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Three new species of *Culex* (*Melanoconion*) (Diptera: Culicidae) from French Guiana based on morphological and molecular data

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Abstract

Culex mosquitoes of the subgenus *Melanoconion* Theobald, 1903 of the genus *Culex* Linnaeus, 1758 include numerous species recognized as vectors of viruses affecting humans. This subgenus is the most speciose among the entire mosquito fauna of the Americas. Despite decades of taxonomic research, many species remain undiscovered, especially in the Amazonian biome. In this article, we provide the description of three new species of *Culex* (*Melanoconion*) recently discovered in a biological reserve in French Guiana. *Culex* (*Mel.*) *sallumae* **n. sp.**, *Cx.* (*Mel.*) *hutchingsae* **n. sp.** and *Cx.* (*Mel.*) *lucakermanni* **n. sp.** are described based on both morphological features of the male genitalia and molecular barcodes obtained from type specimens. Diagnostic characters to assist their identification are provided and their placement within the infrasubgeneric classification of the subgenus *Melanoconion* is discussed.

Key words: barcoding, *Culex*, integrative taxonomy, *Melanoconion*, South America

Introduction

French Guiana is a small French territory (ca. 84,000 km^2) located on the Atlantic coast of South America at the easternmost part of the Guiana Shield, bordered to the west by Suriname and to the east and south by the Brazilian state of Amapá. This territory comprises the coastal floodplain composed of a mosaic of mangroves, marshes, savannas and forests, and the rest of the territory is covered mostly by upland (*terra firme*) forest (Stier *et al.* 2020). This Amazonian biome is recognized as one of the hot spots for mosquito diversity in terms of species density and endemism (Foley *et al.* 2007; Talaga *et al.* 2015). According to a recent review (Talaga *et al*. 2021), the mosquito fauna of French Guiana consists of 242 currently recognized mosquito species, among which 104 species belong to the genus *Culex* Linnaeus, 1758 and 69 species belong to the subgenus *Melanoconion* Theobald, 1903. This subgenus includes 174 currently recognized species, which makes it the most speciose among the entire mosquito fauna of the Americas (Harbach 2022). Most species are morphologically distinguishable based only on features of the male genitalia (e.g. Sallum & Forattini 1996). Owing to this high species richness, the subgenus was divided into nested infrasubgeneric groups that include Sections, Groups and Subgroups (Sirivanakarn 1983; Sallum & Forattini 1996; Harbach 2011). Most species are classified in a group or a subgroup, with the exception of three species for which the infrasubgeneric placement was not formally published, namely *Cx. anoplicitus* Forattini & Sallum, 1989, *Cx. guedesi* da Silva Mattos & Xavier, 1991 and *Cx. herrerai* Sutil Oramas, Pulido Florenzano & Amarista Meneses, 1987.

The subgenus *Melanoconion* includes numerous proven or suspected vector species of viruses affecting humans, especially among the Spissipes Section (Sallum & Forattini 1996; Torres-Gutierrez & Sallum 2015; Talaga *et al.* 2021). Vector competence being often species-specific, accurate delimitation and identification of species is critical. In recent years, DNA barcoding has proven to be a powerful tool for delineating and identifying mosquito species

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in tropical America (Torres-Gutierrez *et al.* 2016; Talaga *et al.* 2017). Nevertheless, descriptions of new mosquito species are still too rarely published with associated DNA barcodes. Yet, sequencing of type specimens remains the best way to accurately associate a barcode with a species name. Despite decades of taxonomic research, many species remain undiscovered, especially in the Amazonian biome. In this article, we provide the description of three new species of *Culex* (*Melanoconion*) from French Guiana, integrating both morphological features of the male genitalia and barcodes obtained from the type specimens.

Materials and methods

Mosquito collection and morphology

This study is based on specimens collected as a part of a mosquito inventory conducted inside the Réserve Naturelle Nationale de La Trinité in French Guiana. This biological reserve is located at approximately 100 km from the coast in a preserved area of ca. 770 km², mostly covered by primary rainforest (Fig. 1A). Adult mosquitoes were collected using Center for Diseases Control (CDC) and CDC-UV light traps placed at 1 m above ground and operated from 1800 to 0600 h. Immature stages were collected in various ground aquatic habitats (e.g. ground pools, streams and crab holes) and kept alive in water from their aquatic habitat until adult emergence. Aquatic habitats were described as precisely as possible and water temperature, pH and conductivity were measured using a Hanna HI991300 multimeter (HANNA Instruments, Woonsocket, RI, USA). Male genitalia were cleared in 10% NaOH for 2 h at 40°C, stained in a 1% solution of acid fuchsin for 5 min at room temperature, dissected in a solution of Marc André (Cohic & Rageau 1955) and mounted in Euparal. Dissected male genitalia were examined and photographed using an inverted EVOS FL-Auto microscope (Thermo Fisher Scientific Inc., Waltham, MA, USA). To increase the depth of field, pictures were Z-stacked using the Helicon Focus processing tool (Helicon Soft Ltd., Kharkiv, Ukraine) and edited in the Photoshop CS3 software (Adobe Inc., San José, CA, USA). Anatomical nomenclature follows Harbach & Knight (1980), as well as the current specific terminology used to describe the male genitalia of *Culex* mosquitoes belonging to the subgenus *Melanoconion* (e.g. Forattini & Sallum 1985, 1989, 1995; Sallum & Forattini 1996; Sallum *et al.* 1997; Sallum & Hutchings 2003; Hutchings & Sallum 2008). This article and the nomenclatural acts it contains have been registered in Zoobank (www.zoobank.org). The Life Science Identifier (LSID): urn: lsid:zoobank.org:pub:02CE72F9-3F0B-4D3A-81CE-B4A6C242123C. LSIDs are also provided in the taxonomic summary of each new species.

