SYSTEMATIC STUDIES OF THE NEARCTIC *Polycentropus SENSU STRICTO* (TRICHOPTERA: POLYCENTROPODIDAE)

By

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To my Mom and Dad, who have always offered unwavering support of my interest in nature and science. Their love and encouragement never faltered, regardless of any insect, reptile, or other critter brought into the house by their son

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LIST OF ABBREVIATIONS

AMNH	American Museum of Natural History, New York City, New York, U.S.A.
BIOUG	Centre for Biodiversity Genomics, University of Guelph, Guelph, Ontario, Canada
BLRI	Blue Ridge Parkway Insect Collection, Asheville, North Carolina, U.S.A.
BYU	Monte L. Bean Life Science Museum at Brigham Young University, Provo, Utah, U.S.A.
CCDB	Canadian Centre for DNA Barcoding, Biodiversity Institute of Ontario, University of Guelph, Guelph, Ontario, Canada
CEI	The Insect Collection of Cole Ecological Inc., Greenfield, Massachusetts, U.S.A.
CUAC	Clemson University Arthropod Collection, Clemson, South Carolina, U.S.A.
CUGA	Cumberland Gap National Historical Park Insect Collection, Middlesboro, Kentucky, U.S.A.
ECC	Etnier Caddisfly Collection of the University of Tennessee, Knoxville, Tennessee, U.S.A.
FAMU	Florida A&M University portion of the Florida State Collection of Arthropods, Tallahassee, Florida, U.S.A.
FSCA	Florida State Collection of Arthropods, Gainesville, Florida, U.S.A.
INHS	Illinois Natural History Survey, Champaign, Illinois, U.S.A.
LIRI	Little River National Preserve, National Park Service, Fort Payne, Alabama, U.S.A.
MACA	Mammoth Cave National Park, National Park Service, Mammoth Cave, Kentucky, U.S.A.
MCZ	Museum of Comparative Zoology at Harvard University, Cambridge, Massachusetts, U.S.A.
NPS	US National Park Service network of collections
ROME	Royal Ontario Museum, Toronto, Ontario, Canada

UMSP	University of Minnesota Insect Collection, St. Paul, Minnesota, U.S.A.
USNMENT	United States National Museum Entomology Collection, Washington D.C., U.S.A.

Abstract of Dissertation Presented to the Graduate School
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SYSTEMATIC STUDIES OF THE NEARCTIC *Polycentropus SENSU STRICTO* (TRICHOPTERA: POLYCENTROPODIDAE)

By

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Twenty-nine nominal species of the genus *Polycentropus* Curtis, 1835 *sensu stricto* (Trichoptera: Polycentropodidae) are recorded from the Nearctic Region. Like most Trichoptera, these species were described from males while taxonomic knowledge of females and immature stages requires substantial investigation. Using molecular and morphological data, this dissertation sought to improve our knowledge of the Nearctic *Polycentropus* species, with an emphasis on females, larvae, and the eastern Nearctic *Polycentropus confusus* Species Group.

Chapter 1 introduces the requisite background information on which the remaining dissertation builds. Chapter 2 describes the use of mitochondrial DNA barcoding using cytochrome oxidase I (COI) to associate unknown females and larvae with known males and to generate a preliminary, species-level phylogenetic analysis of the Nearctic fauna. Chapter 3 and Chapter 4 leverage the results of Chapter 2 to describe and diagnose larvae of the western and eastern *Polycentropus* species, respectively, and discuss limitations and opportunities for diagnosing larvae of species of the *Polycentropus sensu lato*. Chapter 5 revises the *Polycentropus confusus* Species Group based on adults and includes dichotomous keys to males and females. Finally,

Chapter 6 summarizes the findings of the dissertation and discusses the needs and opportunities for future studies of the Nearctic Polycentropodidae.

The larvae of 15 Nearctic *Polycentropus* species are associated for the first time, and the identity of the larva of *P. centralis* Banks 1914 is confirmed. Females of three species are associated for the first time. The 16 larvae are described and diagnosed using diagnostic matrices. Males of all species (n=19) of the *Polycentropus confusus* Species Group are illustrated and redescribed, and the known females (n=14) are illustrated and redescribed or originally described. Two neotypes are designated and keys are provided for males and females of the *P. confusus* Species Group. Numerous new state and province records are documented along with one new country record, and erroneous records are corrected. This dissertation improves our knowledge of the distribution, taxonomy, and phylogeny of the Nearctic *Polycentropus* species and paves the way for basic biological studies and applied water quality tolerance research.

CHAPTER 1
INTRODUCTION

The Trichoptera, or caddisflies, comprise a cosmopolitan and diverse order of primary aquatic insects whose species richness surpasses all other primarily aquatic insect orders combined (Holzenthal et al., 2007; Morse et al., 2019a, b). Caddisfly larvae inhabit a wide variety of lentic and lotic freshwater and brackish systems, including streams, creeks, rivers, ponds, lakes, estuaries, ephemeral pools, and even marine tidal habitats in the case of the Chathamiidae (McCafferty, 1981; Wiggins, 1996; Morse et al., 2019b). As holometabolous insects, caddisflies undergo complete metamorphosis manifest as an egg to larva to pupa to adult ontogeny. As a result, the immature stages are drastically different both ecologically and morphologically relative to the adults.

The larval stage is of particular interest to a variety of fields outside of Trichoptera research, including to local, state, and national governmental and private agencies that use the larvae for monitoring freshwater ecosystem health, the fly-fishing community, and ecologists studying other aspects of freshwater ecosystems (Hamilton, 1986; Morse et al., 2017). Because most species are described based on male genitalia, larvae and females are relatively unknown despite widespread interest in the immature stages of caddisflies. The inability to identify larval Trichoptera prevents determination of species' life histories, habitats, and ecological niches and traits.

Even among the Nearctic Trichoptera, which is among the best globally characterized groups, much work has yet to be done. In fact, the larval identities of most American Trichoptera species remain unknown (Ruiter et al., 2013; Morse et al., 2017), including members of the fixed-retreat-making family Polycentropodidae (Trichoptera:

Annulipalpia). Among the most diverse Trichoptera families, polycentropodids are cosmopolitan in distribution and are represented globally by more than 800 extant nominal species in 14 genera (Chamorro and Holzenthal, 2011, Johanson et al., 2012, Morse, 2022). In the Nearctic Region, 78 species and eight genera have been recorded (Rasmussen and Morse, 2022).

A prime example of the non-male taxonomic knowledge gap is the Nearctic fauna of the genus *Polycentropus* Curtis, 1835 (Trichoptera: Polycentropodidae) *sensu stricto*. The most speciose polycentropodid genus in the Nearctic, almost no *Polycentropus sensu stricto* larvae and only half of the females were previously known. These caddisflies inhabit a variety of lotic habitats ranging from seeps and first-order streams to navigable rivers (e.g., Fig. 1-1, Sams Creek). Like other polycentropodids, members of *Polycentropus sensu stricto* construct funnel-shaped nets or flat tents using silk produced from their labial silk glands (Morse et al., 2019b). Within these silken retreats, the species feed on various small invertebrate organisms and/or filter fine particulate organic matter (FPOM) (Morse et al., 2019b).

The genus has undergone a complex taxonomic history. In his treatment of Illinois Trichoptera, Ross (1944) transferred all members of *Holocentropus* McLachlan, 1878 and *Plectrocnemia* Stephens, 1836 to the genus *Polycentropus* Curtis, 1835 (Trichoptera: Polycentropodidae). In doing so, Ross (1944) did not go so far as to judge the generic status of *Holocentropus* and *Plectrocnemia*, but justified the transfer by stating, “The characters of the larvae and pupae, as well as certain characters of the adults, indicate that these species together form a single unit as contrasted with other generic groups in the family.” After 1944, North American taxonomists adopted Ross’ classification scheme while European taxonomists opted to ignore the classification and instead maintain the pre-1944 scheme distinguishing among the three genera (Nimmo, 1986; Armitage and Hamilton, 1990; Orfinger, 2019). Until fairly recently, little phylogenetic evidence existed to evaluate the rival classification schemes. In 2011, Chamorro and Holzenthal generated a robust phylogeny of the polycentropodids based on larval, pupal, and adult morphological characters. To rectify the conflicting placement of species within the three genera, Chamorro and Holzenthal (2011) reinstated the pre-1944 placement of *Holocentropus* and *Plectrocnemia* species based on phylogenetic evidence. Those North American species described as members of *Polycentropus* after 1944, as listed by Fischer (1972) under *Plectrocnemia* or in Nimmo's (1986) species groups 'A', 'B', 'D' and 'G', were also transferred to *Plectrocnemia* by Chamorro and Holzenthal (2011). Two omissions were subsequently transferred by Orfinger (2019; 2022), namely *Plectrocnemia harpi* (Moulton and Stewart, 1993) and *Holocentropus timesis* (Denning, 1948), respectively. Hereafter, *Polycentropus sensu stricto* will be called simply *Polycentropus*.

Presently, 29 nominal *Polycentropus* species are recorded from the Nearctic Region, defined here as North America north of Mexico. The Nearctic fauna exhibits a largely east-west divide about the states bordering the western bank of the Mississippi River, with the western fauna represented by seven species and the more speciose eastern fauna comprising 22 species. Of the 29 named species, 27 are assigned to Species Groups based on morphological similarity while two remain unplaced (Hamilton, 1986; Armitage and Hamilton, 1990; Orfinger and Moulton, 2021). These Species Groups are the *Polycentropus arizonensis* Species Group (western, one

Nearctic species), the *Polycentropus gertschi* Species Group (western, four Nearctic species), the *Polycentropus colei* Species Group (eastern, three species), and the *Polycentropus confusus* Species Group (eastern, 19 species; = *Polycentropus maculatus* Species Group *sensu* Ross, 1944).

Of the 29 species, the females of only 18 (62%) were previously described while the larva of only one (3%), *P. centralis* Banks, 1914, was previously known (Ross, 1944; Hoffman and Morse, 1990; Orfinger and Moulton, 2021). Although Hamilton (1986) addressed the phylogeny of some of the Species Groups, no effort was made to assess historical relationships within the most speciose group, namely the *P*. *confusus* Species Group. Hamilton emphasized the need for additional larval and female characters prior to such an undertaking.

Unsurprisingly, the lack of knowledge of non-male Nearctic *Polycentropus* members translates to a comparable lack of understanding of those species' ecologies. The overarching goal of this dissertation is, therefore, to bolster the taxonomic and systematic knowledge of the North American *Polycentropus*. Chapter 2 uses mtDNA barcoding to associate larvae, and adult female and males of Nearctic *Polycentropus* species. Chapter 3 subsequently describes and diagnoses the newly associated western larvae and discusses novel findings relating to the identification of larval Nearctic Polycentropodidae. Chapter 4 describes and diagnoses the identifiable eastern larvae. Then, Chapter 5 presents the taxonomic revision of the *Polycentropus confusus* Species Group, treating males, females, and larvae. Finally, Chapter 6 concludes the dissertation with broader conclusions and suggestions for future research directions.

List of Nearctic *Polycentropus* Curtis, 1835, species

For convenience, an annotated list of the 29 nominal species of Nearctic *Polycentropus* fauna is provided below organized by Species Group assignment.

POLYCENTROPUS ARIZONENSIS SPECIES GROUP (WESTERN)

Polycentropus arizonensis Banks, 1905

POLYCENTROPUS COLEI SPECIES GROUP (EASTERN)

Polycentropus barri Ross and Yamamoto, 1965

Polycentropus colei Ross, 1941

Polycentropus rickeri Yamamoto, 1966

POLYCENTROPUS CONFUSUS SPECIES GROUP (EASTERN)

Polycentropus aileenae Orfinger and Moulton, 2021

Polycentropus alabamensis Hamilton, Harris, and Lago, 1990

Polycentropus blicklei Ross and Yamamoto, 1965

Polycentropus carlsoni Morse, 1971

Polycentropus carolinensis Banks, 1905

Polycentropus centralis Banks, 1914

Polycentropus chelatus Ross and Yamamoto, 1965

Polycentropus chenoides Ross and Yamamoto, 1965

Polycentropus confusus Hagen, 1861

Polycentropus dinkinsorum Orfinger and Etnier, 2020

Polycentropus elarus Ross, 1944

Polycentropus floridensis Lago and Harris, 1983

Polycentropus maculatus Banks, 1908

Polycentropus neiswanderi Ross, 1947

Polycentropus pentus Ross, 1941

Polycentropus pixi Ross, 1944

Polycentropus stephani Bowles, Mathis, and Hamilton, 1993

Polycentropus thaxtoni Hamilton and Holzenthal, 1986

Polycentropus vernus Hamilton, Harris, and Lago, 1990

POLYCENTROPUS GERTSCHI SPECIES GROUP (WESTERN)

Polycentropus aztecus Flint, 1967

Polycentropus gertschi Denning, 1950

Polycentropus halidus Milne, 1936

Polycentropus picana Ross, 1947

UNPLACED SPECIES (WESTERN)

Polycentropus denningi Smith, 1962

Polycentropus variegatus Banks, 1900

Figure 1-1. Sams Creek, Great Smoky Mountains National Park, Blount Co., TN, USA, an example of a habitat of Nearctic *Polycentropus* Curtis, 1835, species.

CHAPTER 2

ASSOCIATING LIFE STAGES AND SEXES OF NEARCTIC *Polycentropus* CURTIS, 1835 (TRICHOPTERA: POLYCENTROPODIDAE) USING MITOCHONDRIAL DNA BARCODING

A key challenge in animal taxonomy is associating morphologically distinct life stages and sexes within a species. This is particularly true of the Endopterygota (= Holometabola), 11 insect orders that exhibit complete metamorphosis. The immature stages of holometabolous insects are generally markedly different in both ecology and morphology from adults.

The caddisflies (order Trichoptera) are a prime example of a holometabolous order for which our knowledge of non-males is relatively poor. Among the caddisflies, the taxonomy and identification tools used for aquatic larvae and terrestrial females for most species lag those of terrestrial males, on which alpha taxonomy is mostly based. The inability to identify larvae and females limits detailed study of these organisms' ecology, evolution, and water quality tolerance for development of biomonitoring indices.

There are some notable exceptions to this pattern, however. For example, larvae of the central European caddisfly fauna have been well characterized (Waringer and Graf, 2011). Still, in many regions and for most taxa, male taxonomic knowledge far surpasses that of females, larvae, pupae, and eggs. Our taxonomic understanding of the Oriental and Neotropical Trichoptera faunas typify this generality, with immature and female knowledge poorly resolved relative to male taxonomy (Morse, 2016; Pes et al., 2018, respectively).

Reprinted with permission from Orfinger, A. B., Morse, J. C., & Hix, R. L. (2022a). Associating life stages and sexes of Nearctic *Polycentropus* Curtis, 1835 (Trichoptera: Polycentropodidae) using mitochondrial DNA barcoding. Ecology and Evolution, 12(3), e8741.

The Nearctic caddisfly fauna also exemplifies the disparity of male and non-male caddisfly knowledge (Ruiter et al., 2013). One example of such a taxonomic knowledge gap exists among Nearctic species of the cosmopolitan genus *Polycentropus* Curtis, 1835 (Trichoptera: Polycentropodidae). The genus *Polycentropus* is represented by 29 species in the Nearctic (Rasmussen and Morse, 2022). Larvae of *Polycentropus* construct bag-like silken filter nets in clean, flowing water with which they capture various small invertebrate prey (Wiggins, 1996).

The Nearctic *Polycentropus* fauna can be divided according to geographic distribution, with 22 eastern species and seven western species. This distributional scheme corresponds to the east Nearctic and west Nearctic recognized Trichoptera biogeographical regions (de Moor and Ivanov, 2008) and is reflected in the Nearctic *Polycentropus* species and Species Group distributions (e.g., Hamilton, 1986). The 29 Nearctic *Polycentropus* species are assigned to four monophyletic Species Groups based on synapomorphies of male genital characters (Armitage and Hamilton, 1990; Hamilton, 1986). These include the *Polycentropus arizonensis* Species Group (1 Nearctic species, western), the *P. confusus* Species Group (19 Nearctic species, eastern), the *P. colei* Species Group (3 Nearctic species, eastern), and the *P. gertschi* Species Group (4 species in the Nearctic, western) (Armitage and Hamilton, 1990; Hamilton, 1986). Two additional western species, *P. denningi* Smith, 1962, and *P. variegatus* Banks, 1900, are unplaced (Armitage and Hamilton, 1990).

Of the 29 species, the larva of only one species (3%) and females of only 15 species (52%) were previously described. While larval–adult caddisfly associations have traditionally been achieved by rearing larvae or employing the metamorphotype method (*sensu* Milne, 1938), larval and pupal Nearctic *Polycentropus* are morphologically similar, difficult to sample due to their cryptic nature, and have not yet been successfully reared. Female associations are conventionally based on reared individuals, geographic associations, or individuals sampled *in copula* with identifiable males. Modern molecular techniques offer an alternative solution in the form of DNA barcoding.

DNA barcoding employs the mitochondrial cytochrome c oxidase subunit I (mtCOI) fragment of 658 base pairs to identify species because of the sequence's low intraspecific variability and high interspecific divergence, or barcoding gap, allowing for high success of animal species delineation (Hebert et al., 2003; Ruiter et al., 2013). DNA barcoding has been suggested as an option for associating the different trichopteran life stages and sexes (Barcelos-Silva et al., 2018; Ruiter et al., 2013; Zhou et al., 2007), as well of those of other aquatic insects including stoneflies (e.g., Mynott, 2015; Mynott et al., 2011) and mayflies (e.g., Malakauskas and Zonca, 2018; Molina et al., 2017). The technique has been used successfully to associate larvae and adults in multiple cases, including in a large variety of caddisfly taxa in North America and Asia (Barcelos-Silva et al., 2018; Ruiter et al., 2013; Stroil et al., 2018; Zhou et al., 2007). In fact, barcoding exhibits high sequencing success and >95% success in species assignment, including for Trichoptera (Hajibabaei and McKenna, 2012). Even shorter fragments of the COI barcode region of at least 200 bp can reliably identify species in 95% of cases across a variety of taxa (Meusnier et al., 2008; Yeo et al., 2020). Thanks in part to the Trichoptera Barcode of Life (TBOL) campaign, a robust framework and reference library exist for sequencing, sourcing, and analyzing caddisfly barcoding data (Frandsen et al., 2016; Zhou et al., 2016). By employing DNA barcoding analyses on a wide geographical and morphological variety of larvae and adult males and females, the present study aims to assign species identities to currently unidentifiable larvae and females of the genus *Polycentropus* in the Nearctic. In doing so, this work informs the search for diagnostic morphological characters of larval and female *Polycentropus* species, ultimately making their visual identification possible.

Methods

Specimen Material

Specimens of Trichoptera housed in the Canadian Centre for DNA Barcoding, Biodiversity Institute of Ontario, University of Guelph, Canada (CCDB), the Clemson University Arthropod Collection (CUAC), the portion of the Florida State Collection of Arthropods (FSCA) housed at Florida A&M University (FAMU), the US National Park Service (NPS) network of collections, the Illinois Natural History Survey (INHS), the Monte L. Bean Life Science Museum at Brigham Young University (BYU), privately donated material, and material newly collected for this study were used. Late-instar larvae and adult females were sorted into unique morphotypes. A leg from each of up to 10 specimens of each morphotype was subsampled for DNA. Adult males of each species were also sequenced, or their barcoding sequences sourced from the Barcode of Life Database (BOLD). Finally, available sequences from females, larvae, and pupae identified to genus or species (for some females) were mined from BOLD. Each of the male specimens whose sequences were mined from BOLD has been identified by a taxonomic expert, including the author in most cases, and vouchered in a public natural history collection. Each of the female and immature specimens whose sequences were mined from BOLD have been identified by a taxonomic authority, including the author in many cases, or by Barcode Index Number (BIN) matching *sensu* Ratnasingham and Hebert (2013). Species for which no unknown larval or unknown female sequences of at least 300 base pairs were available were excluded.

DNA Extraction and Sequencing

DNA amplification and alignment generally followed procedures used by Zhou et al. (2007), Baird et al. (2011), Ruiter et al. (2013), and Barcelos-Silva et al. (2018). One leg was subsampled from each specimen, and molecular methods followed standard DNA barcoding protocols (Ivanova et al., 2006). DNA extraction and sequencing were accomplished at the Canadian Centre for DNA Barcoding, Biodiversity Institute of Ontario, University of Guelph, Canada (CCDB). DNA was extracted using an AcroPrep 96-well 3.0-μm glass-fiber plate and eluted with 50 μl of distilled water. Extracted DNA was then amplified targeting the full 658-bp barcoding fragment of COI using polymerase chain reaction DNA amplification and alignment (PCR) in a 12.5 μl reaction volume following the protocol of Ivanova et al. (2006).

The reaction was comprised of 6.25 μl of 10% trehalose (D-(+)-trehalose dehydrate) (per CCDB standard protocols), 2 μl of ddH2O, 1.25 μl 10x of reaction buffer, 0.625 μl of 50 mM MgCl2, 0.0625 μl of 10 mM dNTP, 0.06 μl of 5 U/μl Taq DNA polymerase (Invitrogen), 0.125 μl of 10 μM of both forward and reverse primer, and 2 μl of DNA. The primer used to amplify the full barcoding region was (LCO1490 50-GGTCAACAAATCATAAAGATATTGG-3'/HC02198 50-TAAACTTCAGGGTG ACCAAAAAATCA-30) (Folmer et al., 1994), applied to those specimens preserved in >95% ethanol since collection or preserved in <95% ethanol but that were collected within one year of DNA extraction. For older or more degraded samples, that is, those preserved in <95% ethanol and that were more than 1 year old, the following primers were used to target shorter, overlapping segments of COI: Uni-MinibarF1 (59-TCCACTAATCACAARGATATTGGTAC-39) and Uni-MinibarR1 (59-GAAAATCATAATGAAGGCATGAGC39). These are primers designed for a short fragment at the 5' terminus of the standard barcode region (Meusnier et al., 2008).

Each PCR reaction was thermocycled at 94°C for 1 min; 5 cycles at 94°C for 40 s, 45°C for 40 s, 72°C for 1 min; 35 cycles at 94°C for 40 s, 51°C for 40 s, 72°C for 1 min; held at 72°C for 5 min, and stored at 4°C. Successful PCR reactions were evaluated using an Invitrogen 2% agarose E-gel with an ethidium bromide stain and developed with UV, and if successful, were subsequently bi-directionally sequenced using BigDye and an Applied Biosystems 3730XL DNA analyzer (Hajibabaei et al., 2005). All data associated with each specimen included in this study, including collection information, storing institution, ecological data, taxonomy, photographs, and COI sequences, are available in BOLD under the publicly accessible dataset titled "DS-POLYCSS Nearctic Polycentropodidae (Trichoptera)" (Orfinger et al., 2021).

Sequence Alignment, P-distance Calculation, and Tree Construction

A two-tiered analytical approach was applied to datasets. Initially, a "pooled" dataset including all available sequences was used in executing all tree-and distance-based analyses. Not only were initial associations gathered from the pooled analysis but species for which associations were not currently attainable were also recovered as targets for future association efforts. Following analysis of the pooled dataset, "filtered" datasets composed of only those species for which successful associations were recovered were used in a subsequent iteration of tree-based and distance-based analyses described below.

The filtered datasets were delineated according to biogeographical assignment, with western Nearctic species assigned to the "western" dataset and eastern Nearctic species assigned to the "eastern" dataset following biogeographical patterns recognized in many Nearctic caddisfly taxa, including *Polycentropus* (e.g., Cooper and Morse, 1998; Hamilton, 1986; Lago and Harris, 1987a; Prather and Morse, 2001; Trivette, 1969). The east–west geographic delineation follows a slight variation of the definition of Lago and Harris (1987a) and Cooper and Morse (1998), where "eastern" refers to Manitoba and the US states adjacent to either side of the Mississippi River and eastward, and "western" pertains to the remaining Nearctic region.

A total of 262 sequences of males, females, pupae, and larvae representing 23 total species were included in the initial, pooled analysis. A total of 66 sequences of western species (representing 23 adults and 43 larvae) and 88 sequences of eastern species (representing 48 adults and 40 larvae) were used in the filtered datasets. All sequences used consisted of at least 325 base pairs. All data associated with specimens incorporated in the filtered datasets are publiclly available[1]. COI sequences were aligned using default settings of MUSCLE (Edgar, 2004) in MEGA v. X 10.1.0 (Kumar et al., 2018) for each western and eastern taxon. The alignments were checked manually to avoid stop codons, indels, and amino acid translation frame shifts.

Pairwise divergence distances (p-distances) within- and between-species divergences of COI nucleotides were calculated in MEGA v. X 10.1.0 (Kumar et al., 2018) using the Kimura 2-parameter evolution model (K2P) (Kimura, 1980) and pairwise

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[1] Data accessible in Supplementary File 4 for the western fauna and Supplementary File 5 for the eastern fauna of: Orfinger, A. B., Morse, J. C., & Hix, R. L. (2022). Associating life stages and sexes of Nearctic *Polycentropus* Curtis, 1835 (Trichoptera: Polycentropodidae) using mitochondrial DNA barcoding. Ecology and Evolution, 12(3), e8741.

deletion of missing sites. P-distance describes the proportion of nucleotide sites at which sequences being compared are different and is obtained by dividing the number of nucleotide differences by the total number of nucleotides compared. The number of changes of nucleotides is termed the p-distance (Meyer and Paulay, 2005). Lower pairwise distances are an indication of fewer nucleotide changes, with lower pairwise p-distances expected intraspecifically than interspecifically resulting in a “barcoding gap” (Meyer and Paulay, 2005). Unrooted neighbor-joining (NJ) trees of all available haplotypes, with pairwise deletion of missing sites and K2P distances (Kimura, 1980), were constructed in MEGA v. X 10.1.0 (Tamura et al., 2007). Branch support was calculated using 1,000 bootstrap replicates.

The model-based Maximum Likelihood (ML) method was performed as an additional way to evaluate support for the monophyletic groupings of species. The optimal substitution model was identified using ModelTest-NG v0.1.7 (Darriba et al., 2020) and assessed using AIC, AICc, and BIC criteria. The partitioning scheme was identified and implemented using RAxML version 8 (Stamatakis, 2014) via raxmlGUI 2.0.0 (Edler et al., 2021). Unrooted ML trees were inferred with a TIM2+G4 model using RAxML version 8 (Stamatakis, 2014) via raxmlGUI 2.0.0 (Edler et al., 2021). Bootstrap support was calculated from 1,000 replicates. Resulting trees were visualized and annotated using the Interactive Tree Of Life (iTOL) v5 (Letunic and Bork, 2021) and Adobe Illustrator® version 24.3. Adobe Illustrator version 24.3 was used to make final cosmetic edits, without altering branch lengths, bootstrap values, and topologies.

Larval–Adult and Male–Female Association

The molecular association of larval and female specimens followed criteria proposed by Zhou et al. (2007) and employed by Ruiter et al. (2013) based on a phylogenetic species conceptual approach. Briefly, when the sequence of an individual of an unknown species is identical to that of a confirmed male of a species (i.e., pairwise p-distance is zero), is nested among near-identical COI sequences of males of a species, or is nested in a monophyletic group of specimens of a given species on both trees, the corresponding species name was applied to the unknown female or larval individual.

Results

Tree-Based Associations

Pooled fauna for tree-based associations

A total of 14 novel larval–male associations and two male–female associations were indicated in the pooled analysis. Both the neighbor-joining tree (Figure 2-1) and maximum likelihood tree (Figure 2-2) yielded species-level monophyletic groupings with strong bootstrap support with two notable exceptions. While each species formed a monophyletic group with strong statistical support, *P. alabamensis* Hamilton et al. 1990, was nested among *P. elarus* Ross, 1944, sequences in both NJ and ML trees. Similarly, *P. aileenae* Orfinger and Moulton, 2021, was found to be nested within *P. blicklei* Ross and Yamamoto, 1965, in the NJ tree with low (<50) bootstrap support while the two species formed a single, more admixed clade (*P. aileenae* + *P. blicklei*) with low (<50) bootstrap support in the ML tree.

The identities of four lineages were equivocal. These were termed clades ‘A’, ‘B’, ‘C’, and ‘D’. Clades B and C contained two specimens each, while A and D were each represented by a single unplaced specimen. Clade A occurred only in the ML tree and consisted of a male specimen (NECAD245-08) identified as *P. blicklei* and sister to the *P. aileenae* + *P. blicklei* clade. This specimen is recovered in the NJ tree as a member of the *P. blicklei* clade. Clade A is sister to Clade B, which also occurs only in the ML tree. Clade B consists of two males identified as *P. blicklei* and *P. carolinensis* Banks, 1905 (specimens NECAD247-08 and ORFIN052-20, respectively). Both of these specimens are recovered as members of the *P. carolinensis* clade with strong support in the NJ tree. Clade C was recovered in both trees and is represented by two unassociated larval specimens, ORFIN047-20 and LEPTO1466-13, collected in South Carolina and Pennsylvania, respectively. Finally, Clade D occurs in each tree and is represented by a single, unassociated larva (ORFIN073-20).

Western Fauna for tree-based associations

In total, larvae of four of the seven western species were newly associated with confirmed adults, namely *Polycentropus denningi*, *Polycentropus gertschi* Denning, 1950, *Polycentropus halidus* Milne, 1936, and *Polycentropus variegatus*. Both the neighbor-joining tree (Figure 2-3) and maximum likelihood tree (Figure 2-4) yielded species-level monophyletic groups with strong bootstrap support.

Eastern Fauna for tree-based associations

In total, larvae of 10 of the 22 eastern species were associated for the first time with confirmed adults, namely *P. alabamensis*, *P. blicklei*, *P. carlsoni* Morse, 1971, *P. carolinensis*, *P. colei* Ross, 1941, *P. confusus* Hagen, 1861, *P. elarus*, *P. maculatus* Banks, 1908, *P. pentus* Ross, 1941, and *P. rickeri* Yamamoto, 1966. A notable molecular association confirmed the identity of the previously described larva of *Polycentropus centralis* Banks, 1914, which was known from a single, geographically associated specimen (Ross, 1944). Three novel female associations were also achieved for *P. alabamensis*, *P. carolinensis*, and *P. chelatus* Ross and Yamamoto, 1965.

Both the neighbor-joining tree (Figure 2-5) and maximum likelihood tree (Figure 2-6) yielded species-level monophyletic groupings with strong bootstrap support. While forming a monophyletic group, the *P. alabamensis* clade is nested within the *P. elarus* clade with high bootstrap support in each analysis.

Pairwise Divergence Distances

P-distance for pooled fauna

Summary p-distance data of the pooled fauna are presented in Table 2-1. Maximum intraspecific p-distance values were generally less than minimum interspecific values, indicating the existence of interspecific barcoding gaps. Notable exceptions reflect the ambiguous relationships recovered in the pooled NJ and ML trees. *Polycentropus aileenae* and *P. blicklei* exhibit a minimum distance of 0 and the maximum distance of 0.09. Similarly, *P. blicklei* and *P. carolinensis* exhibit a minimum distance of 0 and the maximum distance of 0.06, reflecting the ambiguity of clade B. Also notable is the small p-distance range between *P. alabamensis* and *P. elarus*, spanning a minimum of 0 and a maximum of only 0.01. The complete pairwise p-distance comparison for pooled fauna publiclly available[2].

P-distance for western fauna

The complete pairwise p-distance matrix for western fauna is publiclly available[3]. P-distance analysis corroborates tree-based analyses with instances of pairwise p-distances between sequences of adults and immature specimens being zero.

[2] Data available in Supplementary File 1 of: Orfinger, A. B., Morse, J. C., & Hix, R. L. (2022). Associating life stages and sexes of Nearctic *Polycentropus* Curtis, 1835 (Trichoptera: Polycentropodidae) using mitochondrial DNA barcoding. Ecology and Evolution, 12(3), e8741.

[3] Data available in Supplementary File 2 of: Orfinger, A. B., Morse, J. C., & Hix, R. L. (2022). Associating life stages and sexes of Nearctic *Polycentropus* Curtis, 1835 (Trichoptera: Polycentropodidae) using mitochondrial DNA barcoding. Ecology and Evolution, 12(3), e8741.

For example, the larval specimen of *P. halidus* with BOLD specimen ID SCCWRP0137008 shared an identical sequence with the male with BOLD specimen ID 09BBTUS-074, indicating that they are the same species. There were no instances of high p-distance values among congeners including immature and female specimens, with all intraspecific values ≤0.01.

Summary p-distance data of the western fauna are presented in Table 2-2. Maximum intraspecific p-distance values were always far less than minimum interspecific values, indicating the existence of interspecific barcoding gaps. Considering the inclusion of larvae in pairwise p-distance comparison, the presence of an interspecific barcode gap supports the specific assignments of larvae obtained from the phylogenetic analyses.

P-distances for eastern fauna

The full pairwise p-distance matrix for eastern fauna is publiclly available[4]. As with the western faunal analysis, p-distance analysis corroborates tree-based analyses with instances of pairwise p-distances between sequences of adults and immature specimens being zero. For example, the female *P. carolinensis* with BOLD specimen ID CCDB-34606-D05 had an identical COI sequence as the male with BOLD specimen ID CCDB-34606-E10 and the larva with BOLD specimen ID CCDB-34606-E07. Similarly, the female *P. chelatus* with BOLD specimen ID CCDB-34606-G06 produced a COI sequence identical to the male with BOLD specimen ID CCDB-34606-E12.

Summary p-distance data of the eastern fauna are presented in Table 2-3.

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[4] Data available in Supplementary File 2 of: Orfinger, A. B., Morse, J. C., & Hix, R. L. (2022). Associating life stages and sexes of Nearctic *Polycentropus* Curtis, 1835 (Trichoptera: Polycentropodidae) using mitochondrial DNA barcoding. Ecology and Evolution, 12(3), e8741.

Maximum intraspecific p-distance values were generally far less than minimum interspecific values, indicating the existence of interspecific barcoding gaps. Considering the inclusion of larvae in pairwise p-distance comparison, the presence of an interspecific barcode gap supports the specific assignments of larvae obtained from the phylogenetic analyses.

Two exceptions from these observations exist in *P. alabamensis* and *P. rickeri*. Specimens of *P. alabamensis* do not demonstrate an interspecific barcode gap with respect to *P. elarus*. This is mirrored in the phylogenies, with the *P. alabamensis* clade nested within the *P. elarus* clade. Meanwhile, *P. rickeri* specimens present high intraspecific pairwise p-distances, with a minimum of 0.04 and a maximum of 0.05 based on only three sequences.

Morphological Corroboration

Like males, female caddisflies are generally identified to species according to morphological aspects of the genitalia. For example, Ross (1944) provided a key to females of the *Polycentropus sensu lato* (i.e., *Holocentropus* McLachlan, 1878, *Plectrocnemia* Stephens, 1836, and *Polycentropus*) based largely on ventral views of cleared genitalia. Initial examination of the three females newly associated here suggests that the ventral plates and internal parts of the gonopods enable separation from other members of the *Polycentropus confusus* Species Group to which they belong.

The newly associated larvae reported here also appear separable by various aspects of their morphology. For example, Figure 2-7 illustrates the apparently consistent interspecific differences in head coloration, roundness, pigment banding, and muscle scar patterning. The degree to which anal claws are curved also appears intraspecifically consistently similar and interspecifically consistently different, with some demonstrating sharply curved anal claws and others possessing gradually curved anal claws.

Discussion

While the work presented here is not intended to infer phylogenetic relationships, some interesting observations arose that merit brief discussion. First, the nested position of *P. alabamensis* relative to the *P. elarus* clade recovered in neighbor-joining and maximum likelihood phylogenies suggests a close relationship between the species, or even that *P. alabamensis* could be a synonym of *P. elarus*. Genetic distances presented in Tables 2-1 and 2-2 suggest the latter conclusion. Still, synonymy is unlikely given the distinct differences readily observable in the male genitalia of each species [e.g., see figures 1A–1F by Hamilton et al. (1990) and figures 245A–245C of *P. elarus* by Ross (1944)]. Instead, it seems more plausible that these two species are closely related sister taxa. A robust phylogenetic study of the *Polycentropus confusus* Species Group, of which these two species belong, will help to resolve this question. Such a study is currently under way, incorporating multiple loci and morphology. So, too, will comparative descriptions of the now-identified larval stages of each species shed light on their relationships.

A second notable observation is the high intraspecific genetic distance observed in *P. rickeri* (Tables 2-1 and 2-2). From sequences of only two male specimens and one immature specimen, pairwise intraspecific distances spanned 0.04 to 0.05, or 4% to 5%. These data more than twice exceed the 2% COI distance threshold often employed to delineate species (Hebert et al., 2003; Meyer and Paulay, 2005; Rivera and Currie, 2009; Sweeney et al., 2011; White et al., 2014). The high values suggest the existence of cryptic species. This species has been reported from seven eastern U.S.A. states (Rasmussen and Morse, 2022). Future sampling and generation of additional molecular data, coupled with morphological study of exemplars from throughout its range, should be performed to test for potential cryptic species. Such an in-depth investigation will be necessary to verify the association proposed here based on the monophyly of the *P. rickeri* clade in the inferred trees.

If combined with investigations of *P. barri* Ross and Yamamoto, 1965, and *P. colei*, a phylogenetic study will also evaluate the relationships of the *P. colei* Species Group, which consists of *P. barri*, *P. colei*, and *P. rickeri*. Among the eastern NJ and ML tree topologies, *P. colei* and *P. rickeri* are recovered as sister taxa with high statistical support. This relationship makes sense within the current classification scheme. While no phylogeny exists yet for this Species Group, the relationship observed in the trees produced here suggests the Species Group is monophyletic. Similarly, among the western taxa, *P. denningi* and *P. variegatus* were recovered as sister taxa with high statistical support within both NJ and ML trees. This suggests close relatedness of these taxa currently unplaced in any Species Group, despite the distinct genitalia of their males (e.g., see figures by Armitage and Hamilton, 1990).

The relationship between *P. aileenae* and *P. blicklei* is similar to that of *P. alabamensis* and *P. elarus*, although with much lower statistical support in separating the former pair. This is not surprising considering a close sister relationship was hypothesized by Orfinger and Moulton (2021) given the morphological similarity of both sexes of the two species. In such cases of closely related Species Groups and sister species, COI is often insufficient by itself in resolving these relationships. For example, while COI was unable to separate the five Finnish members of the *Apatania zonella* (Zetterstedt, 1840) Species Group (Apataniidae), morphology and more than 2 million bp of double digest RAD sequencing (ddRAD-seq) sequence data supported the species hypotheses (Salokannel et al., 2021). A robust phylogeny combining morphology, COI DNA barcoding data, and additional molecular data will be needed to better refine our understanding of the relationship between these two taxa.

Several specimens merit further examination to resolve their specific identity. While ambiguous in the pooled ML tree, the male specimen NECAD247-08 is recovered with strong support as a member of the *P. carolinensis* clade in the pooled NJ tree. The other male specimen of clade B (ORFIN052-20) was examined and agrees with *P. carolinensis*. Therefore, it is likely that specimen NECAD247-08 was misidentified as *P. blicklei* and is in fact *P. carolinensis*, although examination of that specimen is needed to confirm.

The unassociated larvae of clade C are interesting targets for additional scrutiny. Specimen ORFIN047-20 from South Carolina does not completely agree with any of the associated larvae. Examination of the other specimen from Pennsylvania, LEPTO1466-13, is required to confirm that these two unassociated larvae represent the same species. Unfortunately, this specimen was unavailable for examination due to the COVID-19 pandemic but will be examined in the future when possible. Another larva of ambiguous identify is specimen AMII094-08, which may represent *P. aileenae* or *P. blicklei*. Its examination and comparison to *P. blicklei* larvae will be helpful in understanding its identity. As with specimen LEPTO1466-13, however, this specimen is housed in the same collection and was unavailable. The final unassociated larva, ORFIN073-20, comprises clade D and was collected in Washington state. According to the pooled ML and NJ trees, the specimen is closely allied to the *Polycentropus gertschi* Species Group, but is clearly distinct. This specimen is also subtly morphologically different from other associated larvae. It is possible that this specimen and the members of clade C represent undescribed species.

Alternatively, high-quality COI sequence exemplars may not yet be available for adults of the species, precluding molecular association. For example, unidentified members of clade C could represent *Polycentropus barri*, an eastern species and member of the *Polycentropus colei* Species Group along with *P. colei* and *P. rickeri*, for which no sequence data are available. Future adult sampling from near the collection localities of these specimens, coupled with additional COI sequencing to associate the adults, will be required to identify these larvae.

A single pupal specimen (ORFIN384-21) was identified as *P. arizonensis*. Unfortunately, examination of this specimen revealed that the pupal shelter was absent and larval sclerites were lost. Therefore, the specimen is not useful in associating the male and larva of the species via the metamorphotype method (Milne, 1938). Still, while pupal association was not a goal of this study, this is the first reported identification of the pupa of this species, enabling its future morphological study.

Previously, *Polycentropus centralis* was the only Nearctic *Polycentropus* species with an associated larva, based on a presumed geographic association (Ross, 1944). This association and description were based on a single specimen from Illinois and was confirmed with the molecular association of two additional specimens from Missouri and corroborating morphology described in Chapter 4.

In addition to *P. centralis*, the larvae of four western and 10 eastern species are newly associated, bringing the total number of identified Nearctic *Polycentropus* larvae to 15 of 29 species, or 52% of the known fauna. These novel associations pave the way for the morphological description and diagnoses of those species' larvae. The noted morphological characters that appear useful in separating larvae of different species treated here agree with previously published morphological data used to separate polycentropodid larvae, for example, in the former USSR (Lepneva, 1964, 1970), England (Hickin, 1967), and central Europe (Waringer and Graf, 2011). It is likely that these characters, coupled with distinct eastern or western Nearctic geographic distributions, will enable the generation of diagnostic matrices and dichotomous keys to species for identification of the now-associated larvae.

This study constitutes the initial step in this taxonomic process, which aims to culminate in identification tools useful for basic research and applied freshwater bioassessment strategies that utilize caddisfly larvae as sentinels of water quality (Behrens-Chapuis et al., 2021; Resh et al., 1995; Sweeney et al., 2011; White et al., 2014). In addition to novel larval associations, the newly associated females of *P. alabamensis*, *P. carolinensis*, and *P. chelatus* serve to provide material for their descriptions and diagnoses. While historically neglected, identifying female aquatic insects in biological surveys can greatly influence the number of recorded species in an area or at a given time (e.g., Ekrem et al., 2010). From this chapter, females of 17 Nearctic *Polycentropus* species are associated, constituting 59% of the known fauna. In many cases, female associations also allow for recognition and descriptions of the eggs of given species as well (e.g., by Orfinger and Moulton, 2021).

Until the description and diagnoses of the associated larvae and females are complete, and perhaps beyond that point, the newly generated molecular data presented here can serve to identify unknown larvae. Molecular identification of aquatic macroinvertebrates has been increasingly used in concert with traditional morphological identification in freshwater bioassessment (Behrens-Chapuis et al., 2021; Sweeney et al., 2011; White et al., 2014). The data on which the current analyses are based are publicly available and should serve as a reference library for the Nearctic *Polycentropus* species (Orfinger et al., 2021).

Table 2-1. Ranges of pairwise divergence (p-distance) among taxa for the pooled mtCOI dataset. Note that values are rounded to two decimal places. Maximum intraspecific values are displayed in bold.

Species	*P. aileenae*		*P. alabamensis*		*P. arizonensis*		*P. aztecus*		*P. blicklei*	
	min	max	min	max	min	max	min	max	min	max
P. aileenae	0	**0.07**	0.1	0.1	0.1	0.18	0.1	0.18	0	0.09
P. alabamensis			0	**0**	0.1	0.14	0.1	0.15	0.04	0.05
P. arizonensis					0	**0.01**	0.1	0.16	0.11	0.15
P. aztecus							0	**0.01**	0.12	0.14
P. blicklei									0	**0.02**
P. carlsoni										
P. carolinensis										
P. centralis										
P. chelatus										
P. colei										
P. confusus										
P. denningi										
P. elarus										
P. floridensis										
P. halidus										
P. gertschi										
P. maculatus										
P. neiswanderi										
P. pentus										
P. picana										
P. pixi										
P. rickeri										
P. variegatus										

Table 2-1. Continued.

Species	*P. carlsoni*		*P. carolinensis*		*P. centralis*		*P. chelatus*		*P. colei*	
	min	max	min	max	min	max	min	max	min	max
P. aileenae	0.06	0.13	0	0.13	0.1	0.15	0.1	0.14	0.12	0.16
P. alabamensis	0.07	0.07	0	0.04	0.1	0.1	0.1	0.1	0.13	0.16
P. arizonensis	0.09	0.15	0.1	0.15	0.1	0.13	0.1	0.14	0.14	0.17
P. aztecus	0.12	0.14	0.1	0.14	0.1	0.14	0.1	0.14	0.16	0.17
P. blicklei	0.05	0.08	0	0.06	0.1	0.1	0.1	0.09	0.12	0.14
P. carlsoni	0	**0**	0	0.06	0.1	0.1	0.1	0.1	0.12	0.13
P. carolinensis			0	**0**	0.1	0.11	0.1	0.11	0.13	0.16
P. centralis					0	**0.01**	0.1	0.08	0.12	0.15
P. chelatus							0	**0**	0.12	0.13
P. colei									0	**0.02**
P. confusus										
P. denningi										
P. elarus										
P. floridensis										
P. halidus										
P. gertschi										
P. maculatus										
P. neiswanderi										
P. pentus										
P. picana										
P. pixi										
P. rickeri										
P. variegatus										

Table 2-1. Continued.

Species	*P. confusus*		*P. denningi*		*P. elarus*		*P. floridensis*		*P. gertschi*	
	min	max	min	max	min	max	min	max	min	max
P. aileenae	0.07	0.14	0.2	0.19	0	0.1	0.1	0.19	0.13	0.19
P. alabamensis	0.06	0.08	0.1	0.16	0	0.01	0.1	0.13	0.13	0.14
P. arizonensis	0.09	0.16	0.2	0.19	0.1	0.14	0.1	0.18	0.13	0.17
P. aztecus	0.11	0.18	0.2	0.19	0.1	0.15	0.2	0.17	0.06	0.07
P. blicklei	0.07	0.13	0.2	0.16	0	0.06	0.1	0.15	0.12	0.15
P. carlsoni	0.04	0.1	0.1	0.16	0.1	0.07	0.1	0.12	0.14	0.16
P. centralis	0.06	0.12	0.1	0.17	0.1	0.1	0.1	0.11	0.12	0.17
P. chelatus	0.06	0.12	0.2	0.17	0.1	0.09	0.1	0.12	0.14	0.16
P. colei	0.12	0.17	0.1	0.13	0.1	0.15	0.2	0.18	0.14	0.17
P. confusus	0	**0.01**	0.1	0.18	0.1	0.1	0.1	0.15	0.11	0.19
P. denningi			0	**0.01**	0.1	0.16	0.2	0.18	0.16	0.21
P. elarus					0	**0.01**	0.1	0.14	0.11	0.14
P. floridensis							0	**0.01**	0.17	0.19
P. halidus									0.07	0.11
P. gertschi									0	**0.01**
P. maculatus										
P. neiswanderi										
P. pentus										
P. picana										
P. pixi										
P. rickeri										
P. variegatus										

Table 2-1. Continued.

Species	*P. halidus*		*P. maculatus*		*P. neiswanderi*		*P. pentus*		*P. picana*	
	min	max	min	max	min	max	min	max	min	max
P. aileenae	0.12	0.17	0.1	0.12	0.1	0.14	0	0.14	0.12	0.17
P. alabamensis	0.11	0.13	0.1	0.06	0.1	0.08	0.1	0.09	0.11	0.11
P. arizonensis	0.11	0.15	0.1	0.15	0.1	0.14	10	0.15	0.11	0.13
P. aztecus	0.1	0.11	0.2	0.16	0.1	0.15	0.1	0.15	0.1	0.11
P. blicklei	0.11	0.13	0.1	0.08	0.1	0.09	0.1	0.09	0.12	0.13
P. carlsoni	0.09	0.12	0.1	0.06	0.1	0.1	0.1	0.09	0.1	0.12
P. carolinensis	0.1	0.14	0	0.05	0.1	0.11	0.1	0.1	0.09	0.13
P. centralis	0.12	0.17	0.1	0.11	0.1	0.08	0.1	0.09	0.11	0.12
P. chelatus	0.12	0.14	0.1	0.09	0	0.01	0.1	0.07	0.12	0.12
P. colei	0.13	0.17	0.1	0.15	0.1	0.14	0.1	0.16	0.15	0.16
P. confusus	0.07	0.16	0.1	0.1	0.1	0.12	0	0.11	0.08	0.14
P. denningi	0.13	0.18	0.2	0.16	0.2	0.17	0.1	0.17	0.17	0.17
P. elarus	0.1	0.13	0.1	0.06	0.1	0.09	0.1	0.09	0.1	0.12
P. floridensis	0.15	0.17	0.1	0.14	0.1	0.12	0.1	0.11	0.14	0.15
P. halidus	0	**0**	0.1	0.14	0.1	0.15	0.1	0.13	0.09	0.12
P. gertschi	0.08	0.11	0.1	0.16	0.1	0.17	0.1	0.17	0.11	0.13
P. maculatus			0	**0**	0.1	0.11	0.1	0.11	0.11	0.13
P. neiswanderi					N/A	N/A	0.1	0.08	0.12	0.12
P. pentus							0	**0**	0.08	0.13
P. picana									0	**0**
P. pixi										
P. rickeri										
P. variegatus										

Table 2-1. Continued.

Species	*P. pixi*		*P. rickeri*		*P. variegatus*	
	min	max	min	max	min	max
P. aileenae	0.09	0.16	0.1	0.16	0.1	0.19
P. alabamensis	0.09	0.1	0.1	0.13	0.1	0.17
P. arizonensis	0.09	0.14	0.1	0.17	0.1	0.17
P. aztecus	0.14	0.16	0.1	0.18	0.1	0.18
P. blicklei	0.08	0.1	0.1	0.16	0.1	0.18
P. carlsoni	0.08	0.09	0.1	0.14	0.1	0.2
P. carolinensis	0.08	0.11	0.1	0.14	0.1	0.18
P. centralis	0.07	0.1	0.1	0.15	0.1	0.19
P. chelatus	0.07	0.07	0.1	0.13	0.1	0.19
P. colei	0.13	0.15	0.1	0.07	0.1	0.15
P. confusus	0.05	0.13	0.1	0.15	0.1	0.18
P. denningi	0.14	0.15	0.1	0.12	0.1	0.17
P. elarus	0.08	0.1	0.1	0.14	0.1	0.17
P. floridensis	0.1	0.12	0.1	0.15	0.1	0.18
P. halidus	0.12	0.15	0.1	0.17	0.1	0.19
P. gertschi	0.14	0.17	0.2	0.18	0.1	0.19
P. maculatus	0.1	0.11	0.1	0.14	0.1	0.19
P. neiswanderi	0.07	0.07	0.1	0.14	0.1	0.19
P. pentus	0.05	0.06	0.1	0.15	0.1	0.2
P. picana	0.11	0.13	0.1	0.16	0.1	0.15
P. pixi	0	**0.01**	0.1	0.14	0.1	0.2
P. rickeri			0	**0.05**	0.1	0.16
P. variegatus					0	**0.02**

Table 2-2. Ranges of pairwise divergence (p-distance) among taxa for the western mtCOI dataset for which associations were accomplished. Note that values are rounded to two decimal places. Maximum intraspecific values are displayed in bold.

Species	*P. denningi*		*P. halidus*		*P. gertschi*		*P. variegatus*	
	min	max	min	max	min	max	min	max
P. denningi	0	**0.01**	0.13	0.16	0.16	0.19	0.12	0.15
P. halidus			0	**0**	0.07	0.11	0.1	0.17
P. gertschi					0	**0.01**	0.1	0.15
P. variegatus							0	**0.01**

Table 2-3. Ranges of pairwise divergence (p-distance) among taxa for the eastern mtCOI dataset for which associations were accomplished. Note that values are rounded to two decimal places. Maximum intraspecific values are displayed in bold.

Species	*P. alabamensis*		*P. blicklei*		*P. carlsoni*	
	min	max	min	max	min	max
P. alabamensis	0	**0**	0.04	0.05	0.07	0.07
P. blicklei			0	**0.03**	0.05	0.08
P. carlsoni					0	**0**
P. carolinensis						
P. centralis						
P. chelatus						
P. colei						
P. confusus						
P. elarus						
P. maculatus						
P. pentus						
P. rickeri						

Table 2-3. Continued.

Species	*P. carolinensis*		*P. centralis*		*P. chelatus*		*P. colei*	
	min	max	min	max	min	max	min	max
P. alabamensis	0.03	0.04	0.09	0.09	0.09	0.1	0.13	0.14
P. blicklei	0.03	0.06	0.07	0.09	0.07	0.09	0.12	0.14
P. carlsoni	0.05	0.06	0.09	0.1	0.09	0.1	0.12	0.13
P. carolinensis	0	**0**	0.08	0.11	0.07	0.11	0.13	0.14
P. centralis			0	**0.01**	0.07	0.08	0.12	0.13
P. chelatus					0	**0**	0.12	0.13
P. colei							0	**0.02**
P. confusus								
P. elarus								
P. maculatus								
P. pentus								
P. rickeri								

Table 2-3. Continued.

Species	*P. confusus*		*P. elarus*		*P. maculatus*	
	min	max	min	max	min	max
P. alabamensis	0.08	0.08	0	0.01	0.05	0.06
P. blicklei	0.08	0.1	0.04	0.05	0.05	0.06
P. carlsoni	0.08	0.1	0.05	0.07	0.05	0.06
P. carolinensis	0.07	0.1	0	0.04	0.5	0.05
P. centralis	0.07	0.09	0.06	0.09	0.09	0.09
P. chelatus	0.07	0.08	0.07	0.09	0.09	0.09
P. colei	0.13	0.14	0.11	0.14	0.13	0.13
P. confusus	0	**0.01**	0.06	0.08	0.08	0.1
P. elarus			0	**0.01**	0.05	0.05
P. maculatus					0	**0**
P. pentus						
P. rickeri						

Table 2-3. Continued.

Species	*P. pentus*		*P. rickeri*	
	min	max	min	max
P. alabamensis	0.08	0.08	0.12	0.13
P. blicklei	0.07	0.09	0.12	0.16
P. carlsoni	0.07	0.09	0.12	0.13
P. carolinensis	0.06	0.09	0.11	0.13
P. centralis	0.07	0.08	0.12	0.14
P. chelatus	0.06	0.07	0.11	0.13
P. colei	0.13	0.15	0.06	0.07
P. confusus	0.04	0.06	0.1	0.14
P. elarus	0.06	0.08	0.1	0.14
P. maculatus	0.09	0.1	0.12	0.12
P. pentus	0	**0**	0.12	0.14
P. rickeri			0.04	**0.05**

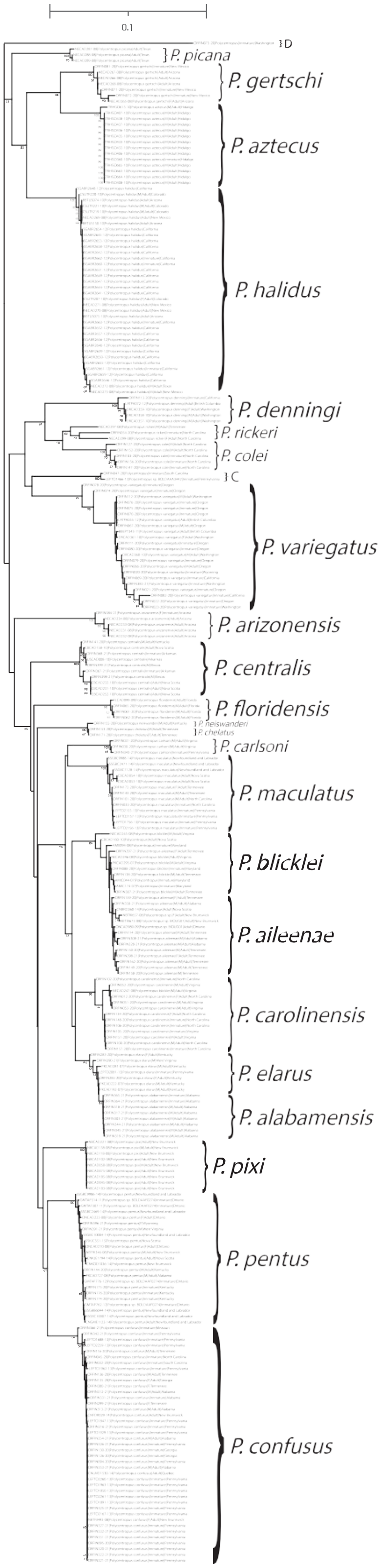

Figure 2-1. Initial neighbor-joining tree for pooled mtCOI barcoding sequence data.

Only bootstrap values ≥50% are shown. Specimen labels at branch tips include taxon, BOLD Sample ID, sex (if adult and available), and life stage. Scale bar indicates genetic distance.

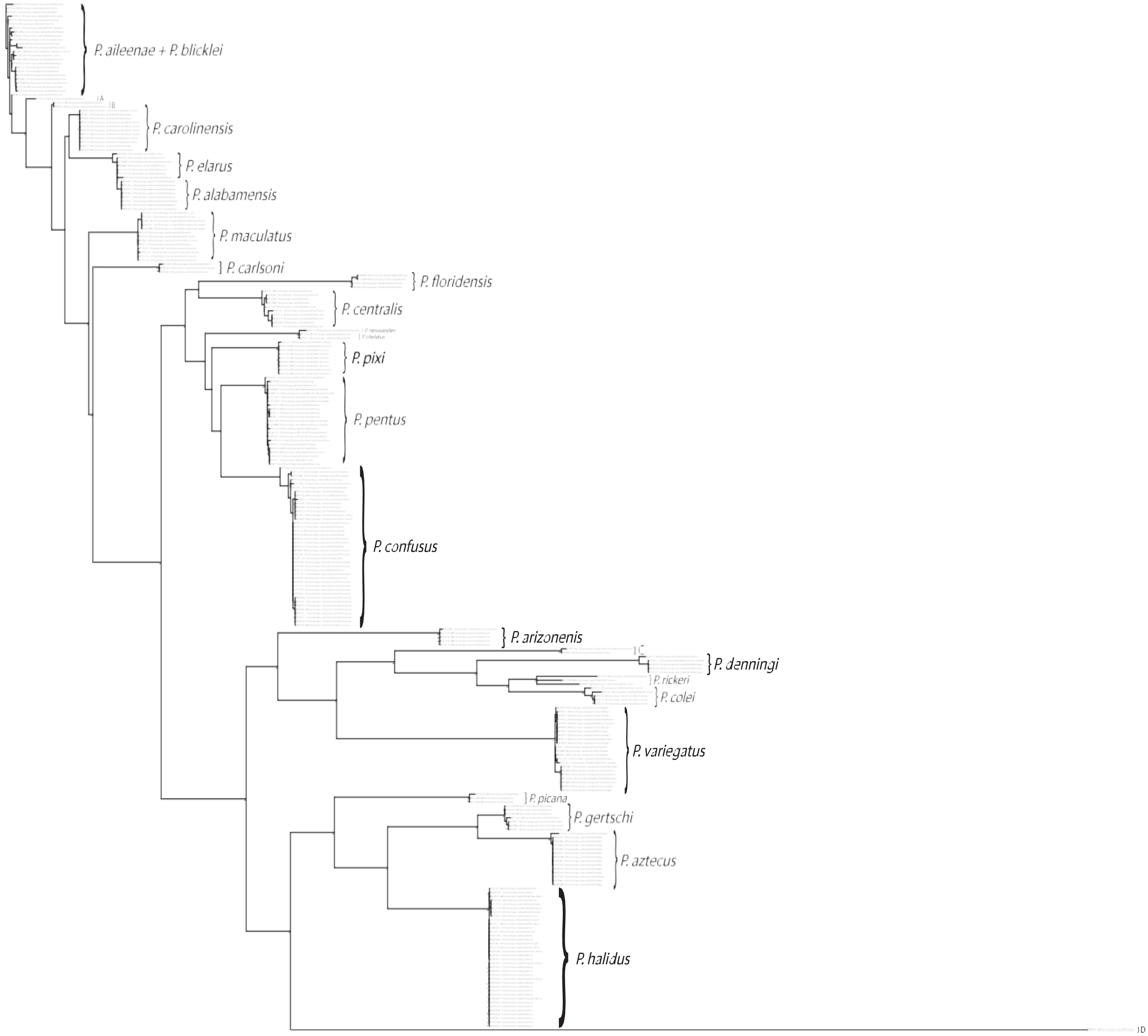

Figure 2-2. Initial maximum likelihood tree for pooled mtCOI barcoding sequence data.

Only bootstrap values ≥50% are shown. Specimen labels at branch tips include taxon, BOLD Sample ID, sex (if adult and available), and life stage. Scale bar indicates substitutions per site.

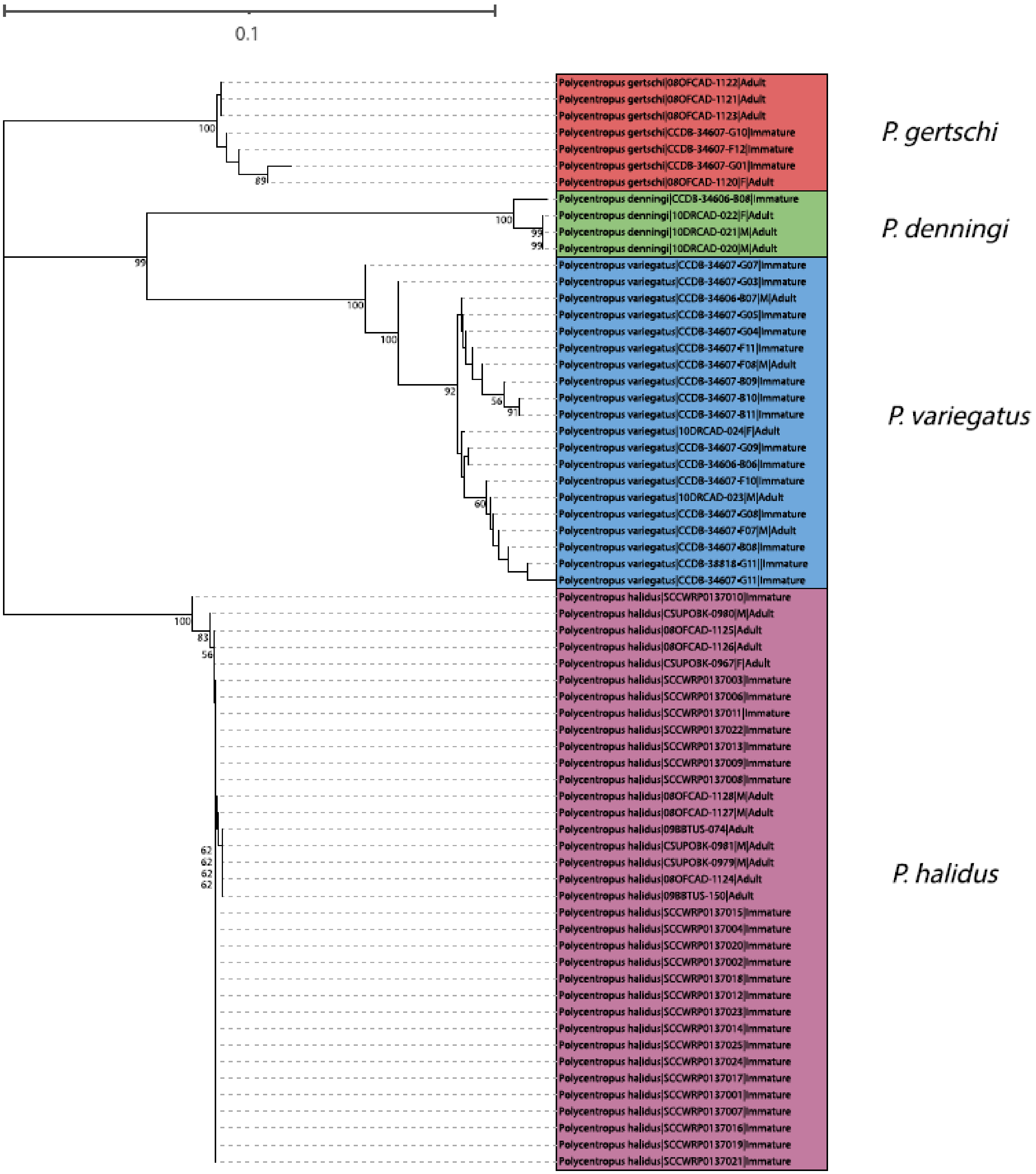

Figure 2-3. Neighbor-joining tree for mtCOI barcoding sequence data of western taxa yielding successful associations.

Only bootstrap values ≥50% are shown. Specimen labels at branch tips include taxon, BOLD Sample ID, sex (if adult and available), and life stage. Scale bar indicates genetic distance.

