Zootaxa 5196 (2): 265–279
https://www.mapress.com/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5196.2.7
http://zoobank.org/urn:lsid:zoobank.org:pub:299E1322-6A5E-4351-95F9-ADD7A9CD10B4

Two new species of small minnow mayfly (Ephemeroptera: Baetidae) from a mine-tailing dam disaster area in Minas Gerais, Brazil

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Abstract

After the worst mine-tailing dam disaster which occurred in Minas Gerais state, Brazil, it was necessary to understand the extent of the biodiversity loss. Thus, based on legal obligations, a monitoring of the fauna and flora along the Rio Doce basin was developed. From this large study, surprisingly, two undescribed psammophilous mayfly species were collected. Given the environmental disaster, and the particular concern with species that inhabiting sandy bottom, the two new species may be already threatened. Considering the reported circumstances, our objective is to described the new species and carry out their extinction risk assessment following the IUCN protocol. *Apobaetis irai* **sp. nov**. can be distinguished by the size and shape of the setae in the distal middle area of the dorsal surface of the labrum, with 3 minutes, blunt spatulate setae and the shape of the labial palp; is likely to be threated, plausibly eligible, at least, as Vulnerable (VU) B2ab(iii)+D2. *Rivudiva watu* **sp. nov**., can be distinguished by the distal shape of glossae, absence of row of setae on ventral margin on the hind tibiae and hypopharynx without distomedial projection; given its distribution, it was not directly impacted by the disaster, however, there is not enough data to accurately estimate the extent of its occurrence (EOO) and area of occupancy (AOO), therefore, it may be eligible for Data Deficient (DD).

Key words: Aquatic insect, mining impacted area, psammophilous, human activities, Rio Doce

Introduction

The Rio Doce basin belongs to the Southeast Atlantic watershed and it is located between the states of Minas Gerais and Espírito Santo. It is the fifth largest basin in Brazil, covering a drainage area of approximately 83,400 km^2 (Coelho 2009). For decades, the basin has been impacted by industrial and agricultural and the dumping of domestic sewage causing the loss of potability of water resources in the basin and adjacent areas, initiating major impacts on the entire aquatic biota (Vieira 2009). In 2015, the Rio Doce basin was severely impacted by iron ore tailings after the Fundão dam collapse, releasing approximately 39.2 billion m^3 of tailings, contaminating several rivers and streams, and soil (Renova Foundation 2016). It is considered one of the biggest environmental disasters in Brazil, causing irreparable damage such as silting of rivers, destruction of riparian vegetation, burying backwaters, puddles, lagoons, mangroves and springs (CEPTA 2015; Lopes 2016), in addition to interrupting the gene flow of species, affecting the entire food chain.

In view of the catastrophic impacts, fauna and flora monitoring was carried out along the Rio Doce to comply with IBAMA's Notification 678322-E and clause 168 of the Transaction Agreement and Conduct Adjustment (TTAC), which establishes programs for the development of plans and procedures in response to the environmental impacts

Accepted by T. Tiunova: 28 Sept. 2022; published: 19 Oct. 2022

resulting from the dam rupture, through the new private and non-profit foundation, the Fundação Renova. From this large study, a total of 63 Ephemeroptera species were recorded and, surprisingly, two undescribed psammophilous species were collected.

Psammophilous mayflies are of particular concern when assessing extinction risk. They are highly specialized, have low population density, low dispersion capacity, limiting their distribution area (Jacobus 2013) (e.g. *Apobaetis, Rivudiva*, *Homoeoneuria*). The sandy habitat is remarkably sensitive due to instability, ranging from non-cohesive to often compacted, immersed in mud and subject to direct impact by silting. This uniqueness of the psammophilous species makes each population unique.

The psammophilous mayflies are also considered one of the most neglected (Cruz & De-Souza 2014; Jacobus 2013; McCafferty 1991). Many species are small, camouflaging themselves in the sand, requiring a lot of time and field effort to collect few specimens (e.g., Cruz & De-Souza 2014). All things considered, the new species may already be threatened (see Lees & Pimm 2015) even before its description.

Considering the reported circumstances, and that the newly discovered species are often threatened with extinction, but in many cases have a limited conservation effort (Liu *et al.* 2022), the aim of this study is to describe two new species *Apobaetis irai* **sp. nov**. and *Rivudiva watu* **sp. nov**., of the Baetidae family, and carry out its extinction risk assessment following the IUCN protocol (IUCN 2012; 2019).

Material and methods

Studied area and Sampling sites

The Rio Doce basin is located between the states of Minas Gerais and Espírito Santo, in the Atlantic Forest biome. It has a drainage area of approximately 83,400 km², with the Rio Doce stretching for 879 km² (Fig. 1).

