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## A NEW SPECIES OF *ISOPERLA* BANKS (PLECOPTERA: PERLODIDAE) FROM THE APPALACHIAN MOUNTAINS, VIRGINIA & WEST VIRGINIA, U.S.A.

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### ABSTRACT

A new species of *Isoperla*, *I. evanescens*, is described from the Appalachian Mountains of Virginia and West Virginia, U.S.A. The new species is proposed based on details of the male aedeagus, paraprocts, vesicle, general body coloration, female subgenital plate, and uniqueness of the chorion of the ovum. Supporting data includes scanning electron micrographs and color images.

**Keywords:** Plecoptera, stonefly, Perlodidae, *Isoperla*, Appalachian Mountains, Virginia, West Virginia

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### INTRODUCTION

The eastern Nearctic species of the genus *Isoperla* were recently reviewed by Szczytko & Kondratieff (2015) recognizing 53 species. A total of 46 species were assigned to twelve species groups, while seven remain unassigned. In their review, species were organized into species groups based primarily on characters of the male aedeagus, female subgenital plate, ovum, and habitus. Recent collecting in the Ridge and Valley Physiographic Province of Virginia (Woodward & Hoffmann 1991) has revealed an additional species belonging to the *I. irregularis* group, which includes *I. decepta* Frison, 1935, *I. irregularis* (Klapálek, 1923), *I. ouachita* Stark & Stewart, 1973, and *I. szczytkoi* Poulton & Stewart, 1987 (Szczytko & Kondratieff 2015, DeWalt et al. 2016). Members of this group are generally characterized by the presence of a posteromedial sclerotized aedeagal structure, an

ovum without a collar, nymphal lacinia with a reduced or absent subapical tooth, marginal lacinial setae continuing to near the base, and a deeply-cleft right mandible with two or three apical teeth (Szczytko & Kondratieff 2015). Previously, 21 species of *Isoperla* had been reported from Virginia and 13 species from West Virginia (Szczytko & Kondratieff 2015), but no members of the *I. irregularis* group have been collected from either state.

### MATERIAL AND METHODS

Adult stoneflies were collected using a beating sheet (BioQuip, Rancho Dominguez, California, catalog # 2840C). Male and female specimens were kept alive in modified ventilated plastic jars (BioQuip, Rancho Dominguez, California, catalog # 8903) for 1-2 days and allowed to fully sclerotize. The male aedeagus was everted from live

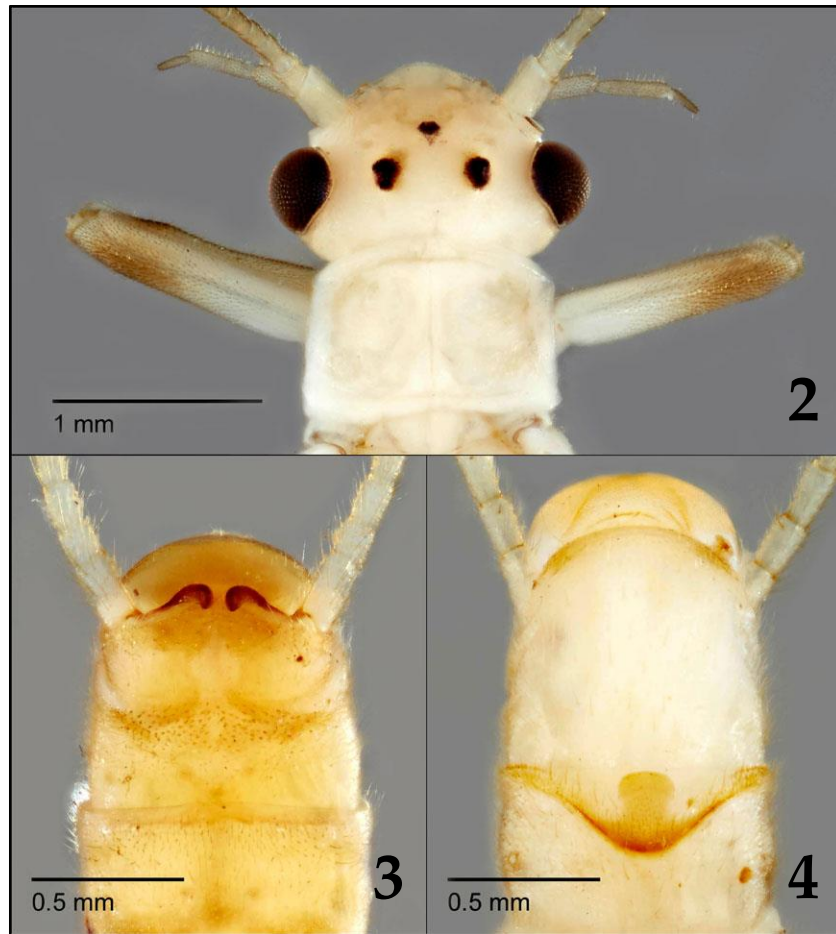


Fig. 1. Dorsal habitus, holotype male, *Isoperla evanescens* sp. n., Virginia, Bland Co., Wolf Creek.

specimens under a stereo dissecting microscope using forceps and fixed in position by briefly submerging them in near boiling water. Additional material was subsequently found among specimens deposited in the C. P. Gillette Museum of Arthropod Diversity, Colorado State University, Fort Collins, Colorado. Data for all currently

known specimens of the new species may be downloaded from a [comma separated values file](#).

