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Description of larva and pupa of *Phylloicus cressae* Prather 2003 (Trichoptera: Calamoceratidae) from a montane forest stream in the peri-urban area of Caracas, Venezuela

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ABSTRACT

Phylloicus is the most species-rich genus of the Neotropical Calamoceratidae. In Venezuela, twelve species are known from the adult stage and, from those, only one has its larval and pupal stages described. Here, we describe and illustrate the larva, pupa, and case of *Phylloicus cressae*, from a first order stream of the heavily disturbed Tuy River watershed in the peri-urban area of Caracas, northcentral Venezuela.

Key words: aquatic insects; caddisfly; Cordillera de la Costa; Neotropical Region; taxonomy

INTRODUCTION

From among 8 extant genera and 5 fossil genera, Calamoceratidae is represented by 2 genera, *Phylloicus* Müller 1880 and *Banyallarga* Navás 1916 in the Neotropical region, with *Phylloicus* being the most species-rich with 60 species described to date (Prather 2003; Holzenthal et al. 2007; Santos & Nessimian 2010; Dumas & Nessimian 2010; Quinteiro et al. 2011; Rueda Martín 2013; Barcelos-Silva et al. 2017; Cavalcante et al. 2018). In Venezuela, twelve *Phylloicus* species have been recorded (Prather 2003), but mostly in the adult stage only; the larva and pupa of *P. lituratus* Banks 1920 have been described (Rueda Martín 2013). *Phylloicus* is characterized by day-active adults, and by larvae with flattened cases made of leaf litter pieces (Prather 2003; Quinteiro et al. 2011). These organisms have been recognized as important shredders in Neotropical streams due to their role in the fragmentation of leaf litter (Rincón & Martínez 2006).

Descriptions of immature stages of aquatic insects further our knowledge of both their taxonomy and their ecological features, which are key for monitoring and assessment of the ecological state of streams and rivers. In this study, we describe for the first time the larva and pupa of *Phylloicus cressae* Prather 2003. We also provide a description of its aquatic and riparian habitats and biological notes.

MATERIALS & METHODS

Study area: The specimens were collected in a first-order forested stream, Quebrada Manantial, at the Instituto Venezolano de Investigaciones Científicas (IVIC), Altos del Pipe, Miranda State, northcentral Venezuela (10°10.34'N, 066°58.12'W; 1600 m a.s.l.). IVIC is located within the heavily disturbed peri-urban area of Caracas. Forests at

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IVIC are composed of interspersed remnant patches of native cloud forests mixed with several exotic tree species such as eucalyptus (*Eucalyptus globulus*), rose apple (*Syzygium jambos*), loquat (*Eriobotrya japonica*), and coffee (*Coffea arabica*), among others. These remnant forest patches are located in the Cordillera de la Costa mountain range at altitudes between 1,500 and 1,700 m a.s.l. Quebrada Manantial runs for approximately 400 m to its receiving stream, Quebrada Guayabal, which is polluted by urban sewage and agricultural runoff. All these streams belong to the Tuy River watershed, that ultimately empties into the Caribbean Sea.

Procedures in laboratory: Larvae were collected directly from the leaf litter in the stream and transported to the IVIC's Laboratory of Aquatic Ecology. Invertebrate collection permits were issued by the Dirección General de Diversidad Biológica of the Venezuelan Ministerio de Ecosocialismo y Aguas under permit number 0004-27760. Association of larvae and adults was accomplished by rearing larvae to obtain pupae and adults in the laboratory, under controlled conditions described below. Identification was based on a comparison of the genitalia of the specimens reared and the original description (Prather 2003). Some of the collected larvae and pupae were stored immediately in Kalhe's solution (Wiggins 1996).

Larvae were placed in aquaria with unfiltered stream water, sterilized sand/gravel mix, and conditioned leaf litter from the stream. Aquaria were maintained on a 12/12 h day/night photoperiod. Aquaria were capped with plastic screen netting to retain adults inside once emergence occurred. Water was aerated with air pumps and water temperature averaged 22 ± 2°C. Larvae were examined daily and were fed *ad libitum* during the rearing period. Emerged adults were pinned, and the genitalia were removed and cleared using 10% KOH solution and stored in Eppendorf tubes with glycerin. Immature specimens were preserved and stored in Eppendorf tubes with Kahle's solution. Larvae and pupae were illustrated with a Leica M125 stereomicroscope and a camera lucida at magnifications up to 100X.

The descriptions of the larva and pupa follow the terminology used by Wiggins (1996) and Prather (2003), respectively.

Specimens examined have been deposited in the University of Minnesota Insect Collection, St. Paul, Minnesota, USA (UMSP), and the insect collection of the Museo del Instituto de Zoología Agrícola of Universidad Central de Venezuela in Maracay, Aragua, Venezuela (MIZA-UCV).

RESULTS

Phylloicus cressae Prather 2003.

Type material: VENEZUELA: Lara: Parque Nacional Dinira, Quebrada Las Pinetas, 09°46'19"N, 70°01'45"W, 1889 m, 22.vi.2001, Holzenthal, Blahnik, Paprocki, & Cressa (UMSP), male.

