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Research Article

Integrative taxonomic revision of *Campylocia* (mayflies: Ephemeroptera, Euthyplociidae)

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A morphological comparison of type and non-type material of species of *Campylocia*, including their junior synonyms, was conducted, in addition to neighbour joining based on K2P distances and Bayesian inference analyses of 376 bp of the mitochondrial gene cytochrome oxidase I (COI) of recently collected specimens. Results revealed the lack of distinguishing characters between *C. bocainensis* and *C. dochmia* supported by the molecular analysis, where the overlap of intra- and interspecific genetic divergences suggested genetic flow among individuals. *Campylocia burmeisteri* is revalidated as a senior synonym of Brazilian south-eastern species *C. bocainensis* and *C. dochmia* and of *E. guntheri*, formerly a synonym of *C. anceps*. *Campylocia burmeisteri* is redescribed based on material from its type-locality, Nova Friburgo, Rio de Janeiro State. Two new species, *C. demoulini* sp. nov. and *C. araca* sp. nov., are described from the Amazon rain forest and a third species, *C. orosi* sp. nov., is described from Costa Rica. Possible cryptic species and the utility of egg morphology in the taxonomy of *Campylocia* are discussed for the first time for the genus. A key to the identification of adult stages of *Campylocia* is provided based on male genitalia and egg morphology

http://zoobank.org/urn:lsid:zoobank.org:pub:6779DC2C-DB98-41DF-8C52-FF97366AAF7A

Key words: cryptic species, identification key, K2P distances, new species, new synonym, redescription of species, scanning electron microscopy

Introduction

The Neotropical mayfly genus *Campylocia* was established by Needham & Murphy in 1924 and currently includes three valid species: *C. anceps* (Eaton, 1883) known from the Amazon rain forest, *C. dochmia* Berner & Thew, 1961, and *C. bocainensis* Pereira & Da-Silva, 1990 from the Atlantic rain forest, south-eastern Brazil. The genus has a complicated taxonomic history and includes the highest number of species synonyms in the family Euthyplociidae. *Campylocia* is considered a senior synonym of *Longinella* Gros & Lestage, 1927 and a single species stands as senior synonym of four of the seven species names placed in *Campylocia*.

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The type species of the genus, *Campylocia anceps*, one of the oldest species known in Euthyplociidae, was described as *Euthyplocia anceps* Eaton, 1883 based on one male imago from Rio Mauhes (probably Maués-Açu River in Amazonas State), Brazil. The same year, *Euthyplocia burmeisteri* Hagen, 1888 was described from two male imagos from Nova Friburgo, Rio de Janeiro State, Brazil. A third species, *E. intercalata* Banks, 1918, was later described based on four females from Colombia, Guyana, and French Guiana. *Euthyplocia guntheri* Navás, 1920 was described from Santos, São Paulo State, Brazil based on a male imago. Needham and Murphy (1924) established the genus with *C. anceps* as the type species and included *E. burmeisteri*, *E. intercalata*, *E. guntheri*, and one new species *C. ampla* Needham & Murphy, 1924, described from three females from Peru and Brazil. Because most of these descriptions

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lacked details and figures, identification of *Campylocia* species was a difficult task and many works discussed the usage of venation characters to diagnose species of the genus (Demoulin, 1952, 1953; Ulmer, 1932, 1939, 1942). Having concluded that wing characters were variable, all existing species were synonymized with *C. anceps.* Ulmer (1920a) firstly synonymized *C. burmeisteri* (Hagen, 1888), but was never sure of this synonym (Ulmer, 1932, 1942). In 1932, Ulmer added *C. ampla*, *C. guntheri* (Navás, 1920), and *C. intercalata* (Banks, 1918) to the list of synonyms of *C. anceps*, which were repeated by Ulmer (1942) and Demoulin (1952), in his comprehensive study of the Euthyplociidae. In all these studies, type material was rarely studied and synonymies were based on the original descriptions and the authors' interpretation of forewing venation. Two other species of *Campylocia* were described later, both from Brazil. *Campylocia dochmia* was described from Minas Gerais State from male and female imagos and *C. bocainensis* from São Paulo State from male and female imagos and nymphs (Berner & Thew, 1961; Pereira & Da-Silva, 1990).

In the present paper, all *Campylocia* species were studied in an integrated approach including a morphological analysis of over 900 specimens and six out of the seven primary types, ultrastructural analyses of the egg chorion, and cytochrome oxidase I sequences of 24 specimens.

Materials and methods

Material examined

The investigated material was preserved in 70–90% alcohol and deposited in the following institutions: Coleção Entomológica Prof. José Alfredo Pinheiro Dutra, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (DZRJ); Museu Nacional do Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ); Coleção Zoológica Norte Capixaba, Universidade Federal do Espírito Santo, São Mateus, Brazil (CZNC); Museu de Zoologia da Universidade Estadual de Santa Cruz, Ilhéus, Brazil (MZUESC); Instituto de Pesquisas da Amazônia, Manaus, Brazil (INPA); Coleção Zoobotânica James Alexander Ratter, Universidade do Estado do Mato Grosso, Nova Xavantina, Brazil (UNEMAT); Museu de Zoologia de São Paulo, Universidade de São Paulo, São Paulo, Brazil (MZSP); Stroud Water Research Center, Avondale, USA (SWRC); Florida Agricultural & Mechanical University, Tallahassee, USA (FAMU); Entomological Research Collection, Purdue University, West Lafayette, USA (PERC). Material was examined either directly through loans or collection visits, or indirectly through photographs as is the case for the holotype and paratypes of *C. ampla* (Insect Collection, Cornell University, Ithaca, USA), one syntype of *C. intercalata* (Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA), and holotype of *C. anceps* (The Natural History Museum, London, UK). A list of the type-material is given with each new species description. Details of specimens used for molecular studies are given in Table SI and further analysed material is given in Table S2 (see online supplemental material, which is available from the article's Taylor & Francis Online page at http://dx.doi.org/10.1080/14772000.2017.1291543)

Molecular analyses

DNA extraction was performed using a modified protocol of the DNeasy® Blood and Tissue kit (QIAGEN, 2011). One or two legs (nymphs) or a leg with thoracic muscles (imagos) were incubated with proteinase k at 55°C for 48 hours without maceration of the specimens. A fragment of the mitochondrial gene cytochrome oxidase I (COI), 376 bp, was amplified using primer sequences published by Simon et al. (1994) for C1-J-1718 (5'-GGAGGATTTGGAAATTGATTAGTTCC) and Folmer, Black, Hoech, Lutz, and Vrijenhoek (1994) for HC0-2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA). PCR was performed as follows: initial denaturation > 94°C for 3 min; 35 cycles > 94°C for 1 min; 50°C for 1 min; 72°C for 2 min; and final elongation > 72°C for 7 min. Electrophoresis of amplified products in a 1% agarose gel to assure the correct size fragment was conducted with successful amplifications sent to Macrogen Inc. (South Korea) for purification and sequencing of both DNA strands using the same primers. Electropherograms were aligned, checked by reading mistakes and manually edited using Sequencher 4.1.4 (Gene Codes Corporation) to produce a consensus sequence. Correct amplification of the targeted gene was confirmed using BLAST (Basic Local Alignment Search Tool) against GenBank sequences (Altschul et al., 1997).

COI sequences were aligned using CLUSTALW (Thompson, Higgins, & Gibson, 1994) implemented on MEGA 5.2.2 (Tamura et al., 2011) using the default parameters of gap opening and extension. A pairwise matrix of Kimura-2-Parameters (K2P, Kimura, 1980) modelled divergences were calculated and submitted to a Neighbour-joining distance analysis. Pairwise divergences matrix can be found in Table SI of the supplemental material. Four independent Bayesian Inference analyses were conducted using MrBayes 3.2.2 (Huelsenbeck & Ronquist, 2001), each with four chains run for 5,000,000 generations with a sample frequency of 500. Sampled parameter distributions were investigated in Tracer v1.6 (Rambaut, Suchard, Xie, & Drummond, 2014) to assess the adequacy of the 25% burn-in. The most suitable evolutionary model for this analysis was investigated in jModelTest 2.1.6 (Darriba, Taboada, Doallo, & Posada, 2012; Guindon & Gascuel, 2003) using the Akaike criterion (Akaike, 1974), the model GTR+G+I was selected.

Scanning electron micrographs

Eggs submitted to scanning microscopy were dehydrated in graded ethanol series, dried by critical point-method and mounted with double-sided tape on SEM stubs prior to coating. Some eggs of *C. anceps* and all eggs of *C. orosi* sp. nov. and females of a population from French Guiana were placed on 100% ethanol without going through a graded series and were not subjected to critical point but left to dry naturally on the stubs prior to coating.

Scanning microscopy photographs were taken at Universidade Federal do Espírito Santo (UFES), Fundação Oswaldo Cruz (FIOCRUZ), and the Division of Plant Industry (DPI), Florida Department of Agriculture and Consumer Services, Gainesville.

Species descriptions

Photographs were taken with a digital camera coupled to a stereomicroscope and images at different focal planes stacked using the program CombineZP (Hadley, 2010). Photos were edited using Adobe Photoshop CS6 (Adobe Systems Inc.), from which vector illustrations were made, and image plates arranged using Adobe Illustrator CS6 (Adobe Systems Inc.). Photographs were taken at Universidade Federal do Rio de Janeiro (UFRJ) and Florida A&M University (FAMU). Egg terminology followed Koss (1974). Measurements, shape, and chorion structure of the egg were given and, when available, the overall shape of the egg mass (oviposition) was also described.