Color images were captured using a Canon EOS 5D digital camera with a Canon MP-E 65 mm macro lens. Images resulted from 7-24 serial photomicrographs taken at progressively deeper focal planes using an automated Camlift, these



Figs. 2-4. *Isoperla evanescens*, sp. n., holotype male. Virginia, Bland Co., Wolf Creek. 2. Head and pronotum, dorsal. 3. Abdomen and paraprocts, dorsal. 4. Abdomen and vesicle, ventral.

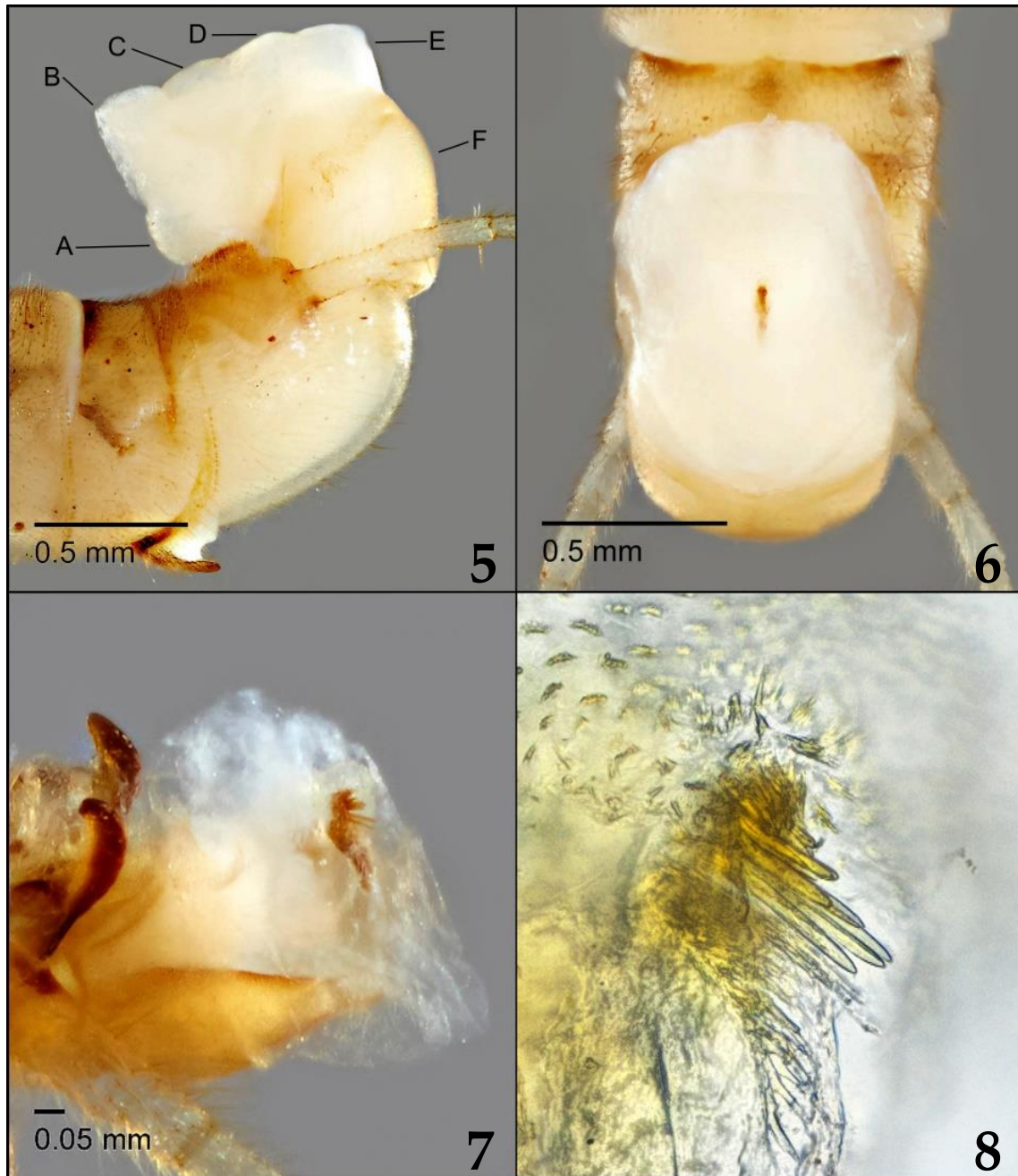
compiled to form a single image using Zerene Stacker version 1.04. Image adjustments and measurements were made using Adobe Photoshop CS6 Extended. Ova were dissected from females and cleaned in alcohol using a fine paint brush to remove tissue adhered to the ovum surface. They were then transferred from 80% ethanol to 100% acetone before being air dried. Ova were mounted to an aluminum stub using double stick copper tape and then sputter coated in 20 nm gold in preparation for scanning electron microscopy. Micrographs of the ova were taken using a JEOL JSM-6500F Field Emission Scanning Electron Microscope at the Central Instrument Facility, Imaging Laboratory, Colorado State University (<http://cif.colostate.edu/imaging-laboratory/>).

Morphology and terminology follow that of Szczytko and Kondratieff (2015). Codens for depositories of specimens are: C.P. Gillette Museum of Arthropod Diversity, Colorado State University, Fort Collins, Colorado (CSUC) and the United States Museum of Natural History, Washington, D.C. (USNM).

