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Taxonomia e Filogeografia de *Blepharopus diaphanus* Kolenati, 1859 (Insecta:
Trichoptera: Hydropsychidae: Macronematinae) no Brasil

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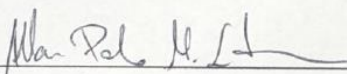
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BRASIL**

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RESUMO

Taxonomia e Filogeografia de *Blepharopus diaphanus* Kolenati, 1859 (Insecta: Trichoptera: Hydropsychidae: Macronematinae) no Brasil

Raquel Almeida Marques

Orientador: Prof. Dr. Allan Paulo Moreira dos Santos

Blepharopus Kolenati, 1859 é um gênero monotípico endêmico da América do Sul. Sua única espécie, *Blepharopus diaphanus* Kolenati, 1859 é registrada na Argentina, Brasil e Venezuela, uma distribuição geográfica ampla. No Brasil, essa espécie ocorre em diferentes biomas, como as florestas tropicais Amazônia e Mata Atlântica, bem como no Cerrado e na Caatinga. Neste estudo, foram utilizadas sequências de um fragmento do gene codificante da citocromo oxidase I (COI) e observadas características de adultos e larvas para analisar sua diversidade morfológica e molecular em sua vasta distribuição no Brasil. 43 sequências de COI foram obtidas para espécimes de *Blepharopus* e 5 sequências foram adquiridas do GenBank®. 58 sequências de outros hidropsíquídeos foram usadas como grupos externos, gerando uma matriz total de 106 sequências e 658 pb. As seguintes análises foram realizadas usando os dados de COI: distâncias par-a-par K2P, neighbor-joining, inferência Bayesiana e dois métodos de delimitação de espécies – Automatic Barcode Gap Discovery (ABGD) e Generalized Mixed Yule Coalescent (GMYC). Além disso, redes de haplótipos foram calculadas usando os biomas, regiões hidrográficas e províncias biogeográficas como unidades geográficas. Os resultados revelaram altas distâncias intraespecíficas entre as sequências de *Blepharopus* (até 22.8%), refletidas na análise de ABGD, que recuperou nove potenciais espécies e um *barcode gap* evidente. As análises de GMYC indicaram sete espécies potenciais. Por outro lado, os grupos definidos com base nas sequências de DNA não foram corroborados por diferenças morfológicas claras em caracteres normalmente utilizados na taxonomia de tricópteros, tais como a venação alar, o padrão de cor e a genitália masculina. Nas análises filogeográficas, não foi observada nenhuma estruturação geográfica para a diversidade genética estudada. Baseando-se nas divergências genéticas, na delimitação de espécies e estudo morfológico, podemos concluir que *Blepharopus diaphanus* representa, de fato, um complexo de espécies crípticas.

Palavras-chave. Insetos aquáticos, DNA barcode, Região Neotropical

ABSTRACT

Taxonomy and phylogeography of *Blepharopus diaphanus* Kolenati, 1859 (Insecta: Trichoptera: Hydropsychidae: Macronematinae) in Brazil

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Blepharopus Kolenati, 1859 is a monotypic genus endemic to the South America. The only known species, *Blepharopus diaphanus* Kolenati, 1859, is recorded from Argentina, Brazil, and Venezuela, a wide geographic distribution. In Brazil, it occurs in different biomes, such as the Amazon and the Atlantic forests, both tropical rainforests, and in Cerrado and Caatinga biomes, both with savanic vegetation. Here, we used sequences of a fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene and observed adult and larval characteristics to study morphological and molecular diversity along vast distribution of *B. diaphanus* in Brazil. 43 COI sequences were generated for specimens of *Blepharopus* and other 5 sequences were gathered from GenBank®. 58 sequences of other hydropsychids were used as outgroups, resulting in a matrix with 106 sequences and 658 bp. K2P pairwise distances, neighbor-joining, Bayesian inference, and two methods for species delimitation: the Automatic Barcode Gap Discovery (ABGD) and the Generalized Mixed Yule Coalescent (GMYC) were performed using the COI dataset. In addition, haplotype networks were calculated using Brazilian biomes, hydrographic regions, and biogeographic provinces as geographic units respectively. The results showed high intraspecific K2P distances among *Blepharopus* sequences (up to 22.8%), reflected in the ABGD analysis, which recovered nine potential species, with a clear barcode gap. GMYC analysis indicated a total of seven potential species. On the other hand, groups based on molecular data were not corroborated by clear morphological differences in characters commonly used in caddisfly taxonomy, such as wing venation, color pattern, and male genitalia. In phylogeographic analysis, it was not observed any geographic structure for the genetic diversity studied. Based on the genetic distances, species delimitation and morphological study we can conclude that *Blepharopus diaphanus* represent, in fact, a complex of cryptic species.

Key words. Caddisfly, aquatic insects, DNA barcode, Neotropics

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INTRODUÇÃO GERAL

Ordem Trichoptera

Os tricópteros compõem a ordem mais diversa dentre os insetos primariamente aquáticos (Neboiss 1991), ocorrendo majoritariamente em ambientes de água-doce, com exceção de poucas espécies marinhas e outras terrestres (Riek 1976). Atualmente, são conhecidas mais de 16.000 espécies no mundo, alocadas em 51 famílias e 618 gêneros (Morse *et al.* 2019). Estão distribuídos em sete regiões biogeográficas, não tendo espécies registradas apenas no continente Antártico. As regiões Oriental e Neotropical detêm juntas mais da metade das espécies conhecidas, esta última registrando mais de 3.000 espécies (De Moor & Ivanov 2008, Morse *et al.* 2019).

A ordem é recuperada como grupo-irmão de Lepidoptera, formando a superordem Amphiesmenoptera, em estudos baseados tanto em caracteres morfológicos quanto moleculares (Wheeler *et al.* 2002, Trautwein *et al.* 2012, Misof *et al.* 2014). Os integrantes desse grupo possuem uma série de sinapomorfias, dentre as quais as asas cobertas por cerdas, modificadas em escamas nas mariposas e borboletas, característica que dá nome à superordem. Além disso, os anfiesmenópteros compartilham a hipofaringe e o pré-lábio fusionados e uma abertura para glândulas produtoras de seda, utilizadas pelas larvas para produzirem casulos, como nos lepidópteros, ou abrigos e redes de captura, no caso dos tricópteros (Kristensen 1984, Holzenthal *et al.* 2007).

Os tricópteros são insetos holometábolos, em que suas formas imaturas (ovo, larva e pupa) vivem em ambientes aquáticos, tanto lóticos quanto lênticos, enquanto o adulto é aéreo-terrestre. Em regiões temperadas, eles geralmente apresentam uma geração por ano (univoltinos) usualmente com período médio de três semanas (Wiggins 1996). Os ovos são normalmente dentro da água e possuem formato esférico ou alongado (Morse 2003).

A fase larval (Fig. 1) constitui a etapa mais longa de vida de um tricóptero, num total de cinco ínstaes. Nesse estágio, o aparelho bucal é bem desenvolvido, as antenas são curtas, alguns grupos possuem brânquias abdominais e no último segmento abdominal está presente um par de falsas pernas anais com garras (Holzenthal *et al.* 2007). A partir das glândulas salivares modificadas é produzida a seda, empregada na construção de abrigos, casas e redes de captura de alimento, junto com detritos minerais, folhas e galhos encontrados no ambiente (Pes *et al.* 2018). A diversificação na utilização da seda pelas larvas de tricópteros permitiu a esses insetos ocuparem diferentes nichos ecológicos

(Mackay & Wiggins 1979). Aqueles que constroem casas portáteis tubulares, mais popularmente conhecidas, apresentam maior eficiência respiratória, através de uma corrente de água constante dentro de seu abrigo promovida por sua ondulação abdominal. Ainda, com a possibilidade de abrigos móveis e redes de captura, as larvas diversificaram seus grupos tróficos, podendo ser predadoras, raspadoras, coletoras ou trituradoras (Cummins 1973, Wiggins 1996).



Figura 1. Larva de *Leptonema* Guérin-Ménéville, 1843 (Hydropsychidae: Macronematinae) Foto:F. Salles.

As pupas são exaratas, possuem escleritos abdominais com ganchos e apesar de alguma semelhança com os adultos, diferem por conta das mandíbulas funcionais e desenvolvidas (Hickin 1967). Os adultos (Fig. 2) compõem a fase de vida mais curta, vivendo associados à vegetação ripária, assemelham-se a pequenas mariposas e a maioria é noturna ou crepuscular. Possuem de 1.5 mm a 4.5 cm e normalmente, com coloração amarronzada, amarelada ou cinzenta, porém algumas espécies apresentam asas cobertas por cerdas e/ou escamas coloridas, formando um padrão distinto. Apesar da pouca duração da fase adulta, em termos taxonômicos, é aquela com maior relevância, uma vez que por meio das genitálias dos machos adultos é que geralmente são identificadas as espécies (Holzenthal & Calor 2017).

Os tricópteros se dividem em duas subordens, consistentemente recuperadas como monofiléticas: Integripalpia e Annulipalpia (Kjer *et al.* 2001, Thomas *et al.* 2020). A maioria dos Annulipalpia constrói abrigos fixos ao substrato usando seda e detritos e/ou outros fragmentos do ambiente, muitas vezes produzindo também redes de captura de alimentos (Fig. 3). Essa rede funciona como um filtro no qual partículas finas ou mais grossas são retidas quando transportadas pela água corrente. Os Integripalpia, por outro lado, fabricam casas tubulares portáteis com seda (Fig. 4), as quais podem ter diversos materiais adicionados, como folhas, grãos minerais, algas etc. Provavelmente essas casas possibilitaram a ocupação de ambientes lênticos, com menores concentrações de oxigênio, à medida que ondulações abdominais geram correntes de água através dos tubos, oxigenando as brânquias (Wiggins 1996, Thomas *et al.* 2020).

Cinco famílias, antigamente classificadas em Spicpalpia, uma terceira subordem, apresentam diferentes tipos de construção de abrigos (Hydroptilidae, Glossosomatidae, Hydrobiosidae, Ptilocolepidae e Rhyacophilidae) e têm posicionamento filogenético ainda incerto. Algumas análises recuperam tais famílias como as primeiras linhagens a divergir dentro da ordem (Malm *et al.* 2013), mas outras análises as recuperam como linhagens dentro de Integripalpia (Kjer *et al.* 2001, Thomas *et al.* 2020), mas em ambos os casos, formando um grupo parafilético.

Os tricópteros possuem extrema importância para os ambientes de água-doce, já que seus imaturos são componentes essenciais do fluxo de energia nesses ecossistemas devido às suas diferentes estratégias alimentares e sua abundância (Morse *et al.* 2019). Eles podem processar grande parte dos recursos do ambiente, deixando-os disponíveis para outros seres, além dos próprios servirem de alimento para animais maiores (Morse 2003). Além disso, devido à diversidade, à abundância e à tolerância diferenciada das espécies à poluição ou outros impactos antrópicos, esses insetos são comumente usados em estudos de biomonitoramento da qualidade da água (Lenat 1993). Como a poluição de rios e córregos vem diminuindo a disponibilidade de água-doce para o consumo humano e a fauna desses ambientes, o uso de larvas de tricópteros, assim como de outros macroinvertebrados, é uma técnica fundamental que deveria ser aprimorada e mais explorada na região Neotropical (Bonada *et al.* 2006). Os imaturos também podem ser potencialmente usados, inclusive, para monitorar poluição por radioatividade após acidentes nucleares (Ueno *et al.* 2018) e para auxiliar em casos forenses de determinação do período pós-morte (Wallace *et al.* 2008, Morse *et al.* 2019).



Figura 2. Adulto de *Marilia* Müller, 1880 (Odontoceridae) Foto: A. Alves.

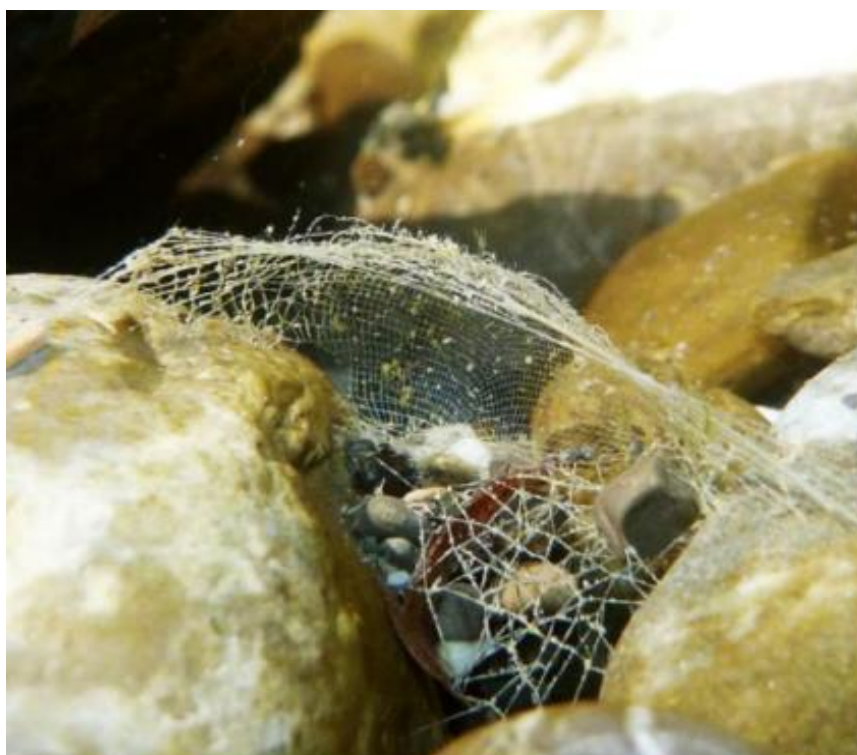


Figura 3. Rede de captura e abrigo de *Hydropsyche* Curtis, 1835 (Hydropsychidae: Hydropsychinae) Foto: W. Graf (Morse *et al.* 2019).



Figura 4. Casa tubular portátil de *Grumichella* Müller, 1879 (Leptoceridae: Grumichellinae) Foto: F. Salles.

Família Hydropsychidae e Subfamília Macronematinae

Inserida na subordem Annulipalpia, a família Hydropsychidae é uma das mais diversas da ordem com mais de 1.900 espécies descritas, as quais estão distribuídas em todas as regiões biogeográficas, exceto a Antártica (Morse *et al.* 2019). Os integrantes da família normalmente vivem em ambientes de água corrente, já que utilizam redes de captura para filtrar seu alimento (Holzenthal & Calor 2017).

Os espécimes adultos da família Hydropsychidae não possuem ocelos, o mesoescutelo ocupa quase a metade do mesoescutro e este não possui verrugas setais. O quinto artigo do palpo maxilar é, normalmente, mais comprido que todos os outros artigos juntos (Neboiss 1991). As larvas possuem a parte dorsal dos segmentos torácicos esclerosadas, brânquias na parte ventral e ventrolateral do abdômen e do meso- e metanoto, além de um tufo de longas cerdas em cada falsa perna anal. As dimensões da malha das redes de captura variam de um gênero para o outro, potencializando ainda mais a especialização dos grupos tróficos (Wiggins 1996).

Os hidropsíquídeos estão divididos em cinco subfamílias: Arctopsychinae, Macronematinae, Hydropsychinae, Diplectroninae e Smicrideinae. Os Arctopsychinae não possuem espécies registradas no Neotrópico, enquanto Hydropsychinae e

Diplectroninae são pouco representados nessa região. Dentre essas, Macronematinae e Smicrideinae possuem a maior riqueza de gêneros e espécies na Região Neotropical, assim como muitos endemismos para essa região (Holzenthal & Calor 2017). Macronematinae atualmente possui 16 gêneros, 8 deles presentes na Região Neotropical: *Blepharopus* Kolenati, 1859, *Centromacronema* Ulmer, 1905, *Leptonema* Guérin-Ménéville, 1843, *Macronema* Pictet, 1836, *Macrostemum* Kolenati, 1859, *Plectromacronema* Ulmer, 1905, *Pseudomacronema* Ulmer, 1905 e *Synoestropsis* Ulmer, 1906.

