**Universidade de São Paulo
Faculdade de Saúde Pública**

**The subgenus *Melanoconion* of *Culex* in South America
(Diptera: Culicidae)**

Carolina Torres Gutierrez

Tese apresentada ao Programa de Pós-graduação
em Saúde Pública para obtenção do título de
Doutor em Ciências.

Área de Concentração: Epidemiologia
Orientadora: Maria Anice Mureb Sallum

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*Nunca seremos recolectores de esbeltas espigas o de flores,
si en los surcos nuestros diarios empeños carecen de la fuerza
que les dan los sueños.*

-Hesíodo-

RESUMO

Torres Gutierrez C. The subgenus *Melanoconion* of *Culex* (Diptera: Culicidae) in South America (tese de doutorado). São Paulo (BR): Faculdade de Saúde Pública da USP; 2015.

Introdução - Algumas espécies de *Culex* (*Melanoconion*) são consideradas vetores de vírus do complexo da Encefalite Equina Venezuelana e do Vírus do Nilo Ocidental. O subgênero *Melanoconion* é considerado grupo taxonômico diverso, amplamente distribuído nos países do continente americano, com exceção do Chile e Canadá. A diferenciação das espécies implica grande dificuldade taxonômica, dependendo de estudos dos caracteres morfológicos da genitália dos machos. Contudo o estudo da genitália masculina demanda destreza na dissecação das respectivas estruturas. A classificação atual do subgênero inclui 160 espécies distribuídas em dois grupos principais, a Seção *Melanoconion* e a Seção *Spissipes*. Estas seções estão subdivididas em agrupamentos não formais que foram propostos de maneira a englobar as espécies morfológicamente semelhantes, em especial por caracteres da genitália masculina. **Objetivos** - Investigar as relações filogenéticas entre as espécies do subgênero, e testar a classificação atual proposta por Sirivanakarn (1983). O estudo avaliou o monofiletismo das seções *Melanoconion* e *Spissipes*, e de alguns agrupamentos incluídos em cada uma. **Métodos** - A partir de amostra composta por 106 espécimes do subgênero (46 espécies) e empregando fragmentos de dois genes nucleares de cópia única (CAD, HB), e do gene mitocondrial (COI), foram propostas hipóteses sobre relações filogenéticas. As análises filogenéticas empregaram os métodos estatísticos de Máxima Verossimilhança e de análise Bayesiana. **Resultados e Discussão** - Apresenta-se a primeira hipótese filogenética de espécies do subgênero *Melanoconion* baseada informações de três genes. Os resultados obtidos corroboram o monofiletismo das duas seções. As espécies incluídas na Seção *Spissipes* estão subdivididas em grupos que refletem a história evolutiva e, portanto, correspondem a grupos naturais que compartilham ancestral comum. Portanto, propomos a revalidação do subgênero *Helcoporpa* Dyar transferindo para ele todas as espécies até agora incluídas na Seção *Sipissipes*, com exceção do *Culex nicaroensis*. Por outro lado,

a Seção *Melanoconion* é formada por grupos monofiléticos e outros grupos polifiléticos ou parafiléticos. Este fato sugere que serão necessários rearranjos taxonômicos na Seção *Melanoconion*, além de estudos mais abrangentes tanto em relação à amostra de espécies como outros genes. Os resultados aqui descritos mostram que o gene COI pode ser utilizado como ferramenta complementar para facilitar a identificação taxonômica das espécies de *Melanoconion*. Adicionalmente, os genes nucleares, quando analisados em conjunto, proporcionaram informação suficiente na diferenciação de seções, grupos não formais e espécies do subgênero. O uso dos três marcadores moleculares é recomendado para estudo da filogenia de *Culex* (*Melanoconion*). Este estudo contribui com informação nova e útil para melhor compreender a classificação de *Melanoconion* e auxiliar na identificação das espécies.

Descritores: *Culex*, *Melanoconion*, *Helcoporpa*, Seção *Spissipes*, Seção *Melanoconion*, sistemática, filogenia molecular.

ABSTRACT

Torres Gutierrez C. The subgenus *Melanoconion* of *Culex* (Diptera: Culicidae) in South America (doctoral thesis). São Paulo (BR): Faculdade de Saúde Pública da USP; 2015.

Introduction - Some of the species of *Culex* (*Melanoconion*) Theobald are recognized as vectors of arboviruses such as Venezuelan Equine Encephalitis Virus complex and West Nile Virus. *Melanoconion* is considered taxonomically diverse and widely distributed in the Americas, with the exception of Chile and Canada. The species of this subgenus pose a real taxonomical challenge as the identification based on morphological traits focuses mainly on the male genitalia that require well-trained skills for dissection of the structures. The current classification of the subgenus recognizes 160 species divided in two major groups, *Melanoconion* Section and *Spissipes* Section. Each one of these sections are further divided into non-formal groupings proposed to include morphologically similar species, mainly based on male genitalia characters. **Research objectives** - To investigate phylogenetic relationships between species of the subgenus *Melanoconion* of *Culex* by testing the current classification by Sirivanakarn (1983). We intended to evaluate the monophyly of the two major sections of the subgenus and some of the non-formal groups included in each section. **Methods** - Our sample taxa included 106 specimens of *Melanoconion* (46 species), from which we used fragments of two single-copy nuclear genes (CAD, HB) and the mitochondrial gene COI. We raised phylogenetic hypotheses about the subgenus *Melanoconion* based on analyses that used statistical methods such as Maximum likelihood and Bayesian inference. **Results and Discussion** - We present the first molecular phylogeny of *Melanoconion* based on information provided by three genes. The obtained phylogenetic trees strongly support that both sections, *Melanoconion* and *Spissipes*, are monophyletic groups. Furthermore the *Spissipes* Section showed phylogenetic lineages consistent with morphological classification. Thus we strongly recommend the resurrection of subgenus *Helcoporpa* Dyar to include species until now considered as *Spissipes* Section, with the exception of *Culex nicaroensis*. As for the *Melanoconion* Section, several non-formal groupings

corresponded to polyphyletic or paraphyletic groups, with only a few having monophyletic origins. This fact suggests that future taxonomic rearrangements should be consider for *Melanoconion* Section, as well as a wider sampling of species and molecular markers. Our results confirmed the use of COI gene as a complementary tool for taxonomical identifications. Additionally, the performance of nuclear genes, when analyzed together, was highly informative, showing resolution between the two sections and also for species level. As for tools to infer phylogeny, our data are in agreement with the use of multi loci approach, in order to propose a phylogenetic hypothesis. Our results contribute with new information that improves the classification of subgenus *Melanoconion* and provides useful data to help with the identification tasks.

Keywords: *Culex*, *Melanoconion*, *Helcoporpa*, Spissipes Section, *Melanoconion* Section, Systematics, molecular phylogeny.

I dedicate this thesis

To Laine Patrick Derr for his endless love, support and generosity during this journey.

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Preamble

This document is intended as a doctoral dissertation consisting of two chapters. Chapter I focuses on the taxonomic history and current distribution of the subgenus *Melanoconion* of genus *Culex* (Diptera: Culicidae), divided in two parts: Part I (Chapter I) informs the history of the subgenus *Melanoconion* as a taxonomic unit of recognized complexity. Part II (Chapter I) consists of a Catalog of the *Melanoconion* species distributed in South America.

Chapter II presents the first comprehensive molecular phylogeny of the subgenus based on DNA sequences of one mitochondrial gene and a couple of single-copy nuclear genes. This dissertation was developed within a period of four years and included an extensive bibliographic review as well as laboratorial work. This document was created using Microsoft Office Word 2007 tools and followed the guidelines of the University of São Paulo, USP.

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Chapter I

Catalog of the subgenus *Melanoconion* of *Culex* for South America

1. Introduction

Species of *Culex* subgenus *Melanoconion* Theobald represent a diverse group widely distributed in the Americas. There are 160 species considered within the subgenus and members of this group are known to occur in the southern part of North America, including United States and Mexico, Central America, some of the Caribbean islands such as Trinidad, Tobago, Jamaica, Martinique and Puerto Rico, among others; and in most of South American countries (Pecor et al. 1992). Despite its wide distribution, the subgenus *Melanoconion* shows greatest diversity in tropical areas, such as the Amazon region and other forested environments of northern and western South America. Although very common in wild areas, *Melanoconion* species have been also recorded in rural and anthropic areas of South America (Forattini et al. 1991; 1993c; 1994).

Plenty of field studies have documented the high abundance of *Melanoconion* species in forest and rural environments (Hutchings et al. 2005; 2010; 2013; Forattini et al. 1991; 1993a), however small portions of the actual surveys reach the species identification level. This fact is explained by the great taxonomic difficulty that this group of mosquitoes poses. When studying *Melanoconion*, the close similarities of female specimens prevent any accurate identification by examining morphological traits only. The most reliable taxonomic characters are found in the male genitalia, and such delicate structures demand specific protocols for slide preparation and well-trained taxonomists for the required dissections (Sallum and Forattini, 1996). Though a complex group of mosquitoes, *Melanoconion* is worth the effort, they represent a group of medically important species. Members of this subgenus are considered vectors of viruses included in the Venezuelan Equine Encephalitis complex (*Togaviridae*), and West Nile Virus (*Flaviviridae*); moreover there are records of isolation of the Eastern Equine Encephalitis Virus and other arboviruses (*Bunyaviridae* and *Flaviviridae*) from species of this subgenus.

A catalog of any insect taxon is a very useful source of information, the most recent catalog for the subgenus *Melanoconion* was published by Pecor et al. (1992), which is considered today as one of the primary sources of valid taxonomic information about the group. Some changes and descriptions of new species have been going on since Pecor et al. (1992) published their Catalog. A recent group of publications have been important contributions to the knowledge of the subgenus (Sallum and Forattini, 1996; Forattini and Sallum, 1992b,c; 1993, 1995; Sallum et al. 1997; Sallum and Hutchings, 2003; Hutchings and Sallum, 2008), however, as it is known today, *Melanoconion* still holds conflicting issues.

One of such difficult issues when studying *Melanoconion* is reaching an accurate geographical distribution of the species. The confusing task of taxonomical identification consequently reflects on the geographical knowledge; which means every misidentification of the species brings along an inaccurate distribution record.

Examples of such cases are explicit in the Revision of the Spissipes Section of the Subgenus *Melanoconion* by Sallum and Forattini (1996), with specific examples such as *Culex taeniopus* and *Cx. jubifer*.

In order to present a current catalog for the subgenus *Melanoconion* we gathered all the bibliographic records of the subgenus in South America, with particular focus on the period of time after the Catalog of Pecor et al. (1992) came available, until the present time. This compilation will clear the way to any future taxonomic revision on the subgenus or one of its non-formal groups. In this catalog we included 139 species occurring in South American countries with all the relevant bibliographic sources.

2. Methods

This chapter is divided in two parts:

Part I is a Taxonomical History of the subgenus *Melanoconion*. It includes the recount of the postulation of the taxon *Melanoconion* and its instability as a taxonomical unit through different periods of time until the formal revisions and consolidation of its subgeneric category; with mention of the recent contributions such as description and redescriptions of species. The current classification is shown for each of the two major Sections in which the subgenus is divided.

Part II is a Catalog of species occurring in South America with corresponding type localities, distribution, synonyms and bibliographical sources. All type localities were taken from Pecor et al. (1992), as well as some taxonomic and geographical references. Information about type specimens were included in the present manuscript with the corresponding authors; however, the complete information on developmental stages known for each species (including existent illustrations) can be found in Pecor et al. (1992).

For some cases such as misspelled names of type localities, corrections were made with corresponding notes of every change. Also, we conducted a geographical corroboration of all type localities from Brazil; adding a proper note where corrections were made. This section presents a compilation of all sorts of studies that have listed species of the subgenus *Melanoconion* as results of any original research. The search for publications was focused on the period of time after the Catalog and illustrated Review of the subgenus *Melanoconion* of *Culex* by Pecor et al. (1992) was published, up to the present time. All geographical records presented are limited to species occurring in South America. A few references from a period of time before 1992 were included given their great importance as sources of either geographical or ecological information for the species of *Melanoconion*.

In order to find all the publications we searched the following websites:

- 1) Web of Science (through CAPES suscription to Thomson Reuters Scientific). The search was intended to find original publications, using key words such as "Culicidae", "Mosquitoes", "Mosquito/Culicidae Inventory" and "Mosquito/Culicidae Diversity".
- 2) Google Scholar website to search for A) Original publications and B) Information related with all the species names. To follow search A, we used "Culicidae", "Mosquitoes", "Mosquito/Culicidae Inventory" and "Mosquito/Culicidae Diversity" as key words in three different languages: English, Spanish and Portuguese. To conduct search B, screening attempts were performed for all 139 species included in this monograph (for 139 species names = 139 independent search attempts).
- 3) Armed Forces Pest Management Board (United States) website (www.afpmb.org). Entering the Literature Retrieval System (<http://www.afpmb.org/content/welcome-literature-retrieval-system>), with specific search attempts using the species name in each attempt (for 139 species).
- 4) We searched the database of the University of São Paulo (Banco de Teses) (<http://www.teses.usp.br/>) in order to include results obtained by graduate students from Master and Doctoral programs.

When specific publications were unavailable using the online platforms listed above, direct contacts to the authors were conducted via email. In other cases, searching for the corresponding publications took place in local libraries such as the library of the Public Health Faculty at the University of São Paulo.

The information provided for each species follows this format:

Name of the species with the corresponding author (i. e. *dureti* Casal and Garcia, 1968:123). Names are listed under alphabetical order.

This means the information presented corresponds to the species *Culex (Melanoconion) dureti* that was described by Casal and Garcia in 1968. Once this reference is found, the species description must be in page 123. All bibliographic references concerning species descriptions are included in the "Literature cited" section, by the end of this chapter.

Holotype information: Informs about the type material used to describe the species (described by a symbol indicating whether the type material is male (♂), female (♀) or an immature stage (L, for larva; P, for pupa). In cases when the type material corresponds to an immature exuviae, the text explicitly informs the specific stage (i.e. larval exuviae or pupal exuviae). In a few cases, the only information provided by the original description consisted of the word "adult" with no further details (i. e. information regarding some of the listed synonyms).

In this section we also inform about the specific geographic locality where each type specimen was collected and the depository (museum or scientific collection) where specimens are currently conserved, using the corresponding acronym (i. e. FSP).

Distribution in South America: Includes names of countries where the given species has been documented with the corresponding bibliographic reference. Countries in bold are indicators of updated information highlighted by this study (i. e. Distribution in South america: Brazil, **Colombia**. This means this catalog is updating Colombia as part of the geographical range of a given species. An update is based on recent information not included by Pecor et al. (1992) or by Sallum and Forattini (1996).

Though in very few cases, we included old records (i.e. Forattini et al. 1970) that were not included in the Catalog by Pecor et al. (1992).

Bibliographic sources: Provides several bibliographic references for further study of the species. Informs about relevant taxonomical studies that include the species. References are given by author name and year, in chronological order.

Synonyms: The complete list of synonyms is provided with reference of the original description and type locality for each case.

For all the species included in this Catalog the name of the type depositories (Entomological collections) were given using acronyms, here listed (as informed in Pecor et al. 1992):

BA	Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina (Formerly Museo Nacional de Ciencias Naturales "Bernardino Rivadavia").
CPRR	Centro de Pesquisas Rene Rachou, Belo Horizonte, Brazil
DERM	Servicio de Entomologia Taxonomica, Division de Endemias Rurales, Maracay, Venezuela.
EUO	Sección de Entomologia, Departamento de Parasitologia y Microbiologia, Escuela de Medicina, Universidad de Oriente, Ciudad Bolivar, Venezuela.
FMRP	Departamento de Parasitologia da Faculdade de Medicina, Ribeirão Preto, São Paulo, Brazil.
FSP	Faculdade de Saúde Pública, Universidade de São Paulo, Brazil.
INM	Instituto Nacional de Microbiologia, Carlos G. Malbran, Buenos Aires, Argentina.
INPA or INPA-CPEN	Entomological Collection of Instituto Nacional de Pesquisas da Amazônia.
ITH	Instituut voor Tropische Hygiene en Geographische Pathologie, Amsterdam, Netherlands.
NHM	Natural History Museum, London, England (Formerly British Museum (Natural History)).
MNHP	Museum National d'Histoire Naturelle, Paris, France.

NE	Type non-existent.
NMNH	National Museum of Natural History, Smithsonian Institution, Washington, DC, USA.
PIG	Institut Pasteur, French Guiana.
PIP	Institut Pasteur, Paris, France.

The following information is listed in the Appendix I of this Catalog:

- 1) Publications listing unclear identifications of specimens called "near" any of the known species (i. e. near *Cx. pilosus*).
- 2) Ambiguous identifications were listed, for example "*Culex gnomatos/vomerifer*", which means the authors could not separate the two species. Therefore such records were only listed in the appendix.
- 3) Misidentifications of species. These cases were documented in some articles or taxonomic revisions of the subgenus.
- 4) Records of *Culex (Melanoconion) aikenii* correspond to a species under *nomina dubia* status (see Belkin, 1970; Pecor et al. 1992: pag. 57). Records previously identified as *Cx. aikenii* could correspond to two different valid species, *Cx. ocosa* and *Cx. panocossa*.
- 5) Some publications listed names of current synonyms of valid species (i. e. *Cx. chrysonotum* is a synonym of *Cx. theobaldi* and *Cx. mistura* is a synonym of *Cx. inadmirabilis*), and thus this Catalog included such references under the corresponding valid species in each case.

2.1 TAXONOMIC HISTORY OF SUBGENUS *MELANOCONION*

Melanoconion as a formal taxonomic name was proposed by F.V. Theobald in 1903 as a new genus of Culicidae. Its etymology from the Greek words "melanos" which means black; and "konis" meaning dust (Black dust). The original description highlighted the "squamosse character of the wings, which are very characteristic" (*squamosse* relates to the latin word *squama* which means "scale"). Furthermore, in the same reference, the six *Melanoconion* species were described as "small black gnats which bite viciously and which occur in swamps and woods" (Theobald, 1903). For several years the status of *Melanoconion* as a taxon suffered frequent changes that altogether have created a considerable level of confusion to this group of mosquitoes.

In 1905, Blanchard changed the designation from *Melanoconion* to *Melanoconium*, though other authors did not follow his version. A year later, Dyar and Knab published a taxonomic contribution to the larvae of Culicidae, in which *Melanoconion* was treated as a synonym of the genus *Culex* and *Mochlostyrax* was created as a new genus with *Mochlostyrax caudelli* Dyar and Knab, 1906 as its type species (Dyar and Knab, 1906a). More than a decade later, through the study of male genitalia's characters, Dyar (1918a) considered 16 subgenera for *Culex*, with *Cacoculex* and *Choeroporpa* as new subgenera, also re-establishing *Melanoconion* (with seven species) and *Mochlostyrax* (with two species) as subgenera. Moreover, in a different paper, same year (Dyar 1918b), he recognized *Helcoporpa* as another subgenus of *Culex*.

Later on, Dyar made new arrangements for *Culex* subgenera, still based on morphological characters of the male genitalia (see "On some of the American subgenera of *Culex*"; Dyar, 1923a). In such paper, he added and corrected to the subgeneric classification of *Culex* (i. e. postulating *Anoedioporpa* instead of *Isostomyia* for six species, and creating *Gnophodeomyia* that included two species). But in reality, this publication added to the confusing state of the genus *Culex* by that time, mainly because the author proposed new groupings for subgenera (Dyar, 1923a), without properly referring to the previous classification presented for the subgeneric level just a few years earlier (Dyar, 1918a,b).

By 1928, Dyar published "The Mosquitoes of the Americas", in which made drastic changes to the subgeneric level of *Culex*. In his monograph, Dyar only kept *Mochlostyrax* and *Melanoconion* as subgenera and transferred previously proposed taxa like *Helcoporpa*, *Anoedioporpa*, *Gnophodeomyia* and *Tinolestes*, as mere sections within subgenera. Moreover, this new arrangement included also two species from the subgenus *Micraedes* as Dyar transferred them to *Melanoconion* Section.

Dyar's classification (1928) can be summarized as follows:

Genus *Culex*

Subgenus *Mochlostyrax*:

Sections: *Dinoporpa*; *Helcoporpa*; *Choeroporpa*; *Mochlostyrax*

Subgenus *Melanoconion*:

Sections: *Tinolestes*; *Gnophodeomyia*; *Melanoconion*; *Anoedioporpa*.

Posteriorly, F.W. Edwards (1932) continued to change the already unstable *Culex* classification. In Edward's proposal, the genus *Culex* consisted of 16 subgenera, being *Mochlostyrax* and *Melanoconion* some of them. Regarding the latter, Edwards made specific comments about Dyar's classification; in Edward's opinion the grouping based on the male style (= gonostylus) were not very sharp and therefore he decided not to adopt Dyar's groupings within the subgenus. Instead, Edwards divided the subgenus *Melanoconion* into three groups (A, B, C) based on the color of hind tarsi, shape of the vertex scales and color of mesonotum. Additionally, Edward's classification treated *Gnophodeomyia*, *Tinolestes*, *Helcoporpa*; *Choeroporpa*; *Dinoporpa* and *Asebeomyia* as synonyms of subgenus *Melanoconion*. For the subgenus *Mochlostyrax* the included species were the same as the ones included by Dyar (1928) in the section under the same name. Furthermore, Edwards considered *Anoedioporpa* as synonym of *Isostomyia*.

Other significant publication by Komp and Curry (1932), proposed *Upsilonporpa* as a new subgenus (for a new species -*haynei*- from Panama). And more importantly, the revision by Komp (1935), approaching the validity of type species of *Melanoconion* and *Mochlostyrax*, at least those deposited in the U.S. National Museum, brought a better light to the taxonomic status of these two subgenera. In Komp's words:

..."Great difficulties were encountered in making determinations, using Dyar's keys, descriptions and figures. This was particularly true in the subgenera *Mochlostyrax* and *Melanoconion*, which have always been accounted difficult

groups. Part of the difficulty lay in the inadequacy and inaccuracy of the descriptions of the male terminalia, which are the final resort in specific determinations in these groups" (Komp, 1935, p.2, 3).

Sharp comments were made by Komp in this review regarding the inadequacy of Dr. Dyar's microscope resources as well as his technique to process the delicate male genitalia parts. And then, after reviewing the U.S. National Museum's type species, 59 out of 75 type species were found as valid; and 16 species were thus indicated as synonyms. One of the findings showed that the recently described, *Culex (Upsiloporpa) haynei*, in reality corresponded to a badly mounted slide of *Cx. (Melanoconion) menytes* (Komp, 1935).

Further taxonomic corrections and revisions for the subgenera *Melanoconion* and *Mochlostyrax* were accomplished by King and Bradley (1937) for some of the species occurring in the United States. Later on, Sevenet and Abonnenc (1939) described new *Melanoconion* species for French Guiana based basically on male genitalia and larvae characters; following Edward's classification of *Culex*. By the same year, Lane (1939) published his Catalog of Neotropical mosquitoes, in which he included 10 *Culex* subgenera occurring in such a region (subgenus *Melanoconion* with 76 species and subgenus *Mochlostyrax* with 6 species). In 1943, Lane and Whitman, also adding to the species of *Culex*, reviewed the taxonomic history of *Culex (Microculex) ocellatus* and transferred the species to subgenus *Melanoconion*, describing as well a new species for the subgenus.

Not until 1950, existed a solid review of the subgenus *Melanoconion* by Lloyd E. Rozeboom and William H. W. Komp, whose work gave stability to this complex taxon. In the words of Rozeboom and Komp:

... "Few if any groups of mosquitoes present greater taxonomic confusion and difficulty than does the subgenus *Melanoconion*. At the present time the species can be recognized with certainty only by the structures of the male terminalia. The females of most species cannot be separated from one another; they are usually small and dark-colored, only a few having a distinctive golden-scaled scutum or white-ringed tarsi. The larvae of many species are readily recognizable but for the most part they are insufficiently known to permit positive identification" (Rozeboom and Komp, 1950; p. 75).

In their review, Rozeboom and Komp provided keys and illustrations for the 91 species recognized as part of the subgenus *Melanoconion*, which meant the removal of *Mochlostyrax* as a valid subgenus, merging all the species as a single subgenus. Also, they thought as artificial and erroneous Edwards's (1932) classification of the subgenus, explicitly adopting Dyar's grouping or sections (except *Anoedioporpa*), which were incorporated in their keys to species; with the keys based on several characters of the male genitalia. Also, a group of 11 species were listed as "position and validity unknown" given the lack of information on the required male characters. The proposed changes by Rozeboom and Komp (1950) were followed by Lane (1953) in his monograph on Neotropical Culicidae (vol. 1); with the exception of *Tinolestes* Section that Lane elevated as a subgenus based on the length of the male palpi.

Subgenus *Melanoconion* by Rozeboom and Komp (1950):

Sections: *Melanoconion*, *Tinolestes*, *Gnophodeomyia*, *Dinoporpa*, *Helcoporpa*, *Choeroporpa* and *Mochlostyrax*.

Unfortunately, the stability gained after Rozeboom and Komp's (1950) solid revision, did not last long. In 1952 and 1954, Foote published taxonomic descriptions and keys for larvae and pupae for the subgenera *Melanoconion* and *Mochlostyrax*; his monographs included great morphological descriptions and display of the immatures's characters. As to the matter of the subgenera, his own words were as follows:

..."After a rather exhaustive study of the morphology and chaetotaxy of the larvae of the subgenus *Melanoconion* (in the sense of Rozeboom and Komp), the writer has concluded that Dyar and Knab's genus *Mochlostyrax* is deserving of subgeneric rank, as suggested by Edwards" (Foote, 1954, p.5).

Foote's taxonomic contribution was a big step to identify the immatures of *Melanoconion* and *Mochlostyrax*, as he listed 58 species for the former and 8 species for the latter (Foote, 1954).

During the 60's and 70's, the most noteworthy contributions to *Melanoconion* were by John N. Belkin (1968) and Belkin et al. (1970). In the monograph by Belkin (1968 - The type specimens of New World Mosquitoes in European museums), he continued the notion of separating *Melanoconion* and *Mochlostyrax*, also re-arranging many of the species included in these and other subgenera of *Culex*. Belkin transferred species from *Tinolestes* to *Melanoconion*; and made other arrangements (either removing or adding species) to different

subgenera such as *Aedinus*, *Anoedioporpa* and *Micraedes*. With the publication of Belkin et al. (1970), the subgenus *Mochlostyrax* came to an end. The referred authors relegated *Mochlostyrax* to a section within *Melanoconion* based on the study of *Mochlostyrax pilosus* (species previously included in *Mochlostyrax*). The entomological community in further publications finally adopted the treatment of *Melanoconion* as consolidated subgenus.

In 1977, a couple of authors published the catalog of the mosquitoes of the world (Knight and Stone, 1977), in which 21 subgenera for *Culex* were considered, keeping the status of *Melanoconion* as Belkin et al. (1970) did; in this catalog 161 species were listed for the subgenus *Melanoconion*. In 1980, Berlin and Belkin published a revision of the subgenera *Aedinus*, *Tinolestes* and *Anoedioporpa* of *Culex*, nonetheless including *Melanoconion* in their taxonomic keys to separate adults, male genitalia, pupae and fourth-instar larvae of the species in the Americas. Moreover, they presented a very pertinent discussion and re-arranged several species that had in earlier works been included in *Melanoconion* or other subgenera.

Many contributions on the subgenus *Melanoconion* were achieved by Sirivanakarn (1978, 1979), Sirivanakarn and Belkin (1980), Sirivanakarn and Degallier (1981), Sirivanakarn and Galindo (1980) and Sirivanakarn and Jacob (1979, 1981a, 1981b). But the most significant of them was indeed the revision of the subgenus *Melanoconion* (Sirivanakarn, 1983 - "A Review of the Systematics and a proposed scheme of internal classification of the New World subgenus *Melanoconion* of *Culex*"). Before the revision by Sirivanakarn there were 165 species known in the subgenus, and after his studies and dedicated analysis to this taxon, he presented 149 species divided into two Sections and several groups and subgroups (see Table 1).

In Sirivanakarn words:

"The first step in this classification is to recognize several primary groups (groups and subgroups). These primary groups are then assembled into major sections on the basis of the type of basal hook of the lateral plate of the male genitalia, the type of decumbent scales in the center of the vertex and other features of the adults, the position of seta 9-VIII of the pupa and certain combinations of larval chaetotaxy as given in the keys". Sirivanakarn (1983).

In his monograph, Sirivanakarn provided keys to separate groups and subgroups for adults, male genitalia, pupae and larvae. Compared to the previous taxonomic revision of the subgenus by Rozeboom and Komp (1950), Sirivanakarn's keys lack information on specific levels which is a significant topic when there is an interest in reaching species-level identification of this complex taxon. Sirivanakarn clearly described all the taxonomic characters used in his

classification scheme, and further discussions on such characters were included for each of the Sections he proposed (*Spissipes*, *Melanoconion* and *Ocellatus*); however the amount and type of illustrations was rather scarce considering the size of the subgenus.

Posterior to Sirivanakarn's revision, most of the taxonomic contributions for the subgenus *Melanoconion* were published by Brazilian specialists that made specific changes to the existent grouping and more importantly added to the knowledge of the species of *Melanoconion*. Examples of significant contributions can be found in Forattini and Sallum (1985; 1987a,b; 1989a,b,c,d; 1990; 1992a), and Peyton and Harbach (1991), which contain redescriptions of several species or even the determination of new ones; with sound taxonomic studies for females, males and in some cases even for immature stages.

Table 1. Scheme of classification for subgenus *Melanoconion* proposed by Sirivanakarn (1983).

