



Stage description, new combination and new records of Neotropical Brachycercinae (Ephemeroptera: Caenidae)

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Abstract

We present taxonomic contributions and new records for Neotropical Brachycercinae based on material from Brazil. We performed a phylogenetic analysis in order to test the relationship between *Alloretochus* Sun & McCafferty, 2008 and *Latineosus* Sun & McCafferty, 2008, and *Alloretochus sigillatus* was recovered in the *Latineosus* clade. Therefore, we propose a new combination, *Latineosus sigillatus* **comb. n.** The nymph of *Latineosus sigillatus* is described and is associated with imago through molecular tools. Moreover, *Alloretochus peruanicus* (Soldán, 1986) is reported for the first time from Brazil.

Key words: *Alloretochus*, *Latineosus*, *A. peruanicus*, *L. sigillatus*, stage association, COI

Introduction

The mayfly subfamily Brachycercinae Lestage, 1924 (Ephemeroptera: Caenidae) has been recently revised by Sun & McCafferty (2008). According to them the group is divided in six tribes, two of which are reported from the Neotropics: Latineosini Sun & McCafferty, 2008, represented by *Latineosus* Sun & McCafferty, 2008, and Cercobranchini Sun & McCafferty, 2008, represented in this area by *Alloretochus* Sun & McCafferty, 2008. Brachycercinae, in general, are rare in collections due to the psammophilous habit of nymphs, because their sand-bottom riverine habitats are generally overlooked by collectors; furthermore, the adults apparently have nocturnal habits (Sun & McCafferty 2008). Because of these facts, most of the species, at least from the Neotropics, are represented by only a small series of specimens and a single life stage.

Latineosus is known exclusively from nymphs and is comprised of three species: *L. colombianus* (Soldán, 1986), the type-species, described based on a single nymph from Tolima, Colombia and then reported from Brazil based on one set of nymphal exuviae (Malzacher 1986); *L. cayo* Sun & McCafferty, 2008, described based on a single nymph from Cayo, Belize; and *L. cibola* Sun & McCafferty, 2008, described based on two nymphs and a set of exuviae from Texas, USA.

Alloretochus, on the other hand, is comprised of two species: *A. peruanicus* (Soldán, 1986), the type-species, described based on nymphs from Peru (Soldán 1986) and later reported from Bolivia, when adults were described (Molineri & Goitia 2006); and *A. sigillatus* Molineri, 2014, recently described based on adults from Bolivia and Ecuador (Molineri 2014) and subsequently reported from Brazil (Angeli *et al.* 2015). According to Molineri (2014), *A. sigillatus* was attributed to *Alloretochus* due to a broadly emarginated apex of the subgenital plate, an autapomorphy proposed by Sun & McCafferty (2008) for the genus.

During a one-year survey of mayflies in the main tributaries of the São Mateus River, Southeastern Brazil, adults of *A. sigillatus* were collected often in Pennsylvania light traps. After an exhaustive search for the corresponding nymphs, candidate specimens representing a previously undescribed form finally were encountered at one of the collection sites. These nymphs, however, showed more affinity with *Latineosus* than *Alloretochus*. Besides the description of these nymphs, the aims of this paper are to correctly associate them with adults based on molecular evidence and to perform a phylogenetic analysis in order to test their relationships with *Alloretochus* and *Latineosus*. Also, we report the discovery of *Alloretochus peruanicus* from the western State of Acre, which is the first record of this species from Brazil.

Material and methods

Phylogenetic analyses. The matrix published in Sun & McCafferty (2008) was used in this study (see Appendix 1). We added one taxon, *Alloretochus sigillatus*, and the egg characters for *A. peruanicus* that were recently described by Molineri (2014). Trees were indistinctly rooted in *Callistellina panda* (Sun & McCafferty, 2004) or *Caenis youngi* Roemhild, 1984, as done by Sun & McCafferty (2008).

Searches were conducted in TNT (Goloboff *et al.* 2008), under prior and implied weights, using the “traditional search” strategy, from 100 Wagner trees and TBR swapping (keeping 10 trees per replication). Two group support values were calculated, relative Bremer (saving trees until 50% longer than best trees) and Symmetric Jackknifing (Goloboff *et al.* 2003). Synapomorphies of the nodes of interest were studied by mapping and listing all synapomorphies found (commands “optimize”, “map synapomorphies” or “list synapomorphies”).

Molecular analysis. DNA was extracted using a Wizard SV Genomic DNA Purification System Kit (Promega) and the protocol for animal tissue. Adult males and nymphs, which could be identified confidently, underwent extractions by two methods. For two adult specimens, the abdomen and wing were removed; all remaining portions were placed in extraction buffer. For two nymph specimens three legs were used; the remaining specimens were retained as vouchers. After incubation, the material was retrieved from the buffer and vouchered at CZNC. A 658-base pair portion of COI was amplified, and PCR was performed in a 25- μ L mixture containing approximately 20 ng/ μ L DNA template; 1X PCR buffer, a 2.0 mM concentration of $MgCl_2$, a 30 μ M concentration of each primer: LCO 1490 (GGTCAACAAATCATAAAGATATTGG) and HCO 2198 (TAAACTTCAGGGTGAC CA A AAAATCA) (Folmer *et al.* 1994), a 100 μ M concentration (each) of dATP, dCTP, dGTP, and dTTP) and 1,5 U *Taq* polymerase. Initial PCR consisted of a preheating at 94°C for 5 minutes; 40 cycles of 94°C for 45 seconds, 48°C of annealing temperature for 45 seconds and 72°C for 45 seconds, and incubation at 72°C for 5 minutes. Negative controls, which contained all elements of the reaction mixture except DNA, were assigned. Successful bands were detected on 1.5% agarose gel in 1X TAE buffer. Products were purified using an ExoSAP-IT for PCR Product Cleanup (GE Healthcare), and cycle sequenced using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems®) with the same primers as employed for amplification and sequenced using an Applied Biosystems 3500 Genetic Analyzer.

