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Preimaginal stages of *Triplectides misionensis* Holzenthal and *Triplectides gracilis* (Burmeister) (Trichoptera: Leptoceridae: Triplectidinae), with notes on the cases occupied by these species

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Abstract

The larvae, pupae and cases of *Triplectides misionensis* Holzenthal and *T. gracilis* (Burmeister) are described from specimens collected in protected areas from northeastern Argentina. The larvae are easily differentiated by the coloration of the cephalic capsule, shape of the ventral apotome and submentum, anterior border of the pronotum, and coloration of the legs, among other characters, while the pupae can be recognized by the chaetotaxy of the head. The larvae occupy empty cases of *Grumicha grumicha* Valot and *Nectopsyche gemma* (Müller), as well as hollow twigs. *Triplectides misionensis* is found almost exclusively in cases of *G. grumicha* while *T. gracilis* prefers twigs of different sizes.

Key words: larva, pupa, larval cases, Neotropics

Introduction

The genus *Triplectides* Kolenati 1859 contains about 70 species worldwide, being the most speciose genus in the subfamily Triplectidinae. It is also the most widespread genus, with species distributed mainly in the Southern Hemisphere and with the highest diversity recorded in the Oceanian Region (Holzenthal 1988, Malm & Johanson 2008). In the Neotropics, 14 species have been recorded from southern Mexico to Patagonia (Flint *et al.* 1999, Dumas & Nessimian 2010). The immature stages of only 2 of these Neotropical species are known: *T. egleri* Sattler 1963, and *T. jaffueli* Navás 1918 (Flint *et al.* 1999).

The larvae of *Triplectides* are found in very diverse habitats including cold and warm, unpolluted to moderately polluted, permanent and temporary lentic and lotic systems (Morse & Neboiss 1982). They build tubular cases from various plant or mineral materials, or entirely from silk, while some species use hollowed-out twigs or empty cases of other caddisflies (Holzenthal 1988, Flint *et al.et al.* 1999, Crisci-Bispo *et al.* 2004).

In this paper the larvae and pupae of *T. misionensis* Holzenthal 1988 and *T. gracilis* (Burmeister 1839) are described and illustrated and information about their cases is provided. In particular the occurrence of larvae of both species in cases of *Grumicha grumicha* Vallot 1855 (Trichoptera: Sericostomatidae) and *Nectopsyche gemma* (Müller 1880) (Trichoptera: Leptoceridae) is analyzed.

Material and methods

The specimens were collected between October 2004 and March 2008, in streams of the Parque Provincial Salto

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Encantado and Área Protegida del Valle del Cuñá-Pirú (between 54° 49’ 45" and 54° 58’ 39" W, and 26° 05’ 48" and 27° 05’ 40" S), and the Reserva Privada de la Universidad Nacional de La Plata (between 54° 54’ 53" and 55° 00’ 52" W, and 27° 04’ 28" and 27° 09’ 51" S) in the province of Misiones, Argentina. The Parque Provincial Salto Encantado and Área Protegida del Valle del Cuñá-Pirú are 2 of the most important conservation units of the province and constitute the southern end of the Misiones’ continuous rainforest (Rolón & Chebez 1998).

Larvae and pupal cocoons were obtained using diverse sampling techniques: with a D-frame net sampler (300-µm pore size), a Surber net sampler (0.09-m^2 area, 250-µm pore size) and manually. Some specimens were preserved in 80% ethyl alcohol in the field, while others were transported alive in a cooler to the laboratory where they were reared to adult or pharate adult stage.

The larvae were associated specifically using the metamorphotype method (Milne 1939). For viewing and drawing, the sclerites of the last instar larva left in the pupal case and entire larvae were used. For the identification of the adults the genital segments were cleared in 10% NaOH heated for several minutes, then the cuticle was rinsed in distilled water, neutralized with phenol, and mounted in Fauré medium or glycerin for examination.

