



New insights into the phylogeny of *Tortopus* Needham and Murphy and *Tortopsis* Molineri (Ephemeroptera, Polymitarcyidae) with description of three new species

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Abstract

The family Polymitarcyidae, with a worldwide distribution, includes mayflies with large burrowing nymphs. South America harbors the highest diversity of this family, including the subfamilies Asthenopodinae and Campsurinae. In this work, three new species belonging to the genera *Tortopsis* and *Tortopus* (Campsurinae) are described based on adults and nymphs from Colombia: *Tortopsis toro* sp. nov., *Tortopsis andaki* sp. nov. and *Tortopus coreguaje* sp. nov. Additionally, *Tortopsis limoncocha* is firstly recorded from Colombia. A cladistic analysis of all the species in these genera is presented, using external morphological characters of adults and eggs. Keys to male and female adults of all the species of both genera are presented.

Key words

Campsurinae, cladistics, new species, South America, systematic, taxonomic key.

1. Introduction

The monophyletic group formed by *Tortopus* Needham and Murphy, 1924 and *Tortopsis* Molineri, 2010 (Campsurinae) gathers very specialized mayflies with burrowing nymphs and extremely short-lived adults (Dominguez et al. 2006). Nymphs present many morphological traits related to their digging habits and food filtering (McCaf-

ferty 1975). Subimagos emerge directly on the surface of the water and have only an aerial stage as short as two hours, to molt to imago and reproduce (McCafferty and Bloodgood 1989; Molineri 2008). Both genera are known from North, Central and South America, but most species are from the Amazon basin (Molineri et al. 2012, 2019),

and a South American origin has been hypothesized for these groups (McCafferty 1998; Molineri et al. 2019).

The role of some Campsurinae species in bioturbation of lentic habitats, with large nutrient and pollutant movements from the sediment by the nymphs had received some attention (Leal et al. 2007). On the contrary, their role in the erosion of the clay-banks of large rivers has not been studied yet, but it may be as important as the effect of the related genus *Asthenopus* in degrading woody debris (Sattler 1967). Another little known aspect in this group is their silk production and use (Sattler 1967; Molineri and Emmerich 2010).

Tortopus was established by Needham and Murphy in 1924 and seven species are currently recognized (Molineri 2010; Molineri et al. 2012): *T. arenales* Molineri, *T. bellus* Lugo-Ortiz and McCafferty, *T. circumfluus* Ulmer, *T. harrisi* Traver, *T. igaranus* Needham and Murphy, *T. pixuna* Molineri, Boldrini and Salles and *T. zottai* (Navás). In Colombia, only *T. igaranus*, the type species of the genus, has been recorded from male and female imago collected in the Peruvian-Colombian border on the Putumayo River (Needham and Murphy 1924; Molineri 2010). *Tortopus* is characterized by male genitalia with flattened and basally fused penis and ninth abdominal sternum with medio-longitudinal notch, short parastyli, female sternum VIII with long furrows anterior to sockets (parastyli receptors), and nymphs with two subapical tubercles on inner margin of mandibular tusks (Molineri 2010).

Tortopsis was established by Molineri (2010) for a group of species previously classified in *Tortopus*. *Tortopsis* is characterized by male genitalia with cylindrical penis, each penean arm separated from the base; ninth abdominal sternum entire, long curved parastyli present; female fore wing without additional veins between R₂ and IR, female sternum VIII with parastyli receptors C or V-shaped, and nymphs with a single subapical tubercle on mandibular tusks (Molineri 2010). Ten species are known from central Argentina to the northern United States of America (Molineri 2010; Gonçalves et al. 2011; Molineri et al. 2012): *T. bruchianus* (Navás), *T. canum* Gonçalves, Da Silva and Nessimian, *T. limoncocha* Molineri, *T. obscuripennis* (Domínguez), *T. parishii* (Banks), *T. primus* (McDunnough), *T. puella* (Pictet), *T. sarae* (Domínguez), *T. spatula* Molineri and *T. unguiculatus* (Ulmer). In Colombia, the genus is represented by only two species (*T. unguiculatus* and *T. spatula*) from several localities in the Amazonas department (Molineri 2010; Molineri et al. 2012).

Molineri (2010) performed a phylogenetic revision of all the species in *Tortopus* and *Tortopsis*, but the taxonomic knowledge has been growing, especially during the last decade, with the description of new species and stages (Gonçalves et al. 2011; Molineri et al. 2012). The aim of the present work is to describe two new species of *Tortopsis* and a new species of *Tortopus* recently collected in a biologically poorly known area in Colombia. Additionally, we amend the phylogeny proposed in Molineri (2010), including all the species presently known. We also present keys to all the species of both genera.

2. Methods

2.1. Study area

The Caquetá River is located in the Amazon region, in the southeastern portion of Colombia and northwestern portion of Brazil. It extends through 2,280 km, with 1,200 km within Colombia and the remaining within Brazil. The river arises in the Colombian Massif known as “Páramo de las Papas”, in the central Andean region of the Cauca department, and flows into the Amazon River in Brazilian territory, where it changes the name to Japurá. It has an extensive water network, and along with the Putumayo River, are the two main tributaries of the Colombian Amazon. The Caquetá River drains a large basin of approximately 267,730 km², shared between the departments of Cauca, Caquetá, Putumayo, Guaviare, Vaupés and Amazonas within Colombia (IGAC 1999). Average flow rate ranges from a minimum of 231.8 m³.sec⁻¹ (December–March) and a maximum of 1,298 m³.sec⁻¹ (May–August), with a medium value of 417.1 m³.sec⁻¹, as recorded for a multi-year average flow in the Andaquiri Hydrological Station (IDEAM 2020). From a limnological point of view, it is a river of white water, due to its Andean origin. It is characterized by high electrolyte content and a significant load of suspended particles from the erosive processes that occur in the surrounding mountain range (Duque et al. 1997). Continuous navigation is very difficult due to the existence of many rapids and riffles along its course, particularly those within the Araracuara region, where the river runs through a deep and long canyon.

The type locality of the collected specimens here described corresponds to the lowlands of the middle basin of the Caquetá River, in the town of Curillo, located within the limits of the dense Amazon jungle in the department of Caquetá. All the material described here was collected at a single station in the town of Curillo, based on authorization 1166 granted by ANLA (National Authority of Environmental Licenses in Colombia) in the project “Trans-Amazon Aquatic Insects: study of potential areas of endemism in Colombia”.

2.2. Collection and descriptions

Nymphs were manually collected on riverbanks, with the help of a blade to separate substrate pieces with the nymphal burrows. Substrate consisted of hard clay, and the pieces picked up were disaggregated on the shore to collect the nymphs. Mature nymphs were settled in rearing cages in the river for a short period and then transported in an ice keeper with river water and a piece of substrate (at ambient temperature). Adults were collected with a light trap at river margin set around sunset. All the collected material was fixed and preserved in ethyl alcohol 96°. Slide mountings were done using Canada Balsam. Photographs were taken with a ZEISS Axio-Cam ICc 5 mounted on a Stemi 508 stereo microscope and a Leica M205C stereomicroscope with an attached

Leica MC–170HD camera. Line drawings were made using a camera lucida mounted on a microscope Olympus BX51. Some photographs are the result of stalking partially focused images with the software CombineZP (Hadley 2010). Institutions housing the studied material: Colección Entomológica del Programa de Biología de la Universidad de Caldas, Colombia (CEBUC); Museo de Entomología de la Universidad del Valle, Cali, Colombia (MUSENUV); Instituto de Biodiversidad Neotropical, Tucumán, Argentina (IBN).

2.3. Phylogenetic analysis

The matrix and characters proposed by Molineri (2010) were amended including four new characters (Appendix 1), the three new species here described, as well as *Tortopsis canum* Gonçalves et al. (2011) and the information provided in Molineri et al. (2012) for *Tortopus igaranus* and *Tortopus ipixuna*. The matrix analyzed here (Table 1) includes 26 taxa and 31 morphological characters. All the species of *Tortopus* and *Tortopsis* were included, together with six outgroups. One of these outgroups (*Ephoron*) was used to root the tree. Searches were conducted in TNT (Goloboff et al. 2008) under parsimony, with implied weights (with different k values, from 3 to 20). Group support was calculated using the symmetric Jackknifing function of TNT, this function repeatedly resamples the character matrix and compares the resultant trees with the shortest tree (we performed 250 resamplings) (Pol and Goloboff 2020). Three poorly known taxa were deactivated during some searches: *Tortopus circumfluus*, *Tortopsis bruchianus*, and *Tortopsis parishi*.

3. Results

3.1. Descriptions

Tortopsis toro sp. nov.

Figs 1–26

<https://zoobank.org/834A4E7E-D5A7-42F5-B786-930927-DOCC43>

Type material. Holotype male imago (CEBUC) from Colombia, Caquetá, Curillo, río Caquetá, 220 m, 01°01'44"N; 75°55'9"W, 20.ix.2018, LG Dias, MC Zúñiga, B Toro, JP Chaux and C Molineri cols. Paratypes: 1 reared male adult with nymphal exuviae (IBN); 5 male imagos, 3 female imagos and 13 nymphs (IBN); 10 males imagos, 5 females imagos and 10 nymphs (CEBUC); 5 male imagos, 3 female imagos and 10 nymphs (MUSENUV), same data as holotype.

