A cladistic approach for generic delimitation of Paracloeodes Day, Rivudiva Lugo-Ortiz & McCafferty, and Varipes Lugo-Ortiz & McCafferty (Ephemeroptera: Baetidae)

3 authors, including:

Paulo Cruz
Universidade Federal de Rondônia
31 PUBLICATIONS **135** CITATIONS

BRILL

A cladistic approach for generic delimitation of *Paracloeodes* Day, *Rivudiva* Lugo-Ortiz & McCafferty, and *Varipes* Lugo-Ortiz & McCafferty (Ephemeroptera: Baetidae)

Paulo Vilela Cruz[a],*, Frederico Falcão Salles[b] and Neusa Hamada[c]

[a]Laboratório de Biodiversidade e Conservação, Universidade Federal de Rondônia – UNIR, CEP: 76940–000, Rolim de Moura, Rondônia, Brazil
[b]Museu de Entomologia, Departamento de Entomologia, Universidade Federal de Viçosa, 36570–900, Minas Gerais, Brazil. E-mail: frederico.salles@ufv.br
[c]Coordenação de Biodiversidade, Instituto Nacional de Pesquisas da Amazônia (INPA), CEP 69067–375, Manaus, Amazonas, Brazil; e-mail: nhamada@inpa.gov.br
*Corresponding author, e-mail: pvilelacruz@gmail.com

Abstract

The taxonomic knowledge of Baetidae has been greatly improved in the last decades in South America. Despite the advances, there are problems that need to be addressed. One of these problems is doubt concerning the systematics of species assigned to the genera *Paracloeodes* Day, *Rivudiva* Lugo-Ortiz & McCafferty, and *Varipes* Lugo-Ortiz & McCafferty, and the evolution of long setae on femora. In the present paper, the monophyly of these three genera is tested using a cladistic approach. The matrix included 53 species and 151 morphological characters: 127 for nymphs and 24 for adults. The dataset was analyzed under equal and implied weights with nine values of *k*. Group support was estimated with relative Bremer and frequency differences. The results corroborate (i) the paraphyly of *Paracloeodes* and *Varipes*, which become monophyletic without *P. caldensis* + *V. singuil,* proposed as a new genus *Rhopyscelis* **gen. n.**, (ii) the long setae on femora as an independent acquisition between *Rhopyscelis* **gen. n.** + *Varipes* and *Rivudiva*, (iii) the transversal rows of setae on femora as an independent acquisition between *Rhopyscelis* **gen. n.** + *Varipes* and *Rivudiva*, (iv) the spine on subgenital plate as an independent acquisition between *Paracloeodes*, *Rivudiva* and Gen. A.

Keywords

aquatic insect; mayfly; new genus; Neotropics; new combination

ZooBank: http://zoobank.org/217D79EE-A577-4B7F-8AB0-1107C9FC4857

Introduction

The taxonomic knowledge of South American Ephemeroptera has been greatly improved in the last decades (Domínguez & Dos Santos 2014). The family Baetidae has been at the center of remarkable number of publications, being responsible, in Brazil alone, for 45% of all scientific publications related to Ephemeroptera between 1992 and 2011 (Shimano et al. 2013). The advances have brought to light a great number of taxa that are endemic to the continent, clarified the taxonomic placement of species described in the last two centuries and allowed the development of phylogenetic studies on the main groups in the family in South America (Nieto 2010). However, at the same time, the increase of the taxonomic knowledge has generated doubts concerning the generic placement of recently collected and/or described species, due to the difficulty of delimiting some genera. At the epicenter of this discussion are *Paracloeodes* Day, *Rivudiva* Lugo-Ortiz & McCafferty, and *Varipes* Lugo-Ortiz & McCafferty, a set of genera belonging to a clade called "clade C" in a South American cladistics analysis provided by Nieto (2010).

Clade C is constituted by *Apobaetis* Day, *Adebrotus* Lugo-Ortiz & McCafferty, *Paracloeodes, Waltzoyphius* McCafferty & Lugo-Ortiz, *Callibaetis* Eaton, *Rivudiva,* and *Varipes*. Nieto (2010) proposed that *Paracloeodes* is the sister group of *Waltzoyphius* + *Callibaetis*, a clade supported by a single synapomorphy, mandibles with inner set of incisors at right angles to the outer set; *Rivudiva* + *Varipes* were placed on a related clade supported by seven synapomorphies, including very long setae on femora. However, when Gutiérrez et al. (2013) described *Paracloeodes caldensis* Gutiérrez, Dias & Salles, an atypical species of *Paracloeodes* with similarities to the species of *Varipes* and *Rivudiva*, they presented a different interpretation of the character "long setae on femora", considering this as convergence. According to these authors, important characteristics clearly exclude *P. caldensis* from *Rivudiva*, but in relation to *Varipes* the distinction is not so obvious, especially considering the similarity between *P. caldensis* and *Varipes singuil* Nieto, an atypical species of *Varipes*. All *Paracloeodes* species and *V. singuil* share many characteristics not observed in the remaining species of *Varipes*, such as glossa not basally broad, inner incisors of both mandibles perpendicular in relation to the outer incisors, and deeply cleft mandibular incisors, especially on the right mandible (Gutiérrez et al. 2013). Based on these characteristics, in the same paper, Gutiérrez et al. (2013) discussed the possibility of *V. singuil* being included in *Paracloeodes* rather than in *Varipes.*

Even though the adult males of the genera mentioned above are poorly known, discussions about generic delimitation include characters for this life stage. Salles & Nascimento (2009) reported that the adult male of *Rivudiva minantenna* Lugo-Ortiz & McCafferty has a small pointed projection on the posterior margin of the subgenital plate and, mainly because of this character, transferred two species only known by adults to *Rivudiva*: *R. coveloae* (Traver) and *R. venezuelensis* (Traver). Recently, Lima et al. (2013) described the same singular projection on the subgenital plate of the adult male of *P. charrua* Emmerich & Nieto. It is difficult to ascertain whether this

projection represents a convergence or if it is an indication that some species of *Rivudiva* would be better placed in *Paracloeodes*, as suggested by Gutiérrez et al. (2013) in the case of *V. singuil*.

Meanwhile, Falcão et al. (2011) reported an atypical species of Baetidae from Roraima state, Brazil, and considered that it should be placed in a new genus, but it was not described (here designated as Gen. A). However, this taxon could be also placed in *Paracloeodes* based on the large spectrum of characteristics attributed to this genus.

Considering that the discussion of supraspecific relationships, evolution of characters, and classifications mentioned above were not based on a cladistic analysis, the objectives of this study are to test the hypothesis that *Paracloeodes*, *Rivudiva,* and *Varipes* are monophyletic and evaluate the evolution of selected characters.

Materials and Methods

Taxon sampling

Whenever possible we included the type-species of the studied genera. Species known at all stages were preferentially included; species without known nymphs were not included; species with type material studied were preferentially included. All selected groups were in accordance with Nieto (2010). Specific criteria for the taxon sample are described below.

Given our limited knowledge of higher-level generic relationships of Baetidae, we decided to root the tree with *Siphlaenigma janae* Penniket (Siphlaenigmatidae). This family has been historically considered as the sister group of Baetidae (Landa & Soldán 1985; Staniczek 1997; Kluge 2004) and has been utilized in recent analyses (Nieto & Richard 2008; Nieto 2010; Salles et al. 2015).

All species of *Paracloeodes*, *Rivudiva,* and *Varipes* with described nymphs were included: *P. atroari* Nieto & Salles*, P. assu* Nieto & Salles*, P. binodulus* Lugo-Ortiz & McCafferty*, P. caldensis* Gutiérrez, Dias & Salles*, P. charrua* Emmerich & Nieto*, P. eurybranchus* Lugo-Ortiz & McCafferty*, P. fleeki* McCafferty & Lenat*, P. ibicui* Lugo-Ortiz & McCafferty*, P. leptobranchus* Lugo-Ortiz & McCafferty*, P. lilliputian* Kluge*, P. lotor* Cruz, Usher & Jacobus*, P. lugoi* Randolph & McCafferty*, P. minutus* (Daggy) (type-species of the genus)*, P. morellii* Emmerich & Nieto*, P. pacawara* Nieto & Salles*, P. peri* Nieto & Salles*, P. poranga* (Salles & Lugo-Ortiz)*, P. quadridentatus* Lima, Salles, Pinheiro & Quinto*, P. waimiri* Nieto & Salles*, P. yuto* Nieto & Salles*, R. coveloae* (Traver)*, R. minantenna* Lugo-Ortiz & McCafferty (type-species of the genus)*, R. trichobasis* Lugo-Ortiz & McCafferty*, R. venezuelensis* (Traver)*, V. cajuato* Nieto*, V. helenae* Salles & Batista*, V. lasiobrachius* Lugo-Ortiz & McCafferty (type-species of the genus)*, V. minutus* Nieto*, V. sancarlos* Castillo & Pérez*,* and *V. singuil* Nieto.

