

## Research article

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Salles & Lugo-Ortiz, 2002 and *Apobaetis kallawayi* Nieto, 2006  
(Ephemeroptera, Baetidae)**Cláudia R.T. DE LIMA <sup>1,\*</sup>, Paulo Vilela CRUZ <sup>2</sup> & Neusa HAMADA <sup>3</sup><sup>1,2,3</sup>Instituto Nacional de Pesquisas da Amazônia (INPA), Coordenação de Pós-Graduação (COPOG), Divisão do Curso em Entomologia (DiEnt), Coordenação de Biodiversidade (CoBio), Laboratório de Citotaxonomia e Insetos Aquáticos, CEP 69067-375, Manaus, Amazonas, Brazil.<sup>2</sup>Universidade Federal de Rondônia (UNIR), Departamento de Biologia, Laboratório de Biodiversidade e Conservação, 76.801-059, Porto Velho, Rondônia, Brazil.\*Corresponding author: [claudia.tavares.lab@gmail.com](mailto:claudia.tavares.lab@gmail.com)<sup>2</sup>Email: [pvilacruz@gmail.com](mailto:pvilacruz@gmail.com)<sup>3</sup>Email: [neusaha@gmail.com](mailto:neusaha@gmail.com)<sup>1</sup>[urn:lsid:zoobank.org:author:18795D2E-427D-4A26-B112-70C929CA5B95](https://zoobank.org/author:18795D2E-427D-4A26-B112-70C929CA5B95)<sup>2</sup>[urn:lsid:zoobank.org:author:34F85F41-2743-4248-813F-0CEEE63FE649](https://zoobank.org/author:34F85F41-2743-4248-813F-0CEEE63FE649)<sup>3</sup>[urn:lsid:zoobank.org:author:0D02F36C-6B09-423C-95A6-6179E9808CD5](https://zoobank.org/author:0D02F36C-6B09-423C-95A6-6179E9808CD5)

**Abstract.** Recently, it has been hypothesized that the wide distribution of *A. fuzai* Salles & Lugo-Ortiz is due to a wide diagnosis and conflicting keys. The same arguments can be applied to *A. kallawayi* Nieto. In order to test this hypothesis, we revised the type material of *A. fuzai*, literature of *A. kallawayi*, and part of the recorded specimens in Brazil. As a result, three new species from previous records are recognized: *Apobaetis pasternakae* sp. nov., former *A. fuzai* in Amazonas, Roraima and Maranhão states; *Apobaetis jaquelineae* sp. nov., former *A. fuzai* in Rondônia State; *Apobaetis luanæ* sp. nov., former *A. kallawayi* in Rondônia State. The new data showed that *A. fuzai* does not occur in the Amazon Biome and *A. kallawayi* does not occur in Brazil. Records of *A. fuzai* that could not be reviewed in this study are treated as putative and should be evaluated in the light of the new evidence. We also studied the intraspecific variation in *A. fuzai* and *A. pasternakae* sp. nov. The pigment pattern of the nymphs was not considered a robust characteristic for the species differentiation due to the wide variation in and between populations, and interspecific similarity of many features. On the other hand, classical morphological characters such as mouthparts, legs, spines of terga, paraproct and caudal filaments have minimal variation, stable and reliable for specific identification.

**Keywords.** Review, corrections, new species, intraspecific variation.

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## Introduction

*Apobaetis* Day, 1955 was originally described based on nymphs and imagoes from California, United States of America. Decades later, Meyer & McCafferty (2003) revised the type species, *Apobaetis indeprensus* Day, 1955, recognizing it as a junior synonym of *Apobaetis etowah* (Traver, 1935), previously allocated to *Pseudocloeon* Klapalek, 1905. Since the recognition and redefinition of *Apobaetis*, 11 species were described, two from North America (*A. etowah* (Traver, 1935), *A. lakota* McCafferty, 2000) and 10 from South America (*A. signifer* Lugo-Ortiz & McCafferty, 1997, *A. fuzai* Salles & Lugo-Ortiz, 2002, *A. kallawayi* Nieto, 2006, *A. niger* Nieto, 2006, *A. insolitus* Nieto, 2006, *A. hamadae* Cruz, Boldrini & Salles, 2011, *A. pucupi* Cruz & De-Souza, 2014, *A. kethepiali* Cruz & De-Souza, 2014, *A. biancae* Boldrini, 2020, *A. jacobusi* Cruz, Boldrini & Hamada, 2020), totaling 12 species.

Nymphs of the genus are psammophilous, inhabiting sandy bottom rivers and streams (Glazaczow 1997; McCafferty 2000), with slow flow or, less frequently, in moderate flow (Day 1955). This habitat was historically overlooked (McCafferty 1991) and, under ideal conditions, requires time and effort to separate the specimens from the sand grains (Cruz & De-Souza 2014; Cruz *et al.* 2020). In addition, species that occupy this habitat generally have a low population density and are often very small (McCafferty 1991; Lugo-Ortiz & McCafferty 1997; Jacobus 2013). Consequently, species have always been described based on a few specimens (e.g., Cruz *et al.* 2011, 2020; Cruz & De-Souza 2014), which limits studies (or considerations) on intraspecific variations.

Once sampled, the proper study of specimens is hampered by their problematic diagnoses and/or incomplete original descriptions (see Cruz 2020; Cruz *et al.* 2020). A broad specific diagnosis can include multiple species and the lack of important information in the original descriptions makes identification difficult (Cruz 2020; Cruz *et al.* 2020). Additionally, the available keys present divergences (see Domínguez *et al.* 2006; Nieto 2006; Cruz & De-Souza 2014), resulting in misidentifications, often between *A. fuzai* and other species (Cruz 2020). To diminish these problems, Cruz (2020) redescribed very similar species, improving their diagnoses and proposed a new identification key. He also hypothesized that the wide distribution of *A. fuzai* is probably due those identification imprecisions. The same arguments can be applied to *A. kallawayi* recorded in Brazil (Boldrini & Cruz 2014). Recently, a similar hypothesis was raised and tested to other psammophilous species (*Rivudiva trichobasis* Lugo-Ortiz & McCafferty, 1998), revealing 5 species hidden behind an easily recognizable one (Cruz *et al.* 2022).

Taking into account the arguments presented by Cruz (2020) and Cruz *et al.* (2020), it is very unlikely that *A. fuzai* and *A. kallawayi* range across different regions, biomes or countries. Therefore, our hypothesis is that most records of *A. fuzai* and the record of *A. kallawayi* from Brazil are, in fact, other species. In order to test our hypothesis, this study aims to review the specimens identified as *A. fuzai* and *A. kallawayi* from Brazil in the light of the new morphological evidence provided in Cruz (2020) and Cruz *et al.* (2020).

## Material and methods

### Specimens, descriptions and illustrations

The descriptions and measurements followed the standardized protocol proposed by Hubbard (1995). The descriptions of the genitalia followed the pattern proposed by Kluge & Novikova (2011). Multi-focal photographs were obtained using a Leica M165C stereo microscope with Leica DFC 420 image-capturing equipment and LED dome lighting for a uniform reflection of light on the specimens (Kawada & Buffington 2016). The final illustrations were prepared following the procedure of Coleman (2006). The material examined is preserved in 80% ethanol and on permanent slides using Euparal® as mounting medium. Part of the slides was made using Acrilex® stained glass varnish diluted with

xylene (Huber & Dos Reis 2011), a method here used for the first time in slides with specimens of Ephemeroptera Hyatt & Arms, 1891.

The identification of the specimens was based on the original descriptions (Nieto 2006; Cruz *et al.* 2011), redescrptions (Cruz 2020), latest identification key (Cruz 2020), consultation of the type material, literature review, specimens used in previous studies and additional material. Type material of *A. fuzai* (photographs) was obtained from the Museum of Entomology of the Federal University of Viçosa, Brazil – UFV; *A. kallawaya* was analyzed through a literature review and consultation of Dr Carolina Nieto (Facultad de Ciencias Naturales and Instituto Miguel Lillo, Tucumán, Argentina); additional material was obtained from the Invertebrate Collection of the Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil (INPA).

Type material and voucher specimens were deposited in the Invertebrate Collection of the Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil (INPA).

#### Abbreviations in figures

A	=	anal
CuA	=	anterior cubital
d.v.	=	dorsal view
gv	=	gonovectes
IMP <sub>2</sub>	=	Intercalary Medius Posterior 2
l.v.	=	lateral view
mb	=	membrane
m.gv	=	gonovectes muscle
ms	=	styligeral muscle
pb	=	penial bridge
Sc	=	subcostal
us	=	unistyliger
v.v.	=	ventral view

#### Institutional abbreviations

UFES	=	Universidade Federal do Espírito Santo, Vitória, Espírito Santo, Brazil
UFV	=	Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil
INPA	=	Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil

#### Results

Class Insecta Linnaeus, 1758  
Order Ephemeroptera Hyatt & Arms, 1891  
Family Baetidae Leach, 1815  
Subfamily Baetinae Leach, 1815 sensu Cruz *et al.* (2021)

Genus *Apobaetis* Day, 1955

The analysis of type material, literature review, consultation of specialist and review of the part of historical records allowed the recognition of three new species: *Apobaetis pasternakae* sp. nov. (former *A. fuzai*), *Apobaetis jaquelineae* sp. nov. (former *A. fuzai*) and *Apobaetis luanae* sp. nov. (former *A. kallawaya*). Consequently, corrections were made to the species distribution records (Fig. 1), restricting *A. fuzai* outside the Amazon biome and *A. kallawaya* to Bolivia. The possibility of examining more than a few specimens allowed considerations of intraspecific variation.

*Apobaetis fiuzai* Salles & Lugo-Ortiz, 2002  
Figs 1–2

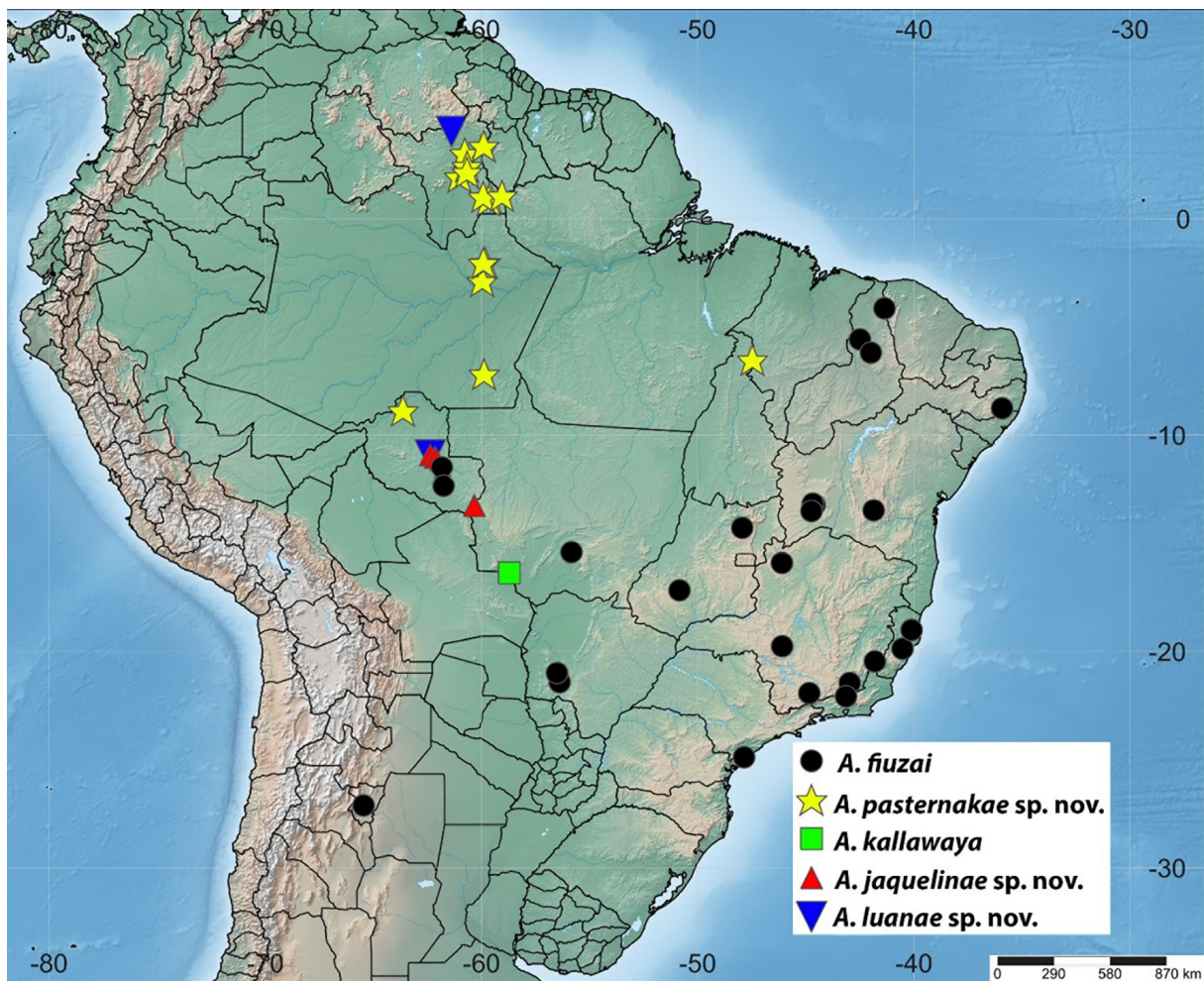
*Apobaetis fiuzai* Salles & Lugo-Ortiz, 2002: 1 (type material).

