Universidade de São Paulo
Faculdade de Saúde Pública

Sistemática de seis espécies de *Anopheles* (*Nyssorhynchus*) Blanchard (Diptera: Culicidae)

Maysa Tiemi Motoki

Dissertação apresentada ao Programa de Pós-Graduação em Saúde Pública para obtenção do título de Mestre em Saúde Pública

Área de concentração: Epidemiologia

Orientadora: Profa. Dra. Maria Anice Mureb Sallum

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RESUMO

Motoki, MT. Sistemática de seis espécies de *Anopheles* (*Nyssorhynchus*) Blanchard (Diptera: Culicidae) [dissertação de mestrado]. São Paulo: Faculdade de Saúde Pública da USP; 2007.

Introdução – Os complexos *An. oswaldoi* e *An. albitarsis* são formados por espécies morfologicamente semelhantes e, algumas delas, são importantes vetores de plasmódios que causam a malária humana. A separação das espécies de cada complexo é problemática devido ao polimorfismo e sobreposição dos caracteres morfológicos utilizados em chaves de identificação, que muitas vezes só é alcançada com o uso de marcadores moleculares. No entanto, a identificação correta das espécies se faz necessária para a avaliação adequada da importância epidemiológica das mesmas. Infelizmente, são poucos os estudos que objetivam a caracterização morfológica dos membros do complexo *An. oswaldoi* e *An. albitarsis*. **Objetivos** – Caracterizar morfologicamente e molecularmente o *An. oswaldoi s.s.*; caracterizar morfologicamente cinco espécies do complexo *An. albitarsis;* estabelecer caracteres morfológicos que permitam a separação entre as espécies do complexo *An. albitarsis*; solucionar problema de nomenclatura de *An. marajoara.* **Métodos** - Foram utilizados espécimes disponíveis nos acervos da Coleção Entomológica do Departamento de Epidemiologia da Faculdade de Saúde Pública (FSP/USP), do Museu Nacional do Rio de Janeiro e do Instituto Oswaldo Cruz, Rio de Janeiro, Brasil e do National Museum of Natural History (NMNH), EUA. Foram descritas e ilustradas as fêmeas adultas, genitálias masculinas, larvas de quarto estadio e pupas de seis espécies de *Nyssorhynchus*, e a larva de primeiro estadio de *An. albitarsis s.s*. Foram utilizados 40 caracteres de fêmeas adultas das cinco espécies do complexo *An. albitarsis*. Nas análises morfométricas foram empregadas técnicas de análises estatísticas multivariadas. **Resultados** – Os adultos machos e fêmeas, larvas de quarto estadio e pupas de *An. oswaldoi s.s., An. albitarsis s.s*., *An. marajoara* e *An.*

deaneorum foram redescritos, e descritos aqueles de *An. albitarsis* B e *An. albitarsis* E. Os resultados das análises multivariadas dos caracteres morfométricos demonstraram que é possível separar as cinco espécies do complexo *An. albitarsis*. Para promover a estabilidade do nome *An. marajoara*, a lâmina da genitália masculina que acompanha o holótipo foi invalidada. **Conclusões** – Foi possível caracterizar *An. oswaldoi s.s*. através de marcadores morfológicos e moleculares. Os resultados das análises morfométricas demonstraram que é possível separar as cinco espécies do complexo *An. albitarsis* e que os espécimes de *An. marajoara* podem pertencer a duas espécies distintas.

Descritores: *Nyssorhynchus, An. albitarsis, An. oswaldoi,* espécies crípticas, morfologia, morfometria.

ABSTRACT

Motoki MT. Sistemática de seis espécies de *Anopheles* (*Nyssorhynchus*) Blanchard (Diptera: Culicidae) / Systematic of six species of the *Anopheles* (*Nyssorhynchus*) Blanchard (Diptera: Culicidae) [dissertation]. São Paulo (BR): Faculdade de Saúde Pública da Universidade de São Paulo; 2007.

Introduction – The *Anopheles oswaldoi* and *An. albitarsis* complexes contain morphologically cryptic species, including some important vectors of human malaria. Species diagnosis within each complex is problematic due to polymorphisms and overlapping morphological characters in identification keys and many times must employ molecular biology methods. Although correct identification is necessary to evaluate the respective epidemiological importance of each species, there exist few studies that have characterized the morphological characters of the *An. oswaldoi* and *An. albitarsis* complexes. **Objectives**– Morphologically and molecularly characterize *An. oswaldoi s.s.*; morphologically characterize five species of the *An. albitarsis* complex*;* establish morphological characters that separate species of the *An. albitarsis* complex; resolve nomenclature problems within *An. marajoara.* **Methods**- Specimens used originated from the entomological collections of the Departamento de Epidemiologia of the Faculdade de Saúde Pública (Universidade de São Paulo), from the Museu Nacional do Rio de Janeiro and from Instituto Oswaldo Cruz (Rio de Janeiro) in Brazil and of the National Museum of Natural History (USA). The adult females, male genitalia, fourth-instar larvae and pupae from six species of *Nyssorhynchus* and the first-instar larva of *An. albitarsis s.s*. were described and illustrated. Forty adult female characters were identified form five species of the *An. albitarsis* complex. Multivariate statistics were used in the morphometric analyses.
Results– Adult males and females, fourth-instar larvae and pupae of *An. oswaldoi s.s., An. albitarsis s.s*., *An. marajoara* and *An. deaneorum* were re-described. Those of *An. albitarsis* B and *An. albitarsis* E were described.

Results from multivariate analyses of morphological characters separated the five species of the *An. albitarsis* complex. To promote the nomenclature stability of *An. marajoara*, the male-genitalia slide associated with the holotype was invalidated. **Conclusions**– It was possible to characterize *An. oswaldoi s.s*. using both morphological characters and molecular markers. Results from morphometric analyses showed that it is possible a morphological distinction among all five species, and that the specimens of *An. marajoara* may belong to two distinct species.

Descriptors: *Nyssorhynchus, An. albitarsis, An. oswaldoi,* criptic species, morfology, morfometry.

ÍNDICE

1 INTRODUÇÃO

1.1 FAMÍLIA CULICIDAE

Os mosquitos, família Culicidae, formam táxon monofilético (WOOD e BORKENT, 1989; MILLER e col., 1997; HARBACH e KITCHING, 1998). Os culicídeos pertencem à ordem Diptera, as moscas de duas asas. A família é grupo grande e abundante que se distribui pelas regiões temperadas e tropicais do mundo, estendendo-se além do Círculo Ártico. Os mosquitos são mais diversos e menos conhecidos nos ambientes das florestas tropicais. Atualmente, são formalmente conhecidas aproximadamente 3.200 espécies, mas estima-se que existam pelo menos duas vezes mais (ZAVORTINK, 1990). Os mosquitos compreendem grupos ecologicamente diversos de invertebrados e são de importância econômica, médica e científica.

Os mosquitos são organismos estreitos, com pernas longas que são facilmente reconhecidos por possuírem probóscida longa e pela presença de escamas em várias partes do corpo. As larvas são distintas das de outros insetos aquáticos pela ausência de pernas, presença de cabeça que apresenta escovas orais e antenas, tórax bulboso que é mais largo do que a cabeça e do que o abdómen, papilas anais posteriores e a presença de par de espiráculos posteriores que se abrem ou diretamente na superfície do corpo (Anophelinae) ou no ápice do sifão respiratório (Culicinae) que está situado na região posterior do abdómen. A identificação das espécies de mosquitos é mais confiavelmente alcançada quando se utiliza caracteres das larvas de quarto estádio e dos adultos. Características da genitália masculina são necessárias para distinguir muitas espécies, isto por que as fêmeas de grupos taxonomicamente próximos são muitas vezes indistinguíveis. Enquanto a taxonomia de múltiplas espécies aparentemente relacionadas é confundida pela similaridade morfológica dos organismos, a

definição dos limites de alguns grupos supra-específicos é dificultada pela diversidade das estruturas anatômicas utilizadas. Alguns gêneros incluem elementos diversos de afinidades evolutivas indefinidas que poderão vir a ser reconhecidos como linhas monofiléticas separadas quando forem estudados em detalhes (BELKIN, 1962; HARBACH e KITCHING, 1998; REINERT e col., 2004).

Muitas espécies de mosquitos são pragas ou vetores de patógenos aos homens e animais. Os agentes infecciosos transmitidos por mosquitos incluem vírus (arbovírus), helmintos (filárias) e protozoários. Em torno de 150 espécies de mosquitos, a maioria dos gêneros *Anopheles*, *Aedes* e *Culex* são causas indiretas de morbidade e mortalidade, mais do que qualquer outro grupo de organismos. Apesar da importância médica e longa história de estudos, a taxonomia dos mosquitos está longe de ser completa e existem sistemas de classificação que são não naturais (BELKIN, 1962; HARBACH e KITCHING, 1998).

1.2 SUBFAMÍLIA ANOPHELINAE

A principal característica da subfamília Anophelinae é a presença de pécten no segmento abdominal VIII da larva de primeiro estadio. Esta estrutura é perdida nos demais estágios de desenvolvimento. Observa-se a existência do pécten no segmento abdominal VIII em todos os estágios larvais dos outros culicídeos (FORATTINI, 2002).

A subfamília Anophelinae apresenta várias sinapormofias no estágio larval como a presença da cerda 13-P, cerda abdominal 1 palmada em um ou em todos os segmentos, cerdas 6,7-I, 7-II plumosas, aparelho espiracular posicionado em lobo, com uma estreita banda posterior esclerotinizada. As sinapormofias presentes na pupa são as cerdas 9-IV-VII inseridas sobre ou

muito próximas ao ângulo do tergito, cerda 9-VIII inserida medianamente entre a superfície dorsal e ventral, cerdas 14-VIII aproximadas e no estágio adulto a ausência de micro cerda basal no pedicelo da antena, ausência de vestimenta da alula e setor radial (veia Rs) da asa com uma veia espúria basal (HARBACH e KITCHING, 1998). As diferenças entre Anophelinae e Culicinae foram corroboradas em estudos sobre estrutura e organização molecular do DNA ribossômico (FARIA e LEONCINI, 1988).

A subfamília Anophelinae é formada pelos gêneros: *Anopheles, Bironella* e *Chagasia*. O *Anopheles* tem distribuição mundial, *Bironella* limita-se à região Australásica e *Chagasia* à região Neotropical (FORATTINI, 2002). Os anofelíneos incluem grande número de complexos de espécies, cujos membros diferem geneticamente e em sua competência vetora, distribuição e características ecológicas. A documentação destas diferenças e a história evolutiva dos seus membros oferecem informações importantes para testar hipóteses evolutivas e ecológicas sobre a transmissão da malária (MUSTERMANN e CONN, 1997).

1.3 GÊNERO *ANOPHELES*

O gênero *Anopheles* inclui 444 espécies que estão amplamente distribuídas, sendo encontradas nas regiões Neártica, Neotropical, Paleártica, Etiópica, Oriental, Australásica (HARBACH, 2004). O gênero *Anopheles* é dividido em sete subgêneros:

- *Anopheles* Meigen, 1818;
- *Baimaia* Harbach, 2005;
- *Cellia* Theobald, 1902;

- *Stethomyia* Theobald, 1902;
- *Nyssorhynchus* Blanchard, 1902;
- *Kerteszia* Theobald, 1905;
- *Lophopodomyia* Antunes, 1937.

SALLUM e col. (2000) propuseram classificação para Anophelinae, baseada nos resultados de análise cladística de caracteres morfológicos. Em resumo, o gênero *Bironella* foi sinonimizado com o gênero *Anopheles* e os subgêneros *Stethomyia* e *Lophodomyia* com o subgênero *Anopheles*. Assim o gênero *Anopheles* ficou composto por 4 subgêneros *Kerteszia*, *Nyssorhynchus*, *Anopheles* e *Cellia*. Recentemente, KRZYWINSKI e col. (2001a, 2001b) discordaram desta proposta, adotando a classificação tradicional da subfamília Anophelinae. Os resultados de SALLUM e col. (2000) foram corroborados por HARBACK E KITCHING (2005), mas a classificação tradicional de *Anopheles* foi mantida.

1.4 SUBGÊNERO *NYSSORHYNCHUS*

Tem como característica do adulto, a marcação dos tarsômeros posteriores 3-5, cobertos inteira ou predominantemente por escamas brancas e o escudo com as áreas acrostical (AA) e dorsocentral (AD) cobertas por escamas. Na larva de quarto estadio as cerdas 5-7-C são plumosas (FORATTINI, 2002).

O subgênero *Nyssorhynchus* é formado por 33 espécies que estão distribuídas em três seções, quatro séries, oito grupos e dois subgrupos

(HARBACH, 2004). Foram registradas 27 espécies de *Nyssorhynchus* no Brasil e 19 no estado de São Paulo.*

O subgênero *Nyssorhynchus* é composto por três seções, Albimanus, Argyritarsis e Myzohynchella. A Seção Albimanus se diferencia da Seção Argyritarsis por apresentar nos adultos evidentes tufos póstero-laterais de escamas nos tergitos abdominais II-VII e o tarsômero posterior 5 com a metade basal escura (FORATTINI, 2002; LINTHICUM, 1988). Enquanto em algumas espécies da Seção Argyritarsis os conjuntos póstero-laterais de escamas são evidentes nos tergitos abdominais II ao VII, na maioria é evidente a partir do tergito IV. O tarsômero posterior 2 é recoberto por escamas escuras na parte basal e brancas na apical e os tarsômeros posteriores 3, 4 e 5 são inteiramente brancos (FORATTINI, 2002; FARAN, 1980). FARAN (1980) reconheceu o Grupo Myzohynchella, porém considerou incerto o status taxonômico. A seção Myzohynchella foi definida como seção de *Nyssorhynchus* por PEYTON e col. (1992) que consideraram esta seção como grupo natural por reunir espécies derivadas de ancestral comum.

Na América Latina, 11 espécies de *Nyssorhynchus* estão envolvidas na transmissão de malária, e pelo menos seis compreendem complexos de espécies crípticas, o *An. aquasalis* (CONN e col., 1993; MALDONADO e col., 1997), *An. benarrochi* (RUIZ e col., 2005), *An. oswaldoi* (MARRELLI e col., 1999b), *An. nuneztovari* (CONN e col., 1993; SIERRA e col., 2004), *An. albitarsis* (WILKERSON e col., 1995) e *An. triannulatus* (SILVA-NASCIMENTO e LOURENÇO-DE-OLIVEIRA, 2002). Segundo MAYR (1969), espécies crípticas são populações naturais morfologicamente semelhantes ou idênticas que são reprodutivamente isoladas. Os membros de complexos apresentam diferenças genéticas, comportamentais, ecológicas e de competência vetora que têm reflexos tanto na distribuição como na dinâmica da transmissão da malária humana (ROSA-FREITAS e

* Comunicação pessoal de SALLUM MAM, 2007.

col., 1998). Na região neotropical o subgênero *Nyssorhynchu*s é o que apresenta maior número de vetores principais ou auxiliares de parasitos da malária, por este motivo nos últimos tempos, a malária tem sido alvo de estudos epidemiológicos (FORATTINI, 2002).

1.5 *ANOPHELES OSWALDOI*

Anopheles (Nyssorhynchus) oswaldoi (PERYASSU, 1922) foi descrita como espécie do subgênero *Cellia*. Os espécimes foram coletados no Vale do Rio Doce, Espírito Santo e na Baixada Fluminense, Rio de Janeiro, Brasil. No ano seguinte, o táxon foi colocado na sinonímia de *An. tarsimaculatus* Goeldi por DYER (1923). Na mesma época, BONNE (1923, 1924) considerou que o táxon *An. tarsimaculatus* incluía duas espécies. A primeira era exofílica e apresentava anel escuro estreito no tarsômero 2 da pata posterior (=*An. oswaldoi*). A segunda era o *An. aquasalis* Curry, encontrado em áreas litorâneas. ROOT (1924), analisando genitálias masculinas de *An. oswaldoi* e *An*. *tarsimaculatus*, considerou as duas espécies morfologicamente idênticas. Por este motivo, colocou *An. oswaldoi* na sinonímia de *An. tarsimaculatus*. LIMA (1928) analisando caracteres dos adultos, larvas e pupas das duas espécies, observou diferenças morfológicas, considerando *An*. *oswaldoi* variedade de *An. tarsimaculatus*.

An. aquasalis Curry foi primeiro descrito como variedade de *An. tarsimaculatus* e depois foi sinonimizado com *An.oswaldoi* (SENEVET e ABONNENC, 1938). GALVÃO e LANE (1937) sugeriram que *An. oswaldoi* consistia em 3 subespécies, *An. oswaldoi oswaldoi*, *An. oswaldoi* var. *metcalfi* Galvão e Lane e *An. oswaldoi* var. *noroestensis* Galvão e Lane. GALVÃO e DAMASCENO (1942) descreveram *An. konderi* a partir de espécimes coletados em Coari, estado de Amazonas, Brasil e consideraram *An. noroestensis* como espécie distinta de *An. oswaldoi* e *An. metcalfi*. Neste

mesmo trabalho *An. metcalfi* foi considerada *nomen dubium*. (possivelmente, por erro, os autores consideraram como *nomen nudum*).