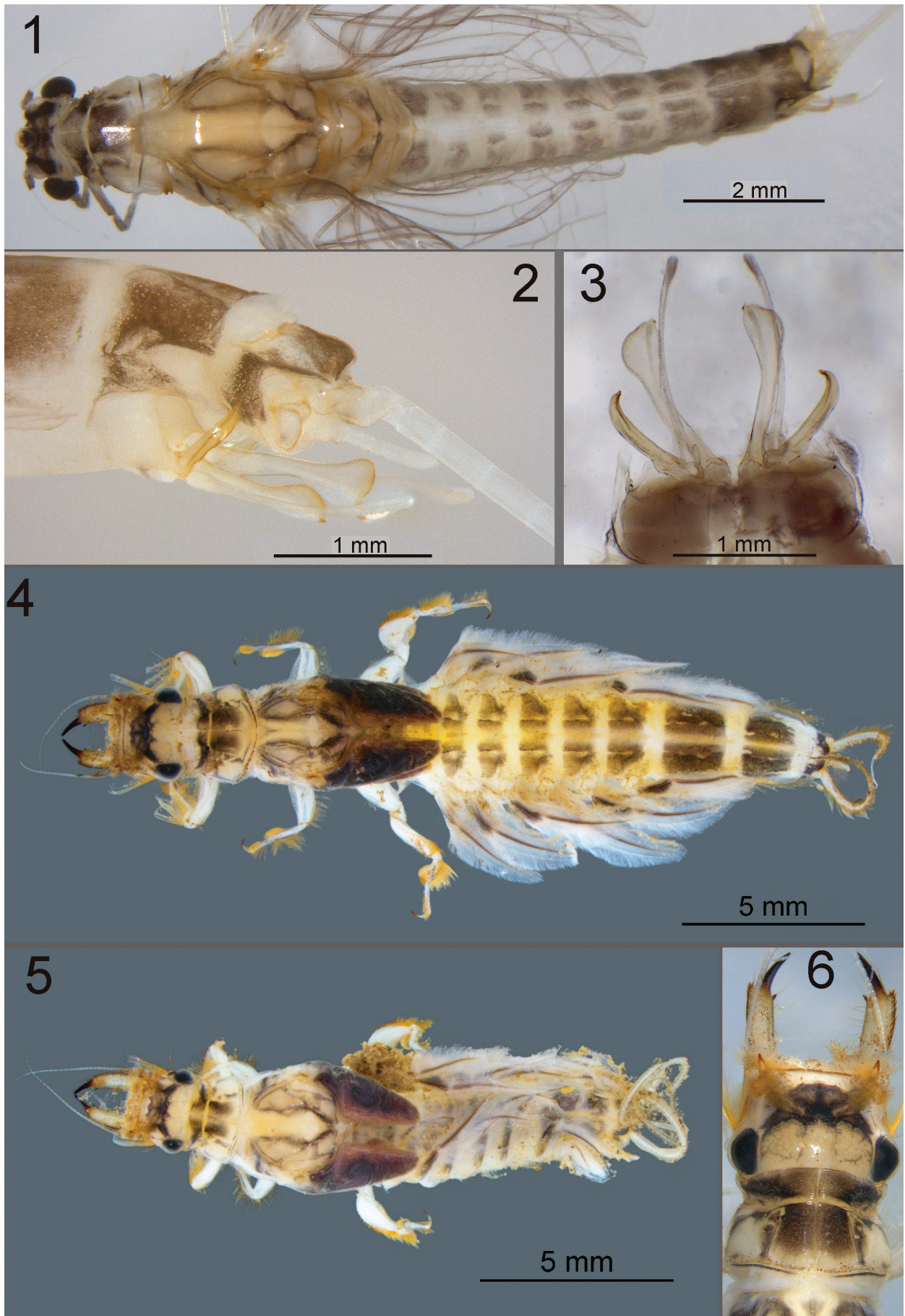
Diagnosis. *Tortopsis toro* sp. nov., known from imagos of both sexes and nymphs, can be distinguished from all other species of the genus by: In adults, 1) forewing length ranges between 12.2–13.2 mm (male) and 15.5–17.0

mm (female); 2) hyaline wings, with brownish veins and costal margin shaded slightly gray; 3) parastyli almost straight in lateral view, slightly curved medially, with a longitudinal ventral furrow (Figs 2–3, 19–21); 4) penis apically widened and flattened, with rounded semicircular apical spine (Fig. 20); 5) female parastyli receptors with rounded and elongated sockets (Fig. 22); 6) head shaded black among ocelli, occiput with very light gray anastomosed lines (Figs 1, 6); 7) coloration as in Fig. 1. The nymphs can be separated from the others of the genus by: 1) mandibles at most with 2 stout small spines on inner margin of tusk (basally to large subdistal tubercle, see arrow in Fig. 24); 2) outer margin of tusk, just before distal spur, with a marked indentation (arrow in Fig. 25); 3) head shaded with gray on occiput as in Fig. 6; 4) developing fore wing buds pigmented with gray slightly along costal margin and base of longitudinal veins (similar to *T. puella* in Molineri 2008: figure 16).

Male imago. Length (mm): body, 14.0–14.5; forewing, 12.2–13.2; hindwing, 5.9–6.1; foreleg, 5.3–5.9; cerci, 32.0–36.0. General coloration yellowish white shaded with gray dorsally (Fig. 1). Head. Coloration yellowish white except black area among ocelli (Fig. 1). Antennae: scape and pedicel yellowish white completely shaded with gray, flagellum hyaline. Thorax. Pronotum hyaline, shaded with gray dorsally (Fig. 1). Mesonotum whitish yellow shaded with gray along sutures but not medially. Metanotum whitish yellow shaded gray medially. Thoracic pleura and sterna whitish yellow. Legs yellowish white, shaded with grayish on fore tibia and tarsus, rest of leg (including femur and claws, not shaded). Wings. Membrane hyaline, shaded slightly gray along C and Sc areas; longitudinal and cross veins brownish. Abdomen. Whitish translucent with gray shading dorsally, except on medial band. Terga I–III shaded gray on submedial rounded areas; terga IV–VII similarly shaded but a longitudinal darker strip is visible submedially (Fig. 1); terga VIII–IX more widely shaded except on small marks and sigilla (Fig. 1); tergum X with whitish median area and blackish margins. Abdominal sterna pale, without dark shadings, except sternum IX with a small median gray mark on posterior margin. Genitalia (Figs 2–3, 19–21): whitish except parastylus yellowish. Parastylus almost straight in lateral view, slightly curved medially, with a longitudinal ventrolateral furrow along its entire length; penis distally widened, semicircular “spine” at apex of penis. Caudal filament whitish translucent.

Female imago. Length (mm): body, 18.0–18.5; forewing, 15.5–17.0; hindwing, 6.5–7.5; cerci, 4.3–4.8. General coloration as in male except darker markings. Head. Coloration black between ocelli, with posterior and submedian grayish lines on occiput (similar to Fig. 4). Thorax. Furcasterna shaded with light gray medially. Wings: Membrane hyaline slightly tinged with yellow. Abdomen. shaded more extensively with gray on terga. Parastyli receptors on sternum VIII with elongated and rounded sockets (Fig. 22). Light gray marks medially on sterna VIII–IX.

Egg. Suboval. Length, 410–420 μ m; width, 320–340 μ m.



Figures 1–6. *Tortopsis toro* sp. nov. Male imago: 1, general view, dorsal; 2, abdominal segments VIII–X, lateral view; 3, genitalia, ventral view. Nymph: 4, mature female nymph; 5, mature male nymph; 6, detail of head and pronotal coloration of not fully-grown male nymph.



Figures 7–14. *Tortopsis toro* sp. nov., nymph. 7, labrum, dorsal view; 8, left mandible, inner view, 9, right mandible, inner view; 10, right mandible, detail of tusk, ventral view (arrow indicates stout spine near inner margin); 11, hypopharynx, dorsal view; 12, labium, ventral view; 13, left maxilla, ventral view (arrow indicates membranous gill); 14, right maxilla, ventral view.