## RESULTS AND DISCUSSION

*Isoperla evanescens* Verdone & Kondratieff, sp. n.  
Vanishing Stripetail  
(Figs. 1-19)

<http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:494849>



Figs. 5-8. *Isoperla evanescens*, sp. n., Virginia, Bland Co., Wolf Creek. 5. Aedeagus, lateral: A. anterodorsal lobe, B. dorsal lobe, C. posterodorsal lobe, D. paired posterolateral lobes, E. posteroventral lobe, F. basal setal band. 6. Aedeagus, posterior. 7. Paraproct, posterior. 8. Posteromedial sclerotized structure of aedeagus with ca. 10 short spines and scallop shaped setae, posterolateral, *Isoperla evanescens* sp. n., Virginia, Russell Co., Carr Creek.

**Material Examined.** USA - Holotype ♂, W 81.26314, 8 June 2016, C. Verdone, B. Kondratieff (USNM). Paratypes: same data as holotype, ♀ (USNM); same data as holotype, 4♂, 5♀ (CSUC);  
**Virginia:**Bland Co., Wolf Creek, Grapefield Rd., Co. Rd. 614, Stephen Levitt Property, N 37.14702,



same location, 4 June 2016, C. Verdone, B. Kondratieff, 5♀ (CSUC); same location, 6 June 2016, C. Verdone, B. Kondratieff, ♂, 2♀ (CSUC); Wolf Creek, Grapefield Rd., Co. Rd. 614, USFS Wolf Creek Picnic Area, N 37.18026, W 81.19496, 8 June 2016, C. Verdone, B. Kondratieff, 2♂, 1♀ (CSUC); same location, 12 June 2016, C. Verdone, B. Kondratieff, ♂, 2♀ (CSUC); **Craig Co.**, Potts Creek, Hwy 18, Steel Bridge Campground, N 37.59800, W 80.22704, 10 June 2016, C. Verdone, B. Kondratieff, ♂, ♀ (CSUC); **Giles Co.**, Sinking Creek, Hwy 42, Newport Community Park, N 37.30289, W 80.48522, 10 June 2016, C. Verdone, B. Kondratieff, ♀ (CSUC). **Additional Material:** **Virginia:** **Giles Co.**, Sinking Creek, Rt. 42, Newport Park, N 37.30289, W 80.48522, 21 May 1979, J. Marshall, 2♀ (CSUC); **Grayson Co.**, New River, Rt. 94 bridge, 3 mi. W of Galax, N 36.64633, W 80.97790, 18 May 1994, B. Kondratieff, F. Kirchner, 2♂, 6♀ (CSUC); **Montgomery Co.**, Mill Creek, Rt. 785, N 37.26135, W 80.34054, 1 June 1978, B. Kondratieff, ♀ (CSUC); same location, 15 April 1980, (emerged 27 April 1980), B. Kondratieff, ♂ (CSUC); Tom's Creek, Rt. 655, N 37.238054, W 80.47339, 11 April 1978, (emerged 11 May 1978), B. Kondratieff, 4♀ (CSUC); same location, 2 May 1978, (emerged 8 May 1978), B. Kondratieff, ♀ (CSUC); same location, 2 May 1978, (emerged 12 May 1978), B. Kondratieff, 2♀ (CSUC); same location, 11 May 1978, B. Kondratieff, ♀ (CSUC); same location, 28 May 1978, B. Kondratieff, ♂ (CSUC); same location, 17 May 1979, C. Parker, ♀ (CSUC); same location, 17 May 1979, Wills, ♀ (CSUC); same location, 7 May 1981, B. Kondratieff, 2N (CSUC); same location, 18 May 1979, B. Kondratieff, ♂ (CSUC); **Smyth Co.**, North Fork Holston River, Rt. 620, N 36.94099, W 81.44901, 8 May 1982, B. Kondratieff, 7♂, 3♀ (CSUC); same location, 1 May 1983, B. Kondratieff, 3♂, 2♀, 2N (CSUC); same location, 11 May 1986, B. Kondratieff, 2♂, 1♀ (CSUC); Saltville, N 36.88150, W 81.76206, 27 May 1980, R. H. Zimmerman, ♀ (CSUC); **Russell Co.**, Carr Creek, Co. Rd. 613, Jct. US 58, Bolton, N 36.80438, W 82.19706, 21 May 1993, B. Kondratieff, F. Kirchner, ♂ (CSUC); **Tazewell Co.**, Little River, at Claypool Hill, N 37.03647, W 81.79749, 21 May 1993, B. Kondratieff,

F. Kirchner, ♀ (CSUC); **West Virginia: Summer Co.**, Indian Creek, Rt. 33, N 37.53020, W 80.80283, 10 May 1982, Burkhardt, 3♂, 7♀ (CSUC).

**Distribution.** USA – VA, WV (Fig. 21)

**Etymology.** The name *evanescens* is derived from the Latin root word *evani* meaning “disappearing” or “vanishing” and the Latin adjectival suffix *escens* meaning “becoming,” or “beginning to” and refers to the seemingly vanishing pigmentation on the proximal portions of the wing veins, head and pronotum of the adult.

