LUCIMAR GOMES DIAS

TAXONOMIA DE *Tricorythopsis* TRAVER E *Tricorythodes* ULMER (EPHEMEROPTERA: LEPTOHYPHIDAE) E AVANÇOS NOS ESTUDOS MOLECULARES DE EPHEMEROPTERA

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de "*Doctor Scientiae*".

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APROVADA: 22 de julho de 2009.

Dr. Pablo Benavides Machado (Co-orientador)	Dr. Carlos Molineri (Co-orientador)
Prof. Lucio Antonio de Oliveira Campos	Prof. Jorge Abdala Dergam dos Santos

Prof. Paulo Sérgio Fiuza Ferreira
(Orientador)

Ao ilustre Professor Fiuza,

Que com sua experiência brindou propostas inovadoras para esta tese.

E, além de orientador se tornou um grande amigo.

Ao bem mais precioso que tenho, minha mãe.

Maria, Maria, é um dom, uma certa magia,
uma força que nos alerta.
Uma mulher que merece viver e amar,
como outra qualquer do planeta.
Maria, maria, é o som, é a cor, é o suor,
é a dose mais forte e lenta,
de uma gente que ri quando deve chorar
e não vive, apenas aguenta.

Mas é preciso ter força, é preciso ter raça,
é preciso ter gana sempre.
Quem traz no corpo a marca,
Maria, Maria, mistura a dor e a alegria.
Mas é preciso ter manha, é preciso ter graça,
é preciso ter sonho sempre,
quem traz na pele essa marca
possui a estranha mania de ter fé na vida.

(Milton Nascimento E Fernando Brant)

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À minha comissão orientadora pela competência e colaboração no desenvolvimento desta tese.

Fiuza, lembra a primeira conversa que tivemos? “Se quiser que eu te oriente, vai ter que trabalhar com taxonomia”. Depois de seis anos trabalhando sob sua orientação com taxonomia, tenho algo para te confessar. Essa foi uma das decisões mais acertada da minha vida!!! Tenho muito que te agradecer.

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Ao Dr. Carlos Molineri, quem desde o mestrado vem compartindo comigo sua experiência na taxonomia de Leptohyphidae e me ensinando coisas novas. Obrigada Carlitos pela competência, orientação e amizade!

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À Universidade Federal de Viçosa e ao Programa de Pós-graduação em Entomologia, pela oportunidade de realizar meu mestrado e doutorado. Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) por financiar meus estudos ao longo dos últimos seis anos.

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decisões profissionais, que são tão importantes na vida de qualquer pessoa. Ephemeroptera foi uma escolha que deu certo!!! Obrigada mesmo.

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Minha grande amiga Nancy Cecília Delgado, minha mudança para Colômbia já valeu a pena pelo fato de conhecer você... Si aprendi a gostar e respeitar o seu país (Colômbia "Tierra querida"), foi graças a você com toda essa sua paixão e comoção pelo seu país e cidade (Manizales). Agora já posso cantar com você, "... Ai Manizales del alma, mi Manizales querido...". Obrigada por todos os seus conselhos e ensinamentos, aprendi muito contigo!!!

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Aos dois Vinícius da minha vida, Vini São Pedro, meu amigo poeta e Vi

Albano, meu amigo cabeção. Inesquecíveis e responsáveis por muitos dos meus sorrisos desses últimos anos.

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BIOGRAFIA

Lucimar Gomes Dias, filha de Walter Ferreira Dias e Neide Maria Gomes Dias, nasceu em Campo Grande, Rio de Janeiro, em 27 de junho de 1980.

Cursou toda sua vida estudantil na rede pública de ensino. Tendo sido aprovada no vestibular em 1999, para o curso ciências biológicas na Faculdade de Formação de Professores da UERJ, onde teve a oportunidade de receber uma bolsa de estudo durante dois anos. Nesse período de graduação realizou estágios no Museu Nacional do Rio de Janeiro e Fundação Oswaldo Cruz, onde também recebeu apoio como bolsista. O título de licenciatura plena em ciências biológicas foi recebido em 2002.

Em julho de 2003 foi aprovada para realizar o mestrado em Entomologia pela Universidade Federal de Viçosa (UFV), o qual foi concluído em julho de 2005. Neste mesmo ano e instituição iniciou o curso de Doutorado em Entomologia, defendendo a tese em julho de 2009. Neste período de pós-graduação recebeu o apoio do CNPq como bolsista.

Como produção acadêmica, tem publicado 13 artigos em revistas indexadas, internacionais na área de sistemática de Ephemeroptera.

Em outubro de 2008, concursou pela primeira vez a uma vaga para professores efetivos de uma Universidade pública na Colômbia (Universidad de Caldas). E em maio de 2009 foi informada oficialmente sobre sua aprovação no concurso. Em agosto deste ano começará a lecionar no curso de ciências biológicas na universidade mencionada, na área de sistemática animal.

ÍNDICE

RESUMO

DIAS, Lucimar Gomes. D.Sc. Universidade Federal de Viçosa, julho de 2009. **Taxonomia de *Tricorythopsis* Traver e *Tricorythodes* Ulmer (Ephemeroptera: Leptohyphidae) e avanços nos estudos moleculares de Ephemeroptera.** Orientador: Paulo Sérgio Fiuza Ferreira. Coorientadores: Pablo Benavides Machado e Carlos Molineri.

Com o objetivo de contribuir para a taxonomia de *Tricorythodes* Ulmer e *Tricorythopsis* Traver, novos taxa foram descritos e técnicas moleculares foram utilizadas como uma ferramenta complementar na sistemática de Ephemeroptera. Como resultados deste trabalho, cinco novas espécies foram descritas, três para o gênero *Tricorythodes* (*T. rondoniensis*, *T. sallesi* e *T. caunapi*) e duas para *Tricorythopsis* (*T. bahyensis* e *T yucupe*), além do primeiro registro de *Tricorythodes quizeri* Molineri para o Brasil. Uma análise filogenética de *Tricorythodes* foi realizada por primeira vez com dados moleculares de três marcadores (18SrDNA, 16SrDNA e COI) combinados a 39 caracteres morfológicos. Esta análise apoiou a monofilía do gênero, com exceção das espécies dos grupos *Asioplax* Wiersema & McCafferty e *Macunahyphes* Dias, Salles & Molineri. Dessa forma, neste trabalho são apresentadas evidencias para a revalidação de *Asioplax* e *Macunahyphes* como gêneros de Leptohyphidae. Os resultados deste trabalho demonstraram também que a técnica molecular de AFLP constitui uma ferramenta útil para a associação de ninfas e adultos de Ephemeroptera. A partir dessa metodologia, o adulto de *Leptohyphes ecuador* Mayo que era desconhecido, foi associado à sua ninfa através do perfil de amplificação do DNA. Além disso, neste trabalho foram apresentados a descrição do adulto de *L. ecuador* e o primeiro registro da espécie para Colômbia.

ABSTRACT

DIAS, Lucimar Gomes. D.Sc. Universidade Federal de Viçosa, July of 2009. **Taxonomy of *Tricorythopsis* Traver and *Tricorythodes* Ulmer (Ephemeroptera: Leptohyphidae) and advancements in the molecular studies of Ephemeroptera.** Adviser: Paulo Sérgio Fiuza Ferreira. Co-advisers: Pablo Benavides Machado and Carlos Molineri.

In order to contribute to the taxonomy of *Tricorythodes* Ulmer and *Tricorythopsis* Traver, new taxa were described and molecular techniques were used as complementary tools in the systematic of the Ephemeroptera. As a result of the work, five new species were described, three for genus *Tricorythodes* (*T. rondoniensis*, *T. sallesi* and *T. caunapi*) and two for *Tricorythopsis* (*T. bahyensis* and *T yucupe*), besides the first record of *Tricorythodes quizeri* Molineri from Brazil. A phylogenetic analysis of *Tricorythodes* was carried out for the first time using molecular data of three markers (18SrDNA, 16SrDNA and COI) combined with 39 morphological characters. This analysis supported the monophyly of Tricorythodes, except for the species of *Asioplax* Wiersema & McCafferty e *Macunahyphes* Dias, Salles & Molineri. Therefore this work presents evidence for *Asioplax* e *Macunahyphes* revalidation as genus of *Leptohyphidae*. The results of this work presented also that the AFLP molecular technique constitute a useful tool to the association of adults and nymphs of Ephemeroptera. From this methodology the unknown adult of *Leptohyphes ecuador* Mayo was associated with its nymph by DNA fingerprint. In addition, the description of the adults of *L. ecuador* and the first record of this species from Colombia were present.

1 - INTRODUÇÃO GERAL

1.1. Aspectos gerais da família de Leptohyphidae (Insecta: Ephemeroptera) e história da sistemática do grupo:

A pan-americana família Leptohyphidae é considerada uma das mais diversas famílias de Ephemeroptera (Domínguez *et al.* 2006). Atualmente a família está representada por aproximadamente 130 espécies, das quais 80 são registradas para América do Sul (Dias & Salles 2005, 2006; Molineri 2006; Molineri & Zúñiga 2006; Emmerich; 2007; Dias *et al.* 2009a, b).

Os integrantes da família são herbívoros, constituindo um importante integrante das cadeias tróficas de ambientes aquáticos, sendo encontrados somente em ambientes lóticos (Domínguez *et al.* 2006). As espécies de Leptohyphidae estão amplamente distribuídas nos rios e cachoeiras e podem colonizar uma série de meso-hábitats, tais como: substratos rochosos, folhiços depositados em áreas de remanso e correnteza, vegetação marginal e areia (Domínguez *et al.* 2006).

As características morfológicas que diferenciam Leptohyphidae das demais famílias de Ephemeroptera são principalmente, em adultos: comprimento do corpo de aproximadamente 2 a 10 mm; tórax robusto; asas anteriores com cerdas na margem posterior; asas posteriores quando presentes são pequenas e com uma longa projeção costal; pênis com forma variável segundo o gênero; fórceps com 2 ou 3 segmentos; filamentos caudais longos e finos, geralmente mais curtos nas fêmeas. Nas ninfas: brânquia do segundo segmento abdominal alargada formando um opérculo; abdômen com brânquias nos segmentos II-VI; filamentos caudais tão longo quanto o corpo; corpo geralmente robusto e coberto por cerdas (Domínguez *et al.* 2006).

Historicamente, Leptohyphidae foi reconhecida por primeira vez como uma subfamília (Leptohyphinae) de Tricorythidae por Edmunds & Traver (1954). Em 1973, Landa elevou Leptohyphinae formalmente à categoria de família (Leptohyphidae), onde foram agrupadas duas subfamílias: Leptohyphinae (com os gêneros panamericanos *Leptohyphes* e *Tricorythodes*) e a monotípica subfamília Dicercomyzinae (com o gênero

africano *Dicercomyzon* Demoulin). Peters & Peters (1993) excluíram esta última subfamília de Leptohyphidae e incluíram o gênero *Coryphorus* estabelecido por Peters (1981). Recentemente, Wiersema & McCafferty (2000) baseando-se principalmente em táxons da América Central, propuseram a divisão de Leptohyphidae em duas subfamílias: Tricorythodinae e Leptohyphinae, mas Molineri & Dominguez (2003) apresentaram evidencias de que estas subfamílias não formam grupos naturais. Molineri (2006) e Baumgardner (2008) realizam uma analise cladística de Leptohyphidae, onde a monofilía da família é fortemente sustentada, entretanto reafirmam que as subfamílias Tricorythodinae e Leptohyphinae não formaram grupos monofiléticos.

1.2. Sistemática do gênero *Tricorythodes* Ulmer (Ephemeroptera: Leptohyphidae) – Histórico resumido:

O gênero *Tricorythodes* Ulmer (Leptohyphidae) possui uma distribuição pan-americana e é considerado um dos mais diversos gêneros de Leptohyphidae, atualmente com 62 espécies, das quais 20 estão registradas para América do Sul (Molineri 2002; Molineri & Zúñiga 2006; Emmerich 2007; Baumgardner 2008; Dias *et al.* 2009a, b).

Tricorythodes é um dos gêneros de Leptohyphidae que tem tido uma classificação um tanto problemática. Diversos taxonomistas têm estudado e proposto diferentes formas de classificação para o grupo (Molineri 2000; Wiersema & McCafferty 2000; Allen & Murvosh 1987; Baumgardner 2008).

O gênero foi proposto por Ulmer (1920) e a primeira revisão foi feita por Allen & Murvosh (1987). Estes últimos autores propuseram três subgêneros para *Tricorythodes*, são eles: *Tricorythodes* Ulmer, *Tricoryhyphes* Allen & Murvosh and *Homoleptohyphes* Allen & Murvosh. Wiersema & McCafferty (2000) elevaram os subgêneros propostos por Allen & Murvosh (1987) ao nível de gênero e alem disso propuseram outros novos gêneros, *Asioplax* e *Epiphrades* para incluir outras espécies de *Tricorythodes* sensu lato. Molineri (2002) em uma analise cladística de *Tricorythodes* mostra que os gêneros propostos por Wiersema & McCafferty (2000) são grupos para e polifiléticos, e por tal razão não reconhece os mesmos. Wiersema & McCafferty (2005),

realizam uma revisão das espécies de *Asioplax*, contrariando o que foi proposto por Molineri (2002).

Em 2005, um outro gênero é proposto para incluir uma espécie de *Tricorythodes*, *Macunahyphes* é descrito para incluir a espécie *T. australis* Traver (Dias *et al.* 2005). Entretanto, na revisão filogenética de Leptohyphidae feita por Molineri (2002), a monofilía de *Macunahyphes* não foi suportada, e o gênero manteve sua posição dentro de *Tricorythodes.*

Baumgardner & Ávila (2006) descreveram um novo gênero baseado em uma espécie da América Central. Segundo estes autores, o gênero proposto é muito próximo de *Tricorythodes,* compartilhando várias características com o mesmo.

Baumgardner (2008), em uma recente revisão filogenética de Leptohyphidae, propõe a sinonimização de todos os gêneros propostos para *Tricorythodes* sensu lato: *Ableptemetes, Cabecar, Epiphrades***,** *Homoleptohyphes***,** *Macunahyphes***,** *Tricoryhyphes***.**

A evolução no número das espécies descritas para *Tricorythodes* pode ser vista na Fig. 1. A figura mostra que o ano 1967 foi o que apresentou maior progresso no conhecimento de *Tricorythodes*, graças ao trabalho de Allen (1967). Entretanto, deve ser observado que após o ano 2000 um avanço progressivo ocorreu, nesse período as maiores contribuições foram de Molineri (1999, 2001a, 2002), Dias & Salles (2006), Baumgardner (2007) e Dias *et al.* (2009a, b).

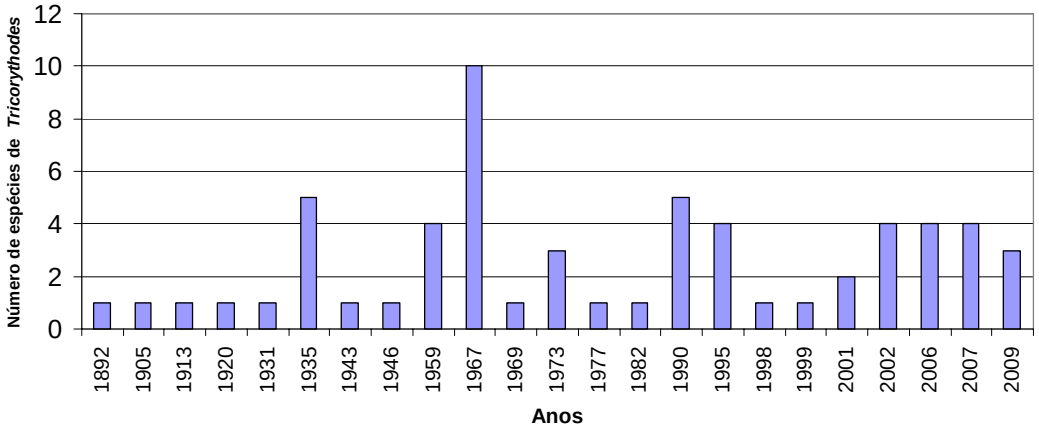

Figura 1. Evolução no número de registros de espécies de *Tricorythodes*, ao longo de 117 anos, desde a descrição da primeira espécie do gênero.

1.3. Sistemática do gênero *Tricorythopsis* Traver (Ephemeroptera: Leptohyphidae) – Histórico resumido:

Tricorythopsis Traver é um pequeno e bem definido sul americano gênero de Leptohyphidae, constituído por 12 espécies, das quais a maioria é reportada para o Brasil (Molineri 1999, 2001; Dias & Salles 2005, Dias *et al.* 2008).

O gênero foi criado por Traver (1958) a partir de adultos da espécie tipo *T. artigas* Traver, cuja localidade tipo pertence ao Uruguai. A primeira revisão de *Tricorythopsis* foi feita por Molineri em 1999, onde foram descritas quatro espécies novas para o gênero e foram feitos novos registros para Argentina, Brasil, Venezuela e Equador. Em 2001, Molineri realiza uma segunda revisão de *Tricorythopsis*, onde propõe novas combinações e sinônimos para o gênero, além de duas novas espécies.

Dias & Salles (2005) descreveram três espécies do gênero para a região Sudeste do Brasil. Em 2008, Dias *et al.* descreveram outras duas espécies para a região Norte do Brasil, que serão apresentadas nesta tese.

A evolução no número das espécies descritas para *Tricorythopsis* pode ser vista na Fig. 2. As maiores contribuições ocorreram a partir do ano 2000, com os trabalhos de Molineri (2001b), Dias & Salles (2005) e Dias *et al.* (2008).

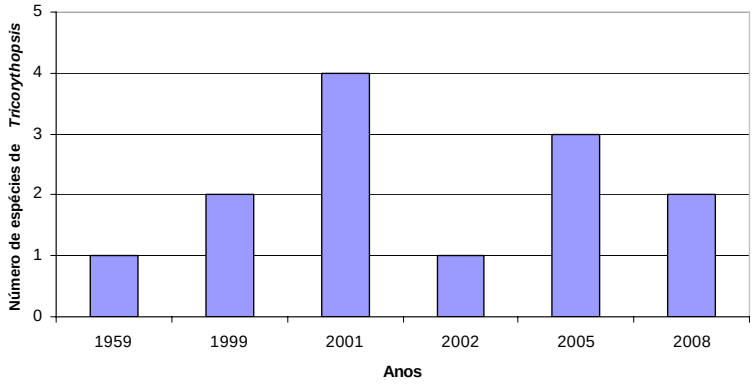

Figura 2. Evolução no número de registros d e espécies de *Tricorythopsis*, ao longo de 49 anos, desde a descrição da primeira espécie do gênero.

1.4. Utilização de evidencias moleculares na taxonomia de Ephemeroptera:

Atualmente, muitas propostas estão sendo apresentadas para a modernização da

taxonomia tradicional, dentre elas está a utilização da biologia molecular como ferramenta (Rapini 2004). A utilização desta ainda é questionada, por ser tratar de uma metodologia relativamente nova (Lipscomb *et al.* 2003, Mallet &, Seberg *et al.* 2003; Willmot 2003). Entretanto, é incontestável o fato de que essa ferramenta vem ganhando espaço na taxonomia devido a sua eficiência na resolução de problemas (Herber *et al.* 2002; Armstrong & Ball 2005; Smith *et al.* 2005).

Na taxonomia da ordem Ephemeroptera, a situação não é diferente e a ferramenta da biologia molecular vem sendo utilizada para resolver problemas na sistemática do grupo (Whiting 2003, 2005; Ball *et al.* 2005; Ogden & Sun *et al.* 2006; O`Donnell & Jockusch 2008; Ogden *et al.* 2009). Obviamente, esta ferramenta não deve ser interpretada como uma forma de substituição da taxonomia baseada em morfologia (Schindel & Miller 2005; Lanteri 2007), mesmo porque essas técnicas também apresentam algumas limitações, entretanto funcionam muito bem como uma ferramenta complementar na taxonomia.

Conforme apresentado anteriormente, o gênero *Tricorythodes* é um exemplo, onde diferentes propostas de classificação foram levantadas nos últimos anos, e pelo menos duas delas seguem em vigor, adotadas por diferentes pesquisadores (Wiersema & McCafferty 2000; Molineri 2006). Para esses casos, onde ambas propostas foram feitas baseadas somente em morfologia, a biologia molecular pode contribuir para um melhor entendimento do grupo em discussão.

Um problema comum na sistemática de Ephemeroptera é a difícil associação entre ninfas e adultos, já que as ninfas geralmente não são criadas com facilidade. Esse problema tem provocado à descrição de espécies, gêneros e até mesmo famílias baseado em apenas ninfa ou adulto. Na América do Sul, por exemplo, apenas 10% das espécies são conhecidas em ambos estados de vida, adulto e imaturo (Domínguez *et al.* 2006). Essa falta de conhecimento dos estados de vida destes insetos, muitas vezes tem prejudicado ou tornado incompleta a definição diagnostica dos taxóns. Para este tipo de problema, a biologia molecular pode ser uma alternativa (Fleck *et al.* 2006; Jeon and Ahn 2007; Miller *et al.* 2005, 2007), a traves do perfil do DNA de uma determinada ninfa e adulto, por exemplo, pode ser determinado facilmente se pertencem a uma mesma espécie.

2. OBJETIVO GERAL

Contribuir a taxonomia de *Tricorythodes* Ulmer e *Tricorythopsis* Traver e apresentar ferramentas moleculares para solucionar problemas na taxonomia de Ephemeroptera.

2.1. Objetivos Específicos:

1. Testar a monofilía do gênero *Tricorythodes* através de evidencias moleculares e morfológicas.
2. Descrever eventuais novos taxa e estágios desconhecidos de *Tricorythodes* e *Tricorythopsis*.
3. Apresentar novos registros de *Tricorythodes* e *Tricorythopsis* para a América do Sul.
4. Utilizar a técnica molecular de AFLP para a associação de adultos e imaturos de Ephemeroptera.

3. MATERIAL E MÉTODOS

3.1. Obtenção e deposição do material

Os exemplares de *Tricorythodes* e *Tricorythopsis* estudados na tese foram provenientes de coletas de campo e empréstimos de várias instituições do Brasil, Argentina, Colômbia e Cuba. As coletas foram realizadas em várias localidades do Brasil e Colômbia, o que será descrito detalhadamente em cada artigo da tese. Os amostradores utilizados nas coletas foram peneiras e redes entomológicas aquáticas com malhas de no máximo 1,0 mm, sendo efetuada a triagem em campo, juntamente com a fixação e conservação em etanol a 90%. As instituições onde foram obtidos os empréstimos foram: Laboratório de Entomologia, Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ – DZRJ; Departamento de Entomologia, Museu Nacional, Universidade Federal do Rio de

Janeiro, RJ – MNRJ; Museu Regional de Entomologia, Universidade Federal de Viçosa, Viçosa, MG – UFVB; Laboratório de Entomologia Aquática, Universidade de São Paulo, Ribeirão Preto, SP – USP; Laboratório de Entomologia Aquática, Universidade Federal de São Carlos, São Carlos, SP – DCBU (Brasil); Instituto-Fundación Miguel Lillo, San Miguel de Tucumán, Tucumán – IFML (Argentina). O material estudado foi depositado nas instituições citadas acima, conforme indicações ao longo do trabalho.

3.2. Estudos taxonômicos

Os espécimes foram identificados utilizando chaves taxonômicas, descrições originais e comparação com o material-tipo, quando disponível. Para as descrições foram utilizados os seguintes trabalhos referentes à taxonomia de *Tricorythodes* e *Tricorythopsis* da América do Sul: Molineri (2001b, 2002), Dominguez *et al.* (2006).

Para a observação de determinadas estruturas, como peças bucais e pernas das ninfas e genitálias dos adultos, foram montadas lâminas temporárias utilizando glicerina e, para o armazenamento de alguns exemplares de séries tipo, foram montadas lâminas permanentes utilizando Euparal MTNG Medium (eucaliptol & resina de aldeído) e Cellossolve (etilen-glicol monoetil éter).

Os desenhos foram elaborados com o auxílio de câmera clara, podendo estar acoplada tanto em microscópio luz Carl Zeiss Jena, com aumento de até 2.000x, quanto a estereomicroscópio Leica MZ-8, com aumento máximo de 200x, todos pertencentes à UFV. Inicialmente as ilustrações foram realizadas a lápis, sendo em seguida passadas para nanquim e posteriormente digitalizadas. Após o processo de digitalização, as figuras foram editadas em computador, utilizando-se o programa "Adobe Photoshop 8.0".

3.3. Analises moleculares

A extração do DNA foi feita de tecidos musculares do tórax e pernas dos espécimes, previamente preservado em ethanol. A técnica de extração foi realizada com o DNeasy Tissue Kit de Qiagen, segundo o protocolo do mesmo. O DNA extraído foi estocado a - 20°C.

Três conjuntos de primer de PCR (Polymerase Chain Reactions) foram usados para amplificar os marcadores COI mtDNA (Folmer et al. 1994), 18S rDNA (Whiting 2002) e 16S rDNA (Ogden and Whiting 2005). As reações de PCR foram realizadas em um volume final de 50µl. O produto PCR foi enviado a *Macrogen Advancing through Genomics – Korea*, que realizou o sequenciamento. Os detalhes da reação de PCR são apresentados no quinto artigo da tese.

A técnica do AFLP foi realizada segundo AFLP Analysis System II desenvolvido Life Technologies (GIBCO Invitrogen Corporation, Carlsbad, CA). A técnica consiste basicamente no corte da fita de DNA em pequenos fragmentos, a traves de enzimas de restrição. Após esse processo são incluídos os adaptadores na reação (que são seqüências que se uniram aos fragmentos DNA). Estes adaptadores são reconhecidos pelos primers na seguinte PCR. Após a amplificação, o padrão de bandeamento do DNA de cada indivíduo foi visualizado após eletroforese em gel de poliacrilamida. As etapas do AFLP são detalhadas no sexto artigo da tese.

3.4. Formatação

A presente tese encontra-se organizada sob a forma de artigos científicos, como disposto no item 2.4 das normas para redação de teses dessa instituição. Cada artigo encontra-se formatado de acordo com as normas da revista a que foi submetido ou publicado, constituindo uma exceção o alinhamento do tipo "justificado" e o espaçamento entre linhas de 1,5, já que usualmente o requerido pelas revistas científicas é o alinhamento do tipo "à esquerda" e o espaçamento de 2,0.

A tese foi organizada em seis artigos científicos, tratando primeiro os novos táxons descritos e em seguida dois artigos onde são estudadas evidências moleculares no estudo sistemático de Ephemeroptera.

4. REFERENCIAS

Allen, R. K. 1967. New species of New World Leptohyphinae (Ephemeroptera: Tricorythidae). Canadian Entomologist, 99:350-375.

Allen, R. K. & Murvosh, C. M. 1987. Mayflies (Ephemeroptera: Tricorythidae) of the Southwestern United States and Northern Mexico. Annals of the Entomological Society of America, 80(1): 35-40.

