A morass of *Isoperla* nymphs
(Plecoptera: Perlodidae)
in North Carolina: a photographic
guide to their identification





DISCLAIMER: This manual is unpublished material. The information contained herein is provisional and is intended only to provide a starting point for the identification of *Isoperla* within North Carolina. While many of the species treated here can be found in other eastern and southeastern states, caution is advised when attempting to identify *Isoperla* outside of the study area. Revised and corrected versions are likely to follow.

The user assumes all risk and responsibility of taxonomic determinations made in conjunction with this manual.

Recommended Citation

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Nymphs used in this study were reared and associated at the NCDENR Biological Assessment Branch lab (BAB) unless otherwise noted.

All photographs in this manual were taken by the Eric Fleek (habitus photos) and Steve Beaty (lacinial photos) unless otherwise noted. They may be used with proper credit.

Keys and Literature for eastern Nearctic Isoperla

Nymphs

- Frison, T. H. 1935. The Stoneflies, or Plecoptera, of Illinois. Illinois Natural History Bulletin 20(4): 281-471.
 - while not containing species of isoperlids that occur in NC, it does contain valuable habitus and mouthpart illustrations of species that are similar to those found in NC (I. bilineata, I richardsoni)
- Frison, T. H. 1942. Studies of North American Plecoptera with special reference to the fauna of Illinois. Illinois Natural History Bulletin 22(2): 235-355.
 - habitus of *I. bilineata*, dark form (similar to *I. poffi*)
 - habitus , maxilla , mandibles of I. burksi, I. dicala, I. lata (so far inseparable from I. pseudosimilis), I. orata, and I. similis/pseudosimilis Groups (as I. similis)
 - habitus, maxilla of *I. holochlora*, *I. slossonae*, and *I. transmarina*
- Hitchcock, S. W. 1974. Guide to the Insects of Connecticut: Part VII. The Plecoptera or Stoneflies of Connecticut. State Geological and Natural History Survey of Connecticut Bulletin 107: 191-211.
 - widely used key for eastern Isoperla nymphs, inadequate as many species not treated
- Pescador, M. L., A. K. Rasmussen, B. A. Richard. 2000. A Guide to the Stoneflies (Plecoptera) of Florida. Florida Department of Environmental Protection, Division of Water Resources Management, Tallahassee. 94 pp. + 72 p. Appendix
 - head, pronotum, and abdominal segments of I. davisi (after Szczytko, unpublished material)
- Poulton, B. C. and K. W. Stewart. 1991. The stoneflies of the Ozark and Ouachita Mountains (Plecoptera). Memoires of the American Entomological Society, No. 38: 1-116.
 - head, pronotum, lacinia, and abdominal segments of I. bilineata, I. burksi, I. davisi (as I. coushatta), I. dicala, and I. namata (currently inseparable from I. kirchneri complex)
- Sandberg, J. B. 2011. The *Isoperla* of California (Plecoptera: Perlodidae); larval descriptions and a key to 17 Western Nearctic species. Illiesia 7(22): 202-258.
 - treats only western species but it contains morphological terminology on which the current study is based; an excellent publication
- Stewart, K. W. and B. P. Stark. 2002. Nymphs of the North American stonefly genera (Plecoptera), Second Edition. The Caddis Press, Columbus, Ohio. xii+510 pp.
 - · chapters on life history, behavior, morphology and on each family
 - the standard for genus level identifications of stoneflies and must have for general aquatic taxonomists
- Stewart, K. W. and B. P. Stark 2008. Chapter 14. Plecoptera. pp 311-384. *In* R. W. Merrit, K. W. Cummins and M. B. Berg (editors). An Introduction to the Aquatic Insects of North America, Fourth Edition. Kendall Hunt Publishing. Dubuque, Iowa. pp 1158.
 - widely used resource for aquatic insect identifications; family and genus level only
- Unzicker, J. D. and V. H. McCaskill. 1982. Plecoptera, Chapter 5 (50 pp.). In A. R. Brigham, W. U. Brigham, and A. Gnilka, editors. *Aquatic Insects and Oligochaetes of North and South Carolina*. Midwest Aquatic Enterprises, Mahomet, Illinois. 837pp.
 - key adapted from Hitchcock for NC and SC species only

Keys and Literature for eastern Isoperla

Adults

- Hitchcock, S. W. 1974. Guide to the Insects of Connecticut: Part VII. The Plecoptera or Stoneflies of Connecticut. State Geological and Natural History Survey of Connecticut Bulletin 107: 191-211.
- Poulton, B. C. and K. W. Stewart. 1991. The stoneflies of the Ozark and Ouachita Mountains (Plecoptera). Memoires of the American Entomological Society, No. 38: 1-116.
 - contains many useful illustrations of the male aedeagus, female subgenital plate and adult head patterns of the species presented in the previous nymph section
- Stewart, K. W. and B. P. Stark 2008. Chapter 14. Plecoptera. pp 311-384. *In* R. W. Merrit, K. W. Cummins and M. B. Berg (editors). An Introduction to the Aquatic Insects of North America, Fourth Edition. Kendall Hunt Publishing. Dubuque, Iowa. pp 1158.
 - widely used resource for aquatic insect identifications; family and genus level only
- Szczytko, S. W. and B. C. Kondratieff. 2015. A review of the Eastern Nearctic Isoperlinae (Plecoptera: Perlodidae) with the description of twenty-two new species. Monographs of Illiesia, No. 1: 1-289.
 - available online at http://illiesia.speciesfile.org/html/monographs 1.html
 - recently published, this excellent work treats males, females and eggs of all previously known eastern Nearctic species as well as 22 new species of which at least 16 are found in NC
- Szczytko, S. W. and B. C. Kondratieff. 2015. A Photographic atlas of the Eastern Nearctic Isoperlinae (Plecoptera: Perlodidae) species. Monographs of Illiesia, No. 2: 1-124.
 - available online at http://illiesia.speciesfile.org/html/monographs 1.html
 - companion to the Isoperlinae monograph above

Online Resources

- DeWalt, R. E., M. D. Maehr, U. Neu-Becker and G. Stueber, D. C. Eades. 2015. Plecoptera Species File Online. Version 5.0/5.0. [17 Aug. 2015]
 - http://plecoptera.speciesfile.org/
 - search taxa, synonyms, and literature; the best all-in-one stonefly resource on the web

Illiesia - International Journal of Stonefly Research

- only online journal with research articles on all things stoneflies
- free access at http://www2.pms-lj.si/illiesia/

NOTE: This list is not complete as there are many publications treating only one or two species or dealing with life histories, etc. An (almost) complete list of literature concerning both eastern and western *Isoperla* is available upon request.

Proposed names based on nymphal patterns

Preliminary identification of *Isoperla* species/groups for North Carolina based on nymphal habitus

Isoperla burksi

<u>Isoperla davisi</u>

Isoperla dicala

Isoperla cf. fauschi

Isoperla frisoni

<u>Isoperla holochlora – light form</u>

<u>Isoperla holochlora – dark form</u>

Isoperla kirchneri complex

Isoperla kirchneri Isoperla montana

. Isoperla siouan

Isoperla tutelo

Isoperla lata/pseudolata

Isoperla orata

Isoperla poffi

Isoperla powhatan

Isoperla similis/pseudosimilis Groups

Isoperla bellona

Isoperla cherokee

Isoperla pauli

Isoperla pseudosimilis

Isoperla reesi

Isoperla starki

Isoperla stewarti

Isoperla slossonae

Isoperla "Collins Cr" n. sp.

Isoperla "Mayo R" n. sp.

Isoperla nr. holochlora

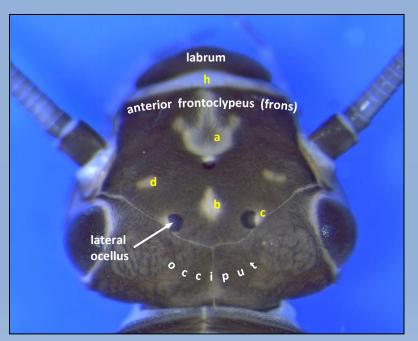
Isoperla nr. transmarina

Isoperla sp. 10

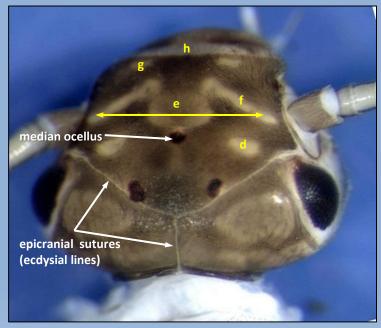
NOTE: The species names and groupings above are based solely on nymphal patterns and morphology. Nymphs of species that are not currently separable are placed together as either a "complex" or a "group". The word "complex" is used to denote cryptic or otherwise very similar looking nymphs that are not necessarily sister species. The word "group" refers to the member status of species that have been described as being aligned in the most recent literature. These groupings differ somewhat from the adult species groups proposed in Szczytko and Kondratieff (2015) and are not meant to replace or compete with those groups. The proposed names simply allow for consistent identifications to be made among regional biologists and taxonomists. As nymphs are the most commonly encountered form of Isoperla, due mainly to their presence in routine aquatic bioassessment samples and their importance as biological indicators, the need for accurate identification and placement cannot be overstated.

Isoperla lenati, I. nelsoni, and I. zuelligi, are not placed above and cannot be easily grouped. The nymphs of these species are currently unknown and, although their identities are suspected, are not treated in this manual. Nymphs of species with a BAB designation (e.g. "Collins Cr", sp. 10) are either currently being described as new species or can usually be separated from other known species. A case is made for separation of the holochlora complex which contains I. holochlora light and dark forms, I. powhatan, and I. nr. holochlora. Identification of these nymphs is relatively easy and, to further investigate and understand the relationship of these species, is necessary to retain distributional and seasonal differences.