SECTION	GROUP	SUBGROUP	Number of species
Ocellatus Section	-	-	4
Spissipes Section			
	Spissipes Group		1
	Taeniopus Group	Taeniopus Subgroup	1
		Vomerifer Subgroup	3
		Pedroi Subgroup	4
	Paracrybda Group	Paracrybda Subgroup	2
		Pereyrai Subgroup	1
	Ocossa Group	-	2
	Jubifer Group	-	2
	Faurani Group	-	1
	Nicaromensis Group	-	1
	Lopesi Group	-	1
Melanoconion Section			
	Atratus Group	-	7
	Distinguendus Group	Putumayensis Subgroup	3
		Distinguendus Subgroup	7
		Chrysonotum Subgroup	1
		Galindoi Subgroup	1
		Rorotaensis Subgroup	1
	Trifidus Group	-	1
	Saramaccensis Group	-	1
	Erraticus Group	Erraticus Subgroup	3
		Clarki Subgroup	1
		Psatharus Subgroup	1
	Educator Group	-	7
	Intrincatus Group	Intrincatus Subgroup	15
		Eastor Subgroup	1
		Idottus Subgroup	5
		Tecmaris Subgroup	1
		Andricus Subgroup	1
		Penai Subgroup	1
	Bastagarius Group	Bastagarius Subgroup	6
		Iolambdis Subgroup	8
	Evansae Group	-	4
	Inhibitor Group	Inhibitor Subgroup	19
		Egcymon Subgroup	4
		Mulrennani Subgroup	1
	Conspirator Group	-	10
	Pilosus Group	Pilosus Subgroup	4
		Caudelli Subgroup	9
	Peccator Group	-	3
TOTAL SPECIES			149

A decade after Sirivanakarn's work, Pecor et al. (1992) published one of the most influential monographs on subgenus *Melanoconion*, their "Catalog and illustrated review of the subgenus *Melanoconion* of *Culex*". This catalog, still a very useful taxonomic guide to the subgenus, contains a complete compilation of all the species of *Melanoconion* described to the year of its publication. Pecor et al. compiled a total of 154 species, with 83 junior synonyms and 13 *nomina dubia*. One of the most important changes to the taxonomic classification of the subgenus was the removal of Ocellatus Section, explained by Pecor et al. as follows: "We have determined that this group of species (*Culex ocellatus* Theobald, 1903; *Cx. nigrimacula* Lane and Whitman, 1943; *Cx. punctiscapularis* Floch and Abonnenc, 1946; *Cx. flochi* Duret, 1969) does not belong in the subgenus *Melanoconion*". To this date, the Ocellatus Section continues to be without any subgeneric placement.

The catalog by Pecor et al. (1992) does not present any keys to the species or groups but instead included reproductions of original drawings of male genitalic structures, with a couple of species having their genitalia drawn for the first time (i.e. *Culex adamesi* Sirivanakarn and Galindo, 1980 and *Culex crybda* Dyar, 1924b). Moreover, for each one of the species included in their catalog, Pecor et al. presented valid names, type species information, geographical distribution (indicated as a country-list) and the complete synonymy records. This catalog represents an invaluable taxonomic tool for any researcher interested in the subgenus.

The most recent taxonomical contributions to the subgenus were published by Forattini and Sallum (1992a) and Sallum and Forattini (1996). In these monographs, the authors studied the Spissipes Section of the subgenus and based their study on morphological characteristics. In the Revision of the Spissipes Section of *Melanoconion*, Sallum and Forattini (1996) recognized 22 species making some taxonomic changes needed such as the designation of a synonym (*Cx. alvarezi* synonymized with *Cx. spissipes*) and the removal of *Cx. nicaroensis* from *Melanoconion*, remarking that the species belonged to *Culex*, without subgeneric assignment. Later, Gonzalez and Rodriguez (2001) proposed the subgenus *Nicaromyia* to include the species *Cx. nicaroensis*. Additionally, Sallum and Forattini (1996) provided descriptions of adult females and males and notes on distribution and bionomics of the species. More importantly, these authors provided taxonomic keys to the species level with corresponding illustrations (for adult characters as well as for male genitalia). The outcome regarding the arrangement of the Spissipes Section included eight groups and three subgroups readily characterized (see Table 2).

Table 2. Classification of the Spissipes Section proposed by Sallum and Forattini (1996).

SECTION	GROUP	SUBGROUP	Number of species
Spissipes Section			
	Spissipes Group	-	1
	Taeniopus Group	-	4
	Crybda Group	Pedroi Subgroup	5
		Paracrybda Subgroup	2
		Pereyrai Subgroup	1
	Vomerifer Group	-	4
	Ocossa Group	-	2
	Jubifer Group	-	2
	Lopesi Group	-	1
	Faurani Group	-	1
TOTAL SPECIES			23

After the catalog published by Pecor et al. (1992), several new species were described mainly from Brazilian regions such as São Paulo and the Amazon states (Forattini and Sallum, 1992b,c; 1993, 1995; Sallum et al. 1997; Sallum and Hutchings, 2003; Hutchings and Sallum, 2008). These papers were based mainly on characters of the male genitalia, with complete descriptions of the male adults and for some species such as *Culex ikelos* Forattini & Sallum, 1995, and *Cx. eknomios* Forattini & Sallum (1992b) the immature forms (larvae and pupae) were fully described as well.

Although Sirivanakarn (1983) did not provide taxonomic keys to the specific level, his scheme of classification was always taken into account by the majority of authors who contributed with descriptions and redescrptions of species. Almost all the taxonomic publications have discussed the position of the newly described species in terms of Sirivanankarn's classification of Sections, Groups and Subgroups. Considering this, the updated classification of the subgenus *Melanoconion* comprises two sections, 21 groups, 23 subgroups and 160 species (Table 3). The species of *Melanoconion* are distributed mainly in the Neotropical region (which includes Central America, the Caribbean islands and South America), with some species occurring in the Nearctic Region (United States and northern Mexico).

Table 3. Current classification of the subgenus *Melanoconion* based on records of the published literature*.

SECTION	GROUP	SUBGROUP	Number of species
Melanoconion Section	Atratus Group	-	7
	Bastagarius Group	-	1
		Bastagarius Subgroup	9
		Iolambdis Subgroup	8
	Conspirator Group	-	10
	Distinguendus Group	Distinguendus Subgroup	7
		Galindoi Subgroup	1
		Putumayensis Subgroup	3
		Rorotaensis Subgroup	1
	Educator Group	-	7
	Erraticus Group	Clarki Subgroup	1
		Erraticus Subgroup	3
		Psatharus Subgroup	1
	Evansae Group	-	4
	Inhibitor Group	-	1
		Egcymon Subgroup	5
		Inhibitor Subgroup	21
		Mulrenani Subgroup	1
	Intrincatus Group	Andricus Subgroup	1
		Eastor Subgroup	1
		Idottus Subgroup	5
		Intrincatus Subgroup	16
		Penai Subgroup	1
		Tecmaris Subgroup	1
	Peccator Group	-	3
	Pilosus Group	Caudelli Subgroup	9
		Pilosus Subgroup	4
	Saramaccensis Group	-	2
	Trifidus Group	-	1
	Undetermined Group		2
	Melanoconion species		137

Table 3 continues in next page.

SECTION	GROUP	SUBGROUP	Number of species
Spissipes Section	Crybda Group	Paracrybda Subgroup	2
		Pedroi Subgroup	5
		Pereyrai Subgroup	1
	Faurani Group	-	1
	Jubifer Group	-	2
	Lopesi Group	-	1
	Ocossa Group	-	2
	Spissipes Group	-	1
	Taeniopus Group	-	4
	Vomerifer Group	-	4
Spissipes species			23
TOTAL			160

* Sources: Mosquito taxonomic inventory (Harbach, 2012. Website/<http://mosquito-taxonomic-inventory.info/>); Sallum and Forattini (1996); Harbach (2011)).

2.2 CATALOG OF THE SUBGENUS *MELANOCONION* OF *CULEX* FOR SOUTH AMERICA

1. *abonnenci* Clastrier, 1970a:468 (♂).

Holotype ♂: Foret du Gallion, French Guiana (MNHP).

Distribution in South America: French Guiana.

Bibliographic sources: Known life stages and taxonomic information registered in Pecor et al. (1992). Ecological notes can be found in Forattini et al. (1986a,b; 1993a).

2. *adamesi* Sirivanakarn and Galindo, 1980:26 (♂, ♀, P, L).

Holotype ♂: across canal from Summit, "Empire Firing Range", Canal Zone, Panama (NMNH).

Distribution in South America: Brazil, Colombia, Ecuador, French Guiana, **Peru**.

Bibliographic sources: The original description recorded the species occurring in Brazil, Colombia, Ecuador, French Guiana and Panama (Sirivanakarn and Galindo, 1980). Taxonomic contributions registered in Pecor et al. (1992), and Sallum and Forattini (1996). This species was included in the taxonomic revision of the *Spissipes* Section by Sallum and Forattini (1996). Recent geographical records in Dickerman et al. (1986); Forattini et al. (1988); Need et al. (1993); Souto et al. (1996); Barrera et al. (2002); Pecor et al. (2000); Moncayo et al. (2001); Jones et al. (2004); Navarro and Weaver (2004); Hutchings et al. (2005; 2008; 2011; 2013); Ferro et al. (2003, 2008); Barbosa et al. (2008); Gomes et al. (2008); Johnson et al. (2008); D'Avila (2011); Confalonieri and Costa-Neto (2012); Andrews et al. (2014).

3. *akritos* Forattini and Sallum, 1995:125 (♂,♀).

Holotype ♂: Experimental Station, Pariquera-Açu County, São Paulo, Brazil (FSP- USP).

Distribution in South America: Brazil.

Bibliographic sources: Taxonomic description and known life stages in Forattini and Sallum (1995); plus an important taxonomic contribution in Sallum and Forattini (1996). Ecological notes can be found in Forattini et al. (1989). Recent geographical records in Forattini and Sallum (1991); Ramos (2009); D'Avila (2011); Laporta (2012); Sá and Sallum (2013) e Sant'Ana (2013) and Chaves et al. (2014).

4. *albinensis* Bonne-Wepster and Bonne, 1920: 173 (♂, ♀, L).

Lectotype ♂: Paramaribo, Republic of Suriname (ITH).

Distribution in South America: Argentina, Brazil, Colombia, French Guiana, Republic of Paraguay, Republic of Suriname, Venezuela.

Bibliographic sources: Known life stages, taxonomic and geographical references in Pecor et al. (1992). Additional geographical records in Forattini et al. (1970; 1973); Sutil (1980); Walder et al. (1984); Suarez et al. (1992); Navarro and Liria (2000); Fé et al. (2003); Alfonzo et al. (2005); Hutchings et al. (2011); Stein et al. (2012); Del Ventura et al. (2013).

Synonyms:

maroniensis Bonne-Wepster and Bonne, 1920: 175 (♂). Holotype ♂: Republic of Suriname (ITH).

gordoni Evans, 1924: 369 (♂). Holotype ♂: Bosque, Manaus, Amazonas, Brazil (NHM).

5. *alcocki* Bonne-Wepster and Bonne, 1920:171 (♂, ♀, L; as *alcocci*).

Lectotype ♂: Kabelstation, Republic of Suriname (ITH).

Distribution in South America: French Guiana, Republic of Suriname.

Bibliographic sources: Known life stages and taxonomic contributions registered in Pecor et al. (1992). Belkin (1968; pag. 12) corrected the name to *alcocki* and designated the Lectotype.

6. *aliciae* Duret, 1953:75 (♂).

Holotype ♂: Misiones, Cerro Azul (Arroyo San Juan), Argentina (NMNH).

Distribution in South America: Argentina, Bolivia, Brazil, Republic of Paraguay.

Bibliographic sources: Known life stages, taxonomic and geographical contributions registered in Pecor et al. (1992). Recent geographical records in Forattini et al. (1987a,b; 1989; 1993a,b,c); Rossi et al. (2006); Demari-Silva et al. (2011); Vesgueiro et al. (2011); Dantur Juri et al.(2012); Laporta (2012); Ribeiro et al.(2012).

7. *alinkios* Sallum and Hutchings, 2003:619 (♂).

Holotype ♂: Itapuan Farm, Itapitangui village, Cananéia, São Paulo, Brazil (FSP-USP).

Distribution in South America: Brazil.

Bibliographic sources: Known life stages and taxonomic descriptions in Sallum and Hutchings (2003). Recent geographical records in Laporta (2012); Ribeiro et al. (2012; 2014).

8. *alogistus* Dyar, 1918:126 (♂,♀).

Lectotype ♂: Republic of Suriname (NMNH).

Distribution in South America: Brazil, Colombia, French Guiana, Republic of Suriname, Venezuela.

Bibliographic sources: Known life stages, taxonomic and geographical contributions registered in Pecor et al. (1992). Other relevant information can be found in Belkin et al. (1965); Xavier and Mattos (1975) and Heinemman and Belkin (1978). Recent geographical records in Hutchings et al.(2002; 2011; 2013).

Synonyms:

megapus Root, 1927: 595 (♂). Holotype ♂ (genitalia only): Angra dos Reis, Rio de Janeiro, Brazil (NMNH).

9. *amitis* Komp, 1936:333 (♂).

Lectotype ♂: Quiriquire (near Maturin), (Monagas), Venezuela (NMNH).

Distribution in South America: Venezuela.

Bibliographic sources: Known life stages and taxonomic contributions registered in Pecor et al. (1992). Geographical records in Forattini et al. (1973); Sutil (1980) and Del Ventura et al. (2013).

10. *andricus* Root, 1927:592 (♂).

Holotype ♂: Lassance, Minas Gerais, Brazil (NMNH).

Distribution in South America: Brazil.

Bibliographic sources: Known life stages and taxonomic references in Pecor et al. (1992). Recent geographical records for Brazil can be found in Gomes et al. (2007).

11. *anoplicitus* Forattini and Sallum, 1989a:1 (♂).

Holotype ♂: Vilarinho farm, Itapitangui village, Cananeia, São Paulo, Brazil (FSP).

Distribution in South America: Brazil.

Bibliographic sources: Known life stages in Forattini and Sallum (1989a).

12. *atratus* Theobald, 1901:55 (♂,♀).

Lectotype ♂: Ferry Swamp, Jamaica (NHM).

Distribution in South America: Brazil, Guyana, Republic of Suriname, Venezuela.

Bibliographic sources: Known life stages and relevant contributions in Pecor et al. (1992). Recent geographical records in Salas et al. (2001); Barbosa et al. (2008); Tissot and Silva (2008) and Suárez-Mutis et al. (2009).

Synonyms:

falsificator Dyar and Knab, 1909c: 257 (Adult). Lectotype ♂: Havana, Cuba (NMNH).

advieri Senevet, 1938: 185 (♂). Holotype ♂: Prise d'Eau de Pointe-a-Pitre, Guadeloupe (MNHP).

13. *aureonotatus* Duret & Barreto, 1956:91 (♂).

Holotype ♂: Monte Alegre Farm, Ribeirão Preto, São Paulo, Brazil (FMRP).

Distribution in South America: Brazil.

Bibliographic sources: Known life stages and taxonomic information in Pecor et al. (1992). Recent geographical and ecological records in Brazil can be found in Lourenço-de-Oliveira (1984); Lourenço-de-Oliveira and Silva (1985); Lourenço-de-Oliveira et al. (1985); Lourenço-de-Oliveira and Heyden (1986); Forattini et al. (1987a,b; 1989a,b; 1993a,b,c); Forattini et al. (1988); Urbinatti et al. (2001); Montes (2005); Gomes et al. (2007); Paula et al. (2007); Ramos (2009); Sugimoto (2009); Laporta (2012); Ribeiro et al. (2012); Chaves et al. (2014).

14. *bahiensis* Duret, 1969b:40 (♂).

Holotype ♂: Uruçuca, Bahia, Brazil (NMNH) (The municipality is here corrected with the corresponding portuguese consonant).

Distribution in South America: Argentina, Brazil, Colombia.

Bibliographic sources: Relevant taxonomic references in Pecor et al. (1992). Description of known life stages in Lestani and Rossi (2012); recent geographical records in Ferro et al. (2008); Hutchings et al. (2011).

15. *bastagarius* Dyar and Knab, 1906b:170 (♂, L).

Lectotype ♂: Laventille, Trinidad (NMNH).

Distribution in South America: Argentina, Brazil, Colombia, Ecuador, French Guiana, Republic of Paraguay, Peru, Republic of Suriname, Venezuela.

Bibliographic sources: Known life stages, geographical and taxonomic references in Pecor et al. (1992). Some ecological notes in Forattini et al. (1989; 1987a,b; 1993a,b,c; 1997). Geographical records in Forattini et al. (1970); Sutil (1980); Forattini and Sallum (1991); Suarez et al. (1994); Luz and Lourenço-de-Oliveira (1996); Quintero et al. (1996); Pecor et al. (2000); Rossi et al. (2002); Fe et al. (2003); Gomes et al. (2007; 2008); Ferro et al. (2008); Sugimoto (2009); Ramos (2009); Suárez-Mutis et al. (2009); Cardoso et al.

(2010); Julião et al. (2010); Viana et al. (2010); Hutchings et al. (2005; 2011); D'Avila (2011); Cardoso et al. (2011); Hoyos et al. (2011); Mendenhall et al. (2012); Ribeiro et al. (2012); Stein et al. (2012); Del Ventura et al. (2013); Cardo et al. (2013); Sá and Sallum (2013); Ribeiro (2014).

Synonyms:

vapulans Dyar, 1920:69 (♂). Lectotype ♂: Paramaribo, Republic of Suriname (NMNH).

alfaroi Dyar, 1921a: 34 (♂, L). Holotype ♂: Atirro, Costa Rica (NMNH).

innominatus Evans, 1924: 363 (♂). Lectotype ♂: Itacoatiara, Amazonas, Brazil (NHM).

cuclyx Dyar and Shannon, 1924a: 48 (♂). Holotype ♂: Cardenas River, Fort Clayton, Canal Zone, Panama (NMNH).

thomasi Evans, 1924: 372 (♂). Holotype ♂: Manaus, Amazonas, Brazil (NHM).

16. *batesi* Rozeboom and Komp, 1948:403 (♂, ♀, L).

Holotype ♂: Villavicencio, Meta, Colombia (NMNH).

Distribution in South America: Colombia, Ecuador, French Guiana.

Bibliographic sources: Known life stages, taxonomic and geographical information in Pecor et al. (1992). Recent geographical records can be found in Hutchings et al. (2011).

17. *bejaranoi* Duret, 1953:75 (♂).

Holotype ♂: Bernardo de Irigoyen, Misiones, Argentina (NMNH).

Distribution in South America: Argentina.

Bibliographic sources: Known life stages and relevant taxonomic references in Pecor et al. (1992).

18. *bequaerti* Dyar and Shannon, 1925:39 (♂, ♀).

Holotype ♂: Sororoca, Rio Branco river, Roraima, Brazil (NMNH) (Type locality is here modified to include a correct geographical information).

Distribution in South America: Brazil.

Bibliographic sources: Known life stages and relevant taxonomic references in Pecor et al. (1992). Recent geographical records can be found in Hutchings et al. (2005; 2010, 2011; 2013).

19. *bifolius* Duret and Barreto, 1956:96 (♂).

Holotype ♂: Fazenda Monte Alegre, Ribeirão Preto, São Paulo, Brazil (FMRP).

Distribution in South America: Brazil.

Bibliographic sources: Known life stages and relevant taxonomic references in Pecor et al. (1992).

20. *brachius* Hutchings and Sallum 2008: 45 (♂).

Holotype ♂: Parque Nacional do Jaú, Jaú River, Novo Airão, Amazonas, Brazil (INPA).

Distribution in South America: Brazil.

Bibliographic sources: Male description, bionomics and taxonomic notes in Hutchings and Sallum (2008). Recent geographical records can be found in Hutchings et al. (2005; 2011; 2013).

21. *caudatus* Clastrier, 1970a: 470 (♂).

Holotype ♂: Forêt de Cabassou, French Guiana (MNHP).

Distribution in South America: Brazil, French Guiana.

Bibliographic sources: Known life stages and relevant taxonomic references in Pecor et al. (1992). Recent geographical records can be found in Hutchings et al. (2005, 2010, 2011; 2013).

22. *caudelli* (Dyar and Knab, 1906a:224) (L; *Mochlostyrax*).

Holotype Le: Arima, Trinidad (NMNH).

Distribution in South America: Brazil, Colombia, French Guiana, Guyana, Republic of Suriname, Peru, Venezuela.

Bibliographic sources: Known life stages and taxonomic and geographical references in Pecor et al. (1992). Ecological contributions can be found in Forattini et al. (1993a,c). Additional geographical records in Forattini et al. (1970; 1973); Sutil (1980); Quintero et al. (1996); Pecor et al. (2000); Barrera et al. (2002); Mendez et al. (2001); Alfonzo et al. (2005); Demari-Silva et al. (2011); Vesgueiro et al. (2011); Hutchings et al. (2011; 2013); Del Ventura et al. (2013).

Synonyms:

multispinosus Bonne-Wepster and Bonne 1920: 177 (♂, ♀, L). Lectotype ♂: Kabelstation, Surinam (ITH).

23. *clarki* Evans, 1924:365 (♂).

Lectotype ♂: Manaus, Amazonas, Brazil (NHM).

Distribution in South America: Argentina, Brazil, Eastern Republic of Uruguay, Republic of Paraguay, Venezuela.

Bibliographic sources: Known life stages, relevant taxonomic and geographical information in Pecor et al. (1992). Recent geographical records in Rossi (2000); Rossi et al. (2002); Rossi and Martinez (2003); Paula and Gomes (2007); Gomes et al. (2007; 2008); Viana et al. (2010); Hutchings et al. (2011; 2013); Stein et al. (2006; 2013); Rossi (2014).

24. *comatus* Senevet and Abonnenc, 1939:103 (♂, L).

Holotype ♂: Crique Mangue, Saut-Tigre, Inini, French Guiana (NE).

Distribution in South America: Brazil, Colombia, French Guiana, **Venezuela**.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Recent geographical records can be found in Suarez et al. (1992); Hutchings et al. (2005, 2010, 2011; 2013).

25. *commevynensis* Bonne-Wepster and Bonne, 1920:176 (♂).

Holotype ♂: Alkmaar, (Commewijne), Republic of Suriname (ITH).

Distribution in South America: **Brazil**, Colombia, French Guiana, Republic of Suriname.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Recent geographical records can be found in Kobayashi (1999); Gomes et al. (2007); Hutchings et al. (2011).

26. *comminutor* Dyar, 1920:70 (♂).

Holotype ♂: near Paramaribo, Republic of Suriname (NMNH).

Distribution in South America: Brazil, Colombia, Ecuador, French Guiana, Republic of Suriname.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992).

27. *confundior* Komp and Rozeboom, 1951:132 (♂).

Holotype ♂: Paramaribo, Republic of Suriname (NMNH).

Distribution in South America: Republic of Suriname.

Bibliographic sources: Known life stages in Pecor et al. (1992).

28. *conspirator* Dyar and Knab, 1906a:217 (L).

Lectotype (larval exuviae and associated pupal exuviae ♀): Almoloya, Oaxaca, Mexico (NMNH).

Distribution in South America: Colombia, Ecuador, Venezuela.

Bibliographic sources: Known life stages and taxonomic and geographical information in Pecor et al. (1992). Recent geographical records in Sutil (1980); Suarez et al. (1994); Alfonzo et al. (2005); Del Ventura et al. (2013).

Synonyms:

holoneus Dyar, 1921a: 35 (♂). Holotype ♂: Orotina, Costa Rica (NMNH).

dysmathes Dyar and Ludlow, 1921: 47 (♂, ♀). Lectotype ♂: Cativa, Canal Zone, Panama (NMNH).

psadaemon Dyar, 1921c: 100 (♂). Holotype ♂: San Jose, Costa Rica (NMNH).

merodaemon Dyar, 1921c:100 (♂). Holotype ♂: Orotina, Costa Rica (NMNH).

fatuator Dyar and Shannon, 1924a:47 (♂). Holotype ♂: Cardenas River, Canal Zone, Panama (NMNH).

meroneus Dyar, 1925a: 22 (♂). Holotype ♂: Barranquilla, (Atlántico), Colombia (NMNH). (type locality corrected).

macaronensis Dyar and Nunez Tovar, 1927:153 (♂). Holotype ♂ (genitalia only): Macaro, Venezuela (NMNH).

inducens Root, 1928 (*in* Dyar, 1928: 307) (♂, ♀). Lectotype ♂: (Maracay, Aragua), Venezuela (NMNH).

29. *contei* Duret, 1968c:121 (♂).

Holotype ♂: São Miguel do Guamá, Pará, Brazil (NMNH).

Distribution in South America: Brazil, French Guiana.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Ecological notes can be found in Forattini et al.

(1993c). Recent geographical records can be found in Gomes et al. (2007; 2008); Hutchings et al. (2010; 2011); Sugimoto (2009).

Synonyms:

seneveti Clastrier, 1970a: 464 (♂). Holotype ♂: Forêt de Cabassou, Cayenne, French Guiana (MNHP).

30. *coppenamensis* Bonne-Wepster and Bonne, 1920:173 (♂, L).

Lectotype ♂: Kabelstation, Republic of Suriname (ITH).

Distribution in South America: Brazil, Colombia, French Guiana, Republic of Suriname, Venezuela.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Ecological notes can be found in Forattini et al. (1986a, b). Taxonomic contributions in Sallum and Hutchings (2003) and Hutchings and Sallum (2008). Additional geographical records in Forattini et al. (1970; 1973); Sutil (1980); Lopes and Lozovei (1995); Lopes (2002); Hutchings et al. (2011; 2013); Del Ventura et al. (2013).

31. *corentynensis* Dyar, 1920:65 (♂).

Lectotype ♂: Republic of Suriname (NMNH).

Distribution in South America: Brazil, French Guiana, Republic of Suriname.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Ecological notes in Forattini et al. (1986a,b; 1993a,b,c; 1997). Recent geographical records in Gomes et al. (1987); Hutchings et al. (2005, 2011; 2013); Ramos (2009).

32. *creole* Anduze, 1949b:149 (♂).

Holotype ♂: Caripito, Monagas, Venezuela (FSP).

Distribution in South America: Brazil, Colombia, French Guiana, Venezuela.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Additional geographical records in Forattini et al. (1970); Sutil (1980); Hutchings et al. (2005, 2010); Del Ventura et al. (2013).

33. *crisovaoi* Duret, 1968a:14 (♂).

Holotype ♂: Caracarai, Roraima, Brazil (NMNH).

Distribution in South America: Brazil, French Guiana.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Recent geographical records in Hutchings et al. (2005, 2011).

34. *crybda* Dyar, 1924b:184 (♂).

Holotype ♂: Atrato River, Murindó (Antioquia), Colombia (NMNH).

Distribution in South America: Brazil, Colombia, Venezuela.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992), and Sallum and Forattini (1996). This species was included in the taxonomic revision of the Spissipes Section by Sallum and Forattini (1996). Recent geographical records in Sutil (1980); Dickerman et al. (1986); Walder et al. (1984); Molina et al. (2000); Barrera et al. (2002); Ferro et al. (2008); Suárez-Mutis et al. (2009); Hutchings et al. (2002, 2005, 2011; 2013).

35. *delpontei* Duret 1969a:8 (♂).

Holotype ♂: Las Palmas, Chaco, Argentina (NMNH).

Distribution in South America: Argentina, Brazil, **Eastern Republic of Uruguay**, Republic of Paraguay.

Bibliographic sources: Known life stages and relevant taxonomic references in Pecor et al. (1992) and Sallum and Forattini (1996). The species was redescribed by Forattini and Sallum (1989b) and included in the taxonomic revision of Spissipes Section by Sallum and Forattini (1996), with significant information on bionomics. Later on Sallum et al. (2001) described the immatures (larva, pupa) of this species.

Ecological contributions in Mitchell et al. (1985; 1987) and Forattini et al. (1989; 1991; 1993b; 1995). Recent geographical records in Brewer et al. (1991); Rossi (2000); Rossi et al. (2002a,b); Oscherov et al. (2007); Gomes et al. (2007; 2008); Ramos (2009); D'Avila (2011); Hoyos et al. (2011); Dantur Juri et al. (2012); Ribeiro et al. (2012); Stein et al. (2004; 2005; 2006; 2013).

36. *distinguendus* Dyar 1928:305 (♂,♀).

Lectotype ♂: Mojinga Swamp, Canal Zone, Panama (NMNH).

Distribution in South America: Brazil, Colombia, Ecuador, French Guiana, **Peru**, **Venezuela**.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Ecological contributions in Forattini et al. (1987a, 1993a,b,c). Additional geographical records in Forattini et al. (1970; 1973); Pecor et al. (2000); Hutchings et al. (2002); Alfonzo et al. (2005); Ramos (2009); Ribeiro et al. (2012); Del Ventura et al. (2013).

37. *dolichophyllus* Clastrier, 1970b:858 (♂).

Holotype ♂: Foret de Cabassou, Cayenne, French Guiana (MNHP).

Distribution in South America: **Brazil**, French Guiana.

Bibliographic sources: Known life stages and taxonomic reference in Pecor et al. (1992). Recent geographical records in Hutchings et al. (2011; 2013).

38. *dunni* Dyar 1918b:123 (♂,♀).

Lectotype ♂: Mandingo River (Canal Zone), Panama (NMNH).