**Male.** Macropterous; forewing length 9.0 - 10.0 mm (N=5). Body length 7.5 - 8.0 mm (N=5). Head and pronotum pale yellow. General body color pale yellow to light brown with light brown markings dorsally (Fig. 1). Dorsum of head unmarked except for dark pigmented ocelli (Fig. 2). Antennal scape pale yellow; flagellum progressively darkened toward apex. Pronotum unmarked; rugosities irregular, raised, pale yellow (Fig. 2). Femora pale yellow on proximal half, light brown on distal half,

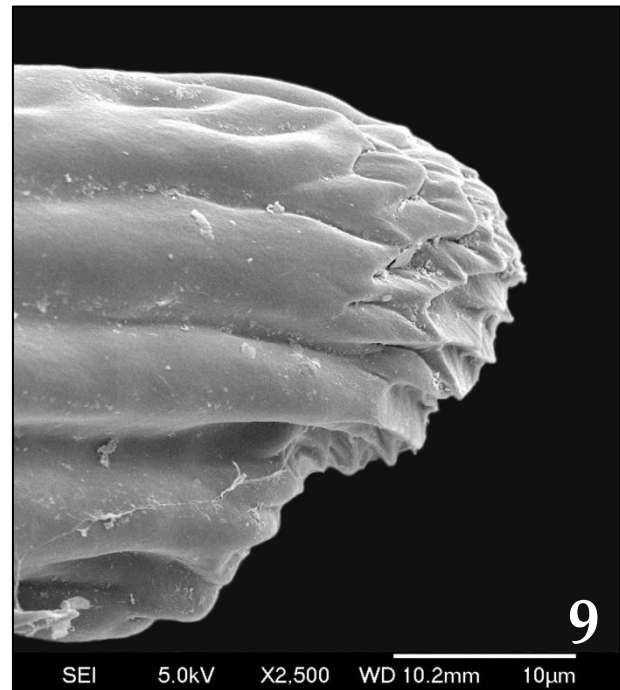


Fig. 9. Male paraproct apex, lateral, *Isoperla evanescens* sp. n. Virginia, Bland Co., Wolf Creek.

darkest on dorsal edge, covered with uniformly spaced, short, brown setae (Fig. 2). Tibia yellow to

light brown, darkest on proximal dorsal edge, covered with uniformly spaced short, brown setae. Tarsi medium brown. Meso- and metanota mostly pale yellow, light brown on posterior margins. Wings pale; veins pale on proximal half, light brown on distal half. Terga pale yellow, posterior  $\frac{1}{3}$  light brown, darkest medially, sometimes forming a stripe; terga 1-8 with uniformly spaced brown setae. Posterior  $\frac{1}{3}$  of tergum 9 with moderately developed patch of sensilla basiconica (Fig. 3). Tergum 10 with a medial, posteriorly directed, pale, spade-shaped marking. Paraprocts short, broadly triangular, slightly incurved (Figs. 3, 7), scattered setae and sensilla basiconica scattered



Fig. 10. Female subgenital plate, ventral, *Isoperla evanescens* sp. n. Virginia, Bland Co., Wolf Creek

over dorsal surface, absent on apex; moderately sclerotized on outer margin; tips bluntly pointed and recurved (Figs. 3, 7, 9). Cerci pale yellow; terminal segments light brown; cercal segments with a single, long, stout, ventral hair at posterior margins. Sterna pale yellow; sternum 8 with a light brown recessed vesicle, 1.4X as long as wide; evenly rounded and expanded posteriorly, extending to the posterior margin of sternum 8

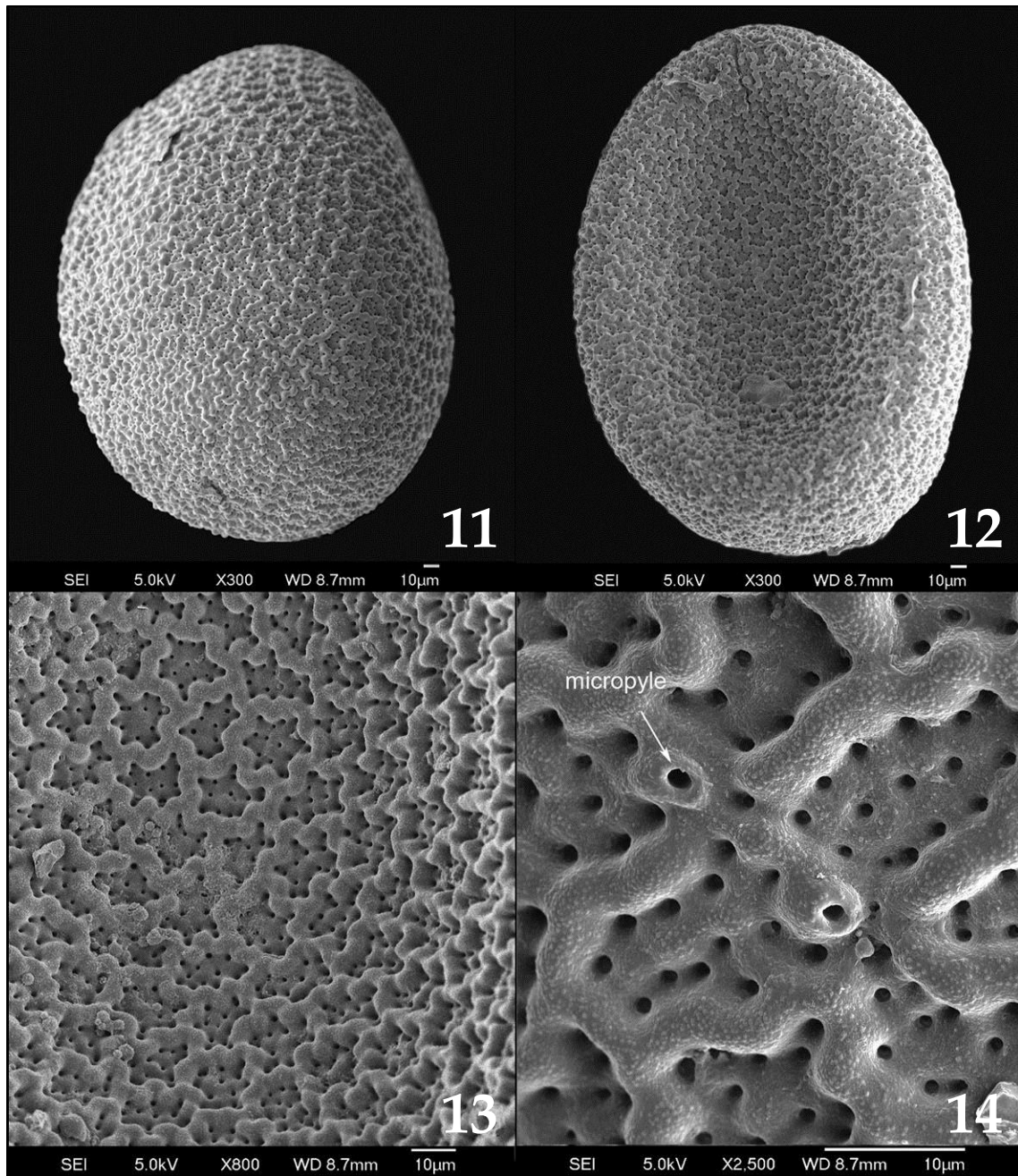
(Fig. 4). Aedeagus with a stout basal stalk, and ridge of short posteromedial spines. Basal stalk completely encircled by dense golden setae (Figs. 5-7); posteromedial area with a small, thin sclerite bearing ca. 10 short spines that are acutely rounded apically; posterior half of sclerite bearing stout setae (Figs. 7-8); area posterodorsal to basal band with small spinulae; areas posterodorsal and anterodorsal to spine bearing sclerite surrounded by scallop-shaped spine plates (Fig. 8).