Armstrong, K. F. & Ball, S. L. 2005. DNA barcodes for biosecurity: invasive species identification. The Philosophical Transactions of the Royal Society B, 360: 1813-1823.

Ball, S. L., Hebert, P. D. N., Burian, S. K. & Webb, J. M. 2005. Biological identifications of mayflies (Ephemeroptera) using DNA barcodes. Journal of the North American Benthological Society, 24:508-524.

Baumgardner, D. E. & Ávila, S. 2006. *Cabecar serratus*, a new genus and species of Leptohyphidae mayfly from Central America, and description of the imaginal stages of *Tricorythodes sordidus* Allen (Ephemeroptera: Leptohyphidae). Zootaxa, 1187:47-59.

Baumgardner, D. E. 2007. New species of Leptohyphidae (Ephemeroptera) from Costa Rica. Proceedings of the Entomological Society of Washington, 109(2):416-426.

Baumgardner, D. E. 2008. Phylogeny and biogeography of the mayfly family Leptohyphidae (Insecta: Ephemeroptera) with a taxonomic revision of selected genera. Ph.D Dissertation, Texas A&M University, College Station, 306 p.

Dias, L. G. & Salles, F. F. 2005. Three new species of Tricorythopsis (Ephemeroptera: Leptohyphidae) from southeastern Brazil. Aquatic Insects 27(4):235-241.

Dias, L. G.; Salles, F. F. & Molineri, C. 2005. *Macunahyphes*: a new genus for *Tricorythodes australis* (Ephemeroptera: Leptohyphidae). Annales de Limnologie - International Journal of Limnology, 41(3): 195-201.

Dias, L. G. & Salles, F. F. 2006. A New Species of *Tricorythodes* (Ephemeroptera: Leptohyphidae) from Minas Gerais, Southeastern Brazil. Neotropical Entomology, 31(1): 56-58.

Dias, L. G., Salles, F. F. & Ferreira, P. S. F. 2008. New species of *Tricorythopsis* Traver (Ephemeroptera: Leptohyphidae) from Northern Brazil. Studies on Neotropical Fauna and Environment, 43: 237-241.

Dias, L. G., Cruz, P. V. & Ferreira, P. F. 2009a. A new species of *Tricorythodes* Ulmer (Ephemeroptera: Leptohyphidae) from Northern Brazil. Annales de Limnologie - International Journal of Limnology, DOI: 10.1051/limn/2009009.

Dias, L. G., Cabette, H. S. R & De Souza, D. 2009b. A new species of *Tricorythodes* Ulmer (Ephemeroptera: Leptohyphidae) and first record of *Tricorythodes quizeri* Molineri from Brazil. Aquatic Insects, *In press.*

Domínguez, E., Molineri, C., Pescador, M., Hubbard, M.D. & Nieto, C., 2006. Aquatic Biodiversity in Latin America. Pensoft, Sofia-Moscow, v.2: Ephemeroptera of South America, 646 p.

Edmunds, G. F., & Traver, J. R. 1954. An outline of a reclassification of the Ephemeroptera. Proceedings of the Entomological Society of Washington, 56(5): 236-240.

Emmerich, D. E. 2007. Two new species of *Tricorythodes* Ulmer (Ephemeroptera: Leptohyphidae) from Colombia. Zootaxa, 1561:63-68.

Fleck, G., Brenk, M. & Misof, B. 2006. DNA Taxonomy and the identification of immature insect stages: the true larva of *Tauriphila argo* (Hagen 1869) (Odonata: Anisoptera: Libellulidae). Annales de la Société Entomologique de France, 42: 91-98.

Hebert, P. D. N., Cywinska, A., Ball, S. L. & Dewaard, J.R. 2003. Biological identifications through DNA barcodes. The Philosophical Transactions of the Royal Society B, 270: 313-321.

Jeon, M.-J., & Ahn, K.-J. 2006. Descriptions of late instars of three littoral *Cafius* species (Coleoptera: Staphylinidae) by association of life stage with DNA sequences. Florida Entomologist, 90: 465-474.

Landa, V. 1973. A contribution to the evolution of the order Ephemeroptera based on comparative anatomy. Pages 155-159 *in* Peters WL; Peters, J. G. Proceedings of the first international conference on Ephemeroptera. E.J. Brill, Leiden.

Lanteri, A. A. 2007. Código de barras del ADN y sus posibles aplicaciones en el campo de la Entomología. Revista de la Sociedad Argentina de Entomología. 66 (3-4): 15-25.

Lipscomb, D., Platnick, N. & Wheeler, Q. 2003. The intellectual content of taxonomy: a comment on DNA taxonomy. Trends in Ecology and Evolution, 18:65-66.

Mallet, J., & Willmott, K. 2003. Taxonomy: renaissance or Tower of Babel?. Trends in Ecology and Evolution, 18 (2): 57-59.

Miller, K. B., Alarie Y., Wolfe, G. W. & Whiting, M. F. 2005. Association of insect life stages using DNA sequences: the larvae of *Philodytes umbrinus* (Motschulsky) (Coleoptera: Dytiscidae). Systematic Entomology, 30: 499-509.

Miller, K. B., Alarie Y. & Whiting, M. F. 2007. Description of the larva of *Notaticus fasciatus* (Coleoptera: Dytiscidae) associated with adults using DNA sequence data. Annals of the Entomological Society of America, 100: 787-797.

Molineri, C. 1999. Revision of the genus *Tricorythopsis* (Ephemeroptera: Leptohyphidae) with the description of four new species. Aquatic Insects, 21(4): 285-300.

Molineri, C. 2001. Una especie nueva de *Tricorythodes* (Ephemeroptera: Leptohyphidae) de la Argentina con notas sobre su Biología. Revista de la Sociedad Argentina de Entomología, 60:61-66.

Molineri, C. 2001. El género *Tricorythopsis* (Ephemeroptera: Leptohyphidae): nuevas combinaciones y descripción de nuevas especies y estadios. Revista de la Sociedad Argentina de Entomología, 60(1-4): 217-238.

Molineri, C. 2002. Cladistic Analysis of the South American species of *Tricorythodes* (Ephemeroptera: Leptohyphidae) with the description of new species and stages. Aquatic Insects, 24: 273-308.

Molineri, C. & Dominguez, E. 2003. Nymph and egg of *Melanemerella brasiliana* (Ephemeroptera: Ephemerelloidea: Melanemerellidae), with comments on its systematic position and the higher classification of Ephemeroptera. Journal of the North American Benthological Society, 22(2): 263-275.

Molineri, C. 2006. Phylogeny of the mayfly family Leptohyphidae (Insecta: Ephemeroptera) in South America. Systematic Entomology, 31:711-728.

Molineri, C. & Zúñiga, M. C. 2006. New species of Leptohyphidae (Insecta: Ephemeroptera) from Colombia with evidence of reproductive time segregation. Studies on Neotropical Fauna & Environment, 41(2):139-151.

O'Donnell, B. C. & Jockusch, E. L. 2008. Phylogenetic relationships of leptophlebiid mayflies as inferred by histone H3 and 28S ribosomal DNA. Systematic Entomology, 33: 651–667.

Ogden, T. H. & Whiting, M. F. 2005. Phylogeny of Ephemeroptera (mayflies) based on molecular evidence. Molecular Phylogenetics and Evolution, 37: 625–643.

Ogden, T. H. & Whiting, M. 2003. The problem with "the Paleoptera problem": Sense and Sensitivity. Cladistics, 19: 432–442.

Ogden, T. H., Osborne, J. T., Jacobus, L. M. & Whiting M. F. 2009. Combined molecular and morphological phylogeny of Ephemerellinae (Ephemerellidae: Ephemeroptera), with remarks about classification. Zootaxa, 1991:28-42.

Peters, W. L. 1981. *Coryphorus aquilus*, a new genus and species of Tricorythidae from the Amazon Basin (Ephemeroptera). Aquatic Insects, 3:209-217.

Peters, W. L., Peters, J. G. 1993. Status changes in Leptohyphidae and Tricorythidae (Ephemeroptera). Aquatic Insects, 15(1): 45-48.

Rapini, A. 2004. Modernizando a taxonomia. Biota Neotropica, 4(1): http://www.biotaneotropica.org.br/v4n1/pt/abstract?point-of-iew+BN00204012004.

Schindel, D. E. & Miller, S. E. 2005. DNA barcoding a useful tool for taxonomists. Nature, 435: 17.

Seberg, O., Humphries, C.J., Knapp, S., Stevenson, D.W., Peteresen, G., Scharff, N. & Andersen, N.M. 2003. Shortcuts in systematics? A commentary on DNA-based taxonomy. Trends in Ecology and Evolution, 18:63-65.

Smith, M. A., Fisher, B. L. & Hebert, P. D. N. 2005. DNA barcoding for effective biodiversity assessment of a hyperdiverse arthropod group: the ants of Madagascar. The Philosophical Transactions of the Royal Society B, 360: 1825-1834.

Sun, L., Sabo, A., Meyer, M. D., Randolph, R. P., Jacobus, L. M., McCafferty, W.P. & Ferris, V.R. 2006. Tests of current hypotheses of mayfly (Ephemeroptera) phylogeny using molecular (18s rDNA) data. Annals of the Entomological Society of America, 99: 241–252.

Traver, J. R. 1958. The subfamily Leptohyphinae (Ephemeroptera: Tricorythidae). Part I. Annals of the Entomological Society of America 51(5):491-503.

Ulmer, G. 1920. Neue Ephemeropteren. Archiv für Naturgeschichte, 85A(11):1-80.

Wiersema, N. A. & McCafferty, W. P. 2000. Generic revision of the North and Central American Leptophyphidae (Ephemeroptera: Pannota). Transactions of the American Entomological Society 126:337-371.

Wiersema, N. A. & McCafferty, W. P. 2005. Contribution to the taxonomy of *Asioplax* (Ephemeroptera: Leptohyphidae: Tricorythodinae) in the New World. Entomological News, 116:147-158.

ARTIGO 1

Studies on Neotropical Fauna and Environment, 43(2008): 237-241.

New species of *Tricorythopsis* Traver (Ephemeroptera: Leptohyphidae) from Northern Brazil

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Abstract

Two new species of *Tricorythopsis* Traver (Ephemeroptera: Leptohyphidae) are described based on nymphs from northern Brazil. *Tricorythopsis yucupe* sp. n. is distinguished by the pronotum with lateral margins expanded; forewing pads elevated just before apex and keel shaped; coxae with projections; femora with short setae; tarsal claws with 3-5 marginal denticles, and two rows of 1-2+1 submarginal denticles; dorsal tubercles present on terga 7-9; color pattern. *Tricorythopsis bahiensis* sp. n. is distinguished by the wide femora bordered with long setae; tarsal claws with 3-4 marginal denticles, and two rows of 5+4 submarginal denticles; dorsal tubercles absent in abdominal segments; color pattern.

Keywords: New species, Leptohyphidae, *Tricorythopsis,* northern Brazil.

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Resumo

Duas novas espécies de *Tricorythopsis* Traver (Ephemeroptera: Leptohyphidae) são descritas baseadas em ninfas do norte do Brasil. *Tricorythopsis yucupe* sp. n. é distinguido pelo pronoto com margens laterais expandidas; extremidade das tecas alares elevadas em forma de quilha; presença de projeção nas coxas; fêmures com cerdas curtas; garra tarsal com 3-5 dentículos marginais e duas fileiras de 1-2+1 dentículos submarginais; tubérculos dorsais presentes nos tergos abdominais 7-9; padrão de coloração. *Tricorythopsis bahiensis* sp. n. é distinguido pelo fêmur largo, bordeado com longas cerdas; garra tarsal com 3-4 dentículos marginais pequenos e duas fileiras de 5+4 dentículos submarginais; ausência de tubérculos abdominais; padrão de coloração.

Palavras-chave: Espécie nova, Leptohyphidae, *Tricorythopsis,* Norte do Brasil.

Introduction

Leptohyphidae (Ephemeroptera) is currently represented in Brazil by seven genera and 26 species (Dias & Salles, 2005, 2006; Domínguez et al., 2006; Siegloch & Froehlich, 2006). Among these, the strictly Neotropical genus *Tricorythopsis* Traver is one of the most representative of the family in South America, specially in the Country. Of the 11 presently described species of *Tricorythopsis*, the following are recorded from Brazil: *T. artigas* Traver, *T. gibbus* (Allen), *T. minimus* (Allen), *T. undulatus* (Allen), *T. sigillatus* Molineri, *T. araponga* Dias & Salles, *T. baptistai* Dias & Salles, *T. pseudogibbus* Dias & Salles, and *T. yacutinga* Molineri (Molineri, 2001; Dias & Salles, 2005; Siegloch & Froehlich, 2006).

The nymphs of this genus are differentiated from the other genera of Leptohyphidae by its small size, generally less than 3 mm, and by the possession of a transversal and poorly sclerotized line in the operculate gills. The adults are differentiated by the presence of two consecutive triads of veins in the cubital and medial sectors of the fore wings, and by the bi-segmented forceps, with the distal segment conical and outside directed (Traver, 1958; Molineri, 2001).

We describe in the present paper two additional new species of *Tricorythopsis* from Brazil, which constituted the first record of the genus from the northern region of country.

Materials and methods

The nymphs were preserved in 80% ethanol. Mouthparts and legs of nymphs were mounted on microscope slides in Euparal and drawn with a camera lucida attached to a stereo microscope. The material is deposited in the following institutions: Invertebrate Collection of the Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil (INPA); Invertebrate Collection of the Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ); Laboratório de Entomologia, Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio Janeiro, Brazil (DZRJ); Instituto-Fundación Miguel Lillo, San Miguel de Tucumán, Tucumán, Argentina (IFML); and Museu

Regional de Entomologia, Universidade Federal de Viçosa, Minas Gerais, Brazil (UFVB).

Results

Tricorythopsis yucupe sp. n.

Nymph. Length of male: body, 3.0-3.2 mm; mesonotum, 1-1.2 mm; caudal filaments, 1.8 mm. Length of female: body, 3.5-3.8 mm; mesonotum, 1.2 mm; caudal filaments, 1.7-1.9 mm.

General. Coloration dark brown, with yellowish marks (Fig. 1). Large size, more than 3 mm (species of the genus often have less than 3.0 mm).

Head. Yellowish brown, except posterior margin, darker brown (Fig. 1). Mouthparts yellowish; maxillary palp bi-segmented with short apical seta (Fig. 2).

Thorax. General coloration dark brown with pale yellowish regions. Pronotum dark brown, with pale yellowish marks in submedian regions and lateral margins; lateral margins expanded (Fig. 1). Mesonotum dark brown, submedian region with pale yellowish marks (Fig. 1). Forewing pads elevated just before apex, keel shaped (Fig. 1, 3). Pleura and sterna dark brown, with yellowish marks. Legs: Coxae with projections, hind coxae with projection absent or reduced (Figs. 4-6). Femora of all legs dark brown, except for a median yellowish zone; fore femora with subdistal transversal row of setae; femora narrow (at least two times longer than wide), and with short setae (Figs. 4-7). Tibiae yellowish with median transversal brown band (Figs. 4-6). Tarsal claws yellow, with 3-5 large marginal denticles, and two rows of 1-2+1 submarginal denticles; and with apical seta (Fig. 8).

Abdomen. Dark brown, except for a yellowish zone on terga 4-7 near operculate gills (Fig. 1). Median dorsal tubercles present on terga 7-9 (Fig. 1). Lateral margins of segments 3-9 laterally expanded; segments 5-9 with posterolateral projections. Sterna yellowish, with brown marks. Operculate gills yellow, base and lateral margins shaded

with brown (Fig. 9); remaining gills completely shaded with light grey. Caudal filaments yellow.

Adults. Unknown.

Diagnosis

1) Pronotum with lateral margins expanded (Fig. 1); 2) forewing pads elevated just before apex and keel shaped (Figs. 1, 3); 3) coxae with projections (Figs. 4-6), hind coxae with projection absent or reduced; 4) femora narrow (at least two times longer than wide), and with short setae (Fig. 7); 5) tarsal claws with 3-5 marginal denticles, and two rows of 1-2+1 submarginal denticles (Fig. 8); 6) tubercles present on terga 7-9 (Fig. 1); 7) abdominal color pattern (Fig. 1).

Discussion

Tricorythopsis yucupe sp. n. is the third species of *Tricorythopsis* in which the nymphal forewing pads is elevated just before the apex; the others are *T. gibbus* and *T. pseudogibbus.* However, the shape of the elevation, the location of the dorsal tubercles on abdominal segments, color pattern and the number of denticles on the tarsal claws, distinguish *T. yucupe* sp. n. from these possibly related species. Besides these characteristics, the reduced maxillary palp of *T. gibbus* and *T. pseudogibbus* and the large size of *T. yucupe* sp. n. (species of the genus often have less than 3.0 mm), allow the differentiation between them.

Material

Holotype: 1 nymph, Brazil, Roraima state, Rio Ereu, riffle litter, 24/x/2004, N. Hamada, F.F. Salles col. (INPA). Paratypes: 2 nymphs, same data as holotype (INPA). 2 nymphs, same data as holotype (DZRJ). 2 nymphs, same data as holotype (MNRJ). 3 nymphs, Brazil, Roraima state, Contigo, 23/x/2004, N. Hamada col (IFML).

Other material studied: 3 nymphs, Brazil, Amazonas state, Presidente Figueiredo, 08/x/2003, N. Hamada, F.F. Salles col. (INPA). 2 nymphs, Brazil, Amazonas state,

Presidente Figueiredo, 07/x/2003, N. Hamada, F.F. Salles col. (INPA). 3 nymphs, Brazil, Roraima state, Surumu, 22/iii/2002, N. Hamada col. (UFVB).

Distribution

Brazil: Roraima and Amazonas.

Etymology

"Yucupe", an indigenous name from the Tupi Guarani language. *Yu*, spine and *cupe*, back; the epithet is an apposition to the forewing pads projection of the new species.

Tricorythopsis bahiensis sp. n.

Nymph. Length of male: body, 2.4-2.7 mm; mesonotum, 0.6-0.9 mm; caudal filaments, 1.1-1.7 mm. Length of female: body, 3.0-3.1 mm; mesonotum, 1.0-1.3 mm; caudal filaments, 1.7 mm.

General. Coloration yellow shaded with grey (Fig. 10).

Head. Yellow with posterior region shaded with grey (Fig. 10). Mouthparts uniformly yellowish; maxillary palp bi-segmented with apical seta (Fig. 16).

Thorax. Pronotum yellow shaded with grey, except for yellowish marks in lateral and submedian region (Fig. 10). Mesonotum and mesoescutellum yellow shaded with light grey, darker between wing pads (Fig. 10). Sterna whitish. Pleura whitish shaded with grey. Legs: Coxae without projections. Femora of all legs yellow shaded with grey, except in the median region, yellowish (Figs. 11-13); fore femora with subdistal transversal row of setae; femora wide, (at least as wide as long), bordered with long setae (Figs. 11-14). Tibiae yellowish with transversal light grey band in median region (Figs. 11-13). Tarsi yellowish with subbasal transversal light grey band; tarsal claws with 3-4 marginal denticles, with two rows of large submarginal denticles, one side with 5 and the other with 3-4 denticles (Fig. 15).

Abdomen. Yellow shaded with grey on terga 1-5; terga 6-9 with darker marks in submedian and median zones (Fig. 10). Sterna whitish yellow, with greyish dorsolateral

marks. Lateral margins of abdominal segments 2-9 expanded; segments 6-9 with posterolateral projections bordered with setae. Operculate gills completely shaded with grey, darker at base, paler toward apex (Fig. 17); remaining gills completely shaded with light grey. Caudal filaments yellowish, basal region grey.

Adults. Unknown.

Diagnosis

1) Femora wide (at least as wide as long), and bordered with long setae (Figs. 11-13); 2) tarsal claws with 3-4 small marginal denticles, and two rows of 5+3-4 submarginal denticles (Fig. 15); 3) dorsal tubercles absent in abdominal segments (Fig. 10); 4) abdominal color pattern as in Fig. 10.

Discussion

Tricorythopsis bahiensis sp. n., as well as *T. yacutinga* and *T. araponga*, presents the nymphal femora bordered with long setae. Nevertheless, while the tarsal claws with marginal denticles distinguish the new species from *T. yacutinga*, the absence of dorsal tubercles in abdominal segments distinguishes it from *T. araponga*.

Material

Holotype: 1 nymph, Brazil, Bahia state, 13°31’25.1’’S, 44°43’30.7’’W, Correntina, Comunidade da Prainha, Rio Arrojado, 05/viii/2003, N. Hamada, F.F. Salles, col. (INPA). Paratypes: 1 nymph, same data as holotype (DZRJ). 1 nymph, same data as holotype (DZRJ). 2 nymphs, same data as holotype (MNRJ). 2 nymphs, same data as holotype (IFML).

Other material studied: 10 nymphs, Brazil, Roraima state, 03°21.0’38’’N 59°54’255’’W, Arraiá, 22/iii/2001, N. Hamada col. (INPA); 3 nymphs, Amazonas state, Presidente Figueiredo, 14/x/2003, N. Hamada, F.F. Salles col (INPA); 1 nymph, same data as holotype, except 03/viii/2003 (UFVB).

Distribution

Brazil: Bahia, Amazonas and Roraima.

Etymology

The epithet of the new species is a reference to Bahia, the state where the holotype was found.

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References

Dias LG, Salles FF. 2005. Three new species of *Tricorythopsis* (Ephemeroptera: Leptohyphidae) from southeastern Brazil. Aquat Insect 24:235-241.

Dias, LG, Salles, FF. 2006. A new species of *Tricorythodes* (Ephemeroptera: Leptohyphidae) from Minas Gerais, southeastern Brazil. Neotrop Entomol 31:56-58.

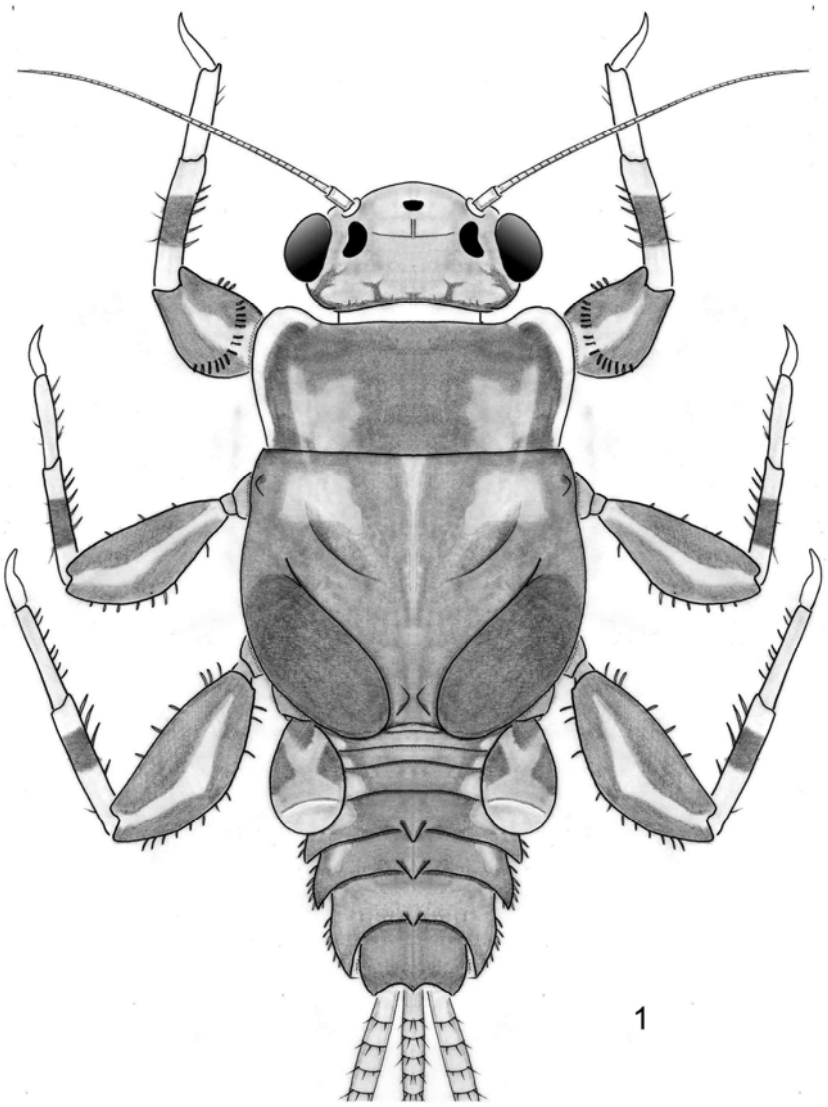
Domínguez E, Molineri C, Pescador M, Hubbard MD, Nieto C. 2006. Ephemeroptera of South America In: Adis J, Arias JR, Rueda-Delgado R, Wantzen KM. Aquatic biodiversity in Latin America, Vol. 2. Sofia-Moscow, Pensoft. 642 p.

Molineri C. 2001. El género *Tricorythopsis* (Ephemeroptera: Leptohyphidae): nuevas combinaciones y descripción de nuevas especies y estadios. Rev Soc Entomol Argent 60:217-238.

Siegloch AE, Froehlich CG. 2006. Insecta, Ephemeroptera, Leptohyphidae, *Tricorythopsis yacutinga*: First record to Brazil. Check List 2(3). Available from: www.checklist.org.br/getpdf?NGD035-06

Traver JR. 1958. The subfamily Leptohyphinae (Ephemeroptera: Tricorythidae). Part I: Ann Entomol Soc Amer 51:491 – 503.

Figure 1. *Tricorythopsis yucupe* sp. n.: Nymphal habitus (dorsal view).



Figures 2-9. *Tricorythopsis yucupe* sp. n.: 2. Maxilla (dorsal view). 3. Thorax (lateral view). 4. Foreleg. 5. Mid leg. 5. Hind leg. 7. Detail of femoral setae. 8. Foreclaw (detail). 9. Operculate gill (dorsal view).