A distinção entre os gêneros dentro de Macronematinae é muitas vezes difícil, sendo ainda mais complicada a distinção entre as espécies de alguns gêneros. Isso decorre, na maioria dos casos pela homogeneidade na morfologia geral da genitália masculina, principal fonte de caracteres para delimitação taxonômica na ordem. Assim, muitas vezes espécies próximas apresentam apenas variações sutis na sua morfologia. Nesse contexto, a adição de características além da genitália masculina, como por exemplo a coloração e a venação das asas é fundamental (Ulmer 1907).

Gênero *Blepharopus* Kolenati, 1859

Blepharopus é um gênero monotípico endêmico da América do Sul, no qual se insere a espécie *Blepharopus diaphanus* Kolenati, 1859. Essa espécie é registrada, até o momento, na Argentina, na Venezuela e no Brasil (para os estados do Acre, do Amazonas, do Pará, de Roraima, da Bahia, do Maranhão, de Pernambuco, do Piauí, do Mato Grosso, do Espírito Santo, de Minas Gerais, do Rio de Janeiro, de São Paulo, do Paraná, e de Santa Catarina) (Pes *et al.* 2021). Indivíduos de *Blepharopus diaphanus*, assim como outros Macronematinae, são geralmente encontrados em grandes rios de águas correntes (Flint & Wallace 1980).

Kolenati (1859) descreveu a espécie, fornecendo uma breve descrição, com um espécime de localidade desconhecida, mas como o coletor, Beske, vivia e trabalhava em Nova Friburgo (Rio de Janeiro, Brasil), supõe-se que essa seja a localidade-tipo. Ulmer (1905), alguns anos depois, fez uma redescrição de um adulto macho. Nos adultos (Fig. 5) é possível notar, principalmente nos machos, uma quilha protuberante na parte dorsal da cabeça, entre as também proeminentes verrugas setais anteriores e a cabeça e o tórax extremamente cerdosos (nos machos); nas larvas, há a presença de uma carena acima da

área dos olhos, menos desenvolvida posteriormente e o corpo é nu, exceto por algumas cerdas longas dispersas (Ulmer 1905, Flint & Wallace 1980).



Figura 5. Adulto de *Blepharopus diaphanus* Kolenati, 1859 (Hydropsychidae: Macronematinae) Foto: F. Salles.

Uma segunda espécie foi descrita no gênero, *Blepharopus reticulatus* Ulmer, 1905, a partir de um espécime de Santa Catarina, Brasil. O novo táxon foi definido com base em algumas diferenças: na asa anterior, manchas escuras no pterostigma e nas veias transversais da anastomose da célula discoidal para a mediana, a terceira célula apical delimitada por uma veia reta e curta e o limite superior da célula mediana mais espesso, e na asa posterior, a segunda célula apical um pouco mais larga que em *B. diaphanus* e número de esporões tibiais em machos de 1,4,4 (Ulmer 1905). Porém, Ulmer (1907) afirmou que as diferenças na venação não eram constantes e usou o nome *B. reticulatus* como variedade (var.) baseado na alteração de cor das asas. De acordo com o Código Internacional de Nomenclatura Zoológica (ICZN 1999, art. 45.6.4) o uso do termo var., em uma publicação anterior a 1961 equivale ao ranque de subespécie. Flint (1978) então afirmou que tais variações de cor poderiam ser apenas um artefato da conservação do material, de forma que os tipos de *B. diaphanus* deveriam ter apenas perdido a cor com o tempo e propôs a sinonímia entre as duas subespécies.

Blepharopus diaphanus apresenta uma ampla distribuição geográfica, abrangendo biomas e ambientes bastante distintos, tais como áreas de floresta úmida como a Amazônia e a Mata Atlântica e áreas áridas como o Cerrado e a Caatinga (Pes *et al.* 2021). De forma geral, os tricópteros, por serem insetos aquáticos e estarem associados a corpos d'água, geralmente rios e riachos, costumam apresentar uma área geográfica restrita de distribuição, especialmente na região Neotropical, onde é encontrado um elevado grau de endemismo para os tricópteros (Flint *et al.* 1999, De Moor & Ivanov 2008, Holzenthal & Calor 2017). Embora a fase adulta e reprodutiva seja terrestre, nessa fase os indivíduos não se afastam muito dos ambientes dulcícolas, onde necessitam depositar seus ovos após a cópula. Na região Neotropical, poucas espécies exibem um padrão parecido, caso de *Oxyethira tica* Holzenthal & Harris, 1992, sendo registrada do México até o sul do Brasil (Souza 2015) e *Cyrtellus fraternus* Banks, 1905 coletado desde os Estados Unidos até grande parte da América do Sul (Holzenthal & Calor 2017). Contudo, esse padrão pode ser resultado de uma dificuldade em reconhecer espécies muito similares morfolologicamente, mas que possivelmente já se encontram isoladas reprodutivamente.

Técnicas moleculares em estudos taxonômicos

Estudos taxonômicos de Trichoptera, normalmente, utilizam-se da morfologia para a identificação de espécies. É observado, principalmente em diferenciação específica, a genitália masculina, mas também podem ser analisadas outras características, como a venação das asas, a coloração corporal e as verrugas setais. Visando facilitar a identificação e a delimitação de espécies, sobretudo naquelas em que somente a morfologia muitas vezes não é suficiente, a utilização de técnicas moleculares, como o marcador molecular popularizado como DNA *barcode* (fragmento do gene mitocondrial codificante da citocromo oxidase I - COI), vêm aumentando (Hebert *et al.* 2003). Esse fragmento de gene tem sido ainda utilizado para delimitação de espécies animais e estudo de espécies crípticas (Pauls *et al.* 2010).

O marcador COI é muito empregado em estudos de taxonomia molecular em animais, pois além de uma alta taxa de mutação, na maioria das vezes, a variação intraespecífica é menor e distinguível da variação interespecífica (Pauls *et al.* 2010, Zhou *et al.* 2016). Grande parte dos trabalhos com taxonomia molecular em Trichoptera foi realizada com foco em outras regiões biogeográficas que não o neotrópico. Na maioria

dos estudos, espécies de Trichoptera ocorrendo em uma área muito ampla geralmente apresentam divergências genéticas altas e possivelmente sugerem ou correspondem a um complexo de espécies (p. ex. Zhou *et al.* 2007, Pauls *et al.* 2010).

OBJETIVOS

Objetivo Geral

Estudar a diversidade morfológica e molecular da espécie *Blepharopus diaphanus* em relação à distribuição geográfica das populações.

Objetivos específicos

- Estudar a morfologia dos indivíduos amostrados nos diferentes biomas, nas regiões hidrográficas e nas províncias biogeográficas propostas por Morrone *et al.* (2022);
- Estimar a variabilidade genética de um fragmento do gene mitocondrial COI entre as populações de *Blepharopus diaphanus* no Brasil;
- Analisar a estruturação geográfica da variação genética de COI entre os indivíduos de *B. diaphanus* das diferentes populações no Brasil;
- Revisar a taxonomia de *Blepharopus diaphanus*, de forma que os táxons definidos reflitam as variações morfológica e genética encontradas.

DIVULGAÇÃO CIENTÍFICA

Com o objetivo de popularizar o conhecimento científico produzido ao longo do projeto e disseminar a produção científica relativa a ele, foram usadas diferentes estratégias de divulgação científica. Na parte inicial do estudo, foi publicado um resumo em simpósio junto a apresentação oral deste (Marques & Santos 2020). Também foi realizado, a partir do laboratório em que a pesquisa é elaborada, a organização de dois eventos online com intuito da disseminação do conhecimento em insetos (Santos *et al.* 2021a, 2021b).

Além disso, no curso da disciplina de Divulgação Científica Aplicada a Biodiversidade foram formulados um pequeno texto, divulgado no site do Programa de

Pós-graduação (PPGBIO) (Marques 2020a) e do laboratório (LabSIN) (<https://sites.google.com/view/labsin>), e ainda um áudio, reproduzido na 72ª Reunião Anual da SBPC (Marques 2020b). Por fim, um site com mais informações sobre a dissertação foi criado, com intuito de divulgação do andamento e resultados da pesquisa (<https://sites.google.com/edu.unirio.br/blepharopus/p%C3%A1gina-inicial>).

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Capítulo 1 - COI sequences reveal cryptic diversity in the caddisfly *Blepharopus diaphanus* Kolenati, 1859 (Trichoptera: Hydropsychidae: Macronematinae) in Brazil

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Abstract. *Blepharopus* Kolenati, 1859 is a monotypic genus endemic to the South America. The only one species, *Blepharopus diaphanus* Kolenati, 1859, is recorded from Argentina, Brazil, and Venezuela, a wide geographic distribution. In Brazil, it occurs in different biomes, such as Amazon and Atlantic forest, both tropical rainforests, and in the South American savanna, the Cerrado biome. Here, we used sequences of cytochrome oxidase subunit I (COI) and observed adult and larval characteristics to study its morphological and molecular diversity along its vast distribution in Brazil. COI sequences of 43 specimens of *Blepharopus* were obtained and other 5 were gathered from GenBank®. K2P pairwise distances, neighbor-joining, Bayesian inference and two methods of species delimitation (Automatic Barcode Gap Discovery–ABGD and Generalized Mixed Yule Coalescent–GMYC) were performed. The results showed high intraspecific K2P distances among the sequences (up to 22.8%), reflected in the ABGD analysis, which recovered a clear barcode gap. ABGD and GMYC analyses indicated between seven and nine potential species, although these groups were not corroborated by clear morphological differences in characters commonly used in caddisfly taxonomy. In phylogeographic analysis, testing for different geographical data, such as Brazilian

biomes, Brazilian hydrographic regions, and biogeographic provinces, it was not observed any geographic structure for the genetic diversity studied. Based on the genetic distances, species delimitation, and morphological study we can conclude that *Blepharopus diaphanus* represent, in fact, a complex of cryptic species.

Key words. Aquatic insects, DNA barcode, Neotropics, integrative taxonomy, phylogeography, caddisflies.

1.Introduction

Trichoptera Kirby, 1813, also known as caddisflies, are holometabolous insects with aquatic immature stages and terrestrial adults. Caddisfly larvae show a wide range of life-history strategies, and they are found in a large variety of freshwater habitats. Among freshwater animal lineages, Trichoptera is the second largest extant monophyletic group (Malm et al. 2013), and much of this diversity can be attributed to the diversified use of silk and building behavior by immature (Mackay and Wiggins 1979). They are known from all biogeographic regions, except for the Antarctic, and have currently more than 16.000 species (Morse et al. 2019).

Hydropsychidae are the most diverse family among the retreat-making caddisflies and the 3rd largest family in the order, with more than 1.900 described species (Morse et al. 2019). Hydropsychid larvae is typically found in fast-flowing water, where they commonly build capture nets to filter their food (Holzenthal and Calor 2017; Morse et al. 2019). Five subfamilies are currently recognized in Hydropsychidae, among which the Macronematinae Ulmer, 1907 represent a mostly Gondwanan group (Uy et al. 2019).

Blepharopus Kolenati, 1859 is a Macronematinae genus endemic to the South America, with only one known species, *Blepharopus diaphanus* Kolenati, 1859. The species is, so far, recorded from Argentina, Brazil, and Venezuela (Pes et al. 2021). *Blepharopus diaphanus* was originally described by Kolenati (1859), based on a specimen from an unknown locality, but as the collector (Beske) lived and worked in Nova Friburgo (Rio de Janeiro State, Brazil), this is assumed to be the type locality. Ulmer (1905) provided a redescription of the species and proposed an additional species, *Blepharopus reticulatus* Ulmer, 1905, based on a specimen from Santa Catarina State, Brazil. Some differences between these two nominal species indicated by Ulmer (1905) were darker patches on the pterostigma region and on the crossveins of forewings; the tibial spur formula 1-4-4; the second apical cell of hindwings wider in *B. reticulatus* than

in *B. diaphanus*. However, two years later, Ulmer (1907) stated that differences in wing venation and color were not constant, so used the name *B. reticulatus* as a variety (var.). Flint (1978) determined that such color variations could be an artifact of material conservation and the types of *B. diaphanus* must have lost color over time, then proposing the synonymy of the two subspecies.

Blepharopus diaphanus has a wide geographic distribution in South America, including areas of humid forests, such as Atlantic and Amazon forests, and dry areas, such as Caatinga and Cerrado biomes (Pes et al. 2021). Although a wide distribution is known for other Neotropical caddisflies, for example, *Cynellus fraternus* (Banks, 1915), *Marilia flexuosa* Ulmer, 1905, and *Oxyethira tica* Holzenthal & Harris, 1992, this is unusual, since they are aquatic insects usually associated with streams and rivers. Also, their dispersal ability is mostly limited to the adult female flying upstream to compensate the larval drift downstream (Holzenthal and Calor 2017). Thus, this pattern may be more a result of the difficulty in recognizing distinct species that are morphologically very similar than a unique widespread species.

The use of sequences of the mitochondrial cytochrome oxidase I gene (COI) for identification and delimitation of species has become common in taxonomic studies and is helpful when morphological distinction is not clear (e.g., Hebert et al. 2003; Pauls et al. 2010; Zhou et al. 2016). Other researchers working on Trichoptera have usually found a clear distinction between intra- and interspecific divergences when analyzing COI sequences, the so-called barcoding gap (Pauls et al. 2010; Santos et al. 2016). These values of genetic divergence are variable among species, as some may have intraspecific divergence greater than other species. In Santos et al. (2016) the maximum intraspecific divergence found is 4.8% for *Metrichia* Ross, 1938 species (Hydroptilidae) in Brazil, while Barcelos-Silva et al. (2018) found a maximum intraspecific divergence of 7.0% among *Synoestropsis* Ulmer, 1906 species (Hydropsychidae).

Our main objective here was to study the morphological and molecular diversity of *B. diaphanus* along its wide distribution in Brazil. For this, we review the taxonomy of *B. diaphanus* and provide a detailed morphological description. We also used a fragment of COI to estimate the genetic variability and to delimit potential taxonomic units within *B. diaphanus* under two methods of species delimitation: ABGD and GMYC. In addition, we calculated a haplotype network to analyze the relationship between genetic groups and geographic distribution.

2.Methods

2.1. Material examined and morphological study

Adults and larvae identified as *Blepharopus diaphanus* from different collections in Brazil were examined. This material represented a total of 146 specimens and were collected in the following states of Brazil: Bahia, Espírito Santo, Mato Grosso, Minas Gerais, Pará, Paraná, Pernambuco, Piauí, Rio de Janeiro, Rio Grande do Sul, Rondônia, Roraima, and Tocantins (Fig. 1 and Fig. 2). Specimens examined are deposited in the following collections: Coleção do Laboratório de Sistemática de Insetos do Departamento de Zoologia da Universidade Federal do Estado do Rio de Janeiro, Rio de Janeiro (DZO/UNIRIO), Coleção Entomológica Professor José Alfredo Pinheiro Dutra, Departamento de Zoologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro (DZRJ/UFRJ), Coleção Entomológica Pe. Jesus Santiago Moure, Departamento de Zoologia, Setor de Ciências Biológicas, Universidade Federal do Paraná, Curitiba (DZUP/UFPR), Coleção de Invertebrados, Instituto Nacional de Pesquisas da Amazônia, Manaus (INPA), Museu de Zoologia da Universidade Federal da Bahia, Salvador (UFBA), University of Minnesota Insect Collection, St. Paul (UMSP). For comparison and to support taxonomic decisions, photographs of type specimens, from both *B. diaphanus* (3 male from original type series) and *B. reticulatus* (holotype) were also analyzed, gently provided by the Naturhistorisches Museum, Vienna (NMW), and the Museum and Institute of Zoology of the Polish Academy of Sciences, Warsaw (PAN).