In this paper, drawings of the pupae of the 2 described species are not provided. Holzenthal (1998) gave excellent line drawings of the pupa of *T. jaffueli* that can be consulted; the pupa of this species differs from the pupae described herein only in the number of setae of the head and abdomen.

Voucher specimens are deposited in the Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

The terminology used in this paper follows that of Wiggins (1996).

Descriptions

Based on the description by Holzenthal (1988) and the characters observed in the 2 species described herein, we propose a new generic diagnosis for the immature stages of Neotropical *Triplectides.*

Genus *Triplectides* Kolenati, 1859

Larva. Head oval, wider posteriorly; antennae long. Chaetotaxy of head typical for family (Figs. 1, 2). Frontoclypeal apotome spatula-like, with a constriction at mid-length. Labrum subtrapezoidal, with anterolateral ends rounded, separated from frontoclypeal apotome by membranous area; labral setae 1–3 peglike; with brush of short, secondary setae on its anteroventral edge (Figs. 1, 13). Mandibles strong, left one with 6 short teeth surrounding central cavity, right one with 5. Ventral apotome longer than wide, triangular to quadrate, completely separating parietal apotomes ventrally. Pair of small, narrow, sclerites placed parallel to posteroventral edge of parietal apotomes. Labium with submentum small, trapezoidal to oval, fused to anterior end of ventral apotome (Figs. 2, 14).

Thorax with pro- and mesonota covered completely by pair of sclerites separated mesally by ecdisyal line. Pronotum with anterior edge generally crenulate, anterolateral corners sometimes sharply pointed; posterior edge dark and rounded. Mesonotum with each hemitergite quadrate, with anterior edge longer than posterior edge; with 3 setal areas (*sa*): *sa*1 with 1 long seta, *sa*2 with 3 setae (2 anteromesal and 1 posterior) and *sa*3 with 5 to 8 setae. Metanotum with 2 or 3 pairs of sclerites: 2 anteromesal, quadrate sclerites each bearing 1 short subapicomesal seta (*sa*1); 2 lateral, oval, longitudinal sclerites (*sa*3), same length to 2 times longer than *sa*1 sclerites, bearing 3 setae; and each *sa*2 position with single, long seta without basal sclerite or with 2 posteromesal, subrectangular, transverse sclerites, each bearing 1 long seta (Fig. 4, 15). Prosternum narrow, membranous, with transverse, mesoposterior sclerite, triangular to rectangular. Mesosternum with 2 long, curved sclerites located at base of each coxa. Metasternum with pair of setal areas at each side of midline, each bearing 6 to 21 long setae, of which variable number of setae each with basal, circular sclerite (Figs. 6, 17). Foretrocantins large, subrectangular, horn-shaped. Forelegs short and robust. Midlegs long (about 2 times as long as forelegs) and slender. Fore- and mid legs each with row of short and pale setae ventrally on tibia and anterior portion of trochanter. Hindlegs long (about 3 times as long as forelegs) and slender; tibiae each 2-segmented (Figs. 5–9, 16–20).

Abdomen membranous, with lateral fringe on each side of segments III to VII; segment VIII with lateral tubercles anteriorly, followed by long fringe. Segment I with dorsal and lateral humps; dorsal hump with 2 dorsolateral setae (1 at each side); lateral humps each with sclerite slightly sclerotized, quadrate, its anterior edge rounded, with anterior half covered by short, fine setae and long, central seta; with 1 long single seta dorsal of the sclerite and other ventral of it. Gills filamentous, simple or 3-branched, arising from anterior end of each of segments I–VIII in dorsolateral, lateral or ventrolateral position, often absent or reduced in length or number on segments I, VII and VIII (Figs. 10, 21). One or 2 pairs of dorsal setae on most segments. Segment IX with dorsal sclerite oval, slightly sclerotized, with 3 to 4 pairs of setae on its posterior margin (Fig. 11). Anal prolegs each with lateral sclerite subtrapezoidal, with 1 short mesoposterior and 1 long ventroposterior seta; basal tuft of 3 long setae; ventral sole plate with transverse black stripe and anterodorsal seta; anal claw with single, large, sharply pointed tooth and 2 or 3 very small dorsal accessory hooks and with 4 long dorsal and 3 short ventral setae (Fig. 12).

