

**UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO
CENTRO DE CIÊNCIAS HUMANAS E NATURAIS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS**

**Filogenia e Biogeografia de Leptohyphidae
(Insecta: Ephemeroptera)**

Paula Malaquias Souto

Vitória, ES

Maior, 2018

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Filogenia e Biogeografia de LeptoHyphidae (Insecta: Ephemeroptera)

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*Foi um rio que passou
em minha vida....*

RESUMO

Leptohyphidae comporta 163 espécies e 15 gêneros válidos, apresentando distribuição pan-americana e provável origem Neotropical. Pertence à infraordem Pannota, onde já foi considerada subfamília de Tricorythidae. No entanto, não existe um consenso entre os pesquisadores quanto às relações entre as famílias de Pannota e qual seria o grupo irmão de Leptohyphidae. Dentre as famílias propostas como mais relacionadas à Leptohyphidae, Tricorythidae e Coryphoridae são dois exemplos. Sendo a primeira restrita ao continente Africano e a segunda endêmica da Amazônia, os dois relacionamentos sugerem diferentes propostas biogeográficas para a origem e evolução de Leptohyphidae. Dentro deste contexto, a presente tese apresenta dois estudos dentro de Leptohyphidae: um acerca da diversidade genética de uma possível espécie críptica com variação na cor dos olhos, *Leptohyphodes inanis*; e outro que investiga as relações filogenéticas de Leptohyphidae com as demais famílias de Pannota, além dos relacionamentos entre gêneros dentro da família e os possíveis processos que contribuíram para o padrão de distribuição atual dos gêneros de Leptohyphidae. Com objetivo de identificar a distância genética dentro de *L. inanis*, sequenciamos um segmento do gene mitocondrial COI de 17 indivíduos de diferentes populações com duas cores de olhos: vermelho e preto. Através das análises moleculares (Inferência bayesiana, *Neighbour Joining* e rede de haplótipos), foi possível encontrar três linhagens evolutivas, contudo as divergências genéticas intraespecíficas modelada pelo Kimura-2 parâmetros mostraram valores muito altos (0 a 30.5%) com 23.3 a 24.9% de média entre as linhagens e 4 a 13% dentro das linhagens, sugerindo uma forte evidência de pelo menos três espécies putativas no complexo de espécies de *L. inanis*. Ainda, os olhos vermelhos foram recuperados como traços plesiomórficos no grupo, não sendo um bom diagnóstico para identificar as espécies. Acerca do segundo estudo, as análises foram feitas com base em caracteres moleculares e morfológicos usando abordagens bayesiana e de parcimônia. Todas as análises recuperaram o monofiletismo da família, enquanto que o grupo irmão de Leptohyphidae variou conforme o método, podendo ser Teloganodidae (Afrotropical), Ephemerythidae (Afrotropical) ou o clado formado por Melanemerella + Coryphoridae + Teloganodidae (Mata Atlântica, Amazônico e Afrotropical, respectivamente). Baseado nas análises de datação molecular, S-DIVA e do VIP (Vicariant Event Program), o ancestral de Leptohyphidae foi encontrado restrito à Subregião Chacoana na América do Sul após um evento vicariante do continente Gondwânico, por volta de 151.9 milhões de anos (120.0 – 184.4).

Palavras-chave: Sistemática, diversidade genética, espécies crípticas, dados combinados

ABSTRACT

Leptohyphidae is a Pan-American mayfly family with 163 species divided into 15 genera. It belongs to the infraorder Pannota, where it was already considered a subfamily of Tricorythidae. However, there is no agreement among researchers as to how exactly pannotan families are related, specially concerning to leptohyphid sister group. Among the families proposed as more related to Leptohyphidae, Tricorythidae and Coryphoridae are two examples. Being the first restricted to the African continent and the second endemic of the Amazon, the two relationships suggest different biogeographic proposals for the origin and evolution of Leptohyphidae. Within this context, the present dissertation presents two studies within Leptohyphidae: one about the genetic diversity of a possible cryptic species with eye color variation, *Leptohyphodes inanis*, and another investigating the phylogenetic relationships of Leptohyphidae with other pannotan families, as well as the relationships between genera within the family and the possible processes that contributed to the current distribution pattern of Leptohyphidae genera. To assess genetic differences among populations and relate them to the colour variation of the eyes, we sequenced a segment of the mitochondrial COI gene from 17 individuals from different populations with two colours variations: red and black. All analyses (Bayesian Inference, Neighbor Joining and haplotype network) found three independently highly supported evolutionary lineages, each one mostly restricted to a Southeastern Brazil mountain range. However, Pairwise divergences modelled by Kimura-2 parameter showed high values of intraspecific genetic divergence (0 to 30.5%), with 23.3 to 24.9% between lineages and 4 to 13% within lineages, providing strong evidence of at least three putative species in the *L. inanis* species complex. In addition, red eyes were recovered as plesiomorphic traits in the group and not a good diagnostic to identify the species. About the second study, the analyses were made based on molecular and morphological characters using Bayesian and parsimony approaches. All analyses recovered the monophyly of the family, whereas the Leptohyphidae sister group varied according to the method, such as Teloganodidae (Afrotropical), Ephemerythidae (Afrotropical) or a clade formed by Melanemerellidae + Coryphoridae + Teloganodidae (Atlantic Forest, Amazonian and Afrotropical, respectively). Based on S-DIVA and divergence time estimation analyses, ancestral Leptohyphidae was found restricted to the Chacoan Subregion in South America after a vicariant event of the Gondwana continent, around 151.9 Mya (120.0 - 184.4). Furthermore, as result three new species were described, two belonging to *Macunahyphes* and one to *Tricorythodes*.

Key-words: Systematics, genetic diversity, cryptic species, combined dataset

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Artigos publicados em anexo no final da tese:

Souto, P.M. & Salles, F.F. 2016. New species of *Macunahyphes* Dias, Salles & Molineri (Ephemeroptera: Leptohyphidae), with taxonomic notes. *European Journal of Taxonomy*, 254, 1–15.

Souto, P.M., Angeli, K.M. & Salles, F.F. 2017. *Tricorythodes tragoedia* sp. nov. (Ephemeroptera: Leptohyphidae), a new species from Rio Doce and surrounding areas, southeastern Brazil. *Zootaxa*, 4341, 555–562.

Introdução Geral e Histórico Taxonômico de Leptohyphidae

“The most important ingredient is a fascination with the wonders of living creatures. And this stays with most biologists for their entire life.”

~Ernst Mayr, **This is Biology: The Science of the Living World.**

Apesar do valor da água doce para a humanidade e a biofilia inerente ao ser humano (Wilson 1984), os corpos hídricos continentais são os ambientes naturais mais impactados por ação antrópica. Atualmente a contaminação da água atinge a maioria dos corpos hídricos continentais (Sarmiento-Soares & Martins-Pinheiro 2017) e, com o contínuo desmatamento da mata ripária, tal fato só tende a aumentar com o tempo. As florestas ripárias interceptam sedimentos, fertilizantes e pesticidas que adentram os rios através de escoamento superficial ou subterrâneo (Naiman *et al.* 2005), sendo essencial para a saúde dos corpos hídricos. As florestas também influenciam as trocas de material orgânico entre o sistema terrestre e aquático (Pusey & Arthington 2003), material este (folhas, troncos, frutos) que serve como substrato, alimento e abrigo para a fauna aquática (Pusey & Arthington 2003; Casatti 2010).

Um exemplo da crise hídrica que assola a humanidade é a situação atual de seca no Espírito Santo (ES), especialmente no Norte do estado. Nesta área geográfica muitos rios perderam sua mata ciliar e encontram-se intensamente assoreados, com vazões reduzidas e muitas nascentes fluviais secas (Sarmiento-Soares & Martins-Pinheiro 2017). Tal fato afeta, inclusive, a capacitação de água de algumas cidades, causando desde falta de água até presença de água salgada no abastecimento. Esse é o caso do rio Cricaré, braço sul do rio São Mateus, com nascente localizada no município São Felix de Minas, no estado de Minas Gerais. O abastecimento de água da cidade de São Mateus no norte do estado, é feita por de captação da água do rio Cricaré, que constantemente encontra-se seco e invadido pela água do mar, fazendo com que o abastecimento da população seja com água salgada.

Outro exemplo é a tragédia do Rio Doce, considerada a maior tragédia ambiental do Brasil. As práticas imprudentes da mineradora Samarco (controlada através pela brasileira Vale S.A. e a anglo-australiana BHP Billiton) causaram uma quebra na barragem de Fundão que descarregou de 55 a 62 milhões de m³ de rejeitos de minério de ferro diretamente na bacia hidrográfica do

rio Doce na cidade de Mariana, estado de Minas Gerais (GTF 2015). Esse volume representa a maior explosão de rejeitos na história moderna da humanidade (IBAMA 2015). A grande onda de lama envolveu o pequeno distrito de Bento Rodrigues, destruindo um patrimônio cultural datado do ano de 1700, deslocando toda a população (600 pessoas) e matando pelo menos 19 pessoas (Marinha do Brasil 2016). A lama encheu as redes hidrológicas ao longo de 663,2 km do rio Doce nos estados de Minas Gerais (MG) e do Espírito Santo (ES), até alcançar o seu estuário, na cidade de Linhares (ES) e ser atirada ao mar (INPE 2015; Fernandes *et al.* 2016). Após dois anos da tragédia, o dano ainda é incontável tanto para as populações ribeirinhas, quanto para a flora e fauna associada ao rio Doce e o ambiente estuarino associado a ele. As consequências em escalas espaciais mais amplas, incluindo as águas internacionais através do movimento transfronteiriço de sedimentos em suspensão, permanecem desconhecidas (Fernandes *et al.* 2016).

Neste cenário encontram-se os insetos da ordem Ephemeroptera, em uma relação de dependência com os frágeis ambientes aquáticos. Os indivíduos da ordem são conhecidos especialmente pelo curto período de vida na fase adulta alada, sendo a fase imatura a maior duração de vida das espécies. Essa existência efêmera é evidente na própria morfologia dos indivíduos, os quais não possuem todo o aparelho digestivo, desde a boca, completamente desenvolvidos; sendo toda a energia acumulada pela fase imatura voltada para a reprodução na fase adulta. Vivendo associados a ambientes aquáticos íntegros tais quais rios e cachoeiras, fazem revoadas sincronizadas após a emergência dos adultos e são muito utilizados como indicadores de qualidade de água por várias espécies serem sensíveis a mudanças antropogênicas no ambiente. A beleza de seu habitat natural, associada ao curto período de vida dos adultos e o comportamento de revoadas despertam a admiração de muitos, fazendo com que as espécies sejam frequentemente representadas na cultura como tema de poesias, contos e músicas, por exemplo. E não é à toa que esses insetos são frequentemente vistos como seres místicos, dada a fragilidade e a nostalgia trazida pelo bucolismo dos ambientes aquáticos. Com o constante desmatamento e ataque principalmente aos corpos hídricos, esses animais encontram-se em severa ameaça.

A água é um dos recursos mais valiosos do planeta e o acesso universal a serviços básicos de água é uma das necessidades fundamentais para o desenvolvimento humano (Gleick 2000). Frente a uma crise da biodiversidade, onde as espécies estão sendo extintas antes que possamos conhecê-las (Wilson 1985) e uma crise hídrica mundial, contribuir para a conservação dos ecossistemas associados aos corpos hídricos é um ato revolucionário. Entender como os sistemas

naturais funcionam e interagem com a vida na terra, a distribuição, composição e relações evolutivas das espécies podem ser importantes ferramentas para definição de *hotspots* de biodiversidade e locais prioritários para conservação. Dentro desse contexto, estudos em taxonomia, ecologia, biogeografia e evolução, por exemplo, permitem um entendimento acerca da biota no planeta e esse conhecimento pode e deve ser usado para desenvolver estratégias para preservar e monitorar essa biota e seus habitats. A má gestão dos sistemas aquáticos coloca a biodiversidade dos rios sob uma crescente ameaça de extinção e esse caminho é sem volta.

O presente estudo tem como objetivo investigar a filogenia, biogeografia histórica e evolução de Leptohyphidae, uma das três famílias mais abundantes de Ephemeroptera em rios neotropicais. Para alcançar esses objetivos, métodos de filogenia e biogeografia usando dados moleculares e morfológicos foram usados. Além disso, foi feito um estudo aprofundado de uma espécie de Leptohyphidae, para investigar se a diversidade molecular reflete a diversidade morfológica das populações, além de prover informações taxonômicas para a espécie. Para finalizar, dois artigos publicados a partir desta tese encontram-se nos anexos. Neste prefácio é dado uma introdução acerca do conhecimento histórico e atual de Leptohyphidae, para servir de base para os dois capítulos, que estão organizados em forma de artigos científicos em inglês.

Histórico taxonômico e Sistemática de Leptohyphidae

Leptohyphidae é uma família da ordem Ephemeroptera (Insecta) com distribuição pan-americana e, apesar de estarem presentes na Região Neártica, é considerada um grupo de origem Neotropical (McCafferty 1998), onde ocorre a maior diversidade em gênero e espécie. Após Baetidae e Leptophlebiidae, é o grupo mais representativo da ordem em rios neotropicais (Salles 2006), sendo formalmente descritas até o momento 163 espécies distribuídas em 15 gêneros (Tabela I). Apesar desta representatividade, estudos acerca da família na Região Neotropical se restringem basicamente à descrição de novas espécies, sendo a última publicação sobre evolução do grupo na América do Sul datada de 2006 (*cf.* Molineri 2006).

Na América do Sul apenas 10% das espécies da ordem são conhecidas em ambos os estágios de vida, adulto e ninfa (Domínguez *et al.* 2006). Assim como ocorre em outros grupos de Ephemeroptera, muitas espécies de Leptohyphidae são descritas baseadas em apenas um estágio de vida. Por exemplo, a taxonomia de *Leptohyphes* é baseada em sua maioria por

indivíduos adultos, sendo 10 das 20 espécies na América do Sul com ninfas descritas (Dias *et al.* 2011). Por outro lado, as espécies do gênero *Tricorythodes* não possuem um padrão das descrições, possuindo espécies descritas a partir de ninfas e outras a partir de adultos, apesar da maioria dos caracteres diagnósticos estarem presentes na fase imatura e a morfologia dos adultos ser pouco variável (Souto *et al.* 2017). Um conhecimento completo dos estágios de desenvolvimento das espécies fornece informações importantes para estudos ecológicos, filogenéticos e evolutivos (Miller *et al.* 2005).

Tabela I. Membros atuais da família Leptohiphidae com sua distribuição e lista sinonímica *sensu* Molineri (2006). Os nomes válidos estão indicados por • (• quando for a espécie tipo do gênero), enquanto que os sinônimos seniores (quando existirem) estão indicados por ◇. Ao lado de cada sinônimo está uma abreviação em parênteses contendo seu *status*. Todos os nomes, válidos ou não, são acompanhados pelo autor e ano da publicação. Os autores dos nomes estão incluídos nesta tabela como primeira menção no texto.

Família: LEPTOHYPHIDAE Edmunds & Traver 1954

Gênero: *Ableptemetes* Wiersema & McCafferty 2004

- *A. dicinctus* (Allen & Brusca, 1973) [México, Guatemala]

- ◇ *Leptohiphes dicinctus* Allen & Brusca 1973 (desig. orig.)

- ◇ *Tricorythopsis dicinctus*: Wiersema & McCafferty 2000 (comb.)

- *A. melanobranchnus* (Allen & Brusca 1973) [Guatemala]

- ◇ *Leptohiphes melanobranchnus* Allen & Brusca 1973 (desig. orig.)

- ◇ *Tricorythopsis melanobranchnus*: Wiersema & McCafferty 2000 (comb.)

Gênero: *Allenhiphes* Hofmann & Sartori (*in* Hofmann & Sartori & Thomas) 1999

- *A. asperulus* (Allen 1967) [Peru]

- ◇ *Leptohiphes asperulus* Allen 1967 (desig. orig.)

- *A. flinti* (Allen 1973) [Antilhas Francesas (Guadalupe, Dominica, Martinica), Venezuela, Panamá]

- ◊ *Leptohyphes flinti* Allen 1973 (desig. orig.)
- *A. spinosus* (Allen & Roback 1969) [Peru]
 - ◊ *Leptohyphes spinosus* Allen & Roback 1969 (desig. orig.)
- *A. vescus* (Allen 1978) [Estados Unidos, México]
 - ◊ *Leptohyphes vescus* Allen 1978 (desig. orig.)

Gênero: *Amanahyphes* Salles & Molineri 2006

- *Am. saguassu* Salles & Molineri 2006 [Brasil, Colômbia*, Venezuela]
- *Am. bahiensis* Molineri & Lima & Knapp & Docio 2015 [Brasil]

Gênero: *Cabecar* Baumgardner & Ávila 2006

- *C. serratus* Baumgardner & Avila 2006 [Costa Rica, Nicarágua, Panamá]

Gênero: *Haplohyphes* Allen, 1966

- *H. aquilonius* Lugo-Ortiz & McCafferty 1995 [Colômbia, Costa Rica]
- *H. baritu* Domínguez 1984 [Argentina, Bolívia]
 - ◊ *Haplohyphes furtiva* Domínguez 1984 (syn.)
- *H. dominguezi* Molineri 1999 [Equador]
- *H. huallaga* Allen 1966 [Peru]
- *H. mithras* (Traver 1958) [Colômbia, Costa Rica, Nicarágua]
 - ◊ *Leptohyphes mithras* Traver 1958 (desig. orig.)
- *H. yanahuicsa* Molineri 2003 [Bolívia]

Gênero *Leptohyphes* Edmunds & Traver 1954

- *L. airuoca* Nascimento, Molineri & Salles 2014 [Brasil]
- *L. albipennis* Molineri & Zuniga 2006 [Colômbia]
- *L. alleni* Brusca 1971 [México]

- *L. apache* Allen 1967 [Estados Unidos, México]
 - ◊ *Leptohyphes hispidus* Allen & Brusca 1973 (syn.)
 - ◊ *Leptohyphes lumas* Allen & Brusca 1973 (syn.)
 - ◊ *Leptohyphes spiculatus* Allen & Brusca 1973 (syn.)
 - ◊ *Leptohyphes succinus* Allen 1978 (syn.)
- *L. beneri* Traver 1958 [México]
- *L. brevissimus* Eaton 1892 [Guatemala]
- *L. carinus* Allen 1973 [Peru]
- *L. coconuco* Molineri & Zuniga 2006 [Colômbia]
- *L. cornutillus* Nascimento, Molineri & Salles, 2014 [Brasil]
- *L. cornutus* Allen 1967 [Argentina, Brasil]
- *L. costaricanus* Ulmer 1920 [Costa Rica]
- *L. ecuador* Mayo 1968 [Equador]
- *L. eximius* Eaton 1882 [Argentina, Bolívia]
 - ◊ *Leptohyphes bruchi* Navás 1913 (syn.)
 - ◊ *Bruchella nigra* Navás 1920 (syn.)
- *L. ferruginus* Allen & Brusca 1973 [Estados Unidos, México]
 - ◊ *Leptohyphes piraticus* Allen 1978 (syn.)
- *L. guadeloupensis* Hofmann & Sartori (*in* Hofmann & Sartori & Thomas) 1999 [Guadalupe]
- *L. hirsutus* Allen & Roback 1969 [Argentina, Bolívia, Peru]
- *L. illiesi* Allen 1967 [Peru]
- *L. invictus* Allen 1973 [Peru]
- *L. jamaicanus* Allen 1973 [Cuba, Jamaica]
 - ◊ *Tricorythodes jamaicanus*: Naranjo 1986 (comb. n.)
- *L. jodiannae* Allen 1967 [Peru]

- *L. lestes* Allen & Brusca 1973 [Estados Unidos, México]
- *L. liniti* Wang, Sites & McCafferty 1998 [Bolivia, Ecuador]
- *L. maculatus* Allen 1967 [Bolivia, Peru]
 - ◊ *Leptohyphes* sp.2 Roback 1966 (syn.)
 - ◊ *Leptohyphes* sp.3 (partim) Roback 1966 (syn.)
 - ◊ *Leptohyphes* sp.4 Roback 1966 (syn.)
 - ◊ *Leptohyphes comatus* Allen 1967 (syn.)
 - ◊ *Leptohyphes myllonotus* Allen & Roback 1969 (syn.)
- *L. mandibulus* Baumgardner 2007 [Costa Rica]
- *L. mollipes* Needham & Murphy 1924 [Brasil]
- *L. murdocki* Allen 1967 [Costa Rica, Panamá]
 - ◊ *Leptohyphes murchisoni* Edmunds & Jensen & Berner 1976 (nom. n.)
- *L. musseri* Allen 1967 [Guatemala, México]
 - ◊ *Leptohyphes brunneus* Allen & Brusca 1973 (syn.)
- *L. nebulosus* Nascimento, Molineri & Salles 2014 [Brasil]
- *L. nigripennis* Molineri & Zuniga 2006 [Bolivia, Colombia]
- *L. nigripunctum* Traver 1943 [México, Venezuela]
 - ◊ *Leptohyphes nigripunctus* Baumgardner & McCafferty 2010 (nom. n.)
- *L. peterseni* Ulmer 1920 [Argentina, Bolivia, Brasil, Guatemala, México]
- *L. petersi* Allen 1967 [Argentina, Brasil, Peru]
 - ◊ *Leptohyphes* nymph no.2 Needham & Murphy 1924 (syn.)
- *L. pilosus* Allen & Brusca 1973 [México]
- *L. plaumanni* Allen 1967 [Argentina, Brasil]
 - ◊ *Leptohyphes pereirae* Da-Silva 1993 (syn.)
- *L. populus* Allen 1973 [Brasil]

- *L. priapus* Traver 1958 [América Central]
- *L. quercus* Kilgore & Allen 1973 [Estados Unidos]
 - ◊ *Homoleptohyphes quercus*: Wiersema & McCafferty 2000 (comb. n.)
- *L. sabinas* Traver 1958 [Estados Unidos, México]
 - ◊ *Leptohyphes castaneus* Allen 1967 (syn.)
 - ◊ *Leptohyphes consortis* Allen & Brusca 1973 (syn.)
 - ◊ *Leptohyphes tarsos*: Allen & Murvosh 1987 (syn.)
- *L. setosus* Allen 1967 [Bolivia, Peru]
 - ◊ *Leptohyphes* sp. 3 Roback 1966 (syn.)
 - ◊ *Leptohyphes* sp. 5 Roback 1966 (syn.)
 - ◊ *Leptohyphes echinatus*: Allen & Roback 1969 (syn.)
- *L. tacajalo* Mayo 1968 [Ecuador]
 - ◊ *Leptohyphes albus* Mayo 1968 (syn.)
- *L. tuberculatus* Allen 1967 [Peru]
 - ◊ *Leptohyphes* sp.6 Roback 1966 (syn.)
- *L. zalope* Traver 1958 [Estados Unidos, Guatemala, Granada, México, Nicaragua, Tobago]
 - ◊ *Leptohyphes zelus* Allen 1978 (syn.)
 - ◊ *Leptohyphes vulturinus* Allen 1978 (syn.)

Gênero: *Leptohyphodes* Ulmer (1919)1920

- *Le. inanis* (Pictet 1843) [Brasil]
 - ◊ *Potamanthus?* *inanis* Pictet 1843 (design. orig.)

Gênero: *Loricypes* Molineri & Mariano 2015

- *Lo. froehlichii* Molineri & Mariano 2015 [Brasil]

Gênero: *Lumahyphes* Molineri 2014

- *Lu. coca* Boldrini, Santos & Oliveira 2015 [Brasil]
- *Lu. guacra* Molineri 2004 [Argentina, Bolívia]
- *Lu. pijcha* Molineri 2004 [Bolívia, Colômbia]
- *Lu. yagua* Molineri & Zuñiga 2004 [Colômbia, Peru]

Gênero *Macunahyphes* Dias, Salles & Molineri 2005

- *M. australis* (Banks 1913) [Argentina, Brasil, Guiana]
 - ◊ *Tricorythus australis* Banks 1913 (desig. orig.)
 - ◊ *Leptohyphodes australis*: Ulmer 1920 (comb. n.)
 - ◊ *Tricorythodes australis*: Traver 1958 (comb. n.)
- *M. araca* Souto & Salles 2016 [Brasil]
- *M. eduardoi* Almeida & Mariano 2015 [Brasil]
- *M. incognitus* Molineri, Grillet, Nieto, Dominguez & Guerrero 2011 [Brasil, Venezuela]
- *M. pemonensis* Molineri & Grillet & Nieto & Dominguez & Guerrero 2011 [Venezuela]
- *M. zagaia* Souto & Salles 2016 [Brasil]

Gênero: *Traverhyphes* Molineri 2001

- *Tr. (Byrsahyphes) nanus* (Allen 1973) [Colômbia, Costa Rica, Guatemala, Nicarágua, Panamá]
 - ◊ *Leptohyphes nanus* Allen 1973 (desig. orig.)
 - ◊ *Allenhyphes nanus*: Wiersema & McCafferty 2000 (comb. n.)
- *Tr. (Byrsahyphes) yuqui* Molineri 2004 [Bolívia, Panamá]
- *Tr. (Mocoihyphes) edmundsi* (Allen 1973) [Argentina, Brasil]
 - ◊ *Leptohyphes edmundsi*: Allen 1973 (desig. orig.)
 - ◊ *Allenhyphes edmundsi*: Wiersema & McCafferty 2000 (comb. n.)
- *Tr. (Mocoihyphes) yuati* Molineri 2004 [Argentina, Brasil]
- *Tr. (Traverhyphes) chiquitano* Molineri 2004 [Bolívia]

- *Tr. (Traverhyphes) frevo* Lima, Salles & Pinheiro 2011 [Brasil]
- *Tr. (Traverhyphes) indicator* (Needham & Muerphy 1924) [Argentina, Brasil, Uruguai]
 - ◊ *Leptohyphes indicator* Needham & Muerphy 1924 (desig. orig.)
- *Tr. (Traverhyphes) pirai* Molineri 2001 [Brasil]

Gênero: *Tricorythodes* Ulmer (1919)1920

- *T. albilineatus* Berner 1946 [Estados Unidos]
- *T. allectus* (Needham 1905) [Canadá, Estados Unidos]
 - ◊ ?*Caenis allecta* Needham 1905 (desig. orig.)
 - ◊ *Tricorythus allectus*: Ulmer (1919)1920 (comb. n.)
 - ◊ *Tricorythodes atratus* McDunnough 1923 (syn.)
 - ◊ *Tricorythodes peridius* Burks 1953 (syn.)
- *T. angulatus* Traver 1959 [México]
- *T. arequita* Traver 1959 [Argentina, Brasil, Uruguai]
- *T. barbatus* Allen 1967 [Argentina, Brasil]
 - ◊ *Tricoryhyphes barbatus*: Wiersema & McCafferty 2000 (comb. n.)
- *T. bullus* Allen 1967 [Argentina, Brasil]
 - ◊ *Epiphraides bullus*: Wiersema & McCafferty 2000 (comb. n.)
- *T. capuccinorum* Emmerich 2007 [Colômbia]
- *T. caunapi* Dias & Bacca & Ferreira 2011 [Colômbia]
- *T. chalaza* Gonçalves, Da-Silva & Nessimian, 2010 [Brasil]
- *T. cobbi* Alba-Tercedor & Flannagan 1995 [Canadá, Estados Unidos]
- *T. comus* Traver 1959 [México]
- *T. condylus* Allen, 1967 [Estados Unidos, México, Nicarágua]
 - ◊ *Tricoryhyphes condyles*: Wiersema & McCafferty 2000 (comb. n.)
- *T. corpulentus* Allen & Murvosh 1987 [Estados Unidos]

- ◇ *Asioplax corpulenta*: Wiersema & McCafferty 2000 (comb. n.)
- *T. cristatus* Allen 1967 [Brasil]
 - ◇ *Epiphraodes cristatus*: Wiersema & McCafferty 2000 (comb. n.)
- *T. cubensis* Kluge & Naranjo 1990 [Cuba]
- *T. curiosus* (Lugo-Ortiz & McCafferty 1995) [Costa Rica]
 - ◇ *Leptohyphes curiosus* Lugo-Ortiz & McCafferty 1995 (desig. orig.)
 - ◇ *Asioplax curiosa*: Wiersema & McCafferty 2000 (comb. n.)
 - ◇ *Asioplax curiosus*: Wiersema & McCafferty 2005
- *T. curvatus* Allen, 1977 [Estados Unidos]
- *T. diasae* Gonçalves, Da-Silva & Nessimian 2010 [Brasil]
- *T. dimorphus* Allen, 1967 [Estados Unidos, México]
 - ◇ *Homoleptohyphes dimorphus*: Wiersema & McCafferty 2000 (comb. n.)
- *T. dolani* Allen, 1967 [Estados Unidos]
 - ◇ *Asioplax dolani*: Wiersema & McCafferty 2000 (comb. n.)
- *T. edmundsi* Allen, 1967 [Canadá, Estados Unidos]
 - ◇ *Asioplax edmundsi*: Wiersema & McCafferty 2000 (comb. n.)
- *T. explicatus* (Eaton, 1892) [Estados Unidos, México]
 - ◇ *Tricorythus explicatus* Eaton, 1892 (desig. orig.)
 - ◇ *Tricorythodes minutus* Traver (*in* Needham & Traver & Hsu) 1935 (syn.)
 - ◇ *Tricorythodes fallax* Traver (*in* Needham & Traver & Hsu) 1935 (syn.)
 - ◇ *Tricorythodes fallacina* McDunnough 1939 (syn.)
- *T. faeculopsis* Belmont, Salles & Hamada, 2011 [Brasil, Venezuela]
- *T. fictus* Traver, 1935 [Estados Unidos, México]
- *T. fugitans* (Needham 1920) [Relato dúbio para a África (*sensu* Gillies 1960)]
 - ◇ *Caenopsis fugitans* Needham 1920 (desig. orig.)

- ◊ *Tricorythafer fugitans*: Lestage 1942 (comb. n.)
- ◊ *Needhamocaenis fugitans*: Lestage 1945 (comb. n.)
- *T. grallator* Kluge & Naranjo 1990 [Cuba]
- *T. griseus* Hofmann & Sartori (*in* Hofmann & Sartori & Thomas) 1999 [Guadalupe]
- *T. hiemalis* Molineri 2001 [Argentina, Brasil]
- *T. isabelia* (Baumgardner & Meyer & McCafferty 2006) [Nicaragua]
 - ◊ *Asioplax isabelia* Baumgardner & Meyer & McCafferty 2006 (desig. orig.)
- *T. kirki* Baumgardner 2007 [Costa Rica]
- *T. lichyi* Traver 1943 [Venezuela]
- *T. mirca* Molineri 2002 [Bolivia, Brasil]
- *T. mirus* (Allen 1967) [Estados Unidos, México]
 - ◊ *Leptohyphes mirus* Allen 1967 (desig. orig.)
 - ◊ *Leptohyphes baumanni* Kilgore & Allen 1973 (syn.)
 - ◊ *Homoleptohyphes mirus*: Wiersema & McCafferty 2000 (comb. n.)
- *T. molinerii* Dias & Salles, 2006 [Brasil]
- *T. montanus* Kluge & Naranjo 1990 [Cuba]
- *T. mosegus* Alba-Tercedor & Flannagan, 1995 [Canadá, Estados Unidos]
- *T. mulaiki* Traver, 1959 [Estados Unidos, México]
 - ◊ *Tricoryhyphes mulaiki*: Wiersema & McCafferty 2000 (comb. n.)
- *T. nicholsae* (Wang, Sites & McCafferty 1998) [Ecuador]
 - ◊ *Leptohyphes nicholsae* Wang, Sites & McCafferty 1998 (desig. orig.)
 - ◊ *Asioplax nicholsae*: Wiersema & McCafferty 2000 (comb. n.)
- *T. notatus* Allen & Brusca 1973 [México]
- *T. numinuh* (Wiersema, McCafferty & Baumgardner 2001) [Estados Unidos, México]
 - ◊ *Asioplax numinuh* Wiersema & McCafferty 2000 (desig. orig.)

- *T. ocellus* Allen & Roback 1969 [Peru]
 - ◊ *Tricorythodes* sp. Roback 1966 (syn.)
- *T. popayanicus* Domínguez 1982 [Argentina, Bolívia]
- *T. primus* Baumgardner 2007 [Costa]
- *T. quizeri* Molineri 2002 [Bolívia, Brasil]
- *T. robacki* (Allen, 1967) [Estados Unidos]
 - ◊ *Leptohyphes robacki* Allen, 1967 (desig. orig.)
- *T. sacculobranhis* Naranjo 1986 [Cuba]
 - ◊ *Asioplax sacculobranhis*: Wiersema & McCafferty 2000 (comb. n.)
- *T. sallesi* Dias & Cabette & De Sousa 2009 [Brasil]
- *T. santarita* Traver 1959 [Argentina, Brasil, Uruguai]
 - ◊ *Asioplax santarita*: Wiersema & McCafferty 2005 (comb. n.)
- *T. sierramaestrae* Kluge & Naranjo 1990 [Cuba]
 - ◊ *Asioplax sierramaestrae*: Wiersema & McCafferty 2000 (comb. n.)
- *T. sordidus* Allen, 1967 [Costa Rica, Guatemala, México, Nicaragua]
- *T. stygiatus* McDunnough, 1931 [Estados Unidos, México]
- *T. tragoedia* Souto, Angeli & Salles 2017 [Brasil]
- *T. texanus* Traver, 1935 [Estados Unidos, México]
 - ◊ *Asioplax texana*: Wiersema & McCafferty 2005 (comb. n.)
- *T. trifasciatus* Molineri & Zuniga 2006 [Colômbia]
- *T. ulmeri* Allen & Brusca, 1973 [México]
- *T. undatus* Lugo-Ortiz & McCafferty 1995 [Costa Rica, Guatemala, Nicaragua]
 - ◊ *Epiphrales undatus*: Wiersema & McCafferty 2000 (comb. n.)
- *T. uniandinus* Emmerich 2007 [Colômbia]
- *T. yapekuna* Belmont, Salles & Hamada, 2012 [Brasil]

- *T. yura* Molineri 2002 [Bolívia, Brasil]
- *T. zunigae* Molineri 2002 [Colômbia]

Gênero: *Tricorythopsis* Traver 1958

- *Th. acara* Belmont, Salles & Hamada 2011 [Brasil]
- *Th. araponga* Dias & Salles 2005 [Brasil]
- *Th. artigas* Traver 1958 [Argentina, Brasil, Uruguai]
 - ◊ *Leptohyphes tinctus* Allen 1973 (syn.)
 - ◊ *Tricorythopsis fictilis* Molineri 1999 (syn.)
 - ◊ *Tricorythopsis minimus* Traver 1958 (partim) (syn.)
- *Th. bahiensis* Dias, Salles & Ferreira 2008 [Brasil]
- *Th. baptistai* Dias & Salles 2005 [Brasil]
- *Th. chiriguano* Molineri 2001 [Bolívia, Brasil]
- *Th. gibbus* (Allen 1967) [Argentina, Brasil]
 - ◊ *Leptohyphes gibbus* Allen 1967 (desig. orig.)
- *Th. intercalatus* Belmont, Salles & Hamada 2011 [Brasil]
- *Th. minimus* (Allen 1973) [Argentina, Brasil, Uruguai]
 - ◊ *Leptohyphes viriosus* Allen 1973 (syn.)
- *Th. pseudogibbus* Dias & Salles 2005 [Brasil]
- *Th. rondoniensis* (Dias, Cruz & Ferreira 2009) [Brasil, Colômbia]
 - ◊ *Tricorythodes rondoniensis* Dias, Cruz & Ferreira 2009 (desig. orig.)
- *Th. sigillatus* Molineri 1999 [Brasil]
- *Th. spongicola* Lima, Salles & Pinheiro 2011 [Brasil]
- *Th. ticuna* Molineri & Zuniga 2006 [Colômbia]
- *Th. undulatus* (Allen 1967) [Brasil]
 - ◊ *Leptohyphes undulates* Allen 1967 (desig. orig.)

- ◊ *Tricorythopsis petersorum* Molineri 1999 (syn.)
- *Th. volsellus* Molineri 1999 [Venezuela]
- *Th. yacutinga* Molineri 2001 [Argentina, Brasil]
- *Th. yucupe* Dias, Salles & Ferreira 2008 [Brasil, Venezuela]
- *Th. yusuaia* Belmont & Cruz & Hamada 2015 [Brasil]

Gênero: *Vacupernius* Wiersema & McCafferty 2000

- *V. packeri* (Allen 1967) [Estados Unidos, Belize, Colômbia, Costa Rica, Guatemala, Honduras, Nicarágua]
 - ◊ *Leptohyphes packeri* Allen 1967 (desig. orig.)
 - ◊ *Leptohyphes phalarobanchus* Kilgore & Allen 1973 (syn.)
 - ◊ *Leptohyphes paraguttatus* Allen 1978 (syn.)
- *V. rolstoni* (Allen 1973) [Cuba, República Dominicana]
 - ◊ *Leptohyphes rolstoni* Allen 1973 (desig. orig.)
 - ◊ *Tricorythodes rolstoni*: Naranjo 1986 (comb. n.)

Gênero: *Yaurina* Molineri 2001

- *Y. mota* Molineri 2001 [Argentina]
- *Y. ralla* (Allen 1967) [Peru]
 - ◊ *Leptohyphes rallus* Allen 1967 (desig. orig.)
 - ◊ *Allenhyphes rallus*: Wiersema & McCafferty 2000 (comb. n.)
- *Y. yapa* Molineri 2001 [Equador]
- *Y. yuta* Molineri 2001 [Argentina]

Historicamente foi reconhecida como uma subfamília (Leptohyphinae) de Tricorythidae (Edmunds & Traver 1954), sendo elevada a família por Landa (1973) a partir de estudos anatômicos comparados dos arranjos dos órgãos internos de diversas famílias de Ephemeroptera.

Originalmente, Edmunds & Traver (1954) consideraram como parte de Leptohyphinae os gêneros *Bruchella* Navás 1920 (= *Leptohyphes*), *Leptohyphes*, *Leptohyphodes*, *Tricorythafer* Lestage 1942 (= *Tricorythodes*) e *Tricorythodes*. Landa (1973) reconheceu duas subfamílias: Leptohyphinae (com os gêneros *Tricorythodes* e *Leptohyphes*); e Dicercomyzinae (com o gênero africano *Dicercomyzon* Demoulin 1954). Peters & Peters (1993) retiraram Dicercomyzinae de Leptohyphidae e passaram para Tricorythidae, com base em observações acerca da morfologia de *Dicercomyzon*, em especial nas asas, concluindo que os indivíduos desse gênero possuem uma venação apomórfica de Tricorythidae (*tricorythid cubital fork*). O único registro africano para Leptohyphidae permanece atualmente como registro dúbio da espécie *Tricorythodes fugitans*, a qual não foi mais encontrada após sua descrição em 1958 baseada em um espécime da Tanzania (Gillies com. pes. Peters & Peters 1993). Tal registro pode ser resultado de uma introdução temporária de *Tricorythodes* na África com posterior extinção, ou uma contaminação na coleção (Peters & Peters 1993). Assim, o que originalmente era conhecido como Tricorythidae foi dividido em duas famílias: Leptohyphidae, estritamente pan-americana; e Tricorythidae, com distribuição restrita ao continente africano.

De acordo com estudos filogenéticos baseados em morfologia, constituem um grupo monofilético (*cf.* McCafferty & Wang, 2000; Molineri, 2006; Ogden *et al.*, 2009) e, junto com outras 10 famílias (Austramerellidae, Ephemerellidae, Ephemerythidae, Caenidae, Machadorythidae, Melanemerella, Neoephemeridae, Teloganellidae, Teloganodidae e Tricorythidae), pertence à infraordem Pannota (*cf.* Ogden & Whiting 2005; Ogden *et al.* 2009). As ninfas de Pannota possuem mais da metade das tecas alares fusionadas (McCafferty & Edmunds 1979), contudo, ainda que altamente fusionadas, as asas pro e mesotorácicas permanecem externamente reconhecíveis através das tecas alares. Ainda, as ninfas da infraordem compartilham a presença de brânquias abdominais altamente modificadas, normalmente dorsais, com lamelas sobrepostas e, frequentemente, brânquias operculares (McCafferty & Wang 2000).

Diferentes autores apresentaram hipóteses filogenéticas com base em caracteres morfológicos que trataram da composição de Leptohyphidae e de sua relação com as demais famílias de Pannota. Domínguez, Hubbard & Peters (1992) trataram *Coryphorus* Peters, 1981 dentro de Leptohyphidae, posição mantida por Wiersema & McCafferty (2000) que propuseram uma classificação filogenética para Leptohyphidae, no entanto sem definir um método de análise dos caracteres. Apesar deles não terem incluído as ninfas conhecidas de *Coryphorus* em sua amostragem, mantiveram o gênero dentro de Leptohyphidae e sugeriram Tricorythidae como

grupo-irmão de Leptohyphidae. No ano seguinte, o adulto de *Coryphorus* foi descrito por Molineri, Peters & Zuñiga (2002) onde estabeleceram a família Coryphoridae e diversas análises cladísticas subsequentes recuperaram a hipótese de que esta seria o grupo-irmão de Leptohyphidae (*e.g.*, Molineri & Domínguez 2003; Jacobus & McCafferty 2006; Molineri 2006; Baumgardner 2008). O grupo-irmão desse clado, Coryphoridae + Leptohyphidae, foi recuperado como sendo Tricorythidae (Molineri & Domínguez 2003; Jacobus & McCafferty 2006; Baumgardner 2008) ou Tricorythidae + Machadorythidae (Molineri 2006), todos táxons de origem africana.

No entanto, em estudo mais recente focado nas relações filogenéticas entre as famílias de Ephemeroptera, utilizando tanto dados morfológicos quanto moleculares, Ogden *et al.* (2009) encontraram um resultado diferente. De acordo com esses autores, Coryphoridae estaria mais relacionado com grupos africanos (Machadorythidae e Ephemerythidae, nas análises com somente dados morfológicos; e Machadorythidae, Teloganodidae e Tricorythidae na inferência total). Porém, um viés desses autores é a falta de representatividade das famílias de Pannota. Além disso, eles não obtiveram sequências de DNA de Coryphoridae e concluem o trabalho sem conseguir propor hipóteses de relações filogenéticas para Pannota, com exceção da recuperação do clado Caenoidea (Neoephemeridae+Caenidae) e de Ephemerelloidea. As relações dentro de Ephemerelloidea, clado que compreende as demais famílias de Pannota, permaneceram desconhecidas. A relação filogenética de Leptohyphidae com outras famílias de Pannota é essencial para entender a origem e, conseqüentemente, a idade da família. Coryphoridae é uma família monotípica restrita ao bioma amazônico, com distribuição para o Brasil, Colômbia, Guiana Francesa e Venezuela. Enquanto que as demais famílias propostas como próximas de Leptohyphidae são todas restritas ao continente africano. Essa relação estreita de Leptohyphidae com famílias africanas, ainda que próxima de Coryphoridae ou não, pode sugerir uma origem da família relacionada ou causada pela separação da Gondwana.

Dentro de Leptohyphidae, Wiersema & McCafferty (2000) dividiram a família em Leptohyphinae (*Allenhyphes*, *Cotopaxi*, *Haplohyphes*, *Leptohyphes*, *Leptohyphodes* e *Vacupernius*) e Tricorythodinae (*Asioplax*, *Coryphorus*, *Epiphraodes*, *Homoleptohyphes*, *Tricorythopsis*, *Tricorythodes* e *Tricoryhyphes*), sendo o monofiletismo dessas duas subfamílias não corroborado pelas análises de Molineri (2006) e Baumgardner (2008) a partir de dados morfológicos, e nem de Ogden & Whiting (2005) com dados moleculares. No entanto, nenhum desses estudos propôs uma classificação alternativa porque as análises somente incluíram gêneros da América do Sul (Molineri 2006), ou não recuperaram clados bem sustentados dentro

de Leptohyphidae (Baumgardner 2008) ou possuíam baixa representatividade de gêneros (Ogden & Whiting 2005). Com base em comparações morfológicas com outras famílias de Pannota, Leptohyphinae possuiria um conjunto grande de caracteres plesiomórficos em comparação à Tricorythodinae (Wiersema & McCafferty, 2000). Porém, além de não terem baseado suas hipóteses de relações filogenéticas em uma análise formal, os autores consideraram ninfas e adultos de uma mesma espécie pertencentes à diferentes subfamílias: o adulto de *Tricorythopsis artigas* foi considerado um Tricorythodinae, enquanto que sua ninfa pertencendo à Leptohyphinae; e as ninfas de *Leptohyphes viriosus* e *L. minimus* (sinônimos de *Tricorythopsis minimus*) consideradas como *Allenhyphes* ("Leptohyphinae"), quando na realidade são *Tricorythopsis* ("Tricorythodinae") (Molineri, 2006). Alguns caracteres sugeridos por Wiersema & McCafferty como diagnósticos e/ou sinapomórficos para Leptohyphinae e Tricorythodinae foram utilizados como caracteres na matriz morfológica das análises filogenéticas do Capítulo 2.

Com 72 espécies descritas, *Tricorythodes* é o gênero com maior número de espécies em Leptohyphidae. Não coincidentemente, é o gênero com mais controvérsia no que diz respeito à taxonomia. Hegemonicamente a diferenciação das espécies é baseada na morfologia da ninfa, em especial as proporções nas medidas das pernas, morfologia da maxila e forma das brânquias abdominais. Ainda que por vezes espécies sejam descritas baseadas somente em adultos, morfologia deles, tanto macho quanto fêmea, é altamente similar, não oferecendo bons caracteres diagnósticos para as espécies (Souto *et al.* 2017).

Diversos autores propuseram diferentes formas de classificação para *Tricorythodes* (*e.g.* Allen & Murvosh 1987; Wiersema & McCafferty 2000; Molineri 2002, 2006; Baumgardner 2008; Dias 2009). O gênero foi proposto por Ulmer (1920) e revisado primeiramente por Allen & Murvosh (1987), que propuseram três subgêneros: *Tricorythodes*, *Tricoryhyphes* e *Homoleptoxyphes*. Em revisão de Leptoxyphidae para América do Norte e Central, Wiersema & McCafferty (2000) elevaram os três subgêneros supracitados a gênero e sugeriram mais dois novos gêneros para incluir outras espécies de *Tricorythodes lato sensu*, *Asioplax* e *Epiphyrades*, desmembrando *Tricorythodes* em cinco gêneros. No livro "Ephemeroptera da América do Sul", Domínguez *et al.* sinonimizam *Epiphyrades*, *Homoleptoxyphes* e *Tricoryhyphes* com *Tricorythodes*. E em análise cladística, Molineri (2002) não recupera *Asioplax* e *Epiphyrades* como grupos naturais. De acordo com o autor, ambos os gêneros seriam clados apotípicos dentro de *Tricorythodes* e, sendo assim, considerou o gênero como uma unidade única até um melhor entendimento das relações entre as espécies justifique o desmembramento do gênero.

A partir da descoberta da ninfa de *T. australis*, Dias *et al.* (2005) propuseram *Macunahyphes* como um novo gênero para agregar essa espécie originalmente descrita para *Tricorythodes*. Desde a descrição da espécie, os adultos de *T. australis* sempre representaram uma dificuldade para os taxonomistas do grupo. A espécie possui uma genitália altamente modificada em relação às descritas para o gênero, não possuindo o que Molineri (2006) considerou como sinapomorfia para *Tricorythodes*: o inchaço no segundo artigo dos fórceps. Entretanto, em análise cladística para Leptohiphidae, Molineri (2006) recupera *Macunahyphes australis* como uma linhagem dentro de *Tricorythodes*, mas decide não tomar nenhuma decisão taxonômica e mantém o gênero como válido até que estudo específico acerca das relações entre espécies do gênero fosse feito. Em 2008, em tese sobre filogenia e biogeografia de Leptohiphidae baseada em dados morfológicos, Baumgardner propõe a sinonimização de todos os gêneros propostos anteriormente para *Tricorythodes sensu lato*, porém o trabalho nunca foi publicado em uma revista científica. Outras cinco espécies de *Macunahyphes* foram descritas recentemente (*c.f.* Molineri *et al.* 2011; Almeida & Mariano 2015; Souto & Salles 2016). Por fim, o último estudo feito com *Tricorythodes* foi a tese de doutorado de Dias (2009), onde a partir de análise utilizando dados morfológicos e moleculares a autora apoia a sinonimização de *Ephiphrades*, *Homoleptohiphes*, *Tricoryhyphes* e *Tricorythodes*, porém mantém a validade de *Asioplax* e *Macunahyphes*. Assim como a tese de Baumgardner (2008), o estudo de Dias (2009) ainda não foi publicado em uma revista científica.

O presente estudo seguiu as propostas taxonômicas de Molineri (2006), que considera todos os gêneros supracitados sinônimos de *Tricorythodes*, exceto *Macunahyphes*.

Morfologia

As ninfas de Leptohiphidae são facilmente identificadas por apresentarem brânquias nos segmentos abdominais II a VI (exceto em *Leptohiphodes* e *Amanahyphes*, de II a V); brânquias operculares de formato variado (ovaladas, arredondadas, triangulares ou sub-retangulares) e geralmente afastadas medianamente; e corpo e pernas geralmente cobertos por finas cerdas (Domínguez *et al.* 2006; Salles 2006). Já os adultos são caracterizados pelos olhos não divididos e geralmente não sexualmente dimórficos (exceto em *Leptohiphodes inanis*, *Amanahyphes* spp. e uma espécie de *Leptohiphes*); tórax robusto e asas dos machos com franjas na margem posterior; asas posteriores geralmente ausentes, podendo estar ausente nos machos e presentes

nas fêmeas, mas quando presentes, reduzidas em tamanho e com uma longa projeção costal; garras tarsais medianas e posteriores dissimilares; genitália masculina de formato variável, tendo os fórceps 2 ou 3-articulados, pênis com espinhos de diversas formas, separados na metade distal ou completamente fusionados, e; filamentos caudais geralmente longos e finos, quase completamente glabros (Domínguez *et al.* 2006). Molineri (2002) propôs como caráter importante a nível genérico o número de lamelas nas brânquias abdominais, o que ele chamou de fórmula branquial (*gill formulae*).

Biologia

As ninfas de LeptoHyphidae são restritas a ambientes lóticos, podendo ser encontradas em áreas de correnteza, associadas à meso-hábitats como seixo, cascalho, areia, folhiço, barranco, hidrófitas e áreas com acúmulo de matéria orgânica finamente particulada (Salles 2006). A presença de um par de brânquias operculares abdominais, cobrindo e protegendo os demais pares, facilita a tolerância das ninfas por ambiente com material orgânico em suspensão (Domínguez *et al.* 2006). Ainda que mais comumente encontradas em áreas de correnteza fraca a moderada, é possível observar às margens dos rios ninfas de *Traverhyphes* e *Tricorythopsis* vivendo em áreas de forte correnteza, associadas às pedras e folhas de Podostemaceae, respectivamente. Ninfas de *LeptoHyphodes inanis*, por sua vez, são bastante comuns em bolsões de folhiço em decomposição em rios da Mata Atlântica brasileira, enquanto que as de *Amanahyphes saguassu* podem ser encontradas em barrancos de igarapés amazônicos, juntamente com ninfas de *Coryphorus aquilus* (Coryphoridae) (Salles 2006).

Os LeptoHyphidae são considerados reptantes por caminharem lentamente sobre o substrato onde vivem (Salles 2006), se alimentando de partículas finas de matéria orgânica depositada no substrato (Domínguez *et al.* 2006). Ainda, ninfas de *Tricorythodes* podem apresentar fungos e matéria orgânica aderidos ao corpo, frequentemente se camuflando em ambientes com grandes quantidade de matéria orgânica depositada ou em suspensão.

Em relação às formas aladas, as subimagos geralmente emergem durante a noite, fazendo a última muda para imago após de 15 minutos a poucas horas. Para a maioria das espécies faltam dados de observação sobre horários do voo nupcial, porém costumam voar pela manhã, podendo a revoada estender até um pouco mais tarde no dia (Domínguez *et al.* 2006). Adultos de

Vacupernius packeri, por exemplo, realizam o voo nupcial somente pela manhã cedo, até por volta das 7 horas, sendo possível encontrá-los após este horário presos em teias de aranhas nas margens dos rios. Francischetti *et al.* (2002) descreveram o vôo nupcial de duas espécies de Leptohyphidae, uma crepuscular e outra matutina, no Rio Campo Belo, no município de Itatiaia, Rio de Janeiro. A partir de observações *in situ*, indivíduos de uma espécie não identificada de *Tricorythopsis* realizam vôo nupcial de cerca de uma hora a partir das 16:30h, formando duas nuvens de revoada de dezenas de milhares de indivíduos (fêmeas em menor quantidade), durando cerca de uma hora e desaparecendo antes de anoitecer. Os indivíduos realizavam movimentos em zigue-zague ascendente até mais de 4 metros acima do nível da água, distando cerca de 1 a 2 metros da margem. Depois de atingirem a altura máxima, os indivíduos descem em zigue-zague e, mais próximo à água, tornavam a formar as grandes nuvens iniciais. A outra revoada observada foi de uma espécie não identificada de *Leptohyphes*, acontecida no início da manhã por volta das 06:20h, durando cerca de 30 minutos e desaparecendo antes do amanhecer. A revoada de machos e fêmeas (em menor quantidade) ocorreu a uma altura de 1,5 a 2 metros da superfície da água, distando cerca de um metro da margem do rio. Durante o vôo havia uma alternância de movimentos ondulatórios ascendentes e descendentes, com uma movimentação lateral (os indivíduos seguiam da direita para a esquerda e depois em sentido contrário).

Biogeografia

Ephemeroptera é considerado um grupo ideal para estudos de biogeografia histórica (Edmunds 1972; 1975; Bae & McCafferty 1991; McCafferty & Wang 1997; Sartori *et al.* 2000) por se tratar de uma ordem antiga de insetos com capacidade limitada de dispersão devido ao curto período de vida das formas aladas, baixa vagilidade e grandes restrições de qualidade de água para a sobrevivência dos imaturos. A dispersão limitada é especialmente interessante por restringir a habilidade dos indivíduos em colonizar grandes áreas rapidamente e de maneira extensiva (Baumgardner 2008), sendo todas essas qualidades muito úteis para estudos de padrões ancestrais afetados por vicariância (Croizat *et al.* 1974). Alguns fatos tornam Leptohyphidae ainda mais interessante para testar hipóteses biogeográficas: 1) são restritos ao Novo Mundo; 2) as espécies são abundantes e amplamente distribuídas por toda América, mostrando uma grande variedade de intervalos de distribuição; e 3) representa um grupo monofilético (McCafferty & Wang 2000; Molineri 2006).

Não há nenhum estudo publicado que analise e discuta hipóteses acerca dos padrões de distribuição em Leptoxyphidae. Contudo, de acordo com dados não publicados usando metodologia cladística, Leptoxyphidae teria se originado na América do Sul, com pelo menos cinco invasões independentes da América do Sul para a América do Norte e Central após a formação do istmo do Panamá (Baumgardner 2008).

