

# Larval taxonomy of eastern Nearctic *Polycentropus* sensu stricto (Trichoptera: Polycentropodidae)

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**Abstract:** Although the alpha taxonomy of male caddisflies (Trichoptera) is largely well resolved in North America north of Mexico, significant work is needed to improve knowledge of larval stages. The genus *Polycentropus* Curtis, 1835 (Polycentropodidae) typifies this fact, with larval descriptions available for only 5/29 Nearctic species. The speciose eastern Nearctic fauna represents 22/29 Nearctic species and exemplifies a larger gap in larval taxonomy, with larval descriptions available for only 1/22 eastern Nearctic species. The inability to identify these larvae to species level precludes studies on these organisms' biology and their inclusion in water-quality bioassessment protocols. Based on molecularly identified larvae, we describe the late- or final-instar larvae of an additional 11/22 eastern species and redescribe the larvae of *Polycentropus centralis* Banks, 1914. Species for which larvae are newly described are *Polycentropus alabamensis* Hamilton, Harris, and Lago, 1990; *Polycentropus blicklei* Ross and Yamamoto, 1965; *Polycentropus carlsoni* Morse, 1971; *Polycentropus carolinensis* Banks, 1905; *Polycentropus confusus* Hagen, 1861; *Polycentropus elarus* Ross, 1944; *Polycentropus floridensis* Lago and Harris, 1983; *Polycentropus maculatus* Banks, 1908; and *Polycentropus pentus* Ross, 1941 of the *Polycentropus confusus* Species Group as well as *Polycentropus colei* Ross, 1941 and *Polycentropus rickeri* Yamamoto, 1966 of the *Polycentropus colei* Species Group. A diagnostic matrix to assist in their identification is provided, and 1 new state record is reported. Future efforts should focus on associating and describing remaining unknown larvae and building upon the data and diagnostic tools provided herein.

**Key words:** Annulipalpia, caddisflies, description, discriminatory matrix, Endopterygota, new record

The Trichoptera, or caddisflies, are widespread holometabolous insects with immature stages typically existing in many kinds of aquatic habitats and alate adults typically inhabiting adjacent riparian areas. Caddisflies are the most diverse primary aquatic insect order, with over 17,000 valid, extant species globally (Morse 2022, Clemson University Arthropod Collection, Clemson, South Carolina, US, personal communication). In light of their abundance, ecological and taxonomic diversity, cosmopolitan distribution, and general sensitivity to pollution, larval caddisflies are used worldwide in biomonitoring programs that evaluate the health of aquatic ecosystems (Morse et al. 2019a). In North America, local, state/provincial, tribal, and federal governments use macroinvertebrates, such as caddisflies, to conduct biomonitoring of freshwaters via standardized regional protocols (e.g., Lenat 1988, 1993, Carter and Resh

2001, Carter et al. 2006, Pilgrim et al. 2011) and recommendations of the United States Environmental Protection Agency (Barbour et al. 1999). It has long been recognized that these biomonitoring programs measure changes in aquatic ecosystem health at better resolution when taxonomic identification is more refined (Lenat and Resh 2001, Jones 2008, Pilgrim et al. 2011).

However, larval identification to the species level is not possible in many caddisfly genera because Trichoptera alpha taxonomy is generally based on adult males, and larvae can be challenging to associate and discriminate at the species level. In North America, larval descriptions have been completed for  $<1/2$  of all caddisfly species (Ruiter et al. 2013, Morse et al. 2017). This substantial knowledge gap prevents identification of most species' larvae and, therefore, precludes greater precision in surface water biomonitoring

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programs. In addition to aiding in bioassessment, resolving species-level larval taxonomy can allow for the study of species' life histories, habitats, ecological niches, and functional traits.

The genus *Polycentropus* Curtis, 1835 (Polycentropodidae) is a particularly good example of the need for research on caddisfly taxonomy in general and, in particular, larval taxonomy. *Polycentropus* and allied genera have been variously treated throughout the years as a single genus or 3 separate genera. In 1944, Ross effectively synonymized *Holocentropus* McLachlan, 1878 and *Plectrocnemia* Stephens, 1836 with *Polycentropus*. European taxonomists did not adopt this classification, but those in North America did, causing a rift in the taxonomic treatment of these genera. Morphological and molecular phylogenies of polycentropodid genera by Chamorro and Holzenthal (2011) and Johanson et al. (2012), respectively, led to the restoration of the pre-1944 classification of the genera in North America. Because of this history, *Holocentropus*, *Plectrocnemia*, and *Polycentropus* are termed collectively as *Polycentropus* sensu lato to distinguish them from the more narrowly defined and originally designated *Polycentropus* sensu stricto (see Orfinger 2019 for additional discussion). *Holocentropus*, *Plectrocnemia*, and *Polycentropus* larvae cannot yet be distinguished from each other and cannot yet be distinguished consistently from larvae of *Cernotina* (Morse et al. 2017, 2019b).

The focus of this paper is the larvae of the *Polycentropus* sensu stricto (e.g., Fig. 1), referred to hereafter simply as *Polycentropus*. Larvae of these animals inhabit clean, cool, lotic waters spanning 1<sup>st</sup>-order streams to navigable rivers.

In the Nearctic region, the genus *Polycentropus* is represented by 29 described species, with most species distributed exclusively either in the eastern or the western portions of the region. Here, we define eastern as consisting of On-

tario, Canada, and the states within the United States adjacent to either side of the Mississippi River and eastward and western as consisting of the complementary Nearctic region (following, e.g., Lago and Harris 1987, Cooper and Morse 1998, Orfinger et al. 2022a, b). The western fauna consists of 7 nominal species, of which larvae of 4 species were recently described by Orfinger et al. (2022a), who also provided a diagnostic character matrix for their identification purposes. The more diverse eastern fauna comprises 22 species and is divided into 2 Species Groups (Armitage and Hamilton 1990), the *Polycentropus confusus* Species Group, represented by 19 species, and the *Polycentropus colei* Species Group, represented by 3 species. Of the 22 eastern species, only the larva of *Polycentropus centralis* Banks, 1914 was described previously, with illustrations of its head and pronotum provided by Ross (1944). The remaining 21 larvae have remained unstudied. Remarkably, the larva of the common and widespread *Polycentropus confusus* Hagen, Yamamoto, 1861 has gone undescribed for the more than 160 y since the species' original description.

The present effort is part of a larger project to resolve the taxonomy of Nearctic *Polycentropus* more completely across life stages and sexes. The goals of this paper are to describe the 11 recently associated larvae from the eastern *Polycentropus* fauna, to redescribe the larva of *P. centralis*, and to provide a diagnostic character matrix for known eastern larvae. We conclude by discussing future research priorities for the identification and description of Nearctic polycentropodid larvae.

## METHODS

Specimens were collected by the authors, donated by other freshwater scientists, or borrowed on loan from various natural history collections as listed in the "Specimens Examined" subsection of each species treatment. Larval-adult association for most species was accomplished with mitochondrial DNA barcoding using the barcoding region of cytochrome oxidase I as described by Orfinger et al. (2022b). Life-stage association for *Polycentropus floridensis* Lago and Harris, 1983 was achieved geographically. Specifically, intense sampling efforts of Eglin Air Force Base in Santa Rosa County, Florida, by one of the authors (AKR) and others targeting immature and adult life stages between 1996 and 2008 allowed for identification of *P. floridensis* from Santa Rosa County, Florida. Within *Polycentropus* sensu lato, we collected only *P. floridensis* and *Plectrocnemia cinerea* (Hagen, 1861) across seasons among 8 sites of the 31 total sites sampled, with larvae of these 2 species readily distinguishable (AKR, unpublished data).