**Female.** Macropterous; forewing wing length 9.5 - 10.5 mm (N=5). Body length 8.6 - 9.2 mm (N=5). Body coloration and morphology similar to male. Sternum 8 with a light brown, broadly triangular, subgenital plate extending approximately  $\frac{2}{3}$  over sternum 9 (Fig. 10). Sternum 10 with a pair of light brown spots on the posterolateral margins.

**Ovum.** General shape oblong (Fig. 11); cross section concave (Fig. 12); collar absent; eclosion line absent (Figs. 11, 12). Color light brown. Length 312 - 333  $\mu\text{m}$ , width 248 - 250  $\mu\text{m}$  (N=2). Chorion covered with reticulate, raised, thickened ridges, some not connected; fine punctations present between reticulations (Fig. 13). Micropyles positioned singularly on top of ridges near anterior third of egg body; openings usually expanded, doughnut shaped (Fig. 14).

**Male nymph.** Body length 6.9 - 7.5 mm, (N=2). Preserved specimen yellow brown with brown markings (Fig. 15). Dorsum of head with a contrasting pigment pattern (Fig. 16); anterior of frontoclypeus unpigmented; anterolateral dark spots near frontoclypeal pale area (Fig. 16); small enclosed pale spot anterior to median ocellus; small, pale, oval interocellar spot anterior to epicranial suture, narrowly open along epicranial Y-stem; interrupting pale spots present on distal edge of lateral ocelli, open along epicranial suture (Fig. 16). Pronotum with dark irregular ring on either side of wide median pale area; a single pale spot within each ring; lateral margins of pronotum pale (Fig. 15). Abdomen with three longitudinal stripes, two wider lateral, one narrow median, each segment with eight dark spots in an anterior transverse row (Fig. 17). Lacinia unidentate (Fig. 18); lacinia tapering evenly from apical tooth and bearing stout marginal setae to near base; medial





Figs. 11-14. *Isoperla evanescens* sp. n. Virginia, Bland Co., Wolf Creek. 11. Ovum. 12. Ovum, showing concave section. 13. Detail of ovum reticulations and punctuations. 14. Detail of ovum chorion and micropyles.

submarginal setae arranged in a close set row below apical tooth (Fig. 18A), no setae on outer surface of apical tooth as in other species of the group; submarginal setae continuous to near base;

gaps between setae increase towards base; apical tooth approximately  $\frac{1}{5}$  as long as palm length. Mandible with 6 teeth and a deep cleft between apical and subapical teeth; most noticeable on

dorsum (Fig. 19); dorsal apical tooth flattened, broadly rounded; interior edge serrated, ventral apical tooth acutely rounded (Fig. 19A).



Fig. 15. Nymphal dorsal habitus, *Isoperla evanescens* sp. n., Virginia, Smyth Co., North Fork Holston River.

