



***Polycentropus aileenae* n. sp. (Trichoptera: Polycentropodidae), a caddisfly species from the eastern Nearctic, with new state records for members of the *Polycentropus confusus* Species Group**

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Abstract

A new eastern Nearctic species of tube-making caddisfly of the genus *Polycentropus* Curtis 1835 (Trichoptera: Polycentropodidae) is described and illustrated based on adult males and females. *Polycentropus aileenae* n. sp., a member of the *Polycentropus confusus* Species Group, is reported from the Canadian province Nova Scotia and the U.S. states Tennessee and Pennsylvania. The species is diagnosed based on multiple aspects of the male genitalia, including inflection of the dorsal processes of the preanal appendages and the slightly recurved dorsum of the apex of the phallus. New U.S. state records of *P. confusus* Hagen 1861 and *P. elarus* Ross 1944 from Florida, and *P. thaxtoni* Hamilton & Holzenthal 1986 from Alabama, are also provided.

Key words: Alpha taxonomy, Annulipalpia, aquatic insects, DNA barcoding, biodiversity

Introduction

The Nearctic fauna of the genus *Polycentropus* Curtis 1835 (Trichoptera: Polycentropodidae) contains 29 previously described species, with the greatest diversity observed east of the Mississippi River (Rasmussen & Morse 2020). Most of the eastern fauna comprises the *Polycentropus confusus* Species Group, which ranges from Manitoba, Canada south to northern Florida, USA, and from the Atlantic coast to the western corridor of the Mississippi River (Hamilton 1986; Rasmussen & Morse 2020; Orfinger *et al.* 2021). With the description of this new species, the species group is currently represented by 19 nominal species.

Members of the *Polycentropus confusus* Species Group are united by the following features of the male terminalia: Each inferior appendage has an elongate, caudally-directed, ventral portion and an erect, dorsobasal process with a rugose mesal point; each preanal appendage has a reduced setose body and a long, slender, caudoventrally-curved dorsomesal process (Armitage & Hamilton 1990).

While a comprehensive revision of the *Polycentropus confusus* Species Group has yet to be performed, multiple works have previously reviewed the group's species. Among these, the two most recent treatments were by Hamilton (1986) and Armitage and Hamilton (1990). Hamilton's (1986) doctoral dissertation described uniting characters of the *P. confusus* Species Group, provided an annotated list of species, described and illustrated the male of "*Polycentropus* new species 12" (= *P. alabamensis* Hamilton, Harris & Lago 1990), and illustrated wing venation of *P. confusus* Hagen 1861 and *P. alabamensis*. Hamilton stressed the need for in-depth study of the group, emphasizing the importance of associating females and larvae and the need to address phylogeny. The atlas by Armitage and Hamilton (1990) later included a species-by-species summary of the group assembling the most relevant diagnostic characters, adult illustrations, some type data, selected references, site-specific maps, and a key to males.

Subsequent to the atlas by Armitage and Hamilton (1990), four additional species have been described: *P. ala-*

bamensis, *P. vernus* Hamilton, Harris & Lago 1990, *P. stephani* Bowles, Mathis & Hamilton 1993, and *P. dinkinsonorum* Orfinger & Etnier 2020.

As with most Trichoptera, the taxonomy of this species group is based on male genital morphology. To date, females of 11 species (including the species described here) and the larva of only one species have been described. The present work constitutes part of a larger effort to fill these taxonomic gaps that will ultimately culminate in a revision of the *Polycentropus confusus* Species Group treating males, females, and larvae. Herein, the male and female of *P. aileenae* n. sp. are described and illustrated.

