New *Cernotina* caddisflies from the Ecuadorian Amazon (Trichoptera: Polycentropodidae)

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Two new species of the caddisfly genus *Cernotina* Ross, 1938 (Polycentropododae) are described from the lowland Amazon basin of Ecuador, *Cernotina tiputini*, new species, and *Cernotina waorani*, new species. These represent the first new species described from this region. We also record from Ecuador for the first time *Cernotina hastilis* Flint, previously known from Tobago, and present new Ecuadorian locality records for *C*. *cygnea* Flint, and *C*. *lobisomem* Santos & Nessimian. The homology of the intermediate appendage of the male genitalia of this genus is established. The region surveyed is under severe environmental threat from logging, mining, and crude oil extraction, making the description of the biodiversity of the region imperative.

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Abstract

Two new species of the caddisfly genus *Cernotina* Ross, 1938 (Polycentropododae) are described from the lowland Amazon basin of Ecuador, *Cernotina tiputini*, new species, and *Cernotina waorani*, new species. These represent the first new species described from this region. We also record from Ecuador for the first time *Cernotina hastilis* Flint, previously known from Tobago, and present new Ecuadorian locality records for *C. cygnea* Flint, and *C. lobisomem* Santos & Nessimian. The homology of the intermediate appendage of the male genitalia of this genus is established. The region surveyed is under severe environmental threat from logging, mining, and crude oil extraction, making the description of the biodiversity of the region imperative.

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Introduction

Trichoptera are an order of insects found in all faunal regions and is comprised of almost 16,000 described species. It is the largest insect order in which all included species live in freshwater during the immature stages (except for a very few semi-terrestrial species and even fewer marine species) (Holzenthal, Thomson & Ríos-Touma., 2015). The Neotropical region (Mexico, Central America, the Caribbean, and South America) is especially diverse in Trichoptera, with more than 3,200 species currently known (Holzenthal & Calor, 2017). Because of their high sensitivity to pollution and environmental changes, caddisflies are considered to be biological indicators of the quality of freshwater (Chang, Lawrence, Ríos-Touma & Resh, 2014). Various biological indices and metrics have been developed incorporating caddisfly diversity and abundance to assess and monitor water quality by many national agencies around the world, including those in South American (Ríos-Touma, Acosta & Prat, 2014).

Among the 39 extant families of Trichoptera, the cosmopolitan family Polycentropodidae contains about 650 species and 15 genera (Chamorro & Holzenthal, 2011). Five genera of polycentropidids occur in the Neotropics: *Cernotina* Ross, 1938, *Cyrnellus* Banks, 1913, *Nyctiophylax* Brauer, 1865, *Polycentropous* Curtis, 1835, and *Polyplectropus* Ulmer, 1905 (Holzenthal & Calor, 2017).

As an exclusively New World genus, *Cernotina* has most of its 70 extant species in the Neotropical region (Holzenthal & Calor 2017), where most occur in the lowlands of the vast Amazon basin (Flint, 1971). One species, *Cernotina pulchra* Wichard, 2007, is known from Dominican amber. No species occur in temperate southern South America (Chile and adjacent patagonian Argentina). In the central and northern Andean countries, *Cernotina* is found exclusively in the Amazonian lowlands (Holzenthal & Calor, 2017). In spite of its diversity and apparently wide distribution, published descriptions and records from South America are few and include those from Argentina (Flint 1983), Brazil (Flint 1971, 1991, Holzenthal & Almeida 2003, Santos & Nessimian 2008, Dumas & Nessimian 2011, Barcelos-Silva, Camargos, Pes & Salles, 2013), Peru (Sykora, 1998), and Uruguay (Angrisano, 1994). The first records of the genus from Ecuador were recently published for *Cernotina cygnea* Flint, 1971 and *C. lobisomem* Santos and Nessimian, 2008 (Ríos-Touma, Holzenthal, Huisman, Thomson & Rázuri-Gonzales, 2017). In North America, *Cernotina* inhabits lotic and lentic freshwaters habitats and the larvae

are considered predators (Morse & Holzenthal, 2008). However, there is no ecological information for the Neotropical species.

Polycentropodidae can be distinguished from other Neotropical caddisflies by a combination of characters (Chamorro & Holzenthal, 2011; Pes, Santos, Barcelos-Silva & Camargos, 2014): absence of ocelli; elongate, flexible segment 5 of the maxillary palp; segment 3 of the maxillary palp inserted subapically on segment 2; pair of distinct, oval, setal warts on the mesoscutum. Adult *Cernotina* can be separated from other Neotropical polycentropodids by the absence of a preapical tibial spur on the foreleg.

