UNIVERSIDADE DE SÃO PAULO

FACULDADE DE FILOSOFIA, CIÊNCIAS E LETRAS DE RIBEIRÃO PRETO

PROGRAMA DE PÓS-GRADUAÇÃO EM ENTOMOLOGIA

Systematic and biogeography of Leptoceridae (Trichoptera) with review of *Achoropsyche* Holzenthal, 1984

Sistemática e Biogeografia de Leptoceridae (Trichoptera) com revisão de *Achoropsyche* Holzenthal, 1984

Everton Santos Dias

Tese apresentada à Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da Universidade de São Paulo, como parte das exigências para obtenção do título de doutor em Ciências, obtido no Programa de Pós-Graduação em Entomologia.

Ribeirão Preto - SP

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Coorientador: Prof. Dr. Adolfo R. Calor (UFBA)

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FICHA CATALOGRÁFICA

Dias, Everton Santos

Systematic and biogeography of Leptoceridae (Trichoptera) with review of *Achoropsyche* Holzenthal, 1984, 1984. Ribeirão Preto, 2020.

vi+ 106 p.

Tese de Doutorado, apresentada junto ao Programa de Pós-Graduação em Entomologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo. Área de Concentração: Entomologia.

Orientador: Bispo, Pitágoras da Conceição.
Coorientador: Prof. Dr. Adolfo R. Calor (UFBA)

1. Inferência Bayesiana. 2.parcimônia. 3. tempo de divergência. 4. morfologia. 5. Bayarea. 6. Parcimônia. 7. Região Neotropical.

Dedico essa tese aos professores que tive ao longo
da minha vida, em especial aos meus orientadores,
aos familiares e amigos, em especial
ao meu irmão Junior Nascimento (1997-2016)
e aminha Avó Ivanilde Santos (1937-2020)

NOTA

Esta tese é parte dos requisitos para obtenção do título de Doutor no programa de Pós-Graduação em Ciências, área de concentração: Entomologia, da Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo. Desta forma, não é considerada uma publicação de acordo com os requisitos do Código Internacional de Nomenclatura Zoológica (ICNZ).

NOTE

This thesis is part of requirements for obtention of the Ph.D´s degree in the Programa de Pós-Graduação em Entomologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo. Therefore, this contribution is not considered a formal publication with the requirements fixed by the International Commission on Zoological Nomenclature (ICNZ)

AGRADECIMENTOS

Eu gostaria de agradecer ao Programa de Pós-Graduação em Entomologia, (Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo) pela oportunidade e por todo suporte ao longo desses 4 anos de doutoramento. Agradeço à Universidade de São Paulo, ao corpo docente e aos funcionários pelo suporte e pelas excelentes condições de trabalho. Um agradecimento especial para Renata Cavallari e Vera de Lucca pela paciência, por toda ajuda e compreensão.

Agradeço também ao departamento de Ciências Biológicas da Faculdade de Ciências e Letras da Universidade Estadual Paulista Júlio de Mesquita Filho, *campus* Assis (UNESP – ASSIS) pelo espaço, suporte e assistência ao longo do desenvolvimento da minha tese junto ao Laboratório de Biologia Aquática (LABIA)

A presente tese de doutorado recebeu o suporte financeiro do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, demanda social: processo 147895/2016-2), tanto através de bolsa de estudos quanto através de taxa de bancada. Eu agradeço imensamente também à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) pelo financiamento do meu estágio na Universidade de Minnesota (EUA) de outubro de 2018 a março de 2019 (CAPES, PDSE-Programa de Doutorado-Sanduíche no Exterior, processo 88881.188558/2018-01).

Agradeço profundamente ao meu orientador Prof. Dr. Pitágoras C. Bispo por me aceitar e ter aberto as portas do Laboratório de Biologia Aquática (LABIA) na UNESP, *campus* de Assis, o qual foi minha segunda casa ao longo desses 4 anos. Serei eternamente grato por todos os ensinamentos, por não medir esforços para nos ajudar, ser sempre muito solicito, pelos conselhos, pelos momentos de descontrações, pelas cobranças, pela liberdade, por ter fornecido toda a infraestrutura necessária e por ter contribuído muito para o desenvolvimento desse projeto.

Agradeço imensamente ao meu coorientador Prof. Dr. Adolfo R. Calor que desde a minha iniciação científica vem investindo energia na minha formação e trajetória acadêmica. Agradeço muito pelas orientações, por toda ajuda (dentro e fora do ambiente acadêmico) pelos ensinamentos, pelos puxões de orelhas, pela paciência e pela

amizade. Tenho certeza de que todo esse tempo de vivência no LEAq e pós UFBA vem agregando na minha formação acadêmica e pessoal. Serei eternamente grato por tudo.

Agradeço a University of Minnesota, *campus* Saint Paul, EUA e ao Prof. Dr. Ralph Wayne Holzenthal por ter aceitado no programa de doutorado sanduíche e por ter aceitado ser meu supervisor durante doutorado sanduíche. Agradeço imensamente ao Dr. Roger Blahnick, Msc. Ernest Razui, Felicia Christy e a curadora do Insect collection of University of Minnesota (EUA) Robin E. Thomson pela assistência, suporte, pelos *happy hours* e empréstimos de material biológico.

Ao Prof. Dr. David Andow (Departamento de Entomologia da University of Minnesota (EUA) e Dra. Débora Pires (EMBRAPA) pelo carinho, afeto, suporte e treinamento de biologia molecular durante meu estágio no exterior.

Agradeço ao Prof. Dr. Dalton S. Amorim (USP), Prof. Dr. Allan P. Santos (UniRio) e ao Prof. Dr. Fábio B. Quinteiro (UFPA) pelas sugestões em diferentes etapas do presente projeto.

Agradeço ao Museu de Zoologia da Bahia (UFBA), ao Laboratório de Entomologia Aquática Professor Claudio Froehlich (LEAq - UFBA), ao Prof. Dr. Frederico Salles (Universidade Federal de Viçosa), ao Instituto de pesquisa de Amazônia (INPA) e ao Msc. Gleison Desidério pela parceria e empréstimo de material biológico.

A todos os integrantes do Laboratório de Biologia Aquática, em especial ao Prof. Dr. Ciro Branco, Msc. Lucas Kortz, Msc. Lucas Almeida (Prego), ao Dr. Jorge Portinho e a Dr. Juliana Pomari pelo compartilhamento dos espações e equipamentos, pelo companheirismo e boas resenhas regadas a café.

Agradeço a todos os familiares, em especial aos meus pilares Jaci Santos (mãe) e Ivanilde Santos (1937-2020), a meus irmãos Crystiane Nascimento e ao Junior Nascimentos (1997 – 2016). Certamente sem a ajuda, o amor, apoio, o sacrifício e carinho de vocês nada disso seria possível. Mandar também um abraço especial para meu primo irmão Ivan Barreto (Bam) e aos meus tios-pai Jair Santos e Jailson Santos. Um agradecimento especial para meus padrinhos Washington Paganelli e Rosa Lordelo pelo amor, pelas palavras de incentivo e pelos carnavais maravilhosos que vem me proporcionando.

Aos irmãos que a vida me proporcionou Prof. Dr. Tácio Duarte e Prof. Msc. Rogério Fields pela irmandade, por segurar os perrengues juntos e não deixar a peteca cair, por ter divido nosso Lar Doce Lar (Bemba House). Sem dúvida vivemos momentos inesquecíveis que geraram crescimentos incalculáveis ao longo de nossa carreira e da vida de todos nós. Ninguém solta a mão de ninguém!

Agradeço ao grande amigo e parceiro Dr. Albane Vilarino pelas colaboraçãos, ensinamentos e companheirismo durante o mestrado em Salvador, doutorado em Assis e o período do doutorado sanduiche nos EUA.

Um agradecimento especial para minha amada e companheira Sarah Guandalini que vem me apoiando e motivando em todos os aspectos. Aos meus sogros Hilda e Marcos Guandalini e toda família, pelo amor, confiança, carinho, acolhimento e pelos excelentes momentos regados a boas cervejas e vinhos. Agradeço por todo apoio e incentivo.

Agradeço a todos os meus amigos, Dr. Victor Gomes, Dr. Fábio Silva, Msc. Larissa Queiroz, Washington Luís (Pop), Ivan Sena pela amizade, Eddy Clayton (Tito), Diana Oliveira pelo incentivo, irmandade, parceria, colaborações e pelos momentos maravilhosos que compartilhamos juntos.

Agradeço aos colegas de programa, em especial a Anne Costa, Felipe Freitas, Levi Barros, Fabiano Steffanelo e Daercio Lucena pela amizade, companheirismo, pelo suporte em dúvidas, pelas companhias nos almoções e jantas do RU, pelo abrigo, pelas conversas acadêmicas regadas a cervejas no Marcão.

Agradeço aos Prof. Dr. Eduardo Almeida e ao Prof. Dr. Fábio Quinteiro por me receber no Laboratório de Biologia Comparada e Abelhas e pelo suporte em algumas análises da tese.

Agradeço aos membros da Minnesota Graduate Club (MGC) em St. Paul, MN, EUA. Um abraço especial para Gabriel Macedo, Thiago Matheus, Sarad Sheresta, Nisha Rao e David Herrera pelo acolhimento, ajuda e companheirismo durante meu doutorado sanduiche nos EUA. Um agradecimento especial para o querido amigo Leo Paixão, conterrâneo que foi um verdadeiro irmão e me ajudou muito durante os meses que morei nos EUA e para todos os brasileiros que conheci lá.

Quando se nasce pobre, ser estudioso

é o maior ato de rebeldia contra o sistema

Peota Sérgio Vaz

Abstract

Our study is divided into two chapters. The first is a phylogenetic proposition based on molecular data for the Leptoceridae family estimating the divergence time between clades and proposing a hypothesis of historical biogeography for family. In order to achieve these objectives, we use the database of the first phylogenetic proposition of the family based on molecular data, which is available on GenBank. This referred proposition used 33 genera and did not estimate the time of divergence. In this context, we added to the matrix the data of five more genera (*Amazonatolica*, *Amphoropsyche*, *Leptecho*, *Neoathripsodes* and *Russobex*), using 38 of the 46 genera of Leptoceridae. The sequences were aligned using the MAFFT platform with the E-INS-i protocol. Models of nucleotide substitution were selected using the *Akaike Information Criterion* (AIC) in jModelTest 2 through the CIPRES platform. The Bayesian inference was implemented in MrBayes through the CIPRES platform. The diversification times was inferred using a relaxed clock through the BEAST 2 platform using fossils and data from the literature as calibration points. A biogeographic analysis was implemented on the R package BioGeoBears. Our results suggested the origin of Leptoceridae about 130 million years ago (MYA) in the supercontinent Gondwana with a first cladogenesis separating two big clades: (Leptorussinae + Triplectidinae), and (Grumichelinae + Leptocerinae). The divergence between these two big lineages is congruent with the division of Gondwana around 135 (MYA) into two blocks: Western Gondwana and Eastern Gondwana. The Leptorussinae + Triplectidinae clade has a possible ancestral range of distribution in Eastern Gondwana, while the Grumichellinae + Leptocerinae clade with a possible ancestral range distribution in Western Gondwana.

The second chapter consists of the reanalysis of specimens of the monotypic genus *Achoropsyche* deposited in some collections. This genus occurs in the Neotropical

region (from Venezuela to Argentina) and has an unusually widespread distribution for an only one species. With the reanalysis of these specimens, we are proposing five new species, four for Brazil and one for Ecuador. In addition, we propose a phylogeny based on morphological data to understand the evolution of the characters and the relationship between species. The data matrix was performed at Mesquite and the phylogenetic analyzes were performed using the Parsimony criterion implemented in the TNT software. Additionally, we perform a scanning electron microscopy (SEM) to analyze ultra-structures of the head, thorax and abdomen. The phylogenetic approach associated with the SEM photos has enabled us to redefine the diagnosis of the genus. In addition, we are proposing an identification key for males of the genus and a map of species distribution.

Key words: Bayesian inference, Divergence time, BioGeoBears, Divergence time, BayArea, Neotropical region, parsimony, Long-horned caddisflies. Scanning Electron Microscopy

Resumo

O nosso estudo está divido em dois capítulos. O primeiro deles consiste numa proposição filogenética baseado em dados moleculares para família Leptoceridae estimando o tempo de divergência entre os clados e propondo uma hipótese de biogeografia histórica para família. Para realização desses objetivos nos utilizamos a base de dados da primeira proposta filogenética da família baseado em dados moleculares disponível no GenBank. Como a referida proposta utilizou somente 33 gêneros e não estimou o tempo de divergência. Nesse contexto, adicionamos na matriz de dados mais cinco gêneros (*Amazonatolica*, *Amphoropsyche*, *Leptecho*, *Neoathripsodes* e *Russobex*), totalizando 38 dos 46 gêneros de Leptoceridae. As sequencias foram alinhadas através da plataforma MAFFT com o protocolo E-INS-i. O modelo de substituição de nucleotídeo para cada gene foi selecionado utilizando o *Akaike Information Criterion* (AIC) implementado no jModelTest 2 através da plataforma CIPRES. A inferência Bayesiana foi implementada no MrBayes através da plataforma CIPRES. Para inferir tempos de diversificação foram inferidos usando um relógio morfológico relaxado através dos plataforma BEAST 2 utilizando fósseis e dados da literatura como ponto de calibração. A análise biogeográfica foi implementada com a plataforma R BioGeoBears. Nossos resultados sugerem que a origem de Leptoceridae ocorreu cerca de 130 milhões de anos atrás (MYA) no supercontinente Gondwana com a primeira cladogênese resultando em dois clados: ((Leptorussinae + Triplectidinae) + (Grumichelinae + Leptocerinae)). A divergência entre essas duas grandes linhagens é congruente com a divisão de Gondwana em dois blocos em torno de 135 (MYA) em: West Gondwana e East Gondwana. O clado Leptorussinae + Triplectidinae tem o possível intervalo ancestral de distribuição no East Gondwana,

enquanto o clado Grumichellinae + Leptocerinae tem o possível intervalo ancestral de distribuição no West Gondwana.

O segundo capítulo consiste na reanalise dos espécimes do gênero monotípico *Achoropsyche* depositados em algumas coleções. Este gênero ocorre na região Neotropical (Venezuela à Argentina) e possuí uma incomum ampla distribuição para uma única espécie. Com a reanalise desses espécimes, estamos propondo cinco espécies novas, quatro para o Brasil e uma para o Equador. Além disso, propomos uma filogenia baseados em dados morfológicos para entender a evolução dos caracteres e a relação de parentesco entre as espécies. A matriz de dados foi feita no Mesquite e as análises filogenéticas foi realizada através do critério de Parcimônia implementada no software TNT. Além disso, realizamos uma microscopia eletrônica de varredura (MEV) para analisar ultra estruturas da cabeça, tórax e abdômen. Essa abordagem filogenética associado com as fotos do MEV nos proporcionou redefinir a diagnose do gênero. Além disso, estamos propondo uma chave de identificação para os machos do gênero e um mapa de distribuição das espécies.

Palavras chave: Inferência Bayesiana, tempo de divergência, BioGeoBears, BayArea, região Neotropical, parcimônia, leptocerídeos. Microscopia Eletrônica de Varredura

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Introduction

Trichoptera Kirby, 1813 comprises the seventh most speciose order of all insects (Adler & Foottit, 2017; Thomas *et al*., 2020) and the most diverse order of exclusively aquatic insects (Holzenthal *et al.*, 2007) with 16 267 extant species, classified in 60 families and 632 genera (Morse, 2020). Additionally, there are 521 fossil species in 133 genera, and 20 families (Morse, 2020). The order has a cosmopolitan distribution occurring in all zoogeographic regions, except polar zone. The region with the largest number of registered species is the Oriental (5 854 species) followed by the Neotropical region (3 309 species). The Afrotropical region has the smallest number of known species (1 251) (Morse *et al*., 2019). The Australian region has the highest genera endemism (73%), followed by Neotropical (69%) and Afrotropical (43%) regions with (de Moor & Ivanov, 2008; Holzenthal *et al.*, 2015).

Trichopterans are holometabolic insects with larvae and aquatic pupae, inhabiting both lentic and lotic environments, including temporary, and semi-aquatic environments (Muñoz-Quesada & Holzenthal, 1997). In Trichoptera, the adults are terrestrial, have wings with bristles that give the group its name (Latin: *trichos* = pelo and *ptera* = wings), a prominent antenna and, with some exceptions, have maxillary and labial palps (de Moor & Ivanov, 2008). They have a fused labium and hypopharynx differentiated in haustellum, used in the absorption of liquids (Morse, 2004; Holzenthal *et al*., 2007) (Fig. 1). The adults are primarily associated with riparian vegetation and are mostly crepuscular or nocturnal (Thomas *et al*., 2020).

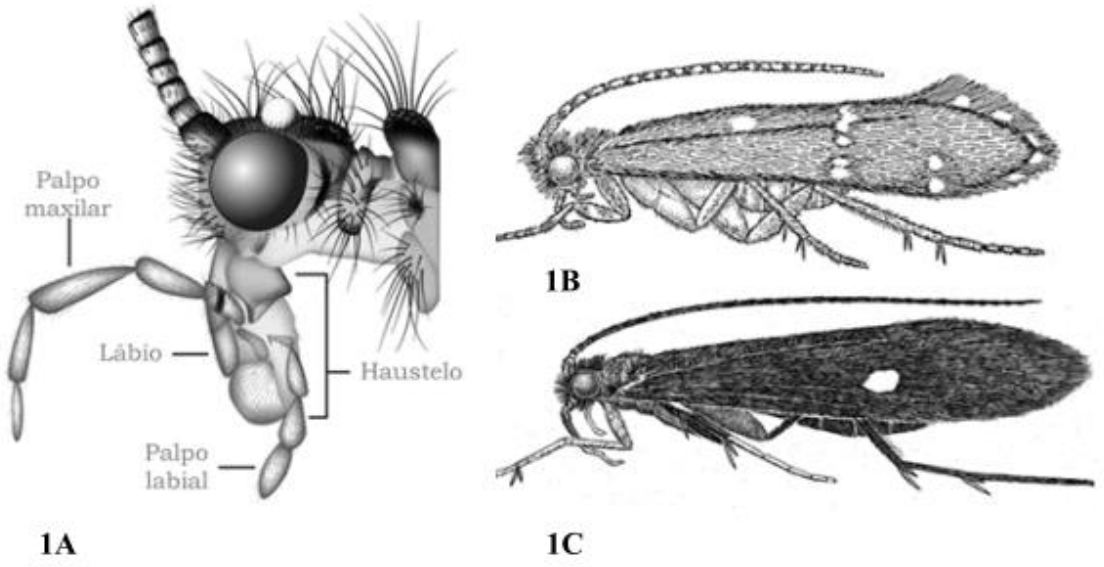

Figure 1: Adult Trichoptera, head and mouth parts. A – *Ptilostomis* sp. (Phryganeidae), B – *Protoptila* sp. (Glossosomatidae); C – *Xiphocentron* sp. (Xiphocentronidae) (modified from Holzenthal *et al*., 2007).

Trichoptera, emerged as sister group of Lepidoptera (butterflies and moths) around 290 million years ago, forming the super order Amphiesmenoptera ("Amphiesmeno" means dressed or covered) (Thomas *et al*., 2020). In the case of Trichoptera, the wings are mostly covered by bristles, while Lepidoptera, the wings are mostly covered by scales (Kristensen, 1984). The caddisflies larvae differ from those of the other orders by following combination of characters: 1) presence of segmented thoracic legs; 2) a pair of anal prolegs, each with a single curved terminal claw and very short, sometimes almost invisible; 3) antennae consisting of a single segment. (Thomas *et al*., 2020).

Initially, Trichoptera were divided into two suborders: Annulipalpia Martynov, 1924 ("retread-makers") and Integripalpia Martynov, 1924 ("case-makers"). Later, Ross (1967) added to this classification, characters of the mouthpieces, behavior of building shelters and preferential habitats of the immature. Annulipalpia (retreat-makers) construct fixe retreats attached to the substrate, while Integripalpia (*sensu* Ross) (case-makers),

construct portable retreat (usually tubular) with leaves, branches, fine sand sediment, small shells (Thomas *et al*., 2020). However, five families present immature that did not construct retreats ("free-living") (Rhyacophilidae, Hydrobiosidae, Glossosomatidae, Ptilocolepidae e Hydroptilidae) and Weaver (1984) proposed the "Spicipalpia" (closed-cocoon-making) for grouping these families. Later, "Spicipalpia" was considered a subfamily by Wiggins & Wichard (1989).

The "Spicipalpia", in turn has been recovered as a nonmonophyletic group (Malm & Johanson, 2013) and its relationship between the other two suborders has been controverse in the literature. Some proposals have positioned "Spicipalpia" as a sister group of the suborder Integripalpia (Ross, 1967; Kjer *et al*., 2002), others as internal group of Integripalpia (Holzenthal *et al*., 2011) or as a group diverged in the first cladogenesis of the order (Wiggins & Wichard, 1989; Malm *et al*., 2013).

The most recent phylogenetic proposition for the order has identified two major lineages in Trichoptera (Annulipalpia and Integripalpia), which emerge as independent clades at the transition between the Permian and Triassic, *ca.* 209 MYA (Thomas *et al.*, 2020). The "closed-cocoon-making" families ("Spicipalpia") appear as internal groups in the first cladogenesis of Integripalpia, except Rhyacophilidae which appears as sister group of Phyganidae (Thomas *et al*., 2020).

Among the Integripalpia, Phyganides (Brevitentoria + Planitentoria) probably diverged in the Jurassic forming a lineage of portable tube-case makers caddisflies in the Pangea (Thomas *et al.*, 2020). In Brevitentoria lineage, the long-horned caddisflies (Leptoceridae) diverged as independent lineage nested to Leptoceroidea clade probably between 127-130 MYA, during the Lower Cretaceous (Malm *et al.*, 2013; Thomas *et al.*, 2020). During this period, the Laurasia was separated from Gondwana; and Afrotopical region and Neotropical region were beginning the rift (Sanmartín & Ronquist, 2004)

Systematic and biogeography of Leptoceridae

The family Leptoceridae was established by Leach (1815) including several species described by Linnaeus in his classic study Systema Naturae, 10th edition. However, some species assigned to Leptoceridae by him were transferred to other families (Odontoceridae, Calamoceratidae, Molannidae and Beraeidae) (Holzenthal *et al*., 2007). Leptoceridae, long-horned caddisflies, are the second largest family of Trichoptera with 2 265 species, including 30 represented by fossils, which are classified in 58 genera, nine of them represented by fossils (Morse *et al*., 2019).

According to de Moor & Ivanov (2008), the leptocerids were found in the Late Jurassic in the extratropical, warm temperature latitude of Laurasia (England and Siberia) and dispersed from landmasses including Gondwana (Brazil) during the early Cretaceous. However, recent studies based on molecular data and divergence time showed the origin of Leptoceridae around 125–130 million years ago, during the Lower Cretaceous (Malm *et al*., 2013; Thomas *et al*., 2020). The family presents a cosmopolitan distribution with the Oriental region presenting the greatest number of species (987), followed by the Australian region (324 species). The Neotropical region presents 286 species of Leptoceridae, being the third region in richness of species in this family (Morse *et al*., 2019).

The leptocerids genera were historically classified, based on morphological data, in two subfamilies: Leptocerinae Leach, 1815 (eight tribes and two incertae sedis genera) and Triplectidinae Ulmer, 1906 (three tribes) (Morse, 1981) (Fig. 1A). In the Morse (1981) proposition, the genera were grouped according to an interpretation of the characters evolution, without a formal cladistic analysis *sensu stricto*. Leptocerinae has a cosmopolitan distribution and its monophyly is based in three characters: (i) loss of one branch of the median vein (fork III) in the hind wing; (ii) loss of the sectoral crossvein in

the hind wing; (iii) reduction of the adult tibial spur formula from 2, 4, 4 to 2, 2, 4 (Morse, 1981; Calor & Holzenthal, 2008; Malm & Johanson, 2011). Triplectidinae has the distribution primarily in the Australasian and Neotropical region. The monophyly is based in three characters: (i) absence of the primitive phallic parameres; (ii) reduction of the phallicata; (iii) presence of a large tooth on each pupal mandible (Morse, 1981; Morse & Holzenthal, 1987; Calor & Holzenthal, 2008; Malm & Johanson, 2011).

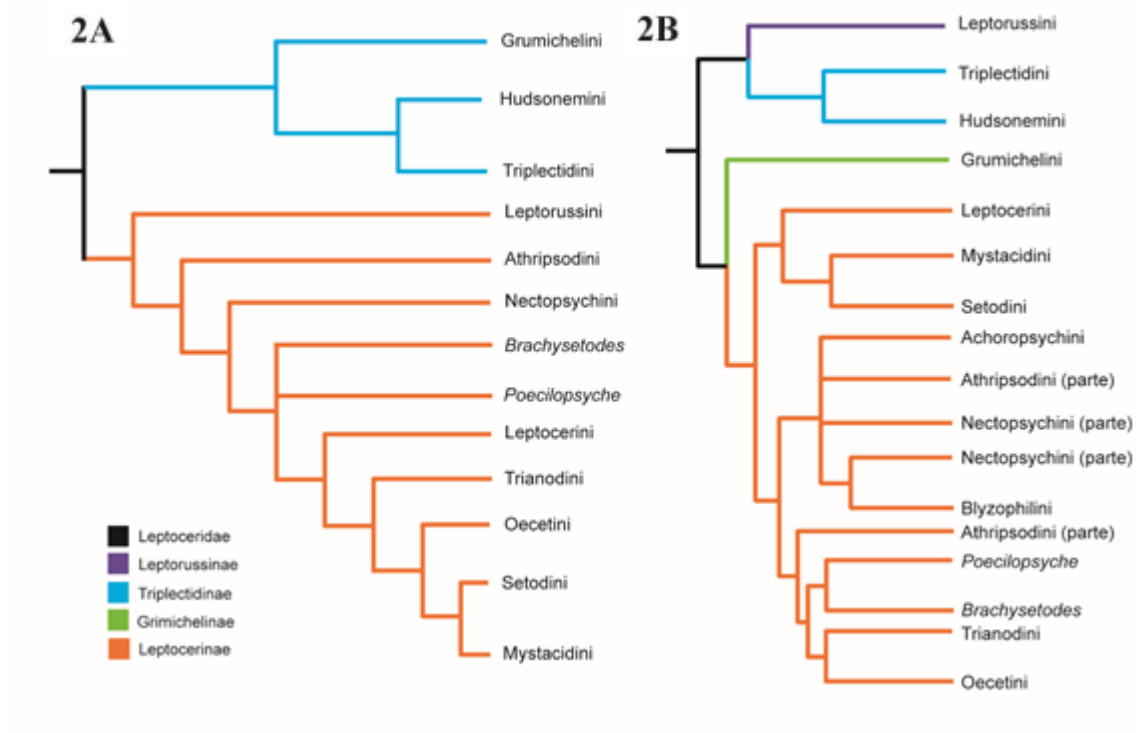

Figure 2. Phylogeny of Leptoceridae, **A.** Proposition of Morse, 1981 + Morse & Holzenthal, 1987. **B.** Proposition of Malm & Johanson, (2011).