Materials and methods

Molecular Association of Sexes

To associate sexes of the new species, the entire 658 base pair barcoding region of cytochrome oxidase I (COI) was sequenced from candidate females collected from two localities in eastern Tennessee, including the type locality. One hind leg from each of 6 female polycentropodid specimens were homogenized and lysed in 150 µl of a Tris-TAPS lysis buffer to which 5 µl of a 20 mg/ml proteinase-K (ThermoFisher Scientific, Waltham, MA) solution was added. Total genomic DNA was extracted from the lysate using the Zymo Research DNA Clean & Concentrator-5 kit; after application of 3.5X volumes binding buffer, DNA was bound to the silica column via centrifugation (8,000 x g for 30s), washed twice with 200 µl wash buffer (13,000 x g for 30s), and eluted in 50 µl of 10 mM Tris (pH = 8.0) (13,000 x g for 30s). The resulting gDNA was stored at minus 20°C until use. PCR amplifications were conducted using TaKaRa Ex Taq Hotstart DNA polymerase (Takara Bio, Shiga, Japan) following the manufacturer's suggested protocol, with 1.0 µL of template DNA and 3 µl each of 20 µM custom forward (5'-GGAACCTTATATTTTATTT-TYGG-3') and reverse (5'-TCATTCKATWRAAGAWTTTMTTTG-3') primers; reactions were accomplished in GenePro (Bioer Technology Co., Hangzhou, China) thermal cyclers. The following PCR regimen was used: 90s denaturing step at 94°C; 4 cycles of 30s at 94°C, 30s at 56°C and 75s at 72°C; 4 cycles of 30s at 94°C, 25s at 52°C, and 75s at 72°C; 9 cycles of 30s at 94°C, 20s at 48°C, and 75s at 72°C; and finally, 38 cycles of 30s at 94°C, 20s at 43°C, and 75s at 72°C. Cycling reactions were completed after a 5-min 72°C soak and were held indefinitely at 15°C.

PCR products were electrophoresed in, and excised from, a 1% agarose gel run at 118V for 30 min. Amplicons were purified using Econospin® silica columns (Epoch Life Science, Sugar Land TX) using 13,000 x g for each centrifugation step. Briefly, 365 µl of 5M guanidium thiocyanate and 100 µl of 100% isopropanol were added to each 1.7 ml Eppendorf tube containing a gel slice and the tubes heated at 55°C until the gel slices were fully dissolved. The resulting lysate was transferred to a silica column (with supplied eluent tube) and the DNA bound via centrifugation for 30s. After discarding the eluent, the column was washed twice via centrifugation for 30s with 350 µl 80% ethanol, dried via centrifugation for 2 min, and eluted into a fresh tube in 75 µl 10mM Tris HCL, pH = 8 via centrifugation for 30s. Silica column-based gel purified PCR products served as template for Sanger® sequencing in both directions in 20 µL reactions using 20-fold diluted (= 0.4 µl) BigDye® v3.1 terminators (Applied Biosystems, Carlsbad, California) using a 5X dilution buffer blend containing ABI 5X dilution buffer, BDX-64 (MCLAB Products, South San Francisco, CA), and BetterBuffer (GelCompany, San Francisco, CA) and a cycling regimen that included 47°C, 45°C, and 43°C touchdown annealing. Sequencing reactions were cleaned using Centrisep columns (Princeton Separations, Adelphia, NJ) and dried in a Centrivap Concentrator (LABCONCO, Kansas City, MO). Dried samples were sent to the University of Tennessee, Knoxville, Genomics Core for analysis using an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequences from opposing strands were reconciled and verified for accuracy using Sequencher 4.7 (Gene Codes Corp., Ann Arbor, MI). All sequences are deposited in BOLD under a dataset titled "DS-POLYCSS Nearctic Polycentropodidae (Trichoptera)" (Orfinger *et al.* 2021). Resulting sequence data were compared to COI data of the male type specimens and other members of the *Polycentropus confusus* Species Group available in the same BOLD dataset. Two females of *Polycentropus aileenae* were associated by the fact that the 658 bp barcoding fragments of COI are identical to sequences derived from male type specimens.

