apically; pedicel brown; scape slightly darker than basal flagellar segments.

Thoracic nota: Pronotum brown, medial stripe obscure (Fig. 10); anterior pronotal flange brown, sometimes medially interrupted; lateral edges pale, with sublateral brown stripes to posterior margin, sometimes obliterated medially; pronotal disk with brown reticulated markings between irregular pale rugosities; posterior pronotal flanges brown; pronotum rimmed with short, closely set, stiff setae, an occasional long seta near posterolateral corners. Meso- and metanota with brown markings, with anterior areas dark brown (Fig. 11); wing pads light brown with medial stripe obliterated posteriorly, often lacking strong patterning; edges of nota fringed with a short, closely set row of stiff setae. All thoracic nota densely covered with dark clothing hairs. Thoracic sterna pale, without distinctive markings.

Legs: Femora brown overall (Fig. 8); ventral face mostly pale; anterior face light brown and densely covered with dark clothing hairs giving it an overall darker appearance, usually with a darker submedial longitudinal brown band; tibiae pale yellow-brown, proximal fourth slightly darker; femora and tibiae with a dorsal fringe of long silky setae; long spinous setae dense on dorsal surface of femora, spines regular but scattered along anterior and posterior faces; tibiae with two dorsal and two ventral longitudinal rows of short spines. Tarsi pale yellow-brown with a ventral row of stiff setae and a sparse dorsal row of silky setae.

Abdomen: Variable, brown overall with two conspicuous, longitudinal dark stripes (Figs. 8, 12); stripes bordered by pale lines; each tergal portion of the lateral stripes usually wider anteriorly and barely reaching posterior edge of respective segment, maculations may be subtriangular on posterior segments but sometimes parallel and reaching posterior edge; stripes will appear contiguous if segments are partially retracted into each other; a faint pair of anteromedial dots on dorsum of each segment may be visible in some specimens; dorsum of abdomen with numerous socketed stout setae with dark origins; posterior edges of terga dark brown and bearing a transverse row of long clear spines; dark clothing hairs abundant on dorsal surface. Abdominal sterna pale, clothing hairs darker on posterior segments, lighter on anterior segments; stout, socketed setae with dark origins on posterior sterna, anterior sterna almost bare; posterior edges bearing a transverse row of long socketed spines, row incomplete medially on anterior segments. Cerci yellow-brown with sparse dorsal fringe of silky setae on distal half.

Etymology. The word *arcanus* refers to “hidden” or “secret” in Latin. The species epithet *arcana* was chosen to signify that the adult of this species remained unknown to science despite the larva being collected for over 30 years in routine water quality assessments.

Diagnosis. The habitus of the adult male *I. arcana* superficially resembles that of *I. holochlora* Klapálek, 1923 and other generally pale members of the *I. signata* group. The paraprocts of *I. arcana* are pointed rather than bluntly rounded as in *I. holochlora*. Also, differences in aedeagal armature will serve to separate *I. arcana* from other *I. signata* group species. The aedeagus is generally similar to that of *I. powhatan*, being mostly membranous and with a wide rectangular spinule patch. However, unlike the sharp spinules of *I. powhatan*, the patch on *I. arcana* is composed of scales with 2–6 apical finger-like projections (Figs. 16, 17). *Isoperla arcana* also has a wide band of sharp spinule around the basal aedeagal stalk.

The subtriangular subgenital plate of female adults of *I. arcana* is similar to that of *I. holochlora*

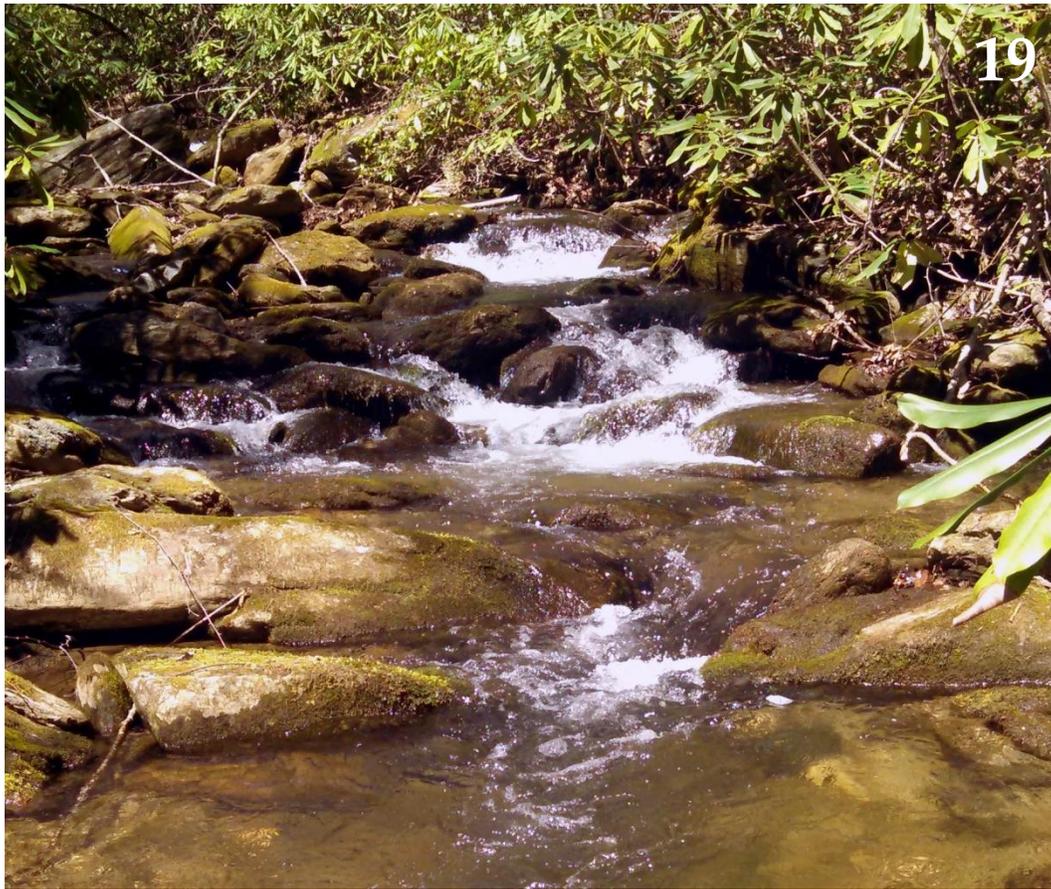


Fig. 19. Curtis Creek near Blue Ridge Parkway, McDowell County, North Carolina. Type locality for *Isoperla arcana*.

and *I. dicala* Frison, 1942. Some variation has been observed in the overall width of the subgenital plate but it is typically shorter and not overlapping as much of segment 9 as in *I. holochlora*. *Isoperla dicala* does not possess a median dorsal abdominal stripe distinguishing it from females of *I. arcana*.

The combination of abdominal stripes, similar to those of *I. holochlora* larvae, and the dark head pattern with a distinct, but pale M-line, shared only by the few known larvae of the *I. similis* and *I. pseudosimilis* groups, will allow separation of *I. arcana* larvae from all other known eastern Nearctic larvae.