The genera present in the clade C of Nieto (2010) were included in the analyses following the criteria listed. All species of *Adebrotus* and *Waltzoyphius* were included: *A. amazonicus* Lugo-Ortiz & McCafferty (type-species of the genus)*, A. lugoi* Salles,

W. fasciatus Lugo-Ortiz & McCafferty (type-species of the genus)*,* and *W. roberti* Thomas & Peru. Cruz et al. (2017) recovered *Callibaetoides caaigua* Cruz, Salles & Hamada (type-species of the genus) as a sister group of the genus *Callibaetis* Eaton, thus we decided to include *Callibaetoides* in the analysis.

The cladistic study of the genus *Callibaetis* (Cruz et al. 2017) recovered four groups of species, treated as subgenera. One species of each subgenus was included in the analyses, all selected based on type specimens availability, plus the type-species of the genus: *C.* (*Abaetetuba*) *capixaba* Cruz, Salles & Hamada*, C.* (*Callibaetis*) *guttatus* Navás*, C.* (*Aiso*) *itannae* Cruz, Salles & Hamada*, C.* (*Cunhaporanga*) *nigracyclus* Cruz, Salles & Hamada*,* and *C.* (*Callibaetis*) *pictus* Eaton (type-species of the genus).

The genus *Apobaetis* has not been analyzed in a cladistics framework. In order to sample its diversity, one species from North and Central America and two from South America were selected based on either type-specimen availability (type availability) or availability of other specimens (specimen availability), plus the type-species of the genus. The following species were included: *A. etowah* (Traver) (type-species of the genus), *A. kallawaya* Nieto (specimen availability), *A. lakota* McCafferty (type availability), and *A. fiuzai* Salles & Lugo-Ortiz (type availability).

The cladistic study of Nieto (2010) recovered other groups of species. From these groups, species were selected based on the availability of type-species, specimens for the type species of the genus, or specimens. The following species were included in the analysis: *Americabaetis alphus* Lugo-Ortiz & McCafferty (type availability), *Aturbina georgei* Lugo-Ortiz & McCafferty (type-species of the genus and type availability), *Cloeodes penai* Morihara & Edmunds (type availability), *Cryptonympha dasilvai* Salles & Francischetti (specimens availability), *Cryptonympha copiosa* Lugo-Ortiz & McCafferty (type species of the genus and type availability), and *Spiritiops silvudus* Lugo-Ortiz & McCafferty (type-species of the genus and type availability).

Two species considered related to *Paracloeodes* and *Varipes* were included in order to test the monophyly of both genera. Gen. A (Falcão et al. 2011) was included based on the shape of labrum, maxilla, labium, and the presence of subgenital spine on male imago; *Kirmaushenkreena zarankoae* McCafferty (type-species of the genus) was included based on length of setae on dorsal margin of femora and denticulation of the claws (Jacobus, L. personal communication).

A list of the material examined, including institutions, stages examined, origin and how the material was studied are presented in the Supplementary Material.

Characters

The matrix included 151 external morphological characters, 127 for nymphs and 24 for adults; all were treated as non-additive (unordered). The characters were sampled from direct observations of specimens and were adapted from the cladistic analysis of Nieto (2010). To effectively separate neomorphic and transformational components, for taxa that lack a particular structure, transformational character states are coded as inapplicable, with a '-' (Sereno 2007).

Definition of characters and matrix are presented in the Supplementary Material.

Analysis

The matrix (Table 2 in the supplementary material) was analyzed with TNT (Goloboff et al. 2008) under equal and implied weights with nine values of *k* (4, 6, 8, 10, 12, 14, 16, 18, and 20). This procedure was used to assess the potential changes in topology (e.g., Salles et al. 2013). Changes in the values of *k* stopped when recovered topologies were repeatedly similar (five times) to the topology recovered under equal weights. This is because, for high values of *k*, the fitted function approaches linearity (Goloboff 1993).

A traditional search was conducted with 500 replicates of random-addition sequences followed by bisection–reconnection of the tree, saving 10 trees per replication, and collapsing rules min. length = 0 and later with none (memory = 1000). Group support was estimated using Relative Bremer support (Goloboff & Farris 2001) and frequency differences (GC) (Goloboff et al. 2003). Bremer support was calculated for the most parsimonious trees and for 1000 suboptimal trees up to 10 steps longer than the shortest tree. Frequency differences (GC) were calculated with 300 replicates by symmetrical resampling (Goloboff et al. 2003) on the shortest tree. Each replicate of symmetrical resampling was calculated by 10 random-addition sequences plus TBR (tree bisection and reconnection), saving 10 trees per replicate. Consistency index and retention index were calculated using script stats.run.

Characters and states were traced over trees using software WinClada ver. 1.00.08 software.

The topology, list of synapomorphies and support values presented in Fig. 1 corresponds to the strict consensus tree obtained through the implied weight analysis with *k* = 4, using collapsing rules: min. length = 0.

Results

Cladistic characterization

The analysis of the dataset used collapsing rules with min. length = 0 or using collapsing rules = none yielded the same 10 trees. All 10 trees had a length of 435 steps (total fit = 95.2, adjusted homoplasy = 34.7, consistency index = 43.7, and retention index = 75.2).

The strict consensus tree (implied weight analysis with *k* = 4, using collapsing rules with min. length = 0) (Fig. 1) had a length of 438 steps (total fit = 95.0, adjusted homoplasy = 34.9, consistency index = 43.4, and retention index = 74.8).

Gen. A was recovered as a sister group of ((*P. caldensis* + *V. singuil*) + *Varipes*) (GC = 0 and Relative Bremer = 32) sharing (i) frons with two keels (Fig. 2) (Char. 2:2), (ii) bifid setae on distal arc of labrum (Char. 7:1), (iii) labrum with mediodorsal arc of setae extending laterally (Fig. 4) (Char. 16:1), (iv) two rows of spine-like setae on distal margin of paraglossa (Char. 61:1), and (v) apex of subgenital plate projection concave (Fig. 34) (Char. 148:1).

The generic status of Gen. A was corroborated by phylogenetic position and the following autapomorphies: (i) lingua projection pointed (Fig. 163 in Falcão et al. 2011)
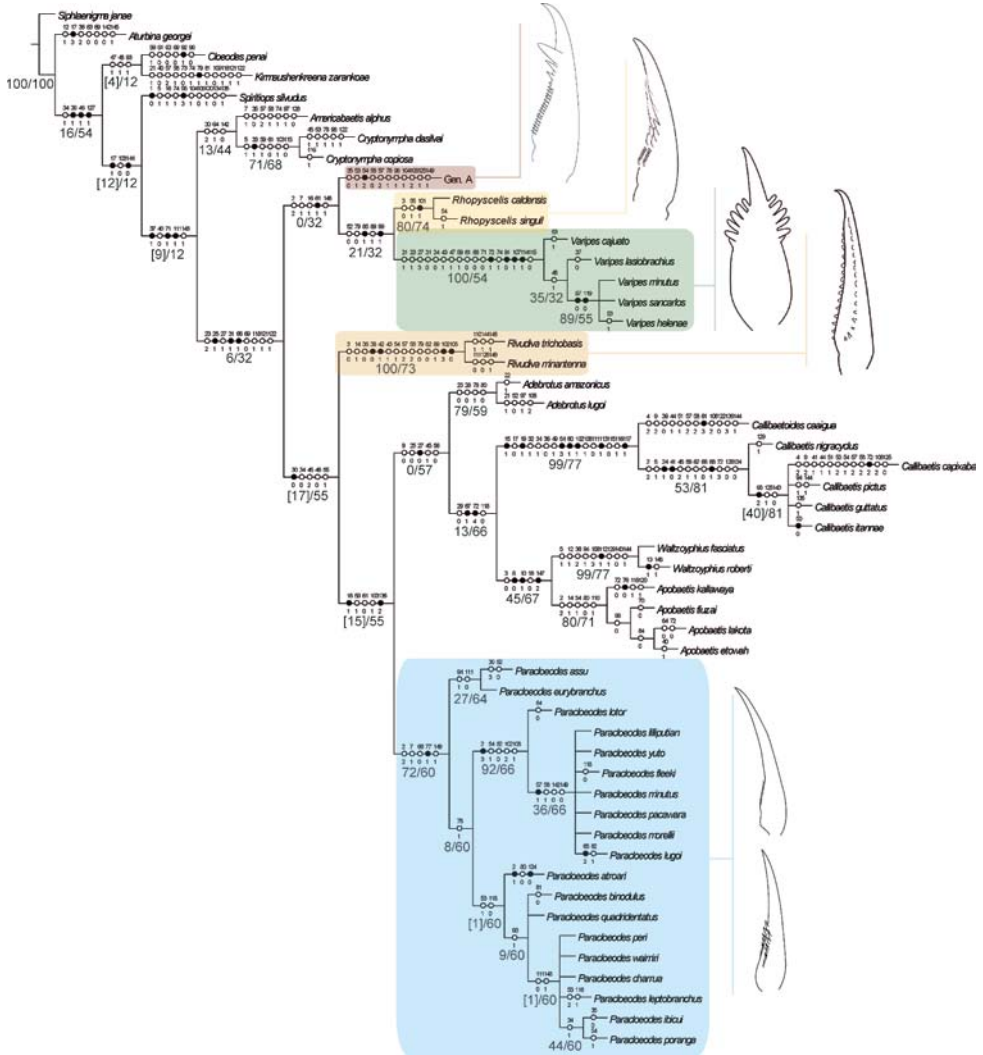
Fig. 1. Phylogenetic hypotheses with k = 4 using collapsing rules: min. length = 0. Black circles indicate synapomorphies, white circles represent non-unique changes in the tree, either forward or reverse; above are the character numbers and below are the character states. The values below branches correspond to GC (left)/Relative Bremer (right).