Molecular barcoding

DNA was extracted from two legs of each specimen using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA), and eluted in 50 µL. The standard 658 bp barcode of the mitochondrial cytochrome c oxidase subunit I gene (*COI*) was amplified using the primers LCO1490: 5′-GGTCAACAAATCATAAAGATATTGG-3′ and HCO2198: 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ (Folmer *et al.* 1994). The total PCR volume was 25 µL and consisted of 2.5 µL of 10X reaction buffer, 2 µL of 2.5 mM dNTPs, 2 µL of 25 mM $MgCl_2$, 0.5 µL of each 10 µM primer, 0.2 µL of 5U/L AmpliTaq Gold DNA polymerase, 15.3 µL of H_2O and 2 µL of template DNA. PCR cycles were as follows: 94°C for 2 min, 40 cycles at 94°C for 30 s, 49°C for 45 s and 72°C for 45 s, and then a final extension at 72°C for 1 min. The PCR products were verified on 2% agarose gel and were commercially sequenced on an ABI3730 by Genewiz (Leipzig, Germany). Forward and reverse sequences were assembled and edited using CodonCode Aligner (CodonCode Corp., Centerville, MA, USA). Consensus sequences were compared using a Maximum Likelihood (ML) phylogenetic analysis under the Kimura 2-parameter substitution model and default parameters in Mega X software (Kumar *et al.* 2018). Node support values were obtained using the Bootstrap method with 500 replications. A *COI* sequence of a specimen of *Aedeomyia squamipennis* (Lynch Arribálzaga, 1878) from French Guiana (specimen number MB2#00075) was used as outgroup (Fig. 1B). All sequences were uploaded to the Barcode of Life Data Systems (BOLD) (Ratnasingham & Hebert 2007) as part of the Mosquitoes of French Guiana (FGMOS) project, which gathers all the barcoding data available on the mosquitoes of French Guiana. BOLD accession numbers of specimens and Barcode Index Numbers (BINs) are provided in the taxonomic summary of each new species. Type specimens, as well as DNA vouchers, are stored in the S. Talaga mosquito collection, currently deposited at the Institut Pasteur de la Guyane, Cayenne (IPG).

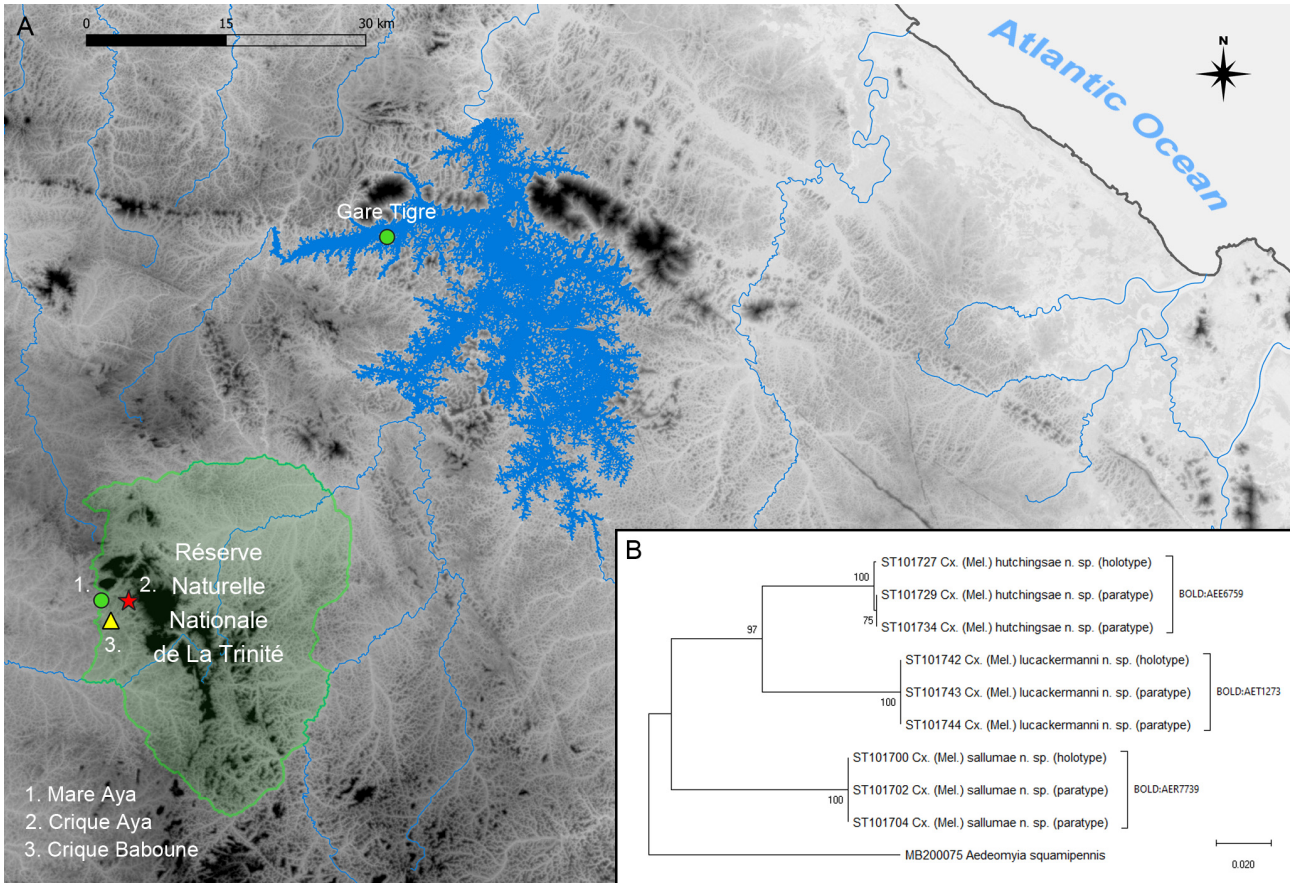

FIGURE 1. A, Map showing the distribution of *Culex* (*Melanoconion*) *sallumae* **n. sp.** (red star), *Cx.* (*Mel.*) *hutchingsae* **n. sp.** (green dots) and *Cx.* (*Mel.*) *lucackermanni* **n. sp.** (yellow triangle) in French Guiana; B, Maximum Likelihood (ML) tree of the *COI* barcodes obtained from the type specimens of the three new species. Bootstrap support values under 500 replications are indicated near nodes. The specimen number, species name and Barcode Index Number (BIN) provided by the Barcode of Life Data Systems (BOLD) are indicated for each barcode sequence.