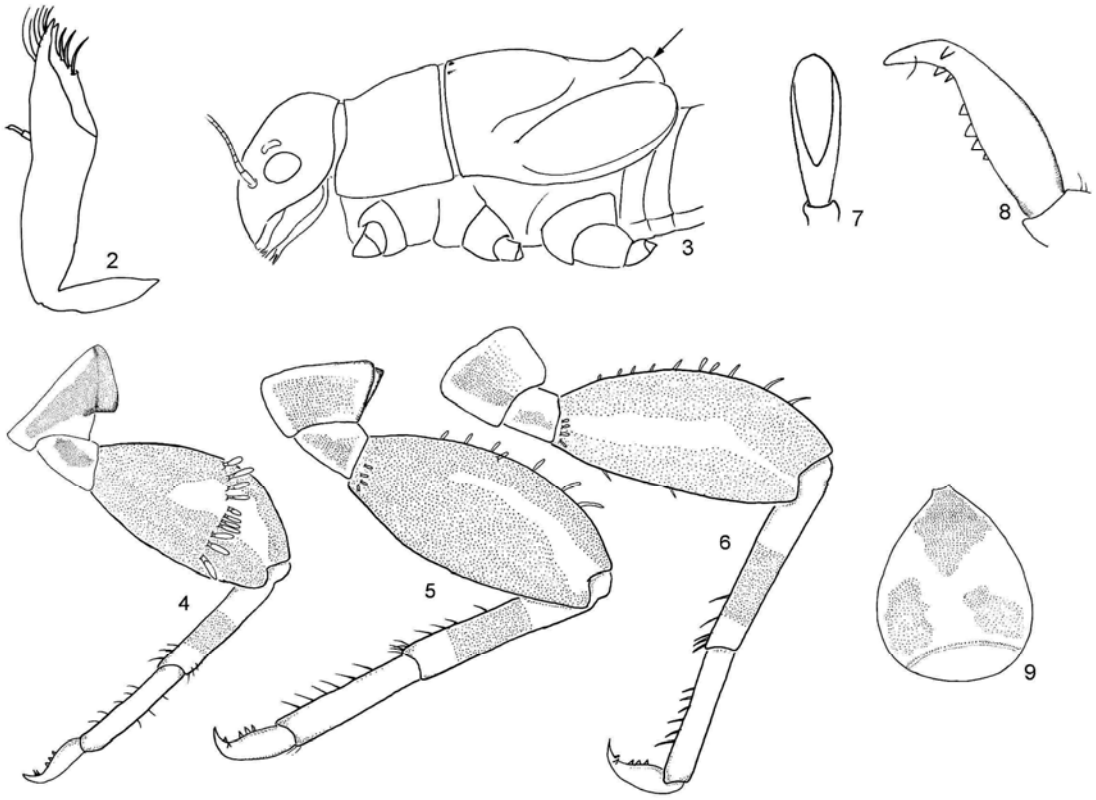
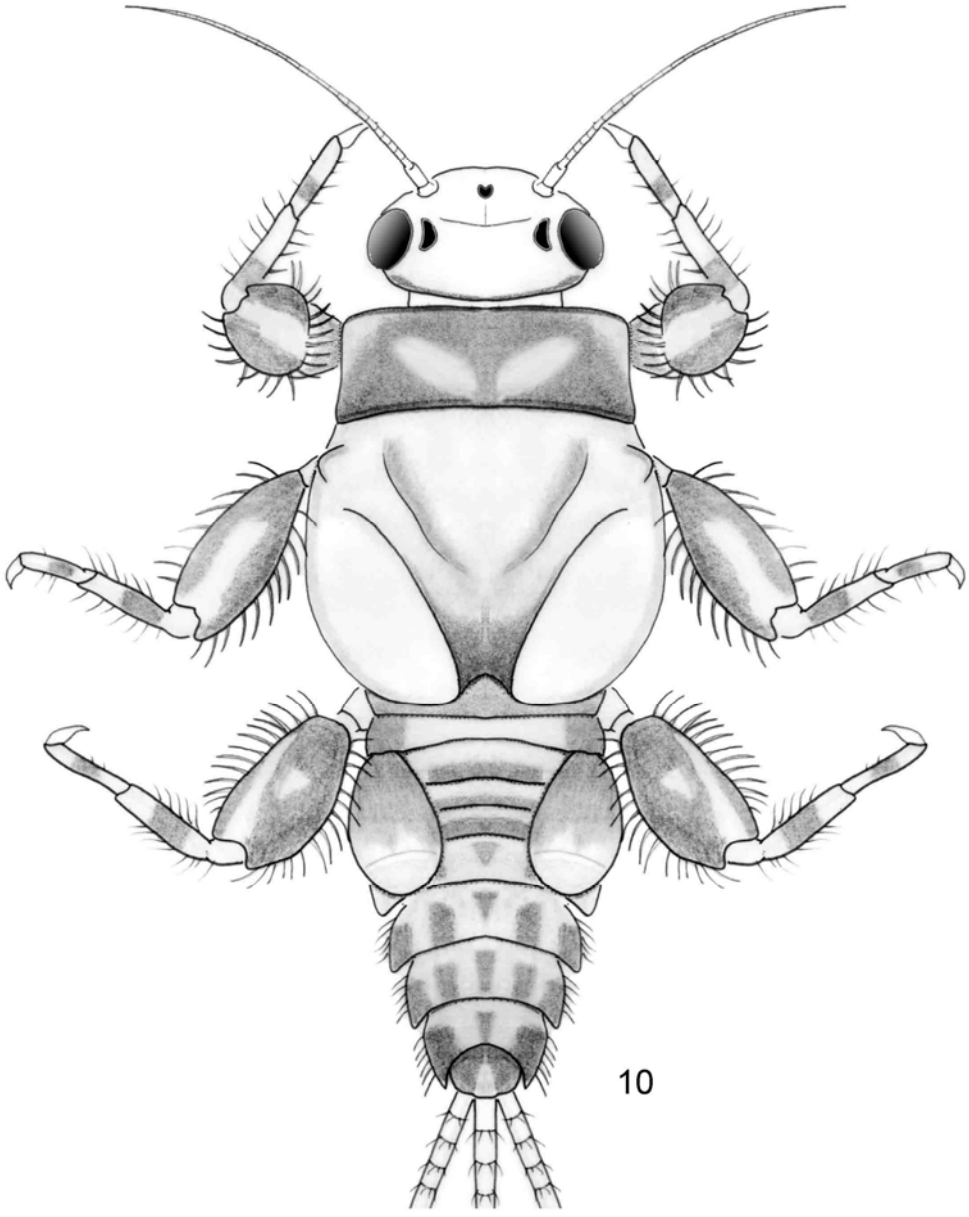
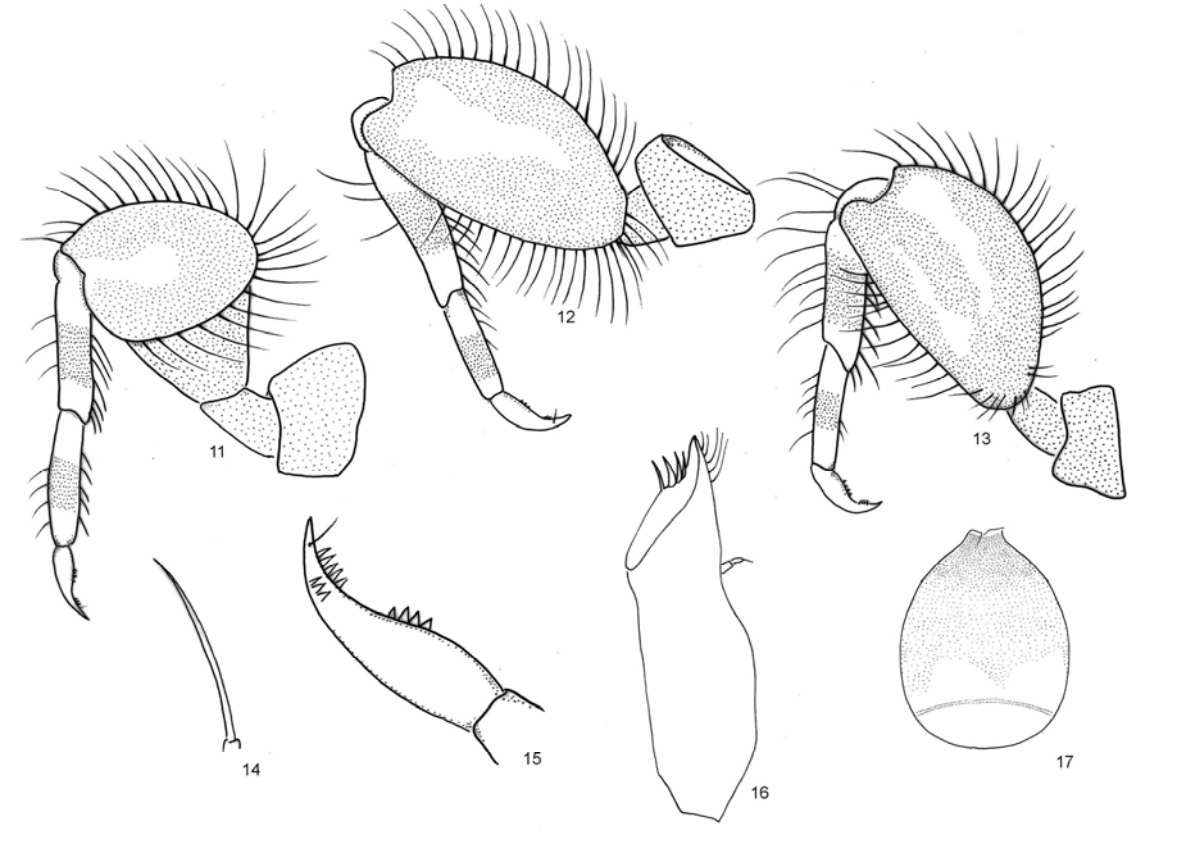


Figure 10. *Tricorythopsis bahiensis* sp. n.: Nymphal habitus (dorsal view).



Figures 11-17. *Tricorythopsis bahiensis* sp. n.: 11. Foreleg. 12. Mid leg. 13. Hind leg. 14. Detail of femoral setae. 15. Foreclaw (detail). 16. Maxilla (dorsal view). 17. Operculate gill (dorsal view).



ARTIGO 2

Annales de Limnology – International Journal of Limnology, 45 (2009): 127–129.

A New species of the *Tricorythodes* Ulmer (Ephemeroptera: Leptohyphidae) From Northern Brazil

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Abstract

A new species of *Tricorythodes* Ulmer (Ephemeroptera: Leptohyphidae) is described based on nymphs from the Northern Region of Brazil. *T. rondoniensis* sp. n. can be distinguished from other species in the genus by the following characters: wide femora, nearly circular and with the margins covered with very long setae; tarsal claws with 5 – 6 marginal denticles; terga III-VII with an acute median tubercle; operculate gill ovoid and abdominal colour pattern.

Keywords: Ephemeroptera, Pannota, *Tricorythodes*, new species, South America.

Introduction

Tricorythodes Ulmer (Leptohyphidae) is a Pan American genus with wide distribution in South America. In the last 6 years, the number of species of *Tricorythodes* recorded from this region has doubled; more of 10 species of this genus were described in this period (Molineri 2002, Dias & Salles 2006, Molineri & Zúñiga 2006, Emerich 2007). Currently in South America, *Tricorythodes* is constituted by the following species: *T. arequita* Traver, *T. barbus* Allen, *T.bullus* Allen, *T. capuccinorum* Emmerich, *T. cristatus* Allen, *T.curiosus* (Lugo-Ortiz & McCafferty), *T. hiemalis* Molineri, *T. lihyi* Traver, *T. mirca* Molineri, *T. molinerii* Dias & Salles, *T. nicholsae* (Wang et al.), *T. ocellus* Allen & Roback, *T. popayanicus* Domínguez, *T. quizeri* Molineri, *T. santarita* Traver, *T. trifasciatus* Molineri & Zúñiga, *T. uniandinus* Emmerich, *T. yura* Molineri, *T. zunigae* Molineri (Domínguez et al. 2006, Dias & Salles 2005, Molineri & Zúñiga 2006, Emmerich 2007). In this work, we describe a new species of *Tricorythodes* based on nymphs.

According to the classification proposed by Wiersema & McCafferty (2000), based on morphological characters the new species should be included in the genus *Asioplax* Wiersema & McCafferty, one of the genera established by these authors to include some species of *Tricorythodes*. Nevertheless, we prefer the cladistic-based classification proposed by Molineri (2002), which considers *Tricorythodes* (sensu lato) as a unity. The material studied of *Tricorythodes rondoniensis* sp. n. originates from the Brazilian states of Rondonia and Roraima, both belong to the north region of Brazil.

Material and methods

The type material is deposited in the following institutions: Entomological Collection of the Instituto Nacional de Pesquisas da Amazônia, Amazonas, Brazil (INPA); Invertebrate Collection of the Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ); Instituto-Fundación Miguel Lillo, San Miguel de Tucumán, Tucumán, Argentina (IFML); and Entomological Collection of the Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (DZRJ).

The length of the body, mesonotum and caudal filaments were measured in mature nymphs. Drawings were made on white paper with the aid of a camera lucida attached to a MZ8 Leica microscope.

***Tricorythodes rondoniensis* sp n.**

Nymph. Length of male: body, 1.9 – 2.5 mm; mesonotum, 0.50 – 0.70 mm; caudal filaments, 1.0 – 1.2 mm. Length of female: body, 2.3 – 2.5 mm; mesonotum, 0.60 – 0.70 mm; caudal filaments, 1.3 – 1.5 mm.

General aspect: small body slightly depressed; wide femora covered with long setae. General coloration yellowish with brown and reddish marks in the dorsal region (Fig. 01), ventrally whitish.

Head yellowish with reddish marks on posterior margin, between ocelli and antennae (Fig. 01). Antennae yellowish translucent. Mouthparts yellowish. Maxillary palp 2-segmented with short apical seta (Fig. 02).

Pronotum yellowish almost completely shaded with brown and reddish, except anterolateral margin and median region, yellowish. Mesonotum yellowish with diffuses brown marks, sometimes with median region completely yellowish. Developing wings blackish, with internal margin reddish. Legs with wide femora, nearly circular and with the margins covered by very long, fine and pectinate setae (Figs. 03, 05-07); dorsal region of femora yellowish with circular small whitish marks distributed irregularly and longitudinal marks brownish (Figs. 05-07); tibiae and tarsi yellowish, with a reddish band on median zone; tarsal claws with 5 – 6 marginal denticles, submarginal denticle absent, and with subapical seta.

Abdomen terga I-V yellowish shaded with brown (Fig. 01); lateral margin of terga III-V whitish; terga VI–VIII yellowish brown, with median triangular region whitish, this whitish region increasing in size from terga VI to the VIII (Fig. 01); terga IX yellowish brown with submedian marks whitish and terga X yellowish brown with median marks whitish. Lateral margins of abdominal segments III-IX expanded, posterolateral spines present on segments VII-IX bordered with setae (Fig. 01). Terga III-VII with acute median tubercle, larger on terga VI-VII (Fig. 01). Operculate gill ovoid (Fig. 08), brown with whitish marks on median and distal region; ventral lamellae of operculate gill long.

Gill formula: 2/3/3/3/2 (Figs. 09-13). Gills III–VI with a small blackish mark at base (10-13). Caudal filaments brownish or grayish, with posterior region yellowish-translucent, with short setae at joinings.

Material. Holotype: 1 nymph, Brazil, Rondônia state, Porto Velho, Rio Mamãe Quinó, BR 364, 27-viii-2006, Cruz, P.V. (INPA). **Paratype:** 1 nymph, same data as holotype (IFML). 2 nymphs, Roraima state, Boa vista, Rio Cauamé, 3.ii.2007, Jesine Falcão col. (INPA). 1 nymph, Roraima state, Boa Vista, Rio Marupú, 10.xi.2006, Jesine Falcão col. (DZRJ). 1 nymph, Roraima state, Amajari, Igarapé do Pedral, Serra do Tepequém, 16.xi.2006, Jesine Falcão col. (MNRJ).

Diagnosis. 1) Maxillary palp 2-segmented, with apical seta (Fig. 2); 2)wide femora, nearly circular and with the margins covered with very long setae (Figs. 05-07); 3) tarsal claws with 5 – 6 marginal denticles and without submarginal denticles (Fig. 04); 4) terga III-VII with a single acute median tubercle (Fig. 01); 5) lateral margins of abdominal segments III-IX expanded, posterolateral spines present on segments VII-IX (Fig. 01); 6) abdominal colour pattern (Fig. 01); 7) operculate gill ovoid, with a long ventral lamellae (Fig. 9).

Discussion. The nymphs of the new species possess operculate gill ovoid and body relatively small as the species included in *Asioplax* genus by Wiersema & McCafferty (2000, 2005) and Baumgardner et al.(2006), they are: *Asioplax corpulenta* (Kilgore & Allen), *A. dolani* (Allen), *A. edmundsi* (Allen), *A. nicholsae* (Wang et al.), *A. sacculobranchis* (Kluge & Naranjo), *A. sierramaestrae* (Kluge & Naranjo), *A. curiosus* (Lugo-Ortiz & McCafferty), *A. numinuh* (Wiersema et al.), *A. santarita* (Traver), *A. texana* (Traver), *A. zunigae* (Molineri) and *A. isabelia* (Baumgardner et al.). In addition, *T. rondoniensis* sp. n. shows close affinities with *T.santarita* Traver and *T. nicholsae* (Wang et al.), due to that all these species possess femora with long setae, maxillary palp reduced and the presence of median tubercles on abdominal terga. However, the differences among these three species are the number of denticles on tarsal claws, localization of the abdominal tubercles on the terga and abdominal colour pattern.

Etymology

The epithet of the new species is a reference to Rondônia, the state where the holotype was found.

Acknowledgments

We thank the CNPq and CAPES for providing funds to LGD and PVC to conduct postgraduate studies at the Universidade Federal de Viçosa and Instituto Nacional de Pesquisas da Amazônia, respectively. We also thank to Dr. Neusa Hamada and Jesine Falcão (Instituto Nacional de Pesquisas da Amazônia) for supplying the specimens from Roraima.

References

Baumgardner, D.E.; Meyer M.D. & McCafferty, W.P. 2006. — A new species of Asioplax (Ephemeroptera: Leptohyphidae) from Costa Rica and Nicaragua. *Pan-Pacific Entomol.,* 82(3/4):346-350.

Dias L.G. & Salles F.F. 2006. — A new species of *Tricorythodes* (Ephemeroptera: Leptohyphidae) from Minas Gerais, Southeastern Brazil. *Neotrop. Entomol.*, 31:56-58.

Domínguez E., Molineri C., Pescador M., Hubbard M.D. & Nieto C. 2006. *Aquatic Biodiversity in Latin America.* Pensoft, Sofia-Moscow, v.2: Ephemeroptera of South America, 646 p.

Emmerich D.E. 2007. — Two new species of *Tricorythodes* Ulmer (Ephemeroptera: Leptohyphidae) from Colombia, *Zootaxa*, 1561: 63–68.

Molineri C. 2002. — Cladistic analysis of the South American species of *Tricorythodes* (Ephemeroptera: Leptohyphidae) with the descriptions of new species and stages, *Aquat. Insects*, 24: 273-308.

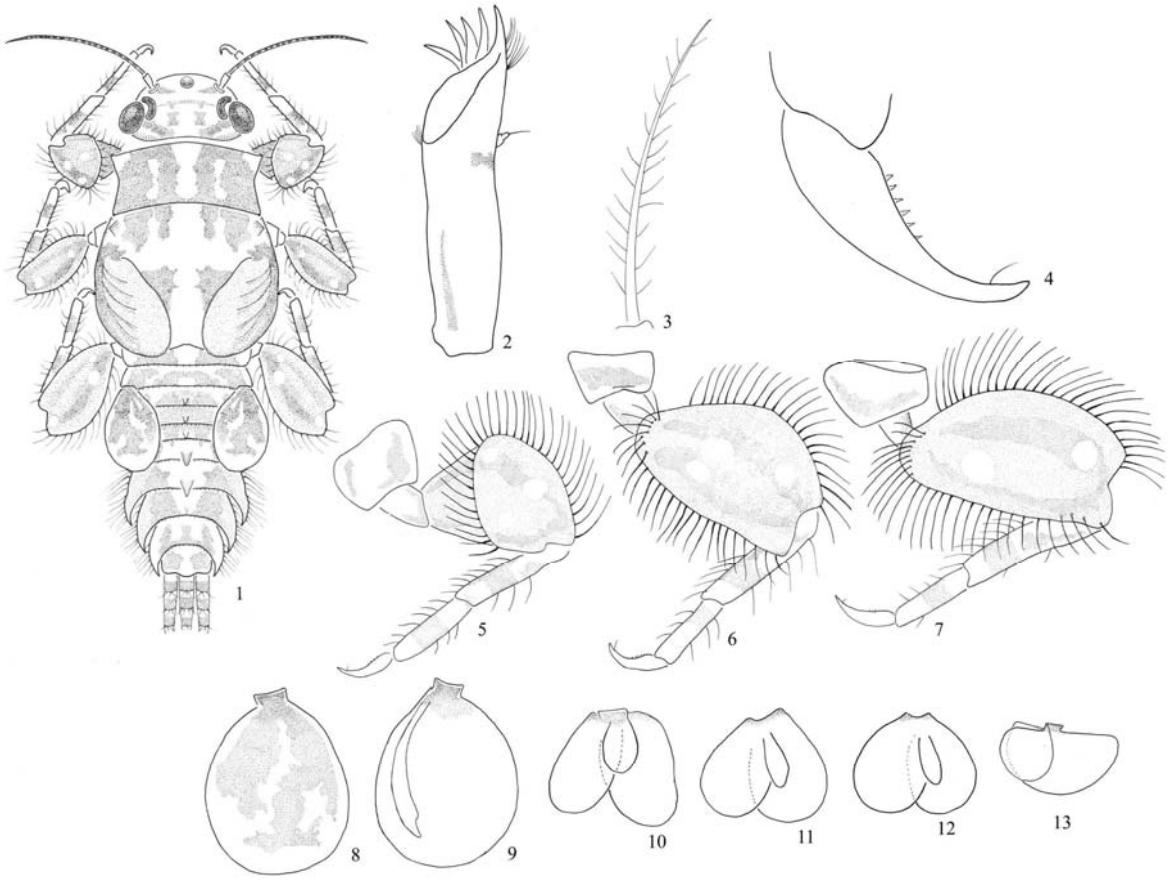
Molineri C. & Zuñiga M.C. 2006. — New species of Leptohyphidae (Insecta: Ephemeroptera) from Colombia with evidence of reproductive time segregation, *Stud. Neotrop. Fauna Environ.*, 41(2): 139 – 151.

Wiersema N.A. & McCafferty W.P. 2000. — Generic Revision of the North and Central American Leptohyphidae (Ephemeroptera: Pannota). *Trans. Amer. Entomol. Soc.*, 126:337- 371.

Wiersema, N.A. & McCafferty, W.P. 2005. — Contribution to the taxonomy of Asioplax (Ephemeroptera: Leptohyphidae: Tricorythodinae) in the New World. *Entomol. News.*, 116:147-158.

Figure legends

Figures 1-13. *Tricorythodes rondoniensis* sp. nov.: 1. Nymphal habitus (dorsal view). 2. Maxilla (dorsal view). 3. Detail setae of femora. 4. Foreclaw (detail). 5. Foreleg. 6. Mid-leg. 7. Hind leg. 8. Operculate gill (dorsal view). 9. Operculate gill (ventral view). 10-13. Gills III-VI (ventral view).



ARTIGO 3

Aquatic Insects (2009), In Press

A new species of *Tricorythodes* Ulmer (Ephemeroptera: Leptohyphidae) and first record of *Tricorythodes quizeri* Molineri from Brazil

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A new species of *Tricorythodes* Ulmer (Ephemeroptera: Leptohyphidae), is described and illustrated based on larvae from Brazil. *T. sallesi* sp. nov. is distinguished from other species of the genus due to the presence of a distinctive spine-like anterolateral projection of the pronotum, besides its tarsal claws morphology and colouration pattern. In addition, the species *Tricorythodes quizeri* Molineri is recorded for the first time from Brazil. Habitat and ecological information associated with these two species are commented.

Keywords: Taxonomy, Pannota, new species, new record, Ephemerelloidea, *Tricorythodes*.

Introduction

Tricorythodes Ulmer is a diverse Pan-American genus of the Leptohyphidae. The genus is represented by eighteen species in South America (Dias & Salles 2006, Molineri 2002, Molineri & Zúñiga 2006, Emmerich 2007). There are six species recorded from Brazil: *T. arequita* Traver, *T. barbus* Allen, *T. bullus* Allen, *T. cristatus* Allen, *T. molinerii* Dias & Salles, and *T. santarita* Traver (Dias et al. 2007).

The larvae of this genus are differentiated from the other genera of the family by their body, legs and operculate gills covered with long setae, and by the operculate gills that generally have a triangulate shape. The adults are dipterous mayflies with very broad wings and with a basal swelling in the second joint of the forceps of the male (Molineri 2002). In the present paper, we describe a new species of *Tricorythodes* based on larvae and record for the first time *Tricorythodes quizeri* Molineri from Brazil.

Material and Methods

Material was preserved in 80% ethanol. Body parts of larvae were mounted on microscope slides in euparal and drawn with a camera lucida attached to a stereo microscope. The length of the body, mesonotum and caudal filaments were measured in mature larvae.

The material is deposited in the following institutions: Invertebrate Collection of the Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ); Instituto-Fundación Miguel Lillo, San Miguel de Tucumán, Tucumán, Argentina (IFML); Entomological Collection of the Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (DZRJ); Museu Regional de Entomologia, Universidade Federal de Viçosa, Minas Gerais, Brazil (UFVB); and Coleção Zoobotânica James A. Ratter, Universidade do Estado de Mato Grosso, Nova Xavantina, MT, Brazil (CZNX).

Systematic account

***Tricorythodes sallesi* sp. nov.** (Figures 1-12)

Material examined Holotype: 1 larva, Brazil, Mato Grosso state, Nova Xavantina, Córrego do Papagaio, S 15º 27' 32" - W 52º 24' 42", 14/vii/2005, De Souza, D.P. and

Cabette, H.S.R. cols. (MNRJ). Paratypes: 1 larva, same data as holotype (MNRJ). 2 larvae, same data as holotype (DZRJ). 2 larvae, same data as holotype (IFML). Other material. 2 larvae, same data as holotype, except, 20/xi/ 2005 (UFVB). 2 larvae, same data as holotype, except, 20/xi/ 2005 (CZNX).

Mature larva

Length: body, 4.0-5.5 mm; mesonotum, 1.3-2.0 mm; caudal filaments, 2.6-3.0 mm (n=5). General colouration yellowish with blackish diffuses marks (Fig. 1).

Head. Genal projection slightly developed. Colouration yellowish with blackish diffuse marks (Fig. 1). Antennae yellowish-translucent. Mouthparts yellowish; maxillary palp three-segmented without apical seta (Figs. 2, 3); median region of mentum and labrum shaded with black.

Thorax. General colouration yellowish with blackish diffuse marks (Fig. 1). Pronotum with anterolateral spine-like projection (Fig. 1); colouration yellowish with blackish diffuse marks, more intense on submedian regions (Fig. 1). Mesonotum yellowish with blackish diffuse marks, except in forewing pads, whitish (Fig. 1); sometimes with a reddish mark in the base of the forewing pads. Metanotum, pleurae and sterna yellowish with blackish marks. Legs narrow and long; colouration yellowish, dorsal region of all femora with blackish diffuse marks; with a large blackish mark on subapical region of all femora and tibiae (Figs. 5-6); tarsi without marks; dorsum of forefemora with a transverse row of setae on submedian region (Fig. 6); tarsal claws with 10-12 marginal denticles and without submarginal denticles (Fig. 4).

