JHON FABER MARULANDA LOPEZ

REVISING THE NEOTROPICAL GENUS *Americabaetis* KLUGE, 1992 (EPHEMEROPTERA: BAETIDAE): PHYLOGENETIC CLASSIFICATION, NEW SPECIES DESCRIPTION, IDENTIFICATION TOOLS, AND ADDITIONAL RECORDS

Thesis submitted to the Entomology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Gustavo Ferreira Martins

VIÇOSA - MINAS GERAIS
2024

Ficha catalográfica elaborada pela Biblioteca Central da
Universidade Federal de Viçosa - Campus Viçosa

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2024

Marulanda Lopez, Jhon Faber, 1994-
Revising the neotropical genus *Americabaetis* Kluge, 1992 (Ephemeroptera: Baetidae) : phylogenetic classification, new species description, identification tools and additional records / Jhon Faber Marulanda Lopez. - Viçosa, MG, 2024.
1 tese eletrônica (152 f.): il.

Texto em inglês.
Inclui anexos.
Orientador: Gustavo Ferreira Martins.
Tese (doutorado) - Universidade Federal de Viçosa, Departamento de Entomologia, 2024.
Inclui bibliografia.
DOI: https://doi.org/10.47328/ufvbbt.2024.478
Modo de acesso: World Wide Web.

1. Baetinae - Filogenia. 2. Baetinae - Classificação. 3. Aprendizado do computador. I. Martins, Gustavo Ferreira, 1980-. II. Universidade Federal de Viçosa. Departamento de Entomologia. Programa de Pós-Graduação em Entomologia. III. Título.

CDD 22. ed. 595.734

Bibliotecário(a) responsável: Alice Regina Pinto Pires CRB-6/2523

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APPROVED: July 31, 2024.

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To God, to my parents and to my teachers

ACKNOWLEDGEMENTS

First of all, I want to thank God for lighting my path, giving me the wisdom and patience necessary to make the best decisions and for the opportunities and people he puts in my path to help me and show me that your hand is always holding mine.

I would like to thank my family for supporting and encouraging me to study since I was a child. In fact, my educational process has always been and still is a priority for you, and I will value this for the rest of my life. I would also like to thank you for supporting my decision to come and study in Brazil, even though it meant being away from you until today. Your unconditional support has always been my driving force and motivation to continue pursuing our family dreams.

To my colleagues and fellow students at the Entomology Museum who were part of this process. I am grateful for the work we did together during the different disciplines. I am also grateful for the chats and barbecues that helped me relax and recharge my batteries. I would also like to extend a special thank you to a woman who changed my life for the better, Kelly Ramirez. Thank you very much for your unconditional support, always encouraging me to become a better person and improve myself every day. It is a privilege for me to share my achievements with you, and even more so knowing that you are part of them. Thank you.

I would like to extend my special thanks to the Federal University of Viçosa, the Graduate Program in Entomology, and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) for allowing me to study at this institution and obtain my degree. This work was carried out with support from (CAPES) – Funding Code # 88887.510807/2020-00. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

I would also like to thank MSc. Hector Jaime Rodriguez Currea, current Coordinator of the Biotechnology Laboratory of Embrujo Specialty-Coffee, my high school teacher, mentor in the world of insects, research and participation in scientific events. Thank you very much for the trust, opportunities and teachings. Knowing you has certainly been inspiring for me and positive not only for me, but also for my entire family.

I would also like to thank Dra. Lucimar Gomes Dias, a professor at the University of Caldas, Colombia, a dear professor and advisor for my undergraduate studies and a valuable friend, for teaching me and delving deeper into the world of fascinating aquatic insects, especially Ephemeroptera, and encouraging me to come and study in her country, Brazil. I would also like to thank her for introducing me to her colleagues and experts in Ephemeroptera, such as Dr. Carlos Molineri, Dr. Eduardo Domínguez and my former advisor, Dr. Frederico Salles, who contributed and continue to contribute positively to my educational process.

I would like to extend my gratitude to my former advisor, Dr. Frederico Falcão Salles, for his guidance, support and trust since I met him in Colombia and until I had the privilege of being his student. Thank you very much, Fred. Meeting you in Colombia was certainly a turning point in my life. It encouraged me even more to come to Brazil, especially knowing the quality of the person and professional who would guide me and contribute to my education, not only academically but also personally. I thank you for accepting me as your student, helping me come to Brazil and reminding you that it was an honor for me to be your student. I would also like to thank you for always encouraging me to become not only an excellent scientist, but also and most importantly, a better and more productive person for society.

Finally, and no less importantly, I would like to extend a special thank you to my current advisor, Dr. Gustavo Ferreira Martins. Thank you very much, dear professor. I have nothing but words of gratitude for you and I wish you success in your personal, family and professional life. I turned to you, professor, at a very difficult time in my life. I was facing a series of difficulties that I had managed myself. I am grateful, from the very first time I met you, for your willingness to listen to me and for calming me down and giving me peace of mind. I left your office in the best mood, thinking that if I were to continue as a student, I would continue to be one and that in the end it would work out. Today, I am pleased to share these words of gratitude with you and to thank you once again for taking on my guidance and for contributing to my learning process and personal development to this day with your valuable knowledge and experience.

Finally, I want to thank myself for never giving up on myself, on my childhood goals and dreams, for continuing to overcome my difficulties, working on my defects and insecurities and for all the effort and investment in becoming the man I am today and what I am becoming for my life and the well-being of my family.

“O único lugar onde o sucesso vem antes do trabalho é no dicionário”.

(Albert Einstein)

ABSTRACT

MARULANDA-LOPEZ, Jhon Faber, D.Sc., Universidade Federal de Viçosa, July, 2024. **Revising the Neotropical genus *Americabaetis* Kluge, 1992 (Ephemeroptera: Baetidae): phylogenetic classification, new species description, identification tools, and additional records**. Adviser: Gustavo Ferreira Martins.

Americabaetis is a mayfly genus of Baetidae family widely distributed in the neotropical region. The species are found in diverse habitats, in a broad altitudinal range, and are very abundant in lotic freshwater environments. Currently the genus is composed of 20 species, of which 13 are recorded from South America. The phylogenetic relationships between the species of *Americabaetis* are unknown, some Argentine species were poorly described and their assignment in the genus remains uncertain. On the other hand, the information of Baetidae and *Americabaetis* are scattered in the literature and the biological and ecological aspects remain incipiently studied. In addition, we have limited tools to aid in the identification of *Americabaetis* species by students, non-specialist and specialists. Our aim was to revise the genus *Americabaetis* and propose a classification for the genus using a phylogenetic hypothesis based on morphological data. Historic and current information of Baetidae and Americabaetis as well as the occurrence maps of the species were compiled in a literature review. A new species from Itatiaia National Park on the borders of Minas Gerais and Rio de Janeiro was described. A machine learning approach using nymphal morphological characteristics to aid in the identification of South American species was proposed as well as an illustrated interactive taxonomic key to identify the nymphs. The monophyly of *Americabaetis* genus was tested and the relationships among the species of the genus and related genera were hypothesized. A taxonomic classification consistent with the evolution hypothesis of the group was proposed. To finalize, the historic and additional records of *Americabaetis* from Colombia were updated.

Keywords: Baetinae. Phylogeny. Taxonomy. Machine learning.

RESUMO

MARULANDA-LOPEZ, Jhon Faber, D.Sc., Universidade Federal de Viçosa, julho de 2024. **Revisando o gênero Neotropical *Americabaetis* Kluge, 1992 (Ephemeroptera: Baetidae): classificação filogenética, descrição de nova espécie, ferramentas de identificação, e registros adicionais**. Orientador: Gustavo Ferreira Martins.

Americabaetis é um gênero de efemérides da família Baetidae amplamente distribuído na região neotropical. As espécies são encontradas em diversos habitats, em uma ampla faixa altitudinal, e são muito abundantes em ambientes lóticos de água doce. Atualmente o gênero é composto por 20 espécies, das quais 13 são registradas para a América do Sul. As relações filogenéticas entre as espécies de *Americabaetis* são desconhecidas, algumas espécies da Argentina foram pobremente descritas e sua atribuição no gênero permanece incerta. Por outro lado, as informações de Baetidae e *Americabaetis* estão dispersas na literatura e os aspectos biológicos e ecológicos permanecem pouco estudados. Além disso, possuímos limitadas ferramentas para auxiliar na identificação das espécies de *Americabaetis* por estudantes, não especialistas e especialistas. Nosso objetivo foi revisar o gênero *Americabaetis* e propor uma classificação para o gênero utilizando uma hipótese filogenética baseada em dados morfológicos. Informações históricas e atuais de Baetidae e *Americabaetis*, bem como os mapas de ocorrência das espécies foram compilados em uma revisão bibliográfica. Uma nova espécie do Parque Nacional de Itatiaia, na divisa de Minas Gerais e Rio de Janeiro, foi descrita. Foi proposta uma abordagem de aprendizado de máquina utilizando características morfológicas das ninfas para auxiliar na identificação de espécies sul-americanas, bem como uma chave taxonômica interativa ilustrada para identificar as ninfas. A monofilia do gênero *Americabaetis* foi testada e as relações entre as espécies do gênero e gêneros relacionados foram hipotetizadas. Foi proposta uma classificação taxonômica consistente com a hipótese de evolução do grupo. Para finalizar, foram atualizados os registros históricos e adicionais de *Americabaetis* da Colômbia.

Palavras-chave: Baetinae. Filogenia. Taxonomia. Aprendizado de máquina.

SUMMARY

1. GENERAL ORGANIZATION

This work is divided into four chapters, the first covers a bibliographical review which compiles information on the Baetidae family and the *Americabaetis* genus highlighting taxonomic, phylogenetic, biological and ecological aspects, as well as distribution maps for all species with some additional records of *A. alphus* from Colombia. The second chapter is an already published article describing a new species of *Americabaetis* from INP (10.1590/S1984-4689.v40.e22054). The third chapter is a manuscript approved for publication in Neotropical Entomology which proposes a machine learning technique to differentiate species of *Americabaeits* using morphological characters and an illustrated interactive key. The fourth chapter proposes a phylogenetic hypothesis for the species of *Americabaetis* and the diagnoses of all species. Additionally, the covers of four annexes are presented that refer to taxonomy articles with other groups of Ephemeroptera and other insects in which I participated as co-author and which were developed during my doctorate: The first annex addresses the first record of the species *Vacupernius packeri* (Ephemeroptera: Leptohyphidae) to Colombia with additional notes on the morphology of its eggs (10.18257/raccefyn.1018), the second the diversity of aquatic insects in the Selva de Florencia national natural park (PNNSF), Norcasia, Caldas (10.18257/raccefyn. 1027), the third annex is the description of a gynandromorph individual of the species *Spodoptera frugiperda* (Lepidoptera: Noctuidae) from a reared individual from Brazil (10.1590/1806-9665-RBENT-2020-0116) and the fourth is the description of the egg of a new species of *Hagenulopsis* (Ephemeroptera: Leptophlebiidae) from Brazil (10.11646/ZOOTAXA.5100.1.3). The references cited throughout each chapter, as well as their images, are compiled at the end of each section.

2. INTRODUCTION

General aspects of Ephemeroptera

Ephemeroptera is an order of amphibiotic insects, in which nymphs are aquatic and the winged stages have an ephemeral free living (Flowers & De la Rosa, 2010). These insects present an intermediary winged life stage between the nymph and adults called subimago and the adults perform a characteristic nuptial flight (Domínguez et al. 2006). Mayflies are nearly cosmopolitan with the exception of Antarctica and some remote islands (Sartori & Brittain, 2015). Currently, mayflies are represented by about 3,500 species associated with freshwater habitats worldwide (Salles et al. 2018), among these 757 species are recorded from South America (Molineri et al. 2024).

The phylogenetic relationship of Ephemeroptera with other insect orders is still discussed and the hypothesis proposed in the literature exhibits controversial results (Kristensen, 1991; Wheeler et al. 2001; Sroka et al. 2015), some authors proposed and accepted the phylogenetic relationship of Ephemeroptera as sister group of Odonata, a monophyletic group called Palaeoptera, supported by morphologic (Blanke et al. 2012) and molecular (Misof et al. 2014) characters. Mayflies are an important component of the trophic network of aquatic ecosystems (Flowers & De la Rosa, 2010) and are insects traditionally used in biomonitoring programs of hydric resources (Firmiano et al. 2017), used as laboratory model organisms (Almundi et al. 2019), and participate in numerous ecosystem and cultural services (Jacobus et al. 2019), as well biotechnological applications (Tan et al. 2018).

Baetidae

Baetidae are the most diversified group of Ephemeroptera with approximately 110 genera and 1,100 species worldwide (Sartori & Brittain, 2015). In South America, the family is represented by 30 genera and 222 species (Salles et al. 2022). The first fossil record of the family is dated to Early Cretaceous (Poinar, 2011). Its members currently inhabit both lentic and lotic freshwater habitats and are present worldwide with exception of Antarctica, New Zealand, and some remote oceanic islands (Edmunds et al. 1976).

Historically, Baetidae was considered sister group of the New Zealand family Siphlaenigmatidae (Staniczek, 1997), both included in a larger clade called Pisciforma (McCafferty, 1991) or Tridentiseta (Kluge, 2004). The phylogenetic relationships of Baetidae with other families are still not well understood, however, analysis using morphologic combined with molecular data recovered Baetidae as sister group to all other mayfly families except for Siphluriscidae (Ogden & Whiting, 2005; Ogden et al. 2009). Recently, Ogden et al. 2019 using phylogenomic data also recovered these relationships. The intrafamilial relationships of Baetidae are discussed until today (Monaghan et al. 2005; Gatolliat et al. 2008; Nieto, 2010; Smith et al. 2011).

***Americabaetis* taxonomy and phylogeny**

The genus *Americabaetis* was originally proposed by Kluge, 1992 as a subgenus of Baetis Leach, 1815 including a single species from Cuba. Two years later, Lugo-Ortiz & McCafferty (1994) synonymized *Americabaetis* with the genus *Acerpenna* Waltz & McCafferty. Posteriorly, Lugo-Ortiz & McCafferty (1996) reinstated its generic rank, transferred three species of *Acerpenna* to *Americabaetis* and described five new species from South America. Currently, the genus is represented by 20 neotropical species. The phylogenetic relationships of *Americabaetis* with other Baetidae genera exhibits controversial results, some authors proposed the *Americabaetis* genus as paraphyletic (Nieto, 2010), as sister group of the remaining Baetidae with exception of *Callibaetis* Eaton, 1881 (Smith et al. 2011), and as a monophyletic group within the Baetinae subfamily (Cruz et al. 2020). In addition, some morphological characteristics proposed by the author of the genus (Kluge, 1992), exhibit until today unclear phylogenetic significance, due to these characteristics have not been implemented in phylogenetic studies focused in the *Americabaetis* genus.

***Americabaetis* species ecology**

Some species, as *Americabaetis alphus* Lugo-Ortiz & McCafferty, 1996 and *Americabaetis longetron* Lugo-Ortiz & McCafferty, 1996 are adapted to live in areas of slow current, such as marginal vegetation and leaf deposited in the bottom, while others, as *Americabaetis titthion* Lugo-Ortiz & McCafferty, 1996 and *Americabaetis labiosus* Lugo-Ortiz & McCafferty, 1996, live in areas with strong current on slabs or large stones (Salles et al. 2022), and has been reported in macrophytes of lotic systems (Rodríguez et al. 2017). The specimens of *Americabaetis* were found in a broad altitudinal range and the genus presented positive associations with physicochemical variables of water environments (Forero-Céspedes et al. 2016; Melo et al. 2018; Arana-Maestre et al. 2021). Streams flowing through pastures presented high abundance of *Americabaetis* (Amaral et al. 2015) and some authors reported that the genus is more tolerant than other genera of the family to environmental pollution (Souza et al. 2011). The *Americabaetis* genus inhabit in many habitats, from sites without evident anthropic impact to highly impacted (Braun et al. 2014), in this context, when only the genus-resolution is used in biomonitoring and the individuals do not are identified to species level, impacts more subtle and specific are missed because the loss of species can be masked by the replacement by more tolerant species (Buss & Vitorino, 2010), such as *A. labiosus* was replaced by *A. alphus* when sewage effluents increased (Buss & Salles, 2007).

Information gaps

The phylogenetic relationships between the species of the genus are unknown. Some species need to be redescribed and also complement the available descriptions of remaining species. Although we have useful characters to differentiate some species of *Americabaetis*, the species *A. bridarollii* (Navás, 1933), *A. jorgenseni* (Esben-Petersen, 1909), *A. oldendorffii* (Weyenbergh, 1883), *A. peterseni* (Hubbard, 1974), and *A. weiseri* (Navás, 1926) present incipient descriptions (Bardavid & Nieto, 2012). In addition, these species were placed in *Americabaetis* by the absence of hind wings, body size and geographical data, and the type material is unable to locate, apparently lost or very deteriorated, therefore, the placement of these species in *Americabaetis* remains uncertain (Alba-Tercedor & Peters, 1985;

Lugo-Ortiz & McCafferty, 1999). On the other hand, useful ultrastructural characters as one's oological are incipiently studied in the group, with only three species with its egg morphology described (Kluge, 1992; Hofmann et al. 1999). Furthermore, we have limited identification tools as keys to aid in the identification of *Americabaetis* species described so far. In addition, the current records of all species in the neotropical region have not been mapped.

In this context, the revision of the species, the proposal of additional characters to complement the characters traditionally used in the group, the elaboration of hypotheses concerning the phylogenetic relationships of the species, occurrence maps and illustrated keys and bioinformatic tools to aid in the identification are necessary in way to contribute with the knowledge of the genus in the neotropical region.

Objectives

General

To revise the genus *Americabaetis* and to propose a classification for the species of the genus according to the phylogenetic hypothesis based on morphological data.

Specifics

Compile the information of Baetidae and *Americabaetis* in a literature review including maps with the actual occurrence of the species of the genus;

Describe a new species of *Americabaetis* from Rio de Janeiro, Brasil;

Propose an machine learning approach using nymphal morphological data as a taxonomic key to support the identification of South American species of *Americabaetis* as well as an illustrated interactive taxonomic key for nymphs;

Test the monophyly of the South American species of *Americabaetis* and hypothesize the phylogenetic relationships between the species of the group and other related genera using morphological data;

Compile the historic and actualize with additional records of *Americabaetis* from Colombia.

3. CHAPTER I

Exploring the biology of mayflies: a comprehensive review of Baetidae Leach 1815 and *Americabaetis* Kluge 1992 (Ephemeroptera: Baetidae) in the Neotropical region

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Short title: A review of Baetidae and *Americabaetis* in the Neotropical region

Abstract

Mayflies play a crucial role in freshwater trophic networks, impacting energy flow and serving as bioindicators of water quality in biomonitoring studies. The Baetidae family, particularly prominent in the Neotropical region, includes intriguing groups like *Americabaetis*, which require revision. This study provides a comprehensive literature review of Ephemeroptera, with a specific focus on the Baetidae family and the *Americabaetis* genus. Within Ephemeroptera, while Baetidae ranks second in diversity after Leptophlebiidae, their phylogenetic relationships remain debated. Some Baetidae species exhibit parthenogenesis, while others are ovoviviparous, making them valuable study models for reproductive biology and for understanding the costs and benefits of these sex strategies in the evolutive history of mayflies. The genus *Americabaetis* boasts twenty described species, in the Neotropical region, and showing high diversity in South America with thirteen species occurring in this region. *Americabaetis* species tolerate low oxygen levels, inhabit a wide altitudinal range, and occur in sites with varying anthropic pollution. Although water quality biomonitoring studies are the primary anthropological application of mayflies, these insects inspire artists and scientists across disciplines, continuing to impact society positively. The Baetidae family and *Americabaetis* species serve as excellent models for biogeography, biology, ecology, and biomonitoring due to their wide distribution, diversity, and abundance. Understanding and conserving mayfly diversity is essential for maintaining healthy freshwater ecosystems and sustaining the services they provide.

Key words: mayflies, Neotropic, biomonitoring, baetids, anthropological applications.

Introduction

Ephemeroptera is an order of amphibiotic insects, with aquatic nymphs and free-living adults. The winged stages of these insects can have a lifespan ranging from a few hours to a few days (Flowers & De la Rosa, 2010). An intermediate winged stage, known as the subimago, exists between the nymph and adult stages, playing a crucial role in the development of forelegs (Kamsoi et al. 2021). Mayflies are nearly cosmopolitan, except for Antarctica and some remote islands (Sartori & Brittain, 2015). They comprise 42 families, 450 genera, and about 3,500 species associated with freshwater habitats (Salles et al. 2018). Among these, 14 families, 117 genera, and 765 species are recorded in South America (Salles et al. 2022).

Mayflies play a crucial role in establishing, maintaining, and regulating the energy flow within aquatic ecosystems (Flowers & De la Rosa, 2010). Nymphs, in particular, are integral to processing organic matter in aquatic environments, representing diverse functional groups including collectors/gatherers, filterers, scrapers/grazers, shredders, and predators (Merritt & Cummins, 1996). They contribute significantly to nutrient cycling and serve as a vital food source for fish, amphibians, and other aquatic macroinvertebrates (Domínguez et al. 2006). On the other hand, adult mayflies serve as a food source for aerial predators such as insects, spiders, birds, and bats (Grant, 2001).

The substantial emergence of winged stages from aquatic environments also contributes to the removal of contaminants in freshwater systems, the aquatic nymphs bioaccumulate nitrates and sulfates from water and these compounds return to terrestrial environments when the subimagos emerges for free terrestrial life (Edmunds et al. 1976). Furthermore, there are reports of bioaccumulation of metals such as mercury, lead, copper, and zinc in nymphs of species like *Baetis pavidus* Müller-Liebenau, 1969 (Bouchelouche & Arab, 2020).

The phylogenetic relationship of Ephemeroptera with other insect orders remains a subject of debate. Some authors place Ephemeroptera as a sister group of Odonata (Misof et al. 2014), while others suggest it as a sister group of Neoptera (Kristensen, 1991), or even of Odonata+Neoptera (Wheeler et al. 2001). Additionally, considering extinct lineages, studies propose a relationship with Coxoplectoptera (Sroka et al. 2015). Conversely, our understanding of the intrafamilial relationships of Ephemeroptera (Figure 1) is hindered by numerous evolutionary convergences or homoplasies in many characters, resulting in two polytomies in the tree, with relationships between families not clearly defined, and remaining a topic of debate (Ogden et al. 2009; Sartori & Brittain, 2015).

Among the 42 families of Ephemeroptera, Baetidae, Leptophlebiidae, and Heptageniidae stand out as the most diversified. Particularly, Baetidae is a globally distributed family that exhibits high taxonomic and ecological diversity in the Neotropics (Sartori & Brittain, 2015). Baetidae is considered the sister group of all other families of Ephemeroptera except for Siphluriscidae (Figure 1) (Ogden et al. 2019). The intrafamilial relationships within Baetidae remain a topic of discussion; the Baetinae and Cloeoninae subfamilies have been recognized based on morphological characters (Kazlauskas, 1972; Riek, 1973), other authors do not recovery these subfamilies (Nieto, 2010; Smith et al. 2011), and although the division of the family into only two subfamilies is likely oversimplified and may include genera that require further revision (Gattolliat et al. 2008).

Nymphs of Baetidae inhabit both lotic and lentic environments and can be found across a wide range of freshwater habitats and microhabitats (Salles et al. 2018). They exhibit diverse feeding habits, with some nymphs consuming organic matter, while others graze or scrape, algae and bacteria (Domínguez et al. 2006). Additionally, certain genera within Baetidae are predatory or carnivorous, preying on small Baetidae nymphs, Simuliidae, and Chironomidae larvae (Agnew, 1962; Mol, 1986).

Among the genera still awaiting revision, *Americabaetis* merits particular attention. For the twenty described species, 13 occurring in South America, four from Central America, and three from the Caribbean region (Table 1). The phylogenetic relationships of *Americabaetis* with other genera within Baetidae remain unclear (Nieto, 2010; Cruz et al. 2020), and the interrelationships among *Americabaetis* species are yet to be elucidated.

The genus *Americabaetis* is prevalent in lotic freshwater environments and shows positive associations with various physicochemical water variables, useful in the evaluation of water quality such as the electrical conductivity, oxygen availability, total solids and phosphates (Forero-Céspedes et al. 2016; Melo et al. 2018; Arana-Maestre et al. 2021). Additionally, *Americabaetis* species display varying degrees of tolerance to anthropogenic pollution (Bruss & Salles, 2007). Due to its wide distribution, abundance, and diversity, *Americabaetis* serves as a valuable model for biogeographic and biomonitoring studies. However, biological and ecological aspects of its species remain poorly studied.

Despite their crucial roles in numerous ecosystem services and their utility in biomonitoring studies, mayflies remain relatively obscure among non-ephemeropterologists. The limited familiarity with this group among non-specialist academic communities hinders the development of novel interdisciplinary research that could contribute to a better understanding of poorly studied aspects of mayflies, such as their biology and ecology. The primary objective of this study was to provide a literature review to summarize the current knowledge of Ephemeroptera, with a particular focus on the Baetidae family and *Americabaetis* genus. Additionally, the study aimed to identify information gaps and propose future interdisciplinary research avenues that could enhance taxonomic and systematic knowledge of mayflies.

Material and methods

The cited articles were sourced from the websites Scopus, Web of Science, ScienceDirect, and Google Scholar using specific search terms: reproduction, taxonomy, evolution, phylogeny, biology, ecology, Ephemeroptera, Baetidae, *Americabaetis*, trade-off, and fitness. These terms were considered in the article title, keywords, summary, and body. The articles were published in English until 2024. The article selection process involved thorough reading, with inclusion criteria focused on the traditional knowledge, the relevance, innovation, and citation. The geographic coordinates used in the maps were obtained from the following publications: Kluge, 1992; Lugo-Ortiz & McCafferty, 1994; Lugo-Ortiz & McCafferty, 1996a; McCafferty et al. 2004; Hofmann et al. 1999; Waltz & McCafferty, 1999; Wiersema & McCafferty, 1999; Randolph & McCafferty, 2000; Randolph & McCafferty, 2001; McCafferty et al. 2004; Lopes & Lazo, 2005; Meyer et al. 2008; McCafferty, 2011; Molineri et al. 2020; Rojas Peña et al. 2021; Cruz et al. 2023; Marulanda-Lopez & Salles, 2023. The additional records from Colombia of the species *Americabaetis alphus* Lugo-Ortiz & McCafferty, 1996b were obtained of identified material at genus level deposited in the Colección de Insectos de la Universidad del Quindío (CIUQ).

Traditional taxonomy

The study of mayflies benefits greatly from examining both their aquatic nymphs and aerial adults, each exhibiting distinct morphological characteristics valuable for taxonomic analysis. Nymphs, being long-lived and more abundant in biological collections compared to the short-lived adults, serve as a rich resource for taxonomic characters, particularly evident in groups like Baetidae (Edmunds & Allen, 1966). However, in certain families such as Leptophlebiidae, adult stages exhibit more discernible traits (Edmunds & Allen, 1966). Nymphal features like mouthparts, legs, abdominal gills, and, in some cases, the shape of paraprocts in groups like Baetidae, serve as key taxonomic characters (Edmunds & Allen, 1966). Conversely, male adult characteristics including eyes, wings, abdominal color patterns, and genitalia structure are pivotal for taxonomic delineation (Zhang et al. 2020). Understanding both nymphs and adults of a species is crucial for validating the species concept, proposing phylogenetic relationships, and establishing a classification aligned with group phylogeny (Edmunds & Allen, 1966). Various approaches, such as implementing linear measurements for morphological variation analysis (Funk et al. 2008) and utilizing geometric morphometry to define body-shape traits, have been instrumental in refining taxonomy within aquatic macroinvertebrates, including mayflies (Orlofske & Baird, 2014).