Pupa. Head with 1–3 pairs of setae on vertex, 4–5 setae on each antennal scape, 2 pairs of long setae on frons and 1 pair below each eye; labrum with 3 long setae at each basolateral corner and 3 groups of 2–3 long setae along its distal margin. Mandibles long, with large apical tooth bearing fine serrations along inner margin and with smaller mesal subapical tooth (Holzenthal 1988, fig. 2C).

Thorax with 3 pairs of dorsal setae on each segment.

Abdomen with 1 pair of dorsolateral, longitudinal sclerotized bars from segment II to VIII. Segment I with spinulose tubercles on posterodorsal angles. Segments III–VI with pair of anterior, subtriangular, dorsal hook plates, each bearing 4 to 6 teeth; segment V with additional posterior pair of subquadrangular plates, each bearing 6 retrorse teeth. Lateral fringes on each side of segments III–VIII, continuing ventrally on last segment. All abdominal terga with dorsal setae. Abdominal gills distributed as in larva, but usually shorter (Holzenthal 1988, fig. 2B). Segment IX with 2 dorsal anterolateral protuberances bearing long setae; its apicolateral portion with 3 to 6 pairs of long setae; anal processes long and slender, each with apex upturned and pointed, bearing small, flat setae and 4 long setae; ventrolateral lobes prominent on mature male pupa, less well developed on female and immature pupae (Holzenthal 1988, figs. 2B, D).