General features of adult morphology were studied in detail, such as: coloration pattern, wing length and venation, head keel and setal warts. In addition, as traditional in insect taxonomy, a detailed study of the male genitalia was performed. For this, the abdomen was removed and clarified in a heated KOH solution (10%), allowing a better anatomical observation. The abdomen was observed in glycerin, with a stereomicroscope and/or a microscope. After observation, the abdomen was placed in a microtube with 96% ethanol along with the rest of the body, in the case of specimens in alcohol, or in a microtube with glycerin and pinned together with the rest of the body, in the case of dry specimens. The morphological structures important for the identification of the species and potential variations observed were illustrated through photographs and/or drawings. The drawings were made using a microscope equipped with a camera lucida and then were used as templates to trace graphic vectors in Adobe Illustrator CC 2018 (Adobe Inc.). To add more information about the morphology of this species, a full redescription

of adult is provided here. Because the larva was described in detail by Flint & Wallace (1980), we provided new illustrations, but for a complete description of immatures we indicated seeing Flint & Wallace (1980). Terminology follows that presented by Flint et al. (1987) and França et al. (2013).

2.2. DNA extraction, amplification, sequencing, and alignment

DNA extraction was performed in a non-destructive way, with the Qiagen® kit (DNeasy tissue kit) using one leg of the specimen, based on the protocol recommended by the manufacturer, except for the extension of the period of initial lysis to 48 hours. The DNA was extracted from up to five specimens per location, if available, and the extractions were deposited in a freezer at the DZRJ (UFRJ). Sequences of the mitochondrial cytochrome oxidase I gene (COI) were amplified by polymerase chain reactions (PCR) using primers LCO-1490 paired with HCO-2198 (Folmer et al. 2004) or, alternatively, C1-J-1718 paired with HCO-2198, or CI-J-1751 paired with CI-N-2191 (Simon et al. 1994) (Table 1).

PCR conditions were the following: with primers LCO-1490/HCO-2198 : initial denaturation cycle at 95°C for 2 min; followed by 5 cycles of denaturation at 95°C for 1 min, annealing at 45°C for 1.5 min and extension at 72°C for 1 min; 35 cycles of denaturation at 95°C for 40 s, annealing at 51°C for 1 min and extension at 72°C for 1 min; and final extension cycle at 72°C for 5 min; and for C1-J-1718/HCO-2198 and CI-J-1751/CI-N-2191 primers – initial denaturation cycle at 94°C for 3 min; followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min and extension at 72°C for 2 min; and final extension cycle at 72° for 7 min. PCR products were analyzed in agarose gel (1%) electrophoresis reactions using GelRed to stain the DNA. PCR products with a positive result were then sent to Macrogen Inc. (Seoul, South Korea) for purification and sequencing.

Electropherograms of forward and reverse strands were assembled and manually edited to generate consensus sequences, using the GeneStudio v. 2.0 (GeneStudio, Inc). These were submitted to the BLAST® tool in GenBank® to verify eventual contaminations. In total, 43 COI sequences were generated here for *Blepharopus* and other five sequences were obtained from GenBank®. In addition, 51 COI sequences from other Macronematinae species and from five *Smicridea* species (Hydropsychidae: Smicrideinae) were included as outgroups (Supplementary Material 1).



Figure 1. South America map with localities of the analyzed specimens of *B. diaphanus* distributed on the biogeographic provinces proposed by Morrone et al. (2022).

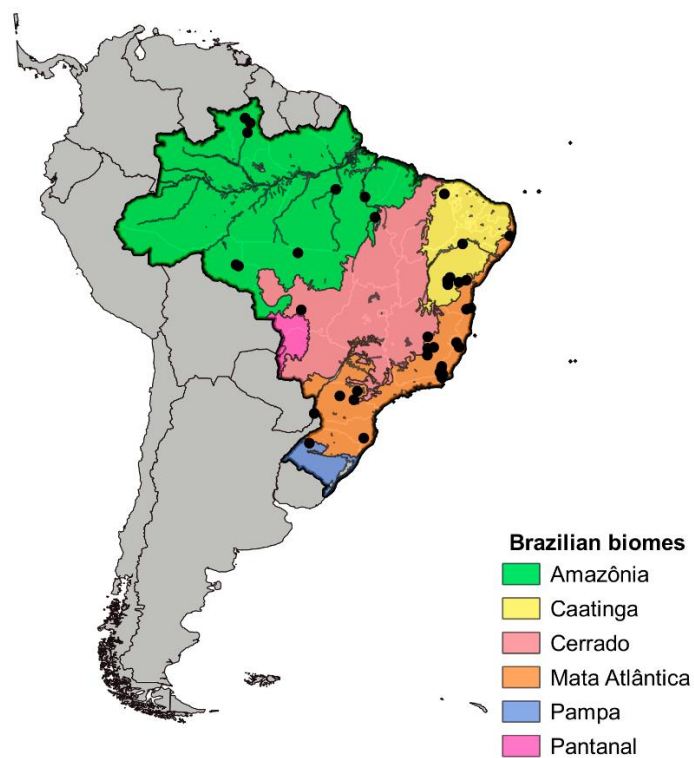


Figure 2. South America map with localities of the analyzed specimens of *B. diaphanus* distributed on the Brazilian biomes.

Table 1. Primers used in the present work and their reference.

Primer	Sequence (5'-3')	Reference
LCO-1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al. 1994
HCO-2198	TAAACTTCAGGGTGACCAAAAATCA	Folmer et al. 1994
C1-J-1718	GGAGGATTTGGAAATTGATTAGTTCC	Simon et al. 1994
CI-J-1751	GGATCACCTGATATAGCATTCCC	Simon et al. 1994
CI-N-2191	CCCGGTAAAATTAAAATATAAACTTC	Simon et al. 1994

The sequences were aligned with MEGA X (Kumar et al. 2018), using ClustalW algorithm with default parameters for pairwise and multiple alignments (gap opening cost of 15 and a gap extension cost of 6.66) and nucleotide sequences were translated into amino acid sequences to ensure amplification of the correct fragment. The final matrix included 106 sequences, of which 48 were from *Blepharopus diaphanus*, and 658 bp long.

2.3. Genetic and phylogenetic analyses

Pairwise genetic distances were calculated using Kimura 2-parameter (K2P) (Kimura 1980) model. Since this model is widely used in molecular taxonomy, we used here in order to compare our results with previous works on caddisfly molecular taxonomy. A neighbor-joining tree was calculated, also using K2P distances, with 500 bootstrap pseudoreplicates. Both analyses were calculated in MEGA X.

For phylogenetic analyses, the most suitable evolutionary model for nucleotide substitution was selected using the Akaike Information Criterion (AIC) (Akaike 1947) in the program jModelTest 2 (Darriba et al. 2012), which resulted in selection of the GTR + I + G model. Bayesian inference analyses were performed with MrBayes 3.2 (Huelsenbeck and Ronquist 2001), via Cypres Science Gateway portal (Miller et al. 2010). It was performed with four independent runs, each with four MCMC chains (Markov chain Monte Carlo) of 10,000,000 generations and sample frequency of 1,000 generations, and with 10% of trees discarded as burnin. Convergence of the chains was examined with Tracer 1.7 (Rambaut et al. 2018). Branch support was estimated by posterior probability (PP) and a 50% majority consensus tree was generated.

BEAST 2.5 (Bouckaert et al. 2019) was used to calculate a calibrated ultrametric tree (Supplementary Material 2) to be used in the GMYC analysis. This tree was obtained using a relaxed uncorrelated log-normal molecular clock (Drummond et al. 2006). The calibration was performed: (1) at the node including all *Macronema* species included,

based on the fossil of *Macronema hispaniola* Wichard, 2007 (15-20 million years ago) (Wichard 2007) with a lognormal distribution with offset=15.0, mu=1.0 and sigma=1.5, to represent probable age older than 47 million years; and (2) in Macronematinae, based on estimated age of the clade by Thomas et al. (2020), with a normal distribution, with mean=116 and standard deviation=1.0, to represent approximately between 114 and 117 million years ago. These analyses were performed for 200,000,000 generations and sampled every 5,000 and, again, Tracer 1.7 was used to assess the convergence of the chains. The results were summarized in a maximum clade credibility (MCC) tree in TreeAnnotator 2.6 (available in the BEAST package), with a burnin of 20%.

2.4. Species delimitation and phylogeographic analyses

ABGD analysis was performed in the online version (<https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html>), using as initial file the alignment only with the sequences of *B. diaphanus*. The settings were defined as Pmin=0.001, Pmax=0.01, steps=20, relative gap width=1.0 and based on a K2P model. GMYC analysis run in SPLITS package (Ezard et al. 2009) within the R platform (R CORE TEAM 2019). The MCC tree from BEAST analysis was used as input and the single-threshold method was applied.

Haplotypes networks were constructed in PopART (Leigh and Bryant 2015) using the Median-joining method (Bandelt et al. 1999), with the exclusion of outgroup sequences. The geographical data used was the biogeographic provinces proposed by Morrone et al. (2022). Maps were created using QGIS 3.20.1 and all figures were edited in Adobe Illustrator CC 2018 (Adobe Inc.).

3. Results

3.1. Taxonomy

Blepharopus Kolenati, 1859

Type species: *Blepharopus diaphanus* Kolenati, 1859; by monotypy.

Blepharopus diaphanus Kolenati, 1859

Type material. Brazil [specific locality unknown; collector Beske lived and worked in the vicinity of Nova Friburgo, Rio de Janeiro state; NMW; ♂].

Blepharopus diaphanus Kolenati, 1859:242 [NMW; ♂]. —Ulmer, 1907c:42 [♂; wings; head]. —Navás, 1932:79 [checklist]. —Fisher, 1963:33 [checklist]. —Flint and

Wallace, 1980:179 [distribution; larva; pupa]. —Flint, 1991:63 [distribution]. —Marinoni and Almeida, 2000:286 [distribution; biology]. —Cohen, 2004:74 [distribution]. —Blahnik et al., 2004:4 [distribution]. —Paprocki et al., 2004:7 [checklist]. —Dumas et al., 2009:357 [distribution]. —Calor, 2011:320 [checklist]. —Nogueira and Cabette, 2011:351 [distribution]. —Barcelos-Silva et al., 2012:1277 [distribution]. —Souza et al., 2013:4 [distribution]. —Costa et al., 2014:218 [distribution]. —Paprocki and França, 2014:22 [checklist]. —Desidério et al., 2017:151 [distribution]. —Uy et al., 2019 [phylogeny]. —Moreno et al., 2020:257 [distribution]

—*reticulatus* Ulmer, 1905a:52 [Type locality: [Brazil], Santa Catharina [sic]; PAN; ♂]. —Ulmer, 1907:43 [♂; as *B. diaphanus* Kolenati (var. *reticulatus* Ulmer)] —Flint, 1978:395, 404 [♂; wings; to synonymy].

Distribution: Argentina, Brazil, and Venezuela.

Remarks. *Blepharopus* is a monotypic genus close related to *Pseudomacronema* Ulmer, 1905 and *Macrostemum* Kolenati, 1859 as recovered in the phylogenetic hypothesis proposed by Uy et al. (2019). *Blepharopus* morphology resembles that of *Macrostemum*, with adults of both genera presenting broad discoidal cell and projecting gland of abdominal sternum V as a long filament. These two genera also have very similar larvae, in both cases with a typical sharp dorsolateral carina and the labrum with lateral brushes. However, they can be distinguished, in the adult, by the keel on the head, more prominent in *Blepharopus*; the different wing patterns, the contrasting dark and light colors in *Macrostemum* and a reticulated pattern in *Blepharopus*, and the tibial spur formula, for *Blepharopus* 0, 4, 4 and for *Macrostemum* 1-2, 4, 4. Diagnostic characters of adult males include the pair of large oval anteroposterior setal warts with a protruding keel between these warts, midleg tibia and tarsus slightly extended and the head and prothorax extremely setose. In larval stages, the head has a low and sharp dorsolateral carina extending almost to posterior region, diverging from the frontoclypeal suture posteriorly.

A complete morphological redescription is provided below for adult male and female. The descriptions and illustrations of adults below are based on specimens from Rio de Janeiro State, since the probable origin of the type defined by Kolenati is Nova Friburgo (Rio de Janeiro State). Small morphological variations observed in specimens from other localities are also mentioned. Additional photographs of larval structures (Fig. 6) are also provided, but since larvae from Rio de Janeiro were not available, we used larval specimens from Rondônia State.

Material. Holotype. BRAZIL • 1 ♂, Santa Catharina [sic], photograph only, PAN [for *B. diaphanus* Kolenati (var. *reticulatus* Ulmer)]. Syntype. BRAZIL • 3 ♂; [specific locality unknown; collector Beske lived and worked in the vicinity of Nova Friburgo, Rio de Janeiro state]; photograph only; NMW. Other material. BRAZIL • 1 ♂, Rio de Janeiro. Trajano de Moraes, Rio Carogango, Cachoeira da Amorosa, S21°22'42.1'', W41°57'44.2'', 90m, 24.iv.2017, LL Dumas, JL Nessimian, LF Duque & JF Barbosa leg., DZRJ • 2 ♀, same data except Conceição de Macabu, Rio Macabu, Poço do Bambuzal, S22°04'18.1'', W41°56'50.8'', 47m, 24.iv.2017, DZRJ • 3 ♀, same data except São Fidélis, Ponte Preta, Rio do Colégio, S21°47'2.8'', W41°50'48'', 314m, 23.x.2016, DZRJ • 1 ♂ 1 ♀, same data except Itaperuna, Rio Carangola, Cachoeira do Galeno, S21°07'23.8'', W41°55'04'', 139m, 07.xii.2018, DZRJ • 3 ♂ 1 ♀, same data except Retiro de Muriaé, Rio Muriaé, Cachoeira da Fumaça, S21°11'3.6'', W41°59'36.3'', 141m, 07.xii.2018, DZRJ • 2 ♀, same data except Santo Antônio de Pádua, Baltazar, Rio Pomba, Cachoeira dos Macacos, S21°35'49.5'', W42°08'34.1'', 75m, 03.xii.2018, DZRJ • 2 ♀, same data except Natividade, estrada Natividade-Purilândia, Fazenda Taboca, Rib. São Sebastião, S21°0'41.6'', W41°57'15'', 198m, 02.xii.2018, DZRJ • 7 ♂ 2 ♀, Roraima, Amajari, Amajari, Estação Ecológica de Maracá, Rio Uraricoera, N03°20'59'', W61°25'22'', 83m, 24.iii.2016, UNIRIO • 2 ♂, same data except Rio Amajari, N03°39'50'', W61°21'34'', 92m, 30.iii.2016, UNIRIO • 3 larvae, same data except Fazenda São Sebastião, S04°02'2.2'', W61°23'11.8'', 231m, 25.iii.2002, AMO Pes, N Hamada, CAS Azevedo e JO da Silva leg., INPA • 3 ♂ 1 ♀, same data except Rio Branco, Cachoeira Bem Querer, 18-21.ii.2002, INPA • 1 ♂, same data except Boa Vista, Rio Caumé, 09.iii.2009, INPA. • 1 ♂, Pará, Tucuruí, Chiqueirão, Rio Tocantins, S04°23'18'', W49°29'27.6'', 69m, 10.iv.1984, BM Mascarenhas, DZRJ • 5 ♂, Espírito Santo, Sooretama, Patrimônio da Lagoa, Lagoa Juparanã, 23.x.2011, INPA • 2 ♂ 1 ♀, same data except Rio São José, S19°07'33.1'', W40°14'26.1'', 24m, 09.v.2010, P. Barcelos-Silva leg., INPA • 1 ♂ 1 ♀, same data except 06 Out 2011, INPA • 1 ♀, same data except Nova Venécia, Santa Rita do Pip Nuck, Rio Cricaré, S18°39'51.4'', W40°30'44.9'', 74m, 25-26.iv.2012, INPA • 1 larvae, Rio Grande do Sul, Bossoroca, Barra do Angico, Rio Piratinim, S28°32'06'', W54°57'29.9'', 112m, 28.vi.2009, AMO Pes, INPA • 3 larvae, same data except 08-09.vii.2012, INPA • 1 ♀, same data except 11-14.ii.2014, INPA • 1 ♂, Pernambuco, Amaraji, Cachoeira de Guariba, 12.v.2013, WRM Souza leg., DZRJ • 1 ♀, Piauí, Piracuruca, PN de Sete