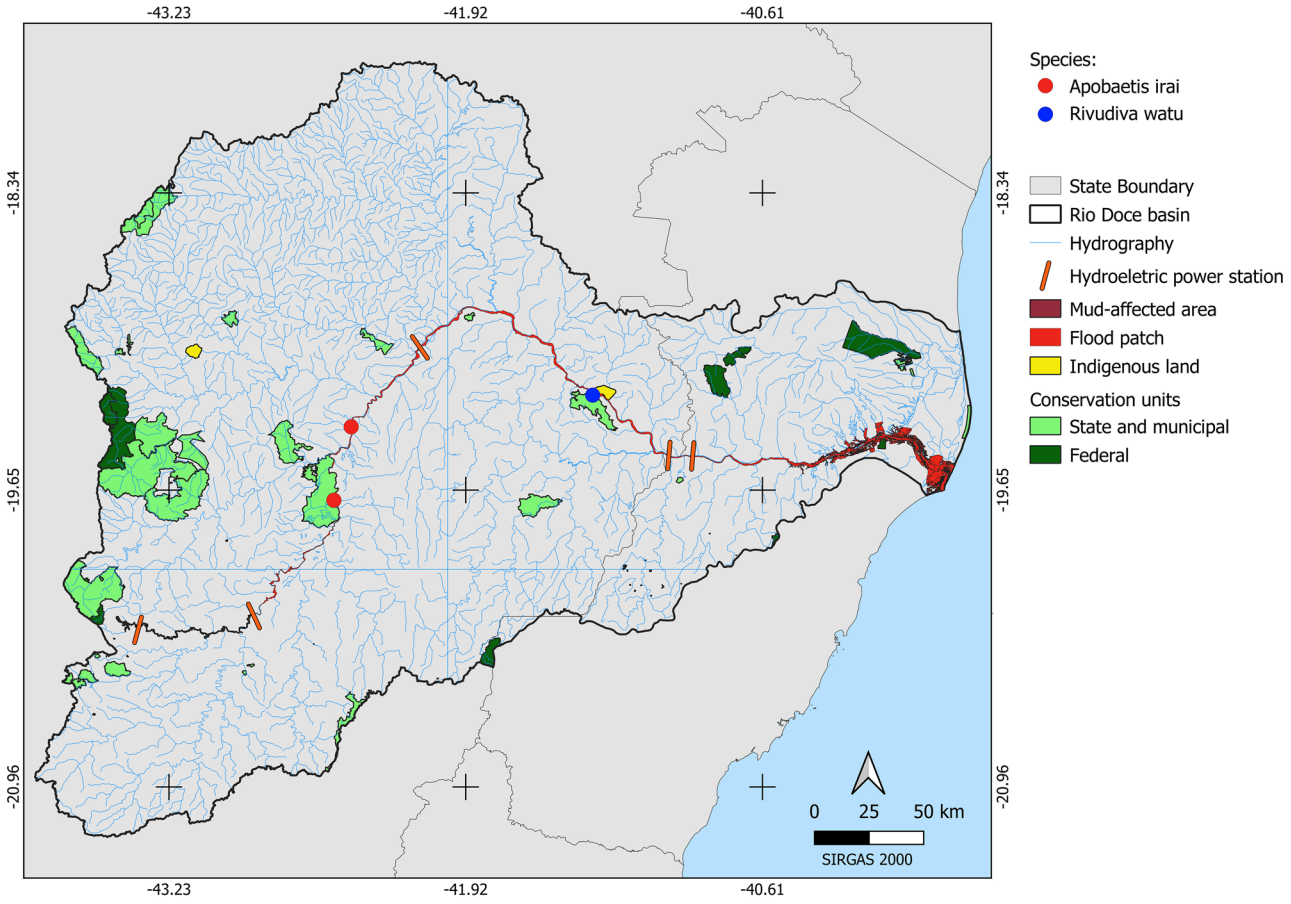

FIGURE 1. Map of species distribution in Rio Doce basin. Red circle *Apobaetis irai* **sp. nov**.; Blue circle *Rivudiva watu* **sp. nov**.

Apobaetis irai **sp. nov.** was collected in the sandbanks and/or bottom of Rio Doce **(**Figs. 1; 2 A, B); *Rivudiva watu* **sp. nov.** was collected in sand bottom in a small stream 180 meters from Rio Doce, between two protected areas, Parque Estadual Sete Salões e Indigenous territory Krenak **(**Figs. 1; 2 C, D).

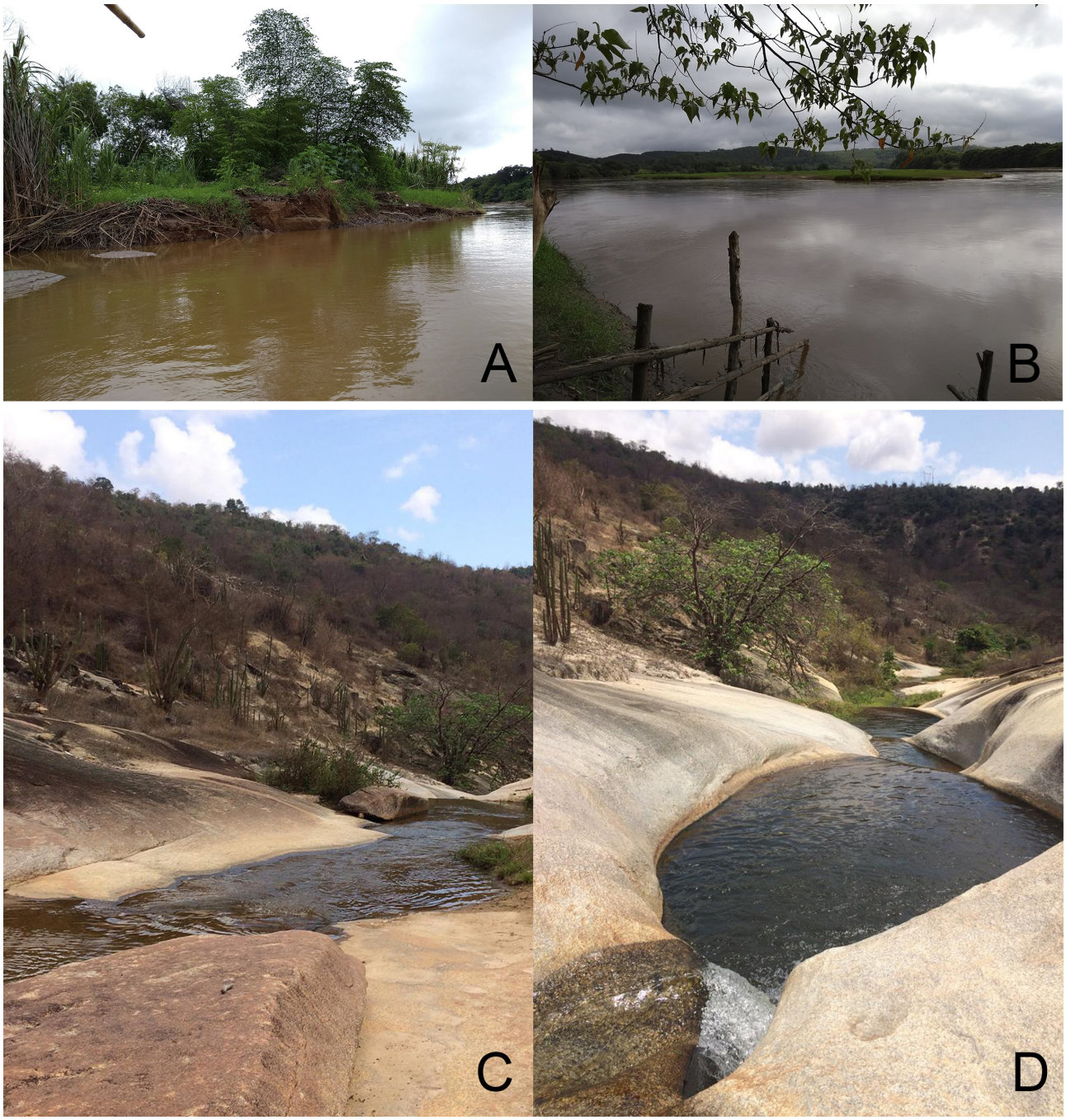

FIGURE 2. Sample sites. A and B sample sites of *Apobaetis irai* **sp. nov**.; C and D sample sites of *Rivudiva watu* **sp. nov**.

Sampling procedures

The immature collections were performed in 50-m stretches of river. In each section ten samples were collected with a D-shaped net (mesh opening less than 1mm) in which a sweeping of the shadow area of net was performed. The sampling distribution in each stretch was made according to substrate availability, such as macrophytes, bottom litter, riffle, litter, stone, sand, marginal vegetation, root, and gravel. All samples collected were fixed in 80% ethanol.

Specimens, description, and illustration

The morphological characteristics of the specimens were used as species delimitation criteria. Nymphs were

mounted on slides with Euparal® as mounting medium; part of the samples was preserved in 80% ethanol. The descriptions and measurements were based on the standardized protocol proposed by Hubbard (1995). Multi-layer digital photographs of the examined specimens were taken using a Leica (M165C) microscope with a DFC420 digital camera and LED dome lighting for uniform reflection of light on the specimens (Kawada & Buffington 2016). The final images were generated using Leica Application Suite ver. 3.8.0 software. Final illustrations were prepared according to Coleman (2006).

Abbreviations in figures
d.v. = dorsal view
v.v. = ventral view
l.v. = lateral view

Institutional abbreviations
Type specimens are deposited in:
INPA = Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil.

Conservation status
The assessment of the risk of extinction for the two new described species followed the IUCN protocol (IUCN 2012; 2019). The same method was adopted to access the risk of extinction of the aquatic biota of the Rio Doce basin (see local red list in Biodiversitas 2021).

The potential Extent of Occurrence (EOO) was estimated based on the minimum convex polygonum between the occurrence points in the river, land was excluded. The potential Area of Occupancy (AOO) was estimated based on the sum of the area around the records that have visible sandbanks.

The maps, evaluation criteria and discussion followed the model padronized in Biodiversitas (2021). The free software QGIS (3.10.9-A) and Google Earth Pro were used to produce the final map and calculate the potential area of occupation and occurrence.