Results

Molecular analysis

Pairwise K2P divergences between all 24 *Campylocia* specimens ranged from 0.0% to 20.8% (mean = 13.2%). Bayesian inference and Neighbour-joining analyses recovered similar topological results. Specimens were grouped into lineages based on the congruence between the two phylogenetic methods, intra- and inter-lineage K2P genetic divergences, and morphological homogeneity. Thus, analysis of the COI sequences recovered eight lineages of *Campylocia* (Fig. 1) with maximum intralineage divergence of 10% (Table 1) and interlineage divergence within the genus of 8.7–20.8% (mean = 14.7%). Most lineages are distributed in the Amazon forest (two of them also occurring in the Cerrado), while one lineage included specimens restricted to the Atlantic forest of

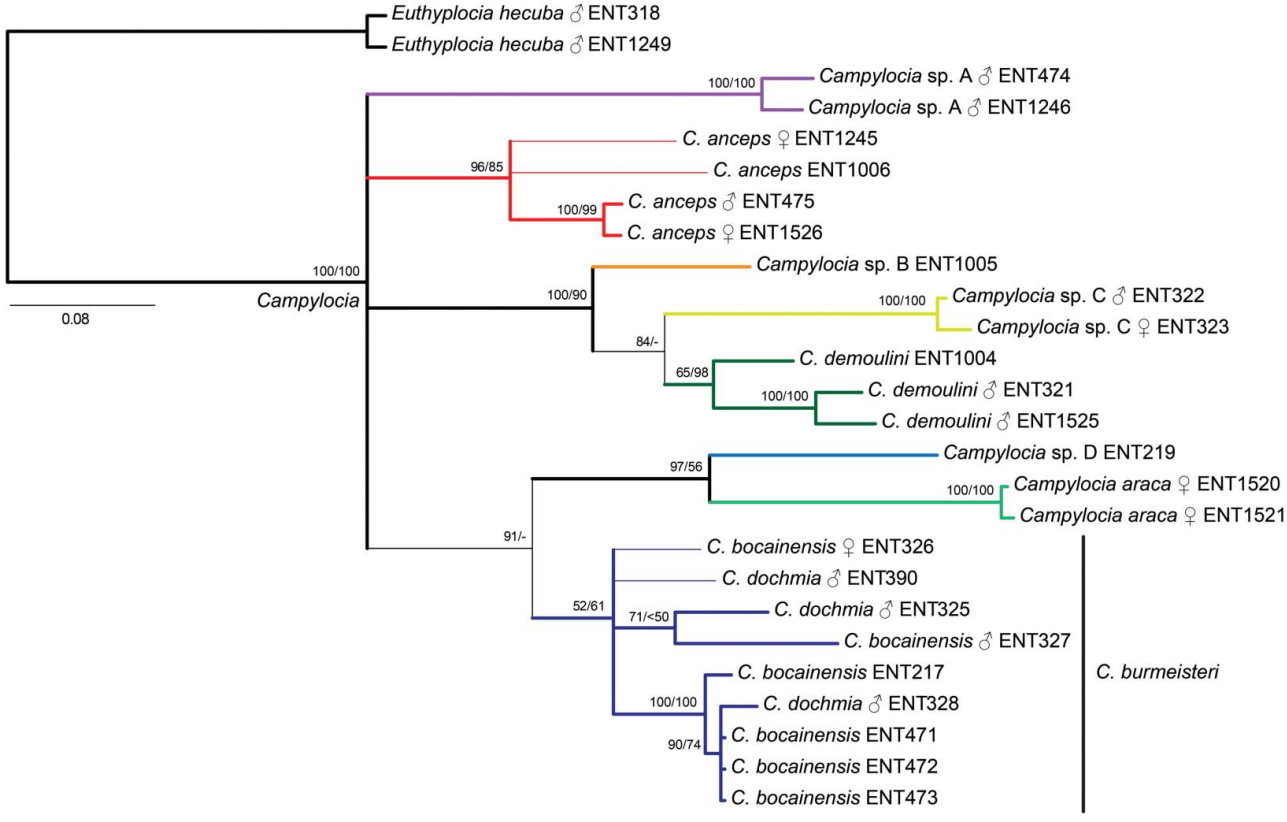

Fig. 1. Bayesian phylogram of COI sequences (376 bp) amongst individuals of *Campylocia*, values of posterior probability/bootstrap are given above branches. Branches in bold were also recovered on the neighbour joining dendrogram of K2P distances. Specimens whose gender is not specified correspond to nymphs. For further information on the recovered *Campylocia* lineages see Tables 1 and S1.

Table 1. Lineages recovered with the Bayesian and NJ phylogenetic analysis of COI sequences of *Campylocia*, with respective range of intralineage divergence, identification, and distribution.

mtDNA lineage	Intralineage K2P divergence	Identified species	Distribution
I	3.7%	*Campylocia* sp. A	Atlantic forest (BA)
II	1.1–9.0% (mean = 7.1%)	*C. anceps*	Amazon forest (AM) and Cerrado (MT, GO)
III	-	*Campylocia* sp. B	Cerrado (MT)
IV	1.4%	*Campylocia* sp. C	Amazon forest (AM)
V	3.3–7.2% (mean = 5.8%)	*C. demoulini* sp. nov.	Amazon forest (AM)
VI	-	*Campylocia* sp. D	Amazon forest (RR)
VII	0.3%	*C. araca* sp. nov.	Amazon forest (AM)
VIII	0.0–10.0% (mean = 5.8%)	*C. burmeisteri*	Atlantic forest (RJ, SP)

south-eastern Brazil (lineage VIII) and another from the Atlantic forest of north-eastern Brazil (lineage I).

Species descriptions and redescriptions

The redescription of *C. burmeisteri* is based on material from its type locality, Nova Friburgo, Rio de Janeiro State, although material from several other localities was also available for study and variations regarding these populations are given. *Campylocia demoulini* sp. nov. is a widespread species and abundant material was available for study; hence, for the type series only specimens from the same locality, Reserva Florestal Adolpho Ducke, Manaus, Amazonas State, were chosen. Two nymphs from Presidente Figueiredo associated to this species by the molecular analysis were also added as paratypes and used on the nymph description. New geographic records for species are marked with asterisk (*) and a map of *Campylocia* species distribution is given on Fig. 2. A list of additional analysed material is available at Table S2 (see online supplemental material).

Although nymphs of *Campylocia* are easily identified to genus, they are difficult to identify at the species level, so a diagnosis is not given. Species identification can only be made with certainty in winged stages.

Campylocia Needham & Murphy, 1924

Campylocia Needham & Murphy, 1924:25 (Type: *Euthyplocia anceps*); Ulmer, 1942:103 (= *Longinella*); Berner & Thew, 1961:329 (literature review); Domínguez, Molineri, Pescador, Hubbard & Nieto, 2006:235 (literature review)
Longinella Gros & Lestage, 1927:158 (Type: *Euthyplocia guntheri*)

Diagnosis

Imago: (1) Sexual dimorphism on sectors R and M of forewings; (2) Forewings with at least one long ICu vein (Figs 9, 11, 13, 15, 17); (3) Base of long ICu connected to CuA (Figs 9, 11, 13, 15, 17); (4) Hindwings triangular, about half the length of forewings (Figs 10, 12, 14, 16, 18); (5) Hindwings with fork of MP on basal region of wings (Figs 10, 12, 14, 16, 18); (6) Forceps with only one segment (Figs 27–33); (7) Median filament of male developed.

Nymph: (1) Head wider than long (Fig. 7); (2) Antennae short, shorter than 3× length of tusks (Fig. 7); (3) Clypeal projection short, not pronounced (Fig. 7); (4) Lateral margins of labrum divergent (Fig. 39); (5) Right mandible

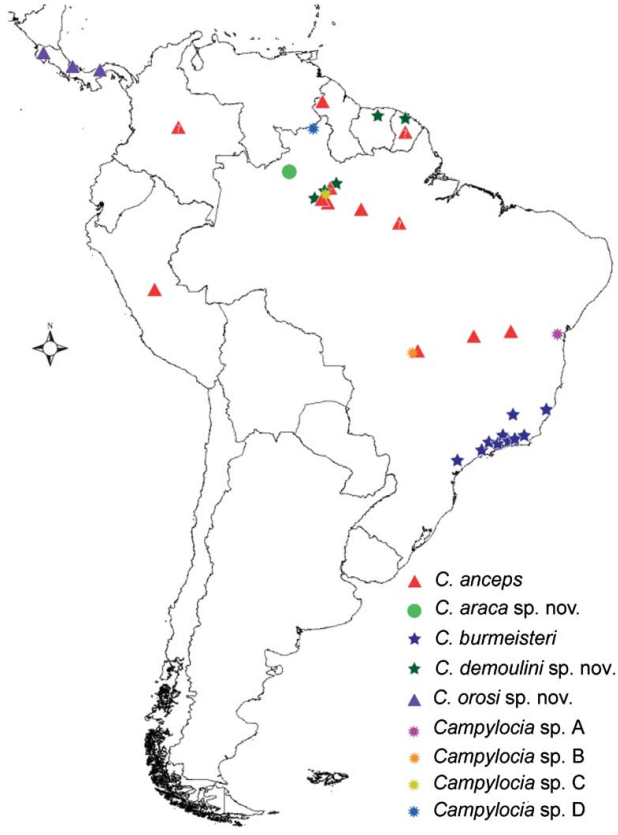

Fig. 2. Distribution of *Campylocia* on America. Country records with unknown locality marked with '?'. Colours of species and lineages (except for *C. orosi* sp. nov.) are according to Fig. 1.

with 4 denticles on outer incisors and 2 denticles on inner incisors and left mandible with 4 denticles on outer incisors and 3 denticles on inner incisors (Figs 42, 43); (6) Tusks long, about 3× or more length of head (Fig. 7); (7) Tusks slender, width about 1/10 of length (Fig. 7); (8) Tusks without distal sinuosity (Fig. 7); (9) Spine-like setae spread throughout dorsal area and external margin of tusks (Fig. 7); (10) Apex of tusks truncate (Fig. 7); (11) Maxillary palp with 3rd segment 7–9× longer than wide (Fig. 45); (12) Maxillary palp with 3rd segment without apical projection (Fig. 45); (13) Labial palpi with 3rd segment apically projected (Fig. 44); (14) Pronotum with anterolateral projections long, acute and narrow, reaching more than half length of eyes (Fig. 7); (15) Pronotum with anterior margin of flanges forming a long and acute projection (Fig. 7); (16) Tarsi without projection; (17) Tibia I with short projection, not reaching half the length of tarsi (Fig. 7); (18) Tibia I with setae on margins of projection; (19) Gill I with two lamellae.

Male Imago

Head (Figs 3–6): Eyes black; ocelli white surrounded by black ring.

Thorax (Figs 3–6): Pronotum wider than long; presence of a membranous elevated ridge on posterior margin lightly tainted with black. Prosternum with a median crest; crest dividing into two oblique ridges distally, forming a triangle with the posterior margin of prosternum.

Wings (Figs 9–18): Membrane translucent, veins purplish. Forewings with cubital area with 1–3 ICu veins; fork of MA on same level or distal to fork of Rs. Hindwings with vein C folding over Sc, forming a groove to effect coupling with forewings; R1 rising from base of the wing; MP forked.

Legs: Forelegs with tarsi II–V long. Middle legs with coxae shaded with black; middle and hind femora shaded with black.