Distribution in South America: **Argentina**, Brazil, Colombia, Ecuador, French Guiana, Republic of Paraguay, **Peru**, Republic of Suriname, Venezuela.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Some ecological contributions in Forattini et al. (1987b; 1993a,b,c) and Gabaldon et al. (1977). Additional geographical records in Forattini et al. (1970); Sutil (1980); Walder et al. (1984); Groot et al. (1996); Rossi (1997);

Kobayashi (1999); Pecor et al. (2000); Mendez et al. (2001); Moncayo et al. (2001); Salas et al. (2001); Barrera et al. (2002); Rossi et al. (2002b); Jones et al. (2004); Alfonzo et al. (2005); Gomes et al. (2007; 2008); Barbosa et al. (2008); Ferro et al. (2008); Johnson et al. (2008); Ramos (2009); Sugimoto (2009); Hutchings et al. (2011); Berti et al. (2013); Del Ventura et al. (2013); Andrews et al. (2014).

Synonyms:

ruffinis Dyar and Shannon, 1924b:143 (♂). Holotype ♂: Barro Colorado Island, Gatun Lake, Canal Zone, Panama (NMNH).

exedrus Root, 1927:580 (♂,♀). Lectotype ♂: Porto das Caixas, Rio de Janeiro, Brazil (NMNH).

39. *dureti* Casal and Garcia, 1968b:123 (♂).

Holotype ♂: San Ignacio, Misiones, Argentina (INM).

Distribution in South America: Argentina, Brazil, Republic of Paraguay, Venezuela.

Bibliographic sources: Known life stages and taxonomic references in Pecor et al. (1992). Geographical records in Sugimoto (2009); D'Avila (2011); Laporta (2012); Del Ventura et al. (2013); Ribeiro (2014).

40. *dyius* Root 1927:587 (♂).

Holotype ♂: Brazil ("Probably obtained in the costal lowlands of the state of Rio de Janeiro") (NMNH).

Distribution in South America: Brazil, French Guiana.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Recent geographical records in Demari-Silva et al. (2011); Vesgueiro et al. (2011); Hutchings et al. (2005; 2011).

41. *eastor* Dyar 1920a:71 (♂).

Holotype ♂: Republic of Suriname (NMNH).

Distribution in South America: Brazil, Colombia, Ecuador, French Guiana, Republic of Suriname, Venezuela.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Additional geographical records in Forattini et al. (1970; 1973; 1988); Xavier and Mattos (1970); Panday (1975); Walder et al. (1984 - documented the species with a misspelled mistake as "*Cx. castor*"); Groot et al. (1996); Pecor et al. (2000); Barbosa et al. (2008); Gomes et al. (2008); Hutchings et al. (2005; 2010; 2011; 2013); Linton et al. (2013); Sant'Ana (2013).

Synonyms:

manaosensis Evans, 1924: 370 (♂). Holotype ♂: Manaus, (Amazonas), Brazil (NHM) (locality corrected).

42. *educator* Dyar and Knab, 1906a:217 (L).

Syntype (Larval exuviae with associated ♀): Rio Aranjuez, near Puntarenas, Costa Rica (NMNH).

Distribution in South America: Argentina, Bolivia, Brazil, Colombia, Ecuador, French Guiana, Peru, Republic of Suriname, Venezuela.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). This species is well reviewed with all stages described and notes on distribution and bionomics in Forattini and Sallum (1993b). Some ecological contributions in Forattini et al. (1991). Additional geographical records in Forattini et al. (1970); Sutil (1980); Peyton et al. (1983); Lopes (2002); Rossi et al. (2002b); Visintin et al. (2009; 2010); Hoyos et al. (2011); Parra-Henao and Suárez (2012); Stein et al. (2004; 2005; 2006; 2012; 2013); Del Ventura et al. (2013).

Synonyms:

apeteticus Howard, Dyar and Knab, 1913: Fig. 91 (♂). Lectotype ♂: Upper Pequini River, Panama (NMNH).

aneles Dyar and Ludlow, 1922:63 (♂,♀). Lectotype ♂: Cardenas River, Canal Zone, Panama (NMNH).

keenani Galindo and Mendez, 1961: 2 (♂). Holotype ♂: Pacora, Panama (NMNH).

43. *egcymon* Dyar, 1923b:67 (♂,♀).

Lectotype ♂: Tabernilla, Canal Zone, Panama (NMNH).

Distribution in South America: Colombia.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992).

44. *eknomios* Forattini and Sallum, 1992:265 (♂, ♀, L, P).

Holotype ♂: Guaporé River, Costa Marques, Rondonia, Brazil (NMNH).

Distribution in South America: Brazil, Ecuador.

Bibliographic sources: Description of life stages in Forattini and Sallum (1992) with notes on distribution. Recent geographical records in Hutchings et al. (2005; 2008; 2010; 2011; 2013).

45. *elephas* Komp, 1936:328 (♂).

Holotype ♂: Juan Diaz, Panama (NMNH).

Distribution in South America: Colombia, Venezuela.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Geographical records in Sutil (1980); Groot et al. (1996); Suarez et al. (1994); Del Ventura et al. (2013).

46. *elevator* Dyar and Knab, 1906a:217 (L).

Lectotype (Larval exuviae with associated pupal exuviae, ♀): Puerto Limon, Costa Rica (NMNH).

Distribution in South America: Argentina, Brazil, Colombia, Ecuador, French Guiana, Venezuela.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Additional geographical records in Forattini et al. (1970); Xavier and Mattos (1970); Sutil (1980); Groot et al. (1996); Stein et al. (2004; 2012); Linton et al. (2013); Del Ventura et al. (2013).

Synonyms:

dornarum Dyar and Shannon, 1924a: 46 (♂). Holotype ♂: Sweet Water Reservoir, Fort Sherman, Canal Zone, Panama (NMNH).

curryi Dyar, 1926: 112 (♂, ♀, L). Lectotype ♂: Mojinga River Swamp, Canal Zone, Panama (NMNH).

bonneti Senevet, 1938: 187 (♂). Holotype ♂: Pointe-a-Pitre, Guadeloupe Island, Lesser Antilles (MNHP).

vogelsangi Anduze, 1949a:61 (♂). Holotype ♂ (genitalia only): Caripito, Monagas, Venezuela (FSP).

47. *ensifomis* Bonne-Wepster and Bonne, 1920:176 (♂, ♀, L).

Lectotype ♂: Dam, Republic of Suriname (ITH).

Distribution in South America: Brazil, Bolivia, French Guiana, Republic of Suriname.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Some ecological contributions in Forattini et al. (1987b; 1991; 1993a,c). Geographical records in Peyton et al. (1983); Kobayashi (1999); Natal et al. (2007); Ramos (2009); Hutchings et al. (2011); Ribeiro (2014).

48. *epanastasis* Dyar, 1922: 191 (♂).

Holotype ♂: Arenal River, Toro Point, Canal Zone, Panama (NMNH).

Distribution in South America: French Guiana.

Bibliographic sources: Known life stages and relevant information in Pecor et al. (1992) and Sallum and Forattini (1996). Sirivanakarn and Jakob (1979) registered the species in Brazil, however this record was considered to be a misidentification of *Culex ribeirensis* by Forattini and Sallum (1985) in their description of *Culex (Melanoconion) ribeirensis* Forattini and Sallum, 1985; also from Brazil. This species was included in the taxonomic revision of Spissipes Section by Sallum and Forattini (1996).

Synonyms:

pseudotaeniopus Galindo and Blanton, 1954:240 (♂, P, L). Holotype ♂: Mojinga Swamp, Canal Zone, Panama (NMNH).

49. *equinoxialis* Floch and Abonnenc, 1945a:3 (♂).

Holotype ♂: Camp Rochambeau, French Guiana (NE).

Distribution in South America: Brazil, French Guiana.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Additional geographical records in Forattini et al. (1973; 1988).

50. *ernanii* Duret, 1968e:74 (♂).

Holotype ♂: Boa Vista, Roraima, Brazil (NMNH).

Distribution in South America: Brazil.

Bibliographic sources: Known life stages and taxonomic information in Pecor et al. (1992).

51. *ernsti* Anduze, 1949a:63 (♂).

Holotype ♂: Caripito, Monagas, Venezuela (FSP).

Distribution in South America: Venezuela.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Geographical records in Sutil (1980); Del Ventura et al. (2013).

52. *erraticus* (Dyar and Knab, 1906a: 224) (L; *Mochlostyrax*).

Lectotype (Larval exuviae): Baton Rouge, Louisiana, United States (NMNH).

Distribution in South America: Brazil, Colombia, Ecuador, French Guiana, Republic of Paraguay, Peru, Republic of Suriname, Venezuela.

Bibliographic sources: Known life stages and geographical information in Pecor et al. (1992). Additional geographical records in Forattini et al. (1970); Sutil (1980); Walder et al. (1984); Salas et al. (2001); Alfonzo et al. (2005); Mendenhall et al. (2012); Parra-Henao and Suárez (2012); Del Ventura et al. (2013).

Synonyms:

leprincei Dyar and Knab, 1907a:202 (♂,♀). Lectotype ♂: Tabernilla, Canal Zone, Panama (NMNH).

egberti Dyar and Knab, 1907b: 214 (♀). Lectotype ♂: Warner's Camp, Lake Okeechobee, Florida, United States (NMNH).

trachycampa Dyar and Knab, 1909b: 101 (♂,♀). Lectotype ♂: Las Cascadas, Canal Zone, Panama (NMNH).

peribleptus Dyar and Knab, 1918: 181 (♂,♀). Lectotype ♂: Parr Shoals, South Carolina, United States (NMNH).

pose Dyar and Knab, 1918a:182 (♀). Holotype ♂: Dallas, Texas, United States (NMNH).

moorei Dyar, 1918a:108 (♀). Lectotype ♂: Plantation Plaisance, Demerara, British Guiana (Georgetown, Guyana) (NMNH).

degustator Dyar, 1921b:39 (♂). Lectotype ♂: Scott, Arkansas, United States (NMNH).

homoepas Dyar and Ludlow, 1921:46 (♂). Holotype ♂: Jackson Barracks, New Orleans, Louisiana, United States (NMNH).

borinqueni Root, 1922: 400 (♂, ♀, L). Lectotype ♂: Rio Piedras, (San Juan), Puerto Rico (NMNH).

tovari Evans, 1924:367 (♂). Holotype ♂: Palo Negro, (Aragua), Venezuela (NHM).

53. *evansae* Root, 1927: 593 (♂, L).

Lectotype ♂: Magé, Rio de Janeiro, Brazil (NMNH).

Distribution in South America: Brazil, Ecuador, French Guiana.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Ecological contributions in Forattini et al. (1981; 1986a,b; 1987b; 1989a,b; 1991; 1993b,c); Gomes et al. (1987). Geographical records in Forattini et al. (1970); Xavier and Mattos (1970); Gomes et al. (1987; 2007); Linton et al. (2013); Sant'Ana (2013).

54. *fairchildi* Galindo and Blanton, 1954: 234 (♂).

Holotype ♂: Patino Point, Darien, Panama (NMNH).

Distribution in South America: Brazil, Venezuela.

Bibliographic sources: Known life stages and relevant taxonomic references in Pecor et al. (1992). Geographical records in Suarez et al. (1994); Hutchings et al. (2005; 2010; 2011; 2013); Del Ventura et al. (2013).

55. *faurani* Duret, 1968e: 77 (♂).

Holotype ♂: Manaus, Amazonas, Brazil (NMNH) (Type locality corrected from "Manaos" to "Manaus").

Distribution in South America: Brazil, French Guiana.

Bibliographic sources: Known life stages and some taxonomic references in Pecor et al. (1992) and Sallum and Forattini (1996). This species was included in the taxonomic revision of Spissipes Section by Sallum and Forattini (1996). Ecological notes in Forattini et al. (1995). Geographical records in Laporta (2012); Sá and Sallum (2013); Sant'Ana (2013); Chaves et al. (2014).

56. *ferreri* Duret, 1968e: 79 (♂).

Holotype ♂: Vereda Agualasal, Cúcuta, Norte de Santander, Colombia (NMNH) (Type locality corrected as to include "vereda", according to corresponding geographical data).

Distribution in South America: Colombia, Venezuela.

Bibliographic sources: Known life stages and relevant taxonomic references in Pecor et al. (1992). Geographical records in Moncayo et al. (2001); Ferro et al. (2003; 2008); Del Ventura et al. (2013).

57. *flabellifer* Komp, 1936: 323 (♂).

Holotype ♂: Santa Rosa, Colon, Panama (NMNH).

Distribution in South America: Brazil, French Guiana, Venezuela.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Recent geographical records in Gomes et al. (2008); Sugimoto (2009); Hutchings et al. (2011); Del Ventura et al. (2013).

58. *foliafer* Komp and Rozeboom, 1951: 121 (♂).

Holotype ♂: Republic of Suriname (NMNH).

Distribution in South America: Brazil, French Guiana, Republic of Suriname.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Recent geographical records in Hutchings et al. (2005; 2010; 2011; 2013).

59. *galindoi* Komp and Rozeboom, 1951: 131 (♂).

Holotype ♂: Quebrada Escondida, Rio Pequeni, Colon, Panama (NMNH).

Distribution in South America: Brazil.

Bibliographic sources: Known life stages and relevant taxonomic information in Pecor et al. (1992). Recent geographical records in Hutchings et al. (2005; 2011).

60. *galvaei* Duret 1968b: 60 (♂).

Holotype ♂: Xerém, Duque de Caxias, Rio de Janeiro, Brazil (NMNH) (Type locality corrected).

Distribution in South America: Brazil.

Bibliographic sources: Known life stages and relevant taxonomic references in Pecor et al. (1992). Ecological notes of the species can be found in Forattini et al. (1986a,b; 1993c,b). Recent geographical records in Laporta (2012).

61. *garcesi* Duret, 1968b: 63 (♂).

Holotype ♂: Quibdo, Choco, Colombia (NMNH)

Distribution in South America: Colombia.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992).

62. *glyptosalphinx* Harbach, Peyton and Harrison, 1984: 185 (♂, ♀, L, P).

Holotype ♂: Santa Cruz, Sandoval, Rincón del Tigre, Bolivia (NMNH).

Distribution in South America: Argentina, Bolivia, Brazil, Republic of Paraguay.

Bibliographic sources: Relevant taxonomic references in Pecor et al. (1992). Geographical records in Forattini et al. (1988); Stein et al. (2004, 2005); Ribeiro et al. (2012).

63. *gnomatos* Sallum, Hutchings and Ferreira, 1997:215 (♂, ♀).

Holotype ♂: Miratucu River, Parque Nacional do Jaú, Novo Airão, Amazonas, Brazil (INPA-CPEN).

Distribution in South America: Brazil, Peru, Venezuela.

Bibliographic sources: Description of the species (female and male) in Sallum et al. (1997), including taxonomic diagnosis to include in the keys previously provided by the revision of Sallum and Forattini (1996). Geographical records in Pecor et al. (2000); Salas et al. (2001); Jones et al. (2004); Navarro and Weaver (2004); Yanoviak et al. (2005); Hutchings et al. (2005; 2008; 2010; 2011; 2013); Turrell et al. (2005; 2006; 2008); Kondig et al. (2007); Andrews et al. (2014); Lawrence et al. (2014).

64. *guedesi* da Silva Mattos and Xavier, 1991: 193 (♂).

Holotype ♂: Lagoa dos Mares, Lagoa Santa, Minas Gerais, Brazil (FSP).

Distribution in South America: Brazil.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992).

65. *herrerae* Sutil Oramas, Pulido Florenzano and Amarista Meneses, 1987: 81 (♂).

Holotype ♂: Jabilla, Queseras del Medio, Apure, Venezuela (DERM).

Distribution in South America: Venezuela.

Bibliographic sources: Known life stages and relevant taxonomic information in Pecor et al. (1992).

66. *idottus* Dyar, 1920a: 77 (♂).

Holotype ♂: Republic of Suriname (NMNH).

Distribution in South America: Argentina, Bolivia, Brazil, Colombia, French Guiana, Republic of Paraguay, Republic of Suriname, Eastern Republic of Uruguay, Venezuela.

Bibliographic sources: Known life stages and taxonomic and geographical information in Pecor et al. (1992). Ecological notes in Lourenço de Oliveira (1984) and Gabaldon et al. (1977). Geographical records in Sutil (1980); Dickerman et al. (1986); Peyton et al. (1983); Ronderos et al. (1992); Suarez et al. (1992); Rossi (1995; 2000); Rossi et al. (2002a,b); Natal et al. (2007); Paula and Gomes (2007); Gomes et al. (2007; 2008); Sugimoto (2009); Viana et al. (2010); Dibo et al. (2011); Hutchings et al. (2011); Del Ventura et al. (2013); Rossi (2014).

Synonyms:

terepaima Anduze, 1949a:65(♂). Holotype ♂: El Valle, Distrito Federal, Venezuela (FSP).

67. *ikelos* Forattini and Sallum, 1995: 132 (♂, ♀, L, P).

Holotype ♂ (with associated larval and pupal exuviae): Campolim district, Sorocaba, São Paulo, Brazil (FSP).

Distribution in South America: Brazil.

Bibliographic sources: The description of the species can be found in Forattini and Sallum (1995), with the complete description and diagnosis of all life stages (adults, larvae and pupa) and notes on bionomics. The species is included in the revision of the Spissipes Section by Sallum and Forattini (1996) with notes on bionomics.

68. *inadmirabilis* Dyar, 1928: 297 (♂).

Holotype ♂: São Paulo, Brazil (NMNH).

Distribution in South America: Argentina, Brazil, Colombia, French Guiana, Venezuela.

Bibliographic sources: Relevant taxonomic and geographical information in Pecor et al. (1992). The species was redescribed by Forattini and Sallum (1993a) with complete descriptions of adults, larva and pupa; as well as notes on bionomics. Ecological contributions in Forattini et al. (1989b; 1990; 1991; 1993a,b). Other geographical records in Sutil (1980); Gomes et al. (1987); Rossi (1997); Rossi et al. (2002b); Ramos (2009); Laporta (2012); Berti et al. (2013 -as *Culex mistura*); Del Ventura et al. (2013); Medeiros-Sousa et al. (2013a,b).

Synonyms:

mistura Komp and Rozeboom, 1951:124 (♂, ♀, L). Holotype ♂: Laguna de la Palmita, Villavicencio, Meta, Colombia (NMNH).

69. *inhibitor* Dyar and Knab, 1906a: 216 (L).

Syntype Larval exuviae with associated ♀: San Francisco mines, Santo Domingo, Dominican Republic (NMNH).

Distribution in South America: Brasil, Colombia, French Guiana, Republic of Suriname, Venezuela (See Appendix I concerning distribution in Brazil).

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Geographical records in Sutil (1980); Lopes (2002); Del Ventura et al. (2013).

Synonyms:

investigator Dyar and Knab, 1906a: 216 (L). Syntypes L: Santa Lucrecia (Veracruz), Mexico (NE).

70. *innovator* Evans, 1924: 373 (♂).

Lectotype ♂: Itacoatiara, Amazonas, Brazil (NHM).

Distribution in South America: Brazil, French Guiana.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Ecological notes in Forattini et al. (1993a,b,c; 1994). Geographical records in Barbosa et al. (2008); Hutchings et al. (2011; 2013).

71. *intrincatus* Brethes, 1916: 214 (♂).

Holotype ♂: San Isidro (8 miles north of Buenos Aires), Argentina (BA).

Distribution in South America: Argentina, Bolivia, Brazil, Eastern Republic of Uruguay, French Guiana, Republic of Suriname, Venezuela.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). Ecological notes in Forattini et al. (1981; 1986a,b; 1987b; 1991; 1993a,c; 1997) and Gabaldon et al. (1977). Recent geographical records can be found in Sutil (1980); Sirivanakarn and Jakob (1981); Brewer et al. (1991); Forattini and Sallum (1991); Ronderos et al. (1992); García et al. (1994); Lopes and

Lozovei (1995); Maciá et al. (1996); Pecor et al. (2000); Rossi (2000); Urbinatti et al. (2001); Lopes (2002); Rossi et al. (2002a,b); Stein et al. (2004, 2005); Alfonzo et al. (2005); Stein et al. (2006); Ramos (2009); Laporta (2012); Ribeiro et al. (2012); Cardo et al. (2011a,b; 2012a,b; 2013); Del Ventura et al. (2013); Medeiros-Sousa et al. (2013a,b); Stein et al. (2011; 2012; 2013); Ribeiro (2014).

Synonyms:

cenus Root, 1927:590 (♂). Lectotype ♂: Mage, Rio de Janeiro, Brazil (NMNH).

xivylis Dyar, 1920a:78 (♂, L). Holotype ♂: Republic of Suriname (NMNH).

72. *iolambdis* Dyar, 1918a: 106 (♂).

Holotype ♂: Panama (NMNH).

Distribution in South America: Colombia, Ecuador, Venezuela.

Bibliographic sources: Known life stages and relevant taxonomic and geographical information in Pecor et al. (1992). The pupa of the species was redescribed by Darsie (2002). Geographical records in Forattini et al. (1970); Sutil (1980); Del Ventura et al. (2013); Linton et al. (2013).

73. *isabelae* Duret, 1968e: 72 (♂).

Holotype ♂: Caracarai, Roraima, Brazil (NMNH).

Distribution in South America: Brazil.

Bibliographic sources: Known life stages and taxonomic reference in Pecor et al. (1992). Geographical records in Pecor et al. (2000); Hutchings et al. (2005; 2011).

74. *johnnyi* Duret, 1968d:17 (♂).

Holotype ♂: Rio Preto da Eva, Amazonas, Brazil (NMNH).

Distribution in South America: Brazil.

Bibliographic sources: Known life stages and original type locality was given in Pecor et al. (1992), however, this publication follows the corrections made by Hutchings et al.

(2011) on the type locality of this species. Recent geographical records can be found in Hutchings et al. (2010; 2011).

75. *johnsoni* Galindo and Mendez, 1961:1 (♂).

Holotype ♂: Pacora, Panama (NMNH).

Distribution in South America: Brazil, Colombia.

Bibliographic sources: Known life stages and taxonomic reference in Pecor et al. (1992). Recent geographical records in Hutchings et al. (2005; 2011; 2013).

76. *kummi* Komp and Rozeboom, 1951: 130 (♂).

Holotype ♂: Chino Swamp, Almirante, Bocas del Toro, Panama (NMNH).

Distribution in South America: Colombia.

Bibliographic sources: Known life stages and relevant information in Pecor et al. (1992).

77. *lacertosus* Komp and Rozeboom, 1951: 123 (♂).

Holotype ♂: Almirante, Bocas del Toro, Panama (NMNH).

Distribution in South America: Brazil, French Guiana.

Bibliographic sources: Known life stages and geographical references in Pecor et al. (1992). Recent geographical records in Hutchings et al. (2002; 2011).

78. *lopesi* Sirivanakarn and Jakob, 1979: 139 (♂).

Holotype ♂: Porto do Rio Ribeira, Iguape, São Paulo, Brazil (NMNH).

Distribution in South America: Brazil.

Bibliographic sources: Known life stages and geographical references in Pecor et al. (1992). The redescription of the species was published by Forattini and Sallum (1990) with complete description of adults and immatures. The species was included in the

revision of the *Spissipes* Section by Sallum and Forattini (1996) with information on bionomics. Ecological contributions in Forattini et al. (1986b; 1987a; 1989a; 1991; 1993; 1995). Geographical records in Sirivanakarn and Jakob (1981); Calisher et al. (1982); Gomes et al. (1987); Forattini et al. (1988); Ramos (2009); Hutchings et al. (2011).

79. *Lucifugus* Komp, 1936: 331 (♂).

Holotype ♂: Quiriquire (near Maturin), Monagas, Venezuela (NMNH).

Distribution in South America: Argentina, Colombia, Ecuador, Venezuela.

Bibliographic sources: Known life stages and taxonomic reference in Pecor et al. (1992). Additional geographical records in Sutil (1980); Groot et al. (1996); Del Ventura et al. (2013).

80. *martinezi* Casal and Garcia, 1968a: 455 (♂, L, P).

Holotype ♂: Vespucio-San Pedrito Road, km 34, Salta, Argentina (INM).

Distribution in South America: Argentina.

Bibliographic sources: Known life stages and taxonomic reference in Pecor et al. (1992).

81. *maxinocca* Dyar, 1920a: 71 (♂, L).

Lectotype ♂: Republic of Suriname (NMNH).

Distribution in South America: Brazil, French Guiana, Republic of Suriname.

Bibliographic sources: Known life stages and taxonomic and geographical references in Pecor et al. (1992). Recent geographical records in Medeiros-Sousa et al. (2013b).

Synonyms:

tosimus Dyar, 1920a:72 (♂, L). Holotype ♂: Republic of Suriname (NMNH).

82. *mesodenticulatus* Galindo and Mendez, 1961: 2 (♂).

Holotype ♂: Almirante, Bocas del Toro, Panama (NMNH).

Distribution in South America: Brazil.

Bibliographic sources: Known life stages and taxonomic reference in Pecor et al. (1992). Recent geographical records in Hutchings et al. (2010).

83. *milwardi* Xavier and da Silva Mattos, 1972: 569 (♂).

Holotype ♂: Ressaca district, Belo Horizonte, Minas Gerais Brazil (FSP).

Distribution in South America: Brazil.

Bibliographic sources: Known life stages and taxonomic reference in Pecor et al. (1992).

84. *missionensis* Duret, 1953: 75 (♂).

Holotype ♂: Aristobulo del Valle, Misiones, Argentina (NMNH).

Distribution in South America: Argentina, Brazil.

Bibliographic sources: Known life stages and taxonomic and geographical references in Pecor et al. (1992). Ecological contributions on the species in Forattini et al. (1981; 1986a; 1987a,b; 1989a; 1990; 1993b,c). Geographical records in Sirivanakarn and Jakob (1981); Calisher et al. (1982); Gomes et al. (1987); Rossi et al. (2002b); Santos-Neto and Lozovei (2008); Tissot and Silva (2008); D'Avila (2011); Laporta (2012); Sant'Ana (2013); Ribeiro et al. (2012); Ribeiro (2014).

85. *nicceriensis* Bonne-Wepster and Bonne, 1920: 174 (♂, ♀, L).

Lectotype ♂: Kabelstation, Republic of Suriname (ITH).

Distribution in South America: Republic of Suriname, Venezuela.

Bibliographic sources: Known life stages and taxonomic and geographical references in Pecor et al. (1992). Other geographical records in Sutil (1980); Navarro and Liria (2000).

86. *ocossa* Dyar and Knab, 1919: 6 (♂).

Lectotype ♂: British Guiana (Georgetown, Guyana) (NMNH).

Distribution in South America: Argentina, Brazil, Colombia, Ecuador, Guayana, **Peru**, Republic of Suriname, Venezuela.

Bibliographic sources: Relevant references in Pecor et al. (1992). This species was included in the Revision of the Spissipes Section by Sallum and Forattini (1996) with notes on bionomics. Sallum et al. (2001) provided full description of immatures (larva, pupa) for this species. Some ecological notes of this species can be found in Forattini et al. (1987b; 1989b; 1991; 1993b,c; 1995).

Geographical records in Sutil (1980); Pecor et al. (2000); Mendez et al. (2001); Salas et al. (2001); Barrera et al. (2002); Barghini et al. (2004); Stein et al. (2004; 2005; 2006; 2013); Jones et al. (2004); Santos et al. (2005); Yanoviak et al. (2005); Turrell et al. (2005); Gomes et al. (2007, 2008); Ferro et al. (2008); Johnson et al. (2008); Hutchings et al. (2008; 2010; 2011); Morrison et al. (2008); Ramos (2009); Sugimoto (2009); D'Avila (2011); Laporta (2012); Del Ventura et al. (2013); Evangelista et al. (2013); Sá and Sallum (2013); Andrews et al. (2014).

87. *oedipus* Root, 1927: 588 (♂).

Lectotype ♂: Magé, Rio de Janeiro, Brazil (NMNH).

Distribution in South America: Argentina, Brazil, **Eastern Republic of Uruguay**, Ecuador.

Bibliographic sources: Known life stages and taxonomic and geographical references in Pecor et al. (1992). Redescription of the species in Forattini and Sallum (1987a) with full description of adults and immature stages. Ecological studies with records of this species can be found in Forattini et al. (1981; 1986a,b; 1987a,b; 1989a,b; 1990; 1993b,c).

Additional geographical records in Lourenço de Oliveira (1984); Lourenço de Oliveira et al. (1986); Forattini et al. (1988); Rossi (1996; 2000; 2002); Rossi et al. (2002b); Gomes et al. (2008); Ramos (2009); Dantur Juri et al. (2012); Laporta (2012); Ribeiro et al. (2012); Sant'Ana (2013); Rossi (2014).

88. *olimpioi* Xavier, da Silva and da Silva Mattos, 1970: 183 (♂).

Holotype ♂: Rio Branco, Acre, Brazil (CPRR).

Distribution in South America: Brazil, Peru.

Bibliographic sources: Known life stages and taxonomic and geographical references in Pecor et al. (1992). Other geographical records in Pecor et al. (2000); Jones et al. (2004).

89. *orfilai* Duret, 1953: 74 (♂).

Holotype ♂: Iguazú, Villa Tacuara, Misiones, Argentina (NMNH).

Distribution in South America: Argentina, Brazil, Eastern Republic of Uruguay.

Bibliographic sources: Known life stages and taxonomic and geographical references in Pecor et al. (1992). Ribeiro et al. (2012); Ribeiro (2014); Rossi (2014).

90. *palaciosi* Duret, 1968d: 15 (♂).

Holotype ♂: Boa Vista, Roraima, Brazil (NMNH).

Distribution in South America: Brazil, French Guiana.

Bibliographic sources: Known life stages and taxonomic and geographical references in Pecor et al. (1992). Other geographical records in Ramos (2009).

91. *panocossa* Dyar, 1923c: 120 (♂).

Lectotype ♂: Bas Obispo, Canal Zone, Panama (NMNH).