LANE (1953) baseando-se em características dos ovos, larvas de quarto estadio, pupas e fêmeas adultas de *Anopheles konderi* e *Anopheles oswaldoi* considerou que os táxons eram morfologicamente idênticos. Dessa maneira, sinonimizou *An. konderi* com *An. oswaldoi.* Recentemente, *Anopheles konderi* foi removido da sinonímia de *An. oswaldoi* por FLORES-MENDOZA e col. (2004b) que redescreveram todos os estágios de desenvolvimento e designaram o neótipo. Este estudo mostrou que os machos das duas espécies podem ser reconhecidos pela forma apical do edeago, porém ainda permanece a dificuldade em identificar as adultas fêmeas. As informações em relação à diversidade na morfologia, sequências de DNA, isoenzimas, preferência por hospedeiro, competência vetora e bionomia além da ampla distribuição sugerem que *An. oswaldoi* compreende complexo de espécies na América do Sul (CAUSEY e col., 1946; KLEIN e LIMA, 1990; CONSOLI e LOURENÇO-de-OLIVEIRA, 1994; ROSA-FREITAS e col., 1998; MARRELLI e col., 1999b; SCARPASSA, 2005; SCARPASSA & CONN, 2006; RUIZ e col., 2005; QUIÑONES e col., 2006). MARRELLI e col. (1999b) sugeriram a presença de quatro formas de *An. oswaldoi s.l.*, baseando-se em sequências de bases nitrogenadas da região ITS2 do DNA ribossômico de populações das espécies do Brasil, Peru e Venezuela. Os autores consideraram que uma das populações poderia representar *An. konderi*. Estudos posteriores demonstraram que o espécime do Espírito Santo utilizado como sendo de *An. oswaldoi* era na verdade de *An. evansae* (MARRELLI e col., 2006). Acresce considerar que o espécime de Yurimaguas pertence à *An. benarrochi* B que é encontrado no sul da Colômbia (RUIZ e col., 2005) e em regiões do Peru (WILKERSON, FLORES-MENDOZA & LINTON, dados não publicados). Nenhuma análise de DNA foi incluída quando *An. konderi* foi formalmente removido da sinonímia com *An. oswaldoi* (FLORES-MENDOZA e col. 2004b). Dessa maneira a identidade molecular do *An. konderi* é desconhecida.

Anopheles oswaldoi s.l. tem sido encontrado por toda América do Sul, leste dos Andes e ao sul e norte das Províncias da Argentina (FARAN, 1980; FARAN & LINTHICUM, 1981). Foi encontrado também na Bolívia (PEYTON e *col*. 1983), Brasil (PERYASSU, 1922; FONSECA e FONSECA, 1942; DEANE e col., 1948; STEINER e col., 1982; ARRUDA e col., 1986; LOURENÇO-de-OLIVEIRA e col., 1989; KLEIN e LIMA, 1990; OLIVEIRA-FERREIRA e col., 1990; KLEIN e col., 1991a, b; NATAL e col., 1992; BRANQUINHO e col., 1993; FORATTINI e col*.*, 1993; LOPES e LOZOVEI, 1995; BRANQUINHO e col., 1996; LOURENÇO-de-OLIVEIRA e LUZ, 1996 NAVES e col., 1996; REBÊLO e col., 1997; MARRELLI e col., 1998; PERERA e col., 1998; TADEI e col., 1998; MARRELLI e col., 1999a, b; GUIMARÃES e col*.,* 2000; PÓVOA e col., 2001; LOPES e col., 2002 PÓVOA e col., 2003; GUIMARÃES e col., 2004; SANTOS e col., 2005; SCARPASSA e col., 2005; PÓVOA e col., 2006; SCARPASSA e CONN, 2006; SILVA e col., 2006), Colômbia (RUIZ e col., 2005; QUIÑONES e col., 2006), Costa Rica (FARAN, 1980), Equador (SAN SEBASTIÁN e col., 2000), Guianas (RAMBAJANL, 1987; LAUBACH e col., 2001), Panamá (SIMMONS, 1979), Paraguai (FARAN, 1980), Peru (HAYES e col., 1987; FLORES-MENDOZA e col., 2004a), Suriname (LOUNIBOS E CONN, 2000), Trinidad (ROZEBOOM, 1942; CHADEE e BEIER, 1996) e Venezuela (RUBIO-PALIS e CURTIS, 1992; GRILLET, 2000).

De acordo com diversos autores, *An. oswaldoi s.l.* é tipicamente exofílico e zoofílico (DEANE e col*.,* 1948; FARAN, 1980; CONSOLI e LOURENÇO-de-OLIVEIRA, 1994; LOURENÇO-de-OLIVEIRA e LUZ, 1996). No entanto, foram capturados exemplares de *An. oswaldoi* picando humanos, nos ambientes intra e peridomiciliares (RUBIO-PALIS e CURTIS, 1992; BRANQUINHO e col.,1996; QUIÑONES e col., 2006). Vale assinalar que foi registrado o possível envolvimento da espécie na dinâmica da transmissão da malária (BRANQUINHO e col., 1993; BRANQUINHO e col., 1996), indicando plasticidade na bionomia do inseto. No Brasil, *An. oswaldoi s.l.* foi encontrado no interior de floresta tropical primária em Balbina, estado

do Amazonas (LOURENÇO-de-OLIVEIRA e LUZ, 1996), em regiões de floresta próxima a barragem hidroelétrica na Serra da Mesa, estado de Goiás (GUIMARÃES e col., 2004), e em área de floresta preservada no Vale do Rio Doce, estado do Espírito Santo. Em contraste, estudos realizados no oeste da Venezuela, demostraram que 42% de 1.000 indivíduos de *An. oswaldoi,* foram capturados com isca humana no interior de cabana experimental (RUBIO-PALIS e CURTIS, 1992). Em área da bacia do Rio Purus, estado do Acre, Brasil, *An. oswaldoi* foi coletado em áreas abertas e em ambiente peri-domiciliar (NATAL e col., 1992). Na Colômbia, *An. oswaldoi* foi encontrado no ambiente interno de casas e com preferência para repousar a mais de 1,5 m de altura nas paredes das casas (QUIÑONES e SUAREZ, 1990). KLEIN e LIMA (1990) observaram que *An. konderi* é muitas vezes confundido com *An. oswaldoi*. Mesmo assim, os autores sugeriram que *An. konderi* está presente em ambiente humano impactado, enquanto *An. oswaldoi* é restrito a áreas de florestas. Foi observado o pico de atividade hematofágica entre 18:00 - 19:00h (DEANE e col., 1948), e entre 18:00 - 20:00h em Rondônia, Brasil, diminuindo sua atividade depois das 21:00h (TADEI e col., 1998). Entretanto, na Venezuela foi observado que em ambiente intra-domiciliar as espécies continuam a se alimentar até próximo a meia-noite (RUBIO-PALIS e CURTIS, 1992; RUBIO-PALIS e col., 1994).

Embora, tenham sido encontrados espécimes naturalmente infectados na Colômbia (QUIÑONES e col,. 2006), Peru (HAYES e col., 1987; FLORES-MENDOZA e col., 2004) e na Venezuela (RUBIO-PALIS e CURTIS, 1992), *An. oswaldoi* não foi considerado vetor importante nesses países devido a baixa densidade. Em estudos realizados no Brasil, foram encontrados exemplares de *An. oswaldoi* naturalmente infectados com *Plasmodium* (FONSECA e FONSECA, 1942; ARRUDA e col., 1986; OLIVEIRA-FERREIRA e col., 1990; KLEIN e col., 1991a, b; BRANQUINHO e col., 1993; BRANQUINHO e col., 1996; LOURENÇO-de-OLIVEIRA e LUZ, 1996; MARRELLI e col., 1999a; SANTOS e col., 2005). Por esse motivo foi

considerado como vetor primário em algumas regiões, e secundário ou sem importância vetora em outras. No estado do Acre, *An. oswaldoi* é encontrado em áreas intra e peri-domiciliares, sendo considerado espécie antropofílica e consequentemente, vetor de plasmódios (BRANQUINHO e col.,1993; BRANQUINHO e col., 1996; MARRELLI e col.,1999a). Mais de 7% (190/2610) dos espécimes testados por ELISA eram positivos, a saber: 3,41% para *P. falciparum*, 2,26% para *P. vivax* VK210, 1,22% para *P. vivax* VK247, e 0,42% para *P. malariae* (BRANQUINHO e col., 1993). Em estudo posterior realizado na mesma área, 29% dos espécimes (1/34) foram encontrados positivos através da dissecção do intestino e glândulas salivares (BRANQUINHO e col.,1996). No estado do Pará, ARRUDA e col., (1986) analisaram 442 exemplares de *An. oswaldoi* (de 962 coletados) e através do ensaio imunoabsorvente de ligação de enzima (ELISA) registraram 10 espécimes positivos para *P. falciparum*. Em estudo posterior, espécimes da mesma população foram encontrados infectados por *P. vivax* em ensaio imunoradiométrico (IRMA) (OLIVEIRA-FERREIRA e col., 1990). O teste de ELISA com a glândula salivar de 417 *Anopheles* do estado do Pará apresentou dois espécimes de *An. oswaldoi* infectados, um com *P. vivax* VK247 e o outro com *P. malariae* (SANTOS e col., 2005). Em Costa Marques, estado de Rondônia, KLEIN e col. (1991a, 1991b) relataram baixa taxa de infecção natural por *P. vivax* e baixo número de esporozoíto nas glândulas salivares de espécimes de *An. oswaldoi* selvagens. Sob condições de laboratório, populações de *An. oswaldoi* de Trinidad apresentaram ser suscetíveis à infecção por *P. vivax* (ROZEBOOM, 1942) e as populações do estado de São Paulo por *P. vivax* e *P. falciparum* (FONSECA & FONSECA, 1942). Em áreas de malária simiana na Amazônia, *An. oswaldoi* foi encontrado nas copas de árvores e no solo, e conseqüentemente foi incriminado como vetor potencial de *P. brasilianum* (LOURENÇO-de-OLIVEIRA e LUZ, 1996).

Imaturos de *An. oswaldoi* foram coletados em criadouros permanentes ou temporários, de água doce, situado no interior ou na

margem de florestas tropicais (FARAN, 1980). Em estudos mais detalhados sobre aspectos da bionomia das larvas de *An. oswaldoi* do estado de Sucre, Venezuela, indivíduos foram encontrados em pouca quantidade, porém, no final da estação seca a presença foi maior. Foram encontradas larvas em reservatórios de água doce permanente (tanque, canal, brejo e floresta de pântano). A ocorrência e a abundância de *An. oswaldoi* foram relacionadas ao alto nível de oxigênio dissolvido na água (GRILLET, 2000). Em região litorânea e de pântano na Venezuela, BERTI e col. (1993) encontrou *An. oswaldoi* em estação chuvosa quando a salinidade das águas era baixa.

Como Peryassú não designou o holótipo de *An. oswaldoi*, os espécimes utilizados na descrição são síntipos (BELKIN *e col*., 1971). Considerando que *An. oswaldoi* compreende complexo de espécies, e algumas destas podem ser vetores de plasmódios humanos, é crucial a redescrição dos diversos estádios de desenvolvimento da espécie e a designação do lectótipo deste táxon para estabelecer a identidade dos membros do complexo Oswaldoi .

1.6 COMPLEXO *ANOPHELES ALBITARSIS*

Estudos epidemiológicos, comportamentais, morfológicos, moleculares sugerem que *Anopheles albitarsis* compreende complexo de espécies crípticas (GALVÃO e DAMASCENO, 1944; KREUTZER e col., 1976; STEINER e col., 1984; LINTHICUM, 1988; ROSA-FREITAS, 1989; NARANG e col., 1993; WILKERSON e col., 1995a; WILKERSON e col., 1995b). Algumas espécies do complexo são consideradas importantes vetores de *Plasmodium* causador da malária humana em determinadas localidades, e vetores secundários ou sem nenhuma importância em outras (PÓVOA e col., 2000; CONN e col., 2002; KLEIN e col, 1991c, d).

Anopheles albitarsis Lynch-Arribálzaga (1878) foi descrito baseado em fêmeas adultas coletadas em Baradero, estado de Buenos Aires, Argentina.

Anopheles marajoara Galvão e Damasceno (1942) foi descrito baseando-se em larva de quarto estadio, pupa e adulto macho, incluindo a genitália masculina. Naquela ocasião, os autores designaram o holótipo. Trata-se de um macho adulto. SENISE e col. (2006) invalidaram a lâmina da genitália masculina do holótipo, por considerarem que se tratava da genitália masculina de um espécime de *Anopheles aquasalis* e não de *An. marajoara*. O adulto e as formas imaturas, estas montadas em lâmina distinta daquela da genitália masculina, pertencem a *An. marajoara*. Dessa maneira, os autores excluíram a lâmina da genitália masculina da série tipo.

GALVÃO e DAMASCENO (1944) compararam espécimes de *An. albitarsis* coletados na Ilha de Marajó, estado do Pará, com amostras do estado de São Paulo e de Tucuman, norte da Argentina. Dessa maneira, os autores demonstraram a existência de diferenças morfológicas e ecológicas. Assim, eles descreveram três subespécies, a saber: *An. albitarsis limai* (GALVÃO e LANE, 1937) baseado em populações do bairros Pinheiros e Butantan, estado de São Paulo; *An. albitarsis imperfectus* (CORRÊA e RAMOS, 1943) em populações de Vera Cruz, estado de São Paulo e *An. albitarsis domesticus* (GALVÃO e DAMASCENO, 1944) em populações da Ilha de Marajó. LANE (1953) considerou *An. albitarsis limai, An. albitarsis imperfectus* e *An. marajoara* variantes morfológicas de *An. albitarsis*, transferindo-as para a sinonímia de *An. albitarsis albitarsis.*

LINTHICUM (1988), em revisão taxonômica da série Argyritarsis do subgênero *Anopheles (Nyssorhynchus*), removeu *An. marajoara* da sinonímia de *An. albitarsis*. Adicionalmente, transferiu *An. albitarsis domesticus* para a sinonímia de *An. marajoara* e apontou caracteres morfológicos dos adultos machos e fêmeas, larvas e pupas para a

separação entre *An. albitarsis* e *An. marajoara*, as únicas espécies válidas naquela oportunidade.

KREUTZER e col. (1976) estudaram espécimes de *An. albitarsis s.l.* da Colômbia, Venezuela e de várias localidades do Brasil e demonstraram a existência de três populações que apresentavam cromossomos politênicos distintos. Em seguida, STEINER e col. (1982) analisaram o padrão de isoenzimas de populações de *An. albitarsis* coletadas ao longo de transecto entre São Paulo e o oeste da Ilha do Marajó, estado do Pará. Dessa maneira, observaram três populações geneticamente diferenciadas. Os resultados dos dois estudos indicaram que *An. albitarsis* poderia ser complexo de três ou mais espécies simpátricas e alopátricas.

Para manter a estabilidade da nomenclatura de *An. albitarsis*, ROSA-FREITAS e DEANE (1989) redescreveram o táxon e designaram o neótipo. Para isso utilizaram espécimes coletados em Baradero, Argentina. A seguir, ROSA-FREITAS (1989) descreveu o *An. deaneorum* a partir de espécimes coletados em Guarajá-Mirim, Rondônia e Rio Branco, Acre, e apontou caracteres morfológicos e analisou o padrão isoenzimático para separar *An. deaneorum* de *An. albitarsis s.s.*

NARANG e col. (1993) analisaram o padrão de isoenzimas e o polimorfismo de comprimento de fragmentos de restrição do DNA mitocondrial (RFLP) para examinar diferenças genéticas entre as populações de *An. albitarsis s.l.* de várias localidades do Brasil. Consequentemente, foram identificados dois grupos distintos: *An. deaneorum* e um complexo reconhecido como *An. marajoara* que inclui pelo menos três formas crípticas. A seguir, WILKERSON e col. (1995a, 1995b) empregaram o método de polimorfismo de DNA amplificado ao acaso – reação em cadeia de polimerase (RAPD-PCR) para estudar diversas populações de *An. albitarsis s.l.* do Brasil, do Paraguai, da Argentina e da Venezuela. Dessa maneira, corroboraram a existência de complexo de espécies crípticas e reconheceram a presença de um quarto táxon que

designaram como *An. albitarsis* B. Consequentemente, o complexo *An. albitarsis* ficou formado pelos táxons, a saber:

- *An. albitarsis s.s.* Lynch – Arribálzaga, 1878;
- *An. albitarsis* B ainda não descrita;
- *An. marajoara* Galvão & Damasceno, 1942;
- *An. deaneorum* Rosa-Freitas, 1989.

LEHR e col. (2005), baseando-se em sequências de nucleotídeos do gene citocromo c oxidase, subunidade I (COI) do genoma mitocondrial (mtDNA), levantaram a hipótese da existência de uma quinta espécie que designaram como *An. albitarsis* E. A hipótese de LEHR e col. (2005) foi corroborada pelos resultados de Merritt e col. (2005) usando seqüências de nucleotídeos do gene nuclear "*White*". Recentemente, Brochero e col. (2007) analisando a região do segundo espaçador interno transcrito (ITS2) de exemplares de *An. marajoara* coletados na Colômbia, reconheceram uma nova espécie designada *An. albitarsis* F.