After 30 years of Morse (1981) phylogenetic proposition, Malm & Johanson (2011) proposed the first molecular phylogenetic inference to Leptoceridae using five genes: one mitochondrial (COI) and four nuclear: (CAD, EF-1α, IDH, POL II) (Fig. 1b). Their phylogenetic result presented a substantial change in the family classification, increasing the number of subfamilies (Fig. 2B), named: Grumichelinae (6 genera), Leptocerinae (24

genera and nine tribes), Leptorussinae (one genus). and Triplectidinae (11 genera and two tribes) (Malm & Johanson, 2011). In addition, two genera were considered synonymy: (i) *Condocerus* was a new synonymy of *Hudsonema*; and (ii) *Ptochoecetis* was synonymy of *Oecetis*.

The phylogeny proposed by Malm & Johanson (2011) was an important contribution to the Leptoceridae systematic. Although, some genera were not included in their analysis and the phylogeny was not dated. The lack of phylogenetic dating of the makes it difficult to understand the family diversification process in view of the different biogeographic events. In this context, we proposed a phylogeny of the Leptoceridae including other genera to the Malm & Johanson (2011) original data sample and estimating the divergence time among the Leptoceridae clades. In addition, we estimated the ancestral range of distribution of the clades in order to understand the process of temporal and spatial diversification of Leptoceridae lineages.

Systematic of *Achoropsyche* Holzenthal, 1984

Among the Neotropical leptocerids, several genera need to be revisited, even the monotypic genera. Here, we studied the monotypic genus *Achoropsyche,* which has wide geographical distribution in Neotropical region. This genus was erected by Holzenthal (1984) to include one species previously classified as *Brachysetodes* Schmid, 1955, *B. duodecimpunctata* (Návas, 1916). The genus has been recorded in Argentina, Bolivia, Brazil, Colombia, Ecuador, Guyana, Paraguay, Peru, Suriname, Uruguay, Venezuela (Holzenthal, 1984; Holzenthal & Calor, 2017).

When the genus was described, a new tribe Achoropsychini was erected to include it (Holzenthal, 1984). In Malm and Johanson (2011), Achoropsychini appears in a polytomy with *Parasetodes* McLachlan, 1880 (Nectopsychini) and *Leptocerina* Mosely, 1932

(Athripsodini) as sister group of all Leptocerinae, except *Nectopsyche* Mueller, 1879. The interesting and unusual widespread distribution for a single caddisfly species as well as the remarkable morphological differences of some specimens drew our attention and solved to analysis the material deposited in some collections. Analysis of these specimens revealed five new species, which are described here. Additionally, we are proposing a morphological phylogeny for the genus.

Objectives:

The present study has the following objectives: to propose a dating phylogeny of Leptoceridae (Trichoptera) and assess the role of biogeographic events on family diversification; 2) to understand the diversity and to propose a morphological phylogeny of the genus *Achoropsyche* (Trichoptera: Leptoceridae:).

In order to achieve these objectives, the thesis is presented in two chapters:

Chapter 1: Diversification and historical biogeography of long-horned caddisflies (Trichoptera: Leptoceridae).

Chapter 2: Redefinition of *Achoropsyche* Holzenthal, 1984 (Trichoptera: Leptoceridae) under phylogenetic approach, with description of five species and identification key

CHAPTER 1

TITLE:

Diversification and historical biogeography of long-horned caddisflies (Trichoptera: Leptoceridae)

AUTHORS:

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Abstract

The first phylogeny proposed for the family from the 1980's and identified two lineages (subfamilies), Leptocerinae and Triplectidinae. 30 year after the first phylogenetic proposition based on molecular data was proposed and changed the paradigm of Leptoceridae systematics, both for innovative phylogenetic proposition, but also for the classification reflecting such relationships. Although, the taxon sampling did not include some genera, and the phylogeny was not dated. In this context, we proposed a dating phylogeny for Leptoceridae considering 38 of the 46 genera of the family, including five additional genera to Malm & Johanson (2011) original data sample (*Amazonatolica*, *Amphoropsyche*, *Leptecho*, *Neoathripsodes* and *Russobex*). Here, we estimated the divergence time between the Leptoceridae clades and its ancestral range of distribution to understand the role of biogeographical events in the family diversification process. Our

phylogenetic analysis revealed that Leptoceridae diverged as an independent lineage around 135–145 MYA (Lower Cretaceous) and has since gradually diversified. The first cladogenesis within the family occurred around 125–135 MYA and resulted in two clades, one formed by Leptossinae and Triplectidinae, and other formed by Leptocerinae and Grumichelinae, both with high branch support. Our results suggest the origin of Leptoceridae in the supercontinent Gondwana with the first cladogenesis of Leptoceridae resulted into two sister groups ((Leptorussinae + Triplectidinae) + (Grumichelinae + Leptocerinae)), which were congruent with the Gondwana break up in two blocks around 135 MYA: West Gondwana and East Gondwana. The clade Leptorussinae + Triplectidinae was estimated about 115 MYA (Lower Cretaceous) and has a possible ancestral range in East Gondwana, while the clade Grumichellinae + Leptocerinae was estimated in the Lower Cretaceous (*ca*. 120 MYA) from a possible ancestral with distribution in West Gondwana. This result is compatible with those found in biogeographic studies based on other organisms, which have shown that many cladogenesis observed are compatible with events of Cretaceous break up of Gondwana continent.

Key words: Systematic, Biogeography, Bayesian inference, BioGeoBears, BAYAREA,

Introduction

Currently, there has been a great effort to increase knowledge about phylogenetic relationships between organisms, including insects (e.g. Henning, 1981; Wheeler *et al.*, 2001, Kukalova-Peck, 1997; Trautwein *et al.*, 2012; Misof *et al.*, 2014). This effort has made it possible to understand the process of biological diversification, to reconstruct the traits evolution and to create a robust framework for comparative evolutionary biology (Peters *et al.*, 2014; Rainford *et al.*, 2016; Wipfler *et al.*, 2019). In addition, the methods and types of data used to estimate phylogenies have changed over time (Kjer *et al.*, 2016). In this scenario, the search to understand the phylogeny of Trichoptera (Insecta) has become a highly motivating topic for many researchers. In Trichoptera, morphological and molecular data, in isolated or integrated way, have been used to propose the phylogeny of the order (Ross, 1967; Weaver & Morse, 1986; Frania & Wiggins, 1997; Ivanov, 1997; Wiggins & Wichard, 1989; Kjer *et al.*, 2002; Malm *et al.*, 2013, Zhou *et al.*, 2016; Thomas *et al.*, 2020). The proposed phylogenies have allowed, among other things: the identification of the main lineages of Trichoptera and the understanding the evolution of the case building and feeding behaviors (Ross, 1967; Weaver & Morse, 1986; Frania & Wiggins, 1997; Ivanov, 1997; Wiggins & Wichard, 1989; Holzenthal *et al.*, 2007; Malm *et al.*, 2013); and understanding of the diversification process over time and space, through a phylogeographic and biogeographic approaches (Johanson, 2001; Wiggins, 2004; Strandberg & Johanson, 2011; Wahlberg & Johanson, 2014; Saito *et al.*, 2016; Thomas *et al.*, 2020).

The findings obtained so far have revealed that around in the Lower Permian (Thomas *et al.*, 2020), Trichoptera diverged from a common ancestral with Lepidoptera and conquered the aquatic habitats. In that period, land masses formed a single continent (Pangea). Since then, the Earth configuration has changed a lot and the fauna of Trichoptera

has diversified, occupied different aquatic habitats (mainly in freshwater ecosystems, both lentics and lotics), and became the order of primary aquatic insects with higher richness (16 267 species, Morse, 2020). The occupation of different aquatic niches by trichopterans has reflected a wide variation in body size and shape, feeding modes (e.g. shredder, collector, scraper, piercing, and predator) and use of habitats (e.g. different types of substrates, water velocity, etc.) (Wiggins, 2004).

In this diversification process, the production of silk and the construction of cases by the larvae may have played a fundamental role, since it allowed the expansion of feeding behaviors, defense strategies against predators (mechanical defense and camouflage), and increase of efficiency in gas exchange (Wiggins, 2004). This may have enabled these organisms to use different resources and occupy habitats with different conditions in aquatic environments.

Despite the intense discussion and slightly conflicting phylogenies (Ross, 1967; Weaver & Morse, 1986; Frania & Wiggins, 1997; Ivanov, 1997; Wiggins & Wichard ,1989; Kjer *et al.*, 2002; Holzenthal *et al.*, 2007; Malm *et al.*, 2013; Zhou *et al.*, 2016; Thomas *et al.*, 2020), it is now considered that Trichoptera evolved into two major lineages (Annulipalpia and Integripalpia), which emerge as an independent clades at the transition between the Permian and Triassic, *ca.* 209 MYA (Thomas *et al.*, 2020). Among the Integripalpia, Phyganides probably diverged in the Jurassic forming a lineage of portable tube-case makers caddisflies (Thomas *et al.*, 2020). This type of case, associated with undulating movements of the body, allowed the flow of water through the tube even in still waters, maintaining gas exchange at adequate levels (Wiggins, 2004). Therefore, Phyganides are able to occupy a wide variety of aquatic habitats, from very still waters (where they can control the flow of water inside the tube, maintaining adequate levels of oxygenation) to places with high water velocity (where they can anchor their tubular cases

on the substrate using silk) (Wiggins, 2004). Phyganides diverged in two lineages in the Pangea around 175 MYA: Plenitentoria (majority in northern hemisphere) and Brevitentoria (majority in northern hemisphere) (Thomas *et al.*, 2020).

In Brevitentoria lineage, the long-horned caddisflies (Leptoceridae) diverged as independent lineage nested to Leptoceroidea clade probably between 127-130 MYA, during the Lower Cretaceous (Malm *et al.*, 2013; Thomas *et al.*, 2020). During this period, the Laurasia was separated from Gondwana; Africa and South America were beginning the rift (Sanmartín & Roquist, 2004); the global temperature was relatively warm; the sea level was high; there was a great diversification of Angiosperms; and Antarctica, southern Australasia and southern South America were still connected and covered by a temperate rainforest biome (Dettmann, 1989; Crisp & Cook, 2013). Since the Lower Cretaceous, the long-horned caddisflies have diversified, becoming the richer family among the Brevitentoria and today it is the second most diverse family among the caddisflies. Currently, the family has about 2 235 species in 47 genera, two of them fossils, and has a cosmopolitan distribution, occurring in all continents, except Antarctica (Morse *et al.*, 2019)

The first phylogeny proposed for the family identified two lineages, which reflected in the proposition of two subfamilies, Leptocerinae Leach, 1815 (eight tribes and two *incertae sedis* genera) and Triplectidinae Ulmer, 1906 (three tribes) (Morse, 1981) (Fig. 1A; Tab 1A). After 30 years of Morse (1981) phylogenetic proposition, Malm & Johanson (2011) proposed the first molecular phylogenetic inference to Leptoceridae (Fig. 1B). In this phylogeny, systematic adjustments were necessary, and Grumichellini and Leptorussini tribes were elevated to subfamily status. In this context, the Leptoceridae genera are currently classified into four subfamilies: Grumichelinae (six genera), Leptocerinae (24

genera and nine tribes), Leptorussinae (one genus) and Triplectidinae (11 genera and two tribes) (Malm & Johanson, 2011) (Fig. 1B, Tab 1B).

Malm & Johanson (2011) changed the paradigm of Leptoceridae systematics, both for innovative phylogenetic proposition, but also for the classification reflecting such relationships. Although, the taxon sampling did not include some genera, and the phylogeny was not dated. The lack of dating phylogeny makes it difficult to understand the family diversification process. For example, a dating phylogeny would facilitate the understanding of the evolution of biological traits and allow the understanding of the role of biogeographical events in the diversification process of the family. In this context, we proposed a dating phylogeny for Leptoceridae considering 38 of the 46 genera of the family, including five additional genera to Malm & Johanson (2011) original data sample (*Amazonatolica*, *Amphoropsyche*, *Leptecho*, *Neoathripsodes* and *Russobex*). Here, we estimated the divergence time between the Leptoceridae clades and its ancestral range of distribution to understand the role of biogeographical events in the family diversification process. In addition, small systematic adjustments have been proposed.

Material and methods

Taxon sampling, DNA extraction and sequencing

The total dataset comprised 101 species: 1) an outgroup with 21 genera into 13 families; and 2) an ingroup with 77 species in 38 genera (S1). The Leptoceridae genera *Axiocerina*, *Leptoceriella*, *Fernandoschmidia*, *Hemileptocerus*, *Osflintia*, *Symphitoneurina*, *Westriplectes*, and *Nietnerella* were not included in our analysis since the specimens were not evaluable, and *Osflintia* was old to extract DNA. For phylogenetic analysis, five genes were considered, one mitochondrial: Cytochrome oxidase subunit I (COI); and four

nuclear: Cadherin-like gene (CAD), Elongation Factor -1〈 (EF-1〈), Isocitrate dehydrogenase (IDH), and RNA polymerase II (POL).

Most of the DNA sequences were those used by Malm & Johanson (2011), which were obtained from GenBank platform (https://www.ncbi.nlm.nih.gov/genbank/). We also used the COI sequences of *Amphoropsyche woodruffi* Flint & Sykora, 1993, *Leptecho* sp., and *Russobex corneatus* St. Clair, 1988 which was obtained from Bold System (www.boldsystems.org/). In addition, we extracted, amplified and sequenced three genes (COI, CAD and IDH) of *Neoathripsodes* sp. Holzenthal, 1989, and *Amazonatolica hamadae* Holzenthal and Pes 2004. In this case, the extractions were made using DNeasy Blood & Tissue kit (QIAGEN). For extraction, first, we removed the abdomen and put it in tubes (0.2 ml) without macerating with 180 µl of ATL buffer and 20 µl of K proteinase. After 24 hours in water 56° C, the samples were purified following the QIAGEN manufacturer's protocol. The quantification of DNA was made using Qubit® 3.0 Fluorometer using the manufacturer's protocol.

The amplification was made using GoTaq(R) Hot Start Green Master Mix follow the protocol: 12.5µl of TAq master mix, 8.5 µl of water, 2 µl of DNA, 1µl of primer. The primers used are listed in table 2. The best temperature of annealing for each gene was identify using a gradient analysis following: initial denaturation 94° C for two minutes; 35 cycles of denaturation 94° C for one minute; annealing 51°C to 61° C for one minute; final extension de 72°C for one minute; and one cycle of final extension of 72° C for 7 min. All genes worked in the temperature of 51°, 52° and 53° C, except EF1〈. The DNA was sequenced at the BMGC Sequencing and Analysis Facility, University of Minnesota, St. Paul, EUA and at the Centro de Recursos Biológicos e Biologia Genômica, FCAV-UNESP - Campus de Jaboticabal, São Paulo State, Brazil.

Phylogenetic analysis and divergence time estimation

All genes were aligned by using MAFFT 7.429, following the E-INS-i protocol on https://mafft.cbrc.jp/alignment/software/ (Katoh & Standley, 2013). The alignments were analyzed separately and subsequently concatenated. The genes were concatenated by Mesquite v.3.4 software (Maddison & Maddison, 2018). The matrix concatenated with the five genes included 4,135 bp: COI 658 bp, CAD 850 bp, EF1〈 1,099, IDH 720 bp and POLII 802 bp. Models of nucleotide substitution were selected using the *Akaike Information Criterion* (AIC) in jModelTest 2 (Darriba *et al.*, 2012) implemented on the CIPRES SCIENCE GATEWAY platform (Miller *et al.,* 2010). The DNA replacement model for all genes was GTR + I + G.

The Bayesian Inference (BI) was implemented using MrBayes 3.2.6 ((Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003; Ronquist *et al.,* 2012) on CIPRES in two runs with Markov chain Monte Carlo (MCMC), each chains with 100 million generation. For the nodes with posterior probabilities < 50% we used the option 'allcompat' in the command 'sumt', for the other parameters we used default ones. The convergence and stationary frequencies were examined on Tracer v.1.6 (Rambaut *et al.,* 2018) checking if the Effective Sample Sizes (ESS) were all > 200.

The divergence analyses were inferred in BEAST 2 (Bouckaert *et al.*, 2014) using the topology obtained from BI analysis with GTR+I+G models for each partition. Analyses were run under a relaxed molecular clock, with the *Yule* speciation process. Six fossil records taken from the Palaeobiology Database (accessed at www.fossilworks.org) were used as calibration points (Table 3). In addition, indirect data from previous studies (*e.g.*, Malm *et al.*, 2013 and Thomas *et al.*, 2020) were also used. BEAST 2 analysis was implemented on CIPRES. A maximum clade credibility tree was computed using TreeAnnotator v.2.4 (Drummond *et al.*, 2012). The tree topology was visualized in FigTree

v.1.4.3 (Rambaut, 2016), exported in SVG and the final edition was made in Adobe Illustrator CS6. Lineage diversification over time was evaluated graphically using the LTT (lineages through time) plot, which was obtained using the ltt.plot function of the R Package APE (Paradis & Schliep, 2019).

Biogeographic analysis

The ancestral range of distribution of Leptoceridae was reconstructed using seven areas: Afrotropical; Australasian; East of Laurasia; Nearctic; Neotropical; Oriental; and West Laurasia) proposed for Morse *et al*., 2019) based in the biogeographic Region by Wallace (1876), except the division of Laurasia in East and West.

The R package BioGeoBEARS (Matzke, 2013, 2014) was used to compare biogeographical models and estimate ancestral areas during the diversification of Leptoceridae. We used the Maximum Likelihood implementation of BioGeoBEARS package and AIC test values to compare the best fit to the our data: DEC (Dispersal – Extinction–Cladogênese, Ree & Smith, 2008), DIVA (Dispersal-Vicariance Analysis, Ronquist, 1997), and BayArea (Bayesian Inference of Historical Biogeography for Discrete Areas, Landis *et al.*, 2013). For the implementation of BioGeoBEARS analysis, we used the BEAST maximum clade credibility tree and an area matrix of presence/absence (Table S2).

Results

Phylogenetics and divergence time estimates

Our results corroborated the monophyly of the family and subfamilies with high support values, as well as most of the tribes and genera. Our phylogenetic analysis revealed that Leptoceridae diverged as an independent lineage around 135–145 MYA (Lower

Cretaceous) and has since gradually diversified. The first cladogenesis within the family occurred around 125–135 MYA and resulted in two clades, one formed by Leptossinae and Triplectidinae, and other formed by Leptocerinae and Grumichelinae, both with high branch support (Fig. 2).

Leptorussinae emerged as sister group of Triplectidinae approximately in 105–120 MYA (Lower Cretaceous) and is represented by a single genus, *Leptorussa*. Triplectidinae, in turn, presented a great diversification from 85–95 MYA (Upper Cretaceous) resulting in two clades (tribes) with a well supported value: Triplectidini and Hudsonemini. Triplectidini started its diversification around 65–75 MYA (Paleocene), resulting in the following internal relationships: *Notoperata* (*Triplectides* + (*Symphitoneura sabaensis* + *S. opposita*)) + (*Triplectidina moselyi* (*Lectrides* + (*Triplectidina nigricornis* + (*Symphitoneuria traingulata* + *S. clara*))))). On the other hand, Hudsonemini started its diversification around 50–60 MYA, in the transition Paleocene–Eocene, resulting in the following internal relationship: *Hudsonema* (*Notalina* + *Russobex*). It is important to note that inside Triplectidini, *Symphitoneura* and *Triplectidina* appeared as non-monophyletic genera (Fig. 2).

In the other clade, Grumichellinae emerged as a sister group to Leptocerinae around 115–125 MYA (Lower Cretaceous). Grumichellinae started its diversification around 70 MYA (end of the Upper Cretaceous), which resulted in a clade with a well supported value: *Grumichella* (*Atanatolica* (*Amazonatolica* (*Triplexa* + *Gracicilipsodes*))). Leptocerinae, in turn, started its diversification around 105–115 MYA, during the transition from Lower to Upper Cretaceous, becoming the most diverse subfamily in Leptoceridae.

The first cladogenesis within Leptocerinae (around 100 MYA) resulted in two well supported clades. In the first clade, Leptocerini emerged as sister group to Mystacidini + Setodini between 85–95 MYA (Upper Cretaceous). Later, around 55–65 MYA (Paleocene),

Mystacidini and Setodini diverged from the common ancestor. In Setodini, the genus *Setodes* was recovered as paralyphyletic with *Trichosetodes* nested inside. The second clade with remaining Leptocerinae was divided in two sister groups around 100–90 MYA: one formed by a well supported clade Oecetini + Trieanodini; and other formed by a weakly supported clade, which includes Athiripsodini (*Athripsodes*, *Ceraclea* and *Homilia*), Blyzophilini, Achoropsychini, Nectopsychini (*Nectopsyche*) and the *incertae sedis* genera (Fig. 2).

Among the lineages of Leptocerinae, the tribes Athiripsodini (75–85 MYA), Nectopsychini (63–77 MYA), Achoropsychini (55–65 MYA), and Blyzophilini (40–60 MYA) emerged as independent lineages from Upper Cretaceous to Eocene. Considering the *incertae sedis* genera, our results are: (i) a low supported clade, *Poecilopsyche* (*Neoathripsodes* + *Brachysetodes*), which diverged as an independent lineage between 77–92 MYA; (ii) Around 70–80 MYA, *Poecilopsyche* diverged from the well supported clade, *Neoathripsodes* + *Brachysetodes*; (iii) a medium supported clade, *Parasetodes* (*Amphoropsyche* + Blyzophilini), which emerged as an independent lineage around 65–75 MYA; and (iv) a clade with high support formed by *Leptocerina* + *Leptecho* dated from Eocene (Fig. 2).

Biogeography of Leptoceridae

The results of the tests AIC and likelihood-ratio obtained in *BioGeoBEARS* indicated BayAreas as a model that better fit our data. Our biogeographic analysis revealed the Leptoceridae diversification started during the Lower Cretaceous, with all four subfamilies already defined in this period. At this time, the Laurasia and Gondwana were already separated (Sanmartín & Ronquist, 2004; McIntyre *et al.*, 2017). Our results suggest the origin of Leptoceridae in the supercontinent Gondwana (Fig. 3).

The first cladogenesis of Leptoceridae resulted into two sister groups ((Leptorussinae + Triplectidinae) + (Grumichelinae + Leptocerinae)), which were congruent with the Gondwana break up in two blocks around 135 MYA: West Gondwana (Africa and South America) and East Gondwana (India, Madagascar, Australia, Antarctica and New Zealand) (Sanmartín & Ronquist, 2004; McIntyre *et al.*, 2017).

The clade Leptorussinae + Triplectidinae was estimated about 115 MYA (Lower Cretaceous) and has a possible ancestral range in East Gondwana. The subfamily Triplectidinae started its diversification from Upper Cretaceous, but it was intensified during the Paleogene. Throughout the diversification of the subfamily, the ancestors of all nodes seem to originate in East Gondwana, especially in the Australasian region. Currently, in addition to Australasian region, the genera *Hudsonema* and *Notalina* also occur in the Neotropical region, *Symphitoneuria* in the Oriental region, and *Triplectides* in several other regions (Oriental, Neotropical, East Paleartic, and Neartic).

The divergence between Grumichellinae + Leptocerinae was estimated in the Lower Cretaceous (*ca*. 120 MYA) from a possible ancestral with distribution in West Gondwana. This cladogenesis event is compatible with the break up in process between Africa and South America (Sanmartín & Ronquist, 2004; McIntyre *et al.*, 2017). Our results revealed that the ancestral distribution of Grumichellinae was probably in the Neotropical region, with subsequent expansion to the Australasian region (*Triplexa* + *Gracilopsodes*). The first cladogenesis of the subfamily Grumichelinae occurred in Upper Cretaceous, but the main process of diversification occurred between 30–65 MYA, during the Paleogene. Currently, this subfamily comprises four genera endemic of Neotropical region (*Amazonatolica*, *Atanatolica*, *Grumichella* and *Osflintia*, this latter not included in this analysis) and other two endemic to Australasian region (*Gracilipsodes* and *Triplexa*) (Fig. 3)

The ancestral distribution of Leptocerinae was probably the Afrotropical + Neotropical region. The first cladogenesis of the subfamily Leptocerinae occurred at the beginning of the Lower Cretaceous (*ca* 120 MYA), whereas the two landmassas were still connected. A high diversification process occurred from the end of Upper Cretaceous to Oligocene and several genera dispersed to other regions. The widespread distribution of some genera (e.g. "*Setodes"*, *Oecetis*, *Triaenodes*, *Athripsodes*, and *Ceraclea*) revealed some internal nodes with high ambiguity (Fig. 3). On the other hand, some genera presented the current distribution endemic to Afrotropical region (*Blyzophilus*, *Homilia*, *Magadacerina*, *Leptecho*, *Leptocerina*, and *Sericodes*), Neotropical region (*Achoropsyche*, *Amphoropsyche*, *Brachysetodes*, and *Neoathripsodes*) and Oriental region (*Poecilopsyche*).