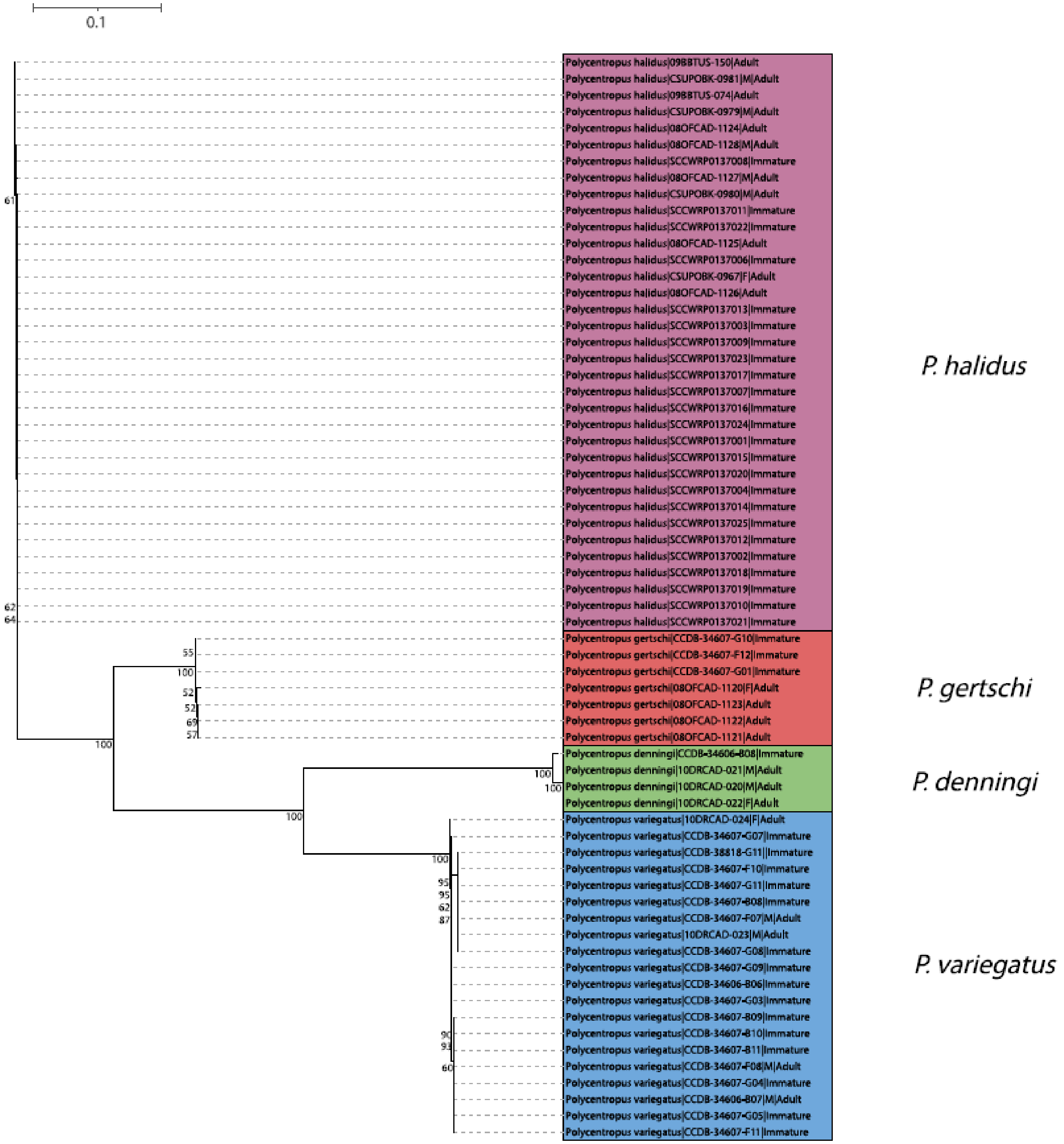

Figure 2-4. Maximum likelihood tree for mtCOI barcoding sequence data of western taxa yielding successful associations.

Only bootstrap values ≥50% are shown. Specimen labels at branch tips include taxon, BOLD Sample ID, sex (if adult and available), and life stage. Scale bar indicates substitutions per site.

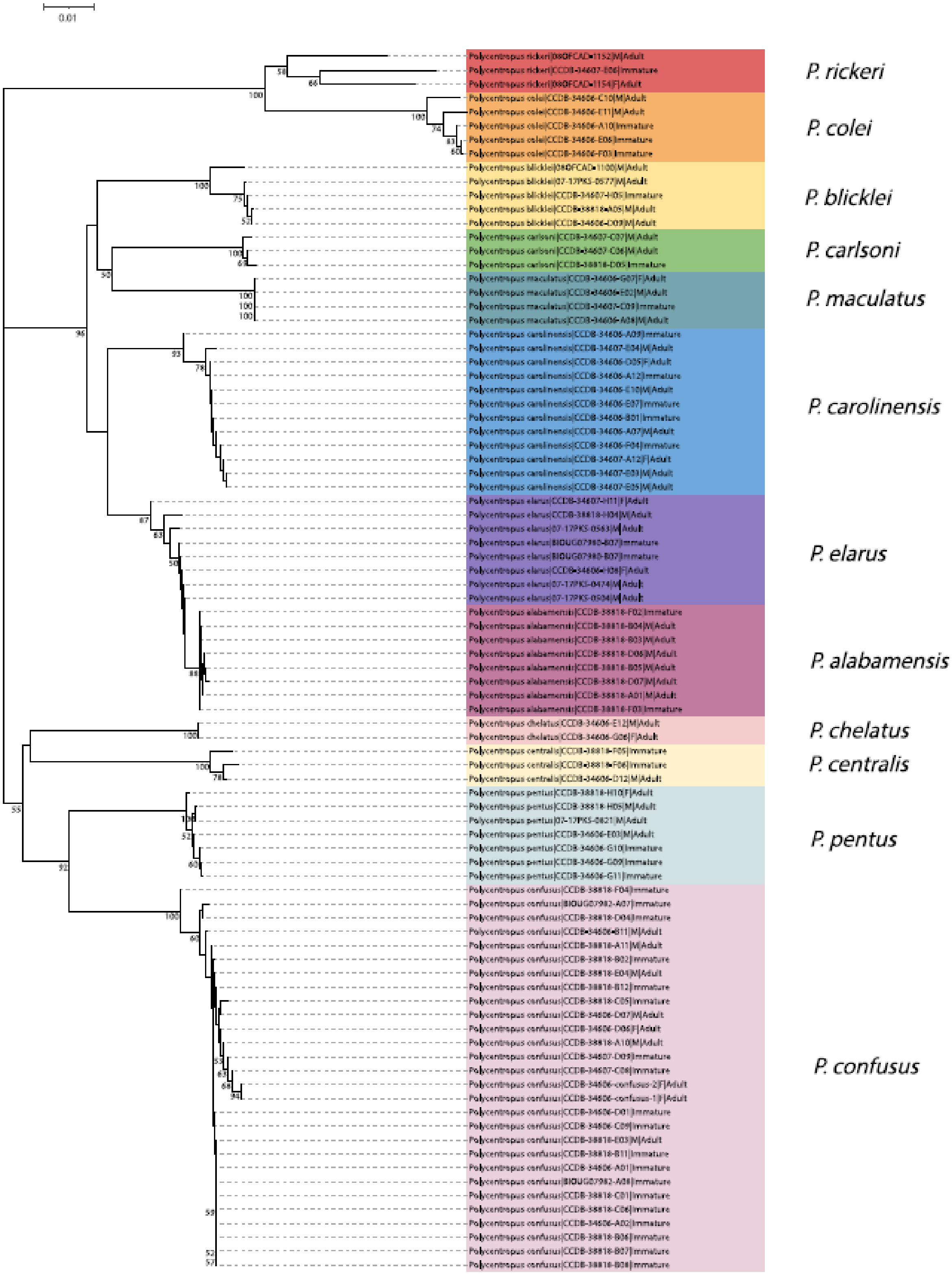

Figure 2-5. Neighbor-joining tree for mtCOI barcoding sequence data of eastern taxa yielding successful associations.

Only bootstrap values ≥ 50% are shown. Specimen labels at branch tips include taxon, BOLD Sample ID, sex (if adult and available), and life stage. Scale bar indicates genetic distance.

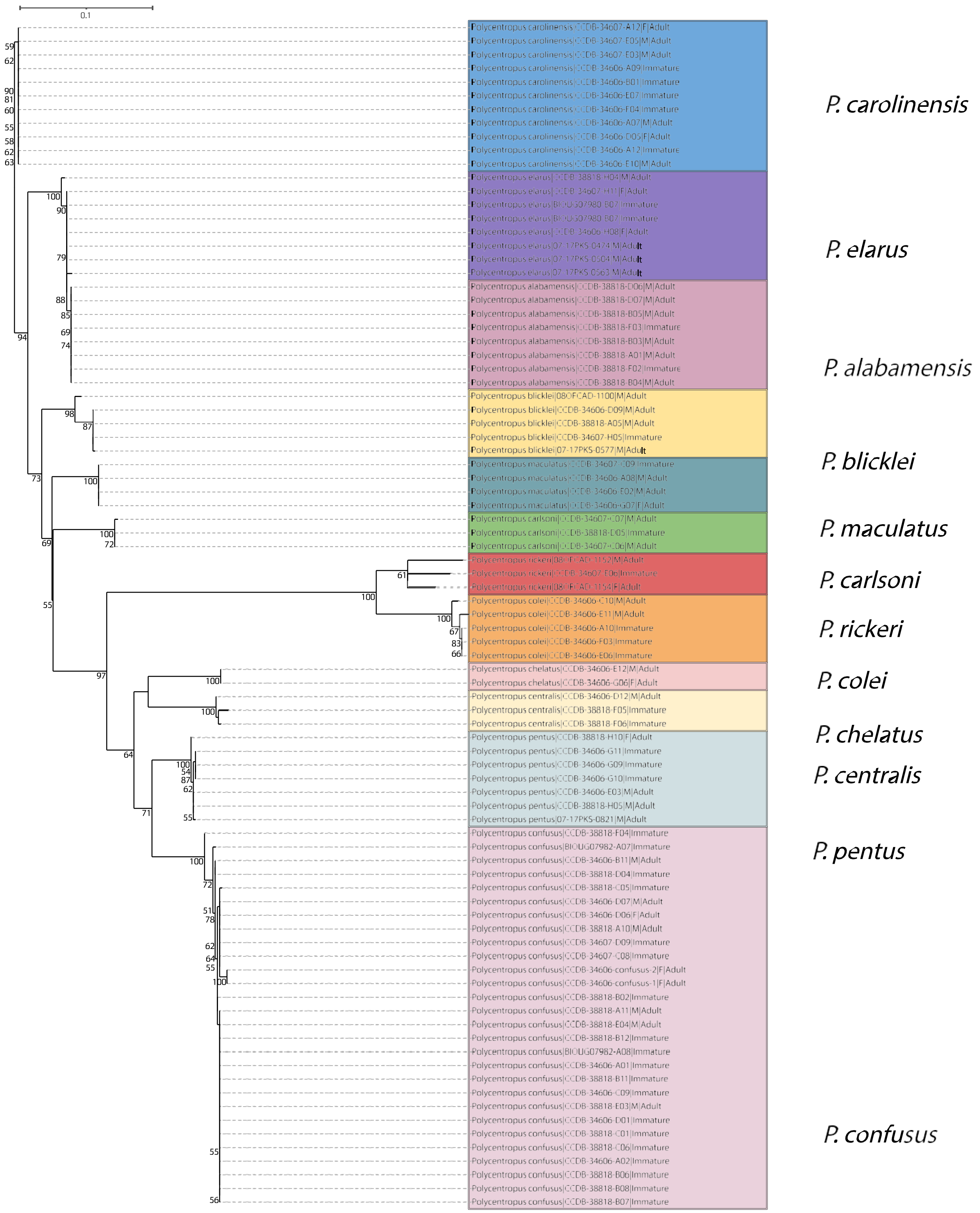

Figure 2-6. Maximum likelihood tree for mtCOI barcoding sequence data of eastern taxa yielding successful associations.

Only bootstrap values ≥50% are shown. Specimen labels at branch tips include taxon, BOLD Sample ID, sex (if adult and available), and life stage. Scale bar indicates substitutions per site.

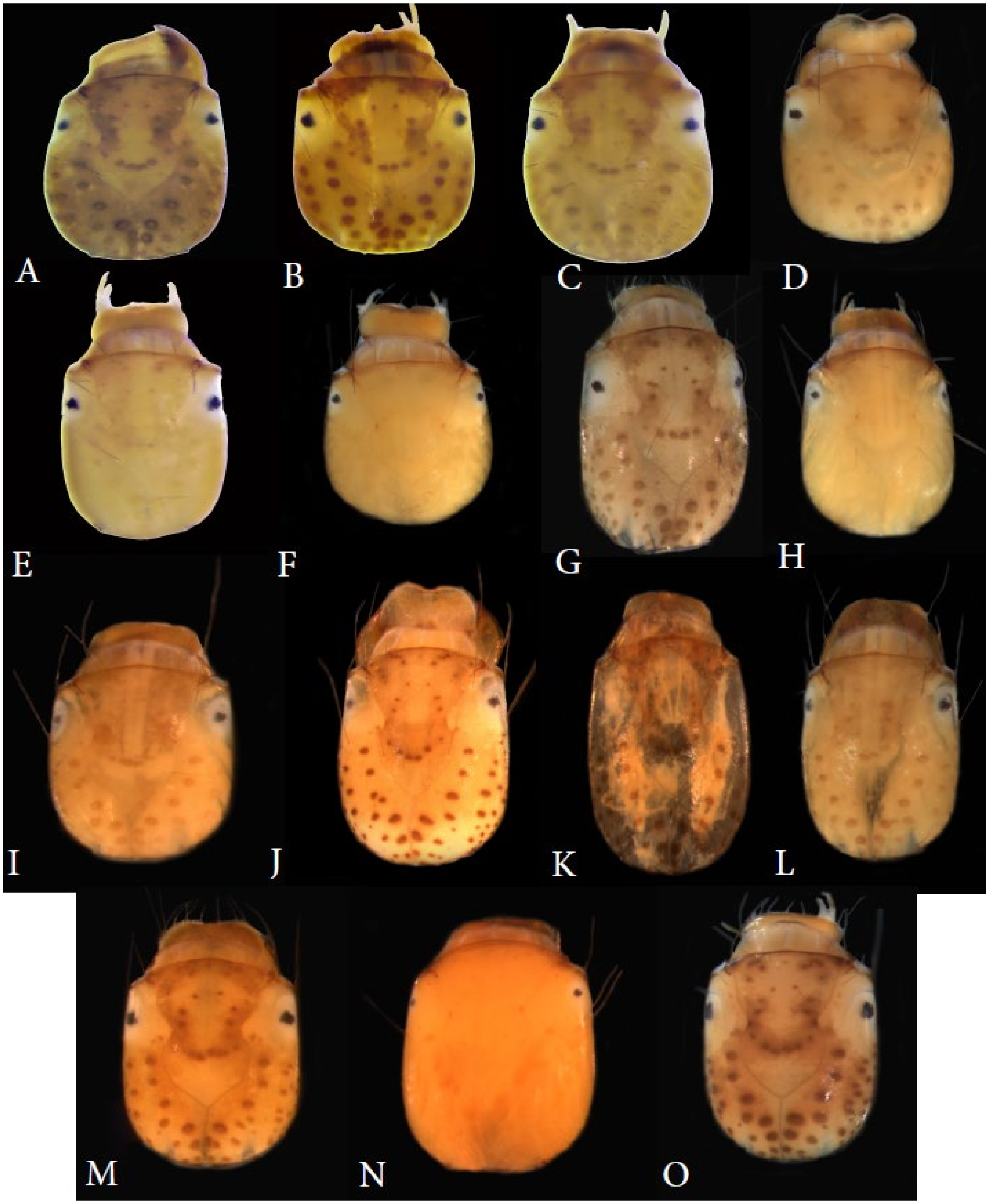

Figure 2-7. Head capsules of associated Nearctic species of *Polycentropus* Curtis, 1835 larva, dorsal views.

A. *P. alabamensis* Hamilton, Harris, and Lago, 1990; B. *P. blicklei* Ross and Yamamoto, 1965; C. *P. carlsoni* Morse, 1971; D. *P. carolinensis* Banks, 1905; E. *P. centralis* Banks, 1914; F. *P. colei* Ross, 1941; G. *P. confusus* Hagen, 1861; H. *P. denningi* Smith, 1962, I. *P. elarus* Ross, 1944; J. *P. gertschi* Denning, 1950; K. *P. halidus* Milne, 1936; L. *P. maculatus* Banks, 1908; M. *P. pentus* Ross, 1941; N. *P. rickeri* Yamamoto, 1966; O. *P. variegatus* Banks, 1900. Note that the head capsule of the *P. halidus* specimen underwent lysis during DNA extraction, removing soft tissue but maintaining the sclerotized head capsule before it was photographed.

CHAPTER 3

LARVAL TAXONOMY OF WESTERN NEARCTIC *Polycentropus* CURTIS, 1835 (TRICHOPTERA: POLYCENTROPODIDAE), WITH COMMENTS ON THE IDENTIFICATION OF IMMATURE NEARCTIC *Polycentropus SENSU LATO*

Trichoptera, or caddisflies, are a cosmopolitan order of holometabolous insects with immature stages found in a variety of aquatic habitats and alate adults in riparian habitats. With more than 17,000 described species, the order is more diverse than all other primarily aquatic orders of insects combined (Morse et al., 2019a; J. Morse, personal communication). Alpha taxonomy is well-developed and primarily based on adult males, while larvae of less than half of Nearctic species are identifiable (Ruiter et al., 2013). This inability to identify immatures hinders studies of life histories, ecology, evolution, and pollution tolerance.

Within the suborder Annulipalpia, superfamily Psychomyioidea, the family Polycentropodidae comprises fixed-retreat-making caddisflies that construct silken tubes or capture nets. One of the most diverse families, Polycentropodidae is represented globally by more than 800 extant nominal species in 14 genera (Chamorro and Holzenthal 2011; Johanson et al., 2012; Morse, 2022). Of these, 78 species in eight genera are recorded from the Nearctic (Rasmussen and Morse, 2022). While historically well-studied in Europe (e.g., Lepneva, 1964; Urbanič, 2006; Waringer and Graf, 2011; Karaouzas and Waringer, 2017), immature stages of the North American polycentropodid fauna are largely unknown beyond genus level. Even identification to genus of Nearctic *Polycentropus sensu lato* (i.e., *Holocentropus* McLachlan, 1878, *Plectrocnemia* Stephens, 1836, and *Polycentropus* Curtis, 1835) is based on only a few

Reprinted with permission from Orfinger, A. B., Hix, R. L., Gerth, W. J., & Rasmussen, A. K. (2022b). Larval taxonomy of western Nearctic *Polycentropus* Curtis, 1835 *sensu stricto* (Trichoptera: Polycentropodidae), with comments on the identification of immature Nearctic *Polycentropus sensu lato*. The Pan-Pacific Entomologist, 98(4), 245–261.

larval representatives from each genus and therefore should be reevaluated when describing additional larvae. To date, existing Nearctic keys to polycentropodid larvae (e.g., Wiggins, 1996; Morse et al., 2017, 2019b) have relied on the same characters used to separate the European *Polycentropus sensu lato* genera without robust examination of the efficacy of the keys in North America.

Nearctic larvae of the genus *Polycentropus* (e.g., Fig. 2-1) are particularly poorly known, with the larva of only one of the 29 nominal *Polycentropus* species described. Ross (1944) provided a brief and somewhat vague description along with an illustration of the head for the larva of *Polycentropus centralis* Banks, 1914. In the Nearctic, the genus *Polycentropus* exhibits a generally East-West distributional divide (Hamilton, 1986). Here, I consider the East as consisting of Manitoba and the U.S.A. states adjacent to either side of the Mississippi River and eastward, and the West consisting of the complementary Nearctic region (following e.g., Lago and Harris, 1987a; Cooper and Morse, 1998; Chapter 2). This scheme roughly translates to eastern species being found east of 100 degrees West longitude and western species being found west of this meridian. The eastern diversity of the genus is much higher, with 22 named species, versus seven for the western fauna. The present paper is part of a series in an ongoing effort to resolve the taxonomy of larvae of the Nearctic *Polycentropus* species.

Currently, no larvae of any of the seven western *Polycentropus* species are described. Using larvae associated using mtDNA from Chapter 2, the aims of this chapter are to describe and illustrate the late-instar larvae of four western Nearctic *Polycentropus* species recently associated with identifiable adults and provide a discriminatory matrix for their identification. Finally, I conclude with a brief discussion on the efficacy of current keys to larval Nearctic polycentropodids considering new findings.

Methods and Materials

Life-stage association was accomplished using mtDNA barcoding as described in Chapter 2. Specimens were observed under a Unitron Z10 stereomicroscope with up to 120x magnification. Measurements were obtained using a calibrated ocular micrometer. Length of each specimen refers to total length, i.e., anterior margin of head to posterior margin of anal claw. Because these larvae are frequently preserved in a curled position, careful straightening of specimens using two pairs of forceps was often necessary when performing length measurements. For morphometric measurements, head width refers to the width of the head measured dorsally at the widest point, while head length describes the length of the head measured dorsally at the longest point excluding the labrum. Terminology follows Waringer and Graf (2011) and Karaouzas and Waringer (2017).

Among the numerous morphological characters examined were several that have been useful in separating larvae of polycentropodid genera and species (e.g., see Waringer and Graf, 2011). The relative length and broadness of the foretarsi and foretibiae were among these characters. Character states include, on each side, a short, broad foretarsus that is no more than 1/2 the length of the broad foretibia (Fig. 3-2A), and a long, more-tubular foretarsus that is at least 2/3 the length of the narrow foretibia (Fig. 3-2B). Anal claw curvature was recorded as either obtuse (Fig. 3-2C) or right-angled (Fig. 3-2D). Anal proleg segments were examined to compare relative proportions of the basal and distal segments, being classified as either the distal segment subequal in length to the basal segment (Fig. 3-2E) or the basal segment being longer than the distal segment (Fig. 3-2F). The arrangement of posterior muscle scars (when present) of the frontoclypeal apotome form either a shallow arc (Fig. 3-3A) or a trapezoidal pattern (Fig. 3-3B). Finally, the distance of muscle scar ‘m’ (*sensu* Waringer and Graf, 2011) relative to the frontoclypeal suture was classified as distant (Fig. 3-3C; “set back” according to Waringer and Graf 2011) or close (Fig. 3-3D).

Unlike the above-mentioned characters, setal arrangements were not illustrated because setal characters were not found to be informative in diagnosing species. In addition, it is common for specimens to have damaged or missing setae. For example, all available specimens of *Polycentropus halidus* Milne, 1936 had previously undergone full-body lysis for DNA extraction, rendering many setae broken or removed altogether. Characters described above, such as anal claw curvature and muscle scar patterning, are consistency available regardless of deterioration due, for example, to poor preservation or lysis during DNA extraction, and were highlighted in diagnosing species. In addition, because neither metamorphotype specimens nor continuous size series were available for described larvae, instar determination could not be definitively made. Given that the specimens described are large sizes (i.e., 12.5–20.0 mm), it is likely that most or all represent final instar larvae. However, because this cannot be stated with certainty, the term “late-instar” is used instead.

For stacked photography of heads, heads were excised and placed in glycerin or hand sanitizer in a depression slide. Between six and 16 photographs of each larval head were taken at different depths of field using a Levenhuk M1400 Plus Digital Camera mounted to a Unitron Z10 dissecting microscope. Photographs were digitally stitched together using Helicon Focus version 7.7.4. Line drawings were produced using a 10 × 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.

Specimens are deposited at the Florida A&M University portion of the Florida State Collection of Arthropods (FAMU) in Tallahassee, Florida, U.S.A., the Centre for Biodiversity Genomics (BIOUG) in Guelph, Ontario, Canada, and the Monte L. Bean Life Science Museum at Brigham Young University, Provo, Utah, U.S.A. (BYU). Only larval specimens examined are listed herein, except for the case of a new state record based on adults. The presence of an asterisk (*) following a state name signifies a new state record.

Point maps reflect adult and larval specimens of all seven western Nearctic *Polycentropus* species examined by the author during this study**.**

Western Larvae Taxonomy Results

Polycentropus denningi Smith, 1962

(Figs. 3-4A, 3-4B, 3-5; Table 3-1)

Description of Late-instar Larva. Larval length 19 mm (n = 1).

Head. Length 1.8 mm, width 1.7 mm (n = 1). Dorsal area of head uniformly yellow with pair of symmetrically positioned, small, well-defined dark muscle scars on incurvate center of frontoclypeal sutures. Dorsal head surface without dark bands and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome lacking muscle scars. Pair of muscle scars ‘m’ faint and nearly invisible, small, and positioned close to frontoclypeal suture. Ventral area of head also uniformly yellow, lacking muscle scars. Ventral apotome long and narrow.

Prothorax. Pronotum same color as head, with no pigmentation or scarring. Prothoracic tarsi tubular, each about two-thirds tibial length and slightly narrower than its tibia (Fig. 3-2B). Faces of femora covered with numerous long setae. Distal section of each tarsus ventrally with well-developed pectinate setae.

Abdomen. Basal segment of each anal proleg subequal in length of distal sclerotized section (Fig. 3-2E), and with numerous long setae. Anal claws obtuse-angled and lacking spines on the concave margins (Fig. 3-2C).

Distribution. Canada: British Columbia; U.S.A.: California*, Idaho, Montana, Oregon, Washington.

Specimens Examined. U.S.A.: California: Humboldt County, Upper Tectah Creek, J. Lee coll., 26/xi/2019, 1 larva. (FAMU). Sierra County, Highway 49, 2 miles east of Yuba Pass, Tahoe National Park, [N39°37'40.8", W120°28'22.8"], D. Ruiter coll., 29/v/1991, 1 male. **New State Record**.

Polycentropus gertschi Denning, 1950

(Figs. 3-4C, 3-4D, 3-6; Table 3-1)

Description of Late-instar Larva. Mean larval length 15.7 mm (14–18, n = 10).

Head. Mean length 1.8 mm (1.5–2.0), width 1.4 mm (1.1–1.6, n = 10). Dorsal area of head yellow-tan with numerous consistently positioned, variously sized, well-defined dark muscle scars; muscle scarring on head occasionally slightly asymmetrical. Dorsal head surface with subtle darker brown bands and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome with muscle scar arrangement trapezoidal (Fig. 3-3B). Pair of muscle scars ‘m’ small, faint, and positioned close to frontoclypeal suture (Fig. 3-3D). Ventral area of head also yellow-tan, with anterior half lacking muscle scars and posterior half with multiple dark muscle scars. Ventral apotome long and narrow.

Pronotum. Same color as head, lacking pigment banding and with multiple small, dark muscle scars. Prothoracic tarsi tubular, each about two-thirds tibial length and slightly narrower than tibiae (Fig. 3-2B). Faces of femora covered with numerous long setae. Distal section of each tarsus ventrally with well-developed pectinate setae.

Abdomen. Basal segment of each anal proleg longer than distal sclerotized section (Fig. 3-2F), and with numerous long setae. Anal claws obtuse-angled and lacking spines on the concave margins (Fig. 3-2C).

Distribution. U.S.A.: Arizona, Colorado, New Mexico*, Texas; Mexico: Chihuahua.

Specimens Examined. U.S.A.: New Mexico: [Taos County], Taos Pueblo, Rio Pueblo, Aquatic Biology Associates, Inc. coll., 12/ ix/2012, 28 larvae. (FAMU). Same data but Taos Pueblo Environmental Office, Rio Lucero Site 16, 4/x/2010, 30 larvae. (FAMU). **New State Record**.

Polycentropus halidus Milne, 1936

(Figs. 3-4E, 3-4F, 3-7; Table 3-1)

Description of Late-instar Larva. Mean larval length 12.9 mm (12.5–14, n = 10).

Head. Mean length 1.6 mm (1.5–1.8), width 1.4 mm (1.2–1.6, n = 10). Dorsal area of head tan with numerous symmetrically positioned, variously sized, well-defined dark muscle scars. Dorsal head surface with distinct darker brown bands and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome with muscle scar arrangement forming shallow arc (Fig. 3-3A).

Pair of muscle scars ‘m’ large, distinct, and distant from frontoclypeal suture (Fig. 3-3C). Ventral area of head also tan, with anterior half lacking muscle scars and posterior half with multiple dark muscle scars. Ventral apotome long and narrow.

Pronotum. Same color as head, lacking pigment banding and with multiple dark muscle scars. Prothoracic tarsi tubular, each about two-thirds tibial length and slightly narrower than its tibia. Faces of femora covered with numerous long setae. Distal section of each tarsus ventrally with well-developed pectinate setae.

Abdomen. Basal segment of each anal proleg longer than distal sclerotized section (Fig. 3-2F), and with numerous long setae. Anal claws right-angled and lacking spines on the concave margins (Fig. 3-2D).

Distribution. Canada: British Columbia; U.S.A.: Alaska, Arizona, California, Colorado, Montana, New Mexico, Oregon*, Texas, Utah, Washington, Wyoming; Mexico: Chihuahua, Durango, Sinaloa.

Notes. Figures of *P. halidus* and *P. variegatus* were inadvertently interchanged by Denning (1956; p. 249, Figs. 10:17a, b), leading to subsequent misidentification of these two species in many instances.

Specimens Examined. U.S.A.: California: Los Angeles County, 334 m a.s.l., [N34°10'15.6", W117°53'16.8"], B. Isham coll., 18/xi/2018, 24 larvae. (SGABR2639-12 - SGABR2663-12 (BIOUG)). Oregon: Josephine County, Whiskey Creek, at confluence with West Fork Illinois, at Forest Service Road 4402, At Light, [N42°06'08.2", W123°46'23.7"], C.D. Kerst coll., 15/viii/2015, 5 females (BYU). [Josephine County], Whiskey Creek at Lone Mountain Road, 1738 m a.s.l., [N42°01'19.9", W123°46'27.8"], 5/viii/2020, 2 males, 22 females (BYU) **New State Record**.

Polycentropus variegatus Banks, 1900

(Figs. 3-1, 3-4G, 3-4H, 3-8; Table 3-1)

Description of Late-instar Larva. Mean larval length 17.5 mm (14.9–20.0 mm (n = 10).

Head. Mean length 1.8 mm (1.5–1.9, n = 10), width 1.4 (1.1–1.6, n = 10). Dorsal area of head tan with numerous symmetrically positioned, variously sized, well-defined dark muscle scars. Dorsal head surface with distinct darker brown bands and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome with muscle scar arrangement forming shallow arc (Fig. 3-3A). Pair of muscle scars ‘m’ large, distinct, and distant from frontoclypeal suture (Fig. 3-3C). Ventral area of head also tan, with anterior half lacking muscle scars and posterior half with multiple dark muscle scars. Ventral apotome long and narrow.

Pronotum. Same color as head, lacking pigment banding and with multiple dark muscle scars. Prothoracic tarsi tubular, each about two-thirds tibial length and slightly narrower than tibiae (Fig. 3-2B). Faces of femora covered with numerous long setae. Distal section of each tarsus ventrally with well-developed pectinate setae.

Abdomen. Basal segment of each anal proleg longer than distal sclerotized section, and with numerous long setae. Anal claws right-angled and lacking spines on the concave margin as (Fig. 3-2D).

Distribution. Canada: Alberta, British Columbia; U.S.A.: Alaska, Arizona, California, Colorado, Montana, Oregon, Utah, Washington, Wyoming.

Notes. This species was erroneously reported from Mexico by Wevers and Wisseman (1987). In addition, figures of *P. variegatus* and *P. halidus* were inadvertently interchanged by Denning (1956; p. 249, Figs. 10:17a, b), leading to subsequent misidentification of these two species and likely the inaccurate report of P. variegatus from Mexico.

Specimens Examined. U.S.A.: California: Placer County, Echo Creek, Tahoe Regional Planning Agency, 21/vii/2010, 7 larvae. (FAMU). [Placer County], Tahoe Regional Planning Agency, McKinney Creek, 18/vii/2013, 3 larvae. (FAMU). Oregon: Douglas County, Umpqua National Forest biomonitoring sites, Slide Creek, Aquatic Biology Associates, Inc. coll., 6/x/2012, 8 larvae. (FAMU). 4/x/2012, 4 larvae. (FAMU). 6/x/2012, 9 larvae. (FAMU). [Douglas County], Umpqua National Forest biomonitoring sites, Upper Cavitt Creek (margin), 8/x/2015, 5 larvae. (FAMU). Hood River County, Cabin Creek below falls and above bike path, 60 mm, [N45°41’13.9”, W121°41’49.2”], R. Wisseman coll., 9/v/2017, 1 larva. (FAMU). Lane County, McKenzie River side-channel restoration, Aquatic Biology Associates, Inc. coll., 7/x/2019, 57 larvae. (FAMU). [Lane County], South Fork Mckenzie River, [N44°09’27.7”, W122°16’58.8”], W.J. Gerth coll., 22/vii/2020, 1 larva. (FAMU). Multnomah County, Eagle Creek, Columbia River Gorge National Scenic Area, 76 m a.s.l., [N45°38’10.7”, W121°55’12.0”], R. Wisseman coll., 16/v/2015, 2 larvae. (FAMU). [Hood River County], Ruckel Creek, Columbia River Gorge National Scenic Area, 60 m a.s.l., [N45°38’42.0”, W121°55’04.8”], 15/v/2015, 1 larva. (FAMU). Washington: [Okanogan County], Mt. Rainier National Park, Blue Lake, 26/viii/2014, 17 larvae. (FAMU). Wyoming: [Park County], Yellowstone National Park, Gibbon River above Virginia Cascade, [N44°42’48.8”, W110°38’59.3”], R. Cantrell coll., 11/viii/2019, 4 larvae. (FAMU).

Unassociated *Polycentropus* larvae

Western Nearctic *Polycentropus* larvae that remain unknown include those of *P. arizonensis* Banks, 1905 (recorded in the Nearctic from Arizona and New Mexico and the Mexican states of Chihuahua, Durango, and Michoacan), *P. aztecus* Flint, 1967 (recorded in the Nearctic from Arizona and from the Mexican states of Chiapas, Chihuahua, Durango, Hidalgo, Mexico, Michoacan, Morelos, and Oaxaca), and *P. picana* Ross, 1947 (recorded in the Nearctic from Texas and from the Mexican states of Durango, Jalisco, Neuvo Leon, San Luis Potosi, Tamaulipas, and Veracruz). Figures 9-11 present distribution data for specimens of these species examined during this study.

In addition to the remaining unassociated Nearctic species, numerous *Polycentropus* species are reported from central and northern Mexico (Holzenthal and Calor, 2017) and may eventually be recorded from the southern United States. Once larvae of these additional taxa are associated and described, the descriptions and diagnostic matrix generated here can serve as a framework to build upon for the identification of all western Nearctic *Polycentropus* species.

Western Larval Taxonomy Discussion

This chapter provides descriptions and a diagnostic matrix (Table 3-1) for larvae of four of seven nominal western Nearctic *Polycentropus* species. Characters shared by the four species include the absence of abdominal gills, prothoracic tarsi being tubular and about two-thirds tibial length and slightly narrower than tibiae, and a lack of spines on the concave margin of the anal claw. Characters useful for separation of species are head color, head pigmentation (banding and muscle scar patterns), head width to head length ratio, head width to body length ratio, ratios of basal and distal segments of anal prolegs, and anal claw curvature. Each of the character states are easily viewable and consistent within each species based on the material available. Since the description of *P. denningi* is based on a single available specimen, future examination of additional specimens, once collected and identified, will provide additional information on intraspecific variation of this species.

Using these available descriptions and the provided diagnostic matrix, species-level identification of these four western Nearctic *Polycentropus* species is now possible, with caution concerning the yet-unknown larvae. Workers in caddisfly taxonomy and systematics, aquatic ecology, aquatic entomology, and water resource monitoring can now begin to study the immature stages of these organisms at the species-level. In addition, new state records and corrections to previously published erroneous records provided here help to resolve the distributions of these four species. Future work should focus on collecting additional specimens of various size classes for use in morphometric studies to delineate larval instars and association of the remaining, yet unknown western Nearctic *Polycentropus* larvae.

Issues related to separating Nearctic polycentropodid genera

During the course of this study, it became apparent that the existing keys (e.g., Wiggins, 1996; Morse et al., 2017, 2019b) to the Nearctic genera comprising *Polycentropus sensu lato* (i.e., *Holocentropus*, *Plectrocnemia*, and *Polycentropus*) are inadequate in separating these genera. This possibility was also suggested by Wiggins (1996), who urged caution in separating these genera given that the larvae of so few Nearctic species are known.

Currently, the character for separating *Polycentropus* from *Cernotina* Ross, 1938, *Holocentropus*, and *Plectrocnemia* is: *Polycentropus* larvae have prothoracic tarsi that are broad and only one-half as long as the prothoracic tibiae (Fig. 3-2A), versus prothoracic tarsi narrow and at least two-thirds as long as the prothoracic tibiae (Fig. 3-2B) in the remaining genera. Based on the newly described material, all of the now-known Nearctic *Polycentropus* larvae exhibit narrower and more tubular prothoracic tarsi that are at least two-thirds as long as the prothoracic tibiae (Fig. 3-2B). Ross (1944) neither mentioned nor illustrated the forelegs of the single *P. centralis* larva he described, but examination of the specimen by the author uncovered the same character state seen in the known western larvae. So, while foreleg segment ratios work in separating the European representatives of *Polycentropus* from other *Polycentropus sensu lato* genera (e.g., Waringer and Graf, 2011), these characters are not useful in distinguishing *Polycentropus* from allied genera for the Nearctic fauna.

Similarly, the basal and distal segments of the anal prolegs of the single larval specimen of *P. denningi* being roughly equal in length (Fig. 3-2E) violates the existing keys (Wiggins, 1996; Morse et al., 2017, 2019b). In these keys, the genus *Neureclipsis* McLachlan, 1864 is separated from *Cyrnellus* Banks, 1913, *Cernotina* Ross, 1938, and *Polycentropus sensu lato* by possessing basal and distal segments of the anal prolegs of approximately equal length (Fig. 3-2E), versus basal segments distinctly longer than the distal segments in the remaining genera (Fig. 3-2F). While three of the four larvae described here conform to the character state previously accepted for these anal proleg segment ratios in *Polycentropus* (Fig. 3-2F), *P. denningi* possesses segments of roughly equal length, resembling known *Neureclipsis* larvae (Fig. 3-2E). Larvae of *Neureclipsis* are still readily separated from *Cernotina*, *Cyrnellus*, and *Polycentropus sensu lato* by the presence of tiny spines on the concave margin of the anal claw observable in *Neureclipsis* versus the absence of these spines in the remaining genera.

Likewise, despite the shared character state of the relative length of the anal proleg segments, *Neureclipsis* larvae have only two to three apicoventral setae versus numerous setae on the dorsal and ventral surfaces of the basal segments, enabling separation. Still, the above-mentioned violation highlights the unreliability of the existing keys for the larvae of *Polycentropus sensu lato* considering additional descriptions.

Characters useful for separating the Nearctic *Polycentropus sensu lato* genera could not be identified despite extensive examination of available material by the author. While efforts will continue to attempt to identify and describe diagnostic characters, an alternative and potentially necessary solution is the generation of a species-level key or matrix inclusive of all known Nearctic *Polycentropus sensu lato* larvae. Currently, this includes 29 *Polycentropus* species with 16 described larvae (Ross, 1944; current study; Chapter 4), 14 *Plectrocnemia* species with three described larvae (Ross, 1944; Moulton and Stewart, 1996), and nine *Holocentropus* species with two described larvae (Ross, 1944; Lepneva, 1964; Hickin, 1967). The new data provided here constitute a necessary step in that direction on which Chapter 4 builds.

Table 3-1. Discriminatory matrix to the known late-instar larvae of western Nearctic *Polycentropus* species.

Character/Species	*Polycentropus denningi*	*Polycentropus gertschi*	*Polycentropus halidus*	*Polycentropus variegatus*
Presence of Muscle Scars on Head	Yes	Yes	Yes	Yes
Muscle Scars on Head Distinct	No	Yes	Yes	Yes
Pigment Banding on Head	Absent	Subtle	Distinct	Distinct
Anal Claw Curvature	Obtuse-angled	Obtuse-angled	Right-angled	Right-angled
Frontoclypeus Concolorate	Yes	No	No	No
Position of Muscle Scar "m" Relative to Frontoclypeal Suture	Close	Close	Distant	Distant
Head Width : Head Length Ratio	0.944	0.733-0.800	0.800-0.899	0.733-0.842
Head Width : Body Length Ratio	0.089	0.078-0.089	0.096-0.114	0.074-0.084
Head Color	Yellow	Yellow-Tan	Tan	Tan
Arrangement of Posterior Scars on Frontoclypeal Apotome	Absent	Trapezoidal	Shallow Arc	Shallow Arc
Foretarsus Length Relative to Foretibia Length	≥2/3	≥2/3	≥2/3	≥2/3
Basal Segment Length Relative to Distal Segment Length of Anal Proleg	Subequal	Longer	Longer	Longer
Geographic Range	CA: British Columbia; USA: California, Idaho, Montana, Oregon, Washington	USA: Arizona, Colorado, New Mexico, Texas; MX: Chihuahua	CA: British Columbia; USA: Alaska, Arizona, California, Colorado, Montana, New Mexico, Oregon, Texas, Utah, Washington, Wyoming; MX: Chihuahua, Durango, Sinaloa	CA: Alberta, British Columbia; USA.: Alaska, Arizona, California, Colorado, Montana, Oregon, Utah, Washington, Wyoming

Figure 3-1. Left lateral habitus of a late-instar larva of *Polycentropus variegatus* Banks, 1900.

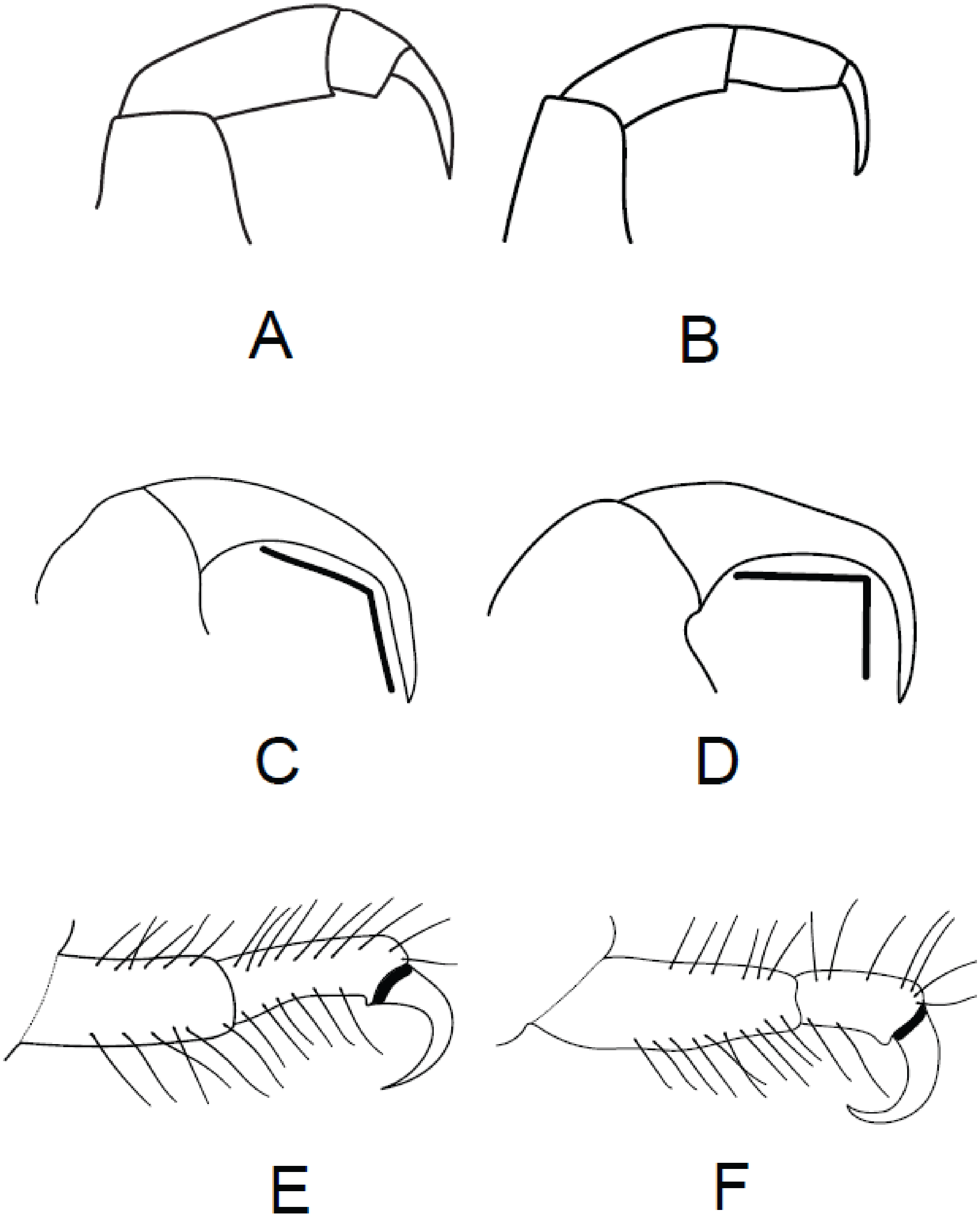

Figure 3-2. Relative proportions of the foretarsi and foretibiae, anal claw curvatures, and relative proportions of anal proleg segments.

A. Foreleg exhibiting a short, broad tarsus that is less than half the length of its tibia; B, Foreleg exhibiting a longer, narrower, and more tubular tarsus that is at least 2/3 the length of its tibia; C, Obtuse-angled anal claw; D, Right-angled anal claw; E, Anal proleg segments roughly equal in length; F, Basal segment of anal proleg longer than distal segment.

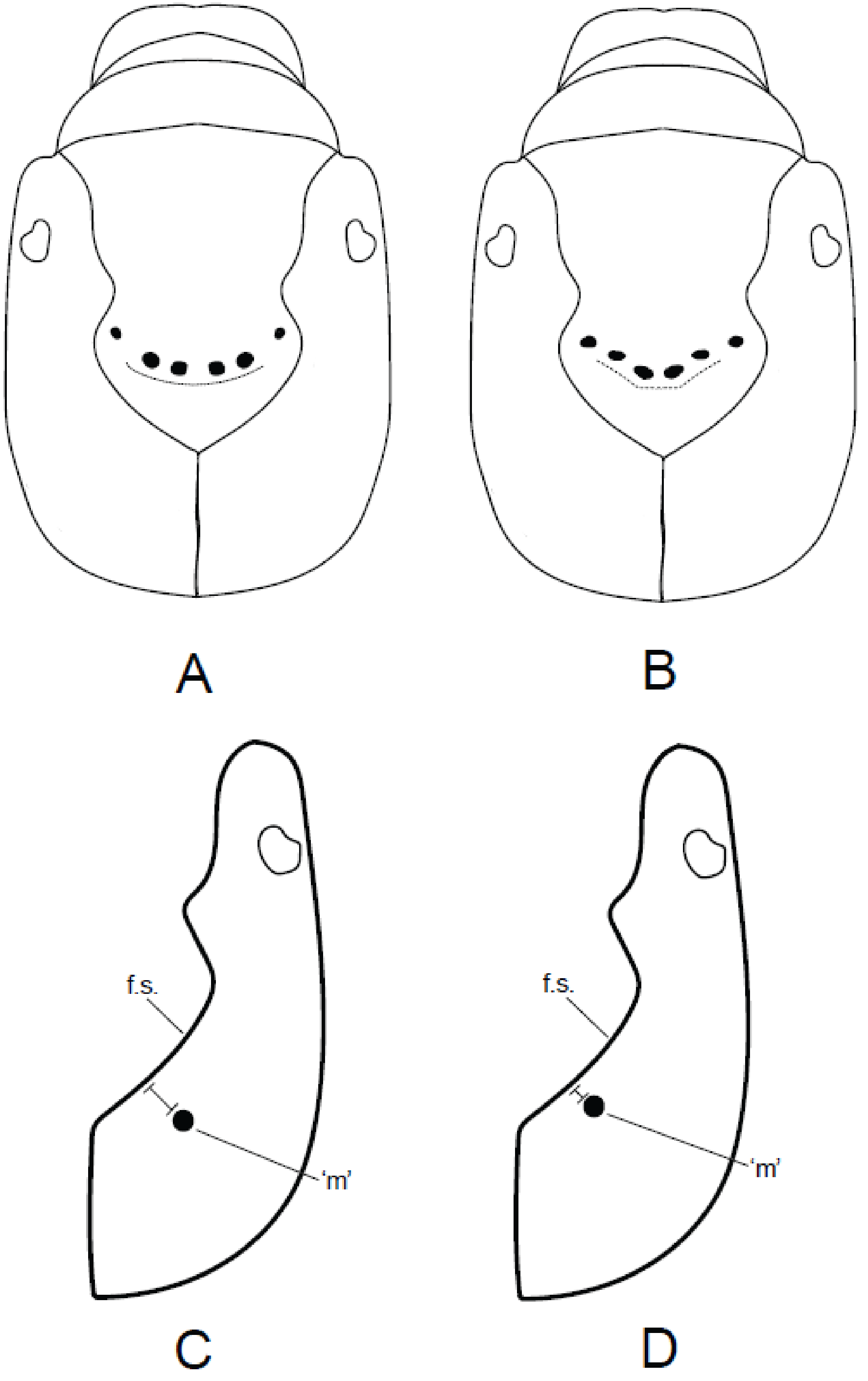

Figure 3-3. Types of muscle scar arrangements of posterior frontoclypeal apotome exhibited by known western Nearctic *Polycentropus* larvae, if present.

A. shallow arc; B. trapezoid; Below, distances of muscle scar 'm' relative to frontoclypeal suture (f.s.). C. distant; D. close.

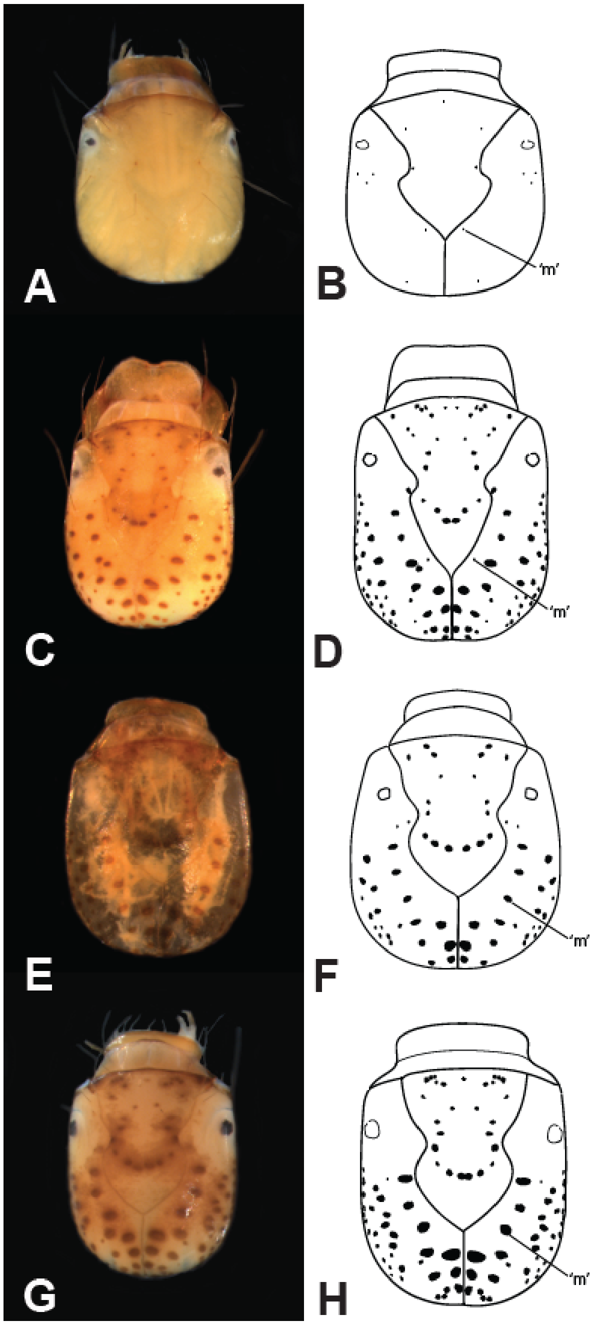

Figure 3-4. Head photographs (black background, dorsal view) and muscle scar maps (white background, dorsal view) of late-instar larvae of associated western Nearctic *Polycentropus* species.

A, B, *P. denningi* Smith, 1962; C, D, *P. gertschi* Denning, 1950; E, F, *P. halidus* Milne, 1936; G, H, *P. variegatus* Banks,1900. Note that the *P. halidus* specimen underwent whole body lysis during DNA extraction and therefore the sclerotized head capsule remains (Fig. 3-4E) but the internal soft tissue of the head is absent.

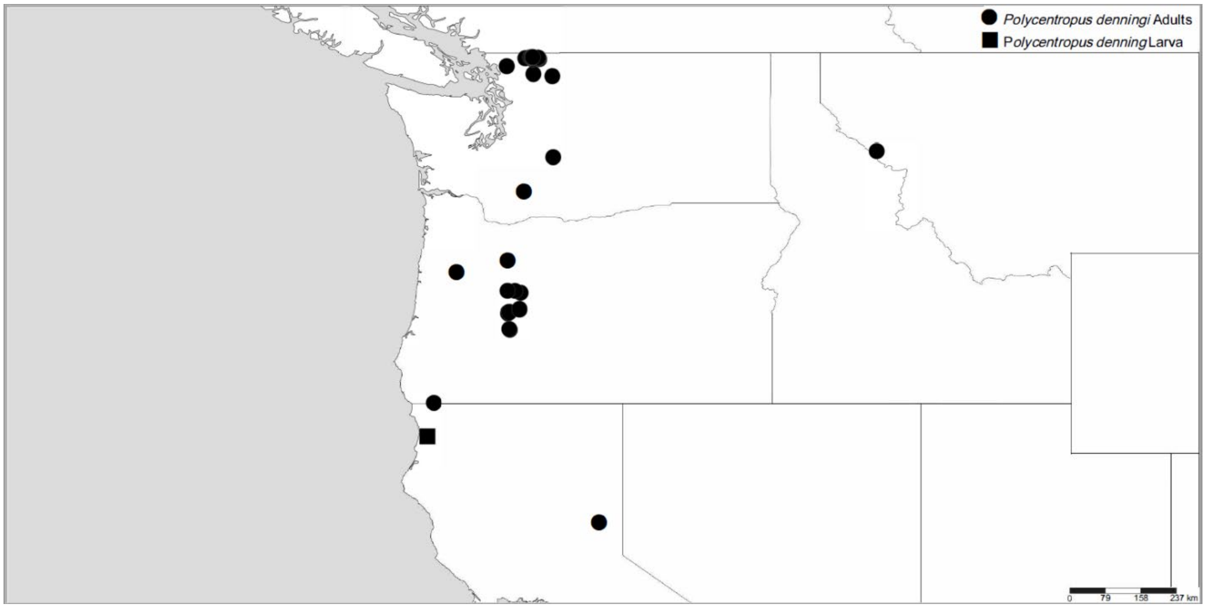

Figure 3-5. Point map of *Polycentropus denningi* Smith, 1962, specimens examined during this study.

Circles represent adults and the square represents the single larval specimen. Any one circle may represent multiple specimens collected from a single location.

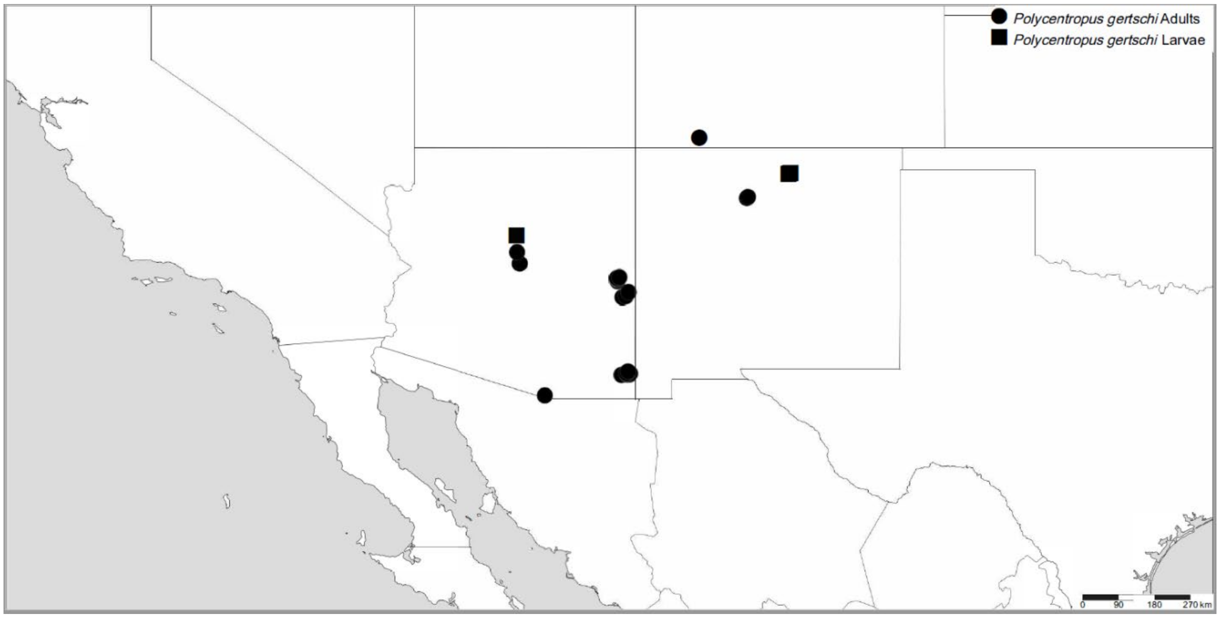

Figure 3-6. Point map of *Polycentropus gertschi* Denning, 1950, specimens examined during this study, reflecting adults and larvae.

Circles represent adults and the square represents the single larval specimen. Any one circle may represent multiple specimens collected from a single location.

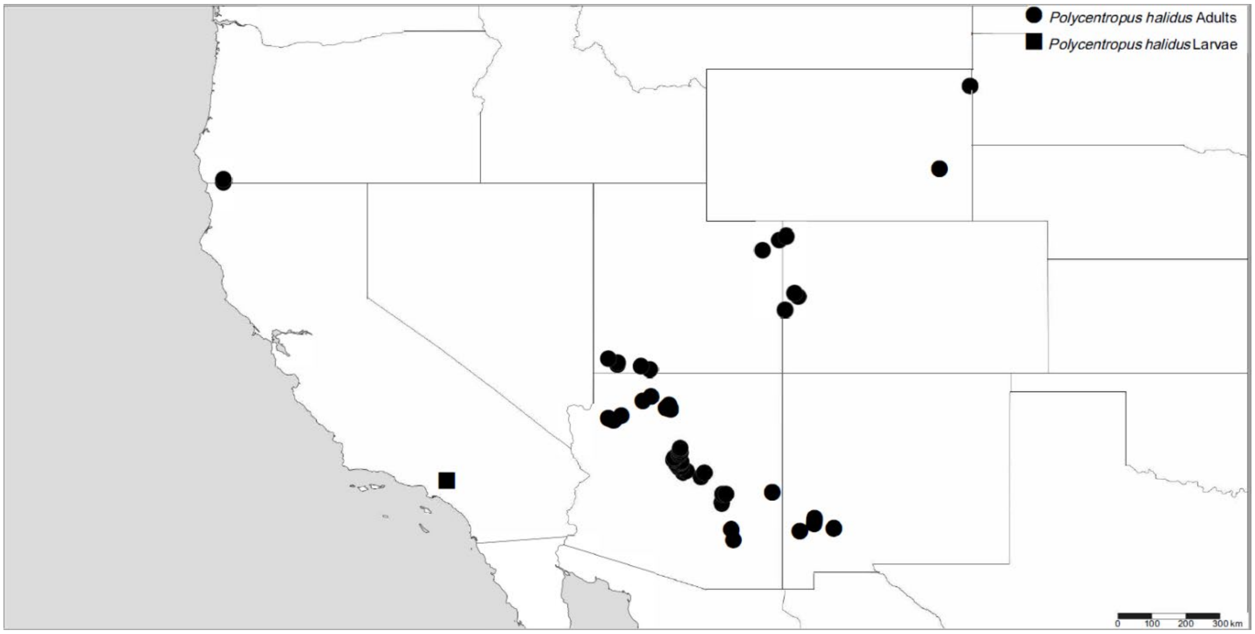

Figure 3-7. Point map of *Polycentropus halidus* Milne, 1936, specimens examined during this study, reflecting adults and larvae.

Circles represent adults and squares represent larvae. Any one symbol may represent multiple specimens collected from a single location.

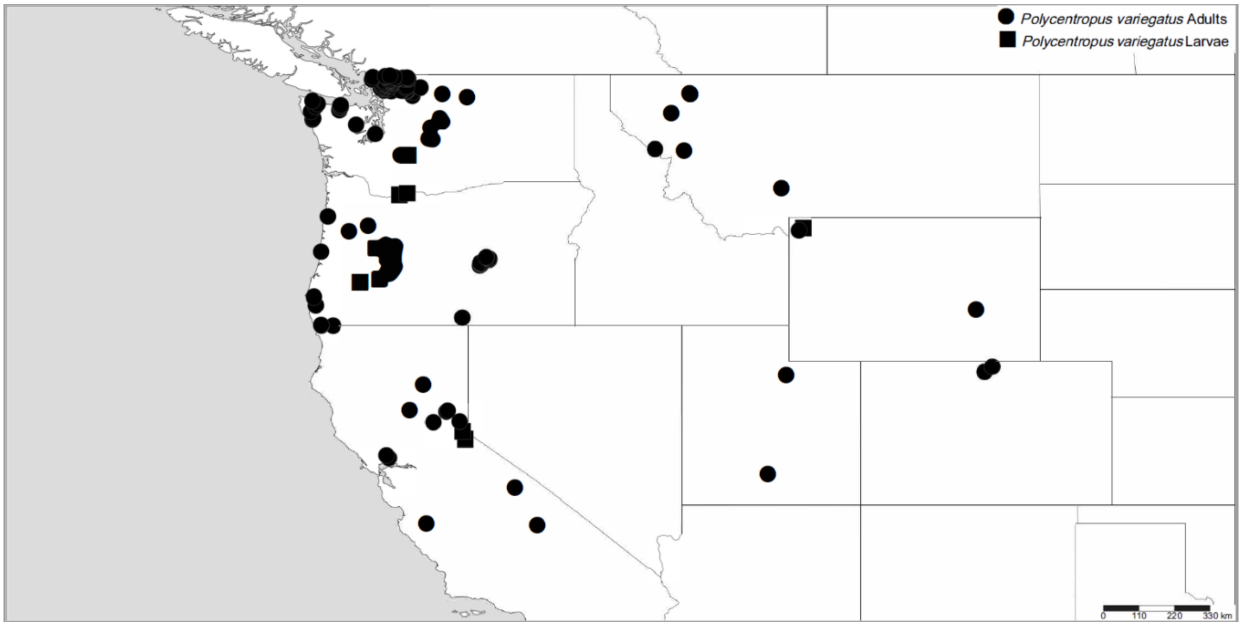

Figure 3-8. Point map of *Polycentropus variegatus* Banks, 1900, specimens examined during this study, reflecting adults and larvae.

Circles represent adults and squares represent larvae. Any one symbol may represent multiple specimens collected from a single location.

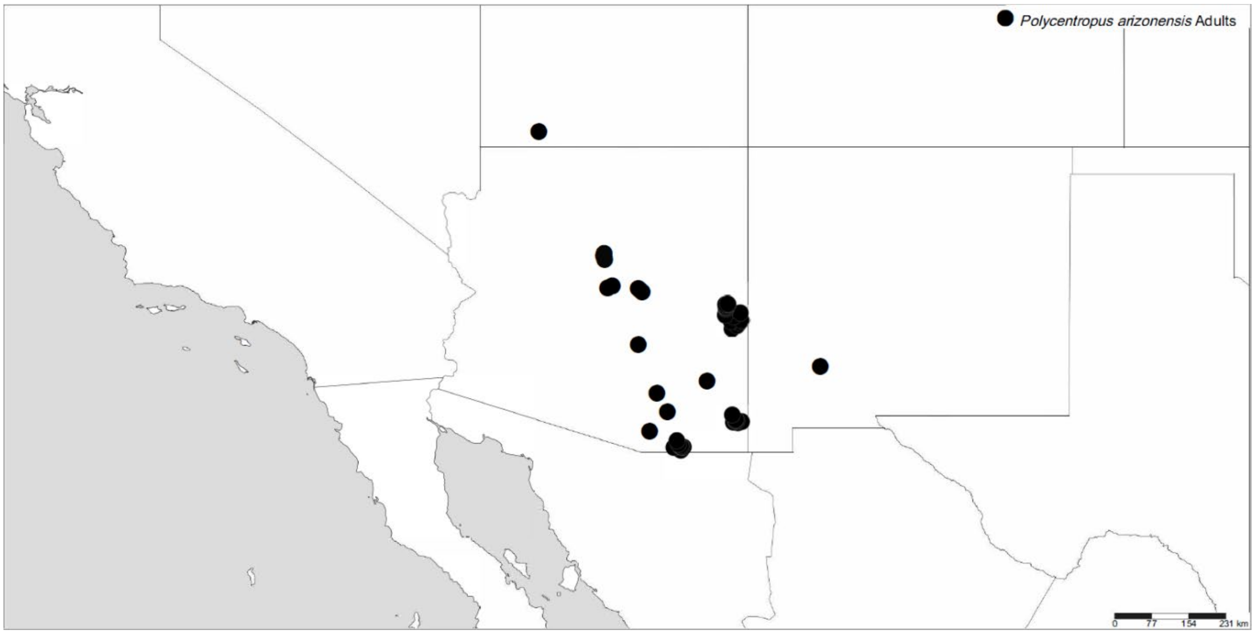

Figure 3-9. Point map of *Polycentropus arizonensis* Banks, 1905, specimens examined during this study, reflecting only adults.