The alignment of sequences was relatively unambiguous as all specimens were length invariable. Sequences were aligned and trimmed to length using Geneious R8, resulting in 611 characters. Additionally, sequences from GenBank of *Caenis* sp. AMI1 (HQ151303), *Brachycercus harrisella* (JQ662051, LN734718 and LN734719) and *Cercobrachys cree* (JQ661558 and JQ662593) were used. Pairwise numbers of nucleotide differences were calculated with the program Mega using the ‘Calculate distances’ option and ‘Nucleotide: *p*-distance’ model option for distances. The *p*-distance, the proportion of sites in which two sequences differ, was calculated by dividing the number of nucleotide differences by the total number of nucleotides (Kumar *et al.* 2001). We used 3% as the maximal value for intraspecific sequence divergence (Hebert *et al.* 2003) in order to establish any positive association between nymphs and adults.

Voucher specimens. The specimens examined are deposited in the Coleção Zoológica Norte Capixaba (CZNC), Universidade Federal do Espírito Santo, São Mateus, Brazil and in Museu de Zoologia da Universidade Estadual de Santa Cruz, Ilhéus, Brazil (MZUESC). Dissected parts were permanently mounted on microscope slides with Euparal.

Description. The taxonomic descriptions presented herein followed a system of characters employed by Sun & McCafferty (2008). Photos were taken using a Stemi-200C ZEISS stereomicroscope with Axiocam ERc 5s digital camera. The free program DIVA-GIS (<http://www.diva-gis.org>) was used to make the distribution map of species.

Results and discussion

Phylogenetic analysis. Prior weights (all characters weight = 1) obtained 12 shortest trees, with the strict consensus of these showing the same relationships (concerning *A. sigillatus* and most-related taxa) as the only most-parsimonious tree found with implied weighting (IW). Further results reported here are from IW tree: best tree was hit 85 from 100 times, and is a fully resolved cladogram (Figure 1) with a length (soft polytomies) of 327 steps, a total fit of 66.11 and adjusted homoplasy (soft polytomies) of 25.89, CI= 41, RI= 76.

Our focus species, *Alloretochus sigillatus*, was grouped inside the *Latineosus* clade with medium support, as a sister group to *L. cibola* from Texas. *Alloretochus peruanicus* was shown to be a sister group to the *Cercobranchys* Soldán, 1986 clade. Four synapomorphies (not unique, i.e. reversed in other taxa) were found for *Latineosus*: 1) character 2 (compound eye elevation), changed from state 0 (not strongly produced dorsally) to state 1 (strongly produced dorsally); 2) character 3 (occiput posterior shape) changed from 0 (lateral margin posterior to compound eye distinctly curved inward) to 1 (lateral margin posterior to compound eye approximately parallel with long axis of body); 3) character 13 (middle ocellar tubercle length relative to lateral tubercle length) changed from 0 (approximately subequal) to 1 (one-half to two-thirds lateral tubercle); and 4) character 33 (labial palp segment 1 ventral setation) changed from 1 (with long fine setae, one-half ore more length of palp segment 1) to 0 (with no apparent setae or very short setae).