Material examined: Venezuela, Miranda, Altos de Pipe, IVIC, Quebrada Manantial, 10°10.34' N, 66°58.12' W, 1600 m a.s.l., (05.v.2017), M. Barrios, M.D. Mendoza, J.V. Montoya, 9 encased larvae, 2 pupae (UMSP); same, 6 encased larvae, 3 pupae (MIZA-UCV).

5th instar larva: Body length mean = 16.19 mm (range 14.9–16.9 mm; n = 15).

Head capsule: Rugose, elongated in dorsal view, amber with yellow, smooth, ovoid muscle scars. Antennae short, 2-segmented, located between anterior margin of head capsule and eyes. Frontoclypeal apotome subtriangular, wider anteriorly, with weak constriction in middle and with 1 large ovoid yellow muscle scar centered in posterior half. Labrum with transverse row of 32 setae (Fig. 1A). Triangular ventral apotome nearly reaching posterior end of head. Mandibles each with 3 teeth, 1 large central apical tooth and 2 smaller subapicolateral ones bordering a mesal groove; inner side with median brushes (Fig. 2). Labium wide basally, with fused submental sclerite subtriangular near base and 3 rounded apical lobes, pair of lateral palpigers bearing labial palps, central one larger than other two and terminated by silk gland opening, labial palps 3-segmented (Fig. 1B). Head chaetotaxy distributed as Fig. 1A as defined by Mathis (1997).

Thorax: Pronotum amber, with smooth, yellow, ovoid muscle scars posteriorly and posterolaterally; anterolateral extensions of pronotum elongated as pair of strong sclerotized hooks in dorsal view; anterior margin serrate; posterior and posterolateral edges black; 3 pairs of setae on anterior edge; 2 pairs on anterolateral extensions; 5 pairs of setae near lateral edges; and 2 pairs of setae near mid-dorsal ecdysial line (Fig. 3A). Mesonotum with central pair of irregular amber sclerites with yellow muscle scars anteriorly, anteromedial margins strongly sclerotized, posterior

margins not clearly delimited. Paired setal areas (*DSa*) present, on each half *DSa*1 with 1 short seta, *DSa*2 with 1 long ($DSa2_1$) and 1 short seta ($DSa2_2$), *DSa*3 on prominent anterolateral sclerite, with 6 long and 4–6 short setae (Fig. 3B). Metanotum membranous with paired setal areas, on each half *DSa*1 with 1 short seta, *DSa*2 with 1 long seta ($DSa2_1$) and 2 short setae ($DSa2_2$, $DSa2_3$), *DSa*3 with 2 short and 6 long setae (Fig. 3C) as defined by Mathis (1997). Legs: Forelegs each with well-developed trochantin, tapered anteriorly, with 1 seta on basal lobe. Each tibia with stout subapicoventral spine. Tarsal claws each with stout basal seta, such that claw and seta appearing as 2 claws. Leg chaetotaxy in Fig. 4 as defined by Williams & Wiggins (1981).