The eastern part of the Ecuadorian Amazon includes the vast Yasuní National Park (ca. 10,000 km^2) and the adjacent, much smaller and private Tiputini Biodiversity Station (6.5 km^2). These conservation areas harbor a great diversity of amphibians, mammals, birds, and plants (Bass et al., 2010). In contrast, most insects, including Trichoptera have not been intensively studied in this area. However, while existing records are scarce, they suggest a diverse fauna (Ríos-Touma, Holzenthal, Huisman, Thomson & Rázuri-Gonzales, 2017). Oil concessions and logging have been threatening the biological diversity of this region for more than five decades (Bass et al. 2010, Sierra, 2000; Viña, Echavarria & Rundquis, 2004, O'Rourke & Connolly, 2003). Further, since freshwater biodiversity is among the world's least known (Dudgeon et al., 2006; Esteban & Finlay, 2010), and the Amazon is among the greatest global freshwater ecosystems, it is imperative to study well preserved areas like Yasuní and Tiputini. In our recent effort to record species of Trichoptera from Tiputini, we found new species and records of *Cernotina* (Ríos-Touma, Holzenthal, Huisman, Thomson & Rázuri-Gonzales, 2017) among other caddisflies. In this paper, we describe two new species of *Cernotina* from Tiputini and record a previously described species for the first time in Ecuador.

Materials and Methods

Collecting was accomplished at three sites in the Tiputini Biodiversity Station in October, 2011. The station is located on the northern bank of the Río Tiputini, an easterly flowing southern tributary of the much larger Río Napo (supplementary file 1). We sampled two small waterways and the Tiputini river using ultraviolet lights for approximately 2.5 hours (17:30-20:00 h). (Fig 1). To collect dry specimens for subsequent pinning, ultraviolet and white fluorescent lights were

hung in front of a white bed sheet placed by the margin of the streams (Fig. 1D). Adult Trichoptera attracted to the lights were captured in jars containing ammonium carbonate as the killing agent. In addition, a small UV light was placed over a white tray containing 80% ethanol and left for about 2.5 hours at streamside. Caddisflies collected in the tray were sorted later in the laboratory from other insects and were stored in 80% ethanol.

For examination and description, the male genitalia were prepared using warm 85% lactic acid to macerate soft tissue following the procedures of Blahnik, Holzenthal & Prather . (2007). Pencil sketches were rendered with the aid of a drawing tube attached to an Olympus BX 41 compound microscope. Pencil sketches were imported into Adobe Illustrator CC to produce final digital illustrations. Terminology for male genitalic structures follows that of Chamorro & Holzenthal (2011) for Polycentropodidae.

The specimens examined in this work are deposited in the University of Minnesota Insect Collection, St. Paul, Minnesota, USA (UMSP), the Museo Ecuatoriano de Ciencias Naturales, Quito, Ecuador (MECN), and the Museo de Ecología Acuática de la Universidad San Francisco de Quito, Ecuador (USFQ) as indicated below. All collections were performed under the Environmental Ministry of Ecuador study permit No 0032 MAE-DPO-PNY -2011.

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: urn:lsid:zoobank.org:pub:5CE7AFEF-5077-4930-96BA-5B746FF12250. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central and CLOCKSS.

Results

Species descriptions

116

117 ***Cernotina tiputini*, new species**

118 urn:lsid:zoobank.org:act:E254D21B-7FA0-47CA-AF34-BE437CEE71CE

119

120 Figure 2

121

122 This species is very similar to *C. chelifera* Flint, 1972 from Argentina in the 2 apical spines of
123 the dorsolateral process of the preanal appendage and the general shape of the appendage. It
124 differs from the Argentinian species by the overall shape of tergum X and the intermediate
125 appendage, its relative size shorter than the inferior appendage, a broader dorsolateral process in
126 dorsal aspect, a narrower inferior appendage, and by having 2 internal spines instead of only 1
127 long spine in the phallus.