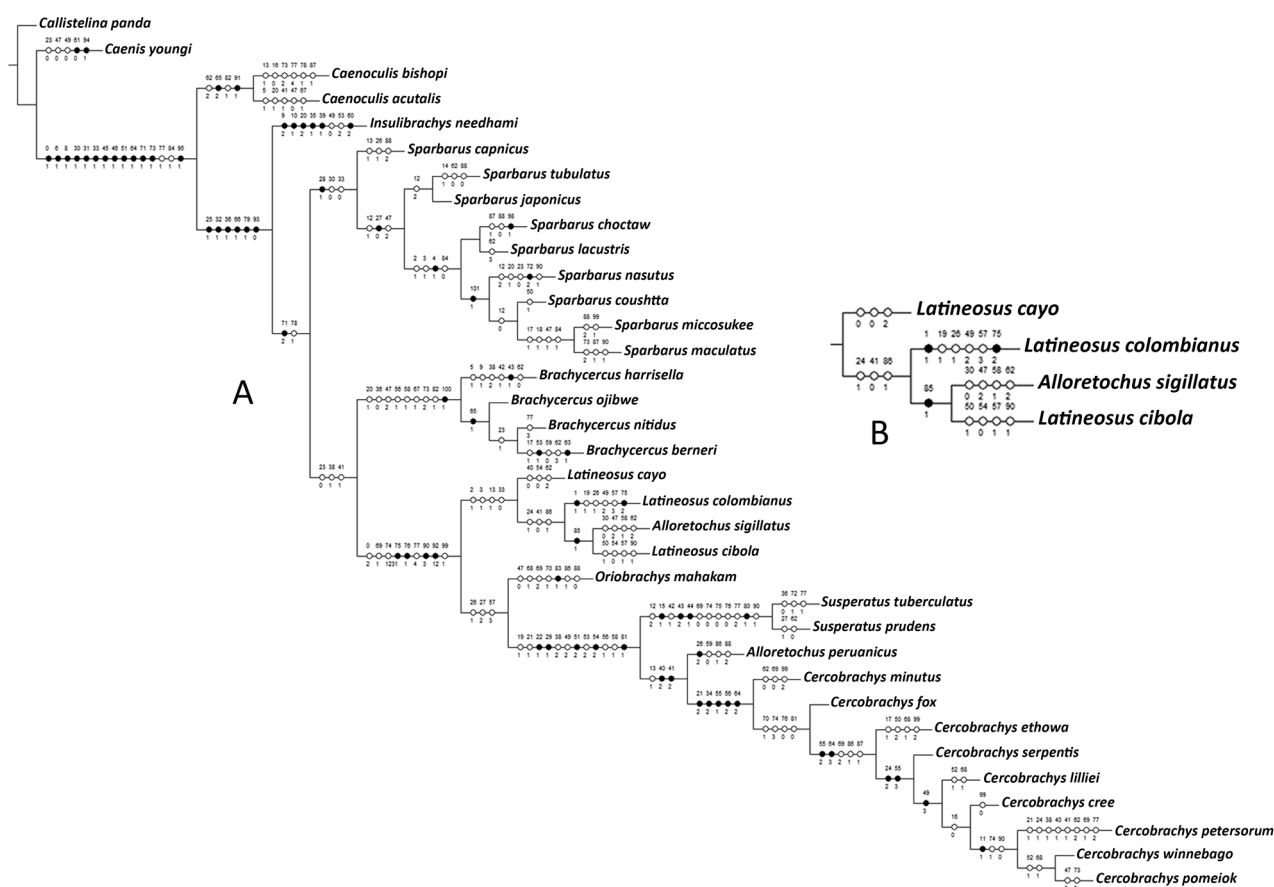


FIGURE 1. A. Most parsimonious cladogram found with implied weighting (327 steps, CI= 41, RI= 76). B. Branch of *Latineosus*.

Alloretochus sigillatus grouped as sister to *L. cibola* because they shared a unique synapomorphic change on character 85 (operculate gill dorsal flat setation, present). Both species are sister to *L. colombianus* by three synapomorphies: 1) character 24 (galealacinia length to width ratio) changed from state 0 (2.3–2.6) to state 1 (ca. 2.0); 2) character 41 (mesosternum anterior margin setation), changed from state 1 (row of setae measuring less than one-fourth length of midfemur) to state 0 (none apparent, or with scattered shorter setae); and 3) character 86 (operculate gill Y-ridge development) changed from state 0 (strong) to state 1 (weak).

The autapomorphies of *A. sigillatus* are three: 1) character 30 (maxillary palp terminal segment outer distal number of long, fine setae), changed from state 1 (15 or more) to state 0 (10 or fewer); 2) character 47 (hindleg length to foreleg length ratio), changed from state 1 (1.3–1.5) to state 2 (1.6–1.8); and 3) character 58 (hindfemur dorsal setae length) changed from state 0 (subequal to, or shorter than, $1.5 \times$ width of hindfemur) to state 1 (subequal to, or longer than, $2.0 \times$ width of hindfemur).

Based on these results, *Alloretochus sigillatus* is more properly placed in the genus *Latineosus*, as *Latineosus sigillatus* (Molineri) **comb. nov.** The relationships proposed by Sun & McCafferty (2008) inside *Latineosus* (*colombianus* (*cayo* + *cibola*)) changed in our analysis to (*cayo* (*colombianus* (*cibola* + *sigillatus*))).

Oriobrachys mahakam Sun & McCafferty, 2008 was not recovered as the sister group to *Latineosus* as in shortest trees by Sun & McCafferty (2008), but this monotypic genus is hypothesized as a sister group to (*Susperatus* (*Alloretochus* + *Cercobrachys*)). These results may have implications for Brachycercinae tribal classification, but that is beyond the scope of this paper.

The male imagoes of *Alloretochus* and *Latineosus* present only a few characters to differentiate them: abdominal coloration (body with gray shading widely extended mainly on abdomen and thoracic sterna in *Alloretochus* and abdominal terga with characteristic blackish stripe in *Latineosus*), ratio of forceps length/subbasal width (6.8–8.0 in *Alloretochus*, 8.9 in *Latineosus*) and ratio of lengths of male foretarsi/forefemur (1.5–1.7 in *Alloretochus*, 1.2–1.3 in *Latineosus*) (Molineri 2014). Since so few species of both genera are known from adults and nymphal stages, descriptions of additional new species and associations of life stages may be necessary to confirm the value of these characters. Therefore, our results suggest that the knowledge of the nymphal stage is of prime importance at the moment of assigning new Neotropical Brachycercinae to genus.

COI intra- and interspecific divergence. The Genbank Accession numbers are given in Table 1, and values of the p-distances between all sequences are given in Table 2. Within the four *L. sigillatus* specimens studied, minimum intraspecific divergences were 0.2%, and maximum intraspecific divergences were 0.5%, with mean = 0.4% (+/- 0.2%). Interspecific divergences ranged from 15.2% between *L. sigillatus* and *C. cree* to 18.6% between *L. sigillatus* and *Caenis* sp., with mean = 17.1%. The undescribed nymph from the São Mateus River system grouped unambiguously with the adult specimens of *A. sigillatus*. Large pairwise distances among congeners reflect the ability of mtDNA markers to separate well-defined genera, revealing highly divergent clades that correspond to currently accepted Brachycercinae taxonomy.