*Apobaetis fiuzai* – Salles *et al.* 2003: 5 (treated here as putative); 2004: 727 (treated here as putative); 2010: 302 (treated here as putative). — Lima *et al.* 2012: 306 (treated here as putative). — Cruz 2020: 322, figs 4a–f, 5a–c (re-description).

non *Apobaetis fiuzai* – Cruz *et al.* 2011: 89. — Falcão *et al.* 2011: 519. — Boldrini *et al.* 2012: 92. — Boldrini & Cruz 2014: 4.

**Diagnosis** (adapted from Cruz 2020)

NYMPH. Characterized by a combination of the following characters: 1) labrum rectangular, distal medial margin without shallow emargination, dorsal surface with 3 to 4 elongated and blunt medial setae near distal margin (Cruz 2020: fig. 4a); 2) hypopharynx with lingua subquadrangular, one medial protuberance, apical tuft of setae, length subequal to superlingua (Cruz 2020: fig. 4d); 3) maxillary



**Fig. 1.** Map with distribution of *Apobaetis fiuzai* Salles & Lugo-Ortiz, 2002 (including putative records) (black circles), *Apobaetis pasternakae* sp. nov. (yellow stars), *Apobaetis kallawayae* Nieto, 2006 (green square), *Apobaetis jaquelineae* sp. nov. (red triangles), and *Apobaetis luanae* sp. nov. (blue triangles).

palp long  $1.5 \times$  length of galea-lacinia, segment II without apical constriction (Cruz 2020: fig. 4e); 4) labial palp with segment II with pointed triangular distomedial projection, laterally directed; segment III rectangular, distal margin almost straight (Cruz 2020: fig. 4f); 5) foreleg with dorsal margin of femur with one row of 11 short concave and blunt setae; claw  $0.6\text{--}0.8 \times$  length of tarsus, without row of denticles (Cruz 2020: fig. 5a); 6) posterior margin of the tergum IV with triangular and pointed spines in regular sizes (Cruz 2020: fig. 5b); 7) paraproct with marginal spines, posterolateral extension with triangular and pointed spines (Cruz 2020: fig. 5c).

### Material examined

#### Holotype

BRAZIL • nymph on slide (photographs); Minas Gerais, Córrego Danta, Fazenda Prata, Rio Prata; 19°45.29' S, 46°5.53' W; 9 Aug. 2001; C.R. Lugo-Ortiz and F.F. Salles leg.; UFES.

#### Paratypes

BRAZIL • 2 nymphs on slide (photographs); same collection data as for holotype; INPA.

#### Additional material

BRAZIL – **Piauí** • 3 nymphs on slide; Rio Grande do Piauí, Povoado Araticum; 04°08'43.1" S, 41°21'59.7" W; 31 May 2011; N. Hamada, P.V. Cruz and R.B. Querino leg.; INPA • 3 nymphs on slide; Monsenhor Gil, Povoado Olho D'água, Riacho calça na mão; 05°34'38.6" S, 42°29'59.5" W; 24 Jun. 2011; N. Hamada, P.V. Cruz and R.B. Querino leg.; INPA • 2 nymphs on slide; Valença do Piauí, Dam Mesa de Pedra; 06°11'31.6" S, 41°59'35.0" W; 8 Jun. 2011; N. Hamada, P.V. Cruz and R.B. Querino leg.; INPA. – **Bahia** • 3 nymphs on slide; Correntina, Comunidade do Val, Rio do Meio; 13°13'35.2" S, 44°35'37.3" W; 7 Jun. 2012; N. Hamada, P.V. Cruz and J.O. Silva leg.; INPA • 1 nymph on slide; Correntina, Comunidade Santo Antônio, Riacho Santo Antônio; 13°10'17.0" S, 44°40'56.6" W; 7 Jun. 2012; N. Hamada, P.V. Cruz and J.O. Silva leg.; INPA • 1 nymph on slide; Correntina, Tributário do Arrojado, Córrego Bonito; 13°30'59.4" S, 44°44'17.2" W; 8 Jun. 2012; N. Hamada, P.V. Cruz and J.O. Silva leg.; INPA. – **Goiás** • 3 nymphs on slide; Colinas do Sul, Rio Tocantinzinho; 14°17'05.7" S, 47°56'29.4" W; 13 Jun. 2012; N. Hamada, P.V. Cruz and J.O. Silva leg.; INPA • 2 nymphs on slide; Paraúna, Tributário do Rio Verdão; 07°10'24.1" S, 50°50'43.9" W; 4 Jun. 2012; N. Hamada, P.V. Cruz and J.O. Silva leg.; INPA • 2 nymphs on slide; Colinas do Sul, Morro Vermelho, road of the São Jorge; 14°13'34.2" S, 47°55'15.4" W; 13 Jun. 2012; N. Hamada, P.V. Cruz and J.O. Silva leg.; INPA. – **Mato Grosso do Sul** • 3 nymphs on slide; Bonito, Rio da Prata, Municipal Bathhouse of Jardim; 21°25'04.3" S, 56°23'24.0" W; 15 Apr. 2012; N. Hamada, P.V. Cruz and N. Zampiva leg.; INPA • 1 nymph on slide; Bonito, Rio Mimoso, Estância Mimosa; 20°59'57.4" S, 56°30'38.7" W; 16 Mar. 2012; N. Hamada, P.V. Cruz and N. Zampiva leg.; INPA. – **Rondônia** • 2 nymphs on slide; Alto Alegre dos Parecis, Road 490; 11°58'59.4" S, 61°51'08.1" W; 26 Jul. 2016; P.V. Cruz and N. Hamada leg.; INPA • 1 nymph on slide; Rolim de Moura, Road 267, Rio Palha; 11°29'52.7" S, 61°50'08.4" W; 17 Jun. 2016; P.V. Cruz and N. Hamada leg.; INPA • 1 nymph on slide; Alto Alegre dos Parecis, Road 370; 12°20'37.4" S, 61°45'26.3" W; 9 Jul. 2016; P.V. Cruz and N. Hamada leg.; INPA. – **Minas Gerais** • 2 nymphs on slide; Juiz de Fora, BR 267, bridge over the Rio Furnas; 21°55'58.7" S, 44°50'26.9" W; 26 Dec. 2011; P.V. Cruz leg.; INPA. – **Espírito Santo** • 2 nymphs on slide; Linhares, Sooretama Reserve, Rio Quirinão; 19°00'39.9" S, 40°06'30" W; 4 Nov. 2011; P.V. Cruz leg.; INPA.

### Intraspecific variation of *Apobaetis fiuzai*

#### Nymph

THORAX. Light yellow, with short brown marks (Fig. 2A, I); lateral brown mark on pronotum; sublateral brown mark on mesonotum; basal half brown on forewing pads (Fig. 2G); brown mark partially covering the thorax (Fig. 2L); femur without mark (Fig. 2F, H).

ABDOMEN. Tergum V with lateral brown mark (Fig. 2A, C, G, I, L); terga I–X with light brown spots (Fig. 2A, C, E, L N); terga II and VI with medial brown mark, III–V and VII, VIII with brown spots (Fig. 2I); terga I–IX brown (Fig. 2L); terga I, II, VI, VII with medial brown mark, tergum VIII almost brown completely, tergum IX with lateral brown mark (Fig. 2G); sternum IX with brown mark on anterior margin (Fig. 2B, D); sternum VIII light brown, sterna VIII and IX with lateral brown mark (Fig. 2H); sternum X light brown (Fig. 2M).

### Morphological variation

LENGTH. Claws  $0.6\text{--}0.8\times$  length of tarsus; body  $2.1\text{--}2.9$  mm.



**Fig. 2.** *Apobaetis fuzai* Salles & Lugo-Ortiz, 2002, habitus of nymph, from different populations, showing pigment variation. **A–F.** Goiás State. **A.** Nymph female (d.v.). **B.** Nymph female (v.v.). **C.** Nymph female (d.v.). **D.** Nymph female (v.v.). **E.** Nymph male (d.v.). **F.** Nymph male (v.v.). – **G–H.** Piauí State. **G.** Nymph female (d.v.). **H.** Nymph female (v.v.). – **I–O.** Bahia State. **I.** Nymph female (d.v.). **J.** Nymph female (v.v.). **L.** Nymph female (d.v.). **M.** Nymph female (v.v.). **N.** Nymph female (d.v.). **O.** Nymph female (v.v.). Abbreviations: d.v. = dorsal view; v.v. = ventral view. Not to scale.

### Comments

Based on the study of the type material, records from Amazonas (Cruz *et al.* 2011), Roraima (Falcão *et al.* 2011), Rondônia (Boldrini & Cruz 2014) and Maranhão (Boldrini *et al.* 2012), are not *A. fuzai*. In the type material of *A. fuzai* the hypopharynx has lingua subquadrangular, with medial protuberance; maxillary palp  $1.5 \times$  length of galea-lacinia and posterolateral extension of paraproct with pointed spines. The specimens recorded as *A. fuzai* from Amazonas, Roraima and Maranhão (described here as *Apobaetis pasternakae* sp. nov.) have lingua subcircular, without medial protuberance; maxillary palp  $2.0 \times$  or longer than galea-lacinia and posterolateral extension of paraproct without spines. The specimen assigned to *A. fuzai* from Rondônia (described here as *Apobaetis jaquelinae* sp. nov.) has lingua subcircular, posterolateral extension of paraproct with blunt spines, and has claws with two row of denticles.

### Distribution

Brazil – Bahia: Rio de Contas (putative), Correntina; Espírito Santo: Alto Caparaó (putative), Santa Teresa (putative), Linhares; Goiás: Colinas do Sul, Paraúna; Mato Grosso: Chapada dos Guimarães; Mato Grosso do Sul: Bonito; Minas Gerais: Arinos, Descoberto, Córrego Danta, Juiz de Fora; Pernambuco: São Benedito do Sul (putative); Piauí: Monsenhor Gil, Rio Grande do Piauí, Valença do Piauí; Rio de Janeiro: Comendador Levy Gasparian (putative); Rondônia: Alto Alegre dos Parecis, Rolim de Moura; São Paulo: Cananeia (putative). Argentina – Acherai: Tucumán (Nieto 2006) (putative). All records treated here as putative must be evaluated.