Preliminary Identification- head morphology



- a. median pale area
- b. pale ocellar spot
- c. outer lateral ocellar pale area (OLOP)
- d. mediolateral spot (to ocellar triangle)



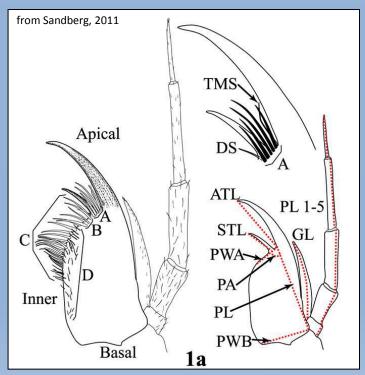
- e. pale M-shaped pattern (width of arrow; median pale area included)
- f. lateral arms to M pattern
- g. anterolateral spot
- h. anterior frontoclypeal area (AFA always pale)

NOTE: It is assumed that the user of this document has a general knowledge of stonefly morphology. Therefore, additional diagrams depicting other morphological characters of the thorax and abdomen are not presented here. However, significant taxonomic characters specific to certain species/groups are highlighted on the relevant species/group page. One caveat: *The following taxonomic notes are based on late to ultimate instar nymphs.* If you cannot see posteriorly developed wingpads (they need not be black wing pads) it may be necessary to leave identifications of some specimens at genus level. Refer to Stewart and Stark (2002) for a comprehensive review of stonefly morphology.

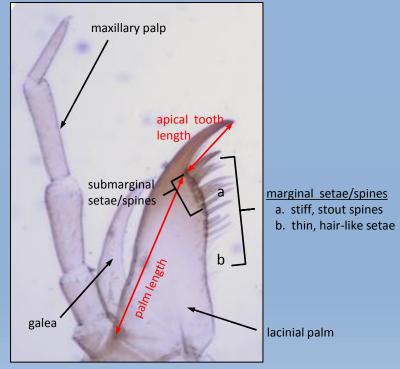
In the following species/group treatments, taxonomic characters are presented in this order: head, thorax, abdomen, mouthparts, and overall size. Important diagnostic characters are bolded and italicized. Additional information is presented under "Other Considerations" and a distributional map follows. Hyperlinks back to the nymphs and morphology pages are located at the top of each treatment page. tolerance values refers to those used in North Carolina Biotic Index (updated in 2010 and based ≥50 records). Distribution maps are based on up to 35 years of BAB benthic collection data.

Descriptions that are in progress for some species not treated in Szczytko and Kondratieff (2015) are noted. Also, a formal dichotomous key for *Isoperla* nymphs of NC is planned (after Sandberg, 2011) and is awaiting genetic analysis of nymphs and adults and more *Isoperla* rearings.

Preliminary Identification- lacinial morphology



1a. Submarginal row groups (A) and (B), Marginal row (C), Ventral surface setae (D), Dorsal surface setae (DSS) not illustrated, Thin marginal seta (TMS), and Dorsal seta (DS) not included in submarginal row counts (Table 1). Maxilla measurements (red lines): Apical tooth length (ATL), Subapical tooth length (STL), Apical palm width (PWA), Palm angle (PA), Palm length (PL), Basal palm width (PWB), Galea length (GL), and Palp segment lengths (PL 1–5).



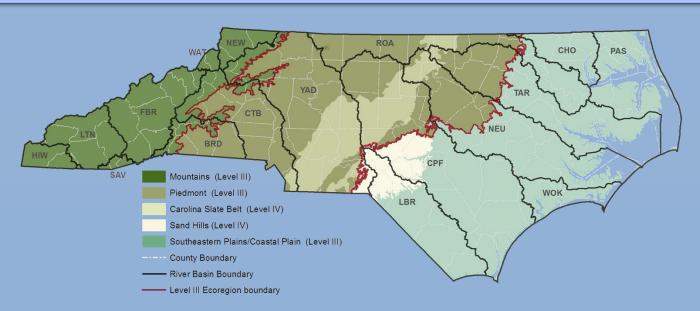
2. Maxilla of Isoperla "Collins Cr" n. sp.

NOTE: Do not confuse submarginal setae with marginal setae. Submarginal setae are removed from the palm edge and typically start near the base of the apical tooth and run beside both the subapical tooth and the stiff marginal setae. As laciniae are 3-D structures, the submarginal setae originate close to the margin but diverge from the marginal setae as one moves apically along the length of a seta. Submarginal setae are mostly ignored in the following species treatments although preliminary analysis suggest they may be valuable in separating some cryptic species.

Lacinial shape is an effective diagnostic character for species determinations. Most species have lacinia that recede from the base and narrow in width towards the apical tooth. A lacinia that does not recede maintains its basal width for most of the palm length and then abruptly narrows to apical tooth. A lacinia that recedes evenly maintains a relatively straight line from the palm base to the apical tooth (although some undulations may be present).

Accurate counting of marginal and submarginal setae requires slide mounted specimens and patience. The difference between stiff, stout setae and thin, hair-like setae is sometimes hard to differentiate. A good rule-of-thumb is to count only those setae that are readily visible under 20X as stiff stout setae (after slide mounting, of course).

Distributions Maps



Approximate Level III and select Level IV ecoregions within North Carolina. The selected Level IV ecoregions are significant with regards to aquatic insect distributions.

Distribution maps are based on both NC DWR larval records and include data as far back as 1978 for some species. Additional records such as those from rearing efforts are also included on the maps, and in some cases, may be the only records available. Some distribution maps depict only specimens with a cryptic habitus form (i.e. a group or complex) and do not represent individual species ranges. This may be due to the historical nature of the distribution data and/or old or dubious species designations as well as the difficulty in separating some species.

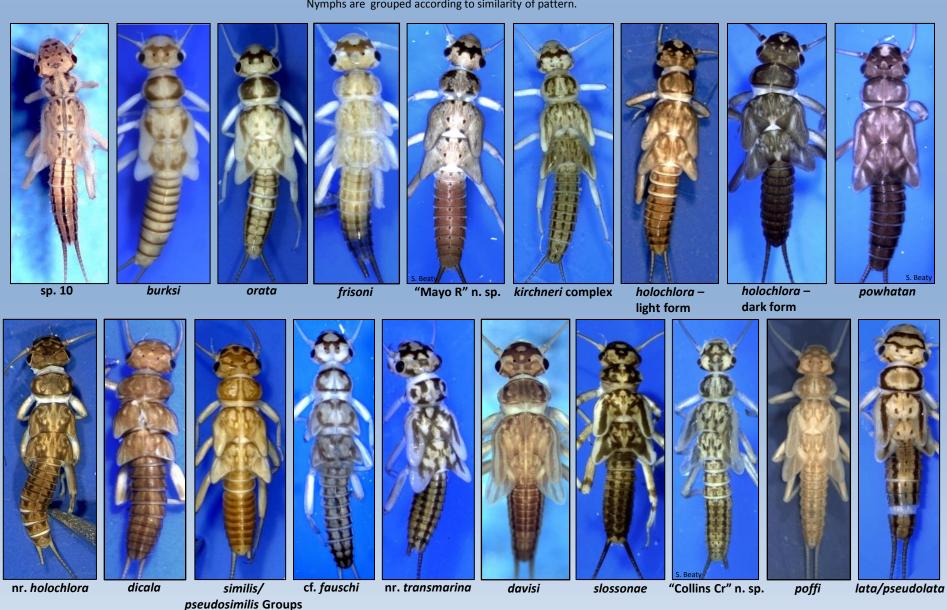
Outlier records were analyzed by re-examining the specimens and were either validated or rejected. In the cases where the specimens were not able to be located the records in question were left in the analysis.

Ecoregions are modified from Griffith et al. (2002). Two Level III ecoregions, Southeastern Plains and the Middle Atlantic Coastal Plain, were combined simply into Coastal Plain by DWR biologists. Both the Southeastern Plains and Middle Atlantic Coastal Plain are east of the Fall Line which appears, based on distributional data, to be the best biological separator between the Piedmont and the geographical areas to the east.

Note on tolerance values: Tolerance values for each species are included when available and range from 0-10. The lower the value the more sensitive a species is to various forms of pollution. Organisms with high tolerance values can withstand and function in more highly disturbed waters. However, many organisms are sensitive to only certain forms of disturbance and tolerant of others. Tolerance value, as measured here, refers to overall sensitivity and does not discriminate between the various forms of water pollution. "Undefined" indicates too few verified records are available to generate a rigorous tolerance value. For details on the derivation of tolerance values and the North Carolina Biotic Index (a modified Hilsenhoff B.I.) please reference Lenat, 1993. An updated list of North Carolina's macroinvertebrate tolerance values can be obtained online at http://portal.ncdenr.org/web/wq/ess/bau.

Preliminary Isoperla Identification - nymphs

Nymphs are grouped according to similarity of pattern.



Isoperla burksi









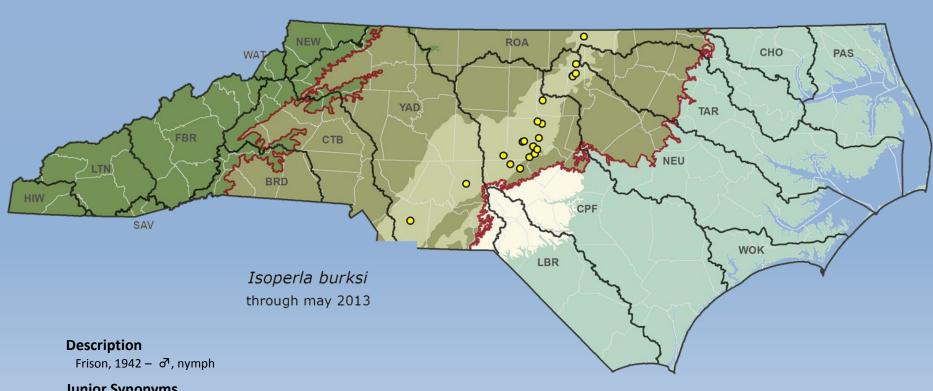
Taxonomic Characters of Nymphs

- head with irregular transverse medial band extending back towards the lateral ocelli and partially onto occiput
- pale ocellar spot typically enclosed behind but may have a narrow pale opening along epicranial Y-stem
- o pronotum with large diamond shaped median pale area
- distinctive transverse abdominal bands on posterior edge of each tergum, approximately 1/3 the width of each respective segment
- o cerci with dense dorsal fringe of silky setae on at least apical 3/4
- o lacinia broad at base and receding evenly; with small, barely conspicuous knob bearing 2-3 thin marginal setae
- o length of apical tooth of lacinia longer than or subequal to palm length and palm width
- $\circ\quad$ Subapical tooth about $\mbox{\em \%}$ length of apical tooth
- o pre-emergent nymphs 9.0-11.0