Any one symbol may represent multiple specimens collected from a single location.

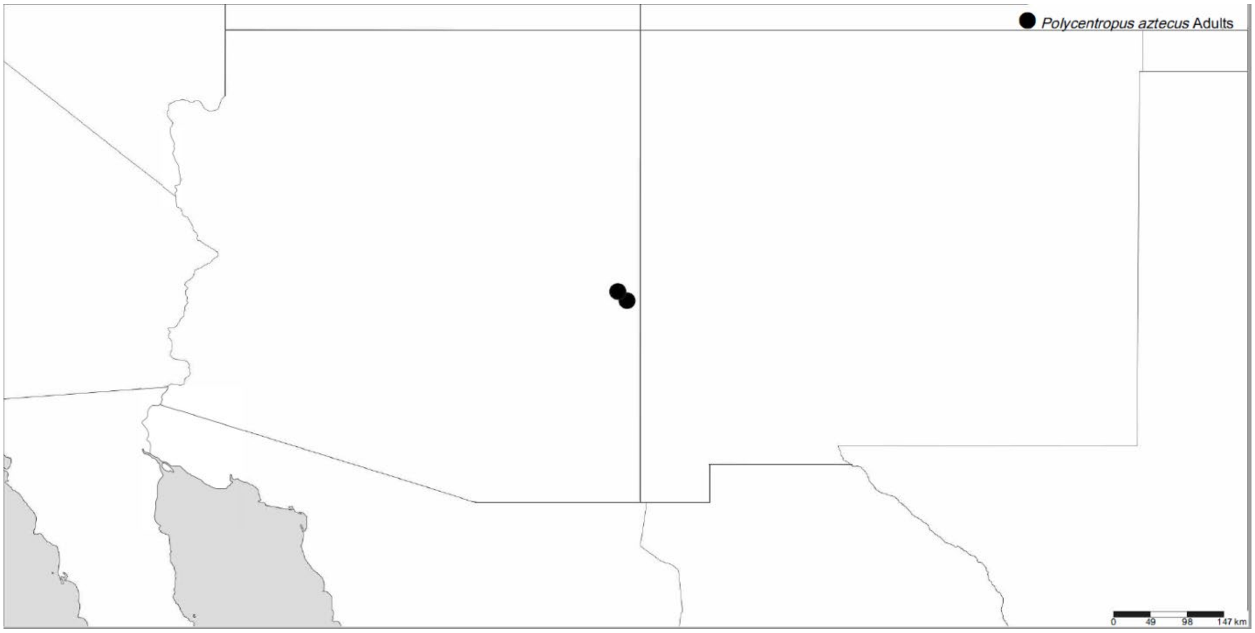

Figure 3-10. Point map of *Polycentropus aztecus* Flint, 1967, specimens examined during this study, reflecting only adults.

Any one symbol may represent multiple specimens collected from a single location.

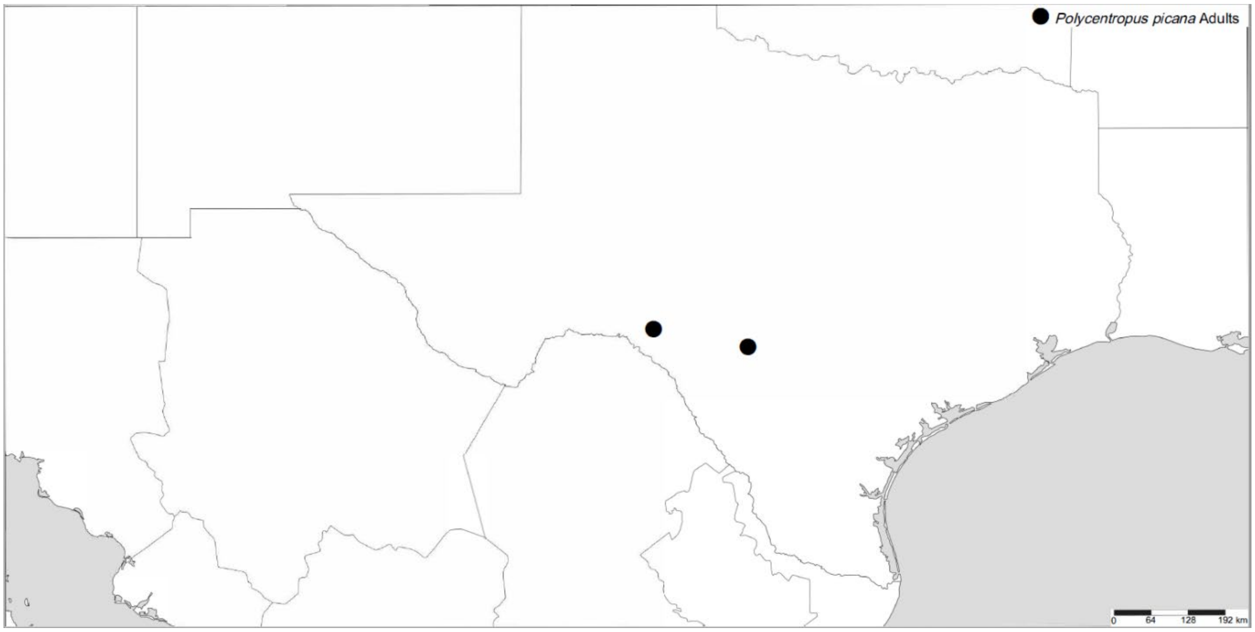

Figure 3-11. Point map of *Polycentropus picana* Ross, 1947, specimens examined during this study, reflecting only adults.

Any one symbol may represent multiple specimens collected from a single location.

CHAPTER 4
LARVAL TAXONOMY OF EASTERN NEARCTIC *Polycentropus SENSU STRICTO* (TRICHOPTERA: POLYCENTROPODIDAE)

The Trichoptera, or caddisflies, are widespread holometabolous insects with immature stages typically existing in many kinds of aquatic habitats and alate adults usually inhabiting adjacent riparian areas. These insects are the most diverse primary aquatic insect order, with over 17,000 valid, extant species globally (J. Morse, personal communication). Considering 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 eosystems (Morse et al., 2019b). In North America, local, state/provincial, tribal, and federal governments conduct biomonitoring of freshwaters using macroinvertebrates such as caddisflies via standardized regional protocols (e.g., Lenat, 1988, 1993; Carter and Resh, 2001; Carter et al., 2006; Pilgrim et al., 2011) based on recommendations of the US Environmental Protection Agency (Barbour et al., 1999). It has long been recognized that these biomonitoring programs exhibit better resolution in measuring changes of aquatic ecosystem health with more-refined taxonomic identifications (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 the fact that larvae can be challenging to associate and discriminate at the species level. In North America, larval descriptions have been completed for fewer than half of all caddisfly species (Ruiter et al., 2013; Morse et al., 2017).

Reprinted with permission from Orfinger, A. B., Hix, R. L., & Rasmussen, A. K. (2023). Larval taxonomy of eastern Nearctic *Polycentropus sensu stricto* (Trichoptera: Polycentropodidae). Freshwater Science, 42(2), 176–189.

This substantial knowledge gap prevents identification of most species' larvae and therefore precludes greater precision in surface water biomonitoring 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 attention in caddisfly taxonomy in general and, in particular, larval taxonomy. *Polycentropus* and allied genera have been variously treated throughout the years as either a single genus or three separate genera. In 1944, Ross effectively synonymized *Holocentropus* McLachlan, 1878, and *Plectrocnemia* Stephens, 1836, with *Polycentropus*. European taxonomists did not adopt this classification, but North American workers 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 collectively termed the *Polycentropus sensu lato* in order to distinguish them from the more narrowly defined and originally designated *Polycentropus sensu stricto* (see Orfinger, 2019 for additional discussion). The larvae of those three genera cannot yet be distinguished from each other and cannot yet be distinguished consistently from larvae of *Cernotina* (Morse et al., 2017, 2019a).

The focus of this chapter is the larvae of the *Polycentropus sensu stricto* (e.g., Fig. 4-1), referred to hereafter simply as *Polycentropus*. Larvae of these animals inhabit clean, cool, lotic waters spanning first order streams to navigable rivers. In the Nearctic

Region, 29 named *Polycentropus* species are recorded, with most species distributed exclusively either in the East or the West. Here, I define the East as consisting of Manitoba and the US states adjacent to either side of the Mississippi River and eastward and the West consisting of the complementary Nearctic region (following e.g., Lago and Harris, 1987a; Cooper and Morse, 1998; Chapter 2; Chapter 3). The western fauna consists of seven nominal species, of which larvae of four species were in the preceding chapter along with a diagnostic character matrix for their identification purposes. The more diverse eastern fauna comprises 22 species and is divided into two species groups (Armitage and Hamilton, 1990), the *Polycentropus confusus* Species Group represented by 19 species and the *Polycentropus colei* Species Group represented by three species. Of the 22 eastern species, only the larva of *Polycentropus centralis* Banks,1914, was previously described and its head and pronotum illustrated by Ross (1944). The remaining 21 larvae have remained unstudied. Remarkably, for example, the larva of the common and widespread *Polycentropus confusus* Hagen, 1861, has gone undescribed for the more than 160 years since the species' description.

The goals of this chapter are to describe the 11 associated larvae from Chapter 2 from the eastern *Polycentropus* fauna, to redescribe the larva of *P. centralis*, and to provide a diagnostic character matrix to the known eastern larvae of the genus. I conclude by discussing future research priorities for the identification and description of Nearctic polycentropodid larvae.

Materials and Methods

Larval-adult association for most species was accomplished with mtDNA barcoding using the barcoding region of cytochrome oxidase I (COI) as described in

Chapter 2. Life stage association for *Polycentropus floridensis* Lago and Harris, 1983, was achieved geographically. Specifically, intense sampling efforts of Eglin Air Force Base by Dr. Andrew Rasmussen and others targeting immature and adult life stages between 1996 and 2008 allowed for identification of *P. floridensis* in Santa Rosa County, Florida. Within *Polycentropus sensu lato*, only *P. floridensis* and *Plectrocnemia cinerea* (Hagen, 1861), were collected across seasons among eight sites of the 31 total sites sampled, with larvae of these two species readily distinguishable (A. Rasmussen, unpublished data).

Morphological methods followed those of Chapter 3. Specimens were observed under a Unitron Z10 stereomicroscope with maximum 120x magnification. Measurements were taken using a calibrated ocular micrometer and are presented to the nearest 0.1 mm. Specimen length refers to total length (i.e., anterior margin of head to posterior ends of anal claws). Because specimens are frequently preserved in a curled position, careful straightening of larvae using two pairs of fine forceps was often necessary when performing length measurements. For morphometric measurements, “head width” describes the width of the head measured dorsally at the widest point, while “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 Chapter 2.

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; Chapter 2): (1) The relative lengths and thicknesses of the foretarsi and foretibiae (for example, comparing dimensions in Figs 4-2A and 4-

2B); (2) curvature of the anal claw [classified as either obtuse (Fig. 4-2C) or right-angled (Fig. 4-2D)]; (3) relative proportions of the two anal proleg segments (for example, comparing dimensions in Figs 4-2E and 4-2F); (4) the arrangement of the posterior muscle scars (when present) of the frontoclypeal apotome, forming either a shallow arc (Fig. 4-3A), a roughly straight line (Fig. 4-3B), or a trapezoidal pattern (Fig. 4-3C); and (5) the distance of muscle scar ‘m’ (*sensu* Waringer and Graf, 2011) from the frontoclypeal suture [classified as “distant” (Fig. 4-4A; termed “set back” by Waringer and Graf, 2011) or “close” (Fig. 4-4B), following Chapter 2.

In most instances, instar number (e.g., 3rd instar, 4th instar, 5th instar) could not be determined confidently due to a lack of larval size class series and/or metamorphotype specimens (*sensu* Milne, 1938). Given that the vast majority of specimens examined are large (i.e., >10 mm 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, the term “late-instar” is used instead.

For stacked photography, heads were excised and placed in glycerine in a depression slide. Between six and 16 photographs of each larval head were taken at different depths of field using a Levenhuk M1400 Plus Digital Camera mounted on a Unitron Z10 microscope. The photographs of each subject were subsequently digitally stitched together using Helicon Focus version 7.7.4 to form a single composite image. Line drawings were produced using a 10 x 10 mm 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.

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

Eastern Larval Taxonomy Results

Presented below are original descriptions of the larvae of 11 eastern Nearctic *Polycentropus* species beginning with nine of 19 members of the *Polycentropus confusus* Species Group and followed by two of three members of the *Polycentropus colei* Species Group. Also presented is a redescription of the larva of *Polycentropus 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 asterisk (*). Descriptions emphasize characters for which interspecific variation was observed, whereas the following suite of character states was consistent among all larvae:

- Muscle scarring present on head
- Foretarsi tubular and with length ≥2/3 foretibiae length (Fig. 4-2B)

- Basal segments of anal proleg longer than distal sclerotized segments of anal proleg (Fig. 4-2F)
- Basal segments of anal proleg with numerous long setae (Fig. 4-2F)
- Abdominal segments lacking gills (Fig. 4-1)
- Anal claws lacking spines on concave margins (Figs. 4-2C, 4-2D)
- Ventral apotome broad and V-shaped
- Mesal faces of femora covered with numerous long setae
- Distal section of each tarsus ventrally with well-developed pectinate setae

Finally, a diagnostic matrix to the 12 known eastern Nearctic *Polycentropus* larvae is presented (Table 4-1). This matrix facilitates identification of each species for which the larva is known based on eight categorical characters, two 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

(Figs. 4-5A, 4-5B; Table 4-1)

Description of Late-instar Larva. Larval length 12.0–12.5 mm (mean = 12.3 mm; n = 2).

Head. Length 1.5 mm, width 1.0–1.1 mm (n = 2). Dorsal area of head yellow-tan with numerous symmetrically positioned, variously sized, well-defined dark muscle scars. Dorsal head surface with distinct darker brown bands laterally in anterior 2/3 of frontoclypeus and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome pale with muscle scar arrangement forming shallow arc (Fig. 4-3A). Pair of muscle scars ‘m’ subtle, small, and positioned close to frontoclypeal suture (Fig. 4-4B). Ventral area of head also yellow-tan, with anterior half lacking muscle scars and posterior half 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. 4-2C).

Distribution.

USA: Alabama

Specimens Examined.

USA. Alabama: Morgan County, Spring outside of Curry Cave, 6-7/iv/2020, 2 larvae. (FAMU).

Polycentropus blicklei Ross and Yamamoto, 1965

(Figs. 4-5C, 4-5D; Table 4-1)

Description of Late-instar Larva. Larval length 9.0–11.8 mm (mean = 11.1 mm; n = 3).

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

Distribution.

Canada: New Brunswick, Newfoundland and Labrador, Nova Scotia, Ontario, Quebec; U.S.A.: Alabama, Delaware, Florida, Georgia, Kentucky, Maine, Maryland, Mississippi, New Hampshire, North Carolina, New Jersey, New York, Ohio, Pennsylvania, Rhode Island, South Carolina, Tennessee, Virginia.

Specimens Examined

USA. Maryland: Anne Arundel County, Sewell Spring Branch, site SR-01-18, [N39°04'34.93", W76°37'05.88"], M. Cole coll., 3/iv/2018, 3 larvae. (Cole Ecological, Inc. 7670).

Polycentropus carlsoni Morse, 1971

(Figs. 4-5E, 4-5F; Table 4-1)

Description of Late-instar Larva. Larval length 10.2 mm (n = 1).

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

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 coll., 24/x/2021, 1 larva. (FAMU). **New State Record**

Polycentropus carolinensis Banks, 1905

(Figs. 4-5G, 4-5H; Table 4-1)

Description of Final-instar Larva. Larval length 11.0–16.4 mm (mean = 13.7 mm; n = 5).

Head. Length 1.1–1.5 mm, width 0.9–1.2 mm (n = 5). Dorsal area of head yellow-tan with numerous symmetrically positioned, variously sized, well-defined dark muscle scars. Dorsal head surface with subtle darker brown bands in anterior 2/3 of frontoclypeus and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome pale with muscle scar arrangement trapezoidal (Fig. 4-3C). Pair of muscle scars ‘m’ subtle, small, and positioned close to frontoclypeal suture (Fig. 4-4B). Ventral area of head also yellow-tan, with anterior half lacking muscle scars and posterior half 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. 4-2C) 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 coll., 17/viii/2005, 3 larvae. (FAMU). Watauga County, Spring seep below spring house, Moses H. Cone Estate, 1146 masl, J. Robinson and C.R. Parker coll., 29/iii/2007, 5 larvae. (BLRI 21955). **Tennessee:** Fentress County, Cave Creek, Tributary of Pogue Creek, at end of ATV road off Williams Cr. Rd, 0.5 km east of Delk Cr. Rd., D. Etnier et al. coll., 21/v/2005, 1 metamorphotype male, 2 larvae. (ECC 3.431). **Virginia:** Augusta County, Spring at Dripping Rock pullout at MP 009.7, left, by Hand, J. Robinson and C.R. Parker coll., 17/viii/2007, 1 larva. (BLRI 25619).

Polycentropus centralis Banks, 1914

(Figs. 4-5I, 4-5J; Table 4-1)

Description of Late-instar Larva. Larval length 10.5–11.5 mm (mean = 11.1 mm; n = 5).

Head. Length 1.0–1.3 mm, width 0.9–1.0 mm (n = 5). Dorsal area 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. Dorsal head surface with subtle darker brown bands in anterior 2/3 of frontoclypeus and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome pale with muscle scar arrangement linear (Fig. 4-3B). Pair of muscle scars ‘m’ faint and nearly invisible, small, and positioned close to frontoclypeal suture (Fig. 4-4B). Ventral area of head yellow, with anterior half lacking muscle scars and posterior half 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. 4-2C).

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"], 38/iii/2021, 11 larvae. (FAMU). **Illinois:** Union County, La Rue, Running Lick Creek, [N37°32'44.02", W89°27'12.99"], B.D. Burks, G.T. Riegel coll., 12/v/1939, 1 larva. (INHS Trichoptera 53462). Wolf Lake, Hutchins Creek, [N37°30'40.86", W89°22'38.75"], Frison and Ross coll., 5/x/1939.

Polycentropus confusus Hagen, 1861

(Figs. 4-1, 4-5K, 4-5L; Table 3-1)

Description of Final-instar Larva. Larval length 12–16.5 mm (mean = 13.6 mm; n = 6).

Head. Length 1.3–2.0 mm, width 1.1–1.5 mm (n = 6). Dorsal area of head tan with numerous symmetrically positioned, variously sized, well-defined dark muscle scars. Dorsal head surface with distinct darker brown bands in anterior 2/3 of frontoclypeus and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome pale with muscle scar arrangement linear (Fig. 4-3B). Pair of muscle scars ‘m’ distinct, small, and positioned close to frontoclypeal suture (Fig. 4-4B). Ventral area of head tan to yellow-tan, with anterior half lacking muscle scars and posterior half 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. 4-2C).

Distribution.

Canada: New Brunswick, Newfoundland and Labrador, Nova Scotia, Ontario, Quebec; USA: Alabama, Arkansas, Connecticut, District of Columbia, Delaware, 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, R. O`Connor coll., 28/v/2018, 1 larva. (BIOUG43980-E07). **USA. Alabama:** Lawrence County, Hubbard Creek below Kinlock Falls, [N34°18'31.68", W87°30'06.84"], 17/iv/2021, 1 larva. (FAMU). **Georgia/South Carolina:** Rabun/Oconee Counties, Chatooga River at SR 28, 24 km S Highlands, NC, 770 masl, [N34°55'09.84", W83°10'06.96"], B. Schmidt coll., 19/vi/2019, 1 larva. (CUAC000088819). K. Hecke coll., 31/vii/2019, 1 larva. (CUAC). **Missouri:** Stone County, Wire Road Conservation Area, Crane Creek, [N36°55'31.80", W93°35'25.80"], D.E. Bowles coll., 14/iii/2021, 1 larva. (FAMU). **North Carolina:** Haywood County, Pigeon River, [N35°44'01.68", W83°01'30.72"], R.W. Smith coll., 21/viii/2019, 2 larvae. (FAMU). **Pennsylvania:** Bucks County, Mill Creek, [N40°10'01.56", W75°00'07.56"], N. Macelko coll., 14/vi/2020, 1 larva. (FAMU). Centre County, Spring Creek, [N40°52'51.24", 77°47'36.60"], 2/v/2021, 1 larva. (FAMU). Chester County, Exton, Valley Creek West, [N40°01'47.30", W75°37'20.30"], 13/iv/2021, 3 larvae. (FAMU). Tributary of Little Neshaminy Creek, [N40°06'02.40", W75°32'33.96"], 26/xi/2020, 1 larva. (FAMU). Valley Creek West, [N40°01'47.28", W75°37'20.28"], 13/iv/2021, 4 larvae. (FAMU). Delaware County, Chester Creek, [N39°54'07.06", W75°28'09.78"], 6/xii/2020, 1 larva. (FAMU). Montgomery County, Pennypack Creek, [N40°08'15.36", W75°04'26.40"], 26/iv/2020, 1 larva. (FAMU). Northampton County, Bush Kill Creek, [N40°44'29.04", W75°14'53.52"], 1/v/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, K. Rondollo coll., 19/vi/2013, 1 larva. (BIOG06971-F10). 1 larva. (BIOUG06971-G01). 1 larva. (BIOUG07978-B07). 1 larva. (BIOUG07982-A07). Cold Run, [N40°42'14.40", W75°59'52.80"], 18/vi/2013, 1 larva. (BIOUG07978-E09). Philadelphia County, Birch Run, off 5 Point Road, 163 masl, [N39°55'44.40", W75°51'39.60"], T. Bringloe, V. Harvey, S. Ripley, and K. Rondollo coll., 19/vi/2013, 1 larva. (BIOUG07981-A10). 1 larva. (BIOUG07982-A08). 1 larva. (BIOUG07982-A09). Philadelphia, Cold Run, 211 masl, [N40°42'14.40", W75°59'52.80"], 18/vi/2013, 1 larva. (BIOUG07978-E09). 1 larva. (BIOUG07978-H06). Philadelphia County, Tributary of Brandywine Creek, 29 masl, [N39°55'04.80", W75°43'22.80"], 19/vi/2013, 1 larva. (BIOUG07978-H07). Chester County, White Clay Creek, [N39°51'46.80", W75°47'02.40"], T. Bringloe, V. Harvey, S. Ripley, K. Rondollo coll., 19/vi/2013, 1 larva. (BIOUG06970-A06). 1 larva.

(BIOUG07063-B11). **South Carolina:** Pickens County, Wildcat Creek, [N34°45'34.20", W82°51'03.24"], C.M. Slack coll., 25/viii/2009, 1 larva. (CUAC000107326).

Polycentropus elarus Ross, 1944

(Figs. 4-5M, 4-5N; Table 4-1)

Description of Late-instar Larva. Larval length 12.3 mm (n = 1).

Head. Length 1.3 mm, width 1.1 mm (n = 1). Dorsal area of head yellow-tan with numerous symmetrically positioned, variously sized, well-defined dark muscle scars. Dorsal head surface with subtle darker brown bands in central 1/3 of frontoclypeus and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome pale with muscle scar arrangement trapezoidal (Fig. 4-3C). Pair of muscle scars ‘m’ distinct, large, and positioned close to frontoclypeal suture (Fig. 4-4B). Ventral area of head yellow-tan, with anterior half lacking muscle scars and posterior half 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. 4-2C).

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, K. Rondollo coll., 13/vi/2013, 1 larva. (BIOUG07980-B07).

Polycentropus floridensis Lago and Harris, 1983

(Figs. 4-5O, 4-5P; Table 4-1)

Description of Late-instar Larva. Larval length 6.5–12.3 mm (mean = 8.2 mm; n = 4).

Head. Length 0.9–1.3 mm, width 0.6–1.1 mm (n = 4). Dorsal area of head orange-tan with numerous symmetrically positioned, variously sized, well-defined dark muscle scars. Dorsal head surface 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. 4-3A). Pair of muscle scars ‘m’ distinct, small, and distant from frontoclypeal suture (Fig. 4-4A). Ventral area of 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. 4=3B).

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 coll., 22/iii/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 coll., 27/v/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 coll., 11/i/2006, 2 larva. (FAMU).

Polycentropus maculatus Banks, 1908

(Figs. 4-5Q, 4-5R; Table 4-1)

Description of Late-instar Larva. Larval length 11.0–15.4 mm (mean = 12.5 mm; n = 5).

Head. Length 1.3–1.5 mm, width 1.2–1.3 mm (n = 5). Dorsal area of head yellow-tan with numerous symmetrically positioned, variously sized, well-defined dark muscle scars. Dorsal head surface 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. 4-3C). Pair of muscle scars ‘m’ subtle, small, and distant from frontoclypeal suture (Fig. 4-4A). Ventral area of head yellow-tan, with anterior half lacking muscle scars and posterior half 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. 4-2C).

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 masl, [N35°01'04.44", W83°14'42.36"], R.L. Heth coll., 23/vii/2018, 1 larva. (CUAC000107327).

Pennsylvania: Schuylkill County, Owl Creek, [N40°46'58.80", W75°57'39.60"], T. Bringloe, V. Harvey, S. Ripley, K. Rondollo coll., 18/vi/2013, 1 larva. (BIOUG06970-G02). Rattling Run, [N40°35'02.40", W76°01'01.20"], 1 larva. (BIOUG07980-H09). 1 larva. (BIOUG07980-H11). 1 larva. (BIOUG07980-H10).

Polycentropus pentus Ross, 1941

(Figs. 4-5S, 4-5T; Table 4-1)

Description of Late-instar Larva. Larval length 9.5–11.0 mm (mean = 10.2 mm; n = 5).

Head. Length 1.1–1.4 mm, width 1.0–1.2 mm (n = 5). Dorsal area of head orange-tan with numerous, variously sized, well-defined dark muscle scars; muscle scarring on head occasionally slightly asymmetrical. Dorsal head surface with distinct darker brown bands in anterior 2/3 of frontoclypeus and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome pale with muscle scar arrangement linear (Fig. 4-3B). Pair of muscle scars ‘m’ distinct, large, and distant from frontoclypeal suture (Fig. 4-4A). Ventral area of head orange-tan, with anterior half lacking muscle scars and posterior half 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. 4-2D).

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 masl, [N46°04'51.60", W78°26'19.68"], C. Freutel and G. Martin coll., 12/vii/2011, 1 larva. (BIOUG00331-B10). 1 larva. (BIOUG00331-C05). 1 larva. (BIOUG01723-C03). 1 larva. (BIOUG01723-D05). **USA. Kentucky:** Bell County, Cumberland Gap National Historical Park headwaters of Shilalah Creek, J. Robinson coll., 4/iv/2007, 5 larvae. (CUGA 4588).

Polycentropus colei Species Group

Polycentropus colei Ross, 1941

(Figs. 4-6A, 4-6B; Table 4-1)

Description of Late-instar Larva. Larval length 10–20 mm (mean = 15.3 mm; n = 3).

Head. Length 1.1–2.0 mm, width 1.0–1.7 mm (n = 3). Dorsal area of head uniformly yellow with two symmetrically positioned, small, well-defined dark muscle scars on incurvate center of frontoclypeal sutures and additional symmetrically positioned, variously sized subtle muscle scars throughout. Dorsal head surface lacking pigment bands and with pale areas around eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome with muscle scars absent. Pair of muscle scars ‘m’ subtle, small, and positioned close to frontoclypeal suture (Fig. 4-4B). 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. 4-2D).

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 masl, J. Robinson and C.R. Parker coll., 27/iii/2007, 1 larva. (BLRI 21962). Transylvania County, Devil`s Courthouse at Blue Ridge Parkway, J.K. Moulton coll., 17/viii/2005, 2 larvae. (FAMU).

Polycentropus rickeri Yamamoto, 1966

(Figs. 4-6C, 4-6D; Table 4-1)

Description of Late-instar Larva. Larval length 11.0–11.5 mm (mean = 11.3 mm; n = 2).

Head. Length 1.5 mm, width 1.4 mm (n = 2). Dorsal area of head uniformly orange-yellow to yellow with two 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. Dorsal head surface lacking pigment bands and with pale areas around small eyes. Anterior margin of frontoclypeal apotome brown. Posteriorly, frontoclypeal apotome with two subtle muscle scars in linear arrangement (Fig. 4-3B). Pair of muscle scars ‘m’ subtle, small, and positioned close to frontoclypeal suture (Fig. 4-4B). 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. 4-2D).

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 masl, 21/x/2006, 2 larvae. (BLRI 22827).

Discussion on Eastern Nearctic *Polycentropus* Larval Taxonomy

The original descriptions of 11 eastern Nearctic *Polycentropus* species and the re-description of *P. centralis* herein brings the total number of described Nearctic *Polycentropus* species to 16. Chapter 2 presented the description of larvae of four of the seven known western Nearctic species. In that chapter, I 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 chapter is a significant step towards that goal.

Two notable instances of differences in character states of the eastern and western fauna were encountered. The first is the relative length of the basal and distal segments of the anal prolegs. In the western larvae, Chapter 2 noted that the larva of *P. denningi* Smith,1962, exhibits anal proleg segments of roughly the same length (Fig. 4-2E), a character state previously unseen in *Polycentropus* larvae. No eastern Nearctic larvae demonstrated this character state. Instead, all eastern Nearctic larvae were found to have basal segments distinctly longer than the distal segments (Fig. 4-2F). The second 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 pattern is present, western larvae were found to possess either a shallow arc (Fig. 4-3A) or a trapezoidal (Fig. 4-3C) configuration. The eastern Nearctic fauna also exhibits these muscle scar patterns of the posterior frontoclypeal apotome, with some species also having a linear configuration (e.g., *P. confusus*; Fig. 4-3B) not yet seen among western species.

The eastern Nearctic *Polycentropus* fauna is divided into two species groups . The *Polycentropus colei* Species Group consists of three species (*P. barri* Ross and Yamamoto, 1965, *P. colei*, and *P. rickeri*). The larvae of the latter two species are described for the first time here. Each exhibits large, rounded, yellow to orange-yellow heads with little (i.e., *P. rickeri*) to no (i.e., *P. colei*) muscle scarring on the posterior half of the frontoclypeal apotome. It would be unsurprising 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 half of the frontoclypeal apotome. The phylogenies presented in Chapter 1 based on mitochondrial cytochrome oxidase I (mtCOI) 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 half of the frontoclypeal apotome is a synapomorphy of this putative monophyletic grouping. A more robust phylogeny of the *Polycentropus colei* Species Group would be useful in testing this hypothesis.

Unlike the *Polycentropus colei* Species Group, known larvae of the *Polycentropus confusus* Species Group possess a series of muscle scars on the posterior half of the frontoclypeal apotome. Three muscle scar patterns are evident, including shallow arc (Fig. 4-3A), linear (Fig. 4-3B), and trapezoidal (Fig. 4-3C) configurations. Among the known larvae, the occurrence of muscle scar patterns appears to be distributed fairly evenly, with three species (*P. alabamensis*, *P. blicklei*, and *P. floridensis*) exhibiting a shallow arc, three species (*P. centralis*, *P. confusus*, and *P. pentus*) exhibiting a linear pattern, and four 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 in Chapter 1, forming a monophyletic grouping along with *Polycentropus chelatus* Ross and Yamamoto, 1965. Alternatively, the correlation of these character states among species exhibiting 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 *Polycentropus confusus* Species Group, the larvae of nine remain unassociated and undescribed, namely *P. aileenae* Orfinger and Moulton, 2021, *P. chelatus*, *P. chenoides* Ross and Yamamoto, 1965, *P. dinkinsorum*, *P. neiswanderi*, *P. pixi* Ross, 1944, *P. stephani* Bowles, Mathis, and Hamilton, 1993, *P. thaxtoni* Hamilton and Holzenthal, 1986, and *P. vernus* Hamilton, Harris, and Lago, 1990.

While this chapter and Chapter 2 have both increased our understanding of the larval taxonomy of Nearctic *Polycentropus* and the distributions of the Nearctic fauna, much work is still 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. This is particularly important for some species, such as *P. carlsoni* and *P. elarus*, for which only one 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. Though this species had been recorded nearby in northern Virginia and Maryland, it had gone undetected in Pennsylvania until now.

Finally, while 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 (A. Orfinger, 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 of those species. Future efforts should attempt to associate and describe remaining larvae, pupae, and females using molecular methods (e.g., as in Chapter 1), the metamorphotype method (Milne, 1938), or by rearing of pupae. This 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 *Cernotina*, such efforts will be especially valuable to the goal of describing and diagnosing the larvae of all Nearctic species of these genera.

Table 4-1. Discriminatory matrix to the known late-instar and final-instar larvae of eastern Nearctic *Polycentropus* species. * = Occasionally intermediate. ** = Two scars only.

Species/ Character	**Muscle Scars on Head Distinct**	**Pigment Banding 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**	**Geographic Range**
Polycentropus alabamensis	Yes	Distinct	Obtuse-Angled	No	Close	0.667–0.733	0.0800–0.092	Yellowish Tan	Shallow Arc	US: AL
Polycentropus blicklei	Yes	Distinct	Right-Angled	No	Close	0.720–0.800	0.110–0.133	Yellowish Tan	Shallow Arc	CA: NB, NS, NLr, ON, QC; US: AL, DE, FL, GA, KY, ME, MD, MS, NC, NH, NJ, NY, OH, PA, RI, SC, TN, VA
Polycentropus carlsoni	Yes	Distinct	Right-Angled	No	Close	0.912	0.108	Yellowish Tan	Trapezoidal	US: AL, MD, NC, PA, SC, VA
Polycentropus carolinensis	Yes	Subtle	Obtuse-Angled*	No	Close	0.667–0.857	0.073–0.082	Yellowish Tan	Trapezoidal	CA: ON, QC; US: KY, MS, NC, NH, OH, PA, TN, VA, VT, WV
Polycentropus centralis	No	Subtle	Obtuse-Angled	No	Close	0.692–0.917	0.080–0.096	Yellow to Yellowish Tan	Linear	CA: NL, NS, ON; US: AL, AR, IA, IL, IN, KS, KY, MN, MO, MS, NY, OH, OK, PA, TN, TX, VA, WI, WV

Table 4-1. Continued.

Species/ Character	**Muscle Scars on Head Distinct**	**Pigment Banding 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**	**Geographic Range**
Polycentropus confusus	Yes	Distinct	Obtuse-Angled	No	Close	0.733–0.923	0.073–0.108	Tan	Linear	CA: NB, NL, NS, ON, QC; US: AL, AR, CT, DC, DE, FL, GA, IA, IN, KY, ME, MA, MI, MS, MO, NH, NJ, NY, NC, OH, OK, PA, SC, TN, VA, VT, WI, WV
Polycentropus elarus	Yes	Subtle	Obtuse-Angled	No	Close	0.846	0.089	Yellowish Tan	Trapezoidal	CA: ON, QC; US: AL, FL, GA, IN, KY, MA, NH, NY, NC, OH, PA, TN, VA, WV
Polycentropus floridensis	Yes	Absent	Right-Angled	No	Distant	0.600–0.846	0.089–0.108	Orangeish Tan	Shallow Arc	US: AL, FL

Table 4-1. Continued.

Species/ Character	Muscle Scars on Head Distinct	Pigment Banding 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	Geographic Range
Polycentropus maculatus	Yes	Absent	Obtuse-Angled	Yes	Distant	0.8571–0.923	0.078–0.109	Yellowish Tan	Trapezoidal	CA: NL, NS, ON, QC; US: CT, KY, ME, MA, NH, NY, NC, OH, PA, SC, TN, VA, VT, WV
Polycentropus pentus	Yes	Distinct	Right-Angled	No	Distant	0.833–0.917	0.100–0.110	Orangeish Tan	Linear	CA: MB, NS, NL, ON, QC; US: AL, CT, IL, KY, ME, MI, MN, NH, NJ, NY, OH, PA, TN, VA, VT, WV, WI, WY
Polycentropus colei	No	Absent	Right-Angled	Yes	Close	0.842–0.909	0.085–0.101	Yellow	Absent	CA: QC; US: KY, NC, PA, TN, VA, WV
Polycentropus rickeri	No	Absent	Right-Angled	Yes	Close	0.933	0.122–0.127	Yellow to Orangeish Yellow	Linear**	US: AL, KY, NC, PA, TN, VA, WV

Figure 4-1. Left lateral habitus of a final-instar larva of *Polycentropus confusus* Hagen, 1861.

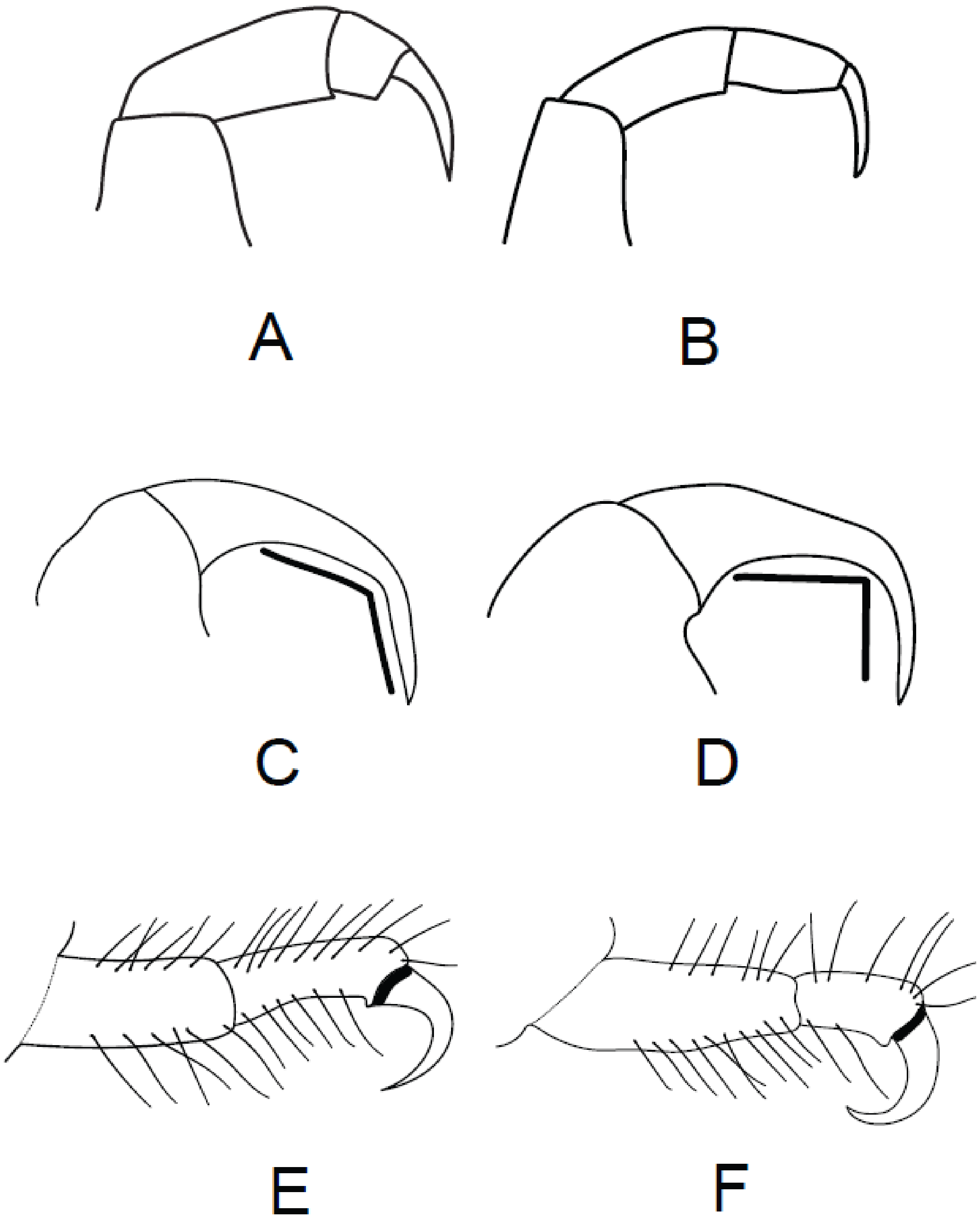

Figure 4-2. Relative proportions of the foretarsi and foretibiae, anal claw curvatures, and relative proportions of anal proleg segments.

A. Foreleg exhibiting a short, broad tarsus that is less than half the length of its tibia; B. Foreleg exhibiting a longer, narrower, and more tubular tarsus that is at least 2/3 the length of its tibia; C. Obtuse-angled anal claw; D. Right-angled anal claw; E. Anal proleg segments roughly equal in length; F, Basal segment of anal proleg longer than distal segment.

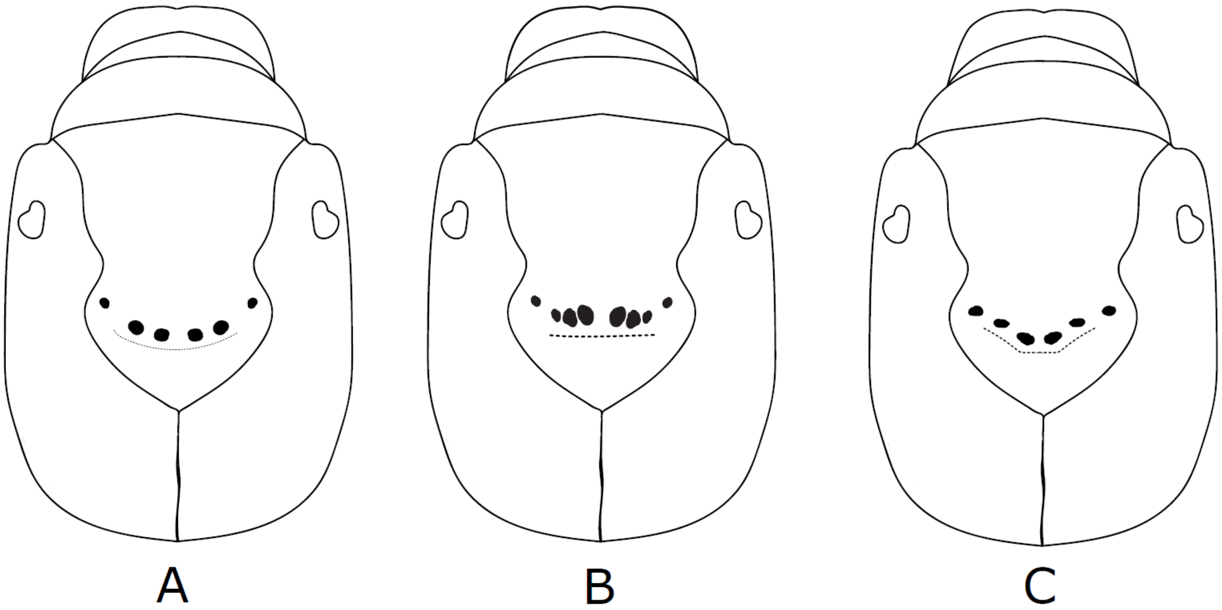

Figure 4-3. Types of muscle scar arrangements of posterior frontoclypeal apotome exhibited by known eastern Nearctic *Polycentropus* larvae, if present.

A. shallow arc; B. linear; C. trapezoidal.

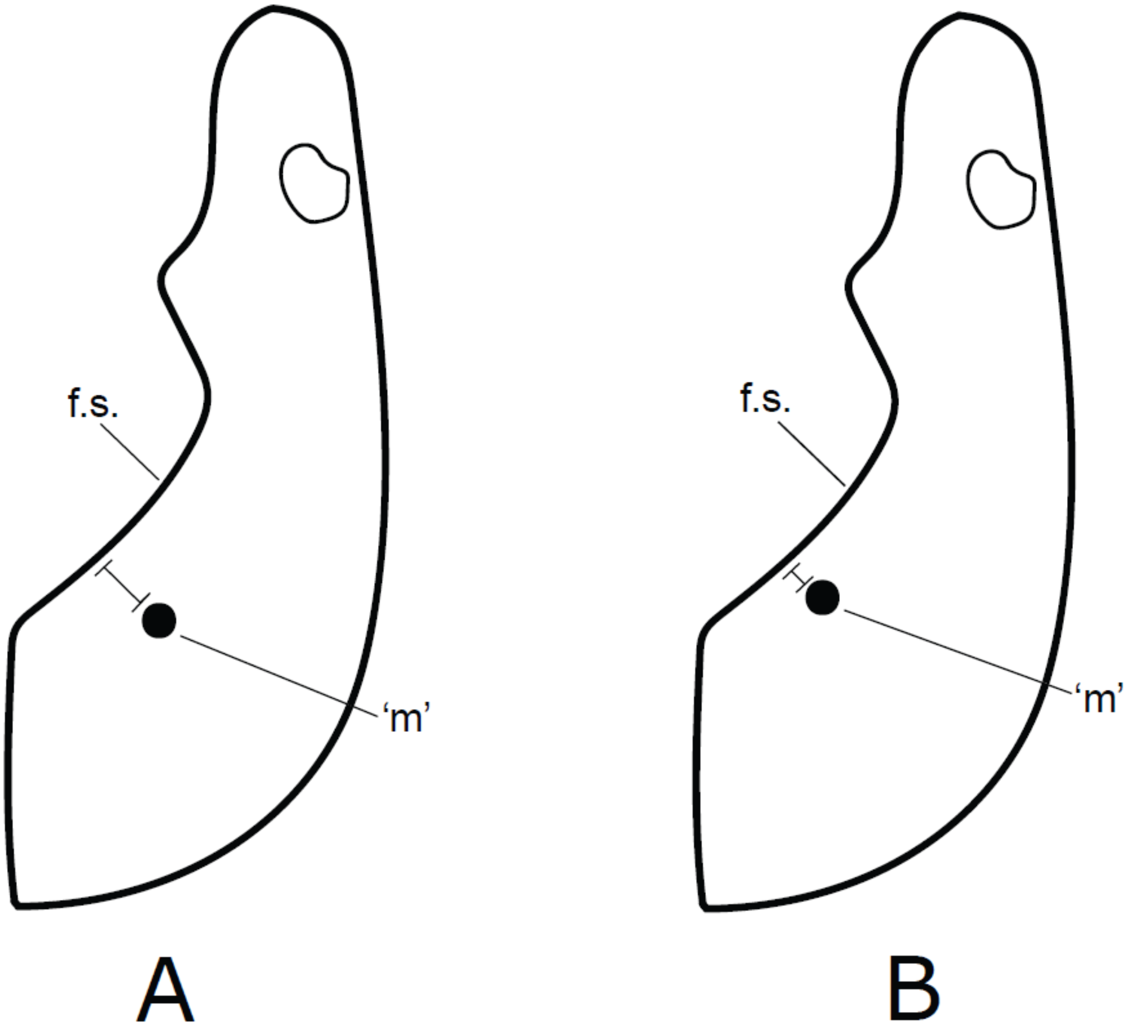

Figure 4-4. Distances of muscle scar 'm' relative to frontoclypeal suture (f.s.). A, distant; B, close.

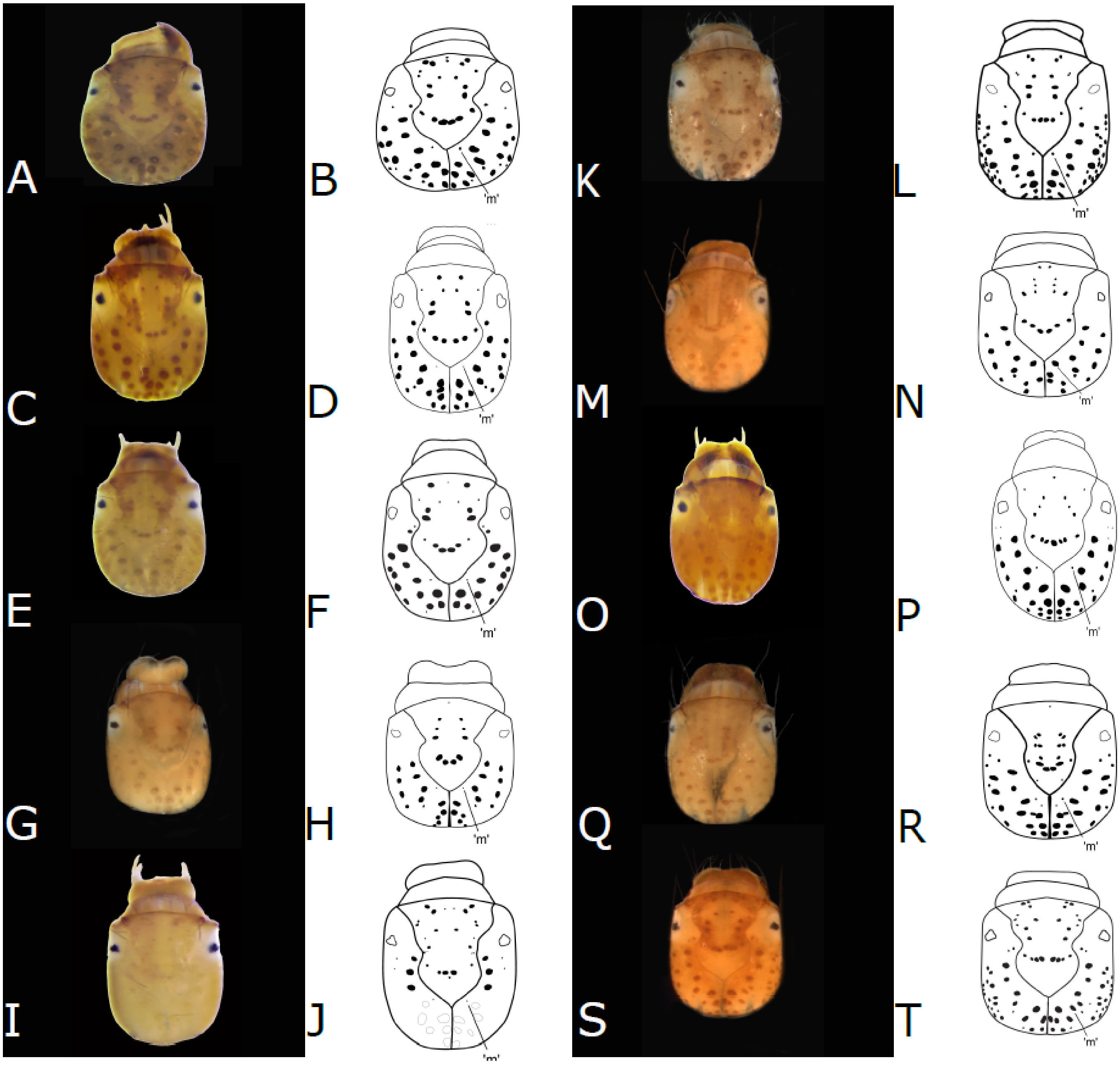

Figure 4-5. Head photographs (black background, dorsal view) and muscle scar maps (white background, dorsal view) of late-instar or final-instar larvae of associated members of the *Polycentropus colei* Species Group, where 'm' denotes the position of muscle scar 'm'.

A, B, *P. alabamensis* Hamilton, Harris and Lago, 1990; C, D, *P. blicklei* Ross and Yamamoto, 1965; E, F, *P. carlsoni* Morse, 1971; G, H, *P. carolinensis* Banks, 1905; I, J, *P. centralis* Banks, 1914; K, L, *P. confusus* Hagen, 1861; M, N, *P. elarus* Ross, 1944; O, P; *P. floridensis* Lago and Harris, 1983; Q, R, *P. maculatus* Banks, 1908; S, T, *P. pentus* Ross, 1941.

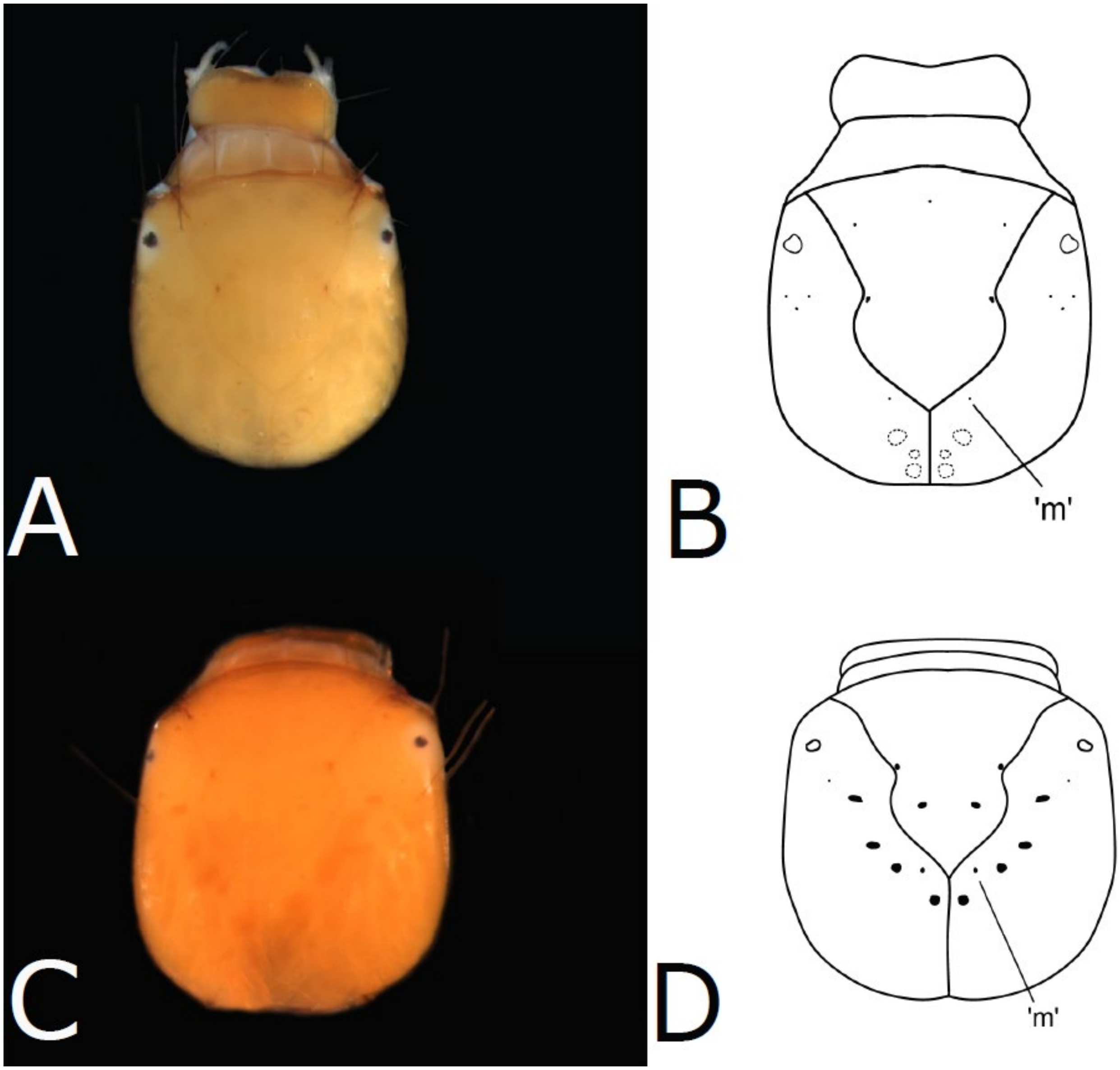

Figure 4-6. Head photographs (black background, dorsal view) and muscle scar maps (white background, dorsal view) of late-instar or final-instar larvae of associated members of the *Polycentropus colei* Species Group, where 'm' denotes the position of muscle scar 'm'.

A, B, *P. colei* Ross, 1941; C, D, *P. rickeri* Yamamoto, 1966.

CHAPTER 5

TAXONOMIC REVISION OF THE *Polycentropus confusus* SPECIES GROUP (TRICHOPTERA: POLYCENTROPODIDAE)

Biological taxonomy and systematics are necessary prerequisites for all of the biological sciences. While systematics brings together all that is known of species to glean their evolutionary relationships and predict yet-unknown traits, taxonomy serves as a framework for archiving and retrieving such information in the modern biological record. Taxonomic revisions serve a key role in synthesizing, updating, and summarizing our knowledge of particular groups of organisms, each manifest as a primary resource for those working on the group in question.

The overarching goal of this chapter is to revise the *Polycentropus confusus* Species Group. Like other polycentropodids, members of the genus *Polycentropus* Curtis, 1835, lack ocelli and have five maxillary palp segments in each sex. The genera of *Polycentropus sensu lato* (i.e., *Holocentropus* McLachlan, 1878, *Plectrocnemia* Stephens, 1836, and *Polycentropus*) are separable from other polycentropodid genera by exhibiting a 3-4-4 spur pattern (i.e., the presence of a preapical spur on the foretibia), the presence of fork I in the forewing (i.e., R2+3 branched), and the absence of fork III in the hind wing (i.e., M is two-branched).

The genus *Polycentropus* differs from allied polycentropodid genera of the *Polycentropus sensu lato* based on solely wing venation. Adults of *Polycentropus* have an open discal cell of the hind wing (i.e., discal or sectoral crossvein absent) and possess a cubito-anal crossvein of the hind wing, versus a closed discal cell and absence of a cubito-anal crossvein of the hind wing in *Plectrocnemia*. From *Holocentropus*, adults of *Polycentropus* are distinguished by the presence of fork I, an open discal cell, and typically the presence of a cubito-anal crossvein, all of the hind wing (e.g., Fig. 5-2; Roy et al., 1980; Schmid, 1980; Hamilton, 1986; Chamorro and Holzenthal, 2011).

While Hamilton (1986) revised several New World *Polycentropus* species groups, he did not revise the most speciose Nearctic species group. That group, the *Polycentropus confusus* Species Group (= *Polycentropus maculatus* Species Group *sensu* Ross, 1944), is one of five New World species groups and is native to the eastern Nearctic Region. These species groups are defined by male genitalic characters. In the case of the *Polycentropus confusus* Species Group, these are the long, thin, curved dorsal processes (d.p.) of the preanal appendages (pre. app.), the shorter, slightly curved intermediate appendages (int. app.), the membranous combined segments IX and X (IX+X), the erect dorsobasal process on the inferior appendages (inf. app.), and the relatively simplified phallus containing a phallic sclerite (ph. sc.) (e.g., Fig 5-3).

The first species of the *P. confusus* Species Group to be described was *Polycentropus confusus* by Hagen (1861). Today, 19 nominal species are recognized and were originally described by Bowles, Mathis, and Hamilton (1993; n=1), Hagen (1861; n=1), Hamilton and Holzenthal (1986; n=1), Lago and Harris (1983; n=1), Morse (1971; n=1), Orfinger and Etnier (2020; n=1), Orfinger and Moulton (2021; n=1), Hamilton, Harris, and Lago (1990; n=2), Ross and Yamamoto (1965; n=3), Banks (1905, 1908, 1914; n=3), and Ross (1941, 1944, 1947; n=4). The taxonomy of known larvae of the *P. confusus* Species Group was treated in the preceding chapter as a component of the treatment of all known eastern Nearctic *Polycentropus* larvae. While males are known from all species, females of only 12 species have been described to date (Hoffman and Morse, 1990; Bowles et al., 1993). Many of the existing descriptions are inadequate, and a synoptic treatment of the entire group is needed. In addition, no comprehensive keys exist. Several incomplete keys have been published (Milne 1936; Ross, 1941; 1944; Marshall, 1974; Marshall and Larson, 1982; Nimmo, 1986; Armitage and Hamilton, 1990). Of these partial keys, only Ross (1944) provides a key to females of the *P. confusus* Species Group.

Therefore, there is a clear need for a revision based on adults of the *P. confusus* Species Group. Here, the *P. confusus* Species Group is revised including redescriptions and illustrations of males of all species, redescriptions and illustrations of the 12 currently described females, original descriptions and illustrations of three additional females, designations of two neotypes, synoptic distribution and biological data and notes, and dichotomous keys to males and known females of the group.

Methodology

Specimens were observed under a Unitron Z10 stereomicroscope with magnifications up to 120x. Measurements were obtained using a calibrated ocular micrometer. Reported lengths were measured to the nearest 0.1 mm from the anterior of the head (excluding antennae) to the tip of the forewing. Association of newly described females was accomplished using mtDNA barcoding as described in Chapter 2. To evaluate internal morphology, genitalia were cleared with a heated solution of 85% lactic acid following Blahnik et al. (2007), then rinsed with ethanol. Some genitalia (e.g., of the holotypes) were previously cleared. When removed from ethanol-stored specimens, genitalia were viewed in glycerine, and then stored in a microvial within a vial of 80% ethanol with the rest of each respective specimen. Genitalia of pinned specimens were stored in microvials which were pinned through the rubber top to the corresponding specimen pin. The phallus was excised from some specimens for illustration. Line drawings were produced using a 10 x 10 mm 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. Habitus and genitalia photographs were taken with a Levenhuk M1400 Plus Digital Camera mounted to a Unitron Z10 and focus-stacked using Helicon Focus version 7.7.4. Genitalia photographs were useful in adding details during final illustration rendering. Morphological terminology follows Hamilton et al. (1990), Hoffman and Morse (1990), and Orfinger and Moulton (2021).

Institutional abbreviations where specimens examined for this study are housed are as follows: American Museum of Natural History, New York City, New York, U.S.A. (AMNH); Centre for Biodiversity Genomics, University of Guelph, Guelph, Ontario, Canada (BIOUG); Blue Ridge Parkway Insect Collection, Asheville, North Carolina, U.S.A. (BLRI); Monte L. Bean Life Science Museum at Brigham Young University, Provo, Utah, U.S.A. (BYU); Canadian Centre for DNA Barcoding, Biodiversity Institute of Ontario, University of Guelph, Guelph, Ontario, Canada (CCDB); Clemson University Arthropod Collection, Clemson, South Carolina, U.S.A. (CUAC); Cumberland Gap National Historical Park Insect Collection, Middlesboro, Kentucky, U.S.A. (CUGA); Etnier Caddisfly Collection of the University of Tennessee, Knoxville, Tennessee, U.S.A. (ECC); Florida A&M University portion of the Florida State Collection of Arthropods, Tallahassee, Florida, U.S.A. (FAMU); Florida State Collection of Arthropods, Gainesville, Florida, U.S.A. (FSCA); Illinois Natural History Survey, Champaign, Illinois, U.S.A. (INHS); Little River National Preserve, National Park Service, Fort Payne, Alabama, U.S.A. (LIRI); Mammoth Cave National Park, National Park Service, Mammoth Cave, Kentucky, U.S.A. (MACA); Museum of Comparative Zoology at Harvard University, Cambridge, Massachusetts, U.S.A. (MCZ); Royal Ontario Museum, Toronto, Ontario, Canada (ROME); University of Minnesota Insect Collection, St. Paul, Minnesota, U.S.A. (UMSP); United States National Museum Entomology Collection, Washington D.C., U.S.A. (USNMENT).

Taxonomic Results

Each species profile below is comprised of a taxonomic history, diagnoses of males and females when known, an adult general description, descriptions of male and female genitalia when known, notes of larval and pupal descriptions, biological data, distribution data, general notes when relevant, and a list of material examined. The taxonomic history (synonymy) of each species includes literature that treats the taxonomy of the given species and literature that includes any nomenclatural synonyms. When present with a figure number, the symbol "♂" or "♀" or a morphological term (e.g., larval head) denotes that the cited source illustrated the male or female, respectively, or other aspects of the morphology of the species. The presence of an asterisk (*) following a country, state, or province indicates a new country, state, or province record for the corresponding species.

The general habitus of all members of the *P. confusus* Species Group exhibits the following attributes: in ethanol, eyes brown to purple and glazed; dorsum of head, prothorax, mesothorax, and tegulae brown with tan to pale yellow setae; antennae, mouthparts, remainder of thorax, legs are brown to pale yellow; femora, tibiae, and tarsi have brown setae; abdominal sclerites tan. Wings are brown to light brown with scattered brown setae and clear-white to pale yellow maculation interspersed on forewings (e.g., Fig. 5-1); venation as in Figure 5-2.

Species Profiles

<u>*Polycentropus aileenae* Orfinger and Moulton</u>

(Figs 5-1; 5-3 — 5-4; Fig. 5-36)

Polycentropus aileenae Orfinger and Moulton, 2021: 355-359, fig. 1, habitus; fig. 2, ♂, fig. 3, ♀; eggs; type locality “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, [N36°02’56.2”, W84°04’07.6”]” (USNM).

Polycentropus carlsoni Morse, 1971—Moulton, 2007: 209-210.

Diagnosis: The male genitalia of *Polycentropus aileenae* are most similar to those of males of *P. blicklei*, *P. carlsoni*, *P. carolinensis*, and *P. elarus*. *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*, *P. aileenae* 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 round 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 round at the posterior apices versus the sharper posterior apices resulting in each having a blade-like appearance in *P. blicklei*. In *P. aileenae*, the posterior apices of the ventral plates are acute in lateral view, versus round in *P. blicklei*. In ventral view, the ventral plates are convergent along the entire length, versus convergent only beyond about ¾ length in *P. blicklei*.

Adult Description:

General (Fig. 5-1). Length of male 6.1—7.0 mm (mean = 6.6 mm; n = 5). Length of female 7.5—8.8 mm (mean = 8.1 mm; n = 2).