*Apobaetis kallawaya* Nieto, 2006

Fig. 1

*Apobaetis kallawaya* Nieto, 2006: 195 (type material).

non *Apobaetis kallawaya* – Boldrini & Cruz 2014: 4.

### Diagnosis (adapted from Nieto 2006)

NYMPH. Characterized by a combination of the following characters: 1) labrum rectangular, distal medial margin without emargination; dorsal surface with 4 to 5 bifid medial spine-like setae near distal margin (Nieto 2006: fig. 19); 2) hypopharynx with lingua subcircular, apical tuft of setae, length subequal to superlingua (Nieto 2006: fig. 22); 3) maxillary palp short, length subequal to galea-lacinia; segment II without apical constriction (Nieto 2006: fig. 23); 4) labial palp with segment II with slender triangular distomedial projection, apically directed; segment III triangular (Nieto 2006: fig. 25); 5) foreleg with dorsal margin, anterior and posterior surface of femur without setae; claw I  $0.7 \times$  length of tarsus I, with tow row of denticles (Nieto 2006: figs 26–27); 6) posterior margin of the tergum IV with apex rounded spines (Nieto 2006: fig. 28); 7) paraproct with marginal spines, posterolateral extension with apex rounded spines (Nieto 2006: fig. 30).

### Material examined

None, analysis based on literature and consultation of Dr Carolina Nieto.

### Comments

Fundamental differences were found between the original description of *A. kallawaya* and the Brazilian specimen (Boldrini & Cruz 2014). In the original description the labrum has dorsal surface with 4 to 5 bifid medial spine-like setae near distal margin; maxillary palp short, length subequal to galea-lacinia, and paraproct with posterolateral extension with apex rounded spines. The specimen of *A. kallawaya* from Rondônia (described here as *Apobaetis luanæ* sp. nov.) has labrum with 4 simple medial spine-like setae near distal margin; maxillary palp long  $2.0 \times$  length of galea-lacinia and paraproct with posterolateral extension without spines.

## Distribution

Bolivia – Acherai, San Matías.

*Apobaetis pasternakae* sp. nov.

[urn:lsid:zoobank.org:act:CA9DDFA5-45BE-44FC-9EC5-A92B09F133B8](https://zoobank.org/urn:lsid:zoobank.org:act:CA9DDFA5-45BE-44FC-9EC5-A92B09F133B8)

Figs 1, 3–5

*Apobaetis fiuzai* – Cruz *et al.* 2011: 89. — Falcão *et al.* 2011: 519. — Boldrini *et al.* 2012: 92.

## Diagnosis

**MALE IMAGO** (adapted from Cruz *et al.* 2011). Wing (Cruz *et al.* 2011: fig. 8). hyaline, veins light brown; stigmatic area with eight veins not touching Sc vein; marginal intercalary veins paired, except single between veins IMP2 and CuA, absent between CuA and A; length of each intercalary vein  $0.6 \times$  distance between adjacent longitudinal vein; length of forewing about  $2.5 \times$  width. External genitalia (Cruz *et al.* 2011: fig. 10a–b). Area between unistyligers with a deep V emargination. Internal genitalia (Fig. 5H). A pair of sclerotized gonovectes V-shaped, broad apex (gv); retractor muscle of the gonovectes fixed in the angle region of the gonovectes (m.gv); sclerotized penial bridge (pb); a pair of styliger muscle (ms).

**NYMPH.** Characterized by a combination of the following characters: 1) labrum rectangular, distal medial margin with one protuberance; dorsal surface with 3 to 5 elongated and blunt medial setae near distal margin (Fig. 4A–B); 2) hypopharynx with lingua subcircular, with apical tuft of setae, length subequal to superlingua (Fig. 4E); 3) maxillary palp long, greater than or equal to  $2.0 \times$  the length of galea-lacinia; segment II tapering abruptly from the apical half to the apex, without apical constriction (Fig. 4F); 4) labial palp segment II with triangular pointed distomedial projection, apically directed; segment III rectangular, distal margin slightly concave (Fig. 4G); 5) foreleg with dorsal margin of femur with one row of 11 to 13 concave and blunt long setae; claw  $0.6–0.8 \times$  length of tarsus, without row of denticles (Fig. 5A); 6) posterior margin of tergum IV with triangular and pointed spines in irregular sizes (Fig. 5C); 7) paraproct with several marginal spines, posterolateral extension without spines (Fig. 5E).

## Etymology

The species name is a tribute to Dr Natália Pasternak, founder of the Instituto Questão de Ciências in São Paulo. During the Coronavirus Pandemic, she acted fighting fake news.

## Material examined

### Holotype

BRAZIL • nymph on slide; Amazonas State, Manaus, Reserva Florestal Adolpho Ducke, stream Barro Branco; 02°53' S, 59°58' W; 10 Mar. 2009; R. Boldrini and P.V. Cruz leg.; INPA.

### Paratypes

BRAZIL – Amazonas • 2 nymphs on slide; same collection data as for holotype; INPA • 5 nymphs in alcohol 80%; same collection data as for holotype; INPA.

### Additional material

BRAZIL – Amazonas • 12 ♂♂ imagoes in 80% alcohol; Manaus, Reserva Florestal Adolpho Ducke, stream Barro Branco; 02°53' S, 59°58' W; 8 Apr. 2009; R. Boldrini leg.; INPA • 5 males imagoes genitalia on slide; same collection data as for preceding; INPA • 6 nymphs on slide; Presidente Figueiredo, Corredeira da Pantera; 02°02' S, 59°50' W; 8 Nov. 2009; R. Boldrini and P.V. Cruz leg.; INPA • 1 nymph on slide; same collection data as for preceding; 12 Nov. 2009; INPA • 3 nymphs on slide; Presidente Figueiredo, Cachoeira do Santuário; 02°03' S, 59°55' W; 13 Nov. 2009; R. Boldrini and P.V. Cruz leg.; INPA • 1 nymph on slide; Apuí; 07°11' S, 59°53' W; 2 Jul. 2018; P.V. Cruz, G. Desidério and N. Hamada



leg.; INPA. – **Roraima** • 2 nymphs on slide; Caroebe, Rio Caroebe, ramal 37, Cachoeirinha farms; 00°57'09.2" N, 59°37'00.5" W; 23 Mar. 2012; N. Hamada, P.V. Cruz, G. Dantas and R. Boldrini leg.; INPA • 12 nymphs in alcohol 80%; same collection data as for preceding; INPA • 1 nymph in alcohol 80%; Caroebe, Lago Jacundá, Vicinal 02; 00°50'59.8" N, 59°40'48.2" W; 29 Nov. 2006; J.N. Falcão leg.; INPA • 2 nymphs on slide; São João da Baliza, sítio do igarapé; 01°00'59.7" N, 59°55'53.1" W; 24 Mar. 2012; N. Hamada, P.V. Cruz, G. Dantas and R. Boldrini leg.; INPA • 3 nymphs in alcohol 80%; same collection data as for preceding; INPA • 3 nymphs on slide; Caroebe, Igarapé do Jacaré, vicinal 05; 01°03'58.2" N, 59°3'06.8" W; 19 Mar. 2013; N. Hamada, P.V. Cruz, G. Dantas and R. Boldrini leg.; INPA • 4 nymphs on slide; Caroebe, Rio Caroebe, vicinal 05; 01°03'58.2" N, 59°3'06.8" W; 12 Mar. 2018; P.V. Cruz and I.O. Fernandes leg.; INPA • 1 nymph on slide; Pacaraima, Rio Ereú; 01°56'01.3" N, 61°01'38.4" W; 26 Mar. 2012; N. Hamada, P.V. Cruz, G. Dantas and R. Boldrini leg.; INPA • 1 nymph on slide; Boa Vista, Rio Murupu; 03°01'16.3" N, 60°46'32.9" W; 19 Dec. 2004; N. Hamada and F.F. Salles leg.; INPA • 1 nymph on slide; Bonfim, Rio Arraia; 03°21'01.6" N, 59°54'14.5" W; 5 Nov. 2006; J.N. Falcão leg.; INPA • 1 nymph in alcohol 80%; Caroebe, Lago Jacundá, first bridge, Vicinal 2; 00°50'59.8" N, 59°40'48.2" W; 29 Nov. 2008; J.N. Falcão leg.; INPA • 2 nymphs on slide; Bonfim, Rio Arraia; 03°21'04.0" N, 59°54'13.5" W; 27 Mar. 2012; N. Hamada, P.V. Cruz, G. Dantas and R. Boldrini leg.; INPA • 2 nymphs in alcohol 80%; same collection data as for preceding; INPA • 2 nymphs on slide; Cantá, Rio do Cachorro bridge; 02°25'20.2" N, 60°40'00.9" W; 28 Mar. 2012; N. Hamada, P.V. Cruz, G. Dantas and R. Boldrini leg.; INPA • 2 nymphs on slide; BR 170; 02°08'59.9" N, 60°40'39.9" W; 28 Mar. 2012; N. Hamada, P.V. Cruz, G. Dantas and R. Boldrini leg.; INPA. – **Rondônia** • 3 nymphs on slide; Candeias do Jamarí, Igarapé da Onça; 08°52'40.0" S, 63°38'02.2" W; 9 Jul. 2016; N. Hamada and P.V. Cruz leg.; INPA. – **Maranhão** • 2 nymphs on slide; Estreito, BR 010, near the bridge, Rio Farinha; 06°31'47.3" S, 47°28'11.4" W; 22 Jul. 2010; N. Hamada, P.V. Cruz, G. Dantas and R. Boldrini leg.; INPA.

## Description

### Nymph

LENGTH. Body: 2.9–3.1 mm.

HEAD. Antenna. Flagellum with minute spines at apex of each flagellomere. Labrum (Fig. 4A–B). Length about  $0.5 \times$  maximum width; distal medial margin with one protuberance; dorsal surface with 3 to 5 elongated and blunt medial setae near distal margin; medially with one row of long and thin setae near distal margin; long, thin and simple setae covering dorsal surface; ventral surface with spine-like setae on distolateral and distal margins. Left mandible (Fig. 4C). Incisors not fused; outer and inner set of incisors with 4 and 3 denticles, respectively; prostheca robust, bifurcated at apex, outer lobe robust, inner lobe slender with medial tuft of setae; margin between prostheca and mola concave; subtriangular process wide with short protuberance on distal margin; tuft of setae at base of subtriangular process; denticles of mola not constricted; lateral margin convex. Right mandible (Fig. 4D). Incisors not fused; outer and inner set of incisors each with 3 denticles; prostheca slender, bifurcated at apex; margin between prostheca and mola concave; tuft of setae at base of mola absent; denticles of mola not constricted; lateral margin convex. Hypopharynx (Fig. 4E). Lingua subcircular, with apical tuft of setae, length subequal to superlingua; superlingua not expanded, with short, fine and simple setae scattered over distolateral and distal margin. Maxilla (Fig. 4F). Maxillary palp long, greater than or equal to  $2.0 \times$  length of galea-lacinia; segment I  $0.7 \times$  length of galea-lacinia; segment II tapering abruptly from apical half to apex, with fine and simple setae scattered over surface, without apical constriction. Labium (Fig. 4G). Glossa subquadrangular, medially broad, subequal to paraglossa; dorsal surface with one longitudinal row of 4 short spine-like setae near inner margin; apex with 3 short spine-like setae; longitudinal row of 9 robust spine-like setae near outer margin; ventral surface covered with long, thin and simple setae. Paraglossa curved inward; dorsal surface with long and robust setae on apex; outer margin with one longitudinal row of 16 long spine-like setae to base and one longitudinal row of 6 long and robust spine-like setae to

base near inner margin; ventral surface with one longitudinal row of 7 long and robust spine-like setae at middle, at apical half. Labial palp with segment I  $0.8 \times$  length of segments II and III combined, covered with micropores (not illustrated); segment II with outer margin covered with thin, long and simple setae, inner margin bare; segment II with triangular pointed distomedial projection apically directed; ventral surface of distomedial projection with tuft thin, long and simple setae; segment III rectangular, distal margin slightly concave, length  $0.6 \times$  width, covered with fine, long and simple setae on outer margin, dorsally with a row of 11 to 14 robust setae in different sizes near distal margin, ventrally with 10 to 12 robust spine-like setae in different sizes near distal margin.