Morphological methods followed those of Orfinger et al. (2022a). We observed specimens under a Z10 stereomicroscope (Unitron<sup>®</sup>, Commack, New York) with maximum 120× magnification. We took measurements to the nearest 0.1 mm with a calibrated ocular micrometer. Specimen



Figure 1. Left lateral habitus of a final-instar larva of *Polycentropus confusus*.

length refers to total length (i.e., anterior margin of head to posterior ends of anal claws). Because specimens were frequently preserved in a curled position, we often needed to use 2 pairs of fine forceps to carefully straighten larvae when measuring length. For morphometric measurements, head width describes the width of the head measured dorsally at the widest point, and head length refers to the length of the head measured dorsally at the longest point and excluding the labrum. Terminology otherwise follows Waringer and Graf (2011), Karaouzas and Waringer (2017), and Orfinger et al. (2022a).

Character examination was informed by the following morphological characters that have proven useful in separating larvae of polycentropodid genera and species (e.g., see Waringer and Graf 2011, Orfinger et al. 2022a): 1) The relative lengths and thicknesses of the foretarsi and foretibiae (e.g., comparing dimensions in Fig. 2A, B); 2) curvature of the anal claw (classified as either obtuse [Fig. 2C] or right angled [Fig. 2D]); 3) relative proportions of the 2 anal proleg segments (e.g., comparing dimensions in Fig. 2E, F); 4) the arrangement of the posterior muscle scars (when present) of the frontoclypeal apotome, forming either a shallow arc

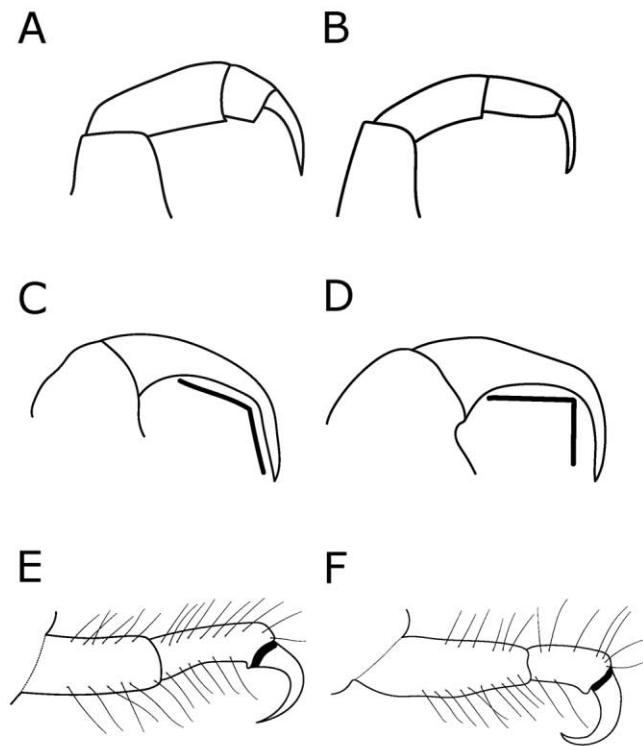


Figure 2. Various characteristics of eastern Nearctic *Polycentropus* larvae: foreleg exhibiting a short, broad tarsus that is  $< \frac{1}{2}$  the length of its tibia (A); foreleg exhibiting a longer, narrower, and more tubular tarsus that is  $\geq \frac{2}{3}$  the length of its tibia (B); obtuse-angled anal claw (C); right-angled anal claw (D); anal proleg segments roughly equal in length (E); basal segment of anal proleg longer than distal segment (F). Modified from Orfinger et al. (2022b).

(Fig. 3A), a roughly straight line (Fig. 3B), or a trapezoidal pattern (Fig. 3C); and 5) the distance of muscle scar ‘m’ (sensu Waringer and Graf 2011) from the frontoclypeal suture (classified as distant [Fig. 4A], also termed set back by Waringer and Graf 2011, or close [Fig. 4B], following Orfinger et al. 2022a).

In most instances, we could not confidently determine instar number (e.g., 3<sup>rd</sup>, 4<sup>th</sup>, or 5<sup>th</sup> instar) because of a lack of larval size class series or metamorphotype specimens (sensu Milne 1938). Given that most specimens examined were large (i.e., >10 mm in length), it is likely that most or all represent final-instar larvae. However, in the many instances for which this cannot be stated with certainty, we use the term late instar.

For stacked photography, we excised heads and placed them in glycerin in a depression slide. Using an M1400 Plus Digital Camera (Levenhuk, Tampa, Florida) mounted on a Unitron Z10 microscope, we took between 6 and 16 photographs of each larval head at different depths of field. We subsequently used Helicon Focus software (version 7.7.4; Helicon Soft, Kharkiv, Ukraine) to digitally stitch together the photographs of each subject to form a single composite image. We produced line drawings with a 10 × 10-mm gridded ocular lens in conjunction with a gridded guide and pencil. We then scanned the drawings and used them as templates in Adobe Illustrator (version 24.3; Adobe, San Jose, California) for the final illustrations.

We deposited voucher specimens at the Florida A&M University portion of the Florida State Collection of Arthropods (FAMU) in Tallahassee, Florida; the Centre for Biodiversity Genomics (BIOUG) in Guelph, Ontario; the insect collection of Cole Ecological Inc in Greenfield, Massachusetts; the Blue Ridge Parkway Insect Collection (BLRI) in Asheville, North Carolina; the Cumberland Gap National Historical Park Insect Collection in Middlesboro, Kentucky; the Clemson University Arthropod Collection (CUAC) in Clemson, South Carolina; the Illinois Natural History Survey in Champaign, Illinois; and the Etnier Caddisfly Collection of the University of Tennessee, Knoxville, Tennessee. Mitochondrial DNA barcode sequence data and associated metadata used for the identification of larval specimens are available in BOLD under the publicly accessible dataset titled “DS-POLYCSSL Nearctic Polycentropodidae (Trichoptera)” (Orfinger et al. 2021).

## RESULTS

Presented below are original larval descriptions of 11 eastern Nearctic *Polycentropus* species, beginning with 9/19 members of the *P. confusus* Species Group, followed by 2/3 members of the *P. colei* Species Group. Also presented is a redescription of the larva of *P. centralis* (*P. confusus* Species Group) based on historical and newly collected material. Each description is followed by distributional data and a list of material examined. New state records are denoted by an

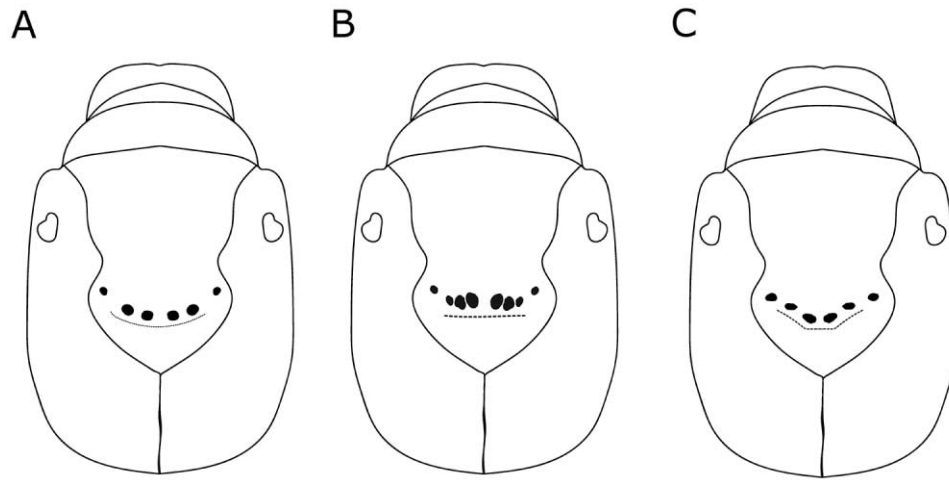


Figure 3. Types of muscle scar arrangements of posterior frontoclypeal apotome exhibited by known eastern Nearctic *Polycentropus* larvae, if present: shallow arc (A), linear (B), trapezoidal (C).

