Zoologischer Anzeiger 284 (2020) 30–42

Contents lists available at ScienceDirect

Zoologischer Anzeiger

journal homepage: www.elsevier.com/locate/jcz

Research paper

Diamantina: An endemic new genus of Neotropical Atalophlebiinae (Ephemeroptera: Leptophlebiidae) evidenced by morphological and molecular data

Frederico Falcão Salles [a, *], Jeane Marcelle C. Nascimento [b], Marina Monjardim [c], Roberta Paresque [c, d], Neusa Hamada [b], Eduardo Dominguez [e]

[a] Museu de Entomologia, Departamento de Entomologia, Universidade Federal de Viçosa, 36570-900, Viçosa, Brazil
[b] Coordenação de Biodiversidade—CoBio, Programa de Pós-Graduação em Entomologia (PPGEnt), Instituto Nacional de Pesquisas da Amazônia, 69067-375, Manaus, AM, Brazil
[c] Programa de Pós-graduação em Biologia Animal, Universidade Federal do Espírito Santo, CEP 29.075-910, Vitória, ES, Brazil
[d] Depto. de Ciências da Saúde, Universidade Federal do Espírito Santo, 29.933-415, São Mateus, ES, Brazil
[e] Instituto de Biodiversidad Neotropical (IBN), CONICET-U.N.T. & Facultad de Ciencias Naturales, Universidad Nacional de Tucumán, San Miguel de Tucumán, 4.000, Tucumán, Argentina

ARTICLE INFO

Article history:
Received 2 March 2019
Received in revised form
24 September 2019
Accepted 28 October 2019
Available online 14 November 2019

Corresponding Editor: S. Bradler

Keywords:
Aquatic insects
Systematics
Mayflies
Ulmeritus
Ulmeritoides
Atopophlebia

ABSTRACT

A new genus and species is described based on nymphs and adults collected exclusively at the Chapada Diamantina National Park, in Brazil. The generic category of this new taxon was tested by reanalyzing a morphological dataset used to access the phylogenetic relationships of species of *Ulmeritus* and *Ulmeritoides* and by the use of molecular analysis, using two different genes. Both analysis corroborated the new taxon belongs to a new genus and species, sister group of *Ulmeritus-Ulmeritoides*, herein described as *Diamantina ulmeri* gen et sp. nov. Among members of this complex, *Diamantina* gen nov. presents a unique apomorphy: absence of setae on lateral margins of abdominal segments. Diagnostic features include the anterior position of the proximal row of setae on dorsal surface of labrum, the U shaped emargination of labrum, eyes of male imago widely separated and shape of penis lobes.

1. Introduction

Ulmeritus Traver and *Ulmeritoides* Traver are two closely related genera of Atalophlebiinae (Ephemeroptera: Leptophlebiidae) from the Neotropics. While *Ulmeritus* is currently composed of three species with a distribution restricted to Uruguay and Southeastern Brazil, *Ulmeritoides* is composed of 18 species and is widely distributed in South and Central America, from Argentina (Misiones Prov.) to Costa Rica (Avila & Flowers 2005; Salles & Domínguez 2012; Gama-Neto & Hamada 2014; Souto et al. 2016; Gama-Neto & Passos 2016; Boldrini & Lima 2017).

Ulmeritoides was originally considered a subgenus of *Ulmeritus*, but Domínguez (1991) raised it to the genus level after associating nymphs and adults. Later, the phylogenetic relationships of the species of *Ulmeritus* and *Ulmeritoides* were revised by Domínguez (1995) and Salles & Domínguez (2012). According to Flowers & Domínguez (1991), these two genera are related to *Atopophlebia* Flowers, another Neotropical Atalophlebiinae whose nymphs possess fimbriate gills and adults share a slanting crossvein above a symmetrical MA fork and penes presenting an apical ventrally oriented lobe (a characteristic secondarily lost in *Ulmeritoides* and not homologous to the telopenis present in Hermanellonota, i.e. Kluge 2007).

During the past 10 years, a large and enigmatic nymph resembling those of *Ulmeritus-Ulmeritoides*, with fimbriate gills, have been collected by us at the Chapada Diamantina National Park, Brazil. Recently, after successive attempts, we were finally able to

* Corresponding author.
E-mail address: frederico.salles@ufv.br (F.F. Salles).

https://doi.org/10.1016/j.jcz.2019.10.005
0044-5231/

collect male and female imagos and subimagos. Besides matching size and coloration of the nymphs, male imagos have a ventrally oriented lobe on the apex of penis, a symmetrical MA fork and a hind wing similar to those of *Ulmeritus* and *Ulmeritoides*. Since this material clearly belongs to the Ulmeritus-Ulmeritoides generic complex, we considered necessary to analyze the relationship of the new species to determine its generic position. To do so we re-analyzed the morphological dataset from Salles & Domínguez (2012) and performed an analysis based on the DNA sequence of portions of two different genes: mitochondrial cytochrome oxidase subunit I (COI) and mitochondrial 16S rRNA. As a result, we herein propose *Diamantina ulmeri*, a new genus and species of Atalophlebiinae.

2. Material and methods

2.1. Cladistic analysis

For the analysis, we based on the matrices generated in the previous studies (Domínguez 1995; Salles & Domínguez 2012). We included the same species treated in Salles & Domínguez (2012), plus the new taxon. We conserved most of the characters and treated them in the same way, although we had to recode some of them to include the new evidence, which are discussed later. Binary characters were coded as 0 and 1. Multistate characters were assigned different numbers and treated in two different ways: additive or non-additive (see list of characters). The program WinClada (Nixon 2002) was used to compile the matrix (Appendix 1) and the program TNT (Goloboff et al. 2008) was used to analyze it. The data were analyzed under "implied weight" (k = 3) and an exhaustive search was performed (with the command "implicit enumeration"). To establish group support, symmetric resampling was estimated because the resulting values obtained under this procedure are not distorted by character weighting. As an additional support Jackknife values were also calculated.

2.2. Characters and coding

Characters are listed in Appendix 2. Only characters treated differently from Salles & Domínguez (2012), due to the new information available from the discovery of the new taxon will be discussed here.

Character 0 (new): number of bullae on fore wings of both sexes. Several aspects of the bullae were recently studied by Domínguez & Abdala (2019). The number of bullae among the South American Leptophlebiidae seems to be fairly stable, being the presence of four bulla in both sexes the common situation. The exceptions are the species of *Ulmeritus* and *Ulmeritoides* in which only three bullae are in both sexes, and *Miroculis* with males of all the species and females of some species presenting three bullae, while the females of other present four. The new material available from *Diamantina* provided more information about the distribution of this character, which is as far as we know used for the first time in the taxonomy of Ephemeroptera.

Character 4. (Spots around cross veins in fore wing): it was modified from presence of bands (present only in *Ulmeritus* species) to width of spots around cross veins, informative to all species.

Characters 14 and 15 [new]: presence and shape of anterior emargination of labrum were added.

Characters 16 and 17: presence and size of denticles on anteromedian emargination of labrum. Former character 14 was divided following Sereno (2007) coding proposal.

Characters 30 and 31: size and setation of abdominal posterolateral projections. Former character 27 was divided into what we consider different characters with the new evidence from *Diamantina*.

