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# TAXONOMIA DE EPHEMEROPTERA (INSECTA) DO ESTADO DE PERNAMBUCO, COM ANÁLISE FILOGENÉTICA E BIOGEOGRÁFICA DE *Brasilocaenis* PUTHZ, 1975 (CAENIDAE)

Recife
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Tese apresentada à Coordenação do Programa de Pós-Graduação em Biologia Animal, como parte dos requisitos para obtenção do título de Doutor em Zoologia.

Orientador: Prof. Dr. Ulisses dos Santos Pinheiro

Co-orientador: Prof. Dr. Frederico Falcão Salles

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*"Tradicionalmente, alguém se torna biólogo ou por formação na área médica ou por ter sido um jovem naturalista. Hoje, é comum para um jovem se empolgar com as ciências da vida através dos meios de comunicação, sobretudo por filmes sobre a natureza na televisão, de visitas a um museu, (geralmente à sala dos dinossauros) ou de um professor inspirador. Há também milhares de jovens observadores de pássaros, alguns dos quais acabarão se tornando biólogos profissionais (como foi o meu caso). O ingrediente mais importante é a fascinação diante das maravilhas das criaturas vivas. E isso permanece com a maioria dos biólogos para o resto da vida. Eles nunca perdem a empolgação com a descoberta científica, seja ela empírica ou teórica, nem o amor pela perseguição de novas ideias, novos vislumbres, novos organismos. E muita coisa na biologia tem uma relação direta com as circunstâncias e com os valores pessoais do biólogo. Ser um biólogo não significa ter um emprego; significa escolher um estilo de vida."*

Ernst Mayr – Isto é biologia: a ciência do mundo vivo (2008).

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## RESUMO

Ephemeroptera é um grupo de insetos aquáticos e importante componente de ambientes dulciaquícolas. O conhecimento sobre esse grupo no Brasil aumentou nos últimos anos, principalmente na Região Nordeste, contribuindo para reduzir lacunas de conhecimento. Das 10 famílias de Ephemeroptera reportadas para o Brasil, Caenidae é uma das mais amplamente distribuídas no mundo. *Brasilocaenis* Puthz, 1975, juntamente com outros três gêneros, é um dos representantes sulamericanos da família. Exceto pela morfologia bem diferenciada da genitália masculina, as espécies desse gênero são muito similares a algumas espécies sulamericanas de *Caenis* Stephens, 1836 (Caenidae). Este trabalho apresenta uma lista atualizada das espécies de Ephemeroptera do Estado de Pernambuco, Nordeste Brasileiro, e realizar a primeira proposta filogenética para o gênero *Brasilocaenis*. Baseado na bibliografia e coletas em vários rios e riachos de 27 municípios e 74 localidades, 16 espécies e 10 gêneros são reportados pela primeira vez para Pernambuco, aumentando de 43 para 59, o número de espécies para o Estado. Com estes resultados, Pernambuco se torna o Estado com o maior número de espécies na Região Nordeste do Brasil. Além disso, *Caenis chamie* Alba-Tercedor & Mosquera, 1999 é reportado pela primeira vez para o Brasil. Para analisar a relação de *Brasilocaenis* e gêneros relacionados, foram examinados aparentes subgrupos por meio caracteres morfológicos. A primeira análise filogenética de *Brasilocaenis* é proposta baseada em uma matriz de 10 caracteres contínuos e 24 discretos analisados sob pesagem implícita. O gênero é recuperado como monofilético e redefinido baseado nas redescrições de adultos machos e ninfas do material tipo ou recém coletados, e de três novas espécies descritas a partir de imagos de ambos os sexos e ninfas. Baseada nesses resultados, uma análise biogeográfica foi conduzida baseada nos registros distribucionais de cada espécie usada na análise filogenética, encontrando dois eventos vicariantes.



**Keywords:** Insetos aquáticos. Efemerópteros. Taxonomia. Filogenia.

## ABSTRACT

Ephemeroptera is a group of aquatic insects and important biotic component of freshwater ecosystems. The knowledge about the distribution of Ephemeroptera in Brazil has increased in recent years, contributing to reduce the gaps of distribution. Of the ten families of Ephemeroptera recorded for Brazil, Caenidae is one of the most widespread in the world. *Brasilocaenis* Puthz, 1975 is a representative South American genus of Caenidae. Except the very differentiate male genitalia, the species of this genus are very similar to some South American species of *Caenis* Stephens, 1835. This paper presents an updated checklist of the species of Ephemeroptera from Pernambuco State, Northeastern Brazil and performs the first phylogenetical proposal to genus *Brasilocaenis*. Based on bibliography and collections from numerous rivers and streams of 27 counties and 74 localities, 16 species and 10 genera are recorded for the first time from Pernambuco State, increasing from 43 to 59 the number of known species. With these results, Pernambuco becomes the state with highest number of species in Northeastern Region of Brazil. Moreover, *Caenis chamie* Alba-Tercedor & Mosquera, 1999 is reported for the first time from Brazil. To analyse the relationship within *Brasilocaenis* and related genera, we examined apparent subgroupings by morphological characters. This first phylogenetic analysis of *Brasilocaenis* is proposed based on a matrix of ten continuous and 24 discrete characters analysed under implied weights. The genus is recovered as monophyletic and redefined based on redescription of male imagos and nymphs from the holotype and/or fresh material, and from three new species described from imagos of both sexes and nymph. Based on these results, a biogeographical analysis was conducted, based on distributional records for each species of the phylogenetic analysis, finding two main vicariant events.



**Keywords:** Aquatic insects. Mayflies. Taxonomy. Phylogeny.

**SUMÁRIO**

## 1 INTRODUÇÃO

A ordem Ephemeroptera Hyatt & Arms, 1891 é uma linhagem de insetos alados viventes, com registro fóssil que data do Carbonífero (mais de 280 m.a) (BRITTAIN; SARTORI, 2003). Ela é composta por insetos aquáticos hemimetábolos, apresentando diferentes estágios de vida (ovo, ninfa, subimago e adulto), passando a maior parte de vida no meio aquático. O nome (*Ephemeros* – breve, *ptera* – asa) é uma alusão ao curto período de vida dos adultos.

A ordem Ephemeroptera, composta por cerca de 4.000 espécies, 400 gêneros e 42 famílias (BARBER-JAMES et al., 2014), os quais podem ser encontrados em todos os continentes, exceto na Antártida, no extremo Ártico e em algumas ilhas oceânicas (EDMUNDS et al., 1976). Esta ordem é um dos grupos de insetos aquáticos mais dominantes e diversos em ambientes aquáticos, sendo um importante componente biótico dos ecossistemas dulciaquícolas. São de grande importância para a dinâmica de rios e riachos, onde realizam a ciclagem de nutrientes e participam da cadeia trófica como item alimentar na dieta de vários predadores como aves, peixes e outros insetos (BRITTAIN, 1982). Muitas espécies de Ephemeroptera são utilizadas como bioindicadoras de qualidade da água devido a sua sensibilidade as alterações do ambiente aquático, sendo inclusas em índices bióticos de monitoramento ambiental (ARMITAGE et al., 1983; RESH; JACKSON, 1993; BUSS; SALLES, 2007).

Como dito anteriormente, as ninfas representam o estágio dominante na história de vida desses insetos, enquanto que os adultos possuem geralmente um curto período de vida que pode variar de algumas horas a poucos dias. De uma maneira geral, tanto ninfa quanto adultos apresentam baixa vagilidade e pouco poder de dispersão. As ninfas podem dispersar por deriva por toda bacia de drenagem do rio, mas estão limitados às partes interligadas da bacia acima da influência do mar; já o sucesso na dispersão pelo vento através de barreiras naturais, como faixas de mar ou montanha é limitada pelo curto período de vida dos adultos (DOS SANTOS et al., 2012).

Duas áreas biogeograficas foram reconhecidas dentro América do Sul: a Andina-Patagônica e a Neotropical (CABRERA & WILLINK, 1973; MORRONE, 2001). A área Andina-Patagônica inclui espécies que são restritas a corpos de águas frias e oxigenadas de regiões montanhosas do Chile e da porção Central e Sul da Argentina. A área Neotropical inclui espécies que são amplamente distribuídas na região subtropical e tropical da América do Sul. Savage

(1987) propôs uma classificação biogeográfica para as espécies neotropicais de Leptophlebiidae (Ephemeroptera), utilizando dados geológicos, e encontrou um padrão similiar ao proposto acima.

No Brasil, são conhecidas atualmente 319 espécies de Ephemeroptera, distribuídas em 72 gêneros e 10 famílias. O conhecimento taxonômico desse grupo, ainda pode ser considerado fragmentado, com uma considerável defasagem de dados para alguns estados brasileiros. Com base na publicação da Lista das espécies de Ephemeroptera registradas para o Brasil (SALLES et al., 2004), a Região Nordeste aparecia como a região menos estudada do país, com apenas duas espécies registradas, enquanto que as Regiões Norte e Sudeste, mais estudadas, apareciam com aproximadamente 60 espécies registradas. Desde então, houve um aumento de levantamentos faunísticos e trabalhos descritivos em todo o Brasil, principalmente no Centro-Oeste e Nordeste do Brasil. Porém, apesar desse crescente aumento de esforço amostral, ainda há lacunas de distribuição como no Acre (1 registro), Amapá (2), Tocantins (2) na Região Norte, e Rio Grande do Norte (1), Alagoas (3) e Sergipe (1) na Região Nordeste. No Estado da Paraíba, nenhuma espécie de Ephemeroptera foi registrada até o momento.

Estudos taxonômicos recentes, têm contribuído de forma significativa para a ampliação do conhecimento da distribuição do grupo no Nordeste Brasileiro, aumentando de 4 para 72, o número de registros de espécies nessa região a partir de 2010 (BOLDRINI et al, 2012; CRUZ et al, 2011; LIMA et al., 2010, 2012; MARIANO, 2010). Dentre os estados dessa região, Pernambuco passou a ter um grande destaque em acréscimo de números de registros. Até 2011, nenhuma espécie havia sido registrada para esse Estado, e desde então, passou a ter 43 registros de espécies de Ephemeroptera, sendo atualmente o estado com maior número de espécies da Região Nordeste (LIMA et al. 2011, 2012, 2013a, 2013b, 2014).

Grande parte dos estudos, com Ephemeroptera na Região Nordeste, têm se concentrado em áreas de Mata Atlântica e Cerrado, enquanto que para o bioma da Caatinga ainda há uma grande escassez de dados sobre esse grupo. Dessa forma, o levantamento de espécies, em áreas pela primeira vez exploradas, poderá ser um ponto chave para o entendimento do relacionamento filogenético de alguns grupos. Isso também tem possibilitado um melhor entendimento não apenas em nível regional, como também um estudo mais amplo do inter-relacionamento de bacias hidrográficas.

Das dez famílias de Ephemeroptera registrada para o Brasil, Caenidae é uma das que apresenta distribuição mais ampla no mundo, estando ausente apenas na Nova Zelândia, Antártica e algumas ilhas oceânicas (EDMUNDS et al., 1976). Para a Região Neotropical estão registrados cinco gêneros e 32 espécies, sendo que para a América do Sul 29 espécies foram reportadas e estão distribuídas em quatro gêneros: *Alloretochus* Sun & McCafferty, 2008; *Brasilocaenis* Puthz, 1975; *Caenis* Stephens, 1836; e *Latineosus* Sun & McCafferty, 2008 (BARBER-JAMES et al., 2014). No Brasil, a família Caenidae teve seu conhecimento significativamente incrementado até o final da década de 1990, e desde então, trabalhos taxonômicos e sistemáticos a respeito do grupo são praticamente inexistentes.

Os Caenidae fazem parte da infraordem Pannota, que integra em conjunto com outras famílias (e.g. Leptophlebiidae, Leptohyphidae, Ephemeridae), a subordem Furcatergalia (OGDEN et al., 2009). Dentro de Pannota integra a superfamília Caenoidea, cuja única outra família integrante é Neoephemeridae. Estudos filogenéticos têm indicado a presença de três subfamílias dentro de Caenidae: Caeninae, Brachycercinae e Madecocercinae – sendo que apenas as duas primeiras possuem representantes no continente americano (JACOBUS; McCAFFERTY, 2006; McCAFFERTY; WANG, 2000). Dentro de Caeninae, Malzacher (2013) propôs a criação de três tribos: Caenini, Clypeocaenini e Tasmanocaenini. A tribo Caenini é composta pelos gêneros *Afrocaenis* Gillies, *Brasilocaenis* Puthz, *Caenis* Stephens, *Caenopsella* Gillies, sendo que desses quatro gêneros, *Brasilocaenis* e *Caenis* são os mais representativos com seis e 158 espécies respectivamente (BARBER-JAMES et al., 2013; MALZACHER 2011, 2012, 2013).

O gênero *Brasilocaenis* foi estabelecido para apenas uma espécie, *B. irmleri*, a partir de imagos de ambos os sexos e exúvia ninfal (PUTHZ, 1975). Em seguida, Malzacher (1986, 1990, 1998) revisou o gênero e descreveu mais cinco espécies. Malzacher (1998) dividiu o gênero em 2 grupos de espécies: o grupo "irmleri" (*B. irmleri* Puthz, 1975; *B. puthzi* Malzacher, 1986; *B. renata* Malzacher, 1986) e o grupo "septentrionalis" (*B. septentrionalis* Malzacher, 1990; *B. mendesi* Malzacher, 1998). Este mesmo autor também afirmou que a identidade de *B. intermedia* Malzacher, 1986, conhecida unicamente a partir de exúvias ninfais é duvidosa.

Trabalhos anteriores já haviam indicado a possibilidade de que as espécies de *Brasilocaenis* fossem parte de *Caenis*, o que tornaria este último um grupo parafilético (DOMÍNGUEZ et al, 2006; MALZACHER, 1990, 1998). Essa dúvida surge principalmente devido aos adultos de *Brasilocaenis* serem diferenciados, dos outros Caenidae, apenas por

caracteres da genitália masculina, e por poucos caracteres significativos nas ninfas. Além disso, alguns *Brasilocaenis* são similares ao grupo de espécies de *Caenis* com fórceps afilados apicalmente. Vale ressaltar ainda, que nenhuma análise filogenética formal foi realizada para testar a hipótese de relacionamento entre espécies desses dois gêneros. Neste sentido, seria crucial a revisão e filogenia de *Brasilocaenis*, trazendo uma maior compreensão para o padrão evolutivo da subfamília Caeninae na América do Sul.

Devido às atuais taxas de degradação e destruição dos hábitats de água doce por todo o Brasil, torna-se prioridade documentar o mais rápido possível, novos registros de ocorrência de Ephemeroptera. Como grande parte dos estudos pretéritos no estado de Pernambuco, se concentraram em ambientes de Mata Atlântica, torna-se prioritário conhecer a diversidade da região semi-árida, para fins comparativos entre essas áreas.

Diante disso, no presente trabalho é apresentado as descrições de novas espécies e estágios de vida, além de uma análise filogenética, para o gênero *Brasilocaenis*. Também é apresentado uma lista atualizada das espécies ocorrentes para o Pernambuco, com novos registros para o Estado e também para o Brasil.

## 1.1 Objetivos

### 1.1.1 Objetivo Principal

Apresentar uma lista de espécies atualizada de Ephemeroptera, com novos registros e espécies novas para o Estado de Pernambuco e para o Brasil, e propor uma análise filogenética e biogeográfica de *Brasilocaenis* Puthz, 1975.

### 1.1.2 Objetivos específicos

- Apresentar a descrição de novas espécies e novos registros para o Estado de Pernambuco;
- Propor hipóteses de relacionamento filogenético entre as espécies de *Brasilocaenis*, afim de testar sua monofilia, com base em caracteres morfológicos;
- Adequar e/ou redescrever as descrições das espécies do gênero *Brasilocaenis*;
- Aplicar uma análise de relações históricas biogeográficas, baseada no cladograma encontrado da análise anterior, a partir do exame de possíveis barreiras de vicariância.

## 1.2 Material e Métodos

### 1.2.1 Obtenção de Material e Procedimentos em campo

O material utilizado no presente estudo foi obtido através de empréstimo de exemplares ou visitas a coleções entomológicas, e também por meio de coletas dentro do estado de Pernambuco e de outros estados. Quando o empréstimo ou coleta não era possível, fotografias dos espécimes-tipos foram solicitadas. O material obtido analisado em coleções, veio predominantemente do Instituto de Biodiversidad Neotropical, da Universidad Nacional de Tucumán, Argentina (IBN). Outras instituições que contribuíram com material foram: Coleção Entomológica Norte Capixaba, Universidade Federal do Espírito Santo, São Mateus, Brasil (CZNC); Coleção Entomológica Prof. José Alfredo Pinheiro Dutra, Departamento de Zoologia da Universidade Federal do Rio de Janeiro, Brasil (DZRJ); Museo de la Universidad del Valle, Cali, Colômbia (MEUV); Museu Paraense Emilio Goeldi, Belém, Brasil (MPEG); Museu de Zoologia, Universidade Estadual de Santa Cruz, Ilhéus, Brasil (MZUESC).

As coletas foram realizadas nos seguintes estados: Acre, Bahia, Ceará, Pará, Paraíba, Pernambuco, Piauí e Rio Grande do Sul. As coletas aquáticas foram realizadas em cinco substratos principais de ambientes lóticos e lênticos (folhiço depositado no fundo do leito, folhiço retido em áreas de correnteza, areia, pedra e raízes/vegetação marginal em barranco). A amostragem foi feita utilizando uma rede D (MERRIT et al., 1996), com abertura de malha de no máximo 1mm, conforme. As ninfas coletadas foram fixadas ainda no campo, e conservadas em etanol a 80%. Quando possível, algumas ninfas (as que apresentavam teca alar escura caracterizando ninfas que estão próximas da emergência) eram criadas em campo ou em laboratório para emergirem, e com isso obter o estágio adulto da espécie para sua descrição ou confirmação. Após a emergência, a subimago era recolhida e armazenada viva em pequenos potes plásticos, e a correspondente exúvia fixada em etanol a 80%. Esta metodologia seguiu Edmunds et al. (1976), com algumas modificações.

Coletas noturnas também foram feitas com o auxílio de armadilhas luminosas para captura dos adultos, a fim de se obter todos os estádios das espécies. Utilizou-se um lençol

branco iluminado com lâmpadas de emergência ou de luz negra de 15 W ligada a uma bateria de 7V, no período entre 17h30min às 20h00min, e também no período de 04h00min às 05h30min. Além do lençol, foi utilizada uma armadilha do tipo Pensilvânia, ligada a uma bateria, a qual permanecia ligada até a manhã seguinte. Esses adultos eram utilizados para trabalhos descritivos e para comparação com aqueles obtidos por criação.

### 1.2.2 Identificação e descrições

A identificação das espécies foi realizada com base na chave de Dominguez et al. (2006). Para confirmação das espécies foram utilizados os artigos das descrições originais ou redescrições de cada espécie. As descrições das espécies ou estágios desconhecidos seguiram a padronização proposta por Hubbard (1995) para posição e nomes das estruturas corporais, como pernas, asas, brânquias e genitália. Os termos referentes ao tórax do adulto são os estabelecidos por Kluge (1994). Para a descrição dos ovos, foi utilizado as terminologias propostas por Koss & Edmunds (1974) e Malzacher (1982).

As ninfas e adultos foram dissecados e examinados com auxílio de microscópio estereoscópico e posterior montagem das estruturas em lâmina. Os indivíduos foram dissecados em álcool etílico a 100% e montados em lâminas permanentes com fixador Euparal® ou Bálsamo do Canadá. No caso dos adultos, as asas foram montadas entre lâmina e lamínula, sem meio de montagem, e as laterais da lamínula foram vedadas com fita adesiva. As genitálias eram normalmente montadas em lâminas permanentes. Contudo, quando era necessário a observação em vista lateral, as genitálias eram observadas em lâmina escavada com glicerina, e armazenadas em microtúbulo com álcool etílico a 80%.

Para o estudo morfológico das ninfas foram utilizadas as seguintes estruturas: antena, labro, mandíbula esquerda e direita, hipofaringe, lábio, maxila, pernas I, II e II, brânquias, tergito IV, paraprocto e cercos. Para adultos: asas anterior e posterior, pernas (I, II e III) e genitália masculina. As lâminas feitas com Euparal® foram secas em estufas a aproximadamente 60°C por dois ou três dias. Depois de secas, as lâminas foram examinadas utilizando microscópio óptico.

Microscopia Eletrônica de Varredura (MEV) foi usada para exame e produção de micrografias de ultraestrutura (ovos, brânquias e cerdas) de Caenidae, para a análise filogenética, utilizando um microscópio eletrônico Zeiss Supra 55VP. Ovos foram desidratados em várias

concentrações álcool etílico (80, 90 e 100%), secados ao ar livre, montados em *stubs* de MEV com fita dupla-face, e depois banhados a ouro.

1.2.3 Análise Filogenética

A análise filogenética do gênero *Brasilocaenis*, foi feita por métodos morfológicos, usando estados de caracteres contínuos e discretos não-ordenados. Para a listagem dos caracteres morfológicos foram utilizados dados disponíveis na literatura, observação de exemplares depositados em coleções científicas e material obtido por meio de coletas. Os estados de caracteres foram propostos, baseados na comparação de sete espécies, pertencentes ao gênero *Caenis* (seis espécies) e *Allorethocus* (uma espécie), incluídas como grupos externos. Para o enraizamento da árvore encontrada foi utilizada a espécie *Allorethocus peruanicus* (Sóldan, 1986).

Os caracteres foram codificados numericamente e registrados numa matriz, utilizando o Microsoft Excel (2013). Na matriz, os caracteres indicados com o símbolo [-] correspondem a dados não aplicáveis e o símbolo [?] corresponde a dados não observados. Logo depois foi gerado um arquivo Nexus, o qual foi analisado posteriormente, usando o programa TNT (Tree Analysis Using New Technology) (GOLOBOFF et al, 2008). Subsequente a cada caráter foram incluídos os números de passos (L) e os índices de consistência (IC) e retenção (IR), com os mesmos índices também sendo atribuído para a árvore encontrada.

A editoração das árvores foi feita através do programa Adobe Photoshop CS5®. No cladograma apresentado, os círculos brancos representam as transformações múltiplas (homoplasias) e os círculos pretos representam transformações únicas (apomorfias).

1.2.4 Análise Biogeográfica

Uma Análise Espacial de Vicariância (ARIAS et al., 2011) foi utilizada para analisar a distribuição dos *Brasilocaenis* na América do Sul. Este método, implementa as idéias de Hovenkamp (1997, 2001) e tenta reconstruir a história biogeográfica de táxons pelo exame de grupos irmãos disjuntos (i.e barreiras) vicariantes, através de um cladograma e dados distribucionais dos terminais. A análise foi implementada usando o software VIP (Vicariance

Inference Program), disponível no site http://www.zmuc.dk/public/phylogeny/vip (ARIAS, 2010).

1.2.5 Depósito

Os espécimes coletados encontram-se depositados ou serão encaminhados posteriormente para as seguintes coleções: Coleção Zoológica Norte Capixaba, Universidade Federal do Espírito Santo, Brasil (CZNC); Coleção Entomológica Prof. José Alfredo Pinheiro Dutra, Departamento de Zoologia da Universidade Federal do Rio de Janeiro, Brasil (DZRJ); Coleção Entomológica da Universidade Federal de Pernambuco, Brasil (CEUFPE); Instituto de Biodiversidad Neotropical, Universidad Nacional de Tucumán, Argentina (IBN); Museo de la Universidad del Valle, Cali, Colômbia (MEUV).

## 1.3 Resultados

Os resultados da presente tese são apresentados na forma de artigos e organizados em duas partes. A primeira parte, denominada **"Taxonomia de Ephemeroptera no Estado de Pernambuco"**, trata do levantamento dos efemerópteros ocorrentes para o Estado, juntamente com a descrição de novos táxons e/ou redescrição de táxons antigos. A segunda parte, **"Análise Filogenética e Biogeográfica de *Brasilocaenis* Puthz"**, tem como objetivo principal apresentar hipóteses de relacionamento filogenético entre as espécies de *Brasilocaenis*.

Do primeiro ao terceiro artigo da primeira parte, tratam-se exclusivamente da família Baetidae. O primeiro ARTIGO, denominado **"Imagos of *Camelobaetidius cayumba*" (Traver & Edmunds, 1968) (Ephemeroptera: Baetidae)"** (artigo publicado na Zootaxa, 2012, 3401: 63-65) e o segundo **"The adult stage of *Paracloeodes charrua* Emmerich & Nieto, 2009 (Ephemeroptera: Baetidae)"** (artigo publicado na Zootaxa, 2013, 3640(4): 597-600) são referentes a descrições dos estágios adultos de *C. cayumba* e *P. charrua*, além do primeiro registro da última espécie para o Brasil. No terceiro artigo **"First description of imago and redescription of nymph for *Cloeodes irvingi* Waltz & McCafferty, 1987 (Ephemeroptera:**

**Baetidae)"** (artigo publicado na Zootaxa, 2014, 838(5): 575-582), a imago macho de *C. irvingi* é descrita pela primeira vez baseada em ninfas criadas. Além da descrição do estágio adulto, também é apresentada uma redescrição da ninfa dessa espécie, baseada no material tipo e também em material recentemente coletado. Essa redescrição era necessária, uma vez que a descrição original estava incompleta e isso dificultava a comparação com outras espécies do gênero.

Os dois artigos subsequentes são relacionados a descrições de novas espécies da família Leptophlebiidae. O quarto artigo, **"New species for *Thraulodes* Ulmer, 1920 (Ephemeroptera: Leptophlebiidae: Atalophlebiinae) and the first key to adults from Brazil"** (artigo publicado na Zootaxa, 2013, 3709(3): 230-242), apresenta a descrição de quatro novas espécies do gênero *Thraulodes* para o Brasil, com duas delas sendo do Estado de Pernambuco. Além das descrições também foi elaborada uma chave de identificação de adultos para as espécies do Brasil. O quinto artigo, **"A new species of *Miroculis* Edmunds, 1963 (Ephemeroptera: Leptophlebiidae) from Northeastern Brazil"** (artigo publicado na Zootaxa, 2014, 3795 (4): 441-448), apresenta uma descrição de uma nova espécie para o gênero *Miroculis*, baseada em ninfas e adultos, a qual apresenta um formato de asa incomum para o gênero.

O sexto e último capítulo da primeira parte denominado, **"New records of mayflies (Ephemeroptera: Insecta) from Pernambuco State, Northeastern Brazil"** (artigo publicado na Check List, 11 (3): 1652), encontra-se uma lista atualizada das espécies ocorrentes no Estado de Pernambuco, com novos registros para o Estado e também para o Brasil.

A segunda parte, apresenta um único artigo denominado **"Phylogeny, taxonomy and biogeography of *Brasilocaenis* Puthz, 1975 (Caenidae: Ephemeroptera)"**. Este artigo trata da descrição e redescrição de espécies do gênero *Brasilocaenis*, além de apresentar uma análise filogenética e biogeográfica para o grupo.

Além da **Introdução**, ao final das duas seções são apresentadas as **conclusões gerais** sobre a tese. Todos os artigos encontram-se formatados de acordo com as normas da revista aos quais serão submetidos ou publicados, constituindo uma exceção as normas da ABNT.

# 2 TAXONOMIA DE EPHEMEROPTERA NO ESTADO DE PERNAMBUCO

Zootaxa (2012). 3401: 63–65.

## 2.1 Imagos of *Camelobaetidius cayumba* (Traver & Edmunds, 1968) (Ephemeroptera: Baetidae)

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## INTRODUCTION

The genus *Camelobaetidius* Demoulin (Ephemeroptera: Baetidae) is represented in South America by 28 species: 19 of them are described based on nymphs, three based solely on adults, and only six based on nymphs and adults (Salles & Serrão, 2005; Dominguez *et al*., 2006; Boldrini & Salles, 2009; Salles & Nascimento, 2009; Boldrini *et al.*, 2010). *Camelobaetidius cayumba* (Traver & Edmunds, 1968) was described from nymphs and can can be distinguished, among other characteristics, by the inner margin of the second segment of labial palp pointed, thoracic gill absent at base of forelegs, claws with 15 –18 denticles, and terminal filament about as long as cerci. It has been reported from Peru (Traver & Edmunds, 1968), French Guiana (Thomas *et al*., 2003), and Brazil (Salles & Serrão, 2005). The aim of the present paper is to describe the adults of *Camelobaetidius cayumba* from reared nymphs. The specimens were collected on rocky substrate of streams, and the adults were obtained by rearing these nymphs in the laboratory. Terms used in descriptions of thorax are in accordance to Kluge (1994). The material examined is housed in the Zoological Collection Norte Capixaba, Universidade Federal do Espírito Santo, São Mateus, ES (CZNC), Instituto Nacional de Pesquisas da Amazônia, Manaus, AM (INPA) and Entomological Collection of the Universidade Federal de Pernambuco, Recife, PE (UFPE).

### *Camelobaetidius cayumba* (Traver & Edmunds, 1968) (Figs. 1–7)

**Diagnoses**. Male: (1) anteronotal and metascutellar protuberance rounded (Fig. 2); (2) terga of abdominal segments II–III and VI medially with remarkable dark marks, segment II with a subrectangular mark, segment III with a mark in W-shaped, and segment VI with spots without form (Fig. 1); (3) forceps segment I without distomedial projection; (4) posterior margin of subgenital plate convex, posteriorly projected (Fig. 3). Female: (1) anteronotal and metascutellar

protuberance rounded; and (2) abdominal terga with segments I–II and V medially with dark spots without form. (Fig. 4).

**Male imago**. Body: 4.0–4.5 mm; cerci broken; forewing: 4.1–4.2 mm; hind wing: 0.8 mm; tibia I: 1.4 mm; tibia II: 1.0 mm; tibia III: 0.8–0.9 mm (n=2).

**Head** (Figs. 1, 2). Coloration brown; compound eyes yellowish black, turbinate portion reddish. Antenna with scape and pedicel light brown, flagellum light yellow.

**Thorax** (Fig. 1, 2). Pronotum dark brown; mesonotum brown with medioparapsidal suture darker, sublateroscutum light brown, posterior scutal protuberance dark brown; metanotum dark brown; prosternum whitish. Anteronotal protuberance rounded. Metascutellar protuberance rounded. Legs. Yellowish. Tarsi segments II of middle and hind legs with a small apical spine (Fig. 3). Leg I: tibia 2.1× length of femur; tarsi 1.3× length of femur. Leg II tibia 1.4× length of femur; tarsi 0.2× length of femur. Leg III tibia 1.5× length of femur; tarsi 0.2× length of femur. Forewing membrane hyaline; veins light brown; stigmatic area with four cross veins not touching subcostal vein; marginal intercalary veins paired, except between veins $ICu_2$ and A; length of forewing about 2.6× width. Hind wing membrane and veins hyaline with two complete longitudinal veins; costal process pointed, located on basal third.

**Abdomen** (Fig. 1). Terga light brown; segments II–III and VI medially with remarkable dark marks, segment II with a subrectangular mark, segment III with a mark in W-shaped, and segment VI with spots without form; and segments IV–V lighter. Tracheation black. Sterna yellowish, with segments VII–IX whitish. Caudal filaments white washed with brown. Genitalia (Fig. 4). Segments of forceps whitish washed with brown. Forceps segment I without distomedial projection; 0.2×length of segment II; distance between internal margins of forceps 0.6× distance between lateral margins of forceps. Forceps segment II narrow submedially. Forceps segment III elongate, 4.2× as long as wide; 0.2× length of segment II. Posterior margin of subgenital plate convex, posteriorly projected.

**Female imago**. Body: 4.0–4.5 mm; cerci: 6.4 mm; forewing: 4.8 mm; hind wing: 0.7 mm; tibia I: 1.0 mm; tibia II: 0.9–1.1 mm; tibia III: 0.8–0.9 mm (n=2).

**Head** (Fig. 5). Antenna with scape, pedicel and flagellum light brown.

**Thorax** (Fig. 5). Pronotum brown (lighter than male); mesonotum brown with margin of medioparapsidal suture darker, sublateroscutum brown, posterior scutal protuberance whitish; metanotum brown; prosternum whitish. Leg I: tibia 1.5× length of femur; tarsi 0.5× length of femur. Leg II tibia 2.0× length of femur; tarsi 0.4× length of femur. Leg III tibia 0.5× length of femur; tarsi 0.3× length of femur. Forewing (Fig. 6) , stigmatic area with five cross veins not touching subcostal vein; length of each intercalary vein 0.5× distance between adjacent longitudinal veins; length of forewing about 2.5× width. Hind wing (Figs. 7a, 7b).

**Abdomen** (Fig. 5). Terga orangish brown, segments I–II and V medially with dark spots, without form. Sterna yellowish, with segments VII–IX whitish.

**Material examined.** Four female imagos (reared), BRAZIL, Pernambuco State, Recife, Rio Capibaribe, 8°02'47.4"S, 34°59'41.9"W, 10/viii/2011, Lima LRC, Souza WRM cols. (2 UFPE, 1 CZNC and 1 INPA); two male imagos, same data except 15/ix/2011 (1 INPA, 1 CZNC).

**Comments.** The shape of the anteronotal protuberance has been regarded as an important character in the genus *Camelobaetidius* (Salles & Dias, 2004; Boldrini *et al*., 2010; Kluge & Novikova, 2011). Unlike many other Baetidae, the anteronotal protuberance in this genus is pointed, as stated at least for *C. billi* Thomas & Dominique, 2001 and *C. francischettii* Salles, Andrade & Da-Silva, 2005 (Salles & Dias, 2004; Boldrini *et al.* 2010). In *C. cayumba*, however, the anteronotal protuberance is not as developed and pointed as in these species. Eigth other species of the genus were described in the adult stage, but this characteristic has not been addressed in any of the descriptions. Because of this variation, becomes necessary to evaluate these characters in a broader sense among species of the genus.

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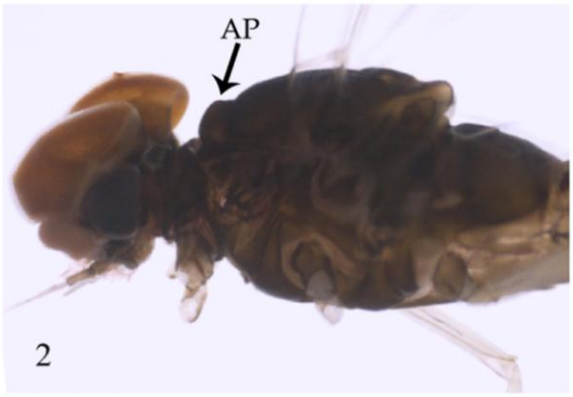





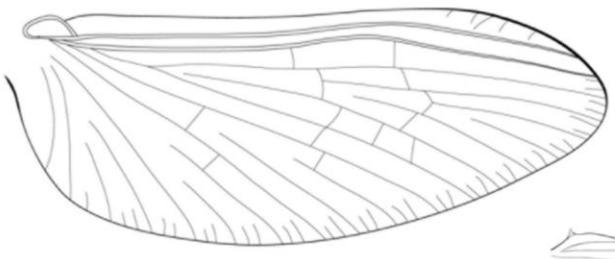



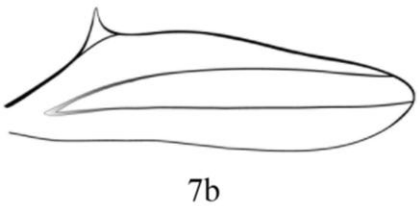


**Figure 1–7.** . Adults of *Camelobaetidius cayumba*. 1–4, male imago. 5–7, female imago. 1, body (dorsal view). 2, body (lateral view). 3, tarsi. 4, genitalia (ventral view). 5, body (dorsolateral view). 6, forewing. 7a, hind wing. 7b, hind wing enlarged (AP: Anteronotal protuberance).

Zootaxa (2012). 3640: 600–990.

## 2.2 The adult stage of *Paracloeodes charrua* Emmerich & Nieto, 2009 (Ephemeroptera: Baetidae)

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## INTRODUCTION

The genus *Paracloeodes* Day, 1955, included originally one new species, *Paracloeodes abditus* Day, 1955, known from nymphs and adults from the USA and one species transferred from *Cloeodes* Traver, 1938, *P. portoricensis* (Traver, 1938) from Puerto Rico. Later, McCafferty & Waltz (1990) synonymized the type species with *Paracloeodes minutus* (Daggy, 1945). Currently, the genus is represented in the New World by 19 species with 14 species reported from South America. In this paper, *Paracloeodes charrua* Emmerich & Nieto, 2009 (previously described from Uruguay) is reported for the first time from Brazil and their adult stages are described.Nymphs of *P. charrua* were collected on marginal vegetation of the streams and rocky substrate. The adults were obtained by rearing these nymphs in the laboratory and catch by light traps. Terms used in descriptions of thorax are from Kluge (1994). The descriptions were made in the free program DELTA (DEscription Language for TAxonomy) (Dallwitz, 1980). The material examined is housed in the Coleção Zoológica Norte Capixaba (CZNC), Instituto Nacional de Pesquisas da Amazônia (INPA) and Coleção Entomológica da Universidade Federal de Pernambuco (UFPE).

### *Paracloeodes charrua* Emmerich & Nieto, 2009 (Figs. 1–6)

**Diagnosis**. The male imagos of *P. charrua* can be distinguished from the other species of genus by the following combination of characters: (1) hind wings present; (2) tibia of forelegs almost twice the length of femur; (3) abdominal terga light brown, submedially with a pair of small red spots; (4) posterior margin of subgenital plate convex, posteriorly with a small projection.

#### Male imago

**Maximal length.** Body: 3.3–3.8 mm; cerci broken; forewing: 3.3–4.2 mm; hind wing: 0.54–0.8 mm; tibia I: 1.3 mm; tibia II: 0.8 mm; tibia III: 0.7 mm.

**Head** (Figs. 1–2). Coloration brown; compound eyes blackish, turbinate portion light brown. Antenna with scape and pedicel light brown, flagellum lighter. Dorsal portion of turbinate eyes oval; length 1.8x width; stalk height 1.1x width of dorsal portion; inner margins not parallel, divergent anteriorly.

**Thorax** (Figs. 1–2). Pronotum brown; mesonotum brown with medioparapsidal suture darker, sublateroscutum brown, posterior scutal protuberance brown; metanotum brown; prosternum brown. Anteronotal protuberance rounded. Legs yellowish with a blackish line on posterior face. Leg I: tibia 1.9× length of femur; tarsi 1.7× length of femur. Leg II tibia 1.4× length of femur; tarsi 0.5× length of femur. Leg III tibia 1.4× length of femur; tarsi 0.5× length of femur. Forewing (Fig. 3): membrane hyaline, veins light yellow, stigmatic area with five cross veins, not touching subcostal vein. Marginal intercalary veins paired, except between veins $ICu_2$ and A; length of forewing about 2.2× width. Hind wing hyaline with two complete longitudinal veins; costal process quadrangular located on basal third (Figs. 4a–4b).

**Abdomen** (Figs. 1–2). Terga light brown, submedially with a pair of small red spots; segments VII–X darker and segments II–VI lighter. Tracheation black. Sterna yellowish, medially with two pairs of small red spots. Genitalia (Fig. 5) with segments of forceps whitish, basally washed with brown. Forceps segment I cylindrical, without distomedial projection; 0.3× length of segment II; distance between base of forceps 0.4× distance between lateral margins of forceps. Forceps segment II narrow submedially. Forceps segment III elongate, length 2.9× the width; 0.2× length of segment II. Posterior margin of subgenital plate convex, posteriorly with a small projection (Fig. 5).

**Female imago**

**Maximal length.** Body: 3.6–4.0 mm; cerci: broken; forewing: 3.4–3.8 mm; hind wing: 0.45–0.52 mm; tibia I: 0.6 mm; tibia II: 0.7 mm; tibia III: 0.6 mm.

**Head** (Fig. 6). Coloration light brown; compound eyes blackish. Antenna with scape, pedicel and flagellum light brown.

**Thorax** (Fig. 6). Pronotum light brown; mesonotum brown with medioparapsidal suture darker, sublateroscutum brown, posterior scutal protuberance brown; metanotum brown; prosternum brown. Anteronotal protuberance rounded. Leg I: tibia 1.2× length of femur; tarsi 0.4× length of femur. Leg II tibia 1.4× length of femur; tarsi 0.4× length of femur. Leg III tibia 1.2× length of femur; tarsi 0.4× length of femur. Forewing hyaline,veins light yellow, stigmatic area with five cross veins, two touching subcostal vein. Marginal intercalary veins paired, except between veins $MP_2$ and $ICu_2$; length of forewing about 2.6× width. Hind wing hyaline with two complete longitudinal veins; costal process quadrangular located on basal third.

**Abdomen** (Fig. 6). Terga light brown, submedially with a pair of small reddish spots. Tracheation black. Sterna yellowish, medially with two pairs of small reddish spots.

**Material examined.** BRAZIL, Pernambuco State, Amaraji, Rio Amaraji, 8°21'48.9"S, 35°28'49.0"W, 20/i/2011, 320m, Lima L.R.C., Nicacio G. cols, three male imagos (light trap) (UFPE); same data as preceding except 21/iv/2012, one male imago (CZNC); BRAZIL, Pernambuco State, Amaraji, Poço do Carreiro, 8°22'1.94"S, 35°28'8.73"W, 28/iv/2012, 269m, Lima L.R.C. coll., one male imago, (light trap) (INPA); BRAZIL, Pernambuco State, Jaqueira, Rio Pirangi, 8°44'53.0"S, 35°48'51.1"W, 23/v/2012, 189m, Lima L.R.C. coll., two male imagos (INPA); same data as preceding except 13/vii/2012, two male imagos (reared) (CZNC); BRAZIL, Pernambuco State, Ribeirão, Rio Amaraji, 8°26'34.1"S, 35°24'22.1"W, 21/i/2011, 124m, Lima LRC, Nicacio G. cols., one female imago (UFPE); BRAZIL, Pernambuco State, Flores, Rio Pajeú, 7°52'35.7"S, 38°00'04.0"W, 19/vii/2011, 473m, Lima LRC coll., two female imagos (reared) (1 INPA, 1 UFPE).

**Comments.** *P. charrua* male imago can be distinguished from other male described by the ratio of tibia/femur of forelegs which is almost twice longer in *P. charrua* than *P. minutus* and *P. portoricensis* (about 1.4x); the abdominal color pattern; the presence/absence of hind wings and one conspicuous characteristic, the presence of a small pointed projection on the posterior margin of subgenital plate (Fig. 5). This projection is for the first time related to the genus, since it is not present in male imagos of *P. minutus* and *P. portoricensis*. Although uncommon, the projection has also been reported for males adults of *Rivudiva minantenna* Lugo-Ortiz and McCafferty, 1998, and because of that, two species described based only on adults were transferred to this genus: *Rivudiva coveloae* (Traver, 1971) and *R. venezuelensis* (Traver, 1943) (Salles & Nascimento, 2011). Other similarities occurs between the subgenital plates of *P. portoricensis* and the recently described male imago of *Rivudiva trichobasis* Lugo-Ortiz & McCafferty, 1998 (Cruz et al. 2011): both possess the subgenital plate concave, though much deeper in *R. trichobasis* than in *P. portoricensis*. Given the few number of species described based on nymphs and adults, it is difficult to ascertain at this time if these similarities are an example of convergence or an indicative of close relationship of both genera.