**THORAX.** Holotype pigment (Fig. 3A–C). Light yellow; femur with rounded brown mark on apical third. Foreleg (Fig. 5A–B). Femur: dorsal margin with one row of 11 to 13 concave and blunt long setae; apex with 2 concave and blunt long setae; ventral margin with one row of 6 to 8 elongated spine-like setae; posterior surface with one row of 8 to 10 elongated spine-like setae near ventral margin. Tibia: dorsal margin bare, ventral margin with one row of 8 to 10 spine-like setae. Patella-tibial suture present, from dorsal to ventral margin. Tarsus: dorsal margin bare, ventral margin with one row of 12 to 15 spine-like setae. Claw:  $0.6\text{--}0.8 \times$  length of tarsus, row of denticles absent. Middle and hindleg similar to foreleg.

**ABDOMEN.** Holotype pigment (Fig. 3A–C). Light yellow; terga I and II with medial brown mark; tergum V with lateral brown mark; tergum IX with brown mark on anterior margin; sterna VI–VIII with anterolateral light brown mark almost imperceptible; sternum IX with brown mark on lateral and anterior margin. Tergum IV (Fig. 5C). Terga surface covered by triangular scale-like projections and by micropores; posterior margin of tergum IV with triangular and pointed spines, in irregular sizes. Gill (Fig. 5D) apex rounded, trachea conspicuous; median length, extending to half of second subsequent tergum. Paraproct (Fig. 5E) with several marginal spines; posterolateral extension without spines. Cerci (Fig. 5F) short lateral spines on all segments. Paracercus (Fig. 5G) short lateral spines on all segments.

#### **Male imago**

**INTERNAL GENITALIA** (Fig. 5H). A pair of unistyligers cylindrical, completely separated one from another (us); sclerotized penial bridge articulated with the gonovectes and the X tergum abdominal (pb); pair of sclerotized gonovectes V-shaped, broad apex (gv) with an attached membrane on lateral parts (mb); a pair of gonovectal muscle goes from gonovectes apex to lateral parts of IX sternum (m.gv); a pair of median styligeral muscle goes from lateral parts of membrane to posterior margin of IX sternum (ms).

#### **Intraspecific variation of *Apobaetis pasternakae* sp. nov.**

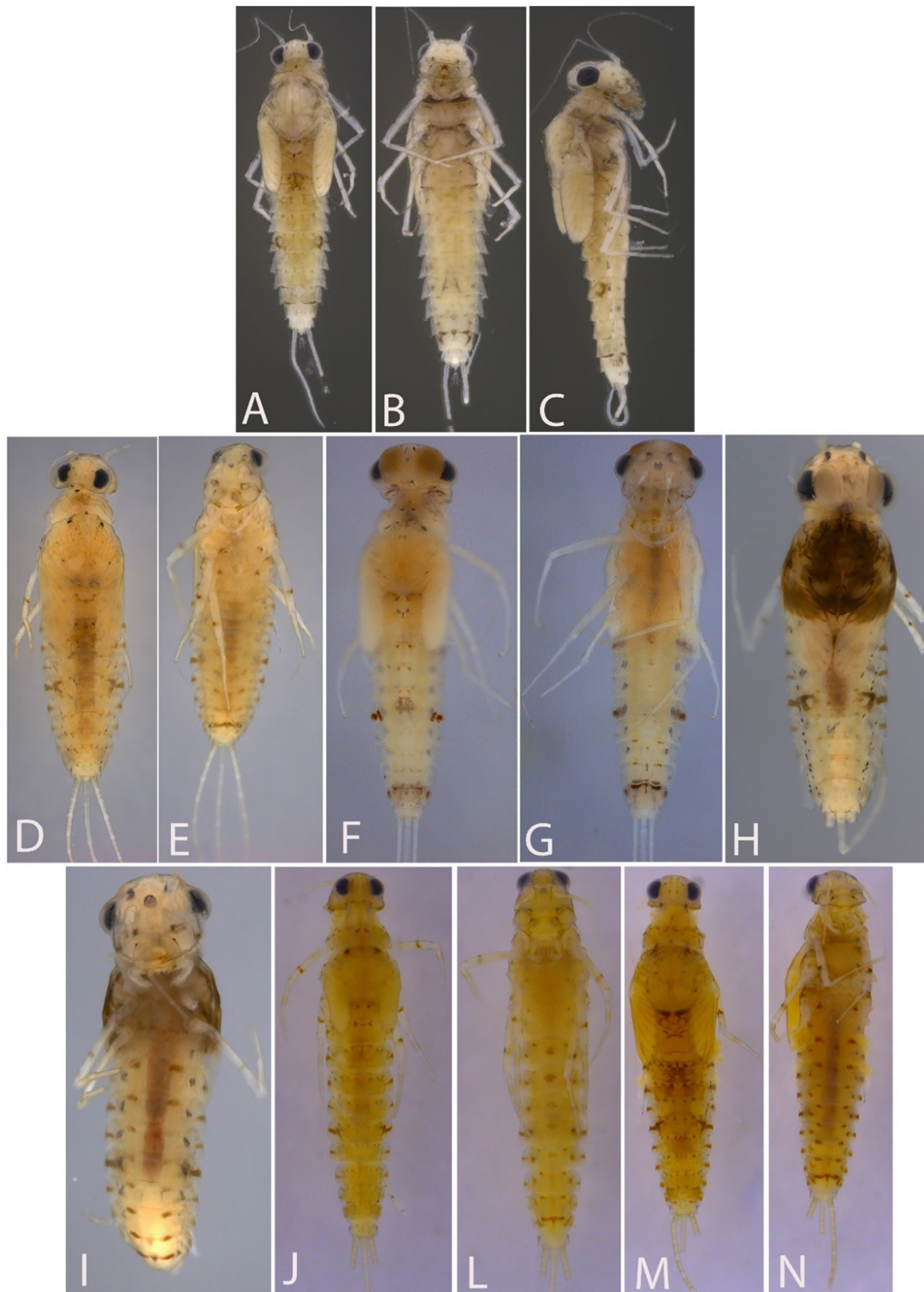
##### **Pigment variation**

**THORAX.** Light yellow, with short brown spots (Fig. 3D, F, 3J, M); brown mark covering almost completely the thorax (Fig. 3H); femur without marks (Fig. 3G); forewing pads with brown mark on basal third (Fig. 3H).

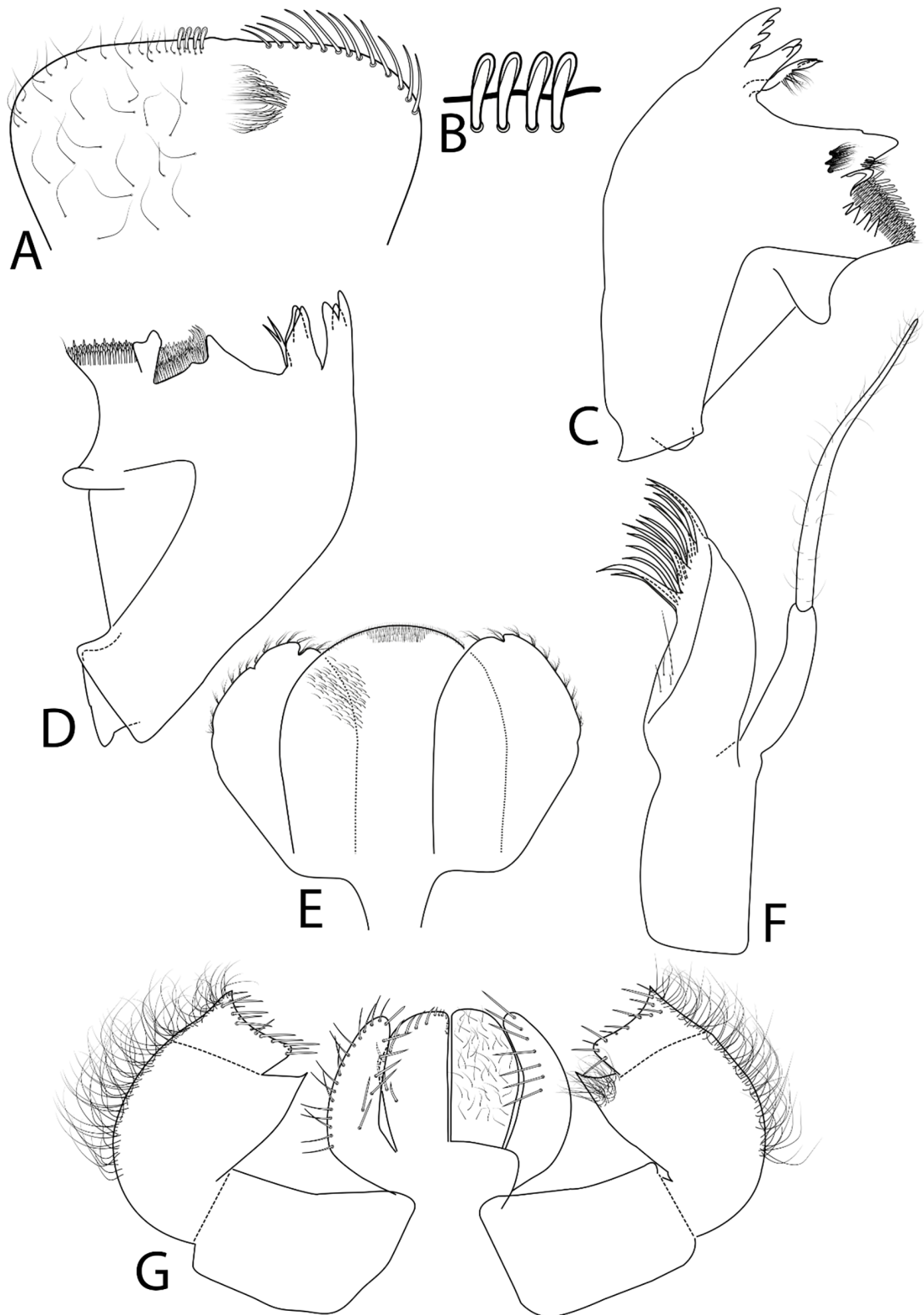
**ABDOMEN.** Terga I–III with brown spots (Fig. 3D, F); terga I–X with short light brown marks (Fig. 3J); tergum I with medial brown mark, terga II and III with brown marks, terga IV–X with light brown spots (Fig. 3M); sterna VII and VIII with anterolateral brown mark (Fig. 3G); sterna I–VIII with anterolateral brown mark, sterna I–VIII with lateral brown mark (Fig. 3L, N); cerci e paracercus with light brown trio marks, alternating over of length (Fig. 3D, J, M).