(Char. 35:0), (ii) glossae subequal in length to paraglossae (Char. 53:1), (iii) glossae subrectangular (Char. 54:2), (iv) row of setae on inner margin of glossa absent (Char. 56:0), (v) paraglossae subrectangular (Char. 57:2), (vi) forefemur setae on dorsal margin blunt (Char. 78:1), (vii) one of the rows of denticles on tarsal claw reduced (Fig. 30) (Char. 98:1), (viii) apical denticles of foreclaw much longer than the others (Fig. 30) (Char. 104:1), (ix) row of denticles of tarsal claw longer than half the length of the claw, but not reaching the apex (Fig. 30) (Char. 106:2), (x) long spines on terminal filament every one segment (Char. 125:1), and (xi) spine on process of subgenital plate present (Char. 149:1).

The *Paracloeodes caldensis* + *Varipes singuil* clade was recovered as a sister group of *Varipes* (GC = 21 and Relative Bremer = 32) sharing: (i) mentum elongate (Char. 52:0), (ii) forefemur setae on dorsal margin long (Figs. 19–24) (Char. 79:0), (iii) forefemur transversal row of long setae present (Figs. 19 and 21) (Char. 85:1), (iv) mid and hind femora transverse row of long state present (Figs. 20 and 22) (Char. 89:1), and (v) irregular row of foreclaw denticles present (Figs. 25–27) (Char. 99:1).

The *Paracloeodes caldensis* + *Varipes singuil* clade (GC = 80 and Relative Bremer = 74) was corroborated as monophyletic by phylogenetic position and by the following synapomorphies: (i) medial lobe on distal margin of labrum absent (Char. 3:0), (ii) base of glossa not overlapping base of paraglossa (Fig. 7 in Gutiérrez et al. 2013) (Char. 55:1), and (iii) foreclaw denticles (apical) apically directed (Figs. 25–27) (Char. 101:1).

The genus *Varipes* (without *V. singuil*) (GC = 100 and Relative Bremer = 54) was corroborated as monophyletic by phylogenetic position and by the following syna- pomorphies: (i) external margin of mandibles straight (Fig. 19 in Nieto 2004) (Char. 21:1), (ii) prostheca of right mandible seta-like (Fig. 4 in Nieto 2004) (Char. 23:1), (iii) left mandible incisors completely fused (Fig. 3A in Nieto 2004) (Char. 27:3), (iv) mandible incisors in the same plane (Char. 31:0), (v) distomedial projection of lingua absent (Char. 34:0), (vi) small lobe on apex of the last segment of maxillary palp present (Fig. 9) (Char. 43:1), (vii) basal projection of first dentiseta coupling with canine present (Fig. 10) (Char. 47:1), (viii) row of setae on outer margin of paraglossa present (Fig. 11) (Char. 59:1), (ix) one row of spine-like setae on distal margin of paraglossa (Char. 61:0), (x) many (10–20) long, thin simple setae on outer margin of labial palp segments II and III absent (Fig. 21 in Nieto 2004) (Char. 66:0), (xi) ventral insertion of segment III of labial palp on segment II (Char. 71:0), (xii) labial palp segment III globose (Fig. 21 in Nieto 2004) (Char. 72:1), (xiii) dorsal margin of femora convex to the ventral (Fig. 22 in Nieto 2004) (Char. 74:1), (xiv) setae sockets on the mid and hind femora transverse row of long setae very near, touching each other (Fig. 24) (Char. 91:0), (xv) row of denticles of foreclaw starts at medial third (Fig. 29) (Char. 107:1), (xvi) gill I racquet-shaped (Fig. 25 in Nieto, 2004) (Char. 114:1), and (xvii) gill I asymmetric (Char. 115:0).

The genus *Rivudiva* was recovered as a sister group of all remaining genera in clade C (Nieto 2010) (GC = [17] and Relative Bremer = 55) by sharing (i) right mandible incisors cleft (Char. 30:0), (ii) distomedial projection of lingua absent (Char. 34:0), (iii) maxillary canines robust and apically pointed (Char. 45:2), (iv) first dentiseta with same width as others (Char. 46:0), and (v) base of glossa not overlapping base of paraglossa (Char. 55:1).

The genus *Rivudiva* (GC = 100 and Relative Bremer = 73) was corroborated as monophyletic by phylogenetic position and by the following synapomorphies: (i) medial lobe on distal margin of labrum absent (Char. 3:0), (ii) sub-distomedial arc of setae on dorsal surface of labrum present (Fig. 5) (Char. 14:1), (iii) lingua longer than superlinguae (Char. 36:0), (iv) inner-ventral setae near maxillary canines curved upon denticles (Char. 38:0), (v) distal margin of last segment of maxillary palp curved
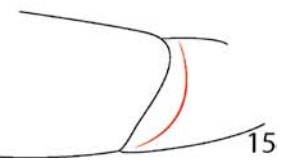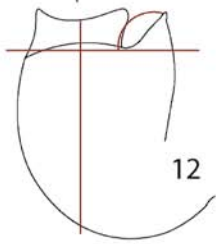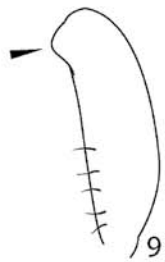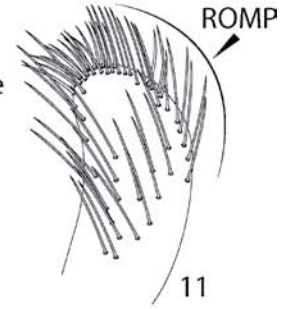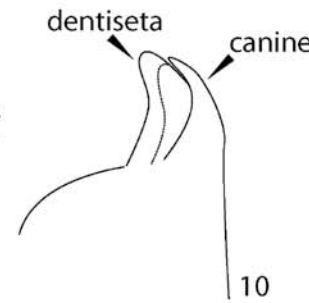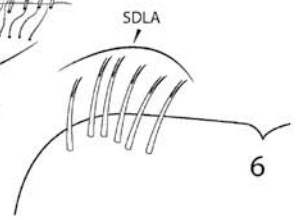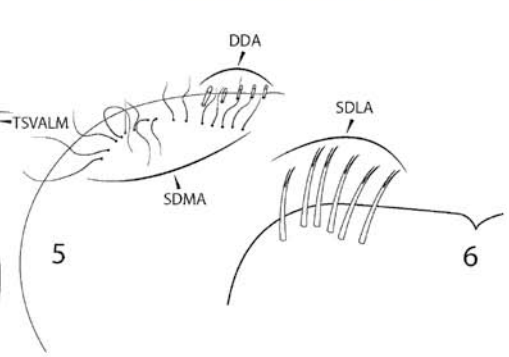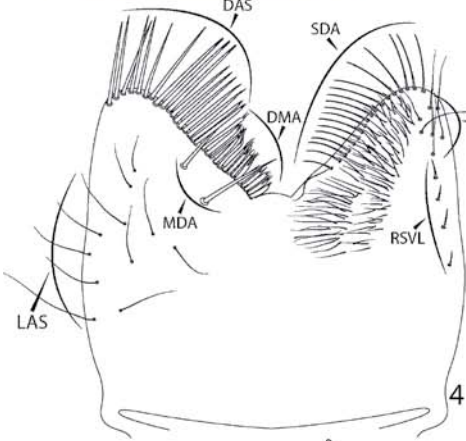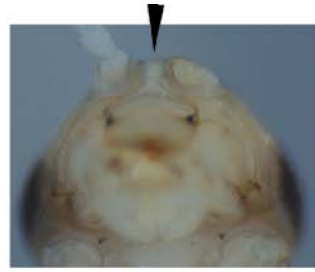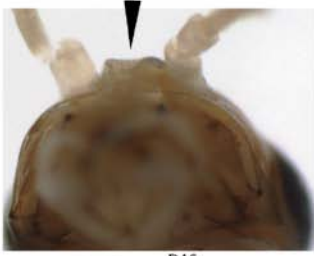
inwardly (Char. 42:1), (vi) small lobe on apex of last segment of maxillary palp present (Fig. 9) (Char. 43:1), (vii) glossa semicircular (Char. 54:1), (viii) paraglossa subrectangular (Char. 57:2), (ix) paraglossa apex truncated (Char. 58:2), (x) forefemur setae on dorsal margin very long (Figs. 19 and 21) (Char. 79:0), (xi) forefemur longitudinal row of setae at middle on anterior surface present (Fig. 18) (Char. 82:0), (xii) mid and hind femora transverse row of long state present (Figs. 20 and 22) (Char.89:1), (xiii) foreclaw denticles conical (Fig. 28) (Char. 102:3), and (xiv) rows of foreclaw denticles distributed from base to apex (Fig. 28) (Char. 106:0).