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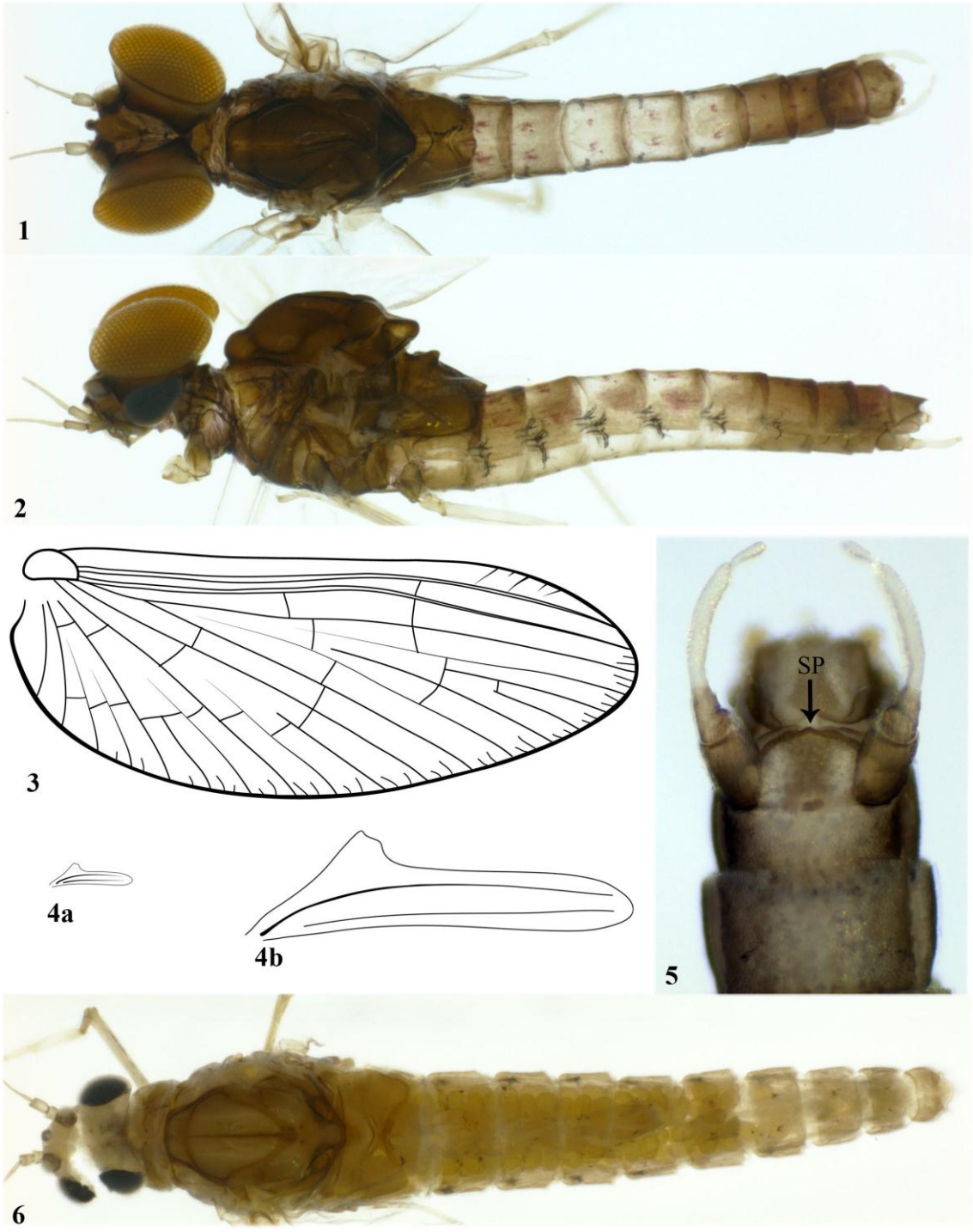


**Figure 1–6.** Adults *Paracloeodes charrua*. 1, male imago (dorsal view). 2, male imago (lateral view). 3, forewing. 4a, hind wing. 4b, hind wing (enlarged). 5, genitalia. 6, female imago (dorsal view) (SP = small projection).

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## 2.3 First description of imago and redescription of nymph for *Cloeodes irvingi* Waltz & McCafferty, 1987 (Ephemeroptera: Baetidae)

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**ABSTRACT**

The male imago of Cloeodes irvingi Waltz & McCafferty, 1987 is described for the first time based on reared nymphs collected from the state of Pernambuco, northeastern Brazil. It is differentiated from Neotropical congeners, among other characteristics, by the marginal intercalary veins being paired, except between veins ICu1–ICu2 and ICu2–CuP where they are single and between Sc–R1 and CuP–A where they are absent; segment II of forceps with a medial constriction; and the posterior margin of the subgenital plate being rounded. The nymph of this species is redescribed based on new and original specimens. It is differentiated from Neotropical congeners, among others characteristics, by having a labrum with a dorsal arc composed of 2 + 0 + 2 long, spine-like setae, a labial palp segment III that is subquadrangular, and the fore femur with an apex that is not projected, with 2 blunt setae.

**Key words:** aquatic insects, mayflies, taxonomy, Brazil, Neotropical Region

## INTRODUCTION

*Cloeodes* Traver (1938) has a widespread pantropical distribution with representatives in Africa (Waltz & McCafferty 1994; Jacobus *et al.* 2006), Madagascar (Lugo-Ortiz *et al.* 1999), Southeast Asia (Soldán & Yang 2003) and the Americas (e.g., Traver 1938; McCafferty & Lugo-Ortiz 1996; Wiersema & Baumgardner 2000; McCafferty *et al*. 2004; Nieto & Richard 2008; McCafferty *et al.* 2012). In South America, there are 23 species known: seven from nymphs, five from adults, and ten from both nymphs and adults (Nieto & Richard 2008; Gonçalves *et al*. 2010; Massariol & Salles 2011; Nieto & Emmerich 2011; Massariol *et al.* 2013). In Brazil, 11 species are reported (Salles *et al.* 2014).

*Cloeodes irvingi* Waltz & McCafferty, 1987 was described based only on nymphs from Paraguay, and later reported from Brazil (Salles *et al.* 2003, 2004, 2010; Boldrini *et al.* 2012; Lima *et al*. 2012). With the increase in knowledge about species diversity within *Cloeodes*, the original description of *C. irvingi* has become inadequate for differentiating it from other congeners in South America, which led us initially to misidentify some recently collected specimens of *C. irvingi*. However, the comparison of these specimens with *C. irvingi* type material deposited at the Purdue University Entomological Research Collection, West Lafayette, Indiana, USA (PERC), revealed them to be *C. irvingi*.

The aim of this paper, therefore, is to describe for the first time the male imago of *C. irvingi*, which we have associated through rearing, and to redescribe the nymph based on the original material and an expanded collection of materials from several localities in state of Pernambuco, northeastern Brazil.

## MATERIALS AND METHODS

The material used in this study was preserved in 80% ethanol. Legs and genitalia of male imago were dissected and mounted in slides with Euparal. Wings were mounted dry. The material is deposited in the following institutions: Coleção Entomológica da Universidade Federal de Pernambuco, Recife, Brazil (UFPE), Coleção Zoológica Norte Capixaba, São Mateus, Brazil (CZNC), Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil (INPA), and PERC.

***Cloeodes irvingi* Waltz & McCafferty, 1987 (Figs. 1–17)**

**Diagnosis**. *Nymph*: 1) antenna about 1.5 × length of head capsule; 2) labrum with dorsal arc of setae composed of 2 + 0 + 2 long, spine-like setae (Fig. 6); 3) segment III of labial palp subquadrangular (Fig. 11); 4) fore femur with apex not projected, with 2 blunt setae (Fig. 13); 5) tarsal claw 0.4 × length of tarsi; 6) abdominal segments I–II brown with blackish transversal mark on the posterior margin; segments III, V–VII and IX–X brown with whitish marks (Fig. 5); 7) paraproct with 10−13 marginal spines (Fig. 17); 8) caudal filaments with posterior margin of segments with short spines on each segment and long spines on every four segments.

*Male imago*: 1) turbinate eyes with inner margins parallel (Fig. 1); 2) abdominal terga I–II brown with blackish transversal mark on the posterior margin; segments III, V–VII and IX–X brown with whitish marks; 3) marginal intercalary veins paired, except between veins ICu1–ICu2, ICu2–CuP single, and between Sc–R1 and CuP–A absent (Fig. 2); 4) segment II of forceps with medial constriction (Fig. 4); 5) segment III of forceps elongated; 6) posterior margin of subgenital plate rounded.

**Descriptions.** *Male imago*: Length. Body: 3.3–4.2 mm; antenna: 0.7–0.8 mm; fore wing: 3.8 mm; tibia I: 1.6 mm; tibia II: 0.7 mm; tibia III: 0.6 mm; caudal filament: broken off.

Head (Fig. 1). Brownish. Turbinate portion of compound eyes dorsally dark brown, stalk reddish brown. Antennae light brown. Dorsal portion of turbinate eyes oblong; length 1.6 × width; stalk height 0.8 × length of dorsal portion; inner margins parallel (Fig. 1).

Thorax (Fig. 1). General coloration brown. Metanotum with grayish margins. Anteronotal protuberance rounded. Metascutellar protuberance pointed and projected dorsally.

Legs. General coloration translucent brown; all femora with transversal blackish mark. Leg I: tibia 2.7 × length of femur; tarsi 2.5 × length of femur and four segments decreasing on length apically. Leg II: 1.4 × tibia length of femur, tarsi 0.8 × length of femur. Leg III: tibia 1.0 × length of femur, tarsi 0.8 × length of femur.

Fore wing (Fig. 2). Membrane hyaline. Longitudinal and cross-veins pale yellow. Stigmatic area with four cross veins touching subcostal vein. Marginal intercalary veins paired, except between veins ICu1–ICu2 and ICu2–CuPsingle, and between Sc–R1 and CuP–A absent;

length of each intercalary vein between IMA and IMA2 0.5 × distance between adjacent longitudinal veins; length of fore wing about 2.3 × width.

Hind wing present (Fig. 3). Membrane hyaline; veins pale yellow; with 2 complete longitudinal veins. Costal process hooked; located on basal third.

Abdomen (Fig. 1). Tergum I brown; terga with segments II−VI whitish translucent and VII−X brown. Tergum I with transversal blackish line on posterior margin; terga II and IV–VII with blackish line interrupted at middle on posterior margin. Tergum IV with medio-postero transversal spots. Tracheation blackish. Sterna with segments II−VI whitish translucent, I and VII−X light brown.

Genitalia (Fig. 4). Light brown. Forceps segment I sub-rectangular; 0.3 × length of segment II; distance between the base of forceps 0.3 × distance between lateral margins of forceps. Segment II of forceps with medial constriction; inner margin undulated. Forceps segment III 1.7 × longer than wide, 0.1 × length of segment II. Posterior margin of subgenital plate rounded.

*Nymph:* Length. Body: 4.1−5.5 mm; antenna: 1.3−1.6 mm; cerci: 1.0−1.2; terminal filament: 1.3−1.5.

Head. Light brown with a narrow median longitudinal band brown along the length of vertex; compound eyes and ocellus of male surrounded yellow. Frons with area between antennal bases yellowish. Turbinate portion of compound eyes of male dark brown. Antenna light brown; 1.5 × length of head capsule.

Labrum (Fig. 6). Rectangular, broader than long, length about 0.7 × maximum width; dorsal surface flat; distal margin with medial emargination and small process. Dorsally with many short, fine, simple setae scattered over surface; dorsal arc of setae composed of 2 + 0 + 2 long, spine-like setae. Ventrally with submarginal row of setae decreasing in length toward medial region, composed of lateral and anterolateral bifid and frayed setae, medial setae simple and bifid; ventral surface with 3 short, blunt setae near the anterolateral margins; lateral margin with fine simple setae.

Right mandible (Fig. 7). Inner and outer set of incisors respectively with 3 + 3 denticles. Prostheca slender, bifurcated at middle, inner lobe long, pectinate, outer short and frayed. Margin between prostheca and mola almost straight; tuft of setae between prostheca and mola absent; tuft

of spine-like setae at base of mola present; tuft of setae at apex of mola present, reduced to bifid seta. Lateral margins almost straight and bare; basal half bare.

Left mandible (Fig. 8). Inner and outer set of incisors respectively with 3+ 3 denticles. Prostheca robust, apically denticulate and with comb-shape structure at apex. Margin between prostheca and mola almost straight, without crenulations; tuft of setae absent; tuft of spine-like setae at base of mola present; subtriangular process wide, above level of area between prostheca and mola; denticles of mola not constricted; tuft of setae at apex of mola absent. Lateral margins almost straight and bare; basal half bare.

Hypopharynx (Fig. 9). Lingua subequal in length to superlingua; apex with anteromedial lobe rounded, with short, fine, simple setae; medial tuft of short setae present; distal half laterally expanded. Superlingua not expanded; fine, simple setae scattered over lateral and distal margin and basal half of lateral margin with short, spine-like setae.

Maxilla (Fig. 10). Crown of galea-lacinia with 3 denticles and one denti-seta; inner dorsal row of setae with 3 pectinate denti-setae. Medial protuberance of galea with 1 short, spine-like setae + 5 long setae. Maxillary palp reaching apex of galea-lacinia; two-segmented; setae on maxillary palp, short, fine and simple, scattered over surface, a simple setae at apex of segment II; palp segment II 1.3 × length of segment I; apex of last segment not constricted at base.

Labium (Fig. 11). Glossa basally broad, narrowing apically and subequal in length to paraglossa; base of glossa well expanded (reaching more than 2/3 of paraglossa); inner margin with 11−14 spine-like setae increasing in length apically, outer margin with 10−12 long, spine-like setae increasing in length apically; ventral surface with a row of 5−6 spine-like setae near inner.Paraglossa sub-rectangular, curved only at apex; apex with 2 rows of spine-like setae; outer margin with a row of 15−16 long and spine-like setae; ventrally with a row of 5 spine-like setae near to inner margin; dorsal surface bare. Labial palp with segment I 0.9 × length of segments II and III combined; segment I covered with short, simple setae and micropores near to outer margin; inner margin of segment II bare; outer margin with short, fine setae; dorsally with row of 4−5 spine-like, simple setae; ventrally with short, fine, simple setae scattered over surface; segment III subquadrangular length 1.2 × width; covered with spine-like simple setae on ventral surface; dorsally with a row of blunt, spine-like, long setae near to apex and inner margin.

Thorax (Fig. 5). General coloration brown with yellow marks. Fore wing pads light brown.

Hind wing pad. Present; light brown.

Fore leg (Fig. 12). General coloration yellowish; femora with a central light brown spot on anterior surface; tarsal claw yellowish brown. Ratio of fore leg 1.4:1.0 (0.5 mm):1.0:0.2.

Fore femur. Length about 3.9 × maximum width; dorsally with a row of 7 blunt setae (in lateral view they look like spine-like setae); length of setae about 0.1 × maximum width of femur; apex not projected with 2 blunt setae (Fig. 13); ventrally with 5 simple setae; anterior surface with abundant scale-bases and scales near to ventral margin.

Tibia. Dorsally with row of abundant, long, fine, simple setae; ventrally with a row of 6 short, spine-like setae; two lanceolate setae subapically; anterior surface with abundant scale bases and scales near to dorsal margin; tibio-patelar suture present. Subtending bristle present (Fig. 14).

Tarsus. Dorsally with a row of abundant long, fine setae; ventrally with row of 11 spine-like setae and a lanceolate setae near the apex; anterior surface with scale bases and scales scattered over surface; tarsal claw bare, 0.4 × length of tarsi.

Mid and hind leg similar to fore leg.

Abdomen (Fig. 5). General coloration brown. Segments I–II brown with blackish transversal mark on the posterior margin. Segments III, V–VII, and IX–X brown with whitish marks. Segments IV and VIII clearer; male mature nymphs showing two spots in medial area of segment IV. Terga surface with abundant scale bases and micropore; posterior margin of terga with regular spines, 1.7 × as long as wide (Fig. 15). Spines present in posterior margin of segments III−X (in some species present spines on segments II–X, but weakly developed on segment II). Sterna with spines present in posterior margin of segments III−IX.

Gills (Fig. 16). Opaque, trachea dark gray; inner and outer margins light brown. Margin with broad spines and short, fine, simple setae on the apex. Trachea extending from main trunk to inner margin. Gill I about 1.0 × length of segment II; oval. Gill IV about 0.5 × length of the segment V and VI combined; oval. Gill VII about 1.0 × length of segment VIII, oblong.

Paraproct (Fig. 17). With 10−13 marginal spines; surface with abundant scale-bases and micropores; postero-lateral extension with blunt marginal spines and scale-bases scattered on surface.

Caudal filaments. Yellowish-brown. Posterior margin of segments with short spines on each segment, and long spines on every four segments.

**Life cycle association.** Rearing male imagos from nymphs.

**Distribution.** PARAGUAY. BRAZIL: states of Ceará, Goiás, Minas Gerais, Pernambuco, Rio de Janeiro, and São Paulo.

**Material examined.** 14 nymphs, Brazil, state of Pernambuco, Amaraji, Rio Amaraji, 20.i.2011, 8°21'48.9"S, 35°28'49.0"W, 320 m, Lima LRC, Nicacio G cols. (PERC); five nymphs, same as preceding except 21.iv.2012, Lima LRC coll. (CZNC); six nymphs and three male imagos (reared), same as preceding except 28.iv.2012 (six nymphs CZNC, two male imagos PERC, and one male imago INPA); three nymphs and one male imago (light trap), Brazil, state of Pernambuco, Amaraji, Rio Amaraji, Poço do Carreiro, 28.iv.2012, 8°22'1.94"S, 35°28'8.73"W, 269 m, Lima LRC coll. (UFPE); one male imago (light trap), Brazil, state of Pernambuco, Correntes, Rio Correntes, Cachoeira Encontro das Águas, 14.ii.2012, 9°11'04.5"S, 36°21'19.7"W, 513 m, Lima LRC, Souza WRM cols. (UFPE); two nymphs, Brazil, state of Pernambuco, Correntes, Rio Correntes, Barragem da Compesa, 15.ii.2012, 9°08'13.0"S, 36°19'57.5W", 386 m, Lima LRC, Souza WRM cols. (INPA); one nymph, Brazil, state of Pernambuco, Cortês, Sirinhaém, Banho da Ilha, 05.ii.2011, 8°30’06.5”S, 35°31’28.6”W, 223 m, Lima LRC, Nicacio, G cols. (PERC); same as preceding except 20.iii.2011 (UFPE).

**Additional material.** Holotype, male larva, Paraguay, Paraguari, Parque Nacional Ybicui, Arroyo Mina, edge, 02.x.1984, Bonace RT (PERC); paratype, slide mounted in Euparal (head capsule and fore leg missing), Paraguay, Departamento. Amambay, Parque Nacional, Cerro Cora, Arroyo Panamy, riffle sample, 05.xi.1983, Bonace RT (PERC).

## DISCUSSION

There are 15 South American species of *Cloeodes* with adult stages described, however only three species, *Cloeodes aymore* Massariol & Salles, 2011, *C.loeodes hydation* McCafferty & Lugo-Ortiz, 1995, *Cloeodes itajara* Massariol & Salles, 2011, and *Cloeodes jaragua* Salles & Lugo-Ortiz, 2003 share similar characteristics with the imago of *C. irvingi*: segment II of forceps with constriction (Fig. 4); and hind wing with costal process hooked, located on basal third and with two longitudinal veins (Fig. 3). The male imago of *C. irvingi* can be distinguished of *C. aymore* by the posterior margin of the subgenital plate being rounded (Fig. 4) instead of truncate.

In relation to *C. hydation*, *C. irvingi* has a medial constriction on segment II (basal in *C. hydation*) (Fig. 10) and besides do not have small spines on costal margin of forewing. *Cloeodes itajara* can be distinguished from *C. irvingi* by the abdominal color pattern that shows anterolateral triangular black marks on terga V−VII and a body size that is almost the double of *C. irvingi*. Finally, the imago of *C. jaragua* can be readily distinguished from *C. irvingi* by the presence of a distolateral projection adjacent to the constriction at the second segment and by its brownish abdominal color pattern.

The nymphs of *C. irvingi* together with *C. incus* (Waltz & McCafferty 1987) from Bolivia, *C. hydation* from Brazil, and *C. penai* (Morihara & Edmunds 1980) from Argentina do not have an apical projection on the fore femora. However, *C. irvingi* can be distinguished from *C. incus* because the latter has a tarsal claw with minute denticles basally. *Cloeodes irvingi* is distinguished from *C. hydation* by the length of the antennae being about 1.5 × length of the head capsule (as compared to two times the length in *C. hydation*), by the absence of spines on posterior margin of terga I–II (as compared to terga I–III with spines in *C. hydation*), and by the maxilla with last segment not constricted (as compared to maxilla with last segment constricted in *C. hydation*). Finally, *C. penai* can be distinguished from *C. irvingi* by the length of the antennae being 3 × length of head capsule, the apex of the femur having pointed spines, and the paraproct having 20−30 marginal spines (compared to 10–13 in *C. irvingi*).

We observed seemingly significant morphological variation on the nymphal armature in type material of *C. irvingi*. The holotype has spines only on segments II–X, with segment II having only a few weakly developed spines. One paratype, however, has spines on the posterior margins of abdominal terga I–X (with many distinctly developed spines on tergum I). We note that our newly reported material all corresponds to the holotype variant. We do not know yet whether the apparently aberrant paratype variant falls within a wider range of variation of *C. irvingi*, or whether this variant represents a different species. This question can be explored only when more specimens are available for study.

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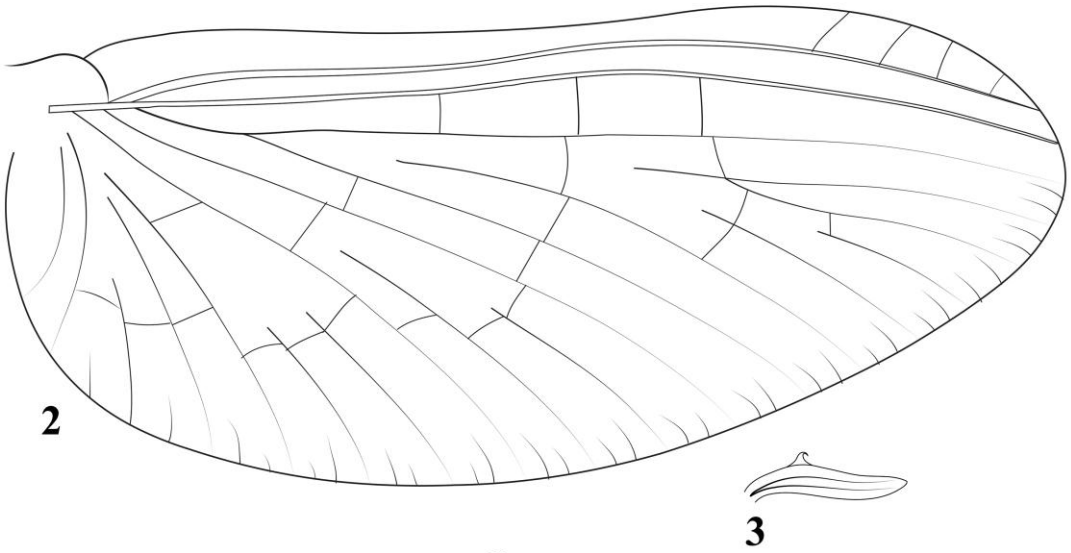
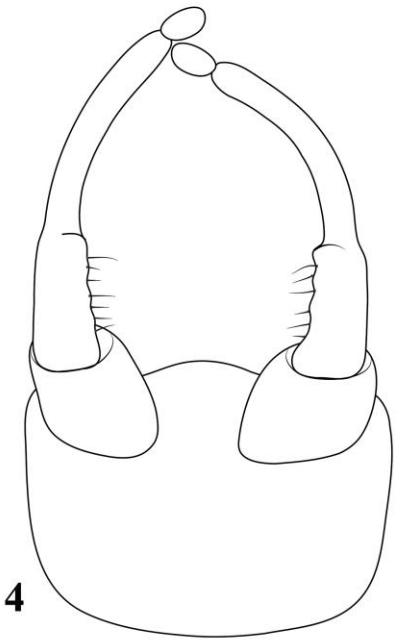
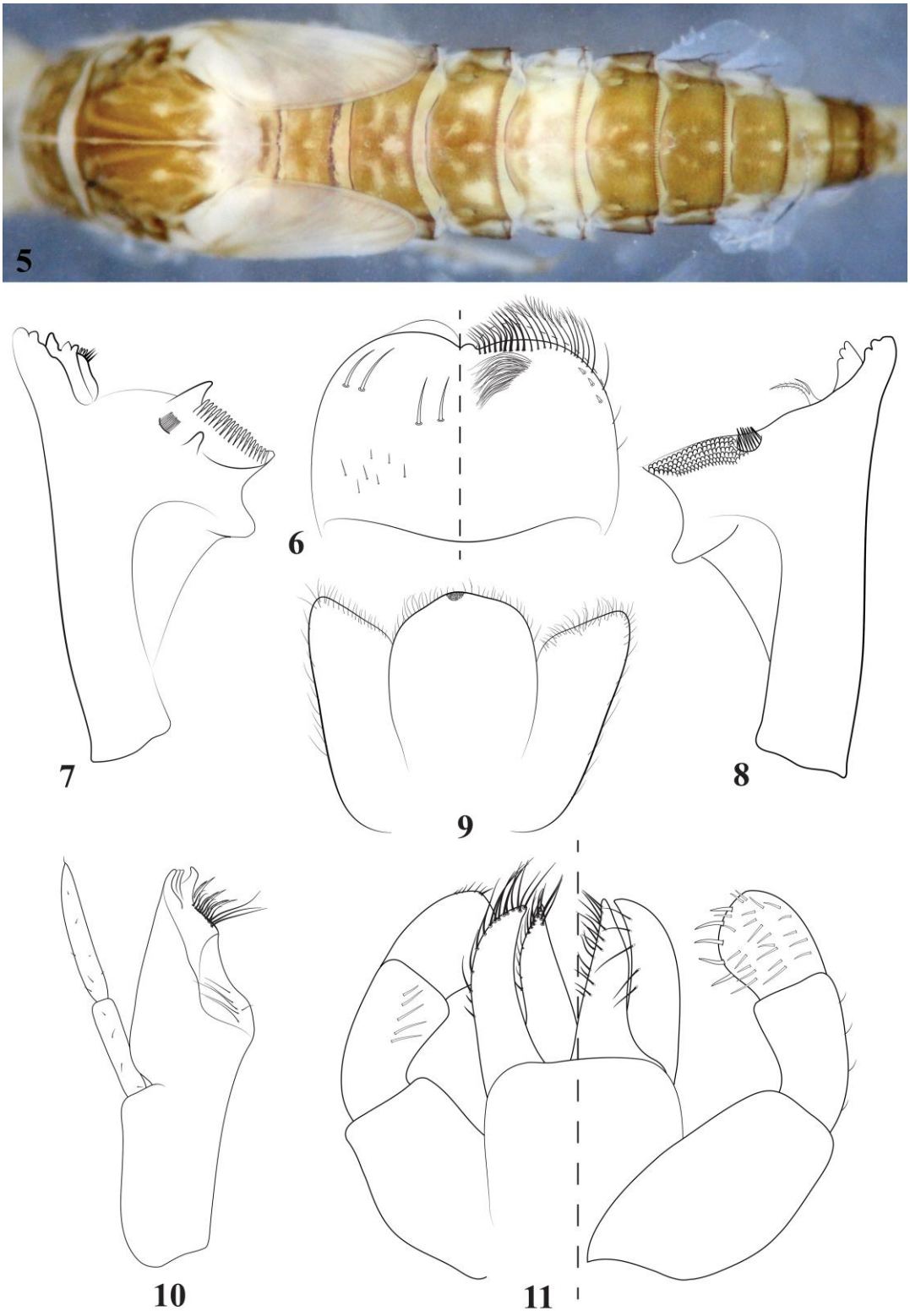
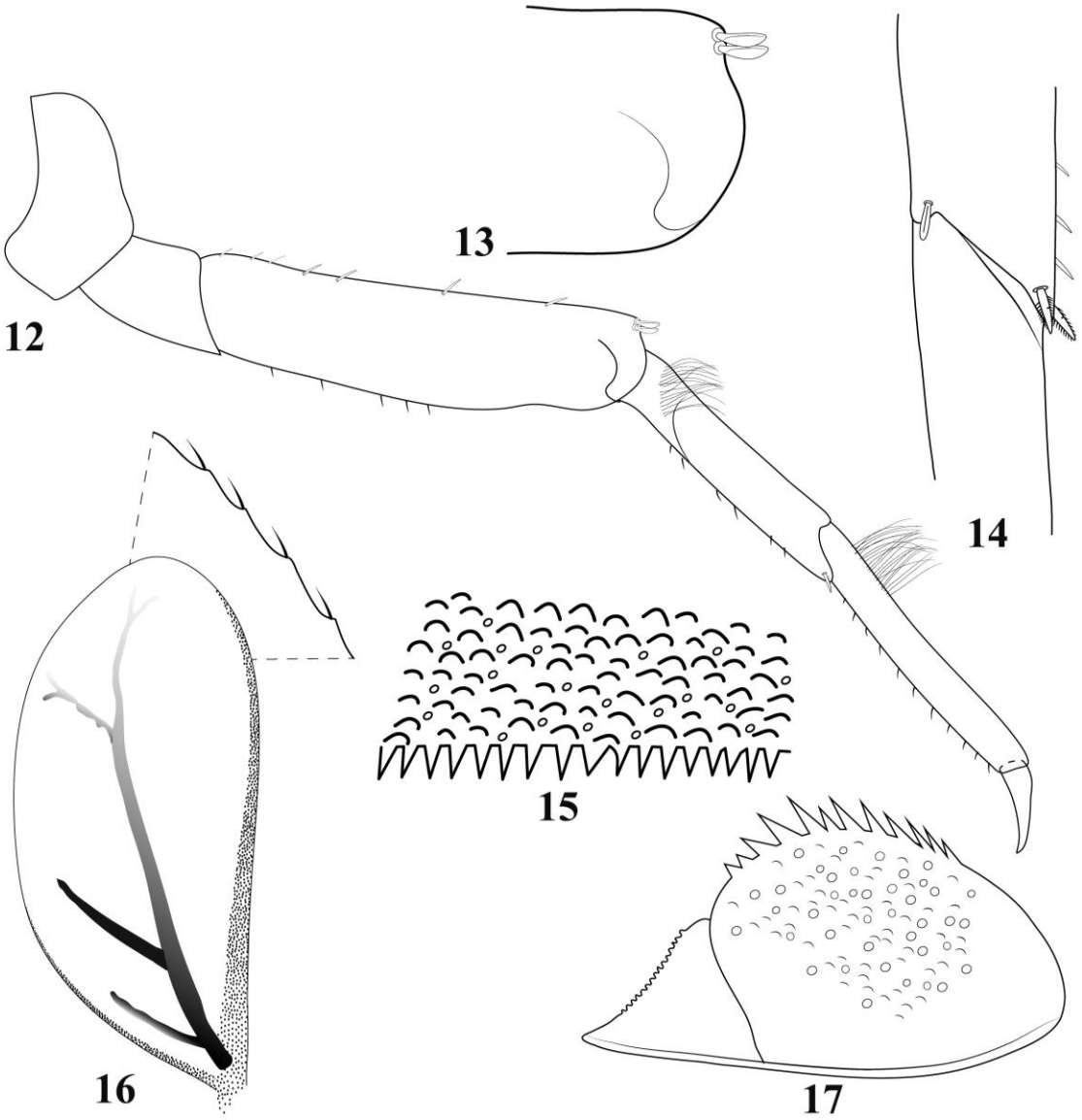



**Figures 1–4.** Male imago of *Cloeodes irvingi*. 1. Dorsal habitus; 2. Fore wing; 3. Hind wing; 4. Genitalia (ventral view).




**Figures 5–11.** Nymph of *Cloeodes irvingi*. 5. Dorsal habitus; 6. Labrum (right: ventral view; left: dorsal view); 7. Right mandible (ventral view); 8. Left mandible (ventral view); 9. Hypopharynx (ventral view); 10. Maxilla (ventral view); 11. Labium (right: ventral view; left: dorsal view).




**Figures 12–17.** Nymph of *Cloeodes irvingi*. 12. Fore leg (anterior surface); 13. Apex of fore femur (anterior surface); 14. Subtending bristle (anterior surface); 15. Posterior margin of tergum IV; 16. Gill IV, with detail of margin; 17. Paraproct.

*Zootaxa* (2013). 3709(3): 230–242

## 2.4 New species for *Thraulodes* Ulmer, 1920 (Ephemeroptera: Leptophlebiidae: Atalophlebiinae) and the first key to adults from Brazil.

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**ABSTRACT**

Four new species of the mayfly genus Thraulodes Ulmer are described based on imagos from Northeastern and Southern Brazil: *Thraulodes luizgonzagai* sp. nov., *Thraulodes sternimaculatus* sp. nov., *Thraulodes alapictus* sp. nov., and *Thraulodes pinhoi* sp. nov. The new species can be easily differentiated by the abdominal color pattern and genitalia morphology. The first key to adults from Brazil is provided.

**Key words:** aquatic insects, mayflies, taxonomy, Brazil, Neotropical Region

## INTRODUCTION

*Thraulodes* is one of the most abundant and the most widespread leptophlebiid genera (Ephemeroptera: Leptophlebiidae) in the Neotropics and its distribution extends from Central Argentina to Neartic Region (Southern USA). Ulmer (1920) established *Thraulodes* from a male imago from Colombia originally described as *Thraulus laetus* Eaton, 1883. Traver & Edmunds (1967) and Allen & Brusca (1978) revised the genus and proposed two species groups: the first based on adult genitalia characters and the second based on the gill structure of the nymphs.

Currently, there are 56 known species, with 35 species reported from South America and 22 from Central and North America (Barber-James *et al.* 2013). *Thraulodes marhieus* Dubey, 1970 was described from Northwest Hymalaya, based on a female imago, however Hubbard & Peters (1978) and Kluge (2004) treat this species as *incertae sedis*. In Brazil 13 species are reported: *T. amanda* Mariano & Froehlich, 2011; *T. bonito* Gonçalves, Da-Silva & Nessimian, 2013; *T. cochunaensis* Domínguez, 1987; *T. daidaleus* Thew, 1960; *T. itatiajanus* Traver & Edmunds, 1967; *T. jones* Gonçalves, Da-Silva & Nessimian 2010; *T. limbatus* (Navás, 1936); *T. pelicanus* Mariano & Froehlich, 2011; *T. schlingeri* Traver & Edmunds, 1967; *T. subfasciatus* Navás, 1924; *T. traverae* Thew, 1960; *T. ulmeri* Edmunds, 1950; and *Thraulodes xavantinensis* Mariano & Froehlich, 2011.

Nevertheless, the mayfly fauna from Brazil is still poorly known. Most species of *Thraulodes* in Brazil were registered from Southeastern and Southern regions, and the majority is known only based on its adult stage, which is a problem found in many groups of mayflies. The aim of this paper is to describe four new species of *Thraulodes* based on imagos and to elaborate the first key to adults from Brazil.

## MATERIAL AND METHODS

The adults were caught on light traps right after sunset in three localities from Northeastern and Southern Brazil (Fig. 1). The material used in this study is preserved in alcohol 80%. Male legs and genitalia were dissected and then mounted in Euparal®, wings were dry mounted. In the following descriptions, terms referring to the adult thorax follow Kluge (1994) and terms referring to the genitalia follow Traver and Edmunds (1967). Drawings were made

with the aid of camera lucida. The material is deposited in the following institutions: Coleção Entomológica da Universidade Federal de Pernambuco (UFPE), Recife, Brazil; Coleção Zoológica Norte Capixaba (CZNC), São Mateus, Brazil; Instituto Miguel Lillo (IML), San Miguel de Tucumán, Argentina; and Museu de Zoologia da Universidade Estadual de Santa Cruz (MZUESC), Ilhéus, Brazil.

**Key to the male imagos of *Thraulodes* from Brazil**[1]

1. Abdomen entirely pigmented (dark brown to orange-brown)…2

1’ Abdomen much lighter, with some segments whitish or translucent… 3

2(1). Penes with a red spot on ventrolateral region; apicolateral area not forming an “ear (Mariano *et al.*, 2011: Fig. 9)…***T. amanda***

2’. Penes without spot; apicolateral area forming an “ear” (Mariano *et al.*, 2011: Fig. 5)...***T. xavantinensis***

3(1’). Costal cross veins basal to bulla present…4

3’. Costal cross veins basal to bulla absent…6

4(3). Penes long and slender, without lateral pouch...5

4’. Penes of moderate length and width, with lateral pouch (Edmunds, 1950: Figs. 4c, 4d)...***T. ulmeri***

5(4). Penes outcurved from base; spine of each lobe of penes long and narrow; recurved folds present only on the basal half of penes (Traver & Edmunds, 1967: Fig. 43)...***T. itatiajanus***

5’. Penes not outcurved from base; spine of each lobe of penes long and directed internally and posteriorly; recurved folds present from the base to the apex of penes (Gonçalves *et al.*, 2010: Fig. 5)...***T. jones***

6(3’). Forewing membrane with cross veins tinged with brown, with a central brown spot on MA fork (Figs. 6, 27)...***T. alapictus* sp. nov.**

6’. Forewing membrane hyaline... 7

7(6’). Penes with short spines (1.2x the width of penes)... 8

7’. Penes with long spines (2.0–2.8x the width of penes)... 10

8(7). Spine of each lobe of penes narrow and straight...9

8’. Spine of each lobe of penes narrow and curved (Traver, 1967: Fig. 45)... ***T. schlingeri***

9(8). Penes without lateral pouch; posterior margin of subgenital plate triangular with large medial projection (Domínguez, 1987: Fig. 10)...***T. cochunaensis***

9’. Penes with lateral pouch; posterior margin of subgenital plate rounded without projection (Figs. 12, 13)…***T. sternimaculatus* sp. nov.**

10(7’). Presence of an outer lateral projection at base of each penis lobe; posterior margin of subgenital plate truncated (Thew, 1960: Fig. 1)...***T. daidaleus***

10’. Absence of an outer lateral projection at base of each penis lobe; posterior margin of subgenital plate triangular or almost straight... 11

11(10’). Posterior margin of subgenital plate triangular with a medial narrow projection (Thew, 1960: Fig. 2)...***T. traverae***

11’ Posterior margin of subgenital plate almost straight with a medial rounded projection…12

12(11’). Penes with spines 2.8x the width of penes; lateral pouch small, 1/5 the length of penes (Figs. 16, 17)...***T. pinhoi* sp. nov.**

12’ Penes with spines 2.0x the width of penes; lateral pouch large, 3/5 the length of penes…13

13(12’). Abdominal color pattern with terga II–VI with dorsal dark brown spots…14

13’. Abdominal color pattern with terga II–VI without dark brown spots (Fig. 20)… ***T. luizgonzagai* sp. nov.**

14(13). Terga II–VI with four black spots, two anterolateral and two medial; body length 9.3 mm; subgenital plate straight with a large medial projection (Mariano *et al.*, 2011: Figs. 15, 25)…***T. pelicanus***

14’. Terga II–VI with a pair of sublateral apical dark brown spots and a pair of diffused median markings; body length between 5.8–7.2 mm; subgenital plate triangular with a short medial projection (Gonçalves *et al.*, 2013: Fig. 5)…***T. bonito***

1. We did not include in this key *T. limbatus* and *T. subfasciatus* because of insufficient descriptions.

***Thraulodes luizgonzagai* sp. nov., Lima, Mariano & Pinheiro**

**(Figs. 2–3; 10–11; 18–22)**

**Diagnosis.** The male adult of this species can be separated from all the other congeners by the combination of the following characters: (1) costal membrane basal to bulla without cross veins (Fig. 2); (2) costal and subcostal zones of forewing translucent (Fig. 2); (3) two dark brown bands on femora (Fig. 22); (4) terga in dorsal view predominantly light brown, in lateral view translucent whitish, with three distinct spots on terga II–VI and one spot on terga VII–VIII (Figs. 19–20; (5) subapical spines of penes long and narrow, apicolateral area not forming an "ear", lateral pouch present, and without recurved folds (Fig. 10–11).

**Male imago.** *Length*: body, 5.5 mm; forewing, 5.8 mm; hind wings, 1.1 mm; general coloration brown.

*Head* (Figs. 18–19): dark brown; antenna yellowish brown with scape lighter; upper portion of eye yellowish brown, lower portion black; ocelli white with a black ring at base.

*Thorax* (Figs. 19–21): general color of pro-, meso and metanotum chestnut, with sutures dark brown. Mesonotum with whitish cup-shaped spot on mesoscutelum, posterior region with scuto scutelar suture whitish. Pleura whitish washed with red. Pro- and mesosternum whitish; furcasternum dark brown.

*Wings* (Figs. 2–3): forewing membrane hyaline, with dark spot near base; costal and subcostal zones translucent; veins ICu2 and CuP not joined basally. Costal membrane basal to bulla without cross veins and 8 cross veins distal to bulla. Hind wing hyaline, with dark spot near base and 4 cross veins.

*Legs* (Fig. 22): femur with two dark brown bands; apical 1/3 of femur tinged with light brown and inner margin washed with black. Tibiae and tarsi light brown. Tarsi pale yellow.

*Abdomen* (Figs. 18–20): terga predominantly light brown. In lateral view translucent brown, with two isolated spots and two stigmatic dots on terga III–VI, and one isolated spot and a blackish stripe on terga VII–IX. In dorsal view, terga segments I, VII–IX brown and II–VI translucent with posterior margin light brown. Terga segments I–III and VI–VII medially with blackish dorsal mark. Sterna I–VI translucent and VII–X brown.

*Genitalia* (Figs. 10–11): forceps pale yellow, segment II washed with black. Posterior margin of subgenital plate almost straight, with a medial rounded projection. Penes pale yellow; 2/3 of penes divided apically, with a pair of long and narrow subapical spines, 2.0x the width of penes;

apicolateral area not forming an "ear"; external margin forming lateral pouch and without recurved folds parallel to inner margin. Terminal filament whitish, basal area of segments black.

*Variations*: Some specimens show color patterns overall darker (Fig. 18) or lighter (Fig. 19), due to alcohol fixation period making them paler.

**Female and nymph.** Unknown.

**Type material. Holotype:** one male imago, Brazil, Bahia State, Juazeiro, Rio São Francisco, 9°25'04.3"S, 40°28'50.9"W, 362m, 05.x.2011, L.R.C. Lima coll. (MZUESC). **Paratype**: same data as holotype, three male imagos (1 CZNC and 2 MZUESC); one male, Brazil, Pernambuco State, Petrolina, Base Fluvial Ilha do Fogo, Rio São Francisco, 9°24'22.9"S, 40°30'17.4"W, 362m, 09.x.2011, L.R.C. Lima coll. (UFPE); two male imagos, Brazil, Pernambuco, Petrolina, Rio da Vitória, afluente do Rio São Francisco, 09°21'814"S, 40°35'409"W, 440 m, 22.x.2008 - luz UV e branca, Calor, Mariano & Mateus colls.

**Etymology.** The specific epithet is a tribute to Luiz Gonzaga, famous singer from the state of Pernambuco, who sang beautiful songs about the semi-arid region of Pernambuco, collection site of the new species.

**Remarks.** *Thraulodes luizgonzagai* sp. nov. appears to be more related to *T. paysandensis* Traver, 1964. Both are small species (with body length up to 8 mm), with femora twice banded, and lobes of penes showing lateral pouch and long and narrow subapical spines. Nevertheless, the new species differs from *T. paysandensis* by the abdominal color pattern, the shape of medial projection of styliger plate (narrow and rounded in the new species, dome-shaped in *T. paysandensis*), and by the absence of recurved folds on penes.

