A DNA barcode library for *Culex* mosquitoes (Diptera: Culicidae) of South America with the description of two cryptic species of subgenus *Melanoconion*

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[$] *In memoriam*

Abstract

The genus *Culex* is one of the most diverse in the world and includes numerous known vector species of parasites and viruses to humans. Morphological identification of *Culex* species is notoriously difficult and rely mostly on the examination of properly dissected male genitalia which largely prevents female and immature identification during entomological, ecological or arboviral surveys. The aims of this study were (i) to establish a DNA barcode library for *Culex* mosquitoes of French Guiana based on the mitochondrial gene cytochrome c oxidase I (COI) marker, (ii) to compare three approaches of molecular delimitation of species to morphological identification, and (iii) to test the effectiveness of the COI marker at a broader geographical scale across South America. Mosquitoes used in this study were sampled in French Guiana between 2013 and 2023. We provide 246 COI sequences for 90 morphologically identified species of *Culex*, including five new country records and two newly described species. Overall, congruence between morphological identification and molecular delimitations using the COI barcode were high. The Barcode of Life Data clustering approach into Barcode Index Numbers gives the best result in terms of species delimitation, followed by the muti-rate Poisson Tree Processes and the Assemble Species by Automatic Partitioning methods. Inconsistencies between morphological identification and molecular delimitation can be explained by introgression, incomplete lineage sorting, imperfect taxonomy or the effect of the geographical scale of sampling. This increases by almost two-fold the number of mosquito species for which a DNA barcode is available in French Guiana, including 75% of the species of *Culex* currently known in the territory. Finally, this study confirms the usefulness of the COI barcode in identifying *Culex* mosquitoes of South America, but also points the limits of this marker for some groups of species within the subgenera *Culex* and *Melanoconion*.

Introduction

The genus *Culex* is one of the most diverse in the world and includes numerous known vector species of parasites and viruses to humans. *Culex* mosquitoes are also of particular concern in view of the threat of emerging diseases in relation to global warming and environmental change [1]. In tropical America, this genus surpasses any other one in term of diversity and several arboviral studies pointed out its importance as vector of viruses. For example, a ten-years arboviral survey in Trinidad resulted in the isolation in *Culex* mosquitoes of 320 out of the 473 (68%) virus isolates [2]. In the Peruvian Amazon, species of *Culex* accounted for 57% of the mosquitoes collected, but 87% of the virus isolations were made from this genus [3]. In northeastern Amazonia, no fewer than fifteen arboviruses have been detected among *Culex* species of French Guiana [4]. To date, this oversea territory of France account for 113 nominal species of *Culex* classified among eight subgenera, representing more than 45% of the total number of recorded mosquito species [5; 6; 7; 8]. The most speciose subgenera of *Culex* were divided into informal infrasubgeneric groups that variously include Sections, Groups, Subgroups, Series and Complexes [1]. Morphological identification of *Culex* species is notoriously difficult and rely mostly on the examination of properly dissected male genitalia which largely prevents female and immature identification during entomological, ecological or arboviral surveys [9; 10; 11; 12].

Twenty years ago, Hebert et al. [13] established that the mitochondrial gene cytochrome c oxidase I (COI) can serve as the core of a global bioidentification system for animals. Since then, this taxon “barcode” was widely used successfully in diverse taxonomic groups (reviewed in [14]), including mosquitoes [15]. In South America, studies that have documented COI sequences for *Culex* mosquitoes are relatively scarce and gave mixed results as regard to delimitation and identification of species. One of the first study providing COI barcodes for South American *Culex* was part of an inventory of the mosquitoes of the Yasuni National Park of eastern Amazonian Ecuador [16]. Only five *Culex* species were included in this study but all of them were successfully delineated by this marker. In a series of articles dealing with the taxonomy of *Culex* (*Culex*) in South America, Laurito et al. [17; 18; 19] provided and analyzed COI sequences for 24 nominal species collected in Argentina and Brazil. Among this subgenus, the COI barcode barely identified 70% of the included species. In Colombia, sequences of 15 species/morphospecies of *Culex* from three ecosystems of the Andes permitted to delimit most taxa, except in the subgenus *Culex* [20]. Another important contribution was made available by Torres-Gutierrez et al. [21], which provided 120 COI sequences for 48 species/morphospecies of *Culex* (*Melanoconion*) from Brazil. These authors obtained coherent delimitation except in few cases where morphological species/morphospecies were split in more than one molecular unit [21]. Unfortunately, their dataset contains approximately 40% of female specimens for which the identification is inherently questionable. Recently, the barcode library for mosquitoes of Argentina was updated with additional species, including *Cx. amazonensis* (Lutz) and four species of *Culex* (*Melanoconion*) from the north-central part of the country [22]. All of them were correctly delimited by their DNA barcode and species of subgenus *Melanoconion* also clustered with specimens from Brazil.

A few years ago, we initiated a molecular database for barcoding and metabarcoding of mosquito species in French Guiana [23]. Overall, this study confirmed the effectiveness of both the COI and 16S markers in delimiting and identifying Guianese mosquitoes. Nevertheless, the genus *Culex* was largely overlooked as only 12% of the *Culex* species known in the territory were included. The aims of the present study were (i) to improve the taxonomic coverage of *Culex* in the barcode library of mosquitoes of French Guiana,

(ii) to compare three approaches of molecular delimitation of species to morphological identification, and (iii) to test the effectiveness of the COI marker at a broader geographical scale across South America.

Material and Methods

Sampling and a priori identification

Culex mosquitoes were sampled using a wide range of methods and traps between 2013 and 2023 in French Guiana. A great diversity of natural habitats was sampled from the coastal plain to the upland terra firme forest, including inhabited and variously anthropized areas. Immature individuals were individually reared in the laboratory until emergence and associated larval and pupal exuviae were conserved in 70% alcohol whenever possible. In most cases, morphological identification has been done by microscopic observation of properly prepared, dissected, and mounted genitalia of males. After separating the last abdominal segments from the rest of the body, male genitalia were cleared in a 10% KOH solution for 2 hours at 40 °C, then stained in a 1% acid fuchsin solution for 5 minutes at room temperature, and finally dissected in a solution of Marc André [24]. Once mounted in Euparal between slide and coverslip, male genitalia were examined using an EVOS FL-Auto inverted microscope (Thermo Fisher Scientific Inc., Waltham, MA, USA). Identification was made using an extensive amount of taxonomic literature, including original description of species and taxonomic revisions like Bram [9], Duret [25], Valencia [10], Berlin and Belkin [11], Sirivanakarn [12], Sallum and Forattini [26], Pecor et al. [27], Sá et al. [28], and Sá et al. [29]. Specimens were selected to increase as much as possible the taxonomic and geographic coverage of the dataset.

DNA extraction and sequencing

Total DNA of selected specimens was extracted from three legs or the abdomen (except male genitalia) of each adult, or the head of larval specimens. PCR amplification was performed using LCO1490 and HCO2198 primers [30], which are the standard for amplifying the 658-bp barcode region at the 5′ end of the COI gene [13]. The detailed protocol of amplification and sequencing that we used can be found in previous works [7; 23]. This article and its nomenclatural acts were registered in Zoobank (https://www.zoobank.org/). The life science identifier (LSID) of the article is: urn:lsid:zoobank.org:pub:C5D9E279-8B6E-45F3-86A1-8D6B78394715. Voucher specimens and DNA templates (including holotypes and paratypes of new species) are stored at the Institut Pasteur de la Guyane (IPG).

Forty-two specimens of *Culex* belonging to 14 morphologically identified species/morphospecies were already included in Talaga et al. [23]. These specimens were re-examined with regard to the knowledge acquired during a recent review of the *Culex* mosquitoes of French Guiana [6]. As a result, specimens MB1#0038, 0039, MB1#0225–0227, and MB1#0810, 0811 turned out to be misidentifications of *Cx. urichii* (Coquillett), *Cx. nigripalpus* Theobald and *Cx. originator* Gordon & Evans, instead of *Cx. infoliatus* Bonne-Wepster & Bonne, *Cx. mollis* Dyar & Knab, and *Cx. imitator* Theobald, respectively. In addition, morphospecies named *Culex* sp.stI, sp.stJ, sp.stK and sp.stL have been respectively identified as the following nominal species: *Culex secundus* Bonne-Wepster & Bonne, *Cx. comminutor* Dyar, *Cx. imitator* and *Cx. putumayensis* Matheson. The taxonomic identification of these voucher specimens has been modified accordingly in BOLD and GenBank databases. Finally, five specimens initially identified as *Cx. imitator* (ST1#0310, 0311) and *Cx. stonei* Lane & Whitman (MB1#0173, 0241, 0242) were not included here because their identification could not be ascertained by any male genitalia.

Molecular delimitation of species

Additional contig sequences were built with CodonCode before to be uploaded to the Barcode of Life Data Systems (BOLD) [31] as part of the 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 throughout the manuscript. A Maximum Likelihood (ML) phylogenetic analysis of the COI marker using the Kimura's two-parameter model with defaults settings was conducted in Mega X [32]. A sequence of a specimen of *Chagasia bonneae* Root (WRBUE110-10) was used as outgroup, and nodal support was assessed using a bootstrap procedure under 1,000 replications. Afterward, morphological identification of species was compared with molecular delimitation using the BOLD BINs method and two standalone methods: the Assemble Species by Automatic Partitioning (ASAP) distance-based method [33] and the multi-rate Poison Tree Processes (mPTP) tree-based method [34]. ASAP divides species partitions based on pairwise genetic distances. ASAP also computes a probability of panmixia (p-val), a relative gap width metric (W), and ranked results by the ASAP score: the lower the score, the better the partitioning [33]. The PTP method is a phylogeny-aware approach that take the evolutionary relationships of the sequences into account. The multi-rate PTP (mPTP) model incorporate the potential divergence in intraspecific diversity and thereby it can better accommodate the sampling- and population-specific characteristics of a broader range of empirical datasets [34].

Finally, our dataset was compared to the others COI sequences of *Culex* available in South America using the BIN delimitation in BOLD. Sequences from Argentina (Laurito et al. [17; 22], Brazil [17; 21], Colombia [20] and Ecuador [16] were included from BOLD.