The genus *Paracloeodes* was recovered as a sister group of (*Adebrotus* + ((*Callibaetoides* + *Callibaetis*) + (*Waltzoyphius* + *Apobaetis*))) (GC = [15] and Relative Bremer = 55) sharing (i) pair of denticles on medial emargination of labrum present (Char. 18:1), (ii) row of setae on outer margin of paraglossa present (Fig. 11) (Char. 59:1), (iii) one row of spine-like setae on distal margin of paraglossa (Char. 61:0), (iv) foreclaw denticles without irregularity and slightly increasing towards apex (Char. 103:1), and (vi) costal process of hind wing quadrangular, when hind wing present (Char. 136:2).

The genus *Paracloeodes* (without *P. caldensis*) (GC = 72 and Relative Bremer = 60) was corroborated as monophyletic by phylogenetic position and by the following synapomorphies: (i) frons with two keels (Char. 2:2), (ii) setae on distal arc of labrum bifid (Char. 7:1), (iii) absence of many (10–20) long, thin simple setae on outer margin of labial palp segments II and III (Char. 66:0), (iv) forefemur setae on dorsal margin concave (Fig. 16) (Char. 77:1), and (v) spine present on subgenital plate (Char. 149:1) [changed to absent in the second most diverse clade].

The genus *Paracloeodes* was divided in three main clades, species in the most diverse clade of *Paracloeodes* share: (i) glossae subequal to paraglossae (Char. 53:1) and (ii) gills with pigmented traquea (Char. 118:0). The species in the second most diverse clade

---

Figs. 2–15. 2, elevation of frons with two keels (*Paracloeodes charrua*), Chars 1 and 2, black arrow indicates frons; **3**, elevation of frons with one keel (*Paracloeodes atroari*) Chars 1 and 2, black arrow indicates frons; **4**, organization of setae on labrum (*Callibaetis capixaba*, left = dorsal, right = ventral) Chars 5, 6, 8, 15, 16, 19, 20, LAS = lateral arc of setae, DAS = distal arc of setae, DMA = distomedial arc of setae, SDA = subdistal arc of setae on ventral surface, MDA = mediodorsal arc of setae, RSVL = ventral row of spine-like setae on lateral margin of labrum, TSVALM = tuft of setae ventrally on anterolateral margin of labrum; **5**, organization of setae on labrum (*Apobaetis* sp.) Chars 10, 14, DDA = distodorsal arc of setae, SDMA = sub-distomedial arc of setae on dorsal surface; **6**, organization of setae on labrum (*Waltzoyphius* sp.) Char. 12, SDLA = sub-distolateral arc of setae on dorsal surface; **7**, detail of deep cleft incisors of right mandible (*Callibaetis* sp.) Char. 30, black arrow indicates the incisors; **8**, detail of partially fused incisors of right mandible (*Paracloeodes* sp.) Char. 30, black arrow indicates the incisors; **9**, detail of a small lobe on apex of last segment of maxillary palp (*Rivudiva* sp.) Char. 43, back arrow indicates the lobe; **10**, detail of basal projection of first dentiseta coupling with canine on maxilla (*Varipes lasiobranchius*) Char. 47; **11**, detail of row of setae on outer margin of paraglossa (ROMP) (*Callibaetis* sp.) Char. 59; **12**, detail of labial palp with red lines showing axes for angle measure (*Apobaetis* sp.) Char. 65; **13**, detail of labial palp segment II and III, black arrow indicates the medial concavity on segment III (*Callibaetis calloventer*) Char. 68; **14**, detail of trochanter inserted on ventral margin of femur, red line shows the connection (*Varipes lasiobrachius*) Char. 73; **15**, trochanter inserted along entire base of femur, red line shows the connection (*Rhopyscelis caldensis* **comb. n.**) Char. 73.
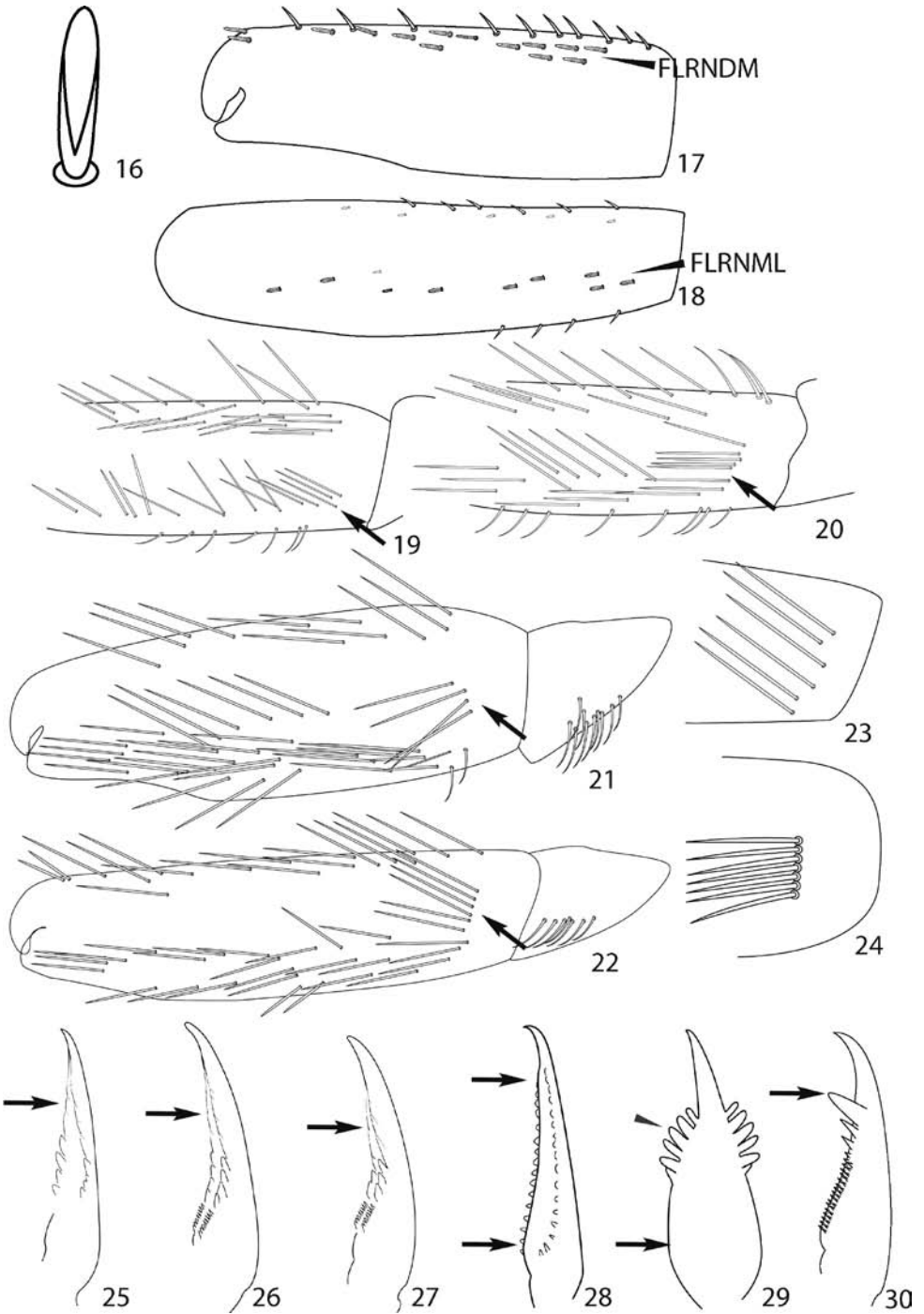
(GC = 92 and Relative Bremer = 66) share: (i) frons with two keels fused in mid length (Char. 2:3), (ii) glossa semicircular (Fig. 101a in Nieto & Salles 2006) (Char. 54:1), (iii) forefemur with one longitudinal row of setae at middle on anterior surface (Char. 82:0), (iv) claws with minute denticles (Char. 102:2), and (v) mid and hind tarsal claw denticles smaller than foreclaw (Char. 108:1). Species in the less-diverse clade of *Paracloeodes* share: (i) presence of setae on foretarsus anterior surface (Char. 94:1) and (ii) hind wing pads present (Char. 111:0).