***Thraulodes sternimaculatus* sp. nov., Lima, Mariano & Pinheiro**

**(Figs. 4–5; 12–13; 23–26)**

**Diagnosis.** The adult of this species can be separated from all the other congeners by a combination of the following characters: (1) costal membrane basal to bulla without cross veins (Fig. 4); (2) costal and subcostal zones of the forewing translucent (Fig. 4); (3) one band on femora (Fig. 23); (4) abdominal segments in dorsal view predominantly translucent, with three lateral dark spots on terga II–VII (Figs. 23, 26); (5) subapical spines of penes short and narrow, apicolateral area not forming an "ear", lateral pouch present and without recurved folds (Figs. 12–13).

**Male imago.** Length: body, 6.5 mm; forewing, 6.5 mm; hind wings, 1.2 mm; general coloration translucent.

*Head* (Fig. 23)*:* black; antenna pale with scape dark brown; upper portion of eye reddish brown, lower portion black; ocelli white with a black ring at base.

*Thorax* (Figs 23–25)*:* general color of pro-, meso and metanotum brown. Pronotum whitish, medial region with a triangular light brown macula. Meso and metanotum brown with sutures darker; scuto-scutelar suture whitish. Prosternum whitish with brown maculae tinged with black on the corners; mesosternum whitish with furcasternum dark brown.

*Wings* (Figs. 4–5): membrane of forewing hyaline, with dark spot near the base; costal and subcostal zones translucent; veins ICu2 and CuP not joined basally. Longitudinal and cross veins pale yellow. Costal membrane basal to bulla without cross veins, 11 cross veins distal to bulla. Hind wing hyaline with dark spot near base and 6–8 cross veins.

*Legs* (Fig. 23): legs with femur pale yellow, apical 1/3 of femur with dark brown band followed by a blackish spot in the median region, and inner margin tinged with black on apical ½; basal half with a light brown spot. Tibiae yellowish with base brown; tarsi pale yellow.

*Abdomen* (Figs. 23–24; 26): segments in dorsal view predominantly translucent, with three lateral dark spots on terga II–VII. Terga I–II brown washed with black on anterior margin; terga III–VI translucent with a medial brown spot on posterior margin; tergum VI with anterior area translucent and posterior brown; terga VII and IX–X brown; tergum VIII translucent whitish, with medial line brown. Sterna II–VI translucent with yellowish spots on medial area and VII–X whitish with lateral margins reddish.

*Genitalia* (Figs. 12–13): forceps whitish, segment II–III washed with black. Posterior margin of subgenital plate rounded, without projection. Penes yellowish washed with black on ventral lateral region; 2/3 of penes divided apically, with a pair of short and narrow subapical spines, 1.2x the width of penes; apicolateral area not forming an “ear”; external margin forming lateral pouch and without recurved folds parallel to inner margin. Terminal filaments whitish, basal area of segments black.

**Female and nymph.** Unknown.

**Type material. Holotype**: one male imago, Brazil, Pernambuco State, Jaqueira, Rio Pirangi, 8°44'53.0"S, 35°48'51.1"W, 189m, 23.v.2012, L.R.C. Lima coll. (CZNC). **Paratype**: one male,

Brazil, Pernambuco State, Amaraji, Rio Amaraji, 8°21'48.9"S, 35°28'49.0"W, 320m, 20.i.2011, L.R.C. Lima coll. (IML); same as preceding except one male, 21.iv.2012 (IML).

**Etymology.** The specific epithet is due to maculae on the corners of prosternum.

**Remarks.** In addition to *T. sternimaculatus* sp. nov., only four species have the subgenital plate with posterior margin medially rounded and without medial projection: *Thraulodes laetus* (Eaton, 1883) and *T. papilionis* Traver & Edmunds, 1967 from Colombia, *T. mucuy* Chacón & Domínguez, 1999 from Venezuela, and *T. regulus* Traver & Edmunds, 1967 from Peru. *Thraulodes sternimaculatus* sp. nov. seems to be more related to *T. laetus* and *T. papilionis,* i.e both are small species (body length up to 8.0 mm), with abdominal segments lighter (whitish or translucent), forewing membrane hyaline and without costal cross veins basal to bulla. The new species can be separated from them by the presence of a lateral pouch, penes short and narrow, apicolateral area of penes not forming an "ear" and recurved folds absent.

***Thraulodes alapictus* sp. nov., Mariano & Lima**

**(Figs. 6–7; 14–15; 27–30)**

**Diagnosis.** The male imago of this species can be separated from all the other congeners by a combination of the following characters: (1) costal membrane basal to bulla with 10 cross veins (Fig. 6); (2) costal and subcostal zones of the forewing translucent (Fig. 6); (3) forewing hyaline with cross veins tinged with brown, with a central brown spot on MA fork (Fig. 6, 27); (4) two dark brown bands in median region of femora (Fig. 27, 30); (5) spines of penes long and narrow, apicolateral area forming an "ear", lateral pouch absent, and without recurved folds parallel to inner margin (Figs. 14–15).

**Male imago.** Length: body, 11.5 mm; forewingforewings, 11.1 mm; hind wings, 2.8 mm; general coloration light brown.

*Head* (Figs. 27–28): black; antenna light brown with scape brown; upper portion of eye reddish brown, lower portion black; ocelli white with a black ring at base.

*Thorax* (Figs. 28–29): pro-, meso and metanotum brown. Pronotum with a medial triangular dark spot. Meso and metanotum light brown with sutures dark brown; recurrent scuto-scutellar suture brown. Pleura whitish washed with some red spots; episternum of prothorax white tinged with purple; mesosternum dark brown with a medial white macula; metasternum dark brown.

*Wings* (Figs. 6–7): membrane of forewing hyaline, tinged with light brown on fork of MA vein, and with dark spot near base; costal and subcostal zones translucent. Longitudinal veins light brown; costal and subcostal cross veins blackish, lighter towards apex. Veins ICu2 and CuP joined basally. Costal membrane with 10 cross veins basal to bulla and 15 cross veins distal to bulla. Hind wing hyaline with 13 light brown cross veins, with dark spot near base.

*Legs*: legs with femur yellowish, apical 1/3 of femur tinged with reddish followed by two black marks in median region, apex black; tibiae yellowish with apex black and a small blackish spot on the base; tarsi yellowish tinged with black with the last tarsomere darker.

*Abdomen* (Figs. 27–28): general coloration translucent brown; tergum I brown washed with black; terga II–VI translucent brown with anterolateral dark spots and a medial light brown macula decreasing toward tergum VI; terga IV–VI with minute medial dark spots; terga VII brown; VIII–X reddish brown. Sternum I–II brown; sterna III–VI translucent, VII–IX whitish; four minute marks on sterna III–VIII.

*Genitalia* (Figs 14–15): forceps whitish with inner margin washed with black. Posterior margin of subgenital plate rounded with a minute medial projection. Penes yellowish washed with black on ventral inner region; 2/3 of penes divided apically, with a pair of long and narrow spines, 3.0x the width of penes; apicolateral area forming an “ear”; external margin not forming lateral pouch and without recurved folds parallel to inner margin. Terminal filament whitish with base of segments black.

**Female imago** (Fig. 30). Length: body, 11.9 mm; forewings, 11.1 mm; hind wings, 2.3 mm; general coloration brown. *Head*: black ventrally and whitish tinged with purple dorsally. *Thorax*: Pronotum brown with a medial dark line. Mesonotum and metanotum light brown with sutures whitish. Prosternum purple with median region whitish. Mesosternum purple with episternum and furcasternum brown. Metasternum brown. *Legs*: Same as male, except by general coloration of femora and tibiae darker. Abdomen entirely dark brown without segments translucent.

**Nymph.** Unknown

**Type material. Holotype**: one male imago, Brazil, Santa Catarina State, Urubici, Cachoeira Avental, 28°02.798’S, 49°37.070’W, 1229m, 08.i.2010, light trap, L.C. Pinho, H.F. Mendes & R. Moreto colls (MZUESC). **Paratype**: same as preceding, one male and four female imagos (CZNC).

**Etymology.** From the latin words, ala (wing) and pictus (point), due to macula on the central region of forewings.

**Remarks.** *Thraulodes basimaculatus* Giordano & Domínguez, 2005 from Bolivia is another species of this genus that has the forewing membrane pigmented. However, in the new species this pigmentation is a central spot restricted to fork of MA vein, whereas in *T. basimaculatus* most of the basal half and the entire membrane of the hind wing is pigmented. *Thraulodes alapictus* sp. nov., *T. liminaris* Domínguez, 1987 from Argentina, *T. brunneus* Koss, 1966 from USA (New Mexico), Honduras and Guatemala, and *T. mexicanus* (Eaton, 1884) from Mexico and Central America are the only ones that have the vein ICu2 of forewing joined at base to vein CuP. However, *T. liminaris* seems more related to *T. alapictus* sp. nov. by the following characteristics: large body length (over 10 mm), the costal area of forewing with numerous cross veins basal to bulla (over 10 cross veins), and a pair of long and narrow subapical spines on penes. The new species is easily distinguished from it by the abdominal color pattern with translucent segments, the presence of a central brown spot on forewing, with costal and subcostal areas hyaline, and the posterior margin of subgenital plate rounded (almost straight with medial rounded projection in *T. liminaris*).

***Thraulodes pinhoi* Mariano & Lima, sp. nov.**

**(Figs. 8–9; 16–17; 31–34)**

**Diagnosis.** The male adult of this species can be separated from all the other congeners by a combination of the following characters: (1) costal membrane basal to bulla without cross veins (Fig. 8); (2) costal and subcostal zones of the forewing translucent (Fig. 8); (3) two brown bands tinged with black (Fig. 34); (4) abdominal segments translucent brown, with anterolateral dark spots and two medial black spots on segments II–VI (Figs. 31–32; (5) spines of penes long and narrow, apicolateral area forming an “ear”, lateral pouch present, and recurved fold parallel to inner margin (Figs. 16–17).

**Male imago.** Length: body, 8.9 mm; forewings, 8.2 mm; hind wings, 2.0 mm; general coloration light brown.

*Head*: black; antenna light brown with scape brown; upper portion of eyes reddish brown, lower portion black; ocelli white with a black ring at base.

*Thorax* (Fig. 31–33): pro-, meso- and metanotum brown. Pronotum with medial region brown and lateral margins whitish. Meso- and metanotum light brown with sutures dark brown; recurrent scuto-scutellar suture whitish; episternum of prothorax whithish with purple maculae; mesosternum dark brown with a medial white macula; metasternum dark brown.

*Wings* (Figs. 8–9): membrane of forewing hyaline, with dark spot near base; costal and subcostal zones translucent. Longitudinal and cross veins faintly brown. Veins ICu2 and CuP not joined basally. Costal membrane without cross veins basal to bulla and with 11 cross veins distal to bulla, some of these anastomosed. Membrane of hind wings hyaline with seven cross veins and a dark spot near base.

*Legs* (Fig. 34): legs with femur pale yellow, two brown bands tinged with black; tibiae pale yellow with apex black; tarsi yellow with the last tarsomere brownish black.

*Abdomen* (Fig. 31–32): general coloration translucent brown; tergum I brown with a medial black spot; terga II–VI translucent brown with anterolateral dark spots and two medial black spots, tergum VII light brown with an anterolateral dark spot and two medial black spots; terga VIII–X light brown. Sternum I light brown; sterna II–VI translucent; sternum VII with anterior margin light brown and posterior margin whitish; sternum VIII whitish with lateral margins purple; sternum IX yellow with whitish spots on lateral margins with a purple spot on posterior margin.

*Genitalia* (Figs 16–17): forceps whitish, apex of segment I and segment II washed with black. Posterior margin of subgenital plate almost straight with medial rounded projection. Penes yellowish; penes 2/3 divided apically, with a pair of long and narrow spines, 2.8x the width of penes; apicolateral area forming an "ear"; external margin forming lateral pouch and recurved folds parallel to inner margin. Terminal filament whitish with base of segments black.

**Female and nymph.** Unknown.

**Type material. Holotype**: male imago, Brazil, Santa Catarina State, Urubici, Cachoeira Avental, 28°02.798'S, 49°37.070'W, 1229m, 08.i.2012, light trap, L.C. Pinho, H.F. Mendes & R. Moreto colls (MZUESC). **Paratype:** same data as holotype, 06 male imagos (01 MZUESC, 02 IML, and 03 CZNC).

**Etymology.** Dedicated to our friend Dr. Luis Carlos de Pinho, Chironomidae specialist, one of the collectors of the type material.

**Remarks.** *Thraulodes pinhoi* sp. nov. is similar to *T. luizgonzagai* sp. nov and *T. paysandensis* Traver, 1964 from Argentina and Uruguay in the following characteristics: small species,

forewings membrane hyaline, subgenital plate with medial projection, and penes with a pair of long and narrow subapical spines. *Thraulodes pinhoi* sp. nov. differs from both by the penes with spines very long (2.8x the width of penes) and apicolateral area forming an "ear", and subgenital plate with a triangular rounded projection (narrow and rounded in *T. luizgonzagai* sp. nov. and dome-shaped in *T. paysandensis*).

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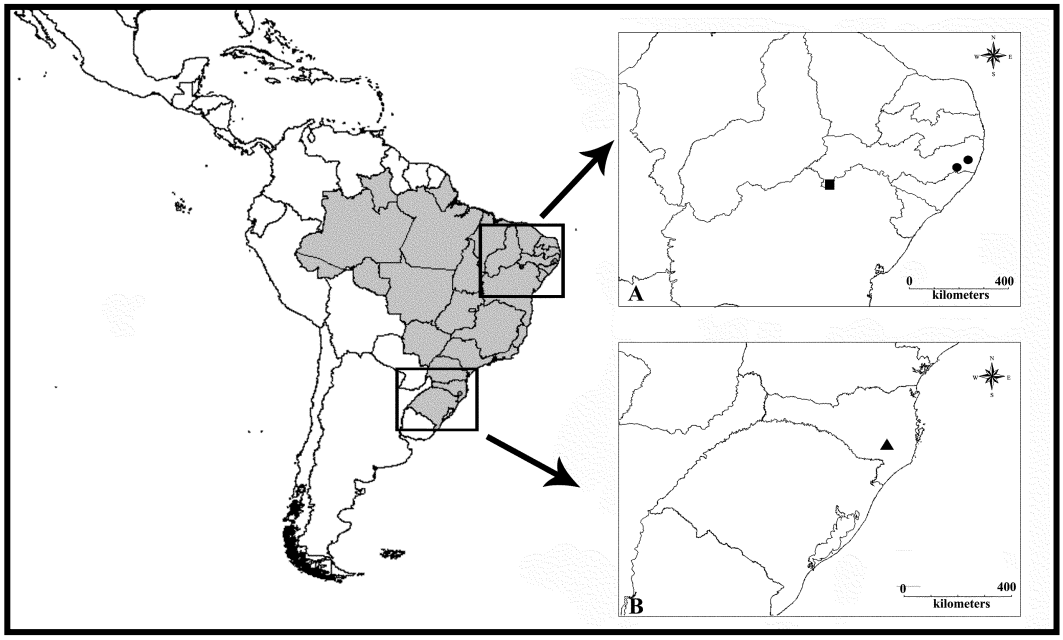
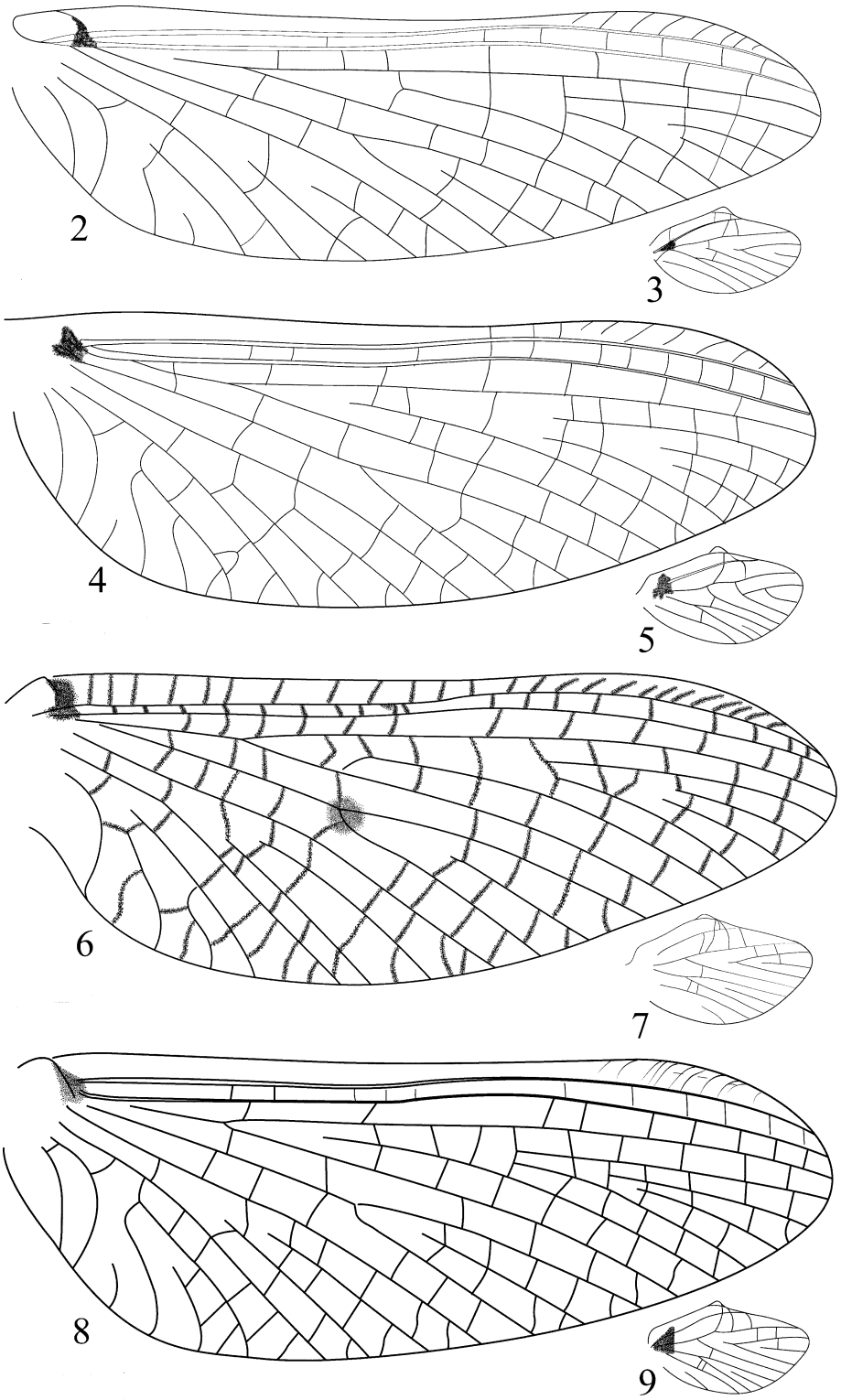
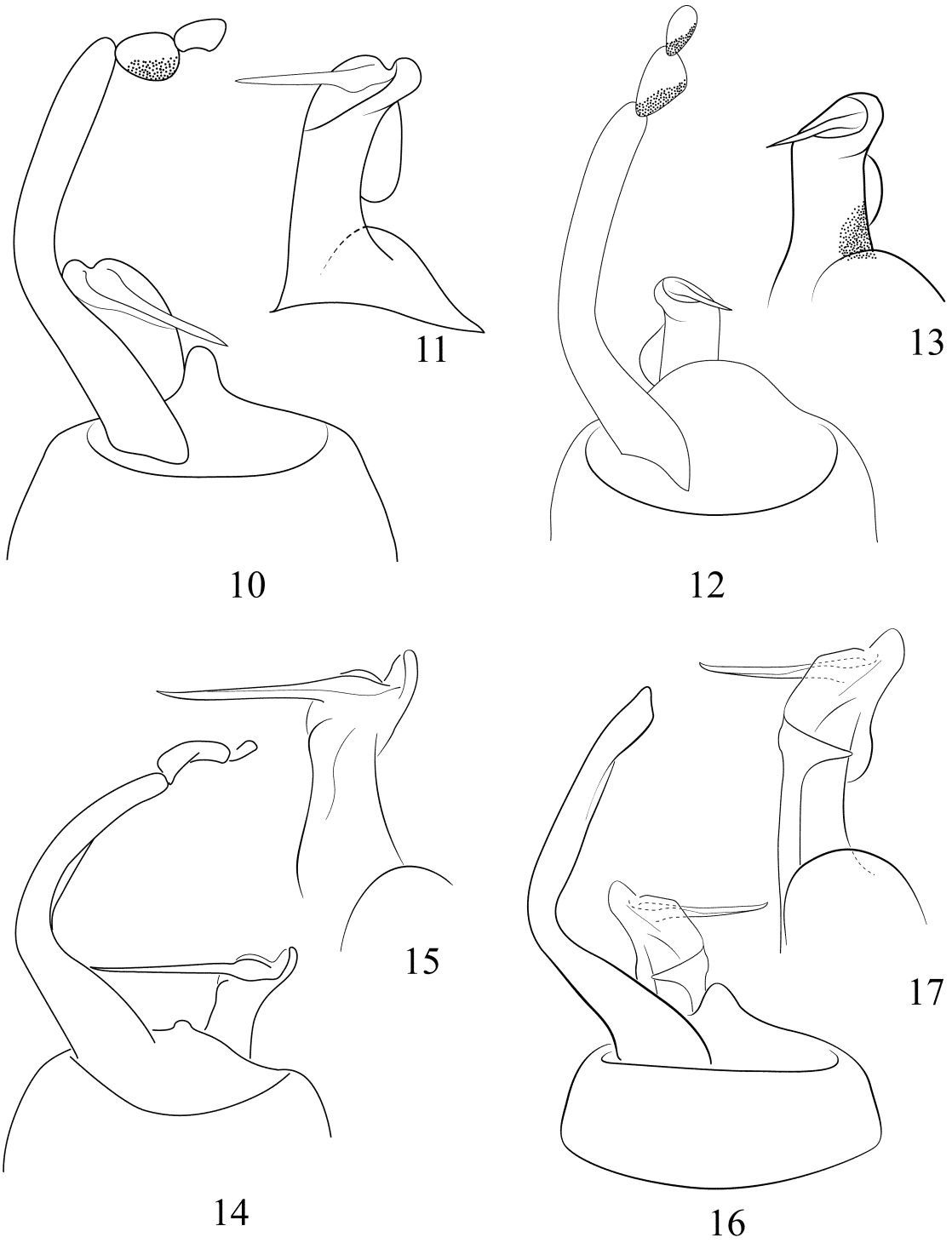


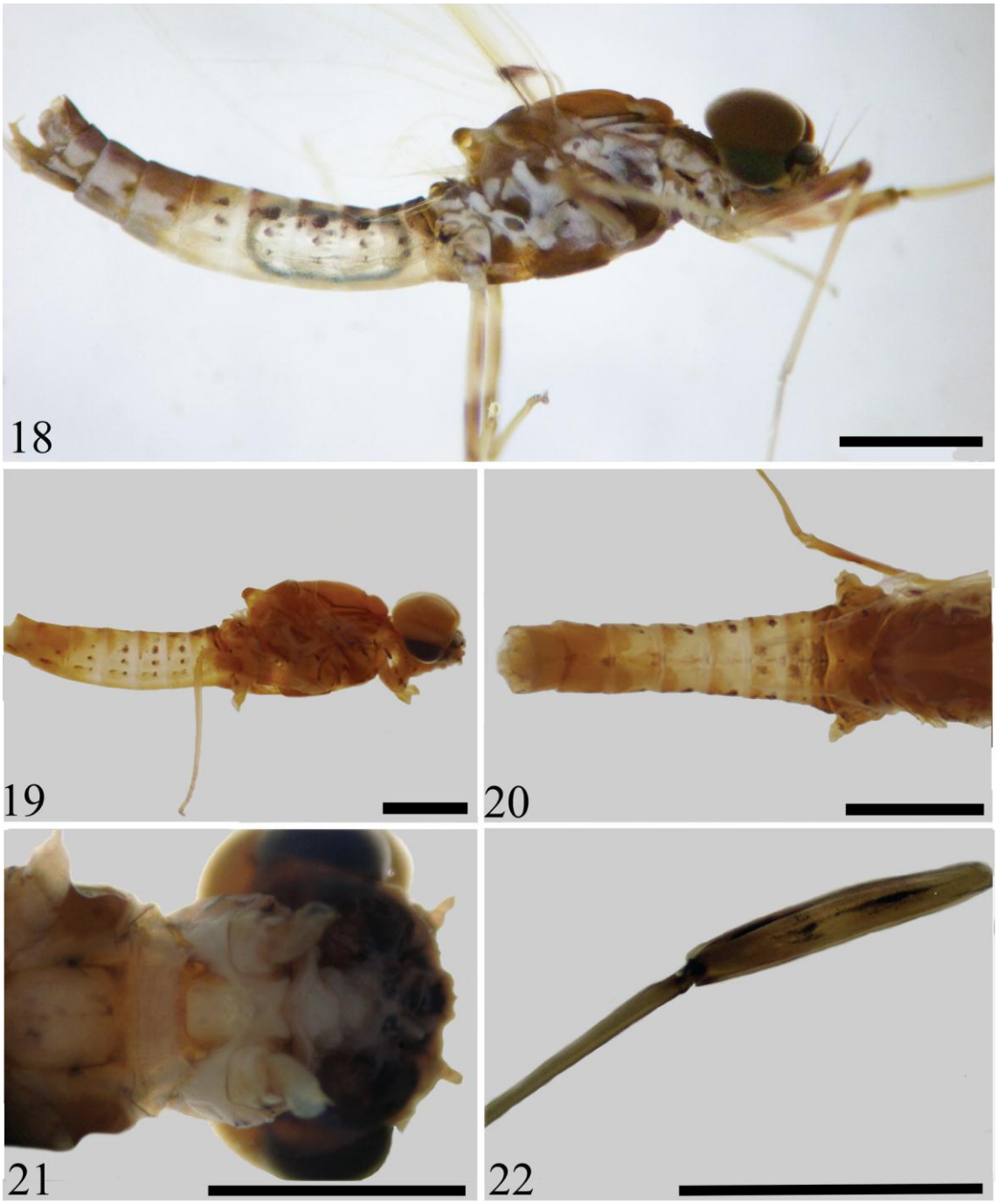

**Figure 1.** Records of the new species of *Thraulodes* from Brazil. A) *T. luizgonzagai* sp. nov. (square) and *T. sternimaculatus* sp. nov. (circles); B) *T. alapictus* sp. nov. and *T. pinhoi* sp. nov. (triangle).



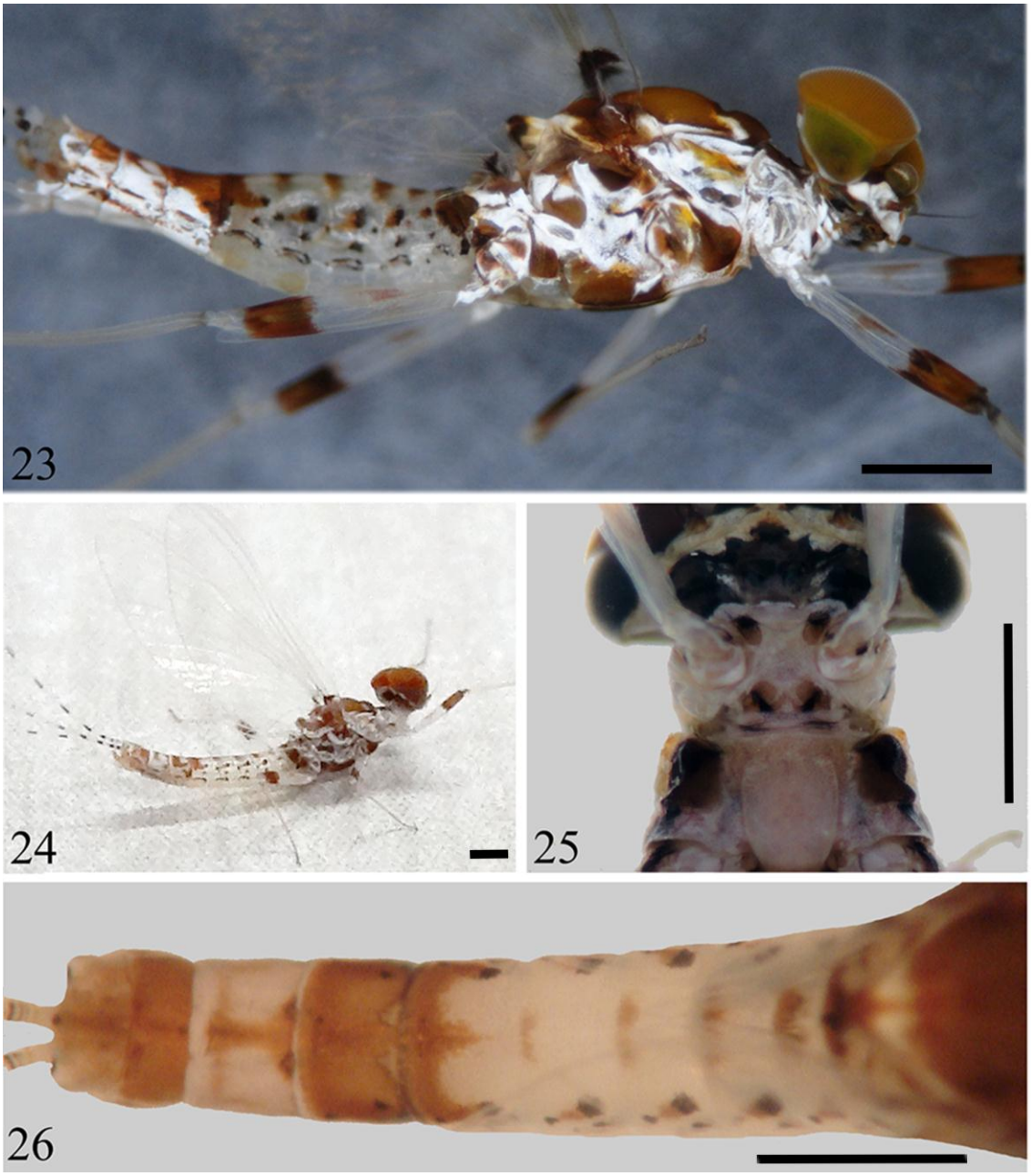

**Figures 2–9.** Wings of male imagos. 2, *Thraulodes luizgonzagai* sp. nov., forewing; 3, idem, hind wing; 4, *T. sternimaculatus* sp. nov., forewing; 5, idem, hind wing; 6, *T. alapictus* sp. nov., forewing; 7, idem, hind wing; 8, *T. pinhoi* sp, nov., forewing; 9, idem, hind wing.




**Figures 10–17.** Genitalia of male imagos. 10, *Thraulodes luizgonzagai* sp. nov., genitalia (ventral view); 11, idem, penes (ventral view); 12, *T. sternimaculatus* sp. nov., genitalia (ventral view); 13, idem, penes (ventral view); 14, *T. alapictus* sp. nov., genitalia (ventral view); 15, idem, penes (ventral view); 16, *T. pinhoi* sp, nov. genitalia (ventral view); 17, idem, penes (ventral view).




**Figures 18–22.** *Thraulodes luizgonzagai* sp. nov.: 18, lateral view (freshly killed specimen); 19, lateral view (fixed specimen); 20, dorsal view (fixed specimen); 21, detail of prosternum; 22, detail of foreleg (scales: 1mm)




**Figures 23–26.** *Thraulodes sternimaculatus* sp. nov.: 23, lateral view; 24, living male imago; 25, detail of prosternum; 26, dorsal view of abdomen (scales: 1mm).


**Figures 27–30.** *Thraulodes alapictus* sp. nov.: 27, lateral view; 28, dorsal view; 29, detail of prosternum; 30, lateral view of female imago (scales: 1mm).


**Figures 31–34**. *Thraulodes pinhoi* sp. nov.: 31, lateral view; 32, dorsal view; 33, detail of prosternum; 34, detail of foreleg (scale: 1mm).

*Zootaxa* (2014). 3795 (4): 441–448

## 2.5 A new species of *Miroculis* Edmunds, 1963 (Ephemeroptera: Leptophlebiidae) from Northeastern Brazil.

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**ABSTRACT**

*Miroculis stenopterus*, sp. nov., is described based on material from State of Pernambuco, Northeastern Brazil. The imago of the new species can be distinguished, among other characteristics, by an uncommon forewing shape, long and narrow, similar to species of genus *Microphlebia*. The tentatively associated nymph can be distinguished from other species of the genus mainly by the abdominal color pattern and the ratio of labial palp segment 2 to segment 1 (1.01–1.04 x).

**Key words:** aquatic insects, mayflies, taxonomy, Brazil, Neotropical Region

## INTRODUCTION

The genus *Miroculis* was established by Edmunds (1963) for the species *Miroculis rossi* Edmunds, 1963 from Peru. The genus is currently composed of 17 described species, ranging from Trinidad to Northeastern Argentina (Edmunds 1963; Savage & Peters 1983; Savage 1987; Domínguez 2007; Peters *et al.* 2008; Salles & Lima 2011; Costa & Mariano 2013; Gama Neto & Hamada 2013).

The genus is distinguished by several apomorphies. In the imago, the most notable are the tent-shaped costal projection of the hind wing and the finger-like projection on the male fore claw. In the nymph, the combination of a long distal filament and lateral lobes on the gills, glossae curved over ventrally and a thick row of pectinate setae on the maxillae (Savage & Peters 1983). Based mainly on the male's eyes, Savage and Peters (1983), with later modifications by Savage (1987) and Peters *et al.* (2008), divided the genus into four subgenera: *Miroculis*, *s.s.* (long dorsally-directed stalks with length greater than width, 5–20 facets in longest row of dorsal portion), *Yaruma* Savage & Peters, 1983 (short wide stalks), *Ommaethus* Savage & Peters, 1983 (without stalks), and *Atroari* Savage & Peters, 1983 (upper portion of compound eye large, without stalk, and with 30–40 medium facets in longest row).

In Brazil, *Miroculis* is one of the Leptophlebiidae genera with the greatest number of species, currently with 11 recorded (Salles *et al.* 2014). The genus, however, has been considered poorly studied in the country because most of these records are based on the original description, and only *M.* (*M.*) *caparaoensis* Salles & Lima, 2011, *M.* (*M.*) *fazzariensis* Costa & Mariano, 2013, and *Miroculis* (*M.*) *tepequensis* Gama Neto & Hamada, 2013 have been described since Savage & Peters (1983). Including Brazil and beyond, the majority of species of the genus (nine) are known only based on the adult stage; two are based only on the nymph; and six are based on both nymphs and imagoes. In the present paper, as part of an effort to study the mayfly fauna of the State of Pernambuco, we describe a new species of *Miroculis* (*Miroculis*) based on the male imago and nymph stages.

## MATERIAL AND METHODS

The material used in this study is preserved in 80% ethanol. Male legs and genitalia were dissected and mounted in Euparal. Wings were mounted dry. Drawings were made with the aid of a camera Lucida. The material is deposited in the following institutions: Coleção Zoológica Norte Capixaba-UFES, São Mateus (CZNC) and Coleção Entomológica de Pernambuco, Recife (UFPE). Terms referring to description of the adult thorax follow Kluge (1994). Descriptions use the following acronyms: **FWW –** Fore Wing Width; **FWL –** Fore Wing Length; **HWW –** Hind Wing Width; **HWL –** Hind Wing Length; **FSI** (**II**, **III**) – Forceps Segment I (II, III); **PL –** Penis Length; **S –** Segment. Angularity of fore wing was taken by joining the following points: a) joint between apical costal arc and Sc, b) apical point of CuA and c) apical point of R1 (**Fig. 3**). The ratios of foreleg segments was expressed as ratios of each segment to the tibia length according Hubbard (1995). All lengths were taken on software ImageJ 1.47v (Rasband, 1997–2012).

## RESULTS AND DISCUSSION

***Miroculis* (*Miroculis*) *stenopterus*, sp. nov.**
**(Figs. 1–17)**

**Diagnosis.** *Miroculis* (*M.*) *stenopterus*, sp. nov., can be separated from all congeners by the following combination of characteristics. *Male imago:* 1) 13–14 facets in the longest row of upper portion of compound eye; 2) fore and hind wings hyaline washed with brown basally; 3) $MP_2$ free basally; 4) cubitoanal margin on forewing poorly developed; 5) styliger plate short, ½ length of sternite IX. *Nymph:* 1) anterolateral margin of labrum slightly flattened; 2) SII of labial palpi 1.01–1.04 x length of SI; 3) terga I–X with posterior blackish brown band and lateral markings; 4) terga VI–VII medially with a triangular, blackish macula; 5) posterolateral spines on terga VI–IX.

**Male imago**. Lengths. Body: 3.3–3.5 mm; forewing: 4.0–4.5 mm; angularity of cubitoanal region: 108.34°; hind wing: 0.9 mm; foreleg: 2.4 mm; cercus: 8.5 mm.

**Ratios**. *Legs*: segments of foreleg: 0.89: 1.00 (0.90 mm): 0.06: 0.20: 0.14: 0.09: 0.26. *Wings*: FWW 0.36 x FWL; HWL 0.20 x FWL; HWW: 0.62 x HWL; fork of $MA_2$ of forewing 0.54 x

total length of MA. *Genitalia*: maximum length along median line 0.55 x maximum width; lateral margin 0.57 x maximum width; FSIII 1.11 x FSII; FSIII: 0.33 x FSI; PL: 0.86 x FSI.

**Coloration**. *Head*: orangish-brown at base of eyes, washed with dark gray anteriorly, pale between ocelli **(Fig. 1)**. Lower portion of compound eye black; stalk of upper portion light orange, with facets light yellow separated by dark brown grooves **(Figs. 1–2)**. Ocelli white surrounded with black **(Fig. 1)**. Antenna brown.

*Thorax:* pronotum orangish-brown heavily washed with black on lateral margins. Meso- and metanotum dark orangish-brown with a V-shaped blackish mark between the posterior scutal projections. Pleura and sterna light-brown with margins dark-brown.

*Wings:* forewing with membrane hyaline washed with light brown near base; longitudinal veins brown with C, Sc and R darker, cross-veins weakly developed and brown **(Fig. 3)**. Hind wing with membrane hyaline washed with light blackish brown near base; longitudinal and cross veins brownish **(Fig. 4)**.

*Legs:* foreleg yellowish translucent; anterior surface of femur with central blackish line, subapical blackish mark and apical brown band. Ventral margin of tibia with sub-basal gray mark and subapical blackish band, and tarsi with apical gray mark. Middle and hind legs with anterior surface of femora with blackish marks on apex of dorsal margin and on median region, and subapical blackish band. Subapical grayish band on tibiae. Dorsal margin of tibiae with apical grayish mark.

*Abdomen*: terga pale washed with gray. Terga I and VIII–X completely washed with gray; anterior region of terga II–VII with submedial pale mark; third basal of terga II–VIII with transversal brownish mark; medial portion of terga II–VIII with longitudinal brownish stripe. Sterna pale; third basal of sterna I–IX with transversal grayish mark.

*Genitalia*: styliger plate brown on posterior margin; forceps brown; dark brown on apical third of SI, on SII and on basal third of SIII; apex of SIII whitish **(Fig. 5)**. Penis dark brown **(Fig. 6)**.

*Caudal filaments:* broken off and missing.

**Morphology**. *Head*: upper portion of compound eye on a short stalk, separated medially by a distance approximately 0.7–0.8 x maximum width of an upper portion **(Fig. 2)**. Medial projection on dorsal surface absent. Dorsal surface circular with 13–14 facets on longest row **(Fig. 1)**.

*Wings*: forewing with bulla visible; 5 cross-veins between C and Sc; fork of $MA_2$ slightly

asymmetric; cross-vein connecting MP and CuA basal to the fusion of MP1 and $MP_2$ absent; MP, IMP, $MP_2$ and CuA free basally; two A veins slightly straight **(Fig. 3)**. Hind wing with apex rounded; costal projection poorly developed; veins Sc ending distant to apex of costal projection; fork of R and MA asymmetric; MP free basally; CuP and A vein absent. *Legs*: claws of foreleg dissimilar, with one claw ending in hook-like projection and one blunt.

*Genitalia*: posteromedial portion of styliger plate smoothly straight; median invagination present and medial incision absent; apex of FSII subequal in width to base of its segment; basal ¼ of inner margin of FSI narrowing abruptly; inner margin undulated and ¼ distal margin developed and outer margin smooth **(Fig. 4)**. Penis wider basally, narrowing toward apex with apex rounded; row of spines absent **(Fig. 5)**.

**Female Imago.** Unknown.

**Mature nymph** (tentative). Lengths. Body: 3.3–3.5 mm. Foreleg: 2.75 mm; middle leg: 2.54 mm (coxa and trochanter are left); hind leg broken off and missing. Cercus: 5.40 mm.

**Ratios.** *Head*: width of mandible 0.60 x length. Width of SI of labium 0.52x length; SIII of labial palp 0.39 x SII; SIII 0.52 x SI; SII 1.33 x SI; length of paraglossa 0.29 x width. Maxilla: SIII of maxillary palp 0.39 x SII; SIII 0.44 x SI; SII 1.13 x SI. *Legs*: foreleg: width of femur 0.33 x length; width of tarsal claw 0.29 x length; middleg: width of femur 0.33 x length; hind leg: width of femur 0.29 x length. *Gill*: length of medial filament 0.64 x length of body of gill.

**Coloration.** *Head*: brown with pale area surrounding ocelli and eyes; lateral edges of genae blackish gray; antenna with scape and flagellum pale yellow and pedicel blackish gray **(Fig. 7)**. Eye: upper portion of turbinate eye dark brown and lower portion blackish; ocelli apically purplish and basally blackish **(Fig. 7)**. Mouthparts: mandibles brown **(Fig. 11–12)**; maxillae light brown **(Fig. 13)**; hypopharynx light brown; superlingua as in **Fig. 14**; labium light brown **(Fig. 15)**; submentum light brown.

*Thorax*: pro-, meso-, and metanotum brown with medial pale yellow line; meso- and metanotum with distinct "V" marks on medial area **(Fig. 7)**. Thoracic sterna whitish, except metasternum with anterior margin dark.

*Legs:* pale yellowish brown with femora with sub-apical and medial blackish bands **(Fig. 16)**; ventral margin of tibia with blackish mark and subapical blackish band.

*Abdomen*: abdominal terga I–X with posterior blackish brown band and lateral markings; terga VI–VII medially with a triangular, medial blackish macula; terga IX–X light brown with lateral minute markings; sterna pale yellowish brown **(Figs. 7–9)**. Gills gray and trachea blackish gray **(Fig. 17)**. Caudal filaments pale yellow; basal portion of joint darker.

**Morphology.** *Head*: clypeus concave anteriorly; labrum with anterolateral margin slightly flattened and lateral margins rounded to angular **(Fig. 10)**; outer margin of mandibles curved **(Figs. 11–12)**; inner margin of apex on SII of labial palp not acute.

*Legs*: subapical denticle of tarsal claw of foreleg larger than the remaining denticles **(Fig. 17)**.

*Abdomen*: posterolateral spines on segments VI–IX **(Fig. 11)**; spines well-developed on segments VIII–IX. Gill: trachea unbranched **(Fig. 18)**.

**Life cycle associations.** The tentative association between male imago and nymph was made by shared abdominal color pattern. Besides that, both stages were collected in the same locality. Note that the holotype is a male imago.

**Etymology.** The epithet *stenopterus* (from greek words *stenos* – narrow and *pterus* – wing) is due the uncommon shape of forewing for species of the genus *Miroculis*.