Type Locality. Curtis Creek (Fig. 19) is a first to third order, high gradient tributary of the Catawba River in McDowell County, North Carolina. The watershed of Curtis Creek lies on the south side of

the Blue Ridge Escarpment in the Southern Crystalline Ridges and Mountains Level IV ecoregion (Griffith et al. 2002) and is contained almost entirely within the Pisgah National Forest. At the site of collection, about 5 km below the Blue Ridge Parkway (elevation 787 m), the stream is about 1–3 m wide and has a drainage area of approximately 2.1 km². In-stream habitat consists of bedrock slides, boulder and cobble cascades, and small areas of sand at the bottom of plunge pools and at stream edges. Well-conditioned leaf packs were abundant at the time of sampling as were several debris dams. While water quality at the type locality has never been assessed using North Carolina Biotic Index protocols (NCBI; Lenat 1993, NCDEQ 2015), a larger, long term monitoring site approximately 6 km downstream on Curtis Creek

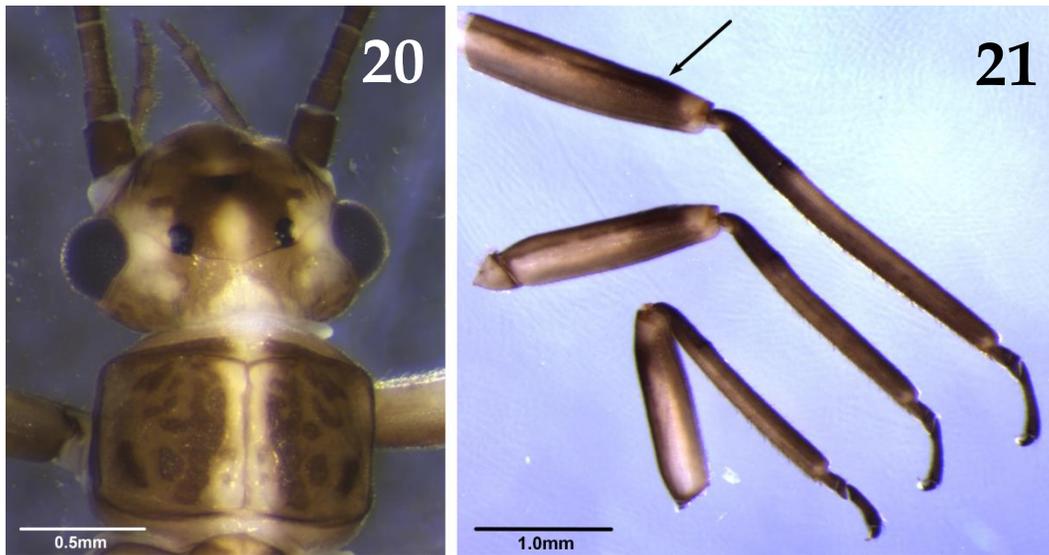


Fig. 20-21. *Isoperla borisi*, adult male. 20. Head and pronotum. 21. Legs, arrow indicates dark subapical banding on metafemur.

has rated Excellent since 2007 with a macroinvertebrate community dominated by intolerant Ephemeroptera, Plecoptera, and Trichoptera (EPT).

Curtis Creek is typical of minimally disturbed, high gradient headwater streams in the Appalachian Mountains. Macroinvertebrate species richness routinely exceeds 40 EPT taxa during the summer months when routine bioassessments are conducted, with even higher species richness during the spring months.

Additional Remarks. The North Carolina Division of Water Resources Biological Assessment Branch has over 150 verified larval records of *Isoperla arcana* (as *I. nr. holochlora*) dating back to 1988. All of these records are from mountain streams (Fig. 45) with a majority (82%) in streams with a drainage area of less than 13 km² (5 mi²) and with 75% in streams of less than 5 m wide. There are no verified North Carolina records of this species occurring in streams over 100 km² or wider than 25 m. In addition, 91% of all *I. arcana* records occur from streams either rated Excellent or Good by North Carolina water quality assessment methods (NCDEQ 2015) indicating that this species is highly intolerant to disturbance. The pollution tolerance

value assigned to *I. arcana* (as *I. nr. holochlora*) and used in the NCBI is 0.0, the lowest value possible.

Little is known of the habitat or life history of *I. arcana*. Larvae are abundant in leaf packs but are readily collected from riffle habitat with little detrital substrate. Feeding habits are unknown, although carnivory seems likely at least in later instars as larger larval specimens have been collected with partially digested chironomid larvae. Adults emerge as early as mid-April in lower elevations to late-June in higher elevation streams. There are no previously published records of adults collected in the wild suggesting that they may be active in the stream canopy out of reach of typical collecting methods. A univoltine-slow life cycle is presumed for this species.

***Isoperla borisi* sp. nov.**

<http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:>

[TaxonName:501184](http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:501184)

Slate Belt Stripetail

(Figs. 20–44)

Material examined. USA – *Holotype* ♂ and larval exuvia, **NORTH CAROLINA, Orange Co.**, Collins Creek, NC 54, 35.93139, -79.20590, 30/ IV/2014, D.