Morphological Study

Specimens were observed under a Unitron Z10 stereomicroscope with magnification up to 120x. Measurements were obtained using a calibrated ocular micrometer. Reported lengths were measured from the anterior of the head

(excluding antennae) to the tip of the forewing. To evaluate internal morphology, genitalia were cleared with a heated solution of 85% lactic acid following Blahnik *et al.* (2007), then rinsed with ethanol. Genitalia were viewed in glycerine, and then stored in a microvial within a vial of 80% ethanol with the rest of each respective specimen. The phallus was excised from some specimens for illustration. Line drawings were produced using a 10 x 10 gridded ocular lens in conjunction with a gridded guide and pencil. Drawings were then scanned and used as templates for the final illustrations using Adobe Illustrator® version 24.3. Morphological terminology follows Hamilton *et al.* (1990) and Orfinger and Etnier (2020) for males, and Nielsen (1980) and Hoffman and Morse (1990) for females. Representative males and females of the other members the *P. confusus* Species Group were examined and compared with those of *P. aileenae*.

The holotype and a single male paratype have been deposited at the US National Museum of Natural History, Smithsonian Institution, Washington, District of Columbia, USA (NMNH). Other paratypes and non-type specimens have been deposited in the Centre for Biodiversity Genomics, University of Guelph, Ontario (BIOUG); Clemson University Arthropod Collection, Clemson, South Carolina (CUAC); Illinois Natural History Survey, Champaign, Illinois (INHS); Florida State Collection of Arthropods at Florida A&M University, Tallahassee, Florida (FAMU); the Royal Ontario Museum, Toronto, Ontario (ROM); the Trichoptera Collection at the University of Tennessee, Knoxville, Tennessee (UTK).

Taxonomy

Polycentropus aileenae Orfinger & Moulton, n. sp.

Figures 1, 2A–2E, 3A, 3B.

Polycentropus carlsoni Morse 1971 (Moulton 2007: 209–210, nec Morse 1971)

Description. General Structure (Fig. 1). Length of male 6.1–7.0 mm (n = 5). Length of female 7.5–8.75 mm (n = 2). General habitus typical of males of the *Polycentropus confusus* Species Group. In ethanol, eyes brown to purple and glazed; body color various shades of brown, with subtle spots of lighter brown and yellow forming irrorate pattern on front wings; hind wings uniformly brown; legs tan to pale yellow.



FIGURE 1. Left lateral habitus of the male of *Polycentropus aileenae* n. sp. Scale bar = 1 mm.

Male Genitalia (Figs 2A–2E): Abdominal segment VIII annular. Segment IX tergum fused with segment X, membranous, caudally extended as short, horn-like projection. Segment IX sternum ovoid in lateral view, posterior margin slightly sinuous. Intermediate appendages positioned beneath terga IX+X, distally extending beyond tergum X, apices each bearing 3 small setae; in lateral view curved slightly ventrad; in dorsal view apices proximate, subparallel. Bodies of preanal appendages (pre.app.) slightly produced, each with small posteroventral lobate process; in lateral view dorsal process long, whip-like, curved ventrad; in dorsal view slender, elongate, strongly inflected and converging apically. Inferior appendages in lateral view each with ventral portion gradually tapered, extended posterad slightly beyond intermediate appendages, ventral margin nearly straight; in ventral view, basal half wide, distal half abruptly narrowed, inner margin curved outward; dorsobasal process in lateral view erect, with long neck terminating in short, rounded head projecting downward, in ventral view obscured completely by ventral portion of clasper. Phallus in lateral view curved ventrad, apical section rectangular, dorsum of apex slightly recurvate (subtle in some specimens), spinules absent, phallic sclerite moderately elongate.

Female Genitalia (Figs 3A, 3B): Venter VIII in ventral view with ventral plates (v.pl.) narrow, tapering apically, convergent, and rounded at posterior apices in ventral view, covered in setae; in lateral view similar but each terminating in more-acute posterior apex; posterior apex of external parts of gonopods VIII (p.ap.e.gon.VIII) with posterior margin slightly triangular and rounded mesally in ventral view; in lateral view extending beyond ventral plates in rounded process (e.gon.VIII); internal parts of gonopods VIII (i.gon.VIII) in ventral view visible through venter VIII, appearing longitudinally wrinkled, darker than surrounding tissue, parallel, semi-elliptical with rounded posterior apices distinctly anterior of apices of ventral plates, anterior ends oblique, extending to sclerotized external part of gonopods VIII at basal third of ventral plates; in ventral view anterior part of genital chamber (g.ch.a) sclerotized, semicircular; processus spermathecae (pr.sp.) subovoid with central elevation bearing opening of ductus spermathecae (op.dt.sp.).