Abdomen. General colouration yellowish with blackish diffuse marks. Terga with more intense marks on median and lateral regions of all segments (Fig. 1). Sterna yellowish shaded with blackish marks, lateral regions of sterna III-VI shaded with grey. Lateral margins of abdominal segments III-VII expanded; segments VII-IX with posterolateral projections and with a row of setae on posterior margin (Fig. 1). Operculate gills triangular, yellowish with blackish or reddish diffuse marks (Fig. 7), operculate gills formed by three lamellae (fig. 8); remaining gills as in Fig. 9-12. Caudal filaments yellowish with whorls of setae at joints.

Adults. Unknown

Etymology

This species is dedicated to Dr. Frederico Falcão Salles, Universidade Federal do Espírito Santo, in recognition of his numerous important contributions to the study of Ephemeroptera from Brazil.

Diagnosis

T. sallesi sp. nov. can be distinguished from other species of the genus by the following combination of characters: 1) maxillary palp three-segmented without apical setae (Figs. 2-3); 2) pronotum with spine-like anterolateral projection (Fig. 1); 3) legs with subapical blackish marks on femora and tibia, tarsi without marks (Figs. 5, 6); 4) dorsum of fore femora with a transversal row of setae in the submedian region (Fig. 6); 5) tarsal claws with 10-12 marginal denticles and without submarginal denticles (Fig. 4); 6) operculate gills triangular, yellowish with blackish diffuse marks (Fig. 7); 7) lateral margins of abdominal segments 3-7 expanded, segments 7-9 with posterolateral projections (Fig. 1).

Discussion

Larvae of *T. sallesi* sp. nov. show affinities with *T. mirca* Molineri, 2002 and *T. arequita* Traver, 1959, these species possess similar colour pattern, formed by pigmented marks distributed irregularly and present blackish marks on the apex of femora and tibia. Based on the general morphologic aspects of the body, as legs relatively elongate, femora narrow-elongate, more than twice as long as broad, tarsal claw morphology, the new species appears more related with *T. bullus* Allen, 1967 and *T. cristatus* Allen, 1967. However, *T. sallesi* sp. nov. can be easily separated from these related species (and all other of the genus) by the presence of the unique spine-like anterolateral projections on the pronotum and absence of dorsal tubercles in the head an thorax. An additional difference between these three species is the number of denticles on the tarsal claws.

***Tricorythodes quizeri* Molineri, a new record from Brazil**

Tricorythodes quizeri Molineri 2002:290

Material examined. 3 larvae, Brazil, Mato Grosso state, Nova Xavantina, Córrego da Mata, S 14º 59' 59" - W 52º 26' 29", 12/i/2005, De Souza, D.P. and Cabette, H.S.R. cols. (MNRJ). 2 larvae, Brazil, Mato Grosso state, Nova Xavantina, Córrego do Papagaio, S 15º 27' 32" - W 52º 24' 42", 14/vii/2005, De Souza, D.P. and Cabette, H.S.R. cols. (DZRJ).

Tricorythodes quizeri was described by Molineri (2002) from Santa Cruz in Bolivia based on larvae and adults. We studied larvae of *T. quizeri* from Mato Grosso, a Brazilian State close to the type locality of this species in Bolivia. These larvae do not show marked differences with the type material and can be distinguished from all other species of the genus by the same original characters described in Molineri (2002).

Ecological data

The two species were collected in streams at Cerrado biome, Nova Xavantina, Mato Grosso state. This zone is of hot and sub-humid tropical climate, with mean air temperatures of 25°C and mean annual precipitation of 1750 mm. The sampling points have original riparian vegetation (between 10-15m of width), despite some areas influenced by grass with cattle exploitation. The water temperature of the streams varied between 20-24 °C, values for dissolved oxygen ranged from 5.9 to 7.2 mg/L and pH varied between 6.6 to 7.5. Mean values for the Habitat Integrity Index (Nessimian et al. 2008), varied between 0.66 to 0.89, corresponding to streams with relatively good water quality. *T. sallesi* sp. nov. and *T. quizeri* were found frequently associated to the marginal bank with submerged roots substrate deposited in zones with some detritus. So as in many species of *Tricorythodes* Ulmer, the collecting specimens also have detritus retained among the setae of body.

Acknowledgements

We are grateful to Dr. Carlos Molineri (Facultad de Ciencias Naturales e Instituto Miguel Lillo – San Miguel de Tucumán, Argentina) for their helpful review of the manuscript. We thank the CNPq for providing funds to LGD to conduct post-graduate studies at the Universidade Federal de Viçosa.

References

Allen, R.K. (1967), "New Species of New World Leptohyphinae (Ephemeroptera: Tricorythidae)". *The Canadian Entomologist*, 99:350-375.

Dias, L.G., Salles, F.F. (2006), **"**A new species of *Tricorythodes* (Ephemeroptera: Leptohyphidae) from Minas Gerais, Southeastern Brazil", *Neotropical Entomology*, 31, 56-58.

Dias, L.G., Molineri, C., Ferreira P.S.F. (2007), "Ephemerelloidea (Insecta: Ephemeroptera) do Brasil", *Papéis Avulsos de Zoologia*, 47, 213-244.

Emmerich, D.E., (2007), **"**Two new species of *Tricorythodes* Ulmer (Ephemeroptera: Leptohyphidae) from Colombia", *Zootaxa*, 1561, 63–68.

Molineri, C., (2002), "Cladistic analysis of the South American species of *Tricorythodes* (Ephemeroptera: Leptohyphidae) with the descriptions of new species and stages", *Aquatic Insects*, 24, 273-308.

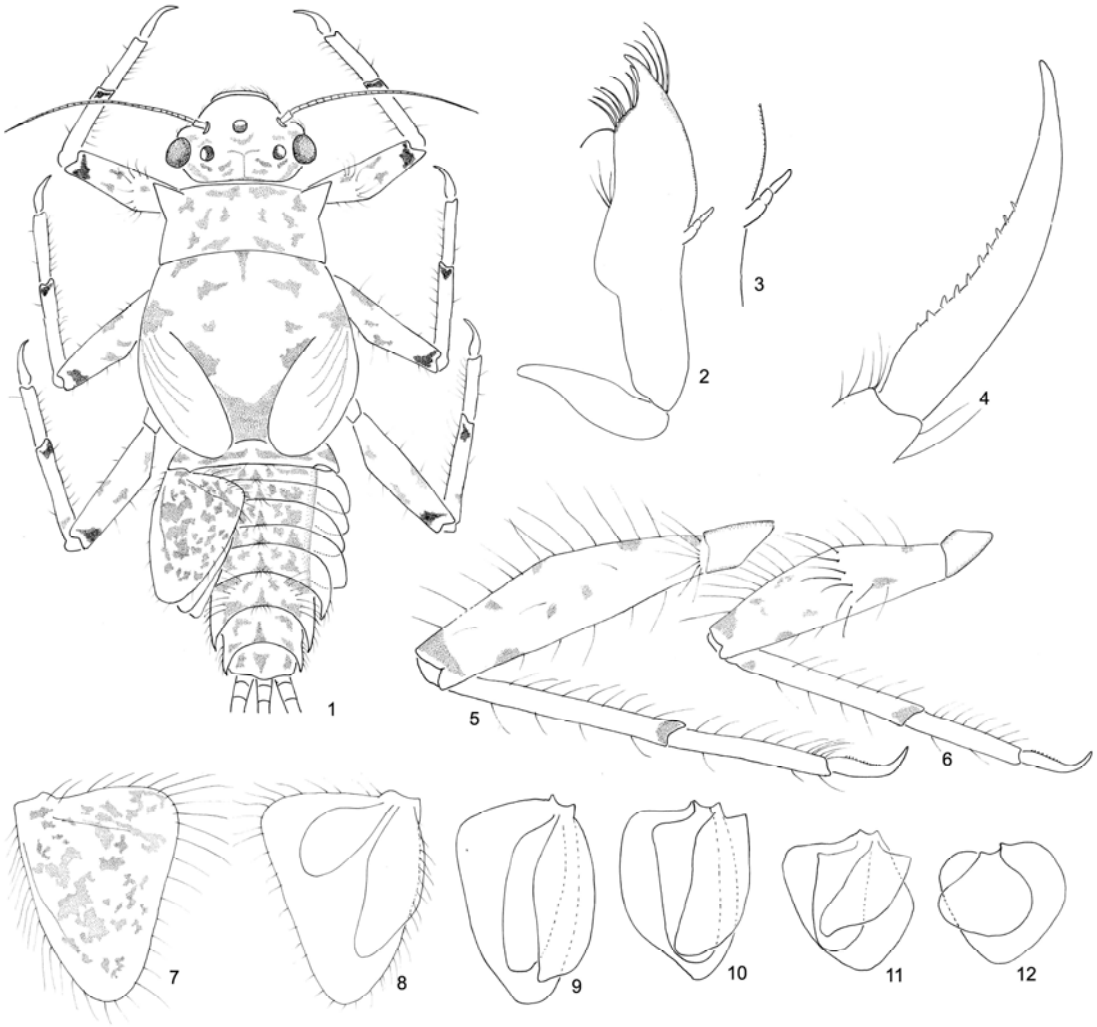
Molineri, C, Zuñiga, M.C. (2006), **"**New species of Leptohyphidae (Insecta: Ephemeroptera) from Colombia with evidence of reproductive time segregation," *Studies on Neotropical Fauna and Environment*, 41(2), 139–151.

Nessimian, J.L., Venticinque, E.M., Zuanon, J., De Marco Jr., P., Gordo, M., Fidelis, L., Batista, J.D., Juen, L. (2008), "Land use, habitat integrity and aquatic insect assemblages in central Amazonian streams", *Hydrobiologia,* 614:117–131.

Traver, J.R. (1959), "The Subfamily Leptohyphinae. Part II: Five New Species of Tricorythodes (Ephemeroptera: Tricorythidae)". *Proceedings of the Entomological Society of Washington,* 61:121-131.

Figure legends

Figures 1-12. *Tricorythodes sallesi* sp. nov. **(**1) Larval habitus (dorsal view), (2) maxilla (dorsal view), (3) maxillary palp (detail), (4) foreclaw (detail), (5) hind leg, (6) foreleg, (7) operculate gill (dorsal view), (8) operculate gill (ventral view), (9-12) gills III-VI (ventral view).



ARTIGO 4

Tricorythodes caunapi a new species from the Rain Forest of the Pacific Colombian (Ephemeroptera: Leptohyphidae).

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Abstract

Tricorythodes caunapi sp n. is described and illustrated from nymphs and adults of both sexes. The name refers to Caunapí River in the Pacific Colombian, where the type-material was collected. This species can be recognized by the following combination of characters in adults: male genitalia form, tibiae and tarsi with blackish bands, abdominal colour pattern and by small size (3 mm approximately); in nymphs: maxillary palp absence, tibia and tarsi with the same coloration of the adult, tarsal claws with 7-8 marginal denticles and double row of 1-2 + 2-3 submarginal denticles, abdominal colour pattern, and triangular operculate gills.

Key words: Ephemeroptera, *Tricorythodes*, Tumaco, Pacific Colombian, new specie.

Resumo

Tricorythodes caunapi sp n. é descrito e ilustrado a partir de ninfas e adultos de ambos sexos. O nome da nova espécie refere-se ao rio Caunapí, localizado na região do Pacífico na Colômbia, onde o material tipo foi coletado. Esta espécie pode ser diferenciada das demais do gênero pelos seguintes caracteres diagnósticos: nos adultos: forma da genitália do macho, tíbia e tarso com bandas escuras, padrão de coloração abdominal e pequeno tamanho do corpo (3mm aproximadamente); nas ninfas: palpo maxilar ausente, tíbia e tarso com o mesmo padrão de coloração dos adultos, garra tarsal

com 7-8 dentículos marginais e uma dupla fileira de 1-2 + 2-3 dentículos submarginais, padrão de coloração abdominal e brânquia opercular triangular.

Palavras-chave: Ephemeroptera, *Tricorythodes*, Tumaco, Pacífico Colombiano, nova espécie.

Introduction

Tricorythodes Ulmer (Leptohyphidae) is a specious genus that belongs to the Leptohyphidae family. The genus was described by Ulmer (1920), and its is widely distributed in the Neotropical region.

The genus is represented by nineteen species in South America. In Colombia four species are known; *T. zunigae* Molineri, *T. capuccinorum* Emmerich, *T. uniandinus* Emmerich and *T. trifasciatus* Molineri & Zúñiga (Domínguez 2006, Emmerich 2007, Molineri 2002 and Molineri & Zúñiga 2006).

The municipality of Tumaco is located in the Pacific Coast of Colombia. Tumaco belongs to the Chocó Biogeographical Region, which shows high levels of diversity and endemism (García-Kirkbride 1986, Gentry 1986). Collecting and conducting studies in this area is difficult due to armed conflicts, illegal crops and intensive oil palm plantations. Sampling of aquatic insects in this region resulted in the finding of a new species of *Tricorythodes* genus.

In this paper, the authors describe and illustrate *Tricorythodes caunapi* sp. n. based on nymphs and adults of both sexes.

Material and Methods

Both nymphs and adults were preserved in 90% ethanol. Mouthparts and legs of nymphs as well as male genitalia and wings of the adults were mounted on microscope slides using Euparal substance. The illustrations were made with a camera lucida attached to a stereo microscope. The specimens are deposited in the following Institutions: de Entomología de la Universidad del Valle, Cali, Colombia (MEUV); Colección Zoológica de la Universidad de Nariño (PSO-CZ); Instituto-Fundación Miguel Lillo, Tucumán, Argentina (IFML). Adults were obtained and associated each

other by rearing method (Fig. 1) in the Caunapi Colombian river (Fig. 2). All measurements in milimeters; the numbers correspond the minimum and maximum values.

***Tricorythodes caunapi* sp. n.**

Figs. 3-25

Male imago (Figs. 3-6) (measurements taken from 5 specimens): Length: body, 2.5 – 2.8 mm; forewing, 2.8 – 3.0 mm. General coloration yellowish with black and brown marks.

Head: yellowish with small black marks on posterior margin of eyes and median region of occipital; antennae yellow.

Thorax: Pronotum uniformly dark, except two small yellowish marks on anterior region; mesonotum yellowish, shaded with black; mesoscutellum brownish with black marks, lateral zones of mesoscutellum yellowish and membranous filaments absence; metanotum yellowish shaded with black; pleural and sternal sclerites whitish. Legs (Fig. 5): coxae and trochanters whitish shaded with black; femora whitish with dark marks on dorsal and subapical regions; tibiae yellowish, shaded with sub apical dark band, besides with a basal small darker mark; tarsi whitish, except fourth tarsal segment blackish. Wings (Fig. 4): membrane hyaline, base and sectors C and Sc shaded with grey; longitudinal veins blackish; cross veins translucent, darkening toward costal margin; CuP absence.

Abdomen: terga uniformly shaded with black, except submedian region of the terga I-IV, and along gill borders; sterna whitish shaded with pale gray. Genitalia (Fig. 6) whitish, except lateral margins of penes brownish; penes pyramidal and somewhat fused; segment I of the forceps subequal segment II; caudal filaments whitish, translucent.

Female imago. (measurements taken from 2 specimens) Length: body, 2.8–3.0 mm; forewings, 3.0 mm.

Female similar to male in morphology and color pattern, except length of wings, longer than male and CuP vein curved, weak and sometimes incomplete (Fig. 3). Abdomen yellowish, black shaded (similar fig. 7).

Nymph (Figs. 7-24)**.** (measurements taken from 6 specimens) Male length: body, 2.0 – 2.5 mm, mesonotum, 0.50 – 0.70 mm; cerci 1.2 mm. Female length: body, 2.5 - 2,8 mm; mesonotum, 0.60 – 0.70mm; cerci 1.0 mm.

General coloration yellowish with blackish marks. Small specimens with stout body.

Head (Fig. 7): yellowish, with small marks on posterior margin of eyes and median region of occipital black; antennae yellowish, mouthparts yellowish with black marks (Figs. 8-14); frontal shelf absence and genal projection slightly developed; maxillae (Figs. 12, 13) with stipes subequal to galea-lacinia length; inner proximal margin of the galea-lacinia with 1 or 2 setae; maxillary palp absence.

Thorax (Fig. 7): Pronotum uniformly black shaded, except two small yellowish marks on anterior region and sometimes in the anterolateral margins; mesonotum yellowish with black shade, darker between wing pads; anterolateral region of the mesonotum with a whitish mark; wing pads yellowish with dark veins; sterna whitish with grey shade. Legs (Figs. 15-17) whitish, with blackish marks on dorsal region of the femora; tibiae with basal and sub apical blackish bands, and tarsi with sub basal blackish band; tarsal claws with 7–8 marginal denticles and a pair of 1-2 + 1-3 submarginal denticles near apex; distal setae presents (Fig. 18).

Abdomen (Fig. 7): uniformly black shaded, except submedian region of the terga I-IV and lateral expansions of the abdomen (Fig. 7); lateral margins of segments III–VII expanded with posterolateral spines on segments VII–IX, very short on IX (Fig. 7); posterior margins of abdominal terga with rows of setae; abdominal sterna whitish shaded with grey. Operculate gills (Fig. 19) triangular, black shaded, except whitish mark in median and distal regions, with two dorsal ridges and with a pair of ventral membranous lamellae (Fig. 20); remaining gills whitish, translucent and shaded with grey (Figs. 21–24); Gill formula 3-3-3-3-2. Caudal filaments yellowish, translucent shaded with grey, with setae in the joints.

Diagnosis. In adults: (1) colour pattern similar Fig. 7; (2) penes pyramidal as in Fig. 6; (3) segment 1 of the forceps subequal than segment 2 (Fig. 6). In nymphs: 1) maxillary palp absence (Figs. 12, 13); 2) tarsal claws with 7-8 marginal denticles and double row of 1-2 + 1-3 submarginal denticles (Fig. 18); 3) opercular gills triangular and blackish, except whitish mark in median and distal zones (Fig. 19); 4) legs with dorsal blackish marks on femora, tibiae with basal and subapical bands, tarsi with sub basal band (Figs. 15-17); 5) transversal row of setae on fore femora in the submedian region (Fig. 15); 6) colour pattern, shaded with black almost completely (Fig. 7); 6) small size (3mm).

Type Material:**.** Holotype male imago, from Colombia, Tumaco, Tangareal, Río Caunapi, 06-XI-07, Dias L. G., Bacca. T., Angulo. D., Estacio. J. (IFML). Paratypes: 5 nymphs, same data as holotype, except, 05-XI-07 (MEUV). 1 male and 1 female imago, same data as holotype (MEUV). 5 nymphs, same data as holotype, except, 05-XI-07 (IFML). Other Material: 5 nymphs, same data as partypes (PSO-CZ). 1 male and 1 female imago, same data as paratypes (PSO-CZ).

Etymology. The new species name is a reference to Caunapi River, where the type specimens were collected.

Distribution. Colombia: Nariño Departament: Tumaco.

Acknowledgments

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References

Domínguez, E., Molineri, C., Pescador, M., Hubbard, M. D. and Nieto, C. 2006. *Aquatic Biodiversity in Latin America.* Pensoft,Sofia-Moscow, v.2: Ephemeroptera of South America, 646 p.

Emmerich, D. E. 2007. Two new species of Tricorythodes Ulmer (Ephemeroptera: Leptohyphidae) from Colombia. Zootaxa, 1561: 63–68.

García-Kirkbride, M. C. 1986. Biological evaluation of the Chocó Biogeographic Region in Colombia. WWF-US, Washington, D.C. 61 pp.

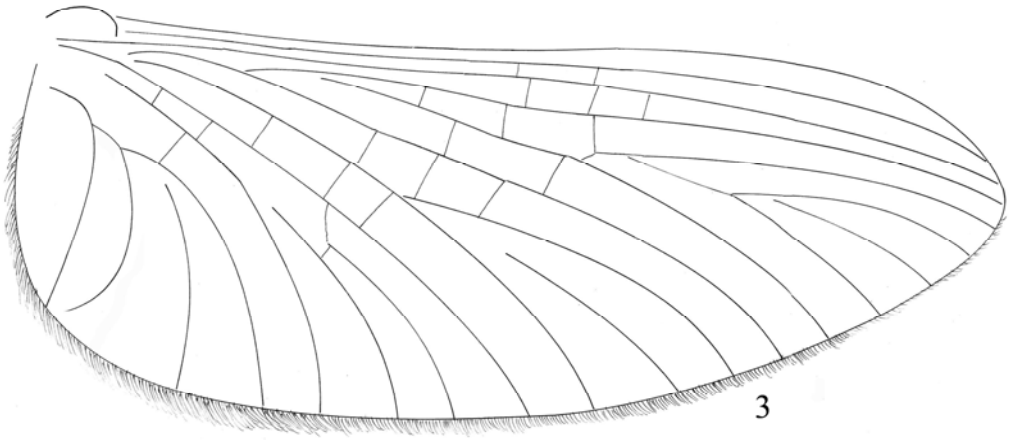
Gentry, A. H. 1986. Species richness and floristic composition of Chocó region plant communities. Caldasia, 15: 71-91.

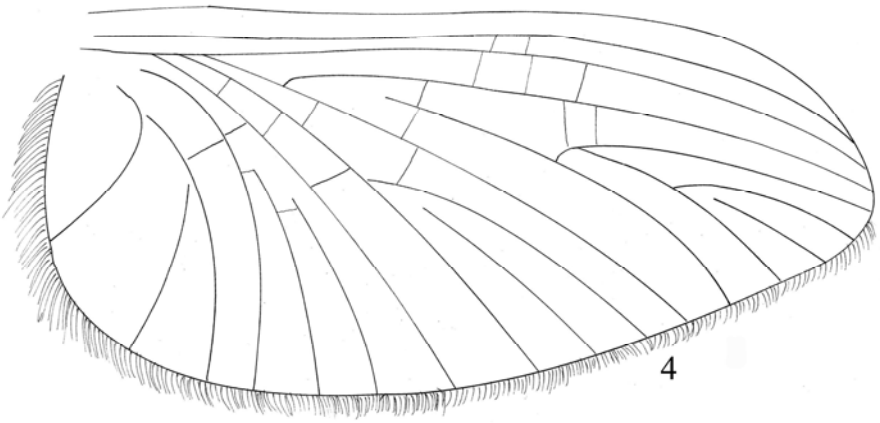
Molineri, C. 2002. Cladistic analysis of the South American species of *Tricorythodes* (Ephemeroptera: Leptohyphidae) with the descriptions of new species and stages. Aquatic Insects, 24: 273-308.

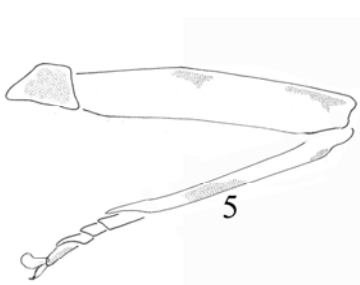
Molineri, C. and Zuñiga, M. C. 2006. New species of Leptohyphidae (Insecta: Ephemeroptera) from Colombia with evidence of reproductive time segregation. Studies on Neotropical Fauna and Environment, 41(2): 139 – 151.

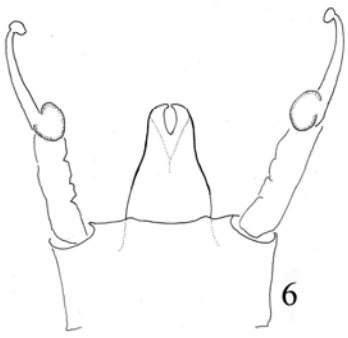
Figures 1-2. Type locality of *Tricorythodes caunapi* sp. n.: rearing flotation method in field; 2. view of the Caunapí river of Tumaco, Colombia.

Figures 3-6. *Tricorythodes caunapi* sp. n.: 3. female fore wing; 4. male fore wing; 5. male mesothoracic leg; 6. male genitalia.

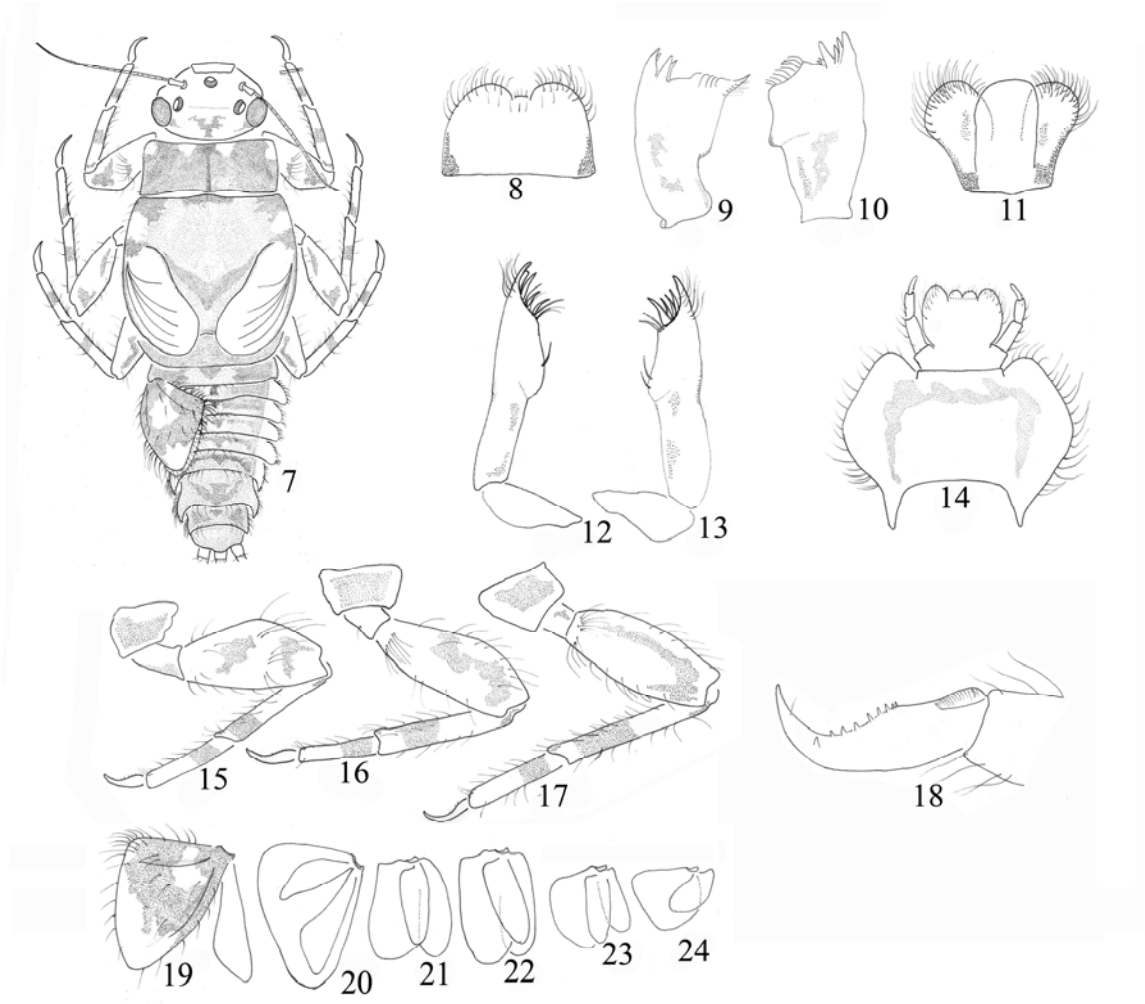








Figures 7-24. *Tricorythodes caunapi* sp. n.: 7. nymphal habitus; 8. labrum; 9. left mandible (dorsal view); 10. right mandible (dorsal view); 11. hypopharynx dorsal view); 12. left maxilla (dorsal view); 13. right maxilla (dorsal view); 14. labium (dorsal view); 15. foreleg; 16. mid leg; 17. hind leg; 18. tarsal claw; 19. operculate gill (dorsal view); 20. operculate gill (ventral view); 21-24. gills III-VI (ventral view).



