

Article

Larval Taxonomy of the Caddisfly *Cernotina truncona* Ross, 1947 (Trichoptera: Polycentropodidae)

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Abstract: The genus *Cernotina* Ross, 1938 is represented in the southeastern United States by three nominal species: *Cernotina calcea* Ross, 1938, *Cernotina spicata* Ross, 1938, and *Cernotina truncona* Ross, 1947. Of all *Cernotina* species, only the larva of *C. spicata* has been described to date. The goal of this paper is to describe, illustrate, and diagnose the larva of *C. truncona* using ecologically associated specimens. In addition, we used publicly available mitochondrial DNA barcoding data to evaluate the genetic relationships of these species. The larvae of *Cernotina truncona* can be distinguished from those of *C. spicata* by differences in setal placement and number on the meso- and metanota, mandibular morphology, head width, and distal setation of the tarsi. The ultrastructure of the anal claw is figured, highlighting the novel finding of small spines on the concave margin of a larva of *Cernotina*. With this new description, just the second described larva from this genus, only *C. calcea* remains unknown in the southeastern United States. The information provided herein enables the in-depth study of the ecology and life history of this diminutive caddisfly.

Keywords: aquatic insects; biomonitoring; chaetotaxy; COI; diagnosis; dichotomous key; DNA barcoding; morphology; Nearctic; scanning electron microscopy



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1. Introduction

Benthic macroinvertebrates are routinely used in biomonitoring programs worldwide to assess freshwater ecosystem health e.g., [1,2]. This process involves collecting freshwater insects using established protocols, identifying target organisms, and subjecting the resulting pool of taxa to various indices to measure water quality. One of the most commonly used groups of insects is the Trichoptera, or the caddisflies. Caddisflies represent the most diverse primary insect order, with more than 17,000 nominal species occurring globally in both lentic and lotic systems [3].

The passing of the Clean Water Act in 1972 sparked the intensive study of larval caddisflies in the United States to develop new, and improve existing, biomonitoring protocols. Since the early 1970s, it has been known that freshwater biomonitoring efficacy is limited by achievable taxonomic resolution, with lower taxonomic resolution yielding more precise evaluations of freshwater ecosystem health [4,5]. Despite this fact, more than 50% of Nearctic caddisfly larvae remain undescribed at species level [6], precluding maximum biomonitoring resolution.

An example of this taxonomic impediment exists in the diminutive (<9 mm) larvae of the New World genus *Cernotina* Ross, 1938 [7] (Trichoptera: Polycentropodidae; Figure 1). Of the roughly 80 known extant species [8], only a single larva has been described to date [9]. Another example of a *Cernotina* larva might exist from Puerto Rico, as described and discussed by Flint [10,11]. However, this association is unconfirmed and additional evidence is required to strengthen this hypothesis.

Most of the genus' diversity occurs in the Neotropics [8,9], with just seven species recorded from the Nearctic all in the eastern half of the region [12]. Of these seven, three species occur in the southeastern United States, namely, *C. spicata* Ross, 1938, *C. calcea* Ross,

1938, and *C. truncona* Ross, 1947. To date, *Cernotina spicata* is the only described larva of the genus *Cernotina* worldwide [9]. It was first described and illustrated by Hudson et al. [13] and later re-described and re-illustrated by Wiggins [14]. The three southeastern species inhabit various aquatic habitats: *Cernotina calcea* inhabits lotic systems (i.e., streams, rivers), *C. truncona* occurs in lentic habitats (i.e., ponds, lakes), and *C. spicata* has been recorded from both flow regimes [13,14] (A. Rasmussen, personal communication).



Figure 1. Left lateral habitus of a final-instar larva of *Cernotina truncona* Ross, 1947. Scale bar indicates 1 mm.

Of the three southeastern Nearctic *Cernotina* species, the larvae in this study were determined to be *C. truncona* according to three factors: 1. their collection in lentic habitats, thus excluding the lotic *C. calcea*; 2. their morphological differentiation from the previously described larva of *C. spicata*; and, 3. light trapping collection over several years, from all included localities, yielding only adults of *C. truncona* and no other *Cernotina* species (A. Rasmussen, D. Denson, personal communications).