As espécies do complexo *An. albitarsis* apresentam grande potencial de adaptação às condições ambientais criadas pelo homem (FORATTINI e MASSAD, 1998; TUBAKI e col., 1999). As evidências apontam que as culturas irrigadas de arroz têm favorecido o aumento da densidade das populações do *Anopheles* (*Nyssorhynchus*) *albitarsis s.l.* na região do Vale do Ribeira, estado de São Paulo (FORATTINI e col., 1995).

A avaliação do envolvimento do *An. albitarsis* na transmissão dos parasitas causadores da malária humana é falha. Recentemente, PÓVOA e col. (2000) observaram que *An. marajoara* é vetor competente do *P. vivax* no Amapá. Paralelamente, CONN e col. (2002) demonstraram que as alterações antrópicas do ambiente, o uso do solo e os movimentos migratórios, no Amapá, têm favorecido o envolvimento do *An*. *marajoara* na transmissão da malária humana. Em decorrência do envolvimento do *An.*

marajoara na transmissão da malária, levantou-se hipótese de que esta espécie está substituindo as populações de *An. darlingi*, até então considerada como principal vetor da enfermidade naquela região. O *An deaneorum* foi demonstrado ser competente para se infectar e transmitir *P. vivax* e *P. falciparum* em laboratório (KLEIN e col.,1991c, 1991d). Em contraste, SANTOS (2001) considerou que as populações de *An. albitarsis s.l.* da região sudeste do estado de São Paulo apresentam baixa capacidade vetora para transmitirem o *P. vivax*. Infelizmente, este último estudo é inconclusivo, pois os parâmeros utilizados para a estimativa da capacidade vetora referem-se, provavelmente a mistura de duas espécies.

Como mencionado anteriormente, a diferenciação das espécies do complexo *An. albitarsis* é difícil. Obviamente, a falta de amostras confiáveis, caracterizadas geneticamente, vem dificultando ainda mais a distinção morfológica das espécies. Acresce considerar o fato das espécies do complexo *An. albitarsis* serem simpátricas e, por este motivo, a probabilidade das amostras disponíveis para estudos morfológicos corresponderem à mistura de táxons é elevada. Assim o uso de exemplares corretamente identificados é indispensável para poder avaliar se os caracteres são polimórficos ou se correspondem a espécies distintas (ROSA-FREITAS e col, 1998). LOUNIBOS e col. (1998a) observaram que o *An. trinkae* e o *An. dunhami* são morfologicamente semelhantes e que a separação das fêmeas é problemática. Empregando caracteres moleculares, cromossômicos e morfológicos, os autores demonstraram a importância de pesquisas multidisciplinares no estudo dos vetores. Diante disso, LOUNIBOS e col. (1998a) observaram que a diferenciação morfológica do *An. trinkae* e do *An. dunhami* é possível e que os ovos oferecem caracteres mais seguros para o reconhecimento específico. Ao contrário, os ovos das espécies do complexo *An. albitarsis* são praticamente inseparáveis (ROSA-FREITAS e DEANE 1989; MARUCCI, 1995).

Objetiva-se que os resultados deste estudo permitam a definição de caracteres morfológicos que possibilitem a distinção das espécies do complexo *An. albitarsis*. Dessa maneira, pretende-se facilitar as pesquisas epidemiológicas e a adoção de medidas adequadas de controle das espécies e, conseqüentemente, as de prevenção dos agentes infecciosos veiculados por elas.

1.7 JUSTIFICATIVA

Espécies morfologicamente semelhantes podem exibir comportamentos e competências vetoras diferentes e não estarem igualmente envolvidas na transmissão de agentes infecciosos. Assim, fica claro que um dos maiores problemas no estudo dos vetores é a dificuldade para a diferenciação das espécies. No entanto, esta é necessária tanto para a avaliação da importância epidemiológica das espécies como para a adoção de medidas de controle.

O subgênero *Nyssorhynchus* tem enorme importância epidemiológica por apresentar espécies vetoras de parasitas como os plasmódios causadores da malária humana. Por isso, se faz necessário a identificação correta das espécies, para que os conhecimentos adquiridos em estudos biológicos, ecológicos e comportamentais possam contribuir para a avaliação adequada da importância epidemiológica dessas populações de vetores.

Em relação aos complexos *An. albitarsis* e *An. oswaldoi*, os estudos taxonômicos, objetivando a caracterização morfológica das espécies, são escassos. Outros estudos referem-se a aspectos ecológicos. Como exemplo, pode-se assinalar aqueles relativos ao *An. albitarsis s.l.* (FORATTINI e col, 1995, 1996; FORATTINI e MASSAD, 1998; SANTOS e

FORATTINI, 1999) e ao *An. oswaldoi s.l.* (KLEIN e col., 1991c; LOUNIBOS e col., 1997). Como os resultados desses estudos são, possivelmente, relativos a mais de uma espécie, o conhecimento do processo de adaptação das populações ao ambiente humano fica prejudicado o que dificulta a incriminação das populações na transmissão de agentes infecciosos.

2. OBJETIVOS

Geral

Contribuir para sistemática de *Anopheles* (*Nyssorhynchus*).

Específico

Caracterizar morfologicamente e molecularmente o *An. oswaldoi s.s.*;

Redescrever as formas imaturas e os adultos de *Anopheles oswaldoi s.s.*;

Designar do lectótipo de *An. oswaldoi s.s.*;

Caracterizar morfologicamente cinco espécies do complexo *Anopheles albitarsis*;

Redescrever os imaturos e os adultos de *Anopheles albitarsis s.s.*;

Redescrever os imaturos e os adultos de *Anopheles marajoara*;

Redescrever os imaturos e os adultos de *Anopheles deaneorum*;

Descrever os imaturos e os adultos de *Anopheles albitarsis* B;

Descrever os adultos de *Anopheles albitarsis* E;

Solucionar problema de nomenclatura de *An. marajoara.*

3. MATERIAL E MÉTODOS

Anopheles oswaldoi s.s.

3.1 MATERIAL BIOLÓGICO E ESTUDO MORFOLÓGICO

Fêmeas de *Anopheles oswaldoi* foram coletadas na Lagoa do Macuco, Fazenda Marianelli, Jaguaré, estado do Espírito Santo, BRASIL, (19º2'5.36''S 39º56'54.54''O), utilizando barraca de Shannon. Em laboratório, as fêmeas identificadas através da chave de identificação de FARAN (1980) foram alimentadas e submetidas a stress, retirando uma das asas para induzir o processo de oviposição, e mantidas separadamente em potes de plástico com água destilada até o momento da oviposição. As larvas de primeiro a quarto estádio foram alimentadas com comida de peixe triturada. As pupas foram transferidas separadamente em pequenos copos de plástico com água tampados, mantidas até a eclosão dos adultos. As exúvias das larvas e pupas foram preservadas em álcool 80% para posterior montagem em lâmina. As lâminas de exúvias de larvas de quarto estadio e pupas foram montadas com bálsamo do Canadá. Adicionalmente, algumas larvas de quarto estadio e genitálias masculinas foram dissecadas para montagem em lâmina. Adultos macho e fêmea foram fixados com alfinete entomológico.

Foram examinados também dois síntipos de *An. oswaldoi* depositados no Museu Nacional do Rio de Janeiro (MNRJ), espécimes não-tipo que são mantidas na Coleção Entomológica da Faculdade de Saúde Pública, Universidade de São Paulo, Brasil (FSP-USP), e do Instituto Oswaldo Cruz, Rio de Janeiro, Brasil (IOC-RJ).

Caracteres morfológicos de adultos macho e fêmea e genitálias masculinas foram examinados e medidos. Larvas e pupas foram

examinadas, medidas, e as cerdas foram contadas para a descrição. Para os adultos foi utilizada a nomenclatura de HARBACH e KNIGHT (1980), para as manchas das asas, WILKERSON e PEYTON (1990) e para as veias das asas BELKIN (1962).

3.2 EXTRAÇÃO DE DNA

Foram obtidas amostras do DNA genômico de 10 indivíduos de *Anopheles oswaldoi* provenientes de progênies de duas fêmeas obtidas em Linhares Vale do Rio Doce, Espírito Santo (19º13'00''S, 40º08'00''O), e de dois espécimes adicionais de Pariquera-Mirim, Pariquera-Açu, Vale do Ribeira, São Paulo (24º43'60''S47º49'00''O). Para as extrações utilizou-se o protocolo modificado baseado no kit QIAgen DNeasy Blood and Tissue (QIAgen Ltd., Crawley, UK). Todos os tampões foram fornecidos com o kit. Mosquitos inteiros foram macerados (AnaChem Ltd., Bedfordshire, UK) em tubos de plástico de 1.5 ml contendo 180 µl do tampão AL. Foram adicionados 20 µl de proteinase K e as amostras foram incubadas a 56ºC por até três horas. Em seguida, as amostras foram mantidas a -20ºC durante a noite e no dia seguinte foram centrifugadas a 8000 rpm por 10 minutos. O sobrenadante foi removido e transferido para outro frasco limpo de 2 µl limpo, contendo 200 µl do tampão AW1.

A seguir, foram adicionados 200 µl de etanol absoluto e a solução foi homogeneizada, pipetando-se a mistura. A amostra foi transferida para DNeasy Mini spin em tubo coletor de 2 ml. As colunas foram centrifugadas por 1 minuto a 8000 rpm, e o líquido contido no tubo coletor foi descartado. Foram adicionados 500 µl do tampão AW1 e a coluna foi novamente centrifugada a 8000 rpm por 1 minuto. O líquido contido no tubo coletor foi descartado. Esse último passo foi repetido com 500 µl do tampão AW2,

porém a coluna foi centrifugada 14000 rpm for 1 minuto. As colunas foram transferidas para tubo coletor limpo e adicionadas 200 µl do tampão AE. As colunas foram incubadas em temperatura ambiente por 1-3 minutos e o DNA foi eluído por centrifugação a 8000 rpm por 1 minuto. A etapa de eluição foi repetida e o DNA foi estocado em tubo separado. Foi verificado que parte do DNA frequentemente permanece na membrana após a primeira eluição.

Dois µl de DNA da primeira eluição foram utilizados nos PCRs. Amplificação da região ITS2 foi conduzida utilizando os iniciadores 5.8SF e 28SR recomendados por COLLINS e PASKEWITZ (1996). Nas preparações das reações de PCR utilizou-se o protocolo, a saber. As reações foram em volume final de 50 µl: 2 µl de DNA; 22.5 µl de H_2O bidestilada, 0.5 µl de tampão QIAgen PCR , 10 µl de solução QIAgen Q, 3 µl de 25 mMMgCl2, 1µl de cada iniciador a 5 µM, 5 µl de 2 mM dNTPs (PE Applied Biosystems, Warrington, England) e 0.5 µl de Taq QIAgen. O ciclo de amplificação consistiu de desnaturação a 94º C por 2 minutos, 34 ciclos de 94ºC, 57ºC e 72ºC por 30 segundos cada, seguidos de 10 minutos de extensão final a 72ºC. Os produtos de PCR foram visualizados em gel de agarose 1%, corados com brometo de etídio. Os produtos foram purificados utilizando Millipore MultiScreen PCR e quantificados em espectrofotômetro NanoDrop ND-1000, antes de serem diluídos a 2ng/µl/100pb para sequenciamento. As reações de sequenciamento foram conduzidas em ambas as direções utilizando os mesmos iniciadores empregados na PCR e Big Dye Terminator Kit v. 1.1. (PE Applied Biosystems, Warrington, England). Os cromatogramas foram lidos em seqüenciador automático ABI 3730 (PE Applied Biosystems).

As seqüências foram editadas utilizando Sequencer versão 4.5 (Genes Codes Corporation, Ann Arbor, Michigan) e alinhadas no CLUSTAL X (THOMPSON e col., 1997). A similaridade entre as sequências geradas nesse estudo e aquelas previamente disponíveis no GenBank foi acessada utilizando FASTA (http://www.ebi.ac.uk/fasta33/). Variação intra-específica foi estimada utilizando MEGA 3.1 (KUMAR e col., 2004). O DNA genômico

utilizado nesse estudo está depositado a -70ºC no Molecular Systematic Laboratory of Entomology e na FSP-USP.

Complexo *Anopheles albitarsis*

3.3 MATERIAL BIOLÓGICO E ESTUDO MORFOLÓGICO

Em 1992, WILKERSON e col. coletaram fêmeas do complexo *Anopheles albitarsis* no sul do Brasil, Paraguai, norte da Argentina e na Venezuela. Em laboratório entre 48 a 72 horas depois de serem alimentadas, as fêmeas foram separadas em potes de plástico com água e induzidas a ovipor retirando uma das asas. Os ovos foram mantidos com água até a eclosão das larvas. As exúvias de larvas foram preservadas em álcool 80% para posterior montagem em lâmina. As pupas foram separadas uma a uma em pequenos copos de plástico com água, tampados e foram mantidas até a eclosão dos adultos. Foram montadas lâminas de exúvias de larvas de 4^{o}.estádio, pupas e genitálias masculinas, adultos macho e fêmea foram fixados com alfinete entomológico. Cada adulto montado foi associado com suas respectivas lâminas de exúvias de larva e pupa. Foi colocado um código em forma de número, para possibilitar a verificação de cada geração e se é da mesma fêmea ou não. Pelo menos um adulto macho ou fêmea de cada geração foi utilizado para identificar a espécie através de RAPD-PCR como feito por Wilkerson e col. (1995a). Esse material foi depositado no National Museum of Natural History (NMNH) - EUA, Army Medical Research - Rio de Janeiro, Instituto Oswaldo Cruz - Rio de Janeiro e na Coleção Entomológica de Referência da Faculdade de Saúde Pública/USP (FSP-USP).

Neste estudo, foram utilizados espécimes adultos macho e fêmea, exúvias de larvas de quarto estádio e de pupas que se encontram

disponíveis nos acervos da Coleção Entomológica de Referência da Faculdade de Saúde Pública/USP (FSP-USP) e do National Museum of Natural History (NMNH) EUA.

Foram estudadas populações identificadas através de RAPD-PCR das seguintes localidades:

An. albitarsis s.s.	→	Brasil	Bahia, São Paulo, Santa Catarina, Paraná.
	→	Argentina	Buenos Aires, Corrientes, Missiones.
	→	Paraguai	Alto do Paraná.
***An. albitarsis* B**	→	Brasil	Bahia, Ceará, Espírito Santo, Rio de Janeiro, Pará, Paraná, São Paulo, Mato Grosso.
	→	Paraguai	Alto do Paraná.
An. marajoara	→	Brasil	Pará, Amazonas, Mato Grosso, São Paulo.
	→	Venezuela	Zulia.
An. deaneorum	→	Brasil	Rondônia, Paraná.
	→	Argentina	Corrientes, Missiones.
***An. albitarsis* E**	→	Brasil	Roraima.

Dentre as inúmeras atividades relacionadas aos estudos morfológicos clássicos de Culicidae está a medição de caracteres e a elaboração de ilustrações a traço que são esquemas representativos das quetotaxias das larvas de quarto estádio, das pupas, dos adultos macho e fêmea e das estruturas anatômicas das genitálias masculinas. Objetiva-se com isso o encontro de caracteres morfológicos que permitam diferenciar as espécies nos diferentes estádios de desenvolvimento.

Foram feitas ilustrações das larvas, pupas e genitálias, com auxílio de microscópio óptico acoplado a uma câmara clara. As ilustrações estão apresentadas nos anexos dos artigos.

As cerdas das larvas, pupas e genitálias masculinas foram contadas e algumas foram medidas, assim como algumas estruturas da genitália masculina com o auxílio de microscópio óptico. As contagens das cerdas das larvas e pupas são apresentadas em forma de tabela nos anexos dos artigos.

As estruturas dos adultos macho e fêmea foram observadas e medidas com auxílio de estereomicroscópio marca Wild acoplado a uma ocular micrométrica digital, marca WILD.

Os caracteres analisados a partir das observações, medidas e contagens, da larva de quarto estádio, da pupa, do adulto macho e fêmea e da genitália masculina das espécies do complexo *An. albitarsis* foram apresentados em forma de redescrição e descrição, sendo que a espécie *Anopheles albitarsis* E foi descrita apenas a forma adulta (macho e fêmea) e a genitália masculina.

3.4 COLETA DE DADOS MORFOMÉTRICOS E ANÁLISE ESTATÍSTICA

Para cada população de mosquitos foram medidos vários caracteres selecionados da asa. Foram utilizados 30 espécimes de cada espécie, o complexo é formado por quatro espécies e uma possível quinta espécie, totalizando 150 espécimes. As medidas foram obtidas em estereomicróscópio, com o uso de uma escala de 2 mm. Foi selecionada a distância entre os olhos e 39 caracteres das asas, portanto, nas análises estatísticas as variáveis a serem estudadas são medidas.

Observou-se que dentro da mesma espécie algumas manchas da veia costa podiam estar ausentes ou presentes. Por isso não foi possível

caracterizar as cinco espécies a partir das manchas que apresentavam polimorfismos em relação à presença e ausência. Nas veias das asas observa-se sequência de manchas: clara, escura, clara, escura e assim suscetivamente. Nesse sentido, observou-se que uma mancha ausente influencia nas manchas vizinhas (por exemplo, uma mancha clara ausente entre duas escuras). Assim, apesar de ser possível avaliar que a mancha clara está ausente, não é possível medir o comprimento das duas manchas escuras. Por este motivo, as medidas ausentes e as vizinhas foram consideradas dados faltantes (missing data).