A presente tese representa o estudo com maior representatividade de espécies de Leptoxyphidae da América do Sul, Central e, ainda que menos representado, da América do Norte. Ainda, é o estudo com a maior representatividade de famílias de Pannota, com espécies de todas as famílias, exceto quatro: Austramerellidae (América do Norte e Ásia), Ephemerithidae (África), Teloganellidae (África) e Machadorythidae (África), sendo a última presente na matriz morfológica. Dos 15 gêneros reconhecidos para Leptoxyphidae, 12 foram representados na matriz molecular e 13 na matriz morfológica (incluindo as espécies tipo), totalizando um total de 121 táxons na matriz combinada (105 do grupo interno), sendo até o momento o estudo evolutivo mais bem representado para família e para a infra-ordem.

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Capítulo 1

DNA barcoding reveals cryptic species in the Neotropical mayfly *Leptohyphodes inanis* (Pictet) (Ephemeroptera: Pannota)

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Hydrobiologia

Em preparação.

DNA barcoding reveals cryptic species in the Neotropical mayfly *Leptohyphodes inanis* (Pictet) (Ephemeroptera: Pannota)

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ABSTRACT

Leptohyphodes inanis (Pictet) is an enigmatic taxon, possessing a rare feature in Leptohyphidae: males with large and divided eyes that can have black or red colour. To assess genetic differences among populations and relate them to the colour variation of the eyes, we have sequenced the DNA barcode (a fragment of the mitochondrial CO1 gene) of 17 individuals. Measurements and morphology of 1,252 individuals was evaluated, in addition to photographs of the type series. Bayesian Inference and the Neighbour Joining analyses supported the monophyly of *L. inanis* and found three independently highly supported evolutionary lineages (=haplogroups), each one mostly restricted to a Southeastern Brazil mountain range. Phylogenetic analyses suggest that red eyes are a plesiomorphic trait in the group, not being a good diagnostic character to separate the putative species alone, but it is important in characterizing populations. Pairwise divergences modelled by Kimura-2 parameter showed high values of intraspecific genetic divergence (0 to 30.5%), with 23.3 to 24.9% between haplogroups and 4 to 13% within haplogroups. Morphological characters used in Ephemeroptera taxonomy are ineffective in separation of the haplogroups, thus our results provide strong evidence for at least three putative species in the *L. inanis* species complex.

Keywords: Barcode, systematics, genetic divergence, cryptic species, Bayesian inference, neighbour joining.

INTRODUCTION

Most taxonomic descriptions and species delimitation are made based on morphological characters, but sometimes these characters are not informative and morphological differentiation can be a difficult task for researchers. In addition to the biodiversity crisis, where many species will be extinct even before they are known to science (Wilson, 1985), the true number of biological species is likely to be greater than the current tally of nominal species, since speciation is not always accompanied by morphological changes (Cardoni *et al.*, 2015). With the advent of molecular studies and subsequent increase in availability of DNA sequences, molecular techniques and analysis methods, there has been an increase in genetic diversity, DNA barcoding, and cryptic species studies of insects (*e.g.*, Bickford *et al.*, 2007; Cardoni *et al.*, 2015; Cook *et al.*, 2008; Fujita *et al.*, 2012; Hebert *et al.*, 2003; Hendrich *et al.*, 2015; Macher *et al.*, 2016; Ossa-López *et al.*, 2017; Petit & Excoffier, 2009; Silveira *et al.*, 2016). Cryptic species (two or more distinct species classified as a single species) represent a challenge to taxonomists, but at the same time the discovery of them can contribute towards defining biodiversity patterns, with implications for evolutionary theory, biogeography and conservation planning (Bickford *et al.*, 2007).

Different studies on genetic distances, often associating morphological and geographic data for example, revealed the existence of cryptic species in several groups of animals and plants, in most types of habitats (*e.g.* Dawson & Jacobs, 2000; Feulner *et al.*, 2006; Gómez *et al.*, 2002; Grundt *et al.*, 2006; Hebert *et al.*, 2004; de Rezende Dias *et al.*, 2018; Vrijenhoek *et al.*, 1994). In this context, Arthropoda seems to be one of the most well represented groups in the cryptic species literature, being the description and recognition of them of great implication for human health (*e.g.* *Anopheles* malaria-transmitting mosquitoes), pest management (different species have variable pesticide resistance) and studies of coevolution and species interaction (Bickford *et al.*, 2007). More recently, several studies using molecular tools have drawn attention to the existence of cryptic species complexes in Ephemeroptera (*e.g.*, Gill *et al.*, 2016; Macher *et al.*, 2016; Ossa-López *et al.*, 2017; Pereira-da-Conceicao *et al.*, 2012; Rutschmann *et al.*, 2014; Williams *et al.*, 2006), specially with information from the mtDNA cytochrome C oxidase subunit I (COI) gene.

The genus *Leptohyphodes* Ulmer, 1920 (Ephemerelloidea: Leptohyphidae) was established for eight males imagoes from Brazil described as "*Potamanthus ? inanis*" by Pictet (1843) (Ulmer 1920). Ulmer (1921) placed in this genus another enigmatic taxon, *Tricorythus australis* Banks (1913) known from male and female imagoes, transferred later to *Tricorythodes* by Traver (1958). The genus is endemic to Southeastern Brazil, monotypic, and its phylogenetic relationships within Leptohyphidae remain unknown. However, Salles & Molineri (2006) described *Amanahyphes* Salles & Molineri, 2006, a genus endemic to the Amazon basin and discussed its relationship close to *Leptohyphodes*. Both genera share similar forewing shape and venation, similarities in the male genitalia (styliger plate posteriorly projected and forceps shape), and, most notably, large divided eyes in males (Salles & Molineri, 2006). The latter is a rare feature in Leptohyphidae, present only in these two genera and in *Leptohyphes populus* Allen (1973) known from a male nymph from Amazonas State (Brazil). Despite the impossibility of including adults and nymphs of *Amanahyphes* in his cladistic analysis of Leptohyphidae, Molineri (2006) found high support for the group formed by *Leptohyphodes* + *Haplohyphes* + *Tricorythodes*.

Although the original description of *Leptohyphodes inanis* (Pictet, 1843) does not provide a specific type locality nor the coloration of male eyes, Molineri (2005) redescribed it based on adults and nymphs from Campos do Jordão, São Paulo State (Brazil). These specimens have a cream-coloured upper portion of male compound eyes. However, representatives of different populations of this species in Southeastern Brazil show the upper portion of compound eyes varying also in shades of red to black, with apparently no other morphological feature. Within this context, the objective of the present work was to investigate whether these morphological variants in the eye can diagnose mtDNA lineages of *L. inanis*.

MATERIAL AND METHODS

Morphology and taxonomy

The material studied was collected in the states of Espírito Santo, Minas Gerais, Rio de Janeiro, and São Paulo, Southeastern Brazil, mostly from the domain of the tropical Atlantic Rain Forest, but some from Brazilian Cerrado. The sampling area comprises three Southeastern

Brazil mountain ranges: Serra do Mar, Serra da Mantiqueira, and Serra do Espinhaço. The species distribution map was made using the program QGIS 2.18.10 (QGIS 2017).

Specimens are deposited at Coleção Entomológica Prof. José Alfredo Pinheiro Dutra, Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (DZRJ) and Coleção Zoológica do Norte Capixaba, Universidade Federal do Espírito Santo, São Mateus, Brazil (CZNC). The material is preserved in 93% ethanol; wings and genitalia were slide-mounted in Euparal®. Photographs and measurements were made with the Leica Application Suite CV3 Automontage Software and later edited using the Adobe Photoshop CC 2018 software. Terminology follows Molineri (2002, 2006). Syntypes of *Leptohyphodes inanis*, deposited at Naturhistorisches Museum Wien, Vienna, Austria (NMW), were observed by photographs. Furthermore, additional material from Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil (MZUSP) were also analysed.

Only fully mature nymphs and imagos were included in the measurements, leading to 13 female nymphs, 11 male nymphs, 10 female imago and 14 male imagos. Despite the vast material available, only a small portion of it is in good condition of preservation and at the same development stage for comparison.

DNA sequences and genetic analyses

Genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following a modified protocol of the manufacturer's of imagoes and/or nymphs of the following members of Leptohyphidae: *Leptohyphodes* species, *Macunahyphes eduardoi* Almeida & Mariano, 2015, *Tricorythopsis chiriguano* Molineri, 2001, *Tricorythopsis gibbus* (Allen, 1967), and *Tricorythopsis spongicola* Lima, Salles & Pinheiro, 2011 (Table I). Partial sequences of the mitochondrial cytochrome oxidase I (COI) gene were amplified by polymerase chain reaction (PCR) using the primers LCO-1490 or C1-J-1718 in combination with HCO-2198 (Folmer *et al.*, 1994; Simon *et al.*, 1994). We used a two-part PCR program with five initial cycles with annealing temperature of 45 °C followed by 35 cycles with annealing temperature of 49 °C. Amplicons were purified and sequenced by Macrogen (Seoul, Korea). Resulting electropherograms from both DNA strands were analysed using Geneious 8.1.7

(<http://www.geneious.com>, Kearsse *et al.*, 2012), adjusted manually to generate a consensus sequence for each specimen. Sequences were checked with Basic Local Alignment Search Tool (BLAST; Altschul *et al.*, 1997) against the GenBank nucleotide database to ensure that the amplified product was correct and not contaminated. Individual sequences were aligned using ClustalW (Thompson, Higgins & Gibson, 1994) implemented in Geneious and translated into amino acids to ensure nonamplification of pseudogenes. Final alignment included 299 bp.

Table I: Specimen information including voucher specimen codes, respective collecting localities, and GenBank accession codes for COI sequences of *Leptohyphodes inanis* and related species.

Species	Voucher	Locality	GenBank accession number
<i>Macunahyphes eduardoi</i>	ENT2863	Espírito Santo, Nova Venécia, Rio Cricaré, 18°39' 51"S, 40°30'45"W, 25-26.vii.2012	
	ENT2872		
<i>Tricorythopsis chiriguano</i>	ENT1837	Roraima, Caracaraí, Balneário Bem Querer, 13.III.2014	
	ENT2239	Pernambuco, Jaqueira, Rio Pirangi, 8°44'53"S, 35°48'51"W, 23.v.2012	
<i>Tricorythopsis gibbus</i>	ENT2239	Espírito Santo, Santa Teresa, 19°56'13"S, 40°28'44", 30.vii.2012	
<i>Tricorythopsis spongicola</i>	ENT1722	Pernambuco, Amaraji, Rio Amaraji, 8°21'49"S, 35°28'49"W, 15.x.2013	
<i>Leptohyphodes inanis</i>	ENT1724	Minas Gerais, P. E. da Serra do Intendente, Rio Peixe Tolo, 19°0'14"S, 43°36'45"W, 07.ix.2012	
	ENT1737	São Paulo, Ubatuba, P. E. da Serra do Mar, 23°21'14"S, 44°46'4"W, 09.ix.2011	
	ENT1739	São Paulo, Ubatuba, Poço do Amor, 23°21'36"S, 44°46'59"W,	
	ENT2063	Minas Gerais, P. E. da Serra do Intendente, Rio Peixe Tolo, 19°0'14"S, 43°36'45"W, 08.ix.2012	
	ENT2248		
	ENT2249	Minas Gerais, P. N. da Serra da Canastra, Cachoeira Casca D'anta, 20°10'08"S, 46°40'13"W, 16.xi.2014	
	ENT2543	Minas Gerais, P. N. da Serra do Caparaó, Pedra Menina, 20°37'30"S, 41°49'27"W, 14.x.2011	
	ENT2545	Rio de Janeiro, P. N. da Serra dos Órgãos, Rio Bonfim, 22°27'51"S, 43°5'21"W, 19.xii.2011	
	ENT2546	Rio de Janeiro, P. N. da Serra dos Órgãos, Rio Bonfim, 22°27'55"S, 43°5'16"W, 19.xii.2011	
	ENT2554	Rio de Janeiro, Visconde de Mauá, Rio Preto, 22°19'50"S, 44°16'55W, 26.i.2012	
		Rio de Janeiro, P. N. da Serra dos Órgãos, tributary of the river Beija-flor, 22°26'54"S, 43°0'27"W, 14.xi.2011	

ENT2558	Rio de Janeiro, Itatiaia, Vale do Pavão, Rio Marimbondo, 22°21'43"S, 44°35'15"W, 28.o.2012
ENT2559	Rio de Janeiro, P. N. do Itatiaia, Córrego Maromba, 22°25'39"S, 44°37'10", 10.i-02.ii.2015
ENT2568	Espírito Santo, P. N. da Serra do Caparaó, Rio Pedra Roxa, 20°23'48"S, 41°44'8"W, 01.vi.2011
ENT2569	Espírito Santo, P. N. da Serra do Caparaó, Rio Pedra Roxa, 20°23'48"S, 41°44'8"W, 01.vi.2011
ENT2883	São Paulo, P. N. da Serra da Bocaina, Ribeirão da Prata, 22°46'49"S, 44°36'40"W, 21.viii.2015
ENT2993	São Paulo, P. E. do Campos do Jordão, 04.viii.2013
ENT2998	São Paulo, P. E. da Serra do Mar, Núcleo Santa Virgínia, 28.vii.2012

Pairwise divergences between COI sequences of specimens of *Leptohyphodes* and related species were calculated modelled by Kimura-2 parameter (K2P, Kimura, 1980). Clade support was evaluated based on 1000 non-parametric bootstrap (bs) pseudoreplicates of data matrices (Felsenstein, 1985) and dendrograms were constructed with a neighbour-joining (NJ) algorithm in MEGA 6 (Tamura *et al.*, 2013). The K2P model accounts for different transition (purine-purine and pyrimidine-pyrimidine exchanges) and transversion (purine-pyrimidine interchanges) rates and has been used extensively in DNA barcoding studies. Moreover, this model is widely used for studies of cryptic species and intra and interspecific variation in Ephemeroptera (Ball *et al.*, 2005; Webb *et al.*, 2007; Alexander *et al.*, 2009; Zhou *et al.*, 2009; Hwang *et al.*, 2013; Ossa-López *et al.*, 2017) and we used it in order to be able to compare our results with other K2P divergences cited in the literature.

The Bayesian inference (BI) approach were performed using MrBayes version 3.2.2 (Ronquist *et al.*, 2012) at the CIPRES portal (Miller *et al.*, 2010). The best-fit evolutionary model for each molecular partition was identified using jModelTest 2.1.7 (Posada, 2008). The Akaike information criterion (AIC) favoured the HKY+I+G model. Four independent Metropolis Coupled Markov Chain Monte Carlo (MCMCMC) analyses each with four chains were ran for 50,000,000 generations, sampling trees every 5000 generations. The initial 25% of sampled trees were discarded as burnin. Convergence among independent analyses was assessed by monitoring the values of standard deviation of split frequencies (<0.05) in MrBayes and parameter sampling was assessed with Tracer version 1.6 (Rambaut *et al.*, 2014) by the effective sample size (ESS)

criterion (>200). A 50% majority-rule consensus post-burnin tree was constructed and values of posterior probabilities (pp) were calculated.

Clades with bootstrap and posterior probabilities greater than 90 were referred to as strongly supported, 70–90 as moderately supported, and lower than 70 as being poorly supported, respectively. Final trees, including both BI and NJ approaches, were previewed at Figtree version 1.4.0 (Rambaut, 2012) and posteriorly edited in Adobe Illustrator CC 2017.

Parsimony haplotype networks were constructed for the same COI dataset using the Median-joining method (Bandelt *et al.*, 1999) implemented in PopART version 1.7 (Population Analysis with Reticulate Trees, Leigh & Bryant, 2015), with epsilon set to 0. Haplotype networks are an intuitive method for visualizing relationships between individual genotypes at the intraspecific level, as well as, to infer the biogeographical history of populations (Leigh & Bryant, 2015). The final figure was edited in Adobe Illustrator CC 2017.

RESULTS

Genetic diversity

The BI and NJ analysis of COI sequences recovered all *Leptohyphodes* sequences grouped together with moderate posterior probability value (pp=0.76) and high bootstrap support value (bs=99) (Figs. 1 and 2). Three lineages were recovered, all strongly supported: Clade 1, comprising sequences of specimens from the Serra da Mantiqueira, all with eyes coloured within the spectrum of red (bs=100; pp=0.95) (except one sequence from Serra do Mar with red eyes); Clade 2, comprising sequences of specimens from the Serra do Espinhaço, all with black eyes (bs=91; pp=0.99); and Clade 3, comprising sequences of specimens from the Serra do Mar, all with eyes coloured within the spectrum of red (bs=100; pp=1.0). Pairwise K2P divergences between all 17 *Leptohyphodes* sequences ranged from 0 to 30.5% (Table S1, see supplemental material). Considering individuals belonging to the three above mentioned lineages, interlineage K2P divergences ranged 23.3 to 24.9%, while estimates of average evolutionary divergence within each clade were: 10% for Clade 1; 13% for Clade 2; and 4% for Clade 3, displaying a clear barcoding gap between lineages. Low divergences were found only when comparing sequences from the same population (*e.g.*, sequences from Caparaó and Itatiaia), which is can be an artefact of a restricted geographic scale of sampling, as species

sampled throughout a larger geographic range will display higher genetic divergences (Bergsten *et al.*, 2012). As a comparison, the K2P divergence between the two sequences of *Macunahyphes eduardoi* was 17.6% and were sampled from individuals collected in very distant geographic localities, one from Roraima State (Northern Brazil) and the other from Espírito Santo State (Southeastern Brazil).

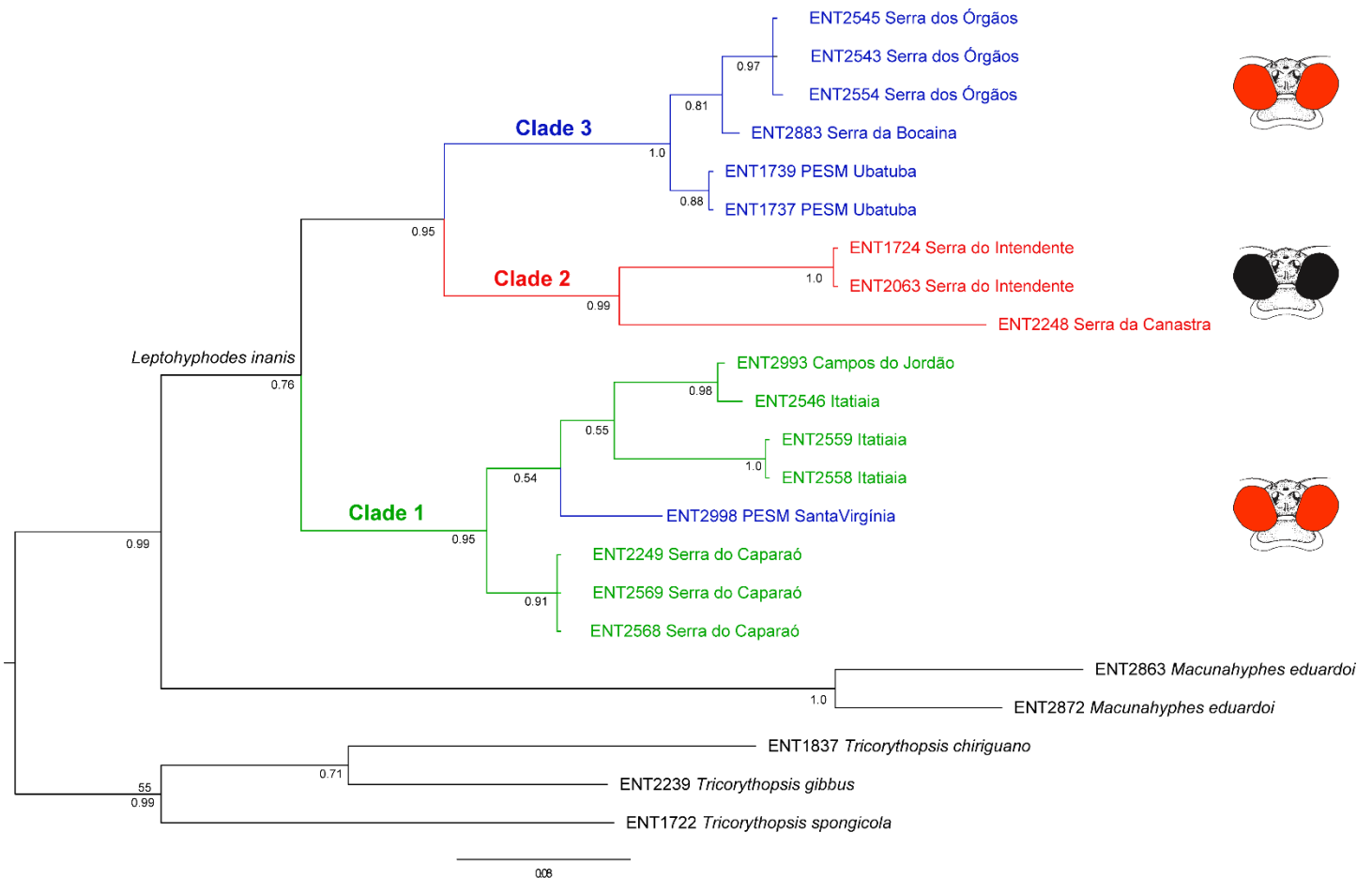


Figure 1: Bayesian inference consensus of COI sequences from different individuals of *Leptohyphodes inanis* (HKY+I+G model, harmonic mean $-\ln L = 2137.66$). Posterior probability values are given below branches. Figure modified from Molineri (2005).

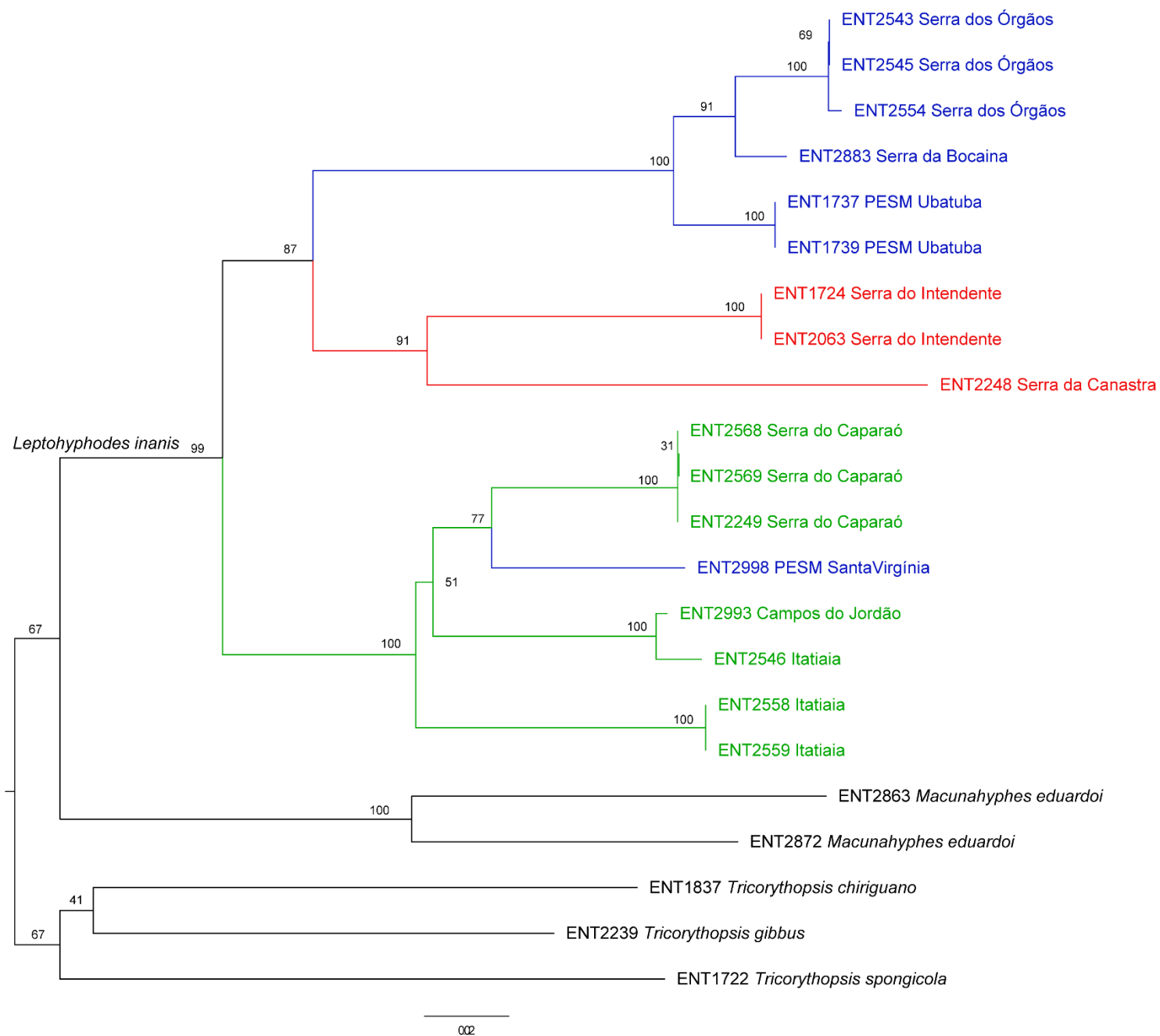
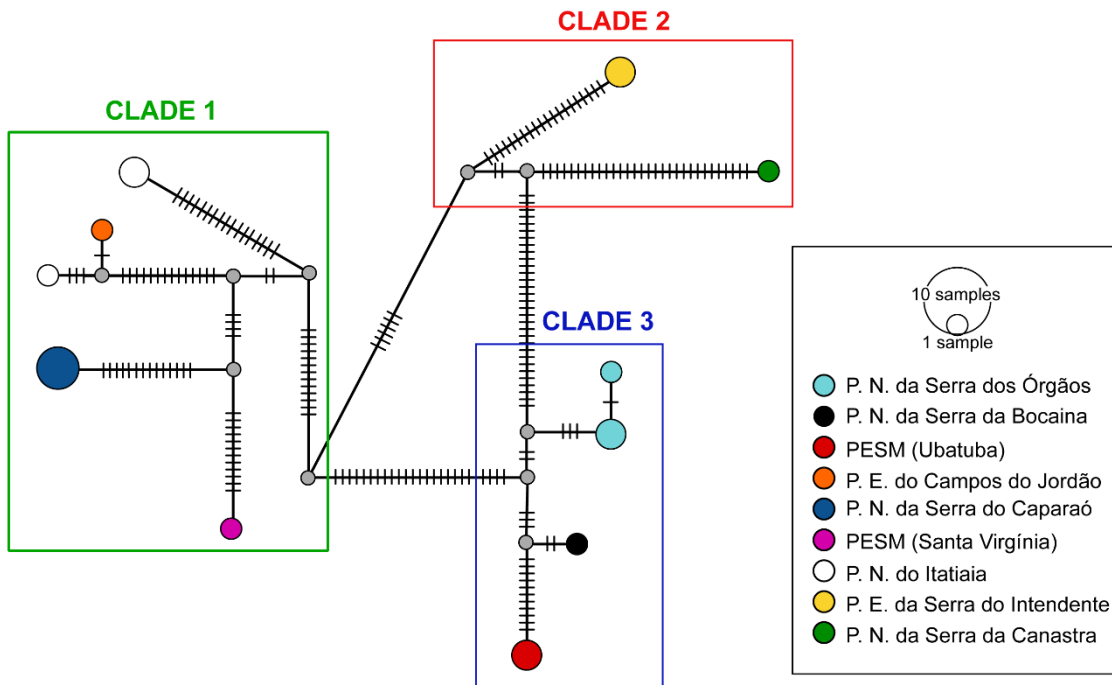


Figure 2: Neighbour joining tree based on K2P-corrected COI distances from different individuals of *Leptohyphodes inanis*. Bootstrap values are given above branches.

Haplotype network

The COI haplotype network consists of 11 haplotypes, which can be divided into three haplogroups similar to the three clades in the phylogenetic analyses (Fig. 3). Two different haplotypes were sampled from populations from Serra dos Órgãos and Itatiaia, and one of the Itatiaia haplotypes appears to be more similar to the Campos de Jordão haplotype.

Figure 3: Haplotype network of COI sequences of *Leptohyphodes inanis*. Colours indicate collecting



locality of haplotypes according to legend. Size of circles related to the frequency of haplotypes and dashes in the branches represent the number of mutations between haplotypes.

Morphology

Morphological characters commonly used in Leptohiphidae taxonomic studies were observed in nymphs and imagoes. Generally, diagnostic characteristics of the nymphs are: absence or presence of maxillary palp; when present, its number of segments; absence or presence of apical setae on the maxillary palp; proportion of the width and height of femora; presence and arrangement of setae on the dorsum of femora; number and arrangement of denticles on tarsal claws; and colour pattern. Adult characteristics more commonly used are: colour pattern; wing venation; and morphology of the male genitalia. In addition, body lengths were compared among populations to see if there is a separation of populations by size (Table II).

Table II: Mean (and standard deviation) of body length measurements (mm) of nymphs and imagoes (females and males) from different *L. inanis* populations. CJ: Parque Estadual Campos do Jordão. SV: Parque Estadual da Serra do Mar, Núcleo Santa Virgínia. ITA: Itatiaia (national park and surroundings). BOC: Parque Nacional da Serra da Bocaina. CAN: Parque Nacional da Serra da Canastra. SO: Serra dos Órgãos (national park and surroundings). BIR: Itabirito (Minas Gerais). N: total number of specimens measured. *: measurement based on only one specimen.

Locality	Nymph				Imago				
	♀		♂		♀		♂		
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	
CLADE 1	CJ	10.13	0.21	8.58	0.53	-	-	8.49*	-
	SV	8.25	0.07	6.90	0.14	-	-	-	-
	ITA	9.04	0.48	7.42	0.58	6.13	0.23	5.79	0.28
CLADE 2	INT	8.38*	-	7.18*	-	7.89	0.70	-	-
	CAN	-	-	-	-	7.49*	-	8.86	0.70
	BIR	-	-	-	-	-	-	8.20	0.61
CLADE 3	BOC	7.15	0.07	6.71*	-	8.32*	-	7.65	0.21
	SO	-	-	-	-	6.04	0.35	7.53	0.15

All material examined agree with the species redescription by Molineri (2005), with no variation in the diagnostic characters, except for the colour of the upper portion of male compound eyes. Individuals of *L. inanis* show variation in the colour pattern (Fig. 4 and 5), body length, and amount of bristles throughout the body; but these features overlap in

different populations. One example is the population of Campos do Jordão, with specimens showing two colour variations of the upper portion of male compound eyes: most specimens (n=31) in shades of orange, including red (Fig. 2A) and fewer specimens (n=10) in shades of grey, including cream (Fig. 2B). Both variations were treated herein as with colour in the red spectrum, because they were difficult to tell apart.



Figure 4: *Leptohyphodes inanis*, male nymph habitus, showing morphological geographical diversity. A and B, Parque Estadual do Campos do Jordão (São Paulo State). C, Parque Nacional do Itatiaia (Minas Gerais State). D, Parque Nacional da Serra da Bocaina (São Paulo State). E, Parque Estadual da Serra do Intendente (Minas Gerais State). F, Reserva Biológica Augusto Ruschi (Espírito Santo State).

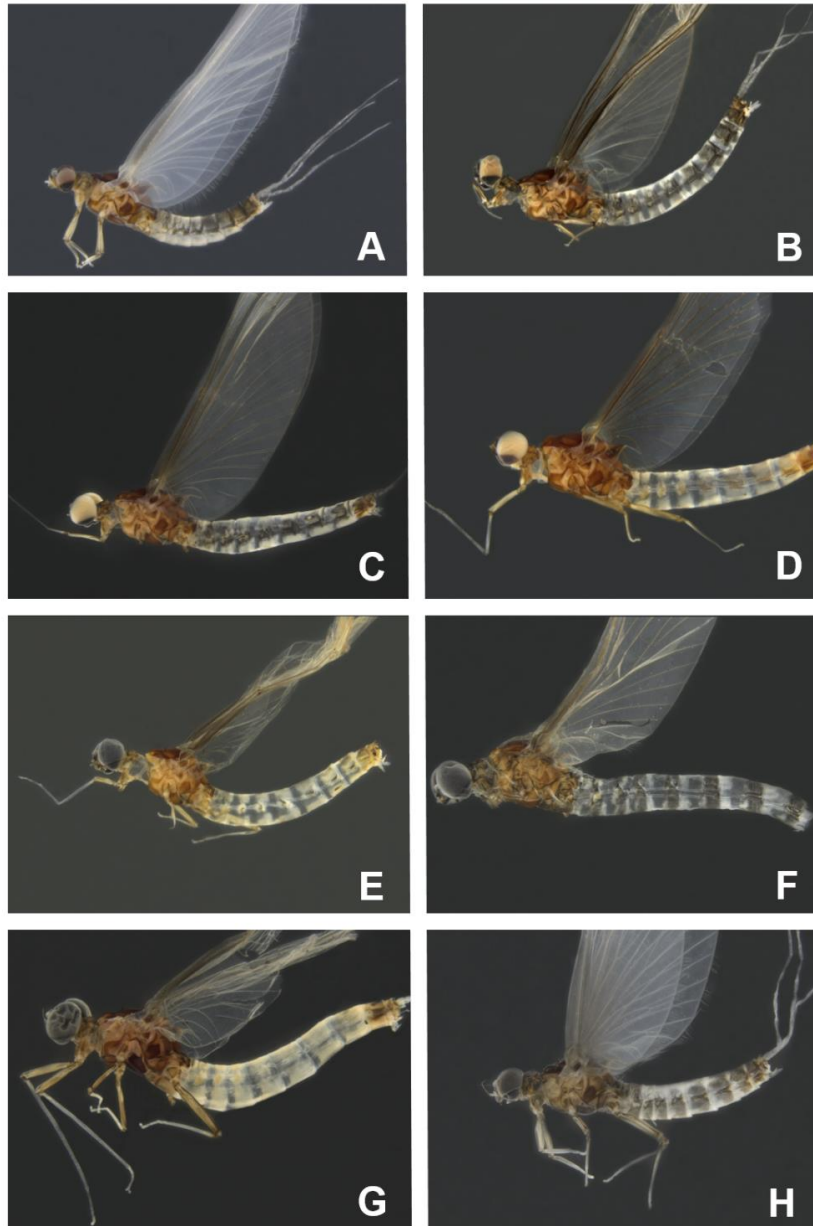


Figure 5: *Leptohyphodes inanis*, male habitus, showing the morphological geographical diversity. A, subimago from Parque Nacional do Itatiaia (Minas Gerais State). B, imago from Parque Nacional da Serra da Bocaina (São Paulo State). C, imago from Parque Nacional da Serra dos Órgãos (Rio de Janeiro State). D, imago from Parque Estadual do Campos do Jordão (São Paulo State). E, imago from Itabirito (Minas Gerais State). F, imago from Parque Estadual da Serra do Intendente (Minas Gerais State). G, imago from Parque Nacional da Serra da Canastra (Minas Gerais State). H, subimago from Reserva Biológica Augusto Ruschi (Espírito Santo State).

Taxonomy

***Leptohyphodes inanis* (Pictet, 1843)**

Potamanthus? inanis. Pictet, 1843: 232 (orig. descr.); Eaton, 1886: 296 (male).

Potamanthus inanis. Walker, 1853: 544, 547; Eaton, 1871: 91 (male).

Leptohyphodes inanis. Ulmer, 1920: 51; Lestage, 1931a: 74; Lestage, 1931b: 60; Traver, 1958: 496 (male, female, nymph); Hubbard, 1982: 274; Molineri, 2005: 250 (redescription).

Leptohyphodes sp. Traver, 1944; Molineri, 2005: 250.

Measurements. Total length: nymph ♂ 5.5–9.0 mm (n=11), nymph ♀ 6.4–10.3 mm (n=13); imago ♂ 5.6–9.4 mm (n=14), imago ♀ 6.0–7.5 mm (n=10).

Diagnosis. According to Molineri (2005), *Leptohyphodes* can be distinguished from all other genera of Leptohiphidae by the following combination of characters. Imago: (1) male fore wings without extended cubitoanal lobe; (2) hind wings absent in both sexes; (3) female caudal filaments relatively long; (4) membranous filaments of mesoscutellum long and slender; (5) male forceps two-segmented, distal segment leaf-like; (6) penes plate-like, fused except on apical excavation; (7) male eyes big and divided. Nymph: (1) hind wing pads absent in both sexes; (2) operculate gills subtriangular, dorsally with two ridges and a median weaker band; (3) number of lamellae per gill (II–V. 2-3-3-2, lamellae subtriangular); (4) 3–4 small imbricated lobes on ventral lamellae of gills II–IV; (5) femora I with a subdistal transverse row of long setae; (6) femora II and III with a subdistal transverse row of setae; (7) maxillary palp small, setiform; (8) labrum with a deep median cleft; (9) tarsal claw with 5–6 slightly marked and blunt marginal denticles on basal half and with a double row of 2–3 submarginal denticles on apical 1/3; (10) frontal and genal projections present; (11) body elongated, not heavily sclerotized and covered by numerous long setae.

Comment. As *Leptohyphodes* is a monotypic genus, the generic diagnostic features of both nymphal and adult stages are maintained for *L. inanis*.

Type material. Syntypes (Fig. 6): seven ♂ imagos (NMW), Brazil. One syntype, left wing missing, bearing labels: 1) blue and rectangular handwritten "Shtt." for Heinrich Wilhelm Schott, who collected the specimens; 2) white rectangular handwritten "Pictet vidit"; 3) white rectangular in Ulmer's handwriting "Leptohyphodes (Ulm.) inanis Pict. Typus". Five syntypes, each one bearing two labels: 1) blue and rectangular handwritten "Shtt."; and 2) white rectangular handwritten "Pictet vidit". One syntype bearing the label "Pictet vidit"

Type locality. Brazil.

Distribution (Fig. 7). Southeastern Brazil (Espírito Santo, Minas Gerais, Rio de Janeiro, and São Paulo) within the domains of the tropical Atlantic Rain Forest and Brazilian Cerrado, occurring between 46 to 1,550 m of altitude. The records in Serra da Canastra (MG) are new records for the species in the Cerrado biome.

Material examined. Appendix 1.

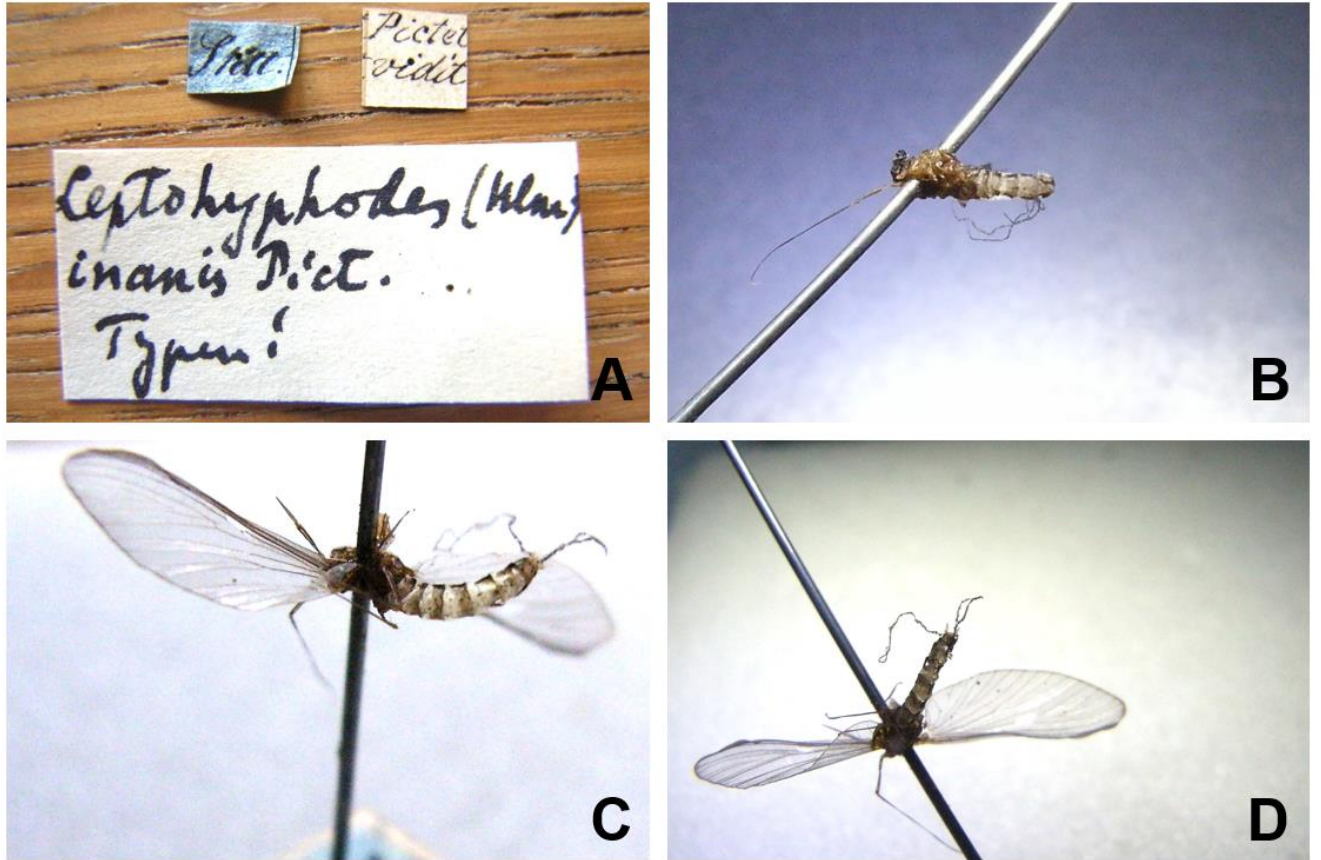


Figure 6: *Leptohyphodes inanis*, male imago, syntypes. A, labels of an unphotographed individual. B, dorsal view from one individual showing the head. C, lateral view from a different individual showing the left wing and abdomen. D, ventral view from the same individual in C showing the male genitalia. Photos by Ernst Bauernfeind.

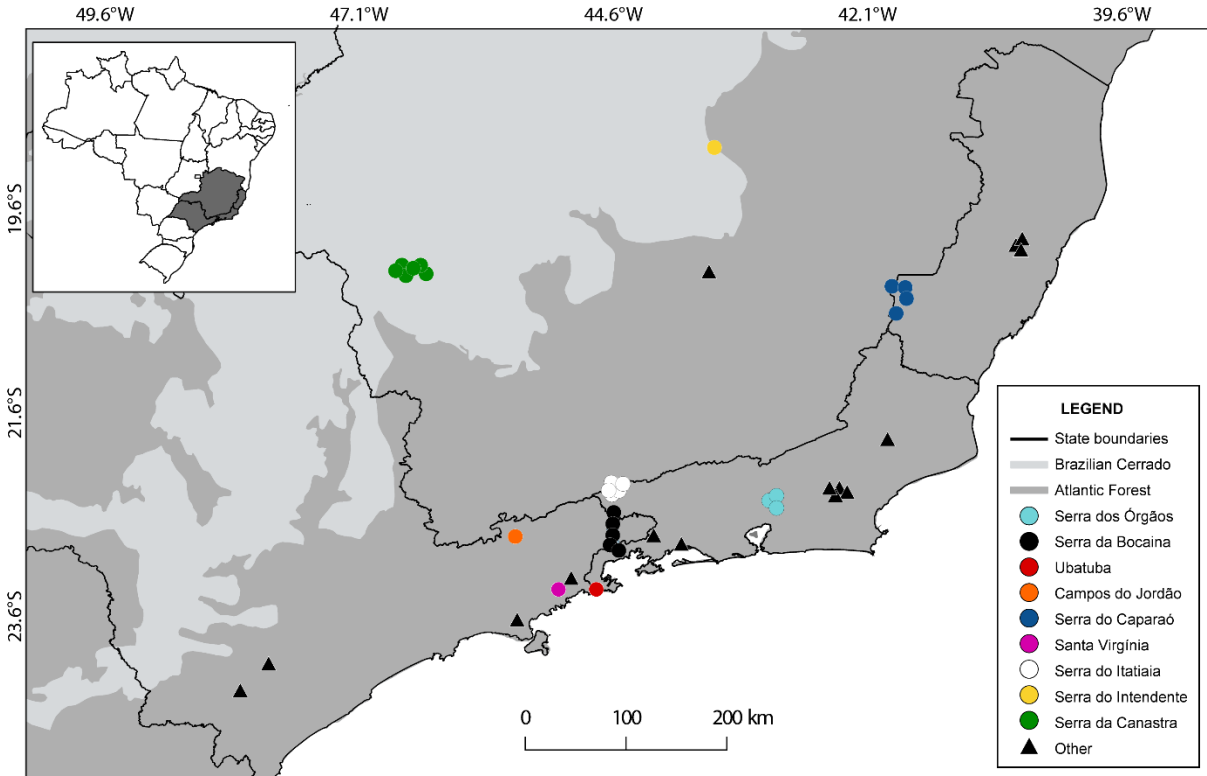


Figure 7: Physiographic map of Southeastern Brazil. *Leptohyphodes inanís* is endemic to Southeastern Brazil, with most records for the Brazilian Atlantic Rainforest, but its distribution herein extends to the Brazilian Cerrado. Colored circles refer to populations with sequences used in molecular analyses. Black triangle refers to the other populations used in the studies, but we do not have sequences (See "Material examined" in Appendix 1).

DISCUSSION

According to the original description of the genus, Ulmer (1921) quoted eight specimens carrying Pictet's label, who originally described *L. inanis*. However, one specimen may be lost since Ulmer's revision of the NMW collection (Ernst Bauernfeind pers. comm). On a visit to the Hamburg Zoological Museum (ZMH), FFS found an adult specimen of *L. inanis* with black eyes, with a "Pictet vidit." label. The ZMH has a large part of the material studied by Ulmer and this specimen may be what is lacking in the syntypes series. Although, the precise collecting locality of syntypes is not stated anywhere, it is assumed that it is Rio de Janeiro (Brazil), because Heinrich Wilhelm Schott collected in Rio de Janeiro between 1817 to 1921, staying practically all the time restricted to the direct vicinity of the city (Schott, 1822). However, the eye colour of all syntypes is black (Fig. 6 and Ernst Bauernfeind pers. comm.). After the observation of 1,252 specimens of *L. inanis*, black eyes were only found in four populations, all restricted to distant areas of Rio de Janeiro: three from the Serra do Espinhaço (Serra do Capanema, Serra da Canastra, and Serra do Intendente in Minas Gerais State), in or near the Brazilian Cerrado biome; and one from Reserva Biológica Augusto Ruschi (Espírito Santo), part of Serra da Mantiqueira, in the Brazilian Atlantic Rainforest. The latter, unfortunately, could not be added to the molecular dataset. It is possible that the eye colour of syntypes may have changed as an artefact of the dry preservation and/or action of time or that the collection site is not Rio de Janeiro.

High values of intra and interspecific K2P divergences appear to be common within the order Ephemeroptera. In a study on DNA barcode of mayflies, Ball *et al.* (2005) found mean values of intra- and interspecific genetic diversity of 1% and 18%, respectively. For example, these values appear to be much higher in mayflies when compared to studies in Lepidoptera (0.25 and 6.8%, respectively; Hebert *et al.*, 2003). These high values found in Ephemeroptera may be related to their biology. In general, they have limited dispersal capacity due to the short life span of winged forms, low vagility, and high restrictions of water quality for immature survival. However, genetic distances found for *L. inanis* (maximum intraspecific = 30.5%) are much higher than expected for conspecific individuals. Furthermore, the haplotype network showed a high haplotype diversity, with many mutational steps even within the three haplogroups.

Based on previous DNA barcode studies, low values of genetic divergence are expected when conspecific sequences are compared, while high values may indicate the existence of multiple species. For Ephemeroptera, Ball *et al.* (2009) found maximum intraspecific divergence of 3.4% and Zhou *et al.* (2009) reported a 2% divergence criterion. Williams *et al.* (2006) found seven significantly divergent haplogroups of *Baetis rhodani* (Pictet, 1843) in Western Europe, constituting many putative species with haplogroup divergence ranging from 0.2–3% (within) to 8–19% (among). After that, other authors found similar results and added other haplogroups, however morphological characters do not allow for a reliable distinction among them, providing strong evidence for cryptic species in the *B. rhodani* complex (Lucentini *et al.*, 2011; Sroka, 2012; Gattolliat *et al.*, 2015). In the Neotropics, at least three species of *Campylocia* Needham & Murphy, 1924 also show high maximum intraspecific barcode divergences between 7.2 to 10% (Gonçalves *et al.* 2017), suggesting cryptic species complexes. Similarly, Ossa-López *et al.* (2017) supported that the Andean species *Andesiops peruvianus* (Ulmer, 1920) is a species complex with genetic distances between 0 and 24.5% for the COI gene, supporting the existence of four putative species in the Chinchiná River Basin (Caldas department, Colombia).

Naturally, there is no magic threshold of genetic distance above which species status can be postulated (Buckley *et al.*, 2001). Nevertheless, the level of sequence divergence between the three lineages found in our analyses exceeds that between other Ephemeroptera species that are well established on morphological criteria, including when compared to species of other genera of Leptohiphidae used in our dataset and in previously studies (Ball *et al.*, 2005: *Tricorythodes* Ulmer, 1920 with interspecific distances of 18.3–25.8%). In addition, our phylogenetic analyses recognize these three distinct evolutionary lineages with high clade support and mostly restricted on different mountain chains in Southeastern Brazil. Considering that red eyes seem to be a plesiomorphic state in the evolution of the group, it is not a good diagnostic character to separate the putative lineages. However, eye colour may be important in characterizing populations, because within each lineage eye colour seems to be consistent.

From the material examined, it is not possible to differentiate the lineages using the diagnostic characters commonly used in Ephemeroptera taxonomy. The high genetic divergences found may be more related to the geographic distance between populations, which would act in the absence of gene flow between them, than in obvious morphological differences. Thus, *Leptohiphodes inanis* represent a group of cryptic species with at least three

putative species in a process of speciation where, with available material and current knowledge, we cannot differentiate morphologically each of the genetic lineages.

Supplementary Table S: Pairwise divergence between COI nucleotide sequences of *Leptohyphodes inanis* and related species using the Kimura 2-parameter model.

Appendix 1. List of material examined during the study. The list is organized by Brazilian states.

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Appendix 1. List of material examined during the study. The list is organized by Brazilian states.