- o In North Carolina this species occurs only in Slate Belt Level IV Ecoregion
- o nymphs occur in drought susceptible, low flow, and often silty streams (characteristic of Slate Belt streams)
- tolerance value currently undefined
- o reared and positively associated with adult males



Isoperla burksi



Junior Synonyms

none

Adult placement

burksi Group



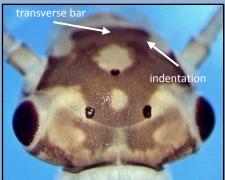






- previously identified as part of *I. transmarina* group
- o widespread but more common in Piedmont and Inner Coastal Plain (map currently unavailable it is currently impossible to extract historical BAB I. davisi records)
- tolerance value currently undefined
- reared and positively associated with adult males and females







Taxonomic Characters of Nymphs

- o head with small pale oval ocellar spot and a larger enclosed median pale area anterior to median ocellus
- o transverse brown bar on anterior frontoclypeus wider than brown area that encloses the pale median area thus creating a slight pale indentation which is confluent with pale anterior frontoclypeal area
- o occiput with irregular brown areas posterior to epicranial suture and on either side of the epicranial stem.
- o oblique dark stripes originating behind eyes and extending to postoccipital margin
- Dorsum of abdomen with distinctive longitudinal "5-lined" banding (not including stripes seen only in lateral aspect); median and sublateral bands darker and usually narrower than submedial bands with intervening pale narrow lines (variably developed)
- cerci with dorsal fringe of silky setae on distal half
- lacinia receding with 6-8 stout marginal spines and 6-8 submarginal setae
- apical tooth about 1/3 palm length and subequal to palm width
- pre-emergent nymphs 7.0-8.0 mm



Description

James, 1974 - ♂, ♀

nymph (as I. coushatta, Szczytko and Stewart, 1977)

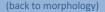
Junior Synonyms

Isoperla coushatta

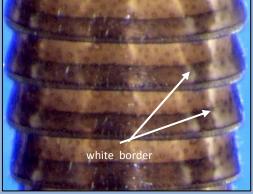
Adult placement decolorata Group

(back to nymphs)

Isoperla dicala





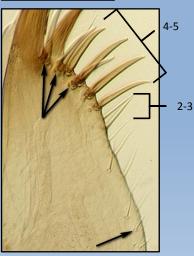










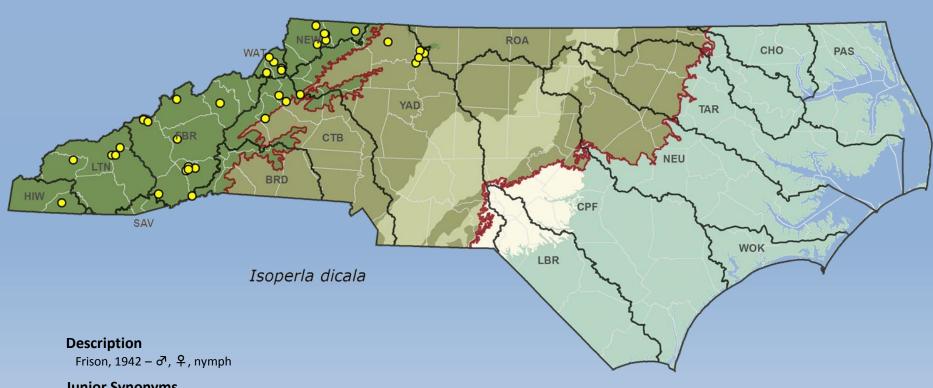


Taxonomic Characters of Nymphs

- head with small pale ocellar spot and pale crown-shaped area anterior to median ocellus; head sometimes with dark speckling and dark edges to pale areas (M-shaped)
- o small pale ocellar spot enclosed.
- O Dorsum of abdomen with 3 dark longitudinal stripes; median and lateral bands narrower than intervening spaces and edged with pale narrow lines (although these can be inconspicuous); with 8 dark dots transversely, 3 pairs sublateral to lateral and 1 pair submedially (these 8 dots sometimes obscured by speckling)
- o abdomen with many spicules with dark origins giving abdomen a freckled appearance, both dorsally and ventrally
- o cerci with dorsal fringe of silky setae on distal half
- o lacinia typical with 4-5 (usually 4) stout marginal spines and 2-3 thin hair-like marginal setae
- o pre-emergent nymphs 7-8 mm

- o occur in small, cold, higher elevation streams of good or excellent water quality; uncommonly collected
- o tolerance value currently undefined

Isoperla dicala



Junior Synonyms

none

Adult placement

bilineata Group

arcs

Isoperla cf. fauschi

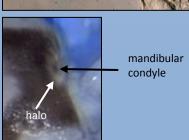


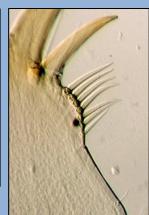










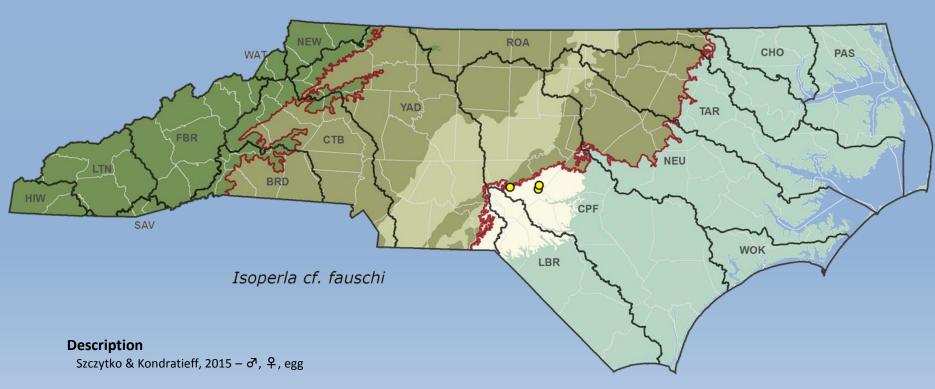


Taxonomic Characters of Nymphs

- o head with small pale ocellar spot open behind and with a large sub-oval pale median area anterior to median ocellus which is always open medially to the anterior frontoclypeal pale area (AFA)
- mandibular condyle dark and visible through integument at antennal-frontoclypeal junction, frontoclypeus above this area pale giving the condyle a "halo"
- o brown markings on occiput posterior the epicranial suture short and *not* extending to postoccipital margin
- labrum with a pair of diffuse brown spots laterally
- oblique dark stripes originating behind eyes and extending to post-occipital margin
- meso- and metanota with distinctive comma-shaped anterior and posterior arcs
- 3 longitudinal dark bands with each tergal segment portion tending towards an hour-glass shape
- cerci with dorsal fringe of silky setae on distal half
- lacinia with low hump below subapical tooth and bearing 6-7 closely set stout marginal spines
- apical tooth approximately 3/4 as long as palm length and subequal to palm width
- pre-emergent nymphs 9.0-10.5 mm

- nymphs collected only from Sand Hills streams (Level IV Ecoregion)
- habitus similar to *Isoperla richardsoni* (as illustrated in Frison, 1935)
- tolerance value currently undefined
- reared and tentatively associated with 2 females; males needed for positive identification

Isoperla cf. fauschi



nymph undescribed

Junior Synonyms

none

Adult placement

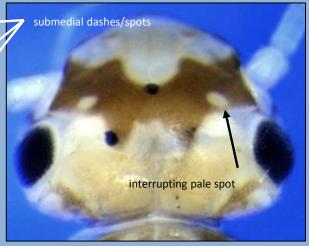
bilineata Group

Isoperla frisoni











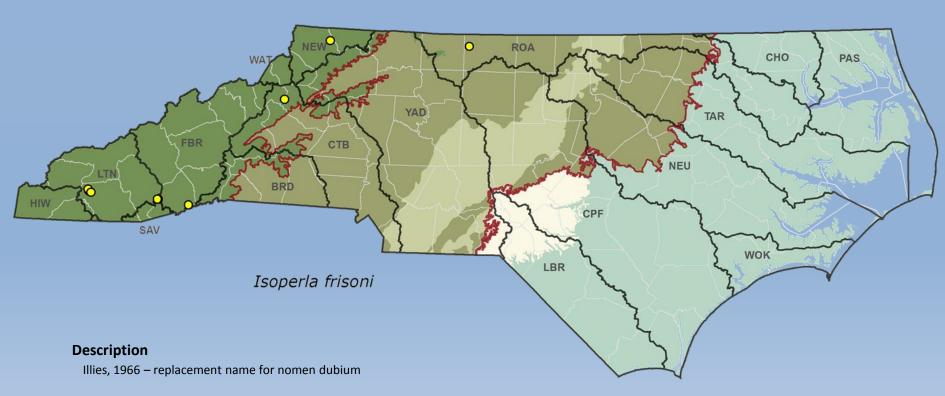
Taxonomic Characters of Nymphs

- o head with irregular transverse medial band with extensions back towards to lateral ocelli
- o **small oval to round pale mediolateral spots which interrupt the posterior edge of medial band;** spots may be almost completely enclosed
- o pronotal edge lined with long stiff setae, particularly abundant and long at posterolateral corners
- thoracic nota with distinctive paired, dark, anterior and posterior submedial dashes (or spots)
- o **longitudinal "3-lined" banding** edge with obscure pale narrow lines on either side of the stripes; medial and lateral stripes often very regular (some darkening may appear in the intervening areas between the stripes)
- o cerci with both dorsal and ventral fringe of silky setae on distal half
- lacinia with low hump basal to subapical tooth and bearing 3-4 stout marginal spines and 2-3 thinner hairlike setae
- o apical tooth approx. 2/3 as long as palm length and subequal to palm width
- o pre-emergent nymphs 8.0-9.5 mm

