


# The *Cricotopus* (*Oliveiriella*) (Diptera: Chironomidae) of the High Altitude Andean Streams, with Description of a New Species, *C. (O.) rieradevallae*

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## Abstract

The genus *Oliveiriella* (Chironomidae, Orthocladiinae) was erected by Wiedenbrug & Fittkau (1997). The adults have characteristic black spots on their wings and other characteristics similar to the genus *Cricotopus*. Pupal skins are very characteristic with strong short spines in the anal lobe instead of setae, while larvae are distinguishable by the long anal papillae and the intense blue color of their body. However, Andersen *et al* (2013) consider *Oliveiriella* as a subgenera of *Cricotopus*. In this paper, using the sequences of the *cox1* gene, we conclude that *Oliveiriella* should be considered a subgenus within *Cricotopus*, confirming its status in Andersen *et al* (2013). Furthermore, we describe *Cricotopus (Oliveiriella) rieradevallae* Prat & Paggi sp. n. from the Saltana river (Ecuador). The adult males, females, and preimaginal stages of the two species of subgenus *Oliveiriella* known from South America *Cricotopus (O.) almeidai* n. comb. from Peru, Brazil, and Argentina and *Cricotopus (O.) sanjavieri* n. comb. from Argentina are compared with those of *Cricotopus (O.) rieradevallae* sp. n. from Ecuador. The differences allow the distinction of the three species. The *cox1* gene reveals that at least three different undescribed species of the same subgenus are present in the high-altitude tropical Andes. The morphology of the available pupae and pupal exuviae reveals the presence of several morphotypes that are candidates to be described as new species. A key used to distinguish these pupal morphotypes is provided, including the three described species. Additionally, the distribution of the subgenus is discussed.

## Introduction

The genus *Oliveiriella* was erected by Wiedenbrug & Fittkau (1997) from material studied from Rio Grande do Sul (southern Brazil) by Oliveira (1946), described originally as

*Spaniotoma (Stictocladus) almeidai*. Years later, Tejerina & Paggi (2009) described a new species from Argentina, *Oliveiriella sanjavieri*, in all stages. In the same year, the range of *Oliveiriella* was extended to North America by Krestian *et al* (2009), which found larvae and pupae of this

species in Arizona and New Mexico. Today, due to morphological similarities with *Cricotopus*, *Oliveiriella* is considered a synonym of *Cricotopus* but is maintained as a separate subgenus according to Andersen *et al* (2013). Therefore, in this paper, we will use *Oliveiriella* as a subgenus of *Cricotopus*. Andersen *et al* (2013) provided a key to distinguish between the different *Cricotopus* subgenera larvae. Prat *et al* (2014) provided a key for the pupae of the high-altitude Andean Chironomidae genus where *Oliveiriella* can be easily distinguished from other genera and subgenera of Orthoclaadiinae.

Larvae of *Oliveiriella* have been found in lotic habitats of montane and submontane streams from southern to south-eastern Brazil, and on the eastern slopes of the Andes in Ecuador and Peru by Wiedenbrug & Fittkau (1997), but the authors do not provide the altitude at which the larvae were found. *Oliveiriella sanjavieri* was found by Tejerina & Paggi (2009) and Tejerina & Malizia (2012) from several streams in the region of Tucumán (Argentina) in altitudes ranging from 825 to 1275 m a.s.l. and in two streams located at 411 and 505 m a.s.l. in the region of Jujuy (Argentina). Finally, pupae of *Oliveiriella* were collected by Krestian *et al* (2009) in Arizona and New Mexico, from two rivers at elevations between 1180 and 1449 m a.s.l. In all cases, streams where the larvae have been found until now are of clear and oligotrophic waters. During our studies in the high-altitude tropical streams in Colombia, Ecuador, and Peru, we found larvae, pupae, and pupal exuviae of *Cricotopus* (*Oliveiriella*) in Peru, Ecuador, and Colombia. In this paper, we used all this material to (1) present a phylogenetic tree of the *Oliveiriella* species using COI sequences that support that *Oliveiriella* is a subgenus of *Cricotopus*; (2) describe a new species of this subgenus found in Saltana creek (Pichincha, Ecuador); (3) report the distribution of the subgenus in the high-altitude Andean rivers; (4) study the morphological variation of larvae and pupae of this subgenus; and (5) provide a preliminary key of the different morphotypes of pupal exuviae.

## Material and Methods

### Study area

Samples were taken in the tropical highland Andean streams (over 2500 m a.s.l.) from northern Colombia to southern Peru. A total of 185 sites were studied in four different research projects from 2007 to 2011 (Table 1). From all the sites sampled, the subgenus *Oliveiriella* was found only in eight sites. The sites with *Oliveiriella*, the date, country, basin, stream, position, and altitude are listed in Table 1. In this table, we also indicate the research project in which the site was studied. Details of the projects and sites may be found at <http://www.ub.edu/riosandes/>. Each site was visited once, except site GUAY O4 from the FUCARA project when samples were taken from April 2009 to November 2010. Physicochemical, habitat (IHF

index), and riparian vegetation (QBR index) characteristics of the sampled sites are listed in Table 2. Material collected from one site in Colombia (VR-6; Table 1) by two of the authors (FR, PO) has been included in the molecular studies. The two species currently known, *Cricotopus* (*O.*) *almeidai* and *Cricotopus* (*O.*) *sanjavieri*, have also been used for comparison with the description of *Cricotopus* (*O.*) *rieradevallae* sp. n. Larvae and pupae of *C. (O.) almeidai* were collected from Los Sosa river, Tucumán, Argentina. We were unable to amplify the DNA of the larvae of *C. (O.) sanjavieri* sampled at San Javier stream, Tucumán, Argentina. For this reason, it was not possible to include this species in the phylogenetic tree.

### Sampling, sorting, and preservation of material

Sampling of larvae and pupae or pupal exuviae was done using a Surber sampler in the CERA and BIQUARA projects (Villamarín *et al* 2013, Prat *et al* 2013) and a Hess sampler at Saltana creek in the FUCARA project (Ríos-Touma *et al* 2011). Surber samples were taken in different substrates following the protocol described by Villamarín *et al* (2013). To enhance the collection of pupae and pupal exuviae, drift samples were also taken. At least two drift nets were left in the river to filter the water in each site during the time used for sampling of benthos with the Surber sampler (2–3 h). In all cases, the net mesh was 250 µm. In the case of the site GUAYO4 (Saltana creek, FUCARA project), Hess samples were taken randomly, every month from April 2009 to August 2010 using the methods reported in Ríos-Touma *et al* (2012).

Except in the BIQUARA project, samples were fixed with formalin in the field, taken to the laboratory, where, after elimination of formalin from the sample, Chironomidae were sorted. At least 100 individuals per sample were collected. In all cases, chironomid larvae were identified at genera or morphotype level under the stereoscope following the key of Prat *et al* (2012). Up to 10 individuals of each genera or morphotype were prepared for microscope examination. The larvae were digested in hot 10% KOH, washed, dehydrated, and mounted in Euparal® on a slide following the usual techniques for optical microscopy described by Epler (2001).

In the BIQUARA project, the main objective was to obtain the barcode of the maximum taxa possible. Live larvae were collected in the field (at least 100 individuals) picking the organisms from a combined sample of 12 Surbers including all the inhabitants found in the stream. The midges were then fixed in the field using 96% alcohol that was changed at least once before the DNA analyses were carried out. The body of the larvae was used for DNA extraction, while the head capsule was prepared following the methods previously described. The pupae (or pupal exuviae) were prepared after the DNA extraction (described further on in the text) in Euparal® using the same protocol of larvae. All the larvae were classified using the key of Prat *et al* (2012) and other papers referred to by the authors. For pupae, the key of Prat *et al* (2014) was used.

**Table 1** High-altitude Andean sites and basins where *Cricotopus (Oliveiriella)* have been found. In the last column, we indicate the number of individuals examined as larvae (L) or pupae (P). In brackets, number of individuals used the molecular analysis. As the individuals of CERA project were fixed in formaldehyde, they cannot be included in the molecular analysis. More information about the research projects and each site can be found in Table 2 and at <http://www.ub.edu/riosandes/>.