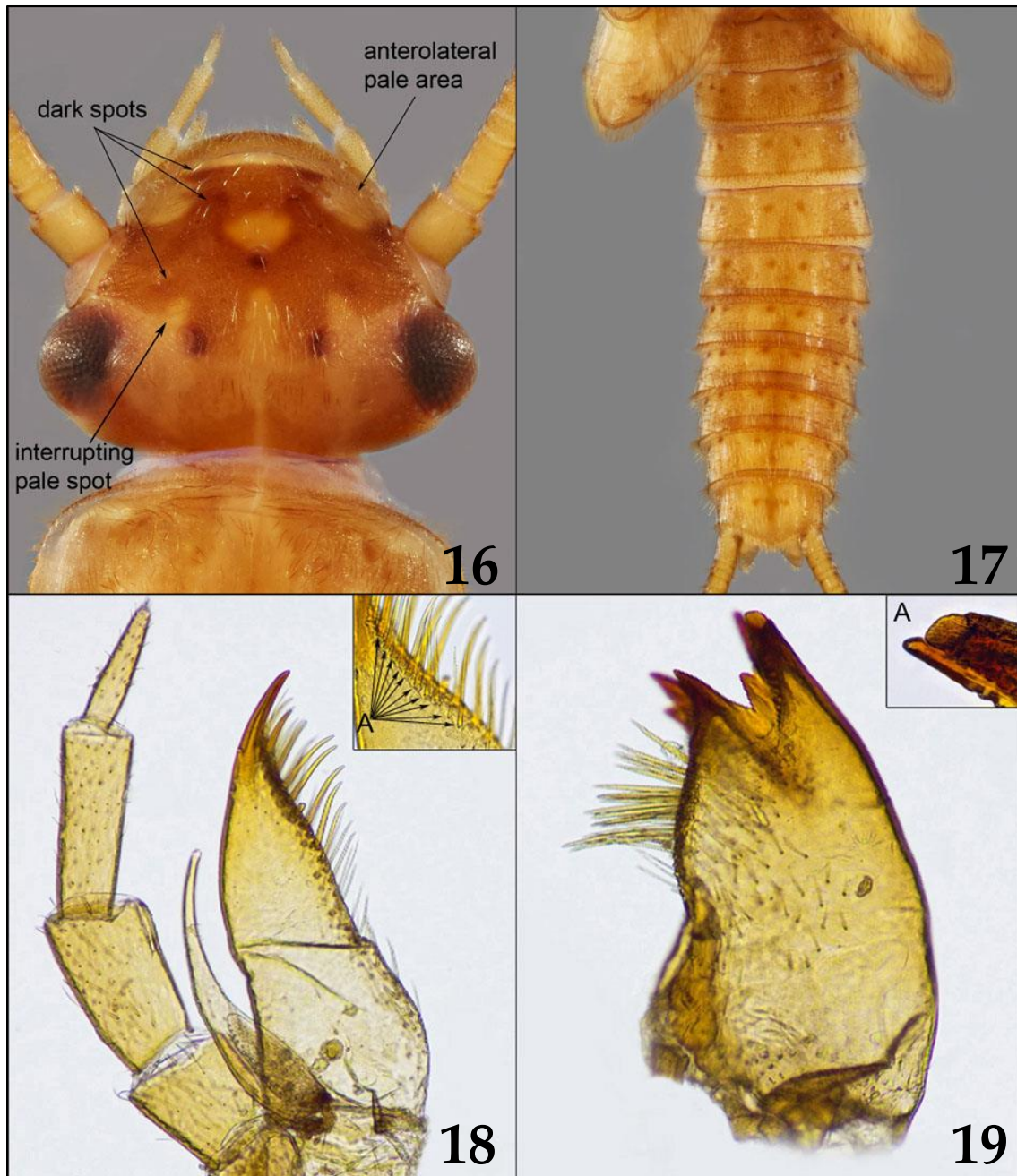
**Diagnosis.** *Isoperla evanescens* is placed in the *I. irregularis* group and is the only member of the group currently known to occur east of the Appalachian Plateau Physiographic Province (Woodward & Hoffmann 1991). *Isoperla evanescens* is superficially similar to *I. decepta* in general habitus, which also lacks all dark markings on the head, (fig. 12.1, Szczytko & Kondratieff 2015). However, males of the two species are separable by the shape of the paraprocts, vesicle, details of the

aedeagus, and pronotal rugosity pigmentation. The paraprocts of *I. evanescens* are broadly triangular and extend only slightly over the posterior margin of tergum 10 (Figs. 3, 7); the vesicle is expanded posteriorly (Fig. 4); the posteromedial sclerotized structure of the aedeagus is composed of a thin sclerite bearing setae and ca. 10 short spines (Fig. 8); additionally, *I. evanescens* lacks dark pigmentation on the pronotum (Fig. 2). Whereas, in males of *I. decepta*, the paraprocts are elongate and extend  $\frac{1}{4}$  to  $\frac{1}{2}$  over tergum 10 (fig. 12.3, Szczytko & Kondratieff 2015); the vesicle is not expanded posteriorly (fig. 12.2, Szczytko & Kondratieff 2015); the posteromedial sclerite of the aedeagus is a lobed structure that is deflected ventrally and is scattered with concentrations of stout sharp spinulae (figs. 12.6, 12.10, 2.11, Szczytko & Kondratieff 2015). Additionally, *I. decepta* has medium to pale brown rugosities on the pronotum (fig. 12.1, Szczytko & Kondratieff 2015).

Females of *I. evanescens* can be separated by the unique combination of a pale yellow head in life, whitish in ethanol preserved specimens, pronotum without dark markings, and a broadly triangular subgenital plate (Fig. 10). Whereas, females of *I. decepta* have medium to pale brown rugosities on the pronotum and the subgenital plate is broadly rounded (fig. 12.4, Szczytko & Kondratieff 2015). Ova of the two species can be separated on the basis of cross sectional shape and the presence of reticulate ridges. The ovum of *I. evanescens* has a concave cross section and possesses reticulate, raised, thickened ridges (Figs. 11-14). Whereas, the ovum of *I. decepta* is circular in cross section and lacks reticulate ridges (figs. 12.18, 12.19 Szczytko & Kondratieff 2015). The ovum of *I. evanescens* is most similar to *I. nana* (Walsh, 1862), but can be separated by the presence of inter-reticulate punctations (Fig. 13), which *I. nana* lacks (figs. 35.13-35.15, Szczytko & Kondratieff 2015).