Abdomen (Figs 19–23): Terga with dark tinge, except two pairs of drop-shaped spots, one on medioapical region and one on medial region; a medial longitudinal line is usually present. Caudal filaments white shaded with black; setae on apical portion of cerci.

Genitalia (Figs 27–38): Forceps with one segment, inner margin and distal portion with several short simple setae.

Female imago. Similar to male imago, except: forelegs absent in females of all analysed species of *Campylocia*. Legs are probably lost during the moult from subimago to imago. Pronotum without elevated ridge on posterior margin. Sexual dimorphism in wings, females usually with additional pairs of veins on radial and medial sectors of forewings.

Egg. Usually barrel shaped except for *C. demoulini* sp. nov. in which it is shaped as an erythrocyte (Figs 46, 48, 50, 52–54, 56). Chorion tuberculate and/or with raised ridges (Figs 46–57). Two type III polar caps (Figs 46, 48, 50, 52–54, 56).

Nymph

Head (Fig. 7): Fronto-clypeal suture concave; clypeus projected anteriorly. Eyes black, ocelli white with inner margin black. Antennae shorter than tusks.

Mouthparts (Figs 39–45): Labrum: lateral margins divergent, anterior margin with median shallow emargination; longitudinal sub-basal row of simple long setae; bordered by simple setae, more so on distal margin; medial subapical row of thinner and shorter simple setae near distal margin. Mandibles: light coloured, tusks, incisors and mola darker. Tusks long, sickle shaped, apex truncated, length almost 3× that of head; several long slender setae on lateral margins, denser on outer margin, rows of setae forming a filtering network on inner margin, dorsal surface and outer lateral margin with several spine-like setae. Left mandible: 4 denticles on outer and 3 on inner incisors; prosthecae sclerotized, bifid. Right mandible: 4 apical denticles on outer and 2 on inner incisors; prosthecae absent. Maxillae: 3 apical and one subapical strong longer denticle, inner margin distally with 2 rows of not so strong setae; lateral margins with row of simple setae, more so on inner margin; apical margin with inner half covered by dense rows of shorter simple setae; palpi with many long simple setae, particularly on inner margin of 2nd and 3rd segments; 3rd segment long, at least 6× longer than wide. Labium: glossa and paraglossa densely covered with simple long setae ventrally; glossa teardrop shaped; paraglossa meet above glossa. Palpi with long simple setae, particularly on outer margins; 3rd segment of palpi with several short strong setae dorsally, segment projected on outer apical margin. Hypopharynx: lingua cordiform with deep medial emargination on distal margin; distal margin with rows of short simple setae; superlingua with simple long setae on distal half of lateral margin; dense setae on distal margin; ventral surface covered with setae.

Thorax (Fig. 7): Collar on pro- and mesonotum. Pronotum with anteroapical projections long, acute and narrow, reaching more than half the length of the eyes; pronotum with flanges – lateral extension of body – with anterior margin forming an acute projection. Prosternum with a pair of oblique ridges forming a triangle with posterior margin.

Legs (Fig. 7): Foretibiae with short anterior projection. Tarsal claws without denticles.

Abdomen (Figs 24, 25): Terga almost completely shaded with dark tinge, except for a pair of drop-shaped spots on medioapical region of terga, a pair on median region, and a pair near lateral margins; a longitudinal

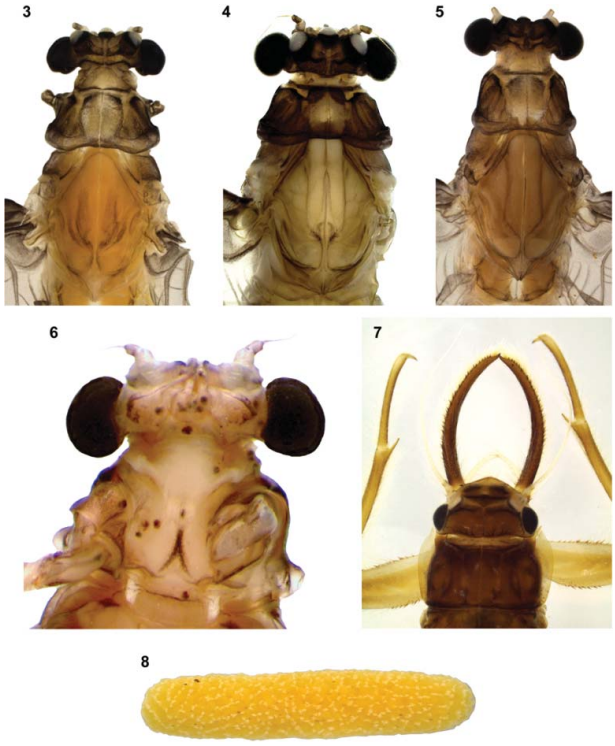

Fig. 3–8. Head and thorax of male imago: (3) *C. anceps*, (4) *C. burmeisteri*, (5) *C. demoulini* sp. nov., (6) *C. orosi* sp. nov. Head and thorax of mature nymph: (7) *C. demoulini* sp. nov. Egg mass: (8) *C. anceps.*

medial line is also usually present. Gills purplish; gills I bifurcated, vestigial; dorsal lamellae wider and longer than ventral lamellae; apex of dorsal lamellae rounded, ventral lamellae filiform. Gills II–VII long, longer than 1.5× length of subjacent abdominal segment, narrow, lanceolate, fringed throughout extension; both lamellae of same length. Caudal filaments light coloured shaded with black; with setae throughout extension, setae more dense basally.

Campylocia anceps (Eaton, 1883)
(Figs 3, 8–10, 19, 27, 34, 46, 47)

Campylocia anceps Needham & Murphy, 1924:26 (male, female); Gros & Lestage, 1927:158; Ulmer, 1932 (= *Campylocia ampla*, = *E. intercalata*); Ulmer, 1942:101 (male, female); Kimmins, 1960:307 (type). *Euthyplocia anceps* Eaton, 1883:38 (male); Ulmer, 1920a:3 (nymph)(= *Euthyplocia burmeisteri*); Ulmer, 1920b:106; Lestage, 1924:121. *Campylocia ampla* Needham & Murphy, 1924:25 (female); Gros & Lestage, 1927:158. *Euthyplocia intercalata* Banks, 1918:10 (female). *Campylocia intercalata* Gros & Lestage, 1927:158.

Diagnosis

Imago: (1) Forewings usually with 1 ICu vein (Fig. 9); (2) Abdominal colour pattern with terga III–VIII with two pairs of drop-shaped spots, one on medioapical region, one on medial region and a medial longitudinal line on terga II–IX with lateral margins with darker black shade (Fig. 19); (3) Styliger plate rounded (Fig. 27); (4) Penes narrow with distal half smoothly curved outwards, shaped as an 'Y' (Figs 27, 34); (5) Eggs barrel shaped (Fig. 46); (6) Egg chorion sculpture tuberculate (Figs 46, 47).

Male imago. Length (mm): Body = 10–16; Forewings = 10–13; Hindwings = 4–5; (5 specimens measured and Eaton, 1883/1888 information).

Head (Fig. 3): Whitish with darker areas particularly on posterior margin and near lateral ocelli. Antennae whitish, pedicel tainted with black, flagellum lightly shaded with dark-brown.

Thorax (Fig. 3): Pronotum whitish, meso- and metanotum yellowish. Pronotum shaded with black; collar margin and lateral margins black; pair of black oblique anterolateral lines. Mesonotum with parascutellum shaded with black; black shade around parapsidal sutures; scutellum whitish-yellow. Prosternum with median crest tainted black; triangle of prosternum with equilateral margins or with wider base. Mesosternum yellow.

Wings (Figs 9, 10): Forewings with membrane between C, Sc, and R_1, purplish on basal 2/3 and whitish on distal 1/3; cubital area with one ICu vein; fork of MA distal to fork of Rs; one intercalary vein between IMP/MP_2 and one between MP_2/CuA.

Legs: Whitish. Forelegs with coxae, trochanters, femora, and tibiae heavily shaded with black.

Abdomen (Fig. 19): Whitish tinged with black, except for two pairs of drop-shaped spots, one on medioapical region, one on medial region on terga III–VIII and a medial longitudinal line on terga II–IX, lateral margins of medial line with darker black shade; tergum I only lightly shaded with black, tergum II slightly more shaded than tergum I, both with posterior margins blackish; terga IX–X whitish yellow tainted with black. Sterna whitish, sternum IX whitish yellow.

Genitalia (Figs 27, 34): Styliger plate rounded, whitish. Forceps whitish.. Penes whitish, basal half narrow and quadrangular; distal half narrower than 1/2 length of base, and smoothly curved outwards; apex somewhat sclerotized laterally; membranous lobes absent.

Variations: One male (ENT1248) with head darker.

Female imago. Length (mm): Body = 12–18 (values approximate, all specimens with abdomen bent); Forewings = 16–25.2; Hindwings = 6–8.9; (4 specimens measured).

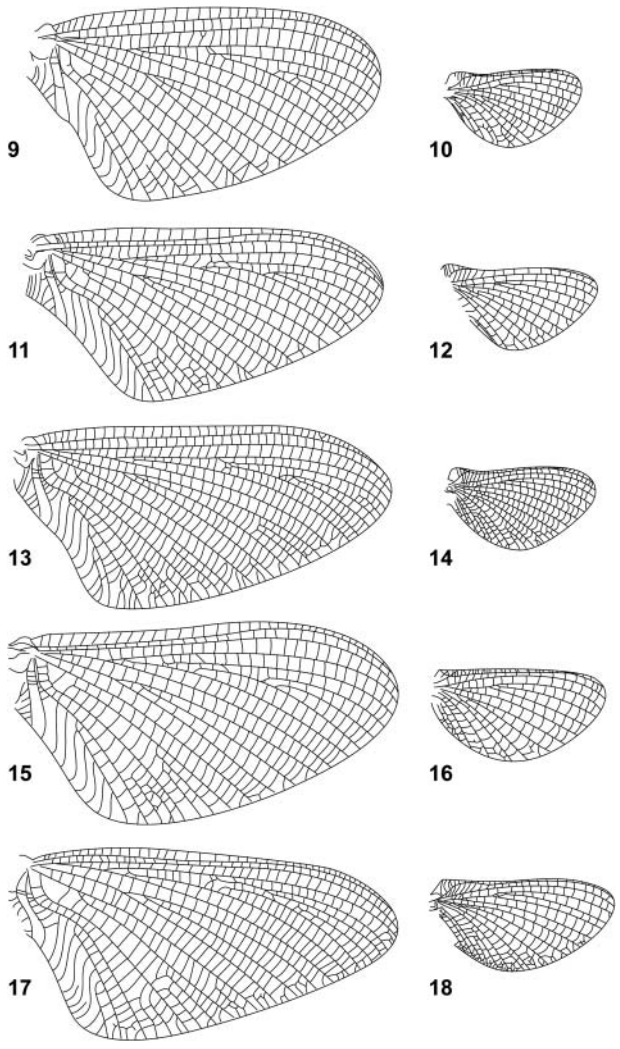

Fig. 9–18. Male imago wings: (9) and (10) *C. anceps*, (11) and (12) *C. araca* sp. nov., (13) and (14) *C. burmeisteri*, (15) and (16) *C. demoulini* sp. nov., (17) and (18) *C. orosi* sp. nov.