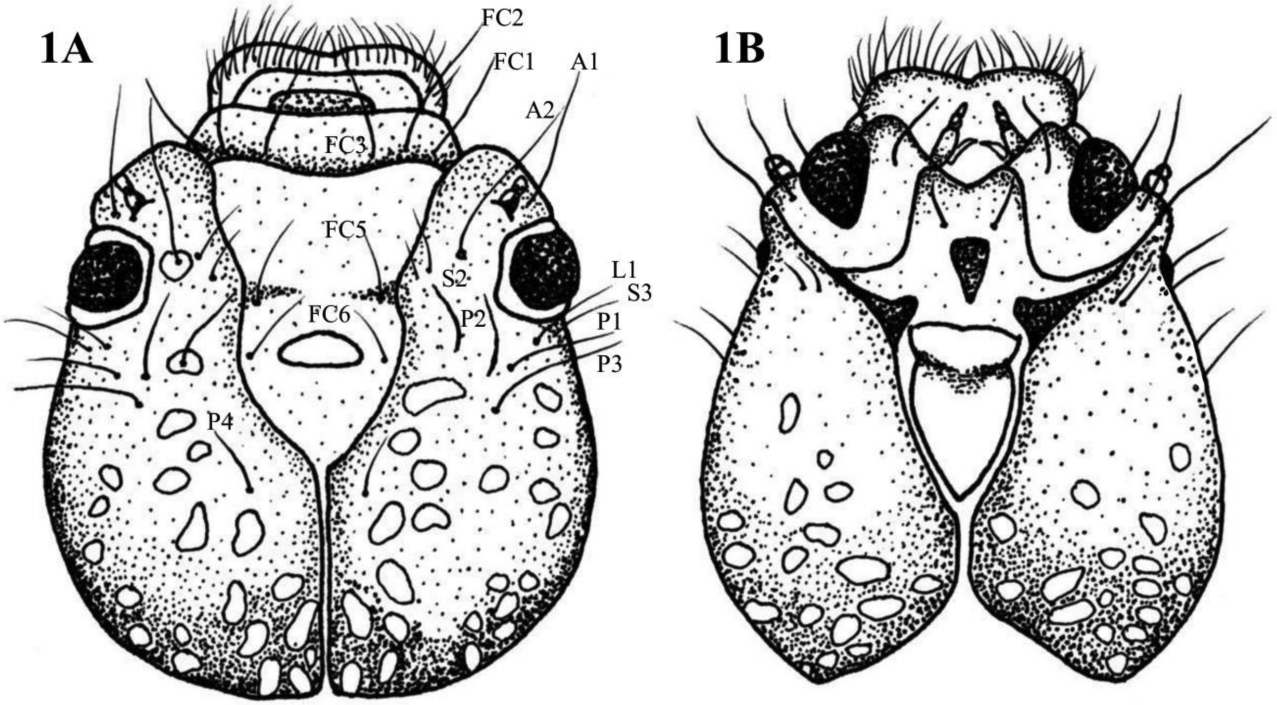

FIGURE 1: *Phylloicus cressae*, larva; head capsule. **1A,** dorsal; **1B,** ventral. Head chaetotaxy in dorsal view, **1A**: FC1–FC6 = frontoclypeal tactile setae, A1–A2 = anterior tactile seta, L1 = lateral tactile seta, posterolateral to stemmata, S2 and S3 = stemmatal tactile seta, P1–P4 = posterior tactile seta.

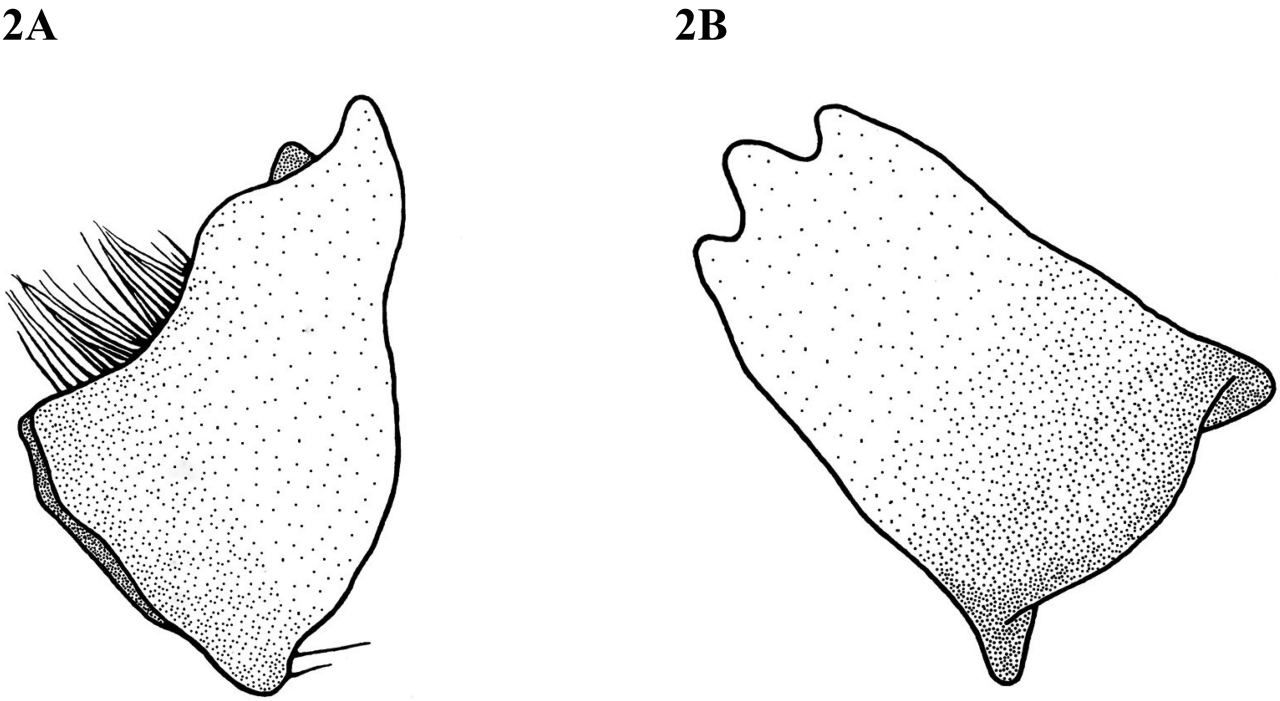

FIGURE 2: *Phylloicus cressae*, larva, mandibles. **2A**, lateral view; **2B**, ventral view.