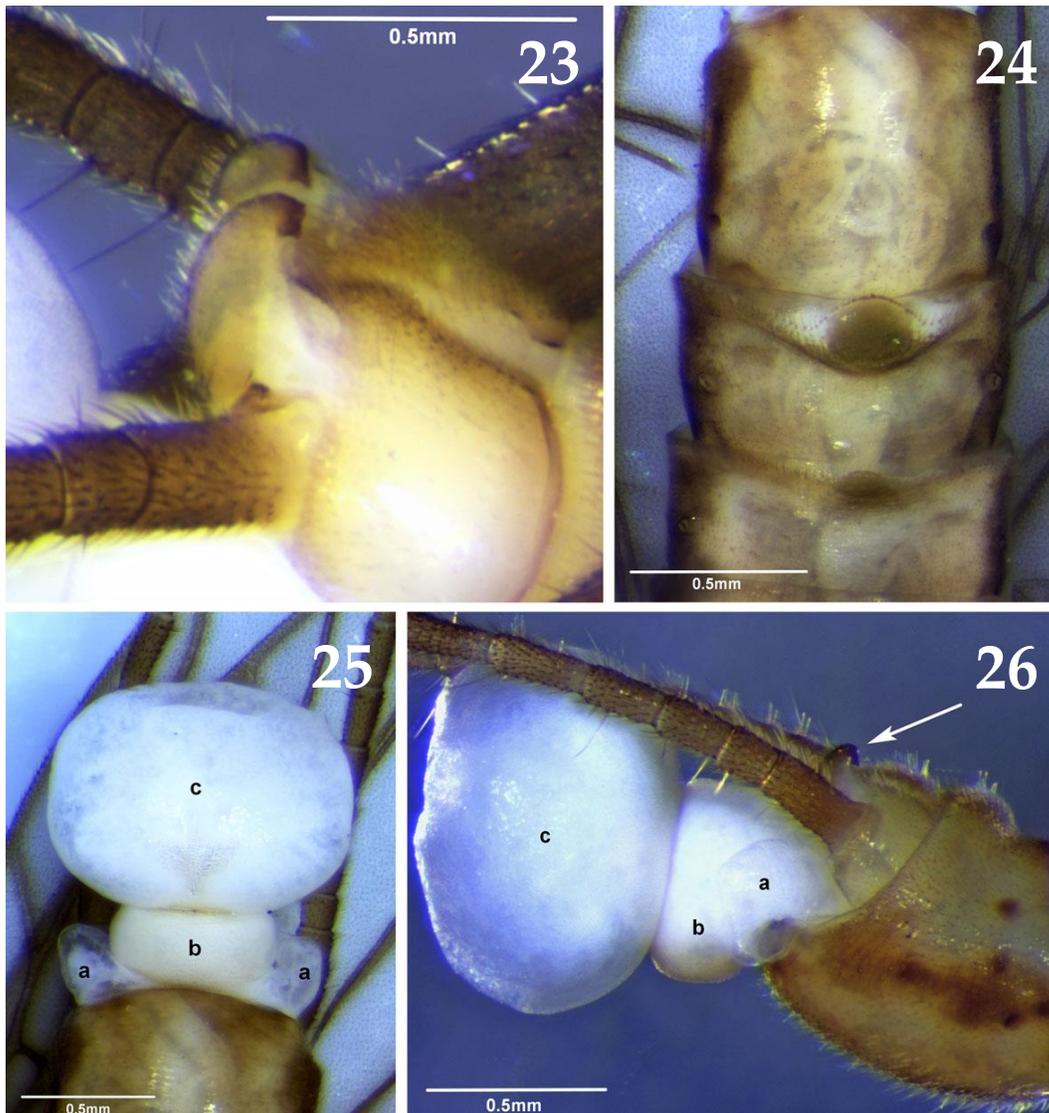


Fig. 22. *Isoperla borisi*, adult male posterior abdominal terga, paraprocts and terminal aedeagal lobe, arrow indicates stout spinulae.

R. Lenat (NMNH). *Paratypes*: same locality as holotype, **Chatham Co.**, Georges Creek, SR 2142, 35.60250, -79.25833, 18/IV/2015, S.R. Beatty, D.R. Lenat, 1♀ (NCDWR); Terrells Creek, NC 87, 35.82166, -79.25555, 30/III/2012, S.R. Beatty, 1♀ (reared) and exuvia (NCDWR). **Moore Co.**, Killlets Creek, Bethlehem Church Rd., 35.35431, -79.45444, 13/IV/2015, D.R. Lenat, 1♀ (reared) (NCDWR). **Orange Co.**, Collins Creek, NC 54, 35.9314, -79.2059, 23/IV/2014, D.R. Lenat, 3♂ (reared) and exuvia, 3♀ (reared) and exuvia (NCDWR), 9 larvae (NMNH); 25/IV/2014, D.R. Lenat, 3♀ (reared) and exuvia (NCDWR); 30/IV/2014, D.R. Lenat, 12 larvae, 2♂ (reared), 21♀ (reared) and exuvia (NCDWR); 6/V/2014, D.R. Lenat, 1♀ (reared) and exuvia (NCDWR); 13/IV/2015, V.B. Holland, 1♂ (reared) and exuvia (CSUIC); 13/IV/2015, V.B. Holland, 2♂ (reared) and

exuvia, 10♀ (reared) and exuvia (NCDWR); 25/IV/2015, S.R. Beatty, V.B. Holland, 5♀ (reared) and exuvia (CSUIC); 25/IV/2015, S.R. Beatty, V.B. Holland, 20♀ (reared) and exuvia, 2 larvae (NCDWR); 30/IV/2015, D.R. Lenat, 2♂ (reared) and exuvia (NCDWR); 5/V/2015, D.R. Lenat, 1♂ (reared) and exuvia (NCDWR); 9/V/2015, S.R. Beatty, V.B. Holland, 1♀ (NMNH), 1♀ w/eggs (NCDWR); 16/V/2015, V.B. Holland, 1♂ (reared), 1♀ (reared) and exuvia (NCDWR).

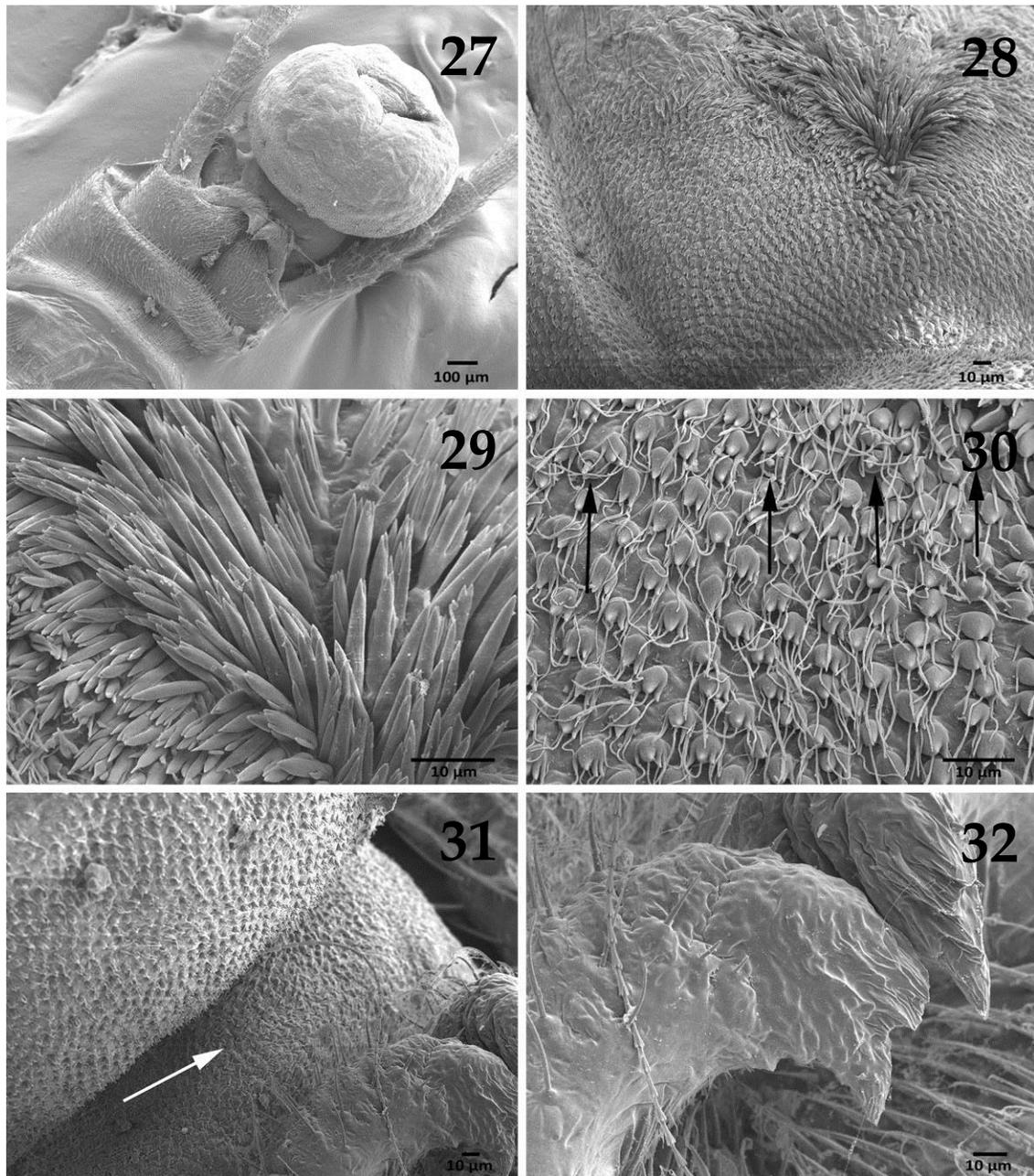
Additional material: **NORTH CAROLINA, Chatham Co.**, Bear Creek, SR 2155, 35.63194, -79.2367, 23/III/2009, 2 larvae (NCDWR); Georges Creek, SR 2142, 35.60250, -79.25833, 25/II/1993, 4 larvae (NCDWR); 10/III/2003, 4 larvae; Terrells Creek, NC 87, 35.82166, -79.25555, 19/III/2009, E.D. Fleek, V.B. Holland, T.L. Morman, 13 larvae (NCDWR); White Oak Creek, NC 751,