Similar to male imago, except: Sexual dimorphism on wings: forewings – 2 intercalary veins between MA_1/IMA; 2 between MA_2/MP_1; 2 between IMP/MP_2; 2 between MP_2/CuA; and one short vein between CuA/ICu; on hindwings – 2 between CuP/A_1. A female from Goiás state also had intercalary between R_3/R_{4+5} of forewing.

Egg. Measurements (μm): Length = 226–257; Width = 168–184. Barrel shaped; chorion tuberculate, diameter 1.3–2.3 and height 1.0–1.6 (Figs 46, 47). Egg mass long, elliptical (Fig. 8).

Nymph. No mature nymphs have been studied; therefore, measurements are not provided.

Head: Yellow with basal half tinged with light brown, wide band tainted with black along lateral branches of epicranial suture, black taint extending to inner margin of eyes; epicranial suture yellow. Antennae yellow.

Fig. 19–26. Abdomen of male imago: (19) *C. anceps*, (20) *C. araca* sp. nov., (21) *C. burmeisteri*, (22) *C. demoulini* sp. nov., (23) *C. orosi* sp. nov.. Abdomen of mature nymph: (24) *C. burmeisteri*, (25) *C. demoulini* sp. nov., (26) *Campylocia* sp. B, lineage III.

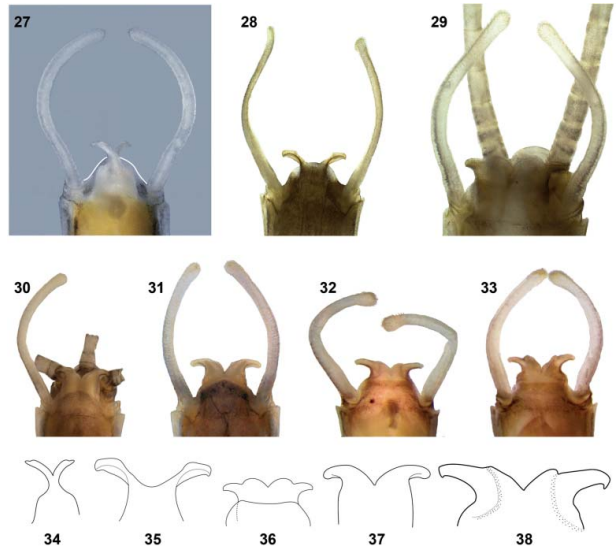

Fig. 27–38. Genitalia of male imago: (27) and (34) *C. anceps*, posterior margin of styliger plate highlighted, (28) and (35) *C. araca* sp. nov., (29) and (36) *C. burmeisteri*, (30), (31) and (37) *C. demoulini* sp. nov., respectively paratype and holotype, (32), (33) and (38) *C. orosi* sp. nov., respectively holotype and paratype.

Thorax: Light brown with black markings. Flanges and anteroapical projections of pronotum yellow; Sterna yellow; triangle on prosternum with lateral sides longer than base.

Legs: Yellow with black markings. Forelegs: coxae shaded with black; femora with longitudinal V-shaded black mark; femora wider on submedial region; several simple long setae; spine-like setae on margins, forming a distinct row on inner margin. Apical projection of tibiae short, not reaching half the length of tarsus, with strong setae on lateral margins of projection; tibiae and tarsi densely covered with simple setae. Middle legs: coxae shaded with black; femora with black subapical marking extending to medial region near the outer margin; long simple setae on dorsal surface and lateral margins; distribution of setae denser on tibiae and tarsi, inner margin of tibiae and tarsi with dense concentration of short plumose setae apically; tibial suture present; tibiae with short apical projection with strong setae on lateral margins. Hind legs: lateral margins and subapical region of femora shaded with black possessing several long simple setae; distoventral surface with area covered by short setae. Tibiae usually with longitudinal black stripe; tibiae and tarsi densely covered by long simple setae; tibiae with wide longitudinal row of shorter simple setae.

Abdomen: Terga yellow shaded with black except for a pair of drop-shaped spots on medioapical margin on terga I–IX; a pair of median region on terga II–IX, on terga X these spots fuse into a pair of longitudinal lines; and a pair of spots near lateral margins; presence of a longitudinal medial line on terga II–IX, line widening toward posterior margin on each terga; lateral margins of this line usually more heavily shaded with black than rest of the terga; posterior margins of terga whitish to translucent. Sterna yellow with lateral margins whitish yellow. Gills purplish white. Caudal filaments yellow, basal segments shaded with black.

Distribution. Colombia, Costa Rica*, Guyana, French Guiana, Peru, and Brazil on the states of Amazonas, Bahia*, Goiás*, Mato Grosso*, and Pará (Banks, 1918; Eaton, 1883/1888; Needham & Murphy, 1924).

Material examined. Table S2 (see supplemental material online).

Discussion. *Campylocia anceps* seems to have been a common and widely spread species on the Amazon region due to its different confirmed synonyms and the extensive bibliography regarding the species. Nevertheless, it seems that this species became more and more scarce during the decades and now could be considered a rare, maybe endangered, species. This species has been misidentified in the past. See discussion under *C. demoulini* sp. nov.

Biology. *Campylocia anceps* was found to co-occur with the new species *Campylocia demoulini* sp. nov. at least in one collecting site (Rio Branquinho, Amazonas State).

Campylocia araca sp. nov. Gonçalves & Salles
(Figs 11, 12, 20, 28, 35, 48, 49)

Diagnosis

Imago: (1) Forewings usually with 1 ICu vein (Fig. 11); (2) Abdominal colour pattern with two pairs of drop-shaped spots, one on mediapical region of terga I–IX and one on medial region of terga II–IX; medial longitudinal line on terga I–IX (Fig. 20); (3) Styliger plate semi-rounded (Fig. 28); (4) Penes narrow with same width from base to lateral projections, strongly curving outwards, lateral projections with sclerotized medial line (Figs 28, 35); (5) Eggs barrel shaped (Fig. 48); (6) Chorion sculpture with tubercles, some elongate almost forming a ridge but never forming a mesh (Figs 48, 49).

Male imago. Length (mm): Body = 13; Forewings = 12; Hindwings = 5 (1 specimen measured).

Head: Yellowish shaded with black. Antennae with scape and pedicel shaded with black, flagellum missing.

Thorax: Yellowish. Pronotum heavily shaded with black; collar and lateral margins black; longitudinal notal suture marked in white. Mesonotum with parascutellum shaded with back; faint black shade along sutures; scutellum whitish yellow. Triangle on prosternum with equal sides. Mesosternum yellow.

Wings (Figs 11, 12): Forewings with membrane between C, Sc and R_1 purplish on basal 1/4; cubital area with one ICu vein; a pair of intercalary veins between IMP/MP_2 and a pair between MP_2/CuA.

Legs: Only one broken foreleg. Leg whitish, heavily shaded with black on femur, tibia and 1st tarsal segment; remaining tarsal segments with faint black shade.

Abdomen (Fig. 20): Whitish shaded with black except for two pairs of drop-shaped spots, one on mediapical region of terga I–IX and one on medial region of terga II–IX; medial longitudinal line on terga I–IX. Tergum X yellowish. Sterna whitish with faint yellowish and blackish shade, lateral margins black. Only one broken cerci, whitish with faint black shade.

Genitalia (Figs 28, 35): Styliger plate semi-rounded, whitish shade with black. Forceps long and slender. Penes whitish, narrow, with same width from base to lateral projections; strongly curving outwards; lateral projections with sclerotized medial line.

Female imago. Length (mm): Body = 16–25.5; Forewings = 18–24; Hindwings = 6.5–9 (5 specimens measured, shorter body measurements are from females with bent abdomens due to oviposition).

Similar to male imago, except: Additional veins: on forewings – a pair between R_3/R_{4+5}, a pair between MA_1/IMA and a pair between MA_2/MP_1. Three females had drop-shaped spots on tergum X.

Egg. Measurements (μm): Length = 240–254; Width = 172–200. Barrel shaped; chorion sculpture with tubercles, some elongate almost forming a ridge but never forming a mesh (Figs 48, 49). Egg mass unknown.

Nymph. Unknown.

Etymology. In apposition to Serra do Aracá, type locality of this species.

Distribution. Brazil, Amazonas State.

Type material. Holotype: 1 male imago: Brazil, Amazonas State, Barcelos, Serra do Aracá, Igarapé do Cobra, BO1, 25.vii.2009, A.P.M. Santos *leg.* (INPA); **Paratypes**: 1 female imago, same data as holotype (CZNC) (DNA voucher ENT1520); 4 female imagos: same data, 27.vii.2009 (CZNC) (DNA voucher ENT1521).

Discussion. Unfortunately, only one male is known and it is possible for the genitalia to be deformed. Because male genitalia and egg morphology are unique between other *Campylocia* and molecular data recovered this population as a different lineage it was decided to describe it as a new species although better understanding of male morphology will only be elucidated after more specimens are collected.

Biology. Specimens were caught in light traps, no further information is available.

Campylocia burmeisteri (Hagen, 1888)
(Figs 4, 13, 14, 21, 24, 29, 36, 50, 51)

Campylocia burmeisteri Needham & Murphy, 1924:25; Ulmer, 1920a:3 (= *Euthyplocia anceps*)

Euthyplocia burmeisteri Hagen, 1888:226 (male, female); *Euthyplocia guntheri* Navás, 1920:414 (male) (NEW SYNONYM); *Campylocia guntheri* Needham & Murphy, 1924:25 (NEW SYNONYM) *Longinella guntheri* Gros & Lestage, 1927:158 (NEW SYNONYM); *Campylocia dochmia* Berner & Thew, 1961:332 (male, female) (NEW SYNONYM); *Campylocia bocainensis* Pereira & Da-Silva, 1990:2 (male, female, nymph) (NEW SYNONYM)

Diagnosis

Imago: (1) Forewings usually with 2–3 ICu veins (Figs 13, 14); (2) Abdominal colour pattern two pairs of drop-shaped spots, one on medioapical region on terga I–IX and one on medial region on terga II–VIII, and a medial longitudinal line on terga I–X (Fig. 21); (3) Styliger plate truncated (Fig. 29); (4) Penes wide, distal half strongly curved outwards and presence of apical membranous lobes (Figs 29, 36); (5) Eggs barrel shaped (Fig. 50); (6) Chorion with raised ridges forming an irregular mesh of 3.0–12.3 μm (Figs 50, 51).