2.3. Molecular data

Four specimens of the new species were used for DNA extractions: one nymph and three adults (1 male and 2 females). Mitochondrial cytochrome c oxidase subunit 1 (COI) and a portion of the mitochondrial 16S rRNA gene sequence data were obtained from all four specimens and, additionally, 2 specimens from *Ulmeritus saopaulensis* Traver, *Ulmeritoidesangelus* Souto et al. and *Ulmeritoides* sp., and 1 specimen from *Askola* sp. and 1 from *Meridialaris tintinnabula* Pescador & Peters. Two species of *Paraleptophlebia* were used as an outgroup taxon, *P. submarginata* (Stephens) and *P. vaciva* (Eaton). Due to the lack of fresh material and its absence in GenBank, we were unable to include *Atopophlebia*. GenBank accession numbers with corresponding sequence data obtained in this study are listed in Table 1. For DNA extraction, amplification of the COI marker and sequencing, see Salles et al. (2016). The primers for the amplification of a portion of the 16S rRNA gene were forward 5′-CCGGTYTGAACTCARATCA-3′ (Takiya et al. 2006) and reverse: 5′-TAAGTGTGCAAAGGTAGC-3′ (Malm & Johanson 2008). Polymerase chain reaction (PCR) was set up in 25 μl volume. The following steps were used for amplification: (1) 94 °C for 3 min, (2) 94 °C for 1 min, (3) 50 °C for 1 min, (4) 72 °C for 2 min, (5) 72 °C for 7 min. Steps (2) to (4) were repeated thirty-nine more times for a total of forty cycles. In all cases, both strands of the amplified fragment were sequenced. The programs used for creating the data

Table 1
Ephemeroptera specimens used in this study: voucher numbers, family, genus, species and GenBank numbers to genes amplified: Cytochrome Oxidase I (COI) and 16S.

Code	Family	Genus	Specific name	COI	16S
	Leptophlebiidae	*Paraleptophlebia*	*submarginata*	LN734754.1	GQ118290.1
	Leptophlebiidae	*Paraleptophlebia*	*vaciva*	JQ663110.1	AY749759.1
Ep6672a	Leptophlebiidae	*Meridialaris*	*tintinnabula*	MG791797	MG791785
Ep5510a	Leptophlebiidae	*Askola*	sp.	MG791798	MG791786
Ep5736a	Leptophlebiidae	*Ulmeritoides*	*angelus*	MG791799	MG791787
Ep5736c	Leptophlebiidae	*Ulmeritoides*	*angelus*	MG791800	MG791788
Ep5728b	Leptophlebiidae	*Ulmeritoides*	sp.	MG791801	MG791789
Ep5728c	Leptophlebiidae	*Ulmeritoides*	sp.	MG791802	MG791790
Ep5729a	Leptophlebiidae	*Ulmeritus*	*saopaulensis*	MG791803	MG791791
Ep5730a	Leptophlebiidae	*Ulmeritus*	*saopaulensis*	MG791804	MG791792
Ep5733a	Leptophlebiidae	*Diamantina*	*ulmeri*	MG791805	MG791793
Ep6702a	Leptophlebiidae	*Diamantina*	*ulmeri*	MG791806	MG791794
Ep5734a	Leptophlebiidae	*Diamantina*	*ulmeri*	MG791807	MG791795
Ep5734b	Leptophlebiidae	*Diamantina*	*ulmeri*	MG791808	MG791796

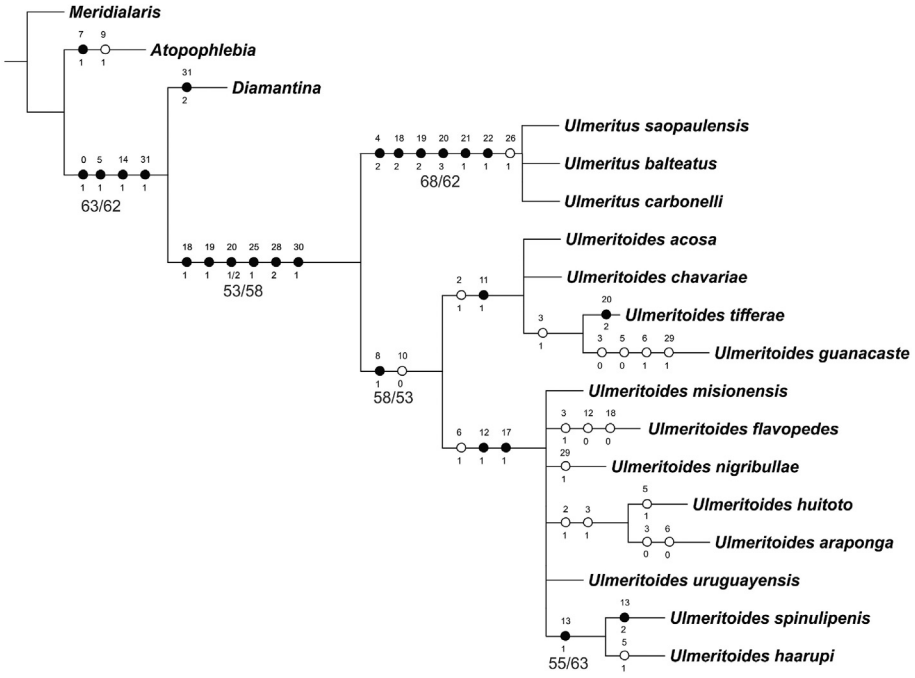

Fig. 1. Strict consensus tree of *Diamantina ulmeri* gen et sp. nov. (Ephemeroptera: Leptophlebiidae) and related taxa. Black circles = apomorphies, empty circles = homoplasies. Numbers above circles are character numbers, numbers below circles are character states. First number below branches correspond to Symmetric Resampling values, second number are Jackknife values, only values above 50% included.

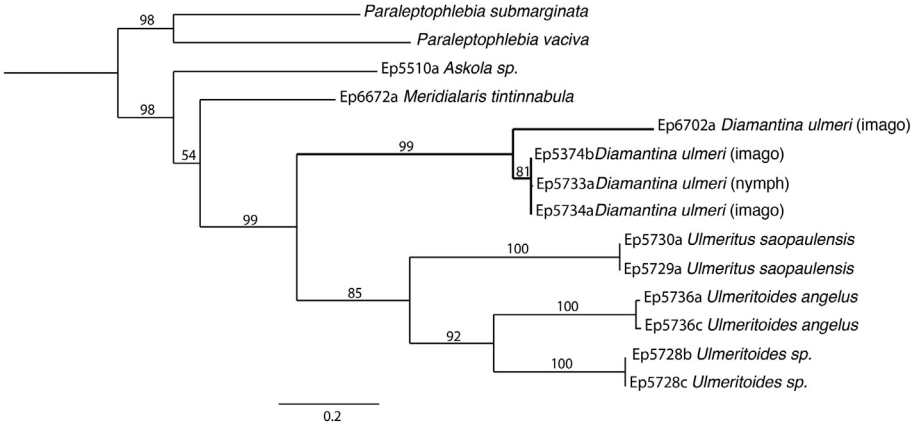

Fig. 2. Phylogenetic relationships of *Diamantina ulmeri* gen et sp. nov. (Ephemeroptera: Leptophlebiidae) and related taxa obtained by maximum-likelihood analysis of Cytochrome Oxidase I (COI) and 16S from DNA mitochondrial. Bootstrap percentages (BP) are shown on the branches.

matrix, calculate the intra and interspecific distances and running the phylogenetic analyses include Geneious, MEGA6 (Tamura et al. 2013), and RAxML ver. 8.2.4 (Stamatakis 2014), respectively. Genetic distances (COI marker) were calculated using the Kimura 2-parameter (K2P) model (Kimura 1980) in MEGA 6. The analyses were performed as follows: (*i*) maximum intraspecific distances, encompassing nymph and adult specimens of the new species, and minimum interspecific distances of individuals were calculated; and (*ii*) distances K2P were calculated at the species and genus level, respectively, based on the original dataset.

Table 2
Mean genetic divergences at interspecific levels inferred from nucleotide sequences of COI along six Leptophlebiidae genera based on the Kimura-2-parameter (K2P) distances.