Taxonomy

***Apobaetis irai* sp. nov.**
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(Figures 3A–C; 4A–G; 5A–G)

Material examined. **Holotype.** Larva (on slide), **BRAZIL,** Minas Gerais State, Ipaba, Rio Doce 42º25′33″W, 19º22′20″S, 26.VIII.2018, 210 m.a.s.l., coll. Bramuth A.C. (INPA). **Paratypes:** 2 larvae (on slide), same data as holotype; 3 larvae (on slide), same data as holotype, except 13.II.2019; 2 larvae (on slide), Minas Gerais State, Marliéria, Rio Doce, 42º30′08″W, 19º41′42″S, 31.I.2019, 247 m.a.s.l., coll. Bramuth A.C., Massariol F.C., (INPA).

Description. ***Larva.*** Body length 3.1–3.4 mm. Coloration of light yellow with shorts brown marks over terga.

Head. *Antenna*: flagellum with minute spines on apex of each flagellomere. *Labrum* (Figs 4A, B): rectangular, length about 0.6 × maximum width; distal margin without medial emargination; ventral surface with one row of 14 spine-like setae on distolateral and distal margins, distal margin with medial spine-like setae; dorsal surface, at distal medial margin, with 3 minute, blunt and spatulate setae, with one medial row of long thin setae and many thin setae over surface. *Left mandible* (Fig. 4C): incisors cleft, outer and inner set of incisors respectively with 4+3 denticles; prostheca robust, bifid, inner lobe with outer margin pectinate; margin between prostheca and mola concave, subtriangular process wide and pointed, with large spine on outer margin; tuft of spine-like setae at base of mola present; denticles of mola not constricted; mola with two large denticle; outer margin convex. *Right mandible* (Fig. 4D): incisors cleft; outer and inner set of incisors respectively with 3+2 denticles; prostheca thin, bifurcated at apex, outer lobe pectinate; margin between prostheca and mola concave; tuft of spine-like setae at base of mola absent; denticles of mola not constricted; apex of mola with one simple setae; process of mola triangular; outer margin convex. *Hypopharynx* (Fig. 4E): lingua subquadrangular, longer than superlingua, without distomedial projection, tuft of setae at apex; superlingua rounded, with short, thin, simple setae scattered over distal margin.

Maxilla (Fig. 4F): maxillary palp 2.3 × length of galea-lacinia; segment I subequal in length of galea-lacinia; segment II robust, covered by thin setae; inner margin of galea-lacinia with two spine-like setae. *Labium* (Fig. 4G): glossa with parallel margins, apex with inner margin concave, same length of paraglossa; dorsal surface with 3 small spine-like setae at apex, one transverse row of 13 robust spine-like setae on apical third; ventral surface covered by thin long setae. Paraglossa curved inward; dorsal surface with 1 long and robust spine-like setae at apex, apical third with one row of 3 long and robust spine-like setae, near inner margin with one row of 7 long and robust spine-like setae; outer margin with one row of 24 long and thin spine-like setae from base to apex; ventral surface with one row of 6 long and robust spine-like setae at middle. Labial palp with segment I 0.6 × length of segments II and III combined; segment I covered by micropores (not illustrated); segment II with outer margin covered by long thin setae, inner margin bare, inner distal protuberance of segment II rounded and expanded, covered with thin, long simple setae; segment III triangular, covered by thin simple setae, length 0.8 × width, outer margin covered by long thin setae; dorsal surface with 7–9 robust spine-like setae on distal margin; ventral surface with 5 robust spine-like setae on distal margin.

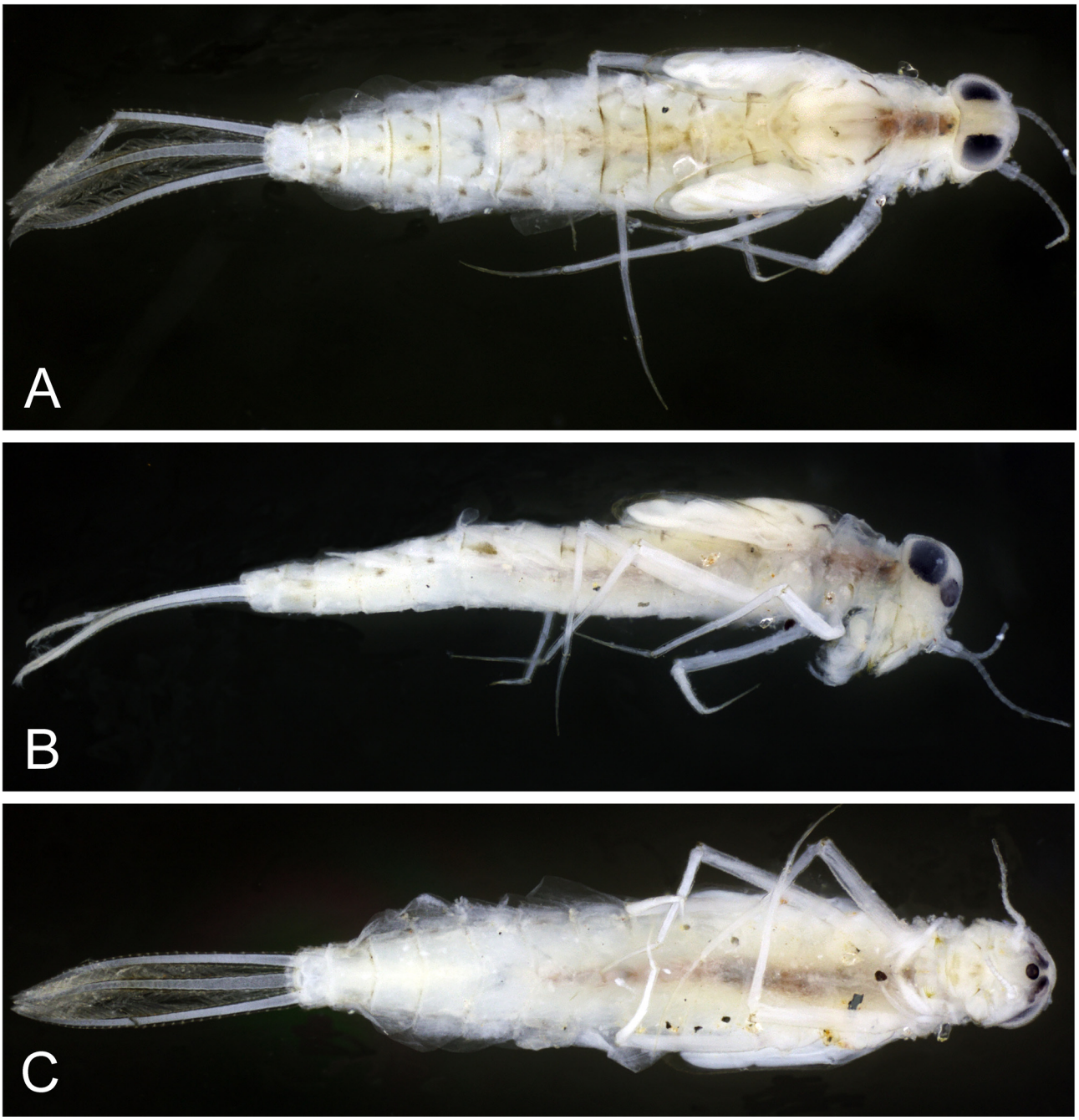

FIGURE 3. *Apobaetis irai* **sp. nov**. holotype (INPA). A, dorsal habitus of larvae female; B, lateral view; C, ventral view.