Pupa and larva: Unknown

Eggs: In ethanol, white, smooth in appearance, each roughly spherical, 0.22 mm to 0.30 mm in diameter; extruded linearly in a moniliform chain.

Diagnosis. The male genitalia of *Polycentropus aileenae* are most similar to those of males of *P. blicklei* Ross & Yamamoto 1965, *P. carlsoni*, *P. carolinensis* Banks 1905, and *P. elarus* Ross 1944. *Polycentropus aileenae* can be separated from the above-mentioned species by the strongly inflected apices of the dorsal processes of the preanal appendages in dorsal view versus straighter dorsal processes in the other species, and the slightly recurved dorsum of the apex of the phallus in lateral view absent in other species. From *P. blicklei*, *P. carlsoni*, and *P. carolinensis*, the new species differs in the shape of the ventral portion of each inferior appendage in which the lateral and ventral margins are nearly straight versus moderately curved in *P. carolinensis* and *P. carlsoni*, and by the long rectangular basal half that in ventral view narrows abruptly from middle to apex versus gradual narrowing along length in *P. blicklei*. *Polycentropus aileenae* differs in the shallower and more rounded body of each preanal appendage versus the more broadly produced and more acutely terminated body of each preanal appendage in *P. blicklei*, *P. carolinensis*, and *P. elarus*.

Female genitalia of *Polycentropus aileenae* are most similar to those of *P. blicklei*, for which only subtle differences are apparent in separating females of the two species. The internal parts of gonopods VIII are rounded at the posterior apices versus the sharper posterior apices resulting in each having a blade-like appearance in *P. blicklei*, and the internal parts of gonopods VIII are parallel in *P. aileenae* versus subparallel in *P. blicklei*. In addition, the processus spermathecae is slightly more rounded in *P. aileenae* relative to the more ovoid shape in *P. blicklei*.

Material Examined. Holotype: USA, Tennessee: Knox County, Fowler Spring Branch, first-order tributary to Beaver Creek, Bell Campground Road, 0.3 km N of jct. with W. Brushy Valley Drive, UV Light Trap, 277 m elevation, [N36°02'56.2", W84°04'07.6"], A. Orfinger and A. Perilla coll., 6-vi-2020, 1 male (USNMENT01445176).

Allotype: USA, Tennessee: Same data as for holotype except J.K. Moulton coll., 9-x-2005, 1 female (FAMU).

Paratypes: USA, Tennessee: Same data as for holotype except J.K. Moulton coll., 9-x-2005, 1 male (USNMENT01445177). Same data except 18-xi-2006, 1 male (CUAC000107331). Jefferson County, Dumplin Creek at Hebron Church Road, UV Light Trap, 337 m elevation, [N36°03'38.8", W83°27'20.6"], J.K. Moulton coll., 30-v-2006, 5 males (INHS Insect Collection 923903). Same data except 9-viii-2006, 1 male, (FAMU). Jefferson County, Tributary of Holston River at Good Hope Church Road, UV Light Trap, 280 m elevation, [N36°09'58.3", W83°33'49.7"], G. Curler coll., 28-v-2005, 1 male (ROME187177). Jefferson County, Dumplin Creek at old TN Rt.

92, UV Light Trap, [N36°03'38.8", W83°27'20.6"], J.K. Moulton coll., 23-v-2005, 1 female (CUAC000107332).

Non-Type Material: **Canada, Nova Scotia:** Cape Breton Highlands National Park, Clyburn Valley Road, near golf course, Malaise Trap, 21 m a.s.l., [N46°39'19.1", W60°25'42.6"], CBHNP Staff coll., 12/VII/2013, 1 male (BIOUG09994-A03). **USA, Tennessee:** Jefferson County, Dumplin Creek at old TN Rt. 92, UV Light Trap, [N36°03'38.8", W83°27'20.6"], J.K. Moulton coll., 26-v-2006, 5 males (UTK).