BRAZIL, MINAS GERAIS, Conceição do Mato Dentro, Parque Estadual Serra do Intendente, S19°0'14", W43°36'45", 07.ix.2012, Salles, Rocha & Braga *leg.*, 4 male imagoes; same locality, 08.ix.2012, same collectors, 11 subimagoes (DZRJ); same data, 6 imagoes (DZRJ). Itabirito, Serra do Capanema, Vale do Catana, Cachoeira do Cascalho, S20°12'26", W43°38'34"W, 9.x.2010, Ferreira-Jr. *leg.*, 5 subimagoes (DZRJ); same locality, 10.x.2010, Ferreira-Jr. *leg.*, 9 subimagoes (DZRJ); same data, 3 subimagoes (DZRJ); Cachoeira da Carranca, S20°12'28", W43°38'26", 10.x.2010, Gonçalves *leg.*, 24 subimagoes (DZRJ); Cachoeira dos Cruzados, S20°12'17", W43°38'10", 10.x.2010, Clarkson & Dumas *leg.*, 8 subimagoes (DZRJ); same locality, 9.x.2010, Clarkson & Dumas *leg.*, 4 subimagoes (DZRJ); São João Batista da Canastra, Parque Nacional da Serra da Canastra, S20°9'12", W46°39'40", 1,231 m, 15.xi.2014, Nessimian, Oliveira, Rocha & Souto *leg.*, 11 subimagoes (DZRJ); same locality, 02.x.2015, Nessimian, Dumas, Rocha & Souto *leg.*, 15 imagoes (DZRJ); Cachoeira do Jota, Rio Araguari, S20°5'50", W46°40'13", 1,141 m, 16.xi.2014, 15 imagoes (DZRJ); same locality, 02.x.2015, Nessimian, Dumas, Rocha & Souto *leg.*, 1 imago (DZRJ); São Roque de Minas, Rio São Francisco, Casca D'Anta (high part), S20°14'37", W46°38'43", 956 m, 16.xi.2014, 6 subimagoes (DZRJ); Parque Nacional da Serra da Canastra, spring of São Francisco river, S20°14'37", W46°26'47", 1,364 m, 15.xi.2014, Nessimian, Oliveira, Rocha & Souto *leg.*, 4 subimagoes (DZRJ); Cachoeira do Rolinho, Ribeirão da Mata, S20°10'34", W46°33'35", 1,100 m, 16.xi.2014, Nessimian, Oliveira, Rocha & Souto *leg.*, 6 subimagoes (DZRJ); Alto Caparaó, Parque Nacional do Caparaó, Rio José Pedro, Cachoeira das Andorinhas, S20°22'29", W41°51'28", 06.x.2010, Ferreira-Jr & Clarkson *leg.*, 1 subimago (DZRJ); Espera Feliz, Parque Nacional do Caparaó, Pedra Menina, S20°37'30", W41°49'27", 14.x.2011, Massariol & Raimundi *leg.*, 5 nymphs (CZNC Ep-4194); same locality, 14.x.2010, 1 nymph (CZNC). Bocaina de Minas, Córrego do Morro Cavado, Cachoeira Santa Clara, S22°18'54", W44°35'45", 27.i.2012, 1 subimago (DZRJ); Itamonte, Parque Nacional do Itatiaia, 20.xi.2004, 1 subimago (DZRJ); same locality, 20.xi.2009, 8 nymphs (DZRJ); first order stream, 21.ix.2007, Jardim, Santos, Dumas & Nessimian *leg.*, 1 nymf (DZRJ 3168); Rio Aiuruoca, S22°20'59", W44°41'36", 20.xi.2004, Nessimian & Ferreira-Jr *leg.*, 1 subimago (DZRJ); Itatiaia, 03.xi.2007, 12 nymphs (DZRJ 3169); Santa Clara, Rio Preto, Poção do Maromba, 16.xii.2006, Moreira, Braga, Alecrim & Vanini *leg.*, 2 nymphs (DZRJ 2652). ESPÍRITO SANTO, Pedra Roxaa, Parque Nacional da Serra do Caparaó, 01.vi.2011, 2 nymphs (CZNC); same data, 1 nymph (CZNC Ep-6433); same data, 1 nymph (CZNC Ep-6434); Ibitirama, Parque Nacional da Serra do Caparaó, Rio Pedra Roxa and tributary, S20°23'48", W41°44'8", 1,063 m, 20.iv.2008, Salles, Massariol, Lima, Boldrini & Brito *leg.*, 2 nymphs (CZNC Ep-218); same data, 1 nymph (CZNC Ep-249); river from Tecnotruta, "Sonho Meu" property, S20°28'9", W41°43'22", 959 m, Salles, Massariol, Lima, Boldrini & Brito *leg.*, 2 nymphs (CZNC Ep-222); same data, 1 nymph (CZNC Ep-226); Santa Marta, 31.v.2011, 1 nymph (CZNC); Santa Teresa, Nova

Lombardia, Capitel de Santo Antônio, Córrego Escavado, S19°52'32", W40°31'47", 705 m, 19.i.2008, Salles, Massariol, Lima, Boldrini & Angeli *leg.*, 1 nymph (CZNC Ep-339); same locality, 26.x.2008, 1 nymph (CZNC Ep-976); Capitel de Santo Antônio, S19°52'31", W40°31'49", 768 m, 24–26.x.2008, Salles, Lima, Brito, Soares, Rúbio & Silva *leg.*, 1 nymph (CZNC Ep-950); Reserva Biológica Augusto Ruschi, S19°55'22", W40°33'13", 20.ii.2009, 1 nymph (CZNC); same data, 2 nymphs (CZNC Ep-1135); Córrego Bragacho, S19°52'3", W40°33'34", 28.iv–27.v.2017, Costa & Salles *leg.*, 2 subimagoes (CZNC); same locality and collectors. 26.v–21.vi.2017, 1 subimago (CZNC);); same locality and collectors, 26.vii–23.viii.2017, 2 subimagoes (CZNC);); same locality and collectors, 24.viii–30.ix.2017, 1 subimago (CZNC); same locality and collectors, 21.x.2017–18.xi.2017, 1 subimago (CZNC); same locality and collectors, 17 nymphs (CZNC). RIO DE JANEIRO, Itatiaia, Parque Nacional do Itatiaia, Córrego Simon, S22°25'55", W44°36'25", 1,149 m, 14.iv.2007, Moreira *leg.*, 1 nymph (DZRJ 2712); Rio Campo Belo tributary, S22°26'44", W44°36'27", 900 m, 15.iv.2007, Dumas & Santos *leg.*, 3 nymphs (DZRJ 2714); Itatiaia, S22°35'59", W44°35'58", 17.iv.2007, Dumas, Santos, Fernandes & Nessimian *leg.*, 4 subimagoes (DZRJ 3163); Rio Marimbondo, S22°21'42", W44°35'14", 14.x.2000, Huamantinco & Nessimian *leg.*, 14 nymphs (DZRJ 3170); Rio Campo Belo, trail to Cachoeira Véu da Noiva, S22°25'42", W44°37'11", 16.iv.2007, Dumas, Santos, Ferreira-Jr & Nessimian *leg.*, 3 subimagoes (DZRJ 3171); Córrego Maromba, below Cachoeira Véu da Noiva, S22°25'39", W44°37'10", 10.i–02.ii.2015, Takiya, Santos & Monné *leg.*, 11 subimagoes (DZRJ 3172); Cachoeira Véu da Noiva, S22°25'38", W44°37'6", 12.x.2013, Silva, Santos & Souza *leg.*, 1 subimago (DZRJ 3173); same locality, 16.iv.2007, Dumas, Santos, Ferreira-Jr & Nessimian *leg.*, 1 subimago (DZRJ 3174); Rio Campo Belo, piscina do Maromba, S22°25'46", W44°37'10", 16.iv.2007, Dumas, Santos, Ferreira-Jr & Nessimian *leg.*, 8 subimagoes (DZRJ 3175); Vale do Pavão, Rio Marimbondo, S22°21'43", W44°35'15", 28.i.2012, Sampaio, Oliveira & Gomes *leg.*, 22 subimagoes (DZRJ 3176); Visconde de Mauá, Maromba, Rio Monjola, Cachoeira Véu da Noiva, S22°19'41", W44°36'1", 26.I.2012, Oliveira *leg.*, 1 subimago (DZRJ 3164); Rio Preto tributary, 15.x.2000, 4 nymphs (DZRJ 3166); Rio Preto, Cachoeira do Escorrega, S22° 19'30", W44°36'55", 26.i.2012, Sampaio *leg.*, 1 subimago (DZRJ 3167); Resende, Serrinha do Alambari, Cachoeira dos Amores, S22°23'36", W44°34'10", 1,041 m, 10.ii.2016, Takiya & Santos *leg.*, 32 subimagoes (DZRJ); same data, 1 nymph (DZRJ); Teresópolis, Parque Nacional da Serra dos Órgãos, Rio Beija-flor (pool), 27.x.2007, Azevedo, Dumas & Kaplan *leg.*, 1 nymph (DZRJ 2651); Rio Beija-flor, S22°26'50", W43°0'20", 19.vii.2000, 3 nymphs (DZRJ 2713); same locality, 11.xi.2011, Oliveira, Nessimian & Santos *leg.*, Rio Paquequer, S22°27'23", W42°59'50", 23–24.iii.2010, 1 subimago (DNA Voucher DZRJ ENT2547); same data, 1 subimago (DNA Voucher DZRJ ENT2557) same data, Passos & Nessimian *leg.*, 1 subimago (DNA Voucher DZRJ ENT2550); tributary of Rio Beija-flor, trail to Pedra do Sino, S22°26'54", W43°0'27", 1,332 m, 14.xi.2011, Oliveira *leg.*, 1 nymph (DNA Voucher DZRJ ENT2554); Petrópolis, Bonfim, Parque Nacional da Serra dos Órgãos, Rio Bonfim, S22°27'55", W43°5'16", 1114 m, 19.xii.2011, Oliveira, Dumas, Passos, Gomes & Nessimian *leg.*, 2 subimagoes and 1 imago (DZRJ); same data, 1 subimago (DNA Voucher DZRJ ENT2545); Rio Bonfim,

S22°27'51", W43°5'21", 19.xii.2011, Oliveira, Dumas, Passos, Gomes & Nessimian *leg.*, 1 subimago (DZRJ); Guapimirim, Parque Nacional da Serra dos Órgãos, Rio Soberbo, Poço da Preguiça, S22°29'34", W43°0'04", 388 m, Silva, Nessimian, Dumas & Souto *leg.*, 1 subimago (DZRJ); Serra do Subaio, Rio Varginha, 20.vii.2000, 1 nymph (DZRJ 2688); Nova Friburgo, Rio das Flores, S22°24'36", W42°29'41", 971 m, 30.xi.2008, Sampaio *leg.*, 1 imago (DZRJ 1699); same data, Gonçalves *leg.*, 3 subimagos (DZRJ 1700); same river, S22°25'37", W42°30'26", 1,062 m, 30.xi.2008, Gonçalves *leg.*, 11 subimagos (DZRJ 1702); same locality, 01.xii.2008, Jardim *leg.*, 1 nymph (DZRJ 1701); same river, S22°25'07", W42°29'55", 993 m, 30.xi.2008, Gonçalves *leg.*, 1 imago (DZRJ 1703); same data, Sampaio & Santos *leg.*, 10 subimagos (DZRJ 1704); Rio Macaé, S22°24'46", W42°31'16", 935 m, 14.ix.2008, Alecrim *leg.*, 11 subimagos (DZRJ 1705), same locality and collector, 12.ix.2008, 7 subimagos (DZRJ 1706); same locality and collector, 13.ix.2008, 2 subimagos (DZRJ 1707); same locality and collector, 12.ix.2008, 5 subimagos (DZRJ 1708); same locality and collector, 14.ix.2008, 3 subimagos (DZRJ 1709); same locality, 30.xi.2008, Gonçalves *leg.*, 4 subimagos (DZRJ 1710); same locality and collector, 15.ix.2008, 11 subimagos (DZRJ 1704); Córrego Verdun, S22°25'27", W42°32'08", 1,008 m, Santos *leg.* (DZRJ 1714); first order tributary of the Macaé river, S22°25'52", W42°32'14", 1,055 m, 29.xi.2008, Santos *leg.*, 20 imagos (DZRJ 1716); second order tributary of the Macaé river, S22°25'58"W42°32'24", 1,103 m, 29.xi.2008, Santos *leg.*, 1 subimago (DZRJ 1717); second order tributary of the Macaé river, S22°25'34"W42°32'56", 1,103 m, 29.xi.2008, Santos *leg.*, 6 subimagos (DZRJ 1718); Rio Macaé, S22°23'30", W42°29'6", 944 m, 30.xi.2008, Santos & Sampaio *leg.*, 1 subimago (DZRJ 1719); same data, 1 subimago (DZRJ 1720); second order tributary of the Macaé river, S22°23'39", W42°30'8", 956 m, 01.xii.2008, Sampaio & Santos *leg.*, 1 subimago (DZRJ 1721); Rio Macaé, Cascata da Fumaça, S22°21'56", W42°15'13", 368 m, 08.iii.2009, Gonçalves *leg.*, 1 subimago (DZRJ 1727); Lumiar, Rio Boa Vista, S22°19'1", W42°17'23", 910 m, 14.xi.2008, Gonçalves *leg.*, 3 subimagos (DZRJ 1722); same data, Nessimian & Sampaio *leg.*, 2 subimagos (DZRJ 1723); Lumiar, second order of the Córrego Santa Margarida, S22°20'35", W42°18'0", 844 m, 17.xi.2008, De-Souza *leg.*, 2 nymphs (DZRJ 1724); first order of the Córrego Santa Margarida, S22°20'10", W42°17'34", 970 m, 16.xi.2008, De-Souza *leg.*, 2 nymphs (DZRJ 1725); first order of the Rio Toca da Onça, S22°23'24", W42°20'5", 716 m, 05.iii.2009, Gonçalves *leg.*, 9 subimagos (DZRJ 1726); Sana, Córrego da Ilha (second order tributary of Rio Boa sorte), S22°20'42", W42°11'4", 381 m, 19.ii.2009, Gonçalves *leg.*, 8 subimagos (DZRJ 1728); Sana, São Bento, Córrego do Colégio, S22°20'23", W42°12'13", 294 m, 19.ii.2009, Gonçalves *leg.*, 14 subimagos (DZRJ 1729); São Fidelis, Parque Estadual do Desengano, Morumbeca dos Marreiros, Ribeirão Macapá, S21°52'40", W41°54'30", 1,083 m, Dumas, Nessimian, Portela & Barbosa *leg.*, 13.iv.2016, 1 subimago (DZRJ DNA Voucher ENT3294); same river and collectors, S21°52'36", W41°54'44", 1,111 m, 1 subimago (DZRJ DNA Voucher ENT3295); Santa Maria Madalena, Parque Estadual do Desengano, Morumbeca dos Marreiros, tributary of Ribeirão Macapá, S21°52'39", W41°54'55", 1,110 m, Dumas, Nessimian, Portela & Barbosa *leg.*, 1 subimago (DZRJ DNA Voucher ENT3296); Mangaratiba, BR101, Rio Muriqui, S22°54'56",

W43°56'09", 18.ix.2007, Baptista, Mugnai & Oliveira *leg.*, 4 nymphs (DZRJ 1385); Rio Claro Lídice, Rio Cotia, S22°50'8", W44°12'32", 02.x.2007, Nessimian, Baptista, Mugnai & Oliveira *leg.*, 1 nymph (DZRJ 1435); Angra dos Reis, Parque Nacional da Serra da Bocaina, Trilha do Ouro, Rio Santo Antônio, 03.x.2007, Nessimian, Baptista, Mugnai & Oliveira *leg.*, 1 nymph (DZRJ 1463); Córrego Maitaca, S22°54'58", W44°37'47", 442 m, 09.viii.2003, Oliveira *leg.*, 2 nymphs (DZRJ 4420); same locality and collector, 07.viii.2004, 1 nymph (DZRJ 1146); unnamed stream, S22°55'31", W44°37'31", 318 m, 09.viii.2003, 3 nymphs (DZRJ 428); same data, 2 nymphs (DZRJ 433); same locality and collector, 07.viii.2004, 24 nymphs (DZRJ 1159); same data, 21 nymphs (DZRJ 1168); Córrego do Forno, S22°55'34", W44°37'25", 318 m, 07.viii.2004, Oliveira *leg.*, 6 nymphs (DZRJ 1169); same data, 4 nymphs (DZRJ 1183); tributary of Rio Mambucaba, S22°54'41", W44°37'52", 586 m, 07.viii.2004, Oliveira *leg.*, 1 nymph (DZRJ 1212); Córrego Itapetininga, S22°54'44", W44°33'12", 586 m, 01.ix.2004, Oliveira *leg.*, 4 nymphs (DZRJ 1252); Córrego da Memória (frontier of the Rio de Janeiro and São Paulo states), S22°54'17", W44°37'44", 720 m, 09.viii.2003, Oliveira *leg.*, 22 nymphs (DZRJ 412); same data, 7 nymphs (DZRJ 417); same locality and collector, 07.viii.2004, 11 nymphs (DZRJ 1124); same data, 122 nymphs (DZRJ 1130); same data, 5 nymphs (DZRJ 1205). SÃO PAULO, São José do Barreiro, Parque Nacional da Serra da Bocaina, Córrego das Posses, S22°46'7", W44°36'36", 1,270 m, 17.iii.2003, Oliveira *leg.*, 5 nymphs (DZRJ 9); same data, 1 nymph (DZRJ 21); same locality and collector, 07.viii.2003, 6 nymphs (DZRJ 382); same data, 4 nymphs (DZRJ 384); same locality and collector, 05.viii.2004, 1 nymph (DZRJ 962); same locality, 11.xii.2012, 3 subimagoes (DZRJ); Ribeirão da Prata, S22°46'49", W44°36'40", 2 nymphs (DZRJ); same locality, 01.ix.2012, 3 nymphs (DZRJ); same locality, 19.xii.2010, 1 subimago (DZRJ); same data, 9 subimago (DZRJ); same locality, 18.xi.2012, 1 nymph (DZRJ); same locality, 07.viii.2003, Oliveira *leg.*, 122 nymphs (DZRJ 389); same data, 6 nymphs (DZRJ 465); same locality and collector, 5.viii.2004, 51 nymphs (DZRJ 985); Ribeirão do Boqueirão, S22°45'17", W44°37'6", 1,364 m, 23.ix.2006, Oliveira *leg.*, 1 nymph (DZRJ); same locality, 05.x.2007, Baptista, Mugnai, Nessimian & Oliveira *leg.*, 3 nymphs (DZRJ 1529); tributary of Rio Mambucaba, S22°43'47", W44°37'5", 1,550 m, 17.iii.2003, Oliveira *leg.*, 2 nymphs (DZRJ 37); same locality and collector, 06.viii.2003, 2 nymphs (DZRJ 364); same data, 3 nymphs (DZRJ 463); same locality and collector, 16 nymphs (DZRJ 921); tributary of Rio Mambucaba, S22°44'6", W44°36'58", 1,520 m, 7.viii.2003, Oliveira *leg.*, 12 nymphs (DZRJ 371); same data, 2 nymphs (DZRJ 372); same data, 9 nymphs (DZRJ 374); same data, 1 nymph (DZRJ 377); same data, 1 nymph (DZRJ 747); same locality, 21.iv.2006, 5 nymphs (DZRJ); Fazenda Barreirinha, tributary of Rio Mambucaba, S22°49'23", W44°35'52", 1,200 m, 7.viii.2003, Oliveira *leg.*, 2 nymphs (DZRJ 394); same locality and collector, 05.viii.2004, 1 nymph (DZRJ 1017); same data, 5 nymphs (DZRJ 1027); same data, 2 nymphs (DZRJ 1030); same data, 1 nymph (DZRJ 1035); same locality and collector, 5.viii.2004, 1 nymph (DZRJ 112); Rio Mambucaba, 05.x.2007, Baptista, Mugnai, Nessimian & Oliveira *leg.*, 4 nymphs (DZRJ 1546); Córrego Barra Branca, S22°51'10", W44°36'7", 1,040 m, 07.viii.2003, Oliveira *leg.*, 1 nymph (DZRJ 397); same data, 2 nymphs (DZRJ 464); same locality and collector,

5.viii.2004, 1 nymph (DZRJ 1046); same data, 4 nymphs (DZRJ 1049); Córrego do Moinho, S22°51'19", W44°36'58", 940 m, 08.viii.2003, Oliveira *leg.*, 15 nymph (DZRJ 402); same data, 1 nymph (DZRJ 468); same locality and collector, 06.viii.2004, 2 nymphs (DZRJ 1064); same data, 2 nymph (DZRJ 1069); same data, 1 nymph (DZRJ 1073); same data, 8 nymphs (DZRJ 1077); same data, 3 nymphs (DZRJ 1080); Córrego São Gonçalo, S22°52'29", W44°36'6", 920 m, Oliveira *leg.*, 1 nymph (DZRJ 474); same data, 5 nymphs (DZRJ 475); same data, 1 nymph (DZRJ 796); same locality and collector, 06.viii.2004, 26 nymphs (DZRJ 1094); same data, 37 nymphs (DZRJ 1103); Ubatuba, Parque Estadual da Serra do Mar, Km 2, BR101, 300 m from Cachoeira da Escada, S23°21'14", W44°46'4", 233m, 9.ix.2011, Souto *leg.*, 4 subimagoes (DZRJ); same locality and collector, 08.ix.2011, 10 subimagoes (DZRJ); same data, 2 subimagoes (DZRJ); same locality and collector, 04.vi.2011, Oliveira, Takiya, Nessimian & Souto *leg.*, 5 subimagoes (DZRJ); Poço do Amor, Km 2, BR101, 146 m, 2 subimagoes (DZRJ); Cunha, Parque Estadual da Serra do Mar, 24.vii.2012, 3 nymphs (DZRJ 3193); same data, 6 nymphs (DZRJ 3194); same data, 1 nymph (DZRJ 3195); Santa Virgínia, Parque Estadual da Serra do Mar, 28.vii.2012, 6 nymphs (DZRJ 3198); same data, 1 nymph (DZRJ 3200); same locality, 30.vii.2012, 1 nymph (DZRJ 3199); Caraguatatuba, Parque Estadual da Serra do Mar, 04.viii.2012, 3 nymphs (DZRJ 3196); same data, 4 nymphs (DZRJ 3197); São Miguel Arcanjo, Serra de Paranapiacaba, Parque Estadual Carlos Botelho, 16.vii.2013, 5 nymphs (DZRJ 3181); same data, 1 nymph (DZRJ 3184); same data, 3 nymphs (DZRJ 3186); same data, 3 nymphs (DZRJ 3187); same locality, 15.vii.2013, 8 nymphs (DZRJ 3182); same data, 1 nymph (DZRJ 3183); same data, 1 nymph (DZRJ 3185); Campos do Jordão, Parque Estadual do Campos do Jordão, 04.vii.2013, 6 nymphs (DZRJ 3188); same locality, 05.vii.2013, 1 nymph (DZRJ 3189); same locality, 06.vii.2013, 1 nymph (DZRJ 3190); same data, 1 nymph (DZRJ 3191); same locality, 14–16.xii.1987, 2 imagoes (MZUSP); 16.xii.1987, 7 nymphs (MZUSP); Córrego Galharada, 25.ix.1997, 33 nymphs (MZUSP); same locality, 15.x.1998, 1 subimago (MZUSP); Parque Estadual de Intervales, 06.vii.2012, 1 nymph (DZRJ 3192).

Supplementary Table S: Pairwise divergence between COI nucleotide sequences of *Leptohyphodes inanis* and related species using the Kimura 2-parameter model.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
1 <i>Macunahyphes eduardoi</i> (ENT2863)																						
2 <i>Macunahyphes eduardoi</i> (ENT2872)	0.18																					
3 <i>Tricorythopsis chiriguano</i> (ENT1837)	0.31	0.30																				
4 <i>Tricorythopsis gibbus</i> (ENT2239)	0.33	0.32	0.24																			
5 <i>Tricorythopsis spongicola</i> (ENT1722)	0.35	0.33	0.29	0.26																		
6 <i>Leptohyphodes inanis</i> Bocaina (ENT2883)	0.35	0.33	0.36	0.32	0.36																	
7 <i>Leptohyphodes inanis</i> Campos do Jordão (ENT2993)	0.33	0.29	0.31	0.26	0.32	0.23																
8 <i>Leptohyphodes inanis</i> Canastra (ENT2248)	0.33	0.37	0.36	0.35	0.36	0.27	0.31															
9 <i>Leptohyphodes inanis</i> Caparaó (ENT2249)	0.33	0.29	0.29	0.27	0.31	0.24	0.11	0.28														
10 <i>Leptohyphodes inanis</i> Caparaó (ENT2568)	0.33	0.29	0.29	0.27	0.31	0.24	0.11	0.28	0.00													
11 <i>Leptohyphodes inanis</i> Caparaó (ENT2569)	0.33	0.29	0.29	0.27	0.31	0.24	0.11	0.28	0.00	0.00												
12 <i>Leptohyphodes inanis</i> Intendente (ENT1724)	0.37	0.34	0.36	0.34	0.34	0.22	0.25	0.20	0.23	0.23	0.23											
13 <i>Leptohyphodes inanis</i> Intendente (ENT2063)	0.37	0.34	0.36	0.34	0.34	0.22	0.25	0.20	0.23	0.23	0.23	0.00										
14 <i>Leptohyphodes inanis</i> Itatiaia (ENT2546)	0.33	0.29	0.32	0.28	0.32	0.23	0.01	0.31	0.13	0.13	0.13	0.26	0.26									
15 <i>Leptohyphodes inanis</i> Itatiaia (ENT2558)	0.36	0.31	0.30	0.28	0.35	0.23	0.13	0.27	0.13	0.13	0.13	0.22	0.22	0.13								
16 <i>Leptohyphodes inanis</i> Itatiaia (ENT2559)	0.36	0.31	0.30	0.28	0.35	0.23	0.13	0.27	0.13	0.13	0.13	0.22	0.22	0.13	0.00							
17 <i>Leptohyphodes inanis</i> Parnaso (ENT2543)	0.32	0.34	0.34	0.35	0.37	0.03	0.25	0.25	0.26	0.26	0.26	0.22	0.22	0.25	0.25	0.25						
18 <i>Leptohyphodes inanis</i> Parnaso (ENT2545)	0.32	0.34	0.34	0.35	0.37	0.03	0.25	0.25	0.26	0.26	0.26	0.22	0.22	0.25	0.25	0.25	0.00					
19 <i>Leptohyphodes inanis</i> Parnaso (ENT2554)	0.32	0.34	0.34	0.36	0.38	0.04	0.25	0.24	0.26	0.26	0.26	0.22	0.22	0.25	0.25	0.25	0.00	0.00				
20 <i>Leptohyphodes inanis</i> Santa Virgínia (ENT2998)	0.38	0.29	0.32	0.30	0.29	0.24	0.11	0.28	0.09	0.09	0.09	0.22	0.22	0.12	0.13	0.13	0.26	0.26	0.26			
21 <i>Leptohyphodes inanis</i> Ubatuba (ENT1737)	0.35	0.34	0.36	0.28	0.32	0.04	0.23	0.25	0.24	0.24	0.24	0.23	0.23	0.23	0.25	0.25	0.07	0.07	0.07	0.07	0.24	
22 <i>Leptohyphodes inanis</i> Ubatuba (ENT1739)	0.35	0.34	0.36	0.28	0.32	0.04	0.23	0.25	0.24	0.24	0.24	0.23	0.23	0.23	0.25	0.25	0.07	0.07	0.07	0.07	0.24	0.00

Phylogeny and Biogeography of Leptohyphidae (Ephemeroptera: Pannota).

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Systematic Entomology

Em preparação.

Phylogeny and biogeography of Leptohiphidae (Ephemeroptera: Pannota).

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ABSTRACT

Leptohiphidae is a Pan-American mayfly family with 163 species divided into 15 genera. It is one of the most representative groups of mayflies in Neotropical rivers, after Baetidae and Leptophlebiidae. However, relationships among lineages of this family remain poorly resolved and its biogeographic history remain largely unexplored. Historically, two subfamilies were proposed, Leptohiphinae and Tricorythodinae, but their monophyly was never recognized. Herein, based on 3,532 characters (141 morphological and 3,391 molecular - COI, 12S, 16S, 18S, and 28S) we performed phylogenetic and biogeographical analyses for the family. Five major groups were recovered in the parsimony analysis, while the mixed-model Bayesian analysis recovered six major groups. Subfamilies Leptohiphinae and Tricorythodinae, as defined by Wiersema & McCafferty (2000), were not recovered as monophyletic in the present study. Different datasets and phylogenetic methods have suggested different sister groups for Leptohiphidae, such as Teloganodidae, Ephemerythidae and Melanemerella + Coryphoridae + Teloganodidae. Finally, based on S-DIVA and divergence time estimation analyses, ancestral Leptohiphidae was found restricted to the Chacoan Subregion in South America after a vicariant event of the Gondwana continent, around 151.9 Mya (120.0 - 184.4) .

Key-words: combined analysis, Neotropics, bayesian analysis, parsimony, mayfly

INTRODUCTION

Leptohiphidae is a Pan-American mayfly (Ephemeroptera) family with 163 species divided into 15 genera: *Ableptemetes* Wiersema & McCafferty, 2004; *Allenhyphes* Hofmann & Sartori (in Hofmann & Sartori & Thomas), 1999; *Amanahyphes* Salles & Molineri, 2006; *Cabecar* Baumgardner & Ávila, 2006; *Haplohyphes* Allen, 1966; *Leptohiphes* Eaton, 1954; *Leptohiphodes* Ulmer, 1920; *Lumahyphes* Molineri, (in Molineri & Zuñiga), 2004; *Loricypbes* Molineri & Mariano, 2015; *Macunahyphes* Dias, Salles & Molineri, 2005; *Traverhyphes* Molineri, 2001; *Tricorythodes* Ulmer, (1919)1920; *Tricorythopsis* Traver, 1958; *Vacupernius* Wiersema & McCafferty, 2000; and *Yaurina* Molineri, 2001. All genera have been reported from South America, except *Ableptemetes* recorded from Mexico and Central America (Domínguez *et al.*, 2006). The distribution of *Allenhyphes*, *Tricorythodes*, *Leptohiphes*, and *Vacupernius* extends into North America (Domínguez *et al.*, 2006). Although occurring in the Nearctic Region, the clade is assumed to have arisen in the Neotropics (McCafferty *et al.*, 1992; McCafferty, 1998), where the greatest genus- and species-level diversity occurs. It is one of the most representative groups of mayflies in Neotropical rivers, after Baetidae and Leptophlebiidae (Salles, 2006).

Leptohiphidae belong to the monophyletic Pannota (Ogden & Whiting 2005, Ogden *et al.*, 2009), an infraorder distinguished by nymphs that have more than half length of forewing pads fused (McCafferty & Edmunds, 1979). Although highly fused, the pro- and mesothoracic wings remain externally recognizable through the wing pads. Also, pannote nymphs share the presence of highly modified, usually dorsal abdominal gills with overlapping lamellae, often opercular (McCafferty & Wang, 2000), which increases the toleration of suspended solids (Domínguez *et al.*, 2006). Historically, Pannota was divided into two superfamilies: Caenoidea, composed by the Holarctic and Oriental Neoephemeridae and the Pan-american Caenidae; and Ephemerelloidea, composed by Austramerellidae (Nearctic and Oriental), Coryphoridae (Neotropical), Ephemerellidae (Nearctic, Palearctic, and Oriental), Ephemerythidae (Afrotropical), Leptohiphidae (Nearctic and Neotropical), Machadorythidae (Afrotropical), Melanemerellidae (Neotropical), Teloganodidae (Afrotropical and Oriental), and Tricorythidae (Afrotropical) (Ogden & Whiting 2005, Ogden *et al.*, 2009).

The composition and internal relationships of Neotropical Ephemerelloidea have been a long-standing debate in the literature. Domínguez, Hubbard & Peters (1992) placed *Coryphorus* Peters, 1981 within Leptohiphidae, a position also recovered by Wiersema & McCafferty (2000). Although the latter did not include *Coryphorus* nymphs in their sampling, they maintained the genus within Leptohiphidae,

and suggested Tricorythidae, an Afrotropical family, as the sister group of Leptohiphidae. Posteriorly, the adult stage of *Coryphorus* was described by Molineri, Peters & Zuñiga (2002), in the same paper where they established the family Coryphoridae. Several subsequent cladistic analyses recovered the hypothesis that the latter family would be the sister group of Leptohiphidae (e.g. Molineri & Domínguez, 2003; Jacobus & McCarfferty 2006; Molineri, 2006; Baumgardner, 2008). Later on, Tricorythidae was recovered as the sister group of Coryphoridae + Leptohiphidae (Molineri & Domínguez, 2003; Jacobus & McCarfferty 2006; Baumgardner, 2008) or Tricorythidae + Machadorythidae (Molineri, 2006), all of which supposedly originated in Africa. Coryphoridae is a monotypic family restricted to the Amazon basin, recorded from Brazil, Colombia, French Guiana, and Venezuela, while, in contrast, other families proposed as close to Leptohiphidae are restricted to the African continent. The close relationship of Leptohiphidae with African families, whether it is sister to Coryphoridae or not, raises the interesting possibility of its origin to be related to the gondwana breakup (Sanmartín & Ronquist, 2004).

The most recent study focused on the higher level phylogenetic relationships within Ephemeroptera, using both morphological and molecular data, found a different result (Ogden *et al.* 2009). According to these authors, Coryphoridae would be more related to African families: Machadorythidae and Ephemerythidae in morphological-only; or Machadorythidae, Teloganodidae, and Tricorythidae in combined matrices. However, this study included a low sampling of Pannotan families, which limited the recognition of phylogenetic relationships within the infraorder. Moreover, they did not obtain DNA sequences from Coryphoridae or discussed its phylogenetic affinities to Pannota. Phylogenetic relationship between Leptohiphidae and other Pannotan families is essential to understand the evolution in space and time of not only the family, but of Ephemeroptera as a whole.

Originally, Leptohiphidae was proposed as a subfamily (Leptohiphinae) of Tricorythidae (Edmunds & Traver, 1954), but ranked as family by Landa (1973) after comparative anatomical studies of internal organs of several Ephemeroptera families. Edmunds & Traver (1954) regarded the following genera as part of the Leptohiphinae: *Bruchella* Navas 1920 (= *Leptohiphes*), *Leptohiphes* Eaton, 1882, *Leptohiphodes* Ulmer, 1919, *Tricorythafer* Lestage 1942 (= *Tricorythodes*), and *Tricorythodes* Ulmer, 1920. Landa (1973) recognized two subfamilies in Leptohiphidae: Leptohiphinae (with the species previously recognized as part of *Tricorythodes* and *Leptohiphes*) and Dicercomyzinae (with the African genus *Dicercomyzon* Demoulin 1954). Peters and Peters (1993) noted many morphological similarities between Dicercomyzinae and Tricorythidae, especially in wing morphology, and moved the former to the latter.

It must be noted that the only African record for Leptohiphidae is a dubious record of the species *Tricorythodes fugitans* Needham, 1920, which was never again collected after its description in 1958 based on a specimen from Tanzania (Gillies pers. comm. Peters & Peters, 1993). Such record may be the result of a temporary introduction of *Tricorythodes* in Africa with subsequent extinction or a contamination in the collection (Peters & Peters, 1993). Thus, what was originally known as Tricorythidae was divided into two families: Leptohiphidae, strictly Pan-American; and Tricorythidae, restricted to the African continent (McCafferty & Wang, 2000).

Regarding the relationships within Leptohiphidae, Wiersema & McCafferty (2000) divided the family in two subfamilies: Leptohiphinae (*Allenhyphes* Hofmann & Sartori in Hofmann & Sartori & Thomas, 1999; *Cotopaxi* Mayo, 1968; *Haplohyphes* Allen, 1966; *Leptohiphodes* and *Vacupernius* Wiersema & McCafferty, 2000) and Tricorythodinae (*Asioplax* Wiersema & McCafferty, 2000; *Coryphorus* Molineri & Peters & Zuñiga-de-Cardoso, 2001; *Epiphrales* Wiersema & McCafferty, 2000; *Homoleptohiphodes* Allen & Murvosh, 1987; *Tricorythopsis* Traver, 1958; *Tricorythodes*, and *Tricoryhyphes* Allen & Murvosh, 1987). These two subfamilies were not corroborated by Molineri (2006) and Baumgardner (2008), with a morphological-only dataset using parsimony, nor by Ogden & Whiting (2005), with a molecular-only dataset using parsimony and maximum likelihood. Nevertheless, none of these studies proposed an alternative classification because: (i) analyses included South American genera only (Molineri, 2006); (ii) did not recover well-supported clades within Leptohiphidae (Baumgardner, 2008); or (iii) had few genus-level representatives (Ogden & Whiting, 2005).

Of all Leptohiphidae genera, *Tricorythodes* Ulmer, 1920 is the most diversified and widely distributed (Souto *et al.*, 2017). To date, the genus is represented by approximately 70 species distributed from Canada to Uruguay, including the Greater and Lesser Antilles (Sartori & Britain, 2015; Molineri, 2002; Kluge & Naranjo, 1990; Hoffman *et al.*, 1999; Alba-Tercedor & Flannagan, 1995; Naranjo & Peters, 2017). Not coincidentally, it is the most controversial genus with different classification proposals and synonyms (Allen & Murvosh, 1987; McCafferty & Wang, 2000; Molineri, 2002; Domínguez *et al.*, 2006). Allen and Murvosh (1987) proposed three subgenera of *Tricorythodes*: *Tricorythodes* Ulmer, *Tricoryhyphes* Allen & Murvosh and *Homoleptohiphodes* Allen & Murvosh. These groups were elevated to generic level by Wiersema & McCafferty (2000), also proposing two new genera: *Asioplax* and *Epiphrales*, thus subdividing the genus *Tricorythodes* in five genera. Later, Molineri (2002) did not recover *Asioplax* and *Epiphrales* as natural groups and Domínguez *et al.* (2006) synonymized *Epiphrales*, *Homoleptohiphodes* and *Tricoryhyphes* with *Tricorythodes*.

In this study, we propose the first phylogenetic hypotheses among genera of Leptohiphidae based on both morphological and molecular data. In addition, for the first time for a pannotan family, the phylogenetic inference was associated with estimation of divergence times and biogeographical methods to reconstruct their evolutionary history.

MATERIAL AND METHODS

Taxon sampling

Even though the inclusion of Leptohiphidae within Pannota is non-controversial (e.g. McCafferty & Wang 2000; Ogden & Whiting 2005; Ogden *et al.* 2009), its sister-group is controversial (e.g., Molineri & Domínguez 2003; Jacobus & McCafferty 2006; Molineri 2006; Ogden *et al.* 2009; see above). Therefore, we included as many pannotan families as possible. The only taxa not included in both molecular and morphological datasets were Austramerellidae, for which representatives are difficult to obtain. Ephemerythidae and Machadorythidae were included only in the morphological dataset. Moreover, we also included representatives of other mayfly families, such as, Leptophlebiidae and Oligoneuriidae for rooting. Ingroup sampling included representatives of all Leptohiphidae genera, preferably type-species, and with as many species as possible, in order to represent the morphological variability within each genus. The genus *Loricypbes* was only recently described and could not be included in the molecular dataset. Our final combined dataset included 121 terminals (Table I), of which 105 were leptohiphid species, representing 100% of currently recognized genera and 63% of species. Finally, within the family there is great debate about its generic classification (e.g. McCafferty & Wang, 2000; Molineri, 2002, 2006). For example, several genera, previously attributed to *Tricorythodes* or described in different genera, were considered by some authors as "*Tricorythodes* l. s." (*Ableptemetes* Wiersema & McCafferty 2004, *Asioplax* Wiersema & McCafferty 2000, *Cabecar* Baumgardner & Ávila 2006, *Epiphraodes* Wiersema & McCafferty 2000, *Homoleptohiphes* Allen & Murvosh 1987, *Tricorythodes* s. s.) and was represented by at least one representative of each group in the phylogeny presented here (the only exception is *Ableptemetes*).

Specimens analysed are deposited in the following institutions: Coleção Entomológica Prof. José Alfredo Pinheiro Dutra, UFRJ, Rio de Janeiro, Brazil (DZRJ); Invertebrate Collection of the Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil (INPA); Invertebrate Collection of the Museu de

Zoologia da Universidade de São Paulo , São Paulo, Brazil (MZSP); Coleção Zoológica Norte Capixaba, UFES, São Mateus, Brazil (CZNC); Florida Agricultural & Mechanical University, Tallahassee, United States of America (FAMU); Colección Entomológica del Programa de Biología de la Universidad de Caldas, Manizales, Colômbia (CEBUC); Instituto de Biodiversidad Neotropical, San Miguel de Tucumán, Argentina (IBN); and Albany Museum, Grahamstown, South Africa (AMGS). Genomic DNA vouchers and specimen vouchers were deposited at DZRJ. The material examined is preserved in 93% ethanol; wings, genitalia, opercular gill, legs and mouthparts were mounted on permanent slides using Euparal®.

Morphological coding

As much as possible, species were coded for morphological characters based on direct study of representative specimens. Additional information was obtained from the literature. We are aware of the drawbacks imposed by this methodology (*i.e.*, confidence in drawings and descriptions of a second party), but we preferred to include such information over ending up with a larger amount of missing data in the dataset. Characters were scored from the external morphology of nymphs, adults (both male and female), and eggs. A total of 141 morphological characters (61 from adults, 66 from nymphs, and 14 from eggs) from the dataset of Molineri (2006) morphological matrix were used, with some modifications and additions of characters and / or character states and species. Morphological terminology followed Molineri (2006).

Primary homologies were proposed by similarity criterion and topological correspondence between structures (de Pinna, 1991). Inapplicable or missing characters were coded as "?". The data matrix was constructed using the software Winclada (Nixon 1999–2002) (Appendix 1).

DNA sequences

Genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following a modified protocol from the manufacturer's instructions of imagos and/or nymphs. Because of the body size of most specimens, most DNA extractions were made using the entire specimen. In groups with larger body size (e.g. Melanemerellidae), the extraction was done using the thoracic legs. Five genes were targeted for amplification and sequencing: 18S rDNA, 28S rDNA (D2–D5 regions), 16S

rDNA, 12S rDNA, and partial sequences of the mitochondrial cytochrome oxidase I (COI) gene (Table II).

Polymerase chain reactions (PCR) were carried out in 25µl final volumes using the Platinum® Taq DNA Polymerase (Invitrogen) kit. Three different PCR conditions were used: 1) For COI, we used denaturation at 94°C for 5min, 35 cycles of sequence amplification (94°C for 45s, 45°C for 45s, 72°C for 45s), and final extension of 72°C for 5min; 2) For 16S and 18S, we used denaturation at 94°C for 3min, 35 cycles of sequence amplification (94°C for 1min, 50°C for 1min, 72°C for 2min), and final extension of 72°C for 7min; 3) For 28S, we used denaturation at 94°C for 5min, 35 cycles of sequence amplification (94°C for 1min, 52°C for 1min30s, 72°C for 1min), and final extension of 72°C for 5 min. Whenever necessary, we added 3.5µl (10mg/mL) of Bovine Serum Albumin (BSA) additive in COI, 16S, and 18S amplifications to optimize the reactions. With the same intention, we added 1.0µl Dimethyl Sulfoxide (DMSO) additive in 28S amplifications.

PCR products were submitted to 1% agarose gel electrophoresis to check for positive amplifications of expected sizes. Amplicons were purified and sequenced by Macrogen (Seoul, Korea). Resulting electropherograms from both DNA strands were aligned, analyzed and adjusted manually to generate consensus sequences for each specimen using Geneious 8.1.7 (Kearse *et al.*, 2012). Sequences were checked with Basic Local Alignment Search Tool (BLAST; Altschul *et al.*, 1997) against the GenBank nucleotide database to ensure that the amplified product was the target and not contaminated. Sequences were translated into amino acids to ensure non-amplification of pseudogenes. Sequences of 12S, 16S, 18S, and 28S of *Allenhyphes flinti* (Allen, 1973) and *Lachlania dominguezi* Pereira, 1989 and 16S, 18S, and 28S of *Yaurina mota* Molineri, 2001 from Ogden & Whiting (2005) were downloaded from GenBank and included in the analysis.

The protein-encoding gene COI was aligned with MUSCLE (Edgar, 2004) under default parameters in Geneious version 9.0.5 (Kearse *et al.*, 2015). The ribosomal genes 16S, 18S, and 28S were aligned using MAFFT (Kato *et al.*, 2009) under the alignment method Q-INS-i, which takes into account secondary structure. All alignments were posteriorly manually refined in Geneious version 9.0.5. The final alignment length of each gene fragment was 455 bp for 12S, 590 bp for 16S, 643 bp for 18S, 1308 bp for 28S and 451 bp for COI. GenBank accession numbers are provided in Table I.

Phylogenetic reconstruction

Both partitions (morphology and DNA sequences) were analyzed together and separately. Datasets were analyzed using Bayesian inference (BI) and Parsimony approaches.

Bayesian Inference (BI). The best-fit evolutionary model for each molecular partition was identified using jModelTest 0.1.1 (Posada, 2008). The Akaike information criterion (AIC) favoured GTR+G for 12S and 28S and GTR+I+G for 16S, 18S, and COI.

BI analyses were performed using MrBayes version 3.2.2 (Ronquist *et al.*, 2012) at the CIPRES portal (Miller *et al.*, 2010). Four independent Metropolis Coupled Markov Chain Monte Carlo (MCMCMC) analyses each with four chains (three heated and one cold) were ran for 20,000,000 generations, sampling trees every 2,000 generations. The initial 25% of sampled trees were discarded at the end of the analyses as burn-in. Convergence among independent analyses was assessed by monitoring the values of standard deviation of split frequencies (<0.05) in MrBayes and parameter mixing was assessed with Tracer version 1.6 (Rambaut *et al.*, 2014) by the effective sample size (ESS) criterion (>200). A 50% majority-rule consensus post-burn-in tree was constructed and values of posterior probabilities (pp) were calculated. Clades with pp greater than 0.95 were referred as strongly supported, 0.80–0.95 as moderately supported, and lower than 0.80 as being poorly supported.

Final trees were previewed at Figtree version 1.4.3 (Rambaut, 2016) and posteriorly edited on Adobe Illustrator CC 2017.

Parsimony. Morphological data alone and total inference were analyzed under parsimony with TNT (Goloboff, Farris & Nixon, 2008) using implied weights ($k = 3.0$). We inactivated all non-informative parsimonious sites before conducting the parsimony analysis, because those that show variation in a single taxon, will add steps to any of the considered trees (Matioli & Fernandes, 2012).

Most parsimonious trees were found using a new technology search with algorithms ratchet, tree drifting, tree fusing, and sectorial searches, with 1,000 random initial replicates, random seed set to 0 (Goloboff *et al.*, 2005). Consistency (CI) and retention (RI) indices were calculated on TNT with *stats* and *wstats* script (Goloboff *et al.*, 2006). A second search was conducted with the trees held in the program memory, allowing subsequent collapse, with collapse = 3 (collapses branch if some

optimization lack support). To assess the confidence of the relationships recovered, the original data matrix was randomly re-sampled with replacement to produce pseudo-replicate datasets, method known as bootstrapping. A set of 500 replications of bootstrap was conducted. Bootstrap (BT) values above 70% were considered as strong support for a group (*cf.*, Holder & Lewis, 2003).

Rooting on Oligoneuriidae (combined dataset) and Leptophlebiidae (morphology-only) was made on Figtree version 1.4.3 (Rambaut, 2016) and the resulting trees were posteriorly edited on Adobe Illustrator CC 2017.

Divergence time estimation

Divergence times were estimated with four independent analyses using the program BEAST version 1.8.3 (Drummond *et al.*, 2012) at the CIPRES portal (Miller *et al.*, 2010). The molecular clock type used was the relaxed uncorrelated lognormal (Drummond *et al.*, 2006) and a tree prior using the birth-death incomplete sampling algorithm (Stadler, 2009). Analyses of the concatenated molecular matrix used a mixed model strategy (partitioned by gene), the same selected for the phylogenetic analyses above. For each analysis, 600 millions of generations were run, with parameters sampled every 60,000th generation, yielding 10,000 trees sampled, with initial 5,000 trees discarded as burning. Convergence and mixing of MCMC chains were checked using Tracer version 1.6 (Rambaut *et al.*, 2014) as described in the phylogenetic reconstruction section. Post-burnin posterior 95% credibility intervals of divergence dates for each node were calculated and plotted on the maximum clade credibility tree using the program TreeAnnotator (Drummond & Rambaut, 2007). Resulting tree files were imported into the program Figtree version 1.4.3 (Rambaut, 2016) to view and save the final time calibrated trees and then edited on Adobe Illustrator CC 2017.

The following nodes were calibrated with age priors using available fossil information: (I) root was calibrated to represent the diversification of Ephemeroptera using a normal distribution with mean 263.5 (SD = 13.5) reaching the maximum age of 290.1 Mya based on age of *Protereisma permianum* Sellards 1907 (Protereismatidae), believed to be one of the stems groups of Ephemeroptera (Grimaldi & Engel, 2005); (II) diversification of Pannota using a lognormal distribution with mean 25.0 Mya (SD = 1.0), based on age of 130 Mya given by Grimaldi & Engel (2005); (III) initial divergence of the family Oligoneuriidae with minimum (237 Mya) and maximum (110 Mya) ages based on ages of *Triassonurus doliiformis* and *Colocrus indicum* (Oligoneuriidae) using lognormal prior with mean of 29.5 (SD =

1.0); (IV) initial divergence of Caenoidea using a lognormal distribution with mean 90.0 (SD = 3.97) based on age of *Neophemeridae antiqua* Sinitshenkovan, 1999 (50-49 mya).

Biogeographic analyses

Event-based methods are increasingly being used in historical biogeographic studies during the past few years, due to the exponential increase in phylogenetic studies (Ferretti *et al.*, 2012). A distinctive characteristic of event-based methods, in contrast to pattern-based methods, is the proposal of explicit models for the processes that affect the geographic distribution of living organisms (Crisci *et al.*, 2003). In this approach, different types of processes (*e.g.*, vicariance, dispersal, and extinction) are identified and assigned values of cost-benefit under an explicit model of natural functioning (Crisci *et al.*, 2003). That is, it is assumed that the different processes occur at dissimilar frequencies in the biogeographic history of organisms, and costs are assigned inversely proportional to their probability of occurrence in the past. Here we use two different approaches of event-based methods: one that assumes predefined areas of distribution; and other, in contrast, which uses the spatial component of taxon distributions.

For the first approach we used two methods: (I) Dispersal vicariance analysis (DIVA) in a Bayesian framework, using the S-DIVA (Statistical Dispersal-Vicariance) method in Reconstruction Ancestral State in Phylogenies (RASP) v.3.2 (Yu *et al.*, 2015) for molecular and combined data; (II) Dispersal Extinction Cladogenesis model (DEC) in a likelihood framework, performed with the BioGeoBEARS package (Matzke, 2013) for R Software v.3.4.3 (R Core Team, 2014) for molecular data only. S-DIVA takes into account phylogenetic uncertainty and DEC model considers branch lengths of a given dated tree. As input for S-DIVA all post-burnin trees from phylogenetic BI analysis (from BEAST) were used, and limited compound areas with a maximum of four areas. As input for the DEC analysis, the maximum clade credibility tree obtained from BEAST divergence time estimation analysis was used. The parameters "range constraints" and "dispersal constraints" were not altered to avoid excessive parameterization.

Concerning the second approach, we used the Vicariance Inference Program (VIP) (Arias *et al.*, 2011) as an optimality criterion in order to discover disjunct (allopatric or vicariant) distributions between sister groups in a phylogenetic context. This method is based on Hovenkamp's ideas on historical biogeography (1997, 2001), which uses observed distributions as data, thus requiring neither

predefined areas nor assumptions of hierarchical relations between areas (Arias *et al.* 2011). The main criticism to methods that assumes predefined areas of distribution is that, in the vast majority of cases there are different degrees of overlapping among the distributions of taxa, and not all terminals show congruence in their distributions. Thus, these methods use algorithms and implementations which allow only very limited numbers of areas, making them of little use for most current phylogenies (DIVA allows up to 16 areas, but only counts up to eight; so far, published studies with DEC use no more than ten areas) (Arias *et al.*, 2011). The input tree for VIP was the parsimony combined dataset result.

Geographical distribution data for each species were taken from different sources: original descriptions, taxonomic works, survey papers, and examined specimens in the present paper. The VIP analysis was performed using a grid of 1.0×1.0 (Von Neumann neighborhood) and a maximum fill of 0, and the barrier was represented by Voronoi lines (De Berg, 2008). The cost of distribution removal was 1.5 and the maximum of distribution overlap was 15%.

For S-DIVA analysis, areas proposed by Morrone (2013) were adopted for taxa distributions as follows: (A) Mexican transition zone; (B) Antillean dominion; (C) Mesoamerican dominion; (D) Northwestern South American dominion; (E) Amazonian Subregion; (F) Chacoan Subregion; (G) Parana Subregion; (H) South American transition zone; and (I) Nearctic region. We assigned the distributional data to species, except for the outgroups that we decided to consider the distribution for the whole family, leading us to more four areas as follows: (J) Afrotropical; (K) Palearctic; (L) Oriental; and (M) Australasian. As our goal was to understand the ancestral distribution of Leptohyphidae, we detailed the distribution of the ingroup rather than of the outgroup. As DEC has a limitation of 10 areas maximum and after consider the S-DIVA result, we had to decrease the total area to only nine of Morrone's areas (2014, A to I).

RESULTS

Phylogenetic relationships

Parsimony analyses

Among the 3,532 characters in the combined dataset, 1,392 were parsimony-informative, 1,253 from DNA sequences, and 139 from the morphology. 10 and 54 most parsimonious trees were found in the analyses of the combined and morphology data matrices, respectively (fit combined dataset =

716.48585; fit morphological only = 664). The strict consensus tree with clade supports of the combined dataset analyses is shown in Fig. 1 (length = 10487; consistency index = 30; retention index = 48). The strict consensus tree of the morphological data alone with clade supports is shown in Fig. 2.

LeptoHyphidae was recovered as a monophyletic group with low support in the combined analyses (BT = 44), as well as in the morphology-only analyses (BT = 52). Phylogenetic relationships within LeptoHyphidae were discordant between the two analyses and the resolution of the relationships within the family improved significantly after the addition of the molecular data. The monophyly of LeptoHyphidae was based on 17 synapomorphies, being the following three non-homoplastic apomorphies: 1) basal part of vein CuP absent [18: 1]; 2) four lamellae on gill III [98: 2]; 3) smaller lamellae of gills along entire margin of main lamella [111: 1], and the following 10 homoplastic ones: 1) male imago fore tarsal claw similar (both blunt) [9: 2]; 2) base of vein ICu1 fused with or clearly directed to the base of CuP [17: 0]; 3) marginal intercalaries absent [22: 0]; 4) shape of hypopharynx linguae sub rectangular [66: 1]; 5) maxillary palp with apical seta [70: 1]; 6) absence of group or transverse row of setae at base of inner margin of maxilla [73:1]; 7) third and second segments of labial palp reduced [79: 1]; 8) presence of basal row of spines/setae on dorsum of femora II and III [92: 0]; 9) ventral lamellae of gills not clearly bifid [95: 1]; 10) eggs with adhesive filaments on eggs [135: 1] (Fig. 1).

In both analyses, Ephemerythidae was recovered as sister group of LeptoHyphidae with low support (BT = 4) (Figs. 1 and 2). Based on the combined analysis, we can highlight five main clades of LeptoHyphidae (Fig. 1). Clade A consisted of all *Tricorythopsis* spp., with strong support (BT = 94), supported by 16 synapomorphies, being the following five non-homoplastic apomorphies: 1) female imago forelegs absent [10: 1]; 2) ventrodistal extension of tibiae slightly marked [11: 1]; 3) MA fork (Median Anterior Vein) absent [19: 1]; 4) IMP (Intercalary Median Posterior Vein) and MP2 fused with CuA, with MP2 appearing as an intercalary [20: 2]; 5) female cerci rudimentary [61: 2]. Clade B was composed of *Haplohyphes*, *LeptoHyphodes*, *Amanahyphes* with low support (BT = 11), supported by one homoplastic apomorphy: nymphs with deep anteromedian emargination of labrum. On the other hand, the group formed by *LeptoHyphodes* and *Amanahyphes* was highly supported (BT = 89) by non-exclusive synapomorphies. Within Clade B, the monophyly of *Haplohyphes* and *Amanahyphes* was recovered with strong support (71 and 99, respectively). Clade C was composed of *Tricorythodes* *l. s.* species, with species of *Tricorythodes*, *Macunahyphes*, *Cabecar* and *Loriccyphes*. In addition, within this clade are species previously considered as part of genera later synonymized with *Tricorythodes*, such as: *T. undatus* [= *Epiphraodes undatus*]; and *T. santarita*, *T. zunigae*, *T. curiosus*, *T. nicholsae*

[*Asioplax*]. This clade was poorly supported (BT = 04) by three non-homoplastic apomorphies: 1) ventrodistal extension of imago tibiae long [11: 2]; 2) presence of a basal swelling on forceps segment two [36: 1]; 3) styliger plate of male genitalia with blunt posteromedial projections [37: 1]. Also, relationships recovered among *Tricorythodes* l. s. species received weak or non-significant support. Clade D was composed of *Allenhyphes*, a monophyletic *Yaurina*, a paraphyletic *Lumahyphes*, a paraphyletic *Traverhyphes* in relation to *Vacupernius*, with low support value (BT = 46) and supported by four non-homoplastic apomorphies: 1) males with very large costal projection of hindwings (0.53 or more) [25: 2]; 2) base of costal projection (basal angle) straight [26: 1]; 3) gonopore associated with a hollow spine [41: 2]; 4) penes spine curved in lateral view [42: 1]. Finally, Clade E was composed by all species of *Leptohyphes* with low support value (BT = 47) and supported by two non-homoplastic apomorphies: 1) angle between penean arms of 45–90° (Y-shaped) [55: 1]; and 2) imagos with dark annuli on caudal filament sexually dimorphic [126: 0].