asterisk (\*). Descriptions emphasize characters for which interspecific variation was observed, whereas the following suite of character states was consistent among all larvae: 1) muscle scarring present on head, 2) foretarsi tubular and with length  $\geq \frac{2}{3}$  foretibiae length (Fig. 2B), 3) basal segments of anal proleg longer than distal sclerotized segments of anal proleg (Fig. 2F), 4) basal segments of anal proleg with numerous long setae (Fig. 2F), 5) abdominal segments lacking gills (Fig. 1), 6) anal claws lacking spines on concave margins (Fig. 2C, D), 7) anterior ventral apotome broad and V-shaped, 8) mesal faces of femora covered with numerous long setae, and 9) distal section of each tarsus with well-developed pectinate setae.

Finally, we present a diagnostic matrix for larvae of the 12 known eastern Nearctic *Polycentropus* species (Table 1). This matrix facilitates identification of each species for which the larva is known based on 8 categorical characters, 2 morphometric characters, and provincial and state distributional data. Furthermore, the matrix is a convenient framework to build upon as additional larvae are associated, described, and diagnosed.

#### *Polycentropus confusus* Species Group

##### *Polycentropus alabamensis* Hamilton, Harris, and Lago, 1990 (Table 1, Fig. 5A, B)

*Description of late-instar larva.* Larval length 12.0 to 12.5 mm ( $\bar{x} = 12.3$  mm,  $n = 2$ ).

**Head:** Length 1.5 mm, width 1.0 to 1.1 mm ( $n = 2$ ). Dorsum of head yellow-tan with numerous symmetrically positioned, variously sized, well-defined dark muscle scars. Dorsum of head with distinct darker brown bands laterally in anterior  $\frac{2}{3}$  of frontoclypeal apotome and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome pale with muscle scar arrangement forming shallow arc (Fig. 3A). Muscle scars 'm' subtle, small, and positioned close to frontoclypeal

suture (Fig. 4B). Ventral area of head also yellow-tan, with anterior  $\frac{1}{2}$  lacking muscle scars and posterior  $\frac{1}{2}$  with multiple faint muscle scars.

**Pronotum:** Same color as head, lacking pigment banding, with multiple dark muscle scars posteriorly and lacking muscle scars anteriorly.

**Abdomen:** Anal claws obtuse angled (Fig. 2C).

*Distribution.* USA—Alabama.

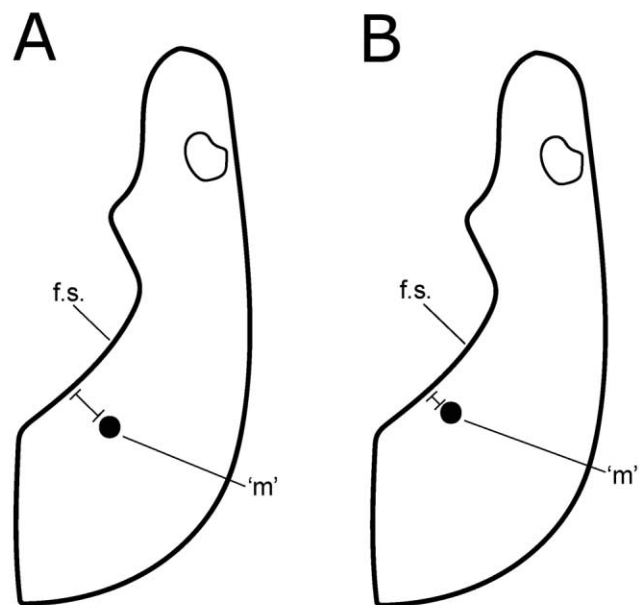


Figure 4. Distances of muscle scar 'm' relative to frontoclypeal suture (f.s.) exhibited by known eastern Nearctic *Polycentropus* larvae: distant (A), close (B). Modified from Orfinger et al. (2022b).



Table 1. Discriminatory matrix to the known late-instar and final-instar larvae of eastern Nearctic *Polycentropus* species. AL = Alabama, AR = Arkansas, CA = Canada, CT = Connecticut, DC = Washington DC, DE = Delaware, FL = Florida, GA = Georgia, IA = Iowa, IL = Illinois, IN = Indiana, KY = Kentucky, MA = Massachusetts, MD = Maryland, ME = Maine, MI = Minnesota, MO = Missouri, MS = Mississippi, NB = New Brunswick, NC = North Carolina, NH = New Hampshire, NJ = New Jersey, NL = Newfoundland and Labrador, NS = Nova Scotia, NY = New York, OH = Ohio, OK = Oklahoma, ON = Ontario, PA = Pennsylvania, QC = Quebec, RI = Rhode Island, SC = South Carolina, TN = Tennessee, TX = Texas, USA = United States of America, VA = Virginia, VT = Vermont, WI = Wisconsin, WV = West Virginia, WY = Wyoming.

Species/ character	Muscle scars on head distinct	Pigment band- ing on head	Anal claw curvature	Frontoclypeus concolorate	Position of muscle scar "m" relative to frontoclypeal suture	Head width: head length ratio	Head width: body length ratio	Head color	Arrangement of posterior scars on frontoclypeal apotome
<i>Polycentropus alabamensis</i>	Yes	Distinct	Obtuse angled	No	Close	0.667–0.733	0.0800–0.092	Yellow-tan	Shallow arc
	Geographic range—USA: AL.								
<i>Polycentropus blicklei</i>	Yes	Distinct	Right angled	No	Close	0.720–0.800	0.110–0.133	Yellow-tan	Shallow arc
	Geographic range—CA: NB, NL, NS, ON, QC. USA: AL, DE, FL, GA, KY, MD, ME, MS, NC, NH, NJ, NY, OH, PA, RI, SC, TN, VA.								
<i>Polycentropus carlsoni</i>	Yes	Distinct	Right angled	No	Close	0.912	0.108	Yellow-tan	Trapezoidal
	Geographic range—USA: AL, MD, NC, PA, SC, VA.								
<i>Polycentropus carolinensis</i>	Yes	Subtle	Obtuse angled <sup>a</sup>	No	Close	0.667–0.857	0.073–0.082	Yellow-tan	Trapezoidal
	Geographic range—CA: ON, QC. USA: KY, MS, NC, NH, OH, PA, TN, VA, VT, WV.								
<i>Polycentropus centralis</i>	No	Subtle	Obtuse angled	No	Close	0.692–0.917	0.080–0.096	Yellow to yellow- tan	Linear
	Geographic range—CA: NL, NS, ON. USA: AL, AR, IA, IL, IN, KS, KY, MN, MO, MS, NY, OH, OK, PA, TN, TX, VA, WI, WV.								