Male Genitalia (Figs 5-3A—5-3E). Abdominal segment VIII annular. Segment IX tergum fused with segment X (IX+X), membranous, caudally extended as short, horn-like projection (hl.p.IX+X). Segment IX sternum (s.IX) ovoid in lateral view, posterior margin slightly sinuous. Intermediate appendages (int.app.) 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. Body of preanal appendages (pre.app.) slightly produced, with small posteroventral lobate process; in lateral view dorsal process long, narrow, curved ventrad; in dorsal view slender, elongate, strongly inflected and converging apically. Inferior appendages (inf.app.) 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 tapered, inner margin curved outward; basodorsal process (d.p.) in lateral view erect, with long neck terminating in short, round head projecting downward, in ventral view completely hidden by main body of inferior appendage, head round with medial projection, in caudal acute, projecting downward. Phallus in lateral view curved ventrad, apical section rectangular, dorsum of apex slightly recurvate (subtle in some specimens), internal spinules absent, internal phallic sclerite (ph.sc.) moderately elongate.

Female Genitalia (Figs 5-4A—5-4B). Venter VIII in ventral view with ventral plates (v.pl.) narrow, tapering apically, convergent, and round at posterior apices in ventral view, covered in setae; in lateral view similar but each terminating in more acute posterad; external parts of gonopods VIII with posterior (p.ap.e.gon.VIII) margin slightly triangular and round mesally in ventral view; in lateral view extending beyond ventral plates in round 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 round 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.).

Larva: Unknown.

Pupa: Unknown.

Notes: This species has also been recorded in abundance from: Destiny Farm, State College, PA, small watercress-choked tributary to Slab Cabin Run, [N40°44’57.9”, W77°52’14.84”], 341 m. The material was lost before collection deposition and is not formally included in this description. However, the existence of this second population suggests a much wider distribution of *P. aileenae* than is currently reported based on deposited material. Additionally, a specimen collected in Exmoor, New Brunswick, Canada and housed at the University of Guelph Centre for Biodiversity Genomics, 08NBEPT-1627, is only 0.47% divergent at the barcoding region of COI than *P. aileenae* (A. Orfinger, unpublished data). This specimen likely represents *P. aileenae*, but it is missing its abdomen and therefore its identity cannot be confirmed. The eggs of this species were briefly described by Orfinger and Moulton (2021).

Biology: Little is known of the biology of *P. aileenae*. Adults of species have been collected from May to October near small (<4 m wide), stenothermic streams with emergent aquatic macrophytes, often watercress, and with rocky and sandy substrates.

Distribution (Fig. 5-36): Canada: Nova Scotia; USA:—Alabama*, Pennsylvania, Tennessee

Material Examined: **Holotype**: **USA**. **Tennessee**: Knox County, Fowler Spring Branch, first-order tributary to Beaver Creek, Bell Campground Rd., 0.3 km N of jct. with W. Brushy Valley Dr., UV Light Trap, 277 m, [N36°02'56.20”, W84°04'07.60"], A. Orfinger and A. Perilla coll., 6-vi-2020, 1 male (USNMENT01445176). **Allotype**: **USA**. **Tennessee**: Knox County, Fowler Spring Branch, first-order tributary to Beaver Creek, Bell Campground Rd., 0.3 km N of jct. with W. Brushy Valley Dr., UV Light Trap, 277 mm, [N36°02'56.20”, W84°04'07.60"], J.K. Moulton coll., 9-x-2005, 1 female (FAMU). **Paratypes**: **USA**. **Tennessee**: Jefferson County, Dumplin Creek at Hebron Church Rd., UV Light Trap, 337 masl, [N36°03'38.83", W83°27'20.64"], J.K. Moulton coll., 30-v-2006, 5 males (INHS Insect Collection 923903). 9-viii-2006, 5 males (FAMU). Dumplin Creek at old TN Rt. 92, 23-v-2005, 1 female (CUAC000107332). Tributary of Holston River at

Good Hope Church Road, 280 masl, [N36°09'58.28", W83°33'49.70"], G. Curler coll., 28-V-2005, 1 male (ROME187177). Knox County, Fowler Spring Branch, first-order tributary to Beaver Creek, Bell Campground Rd., 0.3 km N of jct. with W. Brushy Valley Dr., 277 mals, [N36°02'56.20", W84°04'07.60"], J.K. Moulton coll., 18-xi-2006, 1 male (CUAC000107331). 9-x-2005, 1 male (USNMENT01445177).

Non-type material: **Canada**. **Nova Scotia**: Cape Breton Highlands National Park, Clyburn Valley Road, near golf course, Malaise Trap, 21 masl, [N46°39'19.08", W60°25'42.60"], CBHNP Staff coll., 12-vii-2013, 1 male (BIOUG09994-A03). **USA**. **Alabama**: Fayette County, Wallace Branch at headwaters, [N33°37'44.76", W87°31'15.96"], A. Orfinger coll., 3-v-2021, **2 males** (FAMU) **New State Record**. Morgan County, Hughes Creek, B. Dinkins coll., 19-ix-2020, 1 male (FAMU) **New State Record**.

Polycentropus alabamensis Hamilton, Harris, and Lago

(Figs 5-5 — 5-6; Fig. 5-37)

Polycentropus alabamensis Hamilton, Harris, and Lago, 1990: 363—365, fig.1, ♂; type locality "United States: Alabama: Lawrence Bee Branch below falls, Bankhead National Forest" (USNM, lost).

Polycentropus new species 12—Hamilton, 1986: 70-71, fig. 4.2, ♂.

Polycentropus n. sp. (nr. *elarus*)—Lago and Harris, 1987b: 258.

Diagnosis: Males of this species is most similar to those of *P. elarus* in the apical narrowing of the inferior appendages, the shape basodorsal process of the inferior appendage, the similar shape of the preanal appendages, and the sinuate curvature of the dorsal process of the preanal appendages. *Polycentropus alabamensis* can readily be distinguished from *P. elarus* and all other members of the *P. confusus* Species Group by the following combination of characters: prominent, acute, posteroventral point on each preanal appendage, the apicodorsal emargination forming a caudal spur visible on the dorsobasal process of each preanal appendage, the tapered, incurved apex of the intermediate appendages, and the tapered, strongly incurved apex of the inferior appendages in ventral view.

Females of *P. alabamensis* are separated from those of all other species in the *P. confusus* Species Group by reniform wrinkled interior parts of gonopods VIII, by the posterior apex of external parts of gonopods VIII with a deep mesal emargination and appearing bifid in ventral view, and by the unique shape of segment IX bearing bilateral acute, horn-like processes.

Adult Description:

General Length of male 6.9—7.8 mm (mean = 7.3 mm; n = 5). Length of female 8.2—9.0 mm (mean = 8.5 mm; n = 3).

Male Genitalia (Figs 5-5A—5-5E). Abdominal segment VIII annular. Abdominal sternite IX large, semicircular in lateral view, anterior margin round, posterior margin nearly straight; in ventral anterior margin deeply emarginate. Intermediate appendages originating beneath terga IX+X and extending beyond them, curved gradually caudoventrad, their apices each bearing 5 small setae; distally extending beyond tergum X, apices each bearing 5 small setae; in lateral view curved slightly ventrad; in dorsal view apices proximate, subparallel. Body of preanal appendages short, with prominent sharp point, posteroventral in lateral view; in lateral view dorsal process moderately sinuate, decurved basally, distally, with apicodorsal emargination forming caudal spur; in dorsal view slender, elongate, moderately sinuate, subparallel. Inferior appendages in lateral view each with elongate ventral portions, in lateral view tapering slightly to apex, extending to apex of intermediate appendage, in ventral view greatly tapered beyond mid-length with apex acute, strongly curved mesally; basodorsal process prominent, erect, in lateral view neck long and thin, enlarged apex goose-head shaped with acute ventrally directed point, in caudal view blunt projection projecting downward. Phallus in lateral view tubular, moderately decurved, broadened apically, basally, with mesoventral concavity, internal spinules absent; internal phallic sclerite in lateral aspect moderately long, enlarged slightly distally, with tapered anterior portion.

Female Genitalia (Figs 5-6A—5-6B). Venter VIII in ventral view with ventral plates short, subparallel to subtly divergent, semi-elliptical, tapered, laterally recurved, covered by setae; in lateral view each terminating in round posterior apex; posterior apex of external parts of gonopods VIII in ventral view with posterior margin round with two apical lobes separated by deep mesal emargination; in lateral view extending posterad beyond ventral plates in round process; segment IX in ventral view with diagonal mesolateral emarginations forming acute horn-like processes, in lateral view produced caudodorsad, evenly tapered to acute apex extending beyond ventral plates; internal parts of gonopods VIII in ventral view visible through venter VIII even in uncleared specimen, appearing longitudinally wrinkled, darker than surrounding tissue, subparallel to subtly divergent, reniform, with acute posterior apices distinctly anterior of apices of ventral plates, caudolateral margins sinuous; in ventral view anterior part of genital chamber sclerotized, subtrapezoidal, base overlapping with sclerotized margin of segment IX; processus spermathecae subovoid with central elevation bearing opening of ductus spermathecae.

Larva: Described in Chapter 4.

Pupa: Unknown.

Notes: The type series is lost. Specimen USNMENT01819507 is herein designated as the neotype for *Polycentropus alabamensis*.

Biology: This species is most commonly collected in small streams of the Cumberland Plateau physiographic region and occasionally from the Coastal Plain physiographic region. Adults have been collected using UV light traps from April to September and late-instar larvae take from cool, clear, flowing streams with rocky substrate in April.

Distribution (Fig. 5-37): USA: Alabama.

Material Examined: **Neotype**: **USA**. **Alabama**: Fayette County, Little Tyro Creek, [N33°35'20.76", W87°29'47.04"], A. Orfinger coll., 3-v-2021, 1 male (USNMENT01819507). Non-type material: **USA**. **Alabama**: Bibb County, Rocky Branch, S.C. Harris and S. Hamilton coll., 12-vi-1991, 1 male (FAMU). Rocky Branch at USGS Gage, 1 male (FAMU). Fayette County, Headwaters of Wallace Branch, UV Light, [N33°38'16.80", W87°31'40.80"], A. Orfinger coll., 4-iv-2022, 5 males (FAMU), 3 females (FAMU). Wallace Branch at headwaters, [N33°37'44.76", W87°31'15.96"], 3-v-2021, 3 males (FAMU). Franklin County, Dismal Wonders Garden, Dismal Branch, S. Harris coll., 25-vi-1983, 1 male (FAMU). Morgan County, Hughes Creek, Light Trap, [N34°24'38', W86°37'07'], B. Dinkins coll., 19-ix-2020, 2 males (FAMU). 2 males (FAMU). Spring outside of Curry Cave, 6-7-iv-2020, 2 larvae (FAMU). Winston County, Bankhead National Forest, small tributary of West Sipsey Fork, west side of river 100 m downstream of CR-60 bridge, 9.5 miles north of Double Springs, UV-blacklight pan trap, A.K. Rasmussen coll., 24-v-2003, 1 male (FAMU).

Polycentropus blicklei Ross and Yamamoto

(Figs 5-7 — 5-8; Fig. 5-38)

Polycentropus blicklei Ross and Yamamoto, 1965: 243, fig. 5, ♂; type locality "Plymouth, New Hampshire" (INHS).

Polycentropus blicklei—Marshall and Larson, 1982: 22—24, fig. 49, ♂.

Polycentropus blicklei—Nimmo, 1986: 195, figs 111—114, ♂; figs 115—116, ♀.

Polycentropus blicklei—Armitage and Hamilton, 1990: figs A—B, D, ♂; figs E —F, ♀.

Polycentropus blicklei—Hoffman and Morse, 1990, 276-277, figs 1—4, ♀.

Diagnosis: Males of *Polycentropus blicklei* are most similar to those of *P. aileenae*, *P. chenoides*, *P. elarus*, and *P. maculatus*. From females of *P. aileenae*, those of *P. blicklei* are identified by the only slightly incurvate apices of the dorsal processes of the body of the preanal appendage, versus the strongly inflected dorsal processes of the bodies of the preanal appendages in *P. aileenae*. In ventral view, the ventral portions of the inferior appendages are evenly tapered in *P. blicklei*, versus having basal half wide and distal half abruptly tapered and inner margin curved outward in *P. aileenae* and *P. elarus*. Males of *P. blicklei* differ from *P. chenoides* by the erect dorsobasal processes of the inferior appendage and the slight ventral curvature of the intermediate appendages, versus the deflexed dorsobasal processes of the inferior appendages and the intermediate appendages curved strongly dorsad in *P. chenoides*. From *P. maculatus*, *P. blicklei* is readily separated by the shape of its ventral portions of the inferior appendage in ventral view evenly tapered to a round apex versus the blade-like shape seen in *P. maculatus*, and the phallus curved only slightly ventrad beyond mid-length in *P. blicklei*, versus curved roughly 90° ventrad beyond mid-length in *P. maculatus*.

Females of *P. blicklei* are most similar to those of *P. aileenae*. The internal parts of gonopods VIII are acute at the posterior apices and blade-like in *P. blicklei*, versus round at the apices and semi-elliptical in *P. aileenae*. In addition, the posterior apices of the ventral plates of *P. blicklei* are round in lateral view, versus acute in *P. aileenae*. In ventral view, the ventral plates are convergent only beyond about ¾ length in *P. blicklei*, versus those of *P. blicklei* which are convergent along the length of the ventral plates.

Adult Description:

General Length of male 5.5—7.1 mm (mean = 6.5 mm; n = 9). Length of female 5.8—9.0 mm (mean = 7.7 mm; n = 5).

Male Genitalia (Figs 5-7A—5-7E). Abdominal segment VIII annular. Terga IX and X fused, membranous, extended caudad over bases of intermediate appendages. Sternum IX ovoid in lateral view, posterior margin slightly sinuous. Intermediate appendages originating beneath terga IX+X and extending beyond them, curved slightly ventrad, their apices each bearing 4 small setae; in dorsal view apices proximate, parallel. Bodies of preanal appendages each with triangular posteroventral process with sinuous margins; dorsal process long, curved ventrad, variably terminating above corresponding posteroventral process of body of preanal appendage to extending to head of basodorsal process of corresponding inferior appendage, acute apically; in dorsal view slender, elongate, sinuous, subparallel, incurvate apically. Inferior appendages in lateral view each with capitate basodorsal process erect with moderately long neck and subtriangular head round dorsally, projecting ventrad with anteroventral margin rugose, apex pointed, visible above main body of appendage, main body of appendage with dorsal and ventral margins subparallel along length, subacuminate, apex extended posterad beyond intermediate appendages; in ventral view subparallel, wide basally, distally tapering to round apex, basodorsal process partially hidden by main body of inferior appendage, lateral margins extending beyond lateral margins of each main body of appendage, in cleared specimen broad, with medial pointed projection; in caudal view with wide base and oblong, round medial projection. Phallus a fully sclerotized tube, in lateral view with larger base, curved slightly ventrad beyond mid-length, apical section subrectangular, internal spinules absent, internal phallic sclerite short, oriented dorsoposterad, with round ventral base and tapered, acute dorsal vertex.

Female Genitalia (Figs 5-8A—5-8B). Venter VIII in ventral view with ventral plates subparallel for ¾ length, then convergent for final ¼ length, base broad tapering beyond ¾ length to round apex, covered by setae; in lateral view each terminating in round posterior apex; posterior apex of external parts of gonopods VIII in ventral view with posterior margin subtriangular and round mesally; in lateral view extending posterad beyond ventral plates in round process; internal parts of gonopods VIII in ventral view visible through venter VIII even in uncleared specimen, appearing longitudinally wrinkled, darker than surrounding tissue, subparallel, blade-like, with acute posterior apices distinctly anterior of apices of ventral plates, lateral margins incurvate beyond mid-length; in ventral view anterior part of genital chamber sclerotized, semicircular; processus spermathecae subovoid with central elevation bearing opening of ductus spermathecae.

Larva: Described in Chapter 4.

Pupa: Unknown.

Notes: The figure labeled as depicting the dorsal view of the male genitalia of *P. blicklei* included in the species treatment of *P. blicklei* by Armitage and Hamilton (1990) (Figure C) is incorrect. In fact, the figure is the dorsal view of the male genitalia of *Polycentropus barri* Ross and Yamamoto, 1965, reproduced from Ross and Yamamoto (1965).

Biology: Nimmo (1986) states that this species is found in both standing and flowing waters. I have observed no evidence of lentic examples of the species, and it appears that *P. blicklei* occurs only in flowing, relatively cool waters of various substrates including cobble and sand with benthic deadwood. The single male taken from Illinois was collected adjacent to a wetland with both flowing and still waters, and close to the Vermillion River. It seems most likely based on the record data that the specimen emerged from one of the many lotic habitats in the area. Adults have been collected from March to September, emerging earliest at lower latitudes. The only known larvae are late-instar larvae collected in Maryland in early April.

Distribution (Fig. 5-38): Canada: New Brunswick, Nova Scotia, Newfoundland and Labrador, Ontario, Quebec; USA: Alabama, Delaware, Florida, Georgia, Illinois*, Kentucky, Maine, Maryland, Mississippi, North Carolina, New Hampshire, New Jersey, New York, Ohio, Pennsylvania, Rhode Island, South Carolina, Tennessee, Virginia.

There are several instances of states without records of *P. blicklei* despite being partially or completely surrounded by states or provinces with verified records of the species. These are Connecticut, Indiana, Massachusetts, Michigan, Vermont, West Virginia. It is possible that *P. blicklei* will be found from many or all of these areas, even those that have been well-studied such as Michigan. For example, Ross and Yamamoto (1965) described *P. blicklei* and recorded it from several states. Despite this, it is only from the present study that it has been reported from Illinois, a well-studied state in which both Ross and Yamamoto were based and surveyed extensively. These new record data raise the possibility that improved environmental conditions e.g., resulting from the Clean Water Act and other legislation, have allowed the arrival or return of *P. blicklei* in Illinois. Alternatively, this species may have just been missed in previous surveys, or, less likely, could have arrived by other means (e.g, human-mediated translocation).

Ross and Yamamoto (1965) hypothesized based on the records available at the time that this is a predominantly northeastern species, despite the single paratype specimen recorded from Wall Doxey State Park in northern Mississippi. The authors suggested that this specimen represents a relict population that may have reached the area during cooler periods of the Pleistocene, as evidenced by the cool, fast-flowing stream from which the specimen was taken. Based on the numerous records now available and as far south as Alachua County in north-central Florida, it is evident that this widespread species is an eastern species and is as common in the southeast as in the northeast Nearctic Region.

Material Examined: **Holotype**: **USA**. **New Hampshire**: Grafton County, Plymouth, R.L. Blickle and W.J. Morse coll., 6-viii-1947, 1 male (INHS Insect Collection 38134).

Paratypes: **USA**. **Mississippi**: Marshall County, Holly Springs, Wall Doxey State Park, At Light, Ross and Stannard coll., 21-v-1957, 1 male (INHS Insect Collection 38133). New Hampshire: Strafford County, Lee, W.J. Morse coll., 7-vii-1951, 1 male (INHS Trichoptera 24563).

Non-type material: **Canada**. **New Brunswick**: Exmoor, [N46°58'15.60", W65°51'10.80"], KSH and RB coll., 25-vi-2008, 1 female (08NBEPT-1611). **Ontario**: Grey County, Fay Jensen Farm, Grass field behind barn, UV Light at Sheet, 343 masl, [N44°22'48.00", W80°34'48.00"], J.E. Cossey, N.W. Jeffery, and J.R. Straka coll., 21-vi-2008, 1 female (08ONCAD-0227). **USA**. **Alabama**: Bibb County, Rocky Branch, S. Harris and S. Hamilton coll., 12-vi-1991, 1 male (FAMU). Franklin County, Dismal Wonders Garden, Dismal Branch, UV Light, S.C Harris coll., 25-vi-1983, 1 male (FAMU). Morgan County, Hughes Creek, Light Trap, [N34°24'38', W86°37'07'], B. Dinkins coll., 19-ix-2020, 1 male (FAMU). **Illinois**: LaSalle County, Wetlands South of Iron Bridge, Lone Star Zone, M.A. Repiscak coll., 22-v-2021, 1 male (INHS Insect Collection 932314) **New State Record**. **Maryland**: Anne Arundel County, SR-01-18, Sewell Spring Branch, [N39°04'34.93", W76°37'05.88"], M. Cole coll., 3-iv-2018, 3 larvae (Cole Ecological, Inc. 7670). **Mississippi**: George County, 3 miles north of Lucedale, R. Kergosien coll., 19-31-iii-1996, 1 male (USNMENT01507913), 1 male (USNMENT01507912). 9-14-iv-1996, 1 female (USNMENT01507916), 1 female (USNMENT01507915), 1 male (USNMENT01507914). **New Jersey**: Burlington County, Brendon T Burns State Forest, Mt Misery, [N39°54'58.09", W74°30'52.69"], S. Harris coll., 03-vii-2012, 1 male (FAMU).

Mt. Misery area, Mt. Misery Brook, 6.5 km East jct. Highway 70 and Highway 72, Malaise Trap, 38 masl, [N39°55'11”, W74°31'24”], J. Gelhaus coll., v-30-vi-13-1993, 4 males (FAMU). **New York**: Franklin County, Dutton Brook, Route 3 near Saranac Lake, L. Myers and B.C. Kondratieff coll., 27-vi-2007, 1 male (BYU). **North Carolina**: Transylvania County, S. Mills R. Tributary, US 376 at FS 1206, JLR coll., 12-vi-2008, 1 male (ECC 3.516). **Pennsylvania**: Upper Hedgehog Creek, S. Harris coll., vi-15-2010, 1 male (FAMU). **Tennessee**: Jefferson County, Dumplin Creek at old TN Rt. 92, UV Light Trap, [N36°03'38.83”, W83°27'20.64”], J.K. Moulton coll., 26-v-2006, 1 female (FAMU). Knox County, Fowler Spring Branch, first-order tributary to Beaver Creek, Bell Campground Rd., 0.3 km N of jct. with W. Brushy Valley Dr., 277 masl, [N36°02'56.20”, W84°04'07.60”], A. Orfinger and A. Perilla coll., 6-VI-2020, 2 males (FAMU). J.K. Moulton and A. Orfinger coll., 10-IV-2019, 1 male (FAMU).

Polycentropus carlsoni Morse

(Figs 5-9 — 5-10; Fig. 5-39)

Polycentropus carlsoni Morse, 1971: 78, fig. 3, ♂; type locality “Wildcat Creek, Clemson, S.C." (INHS).

Polycentropus carlsoni—Armitage and Hamilton, 1990: figs A—D, ♂.

Polycentropus carlsoni—Hoffman and Morse, 1990: 277-278, figs 5—9, ♀.

Diagnosis: Males of *P. carlsoni* are most similar to those of *P. aileenae*, *P. blicklei*, *P. carolinensis*, and *P. maculatus*. From males of these species, males of *P. carlsoni* are identified by the shorter, stockier, and deflexed basodorsal process of each inferior appendages, the slightly ventrally recurved apex of the ventral portions of each inferior appendages, and the shape of the body of each preanal appendage which in *P. carlsoni* is round and lacking any posteroventral process.

Females of *P. carlsoni* are most similar to those of *P. aileenae*, *P. blicklei*, and *P. maculatus*. Unlike the blade-like internal parts of gonopods VIII of *P. aileenae*, those of *P. carlsoni* are subrectangular. From *P. maculatus*, females of *P. carlsoni* are separated by the kukri blade-like shape of the ventral plates in ventral view that terminate beyond the internal parts of gonopods VIII, versus the subtriangular ventral plates that terminate anterior to the internal parts of gonopods VIII.

Adult Description:

General Length of male 5.2—6.9 mm (mean = 6.1 mm; n = 5). Length of female 6.1—8.1 mm (mean = 6.9 mm; n = 5).

Male Genitalia (Figs 5-9A—5-9E). Abdominal segment VIII annular. Terga IX and X fused, membranous, extended caudad over bases of intermediate appendages. Sternum IX subovoid in lateral view. Intermediate appendages originating beneath terga IX+X and extending beyond them, curved slightly ventrad, their apices each bearing 4 small setae; in dorsal view apices proximate, subparallel. Bodies of preanal appendages each round, lacking posteroventral process; dorsal process long, curved ventrad, extending to apex of ventral portion of inferior appendage, acute apically; in dorsal view slender, elongate, subparallel. Inferior appendages in lateral view each with capitate basodorsal process deflexed with short neck and stocky subtriangular head round dorsally and projecting ventrad with its pointed apex occasionally visible above main body of appendage, occasionally hidden behind main body of appendage, main body of appendage tapering evenly along length, terminating in round, slightly dorsally recurved apex, terminating at approximately apex of dorsal process of preanal appendage; in ventral view tapering gradually along length, round apices inflected mesally, basodorsal process partially hidden by main body of inferior appendage, with only mesal margins exposed, in cleared specimen broad, with medial pointed projection; in caudal view oblong, with medial projection round. Phallus a fully sclerotized tube, in lateral view with large base, curved slightly ventrad slightly beyond mid-length, apical section subrectangular, internal spinules absent, internal phallic sclerite short, oriented posteroventrally, with acute anterior broadening to a subrectangular posterior apex.

Female Genitalia (Figs 5-10A—5-10B). Venter VIII in ventral view with ventral plates convergent, narrow, blade-like as a kukri sword, tapering apically, covered by setae; in lateral view each terminating in round posterior apex; posterior apex of external parts of gonopods VIII in ventral view with posterior margin mesally produced and round; in lateral view not extending beyond ventral plates in round process; internal parts of gonopods VIII in ventral view visible through venter VIII even in uncleared specimen, appearing longitudinally wrinkled, darker than surrounding tissue, subparallel, subrectangular, with apices distinctly anterior of apices of ventral plates; in ventral view anterior part of genital chamber sclerotized, subovate; processus spermathecae ovoid with central elevation bearing opening of ductus spermathecae.

Larva: Described in Chapter 4.

Pupa: Unknown.

Notes: Moulton's 2007 record of *P. carlsoni* from Tennessee is erroneous and was based on individuals (1 male, 1 female) of *P. aileenae*. In addition, though the holotype was initially deposited at the University of Georgia Collection of Arthropods, it was transferred to the Illinois Natural History Survey where it is currently housed.

Biology: This species is found at first- or second-order cool streams with rocky substrates. Adults of *P. carlsoni* have been collected from April to November while the only known, late-instar larva was collected in October. As noted by others (e.g., Moulton, 2007), collections of this apparently uncommon species are generally comprised of fewer than five individuals. Unlike most *Polycentropus* species, adults of *P. carlsoni* have been collected most frequently using malaise traps or modified emergence traps, while only occasionally being taken in UV light traps. This suggests that perhaps this species flies earlier in the evening than other species or during the day under shaded riparian habitats with dense canopies.

Distribution (Fig. 5-39): USA: Alabama, Maryland, North Carolina, Pennsylvania, South Carolina, Virginia.

Given the known distribution of *P. carlsoni*, it would be unsurprising if it were eventually reported from northern Georgia, southern or eastern Tennessee, southern or eastern Kentucky, and West Virginia.

Material Examined: **Holotype**: **USA**. **South Carolina**: Pickens County, Wildcat Creek, [N34°45'34.20", W82°51'03.24"], P. Carlson coll., 23-iv-1968, 1 male (left inferior appendage broken; INHS Trichoptera 24565). **Paratype**: **USA**. **South Carolina**: Pickens County, Wildcat Creek, [N34°45'34.20", W82°51'03.24"], P. Carlson coll., 23-iv-1968, 1 male (INHS Trichoptera 24564).

Non-type material: **USA**. **Pennsylvania**: Philadelphia County, Tributary of Wissahickon Creek, at seep crossing trail, [N40°02'38.04", W75°12'55.80"], N. Macelko coll., 24-X-

2021, 1 larva (FAMU). **South Carolina**: Pickens County, Clemson, Wildcat Creek, P. Carlson coll., 12-v-1958, 1 female (CUAC0000107319). 15-iv-1968, 1 female (CUAC0000107323). 20-iv-1968, 1 male (CUAC0000107317). 20-iv-1969, 1 male (CUAC0000107316). 24-iv-1968, 1 female (CUAC0000107320). Issaqueena Forest, N. Fork of Creek at Holly Springs Picnic Area, Malaise Trap, 730 masl, K.M. Hoffman coll., 12-20-vi-1988, 3 males (CUAC0000107315). 1 female (CUAC0000107321). 1 female (CUAC0000107322). Wildcat Creek, 770 masl, 27-vi-4-vii-1988, 1 female (CUAC0000107318). **Virginia**: Fairfax County, Gulch Stream, O.S. Flint coll., vi-2008, 13 males, 27 females (CUAC000107325).

Polycentropus carolinensis Banks

(Figs 5-11 — 5-12; Fig. 5-40)

Polycentropus carolinensis Banks, 1905: 217, ♂; type locality "Black Mountains, North Carolina" (AMNH).

Polycentropus carolinensis—Betten, 1934: 220-221.

Polycentropus carolinensis—Milne, 1936: 88 (as junior synonym of *P. confusus* Hagen, 1861)

Polycentropus carolinensis—Nimmo, 1986: 195, figs 117—121, ♂.

Polycentropus carolinensis—Armitage and Hamilton, 1990: figs A—D, ♂.

Diagnosis: Males of *Polycentropus carolinensis* are most similar to those of *P. aileenae*, *P. carlsoni*, and *P. maculatus*. Males of *P. carolinensis* are separated from those of these similar species by the smaller, rounder, head of the basodorsal process of the inferior appendage which appears thumb-shaped laterally. Additionally, the bodies of the preanal appendages in *P. carolinensis* are produced posterad far beyond those of

P. carlsoni and exhibit a posteroventral corner and slightly emarginate ventral margin not seen in *P. carlsoni* but do not possess the posteroventral processes seen in *P. aileenae* and *P. maculatus*.

Females of *P. carolinensis* are most similar to those of *P. aileenae*, *P. blicklei*, *P. carlsoni*, and *P. maculatus*. Females of *P. carolinensis* can be separated from females of the above-mentioned species in ventral view by the narrow, oblong, subparallel ventral plates and semi-trapezoidal anterior part of the genital chamber.

Adult Description:

General Length of male 5.2—7.2 mm (mean = 6.1 mm; n = 5). Length of female 6.1—8.8 mm (mean = 7.3 mm; n = 4).

Male Genitalia (Figs 5-11A—5-11E). Abdominal segment VIII annular. Terga IX and X fused, membranous, extended caudad over bases of intermediate appendages. Sternum IX subovoid in lateral view, posterior margin nearly straight. Intermediate appendages originating beneath terga IX+X and extending beyond them, curved slightly ventrad, their apices each bearing 3 small setae; in dorsal view apices proximate, subparallel. Bodies of preanal appendages each produced posterad, round dorsally with posteroventral corner and slightly emarginate ventral margin; dorsal process long, curved ventrad, extending level to basodorsal process of corresponding inferior appendage, acute apically; in dorsal view slender, short, slightly convergent, bases appearing recurved slightly laterad. Inferior appendages in lateral view each with thumb-like basodorsal process erect with moderately long neck and small head round dorsally and projecting ventrad with its blunt apex visible above main body of appendage, main body of appendage with dorsal and ventral margins tapering evenly along length to dorsally upturned apex extended posterad slightly beyond intermediate appendages; in ventral view subparallel, basal 1/3 wide, distal 2/3 abruptly tapered, inner margin concave, apices inflected mesally, basodorsal process completely hidden by main body of inferior appendage, in cleared specimen small, with medial pointed projection; in caudal view oblong, with medial projection round. Phallus a fully sclerotized tube, in lateral view with larger base, curved about 25° ventrad slightly beyond mid-length, apical section subrectangular, internal spinules absent, internal phallic sclerite short, oriented dorsoposterad, thumb-like with pointed base and round apex curved dorsad.

Female Genitalia (Figs 5-12A—5-12B). Venter VIII in ventral view with ventral plates subparallel, narrow, oblong, tapering basally and apically, covered by setae; in lateral view each terminating in round posterior apex oriented posteroventrad; posterior apex of external parts of gonopods VIII in ventral view with posterior margin nearly linear; in lateral view extending posterodorsad above ventral plates in tapered process with its apex acute; internal parts of gonopods VIII in ventral view visible through venter VIII even in uncleared specimen, appearing longitudinally wrinkled, darker than surrounding tissue, convergent, semi-elliptical, with round posterior apices distinctly anterior of apices of ventral plates, anterior ends round, extending to sclerotized external part of gonopods VIII at basal third of ventral plates; in ventral view anterior part of genital chamber sclerotized, semi-trapezoidal, wrapping around posterior apex of processus spermathecae; processus spermathecae ovoid with central elevation bearing opening of ductus spermathecae.

Larva: Described in Chapter 4.

Pupa: Unknown.

Notes: Ross (1944) speculated that three female specimens from Illinois represented either an undescribed species or the female of *P. carolinensis*. Ross termed these specimens, listed as being housed at the Illinois Natural History Survey but unable to be located, "*Polycentropus* species a" and provided a description and a ventral genitalic illustration (Ross, 1944). Unzicker et al. (1970) reported a specimen of the same species from Big Clifty, Carrol County, Arkansas, which could also not be located. The positive association and description of the female of *P. carolinensis* in the present study indicates that "*Polycentropus* species a" does not represent *P. carolinensis*, which has yet to be recorded from Illinois. The identity of these specimens remains unresolved.

Biology: Adults of *P. carolinensis* have been collected from May to July. Late-instar larvae have been collected from May to August and the few pupae have all been collected in May. This somewhat uncommon species is found in seeps and first- and second-order streams, usually at elevations above 1000 masl. The pupal shelter of *P. carolinensis* is constructed from plant material including sticks and leaves.

Distribution (Fig. 5-40): Canada: Ontario, Quebec; USA: Kentucky, Mississippi, North Carolina, New Hampshire, Ohio, Pennsylvania, Tennessee, Vermont, Virginia, West Virginia.

Nimmo (1986) indicated the fragmentary nature of the known distribution of *P. carolinensis* at the time. Though several new state and province records have since been reported, the known distribution remains patchy. There are several areas in the Midwest (e.g., southern and eastern Indiana), northeast (e.g., Maryland, New York), and in the southeast (e.g., northern Alabama, northern Georgia, and northwestern South

Carolina) where this species may be found. It will most likely be recovered in or around low-order streams at the highest elevations of these areas.

Material Examined: **Holotype**: **USA**. **North Carolina**: Black Mts. 26-v, N. Banks coll., 1 male (AMNH).

Non-type material: **USA**. **North Carolina**: Buncombe County, Seep uphill of Blue Ridge Parkway, By Hand, 1225 masl, J. Robinson and C.R. Parker coll., 2-v-2007, 1 pupa (BLRI 23487). Jackson County, Balsam Mtn. Preserve, 6.6 km E. Sylva, S. Fork Sugarloaf Creek, Malaise-Townes Trap, 1180 masl, [N35°22'17.04", W83°06'37.08"], J.C. Morse coll., 6-13-vii-2013, 3 males, 4 females (CUAC0000107304). Balsam Mtn. Preserve, 6.6 km E. Sylva, S. Fork Sugarloaf Creek, Malaise-Townes Trap, 1180 masl, [N35°22'17.04", W83°06'37.08"], J.C. Morse coll., 6-22-vii-2019, 1 male (CUAC000093253). Swain County, Beetree Ridge. GSM, K. Watson coll., 7-vi-1989, 2 males (one specimen missing genitalia), (ECC 3.264). Transylvania County, Devil`s Courthouse at Blue Ridge Parkway, J.K. Moulton coll., 17-viii-2005, 3 larvae (FAMU). Watauga County, Spring seep below spring house, Moses H. Cone Estate, 1146 masl, J. Robinson and C.R. Parker coll., 29-iii-2007, 5 larvae (BLRI 21955). **Tennessee**: Blount County, Great Smoky Mountains National Park, Cades Cove, Wildcat Branch of Abrams Creek, D. Etnier coll., 16-18-vii-2017, 1 male (ECC 3.735). Fentress County, Cave Creek, Tributary of Pogue Creek, at end of ATV road off Williams Cr. Rd, 0.5 km east of Delk Cr. Rd., D. Etnier et al. coll., 21-v-2005, 1 metamorphotype male pupa, 2 larvae (ECC 3.431). Sevier County, Great Smoky Mountains National Park, Twin Creeks Research Center, Cherokee, Orchard Rd., Malaise Trap, A.J. Mayor et al. coll., 2-vii-2004, 1 male (ECC 3.760). **Virginia**: Augusta County, Spring at Dripping Rock pullout at MP 009.7, left, By Hand, J. Robinson and C.R. Parker coll., 17-vii-2007, 1 larva (BLRI 25619). Bedford County, outflow Abbot Lake in Peaks of Otter Picnic Area, upstream entrance road, 730 masl, M. Geraghty and C.R. Parker coll., 9-vi-2006, 3 males (BLRI 22764). Grayson County, Rivulet at Meadow Beach Rd. , J.K. Moulton coll., 25-v-2005, 1 male (FAMU).

Polycentropus centralis Banks

(Figs 5-13 — 5-14; Fig. 5-41)

Polycentropus centralis Banks, 1914: 258, fig. 67, ♂; type locality "St. Louis, Mo." (MCZ).

Polycentropus centralis—Milne, 1936: 85.

Polycentropus centralis—Ross, 1944: 64-65, fig. 231, larval head and pronotum; fig. 248, ♂; fig. 253, ♀.

Polycentropus centralis—Marshall and Larson, 1982: 24, figs 15—17, protibia, wings, mesonotum; fig. 47, ♂.

Polycentropus centralis—Nimmo, 1986: 196, figs 127—130, ♂; figs 131—132, ♀.

Polycentropus centralis—Armitage and Hamilton, 1990: figs A—D, ♂; fig. E ♀.

Polycentropus centralis—Moulton and Stewart, 1996: fig. 538, wings; figs 556, 564-565, ♂.

Polycentropus centralis—Houghton, 2012: fig. 266, ♂.

Diagnosis: Both sexes of this species are distinct and readily identifiable. Males of *P. centralis* can be separated from those of all members of the *P. confusus* Species Group by the broad, stocky base of the inferior appendage. It can further be distinguished from males of all other species other than *P. pixi* by the nearly straight phallus. From males of *P. pixi*, males of *P. centralis* additionally differs in the triangular head of the basodorsal process of the inferior appendage versus the rounded head seen in *P. pixi*.

Females of *P. centralis* are separated from those of all other members of the *P. confusus* Species Group by the smooth, lanceolate internal parts of gonopods VIII in ventral view and the ventral plates which are of uniform depth for ¾ length, beyond which they taper abruptly to a round apex in lateral view.

Adult Description:

General Length of male 5.3—7.1 mm (mean = 6.6 mm; n = 5). Length of female 6.1—8.9 mm (mean = 7.2 mm; n = 5).

Male Genitalia (Figs 5-13A—5-13G). Abdominal segment VIII annular. Terga IX and X fused, membranous, extended caudad over bases of intermediate appendages. Sternum IX subovoid in lateral view, posterior margin subtriangular in some specimens. Intermediate appendages originating beneath terga IX+X and extending beyond them, oriented subtly ventrad, their apices each bearing 5 small setae; in dorsal view apices proximate, subparallel. Bodies of preanal appendages each emarginate dorsally, produced porsterad in subtriangular process with additional round posteroventral process, or rarely round, produced, lacking round posteroventral process; dorsal process long, curved ventrad, extending above head of basodorsal process of corresponding inferior appendage, acute apically; in dorsal view slender, elongate, nearly straight to curved subtly laterad until mid-length, curved slightly mesad beyond mid-length. Inferior appendages in lateral view each with broad stocky base, capitate basodorsal process erect with narrow neck and triangular head round dorsally and projecting ventrad with its pointed apex visible above main body of appendage, main body of appendage nearly straight, with stocky base, dorsal margin curved strongly ventrad to about mid-length length, ventral margin oriented slightly ventrad to about mid-length, with dorsal and ventral margins subparallel beyond mid-length to round apex extended posterad even with apices of intermediate appendages; in ventral view parallel, wide basally, distally tapering to round apex, lateral margins of basodorsal process usually visible beyond lateral margins of main body of inferior appendage, remaining basodorsal process hidden, in cleared specimen broad, with medial pointed projection; in caudal view oblong, with medial projection round, typically oriented slightly mesoventrad and short, broad, rarely oriented strongly mesoventrad and narrow, elongate. Phallus a fully sclerotized tube, in lateral view with larger base, nearly straight along length, apical section subrectangular, internal spinules absent, internal phallic sclerite long, horizontal, with narrow, round ventral base broadening to subtrapezoidal posterior vertex.

Female Genitalia (Figs 5-14A—5-14B). Venter VIII in ventral view with ventral plates convergent, wide, subovate, tapering basally, covered by setae; in lateral view each broad basally with dorsal and ventral margins parallel for ¾ length, tapering abruptly in final ¼ length, terminating in narrow, round posterior apex; posterior apex of external parts of gonopods VIII in ventral view with posterior margin subtriangular and round mesally; in lateral view extending beyond ventral plates in evenly tapered process with round apex oriented posterodorsad; internal parts of gonopods VIII in ventral view visible through venter VIII even in uncleared specimen, appearing smooth, darker than surrounding tissue, subparallel, lanceolate, with tapered, acute posterior apices distinctly anterior of apices of ventral plates, anterior ends tapered, extending to sclerotized external part of gonopods VIII at basal fourth of ventral plates; in ventral view anterior part of genital chamber stocky, sclerotized, semi-circular, wrapping around posterior apex of processus spermathecae, posterior margin appearing cushioned; processus spermathecae subovoid, appearing flat, bearing opening of ductus spermathecae.

Larva: Described by Ross (1944). Redescribed in Chapter 4.

Pupa: Unknown.

Notes: Males of *P. centralis* exhibit some variation across the species' wide range. Of note, a form with a subtriangular posterior margin of sternum IX and rounded, less produced bodies of the preanal appendages is seen in specimens collected from LaSalle County, Illinois.

Biology: Adults of *P. centralis* have been collected from May to October while late-instar larvae have been collected from March to May. This species occurs in fast flowing, low-order streams with various substrates. This species seems to be more common in the western portion of its range and is the most commonly collected *Polycentropus* species in the interior highlands and is sometime collected in abundance (Moulton and Stewart, 1996; present study).

Distribution (Fig. 5-41): Canada: Newfoundland and Labrador, Nova Scotia, Ontario; USA: Alabama, Arkansas, Illinois, Indiana, Iowa, Kansas, Kentucky, Minnesota, Missouri, Mississippi, New York, Ohio, Oklahoma, Pennsylvania, Tennessee, Texas, Virginia, West Virginia, Wisconsin.

This is a widespread species with few gaps in its known distribution. Of note, this species has been recorded in Newfoundland and Labrador (Marshall and Larson, 1982;

Nimmo, 1986) and Nova Scotia (Nimmo, 1986) but has not been reported from nearby states and provinces such as Maine, Quebec, and New Brunswick. It is possible that these far northeastern records represent relict populations or alternatively that the species exists in nearby areas such as Maine, Quebec, and New Brunswick but has yet to be recorded. Given that *P. centralis* has been recorded from Wisconsin, Indiana, Ohio, and Ontario, it may also occur in Michigan. Similarly, *P. centralis* is known from Texas, Arkansas, and Mississippi, but has yet to be recorded from Louisiana and could well be taken from northern Louisiana in the future.

Material Examined: **Holotype**: **USA**. **Missouri**: St. Louis County, W. St. Louis, Aug. Busch coll., vi-1904, 1 male (MCZ Entomology 11549). **Allotype**: **USA**. **Illinois**: Union County, Wolf Lake, Hutchins Creek, [N37°30'40.86", W89°22'38.7480"], B.D. Burks coll., 31-v-1940, 1 female (INHS Insect Collection 37246).

Non-type material: **USA**. **Alabama**: Franklin County, Dismal Branch at Falls in Dismal Wonders Garden, UV Light, S.C. Harris coll., 29-v-1983, 4 females (FAMU). Lauderdale County, Cypress Creek at County Highway 85, 18-vi-1983, 1 male (FAMU). Marion County, North Fork Creek at Highway 17, LT and S.C. Harris coll., 24-vi-1983, 1 male (FAMU). **Arkansas**: Johnson County, Mulberry River, UV Light, D.E. Bowles coll., 22-ix-1985, 10 males, 2 females (FSCA). Newton County, David Creek at Mt. Hersey, [N36°00'36.39", W97°57'19.85"], 38-iii-2021, 11 larvae (FAMU). **Illinois**: LaSalle County, Wetlands South of Iron Bridge, Lone Star Zone, M.A. Repiscak coll., 22-v-2021, 1 male (INHS Insect Collection 932315). Pope County, Lusk Creek at Dog Hollow Creek, 6.4 km NNE Eddyville, T11S R6E S22 N-W1-4, Site L_03, UV Light Trap, [N37°32'56.04", W88°32'29.76"], J.M. Turner and T. Heatherly coll., 9-vii-2003, 109 males (INHS Insect Collection 39179). Union County, La Rue, Running Lick Creek, [N37°32'44.02”, W89°27'12.99”], B.D. Burks, G.T. Riegel coll., 12-v-1939, 1 larva (INHS Trichoptera 53462). Wolf Lake, Hutchins Creek, [N37°30'40.86”, W89°22'38.75”], Frison and Ross coll., 5-x-1939, 4 females (INHS Trichoptera 48571). **Kentucky**: Marion County, Salt Lick Creek, J.K. Moulton and M.A. Floyd coll., 28-v-2006, 3 male (FAMU). **Oklahoma**: Latimer County, UV Light, K. Stephan coll., vi-1989, 2 males, 1 female (FSCA).

Polycentropus chelatus Ross and Yamamoto

(Figs 5-15 — 5-16; Fig. 5-42)

Polycentropus chelatus Ross and Yamamoto, 1965: 243, fig. 3, ♂; type locality “Sugar Tree, Decatur Co., Tennessee, along Kentucky Lake, tributary of Morgan Creek" (INHS).

Polycentropus chelatus—Armitage and Hamilton, 1990: figs A—D, ♂.

Polycentropus chelatus—Moulton and Stewart, 1996: figs 558-559, ♂.

Diagnosis: Males of *P. chelatus* are separated from all other males of the *P. confusus* Species Group other than *P. neiswanderi* by the thumb-like, digitiform basodorsal process of the inferior appendage. From males of *P. neiswanderi*, those of *P. chelatus* are identified by the lack of a prominent dorsal point and deep emargination of the bodies of the preanal appendages, the longer and more curved basodorsal process of the inferior appendage, and the broader phallus curved less strongly ventrad.

Females of *P. chelatus* are readily separated from all others of the *P. confusus* Species Group by the central ensiform process of the internal parts of gonopods VIII in ventral view and from all females of the *P. confusus* Species Group other than those of

P. stephani by the external parts of gonopods VIII appearing digitiform in lateral view. From females of *P. stephani*, those of *P. chelatus* can be further separated by the subtriangular processus spermathecae versus the subovoid processus spermathecae seen in *P. stephani*.

Adult Description:

General Length of male 4.6—6.5 mm (mean = 5.8 mm; n = 5). Length of female 6.9 mm (n = 1).

Male Genitalia (Figs 5-15A—5-15E). Abdominal segment VIII annular. Terga IX and X fused, membranous, extended caudad over bases of intermediate appendages. Sternum IX subovoid in lateral view, posterior margin subtriangular. Intermediate appendages originating beneath terga IX+X and extending beyond them, curved subtly ventrad, their apices each bearing 5 small setae; in dorsal view apices proximate, subparallel. Bodies of preanal appendages semi-elliptical, each with slightly sinuous posterior margin, lacking posterior process; dorsal process long, curved ventrad, extending just above basodorsal process of corresponding inferior appendage, acute apically; in dorsal view slender, elongate, subparallel, apices appearing recurved mesad. Inferior appendages in lateral view each with digitiform basodorsal process erect, long, thumb-like, oriented dorsoposterad for ½ length, then curved posterad to round apex visible above main body of appendage, main body of appendage with dorsal and ventral margins subparallel for 5/6 length, then convex subapicoventrally and tapering subtly to round apex extended posterad, terminating slightly anterad to intermediate appendages; in ventral view slightly divergent, wide basally, distally tapering to round apex, basodorsal process mostly hidden by main body of inferior appendage, anterior apex projecting beyond medial margins, posterior apex projecting beyond lateral margins, in cleared specimen broad, oblong, apices round; in caudal view oblong, with medial projection round. Phallus a fully sclerotized tube, in lateral view with larger base, curved about 80° ventrad slightly beyond mid-length, apical section broad, round, internal spinules absent, internal phallic sclerite long, horizontal, subrectangular with broad anterior base tapering to narrow posterior apex.

Female Genitalia (Figs 5-16A—5-16B). Venter VIII in ventral view with ventral plates subparallel, narrow, semi-elliptical, round apically, covered by setae; in lateral view each tapering to round posterior apex; posterior apex of external parts of gonopods VIII in ventral view with posterior margin round; in lateral view base large, margins curving mesad for 1/3 length, then digitiform for final 2/3 length, dorsal and ventral margins parallel, extending beyond ventral plates with round apex; internal parts of gonopods VIII in ventral view visible through venter VIII even in uncleared specimen, appearing smooth, darker than surrounding tissue, with central ensiform process extending about ½ length of ventral plates, flanked by two round, thumb-like, convergent processes terminating at about 2/3 length of ventral plates; in ventral view anterior part of genital chamber apparently absent; processus spermathecae subtriangular, flat, anterior apex not extending anterad to sclerotized external part of gonopods VIII, bearing opening of ductus spermathecae.

Notes: The holotype of this species was dissected from the pupal skin, which is stored in the same vial. Unfortunately, most of the larval and pupal sclerites are missing.

Biology: Almost nothing is known of the biology of this uncommon species. Adults have been collected near clear, fast flowing, low-order streams from March to May. The holotype was dissected from its pharate pupal skin collected in May.

Distribution (Fig. 5-42): Alabama, Indiana, Kentucky, Missouri, Tennessee.

P. chelatus has a narrower range than most species, apparently restricted to a more central eastern Nearctic range. Despite being reported from Indiana (Waltz and McCafferty, 1983), Kentucky (Etnier and Schuster, 1979; Moulton and Stewart, 1996; Etnier et al., 2006; Floyd et al., 2012; present study), and Missouri (Mathis and Bowles, 1992; Moulton and Stewart, 1996; present study), *P. chelatus* is not known from Illinois. If this species occurs in Illinois and awaits discovery, it is most likely to occur in the far southern portion of the state.

Material Examined: **Holotype**: **USA**. **Tennessee**: Decatur County, Sugar Tree, along Kentucky Lake, tributary of Morgan Creek, Ross et al. coll., 5-v-1958, 1 male, associated pupal skin (INHS Insect Collection 37258).

Non-type material: **USA**. **Kentucky**: Madison County, Cowbell Creek, Berea College Forest, [N37°32'38.40", W84°13'47.28"], Chapman and Stedenfeld coll., 23-v-2013, 1 male (BYU). **Missouri**: Pulaski County, M. Mathis coll., 25-iv-1986, 12 males (CUAC000107333). **Tennessee**: Montgomery County, Piney Fork at Boiling Spring Rd., Ft. Campbell, [N36°36'58.75", W87°30'51.98"], BHB coll., 29-iv-2004, 8 males (ECC 3.415). Morgan County, Crooked Fork at Petros, Rt. 116 "fire department", J.K. Moulton coll., 20-v-2006, 1 male (FAMU). 8-v-2006, 1 female (FAMU).

Polycentropus chenoides Ross and Yamamoto

(Fig. 5-17; Fig. 5-43)

Polycentropus chenoides Ross and Yamamoto, 1965: 243, fig. 4, ♂; type locality "Oriente, Long Island, New York" (INHS).

Polycentropus chenoides—Armitage and Hamilton, 1990: figs A—D, ♂.

Diagnosis: Males of *Polycentropus chenoides* are readily separable from those of all other species of the *P. confusus* Species Group by the strongly deflexed basodorsal process of the inferior appendage and the intermediate appendages being strongly curved dorsad, in dorsal view crossing to form an “X” shape.

Adult Description:

General Length of male 6.2—7.1 mm (mean = 6.8 mm; n = 5).

Male Genitalia (Figs 5-17A—5-17E). Abdominal segment VIII annular. Terga IX and X fused, membranous, extended caudodorsad over bases of intermediate appendages. Sternum IX subovoid in lateral view, posterior margin sinuous. Intermediate appendages originating beneath terga IX+X and extending beyond them, curved dorsad, their apices each bearing 3 small setae; in dorsal view apices proximate, convergent, crossing to form an “X”. Bodies of preanal appendages each produced posterad with slightly sinuous posterior margin, deep dorsal emargination; dorsal process long, curved ventrad, terminating above head of basodorsal process of corresponding inferior appendage, acute apically, bearing single seta on ventral margin near apex; in dorsal view slender, elongate, divergent, straight, lateral and mesal margins subparallel for ¾ length, tapering evenly to acute apex beyond for final ¼ length. Inferior appendages in lateral view each with capitate basodorsal process strongly deflected, with moderately long neck and subtriangular head round posterad and projecting ventrad with its pointed apex hidden behind main body of appendage, main body of appendage with broad base, tapering evenly to round apex extended posterad slightly beyond intermediate appendages; in ventral view divergent, each semi-elliptical, wide basally, distally tapering to round apex, margins slightly sinuous, basodorsal process nearly completely hidden by main body of inferior appendage, with only medial round tip exposed, in cleared specimen broad, with medial round projection; in caudal view similar, goose-head shaped, with medial projection round. Phallus a fully sclerotized tube, in lateral view with larger base, curved about 15° ventrad slightly beyond mid-length, apical section subrectangular, internal spinules absent, internal phallic sclerite very small, central, horizontal, ovate.

Female Genitalia Unknown.

Larva: Unknown.

Pupa: Unknown.

Biology: Almost nothing is known of the biology of this seldom collected species. Most specimens have been collected in May, with one male specimen collected in Pennsylvania in September (Masteller and Flint, 1992). All specimens were collected near flowing waters of low-order streams to small, navigable rivers.

Distribution (Fig. 5-43): USA: Delaware, New York, Pennsylvania.

This species is apparently endemic to a few northeastern US states. Given that R.W. Lake collected several specimens in Delaware near the Maryland border, it is possible that this *P. chenoides* will eventually be collected from Maryland’s Eastern Shore.

Material Examined: **Holotype**: **USA**. **New York**: Long Island, R. Latham coll., 13-v-1947, 1 male (INHS Insect Collection 37249).

Non-type material: USA. **Delaware**: Sussex County, Blades, UV Light Trap, R.W. Lake coll., 24-25-v-1983, 2 males (CUAC0000107309). 7-8-v-1983, 1 male (CUAC0000107308). Georgetown, Substation, 18-24-v-1976, 1 male (CUAC0000107310). Seaford-Blades, 21-23-v-1983, 1 male (CUAC0000107311). Site #S-27, town of Smith Hill on Rd. 64 near jct. Rd.457, James Br., [N38°29'39.48", W75°30'17.28"], 14-v-1974, 1 male (CUAC0000107307).

Polycentropus confusus Hagen

(Figs 5-18 — 5-19; Fig. 5-44)

Polycentropus confusus Hagen, 1861: 293, ♂; type locality "Trenton Falls, Washington" (MCZ).

Polycentropus confusus—Banks, 1914: 258, fig. 70, ♂.

Plectrocnemia confusa—Betten, 1934: 218.

Polycentropus confusus—Milne, 1936: 85.

Polycentropus confusus—Banks 1936:130, lectotype.

Polycentropus confusus—Ross, 1941: 71, figs 42, 46, 53, ♂; plesiotype (neotype).

Polycentropus confusus—Ross, 1944: 65, fig. 244, ♂; fig. 257, plesio-allotype (allotype) ♀.

Polycentropus confusus— Marshall and Larson, 1982: 24, fig. 46, ♂.

Polycentropus confusus—Nimmo, 1986: 196, figs 133—137, ♂; figs 138—139, ♀.

Polycentropus confusus—Armitage and Hamilton, 1990: figs A—D, ♂; figs E—F, ♀.

Polycentropus confusus—Moulton and Stewart, 1996: figs 562-563, ♂.

Polycentropus confusus—Houghton, 2012: fig. 269, ♂.

Diagnosis: Males of *Polycentropus confusus* can be distinguished from those of other species in the *P. confusus* Species Group by phallus possessing a broad apex bearing numerous microspicules and a ventral lip. Also separating males of this species from those of all the others in the group are the ventral portion of the inferior appendages which, in ventral view, appear slightly divergent with a wide base for ¼ length, then with the lateral margins tapering strongly to round apices for the final ¾ length, and with the majority of the dorsobasal processes visible beyond the lateral margins of the ventral portions of the inferior appendages.

Females of *P. confusus* are separated from those of the remaining *P. confusus* Species Group members by the internal parts of gonopods VIII in ventral view appearing like a cloak with anterior ends divergent, each with acute inner projection and bifid outer projection separated by a deep emargination, with the round “head opening” of the cloak extending just beyond the apices of ventral plates posterad. Also unique to females of *P. confusus* are the ventral plates which, in lateral view, are truncate and possess a broad, sinuous apex with a caudodorsal emargination.

Adult Description:

General Length of male 6.3—8.1 mm (mean = 7.3 mm; n = 5). Length of female 7.3—9.8 mm (mean = 8.5 mm; n = 5).

Male Genitalia (Figs 5-18A—5-18E). Abdominal segment VIII annular. Terga IX and X fused, membranous, extended caudad over bases of intermediate appendages. Sternum IX ovoid in lateral view, posterior margin slightly sinuous. Intermediate appendages originating beneath terga IX+X and extending beyond them, curved slightly ventrad, their apices each bearing 4 small setae; in dorsal view apices proximate, subparallel. Bodies of preanal appendages each with broad, round posteroventral process; dorsal process long, curved only slightly ventrad, extending above posteroventral process of corresponding preanal appendage, acute apically, bearing 2 small setae dorsally visible laterally; in dorsal view slender, elongate, convergent. Inferior appendages in lateral view each with capitate basodorsal process erect with short neck and elongate head round dorsally, concave caudoventral margin with small basoventral process projecting ventrad with pointed apex visible above main body of appendage, main body of appendage with broad base, dorsal margin tapering evenly to subrectangular apex, ventral margin basally slightly convex for approximately 2/3 length, then tapering along final 1/3 length to subrectangular apex terminating anterad of intermediate appendages, surface with dorsomesal excavation for 2/3 length; in ventral view subparallel, subtly divergent distally, each wide basally for 1/4 length, lateral margin strongly tapering distally for final ¾ length to round apex, inner margin curved slightly outward along length, basodorsal process mostly exposed beyond lateral margins of main body of inferior appendage, medial projection hidden behind main body of inferior appendage, in cleared specimen elongate, with medial pointed projection; in caudal view oblong, with medial projection round. Phallus a fully sclerotized tube, in lateral view with larger base, curved about 45° ventrad at apex, apical section broad, round, with extended ventral lip, bearing numerous microspicules apically, internal spinules absent, internal phallic sclerite long, angled caudoventrally, with rectangular anterior base, subrectangular posterior apex, with ventral margin slightly concave, dorsal margin strongly convex.

Female Genitalia (Figs 5-19A—5-19B). Venter VIII in ventral view with ventral plates subtly convergent, broad, sub-elliptical with sinuous medial margin, round apically, covered by setae; in lateral view each truncate, terminating in sinuous apex with caudodorsal emargination; posterior apex of external parts of gonopods VIII in ventral view with posterior margin subtriangular and round mesally; in lateral view extending beyond ventral plates in flat process oriented caudodorsad with tiny apex projected caudad; internal parts of gonopods VIII in ventral view visible through venter VIII even in uncleared specimen, appearing smooth, darker than surrounding tissue, subparallel, elliptical, fused medially, appearing as a cloak, anterior ends divergent, each with acute inner projection and bifid outer projection separated by deep emargination, extending posterad to round "head opening" of cloak extending just beyond apices of ventral plates; in ventral view anterior part of genital chamber apparently absent; processus spermathecae subovoid with central elevation bearing opening of ductus spermathecae.

Larva: Described in Chapter 4.

Pupa: Unknown.

Notes: *Polycentropus confusus* was erroneously reported from Alberta, Canada, by Zhou et al. (2011). In addition, a syntype deposited at the Harvard Museum of Comparative Zoology, MCZ Entomology 648446, was described as *P. confusus* by Hagen (1861) in his initial description of the species. The specimen was later identified by Banks (1936) as *Plectrocnemia* based on the presence of a closed discoidal cell in the hindwing. The specimen is a female *Plectrocnemia crassicornis* (Walker, 1852) and should not be considered a syntype of *Polycentropus confusus*.

Biology: This is among the most common species of the *P. confusus* Species Group. Adults have been collected from April to September and late-instar larvae have been collected from March to December. *Polycentropus confusus* inhabits a wide range of lotic habitats with various hard substrates, spanning small (e.g., second order) streams to navigable rivers such as the Pigeon River in North Carolina.

Distribution (Fig. 5-44): Canada: New Brunswick, Newfoundland and Labrador, Nova Scotia, Ontario, Quebec; USA: Alabama, Arkansas, Connecticut, District of Columbia, Delaware, Florida, Georgia, Indiana, Iowa, Kentucky, Maine, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, New Hampshire, New Jersey, New York, North Carolina, Ohio, Oklahoma, Pennsylvania, South Carolina, Tennessee, Vermont, Virginia, West Virginia, Wisconsin.

This species is perhaps the most widespread of the *Polycentropus confusus* Species Group with a few conspicuous gaps in its known distribution. Ross (1944) indicated that this species is likely eventually to be recorded in Illinois given that it has been taken from every state bordering Illinois. Similarly, it has yet to be reported from Maryland or Rhode Island, despite being recorded from surrounding states.

Material Examined: **Lectotype**: **USA**. New York: Oneida County, Trenton Falls, C. v. Sacken coll., 1858, 1 male (missing head and abdomen) (MCZ Entomology 11038). **Paralectotype**: **USA**. **Washington, D.C.**: District of Columbia, C. v. Sacken coll., 1858, 1 female (MCZ Entomology 648447). **Allotype**: **Canada**. **Ontario**: Algonquin Park, Ontario Fish Research Lab, Costello Lake, Cage No. 1, W.M. Sprules coll., 11-vii-1938, 1 female (INHS Insect Collection 38171).

Non-type material: **Canada**. **New Brunswick**: Exmoor, [N46°58'15.60", W65°51'10.80"], DB and JC coll., 25-vi-2008, 1 female (08NBEPT-1447). **Ontario**: Huron Lake, [N45°19'01.92", W80°06'29.16"], C. Moore, R. O`Connor coll., 28-v-2018, 1 larva (BIOUG43980-E07). **USA**. **Alabama**: Dekalb County, Mentone, Little River at Wester Road (Hwy 165), UV Light Trap, [34°30'30.95", W85°36'30.71"], D. Denson and B. Denson coll., 25-vi-2022, 1 male (FAMU). Fayette County, Confluence of Flat Creek and Cedar Creek, [N33°37'48.00", W87°36'05.04"], A. Orfinger coll., 17-v-2021, 2 males (FAMU). Little Tyro Creek, [N33°35'20.76", W87°29'47.04"], 3-v-2021, 2 males (FAMU). Lawrence County, Hubbard Creek below Kinlock Falls, [N34°18'31.68", W87°30'06.84"], 17-iv-2021, 1 larva (FAMU). Marion County, North Fork Creek at Highway 17, LT and S.C. Harris coll., 24-vi-1983, 6 males (FAMU). **Florida**: Walton County, Natural Bridge Creek at Highway 181, north side, UV Blacklight, [N30°59'18.99", W86°12'16.77"], D. Denson and E. Denson coll., 2-vi-2016, 2 males (FAMU). **Georgia-South Carolina**: Rabun-Oconee Counties, Chatooga River at SR 28, 24 km S Highlands, NC, 770 masl, [N34°55'09.84", W83°10'06.96"], B. Schmidt coll., 19-vi-2019, 1 larva (CUAC000088819). EPT Class coll., 31-vii-2019, 1 female (CUAC). K. Hecke coll., 31-vii-2019, 1 larva (CUAC). **Kentucky**: Marion County, Tributary of Salt Lick Creek at Salt Lick Creek Rd., J.K. Moulton coll., 28-v-2006, 19 males (FAMU). **Minnesota**: Lake County, Finland State Forest, Baptism River, Eckbeck Campground, 320 masl, [N47°22'23.16", W91°13'41.16"], Holzenthal and Huisman coll., 23-vi-1992, 1 male (UMSP 000003727). **Missouri**: Stone County, Wire Road Conservation Area, Crane Creek, [N36°55'31.80", W93°35'25.80"], D.E. Bowles coll., 14-iii-2021, 1 larva (FAMU). **New Jersey**: Hunterdon County, Kingwood, Swampy area near house, [N40°29'07.96", W75°01'01.50"], C. Browne coll., 14-vi-2012, 5 males (FAMU). Sussex County, Stokes State Forest, Beaver pond near Steam Mill Campground, [41°11'54.24", W74°47'05.64"], K. Kjer and J. Kjer coll., 4-vi-2004, 1 male (UMSP 000124763). **North Carolina**: Haywood County, Cataloochee, Great Smoky Mountains National Park, first stream on right past first historical building, J.K. Moulton coll., 27-vii-2005, 9 males (FAMU). Pigeon River, [N35°44'01.68", W83°01'30.72"], R.W. Smith coll., 21-viii-2019, 2 larvae (FAMU). Macon County, Below Dry Falls, 3.6 miles W JCT 64 and 246, At Light, P. Skelley coll., 26-vi-1989, 1 male (FSCA). Swain County, Noland Creek, 430 meters up from Lake Fontana at bridge, Blacklight, 536 masl, [N35°16'21.09", W83°18'59.19"], R.C. Harrington coll., 20-viii-2000, 1 male, 1 female (CUAC0000107306).