Distribution in South America: Colombia, Venezuela.

Bibliographic sources: Known life stages, and several taxonomic and geographical references in Pecor et al. (1992). This species was included in the Revision of the Spissipes Section by Forattini and Sallum (1996) with notes on bionomics. Geographical records in Sutil (1980).

92. *paracrybda* Komp, 1936: 330 (♂).

Holotype ♂: Juan Diaz, Panama (NMNH).

Distribution in South America: Republic of Suriname.

Bibliographic sources: Known life stages and relevant references in Pecor et al. (1992). This species was included in the Revision of the Spissipes Section by Forattini and Sallum (1996).

93. *patientiae* Floch and Fauran, 1955: 1 (♂).

Holotype ♂: (genitalia only) Patience (Haute-Mana), French Guiana (PIG).

Distribution in South America: French Guiana.

Bibliographic sources: Taxonomic reference in Pecor et al. (1992).

94. *pavlovskyi* Casal and Garcia, 1967: 20 (♂).

Holotype ♂: Aeropuerto de Camba Punta, Corrientes, Argentina (INM).

Distribution in South America: Argentina, Brazil.

Bibliographic sources: Known life stages and taxonomic reference in Pecor et al. (1992). Additional geographical records in Forattini et al (1997); Gomes et al. (2007; 2008) and Ramos (2009).

95. *pedroi* Sirivanakarn and Belkin, 1980: 12 (♂, ♀, L, P).

Holotype ♂: Juan Mina (0.5 km S of Rio Chagres), Canal Zone, Panama (NMNH).

Distribution in South America: Argentina, Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, Republic of Suriname, Venezuela.

Bibliographic sources: Important references in Pecor et al. (1992). This species was included in the Revision of the Spissipes Section by Sallum and Forattini (1996), with

notes on bionomics and distribution. Some ecological notes can be found in Forattini et al. (1981; 1986a,b; 1987a,b; 1989a,b; 1991; 1993b,c; 1995).

Additional geographical records in Dickerman et al. (1986); Gomes et al. (1987); Luz and Lourenço de Oliveira (1996); Souto et al. (1996); Pecor et al. (2000); Turrell et al. (2000; 2005; 2006; 2008); Mendez et al. (2001); Moncayo et al. (2001); Salas et al. (2001); Barrera et al. (2002); Hutchings et al. (2002; 2005 2008; 2010; 2011; 2013); Rossi et al. (2002b); Ferro et al. (2003; 2008); Jones et al. (2004); O'Guinn et al. (2004); Navarro and Weaver (2004); Alfonzo et al. (2005); Yanoviak et al. (2005); Kondig et al. (2007); Barreto et al. (2008); Johnson et al. (2008); Barbosa et al. (2008); Santos-Neto and Lozovei (2008); Tissot and Silva (2008); Nunes et al. (2009); Suarez-Mutis et al. (2009); Guedes (2010); Confalonieri and Costa-Neto (2012); Dantur Juri et al. (2012); Laporta (2012); Parra-Henao and Suárez (2012); Ribeiro et al. (2012); Del Ventura et al. (2013); Sá and Sallum (2013); Sant'Ana (2013); Andrews et al. (2014); Chaves et al. (2014); Guedes and Navarro-Silva (2014); Lawrence et al. (2014).

96. *penai* Sirivanakarn, 1979: 135 (♂).

Holotype ♂: Chapare, San Antonio, Bolivia (NMNH).

Distribution in South America: Bolivia, Ecuador.

Bibliographic sources: Known life stages and references in Pecor et al. (1992).

97. *pereyrai* Duret, 1967: 81 (♂).

Holotype ♂: Cecilio Baez, Caaguazu, Republic of Paraguay (NMNH).

Distribution in South America: Brazil, Republic of Paraguay.

Bibliographic sources: Known life stages and relevant references in Pecor et al. (1992). The species was redescribed by Forattini and Sallum (1989b) and included in the Revision of the Spissipes Section by Sallum and Forattini (1996) with notes on bionomics. Later on Sallum et al. (2001) fully described the immature forms (larva, pupa). Some ecological notes can be found in Forattini et al. (1991; 1993b,c; 1995). Additional geographical records in Gomes et al. (1987); D'Avila (2011); Ribeiro et al. (2012).

98. *phlabistus* Dyar, 1920a: 63 (♂).

Holotype ♂: Republic of Suriname (NMNH).

Distribution in South America: Brazil, French Guiana, Republic of Suriname.

Bibliographic sources: Relevant references in Pecor et al. (1992). Recent geographical records in Hutchings et al. (2005; 2011).

Synonyms:

kerri Duret, 1968a:11 (♂). Holotype ♂: Rio Preto, Joao Goulard, Amazonas, Brazil (NMNH).

99. *phlogistus* Dyar, 1920a: 61 (♂, L).

Holotype ♂: Republic of Suriname (NMNH).

Distribution in South America: Brazil, Colombia, French Guiana, Republic of Suriname, Venezuela.

Bibliographic sources: Known life stages, and several taxonomic and geographical references in Pecor et al. (1992). Additional geographical records in Forattini et al. (1970; 1973; 1988); Sutil (1980); Forattini et al. (1988); Groot et al. (1996); Forattini and Sallum (1987a); Hutchings et al. (2005; 2011).

100. *phyllados* Hutchings and Sallum, 2008: 42 (♂).

Holotype ♂: Igarapé Miracutu, Jaú River, Parque Nacional do Jaú, Novo Airão municipality, Amazonas, Brazil (INPA).

Distribution in South America: Brazil.

Bibliographic sources: Complete description of the adult male in Hutchings and Sallum (2008), with notes on distribution and bionomics. Recent geographical records in Hutchings et al. (2010; 2011; 2013).

101. *pifanoi* Anduze, 1949a: 60 (♂).

Holotype ♂: Caripito, Monagas, Venezuela (FSP).

Distribution in South America: Venezuela.

Bibliographic sources: Known life stages, and several taxonomic and geographical references in Pecor et al. (1992). Additional geographical records in Sutil (1980) and Del Ventura et al. (2013).

102. *pilosus* (Dyar and Knab, 1906a: 224) (L, *Mochlostyrax*).

Lectotype Larval exuviae (with associated pupal exuviae, ♂): Santa Lucrecia, (Veracruz), Mexico (NMNH).

Distribution in South America: Argentina, Bolivia, Brazil, Colombia, Ecuador, French Guiana, Republic of Paraguay, Republic of Suriname, Venezuela.

Bibliographic sources: Known life stages and several taxonomic and geographical references in Pecor et al. (1992). Some ecological notes in Forattini et al. (1981; 1986a,b; 1987b; 1990; 1993b,c; 1994; 1997).

Additional geographical records in Forattini et al. (1970; 1973; 1988); Sutil (1980); Peyton et al. (1983); Lourenço de Oliveira (1984); Lourenço de Oliveira et al. (1986); Brewer et al. (1991); Naves et al. (1996; 1998); Pecor et al. (2000); Rossi et al. (2002b); Gomes et al. (2007); Anjos and Navarro-Silva (2008); Barbosa et al. (2008); Tissot and Silva (2008); Pires et al. (2009); Ramos (2009); Sugimoto (2009); D'oria et al. (2010); Cardoso et al. (2010; 2011); D'Avila (2011); Hutchings et al. (2011; 2013); Stein et al. (2011); Laporta (2012); Ribeiro et al. (2012); Del Ventura et al. (2013); Linton et al. (2013); Rossi and Lestani (2014).

Synonyms:

cubensis (Dyar and Knab, 1906a:225) (L; *Mochlostyrax*). Syntypes L: Havana, Cuba (NE).

floridanus (Dyar and Knab, 1906b:171) (L; *Mochlostyrax*). Syntypes L: Estero, Florida, United States (NE).

jamaicensis (Grabham, 1906:318) (♂, ♀, L; *Mochlostyrax*). Lectotype ♂: Kingston, (Surrey), Jamaica (NMNH).

agitator Dyar and Knab, 1907b:100 (*nom. nov.* for *cubensis* Dyar and Knab, non Bigot, 1857).

hesitator Dyar and Knab, 1907c:205 (♂, ♀). Lectotype ♂: Las Cascadas, Canal Zone, Panama (NMNH).

ignobilis Dyar and Knab, 1909a:39 (Adult). Lectotype ♂: San Antonio de los Banos, (Havana), Cuba (NMNH).

reductor Dyar and Knab, 1909c:257 (*nom. nov.* for *jamaicensis* Grabham, *non* Theobald, 1901).

deceptor Dyar and Knab, 1909c:257 (Adult). Lectotype ♂: Fort White, Florida, United States (NMNH).

mastigia Howard, Dyar and Knab, 1913: Fig. 90 (♂). Lectotype ♂: San Antonio de los Banos, (Havana), Cuba (NMNH).

curopinensis Bonne-Wepster and Bonne, 1920:177 (♂). Lectotype ♂: Republic of Suriname (ITH).

colombiensis Dyar, 1924b:184 (♂). Holotype ♂: Colombia (NMNH).

radiatus Senevet and Abonnenc, 1939: 120 (♂, L). Holotype ♂: Cayenne and Saut-Tigre, (Inini), French Guiana (MNHP).

103. *plectoporpe* Root, 1927: 589 (♂).

Lectotype ♂: Bangu district, Rio de Janeiro, Brazil (NMNH).

Distribution in South America: Argentina, Brazil, French Guiana.

Bibliographic sources: Taxonomic and geographical references in Pecor et al. (1992). Forattini and Sallum (1987a) fully redescribed the adults and immatures stages of the species; and provided notes on bionomics. Ecological notes about this species can be found in Forattini et al. (1981; 1987b; 1989a,b; 1991; 1993a,b,c). Additional geographical records in Lourenço de Oliveira (1984); Lourenço de Oliveira et al. (1986); Forattini et al. (1988); Stein et al. (2004); Ramos (2009); Sugimoto (2009).

104. *portesi* Senevet and Abonnenc, 1941: 41 (♂).

Holotype ♂: French Guiana (NE).

Distribution in South America: Brazil, **Colombia**, French Guiana, Guyana, Republic of Suriname, **Peru**, Venezuela.

Bibliographic sources: Known life stages and relevant references in Pecor et al. (1992). This species was included in the Revision of the Spissipes Section by Sallum and Forattini (1996) with notes on bionomics. Additional geographical records in Forattini et al. (1970; 1973); Sutil (1980); Lane (1992); Souto et al. (1996); Pecor et al. (2000); Jones et al. (2004); Navarro and Weaver (2004); Hutchings et al. (2005; 2008; 2010; 2011; 2013); Yanoviak et al. (2005); Turrell et al. (2005; 2006; 2008); Ferro et al. (2008); Johnson et al. (2008); Confalonieri and Costa-Neto (2012); Del Ventura et al. (2013); Andrews et al. (2014); Lawrence et al. (2014).

Synonym:

cayennensis Floch and Abonnenc, 1945b:4 (♂). Holotype ♂: Cayenne, (Guyane), French Guiana (NE).

105. *productus* Senevet and Abonnenc, 1939: 107 (♂, L).

Holotype ♂: Saint-Elie, French Guiana (NE).

Distribution in South America: Brazil, French Guiana.

Bibliographic sources: Known life stages, taxonomic and geographical references in Pecor et al. (1992). Recent records can be found in Hutchings et al. (2011); Ribeiro et al. (2012); Ribeiro (2014).

106. *psatharus* Dyar, 1920b: 173 (♂).

Lectotype ♂: Colon, Canal Zone, Panama (NMNH).

Distribution in South America: Ecuador.

Bibliographic sources: Known life stages, taxonomic and geographical references in Pecor et al. (1992).

107. *putumayensis* Matheson, 1934: 121 (♂).

Holotype ♂: Santo Antonio do Içá, Amazonas, Brazil (NMNH) (Type locality corrected with corresponding portuguese consonant and accent).

Distribution in South America: Brazil, Ecuador, French Guiana, Peru, Republic of Suriname.

Bibliographic sources: Known life stages and taxonomic and geographical references in Pecor et al. (1992). Additional geographical records in Forattini et al. (1970; 1973); Pecor et al. (2000); Hutchings et al. (2005; 2010; 2011; 2013); Ramos (2009); Laporta (2012); Sant'Ana (2013).

Synonyms:

cavernicola Floch and Abonnenc, 1945a:1 (♂; as *cavernicolus*). Holotype ♂: Cayenne, (Guyane), French Guiana (NE).

108. *quasihybridus* Galindo and Blanton, 1954: 232 (♂).

Holotype ♂: Puerto Pilon, Panama (NMNH).

Distribution in South America: Colombia.

Bibliographic sources: Known life stages and relevant references in Pecor et al. (1992).

109. *rabanicola* Floch and Abonnenc, 1946: 1 (♂; as *rabanicolus*).

Holotype ♂: Raban, French Guiana (PIP).

Distribution in South America: French Guiana, Guyana.

Bibliographic sources: Known life stages and taxonomic and geographical references in Pecor et al. (1992).

110. *rabelloi* Forattini and Sallum, 1987a: 130 (♂, ♀, L, P).

Holotype ♂: Pariquera-Mirim district, Pariquera-Açu, São Paulo, Brazil (FSP).

Distribution in South America: Argentina, Brazil, **Eastern Republic of Uruguay.**

Bibliographic sources: Description of adult and immature forms in Forattini and Sallum (1987a). Relevant references in Pecor et al. (1992). Ecological notes in Forattini et al. (1993c). Geographical records in Forattini et al. (1988); Rossi et al. (2002b); Gomes et al. (2007; 2008); D'Avila (2011); Ribeiro et al. (2012); Ramos (2009); Sugimoto (2009); Laporta (2012); Sant'Ana (2013); Rossi (2014).

111. *rachoui* Duret, 1968b: 58 (♂).

Holotype ♂: Paragominas, Pará, Brazil (NMNH) (Type locality corrected with current geographical information).

Distribution in South America: Brazil.

Bibliographic sources: Known life stages and relevant references in Pecor et al. (1992).

112. *ribeirensis* Forattini and Sallum, 1985: 171 (♂, ♀, L, P).

Holotype ♂: Ribeira Valley, Pariquera-Açu, São Paulo, Brazil (FSP).

Distribution in South America: **Argentina**, Brazil.

Bibliographic sources: Relevant references in Pecor et al. (1992). Detailed description of adults and immatures can be found in Forattini and Sallum (1985). This species was included in the Revision of the Spissipes Section by Sallum and Forattini (1996) with notes on bionomics.

Some ecological notes of the species in Forattini et al. (1981; 1986a,b; 1987a,b; 1989a,b; 1991; 1993a,b,c; 1995) and Forattini and Gomes (1988). Geographical records in Lourenço de Oliveira (1984); Lourenço de Oliveira et al. (1985); Lourenço de Oliveira and Silva (1985); Lourenço de Oliveira and Heyden (1986); Natal (1986); Forattini and Sallum (1987a); Gomes et al. (1987; 2007; 2008); Forattini et al. (1988); Lopes et al. (1995); Forattini et al. (1997); Costa (2000); Lopes (2002); Taípe-Lagos and Natal (2003); Barghini et al. (2004); Navarro and Weaver (2004); Cardoso et al. (2005; 2011); Bona and Navarro-Silva (2008); Santos-Neto and Lozovei (2008); Tissot and Silva (2008);

Ramos (2009); Sugimoto (2009); Guedes (2010); Hutchings et al. (2011); D'Avila (2011); Guedes (2012); Ribeiro et al. (2012); Sá and Sallum (2013); Chaves et al. (2014); Guedes and Navarro-Silva (2014); Rossi and Lestani (2014); Silva et al. (2014).

113. *ronderosi* de Linero, 1967: 289 (♂, ♀).

Holotype ♂: Oricopiche, Ciudad Bolivar, Bolivar, Venezuela (EUO).

Distribution in South America: Brazil, Venezuela.

Bibliographic sources: Known life stages and relevant reference in Pecor et al. (1992). Recent geographical records in Hutchings et al. (2005; 2011).

114. *rooti* Rozeboom, 1935: 251 (♂).

Lectotype ♂: Panama City, Panama (NMNH).

Distribution in South America: Argentina, Brazil, Colombia, Venezuela.

Bibliographic sources: Known life stages and relevant references in Pecor et al. (1992). Geographical records in Sutil (1980); Forattini et al. (1994); Rossi et al. (2002b); Del Ventura et al. (2013); Hutchings et al. (2013).

115. *rorotaensis* Floch and Abonnenc, 1946: 3 (♂).

Holotype ♂: Rorota, Guyane, French Guiana (PIG).

Distribution in South America: Brazil, French Guiana, Republic of Suriname.

Bibliographic sources: Known life stages and relevant references in Pecor et al. (1992). Geographical records in Forattini et al. (1970; 1988); Gomes et al. (2007); Hutchingst et al. (2011).

116. *sacchettae* Sirivanakarn and Jakob, 1982: 192 (♂, ♀).

Holotype ♂: Cananéia, São Paulo, Brazil (FSP) (Type locality was corrected).

Distribution in South America: Brazil.

Bibliographic sources: The redescription of the species, including immature forms, was given by Forattini and Sallum (1989d). Relevant references can be found in Pecor et al. (1992). Also, this species was included in the Revision of the Spissipes Section by Sallum and Forattini (1996) with notes on bionomics.

Some ecological notes are included in Forattini et al. (1986a,b; 1987a,b; 1989a,b; 1990; 1991; 1993b,c; 1995). Geographical records can be found in Natal (1986); Gomes et al. (1987); Forattini et al. (1988; 1997; 2000); Forattini and Sallum (1991); Navarro and Weaver (2004); Bona and Navarro-Silva (2008); Ramos (2009); Guedes (2010); Laporta and Sallum (2011); Laporta (2012); Ribeiro et al. (2012); Sá and Sallum (2013); Chaves et al. (2014); Guedes and Navarro-Silva (2014).

117. *saramaccensis* Bonne-Wepster and Bonne, 1920: 172 (♂, ♀, L).

Lectotype ♂: Kabelstation, Surinam River, Republic of Suriname (ITH).

Distribution in South America: Ecuador, French Guiana, Republic of Suriname.

Bibliographic sources: Known life stages, taxonomic and geographical references in Pecor et al. (1992).

Synonym:

implicatus Senevet and Abonnenc, 1939:99 (♂, L). Holotype ♂: Sinnamary, (Guyane), French Guiana (NE).

118. *serratimarge* Root, 1927: 589 (♂).

Holotype ♂: Sant' Anna de Japuiba (locality), Rio de Janeiro city, Brazil (NMNH) (The type locality was corrected with correct geographical data).

Distribution in South America: Argentina, Bolivia, Brazil, Colombia, **Ecuador**, French Guiana, Republic of Paraguay, **Peru**, Venezuela.

Bibliographic sources: Known life stages, taxonomic and geographical references in Pecor et al. (1992). Ecological notes in Forattini et al. (1993c). Additional geographical

records in Forattini et al. (1970); Sutil (1980); Suarez et al. (1994); Rossi (1995); Pecor et al. (2000); Rossi et al. (2002b); Barbosa et al. (2008); Ferro et al. (2008); Visintin et al. (2010); Hutchings et al. (2011); Stein et al. (2012); Del Ventura et al. (2013); Linton et al. (2013).

119. *silvai* Duret, 1968e: 69 (♂).

Holotype ♂: Caracarai, Roraima, Brazil (NMNH).

Distribution in South America: Brazil.

Bibliographic sources: Known life stages and relevant references in Pecor et al. (1992). Recent geographical records in Hutchings et al. (2005; 2011).

120. *simulator* Dyar and Knab, 1906a: 218 (L).

Lectotype Larva: Arima, Trinidad (NMNH).

Distribution in South America: Brazil, Venezuela.

Bibliographic sources: Relevant references in Pecor et al. (1992). This species was included in the Revision of the Spissipes Section by Sallum and Forattini (1996) with bionomics and taxonomic information. Geographical records in Forattini et al. (1970; 1973 -as *Cx. jubifer*- corrections in Forattini and Sallum, 1996); Hutchings et al. (2005; 2011); Del Ventura et al. (2013).

Synonym:

venezuelensis Anduze, 1949a:64 (♂). Holotype ♂: Caripito, Monagas, Venezuela (FSP).

121. *spathulatus* Forattini and Sallum, 1987b: 167 (♂).

Holotype ♂: Santa Helena Farm, São João da Boa Vista, São Paulo, Brazil (FSP).

Distribution in South America: Brazil.

Bibliographic sources: Forattini and Sallum (1987b) provided full description of the male adults. Geographical record in Forattini et al. (1988); Sugimoto (2009).

122. *spissipes* (Theobald, 1903: 242) (♀; Melanoconion).

Holotype ♂: Trinidad (NHM).

Distribution in South America: Bolivia, Brazil, Colombia, Ecuador, French Guiana, **Peru**, Republic of Suriname, Venezuela.

Bibliographic sources: This species was included in the Revision of the Spissipes Section by Sallum and Forattini (1996), with full description of female and male adults and notes on distribution and bionomics. Other relevant references in (Pecor et al. 1992).

Some ecological notes in Forattini et al. (1986a; 1989a,b; 1991; 1993b,c; 1995) and Gabaldon et al. (1977). Geographical records in Forattini et al. (1970; 1973); Sutil (1980); Walder et al. (1984) (These authors listed it as *Culex ferrerii* but later, Weaver et al. 2004, stated it was *Cx. spissipes* and not *Cx. ferrerii*); Dickerman et al. (1986); Roberts et al. (1987); Lane (1992); Groot et al. (1996); Quintero et al. (1996); Souto et al. (1996); Pecor et al. (2000); Mendez et al. (2001); Moncayo et al. (2001); Salas et al. (2001); Barrera et al. (2002); Ferro et al. (2003; 2008); Jones et al. (2004); Hutchings et al. (2005; 2008; 2010; 2011; 2013); Turell et al. (2005; 2006); Yanoviak et al. (2005); Barbosa et al. (2008); Johnson et al. (2008); Nunes et al. (2009); Ramos (2009); Suarez-Mutis et al. (2009); Confalonieri and Costa-Neto (2012); Berti et al. (2013); Del Ventura et al. (2013); Sá and Sallum (2013); Sant'Ana (2013); Andrews et al. (2014).

Synonyms:

fur (Dyar and Knab, 1907c:13) (♀; Melanoconion). Holotype ♀: Colon, Canal Zone, Panama (NMNH).

menytes Dyar, 1918b: 125. Holotype ♂: Trinidad River, Panama (NMNH).

haynei Komp and Curry, 1932: 82 (♂). Holotype ♂ (genitalia only): Mojinga Swamp, lower Chagres River, Canal Zone, Panama (NMNH).

alvarezi Sutil Oramas, Pulido Florenzano and Amaritsta Meneses, 1987:85 (♂). Holotype ♂: Chiricoa, San Camilo, apure, Venezuela (DERM).

123. *sursumptor* Dyar, 1924a: 123 (♂).

Lectotype ♂: Barranquilla, Atlántico, Colombia (NMNH) (Type locality has been corrected after reviewing spelling and corresponding geographical area).

Distribution in South America: Colombia, Ecuador, Venezuela.

Bibliographic sources: Known life stages and relevant references in Pecor et al. (1992). Additional geographical records in Sutil (1980) and Del Ventura et al. (2013).

Synonyms:

ligator Dyar, 1924a: 123 (♂, ♀, L). Lectotype ♂: Barranquilla (Atlántico), Colombia (NMNH) (locality corrected with corresponding geographical area).

124. *symbletos* Sallum and Hutchings, 2003: 620.

Holotype ♂: Carabinani River, Parque Nacional do Jaú, Novo Airão, Amazonas state, Brazil (INPA).

Distribution in South America: Brazil, Ecuador, Peru.

Bibliographic sources: Detailed description of the species in Sallum and Hutchings (2003). Geographical records in Pecor et al. (2000); Hutchings et al. (2005; 2010; 2011; 2013); Linton et al. (2013).

125. *taeniopus* Dyar and Knab, 1907c: 100 (♀).

Holotype ♂: Bluefields, Nicaragua (NMNH).

Distribution in South America: Argentina, Bolivia, Colombia, French Guiana, Peru, Venezuela.

Bibliographic sources: Taxonomic and geographical references in Pecor et al (1992). The species was included in the Revision of the *Spissipes* Section by Sallum and Forattini (1996) with relevant considerations about its distribution.

According to Forattini et al. (1995), Forattini and Sallum (1995) and Sallum and Forattini (1996), there are many misidentifications in the literature concerning *Cx. taeniopus*. This species can be confused with *Cx. cedecei*, *Cx. akritos* or *Cx. ikelos*. In the Revision of the

Spissipes Section of the subgenus *Melanoconion*, the authors did not confirm any record from Brazil despite the existence of some references including the species. The updated geographical records of *Cx. taeniopus* (listed below), include some localities from Brazil, which might fall into the misidentification cases previously documented by Sallum and Forattini (1996), therefore such records should be reviewed.

Geographical records can be found in Forattini et al. (1970; 1973); Sutil (1980); Gomes et al. (1987); Groot et al. (1996); Quintero et al. (1996); Lopes (2002); Rossi et al. (2002b); Ferro et al. (2008); Suarez-Mutis et al. (2009); Confalonieri and Costa-Neto (2012); Guedes (2012); Parra-Henao and Suárez (2012); Del Ventura et al. (2013). Some ecological notes of the species are included in Forattini et al. (1986a; 1987b; 1991; 1993b,c).

Synonyms:

annulipes (Theobald, 1907: 512) (♀; *Melanoconion*). Holotype ♀: Red Hills (Kingston, Surrey), Jamaica (NHM).

ophisthopus Komp, 1926: 44 (♂, ♀). Lectotype ♂: Puerto Castillo River, Honduras (NMNH).

mychonde Komp, 1928 (*in* Dyar 1928: 295) (♂). Holotype ♂ (genitalia only): Almirante, (Bocas del Toro), Panama (NMNH).

126. *tecmarsis* Dyar, 1918b: 124 (♂, ♀).

Lectotype ♂: Trinidad River, Panama (NMNH).

Distribution in South America: Brazil, Colombia, Venezuela.

Bibliographic sources: Known life stages and relevant references in Pecor et al. (1992). Geographical records in Forattini et al. (1970); Sutil (1980).

127. *terebor* Dyar, 1920a: 56 (♂).

Holotype ♂ (genitalia only): Republic of Suriname (NMNH).

Distribution in South America: Republic of Suriname, Venezuela.

Bibliographic sources: Known life stages and relevant references in Pecor et al. (1992). Additional geographical record in Forattini et al. (1970).

128. *theobaldi* (Lutz, 1904 in Bourroul 1904: 70) (♀; Melanoconion).

Lectotype ♀: Lagoão, São Miguel Arcanjo, São Paulo, Brazil, (NHM) (Type locality corrected as informed in the redescription of *Culex theobaldi* by Forattini and Sallum, 1989c).

Distribution in South America: Argentina, Bolivia, Brazil, Colombia, **Eastern Republic of Uruguay**, Ecuador, French Guiana, Peru, Republic of Suriname, Venezuela.

Bibliographic sources: The redescription of the species can be found in Forattini and Sallum (1989c) with complete descriptions of adults (male and female), and notes on distribution and bionomics. Relevant taxonomic and geographical references in Pecor et al. (1992). Ecological notes can be found in Forattini et al. (1986b; 1987a,b; 1989a,b) and Gabaldon et al. (1977) (as *Cx. chrysonotum*).

Geographical records can be found in Forattini et al. (1970); Sutil (1980); Gomes et al. (1987; 2007; 2008); Rossi (1995); Luz and Lourenço de Oliveira (1996); Quintero et al. (1996); Pecor et al. (2000); Hutchings et al. (2002; 2005; 2011; 2013); Rossi et al. (2002b); Rossi and Martinez (2003); Jones et al. (2004); Santos et al. (2005); Turrell et al. (2006); Barbosa et al. (2008); Suarez-Mutis et al. (2009); Julião et al. (2010); Viana et al. (2010); Visintin et al. (2010); D'Avila (2011); Mendenhall et al. (2012); Parra-Henao and Suárez (2012); Del Ventura et al. (2013); Linton et al. (2013); Andrews et al. (2014); Rossi (2014).

Synonyms:

chrysonotum Dyar and Knab, 1908: 57 (♂, ♀). Lectotype ♂: Ancon, Canal zone, Panama (NMNH).

aurilatus Senevet and Abonnenc, 1939: 94 (♂, L). Holotype ♂: French Guiana (NE).

129. *tournieri* Senevet and Abonnenc, 1939: 105 (♂, L).

Holotype ♂: Crique Mangué, Saut-Tigre, (Inini), French Guiana (NE).

Distribution in South America: **Brazil**, French Guiana.

Bibliographic sources: Known life stages and relevant references in Pecor et al. (1992). Recent geographical records in Hutchings et al. (2011).

130. *trigeminatus* Clastrier, 1970a: 473 (♂).

Holotype ♂: Foret du Gallion, French Guiana (MNHP).

Distribution in South America: Brazil, French Guiana.

Bibliographic sources: Known life stages and relevant references in Pecor et al. (1992). Ecological notes can be found in Forattini et al. (1986a; 1993a). Recent geographical records in Kobayahi (1999); Hutchingst et al. (2013); Ribeiro (2014).

131. *trilobulatus* Duret and Barreto, 1956: 93 (♂).

Holotype ♂: Rio Tamandua, Ribeirão Preto, São Paulo, Brazil (FMRP).

Distribution in South America: Brazil.

Bibliographic sources: Known life stages and relevant references in Pecor et al. (1992). Recent geographical record can be found in Ribeiro et al. (2012).

132. *trisetosus* Fauran, 1961: 1 (♂).