Culex (*Melanoconion*) *sallumae* Talaga, n. sp.

Zoobank LSID: urn:lsid:zoobank.org:act:C727E9C3-C13F-40A1-9BF4-279DA8F104D3
BIN: BOLD:AER7739.

Male. Habitus not examined. *Genitalia* (Fig. 2A–G): Tergum VIII with a shallow emargination separating the lateral lobes. Tergum IX lobes small, somewhat triangular in outline, rounded apically, widely separated, bearing 18–21 setae arranged in 2 irregular rows. Gonocoxite obovoid; ventrolateral setae strongly developed; ventromesal surface with small setae scattered from base to level of subapical lobe, setae stronger basally; lateral surface with a well-developed patch of long setae (lsp) at level of subapical lobe; proximal part of ventrolateral surface without scales. Subapical lobe clearly divided into 2 divisions. Proximal division short, columnar, bearing 2 robust, sinuous, apically hooked setae (setae *a* and *b*); seta *a* slightly shorter and thinner than seta *b* and inserted slightly basal to seta *b*; a tight group of 5 enlarged and abruptly pointed setae inserted lateral to proximal division. Distal division long, columnar, with 7 apical setae, 1 long hooked seta (*h*), 1 short saber-like seta (*s*) arising close to seta *h*, 1 relatively long saber-like seta (*s*), 1 broad, petiolate, striate foliform seta (*l*), 3 narrow, appressed flattened setae (*f*) arising from distal side. Gonostylus slender, curved, wider at base, tapering to apex, with a patch of short spicules on dorsal surface extending from basal 0.33 to apical snout, subapical crest weakly distinct before apical snout on ventral side, apical snout forming a small, upturned ridge; gonostylar claw short, leaf-like; 2 small setae near dorsal side before gonostylar claw. Phallosome with lateral plates and aedeagal sclerites equivalent in length; aedeagal sclerite broad, curved in lateral view and broadly connected to base of lateral plate; distal part of lateral plate without median process, sternal and tergal processes present; apical sternal process long and hooked at apex; apical tergal process

very long, nearly pointed, directed dorsolaterally; apical margin markedly concave; base of lateral plate with a markedly distinct tergal process. Aedeagal sclerite not connected by dorsal aedeagal bridge. Proctiger elongate; paraproct narrowed distally, expanded basally, crown a row of about 5 or 6 simple blades. Cercal sclerite long and narrow with 1 or 2 cercal setae. Basal plate and paramere as figured. Tergum X shape as shown in Fig. 2C.