Triplectides misionensis **Holzenthal 1988**

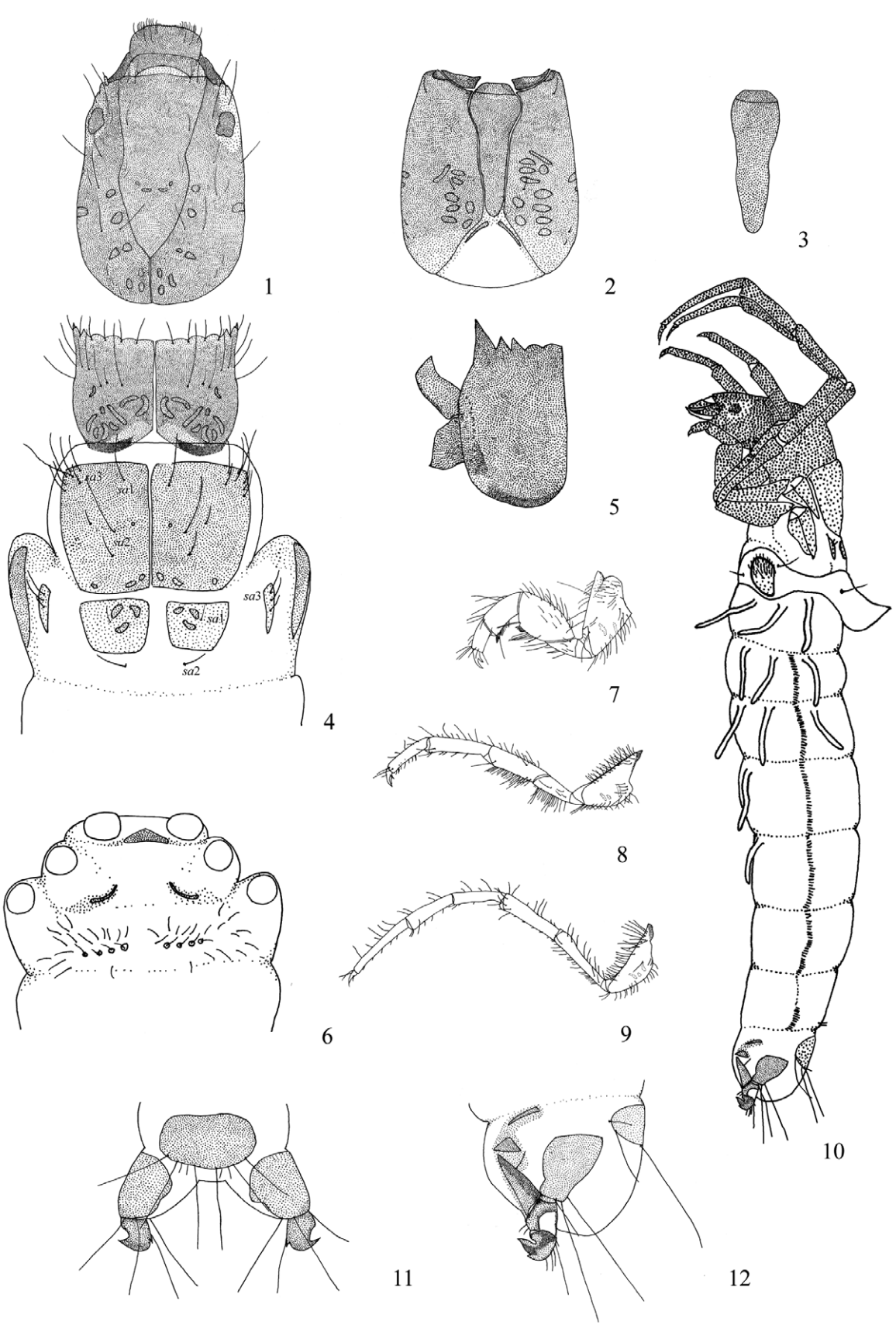
Figs. 1–12, 23

Larva. Length up to 10.5 mm. Head coloration (in alcohol) homogeneous dark reddish brown, with yellowish oval areas around stemmata. Muscle scars of same coloration as rest of head, except that at posterior end somewhat paler, distributed characteristically as in Fig. 1. Labrum dark reddish brown. Ventral apotome triangular, with anterior end wide and laterally rounded in some specimens (Figs. 2, 3). Submentum trapezoidal (Fig. 2).

Thorax with pronotum dark reddish brown; anterior edge crenulate: crenulations rounded, except for anterolateral corners produced and pointed, and the adjacent 2 crenulations slightly pointed (Figs. 4, 5); setal pattern as in Fig. 4. Mesonotum stramineous; *sa*1 each with 1 long seta; *sa*2 each with 1 long anteromesal, 1 short anterolateral and 1 short posterior setae, *sa*3 each with 8 setae (2 long and 6 short) distributed as in Fig. 4; muscle scars as in Fig. 4. Metanotum covered by 2 pairs of sclerites, *sa*1 each with single seta, *sa*2 without sclerites each with single seta, *sa*3 sclerites same length or slightly longer than *sa*1 sclerites, each with 3 setae. Prosternum triangular. Metasternum with setal areas bearing 15 to 21 long setae; pair of mesoposterior rows each of 4 setae with basal, circular sclerite (Fig. 6). Foretrocantins each with dorsal margin excavated, anteroventral corner rounded and anterodorsal corner slightly pointed and upturned (Fig. 5). Legs homogeneous dark reddish brown, setose, with chaetotaxy as shown in Figs. 7–9.

Abdomen with gills simple, present on segments II–VI, segments II–IV with dorsolateral, lateral and ventrolateral gills, V–VI with only ventrolateral gills. Segments II–VII without setae; segment VIII with 2 pairs of short posteromesal setae (Fig. 10). Segment IX dorsal sclerite with 3 pairs of long setae on its posterior margin, 2 lateral and 1 mesal, and 2 pairs of very short setae between those pairs (Fig. 11).

Larval cases. Larvae found only in empty cases of *Grumicha grumicha* (97%) and *Nectopsyche gemma* (3%). For both kinds of cases, larva building cap of little stones covering dorsal portion of anterior opening (Fig. 23). Additionally, for cases of *G. grumicha*, larva adding threads of silk inside anterior end of case. When larva preparing to pupate, dorsal cap removed and stone added to cover opening.