Figs. 23-26. *Isoperla borisi*, adult male. 23. Paraprocts, lateral view, darkened tips are highly sclerotized. 24. Posterior abdominal sterna and vesicle. The vesicle on sternum eight is typical of the species but this specimen has a small aberrant vesicle on sternum seven. 25. Aedeagus, ventral view, a. glabrous basolateral lobes, b. spiculate ventrobasal lobe, c. large terminal lobe with dense golden arrow-like spine patch. 26. Aedeagus and paraprocts, lateral view, a. glabrous basolateral lobes, b. spiculate ventrobasal lobe, c. large terminal lobe. dorsal tooth on paraprocts indicated by arrow.

35.75444, -78.95944, 8/II/1993, D.R. Lenat, L.E. Eaton, G. Coleman, 9 larvae (NCDWR). **Orange Co.**, Collins Creek, NC 54, 5.9314, -79.2059, 15/III/2015, S.R. Beaty, D.R. Lenat, 27 larvae (NCDWR); Jones Creek, Hillsborough Rd., 35.9639, -79.1071, 15/IV/2013, L. E. Eaton, C.

Gregory, L. Montgomery, 2 larvae (NCDWR); 1/III/2009, D.R. Lenat, 4 larvae (NCDWR); Old Field Creek, off Milhouse Rd, 22/IV/2015, D.R. Lenat, 1 larva (NCDWR). **Person Co.**, Crooked Creek, SR 1558, 36.4878, -78.8069, 19/IV/2006, E.D. Fleek, T.F. McPherson, C Tyndall, 5 larvae



Figs. 27-32. *Isoperla borisi*. Scanning electron micrographs. 27. Male posterior abdominal terga and aedeagus. 28. Spinous arrow-like patch. 29. Long bi- and trifurcate spines of the arrowhead-like patch. 30. short bifurcate spinules with subapical and apical hair-like filaments, sensilla basiconica indicated by arrows. 31. Spines on dorsum of terminal lobe (upper left) and dorsum of basal stalk. 32. Male paraprocts.

(NCDWR). **Union Co.**, Richardson Creek, SR 1751, 34.98972, -80.51027, 13/III/1989, T.F. McPherson, L.E. Eaton, D. Penrose, 8 larvae; location unknown,

1/II/1977, 11 larvae (NCDWR).

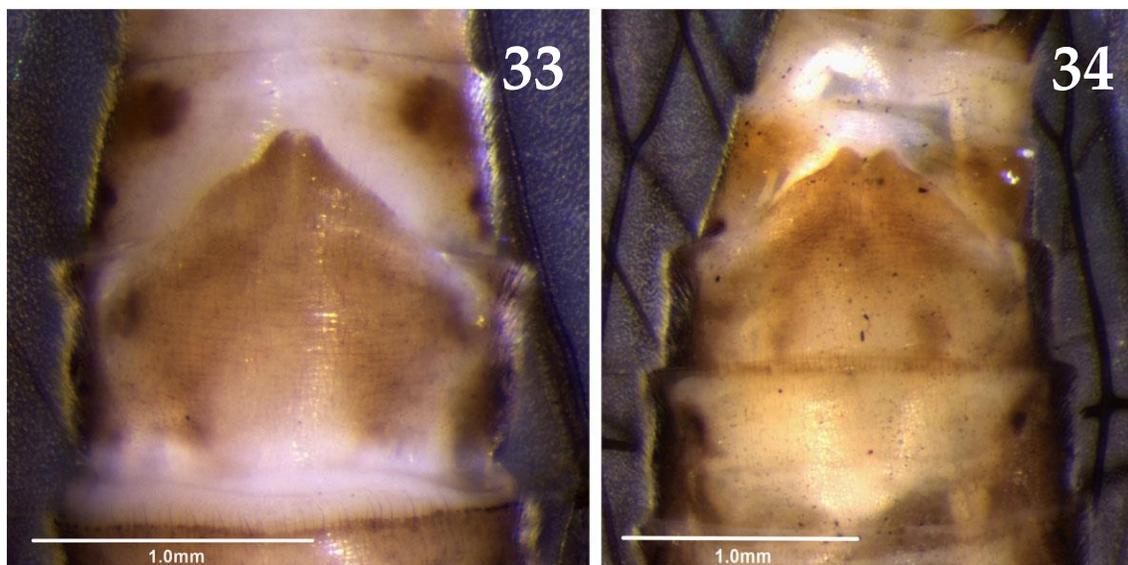
Distribution. USA – NC.

Adult Male — Macropterous. Forewing length 8.3–9.7 mm (n=11), body length 7.5–9.4 mm (n=9). General pattern on head approximately “A”-shaped, or “H”-shaped on lighter specimens. General body color brown to dark brown in life, yellow-brown in alcohol, with darker brown markings.

Head: Palpi brown to medium brown. Labrum with stiff setae of medium length. Dorsum of head (Fig. 20) with wide, dark brown to black bands connecting median ocellus with lateral ocelli; interocellar area yellow-brown and narrowly open posteriorly, ocellar spot may appear closed at epicranial suture in some specimens; tentorial callosities distinct and darkly connected to lateral edge of ocellar bands; pale brown spot anterior to median ocellus oval to subtriangular, rugosities lateral to pale spot usually as dark as ocellar bands and connected to frontoclypeal wrinkles by brown to dark brown pigment; anterior frontoclypeal area brown with posteriorly directed bands on either side of anterior pale spot connected to ocellar bands, paler anteromedially; frontoclypeus pale anterolaterally and anterior to eyes; numerous wrinkles on frontoclypeus near antennal bases; subtriangular pale yellow area on occiput along

vague epicranial stem widest at hind margin of head and congruent with pale pronotal stripe; occiput mostly brown with area near eyes lighter and with brown rugosities and short setae. Antennae medium brown with scape darker brown, basal few segments slightly lighter than remainder of flagellum.

Thorax: Pronotum with median pale hourglass-shaped stripe (Fig. 20); a few small slightly darkened rugosities within posterior half of pronotal stripe; anterior pronotal margin dark brown, narrowly interrupted medially by pale area; posterior pronotum margined with brown, pale medially; lateral pronotal margins barely lighter; mid-dorsal pronotal suture with thin brown line; pronotal disks brown with rugosities darker; rugosities coalesced medially, more isolated laterally, raised. Meso- and metanota brown with dark brown scutal humps, pale yellow-brown stripe medially. Wings dusky with dark brown veins. Meso- and metabasisterna brown; furcal sternites pale with sutures and pits darker. Wings dusky with dark brown venation. **Legs:** Brown overall. Inner and outer faces of femora pale brown, darker dorsally with a thin dark longitudinal sub-dorsal band, with dark brown subapical vertical band and pale at extreme



Figs. 33-34. *Isoperla borisi*, female posterior sterna and subgenital plate, 33. Reared specimen. 34. Wild specimen.

apical margin (Fig. 21); tibiae brown with dark brown band on basal fourth and slightly darker apically; tarsi dark brown.