Site code	Project	Date	Country	Drainage	Basin	Stream name	Longitude	Latitude	Altitude (m.a.s.l.)	Material studied
SA10	CERA	18/10/2007	Peru	Pacific	Santa	Llanganuco bajo laguna	77°25.552'	09°10.886'	3846	7P; 8L
SA12	CERA	18/10/2007	Peru	Pacific	Santa	Río Llullan	77°46.109'	09°01.937'	2746	1P
MO16	CERA	23/10/2007	Peru	Amazon-Atlantic	Mosna	Vaqueria	77°33'23.32"	9°2'37.92"	4010	1L
AGU14	CERA	20/2/2008	Ecuador	Pacific	Napo	S/N	77°41.323'	00°37.169'	3387	1P
NA02	BIQURA	23/10/2011	Ecuador	Amazon-Atlantic	Napo	Papayacta	78°01.675'	00°24.722'	2457	1L (1)
CHIN02	BIQURA	14/9/2011	Colombia	Caribbean-Atlantic	Magdalena	Romerales	75°25.745'	04°59.172'	2485	3
MO08	BIQURA	4/7/2011	Peru	Amazon-Atlantic	Mosna	Purhuay	77°11.513'	09°19.316'	3192	7L (7)
GUAY04	BIQURA FUCARA	22/10/2011 2009/2010	Ecuador	Pacific	Esmeraldas	Saltana	78°13'8.8"	00°19'1.80"	3869	5P (1); 29L (7)
VR-6	EIMAG-REG-MA	09/09/2014	Colombia	Caribbean-Atlantic	Magdalena	Romerales	75°25'58"	04°59'22"	2305	6L (6)

**Table 2** Physicochemical characteristics of the basins where *Cricotopus (Oliveiriella)* larvae or/and pupae have been found in the high-altitude Andean rivers. For BIQURA project, only field data is available. BIQURA streams are reference streams without pollution.

Site code	T (°)	pH	Conductivity (µS/cm)	O <sub>2</sub> (%)	O <sub>2</sub> (mg/l)	Ammonia (mg/l)	Phosphate (mg/l)	Nitrite (mg/l)	Nitrate (mg/l)	QBR	IHF
SA10	12.00	7.84	32.80	73.10	7.93	0.03	0.00	0.02	0.20	95	85
SA12	14.30	7.47	102.00	80.10	8.18	0.11	0.04	0.02	0.10	45	57
MO16	8.2	8.08	17.3	73.9	8.17	0.06	0.00	0.0	0.2	100	50
AG14	8.00	7.87	10.70	77.50	9.16	0.00	0.02	0.00	0.00	100	53
NA02	10.6	7.48	46.1	71.1	7.91					100	69
CHIN02	13.6		61.5							95	59
MO08	11.6	6.64	26.2	75.7	8.2					95	75
GUAY04	5.6	7.46	42.9	63.3	8.02					100	76
	6.3 <sup>a</sup>	7.12 <sup>a</sup>	68.9 <sup>a</sup>	76.2 <sup>a</sup>	9.18 <sup>a</sup>	0.27 <sup>a</sup>	0.07 <sup>a</sup>	0.00 <sup>a</sup>	0.29 <sup>a</sup>	100	100
VR-06	12.5	7.22	67	65.4	4.9	0.11		0.07			

QBR riparian quality index, IHF fluvial habitat index.

<sup>a</sup> Mean values from 15 months.

Adults were collected only at the Saltana creek (FUCARA Project), with insect traps following the methods described in Ríos-Touma *et al.* (2012). Three malaise traps were deployed in the river banks for 24 h on a monthly basis. Table 3 lists the total number of adults of *C. (Oliveiriella) rieradevallae* sp. n. found on different dates. The studied material was preserved in 80% ethanol cleared in 10% KOH and slide-mounted in Euparal® for microscopic observation, following the methods outlined in Paggi (2009). General terminology and abbreviations follow Saether (1980). Total and wing lengths are given in millimeters, and other measurements in micrometers; the measurements are given as range, followed by mean, and the number of specimens in parenthesis. Material examined was deposited at the La Plata Museum of Natural Science (MLP), La Plata, Argentina, and at the Institute of Limnology “Dr. R. A. Ringuelet” (ILPLA), La Plata, Argentina, and in the Museo Ecuatoriano de Ciencias Naturales del Instituto Nacional de Biodiversidad (MECN), Quito, Ecuador.

#### DNA extraction, PCR amplification, sequencing, and phylogenetic analysis

Currently, the molecular identification of species using DNA-based tools represents a successful method to assess macroinvertebrate biodiversity (especially Chironomidae) in high-altitude streams (Montagna *et al.* 2016). In our study, total genomic DNA was extracted from samples preserved in 96% ethanol using the Qlamp® DNA Mini Kit (QIAGEN) or SPEEDTOOLS® Tissue DNA Extraction Kit (BIOTOOLS) following the manufacturer’s protocol. A partial fragment of the cytochrome oxidase I (*cox1*) gene was selectively amplified in polymerase chain reactions (PCR) that were conducted following Krosch *et al.* (2011), Prat *et al.* (2013) and sequenced in the Macrogen sequence center in the Netherlands. Raw sequences were edited and assembled with Geneious 4.6.5 (Drummond *et al.* 2009). All new sequences obtained were deposited in GenBank for localities and GenBank accession numbers of all used samples).

Table 3 Adults of <i>Cricotopus (Oliveiriella) rieradevallae</i> Prat & Paggi sp. n. caught in the Malaise traps of the river Saltana in 10 successive months.	Data	<i>Oliveiriella</i>	Ind/trap
	02/10/2009	3f	30.0
	21/10/2009	1f	20.0
	20/11/2009	0	20.7
	17/12/2009	1m	30.3
	14/01/2010	1m 7f	37.7
	10/02/2010	1f	35.5
	11/03/2010	9m 3f	63.7
	08/04/2010	1m 13f	97.3
	09/05/2010	4f	68.7
	08/06/2010	0	1.0

f female, m male.

Alignment of the *cox1* gene fragments was trivial due to the absence of length polymorphisms. Sequences were translated into amino acids, and no indels or stop codons were found. We obtained the uncorrected pairwise (p)-distance genetic divergences between terminals using MEGA v.7.0.14 (Kumar *et al.* 2016) removing alignment gaps in pairwise comparisons. A maximum likelihood (ML) analysis was performed using RAxML 7.4.2 (Stamatakis 2006) through the graphical front-end RAxMLGUI 1.3 (Silvestro & Michalak 2012).

GTR+G+I nucleotide substitution model was applied, and nonparametric bootstrap support analysis of 1000 pseudoreplicates was conducted. *Limnophyes* species was used to root the tree.

To test if *Oliveiriella* is a valid genus or it is a subgenus of *Cricotopus* according to Andersen *et al.* (2013), we included in the analysis several sequences of *Cricotopus* available at GenBank. In addition, we included in the analysis *Oliveiriella almeidai* from larval and pupal material collected in Argentina (see “Material and Methods” section). In Table 1, we summarized the localities of all the analyzed sites. Because some *Cricotopus* species appear paraphyletic with respect to some *Limnophyes* used as outgroup, a typology of outgroups was enforced.

## Results

### *The species of Oliveiriella in the high-altitude Andean streams and rivers and the identity of Oliveiriella as genus: molecular data*

Specimens, locality, and sequences with corresponding GenBank accession numbers analyzed in the present study are listed in Table 10 in supplementary material. The matrix used in the phylogenetic analysis includes 35 terminals and 454 aligned characters. Figure 1 shows the ML tree obtained. The genus *Cricotopus* appears paraphyletic with respect to the *Oliveiriella* species included, although with moderate bootstrap support. Within the *C. (Oliveiriella)* clade, two clusters are present. The first one is formed by *C. (O.) almeidai* plus the BIQUA samples from Peru (MOO8 samples), together with specimens from Colombia (VR-6). The uncorrected p-distances (Table 4) from *O. almeidai* and MOO8 plus VR-6 (12.5%) suggest that there are two different species, while MOO8 and the sequences from Colombia (VR-6) must be considered as different populations of the same species (uncorrected p-distance = 2.3%). The second cluster shows two putative species, one from river Saltana (GUAY04) described below in this paper as *C. (O.) rieradevallae* sp. n. and another one from Napo River (NAO2) (uncorrected p-distance = 14.4%) (Table 4).