- o probably rare or at least rarely collected
- high quality, cold water streams
- o tolerance value currently undefined



Isoperla frisoni



♂,♀, nymph – Frison, 1937 as *I. truncata*

Junior Synonyms

Isoperla truncata

Adult placement

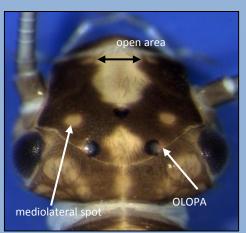
bilineata Group

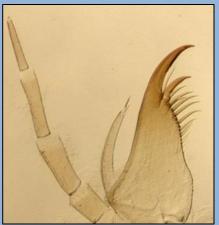
Isoperla holochlora – light form

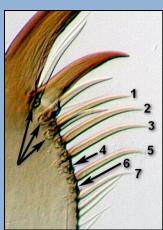
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Taxonomic Characters of Nymphs

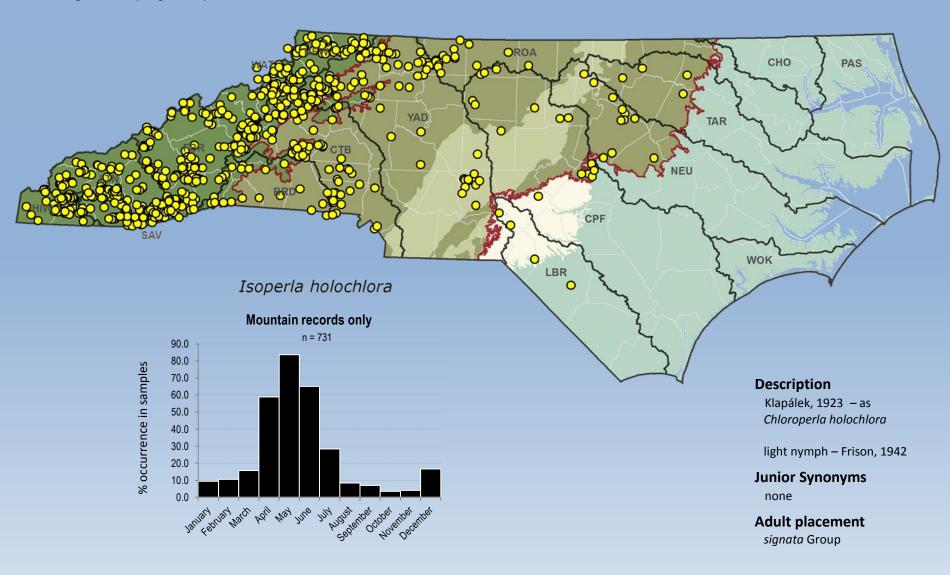
- head with large irregular pale area on frontoclypeus anterior to median ocellus, almost always open to labrum medially (confluent with pale anterior frontoclypeal area); faint lateral M-arms sometimes present, and sometimes pale median area with a lightly darkened center
- o completely enclosed, small and round pale mediolateral spot and outer lateral ocellar pale area (OLOPA)
- ocellar spot usually enclosed and sub-triangular to diamond-shaped, some specimens may have a faint pale area along the epicranial stem which may be confluent with the ocellar spot
- $\circ\quad$ occiput brown, lateral areas with mottled pale reticulations
- abdomen with 2 longitudinal dark stripes laterally and often with an inconspicuous narrow medial stripe; each tergal portion of the stripe sub parallel to wider posteriorly and outside edge of lateral stripe often paler
- o cerci with a dorsal fringe of silky setae on distal half
- o lacinia receding mostly evenly and with 6-7 stout marginal spines and 2-3 thinner hair-like setae, 4 stout submarginal setae
- o apical tooth about half as long as palm length and 2/3 palm width
- o pre-emergent nymphs 6.5-8.5 mm

- o common and widespread across mountains and Piedmont
- o the only NC late emerging Isoperla: early June mid August
- this habitus is exclusively associated with the late emerging populations of holochlora complex (see seasonal histogram).
 It co-occurs with the dark form but pattern and size differences allow for separation.
- Isoperla powhatan appears to have a habitus intermediate between I. holochlora light and dark forms but has a reduced cercal dorsal fringe or lacks it altogether
- o possible cohort splitting (egg diapause?) or cryptic adults?
- o tolerance value = 0.70
- reared and positively associated with both males and females



Isoperla holochlora – light form

NOTE: This map includes probable *Isoperla* cf. *powhatan* specimens. It should be noted that in North Carolina *Isoperla holochlora* appears to be the only *Isoperla* species to emerge in summer (late June-mid August) with sporadic late summer records from the mountains. *Isoperla powhatan* emerges in the spring to early summer



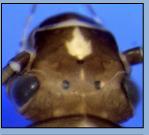
Isoperla holochlora – dark form

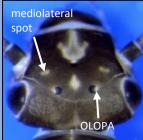
(back to morphology)



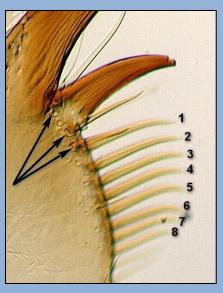












Taxonomic Characters of Nymphs

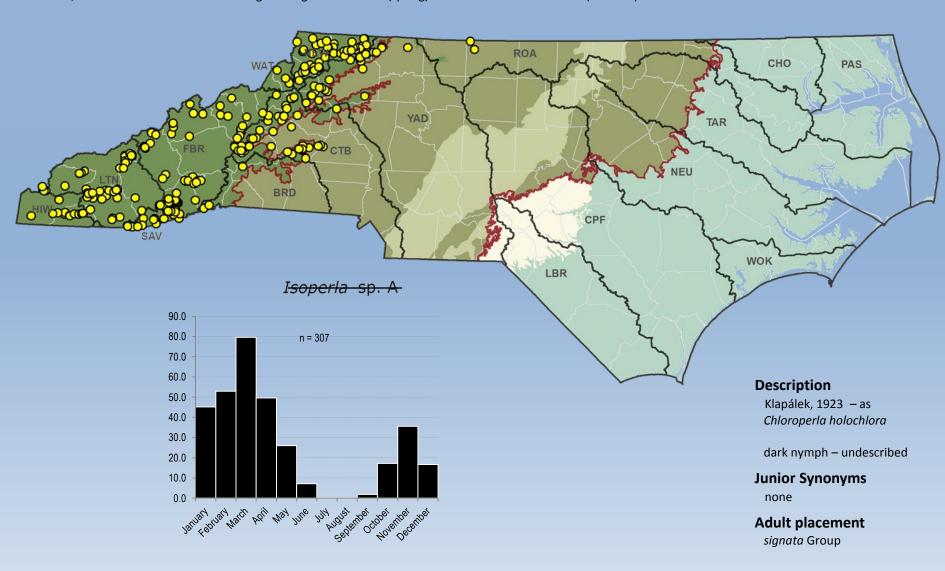
- o an overall very dark nymph with ground color brown to dark brown
- median pale area crown or trident-shaped, variously developed but usually enclosed by a thin brown line, if open to AFA then barely, sometimes with a light brown center; lateral points of crown may be reduced or truncated
- o pale anterolateral spots may or may not be present, if present then small and often obscure
- o ocellar spot present, small and enclosed; mediolateral spot and OLOPA usually present, may be obscure in some specimens
- o abdomen with 2 obscure longitudinal stripes laterally with an inconspicuous narrow medial stripe, fading away altogether on distal segments
- o cerci with a dorsal fringe of silky setae on distal half (Note: younger instars may not have a fully developed fringe and cerci missing several distal segments may appear to not have a fringe)
- o lacinia receding evenly from subapical tooth and with 8-9 stout marginal spines and 2-3 thinner hair-like setae, 3-4 submarginal setae
- o apical tooth at least half as long as palm length (and about 3/4-subequal palm width
- o pre-emergent nymphs 9.5-12.0 mm

- o Previously identified as *Isoperla* sp. A; part of the *holochlora* complex
- o co-occurs with light form *holochlora* but emerges 1-2 months *earlier* and when both forms are collected together, the dark form specimens will be much larger (see seasonal chart)
- o this habitus form only occurs in the mountains
- o tolerance value = 1.20
- o both reared males and females readily identify as *Isoperla holochlora* but nymphs are easily separable based on pattern, morphology (lacinial differences), distribution, and seasonal differences.



Isoperla holochlora – dark form

NOTE: Both the light form and the dark form of *Isoperla holochlora* are sympatric within the North Carolina Appalachians. They regularly occur together within the same stream but are separable based on maturity as well was the outlined taxonomic characters. Depending on collection date, dark form *I. holochlora* will be large and light forms small (spring) or dark forms will be absent (summer).



Isoperla kirchneri complex

(back to morphology)

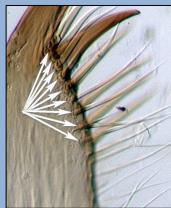




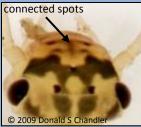












Taxonomic Characters of Nymphs

- head with irregular transverse M-type medial band, extensions back towards posterior ocelli variable; anterior frontoclypeus with 2 pair of small brown markings, some specimens with markings narrowly connected by brown lines or even widely coalesced into a larger brown area
- o brown areas near posterior ocelli on either side of the ecdysial line variable; sometimes extensive, sometimes barely there.
- o abdomen with 3 longitudinal stripes; a transverse row of 6-8 dark spots on each tergum, particularly apparent on posterior segments

Isoperla tutelo

- o cerci with dorsal fringe of silky setae on distal half
- o lacinia bearing 4-5 moderately spaced, stout marginal spines, 8-10 closely set submarginal setae (arrows), and palm edge dense with many thinner hair-like setae along its entire length
- o apical tooth approximately 1/3-1/4 as long as palm length and shorter than palm width
- o pre-emergent nymphs 8.0-9.5 mm