Pennsylvania: Bucks County, Mill Creek, [N40°10'01.56", W75°00'07.56"], N. Macelko coll., 14-vi-2020, 1 larva (FAMU). Centre County, Spring Creek, [N40°52'51.24", 77°47'36.60"], 2-v-2021, 1 larva (FAMU). Chester County, Exton, Valley Creek West, [N40°01'47.30", W75°37'20.30"], 13-iv-2021, 3 larvae (FAMU). Tributary of Little Neshaminy Creek, [N40°06'02.40", W75°32'33.96"], 26-XI-2020, 1 larva (FAMU). Valley Creek West, [N40°01'47.28", W75°37'20.28"], 13-iv-2021, 4 larva (FAMU). White Clay Creek, [N39°51'46.80", W75°47'02.40"], T. Bringloe, V. Harvey, S. Ripley, K. Rondollo coll., 19-vi-2013, 1 larva (BIOUG06970-A06). 1 larva (BIOUG07063-B11). Delaware County, Chester Creek, [N39°54'07.06", W75°28'09.78"], N. Macelko coll., 6-xii-2020, 1 larva (FAMU). Elk County, Birch Run, [N39°55'44.40", W75°51'39.60"], T. Bringloe, V. Harvey, S. Ripley, K. Rondollo coll., 19-vi-2013, 1 larva (BIOG06971-F10). 1 larva (BIOUG06971-G01). 1 larva (BIOUG07978-B07). 1 larva (BIOUG07982-A07). Cold Run, [N40°42'14.40", W75°59'52.80"], 18-vi-2013, 1 larva. (BIOUG07978-E09).

Philadelphia, Birch Run, off 5 Point Road, 163 masl, [N39°55'44.40", W75°51'39.60"], T. Bringloe, V. Harvey, S. Ripley, and K. Rondollo coll., 19-vi-2013, 1 larva (BIOUG07981-A10). 1 larva (BIOUG07982-A08). 1 larva (BIOUG07982-A09). Montgomery County, Pennypack Creek, [N40°08'15.36", W75°04'26.40"], N. Macelko coll., 26-iv-2020, 1 larva (FAMU). Northampton County, Bush Kill Creek, [N40°44'29.04", W75°14'53.52"], 1-V-2021, 3 larvae (FAMU). Philadelphia County, Philadelphia, Cold Run, 211 masl, [N40°42'14.40", W75°59'52.80"], T. Bringloe, V. Harvey, S. Ripley, and K. Rondollo coll., 18-vi-2013, 1 larva. (BIOUG07978-E09). 1 larva (BIOUG07978-H06). Philadelphia, Tributary of Brandywine Creek, 29 masl, [N39°55'04.80", W75°43'22.80"], 19-vi-2013, 1 larva (BIOUG07978-H07). Pickering Creek, N. Macelko coll., 1 larva (FAMU). **South Carolina**: Pickens County, Wildcat Creek, [N34°45'34.20", W82°51'03.24"], C.M. Slack coll., 25-viii-2009, 1 larva (CUAC000107326). **Tennessee**: Knox County, Fowler Spring Branch, first-order tributary to Beaver Creek, Bell Campground Rd., 0.3 km N of jct. with W. Brushy Valley Dr., UV Light Trap, 277 masl, [N36°02'56.20”, W84°04'07.60"], A. Orfinger and A. Perilla coll., 6-vi-2020, 1 male (FAMU). J.K. Moulton coll., 2005, 2 female (FAMU). J.K. Moulton and A. Orfinger coll., 10-iv-2019, 3 females (FAMU). Monroe County, Cherokee National Forest, Bald River at Holly Flats Recreation Area, 667 masl, [N35°17'11.04", W84°10'41.88"], Holzenthal, Blahnik, Hamilton, Chamorro-L., and Robertson coll., 30-v-1999, 1 male (UMSP 000102523). Unico County, Erwin, North Indian Creek at Second Street Bridge, at light, 504 masl, [N36°09'01.55", W82°25'03.58"], C.D. Kerst coll., 22-vii-2015, 6 males, 4 females (BYU).

Polycentropus dinkinsorum Orfinger and Etnier

(Fig. 5-20; Fig. 5-45)

Polycentropus dinkinsorum Orfinger and Etnier, 2020: 127—129, fig. 1, ♂; type locality "North Carolina: Henderson County, Flat Rock, Carl Sandburg Home National Historic Site, [N35°16'16.28, W82°27'01.04"]" (USNM).

Diagnosis: The males of *P. dinkinsorum* can be separated from those of all other members of the *P. confusus* Species Group by the sharp angulate, tooth-like projection of the posteroventral margin on the body of the preanal appendage. Male genitalic structure is most similar to that of *P. carolinensis* and *P. carlsoni*. It can readily be separated from both *P. carolinensis* and *P. carlsoni* by the more pronounced mesoventral swelling on the phallus, the longer necks of the basodorsal process of the inferior appendages, and the more obtusely curved heads of the basodorsal processes of the inferior appendages. Males can also be distinguished from *P. carlsoni* by the slight downward curvature of the phallus, versus the stronger curvature exhibited by *P. carlsoni*. From *P. carolinensis*, it can also be separated by the presence of a distinctly pronounced, horn-like, apical projection of the combined terga IX+X extending caudad over the intermediate appendages. From *P. floridensis*, *P. pentus*, and *P. vernus*, which each exhibit a ventral swelling on the phallobase, males of *P. dinkinsorum* can easily be separated by the more distal location of the mesoventral swelling, and the shape of the basodorsal process of each inferior appendage. From *P. floridensis* and P. *vernus*, *P. dinkinsorum* can be distinguished by the presence of the acute, tooth-like projection of the posteroventral margin of the preanal appendage, and from *P. pentus* by the prominent profile of this projection (set in a concavity in *P. pentus*).

Adult Description:

General Structure: Length of male 6.1—7.2 mm (mean = 6.5 mm; n = 4).

Male genitalia (Figs 5-20A—5-20E). Abdominal segment VIII annular. Tergum IX fused with segment X, membranous, caudally extended as horn-like projection in lateral view. Sternum IX round in lateral view, posterior margin lobate. 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, parallel. Preanal appendages triangular in lateral view, each with posterior angle acute, forming tooth-like projection; in lateral view dorsal process wide basally, distally slender, process curved ventrad terminating in slender spine; in dorsal view, sub-parallel, slender, elongate. Inferior appendages each with ventral portion in lateral view gradually tapered, extended posterad slightly beyond intermediate appendages, ventral margin nearly straight; in ventral view, mesal projection near mid-length, distal half curved inward; basodorsal process in lateral view erect, with long neck terminating in short, round head projecting downward, in ventral view completely hidden by main body of inferior appendage, head round, oriented medially, in caudal view slightly round, curved downward. Phallus in lateral view slightly curved ventrad, mesoventral swelling arising near mid-length, apical section rectangular, internal spinules absent, internal phallic sclerite moderately elongate with narrow distal portion.

Female Genitalia Unknown.

Larva: Unknown.

Pupa: Unknown.

Biology: Almost nothing is known of the biology of this recently described species. *Polycentropus dinkinsorum* has been collected near cool, rocky streams of the southern Appalachian Mountains from May to September. Collection of specimens via both

Townes-style Malaise trap (Townes 1962, 1972) and UV light trap suggests both diurnal and crepuscular flight activity, respectively.

Distribution (Fig. 5-45): USA: North Carolina, Tennessee, West Virginia.

Given that this species is currently known from the Appalachian Mountains of North Carolina, Tennessee, and West Virginia, it is likely to eventually be recorded from eastern Kentucky and western Virginia.

Material Examined: **Holotype**: **USA**. **North Carolina**: Henderson County, Flat Rock, Carl Sandburg Home National Historic Site, Malaise Trap, [N35°16'16.28", W82°27'01.04"], I. Hoff and E. Eleantar coll., 20-ix-2012, 1 male (USNMENT 01350618). **Paratypes**: **USA**. **North Carolina**: Henderson County, Carl Sandberg Home National Historic Site, Flat Rock, Duck Pond, Malaise Trap, [N35°16'15.96", W82°27'01.04"], I. Hoff and E. Eleantar coll., 16-24-v-2012, 1 male (ROME184984). Swain County, Great Smoky Mountains National Park, Kephart Prong Trail near trail head across from bridge over Oconaluftee River, Blacklight Trap, 834 masl, [N35°35'11.52", W83°21'32.87"], B. Sullivan coll., 23-25-vii-2000, 1 male (INHS Insect Collection 923902). 1 male (USNMENT 01350619). 1 male (FAMU). **West Virginia**: Raleigh County, Piney Creek tributary, New River system, [N37°50'39.08", W81°06'48.88"], G.R. Dinkins, B.J. Dinkins, and H.O. Faust coll., 25-vi-2014, 1 male (CUAC000107314).

Non-type material: **USA**. **North Carolina**: Haywood County, Great Smoky Mountains National Park, Mt. Sterling Trail, D. Paulsen coll., 27-vi-1989, 9 males (ECC 3.269). **Tennessee**: Blount County, Sams Creek, 100 m below Thunderhead Creek , D. Etnier coll., 7-vi-1996, 1 male, 1 pupa, 1 larva (includes early instar larva broken in two and pupa) (ECC 3.288). Carter County, Roan Mountain State Park, Black Light, J. Ensminger coll., 6-12-viii-2000, 1 male (ECC 3.357).

Polycentropus elarus Ross

(Figs 5-21 — 5-22; Fig. 5-46)

Polycentropus elarus Ross, 1944: 65-66, fig. 245, ♂; fig. 256, ♀; type locality “Costello Lake, Algonquin Park, Ontario" (INHS, lost).

Polycentropus elarus—Nimmo, 1986: 197, figs 140—144, ♂; figs 145—146, ♀.

Polycentropus elarus—Armitage and Hamilton, 1990: figs A—D, ♂; figs E—F, ♀.

Diagnosis: Males of *Polycentropus elarus* are most similar to those of *P. aileenae* and *P. stephani* in the ventral aspect of the ventral portions of the inferior appendages and to *P. aileenae* also in the size and shape of the head of the basodorsal processes of the inferior appendages. From these and other males of species in the group, *P. elarus* can be separated by the dorsal processes of each preanal appendage being directed caudad and slightly curved dorsad and convergent apically. From males of *P. aileenae*, those of *P. elarus* are separated by the shorter basodorsal processes and ventral portions of the inferior appendages and by the subparallel rather than divergent ventral portions of the inferior appendages. From males of *P. stephani*, males of *P. elarus* are identified by the capitate head of the basodorsal process of the inferior appendage and by the lack of a basoventral spur on the phallus. From all other species, males of *P. elarus* are separated by the size and shape of the small, round heads of the basodorsal processes of the inferior appendages and by the shape of the ventral portions of the inferior appendages as seen in ventral view.

Females of *P. elarus* are separated from female of all other members of the *Polycentropus confusus* Species Group by the combination of the posterior apex of external parts of gonopods VIII in lateral view being slender, almost digitiform, and either oriented caudad or recurved dorsad and the anterior part of genital chamber sclerotized being U-shaped and appearing connected to the smooth internal parts of gonopods VIII that are curved about 90° laterad to apices of ventral plates distally.

Adult Description:

General Length of male 6.0—8.5 mm (mean = 7.2 mm; n = 6). Length of female 8.6—9.5 mm (mean = 9.0 mm; n = 5).

Male Genitalia (Figs 5-21A—5-21E). Abdominal segment VIII annular. Terga IX and X fused, membranous, extended caudad over bases of intermediate appendages. Sternum IX subovoid in lateral view. Intermediate appendages originating beneath terga IX+X and extending beyond them, oriented caudad, their apices each bearing 4 small setae; in dorsal view apices proximate, subparallel to divergent. Bodies of preanal appendages each with small, round subtriangular posteroventral process with subtle ventral emargination; dorsal processes long, each curved ventrad for 2/3 length, curved slightly dorsad for final 1/3 length, extending only to dorsal portion of body of preanal appendage, acute apically; in dorsal view stocky, elongate, subparallel for nearly entire length, convergent apically. Inferior appendages in lateral view each with capitate basodorsal process erect with moderately long neck and small head round dorsally and projecting ventrad with its round, subtriangular apex visible above main body of appendage, main body of appendage with broad base, dorsal and ventral margins tapering evenly to round apex extended posterad slightly beyond intermediate appendages; in ventral view subparallel, wide basally, margins subparallel for ½ length, inner margin curved abruptly outward beyond ½ length to narrow round apex, basodorsal process completely hidden by main body of inferior appendage, in cleared specimen small, with medial round projection; in caudal view oblong, with medial projection round. Phallus a fully sclerotized tube, in lateral view with larger base, curved about 20° ventrad slightly beyond mid-length, apical section slightly enlarged, subrectangular, posterior margin slightly sinuous with ventral lip, with internal spinules absent, internal phallic sclerite long, horizontal, with round slender, acute anterior base and wide subrectangular posterior apex.

Female Genitalia (Figs 5-22A—5-22B). Venter VIII in ventral view with ventral plates convergent, narrow, semi-elliptical, tapering apically, covered by setae; in lateral view each narrow at base, wide at mid-length, ventral margin sinuous beyond mid-length, narrowing to round posterior apex; posterior apex of external parts of gonopods VIII in ventral view with posterior margin subtriangular and round mesally; in lateral view extending beyond ventral plates in flat process with its tiny apex projected caudad to recurved dorsad; internal parts of gonopods VIII in ventral view visible through venter VIII even in uncleared specimen, appearing smooth, darker than surrounding tissue, subparallel basally for 4/5 length, curved about 90° laterad to apices of ventral plates distally, sinuous along length, tapering gradually to apex, anterior ends appearing connected to anterior part of genital chamber at sclerotized external part of gonopods VIII at basal third of ventral plates; in ventral view anterior part of genital chamber sclerotized, U-shaped, strongly sinuous, wrapping around posterior apex of processus spermathecae; processus spermathecae large, subovoid with central elevation bearing opening of ductus spermathecae.

Larva: Described in Chapter 4.

Pupa: Unknown.

Notes: The holotype is listed in Ross (1944) as deposited at INHS but could not be located and is presumed lost. INHS Insect Collection 37255 specimen designated as neotype.

Biology: Adults of this fairly common species is found near cool, clear, flowing streams of with various substrates depending on locality. Adults have been collected from April to September, sometimes in abundance. The single known late-instar larva was collected in June from an unimpaired (Bott et al., 2012), small, cool, fast-flowing stream with rocky substrate.

Distribution (Fig. 5-46): Canada: Ontario, Quebec; USA: Alabama, Florida, Georgia, Illinois*, Indiana, Kentucky, Massachusetts, New Hampshire, New York, North Carolina, Ohio, Pennsylvania, Tennessee, Virginia, West Virginia.

This is a widespread species in the eastern United States and Canada. It is likely that this species will be encountered in additional states and provinces for which it has been taken from neighboring areas. For example, Orfinger and Moulton (2021) recently reported the species from the Florida panhandle based on a single male specimen, and is reported herein for the first time from Illinois based on five male specimens. Similarly, it would be unsurprising if *P. elarus* was eventually recorded from Mississippi, Michigan, South Carolina, Maryland, New Jersey, Vermont, Maine, Delaware, Connecticut, Rhode Island, and New Brunswick.

Material Examined: **Neotype**: **Canada**. **Ontario**: Algonquin Park, Ontario Fish Research Lab, Costello Lake, Cage No. 1, W.M. Sprules coll., 11-vi-1939, 1 male (INHS Insect Collection 37255). **Paratype**: **USA**. **New York**: Adirondack Park, Bear Brook near Blue Mountain Lake, Frison and Ross coll., 19-vi-1941, 1 male (INHS Trichoptera 24573). **Allotype**: **Canada**. **Ontario**: Algonquin Park, Ontario Fish Research Lab, Costello Lake, Cage No. 1, W.M. Sprules coll., 22-vi-1939, 1 female (INHS Insect Collection 37262). Non-type material: **Canada**. **Ontario**: Algonquin Provincial Park, Costello Lake, Ontario Fisheries Research Laboratory, W.M. Sprules coll., 22-vi-1939, 1 female (recorded by Ross as male) (INHS Trichoptera 54986). **USA**. **Alabama**: Dekalb County, Fort Payne, Straight Creek, UV Light Trap, 468 masl, [N34°38'34.98”, W85°36'41.52”], A. Orfinger and A. Perilla coll., 27-28-vi-2020, 116 males (FAMU). **Florida**: Washington County, Econfina Creek Water Management Area, Unnamed ravine stream (lower reach) that flows into Whitewater Lake off Porter Pond Rd., [N30°28'46” W85°33'14”], Rasmussen et al. coll., 6-vi-2009, 1 male (FAMU). **Illinois**: Ford County, Paxton Railroad Prairie, Light, 229 masl, [N40°25'08.33”, W88°06'51.70”], J.D. Unzicker coll., 7-vi-1962, 3 males (INHS Insect Collection 38151) **New State Record**. Pope County, Simmons Creek, War Bluff Valley Audobon Society Sanctuary, 8.7 km N Golconda , Black light trap, [N37°26'43.84”, W88°29'19.64”], J.L. Robinson coll., 29-viii-2015, 2 males (INHS Insect Collection 797581) **New State Record**. **Indiana**: Montgomery County, Shades SP, Crystal Falls Stream, L. Chandler and R. Smith coll., 12-v-1963, 1 female (INHS Trichoptera 54984). **Kentucky**: Bell County, Martins Fork, Cumberland River downstream of upper bog, 735 masl, M. Geraghty and C. Parker coll., 19-vii-2006, 1 female (CUGA 4851). **Pennsylvania**: Schuylkill County, Rattling Run, [N40°35'02.40”, W76°01'01.20"], T. Bringloe, V. Harvey, S. Ripley, K. Rondollo coll., 13-vi-2013, 1 larva (BIOUG07980-B07). **Tennessee**: Greene County, Tusculum College, [N36°10'26.00”, W82°45'39.99"], M. Wright coll., 23-30-v-1946, 1 male, 1 female (INHS Trichoptera 54983). **Virginia**: George Washington National Forest, Passage Creek, G. Washington Nat. For., For. Rt. 274, 396 masl, [38°43'30”, W78°32'24”], Blahnik & Contreras coll., 19-ix-1992, 1 male (UMSP 000048997). **West Virginia**: Hardy County, 3 miles northeast of Mathias, [N38°33'00.00”, W78°29'24.00”], D. Smith coll., 27-vi-2007, 1 male (BYU).

Polycentropus floridensis Lago and Harris

(Fig. 5-23; Fig. 5-47)

Polycentropus floridensis Lago and Harris, 1983: 666, fig. 2, ♂; type locality “Florida, Walton County, Headwaters of Rocky Creek, 4 mi. SW Mossy Head” (USNM).

Polycentropus n.sp.—Harris, Lago, and Scheiring, 1982: 80.

Polycentropus floridensis—Armitage and Hamilton, 1990: A—D, ♂.

Diagnosis: Males of *P. floridensis* are most similar to those of *P. dinkinsorum*, *P. pentus*, and *P. vernus* in regard to the ventral swelling of the phallus, *P. confusus* and *P. thaxtoni* in the size and shape of the basodorsal processes of the inferior appendages. From *P. dinkinsorum*, *P. pentus*, and *P. vernus*, males of *P. floridensis* are separated by the large, round, lobate basodorsal appendage of the inferior appendage and the deep emargination of the bodies of the preanal appendages, which is absent in *P. dinkinsorum* and *P. vernus*; in males *P. vernus*, two deep emarginations of the bodies of the preanal appendages make it appear bifid. Males of *P. floridensis* are separated from those of *P. confusus* and *P. thaxtoni* by the presence of a large mesoventral welling of the phallus and by the shape of the basodorsal process of the inferior appendages which, in ventral view are mostly concealed behind the ventral portions in *P. floridensis* and mostly exposed in *P. confusus* and *P. thaxtoni*.

Adult Description:

General Length of male 5.9—7.0 mm (mean = 6.4 mm; n = 5).

Male Genitalia (Figs 5-23A—5-23E). Abdominal segment VIII annular. Terga IX and X fused, membranous, overlaying bases of intermediate appendages. Sternum IX subovoid in lateral view, posterior margin subtriangular. Intermediate appendages originating under terga IX+X, extending beyond them, oriented dorsocuadad, their apices each bearing 4 small setae; in dorsal view apices proximate, parallel. Bodies of preanal appendages each round, produced caudad with deep dorsal emargination; dorsal process long, curved ventrad, extending just above head of basodorsal process of corresponding inferior appendage, acute apically; in dorsal view slender, sinuous, apices convergent. Inferior appendages in lateral view each with lobate basodorsal process erect with short neck and long, broad head round dorsally and projecting ventrad with its round apex visible above main body of appendage, main body of appendage with wide base possessing basodorsal triangular bump, dorsal and ventral margins tapering along length to round apex terminating anterad of intermediate appendages, surface with dorsomesal excavation along length; in ventral view subparallel, wide basally, medial and lateral margins subparallel for ½ length, medial margin curving outward beyond about ½ length to round apex, basodorsal process mostly hidden by main body of inferior appendage, lateral margins extending beyond main body of inferior appendage to varying degrees, in cleared specimen rounded, subtriangular, with medial blunt projection; in caudal view oblong, with basomedial round bump. Phallus a fully sclerotized tube, in lateral view with larger base, large mesoventral swelling, curved slightly ventrad slightly beyond 2/3 length, apical section subrectangular, sinuous, internal spinules absent, internal phallic sclerite long, horizontal, slender, with subrectangular anterior base and linear posterior apex.

Female Genitalia Unknown.

Larva: Described in Chapter 4.

Pupa: Unknown.

Biology: This is a narrow-range and less common species. This species has been collected from clear, cool, flowing spring-fed streams of various sizes with sand substrate and ample deadwood and aquatic macrophytes. Adults have been collected from March to August and mid- to late-instar larvae have been collected from January to March. This species was classified as a Threatened species by Deyrup and Franz (1994).

Distribution (Fig. 5-47): USA: Alabama, Florida.

Polycentropus floridensis is apparently a narrow-range endemic restricted to the western panhandle of Florida and nearby areas of southern Alabama.

Material Examined: **Holotype**: **USA**. **Florida**: Walton County, Eglin Air Force Base; Headwaters of Rocky Creek; 4 miles southwest of Mossy Head, J.F. Scheiring coll., 11-v-1979, 1 male (USNMENT01507919). **Paratype**: **USA**. **Alabama**: Baldwin County, Pine Log Creek at Hwy. 59, [N31°08'39.90", W87°48'28.30"], S.C. Harris coll., 11-v-1982, 1 male (CUAC000016085).

Non-type material: **USA**. **Florida**: Okaloosa County, Eglin Air Force Base at BR-625, Rogue Creek, UV Blacklight, [N30°33'19". W86°35'51"], A. Rasmussen, M. Pescador,

D. Denson, D. Ray, and B. Albrecht coll., 7-viii-2008, 1 male (UMSP 000173197). Turkey Hen Creek (East Branch) at head, Eglin Air Force Base, 0.3 km W Okaloosa Tower, [N30°38'48", W86°33'23"], R.W. Flowers, M.L. Pescador, A.K. Rasmussen, and B.A. Richard coll., 17-iv-2006, 1 male (FAMU). Unnamed tributary to Turkey Creek N of Eglin 626, T. Thom, T. Dall, and J. Tritt coll., 22-iii-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 coll., 27-v-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 coll., 11-i-2006, 2 larvae (FAMU). UV blacklight, [N30°27'54", W86°53'21"], R.W. Flowers, M.L. Pescador, A.K. Rasmussen, and B.A. Richard coll., 19-iv-2006, 1 male (FAMU). Weaver Creek steephead, Eglin Air Force Base, [N30°30'26", W86°54'42"], R.W. Flowers, A.K. Rasmussen, and B.A. Richard coll., 19-iv-2006, 1 male (FAMU). Walton County, Alaqua Creek East on Hwy. 282, P. Carlson coll., 27-iii-1971, 1 male (CUAC0000107305).

Polycentropus maculatus Banks

(Fig. 5-2; Figs 5-24 — 5-25; Fig. 5-48)

Polycentropus maculatus Banks, 1908: 65, fig. 6, ♂; type locality “Grand Lake, Newfoundland” (MCZ).

Polycentropus maculatus—Betten, 1934: 221.

Polycentropus maculatus—Milne, 1938: 88 (as junior synonym of *P. confusus* Hagen, 1861).

Polycentropus maculatus—Ross, 1944: 65, fig. 243, ♂; fig. 258, allotype ♀.

Polycentropus maculatus—Marshall and Larson, 1982: 25, fig. 48, ♂.

Polycentropus maculatus—Nimmo, 1986: 197, figs 147—150, ♂; figs 151—152, ♀.

Polycentropus maculatus—Armitage and Hamilton, 1990: figs A—D, ♂; figs E—F, ♀.

Polycentropus maculatus—Hoffman and Morse, 1990: 278—280, figs 9—12, ♀.

Diagnosis: Males of *Polycentropus maculatus* are most similar to males of *P. chelatus* and *P. neiswanderi*, and *P. stephani* in the strong downward curvature of the phallus beyond its middle, and *P. carolinensis* and *P. carlsoni* in the blade-like curvature of the ventral portion of each inferior appendage in ventral view. Males of *P. maculatus* are separable from those of *P. chelatus*, *P. neiswanderi*, and *P. stephani* by the posteroventral triangular process of the body of each preanal appendage and by the blade-like shape and breadth of the ventral portion of each inferior appendage in ventral view. From those of *P. carolinensis* and *P. carlsoni*, males of *P. maculatus* are separated by the strong downward curvature of the phallus and shape of the basodorsal process of each inferior appendage.

Females of *P. maculatus* are readily separated from all other females of the *Polycentropus confusus* Species Group other than that of *P. neiswanderi* by the ventrally recurved apex of the external parts of gonopods VIII. From *P. neiswanderi*, females of *P. maculatus* are separated by the shape of the internal parts of the gonopods, which are broad, subrectangular, and tapered anterad in *P. maculatus* versus narrow, oblong, and tapering at the posterior apex in *P. neiswanderi*.

Adult Description:

General (Fig. 5-2). Length of male 5.0—6.2 mm (mean = 5.7 mm; n = 5). Length of female 5.6—8.5 mm (mean = 7.1 mm; n = 5).

Male Genitalia (Figs 5-24A—5-24E). Abdominal segment VIII annular. Terga IX and X fused, membranous, extended caudad over bases of intermediate appendages. Sternum IX ovoid in lateral view, posterior margin slightly sinuous. Intermediate appendages originating beneath terga IX+X and extending beyond them, curved ventrad, their apices each bearing 5 small setae; in dorsal view apices proximate, subparallel. Bodies of preanal appendages each with triangular posteroventral process; dorsal process long, curved ventrad, extending below head of basodorsal process of corresponding inferior appendage, acute apically; in dorsal view slender, elongate, subparallel, apices appearing recurved slightly laterad. Inferior appendages in lateral view each with capitate basodorsal process erect with moderately long neck and subtriangular head round dorsally and projecting ventrad with its pointed apex visible above main body of appendage, main body of appendage with dorsal and ventral margins subparallel for 2/3 length then convex subapicoventrally and tapering to acute upturned apex extended posterad slightly beyond intermediate appendages; in ventral view divergent, each blade-like, wide basally, distally tapering to pointed apex, inner margin slightly sinuous, basodorsal process completely hidden by main body of inferior appendage, in cleared specimen broad, with medial pointed projection; in caudal view oblong, with medial projection round. Phallus a fully sclerotized tube, in lateral view with larger base, curved about 90° ventrad slightly beyond mid-length, apical section subrectangular, internal spinules absent, internal phallic sclerite short, vertical, with round ventral base and tapered dorsal vertex.

Female Genitalia (Figs 5-25A—5-25B). Venter VIII in ventral view with ventral plates convergent, narrow, subtriangular, tapering apically, covered by setae; in lateral view each terminating in round posterior apex; posterior apex of external parts of gonopods VIII in ventral view with posterior margin subtriangular and round mesally; in lateral view extending beyond ventral plates in flat process with its tiny apex recurved ventrad; internal parts of gonopods VIII in ventral view visible through venter VIII even in uncleared specimen, appearing longitudinally wrinkled, darker than surrounding tissue, subparallel, elliptical, with round posterior apices distinctly posterior of apices of ventral plates, anterior ends tapered, extending to sclerotized external part of gonopods VIII at basal third of ventral plates; in ventral view anterior part of genital chamber sclerotized, strongly sinuous, wrapping around posterior apex of processus spermathecae; anterior apex extending anterad beyond sclerotized external part of gonopods VIII, posterior apex with subovoid projection on either side of processus spermathecae; processus spermathecae subovoid with central elevation bearing opening of ductus spermathecae.

Larva: Described in Chapter 4.

Pupa: Unknown.

Biology: Adults have been collected from May to October from riparian habitat of small streams to small rivers, most often with cobble substrate. This species is among the more common eastern Nearctic *Polycentropus* species and is often collected syntopically with other members of the species group, most often *P. confusus*, *P. blicklei*, and *P. carolinensis*.

Distribution (Fig. 5-48): Canada: Newfoundland and Labrador, Nova Scotia, Ontario, Quebec; U.S.A.: Connecticut, Kentucky, Maine, Massachusetts, New Hampshire, New Jersey*, New York, North Carolina, Ohio, Pennsylvania, South Carolina, Tennessee, Vermont, Virginia, West Virginia.

Despite extensive sampling of Illinois by Ross and others and Michigan by Houghton and others, *P. maculatus* has not been recorded from either of these states, even though it is recorded from neighboring Ohio and Kentucky. Similarly, because *P. maculatus* has been recorded from neighboring states and provinces, it likely also occurs in Delaware, Rhode Island, Maryland, and New Brunswick.

Material Examined: **Holotype**: **Canada**. **Newfoundland and Labrador**: Grand Lake, O. Bryant coll., 28-vii-1906, 1 male (MCZ Entomology 11550). **Allotype**: **USA**. **Tennessee**: Sevier County, Great Smoky Mountains National Park, Chimneys Campgrounds, At Light, A.C. Cole coll., 16-vii-1939, 1 female (INHS Insect Collection 37261).

Non-type material: **USA**. **New Jersey**: Burlington County, Brendon T Burns State Forest, Mt Misery, [N39°54'58.09", W79°30'52.69"], S. Harris coll., 3-vii-2012, 5 females (FAMU). **New State Record. New York**: Clinton County, Deep Inlet Brook, Chazy Lake Road, [N44°35'46.32", W73°50'18.60"], L. Myers and C. Binggeli coll., 31-vii-2008, 1 male (BYU). Adirondack Park, Bear Brook near Blue Mountain Lake, Frison and Ross coll., 19-vi-1941, 1 female (INHS Trichoptera 54335). **North Carolina**: Jackson County, Balsam Mtn. Preserve, 6.6 km E. Sylva, S. Fork Sugarloaf Creek, Malaise-Townes Trap, 1180 m asl , [N35°22'17.04", W83°06'37.08"], J.C. Morse coll., 3—6-vi-2019, 3 males, 6 females (CUAC000093025). Balsam Mtn. Preserve, 4.5 km E. Sylva, S Fork Sugarloaf Creek, 1164 m asl , [N35°22'16.86", W83°06'36.11"], M.W. Green coll., 20-ix-2019, 1 male (CUAC000092984). Macon County, E Fk Overflow Cr., 790 m asl , [N35°01'04.44", W83°14'42.36"], R.L. Heth coll., 23-vii-2018, 1 male (CUAC000107327). Ravenel Lake at Highlands Biological Station, 1250 m asl , [N35°03'14.40", W83°11'22.92"], M.A. Floyd coll., 19-vi-2019, 1 male

(CUAC000088820). **Pennsylvania**: Schuylkill County, Owl Creek, [N40°46'58.80", W75°57'39.60"], T. Bringloe, V. Harvey, S. Ripley, K. Rondollo coll., 18-vi-2013, 1 male (BIOUG06970-G02). Rattling Run, [N40°35'02.40", W76°01'01.20"], 1 male (BIOUG07980-H09). 1 male (BIOUG07980-H11). 1 male (BIOUG07980-H10). **Tennessee**: Blount County, Jakes Creek at Elkmont, UV light, J.K. Moulton coll., 27-vii-2005, 1 male (FAMU). Monroe County, Cherokee National Forest, Bald River at Holly Flats Recreation Area, 667 m asl , [N35°17'11.04", W84°10'41.88"], Holzenthal, Blahnik, Hamilton, Chamorro L., and Robertson coll., 31-v-2003, 1 male (UMSP 000102505). Morgan County, Crooked Fork at Petros, Rt. 116 "fire department", J.K. Moulton coll., 10-v-2006, 1 male, 3 females (FAMU). Sevier County, Great Smoky Mountains National Park, Chimneys Campgrounds, Ross and Stannard coll., 1-ix-1948, (missing abdomen), 1 female (INHS Trichoptera 54336). **West Virginia**: Hardy County, 3 miles northeast of Mathias, [N38°33'00.00", W78°29'24.00"], D. Smith coll., 7-vi-2007, 1 male (BYU). Mingo County, Laurel Creek, 2 miles east of Dingess, Kondratieff and Kirchner coll., 27-v-1993, 1 male (BYU).

Polycentropus neiswanderi Ross

(Figs 5-26 — 5-27; Fig. 5-49)

Polycentropus neiswanderi Ross, 1947: 135-136, fig. 10, ♂; ♀; type locality “Shawnee Forest, Ohio" (INHS).

Polycentropus neiswanderi—Nimmo, 1986: 197-198, figs 153—157, ♂; figs 158—159, ♀.

Polycentropus neiswanderi—Armitage and Hamilton, 1990: figs A—D, ♂; figs E—F, ♀.

Diagnosis: Males of *P. neiswanderi* most resemble those of *P. chelatus* in the shape of the basodorsal process of the inferior appendage and *P. maculatus* in the strong ventral curvature of the phallus. From males of *P. chelatus*, those of *P. neiswanderi* can be separated by the shorter, straighter dorsobasal processes of the inferior appendages, the deep emargination at the mid-height the bodies of the preanal appendages, dorsocaudal round process of the bodies of the preanal appendages as seen in lateral view, and by the strong ventral curvature of the phallus. From males *P. maculatus*, those of *P. neiswanderi* are identified by the digitiform basodorsal processes of the inferior appendages, the incurvate apices of the dorsal processes of the preanal appendages, and by the shape of the body of the preanal appendages which, in *P. maculatus*, possesses a subtriangular posteroventral process and lacks any emargination.

Females of *P. neiswanderi* are most similar to those of *P. maculatus* in the tiny, ventrally recurved apex of the posterior apex of external parts of gonopods VIII as viewed in lateral aspect and in having wrinkled, elliptical internal parts of gonopods VIII. Females of *P. neiswanderi* are also somewhat similar to those of *P. pentus* in overall form. Females of *P. neiswanderi* can be separated from females of both *P. maculatus* and *P. pentus* by the shape and orientation of the ventral plates which, in ventral view, appear slender and subparallel rather than broad and convergent. In *P. neiswanderi*, the ventral plates are semi-elliptical in ventral view, versus subtriangular in *P. maculatus*. Also, *P. neiswanderi* females exhibit the tiny, ventrally recurved apex of the posterior apex of external parts of gonopods VIII as viewed in lateral aspect that is absent in *P. pentus*. Finally, from *P. maculatus*, *P. neiswanderi* differs in having a much simpler, slender, semicircular anterior part of genital chamber.

Adult Description:

General Length of male 5.9—6.8 mm (mean = 6.3 mm; n = 5). Length of female 6.1—7.1 mm (mean = 6.5 mm; n = 5).

Male Genitalia (Figs 5-26A—5-26E). Abdominal segment VIII semi-annular. Terga IX and X fused, membranous, extended caudad over bases of intermediate appendages. Sternum IX ovoid in lateral view, posterior margin slightly sinuous with caudoventral lip. Intermediate appendages originating beneath terga IX+X and extending beyond them, curved slightly ventrad, their apices each bearing 5 small setae; in dorsal view apices proximate, subparallel, together appearing bifid. Bodies of preanal appendages each with round ventral half, deep emargination at mid-height, small, round dorsocaudal process ventrad of base of dorsal process; dorsal process long, curved ventrad about 90°, extending below apex of basodorsal process of corresponding inferior appendage, acute apically; in dorsal view slender, elongate, subparallel for basal 2/3 length, distally incurvate for final 1/3 length. Inferior appendages in lateral view each with digitiform basodorsal process erect, finger-like, round dorsally, main body of appendage broad with dorsal and ventral margins subparallel, surface with dorsomesal excavation to about mid-length, round apex extended posterad slightly anterior to intermediate appendages; in ventral view subparallel, wide basally, medial and lateral margins subparallel for 1/3 length, medial margin curving outward beyond about 1/3 length to round apex, basodorsal process mostly hidden by main body of inferior appendage, lateral margins extending beyond main body of inferior appendage near apex, in cleared specimen rounded with medial blunt projection; in caudal view oblong, with medial projection round. Phallus a fully sclerotized tube, in lateral view with larger base, curved about 90° ventrad near apex, apical section subrectangular with basoventral lip, internal spinules absent, two internal phallic sclerites each moderately long, vertical, with round ventral base and round dorsal vertex.

Female Genitalia (Figs 5-27A—5-27B). Venter VIII in ventral view with ventral plates subparallel, narrow, elliptical, subtly tapering apically, covered by setae; in lateral view each terminating in round posterior apex; posterior apex of external parts of gonopods VIII in ventral view with posterior margin round; in lateral view extending beyond ventral plates in tapered process with its tiny apex recurved ventrad; internal parts of gonopods VIII in ventral view visible through venter VIII even in uncleared specimen, appearing longitudinally wrinkled, darker than surrounding tissue, slightly convergent, semi-elliptical, with round posterior apices distinctly anterior of apices of ventral plates, joined by inverted-U-shaped bridge medially, anterior ends wide, extending to sclerotized external part of gonopods VIII at basal third of ventral plates; in ventral view anterior part of genital chamber sclerotized, slender, semicircular, wrapping around processus spermathecae; processus spermathecae subovoid with distomesal elevation bearing opening of ductus spermathecae.

Larva: Unknown.

Pupa: Unknown.

Notes: The paratype with code INHS Insect Collection 38137 has characters intermediate of *P. chelatus* and *P. neiswanderi*, already two morphologically similar species. The phallus curvature and shape of the body of the preanal appendage align more with *P. chelatus*, while the structure of the basodorsal process aligns with *P. neiswanderi*. Upon comparing this specimen to the holotype of *P. chelatus*, Hamilton identified this specimen as *P. chelatus*. This would represent a new state record (Ohio) for the species. Upon my examination of the specimen and comparison with type material, I am cautiously maintaining the *P. neiswanderi* paratype designation. In addition, a male specimen of *P. neiswanderi* listed as collected in Arkansas is included in the NMNH database but could not be found and is lacking an accession code. Arkansas would represent a new state record for the species but without the specimen and with little collection data, this cannot be confirmed. Finally, Houghton et al. (2022) reported *P. neiswanderi* from Illinois, though this record is erroneous and the known distribution of *P. neiswanderi* is restricted to Ohio and Kentucky.

Biology: Almost nothing is known of the biology of this rarely collected species. Adults have been collected using light traps in April, May, and June near small, cool streams. Floyd et al. (2012) classified this species as Threatened in Kentucky.

Distribution (Fig. 5-49): USA: Kentucky, Ohio.

This species is evidently a narrow-range endemic. Dubious or erroneous state records have been reported, as discussed in the Notes subsection above.

Material Examined: **Holotype**: **USA**. **Ohio**: Shawnee Forest, Light Trap, vi-1942, 1 male (INHS Insect Collection 38172). **Paratypes**: **USA**. **Ohio**: Lawrence County, Dean Forest, Light Trap, C. Neiswander coll., v-1939, 1 male (INHS Insect Collection 38137). Shawnee Forest, vi-1942, 1 female (INHS Trichoptera 24579), 4 males (INHS Trichoptera 24578), 1 female (INHS Insect Collection 38135), 12 females (INHS Insect

Collection 38136), 2 males (INHS Trichoptera 24577). **Allotype**: **USA**. **Ohio**: Shawnee Forest, Light Trap, vi-1942, 1 female (INHS Trichoptera 24576).

Non-type material: **USA**. **Kentucky**: Marion County, Tributary of Salt Lick Creek at Salt Lick Creek Rd., J.K. Moulton coll., 28-v-2006, 2 males (FAMU).

Polycentropus pentus Ross

(Figs 5-28 — 5-29; Fig. 5-50)

Polycentropus pentus Ross, 1941: 71-72, fig. 45, ♀; figs 49—51, ♂; type locality "Thunder Bay, Ontario" (INHS).

Polycentropus pentus—Ross, 1944: 65, fig. 242, ♂; fig. 269, ♀.

Polycentropus pentus—Nimmo, 1986: 198, figs 160—164, ♂; figs 165—166, ♀.

Polycentropus pentus—Armitage and Hamilton, 1990: figs A—D, ♂; figs E—F, ♀.

Polycentropus pentus—Houghton, 2012: fig. 277, ♂.

Diagnosis: Males of *P. pentus* are most similar to those of *P. confusus* in general appearance and those species that possess a round ventral swelling of the phallus, namely *P. dinkinsorum*, *P. floridensis*, and *P. vernus*. From males of all of these species, those of *P. pentus* are separated by the mesocaudal processes of each body of the inferior appendages. From males of *P. confusus*, those of *P. pentus* are further separated by the presence of a basoventral swelling of the phallus and by the lack of microspinules on the apex of the phallus. From males of *P. dinkinsorum*, *P. floridensis*, and *P. vernus*, those of *P. pentus* are also separated by the distinct shape of the basodorsal process of the inferior appendage.

Females of *P. pentus* show similarities to females of many species of the *P. confusus* Species Group and are identifiable by the unique shape of the wrinkled internal parts of gonopods VIII and by the sinuous dorsal margin of the posterior apex of external parts of gonopods VIII as seen in lateral aspect.

Adult Description:

General Length of male 8.0—10.0 mm (mean = 9.1 mm; n = 5). Length of female 8.5—9.2 mm (mean = 8.9 mm; n = 5).

Male Genitalia (Figs 5-28A—5-28E). Abdominal segment VIII annular. Terga IX and X fused, membranous, extended caudad over bases of intermediate appendages. Sternum IX subovoid in lateral view, posterior margin slightly sinuous with ventral lip. Intermediate appendages originating beneath terga IX+X and extending beyond them, curved ventrad apically, their apices each bearing 4 small setae; in dorsal view apices proximate, subparallel, together appearing bifid. Bodies of preanal appendages each with apically round, subtriangular mesocaudal process bordered dorsally and ventrally by emarginations, posteroventral process shallow, round; dorsal process long, curved ventrad, extending to apex of basodorsal process of corresponding inferior appendage, acute apically, bearing at least one seta apicoventrally; in dorsal view slender, elongate, subparallel, slightly convergent apically. Inferior appendages in lateral view each with elongate basodorsal process erect with short neck and long digitiform head round dorsally, apex curved caudad, with sharp mesoventral point visible above main body of appendage, main body of appendage with dorsal and ventral margins concave for 1/5 length, tapering evenly beyond 1/5 length to round upturned apex terminating anterad of intermediate appendages; in ventral view subparallel, inner margin curved laterad basally for 1/3 length, curved mesad along middle 1/3 length, reaching widest point about mid-length, curving laterad for final 1/3 length, distally tapering to round apex, lateral margin and distal apex of basodorsal process exposed beyond lateral margins of main body of inferior appendage, apex of medial projection extending beyond medial margin of main body of inferior appendage, in cleared specimen elongate, with medial projection round; in caudal view subtriangular, with wide base and broad posteromesal projection round. Phallus a fully sclerotized tube, in lateral view with larger base bearing basoventral swelling, curved about 30° ventrad slightly beyond mid-length, apical section round with small ventral bump, internal spinules absent, internal phallic sclerite long, horizontal, with broad irregular basal apex deeply emarginate and slender distal apex subrectangular.

Female Genitalia (Figs 5-29A—5-29B). Venter VIII in ventral view with ventral plates convergent, semi-elliptical, tapering apically, covered by setae; in lateral view each terminating in round posterior apex; posterior apex of external parts of gonopods VIII in ventral view with posterior margin subtriangular and round mesally; in lateral view subtriangular, extending beyond ventral plates, dorsal margin sinuous; internal parts of gonopods VIII in ventral view visible through venter VIII even in uncleared specimen, appearing longitudinally wrinkled, darker than surrounding tissue, basally divergent, tapering to triangular base at sclerotized external part of gonopods VIII at basal third of ventral plates. distally subparallel, subrectangular, with mesocaudal corner slightly anterior of apices of ventral plates; in ventral view anterior part of genital chamber apparently absent; processus spermathecae semi-circular with distomesal elevation bearing opening of ductus spermathecae.

Larva: Described in Chapter 4.

Pupa: Unknown.

Biology: Adults of this species have been collected from May to September using UV light traps and Malaise traps. Late-instar larvae have been collected from fast flowing, cool streams with rocky and woody substrates from April to July. Unlike most wide-ranging species, *P. pentus* appears to be absent from the Coastal Plain physiographic region.

Distribution (Fig. 5-50): 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.

This species has a wide distribution. Areas for which it is not known but where it has been taken from surrounding states or provinces are Indiana, Maryland, Delaware, Rhode Island, Massachusetts, and New Brunswick. Whereas all remaining members of the *P. confusus* Species Group are restricted to the eastern Nearctic Region, five specimens (three males, two females) of *P. pentus* were collected in Crook County, Wyoming, suggesting that a disjunct population exists. This could suggest that the species was previously much more widespread, and that the Wyoming specimens represent a relict population. Alternatively, and perhaps less likely, the Wyoming records could be the result of an introduced population.

Material Examined: **Holotype**: **Canada**. **Ontario**: Thunder Bay, H.S. Parish coll., 1-vii-1937, 1 male (INHS Insect Collection 38173). **Paratypes**: **Canada**. **Ontario**: Algonquin Provincial Park, Costello Lake, Ontario Fisheries Research Laboratory, W.M. Sprules coll., 2-vi-1939, 4 males (INHS Trichoptera 24580), 1 female (INHS Insect Collection 37256). 3-vi-1939, 2 males (INHS Insect Collection 37254). 4-vi-1939, 4 females (INHS

Trichoptera 24582). 6-vi-1939, 4 females (INHS Trichoptera 24583). Non-type material: **Canada**. **New Brunswick**: Moose Bog, [N47°15'57.60", W66°02'27.60"], CC, AM, KB and MP coll., 25-vi-2008, 1 male (08NBEPT-1300). **Newfoundland and Labrador**: Gros Morne National Park, James Callaghan Trail (aka Gros Morne Trail), Malaise Trap, 39 masl, [N49°34'06.96", W57°49'48.72"], Anderson coll., 16-vii-2013, 1 male (BIOUG10090-F07). Tablelands Trail, 175 masl, [N49°28'37.20", W57°58'12.00"], BIObus 2013 coll., 20-vii-2013, 1 male (BIOUG14743-A01). 1 male (BIOUG15057-D03). **Nova Scotia**: Kejimkujik National Park, Eel Weir Road, 96 masl, [N44°20'53.52", W65°11'20.40"], BIObus2013 coll., 22-vi-2013, 1 female (BIOUG16546-G04). Jeremy`s Bay Campground, near Amphitheater off of Campfire Circle , 116 masl, [N44°24'23.76", W65°14'43.80"], D. Crossland and K. Rowter coll., 11-vii-2013, 1 female (BIOUG11447-F01). **Ontario**: Algonquin Provincial Park, Small Stream 2.5 km after North River Canoe Launch, 462 masl, [N46°04'51.60", W78°26'19.68"], C. Freutel and G. Martin coll., 12-vii-2011, 1 larva (BIOUG00331-B10). 1 larva (BIOUG00331-C05). 1 larva (BIOUG01723-C03). 1 larva (BIOUG01723-D05). Bruce Peninsula National Park, Cyprus Lake Gate House, UV Light Sheet, [45°13'48", W81°31'48"], BIObus 2008 coll., 14-vi-2008, 1 female (08ONCAD-0079). Gate house light, 12-vi-2008, 1 female (08ONCAD-0066). Nipigon River, D.A. Etnier coll., 2-vii-2004, 2 males, 3 females (ECC 3.432). **Quebec**: Gatineau Park, near Hull, Fortune Creek at Ridge Road, W.E. Ricker coll., 20-vi-1965, 1 female (INHS Trichoptera 54357). **USA**. **Kentucky**: Bell County, Cumberland Gap National Historical Park headwaters of Shilalah Creek, J. Robinson coll., 4-iv-2007, 5 larvae (CUGA 4588). Franklin County, Unnamed tributary at Camp Pleasant, J.K. Moulton coll., 8-v-2007, 1 male (FAMU). **Vermont**: Windsor County, Ascutney Notch, 200 yards from end of road, Weathersfield, T. Ames coll., 11-vi-2007, 1 female (BYU). **West Virginia**: Hardy County, 3 miles northeast of Mathias, Malaise Trap, [N38°55', W78°49'], D. Smith coll., 22-v-7-vi-2007, 7 males, 3 females (BYU). [N38°33'00.00", W78°29'24.00"], 21-v-2007, 1 male (BYU). **Wyoming**: Crook County, 3 ft. wide, clear mountain stream, Black Hills National Forest, R.J. Lavigne coll., 22-vii-1982, 2 males, 2 females (BYU). Sundance, gully at the edge of town, I-90, R.J. Lavigne and M. Pogue coll., 23-vi-1981, 1 male (BYU).

Polycentropus pixi Ross

(Figs 5-30 — 5-31; Fig. 5-51)

Polycentropus pixi Ross, 1944: 66-67, fig. 247, ♂; fig. 255, ♀; type locality “North Woodstock, New Hampshire" (INHS).

Polycentropus pixi—Nimmo, 1986: 198-199, figs 122—126, ♂.

Polycentropus pixi—Armitage and Hamilton, 1990: figs A—D, ♂; figs E—F, ♀.

Diagnosis: Males of *P. pixi* can be separated from those of all remaining species in the *P. confusus* Species Group by the dorsomesal margin of the ventral portion of each inferior appendage being produced into small sharp tooth near mid-length, visible in lateral view. Males of *P. pixi* are most similar to *P. centralis* in the shape and size of the phallus and the shape of the ventral portions of the inferior appendages in ventral view, and to *P. carolinensis* in the shape of the basodorsal process of each inferior appendage. From males of *P. centralis*, those of *P. pixi* can be further separated by the ventral curvature of the apical section of the phallus and the smaller, rounder head of the basodorsal process of each inferior appendage with a less pronounced and more blunt ventral projection. From males of *P. carolinensis*, those of *P. pixi* are further separated by the ventral portions of each inferior appendages being shorter and digitiform as seen in ventral aspect, and by the broader, straighter phallus.

Females of *P. pixi* can be separated from those of all remaining species of the *P. confusus* Species Group by the following combination of characters: the distinct shape of the smooth internal parts of gonopods VIII that is somewhat similar to the females of only *P. stephani*, the anterior parts of the genital chamber being broad and semi-circular with a slightly sinuous posterior margin, and the ventral plates being semi-elliptical and convergent.

Adult Description:

General Length of male 6.5—7.6 mm (mean = 6.9 mm; n = 4). Length of female 7.1—8.0 mm (mean = 7.5; n = 3).

Male Genitalia (Figs 5-30A—5-30E). Abdominal segment VIII annular. Terga IX and X fused, membranous, extended caudad over bases of intermediate appendages. Sternum IX ovoid in lateral view, posterior margin sinuous. Intermediate appendages originating beneath terga IX+X and extending caudad beyond them, their apices each bearing 4 small setae; in dorsal view apices proximate, subparallel. Bodies of preanal appendages each broad, produced caudad, extended furthest caudad at mid-height, ventral half of posterior margin sinuous; dorsal process long, curved posteroventrad, extending roughly even with ventral margin of head of basodorsal process of corresponding inferior appendage, acute apically; in dorsal view slender, elongate, gradually convergent. Inferior appendages in lateral view each with capitate basodorsal process erect with wide, moderately long neck and small head round dorsally and projecting ventrad with its blunt apex visible above main body of appendage, main body of appendage wide basally, dorsomesal margin produced into small sharp tooth near mid-length, surface with shallow dorsomesal excavation along middle 1/3 length, tapering to blunt slightly upturned apex anterior to intermediate appendages; in ventral view basally subparallel to about mid-length, slightly divergent beyond mid-length, digitiform, slender, distally tapering to round apex, lateral margin slightly concave near mid-length, basodorsal process oblong, mostly hidden by main body of inferior appendage, in cleared specimen broad, with medial round projection exposed beyond inner margin of main body of inferior appendage, meeting or nearly meeting medial projection of basodorsal process of other inferior appendage; in caudal view oblong, with medial projection round. Phallus a fully sclerotized tube, in lateral view with larger base, nearly straight along 4/5 length, apical section subrectangular, curved ventrad roughly 25°, internal spinules absent, internal phallic sclerite moderate length, semi-vertical, with slender, acute anterodorsal base and widening to subrectangular posteroventral apex.

Female Genitalia (Figs 5-31A—5-31B). Venter VIII in ventral view with ventral plates convergent, narrow, semi-elliptical, tapering apically, covered by setae; in lateral view each terminating in round posterior apex; posterior apex of external parts of gonopods VIII in ventral view with posterior margin broadly round; in lateral view extending dorsoposterad beyond ventral plates, tapering to round apex; internal parts of gonopods VIII in ventral view visible through venter VIII even in uncleared specimen, appearing smooth, darker than surrounding tissue, subparallel for most of length, with round anterior bases slightly divergent, round posterior apices slightly anterior of apices of ventral plates with sharp caudolateral corner meeting ventral plates, anterior ends tapered, extending to sclerotized external part of gonopods VIII near base of ventral plates, surfaces bearing lateromesal excavation along 4/5 length; in ventral view anterior part of genital chamber sclerotized, broad, semi-circular, posterior margin slightly sinuous, wrapping around posterior apex of processus spermathecae; processus spermathecae circular with distomesal elevation bearing opening of ductus spermathecae.

Larva: Unknown.

Pupa: Unknown.

Notes: One male specimen of *P. pixi* listed as having been collected in Nova Scotia and a second listed as having been collected in New Jersey are included in the NMNH database but could not be found and are lacking accession codes. Nova Scotia and New Jersey would represent new province and state records, respectively, for the species but without the specimens and with little collection data, these cannot be confirmed.

Biology: Almost nothing is known of the biology of this uncommon species. Adults have been collected from May to July near cool lotic systems ranging from low order streams to navigable rivers.

Distribution (Fig. 5-51): Canada*: New Brunswick*; USA: Massachusetts, New Hampshire, New York, Ohio, Pennsylvania, Vermont, Virginia.

This species appears to be restricted to the northeastern United States and far eastern Canada. The two male specimens reported here from New Brunswick, Canada represent new province and country records. Several states and provinces from which *P. pixi* has not been reported despite neighboring territories having confirmed records of the species include West Virginia, Maryland, Delaware, New Jersey, Connecticut, Rhode Island, Maine, Quebec, Prince Edward Island, and Nova Scotia.

Material Examined: **Holotype**: **USA**. **New Hampshire**: Grafton County, North Woodstock, At Light, Frison and Ross coll., 21-vi-1941, 1 male (INHS Insect Collection 38130). **Paratypes**: **USA**. **New Hampshire**: Grafton County, North Woodstock, At Light, Frison and Ross coll., 21-vi-1941, 1 female (INHS Trichoptera 24585). **New York**: Wyoming County, Varysburg, 18-vi-1941, 1 female (INHS Trichoptera 24586).

Non-type material: **Canada**. **New Brunswick**: Northumberland County, Renous River, McGraw Brook, [N46°49'21.00", W66°06'33.12"], X. Zhou and D. Baird coll., 23-vi-2008, 1 male (08NBEPT-0050) **New Country Record**, 1 male (08NBEPT-0138) **New Country Record**. **USA**. **New York**: Greene County, Winter Clove BLT, Maple Lawn Road, [N42°15'25.56", W74°02'24.36"], L. Myers and B.C. Kondratieff coll., 24-vi-2007, 1 male (BYU). **Virginia**: Bath County, Blowing Springs, Route 39, 10 miles west of Warm Springs; Back Creek, [N38°04'12.00", W79°53'17.88"], O. Flint coll., 17-v-2004, 1 male (USNMENT01507896), 1 male (USNMENT01507895), 1 male (USNMENT01507894), 1 male (USNMENT01507893). Highland County, Route 84, Townsend Draft, [N38°17'59.64", W79°46'22.80"], O. Flint and S. Roble coll., 8-vi-2011, 1 female (USNMENT01507903), 1 female (USNMENT0157904), 1 female (USNMENT01507902), 1 female (USNMENT01507901), 1 female (USNMENT01507900), 1 female (USNMENT01507899), 1 female (USNMENT0157905), 1 male (USNMENT01507898), 1 male (USNMENT01507897). Rockingham County, Hone Quarry Camp, [N38°27'43.92", W79°08'06.00"], R.A Flint and O. Flint coll., 17-18-vii-1964, 1 female (USNMENT01507892), 1 male (USNMENT01507891).

Polycentropus stephani Bowles, Mathis, and Hamilton

(Figs 5-32 — 5-33; Fig. 5-52)

Polycentropus stephani Bowles, Mathis, and Hamilton, 1993: 31-32, figs 1—3, ♂; fig. 4, ♀; type locality “Logan Co., Mt. Magazine, Green Beach, Gutter Rock Creek" (NMNH).

Polycentropus species B and C—Bowles and Mathis, 1989: 237

Polycentropus stephani—Moulton and Stewart, 1996: figs 557, 560-561, ♂.

Diagnosis: Males of *Polycentropus stephani* are separated from all other males of the *P. confusus* Species Group by the presence of a caudally directed basoventral spur on the phallus. The basodorsal processes of the inferior appendages are similar to those of *P. chelatus* and *P. neiswanderi* in being somewhat finger-like, but differ from those two species by the small basoventral protuberance.

Females of *P. stephani* are most similar to those of *P. chelatus* in respect to the posterior apex of external parts of gonopods VIII appearing elongate and digitiform in lateral view and in the internal parts of gonopods VIII being smooth, and to females of *P. pixi* in the general shape of the smooth internal parts of gonopods VIII and the ventral plates being convergent. From females of *P. chelatus*, those of *P. stephani* are separated by the sinuous margins of the posterior apex of external parts of gonopods VIII, the convergent and semi-elliptical ventral plates, and the apparent absence of the sclerotized anterior part of genital chamber. From females of *P. pixi*, females of *P. stephani* are separated by the convergent internal parts of gonopods VIII, the posterior apex of external parts of gonopods VIII appearing digitiform in lateral view, and the apparent absence of the sclerotized anterior part of genital chamber.

Adult Description:

General Length of male 5.1—6.0 mm (mean = 5.6 mm; n = 5). Length of female 5.9—7.0 mm (mean = 6.4 mm; n = 4).

Male Genitalia (Figs 5-32A—5-32E). Abdominal segment VIII annular. Terga IX and X fused, membranous, extended caudad over bases of intermediate appendages. Sternum IX subovoid in lateral view, posterior margin slightly sinuous. Intermediate appendages originating beneath terga IX+X and extending beyond them, curved ventrad apically, their apices each bearing 3 small setae; in dorsal view apices proximate, parallel. Bodies of preanal appendages each with round caudal margin, subtriangular posteroventral corner, deep dorsal emargination, dorsal process long, curved ventrad, extending below apex of head of basodorsal process of corresponding inferior appendage, acute apically; in dorsal view slender, elongate, subparallel, apices incurvate. Inferior appendages in lateral view each with digitiform basodorsal process erect, elongate, round dorsally, with basoventral protuberance, curving slightly caudad beyond protuberance, main body of appendage with dorsal and ventral margins subparallel along length to round subtly upturned apex extended posterad, terminating slightly anterad to intermediate appendages; in ventral view subparallel to roughly mid-length, divergent beyond mid-length, each wide basally, inner margin sinuous basally to about mid-length, distally tapering beyond mid-length to round apex, basodorsal process mostly hidden by main body of inferior appendage, anterior apex projecting beyond medial margins, posterior apex projecting beyond lateral margins, in cleared specimen broad, oblong, apices round; in caudal view subtriangular, with medial projection round. Phallus a fully sclerotized tube, depth approximately uniform along length, bearing prominent caudally directed basoventral spur, curved about 80° ventrad at apex, apical section subtriangular, internal spinules absent, internal phallic sclerite long, horizontal, with round apices.

Female Genitalia (Figs 5-33A—5-33B). Venter VIII in ventral view with ventral plates convergent, broad, semi-elliptical, tapering apically, covered by setae; in lateral view each terminating in round posterior apex; posterior apex of external parts of gonopods VIII in ventral view with posterior margin subtriangular; in lateral view digitiform, dorsal and ventral margins sinuous, subparallel, extending beyond ventral plates with round apex; internal parts of gonopods VIII in ventral view visible through venter VIII even in uncleared specimen, appearing smooth, darker than surrounding tissue, convergent, subrectangular, with broad posterior apices slightly anterior of apices of ventral plates, anterior ends tapered, not extending to sclerotized external part of gonopods VIII at basal third of ventral plates; in ventral view anterior part of genital chamber apparently absent; processus spermathecae subovoid with distomesal elevation bearing opening of ductus spermathecae.

Larva: Unknown.

Pupa: Unknown.

Biology: Little is known of the biology of this apparently rare and seldom collected species. Adults have been collected using UV light traps from April to May near intermittent streams and small perennial streams with rocky substrate present. This species is often collected with the much more common species *Polycentropus centralis*.

Distribution (Fig. 5-52): USA: Arkansas.

This species appears to be endemic to the interior highlands of Arkansas.

Material Examined: **Holotype**: **USA**. **Arkansas**: Logan County, Mount Magazine, Green Beach, Gutter Rock Creek, UV Light, R. Leschen coll., 1-v-1987, 1 male (USNMENT01507918). **Paratypes**: **USA**. **Arkansas**: Independence County, unnamed intermittent stream, 18-iv-1987 coll., P.A. Harp, 1 male (USNMENT01507921). Logan County, Mt. Magazine, Green Beach, Gutter Rock Creek, UV Light, R. Leschen coll., 1-v-1987, 1 male, 1 female (FSCA). Washington County, Devils Den State Park, Blacklight, C.E. Carlton coll., 22-iv-1989, 1 male, 1 female (USNMENT01507922), 1 male, 1 female (USNMENT01507923).

Allotype: **USA**. **Arkansas**: Logan County, Mount Magazine, Green Beach, Gutter Rock Creek, UV Light, R. Leschen coll., 1-v-1987, 1 female (USNMENT01507924).

Non-type material: **USA**. **Arkansas**: Polk County, Route 71, north of Mena, O. Flint coll., 15-v-1958, 1 male, 1 pupa (lacking case and shed sclerites) (USNMENT01507925). Saline County, South Alum Creek, [N34°47'00.96”, W93°01'53.04”], A.L. Sheldon coll., 2-v-2001, 2 males, 15 females (ECC 3.478).

Polycentropus thaxtoni Hamilton and Holzenthal

(Fig. 5-34; Fig. 5-53)

Polycentropus thaxtoni Hamilton and Holzenthal, 1986: 163-165, figs 1—7, ♂; type locality “Georgia: Crawford County: Spring Creek above pond at Camp Eunice, approx. five miles SSE of Roberta (ca. 32°40’N, 83°59’W)” (USNM).

Polycentropus thaxtoni—Armitage and Hamilton, 1990: figs A—F, ♂.

Diagnosis: Males of *Polycentropus thaxtoni* are most similar to those of *P. chelatus*, *P. confusus*, *P. floridensis*, *P. neiswanderi*, and *P. pentus*, from which it can be separated by the following combination of characters: a long, dorsomesally excavated ventral portions of the inferior appendages with subrectangular apices, the deep dorsal emarginations of the bodies of the preanal appendages, and the lack of a basoventral swelling of the phallus.

Adult Description:

General Length of male 6.0—6.9 mm (mean = 6.3 mm; n = 4).

Male Genitalia (Figs 5-34A—5-34E). Abdominal segment VIII annular. Terga IX and X fused, membranous, extended caudad over bases of intermediate appendages. Sternum IX subovoid in lateral view, ventral half of posterior margin slightly sinuous, dorsal half of posterior margin concave. Intermediate appendages originating beneath terga IX+X and extending beyond them, curved slightly ventrad, their apices each bearing 3 small setae; in dorsal view apices proximate, subparallel. Bodies of preanal appendages each with subtriangular posteroventral corner, deep dorsal emargination, posterodorsal margin round; dorsal process long, curved roughly 90° ventrad, apex aligned with posterodorsal margin of head of basodorsal process of corresponding inferior appendage, acute apically; in dorsal view slender, subparallel, apices strongly incurvate. Inferior appendages in lateral view each with lobate basodorsal process erect with moderately short neck and broad head round dorsally and projecting caudad with its round apex visible above main body of appendage, main body of appendage with dorsal and ventral margins subparallel along length, dorsal margin curved slightly dorsad at subrectangular apex, surface with dorsomesal excavation along length; in ventral view subparallel, margins subparallel basally, mesal margin curving outward beyond mid-length, lateral margin curving inward apically, appendage distally tapering to round apex, apices oriented caudolaterally, distal half of basodorsal process exposed beyond lateral margins of main body of inferior appendage, medial projection mostly behind main body of inferior appendage, in cleared specimen elongate, with apex of medial round projection exposed mesally; in caudal view oblong, with wide base and slender posteromesal projection round. Phallus a fully sclerotized tube, in lateral view with larger base, curved about 20° ventrad slightly beyond mid-length, apical section rectangular, internal spinules absent, internal phallic sclerite long, horizontal, semi-elliptical.