Other morphological structures, such as the bullae (Domínguez & Abdala, 2019), have attracted attention from researchers and serve as valuable sources for characterizing evolutionary processes (Domínguez et al. 2023). Detailed examination of egg characteristics has been proposed as a means to derive useful taxonomic characters (Koss, 1969). Egg morphology proves invaluable for comparative studies, encompassing characteristics like chorion sculpture, fixation structures, and micropila shape and location (Koss & Edmunds, 1974). Moreover, egg morphology aids in discriminating cryptic species within *Campylocia* Needham & Murphy 1924 (Gonçalves et al. 2017). Conversely, oology serves as a valuable tool for linking nymphs and adults of species such as *Vacupernius packeri* (Allen, 1967) (Bacca et al. 2020), due to in some groups as Terpidinae subfamily (Leptophlebiidae), the egg morphology is similar in the nymphs and adults and constitutes an useful tool to associate stages of the same species (Marulanda-Lopez et al. 2021). Oological characters complement traditional morphological characters in phylogenetic analyses, enriching our understanding of evolutionary relationships (Domínguez & Cuezzo, 2002).

Molecular tools in Ephemeroptera taxonomy

Molecular data has played a pivotal role in bridging the life stages of Ephemeroptera, utilizing techniques such as DNA barcoding (Dias et al. 2011; Molina et al. 2017). This approach has been invaluable for species discrimination (Webb et al. 2012). A notable challenge in Ephemeroptera taxonomy is the little association between nymphs and adults, with only 10% of South American species documented across both stages (Domínguez et al. 2006). This disparity poses significant hurdles in biodiversity studies, potentially resulting in misestimation of diversity. However, the barcoding technique has effectively tackled this issue across various taxa, including the Hermanella complex (Domínguez and Flowers, 1989), *Andesiops peruvianus* (Ulmer, 1920), *Haplohyphes* (Allen, 1966), and *Leptohyphodes inanis* (Pictet, 1843) (Hoyos et al. 2014; Salles et al. 2016; Souto et al. 2021; Mera et al. 2023).

The cytochrome c oxidase I (COI) molecular marker stands out as the most used tool in taxonomic studies due to its standardized DNA region, making it ideal for comparative analysis and enabling taxonomists to compare new taxa with existing ones (Ball et al. 2005). This standardization helps reduce the likelihood of new species being erroneously synonymized (Godfray, 2002). Moreover, the establishment of DNA libraries containing mayfly sequences facilitates various research endeavors, including biomonitoring, biological, and ecological studies, in addition to supporting taxonomic work and phylogenetic hypotheses (Cardoni et al. 2015; Morinière et al. 2017). However, the graphical representations in the form of similarity trees derived using this marker should not be interpreted as phylogenetic trees of species. Nevertheless, DNA-based approaches in taxonomy also pose further limitations, such as the absence of an evolutionary rationale for variations in genetic distances among species within a lineage (Monaghan & Sartori, 2009).

Biology, ecology and ethology

The amphibiotic life cycle of mayflies gives rise to an evolutionary dichotomy, subjecting them to distinct selective pressures in both aquatic and terrestrial habitats. Consequently, winged stages are reduced to serving reproductive and dispersal functions (Edmunds & Allen, 1966; Brittain, 1982; Brittain, 1990) (Figure 2). In mayflies, sexual maturation begins during the final nymphal instars (Domínguez et al. 2006; Sartori & Brittain, 2015), with eggs and sperm becoming physiologically mature within hours or days of emergence as subimagoes (Brinck, 1957; Soldán, 1979). The subimago then undergoes one additional molt (Figure 3) before reaching reproductive adulthood.

Some species exhibit facultative parthenogenesis. The short and fragile adult life stage of mayflies results in limited dispersal capacity and occasional mate limitation, which could favor parthenogenesis. In this context, the trade-off between reproductive modes in mayflies represents a potential evolutionary strategy to combine the advantages of sexual and parthenogenetic reproduction (Liegeois et al. 2021). The incidence of parthenogenesis in Ephemeroptera species is of 1.8 (Liegeois et al. 2021) and is comparable with other groups of invertebrates, such as in orders with haplodiploid sex determination where it varies between 0 to 1.5% (Van der Kooi & Schwander, 2014). This phenomenon also significantly impacts population genetic structure (Monaghan & Sartori, 2009), with its occurrence varying across different families. In certain families, such as Baetidae, Ameletidae, and Ephemerellidae, parthenogenesis occurs more frequently than in families like Leptophlebiidae, Heptageniidae, and Ephemeridae (Liegeois et al. 2021).

The enlarged eyes of male mayflies play pivotal roles in reproduction, enhancing acuity and sensitivity to locate females. Additionally, the greatly elongated forelegs serve a crucial function in grasping and holding females during copulation (Domínguez et al. 2006). The reproductive system of mayflies is distinctive. Unlike other insect groups, males lack accessory glands crucial for sperm activation, nutrition, and providing behavioral stimuli to females (King et al. 2011). Moreover, mayflies lack an ejaculatory duct; instead, the deferent ducts open directly onto paired genital openings. Females, on the other hand, lack spermatheca, and fertilization occurs directly in the lateral oviducts during mating, facilitated by the male’s introduction of the pair of deferent ducts into the female’s tract. Sperm is propelled through the contraction of intrinsic muscles within these ducts, suggesting a brief interval between mating, egg fertilization, and oviposition (Brito et al. 2012; Araújo et al. 2021). In the family Leptophlebiidae, spermatozoa are typically aflagellate and immotile, although exceptions exist such as in the species *Thraulodes* *latinus* Cortes, Dias & Araújo 2021 (Araújo et al. 2021). In contrast, in families like Ephemeridae, Baetidae, and Heptageniidae, spermatozoa are flagellate and mobile (Mencarelli et al. 2014; Brito et al. 2015).

Mayflies typically lay between 400 to 3,000 eggs during oviposition, with individual species exhibiting extremes ranging from as few as 100 eggs to as many as 12,000 eggs. Oviposition methods vary among species: while most lay eggs intermittently, releasing a few at a time until the process concludes, others deposit all eggs at once or scatter them explosively into the water surface. Certain species even swim underwater to complete oviposition (Domínguez et al. 2006). Ovoviviparity is observed in some species of *Callibaetis* Eaton, 1881 in North America and in most *Cloeon* Leach, 1815 species across the Old World, meaning egg development occurs within the female's body until hatching (Sartori & Brittain, 2015).

Ephemeroptera species exhibit a higher degree of temperature dependence compared to other aquatic insect orders such as Plecoptera. Mayflies undergo a variable number of nymphal instars, ranging from 10 to 50 with an average of 10-25, influenced by factors such as species variation, nutritional status, and temperature (Brittain, 1982). Moreover, mayflies rely on algae as a primary food source, particularly thriving in warmer environments and during summer months (Brittain, 1990). This ample food availability leads to accelerated larval growth rates, increased adult body mass, heightened fecundity, larger egg sizes, and shortened emergence times (Scrimgeour & Culp, 1994). Conversely, in species like the Baetidae *Callibaetis ferrugineus* Walsh, 1862, adult body size and emergence times remain unaffected by predator presence (Caudill & Peckarsky, 2003), underscoring the intricate balance between environmental factors and life history traits in mayflies.

Anthropological applications

The mayflies are insects traditionally used in biomonitoring programs of hydric resources (Fikri et al. 2016; Pandiarajan et al. 2019), due to the differential response of their assemblages to anthropic pollution (Firmiano et al. 2017). Mayflies served as a fount of inspiration for a wide spectrum of scientists across various disciplines, transcending the realms of aesthetics and medicine. Whether through their life cycle, ephemeral existence, or intricate morphology, mayflies continue to captivate minds and positively impact society through their contributions to market, art, science, and cultural symbolism. In 2003, Nike introduced a line of ultralight running shoes dubbed Mayfly™, featuring intricate wing venation patterns in their design, further reflecting the fascination with these creatures (Macadam & Stockan, 2015). Beyond fashion, mayflies have also found application in biotechnology. Their deceased bodies have been utilized in synthesizing low-molecular-weight chitosan, exhibiting promising antiproliferative effects in various cancer cell lines, with apoptosis induction identified as the mechanism of cell death (Tan et al. 2018).

Species like *Cloeon dipterum* Linnaeus 1761 serve as laboratory model organisms for studying insect evolution (Almundi et al. 2019). Additionally, mayflies, in general, play crucial roles in various ecosystem services such as serving as food sources, contributing to bioturbation and bioirrigation, as well as cultural services including fishing, serving as inspiration for artists, and being celebrated in festivals worldwide (Jacobus et al. 2019). For instance, *Ephemerella jinghongensis* Xu, You & Hsu, 1984 (Ephemerellidae) is a commonly consumed species in China, providing a protein content of approximately 66% (Chen et al. 2009). Notably, Ephemeroptera species are not only rich in protein but also contain essential minerals, amino acids, B vitamins, and have low fat and moisture content (Bergeron et al. 1988). Expanding into the realm of entomophagy, the abundance of mayflies emerging from rivers like the Mississippi can be harnessed for animal or human consumption (Macadam & Stockan, 2015).

The Tiszavirág Bridge, which spans the River Tisza in Szolnok, Hungary, is a remarkable architectural tribute to the mayfly, designed to emulate the graceful form of the Tisza mayfly species *Palingenia longicauda* (Olivier, 1791), known for its mass emergence in mid-June (Macadam & Stockan, 2015). This species holds a place immortalized in a Hungarian folk song as a symbol of enduring love (Szent-Ivany & Ujházy, 1973).

Baetidae

Baetidae stands out as the most diverse group within Ephemeroptera, boasting approximately 110 genera and 1,100 species worldwide (Sartori & Brittain, 2015). In South America alone, this family is represented by 30 genera and 240 species (Salles et al. 2022). Its evolutionary history traces back to the Early Cretaceous, marking the first fossil record (Poinar, 2011). These mayflies are ubiquitous inhabitants in all types of freshwater ecosystems, thriving across the globe with the exception of Antarctica, New Zealand, and select remote oceanic islands (Edmunds et al. 1976). The nymphs of most genera within the Baetidae are primarily found in lotic environments, although certain genera such as *Cloeon* and *Callibaetis* inhabit lentic waters and they exhibit remarkable adaptability, occupying a diverse array of habitats and microenvironments, and demonstrate tolerance to low oxygen levels in water (Salles et al. 2018).

Baetidae was grouped as a sister lineage to the New Zealand family Siphlaenigmatidae, within the larger clade known as Pisciforma (McCafferty, 1991) or Tridentiseta (Kluge, 2004) using morphological data. Although the precise phylogenetic relationships of Baetidae with other families remain somewhat elusive, combined analyses of morphological and molecular data have consistently positioned Baetidae as the sister group to all other mayfly families, except for Siphluriscidae (Figure 1) (Ogden & Whiting, 2005; Ogden et al. 2009; 2019).

The intrafamilial relationships within Baetidae continue to be a subject of ongoing discussion and investigation. Initially, the family was divided into two subfamilies, Cloeoninae and Baetinae, based on the number of intercalary veins in the fore wings: one for Cloeoninae and two for Baetinae (Kazlauskas, 1972). This traditional division was further supported by additional characteristics, leading to the recognition of the subfamily Callibaetinae (Riek, 1973). However, later studies led to the synonymization of Callibaetinae with Cloeoninae (Landa & Soldan, 1985; Gillies, 1991). Some authors maintain the distinction between Baetinae and Cloeoninae (Landa & Soldan, 1985; Monaghan et al. 2005). Nonetheless, dividing the family into just two subfamilies may oversimplify its complexity, and certain genera may still require revision (Gattolliat et al. 2008). Furthermore, analyses utilizing both parsimony and maximum likelihood methods have not consistently supported the existence of these subfamilies (Nieto, 2010; Smith et al. 2011).

Recently, the first comprehensive global cladistic analysis of the Baetidae family was published, utilizing morphological data. This study revealed that the traditional subfamilies Cloeoninae and Baetinae were polyphyletic and monophyletic, respectively (Cruz et al. 2021). Another classification system proposed for Baetidae is based on a more intuitive approach known as *Cladoendesis*. According to this, Baetidae is classified such as the lineage Liberevenata Kluge, or *Baetis/fg3*, and is divided into two main groups: the extinct plesiomorphon *Palaeocloeon/fg1* and Turbanoculata Kluge or *Baetis/fg4*. Turbanoculata, in turn, is further subdivided into the plesiomorphon Protopatellata Kluge & Novikova and Anteropatellata Kluge or *Baetis/fg5*. Within Anteropatellata, the primary group is Baetovectata Kluge & Novikova or *Baetis/fg6*, characterized mainly by the presence of two intercalary veins in the fore wings (Kluge, 1997; 2004; Kluge & Novikova, 2011).

The feeding habits of Baetidae nymphs are equally diverse. Most nymphs feed on suspended organic matter in the water column or consume organic material deposited in the substrate, while others function as grazers or scrapers, feeding on algae and bacteria (Domínguez et al. 2006). However, there are exceptions to this general trend. For instance, nymphs of *Harpagobaetis* Mol, 1986, are predatory, preying on Diptera larvae (Mol, 1986). Similarly, nymphs of *Centroptiloides bifasciatus* (Esben-Petersen, 1913) exhibit carnivorous behavior, targeting small Baetidae nymphs, Simuliidae, and Chironomidae larvae as their primary prey (Agnew, 1962).

The emergence of subimagos of Baetidae typically occurs in the evening and afternoon, followed by the nuptial flight, which generally takes place in the afternoon, often around midday. Male imagos exhibit a distinctive eye structure, with the dorsal portion hyper-developed, forming a turbinate eye (Figure 4). In some species or among certain sexes, hind wings may be absent. Nymphs of Baetidae exhibit a pisciform body shape, enabling them to navigate water proficiently as skilled swimmers (Domínguez et al. 2006) (Figure 5).

Americabaetis ecology

The *Americabaetis* species display remarkable adaptability across diverse habitat types. Certain species, such as *A. alphus* and *Americabaetis longetron* Lugo-Ortiz & McCafferty, 1996b, are specially adapted to inhabit areas with slow currents, often found amidst marginal vegetation and leaf litter on the riverbed. Conversely, species like *Americabaetis titthion* Lugo-Ortiz & McCafferty, 1996b and *Americabaetis labiosus* Lugo-Ortiz & McCafferty, 1996b prefer habitats with strong currents, commonly found on slabs or large stones (Salles et al. 2022). Moreover, reports indicate the presence of *Americabaetis* within macrophytes (Rodríguez et al. 2017).

Specimens of *Americabaetis* have been collected across an altitudinal range spanning from 398 to 2,411 meters above sea level, with the genus showing positive associations with average river width (Arana-Maestre et al. 2021). Additionally, *A. alphus* exhibits positive correlations with water transparency, depth, and electrical conductivity (Melo et al. 2018), while *Americabaetis robacki* (Lugo-Ortiz & McCafferty, 1994) demonstrates positive associations with various water quality parameters such as alkalinity, hardness, electrical conductivity, oxygen availability, temperature, total solids, and phosphates (Forero-Céspedes et al. 2016).

Studies have also highlighted the abundance of *Americabaetis* in streams flowing through pastures, with some authors noting its lower sensitivity compared to other genera within the family to environmental pollution (Amaral et al. 2015; Souza et al. 2011). This moderate sensitivity and tolerance of *Americabaetis* may explain its widespread distribution across habitats ranging from minimally impacted to highly impacted environments (Braun et al. 2014). However, reliance solely on *Americabaetis* abundance in biomonitoring studies may obscure more subtle and specific environmental impacts, as the loss of species can be masked by the dominance of more tolerant species (Buss & Vitorino, 2010). For example, *A. labiosus* was displaced by *A. alphus* when sewage effluents increased (Buss & Salles, 2007).

***Americabaetis* taxonomy**

The genus *Americabaetis* was initially introduced by Kluge in 1992 as a subgenus of *Baetis* Leach, 1815, encompassing a single species from Cuba, *Baetis* (*Americabaetis*) *naranjoi*. Later, *Americabaetis* was synonymized with the genus *Acerpenna* Waltz & McCafferty, 1987, per the work of Lugo-Ortiz & McCafferty in 1994. Subsequently, *Americabaetis* was revisited, established to its generic status, and three species originally assigned to *Acerpenna* were transferred to *Americabaetis*. Additionally, five new species from South America were described (Lugo-Ortiz & McCafferty, 1996b). In Central America, two new species were described from Guadeloupe in the eastern Caribbean Sea (Hofmann et al. 1999), along with a single species from Costa Rica (Waltz & McCafferty, 1999).

In Brazil, the genus gained attention with initial records of four species in the Rio de Janeiro state (Francischetti et al. 2003). Subsequent studies further enriched our understanding with the description of the female imago of *A. titthion* (Salles et al. 2004), and the male imago of *A. longetron*. Additionally, the first key to identify imagos of the genus was proposed (Salles & Boldrini, 2008).

A noteworthy addition to the taxonomic landscape occurred in southern Brazil with the description of a rare species, marking the debut of the first key for South American nymphs (Salles et al. 2010). Subsequently, the genus received its latest contribution from the region with the description of a species found on the borders between Minas Gerais and Rio de Janeiro states (Marulanda-Lopez & Salles, 2023). Expanding beyond Brazil, Argentina reported the discovery of five species, accompanied by the proposal of keys for both imagos and nymphs (Bardavid & Nieto, 2012).

Americabaetis **distribution**

Currently, the genus comprises a total of 20 neotropical species (see Table 1). These species are classified into three distinct groups based on their geographical patterns. The first group comprises the species with distribution in South America (Figure 6), including *A. alphus*, which holds the distinction of being the genus's most widely distributed species, with numerous records in the Neotropical region, *A. alphus* boasts one of the broadest ranges of distribution among South American species (Molineri et al. 2020). This group also includes the species that are solely recorded in Argentina (Figure 6B) and are among those whose classification within the genus remains uncertain.

The remaining two groups include the type species *A. naranjoi*, found in Cuba, along with other species occurring in other Caribbean countries such as Puerto Rico and Guadeloupe (Figure 7A). The other group comprises the species occurring in Central America (Figure 7B) including the species *Americabaetis pletura* Lugo-Ortiz & McCafferty, 1994. While *Americabaetis* are predominantly found in the Neotropics, an exception to this distribution corresponds to this species cited last, which has been documented in Texas, at the south of the USA and bordering Mexico (Wiersema & McCafferty, 1999). Future biogeographic studies aiming to hypothesize the origin of the genus can significantly contribute to our understanding of the current distribution of *Americabaetis* species. The widely distributed species, such as *A. alphus* serve as excellent model species for such studies.

Additional records of *A. alphus* from Colombia deposited at CIUQ: 15 male and 10 female nymphs, Quindío, Salento, Quebrada Boquia (4°37’28.5’’N 75°35’56.7’’W), 1691 masl; 1 female nymph, Quindío, Filandia, Corredor Colibries (4°40’7.61’’N 75°38’21.5’’W), 1819 masl, 17/v/2016, Red Surber, Lopez-Florez & Hoyos-Cardona cols.; 1 male and 1 female nymphs, Quindío, Armenia, Hojas Anchas (4°33’15.7’’N 75°40’11.5’’W), 1463 masl, 17/ii/2018 and 31/iii/2018, Red Surber, Caicedo V. col.; 3 male and 7 female nymphs, Quindío, Córdoba, Río Verde (4°24’53.6’’N 75°41’50.1’’W), 1281 masl, 13/ix/2014, manual strainer, Lievano A & Cordoba L cols.; 3 male and 4 female nymphs, Quindío, Calarca, Quebrada Negra (4°25’59.9’’N 75°39’0.0’’W), 1531 masl, 21/ii/2015, A. F. Rios col.

Unidentified species of *Americabaetis* have been previously reported from Colombia based on nymphs from the Andean Region (Muñoz & Ospina, 1999; Camargo & Rozo, 2003; Gutiérrez & Reinoso-Flórez, 2010; Forero-Céspedes & Reinoso-Flórez, 2013; Gil-Gonzáles et al. 2023), the Pacific region (Mathuriau & Chauvet, 2002; Rozo & Jimenez, 2016; Mosquera-Murillo & Mosquera-Mosquera, 2017; Mosquera-Murillo, 2017; Mosquera-Murillo, 2018, Mosquera et al. 2019; Mosquera-Murillo et al. 2024), Caribbean region (García et al. 2015; Barragán et al. 2016; Barros-Núñez & Granados-Martínez, 2016), and Orinoquia region (Romero-Lizcano et al. 2023), future studies could be identified this material and confirm the presence of *A. robacki* restricted to the Tolima department (Forero-Céspedes et al. 2014), in a more comprehensive study of *Americabaetis* in Colombia.

***Americabaetis* phylogeny**

The phylogenetic relationships between *Americabaetis* and other genera within Baetidae remain relatively understudied. However, insights have emerged from various cladistic analyses employing both morphological and molecular data. In one study focusing on South American Baetidae, *Americabaetis* was found to be paraphyletic, with certain species exhibiting closer relationships to *Tupiara ibirapitanga* Salles, Lugo-Ortiz, Da Silva & Francischetti, 2003 and *Acerpenna pygmaea* Hagen, 1861 (Nieto, 2010). Another analysis, utilizing molecular data and maximum parsimony, placed *Americabaetis* as a sister group to all other Baetidae genera, excluding *Callibaetis* (Smith et al. 2011). However, a recent hypothesis proposes *Americabaetis* as a monophyletic group within the Baetinae subfamily, based on morphological analyses (Cruz et al. 2020). Despite these advancements, uncertainties persist regarding the precise placement of *Americabaetis* within Baetovectata. Kluge (1992) originally proposed the *Americabaetis* genus without citing specific autapomorphies. However, certain characteristics such as the absence of hind wings, presence of an apical-median projection on the second segment of the labial palp, and the presence of abdominal terga in nymphs with scales in operculate sockets were noted (Kluge, 1992), though their phylogenetic significance remains unclear. Thus, the exact taxonomic position of *Americabaetis* within Baetidae, particularly within Baetovectata, remains uncertain, warranting further investigation and clarification.

Multitools and integrative taxonomy in problematic groups

The integration of morphological and molecular data has significantly enhanced taxonomic research, providing valuable insights into the relationships between individuals from various populations and species of mayflies (Garces et al. 2020; Manko et al. 2023). This multidisciplinary approach has been further bolstered by the expansion and diversification of type datasets, incorporating ultrastructural characters and quantitative analyses. An exemplary case is seen in *Andesiops peruvianus* (Ulmer, 1920), a species widely distributed across the Andean region. Studies have suggested the existence of a species complex characterized by molecular and morphological variations, particularly in the shape and size of abdominal gills and the number of denticles in nymphal tarsal claws (Ossa-López et al. 2018). Recent analyses involving individuals from various localities, including the type locality, have indicated that individuals from the type locality form a single molecular operational taxonomic unit, while suggesting the presence of a cryptic species complex elsewhere in Peru. Moreover, examination of individuals from the type locality has revealed intriguing variations in the pigmentation of tracheation in gills, supporting the hypothesis of phenotypic plasticity at the abdominal gill level (Mera et al. 2023). These findings underscore the importance of combining molecular and morphological approaches in elucidating species boundaries and understanding the complexities of biodiversity within mayfly populations.

The quantification of morphological variation through linear measurements of taxonomically significant structures, in conjunction with genetic and life-history data, provides an alternative and valuable approach for reassessing species complexes (Funk et al. 2008). Geometric morphometrics, a method used to establish body-shape trait criteria for aquatic insects, including Ephemeroptera, offers a powerful tool for this purpose (Orlofske & Baird, 2014). This geometric approach has been applied in studies examining wing shape evolution in the African family Teloganodidae (Pereira-da-Conceicoa et al. 2019). The primary goal of geometric morphometrics is to quantify morphological variation and compare it across taxa, thereby complementing the analytical tools employed by taxonomists and reducing the subjectivity inherent in traditional taxonomy studies (Zelditch et al. 2012).

The aim of integrative taxonomy is to enrich and broaden the dataset utilized in the taxonomy of a species group, integrating various morphological characters alongside molecular, ecological, and/or biological information. An intriguing study focused on *Electrogena* Zurwerra and Tomka, 1985 (Heptageniidae) species compared linear morphometry with Fourier shape descriptor analysis. The authors used Linear Discriminant Analysis, using measures of morphologic structures useful in taxonomic purposes and Fourier analysis as a geometric approach to distinguish among operational taxonomic units defined based on molecular data. This innovative geometric analysis demonstrated its potential to enhance the accuracy of species-level identification, thereby paving the way for future advancements such as computer-aided identification of mayfly taxa using taxonomically significant characteristics (Polášek et al. 2018).

Gaps and future perspectives

Despite *Americabaetis* prevalence in freshwater environments and high representation in biological collections, the phylogenetic relationships between its species remain unknown. Many species require redescriptions, and additional information is needed to complement the descriptions of existing species. Although useful characters have been proposed to differentiate *Americabaetis* species, several species such as *Americabaetis bridarollii* (Navás, 1933), *Americabaetis jorgenseni* (Esben-Petersen, 1909), *Americabaetis oldendorffii* (Weyenbergh, 1883), *Americabaetis peterseni* (Hubbard, 1974), and *Americabaetis weiseri* (Navás, 1926) have incomplete descriptions (Bardavid & Nieto, 2012). Moreover, the type material for these species is apparently lost or deteriorated, unable to locate, and consequently, the placement of these species within *Americabaetis* remains uncertain (Alba-Tercedor & Peters, 1985; Lugo-Ortiz & McCafferty, 1999). Moreover, the arbitrary classification of these species as *Americabaetis* solely based on three criteria, the lack of hind wings, body size, and geographical location (presence in Argentina) further contributes to the ambiguity regarding their classification within the genus. This underscores the need for comprehensive redescriptions and further research to clarify the taxonomy and phylogeny of *Americabaetis* species.

Traditionally, the taxonomy of *Americabaetis* relies on various morphological characters including the shape of turbinate eyes, abdominal color pattern, subgenital plate shape, and forceps shape in adults (Salles & Boldrini, 2008; Bardavid & Nieto, 2012). Nymphs are assessed based on the number of spines in the paraprocts, shape of the labial palp, size and tracheation of gills, body shape, posterior margin of terga shape, and presence of frontal keel (Salles et al. 2010). However, ultrastructural characters such as oocyte morphology remain poorly studied in the group, with egg morphology described for only three species. The shape of the chorionic ridges that confer the chorionic sculpture varies between the three species, pristine cellular sculpture for *A. naranjoi*, low polygonal concavities for *Americabaetis humilis* Hoffman & Thomas 1999 in Thomas et al. 1999, and circular or oval concavities for *Americabaetis spinosus* Hoffman & Thomas 1999 in Thomas et al. 1999, being the egg morphology as a source potential of oological characters with taxonomic value at species level (Kluge, 1992; Hoffmann et al. 1999).

Moreover, taxonomic keys that encompass all described *Americabaetis* species are currently lacking. Available keys for South American species cover only a limited number, including seven nymph species (Salles et al. 2010), six adult species (Salles & Boldrini, 2008), and five argentine species for both nymphs and adults (Bardavid & Nieto, 2012). In light of these challenges, a revision of species, proposal of additional characters to complement traditional ones, formulation of phylogenetic hypotheses, and development of illustrated keys are essential to enhance our understanding of *Americabaetis* in the neotropical region. Future analyses leveraging bioinformatic tools like geometric morphometry and machine learning hold promise for elucidating morphological variation in mayflies, thus complementing taxonomic work and phylogenetic hypotheses put forth by specialists and researchers.