FIGURES 1–12. ***Triplectides misionensis*** larva. 1, head, dorsal; 2, head, ventral; 3, ventral apotome; 4, thorax, dorsal; 5, pronotum, left lateral; 6, thorax, ventral; 7–9, left fore-, mid- and hind legs, posterior; 10, habitus, left lateral; 11, segment IX and anal prolegs, dorsal; 12, segment IX and anal prolegs, left lateral.

Larvae of *Triplectides* sp. in discarded cases of *Nectopsyche* sp. and *Grumicha* sp. were previously reported by Holzenthal (1988) and Crisci-Bispo *et al.* (2004) from Brazil. As opposed to what we observed in this research, Crisci-Bispo *et al.* (2004) found that 57% to 89% of the larvae of *Triplectides* sp. were occupying cases of *Nectopsyche* sp. while only 3 specimens were in cases of *Grumicha* sp.

Pupa. Length up to 13 mm. Head with 1 pair of setae on vertex and 5 setae on each antennal scape (2 dorsal and 3 anterobasal); labrum with 3 groups of setae along its edge, each group with 3 long setae (3 setae in mesal group and 3 in each lateral group).

Abdomen. All abdominal terga with setae. Segment I with 1 anterior pair of dorsal setae; segments II–VIII with 2 pairs (1 anterior and 1 posterior), except for segment VII with 3 pairs (1 anterior and 2 posterior); segments III–VIII with additional pair of lateral setae. Segment IX with anterolateral protuberances each bearing 5 long setae; ventrolateral lobes each with 5 setae.

Material examined. Argentina: Misiones: arroyo Moreno, 26.x.2004, 2 larvae; picada 13, 25–31.x.2004, 4 larvae; arroyo Moreno, 6–11.xii.2004, 3 larvae; arroyo Tamanduá (virgencita), 6–11.xii.2004, 1 larva; arroyo Tateto, 6–11.xii.2004, 12 larvae; arroyo Moreno, 16–27.ii.2005, 18 larvae; arroyo Tamanduá (virgencita), 16–27.ii.2005, 14 larvae; arroyo Tateto, 16–27.ii.2005, 5 larvae; arroyo Cuñá-Pirú (salto), 8–14.i.2006, 4 larvae; arroyo Tamanduá (virgencita), 8–14.i.2006, 20 larvae; arroyo Moreno, 13–20.xi.2006, 5 larvae; arroyo Tamanduá (virgencita), 8–14.i.2006, 2 male metamorphotypes; arroyo Tamanduá (virgencita), 11.iii.2008, 13 larvae, 2 pupae, 1 male metamorphotype.

Triplectides gracilis (Burmeister 1839)

Figs. 13–22, 24

The larva and pupa of *Triplectides gracilis* were originally described by Müller (1921) as *Tetracentron ramulorum*. In his series of notes and letters Müller gave some characters of the immatures of this species, but these characters are so general and isolated that do not allow its identification nowadays. In this paper we provide a complete description of the larva, pupa and cases of this species and compare it with the other described Neotropical species in the genus.

Larva. Length up to 16 mm. Head coloration (in alcohol) homogeneous dark brown, with yellowish oval areas around *stemmata*. Muscle scars somewhat paler than rest of head, distributed characteristically as in Fig. 13. Labrum stramineous, with 2 brown lateral stripes parallel to lateral borders. Ventral apotome subtriangular, with long constriction at mid-length; submentum oval (Fig. 14).