Abdomen: Overall yellow-brown with a broad longitudinal dark brown stripe along each side of the abdomen and dorsal to pleural folds, stripe with an irregular dorsal border and narrowing on posterior segments. Terga 2–9 pale yellow-brown, each with a lateral transverse series of 3 small brown dots, the lateral most dot often obscured by brown longitudinal pleural stripe; each tergum with a mesally divided dense patch of long stout setae, patches on middle segments laterally removed and sparse; tergum 9–10 brown, 9 elevated and with transverse patch of stout spinulae on posterior half, patch divided anteromesally but complete posteromesally (Fig. 22); tergum 10 with a pair of small darkly sclerotized posterior elevations divided by a pale mesal furrow, with stout spinulae on raised areas. Paraprocts stout, heavily sclerotized, apically bifid with a large subapical ventral tooth and a dorsal tooth (Figs. 23, 26, 32), dorsal tooth may appear worn and jagged; dorsum of paraprocts rugose and with spinous setae; paraprocts curve upwards and medially and recurved over the apex of tergum 10. Anterior sterna pale but gradually darkening to brown towards abdominal apex; sterna 2–7 with faint sublateral brown dots; sternum 7 with posterior margin darker; sternum 8 with a large, evenly rounded, and darkly sclerotized brown vesicle, slightly wider than long and continuous with dark posterior margin of sternum 8 (Fig. 24); vesicle with stout marginal hairs and overlapping sternum 9 by up to one-fifth its length, the posterolateral corners of the vesicle usually weakly sclerotized. Cerci medium brown to dark brown, as long as abdomen, each segment with one long ventral seta at posterior margin, an occasional middle segment with 2 posteroventral setae.

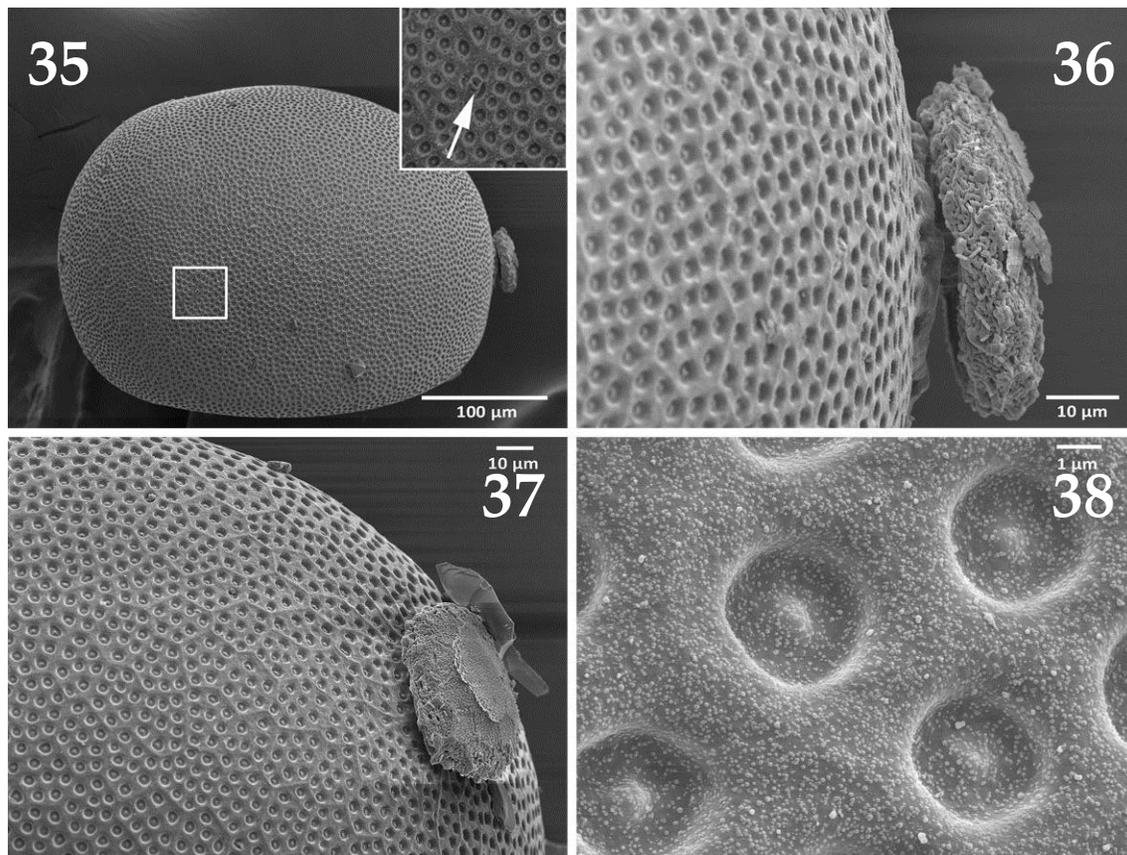
Aedeagus: with paired glabrous basolateral lobes (Figs. 25a, 26a); a heavily spiculate ventrobasal lobe (Figs. 25b, 26b), stout golden spines on ventrobasal lobe decrease in size distally toward terminal lobe and transition dorsally to a densely covered area of golden scale-like spinules and short, sharp spines, most spinules with a fine apical filament (Fig. 31);

large bulbous terminal lobe (Figs. 22, 25c, 26c) with a posterodorsal invagination (Fig. 27) and a small posteromedial lobe below invagination divided by a shallow medial furrow; terminal lobe with a medial longitudinal furrow dorsad of invagination creating a pair of small tuberculate nipple-like points near apex of terminal lobe; lobe with a dense golden brown arrow-shaped patch of spines ventromedially (Fig. 25), long bi- and trifurcate spines within patch decrease in size and transition to short, flat palmate and pectinate spines towards the edges of the patch (Figs. 28, 29); entire anterior half of terminal lobe covered with short spinules with subapical and apical hair-like filaments, some spinules bifurcate or trifurcate (Fig. 30); sensilla basiconica scattered on the ventral surface of the terminal lobe and surrounding the arrow-like spine patch (Fig. 30 - arrows); posterior half of terminal lobe glabrous with some sensilla basiconica near the ventromedial spine patch.

Adult Female — Macropterous. Forewing length 9.5–11.5 mm (n=9), body length 8.5–10.5 (n=9). Head pattern and body color similar to that of the male. Sternum 9 with two posterolateral dark areas, sometimes reduced to dark dots.

Subgenital plate: generally triangular, originating at the distal third of sternum 8 and produced posteriorly approximately half the length of sternum 9; plate basally broad, originating near pleural fold, receding to a somewhat truncate apex, usually with a shallow posterolateral emargination near apex giving the apex large nipple-like appearance (Fig. 33), median emargination may be larger and deeper in some specimens (Fig. 34); plate brown medially, apex usually darker brown, weakly sclerotized medially, and sometimes reflected ventrally.