128

129 Forewing length 3.5 mm male (n=2). Forewing very light brown, apex with small patch of dark
130 setae, white hairs along anal margin; head and thorax with white hair dorsally; antennae
131 stramineous. Forewing with fork V petiolate; hind wing with cross vein Cu2–1A absent, vein 3A
132 absent.
133 *Male genitalia*: Sternum IX with height 3/4ths of entire male genital complex, quadrate,
134 anteroventral margin with deep, broad concavity. Tergum X semi-membranous, divided mid-
135 dorsally; intermediate appendages slightly curved ventrad, thumb-like, about as long as inferior
136 appendage, setose, with 2 thick apical setae, surface with microsetae. Preanal appendages each
137 composed of 2 lobes; dorsolateral process oblong, shorter than inferior appendage, with 2 apical
138 spines; mesoventral process produced dorsolaterally, fused on midline, shorter than inferior
139 appendage, bearing a row of stout setae on posterior margin. Inferior appendages in lateral view
140 slightly fusiform, straight, apex rounded; sclerotized apicomesally, pointed in ventral view;
141 dorsal branch elongate, about as long as body of appendage, oriented posteriad, bearing a row of
142 setae. Phallus slightly bent at mid-length, narrow, with 2 spines; phallotremal sclerite large,
143 ovate, with 2 apparent lateral processes.

144

Holotype male: **ECUADOR:** Orellana, Reserva de Biodiversidad Tiputini, river slough, Numa trail, 00.63954°S, 76.14836°W, el. 260 m, 23.x.2011, Holzenthal and Ríos [pinned] (UMSP000098447) (UMSP).
Paratype: same as holotype, except: 1 male [alcohol] (MECN).

Etymology: The species is named for the Tiputini River and the adjacent biodiversity research station.

***Cernotina waorani*, new species**

urn:lsid:zoobank.org:act:15FD59A3-69F2-4152-B7B8-EE5E34051603

Figure 3

This species has similarities with *C. fallaciosa* Flint, 1983 from Argentina in the bulbous apex of the inferior appendage in lateral aspect and the presence of multiple internal spines in the phallus. However, the absence of apical spines on the dorsolateral process of the preanal appendage, its shape, and the presence of a flap-like median, sub-basal lobe renders this species distinct.

Forewing length 3.5-4 mm (n=6). Forewing stramineous, with slightly darker hairs at apex; head and thorax with lighter hair dorsally; antennae stramineous. Forewing with fork V sessile; hind wing with crossvein Cu2–1A present, vein 3A absent.
Male genitalia: Sternum IX with height about half of entire male genital complex, trapezoidal; anteroventral margin with deep, narrow concavity. Tergum X semi-membranous, divided mid-dorsally; intermediate appendages entire, digitate, about as long as inferior appendage, setose, without spines, surface with microsetae. Preanal appendages each composed of 2 lobes: dorsolateral process elongate, longer than inferior appendage, with flap-like mesal lobe; mesoventral process oblong, not fused on midline, shorter than inferior appendage, bearing a row of stout setae on posterior margin. Inferior appendage subtriangular in lateral view, lateral apex narrow; apex complex, directed mesad, with apicomesal lobe-like processes, mesal process with

sclerotized apex; dorsal branch absent. Phallus straight, narrow, with 2 spines and membranous pouch of 8 small spines; phallotremal sclerite anterodorsal, large, hourglass-shaped.

Holotype male: **ECUADOR:** Orellana, Reserva de Biodiversidad Tiputini, small stream, Harpia trail, 00.63496°S, 76.14602°W, el. 240 m, 22.x.2011, Holzenthal & Ríos [pinned] (UMSP000098911) (UMSP).

Paratypes: same as holotype, except – 4 males [alcohol] (USFQ, MECN); same except: Reserva de Biodiversidade Tiputini, river slough, Numa trail, 00.63954°S, 76.14836°W, el. 260 m, 23.x.2011, Holzenthal & Ríos – 1 male [pinned] (UMSP).

Etymology: This new species in named for the Waorani people, in whose territory, now under severe environmental threat, this species occurs.

Additional species records

Cernotina hastilis **Flint, 1996, NEW RECORD**

Flint, 1996a:75 [original designation]. —Botosaneanu, 2002:95 [checklist]. —Holzenthal & Calor, 2017:415 [catalog].

This species was previously recorded from the island of Tobago.

Material examined: ECUADOR: Orellana, Reserva de Biodiversidad Tiputini, small stream, Harpia trail, 00.63496°S, 76.14602°W, el. 240 m, 22.x.2011, Holzenthal and Ríos – 2 males [pinned] (UMSP); same except: 27 males [alcohol] (UMSP, MECN, USFQ).