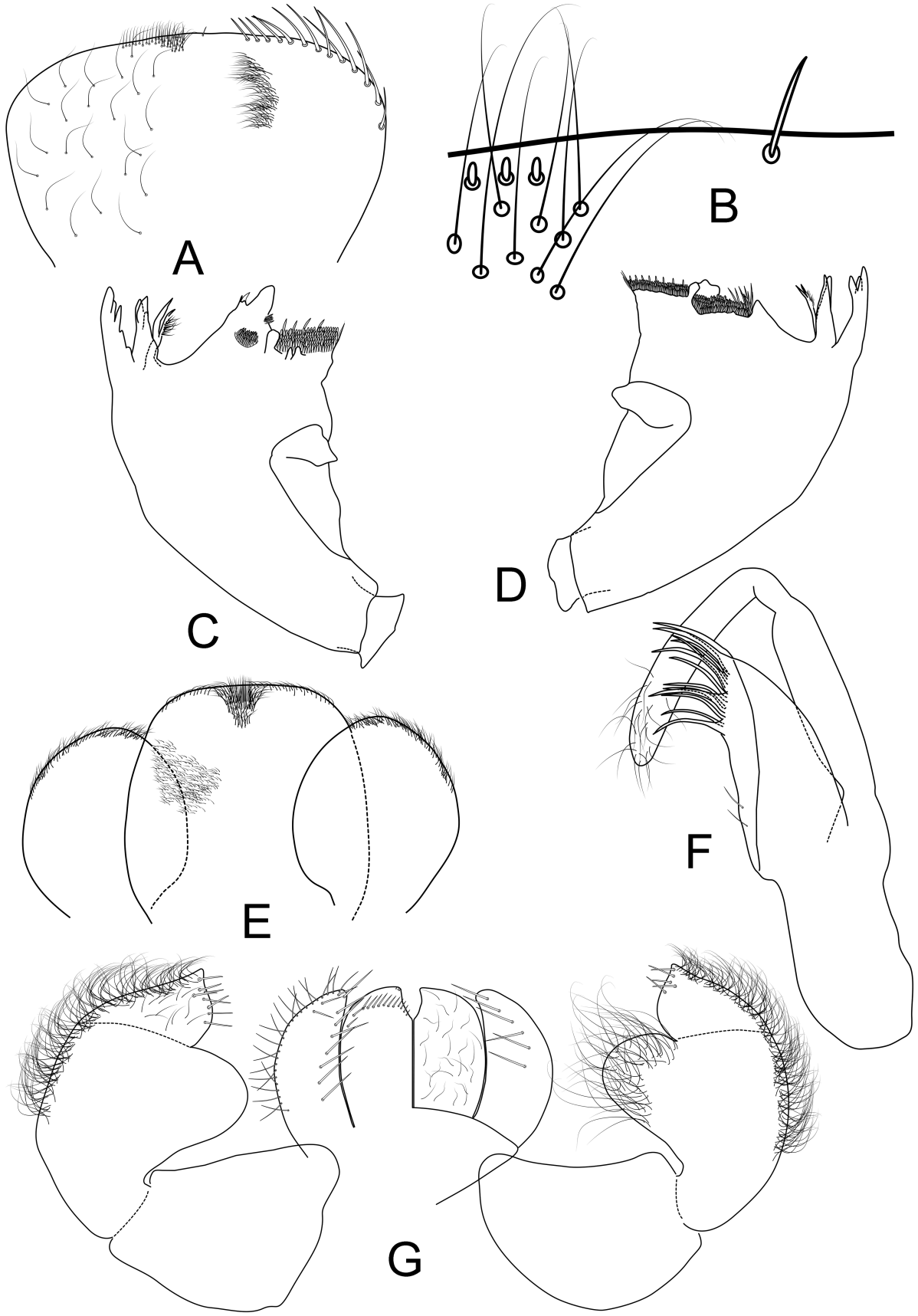

FIGURE 4. *Apobaetis irai* **sp. nov**. holotype (INPA). A, labrum (left d.v., right v.v.); B, labrum (detail of median setae); C, left mandible; D, right mandible; E, hypopharynx; F, left maxilla; G, labium (left d.v., right v.v.).

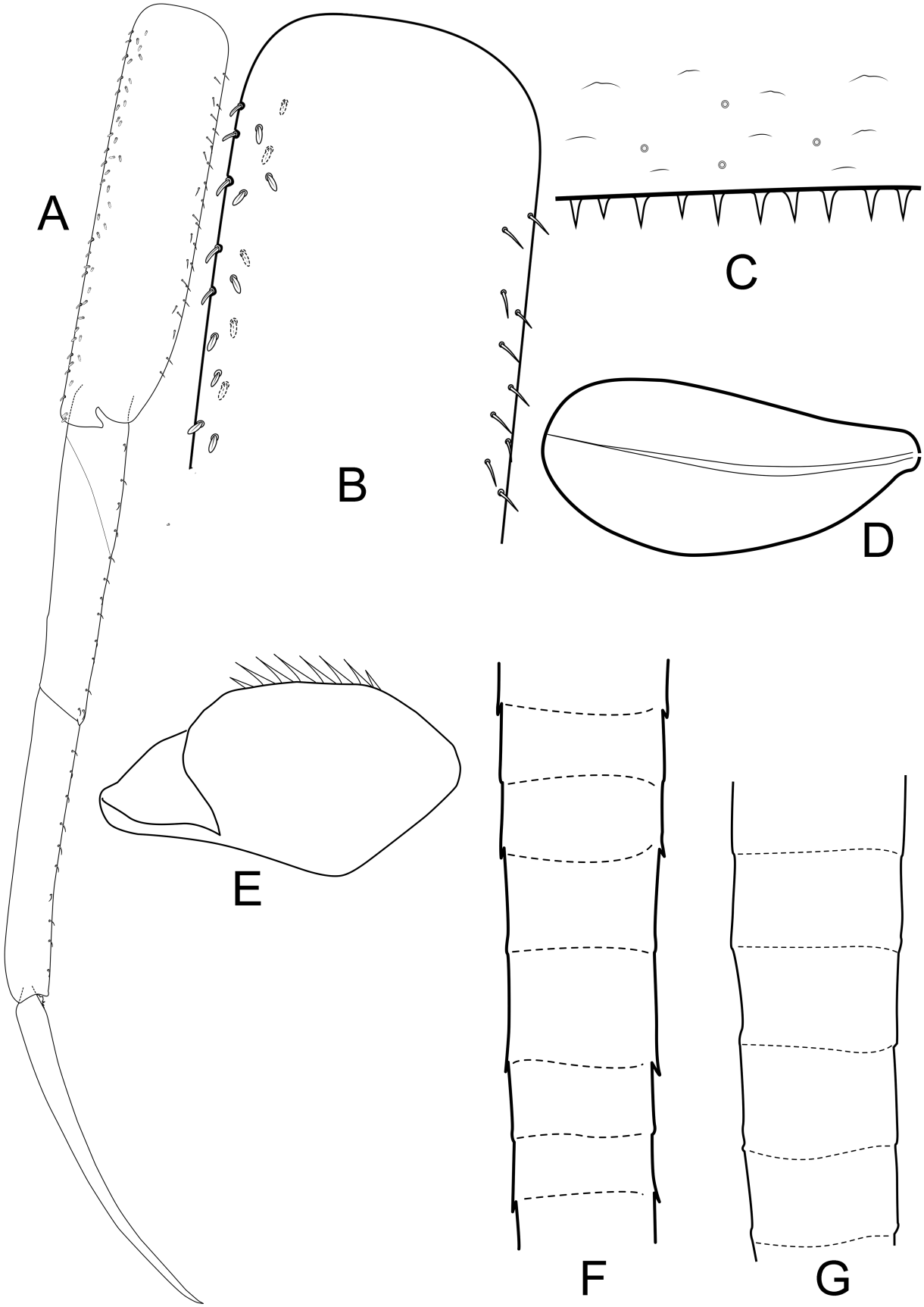

FIGURE 5. *Apobaetis irai* **sp. nov**. holotype (INPA). A, foreleg (anterior surface), detached setae are posterior surface; B, detail of forefemur; C, posterior margin of terga IV; D, gill; E, paraproct; F, cercus; G, paracercus.