Foi elaborada uma matriz de dados das medidas das asas. Cada caráter medido foi considerado uma variável e para facilitar os procedimentos no programa, foi criada sigla para cada caráter. A matriz de dados foi analisada com o programa Minitab 14. Foram realizadas múltiplas análises estatísticas, a saber.

Estatística descritiva é definida como análise exploratória. Esta análise possibilitou verificar a distribuição dos dados (média, mediana, desvio padrão, valor mínimo e máximo observados).

Análise de componentes principais (PCA) é uma técnica de análise multivariada cujo objetivo principal é obter, a partir das variáveis em estudo, um pequeno número de combinações lineares (componente principal) que retenham o máximo de informações possíveis. Os componentes principais são apresentados em ordem decrescente, do mais explicativo para o menos explicativo e o seu processamento pode ter partida na matriz de variâncias e covariâncias ou na matriz de correlação (HUGH e GAUCH, 1982). A análise de componentes principais é uma técnica intermediária, portanto, não é método conclusivo e final. Neste estudo foi utilizada como passo intermediário para a interpretação das outras análises. Foi aplicada para 150 espécimes e 35 variáveis.

Análise de Variância (**ANOVA**) é análise estatística que permite comparar as médias das variáveis estudadas. Assim, foi feita a comparação das médias das cinco espécies para cada variável estudada. Considerando-se nível de significância (α) igual a 5%, adotou-se teste de hipótese de igualdade entre as médias das variáveis para as cinco espécies (H: $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5$). Entre as 35 variáves consideradas na análise, apenas duas não rejeitaram a hipótese H. Por esse motivo essas variáveis foram excluídas da análise discriminante (sem perda importante de informações). Isso pode ser justificado pelo fato de não existirem evidências de diferenças entre as médias das variáveis estudadas para as cinco espécies, o que não ocasionaria prejuízos no poder de discriminação na ausência das variáveis.

Análise discriminante é técnica de análise multivariada que separa populações em grupos com base em suas características mais influentes, e também estabelece regras para alocação de novos elementos (de origem desconhecida) nos grupos anteriormente determinados ou em grupos pré existentes (REIGADA, 2004). Devido ao número de dados faltantes alguns espécimes e variáveis foram excluídos de tal forma que o novo banco de dados ficou completo sem observações faltantes. Dessa maneira, o banco ficou formado por 27 variáveis de 103 espécimes. A análise discriminante foi também realizada considerando o conjunto de 150 espécimes, porém para contornar o problema de muitas observações faltantes, foi feita a transformação dos dados originais padronizados, ponderando-os pelos pesos associados aos dez primeiros componentes principais, de tal maneira que foram obtidas medidas de dez novas variáveis para 150 espécimes.

Análise de agrupamento (**Cluster analysis**) tem como objetivo agrupar objetos semelhantes segundo suas características (variáveis). Deve ser distinguida da análise discriminante, pelo fato de que esta última é aplicada a grupos já estabelecidos, tendo como objetivo a discriminação de um novo indivíduo a um desses grupos. Neste estudo, a análise de

agrupamento não considerou a informação da separação dos espécimes em 5 espécies diferentes. Foi calculada com base na dissimilaridade (distância, (nível = 40), aplicada para 150 espécimes e 35 variáveis. Como forma de validar os resultados da análise de agrupamento, por meio dos dados transformados pelos componentes principais, foi feita análise discriminante.

A coleta dos dados morfológicos foi realizada no Laboratório de Taxonomia e Sistemática do Departamento de Epidemiologia da Faculdade de Saúde Pública da USP. As análises dos dados foram realizadas no laboratório de informática do Instituto de Matemática e Estatística (IME – USP) pelos alunos José Victor Bartol Rodrigues e Robson de Souza Simões sob orientação da profa. Dra. Julia Maria Pavan Sole.

4. RESULTADOS

Como resultados são apresentados 3 manuscritos desenvolvidos no período do Mestrado:

MANUSCRITO 1 **- "Redescription of *Anopheles oswaldoi* (Peryassu, 1922) (Diptera: Culicidae), with formal lectotype designations"**

(aceito para publicação na revista Zootaxa)

MANUSCRITO 2 **- "Revision of five species of the *Anopheles albitarsis* complex (Diptera: Culicidae)"**

(a ser submetido para publicação na revista Cannadian Entomologist)

MANUSCRITO 3 **- "Notes on the holotype of *Anopheles marajoara* Galvão & Damasceno (Diptera: Culicidae)"**

(publicado na Revista Brasileira de Entomologia 50(4): 453-457, dezembro de 2006)

4.1 MANUSCRITO 1

“Redescription of *Anopheles oswaldoi* (Peryassú, 1922) (Diptera: Culicidae), with formal lectotype designations”

(aceito para publicação na revista Zootaxa)

Redescription of *Anopheles oswaldoi* (Peryassú, 1922) (Diptera: Culicidae), with formal lectotype designation

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Abstract

Anopheles (Nyssorhynchus) oswaldoi (Peryassú, 1922) comprises a species complex in South America. To fully characterize other taxa within the Oswaldoi Complex, it is essential to fix the identity of the nominotypical member. Given that the no type was designated in the original description, a lectotype is formally designated from the remaining syntypes in the Museu Nacional do Rio de Janeiro. These and other recently collected specimens from the type locality (Espírito Santo, Brazil) and the State of São Paulo, Brazil were used to redescribe the species using morphological characters of the adult female, male and male genitalia, and the fourth-instar larva and pupa. The larva, pupa, and male genitalia are illustrated. Diagnostic morphological characters of the adult female and male genitalia are provided to distinguish *An. oswaldoi s.s.* from the morphologically similar *An. konderi*, *An. galvaoi,* and *An. ininii*. DNA sequence data from the second nuclear internal transcribed spacer region (ITS2) are included to fix the molecular identity of *An. oswaldoi s.s.*

KEYWORDS: *Anopheles oswaldoi*; lectotype; redescription; *Nyssorhynchus*, ITS2.

Introduction

Anopheles (Nyssorhynchus) oswaldoi (Peryassú, 1922) was first described as a species of the genus *Cellia*, based on adults collected in Vale do Rio Doce, Espírito Santo and Baixada Fluminense, Rio de Janeiro, Brazil. At more or less the same time that Dyar (1923) synonymized *An. oswaldoi* with *An. tarsimaculatus* Goeldi, Bonne (1923, 1924) recognized two "races" of *An. tarsimaculatus*, one exophilic and characterized by a small dark basal ring on the hindtarsal segment 2 (= *An. oswaldoi*) and *An. aquasalis* Curry, which was reported in coastal areas. Root (1924) examined the male genitalia of *An. oswaldoi* and *An. tarsimaculatus* and, considering them identical, once again synonymized the two species. Later, *An. oswaldoi* was resurrected from synonymy with *An. tarsimaculatus* based on morphological differences in the adults, larvae and pupae of both species (Lima 1928). *Anopheles aquacaelestis* Curry was first described as a variety of *An. tarsimaculatus* and later synonymized with *An. oswaldoi* (Senevet & Abonnenc 1938). Galvão & Lane (1937) suggested that *An. oswaldoi* consisted of three subspecies, *An. oswaldoi oswaldoi, An. oswaldoi* var. *metcalfi* Galvão & Lane and *An. oswaldoi* var. *noroestensis* Galvão & Lane. In 1942, Galvão & Damasceno described *An. konderi* from specimens collected in Coari, State of Amazonas, Brazil, and considered *An. noroestensis* to be a distinct species from *An. oswaldoi* and *An. metcalfi*. In the same publication, *An. metcalfi* was considered a *nomen dubium* (mistakenly referred to as a *nomen nudum* in this publication). On the basis of morphological characters of the egg, fourth-instar larva, pupa and adult females, Lane (1953) found *An. konderi* and *An. oswaldoi* to be indistinguishable and again synonymized these taxa. Most recently, Flores-Mendoza *et al.* (2004b) resurrected *An. konderi* from synonymy with *An. oswaldoi*, redescribing all life stages and

designating a neotype. This study clearly showed that males of *An. oswaldoi* and *An. konderi* can be distinguished by the shape of the apical part of the aedeagus, but it remains difficult to reliably identify adult females.

The reported diversity in morphology, DNA sequences, isoenzymes, host preference, vector competence and bionomics across its wide distribution suggest that *An. oswaldoi* comprises a species complex in South America (Causey *et al*. 1946; Klein & Lima 1990; Consoli & Lourenço-de-Oliveira 1994; Rosa-Freitas *et al*. 1998; Marrelli *et al*. 1999b; Scarpassa 2005; Scarpassa & Conn 2006; Ruiz *et al*. 2005; Quiñones *et al*. 2006). Marrelli *et al.* (1999b) suggested there were four forms of *An. oswaldoi s.l.* based on ITS2 sequences from populations across Brazil and in Peru and Venezuela, suggesting that one represents the closely related *An. konderi.* However the purported *An. oswaldoi* specimen from Espírito Santo was in fact a misidentified *An. evansae* (Marrelli *et al*. 2006), and other specimens from Yurimaguas in Peru were later found to correspond to *An. benarrochi* B in southern Colombia (Ruiz *et al*. 2005) and other regions of Peru (Wilkerson, Flores-Mendoza & Linton, unpublished data). As no DNA sequences were included in the formal resurrection of *An. konderi* from synonymy with *An. oswaldoi* (Flores-Mendoza *et al*. 2004b), it still remains unclear which of these DNA identifications, if any, correspond to *An. oswaldoi s.s.*

Given that *An. oswaldoi* comprises a complex of species that vary in vector competence, and that Peryassú (1922) did not designate a type in the original description (Belkin *et al*. 1971), it is now crucial to redescribe and designate a lectotype to fix the identity of the nominotypical member of the Oswaldoi Complex. The aims of this study are: (1) to designate a suitable lectotype and paralectotypes of *An. oswaldoi* from Peryassú's original series; (2) to redescribe the adult and immature stages of *An. oswaldoi* from the original series and new specimens collected at the type locality; and

(3) to generate DNA sequence data from specimens from the type locality to fix the molecular identity of the species.

Materials and Methods

Using a Shannon trap, *Anopheles* species were collected in Lagoa do Macuco, Fazenda Marianelli, Jaguaré, State of Espírito Santo, BRAZIL (19º2'5.36"S, 39º56'54.54"W) in January 2006. Two females of *An. oswaldoi* were identified in the field using the morphological key of Faran (1980). The mosquitoes were blood-fed and induced to lay eggs. The progenies were then maintained in separate dishes with distilled water until hatching and larvae fed with fish food. Fourth-instar larvae were transferred to individual plastic vials with water and kept until adults emerged. Both larval and pupal exuviae were collected and preserved in 80% ethanol, prior to slide-mounting in Canada Balsam. Some whole fourth-instar larvae were preserved. Male genitalia were dissected and slide-mounted in Canada Balsam.

Two syntypes of *An. oswaldoi* deposited in the National Museum of Rio de Janeiro (MNRJ), and a number of non-type specimens housed in the Entomological Collections of the Faculdade de Saúde Pública, Universidade de São Paulo, Brazil (FSP-USP), and in the Instituto Oswaldo Cruz, Rio de Janeiro, Brazil (IOC-RJ), were also examined.

Morphological characters of the adult male and female and the male genitalia were examined and measured. Nomenclature adopted for the adults follows Harbach & Knight (1980), for the wing spots Wilkerson & Peyton (1990), and for the wing veins Belkin (1962). Pupal and larval chaetotaxy were examined, measured and counted for the description. Abbreviations for mosquito life stages are: F, adult female; M, adult

male; G, male genitalia; L, larvae; Le, larval exuviae; P, pupae; Pe, pupal exuviae; E, egg; an asterisk added to the letters means that the life stages was illustrated.

DNA was extracted from ten specimens from the two progeny broods obtained from the females captured at the type locality of Espírito Santo and two additional specimens from São Paulo following a modified protocol based on the QIAgen DNeasy® Blood and Tissue Kit (QIAgen Ltd., Crawley, UK). All buffers were supplied in the kit. Whole mosquitoes were manually ground in 1.5ml Eppendorf® tubes containing 180µl ATL Buffer using plastic pestles (AnaChem Ltd., Bedfordshire, UK). Twenty µl of Proteinase K was added and the samples incubated at 56^{o}C for up to 3 h. Samples were placed at -20^{o}C overnight and, centrifuged the next day at 8,000 rpm for 10 min. Supernatant was removed and placed into clean, labeled Eppendorf® tubes containing 200µl and mixed thoroughly by pipetting, followed by vortexing (15 seconds). Absolute ethanol (200µl) was added and again mixed thoroughly by pipetting, followed by vortexing for 15 sec. The mixture was then transferred into separate DNeasy® Mini spin columns (provided) in a 2ml collection tube. Columns were spun for 1 min at 8,000 rpm, and flow-through discarded. Buffer AW1 (500µl) was added and the columns again centrifuged at 8,000 rpm for 1 min, before discarding the flow-through. This last step was repeated with Buffer AW2 (500µl), but centrifuging at 14,000 rpm for 1 min instead. Spin columns were transferred to clean collection tubes and 200µl of Buffer AE pipetted directly onto the DNeasy® membrane. Columns were incubated at room temperature for 1-3 min and DNA eluted by centrifuging at 8,000 rpm for 1 min. The elution step was repeated and the eluent stored in a separate tube, as we found DNA often remained bound to the membrane following the first elution.

Two µl from the first elution were used as DNA template in the PCR reactions. Amplification of the ITS2 region was carried out using the 5.8SF and 28SR primers

recommended by Collins & Paskewitz (1996). In both cases, PCR products were amplified using the following reaction mix (50µl): 2µl DNA, 22.5µl ddH_20, 5µl QIAgen PCR buffer, 10µl QIAgen Q solution, 3µl 25 mM $MgCl_2$, 1µl of each primer at 5µM; 5µl 2 mM dNTPs (PE Applied Biosystems, Warrington, England), and 0.5µl QIAgen Taq. The PCR thermocycler program consisted of a 2-min denaturation at 94°C, 34 cycles at 94°C, 57°C, and 72°C for 30 sec each, followed by a 10 min extension at 72°C. PCR products were visualized on a 1% agarose gel stained with ethidium bromide. Products were cleaned using the Millipore MultiScreen® PCR_{96} and quantified using a NanoDrop® ND-1000 Spectrophotometer prior to product dilution to 2ng/µl/100bp for sequencing. Sequencing reactions were carried out in both directions using the PCR primers and the Big Dye Terminator Kit v.1.1 (PE Applied Biosystems, Warrington, England). Chromatograms were read on an ABI 3730 automated sequencer (PE Applied Biosystems).

Sequences were edited using Sequencher™ version 4.5 (Genes Codes Corporation, Ann Arbor, Michigan) and aligned in CLUSTAL X (Thompson *et al*. 1997). Sequence similarity of the ITS2 sequences generated in this study with those previously available in GenBank was assessed using FASTA search (http://www.ebi.ac.uk/fasta33/). Intraspecific variation was calculated using MEGA 3.1 (Kumar *et al*. 2004). Template DNA from this study is retained at -70°C in the Molecular Systematics Laboratory, Department of Entomology, the Natural History Museum, London, for future reference (see Material examined for DNA reference numbers).

Results

***Anopheles (Nyssorhynchus) oswaldoi* (Peryassú, 1922)**

Cellia oswaldoi Peryassú, 1922: 179. Syntypes: adult male and female. Vale do Rio Doce, State of Espírito Santo and Baixada Fluminense, State of Rio de Janeiro, Brazil [Museu Nacional do Rio de Janeiro – MNRJ]. LECTOTYPE (hereby designated): Adult male with associated male genitalia dissected and mounted on a microscope slide by one of the authors (CFM), BRAZIL, State of Espírito Santo, Vale do Rio Doce, AG Peryassú coll., 4 - XI -1922.

Anopheles (Nys.) tarsimaculatus var. *aquacaelestis* Curry (1932): 566-571. Syntypes: male with genitalia slide, female and larva. Atlantic side of Canal Zone, Panamá, no type locality cited. Synonymy with *An. oswaldoi* by Senevet & Abonnenc 1938: 487-493.

Anopheles (*Nys*.) *oswaldoi* of Belkin *et al*. 1971: 6 (type info); Faran 1980: 55 (M*, F*, P*, L*); Lounibos *et al*. 1997: 136-155 (scanning electron micro photo, E*).