Cernotina cygnea **Flint, 1971**

Cernotina cygnea Flint, 1971:37 [original description]. —Sykora, 1998:120 [distribution]. —Paprocki, Holzenthal & Blahnik, 2004:15 [checklist]. —Ríos-Touma, Holzenthal, Huisman,

Thomson & Rázuri-Gonzales, 2017:14 [distribution]. —Holzenthal & Calor, 2017:413 [catalog].

This species was previously reported from Brazil, Ecuador, and Peru

Material examined: ECUADOR: Orellana, Reserva de Biodiversidad Tiputini, river slough, Numa trail, 00.63954°S, 76.14836°W, el. 260 m, 23.x.2011, Holzenthal and Ríos - 1 male [pinned] (UMSP).

***Cernotina lobisomem* Santos & Nessimian, 2008**

Cernotina lobisomem Santos & Nessimian, 2008:27 [original description]. —Paprocki & França, 2015:82 [checklist]. —Ríos-Touma, Holzenthal, Huisman, Thomson & Rázuri-Gonzales, 2017:14 [distribution]. —Holzenthal & Calor, 2017:415 [catalog].

Material examined: ECUADOR: Orellana, Reserva de Biodiversidade Tiputini, river slough, Numa trail, 00.63954°S, 76.14836°W, el. 260 m, 23.x.2011, Holzenthal and Ríos - 1 male [alcohol] (UMSP).

This species was previously reported from Brazil.

Discussion

As discussed by Chamorro & Holzenthal (2010), the intermediate appendage in Polycentropodidae is difficult to distinguish in taxa where this structure is fused with tergum X along its mesal margin, a characteristic commonly found in *Cernotina*. This confusion has led to difficulty in determining the homology of the intermediate appendage versus the dorsolateral appendage in previous species descriptions (e.g., Holzenthal & Almeida, 2003). Some species such as *C. perpendicularis* Flint, 1971 has an appendage very distinct from the membranous tergum X, similar to that of some *Polyplectropus*. In those cases, the intermediate appendages

237 are lateral to tergum X, mesal to the dorsolateral process of the preanal appendages, and always
238 setose.
239 In this paper, we used the term "intermediate appendage" to refer to the lateral, setose,
240 lightly sclerotized lobes of tergum X, following the morphological discussions of Chamorro &
241 Holzenthal (2010) for *Polyplectropus* and the character coding from Chamorro & Holzenthal
242 (2011).
243
244 **Conclusions**
245
246 The species of *Cernotina* described and recorded here were collected only adjacent to two small
247 waterways, one a permanent small stream, the other an inundated, separated channel of the
248 Tiputini River. We did not collect any specimen from lights set adjacent to the Tiputuni River.
249 Even though the study consisted of only 3 nights of sampling (one on the Tiputini, two on the
250 small water bodies), we collected 5 species, 3 recorded here and 2 species previously reported
251 from Ecuador by Ríos-Touma, Holzenthal, Huisman, Thomson & Rázuri-Gonzales (2017).
252 Considering the amount of similar freshwater habitats, the potential diversity of this genus in
253 northern Amazonia is enormous. However, several species could become locally extinct due to
254 the effects of environmental degradation from crude oil extraction, mining, and deforestation if
255 current conservation efforts are not maintained. Loss of species diversity could be even greater,
256 especially if regional endemism is also high as might occur with some *Cernotina* (Flint, 1971).
257 The importance of areas such as Tiputini and Yasuní cannot be overstated for the conservation of
258 the largely unknown freshwater insect fauna of the Amazon.
259
260 **Acknowledgements**
261
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265
266

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365 **Figure Legends**

366 Figure 1. **Collecting localities, Tiputini Biodiversity Station, Ecuador.** (A) small stream,
367 Harpia trail, type locality for *Cernotina waorani*, new species. (B) Río Tiputini. (C) river slough,
368 Numa trail, type locality for *Cernotina tiputini*, new species. (D) same, showing UV light
369 collecting method.

370 Figure 2. **Male genitalia of *Cernotina tiputini*, new species.** (A) segment IX and X, lateral. (B)
371 segment X and preanal appendages, dorsal. (C) segment IX and inferior appendages, ventral. (D)
372 phallus, lateral. (E) phallus, dorsal. (F) mesoventral processes of preanal appendages, ventral.