	Paraleptophlebia	*Meridialaris*	*Askola*	*Ulmeritoides*	*Ulmeritus*
Paraleptophlebia					
Meridialaris	0.25				
Askola	0.23	0.22			
Ulmeritoides	0.27	0.23	0.28		
Ulmeritus	0.28	0.27	0.24	0.24	
Diamantina	0.30	0.25	0.28	0.33	0.28

Fig. 3. *Diamantina ulmeri* gen et sp. nov. (Ephemeroptera: Leptophlebiidae) nymphal habitus in the field.

2.4. Images

Pictures of habitus and structures of the body of preserved specimens were taken using a Zeiss Stemi 2000-C stereomicroscope with an Axiocam ERC 5S camera or a Leica M165C stereomicroscope using a LED illumination dome (Kawada & Buffington 2016), in conjunction with the Leica auto montage program and an Olympus digital image acquisition system (DP 72 model using the Cell D program). In order to produce final images with enhanced depth of field, a series of stacked images were processed with the program Helicon Focus®. Living specimens were photographed in the field with a Nikon D800, a 105 mm objective and a Nikon macro flash. SEM photographs were taken with a JEOL 35 CF scanning electron microscope. The free software DIVA-GIS 5.2 (http://www.diva-gis.org/) was used to make the distribution map of the species.

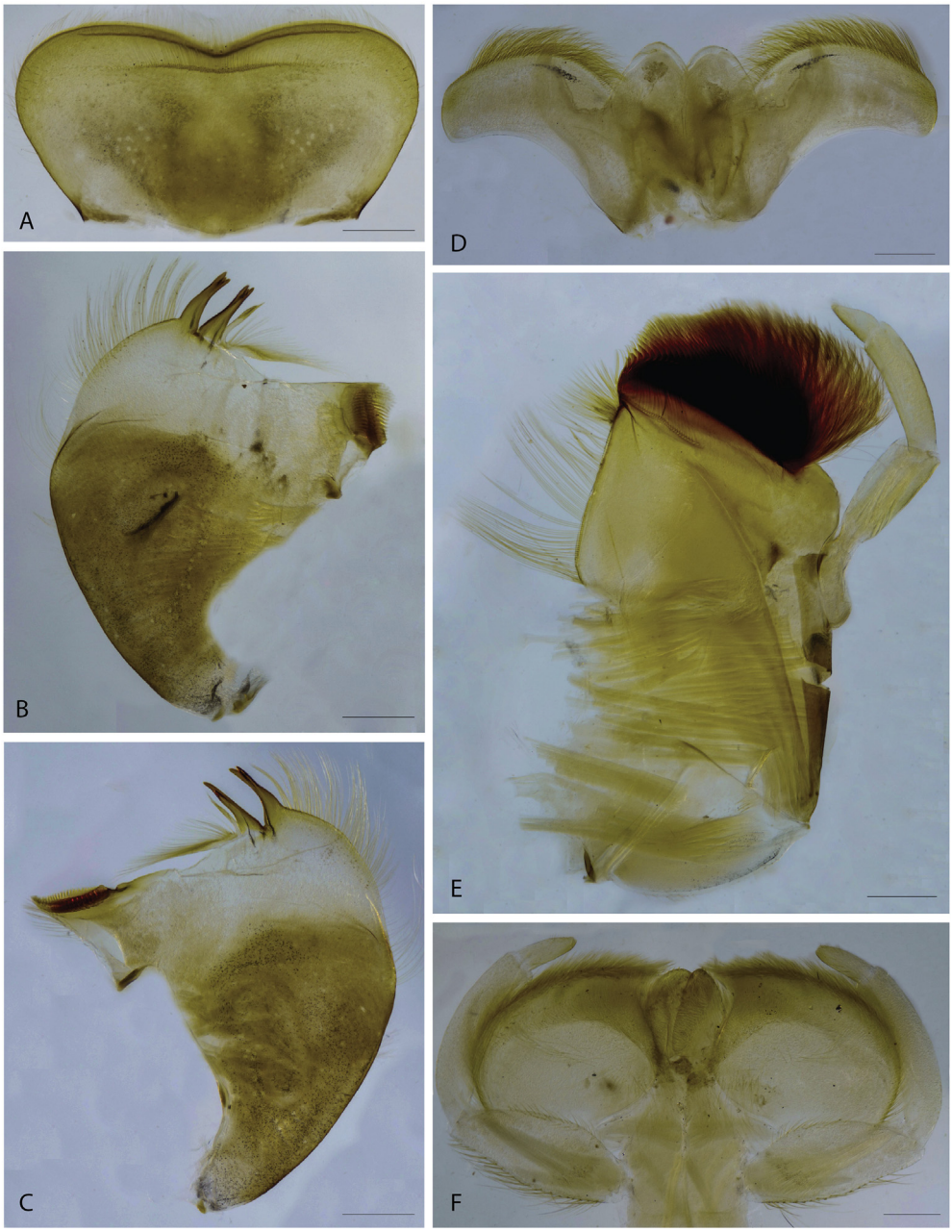

Fig. 4. *Diamantina ulmeri* gen et sp. nov. (Ephemeroptera: Leptophlebiidae) nymphal structures: A. Labrum, dorsal view; B. Left mandible, dorsal view; C. Right mandible, dorsal view; D. Hypopharynx; E. Right maxilla, dorsal view; F. Labium, ventral view.

Fig. 5. *Diamantina ulmeri* gen et sp. nov. (Ephemeroptera: Leptophlebiidae) nymphal structures: A. Foreleg; B. Middle leg; C. Hind leg; D. Abdominal terga I to V; E. Gill IV; F. Abdomen, ventral view.

2.5. Deposition

The material examined is housed in the following institutions: Museu de Entomologia (UFVB), Viçosa, Brazil; Invertebrate Collection of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Brazil; Instituto de Biodiversidad Neotropical (IBN), Tucumán, Argentina.

3. Results and discussion

3.1. Cladistic and molecular analysis

In the morphological analysis we obtained six trees, with a score of 5.34286. The strict consensus tree that summarize them (Fig. 1) depict the same phylogenetic relationship between *Ulmeritus* and *Ulmeritoides* proposed by Salles & Domínguez (2012), with the new taxon appearing as their sister group. The synapomorphies for the clade (*Diamantina* gen. nov. (*Ulmeritus-Ulmeritoides*)) are three bullae present in fore wings of male and female imagos (character 0(1)); C and Sc area in fore wing completely tinged (character 5(1)); anterior emargination of labrum present (character 14(1)); and lateral margins of posterolateral projections on abdominal segments VIII–IX with spines (character 31(1)). The apomorphy of *Diamantina* gen. nov. is lateral margins of posterolateral projections on abdominal segments VIII–IX bare (character 31(2)). The synapomorphies of the clade (*Ulmeritus-Ulmeritoides*) are dorsal row of setae on labrum medial (character 18(1)); dorsal row of setae on labrum entire, sinusoidal (character 19(1)); tusk on inner apical margin of maxilla present, small (character 20(1)) or present medium (character 20(2)), in this case as a polymorphy expressed