Thorax. *Foreleg* (Figs 5A, B). Femur: dorsal margin with one row of 12–14 short, blunt and spatulated setae, apex with a pair short, blunt and spatulated setae; anterior surface with one row of 11–13 short, blunt and spatulated setae near to dorsal margin, and one row of small spine-like setae near to ventral margin; posterior surface with one row of 11–12 short, blunt and spatulated setae near dorsal margin; ventral margin with one row of 11 short spine-like setae. Tibia: dorsal margin bare, ventral margin with one row of 8–10 short spine-like setae; patella-tibial suture present. Tarsus: dorsal margin bare, ventral margin with one row of 9–10 short spine-like setae. Claws 1.0–1.2 × length of tarsus, without denticles. Middle and hind legs similar to foreleg.

Abdomen (Figs 3A–C, 5C–G). Coloration light yellow with tergum IV–X with an anterolateral mark, II–VIII with a medial mark, VII–VIII with an anteromedial mark; sterna light yellow. Terga creased with micropores; posterior margin of tergum IV with regular, triangular and pointed spines (Fig. 5C). Gills (Fig. 5D) with rounded apex, longer than next segment, with one medial trachea pigmented. Paraproct (Fig. 5E) with eight marginal spines, posterolateral extension without spines. Cerci (Fig. 5F) with lateral spines on every segments, longer on every 2nd segment. Paracercus (Fig. 5G) without spines.

Imagoes: Unknown.

Etymology. In the native South American language Tupi-guarani, irai means "honey water", in allusion to the name of the type locality (Doce in Portuguese means "sweet"); the specific epithet should be considered a noun in apposition.

Diagnosis. Larva: 1) labrum rectangular, distal medial margin with 3 minute, blunt and spatulate setae (Fig. 4A); 2) maxillary palp longer than 2.3 × length of galea-lacinia, segment I 1.2 × length of galea-lacinia (Fig. 4F); 3) glossa with parallel margins, apex with concave inner margin (Fig. 4G); 4) inner distal protuberance of segment II rounded and expanded (Fig. 4G); 5) segment III triangular (Fig. 4G); 6) patella-tibial suture present (Fig. 5A); 7) claws 1.0–1.2 × length of tarsus (Fig. 5A); 7) paraproct without spines in posterolateral extension (Fig. 5E).

Comments. *Apobaetis irai* **sp. nov**. is very similar to the species recently described for Rondônia, northern of Brazil, *A. biancae* Cruz, Boldrini & Hamada and *A. jacobusi* Cruz, Boldrini & Hamada (South America), sharing almost all the diagnostic features described in Cruz *et al*. (2020), can also be confused with species *A. lakota* McCafferty of North America (McCafferty 2000), the four species have rounded and expanded medial projection of segment II and triangular segment III of the labial palp.

The new species can be distinguished from *A. lakota* by the hypopharynx, with lingua subquandrangular in *A. irai* **sp. nov**. with tuft of setae at the apex, while *A. lakota* has lingua subcircular with a median lobe, without a tuft of setae; *A. irai* **sp. nov**. has glossae with concave inner margins at apex, inner margin without setae, and subequal in length to paraglossa; in *A. lakota* the glossa narrows slightly in the distal portion, has a rounded apex, with setae on inner margin, and longer than paraglossa.

The *A. irai* **sp**. **nov.** can be distinguished from *A. jacobusi* and *A. biancae* by the setae in the distal middle area of the dorsal surface of the labrum, which are three, minute, blunt and spatulate setae in *A. irai* **sp. nov**., four, strong and pointed in *A. biancae*, while *A. jacobusi* has two protuberances and a row of thin and long setae. *Apobaetis irai* **sp. nov**. has glossa subequal in length to the paraglossa, while in *A. jacobusi* and *A. biancae* the glossa is longer than paraglossa; *A. irai* **sp. nov**. differs from *A. jacobusi* and *A. biancae* by the size of the claws, with 1.0–1.2 × the length of tarsus in *A. irai* **sp. nov**., in *A. biancae* the claw is 1.4 × the length of tarsus and in *A. jacobusi* the claw is subequal to the tarsus; *A. irai* **sp. nov.** has no spines on the paracercus, *A. jacobusi* and *A. biancae* have lateral spines on all segments of the paracercus.

***Rivudiva watu* sp. nov.**

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(Figures 6A–G; 7A–D; 8A–F)

Material examined. Holotype. Larva (on slide), **BRAZIL**, Minas Gerais State, Resplendor, 41º21′35″W, 19º13′57″S, 28.II.2019, 148 m.a.s.l., coll. Bramuth A.C., Massariol, F.C., INPA. **Paratypes:** Larva (on slide), same data as holotype, INPA; 2 larvae (in alcohol), same data as holotype, INPA.

Description. *Larva*. Body length 3.0–4.0 mm. Coloration of light yellow, with light brown mark over terga VI.

Head. *Antenna*: flagellum with minute spines and setae on apex of each flagellomere. *Labrum* (Fig. 6A). Rectangular, length about 0.6 × maximum width; distal margin with medial emargination, one row of robust,

eventually pectinated, setae from lateral to middle; one row of thin bifid setae on distal margin not reaching distolateral margin; dorsal surface with many thin setae near distal margin. *Left mandible* (Fig. 6B): incisors partially cleft in two sets (fused at middle length); outer and inner set of incisors respectively with 4+2 denticles, outer incisor with spine-like process; prostheca robust and apically pectinate; margin between prostheca and mola straight; tuft of spine-like setae at base of mola absent; subtriangular process wide and pointed; denticles of mola constricted; mola with one large denticle; outer margin convex. *Right mandible* (Fig. 6C): incisors cleft in two sets; outer and inner set of incisors respectively with 3+2 denticles, outer incisor with one spine-like process; prostheca stout, bifurcated at middle; margin between prostheca and mola straight; tuft of spine-like setae at base of mola present; denticles of mola not constricted; apex of mola with one simple setae; process of mola triangular; outer margin convex. *Maxilla* (Fig. 6D). Maxillary palp 2.0 × length of galea-lacinia; segment II 1.0 × length of segment I, apex with robust apical lobe; ventral canine expanded, not laterally folded over canines (see Fig. 1E in Cruz *et al*. 2022); two sets of distal setae of inner-ventral, one row clavate and one pectinate; base of maxilla broken. *Hypopharynx* (Fig. 6E): lingua 1.2× longer than superlingua, rounded without distomedial projection or tuft of setae and lateral simple setae; superlingua with rounded outer margin and with short, thin, simple setae scattered over distal margin. *Labium* (Figs 6F–G): glossa slightly broad at base, with parallel margins, distal margin slightly rounded with inner part oblique, with one stout blunt setae, shorter than paraglossa; inner margin with one short spine-like setae on half; ventral surface covered by thin setae; dorsal surface with inner arc close to inner margin, outer arc not sinuous, far from distal margin. Paraglossa curved inward; apex almost straight, with two rows of robust and long spine-like setae; outer margin with 3 long setae; dorsal surface with three longitudinal rows of setae, first row near inner margin longer than half of length, with long robust setae; second with half of length of the inner row, with long robust setae; and third near to outer-distal margin, with long setae; ventral surface with one row of 4 setae near to inner margin. Labial palp with segment I 1.0 × length of segments II and III combined; inner distal protuberance of segment II rounded, covered with thin, long simple setae; segment III narrow and conical, covered by thin simple setae, dorsal surface with robust spine-like setae.