In summary, Fig 1 suggests that *Oliveiriella* cannot be considered as a separate genus in the Orthocladiinae, but just as a subgenus of *Cricotopus* as indicated by Andersen *et al.*

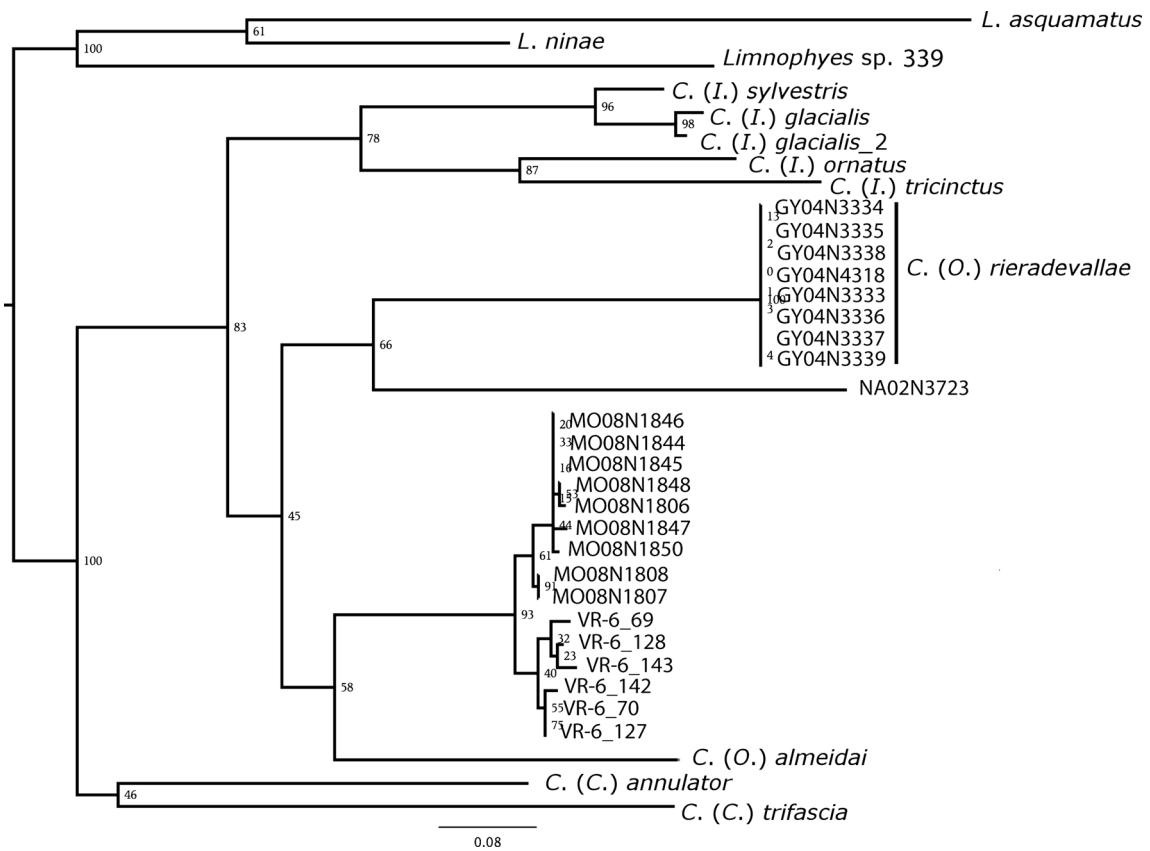


Fig 1 ML tree of the *Oliveiriella* samples using partial *cox1* gene sequences. Numbers in 604 nodes correspond to bootstrap support values. The tree was rooted using three *Limnophyes* species. Sequences of seven species of *Cricotopus* was also included to test the paraphyly of *Cricotopus* species

(2013), and there are at least four different species of the subgenus *Oliveiriella* in the study area.

*Cricotopus (Oliveiriella) rieradevallae* Prat & Paggi new species

**Type material.** *Holotype*, male adult from Ecuador, Saltana river 00°19'1.80"S 78°13'8.8"W, 14.i.2010 leg. Blanca Ríos-

Touma, Malaise trap. Allotype, female 14.i.2010 same data as Holotype. Paratypes: same data as Holotype except for the following data: two adult males Pichincha, Res. Paluguillo, Queb. Saltana 00°19'1.80"S 78°13'8.8"W 3869 m a.s.l. 8.iv.2010; one male adult 11.iii.2010, leg. B. Ríos-Touma & F. González, Malaise trap; one adult female, 14.i.2010; two larvae and pupal exuviae 14.i.2010 (GY04N3334, GY04N3338) and 03.v.2010; two pupae 07.iv.2010 (GY04N4318) and 05.v.2010.

Table 4 Uncorrected p-distances of *cox1* partial sequences between species analyzed in this study.

	GY04	NA02	MO08 & VR-6	<i>O. almeidai</i>	<i>C. glacialis</i>	<i>C. annulator</i>	<i>C. trifascia</i>	<i>C. glacialis_2</i>	<i>C. ornatus</i>	<i>C. sylvestris</i>
GUAYO4										
NA02	0.144									
MO08 & VR-6	0.119	0.150								
<i>C. (O.) almeidai</i>	0.139	0.151	0.125							
<i>C. (I.) glacialis</i>	0.162	0.162	0.129	0.139						
<i>C.(C.) annulator</i>	0.174	0.183	0.153	0.155	0.155					
<i>C.(C.) trifascia</i>	0.178	0.178	0.150	0.182	0.175	0.154				
<i>C. (I.) glacialis_2</i>	0.165	0.162	0.125	0.144	0.014	0.151	0.171			
<i>C. (I.) ornatus</i>	0.162	0.167	0.144	0.158	0.130	0.155	0.168	0.130		
<i>C. (I.) sylvestris</i>	0.160	0.167	0.131	0.128	0.049	0.132	0.175	0.044	0.116	
<i>C. (I.) tricinctus</i>	0.158	0.169	0.142	0.144	0.132	0.146	0.189	0.132	0.102	0.123

**Diagnostic characters.** Male, female, and preimaginal stages of *C. (O.) rieradevallae* sp. n. are differentiated from *C. (O.) almeidai* n. comb. and *C. (O.) sanjavieri* n. comb. by the following characters:

**Adults.** The male can be distinguished from the other two species of subgenus *Oliveiriella* described until now by having a total length of 3.60 mm; 8–9 clypeus setae; 45–60 setae in the scutellum and 7–10 squamal setae (Table 5). Alar spot in the anal cell absent (in *C. (O.) sanjavieri* is present, Fig 2b). Legs with 67–71 comb teeth in hind tibia; fore and mid tibia white band ratio (BR) 0.35 and 0.27, respectively (Fig 2c, e); hind leg completely dark (Fig 2g). This color pattern in the legs is different compared with *C. (O.) sanjavieri* (Fig 2d, f). The female can be distinguished from the other two species by having a total length of 3.42 mm; 11–14 clypeal setae, 20 acrostichal setae; 60–68 scutellar setae; the band ratio on fore and mid tibia and the wings with the same characteristic as in males (Table 5).

**Pupae.** *C. (O.) rieradevallae* from river Saltana is larger than the other two species (Tables 5 and 8). Length of the respiratory organ (OR) 240–273  $\mu$ m and with a clear terminal notch and its entire surface serrated (Fig 3a). The medial precorneal setae are more than 10 times longer than the anterior and posterior (Fig 3a, b) compared with *C. (O.) sanjavieri*, of which the length of the median precorneal setae is at most three times longer than the other two setae, whereas *C. (O.) almeidai* (Brazil) of which all

the setae are of similar size (Table 5). This is also true with the first and third dorsocentral setae of the cephalothorax which are much longer than the second one (Fig 3b) (in *C. (O.) sanjavieri* are of similar length). The abdomen (Fig 3c–g) has much longer lateral setae than those of *C. (O.) almeidai* but shorter than those of *C. (O.) sanjavieri*. *Cricotopus (Oliveiriella) rieradevallae* has characteristic spines in the sternites II and III that lack in the other two species found until now (Fig 3d, e).