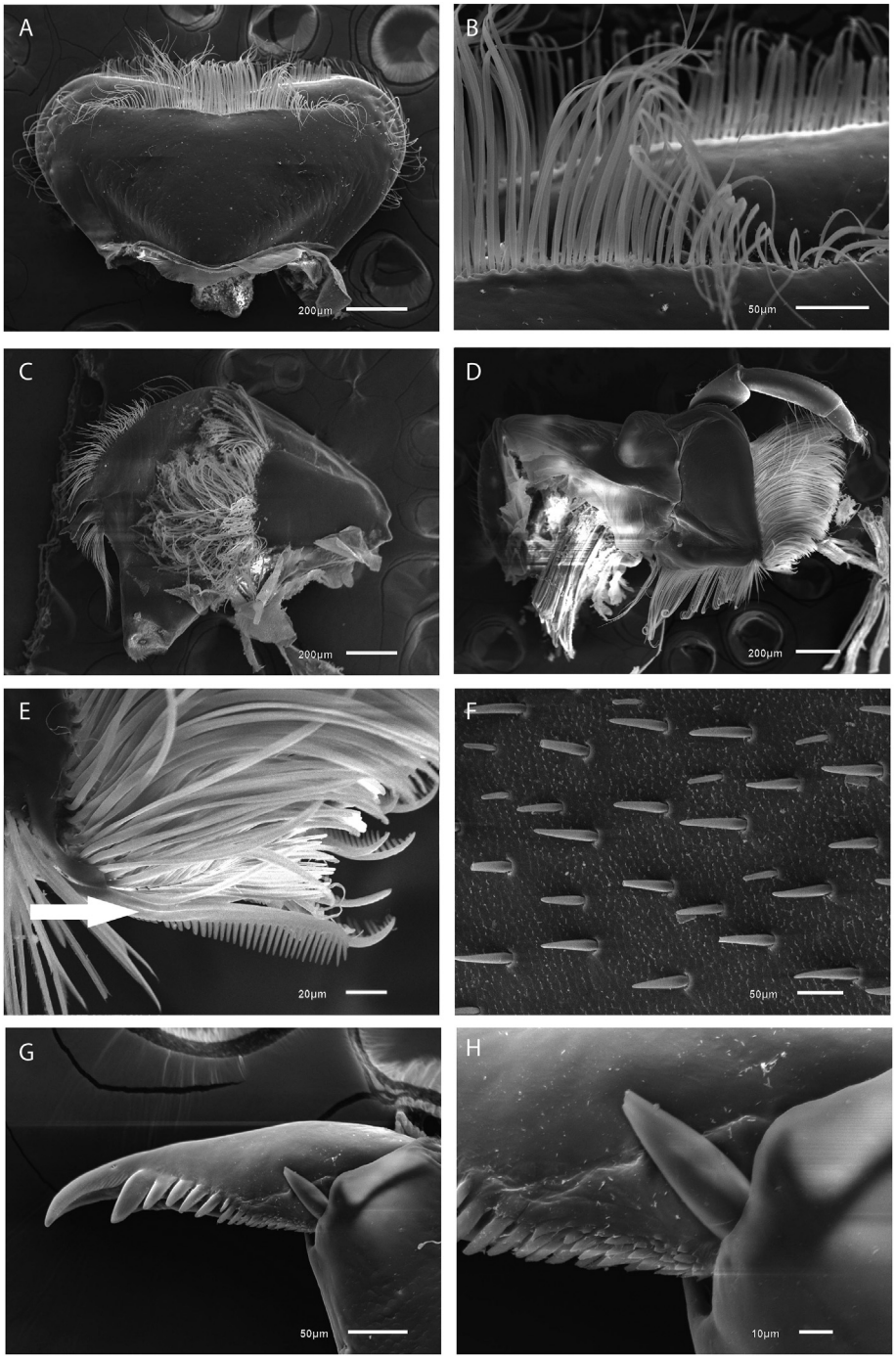

Fig. 6. *Diamantina ulmeri* gen et sp. nov. (Ephemeroptera: Leptophlebiidae) SEM pictures of nymphal structures: A. Labrum, dorsal view; B. Detail of distal margin of labrum; C. Left mandible, ventral view; D. Right maxilla, ventral view; E. Apex of galea-lacinia showing comb-like dentiseta (arrow); F. Detail of setae on dorsal surface of foreleg; G. Tarsal claw; H. Detail of tarsal claw base.

mainly in *Ulmeritoides* (it changes to present, big (20(3)) in *Ulmeritus*); dorsal row of long spines on segments II and III of labial palpi (character 25(1)); one main line, of pectinate setae bifurcated at ½ length, on dorsum of tibia III (character 28(2)); posterolateral projections on abdominal segments VIII–IX wide (character 30(1)) and posterolateral projections on abdominal segments VI-IX. As in Salles & Domínguez (2012), the monophyly of *Ulmeritus* and *Ulmeritoides* is recovered. Within *Ulmeritoides*, the Central American species and the South American species also form two different monophyletic groups.

In the molecular analysis, the sequence data comprised a total of 950 positions (572 bp for COI and 378 for 16S). The sequenced regions contained 136 polymorphic sites with 127 informative sites for COI and 151 polymorphic sites with 128 informative sites for

Fig. 7. *Diamantina ulmeri* gen et sp. nov. (Ephemeroptera: Leptophlebiidae) alate stages in the field: A. Male imago, lateral view; B. Female subimago, dorsolateral view.

16S, and indels were observed just for 16S. The phylogenetic tree shows that *Diamantina* gen. nov. form a clade with *Ulmeritus* and *Ulmeritoides*, being *Diamantina* gen. nov. recovered as sister group of these genera (Fig. 2), the analysis indicated these three taxa are closely related, in a well-supported clade (99% ML bootstrap value). Support values were overall weak at the nodes linking the clade (*Diamantina* gen. nov. (*Ulmeritus* - *Ulmeritoides*)) to *Meridialiaris* and *Askola* (54% ML bootstrap value), indicating some uncertainty in the given topology.

Diamantina specimens showed very low ratios of maximum intraspecific distance and high level of interspecific variation. The genetic distances among individuals of the same species was less than 1%, and the interspecific distances were between 21.1% and 31.3% (Table 2), indicating that the barcode is a sensitive and efficient genetic marker for the delimitation of Ephemeroptera species (Webb et al. 2012; Cardoni et al. 2015).

In conclusion, we considered that the morphological analysis, corroborated by molecular data, allow us to propose *Diamantina* as a new genus belonging to the *Ulmeritus-Ulmeritoides* group.

3.2. *Diamantina Salles, Domínguez and Nascimento, gen. nov.*

Type-species. *D. ulmeri*, here designated by monotypy.

Etymology. The specific epithet is derived from Chapada Diamantina National Park, type-locality of the genus. The gender is feminine.

Distribution. The genus is known from a single locality at the Chapada Diamantina National Park, Bahia state, Brazil (Figs. 10 and 11).

Diagnosis. Nymph. 1) Labrum trapezoidal, proximal row of setae on dorsal surface slightly sinuous and close to distal margin (Figs. 4A and 6A,B); 2) mandibles semicircular (Fig. 4B, C), with dense rows of setae on ventral surface (Fig. 6C); 3) claws with denticles progressively larger apically, base of claws with many small denticles (Fig. 6G, H); 4) gills oval to cordiform, fimbriate along entire margin (Fig. 5D, E); 5) segments II–IX with postero-lateral projections, more developed on segments V–IX; lateral margins bare (Fig. 5F).

Imago. 1) Eyes of male widely separated on meson of head (Fig. 8C); 2) three bullae present in fore wings of male and female imagos; 3) fork of MA symmetrical and fork of MP asymmetrical (Fig. 8A); 4) hind wing with costal projection rounded, located approximately ½ distance from base to apex; vein Sc ¾ distance from base to wing margin (Fig. 8B); 5) penes divided at base, each lobe with short ventrally directed wedge shaped projection (Fig. 9A–C); 6) sternum IX of female with broad indentation (Fig. 8H).