Description

Female. Integument light brown with grayish pollinosity. *Head*: interocular space with a frontal tuft of long, pale-yellow setae and decumbent, curved, white spatulate scales along ocular margin, vertex immediately posterior to frontal tuft with erect, white spatulate scales and few long pale-yellow setae; remainder of vertex and occiput with erect, dark-brown spatulate scales; postgena with tuft of dark-brown spatulate scales and few semi-erect, white spatulate scales at junction of eyes; clypeus bare. Pedicel of antenna dark brown with decumbent, white spatulate scales on dorsal surface; flagellomere 1 with semi-erect white scales on medial and lateral surface, and decumbent white scales at base of dorsal surface. Proboscis dark, length 2.24-2.30 mm (mean=2.27±0.03) (n=4) (Espírito Santo, ES), 2.13–2.93 mm (mean=2.41±0.29) (n=6)

(São Paulo, SP), 1.40–1.46 (mean=1.44±0.03) (n=3) (ES), 1.43–1.51 (mean=1.46±0.03) (n=5) (SP) length of forefemur, 1.40–1.46 (mean=1.44±0.03) (n=3) (ES), 1.43–1.51 (mean=1.46±0.03) (n=5) (SP) length of maxillary palpus. Maxillary palpomere 1 dark, with erect scales on dorsal surface; maxillary palpomere 2 dark, with few pale scales at apex of dorsal surface; maxillary palpomere 3 dark-scaled, with a subapical pale patch on dorsal surface; maxillary palpomere 4 white-scaled, with dark scales at base, apex and lateral surface; maxillary palpomere 5 white-scaled, with dark scales at base; length of palpomere 2 / length of maxillary palpus 0.26–0.28 (mean=0.27±0.01) (n=4) (ES); 0.27–0.28 (mean=0.28±0.06) (n=2) (SP); length of palpomere 3/ length of maxillary palpus 0.37–0.39 (mean=0.37±0.01) (n=4) (ES), 0.37–0.38 (mean=0.37±0.01) (n=2) (SP); length of palpomere 4 / length of maxillary palpus 0.18–0.19 (mean=0.185±0.005) (n=4) (ES), 0.15–0.18 (mean=0.17±0.02) (n=2) (SP); length of palpomere 5 / length of maxillary palpus 0.14 (n=3) (ES), 0.1 (n=2) (SP). *Thorax*: integument with darker area between dorsocentral area and lateral margin, on posterior edge of scutal fossa and posteriorly on prescutellar area; pale, spatulate, decumbent scales on acrostichal and dorsocentral areas, scutal fossa and anteriorly on prescutellar area; supraalar and antealar areas with white, spatulate, decumbent scales; elongate, narrow and erect white scales along lateral margin of antealar area extending posteriorly onto supraalar area; scutum bare anteriorly between acrostichal and dorsocentral areas, posteriorly to scutal fossa, and posteriorly on prescutellar area; anterior promontory with erect, piliform, white scales; lateral anterior end of dorsocentral area with erect, piliform scales; anterior lateral margin of scutum with erect, spatulate scales, white scales dorsally and dark scales ventrally. Scutellum with a few yellowish spatulate scales, posterior margin with long dark setae. Mesopostnotum bare. Antepronotum with dark setae, and a patch of spatulate scales, white dorsally and dark ventrally on upper area, remainder of

antepronotum without scales, with scattered setae. Patch of white scales on lower prealar, prespiracular and lower mesokatepisternum; patch of setae on upper prealar, lower mesokatepisternum and proepisternum. *Wing*: length 3.39–3.63 mm (mean=3.52±0.12) (n=4) (ES), 3.54–3.89 mm (mean=3.70±0.14) (n=4) (SP); wing spot measurements in Table 1; veins with dark areas and spots of pale yellow scales as follow: costa always with basal pale, pre-humeral dark, humeral pale, humeral dark, presector pale, presector dark, distal sector dark, subcostal pale, preapical dark, preapical pale and apical dark; sector pale, proximal sector dark and accessory sector pale present in 25% (ES) and 50% (SP) of wings examined; remigium pale; 0.75–0.79 mm (mean=0.77±0.01) (n=4) (ES), 0.71–0.79 mm (mean=0.75±0.03) (n=5) (SP) distance between basal pale and sector pale spots of vein R; R_S with a patch of pale scales at junction of $R_{4+5,}$ and few pale scales on basal 0.5; R_{2+3} with a patch of pale scales along middle region and few dark scales at apex before furcation of R_2 and R_3; R_{4+5} mostly pale, with small patch of dark scales at proximal 0.2 and distal end; vein M variable, mostly dark, with pale scales from basal end to middle region, or mostly pale with dark spots in middle region and at distal end at furcation of M_{1+2} and M_{3+4}; CuA mostly pale, with small dark spot at distal 0.2 before furcation of CuA_1 and CuA_2; CuA_1 mostly pale, with 2 separate dark spots at basal 0.5 and a small dark spot at distal end; pale fringe spots at apices of veins $R_{2,}$ $R_{4+5,}$ $M_{1+2,}$ $M_{3+4,}$ $CuA_{1,}$ CuA_2 and 1A. *Halter*: pale ventrally and dorsally with proximal and distal dark spots on dorsal surface, capitellum dark ventrally. *Legs*: anterior surface of forecoxa with a patch of white spatulate scales and long, dark setae, posterolateral surface with white spatulate scales, posterior surface with dark spatulate scales; outer surface of midcoxa with white spatulate scales, proximal area with semi-erect scales, posterior and anterior surface with a patch of white spatulate scales; posterior surface of hindcoxa with a small apical patch of white

spatulate scales, distal posterior surface with white spatulate scales. Fore-, mid- and hindtrochanters dark-scaled on anterior and inner surfaces, lateral surface with a few dark scales at base, white scales on posterolateral surface, posterior surface with dark scales at base and white scales distally. Foretarsomere 1 with pale scale at apex; dark base of foretarsomere 2 0.64–0.72 (mean=0.69±0.04) (n=6) (ES), 0.67–0.71 (mean=0.69±0.03) (n=2) (SP) length of tarsomere, dark base of foretarsomere 3 0.16–0.24 (mean=0.20±0.04) (n=6) (ES), 0.34–0.48 (mean=0.41±0.10) (n=2) (SP) length of tarsomere, foretarsomeres 4 and 5 dark-scaled with pale scales at apices, sometimes foretarsomere 5 with a subapical pale patch; midtarsomere 2 with an apical ring of pale scales, dark base of midtarsomere 2 0.87–1.00 (mean=0.92±0.06) (n=6) (ES), 0.83–0.89 (mean=0.86±0.04) (n=2) (SP) length of tarsomere, midtarsomere 3 with a few apical pale scales, dark base of midtarsomere 3 0.77–0.96 (mean=0.89±0.06) (n=6) (ES), 0.95–0.96 (mean=0.96±0.00) (n=2) (SP) length of tarsomere, midtarsomere 4 dark, sometimes with a few pale scales at apex, midtarsomere 5 with pale scales at about apical 0.5; dark base of hindtarsomere 2 0.08–0.15 (mean=0.13±0.03) (n=6) (ES), 0.13–0.24 (mean=0.19±0.05) (n=4) (SP) length of tarsomere, dark base of hindtarsomere 5 0.39–0.42 (mean=0.40±0.01) (n=4) (ES), 0.41–0.51 (mean=0.46±0.04) (n=4) (SP) length of tarsomere, remainder of hindtarsomeres 2, 3, 4 and 5 white-scaled. *Abdomen*: integument light to dark brown; terga II-IV with pale scales, most scales disposed in a sub-triangular pattern on segments II-V, segments VI-VII more equally covered with scales; dark posterolateral scale-tufts large, present on terga II-VII.

Male. Similar to female except for sexual differences. Maxillary palpus pale and dark-scaled; scales semi-erect on basal 0.5 of palpomere 2, decumbent on remainder of palpomere 2 and palpomeres 3-5; palpomere 2 dark-scaled with pale scales at apex,

dorsal surface with a pale spot on basal 0.5; palpomere 3 with pale scales at base, dorsally with a pale spot on medial surface, long setae at apex; palpomere 4 with pale scales on ventral and dorsal surface, dark-scaled at apex and base; palpomere 5 with dark setae along ventral surface, pale scales on dorsal surface, dark scales on ventral surface and base.

Male genitalia (Figures 1A, C; 2B, C). Segment VIII: tergum and sternum narrow, with broad spatulate scales and long setae, scales slightly broader on sternum than on tergum. Segment IX: anterior apodeme sub-trapezoidal; gonocoxite elongate; tergal surface with 4 or 5 setae and subapical surface with 1 or 2 setae; tubercle of parabasal spine large, 0.4 length of parabasal spine; dorsomedian rim 0.11–0.14 (mean=0.12±0.01) (n=2) (ES), 0.13 (n=1) (SP) length of gonocoxite; accessory spines 0.33–0.40 (mean=0.37±0.02) (n=3) (ES), 0.38 (n=1) (SP) length of gonocoxite; internal spine retrorse apically; gonostylar claw spiniform, thin and long. *Dorsal claspette*: pedicel narrow, base rounded, leaflets broad, internally expanded on median area. *Ventral claspette* (Figures 1C; 2C): spiculate, with spicules extending to apex, 0.44–0.55 (mean=0.48±0.04) (n=3) (ES), 0.45 (n=1) (SP) length of gonocoxite, lateral margin tapered toward a narrow apex, basal lobule expanded laterally with long setae on basal margin, setae about 2.0 width of aedeagus, lateral margin of apex rounded and median sulcus shallow, sulcus with sloping sides, preapical plate large, a weakly sclerotized, transparent membranous area basal to preapical plate, refringent structure in shape of an inverted horseshoe or V; aedeagus without subapical leaflets, apex longer than wide, somewhat rounded (Figure 1A).

Pupa (Figure 2A). Position and development of setae as figured; range, number and mode of branches in Table 2. All measurements from 19 or 20 specimens, unless otherwise indicated. *Cephalothorax*: integument yellowish with dark areas in legs and dorsal part, without a pattern of dark areas; trumpet length 0.36–0.43 mm (mean=0.41±0.02) (n=17) (ES), 0.39–0.41 mm (mean=0.41±0.01) (n=3) (SP), pinna moderately to heavily pigmented, light to dark brown, 3.06–4.55 (mean=3.84±0.41) (n=12) (ES), 3.59–4.39 (mean=3.98±0.36) (n=3) (SP) length of meatus, trumpet appearing truncate and flared apically in lateral aspect; seta 2-CT slightly shorter than 1,3-CT, 10-CT usually single (1-2), shorter than 11-CT, 12-CT with 1-4 branches, 1.33–2.00 (mean=1.67±0.24) (n=10) (ES), 1.60–1.87 (mean=1.76±0.13) (n=2) (SP) length of 10-CT. *Abdomen*: integument yellowish with ventral dark areas in intersegmental membrane and laterally on segment II, length 2.58–2.90 mm (mean=2.75±0.09) (n=17) (ES), 2.82–2.88 mm (mean=2.84±0.03) (n=3) (SP); 1-II,III with median branches longer than lateral; 1-IV-VII single and long, slightly longer than following segment; 2-I with 3-9 branches, dendritic, long; 3-I 0.60–1.00 (mean=0.79±0.13) (n=12) (ES), 0.75–1.00 (mean=0.87±0.13) (n=2) (SP) length of 2-I, 3-V normally triple (1-4 branches); 5-IV with 1-4 branches, longer and more accentuated than 5-III, 5-V-VII normally single; 6-I single or double, long, 6-II generally double (1-4 branches), 7-I with 2-5 branches, shorter than 6-I, 7-III-V short, with 5 or fewer branches, generally with 2 or 3 branches, 7-VII single and long; 9-II small and unpigmented, 9-III short, 1.20–2.00 (mean=1.76±0.25) (n=14) (ES), 1.33–1.60 (mean=1.51±0.15) (n=2) (SP) length of 9-II, weakly pigmented, 9-IV 1.25–2.33 (mean=1.69±0.28) (n=17) (ES), 2.00–2.50 (mean=2.24±0.26) (n=3) (SP) length of 9-III, 9-V strong, 1.57–3.29 (mean=2.07±0.30) (n=17) (ES), 1.63–1.90 (mean=1.77±0.09) (n=3) (SP) length of 9-IV, 9-VI strong 0.78–1.36 (mean=1.16±0.11) (n=17) (ES), 1.07–1.43 (mean=1.16±0.14)

(n=3) (SP) length of 9-V, 9-VII strong, weakly curved, 0.71–1.57 (mean=1.19±0.16) (n=16) (ES), 1.27–1.82 (mean=1.47±0.24) (n=3) (SP) length of 9-VI, 9-VIII 0.75–1.5 (mean=0.96±0.15) (n=17) (ES), 0.82–1.15 (mean=0.94±0.13) (n=3) (SP) length of 9-VII, 9-II 0.04–0.06 (mean=0.04±0.01) (n=14) (ES), 0.05–0.07 (mean=0.05±0.01) (n=2) (SP) length of segment II, 9-III 0.04–0.06 (mean=0.05±0.01) (n=17) (ES), 0.05–0.06 (mean=0.06±0.01) (n=3) (SP) length of segment III, 9-IV 0.07–0.12 (mean=0.09±0.01) (n=17) (ES), 0.10–0.15 (mean=0.12±0.02) (n=3) (SP) length of segment IV, 9-V 0.13–0.31 (mean=0.18±0.03) (n=17) (ES), 0.17–0.26 (mean=0.21±0.04) (n=3) (SP) length of segment V, 9-VI 0.15–0.28 (mean=0.21±0.03) (n=17) (ES), 0.17–0.28 (mean=0.24±0,05) (n=3) (SP) length of segment VI, 9-VII 0.15–0.29 (mean=0.23±0.03) (n=17) (ES), 0.21–0.48 (mean=0.34±0.11) (n=3) (SP) length of segment VII, 9-VIII 0.19–0.28 (mean= 0.22±0.02) (n=16) (ES), 0.23–0.44 (mean=0.33±0.09) (n=3) (SP) length of segment VIII; 10-VI absent; Paddle longer than wide, length 0.72–0.82 mm (mean=0.77±0.03) (n=17) (ES), 0.79–0.81 mm (mean=0.80±0.01) (n=1) (SP), width 0.32–0.40 mm (mean=0.36±0.02) (n=17) (ES), 0.33–0.37 (mean=0.35±0.01) (n=3) (SP); refractile index 0.65–0.73 (mean=0.69±0.02) (n=17) (ES), 0.71 (n=1) (SP); subapical area of paddle weakly emarginate at insertion of setae 1-P; midrib distinct basally, indistinct distally; external buttress 0.62–0.76 (mean=0.69±0.04) (n=17) (ES), 0.63 (n=1) (SP) length of paddle; seta 1,2-P single.

Fourth–instar larva (Figure 3). Position and development of setae as figured; range and modes of branches in Table 3. Measurements from 19 or 20 specimens unless otherwise indicated. *Head*: length 0.58–0.65 mm (mean=0.63±0.02) (n=18) (ES); width 0.56–0.57 mm (mean=0.57±0.01) (n=2) (SP); integument weakly sclerotized, somewhat yellowish with dark spots on posterior region of dorsal apotome and lateralia along

dorsal ecdysial line, posterior area of lateralia, ventral area of lateralia, labiogula and along of hypocranial ecdysial line; dorsomentum strongly sclerotized, blackish, median tooth broad, pointed, stronger than lateral teeth. Seta 2-C single, distinctly brushlike on 0.5 distal, 0.95–1.33 (mean=1.10±0.08) (n=17) (ES), 0.80–1.05 (mean=0.96±0.11) (n=2) (SP) length of 3-C; 0.04–0.05 mm (mean=0.04±0.03) (n=18) (ES), 0.05 mm (±0.01) (n=2) (SP) distance between bases of 2-C; 3-C visibly brushlike on 0.5 distal, 0.75–1.06 (mean=0.90±0.07) (n=17) (ES), 0.95–1.25 (mean=1.06±0.14) (n=2) (SP) length of 2-C; clypeal index (distance between bases of 2-C and 3-C one side / distance between bases of 2-C) 1.10–1.63 (mean=1.39±0.11) (n=18) (ES), 1.20–1.30 (mean=1.24±0.08) (n=2) (SP). Seta 4-C with 2-8 short branches, extending half distance to base of 2-C. Collar dark brown, strongly pigmented, dorsomesal region 0.05–0.06 mm (mean=0.05±0.003) (n=17) (ES), 0.05 mm (n=2) (SP). *Antenna*: 0.27–0.31 mm (mean=0.29±0.01) (n=17) (ES), 0.30–0.32 mm (mean=0.31±0.01) (n=2) (SP), darker pigmented than head capsule, mesal margin with numerous, long spicules; 1-A with 4-9 branches, inserted 0.06–0.10 mm (mean=0.08±0.01) (n=17) (ES), 0.09–0.10 mm (mean=0.09±0.01) (n=2) (SP) distance from base. *Thorax*: dark granules under integument, seta 1,2-P on separate tubercles, 1-P with 9-15 narrow, pointed leaflets, 2-P with 11-21 branches, 2.59–4.44 (mean=3.38±0.50) (n=12) (ES), 3.60 (n=1) (SP) length of 1P; 14-P with 7-11 branches, flattened stalk and lateral branches shorter than median branches; 1-M strongly plumose, 22-35 branches; 3-T with 9-15 somewhat transparent leaflets. *Abdomen*: integument similar to that of thorax; seta 0-II-VII usually large; 1-I-VII palmate, 1-I with 8-16 long, narrow, both pointed and truncate leaflets; 2-II with 3-10 large branches, 2-III with 3-8 large branches, 2-IV single; 5-I with 3,4 branches, inserted on lateral margin of abdomen, 13-IV with 4-11 large branches, 13-V with 3-6 branches larger than 13-IV. Pecten plate with 2-5 long spines, 10-16 short spines, long

spines 2.00–3.00 (mean=2.40±0.24) (n=12) (ES), 2.20–3.00 (mean=2.59±0.40) (n=2) (SP) length of short spines. Segment X: covered with fine spicules except anteriorly, spicules stronger posteriorly; seta 1-X as long as saddle, inserted on ventral margin of saddle.