**Larvae.** The only characters that seem to differentiate *C. (O.) rieradevallae* from the other species are the higher AR, major length of the antennal blade, and a higher MR (Tables 5 and 9). The presence of transversal marks in the mentum is characteristic of all the Andean high-altitude river species (Fig 4b). The pattern of small dots of the head capsule is also very conspicuous and both (marks and the pattern) facilitated the distinction of the *Oliveiriella* larvae from the Andean high-altitude rivers from the other subgenera in the *Cricotopus* group and especially from Genus 1 sensu Roback & Coffman (1983).

#### Description (Fig 2)

**Male imago.** ( $n = 4$ , except when otherwise stated). Total length 3.42–3.78, 3.60; wing length 2.00–2.49, 2.44; total length/wing length 1.43–1.52, 1.47; wing length/length of profemur 2.68–2.87, 2.78. General coloration dark brown, almost black. Thorax and abdomen dark brown. Wing with brown areas almost reaching  $M_{3+4}$  and without a spot in the

Table 5 Distinctive characteristics of the three species of *Cricotopus (Oliveiriella)* known from Neotropical region.

	<i>C. (O.) almeidai</i>	<i>C. (O.) sanjavieri</i>	<i>C. (O.) rieradevallae</i>
<b>Male</b>			
Wing length (mm)	1.09	1.9–2.43	2.0–2.49
Clypeus setae	12	14–16	8–9
Scutellum setae	12	18	45–60
Squamal setae	5	7–9	7–10
Comb teeth in the hind tibia		57–60	67–71
Fore and mid tibia with band ratio	<1/2	<1/2	>1/2
Hind leg	Brown to yellow	Brown to yellow	Dark
Alar spot in the anal cell	Absent	Present	Absent
<b>Pupae</b>			
Body size (mm)	2.2–2.8	2.41–3.19	3.22–3.82
RO length ( $\mu$ m)	136–242	220–280	240–273
RO notch	Absent	Absent	Present
Medial/anterior precorneal seta size	1	3	>8
1st and 3rd/2nd dorsocentral setae	1	1	>7
Spines in sternites II and III	Absent	Absent	Present
<b>Larvae</b>			
Size	2.7–3.2	3.4–4.02	3.1–3.73
AR	1.4–1.8	1.16–1.88	1.83–1.89

RO respiratory organ, AR antennal ratio.

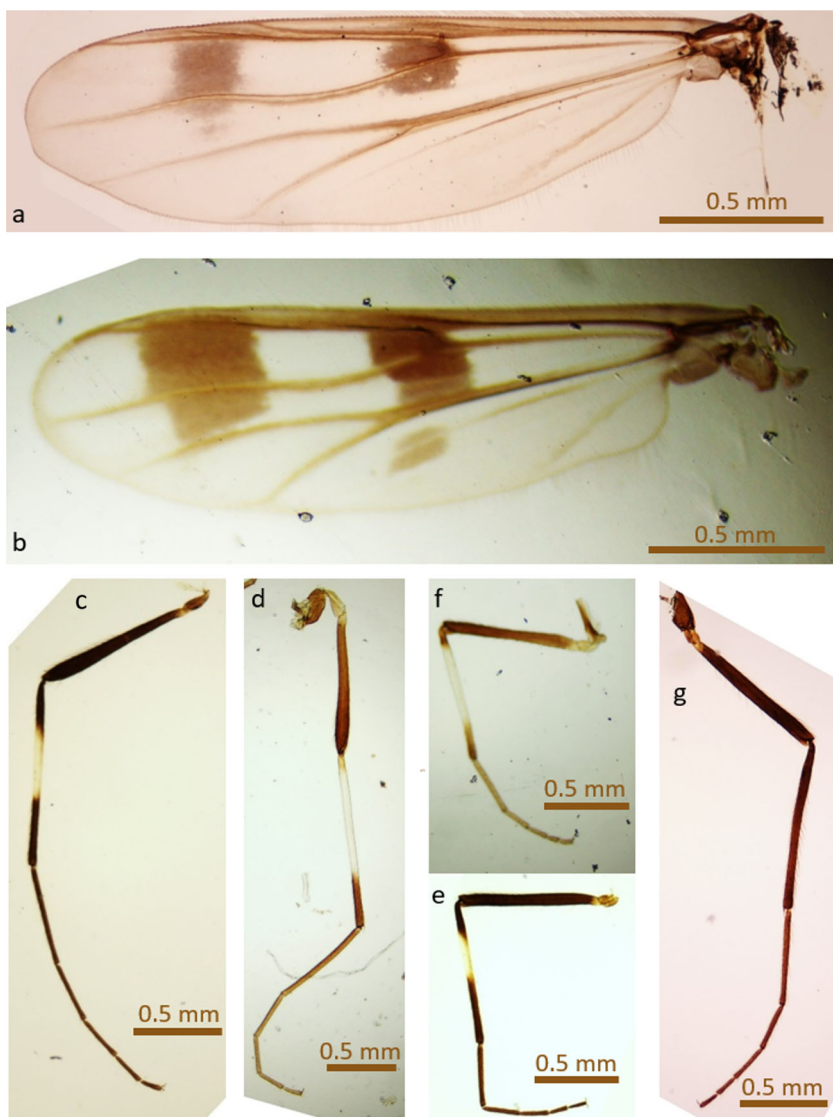


Fig 2 *Cricotopus* (*Oliveiriella*) *rieradevallae* Prat & Paggi sp. n. adult. **a** Wing. **c** PI. **e** PII. **g** PIII. *Cricotopus* (*Oliveiriella*) *sanjavieri* adult. **b** Wing. **d** PI. **f** PII.

anal cell (Fig 2a), abdomen and leg coloration pattern as generic diagnosis.

**Head.** Temporal setae 4–5, 5. Clypeus with 8–9, 8 (3) setae. Tentorium 136–152, 141 long; palpal segment length (1–4): 20, 25, 45, 60, fifth segment not measurable (1).

**Thorax.** Acrostichals 16–18 (2); dorsocentrals 20 (1); prealars 3; scutellars 45–60, 60 (3).

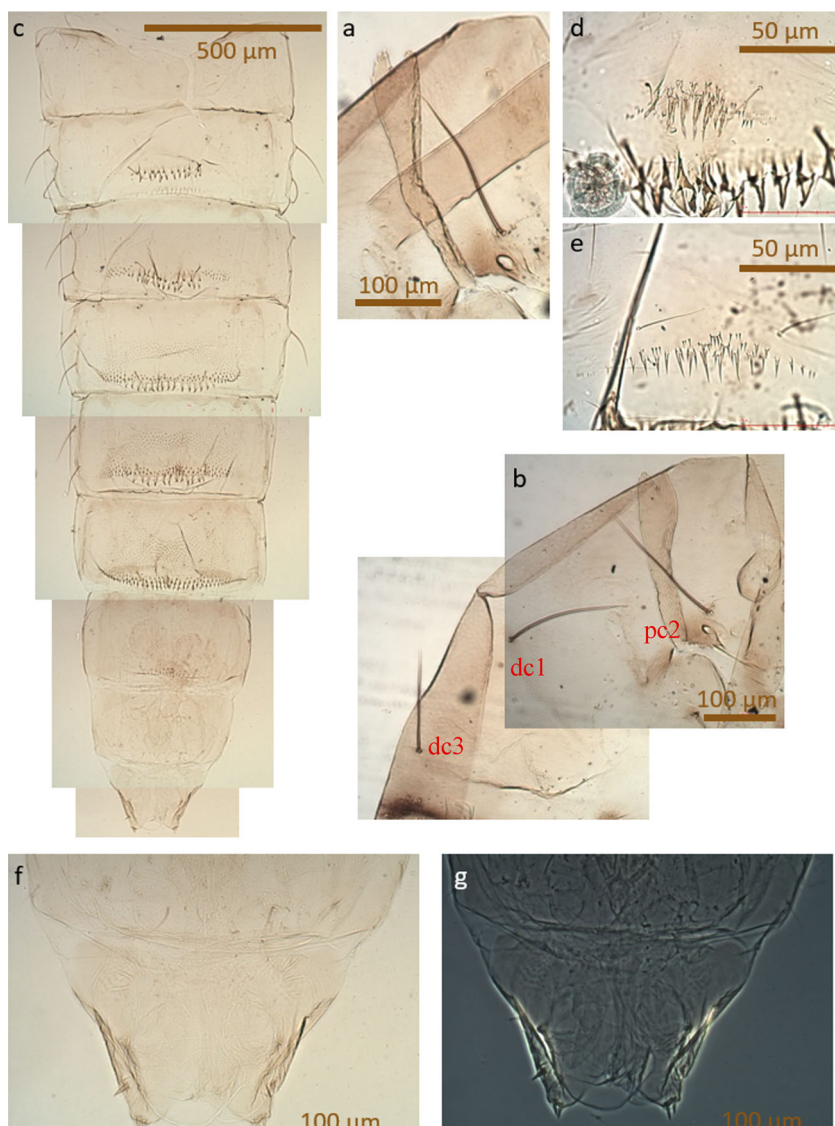
**Wing.** VR 1.14–1.20, 1.17; C/WL 0.90–0.92, 0.91; Cu/WL 0.43–0.44, 0.43. Costa extended beyond  $R_{4+5}$  75–83, 81 Cu slightly curved. Squama with 7–10, 9 setae.