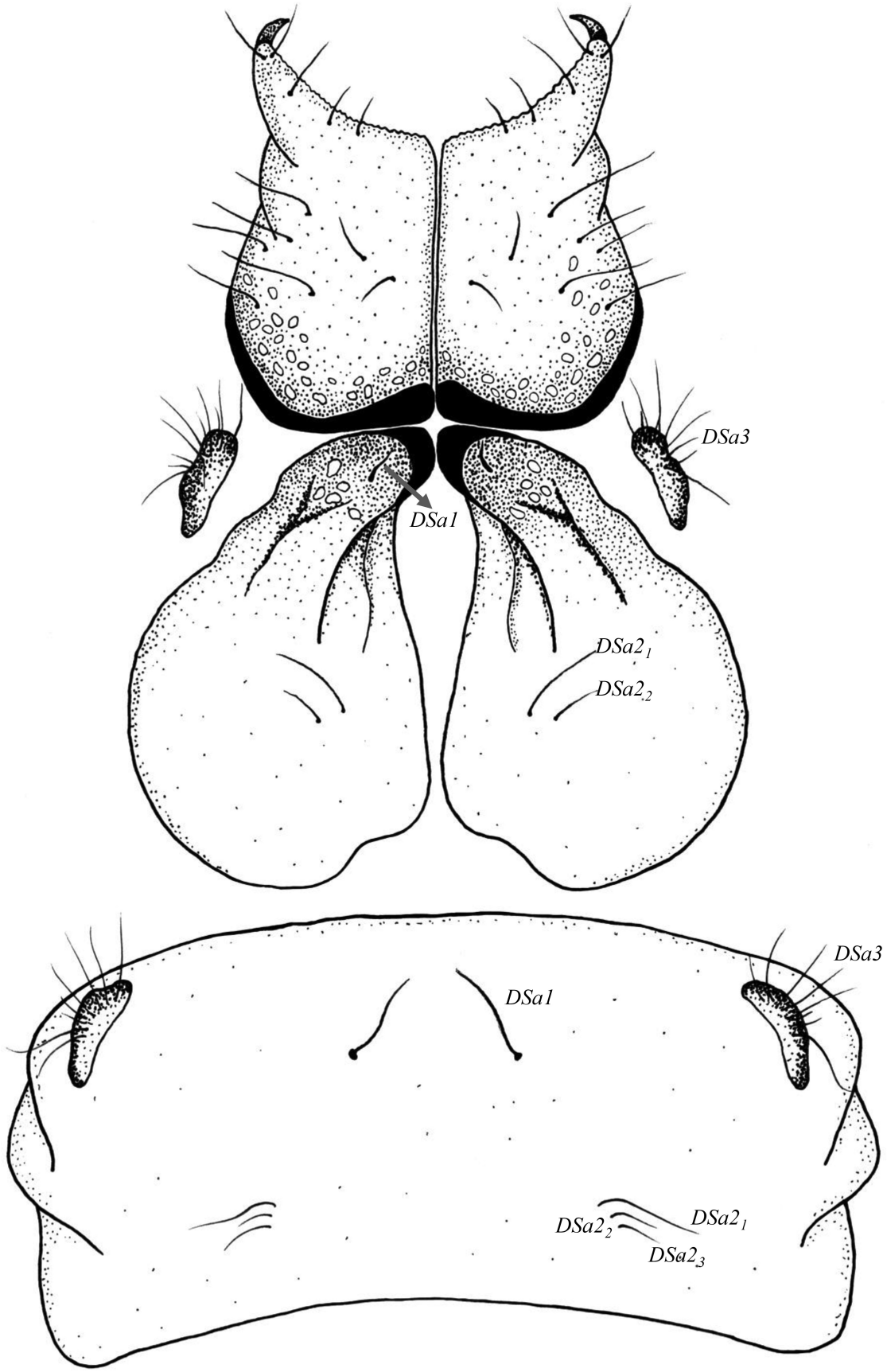

FIGURE 3: *Phylloicus cressae*, larva; thorax, dorsal. **3A,** pronotum; **3B,** mesonotum; **3C,** metanotum. Chaetotaxy of mesonotum and metanotum: *DSa1* = tactile seta in dorsal *Sa1*, *DSa2* = tactile setae (2 or 3) in dorsal *Sa*2, *DSa3* = group of long and short tactile setae in dorsal *Sa3*.