Results

Species identification and delimitation

A total of 246 specimens of *Culex* mosquitoes belonging to 8 subgenera and 90 morphologically identified species from 44 sampling sites were included in the analyses (Figure 1, S1 Table, Supporting information). Readers interested in the authorship of the species sequenced in this study should consult S1 Table. Selected specimens were mostly represented by males with dissected genitalia (78%), followed by larvae (14%) and females (8%). Among them, 80 species were represented by two or more specimens (up to five), but ten species were only represented by one specimen. The dataset included five new country records and two newly described species. The new country records included, *Cx. bibulus* (collected in the Amerindian villages of Camopi and Twenké, and Savanes de Passoura), *Cx. galindoi* (collected along a small river inside the Réserve Naturelle Nationale de La Trinité), *Cx. johnnyi* (collected along the Crique Gabaret, a medium-sized river tributary of the Oyapock), *Cx. longistriatus* (collected along a large river under tidal influence at Roura) and *Cx. ocossa* (collected close by a coastal marsh at Pointe Macouria).

Fig 1. Map showing the distribution of sampling localities of the *Culex* (Diptera: Culicidae) specimens from which the COI barcode was sequenced in this study. French Guiana is coarsely divided into the coastal plain composed of a mosaic of mangroves, marshes, swamps, savannas and forests (dark gray; below 30 m a.s.l.) and upland terra firme forest (light gray; above 30 m a.s.l.). The main rivers are indicated.

The BOLD clustering approach allowed to distinguish 87 BINs out of the 90 morphologically identified species (Figure 2A–C, S2 Table, Supporting information). Among them, 44 BINs were new to BOLD, the 43 remaining BINs included sequences already present in BOLD, including five species of *Culex* recently described from French

Guiana [7; 8]. The results of the clustering approach into BINs were largely congruent with the morphological identification. However, 17% of the species were grouped into BINs with mixed nominal species, preventing their accurate identification. In details, we found five cases where two or more nominal species (up to four) were clustered into a single BIN; namely: BIN ADK0770 clustered sequences of *Cx. batesi* (N=1) and *Cx. evansae* (N=2), BIN AEE2103 clustered sequences of *Cx. contei* (N=3), *Cx. phlogistus* (N=3), and *Cx. serratimarge* (N=3), BIN AAN3636 clustered sequences of *Cx. brevispinosus* (N=2), *Cx. surinamensis* (N=2), and *Cx. usquatus* (N=7), BIN AAF1735 clustered sequences of *Cx. declarator* (N=3), *Cx. mollis* (N=2), and *Cx. nigripalpus* (N=5), and BIN AEE6759 clustered sequences of *Cx. creole* (N=4), *Cx. eastor* (N=4), *Cx. hutchingsae* (N=3), and *Cx. idottus* (N=3). Moreover, in seven cases, nominal species were split into two BINs; namely: *Culex bastagarius* (BINs AEE3102 and AFI9514), *Cx. foliafer* (BINs AEE1181 and AEE1182), *Cx. inadmirabilis* (BINs AFJ0561 and AFJ0562), *Cx. bibulus* (BINs AEE1543 and AFI9809), *Cx. organaboensis* (BINs AFJ0416 and AFT5569), *Cx. rabelloi* (BINs ADE6009 and AEW5154), and *Cx. theobaldi* (BINs ADK5539 and AEE9553).

Fig 2. Maximum Likelihood (ML) phylogenetic analysis of the COI dataset of *Culex* mosquitoes (Diptera: Culicidae) from French Guiana (A–C). For each specimen, we indicated the BOLD specimen code, the morphological identification, the original specimen code, the sampling locality, and the BOLD Barcode Index Number (BIN). Inconsistencies between morphological identification and molecular delimitation using the BOLD BIN, the Assemble Species by Automatic Partitioning (ASAP), and the multi-rate Poison Tree Processes (mPTP) methods are figured in column on the right side. *Culex* specimens are color coded by subgenus as follows: *Aedinus* in orange, *Anoedioporpa* in light green, *Carrollia* in purple, *Culex* in dark red, *Melanoconion* in blue, *Microculex* in green, *Phenacomyia* in red, and *Tinolestes* in grey. *Culex* formerly placed in the Ocellatus Section, presently without subgeneric placement, are colored in light blue. Numbers indicate split specimens belonging to the same species. Bootstrap support values above 50% are indicated near the nodes and a sequence of *Chagasia bonneae* Root was used as outgroup.

The ASAP method was mostly congruent with the results of the BOLD delimitation into BINs. However, the best model retrieved only 76 partitions among the COI dataset with 28% of the species included into partitions with mixed nominal species. Compared to the BIN delimitation, we find four more cases where two or more nominal species (up to four) were grouped in the same partition; namely: *Culex abonnenci* (N=3) and *Cx. rabelloi* (N=3) were grouped in the same subset, *Cx. alinkios* (N=3), *Cx. brachiatus* (N=1), *Cx. rabanicolus* (N=3) and *Cx. ybarmis* (N=3) were grouped in the same subset, *Cx. comatus* (N=3) and *Cx. tournieri* (N=3) were grouped in the same subset, and *Cx. innovator* (N=3) and *Cx. pilosus* (N=3) were grouped in the same subset. On the other hand, this method retrieved *Cx. bibulus*, *Cx. inadmirabilis*, *Cx. organaboensis*, and *Cx. theobaldi* in molecular subsets concordant with their morphological identification, meaning one partition instead of several BINs per species.

The mPTP method was highly congruent with the results of the BOLD delimitation into BINs and retrieved the same number of molecularly delimited species (87). However, 19% of the species were grouped into subsets with mixed nominal species. Compared to the BIN delimitation, we find one more case where two species were grouped in the same subset; namely: *Culex abonnenci* (N=3) and *Cx. rabelloi* (N=3) were grouped in the same molecular unit, as with the ASAP method. In three other cases BINs were split into two or three molecular units; namely: *Culex corentynensis* (BIN AFI9596) was split into two molecular units, *Cx. inadmirabilis* (BINs AFJ0561 and AFJ0562) was split into three molecular units, and *Cx. innovator* (BIN ABZ4907) was split into two molecular units. On the other hand, specimens of *Cx. theobaldi* (BINs AEE9553 and ADK5539) were grouped in the same molecular unit.

Species description

Two cryptic species turned out to be new after COI sequencing and in-depth investigations, including examination of primary type specimens of morphologically close species. In order to help future works, they are formally described and named below.

***Culex* (*Melanoconion*) *carincii* Talaga & Duchemin, sp. nov.**

LSID: urn:lsid:zoobank.org:act:BEA55CAB-4766-408A-9F7B-788CD6768B99

BIN: AAE7955

Male. Habitus briefly examined. Hindtarsomeres 1–5 completely dark. *Genitalia* (Figures 3A–G): Tergum VIII with a deep V-shaped emargination separating the 2 lateral lobes. Tergum IX with 2 distinct lobes, conical to mound-like in shape, widely separated, bearing 6–8 setae. Gonocoxite conical; inner margin moderately concave; ventrolateral setae strongly developed; lateral surface with 14–19 small, scattered setae (lsp) at level of subapical lobe; proximal part of ventrolateral surface with scales. Subapical lobe clearly divided into 2 divisions. Proximal division of subapical lobe with an apical infundibular and hyaline expansion, 2 robust, sinuous, apically hooked setae (setae *a* and *b*) at apex, a subapical hyaline, broad, hooked-falciform seta and 4–6 strong, pointed setae from base to level of insertion of the hooked-falciform seta; a patch of 9–12 short setae inserted mesally at base of distal surface. Distal division of subapical lobe divided into 2 divergent arms, the proximal arm more robust than the distal arm bearing 3 apical setae, 1 long hooked seta (*h*), 1 short saber-like seta (*s*), and 1 long foliform seta (*l*) inserted clearly proximal to setae *h* and *s*; distal arm bearing 5 setae gradually inserted from apex to base, 1 long, curved, saber-like seta (*s*), and 4 appressed flattened setae (*f*), the most basal conspicuously shorter. Gonostylus slender, curved and slightly constricted at midlength, subapical portion barely enlarged on lateral view; 1 long seta inserted at proximal 0.25 on 1 out of the 4 gonostylus of the type series; ventral surface with conspicuous crest extending from apical snout to enlarged subapical portion; apical snout tapered to a truncate apex; gonostylar claw short, leaflike, highly broadened apically; ventral margin with 2 setae before gonostylar claw, distal seta conspicuously longer than proximal seta. 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 short, laterally curved, pointed at apex; apical tergal process longer than apical sternal process, pointed and directed dorsolaterally. Proctiger elongate; paraproct narrowed distally, expanded basally, crown a row of about 7, 8 short simple blades. Cercal sclerite long and narrow with 2 cercal setae. Basal plate, paramere and tergum X as figured.

Fig 3. Male genitalia of *Culex* (*Melanoconion*) *carincii* sp. nov. 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. Morphological structures are abbreviated as follow: 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 our dear colleague Romuald Carinci for his unflagging enthusiasm working on mosquitoes at the Institut Pasteur de la Guyane.

Bionomics. Nothing is known about the bionomics of *Cx. carincii*. Adult males were collected using CDC light traps (supplemented with black light) placed at 1.5 m above ground and operated from 1700 to 0700 h in a lowland forest patch surrounded by swamps and marshes on the coastal plain of French Guiana.

Distribution. *Culex carincii* is only know from the type locality.

Type material. *Holotype*: Adult male in 96% ethanol with dissected genitalia mounted on a microscope slide (specimen number MB2#0290, BOLD: FGMOS2759-20, GenBank: [waiting for accession creation]), FRENCH GUIANA: Guatemala (52.626420° W, 5.136090° N, 3 m above sea level), 22-VI-2017, S. Talaga and R. Carinci, IPG. *Paratypes*: Two adult males in 96% ethanol with dissected genitalia mounted on separate microscope slides (specimen numbers MB2#0296, BOLD: FGMOS2765-20, GenBank: [waiting for accession creation], MB2#0291, BOLD: FGMOS2760-20), same collection data as the holotype, IPG.

Other material examined. Holotype male genitalia of *Cx. crybda* Dyar (specimen number USNMENT01935104), USNM.

Culex (*Melanoconion*) *extenuatus* Talaga & Duchemin, sp. nov.