Thorax. *Foreleg* (Figs 7A–B): femur length about 2.8 × maximum width; dorsal margin with one row of long and robust setae reaching apex; ventral margin with few setae at base; anterior surface with one medial row of elongate setae, one row of long spine-like setae near ventral margin not reaching apex; posterior surface with one medial row of long spine-like setae, and one row of long spine-like setae near ventral margin reaching apex. Tibia dorsally bare; ventral margin with one row of long spine-like setae increasing in length to apex; patella-tibial suture absent. Tarsus: ventral margin with one row of spine-like setae. Claws 0.5 × length of tarsus, with two rows of conical denticles not reaching apex. *Middle and hind legs* (Figs 7C–D): femur anterior surface with one row of spine-like setae near dorsal margin, one row of long spine-like setae near ventral margin, and one medial row of long spine-like setae; posterior surface with one row of long spine-like setae near dorsal margin reaching apex, and one row of spine-like setae near ventral margin not reaching apex. Tibia dorsally bare; ventral margin without row of small blunt setae; patella-tibial suture present. Tarsus. Ventral margin with one row of small blunt setae. Claws 0.5 × length of tarsus, with two rows of small conical denticles reaching apex.

Abdomen (Figs 8A–F). Coloration of light yellow with tergum VI with large medium brown mark; sternum IX posterior margin with transversal dark mark. Posterior margin of terga with triangular spines (Fig. 8C). Gills oblong, longer than next segment, with one medial trachea pigmented. Paraproct (Fig. 8D) with four wide marginal spines, posterolateral extension with spines. Cerci (Fig. 8F) with lateral spines on every segment, longer on every longer on 2nd segment. Paracercus (Fig. 8E) without spines.

Imagoes: Unknown.

Etymology. The Krénak people, the indigenous group who inhabits the region, named the Doce River as Watu; the specific epithet should be considered a noun in apposition.

Diagnosis. Larva: 1) labrum with medial emargination (Fig. 6A); 2) labrum ventral surface with simple robust row of setae on distal margin (Fig. 6A); 3) left mandible with incisors fused at middle length (Fig. 6B); 4) subtriangular process of left mandible pointed (Fig. 6B); 5) ventral canine of maxilla expanded, not laterally folded over canines (Fig. 6D); 6) maxilla with two sets of distal setae on inner-ventral row, one clavate and one pectinate (Fig. 6D); 7) hypopharynx without distomedial projection (Fig. 6E); 8) glossa distal margin with inner part oblique (Fig. 6G); 9) outer arc of setae on glossae not sinuous, far from distal margin (Fig. 6F); 10) labial palp segment III conical (Fig. 6F); 11) ventral margin of hind tibiae without row of setae (Fig. 7C); 12) patella-tibial suture absent (Fig. 7A); 13) paraproct with four spines (Fig. 8D).

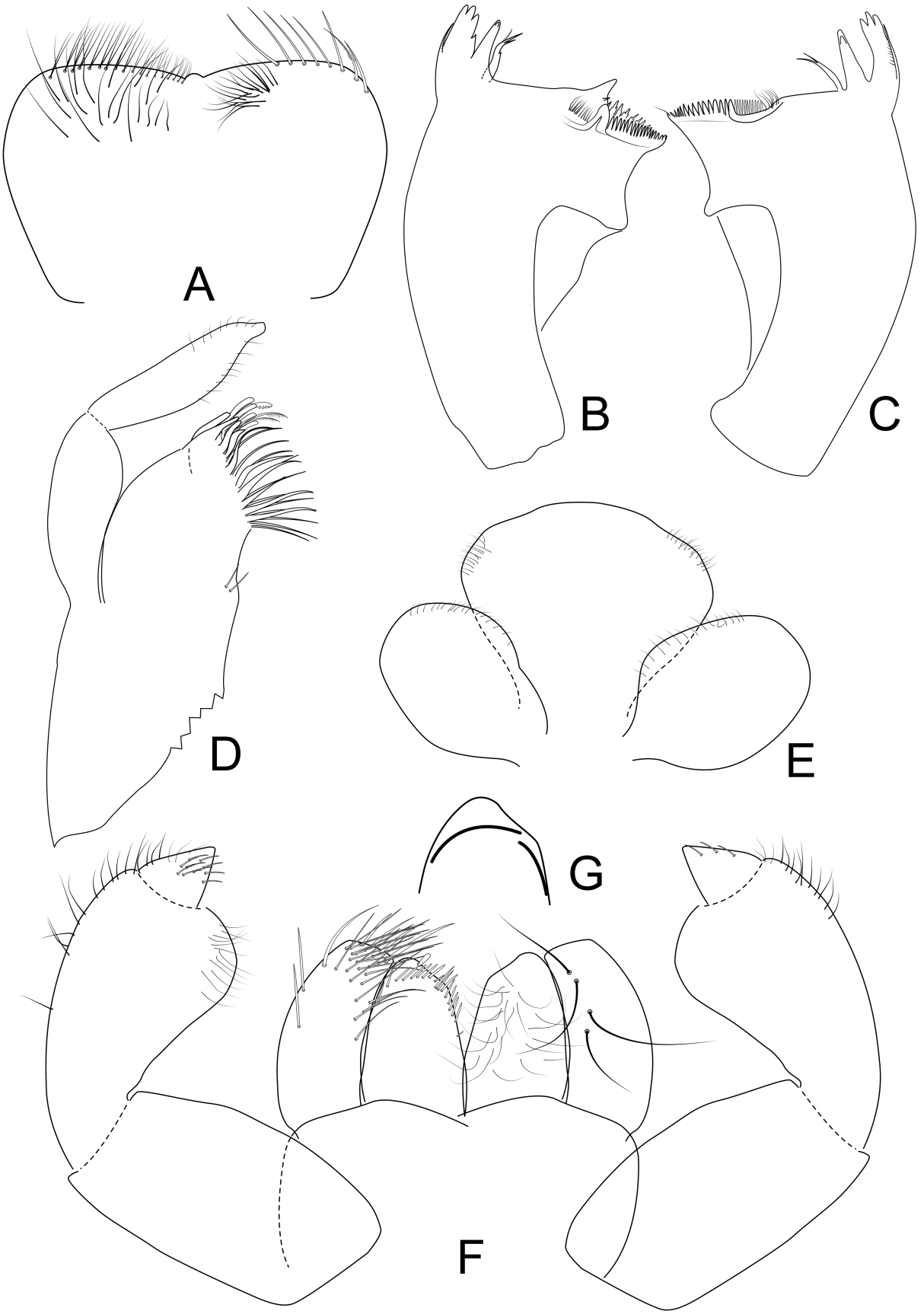

FIGURE 6. *Rivudiva watu* **sp. nov**., holotype (INPA). A, labrum (left d.v., right v.v.); B, left mandible; C, right mandible; D, left maxilla; E, hypopharynx; F, labium (left d.v., right v.v.); G, shape of distal rows of setae of glossa.