Discussion

Phylogeny and systematic adjustments

In general, our phylogeny results implied only small changes in the classification of the family, when compared to that proposed by Malm & Johanson (2011). However, some adjustments at the tribe level were necessary (Table 4). For example, among the five genera added to phylogeny: *Russobex*, which used to be considered Leptocerinae, is actually placed in Triplectidinae. The genera *Neoathripsodes* and *Leptecho* which were classified into Athripsodini appears out of the tribe. In addition, the tribe Mystacidini and Setodini appear as monophyletic in the clade with high supported value. On the other hand, *Amazonatolica* was corroborated as a Grumichelinae and the *incertae sedis* genus *Amphoropsyche* was corroborated as Leptocerinae.

The great novelty in Triplectidinae was the placement of the genus *Russobex* into Hudsonemini as sister group of *Notalina* in a strongly supported clade (Fig. 2; Table 4). *Russobex* was originally classified as *incertae sedis* into Leptocerinae by the absence of

fork III in the posterior wing (St. Clair, 1988). Nevertheless, *Russobex* shares a diagnostic character (presence of the sectoral transversal vein in the posterior wing or closed discoidal cell) with all other members of Triplectidinae. Therefore, following our phylogeny, we are formally transferring of *Russobex* from Leptocerinae to Triplectidinae (Hudsonemini) (Table 4).

The tribe Athripsodini was erected by Morse & Wallace (1976) with the genus *Athripsodes* and *Ceraclea*. After, other six genera were classified as Athripsodini (Table 1). Athripsodini was recovered as a paraphyletic group, but only four genera (*Athripsodes*, *Ceraclea*, *Homilia* and *Leptocerina*) were analyzed by Malm & Johanson (2011). Here, we included three additional genera (*Leptocerina*, *Leptecho* and *Neoathripsodes*), and the tribe members were also recovered as paraphyletic group. In our results, *Athripsodes*, *Ceraclea* and *Homilia* formed a strong support clade (Fig. 2). Therefore, here, we are proposing a new circumscription to the tribe Athripsodini with only these three genera. All others (*Leptocerina*, *Leptecho* and *Neoathripsodes*) are consequently *incertae sedis* now. The clade *Leptocerina* and *Leptecho* emerged as sister group of Nectopsychini + Achoropsychini, but weakly supported. The genus *Neoathripsodes* formed a clade well supported with the Neotropical *Brachysetodes*, an *incertae sedis* genus. Therefore, according to our phylogenetic results, we are removing the genera *Leptecho*, *Leptocerina* and *Neoathripsodes* from Athripsodini tribe (Table 3). A phylogenetic analysis including the genera *Axiocerina* and *Leptoceriella* (Athripsodini according to the classification) should be realized to corroborate this decision.

Among Leptocerinae, the tribe Mystacidini had its monophyly contested by Morse (1981), as well as the genera *Mystacides* and *Tagalopsyche* (primarily Mystacidini) which were transferred to Setodini by Malm & Johanson (2011). In contrast, our analysis showed

Mystacidini (*Mystacides* and *Tagalopsyche*) as a monophyletic group. Both, Mystacidini and Setodini form strongly supported clades.

Trichosetodes (only *T. sisyphos*) was recovered into *Setodes*, and consequently received a synonym proposal by Malm & Johanson (2011), as initially pointed out by Schmid (1987). In our analysis, we included two species of *Trichosetodes* (*T. sisyphos* and *T. japonicus*), and these species formed a well supported clade into *Setodes*. As we did not get the sequences of the type species of *Trichosetodes*, any taxonomic change was proposed.

Considering the Grumichellinae classification, our results showed three congruence with the topology based on adult characters proposed by Calor & Holzenthal (2008): (i) *Amazonatolica* as a Grumichelini member; (ii) *Grumichella* is in the first cladogenesis sister group of other Grumichellinae, and (iii) *Gracilipsodes* as sister group of *Triplexa*.

Amphoropsyche was erected by Holzenthal, 1985, but not classified in any tribe or analyzed in any phylogenetic study until now. Here, we are presenting the first phylogenetic study including *Amphoropsyche* and this genus emerged as a sister group of the clade (*Blyzophilus* + *Magadacerina*), but with weak support value. Blyzophilini, in turn, presented a strong support value. The inclusion of *Madagacerina* nested to *Blyzophilus* was proposed by Malm & Johanson (2011). On that occasion, *Magadacerina* was called “genus novum” and later, it was described by Malm & Johanson (2013). Here, we are corroborating the hypothesis of Malm & Johanson (2011) (Table 4).

Our results reinforce the four subfamilies of Leptoceridae and reveal that, except for *Russobex*, all five Leptoceridae genera included for the first time in a molecular phylogeny were correctly allocated to the expected subfamilies. In relation to the tribes of Leptocerinae, we adopt a more conservative view, so instead of proposing several tribes, we kept several genera as *incertae sedis*, since most of them belong to clades with low

support or genera which we used only one gene sequence (COI for *Leptecho* and *Amphoropsyche*). In this case, the inclusion of the other eight genera that are still missing, and the expansion of source of data (e.g. other genes, and morphological characters) may help to build a more complete phylogeny and more refined classification for Leptocerinae.

Diversification and historical biogeography of Leptoceridae

Leptoceridae is the most successful diversification example among the case-tube makers caddisflies. They occupy different niches (e.g. different habitats, different feeding behaviors, etc) in all biogeographic regions. Understanding the diversification of the family in time and space has been hindered until now by the absence of a dated phylogeny. On the other hand, although the previous studies did not include the time of divergence, they were essential to define the monophyly of the family, establish the main clades, understand the phylogenetic relationships between the genera, and propose a more natural classification scheme.

Although Morse (1981) suggested the origin of the Leptoceridae at 65 MYA, our results revealed an origin twice as old. The origin of the family in Gondwana in the Lower Cretaceous (around 140–130 MYA) had already been estimated by wider phylogenetic studies (Malm *et al.*, 2013; Thomas *et al.*, 2020). However, despite our study suggesting a Gondwanic origin (as suggested by Weaver, 1984), Ivanov (2006) assumed as Leptoceridae a fossil specimen found in the Lower Siberian Cretaceous. That finding would indicate a Laurasic origin of the family, with rapid dispersion to Gondwana still in the Lower Cretaceous (e.g. Lower Cretaceous from Brazil, Martins-Neto, 2001). Our results refute this hypothesis of North-South dispersion, since most of the nodes indicate ancestral areas associated with Gondwana (Fig. 3). The genera that currently occur in regions associated with Laurasia belongs to clades with wide distribution, which have Gondwanic origin, suggesting a predominant dispersion in the South–North direction. Thus, even assuming the

origin of the family in the Late Jurassic (de Moor & Ivanov, 2008), a Laurasic origin would only be possible assuming: (i) dispersion from Laurasia to Gondwana; (ii) extinction of the initial lines in the region of origin; and (iii) diversification of the family from the southern lineages. This scenario can be understood as much less parsimonious than assuming Gondwanic origin. In this context, it is necessary to reassess the identity of the Laurasic older fossil, a putative member of Leptoceridae. This is crucial to understand the initial steps in the diversification of the family.

From the Lower Cretaceous, there was a great proliferation and diversification of case-tube makers caddisflies (Ivanov & Sukatsheva, 2002; Thomas *et al.*, 2020). This diversification was probably benefited by the evolution of angiosperms (Moor & Ivanov, 2008), and the consequent changes in the interactions between terrestrial and aquatic ecosystems. The greater entry of leaves and branches of angiosperms in aquatic environments probably: (i) increased the heterogeneity of habitats; (ii) changed the water characteristics (e.g. water oxygenation, pH, etc.); and (iii) increased the addition of allochthonous organic matter, increasing the importance of detritus food chain. In this scenario, Leptoceridae began its history of diversification.

In the end of Cretaceous (*ca*. 65 MYA), mass extinctions have been observed for several groups, including Trichoptera (de Moor & Ivanov, 2008; Wichard & Wang, 2016; Wichard *et al.*, 2018). The extinction of some groups of Trichoptera may have opened niches for the diversification of Leptoceridae. However, despite this, our results revealed that throughout the history of the family, there was no punctual acceleration in the diversification of the lineages. Instead, the diversification of Leptoceridae has been gradual over time.

Our results reveal that the first cladogenesis of the Leptoceridae family is probably related to the vicariance events resulting from the Gondwana break up. This result is

compatible with those found in biogeographic studies based on other organisms, which have shown that many cladogenesis observed are compatible with events of Cretaceous break up of Gondwana continent (Sanmartín & Ronquist, 2004). These studies include animal and plant organisms with different body sizes, life histories and dispersal capabilities, for example insects (Almeida *et al.*, 2012, 2018); fishes (Maisey, 2000); birds and mammals (Carcraft, 2001; Eiziriki *et al.*, 2001), and plants (McLoughlin, 2001), which reinforces the importance of vicariance in the diversification of Gondwana biota. Therefore, the history of fragmentation of the continents (Sanmartín & Ronquist, 2004; McIntyre *et al.*, 2017) is imprinted in the evolutionary history of several groups, including Trichoptera (Thomas *et al.*, 2020). In this case, the latest comprehensive study on the phylogeny of Trichoptera, although only focusing on understanding of the main clades, identified several signs of fragmentation of Gondwana in the order diversification (Thomas *et al.*, 2020).

Here it is clearly possible to trace the phylogenetic diversification of the Leptoceridae subfamilies in the face of the Gondwana fragmentation process (Fig. 3). In this context, the disjunction process between East and West Gondwana around 130 MYA (Sanmartín & Ronquist, 2004; McIntyre *et al.*, 2017) may be related to the first cladogenesis of Leptoceridae, which generated two major clades: the clade Leptorussinae + Triplectidinae with possible ancestor in East Gondwana (India, Madagascar, Australia, Antarctica and New Zealand); and the clade Grumichelinae + Leptocerinae with the possible ancestral in the West Gondwana (Africa and South America).

Leptorussinae and Triplectidinae diversified mainly from Australasian (part of East Gondwana), with later connection to South America. On the other hand, the most recent ancestor of Grumichelinae + Leptocerinae and the first cladogenesis of the Leptocerinae probably evolved from West Gondwana, approximately 120 MYA and 113 MYA, respectively. Moreover, the first cladogenesis within Grumichelinae occurred after the

fragmentation of West Gondwana, and the most recent ancestor of this subfamily evolved from Neotropical region (South America), with subsequent connection with the Australasian region. Therefore, our findings suggest that vicariance was an important process in the initial diversification of Leptoceridae.

Our results also reveal that in Leptoceridae, in addition to evidence of vicariance, there is also evidence of dispersion events between continents. For example, among the Triplectidinae, *Triplectides* diverged from an ancestor in Australasia as an independent lineage at 50 MYA, since then it has dispersed to the Neotropical, Nearctic, Oriental and East Palearctic regions. Except for *Triplectides*, all other genera with wide distribution, occurring in four or more biogeographic regions, belong to the Leptocerinae clade. In this case, several genera that likely originated in the Afrotropical region after its isolation, approximately 100 MYA, dispersed to other regions. As examples: 1) *Ceraclea* (80 MYA) dispersing to the Oriental, East Palearctic, West Palearctic and Neartic regions; 2) *Parasetodes* (70 MYA) dispersing to the Oriental, East Palearctic and West Palearctic regions; 3) *Mystacides* (45 MYA) dispersing to the Oriental, Western Palearctic and Nearctic regions; 4) *Leptocerus* (90 MYA) dispersing to the Australasian, Oriental and East Palearctic regions; and 6) the clade "*Athripsodes*" + *Homilia* (40 MYA) dispersing to the Oriental, East Palearctic and West Palearctic regions.

In addition to these, it was not possible to identify the ancestral areas of some genera/clades: 1) the clade "*Setodes*" + *Trichosetodes* (50 MYA); 2) *Oecetis* (85 MYA); 3) *Triaenodes* (65 MYA), which occur in all biogeographic regions; and 4) *Adicella* (70 MYA), which occurs in the Afrotropical, Oriental, East Palearctic and West Palearctic regions. Despite the great ambiguity in the ancestral nodes, preventing the definition of the ancestral areas, these lineages belong to large clades of Afrotropical origin. Therefore, our

data suggest that the Afrotropical region was a source of several lineages of Leptoceridae that dispersed to different biogeographic regions.

The history of the fragmentation of the large blocks of Gondwana can bring us some lessons about the importance of vicariance and dispersion in Leptoceridae. One of the most important events in the fragmentation of Gondwana was the separation between the East Gowndwana and West Gondwana (about 100 MYA) (McIntiry *et al.*, 2017). This separation was an important event of vicariance for many organisms (Sanmartím & Ronquist, 2004; Toussain *et al.*, 2017). In the case of Leptoceridae, at least the first vicariance coincides with the final phase of breaking these two continents. However, all other nodes correspond to the periods when the two continents were already separated, in these cases, dispersion events are the only possible explanation.

Another important event was the fragmentation of the trans-Antarctic corridor formed by the junction of Australia, Antarctica and South America, which was important mainly for Grumichelinae and Triplectidinae. The ancestral area of Grumichelinae is the Neotropical region, and around 50 MYA one of lineage with ancestral in Australasia originated the genera *Gracilipsodes* and *Triplexa*. In the case of Triplectidinae, *Hudsonema* (55 MYA) and *Notalina* (40 MYA) currently occur in Australia and South America. Our results revealed that most recent common ancestors of clades that currently inhabit the two continents are prior to rupture of Australia, Antarctica and South America around 40–30 MYA (Paleocene), the physical disruption and cooling of Antarctica isolated the biotas of Australia and South America (Sanmartím & Ronquist, 2004), including Leptoceridae.

Throughout the history of biogeography, there has been a dichotomy between the use of dispersion events or vicariance to explain the patterns of distribution of taxa. With the advent of dated phylogenies and the development of different approaches of analysis, it has become increasingly clear that these two types of events are not exclusive, and that in

addition to vicariance, dispersion events are also usually necessary to explain the current distribution of organisms. Obviously, we must not forget that extinctions and founder event speciation are also part of this process. The congruence between cladogenesis and continental breaks (e.g. divergency between subfamilies of Leptoceridae coincident with Gondwana breakdown) and the interchange fauna between Gondwana landmasses (e.g. Afrotropical, Neotropical and Australasian region), reinforce this view. Therefore, here, we recognize the importance of these two processes as drivers of the Leptoceridae biogeographic history.

Acknowledgements

We thank: 1) Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for fellowships and grants for ESD (number 147895/2016-2) and PCB (number 303260/2018-1); 2) Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for support and grant for ESD internship (CAPES-PSDE, number 88881.188558/2018-01) at the University of Minnesota, USA; and 3) Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the grant for PCB (number 12/21196-8). We also thank: 1) Chico Mendes Institute for Biodiversity Conservation (ICMBio) for issuing collecting permits; 2) Prof. Dr. Ralph Holzenthal, Dr. Roger Blahnick and the Insect collection of University of Minnesota (USA) for support, assistance and loan of biological material; 4) Prof. Dr. David Andow (Department of Entomology, University of Minnesota (USA)) and Dra. Débora Pires (EMBRAPA) for support in molecular biology teachings; 5) Prof. Dr. Frederico Salles of the Universidade Federal de Viçosa, Msc. Gleyson Desidério, and Instituto de Pesquisa de Amazônia (INPA) for the loan of specimens examined in this study. We highly appreciate the valuable manuscript

suggestions of the Prof. Dr. Dalton S. Amorin, Prof. Dr. Allan P. Santos, and Prof. Dr. Fábio B. Quinteiro.

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Legend of figures and tables

Figure 1. Phylogeny of Leptoceridae, **A.** Proposition of Morse, 1981 + Morse & Holzenthal, 1987. **A.** Proposition of Malm & Johanson, 2011.

Figure 2. Bayesian chronogram for Leptoceridae showing the relationship, divergence age and posterior probability. Q = Quaternary; Paleo. = Paleocene; Oligo. = Oligocene; Plio = Pliocene; Plei = Pleistocene. Tribes adjusted according with this new phylogeny.

Figure 3. Reconstructions of the biogeography history of Leptoceridae under BayAreas model as implemented in *BioGeoBAERS*. The pie diagrams at nodes show the relative probability of the possible areas or combinations of areas. The rectangles on the right show the occurrence of the genera. Q = Quaternary; Paleo. = Paleocene; Oligo. = Oligocene; Plio = Pliocene; Plei = Pleistocene.

Table 1. Classification of Leptoceridae with valid generic name. 1A Classification by (Ulmer 1907) + (Morse 1981; 2011). 1B classification by Malm & Johanson, (2011).

Table 2. List of primers used in this study.

Table 3. Calibrated points used for divergence time analysis of Leptoceridae.

Table 4. New classification of Leptoceridae. Genera with "*" were not included in this analysis

Figure 1

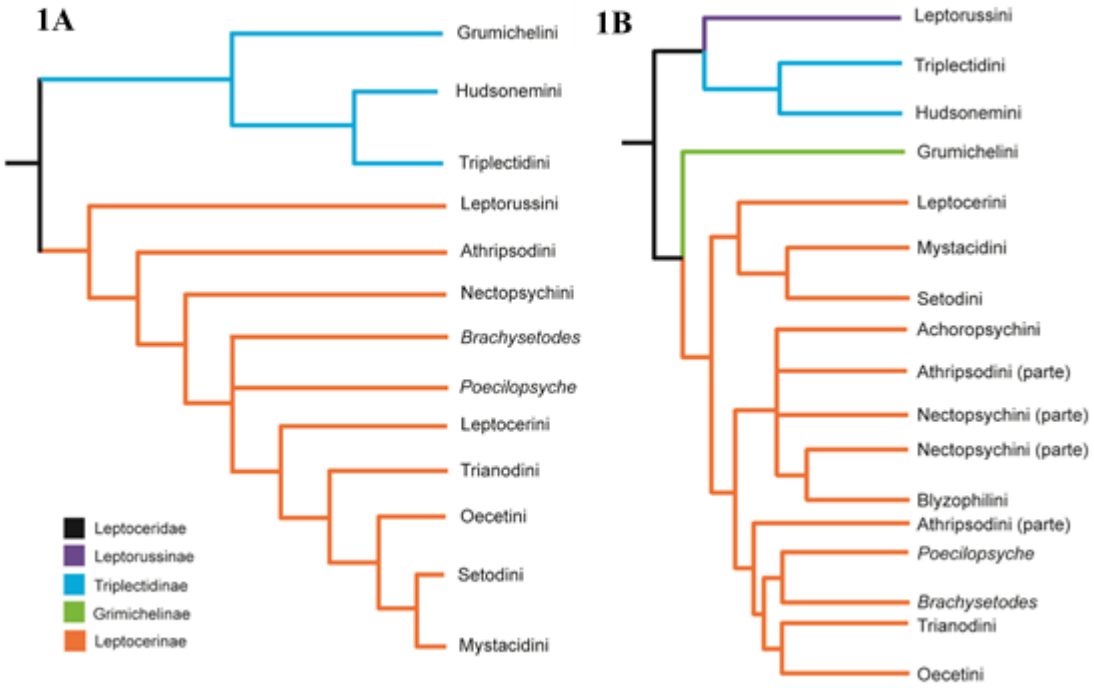
1A
Grumichelini
Hudsonemini
Triplectidini
Leptorussini
Athripsodini
Nectopsychini
Brachysetodes
Poecilopsyche
Leptocerini
Trianodini
Oecetini
Setodini
Mystacidini
Leptoceridae
Leptorussinae
Triplectidinae
Grimichelinae
Leptocerinae
1B
Leptorussini
Triplectidini
Hudsonemini
Grumichelini
Leptocerini
Mystacidini
Setodini
Achoropsychini
Athripsodini (parte)
Nectopsychini (parte)
Nectopsychini (parte)
Blyzophilini
Athripsodini (parte)
Poecilopsyche
Brachysetodes
Trianodini
Oecetini

Figure 2.

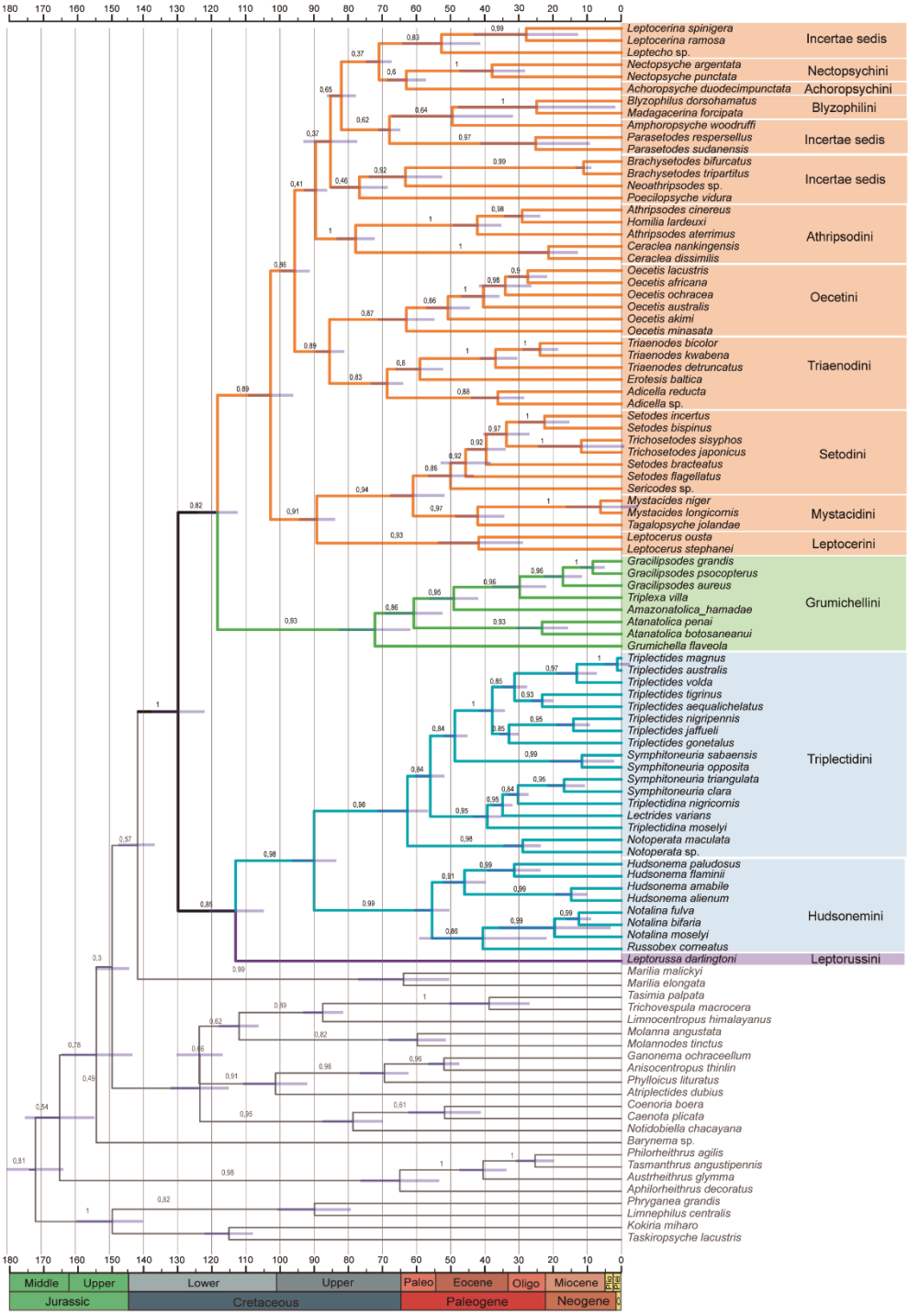
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
Leptocerina spinigera
Leptocerina ramosa
Leptecho sp.
Incertae sedis
Nectopsyche argentata
Nectopsyche punctata
Nectopsychini
Achoropsyche duodecimpunctata
Achoropsychini
Blyzophilus dorsohamatus
Madagacerina forcipata
Blyzophilini
Amphoropsyche woodruffi
Parasetodes respersellus
Parasetodes sudanensis
Incertae sedis
Brachysetodes bifurcatus
Brachysetodes tripartitus
Neoathripsodes sp.
Poecilopsyche vidura
Incertae sedis
Athripsodes cinereus
Homilia lardeuxi
Athripsodes aterrimus
Ceraclea nankingensis
Ceraclea dissimilis
Athripsodini
Oecetis lacustris
Oecetis africana
Oecetis ochracea
Oecetis australis
Oecetis akimi
Oecetis minasata
Oecetini
Triaenodes bicolor
Triaenodes kwabena
Triaenodes detruncatus
Erotesis baltica
Adicella reducta
Adicella sp.
Triaenodini
Setodes incertus
Setodes bispinus
Trichosetodes sisyphos
Trichosetodes japonicus
Setodes bracteatus
Setodes flagellatus
Sericodes sp.
Setodini
Mystacides niger
Mystacides longicornis
Tagalopsyche jolandae
Mystacidini
Leptocerus ousta
Leptocerus stephanei
Leptocerini
Gracilipsodes grandis
Gracilipsodes psocopterus
Gracilipsodes aureus
Triplexa villa
Amazonatolica_hamadae
Atanatolica penai
Atanatolica botosaneanui
Grumichella flaveola
Grumichellini
Triplectides magnus
Triplectides australis
Triplectides volda
Triplectides tigrinus
Triplectides aequalichelatus
Triplectides nigripennis
Triplectides jaffueli
Triplectides gonetalus
Symphitoneuria sabaensis
Symphitoneuria opposita
Symphitoneuria triangulata
Symphitoneuria clara
Triplectidina nigricornis
Lectrides varians
Triplectidina moselyi
Notoperata maculata
Notoperata sp.
Triplectidini
Hudsonema paludosus
Hudsonema flaminii
Hudsonema amabile
Hudsonema alienum
Notalina fulva
Notalina bifaria
Notalina moselyi
Russobex corneatus
Hudsonemini
Leptorussa darlingtoni
Leptorussini
Marilia malickyi
Marilia elongata
Tasimia palpata
Trichovespula macrocera
Limnocentropus himalayanus
Molanna angustata
Molannodes finctus
Ganonema ochracellum
Anisocentropus thinlin
Phylloicus lituratus
Atriplectides dubius
Coenoria boera
Caenota plicata
Notidobiella chacayana
Barynema sp.
Philorheithrus agilis
Tasmanthrus angustipennis
Austrheithrus glymma
Aphilorheithrus decoratus
Phryganea grandis
Limnephilus centralis
Kokiria miharo
Taskiropsyche lacustris
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
Middle
Upper
Lower
Upper
Paleo
Eocene
Oligo
Miocene
Jurassic
Cretaceous
Paleogene
Neogene

Figure 3.