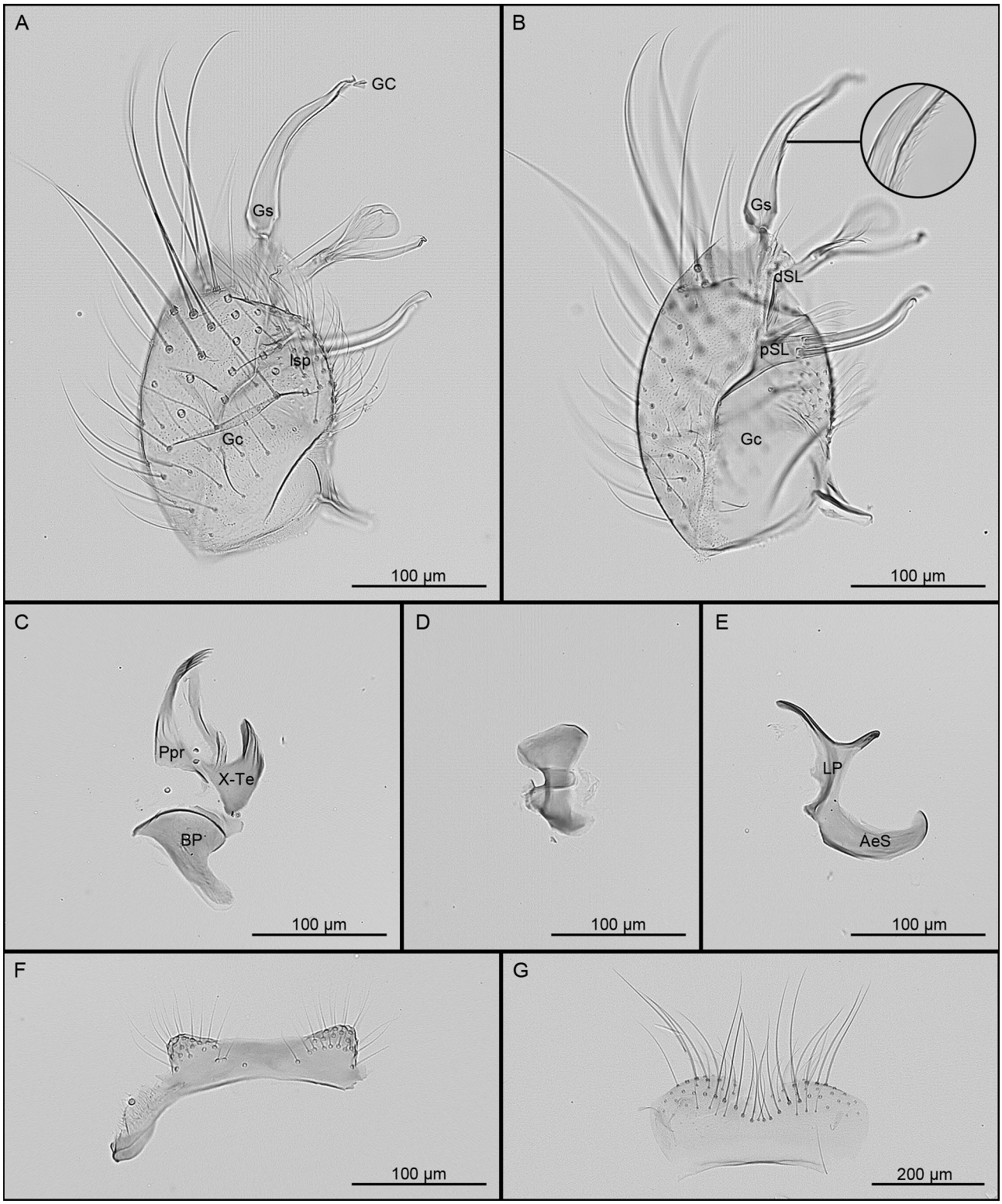

FIGURE 2. Male genitalia of *Culex* (*Melanoconion*) *sallumae* **n. sp.** A, Gonocoxopodite, lateral aspect; B, gonocoxopodite, mesal aspect; C, paraproct, tergum X and basal plate, in lateral views; D, paramere, lateral view; E, lateral plate and aedeagal sclerite, lateral views; F, tergum IX; G, tergum VIII. AeS, aedeagal sclerite; BP, basal plate; dSL, distal division of subapical lobe; Gc, gonocoxite; GC, gonostylar claw; Gs, gonostylus; LP, lateral plate; lsp, lateral setal patch; Ppr, paraproct; pSL, proximal division of subapical lobe; X-Te, tergum X.

Etymology. This species is dedicated to Professor Maria Anice Mureb Sallum for her incommensurable work on mosquito taxonomy in South America, and particularly on the Spissipes Section of *Culex* (*Melanoconion*) (e.g. Forattini & Sallum 1985; Forattini & Sallum 1995; Sallum & Forattini 1996; Sallum *et al.* 1997).

Bionomics. Nothing is known about the bionomics of *Cx. sallumae*. Adult males were collected using CDC and CDC UV-light traps placed at 1 m above ground and operated from 1800 to 0600 h along an oxbow section of a rainforest stream in deep shade.

Distribution. *Culex sallumae* is only known from the type locality (Fig. 1A).

Type material. *Holotype*: Adult male in 96% ethanol with dissected genitalia mounted on a microscope slide (specimen numbers ST1#01700, BOLD: FGMOS2885-22), FRENCH GUIANA: Montagnes de la Trinité, Crique Aya (53.41378° W, 4.60310° N, 115 m above sea level), 3-XI-2020, S. Talaga, IPG. *Paratypes*: Two adult males in alcohol with dissected genitalia mounted on separate microscope slides (specimen numbers ST1#01702, BOLD: FGMOS2887-22 and ST1#01704, BOLD: FGMOS2889-22), same collection data as the holotype, IPG.

Culex (*Melanoconion*) *hutchingsae* Talaga, n. sp.

Zoobank LSID: urn:lsid:zoobank.org:act:359A3B9D-819C-4BA0-86C7-AC3E46513ECC
BIN: BOLD:AEE6759.

Culex (*Melanoconion*) *coppenamensis* Form 2 (in part) of Sallum & Hutchings (2003) (illustration of gonostylus, species distribution).

Male. Habitus not examined. *Genitalia* (Fig. 3A–G): Tergum VIII with a shallow V-shaped emargination separating the 2 lateral lobes, with longer setae forming a lateral concentrated setal group. Tergum IX with 2 distinct lobes, shape as shown in Fig. 3F, bearing 33–35 setae, outer basal setae clearly longer than the others. Gonocoxite globose, outer margin convex, inner margin nearly straight; ventrolateral setae strongly developed; ventromesal surface with small, scattered setae from base to level of distal division of subapical lobe; lateral surface with a well-developed patch of long setae (lsp) at level of subapical lobe, setae longer ventrally; proximal part of ventrolateral surface with numerous scales. Subapical lobe clearly divided into 2 divisions. Proximal division moderately long, columnar, not clearly divided into 2 arms, proximal arm conspicuously shorter than distal arm, each arm bearing 1 long, robust, sinuous, apically hooked seta (setae *a* and *b*), both setae equivalent in length and width; a patch of short setae inserted mesally at base of distal surface. Distal division subdivided into inner and outer arms; inner arm with 2 apical setae, 1 long hooked seta (*h*) and 1 shorter, narrow, saber-like seta (*s*) inserted in a small tubercle at base of seta *h*, both *h* and *s* arise from separate tubercles at proximal side, 3 or 4 subapical setae, 1 long, wide, apically curved saber-like seta (*s*) and 3 indistinct, narrow, appressed flattened setae (*f*) inserted in small tubercles at base of seta *s*; outer arm long, nearly straight with 1 foliform seta (*l*) at apex, seta *l* strongly enlarged, almost symmetrical, striate at base with a well-developed basal expansion. Gonostylus short, strong, with a patch of long spicules at midlength on dorsal surface, distal 0.5 widened and abruptly tapering to apex in lateral view, bearing a conspicuous subapical crest on ventral side restricted to widest part; gonostylar claw long, leaf-like, 2 setae on dorsal side before gonostylar claw, distal seta slightly larger and longer than proximal seta. Phallosome with lateral plates and aedeagal sclerites equivalent in length; aedeagal sclerite narrow, curved in lateral view with anterior margin thickened and sclerotized, narrowly fused to base of lateral plate; distal part of lateral plate with median, sternal and tergal processes; apical median process conical with apex produced into a point tergad, tergal margin of apical process concave; apical sternal process short, somewhat hook-like, pointed, curved laterally; apical tergal process elongate, shorter than apical median process, pointed and directed dorsolaterally; base of lateral plate with short tergal process; aedeagal sclerite not connected by dorsal aedeagal bridge. Proctiger elongate; paraproct narrowed distally, expanded basally, crown a row of about 14 or 15 short simple blades. Cercal sclerite long and narrow with 2 or 3 cercal setae. Basal plate and paramere as figured. Tergum X somewhat rectangular in outline, rounded at apex.

Etymology. This species is dedicated to Rosa Sá Gomes Hutchings for her valuable work on the diversity of mosquitoes in the Brazilian Amazon (e.g. Hutchings *et al.* 2005, 2010, 2013, 2018, 2020), and particularly on species allied to *Culex coppenamensis* Bonne-Wepster & Bonne, 1920, published in Sallum & Hutchings (2003) and Hutchings & Sallum (2008).