Discussion

Phylogenetic relationships, selected characters and evolution

Gutiérrez et al. (2013) placed *P. caldensis* in *Paracloeodes* and suggested that *V. singuil* would be better included in *Paracloeodes* rather than in *Varipes*. They argued that their arrangement was based on characteristics absent in *Varipes* but present in all *Paracloeodes* species, *P. caldensis*, and *V. singuil*, such as the lateral margins of the paraglossa curved inward; glossa not basally broad; inner incisors of both mandibles perpendicular in relation to the outer incisors; and cleft mandibular incisors, especially on the right mandible (Fig. 7). The set of synapomorphies proposed in our study corroborated the set of shared characteristics listed by Gutiérrez et al. (2013) to effectively distinguish *P. caldensis* and *V. singuil* from *Varipes*, but it did not corroborate the inclusion of both species in *Paracloeodes*, since all shared characters listed are shared by all genera of the clade C (as synapomorphies or homoplasies), except for *Varipes* and Gen. A.

In addition to the set of characters excluding *P. caldensis* and *V. singuil* from *Paracloeodes*, our results recovered the absence of long setae on femora (Chars 79 and 81) in the

---

Figs. 16–30. 16, detail of forefemur concave setae on dorsal margin (*Paracloeodes atroari*) Char. 77; **17**, detail of forefemur, black arrow indicates the FLRNDM = forefemur longitudinal row of setae near dorsal margin on anterior surface (*Paracloeodes eurybranchus*) Char. 80; **18**, detail of forefemur, black arrow indicates the FLRNML = forefemur longitudinal row of setae at middle on anterior surface (*Paracloeodes* sp.) Char. 82; **19**, anterior surface of forefemur, black arrow indicates the vestigial row of setae, (*Rhopyscelis singuil* **comb. n.**) Char. 85 and 86; **20**, anterior surface of hind femur, black arrow indicates the transversal row of setae, (*Rhopyscelis singuil* **comb. n.**) Chars 89 and 90; **21**, anterior surface of forefemur, black arrow indicates the vestigial row of setae, (*Rhopyscelis caldensis* **comb. n.**) Chars 85 and 86; **22**, anterior surface of hind femur, black arrow indicates the transversal row of setae, (*Rhopyscelis caldensis* **comb. n.**) Chars 89 and 90; **23**, detail of transversal row of setae on hind femur, (*Rivudiva trichobasis*) Chars 89 and 91; **24**, detail of transversal row of setae on hind femur, (*Varipes helenae*) Chars 89 and 91; **25**, foreclaw, black arrow indicates the denticles apically directed (*Rhopyscelis singuil* **comb. n.**) Chars 96–101; **26**, foreclaw, black arrow indicates the denticles apically directed (*Rhopyscelis caldensis* **comb. n.**) Chars 96–101; **27**, hind claw, black arrow indicates the denticles apically directed (*Rhopyscelis caldensis* **comb. n.**) Chars 96–101; **28**, hind claw, black arrows indicate the regularity of row of denticles, (*Rivudiva trichobasis*) Chars 99 and 102; **29**, foreclaw, black arrow indicates the absence of denticles on base, red arrow indicates the conical and apical denticles, (*Varipes lasiobrachius*) Chars 106 and 107; **30**, detail of foreclaw, black arrow indicates apical denticles on claw extremely higher than the others (Gen. A.) Char. 104.

Paracloeodes lineage, corroborating the exclusion of these two species from *Paracloeodes*. Long setae on femora is not a character (neomorphic), but rather a state related to the length of setae present in rows. Therefore, the correct approach to studying the evolution of setae is to study each of these rows. Five longitudinal rows of setae were recognized here: one on dorsal margin (Char. 76), one on ventral margin, one near dorsal margin on anterior surface (Char. 80), one near ventral margin on anterior surface (Char. 84) and one in the middle on the anterior surface (Char. 83). All rows (when present) in *Rivudiva*, *Varipes*, and *P. caldensis* + *V. singuil* possessed the same change in the length of setae (from short to long, e.g., Char. 79), but were independently acquired in ((*P. caldensis* + *V. singuil*) + *Varipes*) and *Rivudiva*. The independent acquisition recovered here seems to be a case of parallelism, since the same plesiomorphic condition (short setae), in each row, was independently changed to a similar apomorphic condition (long setae) in the closely related groups.

The rows of setae on femora were also important for the hierarchization of taxa proposed by Kluge (2015), where *Varipes* was included in Rivudiva/g1 based, among other features, on the presence of a transversal row of setae on femora (Char. 85). Considering each row as independent, our study recovered the presence of transversal row of setae on forefemora (Char. 85) as a synapomorphy of ((*P. caldensis* + *V. singuil*) + *Varipes*), which was absent in *Rivudiva*. The presence of transversal row of setae on mid and hid femora (Char. 89) was recovered as an independent acquisition between ((*P. caldensis* + *V. singuil*) + *Varipes*) and *Rivudiva*. Therefore, our results did not corroborate one synapomorphy proposed by Kluge (2015) in his hierarchization.

The evolution of the set of characters that make up the claw (Chars 96–109) (Figs. 26–30) is also relevant because they have poorly explored implications for the systematics of the group. The presence of two rows of perpendicular and triangular denticles, starting at base of claw, are plesiomorphies and are present in many lineages in our analysis. Each lineage studied here (*P. caldensis* + *V. singuil*, *Varipes*, and *Rivudiva*) evolved a singular combination of unique apomorphies derived from these plesiomorphies, such as the absence of denticles at base (*Varipes*), conical denticles (*Rivudiva*), and the apical denticles apically directed (not perpendicular) (*P. caldensis* + *V. singuil*).

The reconstructed relationships also took into account the synapomorphies found on male imago, with a special concern for the presence of the subgenital spine (Char. 149). A spine of this type was reported for *Rivudiva*, Gen. A, and for the most diverse group of *Paracloeodes*, and was therefore recovered as an independent acquisition between them. The subgenital spine is a neomorphic character (Char. 149), not a change of state related to the shape of subgenital plate (Chars. 147–148); all three codified states for char. 148 possesses subgenital spine. Considering its independent acquisition, regardless of the shape of the subgenital plate, taxonomic acts based on the subgenital spine are not encouraged.

The analysis corroborated the results of Nieto & Salles (2006), Nieto (2010) and Kluge (2015), supporting the genus *Iguaira* Salles & Lugo-Ortiz as a junior synonym of the genus *Paracloeodes*.

General distribution and phylogenetic relationships

The clade (Gen. A + ((*P. caldensis* + *V. singuil*) + *Varipes*)) is in an isolated position from all genera in clade C. The species in this group are distributed mainly in the Amazonas High Andes, Amazonas Lowlands, North Andean, and Pacific slopes (including Panama and southern Mexico), and are often collected at high altitudes (except *V. singuil* and *V. helenae*). A very similar distribution and phylogenetic detachment from other South American groups is shared with species in the Baetodes complex (see Nieto 2010, Nieto 2015).