**Type material. Holotype:** one male imago, Brazil, Pernambuco State, Amaraji, Rio Amaraji, near Usina Campo Belo, 8°21'48.9"S, 35°28'49.0"W, 320m, 21.iv.2012, L.R.C. Lima coll. (CZNC). **Paratype:** same as holotype except 20.i.2011, one male subimago (CZNC); same as preceding except 28.iv.2012, one male imago (CZNC); same as preceding except 06.xii.2012, three nymphs (CZNC). Two male imagos, Brazil, Pernambuco State, Amaraji, Rio Amaraji, Poço do Carreiro, 8°22'1.94"S, 35°28'8.73"W, 269m, 28.iv.2012, L.R.C. Lima, W.R.M Souza cols. (CZNC).

**Additional material:** One nymph, Brazil, Pernambuco State, Amaraji, Barragem Jaguarana, S8°21'01.6", W 35°24'27.7", 265m, L.R.C. Lima coll (UFPE).

**Comments.** The new species has an uncommon forewing shape for members of the genus *Miroculis* (i.e., long and slender, similar to the species of *Microphlebia* Savage & Peters, 1983).

However, the new species and all species of *Miroculis* have a tent-shaped costal projection on hind wings **(Fig. 4)**, while in *Microphlebia* this projection is well-developed, acute and rounded. Regarding the tarsal claw, the hook-like shape is common to the species of *Microphlebia*, *Miroculitus* Savage & Peters, 1983, *Hermanellopsis* (Spieth, 1943), and in some species of *Miroculis*, such as *Miroculis* (*M.*) *fittkaui* Savage & Peters, 1983 and *M.* (*M.*) *bicoloratus* Savage, 1987..

Among the species of *Miroculis*, *M.* (*M.*) *marauiae* Savage & Peters, 1983 and *M.* (*M.*) *rossi* Edmunds, 1963 share similar characteristics with the imago of the new species, such as the upper portion of the eyes being on narrow, dorsally-directed stalks **(Fig. 2)**; fore and hind wings without maculae around cross veins **(Fig. 3–4)**; and penis not extending beyond forceps **(Fig. 5)**. Nevertheless, the new species differs from *M.* (*M.*) *rossi* by the size of penis (0.8 x the length of SI of forceps in *M.* (*M.*) *stenopterus*, sp. nov., and 2.0–2.2 in *M.* (*M.*) *rossi*), as well as the number of facets in the male compound eyes (14 facets in *M.* (*M.*) *stenopterus*, sp. nov., and six facets in *M.* (*M.*) *rossi*). Regarding *M.* (*M.*) *marauiae*, the shapes of the penis (not apically hooked in the new species) and the posteromedian margin of styliger plate (excavated medially in the new species) clearly distinguish them.

In relation to nymphs, *M.* (*M.*) *stenopterus*, sp. nov., shares some similar characters with *M.* (*M.*) *marauiae*, *M.* (*M.*) *brasiliaensis* Savage & Peters, 1983, *M.* (*M.*) *fittkaui*and *M.* (*Ommaethus*) *mourei* Savage & Peters, 1983, especially by gills with coloration grayish and trachea unbranched **(Fig. 18)**. Among them, *M. (M.) marauiae* is more similar to the new species by having a similar slight flattening of the anterolateral margin of the labrum, terga VI–VII with blackish maculae, and caudal filaments that are brownish-yellow and darker on the joints. However, *M.* (*M.*) *marauiae* can be differentiated from the new species by the following characteristics: SII of labial palpi 1.11–1.25 x length of SI (1.01–1.04 x on the new species), sterna I–IX with posterolateral corners brownish-black (sterna completely pale yellowish brown on new species) and posterolateral spines on terga III–IV (VI–IX on new species).

## ACKNOWLEDGMENTS

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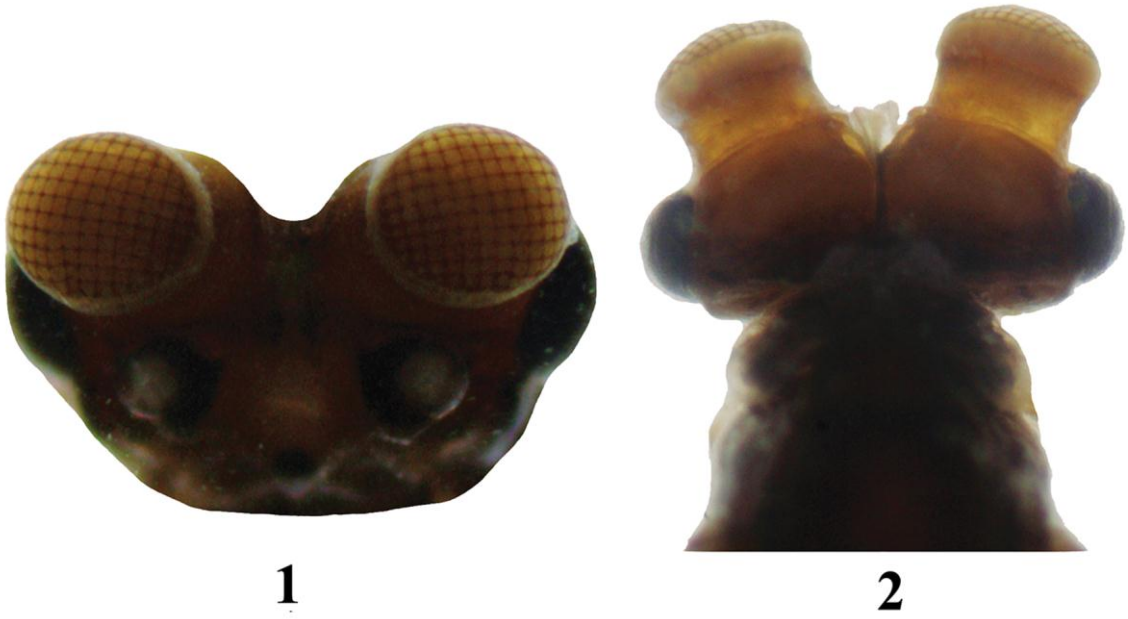
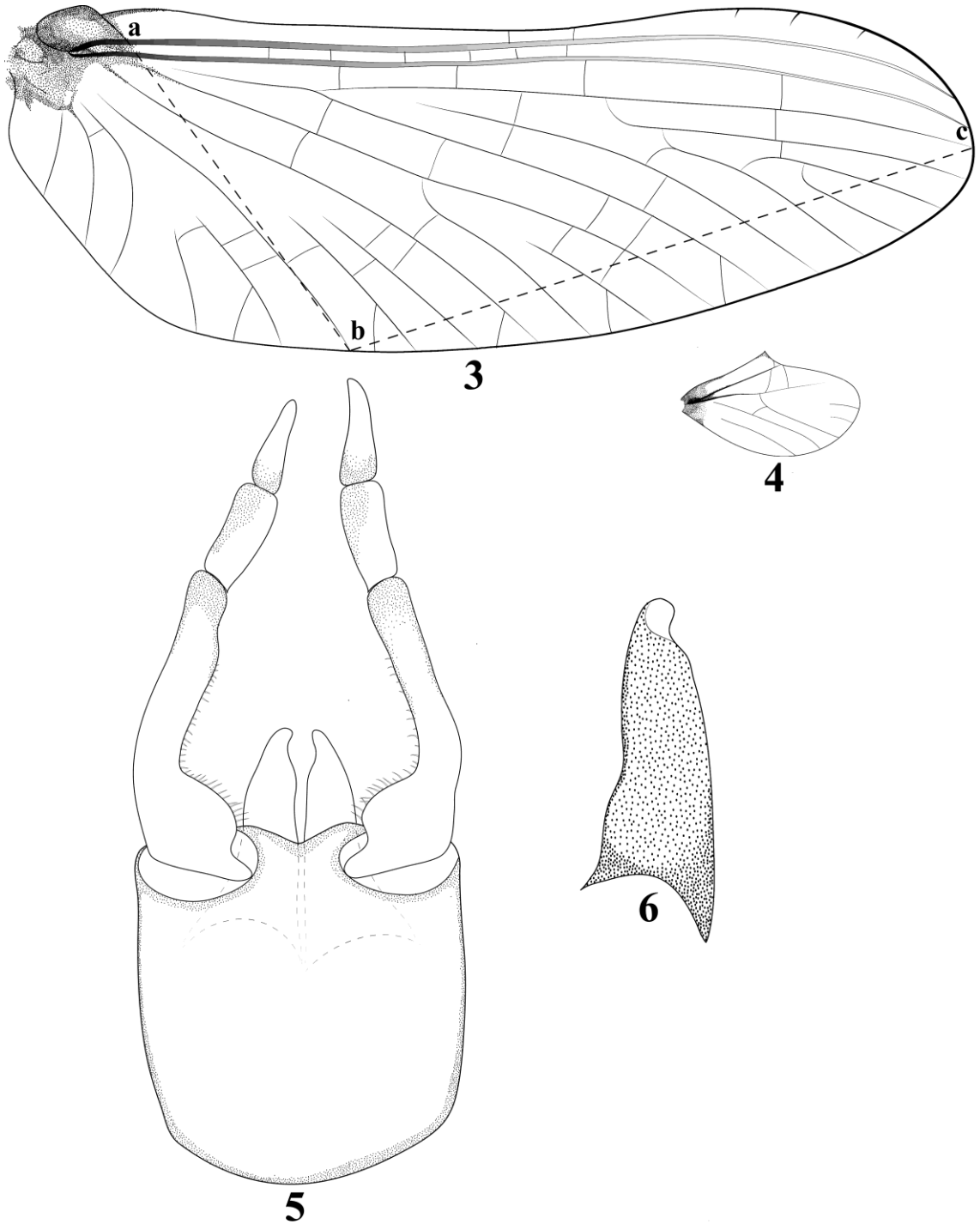
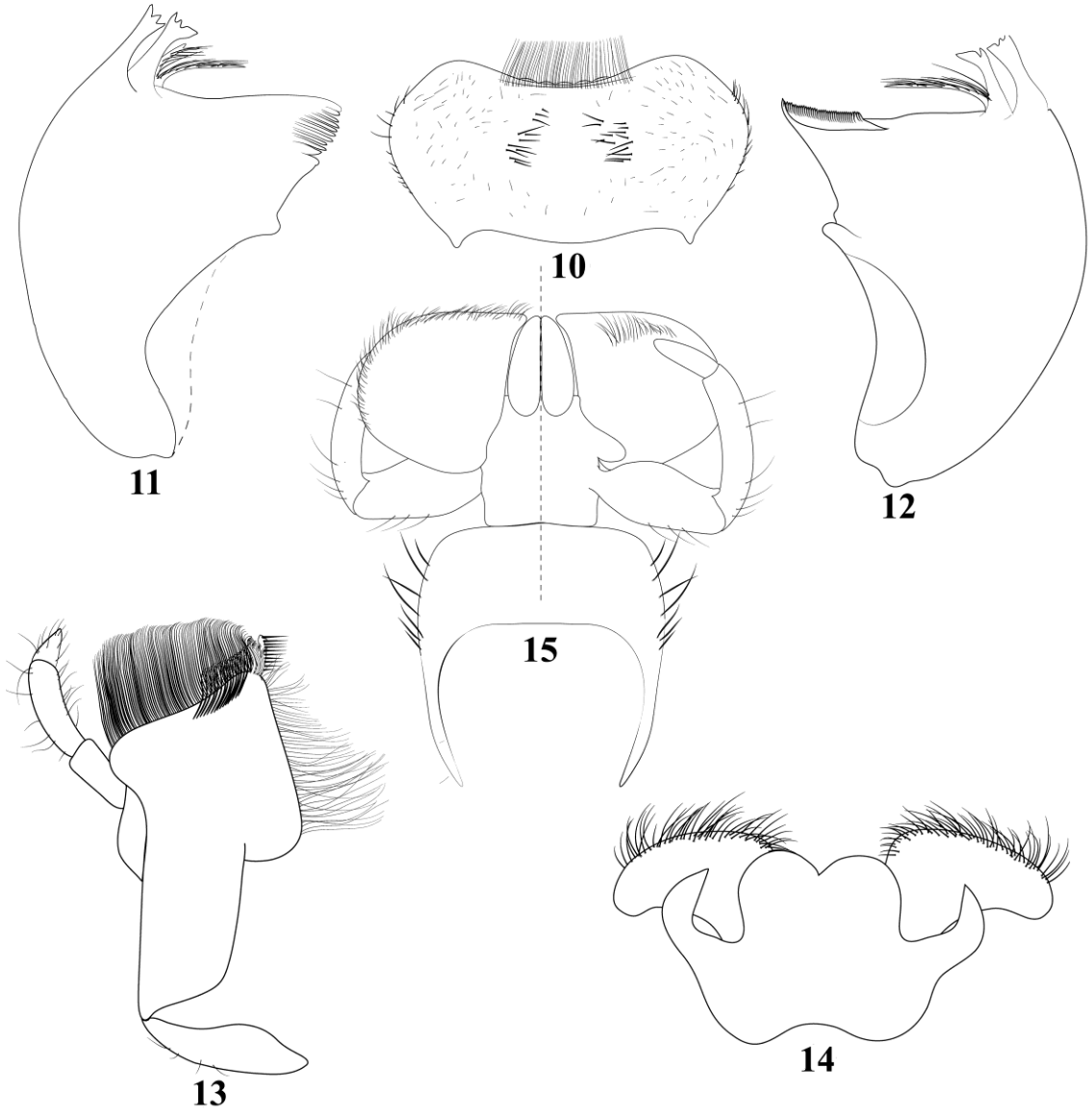



**Figures 1–2.** Male imago of *Miroculis* (*M.*) *stenopterus*, sp. nov. 1. Frontal view of head; 2. Dorsal view of head.

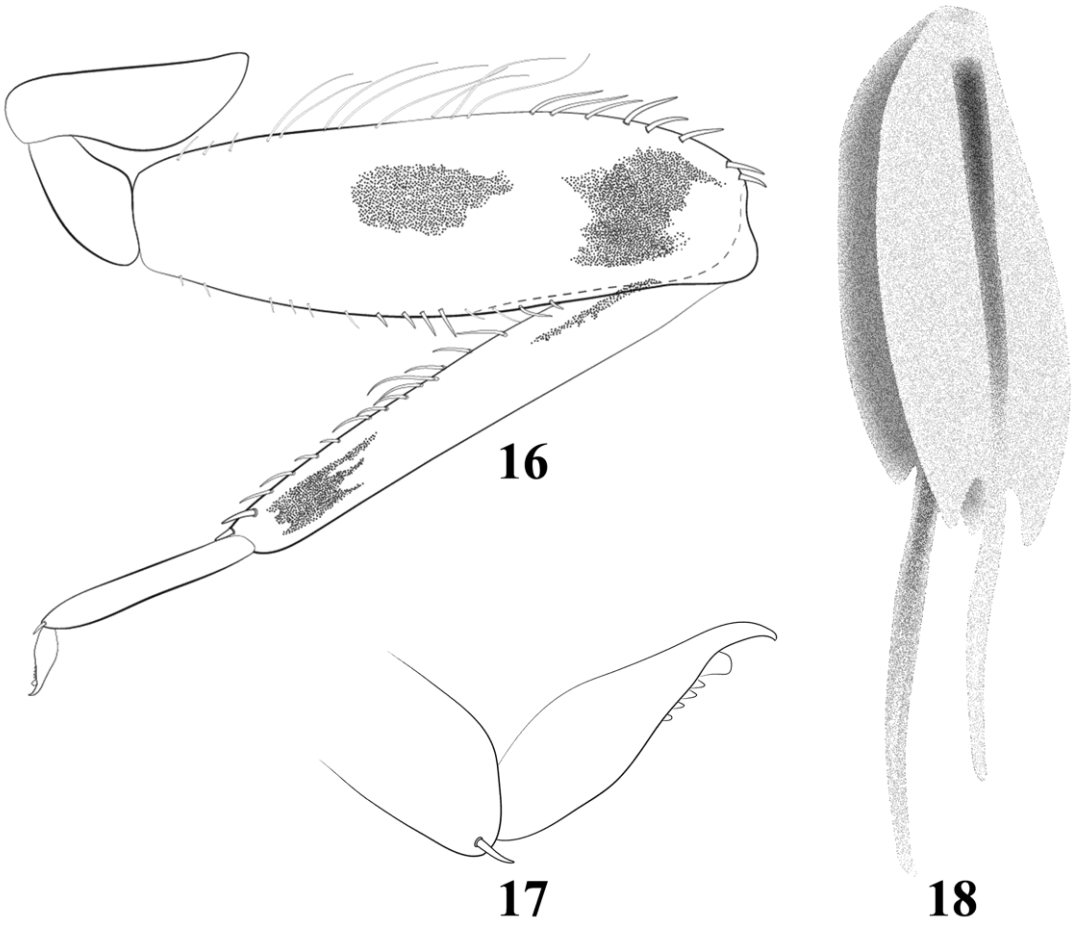



**Figures 3–6.** Male imago of *Miroculis* (*M.*) *stenopterus*, sp. nov. 3. Forewing (**a** = joint between apical costal arc and Sc; **b** = apical point of CuA; **c** = apical point of R1); 4. Hind wing; 5. Male genitalia, ventral view; 6. Detail of penis (dorsal view).


**Figures 7–9.** Putative nymph of *Miroculis* (*M.*) *stenopterus*, sp. nov. 7. Dorsal habitus; 8. Detail of abdominal terga; 9. Detail of abdominal sterna.




**Figures 10–15.** Putative nymph of *Miroculis* (*M.*) *stenopterus*, sp. nov. 10. Labrum (dorsal view); 11. Left mandible (dorsal view); 12. Right mandible (dorsal view); 13. Maxilla (ventral view); 14. Hypopharynx (venral view); 15. Labium: Ventral view (right), dorsal view (left).




**Figures 16–18.** Putative nymph of *Miroculis* (*M.*) *stenopterus*, sp. nov. 16. Foreleg; 17. Detail of tarsal claw of foreleg; 18. Gill III.

*Check List* (2015) 11 (3): 1652

## 2.6 New records of mayflies (Ephemeroptera: Insecta) from Pernambuco State, Northeastern Brazil

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**ABSTRACT**

The knowledge about the distribution of Ephemeroptera in Brazil has increased in recent years, contributing to reduce the gaps about its distribution in this country. This paper presents an updated checklist of the species of Ephemeroptera from Pernambuco State, Northeastern Brazil based on bibliography and collections from numerous rivers and streams of 27 counties and 74 localities. In this study, 18 species and 11 genera are recorded for the first time from Pernambuco State, increasing from 43 to 61 the number of known species. With these results, Pernambuco becomes the state with highest number of species in Northeastern Region of Brazil. Moreover, *Caenis chamie* is reported for the first time from Brazil.

**KEYWORDS**. Mayflies; Neotropical; survey; taxonomy.

## INTRODUCTION

The knowledge about the distribution of Ephemeroptera in Brazil has increased in recent years. According to information provided in the list of species from Brazil from the website "Ephemeroptera do Brasil" (Salles *et al.* 2015), there are 319 species recorded in 10 families and 72 genera. Since the first survey for Brazil performed by Salles et al. (2004a) several local and regional inventories were made, mainly for Southeast and Northeastern Brazil (Boldrini et al. 2012; Cruz *et al.* 2011; Lima *et al.* 2010, 2012; Salles *et al.* 2004b, 2010; Shimano, 2010). These surveys have contributed to reduce the gaps in the knowledge of distribution of Ephemeroptera in Brazil.

The first checklist about mayflies of Pernambuco State was made by Lima et al. (2012) that recorded 38 species, 28 genera, and five families. Later, five species were added: *Cloeodes maracatu* Lima, Pinheiro & Massariol, 2013; *Paracloeodes charrua* Nieto & Emmerich, 2009; *Thraulodes luizgonzagai* Lima, Mariano & Pinheiro, 2013; *Thraulodes sternimaculatus* Lima, Mariano & Pinheiro, 2013 and *Miroculis* (*Miroculis*) *stenopterus* Lima, Raimundi, Pinheiro & Salles, 2014 (Massariol *et al.* 2013; Lima *et al.* 2013a, b; Lima *et al.* 2014).

Based on bibliography and specimens collected in this work, we elaborated an updated checklist of mayflies from Pernambuco State, in order to add new records of species and correct erroneous records.

## MATERIAL AND METHODS

Collections were made between March 2011 and April 2014 in 27 counties and 74 localities **(Figures 1A–E).** Nymphs were collected with an aquatic entomological net and, adults were captured with light traps. When possible, nymphs were reared to obtain the adult stage. Collected specimens were fixed in 80% ethanol. Permanent slides were made using Canada Balsam and Euparal® as the mounting medium. The identifications were based on Domínguez et al. (2006), Salles (2006) and other relevant papers for each taxon. Informations about the geographical distribution of species were consulted in Domínguez et al. (2006), the website "Ephemeroptera do Brazil" updated frequently (Salles et al. 2015), and other relevant papers. In material examined, the symbols "I" and "N" mean "imago" and "nymph", respectively. In Appendix 1, the asterisk (*) after the name refers to a new record of species to Pernambuco State and two

asterisks (**) mean a new generic record. All specimens were collected under the scientific collector's license issued to LRCL (number 34065-3) by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBIO). The material examined is deposited in the following institutions: Instituto de Biodiversidad Neotropical, Tucumán, Argentina (IBN); Coleção Zoológica Norte Capixaba (CZNC), São Mateus, Brazil and Coleção Entomológica da Universidade Federal de Pernambuco (CEUFPE), Recife, Brazil.

## RESULTS

Below we provide a list of new records of mayfly species to Pernambuco State. For each species, we list data on geographical distribution, and comments are made when necessary.

Family Baetidae

***Adebrotus lugoi*** Salles, 2010

MATERIAL EXAMINED. Amaraji, Amaraji River, near to Usina Campo Belo, S8°21'48.9" W35°28'49.0", 320m, 1 N, 21.iv.2012, L.R.C. Lima coll. (CEUFPE)

DISTRIBUTION. BRAZIL: State of Espírito Santo.

REMARKS. Its presence in this study represents the first record of this genus from Northeastern region of Brazil.

***Aturbina georgei*** Lugo-Ortiz & McCafferty, 1996

(Figure 2A)

MATERIAL EXAMINED: Cabrobó, Ilha de Assunção, near to the bridge (Point 2), S8°31'04.9" W39°19'53.3", 322m, 2 I♂ (light trap), 07.x.2011, L.R.C. Lima coll. (CEUFPE); Petrolina, Balneário de Pedrinhas, São Francisco River, S9°16'29.2" W40°19'27", 355m, 1 N, L.R.C. Lima coll. (CEUFPE).

DISTRIBUTION. BRAZIL: States of Acre, Amazonas, Bahia, Espírito Santo, Mato Grosso, Minas Gerais, Pará, Rio de Janeiro, Roraima and São Paulo. COLOMBIA, FRENCH GUIANA and PARAGUAY.

REMARKS. Its presence in this study represents the first record of this species to state of Pernambuco.

***Paracloedes leptobranchus*** Lugo-Ortiz & McCafferty, 1996

(Figure 3A)

MATERIAL EXAMINED: Amaraji, Amaraji River, near to Campo Belo Plant S8°21'48.9" W35°28'49", 320m, 1 N, 21.iv.2012, L.R.C. Lima coll. (CEUFPE).

DISTRIBUTION. BRAZIL: States of Paraná, Rio Grande do Sul, Roraima and Santa Catarina. PARAGUAY.

REMARKS. Its presence in this study represents the first record of this species to state of Pernambuco.

Family Caenidae

***Brasilocaenis septentrionalis*** Malzacher, 1990

MATERIAL EXAMINED. Tamandaré, Mamucabas stream P2, near to Biological Reserve Saltinho S8°43'52.7", W 35°10'24.8", 38m, 1I♂ (light trap), 28.iii.2014 Lima, LRC coll.; same data as preceding except 17.iv.2014, 1I♂.

DISTRIBUTION. BRAZIL: State of Pará.

REMARKS. So far, the species of the genus Brasilocaenis were restricted to the Amazonian basin. Its presence in the Ssate of Pernambuco extends the distribution of the genus outside the Amazonian basin and represents the first record of the genus in coastal basins in Brazil.

***Caenis chamie*** Alba-Tercedor & Mosquera, 1999

(Figures 4A–E)

MATERIAL EXAMINED. Flores, Pajeú River near road to Véia stream, S07°52'35.7" W38°00'04", 473m, 1 I♂ (reared) and 5N, 19.vii.2011, L.R.C. Lima, G. Nicácio, R.P. Salomão cols. (CEUFPE); Triunfo, waterfall near to Canaã, S07°54'20.9" W38°05'46", 464m, 1 N, 19.vii.2011, L.R.C. Lima, G. Nicácio, R.P. Salomão cols. (CEUFPE); Petrolina, Vitória River, near airport, S09°21'43.7" W40°35'25", 380m, 1 N, 06.x.2011, L.R.C. Lima, G. Nicácio cols. (CEUFPE); Vicência, Engenho Embú waterfall, S07°37'22.2" W35°22'51.3", 186m, 93 I♂ and 60 I♀, 13.xi.2011, L.R.C. Lima, W.R.M. Souza cols. (CEUFPE); Vitória de Santo Antão,

Fazenda Boa Esperança, S08°5'59.5" W35°17'49.6", 1 N, 30.viii.2009, L.R.C. Lima, G. Nicácio, R.P. Salomão cols. (CEUFPE).

DISTRIBUTION. COLOMBIA.

REMARKS. Its presence in this study represents the first record of this species to Brazil.

***Caenis cuniana*** Froehlich, 1969

MATERIAL EXAMINED. Orocó, Brígida River, S08°34'54.4" W39°35'03.8", 338m, 3 N, 08.x.2011, L.R.C. Lima, G. Nicácio cols. (CEUFPE); Vitória de Santo Antão, Fazenda Boa Esperança, S08°5'59.5" W35°17'49.6", 1 N, 30.viii.2009, L.R.C. Lima, G. Nicácio, R.P. Salomão cols. (CEUFPE).

DISTRIBUTION. BRAZIL: States of Espírito Santo, Mato Grosso, Rio de Janeiro and São Paulo.

REMARKS. Its presence in this study represents the first record of this species to Northeast region.

***Caenis fittkaui*** Malzacher, 1986

(Figure 3B)

MATERIAL EXAMINED. Araçoiaba, Campo Grande Dam, CIMNIC, S07°50'13.3" W35°6'39.6", 133m, 2 I♀ (reared) and 3 N, 07.xi.2009, L.R.C. Lima coll. (CEUFPE).

DISTRIBUTION. BRAZIL: States of Espírito Santo and Pará.

REMARKS. Its presence in this study represents the first record of this species to Northeast region.

***Caenis*** sp.

MATERIAL EXAMINED. One nymph (slide CEUFPE350113), Itamaracá, stream, estrada para a Lagoa Azul, S7°46'22.3", W34°51'4.6", 21m, 24.v.2009, Lima, LRC coll. (CEUFPE). Six nymphs, Camaragibe, Besouro River, Estrada do Borralho, KM 07, PE-027, S7°59'06.4", W35°00'13.9", 16.vii.2009, 66m, Lima, LRC. coll. (CEUFPE). Seven nymphs (slide CEUFPE350111) and 18 females imagos, Recife, Açude do Prata, Parque Dois Irmãos, S8°00'28.5", W34°56'54.3", 22.viii.2009, 31m, Lima, LRC coll. (CEUFPE); two males and three females imagos (reared) (slide CEUFPE350093), same data as preceding except 12.ix.2009

(CEUFPE); two nymphs and one male imago (reared), same data as preceding except 17.iii.2010 (CZNC). One nymph and three female imagos (reared), Recife, UFRPE stream, S08°00'57.4", W34°56'46.4", 17.iii.2010, 48m, Lima, LRC coll. (CEUFPE); three nymphs and six female imagos (reared), same data as preceding except 06.iv.2010 (CEUFPE); three male imagos (slides CEUFPE350131#001 and CEUFPE350131#002), same data as preceding except 18.xii.2012 (CZNC); one nymph and four imagos (one male and three females) same data except 22.iii.2013 (CZNC); one nymph, four males and 11 females (reared) (slides p.674 and p.675) same data except 14.ii.2014 (IBN). Five nymphs, Igarassu, Jacocá stream, Refúgio Charles Darwin, S07°48'57.5", W34°57'19.1", 22.ii.2010, 37m, Lima, LRC coll. (CEUFPE). One nymph, Goiana, Açude Santa Tereza, S07°35'33.2", W34°59'32.8", 12.xi.2011, 35m, Lima, LRC, Souza, WRM cols. (CEUFPE). Two nymphs, São João, Inhumas Dam stream, S08°58'17.3", W36°21'48.5", 13.ii.2012, 686m, Lima, LRC, Souza, WRM cols. Six males and two females (slides CEUFPE350136 #001 and CEUFPE350136 #002), Amaraji, Barragem Jaguarana, S08°21'01.6", W35°24'27.7", 05.xii.2012, 265m, Lima, LRC coll. (CEUFPE). One female imago (reared), Brazil, Tamandaré, Córrego da sede, Rebio Saltinho, S08°43'48.3", W35°10'35.3", 21.ix.2009, 46m, Lima, LRC coll. (CEUFPE); one nymph, same data except 22.ix.2009 (CEUFPE); one female imago, same data except 08.viii.2010 (CEUFPE). One female imago, Rio Formoso, Fazenda São Manuel, S08°43'21.5", W35°10'12.6", 04.v.2010, 50m, Lima, LRC coll. (CEUFPE). One female imago (reared), Rio Formoso, Gameleira stream, Rebio Saltinho, S08°43'12.3", W35°10'32.9", 22.xi.2012, 47m, Lima LRC, Souza, WRM cols. (CEUFPE). One female imago (reared), Tamandaré, Mamucabas stream Point 1, near dam, S08°43'21.6", W35°11'14.0", 27.iii.2014, 90m, Lima, LRC coll. (CEUFPE). 55 males and 15 females (light trap), Tamandaré, Mamucabas stream Point 2, próximo à sede da Rebio Saltinho, S08°43'52.7", W35°10'24.8", 28.iii.2014, 38m, Lima, LRC coll. (IBN); 92 male and 63 females (slide CEUFPE350188), same data except 17.iv.2014 (CZNC).

REMARKS. *Caenis* sp. probably represents a new species, however it presents some uncommon intermediate characteristics with the genus *Brasilocaenis* in the male imago. Thus, additional studies are necessary to confirm its generic status.

Family Leptohyphidae

***Tricorythodes bullus*** (Allen, 1967)

(Figure 2B)

MATERIAL EXAMINED. Amaraji, Amaraji River, Cachoeira do Amor, S8°26'34.1", W35°24'22.1", 1 I♂ (light trap), 05.ii.2011, L.R.C. Lima, G. Nicacio cols. (DZRJ); Amaraji, Amaraji River, near to Usina Campo Belo, S08°21'48.9" W35°28'49", 320m, 2 I♂ (light trap), 21.iv.2012, L.R.C. Lima, W.R.M. Souza cols. (CEUFPE); Amaraji, Poço do Carreiro, Amaraji River, S08°22'1.9" W35°28'8.7", 269m, 2 I♂ (light trap), 21.iv.2012, L.R.C. Lima coll. (DZRJ).

DISTRIBUTION. BRAZIL: State of Maranhão. BOLIVIA.

REMARKS. The imago shows the penis with length almost equal to basal width (about 1.2x) and groove almost completely fused similar to T. bullus. However, the specimens analyzed here do not show peculiar spots on femur and tibia. This can be a populational variation, because no more relevant differences were found.

***Tricorythopsis chiriguano*** Molineri, 2001

(Figure 2C)

MATERIAL EXAMINED. Amaraji, Amaraji River, near to Usina Campo Belo, S08°21'48.9" W35°28'49", 320m, 4 I♂ (light trap), 21.iv.2012, L.R.C. Lima, W.R.M. Souza cols. (CEUFPE); same data as preceding except 1 I♂ (light trap), 28.iv.2012 (CEUFPE); Amaraji, Poço do Carreiro, Amaraji River, S08°22'1.9" W35°28'8.7", 269m, 10 I♂ (light trap), 21.iv.2012, L.R.C. Lima coll. (DZRJ); Jaqueira, Pirangi River, PE-126 near to the bridge, S08°44'53" W35°48'51.1", 189m, 685 I♂ (light trap), 23.v.2012, L.R.C. Lima, W.R.M. Souza cols. (647 CEUFPE and 38 DZRJ); Moreno, Jaboatão River, near to water treatment plant of COMPESA, S08°06'54.9" W35°07'00.2", 102m, 17 I♂ (light trap), 21.xi.2013, L.R.C. Lima coll. (CEUFPE).

DISTRIBUTION. BRAZIL: State of Maranhão. BOLIVIA.

***Tricorythopsis gibbus*** (Allen, 1967)

MATERIAL EXAMINED. Amaraji, Amaraji River, near to Usina Campo Belo, S08°21'48.9" W35°28'49", 320m, 6 I♂ (light trap), 21.iv.2012, L.R.C. Lima, W.R.M. Souza cols. (DZRJ).

DISTRIBUTION. BRAZIL: States of Espírito Santo, Rio de Janeiro and Santa Catarina. ARGENTINA.

REMARKS. Its presence in this study represents the first record of this species to Northeast region.

***Tricorythopsis sigillatus*** Molineri, 2009

MATERIAL EXAMINED. Correntes, Mundaú River (point 2), S09°07'57.4" W36°19'01.0", 380m, 1 I♂ (light trap), 14.ii.2012, L.R.C. Lima, W.R.M. Souza cols. (DZRJ).

DISTRIBUTION. BRAZIL: State of Rio de Janeiro.

REMARKS. Its presence in this study represents the first record of this species to Northeast region.

***Tricorythopsis yacutinga*** Molineri, 2001

MATERIAL EXAMINED. Amaraji, Amaraji River, Cachoeira do Amor, S8°26'34.1", W35°24'22.1", 9 I♂ (light trap), 05.ii.2011, L.R.C. Lima, G. Nicacio cols. (CEUFPE); Jaqueira, Pirangi River, PE-126 próximo a ponte, S8°44'53.0", W35°48'51.1", 189m, 76 I♂ (light trap), 23.v.2012, L.R.C. Lima, W.R.M. Souza cols. (CEUFPE); Amaraji, Poço do Carreiro, Amaraji River, S08°22'1.9" W35°28'8.7", 269m, 65 I♂ (light trap), 28.iv.2012, L.R.C. Lima coll. (DZRJ). Amaraji, Amaraji River, near to Campo Belo Plant, S08°21'48.9" W35°28'49", 320m, 6 I♂ (light trap), 28.iv.2012, L.R.C. Lima, W.R.M. Souza cols. (CEUFPE).

DISTRIBUTION. BRAZIL: State of Rio Grande do Sul. ARGENTINA.

REMARKS. Its presence in this study represents the first record of this species to Northeast region.

***Tricorythopsis undulatus*** (Allen, 1967)

MATERIAL EXAMINED. Amaraji, Amaraji River, near to Usina Campo Belo, S08°21'48.9" W35°28'49", 320m, 17 I♂ (light trap), 21.iv.2012, L.R.C. Lima, W.R.M. Souza cols. (DZRJ).

DISTRIBUTION. BRAZIL: States of Espírito Santo and Paraná. ARGENTINA.

REMARKS. Its presence in this study represents the first record of this species to Northeast region.

Family Leptophlebiidae

***Simothraulopsis* (*Simothraulopsis*) *demerara*** (Traver, 1947)

MATERIAL EXAMINED. Amaraji, Amaraji River, near to Usina Campo Belo, S08°21'48.9" W35°28'49", 320m, 2 I♂ (light trap), 21.iv.2012, L.R.C. Lima, W.R.M. Souza cols.; same data

except 28.iv.2012, 9 I♂ (light trap); same data except 11.v.2013, 9 I♂ (light trap); Tamandaré, Biological Reserve of Saltinho, Mamucabas stream Point 1, próximo ao açude, S8°43'21.6", W35°11'14.0", 90m,16 I♂ (light trap), 09.viii.2012, L.R.C. Lima & W.R.M. Souza cols.; same data except 27.iii.2014, 6 I♂.

DISTRIBUTION. BRAZIL: States of Amapá, Espírito Santo and Pará. COLOMBIA, FRENCH GUIANA, SURINAME and VENEZUELA.

REMARKS. This species had been misidentified as *Simothraulopsis* (*Maculognathus*) *sabalo* by Lima et al. (2012, figs. 13–18). Comparing our material with reared imagos of S. (M) sabalo by Nascimento (2014) we conclude that previous material belongs to S. (S.) demerara, due mainly to the abdominal color pattern and by the absence of a reentrancy on the base of forceps segment I in the imago. Its presence here represents the first record from Northeastern region of Brazil.

***Ulmeritoides flavopedes*** (Spieth, 1943)

(Figure 3C)

MATERIAL EXAMINED. Moreno, Jaboatão River, near to water treatment plant of COMPESA, S08°06'54.9" W35°07'00.2", 102m, 3 N, I. Arruda coll. (CEUFPE).

DISTRIBUTION. BRAZIL: States of Mato Grosso and Roraima. SURINAME.

REMARKS. Its presence in this study represents the first record of this species from Northeastern region of Brazil.

Family Polymitarcyidae

***Asthenopus*** sp.

MATERIAL EXAMINED. Vicência, Engenho Embú waterfall, S07°37'22.2" W35°22'51.3", 186m, 2 I♀ (light trap), 13.xi.2011, L.R.C. Lima, W.R.M. Souza cols. (CEUFPE).

REMARKS. Due to lack of male adult specimens, species identification cannot be done. Its presence represents the first record of the genus to Northeastern region of Brazil.

***Campsurus truncatus*** Ulmer, 1920 (Figure 2D)

MATERIAL EXAMINED. Belo Jardim, Fazenda Boa Vista do Ipojuca, 2 I♀, 16.iv.2006, M.J.A. Medeiros coll. (CEUFPE); Tamandaré, Biological Reserve of Saltinho, Mamucabas stream, Point

1, near to pond, S8°43'21.6", W35°11'14.0", 90m, 12I♀ and 8 I♂ (light trap), 13.xi.2011, L.R.C. Lima & W.R.M. Souza cols. (CZNC).

DISTRIBUTION. BRAZIL: State of Espírito Santo. BOLIVIA.

REMARKS. Its presence in this study represents the first record of this species from Northeastern region of Brazil.

## DISCUSSION

In this study were record 18 species and 11 genera of Ephemeroptera for the first time from Pernambuco State, increasing from 43 to 61 the number of recorded species. With these results, Pernambuco becomes the state with highest number of known species of Northeastern region of Brazil, followed by the states of Bahia (40), Maranhão (22), and Piauí (9) (Salles *et al*. 2015).

## ACKNOWLEDGMENTS

We thank to LABPOR (Laboratório de Porifera, Universidade Federal de Pernambuco) staff by the logistical support during the fieldwork. We are also grateful to Paula Souto for the help offered to identify the Leptohyphidae specimens. This study received partial financial support from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico).

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APPENDIX 1**.** Species of mayflies (Ephemeroptera: Insecta) recorded to State of Pernambuco (* new record of species; ** new generic records).

**Baetidae**

*Adebrotus* Lugo-Ortiz & McCafferty, 1995

*Adebrotus lugoi* Salles, 2010 **

*Americabaetis* Kluge, 1992

*Americabaetis alphus* Lugo-Ortiz & McCafferty, 1996

*Americabaetis labiosus* Lugo-Ortiz & McCafferty, 1996

*Apobaetis* Day, 1955

*Apobaetis fiuzai* Salles & Lugo-Ortiz, 2002

*Aturbina* Lugo-Ortiz & McCafferty, 1996

*Aturbina beatrixae* Gillies, 2001

*Aturbina georgei* Lugo-Ortiz & McCafferty, 1996 *

*Baetodes* Needham & Murphy, 1924

*Baetodes santatereza* Salles & Polegatto, 2008

*Callibaetis* Eaton, 1881

*Callibaetis gonzalezi* (Navás, 1934)

*Callibaetis guttatus* Navás, 1915

*Callibaetis pollens* Needham & Murphy, 1924

*Camelobaetidius* Demoulin, 1966

*Camelobaetidius billi* Thomas & Dominique, 2000

*Camelobaetidius cayumba* (Traver & Edmunds, 1968)

*Camelobaetidius francischetti* Salles, Andrade & Da-Silva, 2005

*Camelobaetidius lassance* Salles & Serrão, 2005

*Cloeodes* Traver, 1938

*Cloeodes hydation* McCafferty & Lugo-Ortiz, 1995

*Cloeodes irvingi* Waltz & McCafferty, 1987

*Cloeodes maracatu* Lima, Pinheiro & Massariol, 2013

*Cryptonympha* Lugo-Ortiz & McCafferty, 1998

*Cryptonympha dasilvai* Lugo-Ortiz & McCafferty, 1998

*Harpagobaetis* Mol, 1986

*Harpagobaetis gulosus* Mol, 1986

*Paracloeodes* Day, 1955

*Paracloeodes charrua* Emmerich & Nieto, 2009

*Paracloeodes leptobranchus* Lugo-Ortiz & McCafferty, 1996 *

*Waltzoyphius* McCafferty & Lugo-Ortiz, 1995

*Waltzoyphius fasciatus* Lugo-Ortiz & McCafferty, 1995

*Zelusia* Lugo-Ortiz & McCafferty, 1998

*Zelusia principalis* Lugo-Ortiz & McCafferty, 1998

**Caenidae**

*Brasilocaenis* Puthz, 1975

*Brasilocaenis septentrionalis* Malzacher, 1990**

*Caenis* Stephens, 1835

*Caenis chamie* Alba-Tercedor & Mosquera, 1999 *

*Caenis cuniana* Froehlich, 1969 *

*Caenis fittkaui* Malzacher, 1986 *

*Caenis* sp.