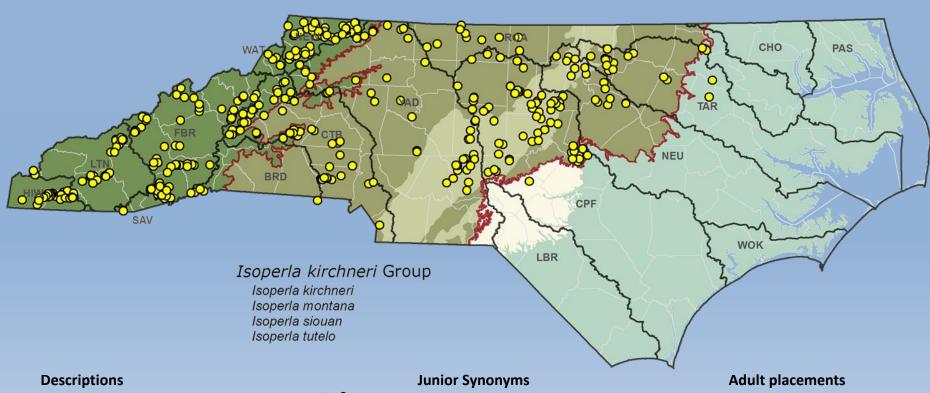
- o *Isoperla kirchneri* Group is a BAB determination based on nymphal pattern and is so named because *I. kirchneri* is, by far, the most common species with this pattern in NC. The grouping is temporary and is not the same as published adult species groups.
- o very common pattern in the NC Mountains and Piedmont
- o preliminary morphological differences between species are unproven but promising
- nymphs of *Isoperla kirchneri, I. montana*, and *I. siouan* have been reared and associated with both males/females. *Isoperla tutelo* reared by B.K.
- o tolerance value currently undefined



edge of lacinial palm

Isoperla kirchneri complex

NOTE: This map references larval DWR records only and therefore individual species distributions cannot be separated out at this time. *Isoperla kirchneri* is widespread while *I. montana* in NC appears to be restricted to the mountains. *Isoperla siouan* may be a Sand Hills species and there is only one known locality for *I. tutelo* (Johns River, Caldwell Co.).



Isoperla kirchneri Szczytko & Kondratieff, $2015 - 3^{1}$, 9, egg Isoperla montana (Banks, 1898) -3^{1} as Chloroperla montana Isoperla siouan Szczytko & Kondratieff, $2015 - 3^{1}$, 9 Isoperla tutelo Szczytko & Kondratieff, $2015 - 3^{1}$, 9

all nymphs undescribed or unknown

none

bilineata Group -Isoperla tutelo

montana Group -Isoperla montana

signata Group -Isoperla kirchneri Isoperla siouan

Isoperla lata/pseudolata

(back to morphology)











Taxonomic Characters of Nymphs

- head with large, wide, median pale area, completely enclosed and appearing somewhat like a stylized bird or wide M pattern
- o ocellar spot open behind and open to median ocellus in some specimens (all North Carolina specimens so far)
- occiput mostly pale but with a narrow oblique line from each eye to post-occipital margin
- pronotum lined by dark oval with large pale area medially (pseudolata only?) but with extreme lateral edges pale
- o abdomen with 2 longitudinal sublateral stripes and with a longitudinal medial row of dashes which are approx. half the length of the respective segment (pseudolata?) or full length of respective segment (lata?)
- abdomen with a sublateral dot near apical margin of sterna 2-8
- cerci with dorsal and ventral fringe of silky setae on distal half
- lacinia unique, broad, not receding from subapical tooth and bearing a dense brush of many long setae
- apical tooth long and thin, about half as long as palm
- pre-emergent nymphs 11-12.5 mm (preliminary)

- found in high cold, Excellent waters (so far)
- relatively rare (3 verified NC records most likely pseudolata)
- may be some pattern differences between lata/pseudolata but are, as of yet, unproven; no confirmed Isoperla lata material has been obtained.
- both species collected from Cataloochee Cr, GSMNP (Szczytko and Kondratieff, 2015)
- tolerance value currently undefined
- Isoperla pseudolata reared (B. Kondratieff)

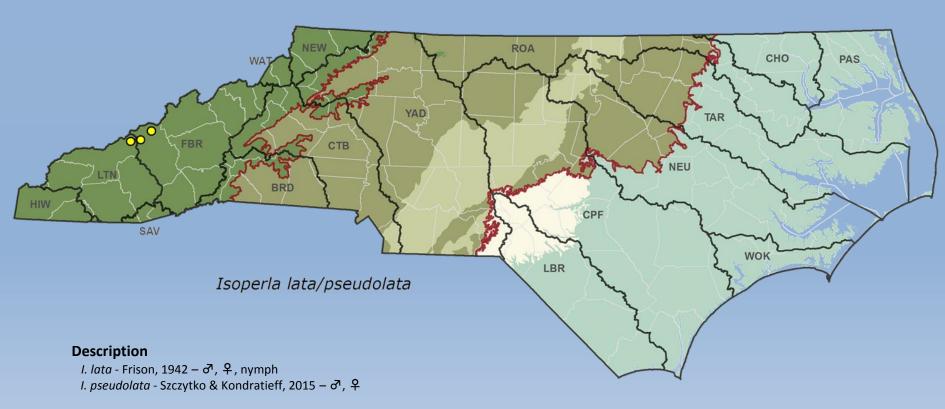






Isoperla pseudolata

Isoperla lata/pseudolata



nymph unknown

Junior Synonyms

none

Adult placement

lata Group

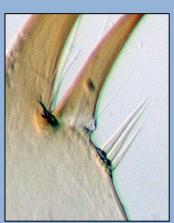
Isoperla orata









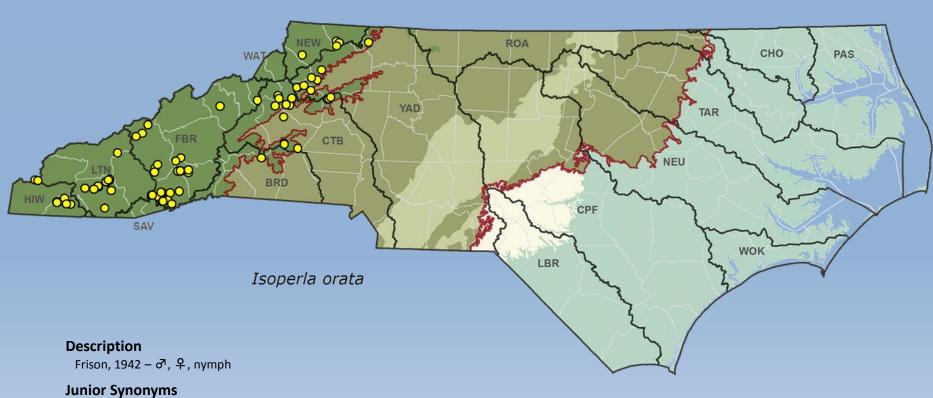


Taxonomic Characters of Nymphs

- head with irregular transverse medial band with extensions back to lateral ocelli
- pale ocellar spot enclosed behind; brown pigment often lighter than "mask"
- o occiput mostly pale but may have small brown area directly posterior to epicranial suture posterior to ocellar spot
- o pronotum mostly dark with irregular pale center area and pale lateral edges
- abdomen with 2 longitudinal lateral stripes and with a median stripe which may vary from approx. half to full length of respective segment, sometimes wider apically (due to incorporation of small dark dots)
- o a pair of small dark spots in apical third sometimes present on each tergum on either side of median stripe (often inconspicuous)
- o cerci with dorsal fringe of silky setae on distal half
- lacinia receding with small barely conspicuous knob at base of subapical tooth bearing 3-4 (usually 3) closely set marginal setae
- o length of apical tooth of lacinia subequal to palm length and width
- pre-emergent nymphs 7.5-8.5 mm

- Relatively uncommon in collections
- High quality, small to medium mid-elevation, cold water streams
- o tolerance value = 0.00
- o reared and positively associated with adult males and females

Isoperla orata



none

Adult placement

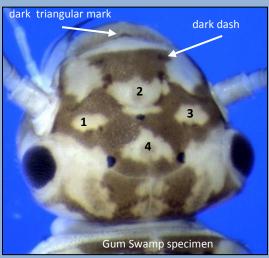
burksi Group

(back to morphology)

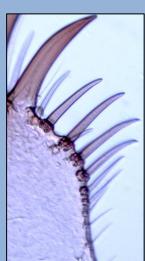












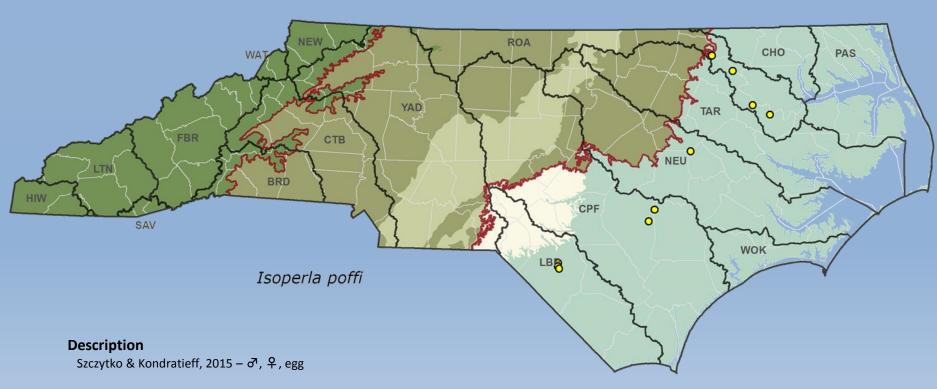
Taxonomic Characters of Nymphs

- o Labrum with a small, dark basomedial triangular mark
- head with 4 enclosed pale spots; the medial spots large (ocellar spot and enclosed median pale area), lateral spots smaller and ovalized.
 Brown areas between the 4 pale spots appearing as an "X".
- o dark brown dashes and sometimes a dark brown "M" pattern superimposed on lighter brown areas (M may be incomplete)
- o occiput with brown areas along epicranial stem although somewhat removed
- abdomen with two lateral and one median longitudinal stripes, stripes may be confluent on anterior segments in some specimens;
 with an anterior, transverse row of 6-8 small dark dots on each tergum; venter slightly speckled with paired median dots
- b head and abdomen may appear somewhat speckled in some specimens
- o cerci with dorsal fringe of silky setae on distal half
- o lacinia bearing 4-5 widely spaced stout marginal spines, 2-3 smaller, thinner setae just below and 3-6 setae along edge of palm
- o length of apical tooth of lacinia approximately 2/3 X palm length subequal to palm width
- o pre-emergent nymphs 9-12.0 mm