Male imago. Length (mm): Body = 15–21; Forewings = 17–22; Hindwings = 7–9 (10 specimens measured).

Head (Fig. 4): Yellowish heavily shaded with black. Antennae yellowish with light black shade.

Thorax (Fig. 4): Pronotum yellowish, meso and metanota yellow. Pronotum with anterior and lateral margins black; posterior margin tainted with black. Mesonotum with parascutellum shaded with black; black shade around medial longitudinal suture and parapsidal sutures black; scutellum lightly shaded with black. Triangle on prosternum with equal sides. Mesosternum with lateral margin of basisternum shaded with black.

Wings (Figs 13, 14): Forewings with membrane between C, Sc, and R_1 purplish on basal 2/3; cubital area with 2–3 ICu veins; fork of MA only slightly distal to fork of Rs; a pair of long intercalary veins MA_1/IMA, a pair between IMP/MP_2, and one pair between MP_2/CuA.

Legs: Yellow. Coxae and trochanters shaded with black, more heavily on fore and median legs; femora shaded with black, shade heavy on forelegs and forming three longitudinal lines on median and hind legs; tibiae and tarsi of forelegs whitish shaded with black, more so on tibiae.

Abdomen (Fig. 21): Yellow tinged with black. Terga heavily shaded with black except for two pairs of drop-shaped spots, one on medioapical region on terga I–IX and one on medial region, on terga II–VIII, and a medial longitudinal line on terga I–X, wider on tergum X. Sterna with lateral and posterior margins slightly shaded with black.

Genitalia (Figs 29, 36): Styliger plate truncated with median emargination, yellowish. Penes wide, quadrangular, with same width from base to lateral projection; distal half of each penes strongly curved outwards; apex with a somewhat sclerotized spine; membranous lobes present.

Variations: Intercalary veins on forewings more variable than other species of *Campylocia*, specimens sometimes with one intercalary between MA_2/MP_1 and one between CuA/ICu_1.

Female imago. Length (mm): Body = 23–28; Forewings = 26–32; Hindwings = 9–12; (4 specimens measured).

Similar to male imago, except: All females analysed showed 3 ICu; fork of MA could be placed on same level, slightly distal or fairly distal to fork of Rs. Females presented all other long interlacaries described for male and

an additional pair of long intercalaries between MA_2/MP_1. Abdomen with tergum I lacking spots; medial longitudinal line not reaching terga X.

Egg. Measurements (μm): Length = 310–331; Width = 188–216; Mesh = 3.0–12.3. Barrel shaped; chorion sculpture with raised ridges sometimes forming an irregular mesh (Figs 50, 51). Egg mass long, elliptical.

Variations: A wider range of body size can be noticed while studying material from other localities. Females ranged from (mm): body = 27–37, forewings = 24–33 and hindwings = 8–11. Male ranged from (mm): body = 17–24, forewings = 16–22.5 and hindwings = 7–9.5. An oddly large female was found at Parque Nacional Serra dos Órgãos with body length of 39 mm, forewings of 40 mm and hindwings of 15 mm.

Mature nymph. Length (mm): Female – Body = 23–33; Male – Body = 16–25 (6 specimens measured each).

Head: Light brown, heavily shaded with black along lateral branches of epicranial suture, tinge extending until inner margin of ocelli; epicranial suture yellow.

Thorax: Flanges and anteroapical projections of pronotum yellow. Sterna yellow.

Legs: Coxae shaded with black; trochanter of fore and middle leg shaded with black; femora shaded with black except for a longitudinal dorsal area, extending from base to near apex. Forelegs: femora with several long setae, wider on basal half, spine-like setae on inner margin absent or few, not forming a distinct row. Tibia and tarsi light brown, densely covered by long setae, apical projection of tibia short not reaching half the length of tarsus with strong setae on lateral margins of projection. Middle legs: long simple setae on dorsal and lateral margin of femora; base of tibiae with black marking; setae denser on tibiae and tarsi, distal inner margin of tibiae and tarsi with dense concentration of short plumose setae apically; tibial suture present; tibiae with short apical projection with strong setae on lateral margins. Hind legs: apex of femora and base of tibiae shaded with black; femora with long setae on lateral margins, distoventral surface with area covered by short setae; tibiae and tarsi densely covered by long simple setae; inner margin of tibiae wide dense row of shorter setae.

Abdomen (Fig. 24): Terga yellow, entirely shaded with black except for a pair of drop-shaped spots on medioapical margin on terga I–X; a pair of narrower also drop-shaped spots on median region on terga II–X, spots narrowing toward tip of abdomen; and a pair of spots near lateral margins; presence of a longitudinal medial line on terga III–IX; posterior margins of terga dark brown. Sterna yellowish. Gills purplish grey. Caudal filaments yellowish shaded with black; median filament of approximate length as cerci, usually longer.

Variations: Apex of dorsal lamellae on gill I sometimes rounded, not truncated. In some specimens, drop-shaped spots are lacking on tergum X. In some specimens from other localities, longitudinal line of abdomen extended to tergum X and/or drop-shaped marks were absent on tergum X. Size variation of such material ranged between 21–35 in females and 18–23 in males. Some female nymphs showed remarkably long tusks, this is the case of the nymphs sheds on *C. bocainensis* type series.

Comparison with type series of *Campylocia bocainensis* Pereira & Da-Silva, 1990

Male imago. Length (mm): Body = 18–22; Forewings = 19–23; Hindwings = 9–11 (Pereira & Da-Silva, 1990).

Female imago. Length (mm): Body = 30–33; Forewings = 30–39; Hindwings = 13–14 (Pereira & Da-Silva, 1990).

Material has an overall discoloured appearance, so colour comparisons could not be performed but colour pattern matched relatively well. Veins yellowish, not purplish, probably due to long time preserved in alcohol. Forewings with 3 ICu veins; fork of MA on same level as fork of Rs or slightly distal. In addition to the pair of long intercalaries described above, the specimens also present a pair between MA_2/MP_1, a variation described for some of the Nova Friburgo specimens. In most specimens, though clearly present, the membranous lobes of penes are starting to deform. Medial longitudinal line of abdomen not reaching terga X.

Comparison with type series of *Campylocia dochmia* Berner & Thew, 1961

Male imago. Length (mm): Body = 15–22; Forewings = 19–22.5; Hindwings = 8.5–9.5 (Berner & Thew, 1961).

Female imago. Length (mm): Body = 22; Forewings = 31–35; Hindwings = 13.8 (Berner & Thew, 1961).

Although level of Rs and MA fork were in same level in males of *C. dochmia*, in females MA fork was usually distal to Rs fork. One male had one forewing with 3 ICu veins. Colour pattern and its variations agreed well with what is described for *C. burmeisteri*. Regarding male genitalia, only four paratypes had some sort of abnormality on apical lobes: the one mounted on a permanent slide and pictured in the original description, one whose genitalia was dissected and kept in glycerin and two that seem to have been subjected to desiccation. Remaining paratypes and the holotype clearly showed apical lobes.

Distribution. Argentina and Brazil, in the states of Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo and probably Rio Grande do Sul, Paraná and Santa

Catarina (Berner & Thew, 1961; Da-Silva, Gonçalves, & De-Souza, 2010; Domínguez et al., 2006; Hagen, 1888; Navás, 1920; Pereira & Da-Silva, 1990; Salles et al., 2010; Ulmer, 1942).

Material examined. Table S2 (see supplemental material online).

Discussion. This is a widely spread species in South-eastern Brazil, it is also likely that it occurs in Southern Brazil given Ulmer's (1942) record of two females with 3 ICu (named by him as *C. anceps*) in Rio Grande do Sul State. Also, two nymphs (not included in the DNA analysis) were studied from Paraná and Santa Catarina states that seemed to belong to this species, although only the study of adults could provide a definite record. Lack of material from the Southern region makes it difficult to know the full range of this species.

Biology. Imagos of *C. burmeisteri* were seen forming small swarms above rivers, early at night. Subimaginal sheds were found on rocks in the middle of rivers and on marginal vegetation. Nymphs inhabit small to medium sized rivers, with clean water and preserved canopy cover. They can be found on leaf litter, gravel, or under rocks and pebbles, using their tusks to dig on those substrates. Detailed information regarding their biology is given by Pereira & Da-Silva (1990).

Campylocia demoulini sp. nov. Gonçalves & Salles (Figs 5, 7, 15, 16, 22, 25, 30, 31, 37, 39–45, 52, 53)

Diagnosis

Imago: (1) Forewings usually with one ICu vein (Fig. 15); (2) Abdominal colour pattern with two pairs of drop-shaped spots, one on medioapical region on terga II–IX and one on medial region, narrower and more subtle, on terga II–VIII (Fig. 22); (3) Styliger plate semi-rounded (Figs 30, 31); (4) Penes wide with distal half strongly curved outwards (Figs 30, 31, 37); (5) Eggs' shape resembling a red blood cell (Figs 52, 53); (6) Chorion sculpture with raised ridges forming a large regular mesh of 15.2–23.5 μm (Figs 52, 53).

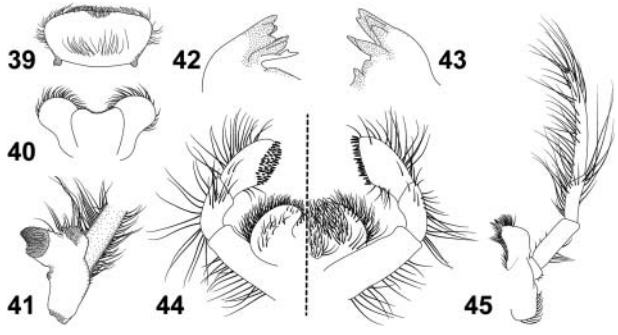

Fig. 39–45. Nymph mouthparts of *C. demoulini* sp. nov.: (39) labrum, d.v., (40) hypopharynx, d.v., (41) left mandible, v.v., (42) detail of incisors and prostechae of left mandible, d.v., (43) detail of incisors of right mandible, d.v., (44) labium in dorsal (left) and ventral (right) views, (45) maxillae.