Bionomics. Very little is known about the bionomics of *Cx. hutchingsae*. Immature stages were collected among roots and dead leaves at the edge of a large ground pool in rainforest (Fig. 5A). Water was highly brown-coloured

with dissolved plant substances, acidic (pH = 5.9), moderately warm (26.4°C) and with a conductivity of 390 µS/cm. Immature stages of *Cx. hutchingsae* were collected together with *Anopheles* (*Anopheles*) *punctimacula* Dyar & Knab, 1906a, *Cx.* (*Mel.*) *rabelloi* Forattini & Sallum, 1987 and *Cx.* (*Mel.*) *serratimarge* Root, 1927b.

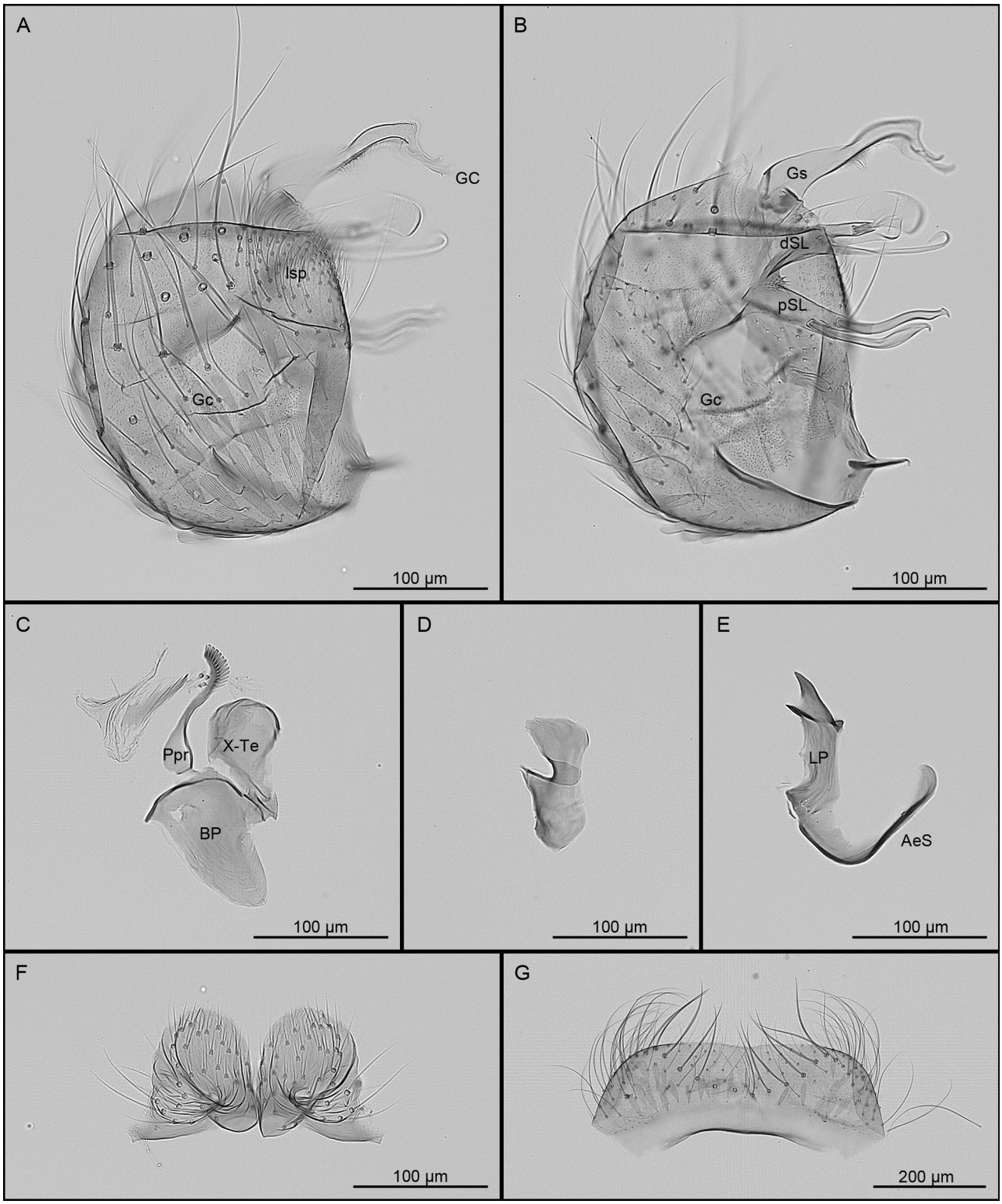

FIGURE 3. Male genitalia of *Culex* (*Melanoconion*) *hutchingsae* **n. sp.** A, Gonocoxopodite, lateral aspect; B, gonocoxopodite, mesal aspect; C, paraproct, tergum X and basal plate, lateral views; D, paramere, lateral view; E, lateral plate and aedeagal sclerite, lateral views; F, tergum IX; G, tergum VIII. AeS, aedeagal sclerite; BP, basal plate; dSL, distal division of subapical lobe; Gc, gonocoxite; GC, gonostylar claw; Gs, gonostylus; LP, lateral plate; lsp, lateral setal patch; Ppr, paraproct; pSL, proximal division of subapical lobe; X-Te, tergum X.

Distribution. *Culex hutchingsae* is known from the type locality and Gare Tigre, French Guiana (Fig. 1A). The latter is located at 45 km north-northeast from the type locality, but this area has been flooded since 1994 following the construction of the Petit Saut dam. Specimens of *Cx. hutchingsae* collected at Gare Tigre on 30 October 1945 were initially identified as *Cx. coppenamensis* by Floch (1946) and Floch & Abonnenc (1947). More recently, they were regarded as *Cx. coppenamensis* Form 2 by Sallum & Hutchings (2003) and as *Cx. coppenamensis* by Talaga *et al.* (2021).