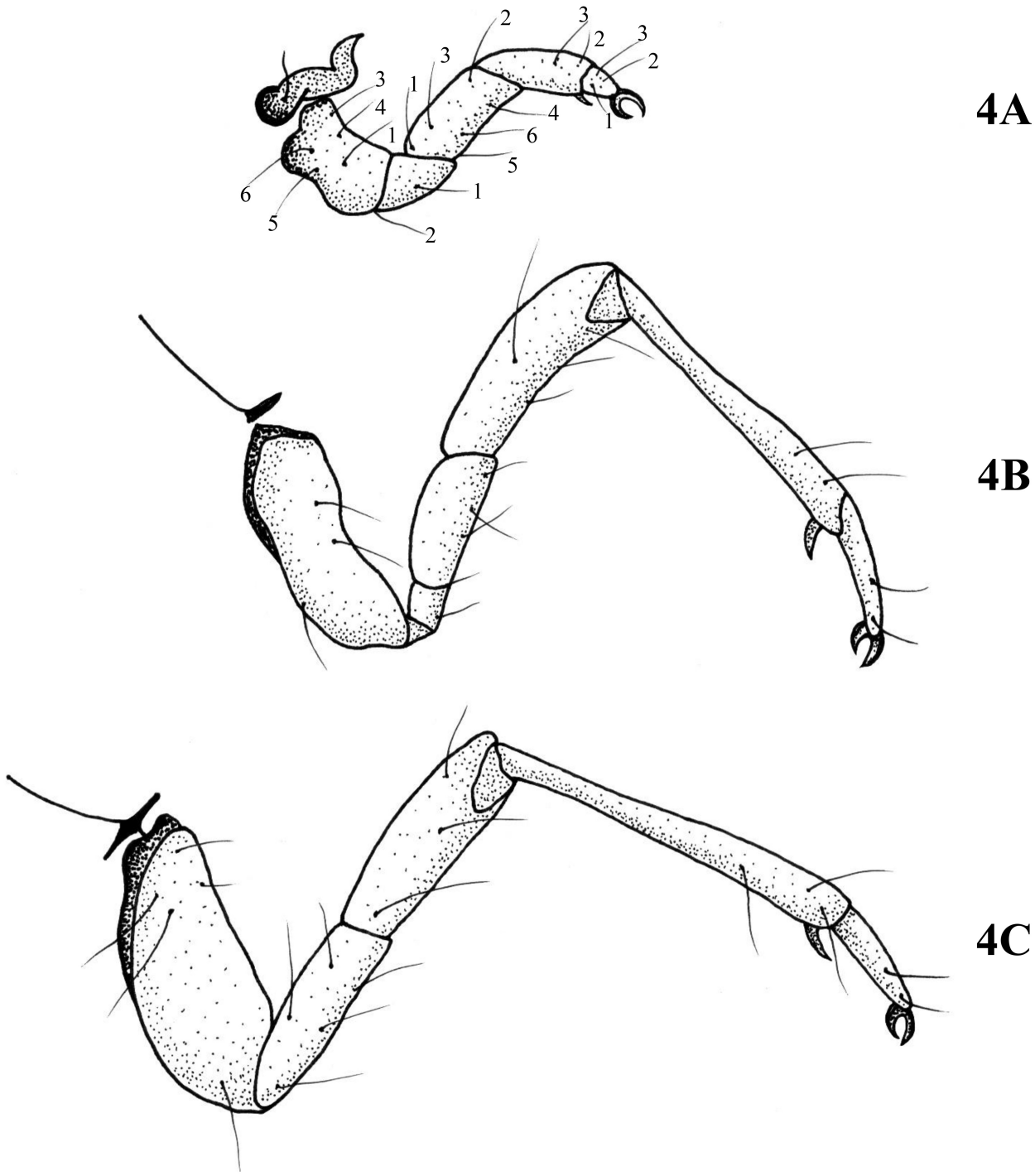

FIGURE 4: *Phylloicus cressae*, larva; right thoracic legs I, II, III, posterior. **4A**, foreleg; **4B**, midleg; **4C**, hind leg. Legs chaetotaxy: **Coxa**: 1 = posterior, dorsal, 3 = anterior, dorsal, proximal, 4 = anterior, dorsal, distal, 5 = anterior, median, 6 = anterior, median. **Trochanter**: 1 = posterior, median, 2 = posterior, ventral, distal. **Femur**: 1 = dorsal, median, 2 = dorsal, distal, 3 = posterior, distal, 4 = posterior, ventral, 5 = anterior, ventral, proximal, 6 = anterior, distal. **Tibia**: 2 = posterior, median, distal, 3 = posterior, dorsal, distal. **Tarsus**: 1 = posterior, ventral, distal, 2 = dorsal, distal, 3 = dorsal, distal.

Abdomen: Membranous, pale yellow. Gills each with 3 or 4 filaments, distributed as in Table 1. Segments III–VII with lateral lines composed of fine, pale setae. Segment VIII with 2 pairs of fine dorsal setae, 1 pair of fine lateral setae, and 1 pair of fine, shorter setae. Segment IX with 1 pair of thick long setae, followed by 3 pairs of fine, short setae in middle of posterior margin, 1 pair of dorsomedial thick setae and 1 pair of lateral setae. Anal prolegs each with very sclerotized hook and long setae.

TABLE 1. *Phylloicus cressae*, distribution pattern of numbers of abdomen gills of the larva and pupa. DL = dorsolateral gills; L = lateral gills; VL = ventrolateral gills.

	Abdomen segments							
	I	II	III	IV	V	VI	VII	VIII
DL	4(l), 0(p)	4	4	4	4	4	4	4
L	0	4	4	4	4(l), 3(p)	3	3	3
VL	0	4	4	4	4(l), 3(p)	3	3	3

(l) = larva; (p) = pupa

Case of 5th instar larva: Length mean = 22.98 mm (range = 21.9–23.5 mm; n = 15) in dorsal side. Flattened, built with fragments of leaves. Each case usually with 3 or 4 dorsal, and 3 ventral rounded or oval fragments (Fig. 5).

Pupae: Body length mean = 12.3 mm (range = 10.3–13.3 mm; n = 5). General color yellowish brown.