LSID: urn:lsid:zoobank.org:act:D0E621BA-3C9A-4928-8965-4C0A63A8C42D

BIN: ADK4497

Male. Habitus briefly examined. Hindtarsomeres 1–4 with conspicuous white rings on base and apex, 5 white. *Genitalia* (Figures 4A–G): Tergum VIII with a deep V-shaped emargination separating the 2 lateral lobes. Tergum IX with 2 distinct lobes, conical to mound-like in shape, widely separated, bearing 8–14 setae. Gonocoxite conical; inner margin moderately concave; ventrolateral setae strongly developed; lateral surface with 13–17 small, scattered setae (lsp) at level of subapical lobe; proximal part of ventrolateral surface with scales. Subapical lobe clearly divided into 2 divisions. Proximal division of subapical lobe with an apical infundibular and hyaline expansion, 2 robust, sinuous, apically hooked setae (setae *a* and *b*) at apex, a subapical hyaline, broad, hooked-falciform seta and 5, 6 strong, pointed setae from base to level of insertion of the hooked-falciform seta; a patch of 10–14 short setae inserted mesally at base of distal surface. Distal division of subapical lobe divided into 2 divergent arms, the proximal arm bearing 3 apical setae, 1 long hooked seta (*h*), 1 shorter saber-like seta (*s*), and 1 long foliform seta (*l*) inserted proximal to setae *h* and *s*; distal arm bearing 5 apical setae, 1 long, curved, saber-like seta (*s*), and 4 appressed flattened setae (*f*). Gonostylus slender, curved at midlength, subapical portion enlarged on lateral view; ventral surface with inconspicuous crest extending from apical snout to enlarged subapical portion; apical snout tapered to a truncate apex; gonostylar claw short, leaflike, broadened apically; ventral margin with 2 setae before gonostylar claw, distal seta conspicuously longer than proximal seta. 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 short, laterally curved, pointed at apex; apical tergal process longer than apical sternal process, pointed and directed dorsolaterally. Proctiger elongate; paraproct narrowed distally, expanded basally, crown a row of about 7, 8 short simple blades. Cercal sclerite long and narrow with 2, 3 cercal setae. Basal plate, paramere and tergum X as figured.

Fig 4. Male genitalia of *Culex* (*Melanoconion*) *extenuatus* sp. nov. 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. Morphological structures are abbreviated as follow: 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. *Culex extenuatus* is named for the thinned arms (proximal and distal) of the distal division of the subapical lobe of the gonocoxite relatively to the other species of the Pedroi Subgroup of the Spissipes Section of *Culex* subgenus *Melanoconion*.

Bionomics. Nothing is known about the bionomics of *Cx. extenuatus*. Adult males were collected using CDC light traps (supplemented with black light or not) placed at 1.5 m above ground and operated from 1700 to 0700 h in the secondary vegetation surrounding the Amerindian village of Elaé on the Lawa River.

Distribution. *Culex extenuatus* is know from the type locality and few inland localities in French Guiana, namely: Cacao, Crique Gabaret, and Grand Santi.

Type material. *Holotype*: Adult male in 96% ethanol with dissected genitalia mounted on a microscope slide (specimen number ST1#1302, BOLD: FGMOS2187-20, GenBank: [waiting for accession creation]), FRENCH GUIANA: Lawa River, Elaé (54.048683° W, 3.380118° N, 100 m above sea level), 22-III-2018, S. Talaga, IPG. *Paratype*: One adult male in 96% ethanol with dissected genitalia mounted on a microscope slide (specimen number ST1#1308, BOLD: FGMOS2193-20, GenBank: [waiting for accession creation]), same collection data as the holotype, IPG.

Other material examined. Three adult males in 96% ethanol with dissected genitalia mounted on separate microscope slides, FRENCH GUIANA: Grand Santi (54.381119° W, 4.270310° N, 50 m above sea level), 28-III-2018, S. Talaga, IPG (specimen number ST1#1325); Cacao (52.466964° W, 4.582039° N, 3 m above sea level), 23-VII-2019, O. Romoli and K. Heu, IPG (specimen number ST1#1567); Crique Gabaret (51.915540° W, 3.925270° N, 25 m above sea level), 19-II-2021, S. Talaga, IPG (specimen number ST1#1873). Holotype male genitalia of *Cx. pedroi* Sirivanakarn & Belkin (specimen number USNMENT01935483), USNM.

COI sequences of *Culex* across South America

Comparison of our dataset with the others COI sequences of *Culex* available in South America (529 sequences of 150 species/morphospecies) revealed accurate matches, close matches and mismatches between nominal species and molecular BINs (S3 Figure, Supporting information). Comparison involving species absent of our dataset and morphospecies were not detailed in order not to lengthen the article too much.

In 18 cases, *Culex* species identified outside French Guiana clustered in the same BIN as our specimens of the same species. For the subgenus *Aedinus*, specimens of *Cx. amazonensis* from Argentina clustered in the same BIN (AAU2664) as our specimens from French Guiana. In *Carrollia*, specimens of *Cx. bonnei* and *Cx. urichii* from Ecuador clustered in the same BINs (AAW1433 and AAG3937, respectively) as our specimens from French Guiana. For the subgenus *Culex*, specimens of *Cx. quinquefasciatus* from Argentina clustered in the same BIN (AAA4751) as our specimens from French Guiana. Specimens of *Cx. declarator* and *Cx. nigripalpus* from Brazil and Colombia, and *Cx. mollis* from

Argentina and Brazil clustered in the same BIN (AAF1735) as our specimens from French Guiana. Specimens of *Cx. surinamensis* from Brazil and a specimen of *Cx. usquatus* from Argentina clustered in the same BIN (AAN3636) as our specimens from French Guiana. For the subgenus *Melanoconion*, a specimen of *Cx. evansae* and a specimen of *Cx. ensiformis* from Brazil clustered in the same BINs (ADK0770 and ADJ7931, respectively) as our specimens from French Guiana. Specimens of *Cx. erraticus* and *Cx. lucifugus* from Colombia clustered in the same BINs (AAG3848 and ACU4075, respectively) as our specimens from French Guiana. Specimens of *Cx. clarki*, *Cx. commevynensis*, *Cx. equinoxialis* and a specimen of *Cx. vaxus* from Brazil clustered in the same BINs (ADK1664, ADK0771, ADK1666, and ADJ7555, respectively) as our specimens from French Guiana. Finally, specimens of *Cx. theobaldi* from Brazil clustered in the same BIN (ADK5539) as a most of our specimens from French Guiana.

In 14 cases we found mismatches where the same nominal species clustered in different BINs. At one exception, all these cases involved species of the subgenus *Melanoconion*. Among the Melanoconion Section, Specimens of *Cx. dunni* from Argentina and Brazil and *Cx. zeteki* from Brazil clustered in different BINs (ADJ7556 and AAZ5313, respectively) from those originating in French Guiana (AEE2793 and AGA9331, respectively). Specimens of *Cx. corentynensis* from Brazil clustered in a distant BIN (ADJ8504) from those originating in French Guiana (AFI9596). Specimens of *Cx. putumayensis* from Brazil clustered in a different but related BIN (ADJ8613) from those originating in French Guiana (ACZ3899). Specimens of *Cx. vaxus* from Argentina and some from Brazil clustered in different BINs (AEB4367, ADM0315, and ADK6634, respectively) than those originating in French Guiana (ADJ7555). Specimens of *Cx. aureonotatus* Duret & Barreto from Brazil clustered in the same BIN (AAG3848) as specimens of *Cx. erraticus* from French Guiana. Specimens of *Cx. serratimarge* from Brazil and Ecuador clustered in different BINs (ADK0466 and AAW1266, respectively) from those originating in French Guiana (AAE2103). Specimens of *Cx. idottus* from Argentina and Brazil and *Cx. eastor* from Brazil clustered in different BINs (ADG8243 and ADJ7929, respectively) from those originating in French Guiana (AEE6759). Specimens of *Cx. pilosus* from Brazil clustered in different BINs (ADJ8438 and ADT4465) than those originating in French Guiana (AAG3858). In the Spissipes Section, a specimen of *Cx. portesi* and specimens of *Cx. spissipes* and *Cx. vomerifer* from Brazil clustered in different BINs (AAZ3500, ADK0011 and ADK2041, respectively) to our specimens from French Guiana (ACS6189, ABY1758 and ADT9229, respectively). In the subgenus *Phenacomyia*, specimens of *Cx. corniger* from Colombia clustered in a different BIN (ABU8489) than those from French Guiana (ADV2314).

Finally, three specimens from other datasets clustered with sequences of different subgenera than their own. Two specimens of *Cx. conspirator* Dyar & Knab from Colombia (GenBank KM593054 and KM593048) clustered with species the subgenus *Culex*, and one specimen of *Cx. rabelloi* from Brazil (GenBank KX779859) clustered with sequences of *Cx. amazonensis* from Argentina and French Guiana. These specimens should be reviewed because their identifications are most likely erroneous.

Discussion

Overall, congruence was high between morphological identification and molecular delimitations for *Culex* of French Guiana using the standard COI marker. The BOLD clustering approach into BINs gives the best result in terms of species delimitation, followed by the mPTP method and the ASAP method. In five cases, the three delimitation approaches grouped more than one nominal species in the same molecular unit: two cases included *Culex* (*Culex*) species (BINs AAF1735 and AAN3636), and three cases included

Culex (*Melanoconion*) species (BINs ADK0770, AEE2103 and AEE6759), for a total of 15 species involved. Although these species are morphologically well-defined, this indicates that the COI marker does not contain enough information to accurately identify these species above their BIN. Such limit was already pointed out on a set of 22 *Culex* (*Culex*) species from Argentina and Brazil from which the COI barcode barely identified 70% of them [17]. Our results show that it can also apply in a lesser extent to the subgenus *Melanoconion* where the COI barcode allowed to accurately identify only 87% of them. On the other hand, some species hardly identifiable among the subgenus *Melanoconion*, even with properly dissected male genitalia, are unambiguously delimited based on the COI barcode. This is particularly true for morphologically close species of the Educator Group of the Melanoconion Section (for example, *Cx. bibulus*, *Cx. longistriatus* and *Cx. vaxus*) or of the Pedroi Subgroup of the Spissipes Section (for example, *Cx. crybda*, *Cx. extenuatus* and *Cx. pedroi*). *Culex bastagarius* and *Cx. foliafer* were the only two species as being split into more than one molecular unit by the three delimitation methods. This result can be a signal of close-related species, but careful examination did not permit to detect any morphological differences. Until more material become available to study, we interpret this delimitation as an artefact linked to sampling geographical scale or proof of separation then further introgression with persistence of mitochondrial DNA signal.