3.3. *Description*

3.3.1. *Nymph*

Head (Fig. 3): prognathous. Clypeus with parallel outer margins. Labrum (Figs. 4A, 6A, B) trapezoidal, widening toward apex where it is 1.2 times wider than clypeus, broadly rounded anterolaterally and with narrow U shaped emargination; proximal row of setae on dorsal surface (third row according to Godunko et al., 2015) slightly sinuous and close to distal margin, setae long; outer margins bordered with setae from base of labrum to anterolateral corner. Mandibles (Figs. 4B, C and 6C) semicircular; two rows of setae present on distal ½ of outer margin, one short, single and with few setae, close to the middle of the margin and on the dorsal surface, the other longer, multiple, with more setae and on the ventral surface; proximal and distal rows of setae separated by a short gap (same length as proximal row); ventral surface (Fig. 6C) with L shaped rows of setae (short row parallel to outer margin, long row perpendicular), setae on perpendicular row long and dense, forming a tuft towards inner margin. Maxilla (Figs. 4E and 6D, E) with apical flange; comb-like dentiseta seta present; tusk very short; segments I and II of palp subequal, segment III 0.4 times the length of segment II; inner margin of segments II and III with long setae; outer corner of galea-lacinia expanded (in a such a way that the apical field of setae is located relatively far from the outer margin). Labium (Fig. 4F) with glossa ventrally expanded, on a plane perpendicular to the paraglossa and triangular in cross section (the base of the triangle on the inner side); inner surface suboval, flat and bare, dorsolateral surface with short spines at apex, and ventral surface with dense tuft of fine setae (these setae are short at apex, but increase in length towards base); paraglossa densely covered with setae along distal and inner margin of ventral surface and with an oblique row of setae; segment I of palp 0.9 times the length of segment II; segment III 0.3 times the length of segment II.

Thorax. Legs (Fig. 5A–C) without patella-tibial suture. Femora with outer margin with two rows of setae, one stout and one fine, fine setae twice the length of stout setae; dorsal surface covered with stout setae, except on apical $^{5}/_{6}$. Claws with denticles progressively larger apically, base of claws with small serrations (Fig. 6G, H).

Abdomen. Gills (Fig. 5D, E) oval to cordiform, fimbriate along entire margin; ventral lamella with inner basal lobe; present on segments I–VII, very small on segment II. Segments II–IX (Fig. 5F) with postero-lateral projections, more developed on segments V–IX; lateral margins bare. Caudal filaments with whorls of spines on each articulation, generally alternating one whorl of long spines, one whorl of short spines.

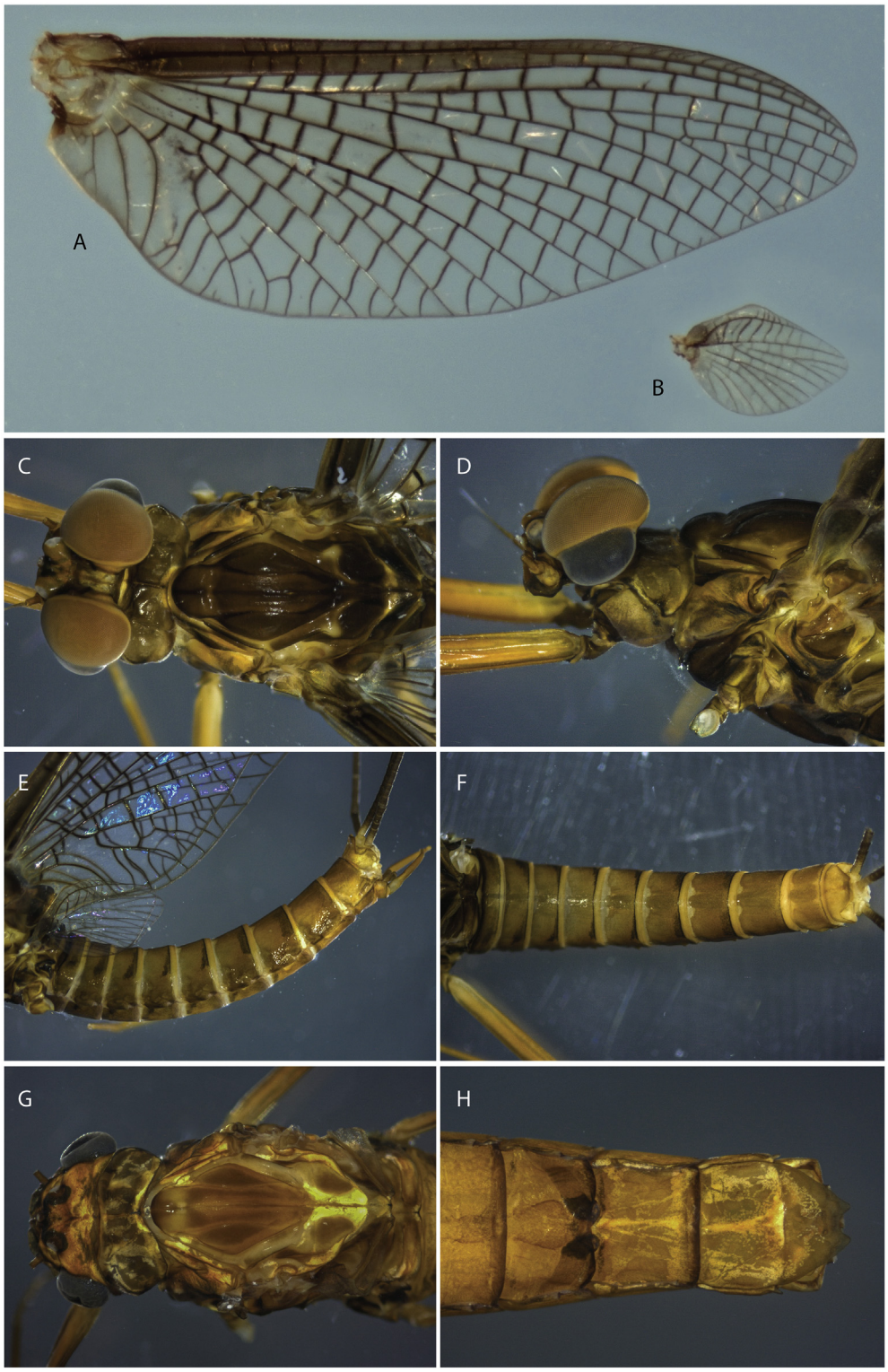

Fig. 8. *Diamantina ulmeri* gen et sp. nov. (Ephemeroptera: Leptophlebiidae) details of imagos: A. Forewing (female); B. Hind wing (female); C. Head and thorax of male imago, dorsal view; D. Head and thorax of male imago, lateral view; E. Abdomen of male imago, lateral view; F. Abdomen of male imago, dorsal view; G. Head and thorax of female imago; H. Abdominal sterna VI to IX of female imago.