Male imago. Length (mm): Body = 11.7–14.5; Forewings = 10–11.5; Hindwings = 3.6–4.7 (10 specimens measured).

Head (Fig. 5): Whitish heavily shaded with black. Antennae whitish, pedicel and flagellum with light black shade.

Thorax (Fig. 5): Pronotum whitish, meso- and metanotum yellowish brown. Pronotum with two thin black lines, one on collar margin, one medial along notal suture. Mesonotum with parascutellum shaded with black; medial longitudinal and parapsidal sutures black; scutellum lightly shaded with black. Triangle on prosternum with equilateral margins or with wider base. Mesosternum with lateral margin of basisternum black.

Wings (Figs 15, 16): Forewings with membrane between C, Sc and R_1 purplish on basal half and whitish on distal half; cubital area with one ICu vein; a pair of intercalary veins present between IMP/MP_2 and a pair between MP_2/ CuA.

Legs: Whitish. Coxae and trochanters shaded with black, more heavily on fore and median legs; femora and tibiae shaded with black, shade lighter on fore tibae. Tarsi of forelegs lightly shaded with black.

Abdomen (Fig. 22): Whitish tinged with black. Tergum heavily shaded with black except for two pairs of drop-shaped spots, one on medioapical region on terga II–IX and one on medial region, narrower and more subtle, on terga II–VIII. Sterna with lateral margins lightly shaded with black; sterna VIII–IX sometimes whitish yellow.

Genitalia (Figs 30, 31, 37): Styliger plate semi-rounded, whitish yellow shaded with black. Penes wide; basal 2/3 quadrangular; distal 1/3 narrower than base with each penes strongly curving outwards; lateral projections somewhat sclerotized; membranous lobes absent.

Female imago. Length (mm): Body = 23.1–24; Forewings = 17–21; Hindwings = 6.2–6.6 (10 specimens measured).

Similar to male imago, except: Additional veins: on forewings – 2 veins between R_3/R_{4+5}, 2 between MA_1/ IMA, 2 between MA_2/MP_1, 2 veins between IMP/MP_2 and 2 between MP_2/CuA; on hindwings – 2 between CuP/ A_1.

Egg. Measurements (μm): Length = 288–311; Width = 227–236; Mesh = 15.2–23.5. Shaped as an erythrocyte; chorion sculpture with raised ridges forming a large regular mesh (Figs 52, 53). Egg mass unknown.

Variations: Despite the fact that the sexual dimorphism on venation described above is true for these females, several females from different localities were found not to follow this pattern of venation. Therefore, wing sexual dimorphism cannot be used as a proper character to identify females of this species as well as females of any *Campylocia* species. A female from Surinam had larger eggs, with 342 μm in length and 269 μm in width, but with mesh size was as described.

Nymph. Length (mm): Body = female: 25.2–28 (two female mature nymph); male: 13–19 (three male non-mature nymphs and one exuvia).

Head (Fig. 7): Brown, suffused with black particularly on anterior half of head; lateral branches of epicranial suture surrounded by yellow. Antennae whitish yellow, scapum and pedicel lightly shaded with brown.

Mouth parts (Figs 39–45): Yellow. Labrum: brown, black on baso-lateral margin. Mandibles: shaded with brown, condyles, inner and outer incisors and mola black, tusks dark brown.

Thorax (Fig. 7): Brown, with black markings. Flanges and anteroapical projections of pronotum yellow. Sterna yellow.

Legs: Coxae and trochanter shaded with black. Forelegs: femora shaded with black, particularly on apical third, width similar throughout length; several simple long setae and spine-like setae on femora particularly on margins, distinct row of spine-like setae on inner margin. Tibiae and basal 3/4 of tarsi yellowish brown; apical projection of tibia short not reaching half the length of tarsus with strong setae on lateral margins of projection; tibiae and tarsi densely covered by simple setae. Middle legs: apex of femora and base of tibiae with dark brown marks; long simple setae on dorsal surface lateral margins of femora; distribution of simple long setae on tibiae and tarsi denser, distal inner margin of tibiae and tarsi with dense concentration of short plumose setae apically; tibial suture present; tibiae with short apical projection possessing strong setae on lateral margins. Hind legs: apex and margins of femora and tibiae shaded with dark brown; femora with scattered simple setae particularly on outer margin, distoventral surface with area covered by short setae; tibiae and tarsi densely covered by long simple setae, tibiae also with wide longitudinal row of shorter simple setae.

Abdomen (Fig. 25): Terga brown, entirely shaded with black except for a pair of drop-shaped spots on medioapical margin on terga II–VIII; a pair of narrower also drop-shaped spots on median region on terga II–VIII; and a narrow longitudinal medial line on terga IV–VII. Posterior margins of terga II–VIII dark brown; terga VIII–IX also shaded with black. Sterna yellow, sterna II–VII with a pair of dark brown spots placed posterolaterally. Gills purplish grey. Caudal filaments yellowish heavily shaded with black; median filament of approximate length as cerci, usually longer.

Variations: Mature nymphs have very dark coloured abdomen, in many of them it was hard or impossible to see the medial line or any spots. In younger nymph this pattern is easier to see, due to its lighter colour.

Etymology. In honour of Georges Demoulin, a great mayfly researcher who dedicated several of his studies to the taxonomy and evolution of Euthyplociidae.

Distribution. Ecuador, Surinam, and Brazil, states of Amazonas, Pará, Mato Grosso, and Distrito Federal.

Type material. Holotype: 1 male imago: Brazil, Amazonas State, Manaus, AM 010 – Km 26, Reserva Florestal Adolpho Ducke, tributary of Igarapé do Barro Branco, 02°55’46.7’’S/59°58’20.8’’W, 04.xi.2008, Neiss, U.G.; Salles, F.F.; Vilela, P. & Laurindo, F. *leg.* (DZRJ 2462). **Paratypes:** 11 male imagos: same data as holotype (INPA) (one male with wings and foreleg on slide); 10 female imagos and 2 female subimagos: same data as holotype (DZRJ 2464) (one female with wings and eggs on slide); 4 female subimagos: Brazil, Amazonas State, Manaus, AM 010 – Km 26, Reserva Florestal Adolpho Ducke, Igarapé Acará, 120 m, 02°56’04.9’’S/59°57’13.5’’W, 08.xi.2008, Neiss, U.G.; Salles, F.F.; Vilela, P. & Laurindo, F. *leg.* (DZRJ 2465); 5 nymphs: same data, 14.i.2007, unknown *leg.* (DZRJ 2466); 1 male imago: Brazil, Amazonas State, Manaus, AM 010 – Km 26, Reserva Florestal Adolpho Ducke, tributary of Igarapé Acará, 43 m, 02°57’07.1’’S/59°57’28.7’’W, 08.xi.2008, Neiss, U.G.; Salles, F.F.; Vilela, P. & Laurindo, F. *leg.* (DZRJ 2467) (DNA voucher ENT321); 1 male imago reared from nymph and nymphal exuvia: Brazil, Amazonas State, Manaus, AM 010 - Km 26, Reserva Florestal Adolpho Ducke, Igarapé do Barro Branco, 62 m, 02°55’46.7’’S/59°58’22.0’’W, 21.i.2012, Salles, F.F. *leg.* (UFES); 2 nymphs, in different vials: Brazil, Amazonas State, Presidente Figueiredo, AM 240 – Km 57, Cachoeira da Pedra Furada, 01°59’34’’S/59°33’26.4’’W, 14.xii.2011, Vilela, P. & De-Souza, M.R. *leg.* (DZRJ 2468 and DZRJ 2469) (DNA voucher ENT1004); 28 males and 18 females imagos and subimagos: Brazil, Amazonas State, Manaus, AM 010- Km 26, Reserva Florestal Adolpho Ducke, tributary of Barro Branco (Ig. 11), 02°55’46,7“S/59°58’20,8”W, 04.xi.2008, Neiss U.G., Sales, F.F. & Cruz, P. V. *leg.* (INPA). For additional analysed material see list in Supplemental Material SII.

Discussion. *Campylocia demoulini* sp. nov. is a very common species in the Brazilian Amazon. It probably has been mistakenly identified as *C. anceps* because of the lack of a detailed illustration of the male genitalia, as well as a mislabel on the original paper (Eaton, 1883/1888;