Cidades, alojamento, S04°05'57'', W41°42'34'', 193m, 18.iv.2012, DM Takiya leg., DZRJ • 3 ♂ 2 ♀, Mato Grosso, Alta Floresta, Rio Jurena, Pousada, 20-21.ix.2017, INPA • 2 ♂, Tocantins, Xambioá, Rio Araguaia, S06°22'12.8''W48°30'41.6'', 132m, 07.v.2017, N Hamada, G. Amora leg., INPA • 1 ♂, Paraná, PN do Iguaçu, Rio São João, S25°37'13.5'', W54°28'35.9'', 178m, 07.ix.2012, ALH Oliveira & B Clarkson leg., DZRJ • 1 ♂, same data except Telemaco Borba, Res. Samuel Klabin, 21.i.1988, DZUP • 1 ♂, same data except Jundial do Sul, Fazenda Monte Verde, 21.i.1988, DZUP • 1 ♂, same data except Fênix, 01.x.1986, DZUP • 1 ♀, same data except 19.iii.1986, DZUP • 4 ♂ 1 ♀ 4 larvae, Rondônia, Nova Londrina, Rio Urupá, S11°02'11'', W62°08'42'', 183m, 31.viii-01.ix.2021, GR Desiderio, AMO Pes, JO Silva leg., INPA • 3 ♂ 2 ♀, same data except Presidente Médici, Rio Urupá, INPA • 4 ♂ 1 ♀, Bahia, Rafael Jambeiro, Rio Paraguaçu, S12°33'52.4'', W39°32'24.4'', 26.iii.2012, Quinteiro, Duarte, Garcia leg., UFBA • 3 ♀, same data except Curaçá, S08°59'57.6'', W39°54'47.2'', 347m, 06.v.2011, AM Silva-Neto leg., UFBA • 4 ♂ 1 ♀, same data except Lençóis, Parque Nacional da Chapada Diamantina, Rio Santo, S12°29'57.9'', W41°19'7.52'', 340m, 26.x.2008, Calor, Mariano & Matheus leg., UFBA • 2 ♂, same data except Mucugé, Chapada Diamantina, Rio Cumbuca, S12°59'26'', W41°21'1.0'', 900m, 07.vi.2007, JA Rafael & FF Xavier leg., UFBA • 4 ♂, same data except Andaraí, Parque Nacional da Chapada Diamantina, Cachoeira Garapa, 340m, 25.x.2008, Calor, Mariano & Matheus leg., UFBA • 6 ♂ 2 ♀, Una, Rio Una, S15°17'30'', W39°06'08'', 21m, 07.viii.2016, APM Santos, DM Takiya, PM Souto, AP Pinto leg., UNIRIO • 2 ♀, same data except Camacan, Rio Panelão, S15°24'28'', W39°32'04'', 176m, 12.viii.2016, UNIRIO • 1 ♂, same data except 01.iii.2012, DZRJ • 2 ♀, Nova Redenção, Rio Paraguaçu, Fazenda Moreno, S12°16'48'', W41°09'10'', 316m, 26.vii.2021, Calor, Quinteiro, França, Lecci, Arantes & Camelier leg., UFBA • 4 ♂ 1 ♀, Iaçú, Balneário "O Pote", Rio Paraguaçu, S12°45'14.6'', W40°16'29.5'', 24.iii.2012, Quinteiro, Duarte, Garcia leg., UFBA • 3 ♂, same data except Fazenda Moreno, Rio Paraguaçu, S12°41'11'', W40°07'08'', 15.v.2010, D França, R Burger leg., UFBA • 3 ♂ 2 ♀, Minas Gerais, Rio Preto, rio abaixo do Parque Estadual do Rio Preto, 723m, 28.x.2000, Paprocki & Isaac leg., UMSP • 1 ♂ 4 ♀, same data except Rio Santo Antônio, rio abaixo do Morro do Pilar, 530m, 17.x.2000, Paprocki & Ferreira leg., UMSP • 3 ♂ 2 ♀, same data except Rio Guanhões, rio abaixo da barragem de Salto Grande, 27.viii.1998, Paprocki leg., UMSP • 5 ♂ 1 ♀, same data except Estação Ecológica de Peti, Rio Santa Barbara & Córrego Brucutu, 23.ix.1998, UMSP.

Adult male (Figs. 3A-B; 4A,B,F). Forewing length 11–15 mm (n=7).

Head (Fig. 4A) golden brown, with dense setae. Pair of large oval anteroposterior setal warts covered with long yellow setae and a protruding keel between these warts. Pair of oval dorsalposterior setal warts smaller than anteroposterior pair, covered with long yellow setae. Compound eyes large, ocelli absent. Antennae longer than the forewings, scape golden, setose. Maxillary palps yellowish brown, 5-articulated, article I short, article II slightly longer, article III longer than article I, article IV similar to article III, article V longer and thinner than all others, striated, densely covered with pale setae. Pronotum (Fig. 4B) brown, densely covered with pale setae, meso- and metanotum brown. Legs golden brown, covered with long pale setae, tibial spur formula 0,4,4; midleg tibia and tarsus slightly extended. Forewings (Figs. 3A, 4F) pale yellow with a reticulated pattern of brown maculae along the entire wing, with darker brown patches in the pterostigma region and close to crossveins. Forks I, II, III, IV, and V present, fork I petiolate, discoidal cell (DC) small, median cell (MC) approximately twice as long as DC, thyridial cell long, approximately three times as long as MC, crossveins *r* and *r-m* present. Nygma and thyridium present. Hind wings (Fig. 3B) hyaline, wider than long, vein Sc thickened and contiguous with R1 near apex; forks II, III, and V present, forks III and V petiolate, crossveins *s*, *m-cu*, *r-m* present. Abdominal segment V with a pair of clear, elongated, and thin processes anterolaterally.

Male genitalia (Figs. 4C-E). Segment IX, in ventral view (Fig. 4C), with a median U-shaped incision on posterior margin, rounded in dorsal view, in lateral view (Fig. 4D), with anterior margin slightly sinuous, posterior margin with a row of very long setae and a slight projection. Segment X, in dorsal view (Fig. 4E), bilobed, each lobe with acuminate apex, with short setae; in lateral view, subtriangular with a pair of warts. Inferior appendages 2-articulated, basal article long and covered with long setae; apical article about half length of basal article, slender, with rounded apex. Phallus tubular, elongate, with a basal curvature; median region slightly constricted; apex, in lateral view, truncate, in ventral view subpentagonal. Small internal sclerites on apical region, in ventral view, two very small anteriorly and a transversal posteriorly.

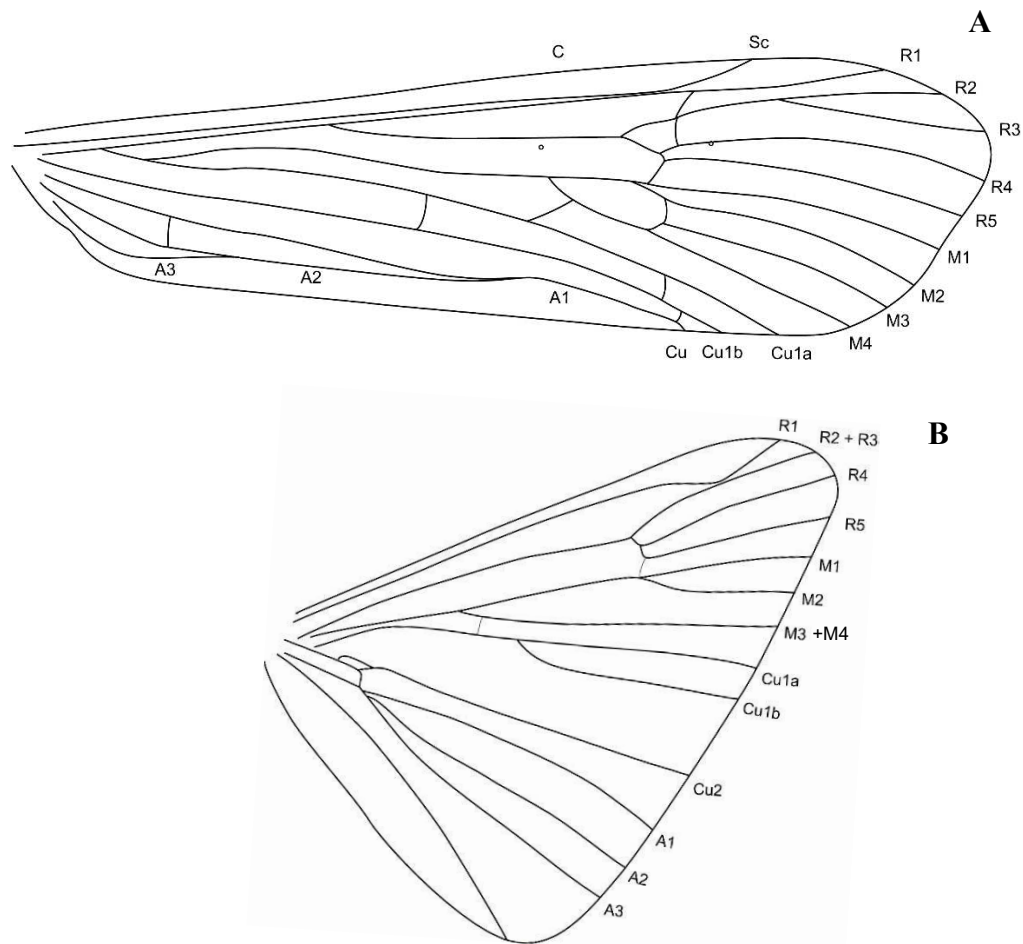


Figure 3. *Blepharopus diaphanus*, male wings. A. Forewing. B. Hind wing.

Adult female (Fig 5A-C). Forewing length 10–13 mm (n=11).

Head brown. Pair of rounded anteroposterior setal warts covered with long pale setae and keel between these warts. Keel smaller than in males and more rounded. Pair of semicircular dorsoposterior setal warts, covered with long pale setae. Pronotum dark brown with dorsal cluster of small setae, meso- and metanotum brown. Midleg tibia and tarsus extended, wider than in male. Forewings usually smaller and lighter than in male. Other characteristics as described for male.

Female genitalia. Segment VIII, in ventral view, completely divided along midline into 2 ventral plates covered with small setae. Segment IX with posterior margin bearing long setae in ventral view. Segment X ending with 3 pairs of processes, two pairs of setose, round, membranous papillae and between them a pair of sclerotized, acuminate cercus is inserted.

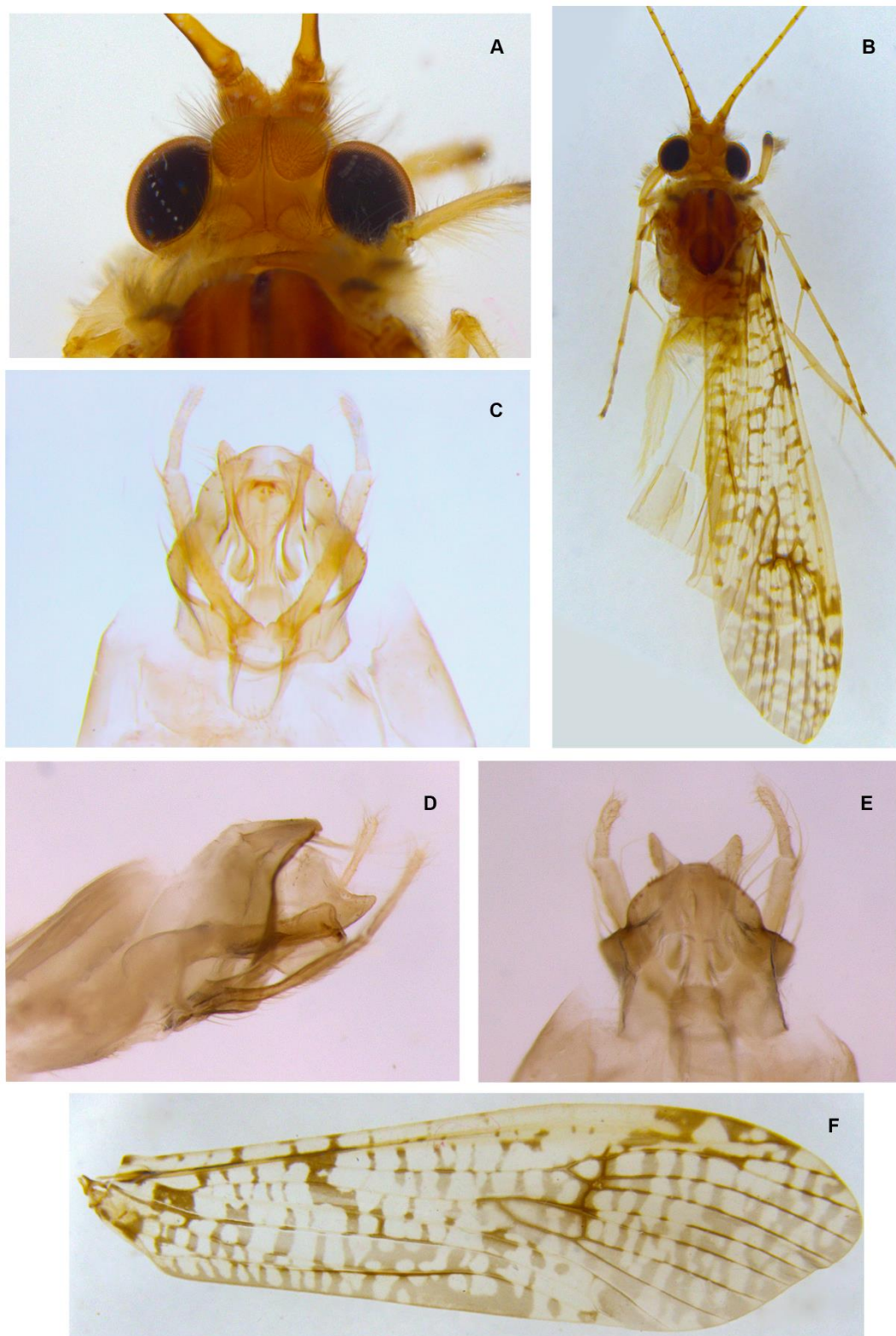


Figure 4. *Blepharopus diaphanus*, adult male (Rio de Janeiro, DZRJ). A. Head, dorsal view. B. Habitus, dorsal view. C. Male genitalia, ventral view. D. Male genitalia, lateral view. E. Male genitalia, dorsal view. F. Forewing.

Variation. In the analyzed material it was observed some variation in the color pattern of the forewings. A lighter reticulated pattern without the darker brown patches was found mainly in specimens from Roraima and in some females from other localities. Also, it was noticed that a few specimens showed an anastomosis or a crossvein between R4-R5 in forewing from one side alone. In addition, the size of the forewings varied over a wide range (9 - 16 mm – n=114). However, after analyzing a large number of specimens, these variations do not reveal a consistent pattern neither match with the grouping pattern indicated by molecular analyses.



Figure 5. *Blepharopus diaphanus*, adult female (Bahia, DZO). A. Habitus, dorsal view. B. Head and thorax, dorsal view. C. Head, lateral view.

Larvae. Additional illustrations of larvae are provided in Figs. 6A-G. For a complete morphological description, see Flint and Wallace (1980).