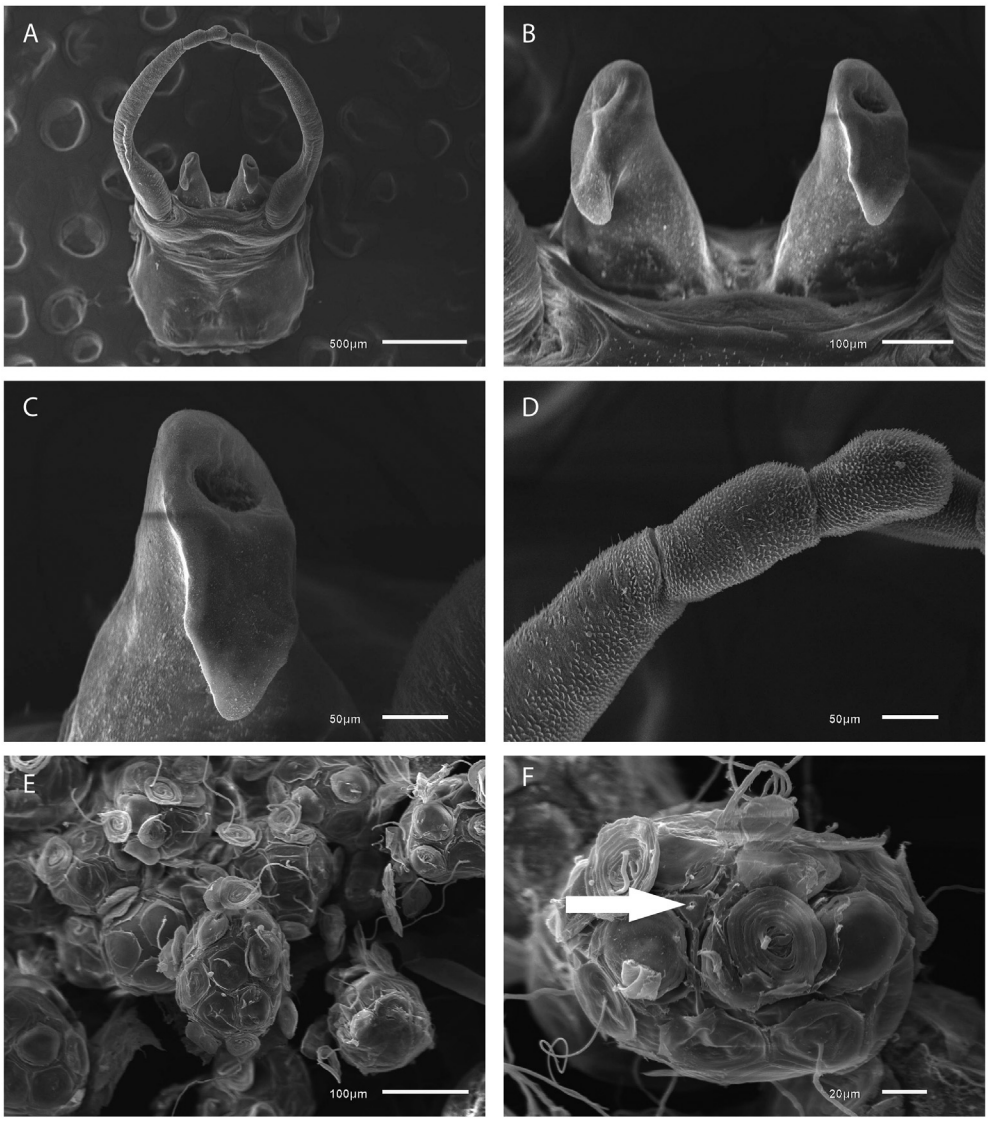

Fig. 9. *Diamantina ulmeri* gen et sp. nov. (Ephemeroptera: Leptophlebiidae) SEM pictures of male genitalia and eggs: A. Ventral view of male genitalia; B. Penes lobes; C. Detail of penis lobe, ventral view; D. Detail of last segments of forceps, ventral view; E and F. Eggs. E general aspect; F details. Arrow: micropyle.

3.3.2. *Imago*

Head (Fig. 8C, D, G): eyes of male widely separated on meson of head, distance about twice the width of a lateral ocelus (Fig. 8C); lower portion of eyes 4/5 length of upper portion (Fig. 8D). Eyes of female (Fig. 8G) separated on meson of head by a distance equal to 6.5 times the width of a lateral ocellus.

Wings (Figs. 7A and 8A, B). Maximum width of forewings 1/3 their maximum length; maximum width of hind wings about $^{2}/_{3}$ their maximum length; maximum length of hind wings 1/5 maximum length of forewings. Forewings (Fig. 8A): three bullae present in male and female imagos; vein Rs forked slightly >1/4 distance from base of vein to margin, fork of vein MA symmetrical and forked at ½ distance from base of vein to margin, cross vein above fork of MA slanted; fork of vein MP asymmetrical and forked 1/3 distance from base of vein to margin; vein ICu_1 attached at base to vein CuA; vein ICu_2 attached to ICu1. Hind wings (Fig. 8B): costal projection rounded, located approximately ½ distance from base to apex; vein MP forked; apex of wings rounded; vein Sc ¾ distance from base to wing margin; ca. 40 cross veins present.

Legs. Ratio of segments of male forelegs, 0.95:1.0 (3.7 mm):0.03:0.1:0.08:0.05:0.08. Tibiae subequal in length to femur on all legs of male and female. Claws on each leg dissimilar, with one apically hooked and one blunt, padlike.

Abdomen. Genitalia (Fig. 9A–D) with segment I curved, slightly broadened at basal $^{1}/_{3}$; segment II of forceps slightly longer than segment III; segment II of forceps 1/6 length of segment I; penes divided at base, each lobe with short ventrally directed wedge shaped projection. Sternum IX of female with broad indentation (Fig. 8H).

3.3.3. *Egg*

Egg (Fig. 9E, F). Size: 130–150 µm in length, 110–120 µm in width. Shape oval; both polar regions convex. Chorionic surface smooth. KCTs evenly distributed and somewhat overlapping, covering completely the chorion when threads are coiled. Collar smooth, with hexagonal outer border, internal area of the collar round. Knob big, (approximately 40 µm) and covering the long

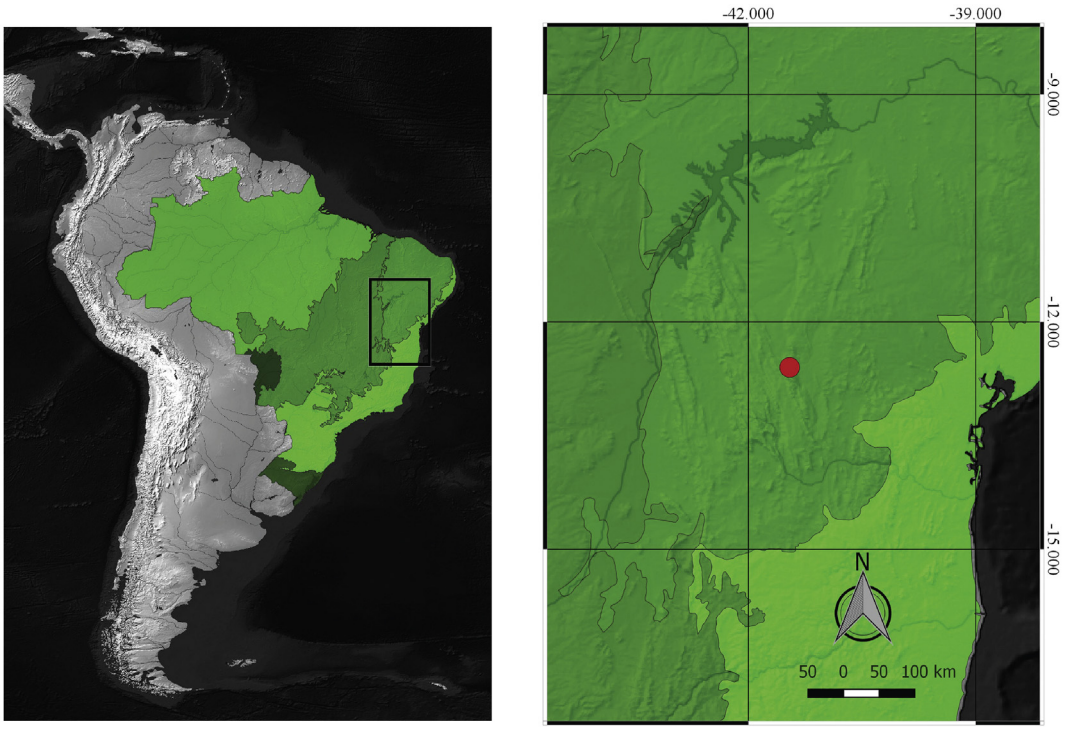

Fig. 10. Map of South America with Brazilian biomes in different shades of green, and detail of Brazil showing the distribution of *Diamantina ulmeri* gen et sp. nov. (red dot). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

thread. Micropyle located in a triangular area, surrounded by 4 KCTs (arrow, Fig. 9F).