By virtue of this habitat fidelity and intensive past collections of adults, the positive association of *C. truncona* larvae was achieved. Using these associated specimens, the goal herein is to describe, illustrate, and diagnose the larva of *C. truncona* for the first time. In addition to these primary aims, we consider morphological similarity relative to genetic distance of the southeastern *Cernotina* species and briefly discuss gaps in distributional knowledge.

2. Materials and Methods

Specimens were examined using a Unitron Z10 stereomicroscope (Unitron[®], Com-mack, New York, NY, USA) with maximum 120× magnification or an Olympus SZH10 stereomicroscope (Olympus Optical[®], Tokyo, Japan) with maximum 140× magnification. Measurements were taken to the nearest 0.01 mm using a calibrated ocular micrometer. Specimen length refers to total length i.e., anterior margin of head to posterior ends of anal claws. Specimens were frequently preserved in a curled position; therefore, it was

often necessary to use pairs of fine forceps to carefully straighten larvae when measuring length. Head width describes the width of the head measured dorsally at the widest point. Morphological terminology follows [13–16].

For stacked photography of the habitus, the specimen was placed in glycerin in a depression slide. Using an M1400 Plus Digital Camera (Levenhuk®, Tampa, FL, USA) mounted on a Unitron Z10 microscope, 15 photographs were taken at different depths of field and subsequently digitally stitched together using Helicon Focus software (version 7.7.4; Helicon Soft®, Kharkiv, Ukraine) to form a single composite image. Pencil line drawings were produced using an Olympus SZH-DA camera lucida drawing tube (Olympus Optical®, Tokyo, Japan) mounted to an Olympus SZH10 stereomicroscope. Pencil line drawings were then scanned and digitally rendered as vector graphics using Adobe Illustrator (version 28.5; Adobe®, San Jose, CA, USA) for the final illustrations.

Anal claws excised for electron microscopy were mounted on stubs using conductive adhesive tape and examined without sputter coating using a Zeiss Evo LS15 scanning electron microscope (Zeiss®, Jena, Germany). Anal claw ultrastructure was visualized under variable pressure using an accelerating voltage of 25 kV.

Specimens are deposited at the Florida A&M University portion of the Florida State Collection of Arthropods (FAMU) in Tallahassee, Florida, at Dalton State College (DSC) in Dalton, Georgia, and at the Clemson University Arthropod Collection (CUAC) in Clemson, South Carolina.

To examine interspecific genetic distances of southeastern *Cernotina* species, all publicly available sequences of the DNA barcoding region of mitochondrial cytochrome oxidase I (COI) of the three species were downloaded from the Barcode of Life Data Base (BOLD) [17] as FASTA sequences. Sequences used all correspond to publicly vouchered male specimens that were identified by a taxonomic expert. Sequences were aligned using default settings of MUSCLE [18] in MEGA v. 11 [19]. Alignments were checked manually to avoid stop codons, indels, and amino acid translation frame shifts. Inter- and intraspecific pairwise divergence distances (p-distances) were calculated in MEGA v. 11 [19] using the Kimura 2-parameter evolution model (K2P) [20] and pairwise deletion of missing sites.

3. Results

3.1. Larval Taxonomy

Cernotina truncona Ross, 1947 [21]

3.1.1. Larval Description

Final-instar mean length 6.99 mm ($\sigma = 1.01$ mm; Table 1). Lengths of other instars and head capsule widths shown in Table 1. Habitus as in Figure 1. Fifth-instar head and pronotum golden brown or straw-colored with darker brown muscle scars arranged posteriorly and laterally on head and variably positioned medially and laterally on each pronotal sclerite (Figure 2A). Frontoclypeal apotome posteriorly with muscle scar arrangement forming shallow arc or trapezoidal pattern (e.g., Figure 2A). Posterior edge of pronotum with narrow, dark brown band (Figure 2B). Membranous mesonotum with short, single sa2 and sa3 setae, lacking sa1 setae; membranous metanotum with short, paired sa2 setae, lacking sa1 and sa3 setae (Figure 2B). Mandibles asymmetrical, with dorsal margins almost entirely overhanging ventral margins; left mandible with two irregular subapical teeth on each margin; dorsal and ventral margins separated by deep mesal groove; right mandible with two irregular dorsal subapical teeth and one ventral subapical tooth (Figure 2C). Foreleg with tarsus about twice as long as broad, nearly as long as tibia, and bearing row of short, fine hairs on ventral margin, lacking distal broad, fringed setae (Figure 2D). Abdomen cream colored in ethanol. Anal proleg with basal segment longer than distal segment and bearing several long setae dorsally and ventrally (Figure 2E). Two dark bands contiguous dorsomesally between lateral sclerite of distal segment and anal claw. Anal claw strongly curved about 90°, with indistinct ventral striae, superficially appearing smooth on concave