TABLE 1. GenBank accession numbers. Abbreviations: N= nymph, I= imago.

Species	Code of Table 2	Accession numbers
<i>Latineosus sigillatus</i>	Ls-N1	KT874510
<i>Latineosus sigillatus</i>	Ls-N2	KT874509
<i>Latineosus sigillatus</i>	Ls-I1	KT874512
<i>Latineosus sigillatus</i>	Ls-I2	KT874511
<i>Brachycercus harrisella</i>	Bh1	JQ662051
<i>Brachycercus harrisella</i>	Bh2	LN734718
<i>Brachycercus harrisella</i>	Bh3	LN734719
<i>Cercobrachys cree</i>	Cc1	JQ661558
<i>Cercobrachys cree</i>	Cc2	JQ662593
<i>Caenis</i> sp. AMI	CAMI1	HQ151303

TABLE 2. Pairwise distances between taxa in analysis expressed as a percentage of nucleotide differences (*p*-distances).

	Ls-N1	Ls-N2	Ls-I1	Ls-I2	Cc2	Cc1	Bh1	Bh2	Bh3
Ls-N2	0,5								
Ls-I1	0,2	0,4							
Ls-I2	0,4	0,5	0,2						
Cc2	15,4	15,2	15,2	15,4					
Cc1	15,6	15,4	15,4	15,6	0,7				
Bh1	18,0	18,0	18,2	18,0	14,5	14,9			
Bh2	17,9	17,9	17,9	17,7	14,0	14,0	5,8		
Bh3	17,5	17,5	17,5	17,3	13,8	14,2	5,6	0,05	
CAMI1	18,4	18,6	18,2	18,0	18,4	18,6	17,7	16,8	16,6

Taxonomic accounts

Latineosus sigillatus (Molineri, 2014), new combination

(Figs. 2–4)

Alloretochus sigillatus Molineri 2014: 142, Angeli *et al.* 2015: 200

Adults and egg. Adequately described by Molineri (2014).

Nymph. Diagnosis. 1) Compound eyes strongly produced dorsally (Figs. 3A, B, C); 2) Anterior margin of compound eyes not extending anterior of base of middle ocellar tubercle (Fig. 3C); 3) Lateral ocellar tubercle with length subequal to basal width, triangulate in lateral view, apex rounded; 4) Labrum trapezoidal with lateral margin nearly straight; 5) Operculate gill ventrally without submarginal microtrichia; 6) Abdominal segment VI with a pair of projections not overlapping medially (Fig. 4B, C); 7) Abdominal segments VII–VIII without projections.

Description. Coloration: General coloration yellowish brown. Head with diffuse dark brown marks on vertex (Fig. 3A); antenna pale. Thorax (Fig. 2B) with pronotum with dark brown mark in medial region; posterior margin of mesonotum dark brown; metanotum shaded with brown; ventrally pale; legs pale. Abdomen (Fig. 2B) dorsally with segments I and II shaded with light brown and with dark brown marks in medial region; segments VII–X with dark brown marks in medial region; ventrally pale; operculate gill with Y-ridge dark brown. Caudal filaments pale with apex shaded with light gray.

Lengths (mm): body, 3.9–4.7; caudal filaments, 1.4–2.2; antenna, 0.9–1.1.

Head: *Occiput* relatively densely covered with long setae. *Compound eye* (Figs. 3A, B, C) strongly produced dorsally; anterior margin not extending anterior of base of middle ocellar tubercle. *Lateral ocellar tubercle* (Fig. 3C, 4A) length subequal to basal width, triangulate in lateral view, apex rounded. *Middle ocellar tubercle* (Fig. 3A, B, 4A) tapered-conical in dorsal view; length $0.7 \times$ of basal width, and $0.7 \times$ length of lateral ocellar tubercle; apex rounded. *Antenna* with pedicel $1.0 \times$ length of scape, with 4 or 5 setae on pedicel. *Labrum* trapezoidal with lateral margin nearly straight. *Hypopharynx* with superlingua subovate with lateral margin moderately and convexly produced. *Maxilla* (Fig. 3D) with galea-lacinia length $2.3 \times$ basal width; palp segment I width $1.6 \times$ width of segment II; segment II $1.4 \times$ length of segment I, with seven long, stout setae along inner margin.

Thorax: *Pronotum* with anterior margin moderately emarginate; lateral margin not forming distinctly convex ridge, without angulate expansion. *Propleuron* partially visible in dorsal view. *Legs*. Ratios of lengths of body: foreleg: midleg: hindleg—3.6: 1.0: 1.7: 1.8. Ratios of lengths of forefemur: tibia: tarsus: claw—2.1: 1.0: 1.1: 0.7. Ratios of length of hindfemur: tibia: tarsus: claw—1.7: 1.0: 1.0: 0.5. *Foreleg*. Femur. Dorsally with few long setae and many short setae; ventrally with sparse row of short setae and many long setae on basal third; length of long setae $0.5\text{--}1.5 \times$ width of femur. Tibia. Ventral margin with row of about seven setae as long as, or shorter than, width of tibia; posterior surface with some scattered setae measuring $1.2\text{--}1.5 \times$ width of tibia, but not arranged in distinct row. Tarsus. Ventral margin with row of about 12 setae as long as, or shorter than, width of tarsus; anterior and posterior surfaces with some setae as long as $1.8 \times$ width of tarsus, not in distinct rows. *Hindleg*. Femur length

7.0 × width; outer margin with row of short setae and few setae 1.5 × longer than width of femur; inner margin with short setae. Tibia. Outer margin with long, fine setae measuring in length about 2.8 × width of tibia; inner margin with few fine, simple setae. Tarsus. Outer margin bare; inner margin with row of about 6 fine, simple setae. Tarsal claw length 7.4 × basal width.