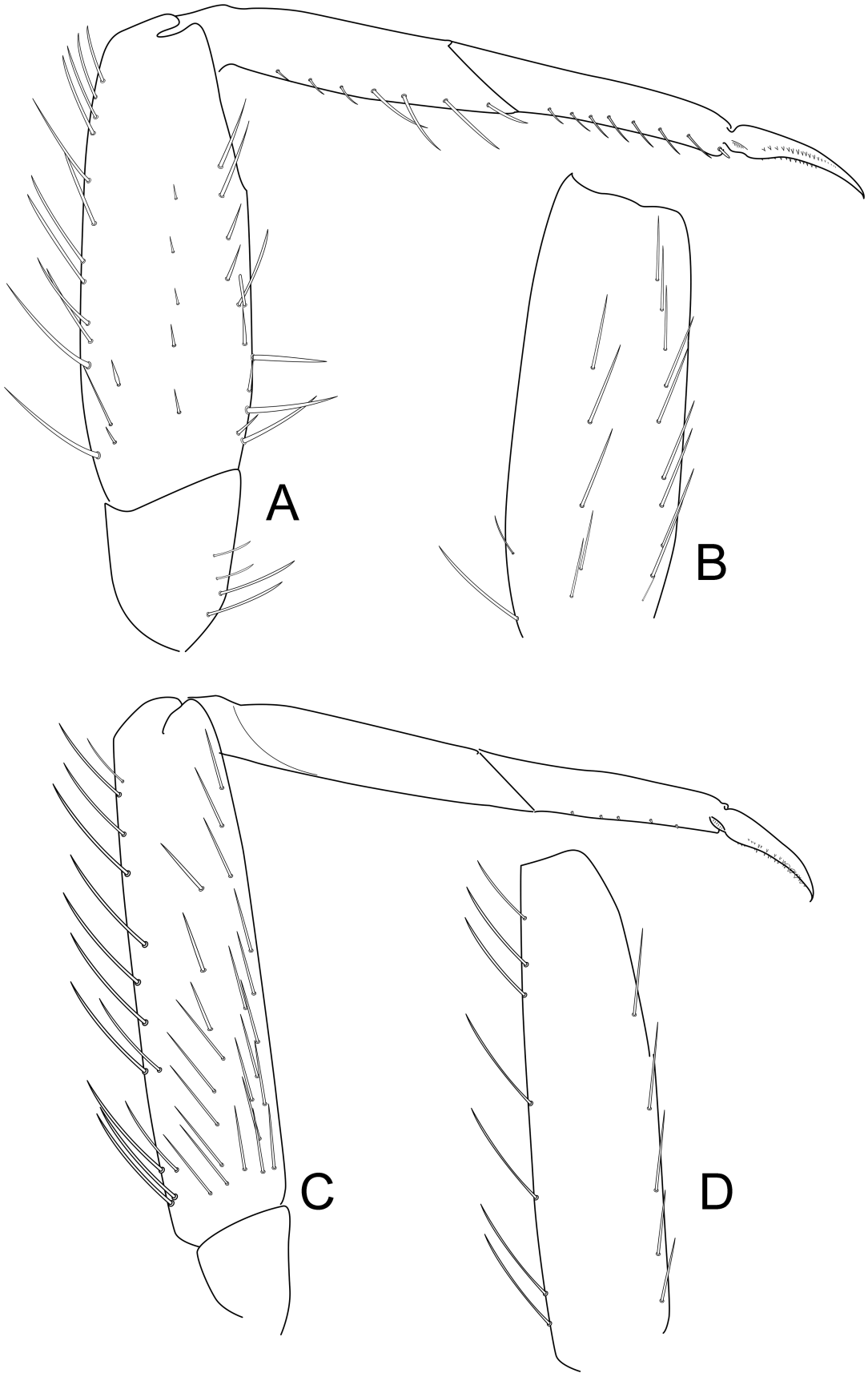

FIGURE 7. *Rivudiva watu* sp. nov., holotype (INPA). A, foreleg (femur on anterior surface); B, posterior surface of forefemur; C, hind leg (femur on anterior surface); D, posterior surface of hind femur.

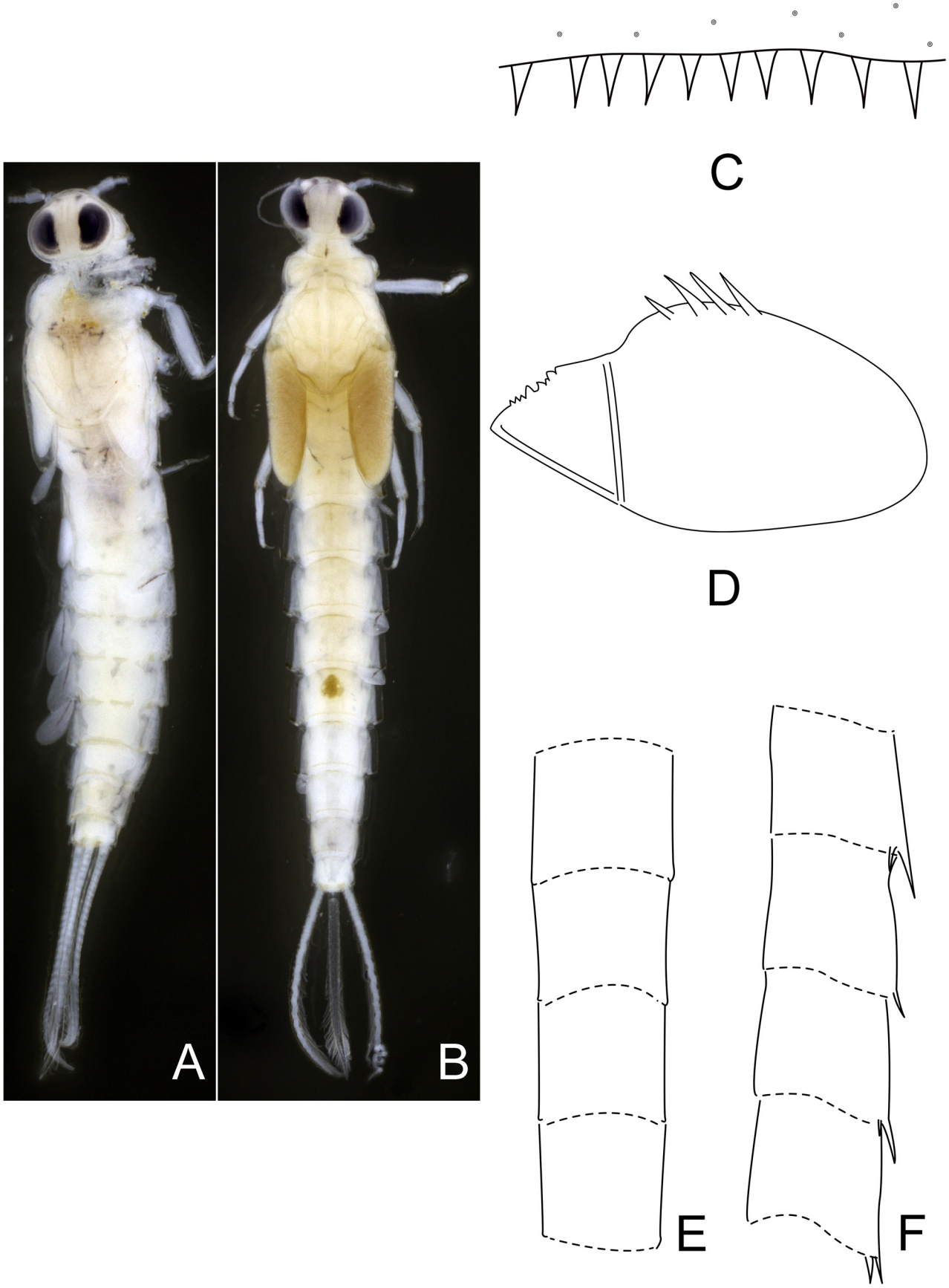

FIGURE 8. *Rivudiva watu* **sp. nov**., (INPA). A, dorsal habitus of immature nymph (holotype); B, dorsal habitus of mature nymph (paratype); C, posterior margin of tergum IV; D, paraproct; E, paracercus; F, cercus.