margin, with single row of small, comb-like spines visible at high magnification ($\geq 350\times$), bearing single subapical dorsal accessory spine (Figures 2E and 3).

Table 1. Total lengths and head capsule widths in millimeters of larvae of *Cernotina truncona* Ross, 1947.

Instar	Sample Size	Overall Length (mm)		Head Capsule Width (mm)	
		Mean	Range	Mean	Range
5th	12	6.99	6.25–8.95	0.82	0.78–0.88
4th	17	5.57	3.30–7.00	0.72	0.68–0.75
3rd	9	3.5	3.05–4.30	0.52	0.50–0.55
2nd	9	2.6	1.90–3.95	0.34	0.30–0.35
1st	1	1.6		0.2	

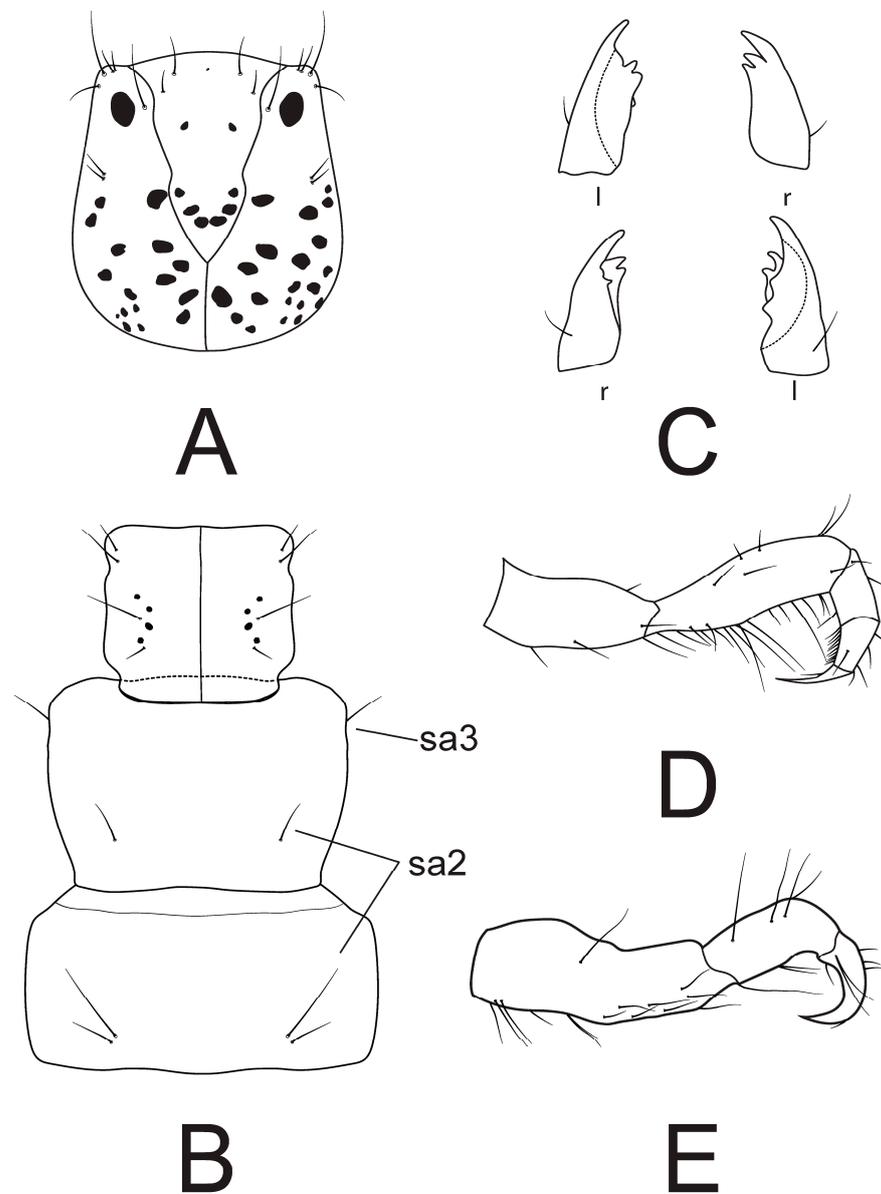


Figure 2. Larval morphology of final instar of *Cernotina truncona* Ross, 1947. (A): Head, dorsal; (B): pro-, meso-, and metanotum, dorsal; (C): mandibles, dorsal (**above**) and ventral (**below**); (D): foreleg, right lateral; (E): anal proleg, right lateral. Abbreviations: r, right mandible; l, left mandible; sa2, sa2 setae; sa3, sa3 setae.

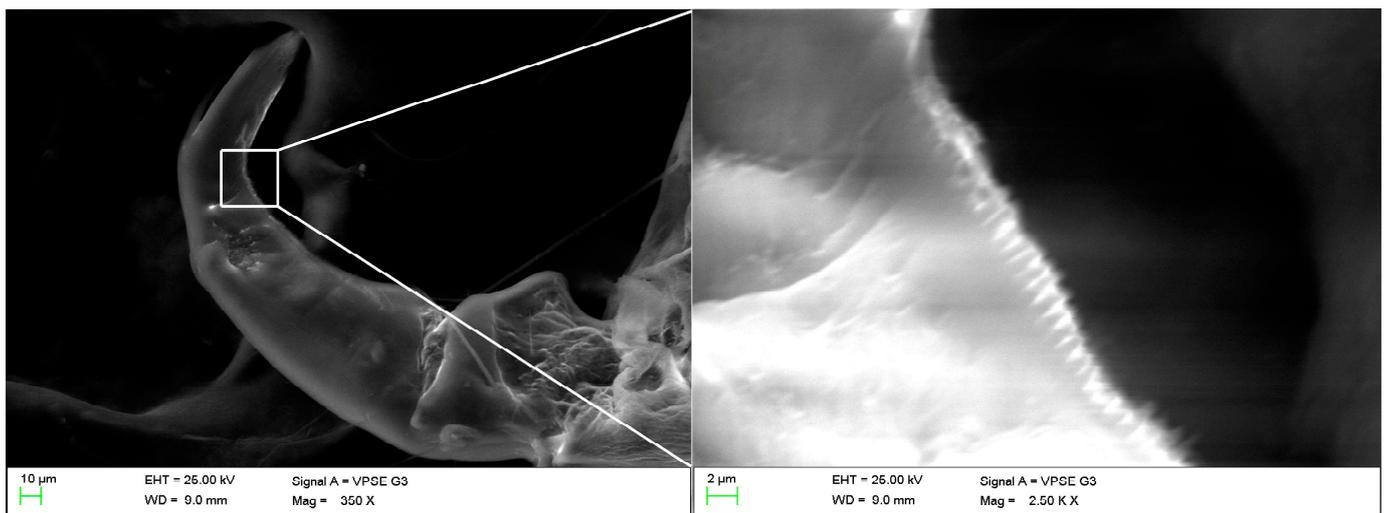


Figure 3. Scanning electron microscopy of anal claw (350X magnification; (left)) highlighting minute spines on concave margin (2500X magnification; (right)).

3.1.2. Material Examined

USA. Florida: Highlands County, Lake Lotela, RPR coll., 13/iii/1986, 3 larvae (FAMU). Leon County, Apalachicola National Forest at Sams Allen Road, Lofton Ponds, Dipnet, [N 30°21'40", W 84°23'25"], D. Denson and A.K. Rasmussen coll., 23-24/i/2007, 7 larvae (FAMU). Orange County, Walt Disney World, EPCOT Lagoon, Petite ponar dredge, [N 28°22'10.9", W 81°32'58.2"], J. Endriss coll., 9/ii/2018, 9 larvae (DSC). Same data as previous entry except S. Duncan coll., 9/ii/2018, 6 larvae (DSC). Same data as previous entry except W. Lavigne coll., 10/ix/2018, 7 larvae (DSC). Same data as previous entry except S. Duncan and J. Endriss coll., 20/xi/2017, 2 larvae (DSC). Same data as previous entry except S. Duncan coll., 10/ix/2018, 14 larvae (CUAC000185394).