Thorax with pronotum reddish brown; mesal half of anterior edge of each pronotal sclerite with 3 smooth, rounded crenulations; lateral half plain, slightly produced and rounded (Figs. 15, 16); setal pattern as in Fig. 15. Mesonotum stramineous; *sa*1 each with single long seta, *sa*2 each with 3 setae (1 long anteromesal, 1 short anterolateral and 1 short posterior) and *sa*3 with 5 or 6 setae (1 long and others short) distributed as in Fig. 15 (when only 5 setae present, anterolateral seta absent); muscle scars as in Fig. 15. Metanotum covered by 3 pairs of sclerites; *sa*1 sclerites each subrectangular with single long seta, *sa*2 with pair of small transverse sclerites (found in *Triplectides* species elsewhere in the world but this is the first record in a Neotropical species—e.g. see St Clair 2002); sclerites *sa*3 slightly longer than *sa*1 and *sa*2 sclerites together (Fig. 15). Prosternum rectangular, slightly produced anteriorly. Mesosternal sclerites subrectangular with both ends curved anteriorly. Metasternum with setal areas bearing 6–9 long setae, with a variable number of basal, circular sclerites (Fig. 17). Foretrocantin with anterodorsal corner pointed and upturned and anteroventral corner rounded (Fig. 16). Legs striped with alternate stramineous and reddish brown bands (Fig. 21); setose (chaetotaxy as shown in Figs. 18–20).

Abdomen with gills simple, present on segments I–VIII (all segments with dorsolateral, lateral and ventrolateral gills, except segment I lacking lateral ones) (Fig. 21). Segments II–VIII each with pair of long posteromesal setae (those of segments III–VII very thin). Segment IX dorsal sclerite with 6 long setae on its posterior margin and 2 pairs of very short, lateral setae anterior to them; with 2 lateroposterior setae at each side of sclerite (Fig. 22).

Larval cases. Larva of this species occupying hollowed-out twigs as well as empty cases of *Grumicha grumicha* and *Nectopsyche gemma*. Contrary to our observations with *T. misionensis*, percentage of larvae occupying empty cases of *G. grumicha* and *N. gemma* representing only 16% of total (9% and 7%, respectively).

For both hollowed twigs and adopted cases, larva building cap of small twigs stuck together with silk threads covering dorsal portion of anterior opening (Fig. 24). Most specimens (84%) occupying hollowed-out twigs of different sizes reaching 35 mm in length.

Pupa. Length up to 17.4 mm. Pupa of this species identical to that of *T. misionensis* Holzenthal (see description above), differing in length and in chaetotaxy of labrum. In *T. gracilis*, labrum similar to that of *T. jaffueli* Navás with 3 long setae at each basolateral corner and 3 pairs of long setae along its distal margin (1 mesal and 2 laterals).

Material examined. Argentina: Misiones: arroyo Moreno, 26.x.2004, 3 larvae; arroyo Azul (puente quemado), 25–31.x.2004, 1 larva; arroyo Tamanduá, 25–31.x.2004, 3 larvae; arroyo Cuñá-Pirú (balneario), 6–11.xii.2004, 4 larvae; arroyo Azul (puente quemado), 6–11.xii.2004, 1 larva; arroyo Moreno, 6–11.xii.2004, 3 larvae; arroyo Tamanduá, 6–11.xii.2004, 3 larvae; arroyo Tamanduá (virgencita), 6–11.xii.2004, 6 larvae; arroyo Azul (puente quemado), 16–27.ii.2005, 5 larvae; arroyo Moreno, 16–27.ii.2005, 3 larvae; arroyo Tamanduá, 16–27.ii.2005, 6 larvae; arroyo Azul, 16–27.ii.2005, 1 larva; arroyo Tateto, 16–27.ii.2005, 2 larvae; arroyo Tamanduá, 8–14.i.2006, 1 larva; arroyo Tamanduá (virgencita), 8–14.i.2006, 1 male metamorphotype.