##### **Morphological variation**

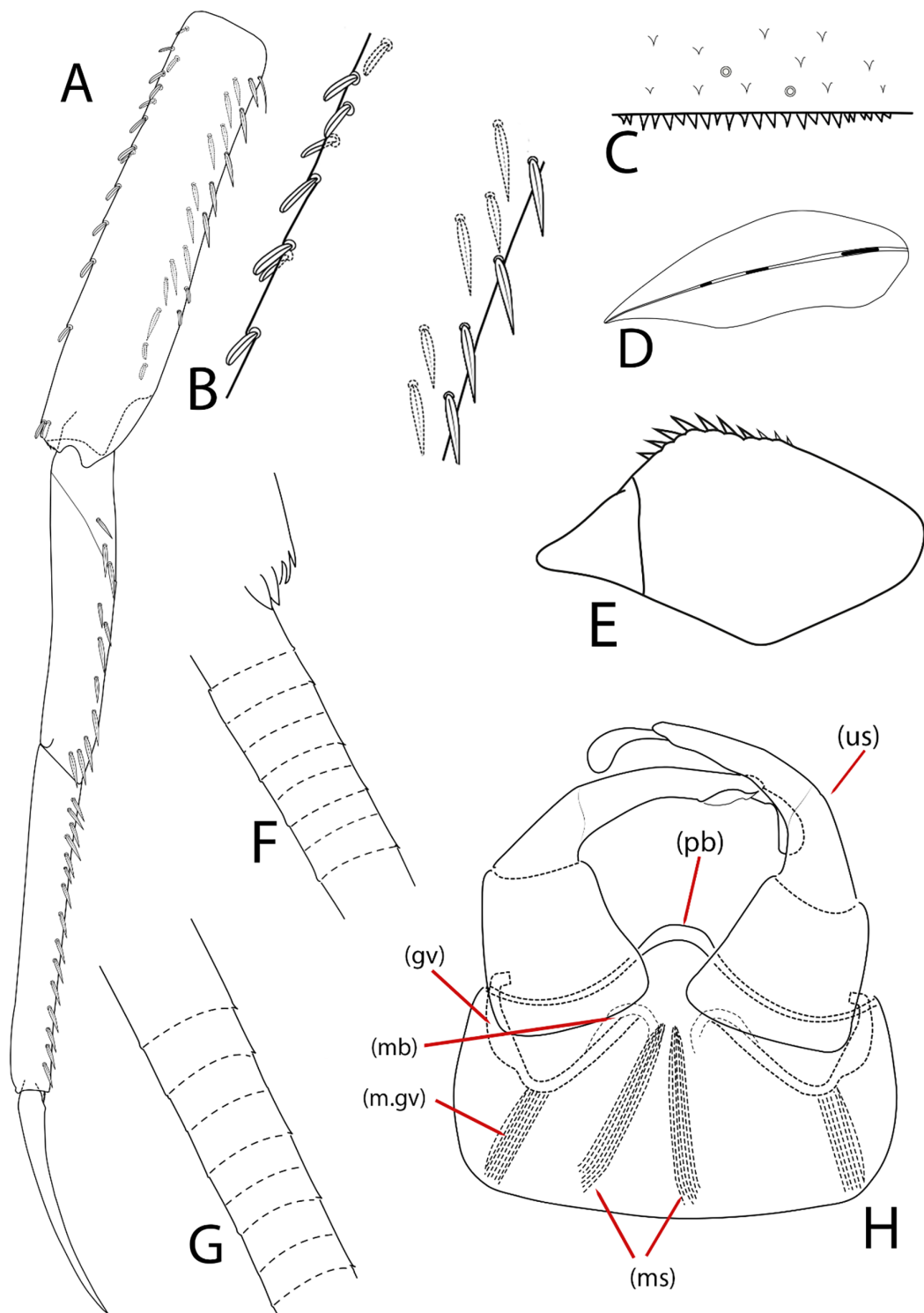
**LENGTH.** Body 2.2–3.1 mm.



**Fig. 3.** *Apobaetis pasternakae* sp. nov., habitus of nymph. **A–C.** Holotype (INPA). **A.** Nymph female (d.v.). **B.** Nymph female (v.v.). **C.** Nymph female (l.v.). **D–N.** Pigment variation in different populations. **D–I.** Amazonas State. **D.** Nymph female (d.v.). **E.** Nymph female (v.v.). **F.** Nymph male (d.v.). **G.** Nymph male (v.v.). **H.** Nymph male (d.v.). **I.** Nymph male (v.v.). **J–N.** Roraima State. **J.** Nymph female (d.v.). **L.** Nymph female (v.v.). **M.** Nymph female (d.v.). **N.** Nymph female (v.v.). Abbreviations: d.v. = dorsal view; l.v. = lateral view; v.v. = ventral view. Not to scale.



**Fig. 4.** *Apobaetis pasternakae* sp. nov., holotype (INPA), mouthparts of nymph. **A.** Labrum (left d.v., right v.v). **B.** Detail of medial spatulate setae on dorsal surface of labrum. **C.** Left mandible. **D.** Right mandible. **E.** Hypopharynx. **F.** Maxilla. **G.** Labium (left d.v., right v.v.). Abbreviations: d.v. = dorsal view; v.v. = ventral view. Not to scale.



**Fig. 5.** *Apobaetis pasternakae* sp. nov., holotype (INPA), thorax and abdomen of nymph. **A.** Foreleg (femur on anterior surface), setae on posterior surface dotted. **B.** Detail of setae on posterior surface of forefemur. **C.** Posterior margin of tergum IV. **D.** Gill. **E.** Paraproct. **F.** Cercus. **G.** Paracercus. Additional material from Cruz *et al.* 2011. **H.** Internal genitalia of male imago (v.v). Abbreviations: gv = gonovectes; mb = membrane; m.gv = gonovectes muscle; ms = styligeral muscle; pb = penial bridge; us = unistyliger; v.v. = ventral view. Not to scale.

*Apobaetis jaquelineae* sp. nov.

[urn:lsid:zoobank.org:act:806D9CCD-9AB3-402E-9D58-FA64C2C1DEF4](https://doi.org/10.21203/rs.3.rs-10000000/v1)

Figs 1, 6–8

*Apobaetis fiuzai* – Boldrini & Cruz 2014: 4.

**Diagnosis**

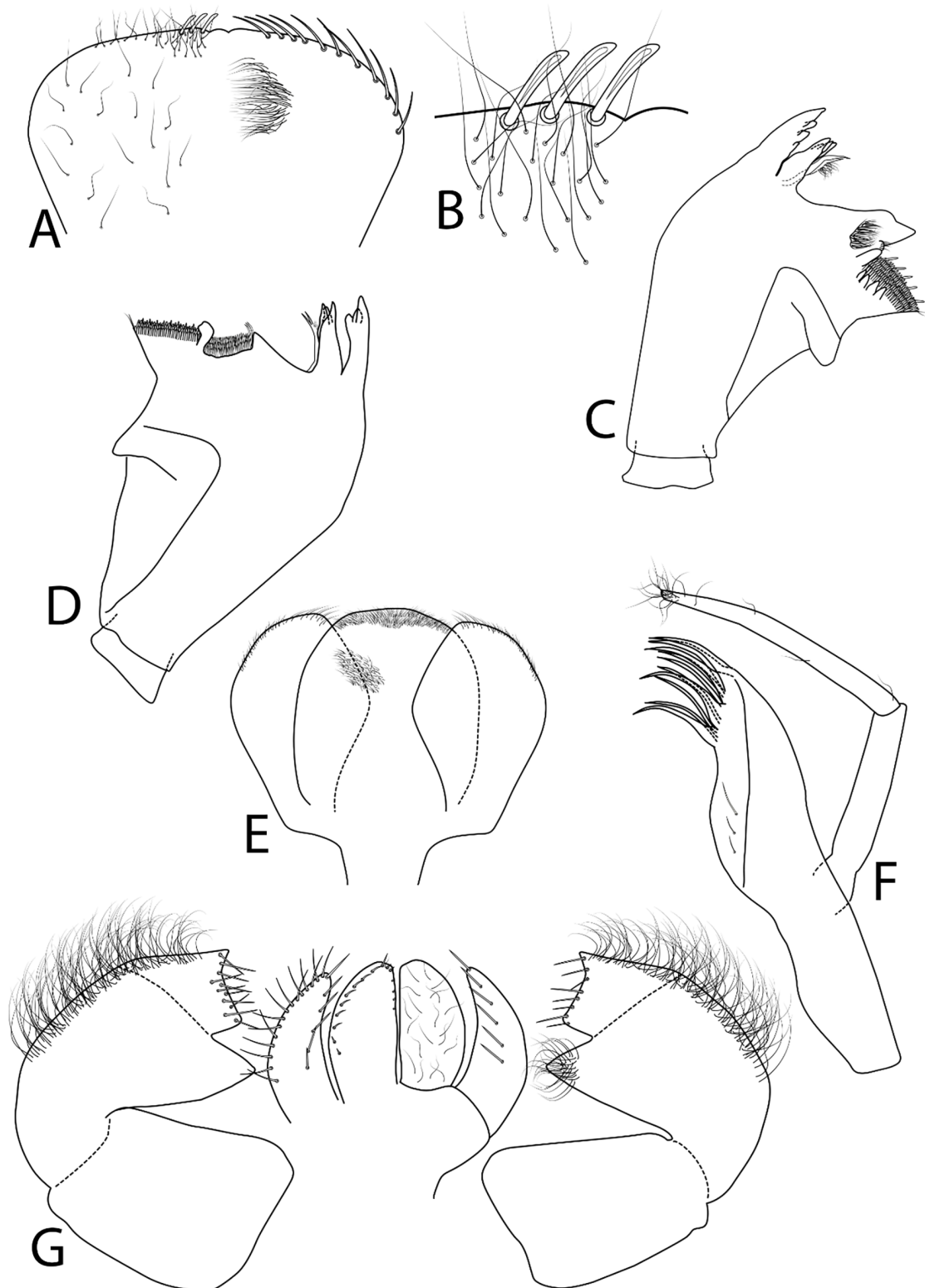
NYMPH. Characterized by a combination of the following characters: 1) labrum rectangular, distal medial margin with one protuberance; dorsal surface with 3 elongated and blunt medial setae near distal margin (Fig. 7A–B); 2) hypopharynx with lingua subcircular, with apical tuft of setae, length subequal to superlingua (Fig. 7E); 3) maxillary palp long  $1.7 \times$  length of galea-lacinia; segment II without apical constriction (Fig. 7F); 4) labial palp with segment II with triangular distomedial projection with rounded apex, laterally directed; segment III rectangular, distal margin concave (Fig. 7G); 5) foreleg with anterior surface of femur with one row of 8 to 9 short concave setae slightly pectinated on apex; apex with 2 short concave setae slightly pectinated on apex; claw  $0.6\text{--}0.7 \times$  length of tarsus, with two row of denticles restricted to middle portion (Fig. 8A–C); 6) posterior margin of tergum IV with triangular and pointed spines in regular sizes (Fig. 8D); 7) paraproct with several marginal spines, posterolateral extension with blunt spines (Fig. 8F).

**Etymology**

The species name is a tribute to Dr Jaqueline Góes, from the Instituto de Medicina Tropical de São Paulo, who was part of the team that developed and improved the viral genome sequencing protocols for the rapid sequencing of the coronavirus (SARS-CoV2).



**Fig. 6.** *Apobaetis jaquelineae* sp. nov., habitus of nymph. **A–B.** Holotype (INPA). **A.** Nymph male (d.v.). **B.** Nymph male (v.v.). **C.** Paratype (INPA), Nymph female (d.v.). Abbreviations: d.v. = dorsal view; v.v. = ventral view. Not to scale.



**Fig. 7.** *Apobaetis jaquelinae* sp. nov., holotype (INPA), mouthparts of nymph. **A.** Labrum (left d.v., right v.v). **B.** Detail of medial spatulate setae on dorsal surface of labrum. **C.** Left mandible. **D.** Right mandible. **E.** Hypopharynx. **F.** Maxilla. **G.** labium (left d.v., right v.v.). Abbreviations: d.v. = dorsal view; v.v. = ventral view. Not to scale.