Holotype ♂: Confluence of Oyac and Conte Rivers, Saint Antoine, French Guiana (MNHP).

Distribution in South America: Brazil, French Guiana.

Bibliographic sources: Known life stages and relevant references in Pecor et al. (1992). Recent geographical records in Hutchings et al (2011).

133. *unicornis* Root, 1928: (in Dyar, 1928: 291) (♂, ♀, L).

Lectotype ♂ (genitalia only): (Maracay, Aragua), Venezuela (NMNH).

Distribution in South America: French Guiana, Venezuela.

Bibliographic sources: Known life stages and relevant references in Pecor et al. (1992). Geographical records can be found in Forattini et al. (1970); Gabaldon et al. (1977); Sutil (1980) and Del Ventura et al. (2013).

134. *vaxus* Dyar, 1920a: 73 (♂, L).

Lectotype ♂: Republic of Suriname (NMNH).

Distribution in South America: Brazil, French Guiana, Republic of Suriname.

Bibliographic sources: Known life stages and relevant references in Forattini and Sallum (1993b) who resurrected the species from synonymy with *Culex educator*. This paper calls attention to previous misidentifications of *Cx. vaxus* with *Cx. educator* and *Cx. theobaldi*, which could consequently misinform about the real distribution of the species.

Geographical records of the species are found in Lopes and Lozovei (1995); Hutchings et al. (2002; 2005; 2010; 2011; 2013); Montes (2005); Paula and Gomes (2007); Gomes et al. (2007; 2008); Ramos (2009); Suárez-Mutis et al. (2009); Sugimoto (2009); D'Avila (2011); Laporta (2012); Müller et al. (2012); Ribeiro et al. (2012); Medeiros-Sousa et al. (2013b); Sá and Sallum (2013); Sant'Ana (2013). Ecological notes in Forattini et al. (1993a; 1997).

Synonyms:

bibulus Dyar, 1920a: 74 (♂, L). Holotype ♂: Republic of Suriname (NMNH).

135. *vidali* Floch and Fauran, 1954: 4 (♂).

Holotype ♂: Moulin-de-Vidal, Ile de Cayenne, French Guiana (PIG).

Distribution in South America: French Guiana.

Bibliographic sources: Known life stages and relevant references in Pecor et al. (1992).

136. *vomerifer* Komp, 1932: 79 (♂).

Holotype ♂: Almirante, Bocas del Toro, Panama (NMNH).

Distribution in South America: Brazil, Colombia, Ecuador, French Guiana, Peru, Venezuela.

Bibliographic sources: Taxonomical and geographical records in Pecor et al. (1992). This species was included in the Revision of the Spissipes Section by Sallum and Forattini (1996), with notes on distribution and bionomics. Ecological notes are found in Forattini et al. (1981).

Additional geographical records in Forattini et al. (1988); Forattini and Sallum (1989); Lane (1992); Souto et al. (1996); Molina et al. (2000); Pecor et al. (2000); Moncayo et al. (2001); Salas et al. (2001); Barrera et al. (2002); Hutchings et al. (2002; 2005; 2008; 2010; 2011); Ferro et al. (2003; 2008); Jones et al. (2004); Navarro and Weaver (2004); Turrell et al. (2005; 2006; 2008); Yanoviak et al. (2005); Suárez-Mutis et al. (2009); Confalonieri and Costa-Neto (2012); Linton et al. (2013); Andrews et al. (2014); Lawrence et al. (2014).

137. *wepsterae* Komp and Rozeboom, 1951: 127 (♂; as *wepsteri*).

Holotype ♂, genitalia only: Paramaribo, Republic of Suriname (NMNH).

Distribution in South America: Republic of Suriname.

Bibliographic sources: Known life stages and relevant references in Pecor et al. (1992).

138. *ybarmis* Dyar, 1920a: 57 (♂, L).

Holotype ♂: Paramaribo, Republic of Suriname (NMNH).

Distribution in South America: Brazil, French Guiana, Republic of Suriname, Venezuela.

Bibliographic sources: Known life stages and relevant references in Pecor et al. (1992). Geographical records in Forattini et al. (1970; 1973); Barbosa et al. (2008); Suarez-Mutis et al. (2009); Hutchings et al. (2010; 2011); Demari-Silva et al. (2011); Vesgueiro et al. (2011).

Synonyms:

jonistes Dyar, 1920a: 79 (♂). Holotype ♂: Republic of Suriname (NMNH).

139. *zeteki* Dyar, 1918b: 122 (♂; as *zeteci*).

Holotype ♂: Gatun, Canal Zone, Panama (NMNH).

Distribution in South America: Brazil, Colombia, French Guiana, Republic of Paraguay, Republic of Suriname, Venezuela.

Bibliographic sources: Known life stages and relevant references in Pecor et al. (1992). Ecological notes of the species can be found in Forattini et al. (1987a,b; 1989a; 1990; 1991; 1993a,b,c).

Geographical records can be found in Forattini et al. (1970); Sutil (1980); Kobayashi (1999); Hutchings et al. (2002; 2011); Gomes et al. (2007; 2008); Santos-Neto and Lozovei (2008); Tissot and Silva (2008); Ramos (2009); Suarez-Mutis et al. (2009); Sugimoto (2009); D'Avila (2011); Demari-Silva et al. (2011); Vesgueiro et al. (2011); Ribeiro et al. (2012); Del Ventura et al. (2013); Sá and Sallum (2013); Sant'Ana (2013); Chaves et al. (2014).

Synonyms:

loturus Dyar, 1925b: 214 (♂). Holotype ♂: Catatumbo River, (Zulia), Venezuela (NMNH).

APPENDIX I

I. Species with ambiguous identifications found during the bibliographic revision

Species listed as "*near*" (when sources were in English) or "*prox*". (when sources were in Spanish or Portuguese) to any of the described species were included below and not considered in the body of the Catalog.

Author(s)/Country	Description of the ambiguous identification
Gabaldon et al. (1977) - Venezuela	Misspelled as <i>Cx. inhibitor</i> , likely a mistake for a valid species such as <i>Cx. inhibitorator</i>
Sirivanakarn and Jakob (1981) - Brazil	near <i>Cx. crybda</i>
Calisher et al. (1982) - Brazil	near <i>Cx. epanastasis</i>
Peyton et al. (1983) -Bolivia	near <i>Cx. intricatus</i>
Lane (1992) - Brazil	near <i>Cx. pedroi</i>
Navarro and Liria (2000) - Venezuela	Listed a species under the name "Grupo Atratus" in one section of the paper and then called it <i>Cx. dunni</i> which seems to be the same specimen.
Turell et al. (2000) - Peru	as <i>Cx.gnomatos/vomerifer</i>
Hutchings et al. (2002) - Brazil	near <i>Cx. coppenamensis</i> near <i>Cx. educator</i> near <i>Cx. evansae</i> <i>Cx. vaxus</i> (morfortype 1) <i>Cx. vaxus</i> (morfortype 2)
Hutchings et al. (2005) - Brazil	near (<i>Cx. batesi</i> , <i>Cx. creole</i> , <i>Cx. serratimarge</i> , <i>Cx. vaxus</i>); " <i>Cx. coppenamensis</i> form 1" was later described as <i>Cx. phyllados</i> by Hutchings and Sallum, 2008.
Turell et al. (2005) - Peru	as <i>Cx.gnomatos/vomerifer</i>
Turell et al. (2006) - Peru	as <i>Cx.gnomatos/vomerifer</i>
Gomes et al. (2007) - Brazil	near <i>Cx. quasihybridus</i>
Johnson et al. (2008) - Peru	as <i>Cx.gnomatos/vomerifer</i>
Guedes (2010) - Brazil	near <i>Cx. pedroi</i>
Hutchings et al. (2010) - Brazil	near <i>Cx. eastor</i> near <i>Cx. vaxus</i>
Hutchings et al. (2011) - Brazil	near <i>Cx. pilosus</i> near <i>Cx. unicornis</i> near <i>Cx. vaxus</i> near <i>Cx. vomerifer</i>
Hutchings et al. (2013a) - Brazil	near <i>Cx. venezuelensis</i>
Medeiros-Sousa et al. (2013a) - Brazil	near <i>Cx. maxinocca</i>
Ribeiro (2014) - Brazil	near <i>Cx. glyptosalpinx</i>

II. Misidentification of species or listing of current synonyms

The following references were included in the content of the Catalog with the corresponding corrections (i. e. the current valid species were included in the Catalog).

Author(s)/Country	Description of taxonomic misidentification
Gabaldon et al. (1977) - Venezuela	Listed as <i>Cx. chrysonotum</i> that is a synonym of <i>Cx. theobaldi</i> (Forattini and Sallum 1989c).
Sutil (1980) - Venezuela	Listed as <i>Cx. aikenii</i> , which was separated into two species, <i>Cx. ocosa</i> and <i>Cx. panoccosa</i> (Belkin 1970). <i>Cx. nigrescens</i> , which is now considered as <i>Nomina dubia</i> by Pecor et al. (1992). <i>Cx. chrysonotum</i> , which is a synonym of <i>Cx. theobaldi</i> (Forattini and Sallum 1989c). <i>Cx. menytes</i> , which is a synonym of <i>Cx. spissipes</i> (Forattini and Sallum, 1996). <i>Cx. mistura</i> , which is a synonym of <i>Cx. inadmirabilis</i> (Forattini and Sallum, 1993a) <i>Cx. jubifer</i> , which might be a misidentification according to Revision of Spissipes Section by Sallum and Forattini (1996).
Lourenço de Oliveira (1984) - Brazil	Listed as <i>Cx. crybda</i> , later on corrected as <i>Cx. ribeirensis</i> by Forattini and Sallum (1985).
Walder et al. (1984) - Venezuela	Listed as <i>Cx. aikenii</i> , which was separated into two species, <i>Cx. ocosa</i> and <i>Cx. panoccosa</i> (Belkin 1970).
Lourenço de Oliveira et al. (1985) - Brazil	Listed as <i>Cx. crybda</i> , later on corrected as <i>Cx. ribeirensis</i> by Forattini and Sallum (1985).
Luz and Lourenço de Oliveira (1996) - Brazil	Listed <i>Cx. chrysonotum</i> , which is a synonym of <i>Cx. theobaldi</i> (Forattini and Sallum 1989c).
Groot et al. (1996) - Colombia	Listed <i>Cx. menytes</i> which is a synonym of <i>Cx. spissipes</i> (Forattini and Sallum, 1996).
Lopes (2002) - Brazil	Listed as <i>Cx. inhibitor</i> , which might be a misidentification given the taxonomic study of mainly immature stages (However, this reference was included in the content of this Catalog). <i>Cx. educator</i> , which should be reviewed considering the studies on Educator Group by Forattini and Sallum (1993b).

	<i>Cx. taeniopus</i> , which should be reviewed after recent descriptions of <i>Cx. akritos</i> and <i>Cx. ikelos</i> (Forattini and Sallum, 1995).
Julião et al. (2010) - Brazil	Listed <i>Cx. chrysonotum</i> which is a synonym of <i>Cx. theobaldi</i> (Forattini and Sallum 1989c).
Parra-Heano and Suárez (2012) - Colombia	Listed as <i>Cx. chrysonotum</i> which is a synonym of <i>Cx. theobaldi</i> (Forattini and Sallum 1989c).
Berti et al. (2013) - Venezuela	Listed as <i>Cx. mistura</i> which is a synonym of <i>Cx. inadmirabilis</i> (Forattini and Sallum, 1993a).

III. New species still undescribed

In Kobayashi (1999), the manuscript (Master degree monograph) lists a number of species named as "species 1", "species 2" and "species 3", with a brief description of the main morphological traits. Each one corresponds to a new and undescribed species of subgenus *Melanoconion* (Atratus Group) (Sallum MAM, personal communication).

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Chapter II

**Phylogenetic analysis of the subgenus *Melanoconion* of *Culex*
(Diptera: Culicidae) based on nuclear genes and COI barcode**

1. Introduction

The subgenus *Melanoconion* Theobald is one of the 26 known subgenera of *Culex*. Historically, it has been documented in the American continent from south of North America, in Central America, in some Caribbean islands and in most of South America (Sirivanakarn, 1983; Pecor et al., 1992; Sallum and Forattini, 1996; Harbach, 2012). The majority of the species occur in the Neotropical region, with documented richness in countries like Argentina, Brazil, Colombia and Venezuela (Pecor et al. 1992; Sallum and Forattini, 1996). In Brazil, many records of the subgenus originated, mainly from two main geographical regions, the Amazon region and the Atlantic forest system (Forattini and Sallum, 1992b, c; 1993, 1995; Sallum et al. 1997; Sallum and Hutchings, 2003; Hutchings and Sallum, 2008). Moreover, Brazil owns one of the most important repositories of *Culex (Melanoconion)* specimens, currently known as the Coleção Entomológica de Referência, Universidade de São Paulo (FSP-USP).

To date, the taxonomic studies on the subgenus include a total of 160 species (Harbach, 2011). According to Sirivanakarn's monograph (1983), one of the most accepted taxonomical studies, *Melanoconion* consists of two major sections: *Melanoconion* Section and *Spissipes* Section. Each one of these sections, in Sirivanakarn's classification, is further divided into several groups and subgroups, known as non-formal hierarchical levels. The classification proposed by Sirivanakarn (1983) was based on morphological features of adults and immatures with a considerable focus on the male genitalia. Though Sirivanakarn's monograph is the most recent extensive study on both major sections of the subgenus, his classification lacks taxonomic keys for the species level, providing keys for the non-formal groups and subgroups only. Comparatively, the *Spissipes* Section has been recently studied, with a taxonomic revision by Sallum and Forattini (1996), in which the authors provided keys to species level, and a comprehensive amount of knowledge presented for each of the species in the section.

In perspective, the taxonomic contributions on the subgenus *Melanoconion* exceed in great proportion the available knowledge on phylogenetic topics about the subgenus. The scarce contributions with significant approaches to the study of the ancestral relationships of *Melanoconion*, considering at least a group of its species, are studies by Miller et al. (1996), Navarro and Liria (2000), Navarro and Weaver (2004), Vesgueiro et al. (2011) and Demari-Silva et al. (2011). Although such authors included either some of the species of *Melanoconion* or some of the non-formal groups within it, there is still insufficient knowledge about the phylogenetic relationships of the two major sections and the species they contain. The current status of the classification of *Melanoconion* is still in need of further research; the complex taxonomic issues well recognized by researches in the past, are in demand of new approaches to better understand the relationships among the species and sections known today.

The subgenus *Melanoconion* is worth studying for several reasons. The species cover a wide range of American countries in geographical distribution. They exhibit a well recognized ecological flexibility that allows them to adapt to an array of different environments from conserved and pristine forests to highly modified and anthropic places (Forattini et al., 1986, 1990; Forattini e Sallum, 1996; Natal et al., 1998). In their behavior as blood feeders *Melanoconion* species display a wide variety of habits, and search for different hosts (rodents, reptiles, mammals), with many species capable of transmitting viral pathogens to a wide animal spectrum that includes humans. Plenty of studies documented many species of the subgenus as potential and effective vectors of Venezuelan Equine Encephalitis Complex (including Venezuelan Equine Encephalitis Virus, Everglades Virus, Mucambo Virus), West Nile Virus and Eastern Equine Encephalitis Virus (Natal, 1981; Walder et al., 1984; Dickerman et al. 1986; Travassos da Rosa et al. 1998; Salas et al. 2001; Weaver et al., 2004; Ferro et al. 2003, 2008; Cupp et al. 2003, 2004, 2007; Yun Young Go et al. 2013).

The search for molecular markers to infer phylogenetic relationships of the target species is an important step of any research (Brower and DeSalle, 1994). Previous phylogenetic studies had recommended nuclear single-copy genes, as a rich source of genetic data, still poorly known (Friedlander et al. 1992; Regier et al., 2008; Wild and Maddison, 2008). Among a considerable number of nuclear genes explored for a different number of invertebrate taxa,

CAD (also called rudimentary) has been referred to as one of the most informative and highest performing gene (Moulton and Wiegmann, 2004; Danforth et al., 2006; Wild and Maddison, 2008). Studies using nuclear genes in Culicidae species have been used mainly on *Anopheles* species (Krzywinski et al., 2001; Foster et al. 2013). Also, the use of nuclear genes on other Culicidae groups (i.e. subfamilies, tribes, etc.), in order to solve phylogenetic questions has been approached by Besansky and Fahey (1997), and more recently by Reidenbach et al. (2009). Among the genes used by Reidenbach et al. (2009), Hunchback (HB) and CAD were considered informative, and priming sequences were provided by these authors.

Furthermore, the mitochondrial gene, COI, also referred as the DNA barcode for species identification, has been extensively used for taxonomic purposes in many invertebrate orders (Hebert et al. 2003a). COI has been used as well to infer phylogenies in species of Culicidae (Mitchell et al. 2002; Lehr et al. 2005; Demari-Silva et al. 2011). The ease to amplify and sequence this mitochondrial fragment is one of the reasons it has become so popular (Fomer et al. 1994). Several authors have used the mitochondrial DNA, COI sequences, to study the cosmopolitan genus *Culex*, in search of an efficient tool that can readily confirm confusing taxonomical issues, a well-documented limitation for the study of this particular taxon (Cywinska et al. 2006; Kumar et al. 2007; Demari-Silva et al. 2011; Wang et al., 2012; Laurito et al. 2013).

In this study we address the current classification of the subgenus, which is proposed based on morphological characteristics (Sirivanakarn, 1983; Sallum and Forattini, 1996). We begin the process of constructing phylogenetic hypotheses for the subgenus *Melanoconion* by considering a set of 46 species of the *Melanoconion* and *Spissipes* sections. We investigate phylogenetic relationships between species of the subgenus *Melanoconion* by testing if the current classification is supported when studying DNA sequences. We evaluated the monophyly of the two major sections of the subgenus and proposed natural groups within each section. This is the first phylogenetic analysis of the subgenus *Melanoconion* based on two single copy nuclear genes (HB and CAD) and the COI barcode sequence data.

2. Research Objectives

2.1 Primary objective

To investigate phylogenetic relationships between species of the subgenus *Melanoconion* of *Culex*.

2.2 Secondary objectives

1. To verify if the current classification proposed by Sirivanakarn (1983) and Sallum and Forattini (1996), is supported by phylogenetic hypotheses based on DNA sequences.
2. To evaluate the monophyly of the two major sections of the subgenus, the Spissipes Section and the Melanoconion Section.
3. To propose monophyletic groups within the subgenus *Melanoconion*
4. To discuss the utility of the genes used to assess the phylogenetic hypotheses.

3. Materials and Methods

Taxa sampled

The ingroup consisted of 107 specimens, and of 46 *Melanoconion* species. Tables 1 and 2 contain a summary of all species included in the study arranged according to the current classification. The outgroup included 7 specimens of 4 species represented by the subgenus *Culex*, genus *Aedes* and genus *Anopheles*. The list of all individuals included in the analysis is shown in the Appendix I (some species are represented by more than one individual), with detailed geographical origins. The specimens used for this study were collected during the development of several research projects at the Public Health Faculty of the University of São Paulo, during the last decade. Some specimens were preserved in ethanol and kept frozen at a temperature of -80°C or kept dried inside plastic boxes with silica gel. Taxonomic identifications

were either performed or confirmed by Dr. M. A. M. Sallum. All male individuals have the corresponding dissected genitalia mounted on microscope slides and deposited in Coleção Entomológica de Referência, Universidade de São Paulo (FSP-USP) as vouchers. The female individuals have supporting information on a database containing the exact geographical information (for most cases geographical coordinates) of collecting sites, as well as records of other individuals of the same species collected at the same localities (such individuals are kept pinned as part of the entomological collection). The aforementioned database belongs to Coleção Entomológica de Referência, Universidade de São Paulo (FSP-USP). Following the current classification of the subgenus *Melanoconion*, our total number of species can be informed as 10 species representing the *Spissipes* Section and 36 species belonging to the *Melanoconion* Section. Additionally, we searched public databases (GenBank) in order to find any representative of the subgenus *Melanoconion* and thus found four sequences to include as ingroup. Moreover, we used other Culicidae sequences available in GenBank as outgroup (see further description in the following sections -Table 1). Including the sample processed by this study and the information from GenBank, this study used 114 specimens of 46 species.

Table 1. List of specimens included in the study as ingroup.

INGROUP					
Current Classification of subgenus <i>Melanoconion</i> of <i>Culex</i>					
Section	Group	Subgroup	Origin	Number of individuals	Species
Melanoconion	Atratus	none	This study	2	<i>Culex (Melanoconion) commevynensis</i>
Melanoconion	Atratus	none	This study	2	<i>Culex (Melanoconion) dunni</i>
Melanoconion	Atratus	none	This study	3	<i>Culex (Melanoconion) ensiformis</i>
Melanoconion	Atratus	none	This study	1	<i>Culex (Melanoconion) trigeminatus</i>
Melanoconion	Atratus	none	This study	2	<i>Culex (Melanoconion) zeteki</i>
Melanoconion	Bastagarius	none	This study	3	<i>Culex (Melanoconion) bastagarius</i>
Melanoconion	Bastagarius	lolambdis	This study	1	<i>Culex (Melanoconion) corentynensis</i>
Melanoconion	Conspirator	none	This study	7	<i>Culex (Melanoconion) aliciae</i>
Melanoconion	Conspirator	none	This study	4	<i>Culex (Melanoconion) dyius</i>
Melanoconion	Conspirator	none	GenBank	1	<i>Culex (Melanoconion) elevator</i>
Melanoconion	Distinguendus	Putumayensis	This study	2	<i>Culex (Melanoconion) putumayensis</i>
Melanoconion	Educator	none	This study	3	<i>Culex (Melanoconion) eknomios</i>
Melanoconion	Educator	none	This study	2	<i>Culex (Melanoconion) theobaldi</i>
Melanoconion	Educator	none	This study	6	<i>Culex (Melanoconion) vaxus</i>
Melanoconion	Erraticus	Erraticus	This study	2	<i>Culex (Melanoconion) aureonotatus</i>
Melanoconion	Erraticus	Clarki	This study	2	<i>Culex (Melanoconion) clarki</i>
Melanoconion	Evansae	none	This study	1	<i>Culex (Melanoconion) evansae</i>
Melanoconion	Inhibitor	Inhibitor	GenBank	1	<i>Culex (Melanoconion) inhibitor</i>
Melanoconion	Inhibitor	Inhibitor	This study	2	<i>Culex (Melanoconion) oedipus</i>
Melanoconion	Inhibitor	Inhibitor	This study	1	<i>Culex (Melanoconion) rabelloi</i>
Melanoconion	Inhibitor	Egcymon	This study	1	<i>Culex (Melanoconion) serratimarge</i>
Melanoconion	Inhibitor	Egcymon	GenBank	1	<i>Culex (Melanoconion) serratimarge_ Linton</i>
Melanoconion	Intrincatus	Eastor	This study	4	<i>Culex (Melanoconion) eastor</i>
Melanoconion	Intrincatus	Intrincatus	This study	3	<i>Culex (Melanoconion) equinoxialis</i>
Melanoconion	Intrincatus	Intrincatus	This study	9	<i>Culex (Melanoconion) intrincatus</i>
Melanoconion	Intrincatus	Idottus	This study	2	<i>Culex (Melanoconion) idottus</i>
Melanoconion	Intrincatus	Intrincatus	This study	3	<i>Culex (Melanoconion) misionensis</i>
Melanoconion	Pilosus	Caudelli	This study	1	<i>Culex (Melanoconion) alogistus</i>
Melanoconion	Pilosus	Caudelli	This study	1	<i>Culex (Melanoconion) lacertosus</i>
Melanoconion	Pilosus	Pilosus	This study	2	<i>Culex (Melanoconion) pilosus</i>
Melanoconion	Unknown		This study	3	<i>Culex (Melanoconion) near aureonotatus</i>
Melanoconion	Unknown		This study	3	<i>Culex (Melanoconion) near corentynensis</i>
Melanoconion	Unknown		This study	1	<i>Culex (Melanoconion) near inhibitor</i>
Melanoconion	Unknown		This study	1	<i>Culex (Melanoconion) near rabelloi_tipo1</i>
Melanoconion	Unknown		This study	1	<i>Culex (Melanoconion) near rabelloi_tipo1.1</i>
Melanoconion	Unknown		This study	1	<i>Culex (Melanoconion) near rabelloi_tipo2</i>
Melanoconion	Unknown		This study	1	<i>Culex (Melanoconion) near rabelloi_tipo3</i>
Melanoconion	Unknown		This study	1	<i>Culex (Melanoconion) near vaxus</i>
Spissipes	Crybda	Pedroi	This study	2	<i>Culex (Melanoconion) pedroi</i>
Spissipes	Crybda	Pedroi	This study	3	<i>Culex (Melanoconion) ribeirensis</i>
Spissipes	Crybda	Pereyrai	This study	2	<i>Culex (Melanoconion) pereyrai</i>
Spissipes	Faurani	none	This study	1	<i>Culex (Melanoconion) faurani</i>
Spissipes	Taeniopus	none	This study	2	<i>Culex (Melanoconion) akritos</i>
Spissipes	Taeniopus	none	GenBank	1	<i>Culex (Melanoconion) taeniopus</i>
Spissipes	Vomerifer	none	This study	2	<i>Culex (Melanoconion) gnomatos</i>
Spissipes	Vomerifer	none	This study	1	<i>Culex (Melanoconion) portesi</i>
Spissipes	Vomerifer	none	This study	3	<i>Culex (Melanoconion) sacchettae</i>
Spissipes	Spissipes	none	This study	3	<i>Culex (Melanoconion) spissipes</i>
Total number of specimens included				107	Total number of species: 46

Table 2. List of specimens included in the study as outgroups.

OUTGROUP					
Family	Subfamily	Tribe	Origin	Number of individuals	Species
Culicidae	Culicinae	Culicini	This study	2	<i>Culex (Culex) mollis</i>
Culicidae	Culicinae	Culicini	This study; GenBank	2	<i>Culex (Culex) quinquefasciatus</i>
Culicidae	Culicinae	Aedini	This study	2	<i>Aedes scapularis</i>
Culicidae	Anophelinae		GenBank	1	<i>Anopheles atroparvus</i>
Total number of specimens included				7	Total number of Species: 4

Molecular methods and phylogenetic reconstruction

I. Molecular markers:

For this study we employed DNA sequence data generated for three protein coding genes, such as two dingle copy nuclear genes and the COI barcode fragment of the cytochrome c subunit I of the mitochondrial gene.

CAD. A single copy nuclear gene. According to Wild and Maddison (2008) "this gene region encodes several enzymes that catalyze pyrimidine biosynthesis". The proteins it encodes are called: carbamoylphosphate synthetase, aspartate transcarbamylase and dihydroorotase (Moulton and Wiegmann, 2004). Previous phylogenetic studies for different groups of insects have used this gene with positive results (Moulton e Wiegmann, 2004; Danforth et al., 2006; and other cited by Wild and Maddison, 2008). It has been further studied for species of *Drosophila* referred as *rudimentary* (Zerges et al. 1992).

Hunchback (HB). A single copy nuclear gene. According to Hancock et al. (1999) and Marques-Souza et al. (2008), this gene is "involved in the determination of anterior-posterior polarity in early development", and its function is presumably conserved in different insect orders. Also, in words of Pinell et al. (2006), hunchback "is a member of the gap class of segmentation genes" first identified in *Drosophila melanogaster*. It encodes a C2H2 zinc finger transcription factor

whose primary function is to regulate the expression of other genes involved in the embryo development.

Cytochrome oxidase c subunit I (COI). A mitochondrial gene. As referred by Lunt et al. (1996), "this gene is involved in both electron transport and the associated translocation of protons across the membrane and it has been shown to contain a range of different types of functional domain". It contains a combination of highly conserved and variable regions that makes it useful for different genetic studies for Metazoan species, especially as an identification tool (Folmer et al., 1994; Hebert et al. 2003a, b; Cameron et al., 2007). These facts turned it into the core fragment for the international project Barcode of life or DNA barcoding (www.barcodinglife.com).

II. Experimental procedures:

All laboratorial procedures were carried out at the Laboratório de Sistemática Molecular at Public Health Faculty of the University of São Paulo, during the period of time between January 2013 until November 2014.

Total genomic DNA extractions were performed using the complete mosquito specimens following a salting out protocol (modified from Miller et al. 1988) (Appendix 2, Protocol 1). All extractions products were routinely conserved at a -20°C freezer. Following extraction the DNA was amplified for fragments of three genes: cytochrome oxidase I (COI); carbamoyl-phosphate synthetase (CAD gene); and Hunchback gene (HB).

Primers used to amplify the mitochondrial (COI) and single-copy nuclear genes (CAD, HB) are listed in Table 3. All Polymerase chain reaction (PCR) reactions were performed with a Mastercycler ep Gradient 5341 (Eppendorf); PCR reactions and Thermo-cycler conditions are listed in Table 4.

Table 3. Primers used for PCR amplifications of the mitochondrial and nuclear genes (See sources below).