Molecular characterization. The ITS2 region was sequenced for 12 individuals, 10 from Espírito Santo and two from São Paulo (GenBank accession numbers EF457228 – EF457239; ES=EF457228-37; SP=EF457238-9). The amplicon length was consistent at 530-bp (including primers), and the 12 sequences revealed a single haplotype. The ITS2 haplotype comprised the following bases: 20.6% T, 28.1% A, 27.2% C, and 24.1% G.

The ITS2 sequences of *An. oswaldoi s.l.* are available in GenBank from Brazil (Acre AF055068, Amapa AF056318, Amazonas AF056317, Rondonia AF055069; Marrelli *et al*. 1999b), Colombia (Putumayo, AY679149-55; Ruiz *et al*. 2005), Peru (Yurimaguas, AF055071; Marrelli *et al*. 1999b), Venezuela (Ocama, AF055070; Marrelli *et al*. 1999b) and from unlisted localities (U92352-3, U92344; Danoff-Burg & Conn direct submission). A FASTA search revealed that the ITS2 sequences of *An. oswaldoi s.s.* (n=12) are unique with regard to those already in GenBank. *Anopheles oswaldoi s.l.* from Rondônia, Brazil (AF055069) shares highest sequence similarity at 97.9% (Figure 4). Along a 486 bp alignment (CCGCGG and GGTACCC removed from 5’ and 3’ end of AF055069, respectively), 10 bases varied, including three 2-bp indels (bases 312-3, 354-5, and 415-6) and 4 singleton polymorphic sites (260, 282, 315, 405) (Figure 4).

Diagnosis. The Oswaldoi Group of *Nyssorhynchus* Blanchard mosquitoes includes 15 formally recognized species divided between two subgroups: the Oswaldoi Subgroup (12 species: *An. anomalophyllus* Komp, *An. aquasalis* Curry, *An. dunhami* Causey, *An. evansae* (Brèthes), *An. galvaoi* Causey, *An. ininii* Senevet & Abonnenc, *An. konderi* Galvão & Damasceno, *An. nuneztovari* Gabaldón (cytotypes A and B/C, Conn *et al.* 1993), *An. oswaldoi*, *An. rangeli* Gabaldón, Cova Garcia & Lopez, *An. sanctielii* Senevet & Abonnec, and *An. trinkae* Faran; and the Strodei Subgroup (3 species: *An. benarrochi* Gabaldón, *An. rondoni* (Neiva & Pinto) and *An. strodei* Root) (Harbach 2004). Six of these species are actively involved in malaria transmission, and at least four, *An. aquasalis* (Conn *et al*. 1993; Maldonado *et al*. 1997), *An. benarrochi* (Ruiz *et al*. 2005), *An. oswaldoi* (Marrelli *et al*. 1999b), and *An. nuneztovari* (Conn *et al*. 1993; Sierra *et al*. 2004), are known to comprise complexes of morphologically indistinct species that exhibit differences in genetics, behavior and vector competence. Herein, the terminology regarding groups, subgroups and complexes follows Harbach (2004): the Oswaldoi Series (= Oswaldoi Group of Faran 1980), Oswaldoi Group (= Oswaldoi Subgroup of Faran 1980), and Oswaldoi Subgroup (= Oswaldoi Complex of Faran 1980). Herein we use the term Oswaldoi Complex to indicate a group of hitherto undifferentiated taxa close to *An. oswaldoi s.s.*

Adult females of the Oswaldoi Series are recognized by having palpomere 4 with the mid-lateral surface pale-scaled, abdominal segment II with posterolateral tuft of dark scales, foretarsomere 5 either entirely pale-scaled or with at least the apex pale-scaled and basally dark. However, in *An. strodei* and *An. triannulatus* the foretarsomere 5 is either entirely pale-scaled or with at least the apex pale-scaled and basally dark or entirely dark-scaled. Based on characters of the male genitalia, members of the Oswaldoi Series are recognized by having sternum IX large, either sub-trapezoidal or

sub-triangular, the ventral claspette with or without spicules and the apex expanded forming large auricular lateral lobes. In the fourth-instar larva, seta 4-C is normally small, except in *An. trinkae* and *An. nuneztovari* where it is long, seta 1-P palmate, seta 9-P,T single, seta 13-I,III,IV varying from small to moderately large, and seta 11-I large with 5-7 branches.

Most species of the Oswaldoi Group can be recognized in the adult female by having palpomere 4 mostly pale-scaled, the absence of an anterior mesepimeral scale-patch, foretarsomere 4 without an apical pale band, except *An. nuneztovari* and *An. ininii* in which foretarsomere 5 is either entirely pale or pale and dark-scaled, and some individuals of *An. strodei* in which foretarsomere 5 is sometimes entirely dark. Additionally, member species possess setae on the basal lobe of the ventral claspette of the male genitalia; the fourth-instar larvae have seta 1-P palmate, usually with 16 narrow or broad branches, seta 14-P with a short or moderately long axis, seta 11-I large with 2-4 branches, seta 13-I short to large, normally triple, seta 13-III normally small with multiple branches, the lateral arm of spiracular apparatus varying from short to moderately long, except for *An. ininii* that has a moderately long lateral arm (Faran 1980).

The Oswaldoi Subgroup is recognized by having the male genitalia with spicules extending toward the apex of the ventral claspette or extending to the apical margin of the preapical plate, the apex of the ventral claspette not laterally expanded, and the parabasal spines short. Based on characters of the male genitalia, Faran (1980) suggested two phyletic lines within the Oswaldoi Subgroup; one formed by *An. oswaldoi*, *An. galvaoi*, *An. evansae* (as *An. noroestensis*), *An. aquasalis*, *An. ininii,* and possibly *An. anomalophyllus*, and with *An. trinkae*, *An. nuneztovari,* and *An. rangeli* in the other.

Anopheles oswaldoi s.l. is morphologically more similar to *An. konderi, An. galvaoi,* and *An. ininii* than to any other species of the Oswaldoi Subgroup. However, these four species can be distinguished based on male genitalia and a few adult female characters. Faran (1980) should be consulted for details to separate *An. oswaldoi s.l. and An. konderi* from *An. galvaoi* and *An. ininii.* Briefly, in *An. oswaldoi s.s.,* setae of the ventral claspette along the basal margin of the basal lobules are about twice as wide as the aedeagus, the preapical plate is moderately sclerotized, and the apex of the aedeagus is longer than broad, whereas in *An. galvaoi* the setae of the ventral claspette along the basal margin of the basal lobules are very long, about three times the width of the aedeagus, the preapical plate is strongly sclerotized and, the apex of the aedeagus is broader than long. *Anopheles ininii* can be recognized by the shape of the ventral claspette, which is somewhat conic, with a very small median sulcus at the apex, and spicules that extends to the apex, whereas in *An. galvaoi* the ventral claspette is somewhat trapezoidal, with a well-developed median sulcus and spicules extending to the apex. Additionally, characters of the female of *An. oswaldoi s.s.* can be distinguished from *An. ininii* by having midtarsomere 4 all dark, foretarsomere 2 pale in the apical 0.20-0.45, foretarsomere 3 pale in the apical 0.50-0.85, dark basal bands on foretarsomeres 3-5 almost completely encircling each segment, foretarsomere 4 dark to least the basal third. In *An. ininii,* midtarsomere 4 has a pale band in the apical 0.15-0.25, foretarsomere 2 pale in the apical 0.35-0.55, foretarsomere 3 pale in the apical 0.70-0.86, foretarsomeres 3-5 predominantly cream-colored to white, foretarsomere 4 all pale to rarely more than the basal third dark.

Lounibos *et al.* (1997) found no differences to separate *An. oswaldoi s.l.* (from Coca, Napo Province and Sucumbios Province, Ecuador, Capanema, State of Pará, Brazil and Brokopondo, Suriname) and *An. konderi* (from Alto Linhares, Cochabamba

Dept., Bolivia) using scanning electron micrographs of the egg exocorion and this, along with lack of morphological or morphometric differences in the pupal stage, was later verified by Flores-Mendoza *et al.* (2004b). However, they reported differences in the dark spots of foretarsomere 2 and hindtarsomere 2, and wing spots that can be used to distinguish the adult females of *An. oswaldoi s.l.* and *An. konderi.* More importantly, these two species can be easily distinguished based on the shape of the apex of aedeagus of the male, which is usually longer than broad and somewhat ovate in *An. oswaldoi s.s.* (Figure 1A), and is broader than long and more conical in *An. konderi* (Figure 1B). Additionally, the lateral surface of the aedeagus is curved into a small lateral projection in *An. konderi* (Figure 1B), whereas it is straight in *An. oswaldoi s.s.* (Figure 1A). The ventral claspette is indistinguishable in these species (Figures 1C, D).

Adult females of *An. oswaldoi s.l.* can be identified by the following characters: foretarsomeres 2 and 3 pale-scaled on 0.28-0.33 and 0.52-0.84 of the apex, respectively, foretarsomere 4 dark-scaled with pale scales at the apex, midtarsomere 4 dark, pale at the apex, hindtarsomere 2 dark-scaled in less than the basal 0.25; basal lobe of the ventral claspette large, laterally expanded, with long setae on the basal margin, twice as long as the width of the aedeagus, preapical plate of the ventral claspette large, poorly sclerotized, aedeagus without apicolateral leaflets; pinna of the pupal trumpet 3.06-4.55 length of the meatus, trumpet truncate and broad at the apex, seta 9-V-VIII short, seta 6-II as long as or longer than 7-II, seta 2-I with 3-9 long branches, dendritic; in the fourth-instar larva, seta 2-C are broadly spaced, clypeal index 1.1-1.6, seta 4-C short, distance between the insertions of 4-C and 2-C equal or longer than distance between insertions of 2-C and 3-C, setae 8,9-C long, 0.17-0.21 distance between 8-C and 5-C, seta 1-X arises from the ventral margin of the saddle, and the anal papillae long, longer than segment X.

Distribution of An. oswaldoi s.s. Based on a comparison of ITS2 sequence data in GenBank (Danoff-Burg & Conn direct submissions; Marrelli *et al.* 1999b; Ruiz *et al*. 2005; Quiñones *et al*. 2006), *An. oswaldoi s.s*. has only been recorded with certainty in Espírito Santo (Peryassú 1922; herein), Rio de Janeiro (Peryassú 1922), and São Paulo (herein).

Anopheles oswaldoi s.l. has been reported throughout South America east of the Andes and as far south as the northern provinces of Argentina (Faran 1980; Faran & Linthicum 1981). It has been reported from Argentina (Faran 1980), Bolivia (Peyton *et al*. 1983), Brazil (see Table 4), Colombia (Ruiz *et al*. 2005; Quiñones *et al*. 2006), Costa Rica (Faran 1980), Ecuador (San Sebastián *et al*. 2000), Guianas (Rambajanl 1987; Laubach *et al*. 2001), Panama (Simmons 1979), Paraguay (Faran 1980), Peru (Hayes *et al*. 1987; Flores-Mendoza *et al*. 2004a), Suriname (Lounibos & Conn 2000), Trinidad (Rozenboom 1942; Chadee & Beier 1996), and Venezuela (Rubio-Palis & Curtis 1992; Grillet 2000).

Bionomics of An. oswaldoi s.s. Due to the restricted knowledge of the true distribution of *An. oswaldoi s.s.* (Peryassú 1922; herein), little is known of the behavior or vector competence of this specie. In this study, *An. oswaldoi s.s.* was collected in a well- preserved forested area in the Vale do Rio Doce, Espírito Santo, Brazil, using a Shannon trap with both light and human attractants, between 18:00-21:00h and accepted a blood meal at 02:00. As only two females were collected, it is not possible infer species behavior from our data.

Immature stages of *An. oswaldoi* have been collected from permanent or temporary freshwater habitats situated in the interior or at the edges of tropical forests

(Faran 1980). In the most detailed study of larval habitats of *An. oswaldoi* to date, Grillet (2000) reported that *An. oswaldoi* was relatively rare, but was the most abundant anopheline at the end of the dry season in Sucre State, northeastern Venezuela. Larvae were found in permanent freshwater (ponds, canals, swamp forests and clear-cut marsh forests) and both the occurrence and abundance of *An. oswaldoi* was closely correlated with high levels of dissolved oxygen. In coastal brackish marshes in Venezuela, Berti *et al*. (1993) found *An. oswaldoi* larvae in the rainy season when salinity was low.

According to most authors, *An. oswaldoi* is typically exophilic and zoophilic (Deane *et al*. 1948; Faran 1980; Consoli & Lourenço-de-Oliveira 1994; Lourenço-de-Oliveira & Luz 1996), but there have also been reports of females being captured biting humans, both indoors and out (Rubio-Palis & Curtis 1992; Branquinho *et al*. 1996; Quiñones *et al*. 2006), and reports of involvement in malaria transmission indicate bionomic plasticity. In Brazil, *An. oswaldoi* has been collected inside primary tropical forest in Balbina, State of Amazonas (Lourenço-de-Oliveira & Luz 1996), in a forested region near hydroelectric dams in Serra da Mesa, State of Goias (Guimarães *et al*. 2004), and in a well-preserved forested area in the Vale do Rio Doce, State of Espírito Santo (herein). In contrast, a study in western Venezuela reported that 42% of 1,000 *An. oswaldoi* were captured on human bait stationed inside an experimental hut (Rubio-Palis & Curtis 1992), whereas in the Purus River Basin, State of Acre, Brazil, *An. oswaldoi* was collected in both open areas and in the peri-domiciliary environment (Natal *et al*. 1992). In Colombia, *An. oswaldoi* was reported resting indoors, with a preferred resting height of >1.5m on walls (Quiñones & Suarez 1990). Klein & Lima (1990) suggested that *An. konderi* is often mistaken for *An. oswaldoi*, proposing that *An. konderi* is present in human impacted environments, whereas *An. oswaldoi* is restricted

to forested areas. Peak biting activity has been reported between 18:00- 19:00h (Deane *et al.* 1948), and between 18:00-20:00h in Rondônia, Brazil, ceasing after 21:00h (Tadei *et al*. 1998). However, in intra-domiciliary environments in Venezuela, the species continues to bite until around midnight (Rubio-Palis & Curtis 1992; Rubio-Palis *et al*. 1994).

Vector competence of An. oswaldoi s.s. No natural infectivity studies appear to have been carried out in Espírito Santo, thus the role of *An. oswaldoi s.s.* in malaria transmission remains unknown. Populations of *An. oswaldoi s.l.* from the State of São Paulo have been shown to be susceptible to infection with *P. vivax* and *P. falciparum* in the laboratory (Fonseca & Fonseca 1942) but laboratory susceptibility does not necessarily indicate that the species is a malaria vector under natural conditions. Sequence data generated in our laboratory suggests that there is another closely related species in sympatry with *An. oswaldoi s.s.* in São Paulo. Whether this species belongs to the Oswaldoi Complex or is a closely related species, e.g. *An. ininii* or *An. galvaoi,* remains to be determined.

Whereas *An. oswaldoi* seems to be involved in the dynamics of malaria transmission in South America, its perceived importance varies geographically. Although the females have been found naturally infected in Colombia (Quiñones *et al*. 2006), Peru (Hayes *et al*. 1987) and Venezuela (Rubio-Palis & Curtis 1992), it is not regarded to be a major vector in these countries due to its low densities. Many studies have detected natural *Plasmodium* infections in *An. oswaldoi* in Brazil (Table 4), yet it is regarded as a primary vector in some regions, and a secondary or unimportant vector in others. In the Brazilian state of Acre, *An. oswaldoi* is found indoors and in peri-domiciliary areas where it is reportedly the most anthropophilic species and acts as an

efficient vector (Branquinho *et al*. 1993; Branquinho *et al*. 1996; Marrelli *et al*. 1999a). More than 7% (190/2610) of specimens tested by ELISA were positive: 3.41% for *P. falciparum*, 2.26% for *P. vivax* VK210, 1.22% for *P. vivax* VK247, and 0.42% for *P. malariae* (Branquinho *et al*. 1993). In a later study in the same area, 29% of specimens (1/34) were found positive by dissection of guts and salivary glands (Branquinho *et al*. 1996). In the State of Pará, Arruda *et al*. (1986) assayed 442 *An. oswaldoi* (of 962 collected) and reported 10 ELISA positive for *P. falciparum*. Later specimens from the same population were found to be infected by *P. vivax* by Immuno Radio Metric Assay (IRMA) (Oliveira-Ferreira *et al*. 1990). ELISA tests on the salivary glands of 417 *Anopheles* from the State of Pará showed two specimens of *An. oswaldoi* to be infected, one with *P. vivax* VK247 and the other with *P. malariae* (Santos *et al.* 2005). In Costa Marques (State of Rondônia, Brazil), Klein *et al.* (1991a,b) reported low natural infection rates with *P. vivax* and low numbers of sporozoites in the salivary glands of wild-caught *An. oswaldoi*. Under laboratory conditions, it was shown that populations of *An. oswaldoi* from Trinidad were susceptible to infection with *P. vivax* (Rozeboom 1942) and those from São Paulo, Brazil could be infected with *P. vivax* and *P. falciparum* (Fonseca & Fonseca 1942). In a monkey malaria area in the Amazon, *An. oswaldoi* was found at both canopy and ground levels, and circumstantially incriminated as a potential vector of *P. brasilianum* to monkeys (Lourenço-de-Oliveira & Luz 1996).