Acknowledgements

We thank the Entomology program of the Federal University of Viçosa for facilitating and promoting the development of this review and the funding agencies CNPq, CAPES, and FAPEMIG for financing and supporting Brazilian science. We thank Pascal Gaudette and Amanda for allowing us to use their images in our manuscript.

Author contributions statement

JFML conducted the literature revision work, wrote the manuscript and elaborated the illustrations. GFM conceived the research, structurate the manuscript, and revised the document. All authors critically review the manuscript and approve the final version of this paper.

Funding

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, #001) to JFML (process # 88887.510807/2020-00) and by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) to GFM (# 402806/2023-9).

Conflict statement

The authors have declared that no competing interests exist.

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Table 1. Current distribution of *Americabaetis* species. Cu: Cuba, Ar: Argentina, Br: Brazil, Co: Colombia, Ch: Chile, Bo: Bolivia, Pa: Paraguay, Ur: Uruguay, Pr: Puerto Rico, Cr: Costa Rica, Ni: Nicaragua, Gu: Guatemala, Me: Mexico, Pe: Peru, Gp: Guadeloupe, USA: United States of America, Be: Belize, Sa: El Salvador, Ho: Honduras, and Pn: Panama.

Species	**Author**	**Distribution**
A. alphus	Lugo-Ortiz & McCafferty, 1996b	Ar, Br, Co, Ch, Bo, and Pa
A. boriquensis	(Lugo-Ortiz & McCafferty, 1994)	Pr
A. bridarollii	(Navás, 1933)	Ar
A. humilis	Hofmann & Thomas, 1999 in Hofmann et al. 1999	Gp
A. intermedia	(Lugo-Ortiz & McCafferty, 1994)	Cr, Gu, Me, and Ni
A. itatiaia	Marulanda & Salles, 2023	Br
A. jorgenseni	(Esben-Petersen, 1909)	Ar
A. labiosus	Lugo-Ortiz & McCafferty, 1996b	Ar, Br, Ur, and Pa
A. longetron	Lugo-Ortiz & McCafferty, 1996b	Ar, Br, Ur, and Pa
A. lugoi	Waltz & McCafferty, 1999	Cr, Me, and Ni
A. maxifolium	Lugo-Ortiz & McCafferty, 1996b	Pa
A. mecistognathus	Salles & Raimundi 2010, in Salles et al. 2010	Br and Ar
A. naranjoi (Type species)	(Kluge, 1992)	Cu
A. oldendorffii	(Weyenbergh, 1883)	Ar
A. peterseni	(Hubbard, 1974)	Ar
A. pletura	(Lugo-Ortiz & McCafferty, 1994)	Gu, Be, Ni, Cr, Me, Ho, USA, Pn, and Sa
A. robacki	(Lugo-Ortiz & McCafferty, 1994)	Pe, Ur, and Co
A. spinosus	Hofmann & Thomas 1999, in Hofmann et al. 1999	Gp
A. titthion	Lugo-Ortiz & McCafferty, 1996b	Ar and Br
A. weiseri	(Navás, 1926)	Ar

Figure legends

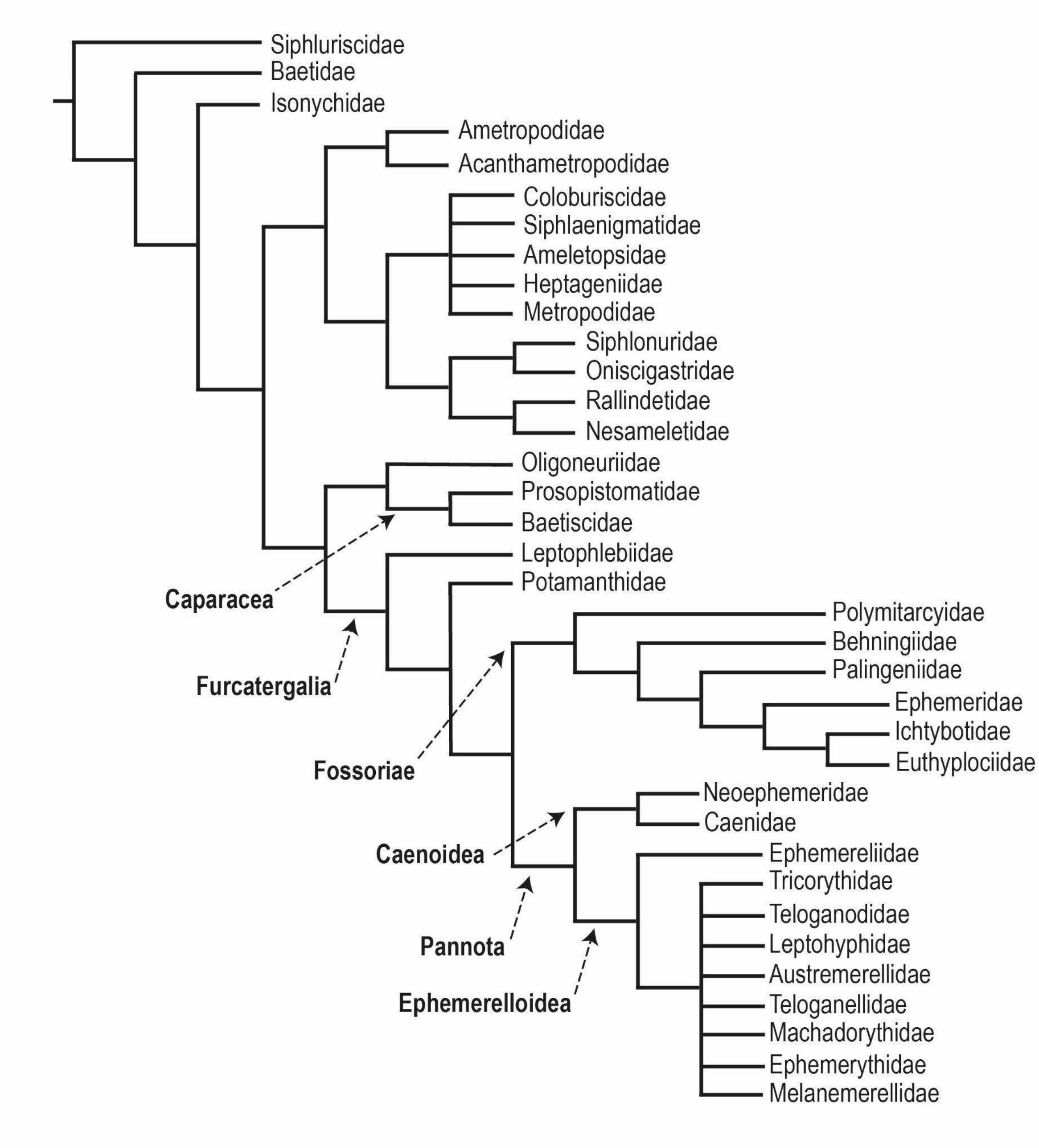

Figure 1 Phylogenetic hypothesis of the evolutive relationships among extant mayflies families; clades supported with former appropriate names are highlighted. Based from Ogden et al. 2009.

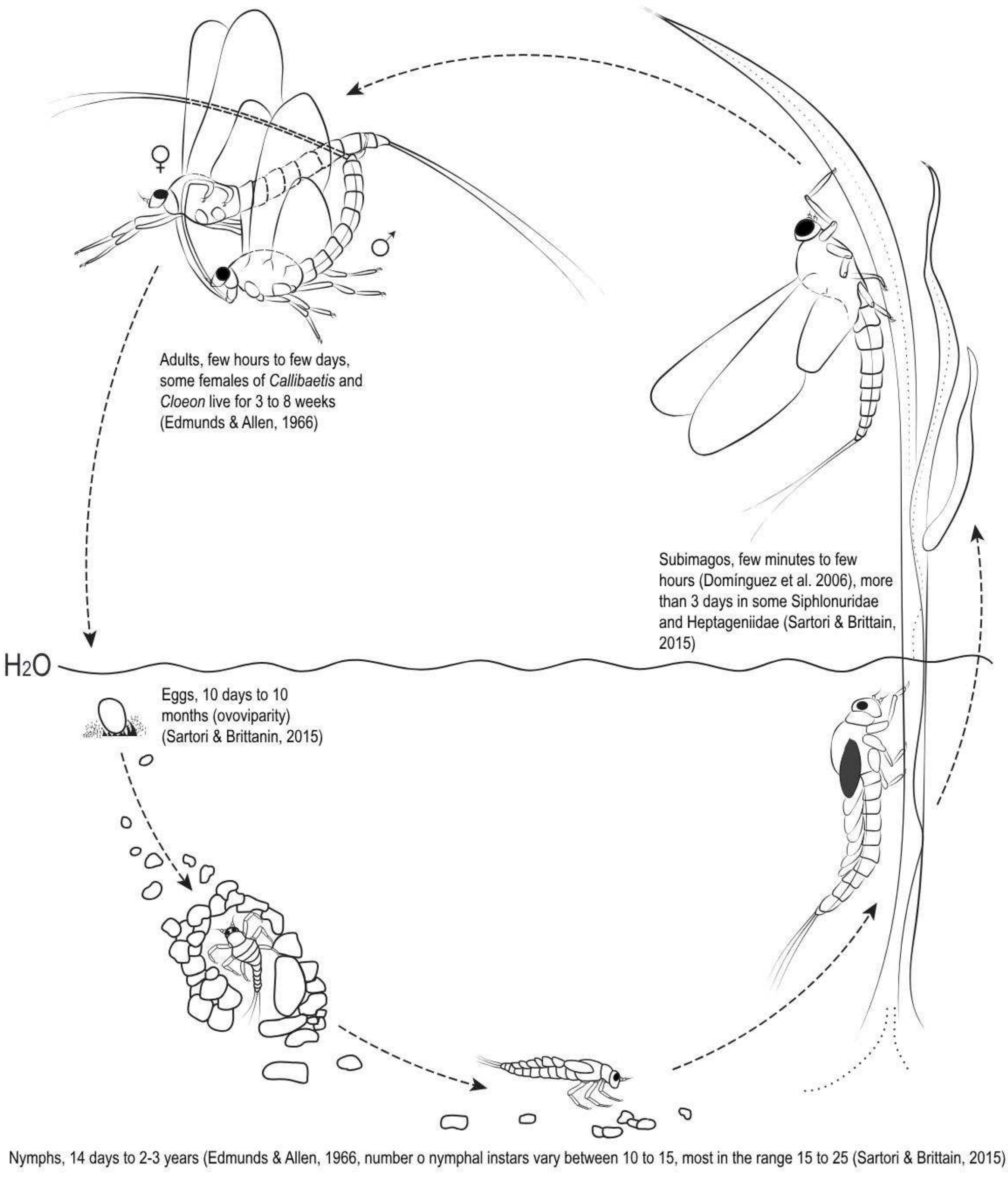

Figure 2 Schematic representation of a mayfly life cycle. Based from Studemann et al. 1992.

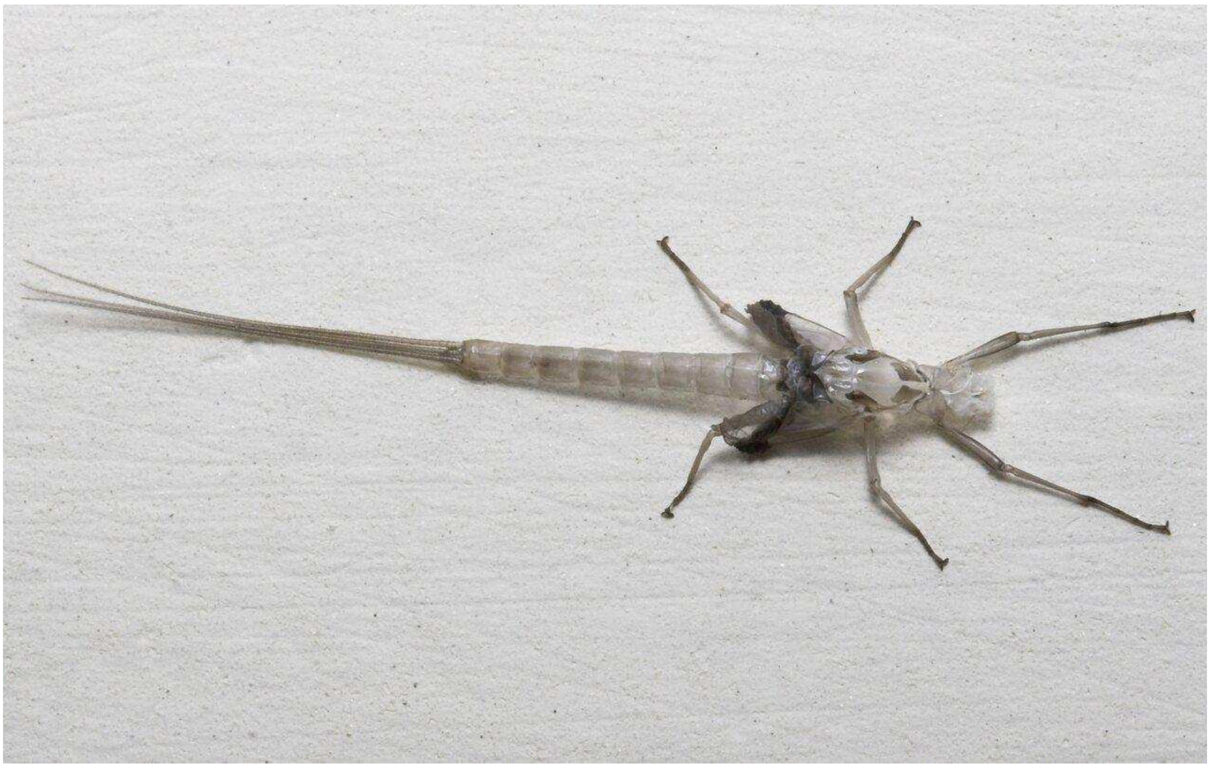

Figure 3 Exuvia of subimago of Ephemeroptera. Photograph with Creative Commons. License provided by Iašalo išnara. https://tyt.lt/picture/8643.

Figure 4 Adult male species of Baetidae, with characteristic turbinate eyes of male baetid species. Photograph with Creative Commons License provided by Pascal Gaudette. https://www.flickr.com/photos/doundounba/19049209870.

Figure 5 Nymph of *Cloeon dipterum* (Baetidae). Photograph with Creative Commons License by S.A 4.0, provided by Amanda 44/Wikimedia Commons. https://denstoredanske.lex.dk/d%C3%B8gnfluer.

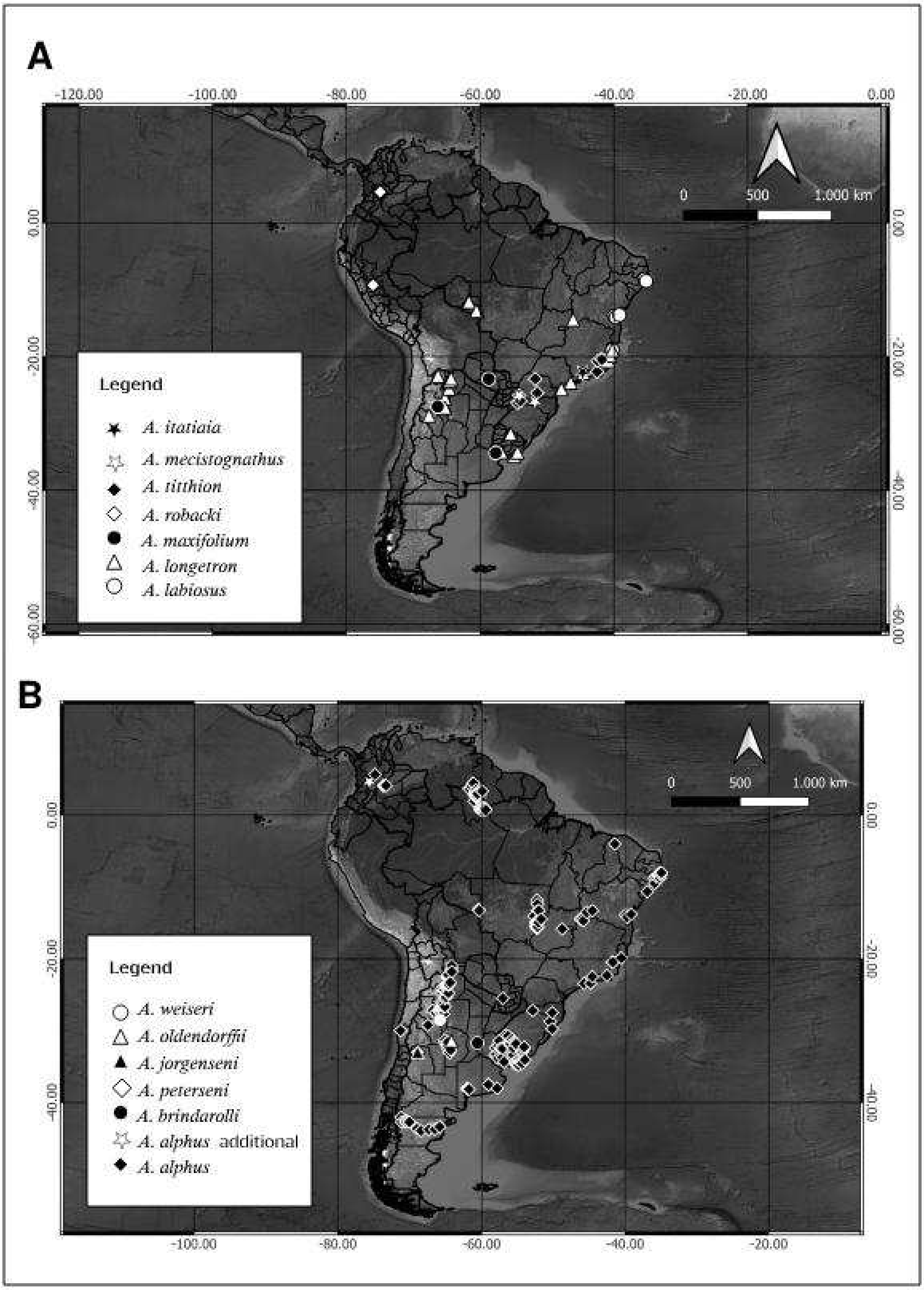

Figure 6 Distributional map showing the occurrence of *Americabaetis* species in South America.

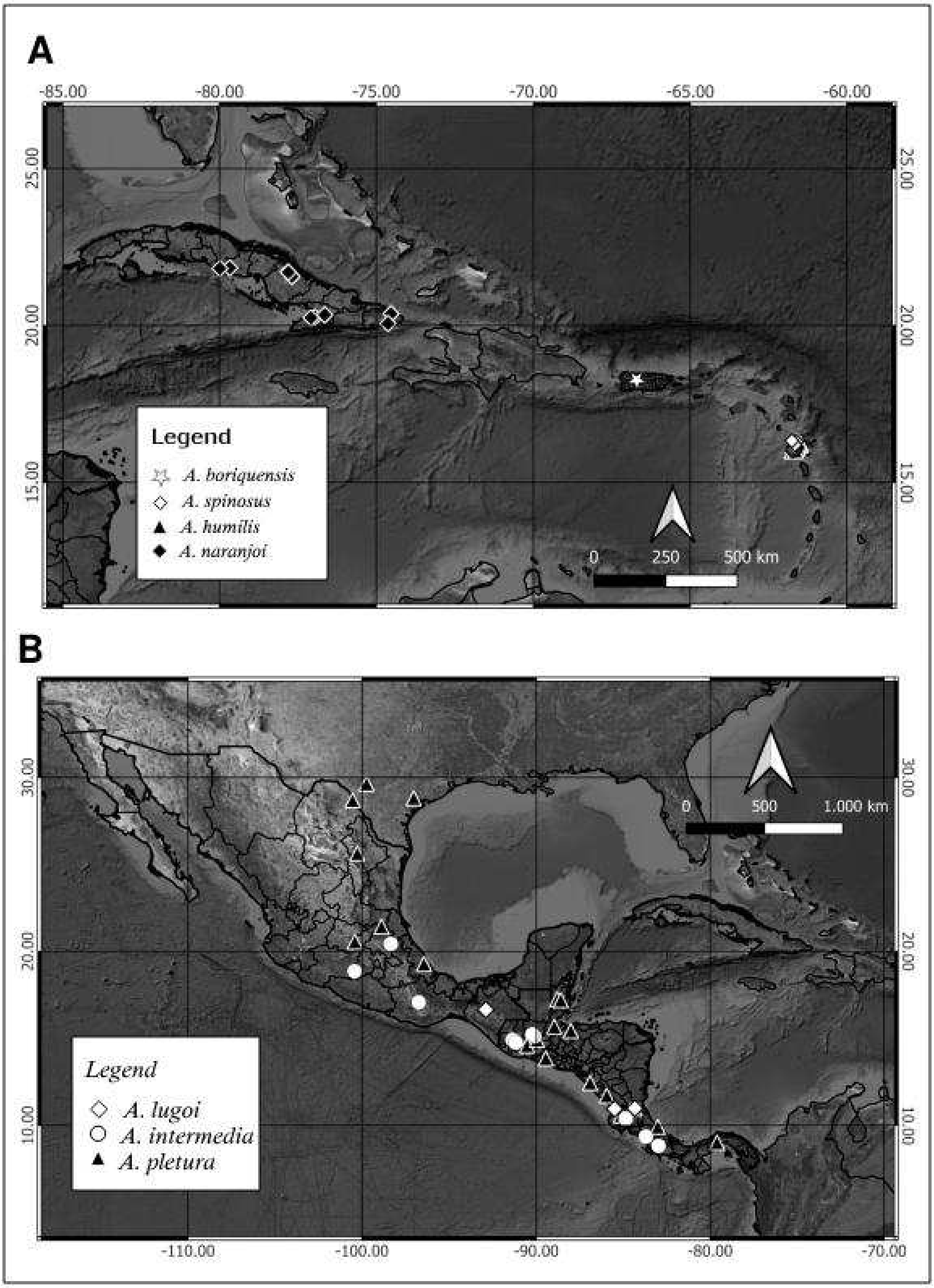

Figure 7 Distributional maps showing the occurrence of additional *Americabaetis* species with distribution in Caribbean countries and Central America. A) Caribbean countries species. B) Central America species.

4. CHAPTER II

A new species of *Americabaetis* Kluge, 1992 (Ephemeroptera: Baetidae), from Itatiaia National Park, Brazil

New species of *Americabaetis*

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Artigo publicado na Revista Brasileira de Entomologia
DOI: 10.1590/S1984-4689.v40.e22054

ZOOLOGIA 40: e22054
ISSN 1984-4689 (online)
scielo.br/zool

RESEARCH ARTICLE

A new species of *Americabaetis* (Ephemeroptera: Baetidae) from Itatiaia National Park, Brazil

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https://zoobank.org/9BC22CAB-FB71-4E9B-A77C-9113CD4A2A54

ABSTRACT. A new species of *Americabaetis* Kluge, 1992 is described based on nymphs and subimagos from Itatiaia National Park, close to the border of Minas Gerais and Rio de Janeiro, Brazil. The new species can be differentiated from the remaining species of the genus based on the following combination of characteristics: (1) frontal keel present, (2) right mandible with angle between incisors and mola obtuse, (3) paraglossa nearly twice longer than wide, (4) fore femur with 40–42 long, apically acute, simple setae in outer margin, (5) gills grayish with pigmented tracheation, (6) distinctive abdominal color pattern (two white posterolateral spots on abdominal terga IV and VII, white medial band running along abdominal terga, and lateral margins washed with brown), (7) posterior margin of abdominal terga with triangular spines, and (8) paraproct marginally with 12–14 spines. Given the development of the labial palp segment III, shape of the apex of the lingua, and robustness of the body, the new species is somewhat similar to *A. labiosus* and *A. titthion*. It can be readily distinguished from *A. titthion* by the absence of meso and metasternal projections and by the shape of spines on the posterior margin of abdominal terga (triangular on the new species, blunt on *A. titthion*). The main differences between the new species and *A. labiosus* are the paraglossa length/width ratio (2.3x in the new species, 2.5x in *A. labiosus*), the relative length of the maxillary palp (longer than galea-lacinia in the new species versus subequal in *A. labiosus*), as well as pigmentation of tracheae on gills (which is absent in *A. labiosus*). The abdominal color pattern of *A. itatiaia* sp. nov. is also very distinctive in relation to *A. labiosus* and *A. titthion*.

KEY WORDS. Baetinae, Insecta, Neotropic, taxonomy.

INTRODUCTION

Americabaetis was originally proposed by Kluge (1992) as a subgenus of *Baetis* Leach, 1815 and included a single species from Cuba. Lugo-Ortiz and McCafferty (1996), two years after proposing the synonym of *Americabaetis* with *Acerpenna* Waltz & McCafferty, 1987 (Lugo-Ortiz and McCafferty 1994), reinstated its generic rank, transferred three species from *Acerpenna* to *Americabaetis*, and described five new species from South America. Hofmann et al. (1999) described two new species from Guadeloupe and in the same year Waltz and McCafferty (1999) described a single species from Costa Rica. Currently, the genus is part of Baetinae (Cruz et al. 2021) and is represented by 19 species, 18 from the Neotropics, and one from the southern limits of the Nearctic region (Wiersema and McCafferty 1999).

Americabaetis can be differentiated from the remaining genera of Baetidae by the following combination of characteristics in the nymphs: (1) mandibles with a tuft of setae between the prostheca and mola, (2) labium with rectangular paraglossa, glossa shorter than paraglossa and labial palp segment II with a variable distomedial process, (3) hindwing pads (and hindwings) absent, (4) apically rounded gills on abdominal segments II–VII only, and (5) abdominal terga with scales in operculate sockets (Lugo-Ortiz and McCafferty 1996).

Lugo-Ortiz and McCafferty (1996) were the first authors to report the presence of *Americabaetis* in Brazil. Of the five species described by them from South America, four were described and/or reported from Brazil. Lugo-Ortiz et al. (2002) and Francischetti et al. (2003) presented the first record of the genus from states of Espírito Santo and Rio de Janeiro, respectively, while Salles et al. (2004) described the female imago stage of *A. titthion* Lugo-Ortiz & McCafferty, 1996. Later, Salles and Boldrini (2008) described the male imago of *A. longetron* Lugo-Ortiz & McCafferty, 1996 and proposed the first key for the adults of the genus. Two years after, Salles et al. (2010) revised the genus in Brazil, described a species from the southern part of the country, the male adult of *A. labiosus* Lugo-Ortiz & McCafferty, 1996; and provided a key for South American nymphs.

In the present contribution, based on nymphs and subimagos from Itatiaia National Park, on the borders of Minas Gerais and Rio de Janeiro, Brazil, we describe a new species of *Americabaetis*.

MATERIAL AND METHODS

The specimens were collected in Itatiaia National Park, close to the border of Minas Gerais and Rio de Janeiro (Fig. 1). The nymphs were collected using manual aquatic nets and some mature nymphs were reared to obtain the subimagos. The collected material was preserved in 80% ethanol. The images of habitus of the specimens were obtained using a stereomicroscope LEICA M205 A with a coupled camera LEICA MC170 HD. Mouth parts, legs, gills, and paraproct were studied and photographed in a OLYMPUS CX31 microscope coupled with a Moticam A5 camera. Morphological characteristics were illustrated on Adobe Illustrator® 2022 based on a series of images following the methodology proposed by Coleman (2003, 2006, 2009). All images and illustrations were finally processed in Adobe Photoshop® 2022.