## Material examined

### Holotype

BRAZIL • nymph on slide; Rondônia, Teixeiraópolis, Vale das Cachoeiras; 10°55'20.4" S, 62°22'34.7" W; 10 Jul. 2018; P.V. Cruz, N. Hamada and G. Desidério leg.; INPA.

### Paratypes

BRAZIL • 2 nymphs on slide; same collection data as for holotype; INPA.

### Additional material

BRAZIL – **Rondonia** • 1 nymph on slide; Colorado do Oeste, Rio Cabixi; 13°15'31.8" S, 60°20'04.8" W; 3 Sep. 2012; N. Hamada and R. Boldrini leg.; INPA • 1 nymph on slide; Nova Londrina, Rio Urupá; 11°02'05.8" S, 62°08'34.1" W; 9 Jul. 2018; P.V. Cruz, N. Hamada and G. Desidério leg.; INPA.

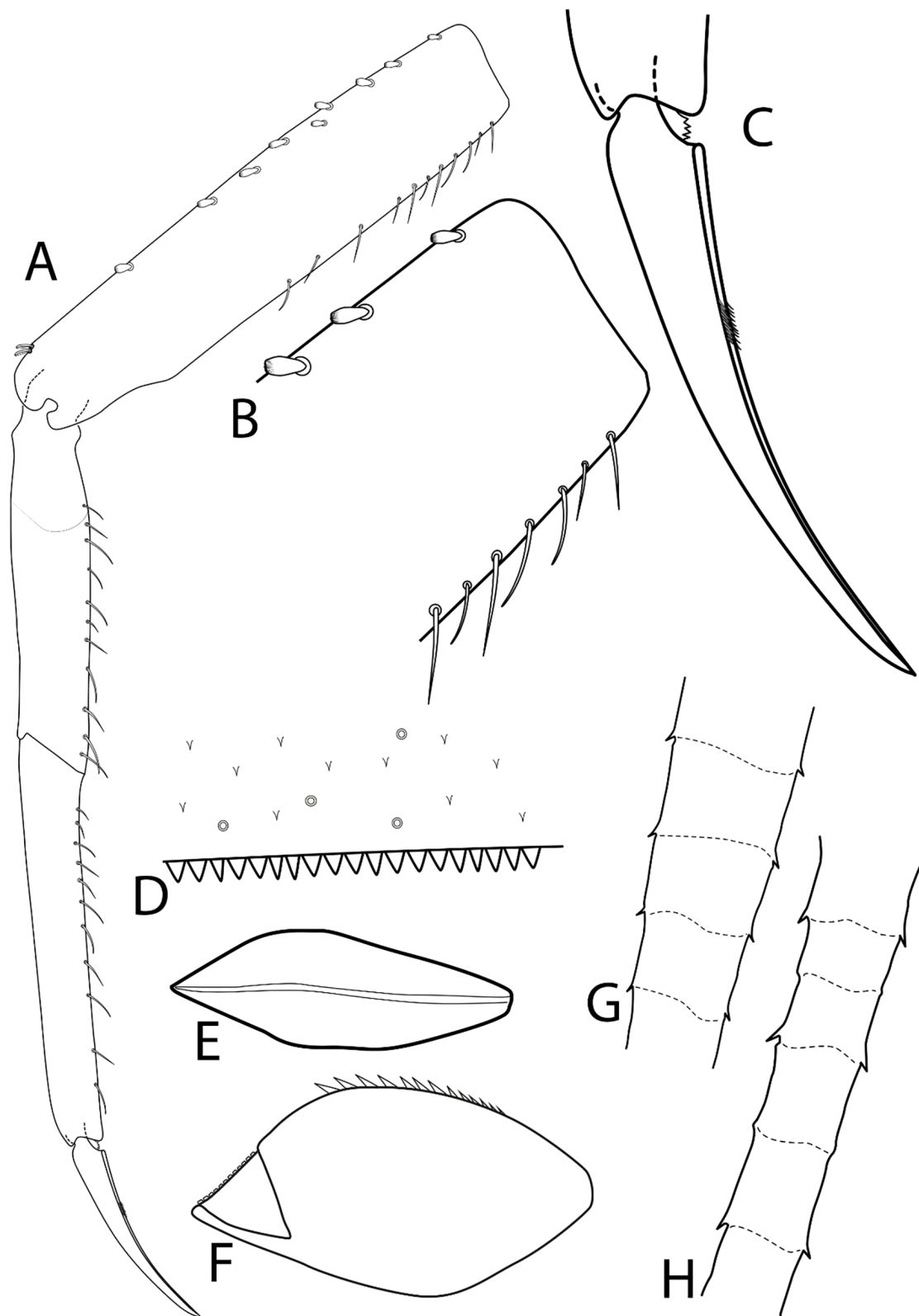
## Description

### Nymph

LENGTH. Body: 2.5–2.8 mm.

HEAD. Antenna: flagellum with minute spines on apex of each flagellomere. Labrum (Fig. 7A–B): length about  $0.6 \times$  of maximum width; distal medial margin with one protuberance, distolateral margin rounded; dorsal surface with 3 elongated and blunt medial setae near distal margin; medially with one row of long and thin setae near distal margin; long, thin and simple setae covering dorsal surface; ventral surface with one row of spine-like setae on distolateral and distal margins. Left mandible (Fig. 7C): incisors not fused; outer and inner set of incisors with 4 and 3 denticles, respectively; prosthema robust, bifurcated at apex, outer lobe robust, inner lobe slender with medial tuft of setae; margin between prosthema and mola concave; subtriangular process wide with short protuberance on distal margin; tuft of setae at base of subtriangular process; denticles of mola not constricted; lateral margin convex. Right mandible (Fig. 7D): incisors not fused; outer and inner set of incisors each with 3 denticles; prosthema slender, bifurcated at apex, tuft medial of minute setae; margin between prosthema and mola concave; tuft of setae at base of mola absent; denticles of mola not constricted; lateral margin convex. Hypopharynx (Fig. 7E): lingua subcircular, apex covered with short setae, subequal to superlingua; superlingua not expanded, with short, thin, simple setae over distolateral and distal margin. Maxilla (Fig. 7F): maxillary palp long,  $1.7 \times$  length of galea-lacinia; segment I  $0.6 \times$  length of galea-lacinia; segment II tapering slightly from base to apex, with thin and simple setae scattered on surface, without apical constriction; medial margin of galea-lacinia with 2 to 3 spine-like setae. Labium (Fig. 7G): glossa subcircular, robust, narrowing apically, subequal to paraglossa; dorsal surface with one longitudinal row of 4 short spine-like setae near inner margin; 2 short spine-like setae near internal margin, thin and simple setae and one robust spine-like setae on apex; longitudinal row of 9 robust spine-like setae on apical  $\frac{2}{3}$  near outer margin; ventral surface covered with long, thin and simple setae. Paraglossa curved inward; dorsal surface with long and robust spine-like setae on apex; longitudinal row of 15 long spine-like setae on apical  $\frac{2}{3}$  near outer margin and one longitudinal row of 5 long and robust spine-like setae on apical  $\frac{2}{3}$  near inner margin; ventral surface with one longitudinal row of 5 robust and long spine-like setae on apical  $\frac{2}{3}$ . Labial palp with segment I  $0.7 \times$  length of segments II and III combined, covered with micropores (not illustrated); segment II with outer margin covered with thin, long and simple setae, inner margin bare; segment II with triangular distomedial projection, with rounded apex, laterally directed; ventral surface of distomedial projection with thin, long and simple tuft of setae; segment III rectangular, with concave distal margin, length  $0.6 \times$  width, covered with thin, long and simple setae on outer margin, dorsally with one row of 11 to 13 spine-like setae in different sizes near distal margin, ventrally with 8 to 10 spine-like setae in different sizes near distal margin.





**Fig. 8.** *Apobaetis jaquelineae* sp. nov., holotype (INPA), thorax and abdomen of nymph. **A.** Foreleg (femur on anterior surface). **B.** Detail of setae on anterior surface of forefemur. **C.** Claw. **D.** Posterior margin of tergum IV. **E.** Gill. **F.** Paraproct. **G.** Cercus. **H.** paracercus. Not to scale.

THORAX. Holotype pigment (Fig. 6A–B): light yellow, with brown marks; forewing pads with basal brown mark. Paratype pigment, nymph female (Fig. 6C): light yellow, with lateral brown mark. Foreleg (Fig. 8A–C). Femur: anterior surface with one row with 8 to 9 short concave setae slightly pectinated on apex near dorsal margin; apex of femur with 2 short concave setae slightly pectinated on apex; ventral margin with one row of 10 to 12 spine-like setae of different sizes. Tibia: dorsal margin bare; ventral margin with one row of 10 to 11 spine-like setae. Patella-tibial suture present, from dorsal margin to ventral margin. Tarsus: dorsal margin bare; ventral margin with one row of 11 to 12 spine-like setae. Claw:  $0.6\text{--}0.7 \times$  length of tarsus, with two rows of denticles restricted to middle portion. Middle and hindleg similar to foreleg.

ABDOMEN. Holotype pigment, nymph male (Fig. 6A–B): tergum V with lateral brown mark; posterior margin of tergum V with medial brown mark and sublateral brown spots; tergum VI with central brown mark; anterior margin of tergum VII with medial brown mark and short spots; terga VIII and IX with lateral brown mark and short spots; sterna II–VII with anterolateral brown mark and with lateral brown mark; sternum VIII brown; sternum IX with anterior margin brown mark. Paratype pigment. Nymph female (Fig. 6C). Tergum II with medial brown mark; terga III and V with lateral brown mark; terga II–IV covered by reddish brown pigmentation. Tergum IV (Fig. 8D): surface covered by triangular scale-like projections and micropores; posterior margin with triangular and pointed spines, in regular sizes. Gill (Fig. 8E): triangular apex, trachea conspicuous; long length, extending to half of third subsequent tergum. Paraproct (Fig. 8F) with several marginal spines. Posterolateral extension with blunt spines. Cerci (Fig. 8G) with spines in all segments. Paracercus (Fig. 8H) with prominent spines in all segments.

*Apobaetis luanae* sp. nov.

[urn:lsid:zoobank.org:act:4DAD1B52-E7CD-42A1-81D5-488EF898A98D](https://doi.org/10.3896/abris.4DAD1B52-E7CD-42A1-81D5-488EF898A98D)

Figs 1, 9–11

*Apobaetis kallawayi* – Boldrini & Cruz 2014: 4.

**Diagnosis**

NYMPH. Characterized by a combination of the following characters: 1) labrum rectangular with rounded distolateral margins, distal medial margin with three protuberances; dorsal surface with 4 short and simple medial spine-like setae near distal margin; ventral surface with short medial spine-like setae near distal margin (Fig. 10A, C); 2) hypopharynx with lingua subquadrangular, elongated, with apical tuft of setae, subequal in length to superlingua (Fig. 10G); 3) maxillary palp long  $2.0 \times$  length of galea-lacinia; segment II without apical constriction (Fig. 10H); 4) labial palp with segment II with robust triangular distomedial projection, apically rounded, laterally directed; segment III triangular (Fig. 10I); 5) foreleg with anterior surface of femur with one row of 4 to 5 minute blunt setae, on basal half, near dorsal margin; claw I  $0.6 \times$  length of tarsus I, with two row of denticles on basal third; 6) posterior margin of tergum IV with triangular spines, wider than long (Fig. 11D); 7) paraproct with several marginal spines, posterolateral extension with minute spines (Fig. 11F).

**Etymology**

The species name is a tribute to Dr Luana Araújo. She spoke out vehemently against the ineffective drugs used to treat COVID-19 in the testimony to the CPI organized by the Brazilian Federal Senate in 2021.