ARTIGO 5

Phylogeny of *Tricorythodes* Ulmer (Leptohyphidae: Ephemeroptera) based on molecular and morphological evidences

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Resumo

Este trabalho constitui a primeira filogenia de *Tricorythodes* Ulmer (Ephemeroptera: Leptohyphidae) usando evidencias moleculares e morfológicas. A análise incluiu 23 espécies da América do Sul, três da América Central e três da América do Norte. A filogenia foi construída baseada nas seqüências de DNA de fragmentos do gene nuclear 18S e dos genes mitocondriais COI e 16S, combinadas com 39 caracteres morfológicos. A monofilia de *Tricorythodes* (lato sensu) foi testada, assim como o suporte para os gêneros recentemente propostos para o grupo. Os resultados deste trabalho, apóiam a sinonimização dos gêneros *Epiphrades, Homoleptohyphes, Tricoryhyphes* e *Tricorythodes*, assim como proposto por outro autores. Entretanto, reconhecemos *Asioplax* e *Macunahyphes* como gêneros de Leptohyphidae.

Palavras-chave: Pannota, *Tricorythodes*, filogenia, evidencias Moleculares.

Abstract

This research constitutes the first phylogeny of *Tricorythodes* Ulmer (Ephemeroptera: Leptohyphidae) using molecular and morphological evidences. The analyses included 23 species from South America, three from North and three from Central America. The phylogeny was constructed based on DNA sequence data from nuclear (18S rDNA) and mitochondrial (COI and 16S rDNA) fragments, and 39 morphological characters. The monophyly of *Tricorythodes* (sensu lato) and the support for generic-level groupings proposed in the last years were tested. The results of this research agree with the synonymization of the genera *Epiphrades, Homoleptohyphes, Tricoryhyphes* and *Tricorythodes*, proposed by other authors and supports to the genus *Asioplax* and *Macunahyphes* as distinct genera of Leptohyphidae.

Keywords: Pannota, *Tricorythodes*, phylogeny, molecular evidence.

Introduction

Tricorythodes Ulmer (Leptohyphidae) is a specious and morphologically diverse Pan American genus. Several taxonomists have contributed to the study of the group, currently *Tricorythodes* (sensu lato) is represented by approximately 62 species (Traver 1959; Allen 1967; Allen and Murvosh 1987; Kluge and Naranjo 1990; Wiersema and McCafferty 2000; Wiersema *et al.* 2001; Molineri 2001, 2002; Baumgardner *et al.* 2006; Dias and Salles 2006; Molineri and Zúñiga 2006; Emmerich 2007; Baumgardner 2007, 2008; Dias *et al.* 2009a, b). The genus described by Ulmer (1920) has had a complex systematic history in the last decades.

In 1987, Allen and Murvosh presented the first revision of the genus and proposed three subgenera: *Tricorythodes* Ulmer, *Tricoryhyphes* Allen & Murvosh and *Homoleptohyphes* Allen & Murvosh.

Wiersema and McCafferty (2000) carried out a generic revision of the Leptohyphidae of North and Central America. These authors, elevated the three subgenera proposed by Allen and Murvosh (1987) to generic level, and proposed two other genera, *Asioplax* Wiersema & McCafferty and *Epiphrades* Wiersema & McCafferty, to include other species of the group.

Molineri (2002) in a cladistic revision of the South American species of *Tricorythodes* (sensu lato) showed that some of the groups proposed by Wiersema and McCafferty (2000) were not monophyletic, and suggested maintaining the genus *Tricorythodes* as a whole. Finally, Wiersema and McCafferty (2005) reviewed *Asioplax*, including some South American species previously classified in *Tricorythodes*.

In 2003, another genus associate with *Tricorythodes* was described, *Ableptemetes* Wiersema & McCafferty based on nymphal from Mexico and Central America (Wiersema and McCafferty 2003).

Dias *et al.* (2005) proposed the genus *Macunahyphes* Dias, Salles & Molineri to include *Tricorythodes australis* Traver, describing for the first time the nymphs of this species. These authors recognized *Macunahyphes* based on morphological characters of the nymphs, mainly the mouthparts, and the atypical penis shape of the male imago.

Molineri (2006) presented a morphological phylogeny for Leptohyphidae, where *Macunahyphes* fell well within *Tricorythodes*.

Baumgardner and Ávila (2006) described the genus *Cabecar* Baumgardner based on nymphs and reared adults from Central America. According to these authors, *Cabecar* shares several characters with *Tricorythodes*, its only distinctive feature being the shape of femoral setae in the nymphs. Furthermore, Baumgardner and Ávila (2006) commented that additional phylogenetic research would be needed to assess the proper position of *Cabecar* within Leptohyphidae.

Baumgardner (2008), in a recent phylogenetic revision of Leptohyphidae based on morphological characters, proposed the following new synonyms for *Tricorythodes*: *Ableptemetes, Cabecar, Epiphrades, Homoleptohyphes, Macunahyphes* and *Tricoryhyphes.* Besides, the genus *Asioplax* was regarded as a subgenus of *Tricorythodes.*

Now, after so many efforts to improve the classification of *Tricorythodes*, we present for the first time the first exploration of molecular evidence in the phylogeny of the group. The molecular evidence was also combined with morphological characters used previously in systematic studies of the group (Kluge 1992; Molineri 2002, 2006; Wiersema and McCafferty 2000; Baumgardner 2008).

The genes (18S rDNA, 16S rDNA and COI) used in this research are commonly used in phylogenetic analyses of mayflies (Ogden and Whiting 2003, 2005; Ball *et al.* 2005; Sun *et al.* 2006). The sequences obtained were used for phylogenetic reconstruction, with the goal of testing the monophyly of *Tricorythodes* (sensu lato) and assessing the support for generic-level groupings proposed in the last years.

Materials and methods

Taxon Sampling (Table 1):

Sixty specimens from 24 species were analysed, representing six of the genera proposed in the last decades for the group (*Tricorythodes, Epiphrades, Asioplax, Macunahyphes, Homoleptohyphes* and *Tricoryhyphes*). Additionally, one species of *Leptohyphes* and *Leptohyphodes* and three of *Haplohyphes* were included. The examined material is deposited in the following institutions: Instituto Miguel Lillo, San

Miguel de Tucumán, Tucumán, Argentina (IML); Museo de Entomologıa de la Universidad del Valle, Cali, Colombia (MEUV); Florida A&M University, Tallahassee, Florida, USA (FAMU); Coleção de Invertebrados do Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ); Coleção Entomológica do Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (DZRJ); Museu Regional de Entomologia, Universidade Federal de Viçosa, Minas Gerais, Brazil (UFVB); Museo Entomológico Marcial Benavides, Centro Nacional de Investigaciones de Café (MEMB) and Coleção Zoobotânica James A. Ratter, Universidade do Estado de Mato Grosso, Nova Xavantina, MT, Brazil (CZNX). The details about the material examined are presented in Table 1.

Morphological Characters (Table 2, Appendix 1):

Thirty nine characters were coded (Table 2), some characters are newly proposed here, but the majority were selected from Molineri (2002, 2006), Baumgardner (2008), Wiersema and McCafferty (2000) and Kluge (1992).

DNA extraction and fragment amplification:

The DNA was isolated from the muscle tissue of the thorax and legs of the specimens previously preserved in pure ethanol. Total DNA was extracted with the DNA Extraction Tissue Kit (Qiagen) following the manufacturer instructions, and stored at -20°C. Three PCR primer sets (Table 3) were used in order to amplify the markers COI mtDNA (Folmer *et al.* 1994), 18S rDNA (Whiting 2002) and 16S rDNA (Ogden and Whiting 2005). The PCR reactions were carried out in a final volume of 50µl. Each reaction consisted of approximately 100ng of total DNA, 0.5µM of each primer (forward and reverse), 0.2mM of each d(AGCT)TP, 1X of 5X taq PCR buffer, 1.25U of Taq polimerase (Go taq flexi Promega), and 2mM of MgCl2 for 16S and 18S, but 1.5mM of MgCl2 for COI. PCR reactions consisted of an initial 5-min denaturation step at 95°C, 35 cycles of 30 s at 94°C, 30 s at the annealing temperature (50°C for 18S, and 45°C for 16S), 45 s at 72°C, and then a final elongation step for 5 min at 72°C. For COI, the procedure reported by Ball et al. (2005) was followed. The PCR products were visualized on 1% agarose gels using ethidium bromide. All the PCR products were then

purified using the QIAquick PCR purification kit (Qiagen), and sequenced in *Macrogen Advancing through Genomics* - Korea. The sequences obtained will be sent to GenBank®.

Sequence alignment and phylogenetic analyses:

The alignment was realized using CLUSTAL-X v.1.82 (Thompson *et al.* 1994). The aligned molecular data were combined with the morphological data and were analyzed using the program TNT (Goloboff *et al.* 2008). The phylogenetic analyses were performed using normal parsimony, 100 Wagner trees were generated and then submitted to TBR (Tree Bisection Reconnection). Seven shortest trees (2999 steps) were found. Similar analyses were carried independently for molecular and morphological databases. Group support was assessed using a technique of symmetric resampling of the original matrix, and then calculating Fequency Differences (Goloboff *et al.* 2003). The support values vary from 0 (minimum support) to 100 (maximum support). The negative values (represented by [] in the tree) show that the contradictory group is more frequent than the group of the shortest tree.

Results and discussion

Several difficulties were found in the amplification of the material used in the molecular analysis; one of them was the conservation in alcohol 70%, another was the age of the samples (more than 3 year old from fixation). The results here presented can be completed with new data, as additional sequence of some species (Table 4) or the utilization of other markers, for the taxonomic formalization of the clade in which the supports are not high.

Our results, the first molecular phylogeny of *Tricorythodes* sensu lato, give support to the monophyly of *Tricorythodes* sensu lato (without *Asioplax* and *Macunahyphes*) node (F) and corroborate other generic groups (Figs. 1, 2), discussed below. Molecular evidence alone found some interesting groups (Fig. 2): node E (*Asioplax*), node B (*Macunahyphes*, *Leptohyphodes*, *Haplohyphes*), etc. However a great polytomy occurs into *Tricorythodes* species (node F), showing that the DNA sequences

used were not enough to separate some taxa. Morphological data provided resolution to this taxonomic level, in combined (molecular and morphology) analysis and in the morphological analysis alone (Figs. 1 and 3).

Among the recently proposed genera of *Tricorythodes* sensu lato (*Asioplax, Epiphrades, Homoleptohyphes, Tricoryhyphes, Tricorythodes* and *Macunahyphes*), only *Asioplax* node (B) and *Macunahyphes* node (G) are supported in the analysis (Fig. 1). The rest of the genera appear as para- or poliphyletic (Figs. 1-3) as proposed by Molineri (2002) and Baumgardner (2008).

Four species were included in a node (B) attributable to *Asioplax* (*A. numinuh* n.comb., *A. rondoniensis* n.comb., *A. tumaco* n.comb. and *A. santarita* n.comb.) (Fig. 1). *T. sierramaestrae,* considered in *Asioplax* by Wiersema and McCafferty (2005), is not related with node A. Wiersema and McCafferty (2005) provisionally placed the Cuban species *T. sierramaestrae* and *T. saculobranchis* in *Asioplax* based only on the incomplete original descriptions. Our results, based on the study of fresh material of *T. sierramaestrae* (morphological and molecular) show that this species is not related with *Asioplax* (Figs. 1-3). In addition, any of both Cuban species show the following apomorphies of *Asioplax*: operculate gills with a single ventral lamellae, wide femora (width more than 50% of max. length), small (approximately 3 mm) and very depressed body, fore femoral transverse row of setae basal in position, and very long and slender femoral setae.

For the reasons mentioned before, the genus *Asioplax* is accepted but with the exclusion of both Cuban species. As mentioned by Kluge (2004) and Baumgardner (2008), it is possible that these Cuban species belong to another basal lineage of *Tricorythodes*; this statement is not be supported in this analysis because these species appear as derived in the tree and the support is not high (Fig. 1 node T). Baumgardner (2008) proposed the genus *Asioplax* as a subgenus of *Tricorythodes* but our results support them as an entirely distinct genus.

The characters used in this work are not informative within *Asioplax* (Figs. 1-3), what denotes the close relationships among these species (*A. rondoniensis, A. tumaco, A. santarita* and *A. numinuh*). A detailed study of these species would be needed to determine relations within *Asioplax*.

The monotypic genus *Macunahyphes* proposed by Dias *et al.* (2005) was supported in this analysis, as previously mentioned (Figs. 1-3). The genus presented the following synapomorphies: penis with a dorsal projection covered with spines; maxillae distally widened, with dentisetae on inner apical margin directed medially; and short setae on the femora. Dias *et al.* (2005) proposed that some affinities existed between *Macunahyphes* and *Haplohyphes*, this statement was corroborated here, since *Macunahyphes* appears as sister to *Haplohyphes* and *Leptohyphodes* (Fig. 1 node E; Fig. 2 node B; Fig. 3 node E).

In addition, these results show *Haplohyphes*, *Leptohyphodes* and *Macunahyphes* are more closely related to *Tricorythodes* (Fig. 1 node E; Fig. 2 node B; Fig. 3 node E) than to *Leptohyphes* (Figs. 1-3), coinciding with Molineri (2006), but contradicting Baumgardner (2008).

Due to the morphologic diversity of *Tricorythodes* together with its wide distribution, it was largely suspected to be formed by many different lineages (Kluge 1992, Wiersema and McCafferty 2000, Baumgardner 2008). Here, some lineages are recovered but their bootstrap values supports are not high, thus, their taxonomic formalization must wait the incorporation of new data.

When examining closely related *Tricorythodes* species, it was necessary to combine molecular and morphologic characters, since separate data gave little useful. Some of the sister taxa consistently recovered were *T. cubensis* and *T. sierramaestrae* (both Cuban species sharing the shape of gills, legs and femoral setae, Kluge & Naranjo 1990) see Fig 1, node T, and *T. molinerii* and *T. montanus* (with well developed anterolateral projection of pronotum, and similar shape of gills, among other characters) see Fig 1, node O.

A clade joining *T. mirus* and *T. dimorphus* was expected, because they are morphologically close species (Allen and Murvosh 1987; Wiersema and McCafferty 2000; Baumgardner 2008), however this was not demonstrated in the trees (Fig. 1). This result is attributed to the fact that *T. mirus* amplified only the COI gene and *T. dimorphus* only the 16S. A more complete amplification is needed to adequately define their relationships.

The node Q constituted by *T. popayanicus*, *T. caunapi*, *T. hiemalis*, *T. mirus* and *T. yura* (Fig. 1) contradict the definition of *Tricoryhyphes*, proposed by Wiersema and McCafferty (2000) to include *T. popayanicus*, *T. barbus* and other North American species. Even though the support of the node Q is not high, these results coincide with Molineri's statement (2002, 2006), in which *T. popayanicus* appears in one group and *T. barbus* in another.

The node K composed by *T. bullus, T. sallesi, T. barbus* and *T. dimorfus*, suggests that *Epiphrades*, *Tricoryhyphes* and *Homoleptohyphes* (Fig. 1) also proposed by Wiersema and McCafferty (2000) are polyphyletic. According with the proposal of these authors *T. bullus* and *T. sallesi* would belong to *Epiphrades*, *T. barbus* to *Tricoryhyphes* and *T. dimorfus* to *Homoleptohyphes*. Nevertheless the support of node K is not high and more data is needed to properly assess the relation of these species.

The species *Tricorythodes* sp. n. (Mato Grosso - MT), in the combined matrix appears apart from *Tricorythodes* (Fig. 1), this might have been caused by the lack of amplification (except for the well conserved 18S). Besides this, the adults of this species are unknown and their morphological data are incomplete (Table 2).

The complementation of the sequences of the species that were amplified only to one gene: *Tricorythodes* sp. n. (MT), *T. dimorphus, T. mirus, T. barbus, M. australis, A. numinuh* and *T. arequita* (Table. 4), can be complemented and give more support to some nodes of the tree.

In conclusion, this work give further evidence for the synonymization of the genera *Epiphrades, Homoleptohyphes, Tricoryhyphes* and *Tricorythodes* (also proposed by Molineri 2002 and Baumgardner 2008) but support the revalidation of *Asioplax* and *Macunahyphes* as distinct genera of Leptohyphidae.

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References

Allen, R. K. (1967). New species of New World Leptohyphinae (Ephemeroptera: Tricorythidae). The Canadian Entomologist, 99(4): 350-375.

Allen, R. K. & Murvosh, C. M. (1987). Mayflies (Ephemeroptera: Tricorythidae) of the Southwestern United States and Northern Mexico. Annals of the Entomological Society of America, 80(1): 35-40.

Baumgardner D. E. (2007). New species of Leptohyphidae (Ephemeroptera) from Costa Rica. Proceedings of the Entomological Society of Washington 109(2):416-426.

Baumgardner, D. E. (2008). Phylogeny and biogeography of the mayfly family Leptohyphidae (Insecta: Ephemeroptera) with a taxonomic revision of selected genera. Ph.D Dissertation, Texas A&M University, College Station, 306 p.

Baumgardner, D. E. & Ávila, S. (2006). *Cabecar serratus*, a new genus and species of leptohyphid mayfly from Central America, and description of the imaginal stages of *Tricorythodes sordidus* Allen (Ephemeroptera: Leptohyphidae). Zootaxa, 1187:47-59.

Ball, S. L., Hebert, P. D. N., Burian, S. K. & Webb, J. M. (2005). Biological identifications of mayflies (Ephemeroptera) using DNA barcodes. Journal of the North American Benthological Society, 24:508-524.

Dias, L. G.; Salles, F. F. & Molineri, C. (2005). *Macunahyphes*: a new genus for *Tricorythodes australis* (Ephemeroptera: Leptohyphidae). Annales de Limnologie - International Journal of Limnology, 41(3): 195-201.

Dias, L. G. & Salles, F. F. (2006). A new species of *Tricorythodes* (Ephemeroptera: Leptohyphidae) from Minas Gerais, Southeastern Brazil. Neotropical Entomology, 31(1): 56-58.

Dias, L. G., Cruz, P. V. & Ferreira, P. F. (2009a). A new species of *Tricorythodes* Ulmer (Ephemeroptera: Leptohyphidae) from Northern Brazil. Annales de Limnologie - International Journal of Limnology, DOI: 10.1051/limn/2009009

Dias, L. G., Cabette, H. S. R & De Souza, D. (2009b). A new species of *Tricorythodes* Ulmer (Ephemeroptera: Leptohyphidae) and first record of *Tricorythodes quizeri* Molineri from Brazil. Aquatic Insects *In press.*

Emmerich, D. E. (2007). Two new species of *Tricorythodes* Ulmer (Ephemeroptera: Leptohyphidae) from Colombia. Zootaxa, 1561:63-68.

Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, 3:294–299.

Goloboff, P. A., Farris, J. S., Kallersjo, M., Oxelman, B., Ramırez, M. J. & Szumik, C. A. (2003). Improvements to resampling measures of group support. Cladistics, 19, 324–332.

Goloboff, P. A., Farris, J. S. & Nixon, K. (2008). T.N.T., a free program for phylogenetic analysis. Cladistics, 24: 774-786.

Kluge, N. & Naranjo, J.C. (1990). Mayflies of the family Leptohyphidae (Ephemeroptera) of Cuba. Opuscula Zoologica Fluminensia 69:564-578.

Kluge, N. Ju. (1992). Redescription of *Leptohyphes eximis* Eaton and diagnoses of the genera *Leptohyphes* and *Tricorythodes* based on the structure of the pterothorax (Ephemeroptera: Tricorythidae, Leptohyphinae). Opuscula Zoologica Fluminensia, 98:1-16.

Kluge, N. Ju. (2004). The phylogenetic system of Ephemeroptera. Kluwer Academic Publishers, Dordrecht.

Molineri, C. (2001). Una especie nueva de *Tricorythodes* (Ephemeroptera: Leptohyphidae) de la Argentina con notas sobre su biología. Revista de la Sociedad Entomológica Argentina, 60:61-66.

Molineri, C. (2002). Cladistic analysis of the South American species of *Tricorythodes* (Ephemeroptera: Leptohyphidae) with the description of new species and stages. Aquatic Insects, 24: 273-308.

Molineri, C. (2003a). Revision of the South American species of *Leptohyphes* Eaton (Ephemeroptera: Leptohyphidae) with a key to the nymphs. Studies on Neotropical Fauna and Environment, 39:47-70.

Molineri, C. (2003b). *Haplohyphes* (Ephemeroptera: Leptohyphidae), new species and stage descriptions with a key to separate the species of the genus. Zootaxa, 263:1-11.

Molineri, C. (2006). Phylogeny of the mayfly family Leptohyphidae (Insecta: Ephemeroptera) in South America. Systematic Entomology 31:711-728.

Molineri, C. and Zúñiga, M. C. (2006). New species of Leptohyphidae (Insecta: Ephemeroptera) from Colombia with evidence of reproductive time segregation. Studies on Neotropical Fauna & Environment, 41(2):139-151.

Ogden, T. H. & Whiting, M. F. (2005). Phylogeny of Ephemeroptera (mayflies) based on molecular evidence. Molecular Phylogenetics and Evolution, 37: 625–643.

Ogden, T. H. & Whiting, M. (2003). The Problem with "the Paleoptera Problem": Sense and Sensitivity. Cladistics, 19: 432–442.

Sun, L., Sabo, A., Meyer, M. D., Randolph, R. P., Jacobus, L. M., McCafferty, W.P. & Ferris, V.R. (2006) Tests of current hypotheses of mayfly (Ephemeroptera) phylogeny using molecular (18s rDNA) data. Annals of the Entomological Society of America, 99: 241–252.

Traver, J. R. (1959). The subfamily Leptohyphinae. Part II: Five new species of Tricorythodes (Ephemeroptera, Tricorythidae). Proceedings of the Entomological Society of Washington, 61:121-131.

Whiting, M. F., 2002. Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. Zoologica Scripta, 31, 93–104.

Wiersema, N. A. & McCafferty, W. P. (2000). Generic Revision of the North and Central American Leptohyphidae (Ephemeroptera: Pannota). Transactions of the American Entomological Society, 126 (3-4): 337-371.

Wiersema, N. A., W. P. McCafferty, & Baumgardner, D. E. (2001). *Asioplax numinuh*, a new species of Ephemeroptera (Leptohyphidae) from Texas and Mexico. Entomological News, 112:301-304.

Wiersema, N. A. & McCafferty, W. P. (2003). *Ableptemetes*: a new genus of Tricorythodinae (Ephemeroptera: Leptohyphidae) from Mexico and Central America. Entomological News, 114:37-40.

Wiersema, N. A. & McCafferty, W. P. (2005). Contribution to the taxonomy of *Asioplax* (Ephemeroptera: Leptohyphidae: Tricorythodinae) in the New World. Entomological News, 116:147-158.