Comments. The distal shape of glossa, distal set of inner-ventral row of maxilla, the absence of row of setae on hind tibiae and hypopharynx without distomedial projection or tuft of setae are unique characteristics from *Rivudiva watu* **sp. nov**., distinguishing it from all other species of the genus.

Rivudiva watu **sp. nov**., in Cruz *et al*. (2022) key, can be identified as *R. trichobasis* Lugo-Ortiz & McCafferty or *R. uiara* Cruz, Boldrini, De Lima & Hamada, 2022. *Rivudiva watu* **sp. nov.** can be differentiated from *R. trichobasis* by left mandible with pointed subtriangular process, rounded in *R. trichobasis*; maxillary palp segment II with small apical lobe, larger in *R. trichobasis*; ventral canine expanded, not laterally folded over canines (Fig. 6D), folded in *R. trichobasis* (Fig. 1E in Cruz *et al*. 2022); maxilla with two sets of distal setae of inner-ventral row, one clavate and one pectinate (Fig. 6D), only clavate in *R. trichobasis* (Fig. 1E in Cruz *et al.* 2022); hypopharynx without distomedial projection or tuft of setae, with projection and tuft of setae in *R. trichobasis*; glossa distal margin with inner part oblique, with one stout blunt seta, distal margin rounded in *R. trichobasis*; row of setae on ventral margin of hind tibiae absent, present in *R. trichobasis*; paraproct with four spines, nine in *R. trichobasis*. The same feature set presented distinguishes *Rivudiva watu* **sp. nov.** from *R. uiara*. Additionally, the new species can be differentiated by the segment III of labial palp, complete conical in *R. watu* **sp. nov**., apex concave in *R. uiara*; and outer arc of setae on glossa not sinuous, far from distal margin in *R. watu* **sp. nov**., sinuous in *R. uiara*.

Conservation status

Extinction risk assessment for newly described species is unusual. However, recent evidence shows that undescribed or newly described species are at greater risk of extinction than known species but have received limited conservation and assessment effort (Lees & Pimm 2015; Liu *et al.* 2022).

In the Rio Doce basin, mayflies follow the pattern observed by Liu *et al.* (2022), of the nine species recognized with some degree of risk (out of the 63 present in the basin), eight were described in the last two decades, and all the oldest species present in the basin are at least of concern. Therefore, accessing the risk of extinction of the species here described is imperative.

Apobaetis irai **sp. nov**. belongs to a group of species known to inhabit the banks and/or bottom of medium to large rivers (Figs 1, 2A, B). Sampling sites suffer the direct impact of toxic tailings and river overflow after the dam failure. The habitats used as shelter and oviposition, such as the sandbanks, were altered, harming the persistence of populations. Feeding habits and food availability for nymphs were affected by the deposition of excess sediment in river beds (Biodiversitas 2021). Also, the species' respiratory processes may have been affected by sediments accumulated in the insect's body. Finally, as this group of species usually occurs in rivers with running water, the large amount of tailings can prevent or reduce the flow of the river and increase pollution in it, making it an unsuitable place for the survival of the species.

Despite systematic samples in the Rio Doce after the disaster, the new species was only collected in the borders with Parque Estadual do Rio Doce natural reserve, and to the north in an area with relatively preserved vegetation. The persistence of the species is probably related to its presence near natural reserves and areas with a certain degree of preservation. Both areas also feature islands with relative preserved flora and sandbars. In the same range, two species of mayflies were elected as threatened, the endemic *Camelobaetidius spinosus* Boldrini & Salles—VU B1ab(iii) and *Paramaka convexa* (Spieth)—VU D2 (Biodiversitas 2021).

Considering the whole relatively preserved area, including the natural reserve, the total potential extent of occupancy (EOO) for *Apobaetis irai* **sp. nov.** is proximally 21.5 km^2 (in river), and the area of occurrence (AOO) is proximally 1.2 km^2. Threats have been identified that indicate a continuing decline in habitat quality, and currently the species is known from only two locations. The river silting is the plausible threatening event capable of rapidly affecting all individuals. This scenario makes *Apobaetis irai* **sp. nov**. eligible to be listed, at least, as Vulnerable (VU) B2ab(iii)+D2.

The sand preference of the genus *Rivudiva* was recently discovered, allowing subsequent fieldwork sampling to be directed, so that a series of discoveries could be made (Cruz *et al.* 2022). Except the habitat preference, there are no information related to biological, ecological and populational aspects.

The new species was sampled in a small stream 180 meters from Rio Doce, between two protected areas, Parque Estadual Sete Salões e Indigenous territory Krenak (Figs. 1, 2C, D). The sample site did not suffer the direct impact of toxic mud that spread down the Doce River.

Besides the psammophilous mayflies in general are considered one of the most threated (Glazaczow 1997; Jacobus 2013; Lillie 1995; McCafferty 1991), the absence of direct impact on sample site, its preference for small streams and the sample site surrounded by reserves, make this species eligible to be listed as Least Concern (LC). However, there are insufficient data to estimate the extent of occurrence (EOO) and area of occupancy (AOO), so it is better eligible to Data Deficient (DD).

The discovery of two new psammophilous species after a mine-tailing dam collapsed gives a glimpse of hope in fields that suffers as much throwbacks as biodiversity and conservation. The persistence of these two species is probably related to the natural reserves and their areas of influence. However, it is not implied a permanent suitable environment. The Rio Doce possess four hydroelectric dams; its riparian vegetation has been continually deforested by agricultural activities and urbanization (Biodiversitas 2021), resulting in river silting events every rainy season; and there are unknown long-term consequences, such as the heavy metals potential risks of cytotoxicity and DNA damage (Gabriel *et al.* 2021; Garcia *et al*. 2016; Segura *et al*. 2016). Therefore, the limits of the disaster are still uncertain, and additional field studies and conservation efforts are necessary.

Acknowledgments

This study was financed by the following projects: Sistemática integrada de insetos aquáticos, com ênfase em Simuliidae (Diptera) na América do Sul (MCTI/INPA), Sistemática das famílias Baetidae e Caenidae (Insecta: Ephemeroptera) em Escala Global, e estudo taxonômico na Amazônia Sul-Ocidental (UNIR); Conselho Nacional de Desenvolvimento Científico e Tecnológico CNPq (processo 133035/2020-4); CAPES/Pro-equipamentos-DCEN; Insetos Aquáticos na América do Sul: taxonomia integrativa, biologia e ecologia, CNPq process n. 308970/2019-5 and FAPEAM/ Programa POSGRAD-2019. We are grateful to Cassio, from Biodiversitas Foundation, for provide the shapefile of area directly affected by mud and overflow area. We would like to thank Bruno Henrique Guimarães, from Prefeitura Municipal de Vila Velha, for his support in the process of elaborating the distribution map of the new species.

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