**Leptohyphidae**

*Leptohyphes* Eaton, 1882

*Leptohyphes petersi* Allen, 1967

*Traverhyphes* Molineri, 2001

*Traverhyphes* (*Mocoihyphes*) *edmundsi* (Allen, 1973)

*Traverhyphes (Traverhyphes) frevo* Lima, Salles & Pinheiro, 2011

*Traverhyphes* (*Traverhyphes*) *pirai* Molineri, 2001

*Tricorythodes* Ulmer, 1920

*Tricorythodes mirca* Molineri, 2002

*Tricorythodes bullus* (Allen, 1967) *

*Tricorythopsis* Traver, 1958

*Tricorythopsis chiriguano* Molineri, 2001 *

*Tricorythopsis gibbus* (Allen, 1967) *

*Tricorythopsis sigillatus* Molineri *

*Tricorythopsis spongicola* Lima, Salles & Pinheiro, 2011

*Tricorythopsis yacutinga* Molineri, 2001 *

*Tricorythopsis undulatus* (Allen, 1967) *

**Leptophlebiidae**

*Farrodes* Peters, 1971

*Farrodes tepui* Dominguez, Molineri & Peters, 1996

*Fittkaulus* Savage & Peters, 1978

*Fittkaulus cururuensis* Savage, 1986

*Hermanella* Needham & Murphy, 1924

*Hermanella froehlichi* Ferreira & Dominguez, 1992

*Hermanella maculipennis* (Ulmer, 1920)

*Hydrosmilodon* Flowers & Domínguez, 1992

*Hydrosmilodon gilliesae* Thomas & Péru, 2004

*Hydrosmilodon plagatus* Lima, Nascimento & Salles, 2012

*Leentvaaria* Demoulin, 1966

*Leentvaaria palpalis* Demoulin (1966)

*Lisetta* Thomas, Dominique & Orth, 2005

*Lisetta ernsti* Thomas & Dominique, 2005

*Miroculis* Edmunds, 1963

*Miroculis fittkaui* Savage & Peters, 1983

*Miroculis stenopterus* Lima, Raimundi, Pinheiro & Salles, 2014

*Needhamella* Domínguez & Flowers, 1989

*Needhamella ehrhadti* (Ulmer, 1920)

*Simothraulopsis* Demoulin, 1966

*Simothraulopsis* (*Simothraulopsis*) *demerara* (Traver, 1947) *

*Simothraulopsis janae* Mariano, 2010

*Terpides* Demoulin, 1966

*Terpides sooretamae* Boldrini & Salles, 2009

*Thraulodes* Ulmer, 1920

*Thraulodes luizgonzagai* Lima, Mariano & Pinheiro, 2013

*Thraulodes sternimaculatus* Lima, Mariano & Pinheiro, 2013

*Ulmeritoides* Traver, 1959

*Ulmeritoides flavopedes* (Spieth, 1943) **

**Polymitarcyidae**

*Asthenopus* Eaton, 1871

*Asthenopus* sp. **

*Campsurus* Eaton, 1868

*Campsurus duplicatus* Spieth, 1943

*Campsurus truncatus* (Ulmer, 1920)*

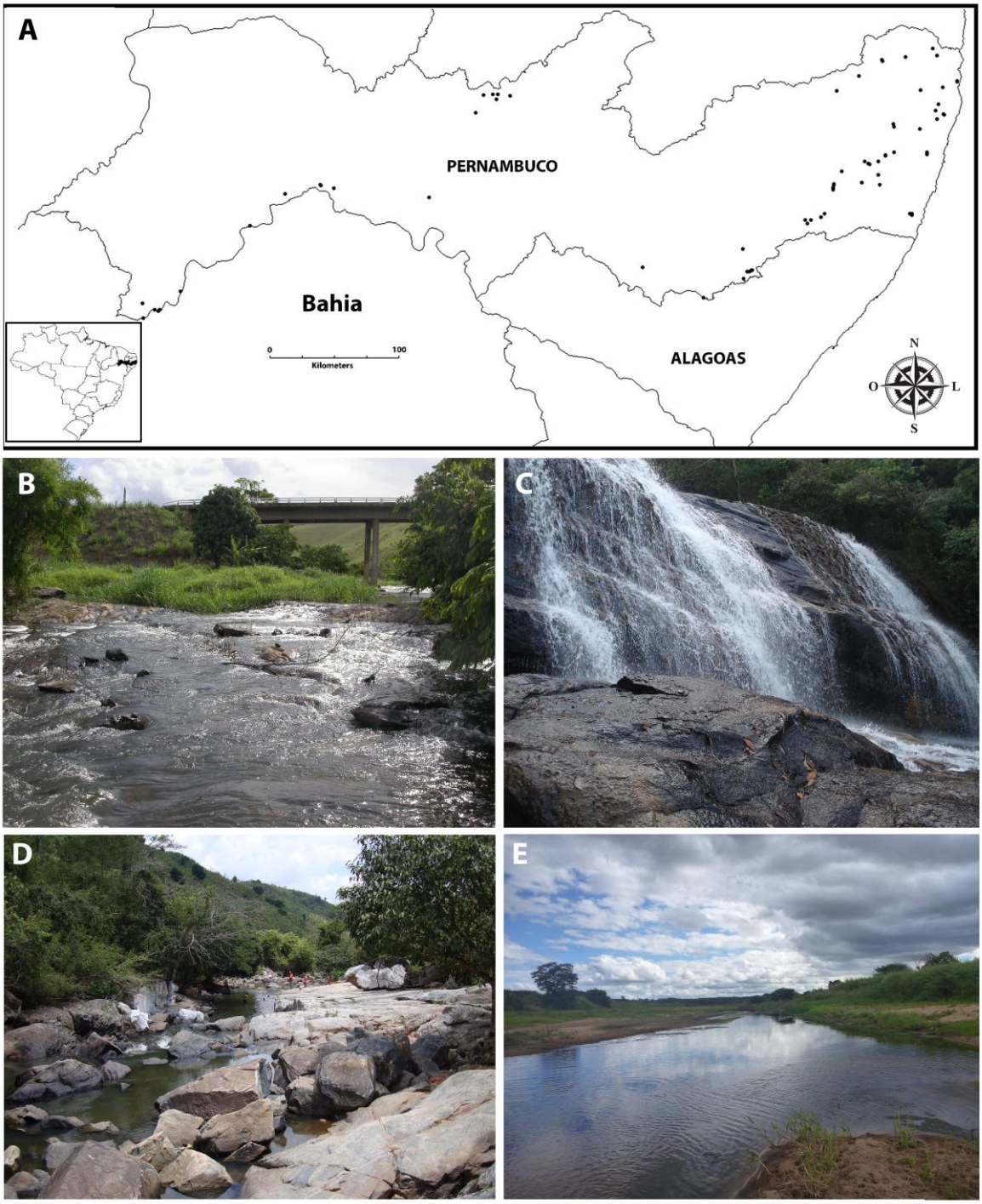


**Figure 1.** Studied area. A) Map with sampling sites in the Pernambuco State where mayflies specimens were collected. Collections localities: B) Rio Piranji, Jaqueira-PE; C) Cachoeira Barra Azul, Bonito-PE; D) E) Poço Verde, Orobó-PE; E) Rio Pajeú, Flores-PE.

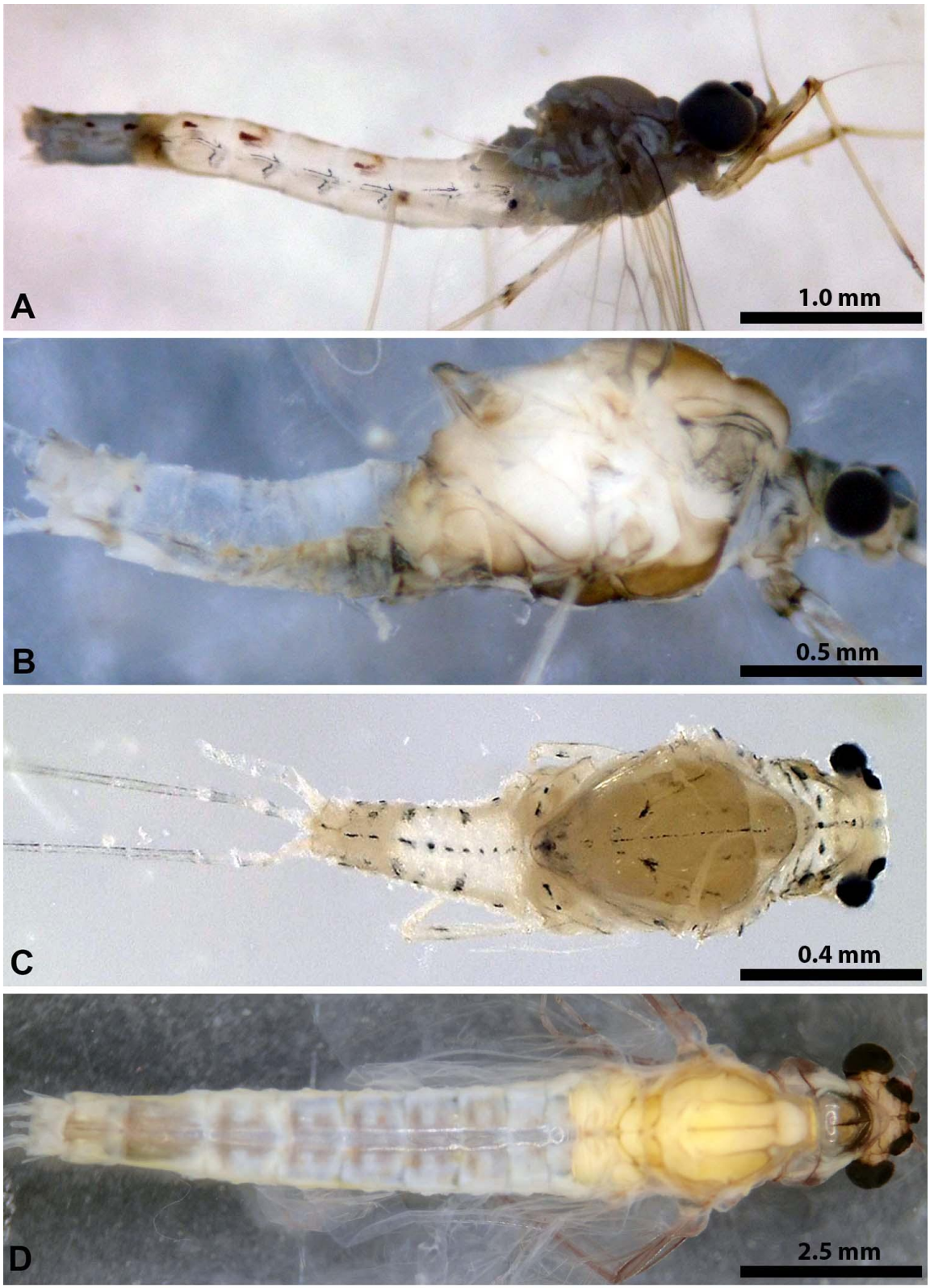


**Figure 2.** Male imagos of Ephemeroptera: **A)** *Aturbina georgei* (Baetidae), lateral view; **B)** *Tricorythodes bullus* (Leptohyphidae), lateral view; **C)** *Tricorythopsis chiriguano* (Leptohyphidae), dorsal view; **D)** *Campsurus truncatus* (Polymitarcyidae), dorsal view.

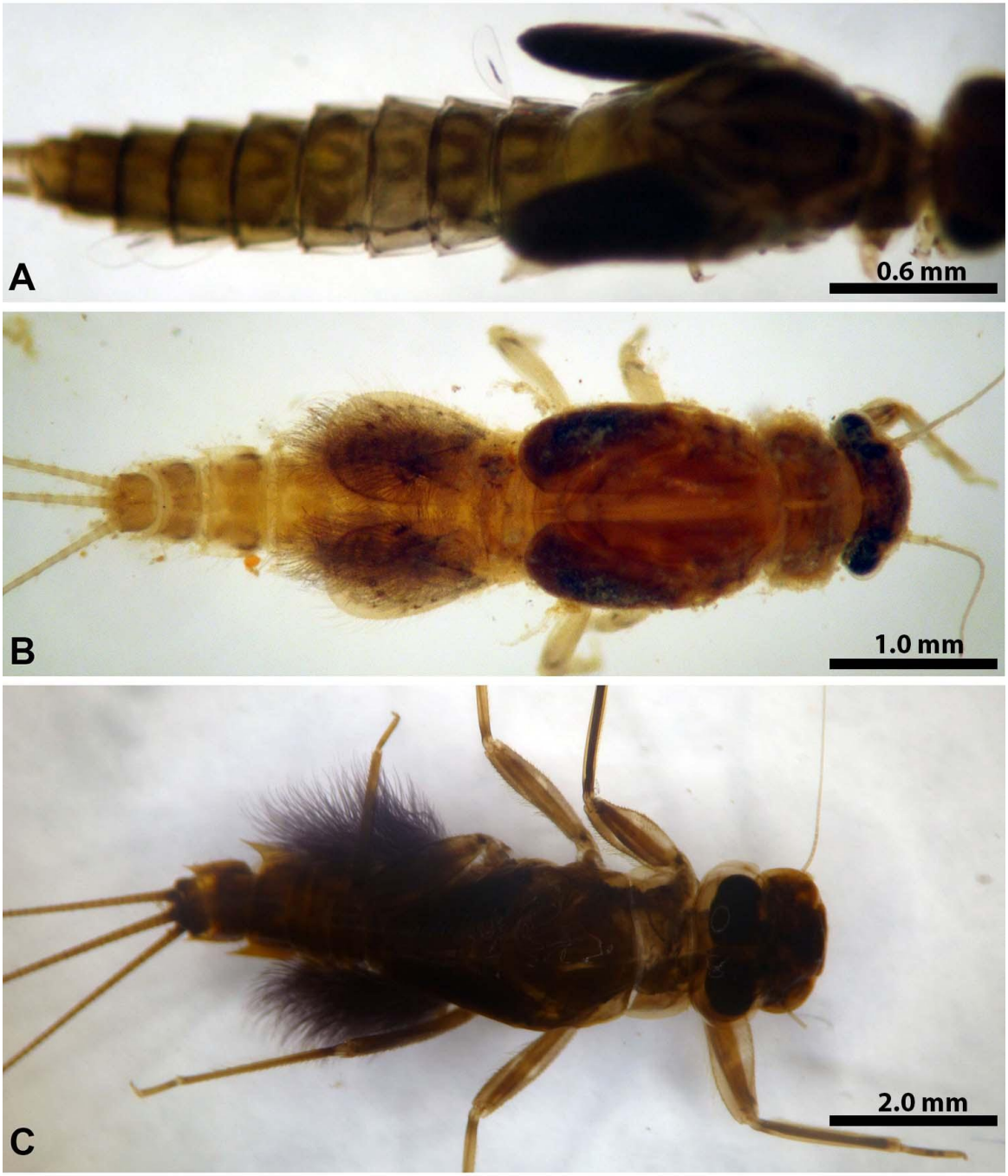


**Figure 3.** Nymphs of Ephemeroptera, dorsal view: **A)** *Paracloeodes leptobranchus* (Baetidae); **B)** *Caenis fittkaui* (Caenidae); **C)** *Ulmeritoides flavopedes* (Leptophlebiidae).

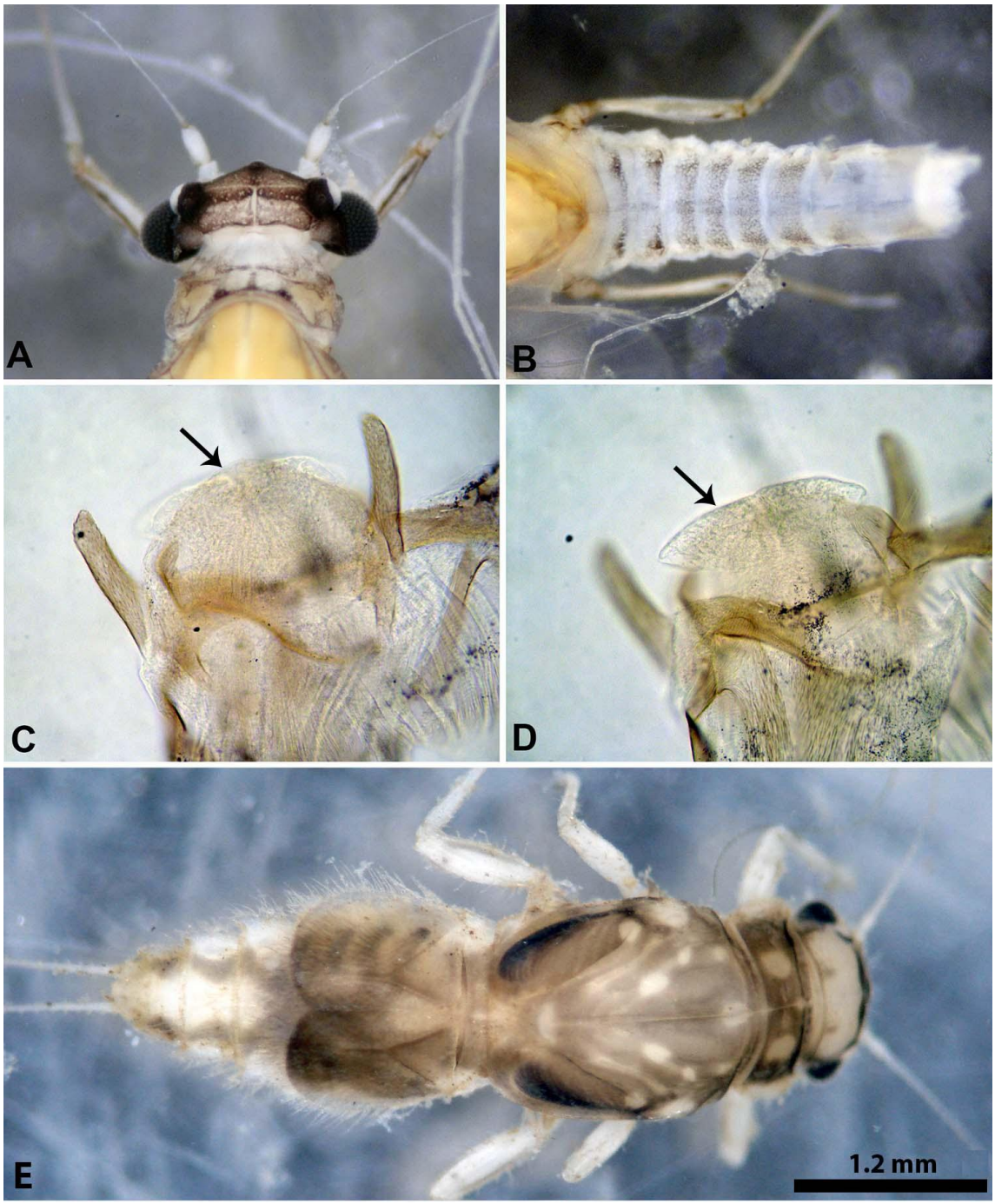


**Figure 4.** *Caenis chamie* Alba-Tercedor & Mosquera, 1999. Male imago: **A)** detail of head; **B)** abdominal color pattern; **C)** genitalia (arrow indicating the styliger plate); **D)** genitalia, (arrow indicating the penis). Nymph: **E)** habitus.

# 3 ANÁLISE FILOGENÉTICA E BIOGEOGRÁFICA DE *Brasilocaenis* PUTHZ, 1975

*A ser submetido a Systematic Entomology*

## 3.1 Phylogeny, biogeography and taxonomy of *Brasilocaenis* Puthz, 1975 (Caenidae: Ephemeroptera)[1]

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**ABSTRACT**

[1] Análise preliminar com resultados apresentados na defesa da tese. Atualmente, está sendo conduzida uma nova análise, acrescida de informações obtidas após a defesa. Os nomes atribuídos às novas espécies descritas neste trabalho não são válidos, de acordo com o Artigo 8 do Código Internacional de Nomenclatura Zoológica (1999).

*Brasilocaenis* Puthz, 1975 is a representative South American genus of Caenidae (Ephemeroptera). Except the very differentiate male genitalia, the species of this genus are very similar to South American *Caenis* species with strong, sclerotized, and apically pointed forceps. To analyse the relationship among *Brasilocaenis* and related genera, was used morphological methods using discrete and continuous character states. This first phylogenetic proposal of *Brasilocaenis* is proposed based on a matrix of seven continuous and 14 discrete characters analysed under implied weights, and includes species of genus *Alloretochus* and *Caenis* as outgroup. The genus is redefined based on redescription of male imagos and nymphs from the holotype and/or fresh material, and three new species: *B. amacayacu* **sp. n.**, *B. atawallpa* **sp. n.** and *B. suassunai* **sp. n.** described from male imagos and nymphs. Based on these results, a biogeographical analysis is conducted (spatial analysis of vicariance) based on distributional records for each species. Two main vicariant events are found: (i) a north–west separation in tropical-subtropical South American lowlands, followed by (ii) a split of two species in the same basin (Amazonia Lowlands basin).

**KEYWORDS**. Mayflies; Caenids; systematics; Neotropics.

## INTRODUCTION

Caenidae (Ephemeroptera) is a widespread group of Ephemeroptera, practically cosmopolitan, except New Zealand, Antarctica and various oceanic islands (Edmunds et al. 1976; Barber-James, 2008). In the Neotropical Region four genera and 31 species are recorded (Barber-James et al. 2013).

The nymphs of this family can be easily distinguished by the operculate gills of tergum II quadrangular, overlapping on median line of body, and with a dorsal Y-shaped ridge; gills on abdominal segment I filamentous; and hind wing pads absent. The nymphs occurs in a variety of ponds, lakes, streams, and rivers, mainly close to the edge on sand with a very thin overburden of silt, also can be found aggregated in vegetation in current zones and in floating lentic plants (Edmunds et al. 1976; Brittain 1982; Edmunds & Waltz 1996; McCafferty et al. 1997; Francischetti et al. 2001; Domínguez et al. 2006).

The adults can be distinguished by the eyes of imagos small and undivided; a median lighter mark (ommation) present on mesonotum; hind wings absent with anal area of fore wings expanded; and forceps 1-segmented (modified from Salles 2006 and Da-Silva & Salles 2012). The subimagos emerge in the early morning in some species and at nightfall in others, molting within minutes; the adult stage is very brief, lasting only a few hours at most (Edmunds et al. 1976). This brief period of life can restrict the long-distance dispersal, and make caenids an excellent object for biogeographic studies.

Caenidae is part of the infraorder Pannota, which integrates together with Ephemerelloidea, Leptophlebiidae and Potamanthidae the suborder Furcatergalia (Ogden et al. 2009). Within Pannota it is part of Caenoidea, whose only other family member is Neoephemeridae. The South American genera are inserted into two subfamilies: Caeninae (*Brasilocaenis* Puthz, 1975 and *Caenis* Stephens, 1836) and Brachycercinae (*Alloretochus* Sun & McCafferty, 2008 and *Latineosus* Sun & McCafferty, 2008).

Malzacher (1991, 2001) distinguished two main species group of the South American Caeninae based on forms of the forceps: one with straight, strongly sclerotized and apically pointed forceps; and another with weakly, sclerotized and apically rounded forceps. Despite of the pointed apex species, this forceps-type can be found all over the Holartic and Neotropical Region, and in South America are included seven species of *Caenis* and six of *Brasilocaenis* (Malzacher 1991). The apically rounded forceps-type is the most representative with 14 species and was subdivided by Malzacher (2001, 2011) into six species-groups: two from Africa (the “elouardi-group” and “vermifera-group”) and four from South and Central

America: the “reissi-group”, the “pflugfelderi-group”, the “grimi-group”, and the “argentina-group”.

*Brasilocaenis* was established for one species, *B. irmleri*, which is known from imagos of both sexes and nymphal exuviae (Puthz 1975). Other five species of the genus *Brasilocaenis* have been described from the male genitalia: *B. intermedia* Malzacher, 1986, *B. mendesi* Malzacher, 1998, *B. puthzi* Malzacher, 1986, *B. renata* Malzacher, 1986 and *B. septentrionalis* Malzacher, 1990.

Although, the *Brasilocaenis* species have not been studied in a phylogenetic context, Malzacher (1998) divided the genus into two groups, based mainly on the morphology of genitalia: “irmleri-group” (*B. irmleri*, *B. puthzi* and *B. renata*) and “septentrionalis-group” (*B. mendesi* and *B. septentrionalis*). These grouping is an assemblage of species within genus that were grouped because by their morphological similarities. Besides, their use has not yet been sufficiently studied, which might or might not indicate phylogenetic relationship.

Except the very differentiate male genitalia, the species of *Brasilocaenis* are very similar to South American *Caenis* species with pointed tip forceps (Malzacher 1986, 1990). Thus, there is a possibility that *Brasilocaenis* actually is part of *Caenis*, which would make the latter paraphyletic or that some species now in *Caenis* should be included in *Brasilocaenis*.

With the increase in knowledge about species diversity in South America, (Malzacher 2001; Molineri 2009, 2011; Molineri & Malzacher 2007), it becomes crucial the need to explore the phylogenetic and biogeographic relationships of these taxa, bringing a greater understanding about patterns of the South American Caeninae.

Thus, the goals of this paper are to perform phylogenetic and biogeographical analyses of *Brasilocaenis*, to redescribe some stages based on fresh material collected in several localities, and describe a nearly related new species from South America.

## MATERIAL AND METHODS

### *Material*

The specimens were examined during the course of this study, including those secured from a number of institutional and personal collections worldwide. Collections of fresh material were made in the following regions of Brazil: Northern (state of Acre and Pará), Northeastern (states of Bahia, Ceará, Paraíba, Pernambuco, and Piauí), and Southern (state of

Rio Grande do Sul). Material examined citations include an indication of life history stage, sex of adult stages, locality, date of collection, collectors, and place of deposition. Materials are listed alphabetically by nation, state or province, and county or equivalent subunit.

The specimens were preserved in 80% ethanol. Legs and genitalia of male imago were dissected and mounted in slides with Euparal. Wings were mounted dry. Characters involving size related characters, color pattern, shape of ocellar tubercles, and gross structure of the thorax and abdomen were examined under dissecting microscopy. Structures associated with appendages, including antennae, mouthparts, legs, and gills usually required slide mounting and examination under compound microscopy, including phase contrast. In conjunction with light microscope usage, illustrations were prepared using an attached camera-lucida, and photographs were taken using an attached digital camera. Scanning Electron Microscope (SEM) was used for examination and producing micrographs of ultrastructure with a Zeiss Supra 55VP. Eggs were dehydrated in a graded ethanol series, dried simply at the air, mounted with double-sided tape on SEM stubs, and sputter coated with gold.

Descriptions and measures (**Table 1** and **Table 2**) were taken following standard descriptions in mayflies and some specific to caenids (Hubbard 1995; Koss & Edmunds 1974; Malzacher 1982, 1990, 1998; Sun & McCafferty 2008). The **Figure 1** and **Figure 2** show schemes of the main structures used. The length of male foreleg does not include the coxa, this measure was taken from the base of the trochanter to the apex of the claws.

*Deposition*

Acronyms used herein for cited institutional collections are as follows: Zoologische Staatssammlung München, Germany (ZSM); Coleção Zoológica Norte Capixaba, Universidade Federal do Espírito Santo (CZNC), Brazil; Coleção Entomológica do Departamento de Zoologia da Universidade Federal do Rio de Janeiro (DZRJ), Brazil; Coleção Entomológica da Universidade Federal de Pernambuco (CEUFPE), Brazil; Instituto de Biodiversidad Neotropical, Universidad Nacional de Tucumán, Argentina (IBN); Museo de la Universidad del Valle, Cali, Colombia (MEUV).

*Taxa*

Were included in the phylogenetic analysis all valid species of genus *Brasilocaenis*. Nine species of *Caenis* composed the outgroup: eight from South America (*C. burmeisteri* Malzacher, 1990 – fresh material; *C. chamie* Alba-Tercedor & Mosquera, 1999 – fresh material and type material; *C. cuniana* Froehlich, 1969 – fresh material; *C. fittkaui* Malzacher,

1986 – fresh material; *Caenis gaucha* Lima, Molineri & Pinheiro, 2015 – type material – type material; *C. ludicra* Návas, 1924 – fresh material*; C. tarapoto* Molineri, 2009 – type material; *C. teipunensis* Molineri, 2011 – type material). Additionally, *Alloretochus peruanicus* (Sóldan, 1986) was also included in the analysis to root the trees (the material studied includes specimens from IBN and fresh material).

*Cladistic methodology*

Presumed homologous morphological characters that were found to have comparative value among outgroups were used for cladistic analysis. Character states were numerically encoded, and recorded in a character matrix (**Table 3** and **Table 4**).

The phylogenetic analysis of the genus *Brasilocaenis* comprised a total of 34 characters scored from external morphological features of male adults, egss and nymphs. Of this total 24 characters were discrete and ten continuous. Except for continuous characters (0–9), all characters were treated as non-additive. Searches were conducted in TNT – Tree Analysis Using New Technology (Goloboff et al. 2008) under implied weights (with different values of k: 3 to 5), and using the 'implicit enumeration' command (under 'Analyse'). By the implicit enumeration, the complete universe of possible trees for the matrix is calculated, then picking the shortest ones. Implied weighting was used because it is a way to decrease the problems of scaling in continuous characters (Goloboff et al. 2006). The analysis of continuous characters as such presents the problem of scaling, since the cost of transformation in characters of different magnitude will not be proportional, so the use of ratios greatly reduced this problem as suggested in Molineri and Salles (2013).

Relative Bremer support was calculated with 3000 suboptimal trees with the commands 'suboptimal' (under 'Analyse') and 'Bremer supports' (under 'Trees'). Frequency Difference (Goloboff et al., 2003), using 300 replications of Symmetric resampling, was also calculated as a measure of group support. This value indicates the difference between the frequency of the group (in the shortest tree) and the frequency of the most common contradictory group obtained through the resampling of the matrix.

Partial analyses were performed activating only nymphal, adult, and eggs characters alone, to compare and test their relative importance in the final tree topology and group support (**Appendix 1 and Appendix 2**).

*Biogeographic analysis*

All available geographical records of the species in *Brasilocaenis* were mapped with diva-gis (version 7.17.2, http://www.diva-gis.org/) using delimitation of the freshwater ecoregions of the world by Abell et al. (2008), and water lines and bodies provided at the cited website (**Appendix 3**).

Spatial analysis of vicariance (Arias et al., 2011), a method that implements the ideas of Hovenkamp (1997, 2001), was used for biogeographical analysis. This method attempts to reconstruct taxon biogeographical history by examining disjunct sister pairs (i.e., barriers), with inputs being a cladogram and distributions of the terminals. The analysis was implemented using the software VIP (Vicariance Inference Program), available at http://www.zmuc.dk/public/phylogeny/vip (Arias, 2010).

The spatial information that VIP requires was obtained by georeferencing the distributional data from literature, collections and a compilation of South American caenids by Domínguez *et al.* 2013. Only data of the ingroup and outgroup species were analysed in VIP (19 species and 138 distributional records). *C. horaria* included in the phylogeny were omitted as they represent very limited geographic sampling for these species groups. The VIP analysis was performed under the default settings, except that overlap was set to 10%, the cost of distribution removal 2.0, and cost of partial removal (activated) 1.0.

A grid of 1◦×1◦ (maximum fill=1) in a NASA World Vegetation map (obtained from http://neo.sci.gsfc.nasa.gov/RenderData?si=1534245&cs=rgb&format=JPEG) was used to represent distributions as absence/presence data in each cell. The heuristic search (1000 iterations) was used to obtain the better reconstructions of disjunct pairs. Searches using different grid size and costs were also conducted for comparative purposes, but the results were similar.

## RESULTS

Follow bellow the definition of continuous and discrete characters and states with Consistency Index (CI) and Retention Index (RI).

*Continuous characters (additive)*

**Male imago characters:**

**0}** Ratio of length foreleg/ hind leg (CI: 0.53; RI: 0.5);

**1}** Ratio of distance between the extreme lateral points of the forceps bases/ the forceps length (**Fig. 1A**) (CI: 0.39; RI: 0.34);

**2}** Ratio of styliger plate length/width (**Fig. 1A**) (CI: 0.54; RI: 0.71);

**3}** Ratio of forceps length/width ½ from base (**Fig. 1B**) (CI: 0.51; 0.43);

**Egg character:**

**4}** Length/width (CI: 0.94; RI: 0.91) (**Fig. 1F**);

**Nymph character:**

**5}** Ratio of length /width of forefemur (**Fig. 2A**) (CI: 0.84; RI: 0.11);

**6}** Ratio of length maxillary palp segment I/ segment II (**Fig. 2C**) (CI: 0.71; RI: 0.26);

**7}** Ratio of length maxillary palp segment I/ segment III (**Fig. 2C**) (CI: 0.59; RI: 0.31);

**8}** Ratio of maximum width maxillary palp segment I/segment II (CI: 0.60; RI: 0.00) (**Fig. 2C**);

**9}** Number of denticles on foretarsal claws (**Fig. 2E**) (CI: 0.39; RI: 0.15);

*Discrete characters (non-additive)*

**Male imago characters:**

**10} Pedicel of antennae:**

0 = twice or more longer than scape **(Figs. 1C–E)**;

1 = almost 1.1 to 1.5 times as long as scape.

**11} Flagellum of antennae:** (CI: 0.33; RI: 0.33)

0 = not dilated basally (**Fig. 1C**)

1 = slightly dilated basally **(Fig. 1D**);

2 = strongly dilated basally (**Fig. 1E**).

**12} Prosternal longitudinal ridges (shape):** (CI: 0.50; RI: 0.33)

0 = forming a rectangular-shaped structure;

1 = forming a triangle-shaped structure **(Fig. 12B)**;

2= forming a trapezoid-shaped structure (**Fig. 8B**)

**13} Posteromedian projection on abdominal tergum II:** (CI: 1.00; RI: 1.00)

0 = absent;

1 = present.

**14} Styliger plate posterior margin shape:** (CI: 0.66; RI: 0.66)

0 = straight or subtriangular (**Fig. 10C**);

1 = rounded (**Fig. 6C–E**);

2 = medially with a deep emargination (**Figs. 8D, 12C**).

**15} Groove on forceps:**

0 = absent;

1 = present.

**16} Forceps shape:** (CI: 0.40; RI: 0.57)

0 = apically pointed, narrowing gradually toward the apex (**Fig. 6C–E**);

1 = apically pointed, sharpening abruptly at apex in a thin and strongly sclerotized tip (**Figs. 8C**);

2 = apically rounded.

**17} Fusion of the forceps with styliger plate:** (CI: 1.00.; RI: 1.00)

0 = forceps not attached nor fused to lateral margins of styliger plate (**Fig. 13C**);

1 = forceps attached to lateral margins of styliger plate (**Fig. 6D**);

2 = forceps strongly fused to the lateral margins of styliger plate (**Figs. 10C, 12D**).

**18} Sculptured part on penis (composed by sclerotized penis folds and tubercles)**: (CI: 1.00; RI: 1.00)

0 = absent;

1 = present (**Fig. 8C**).

**19} Medio-apical portion of penis:** (CI: 0.50; RI: 0.80)

0 = with separate lobes (**Figs. 6C, 8E)**;

1 = fused.

**20} Well-developed dorsolateral portion of penis:** (CI: 1.00; RI: 1.00)

0 = absent (**Fig. 8D**);

1 = present (**Fig. 13C**).

**21} Apophyses of styliger, shape:** (CI: 0.20; RI: 0.50)

0 = wider than long (**Fig. 1F**);

1 = longer than wide (**Fig. 1A**).

**Nymph:**

**22} Ocelar tubercles:**

0 = absent;

1 = present.

**23} Labial palp:**

0 = 2-segmented;

1 = 3-segmented.

**24} Metacoxal process size:** (CI: 0.40; RI: 0.50)

0 = inconspicuous or very short;

1 = short (**Fig. 1C**)

2 = well-developed (**Fig. 1B**).

**25} Metacoxal process shape:** (CI: 1.00; RI: 1.00)

0 = semicircular (**Fig. 1C**);

1 = sharp and triangular (lateral margins converging toward apex) (**Fig. 1B, 7C**);

2 = cylindrical (lateral margins parallels).

**26} Posterolateral projection upcurved on median abdominal segment VI:**

0 = absent;

1 = present.

**27} Short and strong microspines on dorsal surface of gill II:** (CI: 1.00; RI: 1.00)

0 = absent (**Fig. 14E**);

1 = present (**Fig. 7E, 9D**).

**28} Operculate gill:**

0 = longer than wide (length about 1.6x the width);

1 = as long as wide.

**29} Abdominal segments V–VIII (size):** (CI: 0.50; RI: 0.75)

0 **=** with short posterolateral projections;

1 = with long and sharp posterolateral projections (**Fig. 2D**).

**30} Hind margin of sternum IX:** (CI: 0.40; RI: 0.25)

0 = apex with U-shaped or V-shaped emargination;

1 = apex rounded (**Fig. 14F**);

2 = apex truncate (**Fig. 9E**).

**Egg:**

**31} Chorion costa:**

0 = not present (**Fig. 1G, Fig. 9F**);

1 = present.

**32} Polar caps:** (CI: 0.66; RI: 0.00)

0 = one polar cap;

1 = two polar caps (**Fig. 1G**);

2 = absent.

**33} Polar caps type:** (CI: 0.50; RI: 0.80)

0 = cap-shaped (**Fig. 1G**);

1 = coiled-rope shaped (**Fig. 6F**).

The analysis of the dataset with discrete and continuous characters yielded the same single tree for each value of k (3 and 5). The list of synapomorphies and group supports are presented for just one of these values (k=3). The shortest tree presents a length of 114 steps, Consistency Index (I.C.) 0.55, Retention Index (I.R.) 0.55, and group supports are low, with frequency difference and/or relative Bremer values <40 in most of the nodes (**Figure 4**).

The results hypothesize *Brasilocaenis* as a monophyletic group (**Fig. 3**), being supported by the unique synapomorphy: apophyses of styliger plate wider than long (Character 21, state 0). The analysis also recovers two groups of species into *Brasilocaenis*. In the first group, *B. intermedia* is sister to (*B. renata* (*B. puthzi* + *B. irmleri*). Two character state changes define this group: 1) a slightly increase in the ratio of maxillary palp segment I length to segment III (character 7, 1.000–1.040 to 1.050–1.064); 2) abdominal segments V–VIII with short posterolateral projections (character 29, state 1). The node uniting *B. renata* to *B. puthzi* + *B. irmleri* is supported by two sinapomorphies: 1) metacoxal process shape cylindrical with lateral margins parallels (Character 25, state 1); 2) short and strong spines on dorsal surface of gill II present (Character 27, state 1). The group formed by *B. puthzi* + *B. irmleri* is supported by four sinapomorphies: 1) decrease in the ratio of distance between the extreme lateral points of the forceps bases to the forceps length (character 1, 1.350 to 1.060–1.210); 2) an increase in the ratio of styliger plate length to width (character 2, 0.951 to 1.075–1.181); 3) increase in the ratio of forceps length to width ½ from base (character 3, 8.710–9.600 to 12.800); 4) styliger plate with rounded posterior margin (character 14, state 1).

In the second clade, *B. amacayacu* sp. n. is sister to (*B. suassunai* sp. n. (*B. septentrionalis* (*B. atawallpa* sp. n. + *B. mendesi*))) and this group is supported by an unique sinapomorphy: presence of a well-developed dorsolateral portion of penis (character 20, state 1). The clade formed by (*B. suassunai* sp. n. (*B. septentrionalis* (*B. atawallpa* sp. n. + *B. mendesi*))) is also defined by an unique sinapomorphy: presence of a sculptured portion on

penis (character 18, state 1). The group formed by (*B. septentrionalis* (*B. atawallpa* sp. n. + *B. mendesi*) four sinapomorphies: 1) a decrease of the distance between the extreme lateral points of the forceps bases to the forceps length (character 1, 1.410–1.507 to 1.110–1.130); 2) an increase in the ratio of styliger plate length to width (character 2, 0.648–0.862); 3) forceps apically pointed, narrowing gradually toward the apex (character 16, state 0); 4) forceps strongly fused with the lateral margins of styliger plate (character 17, state 2). The node uniting *B. atawallpa* sp. n. + *B. mendesi* is supported by two sinapomorphies: 1) an increase in the ratio of styliger plate length to width (character 2, 0.862 to 0.868–1.037); and 2) posterior margin of styliger plate medially with a deep emargination (character 14, state 2).

The partial analyses with adults, eggs and nymphal characters do not found supported clades. The search with adults character only found 28 trees and consensus with no supported clades (**Appendix 1**). Eggs and nymphal character only, found 10,000 trees with any clade formed. (**Appendix 2**).

### *Biogeographic analysis*

Most records of *Brasilocaenis* species (*B. amacayacu* sp. n., *B. atawallpa* sp. n., *B. intermedia, B. irmleri, B. mendesi, B. puthzi,* and *B. renata*) are from the following basins: Amazonia Lowlands and Amazonas Estuary & Coastal Drainages. Some records of *B. mendesi* are from two other basins: Paraguay, Guapore – Itenez and Amazonas Lowlands. *B. atawallpa* is also recorded from Guapore – Itenez basin. All records of *B. suassunai* are from Northeastern Caatinga & Coastal drainages and São Francisco basins. *B. septentrionalis* is recorded from distant basins: Amazonas Guiana Shield and Northeastern Caatinga & Coastal drainages.

The VIP analysis resulted in six vicariant (without remotions), with a cost of 11 and two of these vicariant nodes concerns the ingroup (**Figure 5**). The first event is the separation between *B. septentrionalis* and *B. atawallpa* + *B. mendesi* (barrier 1) and the second vicariant node is the split between *B. puthzi* and *B. irmleri* (barrier 2).

## DISCUSSION

### *Phylogeny*

This phylogenetic analysis hypothesizes *Brasilocaenis* as a monophyletic group, being supported by an unique synapomorphy (apophyses of styliger plate wider than long), and recovers two groups of species. The use of the combined dataset (discrete/continuous) have increased the support value in some clades, according to same results found by Goloboff et al. (2006), Mello (2011) and Molineri & Salles (2013). However, based on the shortest tree found under parsimony, the sister relationship between the two groups into the genus was not well supported. This weak support can be related to missing data for many species in the matrix. Of the nine species assigned here to *Brasilocaenis* and used in the analysis, only four species have all stages described and the others possess missing characters for some life stage, mainly for eggs and nymphs. In all species (except *B.* intermedia), the adult stages are described, and from an analysis performed with adults characters alone (47% of the entire dataset), similar clades are found as in the complete data set analysis (**Appendix 1**). This can be explained by the taxonomy of Caenidae based mainly in the adult characters and a few species have all life stages known, and so, this decrease the group support. Furthemore, the descriptions for some species are incomplete and the type material is missing or damaged.

Despite this, some groups were relatively well supported: the group formed by *B. septentrionalis* (*B. atawallpa* sp. n. + *B. mendesi*) and the other formed by *B. puthzi* + *B. irmleri*. Both groups with frequency difference and/or relative Bremer values >40, and five and four sinapomorphies, respectively. This suggests that *Brasilocaenis* could have two lineage of species, and this should be tested with the inclusion of more characters or description of unknown life stages. Synapomorphies that define these lineages agree with the groups listed in Malzacher (1998) to define the irmleri and septentrionalis group, except for the forceps fused to lateral margin on styliger plate in the latter (Character 17, state 2).

Regarding the relationship of *Brasilocaenis* and *Caenis*, we observe that it appears as sister group of the *Caenis* with pointed-tips forceps, being this clade supported in the analysis by two synapomorphies: 1) decrease in the ratio of distance between the extreme lateral points of the forceps bases to the forceps length (character 1, 1.694–1.727 to 1.410–1.507); 2) increase in the ratio of forceps length/width ½ from base (character 3, 6.000–6.660 to 8.710–9.250). This suggests that perhaps these *Caenis* species could be include into *Brasilocaenis* or grouped into a new genus. However, we do not include here the pointed-tips *Caenis* into *Brasilocaenis*, because more species (rounded and pointed) should be included in the analysis to confirm this.

Despite the internal resolution of the clades presented above have low resolution and inconsistencies with the older non-phylogenetic proposals presented by Malzacher (1990, 1998), there is a need for new phylogenies involving all South American members of *Caenis*. Futhermore, it is essential to fill the gaps in knowledge about life stages of many species.

***Biogeography of the group***

The genus *Brasilocaenis* is endemic to South America, and according to the VIP results, there are two main vicariant events that seem to have acted on ingroup history. The first represents the split of the following species: *B. septentrionalis* restricted to north and east South America (in the Northeastern Caatinga & Coastal drainages and Amazonas Guiana Shield basins) from the *B. atawallpa* + *B. mendesi* restricted to the west (in the Amazonia Lowlands, Guaporé-Itenez and Paraguay basins). The second vicariant event separated *B. irmleri* from *B. putzhi* in the same basin (Amazonia Lowlands basin). Regarding to this latter event, it is difficult to recognize any allopatric event to separate these species due the proximity of the distributional records between them. This can be a distortion in this node, due the lacking of distributional records of *B. putzhi*, resctricted only this basin, or may indicate a parapatric speciation.

In relation to the first vicariant event (Barrier 1 in **Fig. 5**), we cannot attribute with certainty any paleoclimatic or microtectonic event because the nodes in this analysis are not dated, and there is no paleontological data available to this genus. With respect to biogeographic pattern, it is not possible to recognize a barrier acting today, but this pattern is reported also in other taxonomic groups (Molineri & Salles, 2013). A possible explanation of this pattern can be the successive Atlantic marine transgressions recorded in Middle–Late Miocene (23.8–5.32 Ma) that isolate plants and mammals (Ortiz-Jaureguizar & Cladera, 2006). According, to them, during this time an open seaway separated terrestrial environments of Southern South America from those farther north. In this scenario, the north-western part of South American was connected with the so-called ‘‘Tethys Waterspout’’ in the southern, that covered widespread areas among the Andean Cordillera and the Guayanian and Brasilian Bedrocks.