The distributional map was made on the software QGiS 3.26.2. The collectors of the specimens were Frederico Falcão Salles (FFS), Mellis Layra Soares Rippel (MLSR),

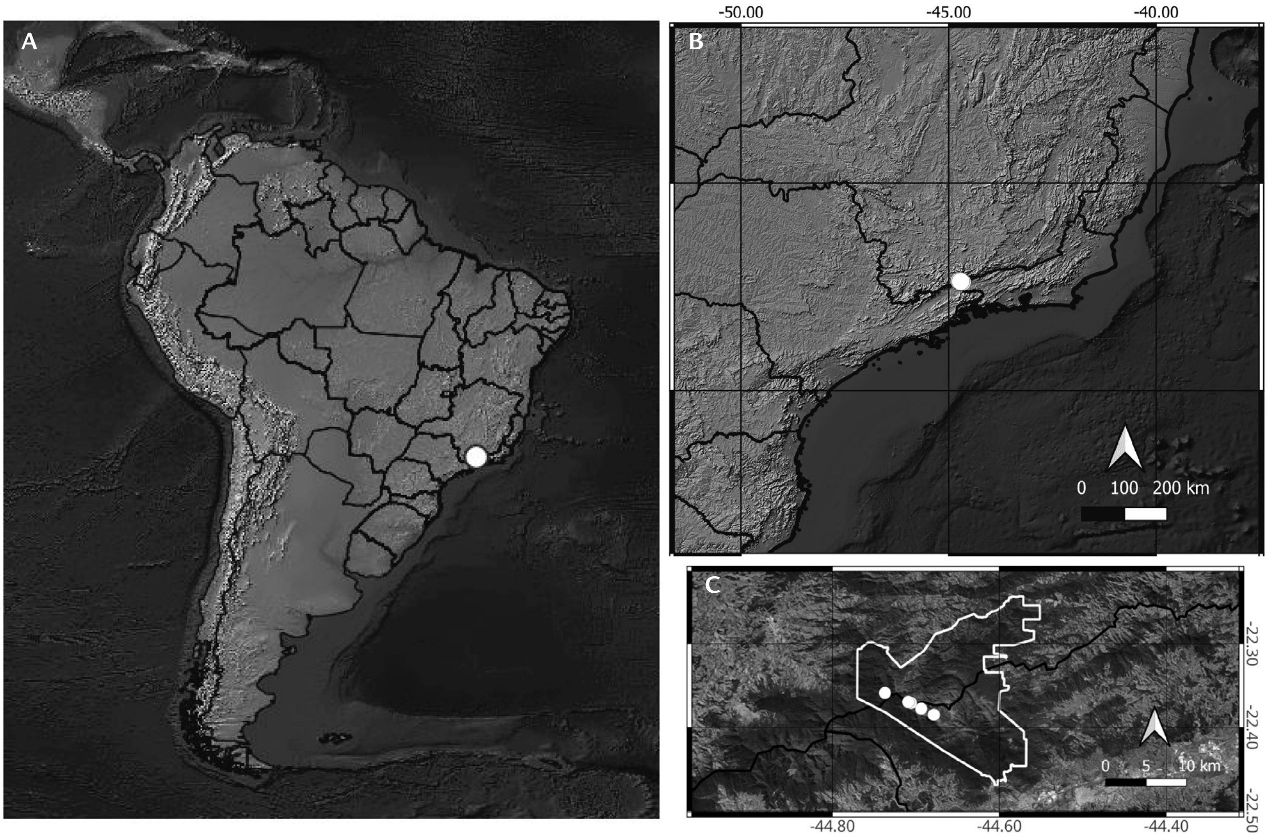

Figure 1. Distributional map showing the occurrence of *Americabaetis itatiaia* sp. nov.: (A) map of South America with detail in Brazil; (B) Minas Gerais and Rio de Janeiro border; (C) Itatiaia National Natural Park, Rio de Janeiro.

Isabel Cristina Hernández Cortes (ICHC), and Pedro Bonfá Neto (PBN). The material examined is housed in the Museu de Entomologia da Universidade Federal de Viçosa (UFVB), Viçosa, Minas Gerais, Brazil and Museu Nacional do Rio de Janeiro (MNRJ), Rio de Janeiro, Brazil.

TAXONOMY

Americabaetis itatiaia Marulanda-Lopez & Salles, sp. nov.

https://zoobank.org/6E5C0DF7-5D10-4077-84F9-7031A2F5E2FE

Diagnosis. Nymphs: (1) frontal keel present, (2) right mandible with angle between incisors and mola obtuse, (3) paraglossa nearly 2x longer than wide, (4) fore femur with 40–42 long, apically acute, simple setae in outer margin, (5) gills grayish with pigmented tracheation, (6) distinctive abdominal color pattern, (7) posterior margin of abdominal terga with triangular spines, and (8) paraprocts marginally with 12–14 spines. Male subimago: (1) turbinate eyes uniformly separated from each other, upper surface ellipsoidal and orangish, (2) abdominal terga brown, except for yellowish brown sigilla and medial stripe, and terga X.

Description

Nymph. Measurements: Body length 5.74 mm; cerci length 2.93 mm, medial terminal filament length 2.0 mm (Terminal filament 0.7x length of cerci).

Head: Coloration brown, except for cream area between inner margin of compound eye and lateral ocelli, and between median ocellus and frontal kell. Antenna with scape and pedicel brown, flagellum light brown and darker toward apex. Epicranial suture with lateral branch sinuous. Frontal keel present. Antenna with scape and pedicel sub-cilindric (Fig. 4A, B).

Labrum (Fig. 2A). Nearly quadrangular; length about 0.8x maximum width. Distal margin with medial emargination and small process, with setae basally bifid and pectinate (not illustrated); dorsally with short, fine, simple setae scattered over surface. Distolateral corner rounded. Dorsolateral arc of setae composed of (3–4) + 1 + 1 long, spine-like setae. Lateral margin with bifid setae (not illustrated). Ventral surface with four short, spine-like setae near distolateral corner.

Left mandible (Fig. 2B). Incisors fused; inner and outer set of incisors with 3 + 3 denticles; prostheca robust, apically denticulate and with comb-shape structure at apex. Angle between incisors and mola obtuse. Margin between prostheca and mola with tuft of setae and with small crenulation close to subtriangular process. Subtriangular process wide, above level of area between prostheca and mola. Apex of mola with strong pectinate setae.

Right mandible (Fig. 2C). Incisors fused; inner and outer set of incisors respectively with 4 + 3 denticles; prostheca robust, apically denticulate. Angle between incisors and mola obtuse. Margin between prostheca and mola with row of setae. Tuft of spine-like setae at base of mola present. Apex of mola with a single pectined setae.

Hypopharynx (Fig. 2D). Lingua longer than superlingua; apex of lingua acute. Superlingua not expanded, distally truncate; short, fine, simple setae scattered over lateral and distal margin.

Maxilla (Fig. 2E). Medial protuberance of galea with 5 + 1 simple setae. Maxillary palp 1.4x length of galea-lacinia, with short, fine simple setae scattered over its surface. Palp segment II subequal to palp segment I.

Labium (Fig. 2F). Glossa broad basally, narrowing distally and shorter than paraglossa (0.8x length of paraglossa); inner margin with 7–8 spine-like setae, outer margin with 7 long spine-like setae increasing in length apically. Paraglossa sub-rectangular with length/width ratio 2.3x; apex with three rows of apically pectinate setae. Labial palp segment I 0.8x the length of segments II and III combined; segment I covered with short, simple setae on outer and inner margins and micropores along outer margin; segment II with moderate and rounded distomedial protuberance (width of distomedial protuberance 0.8x the base of segment III); inner margin with short simple setae, more numerous at apex; outer margin with few, short, simple setae and dorsally with row of 4–6 fine and simple setae; segment III distally rounded, length 1.2x width; covered with spine-like and fine, simple setae.

Thorax (Fig. 4C, D): General coloration brown with light brown marks.

Foreleg (Fig. 3A). Coloration brown. Ratio of foreleg 1.2:1.0:0.6:0.2. Forefemur. Length about 3x maximum width; outer margin with row of 40–42 apically acute, simple setae (length of setae about 0.1x maximum width of femur). Dorsal surface with row of short spine-like setae close to inner margin and micropores close to outer margin. Inner margin with strong spine-like setae in basal half. Tibia. Outer margin with a row of scarce, short, fine simple setae; inner margin with one row of spine-like setae. Tibio-patelar suture present. Tarsus. Outer margin with scarce short simple setae; inner margin with one row of spine-like setae. Tarsal claw with one row of 12–13 denticles, Mid and hind legs similar to foreleg.

Abdomen (Fig. 4C, D): General coloration brown. Terga IV, VII, and X with lateromedial white marks; terga II to VII with sublateral white longitudinal marks; terga II to X with anterior sigilla black; terga II to IX with white longitudinal discontinuous mid stripe (Fig. 4C, D). Sterna.

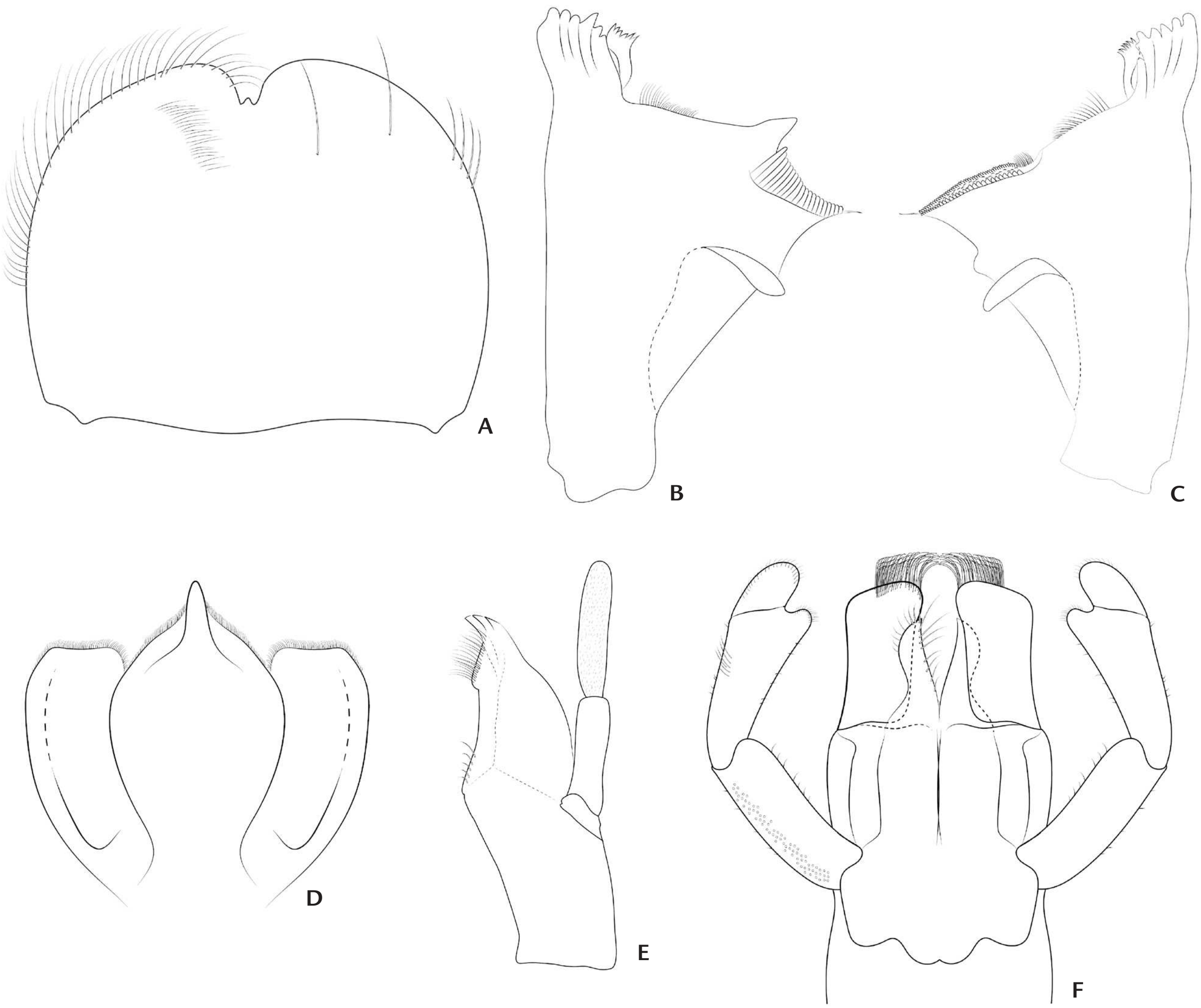

Figure 2. *Americabaetis itatiaia* sp. nov., mouth parts of the holotype: (A) labrum, left ventral view, right dorsal view; (B) left mandible, ventral view; (C) right mandible, dorsal view; (D) hypopharynx, dorsal view; (E) maxilla, dorsal view; (F) labium, left dorsal view, right ventral view.

General coloration light brown; anterior sigilla dark running along the abdominal segment I–VII; lateral margins of abdominal terga washed with brown. Terga (Fig. 3B). Surface with abundant scales and scale-bases; posterior margin with triangular spines. Gills (Fig. 3C). Costal and anal margins with narrow spines intercalating short, fine, simple setae; pigmented tracheation extending from main trunk to both margins. Paraproct (Fig. 3D), with 12–14 marginal spines; surface with scale or scale-bases; posterolateral extension with 12 minute marginal spines. Caudal filaments. Coloration brown. Posterior margin of segments with short spines on each segment. Inner margin of cercus and inner and outer margin of terminal filament with tufts of long, simple setae.

Male subimago (in alcohol). Measurements: Lengths: body, 5.14 mm; fore wing: 5.44 mm.

General coloration brown.

Head (Fig. 5A, B): Light yellowish brown; ocelli surrounded with dark brown. Antennae whitish, scape and flagellum slightly washed with brown. Turbinate eyes uniformly separated from each other, upper surface ellipsoidal, orangish; basal half of stalk light yellowish brown, paler toward apex, apical half light yellowish brown.

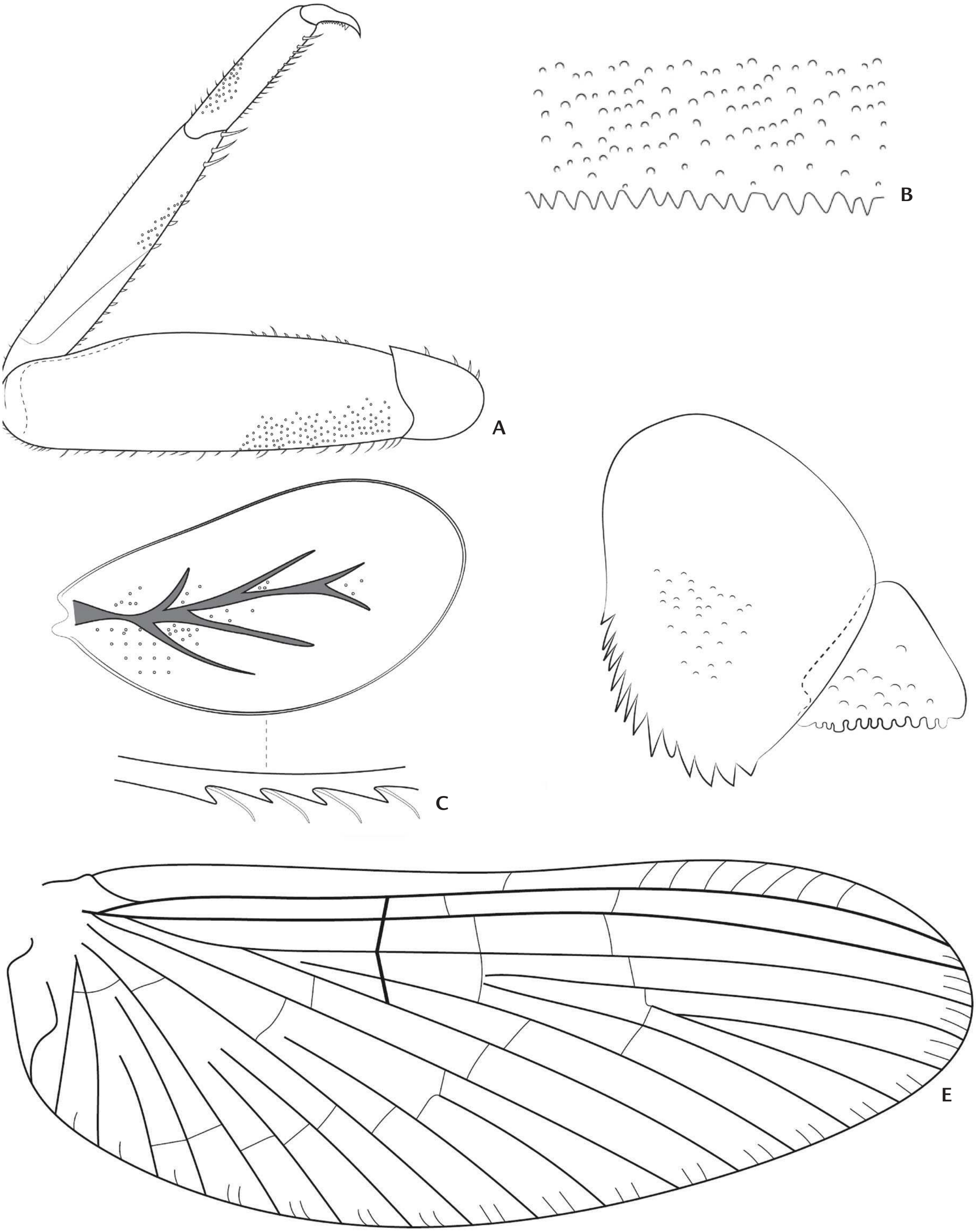

Figure 3. *Americabaetis itatiaia* sp. nov., other structures of the holotype (A-D) and winged stages (E): (A) fore leg; (B) posterior margin of abdominal terga IV; (C) gill IV with margin detail; (D) paraproct; (E) fore wing, black line highlighting the bullae trajectory.

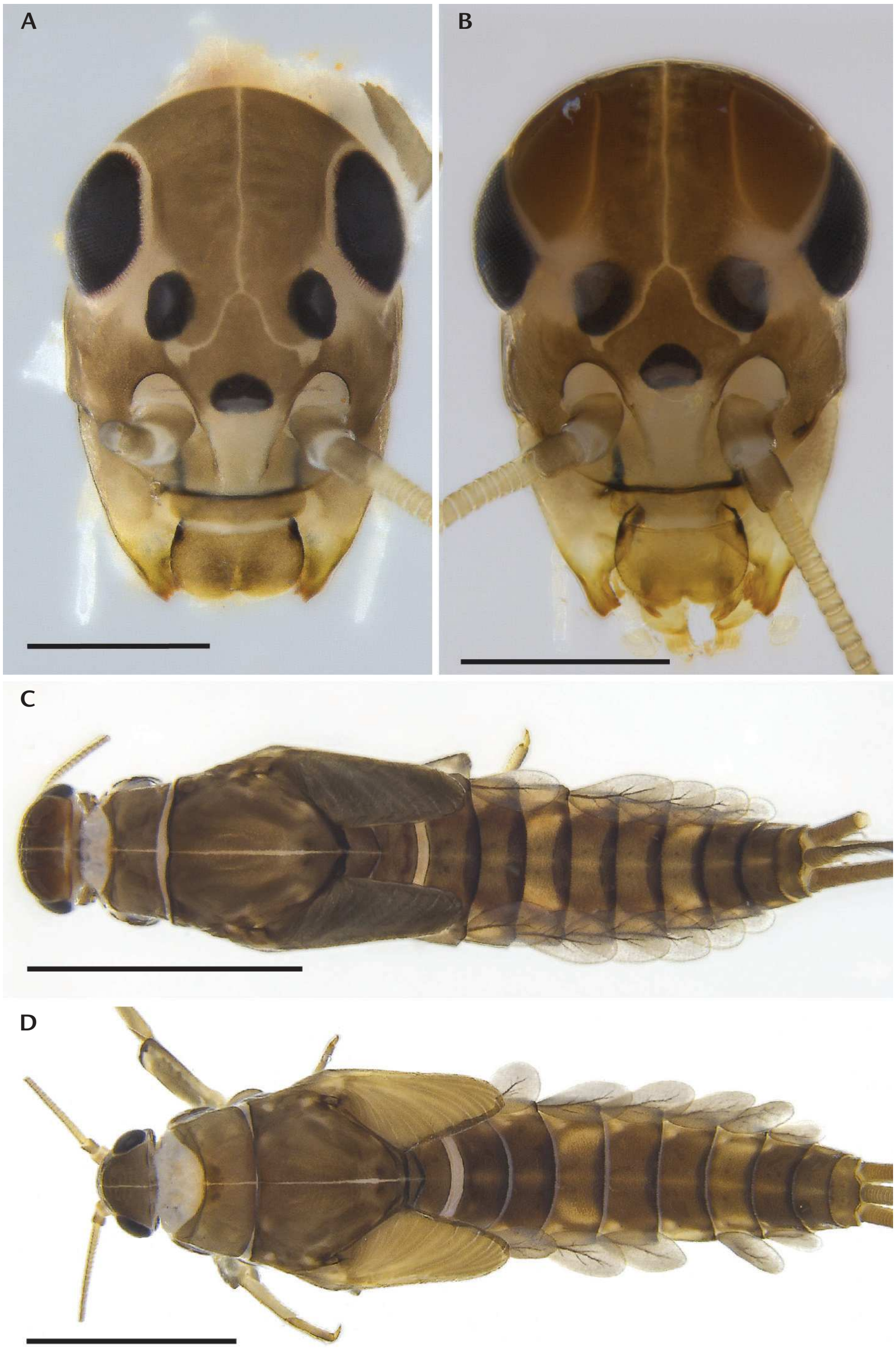

Figure 4. *Americabaetis itatiaia* sp. nov., nymphs: (A) head of female; (B) head of male; (C) habitus of male in dorsal view; (D) habitus of female in dorsal view. Scale bars: A, B = 500 µm, C, D = 2 mm.

Thorax (Fig. 5C): Pro and metanota brown; mesonotum brown with light yellowish brown mark on anteronotal transverse impression, light yellowish brown along lateroparapsidal suture, posterior scutal protuberance dark brown. Legs. Hind tibia 0.5x the length of femur, tarsi 0.3x in length to femur and with four segments decreasing in length

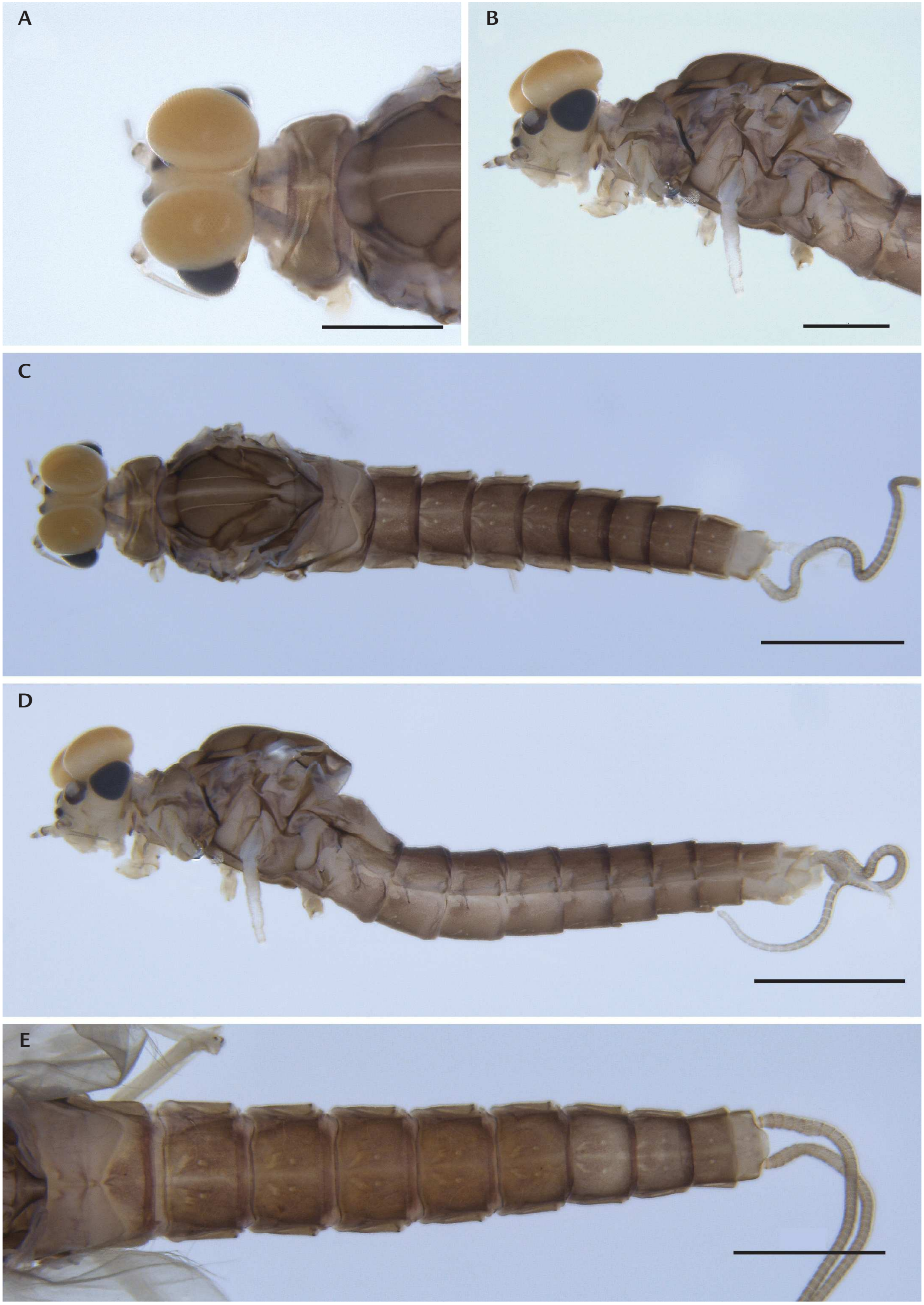

Figure 5. *Americabaetis itatiaia* sp.nov., subimagos, male (A-D), female (E): (A) head, dorsal view; (B) head and thorax, lateral view; (C) habitus, dorsal view; (D) habitus, lateral view; (E) abdomen, dorsal view. Scale bars: A, B = 500 µm, C–E = 1 mm.

Figure 6. General aspect of new species habitat: (A) Campo Belo spring, type locality; (B) Pedra do Camelo stream.

apically. Wing (Fig. 3E): membrane grayish, except for costal and subcostal area grayish brown, costal brace tinged with yellowish brown; longitudinal veins light brown, transversal and intercalary veins whitish. Three bullae in the subcostal veins, RP1 and RP3+4. Angle forming the trajectory of the bullae directed toward the basal region of the wing.

Abdomen (Fig. 5C, D): Terga brown, except for yellowish brown sigilla and medial stripe; posterior margin of segments II to VIII dark brown. Sterna light brown, posterolateral margins dark brown; segments II to VIII yellowish brown sigilla. Forceps light brown, distal segment whitish; cerci whitish.

Female subimago (in alcohol). Measurements: Lengths: body, 6.52 mm; fore wings: folded.

General coloration brown.

Head: Light yellowish brown; ocelli surrounded with dark brown. Antennae whitish, scape and flagellum slightly washed with brown.