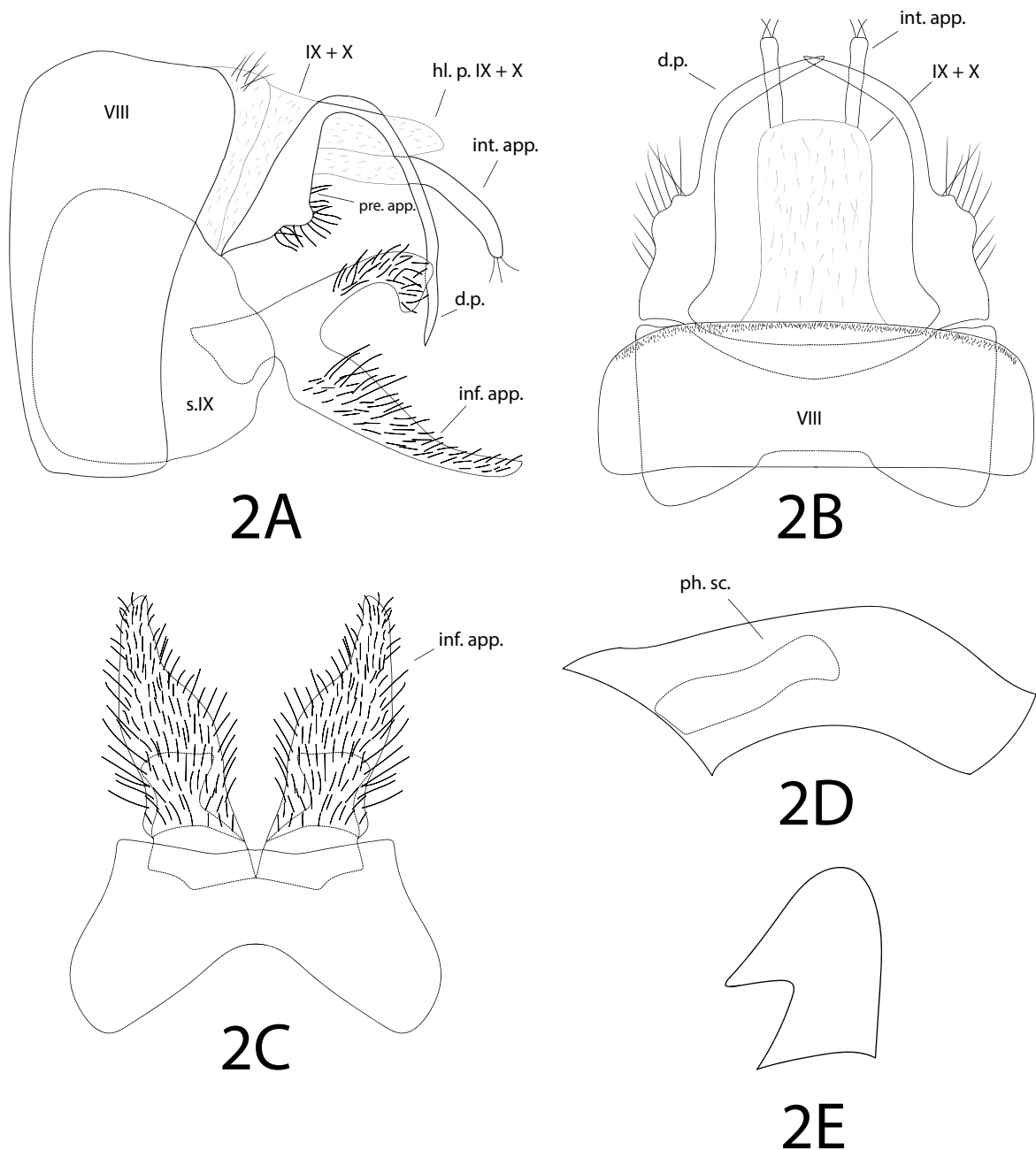


FIGURE 2. *Polycentropus aileenae* n. sp., male genitalia. 2A, left lateral (with phallus removed). 2B, dorsal. 2C, segment IX and inferior appendages, ventral. 2D, phallus, left lateral. 2E, dorsobasal process of right inferior appendage, caudal. Abbreviations: d.p. = dorsobasal process of a preanal appendage (paired); hl. p. = horn-like projection of the combined terga IX and X; inf. app. = inferior appendage (paired); int. app. = intermediate appendage (paired); IX+X = combined terga IX + X; pre. app. = preanal appendage (paired); s.IX = sternum IX.