Table 1. Taxa Sampling for species of Leptohyphidae, collection sites and recent revisions:

TAXA	Species	Material Examined (locality):	Institutional Collection	Recent revisions, description and synonymous
Out-groups	*Leptohyphes ecuador* Mayo, 1968	Colombia, Nariño, Sandoná, Quebrada La Honda, 18/II/07, Dias, L.G. & Bacca, T. cols.	PSO, MEUV	Molineri (2003a)
	Haplohyphes aquilonius Lugo-Ortiz & McCafferty, 1985	Colombia, Nariño, Sandoná, Quebrada La Honda, 18/II/07, Dias, L. G. & Bacca, T. cols.	PSO	Molineri (2003b), Baungardner 2008
	Haplohyphes baritu Domínguez, 1984	Argentina, Tucumán, Rio la Hoyada, 985 m, S26º45'32"-W65º29'28,5", 12/IX/06, Dominguez, E. et al. cols.	IML	Molineri (2003a), Baungardner 2008
	Haplohyphes mithras (Traver, 1958)	Colombia, Nariño, Sandoná, Quebrada La Honda, 18/II/07, Dias, L. G. and Bacca, T. cols.	PSO	Molineri (2003b), Baungardner 2008
	Leptohyphodes inanis Ulmer, 1920	Brazil, Minas Gerais State, Araponga, Serra do Brigadeiro, Vale das Luas, VII/05, Dias, L .G. Col.	UFVB	Molineri (2005b), Baungardner 2008
In-group	*Asioplax. numinuh* Wieserma, McCafferty & Baumgardner, 2001	USA, Texas, Kimble Co. S. Llano River, N38º 28'17,28"-W99º44'3,9", 24/II/98, Bowles col. (Baungardner det.)	IML	Wieserma & McCafferty (2005), as *Asioplax numinuh* Baungardner 2008 as *Tricorythodes (Asioplax) numinuh*
	Asioplax rondoniensis Dias, Cruz & Ferreira, 2009	Brazil, Roraima State, Boa Vista, Rio Marupú, 10/XI/ 06, Falcão, J. col.	UFVB, INPA	Dias *et al.* 2009a
	Asioplax santarita (Traver, 1959)	Brazil, Mato Grosso State, Nova Xavantina, Córrego do Papagaio, S15º27'32"-W52º 24'42", 4/vii/2005, De Souza, D. P. & Cabette, H. S. R. cols.	CZNX	Wieserma & McCafferty (2005), as *Asioplax santarita* Molineri (2002), as *Tricorythodes santarita* Baungardner 2008 as *T. (A.) santarita*
	Asioplax tumaco sp.n.	Colombia, Nariño, Tumaco, Tangareal, Rio Caunapí, 06/XI/07, Dias, L. G., Angulo, D., Estácio, J. and Bacca, T. cols.	PSO	Dias 2009 (undescribed)

Table 1. Taxa Sampling for species of Leptohyphidae, collection sites and recent revisions (Continued):

TAXA	Species	Material Examined (locality):	Institutional Collection	Recent revisions and synonymous
In-group	*Macunahyphes australis* (Bank, 1913)	Brazil, Roraima State, Caracaraí, Rio Branco, C. do Bem Querer, 18-21/ii/02, A.M.O.PESN, LN. Brazil, Roraima, Caracaraí,Cachoeira do Bem Querer, 23/iii/01, Hamada, N. col.	UFVB	Molineri (2002) as *Tricorythodes australis* Baungardner (2008) as *Tricorythodes (Tricorythodes) australis*
	Tricorythodes arequita Traver,1959	Argentina, Misiones, P.P. Urugua-i, Aº Uruzu, 7-11/XII/99, Molineri, C. col.	IML	Molineri (2002) as *Tricorythodes arequita* Baungardner 2008 as *T. (T) arequita*
	Tricorythodes barbus Allen, 1967	Argentina, Misiones, INTA San Vicente, Km 274, 29/xi/86, Dominguez, E. col.	IML	Wiersema & McCafferty (2000) as *Tricoryhyphes barbus* Molineri (2002) as *Tricorythodes barbus* Baungardner 2008 as *T. (T) barbus*
	Tricorythodes bullus Allen, 1967	Argentina, Misiones, Parque Prov. Urugua-í, Aº Uruzu, Ruta Prov. 19, 7-111/XII/99, Molineri, C. col.	IML	Wiersema & McCafferty (2000) as *Epiphrades bullus* Molineri (2002) as *Tricorythodes bullus* Baungardner (2008) as *T. (T) bullus*
	Tricorythodes caunapi Dias, 2009	Colombia, Nariño, Tumaco, Tangareal, Rio Caunapí, 06/xi/07, Dias, L. G., Ângulo, D., Estácio, J. and Bacca, T. cols.	PSO	Dias 2009 (undescribed)
	Tricorythodes cubensis Kluge & Naranjo, 1990	Cuba, Los Morones, 19/iv/07, González-Lazo, D. col.	IML	Baumgardner 2008 *T. (T) cubensis*
	Tricorythodes dimorphus Allen, 1967	USA, New Mexico, Conton Co. Wilow Cr, 7400 ft., N33º29'37" - 108º34'20", Baungardner.	IML	Wiersema & McCafferty (2000) as *Homoleptohyphes dimorphus* Baumgardner 2008 as *T. (T) dimorphus*
	Tricorythodes hiemalis Molineri, 2001	Argentina, Salta, PN El Rey, Aº Los Noques, 905m, S24º44'44"- W64º38'11", 11/xi/05, Molineri, C. col.	IML	Molineri (2002) as *Tricorythodes hiemalis* Baumgardner 2008 as *T. (T) hiemalis*

Table 1. Taxa Sampling for species of Leptohyphidae, collection sites and recent revisions (Continued):

TAXA	Species	Material Examined (locality):	Institutional Collection	Recent revisions and synonymous
In-group	*Tricorythodes molinerii* Dias & Salles, 2006	Brazil, Minas Gerais State, Campos Altos, Rio da Prata, 09/viii/2001, Salles, F. F. & Lugo-Ortiz, C. cols.	UFVB	Dias & Salles (2006)
	Tricorythodes montanus Kluge & Naranjo, 1990	Cuba, Los Morones, 19/vi/07, González-Lazo, D. col.	IML	Baumgardner 2008 as *T. (T) montanus*
	Tricorythodes mirus (Allen, 1967)	USA, Arizona, Santa Cruz Co, Sansita Cocer, nt. Patagonia, 15/iii/97, Slusarte, 8 byrnes cols., Baumgardner col.	IML	Wiersema & McCafferty (2000) as *Homoleptohyphes mirus* Baumgardner 2008 as *T. (T) mirus*
	Tricorythodes popayanicus Domínguez, 1982	Argentina, Tucumán, Acheral, Rio Aranillas , 366 m, S27º06'59,9"- W65º27'43,9", 5/viii/06, Molineri, C. and Nieto, C. cols.	IML	Molineri (2002) as *Tricorythodes popayanicus* Baungardner 2008 as *T. (T) popayanicus*
	Tricorythodes quizeri Molineri, 2002	Argentina, Tucumán, Achera, Rio Aramillas, 28/vii/06, Molineri, C. et al. cols.	IML	Molineri (2002) as *Tricorythodes quizeri* Baungardner 2008 as *T. (T) quizeri*
	Tricorythodes sallesi Dias, Cabette & Souza, 2009	Brazil, Mato Grosso State, Nova Xavantina, Córrego do Papagaio, /vii/2005, Cabette, H. S. R. et al. cols.	UFVB, CZNX	Dias *et al.* 2009b
	Tricorythodes sallesi RR sp.n.	Brazil, Roraima State, Munincipio de Caroebe, N 00°49'28,5"W 59°42'13,7", 29/xi/2006, Falcão, J. col.	IMPA	Dias 2009 (undescribed)
	Tricorythodes sandonensis sp. n.	Colombia, Nariño, Sandoná, Quebrada La Honda, 18/ii/07, Dias, L. G. & Bacca, T. cols.	IML	Dias 2009 (undescribed)
	Tricorythodes sierramaestrae Kluge & Naranjo, 1990	Cuba, Rio Turquino, 22/vi/07, González-Lazo, D. col.	IML	Wieserma & McCafferty (2005) as *Asioplax sierramaestrae* Baumgardner 2008 as *T (T) sierramaestrae*
	Tricorythodes yura Molineri, 2002	Bolivia, La Paz, Aº between Caranavi and Guanai, 500 m, 27/XI/00, Domínguez, E. and Nieto, C. cols.	IML	Molineri (2002) as *Tricorythodes yura* Baumgardner 2008 as *T (T) yura*
	Tricorythodes sp.n. MG	Brazil, Minas Gerais, Paula Cândido, C. Airões, 13/VII/04 , Dias, L. G.	UFVB	Dias 2009 (undescribed)
	Tricorythodes sp.n. MT	Brazil, Mato Grosso State, Nova Xavantina, Córrego do Papagaio, 4/vii/2005, Cabette, H. S. R. et al. cols.	UFVB, CZNX	Dias 2009 (undescribed)

Table 2: Morphological Matrix.

Species	Characters																				
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Leptohyphes ecuador	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	1	0	1	1	0	0
Haplohyphes baritu	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0
Haplohyphes mithras	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0
Haplohyphes aquilonius	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0
Tricorythodes arequita	1	1	1	1	1	1	0	0	1	1	1	1	0	0	1	1	0	1	1	0	0
Macunahyphes australis	1	1	1	1	1	0	0	4	1	1	1	0	1	0	0	0	0	0	0	2	2
Tricorythodes barbus	1	-	-	-	-	-	-	-	-	-	-		0	0	0	0	0	0	1	1	0	0
Tricorythodes bullus	1	1	1	1	1	1	0	1	2	1	1	0	1	1	0	1	0	1	1	0	2
Tricorythodes hiemalis	1	1	1	1	1	1	0	3	1	1	1	0	0	0	0	0	0	1	1	0	1
Tricorythodes popayanicus	1	1	1	1	1	1	0	0	1	1	1	0	1	0	0	0	0	1	1	0	1
Tricorythodes quizeri	1	1	1	1	1	1	0	3	1	1	1	0	0	0	0	1	0	1	1	0	1
Asioplax santarita	1	1	1	1	1	1	1	2	2	1	1	0	2	0	1	1	0	1	1	1	2
Tricorythodes yura	1	1	1	1	1	1	0	0	1	1	1	0	1	0	-	-	0	1	1	0	1
Tricorythodes sallesi	1	-	-	-	-	-	-	-	-	-	-	-	1	0	0	1	0	1	1	0	0
Leptohyphodes inanis	1	0	0	1	0	0	0	0	1	1	1	0	0	0	0	0	0	1	1	0	2
Asioplax tumaco	1	-	-	-	-	-	-	-	-	-	-	-	2	0	1	1	0	1	1	1	2
Asioplax rondoniensis	1	-	-	-	-	-	-	-	-	-	-	-	2	0	1	1	0	1	1	1	1
Asioplax numinuh	1	-	-	1	1	1	1	2	1	1	-		0	2	0	1	-		0	1	1	1	-
Tricorythodes dimorfus	1	1	1	1	1	1	0	0	1	1	0	0	0	0	1	1	0	1	1	0	2
Tricorythodes mirus	1	1	1	1	1	1	0	0	1	1	0	0	0	0	-	-	0	1	1	0	-
Tricorythodes sp. n. MT	1	-	-	-	-	-	-	-	-	-	-	-	0	0	1	1	0	1	1	0	2
Tricorythodes sierramaestrae	1	1	1	1	1	1	0	3	2	1	-		0	1	0	0	1	0	1	1	0	0
Tricorythodes sandonensis sp. n.	1	-	-	-	-	-	-	-	-	-	-	-	1	0	0	1	0	1	1	0	1
Tricorythodes cubensis	1	1	1	1	1	1	0	0	2	1	-		0	1	0	1	1	0	1	1	0	0
Tricorythodes sp. n. MG	1	1	1	1	1	1	0	1	2	1	1	0	1	1	0	1	0	1	1	0	2
Tricorythodes sp. n. RR	1	-	-	-	-	-	-	-	-	-	-	-	0	0	0	1	0	1	1	0	0
Tricorythodes caunapi	1	1	1	1	1	1	0	0	2	1	-	1	1	0	1	1	0	1	1	0	1
Tricorythodes montanus	1	1	1	1	1	1	0	0	2	1	-	0	1	0	0	0	0	1	1	0	1
Tricorythodes molinerii	1	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	1	1	0	1

Table 2: Morphological Matrix (Continued).

Species	Characters																	
	21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**
Leptohyphes ecuador	2	0	0	1	1	0	0	0	1	3	0	0	0	2	2	2	2	1
Haplohyphes baritu	0	0	1	0	1	1	2	0	1	0	1	1	0	0	0	0	0	1
Haplohyphes mithras	0	0	1	0	1	1	-	0	1	0	1	1	0	0	0	0	0	1
Haplohyphes aquilonius	0	0	1	0	1	1	2	0	1	0	1	1	0	0	0	0	0	1
Tricorythodes arequita	1	0	1	1	1	0	1	0	3	2	1	1	1	1	1	0	0	0
Macunahyphes australis	0	0	0	1	1	0	2	0	1	3	1	1	1	0	0	0	0	0
Tricorythodes barbus	0	0	1	0	1	1	1	0	1	1	1	1	1	1	0	0	0	0
Tricorythodes bullus	2	0	1	2	1	0	0	0	2	3	1	1	2	1	0	1	1	0
Tricorythodes hiemalis	1	0	1	1	1	0	1	0	2	2	1	-	1	1	0	0	1	0
Tricorythodes popayanicus	1	0	1	1	1	0	2	0	2	2	1	1	1	1	2	1	1	0
Tricorythodes quizeri	1	0	1	1	1	0	1	0	2	2	1	1	1	1	0	0	1	0
Asioplax santarita	2	1	1	2	1	1	1	1	3	3	1	0	3	2	1	2	2	0
Tricorythodes yura	-	0	1	1	1	0	1	0	2	2	1	1	1	1	2	1	1	-
Tricorythodes sallesi	0	0	1	2	1	0	0	0	2	3	1	1	1	1	1	0	0	0
Leptohyphodes inanis	0	0	1	0	0	0	2	0	0	0	1	1	4	0	0	0	0	0
Asioplax tumaco	2	1	1	2	1	0	0	1	3	3	1	0	3	2	1	2	2	0
Asioplax rondoniensis	2	1	1	2	1	0	0	1	3	3	1	0	3	2	1	2	2	0
Asioplax numinuh	2	1	1	2	1	0	2	0	-	3	1	0	-	2	1	-	-	0
Tricorythodes dimorfus	0	0	1	1	1	0	3	0	3	3	1	1	1	0	0	0	1	0
Tricorythodes mirus	-	0	1	1	1	0	0	0	-	3	1	-	-	-	0	-	-	-
Tricorythodes sp. n. MT	2	0	1	1	1	0	1	0	1	1	1	1	1	1	0	1	1	0
Tricorythodes sierramaestrae	1	0	0	1	1	0	2	0	3	3	1	0	1	2	1	1	1	0
Tricorythodes sandonensis sp. n.	1	0	1	1	1	0	1	0	1	3	1	1	1	1	0	0	0	0
Tricorythodes cubensis	2	0	0	1	1	0	2	0	2	3	1	1	1	2	2	1	1	0
Tricorythodes sp. n. MG	2	0	1	2	1	0	0	0	2	3	1	1	1	1	0	1	1	0
Tricorythodes sp. n. RR	0	0	1	2	1	0	0	0	2	3	1	1	1	1	1	0	0	0
Tricorythodes caunapi	1	0	1	1	1	0	2	0	3	3	1	1	1	1	0	1	1	0
Tricorythodes montanus	1	0	1	1	1	0	1	0	2	3	1	1	1	1	0	0	0	0
Tricorythodes molinerii	0	0	1	1	1	0	1	0	2	3	1	1	1	1	0	0	0	0

Table 2: Morphological Matrix (Continued).

Species	Characters																	
	21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**
Leptohyphes ecuador	2	0	0	1	1	0	0	0	1	3	0	0	0	2	2	2	2	1
Haplohyphes baritu	0	0	1	0	1	1	2	0	1	0	1	1	0	0	0	0	0	1
Haplohyphes mithras	0	0	1	0	1	1	-	0	1	0	1	1	0	0	0	0	0	1
Haplohyphes aquilonius	0	0	1	0	1	1	2	0	1	0	1	1	0	0	0	0	0	1
Tricorythodes arequita	1	0	1	1	1	0	1	0	3	2	1	1	1	1	1	0	0	0
Macunahyphes australis	0	0	0	1	1	0	2	0	1	3	1	1	1	0	0	0	0	0
Tricorythodes barbus	0	0	1	0	1	1	1	0	1	1	1	1	1	1	0	0	0	0
Tricorythodes bullus	2	0	1	2	1	0	0	0	2	3	1	1	2	1	0	1	1	0
Tricorythodes hiemalis	1	0	1	1	1	0	1	0	2	2	1	-	1	1	0	0	1	0
Tricorythodes popayanicus	1	0	1	1	1	0	2	0	2	2	1	1	1	1	2	1	1	0
Tricorythodes quizeri	1	0	1	1	1	0	1	0	2	2	1	1	1	1	0	0	1	0
Asioplax santarita	2	1	1	2	1	1	1	1	3	3	1	0	3	2	1	2	2	0
Tricorythodes yura	-	0	1	1	1	0	1	0	2	2	1	1	1	1	2	1	1	-
Tricorythodes sallesi	0	0	1	2	1	0	0	0	2	3	1	1	1	1	1	0	0	0
Leptohyphodes inanis	0	0	1	0	0	0	2	0	0	0	1	1	4	0	0	0	0	0
Asioplax tumaco	2	1	1	2	1	0	0	1	3	3	1	0	3	2	1	2	2	0
Asioplax rondoniensis	2	1	1	2	1	0	0	1	3	3	1	0	3	2	1	2	2	0
Asioplax numinuh	2	1	1	2	1	0	2	0	-	3	1	0	-	2	1	-	-	0
Tricorythodes dimorfus	0	0	1	1	1	0	3	0	3	3	1	1	1	0	0	0	1	0
Tricorythodes mirus	-	0	1	1	1	0	0	0	-	3	1	-	-	-	0	-	-	-
Tricorythodes sp. n. MT	2	0	1	1	1	0	1	0	1	1	1	1	1	1	0	1	1	0
Tricorythodes sierramaestrae	1	0	0	1	1	0	2	0	3	3	1	0	1	2	1	1	1	0
Tricorythodes sandonensis sp. n.	1	0	1	1	1	0	1	0	1	3	1	1	1	1	0	0	0	0
Tricorythodes cubensis	2	0	0	1	1	0	2	0	2	3	1	1	1	2	2	1	1	0
Tricorythodes sp. n. MG	2	0	1	2	1	0	0	0	2	3	1	1	1	1	0	1	1	0
Tricorythodes sp. n. RR	0	0	1	2	1	0	0	0	2	3	1	1	1	1	1	0	0	0
Tricorythodes caunapi	1	0	1	1	1	0	2	0	3	3	1	1	1	1	0	1	1	0
Tricorythodes montanus	1	0	1	1	1	0	1	0	2	3	1	1	1	1	0	0	0	0
Tricorythodes molinerii	0	0	1	1	1	0	1	0	2	3	1	1	1	1	0	0	0	0

Table 3. Primer sequences and fragment size in pair-bases for nuclear and mitochondrial fragments.

Name	**Primer Sequence (5'-3')**	**Fragment (pb) size approximately**
LCO1490	GGTCAACAAATCATAAAGATATTGG	658
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	
18Sf	AGGGCAAGTCTGGTGCCAGC	620
18Sr	TTTCAGCTTTGCAACCATAC	
16Sa	GCCTGTTTATCAAAAACAT	520
16Sb	CTCCGGTTTGAACTCAGATCA	

Table 4. Species that were amplified for 16S, 18S and COI nuclear and mitochondrial fragments.

Species	18S	16S	COI
Leptohyphes ecuador	*x*	*x*	*x*
Haplohyphes aquilonius	*x*	*x*	*x*
Haplohyphes baritu	*x*	*x*	*x*
Haplohyphes mithras	*x*		
Leptohyphodes inanis		*x*	
Asioplax numinuh			*x*
Asioplax rondoniensis		*x*	*x*
Asioplax santarita	*x*	*x*	
Asioplax tumaco	*x*		*x*
Macunahyphes australis	*x*		
Tricorythodes arequita		*x*	
Tricorythodes barbus		*x*	
Tricorythodes bullus		*x*	*x*
Tricorythodes caunapi	*x*	*x*	*x*
Tricorythodes cubensis	*x*	*x*	
Tricorythodes dimorfus		*x*	
Tricorythodes hiemalis	*x*	*x*	*x*
Tricorythodes popayanicus	*x*	*x*	*x*
Tricorythodes quizeri	*x*	*x*	*x*
Tricorythodes molineri	*x*	*x*	*x*
Tricorythodes montanus	*x*	*x*	*x*
Tricorythodes mirus			*x*
Tricorythodes sallesi	*x*		*x*
Tricorythodes sp. n. RR	*x*	*x*	*x*
Tricorythodes sandonensis	*x*	*x*	*x*
Tricorythodes sierramaestrae	*x*	*x*	*x*
Tricorythodes yura	*x*		*x*
Tricorythodes sp. n. MT	*x*		
Tricorythodes sp. n. MG	*x*	*x*	*x*

(*x* = amplified sequence)

Fig. 1

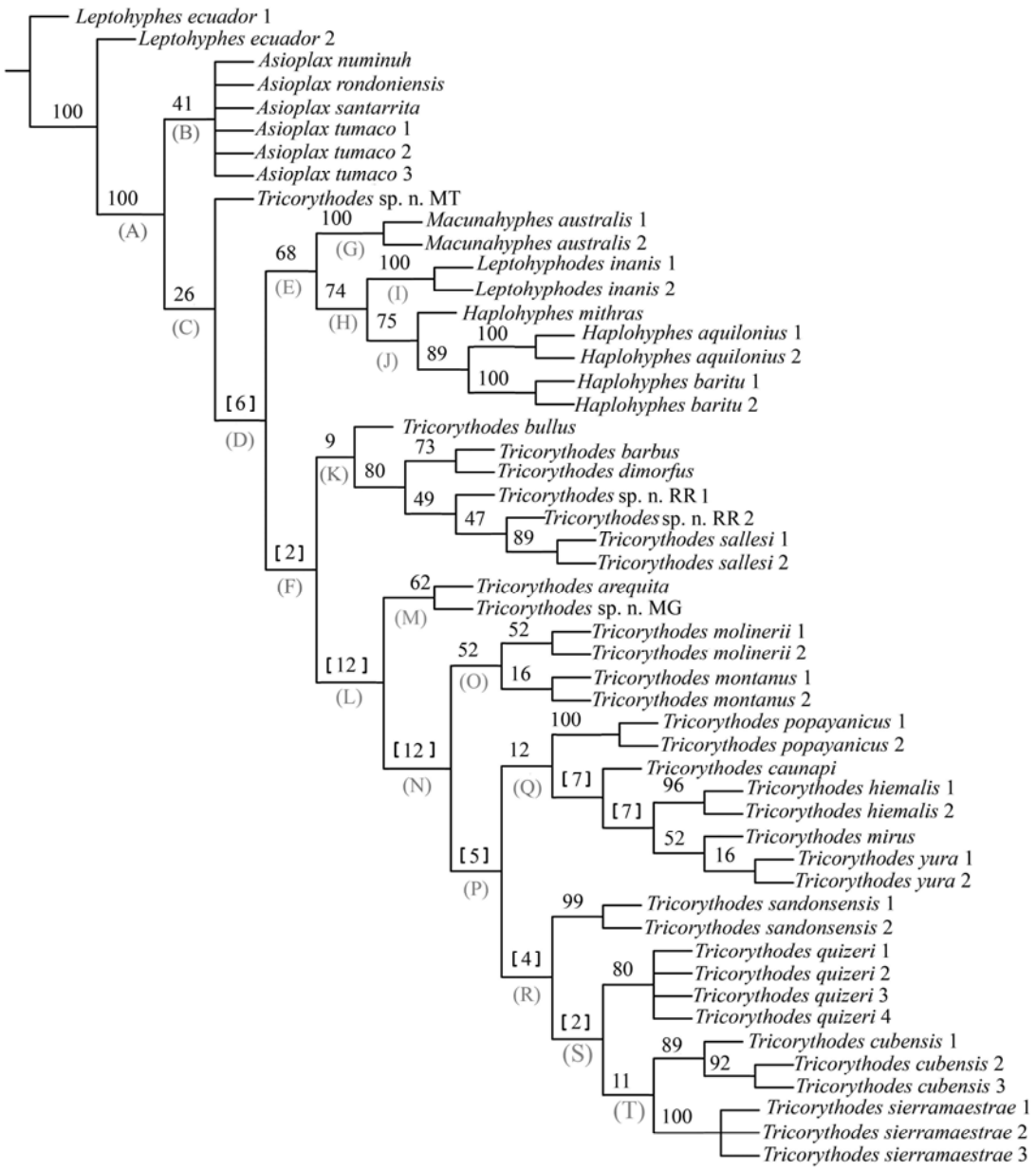


Figure 1. Consensus tree (seven shortest trees – 2999 steps) with combined data (molecular and morphology) mixed model normal parsimony analysis topology computed with TNT program.

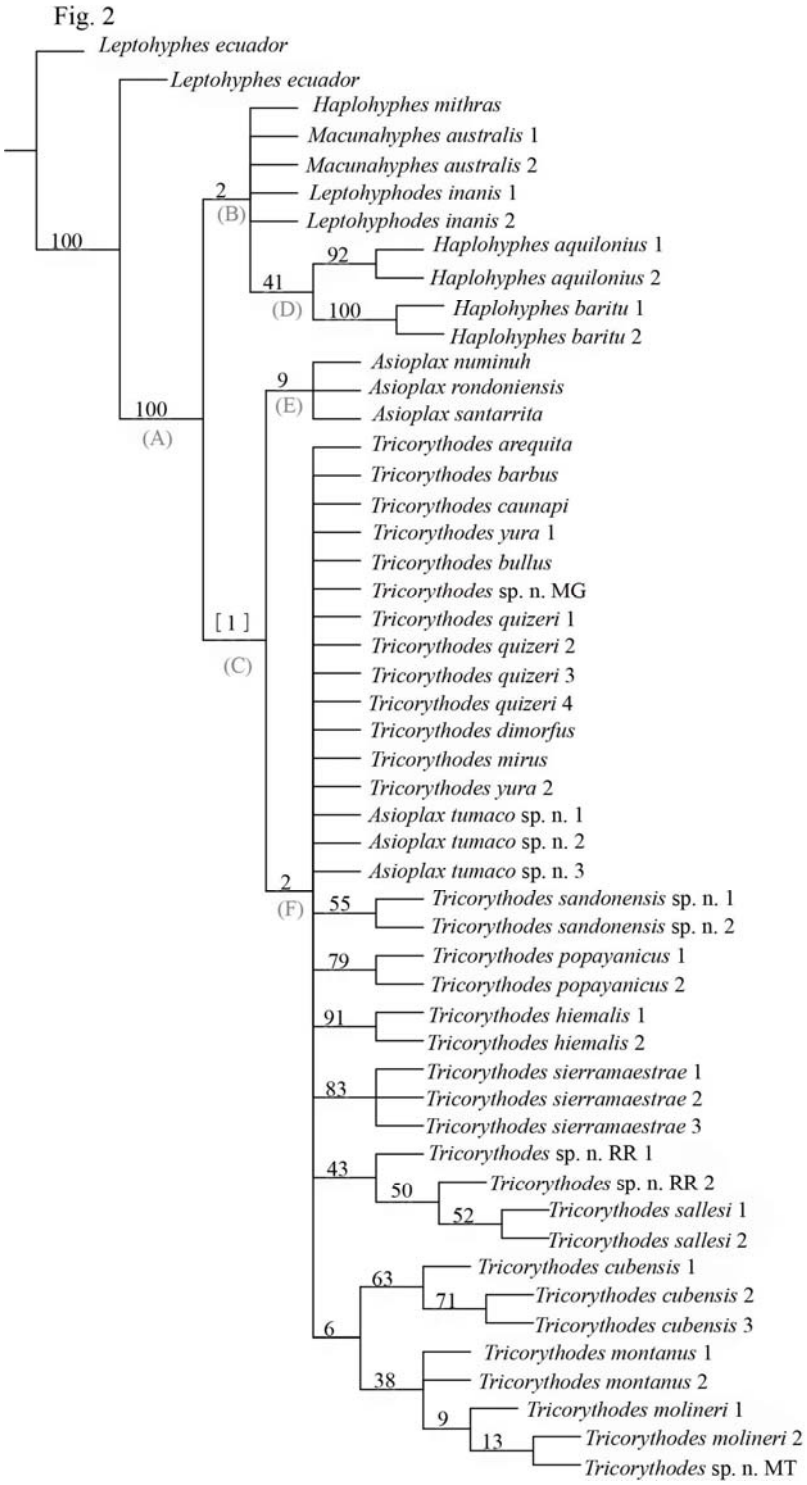


Figure 2. Consensus tree (1232 shortest trees – 2823 steps) with molecular data mixed model normal parsimony analysis topology computed with TNT program.