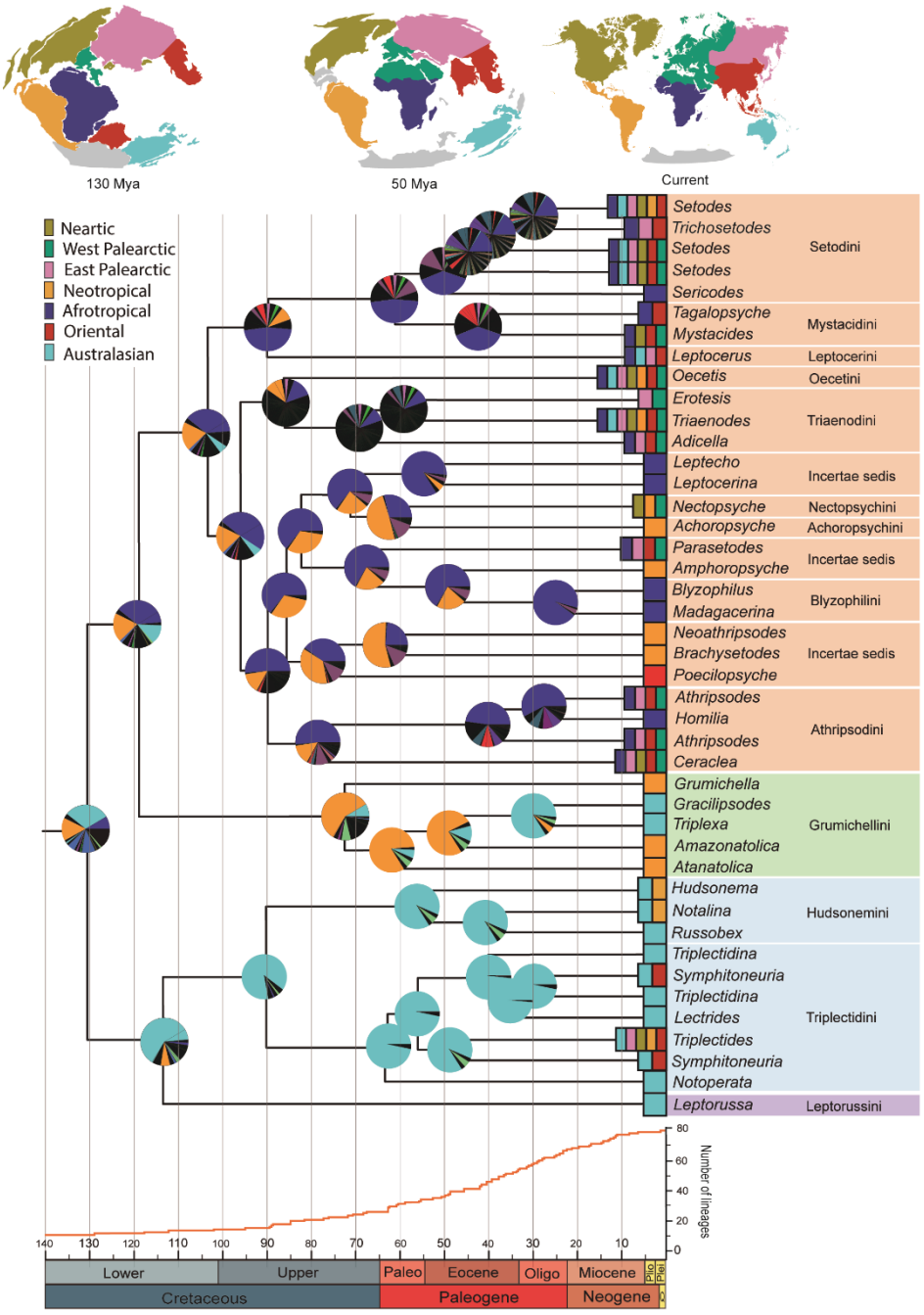
130 Mya
50 Mya
Current
Neartic
West Palearctic
East Palearctic
Neotropical
Afrotropical
Oriental
Australasian
Setodes
Trichosetodes
Setodes
Setodes
Sericodes
Tagalopsyche
Mystacides
Leptocerus
Oecetis
Erotesis
Triaenodes
Adicella
Leptecho
Leptocerina
Nectopsyche
Achoropsyche
Parasetodes
Amphoropsyche
Blyzophilus
Madagacerina
Neoathripsodes
Brachysetodes
Poecilopsyche
Athripsodes
Homilia
Athripsodes
Ceraclea
Grumichella
Gracilipsodes
Triplexa
Amazonatolica
Atanatolica
Hudsonema
Notalina
Russobex
Triplectidina
Symphitoneuria
Triplectidina
Lectrides
Triplectides
Symphitoneuria
Notoperata
Leptorussa
Setodini
Mystacidini
Leptocerini
Oecetini
Triaenodini
Incertae sedis
Nectopsychini
Achoropsychini
Incertae sedis
Blyzophilini
Incertae sedis
Athripsodini
Grumichellini
Hudsonemini
Triplectidini
Leptorussini
Number of lineages
0
20
40
60
80
140
130
120
110
100
90
80
70
60
50
40
30
20
10
Lower
Upper
Paleo
Eocene
Oligo
Miocene
Plio
Ple
Cretaceous
Paleogene
Neogene

Table 1.

A – Ulmer (1907) (Morse 1981; 2011)	**B** – Malm & Johanson (2011)
LEPTOCERINAE	**LEPTOCERINAE**
Achoropsychini	**Achoropsychini**
Achoropsyche Holzenthal, 1984	*Achoropsyche* Holzenthal, 1984
Athripsodini	**"Athripsodini"**
Athripsodes Billberg, 1820	*Athripsodes* Billberg, 1820
Axiocerina Ross, 1957	*Axiocerina* Ross, 1957*
Ceraclea Stephens, 1829	*Ceraclea* Stephens, 1829
Homilia McLachlan, 1877	*Homilia* McLachlan, 1877
Leptecho Barnard, 1934	*Leptecho* Barnard, 1934*
Leptoceriella Schmid, 1993	*Leptoceriella* Schmid, 1993*
Leptocerina Mosely, 1932	*Leptocerina* Mosely, 1932
Neoathripsodes Holzenthal, 1989	*Neoathripsodes* Holzenthal, 1989*
Blyzophilini	**Blyzophilini**
Blyzophilus Anderson & Kjaerandsen, 1999	*Blyzophilus* Anderson & Kjaerandsen, 1999
Leptocerini	**Leptocerini**
Leptocerus Leach, 1815	*Leptocerus* Leach, 1815
Leptorussini	**Mystacidini + Setodini**
Leptorussa Mosely, 1953	*Hemileptocerus* Ulmer, 1922*
Mystacidini	*Fernandoschmidia* Holzenthal & Andersen, 2007*
Mystacides Berthold, 1827	*Mystacides* Berthold, 1827
Fernandoschmidia Holzenthal & Andersen, 2007	*Sericodes* Schmid, 1987
Tagalopsyche Banks, 1913	*Setodes* Rambur, 1842
Nectopsychini	*Tagalopsyche* Banks, 1913
Nectopsyche Müller, 1879	*Trichosetodes* Ulmer, 1915
Oecetini	**"Nectopsychini"**
Oecetis McLachlan, 1877	*Nectopsyche* Müller, 1879
Ptochoecetis Ulmer, 1931	*Parasetodes* McLachlan, 1880
Setodini	**Oecetini**
Hemileptocerus Ulmer, 1922	*Oecetis*McLachlan, 1877
Sericodes Schmid, 1987	**Triaenodini**
Setodes Rambur, 1842	*Adicella* McLachlan, 1877
Trichosetodes Ulmer, 1915	*Erotesis* McLachlan, 1877
Triaenodini	*Triaenodes* McLachlan, 1865
Adicella McLachlan, 1877	***incertae sedis***
Erotesis McLachlan, 1877	*Amphoropsyche* Holzenthal, 1985
Triaenodes McLachlan, 1865	*Brachysetodes* Schmid, 1955
incertae sedis	*Poecilopsyche* Schmid, 1968
Amphoropsyche Holzenthal, 1985	*Russobex* StClair, 1988*
Brachysetodes Schmid, 1955	**GRUMICHELINAE**
Poecilopsyche Schmid, 1968	*Amazonatolica* Holzenthal & Pes, 2004*
Russobex St. Clair, 1988	*Atanatolica* Mosely, 1936
TRIPLECTIDINAE	*Gracilipsodes* Sykora, 1967
Grumichelini	*Grumichella* Mueller, 1879
Amazonatolica Holzenthal & Pes, 2004	*Osflintia* Calor & Holzenthal, 2008*
Atanatolica Mosely, 1936	*Triplexa* Mosely, 1953
Gracilipsodes Sykora, 1967	**LEPTORUSSINAE**
Grumichella Mueller, 1879	*Leptorussa* Mosely, 1953
Osflintia Calor & Holzenthal, 2008	**TRIPLECTIDINAE**
Triplexa Mosely, 1953	**Hudsonemini**
Hudsonemini	*Hudsonema* Mosely, 1936
Condocerus Neboiss, 1977	*Notalina* Mosely, 1936
Hudsonema Mosely, 1936	*Triplexa* Mosely, 1953
Notalina Mosely, 1936	**Triplectidini**
Triplexa Mosely, 1953	*Lectrides* Mosely, 1953
Triplectidini	*Notoperata* Neboiss, 1977
Lectrides Mosely, 1953	*Symphitoneuria* Ulmer, 1906
Notoperata Neboiss, 1977	*Symphitoneurina* Schmid, 1950*
Symphitoneuria Ulmer, 1906	*Triplectides* Kolenati, 1859
Symphitoneurina Schmid, 1950	*Triplectidina* Mosely, 1936
Triplectides Kolenati, 1859	*Westriplectes* Neboiss, 1977*
Triplectidina Mosely, 1936	**Leptoceridae *incertae sedis***
Westriplectes Neboiss, 1977	*Nietnerella* Kimmins, 1963*

Table 2.

Gene	Primer	Direction	Primer sequence (5′- 3′)	References
COI	HCO2198	Foward	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al*. (1994)
	LCO1490	Reverse	GGTCAACAAATCATAAAGATATTGG	Folmer *et al*. (1994)
CAD	CAD743nF-ino	Foward	GGIGTIACIACIGCITGYTTYGARCC	Johanson & Malm (2010)
	CAD743nR-ino	Reverse	TTRTTIGGIARYTGICCICCCAT	Johanson & Malm (2010)
IDH	IDHdeg27F-ino	Foward	GGWGAYGARATGACIAGRATHATHTGG	Malm & Johanson (2011)
	IDHdegR-ino	Reverse	TTYTTRCAIGCCCAIACRAAICCICC	Malm & Johanson (2011)
POL-II	POLFOR2	Foward	TGGGAYGSYAAAATGCCKCAACC	Danforth *et al*. (2006)
	POLREV2	Reverse	TYYACAGCAGTATCRATRAGACCTTC	Danforth *et al*. (2006)
EF-1*a*	Lepto-IF	Foward	TTCGTNCCNATCTCAGGNTGGC	Johanson & Malm (2010)
	aIntR	Reverse	CCAYCCCTTGAACCANGGCAT	Malm & Johanson (2008)
	aF	Foward	ATCGAGAAGTTCGAGAARGARGC	Kjer *et al*. (2001)
	aR	Reverse	GGGAAYTCCTGGAARGAYTC	Kjer *et al*. (2001)

Table 3.

Calibration point	Estimated age (Ma)		Fossil taxa	References
Divergence between *Athripsodes*, *ceraclea* and *Homilia*	85.8 - 84.9	(LN)	*Praeathripsodes jantar*	Botoseneanu & Wichard (1983)
Stem to *setodes*	37.2 - 33.9	(LN)	*Setodes abbreviata*	Scudder (1890)
Stem to *Triplectides*	37.2 - 33.9	(LN)	*Triplectides patens*	Ulmer (1912)
Stem to *Triaenodes*	37.2 - 33.9	(LN)	*Triaenodes fossilis*	Wichard & Barnad (2005)
Divergence between Leptoceridae and Outgroup	130	(N)	———	Thomas *et al*. (2020)
Divergence between *Ganonema* and *Anisocentropus*	40 - 120	(LN)	*Ganonema regularis*	Ulmer (1912)
Divergence between *Mollana* and *Mollanodes*	40 - 130	(LN)	*Molanna crassicornis; Molanna indubius*	Ulmer (1912)

Table 4

LEPTOCERINAE
- **Achoropsychini**
  - *Achoropsyche* Holzenthal, 1984
- **Athripsodini**
  - *Athripsodes* Billberg, 1820
  - *Axiocerina* Ross, 1957*
  - *Ceraclea* Stephens, 1829
  - *Homilia* McLachlan, 1877
  - *Leptoceriella* Schmid, 1993*
- **Blyzophilini**
  - *Blyzophilus* Anderson & Kjaerandsen, 1999
  - *Magadacerina* Malm & Johanson, 2013
- **Leptocerini**
  - *Leptocerus* Leach, 1815
- **Mystacidini**
  - *Hemileptocerus* Ulmer, 1922*
  - *Fernandoschmidia* Holzenthal & Andersen, 2007*
  - *Mystacides* Berthold, 1827
  - *Sericodes* Schmid, 1987
  - *Tagalopsyche* Banks, 1913
- **Nectopsychini**
  - *Nectopsyche* Müller, 1879
- **Oecetini**
  - *Oecetis* McLachlan, 1877
- **Setodini**
  - *Setodes* Rambur, 1842
  - *Trichosetodes* Ulmer, 1915
- **Triaenodini**
  - *Adicella* McLachlan, 1877
  - *Erotesis* McLachlan, 1877
  - *Triaenodes* McLachlan, 1865
- ***incertae sedis***
  - *Amphoropsyche* Holzenthal, 1985
  - *Brachysetodes* Schmid, 1955
  - ***Leptecho* Barnard, 1934**
  - ***Leptocerina* Mosely, 1932**
  - ***Neoathripsodes* Holzenthal, 1989**
  - *Parasetodes* McLachlan, 1880
  - *Poecilopsyche* Schmid, 1968

GRUMICHELINAE
- *Amazonatolica* Holzenthal & Pes, 2004
- *Atanatolica* Mosely, 1936
- *Gracilipsodes* Sykora, 1967
- *Grumichella* Mueller, 1879
- *Osflintia* Calor & Holzenthal, 2008*
- *Triplexa* Mosely, 1953

LEPTORUSSINAE
- *Leptorussa* Mosely, 1953

TRIPLECTIDINAE
- **Hudsonemini**
  - *Hudsonema* Mosely, 1936
  - *Notalina* Mosely, 1936
  - *Triplexa* Mosely, 1953
  - ***Russobex* StClair, 1988**
- **Triplectidini**
  - *Lectrides* Mosely, 1953
  - *Notoperata* Neboiss, 1977
  - *Symphitoneuria* Ulmer, 1906
  - *Symphitoneurina* Schmid, 1950*
  - *Triplectides* Kolenati, 1859
  - *Triplectidina* Mosely, 1936
  - *Westriplectes* Neboiss, 1977*

Leptoceridae *incertae sedis*
- *Nietnerella* Kimmins, 1963*

Supplementary material

Table S1. Trichoptera dataset used.

Espécies	NHRS	Gênero	Localidade	CAD	COI	EF1α	IDH	POL
	voucher			acc. no.	acc. no.	acc. no.	acc. no.	acc. no.
Atriplectididae								
Atriplectides dubius Mosely, 1936	FV7	Female	Australia	FN601132	FN601029	FN600829	FN601242	FN600932
Calamoceratidae								
Anisocentropus thinlin Oláh & Johanson, 2010	FC3	Male	Laos	FN601122	FN601019	FN600819	FN601232	FN600922
Ganonema ochraceellum (McLachlan, 1866)	FC1	Male	Laos	FN601120	FN601017	FN600817	FN601230	FN600920
Phylloicus lituratus Banks, 1920	GE5	Male	Peru	FN601133	FN601030	FN600830	FN601243	FN600933
Calocidae								
Caenota plicata Mosely, 1953	CR9	Male	Australia	FN257690	EF395068	EF395132	FN601147	FN257729
Conoesucidae								
Coenoria boera Mosely, 1953	CQ8	Male	Australia	FN257688	FJ263234	FJ263259	FN601146	FN257727
Kokiiridae								
Kokiria miharo McFarlane, 1964	BP6	Male	New Zealand	FN601036	FN600938	FN600738	FN601140	FN600835
Taskiropsyche lacustris Neboiss, 1977	CS5	Male	Australia	FN257691	FJ263235	FJ263261	FN601149	FN257730
Limnephilidae								
Limnephilus centralis Curtis, 1834	FI9	Male	Norway	FN601123	FN601020	FN600820	FN601233	FN600923
Limnocentropodidae								
Limnocentropus himalayanus Martynov, 1930	FU8	Male	Bhutan	FN601131	FN601028	FN600828	FN601241	FN600931
Molannidae								

Molanna angustata Curtis, 1834	A6	Male	Sweden	FN257671	FJ263247	FJ263247	FN601139	FN257710
Molannodes tinctus (Zetterstedt, 1840)	FT9	Male	Norway	FN601130	FN601027	FN600827	FN601240	FN600930
Odontoceridae								
Barynema sp	CQ7	Male	Australia	FN601040	FN600942	FN600742	FN601145	FN600839
Marilia elongata Martynov, 1912	GE6	Male	Peru	FN601134	FN601031	FN600831	FN601244	FN600934
Marilia malickyi Oláh & Johanson, 2010	FC2	Male	Laos	FN601121	FN601018	FN600818	FN601231	FN600921
Philorheithridae								
Aphilorheithrus decoratus Neboiss, 1977	DE3	Male	Australia	FN601043	FN600945	FN600745	FN601151	FN600842
Austrheithrus glymma Neboiss, 1977	DE4	Male	Australia	FN601044	FN600946	FN600746	FN601152	FN600843
Philorheithrus agilis (Hudson, 1904)	BP7	Male	New Zealand	FN601037	FN600939	FN600739	FN601141	FN600836
Tasmanthrus angustipennis Mosely, 1936	DD8	Male	Australia	FN601042	FN600944	FN600744	FN601150	FN600841
Phryganeidae								
Phryganea grandis Linnaeus, 1758	CF2	Male	Norway	FN601038	FN600940	FN600740	FN601142	FN600837
Sericostomatidae								
Notidobiella chacayana Schmid, 1957	CL5	Male	Chile	FN257686	EF395058	EF395122	FN601143	FN257725
Tasimiidae								
Tasimia palpata Mosely, 1936	CS2	Male	Australia	FN601041	FN600943	FN600743	FN601148	FN600840
Trichovespula macrocera Schmid, 1955	CL7	Male	Chile	FN601039	FN600941	FN600741	FN601144	FN600838
Leptoceridae								
***Amazonatolica hamadae* Holzenthal & Pes, 2004**		male	Brazil					
Achoropsyche duodecimpunctata (Navás, 1916)	FS4	Male	Argentina	FN601103	FN601000	FN600800	FN601213	FN600903
Adicella reducta (McLachlan, 1865)	DN9	Male	Sweden	FN601096	FN600992	FN600792	FN601206	FN600895
Adicella sp.	BS3	Male	Hong Kong	FN601060	FN600960	FN600760	FN601170	FN600859
Atanatolica botosaneanui Flint, 1981	BG8	Female	Venezuela	FN601060	EF42855	EF42852	FN601170	FN600860

				1	1	6	1	
Atanatolica penai Holzenthal, 1988	GE8	Male	Peru	FN601136	FN601033	FN600833	FN601246	FN600936
Athripsodes aterrimus (Stephens, 1836)	DO3	Male	Sweden	FN601099	FN600995	FN600795	FN601209	FN600898
Athripsodes cinereus (Curtis, 1834)	DO2	Male	Sweden	FN601098	FN600994	FN600794	FN601208	FN600897
Amphoropsyche woodruffi Holzenthal, 1985	09OFKMK	Male	Trinidad and Tobago	x	HM381247	x	x	x
Blyzophilus dorsohamatus Andersen & Kjaerandsen, 1999	FT4	Male	Ghana	FN601129	FN601026	FN600826	FN601239	FN600929
Brachysetodes bifurcatus Flint, 1983	CV2	Male	Chile	FN601074	FN600970	FN600770	FN601184	FN600873
Brachysetodes tripartitus Schmid, 1964	CV1	Male	Chile	FN601073	FN600969	FN600769	FN601183	FN600872
Ceraclea dissimilis (Stephens, 1836)	BA5	Female	Sweden	FN601056	FN600956	FN600756	FN601165	FN600855
Ceraclea nankingensis (Hwang, 1957)	DA6	Male	Laos	FN601091	FN600987	FN600787	FN601201	FN600890
Erotesis baltica McLachlan, 1877	EE7	Male	Sweden	FN601102	FN600999	FN600799	FN601212	FN600902
Gracilipsodes aureus Malm & Johanson, 2008	AG9	Male	New Caledonia	FN601047	EF428534	EF428510	FN601155	FN600846
Gracilipsodes grandis Malm & Johanson, 2008	AP1	Male	New Caledonia	FN601048	EF428538	EF428514	FN601158	FN600848
Gracilipsodes psocopterus Sykora, 1967	AM9	Male	New Caledonia	FN257695	EF428536	EF428512	FN601157	FN257734
Grumichella flaveola (Ulmer, 1911)	GE7	Male	Peru	FN601135	FN601032	FN600832	FN601245	FN600935
Homilia lardeuxi Gibon, 1991	EF6	Male	Ghana	FN601110	FN601007	FN600807	FN601220	FN600910
Hudsonema alienum (McLachlan, 1868)	BS2	Male	New Zealand	FN601059	FN600959	FN600759	FN601169	FN600858
Hudsonema amabile (McLachlan, 1868)	BZ4	Male	New Zealand	FN601063	FN600961	FN600761	FN601173	FN600862
Hudsonema flaminii (Navás, 1926)	CU6	Male	Chile	FN601070	FN600966	FN600766	FN601180	FN600869
Hudsonema paludosus Neboiss, 1977	BH7	Larva	Australia	FN257696	FJ263239	FJ263266	FN601166	FN257735
Lectrides varians Mosely, 1953	CW9	Male	Australia	FN601080	FN600976	FN600776	FN601190	FN600879
Leptecho sp. Barnard, 1934	TSR635iiB	Male	South Africa		KX294669			
Leptocerina ramosa (Ulmer, 1912)	EF4	Male	Ghana	FN601108	FN601005	FN600805	FN601218	FN600908
Leptocerina spinigera Mosely, 1932	EF8	Male	Ghana	FN601112	FN601009	FN600809	FN601222	FN600912
Leptocerus ousta Schmid, 1987	CW4	Male	Australia	FN601076	FN600972	FN600772	FN601186	FN600875
Leptocerus stephanei Gibon, 1992	EG6	Male	Ghana	FN601117	FN601014	FN600814	FN601227	FN600917
Leptorussa darlingtoni (Banks, 1939)	EC3	Male	Australia	FN601100	FN600997	FN600797	FN601210	FN600900

Mystacides longicornis (Linnaeus, 1758)	AZ9	Male	Sweden	FN601053	FN600953	FN600753	FN601162	FN600852
Mystacides niger (Linnaeus, 1758)	DO1	Male	Sweden	FN601097	FN600993	FN600793	FN601207	FN600896
Nectopsyche argentata Flint, 1991	CU4	Male	Peru	FN601069	FN600965	FN600765	FN601179	FN600868
Nectopsyche punctata (Ulmer, 1905)	CF1	Male	Peru	FN601065	FN600963	FN600763	FN601175	FN600864
Madagacerina forcipata Malm & Johanson, 2013	DB2	Male	Madagascar	FN601138	FN601035	x	x	x
Neoathripsodes sp. Holzenthal, 1989	-	Male	Brasil					
Notalina bifaria Neboiss, 1977	BH8	Larva	Australia	FN601057	FN600957	FN600757	FN601167	FN600856
Notalina fulva Kimmins, 1953	DA1	Male	Australia	FN601089	FN600985	FN600785	FN601199	FN600888
Notalina moselyi Kimmins, 1953	CX5	Male	Australia	FN601084	FN600980	FN600780	FN601194	FN600883
Notoperata maculata (Mosely, 1953)	EC4	Male	Australia	FN601101	FN600998	FN600798	FN601211	FN600901
Notoperata sp.	CW6	Male	Australia	FN601078	FN600974	FN600774	FN601188	FN600877
Oecetis africana Ulmer, 1931	EG1	Male	Ghana	FN601113	FN601010	FN600810	FN601223	FN600913
Oecetis akimi Gibbs, 1973	EF2	Male	Ghana	FN601106	FN601003	FN600803	FN601216	FN600906
Oecetis australis (Banks, 1920)	FT1	Male	Australia	FN601128	FN601025	FN600825	FN601238	FN600928
Oecetis lacustris (Pictet, 1834)	BA1	Female	Sweden	FN601054	FN600954	FN600754	FN601163	FN600853
Oecetis minasata Mosely, 1953	FS9	Male	Australia	FN601127	FN601024	FN600824	FN601237	FN600927
Oecetis ochracea (Curtis, 1825)	AZ7	Female	Sweden	FN601052	FN600952	FN600752	FN601161	FN600851
Parasetodes repersellus (Rambur, 1842)	CU1	Female	Laos	FN601068	FN600964	FN600764	FN601178	FN600867
Parasetodes sudanensis Ulmer, 1922	EF5	Male	Ghana	FN601109	FN601006	FN600806	FN601219	FN600909
Poecilopsyche vidura Schmid, 1968	DO5	Female	India	x	FN600996	FN600796	x	FN600899
Russobex corneatus St. Clair, 1988	JOS204	Male	Australia	x	KX295783	x	x	x
Sericodes sp.	EE9	Male	Ghana	FN601104	FN601001	FN600801	FN601214	FN600904
Setodes bracteatus Neboiss, 1982	CW5	Male	Australia	FN601077	FN600973	FN600773	FN601187	FN600876
Setodes bispinus Yang & Morse, 1989	DA3	Male	Laos	FN601090	FN600986	FN600786	FN601200	FN600889
Setodes flagellatus Gibbs, 1973	EG4	Male	Ghana	FN601115	FN601012	FN600812	FN601225	FN600915
Setodes incertus (Walker, 1852)	CE8	Male	Canada	FN601064	FN600962	FN600762	FN601174	FN600863
Symphitoneuria clara Ward, 2001	AQ9	Male	New Caledonia	FN601050	FN600950	FN600750	FN601159	FN600849