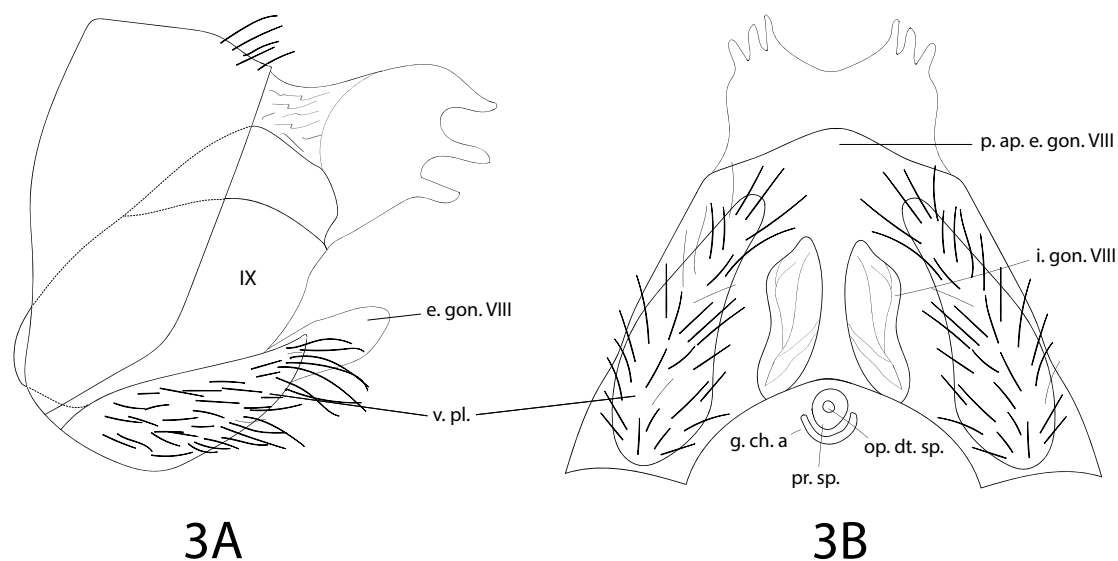


FIGURE 3. *Polycentropus aileenae* n. sp., female genitalia. 3A, left lateral. 3B, ventral. Abbreviations: IX = segment IX; e. gon. VIII = external parts of gonopods VIII; g.ch.a. = anterior part of genital chamber; i. gon. VIII = internal parts of gonopods VIII; op.dt.sp. = opening of ductus spermathecae; p. ap. e. gon. VIII = posterior apex of external parts of gonopods VIII; pr. sp. = processus spermathecae; v. pl. = ventral plate.

Comparative Material Examined. The list of comparative material examined (Table 1) represents the remaining members of the *Polycentropus confusus* Species Group, with the exception of *P. vernus* and the female of *P. neiswanderi* for which the original descriptions and illustrations were used. Type material was examined when available, for most species, and all original descriptions were consulted. The number of females examined is indicated only for those species from which females are known.

TABLE 1. List of comparative material examined.

Species	Author(s)	Males/Females Examined
<i>Polycentropus alabamensis</i>	Hamilton, Harris & Lago 1990	6
<i>Polycentropus blicklei</i>	Ross & Yamamoto 1965	14/3
<i>Polycentropus carlsoni</i>	Morse 1971	21/33
<i>Polycentropus carolinensis</i>	Banks 1905	10
<i>Polycentropus centralis</i>	Banks 1914	125/8
<i>Polycentropus chelatus</i>	Ross & Yamamoto 1965	10
<i>Polycentropus chenoides</i>	Ross & Yamamoto 1965	6
<i>Polycentropus confusus</i>	Hagen 1861 (in Hagen & Uhler 1861)	39/10
<i>Polycentropus dinkinsorum</i>	Orfinger & Etnier 2020	17
<i>Polycentropus elarus</i>	Ross 1944	121/4
<i>Polycentropus floridensis</i>	Lago & Harris 1983	6
<i>Polycentropus maculatus</i>	Banks 1908	7/12
<i>Polycentropus neiswanderi</i>	Ross 1947	3/0
<i>Polycentropus pentus</i>	Ross 1941	5/7
<i>Polycentropus pixi</i>	Ross 1944	3/2
<i>Polycentropus stephani</i>	Bowles, Mathis & Hamilton 1993	3/16
<i>Polycentropus thaxtoni</i>	Hamilton & Holzenthal 1986	2