**Legs.** With a white band (WB) on fore and mid tibiae covering less than half of each tibia in a ratio of (WB/Ti<sub>1</sub>) 0.40–0.29, 0.35 (3) and (WB/Ti<sub>2</sub>) 0.23–0.32, 0.27 (3) (Fig 2c, e, g). Tibial spur of fore leg 45–51, 48 long; spurs

of mid tibiae 20–22 and 16 long; spurs of hind tibia: 67–71, 70 and 20–22, 22 long, comb with 13–15, 14 teeth. Sensilla chaetica 5 (1) on hind Ta<sub>1</sub>. Pulvilli absent. Length and proportions of legs as in Table 6.

**Abdomen.** Chaetotaxy: T1 with an anterolateral group of about 12 setae and distal group of 3–4 dorsal setae, TII–IV with 2 median setae and a transverse row of about 10 dorsal setae; TV–VI with a median longitudinal row of 10–12 setae; TVII–VIII with a central patch of 20–30 irregular distributed setae. TII–VIII each with 5 lateral setae.

**Genitalia.** Hypopygium. Gonocoxite 207–224, 207 long; gonostylus 75–83, 77 long. Tergite IX with 6–10 dorsal setae. Laterosternite 3–4 setae. Megaseta 8–10, 9 long. HR: 2.56–2.78, 2.70. HV: 4.30–5.07, 4.69.



**Fig 3** *Cricotopus (Oliveiriella) rieradevallae* Prat & Paggi sp. n. Pupae: **a** Respiratory organ; **b** Thorax with long dorsocentral and precorneal setae (dc1, dc3 and pc2); **c** Abdomen; **d** Spines of sternite II; **e** Spines of sternite III; **f** Abdominal segment IX; **g** Abdominal segment IX (phase contrast).

**Female imago.** ( $n = 2$  except when otherwise stated). Total length: 3.42; wing length 2.57–2.74; total length/wing length 1.24–1.33; wing length/length of profemur 3. Color pattern as in male.

**Head.** AR = 0.33–0.48. Temporal setae 5–7; clypeals 11–14; tentorium 142–152 long; palpal segment length (1–5): (15–20), (27–33), (41–46), (52–65), (92).

**Thorax.** Anteprenotals 1; acrostichals 20; prealars 3; dorsocentrals 17–19; scutellars 60–68.

**Wing.** VR 1.23; C/WL 0.93–0.94; Cu/WL 0.42–0.44. Costa extended beyond  $R_4 + 5$  108–166. Cu slightly curved; 11 R and 7  $R_4 + 5$  setae; squama with 7–9 setae.

**Legs.** With a white ring on fore and mid tibiae covering less than half of each tibia, in a ratio of (WB/Ti<sub>1</sub>) 0.31 and (WB/Ti<sub>2</sub>)

0.27. Tibial spur of fore leg 34–38 long; spurs of mid tibiae 20 and 14 long; spurs of hind tibia: 61–75 and 22–24 long, comb with 13–14 teeth. Pulvilli absent. Sensilla chaetica 10 on middle Ta<sub>1</sub> and 8–10 on hind Ta<sub>1</sub>. Length and proportions of legs listed in Table 7.

**Abdomen.** Distribution of setae as in male. S VI–VII with a central patch of about 20–22 setae.

**Genitalia.** Gonocoxite IX with 6–8 setae; tergite IX with 7 setae; sternite VIII with 11–16 setae. Seminal capsule ovate, light brown, 112–165 long, 83–122 wide, with long ducts, at least with one loop. Notum 190 long. Cerci 69–81 long.

**Pupa.** ( $n = 4$ , except when otherwise stated, measurements of additional material are in Table 8). Total length 3.22–3.82, 3.48.



Table 6 Lengths (in  $\mu\text{m}$ ) and proportions of legs of *Cricotopus (Oliveiriella) rieradevallae* Prat & Paggi sp. n. (males  $n = 4$ ).

	Fe	Ti	Ta <sub>1</sub>	Ta <sub>2</sub>	Ta <sub>3</sub>	Ta <sub>4</sub>	Ta <sub>5</sub>	LR	BV	SV
P <sub>1</sub>	851–913.882	996–1079.1048	560–581.576	332–353.342	249–291.265	187–208.192	104–125.114	0.54–0.56	2.70–2.90	2.75
P <sub>2</sub>	809–851.835	809–872.851	353–394.374 (3)	187–228.201 (3)	187–145.159 (3)	104 (3)	104 (3)	0.44–0.45	3.40–3.81	3.62 (3)
P <sub>3</sub>	830–892.872	934–1017.991	540 (3)	270–291.277 (3)	228 (3)	104–125.118 (3)	104–125.111(3)	0.53–0.58	3.25–3.26	3.25 (3)

P<sub>1</sub> fore leg, P<sub>2</sub> mid leg, P<sub>3</sub> hind leg, Fe femur, Ti tibia, Ta tarsus, LR leg ratio (length of Ta<sub>4</sub>/length of tibia), BV length of (femur + tibia + Ta<sub>4</sub> + Ta<sub>5</sub>), SV length of (femur + tibia)/length of Ta.

**Cephalothorax.** (Fig 3a, b). Precorneals: anterior 22–24, median 193–207, posterior 18–24  $\mu\text{m}$  long. Dorsocentrals first and third 149–157 long, second and fourth approximately 20 long (2) (Fig 3b). Thoracic horn 240–273 (2) long apically truncated with a median notch and surface serrated as in Fig 3a.

**Abdomen.** (Fig 3c–g). Tergite II with two posterior rows of each 12–15, 13 hooklets. TIII with two rows of each 11–15, 14 hooklets and small spinules on either side of these rows connected to a central shagreen. TIV–V with 1 posterior row of 9–13, 12 hooklets, and small spines anteriorly also connected to shagreen (Fig 3c). TVI with a posteromedian group of small spinules and a row of moderately 12 strong spines. TVII–VIII with a posteromedian patch of spinules. TVI–VII with posteromedian fine shagreen. Abdominal segments: I with one strong lateral setae 121 long; II–V with two strong lateral setae 124–141 long on small tubercles. Tergites II–VIII with a pair of strong D<sub>5</sub> near hind margin. Sternites with ventromedian patches of spinules (Fig 3d, e). Anal lobe with 3 very short spine-like anal macrosetae (Fig 3f, g) length and distance between spines in Table 8).

**Larva.** ( $n = 2$ , except when otherwise stated, measurements of additional material are in Table 9). Total length 3.31–3.73. Body color (live) with intense blue and white bands, dark brown when fixed (Fig 4c).

**Head.** (Fig 4a, b). 415–519 long; 519 wide when mounted on slides. Postmentum 207 long. Head dark brown rugulose with pale spots around the eyes, and the gula more dark, with a pattern of small dots and several transversal marks between and below the mentum.

**Antenna.** (Fig 4c, d). Basal antennal segment 53–55 long; segments (2–5): 14–16; 6; 4; 4 long. AR 1.83–1.89. Blade 41 long. Ring organ at 6.09 from base of basal antennal segment.

**Mentum.** (Fig 4b). Median tooth twice as wide as the first lateral tooth (Table 9). Second lateral tooth smaller and narrower than the rest of the lateral teeth, sixth lateral tooth very small. Seta submenti simple and lateral, close to the base of the mentum.