Type material. *Holotype*: Adult male in 96% ethanol with dissected genitalia mounted on a microscope slide and associated pupal and larval exuviae in 70% ethanol (specimen numbers ST1#01727, BOLD: FGMOS2912-22), FRENCH GUIANA: Montagnes de la Trinité, Mare Aya (53.41445° W, 4.60289° N, 120 m above sea level), 5-XI-2020, S. Talaga, IPG. *Paratypes*: Two individualized adult males in 96% ethanol with dissected genitalia mounted on separate microscope slides and individualized associated pupal exuviae in 70% ethanol (specimen numbers ST1#01729, BOLD: FGMOS2914-22 and ST1#01734, BOLD: FGMOS2919-22), same collection data as the holotype, IPG.

Other material examined. One male genitalia mounted on a microscope slide (IPG1#00639) and associated pupal and larval exuviae mounted on a separate microscope slide (IPG1#00603), original specimen number N°817 (17A), FRENCH GUIANA: Gare Tigre, 30-X-45, E. Abonnenc, IPG.

Culex (*Melanoconion*) *lucackermanni* Talaga, n. sp.

Zoobank LSID: urn:lsid:zoobank.org:act:EBF54E64-DA7C-48CE-9719-5596450631CF
BIN: BOLD:AET1273.

Male. Habitus not examined. *Genitalia* (Fig. 4A–G): Tergum VIII not clearly separated into lateral lobes. Tergum IX with 2 distinct lobes, shape as shown in Fig. 4F, bearing 29–33 setae equally distributed in 2 groups of distinct sizes, outer setae clearly longer than the inner setae. Gonocoxite stocky, ovoid; ventrolateral setae strongly developed; ventromesal surface with small, scattered setae from base to level of distal division of subapical lobe, setae longer basally; lateral surface with a patch of very long setae (lsp) at level of subapical lobe; proximal part of ventrolateral surface with scales. Subapical lobe clearly divided into 2 divisions. Proximal division divided into 2 slightly divergent arms, proximal arm shorter than distal arm, both arms bearing 1 long, robust, sinuous, apically hooked seta (setae *a* and *b*), both setae equivalent in length and width; a patch of short setae inserted mesally at base of distal surface. Distal division subdivided into inner and outer arms; inner arm columnar with 4 apical setae, 3 long, apically expanded, appressed flattened setae (*f*), and 1 shorter, apically truncated, flattened seta (*f*); outer arm with 2 apical setae, 1 long hooked seta (*h*), and 1 short, curved, saber-like seta (*s*); 2 subapical setae, 1 broad, asymmetrical, striated foliform seta (*l*), and 1 long, narrow, saber-like seta (*s*) inserted at base of seta *l*. Gonostylus slender, curved, widened on distal 0.5 in lateral view, tapering to apex, bearing an inconspicuous subapical crest on ventral side, restricted to widest part; ventral surface with small laterally directed lapel-shaped fold, and a small hyaline fold apically with smooth border; gonostylar claw, short, leaf-like, 2 close setae before gonostylar claw, distal seta conspicuously larger than proximal seta. Phallosome with lateral plates longer than aedeagal sclerites; distal part of lateral plate with median and tergal processes, sternal process not developed; apical median process broad and curved on ventral side, apical margin irregular, ending in 1 or 2 points tergad; apical tergal process long, equivalent in length to apical median process, pointed and directed dorsolaterally; base of lateral plate with a markedly distinct tergal process. Proctiger elongate; paraproct narrowed distally, expanded basally, crown a row of about 8 or 9 short simple blades, apical blade conspicuously more sclerotized than basal blades. Cercal sclerite long and narrow with 1 cercal seta. Basal plate and paramere as figured. Tergum X somewhat rectangular in outline, rounded at apex.

Etymology. This species is dedicated to Luc Ackermann, the conservator of the Réserve Naturelle Nationale de La Trinité, for his continuous efforts in documenting the biodiversity of this preserved area of French Guiana.

Bionomics. Very little is known about the bionomics of *Cx. lucackermanni*. Immature stages were collected among roots and dead leaves at the edge of a rainforest stream (Fig. 5B). Water was clear, moderately warm (24.8°C), almost neutral (pH = 7.45), with a conductivity of 180 µS/cm. Immature stages of *Cx. lucackermanni* were collected together with *An.* (*Lophopodomyia*) *squamifemur* Antunes, 1937, *Chagasia bonneae* Root, 1927a and *Cx.* (*Mel.*) *corentynensis* Dyar, 1920.

Distribution. *Culex lucackermanni* is only know from the type locality (Fig. 1A).