Head: Mandibles falciform, well-developed with internal margin finely serrate, apex pointed (Fig. 6A). Each mandible with 2 thick, long basolateral setae. Maxillary palps each 5-segmented, length slightly exceeding bases of forecoxae; labial palps each 3-segmented, short, reaching middle of 3rd segment of maxillary palps. Labrum trapezoidal with base wider than apex, apical area with approximately 12 to 14 pairs of setae; clypeus with 3 pairs of lateral setae. Eyes large, 1 pair of setae in genal area anterior to eyes, another pair on frontal area, 3 pairs of setae posterior to eyes, and another pair on vertex between antennae. Antennal scapes each broad, with group of 5 setae on base and 2 long lateral setae followed by group of 3 short and fine setae on inner side; pedicel without setae; flagellum extremely long, 2x body length and coiled apically (Fig. 6B).

Thorax: Pronotum narrow, bearing 1 pair of setae. Mesonotum wide, with 3 pairs of dorsal setae, 1 anterior and 2 posterior. Metanotum smaller than mesonotum, with 3 pairs of setae, 1 pair on anterior margin, 2 pairs near posterior margin. Middle and hind leg tarsi each with row of fine, long setae, much more numerous on middle tarsi. Wing pads reaching abdominal segment V (Fig. 6B).

Abdomen: Hook plates on anterior part of segments III–VIII, elliptical, each with 2 hooks curved backward, shape varying according to body size and individual; segment I with 1 pair of sclerotized posterolateral lobes each having small hook plate with 3 tiny hooks curved backward; segment V with 1 pair of posterior hook plates, each with 4 hooks curved forward. Segments II–VII each with row of brown setae on posterior margin, these setae longer on segments II–IV, thicker and shorter on segments V–VII. One pair of short setae on anterior part of each segment IV–VII, and 1 pair on posterior part above row of setae on segments V–VII. Segment IX narrow, with 5 pairs of dorsal setae and 2 sclerotized spines apically; apical processes approximately as long as segment IX, each with long seta at midlength and 3 subapical setae (Fig. 6B). Abdominal gills with 3 or 4 filaments on each segment and distributed as in Table 1.

Taxonomic remarks. Some morphological characteristics are noticeable when comparing larvae of *P. cressae* with those described for other species in the genus. The main differences are visible in the color pattern of the head and thorax and their muscle scars, as well as in the mandibles and the chaetotaxy of the mesonotum and metanotum. The color of the larval head and thorax in *P. cressae* is amber with yellow muscle scars, while in other species (*P. obliquus* Navás 1931 (Calvacante et al. 2020)*, P. camargoi* Quintero & Calor 2011 (in Quintero et al. 2011), *P. abdominalis* (Ulmer 1905) (Huamantinco et al. 2005), and *P. lituratus* (Rueda-Martín 2013)) head and thorax are dark brown with yellowish- brown or light brown (*P. camargoi*) muscle scars. The number of teeth on the mandibles also differs between *P. cressae* (3 teeth: 1 central large tooth, and 2 lateral teeth) and *P. obliquus* (2 well-developed teeth), but in *P. camargoi, P. abdominalis*, and *P. pulchrus*, the number and size of teeth are similar. The shape of the central sclerites of the mesonotum in *P. cressae* is triangular with posterior margins rounded, similar to *P. obliquus* and *P. abdominalis,* but in *P. camargoi* the mesonotum tends to be subquadrate and in *P. lituratus* it is subtriangular. Chaetotaxy of the mesonotum also differs from that of other species: *P. obliquus* has *DSa1* with 1 pair of short setae, *DSa2* is without setae, *DSa3* with five pairs of long setae; *P. camargoi, P. abdominalis,* and *P. lituratus* have mesonota with *DSa1* bearing 1 pair of short seta, *DSa2* is with 2 pairs of short and 1 pair of long setae, *DSa3* is with 5 short and 4 long pairs of setae. Metanotal chaetotaxy of *P. cressae* (*DSa*1 is with 1 pair of short seta, *DSa*2 is with 1 pair of long seta and 2 pairs of short setae, *DSa*3 is with 2 pairs of short and 6 pairs of long setae) also differed from *P. obliquus* (*DSa*1 is without setae, *DSa*2 is with 1 pair of long setae, *DSa*3 is with 5 long pairs of setae), *P. camargoi* (*DSa*1 is with 1 pair of long setae, *DSa*2 is with 2 pairs of long setae, *DSa*3 is with 4 pairs of short and 2

pairs of long setae), and *P. abdominalis* (*DSa*1 is with 1 pair of setae, *DSa*2 is with 1 pair of long setae and 2 pairs of short setae, *DSa*3 is with 5 pairs of long setae). Tarsal claws on all legs of *P. cressae* also differed from those of larvae of some species like *P. obliquus* with the tarsal claws simple.