**Mandible.** (Fig 4c). Dark brown with 4 inner teeth, with rugose outer margin, 142–148 long. Premandible simple.

**Abdomen.** (Fig 4e). Long and simple setae (at least half of the segment wide). Posterior parapod 207 long, 116–133 wide. Procerci 14–20 long; 24–28 wide. Anal tubules 331–373 long, longer than posterior parapods.

**Etymology.** This species is named in memory of the late Maria Rieradevall, a distinguished ecologist and

Table 7 Lengths (in  $\mu\text{m}$ ) and proportions of legs of *Cricotopus (Oliveiriella) rieradevallae* Prat & Paggi sp. n. (females  $n = 2$ ).

	Fe	Ti	Ta <sub>1</sub>	Ta <sub>2</sub>	Ta <sub>3</sub>	Ta <sub>4</sub>	Ta <sub>5</sub>	LR	BV	SV
P <sub>1</sub>	851–913	955–1038	498–560	311–353	228–249	166–187	104–125	0.52–0.54	2.75–2.85	3.48–3.63
P <sub>2</sub>	768–851	789–872	332–394	187–208	145–165	104	104	0.42–0.45	3.5–3.64	4.37–4.69
P <sub>3</sub>	809–913	934–1017	498–540	249–270	208–228	104–125	104–125	0.53	3.31–3.38	3.50–3.58

P<sub>1</sub> fore leg, P<sub>2</sub> mid leg, P<sub>3</sub> hind leg, Fe femur, Ti tibia, Ta tarsus, LR leg ratio (length of Ta<sub>1</sub>/length of tibia), BV length of (femur + tibia + Ta<sub>1</sub>)/length of (Ta<sub>2</sub> + Ta<sub>3</sub> + Ta<sub>4</sub> + Ta<sub>5</sub>), SV length of (femur + tibia)/length of Ta.

chironomidologist, and a founding member of the F.E.M. research group.

#### *Distribution of Cricotopus (Oliveiriella) spp. in the high Andean tropical rivers*

Larvae, pupae, or pupal exuviae of *Oliveiriella* were found in a total of nine sites from southern Peru to northern Colombia

(Table 1). The number of larvae and/or pupae found in each site was always low (1 to 20 individuals, Table 1) in streams of low (1 m wide) to medium (8 m) size and in a range of altitudes from 2308 to 4431 m a.s.l. Stones are the dominant substrate of many of the rivers and some of them have a considerable slope (up to 16%).

Conductivity of the streams was low from 10 to 100  $\mu\text{S}/\text{cm}$  temperature between 5, 6, and 14°C and pH mostly neutral

Table 8 Morphometric characteristics of the pupae of *Cricotopus (Oliveiriella)* found in the high tropical Andean rivers. Unless indicated, data are in microns and correspond to the length of the character. Site characteristics are in Tables 1 and 2.

Site	SA10	SA12	AGU14	CHINO2	GUAYO4
n	7	1	1	3	5
Project	CERA	CERA	CERA	BIQURA	BICURA/FUCARA
	Mean			Mean	Mean
Th L	929 (889–945)	863	863	943 (917–992)	1053 (1030–1099)
Ab L	2199 (2173–2274)	2267	2292	1946 (1853–2014)	2663 (2552–2801)
L Dc setae 1	175 (160–194)	201	202	50 (41–62)	178 (160–194)
L Dc setae 2	34 (22–58)	63	18	49 (42–59)	34 (14–52)
L Dc setae 3	159 (143–189)	165	183	41 (34–46)	161 (149–172)
L Dc setae 4	23 (18–28)		33	27 (21–35)	21 (13–39)
L Prec setae 1	49 (41–57)	57	51	69 (59–74)	43 (35–54)
L Prec setae 2	196 (187–207)	209	200	80 (77–83)	216 (184–244)
L Prec setae 3	45 (35–59)	39	42	49 (46–52)	26 (14–39)
RO L	226 (220–232)	193	133	249 (233–275)	296 (273–314)
RO W	43 (26–53)	57	20	42 (24–62)	65 (55–84)
HR T-II (n)	10 (8–11)	6	10	14 (14–15)	13 (12–15)
HR T-III (n)	12 (10–14)	8	10	14 (14–15)	14 (11–15)
HR T-IV (n)	11 (8–14)	9	8	12 (11–13)	13 (9–17)
HR T-V (n)	10 (8–13)	9	11	11 (10–11)	12 (9–13)
L Ab setae S-II	128 (112–137)	138	146	106 (95–112)	147 (138–156)
L Ab setae S-III	122 (114–133)	132	145	118 (115–121)	149 (132–159)
L Ab setae S-IV	127 (112–138)	133	136	118 (122–118)	137 (124–153)
Sp S-II (n)	No/small	Small	8	No	8 (4–13)
Sp S-III (n)	No/small	Small	6	No	9 (8–13)
L AS setae 1	20 (18–22)	12	18	17 (15–20)	16 (14–23)
L AS setae 2	19 (15–21)	11	19	13 (11–16)	20 (18–23)
L AS setae 3	19 (15–21)	11	18	21 (19–25)	18 (16–23)
AS distance 2–3	12 (8–15)	7	11	22 (15–27)	9 (8–11)
AS distance 1–2	26 (17–40)	16	31	36 (29–44)	45 (33–73)

L length, W wide, n number, Th thorax, Ab abdomen, Dc dorsocentral, Prec precorneal, Sp spine, T tergite, S sternite, RO respiratory organ, HR hook row, AS anal segment.

Table 9 Morphometric characteristics of the larvae of *Cricotopus* (*Oliveiriella*) in the high tropical Andean rivers. Unless indicated, data are in microns and correspond to the length of the character. Site characteristics are in Tables 1 and 2.

Site		SA10	MO16	MO08	NAPO02	GUAY04
Project		CERA	CERA	BIQURA	BIQURA	BIQURA/FUCARA
n		8	1		1	
Head cap	L	406 (391–412)	368	397 (373–418) (n = 4)	390	461 (430–493) (n = 16)
	W	311 (293–320)	306	335 (307–359) (n = 4)	291	379 (349–403) (n = 16)
Postmentum		186 (165–198)	184	185 (174–198) (n = 7)	185	220 (201–230) (n = 29)
Antenna	Basal L	44 (41–48)	42	46 (44–50) (n = 7)	43	56 (49–63) (n = 28)
	II L	13 (11–16)	15	14 (12–16) (n = 7)	12	15 (12–16) (n = 28)
	III L	6 (5–7)	7	5 (5–7) (n = 6)	5	6 (4–8) (n = 25)
	IV L	5 (3–5)	5	5 (4–5) (n = 6)	5	4 (3–6) (n = 24)
	V L	3 (2–4)	3	3 (2–4) (n = 4)	2	3 (2–4) (n = 10)
	AR	2 (1–2)	1	2 (1–2) (n = 4)	2	2 (2–3) (n = 24)
	AB L			27 (22–32) (n = 4)		25 (19–33) (n = 10)
	LO L			9 (9–9) (n = 2)		8 (7–10) (n = 5)
Mentum	W	110 (106–125)	106	112 (103–115) (n = 6)	108	118 (104–137) (n = 25)
M1	W	16 (13–18)	17	17 (17–20) (n = 6)	15	21 (18–29) (n = 26)
M2	W	8 (7–9)	8	8 (8–9) (n = 7)	8	8 (7–11) (n = 26)
MR (M1/mentum)		0.14 (0.12–0.17)	0.16	0.157 (0.146–0.162) (n = 5)	0.14	0.17 (0.14–0.25) (n = 25)

L length, W wide, AR antennal ratio, AB antennal blade, LO lauterborn organs.

(Table 2). Oxygen levels were always over 60% saturation with values over 7.91 mg/l. All the streams are oligotrophic with low concentrations of pollutants, including heavy metals. For more details of the physicochemical characteristics of these rivers, see also Villamarín *et al* (2014). Project BIQURA samples were collected from reference areas, sites without or with low human disturbance; therefore, although some parameters were not measured in BIQURA, the water quality of these streams was good. Sometimes the heterogeneity of substrates in the river may be low (as indicated by the values of IHF lower than 50 in Table 2), and the riparian area may be impacted (values of QBR-And lower than 75, Table 2). Values of QBR-And may be lower than expected for a reference site, due to the absence of a true riparian forest in the high-altitude streams (Acosta *et al* 2009, Acosta & Prat 2010).