Ovum — General shape oblong, cross section concave. Color brown and opaque. Length 363 μm ; width 263 μm (Fig. 35). Collar low, disc-like, central stalk with ridges, apex of collar unknown (Fig. 36). Chorionic surface covered with numerous shallow circular punctations, each with a small central spire (Fig. 38), and approximately 3 μm in width; punctations within faint hexagonal follicle cell impressions most visible near poles (Fig. 37). Ecdysis line absent. Micropyles single, located



Figs. 35-38. *Isoperla borisi*. 35. Ovum, detail of micropyle (inset). 36. Detail of egg collar, lateral view. 37. Detail of egg chorion and hexagonal follicle cell impression (FCI). 38. Detail of chorionic punctations.

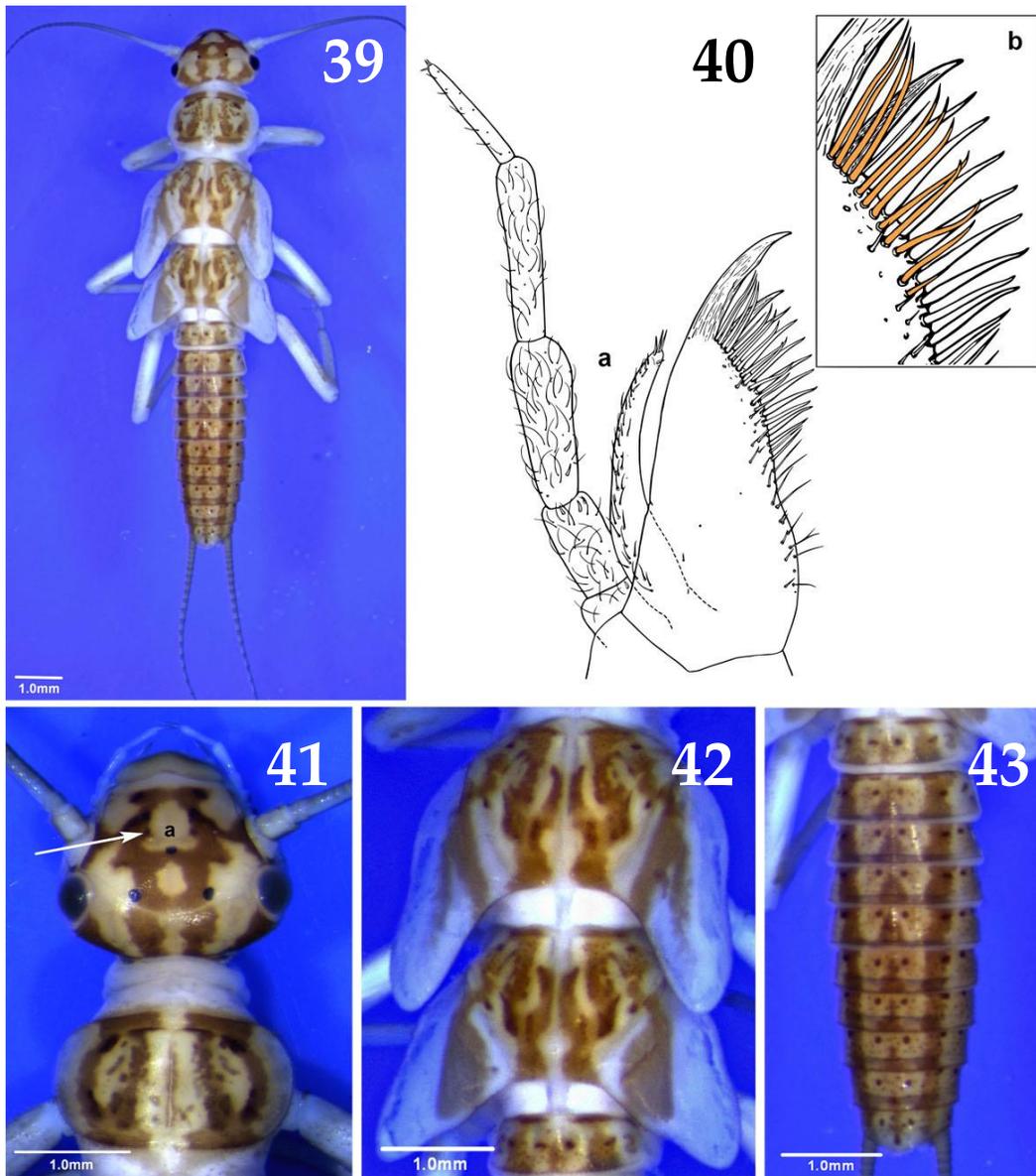
near anterior third; orifices small and flanged, located on chorionic ridges (Fig. 35 inset).

Mature Larva – Pre-emergent larvae 8.2–12.2 mm (n=10). Body slender with contrasting body pattern (Fig. 39). Clothing hairs present but inconspicuous, clear.

Maxilla: Lacinia bidentate; narrowing evenly from base to subapical tooth (Fig. 40a); a thin, long marginal seta present between apical and subapical teeth; 6–9 evenly-spaced, striated, stout marginal setae set below subapical tooth, distal half of marginal setae progressively smaller toward base; an additional 2–9 thin, unevenly-spaced marginal setae along palm edge; a closely-set submarginal row of 7–9 striated, stout setae below apical tooth, an occasional extra thin submarginal seta interrupting row (Fig. 40b); 6–30 short, thin, sometimes bifid setae on ventral surface near palm

edge and approaching base of lacinia; dorsal setae absent. Length of striated apical tooth of lacinia between 0.35–0.42X palm length and slightly shorter than palm width. Subapical tooth 0.50–0.63X the length of the apical tooth. Galea 0.8–0.95X the lacinial palm length, with a ventral row of 8–34 setae and tipped with 2–4 apical spinous setae. Maxillary palp setose, 1.3–1.6X length of lacinia; segments 1–4 successively longer, segments 3 and 5 subequal; segments 1–3 with transverse row of apical spinous setae (Fig. 40a) segment 5 tipped with 4–5 setae.

Mandibles: Left mandible bicuspid; outer cusp with 3 teeth, ventral tooth largest and serrated basally, dorsal tooth smallest; a band of unorganized, long spinous setae on ventral surface from base of teeth to base of mandible; outer cusp with 2–3 teeth, a small tooth may be present at base



Figs. 39-43. *Isoperla borisi*, larva. 39. Dorsal habitus. 40. Right maxilla, ventral, a. lacinia, galea, and maxillary palp, b. marginal and submarginal (colored) setae. 41. Head and pronotal pattern, a. trilobed median pale area, arrow indicates dark extensions into pale area. 42. Meso- and metanotal pattern. 43. Abdominal pattern.

of dorsal tooth (frequently worn or broken); a brush of dense setae basal to cusp near molar ridge; a series of hispid spine-like setae along molar ridge, setae longer and thinner towards base of mandible; a band of unorganized serrated setae on dorsal surface from base of inner cusp towards

base of mandible, setae shorter and thinner towards base. Right mandible similar to left mandible except dense brush of setae at base of inner cusp replaced by a small patch of denticle-like acanthae.

Dorsum of head: Frons with two enclosed pale

spots, an oval to diamond shaped interocellar spot and a large subtriangular median pale area anterior to median ocellus; median pale area almost always trilobed (Fig. 41a), with small dark extensions from the surrounding brown pigment toward but not reaching median ocellus; frons with extensive blackish markings within brown pigmented areas; anterolateral dark spots near anterior frontoclypeal pale area (Fig. 41); occiput with brown areas along epicranial stem somewhat removed; strong oblique dark stripes originating behind eyes and extending to post-occipital margin. Occiput with a transverse row of closely set spicules, spicule origins darkened. Antennae brown, darker apically; scape slightly darker than anterior flagellar segments.