3.4. Notes

Nymphs of *Diamantina* gen. nov., at least in the Neotropics, resemble those of *Ulmeritus*, *Ulmeritoides* and *Atopophlebia* because of the fimbriate gills. The gills of *Diamantina* gen. nov., however, are much smaller than those of *Ulmeritus* and *Ulmeritoides*. Interestingly, while nymphs of *Ulmeritus* and *Ulmeritoides* are usually found in areas with slow current or even in lentic habitats, those of *Diamantina* gen. nov. were always found in areas with moderate current. Besides the size of gills, the anterior position of the proximal row of setae on dorsal surface of labrum, the U shaped emargination of labrum, and the absence of setae on the margins of abdominal posterolateral projections, single apomorphy of *Diamantina* gen. nov., readily distinguishes nymphs of the new genus from all other genera of Leptophlebiidae.

The new genus is also similar in the adult stage to *Atopophlebia*, *Ulmeritus* and *Ulmeritoides*, especially the fore wing venation (slanting cross vein above a symmetrical MA fork and MP fork asymmetrical). The shape and venation of hind wing is extremely similar to those of *Ulmeritus* and *Ulmeritoides*, but not to *Atopophlebia*, which is highly modified. On the other hand, the ventrally oriented projection at the apex of each penis lobe is present in *Ulmeritus* and *Atopophlebia*, but absent in *Ulmeritoides*.

As in *Ulmeritus* and *Ulmeritoides*, the membrane of the subimaginal wings in *Diamantina* gen. nov. is dark, becoming hyaline after the moult to the imago. We suspect that this dark coloration is provided by the high density of microtrichia found in the wings of this group, but careful examination and comparison with other Leptophlebiidae must be done in order to test this hypothesis. The eggs are similar to the eggs of *Ulmeritus carbonelli* Traver in several aspects (see Domínguez & Cuezzo 2002: Fig. 7c–e) but differ from them in the position of the micropyle in a triangular area surrounded by four KCTS and that the knob of the KCT is elevated above the coiled thread.

3.5. Diamantina ulmeri Salles, Domínguez and Nascimento, sp. nov

Diagnosis. This is the only species of the genus known from male imago. Therefore, it is impossible to ascertain at this time the characteristics that will distinguish it from its congeners.

Etymology. The specific epithet is in honor of Dr. Georg Ulmer (1877–1963), german entomologist specialist on mayflies and caddisflies. Dr. Ulmer made several and important contributions to the knowledge of Neotropical Atalophlebiinae and the two genera closely related to the new species, *Ulmeritus* and *Ulmeritoides*, have also being named after him.

3.6. Description

3.6.1. Nymph

Length: body, 11.8–13.0 mm; antenna, 9.0–10.0 mm, filaments: 32–40 mm.

Head (Fig. 3): orange, washed with black anteriorly, compound eyes with lower portion black, upper portion reddish brown. Antenna orangish. Labrum (Fig. 4A) yellowish brown, posterolaterally translucent; with translucent spots and grayish marks submedially. Mandible (Fig. 4B, C): basal 2/3 yellowish brown with grayish marks, apical 1/3 translucent, except by molar area and incisors yellowish brown. Hypopharynx (Fig. 4D) yellowish translucent; lingua with well-developed lateral process; anterior margin with V-shaped cleft; superlingua well developed, with long setae along anterior margin. Maxilla (Fig. 4E). Yellowish, outer margin brownish; with a row of 38–42 subapical pectinate setae. Labium (Fig. 4F): yellowish translucent; glossa with outer margin covered

Fig. 11. *Diamantina ulmeri* gen et sp. nov. (Ephemeroptera: Leptophlebiidae) collection site: A. General view of Fumaça stream close to Cachoeira da Fumaça; B and C. Partial view of the stream.

with fine setae; paraglossa with outer margin rounded; palpus segment I with strong setae on inner and outer margins.

Thorax (Fig. 3): pronotum orangish, posterior margin with a narrow black stripe; pronotal margins expanded and translucent. Mesonotum orangish, with dark brown marks as in Fig. 3. Legs (Fig. 5A–C) orange; each femur with a subapical blackish broad band. Foreleg (Fig. 5A) with dorsal margin of femur with long fine setae and short, broad, apically pointed setae; anterior surface covered by short, broad, apically pointed setae. Fore tibia with a row of short setae more abundant on basal 2/4 of inner surface and with few setae at apex; outer surface with fine setae. Middle and hind leg (Fig. 5B, C) similar to foreleg, anterior surface of femora with setae almost reaching its apex; tibiae without setae on inner margin.

Abdomen (Figs. 3 and 5D, F): orange with black stripes on posterior margins on all segments; terga VII and VIII with antero submedial blackish mark, those on terga VII extending posteriorly. Abdominal sterna (Fig. 5F) yellowish, lateral margins washed with gray. Gills (Fig. 5D, E) light gray, whitish at base; tracheae dark gray; seventh gill reduced. Caudal filaments (Fig. 3) blackish, yellowish translucent apically.

3.6.2. *Male imago*

Lengths: body, 11.8–12.0 mm; fore wings: 11.5–12.0 mm; hind wings: 1.9–2.1 mm.

General coloration: light orange; orange washed with black (in vivo).

Head (Fig. 8C, D): orange, tinged with black between ocelli, frons and at base of antenna; upper portion of eyes orangish, lower portion black.

Thorax (Fig. 8C, D): pronotum light orange with diffuse black pigmentation; mesonotum predominantly dark brown, sutures on medioscutum and submedioscutum darker, remainder yellowish. Wings (Fig. 7A and as in 8A): membranes of fore wing hyaline, base washed with light brown, area between veins C and Sc tinged with orangish brown, paler toward apex. Longitudinal and cross veins tinged with brown, lighter on anal field. Hind wing (Fig. 7A) with hyaline membrane, base of wing and veins tinged with light brown. Foreleg (Fig. 7A) orange, apex and inner margin of femur and base of tibia dark brown; mid and hind legs similar to fore leg, lighter.

Abdomen (Fig. 8E, F): terga orange; tergum I almost completely washed with black; posterior margin of terga II–VII with a blackish stripe, narrower towards middle; tergum VII as in terga II–VI, but pigmentation rather diffuse. Sterna light orange, anterior margins of segments I–III diffusely pigmented, segments II–VII with anterolateral oblique blackish mark. Penes yellowish; spines orangish. Caudal filaments orange strongly washed with black.

3.6.3. Female imago

Lengths: body, 12.2–12.4 mm; fore wings: 12.0–14 mm; hind wings: 2.63–2.8 mm.

Similar to male imago, except for lighter pigmentation on mesothorax (Fig. 8G), absence of black marks on apex of femora and base of tibiae, and sub triangular black mark at base of gonopores (Fig. 8H).

3.7. Notes

As stated previously, the species was found exclusively at the Chapada Diamantina National Park, in an area known as Cachoeira da Fumaça, one of the highest waterfalls in Brazil with 340 m high (Fig. 11). The Cachoeira da Fumaça is formed by a blackwater stream that runs parallel along the trail leading to the waterfall and the material was sampled just above it, in a stretch of about 50 m. One of us (NH) made several collecting trips to the park and sampled in different areas, but no other material was found. At the collecting point altitude is 1290 m.a.s.l., the stream is 2–4 m wide and during one of the expeditions, when most of the nymphs were collected, water temperature was 16 °C, electrical conductivity 30 μS/cm, and pH 4.5. Nymphs were found under rocks in areas with slow to moderate current. Few subimagos, probably recently emerged, were seen floating on the water surface and drifting in the flow, while others were captured perched on the vegetation along the stream. A small swarm of male imagos (10 individuals) was observed around 2:00 PM, flying between four and five meters above the stream.