Systematics. *Anopheles oswaldoi* is considered a species complex based on differences in DNA sequences, bionomics, and vector competence. Herein, the nominotypical member of the complex, *An. oswaldoi s.s.*, is redescribed using morphology and DNA sequence data to fix its identity and form a solid taxonomic foundation from which to describe all other members of the Oswaldoi Complex. A

lectotype is designated herein from Peryassú's original series of syntypes. The internal systematics of the Oswaldoi Complex in South America is currently being studied in our laboratory and the results will be published elsewhere.

Material examined. TYPE SPECIMENS. LECTOTYPE: adult male with dissected male genitalia mounted on a microscope slide, BRAZIL, State of Espírito Santo, Vale do Rio Doce, AG Peryassú coll., 4-XI-1922. PARALECTOTYPES: two females, same collection data as the lectotype; one female, State of Rio de Janeiro, Magé, AG Peryassú coll. The lectotype and two paralectotypes from Espírito Santo reside in the same glass vial, in which there is a paper label and the number 13.206 on the cork in the top of the vial. The specimen collected in Rio de Janeiro, Magé, is placed in a separate vial. The lectotype and three paralectotypes are deposited in Museu Nacional do Rio de Janeiro, Brazil (MNRJ); all are in poor condition.

NON-TYPE SPECIMENS. BRAZIL, State of Espírito Santo, Jaguaré municipality, Fazenda Marianelli, Lagoa do Macuco (19º02'5.36"S 39º56'54.54"W), Natal *et al*., coll., 18-I-2006, progeny broods from females collected landing on human bait, ES08(11)-1 MG [E-12903 at FSP/USP] ; ES08(11)-2 FLePe [E-12884 at FSP/USP]; ES08(11)-3 MLePe [E-12885 at FSP/USP]; ES08(11)-4 MLePe [E-12886 at FSP/USP]; ES08(11)-5 MLePe [E-12887 at FSP/USP]; ES08(11)-6 MLePe [E-12888 at FSP/USP]; ES08(11)-7 LePe [E-12889 at FSP/USP]; ES08(11)-8 LePe [E-12890 at FSP/USP]; ES08(11)-9 MGLePe [E-12904 at FSP/USP]; ES08(11)-10 FLe [E-12891 at FSP/USP]; ES08(11)-11 FLePe [E-12892 at FSP/USP]; ES08(11)-12 F [E-12905 at FSP/USP]; ES08(11)-13 MGLePe [E-12907 at FSP/USP]; ES08(11)-14 FLePe [E-12894 at FSP/USP]; ES08(20)-1 LePe [E-12895 at FSP/USP]; ES08(20)-2 Le [E-12896

at FSP/USP]; ES08(20)-10 Pe [E-12897 at FSP/USP]; ES08(20)-11 LePe [E-12906 at FSP/USP]; ES08(20)-13 FLePe [E-12898 at FSP/USP]; ES08(20)-14 MLePe [E-12899 at FSP/USP]; ES08(20)-15 LePe [E-12893 at FSP/USP]; ES08(20)-16 MLePe [E-12900 at FSP/USP]; ES08(20)-17 FLePe [E-12901 at FSP/USP]; ES08(20)-18 FLePe [E-12902 at FSP/USP]; ES08(20)-19 MLePe [E-12883 at FSP/USP]; ES08(20) Le [E-12908 at FSP/USP]; Linhares municipality, Reserva Florestal de Sooretama (19º13'00"S, 40º08'00"W), C Flores-Mendoza & CB Santos coll., IV-1996, progenies from females collected on animal bait, 1M, 2F, 1MLePe, 2FLePe, 1MG, 1FG. State of São Paulo, Pariquera Açu, P. Mirim, AC Gomes coll., 22-II-1988, E-8351FPe [at FSP/USP]; E-8352MGLePe [at FSP/USP]; E-8353FLePe [at FSP/USP]; State of São Paulo, Pariquera Açú, Experimental Station, OP Forattini coll., Shannon trap, 25-II-1992, E-9461F [at FSP/USP]; State of São Paulo, Iguape, Pq. Fontes, Sucen SR2 coll., 5-IV-1994, human landing, E-11020F [at FSP/USP]; E-11021F [at FSP/USP]. State of São Paulo, Pariquera-Açu municipality, Pariquera-Mirim district (24° 43' 60S 47° 49' 0W), Sallum coll.,SP03-4F; SP03-5F. Newly collected specimens from Espírito Santo used in this study are deposited in the Entomological Collections of the Faculdade de Saúde Pública, Universidade de São Paulo, Brazil.

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References

Arruda, M. de, Carvalho, M.B., Nussenzweig, R.S., Maracic, M., Ferreira, A.W. & Cochrane, A.H. (1986) Potential vectors of malaria and their different susceptibility to *Plasmodium falciparum and Plasmodium vivax* in northern Brazil identified by immunoassay. *American Journal of Tropical Medicine and Hygiene*, 35, 873-881.

Belkin. J.N. (1962) The mosquitoes of the South Pacific (Diptera, Culicidae). Vol. 1. University of California Press, Berkeley and Los Angeles,USA, 620pp.

Belkin, J.N., Schick, R.X. & Heinemann, S.J. (1971) Mosquito Studies (Diptera: Culicidae). XXV. Mosquitoes originally described from Brazil. *Contributions of the American Entomological Institute*, 7(5), 1-64.

Berti, J., Zimmerman, R. & Amarista, J. (1993) Spatial and temporal distribution of Anopheline larvae in two malarious areas in Sucre State, Venezuela. *Memórias do Instituto Oswaldo Cruz***,** 88**,** 353-362.

Bonne, C. (1923) Variability of *Anopheles tarsimaculata* Goeldi (Diptera: Culicidae). *Insecutor Inscitiae Menstruus*, 11, 127-128.

Bonne, C. (1924) Remarques sur les *Cellia* sud-americaines. *Bulletin de la Societe de Pathologie Exotique (Paris)*, 17, 132-137.

Branquinho, M.S., Lagos C.B.T., Rocha, M.R., Natal, D., Barata, J.M.S., Cochrane, A.H., Nardin, E., Nusserzweig, R.S. & Kloetzel, J.K. (1993) Anophelines in the State of Acre, Brazil, infected with *Plasmodium falciparum, P. vivax*, the variant *P. vivax* VK247 and *P. malariae*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 87, 391-394.

Branquinho, M.S., Araújo, M.S., Natal, D., Marrelli, M.T., Rocha, M.R., Taveira, F.A.L. & Kloetzel, J.K. (1996) *Anopheles oswaldoi* a potential malaria vector in Acre, Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 90, 233.

Causey, O.R., Deane, L.M., & Deane, M.P. (1946) Studies on Brazilian anophelines from the northeast and Amazon regions. II. An illustrated key by male genitalia characteristics for the identification of thirty-four species of Anophelini, with a note on dissection technique. *American Journal of Hygiene, Monograph Series*, 18, 21-31.

Chadee, D.D. & Beier, J.C. (1996) Diel oviposition and fecundity of *Anopheles oswaldoi* in Trinidad, West Indies. *Journal of the American Mosquito Control Association*, 12, 402-405.

Collins, F.H. & Paskewitz, S.M. (1996) A review of the use of ribosomal DNA (rDNA) to differentiate among cryptic *Anopheles* species. *Insect Molecular Biology*, 5, 1-9

Conn, J.E., Puertas, Y.R & Seawright, J.A. (1993) A new cytotype of *Anopheles nuneztovari* from western Venezuela and Colombia. *Journal of American Mosquito Control Association*, 9, 294-301.

Consoli, R. & Lourenço-de Oliveira, R. (1994) *Principais Mosquitos de Importância Sanitária no Brasil.* Ed. Fundação Oswaldo Cruz (Fiocruz), 225pp.

Curry, D.P. (1932) Some observations on the *Nyssorhynchus* group of the *Anopheles* (Culicidae) of Panama. *American Journal of Hygiene*, 15, 566-572.

Deane, L.M., Causey, O.R. & Deane, M.P. (1948) Distribuição e biologia dos anofelinos. *Revista do Serviço de Saúde Pública*, 1, 874 – 875.

Dyar, H.G. (1923) The mosquitoes of Panamá (Diptera: Culicidae). *Insecutor Inscitiae Menstruus*, 11,167-186.

Faran, M.E. (1980) Mosquito studies (Diptera: Culicidae) XXXIV. A revision of the Albimanus Section of the subgenus *Nyssorhynchus* of *Anopheles*. *Contributions of American Entomological Institute*, 15(7), 1-215.

Faran M.E. & Linthicum, K.J. (1981) A handbook of the Amazonian species of *Anopheles (Nyssorhynchus*) (Diptera: Culicidae). *Mosquito Systematics*, 13, 1-81.

Flores-Mendoza, C., Fernandez, R., Escobedo-Vargas, K.S., Vela-Perez, Q. & Schoeler, G.B. (2004a) Natural *Plasmodium* infections in *Anopheles darlingi* and *Anopheles benarrochi* (Diptera: Culicidae) from eastern Peru. *Journal of Medical Entomology,* 41, 489-494.

Flores-Mendoza, C., Peyton, E.L., Wilkerson, R.C. & Lourenço-de-Oliveira, R. (2004b) *Anopheles (Nyssorhynchus) konderi* Galvão and Damasceno: neotype designation and resurrection from synonymy with *Anopheles (Nyssorhynchus) oswaldoi* (Peryassú) (Diptera: Culicidae). *Proceedings of the Entomological Society of Washington*, 106, 118-132.

Fonseca, J.A.B. da, & Fonseca, F. da. (1942) Transmissão da malária humana por anofelíneos da série *tarsimaculatus*. *Memórias do Instituto Butantan*, 16, 93-124.

Forattini OP, Kakitani I, Massad E, Marucci D. (1993) Studies on mosquitoes (Diptera: Culicidae) and anthropic environment. 2 – Immature stages research at a rice irrigation system location in South-Eastern Brazil. Revista de Saúde Pública, 27, 227-236.

Galvão, A.L.A. & Lane, J. (1937) Notas sobre os *Nyssorhynchus* de S. Paulo. VII. Estudo sobre as variedades deste grupo com a descrição de *Anopheles (Nyssorhynchus) albitarsis* Arrib., 1878 var. *limai* n. var. *Annais da Universidade de São Paulo Faculdade de Medicina*, 13, 211-238.

Galvão, A.L.A. & Damasceno, R.G. (1942) *Anopheles (Nyssorhynchus) konderi* nova espécie de *Anopheles* do Vale do Amazonas e considerações sobre as espécies do complexo *tarsimaculatus* (Diptera: Culicidae). *Folia Clinica Biológica*, 14, 115-135.

Grillet, M. E. (2000) Factors associated with distribution of *Anopheles aquasalis* and *An. oswaldoi* (Diptera: Culicidae) in a malarious area, northeastern Venezuela. *Journal of Medical Entomology*, 37, 231-238.

Guimarães, A.E., Mello, R.P., Lopes, C.M. & Gentile, C. (2000) Ecology of mosquitoes (Diptera: Culicidae) in areas of Serra do Mar State Park, State of São Paulo, Brazil. II- Habitat distribution. *Memórias do Instituto Oswaldo Cruz*, 95, 17-28.

Guimarães, A.E., Gentile, C., Alencar, J., Lopes, C.M. & Mello, R.P. (2004) Ecology of Anopheline (Diptera, Culicidae) malaria vectors around the Serra da Mesa Reservoir, State of Goiás, Brazil. 1- Frequency and climatic factors. *Caderno de Saúde Pública* 20, 291-302.

Harbach, R.E., Knight, K.L. (1980). *Taxonomists' glossary of mosquito anatomy*. Plexus Publishing, Marlton, New Jersey, 415 pp.

Harbach, R.E. (2004) The classification of genus *Anopheles* (Diptera: Culicidae): a working hypothesis of phylogenetic relationships. *Bulletin of Entomological Research*, 94, 537-553.

Hayes, J., Calderon, G., Falcon, R. & Zambrano, V. (1987) Newly incriminated anopheline vectors of human malaria parasites in Junin Department, Peru. *Journal of the American Mosquito Control Association*, 3, 418-422.

Klein, T.A. & Lima, J.B. (1990) Seasonal distribution and biting patterns of *Anopheles* mosquitoes in Costa Marques, Rondônia, Brazil. *Journal of the American Mosquito Control Association*, 6, 700-707.

Klein, T.A., Lima, J.B.P. & Tada, M.S. (1991a) Comparative susceptibility of anopheline mosquitoes to *Plasmodium falciparum* in Rondônia, Brazil. *American Journal of Tropical Medicine and Hygiene*, 44, 598-603.

Klein, T.A., Lima, J.B.P., Tada, M.S. & Miller, R. (1991b) Comparative susceptibility of anopheline mosquitoes in Rondônia, Brazil to infection by *Plasmodium vivax*. *American Journal of Tropical Medicine and Hygiene*, 44, 598-603.

Kumar, S., Tamura, K. & Nei, M. (2004) MEGA 3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics*, 5, 150-163.

Lane, J. (1953) *Neotropical Culicidae*. Vol. 1. São Paulo, Universidade de São Paulo.

Laubach, H.E., Validium, L., Bonilla, J.A., Agar, A., Cummings, R., Mitchell, C., Cuadrado, R.R. & Palmer, C.J. (2001) Identification of *Anopheles aquasalis* as a possible vector of malaria in Guyana, South America. *West Indian Medical Journal,* 50, 319-321.

Lima, A.C. (1928) Sobre algumas anophelinas encontradas no Brasil. *Memórias do Instituto Oswaldo Cruz*, 3, 91-113.

Lopes, J. & Lozovei, A.L. (1995) Ecologia de mosquitos (Diptera: Culicidae) em criadouros naturais e artificiais de área rural do Norte do Estado do Paraná, Brasil. I – Coletas ao longo do leito de ribeirão. *Revista de Saúde Pública*, 29, 183-191.

Lopes, J., Zequi, J.A.C., Nunes, V., Oliveira, O., Neto, B.P.O. & Rodrigues, W. (2002) Immature Culicidae (Diptera) collected from the Igapó Lake located in the urban area of Londrina, Paraná, Brazil. *Brazilian Archives of Biology and Technology*, 45, 465-471.

Lounibos, L.P. & Conn, J.E. (2000) Malaria vector heterogeneity in South America. *American Entomologist*, 46, 238-249.

Lounibos, L.P., Duzak, D. & Linley, J.R. (1997). Comparative egg morphology of six species of the Albimanus Section of *Anopheles* (*Nyssorhynchus*) (Diptera: Culicidae). *Entomological Society of America*, 34, 136-155.

Lourenço-de-Oliveira, R., Guimarães, A.E., Arlé, M., Silva, T.F., Castro, M.G., Motta, M.A. & Deane, L.M. (1989) Anophelines species, some of their habits and relation to malaria endemic areas of Rondônia State, Amazon Region of Brazil. *Memórias do Instituto Oswaldo Cruz*, 84, 501-514.

Lourenço-de-Oliveira R. & Luz, S.L.B. (1996) Simian malaria at two sites in the Brazilian Amazon – II. Vertical distribution and frequency of Anopheline species inside and outside the forest. *Memórias do Instituto Oswaldo Cruz*, 91, 687-694.

Maldonado, V., Finol, H.J. & Navarro, J.C. (1997) *Anopheles aquasalis* eggs from two Venezuelan localities compared by scanning electron microscopy. *Memórias do Instituto Oswaldo Cruz*, 92, 487-491.

Marrelli, M.T., Branquinho, M.S., Hoffmann, E.H.E., Taipe-Lagos, C.B., Natal, D. & Kloetzel, J.K. (1998) Correlation between positive serology for *Plasmodium vivax*-like/*Plamodium simiovale* malaria parasites in the human and anopheline populations in the State of Acre, Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 92, 149-151.

Marrelli, M.T., Honório, N.A., Flores-Mendoza, C., Lourenço-de-Oliveira, R., Marinotti, O. & Kloetzel, J.K. (1999a) Comparative susceptibility of two members of the *Anopheles oswaldoi* complex, *An. oswaldoi* and *An. konderi*, to infection by *Plasmodium vivax*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 93, 381-384.

Marrelli, M.T., Malafronte, R.S., Flores-Mendoza, C., Lourenço-de-Oliveira, R., Kloetzel, J.K. & Marinotti, O. (1999b) Sequence analysis of the second internal

transcribed spacer of ribosomal DNA in *Anopheles oswaldoi* (Diptera: Culicidae). *Journal of Medical Entomology*, 36, 679-684.