Thorax: Pronotum with pale median stripe; lateral edges pale; pronotal disk with wide, dark brown curvilinear stripes from anterior to posterior margin; irregular brown rugosities on disk, median suture thinly brown; anterior and posterior pronotal flanges brown, interrupted mesally. Meso- and metanota with extensive brown markings, wing pads with longitudinal submedial brown stripes (Fig. 42). Thoracic sterna pale, without distinctive markings.

Legs: Pale brown overall; ventral face with a brown medial obfuscation on distal half; anterior face with a thin brown, longitudinal subdorsal band; femora and tibiae with a dorsal fringe of long silky setae; spinous setae on femora with dark origins giving a speckled appearance; femora with distal fifth pale, lacking dark setal origins and contrasting with a slightly darker subapical area; tibiae with proximal fourth slightly darker; with two longitudinal dorsal rows of short spines; tibiae with a ventral longitudinal row of spines. Tarsi pale brown with ventral row of stiff setae and sparse dorsal row of silky setae.

Abdomen: Three longitudinal stripes, two lateral and one median; median stripe weak, often interrupted; each segment with an anterior transverse row of 8 small dark dots (Fig. 43); terga with stout, socketed spines with dark origins giving abdomen an overall speckled appearance; posterior edges of terga bearing a closely transverse row of short, clear spines. Abdominal sterna pale, freckled with dark spine origins; small,

dark spots visible on sterna 9 and 10; posterior edges of sterna bearing a transverse row of short, socketed spines, row incomplete medially on anterior segments. Cerci brown with sparse dorsal fringe of silky setae on distal half. Body with clear clothing hairs.

Etymology. This species is named in honor of Dr. Boris Kondratieff, of Colorado State University, for his enthusiastic study of aquatic insect systematics, particularly that of Plecoptera and Ephemeroptera. His encouragement of the authors' larval-adult association studies resulted in the discovery of this species of *Isoperla*.

Diagnosis. The adult male *I. borisi* can be separated from most other eastern species by the highly sclerotized and uniquely toothed paraprocts (Figs. 23, 27). It can be further separated from the males of *I. namata* Frison, 1942 *I. signata* (Banks, 1902), *I. slossonae* (Banks, 1911); and other species with toothed paraprocts, by the unique shape and armature of the male aedeagus, particularly the presence of paired basolateral lobes and a central posterior arrow-shaped spine plate, and by the shape of the vesicle. One reared *I. borisi* male had a second, smaller but similarly shaped vesicle-like lobe on the posterior margin of segment 7 (Fig. 24). A similar lobe has been described as unique for *I. maxana* Harden & Mickel, 1952 known only from the holotype male from Minnesota (Szczytko and Kondratieff, 2015a). However, the male aedeagus of *I. maxana* has not been described and additional material of this species has not been collected from the type locality since the original description leading to the supposition that the species may have been extirpated (Szczytko and Kondratieff 2015a). Based on the occurrence of a second lobe on *I. borisi* it is possible that the original description of *I. maxana* (Harden and Mickel 1952) may have been based on an aberrant specimen having an additional sternal lobe.

Female adults of *I. borisi* can be separated from most other eastern species based on the shape of the subgenital plate. Also, both male and females have dark vertical bands at the apices of the femora, a trait shared by *I. slossonae* and *I. jamesae* Grubbs and Szczytko, 2010. Eggs of *I. borisi* have a unique chorionic sculpture of small circular



Fig. 44. Collins Creek, Orange County, North Carolina. Type locality for *Isoperla borisi* and preferred mossy habitat (inset).

depressions with a median raised bump.

Larvae can be separated from all other known eastern larvae by the unique head and abdominal pattern as well as the setation of the lacinia. The overall habitus of this species is similar to what was described as *I. bilineata* by Frison (1935).

Type Locality. *Isoperla borisi* larvae have been extensively reared from Collins Creek, a small tributary of the Haw River, near Carrboro in Orange County, North Carolina. Initial collections of mature larvae were made from the headwaters of Collins Creek. At this location, Collins Creek is a second order stream approximately 2.0–2.5 m wide with a drainage area of approximately 8.5 km² (Fig. 44). Land use in the upper watershed of Collins Creek is primarily forest and agriculture, with few private residences.

As typical of small, permanent Carolina Slate Belt streams, Collins Creek has reduced flows during the summer months and may become a series of disconnected pools during dry years. However, water levels during the winter and spring are typically high and persist through the end of spring to early summer. In-stream habitat consists of riffle-pool sequences of bedrock and cobble with the pools typically silt-bottomed. The substantial amount of in-stream silt appears to originate from adjacent agricultural fields as there is little urban development in the watershed.

Additional Remarks. The larval habitus of *I. borisi* has historically been identified as an *I. bilineata* by taxonomists using Hitchcock (1974) and Unzicker & McCaskill (1982). Re-examination of archived NCDWR benthic macroinvertebrate samples has

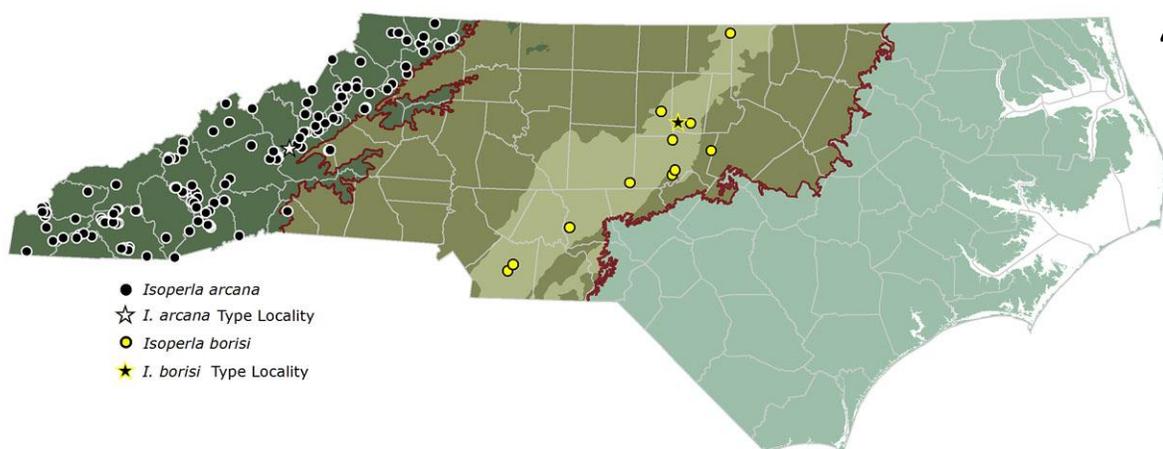
revealed this species to be restricted to the Carolina Slate Belt Level IV ecoregion where it is relatively common (Fig. 45).