Symphitoneuria opposita (Walker, 1852)	CX4	Female	Australia	FN601083	FN600979	FN600779	FN601193	FN600882
Symphitoneuria sabaensis Andersen & Huisman, 1997	FS8	Female	Malaysia	FN601126	FN601023	FN600823	FN601236	FN600926
Symphitoneuria triangulata Malm & Johanson, 2007	M4	Male	New Caledonia	FN601048	FN600949	FN600749	FN601156	FN600847
Tagalopsyche jolandae Holzenthal & Andersen, 2007	ER1	Male	Malaysia	FN601118	FN601015	FN600815	FN601228	FN600918
Tagalopsyche jolandae Holzenthal & Andersen, 2007	ER2	Female	Malaysia	FN601119	FN601016	FN600816	FN601229	FN600919
Triaenodes bicolor (Curtis, 1834)	AZ6	Female	Sweden	FN601051	FN600951	FN600751	FN601160	FN600850
Triaenodes detruncatus Martynov, 1924	BA4	Female	Sweden	FN601055	FN600955	FN600755	FN601164	FN600854
Triaenodes kwabena Andersen & Holzenthal, 2002	EF3	Male	Ghana	FN601107	FN601004	FN600804	FN601217	FN600907
Trichosetodes japonicus Tsuda, 1942	KKCAD-0325		Russia	MN364757	KX104756			
Trichosetodes sisyphos Malicky & Prommi, 2006	DM6	Male	Thailand	FN601093	FN600989	FN600789	FN601203	FN600892
Triplectides aequalichelatus Malm & Johanson, 2008	AE6	Male	New Caledonia	FN601045	FN600947	FN600747	FN601153	FN600844
Triplectides australis Navás, 1934	CY8	Female	Australia	FN601087	FN600983	FN600783	FN601197	FN600886
Triplectides gonetalus Morse & Neboiss, 1982	CY9	Female	Australia	FN601088	FN600984	FN600784	FN601198	FN600887
Triplectides jaffueli Navás, 1918	CU9	Male	Chile	FN601072	FN600968	FN600768	FN601182	FN600871
Triplectides magnus (Walker, 1852)	DM4	Female	Australia	FN601092	FN600988	FN600788	FN601202	FN600891
Triplectides nigripennis Mosely, 1936	CU8	Male	Chile	FN601071	FN600967	FN600767	FN601181	FN600870
Triplectides tigrinus Malm & Johanson, 2008	AF3	Male	New Caledonia	FN601046	FN600948	FN600748	FN601154	FN600845
Triplectides volda Mosely, 1953	CY3	Male	Australia	FN601086	FN600982	FN600782	FN601196	FN600885
Triplectidina moselyi McFarlane & Ward, 1990	BP5	Male	New Zealand	FN601058	FN600958	FN600758	FN601168	FN600857
Triplectidina nigricornis Mosely, 1936	CX2	Male	Australia	FN601082	FN600978	FN600778	FN601192	FN600881
Triplectidina nigricornis Mosely, 1936	DN3	Larva	Australia	FN601094	FN600990	FN600790	FN601204	FN600893
Triplexa villa Mosely, 1953	CP9	Male	Australia	FN601066	EF428553	EF428528	FN601176	FN600865

Tabela S2. Taxons and their respective distribution areas following the Morse (2019) used in BioGeoBears. A = Afrotropical; B= Australiana; C = East Laurasia; D = Neártico; E = Neotropical; F = Oriental; e G = West Laurasia.

Species / Distribution	A	B	C	D	E	F	G
Leptoceridae							
Amphoropsyche woodruffi Holzenthal, 1985	0	0	0	0	1	0	0
Achoropsyche duodecimpunctata (Navás, 1916)	0	0	0	0	1	0	0
Adicella reducta (McLachlan, 1865)	1	0	1	0	0	1	1
Adicella sp.	1	0	1	0	0	1	1
Amazonatolica hamadae Holzenthal & Pes, 2004	0	0	0	0	1	0	0
Atanatolica botosaneanui Flint, 1981	0	0	0	0	1	0	0
Atanatolica penai Holzenthal, 1988	0	0	0	0	1	0	0
Athripsodes aterrimus (Stephens, 1836)	1	0	1	0	0	1	1
Athripsodes cinereus (Curtis, 1834)	1	0	1	0	0	1	1
Blyzophilus dorsohamatus Andersen & Kjaerandsen, 1999	1	0	0	0	0	0	0
Brachysetodes bifurcatus Flint, 1983	0	0	0	0	1	0	0
Brachysetodes tripartitus Schmid, 1964	0	0	0	0	1	0	0
Ceraclea dissimilis (Stephens, 1836)	1	0	1	1	0	1	1
Ceraclea nankingensis (Hwang, 1957)	1	0	1	1	0	1	1
Erotesis baltica McLachlan, 1877	0	0	1	0	0	0	1
Gracilipsodes aureus Malm & Johanson, 2008	0	1	0	0	0	0	0
Gracilipsodes grandis Malm & Johanson, 2008	0	1	0	0	0	0	0
Gracilipsodes psocopterus Sykora, 1967	0	1	0	0	0	0	0
Grumichella flaveola (Ulmer, 1911)	0	0	0	0	1	0	0
Homilia lardeuxi Gibon, 1991	1	0	0	0	0	0	0
Hudsonema alienum (McLachlan, 1868)	0	1	0	0	1	0	0
Hudsonema amabile (McLachlan, 1868)	0	1	0	0	1	0	0
Hudsonema flaminii (Navás, 1926)	0	1	0	0	1	0	0
Hudsonema paludosus Neboiss, 1977	0	1	0	0	0	0	0
Lectrides varians Mosely, 1953	0	1	0	0	0	0	0

Leptecho Barnard, 1934	1	0	0	0	0	0	0
Leptocerina ramosa (Ulmer, 1912)	1	0	0	0	0	0	0
Leptocerina spinigera Mosely, 1932	1	0	0	0	0	0	0
Leptocerus ousta Schmid, 1987	1	1	1	0	0	1	0
Leptocerus stephanei Gibon, 1992	1	1	1	0	0	1	0
Leptorussa darlingtoni (Banks, 1939)	0	1	0	0	0	0	0
Mystacides longicornis (Linnaeus, 1758)	0	0	1	1	0	1	1
Mystacides niger (Linnaeus, 1758)	0	0	1	1	0	1	1
Nectopsyche argentata Flint, 1991	0	0	0	1	1	0	1
Nectopsyche punctata (Ulmer, 1905)	0	0	0	1	1	0	1
Madagacerina forcipata Malm & Johanson, 2013	0	1	0	0	0	0	0
Neoathripsodes sp.	0	0	0	0	1	0	0
Notalina bifaria Neboiss, 1977	0	1	0	0	1	0	0
Notalina fulva Kimmins, 1953	0	1	0	0	1	0	0
Notalina moselyi Kimmins, 1953	0	1	0	0	1	0	0
Notoperata maculata (Mosely, 1953)	0	1	0	0	0	0	0
Notoperata sp.	0	1	0	0	0	0	0
Notoperata sp.	0	1	0	0	0	0	0
Oecetis akimi Gibbs, 1973	1	1	1	1	1	1	1
Oecetis australis (Banks, 1920)	1	1	1	1	1	1	1
Oecetis lacustris (Pictet, 1834)	1	1	1	1	1	1	1
Oecetis minasata Mosely, 1953	1	1	1	1	1	1	1
Oecetis ochracea (Curtis, 1825)	1	1	1	1	1	1	1
Parasetodes repersellus (Rambur, 1842)	1	0	1	0	0	1	1
Parasetodes sudanensis Ulmer, 1922	1	0	1	0	0	1	1
Poecilopsyche vidura Schmid, 1968	0	0	0	0	0	1	0
Russobex corneatus St Clair, 1988	0	1	0	0	0	0	0
Sericodes sp.	1	0	0	0	0	0	0
Setodes bracteatus Neboiss, 1982	1	1	1	1	0	1	1
Setodes bispinus Yang & Morse, 1989	1	1	1	1	0	1	1
Setodes flagellatus Gibbs, 1973	1	1	1	1	0	1	1
Setodes incertus (Walker, 1852)	1	1	1	1	0	1	1

Symphitoneuria clara Ward, 2001	0	1	0	0	0	1	0
Symphitoneuria opposita (Walker, 1852)	0	1	0	0	0	1	0
Symphitoneuria sabaensis Andersen & Huisman, 1997	0	1	0	0	0	1	0
Symphitoneuria triangulata Malm & Johanson, 2007	0	1	0	0	0	1	0
Tagalopsyche jolandae Holzenthal & Andersen, 2007	1	0	0	0	0	1	0
Triaenodes bicolor (Curtis, 1834)	1	1	1	1	1	1	1
Triaenodes detruncatus Martynov, 1924	1	1	1	1	1	1	1
Triaenodes kwabena Andersen & Holzenthal, 2002	1	1	1	1	1	1	1
Trichosetodes sisyphos Malicky & Prommi, 2006	1	0	1	0	0	1	0
Triplectides aequalichelatus Malm & Johanson, 2008	0	1	1	1	1	1	0
Triplectides australis Navás, 1934	0	1	1	1	1	1	0
Triplectides gonetalus Morse & Neboiss, 1982	0	1	1	1	1	1	0
Triplectides magnus (Walker, 1852)	0	1	1	1	1	1	0
Triplectides nigripennis Mosely, 1936	0	1	1	1	1	1	0
Triplectides tigrinus Malm & Johanson, 2008	0	1	1	1	1	1	0
Triplectides volda Mosely, 1953	0	1	1	1	1	1	0
Triplectidina moselyi McFarlane & Ward, 1990	0	1	0	0	0	0	0
Triplectidina nigricornis Mosely, 1936	0	1	0	0	0	0	0
Triplexa villa Mosely, 1953	0	1	0	0	0	0	0

CHAPTER 2

TITLE:

Redefinition of *Achoropsyche* Holzenthal, 1984 (Trichoptera: Leptoceridae) under phylogenetic approach, with description of five new species and identification key

AUTHORS:

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Redefinition of *Achoropsyche* Holzenthal, 1984 (Trichoptera: Leptoceridae) under phylogenetic approach, with description of five new species and identification key

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ABSTRACT

Among the Neotropical leptocerids, the endemic and monotypic genus *Achoropsyche* was erected in 1984. The only known species (*A. duodecimpunctata*) has a widespread distribution and has been recorded in Brazilian and Chacoan subregions (Venezuela to Argentina). Here, we proposed five new species, redefinition of the genus under phylogenetic analysis, presented new records and an identification key. The phylogenetic analyses under equal weight and implicit weight produced one most-parsimonious tree for each method and the monophyly of *Achoropsyche* was corroborated with high support value by six characters: forewing, dark brown spot present; hind wing, veins Sc-R_1 fused before the margin; , proto-tibia, number of apical spurs with one spur; segment IX with triangular process extending to segment present in lateral view; , inferior appendage, keel-like basomesal projection present; and character , inferior appendage, basodorsal process present. *Achoropsyche bifurcata* **sp. nov.** can be diagnosed by segment IX bifid and pointed medially in dorsal view, preanal appendage long and digited, and medial process of inferior appendage with a row of denticles in dorsal margin. *Achoropsyche evaginata* **sp. nov.** can be diagnosed by nine dark brown spots in the forewing, segment X with apex truncated in dorsal view, and presenting a long and narrow process in lateral view, and paramere long with a single apical seta. *Achoropsyche quinteiroi* **sp. nov.** can be diagnosed by a very long medial process of inferior appendage in ventral view, apical process of segment IX subtriangular in dorsal view, phallobase well developed in lateral view, inferior margin of phallicata apical slightly enlarged in lateral view, and segment X with apex truncated in dorsal view. *Achoropsyche robusta* **sp. nov.** can be diagnosed by ten dark brown spots in the forewing, Phallic apparatus long, apical phallicata twice longer than paramere in lateral view, paramere robust with three apical setae in lateral view, and inferior appendage very robust in ventral view; segment IX process constrict in apical

region in dorsal view. *Achoropsyche serrata* **sp. nov.** can be diagnosed by segment X enlarged basally with lateral margin sinuous in dorsal view and with a strong constriction in the ventral margin basally in lateral view; inferior appendage long and narrow and the dorso-basal process with a row of denticles in the ventral margin apically in lateral view. The circumscription of the genus is refined here with the description of five new species, the morphological study using Scanning Electron Microscope and a phylogenetic analysis.

KEYWORDS: Leptocerinae, Achorpsychini, long-horned caddisflies, Neotropical region, taxonomy

INTRODUCTION

Leptoceridae, long-horned caddisflies, are the second largest family of Trichoptera with 2.265 species, including 30 represented by fossils, which are classified in 58 genera, nine of them represented by fossils (Morse *et al.* 2019). The family has a cosmopolitan distribution with the Oriental region presenting the greatest number of species (987), followed by the Australian region (324 species). The Neotropical region presents 286 species of Leptoceridae, being the third region in richness of species in this family (Morse *et al.* 2019). Among the Neotropical leptocerids, the endemic and monotype genus *Achoropsyche* was erected by Holzenthal (1984) to include one species previously classified as *Brachysetodes* Schmid, 1955, *B. duodecimpunctata* (Návas, 1916). *Brachysetodes* is restricted to the Chilean region, west side of Andes mountain ranges (Holzenthal and Calor 2017). On the other hand, *Achoropsyche* presents a widespread distribution, in the east side of the Andes, including the Brazilian and the Chacoan sub-regions. The genus has been recorded from Venezuela to Argentina (Holzenthal 1984; Holzenthal and Calor 2017).

The type locality of the *Achoropsyche duodecimpunctata* is Nova Friburgo, Rio de Janeiro state, Brazil (Parana dominion), and the widespread distribution was used as a reference to name the genus, "from the Greek a*choros* means homeless or without a resting place to both very widespread distribution of the type species and its frequent generic reassignment. Gender feminine" (Holzenthal 1984, p. 181-182). Until the moment, only the male and female are known, usually occurring near large, lowland rivers (Holzenthal 1984; Holzenthal and Calor 2017; Santos and Dumas 2020).

When the genus was described, a new tribe Achoropsychini was erected to include it (Holzenthal, 1984). According with Holzenthal (1984), the new tribe had a possible relationship with Leptocerinae branch Trianodini + Oecetini + Setodini + Mystascidini, as defined by Morse (1981) for shared the fusion of the segment X in the male genitalia. In Malm and Johanson (2011), Achoropsychini appears in a polytomy with *Parasetodes* McLachlan, 1880 (Nectopsychini) and *Leptocerina* Mosely, 1932 (Athripsodini) as sister group of all Leptocerinae, except *Nectopsyche* Mueller, 1879. On the other hand, in our phylogenetic results, *Achoropsyche* appears as a sister group of *Nectopsyche*, around 63 million years ago, nested in a clade with *Leptecho* Barnard, 1934 and *Leptocerina* (Dias *et al.* in prep).

The interesting and unusual widespread distribution for a single caddisfly species as well as the remarkable morphological differences of some specimens drew our attention. In this context, we reanalyzed the material deposited in some collections under a phylogenetic approach, and we are proposing five new species, occurrence map, a new diagnosis and emend in the description of the genus, including characters observed on Scanning Electron Microscope, an identification key for males.

MATERIAL AND METHODS

Material studied

The specimens analyzed are from the Museu de Zoologia da Universidade Federal da Bahia, Salvador, Brazil (UFBA) and UMSP University of Minnesota Insect Collection, Saint Paul, USA (UMSP). The genitalia were cleared using KOH 10% solution and stored in microvials with glycerin. Illustrations were made using a microscope with a drawing tube attached. Digital illustrations were made using the software Adobe Illustrator ® CS6. The SEM (Scanning Electron Microscope) images were taken using a JEOL JSM-661022. Morphological terminology followed Schmid (1980), as implemented by Holzenthal (1984). The holotype and some paratypes will be deposited in Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil (MZSP), UFBA and UMSP. The distribution map (Fig. 1) was generated using the software QGIS version 2.8.2 and edited in Adobe Illustrator CS6. Terminology for biogeography areas followed Morrone (2014). The occurrence points of the type species based on the literature were indicated with a black circle and the references used were indicated in the map legend. Vector files for areas were obtained from the public domain website http://www.neotropico.com.br/shapefile (Löwenberg-Neto, 2014).

Cladistic analysis

The data matrix was produced using MESQUITE 3.61 (Maddison and Maddison 2019) with 33 unordered morphological characters (1, 18 and 14 characters from head, thorax and genitalia, respectively) and 12 taxa (ingroup comprised six species of *Achoropsyche* and outgroup comprises six species from different subfamilies of Leptoceridae). The matrix was analyzed under parsimony using the software TNT (Goloboff *et al*. 2008). The software memory was adjusted to 9.999 to accommodate the highest number of trees in tree space. Inapplicable data assigned "-", and "?" missing were read as missing data. Parsimony

analyses were implemented using implicit enumeration under equal weight (EW) and implied weight (IW). The concavity index (*k* values) was calculated using the script *setk.run* (*k* = 0.996094) (S. Arias, unpublished data). The state transformations were optimized and visualized using WINCLADA (Nixon, 2002), and the final tree figure was edited using Adobe Illustrator CS6.

PHYLOGENETIC RESULTS

The phylogenetic analyses under equal weight (EW) and implicit weight (IW) produced one most-parsimonious tree each method: EW (length = 56 steps, Consistency Index (CI) = 60 and Retention Index (RI) = 69) (Fig. 2A), and IW (length = 57 steps, (CI) = 56 and (RI) = 60) (Fig. 2B).

In both EW and IW, the monophyly of *Achoropsyche* was corroborated with high support value by six synapomorphies: character 10 [1], forewing, dark brown spot present; character 17 [1], hind wing, veins Sc - R_1 fused before the margin; character 19 [0], proto-tibia with one spur; character 23 [1], segment IX with triangular process extending to segment present in lateral view; character 29 [1], inferior appendage, keel-like baso-mesal projection present; and character 30 [1], inferior appendage, baso-dorsal process present. In the first cladogenesis of the genus, *A. duodecimpunctata* appears as sister group of other species of the genus. The characters 7 [1], forewing with the proportional length of thyridial cell longer than the discoidal cell, and character 11 [1], hind wing with vein Sc thickened, appears as synapomorphies in the clade formed by other species of the genus. Moreover, the character 25 [1], segment X with basal region broad in lateral, also appears as synapomorphy for the referred clade in the IW analysis. Another congruence between the approaches is the clado formed by (*A. bifurcata* **n. sp.** + *A. serrata* **n. sp.**) with high support value. This clade is supported by three synapomorphy: character 1 [1], head with

anteromesal setal warts subtriangular in dorsal view, character 5 [0], forewing with crossvein *sc-r* absent, and character 17 [0], hind wing with the veins Sc-R_1 not fused before the margin.

According EW, in the second cladogenesis, *A. evaginata* **n. sp.** appears as a sister group of the clade formed by *A. quinteiroi* **n. sp.** (*A. robusta* **n. sp.** (*A. bifurcata* **n. sp.**+ *A. serrata* **n. sp.**)), while in IW the second cladogenesis of the genus has a polytomy between *A. robusta* **n. sp.**, *A. quinteiroi* **n. sp.** and a clade with weak support formed by *A. evaginata* **n. sp. (***A. bifurcata* **n. sp.** + *A. serrata* **n. sp.).**

TAXONOMY

Achoropsyche Holzenthal, 1984

Achoropsyche Holzenthal, 1984:181

[Type species: *Setodes duodecimpunctata* Navás, 1916: 22, by monotype].

[*Setodes* or *Leptocerus duodecimpunctatus* Fisher, 1966: 45]

[*Brachysetodes duodecimpunctatus* Flint, 1972:244; Flint, 1974:120, male]

Etymology

From the Greek “achoros” meaning homeless or without a resting place in reference to both the very widespread distribution of the type-species and it is frequent generic reassignment. Gender: feminine (Holzenthal, 1984).

Diagnosis

The genus can be distinguished from others by the following characters: antenna scape presents an anterolateral wart, and a concavity in the basodorsal region with a row of small

setae dorsally; the forewing with dark brown spots in the crossveins (*r*; *m-cu*), and in the forks (R_{2+3} and R_{4+5}; R_1 and R_2; M and Cu; Cu1 and Cu2; Cu1B and Cu2; A_2 and A_3; A_1 and A_{2+3}); the segment IX with a dorsal process expanded posteriorly, extending beyond the segment X, and the inferior appendage presents a keel-like baso-mesal projection and a baso-dorsal process.

The following characters should be amended in the original genus description

Head (Fig. 3A–H), color yellowish dorsally with yellow setae, midcranial sulcus absent (Fig. 3A), anteromesal setal warts rounded, anterior setal wart small and rounded; posterior setal warts rounded (Fig. 3B). Antennae, scape slightly constrict basally, setose dorsally (Fig. 3B), with an anterolateral wart (Figs. 3C–D); pedicel rounded, evaginated basodorsally, with thin setae (Figs. 3D–E); flagellum filiform with one to three small openings (Fig. 3F). Maxillary palps yellowish with yellow setae in alcohol (Fig. 3G). Front lateral setal warts sub-rectangular (Fig. 3H). Thorax (Figs. 4A–H), pronotum yellowish in alcohol (Fig. 4A); pronotal setal warts with yellow setae (Fig. 4B). Legs yellowish and setose (Figs. 4C–D); tibial spur formula 1,2,2, with foretibia spur short (Figs. 4C–E); tibia with striated setae, medially enlarged, some setae with jagged edge (Figs. 3F-H); a pair of well developed tarsal claws (Fig. 4H). Forewing with crossveins *sc-r*, r_1-r_2, *r-m* and *m-cu* present, forks I and V present; M 2-branched and petiolate in both sexes; discoidal and thyridial cells equal in length. Hind wing vein R1 fused in the vein Sc before the margin, forks I and V present. *Abdomen* (Figs. 5A–H), segments I to VIII translucid in alcohol (Figs. 5A–B); operculum ovoid (Fig. 5C). Segment IX narrow, sclerotized strips present (Fig. 5D); process of segment IX present (Fig. 5E). Inferior appendage one segmented with a ventral plate and a filiform process dorsal (Figs. 5E–F); basodorsal process presents (Fig. 5G). Phallic apparatus sclerotized strips present (Fig. 5H).

***Achoropsyche duodecimpunctata* Holzenthal, 1984**

(Figures 1-3, p. 1983 in Holzenthal, 1984)

Material examined

BRAZIL, **Minas Gerais**, Parque Estadual do Rio Preto, Rio Preto, 18°06.993'S, 43.20.373'W, el. 650 m, 19.v.1998, Holzenthal & Paprocki, 3 ♂ (pinned, UMSP); same data, except Rio Caraça, near Santa Barbara, 20°01.371S, 43°28.270W, el. 728 m, 9.xi.2001, Holzenthal, Paprocki, Blahnik, Amarante, 3 ♂ (pinned, UMSP). **VENEZUELA**, **Bolivar State**, 4°31.237N, 61°31.591W, el. 869 m, Gran Sabana, E. Pauji, "Rio Curvita", 15-16.vii.2010, UV lights, Holzenthal, Thomson, Cressa. VE100715, 3 ♂ (pinned, UMSP).

Diagnosis

This species differs from its congeners by the thyridaal cell and discoidal cell equal in length, by the preanal appendage with a little evagination in distal margin in dorsal and lateral view, by the segment X acuminate; by the phallic apparatus with a process long and narrow in the pahlobase. *Achoropsyche duodecimpunctata* reseambles *A. evaginata* **sp. nov.,** mainly by general shape of the inferior appendage in lateral view and the general shape of baso mensal projection of the inferior appendage in ventral view. However, the segment X is almost truncated in the apex in dorsal view in *A. evaginata* **sp. nov.**, which in *A. duodecimpunctata* the apex of segment X is acuminated in dorsal view. Additionally, the process of segment IX in *A. duodecimpunctata* is shorter than *A. evaginata* **sp. nov.**.

Distribution

Argentina (Entre Rios, Missiones), Brazil (Amazonas, Bahia, Espírito Santo, Maranhão, Mato Grosso, Minas Gerais, Pará, Paraná, Rio de Janeiro, Roraima, Rondônia, Santa Catarina, and São Paulo), Colombia, Ecuador, Guyana, Paraguay, Peru (Madre de Dios),

Suriname (Sipalliwini), Uruguay, and Venezuela (Bolivar) (Fig. 1) (Holzenthal and Calor, 2017; Santos and Dumas, 2020).

Remarks

In both EW and IW, *Achoropsyche duodecimpunctata* emerger as a sister group of the other species of the genus. The characters 7 [1], forewing with the proportional length of thyridial cell longer than the discoidal cell, and character 11 [1], hind wing with vein Sc thickened, appears as synapomorphies in the clade formed by other species of the genus. in the IW analysis, the character 25 [1], segment X with basal region broad in lateral, also appears as synapomorphy for the referred clade.