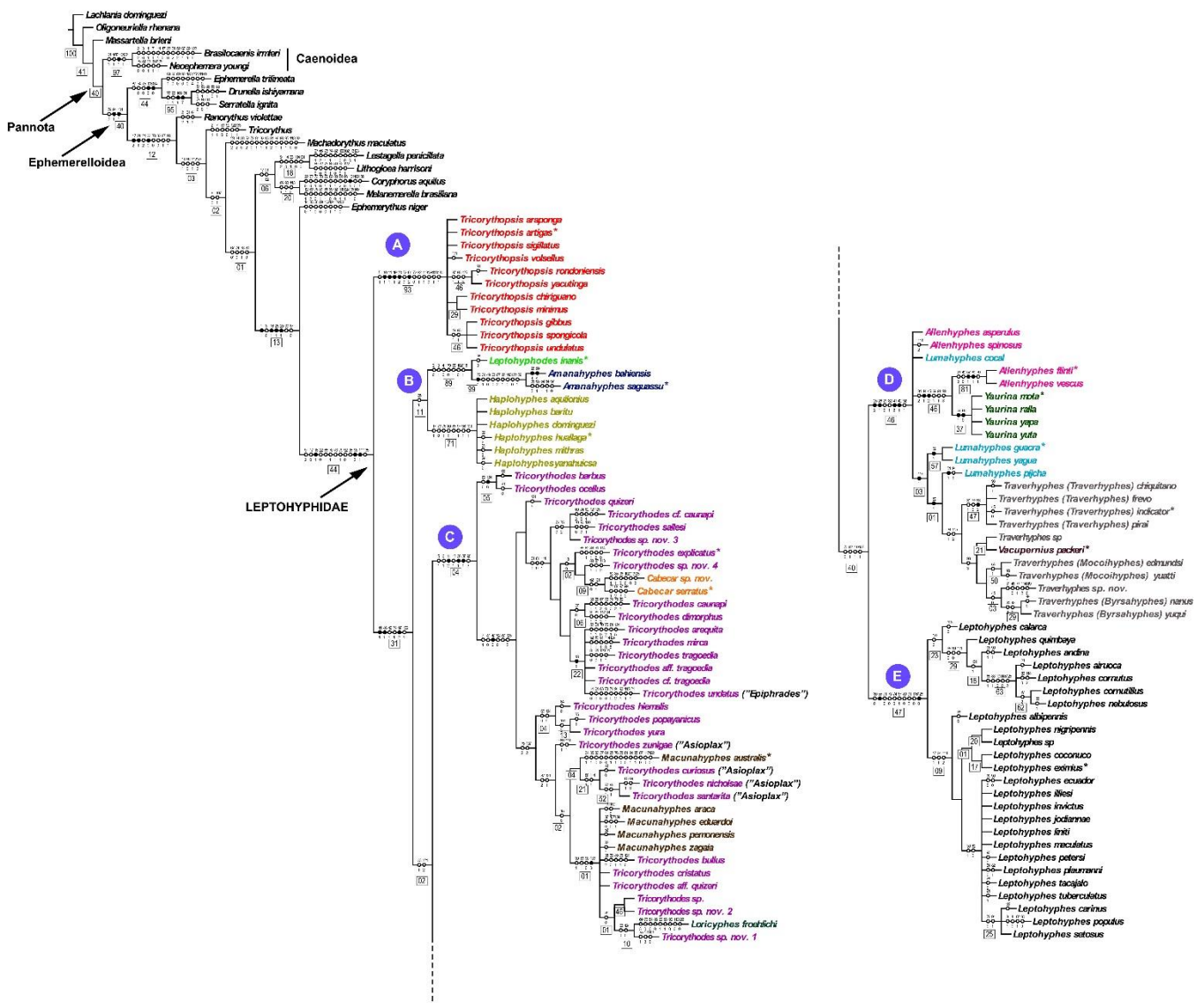


Figure 1. Strict consensus of most parsimonious trees with implied weights ($k = 3$) based on a combined character matrix (12S, 16S, 18S, 28S, COI and morphology) of Leptothyphidae. Bootstrap percentages are boxed below branches. Along branches, black circles are non-homoplastic and white circles are homoplastic apomorphies, with respective character and state numbers. Species marked with an asterisk correspond to the type species. Each color represents a Leptothyphidae genus.

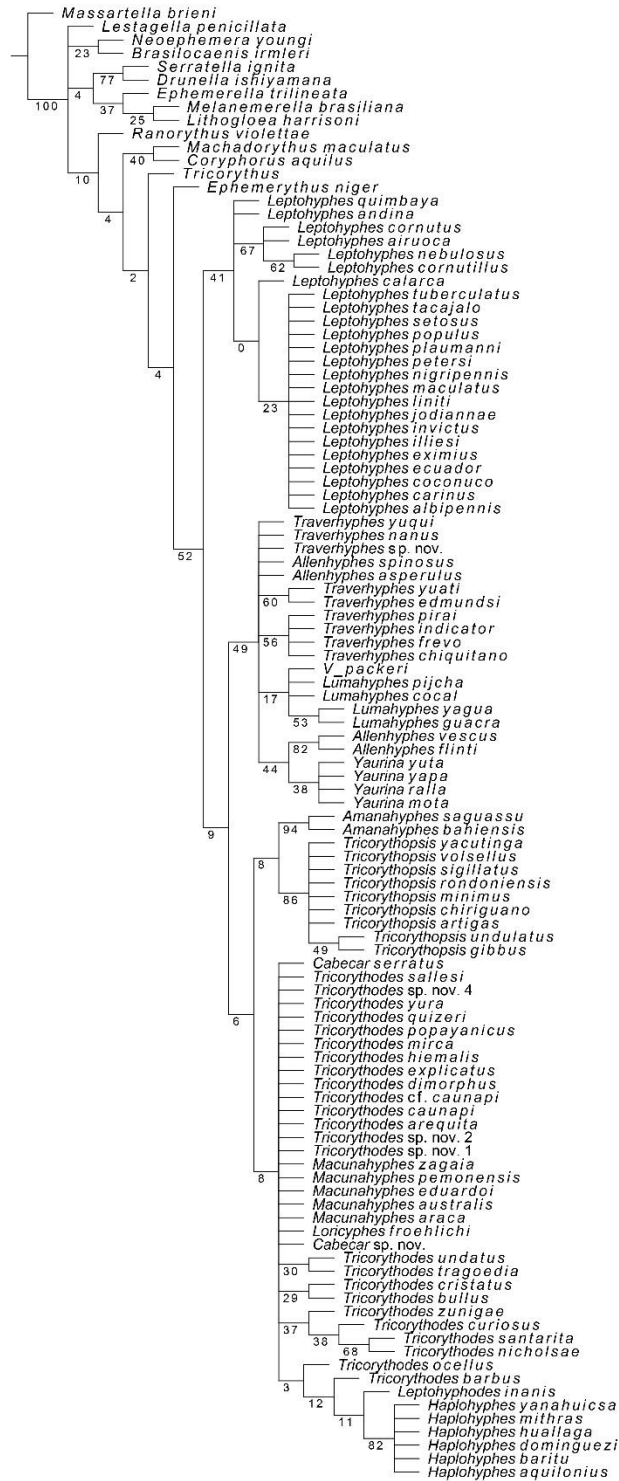


Figure 2. Strict consensus of most parsimonious trees with implied weights (under $k =$ value of 3) based on 141 morphological characters of Leptohiphidae with bootstrap support values

Bayesian analyses

Leptohyphidae was recovered as monophyletic with strong support (pp = 1.0) in the combined dataset analysis (Fig. 3) as well as in the molecular-only analysis (Fig. 4). Both analysis recovered Teloganodidae as sister group of Leptohyphidae with moderate support (0.91 in combined *versus* 0.93 in molecular-only). Considering the combined analysis, Leptohyphidae was divided into six clades (Figs. 3), all of which strongly supported (pp = 1.0). The same clades were found in molecular analysis, but with fewer representatives. Clade A is the same as in the parsimony analysis, composed of all species of *Tricorythopsis*. Unlike the parsimony analysis (Fig. 1, Clade B), *Haplohyphes* (Clade B2) was not recovered as a sister group of *Leptohyphodes* + *Amanahyphes* (Clade B1). Clade C is similar to Clade C in the parsimony analysis, composed of *Tricorythodes lato sensu* species. However, the relationships within this clade varied according to the analysis, for example the BI analysis recovered the monophyly of the clade composed by "*Asioplax*" species within *Tricorythodes* l. s.. Clade D the same as in the parsimony analysis, however the relationships within this clade also varied according to the analysis. Similarly, Clade E is the same as in the parsimony analysis with disagreement in internal relationships.

Divergence time estimation

Divergence time estimates are presented in Fig. 5 and Table 4. We recovered a median age for the origin of Leptohyphidae in the Early Cretaceous at 151.9 Mya (95% HPD: 120.0 - 184.4) when its stem lineage diverged from its sister group. During the Early Cretaceous (around 135.3 Mya, 95% HPD: 107.0 - 165.8) occurred the initial divergence of Leptohyphidae into two main clades - one with all *Tricorythopsis* species and another with all other leptohyphid genera. Within the second clade, the initial divergence into two clades also occurred in the Early Cretaceous, at 121.7 Mya (95% HPD: 93.6 - 148.7). Early splits of this clade occurred by the end of Early Cretaceous up to the transition to the Late Cretaceous: first clade (*Leptohyphodes*, *Amanahyphes* and *Haplohyphes*) at 110.3 Mya (95% HPD: 82.2 - 138.4); the second (*Allenhyphes*, *Yaurina*, *Traverhyphes*, *Lumahyphes*, *Vacupernius*, and *Leptohyphes*) at 101.2 Mya (95% HPD: 77.0 - 126.8); and the third (*Tricorythodes* l. s. species) at 91 Mya (95% HPD: 68.1 - 113.5). The remaining branching events within the family occurred from the Paleocene (Eocene) through the Neogene.

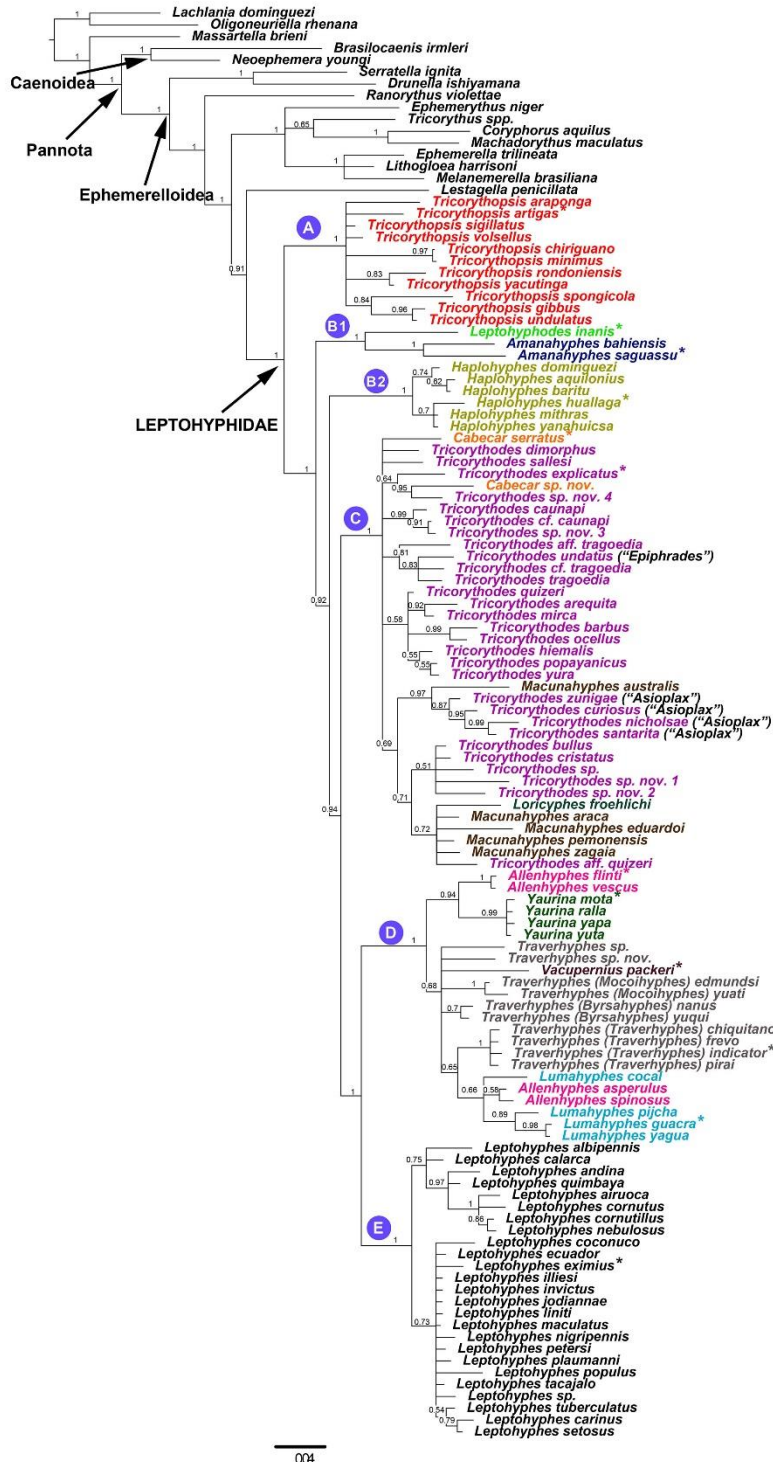


Figure 3. Bayesian consensus of post-burnin trees (average =lnL = -45785.66) resulting from a mixed-model analysis based on a combined character matrix (12S, 16S, 18S, 28S, COI and morphology) of Leptohiphidae. Values above branches are posterior probabilities. Subfamilies are labeled following our proposed classification. Species marked with an asterisk correspond to the type species. Each color represents a Leptohiphidae genus.

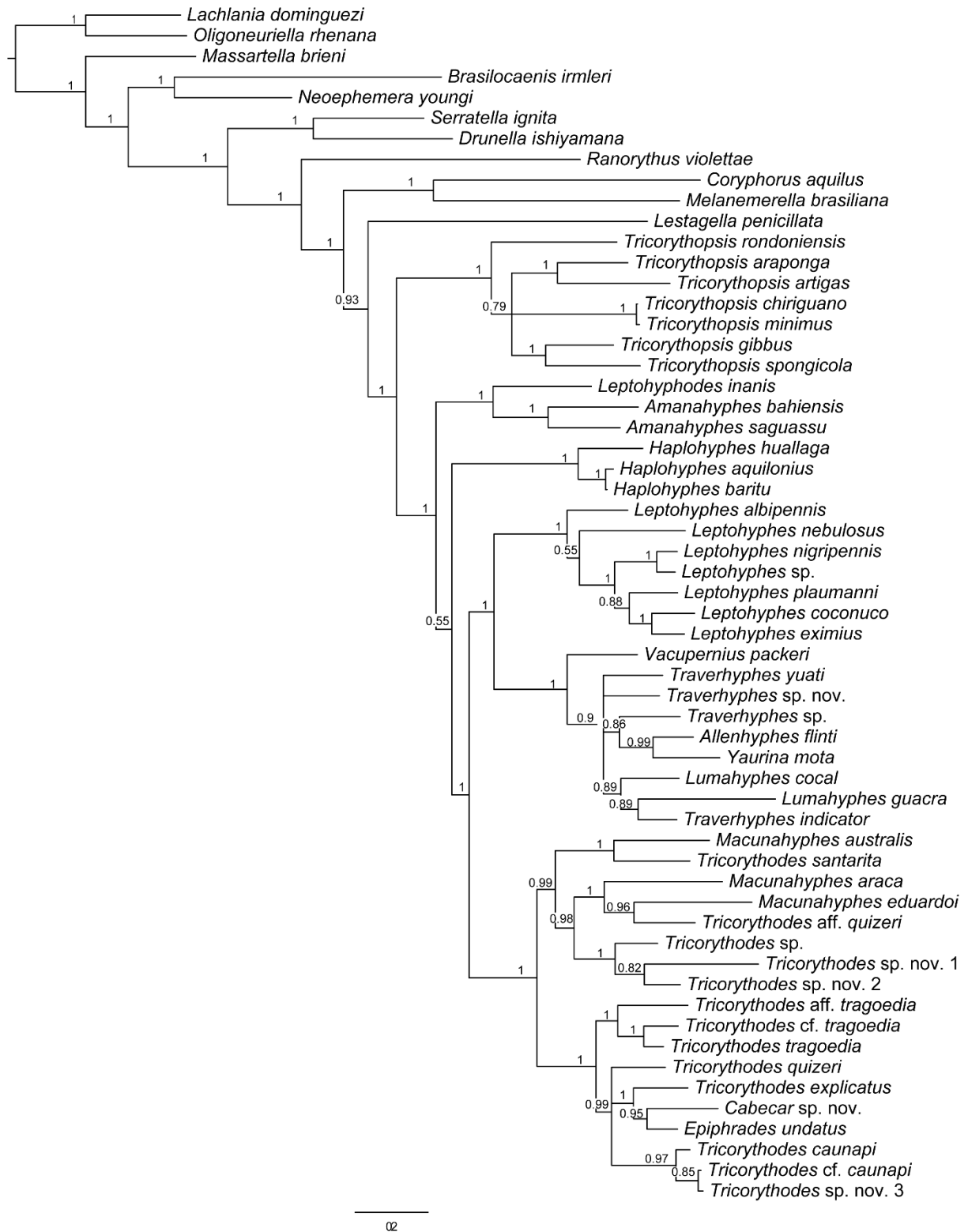


Figure 4. Bayesian consensus of post-burnin trees (average = lnL = -40930.57) resulting from a mixed-model analysis based on a molecular dataset (partitions: 12S, 16S, 18S, 28S, COI) of Leptohiphidae. Numbers above branches indicate posterior probabilities.

Biogeographic analyses

Results of S-DIVA (molecular and combined data) and DEC analyses are summarized in Table 4, Fig. 5, Fig. 6, and Fig. 7. Results of the VIP analysis is shown in Fig. 8. According to S-DIVA analyses, the ancestor of Leptohiphidae was distributed in the Chacoan Subregion (F), with probabilities of 55% and 76% for molecular and combined data respectively. According to S-DIVA molecular-only analysis (Fig. 6), at least three major vicariant events occurred in the family evolution: 1) V1: first split of *Tricorythopsis* with the separation of the Amazonian (E) and Chacoan Subregion (F); 2) V2: first split of the *Tricorythodes tragoedia* group, with the separation of the Amazonian (E) and Parana Subregion (G); and 3) V3: split of a clade containing three unnamed *Tricorythodes* species with the separation of the Amazonian (E) and Parana Subregion (G).

However, with the addition of more species and morphology data, this scenario has changed. According to S-DIVA combined analysis (Fig. 7), at least 12 major vicariant events occurred in the family evolution: 1) V1: first split of *Tricorythopsis* with the separation of the Chacoan Subregion (F) and Parana Subregion (G); 2) V2: first split of *Haplohyphes* with the separation of South American Dominion (D) and Amazonian (E); 3) V3: the split between *Tricorythodes barbuis* and *Tricorythodes ocellus*, with the separation of Amazonian (E) and Parana Subregion (G); 4) V4: the split of *T. sallesi* from sister clade, correlated to the separation of South American Dominion (D) and Chacoan Subregion (F); 5) V5: similar to V2 in the molecular analysis, but with *T. undatus* as part of this clade; 6) V6: split of *Loricophes froehlichii* from sister clade, correlated to the separation of the Amazonian (E) and Parana Subregion (G); 7) V7: split of the ancestor of *Lumahyphes* + *Allenhyphes* restricted to Amazonian (E) from the ancestor of *Traverhyphes* distributed on Parana Subregion (G); 8) V8: split between *Traverhyphes chiquitano* and *Tr. frevo* with the separation of Amazonian (E) and Parana Subregion (G); 9) V9: split between *Traverhyphes nanus* and *Tr. yuqui*, correlated to the separation of South American Dominion (D) and of Amazonian Subregion (E); 10) V10: first split of *Leptohiphys* with the separation of South American Dominion (D) and Amazonian (E); 11) V11: split of *L. andina* from its sister clade with the separation of South American Dominion (D) and Parana Subregion (G); and 12) V12: split of *L. invictus* from its sister clade, with the separation of South American Dominion (D) and Amazonian (E). As DEC has a limitation of 10 areas maximum, we had to consider the S-DIVA result for an ancestral area to Leptohiphidae, leading us to discuss only the results within the family. DEC analysis result (Fig. 6) is similar to S-DIVA morphology-only result, having the same vicariant events.

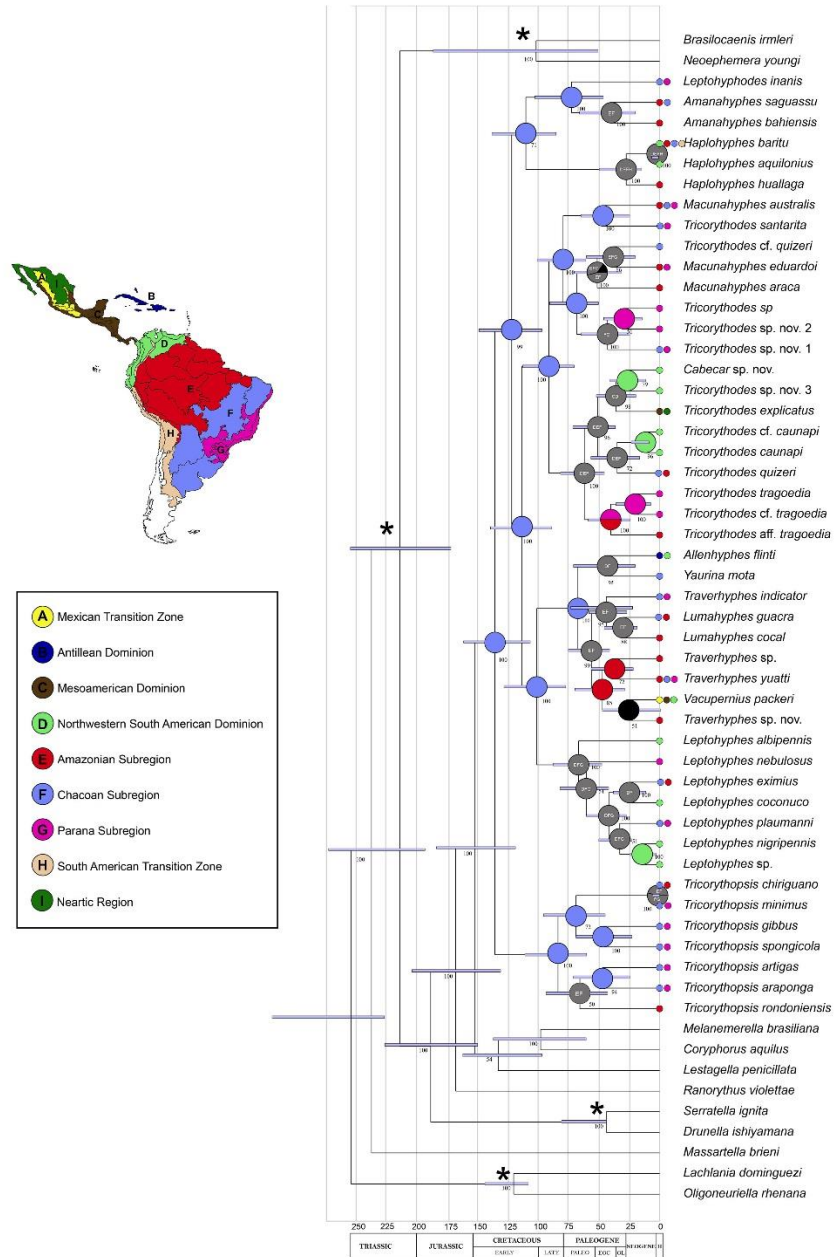


Figure 5. Time-calibrated (millions of years) phylogeny for Leptohiphidae resulting from a mixed-model relaxed uncorrelated lognormal clock analysis of the molecular dataset (partitions: 12S, 16S, 18S, 28S, COI) of Leptohiphidae. At each node, the 95% credible interval of divergence estimates (blue node bars) and results of biogeographical analyses are given. Coloured circles and pie charts at nodes represent geographical distributions, according to Morrone (2013), as showed in the map. Pie charts at nodes represent DEC results: colors correspond to regions, except gray for compound areas and black for numerous ancestral areas with low probabilities. Nodes marked with an asterisk had prior ages calibrated using fossils. Geological time scale used follows Walker *et al.*, 2013.

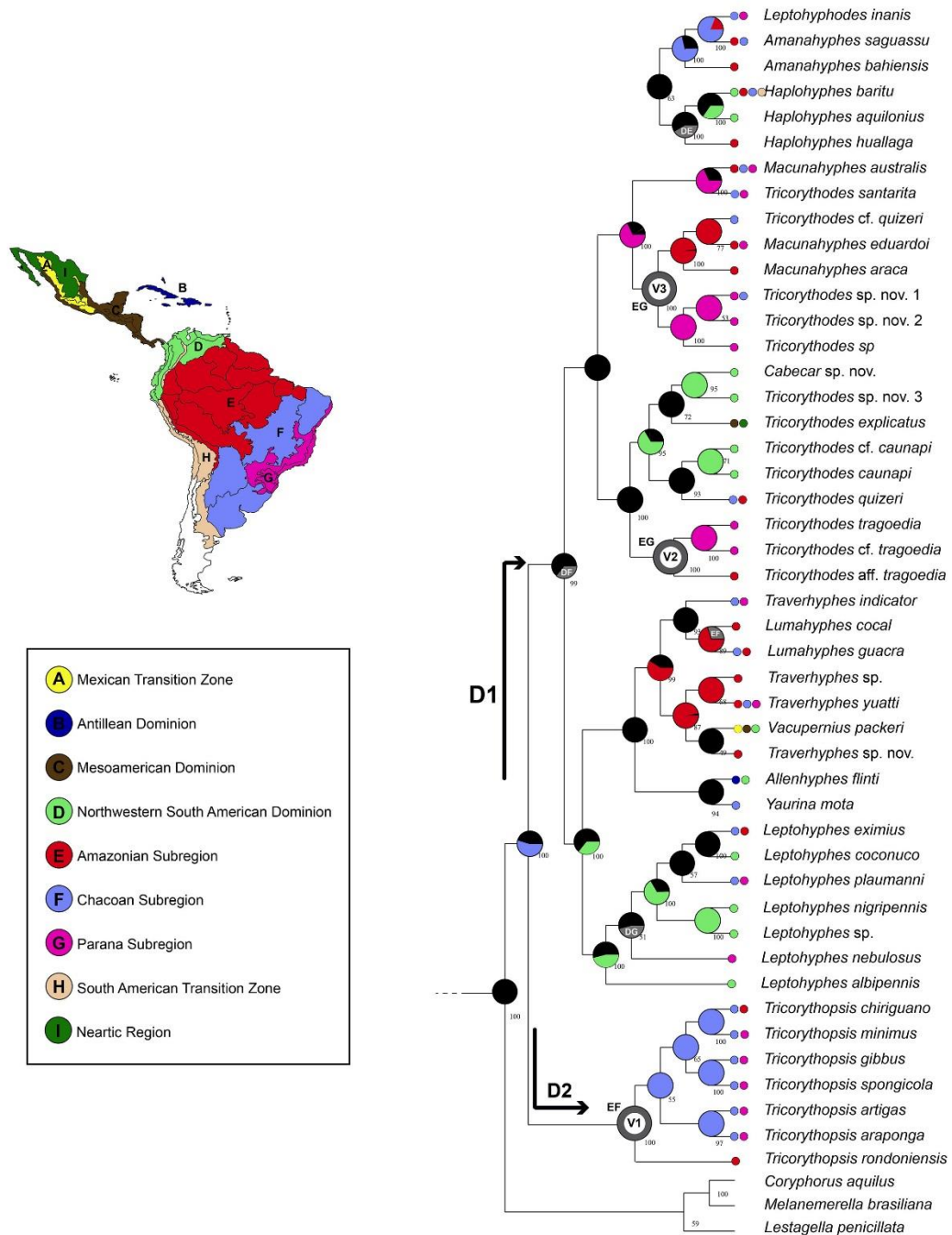
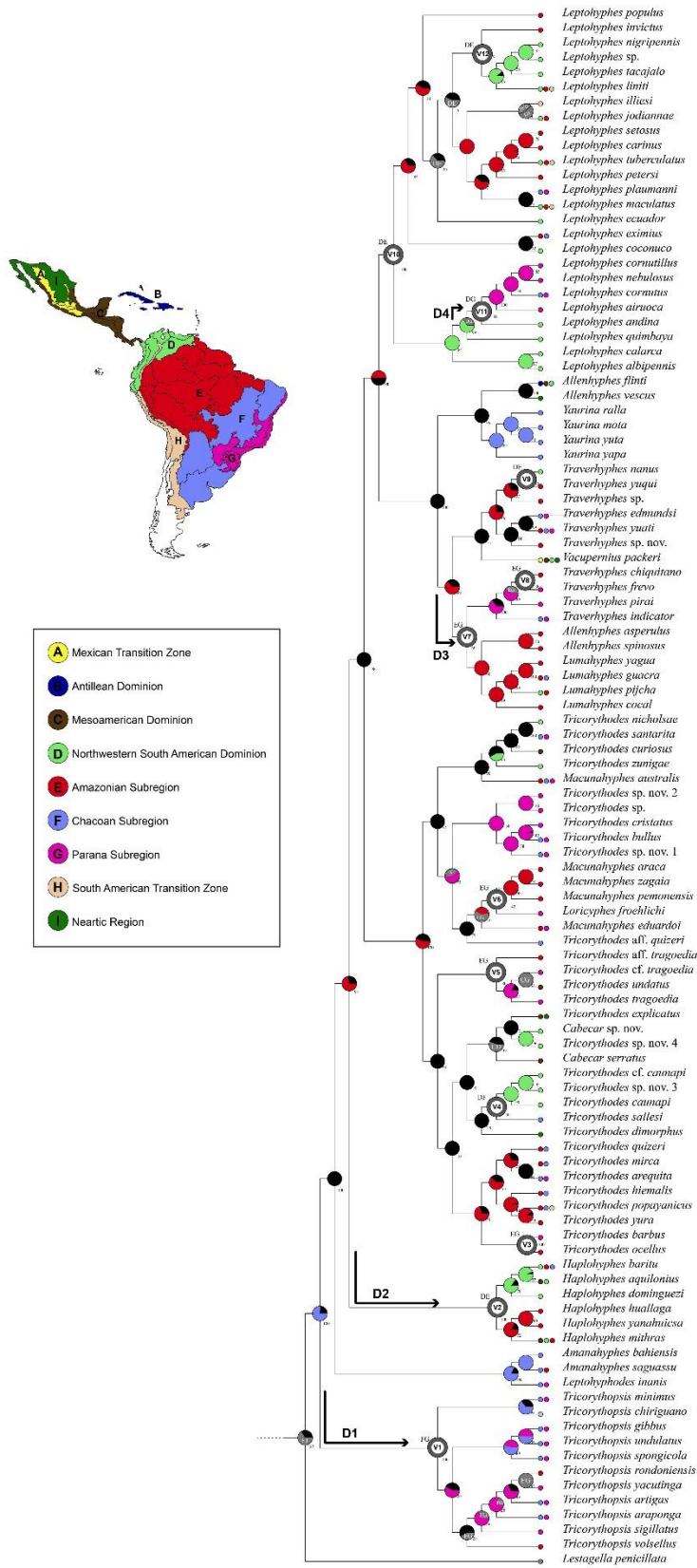


Figure 6. Bayesian consensus from a mixed-model analysis based on the molecular dataset (partitions: 12S, 16S, 18S, 28S, COI) of Leptohiphidae. Coloured circles and pie charts at nodes represent geographical distributions, corresponding to regions proposed by Morrone (2013) as showed in the map above. Pie charts at nodes represent S-DIVA results (based on IB analysis of molecular data): colors correspond to regions, except gray for compound areas and black for numerous ancestral areas with low probabilities (<40%). D= Dispersion. V= Vicariance.



*Legend in the next page

Figure 7. Bayesian consensus from a mixed-model analysis based on the combined dataset (partitions: 12S, 16S, 18S, 28S, COI, morphology) of Leptohyphidae. Coloured circles and pie charts at nodes represent geographical distributions, corresponding to regions proposed by Morrone (2013) as showed in the map above. Pie charts at nodes represent S-DIVA results (based on IB analysis of combined data): colors correspond to regions, except gray for compound areas and black for numerous ancestral areas with low probabilities (<40%). D= Dispersion. V= Vicariance

The VIP analysis resulted in 56 vicariant nodes, from which 18 were considered and discussed (Fig. 8). All vicariant nodes and hypothetical barriers are shown in Fig. 8. The first vicariant node (node 1, Fig. 8) corresponded to the split of the outgroup (*Ephemerythus niger*) and Leptohyphidae. This hypothetical barrier corresponds to the separation of the eastern portion of South America from the western portion of the African continent and was found in the other analyses. Node 2 is related to the split of

Node 3 is related to the split of *Haplohyphes* from *Leptohyphodes* + *Amanahyphes*, which corresponds to the separation of Andean species from central-eastern species of South America. Node 4 indicates the split between two *Haplohyphes* species: *H. mithras*, distributed in the Central America and north of the South America comprising the Mesoamerican Dominion (C), Northwestern South American Dominion (D) and Amazonian Subregion; and *H. yanahuicsa*, restricted to Bolivia in the Amazonian Subregion (E). Node 5 is related to the split of *Leptohyphodes* and *Amanahyphes*, which corresponds to the separation of a species from the Brazilian Atlantic Forest (G, in the S-DIVA and DEC analyses) from other two species from Amazonia and Cerrado biomes. Node 6 corresponds to separation of the *Amanahyphes* species, *A. saguassu* stricted to Amazon biome, and *A. bahiensis*, with distribution to the Chacoan dominion. Node 7 is the same as V11 in S-Diva combined analysis and corresponds to the split of *L. andina* occurring only in Colombia in the Northwestern South American Dominion (D) from its sister clade from the Brazilian Atlantic Forest. Node 8 corresponds to the split of *L. coconuco*, which occurs in Colombia in the Northwestern South American Dominion, and *L. eximius*, and Andean species restricted to Argentina and Bolivia. Node 9 is related to the split between two species of *Lumahyphes*, one Andean species from Argentina and Bolivia (*L. guacra*) and another from Colombia and Peru in the Amazonian Subregion (*L. yagua*). Node 10 is the split of *Allenhyphes* spp. and *Yaurina* spp., corresponding to the separation of North and Central American species with distribution reaching the north of South America and Andean species. Nodes 11, 14 and 18 refers to the same hypothetical barrier, which separates the North and Central America from South America. Node 12 is the same as V3 in S-Diva combined analysis and is related to the split between *T. barbuis* and *T. ocellus*, the first one restricted to Argentina and Brazil in the Parana Subregion (G) and the latter restricted to Peru in the Amazonian Subregion (E). Node 13 separates *T. yura* restricted to Bolivia and *T. popayanicus* with distribution mostly in northwest Argentina reaching Bolivia. Node 15 is the split of two species of *Tricorythodes* that have already been considered belonging to a single genus, *Asioplax* (McCafferty & Wang, 2000), being one widely distributed in the Chacoan (F) and Parana (G)

subregions (*T. santarita*) and the other one restricted to Ecuador in the Northwestern South American Dominion (D).

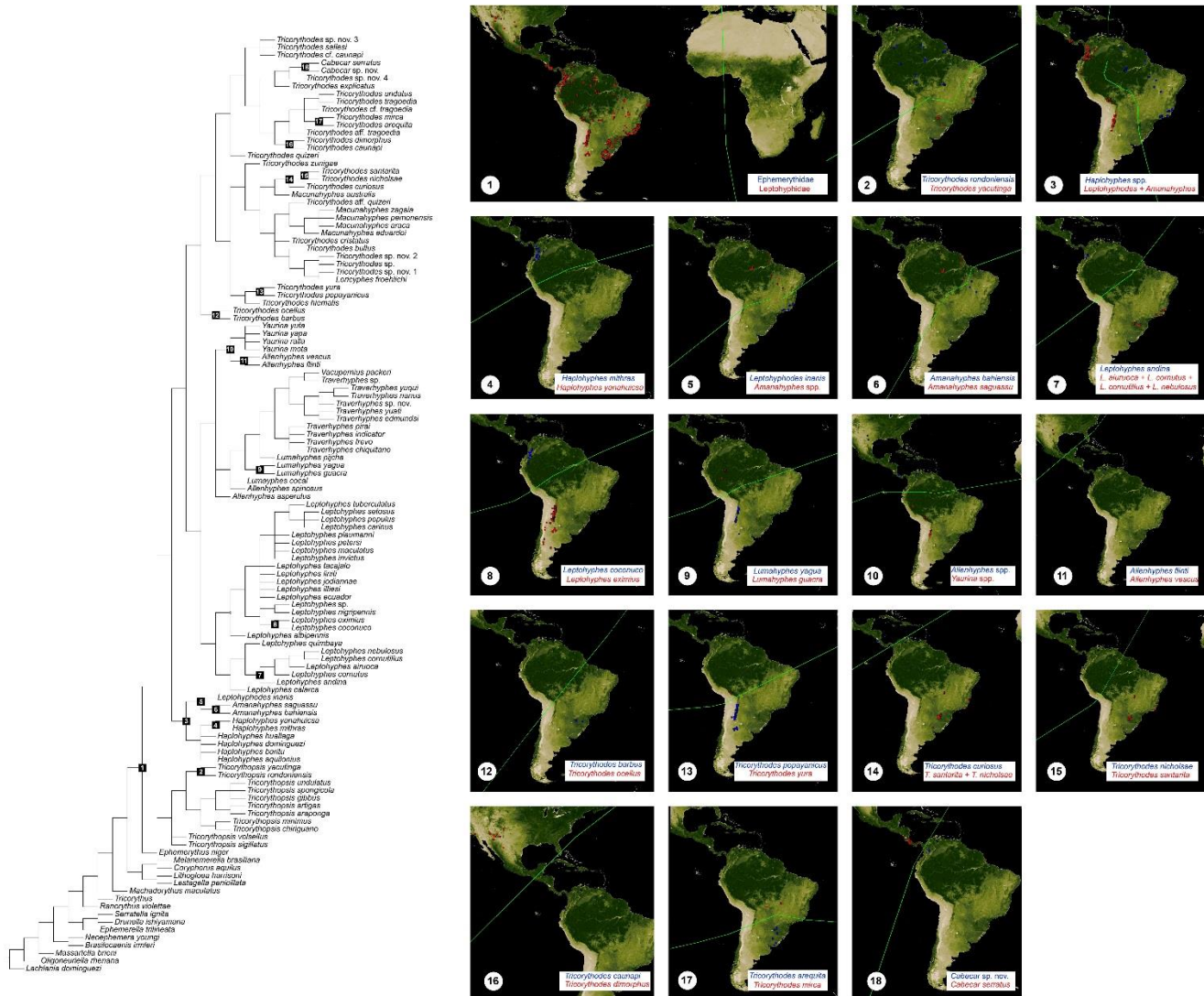


Figure 8. Vicariant nodes 1 to 18 obtained from the VIP analysis using a 1.0×1.0 grid. For each vicariant node there is an image of hypothetical barrier.

DISCUSSION

Phylogenetic relationships and classification

All analyses recovered Leptohiphidae as monophyletic (Figs. 1–5), irrespective of the dataset. Ogden & Whiting (2005) and Ogden *et al.* (2009) included few Leptohiphidae taxa (five and three genera respectively) in their family-level phylogeny of Ephemeroptera, and their analyses also indicated the monophyly of the family. Subfamilies subjectively proposed by McCafferty & Wang (2000) were not recovered in our study. As discussed by Molineri (2006), even though McCafferty & Wang (2000) stated that their classification was cladistically based, they did not include any matrix, or character list, nor a phylogenetic branching diagram. Furthermore, they assigned nymphs and adults of the same species to different subfamilies (Molineri, 2006). However, all present analyses recovered two major monophyletic groups, one with *Tricorythopsis* species and other with all other genera, although relationships within both clades varied according to the analysis. Finally, our results strongly suggest Tricorythodinae as a synonymy with Leptohiphinae, since *Tricorythodes* was recovered in all analysis as part of a clade containing all leptohiphinae genera.

The close relationship between *Amanahyphes* and *Leptohiphodes* found in all analyses in the present study corroborates Baumgardner's cladistic study (2008). The large and sexually dimorphic eyes presented in *Leptohiphodes* and *Amanahyphes* species appears to be a good character to define this group. It is a rare feature in Leptohiphidae, present only in these two genera and in a single species of *Leptohiphes*. In addition, both genera share similar forewing shape and venation, similarities in the male genitalia (styliger plate posteriorly projected and forceps shape). Despite the impossibility of including adults and nymphs of *Amanahyphes* in his cladistic analysis of Leptohiphidae, Molineri (2006) found high support for the group formed by *Leptohiphodes* + *Haplohyphes* + *Tricorythodes*, which was not recognized in the present study. According to the parsimony analyses the relationship between *Haplohyphes* and *Leptohiphodes* + *Amanahyphes* was based only on highly homoplastic characters within the family or even in Ephemeroptera, such as presence of frontal projection and spines on genal projection. Therefore, they are not considered good characters to define this group.

Nymphs of *Yaurina* are known but they are indistinguishable to those of *Allenhyphes* (Domínguez *et al.*, 2006). Furthermore, adult males share a similar genitalia and this was recovered in the parsimony analysis, as two synapomorphies with moderate to high CI: basal insertion of penes spines (44: 1, CI=1.0) and penes spines ventrally positioned (45: 2, CI=0.67). Both features are only

present in this clade, which was recovered in all the approaches used herein. Based on morphology, Molineri (2006) and Baumgardner (2008) found the same result. Regarding this group, both methods recovered a close relationship to the *Traverhyphes* + *Lumahyphes* + *Vacupernius* group, and two mysterious species: *Allenhyphes spinosus*, and *A. asperulus*. Both species were originally described as *Leptoxyphes* (Allen, 1967), subsequently placed in *Allenhyphes* (McCafferty and Wang, 2000). However, as discussed by Molineri (2004) in its *Allenhyphes* + *Traverhyphes* cladistic study, *A. spinosus* and *A. asperulus*, known only from nymph, undoubtedly belong to this group of genera, but their exact position remain unresolved. In addition, the two species share the plesiomorphic nymphs with 3-segmented maxillary palpi with *Lumahyphes*, *Traverhyphes*, or *Vacupernius*. In the combined Bayesian analysis, the two species were not found related to the remaining *Allenhyphes* species, but in a clade poorly supported of *Lumahyphes*, *Traverhyphes*, or *Vacupernius*. Given the non resolution of the trees recovered, it seems better to maintain both species in *Allenhyphes* until the adults are known (Molineri, 2004).

Concerning to *Tricorythodes*, our results based on both methods recovered the genus as highly polyphyletic, suggesting its synonymy with *Asioplax*, *Cabecar*, *Epiphraodes*, *Loricypes*, *Macunahyphes* and *Homoleptoxyphes*. However, the genus is currently the focus of a phylogenetic analysis (Dias *et al.*, in prep.), therefore no taxonomic changes will be proposed herein.

Spatial and temporal evolution of Leptoxyphidae

The complete separation of Africa and South America occurred around 100 Mya ago in the Late Cretaceous. However, South America began to separate from Africa in the Early Cretaceous (135 Mya) with the opening of the South Atlantic Ocean at the latitude of Argentina and Chile. Northern South America and Africa remained connected until the mid-late Cretaceous (110–95 Mya), when a transform fault opened between Brazil and Guinea. As a result, Africa started drifting northeast and collided with Eurasia in the Paleocene (60 Mya), whereas southern South America drifted southwest into contact with Antarctica (Sanmartín & Ronquist, 2004).

Based on present results, the stem lineage of Leptoxyphidae diverged from its sister group around 152 Mya (120.0 - 184.4 Mya) in the Early Cretaceous, possibly caused by the Gondwana breakup (Fig. 5). The Gondwanic origin was suggested earlier by McCafferty (1998) McCafferty & Wang (2000). They proposed the African family Tricorythidae as sister group of Leptoxyphidae, the former is

strictly found in the Western Hemisphere, and the latter with its center of evolution in South America. From this, they hypothesized that the common ancestor of these two clades was in Gondwana after the split of West Gondwana from Laurasia, and after the subsequent split of East and West Gondwana. This vicariant event is generally thought to have been initiated approximately 135 Mya ago (McCafferty & Wang, 2000). Although in the present analyses, Tricorythidae was never recovered as sister to Leptohiphidae, the parsimony and BI analyses recovered Afrotropical taxa as sister to Leptohiphidae. Parsimony results (combined dataset and morphology-only) recovered Ephemerythidae as sister group of Leptohiphidae, while BI analysis (combined dataset and morphology-only) recovered Teloganodidae. The first is restricted to Africa distributed in Angola, Cameroon, Tanzania, Zambia, Congo and Uganda with only one genus, while the second has an Afrotropical and Oriental distribution, with the African groups restricted to South Africa and Madagascar. However, the BEAST BI analyses recovered a clade composed by Teloganodidae + Melanemerellidae + Coryphoridae as sister group of Leptohiphidae

Leptohiphidae ancestor seems to have been distributed at the Chacoan Subregion (Morrone, 2000). This subregion occupies northern and central Argentina, southern and northeastern Brazil. The following provinces integrate the Chacoan Subregion: Caatinga (Northeastern Brazil, in the states of Bahia, Sergipe, Alagoas, Pernambuco, Paraíba, Rio Grande do Norte, Ceará, and portions of Piauí and Minas Gerais), Cerrado (Central western Brazil, in the states of Minas Gerais, Mato Grosso, Goiás, São Paulo, Paraná, Maranhão, and Piauí, and northeastern Paraguay and Bolivia), Chaco (southern Bolivia, western Paraguay, Southern Brazil, and north central Argentina), Pampa (central eastern Argentina, Uruguay, and the southern part of the Brazilian state Rio Grande do Sul), and Monte (central Argentina, from Salta to northeastern Chubut) (Morrone, 2000). The ancestor of the Leptohiphidae may have originated in an area that today has the conformation of the Chacoan Sub-region and this type of distribution was maintained by the *Tricorythopsis* and *Amanahyphes* lineages.

Within the Leptohiphidae diversification we can recognize some important vicariant events such as the division of Central and South American species from North American species (Fig. 8, node 11 and 16) and the division of South American species from North and Central American species (Fig 8, node 10). Unfortunately, these hypothetical barriers could not be dated by molecular dating because of the lack of some species in the molecular matrix. However, although the VIP has not found a hypothetical barrier related to the split between the Nearctic species *T. explicatus* and its related Neotropical species, according to the divergence time estimation they diverged from its sister group around 36.2 Mya (21.4–52.4) in the Paleogene. This event probably occurred prior to the formation of

the Isthmus of Panama *sensu stricto*, which is dated around 2.8 Mya (O’Dea *et al*, 2016). Barriers separating the western and eastern side of South America also appear to be important for evolution within the family (Fig. 8, node 3), as well as the separation of genera and species restricted to certain biomes, such as the *Leptohyphodes* - *Amanahyphes* split (Fig. 8, node 5) and the *A. saguassu* - *A. bahiensis* split (Fig. 8, node 6). However, the ages found for the divergences of these groups were very old, dating from the Cretaceous and Paleogene, being correlated to very old events of landscape evolution within South America.

FINAL REMARKS

This is the most representative study of Leptohyphidae species from South, Central and North America, although the latter was less represented. Still, it is also the most representative study of Pannota, with species representing all families, except for three [Austramerellidae (North America and Asia) and Teloganellidae (Africa)]. Machadorythidae (Africa) was represented in the morphological matrix, but not in the molecular one. Of the 15 genera recognized in Leptohyphidae, 12 were represented in the molecular matrix and 13 in the morphological matrix (including type species), being all genera represented in the combined dataset. Phylogenetic analyses of morphological, molecular, and combined datasets allowed us to corroborate the monophyly of Leptohyphidae. According to biogeographic and divergence time estimation analyses, the initial diversification of Leptohyphidae began at around 152 Mya, with ancestral distribution in the Chocoan Subregion (Morrone, 2013), which include the Cerrado, Caatinga and Chaco domains.

This can be considered a starting point for future studies of phylogeny, biogeography, and evolution of Leptohyphidae and of the order Ephemeroptera as a whole. The incorporation of molecular data for taxa not included in the present study, especially of Nearctic representatives, as well as the addition of new molecular markers may be very important to achieve a better resolution in molecular dating and biogeography of Leptohyphidae. Furthermore, coding of new morphological characters would be desirable in order to increase the resolution of the phylogenetic tree of the family and propose more robust hypotheses on the phylogeny and biogeography of this group of insects.

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Table I. Taxa and character partitions used for the phylogenetic analysis of Leptohiphidae. Different stages (MI= male imago, FI= female imago, N= nymph) studied for morphology coding (CFL= coded from the literature). Depository of material studied and DNA voucher specimen number listed with corresponding gene partition GenBank access number. As soon as we submit the paper "X" will be replaced by access number.

Species	Morphology	Depository	Voucher Number	CO I	12S	16S	18S	28S	
Outgroup									
<u>Caenidae</u>	<i>Brasilocaenis irmleri</i>	N, MI, FI + CFL	DZRJ	ENT1832	X	X	X	X	X
<u>Coryphoridae</u>	<i>Coryphorus aquilus</i>	N, MI, FI	DZRJ, IBN	ENT3285	-	X	X	-	X
<u>Ephemerellidae</u>	<i>Ephemerella trilineata</i>	N, MI, FI	IBN	IBN	-	-	-	-	-
	<i>Drunella ishiyamana</i>	N + CFL	CZNC	ENT2058	-	X	X	X	X
	<i>Serratella ignita</i>	N + CFL	DZRJ	ENT2046	X	X	X	X	X
<u>Ephemerythidae</u>	<i>Ephemerythus niger</i>	N, MI, FI	IBN	-	-	-	-	-	-
<u>Leptophlebiidae</u>	<i>Massartella brienti</i>	N, MI, FI	DZRJ	ENT2983	X	-	X	-	X
<u>Machadorythidae</u>	<i>Machadorythus maculatus</i>	N, MI, FI	IBN	-	-	-	-	-	-
<u>Melanemerellidae</u>	<i>Melanemerella brasiliana</i>	N, FS	DZRJ	ENT2861, ENT2985	X	X	X	-	X
<u>Neoephemeridae</u>	<i>Neoephemera youngi</i>	N, MI, FI + CFL	FAMU	ENT2054	X	X	X	X	X
<u>Oligoneuriidae</u>	<i>Lachlania dominguezi</i>	-	-	-	X	AY749654.1	AY749780.1	AY749856.1	AY749947.1
	<i>Oligoneuriella rhenana</i>	-	-	Ep-5678	X	-	-	X	-
<u>Tricorythidae</u>	<i>Ranorythus violettiae</i>	N + CFL	IBN	ENT2048	-	X	X	X	X
	<i>Tricorythus</i> spp.	N, MI, FI	IBN	-	-	-	-	-	-
<u>Teloganodidae</u>	<i>Lestagella penicillata</i>	N, MI, FI + CFL	AMGS	ENT2045	X	X	X	-	X
	<i>Lithogloea harrisoni</i>	N, MI, FI	IBN	-	-	-	-	-	-
Ingroup									
<i>Allenhyphes asperulus</i>	CFL	-	-	-	-	-	-	-	-
<i>Allenhyphes flinti</i>	N, MI, FI + CFL	-	-	-	-	AY749807.1	AY749880.1	AY749987.1	-
<i>Allenhyphes spinosus</i>	CFL	-	IBN	-	-	AY749677.1	1	1	1

<i>Allenhyphes vescus</i>	N, MI, FI + CFL	IBN		-	-	-	-	-	-
<i>Amanahyphes bahiensis</i>	N, MI, FI	DZRJ	ENT2324		X	X	X	-	-
<i>Amanahyphes saguassa</i>	N, MI, FI	DZRJ	ENT1831, ENT3293		-	X	X	X	-
<i>Cabecar serratus</i>	N, MI, FI	IBN		-	-	-	-	-	-
<i>Cabecar</i> sp. nov.	N	CEBUC	ENT2241		X	X	X	X	X
<i>Haplohyphes aquilonius</i>	N	DZRJ	ENT2240		X	X	X	X	X
<i>Haplohyphes baritu</i>	N	CEBUC	ENT2237		X	X	X	X	-
<i>Haplohyphes dominguezi</i>	MI, FI	-	IBN		-	-	-	-	-
<i>Haplohyphes huallaga</i>	MI	DZRJ	ENT1725		X	X	X	X	-
<i>Haplohyphes mithras</i>	N, MI, FI	IBN		-	-	-	-	-	-
<i>Haplohyphes yanahuicsa</i>	N, MI, FI	IBN		-	-	-	-	-	-
<i>Leptohyphes airuoca</i>	N	IBN		-	-	-	-	-	-
<i>Leptohyphes albipennis</i>	N, MI		ENT2321		X	X	X	X	X
<i>Leptohyphes andina</i>	N	IBN		-	-	-	-	-	-
<i>Leptohyphes calarca</i>	N	IBN		-	-	-	-	-	-
<i>Leptohyphes carinus</i>	N	IBN		-	-	-	-	-	-
<i>Leptohyphes coconuco</i>	N, MI	DZRJ	ENT2320		X	X	X	X	X
<i>Leptohyphes cornutillus</i>	N	CZNC		-	-	-	-	-	-
<i>Leptohyphes cornutus</i>	N	IBN		-	-	-	-	-	-
<i>Leptohyphes ecuador</i>	N, MI, FI	IBN		-	-	-	-	-	-
<i>Leptohyphes eximius</i>	MI + CFL	DZRJ	ENT3007		-	X	X	X	X
<i>Leptohyphes illiesi</i>	N	IBN		-	-	-	-	-	-
<i>Leptohyphes invictus</i>	N	IBN		-	-	-	-	-	-
<i>Leptohyphes jodiannae</i>	N	IBN		-	-	-	-	-	-
<i>Leptohyphes liniti</i>	N	IBN		-	-	-	-	-	-
<i>Leptohyphes maculatus</i>	N, MI, FI	IBN		-	-	-	-	-	-
<i>Leptohyphes nebulosus</i>	N	CZNC	ENT2986		X	X	X	X	X
<i>Leptohyphes nigripennis</i>	N, MI	DZRJ	ENT3291		X	X	X	X	-
<i>Leptohyphes petersi</i>	N, MI, FI	IBN		-	-	-	-	-	-
<i>Leptohyphes plaumanni</i>	N, MI	DZRJ	ENT2047		X	X	X	X	X

<i>Leptohyphes populus</i>	N	IBN	-	-	-	-	-	-
<i>Leptohyphes quimbaya</i>	N	IBN	-	-	-	-	-	-
<i>Leptohyphes setosus</i>	N, MI, FI	IBN	-	-	-	-	-	-
<i>Leptohyphes</i> sp.	MI	DZRJ	ENT2325	-	X	X	X	-
<i>Leptohyphes tacajalo</i>	N	IBN	-	-	-	-	-	-
<i>Leptohyphes tuberculatus</i>	N	IBN	-	-	-	-	-	-
<i>Leptohyphodes inanis</i>	N, MS, MI, FS	DZRJ	ENT2559, ENT2998	X	X	X	X	-
<i>Loricyphe froehlichii</i>	N	IBN	-	-	-	-	-	-
<i>Lumahyphes cocai</i>	MI, FI	DZRJ	ENT2865	-	X	X	X	-
<i>Lumahyphes guacra</i>	N, MI	DZRJ, IBN	ENT3005	-	X	X	X	X
<i>Lumahyphes pijcha</i>	N, MI, FI	IBN	-	-	-	-	-	-
<i>Lumahyphes yagua</i>	N, MI, FI	IBN	-	-	-	-	-	-
<i>Macunahyphes araca</i>	MI	DZRJ, INPA	ENT2059	X	X	X	X	-
<i>Macunahyphes australis</i>	N, MI, FI	DZRJ, CZNC	ENT1732	X	X	X	X	X
<i>Macunahyphes eduardoi</i>	MI, FI	DZRJ, CZNC	ENT2872, ENT2862, ENT2863	X	X	X	X	X
<i>Macunahyphes pemonensis</i>	MI, FI	DZRJ	-	-	-	-	-	-
<i>Macunahyphes zagaia</i>	MI, FI	INPA, DZRJ	-	-	-	-	-	-
<i>Traverhyphes chiquitano</i>	N, MI, FI	IBN	-	-	-	-	-	-
<i>Traverhyphes edmundsi</i>	N, MI, FI	IBN	-	-	-	-	-	-
<i>Traverhyphes frevo</i>	MI	IBN	-	-	-	-	-	-
<i>Traverhyphes indicator</i>	N, MI, FI	DZRJ	ENT2326	X	X	X	X	*
<i>Traverhyphes nanus</i>	N, MI, FI	IBN	-	-	-	-	-	-
<i>Traverhyphes pirai</i>	MI, FI	IBN	-	-	-	-	-	-
<i>Traverhyphes</i> sp. nov.	N, MI, FI	DZRJ	ENT3284	-	X	-	-	-
<i>Traverhyphes</i> sp.	MI	DZRJ	ENT3286	X	X	X	X	-
<i>Traverhyphes yuati</i>	N, MI, FI	DZRJ	ENT1720	X	X	X	X	-
<i>Traverhyphes yuqui</i>	MI, FI	IBN	-	-	-	-	-	-
<i>Tricorythodes</i> aff. <i>quizeri</i>	N	DZRJ	-	-	X	-	-	X

<i>Tricorythodes</i> aff. <i>tragoedia</i>	MI	CZNC	-	-	-	-	-	-
<i>Tricorythodes arequita</i>	N, MI, FI	IBN	-	-	-	-	-	-
<i>Tricorythodes barbuis</i>	N	IBN	-	-	-	-	-	-
<i>Tricorythodes bullus</i>	N, MI, FI	IBN	-	-	-	-	-	-
<i>Tricorythodes caunapi</i>	N, MI	DZRJ	ENT3289	X	-	X	X	-
<i>Tricorythodes</i> cf. <i>tragoedia</i>	N	DZRJ	ENT1721	X	X	X	X	X
<i>Tricorythodes</i> cf. <i>caunapi</i>	N	DZRJ	-	-	-	-	-	-
<i>Tricorythodes cristatus</i>	N	IBN	-	-	-	-	-	-
<i>Tricorythodes curiosus</i>	N, MI, FI	IBN	-	-	-	-	-	-
<i>Tricorythodes dimorphus</i>	N, MI, FI	IBN	-	-	-	-	-	-
<i>Tricorythodes explicatus</i>	N, MI, FI	DZRJ	ENT2925	X	X	X	X	X
<i>Tricorythodes hiemalis</i>	N, MI, FI	IBN	-	-	-	-	-	-
<i>Tricorythodes mirca</i>	N, MI, FI	IBN	-	-	-	-	-	-
<i>Tricorythodes nicholsae</i>	N, MI, FI	IBN	-	-	-	-	-	-
<i>Tricorythodes ocellus</i>	N, MI, FI	IBN	-	-	-	-	-	-
<i>Tricorythodes popayanicus</i>	N, MI, FI	IBN	-	-	-	-	-	-
<i>Tricorythodes quizeri</i>	N, MI, FI	IBN	-	-	-	-	-	-
<i>Tricorythodes sallesi</i>	N	IBN	-	-	-	-	-	-
<i>Tricorythodes santarita</i>	N, MI, FI	DZRJ	ENT2055	X	-	X	X	X
<i>Tricorythodes undatus</i>	N, MS, FS	IBN	-	-	-	-	-	-
<i>Tricorythodes</i> sp.	-	DZRJ	ENT2864	X	-	X	X	-
<i>Tricorythodes</i> sp. nov. 1	N	DZRJ	ENT1733	-	-	-	-	-
<i>Tricorythodes</i> sp. nov. 2	N	DZRJ	ENT1734	X	X	X	X	-
<i>Tricorythodes</i> sp. nov. 3	N	DZRJ	ENT3290	-	X	-	-	-
<i>Tricorythodes</i> sp. nov. 4	N	DZRJ	ENT3281	-	X	X	-	X
<i>Tricorythodes tragoedia</i>	N, MI	CZNC	Ep-7022, Ep-6200, Ep-5387	X	X	X	X	X
<i>Tricorythodes yura</i>	N, MI, FI	IBN	-	-	-	-	-	-
<i>Tricorythodes zunigae</i>	N, MI, FI	IBN	-	-	-	-	-	-
<i>Tricorythopsis araponga</i>		IBN	-	-	-	-	-	-
<i>Tricorythopsis artigas</i>	N, MI, FI	IBN	-	-	-	-	-	-

<i>Tricorythopsis chiriguano</i>	N, MI, FI	DZRJ	ENT1837	X	X	X	X	X
<i>Tricorythopsis gibbus</i>	N, MI, FI	DZRJ	ENT2239	X	X	X	X	X
<i>Tricorythopsis minimus</i>	N, MI, FI	DZRJ	ENT1723	-	X	X	-	-
<i>Tricorythopsis rondoniensis</i>	N	DZRJ	ENT3287	X	X	-	-	X
<i>Tricorythopsis sigillatus</i>	MI, FI	IBN	-	-	-	-	-	-
<i>Tricorythopsis spongicola</i>		DZRJ	ENT1722	X	X	X	X	X
<i>Tricorythopsis undulatus</i>	N, MI, FI	IBN	-	-	-	-	-	-
<i>Tricorythopsis volsellus</i>	MI, FI	IBN	-	-	-	-	-	-
<i>Tricorythopsis yacutinga</i>	N, MI, FI	IBN	-	-	-	-	-	-
<i>Vacupernius packeri</i>	N, MI	DZRJ	ENT2323	X	-	X	X	-
<i>Yaurina mota</i>	N, MI, FI	IBN	-	-	-	AY749777. 1	AY749853. 1	-
<i>Yaurina ralla</i>	N	IBN	-	-	-	-	-	-
<i>Yaurina yapa</i>	N, MI	IBN	-	-	-	-	-	-
<i>Yaurina yuta</i>	N, MI, FI	IBN	-	-	-	-	-	-

Table II. Primer names, sequences, and references used to amplify and sequence selected genes for the phylogenetic analysis of Leptohiphidae.