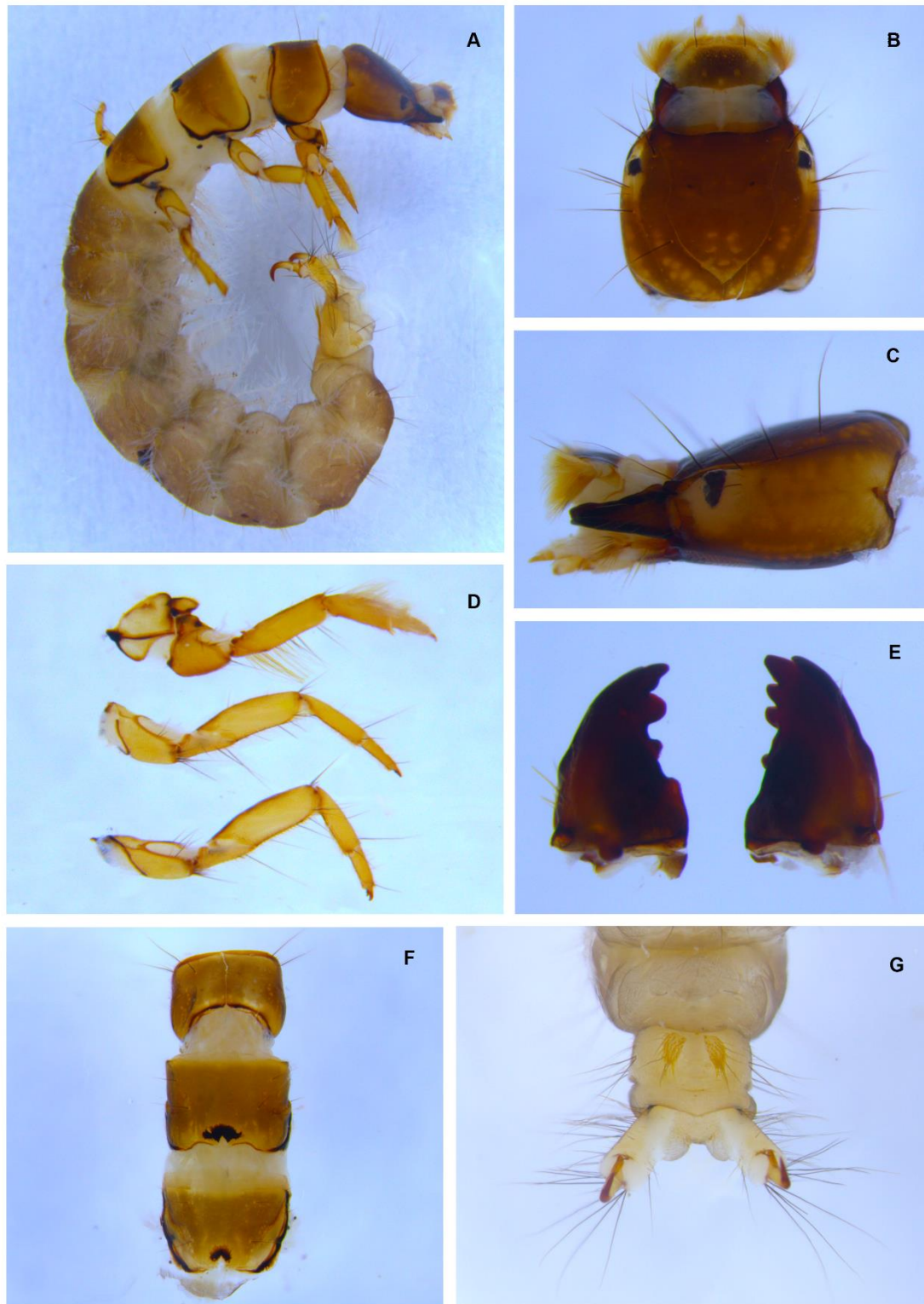


Figure 6. *Blepharopus diaphanus* (Rondônia, INPA) A. Larva, habitus, lateral view. B. Head, dorsal view. C. Head, lateral view. D. Thoracic legs, dorsal view. E. Mandibles, dorsal view. F. Thoracic nota, dorsal view. G. Eighth and ninth sterna, ventral view.

3.2. Molecular analysis

Analyzing COI sequences, we found that specimen of sequence HM424750 is likely a misidentified specimen and correspond to *Macrostemum*, as indicated in NJ tree (Fig. 7). Excluding this sequence, K2P distances were high, ranging up to 22.8% (Supplementary Material 3). Highest distances were found between sequences from Chapada dos Guimarães (Mato Grosso, Brazil - GenBank accession number HQ967713) and Amajari (Roraima State – voucher number ENT5493).

In BI analysis, *Blepharopus* was recovered as monophyletic with maximum support and the same seven groups were found (Fig. 8). BI tree indicated with high support (PP=100) a group of sequences from Rio de Janeiro, Bahia, Espírito Santo, and localities in Minas Gerais State (Braúnas and Morro do Pilar). The second group included all sequences from Roraima (PP=100). The third group included sequences from Mato Grosso State (PP=97). Eight sequences from the states of Rio Grande do Sul, Minas Gerais, Pará and Tocantins got together with low branch support (PP=56). The fifth group formed included all sequences from Rondônia State (PP=100) and two other groups were determined with single sequences (Chapada dos Guimarães, MT and Urubici, SC).

The ABGD analysis, indicated over BI tree (Fig. 8), returned nine potential species, with groups compatible with groups the BI and NJ trees. Distribution of K2P distances revealed a distinct barcode gap between 0.03 and 0.05 distances, potentially corresponding to intra and interspecific distances respectively (Fig. 9). In comparison with the seven previous groups, ABGD analysis split the Mato Grosso group into two groups, the same for sequences from Tocantins State, recovered into its own group. GMYC analysis proposed the seven potential groups, as previous defined based on BI analysis, also indicated over BI tree (Fig. 8).

Blepharopus sequences represented 12 haplotypes. Haplotypes network using biogeographic provinces proposed by Morrone et al. (2022) does not show an evident pattern of geographic structure (Fig. 10). A same result was observed when using Brazilian hydrographic regions or biomes (Supplementary Material 4 and 5). However, we can observe clearly the distinction of the seven potential species within *Blepharopus*.

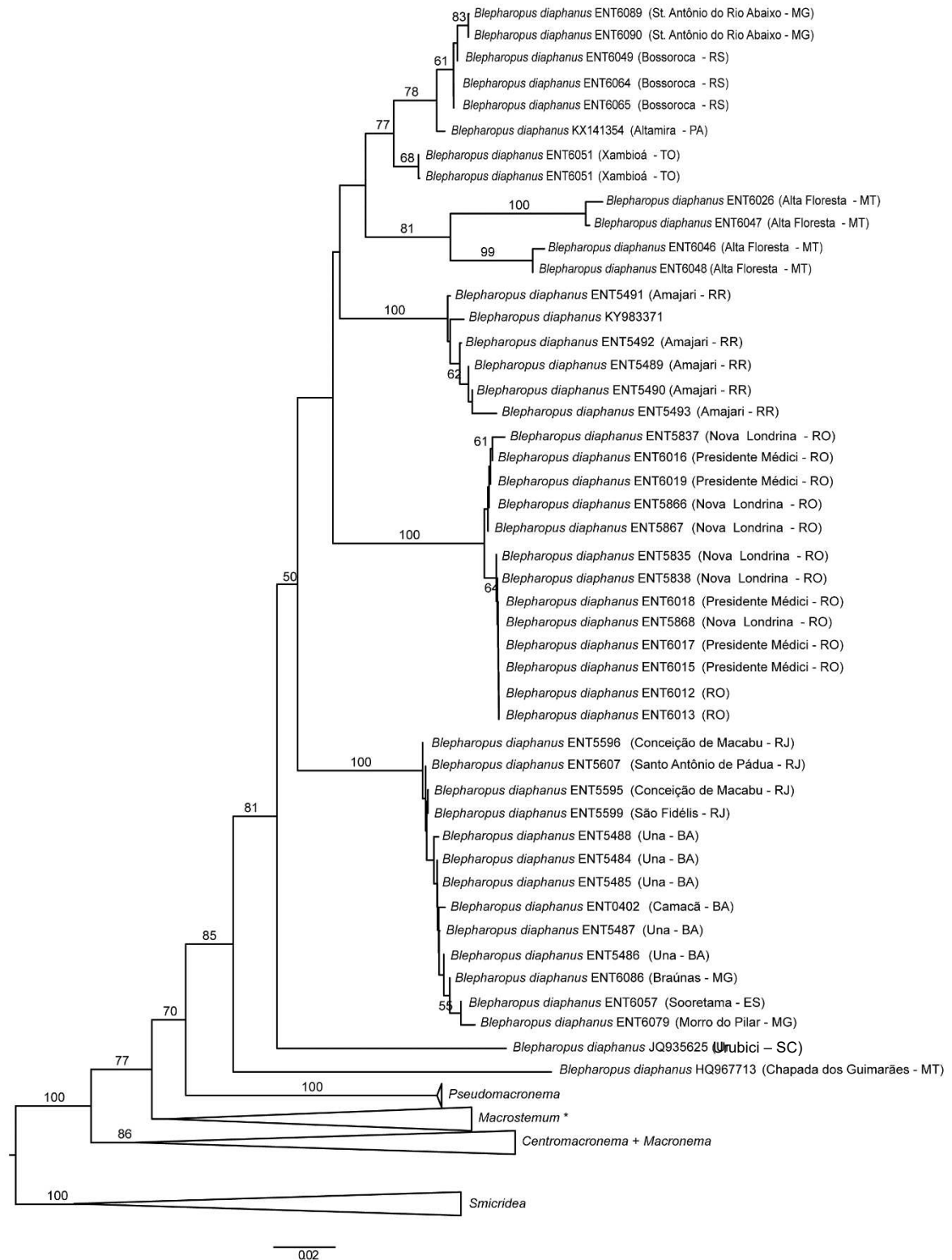


Figure 7. Neighbor joining tree based on K2P distances of COI sequences of *Blepharopus diaphanus* and other Macronematinae species. Bootstrap values above 50% are represented near the branches.

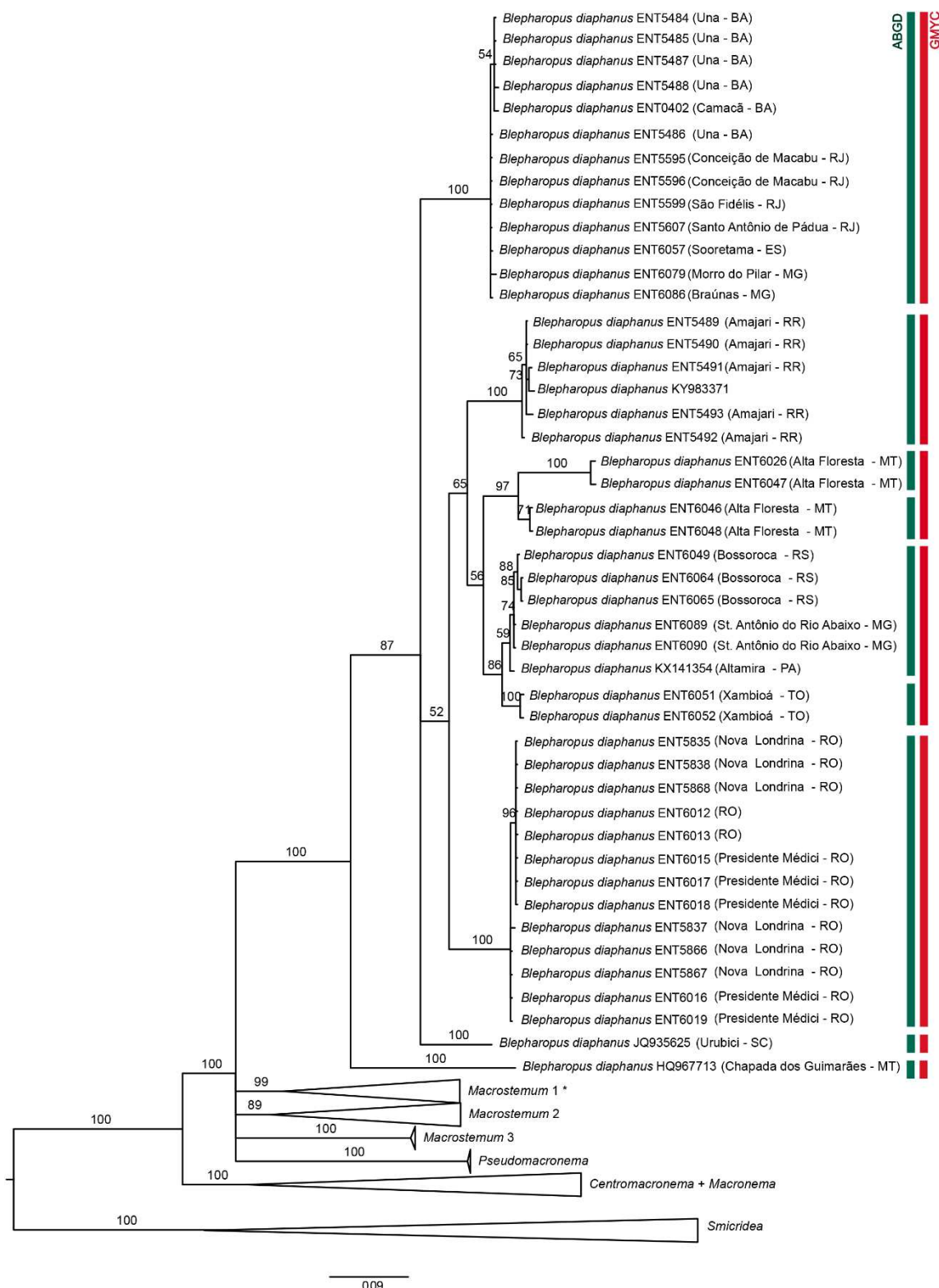


Figure 8. Phylogenetic hypothesis derived from Bayesian inference (50% majority-rule consensus tree, InL = -9052.559) based on COI sequences of *Blepharopus diaphanus* and other Macronematinae species. Posterior probability (PP) values are provided above branches. Bars in the right represent the potential species recovered by ABGD (green bar) and GMYC analysis (red bar), respectively.

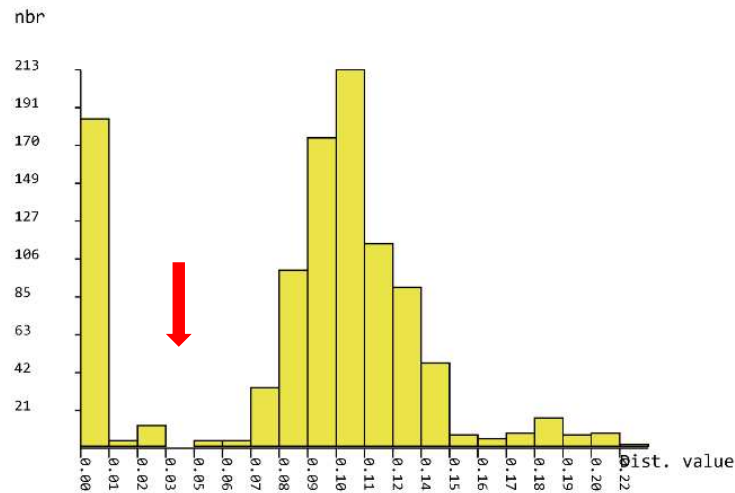


Figure 9. Histogram of the number of pairwise comparisons for K2P divergences among COI sequences of *Blepharopus diaphanus*. The red arrow indicates the (“barcode”) gap between low and high values of K2P distances.