Several specimens of *I. evanescens* that are deposited in the C.P. Gillette Museum of Arthropod Diversity had previously been identified as *I. dicala* Frison, 1942, which is regionally common and is often sympatric with *I. evanescens*. Recently collected material of *I. evanescens* with everted aedeagus has allowed the





Figs. 16-19. *Isoperla evanescens* sp. n. Virginia, Smyth Co., North Fork Holston River. 16. Nymphal head, dorsal. 17. Nymphal abdomen, dorsal. 18. Nymphal left lacinia, dorsal: A. submarginal setae. 19. Nymphal left mandible, dorsal: A. dorsal apical tooth and ventral apical tooth.

separation of these two species, which can be confused by the lack of usually dark dorsal head pigmentation. Males of these species without the aedeagus everted can also be separated by the

differences in pronotal pigmentation and details of the vesicle. *Isoperla dicala* has dark pronotal pigmentation and a vesicle that is 3X as long as wide (figs. 14.1, 14.2, Szczytko & Kondratieff 2015).

Whereas, *I. evanescens* lacks pronotal pigmentation and the vesicle is 1.4X as long as wide. Females can be separated by the differences in the pronotal pigmentation and details of the subgenital plate. The subgenital plate of *I. dicala* usually possesses a

medial nipple (fig. 14.5, Szczytko & Kondratieff 2015), whereas *I. evanescence* lacks it. Eggs of the two species are not similar and can be easily separated by the presence or absence of a collar;



Fig 20. Type locality. Virginia, Bland Co., Wolf Creek, Grapefield Road.

*I. dicala* possesses a collar (fig. 14.13, Szczytko & Kondratieff 2015), whereas *I. evanescens* lacks one.

The following couplets to the keys to males, females, and ova are taken directly from Szczytko & Kondratieff (2015). New couplets are given to include the new species.

#### Males

- 11 Dorsal head pattern pale, usually without dark brown bands or brown markings connecting ocelli (Figs. 14.1, 12.1) ..... 12
- 11' Dorsal head pattern with dark brown bands connecting ocelli (Figs. 17.1, 20.1, 33.1) ..... 13
- 12 Vesicle 3X as long as wide, set in a deep U-shaped depression  $\frac{3}{4}$  as long as length of vesicle (Fig. 14.2); paraprocts lightly sclerotized, bluntly pointed apically, not deflected ventrad

- apically (Fig. 14.4), aedeagus with erect posteromesal sclerotized unicorn-shaped rod (Figs. 14.6, 14.7); head pattern occasionally with thin dark brown bands connecting ocelli distribution—widespread—eastern and midwestern North America ..... *I. dicala*
- 12' Not as above ..... 12a
- 12a Posteromedial sclerotized structure of the aedeagus composed of a thin sclerite bearing 10 short spines and setae (Figs. 6-8); paraprocts broadly triangular extending only slightly over the posterior margin of tergum 10 (Figs. 3, 7, 8) ..... *I. evanescens*
- 12a' Posteromedial sclerotized structure lobe-like, deflected ventrally with scattered concentrations of stout sharp spinulae (figs. 12.6, 12.10, 2.11, Szczytko & Kondratieff 2015);



paraprocts lightly sclerotized, elongate, sharply pointed apically, extending over ca.  $\frac{1}{4}$ - $\frac{1}{2}$  length of tergum 10 (figs. 12.3,12.5, Szczytko & Kondratieff 2015) ..... *I. decepta*

#### Females

- 1 Head without a dark pigment pattern, usually without dark brown bands or brown markings connecting ocelli (Figs. 12.1, 14.1) ..... 2
- 1' Dorsal head pattern with discernible dark brown bands connecting ocelli (Figs. 42.1, 6.1)... 3