- o Nymphs previously identified as bilineata Group in older samples
- Nymphs prefer slower waters; Coastal Plain swamp streams and rivers only
- o early emerger in NC (early March early April)
- o specimens from Roanoke River have variant lacinia: apical tooth slightly shorter and with 6-8 stout marginal setae; nymphs from Lumber Basin are virtually identical to paratype specimens
- o tolerance value currently undefined
- o reared and positively associated by B.K. (Savannah River, SC)



Isoperla poffi



nymph unknown

Junior Synonyms

none

Adult placement

unassigned

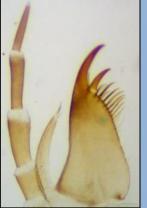
Isoperla powhatan

(back to morphology)







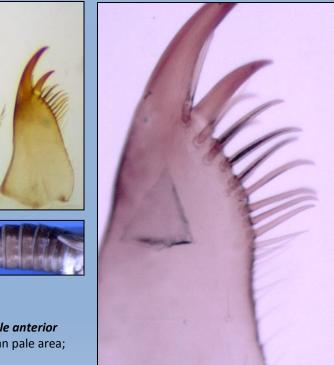






- o an overall very dark nymph with ground color brown to dark brown
- head with large irregular pale open to labrum medially (confluent with pale anterior frontoclypeal area), open area usually narrower than the base of the median pale area; faint lateral M-arms may be present
- pale anterolateral spots may or may not be present, if present then small and often obscure
- ocellar spot present, usually U-shaped, brown posterior invagination variously developed and may not appear on young nymphs. This character may not be stable.
- mediolateral spot and OLOPA usually present, may be obscure in some specimens
- abdomen dark brown with 2 darker longitudinal stripes laterally and an inconspicuous medial stripe
- cerci with reduced dorsal fringe of silky setae of 2-3 hairs on distal half or absent altogether
- lacinia receding evenly from subapical tooth and with 8-9 stout marginal spines and 2-3 thinner hair-like setae, 3-4 submarginal setae
- apical tooth always less than half as long as palm length and about 2/3 palm width
- pre-emergent nymphs 7.5-8.5 mm

- part of holochlora complex; taxonomic characters currently based on 9 nymphs and may not be stable; separation may not be possible with missing or broken cerci
- range extension from PA, VA and only reared and verified from Barnes Cr, Montgomery Co., NC
- earlier emerger than Isoperla holochlora light form
- tolerance value undefined
- reared and positively associated with males

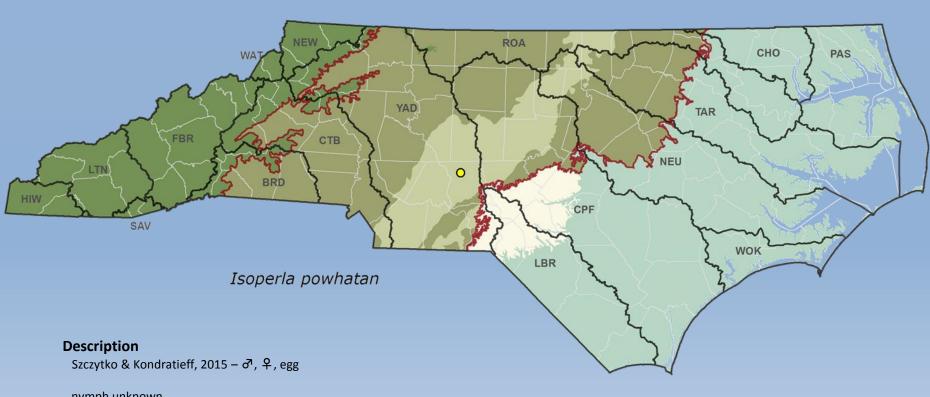






Isoperla powhatan

NOTE: This map reflects records of reared *Isoperla powhatan*. Larval records appear to be more widespread but as of yet are not compiled.



nymph unknown

Junior Synonyms

none

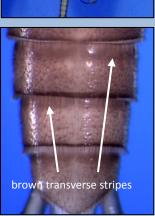
Adult placement

bilineata Group

Isoperla similis/pseudosimilis Groups

(back to morphology)

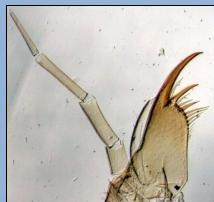












Isoperla similis Group - NC members

Isoperla bellona Isoperla cherokee Isoperla starki

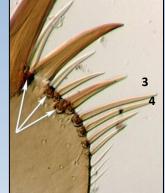
Isoperla pseudosimilis Group - NC members

Isoperla pauli Isoperla pseudosimilis Isoperla reesi Isoperla stewarti

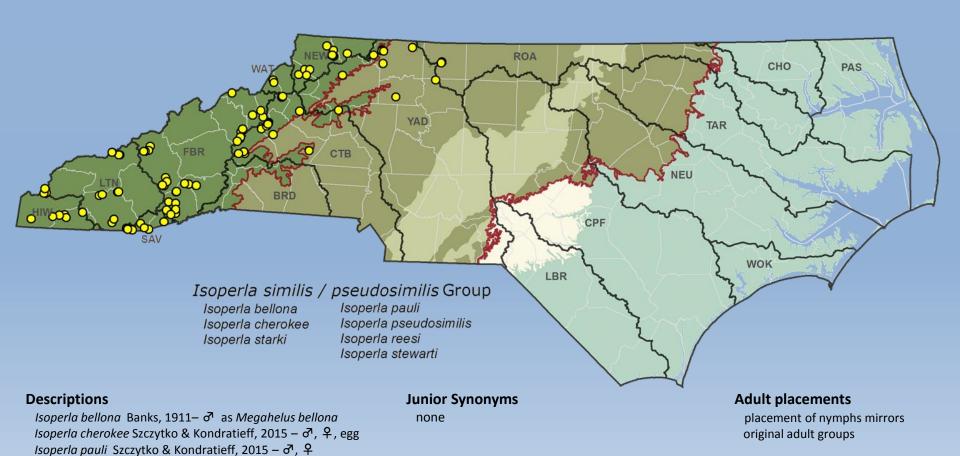
Taxonomic Characters of Nymphs

- o head with distinctive enclosed pale M and with pale anterolateral spots; anterolateral spots may be thin dashes or more ovalized
- o mediolateral spots present, sometimes confluent with pale area beside lateral ocelli (species diff., development, or both)
- o occiput with brown areas along epicranial suture and with pale and brown reticulated pattern behind
- o apparent uniformly brown abdomen with a light, inconspicuous median stripe; stripe with a pair of small pale submedial dots; may be additional small pale dots laterally on each segment
- o anterior 1/4 of each tergum with darker brown transverse stripe, stripe usually not seen as terga are often withdrawn slightly into previous segment (can be confused with the darkening caused by overlapping segments)
- o cerci with very sparse dorsal fringe of silky setae on distal half (1-2 setae) or fringe completely absent
- lacinia barely receding, bearing 6-9 moderately spaced stout marginal spines with middle spines 3 and 4 longest and an additional 3-4 smaller marginal setae; all southern specimens examined in this grouping have 3 stout submarginal spines (arrows); northern specimens (ME) with 1 only.
- o length of apical tooth 2/3 the palm length and subequal to palm width
- o pre-emergent nymphs variable in length (*I. cherokee* 7.5 10 mm, some other species larger)

- o no verified material of *Isoperla similis*, long though to occur in NC, has been found in NC
- o species in these groups appear to be restricted to small, cold, high elevation streams
- o preliminary morphological differences between at least some species are unproven but promising
- o can possibly be confused with the periodid *Malirekus hastatus* (size and lacinia will separate)
- tolerance value(s) = 0.80 (even as a group, this habitus pattern is only found in high quality waters)
 reared and positively associated: *I. cherokee, I. pauli, I. pseudosimilis; I. reesi* (B.K.), *I stewarti* (B.K.)



Isoperla similis/pseudosimilis Groups



Isoperla stewarti Szczytko & Kondratieff, 2015 – ♂, ♀
nymph of each species unknown or undescribed

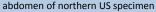
Isoperla reesi Szczytko & Kondratieff, 2015 – ♂, ♀ Isoperla starki Szczytko & Kondratieff, 2015 – ♂, ♀, egg

Isoperla pseudosimilis Szczytko & Kondratieff, 2015 – み, ♀, egg

Isoperla slossonae







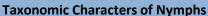






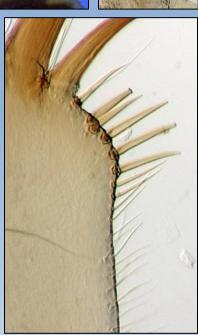




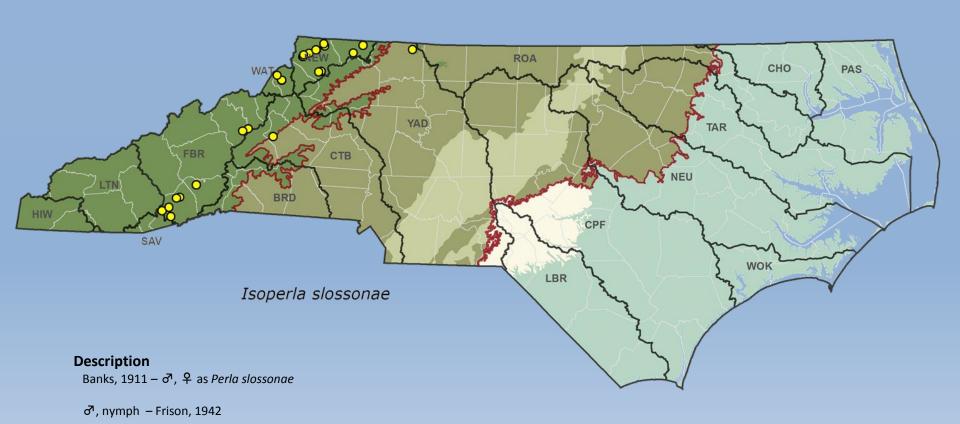


- o extensive dark markings over entire body including cerci
- head with subtriangular enclosed ocellar spot, large median pale area usually with variably developed M-arms; labrum with anteromedial edge pale
- o occiput with small brown areas along epicranial stem; area behind eyes brown with oblique brown stripe to posterior margin of head
- o Legs with a dark band distally on femora and proximally on tibiae
- abdomen with two lateral and one median longitudinal stripes, often confluent on distal segments, median stripe with paired submedial dots; abdomen also highly speckled (dark spicule origins) particularly at stripes but also laterally and ventrally
- o cerci with both dorsal and ventral fringe of silky setae on distal half
- lacinia barely receding, with 6-9 (usually 7) widely spaced stout marginal spines, 3-4 submarginal (although barely) stout setae, and palm edge dense with long hair-like setae to its base
- o pre-emergent nymphs 9.5-12.0 mm