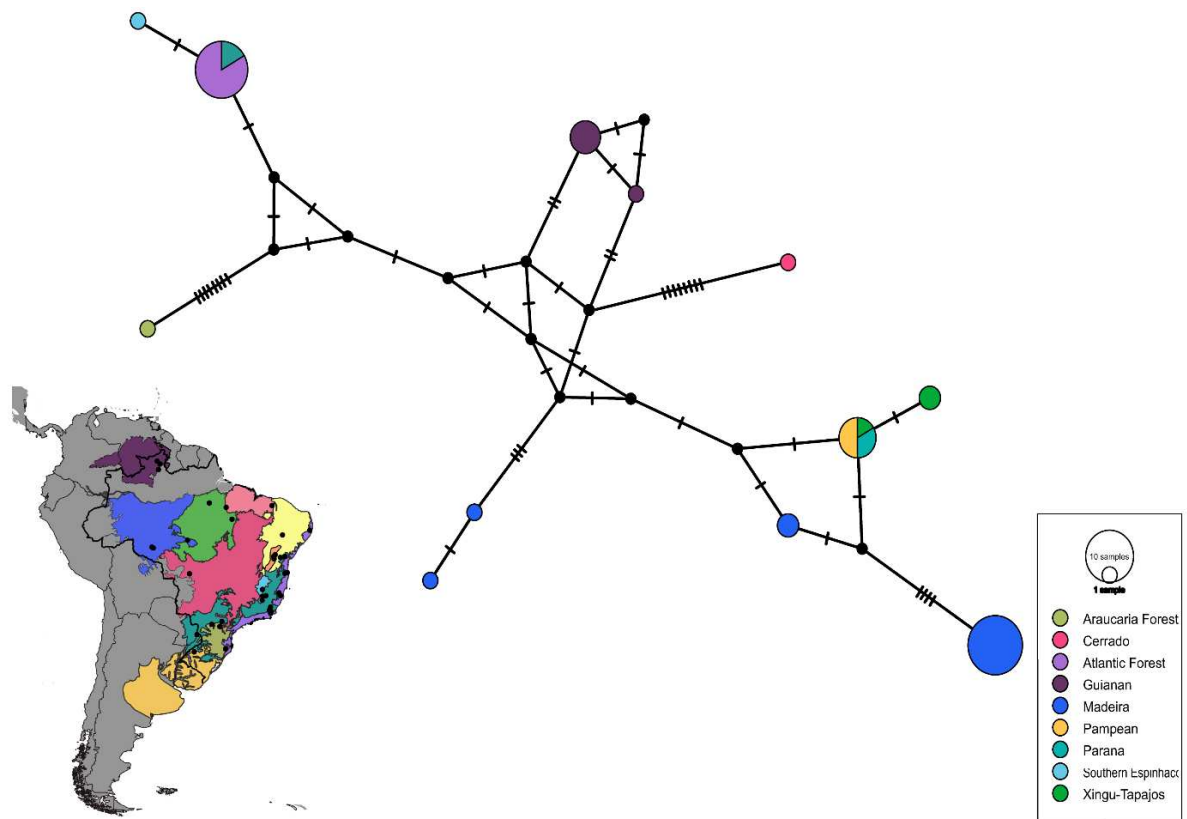


Figure 10. Haplotype network of COI sequences of *B. diaphanus*. Colors indicate biogeographic provinces (Morrone et al. 2022) for occurrence of haplotypes according to legend. Size of circles are related to the frequency of haplotypes and dashes on branches represent the number of mutations between haplotypes.

4. Discussion

4.1. Morphological diversity

As typical for insects, Trichoptera taxonomy is mainly based on male genitalia, since these are the most variable among morphological features (Simmons 2014). When compared with other caddisfly groups, Macronematinae usually show only subtle differences in genitalia between closely related species, in many cases, making it difficult to differentiate them. Sometimes, the taxonomy of this group relies on other features, such as the wing venation and color pattern (e.g., Ulmer 1907, Oláh and Johanson 2012, Dias and Calor 2016). *Blepharopus diaphanus* has a conspicuous color pattern (Fig. 4F) and a distinctive keel (Fig. 4A) on head that distinguish it from any other Neotropical hydropsychid species.

Material analyzed here included specimens from populations that are very distant geographically, for example, *Blepharopus* from Amajari (Roraima State) are separated from those from Bossoroca (Rio Grande do Sul State) by more than 3,600 km. With more and more specimens observed, small variations in morphology were found among them. Those variations included: lighter or darker colors on forewing; forewing length, ranging from 9 mm to 16 mm; wing venation, which a few specimens showed an anastomosis or a crossvein between R4-R5 in forewing.

Color variation in *Blepharopus* was used by Ulmer (1905) to describe a second species, *B. reticulatus*, referred latter as a variety by Ulmer (1907). Flint (1978) suggested that those variations seemed to be due to specimen conservation rather than a true color variation. With specimens preserved, including the types and those recent collected, we can see this variation, but we found them among specimens from the same locality. Another clear distinction, the length, also does not help to distinguish groups within *Blepharopus*, with different sized specimens collected together or, at least, at very close locations. In addition, all morphological variations observed does not follows the genetic pattern that we observed here and could not be used to define nominal species.

4.2. Molecular diversity

Genetic distances of *Blepharopus* COI sequences found here were high, ranging up to 22.8%. These values are much higher than the 3% threshold proposed by Hebert et al. (2003), when delimiting lepidopteran species with DNA barcoding. Although a standard threshold of molecular divergence is not a good choice for delimiting species, when compared with other studies on Trichoptera, this value is still very high. For

example, Santos et al. (2016) found the maximum intraspecific divergence of 4.8% for *Metrichia* Ross, 1938 species (Hydroptilidae) in Brazil,. In a similar way, Barcelos-Silva et al. (2018) found a maximum intraspecific divergence of 7% among *Synoestropsis* Ulmer, 1906 species (Hydropsychidae). More recently, Moreira et al. (2022) used COI sequences to corroborate new species descriptions of Brazilian *Chimarra* Stephens, 1829 (Philopotamidae) and found an apparent high value of intraspecific divergence for *Chimarra odonta* Blahnik, 2002, up to 18.1%, concluding that this represents a complex of cryptic species and morphological differences could distinguish them. Here, although we cannot find consistent morphological features to propose nominal species, the distances obtained for *B. diaphanus*, associated with ABGD and GMYC analyses, indicate at least seven distinct lineages within *Blepharopus diaphanus*.

A sequence from Chapada dos Guimarães (Mato Grosso State, Brazil) was the one with greater genetic divergence among the others. Based on the biogeographic provinces (Morrone et al. 2022) this locality is the only with genetic information in the drier region of Brazil (Cerrado province). The Amazon and the Atlantic forests were once connected but with the climatic variation in the Pleistocene, they became isolated by dry open vegetations (Caatinga, Cerrado, and Chaco) (Antonelli et al. 2010). This dry corridor, besides the different characteristics from humid forests, isolate populations, especially the ones with low dispersibility like caddisflies. This could be observed in Europe, where the fragmentation of forests isolated *Drusus discolor* (Rambur, 1842) populations, that presented high divergence between the populations (Pauls et al. 2006).

It was observed seven lineages in the NJ and delimitation methods. Sequences of Rio de Janeiro, Bahia, Espírito Santo, and localities in Minas Gerais State (Braúnas and Morro do Pilar) grouped. These sequences are from the southeastern part of a former bigger rain forest (Parana Forest and Atlantic provinces) and Southern Espinhaço Province and the maximum COI divergence among them was 0.76%. This third group might be gathered because of the reason cited above. The dry corridor isolated these southeastern forests concentrating the gene flow in this area.

Sequences from Roraima State grouped, revealing low divergence since their location were very near. Other group formed was with the COI sequences from Rondônia State, also with restricted distribution. Despite these two lineages are in the north and Amazon region of Brazil, they are in distinct provinces, Guianan and Madeira, ecoregions divided by climatic, geological, and biotic criteria with evolutionary units (areas of endemism) (Morrone et al. 2022). As demonstrated by Sobral-Souza et al. (2015),

Amazon showed distinct spatial rearrangements in the past, evidencing the diversity of environments in this biome and maybe explain the presence of separated clusters in the North region, as more studies are needed.

The group with sequences from Rio Grande do Sul, Minas Gerais (St. Antônio do Rio Abaixo locality), Pará and Tocantins showed maximum divergence of 3.23%. This value was observed among sequences from Bossoroca (Rio Grande do Sul State) and from Xambioá (Tocantins State), separated by more than 2,800 km and completely different biomes and biogeographic provinces between all states. Although this distance can make it difficult the gene flow between these sites, these sequences were clustered together, requiring further studies. Mato Grosso State sequences grouped, occurring in Madeira province and the single sequence from Santa Catarina State was the only observed in Araucaria Forest province.

Although haplotype networks do not show an evident geographic structure, the seven potential lineages treated within *Blepharopus* can be defined as distinct haplogroups. The absence of geographic structure could be exemplified by the one haplotype found in three distant geographic provinces (Pampean, Parana Forest, and Xingu-Tapajos provinces). Souza (2015) results for *Oxyethira tica* Holzenthal & Harris, 1992 suggest no population structure for the Brazilian populations using the hydrogeographic regions. In Souto et al. (2021), an ephemeroptera complex of cryptic species also show no genetic structure in water basins although it was detected to be mountain-specific.

5. Conclusions

Analyzing the molecular diversity of *Blepharopus diaphanus* in Brazil, we found multiple and distinct lineages within this single nominal species. So far, based on traditional morphological characters we were not able to establish formal species. This observation also resulted in the redescription of the adults of *Blepharopus diaphanus*. Although some lineages seem to be restricted to small geographic locations, other are more widespread. Despite our knowledge about caddisfly, diversity in Neotropics is still limited, this Region is extremely rich in species number. *Blepharopus diaphanus* as a complex of cryptic species show us that diversity can be much higher and the integration of distinct information, such as morphology and DNA sequences is increasingly needed to understand our biodiversity in a broader perspective.

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7. Supplementary material

Supplementary material 1. Specimens used for DNA analysis in this study, with specimen voucher or GenBank ® accession codes, and localities.

		GenBank ®		
Taxon		accession codes	Voucher	Locality
Ingroup				
<i>Blepharopus diaphanus</i>		HM424750		Itatiaia, Minas Gerais, Brazil
<i>Blepharopus diaphanus</i>		HQ967713		Chap.dos Guimarães, Mato Grosso, Brazil
<i>Blepharopus diaphanus</i>		KX141354		Altamira, Pará, Brazil
<i>Blepharopus diaphanus</i>		JQ935625		Urubici, Santa Catarina, Brazil
<i>Blepharopus diaphanus</i>		KY983371		Brazil
<i>Blepharopus diaphanus</i>	♂		ENT0402	Camacã, Bahia, Brazil
<i>Blepharopus diaphanus</i>	♂		ENT5484	Una, Bahia, Brazil
<i>Blepharopus diaphanus</i>	♂		ENT5485	Una, Bahia, Brazil
<i>Blepharopus diaphanus</i>	♂		ENT5486	Una, Bahia, Brazil
<i>Blepharopus diaphanus</i>	♂		ENT5487	Una, Bahia, Brazil
<i>Blepharopus diaphanus</i>	♀		ENT5488	Una, Bahia, Brazil
<i>Blepharopus diaphanus</i>	♂		ENT5489	Amajari, Roraima, Brazil
<i>Blepharopus diaphanus</i>	♂		ENT5490	Amajari, Roraima, Brazil
<i>Blepharopus diaphanus</i>	♂		ENT5491	Amajari, Roraima, Brazil
<i>Blepharopus diaphanus</i>	♂		ENT5492	Amajari, Roraima, Brazil
<i>Blepharopus diaphanus</i>	♂		ENT5493	Amajari, Roraima, Brazil
<i>Blepharopus diaphanus</i>	♀		ENT5595	Conceição de Macabu, Rio de Janeiro, Brazil
<i>Blepharopus diaphanus</i>	♀		ENT5596	Conceição de Macabu, Rio de Janeiro, Brazil
<i>Blepharopus diaphanus</i>	♀		ENT5599	São Fidélis, Rio de Janeiro, Brazil
<i>Blepharopus diaphanus</i>	♀		ENT5607	St. Antônio de Pádua, Rio de Janeiro, Brazil
<i>Blepharopus diaphanus</i>	♂		ENT5835	Nova Londrina, Rondônia, Brazil
<i>Blepharopus diaphanus</i>	♂		ENT5837	Nova Londrina, Rondônia, Brazil

<i>Blepharopus diaphanus</i>	♂	ENT5838	Nova Londrina, Rondônia, Brazil
<i>Blepharopus diaphanus</i>	Larvae	ENT5866	Nova Londrina, Rondônia, Brazil
<i>Blepharopus diaphanus</i>	Larvae	ENT5867	Nova Londrina, Rondônia, Brazil
<i>Blepharopus diaphanus</i>	Larvae	ENT5868	Nova Londrina, Rondônia, Brazil
<i>Blepharopus diaphanus</i>	♀	ENT6012	Rondônia, Brazil
<i>Blepharopus diaphanus</i>	♂	ENT6013	Rondônia, Brazil
<i>Blepharopus diaphanus</i>	♂	ENT6015	Presidente Médici, Rondônia, Brazil
<i>Blepharopus diaphanus</i>	♂	ENT6016	Presidente Médici, Rondônia, Brazil
<i>Blepharopus diaphanus</i>	♂	ENT6017	Presidente Médici, Rondônia, Brazil
<i>Blepharopus diaphanus</i>	♀	ENT6018	Presidente Médici, Rondônia, Brazil
<i>Blepharopus diaphanus</i>	♀	ENT6019	Presidente Médici, Rondônia, Brazil
<i>Blepharopus diaphanus</i>		ENT6026	Alta Floresta, Mato Grosso, Brazil
<i>Blepharopus diaphanus</i>	♂	ENT6046	Alta Floresta, Mato Grosso, Brazil
<i>Blepharopus diaphanus</i>		ENT6047	Alta Floresta, Mato Grosso, Brazil
<i>Blepharopus diaphanus</i>		ENT6048	Alta Floresta, Mato Grosso, Brazil
<i>Blepharopus diaphanus</i>	♀	ENT6049	Bossoroca, Rio Grande do Sul, Brazil
<i>Blepharopus diaphanus</i>	♂	ENT6051	Xambioá, Tocantins, Brazil
<i>Blepharopus diaphanus</i>	♂	ENT6052	Xambioá, Tocantins, Brazil
<i>Blepharopus diaphanus</i>	♀	ENT6057	Sooretama, Espírito Santo, Brazil
<i>Blepharopus diaphanus</i>	Larvae	ENT6064	Bossoroca, Rio Grande do Sul, Brazil
<i>Blepharopus diaphanus</i>	Larvae	ENT6065	Bossoroca, Rio Grande do Sul, Brazil
<i>Blepharopus diaphanus</i>	♀	ENT6079	Minas Gerais, Brazil
<i>Blepharopus diaphanus</i>	♀	ENT6086	Minas Gerais, Brazil
<i>Blepharopus diaphanus</i>	♂	ENT6089	Minas Gerais, Brazil
<i>Blepharopus diaphanus</i>	♂	ENT6090	Minas Gerais, Brazil

Outgroup

<i>Centromacronema apicale</i>		HQ967531		Madre de Dios, Peru
<i>Centromacronema apicale</i>		HQ967716		Ecuador
<i>Centromacronema apicale</i>	♂	KX104110		Alajuela, Costa Rica
<i>Centromacronema apicale</i>		KX104392		
<i>Centromacronema apicale</i>		KX141302		Guanacaste, Costa Rica
<i>Centromacronema auripenne</i>		HQ967714		San Jose, Costa Rica
<i>Centromacronema auripenne</i>		HQ967715		Guanacaste, Costa Rica
<i>Centromacronema auripenne</i>	♂	KX104714		Chiapas, Mexico
<i>Centromacronema auripenne</i>	♂	KX105318		Puntarenas, Costa Rica
<i>Centromacronema auripenne</i>		KX142126		Guanacaste, Costa Rica
<i>Macronema argentilineatum</i>		HQ967728		Camacã, Bahia, Brazil
<i>Macronema argentilineatum</i>		KX142231		Upper Demerara-Berbice, Guyana
<i>Macronema argentilineatum</i>		KX142627		Kanuku Mountains, Guyana
<i>Macronema hageni</i>		HQ967742		Mato Grosso, Brazil
<i>Macronema hageni</i>		HQ967741		Mato Grosso, Brazil
<i>Macronema hageni</i>		HQ967743		Mato Grosso, Brazil
<i>Macronema hageni</i>		HQ967739		Rio Potaro, Guyana
<i>Macronema luteipenne</i>		KX295325		Puntarenas, Costa Rica
<i>Macronema luteipenne</i>		KX103534		Guerrero, Mexico
<i>Macronema luteipenne</i>		HQ967744		Guanacaste, Costa Rica
<i>Macronema percitans</i>		HQ967748		Rio Potaro, Guyana
<i>Macronema variipenne</i>		HQ967757		Heredia, Costa Rica
<i>Macronema variipenne</i>		HQ967755		Puntarenas, Costa Rica
<i>Macronema variipenne</i>	♂	HQ967753		Heredia, Costa Rica
<i>Macrostemum</i> sp.	♀		ENT5597	Rio das Ostras, Rio de Janeiro, Brazil
<i>Macrostemum erichsoni</i>		HQ967761		Rio Essequibo, Guyana
<i>Macrostemum erichsoni</i>		HQ967762		Rio Essequibo, Guyana
<i>Macrostemum erichsoni</i>		HQ967763		Upper Demerara-Berbice, Guyana
<i>Macrostemum erichsoni</i>		HQ967764		Upper Demerara-Berbice, Guyana
<i>Macrostemum erichsoni</i>	♂	KX294870		Manaus, Amazonas, Brazil