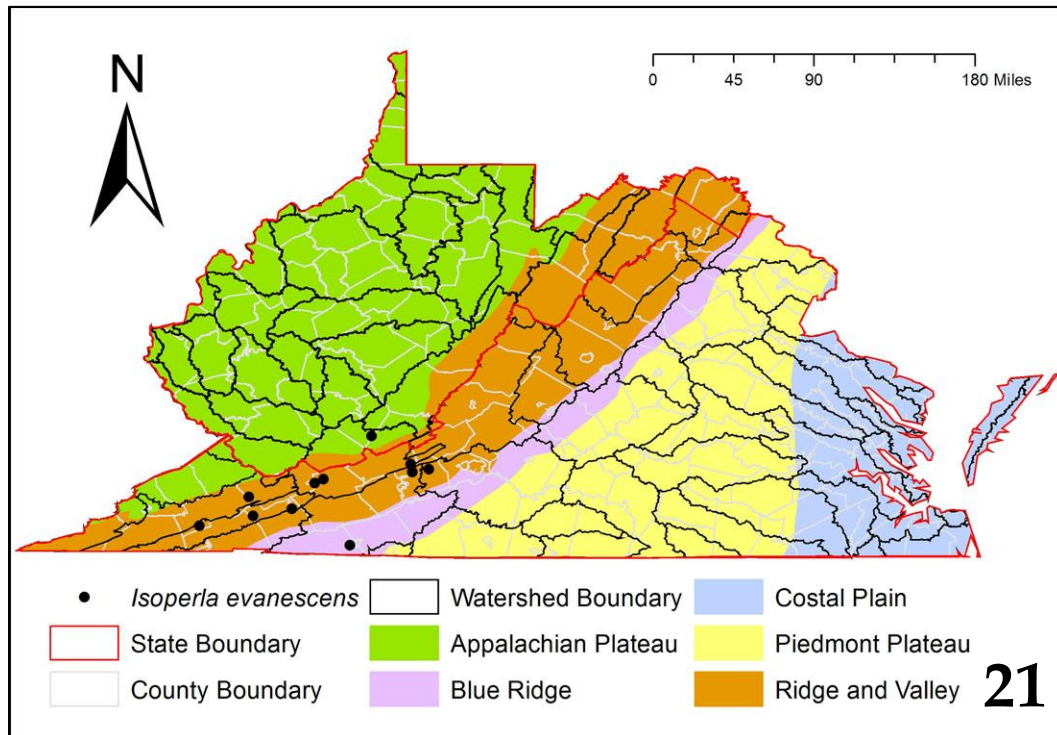


Fig. 21. Distribution of *Isoperla evanescens* sp. n. in Virginia and West Virginia.

- 2 Subgenital plate broadly triangular, extending  $\frac{1}{2}$  length of 9<sup>th</sup> sternum; usually with posteromedian nipple (Fig. 14.5); distribution-widespread, eastern North America through midwestern US ..... *I. dicala*
- 2' Subgenital plate evenly rounded to broadly triangular extending over  $\frac{1}{3}$  or  $\frac{2}{3}$  length of 9<sup>th</sup> sternum (fig. 12.4, Szczytko & Kondratieff 2015; Fig. 9) ..... 2a
- 2a Subgenital plate evenly rounded extending over  $\frac{1}{3}$  length of 9<sup>th</sup> sternum (fig. 12.4, Szczytko & Kondratieff 2015) ..... *I. decepta*
- 2a' Subgenital plate broadly triangular, extending over  $\frac{2}{3}$  length of 9<sup>th</sup> sternum (Fig. 9) ..... *I. evanescens*

#### Eggs

- 1 Collar absent (Figs. 12.18, 22.13, 35.13) ..... 2
- 1' Collar present (Figs. 28.14, 36.9, 41.13, 58.15) ...6
- 2 Chorion covered with raised reticulate thickened ridges, some not connected, eclosion line absent, inter-reticulate punctations absent (figs. 35.13, 35.14 Szczytko & Kondratieff 2015).....*I. nana*
- 2' Not as above ..... 2a
- 2a Inter-reticulate punctations present (Fig. 14) ..... *I. evanescens*
- 2a' Chorion without raised reticulate thickened ridges, eclosion line present or absent (figs. 12.18, 22.13, 22.14 Szczytko & Kondratieff 2015) ..... 3
- 3 Cross section triangular, eclosion line wide, smooth (Figs. 22.13, 22.15) ..... *I. irregularis*



3' Cross section not triangular, either round or concave, eclosion line absent (Figs. 12.18, 23.21, 57.15) ..... 4

**Biological Notes.** There is no information about the biology or life cycle of *I. evanescens*. Based on the above records, the emergence period appears to be from early May to mid-June and, as with most eastern *Isoperla*, a univoltine life cycle is presumed. The type locality, Wolf Creek (Fig. 20), has a diverse stonefly fauna. Other adult stoneflies collected with the new species at the type locality were *Acroneuria abnormis* (Newman, 1838), *A. kosztarabi* Kondratieff & Kirchner, 1993, *Agnatina capitata* (Pictet, 1841), *A. flavescens* (Walsh, 1862), *Amphinemura delosa* (Ricker, 1952), *A. nigrutta* (Provancher, 1876), *A. wui* (Claassen, 1936), *Diploperla morgani* Kondratieff & Voshell, 1979, *I. dicala* Frison, 1942, *I. montana* (Banks, 1898), *I. signata* (Banks, 1902), *Leuctra alexanderi* Hanson, 1941, *L. duplicata* Claassen, 1923, *Neoperla occipitalis* (Pictet, 1841), *Paragnetina media* (Walker, 1852), *Perlesta decipiens* (Walsh, 1862), *P. puttmanni* Kondratieff & Kirchner, 2003, and *P. teaysia* Kirchner and Kondratieff, 1997.

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## REFERENCES

- DeWalt, R.E., M.D. Maehr, U. Neu-Becker & G. Stueber. 2016. Plecoptera Species File Online. Version 5.0/5.0. Available online: <http://plecoptera.speciesfile.org/> (accessed 26 September 2016).
- Szczytko, S.W. & B.C. Kondratieff. 2015. A review of the eastern Nearctic Isoperlinae (Plecoptera: Perlodidae) with the description of twenty-two new species. Monographs of Illiesia, Number 1:1-289. <http://illiesia.speciesfile.org/papers/Monographiae-of-Illiesia.pdf>
- Woodward, S.L. & R.L. Hoffmann. 1991. The nature of Virginia. pp. 23-48. In K. Terwilliger (Ed.). Virginia Endangered Species: Proceedings of a Symposium. McDonald & Woodward Publishing Company, Blacksburg, Virginia. 672 pp.

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