**Material examined**

**Holotype**

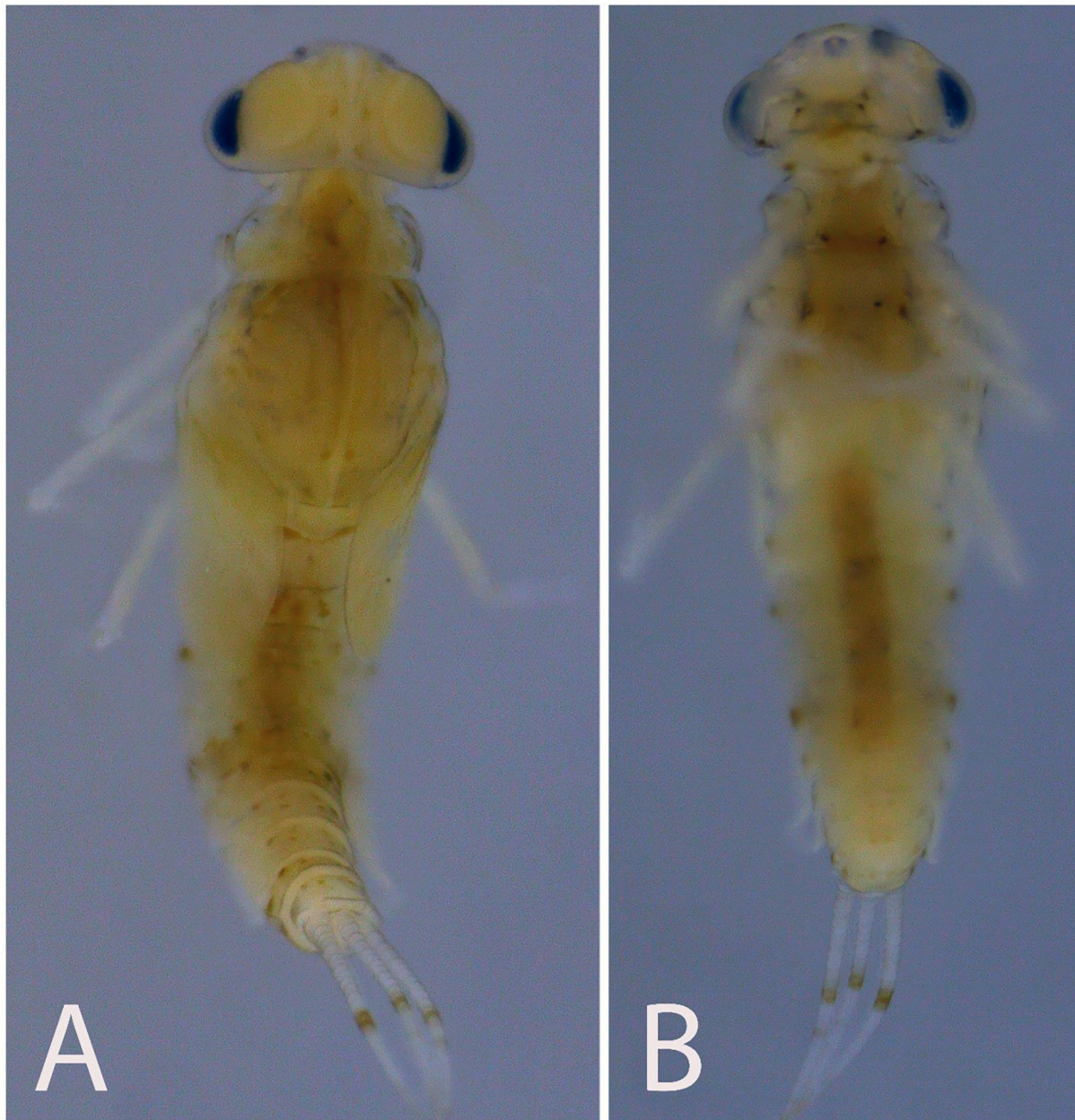
BRAZIL • nymph on slide; Roraima, Amajari, Rio Ereú;  $04^{\circ}02'02.9''$  N,  $61^{\circ}23'09.5''$  W; 26 Mar. 2012; N. Hamada, P.V. Cruz, G. Dantas and R. Boldrini leg.; INPA.

**Paratypes**

BRAZIL – **Roraima** • 2 nymphs on slide; same collection data as for holotype; INPA • 1 nymph in alcohol 80%; same collection data as for holotype; INPA.

**Additional material**

BRAZIL – **Rondônia** • 1 nymph on slide; Teixeiraópolis, Vale das Cachoeiras; 10°55'20.4" S, 62°22'34.7" W; 3 Sep. 2012; R. Boldrini, A.S. Fernandes and N. Hamada leg.; INPA • 1 nymph on slide; same collection data as for preceding, except date 10 Jul. 2018; INPA.



**Fig. 9.** *Apobaetis luanae* sp. nov., holotype (INPA), habitus of nymph. **A.** Nymph male (d.v.). **B.** Nymph male (v.v.). Abbreviations: d.v. = dorsal view; v.v. = ventral view. Not to scale.

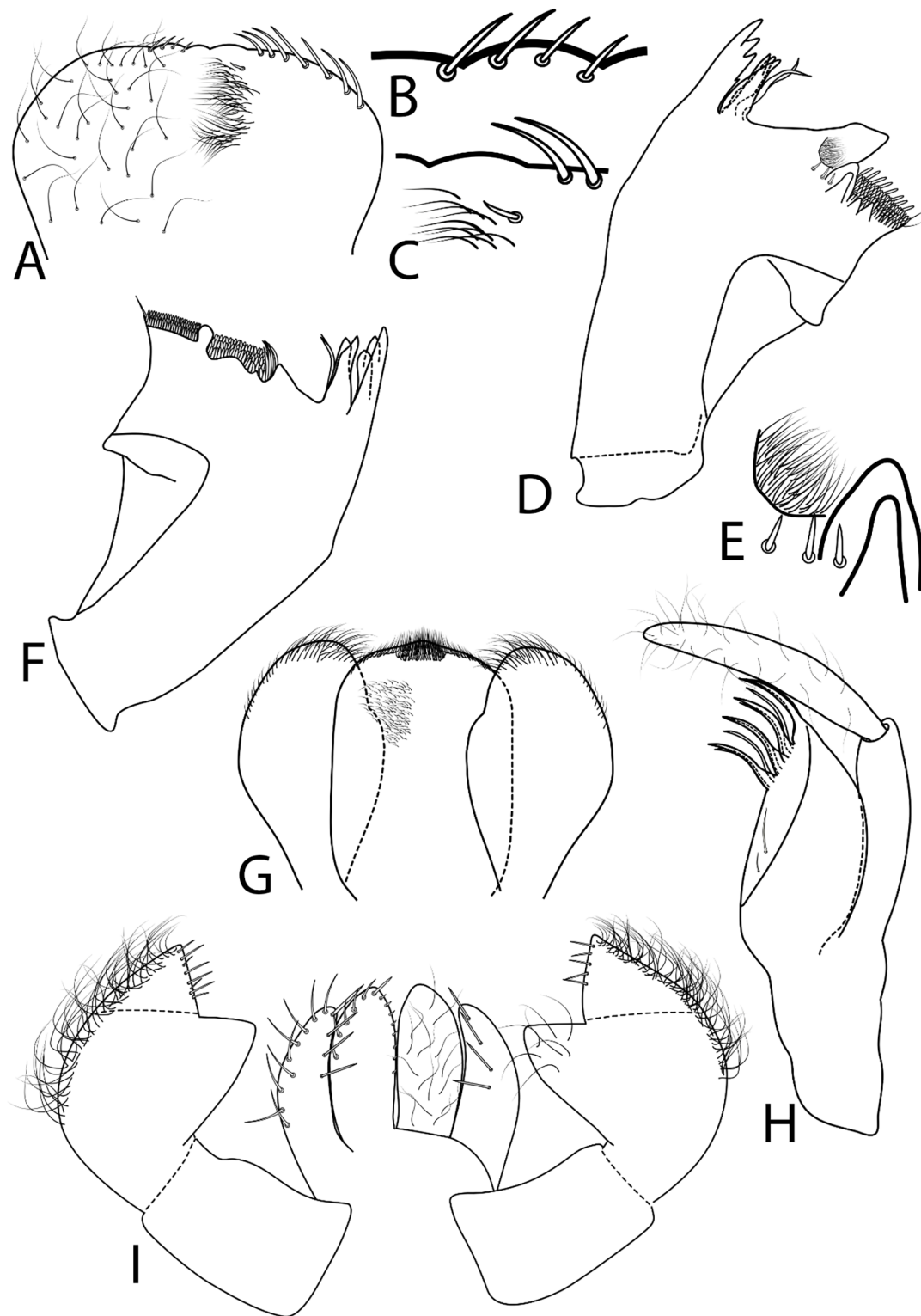
**Description****Nymph**

LENGTH. Body: 3.0–3.3 mm.