Most subgenera and some informal groups within the *Culex* genus are retrieved in well-supported monophyletic clades under the ML phylogenetic analysis of the COI barcode. This is the cases of subgenera *Aedinus* (two species included, 96% bootstrap value), *Anoedioporpa* (only one species included), *Carrollia* (four species included, 79% bootstrap value), *Tinolestes* (two species included, 97% bootstrap value), as well as the former Ocellatus Section presently without subgeneric placement represented here by *Cx. nigrimacula* and *Cx. ocellatus* (98% bootstrap value). Monophyly of the subgenera *Culex*, *Melanoconion* and *Microculex* are not supported statistically by our analysis. Species of *Culex* (*Culex*) included in the analysis clustered together in a clade supported at 71% of bootstrap value but this clade also included *Cx. corniger*, a member of the subgenus *Phenacomyia* Harbach and Peyton [35].

Overall, species of the Melanoconion and Spissipes sections of *Melanoconion* clustered together but their basal nodes were not supported by bootstrap values. Nevertheless, some informal groups within the Melanoconion Section were well-supported. This is the case of the Atratus Group (95%), the Conspirator Group (99%), and the Pilosus Group (83%), comprising the Caudelli Subgroup (97%) and the Pilosus Subgroup (95%). The monophyly of the Atratus and Pilosus groups were already shown by Torres-Gutierrez et al. [21; 36] using the COI barcode and two nuclear markers. The monophyly of the Educator Group as currently interpreted [29] is not sustained by our phylogenetic analysis of the COI marker. Only five out of the eight species included in this study (namely, *Cx. aphyllus*, *Cx. bibulus*, *Cx. eknomios*, *Cx. longistriatus*, and *Cx. vaxus*) were retrieved as monophyletic (51% bootstrap value). However, *Cx. cristovaoi*, *Cx. inadmirabilis* and *Cx. theobaldi* clustered far from this clade which suggests that they not belong to the Educator Group. Some other results are worth noting. A group of ten species originally classified among the Bastagarius Group (namely, *Cx. alinkios*, *Cx. brachiatus*, *Cx. comatus*, *Cx. creole*, *Cx. hutchingsae*, and *Cx. tournieri*) and the Intrincatus Group (namely, *Cx. eastor*, *Cx. idottus*, *Cx. rabanicolus*, and *Cx. ybarmis*) clustered together in a well-supported monophyletic clade (93% bootstrap value). In the male geniatalia, both groups are separated based on the shape of the apical median process of the lateral plate of the phallosome [12]. However, this morphological structure could show a complete intergradation from spinelike to broad quadrate, rendering it ineffective in separating species with intermediate shapes of apical median process. Three pairs of species originally placed in different informal groups were retrieved clustering together in statistically supported clades. This is the case of *Cx. corentynensis* clustering with *Cx. johnnyi* (99% bootstrap value). These species were originally placed in the Iolambdis Subgroup of the Bastagarius Group and Evansae Group, respectively [12]. However, male genitalia of both species show striking morphological similarities, especially regarding the setal arrangement of the distal subapical lobe of the gonocoxite [27]. Similarly, *Cx. cristovaoi* and *Cx. johnsoni* originally placed in the Educator Group and Intrincatus Group, respectively [12] clustered together (52% bootstrap value). The overall morphological similarity of these two species was noted in a recent revision of the Educator Group [29] and supported here by our analysis. Finally, *Cx. bastagarius* of the Bastagarius Subgroup of the Bastagarius Group clustered with two species of the Evansae Group (*Cx. evansae* and *Cx. batesi*) in a well-supported clade (86% bootstrap value). A similar result was found as regard to *Cx. bastagarius* and *Cx. evansae* collected in Brazil [21; 36]. All these results confirm the importance of genital structures in delimitation of *Culex* species but put into perspective the overriding importance of the lateral plate in the infrasubgeneric classification of the Melanoconion Section of the subgenus *Melanoconion*.

Culex carincii and *Cx. extenuatus* described herein belong 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 [12; 26]. Both species belong to the Pedroi Subgroup of the Crybda Group, together with *Cx. adamesi*, *Cx. crybda*, *Cx. epanastasis*, *Cx. pedroi*, and *Cx. ribeirensis*. *Culex carincii* and *Cx. extenuatus* share with them all the distinguishing features of the Pedroi Subgroup [26]. This includes 1) the presence 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 short, pointed, laterally curved; the apical tergal process long, nearly pointed, dorsolaterally directed; 3) the proximal division of the subapical lobe of the gonocoxite with an apical infundibular and hyaline expansion, a subapical broad hooked-falciform seta and few short setae scattered from the base to the level of insertion of the hooked-falciform seta; 4) the distal division of the subapical lobe divided into two arms, the proximal arm with 3 setae, which include a hooked seta (*h*), a saberlike seta (*s*), and a foliform seta (*l*); distal arm with 5 setae, which include a saberlike seta (*s*) and 4 narrow, appressed setae (*f*); 5) the lobes of tergum IX small, cone-shaped, widely separated and with few setae.

In the adults, *Cx. carincii* can be easily separated from *Cx. epanastasis*, *Cx. extenuatus* and *Cx. pedroi* by the hindtarsomeres completely dark-scaled, and from *Cx. adamesi* by the pleural integument with conspicuous pattern of dark spots. *Culex carincii* is morphologically closer to *Cx. crybda* and *Cx. ribeirensis* than to any other species of *Melanoconion*. In the male genitalia, *Cx. carincii* can be separated from all the other species of the Pedroi Subgroup by 1) the distinctive shape of the gonostylus, and 2) the gradual insertion of setae on the distal arm of the distal subapical lobe of the gonocoxite. Furthermore, comparison of the COI sequences of *Cx. carincii* with the ones of *Cx. crybda* and *Cx. ribeirensis* from Brazil [21] showed a mean interspecific divergence of 8.42% and 9.21%, respectively, coherent with three distinct species.

The shape of the gonostylus and IX tergite lobes illustrated for *Cx. crybda* in Sallum and Forattini [26] are very different from the holotype of *Cx. crybda* deposited in the USNM. The distinctive gonostylus and IX tergite lobes contradict the general statement that male genitalia of *Cx. crybda* are identical to the ones of *Cx. adamesi*, *Cx. pedroi*, and *Cx. ribeirensis*. The holotype male genitalia of *Cx. crybda* was never described in detail and the most recent illustrations appear to belong to different species [26; 27]. However, the illustration in Pecor et al. [27] is the one that best matches the holotype male genitalia of

Cx. crybda deposited in the USNM. The Vomerifer Group was not retrieved as monophyletic in the phylogenetic study of Torres-Gutierrez et al. [36], because two females identified as *Culex* near *gnomatos* and *Culex* near *portesi* were though to belong to this group. Based on our analysis, their *Culex* near *gnomatos* clustered in the same BIN of our male specimens of *Cx. ocossa* of the Ocossa Group, and their *Culex* near *portesi* close to the BIN of our male specimens of *Cx. carincii* of the Crybda Group. This strengthens the hypothesis that these females do not belong to the Vomerifer Group, thus explaining why they clustered outside of it in their phylogenetic analyses.

In the adult, *Cx. extenuatus* can be easily separated from *Cx. adamesi*, *Cx. carincii*, *Cx. crybda* and *Cx. ribeirensis* by the hindtarsomeres with conspicuous white rings at joints. In the male genitalia, *Cx. extenuatus* can be separated from *Cx. epanastasis* and *Cx. pedroi* by the longer and thinner arms of the distal subapical lobe of the gonocoxite. While this diagnostic character seems inconspicuous, we found no intergradation among the material examined. In French Guiana, *Cx. pedroi* was only collected along the coastal plain in swampy ecosystems, while *Cx. epanastasis* and *Cx. extenuatus* were collected along more inland riverine ecosystems where *Cx. pedroi* is apparently absent. Furthermore, molecular delimitation of these three species was corroborated by all the methods, *Cx. extenuatus* showing a mean interspecific distance of 8.13% with *Cx. epanastasis* and 8.14% with *Cx. pedroi*. Comparison of our COI sequences with the ones in Torres-Gutierrez et al. [21] suggests that *Cx. extenuatus* also occur in Brazil. The possibility of a cryptic species among *Cx. pedroi* was already proposed based on the study of the ITS2 ribosomal marker [37]. Unfortunately, our data cannot permit to answer if *Cx. extenuatus* described herein is the *Cx. pedroi*-Peru form of Navarro and Weaver [37]. Species of the Pedroi Subgroup have been recognized as vector of several viruses. For example, *Cx. adamesi* is a natural proven enzootic VEEV vector in Colombia [38], *Cx. crybda* was found naturally infected by Bussuquara and Guama viruses in Panama [39], and *Cx. pedroi* is both a natural proven enzootic VEEV vector in Colombia [38] and the main vector of VEEV in the Peruvian Amazon [40]. The existence of more species than initially thought in the Pedroi Subgroup may explain differences in vector competence between populations with possible outcomes regarding transmission and emergence of pathogens.

Conclusions

The COI barcode prove again its usefulness and effectiveness in identifying mosquitoes, including cryptic diversity like exemplified here with *Culex* species of the Pedroi Subgroup of the Spissipes Section of *Melanoconion*. However, this study points the limits of this marker for some groups of species within the subgenera *Culex* and *Melanoconion*, probably because of introgression or incomplete lineage sorting. At the scale of South America, inconsistencies between morphological identification and molecular delimitation most likely the result of imperfect taxonomy and geographical gap in sampling. Nevertheless, the COI barcode remains a powerful tool to mitigate morphological taxonomy across geographical scales and efforts should be pursued to improve the delimitation of Neotropical species. Besides adding more taxa, future work should include additional markers to improve delimitation of species poorly supported by the COI barcode alone, and statistically resolve basal nodes toward a more natural classification. This study represents an important contribution to the barcoding initiative of South American mosquitoes and open exiting perspectives to accurately identify *Culex* female and immature specimens during entomological, ecological, or arboviral surveys.

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Resources: ST JBD.

Software: ST.

Supervision: ID JBD.

Validation: ST.

Visualization: ST.