Gene	Primer	Direction	Sequence (5' - 3')	Reference
12S	SR-J-14233	Forward	AAGAGCGACGGGCGATGTGT	Simon <i>et al.</i> , 1994
	SR-N-14588	Reverse	ARACTAGGATTAGATACCCTAYTAT	Simon <i>et al.</i> , 1994
16S	16Sa	Forward	GCCTGTTTATCAAAAACAT	Ogden & Whiting, 2005
	16Sb	Reverse	CTCCGGTTTGAACCTCAGATCA	Ogden & Whiting, 2005
	16S-Lepto-F	Reverse	TAAGTGTGCAAAGGTAGC	Malm & Johanson, 2008
18S	18Sf	Forward	AGGGCAAGTCTGGTGCCAGC	Ferris <i>et al.</i> , 2004
	18Sr	Reverse	TTTCAGCTTTGCAACCATAC	Ferris <i>et al.</i> , 2004
28S	D2-3665F	Forward	AGAGAGAGTTCAAGAGTACGTG	Hancock <i>et al.</i> , 1988
	D3-4413R	Reverse	TCGGAAGGAACCAGCTACTA	Nunn <i>et al.</i> , 1996
	D5-4749R	Reverse	GTTACACACTCCTTAGCGGA	Hancock <i>et al.</i> , 1988
COI	LCO-1490	Forward	GGTCAACAAATCATAAAGATATTGG	Folmer <i>et al.</i> , 1994
	C1-J-1718	Forward	GGAGGATTTGAAAATTGATTAGTTCC	Simon <i>et al.</i> , 1994
	HCO-2198	Reverse	TAAACTTCAGGGTGACCAAAAAATCA	Folmer <i>et al.</i> , 1994

Table III. Morphological characters and their states coded for the phylogenetic analysis of Leptohiphidae. Character length (number of steps), consistency, and retention indices given based on selected implied weighted trees ($k=3$) resulting from the analysis of morphological characters only (trees with identical metrics).

N°	Character with states	L	CI	RI
Adult				
1	<i>Small sublateral tubercles on hind margin of head:</i> (0) absent; (1) present	2	0.50	0.86
2	<i>Position of compound eyes:</i> (0) laterodorsal; (1) lateral pedunculated; (2) lateral not pedunculated	6	0.33	0.64
3	<i>Size of male compound eyes:</i> (0) small, similar to female; (1) large	6	0.17	0.64
4	<i>Upper and lower division of male compound eyes:</i> (0) present; (1) absent	6	0.17	0.44
5	<i>Mesopleurae, sutures on lateropostnotum:</i> (0) superior suture (SS) and inferior suture (IS) not forming a straight line; (1) SS and IS forming a straight line	3	0.33	0.93
6	<i>Internal and external parapsidal sutures:</i> (0) running independently until fore mesonotal transverse invagination (FMI); (1) fused before or at FMI	3	0.33	0.92
7	<i>Sulcus (dorsal depression between postero–scutal protuberance):</i> (0) present; (1) absent	3	0.33	0.91
8	<i>Membranous filaments of mesoscutellum:</i> (0) absent or not extending beyond tip; (1) short not reaching abdominal segment II; (2) long, reaching abdominal segment II	6	0.25	0.88
9	<i>Male foretarsal claw (imago):</i> (0) similar (both hooked); (1) dissimilar (one hooked and one blunt); (2) similar (both blunt)	3	0.67	0.88
10	<i>Female forelegs (imago):</i> (0) present; (1) absent	1	1.00	1.00
11	<i>Ventrodiscal extension of tibiae:</i> (0) absent; (1) present, slightly marked; (2) present long	3	0.67	0.98
12	<i>Number of tarsal segments:</i> (0) four; (1) five	5	0.40	0.87
13	<i>Black macula on apex of tibiae:</i> (0) absent; (1) present	1	1.00	1.00
14	<i>Fore wings with fringed hind margin (imago):</i> (0) absent; (1) present	5	0.20	0.43
15	<i>Vein CuP (forewing):</i> (0) absent or poorly developed; (1) present	2	0.50	0.86
16	<i>Ratio length of fore and hind wings (male):</i> (0) 0.25 or more; (1) 0.20 or less	1	1.00	1.00
17	<i>Base of vein 1Cu1:</i> (0) Fused with or clearly directed to base of CuP; (10) Free or fused with CuA or CuP by crossveins; (1) Fused with CuP at 1/2 from base (Tricorythid fork)	7	0.29	0.77
18	<i>Base of vein CuP:</i> (0) present (paralleling CuA); (1) absent (ending at vein A)	5	0.20	0.75
19	<i>MA fork (Median Anterior Vein):</i> (0) present; (1) absent, MA1 and MA2 joined by a crossvein	1	1.00	1.00
20	<i>IMP (Intercalary Median Posterior Vein) and MP2:</i> (0) normal, MP fork present; (1) MP2 short and free MP fork absent; (2) fused with CuA, with MP2 appearing as an intercalary	3	0.67	0.94
21	<i>Number of intercalary veins between CuA and CuP:</i> (0) three to four; (1) two; (2) zero	8	0.25	0.14
22	<i>Marginal intercalary:</i> (0) absent; (1) present	4	0.25	0.63
23	<i>Marginal intercalaries:</i> (0) mostly attached; (1) mostly detached; (2) mostly lost in at least one sex	3	0.67	0.50
24	<i>Hindwings:</i> (0) present in both sexes; (1) present in male, absent in female; (2) absent in both	15	0.13	0.77
25	<i>Costal projection of hindwings (male):</i> (0) Short and blunt (<0.2 of wing length); (1) Large (0.3–0.46); (2) Very large (0.53 or more)	2	1.00	1.00
26	<i>Base of costal projection (basal angle):</i> (0) rounded; (1) straight	1	1.00	1.00

27	<i>Hindwing margin (imago):</i> (0) not fringed; (1) fringed on hind margin only; (2) fringed on hind margin and apex of fore margin	3	0.67	0.89
28	<i>Location of costal projection:</i> (0) at half or third from base; (1) at the base	1	1.00	1.00
29	<i>Number of longitudinal veins on male hindwings:</i> (0) more than eight; (1) three; (2) two; (3) one	5	0.60	0.92
30	<i>Hindwings crossveins:</i> (0) present; (1) absent	3	0.33	0.82
31	<i>Posterolateral projections of styliger plate (external):</i> (0) absent; (1) present	2	0.50	0.75
32	<i>Acute posteromedial projections of styliger plate:</i> (0) absent; (1) present	2	0.50	0.95
33	<i>Forceps:</i> (0) two segmented; (1) three segmented; (2) four segmented; (3) one segmented	5	0.60	0.91
34	<i>Length of first forceps segment:</i> (0) long (first and second segments subequal in length); (1) medium (first segment one-third to three-quarters the length of second segment); (2) short (first segment one-fifth to one-quarter the length of second segment).	12	0.17	0.62
35	<i>Distal inner projection of forceps segment one:</i> (0) absent; (1) present	4	0.25	0.63
36	<i>Basal swelling of forceps segment two:</i> (0) absent; (1) present	2	0.50	0.95
37	<i>Blunt posteromedial projections of styliger plate:</i> (0) absent; (1) present	4	0.25	0.81
38	<i>Form of hind margin of styliger plate:</i> (0) strongly projected medially; (1) slightly convex; (2) slightly concave; (3) straight	10	0.18	0.76
39	<i>Styliger plate posteriorly projected forming a columnar base for each forceps:</i> (0) absent; (1) present	3	0.25	0.85
40	<i>Penes with a basal sclerotized ring:</i> (0) absent; (1) present	1	1.00	1.00
41	<i>Gonopore:</i> (0) free, not associated with a spine; (1) associated with an acute and sclerotized structure; (2) associated with a hollow spine	3	0.67	0.98
42	<i>Penes spine curvature in lateral view:</i> (0) absent; (1) present	4	0.25	0.73
43	<i>Penes spine curvature in dorsal view:</i> (0) absent; (1) present	2	0.50	0.90
44	<i>Insertion of spines:</i> (0) apical or subapical; (1) basal	1	1.00	1.00
45	<i>Position of the spines:</i> (0) dorsal; (1) lateral; (2) ventral	3	0.67	0.86
46	<i>Membranous lobes of penes:</i> (0) absent; (1) present	1	1.00	1.00
47	<i>Membranous lobes of penes:</i> (0) present, small; (1) present, large	1	1.00	1.00
48	<i>Additional pair of smaller membranous lobes:</i> (0) absent; (1) present	2	0.50	0.92
49	<i>Lateral margins of penes esclerotized:</i> (0) absent; (1) present	6	0.13	0.71
50	<i>Dorsal extensions of penes:</i> (0) absent; (1) present	2	0.50	0.89
51	<i>Dorsal extensions of penes:</i> (0) present, double; (1) present, single	1	-	-
52	<i>Penes width:</i> (0) wider at base; (1) similar width along their length; (2) wider at apex	11	0.17	0.69
53	<i>Fusion of penes:</i> (0) partial, divided on apical half; (1) total (may be divided on apical third)	7	0.14	0.75
54	<i>Posterolateral margin of penes:</i> (0) rounded; (1) with a lateral notch	2	0.50	0.50
55	<i>Angle between penean arms:</i> (0) 180°(T); (1) 45–90°(Y); (2) <30°(I)	3	0.67	0.90
56	<i>Lateral groove of penes:</i> (0) absent; (1) present	1	1.00	1.00
57	<i>Small internal setae in deep cleft (Allenhyphes kind):</i> (0) absent; (1) present	1	1.00	1.00
58	<i>Dorsal accesory structures of penes:</i> (0) absent; (1) present, single; (2) present, bifid; (3) present, double	5	0.60	0.90
59	<i>Female gonopore with a black macula:</i> (0) absent; (1) present	1	1.00	1.00
60	<i>Male terminal filament with a ventral spine at base:</i> (0) absent; (1) present	1	1.00	1.00
61	<i>Length of female cerci:</i> (0) longer than forewings; (1) shorter than forewings; (2) rudimentary	7	0.25	0.82
Nymph				
62	<i>Frontal projection:</i> (0) present; (1) absent	5	0.20	0.64
63	<i>Genal projections:</i> (0) absent; (1) present	12	0.08	0.27

64	<i>Genal projections</i> : (0) bare; (1) with spines	7	0.14	0.65
65	<i>Anteromedian emargination of labrum</i> : (0) shallow; (1) deep	6	0.14	0.71
66	<i>Hypopharynx, form of linguae</i> : (0) trapezoid or triangular; (1) subrectangular; (2) rounded; (3) alate	7	0.43	0.56
67	<i>Hypopharynx, fore margin of linguae</i> : (0) acute; (1) blunt; (2) concave	11	0.18	0.18
68	<i>Maxillary palpi, form of apical segment</i> : (0) oblong, basally wider (<i>Yaurina</i>); (1) other form	1	1.00	1.00
69	Segments of maxillary palpi: (0) three-segmented; (1) two-segmented; (2) one-segmented; (3) completely reduced	24	0.13	0.70
70	<i>Maxillary palp apical seta</i> : (0) absent; (1) present	7	0.14	0.87
71	<i>Size of maxillary palp</i> : (0) large (almost reaching apex of maxillae); (1) small	6	0.17	0.87
72	<i>Number of long and curved setae on distal brush of galea</i> : (0) less than 20; (1) more than 40	13	0.08	0.46
73	<i>Setae at base of inner margin (group or transverse row)</i> : (0) present; (1) absent	8	0.13	0.81
74	<i>Setae at base of inner margin (longitudinal row)</i> : (0) absent; (1) present	10	0.10	0.78
75	<i>Suture between galea and lacinia</i> : (0) complete; (1) incomplete; (2) absent	21	0.10	0.70
76	<i>General form of maxillae</i> : (0) <i>Leptohyphes</i> kind; (1) <i>Haplohyphes</i> kind; (2) <i>Tricorythus</i> kind	4	0.50	0.67
77	Maxillae, ratio stipes/galea-lacinia length: (0) subequal or stipes shorter; (1) stipes longer than galea-lacinia	4	0.25	0.40
78	<i>Glossae and paraglossae</i> : (0) fused; (1) not fused	4	0.25	0.25
79	<i>Labial palp</i> : (0) third segment reduced; (1) third and second segments reduced; (2) unreduced	5	0.40	0.79
80	<i>Submentum</i> : (0) rounded; (1) not rounded	6	0.17	0.58
81	<i>Prementrum, basal and apical width</i> : (0) similar or wider at apex; (1) wider at base	9	0.09	0.52
82	<i>Anterolateral projections of pronotum</i> : (0) present; (1) absent	20	0.05	0.53
83	<i>Tubercles on head and thorax</i> : (0) absent; (1) present	7	0.14	0.46
84	Form of femoral spines: (0) short length, less than two times width; (1) median, length three to six times width; (2) long, setae-like	15	0.11	0.77
85	<i>Row of stout spines at leading edge of fore femora</i> : (0) absent (setae or bare); (1) present	4	0.25	0.57
86	<i>Transverse row of dorsal setae on forefemur</i> : (0) absent; (1) present	5	0.20	0.00
87	<i>Transverse row on fore femora</i> : (0) subdistal; (1) submedian; (2) subbasal	14	0.13	0.71
88	<i>Femora width</i> : (0) not wider than half length; (1) almost as wide as long	7	0.13	0.22
89	<i>Longitudinal ridge on femora II and III</i> : (0) present; (1) absent	7	0.14	0.79
90	<i>Distal denticles on tarsal claws</i> : (0) absent; (1) present	13	0.07	0.35
91	<i>Distal denticles on tarsal claws</i> : (0) double row; (1) single row; (2) one asymmetric	9	0.18	0.79
92	<i>Basal row of spines/setae on dorsum of femora II and III</i> : (0) present; (1) absent	10	0.10	0.69
93	<i>Dorsal tubercles on abdomen (at least in one abdominal segment)</i> : (0) absent; (1) present	14	0.07	0.26
94	<i>Ventral lamellae of gills</i> : (0) similar to dorsal; (1) different, more complex (double, triple, with flaps)	1	1.00	1.00
95	<i>Ventral lamellae of gills</i> : (0) bifid, with numerous flaps; (1) not clearly bifid, generally without flaps	1	1.00	1.00
96	<i>Form of ventral lamellae of gills</i> : (0) ovoid; (1) subtriangular	6	0.25	0.90
97	<i>Number of lamellae on gill II</i> : (0) five or more; (1) four; (2) three; (3) two; (4) one	10	0.36	0.84
98	<i>Number of lamellae on gills III</i> : (0) seven or more; (1) five; (2) four; (3) three; (4) two	11	0.36	0.90
99	<i>Number of lamellae on gills IV</i> : (0) seven or more; (1) five; (2) four; (3) three; (4) two	12	0.33	0.89
100	<i>Gill V</i> : (0) absent; (1) present	2	0.50	0.00
101	<i>Number of lamellae on gill V</i> : (0) seven or more; (1) four; (2) three; (3) two; (4) one	14	0.29	0.76
102	<i>Gill VI</i> : (0) absent; (1) present	6	0.17	0.29

103	<i>Number of lamellae on gill VI:</i> (0) five or more; (1) two; (2) one	5	0.40	0.83
104	<i>Abdominal gill I:</i> (0) present; (1) absent	2	0.50	0.75
105	<i>Abdominal gill I:</i> (0) normal; (1) filamentous	1	-	-
106	<i>Abdominal gill II:</i> (0) absent; (1) present	1	1.00	1.00
107	<i>Gill II:</i> (0) normal; (1) opercular	2	0.50	0.50
108	<i>Abdominal gill III:</i> (0) normal; (1) opercular	1	1.00	1.00
109	<i>Abdominal gill VII:</i> (0) present; (1) absent	2	0.50	0.67
110	<i>Gill structure, dorsal lamellae margin:</i> (0) smooth; (1) fringed	1	1.00	1.00
111	<i>Gill structure (position of smaller lamellae):</i> (0) along entire margin of main lamella; (1) only at base	1	1.00	1.00
112	<i>Gill structure (pattern of lamellae division):</i> (0) bipinnated (dorsal and ventral); (1) single (ventral or lateral)	3	0.33	0.60
113	<i>Form of gill II:</i> (0) subcuadrate; (1) subtriangular; (2) ovoid	12	0.18	0.73
114	<i>Pigments on gill II:</i> (0) uniform extensive (unpigmented patches may be present); (1) maculated extensive (2) only at base (less than 25% of gill surface)	15	0.13	0.64
115	<i>Ridges on gill II:</i> (0) absent or one slightly marked; (1) one; (2) two	11	0.18	0.81
116	<i>Basal spine of gill II:</i> (0) present; (1) absent	3	0.33	0.92
117	<i>Gill II, ventral inferior lamellae:</i> (0) parallel to dorsal lamellae; (1) perpendicular to dorsal lamellae; (2) reduced	6	0.33	0.50
118	<i>Gill II with a transverse weak line:</i> (0) absent; (1) present	3	0.33	1.00
119	<i>Depigmented macula at posterolateral margin of gill II:</i> (0) absent; (1) present	2	0.50	0.67
120	<i>Basal flap of dorsal lamellae (gills III–V):</i> (0) present; (1) absent	7	0.14	0.78
121	<i>Dorsal projection of ventral lamellae (gills III–V):</i> (0) present; (1) absent	10	0.09	0.50
122	<i>Row of setae on abdominal tergum VII:</i> (0) absent; (1) present	8	0.13	0.75
123	<i>Hind margin of abdominal terga III–V with small spicules:</i> (0) present; (1) absent	6	0.17	0.88
124	<i>Posterolateral spines on abdominal segments:</i> (0) II or III–IX; (1) VI–IX; (2) VII–IX (or VII–VIII or VII); (3) VIII–IX; (4) Absent; (5) VII–VIII; (6) VII IV–IX	20	0.29	0.63
125	<i>Lateral flanges on abdominal segments:</i> (0) absent; (1) III–VI; (2) III–VII; (3) III–VIII; (4) III – IX	16	0.24	0.69
126	<i>Dark annuli on caudal filament (with sexual dimorphism):</i> (0) present; (1) absent	1	1.00	1.00
Egg				
127	<i>Polar cap:</i> (0) absent; (1) present	2	0.50	0.00
128	<i>Number of polar caps:</i> (0) one; (1) two	2	0.50	0.88
129	<i>Conic structure on uncapped pole:</i> (0) absent; (1) present	2	0.50	0.50
130	<i>Sperm guide:</i> (0) absent; (1) present	3	0.33	0.75
131	<i>Position of micropyle:</i> (0) not restringed; (1) polar; (2) equatorial	5	0.33	0.64
132	<i>Chorionic plates:</i> (0) absent; (1) present	5	0.20	0.20
133	<i>Chorionic plates:</i> (0) contiguous; (1) separated by smooth chorion; (2) as an hexagonal netting in longitudinal rows	5	0.60	0.80
134	<i>Normal Knob-terminated coiled threads (KTC):</i> (0) present; (1) absent	1	1.00	1.00
135	<i>Adhesive filaments:</i> (0) absent; (1) present	4	0.25	0.73
136	<i>Adhesive filaments:</i> (0) short; (1) median; (2) long	5	0.40	0.82
137	<i>Chorionic plates (those closest to capped pole):</i> (0) uniform height; (1) wide elevated margin; (2) thin elevated margin	6	0.33	0.69
138	<i>Small groove below KTC:</i> (0) absent; (2) present	3	0.33	0.00
139	<i>Micropylar area (smooth region, as big as a chorionic plate):</i> (0) present; (1) absent	2	0.50	0.00

140	<i>Number of micropyles:</i> (0) three or more; (1) one, rarely two	4	0.50	0.00
141	<i>KTC (or adhesive filaments):</i> (0) inserted inside a groove; (1) inserted outside a groove	1	1.00	1.00

Table IV. Combined results of divergence times estimates and biogeography of Leptohiphidae, showing the median age estimate with 95% HPD based on BEAST time-tree from molecular data.

Taxa	Divergence time estimation		S-Diva (molecular data)		S-Diva (combined dataset)		DEC (molecular data)	
	Age	95% HPD	Range	P	Range	P	Range	P
Leptohiphidae - Outgroup	151.9	120.0 - 184.4	**	**	FJ	0.66	F*	1.00
Clade 1 - Clade 2	135.3	107.0 - 165.8	F	0.55	F	0.76	F*	1.00
<i>Leptohiphes</i> + <i>Traverhiphes</i> + <i>Vacupernius</i> + <i>Lumahyphes</i> + <i>Yaurina</i> + <i>Allenhyphes</i> + <i>Tricorythodes</i> + <i>Cabecar</i> + <i>Macunahyphes</i> - <i>Haplohyphes</i> + <i>Amanahyphes</i> + <i>Leptohiphodes</i>	121.7	93.6 - 148.7	DF	0.36	-	-	F	1.00
<i>Leptohiphes</i> + <i>Traverhiphes</i> + <i>Vacupernius</i> + <i>Lumahyphes</i> + <i>Yaurina</i> + <i>Allenhyphes</i> - <i>Tricorythodes</i> + <i>Cabecar</i> + <i>Macunahyphes</i>	113.1	87.2 - 139.1	G, DEG, DG	0.27	E	0.37	F	1.00
<i>Leptohiphes</i> - <i>Traverhiphes</i> + <i>Vacupernius</i> + <i>Lumahyphes</i> + <i>Yaurina</i> + <i>Allenhyphes</i>	101.2	77.0 - 126.8	D	0.36	E	0.51	F	0.82
<i>Haplohyphes</i> - <i>Leptohiphodes</i> + <i>Amanahyphes</i>	110.3	82.2 - 138.4	**	**	-	-	F	1.00
<i>Leptohiphodes</i> - <i>Amanahyphes</i>	72.7	43.5 - 102.8	F	0.71	F	0.82	F	0.80
<i>A. saguassu</i> - <i>A. bahiensis</i>	39.6	19.8 - 65.2	F	0.81	F	1.00	EF	1.00
<i>Th. chiriguano</i> + <i>Th. minimus</i> + <i>Th. gibbus</i> + <i>Th. spongicola</i> - <i>Th. artigas</i> + <i>Th. araponga</i> + <i>Th. rondoniensis</i>	83.9	57.3 - 112.0	-	-	-	-	F	0.58
<i>Th. rondoniensis</i> - <i>Th. artigas</i> + <i>Th. araponga</i>	65.4	41.3 - 92.6	-	-	-	-	EF	0.75
<i>Th. artigas</i> - <i>Th. araponga</i>	47.0	24.6 - 71.4	F	1.00	-	-	F	0.61
<i>Th. chiriguano</i> + <i>Th. minimus</i> - <i>Th. gibbus</i> + <i>Th. spongicola</i>	68.9	44.4 - 96.2	F	1.00	-	-	F	1.00
<i>Th. chiriguano</i> - <i>Th. minimus</i>	1.4	0.3 - 3.3	F	1.00	F	0.64	EG	0.54
<i>Th. gibbus</i> - <i>Th. spongicola</i>	46.5	24.3 - 71.4	F	1.00	F	0.54	F	0.73

<i>L. albipennis</i> - <i>L. nebulosus</i> + <i>L. eximius</i> + <i>L. coconuco</i> + <i>L. plaumanni</i> + <i>L. nigripennis</i> + <i>L. sp.</i>	66.8	45.5 - 89.3	D	0.46	-	-	DFG	0.65
<i>L. nebulosus</i> - <i>L. eximius</i> + <i>L. coconuco</i> + <i>L. plaumanni</i> + <i>L. nigripennis</i> + <i>L. sp.</i>	60.2	40.5 - 81.7	DG	0.46	-	-	DFG	0.83
<i>L. eximius</i> + <i>L. coconuco</i> - <i>L. plaumanni</i> + <i>L. nigripennis</i> + <i>L. sp.</i>	41.9	26.4 - 58.8	-	-	-	-	DFG	0.70
<i>L. eximius</i> - <i>L. coconuco</i>	24.6	11.9 - 39.7	DEF, DF, DE	0.33	DEF, DE	0.35	DF	0.43
<i>L. plaumanni</i> - <i>L. nigripennis</i> + <i>L. sp.</i>	33.0	20.1 - 49.7	-	-	-	-	DFG	0.69
<i>A. flinti</i> + <i>Y. mota</i> - <i>Tr. indicator</i> + <i>Lu. guacra</i> + <i>Lu. cocal</i> + <i>Tr. sp.</i> + <i>Tr. yuati</i> + <i>V. packeri</i> + <i>Tr. sp. nov.</i>	67.5	49 - 89.6	**	**	-	-	F	0.59
<i>Tr. indicator</i> + <i>Lu. guacra</i> + <i>Lu. cocal</i> - <i>Tr. sp.</i> + <i>Tr. yuati</i> + <i>V. packeri</i> + <i>Tr. sp. nov.</i>	56.0	38.6 - 74.6	E	0.59	-	-	EF	0.85
<i>Tr. indicator</i> - <i>Lu. guacra</i> + <i>Lu. cocal</i>	44.0	28.3 - 62.3	EF	0.31	-	-	EF	0.77
<i>Lu. guacra</i> - <i>Lu. cocal</i>	30.3	16.6 - 46.4	E	0.70	-	-	EF	0.82
<i>Tr. sp.</i> + <i>Tr. yuati</i> - <i>V. packeri</i> + <i>Tr. sp. nov.</i>	47.0	30.3 - 65.9	E	0.97	-	-	E	0.81
<i>A. flinti</i> - <i>Y. mota</i>	43.0	19.9 - 69.1	**	**	-	-	DF	40.00
	91.0	68.1 - 113.5	**	**	-	-	F	0.53
<i>M. australis</i> + <i>T. santarita</i> + <i>T. aff. quizeri</i> + <i>M. eduardoi</i> + <i>M. araca</i> + <i>T. sp.</i> + <i>T. sp. nov. 1</i> + <i>T. sp. nov. 2</i> - <i>C. sp. nov.</i> + <i>T. sp. nov. 4</i> + <i>T. explicatus</i> + <i>T. cf. caunapi</i> + <i>T. caunapi</i> + <i>T. quizeri</i> + <i>T. tragoedia</i> + <i>T. cf. tragoedia</i> + <i>T. aff. Tragoedia</i>	79.6	58.5 - 101.6	G	0.68	-	-	F	0.55
<i>M. australis</i> + <i>T. santarita</i> - <i>T. aff. quizeri</i> + <i>M. eduardoi</i> + <i>M. araca</i> + <i>T. sp.</i> + <i>T. sp. nov. 1</i> + <i>T. sp. nov. 2</i>	46.4	24.9 - 69.4	G	0.68	-	-	F	0.41
<i>M. australis</i> - <i>T. santarita</i>	68.5	48.8 - 89.9	EG	1.00	-	-	F	0.36
<i>T. aff. quizeri</i> + <i>M. eduardoi</i> + <i>M. araca</i> - <i>T. sp.</i> + <i>T. sp. nov. 1</i> + <i>T. sp. nov. 2</i>	61.9	43.3 - 82.1	D	0.66	-	-	DEF	0.48
<i>C. sp. nov.</i> + <i>T. sp. nov. 4</i> + <i>T. explicatus</i> + <i>T. cf. caunapi</i> + <i>T. caunapi</i> + <i>T. quizeri</i> - <i>T. tragoedia</i> + <i>T. cf. tragoedia</i> + <i>T. aff. Tragoedia</i>	26.5	13.3 - 41.9	D	1.00	-	-	D	1.00

Appendix 1. Morphological data matrix coded for the phylogenetic analysis of Leptohiphidae.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	
Brasilocaenis_irmleri	0	2	0	1	0	1	1	0	1	0	0	4	0	1	1	?	0	0	0	0	0	0	?	2	?	?	?	?	?	?	0	0	3	?	0	0	0	2	0	0	
Drunella_ishiyamana	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
Coryphorus_aquilus	0	0	1	1	1	0	0	0	1	0	0	0	0	1	1	?	?	0	0	0	2	0	?	2	?	?	?	?	?	?	0	0	0	1	0	0	0	1	0	0	
Ephemerella_trilineata	0	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	2	0	0	0	1	0	0	
Ephemerythus_niger	1	1	0	1	0	0	0	0	1	0	0	0	0	0	1	1	2	0	0	0	1	1	1	0	1	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	
Lestagella_penicillata	?	0	1	0	0	?	0	0	2	0	0	0	0	0	1	0	1	?	0	0	2	1	1	0	0	0	1	0	0	0	0	1	1	0	0	0	1	0	0		
Lithogloea_harrisoni	0	0	1	0	0	0	0	0	2	0	0	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	
Machadorythus_maculatus	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	?	2	0	0	0	1	1	2	2	?	?	?	?	?	?	0	0	0	2	0	0	0	0	0	0	
Massartella_brieni	0	0	1	0	0	0	0	0	0	0	0	?	?	0	1	0	?	0	0	0	2	1	0	0	0	0	0	0	0	0	0	1	?	0	0	0	2	0	0		
Melanemerella_brasiliana	?	0	1	?	?	?	?	0	?	0	?	1	0	?	1	0	1	0	0	0	0	1	0	0	0	0	?	0	0	0	0	?	?	?	?	?	?	?	?	?	
Neoephemera_youngi	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2	0	0	
Ranorythus_violettae	?	2	1	?	?	?	?	?	1	0	0	0	?	0	1	?	2	0	0	0	1	0	?	2	?	?	?	?	?	?	0	0	0	1	0	0	0	2	0	0	
Serratella_ignita	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
Tricorythus	0	1	1	1	0	1	1	0	1	0	0	0	0	1	1	?	2	0	0	0	1	0	?	2	?	?	?	?	?	?	0	0	0	0	0	0	0	0	1	0	0
A_asperulus	?	?	0	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
A_flinti	1	2	0	1	0	0	0	2	2	0	0	0	0	1	1	1	0	1	0	0	1	0	?	1	2	1	1	1	3	1	0	1	1	2	0	0	0	2	0	0	
A_spinosus	?	?	0	1	?	?	?	?	?	?	?	?	0	?	?	?	?	?	?	?	?	?	?	?	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
A_vescus	1	2	0	1	0	0	0	2	2	0	0	0	0	1	1	1	0	1	0	0	1	0	?	1	2	1	1	1	3	1	0	1	1	2	0	0	0	2	0	0	
Am_bahiensis	?	0	1	0	?	?	?	0	2	2	0	?	?	0	1	1	?	0	1	0	1	1	0	?	2	?	?	?	?	?	0	0	0	1	0	0	0	1	1	0	
Am_saguassu	?	0	1	0	?	?	?	0	2	2	0	?	?	0	1	1	?	0	1	0	1	1	0	?	2	?	?	?	?	?	0	0	0	1	0	0	0	2	1	0	
Cabecar_spn	?	2	0	1	1	1	1	?	2	?	?	1	?	1	1	?	0	?	0	0	?	0	?	2	?	?	?	1	?	?	0	0	1	0	0	1	?	?	?	0	
H_aquilonius	1	2	0	1	0	0	0	0	2	0	0	0	0	1	1	1	1	1	0	0	1	0	?	0	1	0	2	1	1	1	0	0	0	0	1	0	0	1	1	0	
H_baritu	1	2	0	1	0	0	0	0	2	0	0	0	0	1	1	1	1	1	0	0	1	0	?	0	1	0	2	1	1	1	0	0	0	0	1	0	0	1	1	0	
H_dominguezi	1	2	0	1	0	0	0	0	2	0	0	0	0	1	1	1	1	1	0	0	1	0	?	0	1	0	2	1	1	1	0	0	0	0	1	0	0	1	1	0	
H_huallaga	1	2	0	1	0	0	0	0	2	0	0	0	0	1	1	1	1	1	0	0	1	0	?	0	1	0	2	1	3	1	0	0	0	0	1	0	0	1	1	0	
H_mithras	1	2	0	1	0	0	0	0	2	0	0	0	0	1	1	1	1	1	0	0	1	0	?	0	1	0	2	1	2	1	0	0	0	0	1	0	0	1	1	0	
H_yanahuicsa	1	2	0	1	0	0	0	0	2	0	0	0	0	1	1	1	1	1	0	0	1	0	?	0	1	0	2	1	2	1	0	0	0	0	1	0	0	1	1	0	
L_airuoca	?	?	0	1	?	?	?	?	?	?	?	?	0	?	?	?	?	?	?	?	?	?	?	?	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
L_albipennis	1	2	0	1	0	0	0	2	2	0	0	0	0	1	1	1	1	1	0	0	1	0	?	0	1	0	1	1	1	0	0	0	1	1	0	0	0	1	0	0	
L_andina	?	?	0	1	?	?	?	?	?	?	?	?	0	?	?	?	?	?	?	?	?	?	?	?	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
L_calarca	?	?	0	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
L_carinus	?	?	0	1	?	?	?	?	?	?	?	?	?	0	?	?	?	?	?	?	?	?	?	?	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
L_coconuco	1	2	0	1	0	0	0	2	2	0	0	0	0	1	1	1	1	1	0	0	1	0	?	1	1	0	1	1	1	0	0	0	1	1	0	0	0	1	0	0	
L_cornutillus	?	?	0	1	?	?	?	?	?	?	?	?	0	?	?	?	?	?	?	?	?	?	?	?	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
L_cornutus	1	2	0	1	0	0	0	2	2	0	0	0	0	1	1	1	0	0	0	0	0	?	2	0	1	0	1	1	1	0	0	0	1	0	0	0	0	1	0	0	
L_ecuador	?	2	0	1	0	0	0	2	2	0	0	0	0	1	1	1	1	1	0	0	1	0	?	1	1	0	1	1	1	0	0	0	1	1	0	0	0	1	0	0	
L_eximius	1	2	0	1	0	0	0	2	2	0	0	0	0	1	1	1	1	1	0	0	1	0	?	1	1	0	1	1	1	0	0	0	1	1	0	0	0	1	0	0	
L_illiesi	?	?	0	1	?	?	?	?	?	?	?	?	0	?	?	?	?	?	?	?	?	?	?	?	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	

L_invictus	? ? 0 1 ? ? ? ? ? ? ? ? 0 ?
L_jodiannae	? ? 0 1 ? ? ? ? ? ? ? ? 0 ?
L_liniti	? ? 0 1 ? ? ? ? ? ? ? ? 0 ?
L_maculatus	1 2 0 1 0 0 0 2 2 0 0 0 0 1 1 1 1 1 0 0 1 0 ? 1 1 0 1 1 1 1 0 0 1 ? ? 0 0 0 1 0 0
L_nebulosus	? ? 0 1 ? ? ? ? ? ? ? ? ? ? 0 ?
L_nigripennis	1 2 0 1 0 0 0 2 2 0 0 0 0 1 1 1 1 1 0 0 1 0 ? 0 1 0 1 1 1 1 0 0 0 1 1 0 0 0 1 0 0
L_petersi	1 2 0 1 0 0 0 2 2 0 0 0 0 1 1 1 1 1 0 0 1 0 ? 1 1 0 1 1 1 1 0 0 1 ? 0 0 0 1 0 0
L_plaumanni	1 2 0 1 0 0 0 2 2 0 0 0 0 1 1 1 1 1 0 0 1 0 ? 1 1 0 1 1 1 1 0 0 1 0 0 0 0 1 0 0
L_populus	? ? 1 0 ? ? ? ? ? ? ? ? ? 0 ?
L_quimbaya	? ? 0 1 ? ? ? ? ? ? ? ? ? 0 ?
L_setosus	1 2 0 1 0 0 0 2 2 0 0 0 0 1 1 1 1 ? 0 0 1 0 ? 1 1 0 1 1 1 1 0 0 1 ? 0 0 0 1 0 0
L_tacajalo	? ? 0 1 ? ? ? ? ? ? ? ? ? 0 ?
L_tuberculatus	? ? 0 1 ? ? ? ? ? ? ? ? ? 0 ?
Le_inanis	? 0 1 0 0 0 2 2 0 0 1 0 1 1 ? 0 1 0 0 1 0 ? 1 2 ? ? ? ? ? ? ? ? 0 0 0 0 1 0 0 1 1 0
Lo_froehlichii	? ? 0 1 ? ? ? ? ? ? ? ? ? 0 ?
Lu_cocal	? 2 0 1 0 0 0 2 2 ? 0 0 0 1 1 1 0 1 0 0 1 0 ? 1 2 1 1 1 2 1 0 1 1 0 0 0 0 2 0 1
Lu_guacra	1 2 0 1 0 0 0 2 2 0 0 0 0 1 1 1 0 0 0 0 1 0 ? 1 2 1 1 1 2 1 0 1 1 0 0 0 0 2 0 1
Lu_pijcha	1 2 0 1 0 0 0 2 2 0 0 0 0 1 1 1 0 0 0 0 1 0 ? 1 2 1 1 1 2 1 0 1 1 0 0 0 0 2 0 1
Lu_yagua	1 2 0 1 0 0 0 2 2 0 0 0 0 1 1 1 0 0 0 0 1 0 ? 1 2 1 1 1 2 1 0 1 1 0 0 0 0 2 0 1
M_araca	1 2 0 1 1 1 1 0 2 0 2 1 0 1 0 ? 0 1 0 0 ? 0 ? 2 ? ? ? ? ? ? ? 0 0 1 0 0 1 1 1 1 0
M_australis	1 2 0 1 1 1 1 1 2 0 2 1 0 1 0 ? 0 1 0 0 ? 0 1 0 0 ? 0 ? 2 ? ? ? ? ? ? ? 0 0 1 1 0 0 1 0 0
M_eduardoi	? 2 0 1 1 1 1 2 0 2 1 0 1 0 ? 0 1 0 0 ? 0 ? 2 ? ? ? ? ? ? ? ? 0 0 1 0 0 1 0 1 1 0
M_pemonensis	? 2 0 1 1 1 1 0 2 0 ? 1 0 1 0 ? 0 1 0 0 ? 0 ? 2 ? ? ? ? ? ? ? ? 0 0 1 0 0 1 1 1 1 0
M_zagaia	1 2 0 1 1 1 1 0 2 ? 2 1 0 1 0 ? 0 1 0 0 ? 0 ? 2 ? ? ? ? ? ? ? ? 0 1 1 0 0 1 1 1 1 0
Tricorythodes_spn1	? ?
Tricorythodes_spn2	? ?
T_arequita	1 2 0 1 1 1 1 2 0 2 1 1 1 1 ? 0 1 0 0 1 0 ? 2 ? ? ? ? ? ? ? ? 0 0 1 0 0 1 1 2 0 0
T_barbus	? ? 0 1 ? ? ? ? ? ? ? ? ? 0 ?
T_bullus	1 2 0 1 1 1 1 2 0 2 1 0 1 0 ? 0 1 0 0 1 0 ? 2 ? ? ? ? ? ? ? ? 0 0 1 0 0 1 1 2 0 0
T_caunapi	1 2 0 1 1 1 1 2 0 2 1 0 1 0 ? 0 1 0 0 ? 0 ? 2 ? ? ? ? ? ? ? ? 0 0 1 0 0 1 0 1 0 0
T_cfcaunapi	? ?
T_cristatus	? ? 0 1 ? ? ? ? ? ? ? ? ? 0 ?
T_curiosus	? ? 0 1 ? ? ? ? ? ? ? ? ? 0 ?
T_dimorphus	1 2 1 1 1 1 1 2 0 2 1 0 1 ? ? 0 1 0 0 1 0 ? 2 ? ? ? ? ? ? ? ? 0 0 1 0 0 1 0 2 0 0
T_explicatus	? 2 0 1 1 1 1 ? 2 0 ? 1 0 1 1 ? 0 0 0 0 1 0 ? 2 ? ? ? ? ? ? ? ? 0 0 1 0 0 1 ? 2 ? 0
T_hiemalis	1 2 0 1 1 1 1 2 0 2 1 0 1 1 ? 0 1 0 0 1 0 ? 2 ? ? ? ? ? ? ? ? 0 0 1 0 0 1 1 2 0 0
T_mirca	1 2 0 1 1 1 1 2 0 2 1 1 1 ? 0 1 0 0 1 0 ? 2 ? ? ? ? ? ? ? ? 0 0 1 0 0 1 1 2 0 0
T_nicholsae	? ? 0 1 ? ? ? ? ? ? ? ? ? 0 ?
T_ocellus	1 2 0 1 1 1 0 1 2 0 2 1 0 1 1 ? 0 1 0 0 1 0 ? 2 ? ? ? ? ? ? ? ? 0 0 1 0 0 1 1 2 0 0
T_popayanicus	1 2 0 1 1 1 1 2 0 2 1 0 1 1 ? 0 1 0 0 1 0 ? 2 ? ? ? ? ? ? ? ? 0 0 1 0 0 1 1 2 0 0
T_quizeri	1 2 0 1 1 1 1 2 0 2 1 0 1 1 ? 0 1 0 0 1 0 ? 2 ? ? ? ? ? ? ? ? 0 0 1 0 0 1 1 2 0 0
T_santarita	1 2 0 1 1 1 1 2 0 2 1 0 1 0 ? 0 1 0 0 1 0 ? 2 ? ? ? ? ? ? ? ? 0 0 1 0 0 1 1 2 0 0
T_tragoedia	1 2 0 1 1 1 1 0 2 0 ? 1 1 1 1 ? 0 1 0 0 1 0 ? 2 ? ? ? ? ? ? ? ? 0 0 1 0 0 1 1 2 0 0
T_yura	1 2 0 1 1 1 1 2 0 2 1 0 1 1 ? 0 1 0 0 1 0 ? 2 ? ? ? ? ? ? ? ? 0 0 1 0 0 1 1 2 0 0

T_zika	? ?
E_undatus	1 2 0 1 1 1 1 1 ? 0 2 ? 1 0 1 ? 0 1 0 0 1 0 ? ? ? ? ? ? ? ? ? ? 0 0 1 0 0 1 1 2 0 0
T_sallesi	? ?
C_serratus	1 2 0 1 1 1 1 1 2 0 2 1 0 1 1 ? 0 0 0 0 1 0 ? ? ? ? ? ? ? ? ? 0 0 1 0 0 1 1 2 0 0
T_zunigae	1 2 0 1 1 1 1 1 2 ? 2 1 0 1 1 ? 0 1 0 0 1 0 ? 2 ? ? ? ? ? ? ? ? 0 0 1 0 0 1 1 2 0 0
Th_artigas	1 2 0 1 1 0 0 1 2 1 1 0 0 1 1 ? 0 1 1 2 1 0 ? 2 ? ? ? ? ? ? ? ? 0 0 0 0 0 0 1 1 0
Th_chiriguano	1 2 0 1 1 0 0 1 2 1 1 0 0 1 1 ? 0 1 1 2 1 0 ? 2 ? ? ? ? ? ? ? ? 0 0 0 0 0 0 1 1 0
Th_gibbus	1 2 0 1 1 0 0 1 2 1 1 0 0 1 1 ? 0 1 1 2 1 0 ? 2 ? ? ? ? ? ? ? ? 0 0 0 0 0 0 1 1 0
Th_minimus	1 2 0 1 1 0 0 1 2 1 1 0 0 1 1 ? 0 1 1 2 1 0 ? 2 ? ? ? ? ? ? ? ? 0 0 0 0 0 0 1 1 0
Th_rondonienseis	? ? 0 1 ?
Th_sigillatus	1 2 0 1 1 0 0 1 2 1 1 0 0 1 1 ? 0 1 1 2 1 0 ? 2 ? ? ? ? ? ? ? ? 0 0 0 0 0 0 1 1 0
Th_undulatus	1 2 0 1 1 0 0 1 2 1 1 0 0 1 1 ? 0 1 1 2 1 0 ? 2 ? ? ? ? ? ? ? ? 0 0 0 0 0 0 1 1 0
Th_volsellus	1 2 0 1 1 0 0 1 2 1 1 0 0 1 1 ? 0 1 1 2 1 0 ? 2 ? ? ? ? ? ? ? ? 0 0 0 0 0 0 1 1 0
Th_yacutinga	1 2 0 1 1 0 0 1 2 1 1 0 0 1 1 ? 0 1 1 2 1 0 ? 2 ? ? ? ? ? ? ? ? 0 0 0 0 0 0 1 1 0
Traverhyphes_spn	1 2 0 1 0 0 0 2 2 0 0 0 0 1 1 1 0 1 0 0 1 0 ? 1 2 1 1 1 2 1 1 1 1 0 0 0 2 0 1
Tr_chiquitano	1 2 0 1 0 0 0 2 2 0 0 0 0 1 1 1 0 1 0 0 1 0 ? 1 2 1 1 1 2 1 1 1 1 0 0 0 2 0 1
Tr_edmundsi	1 2 0 1 0 0 0 2 2 0 0 0 0 1 1 1 0 1 0 0 1 0 ? 1 2 1 1 1 2 1 0 1 1 1 0 0 0 2 0 1
Tr_frevo	1 2 0 1 0 0 0 2 2 ? 0 0 0 1 1 1 0 1 0 0 1 0 ? 1 2 1 1 1 2 1 1 1 1 1 0 0 0 2 0 1
Tr_indicator	1 2 0 1 0 0 0 2 2 0 0 0 0 1 1 1 0 1 0 0 1 0 ? 1 2 1 1 1 2 1 1 1 1 1 0 0 0 2 0 1
Tr_nanus	1 2 0 1 0 0 0 2 2 0 0 0 0 1 1 1 0 1 0 0 1 0 ? 1 2 1 1 1 2 1 0 1 1 2 0 0 0 2 0 1
Tr_pirai	1 2 0 1 0 0 0 2 2 0 0 0 0 1 1 1 0 1 0 0 1 0 ? 1 2 1 1 1 2 1 1 1 1 1 0 0 0 2 0 1
Tr_yuati	1 2 0 1 0 0 0 2 2 0 0 0 0 1 1 1 0 1 0 0 1 0 ? 1 2 1 1 1 2 1 0 1 1 1 0 0 0 2 0 1
Tr_yuqui	1 2 0 1 0 0 0 2 2 0 0 0 0 1 1 1 0 1 0 0 1 0 ? 1 2 1 1 1 2 1 0 1 1 2 0 0 0 2 0 1
V_packeri	? 2 0 1 0 0 0 2 2 0 0 0 0 1 1 1 0 1 0 0 1 0 ? 1 2 1 1 1 2 1 0 1 1 1 0 0 0 2 0 1
Y_mota	1 2 0 1 0 0 0 2 2 0 0 0 0 1 1 1 0 1 0 0 1 0 ? 1 2 1 1 1 2 1 0 1 1 2 0 0 0 2 0 0
Y_ralla	? ? 0 1 ?
Y_yapa	1 2 0 1 0 0 0 2 2 ? 0 0 0 1 1 1 0 1 0 0 1 0 ? 1 2 1 1 1 2 1 0 1 1 2 0 0 0 2 0 0
Y_yuta	1 2 0 1 0 0 0 2 2 0 0 0 0 1 1 1 0 1 0 0 1 0 ? 1 2 1 1 1 2 1 0 1 1 2 0 0 0 2 0 0