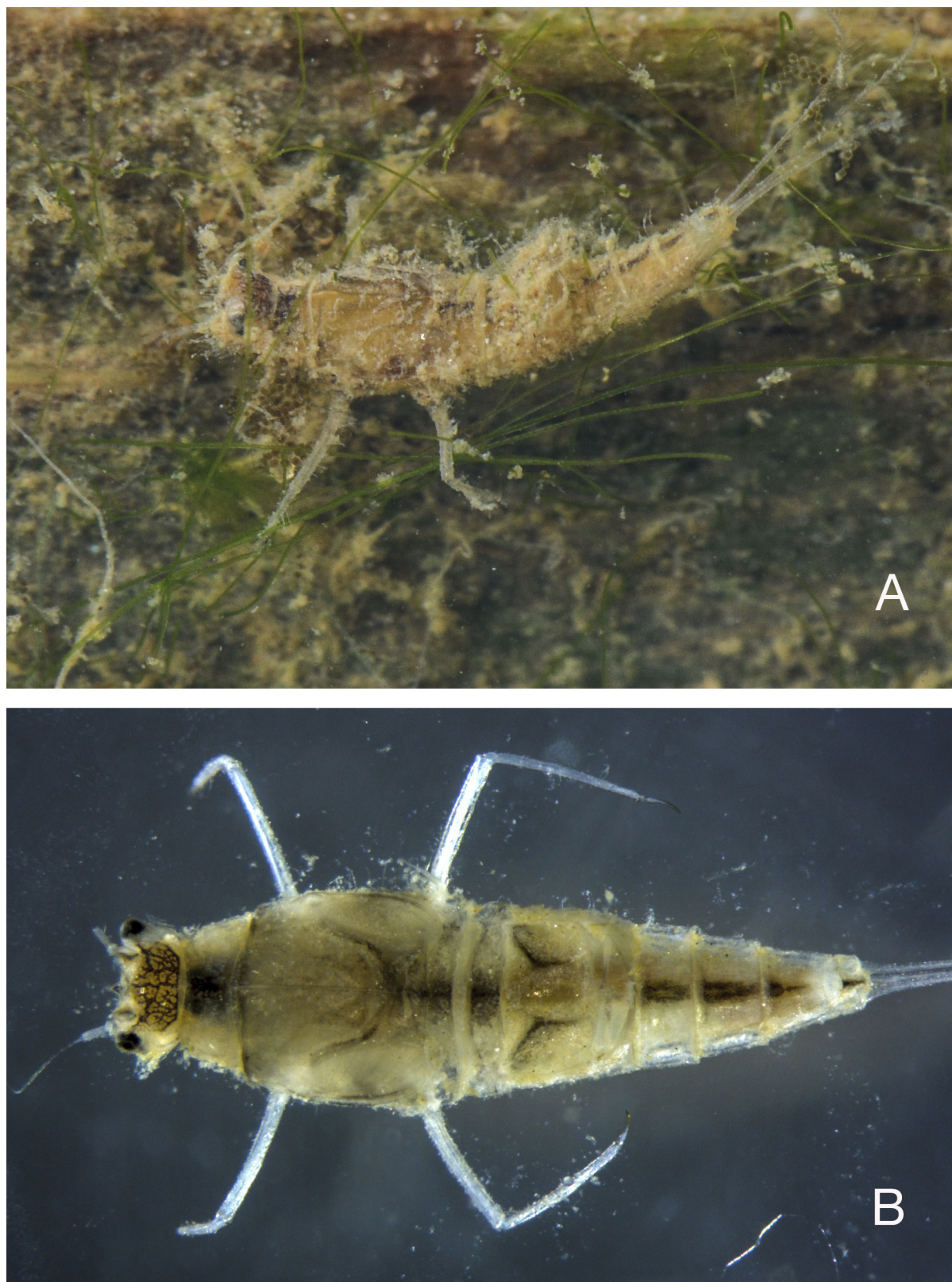


FIGURE 2. *Latineosus sigillatus*. A, B. Habitus, nymph.