Female Genitalia Unknown.

Larva: Unknown.

Pupa: Unknown.

Biology: Virtually nothing is known of the biology of this species. Adults have been collected in May and September using UV light traps. The type locality is a second order blackwater stream with abundant aquatic macrophytes, allochthonous plant matter, and some deadwood with a sand substrate matrix. This locality sits on private property in a rural area with little development and is denslely forested, appearing relatively undisturbed. This is promising when considering that this Spring Creek site is also the type locality and only known locations for *Beraea gorteba* Ross, 1944 (Beraeidae) and *Hydroptila roberta* Hamilton and Holzenthal, 1986 (Hydroptilidae). The single specimen of *P. thaxtoni* collected in Alabama was taken adjacent to a cool, clear, first order stream with rock and sand substrate.

Distribution (Fig. 5-53): USA: Alabama, Georgia.

Until recently, *Polycentropus thaxtoni* was known from only its type locality of Spring Creek in Crawford County, Georgia. Orfinger and Moulton (2021) reported a single male specimen from an unnamed stream in Dekalb County, Alabama.

Material Examined: **Holotype**: **USA**. **Georgia**: Crawford County, Spring Creek above pond at Camp Eunice, approximately 5 miles south-southeast of Roberta, UV Light, S.W. Hamilton and R.W. Holzenthal coll., 8-ix-1983, 1 male (USNMENT01507917).

Paratypes: **USA**. **Georgia**: Crawford County, Spring Creek above pond at Camp Eunice, approx. five miles SSE of Roberta, [N32°040', W83°059'], Hamilton and Holzenthal coll., 8-ix-1983, 1 male (CUAC000016084). Spring Creek above pond at Camp Eunice, approximately 5 miles south-southeast of Roberta, UV Light, S.W. Hamilton and R.W. Holzenthal coll., 29-ix-1983, 1 male (USNMENT01507920).

Non-type material: **USA**. **Alabama**: Dekalb County, unnamed stream crossing Rd 05 c. 220 m south Rd 09, 390 masl, [N34°27'01.42", W85°34'57.66"], J. Robinson and C.R. Parker coll., 11-v-2007, 1 male (LIRI 1098).

Polycentropus vernus Hamilton, Harris, and Lago

(Fig. 5-35; Fig. 5-54)

Polycentropus vernus Hamilton, Harris, and Lago, 1990: 365—367, fig. 2, ♂; type locality “Alabama: Fayette County: small intermittent stream entering Wallace Branch at headwaters, 5.5 mi SE Berry” (USNM, lost)

Polycentropus n. sp. (nr. *chelatus*)—Lago and Harris, 1987b: 258.

Diagnosis: The diminutive size of the male of *Polycentropus vernus* (2.8—3.6 mm forewing length) separates this species from other members of the *P. confusus* Species

Group. The next smallest measured male of the group was a specimen of *P. chelatus*, possessing a forewing length of 4.0 mm. This species is most similar to the *P. confusus* Species Group members with the more elongate head or enlargement on the basodorsal arm of the inferior append age. These species are *P. chelatus*, *P. confusus*, *P. floridensis*, *P. neiswanderi, P. pentus*, *P. stephani*, and *P. thaxtoni*. The species can be separated from the above-mentioned species through a combination of several characters including the shape of the body of the preanal appendage, the size and shape of the dorsal arm of the inferior appendage, the length and shape of the ventral arm of the inferior appendage, and the shape of the phallus. On *P. vernus* the emargination of the body of the preanal appendage is very shallow compared to *P. floridensis*, *P. stephani*, and *P. thaxtoni* and is also different from that of other members of the confusus group. The phallus of the *P. vernus* with its basoventral swelling is only similar to *P. dinkinsorum, P. floridensis* and *P. pentus*. The shape of the inferior appendage can be used to separate these three species from *P. vernus*. In *P. pentus* the basodorsal arm of the inferior appendage is strongly curved, almost sickle-shaped, while in *P. vernus* species it is triangular. Also, in *P. pentus* the mesobasal tooth on the basal arm of the inferior appendage is little developed, versus well developed in both *P. vernus* and *P. floridensis*. While the basodorsal arm of *P. floridensis* is similar to that of the *P. vernus*, the lateral margin of the basal arm is more excavated, exposing the relatively prominent mesobasal tooth. In *P. floridensis* and *P. dinkinsorum*, which have a ventral swelling of the phallus, the basodorsal process of the inferior appendage of these two species form a round head versus the elongate head of the basodorsal process of the inferior appendage of *P. vernus*.

Adult Description:

General Forewing length of male 2.8—3.6 mm.

Male Genitalia (Figs 5-35A—5-35E). Abdominal segment VIII annular. Terga IX and X fused, membranous, extended caudad over bases of intermediate appendages. Sternum IX semicircular in lateral view, posterior margin slightly sinuous. Intermediate appendages originating beneath terga IX+X and extending beyond them, curved ventrad apically, their apices each bearing 3 small setae; in dorsal view apices proximate, subtly divergent. Bodies of preanal appendages in lateral view each with round caudal margin, dorsomesal emargination at about mid-height, dorsal process long, curved ventrad, extending below apex of head of basodorsal process of corresponding inferior appendage, acute apically; in dorsal view slender, elongate, subparallel, apices incurvate. Inferior appendages in lateral view each with prominent basodorsal process erect, neck short, head large, shaped as oblong in-turned blade, apex curved slightly posterad, mesoventral protuberance small, main body of appendage with surface dorsomesal excavation to about mid-length, dorsal margin curved ventrad from base to 1/4 length, subparallel with ventral margin beyond 1/4 length, ventral margin slightly convex along length, tapering evenly to round apex extended posterad, terminating slightly anterad to intermediate appendages; in ventral view subparallel along length, each wide basally, tapering evenly to round apex, basodorsal process mostly hidden by main body of inferior appendage, anterior apex projecting beyond medial margins, in cleared specimen broad, round, anterior apex subtriangular, in caudal view stout, subtriangular, with medial projection subtriangular. Phallus tubular, in lateral view moderately decurved, with basal ventral swelling, apex slightly pointed, internal spinules absent; internal phallic sclerite distal in lateral view, moderately elongate, oblong and uniform in depth along length.

Female Genitalia Unknown.

Larva: Unknown.

Pupa: Unknown.

Notes: The type series represented all known material of this species. Unfortunately, the type series is lost and numerous collecting efforts over multiple years and using several methods at the type locations and nearby streams by the author yielded no new material. During those collection efforts, abundant coal mining and clearcut logging activity was observed at and around the stream sites. Due to the lack of available material, the description and illustrations presented here are based on the original description by Hamilton et al. (1990).

Biology: *Polycentropus vernus* has been collected most often in small intermittent and headwater streams of the Cumberland Plateau physiographic region from March to May.

Distribution (Fig. 5-54): USA: Alabama.

Material Examined: None (see Notes).

Material listed from the original publication is as follows:

Holotype. **United States**: **Alabama**: Fayette County: small intermittent stream entering Wallace Branch at headwaters, 5.5 mi SE Berry, T16S-R10-Sec. 36, sweep net, 16.v.1982, S.C. Harris. **Paratypes**. Fayette Co., same data as holotype, 10 males; same data except 26.iv.1983, 1 male; same data except 26.iv.1983, 1 male metamorphotype; same data except 15.iii.1984, 1 male; same data except 11.iv.1984. 9

males; same data except Wallace Branch at headwaters, 5 mi SE Berry, 16.v.1984, 2 males; Tuscaloosa Co., Wallace Branch, 5 mi S Berry, T17S-R10-Sec. 10, 28.iv.1982, 1 male, S.C. Harris; same data except 25.iv.1982, 1 male; Little Tyro Creek, 6 mi SE Berry, T17S-R10W-Sec. 11, 16.v.1984, 2 males, P.E. O'Neil and R.L. Smith; same data except 19.iii.1982, 1 male; small intermittent stream entering Little Tyro Creek, 6 mi SE Berry,T17S-R10-Sec. 11, 26.iv.1983, 1 male, S.C. Harris; Tyro Creek on unmarked Co. Rd., 3.5 mi E New Lexington, T17S-R10-Sec. 15, 28.iv.1982, 1 male; Walker County: Wolf Creek off Hwy 102, 7 mi S Carbon Hill, 26.iv.1983, 1 male, S.C. Harris and P. E. O’Neil.

Dichotomous Keys

Key to Males of the *Polycentropus confusus* Species Group

1 Apices of ventral portions of inferior appendages curved strongly inward in
ventral view (e.g., figs 5-5C; 5-9C)…2

1’ Apices of ventral portions of inferior appendages not curved strongly inward in
ventral view (e.g., figs 5-3C; 5-7C)…5

2(1) Body of preanal appendage with acute caudoventral projection in lateral view (e.g.,
figs 5-5A; 5-20A)…3

2’ Body of preanal appendage broad, blunt in lateral view (e.g., figs 5-9A; 5-11A)…4

3(2) Phallus with ventral swelling (fig. 5-20D); head of dorsobasal process of inferior
appendage blunt in lateral view (figs 5-20A)…*P. dinkinsorum* Orfinger and Etnier

3’ Phallus lacking ventral swelling (fig. 5-5D); head of dorsobasal process of inferior
appendage pointed in lateral view (fig. 5-5A)…*P. alabamensis* Hamilton,
Harris, and Lago

4(2’) Dorsobasal process of inferior appendage short, deflexed, with broad head in lateral view (fig. 5-9A)…*P. carlsoni* Morse

4’ Dorsobasal process of inferior appendage longer, thumb-like, with smaller, rounded head (fig. 5-11A)…*P. carolinensis* Banks

5(1’) Ventral portion of inferior appendage abruptly narrowing distally in ventral view (figs 5-28C; 5-32C)…6

5’ Ventral portion of inferior appendage gradually narrowing distally or not narrowing distally in ventral view (e.g., figs 5-17C; 5-26C)…12

6(5) Phallus with ventral swelling or spur (e.g., figs 5-23D; 5-32D)…7

6’ Phallus lacking ventral swelling or spur (e.g., figs 5-3D; 5-7D)…9

7(6) Phallus with rounded ventral swelling (e.g., fig. 5-23D)…8

7’ Phallus with basoventral spur (fig. 5-32D)…*P. stephani* Bowles, Mathis, and Hamilton

8(7) Dorsobasal process of inferior appendage narrow with sharp mesal point in lateral view (fig. 5-28A); body of preanal appendage bifid in lateral view (fig. 5-28A)…*P. pentus* Ross

8’ Dorsobasal process of inferior appendage broad with rounded mesal lobe in lateral view (fig. 5-23A); body of preanal appendage broad, rounded in lateral view (fig. 5-23A)…*P. floridensis* Lago and Harris

9(6’) Phallus curved strongly ventrad beyond middle in lateral view (e.g., figs 5-18D; 5-24D)…10

9’ Phallus curved only slightly ventrad beyond middle in lateral view (e.g., figs 5-3D; 5-21D)…11

10(9) Posterior apex of phallus broad, adorned with microspicules (fig. 5-18D); dorsobasal process of inferior appendage extending far beyond outside margins of ventral portion of inferior appendage in ventral view (fig. 5-18C)…*P. confusus* Hagen

10’ Posterior of apex of phallus not broad, lacking microspicules (fig. 5-24D); dorsobasal process of inferior appendage not extending beyond margins of ventral portion of inferior appendage in ventral view (fig. 5-24C)…*P. maculatus* Banks

11(9’) Dorsal processes of preanal appendages strongly incurved in dorsal view (fig. 5-3B; body of preanal appendage shallow, rounded in lateral view (fig. 5-3A); phallic sclerite restricted to anterior half of phallus (fig. 5-3D)…*P. aileenae* Orfinger and Moulton

11’ Dorsal processes of the preanal appendages subparallel, apices only slightly incurved in dorsal view (fig. 5-21B); body of preanal appendage more produced, acute in lateral view (fig. 5-21A); phallic sclerite along entire length of phallus (fig. 5-21D)…*P. elarus* Ross

12(5’) Head of dorsobasal process of inferior appendage acute in lateral view (e.g., figs 5-7A; 5-17A)…13

12’ Head of dorsobasal process of inferior appendage blunt in lateral view (e.g., figs 5-30A; 5-34A)…15

13(12) Dorsobasal process of inferior appendage erect (e.g., figs 5-7A; 5-13A); intermediate appendages parallel to subparallel in dorsal view, curved ventrad in lateral view (e.g., figs 5-7A, B; 5-13A, B)…14

13’ Dorsobasal process of inferior appendage strongly deflexed (fig. 5-17A); intermediate appendages curved dorsad, inflected inward, crossing (fig. 5-17A, B)…*P. chenoides* Ross and Yamamoto

14(13) Phallus nearly straight in lateral view; phallic sclerite large, occupying posterior 2/3 of phallus length in lateral view (fig. 5-13E); dorsal processes of preanal appendages extending posteriorly as far as termination of segment IX+X in dorsal view (fig. 5-13C)…*P. centrali*s Banks

14’ Phallus curved slightly ventral beyond middle in lateral view (fig. 5-7D); phallic sclerite small, restricted to ventral margin of posterior ¼ of phallus in lateral view (fig. 5-7D); dorsal processes of preanal appendage extending posteriorly well past segment IX+X in dorsal view (fig. 5-7B)…*P. blicklei* Ross and Yamamoto

15(12’) Dorsobasal process of the inferior appendage not digitiform, with ventral emargination in lateral view (e.g., figs 5-30A; 5-34A)…16

15’ Dorsobasal process of the inferior appendage digitiform, roughly uniform depth along length in lateral view (e.g., figs 5-15A; 5-26A)…18

16(15) Body of preanal appendage with shallow or no emargination in lateral view (figs 5-30A; 5-35A); dorsobasal process of inferior appendage not extending beyond outside margins of ventral portion of inferior appendage in ventral view (e.g., figs 5-30C; 5-35C)…17

16’ Body of preanal appendage with deep emargination in lateral view (fig. 5-34A); dorsobasal process of inferior appendage extending far beyond outside margins of ventral portion of inferior appendage in ventral view (fig. 5-34C)…*P. thaxtoni* Hamilton and Holzenthal

17(16) Phallus with basoventral swelling (fig. 5-35D); whole animal small in size, forewing length not exceeding 3.6 mm…*P. vernus* Hamilton, Harris, and Lago

17’ Phallus lacking basoventral swelling (fig. 5-30D); whole animal larger in size, forewing length at least 5.5 mm…*P. pixi* Ross

18(15’) Body of preanal appendage with prominent dorsal point, deep emargination in lateral view (fig. 5-26A); phallus strongly curved ventrad apically in lateral view fig. 5-26D); dorsobasal process of inferior appendage approximately straight along length (fig. 5- 26A)…*P. neiswanderi* Ross

18’ Body of preanal appendage lacking dorsal point, not emarginate in lateral view (fig. 5-15A); phallus only moderately curved ventrad apically (fig. 5-15D); dorsobasal process of inferior appendage curved caudad beyond middle (fig. 5-15A)…*P. chelatus* Ross and Yamamoto

Key to the Known Females of Eastern Nearctic *Polycentropus* Curtis, 1835

Accurate identifications using this key are dependent upon viewing cleared genitalia. While some species might be identifiable without clearing genitalia, it is strongly recommended that users first clear the genitlia to best visualize internal morphology.

The females of the following eastern Nearctic *Polycentropus* species remain unknown: *P. chenoides* Ross and Yamamoto, *P. dinkinsorum* Orfinger and Etnier, *P. floridensis* Lago and Harris, *P. thaxtoni* Hamilton and Holzenthal, and *P. vernus*

Hamilton, Harris, and Lago of *the Polycentropus confusus* Species Group, and *P. barri* Ross and Yamamoto and *P. colei* Ross of the *Polycentropus colei* Species Group.

1 Posterior apex of the external parts of gonopods VIII broad and rounded, slightly triangular, or bifid, but not narrow and acute (e.g., figs 5-4B; 5-6B; 5-8B; 5-10B)…2 (*Polycentropus confusus* Species Group)

1’ Posterior apex of the external parts of gonopods VIII terminating in narrow, acute, thorn-like process in ventral view (fig. 1E by Yamamoto (1966))…*P. rickeri* Yamamoto

2(1) Internal parts of gonopods VIII appearing smooth (e.g., figs 5-16B; 5-19B)…3

2’ Internal parts of gonopods VIII appearing wrinkled (e.g., figs 5-4B, 5-6B)…8

3(1) Anterior part of genital chamber well-formed, sclerotized (e.g., figs 5-14B; 5-22B)…4

3’ Anterior part of genital chamber poorly defined or absent (e.g., figs 5-19B; 5-33B)…6

4(3) Anterior part of genital chamber smooth, not appearing cushioned (e.g., figs 5-22B; 5-31B)…5

4’ Anterior part of genital chamber appearing cushioned (fig. 5-14B)…*P. centralis* Banks

5(4) External parts of gonopods VIII terminating posteriorly in tapered, acute, slightly recurved apex in lateral view (fig. 5-22A)…*P. elarus* Ross

5’ External parts of gonopods VIII terminating posteriorly in blunt, rounded apex in lateral view (fig. 5-31A)…*P. pixi* Ross

6(3’) External parts of gonopods VIII digitiform in lateral view with rounded posterior apex (e.g., figs 5-16A; 5-33A; ventral plates elongate, posterior apex rounded (e.g., figs 5-16A, B; 5-33A, B)…7

6’ External parts of gonopods VIII not digitiform, terminating posteriorly in tapered, pointed apex in lateral view (fig. 5-19A); ventral plates truncate, posterior apex sinuous in lateral view (fig. 5-19A)…*P. confusus* Hagen

7(6) Processus spermathecae subovoid, lacking accompanying process in ventral view (fig. 5-33B); ventral plates partially overlaying internal parts of gonopods VIII in ventral view (fig. 5-33B)…*P. stephani* Bowles, Mathis, and Hamilton

7’ Processus spermathecae subtriangular, possessing ensiform process projected posteriorly in ventral view (fig 5-16B); ventral plates not overlaying internal parts of gonopods VIII in ventral view (fig. 5-16B)…*P. chelatus* Ross and Yamamoto

8(2’) Ventral plates convergent in ventral view (e.g., figs 5-10B; 5-25B)…9

8’ Ventral plates parallel to subparallel in ventral view (e.g., figs 5-6B; 5-27B)…13

9(8) Anterior parts of genital chamber semicircular (e.g., figs 5-4B; 5-10B)…10

9’ Anterior parts of genital chamber sinuous or apparently absent (e.g., 5-25B; 5-29B)…12

10(9) Internal parts of gonopods VIII semielliptical or blade-like in ventral view (e.g., figs 5-4B; 5-8B)…11

10’ Internal parts of gonopods VIII subrectangular in ventral view (fig. 5-10B)…*P. carlsoni* Morse

11(10) Internal parts of gonopods VIII semielliptical in ventral view (fig. 5-4B)…*P. aileenae* Orfinger and Moulton

11’ Internal parts of gonopods VIII blade-like in ventral view (fig. 5-8B)…*P. blicklei* Ross and Yamamoto

12(9’) Anterior parts of genital chamber sinuous, elaborate (fig. 5-25B); apex of external parts of gonopods VIII ventrally recurved in lateral view (fig. 5-25A, B)…*P. maculatus* Banks

12’ Anterior parts of genital chamber apparently absent (fig. 5-29B); apex of external parts of gonopods VIII straight, oriented caudally (fig. 5-29A, B)…*P. pentus* Ross

13(8’) Posterior apex of external parts of gonopods VIII rounded (e.g., figs 5-12B; 5-27B)…14

13’ Posterior apex of external parts of gonopods VIII bifid (fig. 5-6)…*P. alabamensis* Hamilton, Harris, and Lago

14(13) Apex of external parts of gonopods VIII blunt, ventrally recurved in lateral view (fig. 5-27A); anterior parts of genital chamber semicircular in ventral view (fig. 5-27B)…*P. neiswanderi* Ross

14’ Apex of external parts of gonopods VIII acute, curved slightly dorsally in lateral view (fig. 5-12A; anterior parts of genital chamber semitrapezoidal in ventral view (fig. 5-12B)…*P. carolinensis* Banks

Discussion and Future Directions

This revision of the nineteen nominal members of the *Polycentropus confusus* Species Group sought to comprehensively treat the taxonomy of all males and known females of the group based on extensive examination of type- and non-type material. The effort here builds upon the previous works aimed at associating larvae and sexes of Nearctic *Polycentropus* Species (Chapter 2) and describing western Nearctic (Chapter 3) and eastern Nearctic (Chapter 4) *Polycentropus* larvae. As a result of this taxonomic treatment, males of all species and females of the 12 species for which females were previously known were redescribed and diagnosed. In addition, females of three species, *P. alabamensis*, *P. carolinensis* and *P. chelatus*, were described and diagnosed for the first time based on material associated using mtDNA barcoding (Chapter 2). Neotypes were designated for *P. alabamensis* and *P. elarus*, and synoptic distribution and biological data and notes were provided for each species. Finally, original dichotomous keys for males of all species and known females of the *P. confusus* Species Group and *P. colei* Species Group were provided.

Females of several eastern Nearctic species of the genus remain unknown, namely *P. chenoides*, *P. dinkinsorum*, *P. floridensis*, *P. thaxtoni*, and *P. vernus* of the *P. confusus* Species Group, and *P. barri* and *P. colei* of the *P. colei* Species Group. Whereas male members of the *P. confusus* Species Group are readily identifiable as members of the group based on the male genitalic characters discussed earlier, characters useful in delimiting females of these species groups are lacking, hence the key to females herein includes eastern Nearctic *Polycentropus* species of both species groups. The descriptions of, and key to, females provided in this chapter serve as a framework on which to build upon as additional females are associated and described.

In addition to continued investigation of females of the *P. confusus* Species Group, several other areas of inquiry are still needed. One such example is the case of *Polycentropus vernus*, for which no known material exists. This diminutive species was described by Hamilton et al. (1990) from several proximate localities from the Cumberland Plateau physiographic of northwest and west-central Alabama during the Spring. I executed three collecting trips over two years at and around the type localities without success. Malaise traps, UV light traps, and beating sheets were all employed. During these excursions, I observed ample evidence of coal mining and clearcutting activity surrounding streams. It is possible that the species is extinct. Perhaps more likely is that it has been locally extirpated from some localities but that populations exist elsewhere still, awaiting discovery. Until that time, no neotype can be designated and our knowledge of the species is restricted to the original description.

Other clear avenues of research include resolving the taxonomy of pupae of the *P. confusus* Species Group, investigating the phylogeny of this group, and sampling areas where gaps in known distribution exist. No pupae have been described from this species group, nor for any Nearctic members of the genus. Similarly, although Hamilton (1986) addressed the phylogeny of two of the Nearctic *Polycentropus* species groups, no effort has been made to assess historical relationships within the *P. confusus* Species Group. Hamilton emphasized the need for additional larval and female characters prior to such an undertaking. Preliminary molecular phylogenies based solely on mtCOI were produced in Chapter 2 and suggest that the *P. confusus* Species Group is monophyletic; however, a more robust phylogeny with rooted outgroups is needed to verify this. The work here, and additional nuclear and mitochondrial molecular data produced in previous studies (e.g., Orfinger et al., 2021) paves the way for this undertaking. Once a robust phylogeny has been produced, questions relating to the monophyly of the group, character mapping, and biogeographic patterns dealing with widespread, narrow-range, and disjunct populations (e.g., those *P. pentus* recovered in Wyoming) can be addressed. Finally, gaps exist in the distributional maps that should be investigated further. In addition to new country, state, and province records in this dissertation, several recent papers have reported new US state and Canadian province records for members of the *P. confusus* Species Group (Orfinger and Etnier, 2020; Houghton et al., 2022; Orfinger and Moulton, 2021). Undoubtedly, some of the gaps in distribution maps represent true gaps in distribution. However, it is likely that many of these state or provincial gaps are artifacts of sampling efforts and/or rare populations or species, particularly in those instances where surrounding territories have verified records of a given species.

It is clear that there is much work to be done regarding the taxonomy, phylogeny, ecology, and distribution of the *Polycentropus confusus* Species Group and the remaining Nearctic *Polycentropus* fauna. Still, the current study has made significant strides in providing a synoptic treatment of the *P. confusus* Species Group, providing identification tools for the known species, and identifying gaps in knowledge that should be prioritized moving forward. Aside from my continued work on the Nearctic *Polycentropus* fauna, it is hoped that future students will use this and previous chapters to springboard their own studies on this fascinating fauna ripe in need of additional attention.

Figure 5-1. Left lateral habitus of the male of *Polycentropus aileenae* Orfinger and Moulton, 2021. Scale bar = 1 mm.

Reproduced from Orfinger and Moulton (2021).

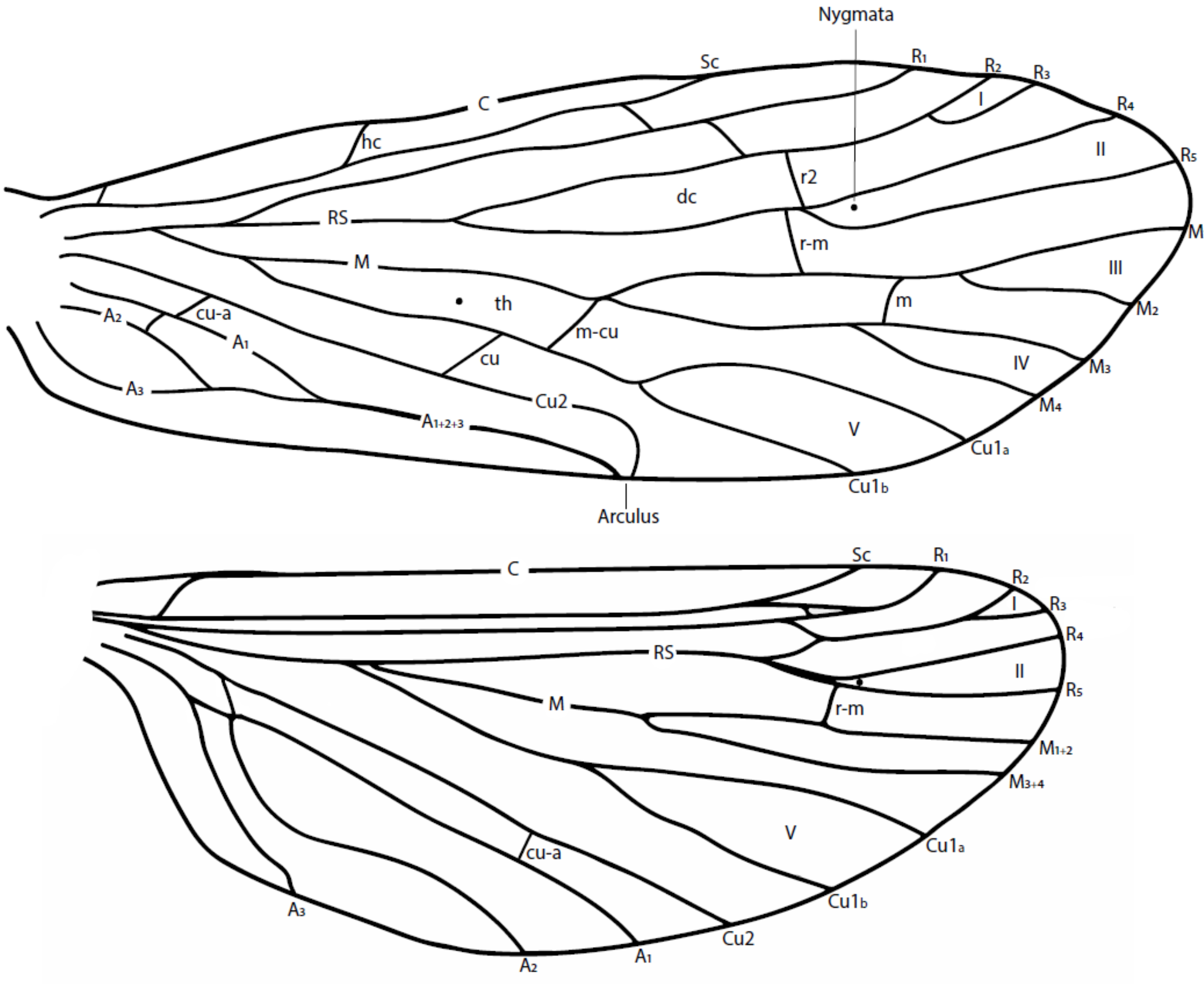

Figure 5-2. Male right forewing (above) and hind wing (below) of *Polycentropus maculatus* Banks, 1908.

Abbreviations: C = costa; Sc = subcosta; R = Radius, radial veins; Rs = radial sector; M = Media, median veins; Cu = cubitus; A = anal veins; dc = discoidal cell; cu-a = cubito-anal crossvein; hc = humeral crossvein; m-cu = median-cubital crossvein; r-m = radial-medial crossvein; th = thyridial cell; I-V = wing forks 1-5.

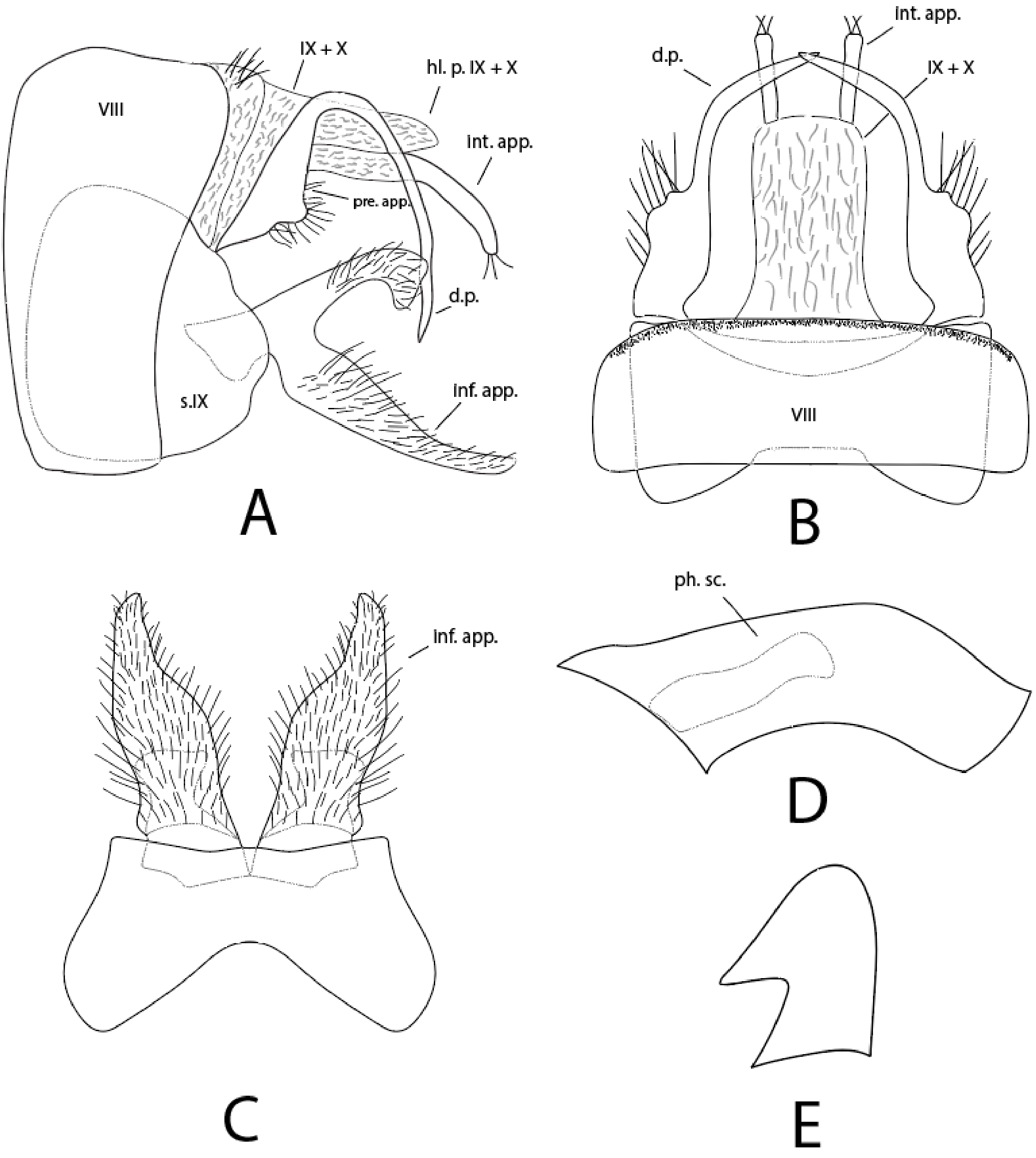

Figure 5-3. *Polycentropus aileenae* Orfinger and Moulton, 2021, male genitalia (holotype).

A. left lateral (with phallus removed); B. dorsal; C. segment IX and inferior appendages, ventral; D. phallus, left lateral; E. 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.

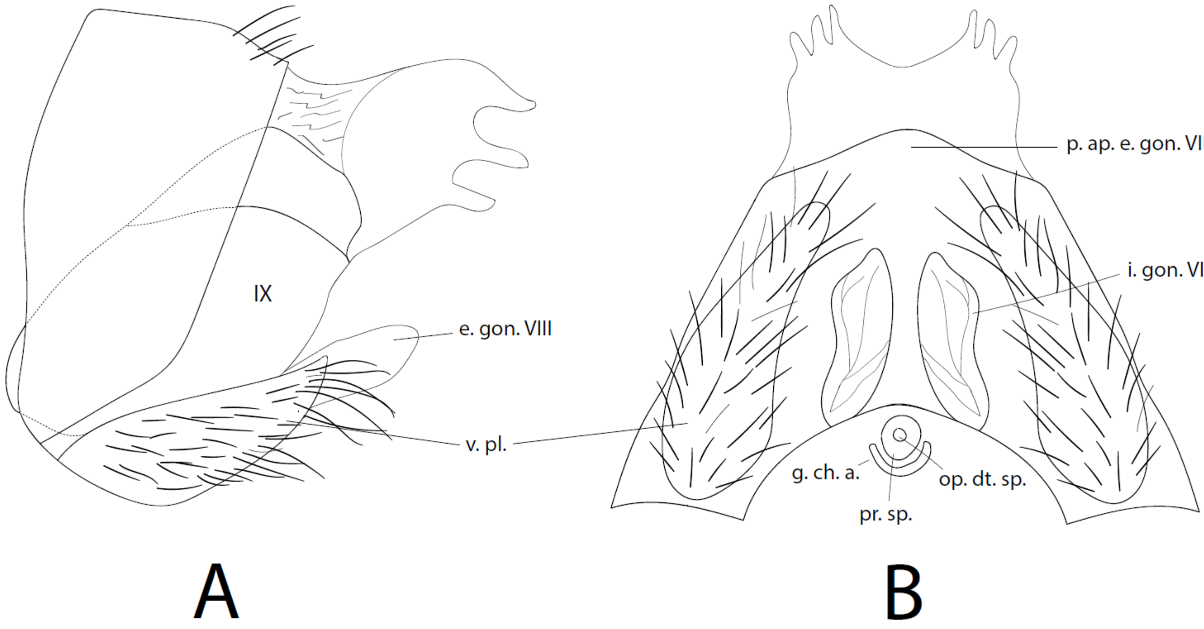

Figure 5-4. *Polycentropus aileenae* Orfinger and Moulton, 2021, female genitalia.

A. left lateral; B. 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.

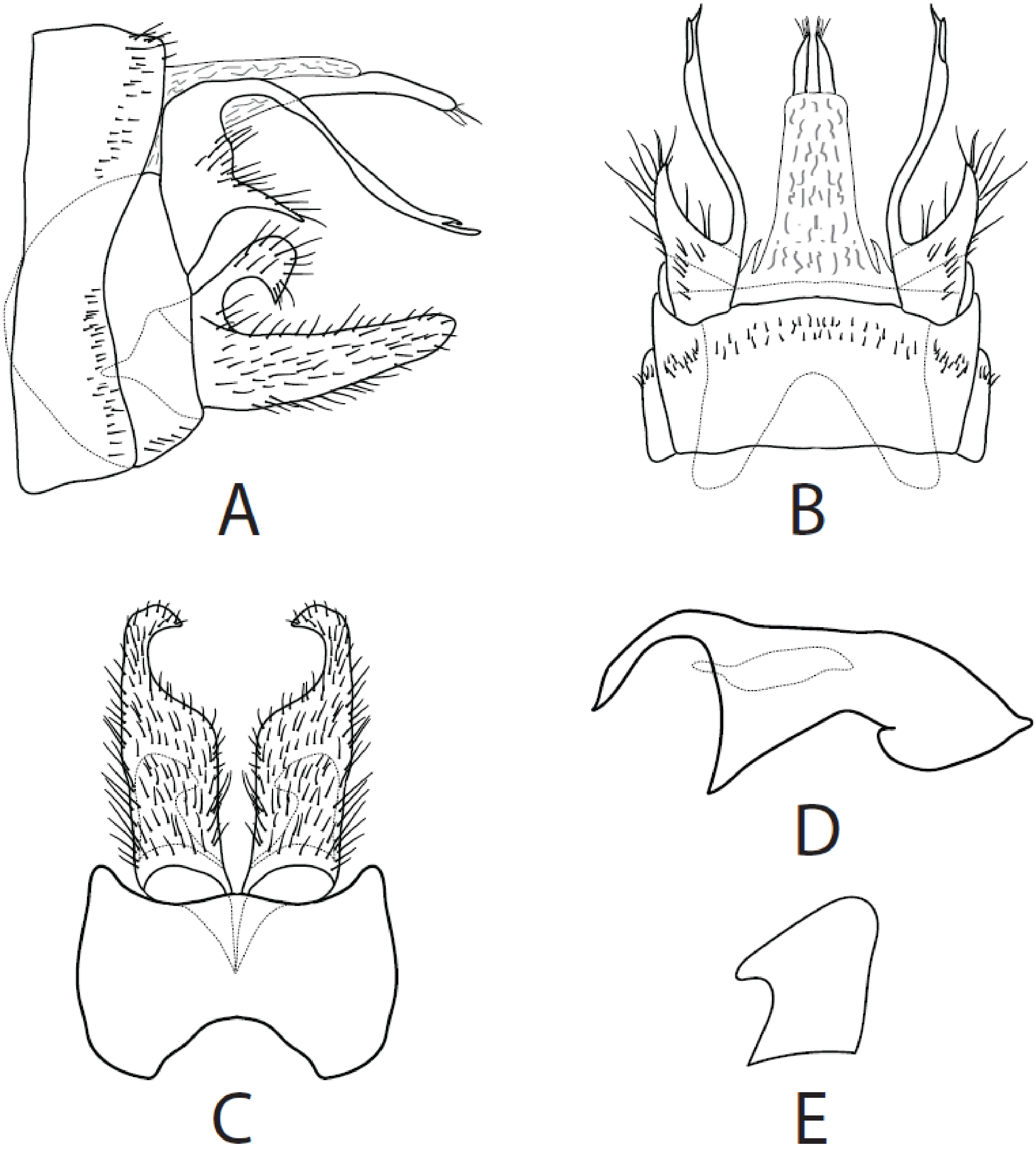

Figure 5-5. *Polycentropus alabamensis* Hamilton, Harris, and Lago, 1990, male genitalia (neotype).

A. left lateral (with phallus removed); B, dorsal; C, segment IX and inferior appendages, ventral; D, phallus, left lateral; E, dorsobasal process of right inferior appendage, caudal.

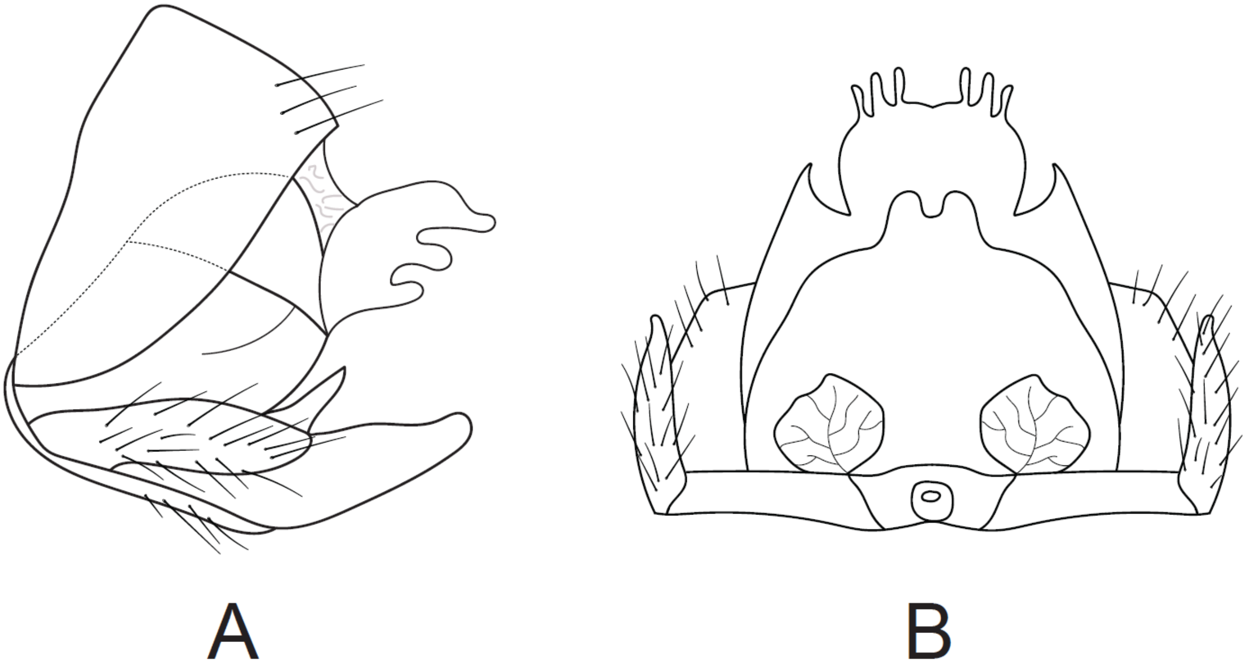

Figure 5-6. *Polycentropus alabamensis* Hamilton, Harris, and Lago, 1990, female genitalia. A. left lateral; B. ventral.

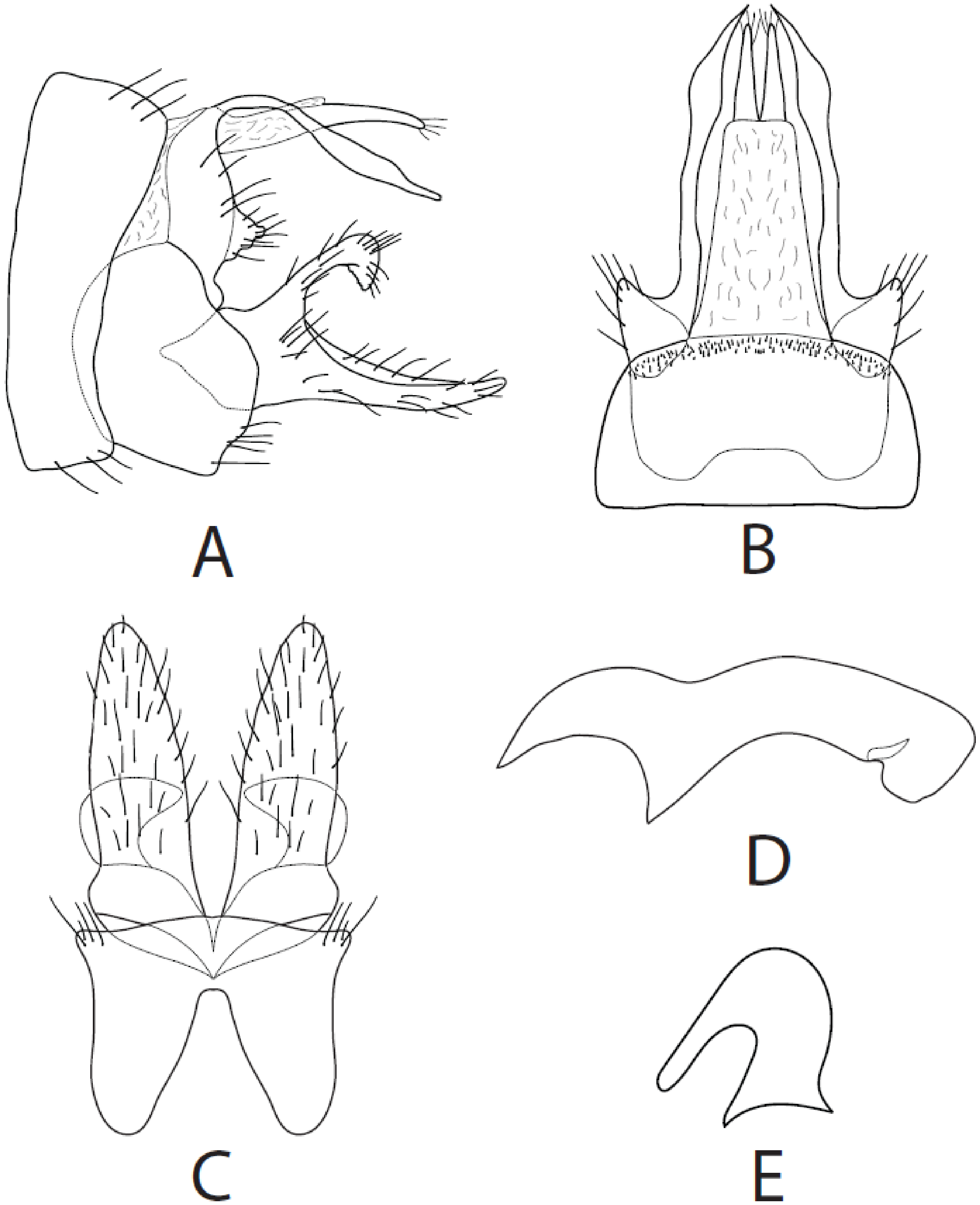

Figure 5-7. *Polycentropus blicklei* Ross and Yamamoto, 1965, male genitalia (holotype).

A. left lateral (with phallus removed); B. dorsal; C. segment IX and inferior appendages, ventral; D. phallus, left lateral; E. dorsobasal process of right inferior appendage, caudal.

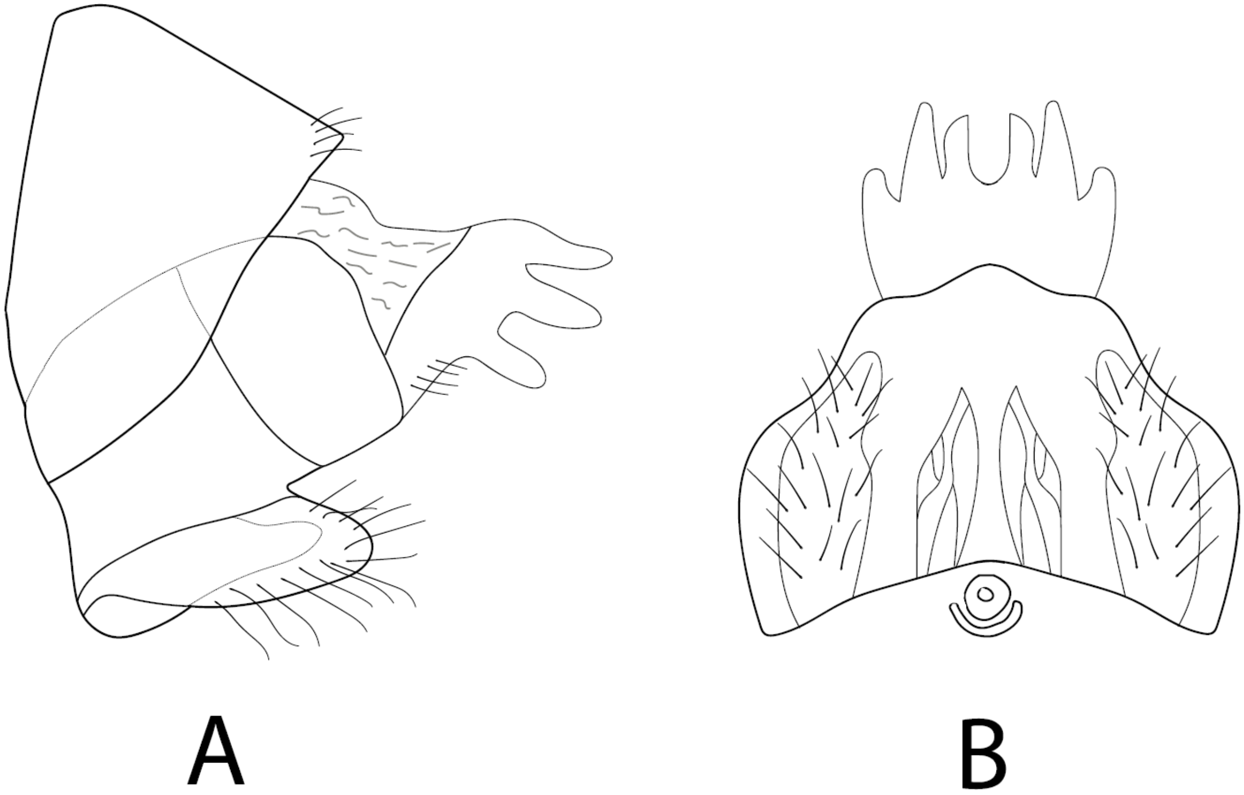

Figure 5-8. *Polycentropus blicklei* Ross and Yamamoto, 1965, female genitalia. A. left lateral; B. ventral.

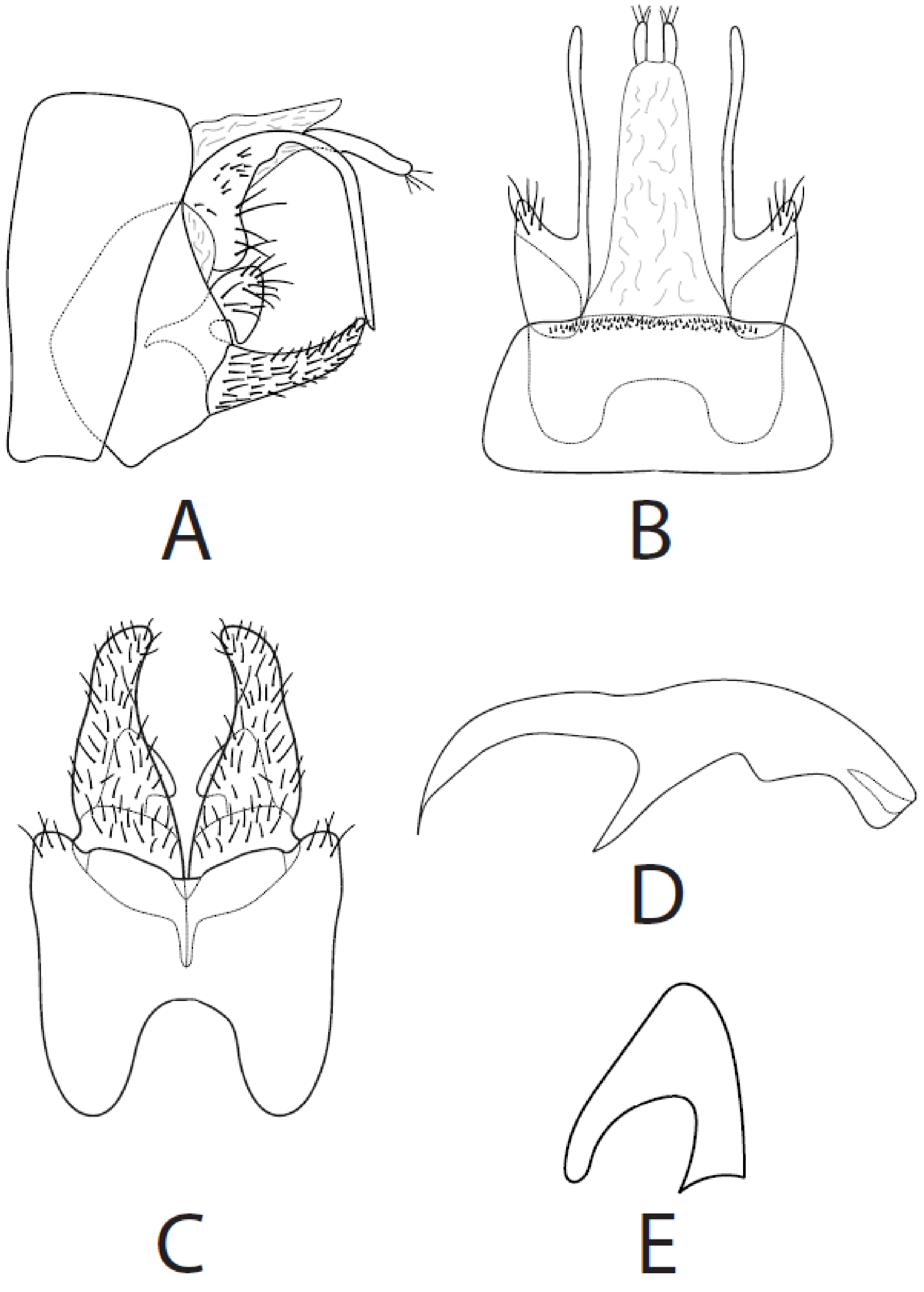

Figure 5-9. *Polycentropus carlsoni* Morse, 1971, male genitalia (holotype).

A. left lateral (with phallus removed); B. dorsal; C. segment IX and inferior appendages, ventral; D. phallus, left lateral; E. dorsobasal process of right inferior appendage, caudal.

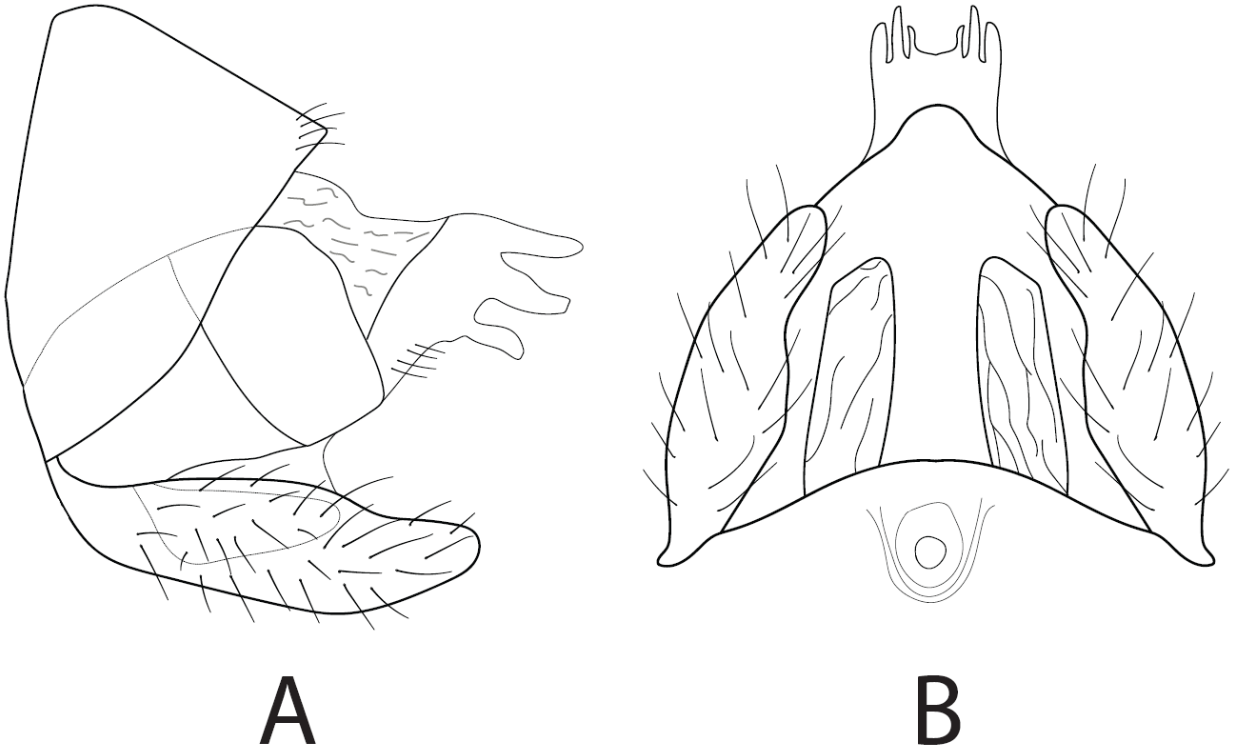

Figure 5-10. *Polycentropus carlsoni* Morse, 1971, female genitalia. A. left lateral; B. ventral.

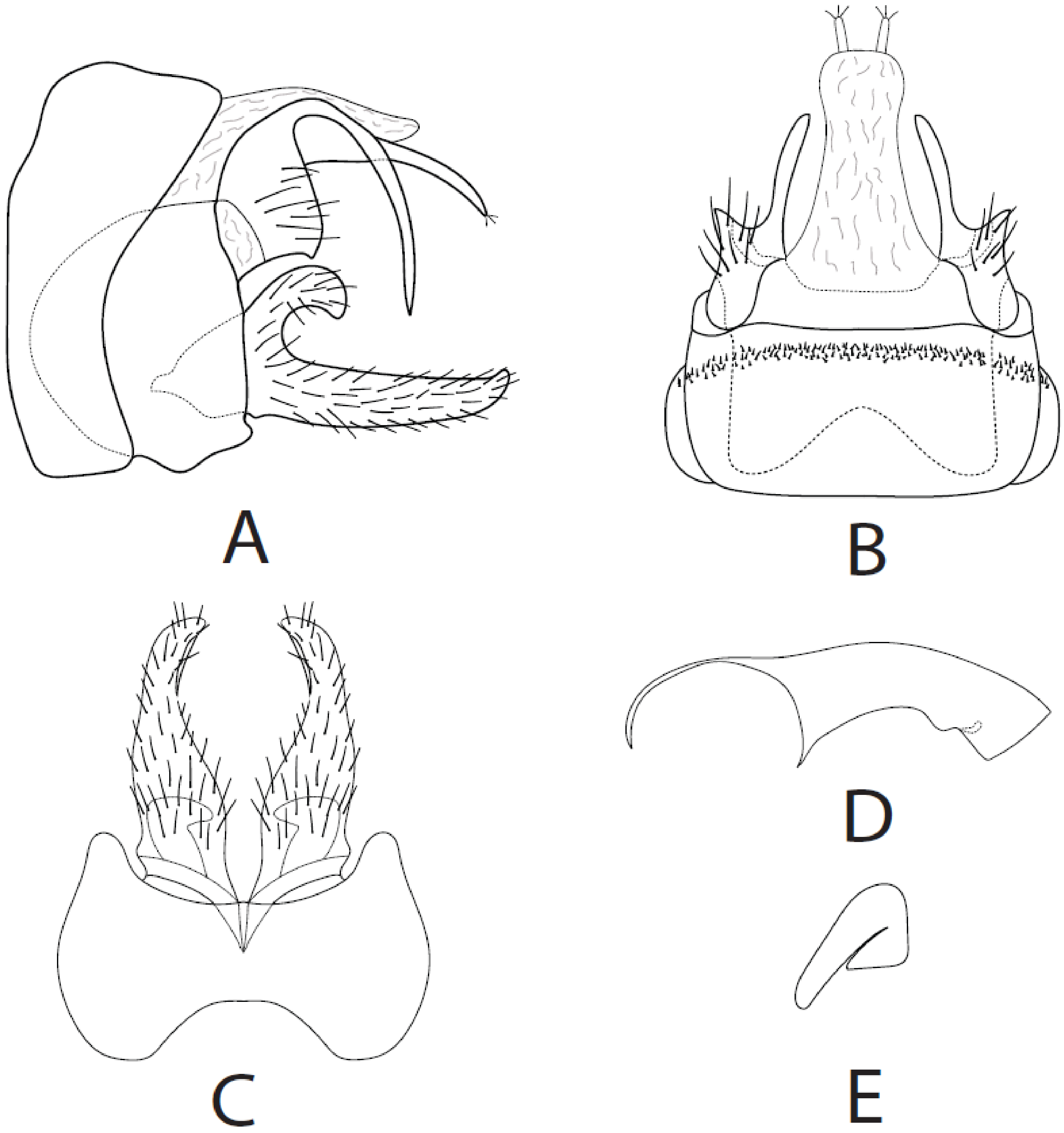

Figure 5-11. *Polycentropus carolinensis* Banks, 1905, male genitalia (holotype).

A. left lateral (with phallus removed). B, dorsal. C, segment IX and inferior appendages, ventral. D, phallus, left lateral. E, dorsobasal process of right inferior appendage, caudal.

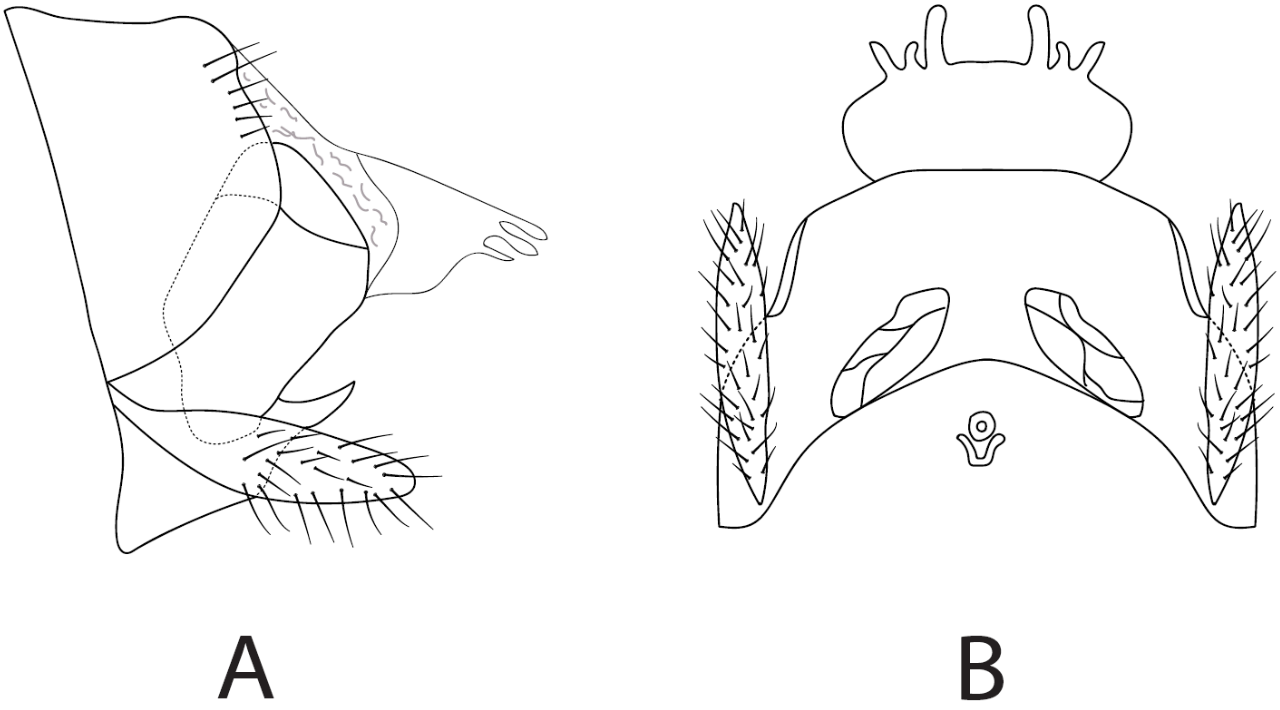

Figure 5-12. *Polycentropus carolinensis* Banks, 1905, female genitalia. A. left lateral. B, ventral.