<i>Macrostemum hyalinum</i>		HQ967765	Ceiba Biol. Station, Guyana
<i>Macrostemum hyalinum</i>		HQ967766	Iwokrama Field Sta., Guyana
<i>Macrostemum hyalinum</i>		HQ967767	Iwokrama Field Sta., Guyana
<i>Macrostemum hyalinum</i>		HQ967768	Venezuela
<i>Macrostemum hyalinum</i>		KX294504	Venezuela
<i>Macrostemum hyalinum</i>		KX295343	Rondonia, Brazil
<i>Macrostemum hyalinum</i>		KX295260	Rondonia, Brazil
<i>Macrostemum ulmeri</i>		HQ967773	Heredia, Costa Rica
<i>Macrostemum ulmeri</i>		HQ967774	Heredia, Costa Rica
<i>Macrostemum ulmeri</i>		HQ967775	Kumu, Guyana
<i>Macrostemum ulmeri</i>		HQ967776	Rio Tiputini, Ecuador
<i>Macrostemum ulmeri</i>		HQ967777	Rio Tiputini, Ecuador
<i>Macrostemum ulmeri</i>		HQ967778	La Paz, Bolivia
<i>Macrostemum ulmeri</i>		HQ967779	Venezuela
<i>Macrostemum ulmeri</i>		HQ967780	Venezuela
<i>Macrostemum ulmeri</i>		HQ967529	Madre de Dios, Peru
<i>Macrostemum ulmeri</i>	♀	HM862457	Heredia, Costa Rica
<i>Macrostemum ulmeri</i>		KX143228	Upper Demerara-Berbice, Guyana
<i>Macrostemum santaeritae</i>		HQ967769	Rio Claro, Mato Grosso, Brazil
<i>Macrostemum santaeritae</i>		HQ967770	Rio Claro, Mato Grosso, Brazil
<i>Pseudomacronema vittatum</i>		KX141004	Beni, Bolivia
<i>Pseudomacronema vittatum</i>	♀	KX105775	Guarico, Venezuela
<i>Smicridea signata</i>		KX141983	Rio Verde, Arizona, USA
<i>Smicridea signata</i>	♂	KX104528	Sucre, Venezuela
<i>Smicridea pucara</i>		HM065420	Chile
<i>Smicridea palifera</i>		HM065392	Cach. de Macacu, Rio de Janeiro, Brazil
<i>Smicridea fasciatella</i>		HM883423	Arizona, USA

Supplementary material 3 Matrix of K2P distances of COI sequences of *Blepharopus* and other Hydropsychidae.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
(1) <i>B.diaphanus</i> ENT5484																				
(2) <i>B.diaphanus</i> ENT5485	0,00%																			
(3) <i>B.diaphanus</i> ENT5486	0,00%	0,00%																		
(4) <i>B.diaphanus</i> ENT5487	0,00%	0,00%	0,00%																	
(5) <i>B.diaphanus</i> ENT5488	0,27%	0,28%	0,00%	0,28%																
(6) <i>B.diaphanus</i> ENT5489	9,67%	9,91%	11,04%	9,78%	10,31%															
(7) <i>B.diaphanus</i> ENT5490	9,91%	10,00%	11,04%	10,02%	10,31%	0,00%														
(8) <i>B.diaphanus</i> ENT5491	9,31%	9,39%	10,33%	9,42%	9,67%	0,34%	0,34%													
(9) <i>B.diaphanus</i> ENT5492	9,67%	9,75%	10,69%	9,77%	9,99%	0,35%	0,34%	0,68%												
(10) <i>B.diaphanus</i> ENT5493	11,72%	11,72%	12,29%	11,87%	11,69%	0,57%	0,57%	1,15%	0,86%											
(11) <i>B.diaphanus</i> ENT5595	0,29%	0,29%	0,00%	0,29%	0,58%	10,01%	10,01%	9,53%	9,53%	12,11%										
(12) <i>B.diaphanus</i> ENT5596	0,27%	0,27%	0,00%	0,27%	0,55%	9,79%	9,79%	9,36%	9,36%	12,10%	0,00%									
(13) <i>Macrostemum</i> sp.	19,13%	19,13%	19,07%	19,40%	19,27%	17,55%	17,55%	16,75%	17,15%	18,37%	19,00%	18,72%								
(14) <i>B.diaphanus</i> ENT5599	0,25%	0,26%	0,00%	0,25%	0,55%	9,84%	10,03%	9,56%	9,60%	12,07%	0,00%	0,00%	18,72%							
(15) <i>B.diaphanus</i> ENT5607	0,25%	0,26%	0,00%	0,25%	0,55%	9,63%	9,63%	9,20%	9,27%	12,07%	0,00%	0,00%	18,72%	0,00%						
(16) <i>B.diaphanus</i> ENT5835	10,62%	10,80%	10,69%	10,74%	10,62%	9,30%	9,43%	8,91%	9,23%	10,40%	9,95%	9,80%	18,74%	10,06%	10,06%					
(17) <i>B. diaphanus</i> ENT5837	12,78%	12,78%	12,84%	12,97%	12,78%	11,62%	11,62%	10,82%	12,03%	12,03%	12,66%	12,37%	18,86%	12,37%	12,37%	1,01%				
(18) <i>B. diaphanus</i> ENT5838	10,52%	10,61%	10,69%	10,64%	10,62%	9,54%	9,54%	8,98%	9,54%	10,22%	10,40%	10,21%	18,74%	10,37%	10,37%	0,00%	1,01%			
(19) <i>B. diaphanus</i> ENT5866	10,52%	10,61%	10,69%	10,64%	10,62%	9,54%	9,54%	8,98%	9,54%	10,22%	10,40%	10,21%	18,33%	10,37%	10,37%	0,48%	0,33%	0,48%		
(20) <i>B. diaphanus</i> ENT5867	10,55%	10,64%	10,69%	10,67%	10,62%	9,21%	9,21%	8,67%	9,21%	10,25%	9,98%	9,83%	18,33%	10,00%	10,00%	0,47%	0,33%	0,48%	0,00%	

Supplementary material 2. Continuation.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
(21) <i>B. diaphanus</i> ENT5868	10,28%	10,37%	10,69%	10,40%	10,62%	9,77%	9,77%	9,19%	9,77%	10,65%	10,29%	10,03%	19,29%	10,03%	10,03%	0,00%	1,01%	0,00%	0,50%	0,49%
(22) <i>B. diaphanus</i> ENT6012	10,98%	10,95%	10,69%	11,10%	10,85%	9,89%	9,83%	9,22%	9,90%	10,40%	10,50%	10,28%	18,74%	10,86%	10,74%	0,00%	1,01%	0,00%	0,54%	0,54%
(23) <i>B. diaphanus</i> ENT6013	12,73%	12,73%	12,96%	12,92%	12,65%	10,03%	10,03%	9,25%	10,43%	10,43%	12,77%	12,33%	18,80%	12,33%	12,33%	0,00%	1,05%	0,00%	0,67%	0,67%
(24) <i>B. diaphanus</i> ENT6015	11,04%	11,04%	10,75%	11,17%	10,59%	9,76%	9,76%	9,12%	10,09%	10,43%	10,57%	10,34%	18,74%	10,71%	10,71%	0,00%	1,01%	0,00%	0,56%	0,56%
(25) <i>B. diaphanus</i> ENT6016	10,87%	10,87%	10,75%	11,01%	10,87%	10,19%	10,19%	9,50%	10,54%	10,93%	10,57%	10,51%	19,00%	10,51%	10,51%	0,60%	0,34%	0,60%	0,00%	0,00%
(26) <i>B. diaphanus</i> ENT6017	11,06%	11,06%	10,69%	11,19%	10,95%	9,81%	9,81%	9,18%	10,13%	10,43%	10,60%	10,37%	18,74%	10,74%	10,74%	0,00%	1,01%	0,00%	0,55%	0,55%
(27) <i>B. diaphanus</i> ENT6018	10,90%	10,90%	10,69%	11,03%	10,95%	9,64%	9,64%	9,00%	9,95%	10,25%	10,60%	10,37%	18,74%	10,57%	10,57%	0,00%	1,01%	0,00%	0,55%	0,55%
(28) <i>B. diaphanus</i> ENT6019	10,81%	10,81%	10,69%	10,94%	10,53%	9,56%	9,56%	8,94%	9,88%	10,40%	10,50%	10,27%	18,33%	10,49%	10,49%	0,54%	0,33%	0,56%	0,00%	0,00%
(29) <i>B. diaphanus</i> ENT6026	13,33%	13,33%	14,15%	13,50%	13,46%	11,35%	11,35%	10,69%	11,02%	11,81%	14,00%	13,84%	19,59%	13,68%	13,68%	14,32%	15,73%	14,28%	13,57%	13,61%
(30) <i>B. diaphanus</i> ENT6046	13,02%	13,02%	13,39%	13,20%	13,07%	9,46%	9,55%	9,55%	8,89%	9,38%	13,21%	12,31%	17,73%	12,64%	12,64%	13,10%	12,59%	12,58%	11,80%	11,84%
(31) <i>B. diaphanus</i> ENT6047	13,44%	13,06%	13,95%	13,64%	13,11%	10,83%	10,79%	9,99%	10,46%	10,62%	13,76%	13,59%	18,58%	13,76%	13,54%	14,47%	14,24%	14,13%	13,27%	13,32%
(32) <i>B. diaphanus</i> ENT6048	12,31%	12,52%	12,81%	12,48%	12,53%	8,87%	9,10%	9,10%	8,45%	8,92%	12,63%	11,79%	17,52%	11,95%	11,95%	12,47%	12,08%	12,06%	11,29%	11,33%
(33) <i>B. diaphanus</i> ENT6049	11,14%	11,14%	11,48%	11,28%	11,25%	7,97%	7,97%	7,97%	7,65%	8,65%	11,26%	10,64%	18,93%	10,81%	10,81%	8,94%	10,16%	8,94%	8,29%	8,32%
(34) <i>B. diaphanus</i> ENT6051	9,14%	9,14%	9,14%	9,14%	9,14%	9,16%	9,16%	9,16%	9,16%	9,16%	9,14%	9,14%	19,61%	9,14%	9,14%	7,43%	9,89%	7,43%	7,43%	7,43%
(35) <i>B. diaphanus</i> ENT6052	8,61%	8,61%	8,61%	8,61%	8,61%	7,23%	7,23%	7,23%	7,23%	7,79%	8,61%	8,61%	19,90%	8,61%	8,61%	7,96%	11,96%	7,96%	7,96%	7,96%
(36) <i>B. diaphanus</i> ENT6057	0,32%	0,32%	0,00%	0,32%	0,33%	12,48%	12,48%	11,71%	12,09%	13,26%	0,00%	0,00%	18,86%	0,00%	0,00%	12,07%	12,42%	12,07%	12,07%	12,07%
(37) <i>B. diaphanus</i> ENT6062	10,55%	10,82%	11,04%	10,67%	11,03%	8,29%	8,51%	8,51%	7,94%	8,80%	10,69%	10,12%	18,80%	10,24%	10,30%	9,26%	10,09%	8,78%	8,15%	8,17%
(38) <i>B. diaphanus</i> ENT6065	10,61%	10,73%	11,04%	10,73%	10,93%	8,27%	8,43%	8,43%	7,87%	8,80%	10,58%	10,03%	18,80%	10,30%	10,30%	9,47%	10,09%	9,00%	8,37%	8,40%
(39) <i>B. diaphanus</i> ENT6079	0,76%	0,76%	0,38%	0,77%	0,76%	12,39%	12,39%	11,47%	11,93%	12,85%	0,38%	0,38%	19,74%	0,38%	0,38%	12,80%	13,27%	12,80%	12,80%	12,80%

Supplementary material 2. Continuation.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
(40) <i>B. diaphanus</i> <i>ENT6086</i>	0,33%	0,33%	0,00%	0,33%	0,33%	11,75%	11,75%	10,97%	11,36%	12,19%	0,00%	0,00%	19,38%	0,00%	0,00%	10,60%	12,34%	10,60%	10,60%	10,60%
(41) <i>B. diaphanus</i> <i>ENT6089</i>	11,32%	11,32%	10,97%	11,48%	11,32%	9,10%	9,10%	9,10%	8,72%	9,27%	10,93%	10,93%	19,70%	10,93%	10,93%	9,51%	10,41%	9,51%	8,74%	8,74%
(42) <i>B. diaphanus</i> <i>ENT6090</i>	11,12%	11,12%	10,78%	11,28%	11,12%	8,95%	8,95%	8,95%	8,57%	9,04%	10,74%	10,74%	19,60%	10,74%	10,74%	9,70%	10,53%	9,70%	8,95%	8,95%
(43) <i>B. diaphanus</i> <i>ENT402</i>	0,25%	0,26%	0,30%	0,25%	0,55%	9,89%	10,12%	9,56%	9,61%	12,07%	0,53%	0,74%	19,54%	0,65%	0,69%	10,76%	13,19%	10,68%	10,68%	10,71%
(44) <i>B. diaphanus</i> <i>HQ967713</i>	20,42%	20,09%	20,03%	20,66%	19,47%	17,83%	17,40%	16,94%	17,70%	22,88%	17,40%	16,37%	24,13%	18,50%	17,28%	19,27%	18,74%	18,78%	18,12%	18,07%
(45) <i>B. diaphanus</i> <i>JQ935625</i>	12,04%	11,81%	12,19%	12,17%	12,35%	12,17%	11,75%	11,13%	11,80%	15,08%	11,75%	11,13%	21,70%	12,62%	11,26%	15,40%	16,29%	14,92%	14,92%	14,67%
(46) <i>B. diaphanus</i> <i>KX141354</i>	10,77%	10,52%	11,04%	10,89%	10,96%	8,54%	8,54%	8,43%	7,98%	8,80%	9,77%	9,36%	18,38%	9,67%	10,02%	9,35%	9,69%	9,32%	8,75%	8,45%
(47) <i>B. diaphanus</i> <i>KY983371</i>	9,60%	9,61%	10,69%	9,70%	10,31%	0,70%	0,71%	0,71%	1,20%	1,15%	10,19%	10,02%	17,15%	9,49%	9,64%	9,74%	11,22%	9,62%	9,62%	9,64%
(48) <i>Blepharopus</i> <i>sp HM424750</i>	18,58%	18,57%	18,30%	18,79%	17,89%	18,39%	17,96%	17,51%	17,37%	18,96%	16,95%	17,34%	14,12%	18,04%	17,51%	18,98%	18,89%	19,12%	18,80%	19,03%

Supplementary material 2. Continuation.