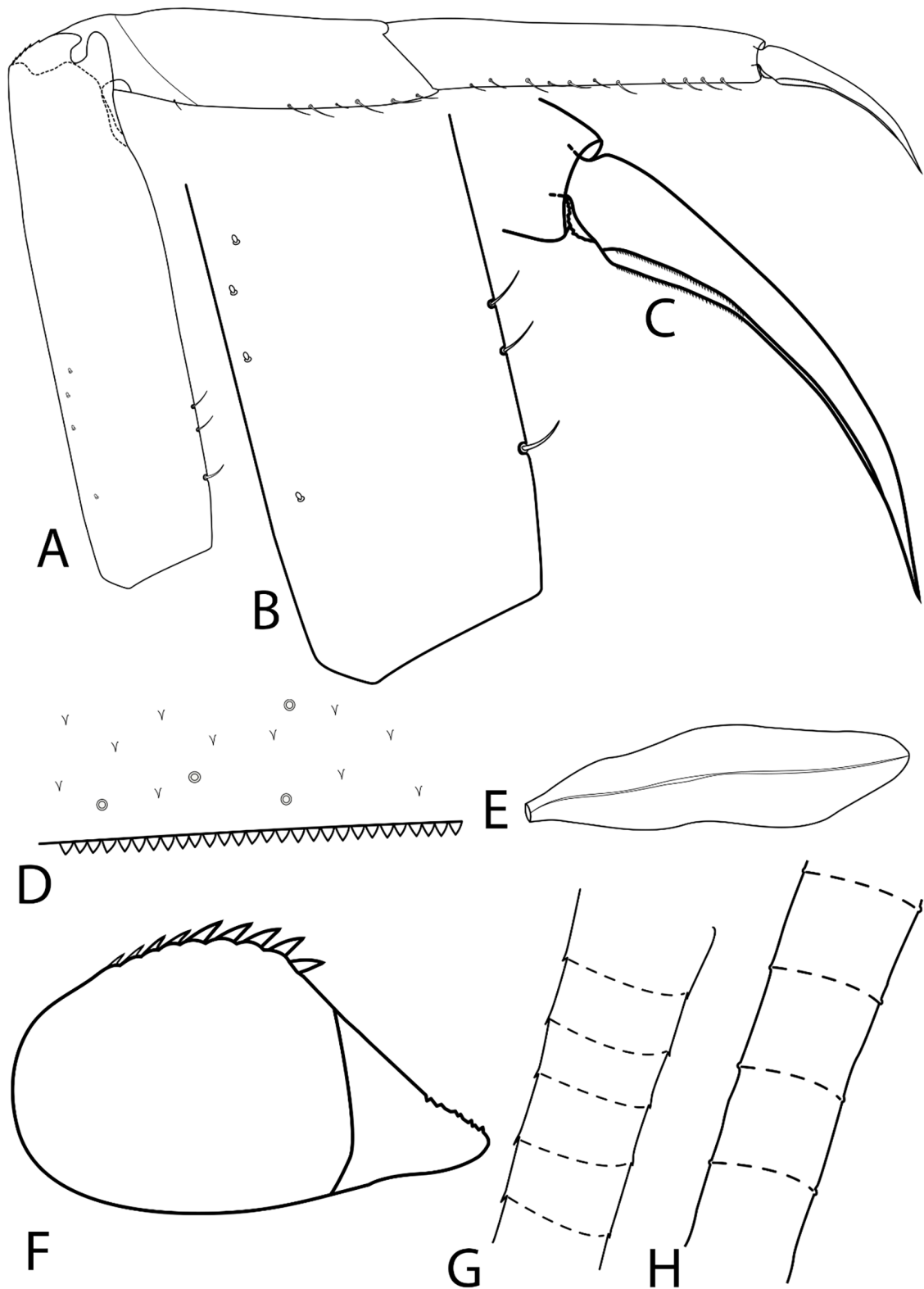
HEAD. Antenna: flagellum with minute spines on apex of each flagellomere. Labrum (Fig. 10A–C): length  $0.6 \times$  maximum width; rounded distolateral margins; distal medial margin with three protuberances; dorsal surface with 4 short and simple medial spine-like setae near distal margin, with one row of long, thin and simple setae near distal margin; dorsal surface covered with long, thin and simple setae; ventral surface with one row of robust spine-like setae near distolateral and distal margins; short medial spine-like setae near distal margin. Left mandible (Fig. 10D–E): incisors not fused; outer and inner set of incisors with 4 and 3 denticles, respectively; prostheca slender, bifurcated at apical middle; margin between prostheca and mola concave; subtriangular process wide with small protuberance on distal margin and 3 short spine-like setae at base; tuft of setae at base of subtriangular process; denticles of mola not constricted, with 4 prominent denticles in irregular sizes; lateral margin convex. Right mandible (Fig. 10F): incisors not fused; outer and inner set of incisors with 3 and 2 denticles, respectively; prostheca slender, bifurcated at apex; margin between prostheca and mola concave; tuft of setae at base of mola absent; denticles of mola not constricted; lateral margin convex. Hypopharynx (Fig. 10G): lingua subquadrangular, longitudinally elongated, apex covered with short setae, subequal to superlingua; superlingua oval, longitudinally elongated, with thin setae of different sizes on distolateral and distal margins. Maxilla (Fig. 10H): maxillary palp long  $2.0 \times$  length of galea-lacinia; segment I subequal to galea-lacinia; segment II with narrow base and apex, with thin and simple setae scattered on surface, without apical constriction; medial margin of galea-lacinia with 2 spine-like setae. Labium (Fig. 10I): glossa subtriangular, longer than paraglossa; dorsal surface with one longitudinal row of 8 short spine-like setae on apical  $\frac{2}{3}$  near inner margin; 1 robust spine-like seta on apex; longitudinal row of 5 robust spine-like setae on apical middle near outer margin; ventral surface covered with long, thin and simple setae. Paraglossa curved inward; dorsal surface with 1 long and robust spine-like seta on apex; longitudinal row of 9 long spine-like setae on apical  $\frac{2}{3}$  near outer margin and longitudinal row of 3 long and robust spine-like setae on apical middle near inner margin. Labial palp with segment I  $0.6 \times$  length of segments II and III combined, covered with micropores (not illustrated); segment II with outer margin covered by thin, long and simple setae, inner margin bare; segment II with robust triangular distomedial projection, apically rounded, laterally directed; ventral surface of distomedial projection with tuft thin, long and simple setae; segment III triangular, length subequal to width, covered by thin, long and simple setae on outer margin, dorsally with one row of 8 robust spine-like setae near inner margin, ventrally with one row of 5 robust spine-like setae in near distal margin.

THORAX. Holotype pigment (Fig. 9A–B): light yellow; femur without mark. Legs (Fig. 10A–C). Femur: anterior surface with one row of 4 to 5 minute blunt setae, on basal half, near dorsal margin; ventral margin with one row of 3 to 4 elongated spine-like setae on basal third. Tibia: dorsal margin bare; ventral margin with one row of 6 to 7 spine-like setae. Patella-tibial suture present, from dorsal margin to ventral margin. Tarsus: dorsal margin bare; ventral margin with one row of 10 to 11 spine-like setae; tarsus I  $1.2 \times$  length of tibia I; tarsi II and III length subequal to tibiae II and III. Claws: two rows of denticles on basal third; claw I  $0.6 \times$  length of tarsus I; claws II and III  $0.7 \times$  length of tarsi II and III.

ABDOMEN. Holotype pigment (Fig. 9A–B): terga II–X covered by light brown marks; cerci and paracercus with medial brown mark. Tergum IV (Fig. 11D): surface covered by triangular scale-like projections and micropores; posterior margin with triangular spines, wider than long. Gills (Fig. 11E): rounded apex, simple trachea without branches; long length, extending to half third subsequent tergum. Paraproct (Fig. 11F) with several marginal spines; posterolateral extension with minute spines. Cerci (Fig. 11G) with short spines on all segments; medial brown mark. Paracercus (Fig. 11H) without spines; medial brown mark.



**Fig. 10.** *Apobaetis luanae* sp. nov., holotype (INPA), mouthparts of nymph. **A.** Labrum (left d.v., right v.v). **B.** Detail of simple medial spine-like setae on dorsal surface of labrum. **C.** Detail of short medial spine-like setae on ventral surface of labrum. **D.** Left mandible. **E.** Detail of setae on process subtriangular base of left mandible. **F.** Right mandible. **G.** Hypopharynx. **H.** Maxilla. **I.** Labium (left d.v., right v.v.). Abbreviations: d.v. = dorsal view; v.v. = ventral view. Not to scale.



**Fig. 11.** *Apobaetis luanae* sp. nov., holotype (INPA), thorax and abdomen of nymph. **A.** Foreleg (femur on anterior surface). **B.** Detail of setae on anterior surface of forefemur. **C.** Claw. **D.** Posterior margin of tergum IV. **E.** Gill. **F.** Paraproct. **G.** Cercus. **H.** Paracercus. Not to scale.

## Discussion

The three new species here recognized corroborate the argument of Cruz (2020), and our hypothesis, that the broad diagnoses of *A. fuzai* and *A. kallawaya* result in the assignment of different species to a single name. In view of this perspective, the specimens assigned to *A. fuzai* that could not be reviewed in this study are treated as putative (Salles *et al.* 2003, 2004, 2010; Nieto 2006; Boldrini *et al.* 2012; Lima *et al.* 2012), and should be evaluated in the light of future evidence.

The distribution of *A. fuzai* after correction does not include the Amazon biome. Its previous records in this biome are recognized here as *A. pasternakae* sp. nov. and *A. jaquelinae* sp. nov. *Apobaetis pasternakae* sp. nov. is widespread in the Amazon biome, while *A. jaquelinae* is restricted of an ecotone area between Amazon and Cerrado (Rondônia). *Apobaetis luanae* sp. nov. presents a disruptive distribution, with records in two patches of Amazonian savanna in the north (Roraima) and south (Rondônia).

*Apobaetis pasternakae* sp. nov. can be differentiated from *A. fuzai* by a hypopharynx, with a subcircular lingua (Fig. 4E) while *A. fuzai* has a subquadrangular lingua with a medial protuberance (Cruz 2020: fig. 4d); a long maxillary palp, longer than or equal to  $2.0 \times$  the length of the galea-lacinia and segment II tapering abruptly from the apical half to the apex (Fig. 4F), while in *A. fuzai* the maxillary palp is  $1.5 \times$  the length of the galea-lacinia and the segment II is robust (Cruz 2020: fig. 4e); a patella-tibial suture is present from the dorsal margin to the ventral margin (Fig. 5A), while in *A. fuzai* it is restricted to the ventral margin (Cruz 2020: fig. 5a); the paraproct has no spines on the posterolateral extension (Fig. 5E), while in *A. fuzai* the posterolateral extension of the paraproct has several pointed spines (Cruz 2020: fig. 5c); the paracercus has short posterolateral spines on all segments (Fig. 5G), while in *A. fuzai* the paracercus has no spines.

The recognition of the species *A. pasternakae* sp. nov. makes the adult male of *A. fuzai* unknown. The internal genitalia of the male imago are described for the first time in the genus. The V-shaped arcuate gonovectes (Fig. 5H) corroborate the genus in the subfamily Baetinae Leach, 1815 or Baetovectata Kluge & Novikova, 2011 (or Baetis/fg6) (Kluge & Novikova 2011; Cruz *et al.* 2021). Unfortunately, as there are no other species with described internal genitalia, it is not possible to determine whether the traits can be used for specific, group, or generic definitions.

*Apobaetis jaquelinae* sp. nov. can be differentiated from *A. fuzai* by having a labial palp with segment II with a triangular distomedial projection with a rounded apex (Fig. 7G), while in *A. fuzai* the distomedial projection of segment II is triangular with a pointed apex (Cruz 2020: fig. 4f); the anterior surface of the femur has one row of 7 to 8 slightly pectinated setae on the apex (Fig. 8A–B), while in *A. fuzai* the setae of the femur have a rounded apex (Cruz 2020: fig. 5a); a claw with two rows of denticles restricted to the medial portion is present (Fig. 8C), while denticles are absent in *A. fuzai* (Cruz 2020: fig. 5a); the spines of the posterolateral extension of the paraproct have a blunt apex (Fig. 8F), while in *A. fuzai* the spines of the posterolateral extension of the paraproct have a pointed apex (Cruz 2020: fig. 5c).

*Apobaetis luanae* sp. nov. is the correction of the record assigned to *A. kallawaya* from Rondônia, in the north of Brazil (Boldrini & Cruz 2014). *Apobaetis luanae* can be differentiated from *A. kallawaya* by the labrum, the simple medial spine-like setae near the distal margin on the dorsal surface (Fig. 10A–B), while in *A. kallawaya* the medial spine-like setae near the distal margin on the dorsal surface are bifid (Nieto 2006: fig. 19); one short spine-like seta medially on the ventral surface near the distal margin of the labrum (Fig. 10C), while this structure is absent in *A. kallawaya*; one row of minute blunt setae on the anterior surface on the basal half of the femur near the dorsal margin (Fig. 10A–B), this row is absent in *A. kallawaya*; the paraproct has marginal pointed spines (Fig. 11F), while in *A. kallawaya* the paraproct has marginal spines with a rounded apex (Nieto 2006: fig. 30); the posterior margin of tergum IV has triangular spines (Fig. 11D), rounded in *A. kallawaya* (Nieto 2006: fig. 28).

The analysis of the intraspecific variation clarifies that the diagnostic characteristics used in the specific identification (mouthparts, legs, tergite spines, paraprot, gills, cerci and paracercus) show minimal variation, thus remaining the most reliable for species identification. The small variation in claw size (vs tarsi) is probably related to the instar. On the other hand, the nymph's pigmentation showed wide intraspecific variation, with more than one species expressing the same characteristics. The most notable is the lateral mark on tergum V, observed in *A. fuzai*, *A. pasternakae* sp. nov. and *A. jaquelineae* sp. nov. The combination of variable elements within species are therefore uninformative for identification (thus ignored), while stable elements between species may have been a facilitator to assign the same name to morphologically distinct populations (here treated as new species). Probably, intraspecific pigmentation variation is related to the feeding and age of the nymph (Cruz P.V. pers. obs.). Younger nymphs, identified by the stage of development of the wing pads, were generally lighter in color, with fewer spots and marks on the terga and sterna. Therefore, the pigment pattern of the nymphs was not considered a robust characteristic for specific identification.

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