Etymology. The specific epithet honors Aileen Perilla in appreciation of her continued support of the first author, her enthusiastic and skillful assistance during field work, and her shared appreciation of the imperiled stream habitats in which these animals occur.

Discussion

The new species has also been collected in abundance from the following locality: USA. Pennsylvania: Centre County, Destiny Farms, small watercress-choked tributary of Slab Cabin Run [N40°44'57.9", W77°52'14.8"], 341 m a.s.l, J.K. Moulton coll., 25-vi-2009, 15 males. Unfortunately, the material was misplaced post identification, is presumed lost, and therefore not listed in the material examined. However, the existence of this Pennsylvania population and the Nova Scotia record indicate a disjunct known distribution of *P. aileenae* (Fig. 4). This disjunct distribution may be an artifact of undercollecting or misidentifications of *P. aileenae* as *P. blicklei*, for example. Additional sampling and review of possibly misidentified museum material will likely serve to elucidate the geographic distribution of the species. Additionally, a specimen collected in Exmoor, New Brunswick, Canada, and housed at the University of Guelph Centre for Biodiversity Genomics (BOLD Sample ID 08NBEP-1627) is only 0.47% divergent at the barcoding region of COI from *P. aileenae* (ABO, unpublished data). While this specimen likely represents *P. aileenae*, it is missing its abdomen so its identity cannot be confirmed.