Gene	Primer used for F strand	Primer used for R strand
COI ^a	LCO1490_F 5'-GGTCAACAAATCATAAAGATATTGG-3'	HCO 2198_R 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'
CAD ^b	CADm-F 5'- TGTAACGACGGCCAGTGTNGTNAARATGCC NMGNTGGGA-3'	CADm-R 5'- CAGGAAACAGCTATGACCAICCIACIGCRCACCART CRAAYTC -3'
HB ^c	HBm-F 5'- TGTAACGACGGCCAGTTGYCCIAARTGYCCI TTYGTIACIG -3'	HBmR- 5'- CAGGAAACAGCTATGACCGCYTGYTGRTICIGCRAA CATYTGRA -3'
HB ^d		5'- GTTGYTKGWGGCBGCRYTYTTGAAC -3'

Bibliographic sources for each set of primers:

- a - Primers referenced by Folmer et al. (1994).
- b - Original primers were used by Reidenbach et al. (2009). We modified them with M13 tails (Graham and Hill, 2001). Notice in gray the M13 tail sequences. Previous experiences at the Laboratory of Molecular Systematics were the main source of information.
- c - Primers included by Reidenbach et al. (2009). We modified them with M13 tails (Graham and Hill, 2001; Foster et al. 2013). Notice in gray the M13 tail sequences. Previous experiences at the Laboratory of Molecular Systematics were the main source of information.
- d - Primer designed by work team at the Molecular Systematics Laboratory in order to sequence the R strand of HB.

Table 4. PCR Reactions and Thermo-cycler conditions for each gene amplification.

PCR reaction	Genes		
	COI^a	CAD^b	HB^c
Buffer concentration	20 mM	20 mM	20 mM
MgCl ₂ concentration	1,5 mM	2,4 mM	2,4 mM
Primer concentration (of each F and R)	25 pmol	100 pmol	112,5 pmol
dNTPs concentration	200 µM	200 µM	200 µM
DNA-Polymerase concentration	0, 625 U	1,25 U	1,25 U
Purified water volume	As needed	As needed	As needed
Extracted DNA volume (1 mosquito)	1 µL (1:20)	2 µL	2 - 4 µL
Dimethyl sulfoxide (DMSO) concentration	-	5%	10%
Final volume of PCR reaction	25 µL	25 µL	25 µL
PCR amplification conditions	94°C x 3mins- 35 cycles of (94°Cx 1 min; 55°C x 1 min; 72°C x 1 min); Annealing and Extension 72 x 7 mins - Final extension.	94°C x 2mins- Enzyme activation; 40 cycles of (94°Cx 30 segs; 55°C x 30 segs; 72°C x 2mins); Annealing and Extension 72 x 5 mins - Final extension.	94°Cx2mins- Enzyme activation; 40 cycles of (94°Cx 30 segs; 55°C x 30 segs; 72°C x 2mins); Annealing and Extension 72 x 5 mins - Final extension.

a - For COI reactions, Platinum® *Taq* DNA Polymerase, High Fidelity (Life Technologies) was used.

b - For CAD reactions, GoTaq® DNA Polymerase (Promega) was used.

c - For HB reactions, GoTaq® DNA Polymerase (Promega) was used.

PCR products for COI and CAD were purified using PEG precipitation protocol (20% polyethylene glycol 8000/2.5 M NaCl) (Appendix 2, Protocol 2). A commercial kit by Zymo Research (DNA Clean and Concentrator) was employed to purify the PCR products generated for the gene HB. PCR products of all genes were electrophoresed in 1% TAE agarose gels stained with GelRed™ Nucleic Acid Gel Stain (Biotium Inc., Hayward, USA), to verify the quality of the amplification and if the amplified fragments were the expected products.

All sequencing reactions were carried out in both directions using ABI Big Dye Terminator Kit v.3.1 (PE Applied Biosystems, Warrington, England). For sequencing COI we employed the same set of primers used for PCR, whereas for CAD gene, we used the M13 primers only. For HB we performed several strategies that involved three different set of primers; thus when the M13 tailed primers were used for PCR reactions, the sequencing for HB used only the M13 tails (Table 2). The sequencing reactions were carried out in a total volume of 10 µL, containing: approximately 20 ng of the PEG purified PCR product; 0,5 µL BigDye Terminator ready reaction mix; 1x sequencing buffer (Applied Biosystems); 3,6 pmol of each primers (for different genes) and the remaining volume of sterile purified water. Once the sequencing reactions were obtained, all products were purified with Sephadex G50 columns (GE Healthcare). Capillary electrophoresis was performed in an Applied Biosystems 3130 DNA Analyser (Applied Biosystems, Foster City, CA, USA).

II. Sequence analyses:

Sequences were edited using licensed Geneious (version R 6.0 - Biomatters Ltd., Auckland, New Zealand) and McClade (Maddison and Maddison, 2005) software. As part of the edition for all the obtained sequences we followed a few steps:

- 1) Removal of primers
- 2) Chromatogram checking in order to determine which sequences were optimal and which needed to be re-processed.
- 3) Search on public databases using BLAST (Basic Local Alignment) tool available on the National Center for Biotechnology Information (NCBI) website. This search was performed

to confirm the homology of the obtained sequences (nucleotides and amino acids) with those sequences deposited in GenBank. The search was considered positive (identity confirmed) when the results consisted of sequences of the same gene for other *Culex* species or other Culicidae species.

4) To determine the corresponding open reading frame for each dataset we searched the Blastx tool at the NCBI site, and this information was considered when checking the amino acid sequences of the different datasets.

Sequences were also corroborated through the construction of alignments using the obtained sequences versus the reference genome for *Culex quinquefasciatus*, using the information available on GenBank (<http://www.ncbi.nlm.nih.gov/>) and Vectorbase (www.vectorbase.org). Below the expected sizes for the target genes:

Gene cytochrome oxidase I (COI)	- 658 base pairs
Gene carbamoyl-phosphate synthetase (CAD)	- 610 base pairs
Gene Hunchback (HB)	- 544 base pairs

Sequence alignments were constructed for nucleotides and amino acids for each one of the three gene datasets. All alignments were constructed using Clustal W, implemented in Geneious R6 (with default parameters), and adjusted manually to remove alignment artifacts.

Each dataset was explored as follows:

- 1) We used jModeltest (version 2) to determine the best-fitting model of nucleotide substitution using the Akaike Information Criterion (AIC) (Darriba et al. 2012) and to calculate nucleotide frequencies for each gene dataset.
- 2) General description, distance matrixes, number of variable sites, conserved sites and parsimony informative sites were determined using MEGA (v. 6) (Tamura et al. 2013).

3) A likelihood mapping analysis was implemented with the Tree puzzle software in order to better understand the phylogenetic content of each data set. This phylogenetic tool was described in Strimmer and von Haeseler (1997).

4) Estimation of intraspecific similarity was performed using Pairwise Identity values for COI dataset. These values were used to corroborate the taxonomical identity of individuals of the same species. We present a unique value when used two individuals of the same species; and a minimum and a maximum values when using more than two individuals of the same species. We used sequence alignments through Geneious (v.6) tools and kept record of the pairwise identity percentage as an indicator of similarity. Geneious definition of pairwise identity is: the percentage of pairwise residues that are identical in the alignment, including gap vs non-gap residues, but excluding gap vs gap residues.

5) All the obtained sequences were translated to amino acid sequences in order to:

A. check their identity (expected protein according to the target gene) and,

B. verify that our sequences were coding sequences (absence of stop codons).

III Phylogenetic Inference:

We used independent datasets per gene, one data set for nuclear fragments only, and one concatenated data set containing all sequences of the three genes (only individuals with the complete set of three genes) (See Appendix 3 to learn which genes were obtained for each individual).

The COI dataset was analyzed under the Kimura 2 Parameter model, following guidelines given by the Barcode of Life project (Herbert et al. 2003a; BOLD Systems, 2013). The phylogenetic tree for COI dataset was estimated using the Neighbor Joining (NJ) algorithm and bootstrap support (obtained with 3.000 replicates). Only support values over 50% are shown. This analysis was performed with MEGA6. For the phylogenetic inference the available GenBank sequences for the subgenus *Melanoconion* were included as part of the ingroup. Only sequences for COI gene were available on GenBank (Table 5).

The phylogenetic inference for *Melanoconion* was performed using two methodologies:

*Maximum Likelihood (ML) analysis with PhyML (v3.0 - Guindon and Gascuel, 2003; Guindon et al. 2010) with approximate likelihood ratio (aLRT-SH) as support values (Anisimova and Gascuel, 2006; Also see the information available on <http://www.atgc-montpellier.fr/phyml/alrt/>).

A ML tree was estimated for CAD, HB and CAD+HB datasets and for a concatenated matrix with three genes (COI+CAD+HB). The nucleotide substitution models used for the nuclear genes were in accordance with the jModeltest output for each dataset. Considering that the best-fitting model for CAD dataset, as informed by jModeltest, was TIM2+I+G, which belongs to the GTR family, we also used GRT+I+G to run the phyML CAD tree, as the phylogenetic software (phyML) has a limited amount of models available. For all the datasets the topology search was performed by using the option "Best of NNIs and SPRs" in phyML, which means the tree topology search operation uses both the Nearest Neighbor interchange (NNI) (faster) and the Subtree Pruning Regraftin (SPR) (slow).

*Bayesian analysis (BA) with Mr.Bayes (v.3.2) (Ronquist and Huelsenbenck, 2003) was used to infer phylogenetic hypotheses of those species with a complete set of multi loci only, using two datasets: nuclear-gene fragments (CAD+HB) and the three-loci dataset (CAD+HB+COI). The datasets were partitioned by gene (for CAD+HB) and by codon (for CAD+HB+COI - all codons considered). Parameters chosen for the estimation of the posterior probability of both datasets follow:

-GTR as substitution model for nucleotides with a gamma-distributed rate variation across sites and a proportion of invariable sites. Parameters were unlinked between all partitions and different transition matrices, proportion of invariant sites and different state frequencies were used. The BA was run for 5.000.000 generations (as it was previously determined by the low value of the standard deviation of split frequencies), using four chains. Chains were sampled every 2500 generations, discarding the first 25%

of the topologies sampled. The consensus tree was calculated using the "allcompat" option.

All ML and BA analyses were developed using the computational resources of the Biology Department at St. Mary's College of Maryland, USA, in charge of Professor Kevin J. Emerson.

Table 5. Available cytochrome oxidase subunit I sequences for species of the subgenus *Melanoconion* retrieved from GenBank.

Species name	Accession number in GenBank	Description	Authors
<i>Culex taeniopus</i>	JX259916	658 bp	Gibson CM, Kao RH, Blevins KK and Travers PD.
<i>Culex inhibitor</i>	JX259908	658 bp	Gibson CM, Kao RH, Blevins KK and Travers PD.
<i>Culex serratimarge</i>	KF671018	658 bp	Linton YM, Pecor JE, Porter CH, Mitchell LB, Garzon-Moreno A, Foley DH, Pecor DB and Wilkerson RC.
<i>Culex * elevator</i>	JX260647	407 bp	Gibson CM, Kao RH, Blevins KK and Travers PD.
<i>Culex* elevator</i>	JX260645	307 bp	Gibson CM, Kao RH, Blevins KK and Travers PD.

* When aligning the two sequences of *Cx. elevator* available in GenBank, they overlap in a small fragment, and together are comparable to the COI sequences processed in this study (which are 658 bp in size).

Phylogenetic trees were edited in FigTree (V. 1.4.2; A. Rambaut) and are shown as cladograms, so the support values are better visualized in the image file. Four species of Culicidae were used as outgroups (Table 2), or groups of comparison, and only for some of them we retrieved sequences from GenBank.

Sequences included as outgroups:

- *Aedes scapularis* - All sequences were processed during the experimental phase of this study. For CAD and HB genes, the datasets include two individuals of the species.
- *Culex mollis* - All datasets (for COI, CAD and HB) include two individuals of this species. All sequences were processed during the experimental phase of this study.
- *Culex quinquefasciatus* - The HB dataset includes two individuals of this species that we processed during the experimental phase of the study. The COI and CAD datasets include GenBank sequences: COI sequences is under the accession number HQ398883 (658 bp), and CAD sequence corresponds to the accession number XM_001848657 (6780 bp); given the size of the CAD sequence for *Cx. quinquefasciatus* only the fragment that aligned with the CAD original primers (primers without the M13 tail - Table 3) was used. The CAD sequence retrieved from GenBank was also aligned to the CAD sequences obtained in this study to verify it corresponded to an homologous sequence.
- *Anopheles atroparvus* - All sequences for this species were retrieved from GenBank. COI sequence is under the accession number LK054515 (767 bp); CAD sequence corresponds to GQ906831 (1467 bp); given the size of this sequence, only the fragment that aligned with CAD original primers was used (primers without the M13 tail - Table 3); additional alignment with CAD sequences processed by this study was also used to verify it corresponded to the expected homologous sequence. The HB sequence corresponding to this species has the accession number GQ906903 (909 bp) in GenBank.

4. Results

Description of Datasets:

Of the 109 specimens processed in this study (ingroup and outgroup), 98,2% were amplified and sequenced for COI gene and 87,167% for CAD gene. The results for Hunchback (HB) were less successful; the obtained sequences correspond to 78%. However this HB value is estimated only for the forward strands as a consequence of the great difficulty encountered during the amplification and sequencing of the reverse strands of this gene fragment. All the different approaches to obtain the R strand for Hunchback failed; therefore we used only the F strands for all the specimens included in the study. The complete report on the number of sequences obtained for each gene fragment is described in Appendix 3.

All datasets were confirmed as highly or somewhat similar to the available gene fragments (nucleotides and amino acids) of several species of Culicidae available on GenBank and VectorBase. A general description of the datasets obtained is explained in Table 6. Fragment sizes for the different datasets were 658 bp in length for COI (with the exception of specimen RJ19_46 for which the R strand could not be obtained, and thus the F strand with 637 bases, was used); 610 bp for CAD sequences and a group of variable sizes for HB gene. The HB dataset consisted only of forward strands, and for some specimens, the beginning of the sequences had bad quality, therefore our sequences are variable in size (470 - 540 bp). Thus HB dataset had most sequences of 477 bp in size (except for three individuals with shorter sequences).

The alignments obtained for the different ingroup datasets and description of sites are in Table 6. The concatenated alignment consisted of 1.850 sites (COI+ plus CAD+ plus HB), this alignment included the outgroups which were described previously (Table 2). The exact correspondence of our datasets in relation to the reference genome (*Culex quinquefasciatus*) is informed in Table 7.

Table 6. General description of the different gene datasets and concatenated matrices (ingroup) employed to infer phylogenetic relationships within *Culex (Melanoconion)*.

Gene	Ingroup	Sequence size in bps	Alignment sizes in bps	Variable sites	Conserved sites	Parsimony informative sites
COI	108*	658	658	271	387	230
CAD	95	610	610	248	362	224
HB	85	Variable - Most of 477	524	200	318	166
CAD+HB¹	87	Variable	1218	554	583	435
CAD+HB² strict multi loci data	81	Variable	1192	572	539	470
Concatenated (COI+CAD+HB) strict multi loci data³	81	Variable	1850	825	944	688

* COI database included sequences from GenBank (*Cx. serratimarge*, *Cx. taeniopus*, *Cx. inhibitor* and *Culex elevator*).

¹ Matrix used for ML analysis.

² This dataset included only individuals with both nuclear-gene fragments. Matrix used in BA.

³ This dataset included only individuals with complete multi loci (CAD+HB+COI) fragments. Matrix used in ML and BA.

Table 7. Correspondence of ingroup sequences to the reference genome of *Culex quinquefasciatus*.

Gene	Reference genome	Position
COI	<i>Culex quinquefasciatus</i> ; mitochondrion, complete genome; 15587 bp DNA; Accession number NC014574.	from 1.487 to 2.144 base positions
CAD	<i>Culex quinquefasciatus</i> ; carbamoyl-phosphate synthase large chain in Vector Base CPIJ007153-RA - Exon 2; 3.557 bp DNA (Complete gene corresponds to 8 exons).	from 1.826 to 2.435 base positions
HB	<i>Culex quinquefasciatus</i> ; hunchback protein, CPIJ012542 in Vector Base. Exon 1 of 1. 1.911 bp DNA.	from 679 to 1.269 base positions

The best-fitting model of nucleotide substitution determined using the jModeltest software for each dataset is in Table 8; where the selected model was unavailable in the statistical method, the most similar model (i. e. GTR) was chosen to run the analysis

Table 8. Best-fit models and criteria by jModeltest software for nucleotide substitution and nucleotide frequencies for each of the datasets (COI, HB, CAD) of *Culex (Melanoconion)*.

Gene	Model	Criterion	Gamma rate variation	Proportion of invariable sites	A	C	T	G
COI	*		0,78	0,59	0,39	0,14	0,42	0,05
CAD	TIM2+I+G	Akaike Information	0,52	0,98	0,21	0,25	0,22	0,31
HB	GTR+I+G	Akaike Information	0,42	0,60	0,24	0,44	0,13	0,20

* The COI dataset was analyzed under the Kimura two-parameters model according to the guidelines by the Barcode of Life project.

A Likelihood mapping analysis is shown in Figure 1. The triangular figures include different quarters showing the partly resolved, unresolved and fully resolved quarters for the different datasets. This is an indicator of the phylogenetic information contained in a sequence alignment (Strimmer and von Haeseler, 1997). Fully resolved quarters, indicated by the quarters marked with numbers 1, 2 and 3 (Figure 1 - corners of the triangle), correspond to moderately high percentages for all the datasets (COI: 31,9%; 31,8% and 32,2%), (CAD: 22,9%; 23,2%; and 23,6%) and (HB: 24,1%, 32,6% and 34,0%). On the other hand, the partly resolved quarters (marked as 4, 5 and 6 -inside the triangle) had low percentages for all datasets. The unresolved quarters were very similar for CAD and HB datasets (below 20% of the data) and very low for COI dataset (Fig. 1).

Figure 1. Likelihood mapping quarter distribution (Seven Basins of Attraction) for the three datasets (COI, CAD, HB). A - Estimated quarter distribution for COI dataset (under HKY model, closest to K2P); B - Estimated quarter distribution for CAD dataset (under GTR model); C - Estimated quarter distribution for HB dataset (under GTR model).

The taxonomical corroboration for COI dataset, using the pairwise identity values showed that most of the species previously identify using morphological characters were in agreement with the percentage of sequence similarity considered common for intraspecific comparison (98% - 100%). However, we found some taxonomical contradictions as well. Some of the individuals identified as *Cx. aliciae*, *Cx. ensiformis*, *Cx. misionensis*, *Cx. pilosus* and *Cx. serratimarge* need further studies given their low pairwise identity values (under 98% of similarity). In Table 9, we present the Pairwise identity values for all species with more than one individual in our sample.

Table 9. Pairwise identity values used to corroborate intraspecific similarity for *Culex* (*Melanoconion*) COI dataset.

Species*	Pairwise identity (%)		
	Unique value**	Minimum value	Maximum value
<i>Culex akritos</i> (2)	99,8	--	--
<i>Culex aliciae</i> (7)	--	95,4	99,8
<i>Culex aureonotatus</i> (2)	99,4	--	--
<i>Culex bastagarius</i> (3)	--	99,1	100
<i>Culex clarki</i> (2)	98,2	--	--
<i>Culex commevynensis</i> (2)	99,2	--	--
<i>Culex dunni</i> (2)	98,9	--	--
<i>Culex dyius</i> (4)	--	--	100
<i>Culex eastor</i> (4)	--	99,7	100
<i>Culex eknomios</i> (3)	--	99,4	100
<i>Culex ensiformis</i> (3)	--	96,8	97,9
<i>Culex equinoxialis</i> (3)	--	99,1	100
<i>Culex gnomatos</i> (2)	99,5	--	--
<i>Culex idottus</i> (2)	100	--	--
<i>Culex intricatus</i> (9)	--	98,8	100
<i>Culex misionensis</i> (3)	--	97,1	99,4
<i>Culex oedipus</i> (2)	99,8	--	--
<i>Culex pedroi</i> (2)	99,4	--	--
<i>Culex pereyrai</i> (2)	98,6	--	--
<i>Culex pilosus</i> (2)	97,4	--	--
<i>Culex putumayensis</i> (2)	99,4	--	--
<i>Culex ribeirensis</i> (4)	--	98,9	99,4
<i>Culex sacchettae</i> (3)	--	--	100
<i>Culex serratimarge</i> (2)	92,6	--	--
<i>Culex spissipes</i> (3)	--	99,8	100
<i>Culex theobaldi</i> (2)	99,2	--	--
<i>Culex zeteki</i> (2)	99,8	--	--
<i>Culex vaxus</i> (6)	--	98,3	100

* Inside parenthesis is given the number of individuals of the same species included in the intraspecific comparison. ** A unique value is given when the pairwise identity was based on only two individuals of the same species.

Obtained Topologies:

The phylogenetic trees estimated with Maximum Likelihood for the different datasets are shown in Figure 2 to Figure 6. Tree scores and general information of the phylogenetic analysis for different datasets can be seen in Table 10. All phylogenetic trees were rooted with an outgroup consisting of *Anopheles atroparvus*, *Aedes scapularis*, *Culex quinquefasciatus* and *Culex mollis* (Methods - Table 2).

Table 10. General information describing the phylogenetic analysis with ML of different datasets inferring phylogenetic relationships within the subgenus *Melanoconion* of *Culex*.

Dataset	Model used	Number of taxa*	Number of Characters	Log-likelihood
COI	Kimura 2P	113	658	Tree with NJ
CAD	GTR	102	610	-8218.65490
HB	GTR	92	524	-6736.13307
CAD + HB	GTR	87	1218	-13946.18229
Concatenated matrix (three genes)	GTR	81	1850	-22463.17958

* The number of taxa includes ingroup+ plus outgroups.

The topology obtained for the mitochondrial gene (COI) showed resolution for most of the included species, to the point of matching the morphological premises of some of the specimens being close but still not considered as same species (i. e. several forms of *Culex* near *rabelloi*; *Culex* near *corentynensis* also referred as *Culex* prox a *corentynensis* and *Culex* near *aureonotatus*). The NJ tree computed and the values of the pairwise identity helped corroborate most of the species and most importantly uncovered a few possible mistakes in the taxonomic identification of a group of species (i. e. *Culex aliciae*, *Cx ensiformis*, *Cx. misionensis* and *Cx. pilosus*). In general, the topology obtained for COI shows very few clusters of species and scant phylogenetic information, though for the species level the resolution was high (Figure 2).

Figure 2.

NJ phylogenetic tree based on Kimura two-parameter for COI dataset. 3.050 replicates. Support values are shown above 50%.

On the other hand, the ML trees obtained for CAD (Figure 3) and HB (Figure 4) datasets (nuclear genes only) showed rich source of information. Both nuclear genes showed topologies in which the positions of Spissipes Section and Melanoconion Section appeared as different lineages, this being more evident in CAD topology. The CAD tree showed the Spissipes Section aside from the rest of the *Melanoconion* as a strongly supported clade (aLRT=12,69). Comparatively, the Melanoconion Section is well defined as a monophyletic group in CAD topology (Support value/ aLRT= 21,98), with only a few of the expected non-formal groupings recovered. A few groups within the Melanoconion Section were constant in both CAD and HB topologies (i. e. Atratus Group, Distinguendus Groups and Pilosus Group). The Inhibitor Group was recovered in HB tree only. The support values given by the aLRT statistics used in ML trees, were considered high when ≥ 5 ; and a low support when < 5 (Anisimova and Gascuel, <http://www.atgc-montpellier.fr/phyml/alrt/>).

In the HB-ML topology the genera and subgenera grouped separate (*Culex* (*Melanoconion*) + *Culex* (*Culex*) + *Aedes* + *Anopheles*), with strong support for the split leading to *Culex* and *Aedes* clade (aLRT=24,19). The same HB tree showed that the species of the two subgenera of *Culex* clustered into two distinct clades. One clade was composed of species of the subgenus *Melanoconion*, whereas the second clade was formed by the species of subgenus *Culex*. However, the statistical support values for both clades were low (aLRT= 0,34). Regarding the species of the Spissipes Section, both of CAD and HB topologies confirmed the monophyly of some of the non-formal groups previously proposed for the section, such as Spissipes Group (*Cx. spissipes*), Vomerifer Group (*Cx. gnomatos* + *Cx. sacchettae*) and Pereyrai Subgroup (*Cx. pereyrai*).

When comparing the branching at the two topologies, CAD and HB, the congruence was not very high between both trees. The total number of sequences obtained for HB dataset compared to the total number available for CAD dataset was an issue. We were not able to generate HB DNA sequences for all individuals included in our sampling taxa. Consequently, this might have caused some differences observed between the CAD and the HB topologies.

The ML topology obtained with the concatenated data sets for the nuclear gene-fragments (CAD+HB) (Figure 5) evidenced well-defined clades, with the Spissipes and Melanoconion Sections separate and the outgroups clustered outside the ingroups. The concatenated topology of CAD and HB corroborated the monophyly of the two sections with high support values. The Spissipes Section was supported by aLRT=11.13, whereas the Melanoconion Section had a stronger support (aLRT=33.95). Within the Spissipes Section, a number of strongly supported groups were recovered. Similarly, within the Melanoconion Section, the monophyly of some non-formal groups were corroborated with strong support values (Pilosus group/aLRT=20.24; Atratus Group/aLRT=55.44; Inhibitor Group/aLRT=21.96; Distinguendus Group/aLRT=119.66).

Finally, the ML tree generated with three genes concatenated (COI+ plus CAD+ plus HB) (Fig. 6) showed lower support values for the Spissipes section (aLRT=7.8) and higher support for the Melanoconion section (aLRT=37.1). The Spissipes Section is marked with an S and a full black bar in front of the terminal taxa. In the same Figure 6, Melanoconion Section is marked with an M in the corresponding clade and an empty bar in front of the terminal taxa (this marking is kept the same for other topologies).

All the non-formal groups of the Spissipes Section were recovered as monophyletic clades (Spissipes Group/aLRT=414.23; Faurani Group -with a single species-; Vomerifer Group/aLRT=61.08 and Pedroi subgroup/Crybda Group/aLRT=32.7). Our sample represented ten non-formal groups within Melanoconion Section, and our phylogenetic hypothesis only recovered three of them as monophyletic clades (Distinguendus Group: *Cx. putumayensis*); Pilosus Group (*Cx. pilosus*, *Cx. alogistus* and *Cx. lacertosus*) and Atratus Group (*Cx. trigeminatus*, *Cx. dunni*, *Cx. ensiformis*, *Cx. zeteki* and *Cx. commevynensis*). The statistical supports for the non-formal groups included in the Melanoconion Section were higher for Distinguendus and Atratus Groups. This topology of concatenated genes did not support the remaining non-formal groups of the Melanoconion Section included in the analysis. Furthermore, it did not recover the same groups and subgroups included in the current classification of the section, which is based on morphological characters. The ML topology showed a whole different phylogenetic

scenario for the Melanoconion Section. It is worth nothing that the sequences of *Cx. quinquefasciatus* (retrieved from GenBank (corresponding to COI and CAD) did not cluster with the two other individuals of the same subgenus (*Cx. mollis*, *Cx. quinquefasciatus* -LESP).

Comparatively, the topologies obtained by the Bayesian inference showed low support for most of the taxa and groupings using both datasets, nuclear-gene fragments and all three genes concatenated (Fig. 7, 8). The topology resulting from the BA with only CAD+HB (Fig. 7) is highly consistent with the branching of the ML topology for the nuclear-gene fragments (Fig. 5). The Spissipes section in both topologies (ML and BA) resulted as a monophyletic group, formed by natural groups such as Pedroi subgroup and Crybda group. The clear difference between the two hypotheses is the lack of support in the outcome by BA. In a similar way, when comparing only the CAD+HB topologies for the Melanoconion section in both analyses, the branching patten is very consistent, but the support is greatly different.

The BA topology obtained for the concatenated matrix (Fig.8), which includes all three gene-fragments, had the least consistent branching pattern when compared to the ML topologies; and showed a characteristic low support values for most of the cladistic groupings. The two main sections of the subgenus lack any resolution in the BA topology, with an additional confusing factor such as the outgroup clustered with the Spissipes Section as ingroup (though with non significant support/ posterior probability =0,3). However the resolution for the species level is overall comparable with the ML concatenated topology.

Figure 3. phyML phylogenetic tree of HB sequences under GTR model for *Culex (Melanoconion)* species. Support values are given by aLRT statistics.

Figure 4. phyML phylogenetic tree of CAD sequences under GTR model for *Culex* (*Melanoconion*). Support values are given by aLRT statistics.

Figure 5. phyML
phylogenetic tree of
CAD+HB sequences
under GTR model for
Culex (Melanoconion)
species. Support
values are given by
aLRT statistics.

Figure 6. phyML phylogenetic tree of all datasets under GTR model for *Culex (Melanoconion)* species. Support values are given by aLRT statistics.

Figure 8. Topology inferred by BA including all datasets for *Culex* (*Melanoconion*) species. Support values correspond to posterior probabilities.

5. Discussion

The molecular data available for the subgenus *Melanoconion* is limited, however a few important and recent contributions can be highlighted. Ribosomal and mitochondrial DNA have been mainly used to solve questions about *Culex (Melanoconion)* species. In the study by Mendenhall et al. (2012), the authors addressed the large distribution of *Cx. erraticus* in the Americas by analyzing the ND4 and IT2 genes. Their research concluded that populations of this species had regional genetic structure, with little exchange among the geographical areas of the study. Furthermore, using a different ribosomal gene, 18S, Williams et al. (2011), developed a useful and practical technique (Multiplex PCR) to identify the eight species occurring in the southern United States.

Another interesting study based on rDNA sequences (ITS-2) is the paper by Navarro and Weaver (2004) intended to study the phylogenetic relationships of the Vomerifer and Pedroi Groups of the Spissipes Section of the subgenus *Melanoconion*. Some other contributions have used as well molecular data of some other *Melanoconion* species (Demari-Silva et al. 2011; Vesgueiro et al. 2011; Gibson et al. 2012; Linton et al. 2013). Despite the several studies mentioned, our sequences only corresponded to the results published by Gibson et al. (2012) and Linton et al. (2013) (sequences of Cytochrome c oxidase subunit I). Therefore our findings can mainly be compared to a number of classification proposals based on morphological characters such as the work by Sirivanakarn (1982) and Sallum and Forattini (1996).