***Taxonomy***

***Brasilocaenis* Puthz, 1975**

*Brasilocaenis* Puthz, 1975: 411; Malzacher, 1986: 84; Malzacher, 1998: 3.
(Type-species: *Brasilocaenis irmleri* Puthz, original designation).

**Diagnosis**. Based on the sinapomorphies from cladistic analysis, we suggest an amended diagnosis to include some species with an atypical characteristic (forceps not attached to lateral margins of styliger plate, character 17, state 0). Thus, the genus can be characterized by the following combination of characters. In the imago: 1) well-developed dorsolateral portion of penis present **(Fig. 8D)**; 2) forceps attached or completely fused to lateral margins of styliger plate **(Figs. 6C, 10C)**; 3) styliger plate posteriorly elongated often covering penes (**Figs.** In the nymph: 1) coxal processes of middle and hind legs long, sharply tapered, and with dentate borders **(Fig. 7C)**; 2) abdominal segments V–VIII with long and sharp posterolateral projections (**Fig. 2D**); 3) short and strong micropines present on dorsal surface of gill II (**Figs. 7E, 9D**).
**Distribution**. Brazil, Colombia, and Bolivia.

***Brasilocaenis intermedia* Malzacher**
*Brasilocaenis intermedia* Malzacher, 1986: 89 (nymph); Malzacher, 1998: 1.

**Diagnosis.** It can be characterized by the following combination of characters in the nymph: 1) length of body of male 2.8–3.0 mm, of female 3.4–4.0 mm; 2) anterolateral margins of pronotum rounded and directed anteriorly; 3) coxal processes of middle and hind legs with parallel sides and rounded apically, with small evenly spaced denticles; 4) posterolateral projections on abdominal segments V–VIII long and pointed; 5) dorsal surface of operculate gills without short and strong spines.
**Ratios.** *Mouthparts:* width of maxillary palp segment I 2.28 × the width of segment II; length of maxillary palp segment I 2.02 × the length of segment II; length of maxillary palp **s**egment I 1.1 × the length of segment III.
**Distribution.** Brazil: Northern region (Tocantins River).
**Comments.** This species was described by Malzacher (1986) based on nymphal exuviae. Fresh material of *B. intermedia* were not available to us for study, only some photos of holotype. In the original description, the nymph has some characters that do not correspond to the generic description of *Brasilocaenis* as coxal processes of middle and hind legs with parallel sides and rounded apically and dorsal surface of operculate gills without short and

strong spines. Thus, the assignment of this species to *Brasilocaenis* must be confirmed when the male imagos are known or fresh material of nymph are analyzed.

***Brasilocaenis irmleri* Puthz (Figures 6–7)**

*Brasilocaenis irmleri* Puthz, 1975: 12 (male, female, nymph); Irmler, 1975: 348; Malzacher, 1986: 85 (male, female, nymph, egg); Malzacher, 1998: 3.

*Brasilocaenis irmeli*, Shimano et al. 2010: 245 (mispelling).

**Diagnosis.** This species can be characterized by the following combination of characters. In the imagos: 1) body length of male 2.50–3.25 mm, of female 3.1–4.3 mm; 2) base of antennal flagellum not dilated; 3) male genitalia, with forceps pointed, enlarged near base and narrow toward the apex, apically curved inwardly, and attached to lateral margins of styliger plate **(Figs. 6C–D)**; 4) styliger plate long with posterior margin rounded; 5) basolateral sclerites parallel, laying apart from the lateral sclerites. In the nymph: 1) body length of male 2.7–3.0 mm, of female 3.4–3.9 mm; 2) anterolateral margins of pronotum forming a very marked angle; 3) coxal processes of middle and hind legs long, sharply tapered, and with dentate borders **(Fig. 7C)**; 4) tarsal claws I–II with 4 minute denticles basally **(Fig. 7D)**; 5) hind margin of the ninth sternite rounded **(Fig. 7D)**; 6) posterolateral projections on abdominal segments V–VIII long and pointed; 7) dorsal surface of operculate gills with short and strong microspines **(Fig. 7E)**. Eggs: 1) two polar caps of coiled-rope type **(Fig. 7F)**; 2) oval shaped with length 1.2–1.5 × the maximum width.

**Male imago.** Body length: 2.50–3.25 mm; forewing length: 2.00–2.37 mm; foreleg length: 1.83–2.00 mm; hind leg length: 1.0–1.5 mm; cercus length: 6.7–8.0 mm.

**Ratios.** *Legs*: body size 1.25– 1.50 × the length of foreleg; foreleg 1.43–2.0x the length of hind leg; first segment of the foretarsus length (micras): $2^{nd}$: $3^{rd}$: $4^{th}$: $5^{th}$ = 3.6–4.1:1.9–2.4:1.5–1.8:1.2. *Genitalia:* styliger plate length 1.08–1.28 × the width; forceps length 17.5–18.8 × the width ½ from base; distance between the extreme lateral points of the forceps bases to forceps length = 0.95–1.21.

**Coloration**. *Head*: whitish shaded with gray; venter of head whitish **(Fig. 6B)**; scape and pedicel of antenna whitish shaded with gray, flagellum hyaline.

*Thorax* **(Fig. 6C)**. Prothorax whitish translucent shaded with black dorsally; prosternum whitish, with blackish lines near base of legs. Meso- and metanotum whitish shaded with gray on carinae; pleurae and sterna paler. Wing membrane hyaline, veins

translucent except C, Sc and Rs grayish. Legs whitish with all coxae, femora and base of foretibiae shaded with gray; median and subapical gray marks present on all femora, and on middle and hind tibiae.

*Abdomen* **(Fig. 6A)**. Whitish shaded with black dorsally, with medial dark spot on terga II–III. Terga I–II and VIII darker laterally, and segments I, VIII–X whitish medially. Sterna translucent white. Pleura with trachea strongly pigmented.

*Genitalia* **(Figs. 6C–E)**. Membranes and sclerites brownish. Forceps brownish. Penis whitish. Sternum IX with chitinous lateral structures strongly colored. Caudal filaments whitish translucent.

**Morphology.** *Antenna*: Base of antennal flagellum not dilated.

*Thorax*. Lateral margins of prothorax straight, not protruding; prosternal longitudinal ridges forming a triangle shaped structure, anteriorly open and laterally with straight margins **(Fig. 6B)**.

*Abdomen*. Lateral filaments present on terga VI–VII, very short; finger-like process on tergum II absent.

*Genitalia.* Styliger plate triangular, posteriorly rounded; central sclerite dome-shaped and elongated; apophyses very short, pointed and, triangular; lateral sclerites tape-shaped, with narrow base, more or less parallel, extended forward to the central sclerite; basolateral sclerites parallel, laying apart from the lateral sclerites **(Figs. 6C–E)**. Forceps pointed, fused to lateral margins of styliger plate, enlarged near base and narrowing gradually toward the apex, apically curved inwardly; and main axis without trichomae or tubercles. Ventral surface of penis with a shallow groove; lobes of the penis separated on apical medial region **(Fig. 6G)**.

**Female imago**. Body length: 3.1–4.3 mm; forewing length: 2.2–3.1 mm; foreleg length: 1.25–1.50 mm; cercus length: 1.6–2.5 mm. *Head*: blackish, venter of head whitish. *Thorax*: mesokatepisternum light brown, rest of mesopleural sclerites whitish. Metanotum brown, with brownish median notal suture darker; other parts light yellowish-brown to white. *Abdomen*: abdominal terga with gray transverse bands, tergum VII–VIII whitish medially; abdominal sterna withish shaded with black. Lateral filaments present on terga V–VIII, very short. Abdominal sternum IX triangular, apically pointed.

**Egg.** Length: 120–135 µm; width: 90–100 µm. Coloration light yellow. Oval shaped with length 1.2–1.5 × maximum width **(Fig. 6F)**. Chorion finely punctured with many filamentous granules on surface **(Fig. 6F)**. Micropyle short and broad, without sperm guide **(Fig. 6G)**.

Two polar caps of coiled-rope type with about ten terminal knobs and a more or less eccentric position in relation to the main axis.

**Nymph.** Length of male: body, 2.7–3.0 mm; cercus, 2.6 mm. Length of female: body, 3.4–3.9 mm; cercus, 5.0–5.6 mm.

**Ratios.** *Mouthparts:* width of maxillary palp segment I 1.80–2.25 × the width of segment II; length of maxillary palp segment I 1.28–1.36 × the length of segment II; length of maxillary palp **s**egment I 0.8–0.9 × the length of segment III. *Leg*: length of forefemur 2.80–3.28 × its maximum width.

**Coloration (Fig. 7A).** *Head*: yellowish, occiput and mouthparts paler, grayish markings forming an irregular net on the occiput, also shaded gray behind eyes. *Antenna*: scape and pedicel brownish, flagellum hyaline.

*Thorax* **(Fig. 7A)**. Nota brownish with black sutures. Pronotum with translucent whitish anterolateral corners, shaded with black on anterior margin and lateral zones; with blackish paramedian dots and median elongated marks. Mesonotum with blackish macula anterior to wingbuds bases, and with a pair of submedian whitish spots at center. Legs yellowish-brown, blackish marks present on coxae, median zones of femora and tibiae. Thoracic sterna paler.

*Abdomen* **(Fig. 7A)**. Terga yellowish-brown, segments I–II with blackish band on medial area, segments VIII–X, paler medial and darker laterally, terga III–VII yellowish-white, shaded with gray. Operculate gills translucent yellowish brown, almost completely maculated with blackish spots. Abdominal sterna much paler with only small grayish sublateral marks. Caudal filaments yellowish-white.

**Morphology.** *Head*: hind margin without setae. *Mouthparts*: lateral margins of labrum rounded, with small submedian protuberances on anteromedian emargination, and long apical setae; mandibles with a dorsolateral row of long setae, lingua of hypopharynx with fore margin straight.

*Thorax*. Pronotum with anterolateral margins pointed, directed laterally (forming a very marked angle); lateral margins denticulate. *Legs:* coxal projections relatively long and triangular (lateral margins converging toward apex), with denticulate margins **(Fig. 7C)**. Dorsal and ventral margins of fore and middle femora with short simple setae, and long simple setae on hind femur; transversal row of spatulate setae on dorsum of forefemora reduced, only with 2–3 setae; strong spine-like setae scattered on anterior surface of hind femur. Foretarsus with one marginal row of simple, spine-like setae; middle with marginal

and ventral rows of simple setae (ventrally with serrated setae on apical third); and hind tarsus with 2 rows of setae: one serrated, the other simple. Claws: tarsal claws I and II with 4 minute denticles basally **(Fig. 7D)**, tarsal claw III with around 30 minute denticles.

*Abdomen.* Tergum II with projection short and wide at base, posteriorly curved. Operculate gills with setae from the submarginal ventral row elongated; Y-ridge complete and well-developed; dorsal surface smooth with short and blunt microspines, and long, simple setae **(Fig. 7E)**. Posterolateral projections on abdominal segments V–VIII long and pointed. Sternum IX with hind margin rounded, laterally denticulate, with simple setae **(Fig. 7F)**.

**Distribution.** Brazil: states of Amazonas and Mato Grosso. Colombia: state of Amazonas. Bolivia: states of Santa Cruz and Beni.

**Material examined. Brazil**: 2 male imagos (light trap), Pará State, Mosqueiro, Rio Murubira, S01°08'03.9", W48°26'38.8", 8m, 27.x.2013, Lima, LRC, Shimano, Y, Cardozo, MN cols. (CEUFPE); 16 nymphs, Pará State, Mosqueiro, Balneário Igaracôco, S01°06'30.4", W48°25'25.9", 8m, 26.x.2013, Lima, LRC, Shimano, Y, Cardozo, MN cols. (IBN); same data as preceding except one nymph (CEUFPE); same data as preceding except three female imago (reared) (CEUFPE); same data as preceding except two male imago (slide CEUFPE350141) (CEUFPE); 9 nymphs (slide CEUFPE350162), Pará State, Mosqueiro, Córrego da ponte, próx. a PA-391, S01°08'49.4, W48°21'42.6", 9m, 26.x.2013, Lima, LRC, Shimano, Y, Cardozo, MN cols. (CEUFPE). **Colombia:** 120 males and 36 females imagos (light trap), Departamento. Amazonas, Parque Nacional Amacayacu, Quebrada Mata-mata, S03°48’28”, W70°15’21”, 02.ii.1999, Zuñiga, MC, Molineri, C, Domínguez, E. cols. (IBN); same data as preceding except 119 males and 19 females imagos (light trap), 2–5.ii.1999 (MEUV); 220 males and 46 females imagos (slides 630, 631 and 632), Departamento Amazonas, Puerto Nariño, S03°43’53” W70°21’59”, 04.II.1999, Zuñiga, MC, Molineri, C, Domínguez, E. cols. (IBN); three nymphs, Depto. Amazonas, Leticia, Laguna Yaguarcaca, 30.i.1999, Zuñiga, MC, Molineri, C, Domínguez, E. cols. (MEUV); two nymphs (slide 375), Leticia, Isla de Ronda, S04°08’23”, W69°59’12”, 08.ii.1999, Zuñiga, MC, Molineri, C, Domínguez, E. cols. (IBN); 49 males and 32 females (ligth trap), Reserva Natural Palmari, Rio Yabari, Muelle Centro Admnistrativo, S04°17’10”, W70°17’49”, 120m, 29.v.2002, Zuñiga, M.C., Emmerich, D., Cardozo-Zuñiga, A.J. & Cardozo-Zuñiga, R.J cols. (IBN). **Bolivia**: 100 males and 43 females imagos (slide 625), Departamento Santa Cruz, La Florida, Parque Nacional Noel Kempff, Casa Guardaparque, S14°36’12.9”, W61°11’38.3”, 180m, 17.vi.2000, Domínguez, E. coll. (IBN); 22 male imagos (light trap), Departamento Beni,

Itenez, Bella Vista, Rio Blanco, S13°14'01.14", W63°'43'22.21", 137m, 07.v.2008, Domínguez, E., Molina, C. cols. (IBN); 31 male imagos (slide 488), Departamento Beni, Versailles, Rio Itenez, 147m, 05.v.2006, Domínguez, E., Molina, C. cols. (IBN).

**Comments.** *B. irmleri* is the type-species of the genus and was described from imagos of both sexes and nymphs from Brazil and posteriorly recorded from Colombia (Domínguez *et al.* 2006). Slight differences were observed in the shape of styliger plate and abdominal color pattern, but we consider here as an intraspecific variations. In addition, this species is for the first time recorded from Bolivia.

***Brasilocaenis mendesi* Malzacher (Figure 8)**

*Brasilocaenis mendesi* Malzacher, 1998: 2 (male).

**Diagnosis.** This species can be characterized by the following combination of characters in the male imago: 1) body length of male 2.12–3.12 mm; 2) base of antennal flagellum not dilated; 3) forceps apically pointed, sharpening abruptly at the apex and slightly curved inwardly, basal half fused to lateral margins of styliger plate **(Fig. 8C)**; 4) styliger plate as wide as long with a deep medial emargination forming two long lateral lobes **(Fig. 8C)**; 5) penis with dorsolaterally protruding lobes and structured median part **(Figs. 8C–D)**.

**Male imago.** Body length: 2.12–3.12 mm; forewing length: 1.75–2.47 mm; foreleg length: 1.75–2.00 mm; hind leg length: 1.15–1.38 mm; cercus length: 5.3–8.0 mm.

**Ratios.** *Leg*: body size 1.14– 1.71 × the length of foreleg; foreleg 1.27–1.62 × the length of hind leg; first segment of the foretarsus length (micras): $2^{nd}$: $3^{rd}$: $4^{th}$: $5^{th}$ = 4.6–5.4:2.2–2.3:1.9–2.1:1.5–1.7. *Genitalia:* styliger plate length 0.87–1.05 × the width; forceps length 7.00–9.41 × the width ½ from base; distance between the extreme lateral points of the forceps bases to forceps length = 1.04–1.54.

**Coloration**. *Head*: blackish, except hind portion whitish dorsally; venter of head whitish **(Fig. 8B)**. *Antenna*: scape and pedicel whitish, flagellum hyaline.

*Thorax.* Prothorax blackish dorsally; prosternum whitish, with anterior and posterior margins blackish. Meso- and metanotum light brown, shaded with gray on carinae, and with blackish sutures; pleura light brown and sterna whitish shaded with black. Wing membrane hyaline, veins translucent except C, Sc and Rs blackish. Legs yellowish; all coxae, femora and base of foretibiae shaded with gray; median and subapical gray marks present on all femora, and middle and hind tibiae.

*Abdomen* **(Fig. 8A)**. Whitish shaded with black on terga I–II and VIII. Sterna translucent white. Pleura with trachea strongly pigmented.

*Genitalia* **(Fig. 8C–D)**. Membranes and sclerites brownish. Forceps brownish. Penis yellowish-white. Sternum IX with chitinous lateral structures strongly colored. Caudal filaments whitish translucent.

**Morphology.** *Antenna*: base of antennal flagellum not dilated.

*Thorax*. Lateral margins straight, not protruding; prosternal longitudinal ridges forming a triangle shaped structure, anteriorly closed and laterally concave **(Fig. 8B)**.

*Abdomen*. Lateral filaments present on terga VII–VIII, very short; finger-like process on tergum II lacking.

*Genitalia.* Styliger plate with posterior margin medially with a deep medial emargination forming two long lateral lobes (bolivian specimens with lateral lobes shorter) **(Fig. 8C)**; central sclerite elongated dome-shaped; apophyses very short, tape shaped (in some specimens nearly invisible); lateral sclerites tape shaped, with narrow base, more or less parallel, extended forward to the central sclerite; basolateral sclerites divergent, laying close to the lateral sclerites. Forceps apically pointed, sharpening abruptly at the apex and slightly curved inwardly; basal half fused to lateral margins of styliger plate **(Fig. 8C)**; main axis straight, without trichomae and denticles. Penis broader than styliger plate; ventral surface with a sculptured part composed by folds not sclerotized and tubercles; lobes of the penis separated on apical medial region, dorsally and laterally protruding **(Fig. 8D)**.

**Female imago and nymph.** Unknown.

**Distribution.** Brazil: Mato Grosso State. Colombia: Amazonas State. Bolivia: states of Santa Cruz and Beni.

**Material examined.** 45 male imagos (slides 633, 634 and 635), Colombia, Departamento Amazonas, Leticia, S04°05’41”, W69°59’01”, 11.ii.1999, 93m, Zuñiga, MC, Domínguez, E., Molineri, C. cols. (30 IBN and 15 MEUV); 09 male imagos (slides 619 and 620), Colombia, Departamento Amazonas, Leticia, Caño Km 11 ruta a Tarapaca, 28.i.1999, 93m, Zuñiga, MC, Domínguez, E., Molineri, C. cols. (IBN). One male imago (slide 623), Bolivia, Departamento Santa Cruz, La Florida, Parque Nacional Noel Kempff, Casa Guardaparque, S14°36’12.9”, W61°11’38.3”, 17.iv.2000, 180m, Domínguez, E. coll. (IBN); Four male imagos (light trap), Bolivia, Departamento Beni, Versailles, Rio Itenez, 05.v.2006, 147m, Domínguez, E., Molina, C. cols. (IBN); 13 male imagos (slide 627), Bolivia, Departamento Beni, Itenez, Bella

Vista, Rio Blanco, S13°14’01.41”, W63°’43’22.21”, 07.v.2006, 137m, Domínguez, E., Molina, C. cols (IBN).

**Comments.** *B. mendesi* was described from male imagos from Brazil (Malzacher 1998), posteriorly recorded from Colombia (Domínguez *et al.* 2006). Some specimens of Bolivia show the lateral lobes of the styliger plate very short. Here, this species is for the first time recorded from Bolivia.

***Brasilocaenis puthzi* Malzacher**

*Brasilocaenis puthzi* Malzacher, 1986: 86 (male, female, nymph, egg); Malzacher, 1998: 3.

**Diagnosis.** This species can be characterized by the following combination of characters. In the male imago: 1) body length of male 2.3 mm; 2) base of antennal flagellum not dilated; 3) male genitalia, with forceps pointed, enlarged near base and narrow toward the apex, apically curved inwardly, and attached to lateral margins of styliger plate; 4) styliger plate long with posterior margin rounded. In the nymph: 1) anterolateral margins of pronotum forming a very marked angle; 3) coxal processes of middle and hind legs long, sharply tapered, and with dentate borders; 4) hind margin of the ninth sternite rounded; 5) posterolateral projections on abdominal segments V–VIII long and pointed; 6) dorsal surface of operculate gills with short and strong microspine. Eggs: 1) two polar caps of coiled-rope type; 2) oval shaped with the length 1.2–1.5 × the width.

**Male imago.** Body length: 2.3 mm; forewing length: 1.9 mm; foreleg length: 1.3 mm; hind leg length: 0.9 mm; cercus broken off.

**Ratios.** *Leg*: body size 1.78 × the length of foreleg; foreleg 1.59 × the length of hind leg; first segment of the foretarsus length (micras): $2^{nd}$: $3^{rd}$: $4^{th}$: $5^{th}$ = 4.5:2.5:2.7:2.1. *Genitalia:* styliger plate length 1.18 ×; forceps length 8.6–12.8 × the width ½ from base; distance between the extreme lateral points of the forceps bases to forceps length = 1.06.

**Male and female imago, eggs and nymphs.** See formal description by Malzacher (1986).

**Distribution.** Brazil: states of Amazonas and Mato Grosso State.

**Material examined.** Paratype, male imago (genitalia and legs on slide mounted in Euparal), Brazil, Amazonas, Rio Itu 10 KM above acampamento, 13.ii.1962, Fittkaui coll. (Malzacher’s Private Collection).

**Comments.** We use only the paratype and some pictures of holotype to analyse this species. Due to the bad conservation state of this material and the damaged genitalia we used the bibliography to complement the analysis of characters.

***Brasilocaenis renata* Malzacher (Figure 9)**

*Brasilocaenis renata* Malzacher, 1986: 88 (male subimago, female, nymph, egg); Malzacher, 1998: 3.

**Diagnosis.** This species can be characterized by the following combination of characters. In the imagos: 1) body length of male 2.8 mm, of female 3.7–4.0 mm; 2) base of antennal flagellum not dilated; 3) with forceps pointed, smoothly curved on external margin and attached to lateral margins of styliger plate; 4) styliger plate as wide as long with posterior margin straight. In the nymph: 1) body length of male 2.4 mm, of female 3.4 mm; 2) anterolateral margins of pronotum rounded; 3) coxal processes of middle and hind legs long, sharply tapered, and with dentate borders (**Fig. 9C**); 4) tarsal claws I–II with six denticles basally; 5) hind margin of the ninth sternite truncate (**Fig. 9E**); 6) posterolateral projections on abdominal segments V–VIII long and pointed; 7) dorsal surface of operculate gill with short and strong microspines (**Fig. 9D**). Eggs: 1) two polar caps of coiled-rope type (**Fig. 9F**); 2) oval shaped with length 1.18–1.31 × maximum width.

**Male subimago and female.** See formal descriptions in Malzacher (1986).

**Egg.** Length: 117.5–127.5µm; width: 97.5–100µm. Coloration light yellow. Oval shaped with the length 1.18–1.31 × the width. Chorion finely punctured with surface slightly reticulate (**Fig. 9F**). Micropyle narrow and long without sperm guide. Two coiled-rope type polar caps with about ten terminal knobs and a more or less eccentric position in relation to main axis.

**Nymph (Fig. 9A).** Length of male: body, 2.4 mm; cercus, broken off. Length of female: body, 3.4 mm; cercus, 4.5 mm.

**Ratios.** *Mouthparts*: width of maxillary palp segment I 1.68–2.25 × the width of segment II; length of maxillary palp segment I 1.83–1.9 × the length of segment II; length of maxillary palp **s**egment I 1.05–1.08 × the length of segment III (**Fig. 9B**). *Leg*: length of forefemur 3.11–3.15 × maximum width.

**Coloration.** *Head*: light brown, occiput and mouthparts paler, a pair of medial grayish markings on posterior margin. *Antenna*: scape and pedicel pale yellow, flagellum hyaline.

*Thorax.* Nota brownish with blackish sutures (**Fig. 9A**). Pronotum with translucent whitish anterolateral corners, shaded with black on anterior margin, and with blackish paramedian dots and median elongated marks. Mesonotum with blackish macula anterior to wingbuds bases, and with a pair of submedian whitish spots at center. Legs yellowish-brown, blackish marks present on coxae, basal and apical part of femora, and medial part of tibiae. Thoracic sterna paler.

*Abdomen.* Terga yellowish, segments I–II with blackish band on area (**Fig. 9A**). Operculate gills translucent yellowish brown, shaded with black on basal area, and with many blackish spots scattered on dorsal surface (**Fig. 9D**). Abdominal sterna paler with only small grayish sublateral marks. Caudal filaments yellowish-white.

**Morphology.** *Head*: hind margin without setae. *Mouthparts*: lateral margins of labrum rounded, with shallow and smooth anteromedian emargination, with long and apical setae; mandibles with short and very thin setae dorsolaterally, lingua of hypopharynx with a median emargination on foremargin.

*Thorax*. Pronotum with anterolateral margins rounded and directed anteriorly; lateral margins denticulate. *Legs:* coxal projections relatively long and triangular (lateral margins converging toward apex) with denticulate margins (metacoxal process smaller than mesocoxal process) (**Fig. 9C**). Ventral and dorsal margins of fore and median femora with short simple setae; hind femur with long thin setae on dorsal margin, and short spine-like setae scattered on anterior surface; transversal row of spatulate setae of forefemora reduced, only with 2–3 setae. Foretarsus with one marginal row of simple pointed setae; middle with marginal and ventral rows of simple setae (ventral with serrated setae on apical third); and hind tarsus with two rows of setae: one with simple, pointed setae and other reduced to apical third with serrated setae. Claws: tarsal claws I–II with six denticles basally, tarsal claw III with around 30 minute denticles.

*Abdomen.* Tergum II with projection long and wide at base, upcurved (one species shows a small process on tergum I). Operculate gill with setae from the submarginal ventral row elongated; Y-ridge complete and well developed; dorsal surface smooth with short and blunt spines, and long, simple setae (**Fig. 9D**). Sternum IX with hind margin truncate, laterally with only simple setae.

**Distribution.** Brazil: states of Amazonas and Mato Grosso.

**Material examined.** One nymph, Brazil, Pará State, Tailândia, P25P15, 2°34'21.60"S 48°34'34.77"W, 47m, 22.xi.2012, Shimano, Y. coll. (GOELDI); 02 nymphs, Pará State,

Gurupá, Baía de Caxiuanã, D08RAP1, S01°41'22.49", W51°28'55.81", 26m, 14.xii.2012, Shimano, Y. coll. (GOELDI). One female imago (reared) (CEUFPE350165), Pará State, Mosqueiro, Córrego da ponte, próx. a PA-391, S01°08'49.4, W48°21'42.6", 9m, 26.x.2013, Lima, LRC, Shimano, Y, Cardozo, MN cols. (CEUFPE).

**Comments.** The identification was made using a male mature nymph with the genitalia pre-formed (**Fig. 9E)**. Therefore, a brief description of nymphs and eggs, from a new material was included, because this species was originally described in German and its original description has become inadequate for differentiating it from other congeners in South America.

***Brasilocaenis septentrionalis* Malzacher (Figure 10)**

*Brasilocaenis septentrionalis* Malzacher, 1990: 37 (male); Malzacher, 1998: 3.

**Diagnosis.** This species can be characterized by the following combination of characters in the male imago: 1) body length 2.6–2.7 mm; 2) base of antennal flagellum dilated; 3) forceps apically pointed, sharpening abruptly at the apex, slightly curved downward, and fused in the lateral margins of styliger plate, except apical part (**Fig. 10B**); 4) styliger plate as wide as long, with posterior margin slightly concave medially (**Fig. 10B**); 5) penis with dorsolaterally protruding lobes and sculptured median portion (**Fig. 10C**).

**Male imago.** Body length: 2.6–2.7 mm; forewing length: 2.0 mm; foreleg length: 1.85 mm; hind leg length: 1.0 mm; cercus length: 5.7–6.1 mm.

**Ratios.** *Leg*: body size 1.46 × the length of foreleg; foreleg 1.85x the length of hind leg; first segment of the foretarsus length (micras): $2^{nd}$: $3^{rd}$: $4^{th}$: $5^{th}$ = 3.9–4.0:1.7–2.3:0.9–1.8:1.0. *Genitalia:* styliger plate length 0.86 × its maximum width; forceps length 11.6 × the width ½ from base; distance between the extreme lateral points of the forceps bases to forceps length = 1.11.

**Coloration**. *Head*: whitish shaded with gray dorsally. Antenna: scape and pedicel whitish, flagellum hyaline basally shaded with black.

*Thorax.* Prothorax light brown shaded with black, laterally and medially with blackish spots; prosternum whitish. Meso- and metanotum brownish with darker sutures; pleura whitish; sterna lighter, medially whitish. Wing membrane hyaline, veins translucent except C, Sc and Rs blackish. Legs whitish; all femora and foretibiae shaded with black; median and subapical gray marks present on apex of femora, and on middle and hind tibiae.

*Abdomen*. Whitish shaded strongly with gray, with terga I–II and VII–X darker (**Fig. 11A**). Sterna translucent white. Pleura with trachea weakly pigmented.

*Genitalia*. Membranes and sclerites light brown (**Fig. 10C**). Forceps dark brown. Penis whitish with a subapical brownish sclerite. Sternum IX with chitinous lateral structures strongly colored. Caudal filaments whitish translucent.

**Morphology.** *Antenna*: Base of antennal flagellum dilated.

*Thorax*. Lateral margins straight, not protruding; prosternal longitudinal ridges forming a triangle shaped structure, anteriorly closed and laterally straight (**Fig. 10A**).

*Abdomen*. Lateral filaments very short on segments VII–VIII; finger-like process on tergum II lacking.

*Genitalia.* Styliger plate with posterior margin straight, slightly concave medially; central sclerite elongated dome-shaped; apophyses very short, tape shaped; lateral sclerites tape shaped, with narrow base, more or less curved, not extended forward to the central sclerite; basolateral sclerites divergent, laying close to the lateral sclerites. Forceps apically pointed, sharpening abruptly at the apex, slightly curved downward, and fused to the lateral margins of styliger plate, except apical portion; main axis straight, with trichomae and without denticles (**Fig. 10C**). Penis broader than styliger plate; ventral surface with a sculptured part composed by sclerotized folds and tubercles; lobes of the penis separated on apical medial region, dorsally and laterally protruding (**Fig. 10B**).

**Female imago and nymph.** Unknown.

**Previous distribution.** Brazil: State of Pará.

**Material examined.** One male imago (slide 673), Brazil, Pernambuco State, Tamandaré, Riacho Mamucabas P2, próximo à sede da Rebio Saltinho S8°43'52.7", W 35°10'24.8", 38m, 28.iii.2014 Lima, LRC coll. (IBN); one male imago (slide 672), same data except 17.iv.2014 (IBN).

**Comments.** Malzacher (1990) described *B. septentrionalis* from a single male from Northern Brazil. Its presence in Pernambuco State extends the distribution outside the Amazonian Basin. In the original description, the only specimen shows the posterior margin of styliger plate with slight medial emargination opposed to straight margin, concave medially in our specimens.

***Brasilocaenis amacayacu* sp. nov. (Figure 11)**

**Diagnosis.** This species can be characterized by the following combination of characters. Male imago: 1) body length of male 2.8 mm; 2) base of antennal flagellum not dilated; 3)

forceps pointed, not close to lateral margins of styliger plate, sharpening abruptly forming a long tip at the apex (**Fig. 11C**); 4) styliger plate as wide as long, with apex straight and two small lateral lobes posteriorly; 5) penis dorsally and laterally protruding, forming two large triangular projections (**Fig. 11C**).

**Male imago.** Body length: 2.8 mm; forewing length: 2.2 mm; foreleg length: 2.3 mm; hind leg length: 1.2 mm; cercus length: 7.4 mm.

**Ratios.** *Leg*: body size 1.22 × the length of foreleg; foreleg 1.88 × the length of hind leg; first segment of the foretarsus length (micras): $2^{nd}$: $3^{rd}$: $4^{th}$: $5^{th}$ = 5.3:2.6:2.1:1.6. *Genitalia*: styliger plate length 0.65× its width; forceps length 12.64 × their width ½ at from base; ratio of distance between the extreme lateral points of the forceps bases to forceps length = 1.65.

**Coloration**. *Head*: whitish shaded with black dorsally, with a pale line medially (**Fig. 11B**); venter of head yellowish. *Antenna:* scape and pedicel whitish, flagellum hyaline shaded with gray in the base.

*Thorax.* Prothorax whitish washed with black dorsally; prosternum whitish. Meso- and metanotum light brown; pleurae and sterna lighter (**Fig. 11B**). Wing membrane hyaline, veins translucent except C, Sc and Rs grayish. Legs whitish, except femora and apical part of tibiae brownish, shaded with black; middle and hind tibiae with blackish marks on apical part of femora and base of tibiae.

*Abdomen*. Whitish with blackish bands dorsally, paler laterally. Terga I–II and VII–IX darker laterally. Sterna whitish, except IX–X blackish. Pleura with trachea weakly pigmented.

*Genitalia*. Sternum IX with chitinous lateral structures strongly colored (**Fig. 11C**). Forceps dark brown. Penis whitish with a ventral V-shaped light brown sclerite. Caudal filaments whitish translucent.

**Morphology.** *Antenna*: Base of antennal flagellum not dilated.

*Thorax*. Lateral margins almost straight, not protruding; prosternal longitudinal ridges forming a broadly triangle-shaped structure, anteriorly truncate and laterally with concave margins (**Fig. 11A**).

*Abdomen*. Lateral filaments present on terga VIII–IX, very short; finger-like process on tergum II lacking.

*Genitalia.* Styliger plate rectangular with apex straight and two small lateral lobes posteriorly; central sclerite elongated dome shaped; apophyses short, triangular and pointed; lateral sclerites tape shaped, with broad base, more or less parallel, extended forward to the central sclerite; basolateral sclerites divergent, laying distant from the lateral sclerites (**Fig.**

**11C**). Forceps pointed, not close to lateral margins of styliger plate, sharpening abruptly forming a long tip at the apex. Ventral surface of penis with a V-shaped sclerotized groove; lobe of penis with an apical incision, dorsally and laterally protruding, forming two large triangular projections laterally (**Fig. 11C**).

**Female imago and nymph.** Unknown.

**Etymology**. The name, noun in apposition, alludes to the type locality (Parque Nacional Amacayacu) where the specimen were collected.

**Type material.** One male imago (slide 658), Colombia, Departamento Amazonas, Parque Nacional Amacayacu, Quebrada Mata-mata, S03°48'28", W70°15'21", 02.ii.1999, Zuñiga, MC, Molineri, C, Domínguez, E. cols. (MEUV).

**Comments.** The presence of large triangular projections in penis is an atypical character not observed in South America caenidae, but resembles some species of African *Caenis* (e.g. *C. jinjanoides* Malzacher, 2011 and *C. kohli* Malzacher, 2011). This shape can have arisen independently in these lineages of both continents.The new species is related to septentrionalis group proposed by Malzacher (1998) and composed by *B. septentrionalis* and *B. mendesi*. In this group, a well-developed dorsolateral portion of penis present and a sculptured portion on penis are the main synapomorphies. Moreover, there is a trend toward to an almost complete fusion of forceps to the lateral margins of styliger plate in this group. However, this trend was not observed in *B. amacayacu* in which the forceps are not attached to the margins of styliger plate.

***Brasilocaenis atawallpa* sp. n. (Figure 12)**

**Diagnosis.** This species can be characterized by the following combination of characters in the male imago: 1) body length of male 2.12–2.4 mm; 2) base of antennal flagellum slightly dilated basally **(Fig. 12B)**; 3) forceps apically pointed, sharpening abruptly, strongly curved downward at apex, fused in the lateral margins of styliger plate, except apical part **(Fig. 12D)**; 4) styliger plate as wide as long with a deep medial emargination forming two short lateral lobes **(Fig. 12D)**; 5) penis with dorsolaterally protruding lobes and a sculptured median part **(Fig. 12C)**.

**Male imago.** Body length: 2.12–2.40 mm; forewing length: 1.70–2.35 mm; foreleg length: 1.57–1.85 mm; hind leg length: 1.00–1.25 mm; cercus length: 4.6–6.0 mm.

**Ratios.** *Leg*: body size 1.32– 1.40 × the length of foreleg; foreleg 1.24–1.80x the length of hind leg; first segment of the foretarsus length (micras): $2^{nd}$: $3^{rd}$: $4^{th}$: $5^{th}$ = 4.8–6.0:2.5–2.9:2.4:1.3–1.5. *Genitalia:* styliger plate length 1.04–1.20 × the width; forceps length 7.80–8.57 × the width ½ from base; ratio distance between the extreme lateral points of the forceps bases to forceps length = 1.03–1.13.

**Coloration**. *Head*: whitish shaded with gray; venter of head whitish **(Fig. 12A)**. Antenna: scape and pedicel whitish, flagellum hyaline **(Fig. 12B)**.

*Thorax.* Prothorax brown shaded with grey, laterally with blackish line; prosternum whitish. Meso- and metanotum brownish with darker sutures; pleurae light brown; and sterna brown, medially whitish. Wing membrane hyaline, veins translucent except C, Sc and Rs blackish. Legs light brown; all femora and foretibiae shaded with black; median and subapical gray marks present on apex of femora, and on middle and hind tibiae.

*Abdomen* **(Fig. 12A)**. Whitish shaded with black, with medial dark spot on terga I–III. Terga I–II and VIII–X darker laterally. Sterna translucent white. Pleura with trachea weakly pigmented.

*Genitalia* **(Figs. 12C–D)**. Membranes and sclerites brownish. Forceps brownish. Penis yellowish-white with a medial transverse light brown sclerite. Sternum IX with chitinous lateral structures strongly colored. Caudal filaments whitish translucent.

**Morphology.** *Antenna*: base of antennal flagellum dilated **(Fig. 12B)**.

*Thorax*. Lateral margins straight, not protruding; prosternal longitudinal ridges forming a triangle shaped structure, anteriorly open and laterally straight **(Fig. 12B)**.

*Abdomen*. Lateral filaments present on terga VII–VIII, very shorts; finger-like process on tergum II lacking.

*Genitalia*. Styliger plate rectangular, with trichomae on surface; posterior margin elongated covering the penis with a deep emargination medially **(Fig. 12D)**; central sclerite dome-shaped; apophyses long, thin, tape shaped; lateral sclerites tape shaped, with base broad, more or less parallel, extended forward to the central sclerite; basolateral sclerites divergent, laying apart from the lateral sclerites. Forceps apically pointed, sharpening abruptly, strongly curved downward at apex, fused in the lateral margins of styliger plate, except apical part **(Fig. 12D)**; main axis enlarged at middle, with trichomae and without denticles. Penis broader than styliger plate; ventral surface with a sculptured part composed by sclerotized folds and tubercles **(Fig. 12C)**; lobes of the penis separated on apical medial region, dorsally and laterally protruding.

**Female imago and nymph.** Unknown.

**Etymology.** Named after *Atawallpa* (in Quechuan spelling), the last Sapa Inca (sovereign emperor) of the Tawantinsuyu (the Inca Empire) before the Spanish conquest.

**Type material. Holotype:** one male imago, Colombia**,** Departamento Amazonas, Parque Nacional Amacayacu, Queda Mata-mata, S03°48'28", W70°15'21", 02.ii.1999, Zuñiga, MC, Molineri, C, Domínguez, E. cols (MEUV). **Paratype:** same data as holotype, four males imagos; same data as preceding except 168 males (slides 659 and 660), 05.ii.1999 (50 CZNC, 70 MEUV and 48 IBN); three males imagos (light trap), Depto. Amazonas, Puerto Nariño, S03°43'53" W70°21'59", 04.II.1999, Zuñiga, MC, Molineri, C, Domínguez, E. cols. (IBN). 98 male imagos (slides 616, 617 and 618), Depto. Amazonas, Leticia, Caño Km 11 ruta a Tarapaca, 28.i.1999, 93m, Zuñiga, MC, Molineri, C, Domínguez, E. cols. (IBN).

**Comments**. The new species share similar characteristics with septentrionalis group proposed by Malzacher (1998), based on the following chracteristics: forceps fused together with lateral margin of styliger plate and a dorsolaterally protruding penis lobes in combination with a sculptured median part of the penis. In this group is more related to *B. septentrionalis* because of the forceps almost completely fused in the lateral margins of styliger plate (except apical part) and curved downward. However, *B. atawallpa* sp. n. can be distinguished by the presence of a posterior margin of styliger plate with a deep medial emargination forming two short lateral lobes and forceps with axis enlarged at middle (straight in *B. septentrionalis*).

***Brasilocaenis suassunai* sp. nov. (Figures 13–14)**

**Diagnosis.** This species can be characterized by the following combination of characters. In the imagos: 1) body length of male 2.1–3.3 mm, of female 3.5–4.3 mm; 2) base of antennal flagellum not dilated; 3) forceps pointed, close to lateral margins of styliger plate, sharpening abruptly forming a long tip at the apex (**Fig. 13C**); 4) styliger plate short with posterior margin straight (**Fig. 13C**); 5) penis dorsally and laterally protruding (**Fig. 13D**). In the nymph: 1) body length of male 2.8–3.3 mm, of female 3.8–4.5 mm; 2) anterolateral margins of pronotum rounded; 3) coxal processes of middle and hind legs well-developed and semicircular, with smooth margins (**Fig. 14C**); 4) tarsal claws I–II without denticles (**Fig. 14D**); 5) hind margin of the ninth sternite rounded (**Fig. 14F**); 6) posterolateral projections on abdominal segments V–VIII short and pointed; 7) dorsal surface of operculate gills without

short and strong microspines (**Fig. 14E**). Eggs: 1) two polar caps of coiled-rope type (**Fig. 13E**); 2) oval shaped length 1.00–1.16 × maximum width.

**Male imago.** Body length: 2.1–3.3 mm; forewing length: 2.0–2.4 mm; foreleg length: 1.8–2.2 mm; hind leg length: 1.1–1.3 mm; cercus length: 7.1–7.4 mm.

**Ratios.** *Leg*: body size 1.17–1.38 × the length of foreleg; foreleg 1.59–1.83 × the length of hind leg; first segment of the foretarsus length (micras): $2^{nd}$: $3^{rd}$: $4^{th}$: $5^{th}$ = 4.6–5.0:2.0–2.5:1.4–1.8:1.2–1.5. *Genitalia*: styliger plate length 0.54–0.62 × the width; forceps length 8.3–9.6 × the width ½ from base; distance between the extreme lateral points of the forceps bases to forceps length = 1.40–1.64.

**Coloration**. *Head*: whitish shaded with black dorsally, except a pale medial line; venter of head whitish. *Antenna:* scape and pedicel whitish shaded with gray, flagellum hyaline shaded with black in the base.

*Thorax.* Prothorax whitish shaded with black dorsally, with two spots near to the middle; prosternum whitish, shaded with black near base of legs. Meso- and metanotum light brown shaded with black on carinae; pleurae and sterna brown shaded with black. Wing membrane hyaline, veins translucent except C, Sc and Rs grayish. Legs whitish with all coxae, femora and foretibiae shaded with black; subapical blackish marks present on all femora, median marks on middle and hind tibiae.

*Abdomen*. Whitish with black bands dorsally, paler laterally (**Fig. 13A**). Terga I–II and VII–IX darker laterally. Sterna whitish, except IX–X blackish. Pleura with trachea strongly pigmented.