<i>Polycentropus confusus</i>	Yes	Distinct	Obtuse angled	No	Close	0.733–0.923	0.073–0.108	Tan	Linear
Geographic range—CA: NB, NL, NS, ON, QC. USA: AL, AR, CT, DC, DE, FL, GA, IA, IN, KY, MA, ME, MI, MO, MS, NH, NJ, NY, NC, OH, OK, PA, SC, TN, VA, VT, WI.									
<i>Polycentropus elarus</i>	Yes	Subtle	Obtuse angled	No	Close	0.846	0.089	Yellow-tan	Trapezoidal
Geographic range—CA: ON, QC. USA: AL, FL, GA, IN, KY, MA, NH, NC, NY, OH, PA, TN, VA, WV.									
<i>Polycentropus floridensis</i>	Yes	Absent	Right angled	No	Distant	0.600–0.846	0.089–0.108	Orange-tan	Shallow arc
Geographic range—USA: AL, FL.									
<i>Polycentropus maculatus</i>	Yes	Absent	Obtuse angled	Yes	Distant	0.8571–0.923	0.078–0.109	Yellow-tan	Trapezoidal
Geographic range—CA: NL, NS, ON, QC. USA: CT, KY, MA, ME, NC, NH, NY, OH, PA, SC, TN, VA, VT, WV.									
<i>Polycentropus pentus</i>	Yes	Distinct	Right angled	No	Distant	0.833–0.917	0.100–0.110	Orange-tan	Linear
Geographic range—CA: MB, NL, NS, ON, QC. USA: AL, CT, IL, KY, ME, MI, MN, NH, NJ, NY, OH, PA, TN, VA, VT, WI, WV, WY.									
<i>Polycentropus colei</i>	No	Absent	Right angled	Yes	Close	0.842–0.909	0.085–0.101	Yellow	Absent
Geographic range—CA: QC. USA: KY, NC, PA, TN, VA, WV.									
<i>Polycentropus rickeri</i>	No	Absent	Right angled	Yes	Close	0.933	0.122–0.127	Yellow to orange-yellow	Linear <sup>b</sup>
Geographic range—USA: AL, KY, NC, PA, TN, VA, WV.									

<sup>a</sup> Occasionally intermediate.

<sup>b</sup> Two scars only.

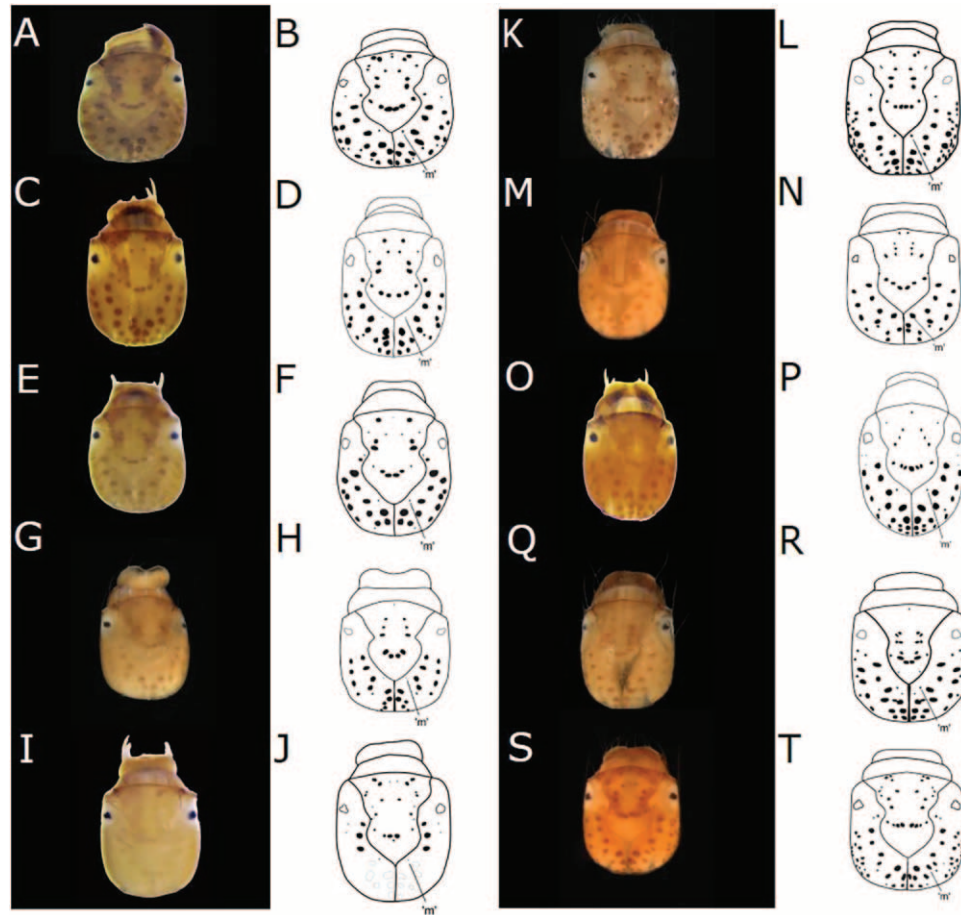


Figure 5. Head photographs (black background, dorsal view) and corresponding muscle scar maps (white background, dorsal view) of late-instar or final-instar larvae of associated members of the *Polycentropus confusus* Species Group, where 'm' denotes the position of muscle scar: *Polycentropus alabamensis* (A, B), *Polycentropus blicklei* (C, D), *Polycentropus carlsoni* (E, F), *Polycentropus carolinensis* (G, H), *Polycentropus centralis* (I, J), *P. confusus* (K, L), *Polycentropus elarus* (M, N), *Polycentropus floridensis* (O, P), *Polycentropus maculatus* (Q, R), and *Polycentropus pentus* (S, T).

*Specimens examined.* USA—Alabama: Morgan County, spring outside of Curry Cave; 6–7 April 2020; 2 larvae (FAMU).

***Polycentropus blicklei* Ross and Yamamoto, 1965** (Table 1, Fig. 5C, D)

*Description of late-instar larva.* Larval length 9.0 to 11.8 mm ( $\bar{x}$  = 11.1 mm,  $n$  = 3).

Head: Length 1.5 to 1.8 mm, width 1.2 to 1.3 mm ( $n$  = 3). Dorsum of head yellow-tan with numerous, variously sized, well-defined dark muscle scars. Muscle scarring on head occasionally slightly asymmetrical. Dorsum of head with distinct darker brown bands laterally in anterior  $2/3$  of frontoclypeal apotome and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome pale with muscle scar arrangement forming shallow arc (Fig. 3A). Muscle scars 'm' distinct, small, and positioned close to frontoclypeal suture (Fig. 4B). Ventral area of head also yellow-tan, with anterior  $1/2$  lacking muscle scars and posterior  $1/2$  with multiple faint muscle scars.

Pronotum: Same color as head, though slightly lighter. Lacking pigment banding, with multiple dark muscle scars posteriorly and lacking muscle scars anteriorly.

Abdomen: Anal claws right angled (Fig. 2D).

*Distribution.* Canada—New Brunswick, Newfoundland and Labrador, Nova Scotia, Ontario, Quebec.

USA—Alabama, Delaware, Florida, Georgia, Kentucky, Maine, Maryland, Mississippi, New Hampshire, New Jersey, New York, North Carolina, Ohio, Pennsylvania, Rhode Island, South Carolina, Tennessee, Virginia.

*Specimen examined.* USA—Maryland: Anne Arundel County, Sewell Spring Branch, site SR-01-18; N39° 04' 34.93", W76° 37' 05.88"; M. Cole collected; 3 April 2018; 3 larvae (Cole Ecological Inc 7670).