3.2. Dichotomous Key to Known Larvae of Southeastern Nearctic *Cernotina*

Note: The larva of *Cernotina calcea* Ross, 1938 is unknown.

1. Mesonotum and metanotum with short sa1 setae; mesonotum with paired sa2 setae and three-grouped sa3 setae; metanotum with paired sa3 setae; tarsi with distal broad, fringed setae; left mandible with three subapical teeth on each margin; right mandible with three ventral subapical teeth; head width < 0.77 mm . . . *Cernotina spicata* Ross, 1938.

1'. Mesonotum and metanotum lacking sa1 setae; mesonotum lacking sa2 setae and with single sa3 setae; metanotum lacking sa3 setae; tarsi lacking distal, broad fringed setae; left mandible with two subapical teeth on each margin; right mandible with one ventral subapical tooth; head width > 0.77 mm . . . *Cernotina truncona* Ross, 1947.

3.3. Genetic Distance

Pairwise genetic distances (p-distances) of southeastern United States *Cernotina* species, based on all the publicly available sequence of the barcoding region of mitochondrial cytochrome oxidase I (COI), are presented in Table 2; this yielded three sequences of *C. calcea*, two of *C. truncona*, and one of *C. spicata*. All sequence lengths are ≥ 495 bp. Intraspecific distances are all substantially lower (0% to 0.013%) than interspecific distances (1.892% to 2.412%), demonstrating consistent barcode gaps. *Cernotina truncona* and *C. spicata* demonstrate the highest overall interspecific p-distance of 2.412%.

Table 2. Pairwise genetic distance (p-distance, expressed as a percentage) of the barcoding region of cytochrome oxidase I between sequences of *Cernotina* species occurring in the southeastern United States, conducted using the Kimura 2-parameter model with pairwise deletion of missing sites. Alphanumeric codes preceding species names represent BOLD sequence IDs.

	FLCAD178-09 <i>C. truncona</i>	FLCAD180-09 <i>C. truncona</i>	FLCAD048-08 <i>C. calcea</i>	FLCAD064-08 <i>C. calcea</i>	PKCAD211-07 <i>C. calcea</i>
FLCAD178-09 <i>C. truncona</i>					
FLCAD180-09 <i>C. truncona</i>	0.000				
FLCAD048-08 <i>C. calcea</i>	2.058	2.004			
FLCAD064-08 <i>C. calcea</i>	2.060	2.006	0.002		
PKCAD211-07 <i>C. calcea</i>	2.007	1.975	0.011	0.013	
MDA465-08 <i>C. spicata</i>	2.412	2.412	1.925	1.927	1.892

Note: Bolded values denote the p-distances between *C. spicata* and *C. truncona*.

4. Discussion

The larva of *Cernotina truncona* is described and illustrated herein for the first time based on ecologically associated specimens from several localities in Florida, USA. This constitutes only the second known *Cernotina* larva [9] and its description enabled a diagnosis distinguishing it from the only previously described *Cernotina* larva, *C. spicata*, based on notal setation and mandibular morphology. The presence of short sa1 setae on the meso- and metanota of *C. spicata*, versus its absence in *C. truncona* (Figure 2B), is consistent and useful in separating larvae of the two species. Likewise, the mesonotum possessing paired sa2 setae and three-grouped sa3 setae and the metanotum with paired sa3 setae in *C. spicata*, versus the mesonotum lacking sa2 setae and exhibiting single sa3 setae and the metanotum lacking sa3 setae in *C. truncona*, are diagnostic. However, these notal setation characters should be used with caution and in conjunction with other informative characters, as setae will sometimes break off with rough collection or handling.