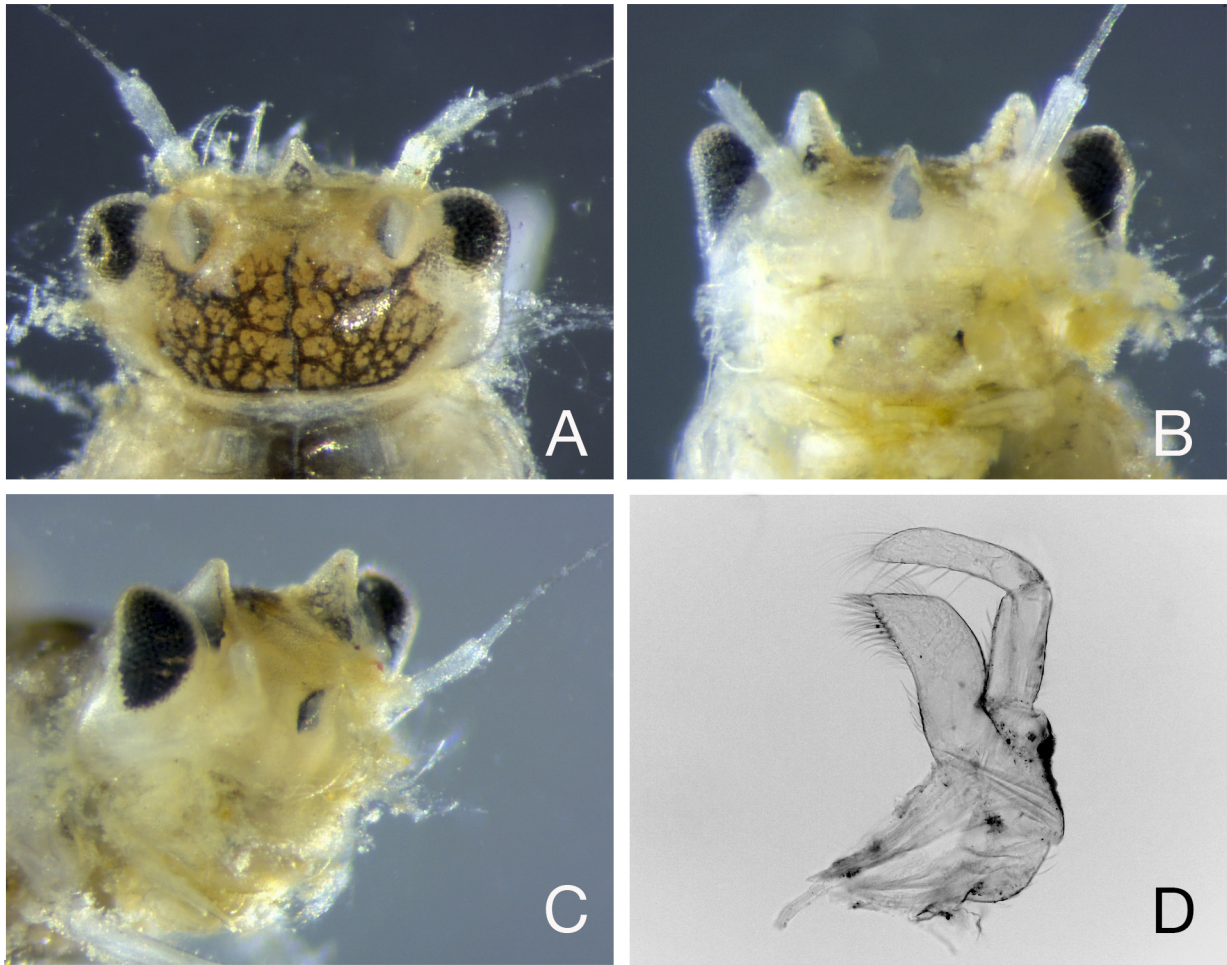


FIGURE 3. *Latineosus sigillatus*. A. Lateral and middle ocellar tubercle, dorsal view. B. Lateral and middle ocellar tubercle, ventral view. C. Lateral and middle ocellar tubercle, lateral view. D. Maxilla.

Abdomen: *Terga*. Posterolateral projection V length $3.4 \times$ basal width (Fig. 4B, C, D); projection VI with distal half with outer margin posterior to inner margin in dorsal view, and with apex rounded, pair of projections not overlapping medially (Fig. 4B, C); segments VII–IX without projections. *Sterna*. Sternum IX with posterior margin truncate. *Operculate gill* (Fig. 4B, C, E). Length $1.2 \times$ width; dorsum with lateral region with some long setae one-fifth to one-third length of gill; posteromedial corner with row of long marginal setae measuring up to one-third length of gill; posterolateral corner with marginal setae one-sixth to one-fourth of gill and not longer than posteromedial corner setae; Y-ridge weakly developed, narrow, indistinct; ventral submarginal microtrichia absent.

Material examined. 8 nymphs, BRAZIL, Espírito Santo, São Mateus, Rio Cotaxé, Japira, S 18°34'39.1" / W 40°16'58.0", 18/iv/2013, F.F. Salles, E. Rosário, cols.; 6 male imagos from Pensilvânia light trap, same data as previous, except: 22–23/xi/2012. 18 male imagos from Pennsylvania light trap, BRAZIL, Espírito Santo, Nova Venécia, Rio Cricaré, Santa Rita do Pip Nuck, S 18°39'51.4" / W 40°30'44.9", 18–19/iv/2012. 94 male imagos from Pennsylvania light trap, BRAZIL, Espírito Santo, São Mateus, Rio Cotaxé, Sr. Gama farm, S 18°37'41.1" / W 40°6'41.6", 20–21/xi/2013. 35 male imagos, BRAZIL, Rio Grande do Sul, Bossoroca, Barra de Angico, Rio Piratinim, 28.vi.2009, S 28° 32' 26" W 54° 57' 30", AMO Pes col.

Alloretochus peruanicus (Soldán, 1986), new country record

Cercobrachys peruanicus Soldán, 1986: 343; Molineri & Goitía, 2006: 64; Domínguez *et al.*, 2006: 223.

Alloretochus peruanicus (Soldán), Sun & McCafferty, 2008: 78; Molineri, 2014: 140.

A. peruanicus, as stated by Molineri (2014), shows a wide range of geographic distribution, including Argentina, Bolivia, Colombia and Peru. The present record, therefore, is not surprising, given the proximity of the locality in Brazil to those from Colombia and Peru (Fig. 5).

Material examined. 3 male imagos, BRAZIL, Acre, Mancio Lima, Recanto Campestre at São Francisco, P. N. Serra do Divisor, Rio Azul, S 08°59'56.7" / W 72°54'56", 15.iii.2006, A.R. Calor col. (2 CZNC and 1 MZUESC).