***Polycentropus carlsoni* Morse, 1971** (Table 1, Fig. 5E, F)

*Description of late-instar larva.* Larval length 10.2 mm ( $n$  = 1).

Head: Length 1.2 mm, width 1.1 mm ( $n = 1$ ). Dorsum of head yellow-tan with numerous symmetrically positioned, variously sized, well-defined dark muscle scars. Dorsum head with distinct darker brown bands in anterior  $2/3$  of frontoclypeal apotome and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome pale with muscle scar arrangement trapezoidal (Fig. 3C). Muscle scars 'm' subtle, small, and positioned close to frontoclypeal suture (Fig. 4B). Ventral area of head also yellow-tan, with anterior  $1/2$  lacking muscle scars and posterior  $1/2$  with multiple faint muscle scars.

Pronotum: Same color as head though slightly lighter, lacking pigment banding, with multiple dark muscle scars posteriorly and lacking muscle scars anteriorly.

Abdomen: Anal claws right angled (Fig. 2D).

*Distribution.* USA—Alabama, Maryland, North Carolina, Pennsylvania\*, South Carolina, Virginia.

*Specimen examined.* USA—Pennsylvania: Philadelphia County, Tributary of Wissahickon Creek, at seep crossing trail; N40°02'38.04", W75°12'55.80"; N. Macelko collected; 24 October 2021; 1 larva (FAMU). **New State Record**

**Polycentropus carolinensis Banks, 1905** (Table 1, Fig. 5G, H)

*Description of final-instar larva.* Larval length 11.0 to 16.4 mm ( $\bar{x} = 13.7$  mm,  $n = 5$ ).

Head: Length 1.1 to 1.5 mm, width 0.9 to 1.2 mm ( $n = 5$ ). Dorsum of head yellow-tan with numerous symmetrically positioned, variously sized, well-defined dark muscle scars. Dorsum of head with subtle darker brown bands in anterior  $2/3$  of frontoclypeal apotome and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome pale with muscle scar arrangement trapezoidal (Fig. 3C). Muscle scars 'm' subtle, small, and positioned close to frontoclypeal suture (Fig. 4B). Ventral area of head also yellow-tan, with anterior  $1/2$  lacking muscle scars and posterior  $1/2$  with multiple faint muscle scars.

Pronotum: Same color as head, though slightly lighter. Lacking pigment banding, with multiple faint muscle scars posteriorly and lacking muscle scars anteriorly.

Abdomen: Anal claws usually obtuse angled (Fig. 2C), though occasionally exhibiting intermediate angulation approaching right angled.

*Distribution.* Canada—Ontario, Quebec.

USA—Kentucky, Mississippi, New Hampshire, North Carolina, Ohio, Pennsylvania, Tennessee, Vermont, Virginia, West Virginia.

*Specimens examined.* USA—North Carolina: Transylvania County, Devil's Courthouse at Blue Ridge Parkway; J. K. Moulton collected; 17 August 2005; 3 larvae (FAMU).

Watauga County, Spring, seep below spring house, Moses H. Cone Estate, 1146 m a.s.l.; J. Robinson and C. R. Parker collected; 29 March 2007; 5 larvae (BLRI 21955).

Tennessee: Fentress County, Cave Creek, Tributary of Pogue Creek, at end of all-terrain-vehicle road off Williams Cr. Rd, 0.5 km east of Delk Cr. Rd.; D. Etnier et al. collected; 21 May 2005; 1 metamorphotype male, 2 larvae (Etnier Caddisfly Collection 3.431).

Virginia: Augusta County, Spring at Dripping Rock, pullout at MP 009.7; left, by hand, J. Robinson and C. R. Parker collected; 17 August 2007; 1 larva (BLRI 25619).

**Polycentropus centralis Banks, 1914** (Table 1, Fig. 5I, J)

*Description of late-instar larva.* Larval length 10.5 to 11.5 mm ( $\bar{x} = 11.1$  mm,  $n = 5$ ).

Head: Length 1.0 to 1.3 mm, width 0.9 to 1.0 mm ( $n = 5$ ). Dorsum of head generally yellow, though rarely yellow-tan, and with numerous symmetrically positioned, variously sized, well-defined dark muscle scars anteriorly and symmetrically positioned, variously sized, subtle muscle scars posteriorly. Dorsum of head with subtle darker brown bands in anterior  $2/3$  of frontoclypeal apotome and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome pale with muscle scar arrangement linear (Fig. 3B). Muscle scars 'm' faint and nearly invisible, small, and positioned close to frontoclypeal suture (Fig. 4B). Ventral area of head yellow, with anterior  $1/2$  lacking muscle scars and posterior  $1/2$  with multiple very faint muscle scars.

Pronotum: Same color as head, lacking pigment banding, with multiple very faint muscle scars posteriorly and lacking muscle scars anteriorly.

Abdomen: Anal claws obtuse angled (Fig. 2C).

*Distribution.* Canada—Newfoundland and Labrador, Nova Scotia, Ontario.

USA—Alabama, Arkansas, Illinois, Indiana, Iowa, Kansas, Kentucky, Minnesota, Mississippi, Missouri, New York, Ohio, Oklahoma, Pennsylvania, Tennessee, Texas, Virginia, West Virginia, Wisconsin.

*Specimens examined.* USA—Arkansas: Newton County, David Creek at Mt. Hersey; N36°00'36.39", W97°57'19.85"; 28 March 2021; 11 larvae (FAMU).

Illinois: Union County, La Rue, Running Lick Creek; N37°32'44.02", W89°27'12.99"; B. D. Burks and G. T. Riegel collected; 12 May 1939; 1 larva (Illinois Natural History Survey Trichoptera 53462). Wolf Lake, Hutchins Creek; N37°30'40.86", W89°22'38.75"; Frison and Ross collected; 5 October 1939, 4 larvae (INHS Trichoptera 48571).

**Polycentropus confusus Hagen, 1861** (Table 1, Fig. 1, 5K, L)

*Description of final-instar larva.* Larval length 12 to 16.5 mm ( $\bar{x} = 13.6$  mm,  $n = 6$ ).

Head: Length 1.3 to 2.0 mm, width 1.1 to 1.5 mm ( $n = 6$ ). Dorsum of head tan with numerous symmetrically



positioned, variously sized, well-defined dark muscle scars. Dorsum of head with distinct darker brown bands in anterior  $\frac{2}{3}$  of frontoclypeal apotome and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome pale with muscle scar arrangement linear (Fig. 3B). Muscle scars 'm' distinct, small, and positioned close to frontoclypeal suture (Fig. 4B). Ventral area of head tan to yellow-tan, with anterior  $\frac{1}{2}$  lacking muscle scars and posterior  $\frac{1}{2}$  with multiple distinct muscle scars.

Pronotum: Same color as ventral area of head, lacking pigment banding, with multiple faint muscle scars posteriorly and lacking muscle scars anteriorly.

Abdomen: Anal claws obtuse angled (Fig. 2C).

*Distribution.* Canada—New Brunswick, Newfoundland and Labrador, Nova Scotia, Ontario, Quebec.

USA—Alabama, Arkansas, Connecticut, Delaware, District of Columbia, Florida, Georgia, Indiana, Iowa, Kentucky, Maine, Massachusetts, Michigan, Mississippi, Missouri, New Hampshire, New Jersey, New York, North Carolina, Ohio, Oklahoma, Pennsylvania, South Carolina, Tennessee, Vermont, Virginia, West Virginia, Wisconsin.