***Achoropsyche bifurcata* sp. nov.**

(Fig. 6)

Material examined

Holotype

♂ (pinned, MZSP). **BRAZIL: São Paulo**, Pedregulho, Ribeirão São Pedro, 20°09.113'S, 47°30.626'W, el. 617 m, 16.ix.2003, Holzenthal, Paprocki & Calor.

Paratypes

Same data as holotype, except 4 ♂ (pinned, USMP). Same data, except 2 ♂, Altinópolis, Fazenda São João da Mata, Rio Bagassu, 21°00.288'S, 47°28.900'W, el. 745 m, 19-21.xi.2003, Holzenthal, Paprocki & Calor (pinned, UMSP).

Diagnosis

This species differs from its congeners by segment IX bifid and pointed medially in dorsal view, by the preanal appendage long and digited, and by the the medial process of inferior appendage with a row of denticles in dorsal margin. *Achoropsyche bifurcata* **n. sp.** resembles *A. serrata* **sp. nov.**, mainly by general shape of the phallic apparatus in lateral view and baso-mesal plate of the inferior appendage in ventral view. However, the medial evagination of the distal margin of the segment IX in *A. bifurcata* **sp. nov** is deeper than in *A. serrata* **sp. nov.** Additionally, both the inferior appendage and the phalotremal sclerite of *A. bifurcata* **sp. nov.** are broader than *A. serrata* **sp. nov.** in lateral view.

Description

Adult male: Forewing length 5.12–5.9 mm (n= 5); hind wing length 3.9–4.15 mm (n= 5). Head, color yellowish dorsally with yellow setae, midcranial sulcus absent, anteromesal setal warts rounded, anterior setal wart small and rounded; posterior setal warts rounded. Antennae, scape slightly constrict basally, setose dorsally, with an anterolateral wart; pedicel rounded, evaginated basodorsally with thin setae; flagellum filiform with one to three small openings. Maxillary palps yellowish with yellow setae (pinned). Front lateral setal warts sub-rectangular. Thorax, pronotum yellowish, pronotal setal warts with yellow setae; mesoscutum and metanotum yellowish with yellow setae. Legs pale yellowish; tibial spur formula 1,2,2. Forewing with 13 dark brown spots; vein Sc slightly thickened; crossveins r_1-r_2, *r-m* and *m-cu* present; forks I and V present (Fig. 6A). Hind wing veins Sc and Cu1A thickened; vein R_1 parallel (no fused) to vein Sc; crossvein *r-m* present; forks I and V present (Fig. 6B).

Male abdomen. Segments I to VIII yellowish. Segment IX bifid and pointed in the middle region in dorsal view (Fig 6D); pointed in ventral region, projected dorsally in lateral view (Fig. 6C); process of segment IX narrow in anterior and middle region, apex broad in dorsal

view (Fig. 6D); narrow medially, apex broad and acuminated in posteroventral margin in lateral view (Fig. 6C). Segment X enlarged basally, apex truncated in dorsal view (Fig. 6D); triangular, broad in basal region, acute apex in lateral view (Fig. 6C). Preanal appendage, long, setose, digited in dorsal view (Fig. 6D); subtriagular in lateral view (Fig. 6C). Inferior appendage apically enlarged, apex acute, medial process of inferior appendage with a row of denticles in dorsal margin in lateral view (Fig. 6C); digited, ventral plate broad, medial projection long and narrow in ventral view (Fig. 6F). Phallic apparatus narrow; paramere long and narrow, with three setae in the apex; phallobase well developed; phallotremal sclerite rounded in lateral view (Fig. 6E).

Etymology

Derived from Latin, *bifurcatus* means bifurcated in reference to distal margin of segment IX in dorsal view.

Distribution

Brazil (São Paulo State) (Fig. 1).

Remarks

Achoropsyche bifurcata **n. sp.** appears as a sister group of *A. serrata* **sp. nov.** in a clade with high support value in both phylogenetic analyses. Theses two species shared three characters, two of then exclusive synapomorphies (1 [1], head with anteromesal setal warts V-shaped in dorsal view, and 5 [0], forewing with crossvein *sc-r* absent), and a non exclusive synapomorphy (18[1], hind wing with veins Sc-R_1 not fused before the margin).

***Achoropsyche evaginata* sp. nov.**

(Fig. 7)

Material examined

Holotype

♂ (alcohol, MZSP). **BRAZIL: Bahia,** Varzedo, Serra da Jiboia, Faz. Baixa Grande, Córrego Cai Camarão, 12°57’40.5’’S, 39°26’54’’W, el. 276 m, 09.ii.2014, UV Light Pan trap, A. R. Calor & A. Vilarino.

Paratypes

Same data as holotype, except 3 ♂, 27.viii.2013, light trap, A. R. Calor, A. Zanata & V. A. Gomes (alcohol, MZSP); same data, except 5 ♂, 24.x.2012, V. A. Gomes, A. Vilarino & R. Campos(alcohol, UFBA); same data, except 3 ♂ 28.vi.2013, A. R. Calor, A. Medeiros & V. A. Gomes (alcohol, USMP). Same data, except 2 ♂, Una, Reserva Biológica Una, 15°10’30’’, 39°03’30’’W, el. 15 m, 03.viii.2017, E. Dias, R. Campos, F. Silva & F. Gudin (alcohol, UFBA).

Diagnosis

This new species differs from its congeners by the nine dark brown spots on the forewing, by the segment X with apex truncated in dorsal view, by the process of segment IX long and narrow in lateral view, and by the paramere long with one setae in the apex. *Achoropsyche evaginata* **sp. nov.**. resembles *A. bifurcata* **sp. nov.**, mainly by general shape of the segment IX, segment X and preanal appendage in dorsal view. However, the medial evagination of the distal margin of the segment IX in *A. bifurcata* **sp. nov.** is deeper than in *A. evaginata* **sp. nov.**. Additionally, the inferior appendage of *A. evaginata* **sp. nov.** is more enlarged than in *A. bifurcata* **sp. nov.** in ventral view. The phallobase of the *A. evaginata* **sp. nov.** is broader than that of *A. bifurcata* **sp. nov.**.

Description

Adult male. Forewing length 5.1–5.2 mm (n= 4); hind wing length 3.9–4.1 mm (n= 4). Head, color yellowish dorsally with yellow setae, midcranial sulcus absent, anteromesal setal warts rounded, anterior setal wart small and rounded; posterior setal warts rounded. Antennae, scape slightly constrict basally, setose dorsally, with an anterolateral wart; pedicel rounded, evaginated basodorsally, with thin setae; flagellum filiform with one to three small openings. Maxillary palps yellowish with yellow setae (in alcohol). Front lateral setal warts sub-rectangular. Thorax, pronotum yellowish, pronotal setal warts with yellow setae; mesoscutum and metanotum yellowish with yellow setae. Legs pale yellowish; tibial spur formula 1,2,2. Forewing with nine dark brown spots; veins Sc, R_1, and A_3 thickened; crossveins *sc-r*, r_1-r_2, *r-m* and *m-cu* present; forks I and V present (Fig. 7A). Hind wind vein Sc and Cu1A thickened; vein R_1 fused in the vein Sc before the margin; crossvein *r-m* present; forks I and V present (Fig. 7B).

Male Abdomen. Segments I to VIII translucid in alcohol. Segment IX slightly evaginated in the middle region in dorsal view (Fig. 7D); pointed in ventral region, with pointed projection in dorsal region in lateral view (Fig. 7C). Process of segment IX narrow in middle region, apex broad in dorsal view (Fig. 7D); narrow in middle region, apex broad, apex pointed in ventral region in lateral view (Fig. 7C). Segment X enlarged basally, apex truncated in dorsal view (Fig. 7D); broad in the basal region, acute in the apex in lateral view (Fig. 7C). Preanal appendage setose, digited in dorsal view (Fig. 7D); subtriagular in lateral view (Fig. 7D). Inferior appendage setose, enlarged in middle region, apex subquadrate in lateral view (Fig. 7C); apex acute, ventral plate broad, medial projection acute in lateral view (Fig. 7F). Phallic apparatus narrow, paramere narrow, one seta in the apex, phallobase well developed, phallotremal sclerite rounded and small in lateral view (Fig. 7E).

Etymology

Derived from Latin, *evagino* means evaginated in reference to distal margin of segment IX slightly evaginated medially.

Distribution

Brazil (Bahia State) (Fig. 1)

Remarks

According to the phylogenetic results under EW, *Achoropsyche evaginata* **sp. nov.** appears in the second cladogenesis as a sister group of the clade (*A. quinteiroi* **sp. nov. +** (*A. robusta* **sp. nov.** + (*A. serrata* **sp. nov. +** *A. bifurcata* **sp. nov.**)). In the results from IW, *A. evaginata* appears as the sister group of the clade (*A. serrata* **sp. nov. +** *A. bifurcata* **sp. nov**). The character synapomorphic for this group is character 5 [0], forewing with vein R1 uniformly thickened.

***Achoropsyche quinteiroi* sp. nov.**

(Fig. 8)

Material examined

Holotype

♂ (alcohol, MZSP). **BRAZIL: Acre**, Mâncio Lima, Parque Nacional da Serra do Divisor, Igarapé Amor, 07º26'46.6''S, 73°40'10.8''W, el. 291 m, 16.iii.2006, Malaise trap, A.R. Calor.

Paratypes

Same data as holotype, except 4 ♂, Rio Azul, light pan trap (alcohol); same data, except 4 ♂, 15.iii.2006 (alcohol, MZSP); same data, except 5 ♂ (alcohol, UMSP).

Diagnosis

This new species differs from its congeners by 14 dark brown spots in the forewing; by inferior appendage bearing a medial process of the basomesal projection very long in ventral view; by process of segment IX subtriangular apically in dorsal view; phallobase well developed in lateral view; phallicata with apical inferior margin slightly enlarged in lateral view, and segment X with apex truncated in dorsal view. *Achoropsyche quinteiroi* **sp**. **nov.** resembles *A. evaginata* **sp. nov.**, mainly by general shape of the preanal appendage and the apex of segment IX in lateral view . However, the inferior appendage of the *A. quinteiroi* **sp. nov.** is thinner than that of *A. evaginata* **sp. nov.** in lateral view. The distal margin of the segment IX in the middle region is rounded in *A. quinteiroi* **sp. nov.** which in *A. evaginata* **sp. nov.** is slightly evaginated in dorsal view. The media region of baso mensal projection of the inferior appendage of the *A. quinteiroi* **sp. nov.** is longer than the *A. evaginata* **sp. nov.** in ventral view.

Description

Adult male. Forewing length 5.15–6.12 mm (n= 4); hind wing length 4.14–4.9 mm (n= 4). Head, color yellowish dorsally with yellow setae, midcranial sulcus absent (Fig. 3A–H), anteromesal setal warts rounded, anterior setal wart small and rounded; posterior setal warts rounded (Fig. 3B). Antennae, scape slightly constrict basally, setose dorsally (Fig. 2B), with an anterolateral wart (Figs. 3C–D); pedicel rounded, evaginated basodorsally, with thin setae (Figs. 3–-E); flagellum filiform with one to three small openings (Fig. 3F). Maxillary palps yellowish with yellow setae in alcohol (Fig. 3G). Front lateral setal warts sub-rectangular (Fig. 3H). Thorax (Figs. 4A–H), pronotum yellowish in alcohol (Fig. 3A);

pronotal setal warts with yellow setae (Fig. 4B). Mesoscutum and metanotum yellowish with yellow setae. Legs yellowish and setose (Figs. 4C–D); tibial spur formula 1,2,2 (Figs. 4C–E); tibia recovery for striated setae and medially enlarged, some setae with jagged edge (Figs. 4F–H); a pair of well developed tarsal claws (Fig. 4H). Forewing with 14 dark brown spots; veins Sc and R_1, slightly thickened; crossveins *sc-r*, r_1-r_2, *r-m* and *m-cu* present; forks I and V present (Fig. 8A). Hind wing vein Sc thickened and Cu1A slightly thickened; vein R_1 fused in the vein Sc before the margin; crossvein *r-m* present; forks I and V present (Fig. 8B).

Male abdomen. Segments I to VIII translucid; segment IX rounded in the middle region in dorsal view (Fig 8D); pointed in ventral region, projected dorsally in lateral view (Fig. 8C); process of segment IX narrow in middle region, apex broad and acute in dorsal view (Fig. 8D); narrow medially, apex broad and pointed ventrally in lateral view (Fig. 8C). Segment X enlarged basally, apex rounded in dorsal view (Fig. 8D); triangular, broad in basal region, acute in the apex in lateral view (Fig. 8C). Preanal appendage setose, digited in dorsal view (Fig. 8D); setose, subtriagular in lateral view (Fig. 8C). Inferior appendage uniformly enlarged, apex acute in lateral view (Fig. 8C); digited, ventral plate broad, medial projection long and narrow in lateral view (Fig. 8F). Phallic apparatus narrow, paramere long and narrow, three setae in the apex, phallobase well developed, phallotremal sclerite rounded in lateral view (Fig. 8E).

Etymology

The species name *quinteiroi* is in honor of Prof. Dr. Fábio B. Quinteiro, who has been instrumental in contributing to the knowledge of Neotropical caddisflies.

Distribution

Brazil (Acre state) (Fig. 1).

Remarks

According to the phylogenetic analysis under EW, *A. quinteiroi* **sp. nov.** appears in the third cladogenesis as a sister group of the clade (*A robusta* **sp. nov.** + (*A. serrata* **sp.nov. +** *A. bifurcata* **sp. nov.**)) sharing the synapomorphy not exclusive characters 25[1] segment X with basal region broad in lateral view. However, the synapomorphy not exclusive characters 13 [0] is present in the referred clade and absent in *A. quinteiroi* **sp. nov.** In IW analysis, *A. quinteiroi* **sp. nov.** appears in a polytomy in the second cladogenesis with *A. robusta* **sp. nov.** and the clade formed by (*A. evaginata* **sp. nov. +** (*A. serrata* **sp. nov. +** *A. bifurcata* **sp. nov.**).

***Achoropsyche robusta* sp. nov.**

(Fig. 9)

Material examined

Holotype

♂ (alcohol, MZSP). **BRAZIL: Mato Grosso,** Ribeirão Cascalheira, Faz. Campina Verde, Rio Suiamissu, 12°56’58.9’’S, 51°49’29.1’’W, el. 364 m, 28.xi.2006, UV Light Pan trap, A. R. Calor, R. Silva & S. Mateus.

Paratypes

Same data as holotype, except 3 ♂ (alcohol, MZSP); same data, except 10 ♂ (alcohol, UFBA).

Diagnosis

This species differs from its congeners by the following characters: ten dark brown spots in the forewing; phallic apparatus long, apical phallicata twice longer than paramere in lateral

view; paramere robust with three setae in the apex in lateral view; inferior appendage very robust in ventral view; segment IX process constrict in the apex in dorsal view. *Achoropsyche robusta* **sp. nov**. resembles *A. evaginata* **sp. nov.**, mainly by the spots in the forewing, the general shape of the preanal appendage in dorsal view and the general shape of inferior in lateral view. However, these two species differ by the shape of the segment IX in dorsal view, distal margin subquadrate in the middle region in *A. robusta* **sp. nov.**, while there is a small evagination in *A. evaginata* **sp. nov.**. Additionally, the phallic paramere in *A. robusta* **sp. nov.** is more enlarged than that in species *A. evaginata* **sp. nov.** in lateral view.

Description

Adult male. Forewing length 5.47–6.12 mm (n= 8); hind wing length 3.69–4.16 mm (n= 8). Head, color yellowish dorsally with yellow setae, midcranial sulcus absent, anteromesal setal warts rounded, anterior setal wart small and rounded; posterior setal warts rounded. Antennae, scape slightly constrict basally, setose dorsally, with an anterolateral wart; pedicel rounded, evaginated basodorsally, with thin setae; flagellum filiform with one to three small openings. Maxillary palps yellowish with yellow setae (in alcohol). Thorax, pronotum yellowish, pronotal setal warts with yellow setae; mesoscutum and metanotum yellowish with yellow setae. Legs yellowish; tibial spur formula 1,2,2. Forewing with ten dark brown spots; vein Sc thickened; crossveins *sc-r*, r_1-r_2, *r-m* and *m-cu* present; forks I and V present (Fig. 9A). Hind wing vein Sc thickened; crossvein *r-m* present; vein R_1 fused in the vein Sc before the margin; crossvein *r-m* present; forks I and V present (Fig. 8B).

Male abdomen. segments I to VIII translucid in alcohol. Segment IX subquadrate medially in dorsal view (Fig. 9D); margin sinuous in ventral region in lateral view (Fig. 9C); process of segment IX constrict in the apex in dorsal view (Fig. 9D), uniformly enlarged, apex pointed in the ventral region in lateral view (Fig. 9C). Segment X enlarged in basal region,

apex slightly acute in dorsal region (Fig. 9D); broad in basal region, abruptly acute in the apex in lateral view (Fig. 9C). Preanal appendage digited in dorsal view (Fig. 9D); setose, rounded in lateral view (Fig. 9C). Inferior appendage enlarged in middle region, apex digited in lateral view (Fig. 9C); very robust, ventral plate enlarged in the middle region in ventral view (Fig. 9F). Phallic apparatus long, apical phallicata 2x longer than paramere; paramere robust with 3 setae in the apex, phallotremal sclerite rounded in lateral view (Fig. 9E).

Etymology

Derived from Latin, *robustus* means robust in reference to inferior appendage very robust in ventral view.

Distribution

Brazil (Mato Grosso state) (Fig. 1).

Remarks

Achoropsyche robusta **sp. nov.**, appear as a sister group of the clade (*A. serrata* **sp. nov.** + *A. bifurcata* **sp. nov.**) in the phylogenetic analysis under equal weight. According to the phylogenetic analysis under implicit weight, this species appears in the polytomy with *A. quinteiroi* **sp. nov.** and the clade (*A. evaginata* **sp. nov.** + (*A. bifurcata* **sp. nov.** + *A. serrata* **sp. nov.**). In both analyses, *A. robusta* **sp. nov.** present the character 22 [2], segment IX, margin distal in dorsal view subquadrate as an autapomorphy.

***Achoropsyche serrata* sp. nov.**

(Fig. 10)

Material examined

Holotype

♂ (pinned, USMP). **ECUADOR: Orellana**, Reserva de Biodiversidad, Tiputini, river slough, numa trail, 00.63954°S, 76.14836° W, el. 260 m, 23.x.2011, Holzenthal & Rios.

Paratypes

Same data as holotype, except 4 ♂ (pinned, USMP).

Diagnosis

This species differs from its congeners by the following characters: segment X enlarged basally with lateral margin sinuous in dorsal view, and with a strong constriction in the ventral margin basally in lateral view, inferior appendage long and narrow, and bearing a basodorsal process with a row of denticles in the ventral margin apically in lateral view. *Achoropsyche serrata* **sp**. **nov.** resembles *A. quinteiroi* **sp. nov.**, mainly by general shape of the first segment of the preanal appendage in lateral view, the apex of process of segment IX and the preanal appendage. However, the inferior appendage of the *A. serrata* **sp. nov.** presents in the dorso-basal process with a row of denticles in the ventral margin apically, absent in *A. quinteiroi* **sp. nov.**. The medial process of the inferior appendage, in ventral view, of *A. quinteiroi* **sp. nov.** is shorter than that of *A. serrata* **sp. nov.**.

Description

Forewing length mm (n= 5); hind wing length mm (n= 5). Head, color yellowish dorsally with yellow setae, midcranial sulcus absent, anteromesal setal warts rounded, anterior setal wart small and rounded; posterior setal warts rounded. Antennae, scape slightly constrict basally, setose dorsally, with an anterolateral wart; pedicel rounded, evaginated basodorsally with thin setae; flagellum filiform with one to three small openings. Maxillary palps yellowish with yellow setae (in alcohol). Thorax, pronotum yellowish, pronotal setal

warts with yellow setae; mesoscutum and metanotum yellowish with yellow setae. Legs pale yellowish; tibial spur formula 1,2,2. Forewing with 11 dark brown spots; vein R_1 slightly thickened; crossveins *r_1-r_2*, *r-m* and *m-cu* present; forks I and V present (Fig. 10A). Hind wing veins Sc thickened and Cu1A slight thickened; vein R_1 parallel (no fused) to vein Sc; crossvein *r-m* present; forks I and V present (Fig. 10B).

Male abdomen. Segments I to VIII yellowish. Segment IX sinuous in middle region in dorsal view (Fig 10D); pointed in ventral region, little projected dorsally in lateral view (Fig. 10C). Process of segment IX narrow in middle region, apex broad and acute in dorsal view (Fig. 10D); uniformly enlarged, apex pointed ventrally in lateral view (Fig. 10C). Segment X strongly enlarged basally, lateral margin sinuous medially, apex rounded in dorsal view (Fig. 10D); triangular, strongly constricted in the ventral margin in the basal region, acute in apex in lateral view (Fig. 10C). Preanal appendage setose, rounded in dorsal view (Fig. 10D); setose, subtriagular in lateral view (Fig. 10C). Inferior appendage almost uniformly enlarged, apex acute, basodorsal process with a row of denticles in the ventral margin apically in lateral view (Fig. 10C); dorso-basal process with a row of denticles in the internal margin apically, ventral plate broad, medial projection long and narrow in ventral view (Fig. 10F). Phallic apparatus narrow, paramere long and narrow, three setae in the apex, phallobase well developed, phallotremal sclerite rounded in lateral view (Fig. 10E).

Etymology

Derived from Latin, *serratis* mean serrated in refer to inferior appendage with dorso-basal process with a row of denticles in the ventral margin apically.

Distribution

Ecuador (Orellana province) (Fig. 1).

Remarks

For taxonomic remarks see *Achoropsyche bifurcata* **n. sp.**.

Identification key to males of *Achoropsyche*

1. Forewing with thyridial cell equal to the discoidal cell in length (fig. 3A of Holzenthal, 1984) ... *A. duodecimpucatata*

— Forewing with thyridial cell longer than the discoidal cell in length (Fig. 6A) ... 2

2. (1) Inferior appendage very robust in ventral view (Fig. 9F); segment IX with distal margin subquadrate mesally (Fig. 9D) ... *A. robusta* **sp. nov.**

— Inferior appendage not robust in ventral view (Fig. 7F); segment IX with distal margin not subquadrate mesally (Fig. 6D) ... 3

3. (2) Distal margin of segment IX slightly evaginated and slight sinuous medially (Fig 7D) ... *A. evaginata* **sp. nov.**

— Distal margin of segment IX not evaginated medially (Fig. 9D) ... 4

4. (3) Forewing with 14 dark brown spots (Fig. 8A); segment IX with distal margin rounded medially (Fig. 8D) ... *A. quinteiroi* **sp. nov.**

— Forewing 13 or less dark brown spots (Fig. 7B); segment IX with distal margin not rounded medially in dorsal view (Fig. 7D) ... 5

5. (4) Inferior appendage with dorsobasal process with a row of denticles in the ventral margin apically in lateral view (Fig. 10C); segment IX with distal margin not bifid medially (Fig. 10D) ... *A. serrata* **sp. nov.**

— Inferior appendage without dorsobasal process with a row of denticles in the ventral margin apically in lateral view (Fig 6C); segment IX with distal margin bifid medially (Fig 6D) ... *A. bifurcata* **sp. nov.**

DISCUSSION

The monophy of *Achoropsyche* was corroborated with well supported value and six characters. Four of them are synapomorphies: character 19 [1], proto-tibia with one apical spurs; character 23 [1], segment IX with a triangular process extending to segment X in lateral view; character 29 [1], inferior appendage with a keel-like baso-mesal projection; and character 30 [1], inferior appendage with basodorsal process present (Fig. 2). Additionally, two characters of the genus appear as homoplastics: character 10 [1], forewing with dark brown spot; and character 17 [1], hind wing with veins Sc-R_1 fused before the margin. The character 10 [1] is also present in *Oecetis punctipennis*, while the character 17 [1] is present in *Grumichella rostrata*, member of subfamily Grumichellinae.

Despite the character 17 [1] appearing as a nonexclusive synapomorphy for the genus in both analysis (EW and IW), our phylogenetic results showed a change of state in the clade (*A. serrata* **sp. nov.** + *A. bifurcata* **sp. nov.**). In the referred clade, the character hind wing with veins Sc-R_1 not fused before the margin indicates a possible reversion to plesiomorphic character state in comparison with outgroup.

Achoropsyche duodecimpunctata appears in the first cladogenesis as a sister group of other species of the genus in both analyses. This species presents the discoidal and thyridial cell subequal in length. Differently from the other species of the genus, the thyridial cell is longer than the discoidal cell showing the thyridial cell elongation happened secondarily within the group. In addition, we identify the pattern of nine black spots on the forewing. However, the number of the spots changed and independently evolved over time (for example: *A. evaginata* **sp. nov.** has nine dark spots, while *A. quinteiroi* **sp. nov.** presents 14).

The delimitation of the Neotropical genus *Achoropsyche* is better comprehended with the description of five new species. The description of these new species, and the

morphological study using Scanning Electron Microscope allowed us to observe characters not previously observed (e.g. antero lateral setal and dorso basal evagination with a row of thin setae) and helped us to redefine the diagnosis for the genus. In addition, the phylogenetic study was crucial to understand the relationship between the species, despite the phylogenetics relationship between *A. evaginata* **sp. nov.**, *A. quinteiroi* **sp. nov.** and *A. robusta* **sp. nov.** changed drastically in comparison the analysis under EW and IW. xx

Our results revealed that the genus *Achoropsyche* constitutes a monophyletic group, which emerged in the transition from Upper Cretaceous to Paleocene (Dias *et al.* in prep) and has a wide distribution in tropical South America. After 36 years being a monotypic genus, with the five new species, and new morphological data, including SEM, the circumscription of genus is refined as well its monophyly is corroborated. Therefore, this study contributes to a better understanding of the diversity of species in Neotropical Leptoceridae.