Thorax: Pro and metanota brown; mesonotum brown with light yellowish-brown mark on anteronotal transverse impression, light yellowish brown along lateroparapsidal suture, and with posterior scutal protuberance dark brown. Legs light yellowish brown.

Abdomen (Fig. 5E): as in male. Cerci broken.

Imagos: unknown.

Material examined. Type material. HOLOTYPE: BRAZIL, 1 male mature nymph; mouthparts, legs and paraprocts in slides); Itatiaia National Park, border of Minas Gerais and Rio de Janeiro; Waterhead of Campo Belo River; S 22°21'40"/W 44°41'37"; 2400 m.a.s.l.; 17/iii/2022; FFS, MLSR, ICHC, PB cols. (UFVB). PARATYPES: BRAZIL, 12 nymphs (5 males and 7 females), same data as holotype (UFVB). 14 nymphs (4 males and 10 females); Itatiaia National Park, border of Minas Gerais and Rio de Janeiro; Rebouças dam; S 22°23'06"/W 44°40'43"; 2380 m.a.s.l.; 17/iii/2022; FFS, MLSR, ICHC, PB cols. (UFVB). 14 nymphs (3 males and 11 females); Itatiaia National Park, border of Minas Gerais and Rio de Janeiro; Pedra do Camelo stream; S 22°22'11"/W 44°42'33"; 2350 m.a.s.l.; 17/iii/2022; FFS, MLSR, ICHC, PB cols. (UFVB). 19 nymphs (6 males and 13 females); Itatiaia National Park, border of Minas Gerais and Rio de Janeiro; stream close to the road Km 12; S 22°22'15"/W 44°42'21"; 2380 m.a.s.l.; 17/iii/2022; FFS, MLSR, ICHC, PB cols. (UFVB).

Forty-four nymphs (9 males and 35 females) and 2 subimagos, wings in slides (1 male with nymphal exuviae and female); Itatiaia National Park, border of Minas Gerais and Rio de Janeiro; Brejo da Lapa; S 22°21'32"/W 44°44'14"; 2140 m.a.s.l.; 16/iii/2022; FFS, MLSR, ICHC, PB cols. (UFVB). 44 nymphs (9 males and 35 females) and 1 subimago (female); Itatiaia National Park, border of Minas Gerais and Rio de Janeiro; Brejo da Lapa; S 22°21'32"/W 44°44'14"; 2140 m.a.s.l.; 16/iii/2022; FFS, MLSR, ICHC, PB cols. (MNRJ).

Etymology. The name of the new species is an allusion to the word itá-ti-aia, name of the National Park and which in *Tupi Guaraní* means wet stone.

Distribution and habitat. Brazil: Itatiaia National Park (Fig. 1). The new species was collected in headwaters of the Campo Belo stream, from Itatiaia National Park, close to the border of Minas Gerais and Rio de Janeiro, in an altitudinal range of 2140–2400 m a.s.l. (Fig. 6) along the Mantiqueira mountain range. The new species was collected exclusively in the Itatiaia Plateau region, also known as "Upper Part", with high fields and hanging valleys with several river sources (ICMBio 2022).

Remarks. Given the development of the labial palp segment III and robustness of the body, the new species is somewhat similar to *A. labiosus* and *A. titthion*. It can be distinguished from *A. titthion* by the absence of meso and metasternal projections and by the shape of spines on the posterior margin of abdominal terga (triangular on the new species, blunt on *A. titthion*). The main differences between the new species and *A. labiosus* are the paraglossa length/width ratio (2.3x in the new species, 2.5x in *A. labiosus*), the relative length of the maxillary palp (longer than galea-lacinia in the new species versus subequal in *A. labiosus*), as well as pigmentation of tracheae on gills (which is absent in *A. labiosus*). The abdominal color pattern of *A. itatiaia* sp. nov., characterized by two white posterolateral spots on abdominal terga IV and VII (as in *A. alphus*), a white medial band running along the abdominal terga (as in *A. longetron*), and lateral margins washed with brown, is also very distinctive in relation to these two species. The imagos are unknown, but the subimagos can be differentiated by the turbinate eyes uniformly separated from each other, upper surface ellipsoidal and orangish; mesonotum brown, with light yellowish brown mark on anteronotal transverse impression, light yellowish brown band along lateroparapsidal suture; and abdominal terga brown, except for yellowish brown sigilla and medial stripe.

ACKNOWLEDGEMENTS

We thank Isabel Cristina Hernandez, Mellis Layra Soares, and Pedro Bonfá for assistance in the field. We also thank Og De Souza (Termitology Laboratory of the Universidade Federal de Viçosa) for facilitating the use of optic equipment and Pedro Bonfá for assistance during the map elaboration. Finally, we thank ICMBio and Parque Nacional de Itatiaia for facilitating the sampling of macroinvertebrates in the park. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior to JFML (Capes process 88887.510807/2020-00) and by the Conselho Nacional de Desenvolvimento Científico e Tecnológico to FFS (CNPq process 309666/2019-8).

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Submitted: November 16, 2022
Accepted: August 3, 2023
Editorial responsibility: Ralph Holzenthal

Author Contributions

FFS conceived the research, conducted the field work and produced the final plates. JFML wrote the document and elaborated the maps and illustrations. All authors critically review the manuscript and approved the final version of this paper.

Competing Interests

The authors have declared that no competing interests exist.

How to cite this article

Marulanda-Lopez JF, Salles FF (2023) A new species of *Americabaetis* (Ephemeroptera: Baetidae), from Itatiaia National Park, Brazil. Zoologia 40: e22054 https://doi.org/10.1590/S1984-4689.v40.e23054

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5. CHAPTER III

Machine learning approach to support taxonomic discrimination of mayflies species based on morphologic data

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Artigo aceito para publicação na revista Neotropical Entomology

NENT-D-2400084R

Manuscript section

Systematics, Morphology and Physiology

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Acknowledgements

We thank Dr. Gustavo Ferreira Martins (Biology Program of the Universidade Federal de Viçosa) for revising the structure and english version of this manuscript. We also thank Dr. Og De Souza (Termitology Laboratory of the Universidade Federal de Viçosa) for facilitating the use of optic equipment to take the images. We thank the associated editors and the anonymous reviewers for their valuable suggestions that improved the quality of the final version of our manuscript. We thank the Museu de Entomologia da Universidade Federal de Viçosa (UFVB) and to Dr. Frederico Salles by permite revise the necessary material to realize this research.

Contribution of the authors

JFML conceived the research, conducted the morphologic study, literature revision work, produced the final plate of the illustrated decision tree, and wrote the document. RSF structurate the research, revised the document and supervised the elaboration of the decision tree and the Google Colab. WBBN perform the decision tree analysis and the Google Colab. All authors critically review the manuscript and approve the final version of this paper.

Machine learning approach to support taxonomic discrimination of mayflies species based on morphologic data

Abstract

Artificial Intelligence (AI) and Machine Learning (ML) offer objective solutions in the elaboration of taxonomic keys, such as the processing of large numbers of samples, aid in the species identification and optimizing the time required for this process. We utilized ML to study the morphological data of eight species of *Americabaetis* Kluge 1992, a diverse genus in South American freshwater environments. Decision trees were employed, examining specimens from the Museu de Entomologia da Universidade Federal de Viçosa (UFVB/Brazil) and literature data. Eleven morphological traits of taxonomic importance from the literature, including frontal keel, shape of the mouthparts, and abdominal color pattern, were analyzed. The decision tree obtained with the Gini algorithm effectively differentiates eight species (40% of the known species), using only eight morphological characters. Our analysis revealed distinct groups within *Americabaetis alphus* Lugo-Ortiz and McCafferty 1996a, based on variations in abdominal tracheae pigmentation. This study introduces a novel approach, integrating AI techniques, biological collections and literature data for aid in the *Americabaetis* species identification. It provides a valuable tool for taxonomic research on contemporary and extinct mayflies.

Key words: Taxonomy, Artificial intelligence, Baetidae, *Americabaetis*, Morphological characters.

Introduction

The order Ephemeroptera, commonly known as mayflies, comprise a group of amphibiotic aquatic insects found worldwide (Sartori and Brittain, 2015). Nymphs inhabit freshwater environments, playing crucial roles in trophic networks as food source for other aquatic organisms and in the processing of organic matter (Flowers and De la Rosa, 2010). The emergence of free-living adults is significant for energy transfer between aquatic and terrestrial environments (Martin-Creuzburg et al. 2017). Mayflies uniquely undergo an intermediate stage, known as the subimago, between the last nymphal instar and adulthood, crucial for foreleg development (Kamsoi et al. 2021). Mayflies are integral to water quality biomonitoring studies due to their assemblages responsiveness to anthropogenic pollution (Firmiano et al. 2017).

The Baetidae family stands out as one of the most diverse groups within Ephemeroptera, both taxonomically and ecologically, boasting a recorded count of 1,100 species distributed across 110 genera and presenting a cosmopolitan distribution (Sartori and Brittain, 2015). In South America, this family is represented by 240 species spanning 30 genera (Salles et al. 2022). Specifically, the *Americabaetis* genus was initially proposed by Kluge in 1992 as a subgenus of the *Acerpenna* genus introduced by Waltz and McCafferty in 1987. Later, Lugo-Ortiz and McCafferty in 1996a elevated it to the status of genus, transferring three species from *Acerpenna* to *Americabaetis* and describing additional five species from South America. Presently, the genus comprises 20 neotropical species, with the exception of *Americabaetis pletura* (Lugo-Ortiz and McCafferty 1994), which has been recorded in various countries of Central America as Panama and Mexico (Lugo-Ortiz and McCafferty, 1996b; Randolph and McCafferty, 2000), Guatemala (McCaffety et al. 2004) and Nicaragua (Meyer et al. 2008), and in Texas, USA (Wiersema and McCafferty 1999). Species within this genus are highly prevalent and abundant in both biological collections and freshwater environments, making them an excellent model for biomonitoring, biogeographic, taxonomic, and phylogenetic studies.

Traditionally, the taxonomy of this genus relies on several key morphological characteristics. In nymphs, these include the shape of the mouthparts, claws and paraprocts, leg setation and spines in abdominal terga (Salles et al. 2010; Bardavid and Nieto 2012). Conversely, adults are identified based on wing venation, abdominal color pattern, and oologic characters (Kluge 1992; Hoffman et al. 1999; Salles and Boldrini 2008). The taxonomy classification and species identification is this genus typically involved qualitative descriptions and keys intuitively elaborated by specialists, utilizing previously defined characteristics (Salles et al. 2010; Barnavid and Nieto 2012). To address this, keys are developed based on detected morphological variations, often illustrated to aid specialists and students in the identification (De and Dey 2019). These taxonomic keys can be generated using specialized software such as Xper3 (https://xper3.fr) and Delta (https://www.delta-intkey.com), either intuitively elaborated by specialists or employing ML techniques.

The AI/ML techniques have been increasingly utilized for automated species recognition, including image-based approaches (Spiesman et al. 2020) and decision tree models for species identification (Swain et al. 2019). Decision trees serve as classifiers that predict class labels for data items. They operate by posing a series of questions about the features associated with the items, with each question contained in a node. These nodes form a hierarchical structure encoded as a tree (Kingsford and Salzberg 2008). Decision trees are valuable tools for constructing taxonomic keys, providing graphical representation that facilitate easy identification of the species and their morphological characteristics (Almeida et al. 2020). They automatically select the most relevant morphological characters to construct the tree, aiding in the species identification. Consequently, decision trees play a crucial role in developing automated taxonomic keys and supporting species identification. In this study, a decision tree was constructed using morphological data, the characteristics employed were illustrated, and the obtained tree was proposed as a taxonomic key to aid the identification of South American species of *Americabaetis*. In addition, an illustrated interactive taxonomic key was also proposed to identify the species.

Material and methods

Morphological study

Samples deposited at the Museu de Entomologia da Universidade Federal de Viçosa (UFV), MG, Brazil, from the states of Santa Catarina, Rio de Janeiro, and

Minas Gerais were examined. Species identification was conducted using taxonomic keys and original descriptions (Lugo-Ortiz and McCafferty 1994, 1996a; Francischetti et al. 2003; Salles et al. 2010; Bardavid and Nieto 2012). In these identifications, we considered the presence of frontal keel, the number of spines in paraprocts, the morphology of mouthparts, and the abdominal color pattern. Eleven morphological characters were proposed using the terminology for an character and character statement by Sereno (2007):

1- Head, frontal keel: absent or present (Figure 1A);

2- Labium, labial palp II, distomedial projection, development: less developed (Figure 50 in Lugo-Ortiz and McCafferty 1996a), moderate developed (Figure 6 in Lugo-Ortiz and McCafferty 1996a), and well developed (Figure 40 in Lugo-Ortiz and McCafferty 1996a) (**ordered character**) (Figure 1B);

3- Mouthparts elongation: only labium elongated (Figure 1C), maxillae and labium robust, all mouthparts elongated, mouthparts not elongated;

4- Meso and metasterna, paired setose projections: absent or present (Figure 1D);

5- Abdomen, posterior margin of terga, shape of spines: rounded or pointed (Figure 1E);

6- Abdomen, color pattern: distinct medial band, tergum I and VII with circular posterolateral spots;

7- Gills, tracheation, pigmentation: Unpigmented tracheation, only the main trachea pigmented, pigmented tracheation (Figure 1F);

8- Paraprocts, spines, number: 6 to 7, 8 to 9, 10 to 14, or 15-18 (**ordered character**) (Figure 1G);

9- Body, length: not elongated (4 - 6 mm) or elongated (6 - 8 mm) (**ordered character**);

10- Hypopharynx, apex of lingua, shape: rounded or pointed (Figure 1H);

11- Gills, size: gills as long as two and one-half or three abdominal segments combined, gills as shorter as two and one-half or three abdominal segments combined, or gills elongated.

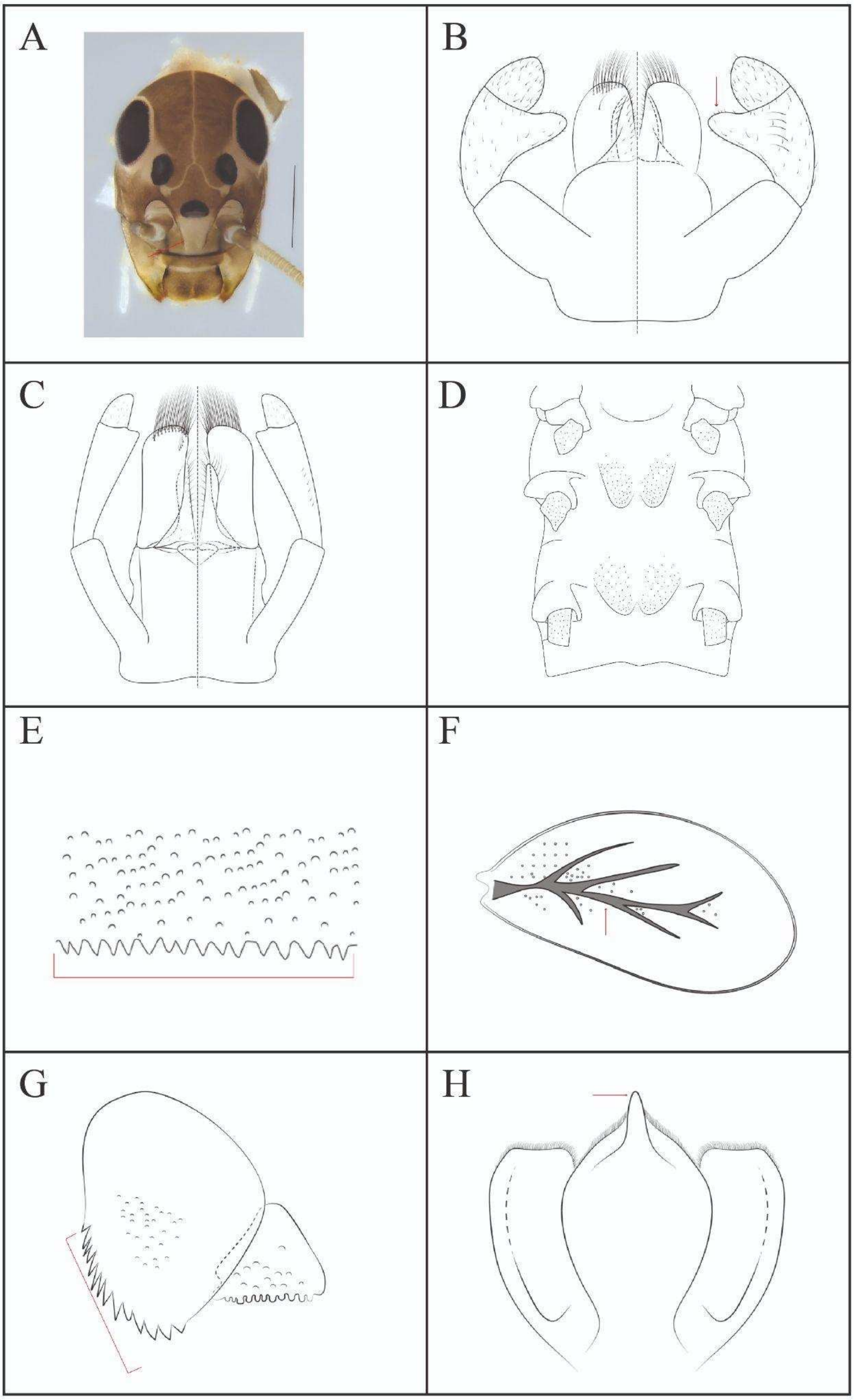

Fig. 1 (A) Frontal keel of *Americabaetis itatiaia* Marulanda-Lopez and Salles 2023. (B) Well developed distomedial process of labial palp II of *Americabaetis maxifolium* Lugo-Ortiz and McCafferty 1996a. (C) elongated labium of *Americabaetis labiosus* Lugo-Ortiz and McCafferty 1996a. (D) Paired setose projections of *Americabaetis titthion* Lugo-Ortiz and McCafferty 1996a. (E) Pointed spines on posterior margin of abdominal segment IV of *A. itatiaia*. (F) Pigmented tracheation of abdominal gills of *A. itatiaia*. (G) Marginal spines of the paraproct of *A. itatiaia*. (H) Pointed lingua of the hypopharynx of *A. itatiaia*. The red arrows and lines signal the characteristics.

Data matrix

A matrix with eight species, 11 characters, and 279 samples that compile the studied characters across South America species was constructed. This matrix was constructed using data from the examined material (Table 1), as well the literature information using the number of individuals provided in the original descriptions of the species and records for other states of Brazil and other countries of South America (Lugo-Ortiz and McCafferty, 1994; 1996a; Salles et al. 2010; Bradavid and Nieto, 2012; Gutiérrez and Llano, 2015; Marulanda-Lopez and Salles, 2023). In instances, where specific morphological characters were not described for certain species in the literature, these characters were encoded as missing data in our matrix. The constructed matrix served as the primary data source for our approach. Initially, our script outlined the composition of the matrix: the first column contained sample numbers, followed by eleven character columns, with the species name listed in the final column and the files corresponding to the samples information. We explicitly defined our data as categorical, with certain characters designated as ordered (2, 8, and 9). Additionally, we accounted for missing data within the matrix.

Decision tree

Initially, was specified in the script the inclusion of species name in the last column and instructed its use for classification purposes. Posteriorly, the decision tree analysis was conducted, partitioning the dataset into a training and validating sets in a 70:30 ratio. ML classification algorithms generate training models, which undergo testing using cross-validation techniques, based on the division and selection of different subsets of the matrix as training and validation sets and conferring the capacity of the model to still correctly identify the species. The Normalized Confusion Matrix and F-1 Score were calculated as accuracy measures, as were included in the script. The Gini algorithm was utilized for the classification, employing the ScikitLearn Library within the Google Colab platform. The scripts employed in the elaboration of the decision tree, as well as an explanation of each step in the process, can be found in the following Google Colab link, including the matrix, the scripts to print the decision tree, an interface to visualize the constructed tree, and a consult option with drop box choice for the eleven morphologic characteristics and submit button to consult the potential species to *Americabaetis* in the obtained tree.

ML_approach_to_support_taxonomic_species_discrimination_based_on_mayfl…

This google Colab can be easily visualize and shared with students, ecologists and specialist interested using the following QR code or use this in notebooks personal, publics and mobile dispositives:

Fig. 2 Quick Response code to access the Google Colaboratory, the Colab contains the matrix, the scripts to print the decision tree, an interface to visualize the constructed tree, and a consult option with drop box choice and submit button to consult the potential species in the obtained tree.

For comparison, classic taxonomic keys proposed by Bardavid and Nieto (2012) and Salles et al. (2010), were compared alongside our illustrated decision tree.

Interactive taxonomic key

An interactive taxonomic key for the nymphs was developed in the Xper3 plattform (http://www.xper3.fr/). For the interactive key were utilized the same eleven characters and states employed in the elaboration of the decision tree.

Illustrations and images

Several morphological characteristics employed by the decision tree and integrated into the key were illustrated using ADOBE ILLUSTRATOR® 2022, following the methods proposed by Coleman (2003, 2006, 2009). Subsequently, all images, illustrations, and the tree were processed in ADOBE PHOTOSHOP® 2022.

Results

Decision tree as illustrated taxonomic key

The Gini algorithm produced a traditional decision tree representation that serves as a taxonomic key. Figure 3 displays the decision tree generated by the Gini algorithm, utilizing all morphological characters and samples of the species. It was illustrated to enhance the comprehension of the characters used. Remarkably, our proposed decision tree effectively discriminates among all eight *Americabaetis* species analyzed, utilizing only eight out of the eleven proposed morphological characters. The accuracy value of the F-1 score for our decision tree was 0.97%. Furthermore, our analysis successfully differentiate two distinct groups within the species *A. alphus*, characterized by variation in the degree of pigmentation of the tracheae in the abdominal gills. Some individuals exhibited pigmented main and secondary tracheae, while others had only the main tracheae pigmented.

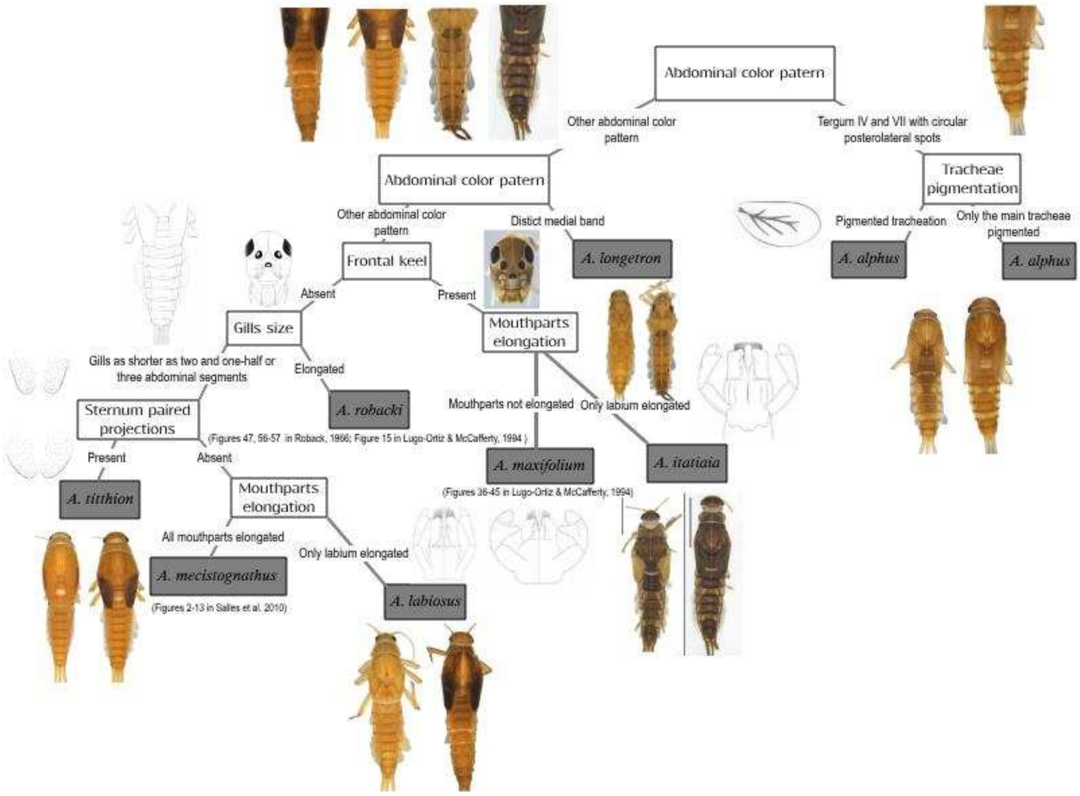

Fig. 3 Decision tree for *Americabaetis* species discrimination using the Gini algorithm and morphological parameters. Gray boxes are the species names, white boxes are the morphological characters, and the characteristics in the lines between the characters. The habitus of the species presents the females on the left side and the males on the right.

The present study creates a dataset that contains 8 out of 20 described species within the genus. The advantage of using this method is due to the facility to process big and diversified datasets and the automated recognition of morphological variation directioning the focus of the researchers on these informative structures with variation and complementing the work of specialists, as well aid in the identification processes to students. This dataset represents a valuable contribution by compiling variation in eleven morphologic characteristics, offering insights into a fraction of species of the true biological diversity of *Americabaetis* in the Neotropics. Furthermore, a portion of the material deposited at UFVB was reviewed, and information from literature sources was utilized for species such as for *Americabaetis mecistognathus* Salles and Raimundi, 2010 in Salles et al. 2010, *Americabaetis robacki* (Lugo-Ortiz and McCafferty 1994) and *A. maxifolium*.

Interactive taxonomic key

The access to the illustrated interactive key generated by the Xper3 platform through the following link:

https://app.xper3.fr:443/xper3GeneratedFiles/publish/identification/6199564990150034100/

Discussion

The taxonomic keys proposed in the literature exhibit similarity with our decision tree. Salles et al. (2010) and Bardavid and Nieto (2012) proposed illustrated taxonomic keys to distinguish South American species of *Americabaetis*. Several of the characters utilized by these authors align with those employed by our automatized decision tree to discriminate species, including the frontal keel, abdominal color pattern, mouthparts elongation, paired projections in the sterna, and size of the abdominal gills. Notably, species such as *A. mecistognathus* and *A. labiosus*, were identified concurrently, with some characters used by Salles et al. (2010) key were employed by our decision tree.

An intriguing outcome of our decision tree was the identification of morphological variation linked with the species *A. alphus*, associated with different localities, particularly regarding the degree of pigmentation in the tracheae of the gills. This species is among the most widely distributed in the Neotropical region (Molineri et al. 2020), suggesting potential implications for future studies. It is plausible that this species constitutes a related species complex or exhibits cryptic species diversity, akin to recent findings in *Andesiops peruvianus* (Ulmer 1920) (Ossa-López et al. 2018). Notably, variation in the degree of pigmentation of the abdominal gills of *A. peruvianus* was reported as a characteristic that supported the hypothesis of phenotypic plasticity at the species level in the pigmentation of the trachea on abdominal gills (Mera et al. 2023). In our study, we also incorporate illustrations and images of taxonomically significant structures into both the decision tree and the interactive key. These graphical representations and figures serve as valuable aids for ecologists and students in species identification.