- color pattern variable over its range: southeastern US specimens tend towards lighter and northern US specimens darker (particularly the abdomen)
- o early instars may be confused with *Isoperla dicala* due to highly speckled bodies and incompletely developed pattern; leg patterns will separate
- o uncommonly collected in NC: from high quality medium streams to medium rivers in Appalachians
- tolerance value currently undefined



Isoperla slossonae



Junior Synonyms

Clioperla annecta

Adult placement

phalerata Group

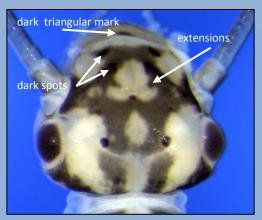
Isoperla "Collins Cr" n. sp.

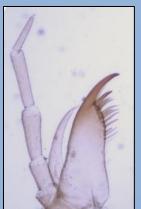












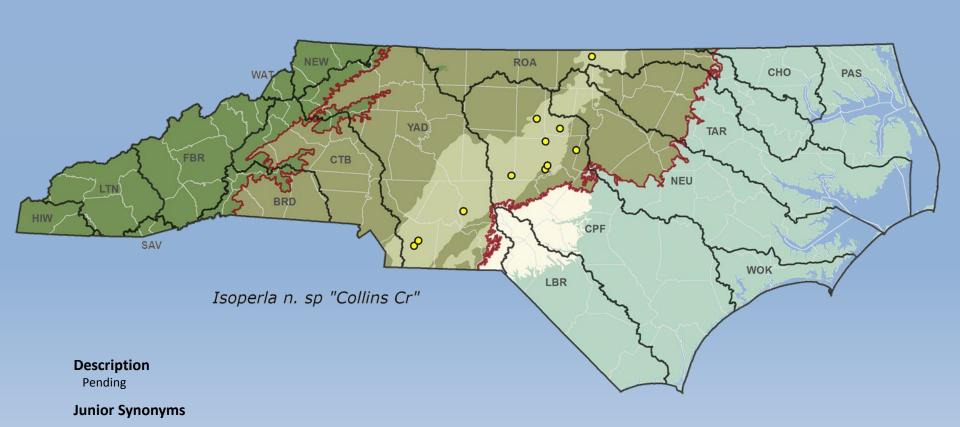


Taxonomic Characters of Nymphs

- o a large but slender species
- head with 2 enclosed pale spots, an oval to diamond shaped ocellar spot and a large median subtriangular pale area
- median pale area almost always with small dark extensions toward but not reaching median ocellus (variously developed)
- frons with extensive blackish spots within brown pattern including anterolateral dark spots near frontoclypeal pale area
- o occiput with brown areas along epicranial stem although somewhat removed
- strong oblique dark stripes originating behind eyes and extending to post-occipital margin
- abdomen with 3 longitudinal stripes, two lateral and one median, and with anterior transverse row
 of 8 small dark dots; median stripe often weak; abdominal spicules with dark origins giving abdomen
 a speckled appearance
- o lacinia receding evenly from subapical tooth and bearing 6-7 stout marginal setae and bearing a closely set submarginal row of 6-8 stout setae from apical tooth
- length of apical tooth of lacinia between 1/3rd to 1/4th palm length and slightly shorter than palm width
- o cerci with sparse dorsal fringe of silky setae on distal half
- o pre-emergent nymphs 9.5-12.0 mm

- o adult males and females distinctive; only 3 previously known eastern species with males with toothed paraprocts (*I. namata, I. signata, I. slossonae*)
- occurs only in Carolina Slate Belt Level IV ecoregion which is comprised of drought susceptible, low flow, often silty streams
- o Type locality: Collins Creek, Orange Co., NC
- o tolerance value currently undefined
- reared and associated (males and females)
- o currently being described by Beaty et al.

Isoperla "Collins Cr." n. sp.



Adult placement

possibly decolorata or phalerata Group

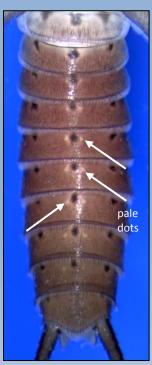
(back to nymphs)

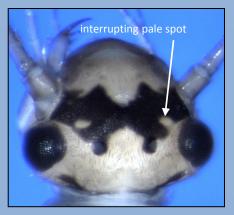
Isoperla "Mayo R" n. sp.

(back to morphology)







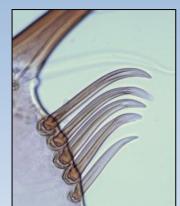




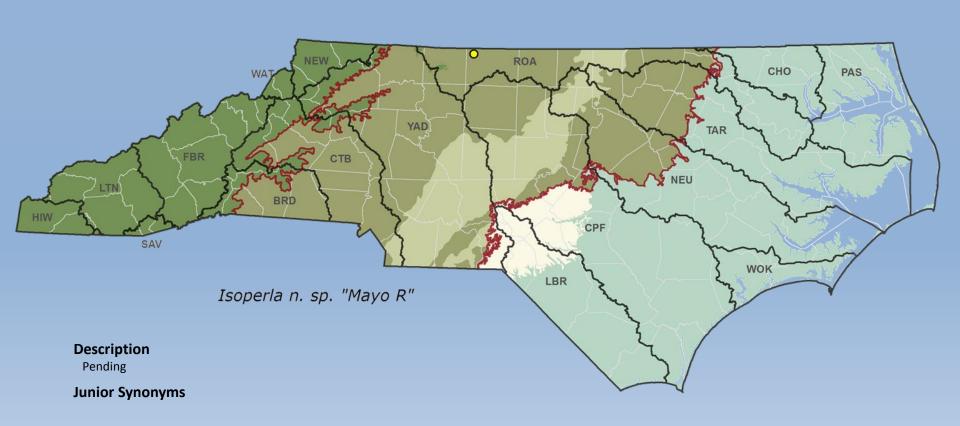
Taxonomic Characters of Nymphs

- o head with irregular transverse medial band with extensions back towards to lateral ocelli
- small oval to round pale spots anterolateral to ocellar triangle which interrupt the
 posterior edge of medial band; spots may be almost completely enclosed
- o pronotum distinctive, with large irregular anterior dark blotches and smaller irregular posterior dark spots; pronotal edge lined with long stiff setae, shorter than in *I. frisoni*
- o thoracic nota with distinctive dark anterior and posterior submedial spots
- o **abdomen vividly reddish-orange in life, each tergum with 3 anterior dark spots;** median spot with small pale lateral dots on either side
- o cerci with a dense long dorsal and ventral fringe of silky setae in distal half
- lacinia evenly receding and bearing 5-6 stout marginal spines, some spines may be slightly widened apically
- o apical tooth approximately 2/3 as long as palm length and subequal to palm width
- o pre-emergent nymphs 7.5-9.0 mm

- o probably rare
- o so far collected only from Mayo River, Rockingham County, NC
- head and pronotal patterns similar to *Isoperla frisoni* but other characters will definitively separate these two species
- o tolerance value currently undefined
- reared and positively associated (males and females)
- $\circ\quad$ currently being described by Beaty et al.



Isoperla "Mayo R" n. sp.



Adult placement

Isoperla nr. holochlora

(back to morphology)













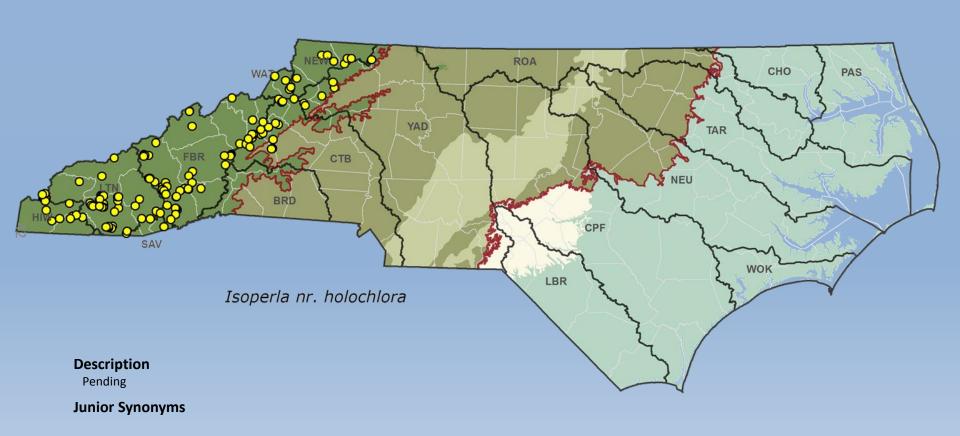


Taxonomic Characters of Nymphs

- head with distinctive pale M-pattern obscured by median pale area and with anterolateral spots (spots may be obscure in some specimens), medial transverse band posterior to the M-pattern but anterior to median ocellus sometimes darker than remainder of head (appearing like a mask)
- ocellar spot obscure to absent, if present then small and faint; mediolateral spots always present
- o occiput with brown areas along epicranial suture, a pale and brown reticulated pattern behind which is enclosed posteriorly by a heavily spiculate curved dark bar from epicranial stem to eye
- o abdomen brown and laterally with 2 conspicuous, longitudinal dark stripes bordered by paler areas, and an obscure narrow median stripe
- o each tergal portion of the lateral stripes usually wider anteriorly and not reaching posterior edge of respective segment (subtriangular on posterior segments) but sometimes parallel and reaching posterior edge; in the former case, stripes will appear contiguous if segments are partially retracted into each other
- o lacinia receding evenly, broad basally, and bearing 5-6 stout marginal setae, 1-2 thinner hair-like marginal setae (not including those along length of the palm), and 4-5 widely spaced thin submarginal setae (arrows)
- o length of apical tooth of lacinia about half the palm length and about 3/4 the palm width
- o cerci with a dense dorsal fringe of long silky setae in distal half (may be present in proximal half but sparse)
- o pre-emergent nymphs 7.5-9.0 mm