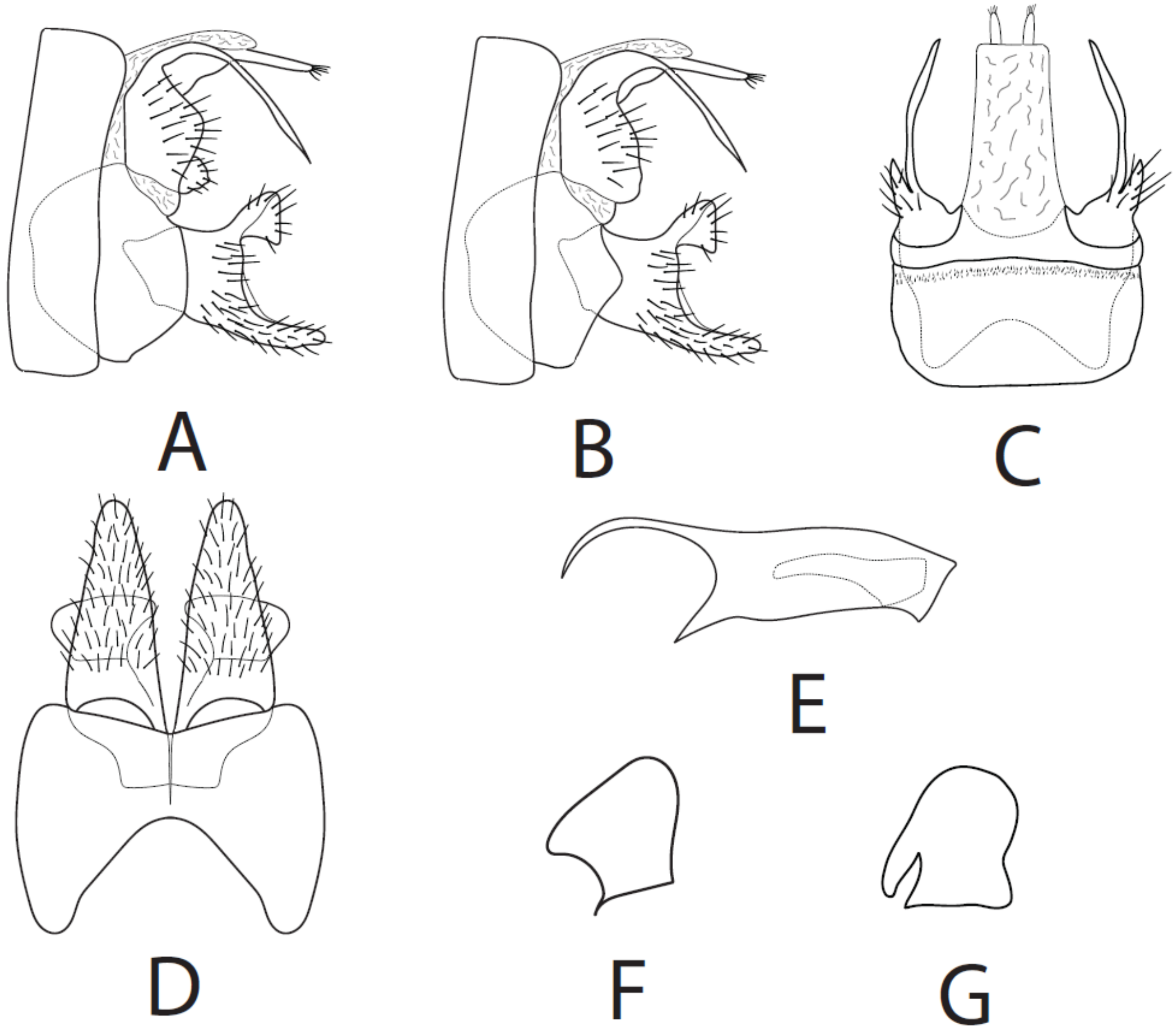

Figure 5-13. *Polycentropus centralis* Banks, 1914, male genitalia (holotype and additional Illinois material).

A. left lateral (with phallus removed); B. variation of left lateral (with phallus removed). C. dorsal; D. segment IX and inferior appendages, ventral; E. phallus, left lateral; F. dorsobasal process of right inferior appendage, caudal; G. variation of dorsobasal process of right inferior appendage, caudal.

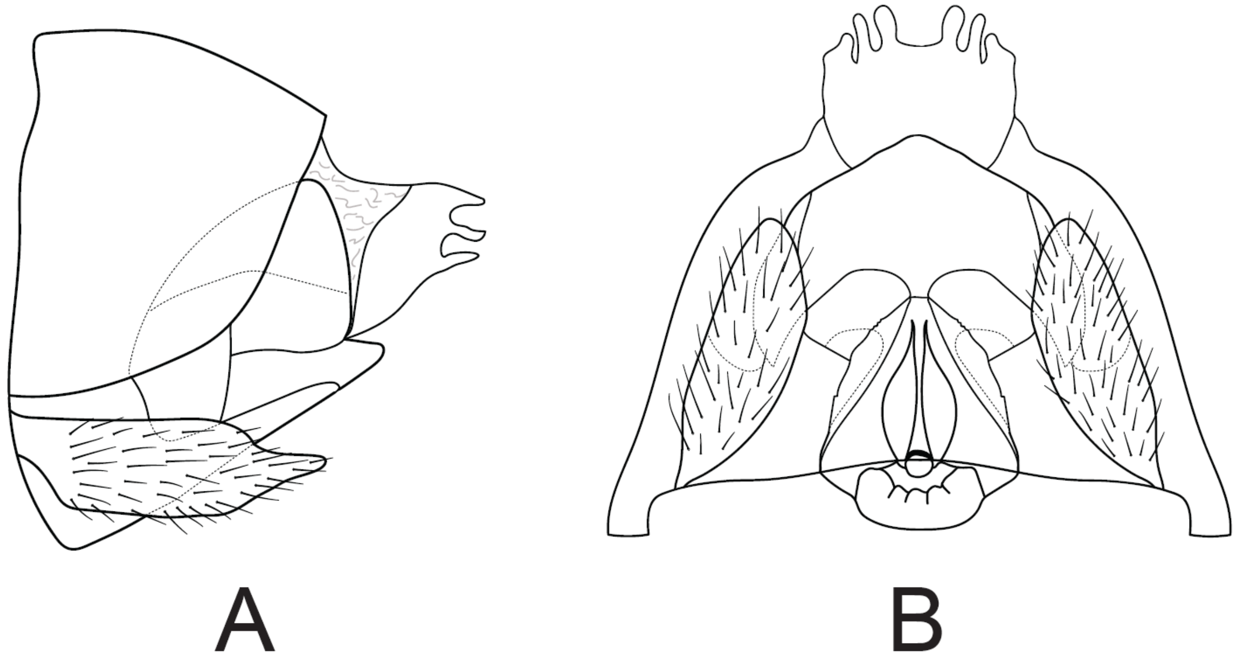

Figure 5-14. *Polycentropus centralis* Banks, 1914, female genitalia. A. left lateral; B. ventral.

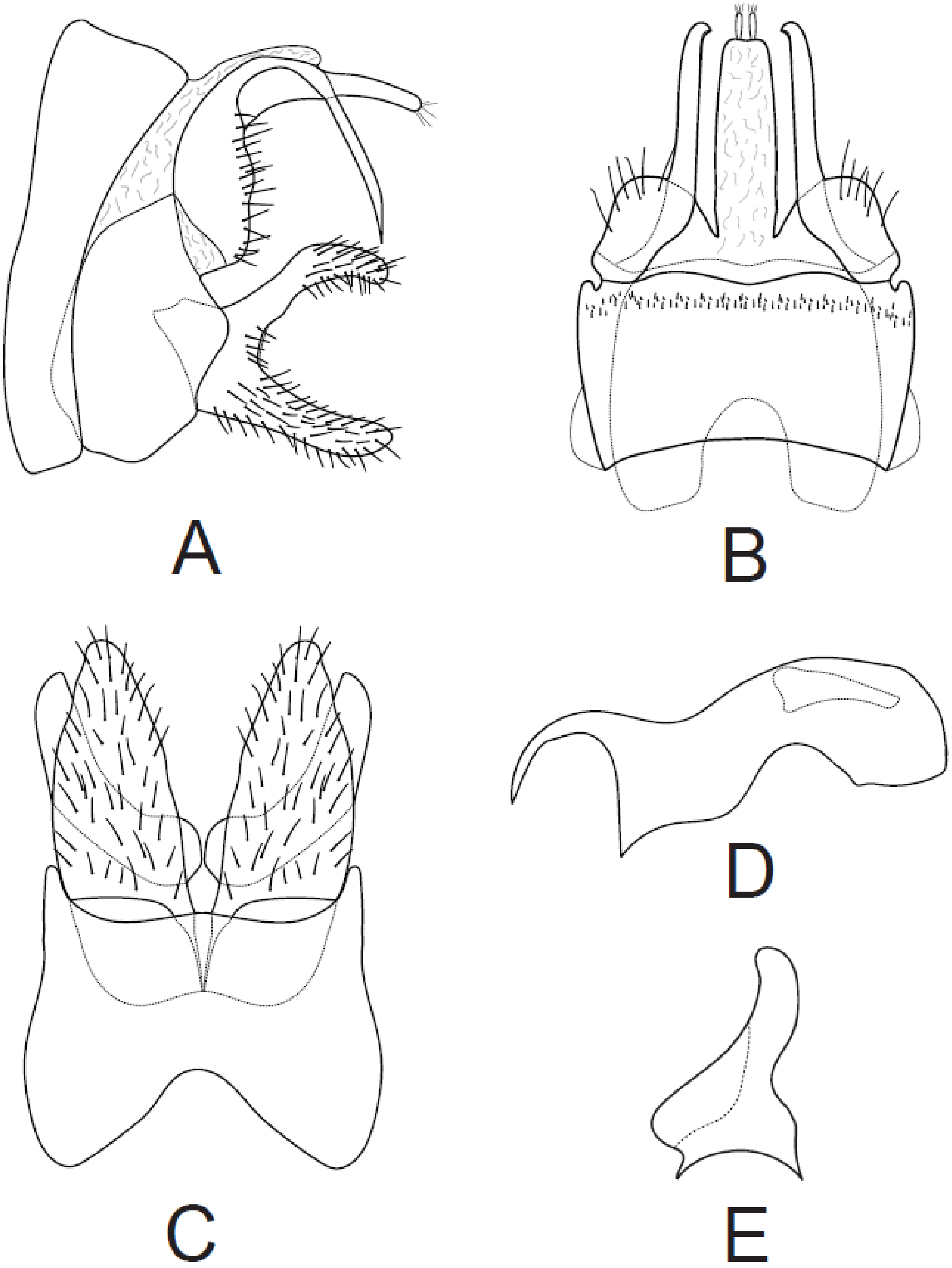

Figure 5-15. *Polycentropus chelatus* Ross and Yamamoto, 1965, male genitalia (holotype).

A. left lateral (with phallus removed); B. dorsal; C. segment IX and inferior appendages, ventral; D. phallus, left lateral; E. dorsobasal process of right inferior appendage, caudal.

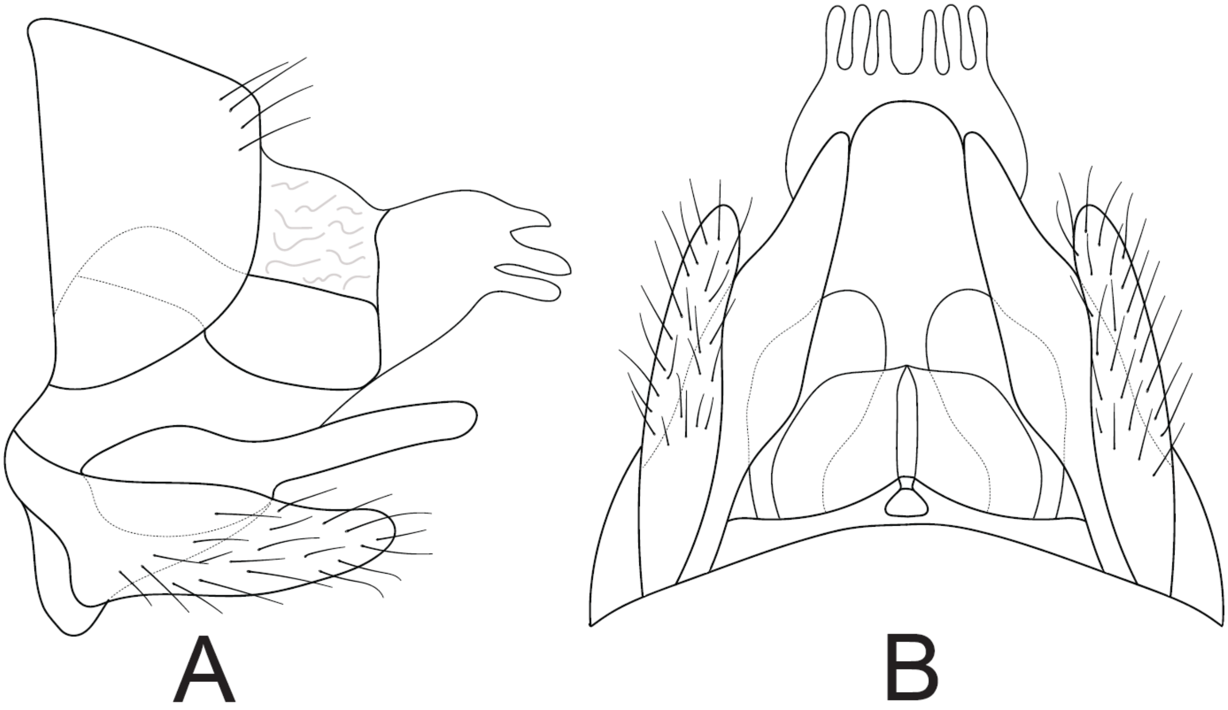

Figure 5-16. *Polycentropus chelatus* Ross and Yamamoto, 1965, female genitalia. A. left lateral; B. ventral.

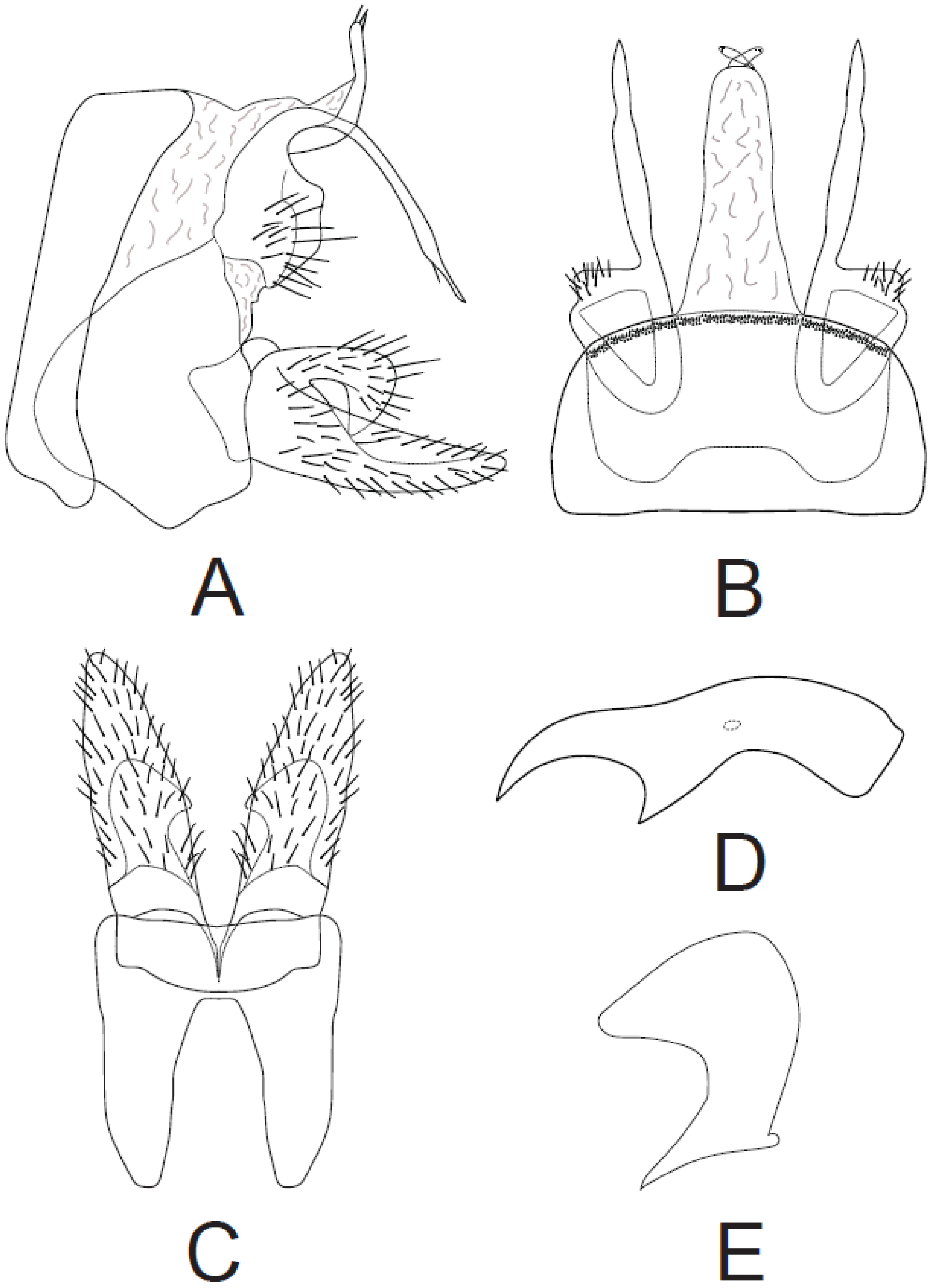

Figure 5-17. *Polycentropus chenoides* Ross and Yamamoto, 1965, male genitalia (holotype).

A. left lateral (with phallus removed); B. dorsal; C. segment IX and inferior appendages, ventral; D. phallus, left lateral; E. dorsobasal process of right inferior appendage, caudal.

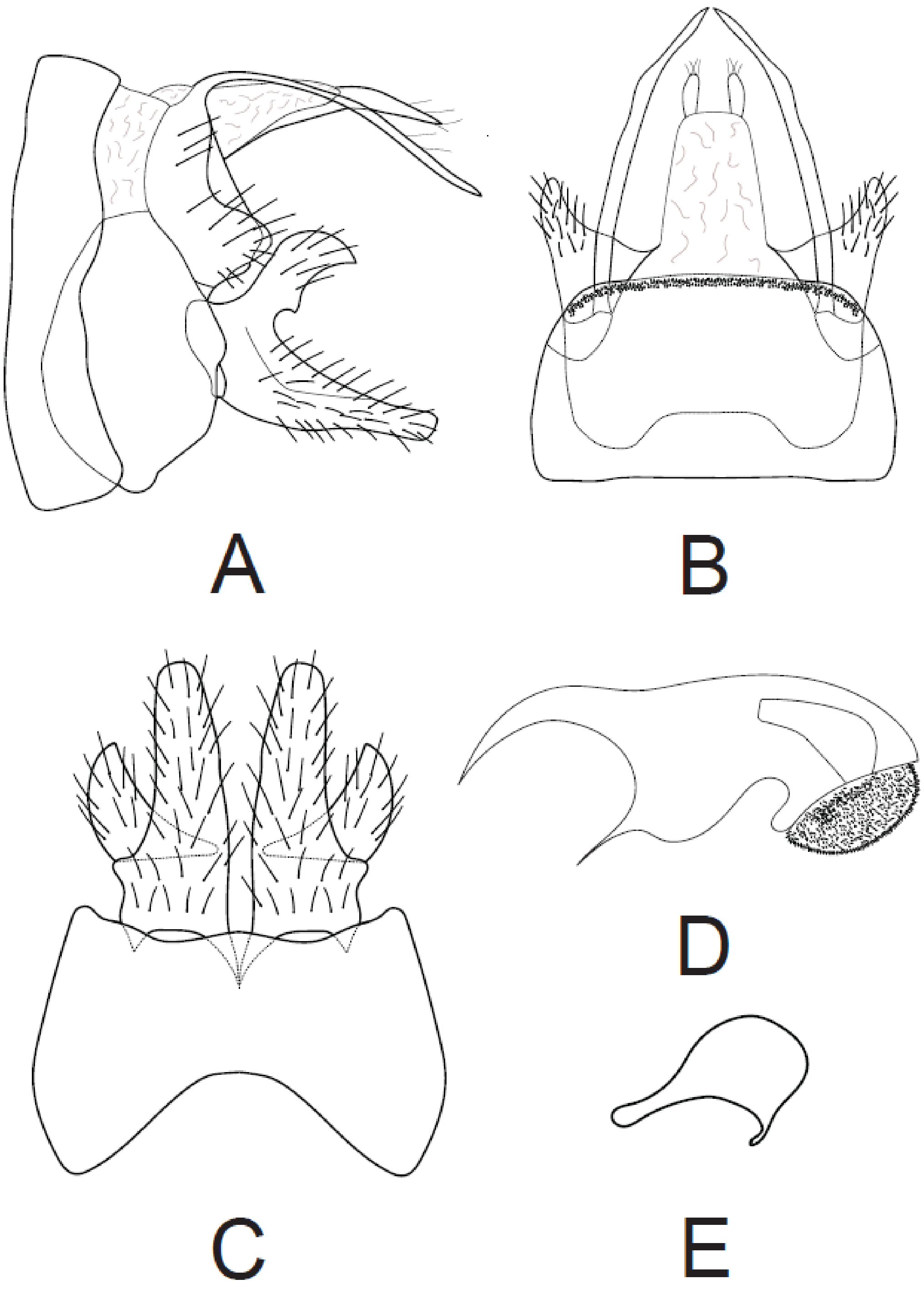

Figure 5-18. *Polycentropus confusus* Hagen, 1861, male genitalia (lectotype).

A. left lateral (with phallus removed); B. dorsal; C. segment IX and inferior appendages, ventral; D. phallus, left lateral. E. dorsobasal process of right inferior appendage, caudal.

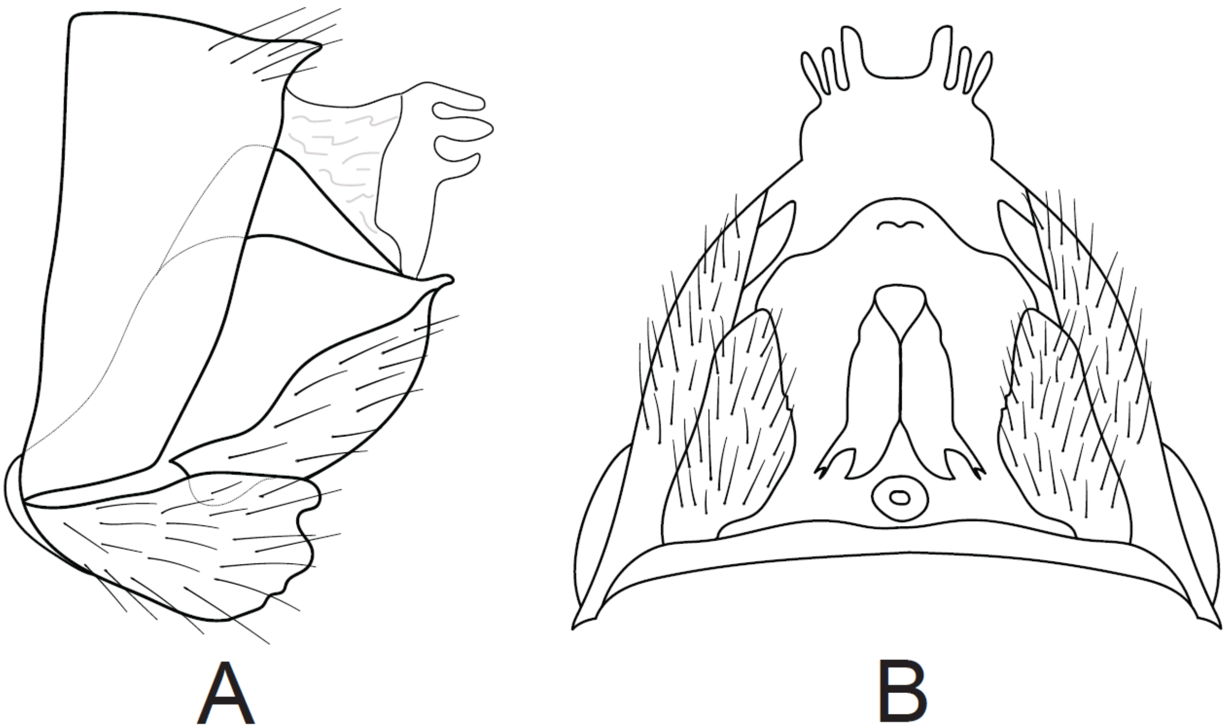

Figure 5-19. *Polycentropus confusus* Hagen, 1861, female genitalia. A. left lateral. B, ventral.

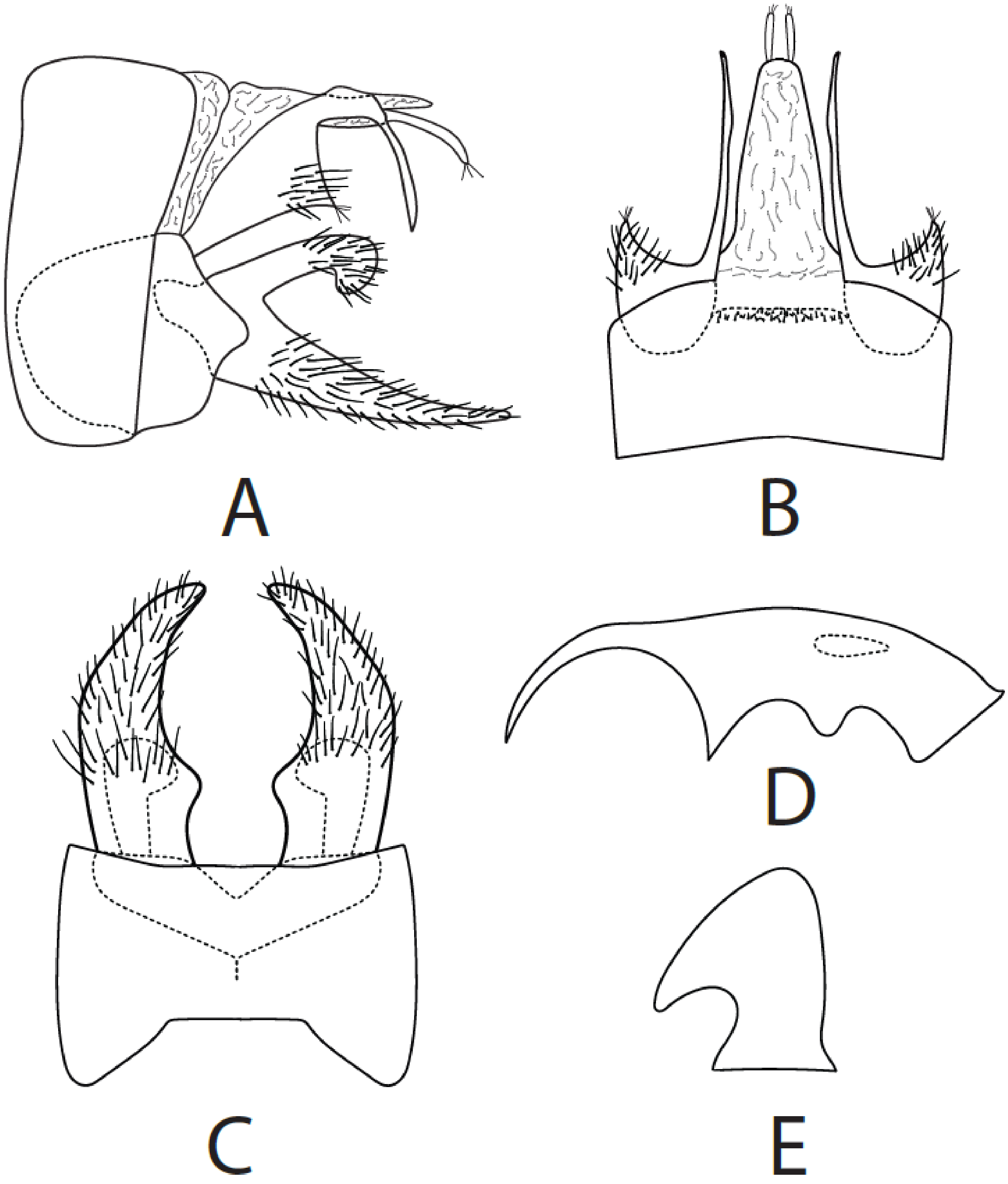

Figure 5-20. *Polycentropus dinkinsorum* Orfinger and Etnier, 2020, male genitalia (holotype).

A. left lateral (with phallus removed); B. dorsal; C. segment IX and inferior appendages, ventral; D. phallus, left lateral; E. dorsobasal process of right inferior appendage, caudal.

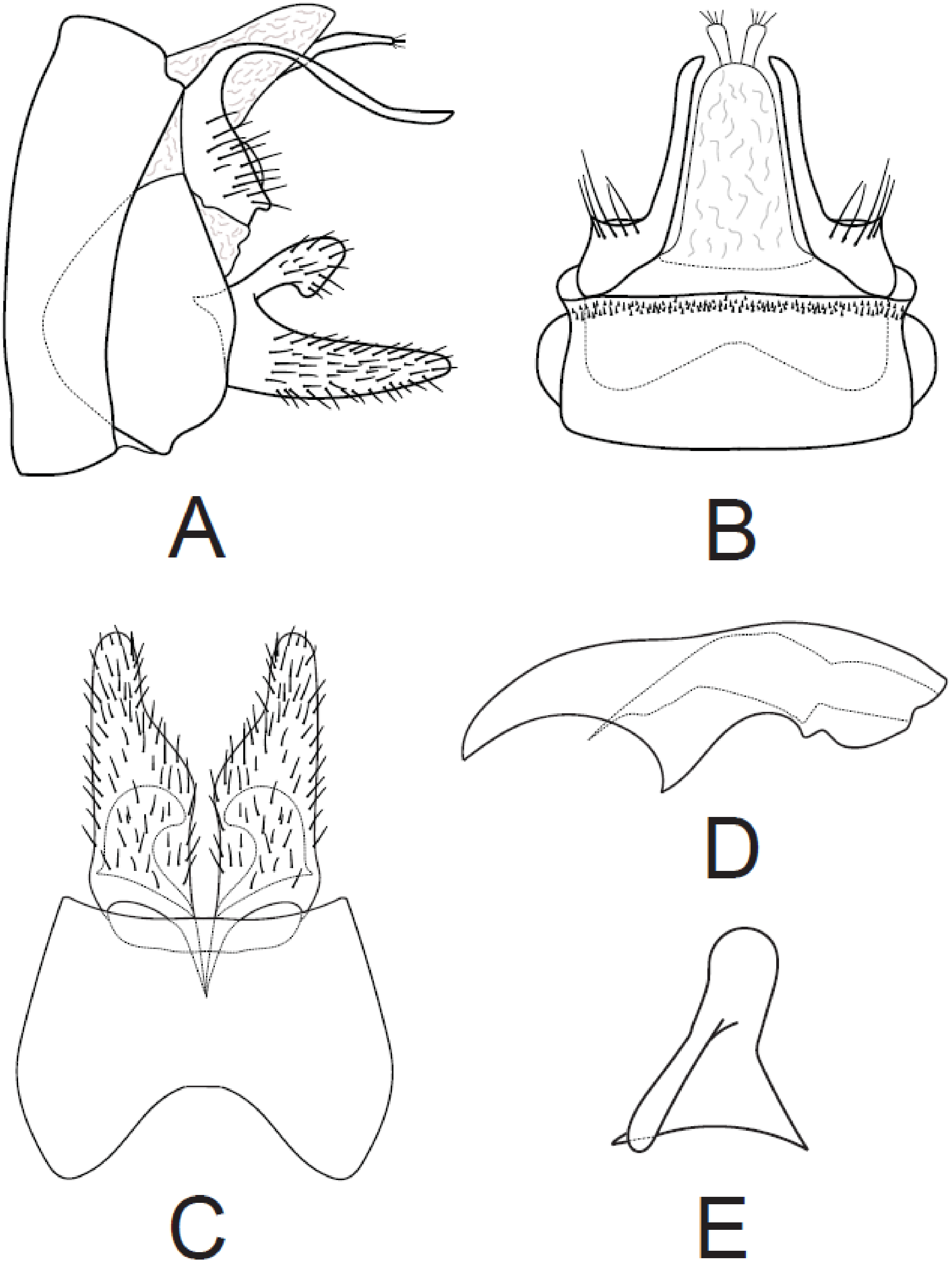

Figure 5-21. *Polycentropus elarus* Ross, 1944, male genitalia (neotype).

A. left lateral (with phallus removed); B. dorsal; C. segment IX and inferior appendages, ventral; D. phallus, left lateral; E. dorsobasal process of right inferior appendage, caudal.

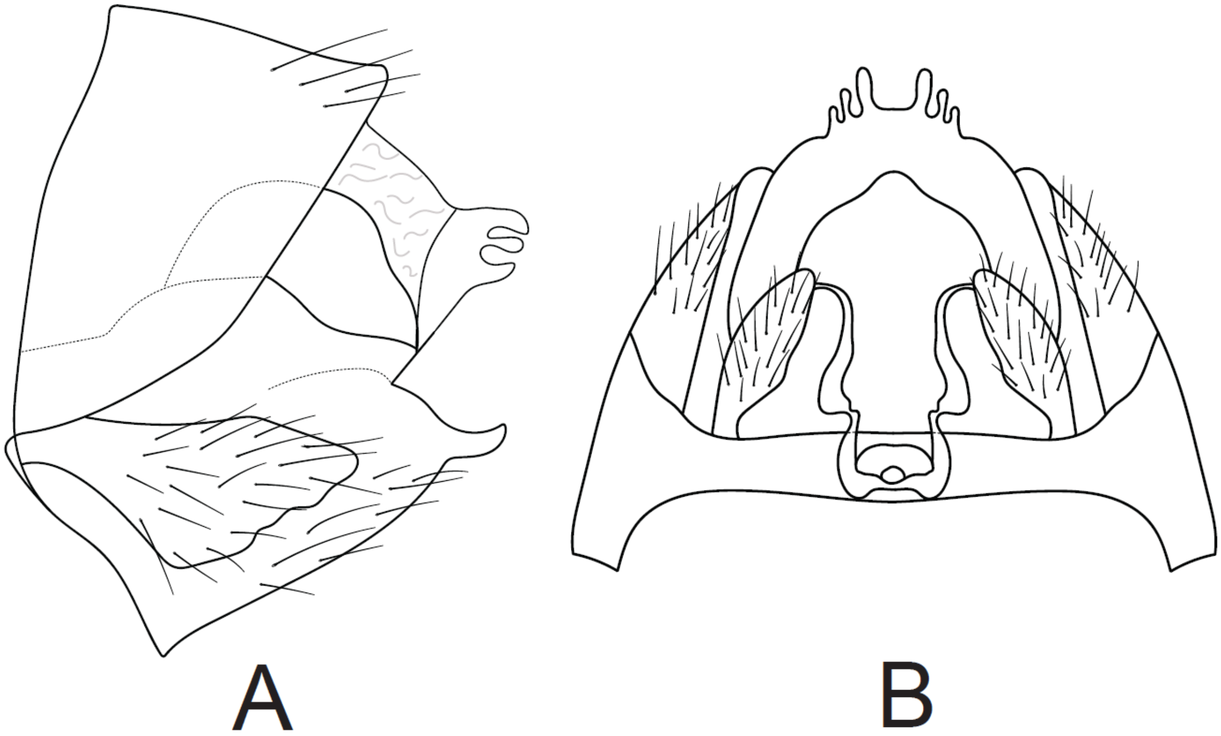

Figure 5-22. *Polycentropus elarus* Ross, 1944, female genitalia. A, left lateral. B, ventral.

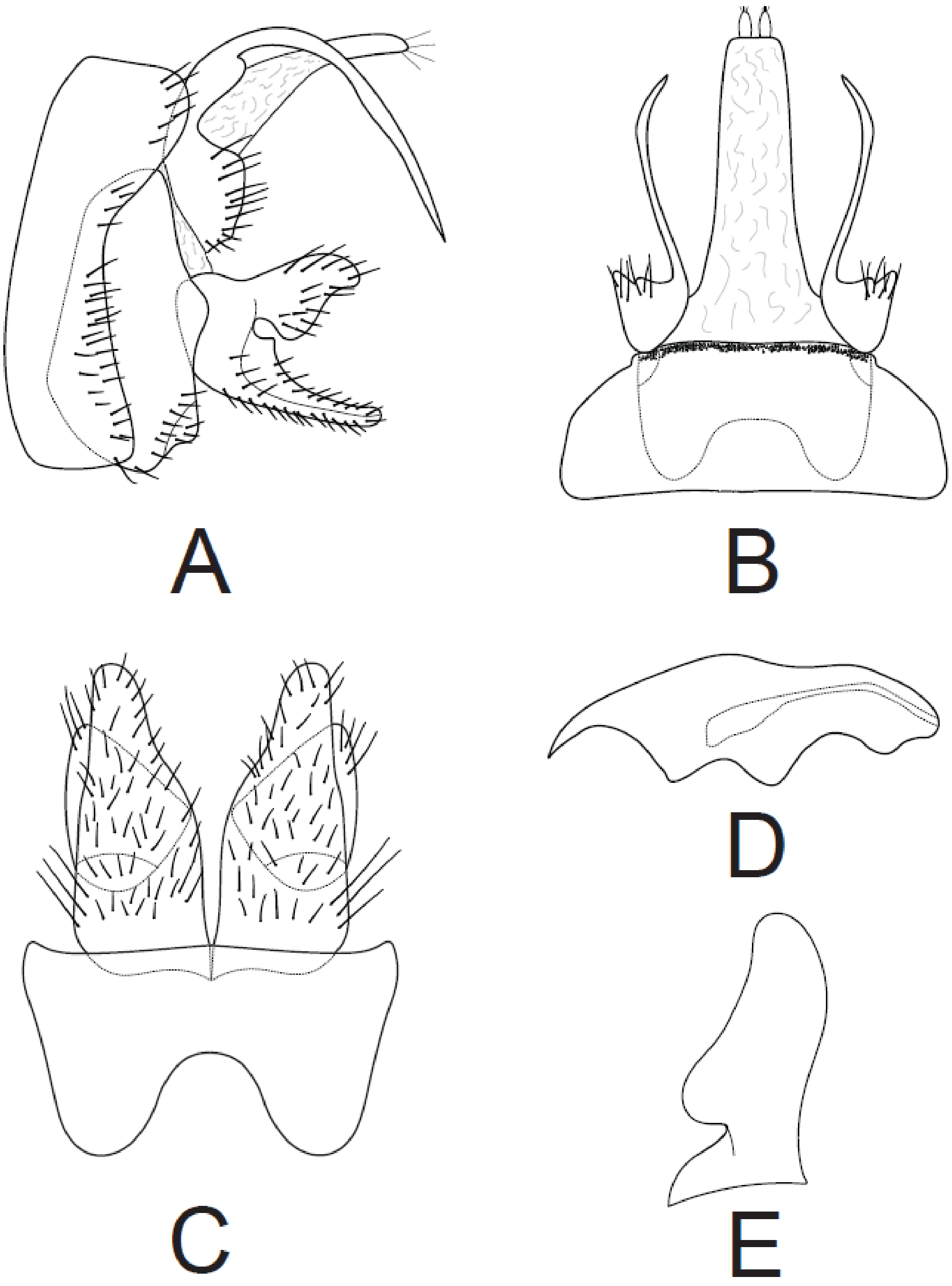

Figure 5-23. *Polycentropus floridensis* Lago and Harris, 1983, male genitalia (holotype). A. left lateral (with phallus removed); B. dorsal; C. segment IX and inferior appendages, ventral; D. phallus, left lateral; E. dorsobasal process of right inferior appendage, caudal.

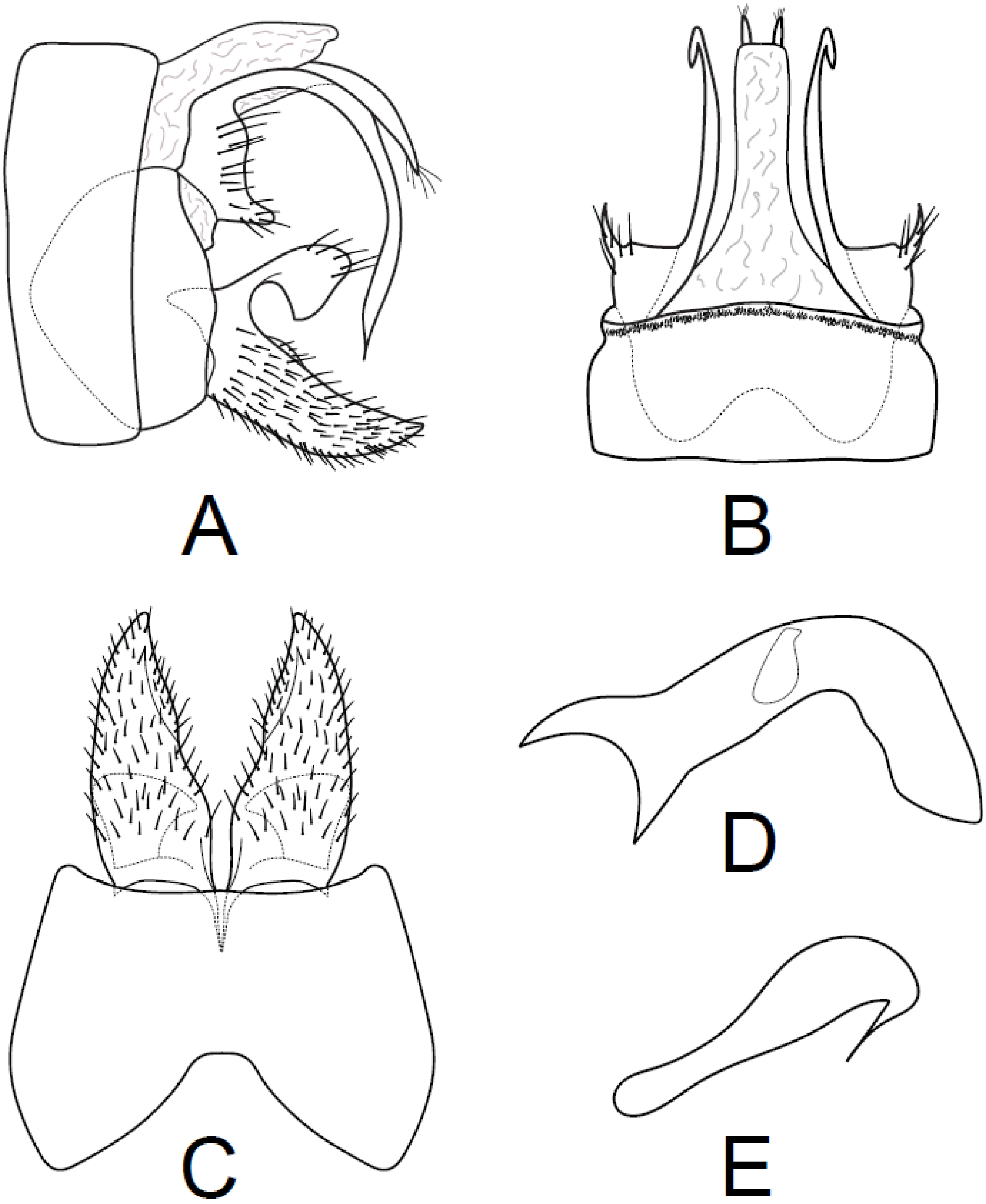

Figure 5-24. *Polycentropus maculatus* Banks, 1908, male genitalia (holotype). A. left lateral (with phallus removed); B. dorsal; C. segment IX and inferior appendages, ventral; D. phallus, left lateral; E. dorsobasal process of right inferior appendage, caudal.

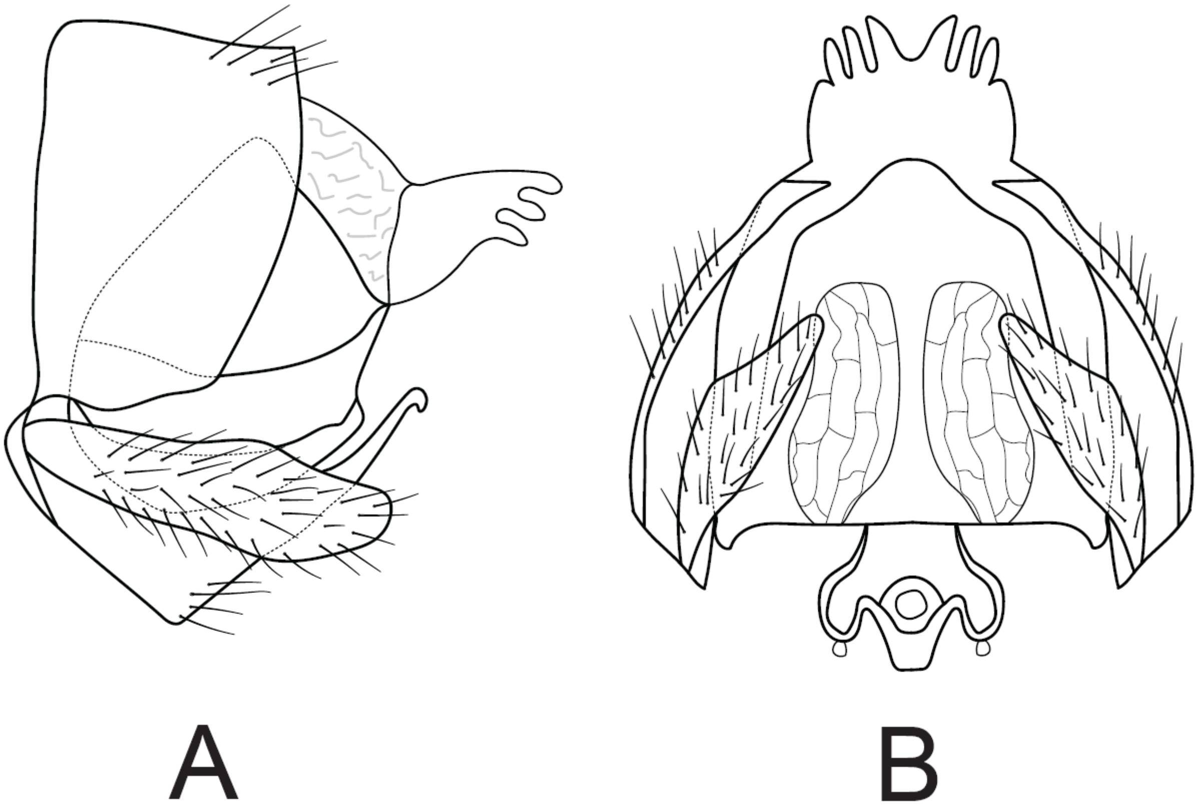

Figure 5-25. *Polycentropus maculatus* Banks, 1908, female genitalia. A, left lateral; B, ventral.

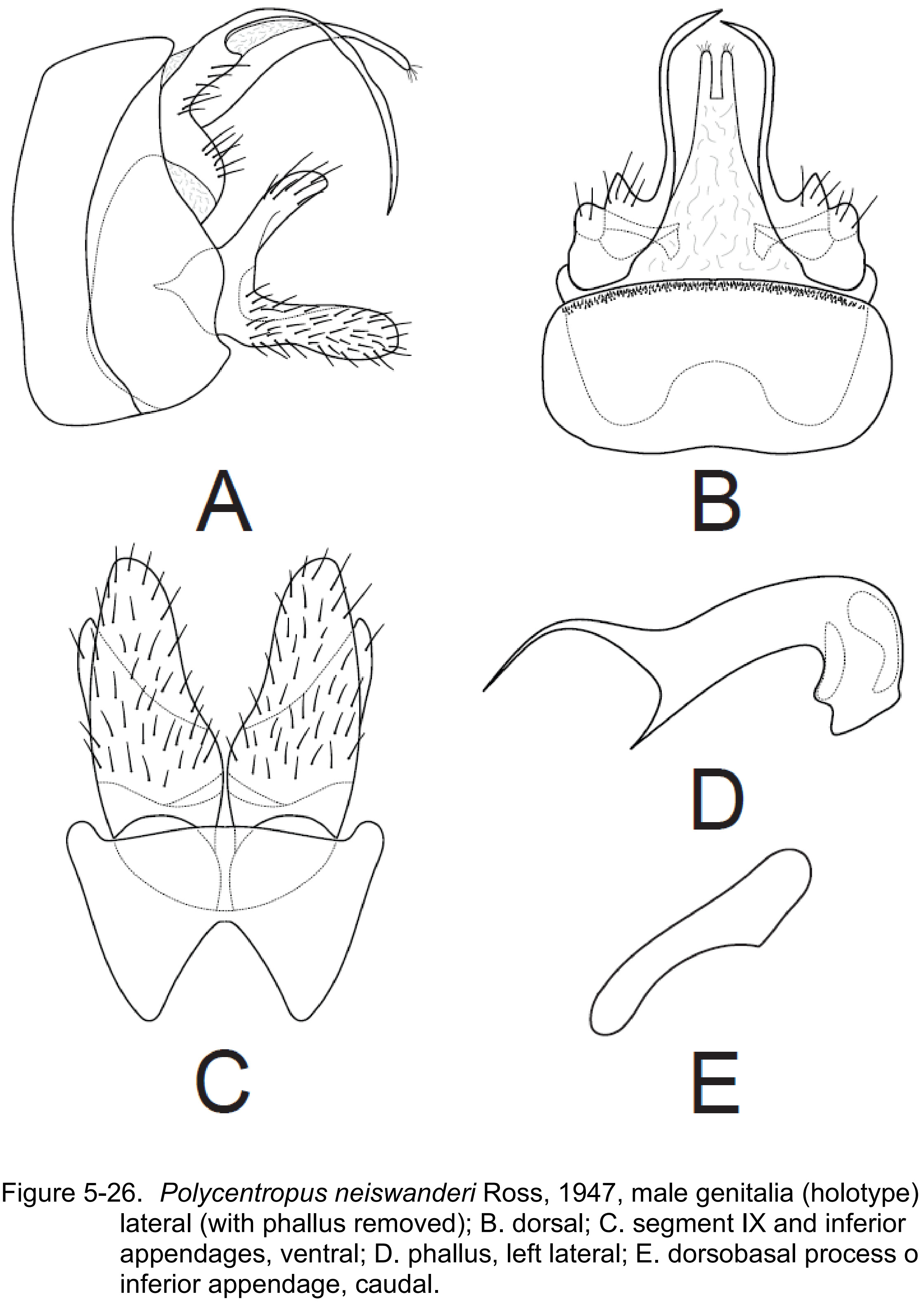

Figure 5-26. *Polycentropus neiswanderi* Ross, 1947, male genitalia (holotype). A. left lateral (with phallus removed); B. dorsal; C. segment IX and inferior appendages, ventral; D. phallus, left lateral; E. dorsobasal process of right inferior appendage, caudal.

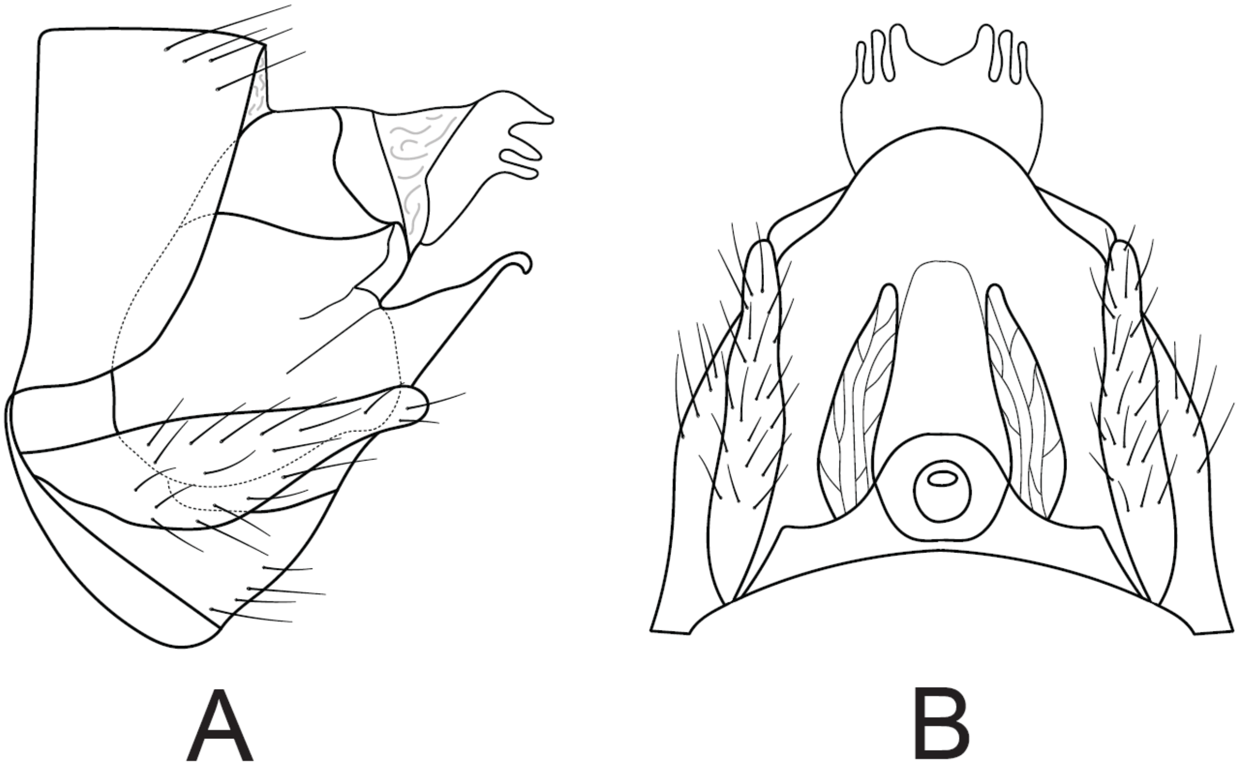

Figure 5-27. *Polycentropus neiswanderi* Ross, 1947, female genitalia. A. left lateral; B. ventral.

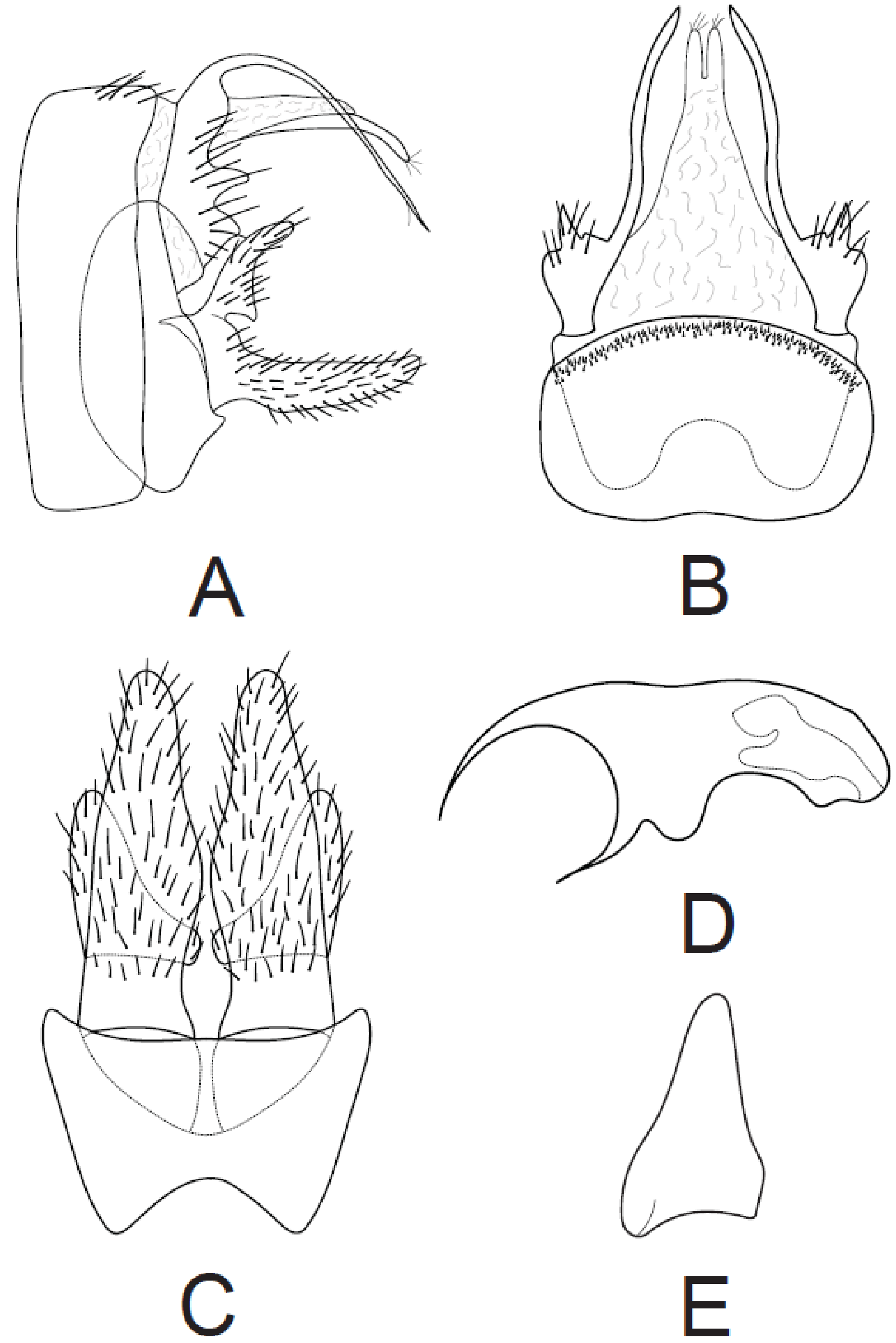

Figure 5-28. *Polycentropus pentus* Ross, 1941, male genitalia (holotype). A. left lateral (with phallus removed); B. dorsal; C. segment IX and inferior appendages, ventral; D. phallus, left lateral; E. dorsobasal process of right inferior appendage, caudal.

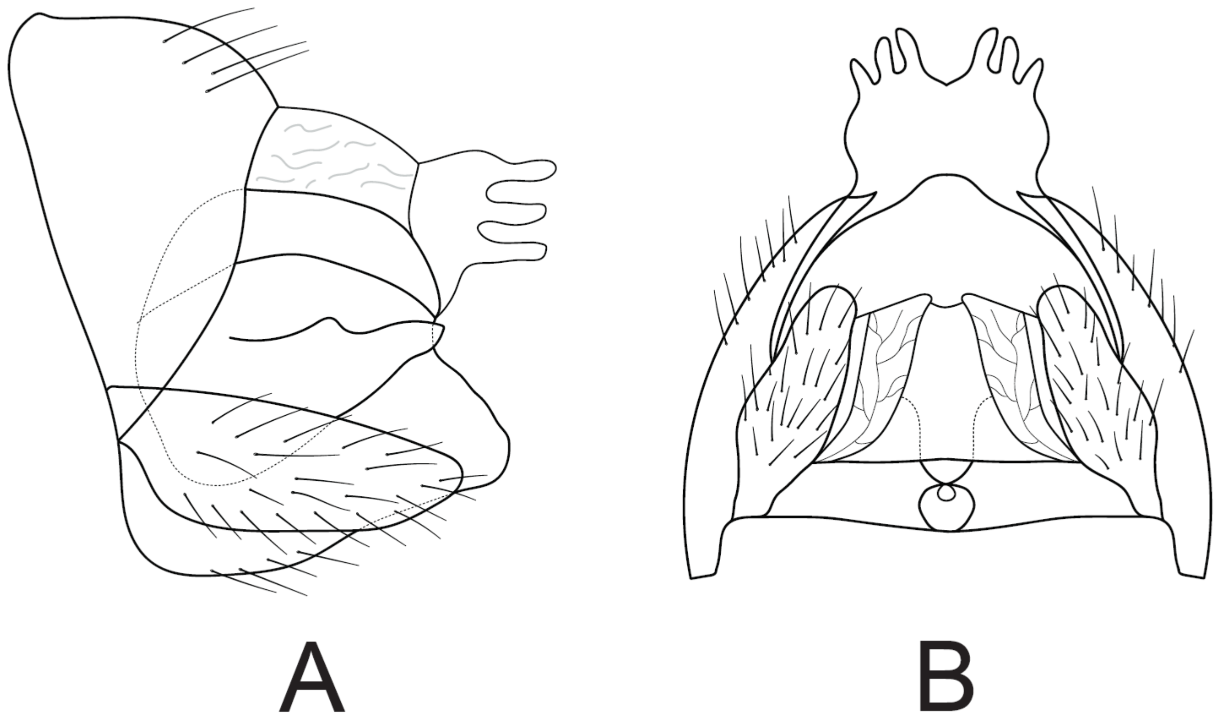

Figure 5-29. *Polycentropus pentus* Ross, 1941, female genitalia. A. left lateral; B. ventral.

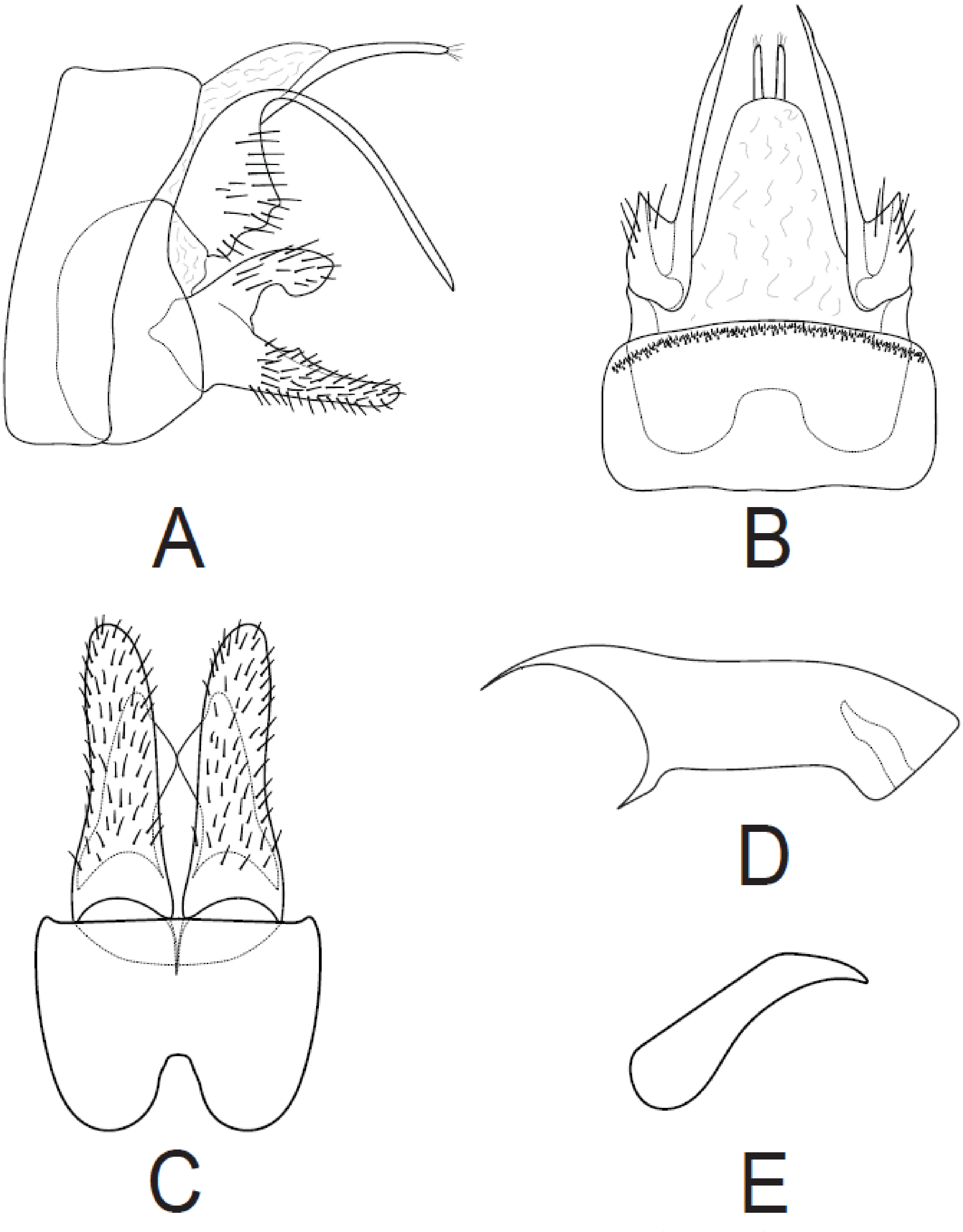

Figure 5-30. *Polycentropus pixi* Ross, 1944, male genitalia (holotype).

A. left lateral (with phallus removed); B. dorsal; C. segment IX and inferior appendages, ventral; D. phallus, left lateral; E. dorsobasal process of right inferior appendage, caudal.

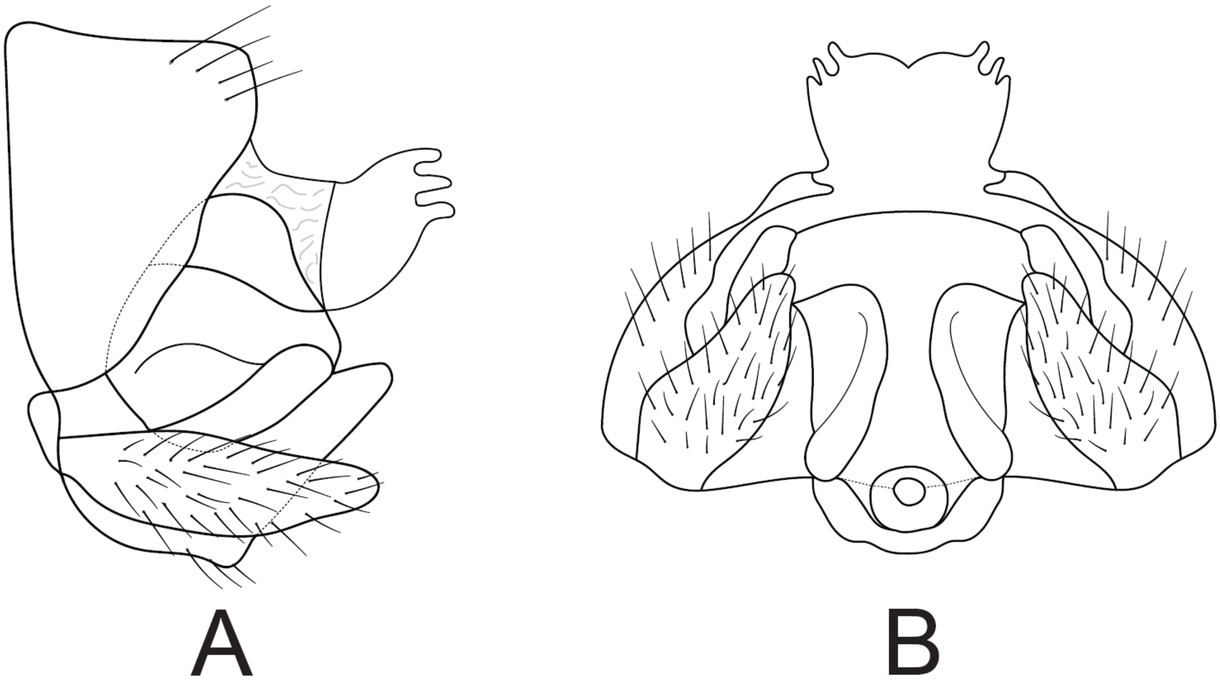

Figure 5-31. *Polycentropus pixi* Ross, 1944, female genitalia. A. left lateral; B. ventral.