*Genitalia*. Sternum IX with chitinous lateral structures strongly colored. Forceps dark brown. Penis whitish with a ventral, semi-circular dark brown sclerite (**Fig. 13D**). Caudal filaments whitish translucent.

**Morphology.** *Antenna*: Base of antennal flagellum not dilated.

*Thorax*. Lateral margins straight, not protruding; prosternal longitudinal ridges forming a triangle shaped structure, anteriorly closed and laterally with straight margin (**Fig. 13B**).

*Abdomen*. Lateral filaments present on terga VII–VIII, very short; finger-like process on tergum II lacking.

*Genitalia.* Styliger plate rectangular with apex slightly subtriangular (some specimens with small tubercles medially on posterior margin); central sclerite elongated dome shaped; apophyses short, triangular and pointed; lateral sclerites with broad base, more or less curved,

not extended forward to the central sclerite; basolateral sclerites divergent, laying distant from the lateral sclerites (**Fig. 13C**). Forceps pointed, close to lateral margins of styliger plate, sharpening abruptly forming a long tip at the apex, main axis with small denticles on inner margin of ventral surface (**Fig. 13C**). Penis with an apical incision, dorsally and laterally protruding (or swollen some specimens) with a sculptured part composed by sclerotized folds and tubercles (**Fig. 13C–D**).

**Female imago**. Body length, 3.5–4.3 mm; forewing length, 2.4–2.6 mm; foreleg length, 1.4–1.8 mm; cercus length, 2.1–2.6 mm. Same coloration as in male except mesokatepisternum dark brown, rest of mesopleural sclerites paler. Lateral filaments present on terga VII–IX, very short. Abdominal sternum 9 rounded, not projected.

**Egg.** Length: 95–105µm; width: 90–95µm. Coloration light yellow. Oval shaped with length 1.00–1.16 × maximum width. Chorion finely punctured. Micropyle funnel-shaped, narrow and long, without sperm guide (**Fig. 13F**). Two coiled-rope type polar caps with about ten terminal knobs and a more or less eccentric position in relation to the main axis **Fig. 13E**).

**Nymph (Fig. 14A).** Length of male: body, 2.8–3.3 mm; cercus, 2.5–2.9 mm. Length of female: body, 3.8–4.5 mm; cercus, 3.7–4.2 mm.

**Ratios.** *Mouthparts*: width of maxillary palp segment I 1.6–2.0 × the width of segment II; length of maxillary palp segment I 1.33–1.81 × the length of segment II; length of maxillary palp **s**egment I 0.83–1.04 × the length of segment III (**Fig. 14B**). *Leg*: length of forefemur 2.83–3.64 × its maximum width.

**Coloration.** *Head*: light brown, occiput and mouthparts paler, grayish markings forming an irregular net on the occiput, also shaded gray behind eyes. *Antenna*: scape and pedicel light brown, flagellum hyaline.

*Thorax.* Nota brownish with black sutures. Pronotum with translucent anterolateral corners, shaded with black on anterior margin and lateral zones; with blackish paramedian dots and median elongated marks. Mesonotum with blackish macula anterior to wingbuds bases, with a pair of medial blackish lines. Legs yellowish, with blackish marks present on subapical zones of femora and basally on tibiae. Thoracic sterna paler.

*Abdomen.* Terga brownish, segments I–II with grayish band on medial area, segments VIII–X, paler medial and darker laterally. Operculate gills translucent yellowish, almost completely shaded with black and apically with 3–4 blackish spots. Abdominal sterna much paler with only small grayish sublateral marks. Caudal filaments yellowish.

**Morphology.** *Head*: hind margin of the head without setae. *Mouthparts*: lateral margins of labrum rounded, with small submedian protuberances on anteromedian emargination, and long apical setae; mandibles with a dorso-lateral row of long setae, lingua of hypopharynx with fore margin almost straight.

*Thorax*. Pronotum with anterolateral margins rounded and directed anteriorly; lateral margins denticulate. *Legs:* coxal projections well-developed and semicircular, with smooth margins (**Fig. 14C**). Ventral and dorsal margins of fore and middle femora with short simple setae; hind femur with long robust setae on dorsal margin; transversal row of spatulate setae of forefemora reduced, only with 3–4 setae. Foretarsus with one marginal row of simple pointed setae, middle with one marginal row of serrated setae, and hind tarsus with 2 rows of setae: one simple and the other serrated. Claws: tarsal claws I and II without denticles (**Fig. 14D**), and tarsal claw III with ca. 20 minute denticles.

*Abdomen.* Tergum II with projection small, wide at base and posteriorly curved. Operculate gills with setae from the submarginal ventral row elongated; Y-ridge complete and well-developed; dorsal surface smooth with long, simple setae (**Fig. 14E**). Posterolateral projections on abdominal segments V–VIII short and pointed. Sternum IX with hind margin rounded, laterally denticulate and posteriorly with long simple setae (**Fig. 14F**).

**Etymology.** This species is named in honor of writer Ariano Vilar Suassuna, a staunch defender of the culture of the Northeastern of Brazil, region where the new species was found.

**Material examined. Holotype:** one male imago (reared), Brazil, Bahia State, Barreiras, Vau da Boa Esperança, Córrego de Primeira Ordem Ponto 2, S12°12'21.40", W45°13'27.28", 16.iii.2014, 556m, Lima, LRC, Knapp, W. colls. (CZNC)**. Paratype:** one nymph, Brazil, Bahia State, Barreiras, Vau da Boa Esperança, Córrego de Primeira Ordem Ponto 1, S12°12'18.26", W45°13'30.09", 14.xi.2013, 548m, Knapp, W. coll (CEUFPE); same data as preceding except, 14 male imagos (11 males and three females) (slide CEUFPE350191) and three nymphs, 16.iii.2014 (CZNC). Four male imagos (slide CEUFPE350194), Brazil, Ceará State, Ubajara, Serra do Ibiapaba, Parque Nacional de Ubajara, S03°50'40.8", W40°54'35", 23.x.2011, 858m, Silva-Neto, A; Xavier, M.; Lima, E. colls. (CEUFPE). Two male imagos (slide CEUFPE350195), Brazil, Paraíba State, Mamanguape, Rebio Guaribas, Rio Cabeça de Boi, 29.vii.2007. One nymph (slide CEUFPE350113), Brazil, Pernambuco State, Itamaracá, Córrego de 1° ordem, estrada para a Lagoa Azul, S7°46'22.3", W34°51'4.6", 21m, 24.v.2009, Lima, LRC coll. (CEUFPE). Six nymphs, Brazil, Pernambuco State, Camaragibe, Rio Besouro, Estrada do Borralho, KM 07, PE-027, S7°59'06.4", W35°00'13.9", 16.vii.2009,

66m, Lima, LRC. coll. (CEUFPE). Seven nymphs (slide CEUFPE350111) and 18 females imagos, Brazil, Pernambuco State, Recife, caminho para o Açude do Prata, Parque Dois Irmãos, S8°00'28.5", W34°56'54.3", 22.viii.2009, 31m, Lima, LRC coll. (CEUFPE); two males and three females imagos (reared) (slide CEUFPE350093), same data as preceding except 12.ix.2009 (CEUFPE); two nymphs and one male imago (reared), same data as preceding except 17.iii.2010 (CZNC). One nymph and three female imagos (reared), Brazil, Pernambuco State, Recife, riacho da UFRPE, S08°00'57.4", W34°56'46.4", 17.iii.2010, 48m, Lima, LRC coll. (CEUFPE); three nymphs and six female imagos (reared), same data as preceding except 06.iv.2010 (CEUFPE); three male imagos (slides CEUFPE350131#001 and CEUFPE350131#002), same data as preceding except 18.xii.2012 (CZNC); one nymph and four imagos (one male and three females) same data except 22.iii.2013 (CZNC); one nymph, four males and 11 females (reared) (slides p.674 and p.675) same data except 14.ii.2014 (IBN). Five nymphs, Brazil, Pernambuco State, Igarassu, Riacho Jacocá, Refúgio Charles Darwin, S07°48'57.5", W34°57'19.1", 22.ii.2010, 37m, Lima, LRC coll. (CEUFPE). One nymph, Pernambuco State, Goiana, Açude Santa Tereza, S07°35'33.2", W34°59'32.8", 12.xi.2011, 35m, Lima, LRC, Souza, WRM cols. (CEUFPE). Two nymphs, Brazil, Pernambuco State, São João, Riacho da Barragem Inhumas, S08°58'17.3", W36°21'48.5", 13.ii.2012, 686m, Lima, LRC, Souza, WRM cols. Six males and two females (slides CEUFPE350136 #001 and CEUFPE350136 #002), Brazil, Pernambuco State, Amaraji, Barragem Jaguarana, S08°21'01.6", W35°24'27.7", 05.xii.2012, 265m, Lima, LRC coll. (CEUFPE). One female imago (reared), Pernambuco State, Brazil, Tamandaré, Córrego da sede, Rebio Saltinho, S08°43'48.3", W35°10'35.3", 21.ix.2009, 46m, Lima, LRC coll. (CEUFPE); one nymph, same data except 22.ix.2009 (CEUFPE); one female imago, same data except 08.viii.2010 (CEUFPE). One female imago, Brazil, Pernambuco State, Rio Formoso, Fazenda São Manuel, S08°43'21.5", W35°10'12.6", 04.v.2010, 50m, Lima, LRC coll. (CEUFPE). One female imago (reared), Brazil, Pernambuco State, Rio Formoso, Riacho Gameleira, Rebio Saltinho, S08°43'12.3", W35°10'32.9", 22.xi.2012, 47m, Lima LRC, Souza, WRM cols. (CEUFPE). One female imago (reared), Brazil, Tamandaré, Rio Mamucabas Ponto 1, próximo ao açude, S08°43'21.6", W35°11'14.0", 27.iii.2014, 90m, Lima, LRC coll. (CEUFPE). 55 males and 15 females (light trap), Brazil, Pernambuco State, Tamandaré, Riacho Mamucabas Ponto 2, próximo à sede da Rebio Saltinho, S08°43'52.7", W35°10'24.8", 28.iii.2014, 38m, Lima, LRC coll. (IBN); 92 male and 63 females (slide CEUFPE350188), same data except 17.iv.2014 (CZNC).

**Comments**. The adult stage of *B. suassunai* sp. nov. shares some characters with septentrionalis group: styliger plate with basolateral sclerites divergent, laying distant from the lateral sclerites, penis dorsally and laterally protruding with a ventral, and a sculptured part composed by sclerotized folds and tubercles. However, as in as *B. amacayacu* sp. nov., the male imago do not have a typical genitalia of the genus, and the forceps are not attached or fused to styliger plate. Besides, the styliger plate is the most short than other species of *Brasilocaenis* (length almost half width).

The nymphal stage of the new species shows characteristics generally associated with those of genus *Caenis* (Domínguez et al. 2006; Malzacher, 1986; Puthz, 1975): absence of pointed microspines on dorsal surface of opercular gill II; coxal projections semicircular; and posterolateral abdominal projections short. The new species can be distinguished in the nymph, from the other species of South American *Caenis* by: body length of male 2.8–3.3 mm and female 3.8–4.5 mm; tarsal claws I–II without denticles; and sternum IX with hind margin rounded, laterally denticulate and posteriorly with long simple setae.

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**Table 1.** Measurements of the adults, eggs and nymphs obtained from the species analyzed and abbreviations to Table 2.

| Measurements | Abbreviation |
|---|---|
| Male imago, body length | BL |
| Male imago, foreleg | FL |
| Male imago, hind leg | HL |
| Male imago, distance between the extreme lateral points of the forceps bases | ELP |
| Male imago, forceps length | FCL |
| Male imago, forceps width ½ from base | FCW |
| Male imago, styliger plate length | SL |
| Male imago, styliger plate width | SW |
| Eggs, length | EL |
| Eggs, width | EW |
| Nymph, length of femur | LF |
| Nymph, width of femur | WF |
| Nymph, maxillary palp I length | MP1 |
| Nymph, maxillary palp II length | MP2 |
| Nymph, maxillary palp III length | MP3 |
| Nymph, maxillary palp I width | MPIW |
| Nymph, maxillary palp II width | MPIIW |

**Table 2.** Quantity of specimens measured by species in the study. The symbol [?] indicates unobservable data.

| Species | BL | FL | HL | ELP | FCL | FCW | SL | SW | EL* | EW* | LF | WF | MP1 | MP2 | MP3 | MPIW | MPIIW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***A. peruanicus**** | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 10 | 10 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| ***B. intermedia*** | ? | ? | ? | ? | ? | ? | ? | ? | 10 | 10 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| ***B. irmleri*** | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 10 | 10 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| ***B. mendesi*** | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| ***B. puthzi*** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 10 | 10 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| ***B. renata*** | ? | ? | ? | ? | ? | ? | ? | ? | 10 | 10 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| ***B. septentrionalis*** | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 10 | 10 | ? | ? | ? | ? | ? | ? | ? |
| ***B. atawallpa*** | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| ***B. suassunai*** | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 10 | 10 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| ***B. amacayacu*** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| ***C. burmeisteri*** | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 10 | 10 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| ***C. chamie*** | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| ***C. cuniana*** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 10 | 10 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| ***C. fittkaui*** | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | ? | ? | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| ***C. horaria*** | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 10 | 10 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| ***C. tarapoto*** | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| ***C. teipunensis*** | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 10 | 10 | ? | ? | ? | ? | ? | ? | ? |
| ***C. ludicra*** | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 10 | 10 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| ***C. gaucha*** | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 10 | 10 | ? | ? | ? | ? | ? | ? | ? |

*****Number of eggs measured.

**Table 3.** Data matrix of continuous characters and state assignation for each species used in the cladistic analysis. The symbol [-] indicates inapplicable data, the [?] indicates unobservable data, and the [*] indicates the taxon used to root the trees.

| | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** |
|---|---|---|---|---|---|---|---|---|---|---|
| ***A. peruanicus**** | 1.652-1.982 | 0.709-0.840 | 0.333 | 7.800-9.500 | 3.000-3.300 | 5.454 | 0.496 | ? | 1.500 | 0.000 |
| ***B. intermedia*** | ? | ? | ? | ? | ? | ? | 2.020 | 1.100 | 2.280 | ? |
| ***B. irmleri*** | ? | 0.950-1.210 | 1.075-1.281 | 17.50-18.80 | 1.200-1.500 | 2.800-3.500 | 1.280-1.360 | | 1.800-2.250 | 4.000-5.000 |
| ***B. mendesi*** | 1.430-2.000 | 1.040-1.540 | 0.868-1.046 | 7.000-9.410 | ? | ? | ? | ? | ? | ? |
| ***B. puthzi*** | 1.270-1.620 | 1.060 | 1.181 | 12.80 | ? | 2.8 | 1.833 | 1.064 | 1.625 | 4.000-5.000 |
| ***B. renata*** | 1.592 | 1.350 | 0.951 | 8.000 | 1.180-1.310 | 2.941-3.150 | 1.830-1.916 | 1.050-1.1500 | 2.112-2.500 | 6.000-7.000 |
| ***B. septentrionalis*** | ? | 1.110 | 0.862 | 11.60 | ? | ? | ? | ? | ? | ? |
| ***B. atawallpa*** | 1.850 | 1.030-1.130 | 1.037-1.204 | 7.800-8.571 | ? | ? | ? | ? | ? | ? |
| ***B. suassunai*** | 1.240-1.800 | 1.410-1.640 | 0.535-0.615 | 8.280-9.600 | 1.000-1.160 | 2.830-3.640 | 1.330-1.830 | 0.830-1.040 | 1.600-2.000 | 0.000 |
| ***B. amacayacu*** | 1.590-1.830 | 1.650 | 0.648 | 12.64 | ? | ? | ? | ? | ? | ? |
| ***C. burmeisteri*** | 1.880 | 1.692-2.100 | 0.441-0.493 | 4.750-7.428 | 1.170-1.370 | 3.000-3.176 | 1.925-2.250 | 0.938-0.962 | 1.66-1.800 | 6.000-7.000 |
| ***C. chamie*** | 1.416-1.634 | 1.333-1.727 | 0.577-0.702 | 5.000-7.500 | 1.440-1.630 | 2.337-2.564 | 1.528-1.744 | 0.952-1.000 | 1.828-2.148 | 0.000 |
| ***C. cuniana*** | 1.327-1.464 | 1.700 | 0.523 | 6.900 | 1.120-1.320 | 2.760-3.550 | 1.420-1.780 | 0.900-1.020 | 1.680-2.500 | 7.000-9.000 |
| ***C. fittkaui*** | 1.330 | 0.990-1.130 | 0.7808-0.7916 | 9.250-10.90 | 1.144 | 2.660-2.850 | 1.650-2.000 | 0.920-1.000 | 1.800-2.150 | 4.000 |
| ***C. horaria*** | 1.720-1.940 | 1.022-1.388 | 0.219 | 7.000-9.000 | 1.585-1.634 | 2.500-2.760 | 1.600-1.647 | 1.037-1.306 | 1.687-1.833 | 5.000-6.000 |
| ***C. tarapoto*** | 1.930-1.938 | 2.121-2.33 | 0.433-0.536 | 6.000-6.660 | ? | ? | ? | ? | ? | ? |
| ***C. teipunensis*** | 1.312-1.551 | 1.405-1.507 | 0.453-0.473 | 8.710-9.250 | 1.240-1.290 | ? | ? | ? | ? | ? |
| ***C. ludicra*** | 1.458-1.608 | 2.032-2.193 | 2.222-2.511 | 0.381-0.420 | 3.909-5.000 | 1.650-1.790 | 2.310-2.607 | 1.675-1.812 | 1.033-1.203 | 1.769-1.923 |
| ***C. gaucha*** | 1.312-1.551 | 1.705-2.157 | 1.694-1.857 | 0.475-0.630 | 4.375-6.000 | 1.660-1.920 | ? | ? | ? | ? |

**Table 4.** Data matrix of discrete characters and state assignation for each species used in the cladistic analysis. The symbol [-] indicates inapplicable data, the [?] indicates unobservable data, and the [*] indicates the taxon used to root the trees.

| | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** | **25** | **26** | **27** | **28** | **29** | **30** | **31** | **32** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***A. peruanicus**** | 1 | 0 | 0 | 1 | 2 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | ? | 1 | 0 | 0 | ? | 2 | 1 | 0 |
| ***B. intermedia*** | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | 0 | 1 | 2 | 2 | 0 | 0 | 1 | 1 | 1 | ? | ? |
| ***B. irmleri*** | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 |
| ***B. mendesi*** | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 2 | 1 | 0 | 1 | 0 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| ***B. puthzi*** | 0 | 1 | 2 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 2 | 1 | 0 | 1 | 1 | 1 | 2 | 0 | 1 |
| ***B. renata*** | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | ? | ? | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 2 | 0 | 1 |
| ***B. septentrionalis*** | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 1 | 1 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| ***B. atawallpa*** | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 2 | 1 | 0 | 1 | 1 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| ***B. suassunai*** | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 2 | 0 | 0 | 1 | 0 | 1 | 0 | 1 |
| ***B. amacayacu*** | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| ***C. burmeisteri*** | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 |
| ***C. chamie*** | 0 | 1 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 2 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| ***C. cuniana*** | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 |
| ***C. fittkaui*** | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 1 |
| ***C. horaria*** | 0 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 2 | 0 | 0 | 1 | 0 | 2 | 0 | 1 |
| ***C. tarapoto*** | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| ***C. teipunensis*** | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | ? | ? | ? | ? | ? | ? | ? | ? | ? | 0 | 2 |
| ***C. ludicra*** | 0 | 0 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 2 | 0 | 0 | 1 | 0 | 2 | 0 | 1 |
| ***C. gaucha*** | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | ? | ? | ? | ? | ? | ? | ? | ? | ? | 0 | 1 |

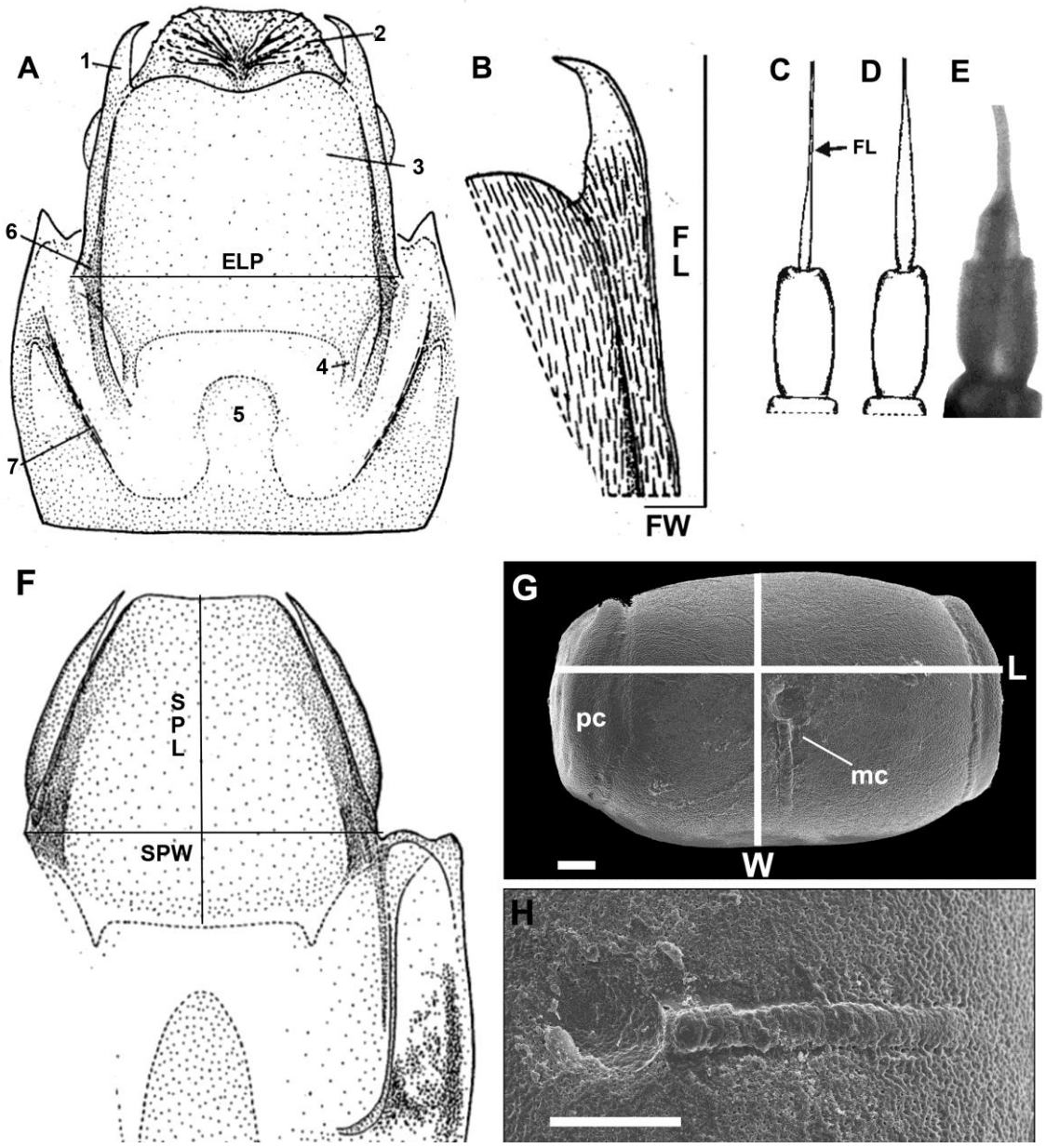



**Figure 1.** Main structures analyzed in adults of *Brasilocaenis*. *B. septentrionalis:* A) genitalia, ventral view; B) detail of forceps. C–E) Male imago antennae: C, *B. irmleri* (modified from *Malzacher*, 1986); D, *B. puthzi* (modified from *Malzacher*, 1986); E, *C. horaria*. H) genitalia, ventral view (modified from *Malzacher*, 1998). Eggs of *Caenis ludicra*: F) general view; G) detail of micropyle. Scale bars: 10 µm. Explications: *1*= forceps, *2*=penis, *3*= styliger plate, *4*=apophyses, *5*= central slcerite, *6*=lateral sclerite, *7*=basolateral sclerite, *ELP*=extreme lateral points between the forceps, *FL*=flagellum of antenna, *W*=width, *L*=length, *SPL*=styliger plate length, *SPW*=styliger plate width, *pc*=polar cap, *mc*=micropyle.

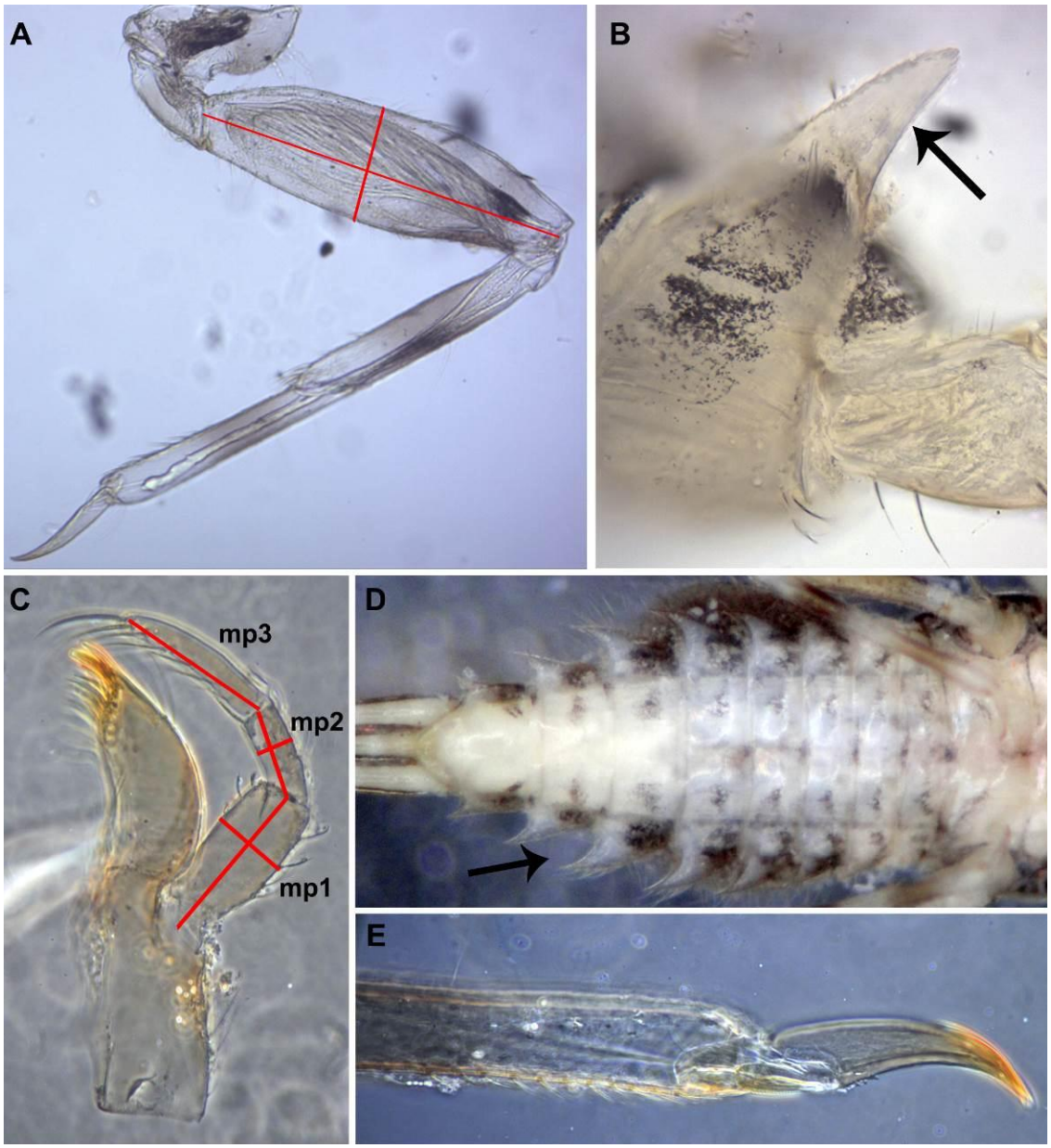



**Figure 2.** Main structures analyzed in the nymphs of *Brasilocaenis*. A) foreleg (red lines used to measure the length/width of forefemur); B) Metacoxal process; C) maxilla (red lines used to measure the maxillay palp segments); D) abdominal posterolateral projections, ventral view; E) foretarsal claw. *Abreviations*: *mp1*=maxillary palp 1, *mp2*=maxillay palp 2, *mp3*=maxillary palp 3.

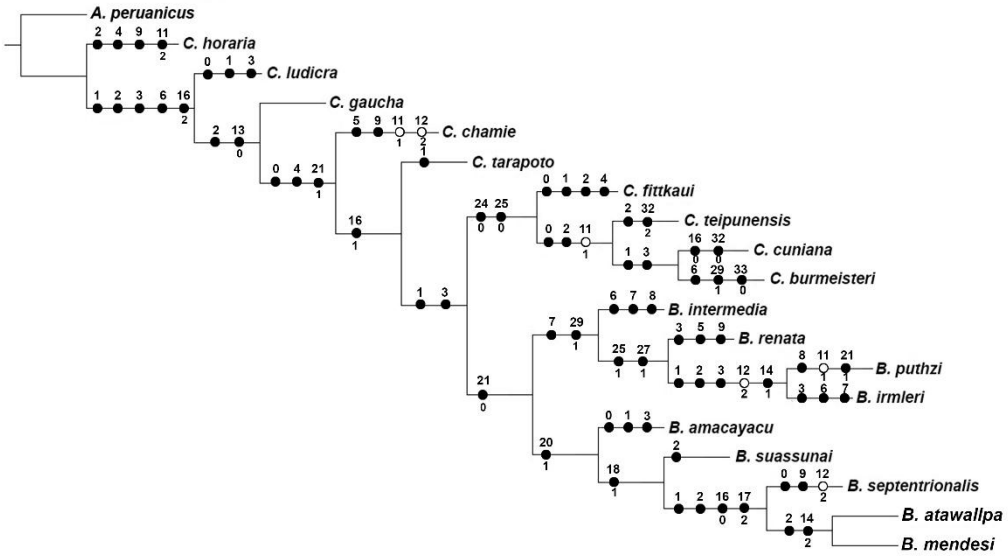



**Figure 3.** Phylogenetic hypothesis (single shortest tree with k = 3 with complete dataset) and supporting synapomorphies.

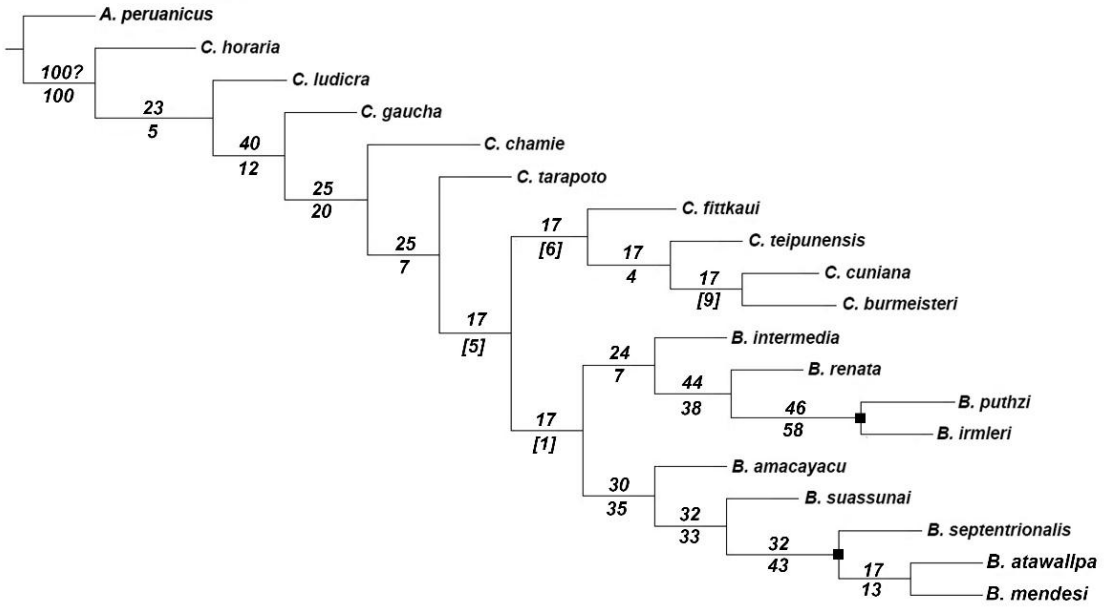



**Figure 4.** Group support and vicariant events. Numbers above and below the nodes indicate frequency difference values and relative Bremer support, respectively. Black squares indicate disjunct sister pairs.

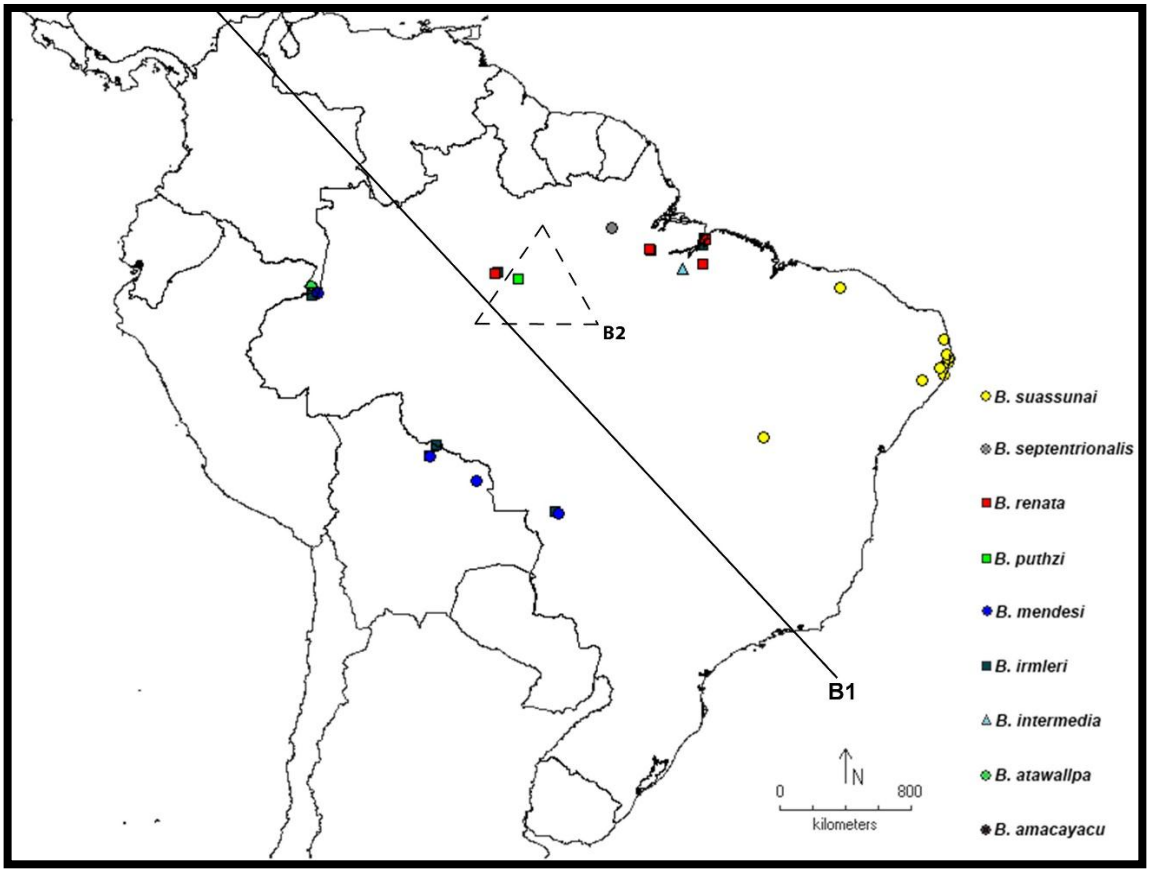



**Figure 5.** Distribution map with species records and the possible barriers (or vicariant events) found in the ingroup.

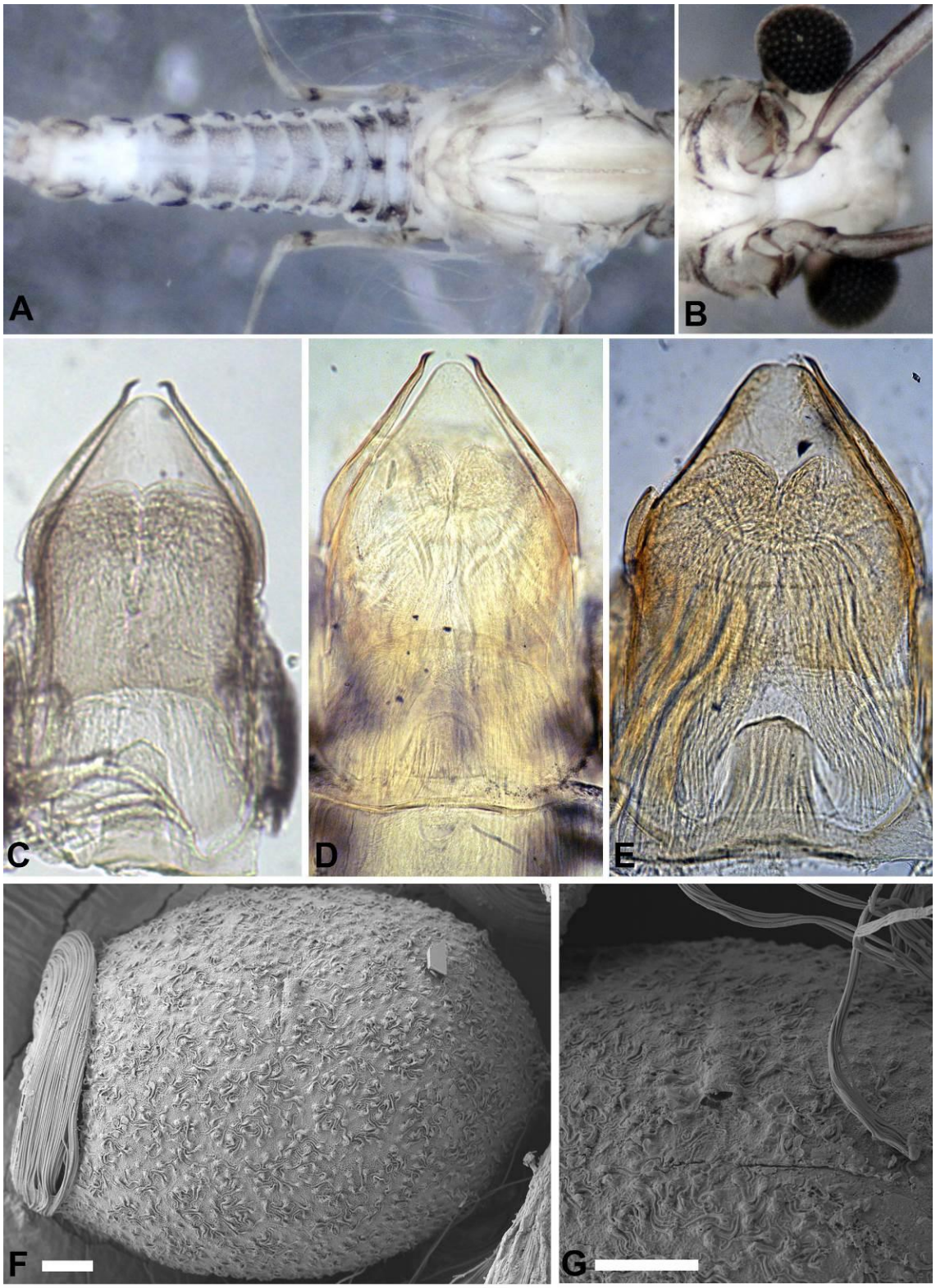



**Figure 6.** *Brasilocaenis irmleri* Malzacher, 1986. Male imago: A) abdomen and thorax, dorsal view; C–E) intraspecific variation of styliger plate. Eggs: F) detail of chorion; G) detail of micropyle. Scale bar: 10µm.

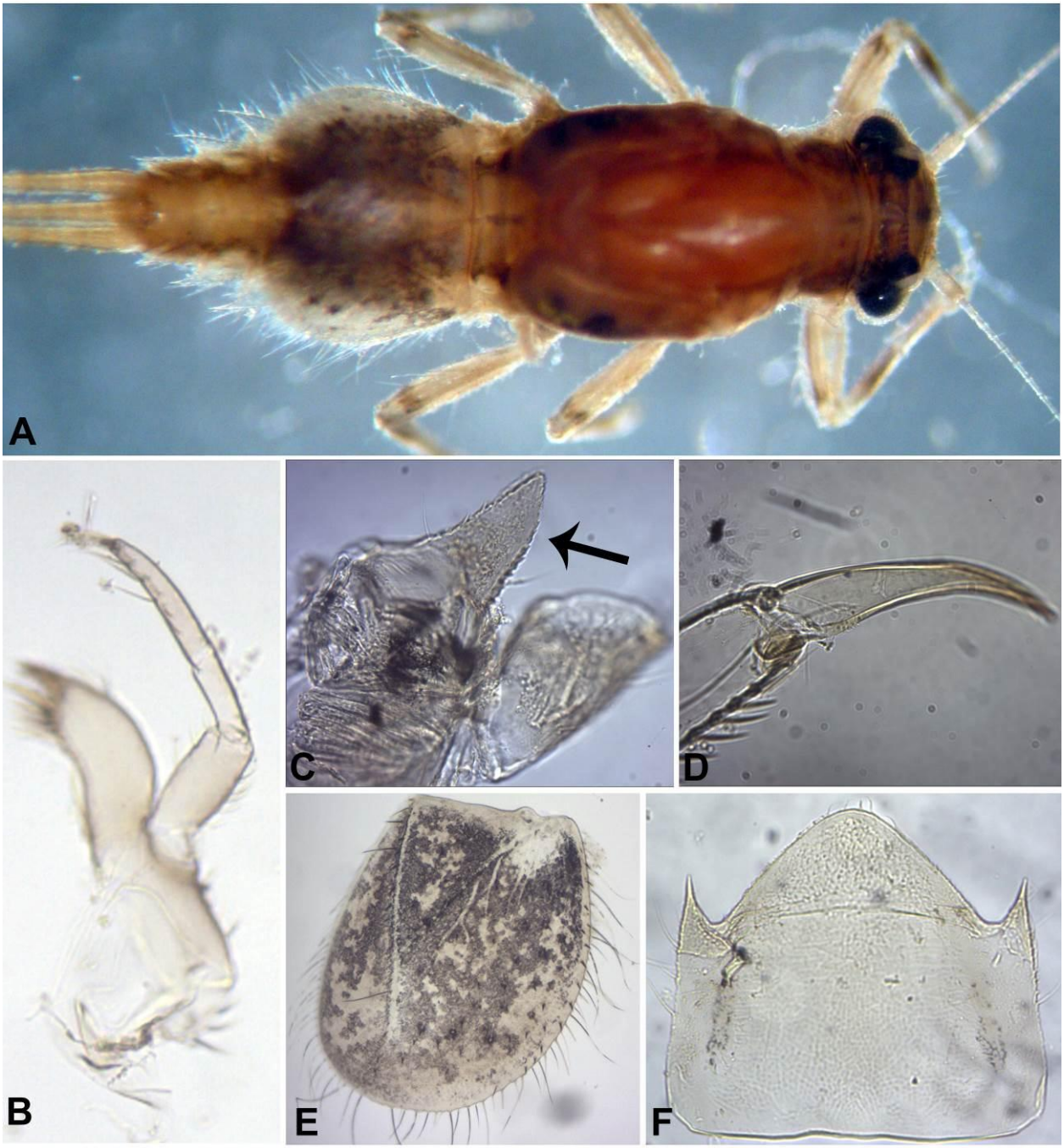



**Figure 7.** *Brasilocaenis irmleri* Malzacher, 1986. Nymph: A) habitus; B) maxilla; C) metacoxal process; D) foretarsal claw; E) operculate gill; F) abdominal sternum IX.