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
(21)B. diaphanus ENT5868																			
(22)B. diaphanus ENT6012	0,00%																		
(23)B. diaphanus ENT6013	0,00%	0,00%																	
(24)B. diaphanus ENT6015	0,00%	0,00%	0,00%																
(25)B. diaphanus ENT6016	0,60%	0,60%	0,70%	0,60%															
(26)B. diaphanus ENT6017	0,00%	0,00%	0,00%	0,00%	0,60%														
(27)B. diaphanus ENT6018	0,00%	0,00%	0,00%	0,00%	0,60%	0,00%													
(28)B. diaphanus ENT6019	0,58%	0,54%	0,67%	0,55%	0,00%	0,55%	0,56%												
(29)B. diaphanus ENT6026	14,88%	14,32%	14,45%	14,45%	14,52%	14,45%	14,32%	13,63%											
(30)B. diaphanus ENT6046	12,77%	13,10%	12,51%	12,80%	12,31%	12,80%	12,63%	12,20%	7,54%										
(31)B. diaphanus ENT6047	14,87%	14,36%	14,47%	14,35%	14,42%	14,35%	14,19%	13,66%	0,68%	7,27%									
(32)B. diaphanus ENT6048	12,23%	12,39%	12,10%	12,28%	11,76%	12,28%	12,11%	11,70%	8,17%	0,30%	7,51%								
(33)B. diaphanus ENT6049	8,96%	8,94%	10,17%	9,02%	8,58%	8,94%	8,96%	8,32%	11,02%	7,11%	11,34%	6,68%							
(34)B.diaphanus ENT6051	7,43%	7,43%	6,47%	7,43%	7,43%	7,43%	7,43%	7,43%	14,05%	4,86%	10,53%	4,73%	3,23%						
(35)B. diaphanus ENT6052	7,96%	7,96%	8,96%	7,96%	7,96%	7,96%	7,96%	7,96%	11,72%	5,37%	10,59%	5,23%	2,56%	0,00%					
(36)B. diaphanus ENT6057	11,80%	12,07%	12,33%	12,07%	12,14%	12,07%	12,07%	12,07%	14,90%	12,52%	13,81%	12,09%	12,55%	10,96%	11,94%				
(37)B. diaphanus ENT6062	8,80%	9,41%	10,10%	9,18%	8,53%	8,99%	8,81%	8,68%	11,06%	7,79%	11,91%	7,25%	0,28%	3,23%	2,56%	12,07%			
(38)B. diaphanus ENT6065	9,03%	9,44%	10,10%	9,18%	8,53%	9,24%	9,06%	8,68%	11,06%	7,79%	11,33%	7,27%	0,28%	3,23%	2,56%	12,07%	0,00%		
(39)B. diaphanus ENT6079	12,80%	12,80%	13,02%	12,80%	12,80%	12,80%	12,80%	12,80%	14,31%	12,85%	14,58%	12,39%	12,94%	10,57%	11,63%	0,38%	12,83%	12,83%	

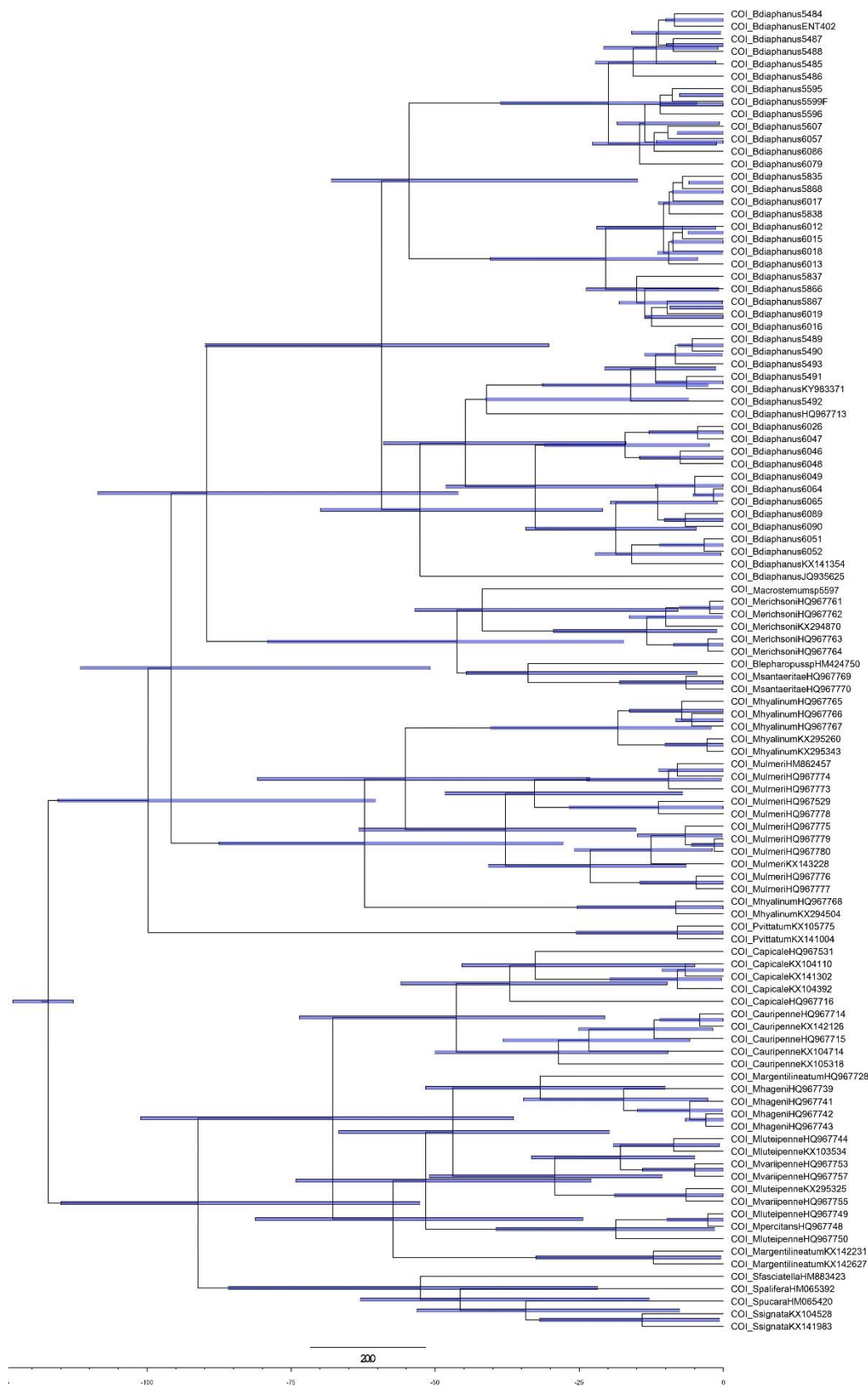
Supplementary material 2. Continuation.

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
(40) <i>B. diaphanus</i> ENT6086	10,60%	10,60%	12,45%	10,60%	10,60%	10,60%	10,60%	10,60%	14,24%	12,89%	13,93%	12,29%	11,44%	9,14%	9,22%	0,00%	11,36%	11,36%	0,38%
(41) <i>B. diaphanus</i> ENT6089	9,51%	9,51%	10,48%	9,51%	8,74%	9,51%	9,51%	8,74%	11,85%	8,83%	12,65%	8,30%	0,33%	3,23%	2,67%	12,52%	0,33%	0,33%	12,42%
(42) <i>B. diaphanus</i> ENT6090	9,70%	9,70%	10,60%	9,70%	8,95%	9,70%	9,70%	8,95%	12,04%	8,59%	12,26%	8,07%	0,32%	3,23%	2,70%	12,18%	0,32%	0,32%	12,36%
(43) <i>B. diaphanus</i> ENT402	10,46%	11,46%	13,14%	11,37%	11,22%	11,38%	11,22%	11,14%	12,99%	13,40%	12,98%	12,68%	11,47%	9,14%	8,61%	0,64%	10,86%	10,92%	0,76%
(44) <i>B. diaphanus</i> HQ967713	18,31%	20,09%	18,71%	19,00%	18,26%	18,80%	19,10%	18,58%	19,82%	18,70%	22,42%	18,20%	18,80%	19,85%	20,37%	19,97%	18,56%	18,31%	20,46%
(45) <i>B. diaphanus</i> JQ935625	14,97%	15,00%	15,87%	14,54%	14,61%	14,46%	14,33%	14,58%	15,19%	14,73%	15,90%	15,11%	13,02%	17,05%	15,67%	12,92%	13,91%	13,99%	13,83%
(46) <i>B. diaphanus</i> KX141354	9,25%	9,46%	9,70%	9,18%	8,53%	9,24%	9,06%	8,68%	11,73%	6,75%	11,89%	6,24%	0,84%	2,40%	2,56%	11,69%	1,30%	1,04%	11,91%
(47) <i>B. diaphanus</i> KY983371	9,95%	9,70%	9,64%	9,44%	9,85%	9,50%	9,32%	9,25%	11,35%	9,46%	11,10%	8,87%	7,97%	10,07%	7,93%	12,09%	8,22%	8,27%	11,93%
(48) <i>Blepharopus</i> sp HM424750	18,97%	19,46%	18,41%	19,46%	19,55%	19,27%	19,57%	19,04%	21,14%	19,28%	20,11%	19,13%	19,31%	21,58%	20,77%	16,98%	19,33%	18,77%	16,85%

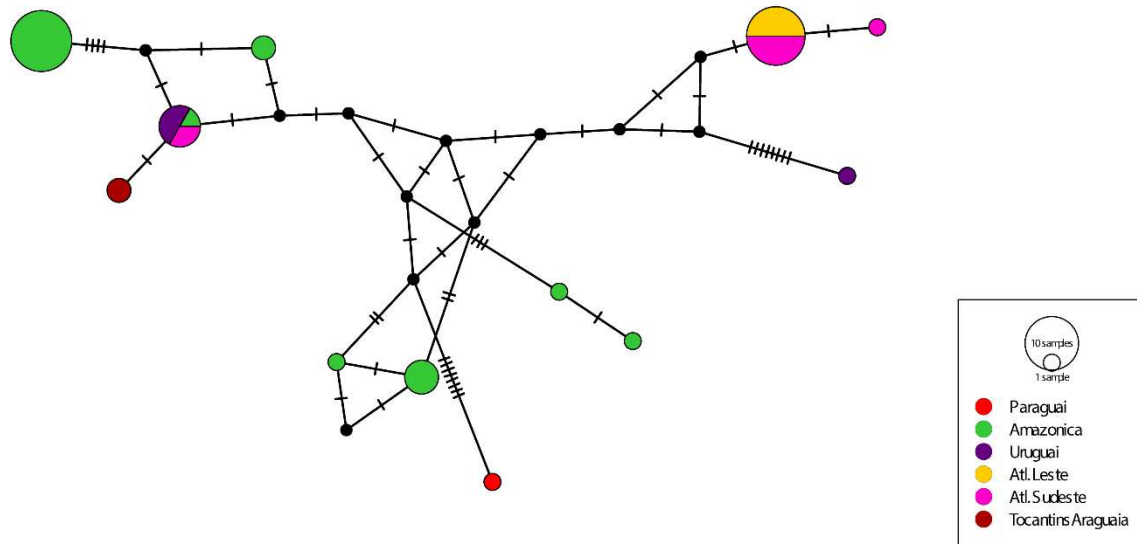
Supplementary material 2. Continuation.

	40	41	42	43	44	45	46	47	48
(40) <i>B. diaphanus</i> ENT6086									
(41) <i>B. diaphanus</i> ENT6089	11,09%								
(42) <i>B. diaphanus</i> ENT6090	10,97%	0,00%							
(43) <i>B. diaphanus</i> ENT402	0,33%	11,32%	11,12%						
(44) <i>B. diaphanus</i> HQ967713	20,84%	21,01%	21,02%	20,42%					
(45) <i>B. diaphanus</i> JQ935625	12,22%	15,23%	14,95%	12,62%	18,08%				
(46) <i>B. diaphanus</i> KX141354	10,97%	0,66%	0,65%	10,46%	16,30%	11,83%			

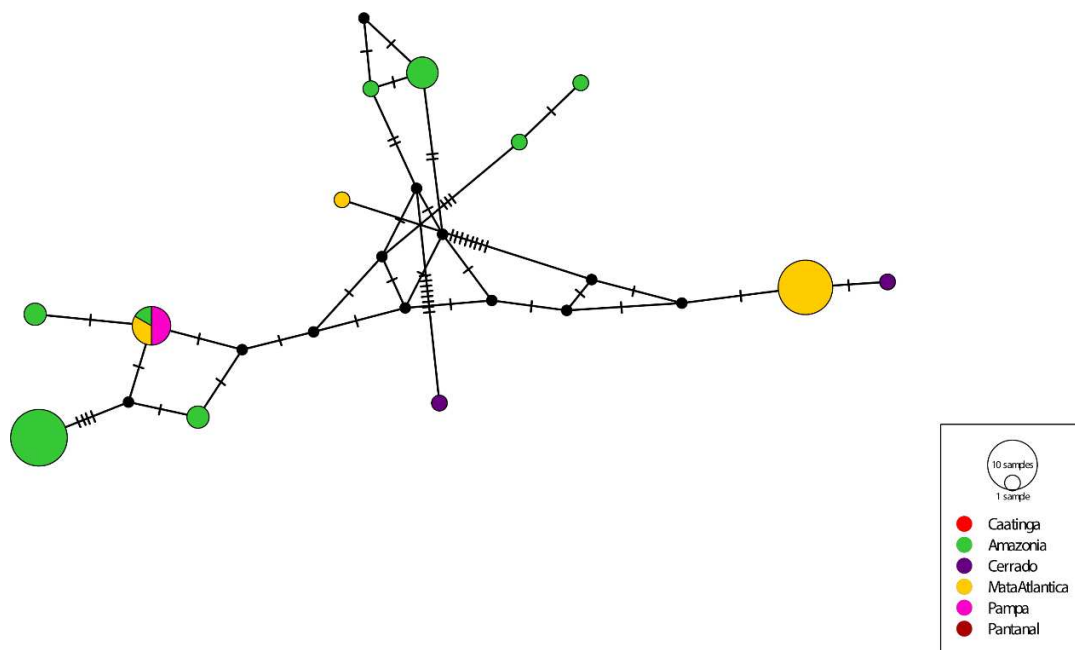
(47) <i>B.diaphanus</i> KY983371	11,36%	9,10%	8,95%	9,74%	18,92%	12,75%	7,82%	
		20,63%	20,25%	18,50%	21,52%	20,32%	18,44%	19,44%
(48) <i>Blepharopus</i> sp HM424750	18,93%							



Supplementary material 4. Haplotype network of COI sequences of *B. diaphanus*. Colours indicate hydrographic regions of haplotypes according to legend. Size of circles related to the frequency of haplotypes and dashes on branches represent the number of mutations between haplotypes.



Supplementary material 5. Haplotype network of COI sequences of *B. diaphanus*. Colours indicate biomes of haplotypes according to legend. Size of circles are related to the frequency of haplotypes and dashes on branches represent the number of mutations between haplotypes.



CONSIDERAÇÕES FINAIS

Com intuito de responder aos objetivos do presente trabalho, a diversidade morfológica e molecular de *Blepharopus diaphanus* no Brasil foi analisada em detalhe. Foi encontrada a presença um complexo de espécies crípticas, uma vez que se observou distinção molecular e separação entre as sequências nas análises, mas embora variações morfológicas tenham sido observadas, elas não foram consistentes com as linhagens detectadas. Para avaliar se populações mais próximas eram mais semelhantes entre si, foram calculadas as distâncias K2P, não sendo possível determinar um padrão. As distâncias intraespecíficas foram altas entre todas as populações e valores baixos foram observados apenas dentro desses grupos.

Não foi observada uma estruturação geográfica clara para a diversidade genética estudada, com algumas linhagens restritas a alguns locais e outras com distribuição mais ampla. Isso indica a necessidade de uma investigação mais abrangente, incluindo dados de mais populações de *Blepharopus diaphanus*, especialmente de localidades adicionais no corredor árido (Caatinga, Cerrado e Chaco).