3.8. Material examined

Holotype. Male imago, BRAZIL, Bahia, Parque Nacional da Chapada Diamantina, Vale do Capão, Cachoeira da Fumaça, 10/iv/2015, 12°36′00.2″S 41°27′19.9″W, 1.270 masl, Salles FF, Paresque R, Almeida T and Monjardin M cols (UFVB). Paratypes. 5 male imagos, 1 male subimago and 1 female imago, same data as holotype (4 male imagos and 1 female imago, UFVB; 1 male imago, 1 male subimago IBN). 3 female imagos, same data as holotype except 09/iv/2015. 1 female imago, 1 male subimago, same data as holotype except 08/iv/2015. 2 nymphs, same data as holotype, except 04/VIII/2005, Hamada N col. (IBN). 2 females, 7 mature nymphs and 6 immature nymphs, same data as holotype except 01.vi.2013, Nascimento JMC, Hamada N and Silva JO cols (INPA).

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgements

We are indebted to Jeferson Oliveira da Silva (INPA), Jardiel (guide from Capão village, in Chapada Diamantina) and Tais A. Barbosa (PPGBAN/UFES) for assistance during the field work. We acknowledge Dr. Marco Guimarães and Jairo Oliveira from the Laboratório de Ultraestrutura Celular Carlos Alberto Redins (LUCCAR), CCS/UFES, as well the edital MCT/FINEP/CT-INFRA – PROINFRA 01/2006 for the SEM images. National Counsel of Technological and Scientific Development from Brazil (CNPq) for a research productivity fellowship awarded to FFS and NH. ICMBio and IBAMA for collection permissions (numbers 38773 and 16719-4). CAPES Pro-Equipamentos for equipment support. This work was partially supported by the Fundação de Amparo a Pesquisa do Estado do Espírito Santo (FAPES/processes #60016604/12, 61938408/13).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcz.2019.10.005.

References

Avila, A.S., Flowers, R.W., 2005. New species and records of *Ulmeritoides* (Ephemeroptera: Leptophlebiidae) from Costa Rica. Zootaxa 1010, 1–14. https://doi.org/10.11646/zootaxa.1010.1.1.

Boldrini, R., Lima, L.R.C., 2017. New species and stages description of *Ulmeritoides* Traver, 1959 (Ephemeroptera: Leptophlebiidae) from Roraima state, Northern Brazil. Zootaxa 4282, 385–394. https://doi.org/10.11646/zootaxa.4282.2.11.

Cardoni, S., Tenchini, R., Ficulle, I., Piredda, R., Simeone, M.C., Belfiore, C., 2015. DNA barcode assessment of Mediterranean mayflies (Ephemeroptera), benchmark data for a regional reference library for rapid biomonitoring of freshwaters. Biochem. Syst. Ecol. 62, 36–50. https://doi.org/10.1016/j.bse.2015.07.035.

Domínguez, E., 1991. The status of the genus Ulmeritus (Ephemeroptera: Leptophlebiidae: Atalophlebiinae) and related taxa. In: Alba-Tercedor, J., Sánchez-Ortega, A. (Eds.), Overview and Strategies of Ephemeroptera and Plecoptera. Sandhill Crane Press, Gainesville, Florida, pp. 157–167.

Domínguez, E., 1995. Cladistic analysis of the Ulmeritus-Ulmeritoides group (Ephemeroptera, Leptophlebiidae), with descriptions of five new species of Ulmeritoides. J.N.Y. Entomol. Soc. 103, 15–38.

Domínguez, E., Abdala, V., 2019. Morphology and evolution of the wing bullae in South American Leptophlebiidae (Ephemeroptera). J. Morphol. 280, 95–102. https://doi.org/10.1002/jmor.20920.

Domínguez, E., Cuezzo, M.G., 2002. Ephemeroptera egg chorion characters: a test of their importance in assessing phylogenetic relationships. J. Morphol. 253, 148–165. https://doi.org/10.1002/jmor.1117.

Flowers, R.W., Domínguez, E., 1991. Preliminary cladistics of the Hermanella complex (Ephemeroptera: Leptophlebiidae: Atalophlebiinae). In: Alba-Tercedor, J., Sánchez-Ortega, A. (Eds.), Overview and Strategies of Ephemeroptera and Plecoptera. Sandhill Crane Press, Gainesville, Florida, pp. 49–62.

Gama-Neto, J.L., Hamada, N., 2014. Leptophlebiidae (Ephemeroptera) of the Serra do Tepequém, Roraima State, Brazil: new records and description of two new species. Zootaxa 3900, 279–286. https://doi.org/10.11646/zootaxa.3900.2.8.

Gama-Neto, J.L., Passos, M.A.B., 2016. Leptophlebiidae (Ephemeroptera) from Roraima state, Brazil: description of two new species of *Ulmeritoides* Domínguez, 1991. Zootaxa 4178, 295–300. https://doi.org/10.11646/zootaxa.4178.2.8.

Godunko, R., Sroka, P., Soldan, T., Bojková, J., 2015. The higher phylogeny of Leptophlebiidae (Insecta: Ephemeroptera), with description of a new species of Calliarcys Eaton, 1881. Arthropod Systematics & Phylogeny 73, 259–279.

Goloboff, P.A., Farris, J.S., Nixon, K., 2008. TNT, a free program for phylogenetic analysis. Cladistics 24, 774–786. https://doi.org/10.1111/j.1096-0031.2008.00217.x.

Kawada, R., Buffington, M.L., 2016. A scalable and modular dome illumination system for scientific microphotography on a budget. PLoS One 11, e0153426. https://doi.org/10.1371/journal.pone.0153426.

Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120. https://doi.org/10.1007/bf01731581.

Kluge, N.J., 2007. A new taxon Hermanellonota, or subtribe Hermanellini subtr.n. (Ephemeroptera: Leptophlebiidae: Hagenulini), with description of three new species from Peruvian Amazonia. Russ. Entomol. J. 16, 385–400.

Malm, T., Johanson, K.A., 2008. Revision of the new Caledonian endemic genus *Gracilipsodes* (Trichoptera: Leptoceridae: Grumichellini). Zool. J. Linn. Soc. 153, 425–452. https://doi.org/10.1111/j.1096-3642.2008.00403.x.

Nixon, K.C., 2002. WINCLADA. Program and Documentation Distributed by the Author. Cornell University, Ithaca, EUA (software).

Salles, F.F., Domínguez, E., 2012. Systematics and phylogeny of *Ulmeritus-Ulmeritoides* revisited (Ephemeroptera: Leptophlebiidae). Zootaxa 3571, 49–65.

Salles, F.F., Dominguez, E., Mariano, R., Paresque, R., 2016. The imagos of some enigmatic members of the Hermanella complex (Ephemeroptera, Leptophlebiidae). ZooKeys 625, 45–66. https://doi.org/10.3897/zookeys.625.9874.

Sereno, P., 2007. Logical basis for morphological characters in phylogenetics. Cladistics 23, 565–587. https://doi.org/10.1111/j.1096-0031.2007.00161.x.

Souto, P.M., Da-Silva, E.R., Nessimian, J.L., Gonçalves, I.C., 2016. Two new species of *Ulmeritoides* Traver (Ephemeroptera: Leptophlebiidae) from Southeastern Brazil. Zootaxa 4078, 127–136. https://doi.org/10.11646/zootaxa.4078.1.11.

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729. https://doi.org/10.1093/molbev/mst197.

Takiya, D.M., Tran, P.L., Dietrich, C.H., Moran, N.A., 2006. Co-cladogenesis spanning three phyla: leafhoppers (Insecta: Hemiptera: Cicadellidae) and their dual bacterial symbionts. Mol. Ecol. 15, 4175–4191. https://doi.org/10.1111/j.1365-294x.2006.03071.x.

Webb, J.M., Jacobus, L.M., Funk, D.H., Zhou, X., Kondratieff, B., 2012. A DNA barcode library for North American Ephemeroptera: progress and prospects. PLoS One 7, e38063. https://doi:10.1371/journal.pone.0038063.