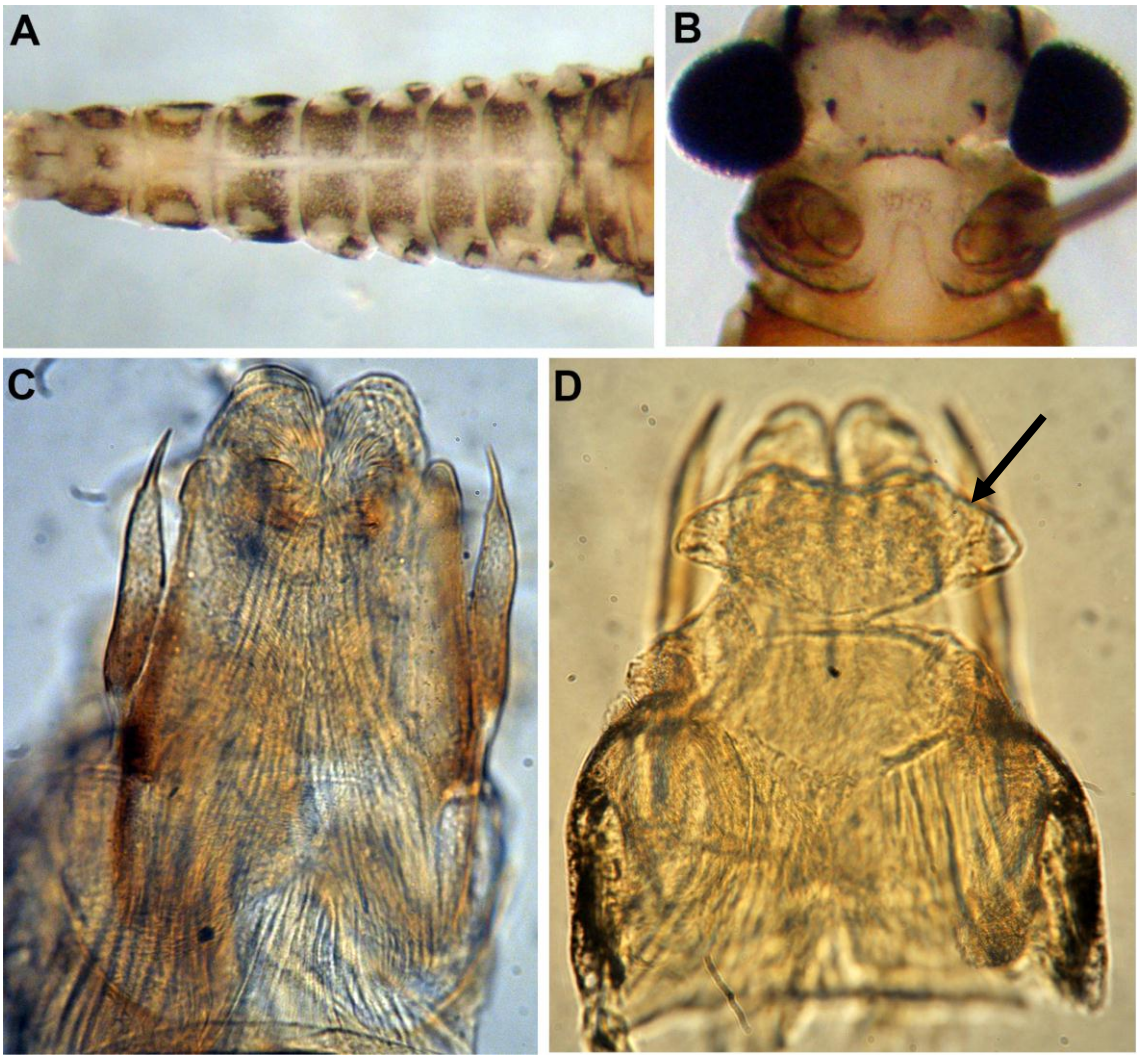



**Figure 8.** *Brasilocaenis mendesi* Malzacher, 1998. Male imago: A) abdominal color pattern; B) prosternum and and prosternal longitudinal ridges; C) genitalia in ventral view; D) genitalia, dorsal view (black arrow indicating the well-developed dorsolateral portion of penis).

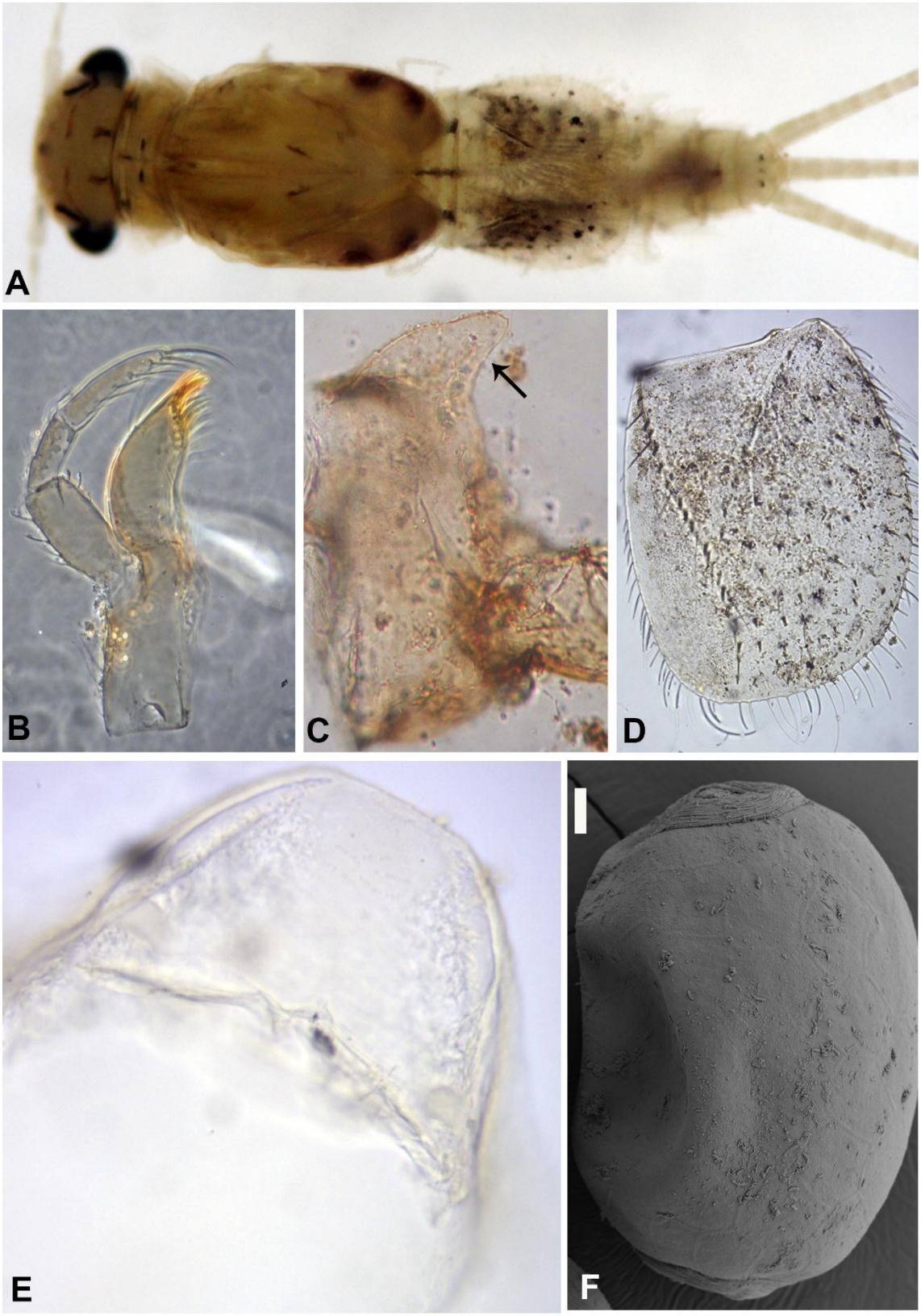



**Figure 9.** *Brasilocaenis renata* Malzacher, 1986. Nymph: A) habitus; B) maxila; C) metacoxal process; D) operculate gill; E) sternum IX (with detail of pre-formed genitalia). Egg: F) chorion and polar cap. Scale bar: 10µm.

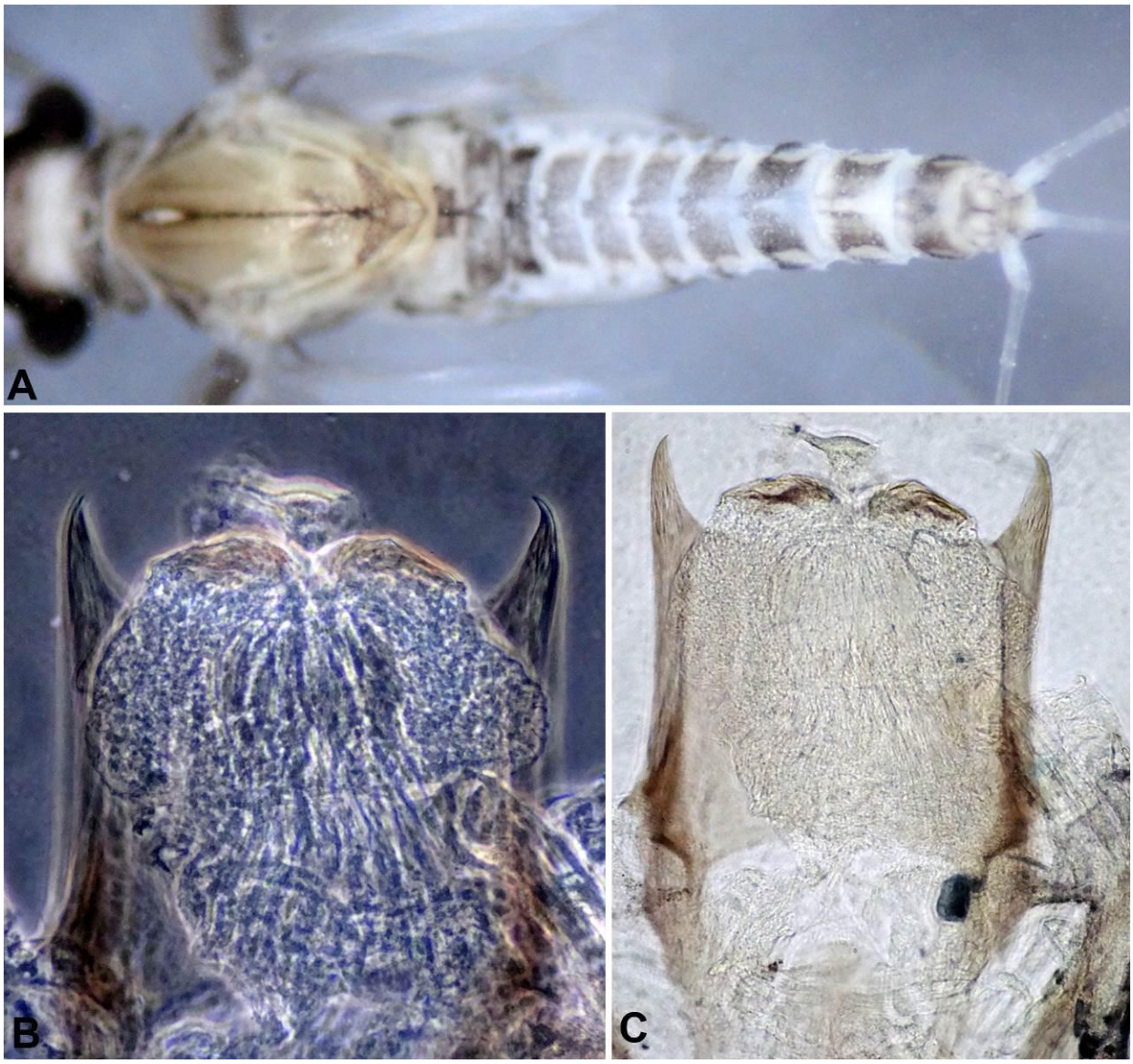



**Figure 10.** *Brasilocaenis septentrionalis* Malzacher, 1990. Male imago: A) abdomen and thorax, dorsal view; B) genitalia, dorsal view; C) genitalia, ventral view.

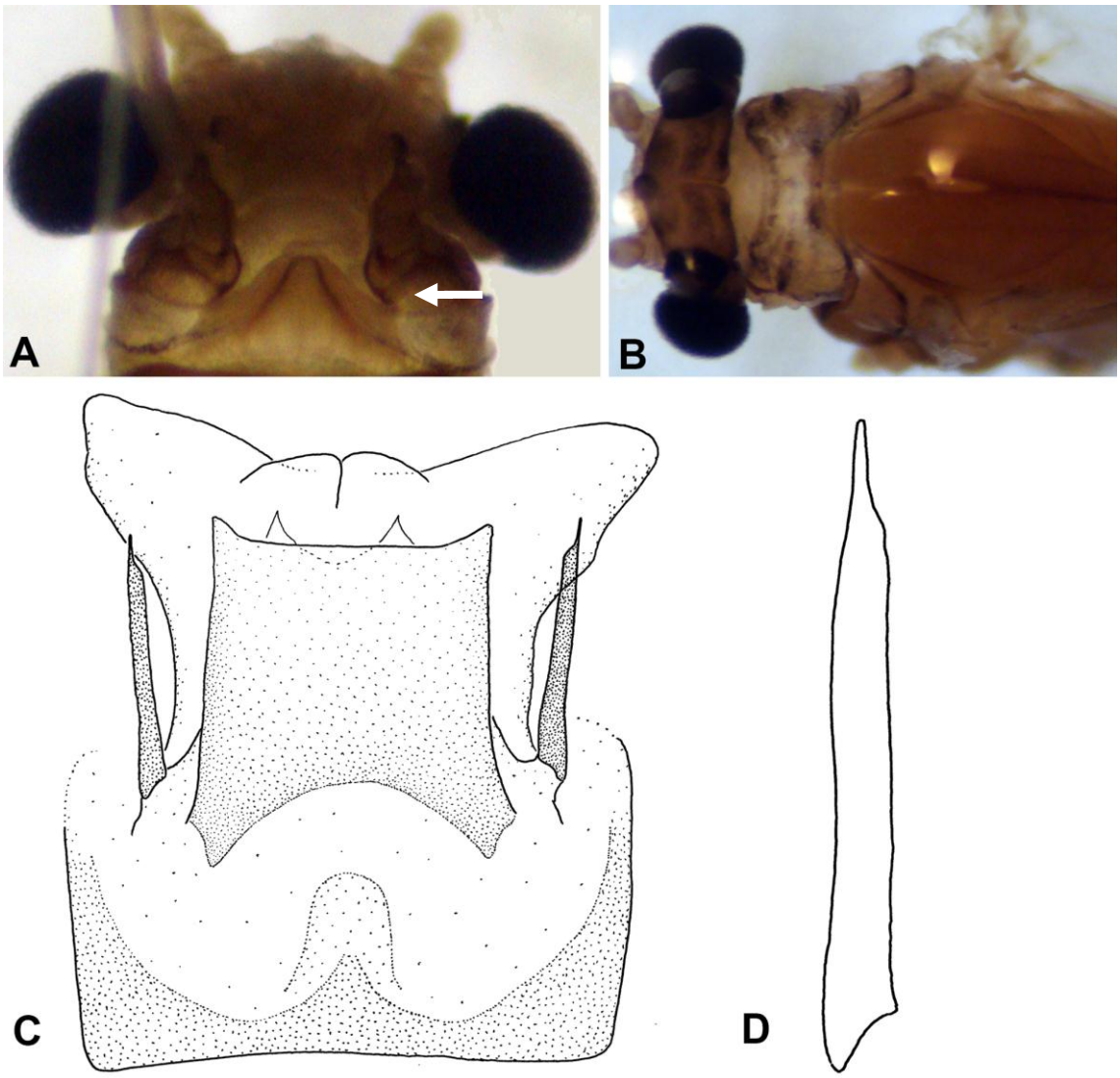



**Figure 11.** *Brasilocaenis amacayacu* sp. nov. Male imago: A) prosternum and prosternal longitudinal ridges (white arrow); B) head and thorax, dorsal view; C) genitalia, ventral view; D) detail of forceps.

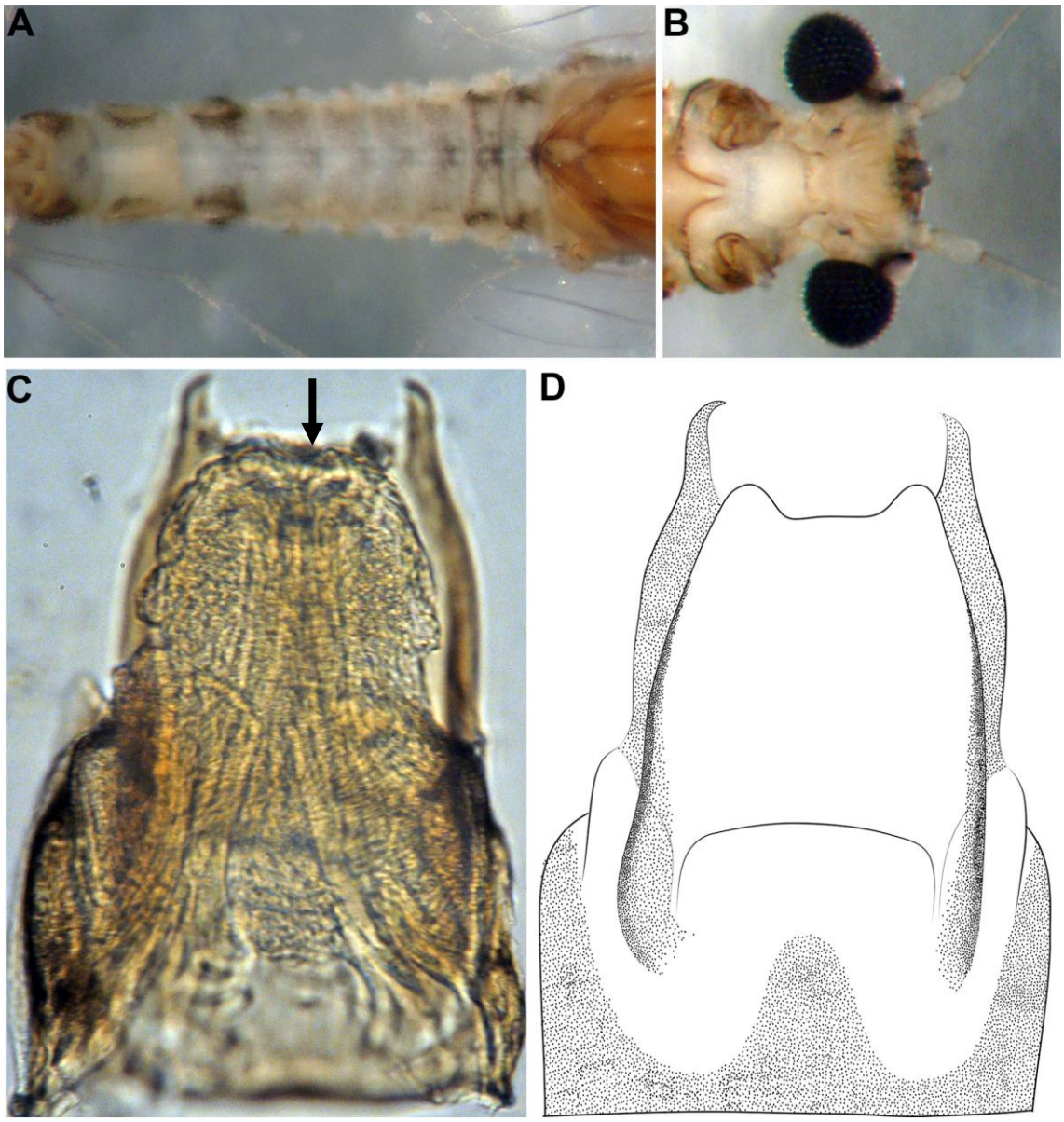



**Figure 12.** *Brasilocaenis atawallpa* sp. n. Male imago: A) abdominal color pattern, dorsal view; B) prosternum and prosternal longitudinal ridges; C) genitalia in dorsal view (black arrow indicating the penis); D) genitalia in ventral view, with details of styliger plate.

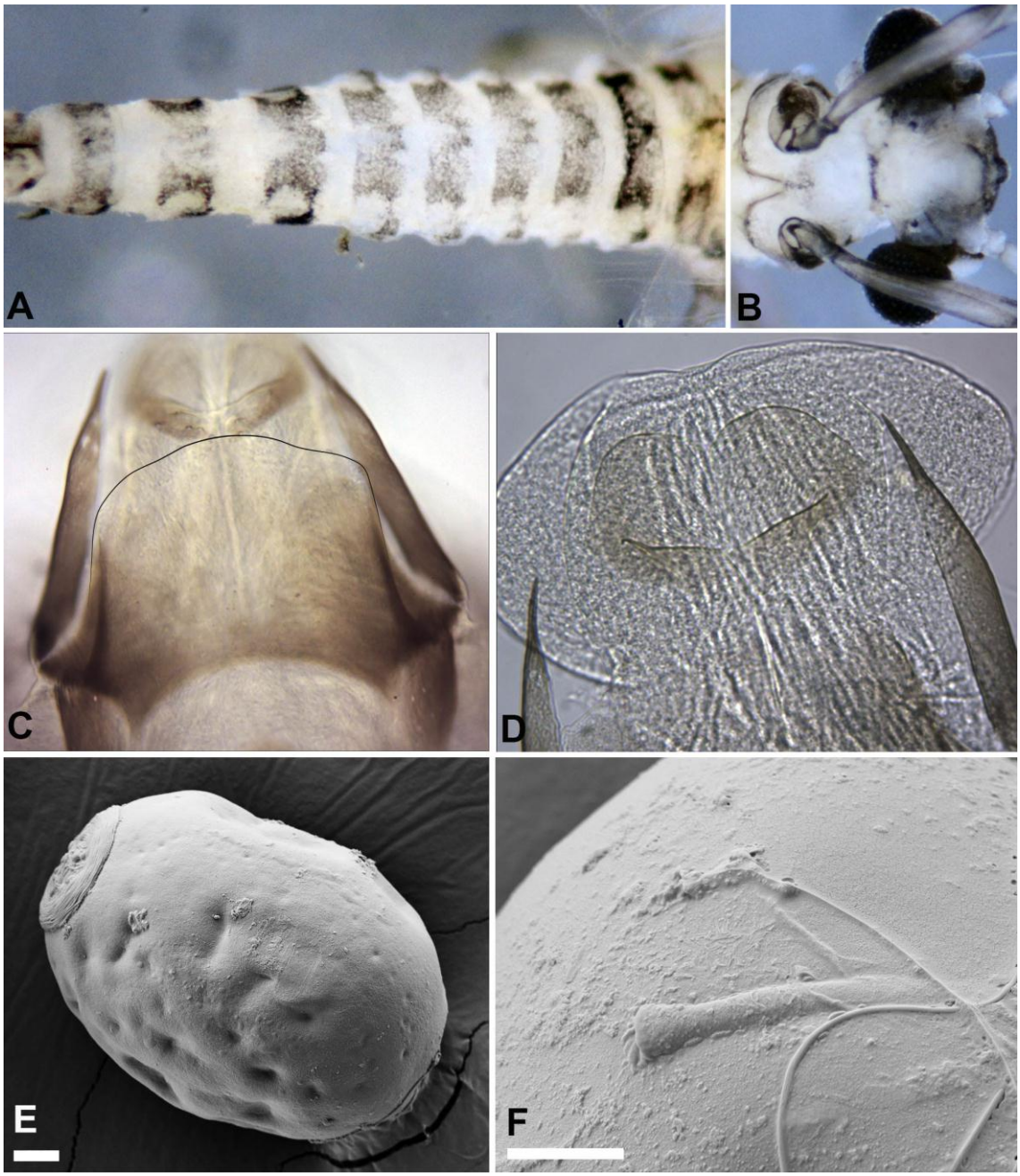



**Figure 13.** *Brasilocaenis suassunai* sp. nov. Male imago: A) abdominal color pattern; B) prosternum and prosternal longitudinal ridges; C) genitalia, ventral view; D) detail of penis, with swollen lobes. Eggs: E) detail of chorion and polar caps; F) detail of micropyle. Scale bar: 10µm.

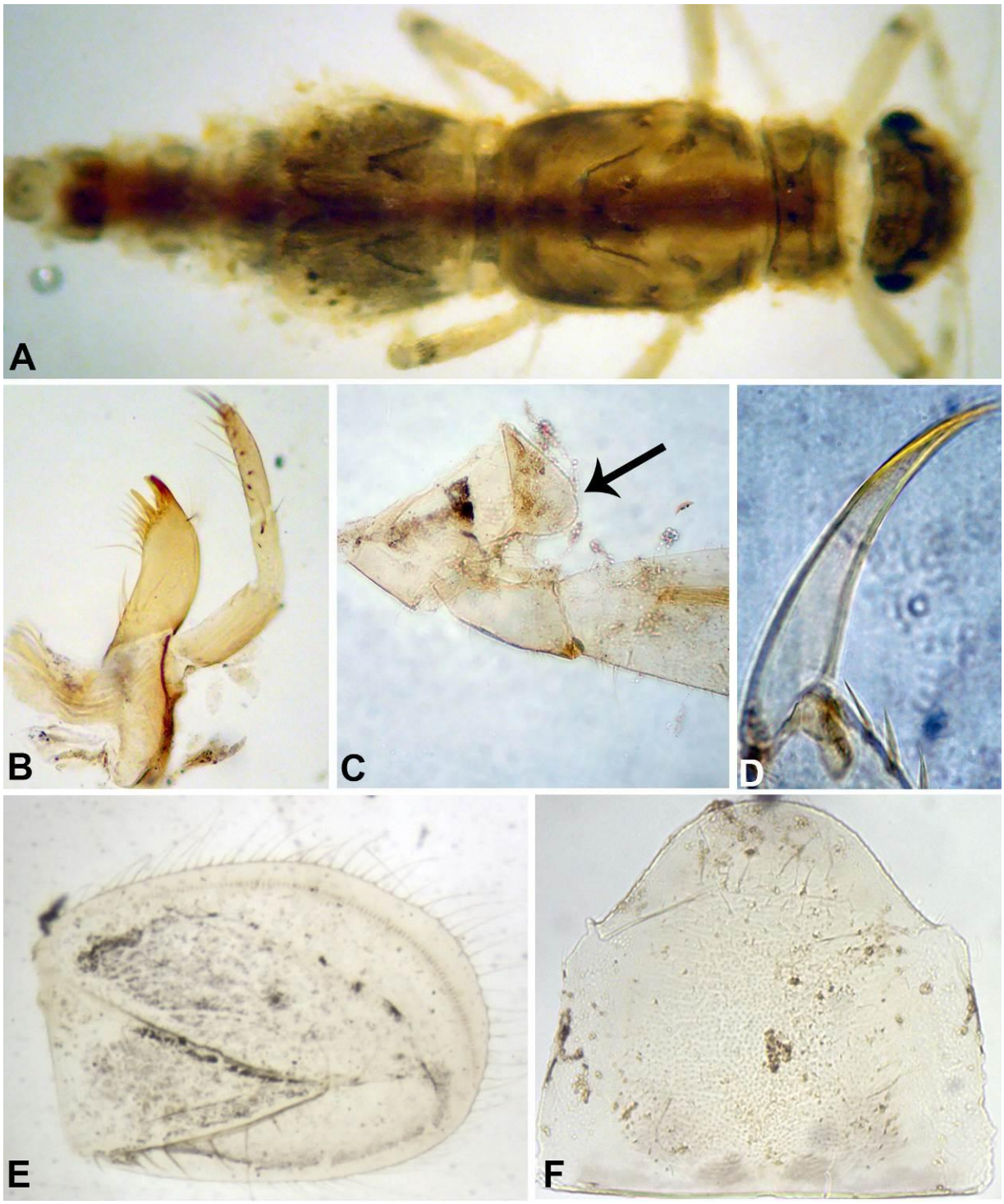
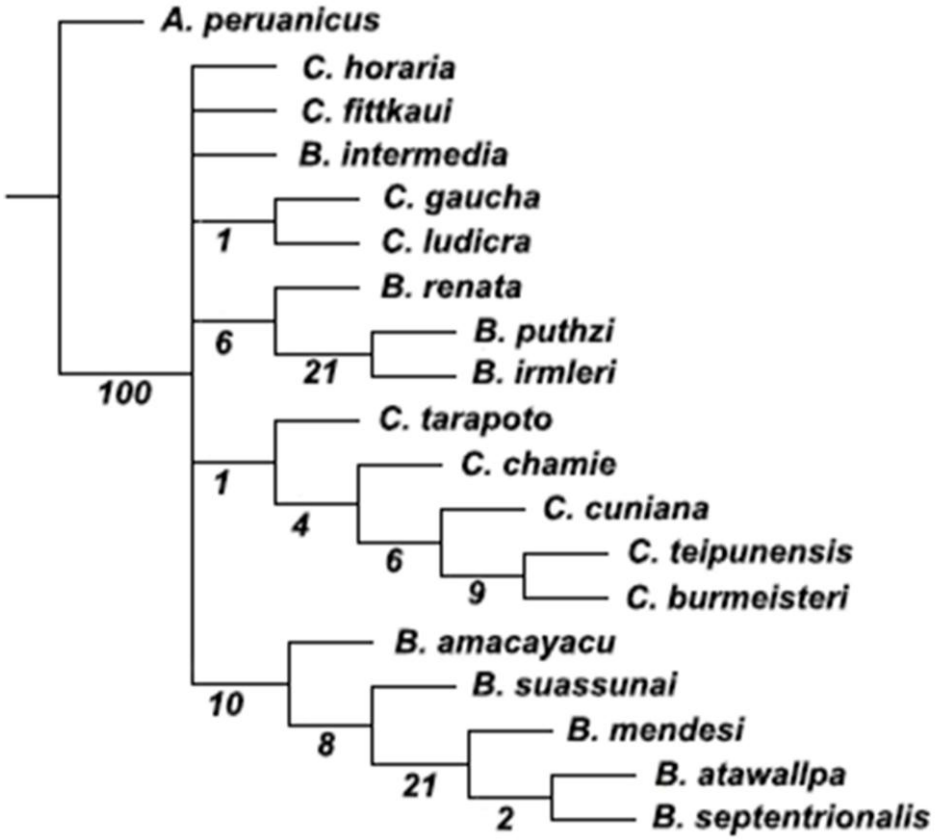
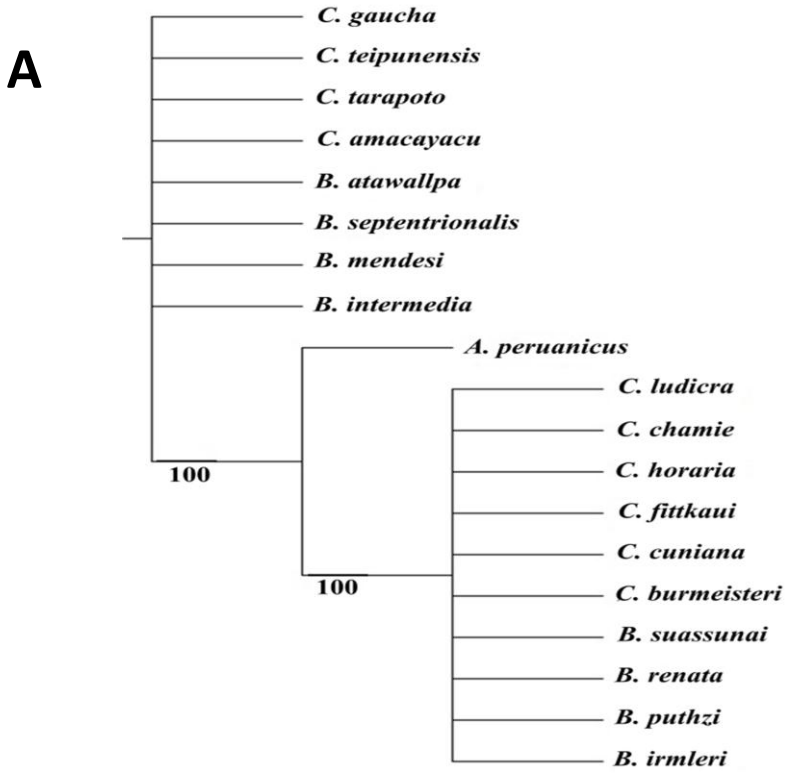


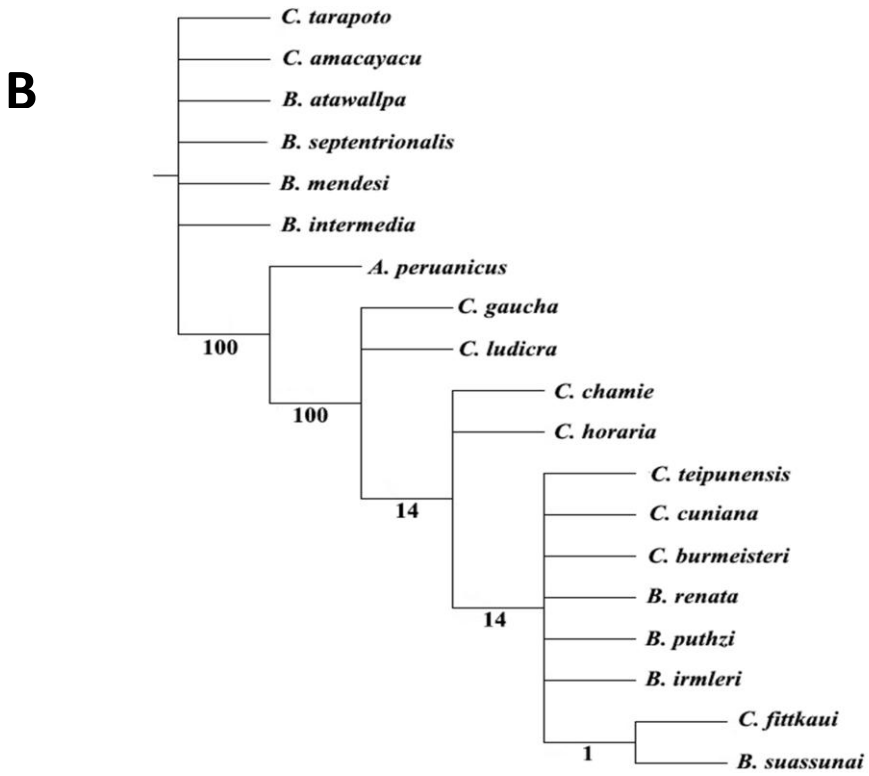

**Figure 14.** *Brasilocaenis suassunai* sp. nov. Nymph: A) habitus; B) maxila; C) metacoxal process; D) foretarsal claw; E) operculate gill; F) abdominal sternum IX.

**Appendix 1.** Partial phylogenetic analysis with adult characters only and group support (frequency difference values only).



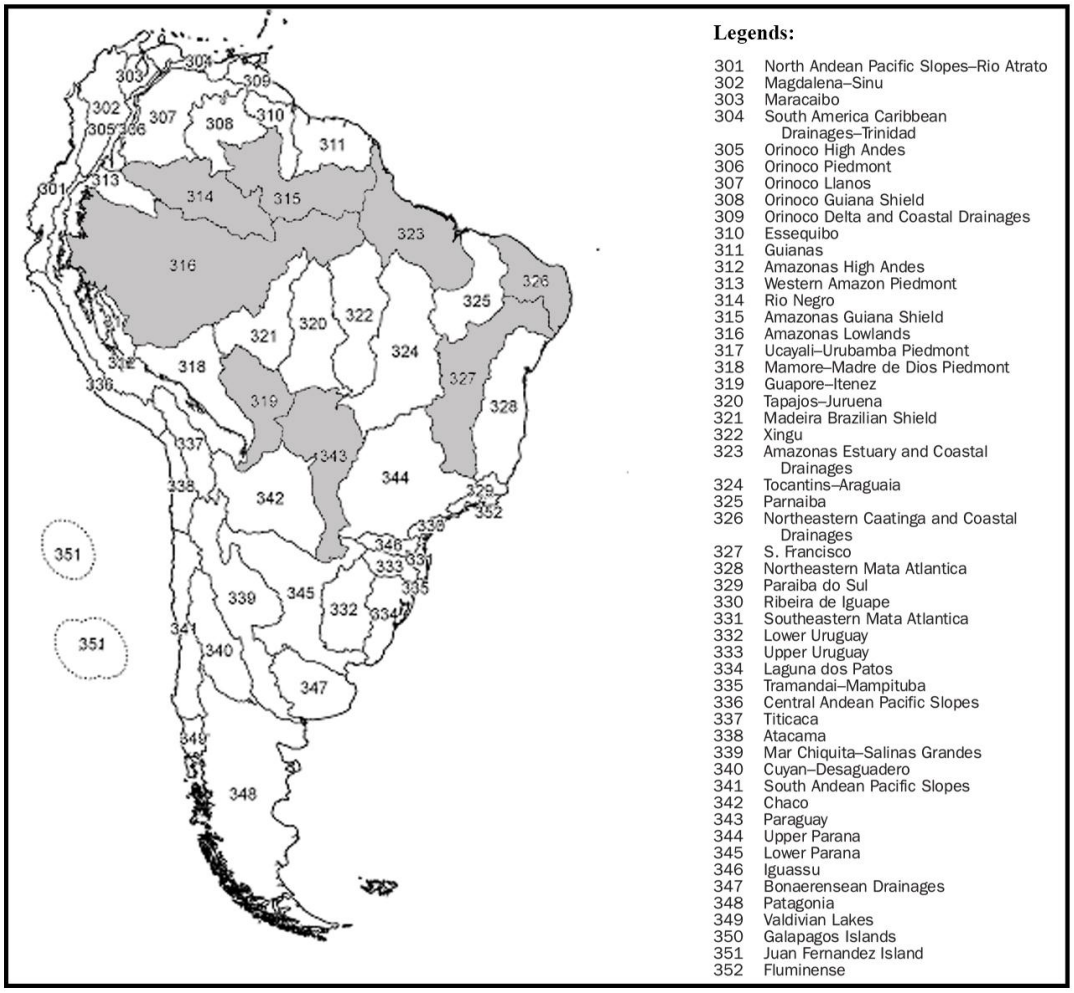
**Appendix 2.** Partial phylogenetic analysis: **A)** nymphal characters only; **B)** egg characters only (frequency differences values only).




**Appendix 3.** Map of freshwater ecoregions of the world, in which 51 ecoregions of South

America are delineated. Highlighted areas show *Brasilocaenis* distribution (modified from Abell et al. 2008).



## 4 CONCLUSÕES GERAIS

Os resultados obtidos do levantamento de espécies para Pernambuco, aumentaram de 43 para 61 o número de registros de espécies de Ephemeroptera para o estado. Com esse resultado, aumenta para 88 o número de espécies registradas para a Região Nordeste, ampliando de forma significativa o conhecimento do grupo para essa região. Pernambuco torna-se também um dos estados do Nordeste Brasileiro com maior número de espécies registradas, seguido pelos estados da Bahia (40), Maranhão (22) e Piauí (9). Comparando a diversidade de Ephemeroptera encontrada em outros estados brasileiros, o maior número de registros está concentrado na Região Sudeste e Sul do país. Nesses estados, as pesquisas com insetos aquáticos são realizadas já relativamente a um longo tempo e/ou estão associadas a grupos de pesquisas consolidados.

Para a maior parte das espécies encontradas nesse estudo, os registros representaram ampliações pouco significativas nos seus padrões de distribuição no país. No entanto, algumas espécies extenderam consideravelmente sua distribuição prévia. É o caso de *Caenis chamie* (Caenidae) previamente registrada para Colômbia e de *Brasilocaenis septentrionalis* (Caenidae) reportada apenas para a Bacia Amazônica. Isso não indica necessariamente numa distribuição disjunta, mas provavelmente um efeito de subamostragem em muitos estados do Nordeste Brasileiro, o que mascara o padrão real de distribuição dessas espécies.

A revisão do gênero *Brasilocaenis*, contribuiu para um acréscimo de 50% no número de espécies descritas para o gênero *Brasilocaenis*, passando de seis para nove espécies descritas. Desde o final da década de 1990, trabalhos taxonômicos e sistemáticos a respeito do grupo, na América do Sul são praticamente escassos, com apenas alguns trabalhos descritivos desde então. O aumento significativo do número de espécies, para um único gênero, corrobora a hipótese de que ainda há um grande número de táxons de Caenidae, a ser descrito para a Região Neotropical.

Os resultados contribuíram também para ampliar a distribuição geográfica das espécies de *Brasilocaenis*, com novos registros de ocorrência para diferentes regiões do Brasil. Contudo, ainda existem lacunas de distribuição, tanto para o gênero quanto para outros representantes da família, em muitas regiões do Brasil, principalmente para a Região Centro-Oeste e Nordeste. Isso provavelmente, reflete o pouco esforço amostral ou talvez a falta de trabalhos específicos para o grupo nessas regiões. Também são descritos estágios de vida não

conhecidos para algumas espécies de *Brasilocaenis*, além de uma nova espécie para o gênero *Caenis*.

Esse conhecimento gerado, incluindo a descrição e inclusão de novos caracteres, não utilizados tradicionalmente na taxonomia de Caenidae, serviram de base para a realização da análise cladística. O método de reconstrução filogenética utilizado corroborou o monofiletismo do gênero e propôs hipóteses de relacionamento entre suas espécies. No entanto, as relações supragenéricas, principalmente entre um grupo de espécies de *Caenis*, necessitam ser avaliadas com maior profundidade, uma vez que qualquer inferência só poderá ser feita a partir de uma análise que inclua mais representantes da América do Sul.

Algumas espécies, ainda necessitam ser redescritas para uma melhor compreensão das relações dentro do gênero *Brasilocaenis*, uma vez que cinco espécies apresentam descrição com base em apenas um dos estágios de vida. Isso influenciou significativamente os baixos valores de suporte dos clados encontrados. Sendo, assim mais esforços precisam ser dispendidos no sentido de fazer associação entre ninfas e adultos, além da associação molecular para futuros estudos populacionais.

A Análise Espacial de Vicariância (VIP) utilizada para analisar a distribuição dos *Brasilocaenis* na América do Sul, encontrou eventos vicariantes pouco explicativos dentro do gênero *Brasilocaenis*. O padrão apresentado por esses eventos podem ter sofrido distorções devido ao efeito de subamostragem, fato comum para a família Caenidae na América do Sul. Muitas espécies, que possuem registros únicos e pontuais podem possuir distribuições mais amplas, e tais registros podem ser fundamentais para esclarecer melhor os eventos vicariantes encontrados na análise.

A respeito, dos padrões biogeográficos, podemos perceber que períodos de transgressões marinhas no Mioceno Médio-Superior (23,8-5,32 maa) podem ter sido a causa do isolamento e grande diversificação do gênero na Bacia Amazônica. Postetiormente, algumas espécies podem ter se dispersado para outras bacias, fato relacionado a presença de *B. septentrionalis* e *B. suassunai*, ambas co-ocorrentes na bacia costeira do Atlântico Nordeste Oriental. Contudo, esses resultados são apenas inferências, uma vez que, que só foram usados uma amostra da diversidade de espécies da América do Sul. Acreditamos, que uma futura análise, com mais representantes de Caenidae, contribuirão para aumentar a robustez da análise e encontrar padrões mais consistentes ou reforçar os padrões encontrados aqui.

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