The study by Vesgueiro et al. (2011) using ITS-2 for a number of *Culex (Culex)* and *Culex (Melanoconion)* species, is an important background but a warning as well, exposing the difficulties encountered with the internal transcribed spacer (ITS2) of ribosomal DNA for these taxa. In the paper by Vesgueiro et al., the authors documented high intraspecific variation when using ITS-2 sequences of *Culex* species and great difficulty for the aligning phase which in turn, prevented the phylogenetic studies originally intended. For this reason, the use of ITS2 was not considered for the current study.

The taxonomic status of the Spissipes Section is better understood than that of the Melanoconion Section. Currently the Spissipes Section includes 23 species separated into eight groups and three subgroups (Sallum and Forattini, 1996). Results of the phylogenetic analyses of all three genes either separate or concatenated supported the monophyly of the non-formal groups of the Spissipes Section. Regarding the subgroups of Spissipes Section, also used in the current classification, we found support for two subgroups. The Pedroi Subgroup (*Cx. pedroi*) and the Pereyrai Subgroup (*Cx. pereyrai*) are well supported on our concatenated three-gene topology, with high aLRT support. The fact that the current taxon sampling includes only two species of the Pedroi Subgroup (composed of five species), somewhat limits the conclusions about the monophyly of the subgroup within the Spissipes Section. However, the topology obtained with the three protein coding genes concatenated (Figure 6) showed that *Cx. pedroi* and *Cx. ribeirensis* are sisters and they form a group that clusters with *Culex pereyrai* lineage. In addition, the fact that *Culex pereyrai* clustered together with species of the Pedroi Subgroup suggests that this subgroup is paraphyletic as defined by Sirivanakarn (1983) and Sallum and Forattini (1996). Consequently, it seems that *Cx. pereyrai* belongs to the Subgroup Pedroi and that the Pereyrai Subgroup is not a natural group.

The phylogenetic study by Navarro and Weaver (2004) discussed extensively on the variability of specimens included in Pedroi Subgroup, collected in Guatemala, Venezuela, Colombia and Peru. Their findings using morphological and molecular data, besides providing evidence of a cryptic pedroi-like species occurring in Venezuela and Peru, also showed evidence of the monophyly of the Pedroi Group. The difference between Navarro and Weaver (2004) phylogeny and our phylogeny is basically the informal treatment of the species. Navarro and Weaver followed Sirivanakarn (1982) in treating *Cx. adamesi*, *Cx. pedroi* and *Cx. ribeirensis*, as Pedroi Group, while we included only two of those species under the premise of being a Subgroup of Spissipes (following Sallum and Forattini, 1986).

Results of the phylogenetic analyses showed support to question the position of the Spissipes Section within the subgenus *Melanoconion*. After considering the phylogenetic evidences that Spissipes Section is a differentiated natural group, other arguments can be raised. Numerous studies have demonstrated the role of species included in the Spissipes Section, as vectors of an array of viral agents (Mitchell et al., 1985, 1987; Vasconcelos et al., 2001; Travassos-da-Rosa et al., 1997, 1998; Natal et al., 1998; Hutchings et al., 2005; Azevedo et al., 2007; Nunes et al., 2009). Also, morphological taxonomy clearly differentiates species of the Spissipes Section from those of the Melanoconion Section by characteristics of the male genitalia, pupa and fourth-instar larvae (Sirivanakarn, 1983; Sallum and Forattini, 1996). The basal sclerite and its connection to the lateral plate of the phalosome of the male genitalia, separates individuals of the species included in the Spissipes or Melanoconion Sections (Sallum and Forattini, 1996, Male genitalia keys, pag. 520). However, the quality of the genitalia dissections must be excellent to avoid distortions and breaking the structure into small pieces.

The presented evidences lead to significant changes to the known taxonomical status of the Spissipes Section. We strongly assume that this Section is a well defined monophyletic group that is supported by distinct sources of evidences, such as morphology, role of some species as vectors of arboviruses, in addition to the results of phylogenetic analyses of two single copy nuclear genes and the COI barcode. Consequently, we are proposing the Spissipes Section as a subgenus of the genus *Culex*.

Here we are proposing the resurrection of the subgenus *Helcoporpa* by Dyar (1923) ("On some of the American subgenera of *Culex*", Dyar, 1923). In his work, Dyar named the subgenus *Helcoporpa* with *Culex (Helcoporpa) menytes* as the type species. However, *Cx. H. menytes* was synonymized with *Cx. (Mel.) spissipes* by Takahashi (1968 -in Galindo 1969). Following, Galindo (1969) designated the Spissipes Group that was further treated as the Spissipes Section by Sirivanakarn (1983) with *Culex spissipes* as type species. Considering that

the type species of the *Helcoporpa* is *Culex spissipes* we are revalidating it as a subgenus of *Culex* and transferring together all the species included in the Spissipes Section to it. It is worth nothing that the species *Cx. nicaroensis*, included in the subgenus *Melanoconion* by Sirivanakarn (1983) but excluded from the subgenus by Sallum and Forattini (1996), has already been placed in the subgenus *Nicaromyia*, proposed by González Broche and Rodríguez Rodríguez (2001).

The taxonomic status of the Melanoconion Section is more challenging; it consists of 137 species and the last revision focused on this group was by Sirivanakarn (1983). In this context, we were expecting that results of phylogenetic analyses of species of the Melanoconion Section to show inconsistencies when compared to the classification based on morphology as it did. Despite the fact that the relationships between the species inside the Melanoconion Section need further study, the validity of the Section as a grouping with a common ancestor was supported by the inferred phylogenetic relationships. Though the use of "Sections" or "Groups", as auxiliary taxonomic tools, is not yet being discussed, the fact that the species of the Melanoconion Section form a historic lineage is an important result.

Our sample data corresponding to Melanoconion Section contained more specimens than the sample included for the Spissipes Section. We included representatives of ten Groups of Melanoconion Section: Distinguendus Group; Conspirator Group, Atratus Group, Educator Group, Bastagarius Group, Intrincatus Group, Inhibitor Group, Erraticus Group, Evansae Group and Pilosus Group. The only categories with evidence of monophyly are Distinguendus Group (1 species included), Atratus Group (5 species included), and Pilosus Group (3 species included). The other groupings correspond to polyphyletic or paraphyletic groups that should be re-arranged in the future, with a bigger sample of taxa and genes. The two monophyletic groups recovered with more than one species sampled (i. e. Pilosus and Atratus Groups) had high support (ML topologies), though just one of them contains the majority of representatives of its category. Atratus Group is formed by seven species, five of which are included in our data. Pilosus Group is formed by 13 species, three of them included in our data. We believe the

heterogeneity in the number of taxa sampled did not prevent our analysis from finding phylogenetic support for the related species. The current classification of the *Melanoconion* Section is composed by 13 Groups, which means, our sample reached 77% of the non-formal categories included in the analysis. However the total number of species need to be increased in the future; our sample corresponds to 25,5% of the valid species.

The phylogenetic findings for the *Melanoconion* Section demand a rearrangement of the non-formal categories created to help the taxonomical classification of the subgenus. Our three-gene phylogeny gives light on how those changes should start. For instance, the *Intrincatus* Group, according to the current classification consists of six subgroups and 25 species. In our sample, the five species we included, turned out to be two different monophyletic groupings. One of them containing *Cx. eastor*, *Cx. idottus* and *Cx. intrincatus* with higher support (aLRT=62,6; ML concatenated topology), indicating a smaller group of tightly close species. Thus we propose rearranging *Intrincatus* Group as to exclude *Cx. misionensis* and *Cx. equinoxialis* that proved not to belong to this category. More information should be gathered as to decide what to do regarding the groups formed by *Cx. misionensis* and *Cx. equinoxialis*.

Several non-monophyletic groups were evident in our phylogenetic inference, such as: *Conspirator*, *Erraticus*, *Educator*, *Inhibitor* and *Bastagarius* Groups. All these categories are in need of a bigger sample and further study. As has been documented for other insect groups (Reidenbach et al., 2009; Ceccarelli et al. 2012), combining morphological and molecular data should gather better information to solve problematic relationships as the ones we found for the *Melanoconion* Section. Despite the existence of an illustrated catalog of the species of the subgenus *Melanoconion* (Pecor et al. 1992), and keys to the non-formal categories included in this Section (Sirivanankarn, 1982), the challenge of reaching an accurate identification of the species is as current as it was in 1950 when Rozeboom and Komp published the first revision on the subgenus. Some of the illustrations contained in Pecor's catalog are poorly made and have

not been corrected in decades. The lack of a taxonomical revision of the *Melanoconion* Section creates a huge gap that should be overcome in the coming future.

The use of gene COI as a complementary tool for taxonomic corroboration has been widely documented in other studies with *Culex* (Kumar et al., 2007; Shaikevich and Zakharov, 2010; Laurito et al., 2013; Demari-Silva et al., 2011). As it has been previously stated, the taxonomic difficulty of subgenus *Melanoconion* demands all possible information in hand, thus we strongly recommend the use of COI sequences to corroborate any identification reached based on morphological characters. Our intraspecific comparisons for COI data showed still, after extensive morphological observation of the species included in the study, a few contradictions. Some of the estimated values for pairwise identity of some group of species (in *Melanoconion* Section) were lower than expected (i.e. less than 98% identical sites). Therefore, we should further review some of the specimens of *Culex aliciae*, *Cx. ensiformis*, *Cx. misionensis* and *Cx. pilosus* that had the higher values. The only information for COI intended for the subgenus *Melanoconion* is given by Demari-Silva et al. (2011). The vast majority of information available on the resolution of COI as a barcode for an easy and accurate identification is about species of subgenus *Culex* (Laurito et al. 2013).

In addition, computed distances of COI intraspecific values have been traditionally estimated through K2P genetic distances, however, the fact of choosing a model that has a certain number of assumptions which not necessarily represent all kinds of data might as well be misleading. An interesting approach to this variance in distance estimation methods is given by Collins et al. (2012). We estimated the K2P distances as well (data not shown) but chose to use the pairwise identity instead.

An important outcome of our COI taxonomical corroboration was the relationship found for *Culex serratimarge* in our analysis. The COI sequence of this species by Linton et al. (2013), available in GenBank, was used and compared to our specimen (Cx. serratimarge_PM1-2X), but we believe the species by Linton et al. (2013) needs taxonomical revision. Our voucher specimen corresponds to mounted genitalia of a male individual that is conserved at Coleção Entomológica de Referência da Faculdade de Saúde Pública, Universidade de São Paulo (FSP-USP).

Other research papers studying Culicidae used some or all of the molecular markers we used in this study (Hemmerter et al. 2009; Reidenbach et al. 2009; Foster et al. 2013; Laurito et al. 2013) and though some of the studies included *Culex* species none of them were focused on *Culex (Melanoconion)*; most studies are dedicated to solve questions regarding the cosmopolitan species of *Culex (Culex)*. The most recent taxonomical contribution that used ribosomal DNA in order to improve the identification tools for species of *Melanoconion* was described before in William and Savage (2011).

The documented difficulty in sequencing the nuclear fragment of the Hunchback gene was experienced by more than one member of our team. Other authors included HB gene in the list of rejected candidates given the short alignable regions (Wild and Maddison, 2008) detected in this coding gene. What we experienced attempting to amplify and sequence this gene fragment might have been caused by a lack of conserved priming sites occurring in our group of species. The original primers (first time used in Culicidae) referenced by Reidenbach et al. (2009) had several degenerate bases (see Methods section). Despite the fact that HB gene is taken as a very conserved gene (Pinell et al., 2006), the region we were trying to sequence resulted in an problematic goal in terms of cost-benefit tradeoff.

Once the datasets were obtained, the most difficult group of data to align was HB gene. This can be explained by the variable sizes of the obtained sequences. As a result, our HB dataset, and consequently the CAD+HB dataset, are the ones with a smaller sample. On the

other hand, COI and CAD datasets were easily aligned and the difficulty observed to sequence them was minimal for COI and medium to high for CAD. The challenging process for obtaining a CAD database was already stated by Danforth et al. (2006) and Wild and Maddison (2008); however, the use of M13 primers improved our results in a significant way. Foster et al. (2013) used M13 primers as well to facilitate the study of CAD gene in *Anopheles* species. The mitochondrial gene COI has been widely used in taxonomic studies in insects, and Culicidae; and lately in systematic studies as well (Sallum et al. 2002; Ciwynska et al. 2006; Demari-Silva et al. 2011; Laurito et al., 2013; Diaz-Nieto et al., 2013). Comparatively, the nuclear genes have been gaining attention as rich sources of genetic data, with potential for phylogenetic analysis (Wiegmann et al. 2000; Wild and Maddison, 2008; Kawahara et al. 2009; Sharanowski et al., 2011).

The Likelihood Mapping analysis (Strimmer and von Haeseler, 1997) of the different datasets showed informative, having most of the data in quarters of fully resolved areas. Therefore the phylogenetic content of the three genes we chose was considered significant. This same indicator was used for (Wiegmann et al., 2009) when studying the phylogeny of holometabolous insects.

The ML analysis of the different datasets was more informative when the datasets were combined (concatenated dataset of three genes), which did not happen with the Bayesian analysis. The concatenated data on which we used BA to infer phylogenetic hypotheses resulted in a less informative topology, when compared to the one obtained with the nuclear-gene fragments only. In the future, a different partition could be tested, as well as different setting regarding the nucleotide model of substitution and other parameters. In Brandley et al. (2005) the authors discussed events of mismodeling when working with partitioned data that might have a meaning as well for our low-support bayesian inference.

With data originated from the two-gene and three-gene datasets, the relationships between the sections, groups and species had more resolution and considerable support for some of the monophyletic groups found; specially in the ML topologies obtained. Therefore we recommend

a multi-gene approach when attempting to infer a phylogenetic hypothesis of members of the genus *Culex*. Other authors have stated as well, the importance of using diverse data to approach challenging insect taxa (Wheeler et al., 1993; Lin e Danforth, 2004).

We presented here the first comprehensive molecular phylogeny of the subgenus *Melanoconion* with evidence to separate Spissipes Section as a new subgenus and corroborate the validity of the Melanoconion Section as a monophyletic clade, still in need of an internal taxonomic rearrangement.

6. Conclusions

1. Our phylogenetic studies found the two major sections of subgenus *Melanoconion* to be well-differentiated monophyletic lineages.
2. All the internal groupings of the Spissipes Section are in agreement with the current classification based on morphological characters.
3. We propose the resurrection of the subgenus *Helcoporpa* Dyar, 1923 to elevate the Spissipes Section to a subgeneric level given the evidence collected with the multi loci topologies.
4. A very few internal groupings of the Melanoconion Section were recovered as monophyletic units, highlighting the need for a rearrangement of the current classification of this major Section.
5. The phylogenetic inference based on two nuclear genes and one mitochondrial gene was informative and generated a number of well defined natural groups that brought a better understanding of *Melanoconion* classification.

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Appendix 1 - Detailed information about specimens used in the study

Species	Class	Code	Analysis	Type of conservation	Geographical Origin Data
Cx. akritos	♀	akri1	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC+CO2+Lurex (ac.lactico)
Cx. akritos	♀	akri2	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC+CO2+Lurex (ac.lactico)
Cx. aliciae_1	♂	PM1-7	Ingroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Bairro Pariqueira Mirim. Riachino dentro da mata A. 06-02-2013.
Cx. aliciae_2	♀	PM1-6	Ingroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Bairro Pariqueira Mirim. Riachino dentro da mata A. 06-02-2013.
Cx. aliciae	♀	MG57_13	Ingroup	Dried, in silica gel	Minas Gerais. Campo Belo. Rodovia Oliveira - Campo Belo, Km33 (15/04/2010) 23K 0487328 7700518
Cx. aliciae	♂	MG57_5	Ingroup	Dried, in silica gel	Minas Gerais. Campo Belo. Rodovia Oliveira - Campo Belo, Km33 (15/04/2010) 23K 0487328 7700518
Cx. aliciae	♂	ES28-117	Ingroup	Freezer -80°C	Espírito Santo. Linhares. Reserva Natural Vale do Rio doce, Estrada da Gávea.
Cx. aliciae	♂	RJ19-46	Ingroup	Dried, in silica gel	Rio de Janeiro. Nova Iguaçu. Parque Municipal (24/08/2014) 22°46'48.9" S 43°27'33.3" W
Cx. aliciae	♂	MG31-1	Ingroup	Freezer -80°C	Minas Gerais. Coronel Pacheco. Fazenda Fortaleza de Sant'Anna (01/12/2008) 21°38'08,95" S 43°19'09,36" W
Cx. alogistus	♂	SG1-2	Ingroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Sítio Galiléia. Buraco na pedra. Mata. Hora 13:00 – 15:00.
Cx. aureonotatus_1	♂	G01-108	Ingroup	Freezer -80°C, box96, tube 33	Goiás. Caçu. ETH Rio Claro
Cx. aureonotatus_2	♂	SP35-1	Ingroup	Freezer -80°C	São Paulo. Inúbia Paulista. Represa Piscina, Fazenda Oroitê (04/02/2008) 21°40'14,6" S 50°57'01,9" W
Cx. near_aureonotatus_1	♀	TO1-12_1	Ingroup	Freezer -80°C	Tocantins. Lagoa da Confusão. Fazenda Trindade (23/07/2009) 10°35'09,3" S 49°41'23,3" W
Cx. near_aureonotatus_2	♀	TO1-12_2	Ingroup	Freezer -80°C	Tocantins. Lagoa da Confusão. Fazenda Trindade (23/07/2009) 10°35'09,3" S 49°41'23,3" W
Cx. near_aureonotatus	♀	T01-3	Ingroup	Dried, in silica gel	Tocantins. Lagoa da Confusão. Fazenda Trindade (10°35'09,3" S 49°41'23,3" W) - 23/07/2009 Bergo et al.
Cx. bastagarius_1	♂	MS2-104	Ingroup	Freezer -80°C, box78, tube40	Mato Grosso do Sul. Aquidauana. Fazenda Santa Emília
Cx. bastagarius_2	♂	SP70-106	Ingroup	Freezer -80°C, box141, tube58	São Paulo. Dourado. SP 255Km - Fazenda Santa Leonor
Cx. bastagarius_3	♂	MsExtra 1-4	Ingroup	Freezer -80°, box80, tub40/MSEExtra	Mato Grosso do Sul. Aquidauana. Pousada Araraúna.
Cx. clarki_2	♂	SP68-133	Ingroup	Freezer -80°C, box141, tube8	São Paulo. Dourado. SP 255Km – Obelisco
Cx. clarki_3	♂	SP67-1	Ingroup	Freezer -80°C	São Paulo. Dourado. SP 255Km - Obelisco (06/05/2009) 22°04'32,5" S 48°26'14,5" W
Cx. commewynensis_2	♂	SP68-139	Ingroup	Freezer -80°C, box141, tube12	São Paulo. Dourado. SP 255Km – Obelisco.
Cx. commewynensis_1	♂	SP70-23	Ingroup	Freezer -80°C, box141, tube47.	São Paulo.Dourado. SP 255Km - Fazenda Santa Leonor
Cx. corentynensis_1	♂	PA1-1	Ingroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Bairro Branco Magro. Lagoa dentro da Mata. Larvas-adultos.12:30-16:00h.
Cx. near_corentynensis_1	♀	SG1-5	Ingroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Sítio Galiléia.Buraco na pedra -Mata. 11-06-2013. 13:00 – 15:00.
Cx. near_corentynensis_2	♂	SG1-6	Ingroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Sítio Galiléia.Buraco na pedra -Mata. 11-06-2013. 13:00 – 15:00.
Cx. near_corentynensis_3	♂	SG1-4	Ingroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Sítio Galiléia.Buraco na pedra -Mata. 11-06-2013. 13:00 – 15:00.
Cx. dunni_1	♂	MGD8-102	Ingroup	Freezer -80°C	Minas Gerais. Planura. Fazenda Natividade, Rod. BR 364 Km 6.
Cx. dunni_2	♂	SP68-35	Ingroup	Freezer -80°C	São Paulo. Dourado. SP 255Km – Obelisco.
Cx. dyius	♂	RJ19-130	Ingroup	Dried, in silica gel	Rio de Janeiro. Nova Iguaçu. Parque Municipal (24/08/2014) 22°46'48.9" S 43°27'33.3" W
Cx. dyius	♂	RJ19-104	Ingroup	Dried, in silica gel	Rio de Janeiro. Nova Iguaçu. Parque Municipal (24/08/2014) 22°46'48.9" S 43°27'33.3" W
Cx. dyius	♀	RJ19-119	Ingroup	Dried, in silica gel	Rio de Janeiro. Nova Iguaçu. Parque Municipal (24/08/2014) 22°46'48.9" S 43°27'33.3" W
Cx. dyius	♂	RJ19-111	Ingroup	Dried, in silica gel	Rio de Janeiro. Nova Iguaçu. Parque Municipal (24/08/2014) 22°46'48.9" S 43°27'33.3" W
Cx. equinoxialis	♂	MG34-101	Ingroup	Freezer -80°C	Minas Gerais. Oliveira. Rodovia Oliveira - Campo Belo (11/04/2010) 20°42'45" S 44°55'28" W
Cx. equinoxialis	♂	MG54_106	Ingroup	Dried, in silica gel	Minas Gerais. São Francisco de Paula. Rodovia Oliveira - Campo Belo, Km17.5 (14/04/2010) 23K 0499313 7708140
Cx. equinoxialis	♀	MG54_107	Ingroup	Dried, in silica gel	Minas Gerais. São Francisco de Paula. Rodovia Oliveira - Campo Belo, Km17.5 (14/04/2010) 23K 0499313 7708140
Cx. evansae_2	♂	ES25-6	Ingroup	Freezer -80°C, box59, tube24	Espírito Santo. Linhares. Reserva Nat. Vale do Rio Doce, Estrada da Gávea (Sul) 19°11'53,0" S 39°57'13,0" W (24/10/2007) Bergo & Motoki
Cx. eastor	♂	east (4)	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC-LT.
Cx. eastor	♂	east (1B)	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC-LT.
Cx. eastor_3	♂	east (1d)	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC-LT (09-10/04/2012) S24.78922°, W047.78045°
Cx. eastor_4	♂	east (7)	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC-LT (12-13/03/12) S24.78922°, W047.78045°
Cx. eknomios_1	♀	SE-9	Ingroup	Dried, in silica gel	Amazonas. PNJ Rio Jaú. Seringalzinho. CDC Peri. 18:00 – 6:00 h.
Cx. eknomios_2	♀	SE-8	Ingroup	Dried, in silica gel	Amazonas. PNJ Rio Jaú. Seringalzinho. CDC Peri. 18:00 – 6:00 h.

Species	Class	Code	Analysis	Type of conservation	Geographical Origin Data
Cx. eknomios_3	♀	SE2	Ingroup	Dried, in silica gel	Amazonas. PNJ Rio Jaú. Seringalzinho. Per 18-19h.
Cx. faurani	♂	faur	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC+CO2+Lurex(ac.lactico)
Cx. ensiformis_1	♂	ES18-109	Ingroup	Freezer -80°C	Espírito Santo. Linhares. Reserva Natural Vale do Rio doce, Estrada Oitica.
Cx. ensiformis_2	♂	(3c)	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC-LT (14-15/03/2012) S24.78875°, W047.77980°
Cx. ensiformis	♂	MG54_16	Ingroup	Dried, in silica gel	Minas Gerais. São Francisco de Paula. Rodovia Oliveira - Campo Belo, Km 17.5 (14/04/2010) 23K 0499313 7708140
Cx. gnomatos_1	♀	PAT -1	Ingroup	Freezer -80°C	Amazonas. Novo Airão. Parque Nacional do Jaú.
Cx. gnomatos_2	♀	PNJ	Ingroup	Freezer -80°C	Amazonas. Novo Airão. Rio Jaú / São Lázaro, Peridomiciliar.
Cx. idottus_1	♀	SP71-117	Ingroup	Dried, in silica gel	São Paulo.Dourado. SP 255Km - Fazenda Santa Leonor (22°04'26.5" S 48°26'41.4" W) - 07/05/2009 Bergo et al
Cx. idottus_2	♀	SP71-119	Ingroup	Dried, in silica gel	São Paulo.Dourado. SP 255Km - Fazenda Santa Leonor (22°04'26.5" S 48°26'41.4" W) - 07/05/2009 Bergo et al
Cx. near_inhibitor	♂	(1g)	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC-LT (09-10/04/2012) S24.78922°, W047.78045°
Cx. intricatus_1	♂	PA1-3	Ingroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Bairro Branco Magro. Lagoa dentro da Mata. Larvas-adultos.12:30-16:00h.
Cx. intricatus_2	♂	PA2-1	Ingroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Sítio J.L. Riachinho represado. Marginal da Mata. 12:30-14:30 h. Larvas-adultos.
Cx. intricatus_3	♂	PA2-2	Ingroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Sítio J.L. Riachinho represado. Marginal da Mata. 12:30-14:30 h. Larvas-adultos.
Cx. intricatus_4	♂	PA2-3	Ingroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Sítio J.L. Riachinho represado. Marginal da Mata. 12:30-14:30 h. Larvas-adultos.
Cx. intricatus_5	♂	SG2-2	Ingroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Sítio Galiléia. Lagoa dentro da Mata. Hora 13:00 – 15:00.
Cx. intricatus_6	♂	PM1-1x	Ingroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Bairro Pariqueira Mirim. Riachino dentro da mata A. 06-02-2013.
Cx. intricatus_7	♂	PM1-5	Ingroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Bairro Pariqueira Mirim. Riachino dentro da mata A. 06-02-2013.
Cx. intricatus_10	♂	PM1-3	Ingroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Bairro Pariqueira Mirim. Riachino dentro da mata A. 06-02-2013.
Cx. intricatus_11	♂	PM1-4	Ingroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Bairro Pariqueira Mirim. Riachino dentro da mata A. 06-02-2013.
Cx. lacertosus	♂	SO2-103	Ingroup	Freezer -80°C	Amazonas. Barcelos. Rio Unini, próximo Comunidade Solimolzinho (09/02/2009) 1°37'41,1" S 62°58'27,9" W
Cx. misionensis	♂	2A (put 1)	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC-LT.
Cx. misionensis_2	♂	SP89-18	Ingroup	Dried, in silica gel	São Paulo / São Paulo / CTL - Aterro Sanitário (23°32'51" S 46°38'10" W) - 26/10/2010 Natal & Sant'Ana
Cx. misionensis_3	♂	SP89-14	Ingroup	Dried, in silica gel	São Paulo / São Paulo / CTL - Aterro Sanitário (23°32'51" S 46°38'10" W) - 26/10/2010 Natal & Sant'Ana
Cx. oedipus	♂	2c (oed1)	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC-LT.
Cx. oedipus_2	♂	IG1-1	Ingroup	Dried, in silica gel	São Paulo. Vale do Ribeira. Iguape. Rio Ribeira de Iguape. 21-08-2013.
Cx. pilosus_1	♂	SP50-14	Ingroup	Freezer -80°C	São Paulo. Pindamonhangaba. Fazenda Saint Clair (22/01/2009) 22°45'50" S 45°30'87" W
Cx. pilosus_2	♂	SP56-107	Ingroup	Freezer -80°C	São Paulo. Mairiporã (02/08/2009) 23°19'08" S 46°35'13" W
Cx. pedroi	♀	ped 1	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC+CO2+Lurex(ac.lactico)
Cx. pedroi	♀	ped 2	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC+CO2+Lurex(ac.lactico)
Cx. pereyrai_1	♂	IG1-2	Ingroup	Dried, in silica gel	São Paulo. Vale do Ribeira. Iguape. Rio Ribeira de Iguape. 21-08-2013.
Cx. pereyrai_2	♂	IG1-3	Ingroup	Dried, in silica gel	São Paulo. Vale do Ribeira. Iguape. Rio Ribeira de Iguape. 21-08-2013.
Cx. portesi	♀	VA3	Ingroup	Dried, in silica gel	Amazonas. PNJ. Rio Unini. Vista Alegre. Isca Humana, Peri.18:00 – 19:00 h.
Cx. putumayensis	♂	2b (mis 1)	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC-LT.
Cx. putumayensis_2	♂	3b	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC-LT (14-15/03/2012) S24.78875°, W047.77980°
Cx. rabelloi	♂	PA1-2	Ingroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Bairro Branco Magro. Lagoa dentro da Mata. Larvas-adultos.12:30-16:00h.
Cx. near_rabelloi_tipo 1	♂	(1e)	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC-LT (09-10/04/2012) S24.78922°, W047.78045°
Cx. near_rabelloi_tipo 1.1	♂	(1c)	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC-LT (09-10/04/2012) S24.78922°, W047.78045°