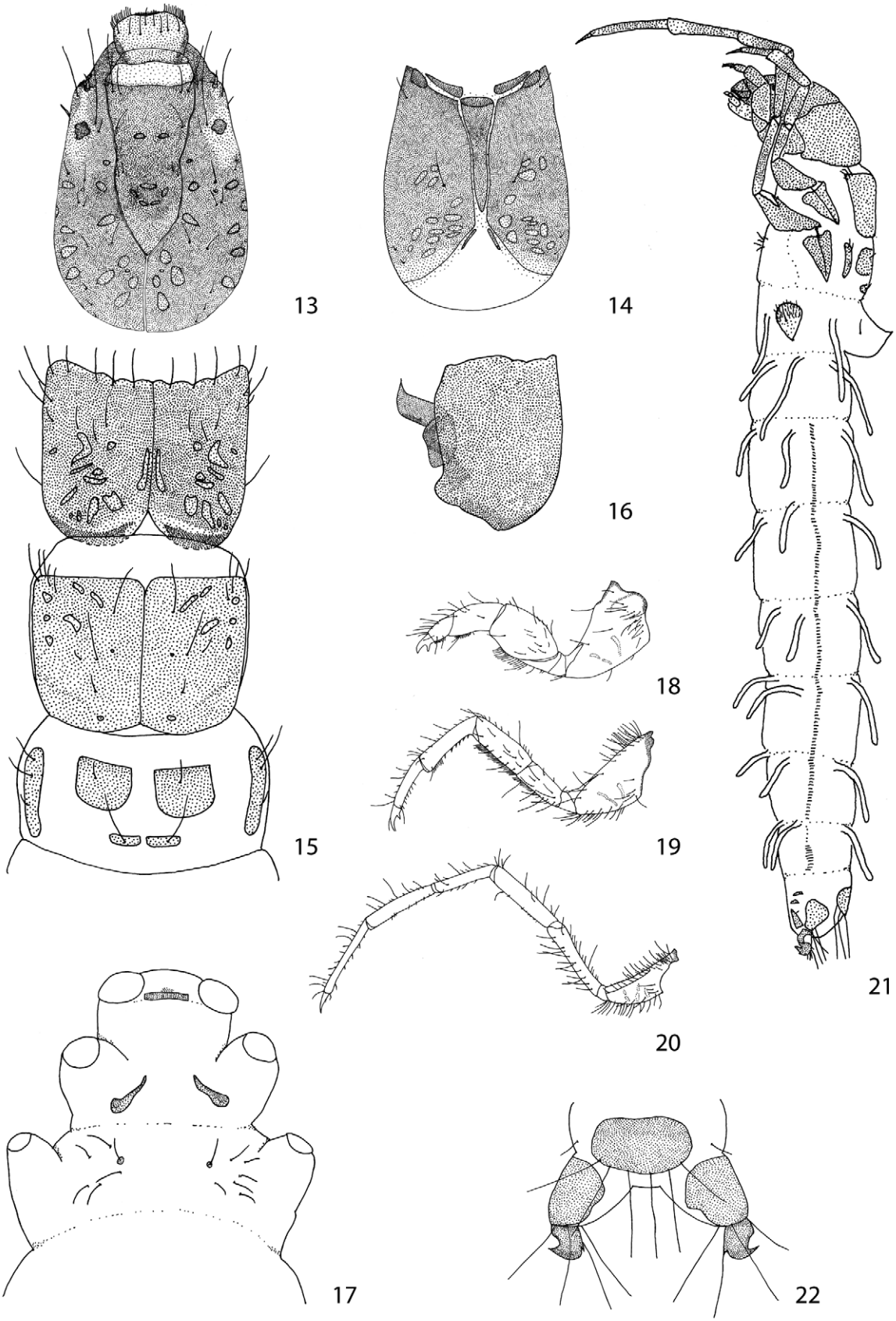
Habitat. The immatures of both species were found in almost all sites sampled, in 1st (arroyo Tamanduá and arroyo Azul) to 4th (arroyo Cuñá-Pirú) order streams that ranged from 161 to 403 m a.s.l. In all streams the substrate was composed of boulders, cobbles, gravel and sand, along with leaves and woody debris that comes from the gallery forest that develops on the margins. The larvae were collected in runs, riffles and pools in areas with accumulations of organic matter, especially woody debris.