To our knowledge, this study marks the first application of AI techniques to taxonomic identification of mayflies, presenting an opportunity for advancements in biodiversity, technology research, and biomonitoring. Notably, Dominguez-Granda et al. (2011) proposed a decision tree model for predicting water quality based on the presence of macroinvertebrates, highlighting the potential of similar approaches in biomonitoring applications. Our study underscores the utility of decision trees as bioinformatic tools capable of generating automated graphical representations, facilitating species discrimination through illustrated morphological characters. The ability of our decision tree to detect morphological variation in *A. alphus* underscores its potential as a tool for automated identification of this variation, thereby complementing the taxonomic efforts of the specialists. In conclusion, our work represents an initial step toward mayfly species identification, utilizing the *Americabaetis* genus as a model, and employing machine learning tools alongside interactive keys. The decision tree proposed in this study and our interactive key serves as bioinformatic tools, both useful as identification tools to aid in identifying South American *Americabaetis* species. These bioinformatic techniques make the identification process of mayflies easier for students and ecology researchers, and could be useful to future approaches as a computer-based identification process. In relation to an illustrated taxonomic key, the decision tree algorithm has the advantage of automatically identifying patterns of variation consistent in a large number of samples and useful for differentiating species. In this context, it constitutes a bioinformatics tool that can complement the studies of specialists, allowing, for example, to locate structures or characters with variation and focus their morphological studies on these sources of variation.

This study underscores the importance of detailed morphological descriptions, which enable the compilation of variation in morphological characteristics and enrich such studies that use literature data sources with the examination of material deposited in biological collections. Moreover, the inclusion of new curated information of other biological collections, such as the Instituto de Biodiversidad Neotropical (IBN), Tucumán, Argentina and Colección Entomológica del Programa de Biología de la Universidad de Caldas (CEBUC), Manizales, Colombia, will further complement this dataset. Such additions will enhance our understanding of morphological variation in *Americabaetis*, thus facilitating future taxonomic work, phylogenetic analysis and ecological studies within the group. Analyzing the morphological variation of *A. alphus* across its distribution range could shed light on its validity as a species.

Statements and declarations

Funding

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, #001) to JFML (process # 88887.510807/2020-00).

Informed Consent

All the authors of this manuscript accepted that the paper is submitted for publication in the Neotropical Entomology journal and reported that this paper has not been published or accepted for publication in another journal and is not under consideration at another journal.

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Competing interests

The authors have declared that no competing interests exist.

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Table 1. Examined material. SC is Santa Catarina, MG is Minas Gerais, FFS is Frederico Falcão Salles, TSR is Tayna Silva Raymundo, Iury Domingues, JFM is Jhon Faber Marulanda, DB is Daniel Beliche, MINES is Maria Ines Gonçalves, MLSR is Mellis Layra Soares Rippel, ICHC is Isabel Cristina Hernández Cortés, PB is Pedro Bonfá.

Species	**Country**	**State**	**Municipality**	**Locality**	**Latitude**	**Longitude**	**Altitude m.a.s.l**	**Collectors**	**Data**	**Individuals**	**Stage**	**Collection**
A. labiosus	Brazil	SC		#12					06.ix. 2009	4	Nymph	UFVB
A. labiosus	Brazil	SC		#2					01.ix. 2009	5	Nymph	UFVB
A. labiosus	Brazil	SC		#3					01.ix. 2009	1	Nymph	UFVB
A. labiosus	Brazil	SC		#8					04.ix. 2009	4	Nymph	UFVB
A. labiosus	Brazil	SC		#2					01.ix. 2009	26	Nymph	UFVB
A. labiosus	Brazil	SC		#12					06.ix. 2009	6	Nymph	UFVB
A. labiosus	Brazil	SC		#1					31.viii .2009	3	Nymph	UFVB
A. titthion	Brazil	SC		#2					01.ix. 2009	3	Nymph	UFVB
A. titthion	Brazil	SC		#1					31.viii .2009	5	Nymph	UFVB
A. titthion	Brazil	MG	Araponga	Cachoei	20°38’49	42°27’29.7	949	FFS, TSR,	11.vii.	4	Nymph	UFVB

				ra do Bone	.25”S	4”W		IDO & JFM	2019			
A. titthion	Brazil	MG	Araponga	Cachoeira do Bone	20°38’49.25”S	42°27’29.74”W	949	FFS, DB, MINES	24.ix.2018	18	Nymph	UFVB
A. titthion	Brazil	MG	Araponga	Cachoeira do Bone	20°38’49.25”S	42°27’29.74”W	949	FFS	24.ix.2021	1	Nymph	UFVB
A. longetron	Brazil	SC		#4					01.ix.2009	2	Nymph	UFVB
A. longetron	Brazil	SC		Farna Araucárias					13.iii.2009	10	Nymph	UFVB
A. longetron	Brazil	MG	Araponga	Cachoeira do Bone	20°38’49.25”S	42°27’29.74”W	949	FFS, DB, MINES	24.ix.2018	2	Nymph	UFVB
A. longetron	Brazil	MG	Araponga	Cachoeira do Bone	20°38’49.25”S	42°27’29.74”W	949	FFS, Pereira & JFM	05.ix.2018	2	Nymph	UFVB
A. alphus	Brazil	SC		#8					04.ix.2009	3	Nymph	UFVB
A. alphus	Brazil	SC		#1					31.viii.2009	2	Nymph	UFVB
A. itatiaia	Brazil	MG	Itatiaia National Park	Campo Belo River	22°21’40”S	44°41’37”W	2400	FFS, MLSR, ICHC & PB	17.iii.2022	13	Nymph	UFVB
A. itatiaia	Brazil	MG	Itatiaia	Rebouç	22°23’06	44°40’43”	2380	FFS,	17.iii.	14	Nymph	UFVB

			National Park	as dam	”S	W		MLSR, ICHC & PB	2022			
A. itatiaia	Brazil	MG	Itatiaia National Park	Pedra do Camelo Stream	22°22’11”S	44°42’33” W	2350	FFS, MLSR, ICHC & PB	17.iii. 2022	14	Nymph	UFVB
A. itatiaia	Brazil	MG	Itatiaia National Park	Stream close to the road Km12	22°22’15”S	44°42’21” W	2380	FFS, MLSR, ICHC & PB	17.iii. 2022	19	Nymph	UFVB
A. itatiaia	Brazil	MG	Itatiaia National Park	Brejo da Lapa	22°21’32”S	44°44’14” W	2140	FFS, MLSR, ICHC & PB	16.iii. 2022	2	Subimago	UFVB
A. itatiaia	Brazil	MG	Itatiaia National Park	Brejo da Lapa	22°21’32”S	44°44’14” W	2140	FFS, MLSR, ICHC & PB	16.iii. 2022	88	Nymph	UFVB

6. CHAPTER IV

Phylogenetic hypothesis of *Americabaetis* Kluge 1992 (Ephemeroptera: Baetidae) species using morphological data

Phylogenetic hypothesis of *Americabaetis*

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Abstract

The phylogenetic relationships between all *Americabaetis* species are unknown and the studies that include some species of the genus proposed controversial results with some genera exhibing closed relationships, using both morphological and molecular data. The aim of our research was to hypothesize the phylogenetic relationships between *Americabaetis* species using morphological data and test the monophyly of the genus. The material deposited in the Museu de Entomologia da Universidade Federal de Viçosa (UFVB) was revised and a matrix of 30 morphological characters and 23 taxa was constructed. The parsimony method was implemented in the tree searches, the implied weighting algorithm and different *K* values. *Americabaetis* was not recovered as a monophyletic group and the species *Tupiara ibirapitanga* and *Acerpenna pygmaea* were recovered within the genus in a polytomy with all species. Within the polytomy three well defined groups were retrieved as monophyletic groups, one composed by the species *Americabaetis labiosus* + *Americabaetis mecistognathus* and *Americabaetis titthion*, supported by four homoplasious characters, apex of the lingua pointed, maxillary palp slightly extended beyond galealaciniae, distomedial process of labial palp II moderately developed, and apex of labial palp III conical. The sister group species *A. mecistognathus* and *A. labiosus*, was supported by one synapomorphy, posterior margin of abdominal terga with rounded spines and one homoplasious character, labial palp I subequal in length to segment II. *Americabaetis spinosus* + *Americabaetis robacki* were recovered such as a monophyletic group supported by three homoplasious characters, maxillary palp slightly extended beyond galealaciniae, turbinate eyes in male imagos divergent anterior and posteriorly, and segment I of the forceps without medioapical constriction. *Americabaetis lugoi* was recovered as a sister group of *Americabaetis maxifolium*, and this relationship was supported by three homoplasious characters, presence of frontal keel, labrum subrectangular, and tarsal claws with 14 to 16 denticles. The subsequent citations, the deposition of type material, diagnoses, and distribution of all species were compiled and some details of biology and ecology aspects of the species.

Key words: Baetinae. Neotropic. morphology. Phylogeny. Taxonomy.

Introduction

Currently, the genus *Americabaetis* is composed of 20 species with Neotropical distribution, with exception of the species *Americabaetis pletura* (Lugo-Ortiz & McCafferty,1994), widely distributed in Central America, and also recorded from Texas in North America (Wiersema & McCafferty, 1999). The genus was described initially as subgenus of *Baetis* Leach, 1815 (Kluge, 1992), latter was transferred to *Acerpenna* Waltz & McCafferty, 1987 (Lugo-Ortiz & McCafferty, 1994) and posteriorly *Americabaetis* was revisited and was established their genus status until today (Lugo-Ortiz & McCafferty, 1996). The biological and ecological aspects of *Americabaetis* remains poorly studied, however, the species are collected across diverse habitats (Salles et al. 2022), in wide altitudinal range (Arana-Maestre et al. 2021), and exhibits differential relationships with physicochemical variables related to water pollution (Forero-Céspedes et al. 2016; Melo et al. 2017). In this context, the species of *Americabaetis* genus constitutes a good model for biomonitoring studies.

The phylogenetic relationships of *Americabaetis* species have been incipiently studied and the phylogenetic hypothesis that included some species of the genus exhibits controversial results. The *Americabaetis* genus was hypothesized as paraphyletic, with some species more related to *Acerpenna pygmaea* Hagen, 1861 and *Tupiara ibirapitanga* Salles, 2003 in Salles et al. 2003 in a phylogenetic analysis of South American Baetidae based in morphological data (Nieto, 2010). Using molecular information, the genus was recovered as a sister group to all other Baetidae genera excluding the genus *Callibaetis* Eaton, 1881 (Smith et al. 2011). More recently, the genus was recovered as a monophyletic group present within the Baetinae subfamily using morphological data, in a more comprehensive study including only three species of the genus. The monophyly of the genus was supported by three homoplasious characters, row of spine-like setae on labial palp III oriented to outer apical margin, row of denticles of claws slightly increasing in size toward to apex, and fusion of rib on gills (Cruz et al. 2021).

Originally, the genus was proposed based on morphological characteristics that until today remain with phylogenetic significance unclear (Kluge, 1992). In addition, the phylogenetic relationships between all *Americabaetis* species are unknown. The aim of our research was to hypothesize the phylogenetic relationships between *Americabaetis* species using morphological data and test the monophyly of the genus.

Material and methods

Material study and preparation

The material deposited in the Museu de Entomologia (UFVB) (Viçosa, Minas Gerais, Brazil) from Rio de Janeiro, Minas Gerais, and Santa Catarina was revised (Table 1). The identification of the species was made using taxonomic keys and original descriptions (Kluge, 1992; Lugo-Ortiz & McCafferty, 1994; Lugo-Ortiz & McCafferty, 1996; Lugo-Ortiz & McCafferty, 1999; Waltz & McCafferty, 1999; Wiersema & McCaffety, 1999; Francischetti, 2003; Salles et al. 2004; Salles & Boldrini, 2008; Salles et al. 2010; Bardavid & Nieto, 2012). In the identifications, the shape of the turbinate eyes, shape of meso and metasternum, wing venation, abdominal color pattern and shape of the forceps were examined in the adults. In the nymphs, the spines present in paraprocts, morphology of mouthparts, gills and claws were considered (Kluge, 1992; Lugo-Ortiz & McCafferty, 1994, 1996; Salles & Boldrini, 2008; Salles et al. 2010).

Permanent slides of mouthparts, gills, legs, and wing were elaborated using Euparal®. All characters were studied under high magnifications as 200x and 400x, visualized in a microscope OLYMPUS CX31 and stereomicroscope LEICA M205 A. The structures of taxonomic interest were photographed using a coupled camera LEICA MC170 HD.

Character coding

The character statements for the cladistic analysis were constructed following Sereno (2007), the author proposed the following components for a character statement: Locator (Ln), Variable (V), Variable qualifier (q), and Character state (vn).

The author defines the Locator as the morphological structure, the Variable as the aspect that varies, the Variable qualifier as the variable modifier and the Character state as mutually exclusive conditions of a character (Sereno, 2007).

The morphological matrix was elaborated using the software Mesquite 3.7 (Maddison & Maddison, 2021). Non applicable characters were scored with dashes (-) and unknown with a question mark (?).

Choice of terminals

All species of *Americabaetis* were included to hypothesize the phylogenetic relationships between the sécies and to test the monophyly of the genus, were included the species *Acerpenna pygmaea* Waltz & McCafferty, 1987, and *Tupiara ibirapitanga* Salles, 2003 in Salles et al. 2003, and *Zelusia principalis* Lugo-Ortiz & McCafferty, 1998. The tree was rooted in the species *Z. principalis*.

Phylogenetic analysis

Memory for 70000 trees was configured as the initial step before the tree searches. The monophyly and the phylogenetic relationships between the species of *Americabaetis* were tested using the maximum parsimony method. Implied weighting was implemented using concavity coefficients *K* in a weighting ratio between no homoplasy and maximum possible steps, in different independent rounds using *K* values of 2 to 9 (Farris, 1969; Goloboff, 1993; Chippindale & Wiens, 1994). The *Implicit Enumeration* algorithm was implemented in the tree searches. The relative Bremmer supports (RBS, Goloboff & Farris, 2001) were calculated as a measure of clade support using 2271 suboptimal trees obtained by Tree Bisection and Reconnection (TBR). In addition, the consistency and retention index for the complete tree was calculated using the script *Stats.run*. The synapomorphies in the obtained tree were mapped using the software WinClada and a search for non informative characters (Nixon, 2002). All analyses and study of the evolution of selected characters traditionally implemented in the systematic of the genus were performed in the software TNT v.1.6 (Goloboff & Morales, 2023).

Illustrations and images

Some morphological characteristics employed in the diagnosis of the species were illustrated using ADOBE ILLUSTRATOR® 2022, following the methods proposed by Coleman (2003, 2006, 2009). Subsequently, all images of species habitus, illustrations, and the tree were processed in ADOBE PHOTOSHOP® 2022.

Results

A total of 30 morphological characters were defined for nymphs and adults, and comprises 23 morphological characters of nymphs and 7 of imagos. The nymphal characters, shape of the apex of the gill VII and the number of denticles in the tarsal claws were included at the final of the matrix. The character statements following below:

Nymph

Head

1. Frontal keel: (0) absent, or (1) present
2. Antennae, scape, setae: (0) absent, or (1) present
3. Epicranial suture, lateral branch, shape: (0) straight or curved, or (1) sinuous
4. Labrum: (0) subrectangular (more longer than wide), or (1) subrectangular (more broader than long), or (2) subcircular
5. Hypopharynx, lingua, shape of the apex: (0) rounded, or (1) pointed
6. Hypopharynx, superlingua, shape: (0) subcircular, or (1) sub-rectangular
7. Maxilla, maxillary palp in relation to galealacinia, size: (0) subequal, or (1) slightly extended beyond galealaciniae, or (2) strongly extended beyond galealaciniae, or (3) 3/4 of galealaciniae
8. Labium, paraglossae, ratio length/width: (0) less than 2.0x longer than wide, or (1) more than 2.0x longer than wide

9. Labium, paraglossae, apex, number of rows of setae: (0) two, or (1) three
10. Labium paraglossae, apex, shape of apex of the row of setae (0) simple, or (1) simple with pectinate apex
11. Labium, glossae, size: (0) subequal to paraglossae, or (1) slightly shorter than paraglossae, or (2) shorter than paraglossae (0.7–0.9x as long as paraglossae)
12. Labium, labial palp I, relation of size: (0) subequal to segment II, or (1) subequal to segments II and III combined, or (2) less than the length of segments II and III combined
13. Labium, labial palp II, distomedial process: (0) absent, or (1) present
14. Labium, labial palp II, distomedial process, size: (0) moderately developed, or (1) well developed
15. Labium, labial palp II, distomedial process, shape: (0) rounded, or (1) conical, or (2) digitiform (thumblike)
16. Labium, labial palp III, shape of the apex: (0) rounded, or (1) conical

Thorax

17. Hindwingpads: (0) absent, or (1) present

Abdomen

18. Abdominal terga, scales, scale bases: (0) absent, or (1) present
19. Posterior margin of terga, spines, shape: (0) rounded, or (1) pointed
20. Gills; presence in abdominal segments: (0) I-VII, or (1) II-VII
21. Paraproct, marginal spines, number: (0) 6-10, or (1) 11-20, or (2) numerous

Imagos

Head

22. Turbinate eyes, distance one eye in relation to the other: (0) diverge only anteriorly, or (1) diverge both anterior and posterior, or (2) contiguous

Abdomen

23. Genitalia, forceps, segment I, medioapical constriction (0) absent, (1) present
24. Genitalia, conical process between the forceps I: (0) absent, or (1) present
25. Genitalia, forceps, last segment, shape: (0) ca. 4-fold longer than wider, or (1) ca. 3-fold longer than wider
26. Genitalia, forceps, first segment, color: (0) whitish, or (1) brownish
27. Abdominal color, pattern: (0) two white spots on abdominal segments II and VII, or (1) longitudinal white mark, or (2) other abdominal color pattern
28. Caudal filaments, dark bands: (0) absence, or (1) presence

Additional characters

29. Gills, seven gill, apex, shape: (0) oval, or (1) pointed or lanceolate
30. Tarsal claws, denticles, number: (0) 6-9, or (1) 10-13, or (2) 14-16

Six uninformative characters were identified in our morphological matrix (Supplementary information I), the presence or absence setae on escape of antennae (2), number of rows of setae on the apex of paraglossae (9), the presence or absence o distomedial process on labial palp II (13), the presence or absence of scales on abdominal terga (18), the shape of the last segment of forceps (25), and the shape of the apex of gill VII (29).

Cladistic analysis

The first implicit enumeration analysis using all terminals and based on prior weights recovered more than 70000 most parsimonious trees with a score of 78. The wildcards were identified in the consensus estimation and corresponded with the five argentine species, *A. bridarollii*, *A. jorgenseni*, *A. oldendorffii*, *A. peterseni*, and *A. weiseri*; their location in the strict consensus is signalized by the letters a, b, c, d, and e (Figure 1a). These species changed location in each round of the tree searches and were closely recovered with *A. alphus*. The topology of the tree was a polytomy including all species of *Americabaetis* + *A. pygmaea* + *T. ibirapitanga*. The Argentine species were allocated in *Americabaetis* by only three characters, the nymphal stages are unknown, the descriptions of the winged stages are poor and only three morphological characters could be codified in our matrix for these species (3% of the characters). Due to the fact that these species exhibit many missing data, change of position in each replication of the tree search and consequently increased the number of equally parsimonious trees, we decided to remove them from subsequent analysis. The second implicit enumeration analysis based on prior weights and excluding the Argentine species recovered five most parsimonious trees, with a score of 78 and the strict consensus was elaborated (Figure 1b).

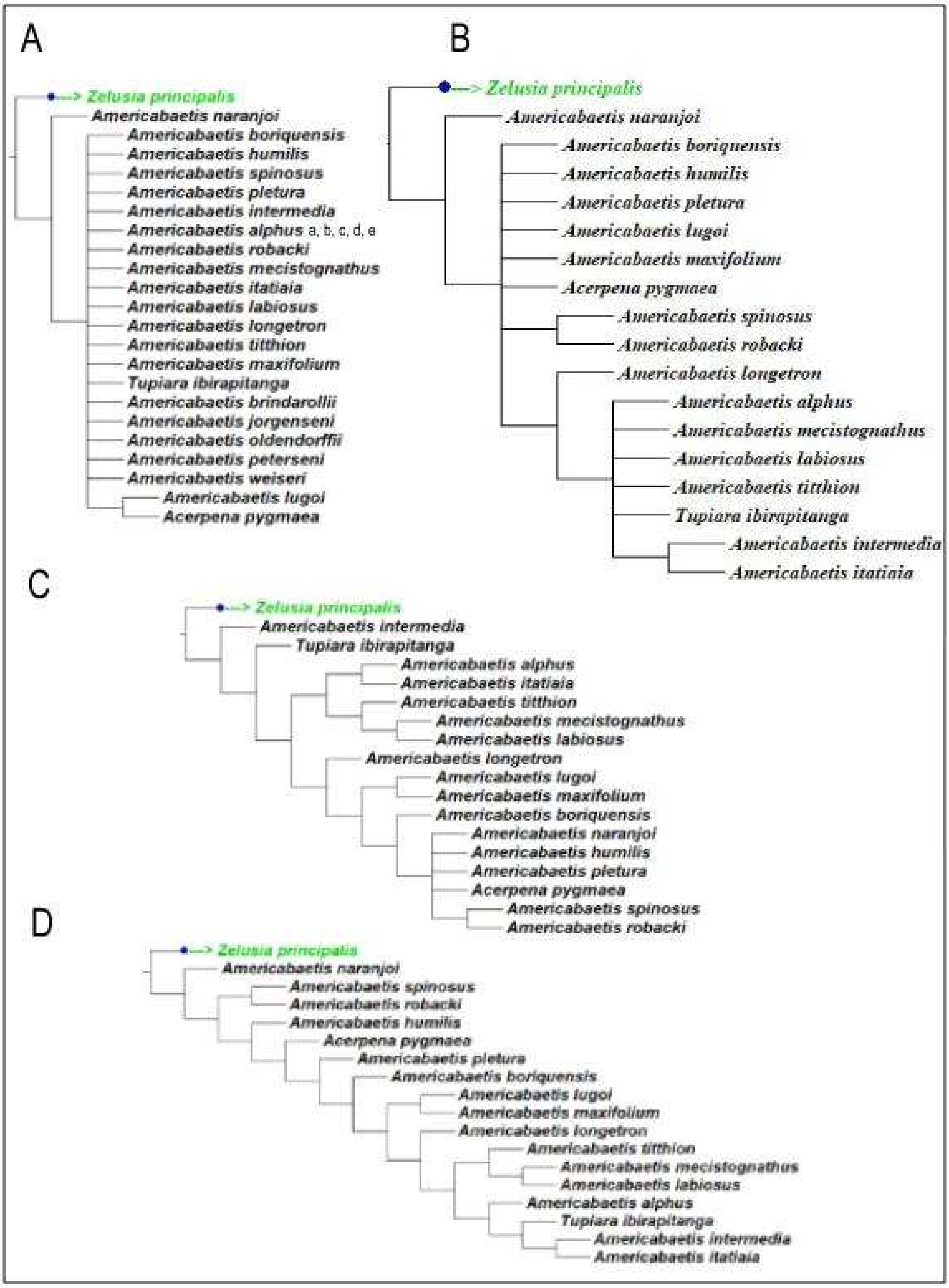

Figure 1. Phylogenetic hypothesis of *Americabaetis*: A) prior weights of all species, the letters a, b, c, d, and e highlight the location of the five Argentine species,

identified as wildcard taxa; B) prior weights excluding the Argentine species; C) implied weighting using *K* = 3; C) implied weighting using *K* = 8.

The implied weighting analysis based on implied weights retrieved the same three most parsimonious trees in each round of analysis using the *K* values of 2, 3, 4 and 5. The consensus was represented, the species *A. pygmaea* was recovered within *Americabaetis* and the species *T. ibirapitanga* was recovered as the sister group of all species of *Americabaetis* + *A. pygmaea* (Figure 1c). The implied weighting analysis using the *K* values of 6, 7, 8 and 9 retrieved one most parsimonious tree (Figure 1d), the species *T. ibirapitanga* and *A. pygmaea* were recovered within *Americabaetis* and the type species *A. naranjoi* was recovered as the sister groped of the remaining *Americabaetis* species + *A. pygmaea* + *T. ibirapitanga*. With the two obtained topologies using the different *K* values (Figure c and c), we estimated a strict consensus, with consistency index of 0.352, retention index of 0.176 and tree length of 108 steps. This topology was used to map the synapomorphies, the characters with homoplasy, supports, study the evolution of the taxonomic characters traditionally implemented in the group, and discuss the phylogenetic relationships between the *Americabaetis* species (Figure 2).

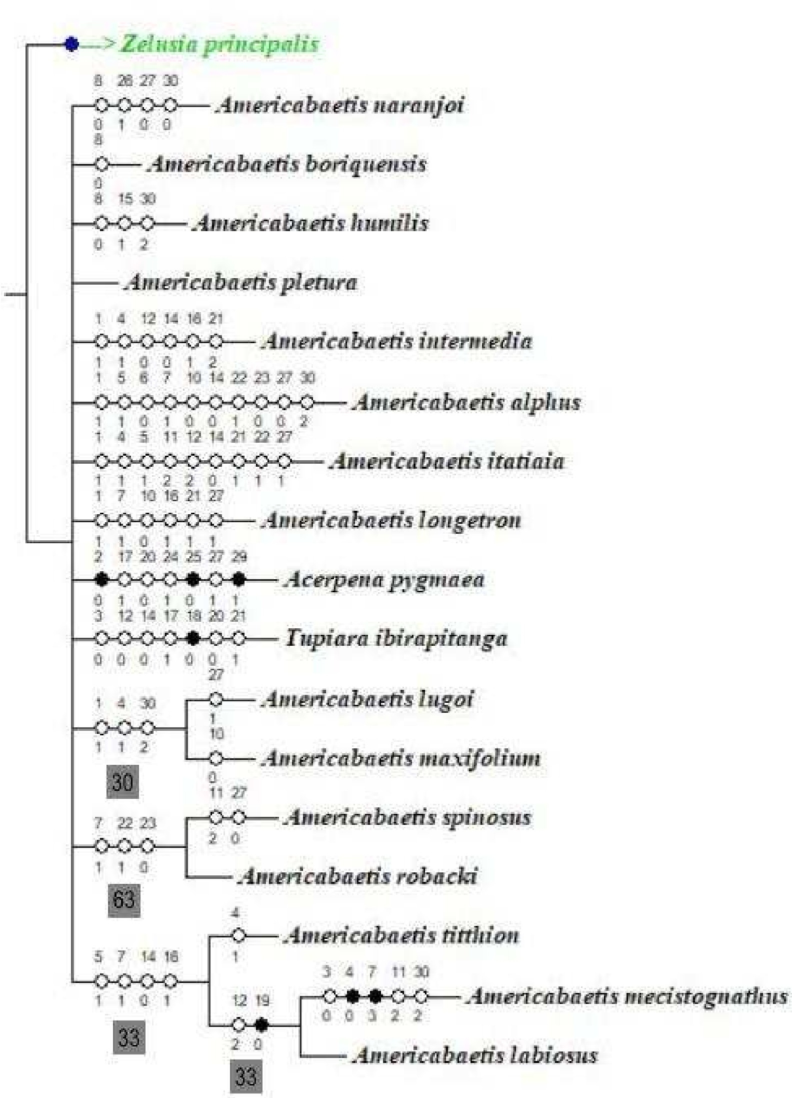

Figure 2. Phylogenetic hypothesis of *Americabaetis* species, this topology was the strict consensus of *K* = 3 and *K* = 8, the supporting synapomorphies are the black circles and the homoplasious characters the white circles. The numbers in the upper part of the circles correspond to the characters and below these to the character states. The gray rectangles are the Relative Bremmer Supports.