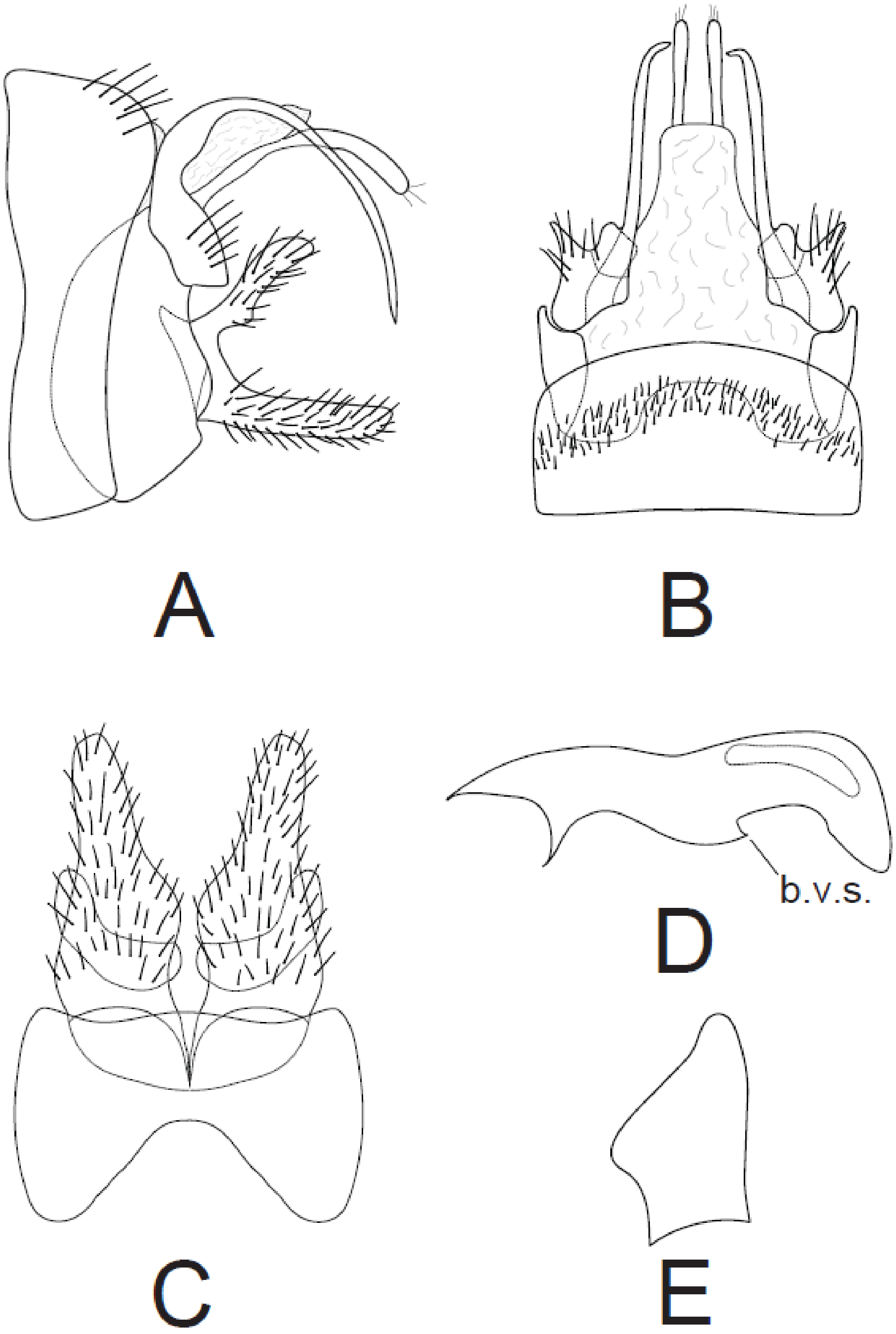

Figure 5-32. *Polycentropus stephani* Bowles, Mathis, and Hamilton, 1993, male genitalia (holotype).

A. left lateral (with phallus removed); B. dorsal; C. segment IX and inferior appendages, ventral; D. phallus, left lateral; E. dorsobasal process of right inferior appendage, caudal. b.v.s. = basoventral spur.

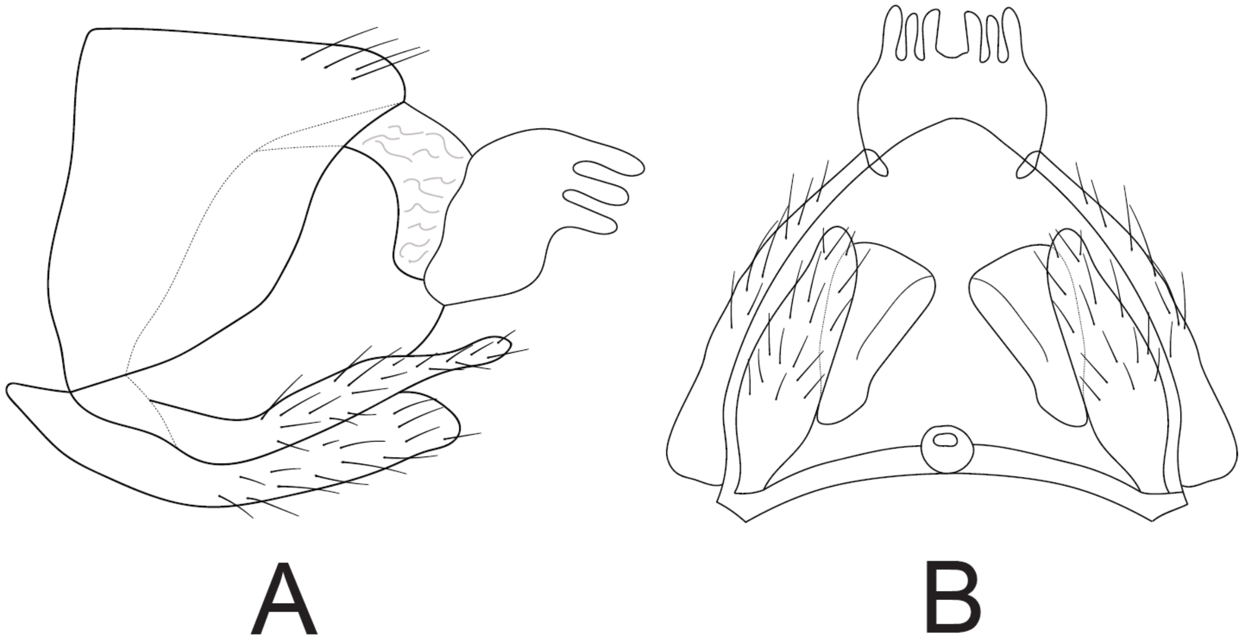

Figure 5-33. *Polycentropus stephani* Bowles, Mathis, and Hamilton, 1993, female genitalia. A. left lateral; B. ventral.

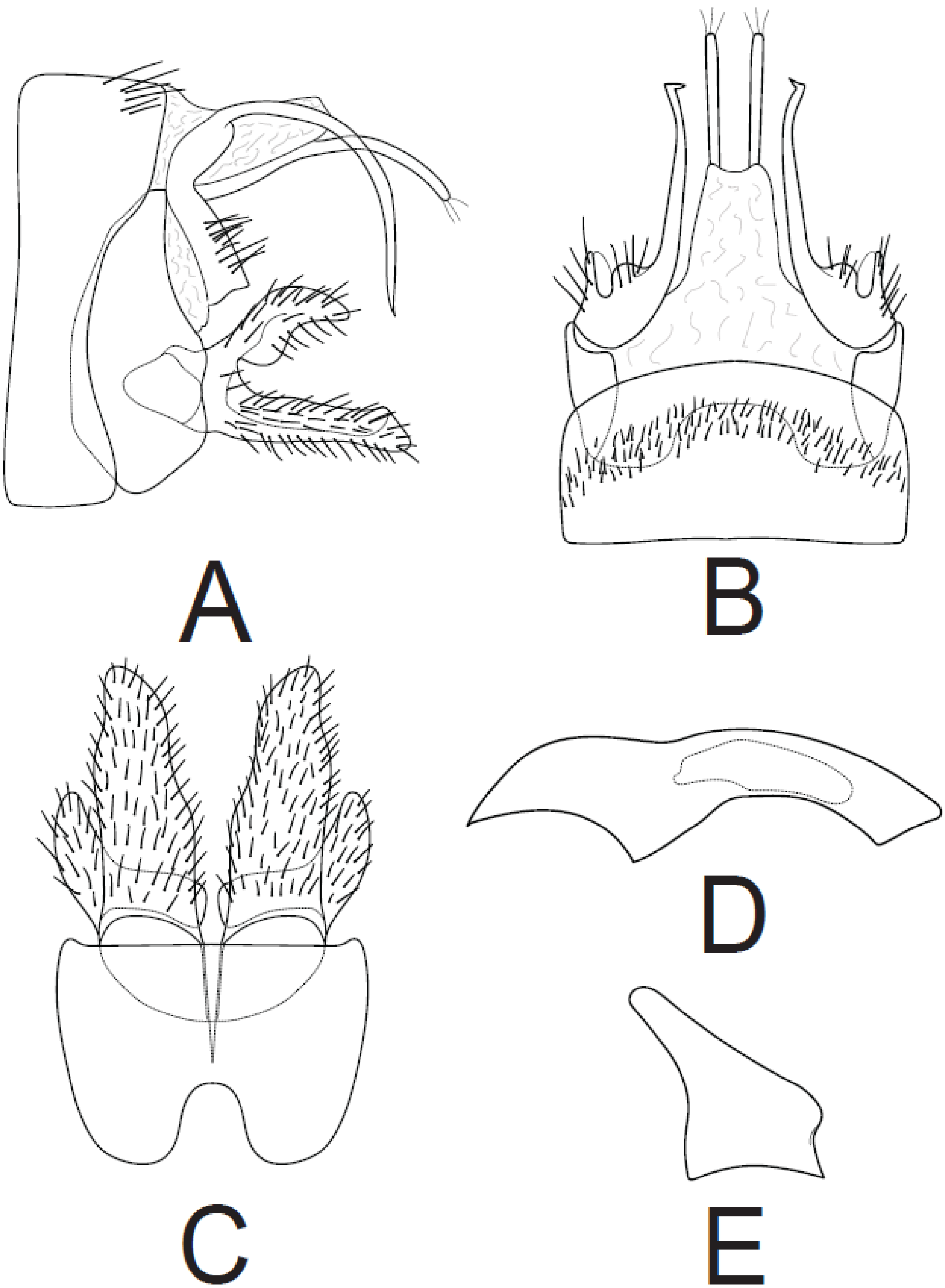

Figure 5-34. *Polycentropus thaxtoni* Hamilton and Holzenthal, 1986, male genitalia (holotype). A. left lateral (with phallus removed); B. dorsal; C, segment IX and inferior appendages, ventral; D. phallus, left lateral; E. dorsobasal process of right inferior appendage, caudal.

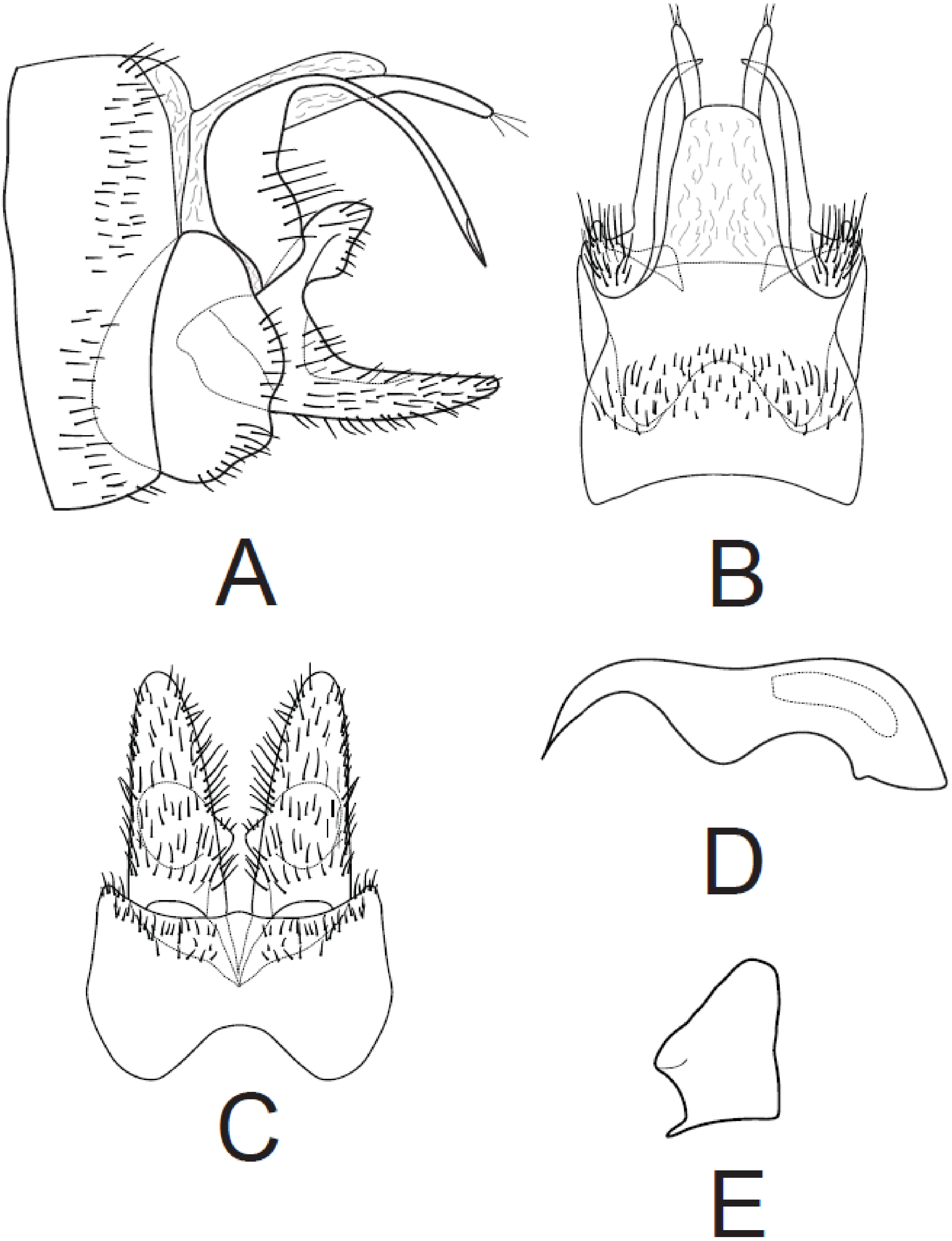

Figure 5-35. *Polycentropus vernus* Hamilton, Harris, and Lago, 1990, male genitalia (re-drawn from original description).

A. left lateral (with phallus removed); B. dorsal; C. segment IX and inferior appendages, ventral; D. phallus, left lateral; E. dorsobasal process of right inferior appendage, caudal.

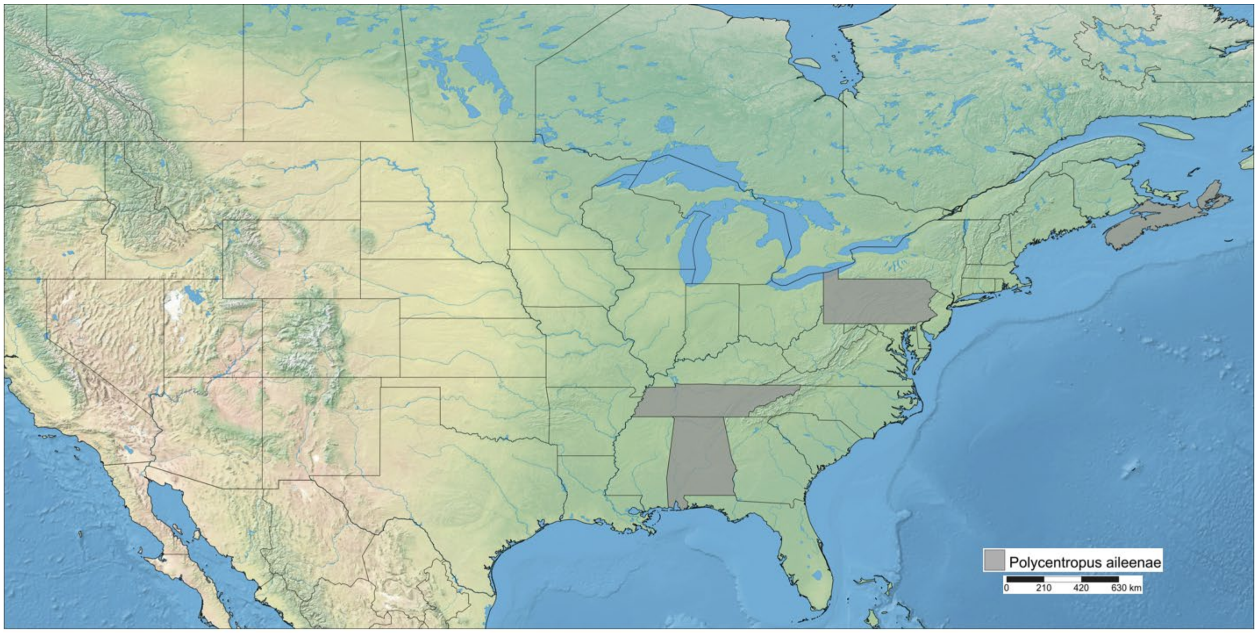

Figure 5-36. Known Canadian province and US state distribution of *Polycentropus aileenae* Orfinger and Moulton, 2021, as indicated by grey shading.

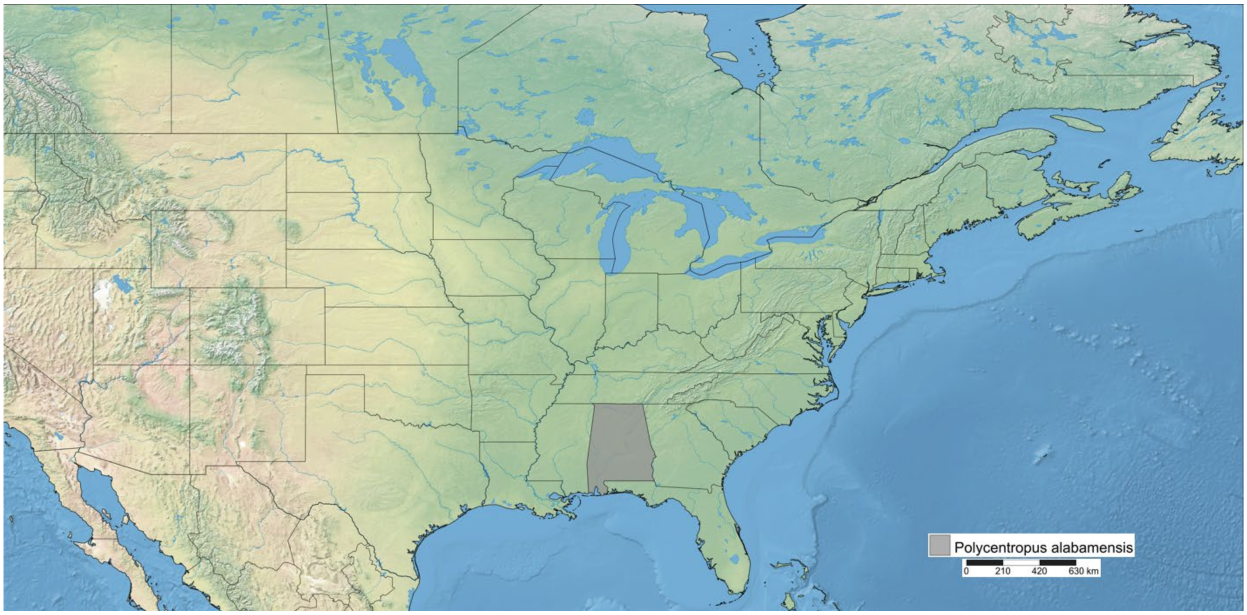

Figure 5-37. Known Canadian province and US state distribution of *Polycentropus alabamensis* Hamilton, Harris, and Lago, 1990, as indicated by grey shading.

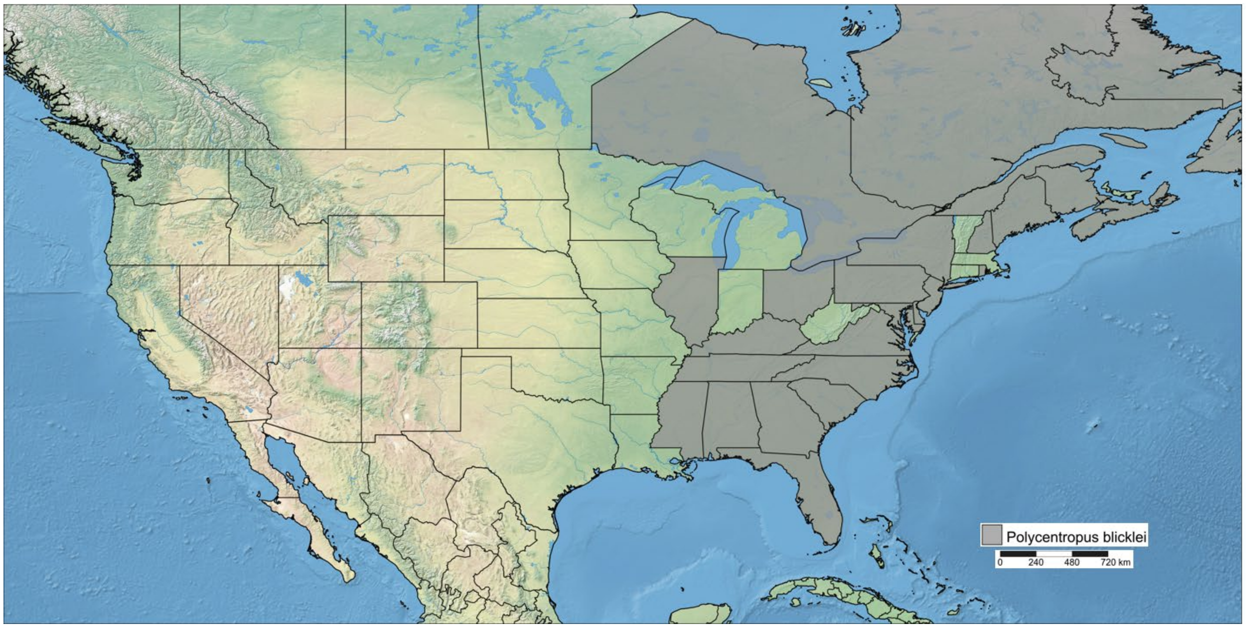

Figure 5-38. Known Canadian province and US state distribution of *Polycentropus blicklei* Ross and Yamamoto, 1965, as indicated by grey shading.

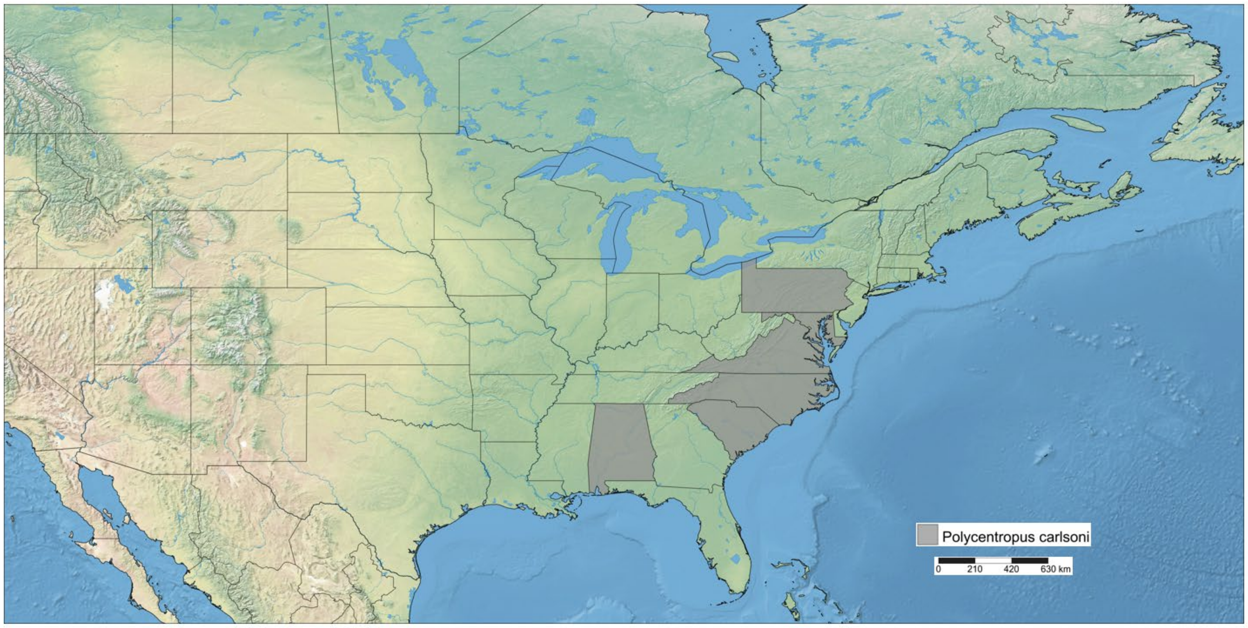

Figure 5-39. Known Canadian province and US state distribution of *Polycentropus carlsoni* Morse, 1971, as indicated by grey shading.

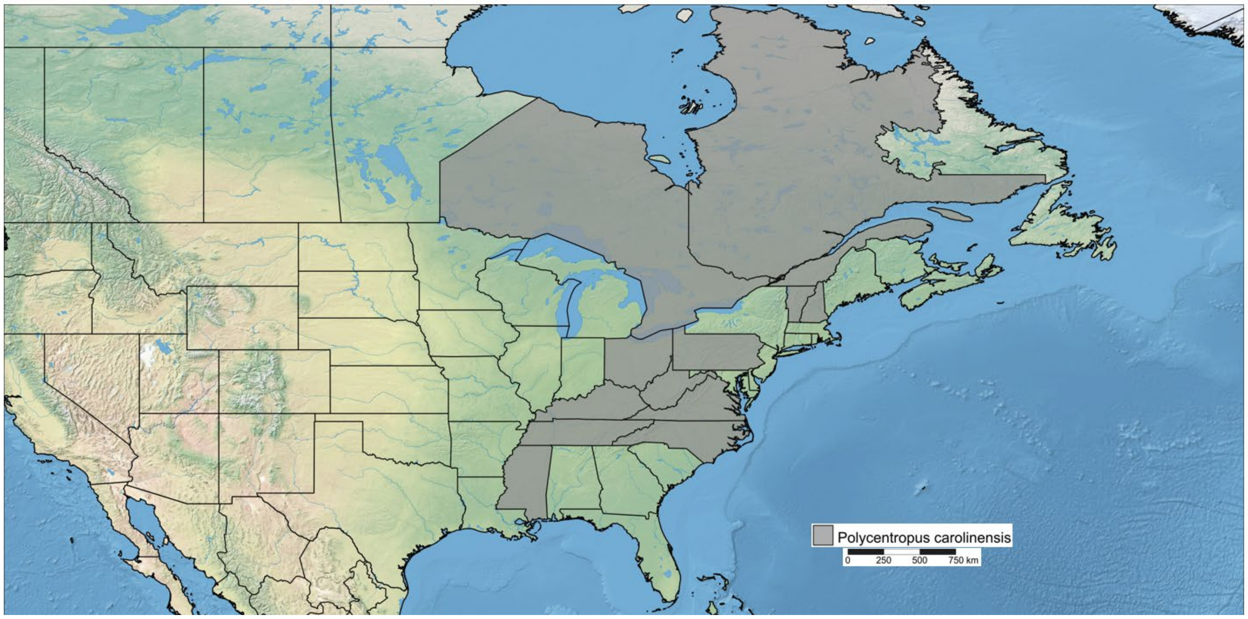

Figure 5-40. Known Canadian province and US state distribution of *Polycentropus carolinensis* Banks, 1905, as indicated by grey shading.

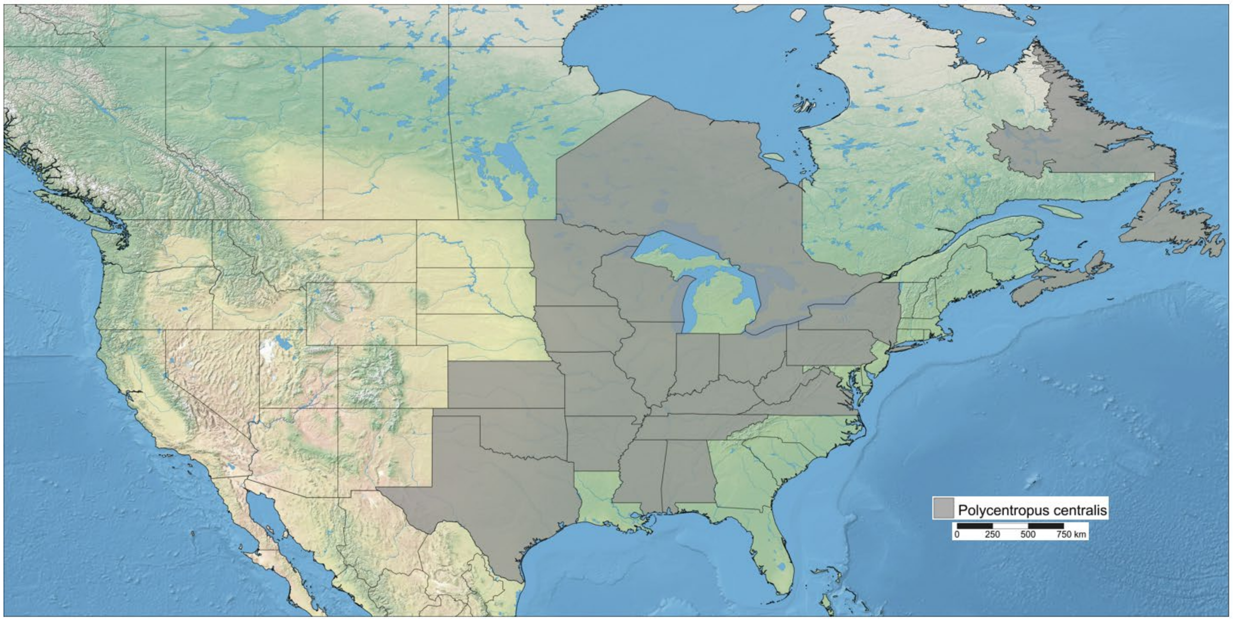

Figure 5-41. Known Canadian province and US state distribution of *Polycentropus centralis* Banks, 1914, as indicated by grey shading.

Figure 5-42. Known Canadian province and US state distribution of *Polycentropus chelatus* Ross and Yamamoto, 1965, as indicated by grey shading.

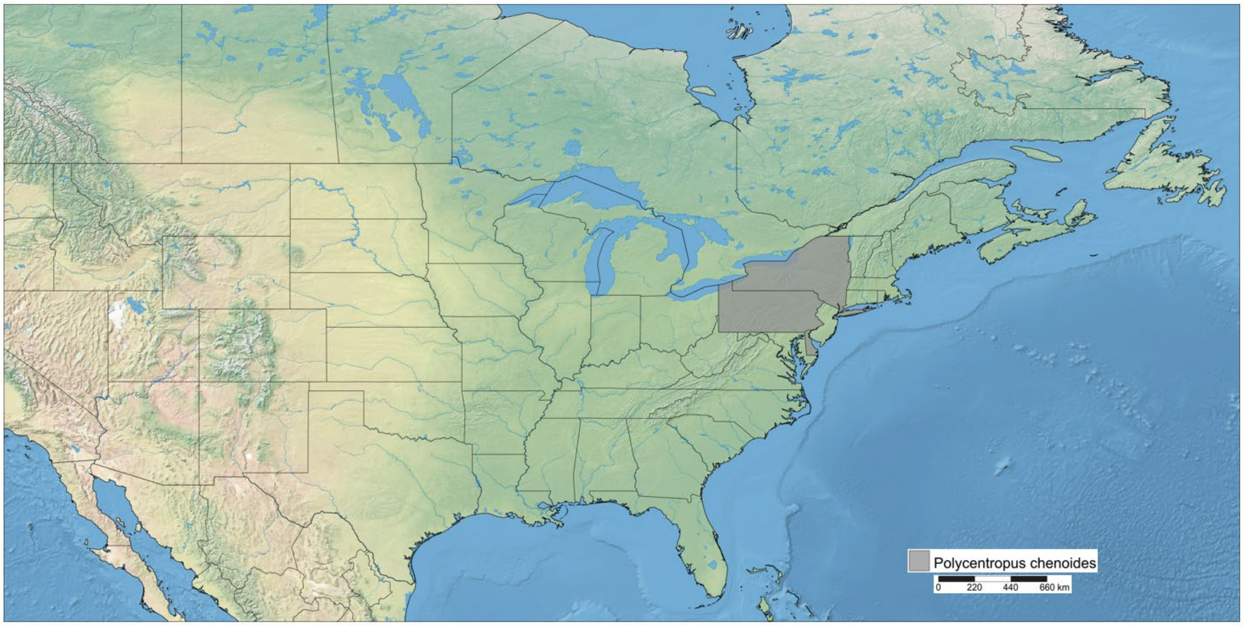

Figure 5-43. Known Canadian province and US state distribution of *Polycentropus chenoides* Ross and Yamamoto, 1965, as indicated by grey shading.

Figure 5-44. Known Canadian province and US state distribution of *Polycentropus confusus* Hagen, 1861, as indicated by grey shading.

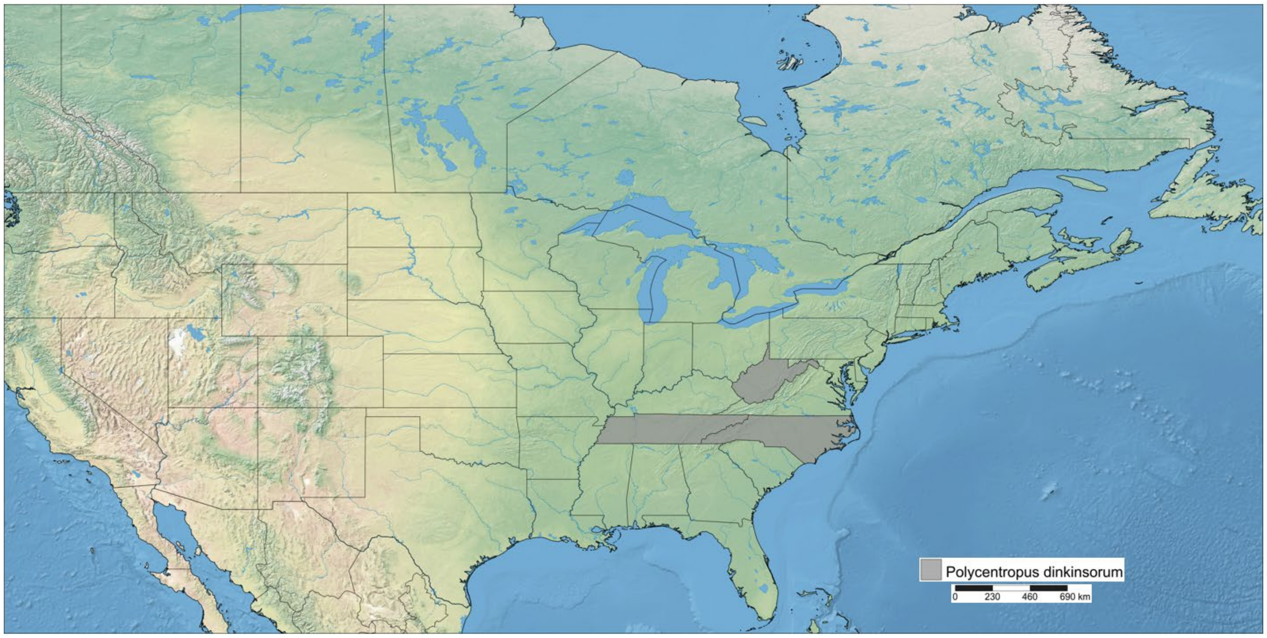

Figure 5-45. Known Canadian province and US state distribution of *Polycentropus dinkinsorum* Orfinger and Etnier, 2020, as indicated by grey shading.

Figure 5-46. Known Canadian province and US state distribution of *Polycentropus elarus* Ross, 1944, as indicated by grey shading.

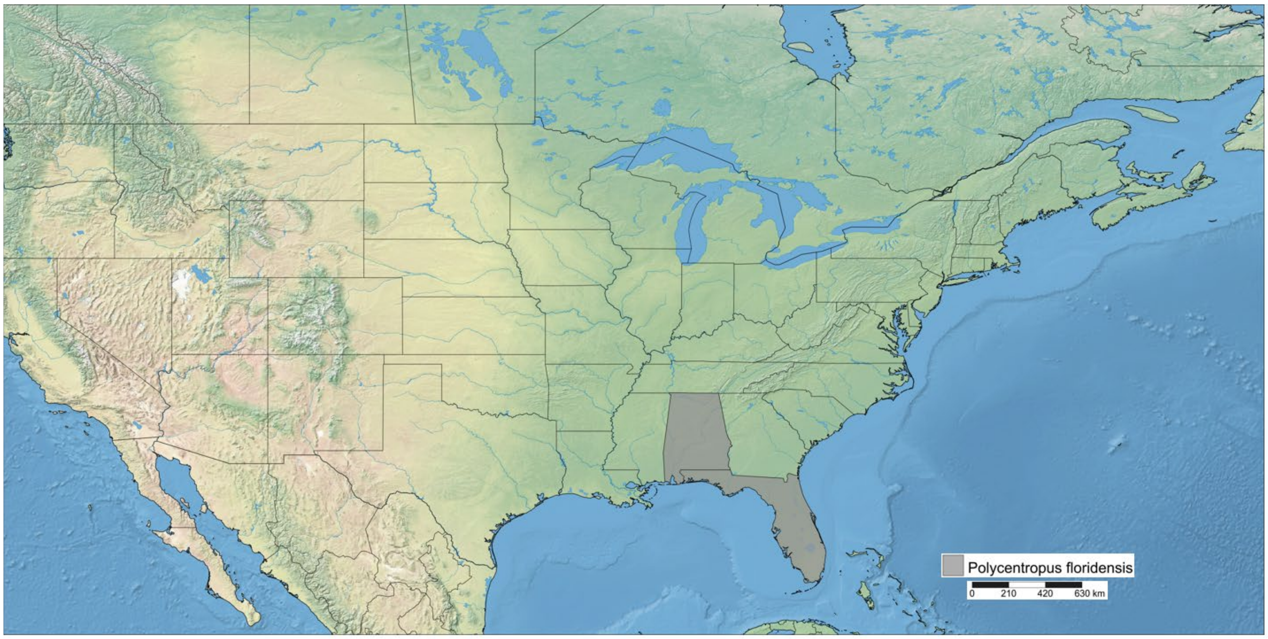

Figure 5-47. Known Canadian province and US state distribution of *Polycentropus floridensis* Lago and Harris, 1983, as indicated by grey shading.

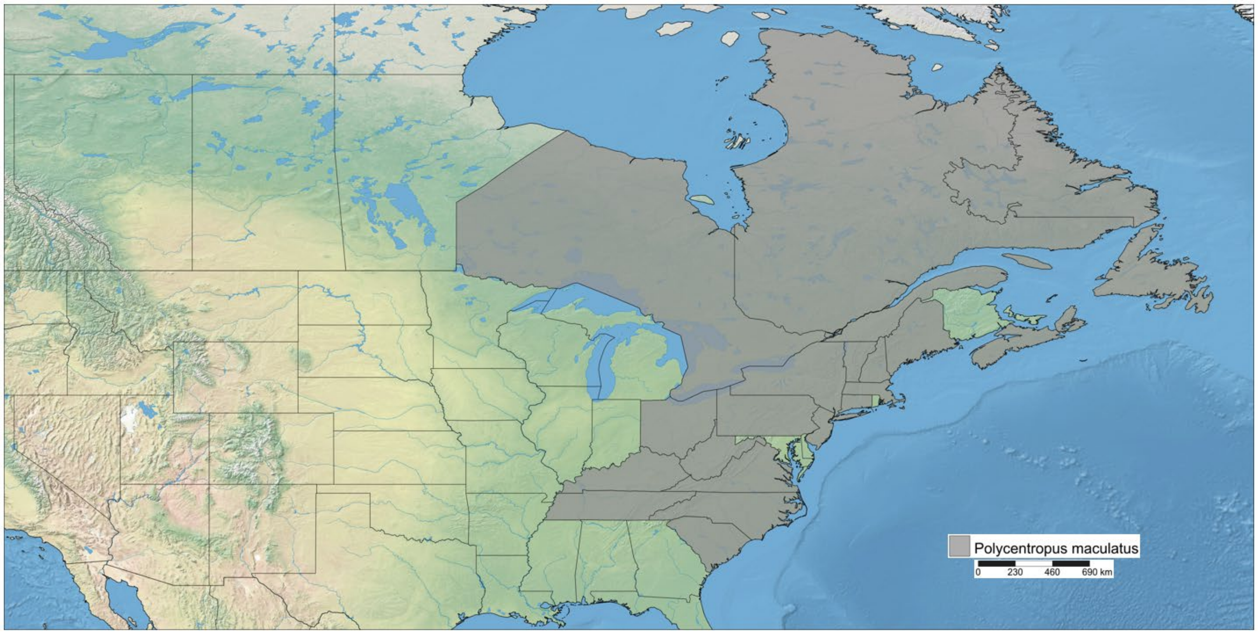

Figure 5-48. Known Canadian province and US state distribution of *Polycentropus maculatus* Banks, 1908, as indicated by grey shading.

Figure 5-49. Known Canadian province and US state distribution of *Polycentropus neiswanderi* Ross, 1947, as indicated by grey shading.

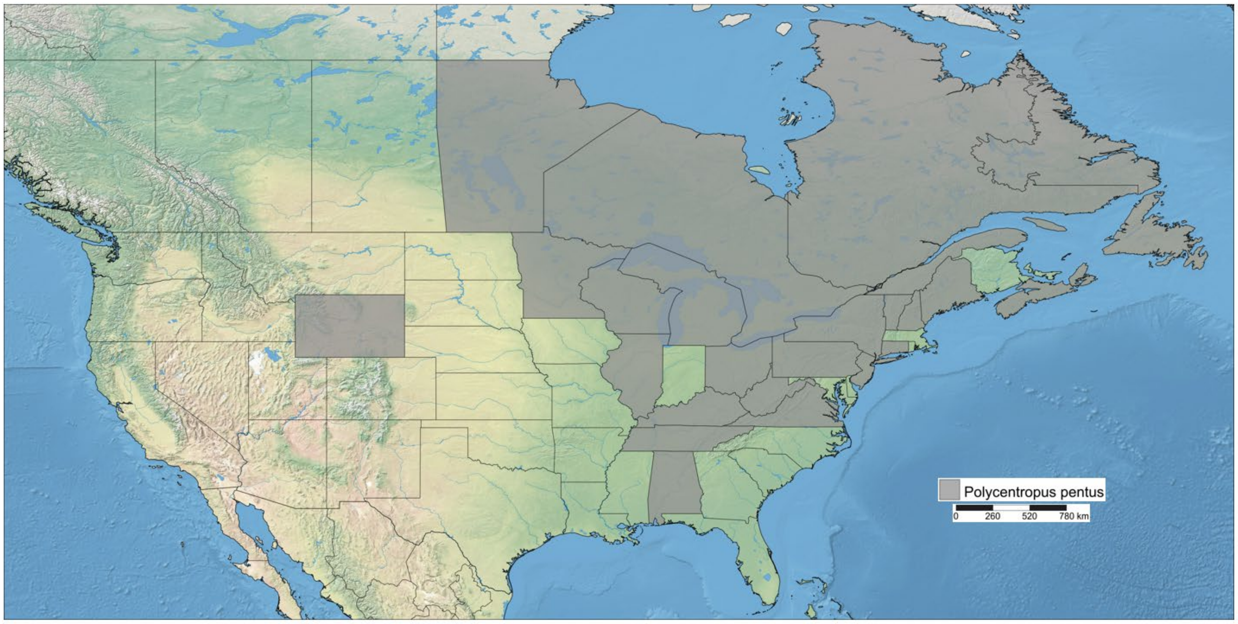

Figure 5-50. Known Canadian province and US state distribution of *Polycentropus pentus* Ross, 1941, as indicated by grey shading.

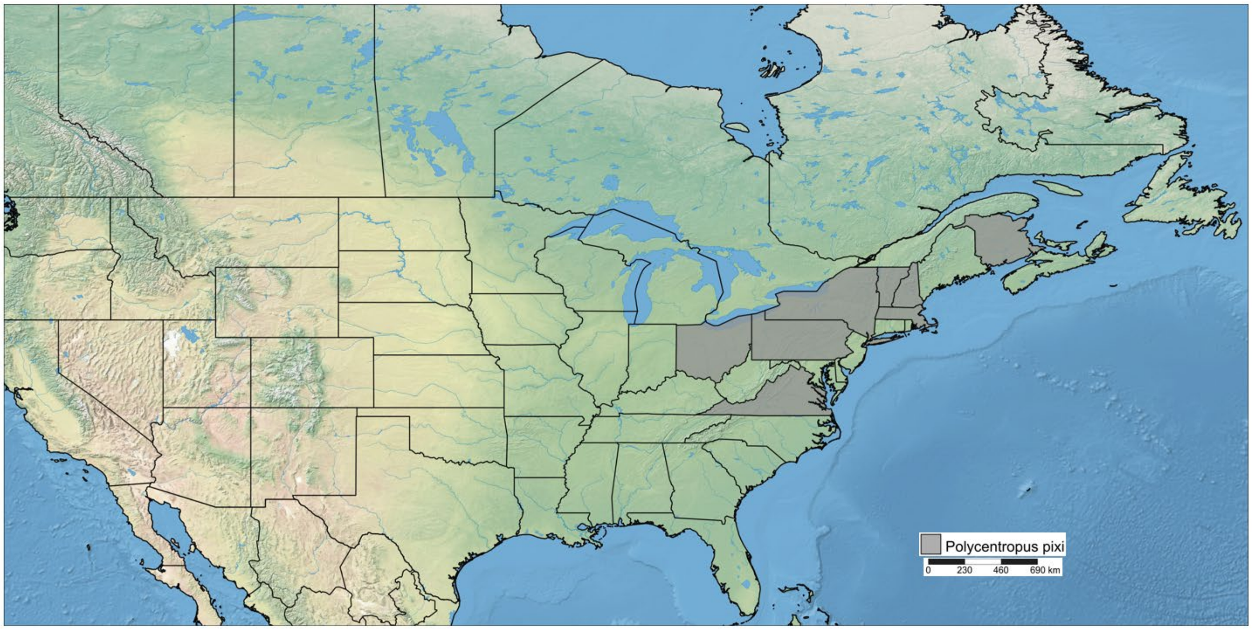

Figure 5-51. Known Canadian province and US state distribution of *Polycentropus pixi* Ross, 1944, as indicated by grey shading.

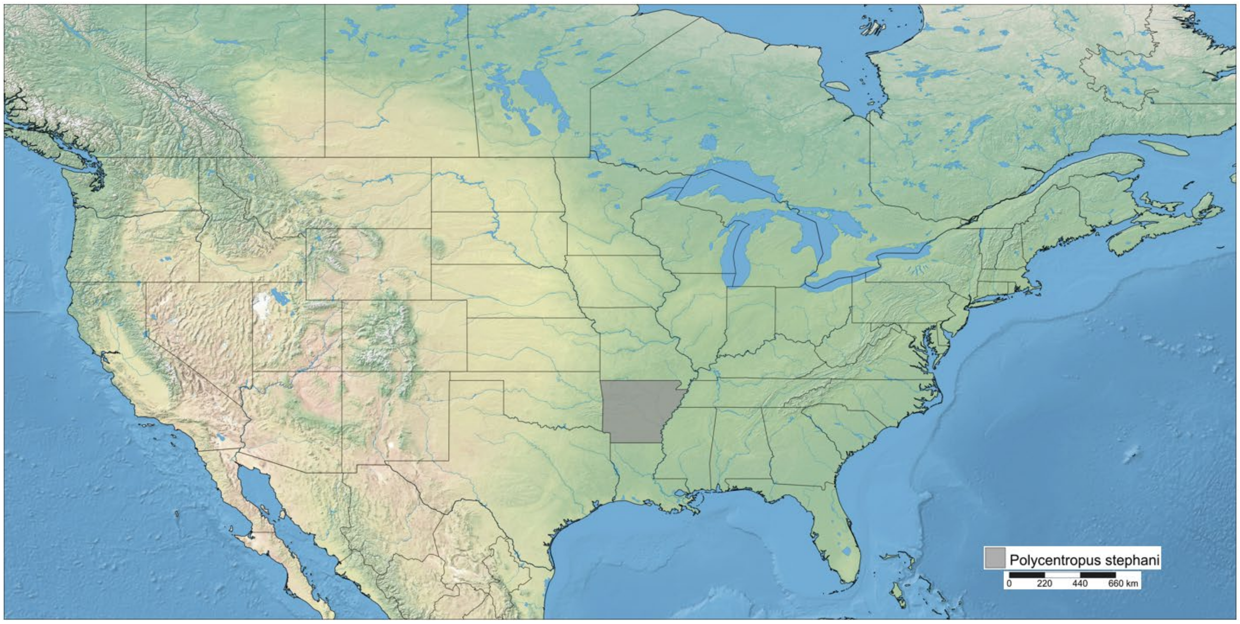

Figure 5-52. Known Canadian province and US state distribution of *Polycentropus stephani* Bowles, Mathis, and Hamilton, 1993, as indicated by grey shading.

Figure 5-53. Known Canadian province and US state distribution of *Polycentropus thaxtoni* Hamilton and Holzenthal, 1986, as indicated by grey shading.

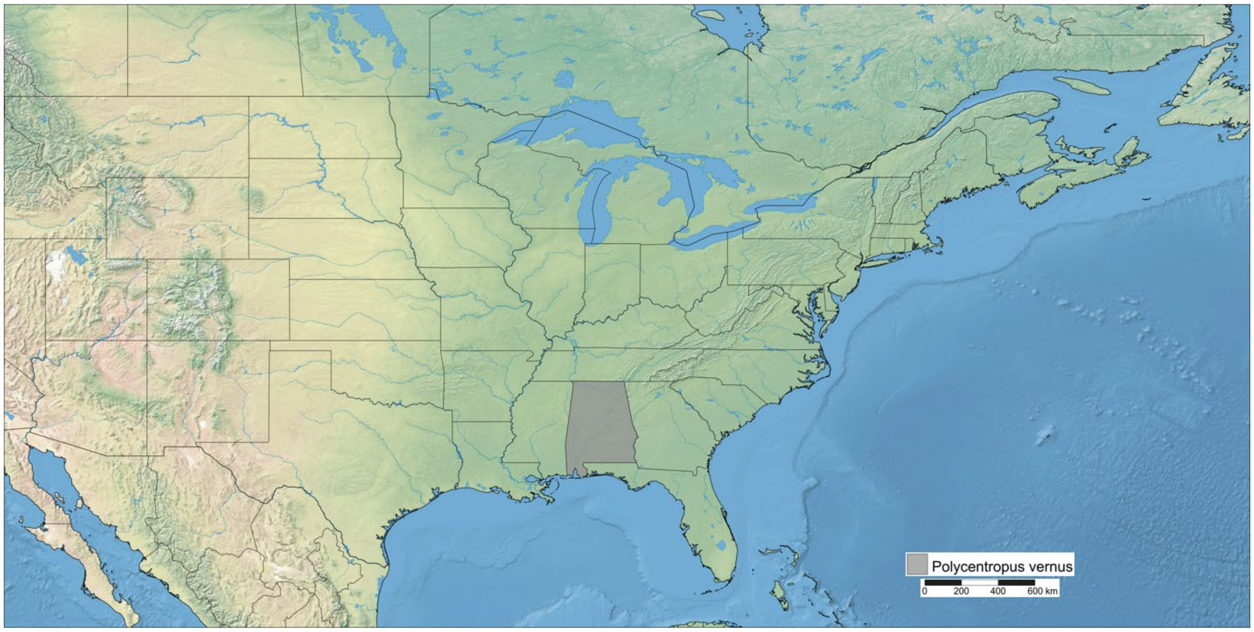

Figure 5-54. Known Canadian province and US state distribution of *Polycentropus vernus* Hamilton, Harris, and Lago, 1990, as indicated by grey shading.

CHAPTER 6
CONCLUSION

This dissertation constitutes the first of several steps towards a comprehensive taxonomic and distributional treatment of all life stages and both sexes of the Nearctic *Polycentropus sensu lato*. This process is necessary to rectify the disparity between our robust knowledge of males of the genera and our relatively limited knowledge of females, pupae, larvae, and eggs of the species in the Nearctic Region. This long-term goal seeks to manifest in the descriptions and identification tools of all of these life history stages and both sexes in the region. The results will pave the way for basic biological studies and applied water quality tolerance research.

Summary

The work presented here is restricted to the *Polycentropus sensu stricto* and treated, to varying extents, all 29 nominal species in the Nearctic Region. First, molecular methods were used to associate females and larvae of *Polycentropus* species represented in the Nearctic. Analysis of mtCOI sequences using distance- and tree-based methods resulted in the association of larvae for 15 species of *Polycentropus* (*P. alabamensis* Hamilton, Harris and Lago, 1990, *P. blicklei* Ross and Yamamoto, 1965, *P. carlsoni* Morse, 1971, *P. carolinensis* Banks, 1905, *P. centralis* Banks, 1914, *P. colei* Ross, 1941, *P. confusus* Hagen, 1861, *P. denningi* Smith, 1962, *P. elarus* Ross, 1944, *P. gertschi* Denning, 1950, *Polycentropus halidus* Milne, 1936, *P. maculatus* Banks, 1908, *P. pentus* Ross, 1941, *P. rickeri* Yamamoto, 1966, and *P. variegatus* Banks, 1900). The larval identify of one additional species (*P. floridensis* Lago and Harris, 1983) was achieved via geographical association. In addition, mtDNA-based association was used to identify females for three species (*P. alabamensis*, *P.*

carolinensis, and *P. chelatus* Ross and Yamamoto, 1965). These results paved the way for the subsequent descriptions and diagnoses of these previously unidentifiable life forms. The tree-based methods leveraged in Chapter 2 also implied interesting phylogenetic relationships, including the sister relationship of *P. blicklei* and *P. aileenae* Orfinger and Moulton, 2021, and of *P. elarus* and *P. alabamensis*. These topologies (e.g., Fig. 2-1) also mirrored the distinct east-west geographic divide observed in Nearctic in the *Polycentropus* fauna.

Building on the results from Chapter 2, Chapter 3 sought to describe and diagnose the newly associated western Nearctic *Polycentropus* larvae. Accordingly, the late-instar larvae of four of seven western Nearctic *Polycentropus* species were described and figured for the first time. These were *P. denningi* Smith, 1962, *P. gertschi* Denning, 1950, *P. halidus* Milne, 1936, and *P. variegatus* Banks, 1900. A diagnostic matrix to assist in their identification was provided (Table 3-1), new state records reported, and previous records clarified. During the examination of the western larvae and comparative material from several Nearctic members of the genera *Cernotina* Ross, 1938, *Holocentropus* McLachlan, 1878, and *Plectrocnemia* Stephens, 1836, it was determined that the current keys (e.g., Wiggins, 1996; Morse et al., 2017, 2019b) to the larvae of the *Polycentropus sensu lato* are unable to separate the genera. Previously, the character for separating *Polycentropus* from *Cernotina, Holocentropus*, and *Plectrocnemia* was: *Polycentropus* larvae have prothoracic tarsi that are broad and only one-half as long as the prothoracic tibiae (Fig. 3-2A), versus prothoracic tarsi narrow and at least two-thirds as long as the prothoracic tibiae (Fig. 3-2B) in the remaining genera. All of the *Polycentropus* larvae examined during the course of this dissertation exhibited prothoracic tarsi that are narrow and at least two-thirds as long as the prothoracic tibiae. This included the previously associated and described larva of *P. centralis*, which Ross treated in his classic 1944 work, but failed to describe the leg morphology of the larva. So, while foreleg segment ratios work in separating the European representatives of *Polycentropus* from other *Polycentropus sensu lato* genera (e.g., Waringer and Graf, 2011), these characters are not useful in distinguishing *Polycentropus* from allied genera for the Nearctic fauna.

Following the treatment of the western Nearctic larvae, the eastern Nearctic *Polycentropus* larvae were then described and diagnosed in Chapter 4. The late- or final-instar larvae of an additional 11 of the 22 eastern species were described and the larva of *Polycentropus centralis* Banks 1914 was redescribed. Species for which larvae were newly described were *P. alabamensis*, *P. blicklei*, *P. carlsoni*, *P. carolinensis* Banks 1905, *P. confusus*, *P. elarus*, *P. floridensis*, *P. maculatus*, and *P. pentus* of the *Polycentropus confusus* Species Group and *P. colei* and *P. rickeri* of the *Polycentropus colei* Species Group. A diagnostic matrix to assist in their identification (Table 4-1) was produced and one new state record reported.

Finally, Chapter 5 was a taxonomic revision of the adults of the *Polycentropus confusus* Species Group, an eastern Nearctic grouping representing 19 of the 22 eastern Nearctic known *Polycentropus* species. The revision was based on thousands of specimens from dozens of public and private natural history collections along with newly collected material. Males of all 19 species were redescribed and illustrated, the 14 known females of group were described and illustrated, including the original descriptions and illustrations for the females of *P. alabamensis*, *P. carolinensis*, and *P.*

chelatus. Neotypes were designated for *P. alabamensis* and *P. elarus*, and dichotomous keys to males and females of the species were provided. New state and province records were reported, one new country record was reported, erroneous records were corrected, and the known distribution of each species summarized.

Adult Identification

In the course of examining thousands of specimens spanning multiple life stages, both sexes, and broad spatiotemporal coverage, I have developed a keen sense of the challenges and opportunities related to the identification of these animals. Characters most useful for male identification within the *P. confusus* Species Group tend to be the size, shape, and orientation of the dorsobasal processes of the inferior appendages as viewed in lateral aspect, the archictecture of the main bodies of the preanal appendages as viewed in lateral aspect, the shape and curvature of the phallus, and the form of the ventral portion of the inferior appendages in ventral view. Indeed, the most recent key to males of the *P. confusus* Species Group by Armitage and Hamilton (1990) treated 14 of the *P. confusus* Species Group males and relied heavily upon the “necks” and “heads” of the dorsal processes of the inferior appendages to separate species early in the key. The key to males presented in Chapter 5 utilizes these characters as well, but instead relies on the curvature and size of the ventral portions of the inferior appendages in ventral view as an early trait by which to broadly separate species. Not only do the ventral portions of the inferior appendages exhibit a great deal of interspecific variation, but these structures are easily viewed, even in uncleared specimens, and provide a good starting point for identification.

Females of the *P. confusus* Species Group can be challenging to identify. Ideally, all specimens should be cleared prior to attempting species-level identification. This is particularly important regarding visibility of the internal parts of gonopods VIII, the shape, orientation, and apparent texture of which are enormously informative in separating species. Though these structures are generally visible in uncleared specimens, the requisite level of detail of the internal parts of gonopods VIII needed for identification typically necessitates clearing of the genitalia.

Presently, the females of the *P. confusus* Species Group are separated from the only known female (*P. rickeri*) of the *P. colei* Species Group by the shape of the posterior apex of external gonopods VIII as viewed in ventral aspect. The female of *P. rickeri* bears a concpicuous narrow and acute thorn-like process absent in any known females of the *P. confusus* Species Group. However, given that several females of the *P. confusus* Species Group remain unknown as do the females of *P. barri* and *P. colei* from the *P. colei* Species Group, the ability to use this character to separate females of the two groups could be nullified if any of those unknown females are found to violate that first couplet of the key presented in Chapter 5. This will remain unknown until the remaining females of the eastern *Polycentropus* fauna are associated and described.

Larval Identification

The larval taxonomy of the Nearctic *Polycentropus* fauna is far from completely resolved, though significant progress has been made. Given the lack of gills, portable cases, and spacing humps, for example, that serve as valulable characters in the identification of many other caddisfly larvae, identification of Nearctic *Polycentrpopus* is often difficult. As with the European fauna, the curvature of the anal claws and the patterning of the posterior muscle scars on the frontoclypeus were particularly useful. Morphometric data characterizing the relative sizes of larval heads was also found to be informative. In one instance, for example, the ratio of head width to body length and close examination of provided muscle scar maps were needed to separate *P. halidus* and *P. variegatus*. Though chaetotaxy was not discussed in this dissertation for several reasons mentioned in Chapter 3, no chaetotaxic characters were found to be informative for identification to the species or genus level. However, numerous specimens used in this study exhibited significant damage from whole-body lysis, long-term bulk sample storage, and/or having been used in water quality studies. Future efforts leveraging additional material may be able to identify chaetotaxic characters for use in taxonomy or phylogenetics of the Nearctic *Polycentropus sensu lato* fauna. In addition, though dozens of larvae were available for examination for some species (e.g., *P. confusus*, *P. variegatus*) some species had few associated larvae available for study including, for example, *P. denningi* (n=1) and *P. carlsoni* (n=1). Examination of additional specimens for those species with little material available will yield a better understanding of intraspecific variation useful in making species-level larval determinations.

Integrative Taxonomy

In cases of uncertainty when using morphology to achieve species-level identification, distributional and molecular data provided in this dissertation are particularly useful. This dissertation resulted in the production of a robust DNA barcode library with all data publiclly available (Orfinger et al., 2021). These molecular data, when applied in a statistically sound framework, provide a powerful tool in identifying various North American polycentropodid taxa. DNA barcoding is not an identification panacea, however, and should not be treated as such. For example, Chapter 2 demonstrates that the barcoding region of COI fails to consistently separate some taxa such as *P. aileenae* and *P. blicklei*, as well as *P. alabamensis* and *P. elarus*. Similarly, other studies have shown that COI DNA barcoding can be insufficient in separating closely related caddisfly species (e.g., Salokannel et al., 2021). As with perhaps all biological taxonomy, the ideal approach to identifying and describing taxa is to apply an integrative framework leveraging various data types such as morphological, mitochondrial, nuclear, ecological, and distributional data (Zamani et al., 2022a, 2022b).

Future Research Needs

Despite the progress made in this dissertation, several gaps persist in our taxonomic knowledge of the Nearctic *Polycentropus* fauna. Larvae of three western Nearctic species (*P. arizonensis* Banks, 1905, *P. aztecus* Flint, 1967, and *P. picana* Ross, 1947) remain unknown while the larvae of 10 eastern Nearctic species remain unknown (*P. aileenae*, *P. barri* Ross and Yamamoto, 1965, *P. chelatus*, *P. chenoides*, *P. dinkinsorum* Orfinger and Etnier, 2020, *P. neiswanderi*, *P. pixi* Ross, 1944, *P. stephani* Bowles, Mathis, and Hamilton, 1993, *P. thaxtoni* Hamilton and Holzenthal, 1986, and *P. vernus* Hamilton, Harris, and Lago, 1990). No material is known from *Polycentropus vernus* since the type series was apparently lost and no new material since recorded, despite exhaustive efforts by the author to collect new material. Pupae and eggs of all species are virtually unknown, though associated material of both for several species awaits formal description (A. Orfinger, unpublished data). Finally, the inability to separate Nearctic genera of the *Polycentropus sensu lato* will hamper efforts to study the biology and phylogeny of these animals and precludes the accurate production and usage of genus-level freshwater biomonitoring indices. To address this, future efforts should seek to produce a larval species-level dichotomous key or diagnostic matrix inclusive of all seven *Cernotina* species, 29 *Polycentropus* species, 14

Plectrocnemia species, and nine *Holocentropus* species recorded from North America north of Mexico. This dissertation works towards that goal.

As a result of this dissertation, our knowledge of the distribution and taxonomy of the Nearctic *Polycentropus sensu stricto* fauna is greatly strengthened. The data and tools generated provide the requisite taxonomic materials necessary for investigations into the biology, biogeography, and phylogeny of these animals, as well as their potential inclusion in freshwater ecosystem health biomonitoring indices. Finally, with waning numbers of taxonomic specialists in a time of unparalleled extinction events (the "insect apocalypse" (Cardoso and Leather, 2019; Cardoso et al., 2020; Samways et al., 2020; Wagner, 2020)), it is perhaps more urgent than ever to foster the skills necessary to catalogue and study Earth's biodiversity. This dissertation constitutes a small but meaningful step towards that goal.

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BIOGRAPHICAL SKETCH

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