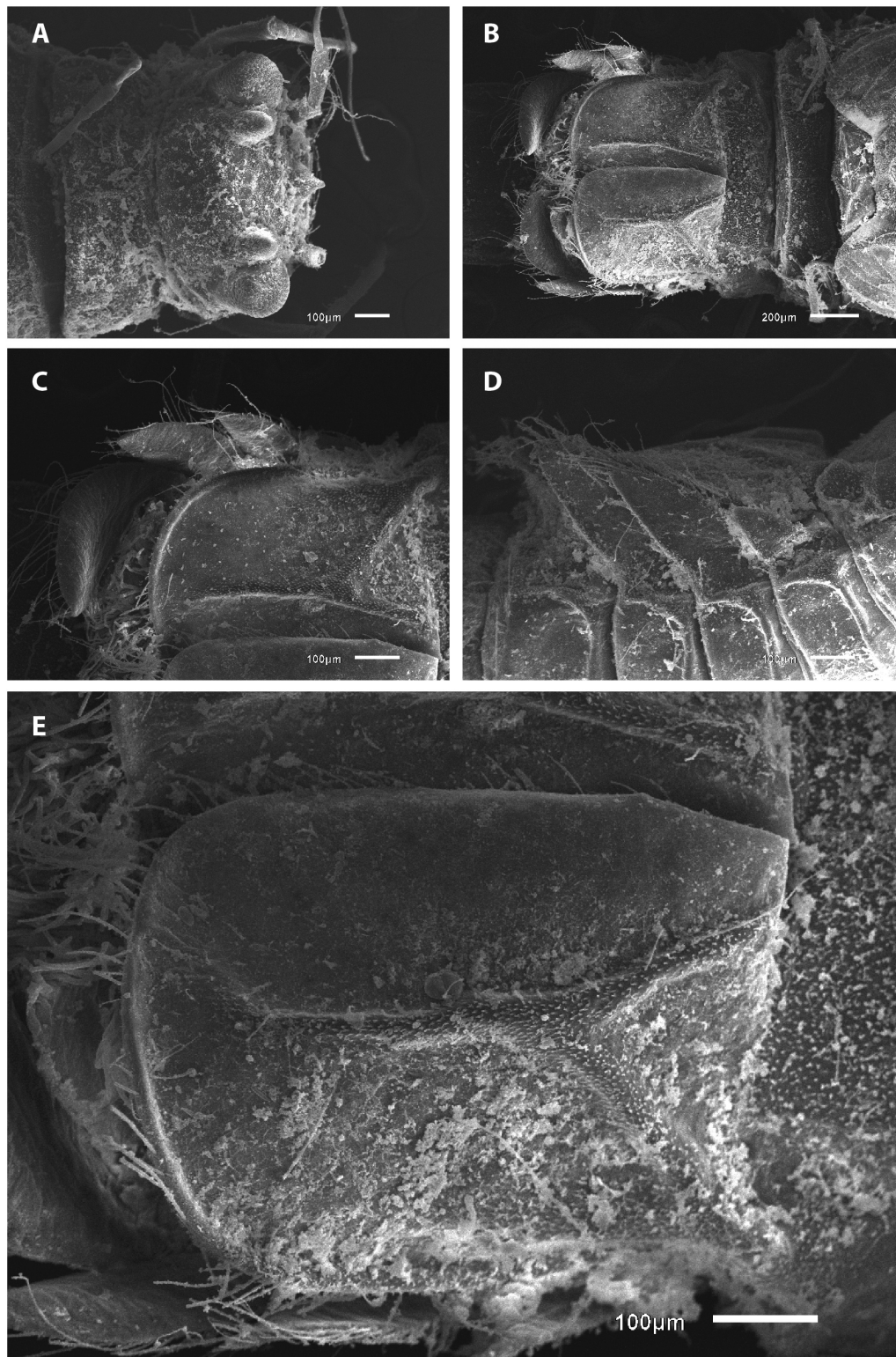


FIGURE 4. *Latineosus sigillatus*. A. Head, dorsal view. B, C. Operculate gills and abdominal posterolateral projections. D. Detail of abdominal posterolateral projections. E. Detail of operculate gill.