*Specimens examined.* Canada—Ontario: Huron Lake; N45°19'01.92", W80°06'29.16"; C. Moore and R. O'Connor collected; 28 May 2018; 1 larva (BIOUG 43980-E07).

USA—Alabama: Lawrence County, Hubbard Creek below Kinlock Falls; N34°18'31.68"; W87°30'06.84"; 17 April 2021; 1 larva (FAMU).

Georgia/South Carolina: Rabun/Oconee Counties, Chattooga River at SR 28, 24 km S Highlands, NC, 770 m a.s.l.; N34°55'09.84", W83°10'06.96"; B. Schmidt collected; 19 April 2019; 1 larva (CUAC 000088819). K. Hecke collected; 31 August 2019; 1 larva (CUAC).

Missouri: Stone County, Wire Road Conservation Area, Crane Creek; N36°55'31.80", W93°35'25.80"; D. E. Bowles collected; 14 March 2021; 1 larva (FAMU).

North Carolina: Haywood County, Pigeon River; N35°44'01.68", W83°01'30.72"; R. W. Smith collected; 21 August 2019; 2 larvae (FAMU).

Pennsylvania: Bucks County, Mill Creek; N40°10'01.56", W75°00'07.56"; N. Macello collected; 14 June 2020; 1 larva (FAMU). Centre County, Spring Creek; N40°52'51.24", 77°47'36.60"; 2 May 2021; 1 larva (FAMU). Chester County, Exton, Valley Creek NWest; N40°01'47.30", W75°37'20.30"; 13 April 2021; 3 larvae (FAMU). Tributary of Little Nesha-miny Creek; N40°06'02.40", W75°32'33.96"; 26 June 2020; 1 larva (FAMU). Valley Creek West; N40°01'47.28", W75°37'20.28"; 13 April 2021; 4 larvae (FAMU). Delaware County, Chester Creek; N39°54'07.06", W75°28'09.78"; 6 July 2020; 1 larva (FAMU). Montgomery County, Pennypack Creek, N40°08'15.36", W75°04'26.40", 26 April 2020, 1 larva (FAMU). Northampton County, Bush Kill Creek; N40°44'29.04", W75°14'53.52"; 1 May 2021; 3 larvae (FAMU). Philadelphia

County, Pickering Creek; 1 larva (FAMU). Elk County, Birch Run; N39°55'44.40", W75°51'39.60"; T. Bringloe, V. Harvey, S. Ripley, and K. Rondollo collected; 19 June 2013; 4 larvae (BIOG 06971-F10, 06971-G01, 07978-B07, and 07982-A07). Cold Run; N40°42'14.40", W75°59'52.80"; 18 June 2013; 1 larva (BIOUG07978-E09). Philadelphia County, Birch Run, off 5 Point Road, 163 m a.s.l.; N39°55'44.40", W75°51'39.60"; T. Bringloe, V. Harvey, S. Ripley, and K. Rondollo collected; 19 June 2013; 3 larvae (BIOUG 07981-A10, 07982-A08, and 07982-A09). Philadelphia, Cold Run, 211 m a.s.l.; N40°42'14.40", W75°59'52.80"; 18 June 2013; 2 larvae (BIOUG 07978-E09 and 07978-H06). Philadelphia County, Tributary of Brandywine Creek, 29 m a.s.l.; N39°55'04.80", W75°43'22.80"; 19 June 2013; 1 larva (BIOUG 07978-H07). Chester County, White Clay Creek; N39°51'46.80", W75°47'02.40"; T. Bringloe, V. Harvey, S. Ripley, and K. Rondollo collected; 19 June 2013; 2 larvae (BIOUG 06970-A06 and 07063-B11).

South Carolina: Pickens County, Wildcat Creek; N34°45'34.20", W82°51'03.24"; C. M. Slack collected; 19 August 2009; 1 larva (CUAC000107326).

### ***Polycentropus elarus* Ross, 1944** (Table 1, Fig. 5M, N)

*Description of late-instar larva.* Larval length 12.3 mm ( $n = 1$ ).

Head: Length 1.3 mm, width 1.1 mm ( $n = 1$ ). Dorsum of head yellow-tan with numerous symmetrically positioned, variously sized, well-defined dark muscle scars. Dorsum of head with subtle darker brown bands in central  $\frac{1}{3}$  of frontoclypeal apotome and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome pale with muscle scar arrangement trapezoidal (Fig. 3C). Muscle scars 'm' distinct, large, and positioned close to frontoclypeal suture (Fig. 4B). Ventral area of head yellow-tan, with anterior  $\frac{1}{2}$  lacking muscle scars and posterior  $\frac{1}{2}$  with multiple distinct muscle scars.

Pronotum: Same color as head, lacking pigment banding, with multiple distinct muscle scars posteriorly and lacking muscle scars anteriorly.

Abdomen: Anal claws obtuse angled (Fig. 2C).

*Distribution.* Canada—Ontario, Quebec.

USA—Alabama, Florida, Georgia, Indiana, Kentucky, Massachusetts, New Hampshire, New York, North Carolina, Ohio, Pennsylvania, Tennessee, Virginia, West Virginia.

*Specimen examined.* USA—Pennsylvania: Schuylkill County, Rattling Run; N40°35'02.40", W76°01'01.20"; T. Bringloe, V. Harvey, S. Ripley, and K. Rondollo collected; 13 June 2013; 1 larva (BIOUG 07980-B07).

### ***Polycentropus floridensis* Lago and Harris, 1983** (Table 1, Fig. 5O, P)

*Description of late-instar larva.* Larval length 6.5 to 12.3 mm ( $\bar{x} = 8.2$  mm,  $n = 4$ ).

Head: Length 0.9 to 1.3 mm, width 0.6 to 1.1 mm ( $n = 4$ ). Dorsum of head orange-tan with numerous symmetrically positioned, variously sized, well-defined dark muscle scars. Dorsum of head without darker brown pigment bands and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome with muscle scar arrangement forming shallow arc (Fig. 3A). Muscle scars 'm' distinct, small, and distant from frontoclypeal suture (Fig. 4A). Ventral area yellow-tan, lacking muscle scars.

Pronotum: Same color as dorsal surface of head, lacking pigment banding, with multiple faint muscle scars posteriorly and lacking muscle scars anteriorly.

Abdomen: Anal claws right angled (Fig. 2D).

*Distribution.* USA: Alabama, Florida.

*Specimens examined.* USA—Florida: Okaloosa County, unnamed tributary to Turkey Creek N of Eglin 626; T. Thom, T. Dall, and J. Tritt collected; 22 March 2001; 1 larva (FAMU). Santa Rosa County, East Branch of Dean Creek at powerline road, Eglin Air Force Base, dipnet; N30°27'54", W86°53'17"; R. W. Flowers, M. L. Pescador, A. K. Rasmussen, and B. A. Richard collected; 27 May 2004; 1 larva (FAMU). Watering Head Branch at powerline road, Eglin Air Force Base; N30°28'22", W86°53'17"; A. K. Rasmussen, B. A. Richard, and M. Tongue collected; 11 January 2006, 2 larva (FAMU).

**Polycentropus maculatus Banks, 1908** (Table 1, Fig. 5Q, R)

*Description of late-instar larva.* Larval length 11.0 to 15.4 mm ( $\bar{x} = 12.5$  mm,  $n = 5$ ).