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Supporting information captions

S1 Table. List of the *Culex* species corresponding to the voucher specimens that were COI sequenced in this study. Species are listed alphabetically by subgenus and the life stage is indicated for each taxa (M: male with dissected genitalia; F: female; L: larva). (DOCX)

S2 Table. List of Barcode Index Numbers (BINs) with their associated *Culex* species obtained from BOLD (last visited august 2024). Species are listed alphabetically by subgenus except when more than one morphological species are included in a BIN. Distances (p-distance) correspond to the percentage of dissimilar pairwise nucleotides and counts correspond to the number of voucher specimens included in this study followed, between brackets, by the total number of specimens (including ours) present in the BOLD database. (DOCX)

S3 Fig. Neighbor Joining tree of 529 COI sequences belonging to 150 species/morphospecies of *Culex* from South America. This dataset is composed of 246 sequences from French Guiana, 164 sequences from Brazil [17; 21], 62 sequences from Colombia [20], 46 sequences from Argentina [17; 22], and 11 sequences from Ecuador [16]. For each specimen, we indicated the morphological identification, the BOLD specimen code, the original specimen code, the sampling country and the BOLD Barcode Index Number (BIN). Identification of eight specimens from Brazil were missing in BOLD. Identifications of these specimens in the original publication [21] were as follows: KX779777: *Culex akritos*, KX779846: *Culex* nr. *pedroi*, KX779796: *Culex dunni*, and KX779874–KX779877 and KX779843: *Culex vaxus*.

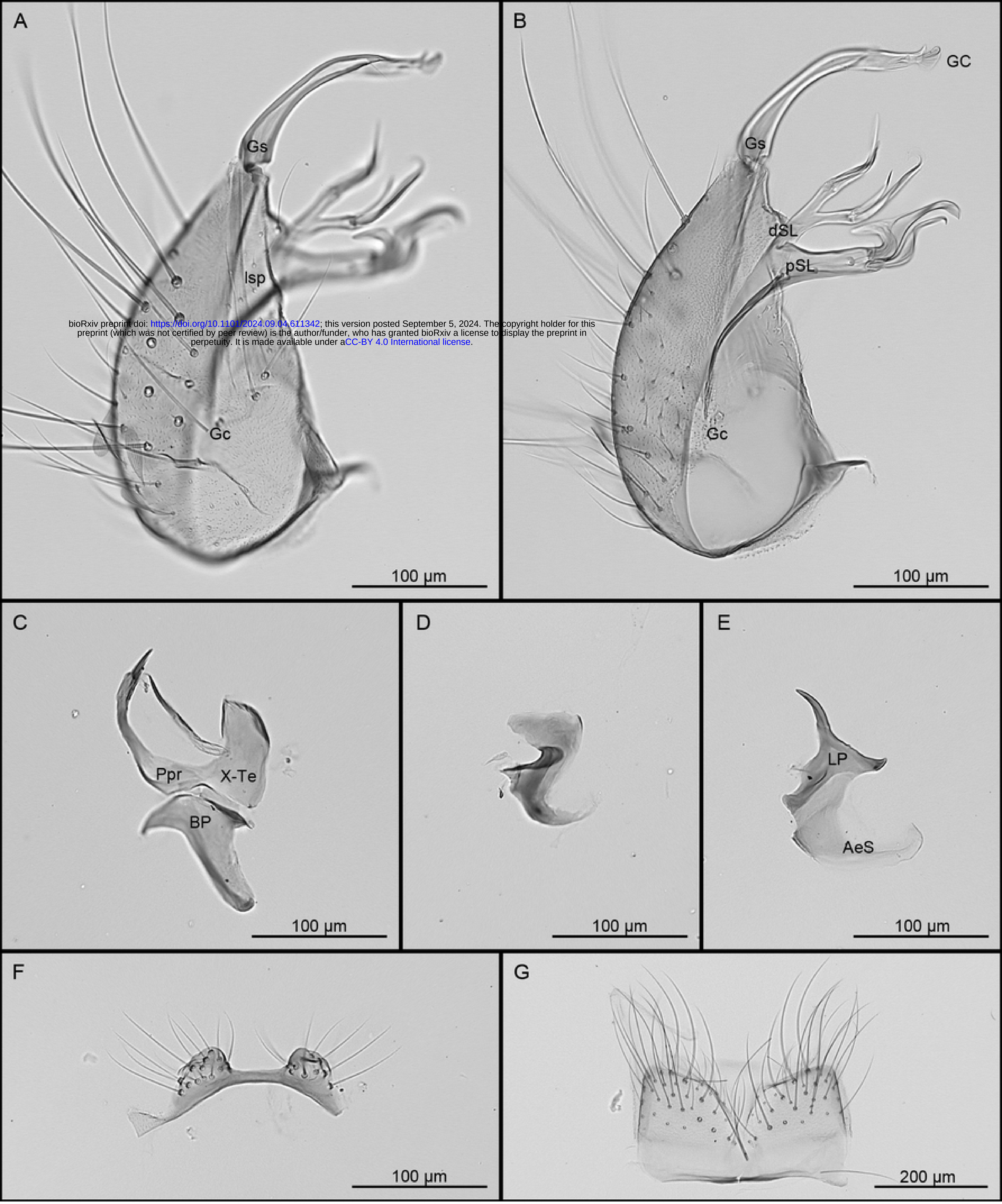
A
Gs
lsp
Gc
100 µm
B
GC
Gs
dSL
pSL
Gc
100 µm
C
Ppr
X-Te
BP
100 µm
D
100 µm
E
LP
AeS
100 µm
F
100 µm
G
200 µm

Figure 4

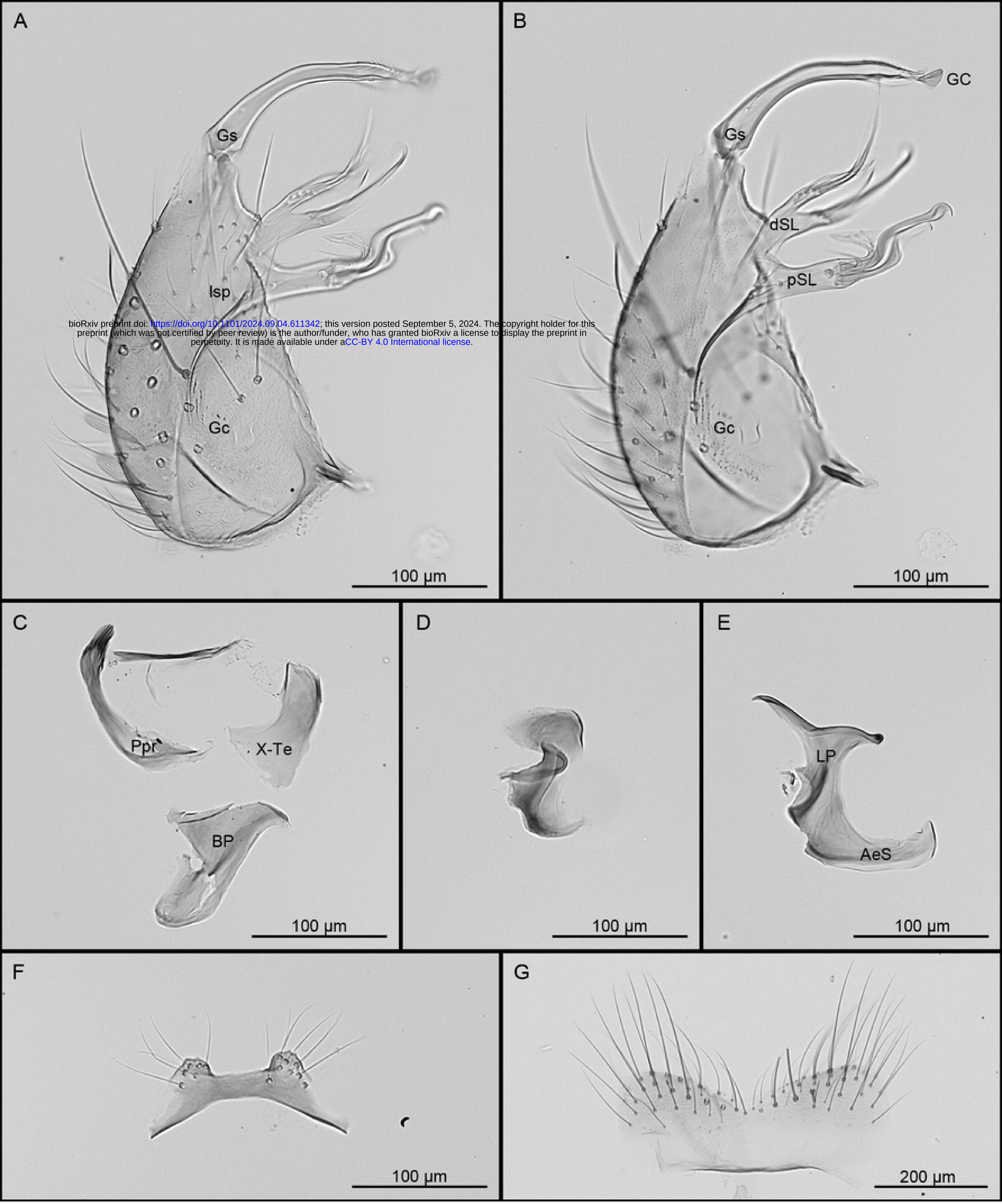
A
Gs
Isp
Gc
100 μm
B
GC
Gs
dSL
pSL
Gc
100 μm
C
Ppr
X-Te
BP
100 μm
D
100 μm
E
LP
AeS
100 μm
F
100 μm
G
200 μm

Figure 3


N
Atlantic Ocean
Maroni
Mana
Kourou
Sinnamary
Mana
SURINAME
Lawa
Approuague
Inini
Oyapock
Litani
Camopi
Marouini
Oyapock
BRAZIL
0
100
200 km