Continuation

	4	4	4	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	8
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0			
Brasilocaenis_irmleri	0	?	?	?	?	0	?	0	0	0	?	2	1	0	2	0	0	0	0	0	1	1	0	?	0	2	1	1	1	0	0	0	1	1	2	0	0	1	2	1			
Drunella_ishiyamana	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	0	?	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	
Coryphorus_aquilus	0	?	?	?	?	0	?	0	0	0	?	2	1	0	2	0	0	0	0	0	0	1	0	?	0	1	1	1	3	0	1	0	1	0	2	0	0	0	1	0			
Ephemerella_trilineata	0	?	?	?	?	0	?	0	0	0	?	1	0	0	2	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1		
Ephemerythus_niger	0	?	?	?	?	0	?	0	0	0	?	1	1	0	2	0	0	0	0	0	0	1	1	0	0	0	0	1	3	0	0	1	0	0	2	0	0	1	0	0			
Lestagella_penicillata	0	0	0	?	?	0	?	0	0	0	?	1	1	0	2	0	0	0	0	0	0	1	0	?	0	2	1	?	3	?	?	1	1	1	2	?	0	1	0	0			
Lithogloea_harrisoni	0	?	?	?	?	0	?	0	0	0	?	1	1	0	2	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	1		
Machadorythus_maculatus	0	?	?	?	?	0	?	0	0	0	?	2	1	0	2	0	0	0	0	0	0	1	0	?	0	0	2	1	1	0	0	0	0	1	2	2	0	0	0	1			
Massartella_brieni	0	?	?	?	?	0	?	0	0	0	?	0	?	?	2	0	0	0	0	0	0	1	0	?	0	3	2	1	0	0	0	1	1	1	2	?	0	1	2	0			
Melanemerella_brasiliana	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	?	0	1	1	0	0	1	0	1	2	0	1	1	0	0	0	0	0	1	0	0		
Neoephemera_youngi	0	?	?	?	?	0	?	0	0	0	?	1	1	0	2	0	0	0	0	0	1	1	0	?	0	2	2	1	0	0	0	0	1	1	0	0	0	1	2	1			
Ranorythus_violettae	0	?	?	?	?	0	?	0	0	0	?	1	0	0	2	0	0	0	0	0	1	1	0	?	0	2	2	1	0	0	0	1	1	1	2	0	0	0	0	1			
Serratella_ignita	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	0	?	0	2	1	1	0	?	0	0	0	0	0	0	0	1	1	2	0		
Tricorythus	0	?	?	?	?	0	?	0	0	0	?	1	1	0	2	0	0	0	0	0	0	1	1	0	0	0	2	1	0	0	0	1	0	1	2	2	0	0	0	1			
A_asperulus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	1	0	0	1	1	1	0	0	1	1	1	1	1	1	0	0	1	1	0		
A_flinti	2	0	0	1	2	1	1	0	1	0	?	1	1	0	2	0	1	0	0	1	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0		
A_spinus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	1	0	0	1	1	1	0	0	1	1	1	1	1	1	0	0	1	1	0		
A_vescus	2	0	0	1	2	1	1	0	1	0	?	1	1	0	2	0	1	0	0	1	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	
Am_bahiensis	1	0	0	0	1	1	1	0	0	0	?	1	0	0	2	0	0	0	0	0	?	1	0	?	1	1	2	1	2	1	1	1	1	1	1	1	0	0	0	1	0	0	
Am_saguassu	1	0	0	0	1	1	1	0	0	0	?	1	1	1	2	0	0	0	0	0	1	1	0	?	1	1	2	1	2	1	1	1	1	1	1	0	0	0	1	0	0		
Cabecar_spn	0	0	0	?	?	1	0	0	?	0	?	?	1	0	2	0	0	0	0	0	?	0	0	?	0	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0			
H_aquilonius	1	0	0	0	1	1	1	0	1	0	?	1	0	0	2	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	2	1	0	1	1	1		
H_baritu	1	0	0	0	1	1	1	0	1	0	?	1	0	0	2	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	2	1	0	1	1	1		
H_dominguezi	1	0	0	0	1	1	1	0	1	0	?	1	0	0	2	0	0	0	0	0	?	?	?	1	?	?	1	1	?	1	?	?	?	?	?	?	?	?	?	?			
H_huallaga	1	0	0	0	1	1	1	0	1	0	?	1	0	0	2	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	2	1	0	1	1	1			
H_mithras	1	0	0	0	1	1	1	0	1	0	?	1	0	0	2	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	2	1	0	1	1	1		
H_yanahuicsa	1	0	0	0	1	1	1	0	1	0	?	1	0	0	2	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	2	1	0	1	1	1		
L_airuoca	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	1	0	1	1	1	1	0	0	0	1	0	0	0	0	0	0	1	1	0		

Tricorythodes_spn1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	0	?	0	1	1	1	2	1	1	1	1	1	1	1	0	0	1	1	0				
Tricorythodes_spn2	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	1	0	0	1	1	1	2	1	1	1	1	0	2	0	0	1	1	0				
T_arequita	0	?	?	0	1	1	0	0	1	0	?	1	1	0	2	0	0	0	0	0	1	1	1	1	0	1	1	1	0	0	1	1	1	1	2	0	0	1	1	0			
T_barbus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	0	1	1	0			
T_bullus	0	?	?	0	1	1	0	0	1	0	?	0	1	0	2	0	0	0	0	0	1	1	1	1	0	1	1	1	2	1	1	1	1	1	1	0	0	1	1	0			
T_caunapi	0	?	?	?	?	1	0	0	1	0	?	0	1	0	2	0	0	0	0	0	?	1	1	0	1	1	2	1	2	1	1	0	1	0	2	0	0	1	1	0			
T_cfcaunapi	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	1	0	0	1	1	1	2	1	1	0	1	0	2	0	0	1	1	0			
T_cristatus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	?	1	?	1	1	0	1	1	1	2	1	1	1	1	1	2	0	0	1	1	0		
T_curiosus	0	?	?	0	1	1	0	0	1	0	?	0	1	0	2	0	0	0	0	0	1	1	1	1	0	1	1	1	2	1	1	0	1	1	2	0	0	1	1	0			
T_dimorphus	0	?	?	0	1	1	0	0	1	0	?	0	1	0	2	0	0	0	0	0	1	1	1	0	0	1	1	1	2	?	1	1	1	1	1	0	0	1	1	0			
T_explicatus	0	?	?	?	?	1	0	0	?	0	?	0	1	0	2	0	0	0	0	0	1	?	?	?	?	1	?	?	1	1	1	1	1	?	?	1	0	0	1	1	0		
T_hiemalis	0	?	?	0	1	1	0	0	1	0	?	0	1	0	2	0	0	0	0	0	1	0	1	0	0	1	1	1	1	1	1	1	1	1	1	2	0	0	1	1	0		
T_mirca	0	?	?	0	1	1	0	0	1	0	?	1	1	0	2	0	0	0	0	0	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0		
T_nicholsae	?	?	?	0	1	1	0	0	1	0	?	0	1	0	2	0	0	0	0	0	1	1	1	1	1	1	1	1	3	1	1	?	?	?	?	?	?	1	1	1	0		
T_ocellus	1	0	0	0	1	1	1	0	1	0	?	1	0	0	2	0	0	0	?	0	?	0	1	1	0	1	1	1	1	0	1	1	1	1	?	0	0	1	1	0			
T_popayanicus	0	?	?	0	1	1	0	0	1	0	?	0	1	0	2	0	0	0	0	0	1	0	1	1	0	1	1	1	1	1	1	1	?	?	1	0	0	1	1	0			
T_quizeri	0	?	?	0	1	1	0	0	1	0	?	0	1	0	2	0	0	0	0	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	
T_santarita	0	?	?	0	1	1	0	0	1	0	?	0	1	0	2	0	0	0	0	0	1	1	1	1	0	1	1	1	2	1	1	1	1	1	1	2	0	1	1	1	0		
T_tragoedia	0	?	?	?	?	1	0	0	1	0	?	0	1	0	2	0	0	0	0	0	0	1	?	?	0	1	1	1	0	1	1	1	?	?	1	0	0	1	1	0			
T_yura	0	?	?	0	1	1	0	0	1	0	?	0	1	0	2	0	0	0	0	0	1	?	1	1	0	1	1	1	1	1	1	1	1	1	1	2	0	0	1	1	0		
T_zika	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	0	?	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	
E_undatus	0	?	?	0	1	1	0	0	?	0	?	0	1	0	2	0	?	?	0	?	0	1	0	?	0	1	1	1	0	1	1	1	0	0	1	0	0	1	0	0	1	1	0
T_sallesi	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	1	0	0	1	1	1	1	0	1	0	1	1	2	0	0	1	1	0			
C_serratus	0	?	?	0	1	1	0	0	1	0	?	0	1	0	2	0	0	0	0	0	?	0	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0		
T_zunigae	0	?	?	0	1	1	0	0	1	0	?	0	1	0	2	0	0	0	0	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	2	0	0	1	1	0		
Th_artigas	0	?	?	?	?	0	?	0	0	0	?	0	1	0	2	0	0	0	0	0	2	1	1	0	0	1	1	1	2	1	1	0	1	0	0	0	0	0	1	1	0		
Th_chiriguano	0	?	?	?	?	0	?	0	0	0	?	0	1	0	2	0	0	0	0	0	2	1	1	0	0	1	1	1	2	1	1	0	1	0	0	0	0	0	1	1	0		
Th_gibbus	0	?	?	?	?	0	?	0	1	0	?	0	1	0	2	0	0	0	0	0	2	1	1	0	0	1	1	1	2	1	1	0	1	0	0	0	0	0	1	1	0		
Th_minimus	0	?	?	?	?	0	?	0	0	0	?	0	1	0	2	0	0	0	0	0	2	1	1	0	0	1	1	1	2	1	1	0	1	0	0	0	0	0	1	1	0		
Th_rondoniense	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	1	0	0	1	1	1	2	1	1	0	1	0	0	0	0	0	1	1	0		
Th_sigillatus	0	?	?	?	?	0	?	0	0	0	?	0	1	0	2	0	0	0	0	0	2	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
Th_undulatus	0	?	?	?	?	0	?	0	1	0	?	0	1	0	2	0	0	0	0	0	2	1	1	0	0	1	1	1	2	1	1	0	1	0	0	0	0	0	1	1	0		
Th_volsellus	0	?	?	?	?	0	?	0	0	0	?	0	1	0	2	0	0	0	0	0	2	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
Th_yacutinga	0	?	?	?	?	0	?	0	0	0	?	0	1	0	2	0	0	0	0	0	2	1	1	0	0	1	1	1	2	1	1	0	1	0	0	0	0	0	1	1	0		

Traverhyphes_spn	2	1	1	0	0	1	1	0	1	1	0	1	1	0	2	0	0	1	0	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	
Tr_chiquitano	2	0	1	0	1	1	1	1	1	1	0	1	0	0	2	0	0	2	0	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0
Tr_edmundsi	2	1	1	0	1	1	1	1	1	1	0	1	1	1	2	0	0	3	0	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0
Tr_frevo	2	0	1	0	1	1	1	1	1	1	0	1	0	0	2	0	0	2	?	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Tr_indicator	2	0	1	0	0	1	1	1	1	1	0	1	0	0	2	0	0	2	0	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	
Tr_nanus	2	1	0	0	0	1	1	1	0	1	0	1	1	0	2	0	0	1	0	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	
Tr_pirai	2	0	1	0	1	1	1	1	1	1	0	1	0	0	2	0	0	2	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Tr_yuati	2	1	1	0	1	1	1	1	1	1	0	1	1	1	2	0	0	3	0	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	
Tr_yuqui	2	1	0	0	0	1	1	1	1	1	0	1	1	0	2	0	0	1	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
V_packeri	2	0	1	0	1	1	1	1	?	?	?	2	1	0	2	0	0	?	?	0	?	1	1	0	0	1	2	1	0	0	0	1	1	1	1	1	0	0	1	1	0			
Y_mota	2	1	0	1	2	1	1	0	1	0	?	1	1	0	2	1	0	0	0	0	0	1	1	0	0	1	1	0	1	0	1	1	1	1	1	1	0	0	1	1	0			
Y_ralla	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	1	0	0	1	1	0	1	0	1	1	1	1	1	1	0	0	1	1	0			
Y_yapa	2	1	0	1	2	1	1	0	1	0	?	1	1	0	2	1	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
Y_yuta	2	1	0	1	2	1	1	0	1	0	?	1	1	0	2	1	0	0	0	0	1	1	0	0	1	1	0	1	0	1	1	1	1	1	1	1	0	0	1	1	0			

Continuation

	8	8	8	8	8	8	8	8	8	9	9	9	9	9	9	9	9	9	9	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	11	11	11	11	11	11	11	11	11	11	11	11	12		
Brasilocaenis_irmlieri	0	1	0	0	0	1	?	0	1	0	?	1	0	0	?	?	?	?	?	?	?	?	?	0	1	1	1	0	1	1	?	?	?	?	0	0	2	1	?	0	0	1									
Drunella_ishiyamana	0	1	1	0	1	0	?	1	1	0	?	1	1	1	0	?	?	?	?	?	?	?	?	1	?	0	?	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1		
Coryphorus_aquilus	0	1	1	2	0	0	?	0	1	0	?	1	1	1	0	0	0	0	0	1	4	0	?	1	?	1	1	0	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1							
Ephemerella_trilineata	0	1	0	0	0	1	0	0	0	?	0	0	1	0	0	0	0	0	0	1	0	1	0	1	?	0	?	0	0	0	0	0	0	2	?	0	1	0	0	0	1										
Ephemerythus_niger	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	?	1	?	1	1	0	1	0	0	1	2	0	0	1	0	0	0	1												
Lestagella_penicillata	0	1	0	0	0	1	1	0	1	1	1	1	0	1	0	0	0	0	?	0	?	0	?	0	1	1	1	0	1	0	0	?	2	0	0	0	0	0	0	0	0	1									
Lithogloea_harrisoni	0	0	0	0	0	1	1	0	0	0	?	0	0	1	0	0	0	0	0	1	0	1	0	0	1	1	1	0	1	0	0	0	2	0	0	1	0	0	0	1											
Machadorythus_maculatus	1	1	1	2	0	0	?	0	1	1	0	1	1	1	1	0	0	0	0	1	0	1	0	1	?	1	1	0	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1							
Massartella_brieni	0	1	0	1	0	0	?	0	1	0	?	1	0	0	?	0	?	?	?	?	?	?	?	?	0	0	1	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1			
Melanemerella_brasiliiana	1	0	0	0	0	1	2	1	0	0	?	0	1	1	0	0	0	0	0	1	0	1	0	1	?	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1								
Neophemera_youngi	0	0	0	1	0	1	0	0	1	0	?	1	1	0	?	0	1	0	0	1	0	1	0	0	1	1	1	0	1	1	0	0	0	0	0	0	1	1	?	0	0	1									
Ranorythus_violettae	0	0	0	1	0	1	1	0	1	1	0	1	0	1	0	0	0	0	0	1	0	1	0	1	?	1	0	0	1	0	0	0	2	0	0	1	0	0	0	1											
Serratella_ignita	0	1	0	?	0	1	0	0	1	1	1	1	1	1	0	0	?	0	0	0	?	0	?	1	?	0	?	1	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	
Tricorythus	0	0	0	0	0	1	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0	1	0	1	?	1	0	0	1	0	0	1	2	0	0	1	0	0	0	1											
A_asperulus	0	1	0	1	0	1	0	0	1	1	0	1	0	1	1	0	2	?	?	?	?	?	?	?	1	?	1	1	0	1	0	1	1	2	0	1	1	1	0	0	1										
A_flinti	0	1	0	1	0	1	0	0	1	1	1	1	0	1	1	0	2	3	3	1	2	1	1	1	?	1	1	0	1	0	1	1	2	0	1	1	1	0	0	1											
A_spinosus	0	1	0	1	0	1	0	0	1	1	0	1	0	1	1	0	2	?	?	?	?	?	?	1	?	1	?	1	1	0	1	0	1	1	2	2	1	1	1	0	0	1									
A_vescus	0	1	0	1	0	1	0	0	1	1	1	1	0	1	1	0	2	3	3	1	2	1	1	1	?	1	1	0	1	0	1	1	2	0	1	1	1	0	0	1											
Am_bahiensis	0	1	0	2	0	1	0	0	1	1	0	1	0	1	1	[0	1]	2	4	4	1	3	0	?	1	?	1	1	0	1	1	0	1	1	0	0	1	0	0	1									
Am_saguassu	0	1	0	1	0	1	0	0	1	1	0	1	1	1	[0	1]	0	2	3	1	3	0	?	1	?	1	1	0	1	0	1	1	1	0	0	0	1	0											
Cabecar_spn	0	1	0	2	1	1	1	0	1	1	0	0	0	1	1	0	2	3	3	1	3	0	?	1	?	1	1	0	1	0	1	1	0	1	2	1	1	0	0	0											
H_aquilonius	1	0	0	2	1	1	0	0	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	?	1	1	0	1	0	1	1	1	0	2	1	1	0	0	0												
H_baritu	1	0	0	2	1	1	0	0	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	?	1	1	0	1	0	1	1	1	0	2	1	1	0	0	0												
H_dominguezi	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
H_huallaga	1	0	0	2	1	1	0	0	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	?	1	1	0	1	0	1	1	1	0	2	1	1	0	0	0												
H_mithras	1	0	0	2	1	1	0	0	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	?	1	1	0	1	0	1	1	1	0	2	1	1	0	0	0												
H_yanahuicsa	1	0	0	2	1	1	0	0	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	?	1	1	0	1	0	1	1	1	0	2	1	1	0	0	0												
L_airuoca	0	1	1	0	0	1	1	0	0	?	0	1	1	1	0	2	0	0	1	0	1	0	1	?	1	1	0	1	0	1	1	2	0	0	0	0	0	0	0	1											
L_albipennis	0	1	0	0	0	1	1	0	0	1	2	0	0	1	1	0	2	2	2	1	2	1	1	1	?	1	1	0	1	0	1	1	2	2	0	0	0	0	0	1											
L_andina	0	1	0	0	0	1	1	0	0	?	0	0	1	1	0	2	1	1	1	1	1	1	1	?	1	1	0	1	0	1	1	2	0	0	0	0	0	0	1												
L_calarca	0	1	0	0	0	1	1	0	0	1	2	0	0	1	1	0	2	2	2	1	2	1	2	1	?	1	1	0	1	0	1	1	2	0	0	0	0	0	0	1											
L_carinus	0	0	0	0	0	1	1	0	0	1	1	0	1	1	1	0	2	2	2	1	2	1	1	1	?	1	1	0	1	0	1	1	2	2	0	0	0	0	0	1											
L_coconuco	0	1	0	0	0	1	1	0	0	1	2	0	0	1	1	0	2	2	2	1	2	1	1	1	?	1	1	0	1	0	1	1	2	2	0	0	0	0	0	1											
L_cornutillus	0	1	1	0	0	1	2	0	0	1	2	0	1	1	1	0	2	0	0	1	0	1	0	1	?	1	1	0	1	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1				

T_zika	0	0	1	2	0	1	0	0	1	0	?	0	0	1	1	1	2	3	3	1	2	1	1	1	?	1	1	0	1	0	1	1	2	1	2	1	1	0	0	0	0	
E_undatus	0	0	0	2	0	1	1	0	1	1	2	0	1	1	1	1	2	3	3	1	2	1	1	1	?	1	1	0	1	0	1	1	[0	1]	1	1	1	1	1	
T_sallesi	0	0	0	2	0	1	1	0	1	0	?	0	0	1	1	1	2	3	3	1	2	1	1	1	?	1	1	0	1	0	1	1	1	1	2	1	1	0	0	1	1	
C_serratus	0	0	0	2	0	1	2	0	1	0	?	0	0	1	1	1	3	3	2	1	1	1	1	1	?	1	1	0	1	0	1	1	1	1	2	1	1	0	0	0	0	
T_zunigae	1	0	0	2	0	1	2	1	1	1	0	0	0	1	1	1	2	3	3	1	2	1	1	1	?	1	1	0	1	0	1	1	2	1	2	1	1	0	0	0	0	
Th_artigas	0	1	0	1	0	1	0	0	1	1	0	0	0	1	1	0	2	2	1	3	1	2	1	?	1	1	0	1	0	1	1	2	0	0	1	0	1	0	1	0	1	
Th_chiriguano	0	1	0	1	0	1	0	0	1	1	0	0	0	1	1	0	2	2	1	3	1	2	1	?	1	1	0	1	0	1	1	2	0	0	1	0	1	0	1	0	1	
Th_gibbus	0	1	0	0	0	1	0	0	1	1	0	0	1	1	1	0	2	2	1	3	1	2	1	?	1	1	0	1	0	1	1	2	0	0	1	0	1	0	1	0	1	
Th_minimus	0	1	0	1	0	1	0	0	1	1	0	0	0	1	1	0	2	2	1	3	1	2	1	?	1	1	0	1	0	1	1	2	0	0	1	0	1	0	1	0	1	
Th_rondoniense	0	1	0	2	0	1	1	1	1	1	0	0	1	1	1	0	2	2	1	3	1	2	1	?	1	1	0	1	0	1	1	2	0	0	1	0	1	0	1	0	1	
Th_sigillatus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Th_undulatus	0	1	0	0	0	1	0	0	1	1	0	0	1	1	1	0	2	2	1	3	1	2	1	?	1	1	0	1	0	1	1	2	0	0	1	0	1	0	1	0	1	
Th_volsellus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Th_yacutinga	0	1	0	2	0	1	1	1	1	1	0	0	0	1	1	0	2	2	1	3	1	2	1	?	1	1	0	1	0	1	1	2	0	0	1	0	1	0	1	0	1	
Traverhyphes_sp	0	1	0	1	0	1	?	0	1	1	0	1	0	1	1	0	2	3	3	1	2	1	1	1	?	1	1	0	1	0	1	1	2	2	?	1	1	0	0	1	1	
n	0	1	0	1	0	1	0	0	1	1	0	1	1	1	1	0	2	2	2	1	2	1	1	1	?	1	1	0	1	0	1	1	2	0	2	1	1	0	1	1	1	
Tr_chiquitano	0	1	0	1	0	1	0	0	1	1	0	1	1	1	1	0	2	2	2	1	2	1	1	1	?	1	1	0	1	0	1	1	2	0	2	1	1	0	1	1	1	
Tr_edmundsi	0	1	0	1	0	1	0	0	1	1	0	1	0	1	1	0	2	3	3	1	2	1	1	1	?	1	1	0	1	0	1	1	2	0	2	1	1	0	1	1	1	
Tr_frevo	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Tr_indicator	0	1	0	1	0	1	0	0	1	1	0	1	0	1	1	0	2	2	3	1	2	1	1	1	?	1	1	0	1	0	1	1	2	0	2	1	1	0	1	1	1	
Tr_nanus	0	1	0	1	0	1	0	0	1	1	0	1	0	1	1	0	2	3	3	1	2	1	2	1	?	1	1	0	1	0	1	1	2	0	1	1	1	0	1	1	1	
Tr_pirai	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Tr_yuati	0	1	0	1	0	1	0	0	1	1	0	1	1	1	1	0	2	3	3	1	2	1	1	1	?	1	1	0	1	0	1	1	2	0	2	1	1	0	1	1	1	
Tr_yuqui	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
V_packeri	0	0	0	1	0	1	1	0	1	1	0	0	0	1	1	0	2	3	3	1	2	1	1	1	?	1	1	0	1	0	1	1	2	0	2	1	1	0	1	0	1	0
Y_mota	0	1	0	1	0	1	0	0	1	1	1	1	0	1	1	0	2	3	3	1	2	1	1	1	?	1	1	0	1	0	1	1	2	0	1	1	1	0	0	1	1	
Y_ralla	0	1	0	1	0	1	0	0	1	1	1	1	0	1	1	0	2	3	3	1	2	1	1	1	?	1	1	0	1	0	1	1	2	0	1	1	1	0	0	1	1	
Y_yapa	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Y_yuta	0	1	0	1	0	1	0	0	1	1	1	1	0	1	1	0	2	3	3	1	2	1	1	1	?	1	1	0	1	0	1	1	2	0	1	1	1	0	0	1	1	

Continuation

	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141
Brasilocaenis_irmleri	1	1	1	0	0	1	1	1	0	0	2	0	?	1	0	?	?	0	0	?	?
Drunella_ishiyamana	1	0	1	7	0	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Coryphorus_aquilus	1	0	0	4	1	1	1	0	0	0	1	1	2	1	0	?	2	?	1	1	?
Ephemerella_trilineata	1	0	0	0	4	1	1	0	0	0	0	1	0	0	0	?	0	1	0	0	0
Ephemerythus_niger	1	0	0	1	0	1	1	0	1	0	?	0	?	1	0	?	?	?	?	?	?
Lestagella_penicillata	1	0	1	0	3	1	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?
Lithogloea_harrisoni	1	1	0	0	4	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Machadorythus_maculatus	1	0	0	4	1	1	1	0	0	0	?	0	?	1	0	?	?	?	?	?	?
Massartella_brieni	1	0	1	0	0	1	0	?	0	0	2	1	2	1	0	?	?	?	0	?	?
Melanemerella_brasiliana	1	0	0	1	4	1	1	0	0	0	0	1	2	1	1	0	2	1	0	0	0
Neoephemera_youngi	1	0	1	0	0	1	0	?	0	1	2	0	?	1	1	0	?	?	0	2	?
Ranorythus_violettae	1	0	1	1	0	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Serratella_ignita	1	0	1	7	0	1	1	0	0	0	2	0	?	0	0	?	?	?	0	1	0
Tricorythus	1	1	0	4	0	1	1	0	0	0	?	1	1	1	0	?	0	?	?	?	?
A_asperulus	1	1	0	2	1	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
A_flinti	1	1	0	2	1	1	1	0	0	0	1	1	1	1	1	0	1	0	0	1	1
A_spinosus	1	1	0	2	1	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
A_vescus	1	1	0	2	1	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Am_bahiensis	1	0	1	1	0	1	4	0	1	1	0	1	?	?	?	?	?	?	?	?	?
Am_saguassu	1	0	1	1	0	1	4	0	1	1	0	1	?	?	1	0	1	1	2	1	0
Cabecar_spn	1	1	1	0	3	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
H_aquilonius	0	1	1	4	1	1	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?
H_baritu	0	1	1	4	1	1	1	0	0	0	1	1	0	1	1	1	2	0	0	1	1
H_dominguezi	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
H_huallaga	0	1	1	4	1	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
H_mithras	0	1	1	4	1	1	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?
H_yanahuicsa	0	1	1	4	1	1	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?
L_airuoca	1	0	0	1	2	0	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?
L_albipennis	1	1	0	2	1	0	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?
L_andina	1	0	0	1	1	0	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?

L_calarca	1	0	0	2	1	0	1	0	0	?	?	?	?	?	?	?	?	?	?
L_carinus	1	1	0	2	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?
L_coconuco	1	1	0	2	1	0	1	0	0	?	?	?	?	?	?	?	?	?	?
L_cornutillus	1	0	0	1	2	0	1	0	0	?	?	?	?	?	?	?	?	?	?
L_cornutus	1	0	0	1	2	0	1	0	0	0	1	1	1	1	1	0	1	0	1
L_ecuador	1	1	0	2	1	0	1	0	0	?	?	?	?	?	?	?	?	?	?
L_eximius	1	1	0	2	1	0	1	0	0	0	1	1	1	1	1	0	1	1	0
L_illiesi	1	1	0	2	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?
L_invictus	1	1	0	2	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?
L_jodiannae	1	1	0	2	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?
L_liniti	1	1	0	2	1	0	1	0	0	?	?	?	?	?	?	?	?	?	?
L_maculatus	1	1	0	2	1	0	1	0	0	?	?	?	?	?	?	?	?	?	?
L_nebulosus	1	0	0	1	2	0	1	0	0	?	?	?	?	?	?	?	?	?	?
L_nigripennis	1	1	0	2	1	0	1	0	0	?	?	?	?	?	?	?	?	?	?
L_petersi	1	1	0	2	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?
L_plaumanni	1	1	0	2	1	0	1	0	0	?	?	?	?	?	?	?	?	?	?
L_populus	1	1	0	?	?	0	?	?	?	?	?	?	?	?	?	?	?	?	?
L_quimbaya	1	0	0	2	1	0	1	0	0	?	?	?	?	?	?	?	?	?	?
L_setosus	1	1	0	2	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?
L_tacajalo	1	1	0	2	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?
L_tuberculatus	1	1	0	2	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?
Le_inanis	0	1	1	4	0	1	1	0	0	0	1	1	0	1	1	1	0	0	0
Lo_froehlichii	1	0	1	0	2	1	1	0	0	?	?	1	3	1	0	?	2	?	?
Lu_cocal	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Lu_guacra	1	1	0	2	1	1	1	0	0	?	?	?	?	?	?	?	?	?	?
Lu_pijcha	1	1	0	2	1	1	1	0	0	?	?	?	?	?	?	?	?	?	?
Lu_yagua	1	1	0	2	1	1	1	0	0	?	?	?	?	?	?	?	?	?	?
M_araca	?	?	?	?	?	?	1	0	0	1	1	0	?	1	0	?	?	0	0
M_australis	1	0	1	2	1	1	1	0	0	0	1	1	0	1	0	?	2	?	0
M_eduardoi	?	?	?	?	?	?	1	0	0	0	?	1	3	1	0	?	0	?	1
M_pemonensis	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
M_zagaia	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Tricorythodes_spn1	0	1	1	0	2	1	?	?	?	?	?	?	?	?	?	?	?	?	?

Tricorythodes_spn2	1	1	1	2	2	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
T_arequita	0	1	1	5	2	1	1	0	0	?	?	?	?	?	?	?	?	?	?	?	
T_barbus	0	1	1	6	1	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
T_bullus	0	1	1	2	2	1	1	0	0	?	?	?	?	?	?	?	?	?	?	?	
T_caunapi	0	1	1	2	2	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
T_cfcaunapi	1	1	1	2	1	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
T_cristatus	?	1	1	?	?	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
T_curiosus	1	1	1	2	3	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
T_dimorphus	0	1	1	1	2	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
T_explicatus	0	1	1	2	?	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
T_hiemalis	0	1	1	5	2	1	1	0	0	0	1	1	0	1	1	2	2	0	0	1	1
T_mirca	0	1	1	5	2	1	1	0	0	?	?	?	?	?	?	?	?	?	?	?	
T_nicholsae	?	1	1	2	3	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
T_ocellus	?	1	1	6	?	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
T_popayanicus	0	1	1	5	2	1	1	0	0	0	1	1	0	1	1	2	2	0	0	1	1
T_quizeri	0	1	1	5	2	1	1	0	0	?	?	?	?	?	?	?	?	?	?	?	
T_santarita	1	1	1	2	3	1	1	0	0	?	?	?	?	?	?	?	?	?	?	?	
T_tragoedia	1	1	1	2	2	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
T_yura	0	1	1	5	2	1	1	0	0	?	?	?	?	?	?	?	?	?	?	?	
T_zika	0	1	1	0	1	1	1	0	0	0	2	1	0	1	1	2	0	?	0	?	?
E_undatus	0	0	0	1	1	1	2	2	1	?	?	?	?	?	?	?	?	?	?	?	
T_sallesi	0	1	1	2	2	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
C_serratus	1	1	1	2	1	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
T_zunigae	1	1	1	2	3	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
Th_artigas	1	0	0	0	2	1	1	1	0	1	2	1	0	1	1	2	0	0	0	1	1
Th_chiriguano	1	0	0	0	2	1	1	1	0	1	2	1	0	1	1	2	0	0	0	1	1
Th_gibbus	1	0	0	0	2	1	1	1	0	1	2	1	0	1	1	2	0	0	0	1	1
Th_minimus	1	0	0	0	2	1	1	1	0	1	2	1	0	1	1	2	0	0	0	1	1
Th_rondoniensis	1	0	0	0	3	1	1	1	0	?	?	?	?	?	?	?	?	?	?	?	
Th_sigillatus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
Th_undulatus	1	0	0	0	2	1	1	1	0	1	2	1	0	1	1	2	0	0	0	1	1
Th_volsellus	?	?	?	?	?	?	1	1	0	1	1	?	?	?	?	?	?	?	?	?	
Th_yacutinga	1	0	0	0	3	1	1	1	0	1	2	1	0	1	1	2	0	0	0	1	1

Traverhyphes_spn	1	1	?	2	1	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
Tr_chiquitano	1	1	0	2	1	1	1	0	0	0	1	1	1	1	1	0	1	0	0	1	1
Tr_edmundsi	1	1	0	2	1	1	1	0	0	0	1	1	1	1	1	0	1	0	0	1	1
Tr_frevo	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Tr_indicator	1	1	0	2	1	1	1	0	0	0	1	1	1	1	1	0	1	0	0	1	1
Tr_nanus	1	1	0	2	1	1	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?
Tr_pirai	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Tr_yuati	1	1	0	2	1	1	1	0	0	0	1	1	1	1	1	0	1	0	0	1	1
Tr_yuqui	?	?	?	?	?	?	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?
V_packeri	0	1	0	2	1	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Y_mota	1	1	0	2	1	1	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?
Y_ralla	1	1	0	2	1	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Y_yapa	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Y_yuta	1	1	0	2	1	1	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?

Appendix 2. Nexus file including concatenated molecular data of four gene partitions (12S, 16S, 18S, 28S and COI) used for the phylogenetic analysis of Leptohyphidae. MrBayes command block at the end of file.

```
#NEXUS

begin data;

dimensions ntax=58 nchar=3390;

format datatype=dna missing=? gap=- interleave=yes;

matrix

[12s ntax=50 nchar=465]

    A_flinti
    ATACC--CTNT--T-ATTCTA-A-----TCTAA--GG-GGG--GTNGTAAAATT-----
    TTTAGAAACCTAAAGAAATTTGGCGGCAGCCT-AAAAAATTAGAGGAACCTGTCTCGTAATTGATAGTCCACATTTAACTGACTTACA-TAAA-----
    AGCTTATATACCGCCGTC AAC-AGGGGATATT-----AT---AA-GATTACCCTC--TTAATT--TT--GA-----AGGAA---AAA-
    ATGTCAGGTCAAGGTGTAGTTAATGTGTAAG-GGAG-GATGGGTACAAA---AAA---TTA---AAAAGTA-AATGA-AGTTCCC--TTG---TAAA--A--G-
    GAGGAGGAA-GGT-GGATTTAAATGTA-----AT----AT-----TTT--TGA-----GAGG-A--AATGT--GATTG-AAATCTAGGCTGTGCACACATCG

    Am_bahiensis
    ATACC--CTAC--T-ATACCA-G-----CGTTAA--G-GGG--GTAGTAACA-T-----
    GGAGGAAACCTAAATTTTTGACGGTGGCTT-ATTCAGTTGGAGGAACCTGCCCTATAATTGATATTACTCATTTAAAATTACTTTTA-AAAA-----
    AGCCTATATACCGTTGTCAAAA-AGGGGGTATT-----AT---AA-AGT-ATTTTC--AGGATT--CC--AT-----AAGGA---ATG-
    ATGTCAAATCAAGGTGTAGCTTATGTAAAAGGAAGA-GGTGTGTTACA---AAA---TTTAT--AAGATAA-CATGG-AAGGTT---TTG---TAAT-----
    ATGGATAAA-AGA-GGATTCGAGGGTA-----AA----AT-----AAA-----

    Am_saguassu
    ATACC--CTAC--T-ATTCTA-A-----TAAGTAG-----GGG--GTAGTAGCG-
    G-----GGGTGAAACCCAAAATTTTTGACGGTAACTT-ACATGATTGGAGGAACCTGCCCTGTGATTGATACTACTCACTAAAATAACTCTAA-TTAA-----
    AGCTTATATACCGTTGTCAAAA-AGGGGATGTC-----AA---AA-GGTGATCTTC--AAGCTT--CA--TA-----AATGA---AGG-
    ATGTCAAATCAAGGTGTAGCCAATTTGGAGGAGGA-GGTGAGTTACA---AAA---TTTAT--TAAATGA-CATGG-AAGTTATT-GTG---TAA---A---
    AGGAATAAA-AGC-GGATTC-----

    Brasilocaenis_irmleri
    TTAA--AAGT--T-ATT-----GC-----CAGAGTAG---T-AACAATTAATT-----
    TTTTGAAACTTAAAGGATTTGACGGTGATTTAACTAACCAGAGGAACCTGTCCCGTGATCGATAATCCTCGATGAACCTTACTTAAA-
    TTTGTTATCAGCTTATATACCGCCGTCATA-AAATTATATT-----AA---AA-AATTATGATT--TAAATA--TGT-AA-----TTAAA-----
    ATGTCAGGTCAAGGTGTAGCTTATTTAAG-AGGA-GATGAGTTACA---ATA---AAA---GTATTTA-TACGG-AATAAA-----AATGAAAAT-TTTTAATAA-
    GGT-GGATTTGGTAGTA-----AA----AT-----ATC--G-----TAGAGAAAG-A--TTTAT--GAATATAGCTCTAAATCATGTACA-----

    Cabecar_spn
    -TACC--CTAC--T-ATAATA-A-----AATAG--CG-AGG--GTAGTAATA-
    A-----ATAAGAAACCCAAAATTTTTGCGGTAATTA-ATCTGATCAGAGGAATTTGTCTAGTAATTGATAGTCCACAATTTAACTTACTTAAG-TCAT-----
    AACTTGTATACCGTCGTTTAT-AGTAAATTTT-----TT---AA-AAATATTTTC--GTTACC--CT--AA-----GAAGG---AGG-
    AAATCAGATCAAGGTGTAGTTGATTTAAG-ATGG-GATGAATTACA---ATAC--TA---TAAAGTA-AACGG-AGGGTAT--TTG---TAAT--A---
    GTAAAGGAAAGGT-GGATTTGAGAGTA-----ATT-ATATATCCCTG-----G-TANAAAAA-T--ANNAN--GANATTANCTCTAAATTTATGTAC-----

    Coryphorus_aquilus
    -----
    ATTTAAACCTAAAGAAATTTGGCGGTGGTAA-ATCTTGTAGAGGAACCTGTTCTATGATAGATACTCCCAATATATCTGACTTGGT-CTGG-----
    GGCTTATATACCGTCGTCATA-AGGAGATATA-----AG---GATATTAATTTTC--TTAATT--TT--AA-----AA-AT--AAAT-
    ATGTCAGATCAAGGTGTAGTTTATGGCCAAG-GAGA-AATGAGTTACA---ATA---AAA---TAATTTA-AACGG-ATGAGGT---TG---AAAT--A---CTTGTGAA-
    GGT-GGATTTAAAGGTA-----AA----TT-----TAA--T-----CAGAGAGT-T--AAGTT--GATGGTGGCTTTTATCATGCACACATCN

    Drunella_ishiyamana
    ATACC--CTAC--T-ATTCTA-GGAGTTAGGA---GAGGTCT-----TGA--GTAGTAAAA-
    GTTATGGGCTTAAACTCAAAAATTTTTGGCGGTAGTTT-AACTAACCAGAGGAATTTGCCCTATAATCGATAATCCCGTTATATTTGCTTAAA-
    CTTGTAGCCAGCTTGTATACCGCCGTCACC-AGGAAATTTT-----AG---GA-AAATATTTTC--AAGATC--CT---T-----TGAGGTAGA---
    ATGTCAGGTCAAGGTGCAGCTAATTTAAG-AAGA-GGTGAATTACA---ATA---AGG---GAACTTA-CACGG-AGTGTTAATGA---AAA---C---
    GAATATGAA-GGT-GGATTTGGGAGTA-----AT----AT-----TTT--AAGCA-A--GAAAAA-T--AAT-----AT--TAGCTCTAAACTGTGCACACATCG

    E_undatus
    ATACC--CTAC--T-ATAATA-A-----AATTG--AG-GGG--GTAGTAATA-
    A-----ATAAGAAACCCAAAATTTTTGCGGTAATTA-ATCTGATCAGAGGAATTTGTCTGAGAATTGATAGTCCCTCATTTAACTAACTTAAA-GTAC-----
    AACTTATATACCGTCGTTTAT-AGTAAATTTT-----TA---AA-AAATATTTTC--TAGAAA--TTA-AC-----AAAAT---AAA-
    AAATCAGATCAAGGTGTAGTTAATTTAAG-GCAA-GATGAATTACA---ATA---TTA---AAAAATA-TACGG-ATAGTGT--TTG---TAAG--A---ACTCGTGAA-
    GGT-GGATTTGAAAGTA-----ATT-ATAT-----TTA--GAACGCG-TAGAAAAA-T--AAAAT--GAAGTTAGCTCTAAATTTATGCACACATCG
```

H_aquilonius ATACC--CTAC--T-ATTCTT-A-----AGTAT---TG-GGG--GTAGTAAAG-A-----
TTTTGAAACTCAAAAATTTTGGCGGTGATTA-ATTTAACCGGAGGAACCTTGTTTTATAATTGATAATCCTCATTATATTTTACTTTAA-TTAA-----
GGCTTGTATACCGTCGTCATC-AGGAAATTTT-----AT---GA-GAAAATTTTCAAGGAATA--GT--TT-----AAAAT---TAA-
ATGTCAGATCAAGGTGTAGTATATGTTAGAG-ATAA-AATGAGTTACA---ATG---TAAG--GGGGATA-GGCGG-ATATTAA--TTG---AAAG--A---
TTAATTTAAA-GAT-GGATTCGGGGGTA-----AA---AT-----TAA--TAG-----AGAGTT--AATTT--GATTGTGACTCTTAATTATGCACACATCG

H_baritu ATACC--CTAC--T-ATTCTT-A-----AGTAT---TG-GGG--GTAGTAAAG-A-----
TTTTGAAACTCAAAAATTTTGGCGGTGATTA-ATTTAACCGGAGGAACCTTGTTTTATAATTGATAATCCTCATTATATTTTACTTTAA-TTAA-----
GGCTTGTATATCGTCGTCATC-AGGAAATTTT-----AT---GA-GAAAATTTTCAAGGAATA--GT--TTA-----AAAT---TAA-
ATGTCAGATCAAGGTGTAGTATATGTTAGAG-ATAA-AATGAGTTACA---ATG---TAAA--GGGGATA-GGCGG-ATATTAA--TTG---AAAG--A---
TTAATTTAAA-GAT-GGATTCGGGGGTA-----AA---AT-----TAA--TAG-----AGAGTT--AATTT--GATTGTGACTCTTAATTATGCAC-----

H_huallaga ATACC--CTAC--T-ATTTTT-A-----AGTAT---TG-GGG--GTAGTAAAA-
T-----TTTTGAAACCCAAAAATTTTGGCGGTGATTA-ATTTAATCGGAGGAATTTGTTTTATAATTGATGATCCTCAATATATTTTACTTTAG-TTAA-----
TGTTTATATACCGTCGTCATC-AGGGAATTTT-----AT---GA-GAAGATTTTTCGAGAGATA--GA--TTA-----AAAT---TAA-
ATGTCAGATCAAGGTGTAGCGTATGTTAAAG-AAAA-AATGAATTACA---ATATTTTAAA--AAAGATA-AGCGG-ATATTAA--TTG---AAAG--A---
TTAATTTAAA-GAT-GGATTCGGGGGTA-----AG---AT-----TAA--TAG-----AGAGTT--NANTT--NNNNNTANCNCTTAATTATGTACGC-TC-

L_albipennis ATACC--CTAC--T-ATTTTG-A-----TTATTTG-----GGG--GTAGTAGAA-T-----
TTTAGAAACCTAAAAATTTTGGCGGTATTTT-ATTGGATTAGAGGAACCTTGCCCTTAATTGATAACCCCAATATAATTTATTTTAA-TTGA-----
AGCTTGTATACCGTCGTCATC-AGAGAATATT-----TT---AA-GGTAATTTTC--GGGGTA--TT--GT-----TAGAT---AAG-
ATGTCAGATCAAGGTGTAGTTTATATTGAAA-GGAG-GGTGAGTTACA---AAA--ATAATTAAGTTA-AACGG-ATCTTTT--TTTTAAAAAT--A---AAGG--
GAA-GGA-GGATTT-----A-----

L_coconuco ATACC--CTAC--T-ATTCTA-A-----TGTGTT---TG-GGG--GTAGTAGAG-
C-----TTTAGAAACCTAAAGAATTAGCGGTATTTT-ATTGAGTTAGAGGAACCTTGCCCTGTAATTGACAGTCTCATTATAATTTATTTTAA-TTAA-----
AACTTGTATATCGTCGTCATC-AGAGAATATT-----AA---AA-GGTAATTTTC--AGGATA--TT--GT-----AAAAT---AGT-
ATGTCAGATCAAGGTGTAGTTAATGTTAAAA-GGAG-GGTGAGTTACA---AAA--ATAATA-AAAATTA-AATGG-AACTTA--TTG---TGAA--A---
AAAGTGAA-AGT-GGATTTAATTGTA-----A-----

L_eximius ATACC--CTAC--T-ATTCTN-A-----AATTAAGG-GGG--GTNGTAAAG-T-----
TTTAGAAACCTAAAAATTTTGGCGGTATTTT-ATTGAATTAGAGGAACCTTGCTCGTAATTGACAACCCCAATATAATTTATTTTAA-TTAA-----
GGCTTGTATATCGTCGTCATC-AGAGAATATT-----AA---GA-GGTAATTTTC--TAAATA--TT--AT-----AAAAT---AGT-
ATGTCAGATCAAGGTGTAGTTAATATTTAAAA-GGAG-GATGAGTTACA---AAA--ATATAAAAAATTA-AATGG-AACTTTT--TTG---AAAAAA-----
AAGAGGGAA-AGT-GGATTTGATTGTA-----A-----AATTAATTAGA-----

L_nebulosus ATACC--CTAC--T-ATTCTA-G-----TTAATTN-----GGG--GTAGTAAAG-
T-----TGTGGAAACCTAAAAAATTTTGGCGGTATTTT-ATTGAGTTAGAGGAATTTGCTCGTAATTGATAATCCCCTATAATTTATTTTGA-TAAA-----
AGCTTATATATCGTCGTCATC-AGAGAATATT-----AA---GA-TATTATTTTC--TTGAAA--TT--GT-----TAAAT---AAG-
ATGTCAGATCAGGGTGTAGTAAATATTTAAAA-GGAA-GATGAATTACAAAATA---ATA---AAAATTA-AACGG-ATTCTTT--TTG---AGAG--A---
AAGGGGGAA-GGA-GGATTTAAGCGTA-----A---AA---TTA-AAA---AAGAGAGT-T-AGTTTGAATTT--TATCTTAAAATATGCACACATCG

L_nigripennis ATACC--CTAC--T-ATTCTATG-----TAATTA-----GGG--GTAGTAAAA-A-----
TTTTGAAACCTAAAAAATTTTGGCGGTACTTT-ATTGGCTTAGAGGAACCTTGCCCTAGTAATTGACAGTCCCTCAATGTAATTTATTTCAA-TTAA-----
AACTTGTATACCGTCGTCATC-AGAGGATATT-----AA---AA-GGTAATTTTC--AAGATA--TT--AT-----AAAAT---AGA-
ATGTCAGATCAAGGTGTAGTAAATGTTGAAA-AAGG-GGTGAGTTACA---AAA--GTAAA-AATAACTA-AACGG-ATGTCTT--TTG---GAAT--A---
GGGGTTGAA-GGT-GGATTTAGTTGTA-----AT---AT-----TAA-----

L_plaumanni ATACC--CTAC--T-ATTCTA-A-----CTAGG---TA-GGG--GTAGTAAAA-
A-----TTTTGAAACCTAAAAAATTTTGGCGGTATTTT-ATTAATTAGAGGAACCTTGCCCTGTAATTGACAGTCTCATTATAATTTATTTTAA-CTAA-----
AACTTGTATATCGCCGTCATC-AGAGAATATT-----AA---GA-GGTTATTTTCA-----GTA--TA--TTA-----TTAAAT---AAA-
ATGTCAGGTCAAGGTGTAGTATATGTTAAAA-GGAG-GGTGAGTTACA---AAAATAATAA---AAATTA-AATGG-AGACTCT--TTT---AGAT--A---
AGGGAAGAA-GGT-GGATT-----TAAT-----

Lachlania_dominguezi ATACC--CTAT--T-ATTCTA-AGTGTAAAA--TTGTATAC---T-TGA--GTAGTATTA-
GTTATGTTCTTAAAACCTCAAAGAAATTTTGGCGGTGTTTTATTCTCCAGAGGAACCTGTTCCGTAATCGATAGTCCAGTTTTACCCAACCTAAT-
TTTTGATTCAGCTTGTATACCTCCGTCATA-AGATTACT-----TA---AA-GGTTATATTC--TAAATT--TATTAT-----TTTTAA---AAA-
ATGTCAGGTCAAGGTGTCAGCTTATGATTAAG-AGGT-AATGGGTTACA---TTA---ATT---TAAATTA-TACGA-ATTATA-----A---
AAATGAAAATA TTATATGAA-GGT-GGATTTGGTTGTA-----AC---GA---AGT--ATG---CCAGAAAAA-T--TTCGT--
GATGGTAGCTCTAAAACATGCACACATCG

Le_inanis ATACC--CTAC--T-ATTCTA-A-----AGAATAA--CC-AGG--GTAGTATAA-G-----
GGTAGAAACCTCAAAAATTTTGGCGGTGATTT-ATTTAATTGGAGGAACCTTGCCCTGTAATTGATGATCCTCACTTAAAATAACTTTAA-TTAA-----
AACTTGTATACCGTTGTCATA-AGGGGATTTT-----TG---AA-AATTACCTCC--AGAAT--TT--ATT-----TAAAGAAAAAT-

A_flinti -AGATCATGTAG-AAATTTATT--GGTCGAACAGACCT-
AACCTTACAGGGGCTGCCCGTAA-T-
TCCTCTAATCCAACATCGAGGTGCGAATCCCTCTCATCAATTTGAAGCTCTCCGAAAGGATTACGCTGTTATCCCTAAGGTAACCTATTCTC-TAT-TT----TC---
TT----GAGATCTTTTAT-CCACCT-AA--A-ATGTTTCTTT-TTTT-AGAAG---TTTATTTATTCTATTATCACCCCAATTTAATAAAC-----ATAATA-T-CGTTA-
AA--ACT--TTT----T-CCA-----A---TTTAA--CAA--GAATTA-----TTT--
ATAAAGCTCTATAGGGTCTTCTCGTCCCTACATCATTATAGCCTTTTCACTATAAAAATAAAATTT---TA-GT-CG--T--
AAAAAGAGACAATATATTTCTCGTTCACCTTTTCAATCCAGTCTCATTTAAAAAACTAATGATTATGCTACCTTTGCACGGTCAAATACCGCGGCCATTTA
AT-----TCTCAG-GGGCAGGTATACCTTTTCATC-CTCTCAAAGGAAAT

Am_bahiensis -AAATCATGTAG-AAAAATAA---GGTCGAACAGACCC-
TAATTTTTAAAAAGCTGCCCTAAAAAT-
TTATTCTAATCCAACATCGAGGTGCGAACCCTTTTATCAATTCGAAGCTCTCCAAAAAGATTACGCTGTTATCCCTAAGGTAATTTAATCTT-TAT-TCA---AT--
-CTAT--TGGAACCTTCAA-TCATA-AT--CAATGTT-ACC-TGAA-AAGAG---TTTTTATATCTTTACGTACCCCAACCAATAAAC-----TCATT-T-TAAAT-
-A-TATG--TTT----A-CCA-----ATC-TTATA---AA--AATTAT-----TTT--
ATAAACTCTATAGGGTCTTCTCGTCTTTCTAAATTATTATAGCTTTTCTCACTATAAAAATAAAATTT---CT-GT---AC--
CCTTAAGAGACAGCTAATTTTTCGTTAAACCCTTCATACAAGTCCCAATTAAGAAGCTAATGATTATGCTACCTG-----

Am_saguassu ---ATCATGTAG-AAATC-CAC--GGTCGAACAGACCC-
TAACCTTTAGAGACTGCCCTAAAGAT-
GTATTCTAATCCAACATCGAGGTGCGAACCCTTTTATCGATTTGAAGCTCTCCAAAAAGATTACGCTGTTATCCCTAAGGTAATTTAATCTT-CAT-TCA---AC--
-CTCT--TGGACCTTCTC-TCATAA-AC--TTATGGA-ACC-TTTA-AGGAG--TTTCTTTTATCCTCGTATCACCCCAACAAATAAAC-----
TTACCCTCAAACA-TATTT----TT---A-CCA-----AAC-CCATA---AA--AATTTA-----TTT--
ATCAAATCTATAGGGTCTTCTCGTCTTTTAAACCATATAGCTTTTACCATAAAAATAAAATTT---TA--C-TA-TC--TAAC-
AGAGACAGCTTATTTTTCGTTAAACCCTTCATCAAGTCCCTATTAAGAAGCTAATGATTATGCTACCT-----

Brasilocaenis_irmieri ---ATCATGTAA-GAATTAAG--GGTCGAACAGACCCAGATTTTTAAGGGGCTGCCCTAAACC-
TATTCTTAGTCCAACATCGAGGTGCGAACCCTTTTATCGATAAGAAGCTCTCCAAAAAATTACGCTGTTATCCCTAAGGTAACCTATTCTT-ATAATC---
CTTATTA---AGGATCATTTTC-CCATAA-AT--TAATGTTA-TTA-AAAG-AAAAG---TTTTATTATTTCTGTACCCCAACATAATTTAA---AAACT-CTC---
AT---ATT-ATA---TT---T-CTAC-TACAA---ACATG---AA--A-A-TA-----
TTTTAAATAAAGCTCTATAGGGTCTTCTCGTCCCTTAGTTTATTTAAGCCTTTTCACTTAAAGGTTAAATTC---G----CAA-T-
TTTTAAATAGACAGCTTGAATTAATGTCGAACCCTTCATCCAGCCTCCAATTAAGAAGCTAATGATTATGCTACCTTTGCACGGTCAAATACCGCGGCCCTTTT
AAA----TAATCAGTGGGGCAGGTATACCCCTAAC-ATATCAGGGGGCTAT

Cabecar_spn ---TCATGTAG-AAATTTAAT--AGTCGAACAGACTT-
AATAACTTAATTTTTGCTCCAGGTAA-
TTTTTCTAATCCAACATCGAGGTGCGAACCCTTTTATCGATTTGAAGCTCTCCAAAAACAATTACGCTGTTATCCCTAAGGTAACCTACTCTT-AAT-GT---ACC--
-CA-----AAGATCTTAAAT-TCACAT-AT--CTATGTTT-ATA-TCAT-AAAAG---TTTTTTTATTTATCATCATCACCCCAATTCATAAATA-TTAGAT-C-----T----
AAC-TAT--TCC---A-ATA---AA---TT-----CCAAC-----ATTT--
ATAAAGCTCTATAGGGTCTTCTCGTCTTTTACTTTTATATAGCTTTTCACTATAAAAATAAAATTC---A-AT-TAA-T--
TACTAAGAGACAAATAAACTTTTCTGTTTGACCATTCATCCAGCCTCAATTAAGAAGCTAATGATTATGCTACCTTCGCACAGTCAATATACTGCGGCTCTTTA
AT-----TCTCAG-TGAGCAGGTCTTACCTCTTATT-TTATCAAGAGGCAAT

Coryphorus_aquilus CAGATCATGTAG-GAATTAATA---GGTCGAACAGACCC-AGCGCCCTAATTTCTGCTTTAAAGCC-
TAATCCTAATCCAACATCGAGGTGCGAACCCTTTATCATCAATTTGAAGCTCTCCGACAAAATTATGCTGTTATCCCTAAGGTAATTTGTTCTT-TTA-ATC-ACAA-
--ATA---AGGCTCACCAAT-TCTTTC-AT--TAAAGTCC-TAT-TATT-AAAAG---TTTTTTTATTTCAACGTACCCCAACTAAACAACCC---CCACATT-----
-AAACAATTCACAT-GCA-----AAT-AT-----GATATA-----TT--
GTAAACTCTATAGGGTCTTCTCGTCTTTTACCCCAACCCAGCCTTTTCACTAGGATGTAATAAATTA---AT--T-AT-AT--
CTGCAAGAGACAGTCAATTTCTCGTTTAAACCTTTCATACCAGCCTCAATCAAAGAGCTAATGATTATGCTACCTTG-----

Drunella_ishiyamana -----TCTCAGCGGCTGCACCTTAGAT-
TACCTTTAATCCAACATCGAGGTGCGAACCCTTTTATCGATATGAAGCTCTCCAAAAAGGATTACGCTGTTATCCCTAAGGTAACCTAATTTT-TTATTC---
ACTATTTA---TGGATCCTTAC-TCACGT-AT--T-ATGTTTCTGT-TAAG-ATAAG---TTAATTTATTACCCCATCACCCCAACGTAATAGAGGTTAAAGA-CAA-
--AC---AAA-ACA--TTT---T-CTA---AA---AGCTT---GA--T--GCTA-----CTCT--
ATAAAGTTCTATAGGGTCTTCTCGTCTTTTAACTTAACTTAACTGAAAGGTTAAATTC-----TT-TCT-T--
TTTATGAGACAGTTTGTACCTGTCCTTCACTCCAGCCTTAAATAGAAAGCTAATGATTATGCTACCTTTGCACGGTCAAATACCGCGGCCCTTA
AAC-----TATCAG-TGGGCAGGTAAGACCTT-----