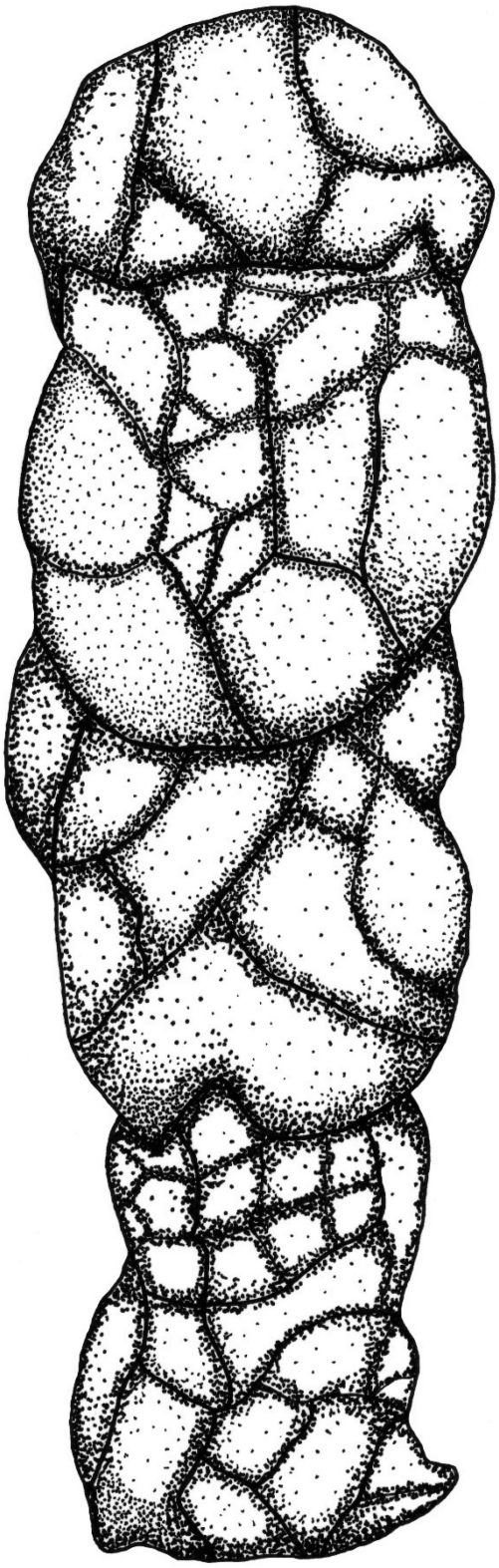

FIGURE 5: *Phylloicus cressae*, 5th instar larval case, dorsal.

The pupa of *P. cressae* can be distinguished from those of other *Phylloicus* species by the presence of 3 hooks on each of the plates on the posterior margin of segment I, 2 hooks on each hook plate on the anterior part of seg-

ments III–VIII, and 4 tiny hooks on each posterior hook plate on segment V, while *P. obliquus* has 3 or 4 small hooks on each hook plate on segment I, 2 hooks on each hookplate on segment III, and 3 hooks on each anterior hook plate on segments IV and V, each posterior hook plate on segment V with 4 or 5 hooks, and on segments VI–VIII with 3 or 4 hooks. In the case of *P. camargoi*, each anterior hook plate of segments III–V, VII, and VIII, with 1–3 hooks (V and VIII usually with 2 hooks), those on segment VI each with 2–3 hooks, and posterior hook plates on segment V with 2–4 hooks. For *P. lituratus,* all segments have each hook plate usually with 2 hooks and posterior hook plates on segment V each with 5 hooks. The pupa of *P. abdominalis* has 3 or 4 hooks on each hook plate.