Figure 1


A
Morph
BOLD
ASAP
mPTP
FGMOS2896-22|Culex ybarmis|ST11711|La Trinite|BOLD:AEE1897
FGMOS2898-22|Culex ybarmis|ST11713|La Trinite|BOLD:AEE1897
FGMOS2044-20|Culex ybarmis|ST11159|Les Nouragues|BOLD:AEE1897
FGMOS2768-20|Culex rabanicolus|MB20299|Guatemala|BOLD:AEE2997
FGMOS2234-20|Culex rabanicolus|ST11349|La Cesaree|BOLD:AEE2997
FGMOS2744-20|Culex rabanicolus|MB20275|Zoo de Montsinery|BOLD:AEE2997
FGMOS2216-20|Culex brachiatus|ST11331|Enfant Perdu|BOLD:AEE7320
FGMOS2938-22|Culex alinkios|ST11753|La Trinite|BOLD:ADJ7929
FGMOS2194-20|Culex alinkios|ST11309|Elae|BOLD:ADJ7929
FGMOS2940-22|Culex alinkios|ST11755|La Trinite|BOLD:ADJ7929
FGMOS2060-20|Culex tournieri|ST11175|Saut Grand Machikou|BOLD:AEE9698
FGMOS2227-20|Culex tournieri|ST11342|Enfant Perdu|BOLD:AEE9698
Perdu|BOLD:AEE9698
11313|Nouveau Wacapou|BOLD:AEE7402
FGMOS2180-20|Culex comatus|ST11295|Twenke|BOLD:AEE7402
FGMOS2186-20|Culex comatus|ST11301|Elae|BOLD:AEE7402
FGMOS3098-23|Culex idottus|ST11913|Pointe Macouria|BOLD:AEE6759
FGMOS2206-20|Culex idottus|ST11321|Enfant Perdu|BOLD:AEE6759
FGMOS2207-20|Culex idottus|ST11322|Enfant Perdu|BOLD:AEE6759
FGMOS2192-20|Culex creole|ST11307|Elae|BOLD:AEE6759
FGMOS2226-20|Culex creole|ST11341|Enfant Perdu|BOLD:AEE6759
FGMOS2695-20|Culex eastor|MB20226|Mana|BOLD:AEE6759
FGMOS2711-20|Culex eastor|MB20242|Guatemala|BOLD:AEE6759
FGMOS2897-22|Culex creole|ST11712|La Trinite|BOLD:AEE6759
FGMOS2899-22|Culex creole|ST11714|La Trinite|BOLD:AEE6759
FGMOS2743-20|Culex eastor|MB20274|Zoo de Montsinery|BOLD:AEE6759
FGMOS2752-20|Culex eastor|MB20283|Zoo de Montsinery|BOLD:AEE6759
FGMOS2912-22|Culex hutchingsae|ST11727|La Trinite|BOLD:AEE6759
FGMOS2914-22|Culex hutchingsae|ST11729|La Trinite|BOLD:AEE6759
FGMOS2919-22|Culex hutchingsae|ST11734|La Trinite|BOLD:AEE6759
FGMOS3034-22|Culex inadmirabilis|ST11849|Savanes Viguie|BOLD:AFJ0561
FGMOS3035-22|Culex inadmirabilis|ST11850|Savanes Viguie|BOLD:AFJ0562
FGMOS3036-22|Culex inadmirabilis|ST11851|Savanes Viguie|BOLD:AFJ0562
FGMOS2217-20|Culex saramaccensis|ST11332|Enfant Perdu|BOLD:AEE1449
FGMOS2886-22|Culex corentynensis|ST11701|La Trinite|BOLD:AFI9596
FGMOS2930-22|Culex corentynensis|ST11745|La Trinite|BOLD:AFI9596
FGMOS3068-22|Culex johnnyi|ST11883|Crique Gabaret|BOLD:AFI9771
FGMOS3066-22|Culex johnnyi|ST11881|Crique Gabaret|BOLD:AFI9771
FGMOS3067-22|Culex johnnyi|ST11882|Crique Gabaret|BOLD:AFI9771
FGMOS2179-20|Culex bastagarius|ST11294|Twenke|BOLD:AEE3102
FGMOS2181-20|Culex bastagarius|ST11296|Twenke|BOLD:AEE3102
FGMOS3299-23|Culex bastagarius|ST12114|Camopi|BOLD:AFI9514
FGMOS3169-23|Culex evansae|ST11984|Savanes dOrganabo|BOLD:ADK0770
FGMOS3342-23|Culex evansae|ST12157|Mont Grand Matoury|BOLD:ADK0770
FGMOS2427-20|Culex batesi|ST11542|Trois Paletuviers|BOLD:ADK0770
FGMOS3037-22|Culex ernanii|ST11852|Savanes Viguie|BOLD:AFI9301
FGMOS3040-22|Culex ernanii|ST11855|Savanes Viguie|BOLD:AFI9301
FGMOS3130-23|Culex ernanii|ST11945|Savanes de Passoura|BOLD:AFI9301
FGMOS3178-23|Culex zabanicus|ST11993|Savane de Counamama|BOLD:AFI9750
FGMOS3182-23|Culex zabanicus|ST11997|Savane de Counamama|BOLD:AFI9750
FGMOS2182-20|Culex bibulus|ST11297|Twenke|BOLD:AEE1543
FGMOS3295-23|Culex bibulus|ST12110|Camopi|BOLD:AEE1543
FGMOS3127-23|Culex bibulus|ST11942|Savanes de Passoura|BOLD:AFI9809
FGMOS3376-23|Culex longistriatus|ST12191|Roura|BOLD:AFI9808
FGMOS3377-23|Culex longistriatus|ST12192|Roura|BOLD:AFI9808
FGMOS3378-23|Culex longistriatus|ST12193|Roura|BOLD:AFI9808
FGMOS3380-23|Culex longistriatus|ST12195|Roura|BOLD:AFI9808
FGMOS3384-23|Culex longistriatus|ST12199|Roura|BOLD:AFI9808
FGMOS2429-20|Culex eknomios|ST11544|Lac de Petit Saut|BOLD:AFI9981
FGMOS2430-20|Culex eknomios|ST11545|Lac de Petit Saut|BOLD:AFI9981
FGMOS2440-20|Culex eknomios|ST11555|Lac de Petit Saut|BOLD:AFI9981
FGMOS1713-20|Culex vaxus|ST10828|Montagne du Mahury|BOLD:ADJ7555
FGMOS2157-20|Culex vaxus|ST11272|La Cesaree|BOLD:ADJ7555
FGMOS2436-20|Culex aphyllus|ST11551|Lac de Petit Saut|BOLD:AFI9537
FGMOS2459-20|Culex aphyllus|ST11574|Saut Leodate|BOLD:AFI9537
FGMOS1396-20|Culex lucifugus|ST10511|Montagne de Kaw|BOLD:ACU4075
FGMOS2001-20|Culex lucifugus|ST11116|La Trinite|BOLD:ACU4075
FGMOS2013-20|Culex lucifugus|ST11128|La Trinite|BOLD:ACU4075
FGMOS2928-22|Culex lucackermanni|ST11743|La Trinite|BOLD:AET1273
FGMOS2927-22|Culex lucackermanni|ST11742|La Trinite|BOLD:AET1273
FGMOS2929-22|Culex lucackermanni|ST11744|La Trinite|BOLD:AET1273
99
97
57
90
65
99
83
70
99
93
81
99
77
93
55
99
87
62
99
86
99
99
99
74
99
98
86
99
97
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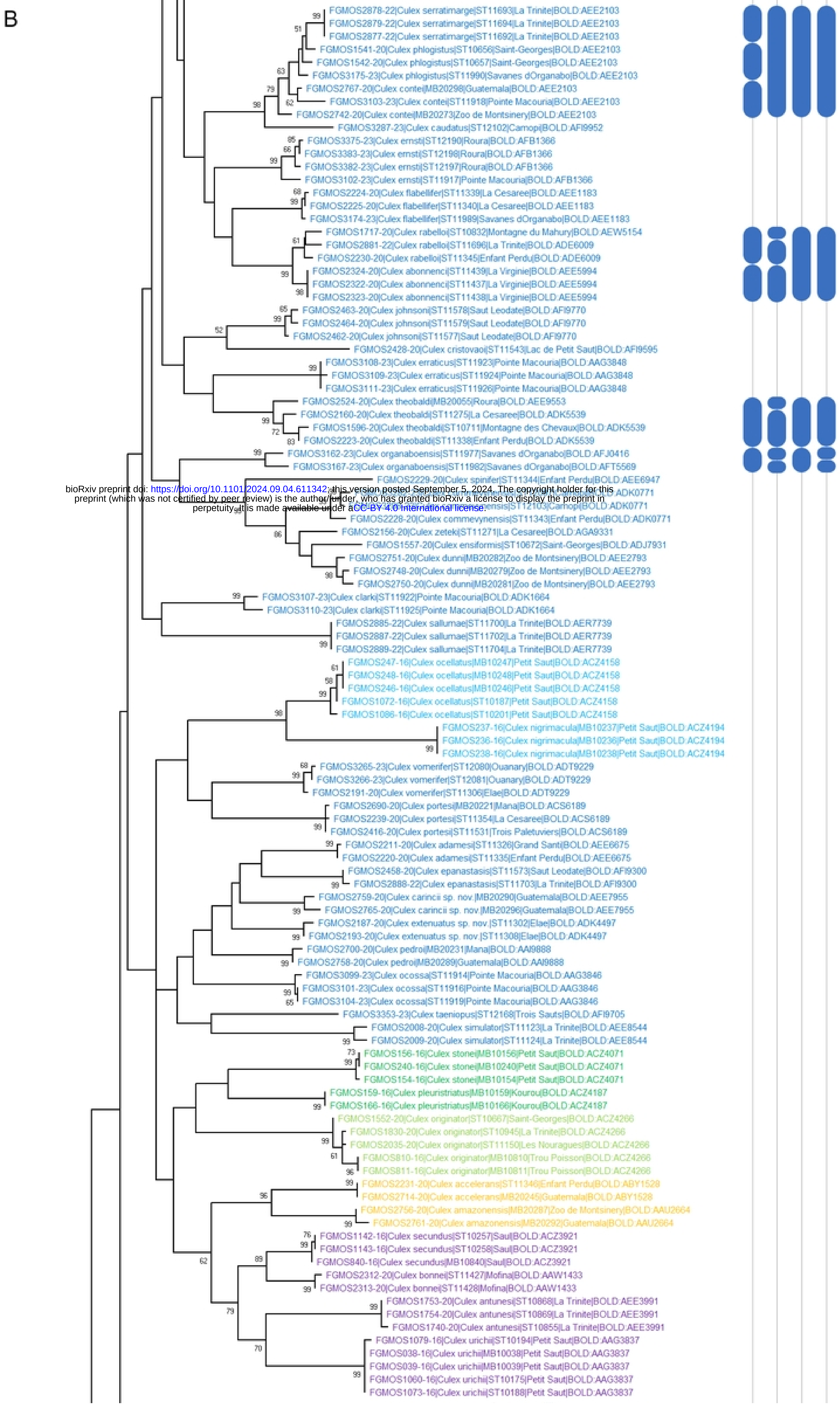
99
99
1
2
1
2