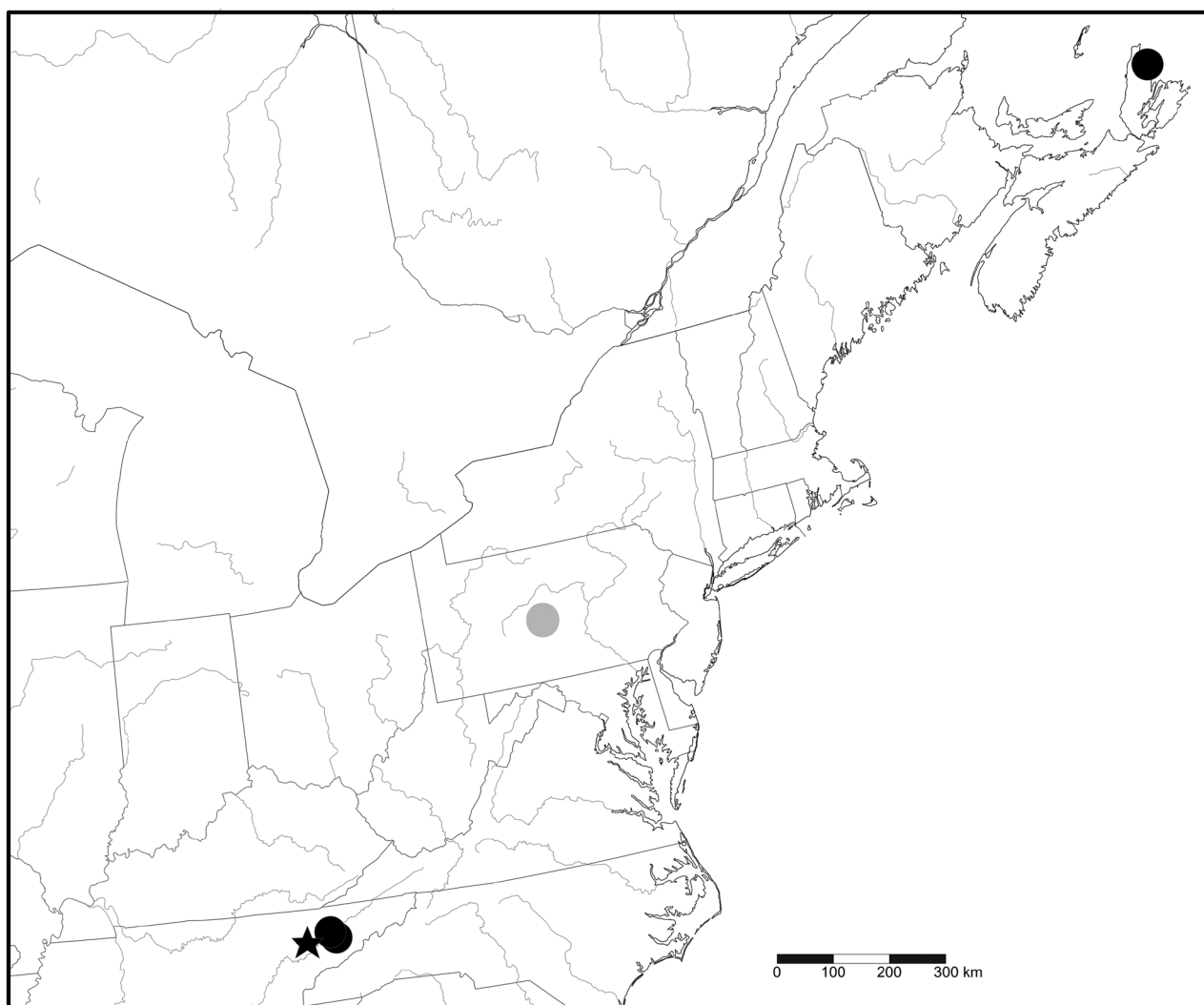


FIGURE 4. Recorded distribution of *Polycentropus aileenae* n. sp. The star represents the locality of the holotype (and some paratypes), black circles represent other collection sites included in the material examined, and the grey circle represents the additional record not included in material examined.

The ecology and phylogenetic affinities of *P. aileenae* are poorly known. All of the known localities from which the species has been collected are small (<4 m wide), stenothermic streams with emergent vegetation, often water-cress, along with rocky and sandy substrates (Fig. 5). Adults have been taken from late May to October. Male genitalic similarities suggest a close relationship to *P. blicklei*, but to a lesser degree also to *P. carlsoni*, *P. carolinensis*, and *P. elarus*. Female genitalic similarities bolster the hypothesis of a close relationship with *P. blicklei*. Determination of the phyletic affinities should be considered unresolved until a comprehensive phylogenomic-morphologic analysis is conducted on the *Polycentropus confusus* Species Group.



FIGURE 5. Fowler Spring Branch, Knox County, Tennessee, USA on 4-x-2019, the type locality of *Polycentropus aileenae* n. sp.

New Records for Group Members

Polycentropus confusus Hagen 1861, New State Record

USA: Florida: Walton County, Natural Bridge Creek at Highway 181, North side, UV Blacklight, [N30°59'19.0", W86°12'16.8"], D. Denson and E. Denson coll., 2-vi-2016, 2 males (FAMU). **Comments:** This is the first published report of this species from Florida.

***Polycentropus elarus* Ross 1944, New State Record**

USA: Florida: Washington County, Econfina Creek Water Management Area, Unnamed ravine stream (lower reach) that flows into Whitewater Lake off Porter Pond Road, [N30°28'46.0", W85°33'14.0"], A. Rasmussen *et al.* coll., 6-vi-2009, 1 male (FAMU). **Comments:** This is the first published report of this species from Florida.

***Polycentropus thaxtoni* Hamilton & Holzenthal 1986, New State Record**

USA: Alabama: Dekalb County, unnamed stream crossing Road 05 c. 220 m south Road 09, mile marker 390, [N34°27'01.4", W85°34'57.7"], J. Robinson and C.R. Parker coll., 11/v/2007, 1 male (LIRI 1098). **Comments:** This is the first published report of this species from Alabama.

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