E_undatus --GATCATGTAG-AAATTTAAT--AGTCGAACAGACTT-
AACAAATTAATTTTTGCTTCAAACAA-
TTTTTCTAATCCAACATCGAGGTGCGAACCCTTTTATCAATTTGAAGCTCTCCAAAAATAATTACGCTGTTATCCCTAAGGTAACCTATTCTT-TAT-GT---AC---

CTA----AAGATATTTTAT-TCATTT-AT---AATGTTT-CCA-TCTT-AAAAG---TTTATTTATTTTCATAATCACCCCAATTCATAAAATA-----
TAAAAATTT----T-GCC-----AAT-ATTT---TTC--TCCGTA-----TTT--
ATCAAGCTCTATAGGGTCTTCTCGTCCTTATTTTTATTATAGCTTTTTCTACTATAAAAATAAAATTA---TA--T-CC-AT--
CCCGGGGAGACAAGTAAATTTTCTGTTGACCATTTCATCCAGCCTTCAATCAAAAAGCTAATGATTATGCTACC-----

H_aquilonius

---TCATGTAG-AACTCCACA--GGTCGAACAGACCT-

TTTACTTTAAATACTACTCCAAGCT-
TTCTTCTAATCCAACATCGAGGTCGCAAACCCCTTTCATCGATTGAACTCTCCAAAAAGATTACGCTGTTATCCCTAAGGTAACCTTATTCTT-TAT-TC---CTA--
-TA----AGGATCCTCTTT-TCATTT-AC--A-ATGTTA-TAA-TTTT-AAAAG---TTTATTTATTTAATATCACCCCAATCAAATAAATT-ATAAAT-TAC---TT---
AA-AAA--TTT----A-CTA-----AT---TTTTAT---AA--T-CCAAA-----ATTT--
ATTAAGCTCTATAGGGTCTTCTCGTCCCTTTAATCATTATAGCTTTTTCTACTATAAAATTAATTC-----TT-TAT-C--
TCCTTGGAGACAGCCAATTTCTCGTTTAAACCATTTCAGCTTTCATTAATAAAACTAATGATTATGCTACCTTTGCACGGTCAAAAATACCGCGGCCCTTTA
AC-----TTGCAG-GGGCAGGCCTTACCTTATAAC-TCCCAACAAGCAA-

H_baritu

-AGATCATGTAG-AACTCCACA--GGTCGAACAGACCT-

TTTACTTTAAATACTACTCCAAGCT-
TTCTTCTAATCCAACATCGAGGTCGCAAACCCCTTTCATCGATTGAACTCTCCAAAAAGATTACGCTGTTATCCCTAAGGTAACCTTATTCTT-TAT-TC---CT---
ATA----AGGATCCTCTTT-TCATTT-AC---AATGTTA-TAA-TTTT-AAAAG---TTTATTTATTTAATATCACCCCAATCAAATAAATT-----ATAAAT---TACTT-
AA-AAA--TTT----A-CTA-----A---TTTTA--TAAT-CCAAA-----TTT--
ATTAAGCTCTATAGGGTCTTCTCGTCCCTTTAATCATTATAGCTTTTTCTACTATAAAATTAATTC---TT-TA-TC--T--C-
CTTGGAGACAGCCAATTTCTCGTTAACCATTTCAGCTTTCATTAATAAAACTAATGATTATGCTACCT-----

H_huallaga

---ATCATGTAG-AAATTTATA--GGTCGAACAGACCT-

CTCACTTCAAATACTACTCCAAGAC-
TTCTTCTAATCCAACATCGAGGTCGCAAACCCCTCCATCGATTGAACTCTCCGAAAAATTACGCTGTTATCCCTAAGGTAACCTTATTCTT-TGT-TC---CT--
-ATT---AGGATCCCTTT-TCACTA-AT---AATGTCA-TAA-TTTT-AAAAG---TTTACTTATTTAATATCACCCCAATCAAATAAATT-----TTATAA-T-TACTT-
AA-AAA--TTT----A-CTA-----A---TTTTA--TAAC-TCAAAA-----TTT--
ATTAAGCTCTATAGGGTCTTCTCGTCCCTTTAATCATTATAGCTTTTTCTACTATAAAATTAATTC---TC-TA-CT--T--T-
CTCAGAGACAGCCAATCTCTCGTTAACCCTTCATCCAGCTTTCATTAATAAAACTAATGATTATGCTACCT-----

L_albipennis

---TCATGTAG-AATTTTAAA--GGTCGAACAGACCT-

AAATTAATAAAATTTGCCCCAGTAAT-
ACTTTCTAATCCAACATCGAGGTCGCAAACCCCTTTCATCAATTTGAACTCTCCGAAAGGATAACGCTGTTATCCCTAAGGTAACCTTAACTC-TC--TC---ACC--
-TA----AAGAATCAT-TCATTT-AT--AATGTTT-AAA-TCTT-AGAAG---TTTATTTATTTCTAATATCACCCCAATAAAAAGAAAT-TTAAAT-ATA---TA----
AAT-AAC--TTT----A-CCA-----A---TTTTA---AA--TAGCTAA-----TTCC--
TTTAAGCTCTATAGGGTCTTCTCGTCCCTTTTACAGTATTATAGCTTTTTCTACTATAAAATTAATTC---TA-AC-CTG-C--
CTAGGAAAGACAGCTTATTTCTCGTTAACCCTTCATCCAGCTTTCATTAATAAAAGACTACTTATTATGCTACCTTTGCACGGTCAAAAATACCGCGGCCATTCA
AT-----TACTCAT-TGGCAGGCCTTACCTTATATT-TTTTCAAAGGCAAT

L_coconuco

-AGATCATGTAG-AAATTAAG--GGTCGAACAGACCC-

AAACTATTAAATTTTGCCCTAATAGT-
GATTTCTAATCCAACATCGAGGTCGCAAACCTTTTTATCGATTGAACTCTCCAAAAGAATAACGCTGTTATCCCTAAGGTAACCTTAACTT-TTT--C---TT---
CTT---TAGATCTTAAAT-TCATTT-AT--ACACGTTT-AAT-TATT-AAAAG---TTTGTTTATTTCAATATCACCCCAACAAAAGAGGT-----AAAAAGTCTA-----
ACTACT--TTT----T-CTA-----AGT-TTAAA--CAG--TCCTAA-----TTC--
TTAAAGCTCTATAGGGTCTTCTCGTCCCTTTTACATTATTTATAGCTTTTTCTACTATAAAATTAATTT---CA-AC-AC-AC--
TAAAAGGAGACAGTTATTTTCTGTTCAACCTTTTCATCCAGCTTTCATTAATAAAAGACTAATTAATGCTACCT-----

L_eximius

--GATCATGTAG-AAATTTATA--GGTCGAACAGACCA-

CAGTTATTAATTTTGCTTTAATAAA-
GATTTCTAATCCAACATCGAGGTCGCAAACCTTTTTATCGATTGAACTCTCCAAAAGAATAACGCTGTTATCCCTAAGGTAACCTTAACTT-TTT-TT-----
CCT---TAGATCTTAAAT-TCACCC-AT--TTATGTCA-AAT-TTTT-AAAAG---TTTATTTATTTAATATCACCCCAATAAAAAGAAAC--GAAAAAAT-----T-----
-AACTACTTTT---T-CTA-----AAT-TTTA--GCC--CCTCAA-----ATC--
CTCAAGCTCTATAGGGTCTTCTCGTCCCTTTTATCTTATTATAGCTTTTTCTACTATAAAATTAATTT---AA-AC-AC-AC--
TAAAAGGAGACAGTTATTTTCTGTTCAACCTTTTCATCCAGCTTTCATTAATAAAAGACTAATTAATGCTACCT-----

L_nebulosus

--GATCATGTAG-AATAATATT--GGTCGAACAGACCA-

TAATCATTAAATTTGCCCTAATGAT-
AATTTCTAATCCAACATCGAGGTCGCAAACCCCTTTCATCAATTTGAACTCTCCAAGAGGATAACGCTGTTATCCCTAAGGTAACCTTAACTT-TCT-TC-----

CCA----AAGAACTTACAT-TCACAA-AT--CAGTGTTC-AAA-TCTT-AGAAG---TTTACTTATTCTAATATCACCCCAATAAAAAGAATA---TCTCCCT-----C-----
AAATAAATT---T-ACC-----AAA-CTTAA--GAA--AAGTTAC-----TTC--
CTAAAGCTCTATAGGGTCTTCTCGTCCTTACTACATTATAGCTTTTTCACTATAAAAATAAAATTC---TA-GA-TC-AC--
TAAAAAGAGACAGTTATTTTTCGTTTAACTTCAATCCAGTCTCAATTAATAAACTAATTATTATGCTACC-----

L_nigripennis

--GATCATGTAG-AAATTTATA--GGTCGAACAGACCC-

TAATTATTAATAATTTACCCTAATAAT-
GATTTCTAATCCAACATCGAGGTCGCAAACCTCTTTCATCGATTTGAACTCTCCAAAAGAATAACGCTGTTATCCCTAAGGTAACCTTTATCT--TAT-TT----AT---
CTT---TAGATCTTTTAT-TCATTT-AC--CTGTGTTT-TAT-TCTT-AAAAG---TTTATTTATTTTCATTATCACCCCAATAAAAAGAAGCTTTTCTAACCTT-----
ACTAAT---TT---T-TCT-----AAA-TTTTAA-TAT-AGATATA-----TTC--
CTTAAGCTCTATAGGGTCTTCTCGTCCTTTTACCTTATTATAGCTTTTTCACTATAAAAATAAAGTTC---CA-AA-TC-AC--
CTCAAAGAGACAGTATATTTTTCGTTTCAACCTTTTCAATCCAGTCTTCAATTAATAAGACTAATTACTATGCTACC-----

L_plaumanni

-AGATCATGTAG-AAATCAAAA--GGTCGAACAGACCC-

TAATTATTAATAATTTACCCTAATAAC-
GTTTTCTAATCCAACATCGAGGTCGCAAACCTCTTTTATCGATTTGAACTCTCCAAAAGAATAACGCTGTTATCCCTAAGGTAACCTAATCTT-AGTTTT---CTC-
-----GAGACCCAAAAT-TCATCT-AT--TTATGTTA-TAT-TTTT-GAAAG---TTTATTTATTTTCATTATCACCCCAATAAAAAGAGA-CCATAC-CCC---TC---
ACT-AAC--TTT---T-CTA-----AA---TTTTAA---AA--A-TATTT-----ATCC--
TTTAAGCTCTATAGGGTCTTCTCGTCCTTTTACATCATTATAGCTTTTTCACTATAAAAATAAAGTTC-----TA-AAT-
CACCTCTTAGAGACAGTTTATTTTTCGTTTCAACCTTTTCAATCAAGTCCCAATTAATAAGACTAATTACTATGCTACCTTTGCACGGTCAATATACCGCGGCCAT
TCAAC-----TCCTCAT-TGGGCAGGTTATACCTTTTCAAT-CTTTGAAAGGAAAT

Lachlania_dominguezi

CAGATCATGTAA-GAATTTAAA--GGTCGAACAGACCT-AAAACCTGAACTTTTGCACCCAAGAT-

TTATCTTAGTCCAACATCGAGGTCGCAAACCTCTTTTATCGATTTGAACTCTCCAAAAGAATAACGCTGTTATCCCTAAGGTAACCTAATCTT-TTA-AT----CG--
-TAAAAACGGATCAAATAT-ACACTC-AT--CTGTGAAA-AAA-AAGA-AAAAGAGTTATTTTTATCTTTCAGTCACCCCAACTAAACA-----C-AATTA-
AATTATT-TTT---T-AAA-----A---TTAAA--TAA--AAATTAATGCTTAT--
GTCAAGCTCTATAGGGTCTTCTCGTCCTTCAATATTTTATAGCTTTTTGACTAAAAAATAAATTC---TA-TTTCAT-T--
ATAAAAAAGACAGTAAATACTTCTCGTCCGCCATTCATACCAGCCTTCAATTAATAAGACTAATGATTATGCTACCTTTGCACGGTCAAATACCGCGGCCCTTCA
AA-----AAATCAG-TGGGCAGGCGATACCTATTATT-TTTACAATAGGCGAT

Le_inanis

----CATGTAG-AAATTCAT--GGTCGAACAGACCT-

AAATTTTTAAATGCTGCCCCAAAAAC-
TTATTCTAATCCAACATCGAGGTCGCAAACCTTTTTATCGATTTGAACTCTCCAAAAGATTACGCTGTTATCCCTAAGGTAATTTAATCTT-TAT-TCATAAA--
---A----TGGATCCAACACACCACTT-AC--AAGTGCC-GAC-TAAT-AAAAG---TTTATTTATTTCCACTATCACCCCAACAAAACAAGT--TCAAAC-TTA-A-AC---
CA-ACT--TTT---T-CTA-----A---TCCTA--CAA--GCCTTAA-----TCTT--
GTGAAACTCTATAGGGTCTTCTCGTCCTTTTAACTTATTATAGCTTTTTCACTATAAAAATAAATTCCTCA-AA-TA-----
AAAAGAGACAGCTAATTCCTCGTTAAACCTTTCATGCCAGTCTTCAATTAATAAACTAATGATTATGCTACCTTTGCACGGTCAAGATACCGCGGCCCTTAAAC
-----TCAG-TGGGCAGGTCCTACCTTAAAT-TTACCTGAAGGCAAT

Leptohyphes_sp

--ATCATGTAG-AAATTTAAA--GGTCGAACAGACCC-

TAATTATTAATAATTTACCCTAATAAT-
GATTTCTAATCCAACATCGAGGTCGCAAACCTTTTTCATCGATTTGAACTCTCCAAAATAAAGCTGTTATCCCTAAGGTAACCTTTATCTT-ATT--T----AT---
CTT---TAGATCTTTTAT-TCATTT-AT--TTGTGTTT-AAC-TCTT-AAAAG---TTTATTTATTTCAATATCACCCCAATAAAAAGAAGCT-----TCTT-T-TATCT-
TATTAAT--TTT---A-CTA-----AGT-TTTAA--TAATAGAGATA-----TTC--
CTTAAGCTCTATAGGGTCTTCTCGTCCTTTTATCACATTATAGCTTTTTCACTATAAAAATAAAGTTC---CA-AA-TC-AC--
CTCAAAGAGACAGTATATTTTTCGTTTAACTTTCAATCCAGTCTTCAATTAATAAGACTAATTACTATGCTACCT-----

Lestagella_penicillata

-AGATCATGTAG-AAATTTAAA--GGTCGAACAGACCT-GGGGGTTGAGCTGCTACACCCGCCCC-

CTATTCTAATCCAACATCGAGGTCACAATCTTTTCTATCGATTTGGACTCTCCAGAAAATATGCTGTTATCCCTAAGGTAACCTAATCTATTAA-TCA-
TTAA---AAA----TGGATCCTAAAT-ACAGAA-AT--CATTGGTT-TCA-TAAA-AAAAG---TTTATTTATTTTATAGTCACCCCAACCAAATACAAG-C---
CTGCTTAA-AA-TAAA-AA---TTT---T-CCA-----GATAAATCAC-CCA--AACTCA-----T--
ATAAAGATCTATAGGGTCTTTTCTCGTCCCAATTCATCTTAGCCTTCTCACTAAAAAGTAAATTT---AT-AG-TT--T--A-
TAATGAGACAGCTTTTGTTCGTCTAACCGTTTATGCCAGCCCTAATTAAGAAGACTAATGATTATGCTAC-----

Lu_cocal

---ATCATGTAG-AAATTTAAC--GGTCGAACAGACCT-AACTTTACAAGGGCTGCCCA-

TAAT-TTTTTCTAATCCAACATCGAGGTCACAATCCCCCTATCAATTTGAACTCTCAAAGGGGATTACGCTGTTATCCCTAAGGTAACCTTACTT-CTT-TT--
--AT---CTA---AAGATCTTTAAT-CCACTT-AA---AATGTTA-TAA-TTAT-AAAAG---TTTATTTATTTTAAATCACCCCAATTTAATAGGAA-----TAGAT-T-
TACCT-TA-ACA--TTT---A-CCA-----A---TTTAG--TAA--AATTTA-----CCT--
ATAAAGCTCTATAGGGTCTTCTCGTCTTATCTCATTATAGCTTTTCACTATAAAAATAAATTC---TA-AA-CC--T--

AAAAAGGAGACAGACTATTTTTTCGTTAAACCCTTCATACCAGTCTCCATTTAAAGACTAATGATTATGCT-----

Lu_guacra ----TCATGTAG-AAAATTATT--GGTCGAACAGACCT-
AACTTTACGAGGGCTACCCCA-TAAT-
TTTTTCTAATCCAACATCGAGGTCGCAACCCCTCTTATCAATTTGAAGTCTCCAAGAAGATTACGCTGTTATCCCTAAGGTAACCTTATTCTT-CTT-GT----AT---
TTT----AAGATCTTTAAT-CCTACT-AA---AATGTTT-ATT-TTTT-AAAAG---TTTATTTATTTTAAATCACCCCAATTAATAGATT-----TAAAC-T-TATTT-
CA--AAA--TTT----A-CCA-----A---TTTAA--TAA--TATTCC-----TCT--
ATAAAGCTCTATAGGGTCTTCTCGTCCTTTGTATAATTATAGCTTTTTCACTATAAGATAAAATTT---TG-AC-TT--T--
AAAAAGGAGACAGGTTATTTTTCGTAAGACCCCTTCATCCAGTCTTCATTTAAAAAACTAATGATTATGCTA-----

M_araca ---ATCATGTAA-AAATT-AAA--GGTCGAACAGACCC-
GCAAACTTAACTTTTGCATCAAGTA--
TAATTTAATCCAACATCGAGGTCGCAAACTTTCTTATCAATTAAGAAGTCCCAAGAAAATTACGCTGTTATCCCTAGGGTAATTT-TAATT-TTTGAT----AT---
CTT----AAGATCTTAAAT-TCATAA-AT--AAATGTTA-TTT-TTAT-AAAAG---TTTGTTTATTTTACTATCACCCCAATCAAAATAGT-----TAATT-T-----
TA----TT----A-CCA-----AAT-AAAA-----TTTAA-----TTT--
ATAAACTCCTTAGGGTCTTCTCGTCCTTTTAAATAATTATAGCTTTTTCACTATAAAATAAAATTT---TA--T-AA-AT--
TATTAAGAGACAATAAACTTACATTATACCTTTCTAGTCTTTAATTATAAACTAATGATTATGCTA-----

M_australis -AGATCATGTAG-ATTTTCAA--GGTCGAACAGACCT-
ATTTACTTAAAAATTTGCCCAATAA-
ATAATCTAATCCAACATCGAGGTCGCAAACTTTTTCTTAAATTTGACTCGCCGAAAAAATTACGCTGTTATCCCAAGGTAACCTAATCTT-TAT-GT----TT---
CTT----TAGACCAAAAAA-TCAATA-AT---TATGTTT-TTT-TAAT-AAAAG---TTTATTTATTTTAGTATCACCCCAATCAATAAAAT-----TGCC-----C---
GCCA----TT----C-CCA-----AAT-TTTC-----T--TATTTT-----TTA--
TTAAGCTTCAAGGGTCTTCTCGTCCTTTTAAAAAATTATAGCTTTTTCACTATAAAATAAAATTT---TA--A-TC-CT--
AATTAAGAGACAATAAAATTTTGTAAATCCCTTCATTTCTAGTCTTCAATTAATAAACTAATGATTATGCTACC-----

M_eduardoi -AGATCATGTAA-AGTTTTAA--GGTCGAACAGACCC-
AACAAATTAATGATTACTCCAAC-T-
TTTTTTAATCCAACATCGAGGTCGCAAACTTTTCTATTAATATGACTCGCCGAAAAAATAACGCTGTTATCCCTAGGGTAATTTATTATT-TAG-GT---AAC--
-CC-----AAGATCTTTAT-TCATTA-AT--AAATGTTA-TAA-TTTA-AAAAG---TTTATTTATTTTCTATCACCCCAATCAATAATTT-TTATTTATTA-C-C-----
-----ATA--CAC--ATTTTTA-----CTTT--
ATAAACTCCATAGGGTCTTCTCGTCCTTTAGTTTATTATAGCTTTTTCACTATAAAATAAAATTTA---TA-AA-TT--T--
TATTTGAGACAATTTTATTTCCGTTATACCTTTCTAGCTTCAATTAATAAAAGCTAATGATTATGCTACCTTTGCACGGTCATATTACCGCGGCCCTTTA
AT-----ATTTTCAG-GGGGAGGTTTTACTTTTTATA-TATCCAAAAAGCAAT

Massartella_brieni --GATCATGTAA-GGTTTTAAT--GGTCGAACAGACCT-TAGATTTAAGCTACTGCACTTAAATT-
TACCCTTAGTCCAACATCGAGGTCACAACCCCTCTTATCGATTAGAAGTCTTAGAAGAGATTATGCTGTTATCCCTAAGGTAACCTTACTCTTATAA-TCA-
TTAT---TAA---TGGATCTTAAAT-TCATA-AG--ATGTGTTT-TAG-AAAA-GAAAG---TTATTTTATTTCTTGTGCCCCAACAAAAAATTTT-C---
TTAGTTAA-AA-GT-AA--AC---TTC---AGCTA-----AG---ACCT-TTA--AGGGAA-----ACT--
GTTAAGATCTATAGGGTCTTCTCGTCCTTTAATGTTATCTCAGCCTTTTAACTGAGAGGTCAAATTT---TA-AA-TATCT--T-
TTAAGAGACAGCTTTTACCTCATCAACCAATTCATCCAGCCTTCAATTAATAAAAGACTAATGATTATGCTAC-----

Melanemerella_brasiliana --GATCATGTAGAAATATTGAA--GGTCGAACAGACCT-AGCATTTAAGCTTCTTCTCTAAATT-
ACTTTCTAATCCAACATCGAGGTCACAACCTTTTATCGATTGAAGTCTCCAAAAGAATAATGCTGTTATCCCTAAGGTAATTTA-TCTA-TAA-TCG-TCA---
-CAA----CGATCCTATAA-CTTTTT-AC--CAAATAAA--AA-TAAA-AAAAG---TTTACTTATTTCCATAACACCCCAATCAAAAA-----TATTTAA-
AACCTTAA--AA--TAC---C-ACA-----CAC---TCGC-CAG-----TAA-----TAT--
TTTAACTCTATAGGGTCTTCTCGTCCTTTTCCCCATTTTCAAGCCTTTTCACTAAAAGGTAATTTCT---TT-AA-AT--T--A-
TTTTAAGACAGACGACCCCTCGTTAACCATTTCATCCAGCCTTCAATCAAAAACTAATGATTATGCTACCT-----

Neophemera_youngi -----ATGTAA-GAATTTAAA--GGTCGAACAGACCT-
AATTTTTAAGTTTCTACTCCTAAAAT-
TATTTAGTCCAACATCGAGGTCGCAATCCTTTTTATCGATTAGGACTCTCCAAAAGATTACGCTGTTATCCCTAAGGTAATTTAATCTT-ATAATC---ATT-
---A--TTATGGATCCTAAAT-TCATTA-AT--TAATGTTAACAG-TAAG-AAAAG---TTTTTTATTTTCTTGTACACCCCAACAAAAATTT--TTAAAT-ATC---CA---
-AAT-AAT--CTTTA--A-CTATTTTTAA--ATATA---TA--A--A-----TT--
ATAAACTCTATAGGGTCTTCTCGTCCTTTTAAAGTTATTTAAGCGTTTTCACTCAAATCTTAAATTT---T--TA-TAA-T--
TTAAACGAGACAGCTAACATTTCTGTCACCCCTTCATGCCAGCCTTAAATAAGAGACTAATGATTATGCTACCTTTGCACGGTCAAAATACCGCGGCCCTTT
ATTACATTTTATCAG-TGGGACAGTTACACCTTAAATTT-AAACCTGAAGGCTAT

TGT----AAGATCTTAAAT-TCAATT-AA---TTTGTTA-TTA-TTTT-AAAAG---TTTACTTATTTTATTATCACCCCAATCAAATAA-----TACT-----T----
ATTTA-----T----T-CTA-----AAT-AATT-----TATTTA-----TT--
ATAAACTCTATAGGGTCTTCTCGTCCAATTTATTTATTATAGCTTTTTCTACTATAAAAATAAAATTC---AA--T-AA-AT--
TAATAAGAGACAAGAAAAATTTTCGTTTAACTTTTCATGCCAGCCTTCAATTAATAAGGCTAATTACTATGCTA-----

T_explicatus -AGATCATGTAG-AAATTTAAT--AGTCGAACAGACTA-
AATAATTTAATTTTACTCCAATAA-
TTTTTCTAATCCAACATCGAGGTCGCAACTCTATTTTATCAATTTGAGCTCTCCAAAATAATTACGCTGTATCCCAAGGTAACCTACTCTT-TAT-GT---AAT--
-CT-----AAGATCTGACAT-TCACCT-AT--A-ATGTTA-CTA-TCTA-AAAAG---TTTATTTATTTTATTATCACCCCAACATAATTAATT-TAGAAATATTA-T-
AATTACCA--ATT--TTT----T-TCA-----TTTTA-----
ATAAAGCTCTATGGGGTCTTCTCGTCCCTTTTACTTCATTATAGCTTTTTCTACTATAAAAATAAAATTA---TA-TA-AA--T--
TACTAAGAGACAATCAAATTTTCGTTGGACCAATTCATCCAGCCTTCAATGAAAAAGCTAATGATTATGCTACCTTCGCACGGTCAATATACCGCGGCTCTTT
AAGAG---ATCTCAG-TGAGCAGGTCATACTTTTTATT-AATACAAAAAGAAAT

T_quizeri --GATCATGTAG-AATTTAAT--AGTCGAACAGACTT-
AATTATTTAATTTTACTTTAAATA--
TCCTTCTAATCCAACATCGAGGTCGCAAACTATTTTATCGATTTGGGCTCTCCAAAATAATTACGCTGTATCCCAAGGTAACCTAATCTT-CAT-GT----AA--
-CTA---AAGATCTTAAAT-TCATTT-AG--CTTTGTTG-TTA-TTAC-AAAAG---TTTATTTATTTTTCGACATCACCCCAATTTAACAAAAT---CTACATT-----T-----
AA-----CT---T-CTA-----AAT-TTT-----TAATTA-----TTT--
GTAAAGCTCTACGGGCTTCTCGTCCCTTTATTTATATAGCTTTTCTACTATAAAAATAAAATTT---AA--T-AA-TT--
CAATAAGAGACAATTAATTTTTCGTTTAACTTTTCATCCAGCCTCAATTAATAAGGCTAATGATTATGCTAC-----

T_santarita ----CATGTAG-AATACAATA--GGTCGAACAGACCC-
AAATATTTAACTTTACCTTAAATAT-
GCTCTCAATTTCAACATCGAGGTCGCAAACTTTTTCATCAATATGAACCTCTGAAAAAATTACGCTGTATCCCTAGAGTAACCTACTCTT-CTTTA-----
A--CTTAAGATCTTCTAC-TCAATT-ATACTTTGTTT-AAC-TCAT-AAAAG---TTTATTTATTTTATTATCACCCCAATCCAATATA---TTATTT-----TA-----
---TTT---A-CTA-----AA-----CAA--T-CTCC-----TTAT--
ATAAAGCTTCAATAGGGTCTTCTCGTCCCTTCTAATTAATTATAGCTTTTTTACTATAAAAATAAAATTT-----TACCAA-T--
TAATAAGAGACAACCAAGTTTACGAAAACTCTTTCATTCAGCCTTCAATTAATAAGGCTAATGATTATGCTACCTTTCACAGTCATAATACTGCGGCCCTTTA
AC-----TTGTTCAG-GGGGCAGGAAGTACCTTTTATC-CTATCAAAAAGCAAT

T_tragedia -AAATCATGTAG-AAATTTAAT--AGTCGAACAGACTC-
CTCCATTTAATTTCTGCTTTAAATA--
TATTTCTAATCCAACATCGAGGTCGCAACCTTTCATCAATTTGAACCTCTCCAAAATAATTACGCTGTATCCCTAAGGTAATTT-TTCTT-TAT-GT---AC---
TTT-----AAGATCTTTAAT-TCAATT-AT---ACTGTTA-TTA-TTTT-AAAAG---TTTACTTATTTTATTATCACCCCAATCAAATAATTT-----TAATT-T-----
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Th_araponga -AGATCATGTA--AAATCTAAA--GGTCGAACAGACCT-
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AACTTTTAATTTCAACATCGAGGTCGCAACCCCTCCCATCGATAAGGACTCTCCGGGAAGATTACGCTGTTATCCCTAAGGTAAGCTTATGATCTT-CAA-TA----AT-
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TGTAT-TA--AC---TTT---A-CCA-----AAA-TTAAAGGTGG-----AG-----GCT--
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Th_artigas -AGATCATGTAA-AATGTTAAA--
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Th_chiriguano ----TCATGTAA-AATTTTAAA--GGTCGAACAGACCT-
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[18s ntax=45 nchar=641]

A. flinti

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Am. bahiensis

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Am. saguassu

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Brasilocaenis_irmli

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Coryphorus_aquilus

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Drunella_ishiyamana

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L_nigripennis ---TGCCAGCAGCCGCGTAATTCCAGCTCCATT-GGC GTA-
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Lachlania_dominguezi -GGTGCCAGCAGCCGCGTAATTCCAGCTCCATT-GGC GTA-
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Le_inanis ---TGCCAGCAGCCGCGTAATTCCAGCTCCATT-GGC GTA-
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Leptohyphes_sp
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T_cfaunapi ---TGCCAGCAGCCGCGGTAATTCCAGCTCCATT-GGCCTA-
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T_cfragoedia ---TGCCAGCAGCCGCGGTAATTCCAGCTCCATT-GGCCTA-
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T_explicatus -----
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T_santarita ---TGCCAGCAGCCGCGGTAATTCCAGCTCCATT-GGCCTA-
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Th_araponga ----CCAGCAGCCGCGGTAATTCCAGCTCCATT-GGCCTA-
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Th_chiriguano -----
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Th_gibbus -----CCAGCAGCCGCGTAATCCAGCTCCATT-GGCGTA-
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Th_minimus
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GGTGGC-C--TTTG--CTCT-----CGGG----CTTTGGCCGTC-----ACC-
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Traverhypes_sp ----
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CTCGCGGCTTTAACTGACCGTTTCCGGACGTCCTGCCGTTGGC-C--TTTG--CTCT-----CGGG----CATTGGCCGTC-----ACC-
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Traverhypes_spn
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Tricorythodes_sp ---TGCCAGCAGCCGCGTAATCCAGCTCCATT-GGCGTA-
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GGTGGT-C--TTCG--CCCG-----CGAGGG-----TGTTGGCCGTC-----ACC-
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TGCATGGAATAATGGAATAGGACCTCTTTCTATTTTGTGGCTTTTCTGAAA-
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Tricorythodes_spn1
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Tricorythodes_spn2 ---TGCCAGCAGCCGCGTAATCCAGCTCCATT-GGCGTA-
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Tricorythodes_spn3
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L_plaumanni

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Lachlania_dominguezi

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Le_inanis

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Leptohyphes_sp

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Lestagella_penicillata

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Lu_cocal

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Lu_guacra

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M_araca

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M_australis

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M_eduardoi

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Massartella_brieni

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Melanemerella_brasiliana

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Neophemera_youngi

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Oligoneuriella_rhenana

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Ranorythus_violettae

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Serratella_ignita

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T_explicatus

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T_quizeri

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T_santarita

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T_tragoedia

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Th_araponga

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Th_artigas

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Th_chiriguano

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Th_gibbus

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Th_minimus

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Th_rondoniensis

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Th_spongicola

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Tr_indicator

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Tr_yuati

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Traverhypes_sp

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Traverhypes_spn

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Tricorythodes_sp

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Tricorythodes_spn1

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Tricorythodes_spn2

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Tricorythodes_spn3

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V_packeri

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Y_mota

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[28S] [ntax=34 nchar=1308]

A_flinti

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Am_bahiensis

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Am_saguassu

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Brasilocaenis_irmleri

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ACG-TT-TCG--ACGT---G-----T--TGACAGATCCCATTTGCCCC-----GGCCGATTGCGCCGACGGTCTCAAAAG-CTG---GAACT--GGCCC--GCGG--TT-----
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GTGC---ATCTCGAGT-AGTTGGT---C-TG-----T-
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Cabecar_spn

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GCGGGCGCCGAGCGCACTTCTCCTAGTAGGACGCCGCGACCCGTCGCT--TTGAACGAACG--CG--GAGCTATGGGCGCGGGCGCA-AGTCCCG-----
GACCC-CCC-GCCTCG--GCGGG--G-----A--CCGGGGGCC-GCTCACCC-----GCTCCGCGG--CCGACGTCGCGAG--G-ACG--GAGGT-NGTCCC--TTCG-----
-AA---TG--AAGCCTCGCGCGCCGGGCGAGCGCGCCGCTG---GTCTCAAGCCGG--CCGCGAC-CT----GGTCCGCGCCG-CCCAG---
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GCAGCGGT-----CAAACCGTGC--GG-TCG-CCGCGCGA-GTTG----T---C-----
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---GG---CGA-GATTG--TCT---G-----C--TGCGAGCCCGGTGTCCT----GGTCCGCTT--CCGACGTCGTCGTTA-ACG--GAGCT--GTCC--GCAA-
CTT-----TGT--TGCGCCGTGCGGGTGCAGCGTCTTCC--GCTA---GTCGG--GCGCGACTTC-----GGTCCGCTCGA-TCGGG---
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ACG--AGAGCTCGTGCCCGCGAACGTTCTG-GAA--GTG----GCCGTGAC-GCGGAC-CT-----GGTCCGCCGCGG-TCCTC----CGGGCGTTGCCGGCG-TG-
 G--GTCT---TTCTCGGGC-AG--GG----A--C-----T-
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 TACCATGA

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 GC---CTCTCGGC-AG--GG---A--C-----T-
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Lachlania_dominguezi

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TACC----

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GGGGAGATTG-AGTATCCATTCTCACTGCCGCT--CT--CTGCC----GCGGGTATAACTCGGG-----C--G--GCCCCCTC--G--GGGG--CTGC-CC-T--
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Melanemerella_brasiliana

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GCC-CGCCCAGCGCGGGTGC-----G--GCCGACCCCGGGAGTCC-----CGCCCGCGC--CCGACGGTCAAC--G-GTG--GAGAT--CTCGC--TCCC-CCT--
AGCGGG--GGAGCCCGCGCCCGCGAGCGACCCGGCG--GTCTAAAGCCGG-AG-GCGGACCC-----TGTCGCCCGCG-CCCGT----
CGGCGCTGCGGCG-GG-G--C-GC--GGATCGGGC-GG--CG----A--G--
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Neophemera_youngi

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AACCATGA

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CACC----

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TGCTGGCCAGGACGAAGTCA-GGGGAAACCTGAT-GGAGGTCCGTAGCGA-TTCTGACGTGC-AAATCGATCGTCAGAGCT-GGGTATAGGGG-CG-
AAAGACTAATC--GAACCATCTAGTAGCTGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGTTGT--
CGAGTCCCATCCGGTAAAGCGAATGATTAGAGGCCTTGGGGTCAAAACGACTTCAACCTATTCTCAAACCTTAAATGGGTGGGAACCGCGGCTTGCCTGAAA
AGTGAAGCTCGCGGTGT-
ATCGGATCGGAGTGCCAAGTGGGCCATTTTTGGTAAGCAGAAGTGGCGCTGTGGGATGAACCAAACGTCGGGTTAAGGCGCCGAATGGATGCTCATTAGA
TACCA---

T_tragoedia

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GTAAACGGGTGGAGCCCTAAATGTGCAACGGGGAGATTC-AGTGTCCGATGCGTGNCGCGGCG--GC--CCG-T----ACGCC-----TCAAGC-----C--G--
GCGGTTTT-----CC-GT-C--GG-TGTGGCGTGCGGG-TG-----C--T-----GTC-
GCGGGCGCCGAGCGCACTTCTCTCTAGTAGGACGCCGCGACCCGTTGGT-----TGNG--TG--GACGTAAGGC-CCCGCGCG-AGNCCCG-----
TCC-TCTTTC-----G--A----G-AGCCGGAGCCGGGTGTCT-----CGTCTCTG--CCGACGGTCTGTA--G-GCG--GAGTC--TTCCC--TCCT-----CG--

Tr_indicator

AAGTACC GTGAGGGAAAGTTGAAA GAAC TTTGAAGAGAGAGTTCAAGAGTACGTGAAACCGTTATGGG-
GTA AACGGGTGAGCCCTAAAGGTGCAACGGGAGAGATTC-AGTGTCCATTGCACGTGCGGCA--GC--GCC-C----GCGCT-----TCAAGC-----T--
GTGGCGGC-----CCGGTCGTCTC--GG-TGT--CGTTCGGG-CGTG---T---G-----GCA-
GCGCGTGTGGAGCGCACTTCTCCTAGTAGGACGTGCGACCCGCCGGGA-----GATCA--AACGTAAGGC-CTGCGCGCG-AGTCTGG-----AG--T-
TTC-GCTTCG--GCGTT--T-----C-CCCAGAGCCGCGGTGCCT-----CGTTTGCC--CCGACGTCGTAG--A-GTG---GAGTT-TCTCC--TCTC-----TG--
TGAGCTCGTGTCTGCGCNAACATCCCG-NA---ACC---GTTGTAG-GCAGGC-CT----GGTCTGCCGAG-CCCTC----TGGGTGTGTCGGTG-CG-G--A-
TC--TTCTCGGC-AG--GG---A-----G---
TTCTCGTGGCGAGCTNAAACTGGGCGGCCACCCGACCGTCTTGAACACGGACCAAGGAGTCTAACATGCGTGGCAGTCATTGGGTG--C-
ACTAAACCCAAAGGCGCATTGAAGAAGAAGTAAGCT-TT--T-GTTTGCCGAGGGAAGATG-GGGG-TTCGTTT-----TCGT-
TCCTCCCGCATTCCCGGGCGTCTTCTTAGCGCGACCCGTTGCGTAAGGTGAGGCGCACCAAGAGCACGCACGTTGGGACCCGAAAGA TGGTGA ACTAT
GCCTGGCCAGCAGAACTCA-GGGAAACCTGTAG-GGAGTCCGTAGCGA-TTCTGACGTGC-AAATCGATCGTCAGAGCT-GGGTATAGGGG-CG-
AAAGACTAATC--GAACCATCTAGTAGTGGTCCCTCCGAAGTTCCCTCAGATAGCTGGCGCTCGTTGT--
CGAGTCCATCCGGTAAAGCGAATGATTAGAGGCC TTTGGGTGAAACGACCTCAACCTATTCTCAA ACTTTAAATGGGTGGAACTGCGGCTTGCTTGAAC
ACTGAAGCTGCGAGT---TTCGGATCGGAGTGCCAAGTGGGCCATTTTGNNAAGCAGAAC-
GGCGCTGTGGATGAACCAAACGCCGAGTAAAGGCGCCGAATAGATGCTCATACGATACC----

Tr_yuati

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Traverhypes_spn

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OUTGROUP Massartella_brieni;
OUTGROUP Melanemerella_brasiliana;
OUTGROUP Neoephemera_youngi;
OUTGROUP Oligoneuriella_rhenana;
OUTGROUP Ranorythus_violettae;
OUTGROUP Serratella_ignita;

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LSET APPLYTO=(5) NST=6 RATES=GAMMA; [GTR+G]
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end;
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Research article

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New species of *Macunahyphes* Dias, Salles & Molineri (Ephemeroptera: Leptohephidae), with taxonomic notes

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Abstract. *Macunahyphes zagaia* sp. nov. and *M. araca* sp. nov. are described based on imagines collected from the Amazon biome in Brazil. Specimens belonging to all species of the genus were examined and a diagnosis for each one is provided. Variation seen in the male imago of *M. eduardoi* is described, as well as the female and egg for the first time. Together with *M. eduardoi*, the two new species share the presence of a basal swelling on segment II of the forceps, which raises important questions concerning the evolution of this character in Leptohephidae. Finally, a re-definition of the genus is proposed.

Keywords. Taxonomy, mayfly, South America, Amazon Rainforest, aquatic insect.

Souto P.M. & Salles F.F. 2016. New species of *Macunahyphes* Dias, Salles & Molineri (Ephemeroptera: Leptohephidae), with taxonomic notes. *European Journal of Taxonomy* 254: 1–15. <http://dx.doi.org/10.5852/ejt.2016.254>

Introduction

The genus *Macunahyphes* Dias, Salles & Molineri, 2005 was erected to include *Macunahyphes australis* (Banks, 1913) originally described by Banks (1913) in the genus *Tricorythus* Eaton, 1868. This species, described solely on adults, was subsequently transferred to *Leptohephodes* Ulmer (Ulmer 1920) and then to *Tricorythodes* Ulmer (Traver 1958). With the discovery and subsequent description of the nymphs, *T. australis* was finally transferred to the new genus *Macunahyphes* (Dias *et al.* 2005). Among the distinctive characteristics of this species, Traver (1958), as well as Molineri (2002), pointed out the absence of the basal swelling of the second joint of the forceps, absence of the longitudinal vein CuP on male fore wings, and the morphology of the penis, with the presence of a ventral projection.

Based on adults from Venezuela and Brazil, respectively, Molineri *et al.* (2011) described two additional species: *M. pemonensis* Molineri *et al.* and *M. incognitus* Molineri *et al.* Despite the absence of forceps in the few available specimens of both species, the presence of a ventral projection on the penis, wing venation and similar color pattern were enough to allocate them to the genus *Macunahyphes* (Molineri *et al.* 2011).

Recently, two additional species were found in Brazil: *M. pemonensis*, recorded from Northern Brazil based on a larger series of male adults, and *M. eduardoi* Almeida & Mariano, 2015, based on male adults from Northeastern Brazil (Belmont *et al.* 2015; Almeida & Mariano 2015). Importantly, forceps of both species were unbroken and the basal swelling, absent in *M. australis* and considered one of the striking characteristics of the genus, was found to be present in *M. eduardoi* and *M. pemonensis*.

In the present paper, based on material from the Amazon and Atlantic Forest biomes in Brazil, two new species of *Macunahyphes* are described. In addition, specimens belonging to all species of the genus are examined, new data are provided for them and a re-definition of the genus is proposed.

Material and methods

Specimens were preserved in 80% ethanol, wings were mounted dry and genitalia were mounted in Euparal[®]. Photographs were taken with a digital camera coupled to a Zeiss Axiocam ERc 5s stereo microscope and combined using Helicon Focus 6[®] software. Some of the photographs were used as templates for trace vector graphics in Adobe Illustrator CS6[®] software to produce the illustrations. Material from the following institutions was studied: Coleção Zoológica Norte Capixaba, Universidade Federal do Espírito Santo, São Mateus, Brazil (CZNC) and Coleção Entomológica Professor José Alfredo Dutra (DZRJ), Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil. Holotypes and part of the paratypes were deposited in the Coleção de Invertebrados of the Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil (INPA). The other paratypes were deposited in CZNC and DZRJ. Wings were mounted on dry slides; as all were identical, there was no need to draw them. Eggs were removed from females of *Macunahyphes eduardoi* Almeida & Mariano, 2015 and *Macunahyphes araca* sp. nov., then mounted with double sided tape on SEM stubs and sputter coated with gold. They were observed and photographed with a Jeol JSM-6510 scanning electron microscope. The terminology proposed by Koss & Edmunds (1974) was used to describe the eggs. The distribution map was made using the program QGIS 2.12.0-Lyon (QGIS 2015).

Results

Class Hexapoda Latreille, 1825
Subclass Insecta Linnaeus, 1758
Order Ephemeroptera Hyatt & Arms, 1890
Suborder Pannota McCafferty & Edmunds, 1979
Family Leptohephidae Landa, 1973

Macunahyphes Dias, Salles & Molineri, 2005

Macunahyphes Dias, Salles & Molineri, 2005: 196 (type species: *Tricorythus australis* (Banks, 1913), by monotypy).

Diagnosis

Genus characterisation: 1) eyes undivided in both sexes; 2) fore wings with well-developed Cu-A lobe, especially in males; 3) longitudinal vein CuP poorly developed in both sexes or absent; 4) hind wings absent in both sexes; 5) membranous filaments of mesoscutellum present or absent; 6) styliger plate

very slightly projected posteriorly as a columnar base for each pair of forceps (see figs 7–9 in Molineri *et al.* 2011) ; 7) basal swelling on forceps segment II present or absent. In the nymph: 1) maxillary palp reduced in size, one-segmented, with apical seta; 2) glossa and paraglossa almost completely fused, with a circular outline; 3) femora with numerous robust serrate setae; 4) tarsal claws long and slender, with 7–10 marginal denticles and a double row of 2–4 submarginal denticles; 5) gills present on abdominal segments II–VI, gill formula 3/3/3/3/2; 6) operculate gills subtriangular; 7) posterior margin of terga II–V smooth, other segments with denticles.

Comments

Due to the presence of membranous filaments on the mesoscutellum in females and males in one species, and to the presence of a basal swelling on forceps segment II in four species, we propose an expansion to the diagnosis of the genus provided by Dias *et al.* (2005) to include characters 5 and 7 above. As *M. australis* is the only species in which nymphs have been described, the generic diagnostic features of the nymphal stage are maintained.

Distribution (Fig. 1)

Argentina, Brazil, Guyana and Venezuela.

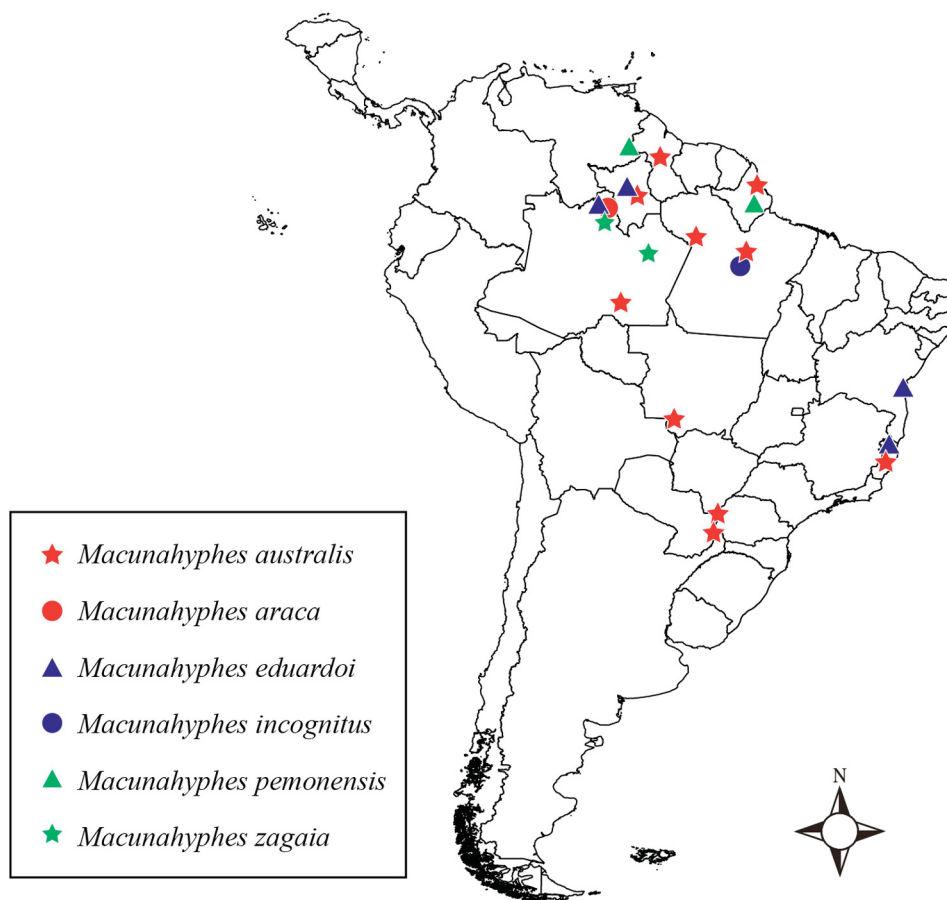


Fig. 1. Geographical distribution of species of *Macunahyphes* in South America and Brazil (per state).

Macunahyphes australis (Banks, 1913)

Fig. 2C

Tricorythus australis Banks, 1913: 85.

Leptohyphodes australis – Ulmer 1920: 50.

Tricorythodes australis – Traver 1958: 501–503. — Hubbard 1982: 274. — Molineri 2002: 278.

Macunahyphes australis – Dias, Salles & Molineri 2005: 199.

Material examined

BRAZIL: Espírito Santo: 113 ♂♂, imagines, Colatina, Itapina, Doce River, 19°31'18.1" S, 40°50'11.1" W, 7–8 Sep. 2014, E.M. Rozário and F.F. Salles leg. (CZNC Ep-6440); 14 ♀♀, imagines, same data (CZNC Ep-6441); 72 ♂♂, imagines, same data (CZNC Ep-6443); 73 ♂♂, imagines, same data (CZNC Ep-6444); 64 ♂♂, imagines, same data (CZNC Ep-6445); 100 ♂♂, imagines, same data (CZNC Ep-6447); 3 ♂♂, imagines, Marilândia, Doce River, 19°31'5.3" S, 40°34'49.9" W, 8–9 Sep. 2014, E.M. Rozário and F.F. Salles leg. (CZNC Ep-6439); 7 ♂♂, imagines, same data (CZNC Ep-6442); 1 ♀, imago, same data (CZNC Ep-6446); IFES Itapina, Doce River, 19°31'15.1" S, 40°46'53.6" W, E.M. Rozário and F.F. Salles leg. (CZNC Ep-6448); 2 ♂♂, imagines, same data (CZNC Ep-6449); 5 ♂♂, imagines, Nova Venécia, city gate, Cricaré River, 18°42'54.91" S, 40°22'33.33" W, 23–24 May 2012, F.F. Salles and K.B. Angeli leg. (CZNC Ep-4523); 1 ♂, 1 ♀, imagines, São Mateus, Cotaxé River, Japira waterfall, 18°34'39.1" S,

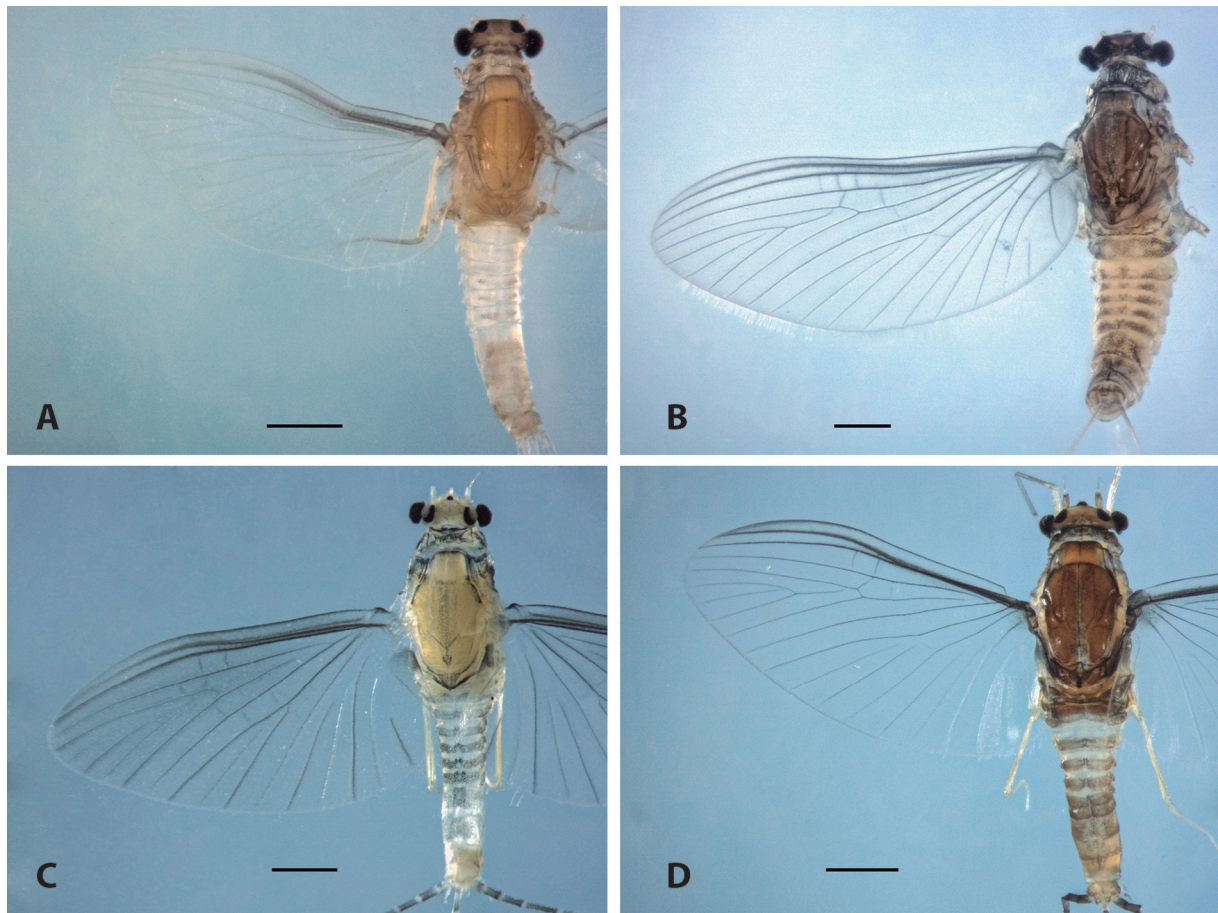


Fig. 2. *Macunahyphes* spp., dorsal view. **A.** *M. zagaia* sp. nov. (♂). **B.** *M. eduardoi* Almeida & Mariano, 2015 (♀). **C.** *M. australis* (Banks, 1913) (♂). **D.** *M. araca* sp. nov. (♂). Scale bars: 0.5 mm.

40°16'58" W, 7–8 Sep. 2012, K.B. Angeli and E.A. Raimundi leg. (CZNC Ep-4524); 1 ♂, imago, São Mateus River, 18°39'17.1" S, 39°59'36" W, 7–8 Sep. 2012, K.B. Angeli and E.A. Raimundi leg. (CZNC Ep-4527); 1 ♂ imago, Pedra d'Água, 18°43'15.2" S, 39°48'50.8" W, 19–20 Apr. 2012, K.B. Angeli and E.A. Raimundi leg. (CZNC Ep-4528); 4 ♂♂, imagines, Cotaxé River, Japira waterfall, 18°34'39.1" S, 40°16'58" W, 6–7 Feb. 2013 (CZNC Ep-6159); 8 ♂♂, 6 ♀♀, imagines, Roraima, Caracarái, Balneário Bem Querer, 01°55'46.3" N, 61°00'06.9" W, 13 Mar. 2014, F.F. Salles, R. Boldrini, E. Dominguez leg. (CZNC Ep-6560); 3 ♂♂, Balneário Bem Querer, 18–21 Nov. 2002, A.M.O. Pes leg. (CZNC Ep-6561).