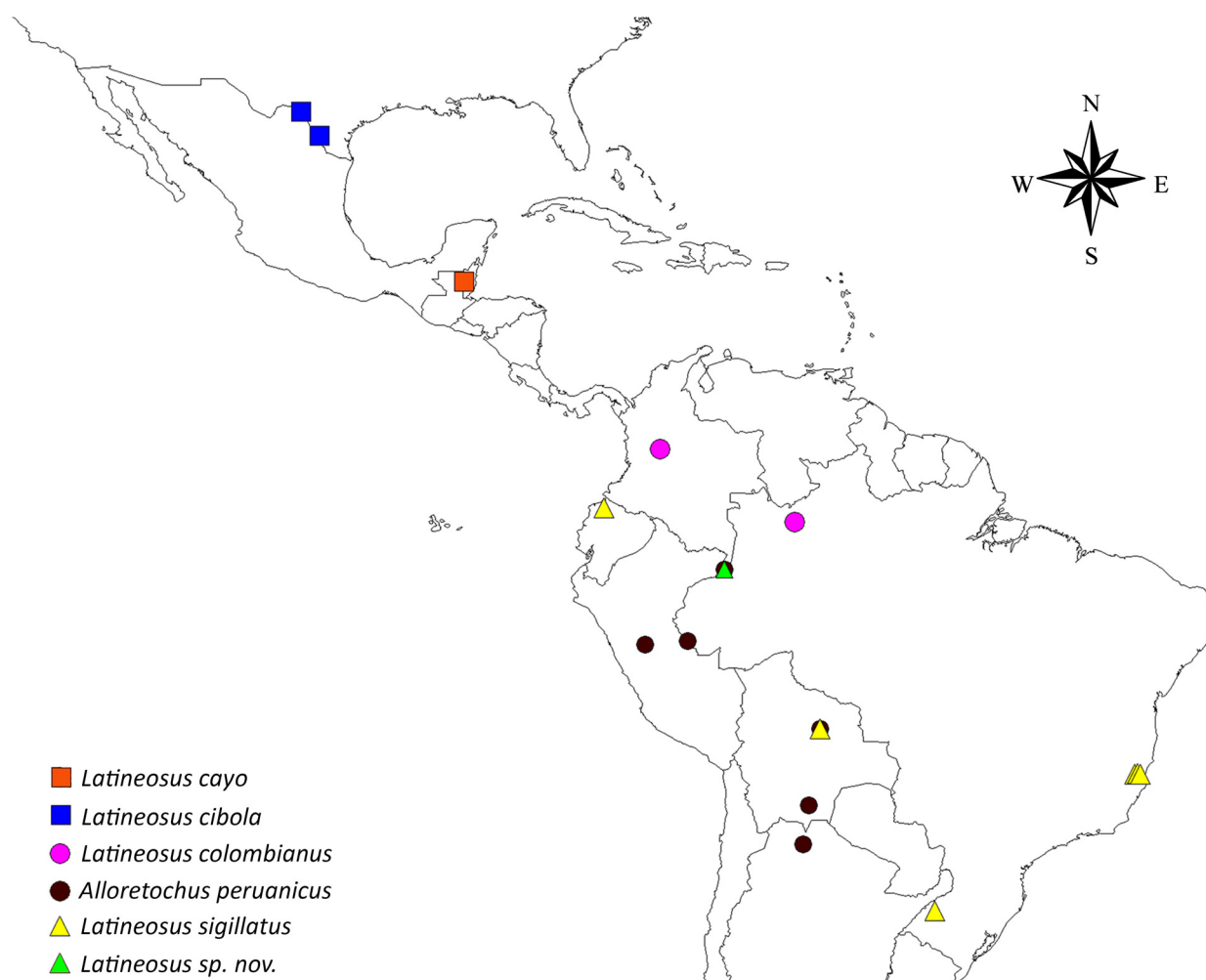


FIGURE 5. Distribution map of *Latineosus* and *Alloretochus* species.

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APPENDIX 1. Matrix of 35 taxa and 102 characters, ready to run in TNT. Modified from Sun & McCafferty (2008); see text for explanation.

[illegible]

cibola
 20110011100001?010000001?101001110101?10100001111110000010011101010010201311411000011111?102???1?????
 cayo
 20110011100001?0100000000101001110101?100100011111210000020011201010010201311411000000011?3?100???????
 choctaw
 201110111000100010000001?100100110101?0000000112112100000110012010100002110001110000000100200001111?00
 ojibwe
 10000011100010001000100001010011111001101100011211210010121111201111000202000211001010001?000000110010
 nitidus
 100000111000000010001001?1010011111001101100011211210010121111101111000202000311001010001?0000001?????
 needhami
 10000011121?0000?0002001?102001111011?011000011110110210010121001010000101000101000010001?000001?????
 nasutus
 1011101110002?20100010000100100110101?000000011211210000011011201010000221000111000000001?100001110001
 minutus
 20000011100001?010?102100112011111201?202200011112120221231111002010000202111411010010001?302???11?200
 miccosukee
 20111011100001?0111?0001?100100110101?000000011111210000010011301010000201000211000010002?20000111?101
 mahakam
 ??00001110000000100000000112001111101?10110001101111001003011110101012120131141100010011003?2?????????
 maculatus
 20111011100001?0111?0001?100100110101?001000011111210000010001201010000202000311000010011?100001110001
 lilliei
 20000011100001?010?1021?2112011111201?202200011113121223230211003010121201210411000000111?302???11?100
 lacustris
 201110111000100010000001?100100110101?000000011211210000011001301010000211000211000000001?200001110000
 japonica
 100000111000200010000001?100100110101?000000011211210000000011101010000211000111000010001?200001110000
 harrisella
 10000111110010001000100001010011111001201111011211210010111111001011000202000211001010001?000000110010
 fox
 20000011100001?010?102100112011111201?202200011112120221231111102010011201310411000000001?302???11?100
 ethowa
 20000011100001?011?102100112011111201?202200011112220222231111103010121201310411000000111?302???11?200
 cree
 20000011100001?000?1021?2112011111201?202200011113120223231111003010021201310411000000111?302???110000
 coushatta
 20111011100001?010000001?100100110101?001000011211110000010001301010000201000311000000001?200001110001
 capnicus
 10000011100001?010000001?111100110101?00000001111110000000001201010000201000211000010002?200001110000
 bishopi
 10000010100001?000000001?0??00110110000000000111110100000000012012000001020004100010100100010101?????
 berneri
 100000111000000011001001?1010011111001101100011211210110121011311111000202000211001010001?0000001????
 acutalis
 100001111100000010001001?0??0011011000001100011001110010000001201201000101000100001010001?0101010????
 ;
 proc/;