Fortunately, the interspecific differences in subapical teeth provide a second character state to separate the taxa. An additional difference exists in terms of size. The final instar larvae of *C. spicata* range in length from 3.4 to 8.0 mm [13,14] while *C. truncona* range from 6.25 to 8.95 mm (Table 1). So, though *C. truncona* larvae appear to attain longer lengths than their known congener, this is not necessarily diagnostic as there is overlap in their length ranges. The head size appears more informative, with no recorded overlap between the fifth-instar head width of *C. spicata* (0.63–0.76 mm; [13]) and that of *C. truncona* (0.78–0.88 mm). The head width is therefore also cautiously considered diagnostic, but given the fine margin, this should be applied in conjunction with other characters such as the mandibular tooth count and notal setation when identifying specimens until the heads of additional larvae of these two species are measured. A final character differentiating the two species' described larvae is the presence or absence of distal, broad, fringed setae on the tarsi of all legs. Though not noted by Hudson et al. [13], Wiggins [14] subsequently described and illustrated these distal, broad, fringed setae on the tarsi of all legs of *C. spicata*, which are absent in *C. truncona*.

These several but somewhat subtle morphological differences are consistent with those found in other studies that have found clear interspecific genetic differentiation (i.e., Table 2; [22]) coupled with little morphological separation in the polycentropodid larvae [15,23] and some other Annulipalpi taxa such as the Hydropsychidae e.g., [24]. Given that congeneric larvae and, sometimes, larvae of different genera (e.g., the *Polycentropus sensu lato*; [23]) are often difficult to separate morphologically despite distinct adults and clear genetic differentiation, using integrated approaches combining multiple lines of evidence such as morphology, DNA, ecology, and geography is ideal when identifying many caddisfly larvae.

A final and noteworthy finding of this study is the ultrastructure of the anal claws of the larva of *C. truncona*. To date, the concave margins of the anal claws of *Cernotina* and the

remaining *Polycentropus sensu lato* have been considered smooth [13,14]. Using scanning electron microscopy, it was determined here that the ventral margin of the anal claw exhibits a single row of minute, comb-like spines (Figure 3). Interestingly, though Wiggins [14] describes the ventral margin of the anal claw of *C. spicata* as being totally smooth, his accompanying illustration seems to indicate the small, comb-like spines described here. The functionality of these spines is unknown, but they may be used for adhesion to hard substrate, in self-grooming, or could simply be a phylogenetic artifact. Regardless of their function, this finding opens up an interesting avenue of inquiry to determine whether other Annulipalpian larvae have undiscovered architecture of their anal claws and, if so, whether these characters could be phylogenetically informative. To investigate this, it would be worthwhile to survey the ultrastructure of a phylogenetically diverse sampling of Annulipalpian taxa, perhaps beginning with other polycentropodid genera.

The present work lends itself to additional areas of investigation as well. First, the taxonomy, ecology, and distribution of *C. truncona* remains incompletely understood. The pupa and female are undescribed, and the species' distribution is almost certainly incompletely mapped. For example, though *C. truncona* is recorded from Florida, South Carolina, North Carolina, Alabama, and Virginia [12], it is not known from Georgia (Figure 4). Additional sampling will almost certainly take *C. truncona* from the state of Georgia, and, likely, from the far southern southeastern coastal plain, given that the species occurs in lentic habitats that are more abundant in that physiographic region. With the description of the larva of *C. truncona*, only one southeastern Nearctic *Cernotina* remains unknown, namely, *C. calcea*. *C. calcea* is lotic and much more widespread than *C. truncona*, having been recorded from Oklahoma, Texas, Alabama, Arkansas, Florida, Illinois, Indiana, Kansas, Kentucky, Louisiana, Missouri, Mississippi, North Carolina, Tennessee, and Virginia from the Nearctic, and into the Neotropical Region from Mexico and Nicaragua [12]. In addition to some apparent distributional gaps for *C. calcea* (e.g., Georgia and Central American countries between Mexico and Nicaragua), the pupal taxonomy of *C. calcea* also remains unresolved. So, while much work has yet to be done to improve our understanding of the taxonomy, ecology, and distribution of the southeastern Nearctic *Cernotina* fauna and, indeed, the entire genus, the novel data provided here enable the identification of the larva of *C. truncona* and offer new tools and questions for future work. This effort constitutes a small step in the larger goal of resolving the taxonomy of all Nearctic polycentropodid larva.



Figure 4. Known U.S. state-level distribution of *Cernotina truncona* Ross, 1947.

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