Species	Class	Code	Analysis	Type of conservation	Geographical Origin Data
Cx. near_rabelloi_tipo 2	♂	(1A)	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC-LT (09-10/04/2012) S24.78922°, W047.78045°
Cx. near_rabelloi_tipo 3	♂	(1H)	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC-LT.
Cx. ribeirensis	♀	rib 1	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC+CO2+Lurex(ac.lactico)
Cx. ribeirensis	♀	rib 2	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC+CO2+Lurex(ac.lactico)
Cx. ribeirensis	♀	IG20-12	Ingroup	Dried, in silica gel	São Paulo. Vale do Ribeira. Iguape. Rio Ribeira de Iguape. Barraca de Shannon – 13/11/2013 (18:00 às 20:00)
Cx. ribeirensis	♀	IG20-13	Ingroup	Dried, in silica gel	São Paulo. Vale do Ribeira. Iguape. Rio Ribeira de Iguape. Barraca de Shannon – 13/11/2013 (18:00 às 20:00)
Cx. sacchettae	♀	sach 1	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Armadilha magnetica mosq.magnet.
Cx. sacchettae	♀	sach 2	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul.Armadilha magnetica mosq.magnet.
Cx. sacchettae	♀	sach 3	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Armadilha magnetica mosq.magnet.
Cx. serratimarge	♂	PM1-2x	Ingroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Bairro Pariqueira Mirim. Riachino dentro da mata A. 06-02-2013.
Cx. spissipes	♀	spis 1	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC-LT.
Cx. spissipes	♀	spis 2	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC-LT.
Cx. spissipes	♀	spis 3	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul. Coleta CDC-LT.
Cx. theobaldi_1	♂	MS6-100	Ingroup	Freezer -80°C	Mato Grosso do Sul. Aquidauana. Pousada Araraúna divisa com Fazenda Santa Maria.
Cx. theobaldi_2	♀	MS7-100	Ingroup	Freezer -80°C	Mato Grosso do Sul. Aquidauana. Pousada Araraúna, Porto do Rio correntoso.
Cx. trigeminatus_1	♂	PA1-4	Ingroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Bairro Branco Magro. Lagoa dentro da Mata. Larvas-adultos.12:30-16:00h.
Cx. vaxus	♂	IG20-1	Ingroup	Dried, in silica gel	São Paulo. Vale do Ribeira. Iguape. Rio Ribeira de Iguape. Barraca de Shannon – 13/11/2013 (18:00 às 20:00)
Cx. vaxus	♂	IG20-2	Ingroup	Dried, in silica gel	São Paulo. Vale do Ribeira. Iguape. Rio Ribeira de Iguape. Barraca de Shannon – 13/11/2013 (18:00 às 20:00)
Cx. vaxus	♂	IG20-3	Ingroup	Dried, in silica gel	São Paulo. Vale do Ribeira. Iguape. Rio Ribeira de Iguape. Barraca de Shannon – 13/11/2013 (18:00 às 20:00)
Cx. vaxus	♂	IG20-4	Ingroup	Dried, in silica gel	São Paulo. Vale do Ribeira. Iguape. Rio Ribeira de Iguape. Barraca de Shannon – 13/11/2013 (18:00 às 20:00)
Cx. vaxus	♂	IG20-5	Ingroup	Dried, in silica gel	São Paulo. Vale do Ribeira. Iguape. Rio Ribeira de Iguape. Barraca de Shannon – 13/11/2013 (18:00 às 20:00)
Cx. vaxus	♂	IG20-6	Ingroup	Dried, in silica gel	São Paulo. Vale do Ribeira. Iguape. Rio Ribeira de Iguape. Barraca de Shannon – 13/11/2013 (18:00 às 20:00)
Cx. near_vaxus_9	♂	TO1-9	Ingroup	Freezer -80°C	Tocantins. Lagoa da Confusão. Fazenda Trindade (23/07/2009) 10°35'09,3" S 49°41'23,3" W
Cx. zeteki_1	♂	zet 1 (5)	Ingroup	Dried, in silica gel	São Paulo. Iguape.Litoral Paulista. Região Vale do Ribeira. Sítio Santa Rosa. Rodovia Estadual SP-222. Via Pariqueira-Açu/Iguape. Km.87. Bairro Serra azul.Armadilha magnetica mosq.magnet.
Cx. zeteki_2	♂	SP42-2	Ingroup	Freezer -80°C	São Paulo. Cananéia. Sítio Gentil, Lagoa.
Cx. (Culex) mollis 2	♂	SG1-1	Outgroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Sítio Galiléia. Buraco na pedra. Mata. Hora 13:00 – 15:00.
Cx. (Culex) mollis 1	♂	SG1-3	Outgroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Sítio Galiléia. Buraco na pedra. Mata. Hora 13:00 – 15:00.
Culex (Cx) quinquefasciatus	♂	LESP1	Outgroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Sítio Galiléia. Buraco na pedra. Mata. Hora 13:00 – 15:00.
Culex (Cx) quinquefasciatus	♂	LESP2	Outgroup	Dried, in silica gel	São Paulo. Pariqueira Açu. Sítio Galiléia. Buraco na pedra. Mata. Hora 13:00 – 15:00.
Aedes scapularis	♂	IG20-10	Outgroup	Dried, in silica gel	São Paulo. Vale do Ribeira. Iguape. Rio Ribeira de Iguape. Barraca de Shannon – 13/11/2013 (18:00 às 20:00)
Aedes scapularis	♀	IG20-11	Outgroup	Dried, in silica gel	São Paulo. Vale do Ribeira. Iguape. Rio Ribeira de Iguape. Barraca de Shannon – 13/11/2013 (18:00 às 20:00)

Appendix 2 - Experimental Protocols

Protocol 1 - DNA extraction - Salting Out

Source: Modified from Miller et al. (1988).

Part 1

- Tissue Digestion

1. Macerate each mosquito inside a 1,5 ml tube with 300 uL of TNES buffer (TNES buffer: 10 mM Tris-HCL, pH 7,5, 400 Mm NaCl, 100 mM EDTA pH 8,0, 0,6% SDS)
2. Add 1µL Proteinase K (18,7 mg/ml) to each tube (with a macerated mosquito in TNES buffer)
3. Incubate at 56°C for 16-18 hours (overnight) in a dry block heater with agitation (600 rpm per 15 sec every 10 mins).

- Precipitation of Protein and Cell Debris

4. Add 85 uL NaCl (5M) to each tube and homogenise
5. Centrifuge at 13.000 rpm for 10 mins at ambient temperature
6. Transfer supernatant into a new tube

- Precipitation of Nucleic Acids

7. Add 300 uL of cold 98% ethanol (4°C) to each tube and homogenise by inverting the tubes upside down, carefully.
8. Incubate at -20°C for ~20 minutes
9. Centrifuge (preferably at 4°C) at 13.000 rpm in a benchtop centrifuge for 10 minutes
10. Flip the tube carefully to discard the supernatant.
11. Add 500 uL of 70% ethanol and repeat from step 3 (Centrifuge...)
12. Allow to air dry inside a speed vacuum for 5 to 10 mins at 60°C
13. Resuspend in 100 uL of TE buffer

Part 2

- Removing contaminating RNA

1. Add 0,1 uL RNase (10mg/ml) to every 10 ul of DNA product of extraction protocol.
2. Incubate at 37°C for 30 mins
3. Store all products in freezer -20°C

Critical steps

- After adding sodium chloride and centrifuging be careful not to transfer any of the white solid (cell debris and SDS) into the fresh tube (Part 1, Step 4).
- Ensure to dry the pelleted DNA completely before attempting to resuspend (Part 1, Step 12).

Appendix 2

Protocol 2 - PEG precipitation (20% polyethylene glycol 8000/2.5 M NaCl)

1. Add equal volumes of PCR product and PEG 20%.
2. Vortex briefly and incubate for 15 min at 37°C.
3. Centrifuge at 13.200 rpm for 15 min at ambient temperature
4. Discard supernatant with pipette, taking away the liquid; introduce the pipette carefully in order to not take in the pellet that contains the DNA.
5. Wash the pellet with 200 μ L of 80% ethanol and flip the tubes up and down, twice, to mix well.
6. Centrifuge at 13.200 rpm for 15 min at 4°C
7. Remove the ethanol flipping the content of each tube at once in a beaker.
8. Repeat wash with ethanol. Go back to Step 5 and this time use cold ethanol.
9. Centrifuge at 13.200 rpm for 15 min at 4°C
10. Remove the ethanol flipping the content at once in a beaker.

Appendix 3 - Species included in the study and gene sequences obtained.

Species	Code	COI - consensus	CAD - consensus	HB - F strand only
Cx. akritos	akri1	OK	OK	OK
Cx. akritos	akri2	OK	OK	X
Cx. aliciae_1	PM1-7	OK	OK	OK
Cx. aliciae_2	PM1-6	OK	OK	OK
Cx. aliciae	MG57_13	OK	OK	OK
Cx. aliciae	MG57_5	OK	OK	OK
Cx. aliciae	RJ19-46	OK	OK	OK
Cx. aliciae	MG31-1	OK	OK	OK
Cx. aliciae	ES28-117	OK	X	X
Cx. alogistus	SG1-2	OK	OK	OK
Cx. aureonotatus_1	G01-108	OK	OK	OK
Cx. aureonotatus_2	SP35-1	OK	OK	OK
Cx. near_aureonotatus_1	TO1-12_1	OK	OK	OK
Cx. near_aureonotatus_2	TO1-12_2	OK	OK	OK
Cx. near_aureonotatus	T01-3	OK	OK	OK
Cx. bastagarius_1	MS2-104	OK	OK	OK
Cx. bastagarius_2	SP70-106	OK	OK	OK
Cx. bastagarius_3	MsExtra 1-4	OK	OK	OK
Cx. clarki_2	SP68-133	OK	OK	OK
Cx. clarki_3	SP67-1	OK	OK	OK
Cx. commevynensis_2	SP68-139	OK	OK	OK
Cx. commevynensis_1	SP70-23	OK	OK	X
Cx. corentynensis_1	PA1-1	OK	OK	OK
Cx. prox a corentynensis_1	SG1-5	OK	X	X
Cx. prox a corentynensis_2	SG1-6	OK	X	X
Cx. prox a corentynensis_3	SG1-4	OK	X	X
Cx. dunni_1	MGD8-102	OK	OK	X
Cx. dunni_2	SP68-35	OK	OK	X
Cx. dyius	RJ19-130	OK	OK	OK
Cx. dyius	RJ19-104	OK	OK	OK
Cx. dyius	RJ19-119	OK	OK	OK
Cx. dyius	RJ19-111	OK	OK	OK
Cx. equinoxialis	MG34-101	OK	OK	X
Cx. equinoxialis	MG54_106	OK	OK	X
Cx. equinoxialis	MG54_107	OK	OK	X
Cx. evansae_2	ES25-6	OK	OK	OK

Species	Code	COI - consensus	CAD - consensus	HB - F strand only
Cx. eastor	east (4)	OK	OK	OK
Cx. eastor	east (1B)	OK	OK	OK
Cx. eastor_3	east (1d)	OK	OK	OK
Cx. eastor_4	east (7)	OK	OK	OK
Cx. eknomios_1	SE-9	OK	OK	OK
Cx. eknomios_2	SE-8	OK	OK	OK
Cx. eknomios_3	SE2	OK	OK	OK
Cx. faurani	faur	OK	OK	OK
Cx. ensiformis_1	ES18-109	OK	OK	OK
Cx. ensiformis_2	(3c)	OK	X	OK
Cx. ensiformis	MG54_16	OK	OK	OK
Cx. gnomatos_1	PAT -1	OK	OK	OK
Cx. gnomatos_2	PNJ	OK	OK	OK
Cx. idottus_1	SP71-117	OK	OK	X
Cx. idottus_2	SP71-119	OK	OK	OK
Cx. near_inhibitor	(1g)	OK	OK	OK
Cx. intricatus_1	PA1-3	OK	OK	OK
Cx. intricatus_2	PA2-1	OK	OK	OK
Cx. intricatus_3	PA2-2	OK	OK	OK
Cx. intricatus_4	PA2-3	OK	OK	OK
Cx. intricatus_5	SG2-2	OK	OK	OK
Cx. intricatus_6	PM1-1x	OK	OK	OK
Cx. intricatus_7	PM1-5	OK	OK	OK
Cx. intricatus_10	PM1-3	OK	OK	OK
Cx. intricatus_11	PM1-4	OK	OK	X
Cx. lacertosus	SO2-103	OK	OK	OK
Cx. misionensis	(2A)	OK	OK	OK
Cx. misionensis_2	SP89-18	OK	OK	OK
Cx. misionensis_3	SP89-14	OK	OK	OK
Cx. oedipus	(2c) oed 1	OK	OK	OK
Cx. oedipus_2	IG 1-1	OK	OK	OK
Cx. pilosus_1	SP50-14	OK	OK	OK
Cx. pilosus_2	SP56-107	OK	OK	OK
Cx. pedroi	ped 1	OK	OK	OK
Cx. pedroi	ped 2	OK	OK	OK
Cx. pereyrai_1	IG1-2	OK	OK	OK
Cx. pereyrai_2	IG1-3	OK	OK	OK

Species	Code	COI - consensus	CAD - consensus	HB - F strand only
Cx. portesi	VA3	OK	X	X
Cx. putumayensis	(2b) mis 1	OK	OK	OK
Cx. putumayensis_2	(3b)	OK	OK	OK
Cx. rabelloi	PA1-2	OK	OK	OK
Cx. near_rabelloi_tipo 1	(1e)	OK	X	OK
Cx. near_rabelloi_tipo 1.1	(1c)	OK	OK	OK
Cx. near_rabelloi_tipo 2	(1A)	OK	OK	OK
Cx. near_rabelloi_tipo 3	(1H)	OK	OK	OK
Cx. ribeirensis	rib 1	OK	OK	X
Cx. ribeirensis	rib 2	OK	OK	X
Cx. ribeirensis	IG20-12	OK	X	X
Cx. ribeirensis	IG20-13	OK	OK	X
Cx. sacchettae	sach 1	OK	OK	OK
Cx. sacchettae	sach 2	OK	OK	OK
Cx. sacchettae	sacj 3	OK	OK	OK
Cx. serratimarge	PM1-2x	OK	OK	OK
Cx. spissipes	spis 1	OK	OK	OK
Cx. spissipes	spis 2	OK	OK	OK
Cx. spissipes	spis 3	OK	OK	OK
Cx. theobaldi_1	MS6-100	OK	OK	OK
Cx. theobaldi_2	MS7-100	OK	OK	OK
Cx. trigeminatus_1	PA1-4	OK	OK	OK
Cx. vaxus	IG20-1	X	OK	OK
Cx. vaxus	IG20-2	OK	OK	OK
Cx. vaxus	IG20-3	X	OK	OK
Cx. vaxus	IG20-4	OK	OK	OK
Cx. vaxus	IG20-5	OK	OK	OK
Cx. vaxus	IG20-6	OK	OK	OK
Cx. near_vaxus_9	TO1-9	OK	OK	OK
Cx. zeteki_1	zet 1 (5)	OK	OK	OK
Cx. zeteki_2	SP42-2	OK	OK	OK
Cx. (Culex) mollis 2	SG1-1	OK	OK	OK
Cx. (Culex) mollis 1	SG1-3	OK	OK	OK
Cx. (Culex) quinquefasciatus	LESP 1	N	N	OK
Cx. (Culex) quinquefasciatus	LESP 2	N	N	OK
Aedes scapularis	IG20-10	OK	OK	OK
Aedes scapularis	IG20-11	X	OK	OK

Carolina Torres Gutierrez

- Endereço para acessar este CV: <http://lattes.cnpq.br/0653223284777321>
- Última atualização do currículo em 06/09/2012

Possui graduação em Biologia pela Pontifícia Universidad Javeriana, Bogotá, D.C., Colômbia (2000). Mestrado em Entomologia pela Universidade Federal de Viçosa UFV Minas Gerais, Brasil (2006). Possui vínculo laboral desde o ano 2006 com a Universidad de Antioquia (Medellín, Colômbia), como docente e pesquisadora, atualmente em período de afastamento para cursar estudos de Doutorado na Universidade de São Paulo. Tem experiência nos seguintes temas: Taxonomia de espécies vetores de malária, dengue, encefalite equina e outras doenças; desenvolvimento de atividades no laboratório e campo relacionadas com o estudo de mosquitos vetores; Elaboração e administração de projetos de pesquisa. **(Texto informado pelo autor)**

Identificação

Carolina Torres Gutierrez

Nome

Torres C.

Nome em citações bibliográficas

Endereço

Universidade de São Paulo, Faculdade de Saúde Pública.
Avenida Dr. Arnaldo 715 / Sala 210
Cerqueira Cesar
01246-904 - São Paulo, SP - Brasil
URL da Homepage: <http://www.fsp.usp.br/site/>

Endereço Profissional

Formação acadêmica/titulação

2011

Doutorado em andamento em Saúde Pública (Conceito CAPES 6).
Universidade de São Paulo, USP, Brasil.
Título: Filogenia Molecular do Subgênero Melanconion do gênero Culex (Diptera Culicidae),

Orientador: Maria Anice Mureb Sallum.
Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.
Palavras-chave: Filogenia; Diptera, Culicidae.
Grande área: Outros / Área: Divulgação Científica / Subárea: Entomologia Médica.
Setores de atividade: Saúde humana e serviços sociais.

2004 - 2006

Mestrado em Entomologia (Conceito CAPES 7).
Universidade Federal de Viçosa, UFV, Brasil.
Título: Desenvolvimento e Reprodução do predador Brontocoris tabidus (Heteroptera: Pentatomidae) no campo após exposição ao piretroida deltametrina., Ano de Obtenção: 2006.

Orientador: Jose Cola Zanuncio.
Bolsista do(a): Conselho Nacional de Desenvolvimento Científico e Tecnológico.
Palavras-chave: Controle Biológico; Brontocoris tabidus; Seletividade de inseticidas.
Grande área: Ciências Biológicas / Área: Biologia Geral / Subárea: Taxonomia / Especialidade: Entomologia.
Setores de atividade: Atividades profissionais, científicas e técnicas.

1993 - 2000

Graduação em Biologia.
Pontifícia Universidad Javeriana.
Título: Bióloga.
Orientador: Fernando Fernandez.

Formação Complementar

Interactive Course on Vector Biology and Control. (Carga horária: 40h). Universidad de Antioquia, UdeA, Colômbia.	2009 - 2009
Pruebas colorimétricas para det. de residualidad. (Carga horária: 40h). Instituto Nacional de Salud.	2009 - 2009
Interactive course on Vector Biology and Control. (Carga horária: 40h). Programa de Estudio y Control de Enfermedades Tropicales.	2009 - 2009
Extensão universitária em Fundamentación Pedagógica y Didáctica Universitari. (Carga horária: 90h). Universidad de Antioquia.	2007 - 2007
Extensão universitária em Enfoques y Dilemas Contemporaneos En Salud. (Carga horária: 45h). Universidad Nacional de Colômbia.	2002 - 2002
Fortalecimiento Gerencial de la Red Nal de Laborat. (Carga horária: 40h). Organización panamericana de la Salud.	2002 - 2002
Curso de capacitación en Entomología Médica. (Carga horária: 30h). Instituto Nacional de Salud.	2002 - 2002
Entomologia Médica II. (Carga horária: 40h). Instituto Nacional de Salud.	2000 - 2000

Atuação Profissional

Universidad de Antioquia, UDEA, Colômbia.	
	Vínculo institucional
	2006 - Atual
Vínculo: Servidor público (Professor), Enquadramento Funcional: Professor Assistente e Pesquisador, Carga horária: 40	
Cargo: Coordenadora Unidade de Entomología Médica Programa de Estudio y Control de Enfermedades Tropicales PECET. Instituto de Investigaciones Médicas, Facultad de Medicina. Áreas de atuação: Biologia, taxonomia, ecologia e controle de mosquitos vetores de doenças tropicais. Ensino em graduação e pósgraduação; Desenvolvimento de projetos de pesquisa básica e aplicada.	Outras informações
	Atividades
Ensino, Ciencias Biológicas, Nível: Pós-Graduação	2/2010 - Atual
Disciplinas ministradas Taxonomia básica de Mosquitos Culicídeos Tópicos na Bionomia de mosquitos vetores	
Ensino, Entorno, Nível: Graduação	6/2009 - Atual
Disciplinas ministradas Entomologia Médica: Aspectos gerais Metodo Ecoepidemiologico	
Extensão universitária , Programa de Estudio y Control de Enfermedades - PECET, .	6/2008 - Atual
Atividade de extensão realizada Avaliação de produtos larvicidas, repelentes e inseticidas, no laboratório e campo, com mosquitos culicídeos. Colômbia..	2/2008 - Atual

Outras atividades técnico-científicas , Programa de Estudio y Control de Enfermedades - PECET, .

Atividade realizada

Orientadora de trabalhos de graduação e pós-graduação.

Instituto Nacional de Salud, INS, Colômbia.

Vínculo institucional

2000 - 2002

Vínculo: Contrato, Enquadramento Funcional: Bióloga, Carga horária: 40, Regime: Dedicação exclusiva.

Outras informações

Programa de Vigilância Entomológica de Doenças Transmitidas por Vetores. Laboratório de Entomologia. Apoio técnico para identificação de insetos vetores e trabalho de campo nas áreas de transmissão de doenças.

Atividades

1/2001 - 12/2002

Serviços técnicos especializados .

Serviço realizado

Identificação de espécies de mosquitos de importância médica em regiões rurais da Colômbia..

9/2000 - 12/2002

Direção e administração, Laboratório de Entomologia, .

Cargo ou função

Estruturação e fortalecimento das Unidades Básicas de Entomologia, nos pontos locais de Saúde, em regiões da Colômbia Rede Nacional Entomologia Médica. Instituto Nacional de Saúde. Bogotá..

Secretaria Departamental de Salud Vichada, SDSV, Colômbia.

Vínculo institucional

2003 - 2003

Vínculo: Contrato, Enquadramento Funcional: Bióloga Coordenadora - Unidade de Entomologia, Carga horária: 55, Regime: Dedicação exclusiva.

Outras informações

Bióloga. Coordenadora Unidade de Entomología Médica. Unidade Local de Saúde. Departamento Vichada, Colômbia. Areas de atuação: Biologia e taxonomia dos insetos vetores de doenças tropicais.

Atividades

5/2003 - 12/2003

Serviços técnicos especializados , Unidade de Entomologia Médica, Oficina local de saúde, .

Serviço realizado

Coordenação de atividades de campo e laboratório para o estudo, prevenção e controle dos vetores de dengue y malária nas zonas rurais do departamento do Vichada, Colômbia..

5/2003 - 12/2003

Treinamentos ministrados , Unidade de Entomologia Médica, Oficina local de saúde, .

Treinamentos ministrados

Capacitação sobre identificação taxonómica dos mosquitos vetores da dengue e malária ao pessoal técnico em localidades rurais de Vichada.

Projetos de pesquisa

2011 - Atual

Mapas de distribución espacio-temporal de especies de Lutzomyia en Antioquia: una aproximación real a la distribución de los vectores de leishmaniasis

Situação: Em andamento; Natureza: Pesquisa.

Integrantes: Carolina Torres Gutierrez - Integrante / Iván Darío Vélez Bernal - Coordinador / Mónica Zuleta - Integrante.

2009 - 2011

Mosquitos Asociados a Guadua en el eje cafetero (Colombia) / Mosquitos associados ao Bambú em zonas de cafezais na Colômbia.

Descrição: Projeto de Pesquisasobre a riqueza de Mosquitos associados ao bambú em áreas rurais..

Situação: Concluído; Natureza: Pesquisa.

Alunos envolvidos: Mestrado acadêmico: (1) .

Integrantes: Carolina Torres Gutierrez - Coordinador / Sandra Inés Uribe Soto - Integrante / Charles Porter - Integrante / Guillermo León Rúa-Urbe - Integrante.

Financiador(es): Centers for Disease Control and Prevention - Cooperação / Fundación para la Promoción de la Investigación y la Tecnología - Auxílio financeiro / Universidad de Antioquia - Remuneração / Universidad Nacional de Colombia - Sede Medellín - Remuneração.

Número de produções C, T & A: 2

2009 - Atual

SCD PROBIOTICS AS BIOLOGICAL CONTROL FOR VECTOR MOSQUITO LARVAE / Avaliação de microorganismos probióticos como estratégia de controle biológico contra mosquitos vetores

Situação: Em andamento; Natureza: Pesquisa.

Alunos envolvidos: Graduação: (1) .

Integrantes: Carolina Torres Gutierrez - Coordinador / Iván D. Vélez - Integrante / Raul L. Rocha Orjuela - Integrante / José David Mojica - Integrante / Margarita M. Correa - Integrante.

Financiador(es): Auroville - Cooperação / Universidad de Antioquia - Remuneração.

Número de produções C, T & A: 1 / Número de orientações: 1

2009 - Atual

Influence of Global Warming on Vector-borne Disease Transmission in Colombia / Influência do cambio climático na transmissão de doenças transmitidas por vetores na Colômbia.

Descrição: Projeto de pesquisa em andamento desenvolvido por três instituições: Centers for Disease Control and Prevention, Universidad Nacional de Colombia e Universidad de Antioquia, Colombia..

Situação: Em andamento; Natureza: Pesquisa.

Alunos envolvidos: Graduação: (1) / Mestrado acadêmico: (2) / Doutorado: (2) .

Integrantes: Carolina Torres Gutierrez - Integrante / Sandra Inés Uribe Soto - Integrante / Iván D. Vélez - Integrante / Charles Porter - Coordinador.

Financiador(es): Centers for Disease Control and Prevention - Auxílio financeiro / Universidad de Antioquia - Remuneração / Universidad Nacional de Colombia - Sede Medellín - Remuneração.

Número de produções C, T & A: 1

2008 - 2011

Reconocimiento e Identificación de Mosquitos asociados a guadua / Reconhecimento e Identificação de Mosquitos (Diptera: Culicidae) associados ao Bambú

Descrição: Pesquisa de espécies de Culicidae (mosquitos) associadas a habitats fitotelmata em regioes rurais da Colômbia..

Situação: Concluído; Natureza: Pesquisa.

Alunos envolvidos: Graduação: (1) / Mestrado acadêmico: (2) .

Integrantes: Carolina Torres Gutierrez - Coordinador / Sandra Uribe Soto - Integrante / Charles H. Porter - Integrante / Guillermo L. Rúa-Urbe - Integrante.

Financiador(es): Departamento Administrativo de Ciencia, Tecnología e Innovación - Auxílio financeiro / Universidad de Antioquia - Remuneração / Universidad Nacional de Colombia - Sede Medellín - Remuneração / Centers for Disease Control and Prevention - Cooperação.

Número de produções C, T & A: 5 / Número de orientações: 1

2008 - 2011

Estudio Ecoepidemiológico para la prevención y control de la leishmaniosis en el departamento del Chocó / Estudo Ecoepidemiologico para a prevenção e controle da leishmaniose no estado do Chocó, Colômbia

Situação: Concluído; Natureza: Pesquisa.

Alunos envolvidos: Mestrado acadêmico: (1) .

Integrantes: Carolina Torres Gutierrez - Integrante / Iván D. Vélez - Integrante / Lina Maria Carrillo - Coordinador.

Financiador(es): Departamento Administrativo de Ciencia, Tecnología e Innovación - Auxílio financeiro / Universidad de Antioquia - Remuneração.

Número de produções C, T & A: 2

2008 - 2011

Identificación de Microhábitats de inmaduros del género *Lutzomyia* / Identificação de microhabitats de imaturos do gênero *Lutzomyia* (Diptera: Psychodidae), vetores de Leishmaniose na Colômbia.

Situação: Concluído; Natureza: Pesquisa.

Alunos envolvidos: Graduação: (2) / Mestrado acadêmico: (2) .

Integrantes: Carolina Torres Gutierrez - Integrante / Marcel Marin Villa - Integrante / Rafael J. Vivero Gomez - Integrante / Eduar E. Bejarano - Integrante / Carlos Enrique Muskus - Coordenador.

Financiador(es): Departamento Administrativo de Ciencia, Tecnología e Innovación - Auxílio financeiro / Universidad de Antioquia - Remuneração / Universidad de Sucre - Remuneração.

Número de produções C, T & A: 10 / Número de orientações: 1

Projetos de desenvolvimento

2003 - 2003

Identificación taxonómica de mosquitos vectores de malaria en comunidades indígenas del Vichada (Colombia) / Identificação taxonómica de vetores de malária em comunidades indígenas de Vichada, Colômbia.

Situação: Concluído; Natureza: Desenvolvimento.

Integrantes: Carolina Torres Gutierrez - Coordenador.

Financiador(es): Secretaria Departamental de Salud Vichada - Remuneração.

Outros Projetos

2000 - 2002

Estruturação e fortalecimento das Unidades Básicas de Entomologia, nos pontos locais de Saúde, em diferentes regiões da Colômbia - Rede Nacional de Entomologia Médica. Instituto Nacional de Saúde. Bogotá. 2000 - 2002.

Situação: Concluído; Natureza: Outra.

Integrantes: Carolina Torres Gutierrez - Integrante / Victor Alberto Olano - Coordenador / Helena Brochero - Integrante.

Revisor de periódico

2009 - 2009

Periódico: Revista Colombiana de Entomología

2008 - 2008

Periódico: Biomédica (Bogotá)

2010 - 2010

Periódico: Revista Facultad de Ciencias Básicas, Universidad Militar Nueva Granada

Áreas de atuação

Grande área: Ciências Biológicas / Área: Biologia Geral / Subárea: Taxonomia/Especialidade: Entomologia.

2.

Grande área: Ciências Biológicas / Área: Biologia Geral.

3.

Grande área: Outros / Área: Divulgação Científica / Subárea: Entomologia Médica.

Idiomas

	Espanhol
Compreende Bem, Fala Bem, Lê Bem, Escreve Bem.	
Compreende Bem, Fala Bem, Lê Bem, Escreve Bem.	Inglês
Compreende Bem, Fala Razoavelmente, Lê Bem, Escreve Razoavelmente.	Português

Prêmios e títulos

Prêmio Nacional de Entomologia Medica, Sociedad Colombiana de Entomología, SOCOLEN..	2010
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Docente e Pesquisadora colombiana, membro do grupo de Pesquisa denominado "Programa de Estudio y Control de Enfermedades Tropicales" - PECET, da Universidad de Antioquia, em Medellín, Colômbia. Docente com vínculo empregatício e licença de afastamento para cursar Programa de Doutorado no Brasil. Interesses científicos e acadêmicos nas áreas da Entomologia Médica e Saúde Pública.