Figure 2A


B
FGMOS2878-22|Culex serratimarge|ST11693|La Trinite|BOLD:AEE2103
FGMOS2879-22|Culex serratimarge|ST11694|La Trinite|BOLD:AEE2103
FGMOS2877-22|Culex serratimarge|ST11692|La Trinite|BOLD:AEE2103
FGMOS1541-20|Culex phlogistus|ST10656|Saint-Georges|BOLD:AEE2103
FGMOS1542-20|Culex phlogistus|ST10657|Saint-Georges|BOLD:AEE2103
FGMOS3175-23|Culex phlogistus|ST11990|Savanes dOrganabo|BOLD:AEE2103
FGMOS2767-20|Culex conte|MB20298|Guatemala|BOLD:AEE2103
FGMOS3103-23|Culex conte|ST11918|Pointe Macouria|BOLD:AEE2103
FGMOS2742-20|Culex conte|MB20273|Zoo de Montsinery|BOLD:AEE2103
FGMOS3287-23|Culex caudatus|ST12102|Camopi|BOLD:AFI9952
FGMOS3375-23|Culex ernsti|ST12190|Roura|BOLD:AFB1366
FGMOS3383-23|Culex ernsti|ST12198|Roura|BOLD:AFB1366
FGMOS3382-23|Culex ernsti|ST12197|Roura|BOLD:AFB1366
FGMOS3102-23|Culex ernsti|ST11917|Pointe Macouria|BOLD:AFB1366
FGMOS2224-20|Culex flabellifer|ST11339|La Cesaree|BOLD:AEE1183
FGMOS2225-20|Culex flabellifer|ST11340|La Cesaree|BOLD:AEE1183
FGMOS3174-23|Culex flabellifer|ST11989|Savanes dOrganabo|BOLD:AEE1183
FGMOS1717-20|Culex rabelloi|ST10832|Montagne du Mahury|BOLD:AEW5154
FGMOS2881-22|Culex rabelloi|ST11696|La Trinite|BOLD:ADE6009
FGMOS2230-20|Culex rabelloi|ST11345|Enfant Perdu|BOLD:ADE6009
FGMOS2324-20|Culex abonnenci|ST11439|La Virginie|BOLD:AEE5994
FGMOS2322-20|Culex abonnenci|ST11437|La Virginie|BOLD:AEE5994
FGMOS2323-20|Culex abonnenci|ST11438|La Virginie|BOLD:AEE5994
FGMOS2463-20|Culex johnsoni|ST11578|Saut Leodate|BOLD:AFI9770
FGMOS2464-20|Culex johnsoni|ST11579|Saut Leodate|BOLD:AFI9770
FGMOS2462-20|Culex johnsoni|ST11577|Saut Leodate|BOLD:AFI9770
FGMOS2428-20|Culex cristovaoi|ST11543|Lac de Petit Saut|BOLD:AFI9595
FGMOS3108-23|Culex erraticus|ST11923|Pointe Macouria|BOLD:AAG3848
FGMOS3109-23|Culex erraticus|ST11924|Pointe Macouria|BOLD:AAG3848
FGMOS3111-23|Culex erraticus|ST11926|Pointe Macouria|BOLD:AAG3848
FGMOS2524-20|Culex theobaldi|MB20055|Roura|BOLD:AEE9553
FGMOS2160-20|Culex theobaldi|ST11275|La Cesaree|BOLD:ADK5539
FGMOS1596-20|Culex theobaldi|ST10711|Montagne des Chevaux|BOLD:ADK5539
FGMOS2223-20|Culex theobaldi|ST11338|Enfant Perdu|BOLD:ADK5539
FGMOS3162-23|Culex organaboensis|ST11977|Savanes dOrganabo|BOLD:AFJ0416
FGMOS3167-23|Culex organaboensis|ST11982|Savanes dOrganabo|BOLD:AFT5569
FGMOS2229-20|Culex spinifer|ST11344|Enfant Perdu|BOLD:AEE6947
ADK0771
ensis|ST12103|Camopi|BOLD:ADK0771
FGMOS2228-20|Culex commevynensis|ST11343|Enfant Perdu|BOLD:ADK0771
FGMOS2156-20|Culex zeteki|ST11271|La Cesaree|BOLD:AGA9331
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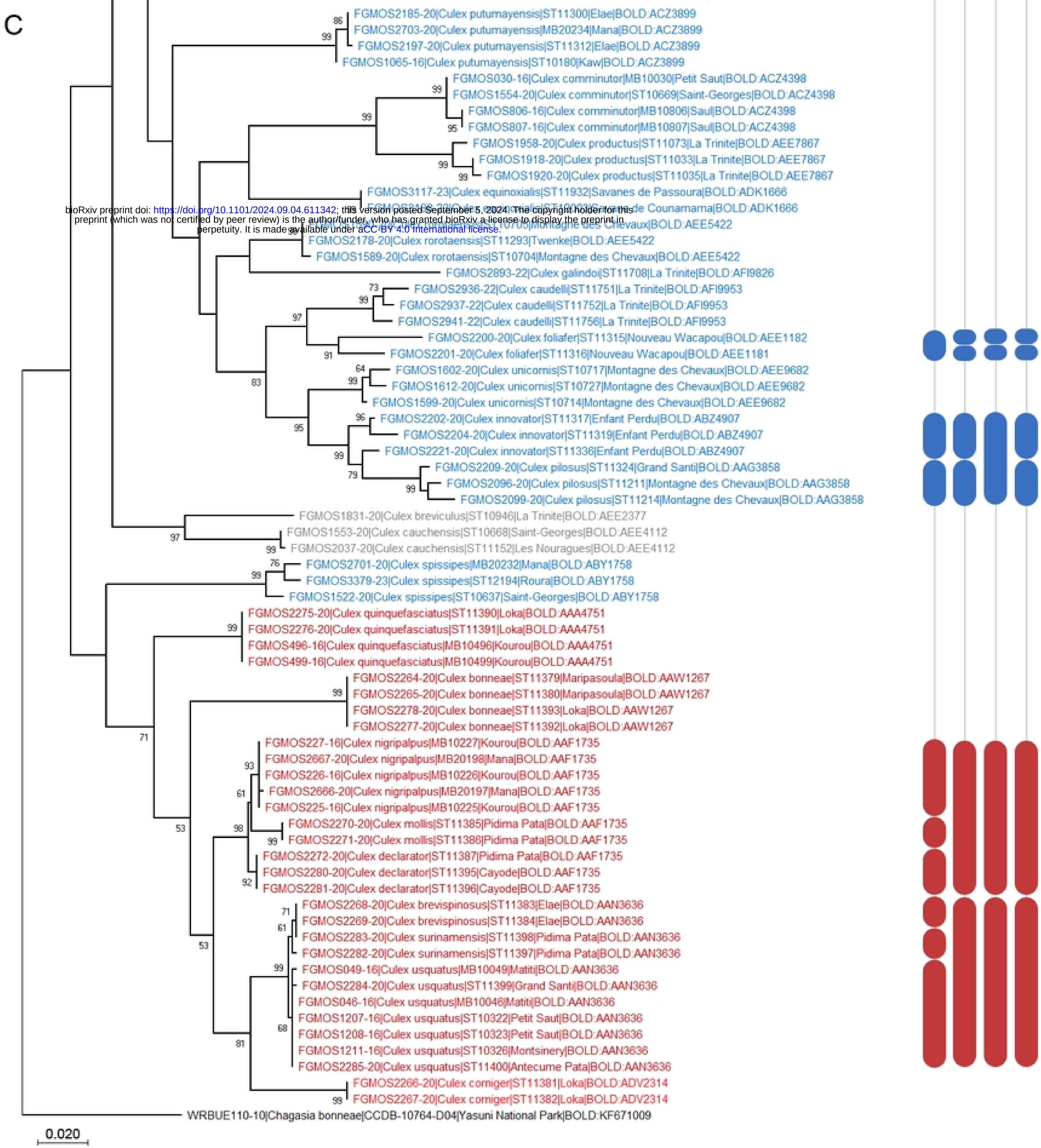
FGMOS2748-20|Culex dunni|MB20279|Zoo de Montsinery|BOLD:AEE2793
FGMOS2750-20|Culex dunni|MB20281|Zoo de Montsinery|BOLD:AEE2793
FGMOS3107-23|Culex clarki|ST11922|Pointe Macouria|BOLD:ADK1664
FGMOS3110-23|Culex clarki|ST11925|Pointe Macouria|BOLD:ADK1664
FGMOS2885-22|Culex sallumae|ST11700|La Trinite|BOLD:AER7739
FGMOS2887-22|Culex sallumae|ST11702|La Trinite|BOLD:AER7739
FGMOS2889-22|Culex sallumae|ST11704|La Trinite|BOLD:AER7739
FGMOS247-16|Culex ocellatus|MB10247|Petit Saut|BOLD:ACZ4158
FGMOS248-16|Culex ocellatus|MB10248|Petit Saut|BOLD:ACZ4158
FGMOS246-16|Culex ocellatus|MB10246|Petit Saut|BOLD:ACZ4158
FGMOS1072-16|Culex ocellatus|ST10187|Petit Saut|BOLD:ACZ4158
FGMOS1086-16|Culex ocellatus|ST10201|Petit Saut|BOLD:ACZ4158
FGMOS237-16|Culex nigrimacula|MB10237|Petit Saut|BOLD:ACZ4194
FGMOS236-16|Culex nigrimacula|MB10236|Petit Saut|BOLD:ACZ4194
FGMOS238-16|Culex nigrimacula|MB10238|Petit Saut|BOLD:ACZ4194
FGMOS3265-23|Culex vomerifer|ST12080|Ouanary|BOLD:ADT9229
FGMOS3266-23|Culex vomerifer|ST12081|Ouanary|BOLD:ADT9229
FGMOS2191-20|Culex vomerifer|ST11306|Elae|BOLD:ADT9229
FGMOS2690-20|Culex portesi|MB20221|Mana|BOLD:ACS6189
FGMOS2239-20|Culex portesi|ST11354|La Cesaree|BOLD:ACS6189
FGMOS2416-20|Culex portesi|ST11531|Trois Paletuviers|BOLD:ACS6189
FGMOS2211-20|Culex adamesi|ST11326|Grand Santi|BOLD:AEE6675
FGMOS2220-20|Culex adamesi|ST11335|Enfant Perdu|BOLD:AEE6675
FGMOS2458-20|Culex epanastasis|ST11573|Saut Leodate|BOLD:AFI9300
FGMOS2888-22|Culex epanastasis|ST11703|La Trinite|BOLD:AFI9300
FGMOS2759-20|Culex carincii sp. nov.|MB20290|Guatemala|BOLD:AEE7955
FGMOS2765-20|Culex carincii sp. nov.|MB20296|Guatemala|BOLD:AEE7955
FGMOS2187-20|Culex extenuatus sp. nov.|ST11302|Elae|BOLD:ADK4497
FGMOS2193-20|Culex extenuatus sp. nov.|ST11308|Elae|BOLD:ADK4497
FGMOS2700-20|Culex pedroi|MB20231|Mana|BOLD:AAI9888
FGMOS2758-20|Culex pedroi|MB20289|Guatemala|BOLD:AAI9888
FGMOS3099-23|Culex ocossa|ST11914|Pointe Macouria|BOLD:AAG3846
FGMOS3101-23|Culex ocossa|ST11916|Pointe Macouria|BOLD:AAG3846
FGMOS3104-23|Culex ocossa|ST11919|Pointe Macouria|BOLD:AAG3846
FGMOS3353-23|Culex taeniopus|ST12168|Trois Sauts|BOLD:AFI9705
FGMOS2008-20|Culex simulator|ST11123|La Trinite|BOLD:AEE8544
FGMOS2009-20|Culex simulator|ST11124|La Trinite|BOLD:AEE8544
FGMOS156-16|Culex stonei|MB10156|Petit Saut|BOLD:ACZ4071
FGMOS240-16|Culex stonei|MB10240|Petit Saut|BOLD:ACZ4071
FGMOS154-16|Culex stonei|MB10154|Petit Saut|BOLD:ACZ4071
FGMOS159-16|Culex pleuristriatus|MB10159|Kourou|BOLD:ACZ4187
FGMOS166-16|Culex pleuristriatus|MB10166|Kourou|BOLD:ACZ4187
FGMOS1552-20|Culex originator|ST10667|Saint-Georges|BOLD:ACZ4266
FGMOS1830-20|Culex originator|ST10945|La Trinite|BOLD:ACZ4266
FGMOS2035-20|Culex originator|ST11150|Les Nouragues|BOLD:ACZ4266
FGMOS810-16|Culex originator|MB10810|Trou Poisson|BOLD:ACZ4266
FGMOS811-16|Culex originator|MB10811|Trou Poisson|BOLD:ACZ4266
FGMOS2231-20|Culex accelerans|ST11346|Enfant Perdu|BOLD:ABY1528
FGMOS2714-20|Culex accelerans|MB20245|Guatemala|BOLD:ABY1528
FGMOS2756-20|Culex amazonensis|MB20287|Zoo de Montsinery|BOLD:AAU2664
FGMOS2761-20|Culex amazonensis|MB20292|Guatemala|BOLD:AAU2664
FGMOS1142-16|Culex secundus|ST10257|Saul|BOLD:ACZ3921
FGMOS1143-16|Culex secundus|ST10258|Saul|BOLD:ACZ3921
FGMOS840-16|Culex secundus|MB10840|Saul|BOLD:ACZ3921
FGMOS2312-20|Culex bonnei|ST11427|Mofina|BOLD:AAW1433
FGMOS2313-20|Culex bonnei|ST11428|Mofina|BOLD:AAW1433
FGMOS1753-20|Culex antunesi|ST10868|La Trinite|BOLD:AEE3991
FGMOS1754-20|Culex antunesi|ST10869|La Trinite|BOLD:AEE3991
FGMOS1740-20|Culex antunesi|ST10855|La Trinite|BOLD:AEE3991
FGMOS1079-16|Culex urichii|ST10194|Petit Saut|BOLD:AAG3837
FGMOS038-16|Culex urichii|MB10038|Petit Saut|BOLD:AAG3837
FGMOS039-16|Culex urichii|MB10039|Petit Saut|BOLD:AAG3837
FGMOS1060-16|Culex urichii|ST10175|Petit Saut|BOLD:AAG3837
FGMOS1073-16|Culex urichii|ST10188|Petit Saut|BOLD:AAG3837