- o part of the *holochlora* complex; probable new species, not treated in Szczytko and Kondratieff (2015).
- o adult habitus similar to *I. holochlora* but aedeagus similar to *I. powhatan* with smaller, shorter spine patch
- o historical name within BAB (in use for over 25 years), so named due to it's overall similar appearance to Isoperla holochlora (sort of)
- o widespread throughout NC Appalachians and relatively common in clean, mountain streams
- tolerance value 0.00
- o reared and associated with males and females

Isoperla nr. holochlora



Adult placement

Isoperla nr. transmarina

(back to morphology)



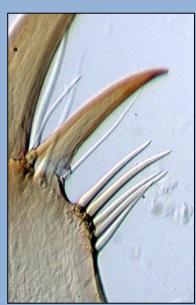










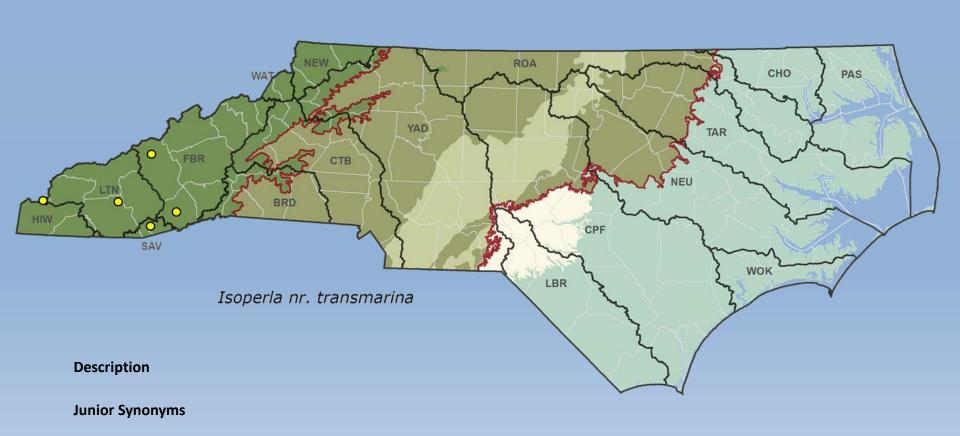


Taxonomic Characters of Nymphs

- head with extensive dark markings, dominated by a transverse median stripe with large irregular extensions forward to the anterior frontoclypeal area and extensions back to lateral ocelli
- o bell-shaped median pale area open to pale anterior frontoclypeal area
- o ocellar spot large, enclosed, and usually roughly triangular
- semi-quadrate pale area anterolateral to bell-shaped median spot; labrum with basolateral dark spots
- o occiput with short, brown areas along epicranial stem directly behind ocellar spot and with strong oblique dark stripes originating behind eyes and extending to post-occipital margin
- o abdomen with 3 longitudinal stripes, two lateral and one median, tergal portions typically hour-glass shaped
- o cerci with a dense dorsal fringe of long silky setae in distal half
- lacinia with low hump below subapical tooth and bearing 5-6 closely set stout marginal spines
- o apical tooth approximately 3/4 as long as palm length and subequal to palm width
- o pre-emergent nymphs 7.0-8.5 mm

- o the nymphal habitus strongly resembles that described for *Isoperla transmarina* by Frison, 1942.
- o the reared female of this species shares the distinctive subgenital plate and head pattern of *Isoperla nelsoni* instead of that of *I. transmarina* or *I. richardsoni*. No confident identification can be made without reared males.
- o nymphs occur in clean, mountain streams although they are rarely collected; true Isoperla transmarina may be a more northerly species and not occur in NC at all
- o tolerance value currently undefined

Isoperla nr. transmarina

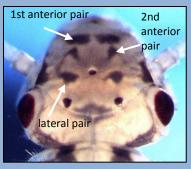


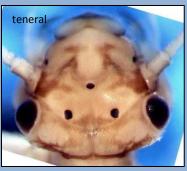
Adult placement

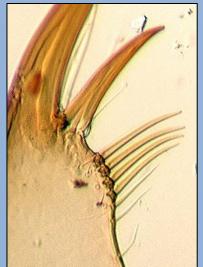
Isoperla sp. 10













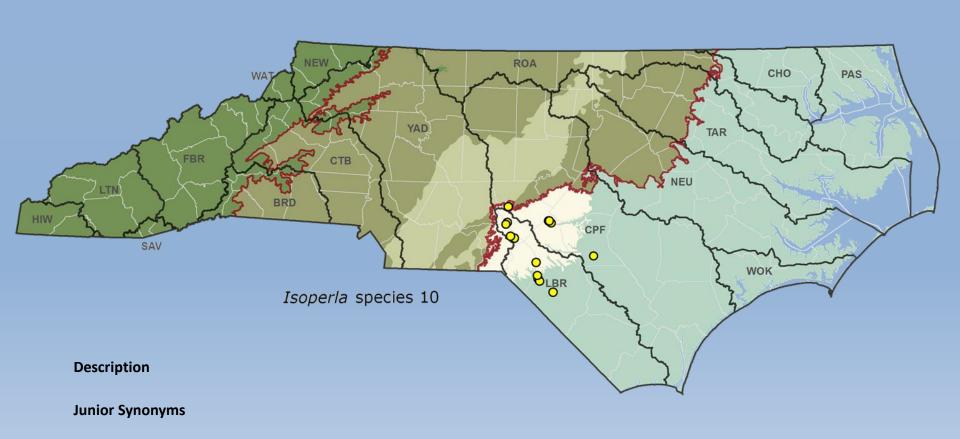
Taxonomic Characters of Nymphs

- o an overall pale species with brown to dark brown markings
- o no enclosed pale areas on head, frontoclypeus with 3 pairs of brown markings in parallel two pairs anterior and one pair lateral to median ocellus, 2nd anterior pair (middle pair) may have brown extensions along the lateral M-arms
- o occiput with brown areas along either side of epicranial stem below ocellar triangle
- o pronotum with large irregular anterior and posterior dark blotches
- o abdomen with two dark lateral and one median longitudinal stripes
- o cerci with a dense dorsal fringe of silky setae in distal half, setae very long on some segments with setae as long as segment itself
- o lacinia with small knob below subapical tooth bearing 4-5 (usually 4) closely set marginal setae, 1-2 thinner marginal setae, and 3-4 submarginal setae
- o length of apical tooth of lacinia 3/4 the palm length and subequal to palm width
- o pre-emergent nymphs 7.5-8.5 mm

- o name is historical BAB designation; origin unknown
- o occurs in tannic streams of the Sand Hills (Level IV ecoregion)
- o may be Isoperla lenati: males appear to be I. lenati, but females do not match I. lenati description and appear more closely aligned with I. frisoni
- o tolerance value currently undefined
- reared with males and females

Adult placement

Isoperla sp. 10



Species unknown as nymphs

Isoperla species currently not associated

Isoperla lenati* Isoperla nelsoni Isoperla zuelligi**

Of course, nymphs of *Isoperla kirchneri gr, I. lata/pseudolata, and I. similis/pseudosimilis* groups need further study and more associations for possible morphological separation of species.

^{*} Identity suspected. See *Isoperla* sp. 10.

^{**} reared from Barnes Cr. ,Montgomery County, NC. Exuvia lost or compromised.

Isoperla sp. VA





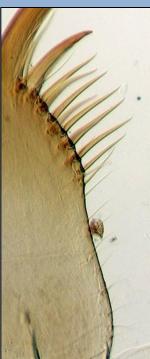


Taxonomic Characters of Nymphs

- o a light brown species
 - head pattern obscure, large M-pattern pale area, may be open medially to anterior frontoclypeal area
- pale ocellar spot narrowly open behind
- Abdomen with obscure median spots that may coalesce into an obscure stripe, areas directly lateral to median stripe paler
- o abdomen may have small dots anterolaterally
- cerci with a sparse and short dorsal fringe of silky setae in distal half
- 5-6 stout marginal spines, 1-2 thin marginal setae, and 6-7 widely spaced submarginal stout setae
- apical tooth approximately half as long as palm length and subequal to palm width
- o pre-emergent nymphs 9.5-11.0 mm







- o not included on identification page as currently unknown from NC but likely to occur
- o BAB determination based on collections from Virginia; not yet collected from NC
- o nymphs so far collected from small headwater streams from VA; along Blue Ridge Parkway (Bedford and Botetourt Co, D. Lenat), White Rocks campground on WV/VA border (Buchanon Co., V. Holland), and in central VA from the northern Piedmont (Albemarle Co., R. Henricks)
- o possibly in NC in high elevation headwater streams or seeps
- o not reared; identity unknown
- o possibly part of similis/pseudopsimilis group?

Acknowledgments

I would like to extend a special thanks to biologists Victor Holland and Dave Lenat for their uncompromised assistance and valuable insights on this project. Their encouragement and unbridled enthusiastic *Isoperla* collecting has allowed for the discovery of new species as well as positive associations with known species. Additionally, Eric Fleek, Mike Walters, and Larry Eaton, taxonomists in the Biological Assessment Branch, collected and contributed nymphs for study, provided comments and discussion on our North Carolina species, and drank beer.

Dr. Boris Kondratieff deserves special mention for encouraging me to proceed with this almost impossible task of pinning down these *Isoperla* identities. He has provided both identifications and confirmations of much of our NC material and loaned nymphs and adults of species recently described by himself and Dr. Stan Szczytko. He is super.

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Stay tuned...

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