Systematic considerations. The larvae of the 2 species of *Triplectides* described herein each have a series of distinctive features that allows their differentiation from those of the other known Neotropical species in the genus, *T. jaffueli* (see Holzenthal 1988) and *T. egleri* (see Sattler 1963) (Table 1). Of this series of characters, the shape of the anterior border of the pronotum, the presence of *sa2* sclerites in the mesonotum, the coloration of the legs and the number and distribution of the gills are the most important features in the identification of the larvae of these species.

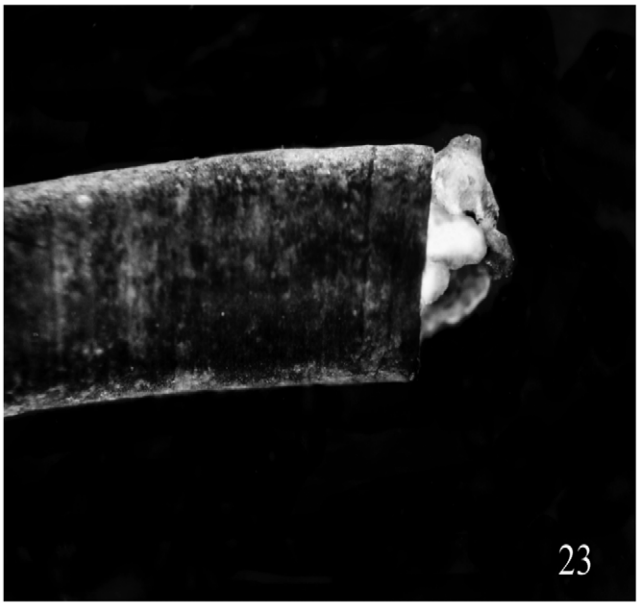
Pupae of these species can be distinguished by the chaetotaxy of the vertex, antennal scape and labrum. The pupa of *T. gracilis* can be differentiated from that of *T. misionensis* by the chaetotaxy of the labrum, which is identical to that of *T. jaffueli*, bearing 3 pairs of long setae along its distal margin, and from *T. jaffueli* by the number of setae on the vertex (1 pair vs. 3) and on the antennal scape (5 setae vs. 4). There are no available data on the pupa *T. egleri*.

TABLE 1. Diagnostic characters for Neotropical species of the known larvae of *Triplectides*. *Sa* = setal area; ? = no data available.

	Triplectides gracilis	*Triplectides misionensis*	*Triplectides jaffueli*	*Triplectides egleri*
Submentum	Oval	Pentagonal	Oval	Pentagonal
Ventral apotome	Subtriangular (constricted at mid-length)	Subtriangular (wide anteriorly in some specimens)	Triangular	Quadrate
Anterior edge of each pronotal plate	Mesal half with smooth crenulations, lateral half plain and slightly produced	Crenulate, anterolateral corners produced	Crenulate, anterolateral corners produced	Crenulate, anterolateral corners produced
Each mesothoracic *sa*3	5–6 setae	8 setae	6 setae	?
Metathoracic *sa*2	With pair of sclerites	Without sclerites	Without sclerites	Without sclerites
Prosternum	Rectangular	Triangular	?	Convex anteriorly, concave posteriorly
Legs	Striped	Homogeneous	Homogeneous	Striped
Abdominal gills	1-branched, on segments I–VIII	1-branched, on segments II–VI	1- to 3-branched, on segments II–VIII	1- to 3-branched, on segments II–VIII



FIGURES 13–22. ***Triplectides gracilis*** larva. 13, head, dorsal; 14, head, ventral; 15, thorax, dorsal; 16, pronotum, left lateral; 17, thorax, ventral; 18–20, left fore-, mid- and hind legs, posterior; 21, habitus, left lateral; 22, segment IX and anal prolegs, dorsal.



FIGURES 23–24. 23, *Triplectides misionensis*, larval case, right lateral; 24, *Triplectides gracilis*, larva and larval case, right lateral.

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