Fig. 3

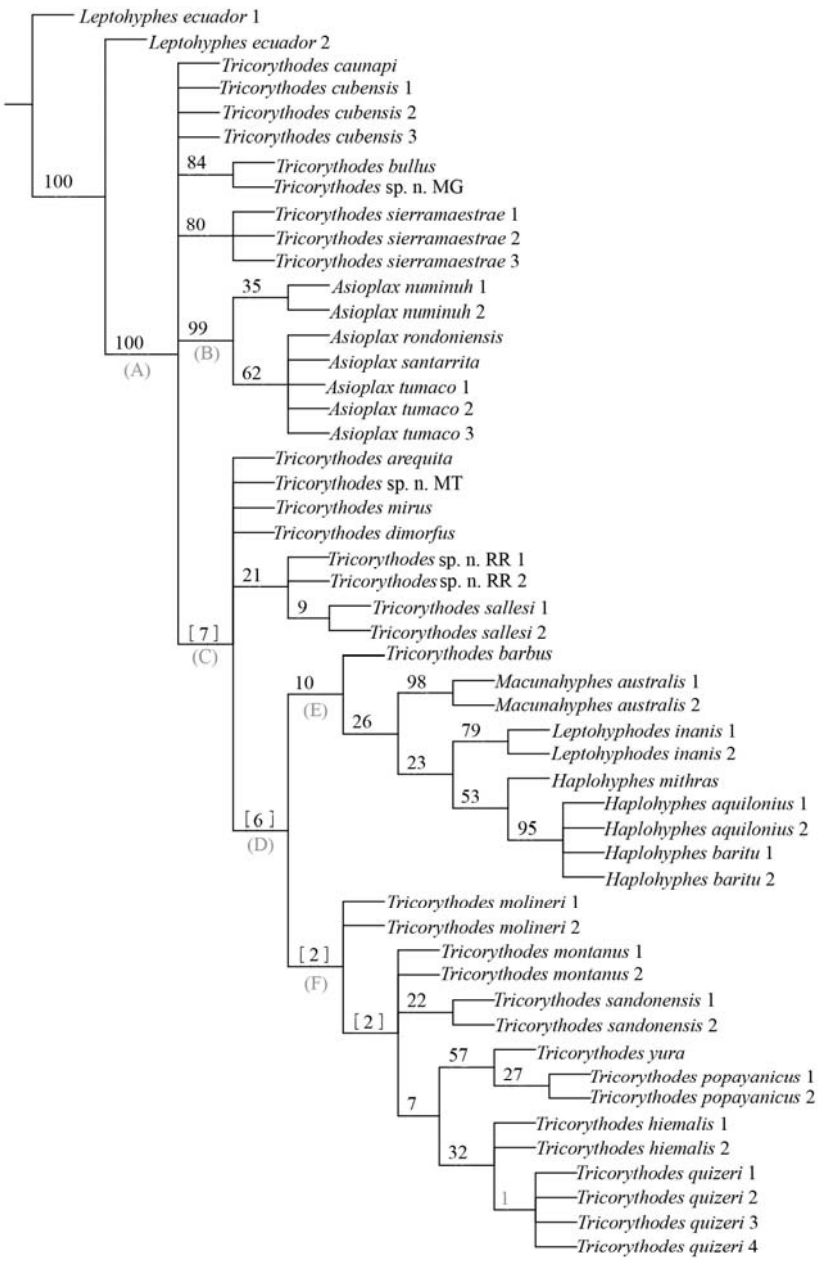


Figure 3. Consensus tree (24 shortest trees – 124 steps) with morphological data mixed model normal parsimony analysis topology computed with TNT program.

Appendix 1. Morphological characters and character states:

Adult characters:

Thorax

0. Hind wings: 0 = present, 1 = absent.

1. Fusion of parapsidal sutures: 0 = medioparapsidal suture (mps) fused with transverse interscutal suture (tis) (Fig. 4), 1 = mps fused with lateroparapsidal suture (lps) (Fig. 5).

2. Membranous filaments of mesoscutellum: 0 = absent (Fig. 5), 1 = present (Fig. 4).

3. Number of tarsal segments: 0 = 4, 1 = 5.

Male genitalia

4. Forceps: 0 = two-segmented (Figs. 6, 7), 1 = tree-segmented (Figs. 8-12).

5. Basal swelling on second segment of forceps: 0 = absent, 1 = present (Figs. 8-11).

6. Relative length of forceps segments 1 and 2: 0 = segment 1 shorter or subequal than segment 2 (Figs. 8,10-12), 1 = segment 1 more than 50% longer (Fig. 9).

7. Form of penis: 0 = plate-like (Figs. 6-8), 1 = pyramidal (Fig. 10), 2 = narrowing slightly on apical 1/3 (Fig. 9), 3 = narrowing abruptly on apical 1/2 or 2/3 (Fig. 11), 4 = “australis”-type, with a ventral projection (Figs. 12a, b).

8. Median lobes of penis: 0 = present (Fig. 6), 1 = reduced (Figs. 7, 8), 2 = absent (Figs. 10).

9. Fusion of penis: 0 = basal half (Fig. 6), 1 = almost completely fused (Figs. 7-12a).

Female

10. Female cerci: 0 = sub equal or longer than forewings, 1 = shorter than wings.

Colour markings (adults and nymphs)

11. Subapical blackish marks on tibiae and tarsi: 0 = absent, 1 = present.

Nymphal characters:

12. General shape of body: 0 = elongated, 1 = stout, 2 = very depressed, circular outlining.

13. Tubercles on head and thorax: 0 = absent, 1 = present.

Head and mouthparts

14. Genal projections: 0 = present (Fig. 13), 1 = absent.

15. Frontal shelf: 0 = present (Figs. 13, 14), 1 = absent.

16. Labial palps, ratio of segment one to segments two and three (lpr): 0 = l < 3 (Fig. 15), 1 = l > 5 (Fig. 16).

17. Maxillae, row of thick setae and dentisetae on inner apical margin: 0=directed medially (Fig. 17), 1= directed apicomedially (Figs. 18-20).

18. Maxillae, form: 0= with a similar width along entire length of galea-lacinia (Fig. 17), 1= gradually getting thinner toward the canines (Figs. 18-20).

19. Maxilla (relative length of stipes and galeolacinia): 0 = stipes subequal than galeolacinia (Figs. 18,19), 1 = stipes longer than galeolacinia (Fig. 20) 2 = galeolacinia longer than stipe (Fig. 17).

20. Maxillary palp (number of segments): 0 = 3 segments, sometimes with an apical seta (Fig. 19), 1 = 2 segments and apical seta (Fig.18), 2 = 1 segment and apical seta (Fig. 17, 20), 3 = palp absent.

Thorax and legs

21. Projections on anterolateral corners of pronotum: 0 = large (Fig. 13), 1 = medium size (Fig. 14), 2 = absent.

22. Width of femora: 0 = thin (maximum width less than 40% of maximum length, Figs. 21a, b), 1 = wide (max. width more than 50% of max. length, Figs. 22a, b).

23. larval femoral setae: 0 = short (Fig. 23), 1 = long (Figs. 21, 22, 24).

24. Position of transverse row of setae on fore femora: 0 = subdistal (Fig. 24), 1 = submedian (Fig. 22a), 2 = subbasal (Fig. 21a).

25. Meso- and metafemora, basal setae: 0 = absent, 1 = present (Fig. 21b, 22b, 23).

26. Tarsal claw (marginal denticles): 0 = present (Fig. 25), 1 = absent (Figs. 26, 27).

27. Tarsal claw (submarginal denticles, near apex): 0 = absent (Fig. 25), 1 = 1 pair (symmetric, Fig. 26), 2 = 3 or more denticles distributed in two asymmetrical rows (Fig. 27).

Abdomen and gills

28. Tubercles on abdomen: 0 = absent, 1 = present.

29. Abdominal segments laterally expanded: 0 = None expanded, 1 = segments III–VI, 2 = III–VII (Fig. 28), 3 = III–VIII.

30. Posterolateral spines on abdominal segment: 0 = absent, 1 = present on segment VII, 2 = on VII–VIII, 3 = on VII–IX (Fig. 28).

31. Basal spine (beak like projection) of gill II: 0 = present (Fig. 29b), 1 = absent.

32. Ridges on operculate gill: 0 = absent, 1 = present (Figs. 30, 31a).

33. Gill formula (number of lamellae per gill, gills II, III, IV, V, VI): 0 = 3-4-4-4-2 or more, 1 = 3-3-3-3-2 (Figs. 31a-f), 2 = 2-3-3-3-2, 3 = 1-3-3-3-2, 4 = 2-3-3-2-0.

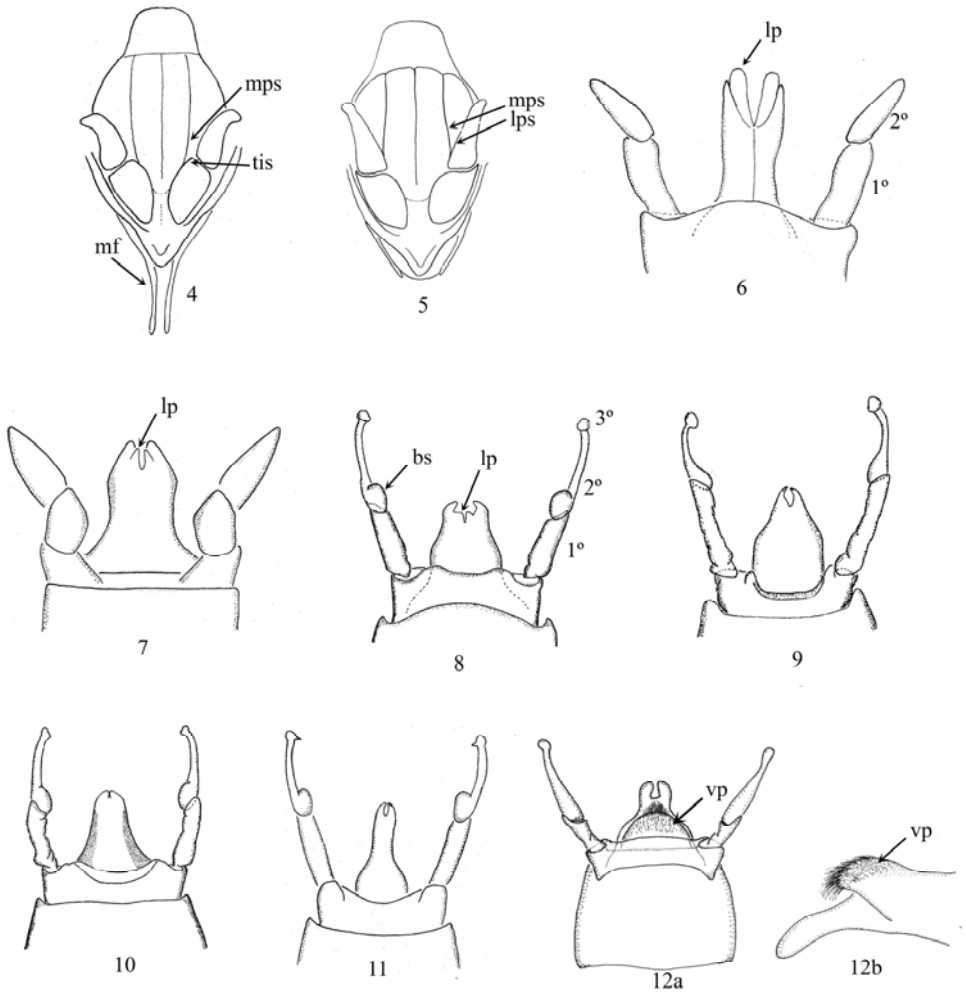
34. Shape of operculate gill: 0 = subtriangular (Fig. 30), 1 = triangular (Fig. 31a), 2 = ovoid (Fig. 29).

35. Pigments on operculate gills: 0 = extense, 1 = restricted to base of gill.

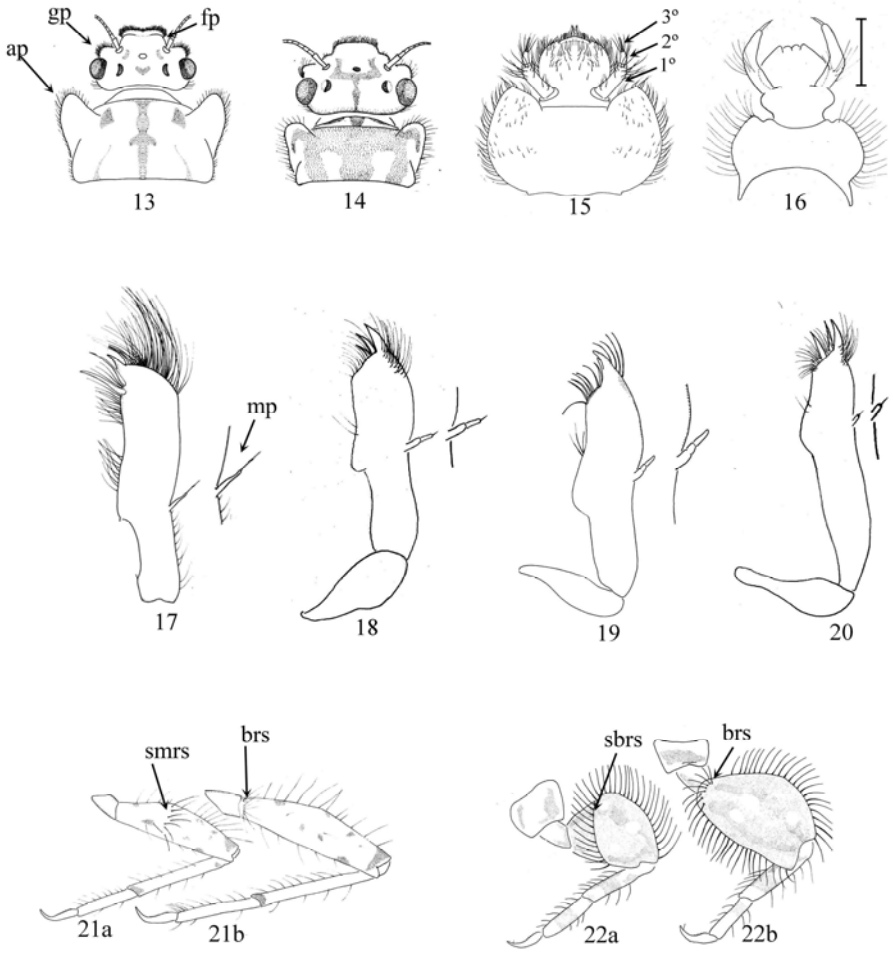
36. Dorsal angle of ventral lamella of gills of segments III–V: 0 = well developed (Figs. 31c-f), 1 = slightly developed, 2 = absent.

37. Basal extension of dorsal lamellae of gills of segments III–V: 0 = well developed (Figs. 31c-f), 1 = slightly, 2 = absent.

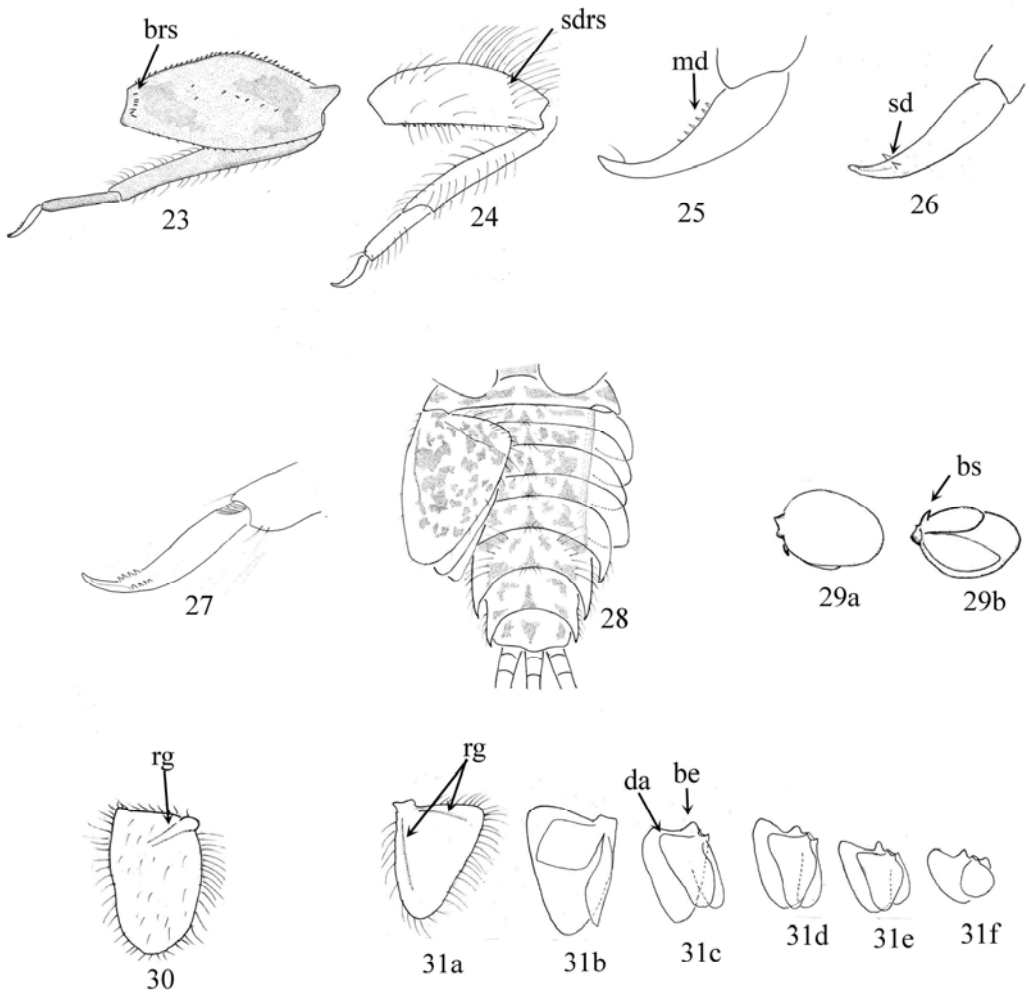
38. Row of setae on abdominal tergum VII: 0 = absent, 1 = present (Fig. 28).



Figures 4-12. 1. Adult characters. Mesonotum: 4. *Leptohyphes*; 5. *Tricorythodes*. Male genitalia: 6. *Haplohyphes*; 7. *Leptohyphodes;* 8. *Tricorythodes arequita*; 9. *Asioplax santarrita*; 10. *Tricorythodes bullus*; 11. *Tricorythodes quizeri*; 12. *Macunahyphes australis,*(a) ventral view and (b) lateral view. (mps = medioparapsidal suture, tis = transverse interscutal suture, mf = Membranous filaments, lps = lateroparapsidal suture, lp = lobes of penis, bs = Basal swelling, vp = ventral projection).



Figures 13-22. Nymphal characters. Head and Pronotum: 13. *Tricorythodes molinerii*; 14. *Tricorythodes barbus*; 15. *Haplohyphes*; 16. *Macunahyphes*. Maxilla (maxillary palp detail): 17. *Macunahyphes*; 18. *T. molinerii*; 19. *Tricorythodes sallesi;* 20. *Tricorythodes santarrita.* Legs: 21. *Asioplax rondoniensis;* 22. *T. sallesi.* (gp = genal projectoion, fp = frontal projection, ap = anterolateral projection of the pronotum, mp = maxillary palp, sbrs = sub basal row of setae, brs = basal row of setae, smrs = submedian row of setae).



Figures 23-31. Nymphal characters. Legs: 23. *Leptohyphes ecuador*; 24. *Haplohyphes*. Tarsal claw: 25. *Tricorythodes rondoniensis*; 26. *Tricorythodes molinerii*; 27. *Haplohyphes*. Abdomen: 28. *Tricorythodes sallesi*. Gills (a, dorsal view and b-f ventral view): 29. *Leptohyphes;* 30. *Leptohyphodes;* 31. *Tricorythodes barbus.* (brs = basal row of setae, sdrs = sub distal row of setae, md = marginal denticles, sd = subapical denticles, rg = ridges of the operculate gill, da = dorsal angle of lamella, be = basal extension of lamella).

ARTIGO 6

Association of nymphs and adults of Ephemeroptera (Insecta) using Amplified Fragment Length Polymorphism (AFLP) technique

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Abstract

Usually many species of Ephemeroptera have been described based on nymphs or adults. Only 10% of Ephemeroptera species in South America are known in both life stages. The current research was carried out in order to molecularly associate immature and adults of Ephemeroptera, using the AFLP technique. We obtained molecular fingerprints by AFLP of five species *Leptohyphes albipennis, L. coconuco, L. ecuador, Baetodes levis* and *Prebaetodes sitesi.* In the first two species the adults and nymphs are known and in the others only the nymphs. The association between nymphs and adults of *L. albipenis and L. coconuco* by this analysis confirmed the effectiveness of the technique. The molecular fingerprints of *B. levis* and *P. sitesi* were generated with the hope that future collections of unknown adults would match the fingerprint and solve an identification problem. We describe here for the first time the adult of *L. ecuador* by matching an adult trapped in the field with the DNA fingerprint of nymphs obtained by means of AFLP.

Keywords: Ephemeroptera, nymphs, adults, association, DNA, AFLP, fingerprint.

Introduction

The order Ephemeroptera is a diverse and important component of freshwater ecosystems and has been considered excellent indicators of quality of water (Resh and Jackson, 1993; Domínguez *et al.,* 2006; Menetrey *et al.,* 2008). The nymphs play a significant role in nutrients recycling; they are involved in processing organic matter and food web in water streams (Merritt and Cummins, 1996; Grant, 2001; Domínguez *et al.,* 2006). The nymphal life span in mayflies varies from 3-4 weeks to about 2.5 years. In contrast, the adults do not feed and rely on reserves built up during their nymphal life and live from a few hours to a few days depending on the species (Brittain, 1982).

Ephemeroptera is a small group of winged insects, represented by over 3.000 described species in 42 families and more than 400 genera. The highest generic diversity occurs in the Neotropics, with correspondingly high species diversity (Barber-James *et al*., 2008). In South America approximately 15 families and 450 species distributed in 100 genera are reported (Domínguez *et al*., 2006). Despite the effort to known of Ephemeroptera in this region, the fauna of several areas remain uncollected and many species and stages of life remain unknown (Domínguez *et al*., 2006).

In taxonomical descriptions of Ephemeroptera species, the lack of association between nymphs and adults is frequent. It occurs because adults are ephemeral and nymphs are sensible to artificial rearing conditions. As a result, new species, genera and even families of Ephemeroptera are described based only on nymph or adult. Domínguez *et al.* (2006), reported rearing methods to successfully associate nymph and adult mayflies stages. However, a controversial arises when they mentioned that only 10% of Ephemeroptera species in South America are known in both life stages.

In the genus *Baetodes* Needham & Murphy, for instance, most species are known only as nymphs (Nieto, 2004). The adults of the monotypic genus *Prebaetodes* are unknown (Domínguez *et al.,* 2006; Lugo-Ortiz and McCafferty, 1996). For these genera and many other mayflies, temperature and oxygenation are critical factors in rearing methods (Domínguez *et al.,* 2006).

Leptohyphes Eaton is another example, this genus have numerous species described based only on one stage of life. In South America, nine species of this genus are known from adults and nymphs, three species are described based on male adult morphology and six were described based on nymphs (Molineri, 2003; Molineri and Zúñiga, 2006).

A complete knowledge of life stages of species provide important information for the progress of ecological, phylogenetic and evolutionary studies (Miller *et al.,* 2005; Jeon and Ahn, 2007). Molecular biology techniques have been applied to identify immature and adults stages of insects (Fleck *et al.*, 2006; Miller *et al.,* 2005, 2007; Jeon and Ahn, 2007). For example, Miller *et al*. (2005) developed strategies for the association of unknown larvae and known adults of Dytiscidae (Coleoptera) using DNA sequence data, with a 806-bp portion of the gene cytochrome oxidase I. In 2007, Jeon and Ahn used the partial cytochrome oxidase II gene (375 bp) to associate unknown larvae and identified adults of the Staphilinidae (Coleoptera). Fleck *et al.* (2006) used mitochondrial SSU rRNA gene fragments to associate unidentified larvae and described adults of Libellulidae (Odonata: Anisoptera). These works have confirmed that molecular techniques constitute useful tools for the determination of unknown stages of life in species of insects.

In this current work, we are proposing the use of the "Amplified fragment length polymorphism" (AFLP), a molecular technique, to solve taxonomical problems of Ephemeroptera species. The objective was to associate known immature and adults of same species using DNA fingerprints and to generate DNA fingerprints of species which were known only for one stage of life. The species used here were *Leptohyphes albipennis* Molineri & Zúñiga, *Leptohyphes coconuco* Molineri & Zúñiga, *Leptohyphes ecuador* Mayo, *Baetodes levis* Mayo and *Prebaetodes sitesi* Lugo-Ortiz & McCafferty. The first two species were used as positive control, since both adults and nymphs were known, when described in original designation (Molineri and Zúñiga 2006). The other species were described only as nymphs (Lugo-Ortiz and McCafferty, 1996; Domínguez *et al.,* 2006). Developing molecular fingerprint of nymphs of these later species would facilitate future associations of unknown adults in systematic collections.

Materials and Methods

Mayflies studied:

Five species containing 78 specimens were analysed in this research. *Leptohyphes albipennis* (ten adults and ten nymphs), *Leptohyphes coconuco* (ten adults and three nymphs), *Leptohyphes ecuador* (eight nymphs and eight possible adults of this species), *Baetodes levis* (20 nymphs) and *Prebaetodes sitesi* (nine nymphs). Larvae and adults were collected from two water streams in Colombia, located in Quebrada Onda, Sandoná, Nariño, 13-18/x/2007, Dias L.G. and Bacca T. cols. Quebrada Genoy Guaico, Genoy, Nariño, S 1°16'24.35''– W 77°20'16.16'', 2.501 msnm. 20-22/IV/2007, Dias, L.G. and Bacca T. cols.

The insect samples were deposited in the Colección Zoológica, Universidad de Nariño, Nariño, Colombia (PSO). The material containing *Leptohyphes ecuador* was deposited at the Museo de Entomología, Universidad del Valle, Cali, Colombia (MEUV); Instituto-Fundación Miguel Lillo, Tucumán, Argentina (IFML) and Florida A & M University, Tallahassee, Florida, USA (FAMU).

Valued body parts of adults and nymphs were mounted on microscope slides in Canada balsam. Drawings were made with a camera lucida attached to a MZ8 Leica microscope.

DNA extraction:

The DNA was extracted from the entire specimen previously preserved in pure ethanol. Total DNA was extracted with the DNA Extraction Tissue Kit (Qiagen) following the manufacture instructions. Extracted DNA was stored at -20 °C.

AFLP technique:

AFLP analysis was performed using the AFLP Analysis System II for small genomes developed by Life Technologies (GIBCO Invitrogen Corporation, Carlsbad, CA) as recommended by the manufacturer. Each sample of genomic DNA (~125 ng) was completely digested with the restriction endonucleases *Mse*I and *Eco*RI. Double stranded DNA adapters provided in the kit were ligated to the ends of the DNA fragments. Pre-selective primers were complementary to the core of the adapter sequences. The new DNA fragments were then pre-amplified by PCR (Polymerase

Chain Reaction) and selective amplification was performed using four primers combinations (AT/CTC, TG/CAA, TG/CAT, TC/CTA) to select the least polymorphic primers for Ephemeroptera. Amplified fragments were separated by gel electrophoresis for 2 h at 50 V in 6% Long Ranger (FMC Bioproducts, Rockland, ME) denaturing polyacrylamide gels. The gels were silver stained and scanned (PowerLook, 2100xl, UMAX) at 300 dpi and grayscale format.