Based on our results, the *Americabaetis* genus was not recovered as a monophyletic group with the species *T. ibirapitanga* and *A. pygmaea* retrieved within *Americabaetis* (Figure 2). One polytomy with all *Americabaetis* species + *A. pygmaea* + *T. ibirapitanga* was the obtained topology of our hypothesis. Within the polytomy three monophyletic groups were recovered one composed by the species *Americabaetis maxifolium* Lugo-Ortiz & McCafferty, 1996 and *Americabaetis lugoi*

Waltz & McCafferty, 1999, other with *Americabaetis robacki* (Lugo-Ortiz & McCafferty, 1994) and *Americabaetis spinosus* Hofmann & Thomas, 1999 in Hofmann et al. 1999 and the other with *Americabaetis labiosus* Lugo-Ortiz & McCafferty, 1996 + *Americabaetis mecistognathus* Salles & Raimundi, 2010 in Salles et al. 2010 and *Americabaetis titthion* Lugo-Ortiz & McCafferty, 1996.

The South American species (*A. labiosus* + *A. mecistognathus*) + *A. titthion* was recovered as a monophyletic group supported by four homoplasious characters, apex of the lingua pointed (5-1), maxillary palp slightly extended beyond the galealaciniae (7-1), distomedial process of labial palp II moderately developed (14-0), and apex of labial palp III conical (16-1). The species *A. mecistognathus* and *A. labiosus*, were recovered as sister groups supported by one synapomorphy, the posterior margin of abdominal terga with rounded spines (19-0) and one homoplasious character, the labial palp I subequal in length to segment II (12-0). Another monophyletic group was recovered and composed by the species *A. spinosus* + *A. robacki*, supported by three homoplasious characters, maxillary palp slightly extended beyond of galealaciniae (7-1), turbinate eyes in male imagos divergent anterior and posteriorly (22-1), and segment I of the forceps without medioapical constriction (23-0). The species *A. lugoi* was recovered as sister group of the species *A. maxifolium*, and this relationship was supported by three homoplasious characters, presence of frontal keel (1-1), labrum subrectangular (more broader than long) (4-1), and tarsal claws with 14 to 16 denticles (30-2).

Discussion

Not monophyly of *Americabaetis* and relationships with other genera

Kluge (1992) did not cited autapomorphies when proposed *Americabaetis*, but documented some characters for the genus with unclear phylogenetic status, such as: absence of hind wings, second segment of labial palp with distomedial projection, and abdominal terga of the nymphs with scales in operculate sockets. Among the character states proposed by the author, the absence of hind wings (character 17-state 0) was recovered such as a plesiomorphic condition and a homoplasious character. The presence of distomedial process on labial palp II (13-1) was recovered as a plesiomorphy, shared by all species of *Americabaetis*. Lugo-Ortiz & McCafferty (1996) proposed the absence of hind wing pads (17-0) and presence of gills in the abdominal segments II-VII as diagnostic characteristics for the genus. In our phylogenetic analysis, these characteristics were not recovered as synapomorphies for the genus.

The species *T. ibirapitanga* and *A. pygmaea* were recovered within *Americabaetis*, without synapomorphies supporting these relationships, and the phylogenetic relationships of *Tupiara* and *Acerpenna* with *Americabaetis* remain unknown. Nieto (2010) recovered the genus *Americabaetis* as paraphyletic, with the species *A. labiosus* more related to *T. ibirapitanga* and the species *A. alphus* sister group of *A. pygmaea*. The focus of the author was not the *Americabaetis* genus, however, our phylogenetic hypothesis contributes with the knowledge of the phylogenetic relationships between these baetid genera. *Tupiara* is a monotypic genus comprising only the species *T. ibirapitanga* distributed in Brazil, with abdominal gills on abdominal segments I-VII and equally that in some species of *Americabaetis*, has distomedial process on labial palp II rounded and moderately developed (Salles et al. 2003).

Phylogenetic relationships among *Americabaetis* species

The species, *A. mecistognathus* and *A. labiosus*, recovered as sister group and exhibits an elongation of the mouthparts, all parts elongated by *A. mecisthognatus* and only the labium elongate for *A. labiosus* (Lugo-Ortiz & McCafferty, 1996; Salles et al. 2010). Another monophyletic group was recovered and composed by the species *A. spinosus* + *A. robacki*. The species *A. spinosus* is distributed in the Guadeloupe, on the other hand, the species *A. robacki* occurs in South America (Kluge, 1992; Lugo-Ortiz & McCafferty, 1994; Hofmann et al. 1999). Both species of this sister group exhibit frontal keel and the distomedial process of the labial palp II is well developed and digitiform in shape (Lugo-Ortiz & McCafferty, 1994; Hofmann et al. 1999). The species *A. lugoi* that occurs in Costa Rica, Mexico and Nicaragua (Waltz & McCafferty, 1999; Randolph & McCafferty, 2000; Meyer et al.

2008) was recovered as sister group of the species *A. maxifolium* recorded from Paraguay (Lugo-Ortiz & McCafferty, 1996). Both species exhibits labial palp I subequal in length to the segments II-III combined (12-1), distomedial process of labial palp II well developed (14-1), apex of labial palp III conical (16-1), and paraprocts with 6 to 10 marginal spines (21-0) (Lugo-Ortiz & McCafferty, 1996; Waltz & McCafferty, 1999).

The species *A. intermedia* present morphological characteristics developed intermediate in relation with the other species of the *Americabaetis*. *A. longetron*, *A. alphus*, and *A. maxifolium* present and well developed distomedial process of the labial palp II, with shape digitiform and apex of labial palp III conical. On the other hand, the species *A. titthion*, *A. mecistognathus*, and *A. labiosus* has a moderately developed distomedial process of labial palp II with rounded shape and rounded apex of the labial palp II, such as in the species *A. intermedia*. The development of these characteristics in this last cited species is intermediate and equal to its distributional pattern recorded from Costa Rica, Guatemala and Nicaragua, in relation with the remaining species of *Americabaetis* distributed on the remaining countries of Central America, Caribbean countries and South America. Lugo-Ortiz & McCafferty, 1994 reported that the mouthparts of *A. intermedia* appear to be somewhat intermediate between those of *Fallceon* Waltz & McCafferty and other known *Acerpenna*. The glossae and paraglossa, however, are broader than those found in *Fallceon*, and the second segment of the labial palps have a moderately developed distal projection that is absent in *Fallceon*.

The species *Americabaetis bridarollii* (Navás, 1933), *Americabaetis jorgenseni* (Esben-Petersen, 1909), *Americabaetis oldendorffii* (Weyenbergh, 1883), *Americabaetis peterseni* (Hubbard, 1974), and *Americabaetis weiseri* (Navás, 1926) were poorly described (Barnavid & Nieto, 2012), the type material of these Argentine species is unable to locate, apparently lost or very deteriorated (Alba-Tercedor & Peters, 1985; Lugo-Ortiz & McCafferty, 1999). Therefore, the placement of these species in *Americabaetis* remains uncertain (Alba-Tercedor & Peters, 1985; Lugo-Ortiz & McCafferty, 1999). All species were recorded from Argentina for unknown localities in Mendoza, Cordoba, Catamarca and Santa Fe and these species were not recorded again. The first round of our phylogenetic analysis including these species recovered more than 70000 trees equally parsimonious and these species by the high amount of missing data, change of location on each replication in the trees search and was not possible to include in the subsequent analysis using implied weighting. Based on these results and the information documented in the literature is proposed the status of *nomen dubium* for these Argentine species.

Our results are not conclusive to resolve more comprehensive questions of *Americabaetis* genus. In addition, the high level of homoplasy on our matrix (even as part of evolutive story of the groups), the low support in the only four monophyletic groups recovered, demonstrates how our phylogenetic hypothesis is fragile and highlights the need of more morphological characters or an independent approach to analysis using molecular data. Only three species of other genera related with *Americabaetis* were included as outgroups, future studies could be use the methodology of outgroup expansion, increasing successively the number of outgroups in each round o phylogenetic analysis, until recovering stable topologies in consecutives rounds increasing the sampling (Grant, 2019). Another interesting approach is to identify the wildcard taxa, species that change the position by the quantity of missing data and remove these from the subsequent phylogenetic analysis and interpret the reduced tree with the obtained relationships and in a critical manner known who are the *Americabaetis* species *incertae sedis*.

Taxonomy

Following subsequent citations, deposition of type material, diagnoses for known stages, and distribution of all species of *Americabaetis*:

Americabaetis naranjoi Kluge, 1992 (Type species)

Subsequent citations. Lopez & Laso, 2005: 236 (additional record from Cuba).

Deposition of the type material. Holotype: imago deposited in N. Kluge personal collection. Paratypes: one imago and two nymphs deposited in the C. Naranjo personal collection, two nymphs deposited in the C. Naranjo personal collection.

Diagnosis.

Based from Kluge, 1992

Nymphs: 1) body slender, elongated, and in the dorsal side, a more or less pronounced uneven light medial stripe stretches along the head and thorax; 2) legs are light, without pattern or with a more or less well-defined rectangular brown spot in the distal half of the thigh; 3) abdominal tergites with pale longitudinal stripe on darker background, or only with pale medial spot at anterior margin; 4) abdominal tergites IV and VII with a pair of large rounded pale spots on the sides, and the tergite X entirely pale; 5) gills on abdominal segments II-VII.

Male imago: 1) turbinate eyes diverge very strongly anteriorly (Figs. 17-18 in Kluge, 1992); 2) abdominal segments VIII-X with rufous spots; 3) first segment of the forceps brownish, the second and third whitish.

Female imago: 1) head as Figs. 14-15 in Kluge, 1992; 2) abdominal tergites and sternites pale in medial part, brown on sides.

Eggs: 1) oval shape without polar capsule; 2) chorion with pristine cellular sculpture (chorionic ridges) (Figs. 5-6 in Kluge, 1992; 3) micropylar area not visible.

Distribution. Cuba: Guantanamo, Camaguey, Sancti Spiritus Provinces.

Americabaetis itatiaia **Marulanda-Lopez & Salles, 2023**

Subsequent citations. None.

Deposition of the type material. Holotype: nymph deposited at UFVB. Paratypes: twelve nymphs deposited at UFVB. Additional material examined: 77 nymphs and 3 subimagos deposited at UFVB.

Diagnosis.

Based from Marulanda-Lopez & Salles, 2023

Nymphs: 1) frontal keel present; 2) lingua pointed apically (Fig. 2D in Marulanda-Lopez & Salles, 2023); 3) paraglossae nearly 2.3x longer than wide, with three rows of long, simple setae apically; 4) segment II of labial palp with a moderate developed distomedial projection rounded in shape (Fig. 3A); 5) fore femur with 40-42 long, apically acute, simple setae in outer margin; 6) abdomen, terga with two lateromedial spots on the segments IV and VII; 7) posterior margin of abdominal terga with pointed spines (Fig. 3B in Marulanda-Lopez & Salles, 2023); 8) paraprocts marginally with 12-14 spines increasing in length apically .

Male subimago: 1) dorsal portion of turbinate eyes ellipsoidal, inner margins widely separated and divergent anteriorly; 2) abdominal terga brown, except by yellowish brown sigilla, medial stripe and terga X; 3) length of body 5.14 mm, fore wing 5.44.

Distribution. Brazil: Minas Gerais and Rio de Janeiro borders.

Americabaetis alphus Lugo-Ortiz & McCafferty, 1996 (Fig. 4A)

Subsequent citations. Francischetti *et al*., 2003: 69 (record from Rio de Janeiro); Meyer *et al*. 2008: 135 (record from Nicaragua: San Juan River); Salles *et al*., 2010: 22 (records from Brazil: Alagoas, Sergipe, Goiás, Bahia, Amazonas, Espírito Santo); Gutiérrez & Llano, 2015: 148 (record from Colombia: Huila and Caldas).

Deposition of the type material. Holotype: nymph deposited at PERC. Paratypes: 39 nymphs deposited at PERC. Additional material examined by the authors of the species: 1758 nymphs deposited at PERC.

Diagnosis.

Based from Lugo-Ortiz & McCafferty, 1996

Nymphs: 1) frontal keel present; 2) lingua pointed apically; 3) paraglossa nearly 2.2x longer than wide; 4) segment II of labial palp with a moderately developed rounded distomedial projection; 5) fore femur with 25-27 long, apically acuted, with simple setae in the outer margin; 6) abdominal terga IV and VII with circular lateral spots; 7) posterior margin of abdominal terga with pointed spines; 8) paraprocts marginally with 6-7 spines.

Male imago: 1) turbinate eyes uniformly and widely separated from each other, upper surface ellipsoidal; 2) forceps with first segment not constricted medioapically; 3) posterior margin of subgenital plate without a small rounded projection; 4) abdominal color without distinctive pattern; 5) length of body relatively small (less than 5.0 mm).

Distribution. Argentina: Córdoba, Tucumán; Paraguay: Paraguarí, Amambay, Cordillera, Guairá; Misiones Brazil: Santa Catarina, Paraná, Rio Grande do Sul, Minas Gerais, Rio de Janeiro, Alagoas, Sergipe, Amazonas, Goiás, Espírito Santo; Chile: Aconcagua, Conquimbo; Nicaragua: San Juan river; Colombia: Huila, Caldas and Quindio.

Americabaetis labiosus Lugo-Ortiz & McCafferty, 1996 (Fig. 4B)

Subsequent citations. Francischetti *et al*., 2003: 69 (record from Rio de Janeiro); Salles *et al*., 2010: 23 (description of male imago and records from Minas Gerais and Espírito Santo); Bardavid & Nieto, 2012: 170 (record from Argentina: Misiones).

Deposition of the type material. Holotype: nymph deposited at PERC. Paratypes: eleven nymphs deposited at PERC. Additional material examined: 437 nymphs deposited at PERC.

Diagnosis.

Based from Lugo-Ortiz & McCafferty, 1996 and Salles et al. 2010

Nymphs: 1) frontal keel absent; 2) lingua pointed apically (Fig. 17 in Lugo-Ortiz & McCafferty, 1996); 3) paraglossae nearly 2.5x longer than wide, with three rows of long, simple setae apically; 4) segment II of labial palp with moderate distomedial projection (Fig. 3B); 5) fore femur with 40-42 long, apically acuted, simple setae in outer margin; 6) abdomen yellow-brown, with no distinct pattern, rarely with cream median longitudinal streak; 7) posterior margin of abdominal terga with rounded spines; 8) paraprocts marginally with 16-18 spines increasing in length apically (Fig. 25 in Lugo-Ortiz & McCafferty, 1996).

Male imago: 1) dorsal portion of turbinate eyes ellipsoidal, inner margins widely separated and divergent anteriorly; 2) abdominal terga light whitish brown, lateral margins darker; tracheation blackish; 3) forceps segment I distinctly constricted medioapically; 4) posterior margin of subgenital plate with rounded projection; 4) length of body 4 mm.

Distribution. Brazil: Santa Catarina, Paraná, Rio Grande do Sul, Rio de Janeiro, Minas Gerais, Espírito Santo; Paraguay: Itapúa, Paraguaí, Maldonado; Argentina: Misiones.

Americabaetis longetron Lugo-Ortiz & McCafferty, 1996 (Fig. 4C)

Subsequent citations. Francischetti *et al*., 2003: 69 (record from Rio de Janeiro); Salles & Boldrini, 2008: 565 (description of male imago and record from Espírito Santo); Salles *et al*., 2010: 25 (record from Espírito Santo).

Deposition of the type material. Holotype: nymph deposited at PERC. Paratypes: 6 nymphs deposited at PERC. Additional material examined: 84 nymphs deposited at PERC.

Diagnosis.

Based from Lugo-Ortiz & McCafferty, 1996

Nymph: 1) frontal keel present; 2) lingua rounded apically; 3) paraglossa nearly 2.3x longer than wide; 4) segment II of labial palp with a well developed digitiform distomedial projection (Fig. 3C); 5) fore femur with 22-25 long, apically acuted, simple setae in the outer margin decreasing in length apically; 6) distinct longitudinal brown medial band; 7) posterior margin of abdominal terga with pointed spines, nearly 2.0x basal width; 8) paraprocts marginally with 11-13 slender spines, increasing in length apically.

Male imago: 1) turbinate eyes uniformly separated from each other, upper surface ellipsoidal; 2) abdominal terga with a light yellowish brown band along midline; 3) forceps with first segment distinctly constricted medioapically; 4) posterior margin of subgenital plate without a small rounded projection; 5) length of body relatively long (6.3 mm).

Distribution. Uruguay: Maldonado; Brazil: Santa Catarina, Paraná, Rio de Janeiro, Minas Gerais, Espírito Santo; Paraguay: Amambai, Guairá, Itapúa, Paraguari; Argentina: Misiones.

Americabaetis maxifolium Lugo-Ortiz & McCafferty, 1996

Subsequent citations. None.

Deposition of the type material. Holotype: one nymph deposited at PERC. Paratypes: three nymphs deposited at PERC. Additional material examined: four nymphs deposited at PERC.

Diagnosis.

Based from Lugo-Ortiz & McCafferty, 1996

Nymphs: 1) frontal keel present; 2) lingua pointed apically; 3) paraglossa nearly 2.1x longer than wide; 4) segment II of labial palp with a pronounced distomedial projection (Fig. 3D); 5) fore femur with 18-20 robust, apically acute, simple setae in the outer margin; 6) posterior margin of abdominal terga with pointed spines, nearly 2.0x basal width (Fig. 42 in Lugo-Ortiz & McCafferty, 1996); 7) gills as long as two and one-half or three abdominal segments combined (Fig. 43 in Lugo-Ortiz & McCafferty, 1996); 8) paraprocts marginally with 10-11 sharp spines, increasing in length apically.

Adults: unknown.

Distribution. Paraguay: Presidente Hayes.

Americabaetis titthion Lugo-Ortiz & McCafferty, 1996 (Fig. 4D)

Subsequent citations. Francischetti *et al*., 2003: 69 (record from Brazil: Rio de Janeiro); Salles *et al*., 2004: 24 (description of female imago and record from Rio de Janeiro); Salles *et al*., 2010: 26 (record from Brazil: Espírito Santo and Minas Gerais); Bardavid & Nieto, 2012: 172 (record from Argentina: Misiones).

Deposition of the type material. Holotype: nymph deposited at PERC. Paratypes: 7 nymphs deposited at PERC. Additional material examined: 156 nymphs deposited at PERC.

Diagnosis.

Based from Lugo-Ortiz & McCafferty, 1996

Nymphs: 1) frontal keel absent; 2) lingua broadly pointed apically; 3) paraglossa nearly 2.2x longer than wide; 4) segment II of labial palp with a moderate developed rounded distomedial projection; 5) meso and metasternum with paired setose protuberances (Fig. 3E); 6) fore femur with 40-42 long apically acute, simple setae in the outer margin decreasing in length apically; 7) posterior margin of abdominal terga with rounded spines; 8) paraproct marginally with 16-18 somewhat irregular spines.

Female imago: 1) posterior margin of head dorsally projected; 2) forewing with costal area reddish-brown; 3) meso and metasterna with small, setose, paired protuberances.

Male imago: unknown.

Distribution. Brazil: Santa Catarina, Paraná, Rio de Janeiro, Rio Grande do Sul, Minas Gerais, Espírito Santo; Argentina: Misiones.

Americabaetis mecistognathus Salles & Raimundi, 2010 in Salles *et al*., 2010

Subsequent citations. Bardavid & Nieto, 2012: 171 (Record from Argentina).

Deposition of the type material. Holotype nymph (UFES), paratypes [4 nymphs, 2 at CEUNES (one mounted on slides), 2 at INPA].

Diagnosis.

Based from Salles *et al*., 2010

Nymphs: 1) frontal keel absent (Fig. 3F); 2) lingua broadly pointed apically (Fig. 6 in Salles *et al*., 2010); 4) paraglossa subrectangular; 5) segment II of labial palp with a moderate developed rounded distomedial projection, distomedial protuberance 0.4x width of the base of segment III (Fig. 8 in Salles *et al*., 2010); 6) mouthparts unusually elongate (Fig. 2 in Salles *et al*., 2010); 7) posterior margin of abdominal terga with rounded spines (Fig. 11 in Salles *et al*., 2010); 8) paraprocts marginally with 8 spines.

Adults: unknown.

Distribution. Brazil: Santa Catarina; Argentina: Misiones.

Americabaetis boriquensis (Lugo-Ortiz & McCafferty, 1994)

Subsequent citations. None.

Deposition of the type material. Holotype female nymph and paratypes female nymph and four male nymph (CU).

Diagnosis.

Based from Lugo-Ortiz & McCafferty, 1994

Nymphs: 1) frontal keel absent; 2) maxilla elongated, maxillary palps extending as far as galealacinia; 3) paraglossae broad, three rows of weakly pectinate setae at apex; 4) segment II of labial palp with a digitiform distomedial projection; 5) fore femur with 21-22 long, apically acute, simple setae in outer margin; 6) posterior margin of abdominal terga with short pointed spines; 7) paraprocts marginally with 5-6 sharp spines (Fig. 5 in Lugo-Ortiz & McCafferty, 1994).

Adults: unknown.

Distribution. Puerto Rico: Utuado.

Americabaetis bridarollii (Navas, 1933)

Subsequent citations. None.

Deposition of the type material. Type material unable to locate, probably lost (Lugo-Ortiz & McCafferty, 1999).

Diagnosis. *Nymph:* unknown.

Male imago: 1) rusty head, with rusty-shelled eyes; 2) thorax in dorsal view with a medial longitudinal band; 3) abdomen yellow with posterior margin terga darker; 4) fore wing with III-V thick transversal veins in the stigmatic field.

Distribution. Argentina: Santa Fe.

Americabaetis jorgenseni (Esben-Petersen, 1909)

Subsequent citations. None.

Deposition of the type material. Material in alcohol in the personal collection of the author (Esben-Petersen, 1909). Type material unknown (Lugo-Ortiz & McCafferty, 1999).

Diagnosis. *Nymph:* unknown.

Male imago: 1) turbinate eyes brownish-red; 2) mesothorax pitchy brown; 3) abdomen light brown; 4) terminal tip of the tibiae brown; 5) fore wing hyaline with a faint yellowish brown tint (Fig. 2 in Esben-Petersen, 1909); 6) forceps without medial constriction (Fig. 1 in Esben-Petersen, 1909).

Female imago: 1) mesothorax pitchy brown; 2) abdomen brown red; 3) femora light brown, tibiae and tarsi yellowish brown; 4) wings as male.

Distribution. Argentina: Mendoza.

Americabaetis oldendorffii (Weyenbergh, 1883)

Subsequent citations. None.

Deposition of the type material. The type material of this species was apparently lost (Lugo-Ortiz & McCafferty, 1999).

Diagnosis. *Nymphs*: unknown.

Male imago: 1) thorax pale gray; 2) fore wings as the figure 6 in Weyenbergh, 1883; 3) abdomen grayish brown.

Distribution. Northern Argentina.

Americabaetis peterseni (Esben-Petersen, 1909)

Subsequent citations. Hubbard, 1974: 357 (*Cloeon peterseni* new name for *Cloeon brunneum*).

Deposition of the type material. The species was described based on an unspecified number of male, female adults and subimagos, the generically diagnostic figure of the male genitalia provided in the description, clearly indicating that this species belongs to *Americabaetis* (Esben-Petersen, 1909).

Diagnosis. *Nymphs*: unknown.

Male imago: 1) turbinate eyes light brown; 2) mesothorax in dorsal view with two grayish yellowish oblong spots, each surrounded by a dark line and separated from one another by a brownish gray longitudinal stripe; 3) first abdominal segment anteriorly brown with a dark brow W-shaped marking, posteriorly white (Fig. 4 in

Esben-Petersen, 1909); 4) wing hyaline with costa, subcostal and radial nervures yellowish; 5) genitalia as the figure 3 in Esben-Petersen, 1909.

Female imago: 1) head brownish red; 2) mesothorax with three longitudinal stripes, separated by two dark brown, posteriorly wavy bands; 3) first abdominal segment anteriorly brown with a dark brow W-shaped marking; 4) wing with a yellowish tint, and the nervures more distinct.

Distribution. Argentina: Mendoza.

Americabaetis weiseri Navás, 1926

Subsequent citations. None.

Deposition of the type material. One specimen labeled as "Typus" (Museo de Zoologia del Ayuntamiento, Barcelona, España) (Lugo-Ortiz & McCafferty, 1999).

Diagnosis**.** *Nymphs*: unknown.

Male imago: 1) eyes in black socket, upper part of the turbinate eyes is depressed; 2) abdomen yellow with posterior region of darker abdominal terga; 3) legs yellowish; 4) wings with cross veins of the brownish stigmatic region of the wing forming a reticulate net, gradually towards the base shorter (Fig. 2 in Navás, 1926).

Distribution. Argentina: Catamarca.

Americabaetis lugoi Waltz & McCafferty, 1999

Subsequent citations. Randolph and McCafferty, 2000: 115 (record from Mexico); Meyer *et al*., 2008: 135 (Record from Nicaragua).

Deposition of the type material. Holotype female nymph and paratypes four female nymph and four adult females (PERC).

Diagnosis.

Based from Waltz & McCafferty, 1999

Nymphs: 1) frontal keel present (Fig. 1 in Waltz & McCafferty, 1999); 2) maxilla elongated, maxillary palp reaching the apex of galealacinia; 3) paraglossa elongated and robust; 4) segment II of labial palp with well developed conical distomedial projection (Fig. 5 in Waltz & McCafferty, 1999); 5) fore femur with 18-20 blunt setae in the outer margin; 6) abdominal terga with broken dorsal medial stripe; 7) posterior margin of abdominal terga with sharp spines, length 1-2x the basal width; 8) paraprocts with 8-10 marginal spines (Fig. 7 in Waltz & McCafferty, 1999).

Female imago: 1) body brick-red to dark reddish brown; 2) legs pale yellow contrasting with dorsal coloration of the body; 3) fore wing with distinctive brown stain at base (Fig. 8 in Waltz & McCafferty, 1999; 4) abdominal segments with thin pale median longitudinal stripe (Fig. 9 in Waltz & McCafferty, 1999).

Distribution. Costa Rica: Guanacaste; Mexico: Chiapas; Nicaragua: San Juan River.

Americabaetis humilis Hoffman & Thomas 1999 in Hoffman *et al*., 1999

Subsequent citations. None.

Deposition of the type material. Holotype and paratypes (personal collection of the author).

Diagnosis.

Based from Hoffman *et al*., 1999

Nymphs: 1) frontal keel absent; 2) lingua pointed apically (Fig. 23 in Hoffman *et al*., 1999); 3) paraglossa nearly 2x longer than wide; 4) segment II of labial palp with a well developed digitiform distomedial projection; 5) fore femur with maximum 10 long, apically acute, simple setae in the outer margin; 6) posterior margin of abdominal terga with pointed spines (Fig. 28 in Hoffman *et al*., 1999); 7) paraprocts marginally with 10-11 spines Fig. 30 in Hoffman *et al*., 1999); 8) caudal filaments sometimes with two brown bands marked.

Male imago: 1) turbinate eyes anteriorly divergents; 2) abdominal segments I-VI whitish-translucent and segments VII-X brown; 3) genitalia with first segment of the forceps brown and in the form of a "staircase" (Fig. 34 in Hoffman *et al*., 1999); 4) maximum body length 3.2 mm.

Female imago: 1) head more or less uniformly brown-red; 2) red-brown thorax; clear paws; 3) red-brown abdomen.

Eggs: 1) length-width = 1.85; 2) oval shape without polar capsule; 3) particular micropylar structure not viewed; 4) chorion formed by ridges relatively spaced delimiting slow polygonal concavities (Figs. 202-203 in Hoffman, 1999).