Nymph (mature). Length of body (from apex of tusks to apex of abdominal tergum X): female, 22.0–26.0 mm; male, 14.0–19.0 mm. General coloration yellowish white with gray markings dorsally (Figs 4–6). Head. Coloration whitish shaded gray among ocelli, occiput with very light gray anastomosed lines submedially as Fig. 6. Antennae and mouthparts whitish except apex of tusk orangeish, and spines yellowish. Finger-like gill present near base of maxillae (arrow in Fig. 13). Mandibular tusks with 0–2 stout spines on inner margin, basal to subdistal tubercle (see arrow in Fig. 24); outer margin of tusk with row of 18–26 stout spines (Figs 8–10, 24–25). Thorax. Pronotum, anterior ring shaded black almost completely including the anterolateral pointed projections, posterior ring patterned as in Fig. 6, both with a median pale line. Meso- and metanotum with gray and black marks, lighter on median area; wing buds whitish, pigmented with gray slightly along costal margin and base of longitudinal

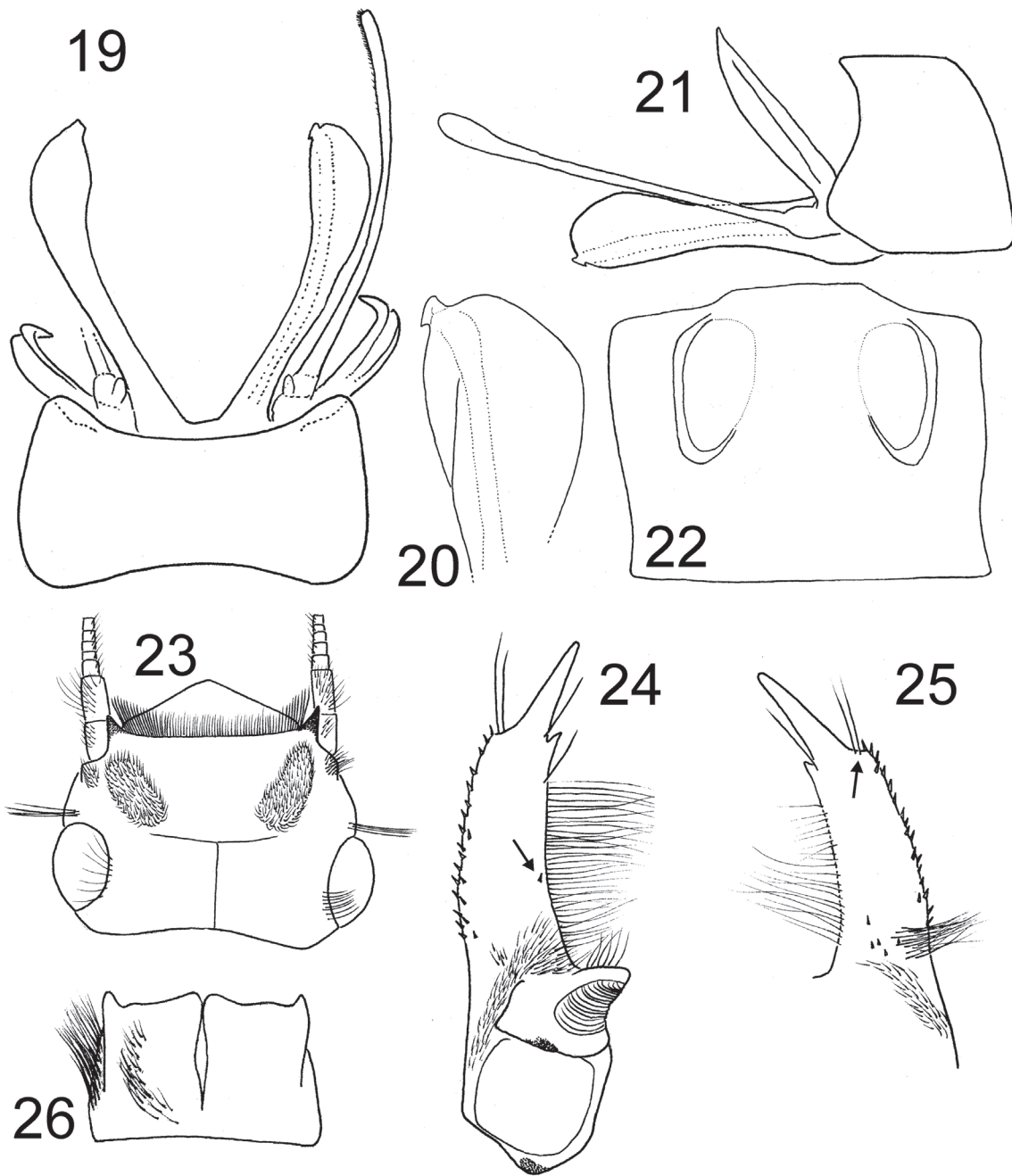
veins. Thoracic pleura and sterna whitish. Legs whitish, with yellowish setae and apex of tarsal claws yellowish (Figs 15–18). Abdomen. Color pattern as described for the adults. Gills: vestigial gills I translucent; gills II–VII well developed, formed by a pair of large whitish lamellae, the outer (dorsal) lamellae of each pair is shaded with black on a medio longitudinal band (thinner on gill II), the inner (ventral) lamellae of each pair show only a thin brownish line along trachea. Genital rudiments of males showing distally widened penis. Caudal filaments yellowish white.

Etymology. This species is dedicated to Dr. Beatriz Toro Restrepo, Universidad de Caldas, in recognition of her work in environmental education, friendship and contributions in fieldwork with aquatic insects of Colombia.

Distribution. Known only from the type locality.



Figures 15–18. *Tortopsis toro* sp. nov., nymph. 15, fore leg, dorsal view; 16, fore tarsal claw; 17, middle leg, dorsal view; 18, hind leg, ventral view.



Figures 19–26. *Tortopsis toro* sp. nov., imago: 19, male genitalia, ventral view; 20, detail of apex of penis, ventrolateral view; 21, male genitalia, lateral view; 22, female abdominal sternum VIII. Nymph: 23, head outline and setation, dorsal view (tusks omitted); 24, left mandible, inner view (arrow indicates stout spine near inner margin); 25, detail of right mandibular tusk, inner view (arrow indicates subapical outer indentation); 26, paraprocts, ventral view (right half setation omitted).

Tortopsis andaki sp. nov.

Figs 27–52

<https://zoobank.org/DFE52BBC-7153-4EA3-89B5-403D069-BF5EE>

Type material. Holotype male imago (CEBUC) from Colombia, Caquetá, Curillo, río Caquetá, 220 m, 01°01'44"N; 75°55'9"W, 20.ix.2018, LG Dias, MC Zúñiga, B Toro, JP Chaux and C Molineri cols. Paratypes: 14 male and 5 female imagos (IBN). Paratypes: 10 male 5 female imagos and 1 nymph (CEBUC); 10 male and 2 female imagos (MUSENUV), same data as holotype.

Diagnosis. *Tortopsis andaki* sp. nov., known from imagos of both sexes and nymph, can be distinguished from all other species of the genus by: In adults, 1) fore wing length 9.2–9.6 mm (male), 9.5–9.7 mm (female); 2) wings hyaline slightly tinted with purplish gray, veins purplish gray; 3) parastylus curved dorsally, more markedly on apical third, with a longitudinal ventral furrow (Figs 33–34); 4) penis slender, apical spine rounded and flattened (Figs 36–38); 5) parastyli receptors on sternum VIII with V-shaped sockets (Figs 39–40); 6) head shaded gray among ocelli, occiput with small gray marks (Fig. 27). The nymphs are characterized by: 1) mandibles with 7 stout small spines on inner margin of tusk see arrows in Fig. 44; 2) occiput whitish without gray markings (Fig. 31); 3) wingbuds completely whitish (Fig. 32).

Male imago. Length (mm): body, 10.8–11.0; fore wing, 9.2–9.6; hind wing, 4.0; foreleg, 4.9–5.0; cerci, 25.0–26.0. General coloration whitish shaded dorsally with light purplish gray (Figs 27). Head. Whitish shaded with light brownish gray among ocelli and on scape and pedicel, flagellum hyaline. Thorax. Pronotum hyaline shaded with purplish gray dorsally as in Fig. 27. Mesonotum whitish yellow shaded with purplish gray along medioparapsidal sutures and contiguous area, medial zone paler. Metanotum whitish yellow shaded gray dorsally. Thoracic pleura and sterna whitish. Legs whitish shaded with light purplish gray on fore tibia and fore tarsi. Wings: Membrane hyaline very lightly tinted with purplish gray, longitudinal and cross veins purplish gray. Abdomen. Translucent white, shaded slightly but extensively with purplish gray on terga, including medial area; some markings are darker (Fig. 27) and a thin medial dark line is present along most terga; terga VIII–X shaded with darker gray. Abdominal sterna whitish; sternum IX shaded very slightly with gray on median area. Genitalia (Figs 28–29, 33–38): whitish except apex of parastylus

yellowish; parastylus curved dorsally more markedly on apical third (in lateral view, Figs 28 and 34–35) and with a longitudinal ventral furrow along its entire length (Fig. 33); forceps and penis translucent white, shaded very slightly gray on forceps; penis slender, spine at apex of penis rounded and flattened (Figs 36–38). Caudal filament whitish translucent.