In contrast to the group mentioned above, the genus *Rivudiva* is widespread in South America, with the exception of high altitudes. In Brazil, the species in this genus (some yet to be described) were collected in almost all biomes (see Boldrini & Cruz 2014, Boldrini et al. 2012, Cruz et al. 2011, Salles & Nascimento 2009). It is likely that future biogeographic investigation will recover these apparent evolutionary patterns, which may corroborate the paraphyletic relationship between each lineage studied here.

Three main clades were recovered in *Paracloeodes*. The second most diverse clade (GC = 92 and Relative Bremer = 66) has all of its species considered to be psammophilous (sand-loving), and is widely distributed on the American continent and outlying areas (*P. lilliputian* from Cuba). Their morphological similarities, which were expressed as a large polytomy (7 out of 8 species), can be explained by sand "choice", since the psammophilous macroinvertebrate communities (especially mayflies) are highly specialized in their morphologies and behaviors (Jacobus 2013, McCafferty 1991, Stagliano 2016).

Cladistic conclusions

The analysis of the dataset with either equal weighting or implied weighting (with all values of *k*) resulted in similar topologies, corroborating: (i) the paraphyly of *Paracloeodes* and *Varipes*, which became monophyletic without *P. caldensis* + *V. singuil*, (ii) the long setae on femora as an independent acquisition (likely parallelism) between ((*P. caldensis* + *V. singuil*) + *Varipes*) and *Rivudiva*, (iii) the transversal rows of setae on femora as an independent acquisition (parallelism or convergence) between ((*P. caldensis* + *V. singuil*) + *Varipes*) and *Rivudiva*, (iv) spine on subgenital plate as an independent acquisition (parallelism or convergence) of a character (neomorphic) between *Paracloeodes*, *Rivudiva,* and Gen. A, not a change of state related to the shape of subgenital plate.

Taxonomic Accounts

Paracloeodes Day, 1955
In addition to recovering synapomorphies, all *Paracloeodes* species were observed to have a rounded distomedial projection on segment II of labial palp and segment III of labial palp conical, with spine-like setae on inner margin.
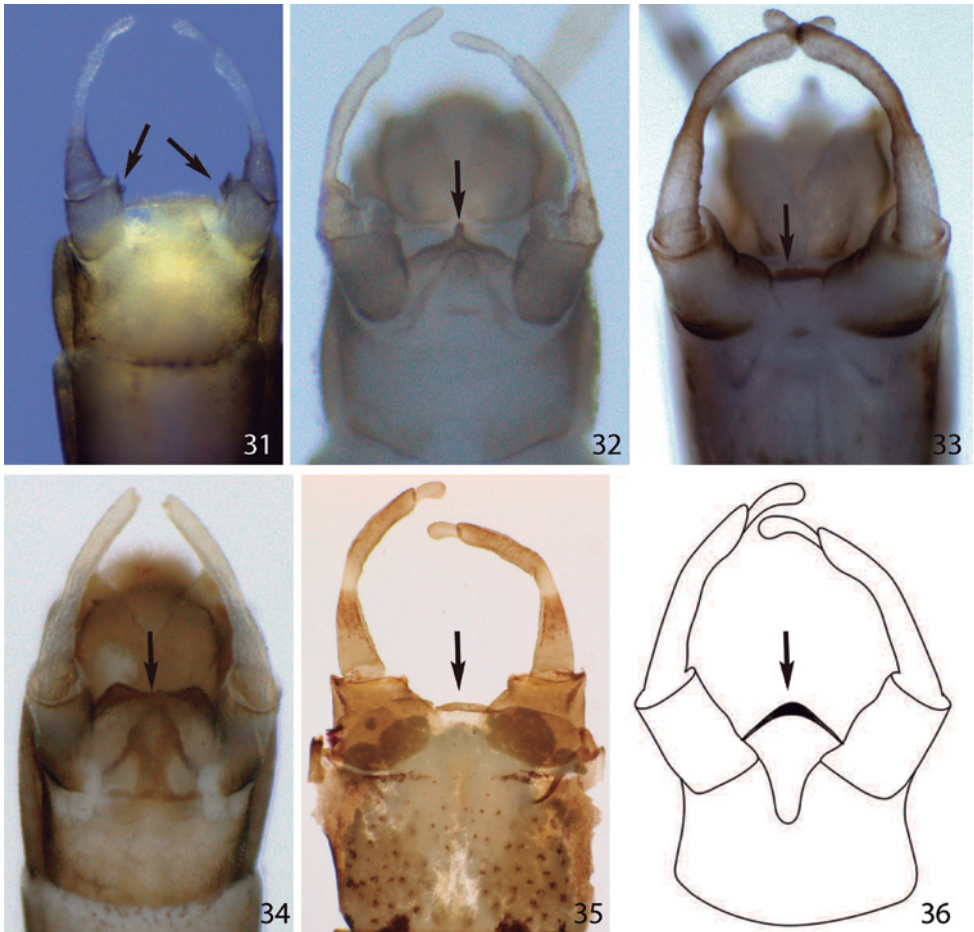
Figs. 31–36. 31, genitalia, black arrows indicate the unistyliger inner projection (*Paracloeodes* sp.) Chars 142–149; **32**, genitalia, black arrow indicates the spine and convex subgenital plate (*Paracloeodes atroari*) Chars 142–149; **33**, genitalia, black arrow indicates the rounded apex of subgenital plate (*Callibaetis* sp.) Chars 142–149; **34**, genitalia, black arrow indicates the concave subgenital plate (*Paracloeodes* sp.) Chars 142–149; **35**, genitalia, black arrow indicates the rounded apex of subgenital plate (*Callibaetis capixaba*) Chars 142–149; **36**, genitalia, black arrow indicates the triangular subgenital plate (*Apobaetis fiuzai*) Chars 142–149, adapted from Cruz et al. 2011.

Rhopyscelis **gen. n.**
ZooBank: http://zoobank.org/46753411-F21E-45AE-BC10-8397138F68A4
Type species: *Varipes singuil* Nieto 2004 (original designation)
Etymology. The generic name is an arbitrary combination of the Greek words *rhop* (brush) and *scelis* (leg), an allusion to the many long femoral setae on both species allocated to the new genus. The gender is feminine.
Composition. Rhopyscelis singuil (Nieto 2004) **comb. n.** (type species) and *Rhopyscelis caldensis* (Gutiérrez, Dias & Salles 2013) **comb. n.**

Diagnosis. **Male imago.** 1) forewing hyaline (Fig. 30 in Nieto 2004); 2) marginal intercalary veins paired (Fig. 30 in Nieto 2004); 3) hind wing absent; 4) subgenital plate without apical spine (Fig. 31 in Nieto 2004); 5) first segment of forceps with inner projection (Fig. 31 in Nieto 2004); 6) last segment of forceps rod-like (Fig. 31 in Nieto 2004).

Mature nymph. 1) prostheca of right mandible slender and bifid (Fig. 34 in Nieto 2004); 2) mandibular incisors partially fused (Fig. 34 in Nieto 2004); 3) inner set of incisors at right angle to the outer set; 4) distomedial projection of labial palp segment II rounded (Fig. 7 in Gutiérrez et al. 2013; Fig. 37 in Nieto 2004); 5) segment III of labial palp conical (apex broadly pointed) (Fig. 7 in Gutiérrez et al. 2013; Fig. 37 in Nieto 2004); 6) trochanter inserted along entire base of femur (Fig. 15; Fig. 11 in Gutiérrez et al. 2013; Fig. 38 in Nieto 2004); 7) many long thin femoral setae (Figs. 19–22); 8) vestigial transverse row of long setae on proximal third of forefemur (Figs. 19 and 20); 9) mid and hind femora with transversal row of long setae on proximal third (Figs. 20 and 22); 10) sockets of transverse row of setae on the mid and hind femora not touching each other (Figs. 23); 11) two rows of denticles on foreclaw apically directed (Figs. 25–27); 12) hind wing pads absent; 13) gill I-VII not racquet-shaped; 14) abdominal terga surface creased, without scale-like setae/socket.

Description

Male imago. Head. Dorsal portion of compound eyes oval (Fig. 29 in Nieto 2004); stalk in lateral view of dorsal portion of compound eye divergent. **Thorax.** Forewing hyaline with paired marginal intercalary veins (Fig. 30 in Nieto 2004); length of each intercalary vein 0.5 × distance between adjacent longitudinal veins (Fig. 30 in Nieto 2004). Hind wing absent. **Abdomen.** Genitalia (Fig. 31 in Nieto 2004). Forceps segment II narrow medially; segment III rod-like.