Distribution. Guadeloupe: Grande Rivière à Goyaves, Petit David Premier Bras River, Baron River, Bonfils River, Ravine Grand Camp, Corossol River (Bras Saint-Jean).

Americabaetis spinosus Hoffman & Thomas 1999 in Hoffman *et al*., 1999

Subsequent citations. None.

Deposition of the type material. Holotype and paratypes (personal collection of the author).

Diagnosis.

Based from Hoffman *et al*., 1999

Nymph: 1) frontal keel absent; 2) lingua pointed apically (Fig. 5 in Hoffman *et al*., 1999); 3) paraglossa nearly 2x longer than wide; 4) segment II of labial palp with a well developed developed digitiform distomedial projection; 5) fore femur with 20-30 long, apically acute, simple setae in the outer margin; 6) posterior margin of abdominal terga with pointed spines (Fig. 11 in Hoffman *et al*., 1999); 7) paraprocts with 6-8 marginal spines Fig. 12 in Hoffman *et al*., 1999); 8) caudal filaments with twot brown bands, one located at the middle and other at the end of the end.

Male imago: 1) well separated turbinate eyes, anteriorly divergent, reddish above, brown below, and oval in shape (Figs. 14-16 in Hoffman, 1999); 2) abdominal segments I-V and anterior part of VI translucent, segments VII-VIII and posterior part of VI yellow-brown, segments IX-X whitish; 3) genitalia with first segment of very pronounced conical shape, much wider at the base than at the distal end (Fig. 17 in Hofffman *et al*., 1999); 4) maximum body length 4.2 mm.

Female imago: 1) head more or less uniformly brown-red.; 2) red-brown thorax; legs pale, with coxa, trochanters and bases of femurs brown-red; 3) red-brown abdominal segments.

Eggs: 1) length-width = 1.7; 2) oval shape without polar capsule; 3) particular micropylar structure not viewed; 4) chorion formed by ridges spaced delimiting circular or oval concavities (Figs. 200-201 in Hoffman *et al*., 1999).

Distribution. Guadeloupe: Pumping station, Goyavier Bridge, Hermitage, Corossol River (Bras Saint-Jean), Grand Matouba, Fond Royal, Montgommier.

Americabaetis robacki (Lugo-Ortiz & McCafferty, 1994)

Subsequent citations. Waltz & McCafferty, 1999: 42 (record from Uruguay); Forero-Cespedes *et al*., 2014: 61 (record from Colombia).

Deposition of the type material. Holotype: female larva deposited at ANSP.

***Diagnosis*.**

Based from Lugo-Ortiz & McCafferty, 1994

Nymphs: 1) frontal keel absent; 2) maxilla robust, maxillary palps slightly extended beyond galealacinia; 3) segment I of labial palp as long as segments II and III combined; 4) segment II of labial palp with well developed digitiform projection; 5) fore femur with 20-21 long, apically acuted, simple setae in outer margin; 6) posterior margin of abdominal terga with short sharp spines (Fig. 11 in Lugo-Ortiz & McCafferty, 1994); 7) paraprocts marginally with 10 long, slender spines (Fig. 15 in

Lugo-Ortiz & McCafferty, 1994); 8) terminal filament with medial brown band and pre-apical brown bands.

Adults: unknown.

Distribution. Peru: Loreto; Uruguay: Maldonado; Colombia: Tolima.

Americabaetis intermedia (Lugo-Ortiz & McCafferty, 1994)

Subsequent citations. Lugo-Ortiz & McCafferty, 1996a: 304 (record from Guatemala and México); Randolph & McCafferty, 2000: 115 (record from México); McCafferty *et al*., 2004: 203 (record from Guatemala); Meyer *et al*., 2008: 135 (record from Nicaragua).

Deposition of the type material. Holotype: male nymph deposited at PERC. Paratypes: male nymph deposited at PERC, one male and female nymphs deposited at FAMU, one male nymph deposited at PERC. Additional material examined: eleven nymphs deposited at PERC.

***Diagnosis*.**

Based from Lugo-Ortiz & McCafferty, 1994

Nymph: 1) frontal keel present; 2) head with triangular process (Fig. 6 in Lugo-Ortiz & McCafferty, 1994); 3) maxillary palp extending so far as galealacinia; 4) segment I and II of labial palp subequal and longer; 5) segment II of labial palp with moderate-developed rounded distomedial projection (Fig. 10 in Lugo-Ortiz & McCafferty, 1994); 6) fore femur with 20-21 long, apically acuted, simple setae in the outer margin; 7) posterior margin of abdominal terga with short sharp spines; 8) paraprocts marginally with numerous minute spines (Fig. 11 in Lugo-Ortiz & McCafferty, 1994).

Adults: unknown.

Distribution. Costa Rica: San Jose and Puntarenas; Guatemala: Totonicapán and Solala; Mexico: Oaxaca, Veracruz; Guatemala: Baja Verapaz; Nicaragua: San Juan river, León.

Americabaetis pletura (Lugo-Ortiz & McCafferty, 1994)

Subsequent citations. Lugo-Ortiz & McCafferty, 1996a: 304 (record from México and Panama); Wiersema & McCafferty, 1999: 36 (male and female adult descriptions and record from USA), Randolph & McCafferty, 2000: 115 (record from México); McCafferty *et al*., 2004: 203 (record from Guatemala); Meyer *et al*., 2008: 135 (record from Nicaragua).

Deposition of the type material. Holotype: male nymph deposited at PERC. Paratypes: two male and three female nymphs deposited at PERC. Additional material examined: two nymphs deposited at FAMU and sixteen nymphs deposited at PERC.

Diagnosis.

Based from Lugo-Ortiz & McCafferty, 1994 and Wiersema & McCafferty, 1999

Nymph: 1) frontal keel absent; 2) maxilla robust, palps extending as far as galealaecinia; 3) segment I of labial palp as long as segments II-III combined; 4) segment II of labial palp with well developed digitiform projection; 5) fore femur with 17-18 long, apically acuted, simple setae in the outer margin; 6) posterior margin of abdominal terga with short sharp spines; 7) paraprocts with 11-13 sharp marginal spines (Fig. 15 in Lugo-Ortiz & McCafferty, 1994); 8) caudal filaments with broad brown band medially.

Male imago: 1) turbinate eyes large, widely divergent anteriorly, and almost contiguous posteriorly (Fig. 4 in Lugo-Ortiz & McCafferty, 1994); 2) abdominal segments I-V with faint orange-red shading; posterior half of terga VI and VII-X orange-red, and segments I-VII with branching spiracular lines laterally and extending dorsally; 3) genitalia with basal segment large and cylindrical, with distinct medio apical constriction (Fig. 1 in Wiersema & McCafferty, 1999); 4) length of body 4.2-5 mm.

Female imago: 1) body reddish brown; 2) abdomen distinctly darker laterally than dorsally and ventrally (with broad, median pale longitudinal area both dorsally and ventrally (Fig. 2 in Wiersema & McCafferty, 1999); 3) dark branching tracheation lines laterally and dorsally.

Distribution. México: Nuevo León, San Luis Potosí, Tamaulipas, Veracruz; Conca river, Piedras Negras river; Panama: George Green Park; EUA: Texas; Guatemala: Baja Verapaz, Izabal, El Progreso; Nicaragua: Granada and San Juan river.

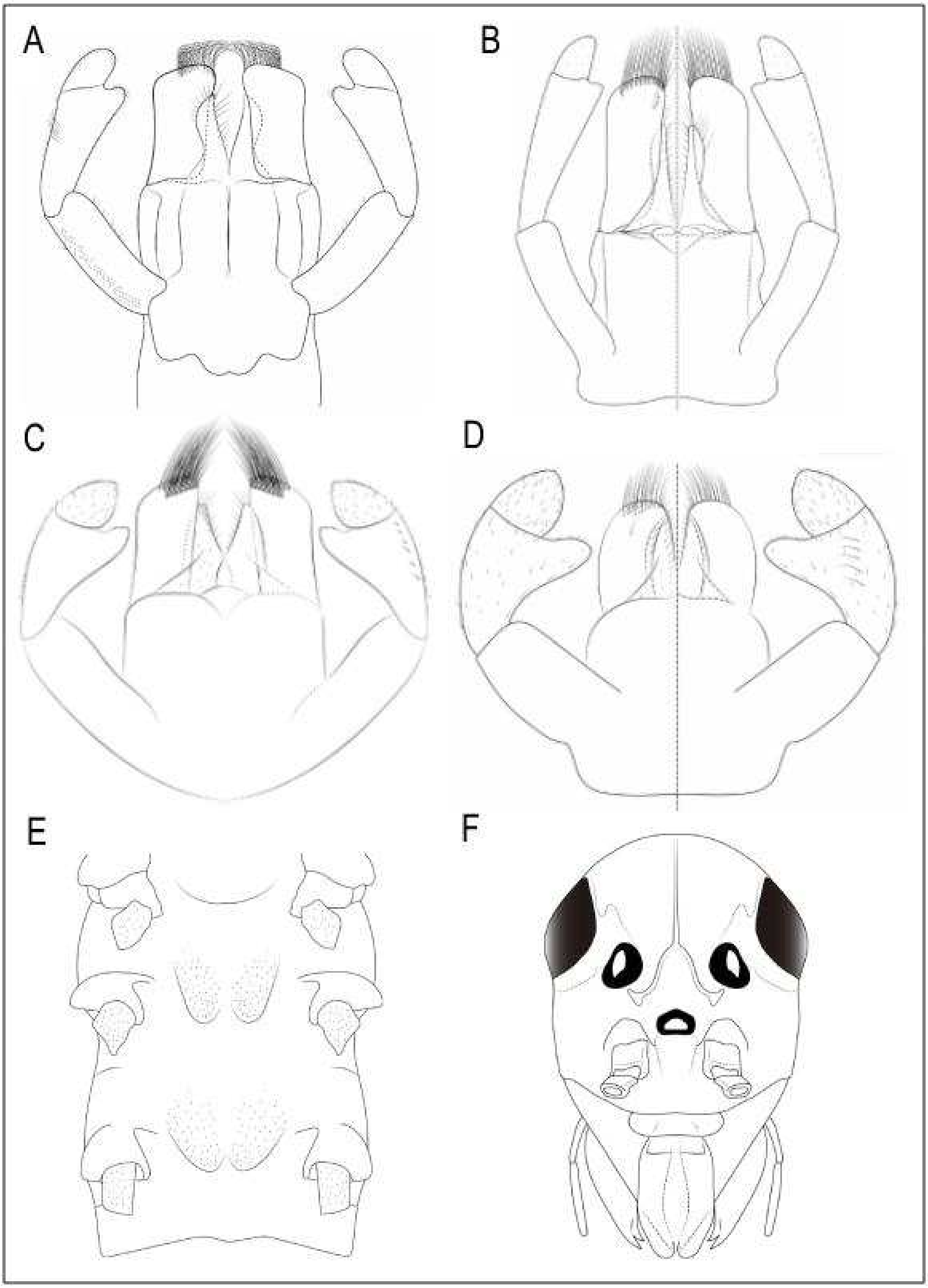

Figure 3. Nymphal characters of *Americabaetis* species: A) labium of *A. itatiaia*, left dorsal view, right ventral view; B) labium of *A. labiosus*, left dorsal view, right ventral

view; C) labium o *A. longetron*; D) labium of *A. maxifolium*, left dorsal view, right ventral view; E) ventral view of *A. titthion*; F) head of *A. mecistognathus* in frontal view.

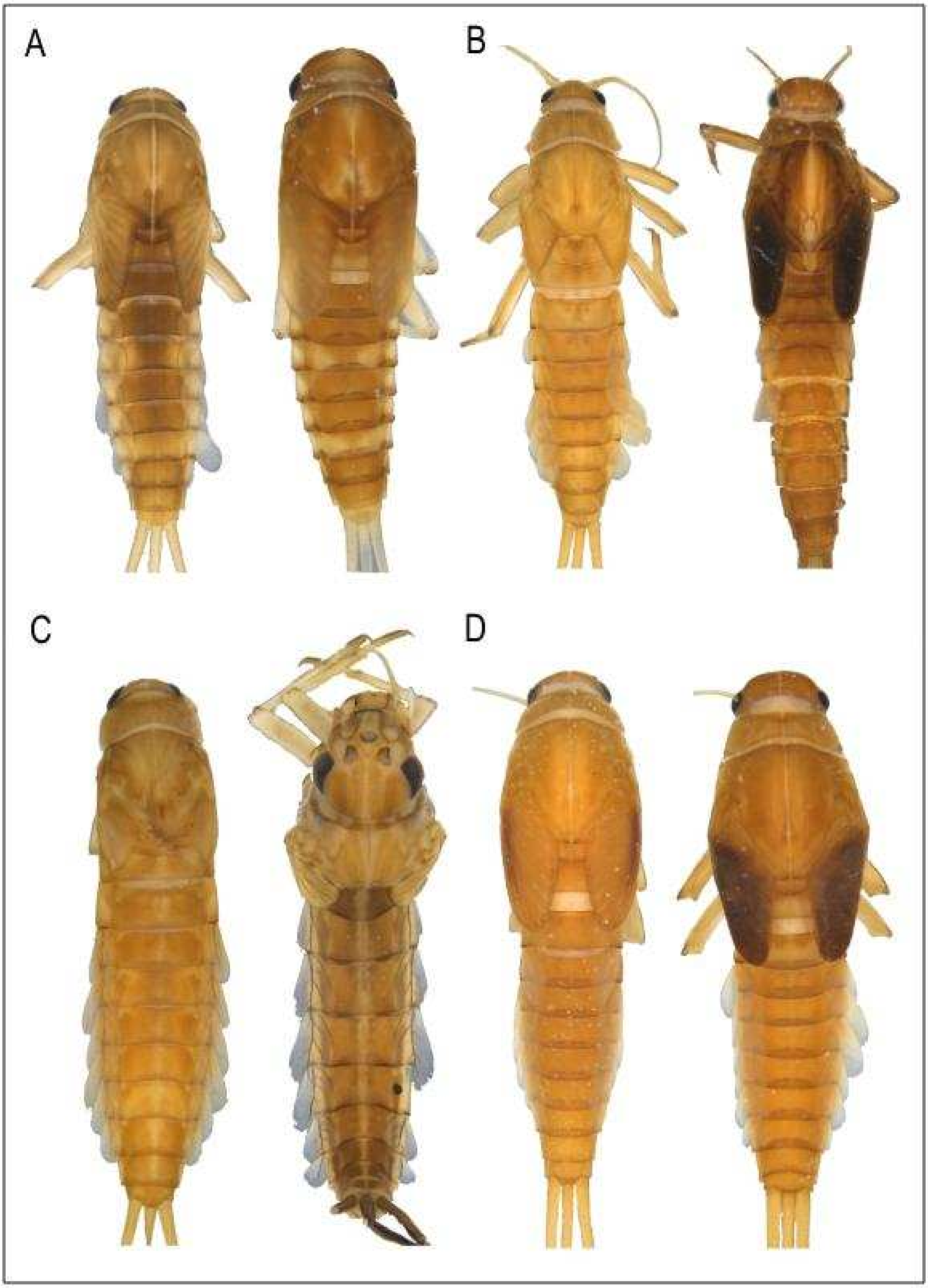

Figure 4. Habitus in dorsal view of *Americabaetis* species. Left side female and right side male: A) *A. alphus*; B) *A. labiosus*; C) *A. longetron*; D) *A. titthion*.

Biology and Ecology

Biological aspects and ecology of *Americabaetis* species remain poorly studied. However, Jackson & Sweeney (1995) discussed some biological aspects of *A. lugoi* (as *Acerpenna* sp.) The authors reported a median developmental time of 23 days for the eggs and 28 days for the nymphs. In the same paper, this species was considered as a recollector of particulate organic matter, since they could successfully complete development on algae diet. In addition, males were not present in a sample of 86 reared specimens, considering these results, Waltz & McCafferty (1999) proposed that the population of Costa Rica of this species as parthenogenetic. Wiersema & McCafferty (1999) reported that the nymphs of *A. pletura* were collected from the lower reaches of the Guadalupe River, where they were found clinging to cobble heavily covered with periphyton in a swiftly flowing riffle. This region of the river is characterized by high clay banks, shallow flat riffle areas offset from the main channel with a substrate of mixed cobble, gravel, and sand, and having shifting sand substrate.

Salles *et al*. (2010) reported that the nymphs of *A. titthion*, *A. labiosus*, and *A. mecistognathus* are present in shallow areas above rocks, in moderate to fast current. The same authors reported that the nymphs of *A. longetron* were present almost exclusively in banks with marginal vegetation and leaf litter in areas with slow to moderate current, and somewhat similar habitat for the species *A. alphus*. In the same publication, the author highlighted that the nymphs of the last cited species were found in several biomes and on small streams to large rivers. The altitudinal range of *A. alphus* is also highly variable; specimens are found from the sea level to 1900 m.a.s.l. They are also encountered in pristine to moderately impacted streams, demonstrating that the species is somewhat tolerant, which, in turn, would explain its wide distribution (Salles *et al*., 2010). Gutiérrez & Llano (2015) recorded the presence of *A. alphus* from Colombia, the specimens were collected in two habitats at 187 and 1555 masl, and associated with leaves and stony substrates.

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Table 1. Examined material. SC is Santa Catarina, MG is Minas Gerais, FFS is Frederico Falcão Salles, TSR is Tayna Silva Raymundo, IDO is Iury Domingues, JFM is Jhon Faber Marulanda, DB is Daniel Beliche, MINES is Maria Ines Gonçalves, MLSR is Mellis Layra Soares Rippel, ICHC is Isabel Cristina Hernández Cortés, PB is Pedro Bonfá and IFA is Igor Ferreira Amaral.

Species	**Country**	**State**	**Municipality**	**Locality**	**Latitude**	**Longitud e**	**Altitude m.a.s.l**	**Collectors**	**Data**	**Individuals**	**Stage**	**Collection**
A. labiosus	Brazil	SC		#12					06.ix. 2009	4	Nymph	UFVB
A. labiosus	Brazil	SC		#2					01.ix. 2009	5	Nymph	UFVB
A. labiosus	Brazil	SC		#3					01.ix. 2009	1	Nymph	UFVB
A. labiosus	Brazil	SC		#8					04.ix. 2009	4	Nymph	UFVB
A. labiosus	Brazil	SC		#2					01.ix. 2009	26	Nymph	UFVB
A. labiosus	Brazil	SC		#12					06.ix. 2009	6	Nymph	UFVB
A. labiosus	Brazil	SC		#1					31.viii .2009	3	Nymph	UFVB
A. titthion	Brazil	SC		#2					01.ix. 2009	3	Nymph	UFVB
A. titthion	Brazil	SC		#1					31.viii .2009	5	Nymph	UFVB
A. titthion	Brazil	MG	Araponga	Cachoei	20°38’49.	42°27’29.	949	FFS, TSR,	11.vii.	4	Nymph	UFVB

				ra do Bone	25”S	74”W		IDO & JFM	2019			
A. titthion	Brazil	MG	Araponga	Cachoeira do Bone	20°38’49.25”S	42°27’29.74”W	949	FFS, DB, MINES	24.ix.2018	18	Nymph	UFVB
A. titthion	Brazil	MG	Araponga	Cachoeira do Bone	20°38’49.25”S	42°27’29.74”W	949	FFS	24.ix.2021	1	Nymph	UFVB
A. longetron	Brazil	SC		#4					01.ix.2009	2	Nymph	UFVB
A. longetron	Brazil	SC		Farna Araucárias					13.iii.2009	10	Nymph	UFVB
A. longetron	Brazil	MG	Araponga	Cachoeira do Bone	20°38’49.25”S	42°27’29.74”W	949	FFS, DB, MINES	24.ix.2018	2	Nymph	UFVB
A. longetron	Brazil	MG	Araponga	Cachoeira do Bone	20°38’49.25”S	42°27’29.74”W	949	FFS, Pereira & JFM	05.ix.2018	2	Nymph	UFVB
A. alphus	Brazil	SC		#8					04.ix.2009	3	Nymph	UFVB
A. alphus	Brazil	SC		#1					31.viii.2009	2	Nymph	UFVB
A. itatiaia	Brazil	MG	Itatiaia National Park	Campo Belo River	22°21’40”S	44°41’37”W	2400	FFS, MLSR, ICHC & PB	17.iii.2022	13	Nymph	UFVB
A. itatiaia	Brazil	MG	Itatiaia	Rebouça	22°23’06”	44°40’43”	2380	FFS,	17.iii.	14	Nymph	UFVB

			National Park	s dam	S	W		MLSR, ICHC & PB	2022			
A. itatiaia	Brazil	MG	Itatiaia National Park	Pedra do Camelo Stream	22°22’11” S	44°42’33” W	2350	FFS, MLSR, ICHC & PB	17.iii. 2022	14	Nymph	UFVB
A. itatiaia	Brazil	MG	Itatiaia National Park	Stream close to the road Km12	22°22’15” S	44°42’21” W	2380	FFS, MLSR, ICHC & PB	17.iii. 2022	19	Nymph	UFVB
A. itatiaia	Brazil	MG	Itatiaia National Park	Brejo da Lapa	22°21’32” S	44°44’14” W	2140	FFS, MLSR, ICHC & PB	16.iii. 2022	2	Subima go	UFVB
Tupiara ibirapitanga	Brazil	MG	Ibitipoca National Park	Megalop tera beach				FFS, MLSR & JFM	25.x.2 022	8	Nymph	UFVB
Tupiara ibirapitanga	Brazil	MG	Ibitipoca National Park	#3				FFS, MLSR & JFM	26.x.2 022	15	Nymph	UFVB
Zelusia principalis	Brazil	MG	Araponga	Cap trail				FFS, ICHC, PB, IFA	09.ix. 2023	1	Nymph	UFVB

7. FINAL CONSIDERATIONS

The species *A. bridarollii*, *A. jorgenseni*, *A. oldendorffii*, *A. peterseni*, and *A. weiseri* were placed in *Americabaetis* by the absence of hind wings, body size and geographical data, and the type material is unable to locate, apparently loss or very deteriorated. The lack of location of the type material originally assigned to these species, the incomplete descriptions and the way in which they were assigned to the genus *Americabaetis* highlight that the establishment of these species in *Americabaetis* remains uncertain (Alba-Tercedor & Peters, 1985; Lugo-Ortiz & McCafferty, 1999; Bardavid & Nieto, 2012). The type localities in Mendoza, Catamarca, Cordoba and Santa Fe for these Argentine species have been sampled in recent years by Argentine researchers, who unfortunately have not been successful in their field collections (C Molineri, **pers. commun.**). The absence in the field in recent years, the conditions to access the information of these species limits their inclusion in future studies and we propose the status of *nomen dubium* for these species.

A. alphus is a widely distributed species in South America (Molineri et al., 2020) and occurs in many localities and subregions of the neotropical region (Morrone et al., 2022), it is possible exhibits phenotypic plasticity or corresponds to a species complex, as recently reported by *Andesiops peruvianus* (Ulmer, 1920) (Mera et al., 2023). A Brazilian researcher is analyzing samples from different locations of *A. alphus*, including those from Colombia (LG Dias, **pers. commun.**), in a more comprehensive morphological and molecular study focused which will soon provide future results of the identity of this species.

Other sources of information such as molecular characters, oological characters and quantitative morphological analysis using bioinformatic tools such as geometric morphometry, could complement the morphological characters traditionally implemented in the reconstruction of phylogenetic hypothesis and taxonomic work, as well the inclusion of these types of characters generate additional support for the well supported clades or change the topology of poorly supported clades such as proposed by other mayfly groups (Dominguez & Cuezzo, 2002; Pereira-da-Conceicoa et al., 2019). In addition, bioinformatic tools as machine learning could help in the analysis of big datasets and the automated recognition of patterns in morphological matrices or images, complementing the taxonomic work of specialists, and aid futurely in the elaboration of a bioinformatic tools such a based-software identification to aid in identify the mayflies species in a automated way by students and non-specialists.

The species of *Americabaetis* are good models to biomonitoring, ecologic and biogeographic studies due to their wide distribution in the neotropical region, abundance in the freshwater environments and biological collections, presence in sites with and without apparent pollution, and their differential response to anthropic contamination and physicochemical variables (Forero-Céspedes & Reinoso-Flórez, 2013). In addition, the replacement of the species due to anthropic pollution (Buss & Salles, 2007; Buss & Vitorino, 2010), is useful in biomonitoring studies when the specific level resolution is used in the biomonitoring studies. In addition, futurely physiologic, genetic, biologic or morphologic biomarkers will be proposed to assess water quality using responses to the species level.

To finalize this study highlights the importance of biological collections as repository information to compile characters and valuable information for species poorly studied and widely distributed. Additionally, studies combining information of literature and of biological collections complement and diversify the dataset to analyze. Interdisciplinary studies can facilitate more comprehensive studies and allow a positive impact on taxonomic and phylogenetic work with a problematic species or a particular group of species, due to the multiple approaches, results and applications after multidisciplinary collaborative work.

8. REFERENCES

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9. Annex I First record of *Vacupernius packeri* (Allen) (Ephemeroptera: Leptohyphidae) from Colombia and South America with additional notes on egg morphology

Rev. Acad. Colomb. Cienc. Ex. Fis. Nat. 44(171):452-457, abril-junio de 2020
doi: https://doi.org/10.18257/raccefyn.1018

Natural Sciences

Original article

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Primer registro del efemeróptero *Vacupernius packeri* en Colombia y América del Sur y descripción de la morfología de sus huevos

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10. Annex II Diversidad de macroinvertebrados acuáticos en el Parque Nacional Natural Selva de Florencia, Colombia

Rev. Acad. Colomb. Cienc. Ex. Fis. Nat. 44(171):560-571, abril-junio de 2020
doi: https://doi.org/10.18257/raccefyn.1027

Ciencias Naturales

Artículo original

Diversidad de macroinvertebrados acuáticos en el Parque Nacional Natural Selva de Florencia, Colombia

Diversity of aquatic macroinvertebrates in the National Natural Park *Selva de Florencia*, Colombia

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11. Annex III Description of a bilateral gynandromorph in *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) from Brazil

Revista Brasileira de Entomologia 65(2):e20200116, 2021

SOCIEDADE BRASILEIRA DE ENTOMOLOGIA FUNDADA EM 1937

REVISTA BRASILEIRA DE Entomologia
A Journal on Insect Diversity and Evolution

Description of a bilateral gynandromorph in *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) from Brazil

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ARTICLE INFO

Article history:
Received 23 November 2020
Accepted 26 April 2021
Available online 12 May 2021
Associate Editor: Héctor Vargas

Keywords:
Fall armyworm
Faxitron X-Ray Corp
Morphology
Gynandromorphic

ABSTRACT

A bilateral gynandromorph of *Spodoptera frugiperda* was reported for the first time from Minas Gerais State, Brazil. The specimen with this trait was found among the progeny of a laboratory rearing population and exhibited dimorphism in the antennae, wings, head, thorax, and abdomen. It had a male left side and a female right side. Both the external and internal genitalia were typically those of a male.

12. Annex IV *Hagenulopsis* Ulmer (Ephemeroptera: Leptophlebiidae): re-description, morphological notes and a new species from South America, Brazil

Zootaxa 0000 (0): 000–000
https://www.mapress.com/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.0000.0.0
http://zoobank.org/urn:lsid:zoobank.org:pub:00000000-0000-0000-0000-00000000000

***Hagenulopsis* Ulmer (Ephemeroptera: Leptophlebiidae): re-description, morphological notes and a new species from South America, Brazil**

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