373 Figure 3. **Male genitalia of *Cernotina waorani*, new species.** (A) segment IX and X, lateral. (B)
374 segment X and preanal appendages, dorsal. (C) segment IX and inferior appendages, ventral. (D)
375 phallus, lateral. (E) phallus, dorsal.

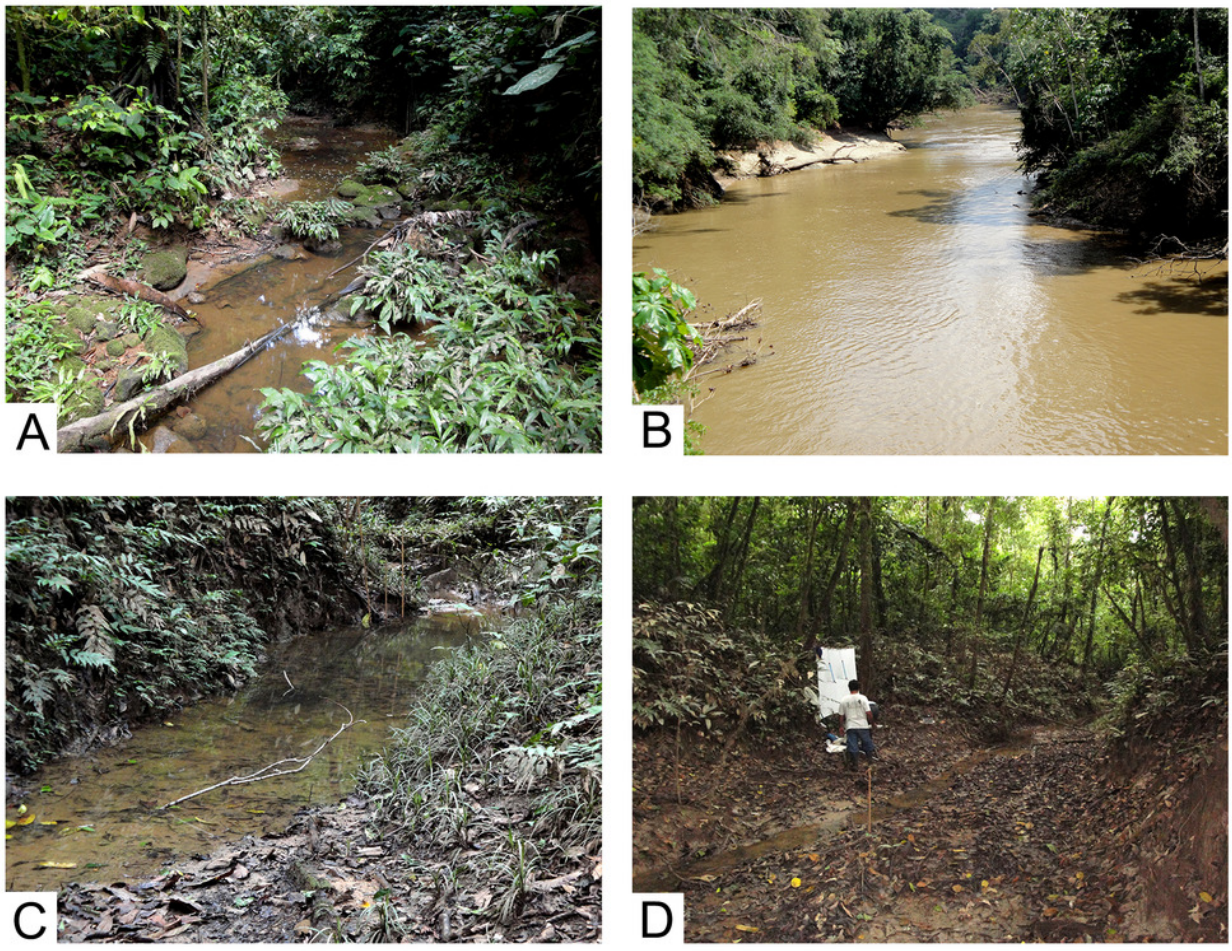
376

377
378

Figure 1

Figure 1. Collecting localities, Tiputini Biodiversity Station, Ecuador.

(A) small stream, Harpia trail, type locality for *Cernotina waorani*, new species. (B) Río Tiputini. (C) river slough, Numa trail, type locality for *Cernotina tiputini*, new species. (D) same, showing UV light collecting method.


A
B
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D

Figure 2(on next page)

Figure 2. Male genitalia of *Cernotina tiputini*, new species.