ACKNOWLEDGEMENTS

We would like to thank: the National Council of Technological Scientific Development for the grants (147895/2016 for ESD; 303260/2018-1 for PCB); the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the grand for ESD internship (CAPES-PSDE, number 88881.188558/2018-01) at the University of Minnesota, USA; and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the grant for PCB (number 12/21196-8). We also thank: to Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB, process APP0017/2009 and process 2642/2013), and the Research Program in Biodiversity of Semiarid (PPBio Semiárido/MCTi) for collects financial support; to the Museu de Zoologia da Bahia (UFBA) and University of Minnesota Insect Collection, especially Dr. Ralph Holzenthal and Dr. Roger Blahnik. To Evandro Nakandakari, Faculdade de Ondontologia de Araraquara (FOAR-UNESP), and Dr. Jader

for MEV assistance. We are grateful to the Prof. Dr. Frederico Salles of the Universidade Federal de Viçosa, Msc. Gleyson Desidério, and Instituto de Pesquisa da Amazônia (INPA) for the loan of specimens examined in this study. We highly appreciate the valuable manuscript suggestions of the Prof. Dr. Dalton S. Amorin, Prof. Dr. Allan P. Santos, and Prof. Dr. Fábio B. Quinteiro.

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LEGENDS OF FIGURES

FIGURE 1. Map of distribution of the *Achoropsyche* species. The type species occurrence point of Achoropsyche duodecimpunctata with a circle represents records from literature (Flint, 1972; 1974; 1996; Almeida & Marinoni, 2000; Angrisano & Sganga; 2007; Manzo *et al.* 2014; Moretto and Bispo, 2015; Desidério *et al.* 2017)

FIGURE 2. Parsimony phylogenetic results: (A) under equal weight; (B) under implicit weight. The black circle is characters with consistency index = 1 and white circle is characters with consistency index < 1. Below each branch in blue the number of bootstrap value.

FIGURE 3. Scanning Electron Microscope of the *Achoropsyche quinteiroi* **n. sp.**, head: 1A head, dorsal view; 1B antennae, scape dorsal view; 1C scape and pedicel, lateral view; 1D antennae, scape, pedicel and the first flagellum segment, anterosuperior view; 1E antennae, pedicel dorsal view; 1F antennae, 2nd flagellum segment; 1G maxillary palp; 1H antero lateral setal warts.

FIGURE 4. Scanning Electron Microscope of the *Achoropsyche quinteiroi* **n. sp.**, thorax: 2A meso and metathorax, dorsal view; 2B thorax and legs, lateral view; 2C thorax and legs, ventral view; 2D 1st pair of leg, coxa, trochanter, femur and tibia, ventral view; 2E 2nd pair of legs, spurs, lateral view; 2F 2nd pair of legs, tibia, lateral view; 2G 1st pair of leg, tarso, lateral view; 2H 1st pair of leg, tarsus claw, lateral view.

FIGURE 5. Scanning Electron Microscope of the *Achoropsyche quinteiroi* **n. sp.**, abdomen: 3A abdomen, lateral view; 3B Segments 3, 4 and5, lateral view; 3C opening of the spiracle, lateral view; 3D genitalia, lateral; 3E inferior appendage, lateral view; 3F genitalia, ventral view; 3G genitalia, ventral view; 3H genitalia, dorsal view.

FIGURE 6. *Achoropsyche bifurcata* **n. sp.**, male: 4, right forewing, dorsal; 4B, right hind wing, dorsal; 4C, genitalia, left lateral; 4D, genitalia, dorsal; 4E, Phallic apparatus, left lateral; 4F, genitalia, ventral. Abbreviations: BDP = baso dorsal process of inferior appendage; BMP = baso mensal projection of the inferior appendage; InAP = inferior appendage; Phal = Phallic apparatus; PRM = phallic paramere; PTE = phallotremal sclerite; PAP = preanal appendage; PSIX = process of segment IX; Seg IX = Segment IX; Seg X = Segment X.

FIGURE 7. *Achoropsyche evaginata* **n. sp.**, male: 5A, right forewing, dorsal; 5B, right hind wing, dorsal; 5C, genitalia, left lateral; 5D, genitalia, dorsal; 5E, Phallic apparatus, left lateral; 5F, genitalia, ventral.

FIGURE 8. *Achoropsyche quinteiroi* **n. sp.**, male: 6A, right forewing, dorsal; 6B, right hind wing, dorsal; 6C, genitalia, left lateral; 6D, genitalia, dorsal; 6E, Phallic apparatus, left lateral; 6F, genitalia, ventral.

FIGURE 9. *Achoropsyche robusta* **n. sp.**, male: 7A, right forewing, dorsal; 7B, right hind wing, dorsal; 7C, genitalia, left lateral; 7D, genitalia, dorsal; 7E, Phallic apparatus, left lateral; 7F, genitalia, ventral.

FIGURE 10. *Achoropsyche serrata* **n. sp.**, male: 4A, right forewing, dorsal; 4B, genitalia, left lateral; 4C, genitalia, dorsal; 4D, Phallic apparatus, left lateral; 4E, genitalia, ventral.

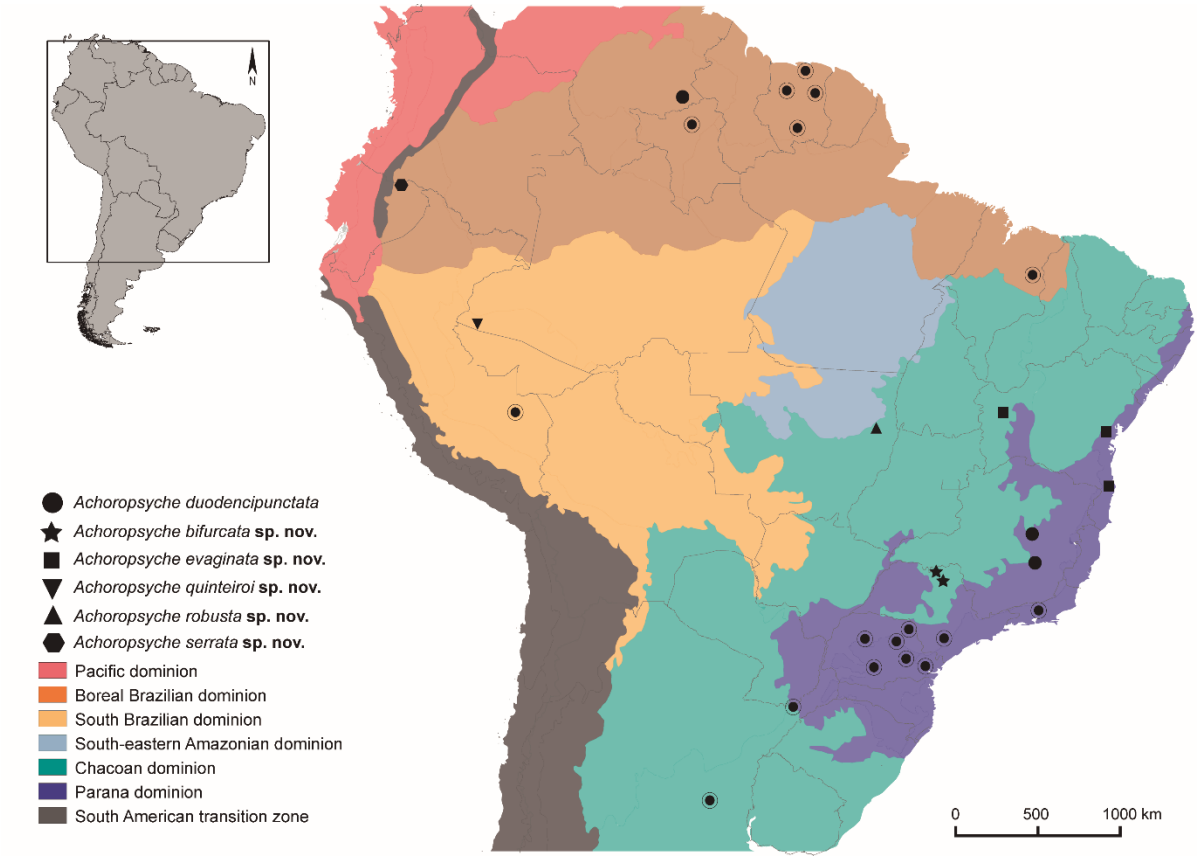

N
Achoropsyche duodencipunctata
Achoropsyche bifurcata sp. nov.
Achoropsyche evaginata sp. nov.
Achoropsyche quinteiroi sp. nov.
Achoropsyche robusta sp. nov.
Achoropsyche serrata sp. nov.
Pacific dominion
Boreal Brazilian dominion
South Brazilian dominion
South-eastern Amazonian dominion
Chacoan dominion
Parana dominion
South American transition zone
0
500
1000 km

Figure 1.

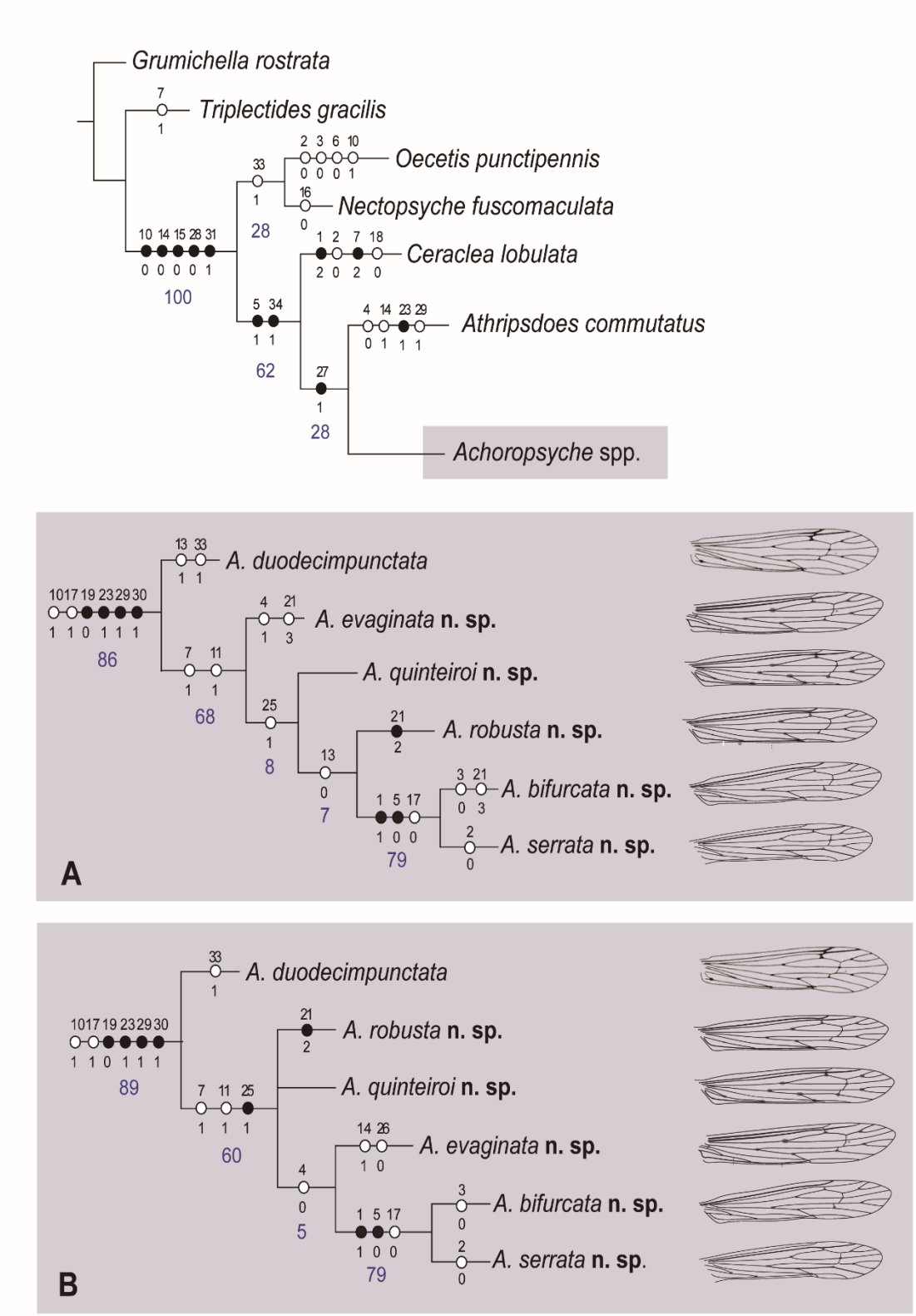
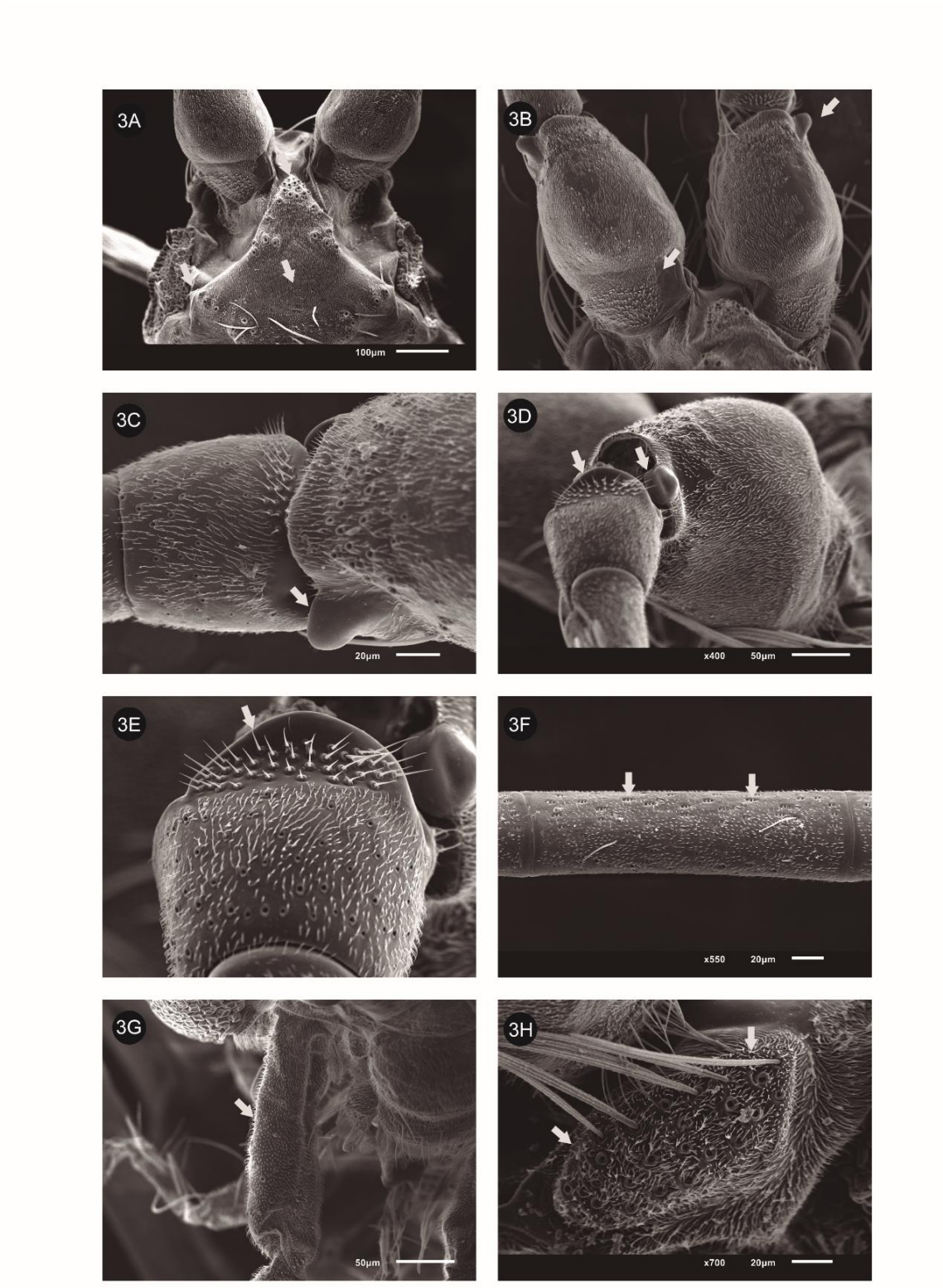

Grumichella rostrata
Triplectides gracilis
Oecetis punctipennis
Nectopsyche fuscomaculata
Ceraclea lobulata
Athripsdoes commutatus
Achoropsyche spp.
A. duodecimpunctata
A. evaginata n. sp.
A. quinteiroi n. sp.
A. robusta n. sp.
A. bifurcata n. sp.
A. serrata n. sp.
A
A. duodecimpunctata
A. robusta n. sp.
A. quinteiroi n. sp.
A. evaginata n. sp.
A. bifurcata n. sp.
A. serrata n. sp.
B

Figure 2.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3B
3C
20µm
3D
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3E
3F
x550
20µm
3G
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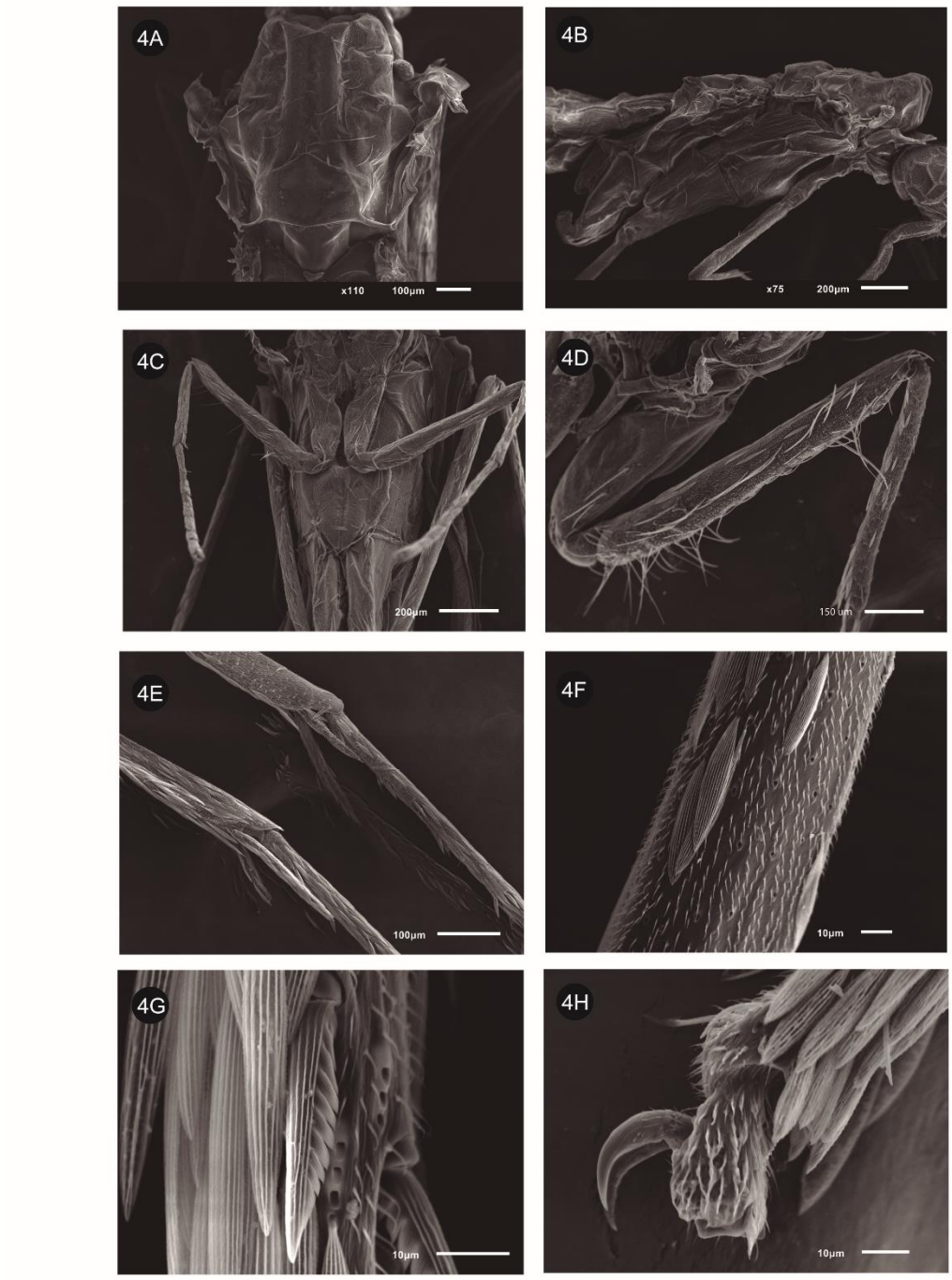
3H
x700
20µm

Figure 3.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x110 100µm
4B
x75 200µm
4C
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4E
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4F
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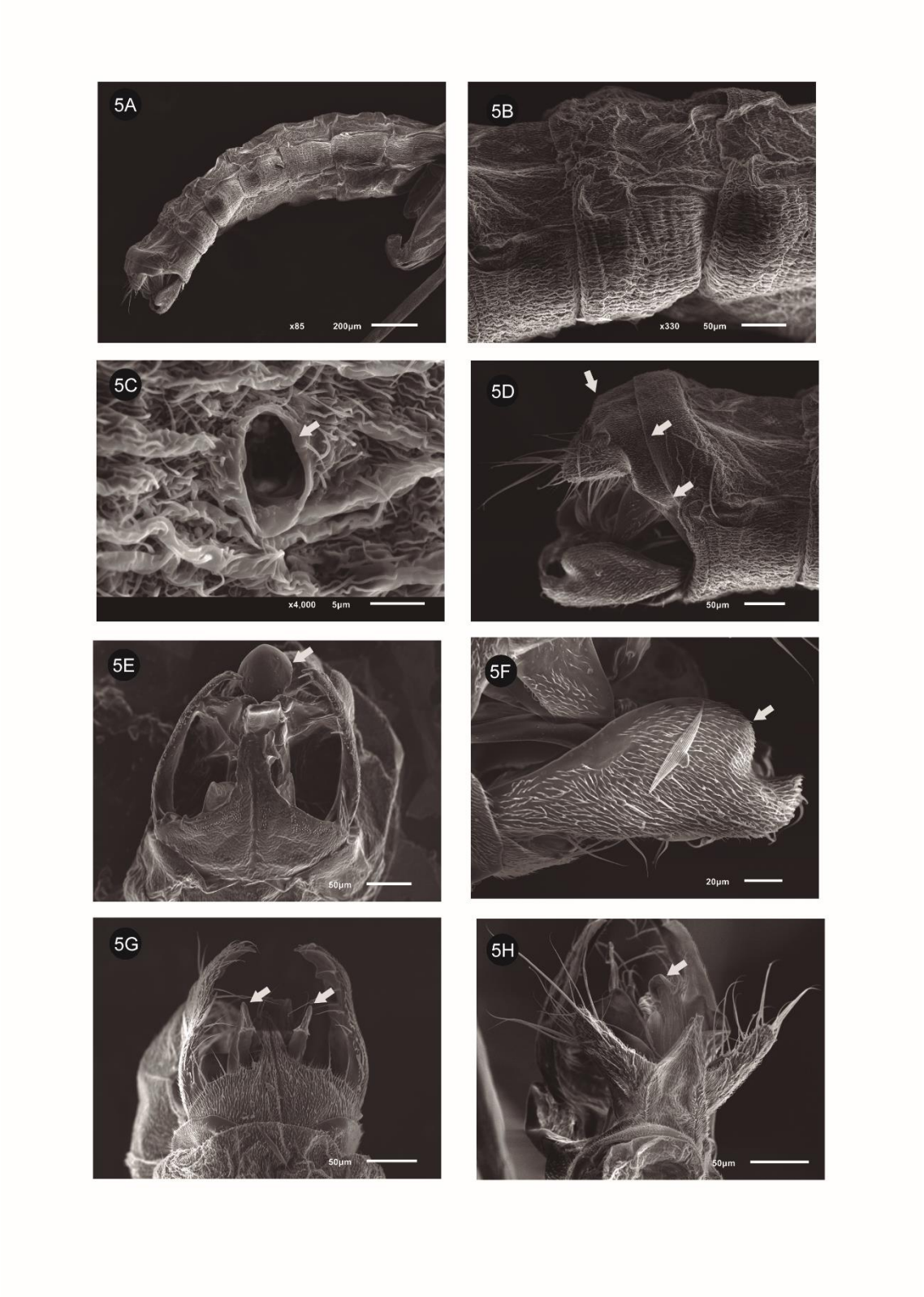
4G
10µm
4H
10µm