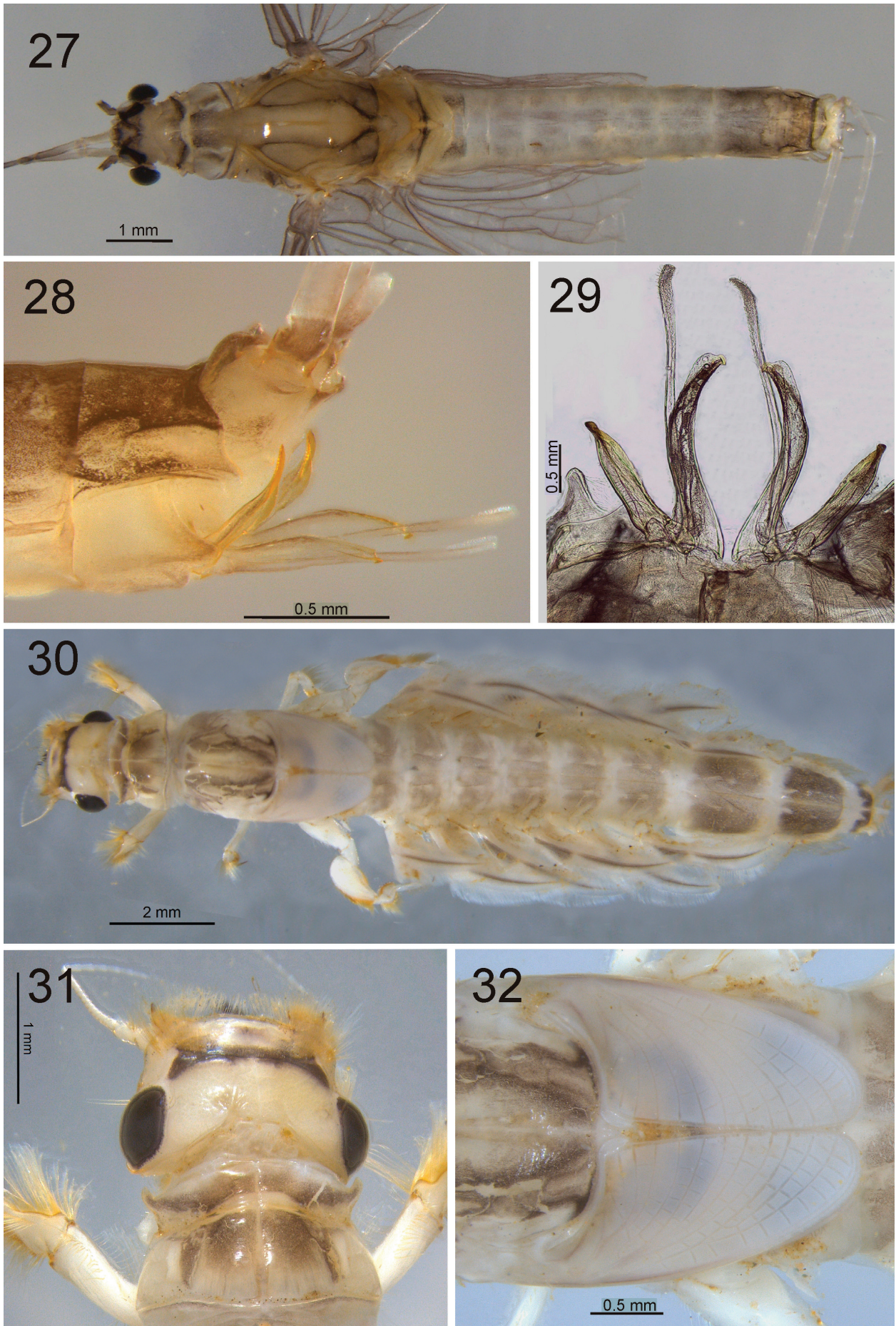
Female imago. Length (mm): body, 10.0–10.1; fore wing, 9.5–9.7; hind wing, 3.9–4.0; cerci, 3.0. General coloration as in male but shading more strongly marked. Head black between ocelli, with small light gray marks on occiput. Wings membrane hyaline slightly tinged with whitish yellow, veins brownish. Abdomen shaded with gray on terga. Parastyli receptors on sternum VIII with sockets (Figs 39–40) with sinuous lateral margin and acute distal corner (V-shaped).

Nymph (mature female). Length: body, 17.0 mm. General coloration yellowish white shaded with gray dorsally (Fig. 30). Head. Whitish shaded with gray among ocelli, occiput completely white without marks (Fig. 31). Antennae and mouthparts whitish except apex of tusks brownish, and spines yellowish. Finger-like gill present near base of maxillae (Figs 47–48). Mandibular tusks with 7 small stout spines on inner margin, basal to subdistal tubercle (see arrows in Fig. 44); outer margin of tusk with row of 16–17 stout spines (Figs 42–44). Thorax. Pronotum, anterior ring shaded gray widely including the anterolateral pointed projections, posterior ring as in Fig. 30, both with a median pale line. Meso- and metanotum with gray and brown marks, lighter on median area; wing buds whitish, longitudinal veins hyaline, except at the basal region, with the anal and costal margins brownish. Thoracic pleura and sterna whitish. Legs whitish, with yellowish setae and apex of tarsal claws yellowish (Figs 49–52). Abdomen. Abdominal color pattern as described for the adults (Fig. 30). Gills: vestigial gills I translucent; gills II–VII well developed, formed by a pair of large whitish lamellae, the outer (dorsal) lamellae of each pair is shaded with gray on a medio longitudinal band (Fig. 30), the inner (ventral) lamellae of each pair present only a thin brownish line along trachea. Caudal filaments yellowish white.

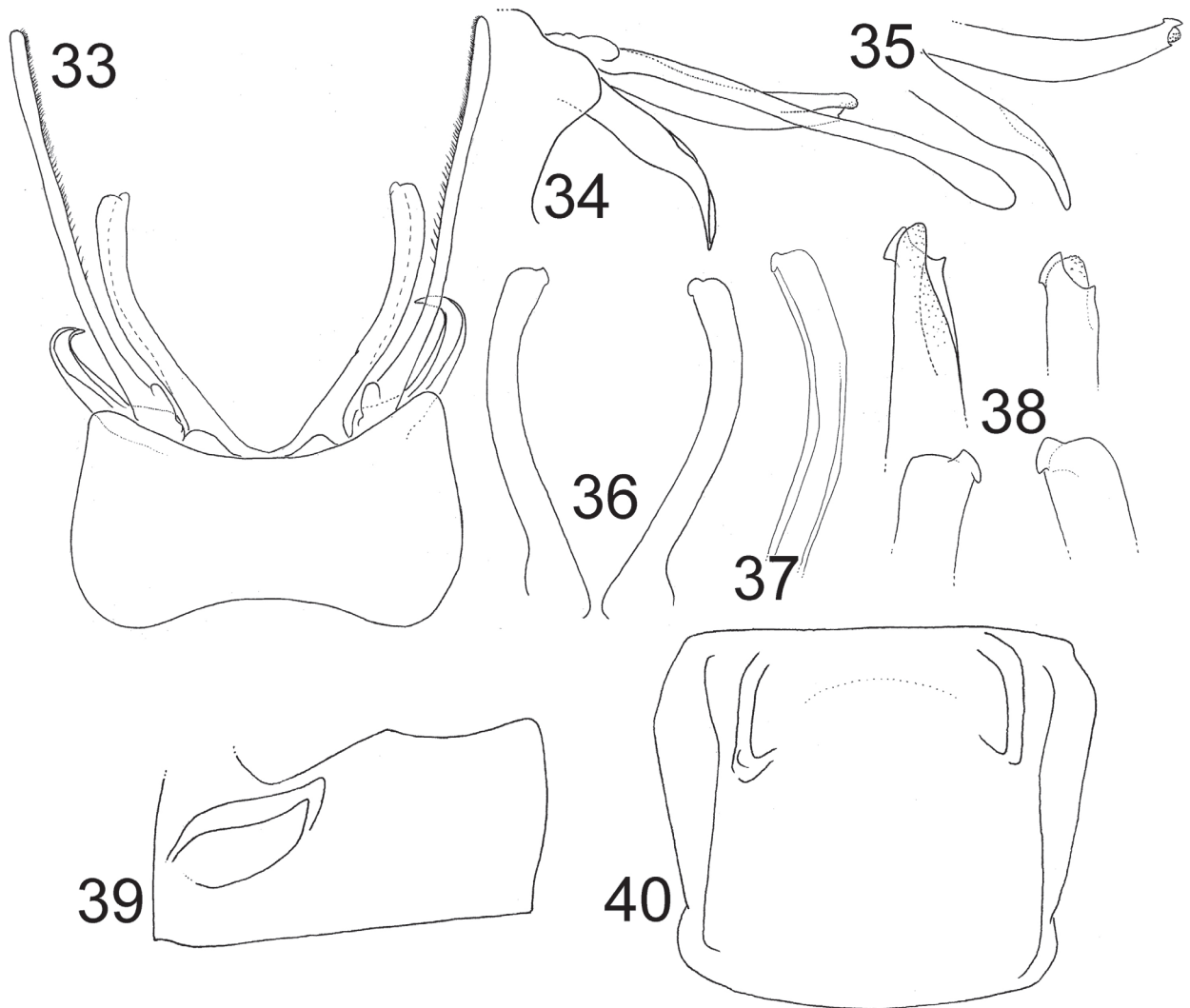
Egg. Subcircular, white. Length, 330–350 µm; width, 280–300 µm.

Etymology. This species is dedicated to the Andaki indigenous people, an American ethnic group that inhabited the upper Caquetá River basin.