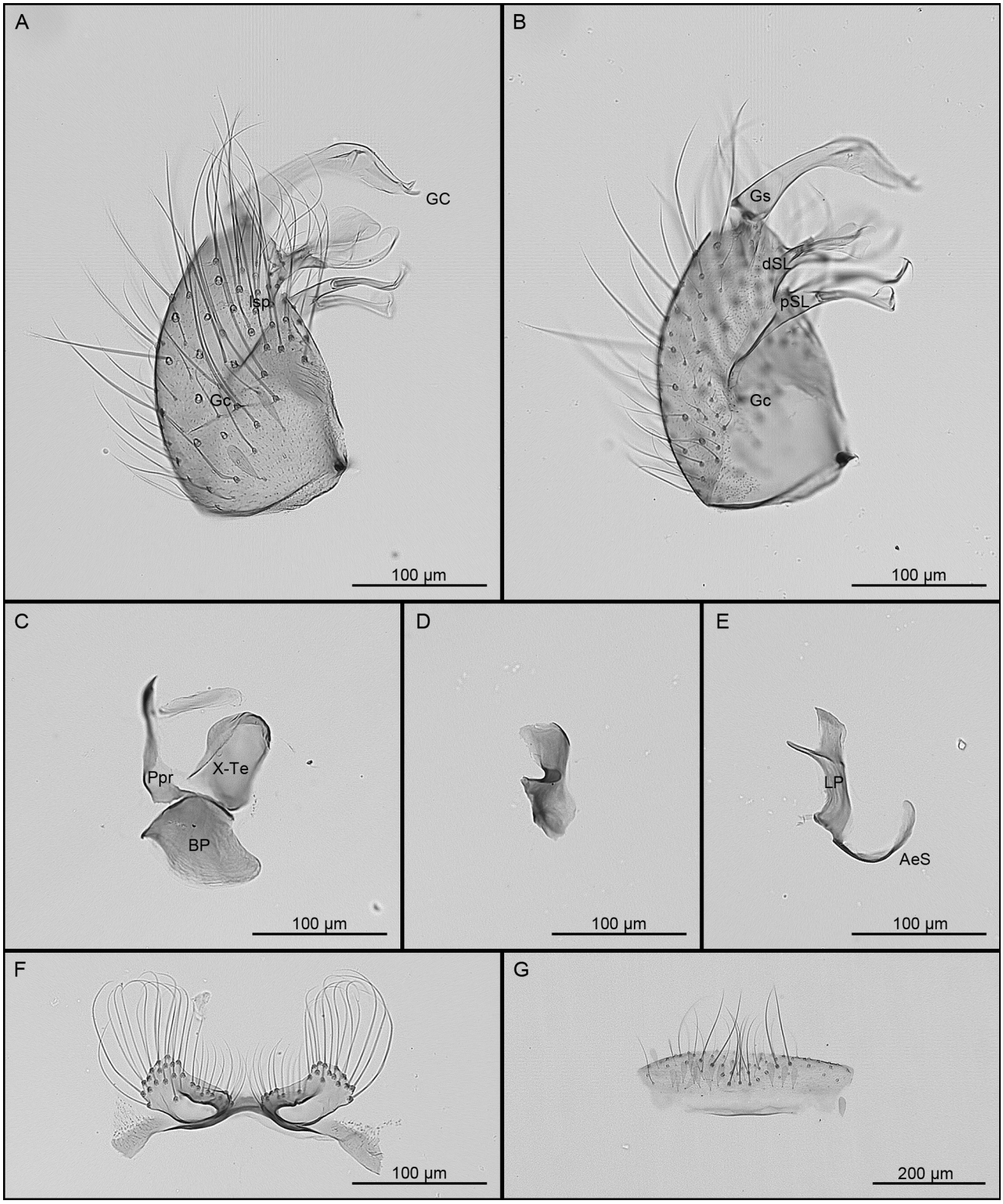

FIGURE 4. Male genitalia of *Culex* (*Melanoconion*) *lucackermanni* **n. sp.** A, Gonocoxopodite, lateral aspect; B, gonocoxopodite, mesal aspect; C, paraproct, tergum X and basal plate, lateral views; D, paramere, lateral view; E, lateral plate and aedeagal sclerite, lateral views; F, tergum IX; G, tergum VIII. AeS, aedeagal sclerite; BP, basal plate; dSL, distal division of subapical lobe; Gc, gonocoxite; GC, gonostylar claw; Gs, gonostylus; LP, lateral plate; lsp, lateral setal patch; Ppr, paraproct; pSL, proximal division of subapical lobe; X-Te, tergum X.

FIGURE 5. Aquatic habitats of (A) *Culex* (*Melanoconion*) *hutchingsae* **n. sp.** and (B) *Cx.* (*Mel.*) *lucackermanni* **n. sp.** in the Réserve Naturelle Nationale de La Trinité, French Guiana.

Type material. *Holotype*: Adult male in 96% ethanol with dissected genitalia mounted on a microscope slide and associated pupal exuviae in 70% ethanol (specimen numbers ST1#01742, BOLD: FGMOS2927-22), FRENCH GUIANA: Montagnes de la Trinité, Crique Baboune (53.40525° W, 4.58359° N, 90 m above sea level), 8-XI-2020, S. Talaga, IPG. *Paratypes*: Two adult males individualized in 96% ethanol with dissected genitalia mounted on separate microscope slides and individualized associated pupal exuviae in 70% ethanol (specimen numbers ST1#01743, BOLD: FGMOS2928-22 and ST1#01744, BOLD: FGMOS2929-22), same collection data as the holotype, IPG.

Discussion

The new species described in this article belong to the subgenus *Melanoconion* of *Culex*, the most diverse subgenus in tropical America. To assist their morphological identification, diagnostic characters are provided below and their placement within the infrasubgeneric classification of the subgenus is discussed. Because adults were stored in ethanol, most of their external features were damaged, therefore no specific effort was made to describe them in the present article.

Culex sallumae belongs to the Spissipes Section within the infrasubgeneric classification of the subgenus based on the broad aedeagal sclerite, curved in lateral view and broadly connected to the base of the lateral plate (Sirivanakarn 1983; Sallum & Forattini 1996). In the male genitalia, *Cx. sallumae* is morphologically more similar to *Cx. gnomatos* Sallum, Hutchings, Leila & Ferreira, 1997 than to any other species of *Melanoconion*. This species belongs to the Vomerifer Group, together with *Cx. portesi* Senevet & Abonnenc, 1941, *Cx. sacchettae* Sirivanakarn & Jakob, 1982 and *Cx. vomerifer* Komp, 1932 according to Harbach (2011). *Culex sallumae* shares with them all of the distinguishing features of the Vomerifer Group (Sallum & Forattini 1996; Sallum *et al.* 1997). This includes 1) the absence of scales on the proximal part of the ventrolateral surface of the gonocoxite, 2) the lateral plate of the phallosome without an apical median process, apical sternal and tergal processes present; the apical sternal process long and hooked at apex; the apical tergal process very long, nearly pointed, dorsolaterally directed; 3) the distal division of the subapical lobe of the gonocoxite columnar, bearing a broad foliform seta (*l*), and 4) the lobes of tergum IX small, apically rounded and widely separated. Nevertheless, C*x. sallumae* can be easily separated from all species of the Vomerifer Group by 1) the presence of a tight group of five enlarged and abruptly pointed setae inserted lateral to the proximal division of the subapical lobe, 2) the proximal and distal divisions of the subapical lobe widely separated, 3) the presence of a patch of short spicules on the dorsal surface of the gonostylus extending from the basal third to the apical snout and 4) the distinctive shape of each lobe of tergum IX bearing 18–21 setae arranged in two irregular rows. In addition, *Cx. sallumae* differs of *Cx. portesi*, *Cx. sacchettae* and *Cx. vomerifer* in not having a hyaline expansion near the middle of the ventral side of the gonostylus. Based on these observations, *Cx. sallumae* is tentatively placed in the Vomerifer Group of the subgenus.