Mature *Isoperla borisi* nymphs were particularly abundant in riffles containing the moss *Fontinalis sphagnifolia* (Müller Hal.) Wijk & Margadant but were relatively scarce in other habitats (e.g. leaf packs). The associated larval EPT macroinvertebrate community collected concurrently with *I. borisi* was largely composed of facultatively intolerant taxa that included the mayflies *Stenacron interpunctatum* (Say, 1839), and *Baetis flavistriga* McDunnough, 1921, the stoneflies *Amphinemura* cf. *nigritta* (Provancher, 1876) and *Perlesta* spp., and the caddisflies *Ironoquia punctatissima* (Walker, 1852) and *Rhyacophila fenestralledra*. An additional slate belt indicator species, the heptageniid mayfly *Stenonema femoratum* (Say, 1823) was also present along with *Neophylax atlanta* Ross, 1947, a thremmatid caddisfly restricted to headwater piedmont streams.

Little is known about the ecology of the larvae or adults. Larvae were collected in abundance during late winter to early spring. A short adult emergence period occurred mid-April to mid-May with peak emergence in late April. Few adults were collected using traditional methods (beating

sheet) and appeared to move immediately into the higher adjacent riparian vegetation after emergence. While *I. borisi* larvae appear to be tolerant of the high silt loads frequently present in Slate Belt streams, their response to other forms of disturbance is unknown as adequate verified records are not available to generate a rigorous tolerance value for use in the NCBI. However, this species does appear to be the only facultatively tolerant *Isoperla* in North Carolina based on the authors' field observations.

Note on Phylogenetic placements. Considering the uniqueness of each life stage, both *I. arcana* and *I. borisi* are not presently assigned to any species group. Preliminary phylogenetic analysis based on mitochondrial COI DNA indicates that each are distinct species. However, we use caution interpreting these barcoding results since both the number *Isoperla* species and the number of individuals assessed was small. Additional and expanded genetic analysis is required to firmly establish the relationships of *I. arcana* and *I. borisi* with their eastern congeners. Preliminary phylogenetic data is available for viewing at BOLD Systems (www.boldsystems.org) under the project name Nearctic Ephemeroptera and Plecoptera (NEAP).



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Fig. 45. North Carolina distribution of *Isoperla arcana* and *I. borisi*. Level III ecoregions (Mountains, Piedmont, and Coastal Plain – see Beaty 2015 for an explanation of ecoregions) are delineated by the red borders. The Carolina Slate Belt Level IV ecoregion is shown to illustrate the restricted range of *I. borisi*.

Table 1. Reported North Carolina species of *Isoperla*. Bold text indicates species reported to occur in North Carolina in this paper. An asterisk indicates recently described species.

	Kondratieff et al. 1995	Szczytko & Kondratieff (2015a)	Beaty et al. 2017
*<i>Isoperla arcana</i> sp. nov.			X
<i>Isoperla bellona</i> Banks, 1911	X	X	X
<i>Isoperla bilineata</i> (Say, 1823)	X		
*<i>Isoperla borisi</i> sp. nov.			X
<i>Isoperla burksi</i> Frison, 1942	X	X	X
<i>Isoperla cherokee</i> Szczytko and Kondratieff, 2015		X	X
<i>Isoperla davisi</i> James, 1974	X	X	X
*<i>Isoperla dewalti</i> Verdone and Kondratieff, 2017			X
<i>Isoperla dicala</i> Frison, 1942	X	X	X
<i>Isoperla distincta</i> Nelson, 1976	X		
<i>Isoperla fauschi</i> Szczytko and Kondratieff, 2015		X	X
<i>Isoperla frisoni</i> Illies, 1966	X	X	X
<i>Isoperla holochlora</i> (Klapálek, 1923)	X	X	X
<i>Isoperla kirchneri</i> Szczytko and Kondratieff, 2015		X	X
<i>Isoperla lata</i> Frison, 1942	X	X	X
<i>Isoperla lenati</i> Szczytko and Kondratieff, 2015		X	X
<i>Isoperla montana</i> (Banks, 1898)		X	X
<i>Isoperla namata</i> Frison, 1942	X		
<i>Isoperla nelsoni</i> Szczytko and Kondratieff, 2015		X	X
<i>Isoperla orata</i> Frison 1942	X	X	X
<i>Isoperla pauli</i> Szczytko and Kondratieff, 2015		X	X
<i>Isoperla poffi</i> Szczytko and Kondratieff, 2015		X	X
<i>Isoperla powhatan</i> Szczytko and Kondratieff, 2015			X
<i>Isoperla pseudolata</i> Szczytko and Kondratieff, 2015		X	X
<i>Isoperla pseudosimilis</i> Szczytko and Kondratieff, 2015		X	X
<i>Isoperla reesi</i> Szczytko and Kondratieff, 2015		X	X
<i>Isoperla similis</i> (Hagen, 1861)	X		
<i>Isoperla siouan</i> Szczytko and Kondratieff, 2015		X	X
<i>Isoperla slossonae</i> (Banks, 1911)	X	X	X
<i>Isoperla starki</i> Szczytko and Kondratieff, 2015		X	X
<i>Isoperla stewarti</i> Szczytko and Kondratieff, 2015		X	X
<i>Isoperla transmarina</i> (Newman, 1838)	X	X	X
<i>Isoperla tuteloi</i> Szczytko and Kondratieff, 2015		X	X
<i>Isoperla zuelligi</i> Szczytko and Kondratieff, 2015		X	X
Number of Species→	14	26	30

NEW STATE RECORD

Isoperla powhatan Szczytko and Kondratieff, 2015
<http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:468708>

Material. USA – North Carolina, Montgomery Co., Barnes Creek, Ophir Rd, 35.43861, -79.99888, 7/V/2014, D.R. Lenat, 4 larvae, 3♂ (reared) and exuvia; 2/V/2015, S.R. Beaty, D.R. Lenat, 3♂ (reared) and exuvia.

Remarks. Males of *I. powhatan*, recently described by Szczytko & Kondratieff, (2015a), were successfully reared from larvae collected from Barnes Creek, a 4th order tributary of the Uwharrie River. Previous to this reporting, *I. powhatan* was recorded only from Pennsylvania and Virginia. Szczytko & Kondratieff (2015a) considered the distribution of this species to be wider than they initially reported but material from adjacent states was unavailable for study.

This first North Carolina record of *I. powhatan* resulted from attempts to rear and associate the larvae of *I. zuelligi* Szczytko & Kondratieff, 2015, another recently described North Carolina species. Whereas *I. zuelligi* was not reared from collected larvae, adult *I. zuelligi* males and females with eggs were collected concurrently from nearby low riparian vegetation.

Isoperla powhatan males exhibit a unique and somewhat box-shaped aedeagus with a long, wide median band of sharp, stout golden-brown spinulae located between a pair of posteromesal lobes (see 42.7 and 42.8 in Szczytko and Kondratieff 2015a). In conjunction with the broadly rounded paraprocts and the unique head pattern, the identity of this species is unmistakable. Associated larvae and exuvia from reared specimens closely resemble a dark-form habitus of *I. holochlora* larvae. However, stable morphological characters for separating larvae of *I. powhatan* and *I. holochlora* remain elusive.

Barnes Creek is the type locality for *I. zuelligi*. The stream lies in the Carolina Slate Belt Level IV ecoregion in the midst of the Uwharrie Mountains, a relict mountain chain located in the North Carolina piedmont. Much of the Uwharries are managed as National Forest and thus the greatest

impacts to local waterbodies are from timber harvest. Similar to most slate belt streams, Barnes Creek experiences reduced summer flows and a heavy silt load.

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