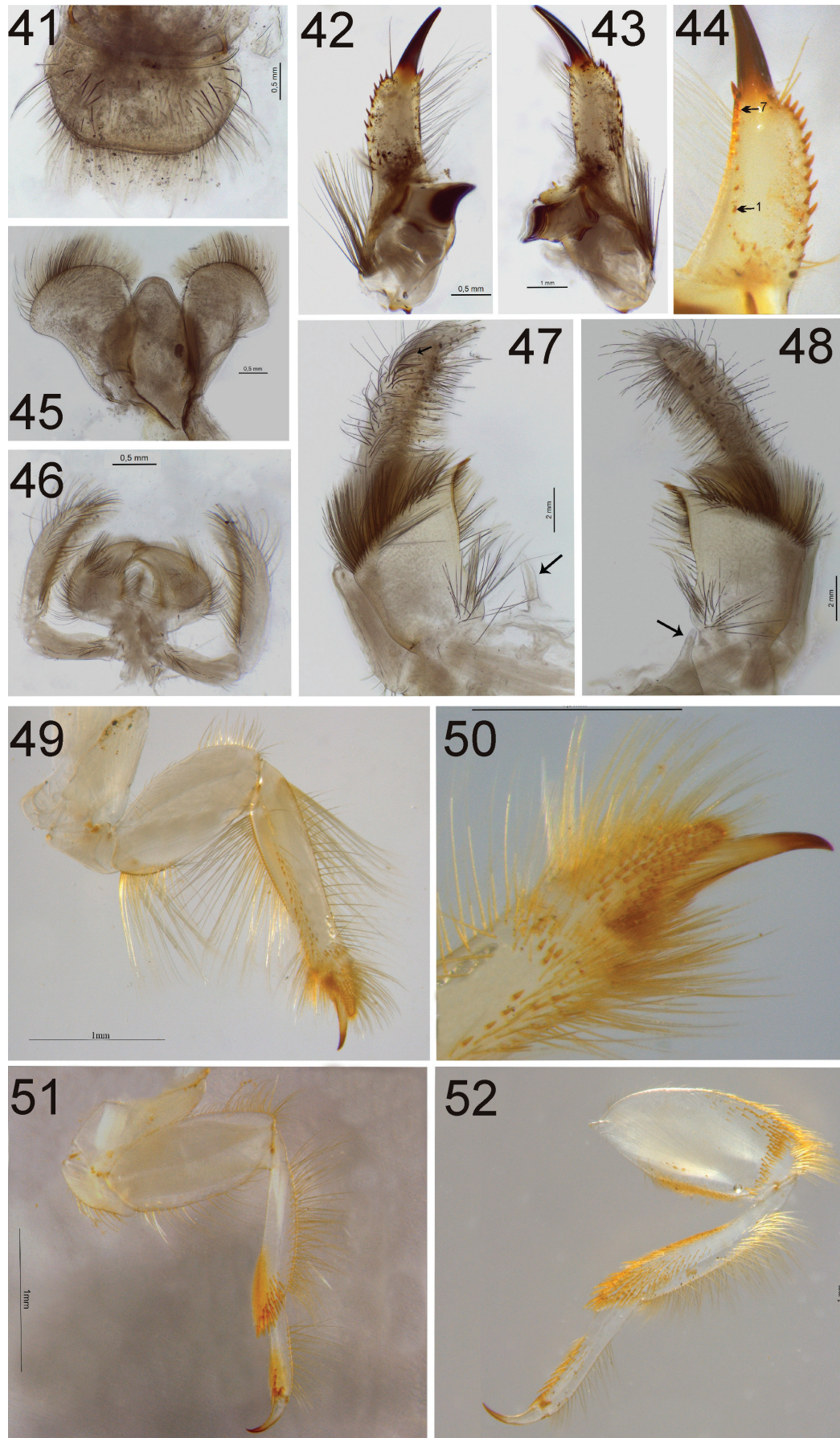
Distribution. Known only from the type locality.



Figures 27–32. *Tortopsis andaki* sp. nov., male imago. 27, general dorsal view; 28, genitalia, lateral view; 29, genitalia, ventral view. Nymph (female): 30, general view; 31, head and pronotum; 32, part of mesonotum and wingbuds.



Figures 33–40. *Tortopsis andaki* sp. nov., imago. Male: 33, male genitalia, ventral view; 34, genitalia, lateral external view; 35, same, inner view; 36, penis detail, dorsal view; 37, penis, detail, ventral view; 38, details of penis apex, dorsal view above, ventral view below. Female: 39, abdominal sternum VIII, lateral view; 40, same, ventral view.



Figures 41–52. *Tortopsis andaki* sp. nov., female nymph. 41, labrum, dorsal view; 42, left mandible, ventral view; 43, right mandible, ventral view; 44, same, detail (arrows indicate first and last spine of the inner marginal row); 45, hypopharynx, ventral view; 46, labium, ventral view; 47, right maxilla, ventral view (arrow indicates membranous gill); 48, left maxilla, ventral view; 49, fore leg, dorsal view; 50, detail of fore tarsal claw, dorsal view; 51, middle leg, dorsal view; 53, hind leg, dorsal view.

***Tortopsis limoncocha* Molineri 2010**

Figs 53–55

Material. 1 male imago (CEBUC) from Colombia, Caquetá, Curillo, río Caquetá, 220 m, 01°01'43.5"N, 75°55'8.6"W, 20.ix.2018, LG Dias,

MC Zúñiga, B Toro, JP Chaux and C Molineri cols. — *Notes:* The single male collected in Caquetá River (Colombia) presents a straight parastyli only curved at the apex (Figs 54–55), a feature that characterizes *T. limoncocha*. Body and wing size, and coloration also fit the diagnosis of this species (Molineri 2010).

Key to *Tortopsis* adults (modified from Molineri 2010 and Gonçalves et al. 2011)

Male adults (Not keyed: *T. bruchianus* (Navás) is only known from female; *T. parishii* (Banks) is poorly known from the holotype male)

- 1 Penis flattened and somewhat widened distally (Molineri 2008: figure 2)2
- Penis cylindrical, long and slender (Molineri 2010: figures 41, 57, 64)4
- 2(1) Penis slightly widening from base to apex *T. puella* (Pictet)
- Penis abruptly widened in distal half3
- 3(2) Parastyli entire, without furrow; apical spine of penis pointed (Molineri 2008: figure 2) *T. obscuripennis* (Domínguez)
- Longitudinal furrow on parastyli (Figs 19–21), apical spine of penis semicircular (Fig. 20) *Tortopsis toro* sp. nov.
- 4(1) Parastyli with a longitudinal ventral furrow (Figs 63–65, 77 in Molineri 2010); apical penial spine rounded and flattened (Molineri 2010: figures 66, 77)5
- Parastyli entire; apical spine hooked (e.g., Figs 54–55)6
- 5(4) Apex of penis simple, with a semicircular sclerotized structure (Molineri 2010: figure 66) .*T. spatula* Molineri
- Apex of penis more complex, with sclerotized two-pointed portion, and a membranous rounded lobe (Figs 36–39) *T. andaki* sp. nov.
- 6(4) Parastyli relatively straight in lateral view, may be curved on apical 1/47
- Parastyli curved from its base (Molineri 2010: figure 42)8
- 7(6) Parastyli curved on apical 1/4 (Figs 54–55) *T. limoncocha* Molineri
- Parastyli straight (Gonçalves et al. 2011: figure 10) *T. canum* Gonçalves et al.
- 8(6) Parastyli strongly curved, the main axis of the apical third forms an angle of 90° with the corresponding axis of the basal third (Molineri 2010: figure 42) *T. unguiculatus* (Ulmer)
- Parastyli curved smoothly from its base 9
- 9(8) Known distribution restricted to southern Bolivia and Northwestern Argentina *T. sarae* (Domínguez)
- Known distribution restricted to North America *T. primus* (McDunnough)

Female adults (female of *T. unguiculatus* is inadequately known and could not be included in the key)

- 1 Wings dark, all veins shaded strongly with gray or black; fore wing length 19.5–20.5 mm; color pattern on head and pronotum as in figure 89 in Molineri (2010) *T. obscuripennis* (Domínguez)
- Wings lighter, yellowish to brownish, some veins may be dark, mainly in costal region, fore wing length variable; color pattern on head and pronotum not as above2
- 2(1) Parastyli receptors on abdominal sternum VIII elongated and with slightly sinuous anterior margin (Molineri 2010: figures 49–52), anterior margin reaching the anterior margin of the sternum; head with a whitish zone behind median ocellus; North American species3
- Parastyli receptors on abdominal sternum VIII generally not elongated (except *T. spatula*), margin not sinuous (Molineri 2010: figures 46–48); head shaded black or gray behind median ocellus; South American species ...4
- 3(2) Head shaded gray to black between lateral ocelli and with few small markings in occiput (Molineri 2010: figure 90); abdomen with thin blackish medial line on terga III–IX; on sternum VIII, anterior and posterior margins of sockets run more or less parallel to each other (Molineri 2010: figures 50–52) *T. puella* (Pictet)
- Head shaded gray in a V-shaped line between lateral ocelli and another medial thin line running anteriorly toward median ocellus, occiput without traceable marks (but material studied was very faded); abdomen with thin blackish medial line on terga I–VIII; on sternum VIII, anterior and posterior margins of sockets are divergent toward medial line (Molineri 2010: figure 49) *T. primus* (McDunnough)
- 4(2) Parastyli receptors V-shaped (Fig. 39)5
- Parastyli receptors C-shaped (Molineri 2010: figure 88)6
- 5(4) Occiput shaded with gray (Molineri 2010: figure 92); parastyli receptors as Figs 61–62, 78 in Molineri 2010, eggs with a long coiled thread *T. spatula* Molineri
- Occiput whitish, without shading; parastyli receptors as in Figs 39–40, eggs without thread.. *T. andaki* sp. nov.



Figures 53–55. *Tortopsis limoncocha* Molineri, male imago. 53, general dorsal view; 54, genitalia, lateral view; 55, same, ventral view.