Diagnosis

1) Longitudinal vein CuP poorly developed in both sexes or absent; 2) membranous filaments of mesoscutellum absent; 3) forceps tri-segmented with first segment, distomedially projecting, 4) penis with very wide base, becoming thinner towards a subapical constriction, and then slightly widening again, penis lobes of each side almost completely fused except apical incision; 5) penis with a ventral projection covered with spines.

As *M. australis* is the only species for which nymphs have been described, the generic diagnostic features of the nymphal stage should also be used for specific diagnosis.

Distribution (Fig. 1)

Argentina (Misiones), Brazil (Amazonas, Espírito Santo, Mato Grosso, Pará, Paraná, Roraima) and Guyana.

Macunahyphes araca sp. nov.

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Figs 2D, 3A, 4A–D

Diagnosis

The male of *M. araca* sp. nov. can be distinguished from all congeners by the following combination of characteristics: 1) longitudinal vein CuP absent; 2) membranous filaments of mesoscutellum absent;

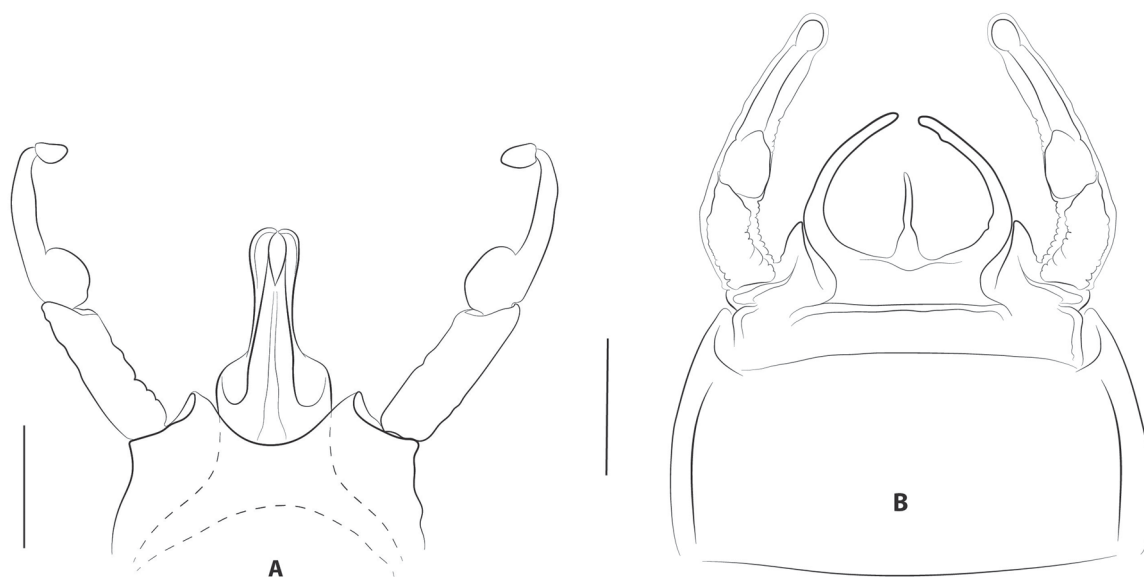


Fig. 3. Male genitalia (ventral view). **A.** *Macunahyphes araca* sp. nov. **B.** *Macunahyphes zagaia* sp. nov. Scale bars: 100 μ m.

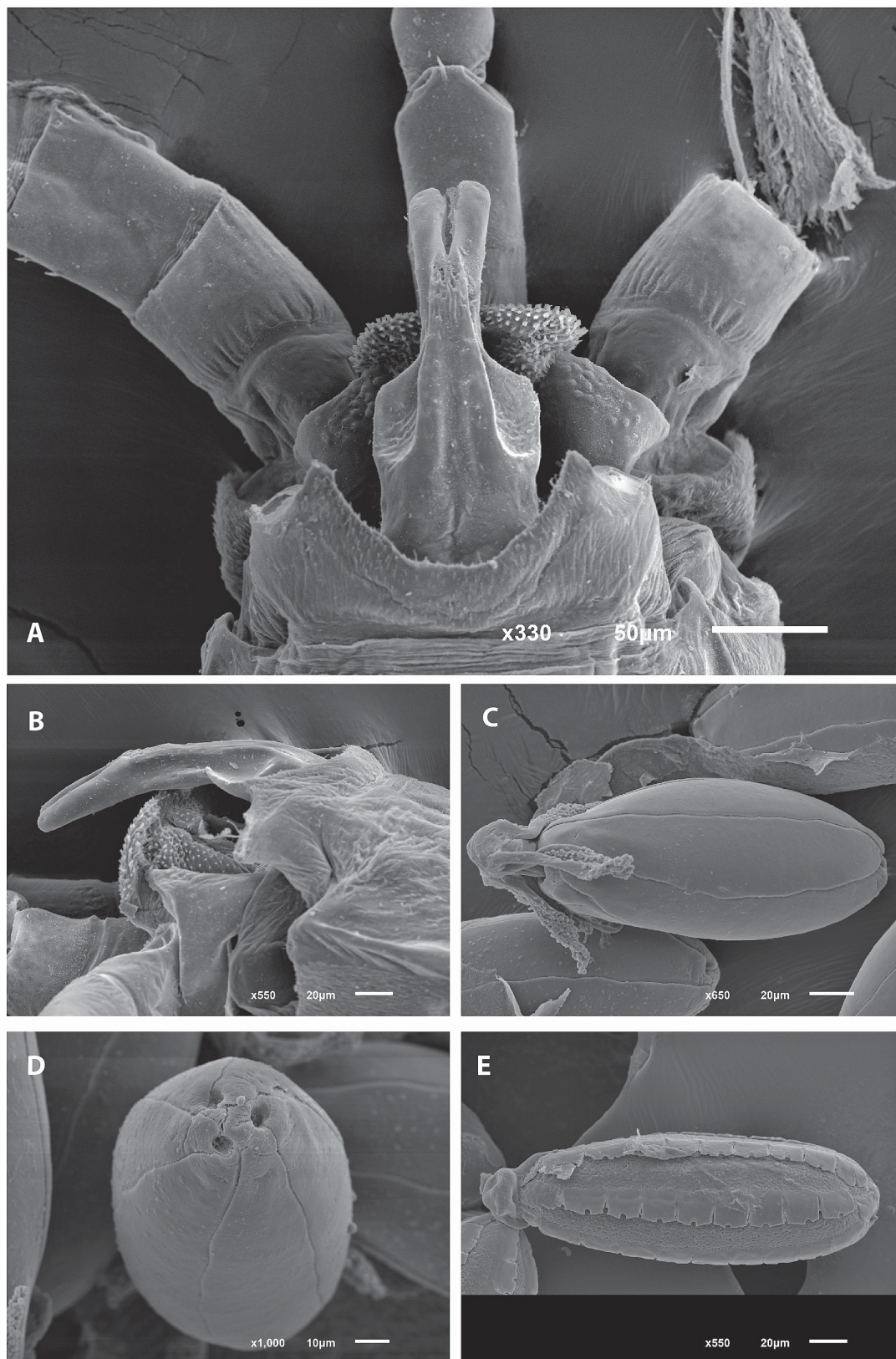


Fig. 4. SEM micrographs. — **A–D.** *M. araca* sp. nov. **A.** Male genitalia (ventral view). **B.** Male genitalia (lateral view). **C.** Egg (general aspect). **D.** Egg showing the micropylar area. — **E.** *M. eduardoi* Almeida & Mariano, 2015, egg (general aspect).

3) segment II of forceps with a basal swelling; 4) spermatic duct dark purple, visible laterally from outside; 5) styliger plate slightly projected posteriorly as a columnar base for each forceps, with sublateral acute projection on hind margin; 6) penis base subquadrate, narrowing abruptly in median zone; 7) penis with deep apical furrow; 8) ventral structure of penis reaching the deep apical furrow.

Etymology

Aracá is the name of the mountain range where this new species was collected.

Type material

Holotype

BRAZIL: ♂, imago, Amazonas, Barcelos, Serra do Aracá, Igarapé do Anta, 0°54'30.38" N, 63°26'24.32" W, 2 Aug. 2009, F.F. Salles, A.P. Santos and N. Ferreira-Jr. leg. (INPA-EPH 000001).

Paratypes

BRAZIL: 23 ♂♂, imagines, same data (CZNC Ep-6351); 10 ♂♂, imagines, same data (INPA-EPH 000002 to INPA-EPH 000011); 13 ♂♂, 1 ♀, imagines, Igarapé do Anta, 0°54'30.38" N, 63°26'24.32" W, 1–3 Aug. 2009, F.F. Salles, A.P. Santos and N. Ferreira-Jr. leg. (DZRJ 3143); 1 ♂, imago, Jauari River, 0°48'0.28" N, 63°29'22.92" W, 21 Jul. 2009, F.F. Salles, A.P. Santos and N. Ferreira-Jr. leg. (CZC Ep-5585); 1 ♂, imago, same data (CZNC Ep-6354); 5 ♂♂ imagines, Igarapé da Serrinha, 0°25'19.96" N, 63°23'47.04" W, 28 Jul.–1 Aug. 2009, F.F. Salles, A.P. Santos and N. Ferreira-Jr. leg. (CZNC Ep-6353); 1 ♂, imago, same data but 19 Jul. 2009 (CZNC Ep-6355); 5 ♂♂, 1 ♀, imagines, Igarapé do Anta, 0°54'30.38" N, 63°26'24.32" W, 1 Aug. 2009, F.F. Salles, A.P. Santos and N. Ferreira-Jr. leg. (CZNC Ep-6356).

Description

Holotype

LENGTH (mm). Body: 2.8; fore wing: 2.6; cerci: 5.8; terminal filament: 7.5. General coloration brown.

HEAD. Yellowish brown; antennae brown.

THORAX. Pronotum translucent, washed with black; mesonotum brown, washed with black, except on lateral region of anterotal protuberance and submesoscutum; metanotum yellowish brown, washed with black; membranous filaments of mesoscutellum absent.

FORE WING. Membrane translucent; base and coastal region washed with dark purple; longitudinal veins black; vein CuP absent.

LEGS. Yellowish, inner apex of trochanter tinged with black; fore tibia and fore tarsi slightly washed with dark purple.

ABDOMEN. Terga II–X with medio-longitudinal dark line, terga I–VII translucent, tinged with dark purple, terga VIII–X yellowish brown, washed dark purple; tergum X with medial dark furrow; spermatic duct dark purple, visible from outside.

GENITALIA. Forceps translucent, washed with dark purple, except on inner margin of segment I, styliger plate and penes translucent yellowish; styliger plate slightly projected posteriorly as a columnar base for each forceps, with sublateral acute projection on hind margin; forceps segment I weakly attached to styliger plate, basal swelling present on segment II; penes long, with subquadrate base, deep apical furrow and a somewhat protruded ventral structure. Caudal filaments translucent, tinged with dark purple at base, becoming lighter towards apex.

Female imago

LENGTH (mm). Body: 3.0; fore wing: 3.0. General coloration brown.

HEAD. Yellowish brown, washed with black, posterior margin darker; scape and pedicel dark brown, flagellum whitish.

THORAX. Pronotum tinged with black, lateral corner brown, washed with black; mesonotum brown, washed with black; metanotum yellowish brown, washed with black; membranous filaments of mesoscutellum absent.

FORE WING. Membrane white, longitudinal and cross-veins black; vein CuP absent.

LEGS. Yellowish white.

ABDOMEN. Translucent, tinged with black; caudal filaments broken off.

Egg (Figs 4C–D)

Yellow; single polar cap, attachment structures with tubercles on surface; three micropylar openings present at opposite pole of polar cap; chorionic surface without sculpture and with six elongated and linear micropylar canals ending at micropylar opening.

Biology

The specimens were collected at light traps during the dry season. The new species was found exclusively at the Serra do Aracá, together with *M. eduardoi* and *M. zagaia* sp. nov. *Macunahyphes araca* sp. nov. was the only species of mayfly found at the base and at the top of the Serra do Aracá. The Serra do Aracá is an elevated area (100 to 1500 m a.s.l.) in the State of Amazonas near the borders of Roraima State and Venezuela. Only five species of mayflies were collected at the top of Serra do Aracá during the same field trip; all of them were new to science at that time. In addition to the *Macunahyphes* species cited above, the other two species were *Askola yanoman* Nascimento, Barcelos-Silva & Salles, 2011 and an undescribed species, also found exclusively at the top, suggesting a high level of endemism in this area.

Remarks

Macunahyphes araca sp. nov. and *M. pemonensis* are the darker species of the genus. *Macunahyphes araca* sp. nov. can also be distinguished from all other species by the unique spermatid duct, which is visible laterally from outside due to its dark purple coloration, a characteristic also found in *M. pemonensis* (see Belmont *et al.* 2015: figs 1, 6). As in *M. pemonensis*, the styliiger plate is slightly projected posteriorly as a columnar base for each forceps, with a sublateral acute projection on the hind margin, unlike the one found in *M. incognitus*. On the other hand, in the new species the penis base is subquadrate, narrowing abruptly in the median zone, as found in *M. incognitus*. Despite the very similar genitalia of *M. araca* sp. nov. and *M. incognitus*, the ventral structure of the new species reaches the deep apical furrow, while the ventral structure of *M. incognitus* ends before the apical furrow and the penis lobes are almost completely fused. According to the original description, *M. incognitus* seems to be similar to *M. australis* regarding the overall coloration, lighter in comparison to *M. araca* sp. nov.

Distribution (Fig. 1)

Brazil (Amazonas).

Macunahyphes eduardoi Almeida & Mariano, 2015

Figs 2B, 4E

Macunahyphes eduardoi Almeida & Mariano, 2015: 498.

Diagnosis

According to the original description, the male of *M. eduardoi* can be distinguished from all congeners by the following combination of characteristics: 1) fore wing translucent, with costal and subcostal area tinged with grey and with longitudinal vein CuP absent; 2) penes opaque yellow, spine elongate with lateral projections.

Material examined

Holotype

BRAZIL: ♂, imago, Bahia, Igrapiúna, Michelin Ecological Reserve, Pacangê River, Pacangê-Sapucaia 2ª Ponte, 13°50'17.1" S, 39°14'27.7" W, 101 m, 21 Sep. 2012, A.R. Calor and Equipe LEAq leg. (CZNC).

Other material

BRAZIL: 1 ♂, imago, Espírito Santo, Nova Venécia, Santa Rita do Pipinuck, Rio Cricaré, 18°39'51.4" S, 40°30'44.9" W, 25–26 Jul. 2012, K.B. Angeli leg. (CZNC Ep-4561); 2 ♂♂, imagines, same data but 18 Apr. 2012 (CZNC Ep-6431); 4 ♂♂, 3 ♀♀, imagines, Amazonas, Barcelos, Serra do Aracá (Base), tributary of Igarapé do Cobra, 00°52'13.22" N, 63°27'13.36" W, 25–26 Jul. 2009, F.F. Salles, A.P. Santos and N. Ferreira-Jr. leg. (CZNC Ep-6437); 1 ♂ imago, Jauari River, 00°48'0.28" N, 63°29'22.92" W, 26 Jul. 2009, F.F. Salles, A.P. Santos and N. Ferreira-Jr. leg. (CZNC Ep-6438); 1 ♂, 3 ♀♀, subimagines, Roraima, Caracaraí, Balneário Bem Querer, 01°55'46.3" N, 61°00'06.9" W, 13 Mar. 2014, F.F. Salles, R. Boldrini, E. Dominguez leg. (CZNC Ep-6562).

Description

Female subimago (Fig. 2B)

LENGTH (mm). Body: 3.0–4.8; fore wing: 3.2–5.0. General coloration dark brown and yellowish.

HEAD. Brown, washed with black; area between lateral ocelli and posterior margin tinged with black.

THORAX. Pronotum brown, tinged with black, except for submedial stripe and anterolateral corner; mesonotum brown, washed with black; metanotum yellowish, washed with black; membranous filaments of mesoscutellum present and longer than in males.

FORE WING. Membrane white, longitudinal and cross-veins black; vein CuP absent.

ABDOMEN. Translucent (yellow because of the presence of eggs), washed with black; terga II–V forming a V-shaped unpigmented area; caudal filaments broken off.

Egg (Fig. 4E)

Yellow. Shape elongate. One polar cap present. Chorion with longitudinal costae formed by plates, each one with a deep emargination.

Remarks

In the description of the male imago of *M. eduardoi* there is no reference to the presence of membranous filaments of the mesoscutellum; this character is evident from the fresh material studied here. After examining the holotype of *M. eduardoi* it was possible to observe the presence of a short filament of about ¼ the length of the mesoscutellum. According to the original description, the hook-shaped lateral

expansions present in the penis projection are distributed from its base to the middle region, whereas in the material examined by us the expansions are present throughout the penis projection, decreasing in size towards the apex. This is the first record of *M. eduardoi* from southeastern (state of Espírito Santo) and northern (state of Amazonas) Brazil.

Macunahyphes eduardoi occurs in areas comprising two disjunct biomes, Amazon and Atlantic Forest. Other species of plants and animals also show this distribution pattern, including some aquatic insects, such as *Asthenopodes chumuco* Molineri, Salles & Peters, 2015 (Ephemeroptera: Polymitarcyidae), *Macrostemum erichsoni* (Banks, 1920) and *Smicridea (Ryachophylax) roraimense* Albino, Pes & Hamada, 2011 (Trichoptera: Hydropsychidae). The presence of disjunct populations of a single taxon in Amazonia and the Atlantic Forest may be evidence of ancient connections between these biomes (see Costa 2003; Santos *et al.* 2007; Fiaschi & Pirani 2009; Buso Junior *et al.* 2013).

Distribution (Fig. 1)

Brazil (Amazonas, Bahia and Espírito Santo).

Macunahyphes incognitus Molineri, Grillet, Nieto, Dominguez & Guerrero, 2011

Macunahyphes incognitus Molineri, Grillet, Nieto, Dominguez & Guerrero, 2011: 45.

Diagnosis

According to the original description, the male imago of *Macunahyphes incognitus* can be distinguished from all congeners by the following combination of characteristics: 1) fore wings with hyaline membrane except around basal half of vein Sc, shaded with gray; 2) styliger with a pair of sublateral acute projections on the hind margin; 3) penes completely fused apically, with subquadrate base, narrowing abruptly in median zone, and with a ventral projection.

Material examined

Holotype

BRAZIL: ♂, imago (genitalia), Pará, rio Xingú, Campament, ca 60 km S of Altamira, 3°39' S, 52°22' W, 1–21 Oct. 1986, P. Spangler & O. Flint leg. (INPA).

Remarks

Macunahyphes incognitus was described based on only one specimen and since then there has been no further record of this species. The forceps are unknown and we only had access to the holotype genitalia. Because of this, the comparisons between species were made based on the genitalia slide and original description.

Distribution (Fig. 1)

Brazil (Pará).

Macunahyphes pemonensis Molineri, Grillet, Nieto, Dominguez & Guerrero, 2011

Macunahyphes pemonensis Molineri, Grillet, Nieto, Dominguez & Guerrero, 2011: 45.

Diagnosis

According to the original description, the male imago of *Macunahyphes pemonensis* can be distinguished from all congeners by the following combination of characteristics: 1) fore wings hyaline, membrane tinged with gray on basal half of C and Sc regions and basally to vein A; 2) styliger plate very slightly

projected posteriorly as a columnar base for each forceps, with a pair of sublateral acute projections on the hind margin; 3) penis long and slender, with apical furrow and a somewhat protruding ventral projection.

Material examined

BRAZIL: 2 ♂♂, imagines, Amapá, Pedra Branca, stream crossing highway BR 210 near Pedra Branca (PT3), 00°37'38.9" N, 51°38'15.2" W, 3 Aug. 2011, P.V. Cruz, A. Pes and A.S. Fernandes leg. (DZRJ 3156).

Remarks

In the original description of the male imago of *M. pemonensis* there is no mention of the presence of a dark purple spermatic duct that is laterally visible from outside, a character also found in *M. araca* sp. nov. and evident from the fresh material of *M. pemonensis* studied here (see Belmont *et al.* 2015: figs 2, 6). In addition, a new interpretation is given to the ventral structure present in the penis described by Molineri *et al.* (2011). The lateral margins of the penis lobe are strongly sclerotized and are ventrally twisted, forming a lateral narrow flap on each side of the penis. The area between these flaps, interpreted as a ventral structure in the original description, is in fact a shallow groove (see Belmont *et al.* 2015: figs 4–6).

Distribution (Fig. 1)

Brazil (Amapá) and Venezuela (Bolívar).

Macunahyphes zagaia sp. nov.

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Figs 2A, 3B

Diagnosis

The male of *M. zagaia* sp. nov. can be distinguished from all congeners by the following combination of characteristics: 1) longitudinal vein CuP absent; 2) membranous filaments of mesoscutellum absent; 3) segment II of forceps with a basal swelling; 4) penes trident-like, with inward curved lateral projections and acute medial projection.

Etymology

Zagaia is a spear with three points used in Amazonian artisanal fishery, reminiscent of the characteristic trident-like penis of the new species.

Type material

Holotype

BRAZIL: ♂, subimago, Amazonas, Manaus, Reserva Adolfo Ducke, Igarapé do Tinga, 2°55'33.53" S, 59°54'1.16" W, 10–13 Jun. 2002, A.M.O. Pes leg. (INPA-EPH 000012).

Paratypes

BRAZIL: 1 ♂, subimago, same data as holotype but 10–15 Jun. 2004, A.M.O. Pes leg. (DZRJ 3145); 1 ♂, subimago, Barcelos, Aracá sierra, Aracá river, before mouth of the igarapé da serrinha, 00°23'57.41" N, 63°22'43.97" W, 1 Feb. 2009, F.F. Salles, A.P. Santos and N. Ferreira-Jr leg. (INPA-EPH 000013); 1 ♂, subimago, Aracá sierra, 2009, F.F. Salles, A.P. Santos and N. Ferreira-Jr leg. (DZRJ 3144); 2 ♂♂, subimagines, Jauari River, 00°48'0.28" N, 63°29'22.92" W, 26 Jul. 2009, F.F. Salles, A.P. Santos and N. Ferreira-Jr leg. (CZNC Ep-6436).

Description

Holotype

LENGTH (mm). Body: 2.75; fore wing: 2.76; cerci: 1.5; terminal filament: 2.14. General coloration yellowish brown and white, abdomen slightly washed with black.

HEAD. Yellowish, washed with black; ocelli white, surrounded with black; eyes black; antennae translucent white.

THORAX. Pronotum yellowish, heavily washed with black, except submedial unpigmented stripe and oblique brownish line; mesonotum yellowish brown, slightly washed with black and darker on carinae, anteronotal protuberance yellowish; metanotum yellowish; pleura yellowish; sterna yellowish.

FORE WING. Membrane whitish, tinged with gray on basal half of C and Sc regions; longitudinal veins and cross veins whitish, except for vein Sc; vein CuP absent.

LEGS. Fore leg lost in holotype; middle and hind legs translucent yellowish, femur with apical black mark.

ABDOMEN. Segments I–VI translucent white, VII–X yellowish white. Terga I–X almost completely washed with black, terga I–VIII with unpigmented sublateral stripe, tergum X with medial dark furrow. Sterna slightly washed with black.

GENITALIA. Forceps whitish, styliger plate and penes yellowish; segment II of forceps with a basal swelling; styliger plate with acute sublateral projection on hind margin; penes trident-like, with inward curved lateral projection and acute medial projection. Caudal filaments whitish, slightly washed with black at base.

Female

Unknown.

Nymph

Unknown.

Biology

The new species was collected during late fall and winter (Jun. 2002 and 2004; Jul. and Aug. 2009).

Variation

Differences were found only in relation to the length of the subimagines, ranging from 2.7 to 3.1 mm (body), 6.0 to 7.6 mm (cerci) and 4.8 mm (terminal filament).

Remarks

The male imago of *Macunahyphes zagaia* sp. nov. can be readily distinguished from all other species in the genus by the unique morphology of the penes.

Distribution (Fig. 1)

Brazil (Amazonas).

Discussion

According to Dias *et al.* (2005), unique characters present in imagines of *Macunahyphes* are: forceps tri-segmented, with first segment distomedially projecting; penis with very wide base, becoming thinner toward a subapical constriction, and then slightly widening again; penis lobes of each side almost completely fused except for apical incision; penis with a ventral projection covered with spines. With the recent discovery of new species, some of these features are actually restricted to *M. australis*.

Concerning the forceps, the presence of a basal swelling in the two new species described here, as well as in adults of *M. pemonensis* and *M. eduardoi*, raises important questions concerning the evolution of this character in Leptohiphidae. According to Molineri (2002), the presence of the basal swelling was considered one of the synapomorphies of the genus *Tricorythodes*. This basal swelling, however, might be a synapomorphy not for *Tricorythodes*, but for the clade leading to *Macunahyphes* and *Tricorythodes* (see Molineri 2002, 2006). In that case, its absence in *M. australis* seems to be an autapomorphic trait.

The ventral projection of the penis covered with spines is clearly an autapomorphy of *M. australis*, since it is not found in any of the remaining species. In fact, unlike other Leptohiphidae, the morphology of the penis in *Macunahyphes* is highly variable among species. Any attempt to provide a diagnostic characteristic for that structure, therefore, is unsuccessful.

Wing venation, especially the absence of the vein CuP, was the main characteristic used for the allocation of the new species in *Macunahyphes*. The shape of the penis, a character somewhat conservative among the genera of Leptohiphidae and historically useful for genus delimitation in mayflies, is surprisingly variable in *Macunahyphes*. Despite the fact that vein CuP is poorly developed in some species of *Tricorythodes* (e.g., *T. bullus*), as well as in some specimens of *M. australis*, the new species described here should not be allocated to *Tricorythodes*. In this genus, one of the most diverse and widely distributed of the family, the shape of the penis is similar in all the species described so far (ca 55 species described). The description of the immature stages of most of the species of *Macunahyphes* will undoubtedly be essential for elucidating the monophyly of the genus and its relationship with *Tricorythodes*.

To improve our current knowledge of the genus, new efforts must be made in order to find more specimens of some rare species, such as *M. incognitus*, and, even more important, to find and describe the unknown nymphs of the species described in the last few years.

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***Tricorythodes tragoedia* sp. nov. (Ephemeroptera: Leptohiphidae), a new species from Rio Doce and surrounding areas, southeastern Brazil**

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Abstract

A new species of *Tricorythodes* Ulmer (Ephemeroptera: Leptohiphidae) is described and illustrated based on nymphs and adults from the Doce River and surrounding areas in southeastern Brazil. *Tricorythodes tragoedia* sp. nov. is related to *T. arequita* Traver, *T. mirca* Molineri and *T. sallesi* Dias, Cabette & De Sousa, but its nymphs can be distinguished from these species by having a three-segmented maxillary palp with apical seta and one pair of submarginal denticles on the tarsal claws. Nymphs were collected from small to large rivers at altitudes of less than 100 m where they inhabit submersed substrates in areas with slow current. Nymphs and adults were found throughout the year.

Key words: mayfly, taxonomy, aquatic insect, South America, Espírito Santo

Introduction

Tricorythodes Ulmer, 1920 is the most diversified and widely distributed genus of the New World family Leptohiphidae (Ephemeroptera: Ephemerelloidea). To date, the genus is represented by approximately 60 species distributed from Canada to Uruguay, including the Greater and Lesser Antilles (Sartori & Britain 2015, Molineri 2002, Kluge & Naranjo 1990, Hoffman *et al.* 1999, Alba-Tercedor & Flannagan 1995, Naranjo & Peters 2017).

In South America, *Tricorythodes* has received considerable attention in the last 15 years. Molineri (2002) revised the representatives of the genus in this area up to that time and described four new species. Subsequently, based on material from Colombia and Brazil, 11 new species have been described (*c.f.* Emmerich 2007; Dias *et al.* 2009a, 2009b, 2011; Gonçalves *et al.* 2011; Belmont *et al.* 2011, 2012).

Numerous new species, however, remain to be described from South America. In the present paper, based on nymphs and adults (male and female), we describe a new species from Northern Espírito Santo, Southeastern Brazil. A formal name for this species is extremely important since we found most of the specimens at the Doce River basin, an area that has been recently impacted by a disastrous dam breach in Mariana (Fernandes *et al.* 2016).

Material and methods

Specimens are preserved in 93% ethanol; wings and genitalia were mounted in Euparal®. Photographs were taken with a digital camera coupled to a Zeiss Axiocam ERc 5s stereo microscope and combined using Helicon Focus 6® software. Some of the photographs were used as templates for trace vector graphics in Adobe Illustrator CC® software to produce the illustrations. Gill formula (number of lamellae per gill: gills II, III, IV, V, VI) follows Molineri (2002). The holotype and some paratypes are deposited in the Coleção Zoológica Norte Capixaba,

Universidade Federal do Espírito Santo, São Mateus, Brazil (CZNC). The other paratypes are deposited in Instituto de Biodiversidad Neotropical-CONICET, Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán, Tucumán, Argentina (IBN). The distribution map was made using the program QGIS 2.14.11 (QGIS 2016).

Results

Tricorythodes tragoedia sp. nov.

(Figures 1–18)

Diagnosis. *Tricorythodes tragoedia* sp. nov. can be distinguished from other species of the genus by the following combination of characters. In the male imago: 1) tibiae with a large blackish mark on the subapical region; 2) abdomen shaded almost completely with black, but with small unpigmented dots (Fig. 1); 3) penes broad and flattened (Fig. 16); 4) forceps segment I subequal in length to segment II (Fig. 16); 5) vein CuP present (Fig. 15). In the nymph: 1) maxillary palp three-segmented with apical seta (Fig. 8); 2) pronotum with pointed anterolateral projection (Fig. 2); 3) legs with subapical blackish marks on femur and tibia, tarsi without marks (Figs. 12–14); 4) dorsum of fore femur with a transversal row of setae on the submedian region (Fig. 12); 5) middle femur with a transverse row of setae; 6) fore claw with 9–12 marginal denticles and one pair of submarginal denticles (Fig. 9); 7) operculate gill triangular, yellowish with blackish diffuse marks (Fig. 11); 8) lateral margins of abdominal segments III–VII expanded, segments VII–IX with posterolateral projections (Fig. 2).

Descriptions. Male imago (Figs 1, 15, 16). Body, 3.4–3.8 mm; forewing, 3.8–4.1 mm; cerci= 7.5–10 mm; median terminal filament= 12.5–14 mm (n=6). General coloration: thorax brown, legs and abdomen whitish with blackish and brownish marks.

Head (Fig. 1). Yellowish-brown, darker on hind margin and with Y-shaped black mark; black around bases of ocelli; antennae yellowish-white.

Thorax (Figs 1, 15). Pronotum yellowish-brown with blackish diffuse marks. Meso- and metanotum brown shaded with grey. Leg: coxa, trochanter and femur of foreleg dark brown; tibia with small subapical blackish mark; tarsus without marks; remaining segments of meso- and hind legs yellowish-white. Wing: membrane of forewing hyaline, longitudinal and cross veins whitish translucent, shaded with grey from costal margin to radial sector; vein CuP present.

Abdomen (Figs 1, 16). Terga yellowish-white, shaded with black. Sterna whitish shaded with black on lateral zones and near posterior margins. Genitalia: styliger plate yellowish-white, forceps white and penes yellowish-translucent; penes broad and flattened; forceps segment I subequal to length of segment II. Caudal filaments white.

Female imago. Body, 3.1–4.1 mm; forewings, 3.4–5.0 mm; cerci= 9 mm; median terminal filament= 12.5 mm (n=6). General coloration as in male. Eggs in abdomen yellowish. Abdominal shading lighter than male. Caudal filaments whitish-translucent, cerci shorter and thinner than median terminal filament.

Nymph (last instar) (Figs 2–11, 17). Body, 4.4–5.8 mm; mesonotum, 1.1–1.6 mm; caudal filaments, 3.8–6.3 mm (n=12).

Head (Figs 2–8, 10). Yellowish-white with blackish diffuse marks. Antennae yellowish-translucent. Mouthparts yellowish; maxillary palp three-segmented with apical seta.

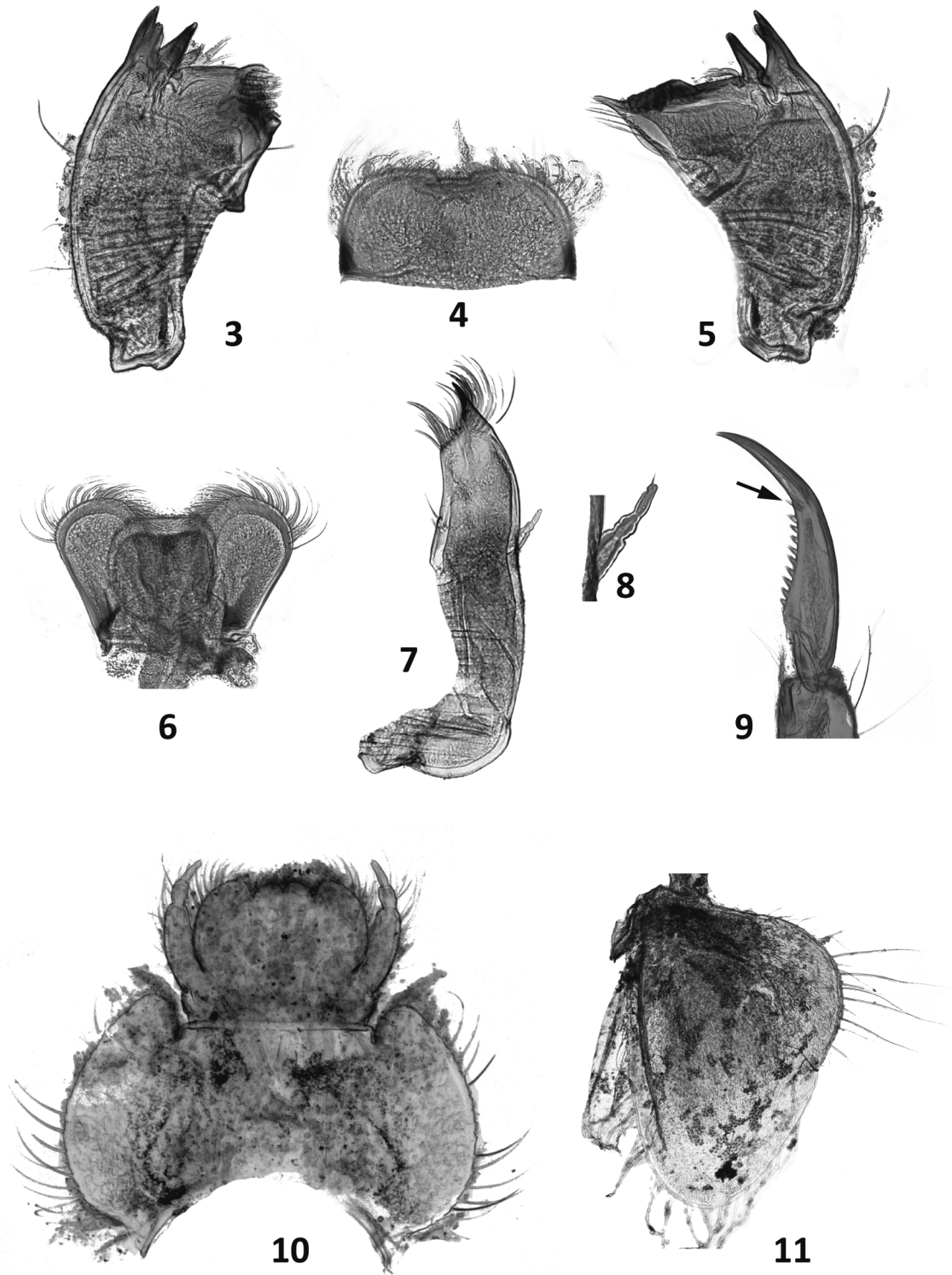
Thorax (Figs 2, 9, 12–14). General colouration yellowish or yellowish-white with blackish diffuse marks. Pronotum with short pointed anterolateral projection. Mesonotum yellowish with blackish diffuse marks, except in forewing pads, whitish or yellowish. Metanotum, pleurae and sterna yellowish with blackish marks. Legs narrow and long; colouration yellowish, dorsal regions of all femora with blackish marks; with large blackish mark on subapical region of each tibia; tarsus without marks; dorsum of fore femur and median femur with transverse row of setae on submedian region; femur subequal to tibia and 2× longer than tarsus and 3× longer than tarsal claw; width/length ratio of fore- and mid femur of 0.3; width/length ratio of hind femur of 0.25; fore claw with 9–12 marginal denticles and with pair of submarginal denticles; median and hind claws with 7–12 marginal denticles and with pair of submarginal denticles.

Abdomen (Figs 2, 11). General colouration yellowish with blackish diffuse marks. Sterna yellowish shaded with blackish marks, lateral regions of sterna III–VI shaded with grey. Lateral margins of abdominal segments



FIGURES 1–2. *Tricorythodes tragoedia* **sp. nov.**, habitus: (1) male imago, paratype, dorsal view; (2) mature nymph, paratype, dorsal view.

III–VII expanded; segments VII–IX with posterolateral projections; segments II–VII with a posteromedial tuft of setae; segments VIII and IX with row of setae on posterior margin. Operculate gill triangular, yellowish with blackish areas; remaining gills whitish-translucent shaded with grey; gill formula 3/3/3/3/2; dorsal lamellae of gills 3–5 without basal flap; ventral lamellae of gills 3–5 subtriangular without dorsal projection. Caudal filaments ranging from yellowish-white to light brown.



FIGURES 3–11. *Tricorythodes tragoedia* sp. nov., mature nymphs, paratypes: (3) left mandible, dorsal view; (4) labrum, dorsal view; (5) right mandible, dorsal view; (6) hypopharynx, ventral view; (7) right maxilla, dorsal view; (8) detail of maxillary palp; (9) tarsal claw of fore leg, dorsal view (arrow indicates the submarginal denticle); (10) labium, ventral view; (11) gill II, dorsal view.



FIGURES 12–14. *Tricorythodes tragoedia* sp. nov., mature nymph, paratype: (12) fore leg, dorsal view; (13) middle leg, dorsal view; (14) hind leg, dorsal view.

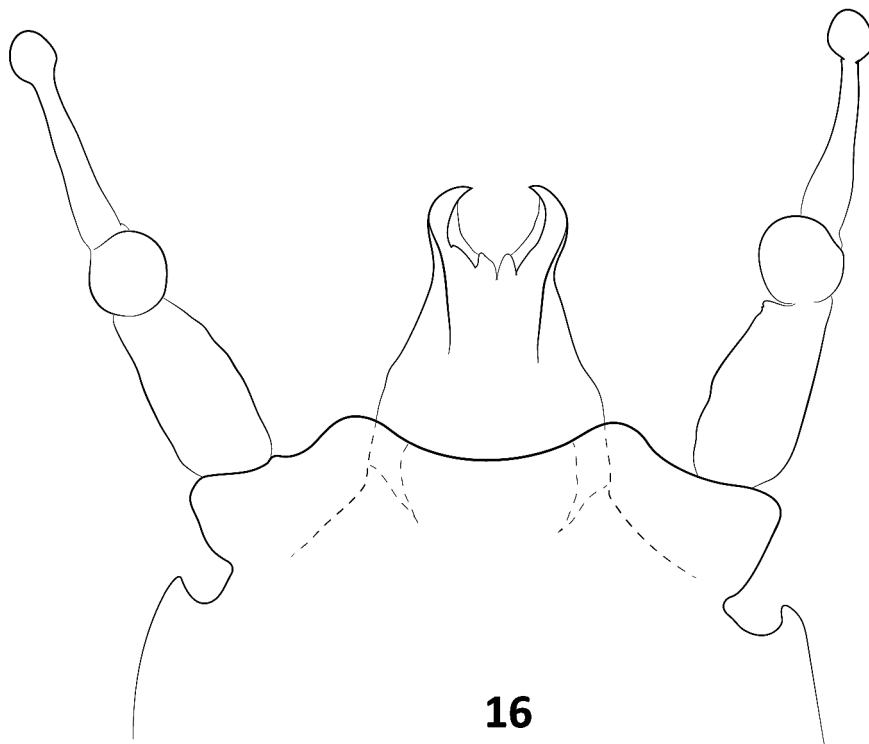
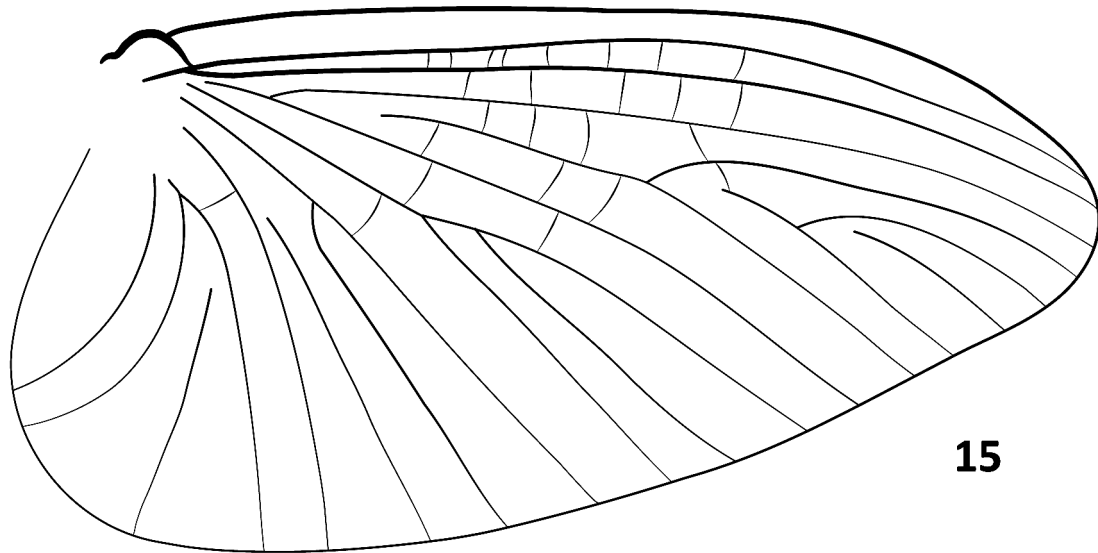
Variation. Some adult specimens from the same population showed darker coloration, with the abdomen almost completely shaded with black. Other specimens showed a black longitudinal dorsal stripe. Some other specimens showed tibiae with orange bands.

Two nymphs showed lighter coloration, with blackish markings forming a darker rounded spot in the middle of the operculate gill. Some specimens had orange diffuse marks on the operculate gills.

Life cycle association. Adults of both sexes and nymphs from close or the same localities were associated by shared color patterns. Two female adults were reared from nymphs to confirm these associations.

Type material. Brazil, Espírito Santo State: HOLOTYPE: São Mateus, Rio Preto, tributary of Rio São Mateus, S18°44'8", W39°47'47", 12.xii.2008, Massariol, F.C. & Angeli, K.B. leg., male nymph (CZNC Ep-7022). **PARATYPES:** Same data as holotype, 3 nymphs (CZNC Ep-669, legs, operculate gill and mouthparts of one nymph on slide); same data as holotype, 2 nymphs (IBN); São Mateus, Fazenda Liberdade, Rio Cricaré, tributary of Rio São Mateus, S18°39'2", W40°7'23", 22-23.v.2012, Salles, F.F. leg., 1 male subimago (CZNC Ep-6163, genitalia and wing on slide); São Mateus, Rio Cotaxé, tributary of Rio São Mateus, S18°37'41", W40°6'41.6", 20-21.xi.2012, Salles, F.F. leg., 3 male imago (CZNC Ep-6200, genitalia and wing of one imago on slide); same locality, 5-6.ii.2013, Salles, F.F. leg., 2 male subimagos (CZNC Ep-6180); Sooretama, Rio São José, tributary of Rio São Mateus, S19°7'33", W40°14'26", 21.ii.2013, Salles, F.F. leg., 1 nymph (CZNC Ep-5387, legs, operculate gill and mouthparts on slide); same locality, 09.ix.2010, Salles, F.F. leg., 2 nymphs (CZNC Ep-1392); same locality, 04.xi.2013, Salles, F.F. leg., 1 nymph (CZNC Ep-5533); same data, 1 nymph (IBN); Sooretama, Reserva Biológica de Sooretama, Cachoeira Bonjardim, S18°59'56", W40°14'01", 29.iv.2009, Salles, F.F. leg., 1 nymph (CZNC Ep-1139); Sooretama, Reserva Biológica de Sooretama, Córrego Rodrigues, S19°01'36", W40°13'39", 30.iv.2009, Salles, F.F. leg., 1 nymph (CZNC Ep-1173); Nova Venécia, Santa Rita do Pip-Nuk, Rio Cricaré, tributary of Rio São Mateus, S18°39'51", W40°30'45", 22.x.2012, Salles, F.F. leg., 2 nymphs (CZNC Ep-5473, legs, operculate gill and mouthparts of one nymph on slide); same locality, 25-26.vii.2012, Salles, F.F. leg., 1 female imago (CZNC Ep-4543); same locality, 22.x.2011, Salles, F.F. leg., 2 female imagos reared from nymph (CZNC Ep-7004); Nova Venécia, Rio Cricaré, tributary of Rio São Mateus, S18°42'55", W40°22'33", 21-22.xi.2012, Salles, F.F. leg., 1 male imago (CZNC Ep-6195, genitalia and wing on slide); same locality, 18-19.ii.2013, Salles, F.F. leg., 4 male imagos (CZNC Ep-6170); Nova Venécia, Patrimônio do Bis, Rio Cotaxé, tributary of Rio São Mateus, S18°33'27", W40°20'6", 20-21.xi.2012, Salles, F.F. leg., 2 female imagos and 2 male imagos (CZNC Ep-6160); same locality, 16-17.iv.2012, Salles, F.F. leg., 2 male subimagos and 1 female subimago (IBN); Pinheiros, Reserva Biológica do Córrego do Veado, Córrego São Roque, S18°19'26", W40°07'34", 25.ii.2010, Salles, F.F. leg., 1 nymph (CZNC Ep-1721); Colatina, Itapina, Rio Doce, S19°31'18", W 40°50'11", 11.ix.2014, Salles, F.F. leg., 1 nymph (CZNC Ep-7010) Same data, 2 nymphs (CZNC Ep-6999, legs, operculate gill and mouthparts of one nymph on slide); same data, 1 nymph (CZNC Ep-7000, legs, operculate gill and mouthparts on slide); same data, 2 nymphs (CZNC Ep-7001, legs, operculate gill and mouthparts on slide); same data, 1

nymph (CZNC Ep-7002, legs, operculate gill and mouthparts on slide); same data as holotype, 2 nymphs (CZNC Ep-7003); same data, 1 nymph (IBN); Colatina, Ipiranga, Rio Doce, S19°59'23", W40°16'41", 19-20.xi.2014, Salles, F.F. leg. (CZNC Ep-7012); Colatina, Baunilha, Rio Doce, S19°30'53", W40°30'59", 19-20.xi.2014, Salles, F.F. leg., 1 male imago (CZNC Ep-6459, genitalia and wing on slide); Linhares, Fazenda Rio Claro, Rio Doce, S19°32'18", W39°52'59", 24.ix.2014, Salles, F.F. leg., 1 male imago (CZNC Ep-7011, genitalia and wing on slide); same data, 1 male imago (IBN); Linhares, Instituto Federal do Espírito Santo, Rio Doce, S19°26'39", W39°57'00", 23-24.ix.2014, Salles, F.F. leg., 1 male subimago (CZNC Ep-6513); Linhares, Fazenda Amparo, Rio Doce, S19°26'51", W39°56'29", 23-24.ix.2014, Salles, F.F. leg., 1 male imago (CZNC Ep-6520); same data, 1 male imago (IBN).



FIGURES 15–16. *Tricorythodes tragoedia* sp. nov., male imago, paratype: (15) fore wing, dorsal view; (16) genitalia, ventral view.



FIGURE 17. *Tricorythodes tragoedia* sp. nov., mature nymph. Photo by Frederico F. Salles.

Biology. *Tricorythodes tragoedia* sp. nov. was found in a variety of habitats in the northern portion of the State of Espírito Santo. This area is completely within the Atlantic Forest biome, but only a few, and usually small, remnants of the original vegetation can be found. Our specimens were collected from a range of small streams to large rivers, but they always were collected at low altitudes of less than 100 meters (Fig. 18). Nymphs inhabit submersed substrates such as leaves, pool litter, macrophytes, marginal vegetation, roots, and algae in areas with slow current. Nymphs and adults were found throughout the year.

Most of the sampling sites are under the influence of agriculture and/or cattle, and do not present well-preserved riparian vegetation. On the other hand, a few of the collected areas are inside federal conservation units, such as Reserva Biológica de Sooretama and Reserva Biológica do Córrego do Veado, and their original riparian vegetation is well-preserved even though the headwaters of these streams are often outside the conservation units, where they are subjected to the same impacts from cattle and other agricultural practices. On top of that, the habitat alterations caused by the recent environmental tragedy of the dam break on the Doce River (Fernandes *et al.* 2016) is a possible threat to populations of this new species and its habitats.

Distribution (Fig 18). Southeastern Brazil, Espírito Santo State (Colatina, Ipiranga, Linhares, Nova Venécia, São Mateus, Sooretama, Pinheiros).

Etymology. The specific epithet, *tragoedia*, is from Latin and means "tragedy", in reference to the social and environmental disaster caused by the rupture in November 2015 of a mining dam controlled by Samarco Mineração S.A., a joint venture between mining BHP Billiton and Vale S.A. in the Doce River, where some of the paratypes were collected.

Discussion

The new species seems to be closely related to *T. arequita* Traver, 1959, *T. mirca* Molineri, 2002 and *T. sallesi* Dias,

Cabette & De Sousa, 2009. All four species possess abdominal color patterns formed by irregularly distributed pigments and legs with subapical blackish marks on tibiae. In both nymph and adult stages of *T. arequita*, the subapical blackish marks are also present. The adults of *T. sallesi* are still unknown, but in the others, the penes are very similar, broad and flattened. The species from this group are usually described based on nymphs because the morphology of the adults is highly similar. However, the new species described here can be separated easily from these other species by the morphology of the maxillary palp, which is 3-segmented with an apical seta in the new species, 3-segmented but without apical seta in *T. arequita* and *T. sallesi*, and 2-segmented with an apical seta in *T. mirca*. In addition, the tarsal claw can be used as a way of differentiating the new species from *T. mirca* and *T. sallesi*, as *T. tragoedia* **sp. nov.** has a fore claw with 9–12 marginal denticles with a pair of submarginal denticles, while *T. sallesi* has 10–12 marginal denticles without submarginal denticles, and *T. mirca* has 8–9 marginal denticles with 2 or 3 submarginal denticles. Besides that, the anterolateral projection in the pronotum of the nymph of *T. sallesi* is larger than in our new species.

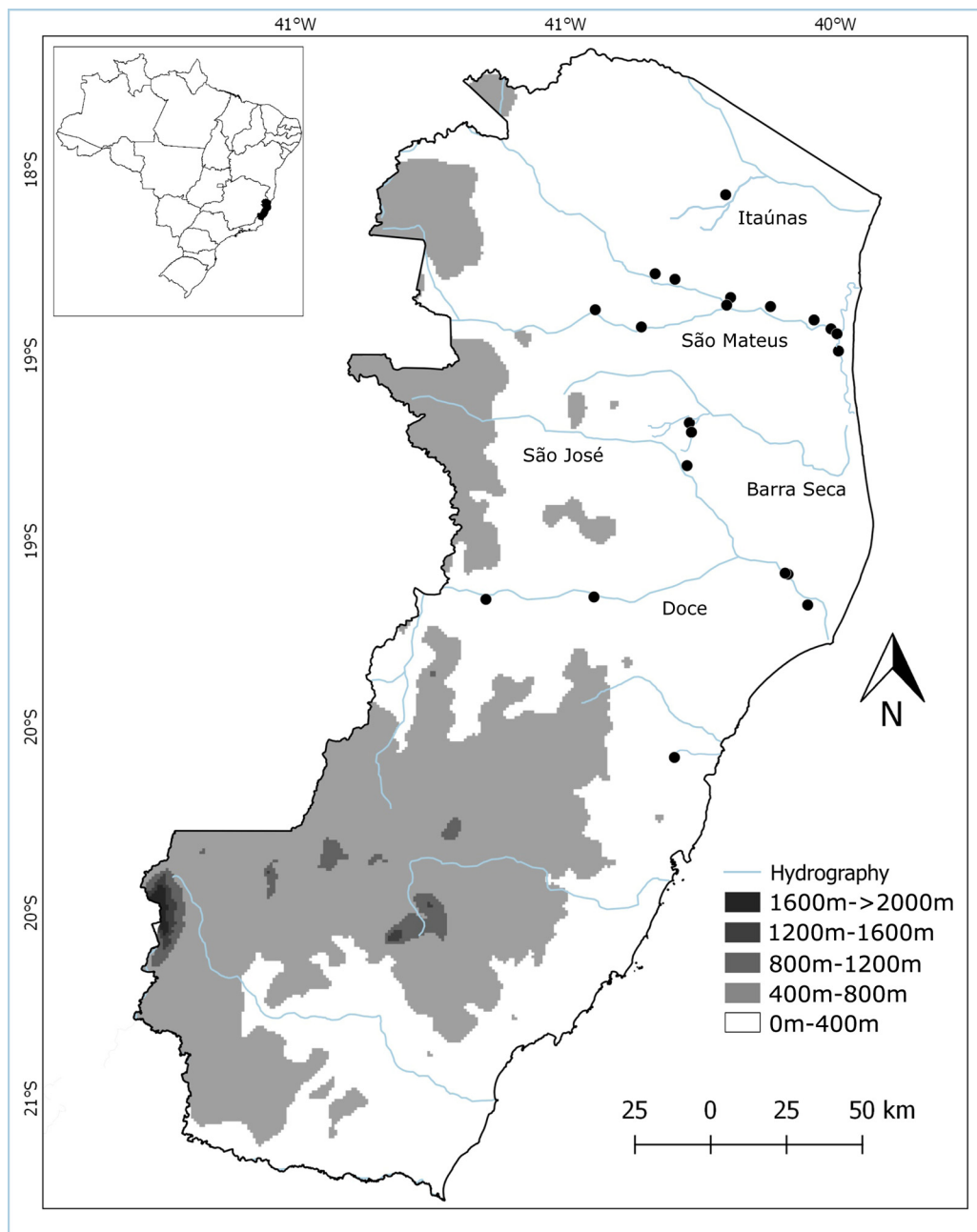


FIGURE 18. Physiographic map of Espírito Santo State showing the main rivers with details of the distribution of the new species according to the altitude, showing that the new species is restricted to low altitudes (less than 100 m).

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