*Morphological variability of larvae and pupae of high-altitude Andean Cricotopus (Oliveiriella) species*

*Larvae.* Live larvae of *Oliveiriella* of river Saltana were quite unique for its body color (blue and white), the intense black head capsule and the very long anal tubules (up to 395) that are more than double the length of the posterior parapods (Fig 4e). In living samples, these two characteristics made *Oliveiriella* easily recognizable under the stereoscope at low (×10) magnification. When fixed, the larvae lost the color but still the long anal tubules and the presence of body setae,

with a length close to half of the segment wide, made the larvae distinctive in the stereoscope. However, the larvae may lose the anal tubules when treated with KOH, which may create some confusion if only slide material is studied. Some of the larval head characteristics are similar to larvae of individuals of the genera comprised in the *Cricotopus* group (including the bifid SII, the crenulations in the mandible, or the general form of the mentum), but the head capsule of *Oliveiriella* offers some characteristic features that allow us to distinguish these larvae from the rest of the *Cricotopus* group. The color of the head is brown with a pattern of small dots. In the mentum and postmentum, there several line marks, which are characteristic (Fig 4b). Distinction of the larvae of different members of the *Cricotopus* complex in the Andes may be found in Prat *et al* (2012).

According to Andersen *et al* (2013), larvae of *Cricotopus* (*Oliveiriella*) have yellow to light brown head capsules, which is not the case of the *C. (Oliveiriella)* larvae of the Andes that have a dark brown head. The same authors include *C. (Oliveiriella)* in the group of species with setal tufts and the outer margin of the larvae completely smooth, which is not true in the larvae from the Andes (that wear simple setae and have a rugose outer margin of the mandible) and that otherwise have transversal marks in the gula that are characteristic as well as are the long anal tubules. In fact, in the paper of Krestian *et al* (2009), there is a figure of the mentum of *Oliveiriella* (Fig 3a) where it can be seen that the

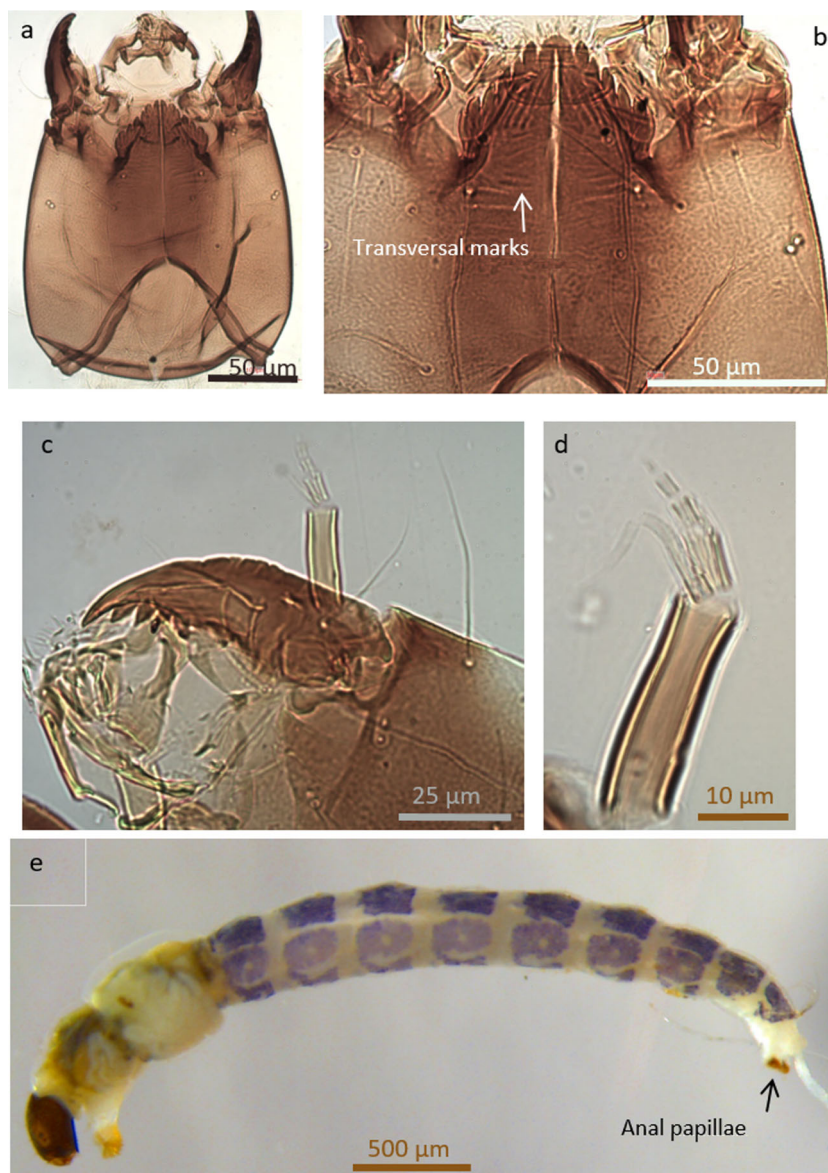


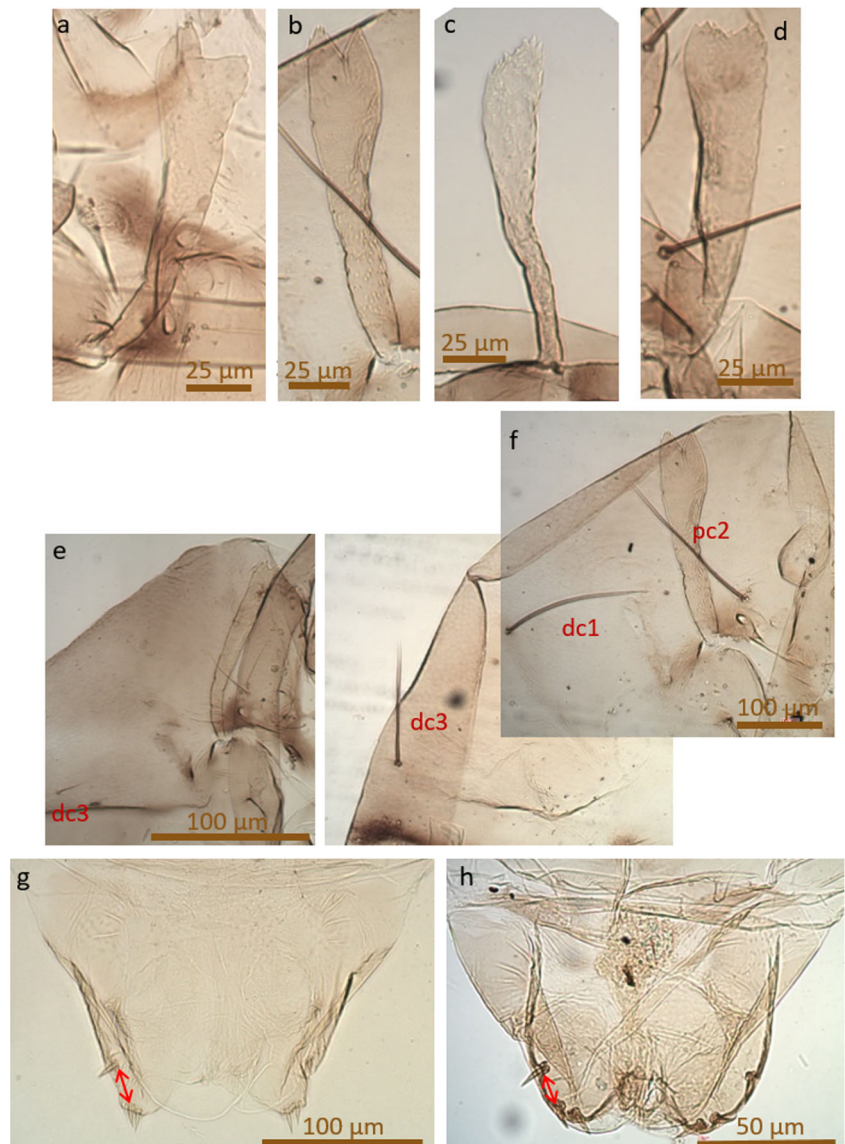
Fig 4 *Cricotopus (Oliveiriella) rieradevallae* Prat & Paggi sp. n. larvae. **a** Head capsule. **b** Mentum. **c** Mandible. **d** Antennae. **e** Larvae showing the long papillae (picture: Paula Ossa).

mentum is dark and with striae similar to those of ours Fig 4b. The key of Andersen *et al* (2013) should be consequently changed to allow the distinction of the *Oliveiriella* subgenus members from the genus *Cricotopus*.