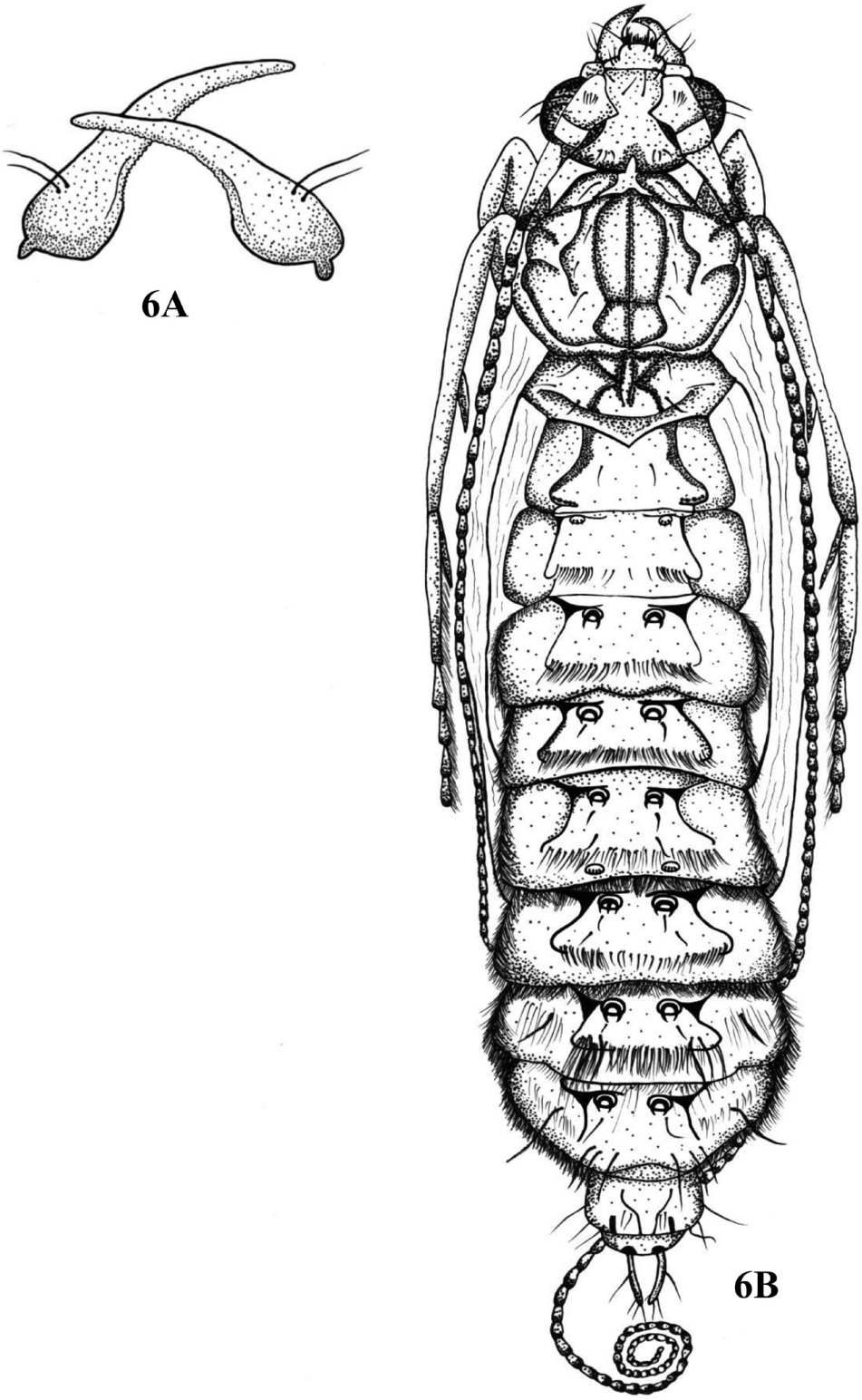

FIGURE 6: *Phylloicus cressae*, pupa. **6A,** mandibles, ventral; **6B,** pupa body, dorsal.

Aquatic and riparian habitat: Larvae and pupae of *Phylloicus cressae* were found in a spring-fed first-order stream named Quebrada Manantial. This is a small mountain stream at 1600 m a.s.l. with a mean discharge of 0.8–1 $L.s^{-1}$. The bottom substrate was characterized by sand and gravel with interspersed leaf litter packets. The ranges of physico-chemical parameters were as follows: Temperature 18.14–21.19°C, dissolved oxygen 3.48–7.10 $mg.L^{-1}$, and 37.4–76.4 %, pH 7.64–8.18, and conductivity 191.40–226.25 $\mu S.cm^{-1}$ (values measured *in situ* using a YSI ProPlus multiparameter sonde). This creek is one of the least-disturbed streams in the region, even though it is located in the heavily impacted peri-urban area of Caracas. We did not find *Phylloicus cressae* in other streams nearby, like Quebrada Fernández Morán or Quebrada Guayabal which is where Quebrada Manantial empties its waters. Quebrada Guayabal is part of the Tuy River basin, one of the most disturbed and polluted watersheds in Venezuela (Rodríguez-Olarte et al. 2018). Most of headwater streams of the peri-urban area of Caracas are strongly affected by direct inputs of untreated urban and industrial sewage and agricultural runoff, making them unfitted habitats for species of aquatic insects intolerant to pollution and habitat degradation, such as species of the genus *Phylloicus*. In Quebrada Manantial, riparian forest is mainly composed of *Croton megalodendron* (Euphorbiaceae), *Bunchosia armeniaca* (Malpigiaceae), *Turpinia occidentalis* (Staphyliaceae), *Piper brederelleri* (Piperaceae), *Toxicodendrum occidentalis* (Anacardiaceae), *Cupania* sp. (Sapindaceae), *Cecropia angustifolia* (Cecropiaceae), *Ficus tonduzii* (Moraceae), *Erythrina poppeyana* (Fabaceae), *Persea caerulea* (Lauraceae), and introduced *Syzygium jambos* (Myrtaceae), *Citrus limon* (Rutaceae), and *Coffea arabica* (Rubiaceae).

Biological notes: *Phylloicus cressae* is one of the most abundant macroinvertebrates in the headwater stream (pers. obs.). Larvae were usually found on or among submerged leaves of *Ficus tonduzii, Erythrina poppeyana, Turpinia occidentalis, Cupania* sp., *Cecropia angustifolia,* and *Syzygium jambos*, which are the most common tree species of the riparian forest of the headwater stream. In the field, pupae were found usually among leaf litter, rocks, dead wood, and fig fruits. Egg masses were found on leaves of *Cupania* sp. and *C. angustifolia* (pers. obs.). *Phylloicus* spp. are characterized by day-active adults; nevertheless, we could not find any evidence of activity of this species during the day. Adults emerged from pupae at the end of the afternoon, from 17h00 to 19h00 (this could be seen only in the laboratory); it is possible that they are active at these hours in nature for copulation, oviposition, or dispersal. While rearing pupae and adults in laboratory conditions, we observed that larval mortality occurred above 25°C (range of stream water temperature: 18.1–21.2°C).

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