- 6(4) Head with gray or black markings on occiput (Fig. 53).....7
- Occiput without marks or with very light gray and small markings near hind margin (Molineri 2010: figures 87, 91).....8
- 7(6) Color pattern on head and pronotum as in Fig. 88 (in Molineri, 2010); parastyli receptors small, not reaching more than half of VIII sternum (Molineri 2010: figure 88)..... *T. limoncocha* Molineri
- Color patten on head and pronotum as in Fig. 1 in Gonçalves et al. 2011; parastyli receptors wide, reaching nearly all extension of sternum VIII..... *T. canum* Gonçalves et al.
- 8(6) Eggs orangeish, known distribution: Central Argentina..... *T. bruchianus* (Navás)
- Eggs yellowish..... 9
- 9(8) Sockets on sternum VIII relatively small (about 1/3 or less of total sternal length); known distribution: NW Argentina and S Bolivia.....*T. sarae* (Domínguez)
- Sockets on sternum VIII relatively large (about 1/2 of total sternal length); known distribution Colombia–Caquetá *T. toro* sp. nov.

***Tortopus coreguaje* sp. nov.**

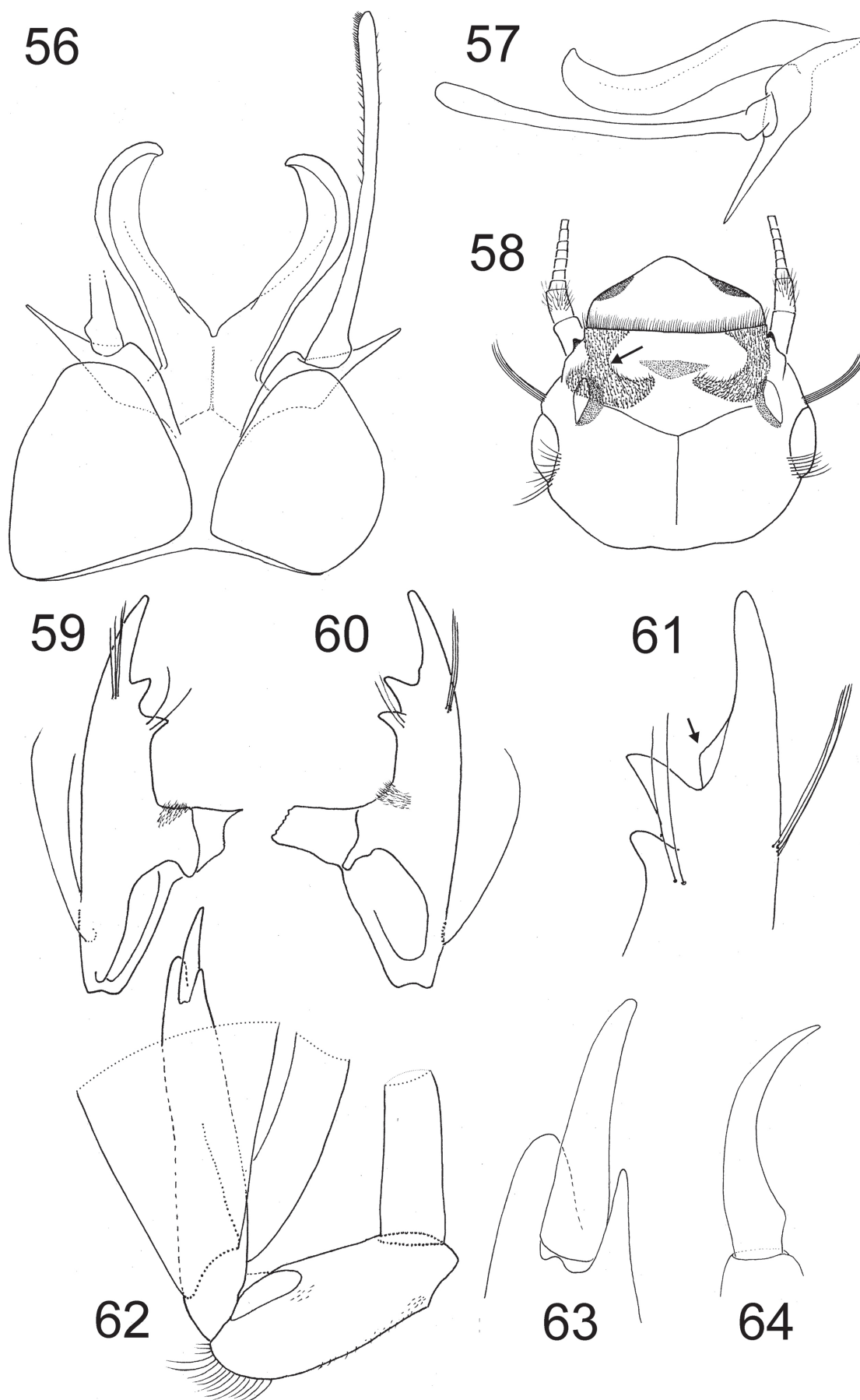
Figs 56–70

<https://zoobank.org/18B80D42-AF18-48C9-8A99-BFB627C-04CA9>

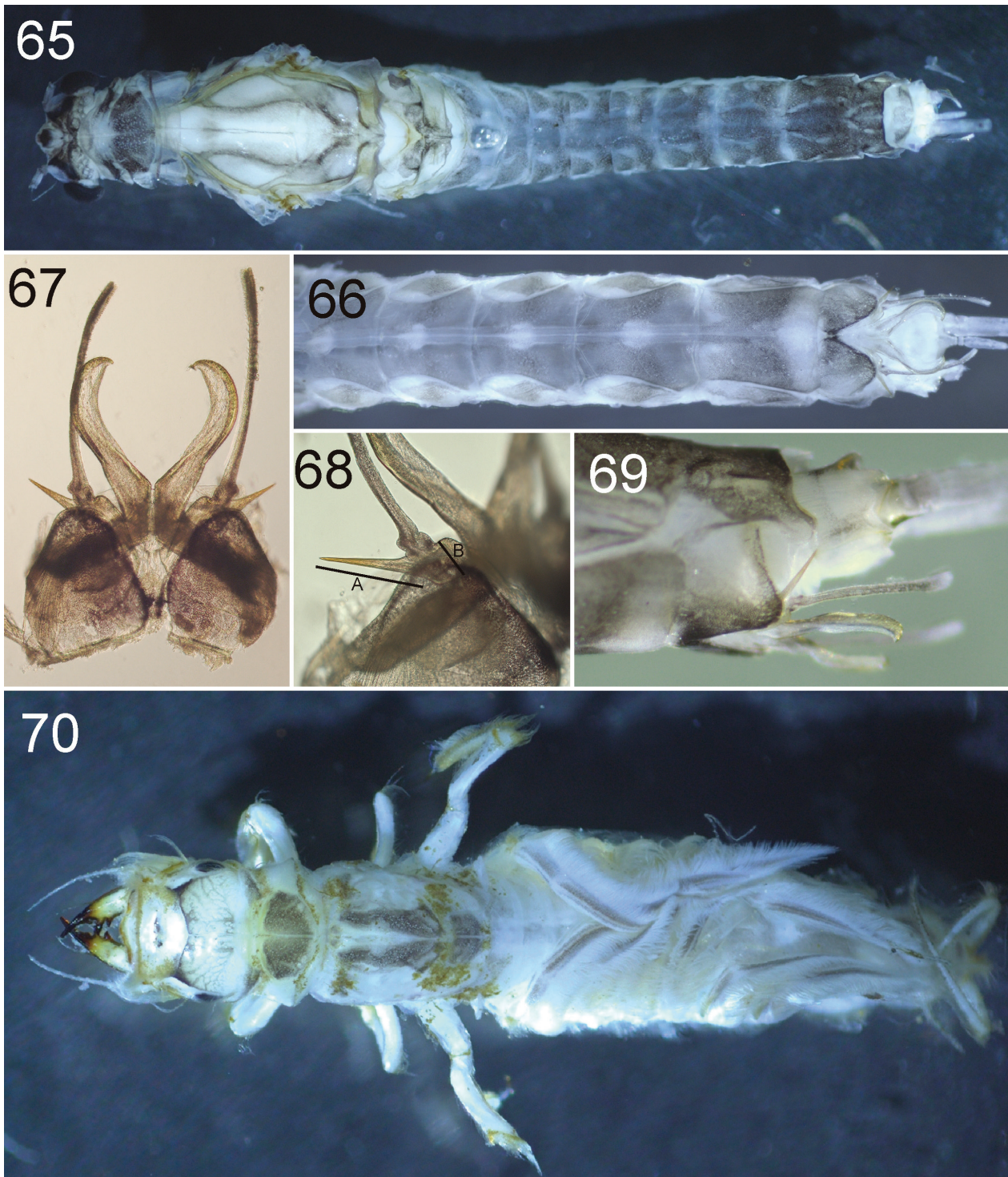
Type material. Holotype male imago (CEBUC) from Colombia, Caquetá, Curillo, río Caquetá, 220 m, 01°01'44"N; 75°55'9"W, 20.ix.2018, LG Dias, MC Zúñiga, B Toro, JP Chaux and C Molineri cols. Paratypes: 1 male imago (IBN) and 1 nymph (CEBUC), same data as holotype.

Diagnosis. *Tortopus coreguaje* sp. nov., known from male imago and nymph, can be distinguished from all other species of the genus by the following combination of charac-

ters: In male imagos, 1) fore wing length 10.9–11.0 mm (male); 2) pale wings, veins translucent gray; 3) pedestal short with relatively large, straight and very long parastyli (Figs 56–57, 67 and 69) (parastylus 2.6 times the length of pedestal base, in lateral view the parastylus reach 0.37 of the length of forceps from base); 3) ventral knob on forceps base relatively small; 4) penis relatively thin at base (fused portion), not strongly expanded distally (Figs 56 and 67); and 5) relatively dark species, head shaded stronger between lateral ocelli, occiput shaded gray except on pale medial line (Fig. 65). In the nymph: 1) inner protuberances on mandibular tusk almost contiguous (Figs 59–61); 2) frontal tuft of setae with irregular sinuous form (arrow in Fig. 58); 3) ventral and dorsal projection at the apex of fore tarsus subequal in length (Fig. 63).