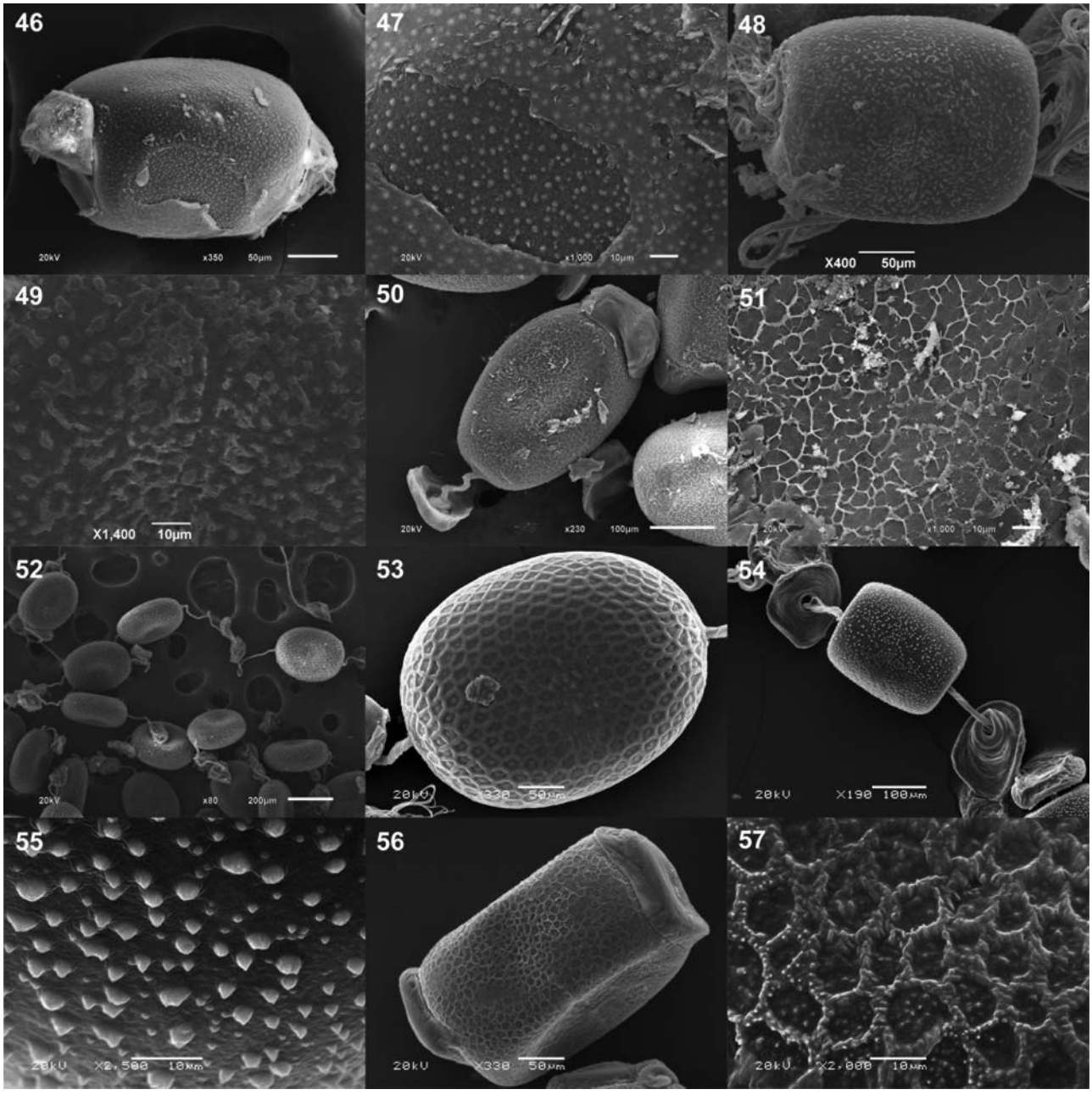

Fig. 46–57. Eggs: (46) and (47) *Campylocia anceps*, (48) and (49) *Campylocia araca* sp. nov., (50) and (51) *Campylocia burmeisteri*, (52) and (53) *Campylocia demoulini* sp. nov., (54) and (55) *Campylocia* population from French Guiana, apparently related to *C. demoulini* sp. nov., (56) and (57) *Campylocia orosi* sp. nov.

Needham & Murphy, 1924; Spieth, 1943; Ulmer, 1920a; Ulmer, 1942). Kimmins (1960) solved this issue by picturing the holotype's genitalia, making clear that these are two different species.

A population of *Campylocia* from French Guiana, outside the Amazon rain forest, seems to be related to this species. Males of this population fit in the description of *C. demoulini* sp. nov. although some of these individuals had a narrow triangle on prosternum, truncate styliger plate and apex of forceps more rounded. Females agree with *C. demoulini* sp. nov. description but show a pair of long intercalary vein between CuP/A. The most striking feature of this population is the shape and chorionic sculpture of the eggs, which are barrel shaped and tuberculate resembling eggs of *C. anceps* (Figs 54, 55). Size of the eggs varies from 228–247 µm in length and 187–229 µm in width, and tubercles in 1.0–3.8 µm in height and 1.1–5.2 µm in diameter. Tubercles in this population show much more variation in size with many elongate tubercles, while apices are also more acute in many of these

tubercles. Barrel-shaped eggs seem to be a plesiomorphic state in *Campylocia* also being common in other genera (e.g., *Afroplocia*, *Exeuthyplocia*, and *Polyplocia*) and related families. Overall body and wings size was also larger in this particular population than in *C. demoulini* sp. nov. Males from French Guiana had the following measurements (mm): Body = 13–16, forewings = 14–15, and hindwings = 6–7. Females: Body = 21–34, forewings = 23–29, and hindwings = 8–11. With the exception of the eggs, no morphological characters would allow separating this population from other specimens of *C. demoulini* sp. nov. They could represent a hybrid between *C. anceps* and *C. demoulini* sp. nov. if it is discovered that these species co-occur or even a different evolutionary lineage diverging from this species. Because material is old, no molecular analysis could be performed.

Biology. Adults were collected with light traps. Most nymphs were collected on small (1–2 m), warm (26–24°C), black water streams, with 60–50% canopy cover and sandy bottom. Specimens were usually found living on roots and litter.

Two mature female nymphs were collected in a larger river at Cachoeira da Pedra Furada, Presidente Figueiredo, Amazonas State, on a semi-submerged trunk. When placed on a tray they showed aggressive behaviour, using their tusks and forelegs to fight each other (see video on SIII). Euthyplociids possess the longest tusks in the mayfly order and are believed to use their tusks for feeding and burrow under leaves and rocks. This is the first record of aggressive behaviour in the group as well as the first report of tusks being used as a defensive mechanism. One of these females had a broken tusk, but since the animal was found this way it is impossible to know if this injury was caused by fighting or something else. Broken tusks were observed in several *Campylocia* nymphs regardless of species, gender, and age. Two possible explanations are that nymphs fight over good feeding/shelter sites on the river or that mature female nymphs fight as a form of sexual selection, perhaps for good moulting/mating sites.

Campylocia orosi sp. nov. Gonçalves & Peters
(Figs 6, 17, 18, 23, 32, 33, 38, 56, 57)

Diagnosis. *Imago*: (1) Forewings with 1 or 2 ICu veins (Fig. 17); (2) Abdominal colour pattern with a medial longitudinal line and two pairs of drop-shaped spots, one on medioapical and one on medial region, on terga I–VIII, medial spots harder to see (Fig. 23); (3) Styliger plate truncate (Figs 32, 33); (4) Penes wide with distal half strongly curved outwards, inner and outer parts of penes on different levels: inner elevated, outer and lateral projections lowered (Figs 32, 33, 38); (5) Eggs barrel shaped (Fig. 56); (6) Chorionic sculpture with tubercles and irregular raised ridges forming a mesh of 05.3–15.4 μm (Figs 56, 57).

Male imago. Length (mm): Body = 16–22; Forewings = 14–18; Hindwings = 5–7 (3 specimens measured).

Head (Fig. 6): Whitish washed with black and a somewhat purplish shade. Antennae whitish, pedicel and flagellum with light black shade.

Thorax (Fig. 6): Yellowish brown, prothorax slightly translucent. Pronotum shaded with black, collar and lateral margins black; longitudinal notal suture faintly marked. Mesonotum with parascutellum lightly shaded with black and three straight brown lines along medial and parapsidal sutures; scuto-scutellar impression shaded black. Metanotum with posterior margin shaded back. Triangle on prosternum narrow with sides longer than base. Mesosternum with lateral margins of basisternum black.

Wings (Figs 17, 18): Forewings with membrane between C, Sc and R_1 purplish on basal 2/3; cubital area with one or two ICu vein; a pair of long intercalary veins between IMP/MP_2 and between MP_2/CuA. Fork of MA distal to fork of Rs.

Legs: Yellowish. Coxae and trochanters shaded with black, more heavily on fore and median legs; femora heavily shaded black; tibiae of forelegs shaded with black; tarsi of forelegs lightly shaded with black.

Abdomen (Fig. 23): Whitish. Tergum heavily shaded with black except for a medial longitudinal line and two pair of drop-shaped spots, one on medioapical and one on medial region, on terga I–VIII; medial spots harder to see. Sterna with lateral margins shaded with black. Caudal filaments whitish, basal segments shaded with black.

Genitalia (Figs 32, 33, 38): Styliger plate truncate, yellowish with light black shade. Apex of forceps wide, drop-shaped. Penes wide, basal half quadrangular; distal half slightly narrower than base, with each penes strongly curved outwards with an apico-lateral projection. Inner portion of each penes elevated, outer portion, where projection is, lowered; membranous lobes absent.

Variations: Two males exhibited 2 ICu on forewings and one exhibit 1 ICu. One individual had one wing with one short intercalary vein between MP_1/IMP. Other specimens exhibited a pair of short intercalary between MA_1/IMA, a pair between MA_2/MP_1 and a pair between MP_1/IMP on both wings. In the non-type material, three males from Panama exhibited 1 ICu vein except for one specimens' forewing. Triangle on prosternum was not narrow on two of these males and they also displayed only one short intercalary between CuP/A of hindwings.

Female imago. Length (mm): Body = 19–29; Forewings = 22–28; Hindwings = 7–9 (body size approximate, all females with abdomen curved due to egg laying).

Similar to male imago except: Head without purplish shade; forewings with only one ICu vein and additional

pairs of long intercalary veins between R_3/R_{4+5}, between MA_1/IMA and between MA_2/MP_1.

Variations: two of the five females lacked intercalary between R_3/R_{4+5}. One female had one wing with 2 ICu, other lacked intercalaries between CuA/CuP of hindwings and another one had this and other intercalary veins shorter. Some females had only 1 – and not 2 – intercalary on hindwings.

Egg. Measurements (μm): Length = 252–285; Width = 71–226; Mesh = 05.3–15.4. Barrel shaped; chorion sculpture with tubercles and irregular raised ridges forming a mesh, mesh also with some tubercles (Figs 56, 57). Egg mass unknown.

Nymph. Unknown.

Etymology. In apposition to the Orosí River, type locality of this species.

Distribution. Costa Rica and Panama.

Type material. Holotype: Costa Rica, Guanacaste, Rio Orosí, Pitilla, 50 m from Biological Station, 10 km south of town of Santa Cecilia, Parque Nacional Guanacaste, South-west of La Cruz, Guanacaste Province, 12.VI.1989, 1 male imago (FAMU); **Paratypes**: same data, 2 male and 5 female imagos (FAMU). For additional material, see Table S2 (supplemental material online).

Discussion. *Campylocia orosi* sp. nov. could be misidentified as *C. demoulini* sp. nov. or even *C. anceps* due to how easily the penes lobes can deform. Therefore, attention is needed when examining specimens. No molecular data could be obtained for this species.

Biology. No information about its biology is available.