After measuring a total of 50 larvae from five sites, some differences appear between the larvae of site GUAY04 (Saltana) and the other sites. Larvae of the GUAY04 site are larger, with a higher AR and MR than the other larvae, which are very similar. We use the measure of postmentum as a measure of size of the larvae (see Table 9) because in the slides, the head capsule larval length and width may depend

on the position of the larvae and the pressure applied during the preparation of the slide.

**Pupae.** The pupae of *Oliveiriella* are very characteristic, as described by Wiedenbrug & Fittkau (1997) and Tejerina & Paggi (2009) and very different from any species of the group *Cricotopus* as can be seen in the key of pupae or Prat *et al* (2014). The presence of hook rows (HR) in the segments II to V is characteristic, as well as the size and shape of the three setae of the anal segment that are transformed in small spines (Fig 3f, g). In Table 8, we compare the characteristics of the *Cricotopus (Oliveiriella)* pupae and pupal exuviae found in the



**Fig 5** *Cricotopus* (*Oliveiriella*) spp. pupae. Respiratory organ: **a** CHINO2 (Colombia), **b** GUAYO4 (Ecuador), **c** AGU14 (Ecuador), **d** SA10 (Peru). Thorax: **e** CHINO2 (short dorsocentral and precorneal setae), **f** GUAYO4. Abdominal segment IX: **g** GUAYO4, **h** SA10. In red the distance between the AS1 and AS2 (see Table 8).

tropical Andean high-altitude streams (5 sites, 19 individuals). There are two different groups of species easily recognizable by the difference of length of precorneal medium setae and the length of dorsal setae 1 and 3 with respect to the dorsal seta 2 (Tables 5 and 8). In the first group, only one specimen appeared in the CHINO2 site (from Chinchiná River). Pupae at this site have both the precorneals and dorsal setae of similar length as in *C. (O.) almeidai* and *C. (O.) sanjavieri*, although the setae are shorter (41 to 49 in CHINO2 vs 109 in *C. (O.) sanjavieri*, Tejerina & Paggi 2009). The second group contains specimens with long dorsal setae 1 and 3 and very long medium precorneal setae (Table 5). In between this large group, two subgroups can also be distinguished, the GUAYO4 samples, with spines in the sternites II and III longer than 5 µm, which correspond to *C. (O.) rieradevallae* sp. n. described in this paper, and the others (*C.*

*(O.)* sp. Santa) that do not have such spines (or only very small ones) and according to the molecular analysis belong to undescribed species. Also, the distance between the AS1 and AS2 in these two subgroups is different (Fig 5g, h). The respiratory organ (RO) is of similar length among all the pupae studied except for the sole pupal exuviae found in the Aguarico stream (AGU14 in Table 8; Fig 5c) which is shorter and without final notch, while in all the other pupae, the RO has a distinctive notch in the terminal part that is serrated (Figs 3a and 5a–b, d). The RO of *C. (O.) almeidai* and *C. (O.) sanjavieri* do not have this notch. The length of the RO is similar in the pupae that we have examined and similar to the length of the other two species described until now. The mean number of hooklets in the tergites is lower than those found in the two other described species (Table 8) but this number is very variable.

The pupal characteristics facilitated the description of a preliminary key of the morphotypes known at present, which most likely belong to different species.

#### Preliminary key of the pupae of *Cricotopus* (*Oliveiriella*)

1. All precorneal and dorsocentral setae short (max 120  $\mu\text{m}$ ) (Fig 5e) ..... 2
- One precorneal setae much longer (over 180  $\mu\text{m}$ ) than the other two (Fig 5f). Dorsocentral setae 1 and 3 longer (over 140  $\mu\text{m}$ ) compared with 2 and 4 (Fig 3b) ..... 4
2. Respiratory organ with a conspicuous notch (Fig 5a). Less than 20 HR spines in abdominal segment III ..... *C. (O.)* sp. Chinchiná
- Respiratory organ without a notch. More than 20 HR spines in abdominal segment III ..... 3
3. RO simple apex, blunt. Length: 181  $\mu\text{m}$  (136–242) ..... *C. (O.) almeidai* (Oliveira)
- RO serrated at tip: Length: 246  $\mu\text{m}$  (220–280) ..... *C. (O.) sanjavieri* Tejerina & Paggi
4. Without spines, or with only very small ones, in the abdominal sternites II and III. RO with a distal notch (Fig 5d) ..... *C. (O.)* sp. Santa
- With apparent spines (between 6 and 12  $\mu\text{m}$ ) in the abdominal sternites II and III (Fig 3d, e). RO with or without a distal notch ..... 5
5. Large species, RO over 3.5 mm and with a notch in the tip (Fig 5b) ..... *C. (O.) rieradevallae* sp. n.
- Smaller species, RO below 3 mm and without a notch (Fig 5c) ..... *C. (O.)* sp. Aguarico

## Discussion

In this paper, we describe a new species of *Cricotopus* (*Oliveiriella*) from all their instars and we include the molecular characteristics. The use of molecular analysis confirms the statement of Andersen *et al* (2013), based on morphological characteristics, that *Oliveiriella* must be included in the genus *Cricotopus* as a subgenus. Our molecular data, thus, support the inclusion of *Oliveiriella* as a subgenus of *Cricotopus*.

*Cricotopus* (*Oliveiriella*) *rieradevallae* sp. n. may be distinguished from the other described species of these subgenera in all three instars. The adult has a different pattern of spots on the wing and different color patterns in the legs and abdomen, as well as a different number of bristles in the squama and clypeus. The pupae may be distinguished by the size and form of the respiratory organ (RO) and the length of the medium setae of precorneals and the length of dorsocentral setae 1 and 3, and the presence of spines in the sternites II and III. Larvae are larger than those of the other species.

In the pupae examined in the sites studied (see Tables 1 and 8), we found very different combinations of the size and form of the RO, in the size of the precorneal and dorsocentral setae, and in the presence or not of spines of sternites II and III. This suggests the possible presence of at least four new species, which is supported by the molecular analysis. All the *Oliveiriella* converge among themselves before converging with other *Cricotopus*. The already known species *C. (O.) almeidai* (from Argentina) is clearly distinguished from the other species and is more similar to our material from Peru and Colombia than those of Ecuador.

There are four distinct populations of the Andean central area: Ecuador (NAO2 and GUAYO4), Peru (MOO8), and Colombia (VR-6). Interestingly, the two late populations seem to be identical despite the fact that they are more distant than the NA and GUAY materials. Note that all the material was collected at high altitude.

In this paper, we enlarged considerably the distribution of the subgenus *Cricotopus* (*Oliveiriella*) to the north and to the high-altitude rivers. We have found larvae and pupae of *C. (Oliveiriella)* at high-altitude Andean streams between 2457 and 3845 m a.s.l., in eight of the 186 sites we studied. Although we found larvae in the Saltana river throughout the year and the adults flying most of the year at this site, the abundance was never as high as other *Cricotopus*. The two species known to be present, i.e., *C. (O.) sanjavieri* and *C. (O.) almeidai*, were found in Argentina and Brazil (Paggi & Donato 2007, Tejerina & Paggi 2009, Tejerina & Malizia 2012, Wiedenbrug & Fittkau 1997). Larvae and pupae of an unknown species have been found in North America (Krestian *et al* 2009) which most likely means that the subgenus has a wide distribution in South and Central America. Although we have described only one species in this paper (*C. (O.) rieradevallae*), the differences found in the pupae and pupal exuviae collected in other high-altitude Andean streams indicate that many species are likely to be present, which is corroborated by the molecular analysis that differentiates *C. (O.) almeidai* of the Andean species while up to three species may be distinguished from the material collected in the central Andes.

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