Figures 56–64. *Tortopus coreguaje* sp. nov., male imago. 56, genitalia, ventral view (left forceps omitted); 57, genitalia, lateral view. Nymph: 58, head outline and setation, dorsal view (tusks omitted); 59, right mandible, inner view; 60, left mandible, inner view; 61, same, detail of apex (arrow indicates blade-like projection); 62, fore leg, ventral view (rows of filtering setae delineated); 63, fore tarsal claw; 64, hind tarsal claw.



Figures 65–70. *Tortopus coreguaje* sp. nov., male imago: 65, general view, dorsal; 66, abdomen, ventral view; 67, genitalia, ventral view; 68, detail of parastylus (A=length of parastylus, B= length of pedestal); 69, genitalia, lateral view. Nymph: 70, general view, dorsal.

Male imago. Length (mm): body, 11.0; fore wing, 10.9–11.0; hind wing, 4.8; cerci, 28.0. General coloration yellowish white shaded with gray (Fig. 65). Head. Coloration yellowish white shaded widely with gray on dorsum, shaded stronger between lateral ocelli, except around median ocellus and hind margin (Fig. 65). Antennae: scape and pedicel yellowish white completely shaded with gray, flagellum hyaline. Thorax. Pronotum with anterior ring hyaline, shaded with black posterolaterally

(Fig. 65); posterior ring shaded gray except on sublateral areas and medial line. Mesonotum whitish yellow shaded with gray along medioparapsidal sutures and shaded with black on a V-shaped mark between posterior scutal protuberances (Fig. 65). Metanotum whitish yellow shaded gray medially. Thoracic pleura and sterna whitish yellow, with grayish small marks medially on furcasterna. Legs yellowish white shaded with grayish (fore legs broken off and lost). Wings: Membrane hyaline except basally

slightly grayish, longitudinal and cross veins translucent shaded slightly with gray. Abdomen. Abdominal segments translucent-hyaline. Tergum I shaded gray anterolaterally (Fig. 65), terga II–IX shaded more extensively towards rear segments, except on: medial line of terga II–VII, and two pale marks (one submedian and one sublateral) in II–IX; tergum X shaded more completely with gray. Abdominal sterna shaded widely with brownish gray except on intersegmental membranes and around gill sclerites (Fig. 66). Genitalia (Figs 56–57, 67–69): sternum IX shaded with black on posterior margin and at both sides of the median furrow; relatively large parastyli orangeish, straight and long (Fig. 56); parastylus 2.6 times the length of pedestal base, in lateral view the parastylus reach 0.37 of the length of forceps from base; forceps and penis yellowish white, shaded gray on forceps. Caudal filament whitish translucent.

Male nymph (immature). Length: body, 9.5 mm; cerci, 2.5 mm; caudal filament, 2.0 mm. General coloration whitish shaded with gray (Fig. 70). Head. Head shaded with gray in net-shaped pattern posteriorly to epicranial suture (Fig. 70); anteriorly to this suture with two small lateral irregular-sinuuous-shaped tufts of short setae (anterior to lateral ocelli, arrow in Fig. 58); frontal ridge relatively straight in dorsal view (Fig. 58); fronto-clypeal region acutely projected medially (Fig. 58); a tuft of ca. 10 long setae basally to antennal condyle; and a group of 3 long setae anterior to eye and 6 long curved setae posterior to eye (Fig. 58). Mandibular tusks straight (Figs 59–61, 70), with 2 subdistal rigid inner setae and ca. 12 weaker but long setae forming a basal arc (Figs 59–61); inner margin with two large and contiguous tubercles, the subdistal directed ventrally and the submedian directed medially (Figs 59–61), also a small blade-like projection present basally to distal spur (arrow in Fig. 61). Maxillae with small triangular ventral gill. Hypopharynx and labium whitish. Thorax. Pronotum with narrow anterior ring (0.28 of total length of pronotum); antero-lateral corners acutely projected; shaded with black laterally on anterior

ring and medially on posterior ring. Meso and metanotum shaded black medially except medial line; wingbuds whitish shaded gray only at base of costal area. Legs. Whitish with yellowish setae and sclerotized portions. Foreleg with tibia-tarsus flattened, remnants of the suture between tarsus and tibia visible, tarsal dorso-distal projection 0.5 of total length of claw (Figs 62–63); ventral surface of fore tibia with rows of long filtering setae as in Fig. 62; fore femur with anterobasal short U-shaped row of filtering setae and an immediately posterior small group of ca. 7 long rigid and simple setae; apex of fore tarsus with ventral and dorsal projections subequal in length (but the ventral is much thinner, Fig. 63). Middle leg with long setae on anterior and posterior (functionally ventral and dorsal, respectively) margins of femur, anterior margin of tibia and tarsus; apical third of tibia and tarsus completely covered with strong setae, apex of tibia with a brush of thick setae ventrally. Hind leg with short strong setae on posterior margin, and transverse subdistal row of short setae on dorsal surface; hind tibia and tarsus with long setae on posterior margin, anterior margin covered with short and strong setae. All tarsal claws slender and curved, without denticles. Abdomen (Fig. 70). Gill I single, small and elongated, remaining gills well developed and double. Terga II–IX with medio-longitudinal row of setae; abdominal sterna with lateral margins strongly covered with setae, increasing in number posteriorly, sterna V–VI also with row of shorter setae on posterior margin; sternum IX with a row of few long setae on posterior half, along medial line. Cerci with rows of setae at each article, mainly on basal fourth; terminal filament much thinner and with whorls of setae almost on its entire length.

Etymology. This species is dedicated to the Coreguaje community inhabiting along the piemont and lower parts of the Caquetá River and its affluents.

Distribution. Known only from the type locality.

Key to males of the species of *Tortopus* (Modified from Molineri 2010 and Molineri et al. 2012)

Male adults (not keyed: *T. circumfluus* Ulmer is only known from female)

- 1 Pedestal longer than wide, with small basal extension; parastylus reduced, expressed only as an acute point (Molineri et al. 2012: figure 7) ***T. ipixuna* Molineri et al.**
- Pedestal wider than long (not including the parastylus), with a well-developed basal extension, parastylus at least 1/2 the length of pedestal (Molineri et al. 2012: figure 2) **2**
- 2(1)** Parastylus length shorter than length of its base (Molineri 2010: figures 10, 19) **3**
- Parastylus length subequal to base to more than 2 times length of base (Molineri 2010: figures 2, 24, 36) **4**
- 3(2)** Abdominal terga and sterna shaded brownish gray; parastylus relatively short (Molineri 2010: figure 12) ***T. bellus* Lugo-Ortiz & McCafferty**
- Abdominal terga shaded gray, sterna much less marked; parastylus slightly longer (Molineri 2010 figure 22) ***T. harrisi* Traver**
- 4(2)** Parastylus relatively thin from base (Molineri 2010: figures 2–3) **5**
- Parastylus thick at the base (Molineri 2010: figures 24, 36–37) **6**
- 5(4)** Parastylus 1.5–2.5 the length of pedestal base (Molineri 2010: figures 2–3); in lateral view the parastylus reach at most 0.27 of the length of forceps from base; penis relatively thin ***T. igaranus* Needham & Murphy**