Key to imagos of *Campylocia*:

1. Styliger plate rounded (Fig. 27); penes long, Y-shaped (Figs 27, 34); egg chorion sculpture with distinct tubercles, not elongate, and without raised ridges (Figs 46, 47) . . . . . *Campylocia anceps*
   – Styliger plate truncate or semi-rounded (Figs 28–33, 36); penes short, not Y-shaped (Figs 28–33, 35–38); egg chorion sculpture with raised ridges or with elongate tubercles almost forming a ridge by themselves (Figs 48–53, 56, 57) 2
2. Penes narrow (Figs 28, 35); egg chorion with tubercles, some elongate almost forming a ridge (Figs 48, 49) . . . . . *Campylocia araca* sp. nov.
   – Penes wide (Figs 29–33, 36–38); egg chorion with raised ridges (Figs 50–53, 56, 57) … 3
3. Forewings usually with 2 or 3 ICu veins (Fig. 13); penes with apical membranous lobes (Figs 29, 36); egg chorion sculpture with raised ridges that sometimes connect (Figs 50, 51) . . . . . *Campylocia burmeisteri*
   – Forewings usually with 1 or 2 ICu (Figs 15, 17); penes without apical lobes (Figs 30–33, 37, 38); egg chorion sculpture with raised ridges always connecting and forming a mesh (Figs 52, 53, 56, 57) 4
4. Large specimens, males about 16–22 mm; styliger plate truncate (Figs 32, 33); penes with inner half elevated and outer half (including projections of penes) lowered (Figs 32, 33, 38); eggs barrel shaped (Fig. 56); egg chorion sculpture with scattered tubercles and irregular raised ridges forming a mesh of 5.3–15.4 μm (Fig. 57) . . . . . *Campylocia orosi* sp. nov.
   – Small specimens, males about 12–15 mm; styliger plate semi-rounded (Figs 30, 31); penes without distinction between elevated and lowered areas (Figs 30, 31, 37); eggs erythrocyte shaped (Figs 52, 53); egg chorion sculpture with raised ridges forming a large regular mesh of 15.2–23.5 μm (Fig. 53) . . . . . *Campylocia demoulini* sp. nov.

Discussion

Molecular analysis

Specimens belonging to three of the Amazonian lineages were identified based on morphological characters as *Campylocia anceps* (lineage II) and two new species, described as *Campylocia demoulini* sp. nov. (lineage V) and *Campylocia araca* sp. nov. (lineage VII). However, specimens from the Atlantic forest in South-eastern Brazil identified based on the number of intercubital veins of forewings as *C. bocainensis* and *C. dochmia* were not recovered as independent monophyletic lineages, but all clustered in lineage VIII (Fig. 1). K2P divergences between *C. bocainensis* individuals ranged from 0.0% to 8.7% and between *C. dochmia* from 7.5% to 8.7%. In addition, divergences between individuals of *C. dochmia* and *C. bocainensis* were 0.0% to 10.0% (mean = 5.8%). The non-recovery of individuals identified as these species as monophyletic groups and overlap between intra- and interspecific genetic divergences suggest that there is only a single species of *Campylocia* in South-eastern Brazil. Thus it is proposed herein that these two species are conspecific and treated as *C. burmeisteri* (see below).

All lineages delimited herein show high values of maximum intraspecific K2P divergences with a maximum of 10.0%, however there is no clear 'barcoding gap' between

maximum intra- and minimum interspecific gap, to justify delimitation of all lineages based on genetic K2P divergences. Overlapping values are caused by the apparent small divergences found between the representative specimen of lineage III and all specimens of lineage V, which range between 3.3% and 9.6%. However, lineage III was tentatively considered herein as a separate species because the close phylogenetic relationship of both lineages was not recovered in the Bayesian analysis. Except for lineage III, there is a clear 'barcoding gap', as the minimum interspecific divergence rises to 10.5%. High intraspecific divergences, appear to be a common scenario amongst mayflies, with Baetidae species showing values of 26.7% in *Fallceon quilleri* (Dodds, 1932), 26.2% in *Acerpenna pygmaea* (Hagen, 1861), and 22.9% in *Acentrella insignificans* (McDunnough, 1926) (Webb et al., 2012). Divergences similar to those found in *Campylocia* are also seen in other Fossoriae (Webb et al., 2012), such as 10.1% in *Hexagenia limbatta* (Guerin, 1829–1843) and 8.9% in *Ephemera varia* Eaton, 1883/1888 (Ephemeridae), 9.0% in *Ephoron album* (Say, 1823) (Polymitarcyidae), and 7.9% in *Dolania americana* Edmunds & Traver, 1959 (Behningiidae). It is also likely that the high values of genetic distance found in *Campylocia* and other Fossoriae are a result of their low dispersal ability. Euthyplociidae are large mayflies that swarm near rivers and are not usually found far from them. The lack of vagility may contribute to the genetic isolation of lineages and consequent high maximum intraspecific divergences.

Phylogenetic results also reveal the existence of two unrelated lineages, lineage I from the Atlantic forest of north-eastern Brazil and lineage IV from the Amazon forest that include male individuals that cannot be distinguished from males of *C. burmeisteri*, restricted to the Atlantic forest of south-eastern Brazil, but were not found to be related to this species. Both lineages are separated from individuals of its sister-lineage by at least 10% genetic divergence, the maximum value found among individuals considered as conspecific in *Campylocia* (as discussed above). Due to lack of morphological characters to diagnose specimens included in these lineages, they are not described herein, although are considered as independent evolutionary lineages. The inclusion of more specimens, especially females with eggs, is needed to justify the nomenclatural status of these lineages in the future. They might either be described as separate species or, if no additional morphological characters are found, included in the present delimitation of *C. burmeisteri*, which could be treated as a complex of cryptic species. Finally, no taxonomic comments can be made about lineages III and VI, both represented by nymphs in this study. Lineage VI is represented by a single nymph from Roraima State, Brazil, indistinguishable from most other *Campylocia* species nymphs but distant by an average 12.5% divergence from its sister lineage. On the other hand, lineage III (possibly conspecific to members of lineage V, see discussion above) is represented by a nymph, which represents the first record of colour variation in Euthyplociidae. The abdominal colour pattern of this oddly coloured nymph can be seen in Fig. 26 next to a typical abdominal colour pattern in Figs 24, 25. In these specimens, the two pairs of drop-shaped spots seem to have fused so that terga IV–X show a pair of median oblique longitudinal lines. Adults and nymphs of Euthyplociidae display a very similar abdominal colour pattern with the presence of drop-shaped markings and a medial line. This is the first record of such an abdominal colour variation in the family.

Morphological analysis

Individuals of different species of *Campylocia* show a clear sexual dimorphism particularly in the forewings, with females having more intercalary veins. Such dimorphism was named gemination (Demoulin, 1970; Kluge, 2004) and is also known from other Euthyplociidae genera such as *Polyplocia* Lestage, 1921. Location of such veins is the same regardless of species but there is also great individual variation. As a consequence, according to the present study, the only reliable way to identify females to the species level is based on the chorionic sculpture of the eggs. Egg shape is also important since *C. demoulini* sp. nov. has erythrocyte-shaped eggs instead of the plesiomorphic barrel-shape.

According to the literature, *Campylocia dochmia* and *C. bocainensis* are distinguished by the number of intercubital (ICu) veins on forewing – three in *C. bocainensis* and two in *C. dochmia* – and shape of penis – with median membranous lobes present in *C. bocainensis* and absent in *C. dochmia*. Study of the type series and vast additional material has led to the conclusion that these characters are either too variable (number of ICU) or not variable at all (median lobes on penes), and the two species are morphologically indistinguishable. When examining large series of these individuals it is not unusual for one specimen to exhibit two ICu in one wing and three ICu on the other. Based on 78 individuals studied, nearly 16% of specimens had unequal number of ICu veins from one wing to the other whereas 65% had 3 ICu on both wings. Number of ICu veins varied regardless of period of the year and altitude of collecting sites but males exhibited relatively larger number of variations than females. Over 80% of females had 3 ICu in both wings, 11% exhibited unequal number of veins and 9% had 2 ICu on both wings. On males, only 44% displayed 3 ICu veins, with 28% having unequal number of ICu and 28% with 2 ICu on both wings. Females have larger body size than males: hence, differences in number of ICu veins may be related to body size and the need of improved wing support during flight. Domínguez et al. (2006) reported *Campylocia* males from Misiones, Argentina, which can be identified as *C. bocainensis* based on the genitalia, but with an intermediate wing venation of *C. dochmia* and suggested that the species could be synonyms.

Membranous lobes on penes were found to be highly deformable; having their shape altered depending on fixation and/or preservation of the specimens. Specimens pinned or not immediately preserved in alcohol after collecting had median lobes deformed, as if absent. Specimens preserved in alcohol but subjected to evaporation also presented deformed lobes, as well as did specimens from which genitalia had been dissected and mounted onto a permanent slide. This deformity was also observed when analysing *C. dochmia* types. Lobes cannot be seen in the mounted genitalia of the paratype illustrated by Berner and Thew (1961) and in three other paratypes, which appears to be deformed, but lobes can be easily observed in the holotype and remaining paratypes.

Molecular data and the lack of diagnostic characters point to the conclusion that the South-eastern Brazil specimens of *Campylocia* should be treated as a single species, *C. burmeisteri*, one of the names synonymized under *C. anceps*, but revalidated herein.

Hagen (1888) described *Euthyplocia burmeisteri* based on two males collected in Nova Friburgo, Brazil. Ulmer (1932) then transferred the species to *Campylocia* regarding it as a synonym of *C. anceps*. Berner & Thew (1961) accept Ulmer's synonymy, but oddly state that Hagen's specimen belonged to the new species they were describing, *C. dochmia*. A letter written by Berner was made available to the senior author, where he states that Thew analysed the type of *E. burmeisteri* and noticed it was very similar to *C. dochmia*, probably belonging to the same species (Berner *in litt.*). The distribution of *C. burmeisteri* is also informative as its type locality, Nova Friburgo in Rio de Janeiro State, is an area where specimens identified as *C. bocainensis* are commonly collected. Unfortunately, types of *C. burmeisteri* could not be located and are probably lost. Another synonym of *C. anceps*, *Euthyplocia guntheri*, is described based on a male from Santos, São Paulo State, Brazil. Analysis of the pinned holotype, despite its poor condition, confirms it belongs to the south-eastern species and not to *C. anceps.* As a consequence, the name *Campylocia burmeisteri* (Hagen, 1888) is revalidated and *C. guntheri*, *C. dochmia*, and *C. bocainensis* are placed as its junior synonyms.

The remaining synonyms were described based only on females. Both with only 1 ICu vein and barrel-shaped eggs. When describing *Campylocia ampla* from Brazil and Peru, Needham and Murphy (1924) illustrate a barrel-shaped egg with numerous tubercles, such as those found in *C. anceps* confirming the synonym. *Campylocia intercalata*, from Guiana, French Guiana, and Colombia, was briefly described without illustrations (Banks, 1918). The shape of the eggs and their distribution overlap with that of *C. anceps* supporting the synonymy; also, they could not belong to *C. demoulini* sp. nov., since the new species has erythrocyte-shaped eggs. This synonym is based on literature and photographic material of the holotype and paratypes of *C. ampla*, and a syntype of *C. intercalata*.

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Supplemental data

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