Data analyses

The presence/absence of scorable fragments was recorded in a binary data matrix. Data from the two primer combinations selected were combined. A similarity matrix was obtained using UPGMA cluster analysis performed using the software Phylogenetic Analysis Using Parsimony (PAUP 4.0b10 Altivec) (Swofford, 1998). Bootstraping was performed with 1.000 replications.

Results and discussion

The UPGMA analysis showed that nymph and adult individuals of a single species were grouped together, and every terminal branch at the species level was supported by 100% regardless of the location where they were collected (Fig. 1). This result was in agreement with the association between larva and adult stages in other insects, when gene sequences were used (Miller *et al.,* 2005, 2007; Fleck *et al.,* 2006; Jeon and Ahn, 2007).

In this research, the AFLP technique revealed advantages in relation to other molecular technique; for instance, previous knowledge about gene sequence of the organisms studied was not necessary and allowed exploration and comparison of the whole the genome, despite its moderate cost (Fernández-Cuenca, 2004; Meudt and Clarke, 2007;). In contrast, the association using sequences required previous knowledge about the entire extent of variation in the target gene, mainly to compare or associate the life stages of closely related species (Miller *et al.,* 2005).

The AFLP technique has usually been used to establish difference among species or populations based on molecular polymorphisms (Najimi *et al.*, 2002; Zhong *et al.,* 2002; Benavides *et al*., 2003; Suinaga *et al.,* 2004). However, this was not the case of this research. We associated nymphs and adults of the same species by getting molecular similarities among individuals. So, in order to minimize

polymorphism effects in the association, we selected the least polymorphic primers for the analysis (TG-CAA and TG-CTA). Even though we found some polymorphisms in some individuals (Figs. 2, 3), the branch of all individuals of a same species was well supported (Fig. 3). Besides, a considerable number of individuals were used to guarantee reliability in the associations.

As expected, adults and nymphs of *L. coconuco* showed similar molecular fingerprints and so was for *L. albipennis* (Figs. 2, 3). These species were described in both life stages (Molineri and Zúniga, 2006). These results confirmed the efficacy of the AFLP technique in associating immature and adults of Ephemeroptera.

We generated for the first time the molecular fingerprint of *Prebaetodes sitesi* and *Baetodes levis* (Figs. 2, 3). These DNA fingerprints would be able to be used in further research aiming to match an unknown adult found in different regions with the nymphs already described, since the AFLP technique is totally reproducible (Meudt and Clarke, 2007).

In fact, an adult of *L. ecuador* was associated with its nymph for the first time (Figs. 1-3). This is the first time that *L. ecuador* has been reported after its description by Mayo (1968) from species collected in Cotopaxi Province in Ecuador. Our findings were made from specimens collected in Colombia at the department of Nariño.

The association between nymphs and adults of *L. ecuador* would has been difficult to determine without using molecular techniques, because the morphology of the adults of this species was very similar to the adults of *L. coconuco.* In addition, these species (*L. coconuco* and *L. ecuador*) together with *L. albipennis* were sympatric, sharing the same hour of adult activity and the same microhabitats as nymphs, as reported by Molineri (2003) and Molineri and Zúñiga (2006) for other species of the *Leptohyphes*.

In many other orders of aquatic insects, such as Plecoptera and Odonata similar association problems occur. In these orders, the species identification is usually based on adults; the nymph does not present sufficient morphological characters for the identification at the species level, and the identification depends upon an established link to unequivocally identified imagines (Fleck *et al.,* 2006; Stark *et al.,* 2009). Given the successful association of these specimens through AFLPs technique, similar success may be expected in associating of the adults and inmatures of other insect groups.

In conclusion, the association of nymphal and adults stages of Ephemeroptera through AFLPs technique constituted a useful tool for the taxonomic determination of the group. Besides, this technique is also efficient in the differentiation of close related species of *Leptohyphes* genus.

A description of the adults of *L. ecuador* and additional proposed characters to complement the nymphs' description are as follow:

Description of the adult of *L. ecuador* Mayo, 1968

Male imago

Length: body, 3.2 – 4.5 mm; forewings, 5.2 – 6.0 mm; hind wings, 0.6 – 1.0 mm. General coloration yellowish-brown shaded with gray or black.

Head: shaded with black between ocelli, small whitish mark posterior to median ocellus; occiput whitish. Eyes blackish, ocelli whitish rounded with black. Antennae yellowish.

Thorax: pronotum yellowish shaded with gray, except on sublateral region and median longitudinal line; meso- and metanotum yellowish brown shaded with gray; membranous filaments of mesoscutellum completely blackish; mesopleurae, mesosternum and metathorax yellowish, shaded with darker gray on sclerites. Legs yellowish; forefemora, foretibiae and tarsi shaded with gray on dorsal margin; middle and hind femora shaded with grayish black on dorsal marks, on subbasal and subapical regions; tibiae and tarsi yellowish with brownish margins. Wings (Figs. 4-6): membrane of wings hyaline lightly tinged with yellow, shaded with gray at base and on costal margin; longitudinal veins brownish, cross-veins yellowish. Hind wings with tree longitudinal veins (Figs. 5, 6).

Abdomen: translucent yellowish-white shaded with gray, shading darker on pair of submedian longitudinal lines on terga II – VI, and a mediolongitudinal band on terga VII –X darker; abdominal sterna whitish shaded with gray; pleural folds whitish. Genitalia (Fig. 7): penis Y-shaped (Fig. 7); styliger plate whitish with yellowish margins; forceps paler; penis whitish; basal region of the penis slightly expanded,

with curved external margin as Fig. 7. Cerci white shaded with light gray; terminal filament whitish-translucent.

Female imago

Length: body, 4.0 – 5.0 mm; forewings, 6.0 – 7.0 mm. Head and thorax as in male, but darker; membranous filaments of mesoscutellum shaded gray. Wings as in male except usual sexual dimorphism (Fig. 8), hind wings absent. Abdomen as in male. Ninth sternum yellowish brown with anterolateral margins yellowish, apex with a small median notch. Caudal filaments whitish-translucent shaded with light gray. Eggs yellowish with greyish polar cap.

Diagnosis

Leptohyphes ecuador can be distinguished from the other species of the genus by the following combination of characters. In the imago: (1) occiput whitish; (2) thorax yellowish shaded with gray; (3) veins of wings brownish-yellow; (4) hind wings absent in female; (5) membranous filaments of mesoscutellum blackish in male and grayish in female; (6) penis Y shaped, with basal region slightly expanded (Fig. 7).

Nymph remarks.

An additional characteristic to nymphs of *L. ecuador* is mentioned: dorsum of middle and hind femora with mediolongitudinal row of 5-7 spines (Fig. 9).

The presence of this characteristic was confirmed on the holotype of this species deposited in FAMU, and all specimens from Colombia studied in this current work have this characteristic.

Biology and ecology

The nuptial flight was observed near the stream, 2-3 m above the ground or water. Swarms were numerous and contained hundreds of males; each male moved in the usual up and down manner. The flight was observed before sunrise at 6:00 am and disappeared at 8:30 am.

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References

Barber-James H.M., Gattolliat J-L., Sartori M, Hubbard M.D. 2008. Global diversity of mayflies (Ephemeroptera, Insecta) in freshwater. Hydrobiol., 595:339-350.

Benavides P.M., Vega F., Stuart J., Bustillo A.E.P. and Everson J.R. 2003. Biodiversity and biogeography of an important inbred pest of coffee, the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae). Ann. Entomol. Soc. Am., 98, 359-366.

Brittain J.E. 1982. Biology of Mayflies. Annu. Rev. Entomol., 27, 119-147.

Domínguez E., Molineri C., Pescador M., Hubbard M.D. and Nieto C. 2006. Aquatic Biodiversity in Latin America. Pensoft, Sofia-Moscow, v.2: Ephemeroptera of South America, 646 p.

Fernández-Cuenca F. 2004. Aplicaciones de las técnicas de PCR a la epidemiología molecular de las enfermedades infecciosas. Enferm. Infecc. Microbiol. Clin., 22, 355-360.

Fleck G., Brenk M. and Misof B. 2006. DNA Taxonomy and the identification of immature insect stages: the true larva of *Tauriphila argo* (Hagen 1869) (Odonata: Anisoptera: Libellulidae). Ann. soc. entomol. Fr., 42, 91-98.

Grant P. M. 2001. Mayflies as food. *In*: Domínguez E (ed.). Trends in Research in Ephemeroptera and Plecoptera, Kluwer Academic / Plenum Publishers, New York, 107-124.

Jeon M.-J. and Ahn K.-J. 2007. Descriptions of late instars of three littoral *Cafius* species (Coleoptera: Staphylinidae) by association df life stage with DNA sequences. Flor. Entomol., 90, 465-474.

Lugo-Ortiz C.R. and McCafferty W.P. 1996. Phylogeny and classification of the Baetodes complex (Ephemeroptera:Baetidae),with description of a new genus. J. N. Am. Benthol. Soc., 15, 367-380.

Mayo V.K. 1968. Some new mayflies of the subfamily Leptohyphinae (Ephemeroptera: Tricorythidae). Pan. Pacific. Entomologist., 44, 301-308.

Menetrey N., Oertli B., Sartori M., Wagner A. and Lachavanne J.B. 2008. Eutrophication: are mayflies (Ephemeroptera) good bioindicators for ponds?. Hydrobiol., 597,125-135.

Merritt R.W. and Cummins K.W. 1996. An introduction to Aquatic Insects of North America. Third edition. Kendall/Hunt Publishing Company. Dubuque, 862 p.

Meudt H.M. and Clarke A.C. 2007. Almost Forgotten or Latest Practice? AFLP applications, analyses and advances. Trends Plant Sci., 12, 106-117.

Miller K.B., Alarie Y., Wolfe G.W. and Whiting M.F. 2005. Association of insect life stages using DNA sequences: the larvae of *Philodytes umbrinus* (Motschulsky) (Coleoptera: Dytiscidae). Syst. Entomol., 30, 499-509.

Miller K.B., Alarie Y. and Whiting M.F. 2007. Description of the larva of *Notaticus fasciatus* (Coleoptera: Dytiscidae) associated with adults using DNA sequence data. Ann. Entomol. Soc. Am., 100, 787-797.

Molineri C. 2003. Revision of the South American species of Leptohyphes (Ephemeroptera: Leptohyphidae) with a key for the nymphs. Stud. Neotrop. Fauna Environ., 38, 47 – 70.

Molineri C. and Zúñiga M.C. 2006. New species of Leptohyphidae (Insecta: Ephemeroptera) from Colombia with evidence of reproductive time segregation. Stud. Neotrop. Fauna Environ., 41, 139-151.

Najimi B., Boukhatem N., El Jaafari S., Jlibène M. and Paul R, Jacquemin J.-M. 2002. Amplified fragment length polymorphism (AFLP) analysis of markers associated with H5 and H22 Hessian fly resistance genes in bread wheat. Biotechnol. Agron. Soc. Environ. 6,79–85.

Nieto C., 2004. The genus *Baetodes* (Ephemeroptera: Baetidae) in South America with the description of new species from Argentina, Bolivia and Peru. Stud. Neotrop. Fauna Environ., 39, 63–79.

Resh V.H. and Jackson J.K. 1993. Rapid assessment approaches to biomonitoring using benthic macroinvertebrates. In: Rosenberg, D. M. & V. H. Resh (eds), Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapmann and Hall, V. H. New York: 159-194.

Stark B., Froehlich C. and Zuñiga M.C., 2009. South American Stoneflies (Plecoptera). Aquatic Biodiversity in Latin America Vol. 5, Pensoft, Sofia, 154 p.

Suinaga F.A., Casali V.W.D., Picanço M. and Foster J., 2004. Genetic divergence among tomato leafminer populations based on AFLP analysis. Pesq. Agropec. Bras., 39, 645-651.

Swofford D.L. 1998. Phylogenetic analysis using parsimony computer program, version 4. D. L. Swofford Sunderland, MA.

Zhong M., McCarty J.C., Jenkins J.N. and Saha S. 2002. Assessment of day-neutral backcross populations of cotton using AFLP markers. J. Cotton Sci., 6, 97-103.

Figure 1. UPGMA tree based on DNA fingerprints. The number at the right side of the individuals represents the order in the gel. Bootstrap values are indicated.

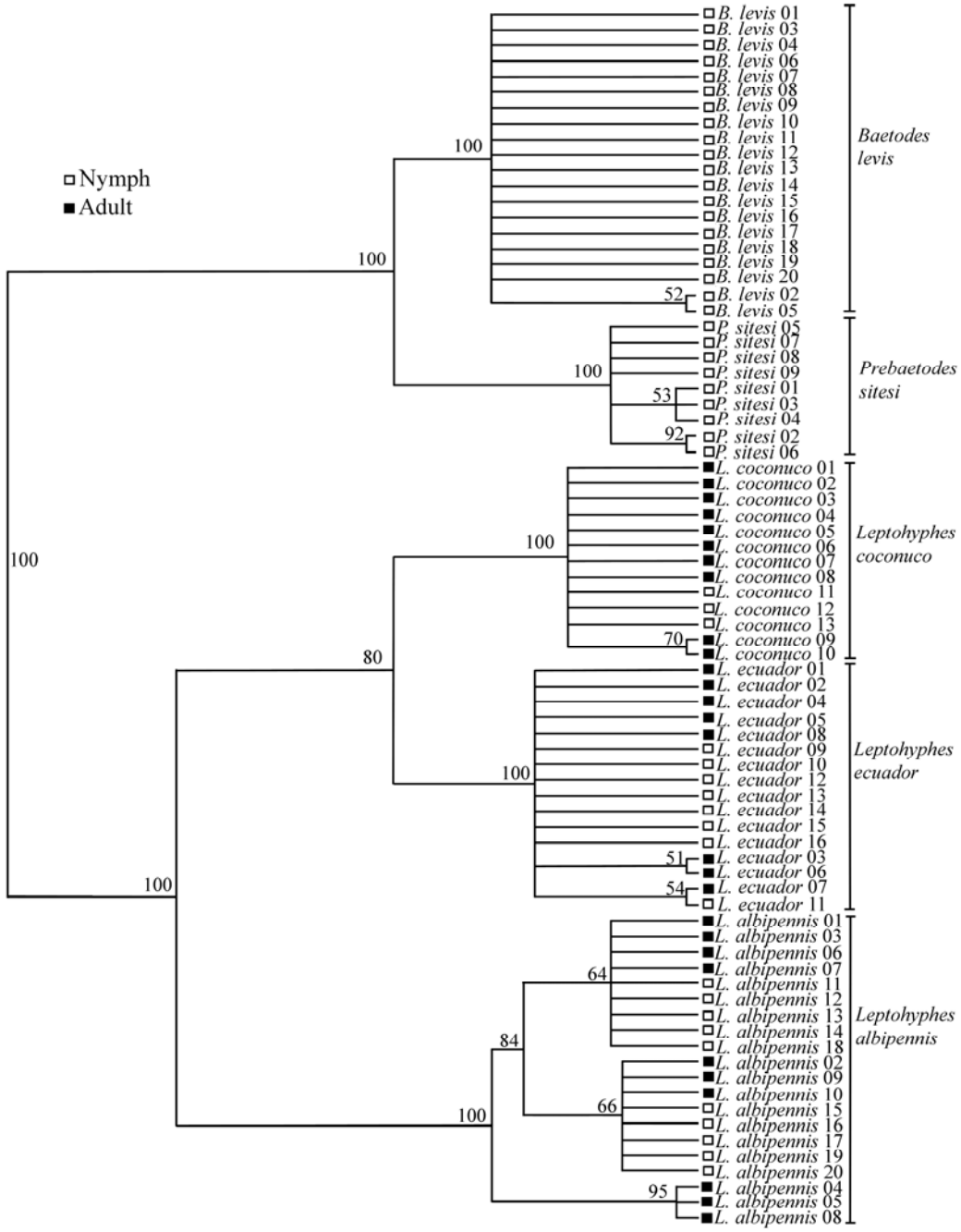


Figure 2. Gels showing AFLP-based DNA fingerprints of *B. levis, P. sitesi, L. coconuco, L. ecuador, L. albipennis* using TG/CAA primer combination (N = nymphs, A = adults, M = molecular weight marker).

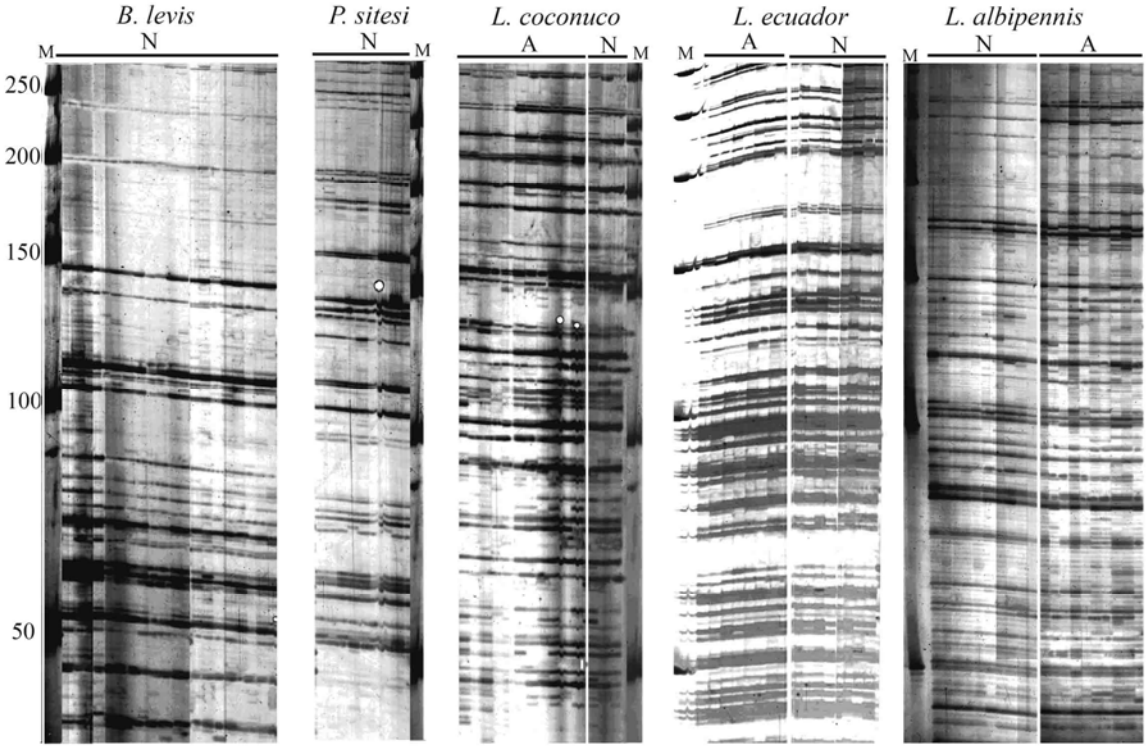
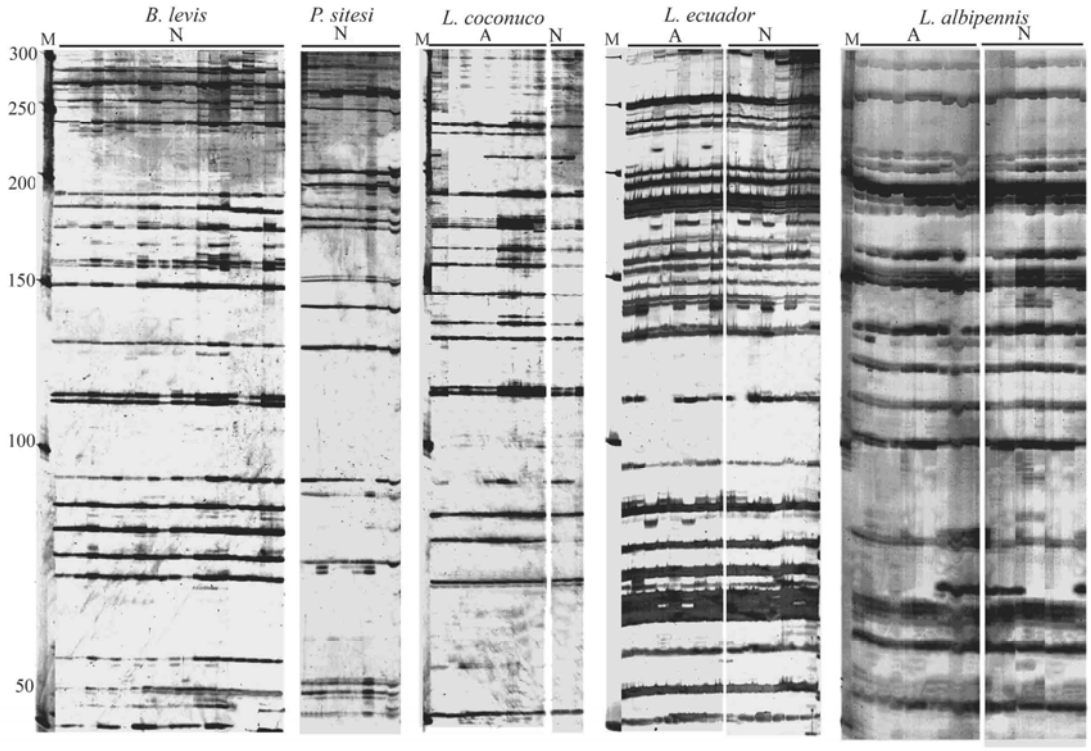
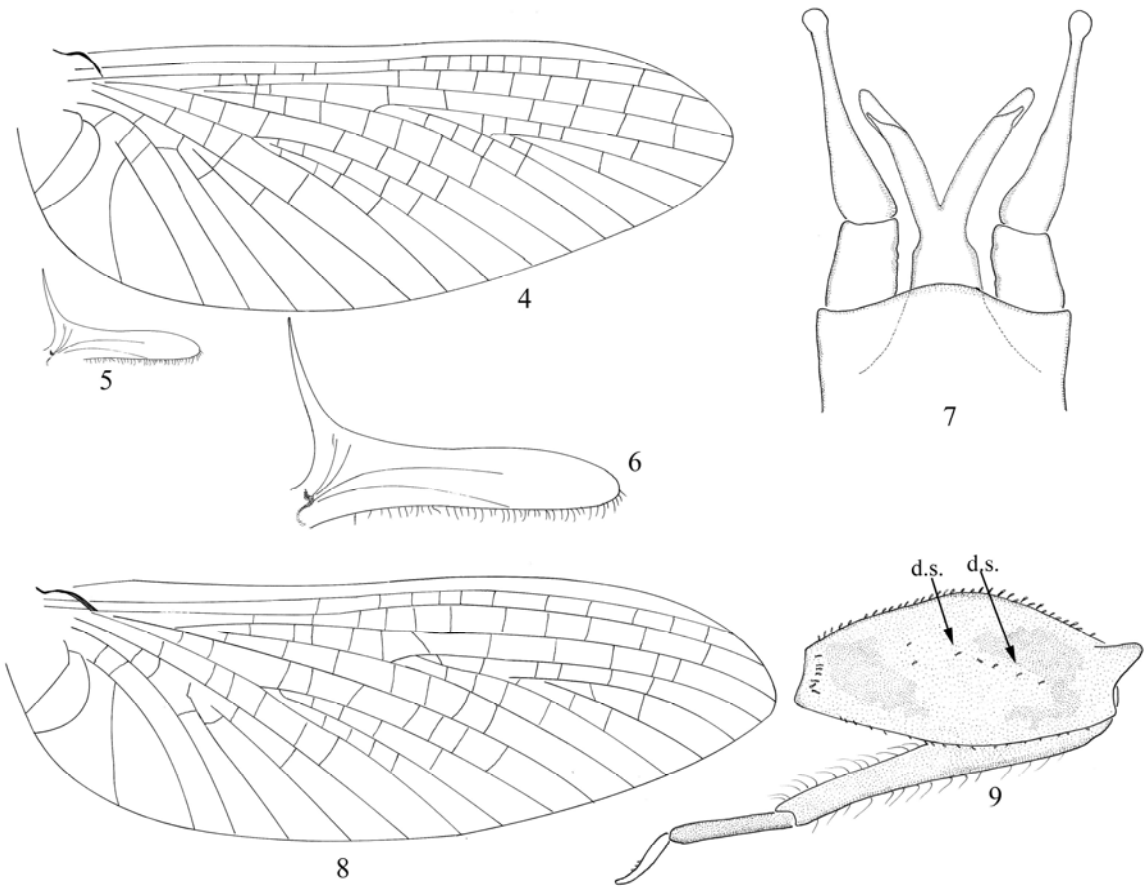


Figure 3. Gels showing AFLP-based DNA fingerprints of *B. levis, P. sitesi, L. coconuco, L. ecuador, L. albipennis* using TC/CTA primer combination



Figures 4-9. *Leptohyphes ecuador*. Adult : (4) male forewing; (5) male hind wing; (6) the same, enlarged; (7) male genitalia, ventral view. (8) female forewing; Nymph: (9) hind leg (d.s. = dorsal spine).



CONCLUSÕES GERAIS

A descrição de duas novas espécies para *Tricorythopsis* Traver (*T. bahyensis* e *T. yucupe*) e três espécies para *Tricorythodes* (*T. sallesi, T. rondoniensis* e *T. caunapi*), além das outras cinco espécies novas citadas na tese que ainda não foram descritas (*Tricorythodes* sp. n. MG, *Tricorythodes* sp. n. MT, *Tricorythodes* sp. n. RR, *Tricorythodes sandonensis* sp. n. e *Asioplax tumaco* sp. n.), corroboram com a hipótese de que uma grande quantidade de taxa de Ephemeroptera permanece por ser descoberta na região Neotropical. O que reforça a necessidade da realização de trabalhos taxonômicos e ampliação das coletas nesta região, afim de que a riqueza faunística seja melhor documentada.

A primeira analise filogenética de *Tricorythodes* Ulmer utilizando dados moleculares e morfológicos, apresentou evidencias para a sinonimização dos gêneros: *Epiphrades* Wiersema & McCafferty, *Homoleptohyphes* (Allen & Murvosh), *Tricoryhyphes* (Allen & Murvosh) e *Tricorythodes* (Allen & Murvosh). As análises apontaram também evidencias para a revalidação de *Asioplax* Wiersema & McCafferty e *Macunahyphes* Dias, Salles & Molineri, como gêneros de Leptohyphidae. Entretanto, seria necessária a complementação desta analise através de outros marcadores e/ou caracteres morfológicos para a formalização destes resultados.

A associação de ninfas e adultos de Ephemeroptera através da técnica de AFLP constitui uma ferramenta útil para a determinação taxonômica do grupo. A descrição do adulto da espécie *Leptohyphes ecuador* Mayo, além do primeiro registro desta espécie para Colômbia contribuem para a expansão do conhecimento faunístico na América do Sul.