Head: Length 1.3 to 1.5 mm, width 1.2 to 1.3 mm ( $n = 5$ ). Dorsum of head yellow-tan with numerous symmetrically positioned, variously sized, well-defined dark muscle scars. Dorsum of head without darker brown pigment bands and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome with muscle scar arrangement trapezoidal (Fig. 3C). Muscle scars 'm' subtle, small, and distant from frontoclypeal suture (Fig. 4A). Ventral area of head yellow-tan, with anterior  $\frac{1}{2}$  lacking muscle scars and posterior  $\frac{1}{2}$  with multiple distinct muscle scars.

Pronotum: Same color as head, lacking pigment banding, with multiple subtle muscle scars posteriorly and lacking muscle scars anteriorly.

Abdomen: Anal claws obtuse angled (Fig. 2C).

*Distribution.* Canada—Newfoundland and Labrador, Nova Scotia, Ontario, Quebec.

USA—Connecticut, Kentucky, Maine, Massachusetts, New Hampshire, New York, North Carolina, Ohio, Pennsylvania, South Carolina, Tennessee, Vermont, Virginia, West Virginia.

*Specimens examined.* USA—North Carolina: Macon County, E Fk Overflow Cr., 790 m a.s.l.; N35°01'04.44", W83°14'42.36"; R. L. Heth collected; 23 July 2018; 1 larva (CUAC 000107327).

Pennsylvania: Schuylkill County, Owl Creek; N40°46'58.80", W75°57'39.60"; T. Bringloe, V. Harvey, S. Ripley, and K. Rondollo collected; 18 June 2013; 1 larva (BIOUG06970-G02). Rattling Run; N40°35'02.40", W76°01'01.20"; 3 larvae (BIOUG 07980-H09, 07980-H11, and 07980-H10).

**Polycentropus pentus Ross, 1941** (Table 1, Fig. 5S, T)

*Description of late-instar larva.* Larval length 9.5 to 11.0 mm ( $\bar{x} = 10.2$  mm,  $n = 5$ ).

Head: Length 1.1 to 1.4 mm, width 1.0 to 1.2 mm ( $n = 5$ ). Dorsum of head orange-tan with numerous, variously sized, well-defined dark muscle scars. Muscle scarring on head occasionally slightly asymmetrical. Dorsum of head with distinct darker brown bands in anterior  $\frac{2}{3}$  of frontoclypeal apotome and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome pale with muscle scar arrangement linear (Fig. 3B). Muscle scars 'm' distinct, large, and distant from frontoclypeal suture (Fig. 4A). Ventral area of head orange-tan, with anterior  $\frac{1}{2}$  lacking muscle scars and posterior  $\frac{1}{2}$  with multiple distinct muscle scars.

Pronotum: Same color as head, lacking pigment banding, with multiple distinct muscle scars posteriorly and lacking muscle scars anteriorly.

Abdomen: Anal claws right angled (Fig. 2D).

*Distribution.* Canada—Manitoba, Newfoundland and Labrador, Nova Scotia, Ontario, Quebec.

USA—Alabama, Connecticut, Illinois, Kentucky, Maine, Michigan, Minnesota, New Hampshire, New Jersey, New York, Ohio, Pennsylvania, Tennessee, Vermont, Virginia, West Virginia, Wisconsin, Wyoming.

*Specimens examined.* Canada—Ontario: Algonquin Provincial Park, small stream 2.5 km after North River Canoe Launch, 462 m a.s.l.; N46°04'51.60", W78°26'19.68"; C. Freutel and G. Martin collected; 12 July 2011; 4 larvae (BIOUG 00331-B10, 00331-C05, 01723-C03, and 01723-D05).

USA—Kentucky: Bell County, Cumberland Gap National Historical Park headwaters of Shilalah Creek; J. Robinson collected; 4 April 2007; 5 larvae (Cumberland Gap National Historical Park Insect Collection 4588).

**Polycentropus colei Species Group**

**Polycentropus colei Ross, 1941** (Table 1, Fig. 6A, B)

*Description of late-instar larva.* Larval length 10 to 12 mm ( $\bar{x} = 15.3$  mm,  $n = 3$ ).

Head: Length 1.1 to 2.0 mm, width 1.0 to 1.7 mm ( $n = 3$ ). Dorsum of head uniformly yellow with 2 symmetrically

positioned, small, well-defined dark muscle scars on incurvate center of frontoclypeal sutures and additional symmetrically positioned, variously sized, subtle muscle scars throughout. Dorsum of head lacking pigment bands and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome with muscle scars absent. Muscle scars 'm' subtle, small, and positioned close to frontoclypeal suture (Fig. 4B). Ventral area of head yellow, lacking muscle scars.

Pronotum: Same color as head, lacking pigment banding, with multiple very faint muscle scars posteriorly and lacking muscle scars anteriorly.

Abdomen: Anal claws right angled (Fig. 2D).

*Distribution.* Canada—Quebec.

USA—Kentucky, North Carolina, Pennsylvania, Tennessee, Virginia, West Virginia.

*Specimens examined.* USA—North Carolina: Haywood County, pool at base of roadcut seep at MP 422.5, parkway left, 1660 m a.s.l.; J. Robinson and C. R. Parker collected; 27 March 2007; 1 larva (BLRI 21962). Transylvania County, Devil's Courthouse at Blue Ridge Parkway; J. K. Moulton collected; 17 August 2005; 2 larvae (FAMU).

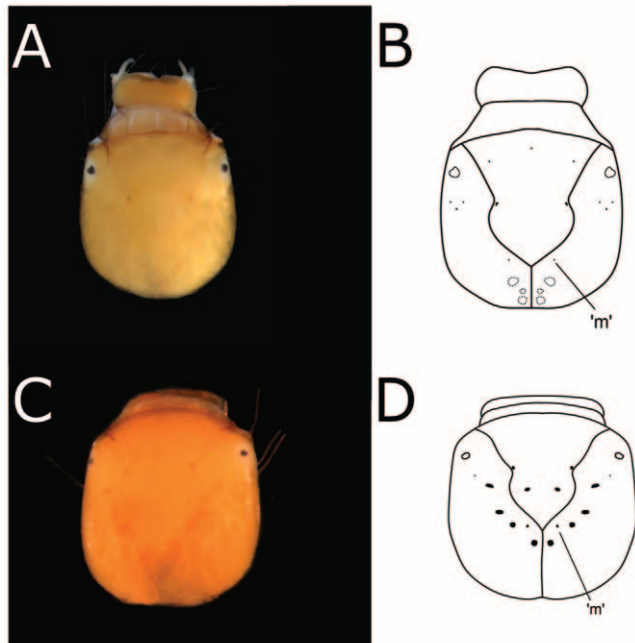


Figure 6. Head photographs (black background, dorsal view) and corresponding muscle scar maps (white background, dorsal view) of late-instar or final-instar larvae of associated members of the *Polycentropus colei* Species Group, where 'm' denotes the position of muscle scar: *P. colei* (A, B), *Polycentropus rickeri* (C, D)

***Polycentropus rickeri* Yamamoto, 1966** (Table 1, Fig. 29, 30; 6C, D)

*Description of late-instar larva.* Larval length 11.0 to 11.5 mm ( $\bar{x}$  = 11.3 mm,  $n$  = 2).

Head: Length 1.5 mm, width 1.4 mm ( $n$  = 2). Dorsum of head uniformly orange-yellow to yellow with 2 symmetrically positioned, small, well-defined, dark muscle scars immediately anterior to incurvate center of frontoclypeal sutures and additional symmetrically positioned, variously sized, subtle muscle scars throughout. Dorsum of head lacking pigment bands and with pale areas around small eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome with 2 subtle muscle scars in linear arrangement (Fig. 3B). Muscle scars 'm' subtle, small, and positioned close to frontoclypeal suture (Fig. 4B). Ventral area of head orange-yellow to yellow, lacking muscle scars.