(A) segment IX and X, lateral. (B) segment X and preanal appendages, dorsal. (C) segment IX and inferior appendages, ventral. (D) phallus, lateral. (E) phallus, dorsal. (F) mesoventral processes of preanal appendages, ventral.

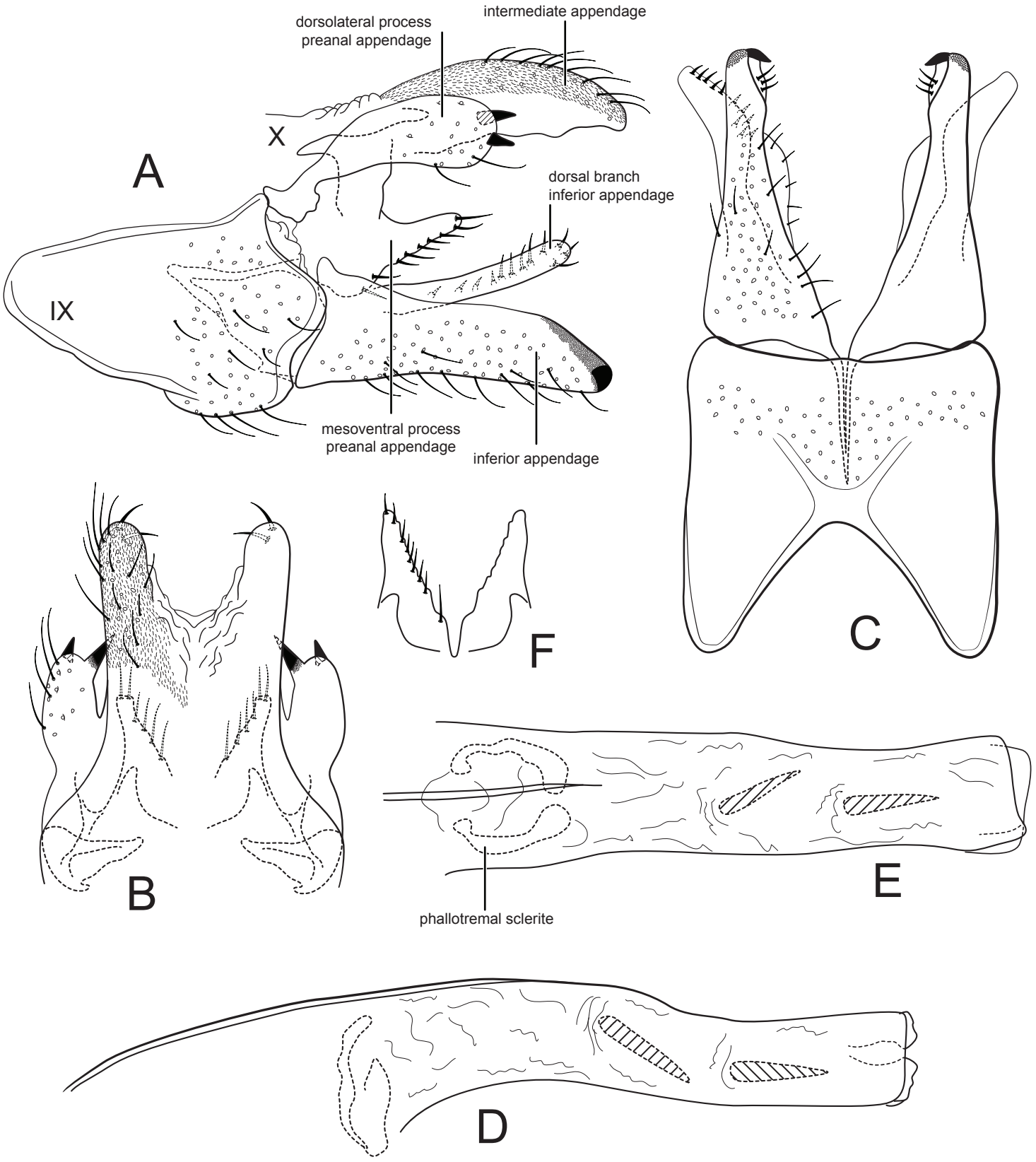
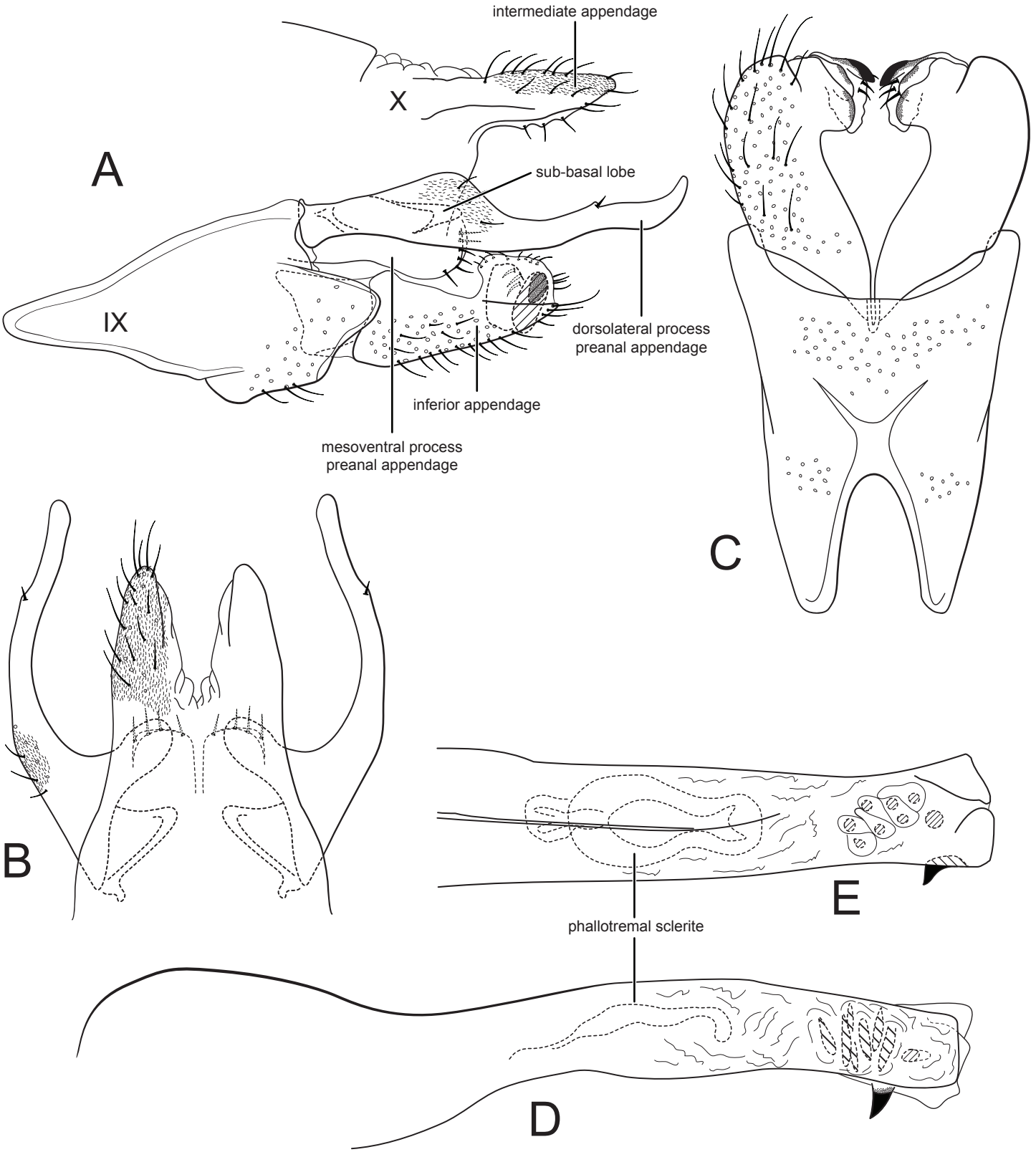

dorsolateral process
preanal appendage
intermediate appendage
X
A
dorsal branch
inferior appendage
IX
mesoventral process
preanal appendage
inferior appendage
C
B
F
E
phallotremal sclerite
D

Figure 3(on next page)

Figure 3. Male genitalia of *Cernotina waorani*, new species.

(A) segment IX and X, lateral. (B) segment X and preanal appendages, dorsal. (C) segment IX and inferior appendages, ventral. (D) phallus, lateral. (E) phallus, dorsal.


intermediate appendage
X
A
sub-basal lobe
IX
dorsolateral process
preanal appendage
inferior appendage
mesoventral process
preanal appendage
C
B
E
phallotremal sclerite
D