Figure 2B


C
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FGMOS2703-20|Culex putumayensis|MB20234|Mana|BOLD:ACZ3899
FGMOS2197-20|Culex putumayensis|ST11312|Elae|BOLD:ACZ3899
FGMOS1065-16|Culex putumayensis|ST10180|Kaw|BOLD:ACZ3899
FGMOS030-16|Culex comminutor|MB10030|Petit Saut|BOLD:ACZ4398
FGMOS1554-20|Culex comminutor|ST10669|Saint-Georges|BOLD:ACZ4398
FGMOS806-16|Culex comminutor|MB10806|Saul|BOLD:ACZ4398
FGMOS807-16|Culex comminutor|MB10807|Saul|BOLD:ACZ4398
FGMOS1958-20|Culex productus|ST11073|La Trinite|BOLD:AEE7867
FGMOS1918-20|Culex productus|ST11033|La Trinite|BOLD:AEE7867
FGMOS1920-20|Culex productus|ST11035|La Trinite|BOLD:AEE7867
FGMOS3117-23|Culex equinoxialis|ST11932|Savanes de Passoura|BOLD:ADK1666
de Counamama|BOLD:ADK1666
Montagne des Chevaux|BOLD:AEE5422
FGMOS2178-20|Culex rorotaensis|ST11293|Twenke|BOLD:AEE5422
FGMOS1589-20|Culex rorotaensis|ST10704|Montagne des Chevaux|BOLD:AEE5422
FGMOS2893-22|Culex galindoi|ST11708|La Trinite|BOLD:AFI9826
FGMOS2936-22|Culex caudelli|ST11751|La Trinite|BOLD:AFI9953
FGMOS2937-22|Culex caudelli|ST11752|La Trinite|BOLD:AFI9953
FGMOS2941-22|Culex caudelli|ST11756|La Trinite|BOLD:AFI9953
FGMOS2200-20|Culex foliafer|ST11315|Nouveau Wacapou|BOLD:AEE1182
FGMOS2201-20|Culex foliafer|ST11316|Nouveau Wacapou|BOLD:AEE1181
FGMOS1602-20|Culex unicornis|ST10717|Montagne des Chevaux|BOLD:AEE9682
FGMOS1612-20|Culex unicornis|ST10727|Montagne des Chevaux|BOLD:AEE9682
FGMOS1599-20|Culex unicornis|ST10714|Montagne des Chevaux|BOLD:AEE9682
FGMOS2202-20|Culex innovator|ST11317|Enfant Perdu|BOLD:ABZ4907
FGMOS2204-20|Culex innovator|ST11319|Enfant Perdu|BOLD:ABZ4907
FGMOS2221-20|Culex innovator|ST11336|Enfant Perdu|BOLD:ABZ4907
FGMOS2209-20|Culex pilosus|ST11324|Grand Santi|BOLD:AAG3858
FGMOS2096-20|Culex pilosus|ST11211|Montagne des Chevaux|BOLD:AAG3858
FGMOS2099-20|Culex pilosus|ST11214|Montagne des Chevaux|BOLD:AAG3858
FGMOS1831-20|Culex breviculus|ST10946|La Trinite|BOLD:AEE2377
FGMOS1553-20|Culex cauchensis|ST10668|Saint-Georges|BOLD:AEE4112
FGMOS2037-20|Culex cauchensis|ST11152|Les Nouragues|BOLD:AEE4112
FGMOS2701-20|Culex spissipes|MB20232|Mana|BOLD:ABY1758
FGMOS3379-23|Culex spissipes|ST12194|Roura|BOLD:ABY1758
FGMOS1522-20|Culex spissipes|ST10637|Saint-Georges|BOLD:ABY1758
FGMOS2275-20|Culex quinquefasciatus|ST11390|Loka|BOLD:AAA4751
FGMOS2276-20|Culex quinquefasciatus|ST11391|Loka|BOLD:AAA4751
FGMOS496-16|Culex quinquefasciatus|MB10496|Kourou|BOLD:AAA4751
FGMOS499-16|Culex quinquefasciatus|MB10499|Kourou|BOLD:AAA4751
FGMOS2264-20|Culex bonneae|ST11379|Maripasoula|BOLD:AAW1267
FGMOS2265-20|Culex bonneae|ST11380|Maripasoula|BOLD:AAW1267
FGMOS2278-20|Culex bonneae|ST11393|Loka|BOLD:AAW1267
FGMOS2277-20|Culex bonneae|ST11392|Loka|BOLD:AAW1267
FGMOS227-16|Culex nigripalpus|MB10227|Kourou|BOLD:AAF1735
FGMOS2667-20|Culex nigripalpus|MB20198|Mana|BOLD:AAF1735
FGMOS226-16|Culex nigripalpus|MB10226|Kourou|BOLD:AAF1735
FGMOS2666-20|Culex nigripalpus|MB20197|Mana|BOLD:AAF1735
FGMOS225-16|Culex nigripalpus|MB10225|Kourou|BOLD:AAF1735
FGMOS2270-20|Culex mollis|ST11385|Pidima Pata|BOLD:AAF1735
FGMOS2271-20|Culex mollis|ST11386|Pidima Pata|BOLD:AAF1735
FGMOS2272-20|Culex declarator|ST11387|Pidima Pata|BOLD:AAF1735
FGMOS2280-20|Culex declarator|ST11395|Cayode|BOLD:AAF1735
FGMOS2281-20|Culex declarator|ST11396|Cayode|BOLD:AAF1735
FGMOS2268-20|Culex brevispinosus|ST11383|Elae|BOLD:AAN3636
FGMOS2269-20|Culex brevispinosus|ST11384|Elae|BOLD:AAN3636
FGMOS2283-20|Culex surinamensis|ST11398|Pidima Pata|BOLD:AAN3636
FGMOS2282-20|Culex surinamensis|ST11397|Pidima Pata|BOLD:AAN3636
FGMOS049-16|Culex usquatus|MB10049|Matiti|BOLD:AAN3636
FGMOS2284-20|Culex usquatus|ST11399|Grand Santi|BOLD:AAN3636
FGMOS046-16|Culex usquatus|MB10046|Matiti|BOLD:AAN3636
FGMOS1207-16|Culex usquatus|ST10322|Petit Saut|BOLD:AAN3636
FGMOS1208-16|Culex usquatus|ST10323|Petit Saut|BOLD:AAN3636
FGMOS1211-16|Culex usquatus|ST10326|Montsinery|BOLD:AAN3636
FGMOS2285-20|Culex usquatus|ST11400|Antecume Pata|BOLD:AAN3636
FGMOS2266-20|Culex corniger|ST11381|Loka|BOLD:ADV2314
FGMOS2267-20|Culex corniger|ST11382|Loka|BOLD:ADV2314
WRBUE110-10|Chagasia bonneae|CCDB-10764-D04|Yasuni National Park|BOLD:KF671009
0.020

Figure 2C