Mature nymph. Head. Frons with two longitudinal keels. Antenna with spines and simple setae. Labrum (Fig. 32 in Nieto 2004; Fig. 2 in Gutiérrez et al. 2013) with bifid setae on distoventral arc; bifid setae on distomedial arc; mediodorsal arc of setae present and extending laterally; and absence of pair of denticles on the medial emargination. Right mandible (Fig. 34 in Nieto 2004; Fig. 4 in Gutiérrez et al. 2013) with external margin convex; prostheca slender and bifid; incisors partially fused; and inner set of incisors at right angle with outer set. Left mandible (Fig. 33 in Nieto 2004; Fig. 3 in Gutiérrez et al. 2013) with external margin of mandibles convex; prostheca stout; molars with constriction; incisors partially fused; inner set of incisors at right angle with outer set. Maxilla (Fig. 36 in Nieto 2004; Fig. 6 in Gutiérrez et al. 2013) inner-dorsal margin with two rows of setae; first dentiseta more robust than others; first dentiseta not coupling with canine at base (Fig. 10); palp with two segments. Labium (Fig. 37 in Nieto 2004; Fig. 7 in Gutiérrez et al. 2013) with elongate mentum; base of glossa not overlapping the base of paraglossa; paraglossa subtriangular; distomedial projection of labial palp segment II rounded; segment III of labial palp conical (apex broadly pointed). **Thorax.** Hind wing pad absent. Legs (Fig. 22) with trochanter inserted along entire base of femur; many long and thin setae on femora; vestigial transverse

row of long setae on proximal third of forefemur; mid and hind femora with transverse row of long setae on proximal third; sockets of setae on the mid and hind transverse rows not touching each other; two rows of denticles on the claws; foreclaw denticles apically directed; denticles triangular. **Abdomen.** Gills on segment I–VII without folds, gill I not racquet-shaped; posterior margin of terga with spines; abdominal terga surface creased, without scale-like setae/socket; terminal filament subequal in length than cerci; caudal filaments with spines on each segment.

Distribution: Argentina and Colombia.

Comments

Considering the topology, the position of (*P. caldensis* + *V. singuil*) corroborated its placement in *Varipes* as subgenus or a new genus; in a broad sense, Gen. A could also belong to *Varipes*. However, evaluating the stability of the clades, *Varipes* (narrow sense) + (*P. caldensis* + *V. singuil*) is a weakly supported clade, while (*P. caldensis* + *V. singuil*) and *Varipes* have high values of the indices and, in the case of *Varipes*, a large number of synapomorphies. Gen. A + (*P. caldensis* + *V. singuil*) + *Varipes*) is a weakly supported clade, while Gen. A, (*P. caldensis* + *V. singuil*) and *Varipes* are three well-supported groups. Analytical settings were varied to verify the robustness of these monophyletic groups (see Materials and Methods section). All topologies resulting from these exploratory searches recovered Gen. A, (*P. caldensis* + *V. singuil*), *Rivudiva*, *Varipes*, and *Paracloeodes* as well-supported and stable monophyletic groups.

In addition to the highly stable monophyletic groups and the understanding that the long setae state is an independent acquisition between groups, a large number of generic characteristics was obtained in morphological analyses (some of them treated as characters and states in the matrix). Those attributed to *P. caldensis* and *V. singuil* have proved to be very effective in differentiating both of these species, not only from *Varipes*, but also from all other Neotropical and Nearctic genera as follows: scape and pedicel without scale-like tubercles in *P. caldensis* + *V. singuil*, while present in *Varipes*; mandibular incisors partially fused in *P. caldensis* + *V. singuil*, while fused in *Varipes*; inner set of incisors at right angle to the outer set in *P. caldensis* + *V. singuil*, while in the same plane in *Varipes*; maxilla with the first dentiseta with the same robustness as the others and with its basal projection not coupling with canine in *P. caldensis* + *V. singuil*, while the first dentiseta is more robust than the others and with base coupling with canine in *Varipes*; segment III of labial palp semi conical (apex broadly pointed) in *P. caldensis* + *V. singuil*, while globose in *Varipes*; base of glossa not overlapping base of paraglossa in *P. caldensis* + *V. singuil*, while overlapping in *Varipes*; lingua with distomedial projection in *P. caldensis* + *V. singuil*, while absent in *Varipes*; dorsal margin of femora parallel to ventral in *P. caldensis* + *V. singuil*, while convex in *Varipes*; trochanter inserted along entire base of femur in *P. caldensis* + *V. singuil*, while at the base of femur in *Varipes*; vestigial transverse row of long setae on proximal third of forefemur in *P. caldensis* + *V. singuil*, while not vestigial in *Varipes*; mid and hind femora with transverse row of long setae on proximal third with sockets not touching each other in *P. caldensis* + *V. singuil*, while touching each other in *Varipes*; tibio-patelar suture with length equal

or less than one third of tibia in *P. caldensis* + *V. singuil*, while equal or more than a half of tibia in *Varipes*; claws with triangular and apically directed denticles starting at base in *P. caldensis* + *V. singuil*, while conical, perpendicular and starting at medial third in *Varipes*; gill I symmetrical in *P. caldensis* + *V. singuil*, while racquet-shaped in *Varipes*.

P. caldensis + *V. singuil* could also be confused with the Nearctic genus *Kirmaushenkreena* McCafferty, mainly based on long setae on forefemur and claws with symmetrical rows of denticles (key of Waltz & Burian, 2008). The genus *Kirmaushenkreena* can be differentiated from *P. caldensis* + *V. singuil*, among other characteristics, by the presence of a subapical pair of setae on claws, while is absent in *P. caldensis* + *V. singuil*; the first dentiseta with its base coupling with canine, while not coupling in *P. caldensis* + *V. singuil*; and transverse row of setae on femora absent, while present in *P. caldensis* + *V. singuil*.

Taking into account the phylogenetic position, the number of synapomorphies, clade stability, and the great number of generic characteristics that differentiate the *P. caldensis* + *V. singuil* clade from other genera in the Nearctic and Neotropical realms, we propose this clade as a new genus, *Rhopyscelis* **gen. n.**, described and diagnosed above.

Rhopyscelis singuil (Nieto 2004) **comb. n.**
Varipes singuil Nieto 2004: 169; Domínguez et al. 2006: 188; Castillo & Pérez 2011: 348; Gutiérrez et al. 2013: 295.
Material examined. One nymph on slide (paratype), ARGENTINA, Escaba, State of Tucuman, Rio Singuil, 5.viii.1999, Molineri C. col. One nymph on slide (paratype), ARGENTINA, Salta, Dpto. Guachipas, Rio Grande de El Sauce, 15.ix.1998, Domínguez E., Molineri C. and Ubero cols, deposited at Museo Miguel Lillo – Argentina. *Additional material not examined:* Holotype (male nymph), Tucuman, ARGENTINA, river Medina, S26°32'27". W65°01'45", 860 m. 14.vi. 2001, Moiineri, Manzo, Orce & Nieto cols, deposited at Museo Miguel Lillo - Argentina.
Diagnosis. **Male imago.** The characters used in the generic section can also be used for specific identification.

Mature nymph. 1) antennae long, 1.5 × length of head capsule; 2) presence of medial decline on distal margin of labrum; 3) maxillae with palp 1.5 × length of galea-lacinia; 4) lingua with a small rounded apical projection (Fig. 35 in Nieto 2004); 5) glossa subcircular (Fig. 37 in Nieto 2004); 6) tarsal claws 0.36 × length of tarsi; 7) abdominal terga I and VIII dark brown.
Distribution. Argentina.

Rhopyscelis caldensis (Gutiérrez, Dias & Salles 2013) **comb. n.**
Paracloeodes caldensis Gutiérrez, Dias & Salles 2013: 291
Material examined. Three nymphs, two on slides (paratypes), COLOMBIA, Caldas, Neira, Qda. Collected on the highway, 5° 10,586'N/75° 31,532'W, 10.ii.2011, Gutiérrez Y., Dias L., Walteros Y., Montaño M., Tabares F. and Hoyos D. cols, deposited at Instituto Nacional de Pesquisas da Amazônia – Brazil. *Additional material not examined:* Holotype (mature nymph), COLOMBIA, Caldas, Neira, Qda. Sobre la carretera.

5°10,586'N 75°31, 532'W, 10.ii.2011, Gutiérrez, Y., Dias, L., Walteros, Y., Montaño, M., Tabares, F., Hoyos D. cols, deposited at Colección Entomológica del Programa de Biología, Universidad de Caldas, Manizale – Colombia.

Diagnosis. **Male imago.** Adults are not known.