Culex hutchingsae belongs to the Melanoconion Section within the infrasubgeneric classification of the subgenus based on the narrow aedeagal sclerite, curved in lateral view and narrowly connected to the base of the lateral plate (Sirivanakarn 1983; Sallum & Forattini 1996). In the male genitalia, *Cx. hutchingsae* is more similar to *Cx. alinkios* Sallum & Hutchings, 2003, *Cx. brachiatus* Hutchings & Sallum, 2008, *Cx. coppenamensis*, and *Cx. phyllados* Hutchings & Sallum, 2008 than to any other described species of *Melanoconion*. All of these species are included in the Bastagarius Subgroup of the Bastagarius Group (Sallum & Hutchings 2003; Hutchings & Sallum 2008; Harbach 2011). *Culex hutchingsae* shares with those species a unique combination of morphological characters, which include 1) the gonocoxite globose with a patch of long setae (lsp) on the lateral surface at the level of the subapical lobe, 2) the proximal division of the subapical lobe of the gonocoxite columnar, not clearly divided into two arms, 3) the distal division of the subapical lobe of the gonocoxite subdivided into inner and outer arms and 4) the outer arm of the distal division with a foliform seta (*l*) inserted at the apex of a tubercle that arises separately on the outer side of the division. Based on these observations, *Cx. hutchingsae* is placed in the Bastagarius Subgroup of the Bastagarius Group of the subgenus. Nevertheless, *Cx. hutchingsae* is easily separated from all of these species by 1) the conical, apical median process of the lateral plate of the phallosome (similar to species of the Intrincatus Group) and 2) the widened part of the gonostylus abruptly tapering to the apex in lateral view. In addition, *Cx. hutchingsae* is distinguished from *Cx. alinkios* in having the outer arm of the distal division nearly straight, and tergum VIII with a shallow V-shaped emargination separating two lateral lobes; from *Cx. coppenamensis sensu*

Hutchings & Sallum (2008) in having the inner arm of the distal division bearing one long, broad, apically curved saber-like seta (*s*) and three indistinct, narrow, appressed flattened setae (*f*) inserted in small tubercles at the base of seta *s*; from *Cx. brachiatus* in having a patch of long spicules at midlength on the dorsal surface of the gonostylus, and tergum VIII with scattered setae forming a lateral concentrated setal group; and from *Cx. phyllados* in having the tergomesal surface without foliform setae at the level of the subapical lobe, and the outer arm of the distal division with an almost symmetrical foliform seta (*l*) inserted apically.

Culex lucackermanni belongs to the Conspirator Group of the Melanoconion Section of the subgenus based on the narrow aedeagal sclerite and the lateral plate of the phallosome with a sternal process not developed (Sirivanakarn 1983; Sallum & Forattini 1996). The Conspirator Group includes 10 valid species: *Cx. aliciae* Duret, 1953, *Cx. conspirator* Dyar & Knab, 1906b, *Cx. dyius* Root, 1927b, *Cx. elevator* Dyar & Knab, 1906b, *Cx. jocasta* Komp & Rozeboom, 1951, *Cx. lucifugus* Komp, 1936, *Cx. madininensis* Senevet, 1936, *Cx. martinezi* Casal & García, 1968, *Cx. olimpioi* Xavier, da Silva & da Silva Mattos, 1970 and *Cx. terebor* Dyar, 1920 according to Harbach (2011). In the male genitalia, *Cx. lucackermanni* is easily separated from those species by the distinctive shape of the lobe of tergum IX and the arrangement of setae in two groups of different sizes. In addition, *Cx. lucackermanni* is distinguished from *Cx. aliciae*, *Cx. dyius*, *Cx. martinezi*, *Cx. olimpioi* and *Cx. terebor* in having a broad foliform seta (*l*) inserted on the distal subapical lobe of the gonocoxite; and from *Cx. conspirator*, *Cx. elevator*, *Cx. jocasta*, *Cx. lucifugus* and *Cx. madininensis* by the distinctive shape of the apical median and tergal processes of the lateral plate of the phallosome. Nevertheless, the male genitalia of *Cx. lucackermanni* also bears striking similarities with the genitalia of *Cx. anoplicitus*. *Culex lucackermanni* only differs from *Cx. anoplicitus* in having 1) the long seta (*s*) of the distal subapical lobe of the gonocoxite inserted at the base of seta (*l*), 2) the apical tergal process of the lateral plate of the phallosome without serration, 3) the hyaline apical fold of the gonostylus with a smooth margin and 4) the lobe of tergum IX bearing 29–33 setae. Originally, *Cx. anoplicitus* was not formally placed in any of the informal groups of the Melanoconion Section (Forattini & Sallum 1989). To be consistent with the internal classification of *Melanoconion*, both *Cx. anoplicitus* and *Cx. lucackermanni* are here placed in the Conspirator Group of the Melanoconion Section. The immature stages of *Cx. lucackermanni* were collected together with the immature stages of three other mosquito species, including *Cx. corentynensis* Dyar, 1920. This species was originally described from the neighbouring Suriname and constitutes a new country record for French Guiana (see Talaga *et al.* 2021).

Barcode sequences obtained from the type specimens of each new species clustered together in the ML phylogenetic analysis and within three distinct new BINs (Fig. 1B). This indicates that these species are molecularly distinct from each other and from all the other species for which *COI* barcodes are currently available. Beyond the description of the three new species, this article represents a step towards an integrative approach to the alpha taxonomy of mosquitoes. In the future, we strongly recommend that taxonomists should provide barcode sequences obtained from type specimens when describing new mosquito species.

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