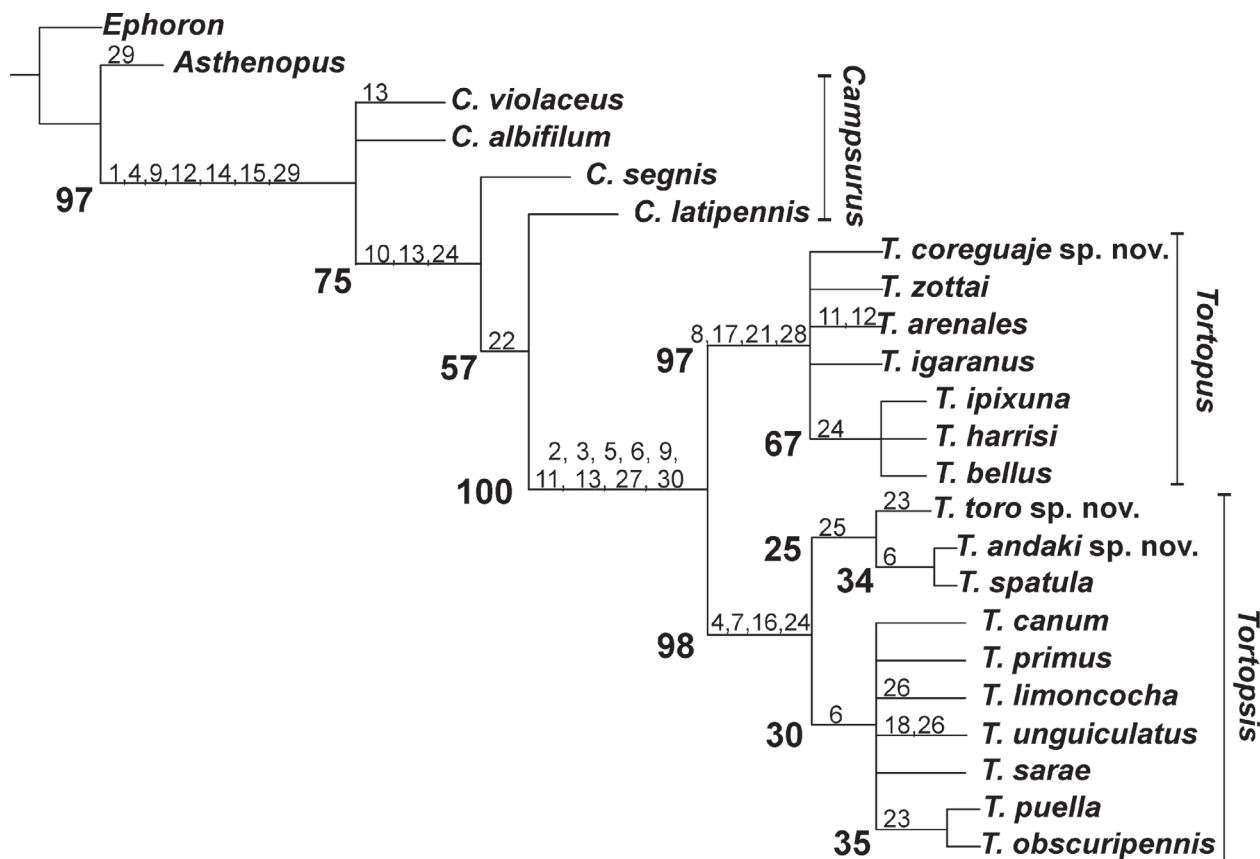


Figure 71. Unique tree found under implied weighted parsimony (k=3). Three taxa are not included (*Tortopus circumfluus*, *Tortopsis bruchianus*, and *Tortopsis parishii*). Numbers above nodes are character changes (Appendix 1). Numbers on nodes indicate support values (symmetric Jackknifing).

- Parastylus 2.6 the length of pedestal base (A/B in Fig. 68), in lateral view the parastylus reach 0.37 of the length of forceps from base, penis wider..... ***T. coreguaje sp. nov.***
- 6(4)** Abdomen shaded with gray on dorsum, ventrally paler; penis relatively thin basally (Molineri 2010: figure 24).
..... ***T. zottai* (Navas)**
- Abdomen shaded strongly with brownish gray, dorsally and ventrally; penis with a wider base (Molineri 2010: figure 36)..... ***T. arenales* Molineri**

3.2. Phylogenetic relationships

Searches including all the taxa (under both, equal and implied weights) resulted in many possible resolutions with their strict consensus showing a large polytomy that included all the species in *Tortopus* and *Tortopsis*. To study the relationship among species in *Tortopus* and *Tortopsis*, we deactivated three of them (*Tortopus circumfluus*, *Tortopsis bruchianus* and *Tortopsis parishii*). These species present many missing entries in the matrix, as they are poorly known from their original descriptions and were not collected again. When deactivated, a unique and more resolved tree is found under implied weights (Fig. 71), with high support for the sister relationship between *Tortopus* and *Tortopsis*, and for each genus as separate monophyletic groups. Equal weights found both genera as monophyletic but relationships inside them were poorly resolved.

Equal and implied weighting strategies found the same synapomorphies for the group *Tortopsis* + *Tortopus*, and

for each genus, as detailed below. Synapomorphies defining the clade *Tortopus* + *Tortopsis*: Female wing veins thickened (character 2:1); female hind wing with anastomosed anal sector (character 3:1); female parastyli receptors on abdominal sternum VIII, formed by paired sockets (character 5:2); female parastyli receptors, U-shaped (character 6:1, later change to other forms); legs of imagos of both sexes (except male forelegs), reduced and distorted (character 9:1); male genitalia with two segments on forceps (character 11:1); with outer projected pedestals, forming a dorsal and pointed parastylus (character 13:3); knob at forceps base present (character 27:1); and egg without polar caps (character 30:0).

The synapomorphies of *Tortopus* are: Female parastyli receptors with long furrows anterior to sockets (character 8:1); male gonopore associated with a sclerotized margin (character 17:1); penis flattened (character 21:1); and male abdominal sternum IX almost separated in two portions by a median notch (character 28:2). Finally, the synapomorphies of *Tortopsis* are: Female fore wing veins



Figures 72–75. 72, Caquetá River at Curillo; 73, detail of a U-shaped tunnel, broken along its longitudinal axis; 74, detail of river shore with tunnel's openings; 75, a nymph in a broken burrow.

on R sector without longitudinal or intercalary veins between IR and R_1 (some fused crossveins may be present, forming 1 or 2 short attached marginal intercalaries) (character 4:2); female parastyli receptors with sockets opening towards median line (character 7:1); male gonopore associated with a claw-like or spatulated spine (character 16:1); and male genitalia with long parastyli (character 24:3).

Some species groupings were found inside each genus. For example in *Tortopus*, three species (*T. ipixuna*, *T. harrisi* and *T. bellus*) share male genitalia with very short parastyli (character 24:1). In *Tortopsis*, *T. toro sp. nov.* is sister to the pair *T. andaki sp. nov.* + *T. spatula*, because the three species present a longitudinal furrow on parastyli (character 25:1). The remaining species in *Tortopsis* (*T. canum*, *T. primus*, *T. limoncocha*, *T. unguiculatus*, *T. sarae*, *T. puella* and *T. obscuripennis*) form a monophyletic group defined by female parastyli receptors C-shaped (character 6:0). Finally, two species (*T. puella*

+ *T. obscuripennis*) are joined by their apically wide penis (character 23:1), a feature independently acquired by *T. toro sp. nov.*

4. Discussion

4.1. Taxonomy

The form of the penis in *T. toro sp. nov.* is similar to *T. obscuripennis* and *T. puella*, because of the abrupt distal widening. On the contrary, the semicircular spine at the apex of penis is similar to *T. spatula*, as well as the presence of a furrow along the parastyli (this last feature also shared with *T. andaki sp. nov.*). The nymph of *T. toro sp. nov.* is similar to *T. obscuripennis* in the shape and setation of tusks, but the subapical outer indentation on

the tusks of *T. toro* **sp. nov.** is more marked (Figs 6, 10, 25) if compared with the other species (Molineri 2010: figure 96). Additionally, *T. obscuripennis* presents much darker wingbuds, with more extensive gray shading on developing veins (Molineri 2008).

Tortopsis andaki **sp. nov.** is similar to *T. spatula*, but can be readily distinguished because *T. andaki* **sp. nov.** presents a more complex penis apex, with a rounded membranous lobe and two sclerotized projections (Figs 36–37). The nymph of *T. spatula* is unknown and the nymph here attributed to *T. andaki* **sp. nov.** has 7 stout small spines on the inner margin of the tusk and wingbuds completely whitish, the finding of the nymph of *T. spatula* will allow further comparisons between these species.

Tortopsis limoncocha was previously known from a single locality in the Ecuadorian portion of the Amazonas basin (Molineri 2010) and another locality in Brazilian Amazon (Molineri et al. 2012), so here is firstly recorded for Colombia.

The nymph here described for *Tortopus coreguaje* **sp. nov.** is the second nymph known for the genus; the nymph of *T. harrisi* (Molineri et al. 2010) presents the inner protuberances on mandibular tusk more separated (by a distance subequal to tusk width at this zone), the frontal tuft of setae is rounded in outline, and the fore tarsal distal projections are of different length (the ventral is much shorter). The male imago of *T. coreguaje* **sp. nov.** is similar to *T. igaranus*, because of the long and slender parastyli, nevertheless *T. coreguaje* **sp. nov.** is a larger and darker species, the penis are wider and the parastyli are longer (see key).

4.2. Phylogenetic relationships

Our results improved the resolution reached in the last published phylogenetic hypothesis (Molineri 2010), by adding six more species and four characters. Also, the few sister species pairs obtained by Molineri (2010), were not recovered again. Other interesting relationships were recovered instead, that are sustained on the evolution of some characters in male genitalia. For example, in *Tortopus*, the extreme shortening of the parastylus defines the politomy containing three species (*T. ipixuna*, *T. harrisi*, *T. bellus*). On the other hand, it was surprising that the extremely wide penis present in three species of *Tortopsis* (*T. toro* **sp. nov.**, *T. obscuripennis* and *T. puel-la*) was not recovered as a synapomorphy for these three species. We expect that future knowledge of the nymphs of the species known only from adults will allow the inclusion of numerous new characters, but for the moment, this was not possible.

5. Conclusions

The biodiversity study in the Caquetá region (Figs. 72–75) has been restricted for approximately 50 years, since many locations remained inaccessible because of public

order problems. This fieldwork was carried out precisely in the post–conflict period in Colombia, and shows the richness of the region and the need to expand the knowledge of its biodiversity as the first step for the implementation of future conservation and management proposals.

6. Authors' contributions

The authors have no conflicts of interest to declare that are relevant to the content of this article. All authors contributed equally to the manuscript.

7. Acknowledgements

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Supplementary material

File 1

Authors: Molineri C, Dias LG, Zúñiga MC (2021)

Data type: .pdf

Explanation note: Matrix, list of characters and states (modified from Molineri 2010), and list of synapomorphies defining the unique shortest tree (Fig. 71) found under implied weighting parsimony..

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