Pronotum: Same color as head, lacking pigment banding and muscle scars.

Abdomen: Anal claws right angled (Fig. 2D).

*Distribution.* USA—Alabama, Kentucky, North Carolina, Pennsylvania, Tennessee, Virginia, West Virginia.

*Specimens examined.* USA—North Carolina: Buncombe County, small seep run at MP 357.6, parkway right, 1691 m a.s.l.; 21 October 2006; 2 larvae (BLRI 22827).

## DISCUSSION

The original descriptions of 11 eastern Nearctic *Polycentropus* species and redescription of *P. centralis* herein brings the total number of described Nearctic *Polycentropus* species to 16. Previously, Orfinger et al. (2022a) described larvae of 4/7 known western Nearctic species. In that paper, the authors discussed the current inability to separate larvae of the *Polycentropus* sensu lato genera and suggested that a key or diagnostic matrix to all Nearctic species of the larvae of *Polycentropus* sensu lato may be required. The present study is a significant step towards that goal.

We encountered 2 notable instances of differences in character states of the eastern and western fauna. The 1<sup>st</sup> is the relative length of the basal and distal segments of the anal prolegs. In the western larvae, the larvae of *Polycentropus denningi* Smith, 1962 has basal anal proleg segments of roughly the same length as the distal segments (Fig. 2E; Orfinger et al. 2022a), a character state previously unseen in *Polycentropus* larvae. No eastern Nearctic larvae demonstrated this character state. Instead, all eastern Nearctic larvae have basal segments distinctly longer than the distal segments (Fig. 2F). The 2<sup>nd</sup> notable character state that differentiates the known eastern and western *Polycentropus* larvae pertains to the posterior muscle scar patterns of the frontoclypeal apotome. If a scar pattern is present, western larvae possess either a shallow arc (Fig. 3A)



or a trapezoidal (Fig. 3C) configuration (Orfinger et al. 2022a). The eastern Nearctic fauna shared these scar patterns, with some species also having a linear configuration (e.g., *P. confusus*; Fig. 3B) not yet seen among western species.

The eastern Nearctic *Polycentropus* fauna is divided into 2 species groups. The *P. colei* Species Group consists of 3 species (*Polycentropus barri* Ross and Yamamoto, 1965; *P. colei*; and *P. rickeri*). The larvae of the latter 2 species are described for the 1<sup>st</sup> time here. Both exhibit large, rounded, yellow to orange-yellow heads with little (i.e., *P. rickeri*) to no (i.e., *P. colei*) muscle scarring on the posterior ½ of the frontoclypeal apotome. It would not be surprising if the unknown larva of *P. barri* also has a large, round, yellow to orangish-yellow head with little or no muscle scarring on the posterior ½ of the frontoclypeal apotome. Recent phylogenies published by Orfinger et al. (2022b) based on mitochondrial cytochrome oxidase I DNA barcoding data support the close relationship of *P. colei* and *P. rickeri*. It is possible that the reduction of muscle scarring of the posterior ½ of the frontoclypeal apotome is a synapomorphy of this putative monophyletic grouping. A more robust phylogeny of the *P. colei* Species Group would be useful in testing this hypothesis.

Unlike the *P. colei* Species Group, known larvae of the *P. confusus* Species Group possess a series of muscle scars on the posterior ½ of the frontoclypeal apotome. Three muscle scar patterns are evident, including shallow arc (Fig. 3A), linear (Fig. 3B), and trapezoidal (Fig. 3C) configurations. Among known larvae, the occurrence of muscle scar patterns appears to be distributed fairly evenly, with 3 species (*P. alabamensis*, *P. blicklei*, and *P. floridensis*) exhibiting a shallow arc, 3 species (*P. centralis*, *P. confusus*, and *P. pentus*) exhibiting a linear pattern, and 4 species (*P. carlsoni*, *P. carolinensis*, *P. elarus*, and *P. maculatus*) exhibiting a trapezoidal pattern. It is unclear whether these character states are correlated with phylogeny. For example, species exhibiting a linear pattern appear to be closely related, according to the preliminary trees presented by Orfinger et al. (2022b), forming a monophyletic grouping along with *Polycentropus chelatus* Ross and Yamamoto, 1965. Alternatively, the correlation of these character states among species with shallow arcs and trapezoidal patterning is less apparent. A robust phylogeny, ideally generated from additional mitochondrial and nuclear molecular data potentially coupled with male, female, and larval morphological characters, will be useful in determining the extent of the phylogenetic signal of this and other morphological characters. Once such a phylogeny is available, mapping of morphological and distributional data onto the topology, complemented by additional ecological studies, should shed light on morphological synapomorphies, biogeography, and functional morphology of these animals.

Of the 19 nominal species of the *P. confusus* Species Group, the larvae of 9 remain unassociated and undescribed,

namely *Polycentropus aileenae* Orfinger and Moulton, 2021; *P. chelatus*; *Polycentropus chenoides* Ross and Yamamoto, 1965; *Polycentropus dinkinsorum* Orfinger and Etnier 2020; *Polycentropus neiswanderi* Ross, 1947; *Polycentropus pixi* Ross, 1944; *Polycentropus stephani* Bowles, Mathis, and Hamilton, 1993; *Polycentropus thaxtoni* Hamilton and Holzenthal, 1986; and *Polycentropus vernus* Hamilton, Harris, and Lago, 1990.

Although this and other recent works (e.g., Orfinger et al. 2022a) have increased our understanding of the larval taxonomy of Nearctic *Polycentropus* and the distributions of the Nearctic fauna, much work is needed. First, during the search for undescribed larvae, additional exemplars of described larvae will be encountered that better capture the intraspecific morphological and molecular variation. Additional characterization of intraspecific variation is particularly important for some species, such as *P. carlsoni* and *P. elarus*, for which only 1 specimen or few larval specimens are known. Future efforts targeting unknown larvae, pupae, and females will also likely uncover new distributional records, as exemplified by the new state record presented here of *P. carlsoni* from southern Pennsylvania. Although this species had been recorded nearby in northern Virginia and Maryland, it had gone undetected in Pennsylvania until now.

Finally, although our understanding of the larval taxonomy of the Nearctic *Polycentropus* fauna is much improved, our knowledge of the pupae and females and the ecology of these animals is limited. No Nearctic *Polycentropus* pupae have been described to date, and only a few are associated (ABO, unpublished data). The same is true for *Polycentropus* females. Likewise, detailed studies of the ecology and life histories of most species are lacking, due in large part to our inability to identify many larvae to species. Descriptions of larvae provided by this research will help facilitate those studies for more eastern Nearctic *Polycentropus* species. Future efforts should attempt to associate and describe remaining larvae, pupae, and females by using molecular methods (e.g., as by Orfinger et al. 2022b), the metamorphotype method (Milne 1938), or by rearing of pupae. Such additional associations will in turn enable detailed studies of these animals' biology and, ideally, their ultimate incorporation into existing bioassessment programs at the species level. Given the continuing inability to diagnose larvae of Nearctic *Polycentropus*, *Holocentropus*, *Plectrocnemia*, and *Cermotina*, such efforts will be especially valuable to the goal of describing and diagnosing the larvae of all Nearctic species of these genera.

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and editing. AKR: conceptualization, resources, supervision, validation, writing—review and editing.

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