Mature nymph. 1) antennae long, 2.0 × length of head capsule; 2) absence of medial decline on distal margin of labrum; 3) maxillae with palp twice the length of galea-lacinia; 4) lingua with a subconical apical projection (Fig. 5 in Gutiérrez et al. 2013); 5) glossa subtriangular (Fig. 7 in Gutiérrez et al. 2013); 6) tarsal claws 0.33 × length of tarsi; 7) abdominal terga I, VII and VIII darker than others.

Distribution. Colombia.

Rivudiva Lugo-Ortiz & McCafferty 1998
In addition to recovering synapomorphies, all *Rivudiva* species were observed to have lingua with apical tuft of setae, absence of transverse row of setae on forefemur and presence of transverse row of setae on mid and hind femora at proximal third with sockets not touching each other (Fig. 23).

Varipes Lugo-Ortiz & McCafferty 1998
In addition to recovering synapomorphies, all *Varipes* species were observed to have base of paraglossa overlapping base of glossa (Fig. 21 in Nieto 2004).
Gen. A.
In addition to recovering synapomorphies, Gen. A were observed to have glossa and paraglossa with strong pectinated setae (Fig. 159 in Falcão et al. 2011).

Acknowledgments

Supplementary material

Supplementary material is available online at https://doi.org/10.6084/m9.figshare .6840119.v1

References

Boldrini, R. & Cruz, P.V. (2014) Baetidae (Insecta: Ephemeroptera) from the state of Rondônia, Northern Brazil. *Bol. Mus. Int. de Roraima* **8**(1): 1–9.

Boldrini, R., Cruz, P.V., Salles, F.F., Belmont, E. L. & Hamada, N. (2012) Baetidae (Insecta: Ephemeroptera) from northeastern Brazil. *Check List* **8**(1): 088–094.

Castillo, M.M. & Pérez, B. (2011). Varipes (Ephemeroptera: Baetidae) em Venezuela: descripción de nueva especie. *Revista Colombiana de Entomología* **37**(1): 346–349.

Cruz, P.V., Boldrini, R., Salles, F.F. & Hamada, N. (2011) The male imago of *Rivudiva trichobasis* Lugo-Ortiz & McCafferty from Amazonas state, Brazil. *Zootaxa* **2907**: 60–62.

Cruz, P.V., Salles, F.F. & Hamada, N. (2017) Phylogeny of *Callibaetis* (Ephemeroptera: Baetidae) based on morphology of nymphs and adults. *Zoologica Scripta* **46**(2):138–149. doi:10.1111/zsc.12197

Day, W.C. (1955) New genera of mayflies from California (Ephemeroptera). *Pan-Pacific Entomologist* **31**(3): 121–137.

Domínguez, E.D. & Dos Santos, A. (2014) Co-authorship networks (and other contextual factors) behind the growth of taxonomy of South American Ephemeroptera: A scientometric approach. *Zootaxa* **3754**: 059–085.

Domínguez, E., Molineri, C., Pescador, M.L., Hubbard, M.D. & Nieto, C. (2006*) Aquatic Biodiversity of Latin América, Ephemeroptera of South America*. Vol. 2. Pensoft, Sofia-Moscow, 646 pp.

Falcão, J.N., Salles, F.F. & Hamada, N. (2011) Baetidae (Insecta: Ephemeroptera) ocorrentes em Roraima, Brasil: novos registros e chaves para gêneros e espécies no estágio ninfal. *Revista Brasileira de Entomologia* **4**: 516–548.

Goloboff, P.A. (1993) Estimating character weights during tree search. *Cladistics* **9**: 83–91.

Goloboff, P.A. & Farris, J. (2001) Methods for quick consensus estimation. *Cladistics* **17**: 26–34.

Goloboff, P.A., Farris, J.S. & Nixon, K. (2008) TNT, a free program for phylogenetic analysis. *Cladistics* **24**: 774–786.

Goloboff, P.A., Farris, J.S., Kallersjo, M., Oxelman, B., Ramirez, M.J & Szumik, C.A. (2003) Improvements to resampling measures of group support. *Cladistics* **19**: 324–332.

Gutiérrez, Y., Dias, L.G. & Salles, F.F. (2013) *Paracloeodes caldensis* (Ephemeroptera: Baetidae), an atypical new species from the Colombian Andes. *Zootaxa* **3721**: 291–295.

Jacobus, L.M. (2013) South Carolina mayflies (Insecta: Ephemeroptera) of Conservation Concern. *Journal of the South Carolina Academy of Science* **11**: 21–26.

Kluge, N.J. (2004) *The Phylogenetic System of Ephemeroptera*. Kluwer Academic, Dordrecht.

Kluge, N.J. (2015). Phylogeny of Ephemeroptera. http://www.insecta.bio.spbu.ru/z/Eph-spp/))Rivudiva.htm. (accessed 22 Fev 2017).

Landa, V. & Soldán, T. (1985) *Phylogeny and Higher Classification of the Order Ephemeroptera: A Discussion from the Comparative Anatomical Point of View*. Studie Ceskoslovenská Akademie Ved, Prague, 121 pp.

Lima, L.R.C., Cruz, P.V. & Pinheiro, U.S. (2013) The adult stages of *Paracloeodes charrua* Emmerich & Nieto, 2009 (Ephemeroptera: Baetidae). *Zootaxa* **3640**: 597–600.

Lugo-Ortiz, C. R. & McCafferty, W. P. (1998) Five new genera of Baetidae (Insecta: Ephemeroptera) from South America. *Annales de Limnologie* **34**: 57–73.

McCafferty, W.P. (1991) Comparison of Old and New World Acanthametropus (Ephemeroptera: Acanthametropodidae) and other psammophilous mayflies. *Entomological News*, **102**: 205–214.

Nieto, C. (2004) Redescription of *Varipes* (Ephemeroptera: Baetidae), with the description of new species from Bolivia and Argentina. *Aquatic Insects* **26**: 161–173.

Nieto, C. (2010) Cladistic analysis of the family Baetidae (Insecta: Ephemeroptera) in South America. *Systematic Entomology* **35**: 512–525.

Nieto, C. (2015) The Baetodes complex (Ephemeroptera: Baetidae),phylogeny, biogeography, and new species of Mayobaetis. *Freshwater Science* **35**(1): 55–64.

Nieto, C. & Richard, B. (2008) The genus *Cloeodes* (Ephemeroptera: Baetidae) in Argentina with new generic synonymy and new species. *Zootaxa* **1727**: 1–21.

Nieto, C. & Salles, F.F. (2006) Revision of the genus *Paracloeodes* (Ephemeroptera: Baetidae) in South America. *Zootaxa* **1301**: 1–33.

Salles, F.F. & Nascimento, J.M.C. (2009) The genus *Rivudiva* Lugo-Ortiz and McCafferty (Ephemeroptera: Baetidae): First generic description of adults, new combinations, and notes on the nymphs. *Annales de Limnologie - International Journal of Limnology* **45**: 231–235.

Salles, F.F., Gattolliat, J.-L. & Sartori, M. (2015) Phylogenetic analyses of *Cloeodes* Traver and related genera (Ephemeroptera: Baetidae). *Systematic Entomology* **41**(1): 93–111.

Salles, F.F., Soares, E.D.G., Massariol, F. & Faria, R.R.L. (2013) *Oligoneuria* Pictet: phylogenetic analysis and description of three new species from Brazil (Ephemeroptera: Oligoneuriidae). *Systematic Entomology* **39**: 223–241.

Sereno, P. (2007) Logical basis for morphological characters in phylogenetics. *Cladistics* **23**: 565–587.

Shimano, Y., Salles, F.F. & Juen, L. (2013) Study of the mayfly order Ephemeroptera (Insecta) in Brazil: a scienciometric review. *Revista Brasileira de Entomologia* **57**: 359–364.

Stagliano, D.M. (2016) Mayflies (Insecta: Ephemeroptera) of conservation concern in Montana: status updates and management needs. *Western North American Naturalist* **76**: 441–451.

Staniczek, A.H. (1997) The morphology of *Siphlaenigma janae* Penniket (Ephemeroptera, Siphlaenigmatidae), and its signifiance for the ground plan of the Baetoidea. *Ephemeroptera & Plecoptera Biology-Ecology-Systematics* (ed. by P. Landolt and M. Sartori), pp. 536–549. Mauron+Tinguely & Lachat SA, Fribourg.

Waltz, R.D. & Burian, S.K. (2008) Ephemeroptera. In: Merritt, R.W., Cummins, K.W. & Berg, M.B. (Eds.), *An Introduction to the Aquatic Insects of North America*. Kendall/Hunt, Dubuque, Iowa, pp. 181–236.