Figure 4.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5B
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5C
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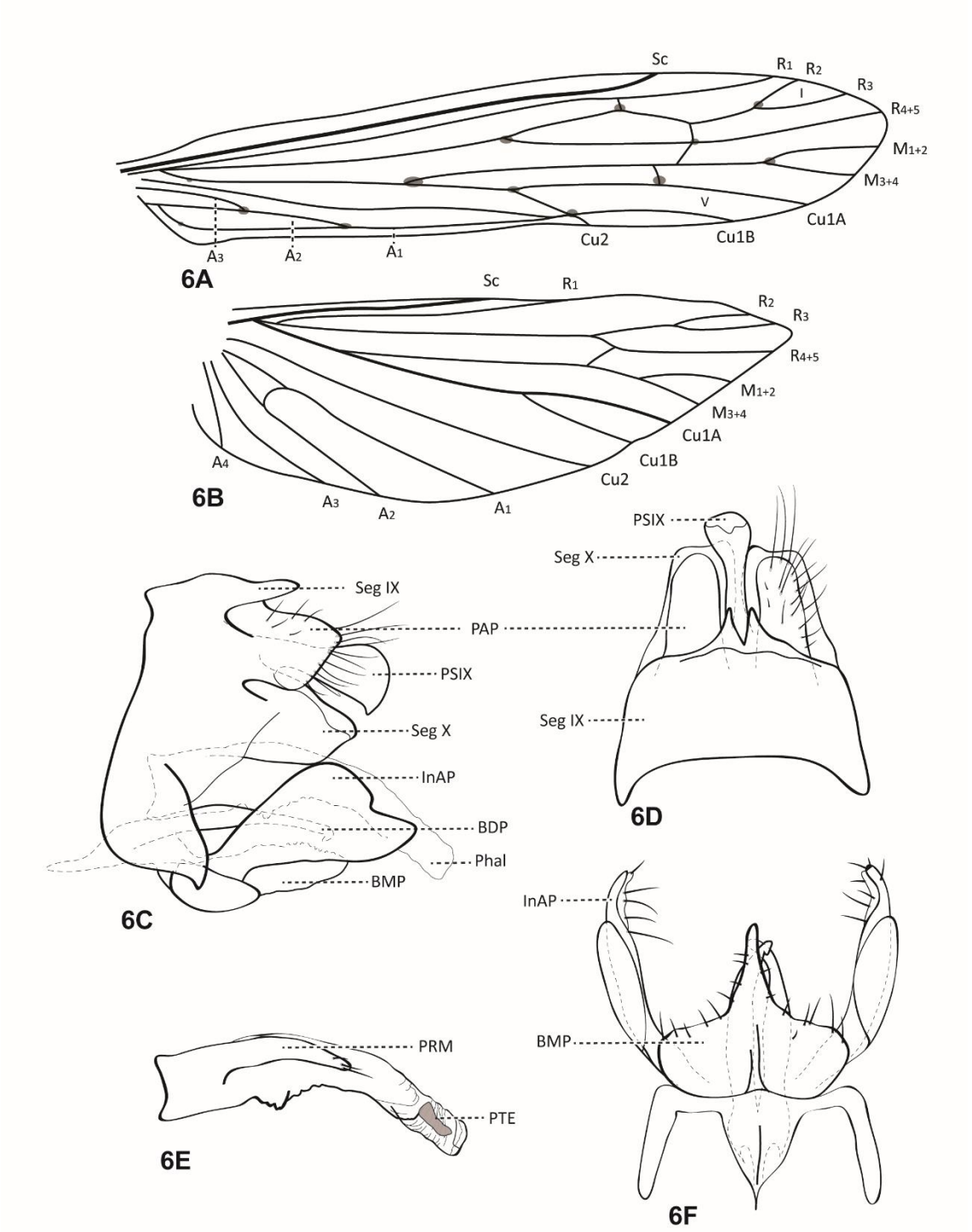
Figure 5.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R1
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R3
I
R4+5
M1+2
M3+4
V
Cu1A
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A3
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A1
6A
Sc
R1
R2
R3
R4+5
M1+2
M3+4
Cu1A
Cu1B
Cu2
A4
A3
A2
A1
6B
Seg IX
PAP
PSIX
Seg X
InAP
BDP
Phal
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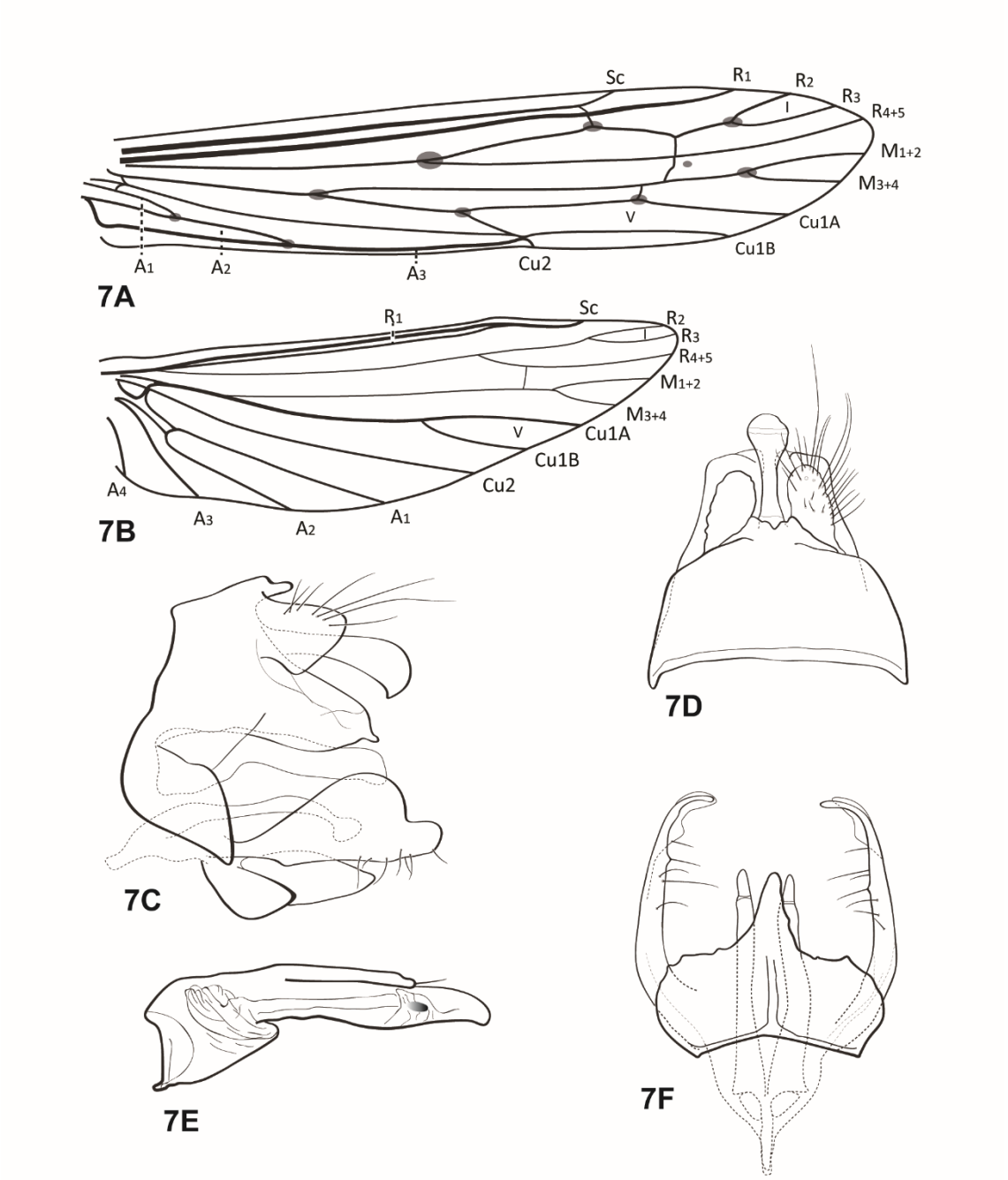
6C
PSIX
Seg X
Seg IX
6D
PRM
PTE
6E
InAP
BMP
6F

Figure 6.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R2
R3
R4+5
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Cu2
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A2
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Sc
R1
R2
R3
R4+5
M1+2
M3+4
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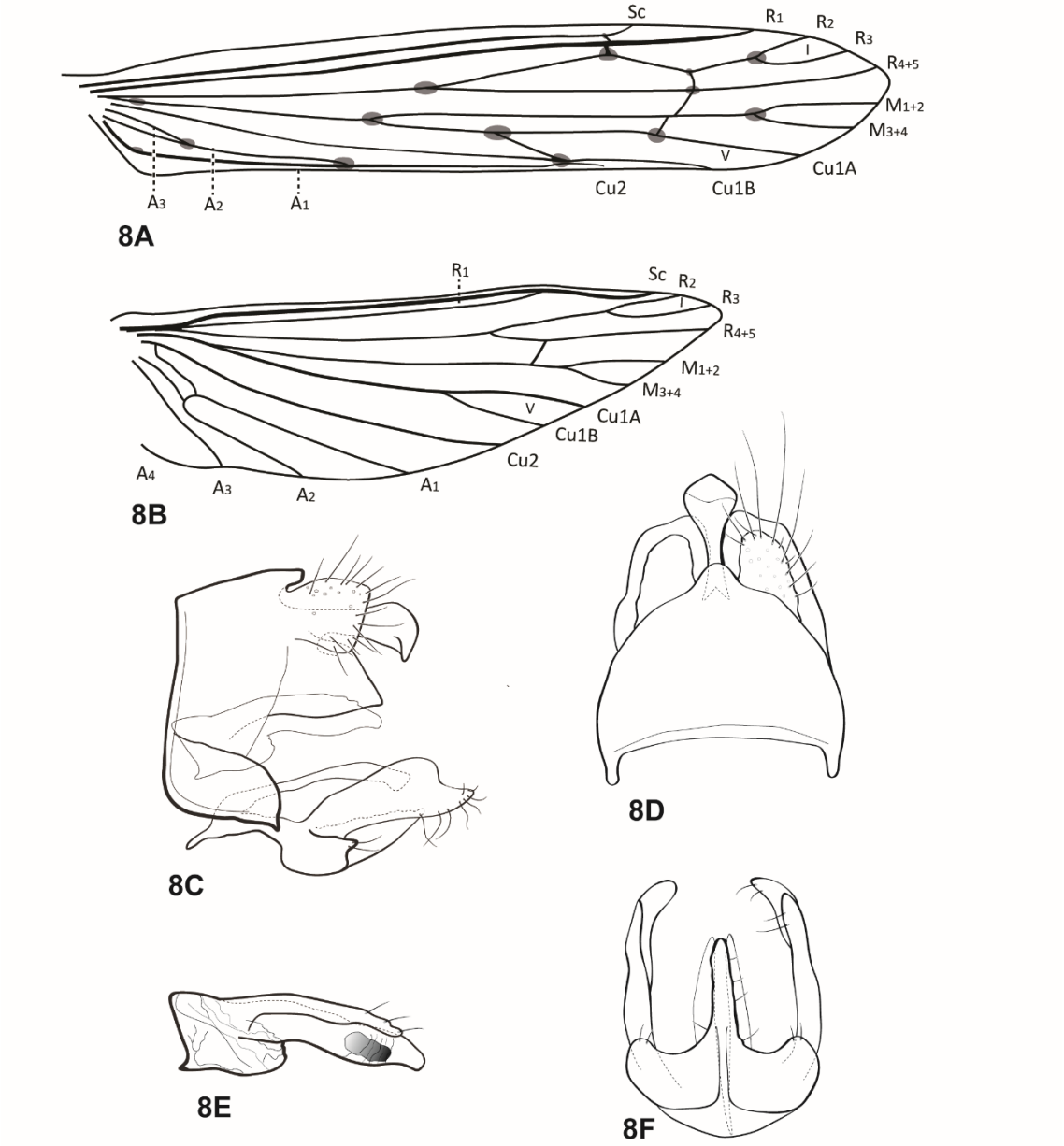
Cu2
A4
A3
A2
A1
7B
7C
7D
7E
7F

Figure 7.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Cu2
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8A
R1
Sc
R2
R3
I
R4+5
M1+2
M3+4
V
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8E
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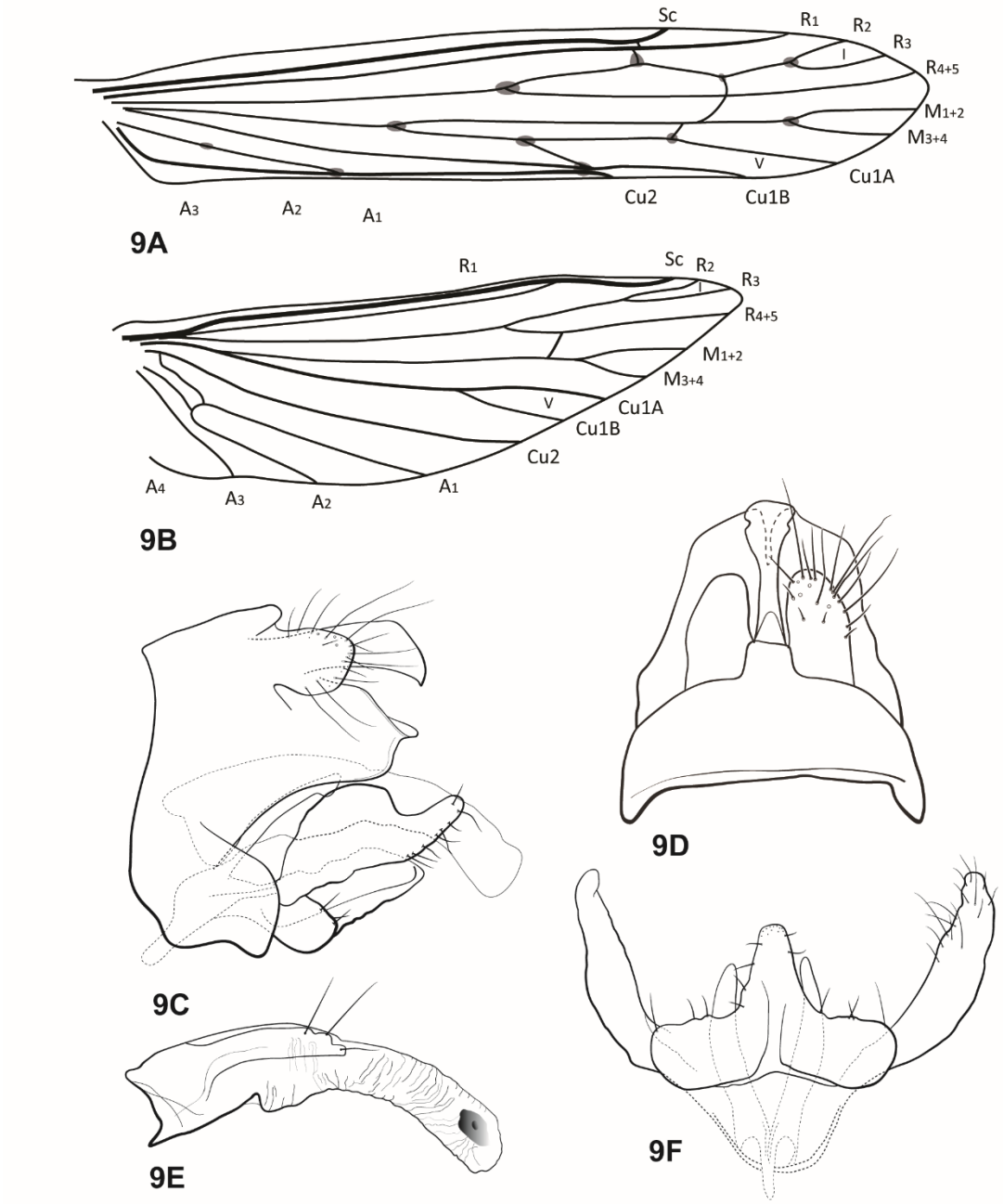
Figure 8.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R1
R2
R3
R4+5
M1+2
M3+4
V
Cu1A
Cu1B
Cu2
A3
A2
A1
9A
R1
Sc
R2
R3
R4+5
M1+2
M3+4
V
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A4
A3
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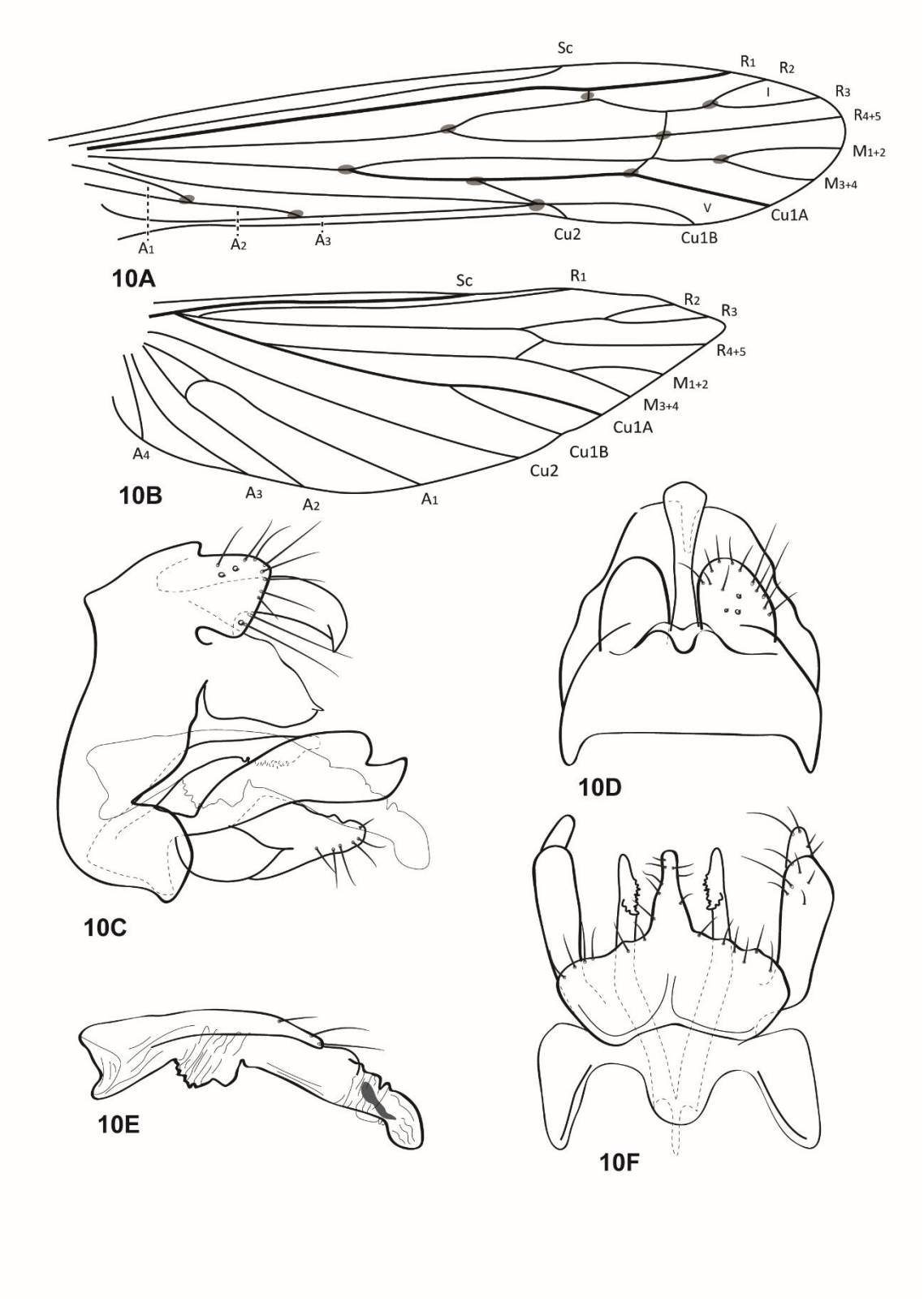
A1
9B
9C
9D
9E
9F

Figure 9.


Sc
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R2
I
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V
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10A
Sc
R1
R2
R3
R4+5
M1+2
M3+4
Cu1A
Cu1B
Cu2
A4
A3
A2
A1
10B
10C
10D
10E
10F

Figure 10.

SUPPLEMENTARY MATERIAL

S1. Characters list, including notes about the characters history

1. Head, anteromesal setal warts in dorsal view: 0 – rounded; 1 – V-shaped; 2 – subtriangular

2. Forewing, vein Sc: 0 – not thickened; 1 – thickened

3. Forewing, vein R_1: 0 – not thickened; 1 – thickened

4. Forewing, thickened of vein R_1: 0 – uniformly thickened; 1 – especially thickened after discal cell

5. Forewing, crossvein *sc-r*: 0 – absent; 1 – present

6. Forewing, crossvein *r*: 0 – absent; 1 – present

7. Forewing, proportional length of thyridial cell/discoidal cell: 0 – subequal; 1 – thyridial cell longer than the discoidal cell

8. Forewing, fork I: 0 – sessile; 1 – petiolate

9 Forewing, fork III: 0 – absent; 1 – present

10. Forewing, dark brown spot: 0 – absent; 1 – present

11. Hind wing, vein Sc: 0 – not thickened; 1 – thickened

12 Hind wing, vein R_1: 0 – not thickened; 1 – thickened

13. Hind wing, fork I: 0 – petiolate; 1 – sessile

14. Hind wing, fork III: 0 – absent; 1 – present

15. Hind wing, sectoral crossvein: 0 – absent; 1 – present

Note. The absence (or loss) of the hind wing sectoral crossvein was proposed as a synapomorphy of Leptocerinae by Morse (1981) and Morse & Holzenthal (1987, character 10).

16. Hind wing, crossvein *r-m*: 0 – absent; 1 – present

17. Hind wing, veins Sc-R_1: 0 – not fused before the margin; 1 – fused before the margin

18. Proto-tibia, apical spurs: 0 – absent; 1 – present

19. Proto-tibia, number of apical spurs: 0 – one spur; 1 – two spurs

Note. The proto-tibia with one apical spur was proposed as synapomorphy of Nectopsychini by Morse (1981).

20. Preanal appendage, shape in dorsal view: 0 – rounded; 1 – triangular; 2 – digitated

21. Segment IX, middle regions of the distal margin in dorsal view: 0 – rounded; 1 – pointed; 2 – subquadrate; 3 – evaginated

22. Segment IX, ventral region in lateral view: 0 – narrow; 1 – broad

23. Segment IX, triangular process extending to segment in lateral view: 0 – absent; 1 – present

24. Segment X, apex in dorsal view: 0 – not bifid; 1 – bifid

25. Segment X, basal region in lateral view: 0 – narrow; 1 – broad

26. Inferior appendage, 1st article in lateral view: 0 – not bifid; 1 – bifid

27. Inferior appendage, length of basoventral lobe: 0 – short; 1– medium; 2 – long

28. Inferior appendage, harpago: 0 – absent (or fusion of vestige); 1 – present

29. Inferior appendage, keel-like baso-mesal projection: 0 – absent; 1 – present

30. Inferior appendage, baso-dorsal process: 0 – absent; 1 – present

31. Apical phallicata: 0 – reduced; 1 – developed

32. Phallic apparatus, parameres: 0 – absent; 1 – present

Note. The loss of phallic parameres was proposed as a synapomorphy of Triplectidinae by Morse (1981) and Morse & Holzenthal (1987, character 13).

33. Phallotremal sclerite, shape in lateral view: 0 – triangular; 1 – digitated; 2 – rounded

S2. Data matrix used in phylogenetic analyses. The symbol "–" refers to non-applicable characters.

Taxa	**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**
Grumichela rostrata	0	1	1	0	1	1	0	1	1	0	0	0	1	1	1	1	1	0	-	2	0	0	0	0	0	-	1	0	0	0	0	0	2
Triplectides gracilis	0	1	1	0	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	2	1	0	1	1	0	-	1	1	0	0	0	0	2
Oecetis punctipennis	0	0	0	-	1	0	0	1	0	1	0	0	0	0	0	1	0	1	1	2	1	0	1	1	0	-	0	-	0	0	1	0	1
Nectopsyche fuscomaculata	0	1	1	0	1	1	0	1	0	0	0	0	1	0	0	0	-	1	1	2	1	0	0	1	0	-	0	-	0	0	1	0	1
Ceraclea lobulata	2	0	1	1	1	1	2	1	0	0	0	0	0	0	0	1	0	0	-	2	0	0	0	0	0	-	0	-	0	0	1	1	2
Athripsodes commutatus	0	1	0	-	1	1	0	1	0	0	0	0	1	0	0	1	0	1	1	1	0	0	1	0	1	0	1	0	0	0	1	1	-
Achoropsyche duodecimpunctata	0	1	1	1	1	1	0	1	0	1	0	0	0	0	0	1	1	1	0	2	0	0	1	0	1	0	0	-	1	1	1	1	1
Achoropsyche robusta **n. sp.**	0	1	1	1	1	1	1	1	0	1	1	0	0	0	0	1	1	1	0	2	2	1	1	1	1	0	0	-	1	1	1	1	2
Achoropsyche evaginata **n. sp.**	0	1	1	0	1	1	1	1	0	1	1	0	1	0	0	1	1	1	0	2	3	1	1	0	1	0	0	-	1	1	1	1	2
Achoropsyche quinteiroi **n. sp.**	0	1	1	1	1	1	1	1	0	1	1	0	1	0	0	1	1	1	0	2	0	1	1	1	1	0	0	-	1	1	1	1	2
Achoropsyche bifurcata **n. sp.**	1	1	0	-	0	1	1	1	0	1	1	0	1	0	0	1	1	1	0	2	3	1	1	1	1	0	0	-	1	1	1	1	2
Achoropsyche serrata **n. sp.**	1	0	1	0	0	1	1	1	0	1	1	0	1	0	0	1	1	1	0	2	0	1	1	1	1	0	0	-	1	1	1	1	2

Final considerations

We are proposing the first dating phylogeny estimating the divergence time to the family Leptoceridae and the first hypothesis of historical biogeography conducted using an objective method (BayArea implemented on BioGeoBears). Our results revealed that the first cladogenesis separated the family in two major lineages ((Leptocerinae + Grumichellinae) + (Triplectidinae + Leptorussinae)). This cladogenesis occurred in *ca*. 130 million years ago (MYA) and is congruent with the Gondwana break up in two blocks: West Gondwana and East Gondwana. The lineages Leptorussinae + Triplectidinae has a possible ancestral distribution in the West Gondwana, while the possible ancestral distribution of Grumichellinae + Leptocerinae is the West Gondwana. In our phylogeny, we included 38 of the 46 Leptoceridae genera, five genera more than Malm & Johanson (2011). This is the first time that the genera *Amazonatolica*, *Amphoropsyche*, *Leptecho*, *Neoathripsodes* and *Russobex* have been included in a phylogenetic proposition for the family.

After 36 years as a monotypic genus, *Achoropsyche* will have six species, five of them are being described here. The description of these new species under a morphological study using Scanning Electron Microscope and a phylogenetic analysis allowed us to study characters not previously observed. These new data allowed us to better understand the diversity and circumscription of the genus, and to propose a morphological phylogeny. Additionally, our study revealed that the genus *Achoropsyche* constitutes a monophyletic group, which emerged around 63 million years old. The results presented in the two chapters allowed us to have a better view on

the diversification process and the diversity of the trichopterans of the Leptoceridae family.

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