Marrelli, M.T., Sallum, M.A.M. & Marinotti, O. (2006) The second internal transcribed spacer of nuclear ribosomal DNA as a tool for Latin American anopheline taxonomy – a critical review. *Memórias do Instituto Oswaldo Cruz,* 101, 817-832.

Natal, D., Barata, J.M.S., Taipe-Lagos, C.B. & Rocha, R.M. (1992) Nota sobre culicídeos (Diptera: Culicidae) da bacia do rio Purus, Acre, Amazônia (Brasil). *Revista de Saúde Publica*, 26, 129-131.

Naves, H.A.M., Carvalho, M.E.S.D., Carneiro, E. & Sales, K.P. (1996) Espécies de Culicidae de Goiânia–Goiás-Brasil. *Revista de Patologia Tropical*, 25, 31-42.

Oliveira-Ferreira, J. de, Lourenço-de-Oliveira, R., Teva, A. & Deane, L.M. (1990) Natural malaria infections in anophelines in Rondônia State, Brazilian Amazon. *American Journal of Tropical Medicine and Hygiene*, 43, 6-10.

Perera, O.P., Cockburn, A.F., Mitchell, S.E., Conn, J. & Seawright, J.A. (1998) Species-specific repeat units in the intergenic spacer of the ribosomal RNA cistron of *Anopheles aquasalis* Curry. *American Journal of Tropical Medicine and Hygiene*, 59, 673-678.

Peryassú, A. (1922) Duas novas espécies de mosquitos do Brasil. *A folha médica*, 3, 179.

Peyton, E.L., Roberts, D.R., Pinheiro, F.P., Vargas, R. & Balderama, F. (1983) Mosquito collections from a remote unstudied area of southeastern Bolivia. *Mosquito Systematics*, 15, 61-89.

Póvoa, M.M., Wirtz, R.A., Lacerda, R.N.L., Miles, M.A. & Warhurst, D. (2001) Malaria vectors in Municipality of Serra do Navio, State of Amapá, Amazon Region, Brazil. *Memórias do Instituto Oswaldo Cruz*, 96, 179-184.

Póvoa, M.M., Conn, J.E., Schlichting, C.D., Amaral, J.C., Segura, M.N., Silva A.N. da, Santos C. C. dos, Lacerda R.N., Souza R.T. de, Galiza, D., Santa Rosa, E.P. & Wirtz, R.A. (2003). Malaria vectors, epidemiology, and the re-emergence of *Anopheles darlingi* in Belém, Pará, Brazil. *Journal of Medical Entomology*, 40, 379-386.

Póvoa, M.M., Souza, R.T.L., Lacerda, R.N.L., Rosa, E.S., Galiza, D., Souza, J.R., Wirtz, R.A., Schlichting, C.D. & Conn, J.E. (2006) The importance of *Anopheles albitarsis* E and *Anopheles darlingi* in human malaria transmission in Boa Vista, state of Roraima, Brazil. *Memórias do Instituto Oswaldo Cruz*, 101, 163-168.

Quiñones, M.L. & Suarez, M.F. (1990) Indoor resting heights of some anophelines in Colombia. *Journal of the American Mosquito Association*, 6, 602-604.

Quiñones, M.L., Ruiz, F., Calle, D.A., Harbach, R.E., Erazo, H.F. & Linton, Y.-M. (2006) Incrimination of *Anopheles* (*Nyssorhynchus*) *rangeli* and *An.* (*Nys.) oswaldoi* as natural vectors of *Plasmodium vivax* in southern Colombia. *Memórias do Instituto Oswaldo Cruz*, 101, 617-623.

Rambajanl, I. (1987) An annotated checklist of the *Anopheles* of Guyana, South America. *Mosquito Systematics*, 19, 146-161.

Rebêlo, J.M.M., Silva, A.R., Ferreira, L.A. & Vieira, J.A. (1997) *Anopheles* (Culicidae, Anophelinae) e a malária em Buricutu – Santa Luzia, Pré-Amazônia Maranhense. *Revista da Sociedade Brasileira de Medicina Tropical*, 30, 107-111.

Root, F.M. (1924) Further notes on the male genitalia of American *Anopheles*. *American Journal of Hygiene*, 4, 456-465.

Rosas-Freitas, M. G., Lourenço-de-Oliveira, R., Carvalho-Pinto, C.J., Flores-Mendoza, C. & Silva-do-Nascimento, T.F. (1998) Anopheline species complexes in Brazil.

Current knowledge of those related to malaria transmission. *Memórias do Oswaldo Cruz*, 93, 651-655.

Rozeboom, L.E. (1942) Subspecific variations among Neotropical *Anopheles* mosquitoes, and their importance in the transmission of malaria. *American Journal of Tropical Medicine*, 22, 235-255.

Rubio-Palis, Y. & Curtis, C.F. (1992) Biting and resting behaviour of anophelines in western Venezuela and implications for control of malaria transmission. *Medical and Veterinary Entomology*, 6, 325-334.

Rubio-Palis, Y., Curtis, C.F., Gonzáles, C. & Wirtz, R.A. (1994) Host choice of anopheline mosquitoes in a malaria endemic area of western Venezuela. *Medical and Veterinary Entomology*, 8, 275-280.

Ruiz, F., Quiñones, M.L., Erazo, H.F., Calle, D.A., Alzate, J.F. & Linton, Y.-M. (2005) Molecular differentiation of *Anopheles (Nyssorhynchus*) *benarrochi* and *Anopheles (Nys.) oswaldoi* from southern Colombia. *Memórias do Instituto Oswaldo Cruz*, 100, 155-160.

San Sebastián, M., Jativa, R. & Goicolea, I. (2000) Epidemiology of malaria in the Amazon basin of Ecuador. *Revista Panamericana de Salud Pública*, 7, 24-28.

Santos, R.L.C., Sucupira, I.M.C., Lacerda, R.N.L., Fayal, A.S. & Póvoa, M.M. (2005) Inquérito entomológico e infectividade durante epidemia de malária no município de Anajás, Estado do Pará. *Revista Brasileira de Medicina Tropical*, 38, 202-204.

Scarpassa, V.M. (2005) Isozyme similarity in *Anopheles oswaldoi sensu lato* (Diptera; Culicidae) from the Amazon region. *Journal of Medical Entomology*, 42, 319-326.

Scarpassa, V.M. & Conn, J.E. (2006) Molecular differentiation in natural populations of *Anopheles oswaldoi sensu lato* (Diptera: Culicidae) from the Brazilian Amazon,

using sequences of the COI gene from mitochondrial DNA. *Genetics and Molecular Research*, 5, 493-502.

Senevet, G. & Abonnenc, E. (1938) Quelques anophelines de la Guyane Francaise. *Archives of the Institute Pasteur Algeria*, 16, 486-512.

Sierra, D.M., Velez, I.D. & Linton, Y.-M. (2004) The malaria vector *Anopheles* (*Nyssorhynchus*) *nuneztovari* Gabaldón comprises one genetic species in Colombia based on homogeneity of nuclear ITS2 rDNA. *Journal of Medical Entomology,* 41, 302-307.

Silva, A.N.M. da, Fraiha-Neto, H., Santos, C.C.B. dos, Segura, M.N.O., Amaral, J.C.O.F., Gorayeb, I.S., Lacerda, R.N.L., Sucupira, I.M.C., Pimentel, L.N., Conn, J.E. & Póvoa, M.M. (2006) Fauna anofélica da cidade de Belém, Pará, Brasil: dados atuais e retrospectivos. *Caderno de Saúde Publica*, 22, 1575-1585.

Simmons, J.S. (1979) Malaria in Panama. www.books.google.com

Steiner, W.W.M., Narang, S., Kitzmiller, J.B. & Swofford, D.L. (1982) Genetic diversity and evolution in Neotropical *Anopheles* (subgenus *Nyssorhynchus*). In: W.W.M. Steiner, W.J. Tabachnick, K.S. Rai & S.K. Narang (eds), *Recent Developments in the Genetics of Insect Disease Vectors*, Stipes, Champaign, Illinois, p. 523-550.

Tadei, W.P., Santos, J.M.M., Costa, W.L.S. & Scarpassa, V.M. (1988) Biologia de anofelinos amazônicos. XII. Ocorrência de espécies de *Anopheles*, dinâmica na transmissão e controle da malária na zona urbana de Ariquemes (Rondônia I). *Revista do Instituto de Medicina Tropical de São Paulo,* 30, 221-251.

Tadei, W.P., Thatcher, B.D., Santos, J.M.M., Scarpassa, V.M., Rodrigues, I.B. & Rafal, M.S. (1998) Ecologica observations on Anopheline vectors of malaria in the

Brazilian Amazon. *American Journal of Tropical Medicine and Hygiene*, 59, 325-335.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 24, 4876-4882.

Wilkerson, R.C. & Peyton, E.L. (1990) Standardized nomenclature for the costal wing spots of the genus *Anopheles* and other spotted-wing mosquitoes (Diptera: Culicidae). *Journal of Medical Entomology*, 27, 207-224.

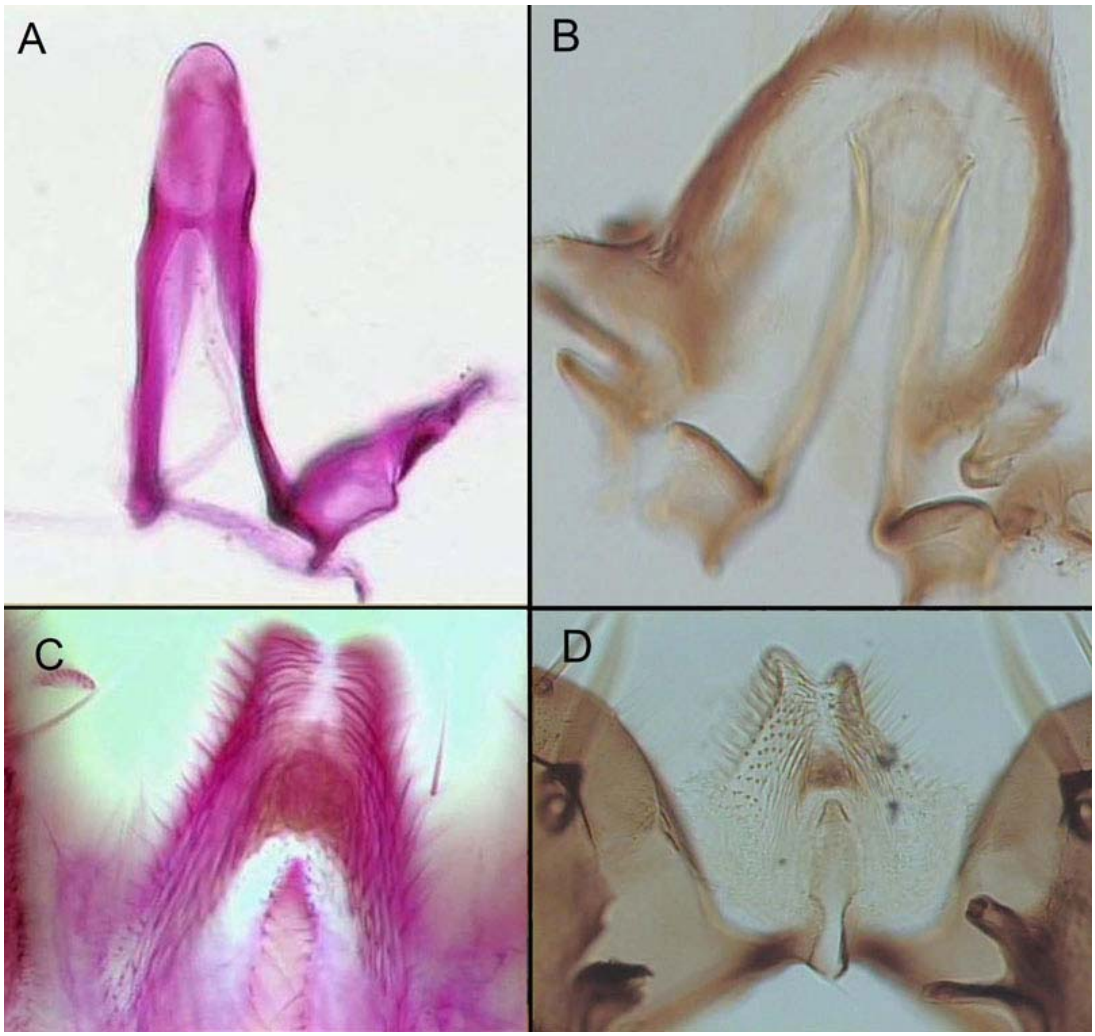

Figure 1. Photographs depicting the morphological differences between the male genitalia of *Anopheles oswaldoi* and *An. konderi*. A: Aedeagus of the lectotype of *An. oswaldoi*; **B:** Aedeagus of *An. konderi* (from Coari, State of Amazonas, Brazil); **C:** Ventral claspette of the lectotype of *An. oswaldoi*; **D:** Ventral claspette of *An. konderi* (from Coari, State of Amazonas, Brazil).

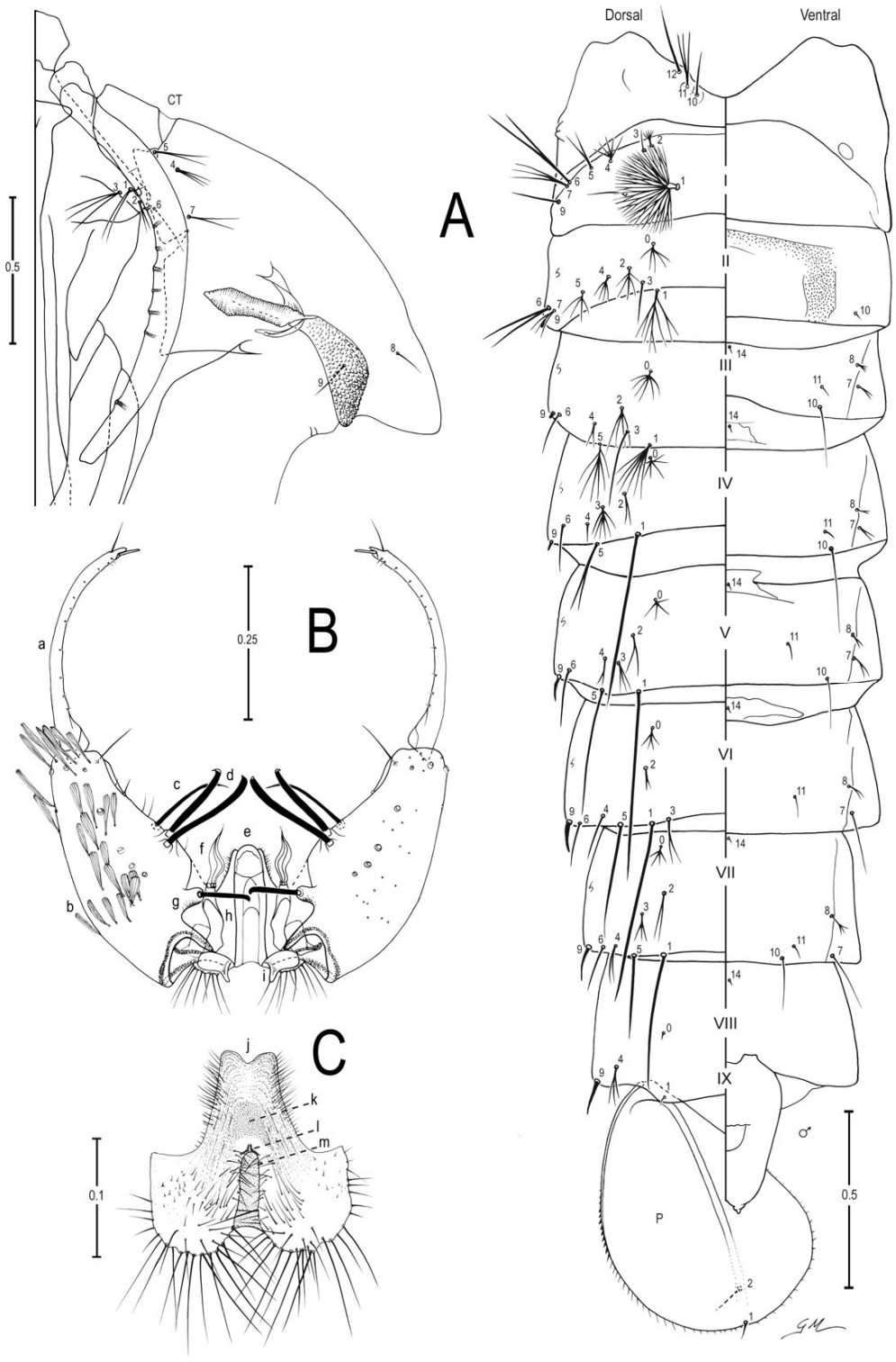

Figure 2. Pupa and male genitalia of *Anopheles oswaldoi*. A: Pupa - CT: cephalothorax; P: paddle; I-IX: abdominal segments. **B**: **Male genitalia -** a: gonostylus; b: gonocoxite; c: internal seta; d: accessory setae; e: aedeagus; f: dorsal claspette; g: tubercle of parabasal spine; h: ventral claspette; i: parameter. **C**: **Ventral claspette –** ventral view, j: median sulcus; k: preapical plate; l: refringent structure; m: mesal cleft. Scales in mm.

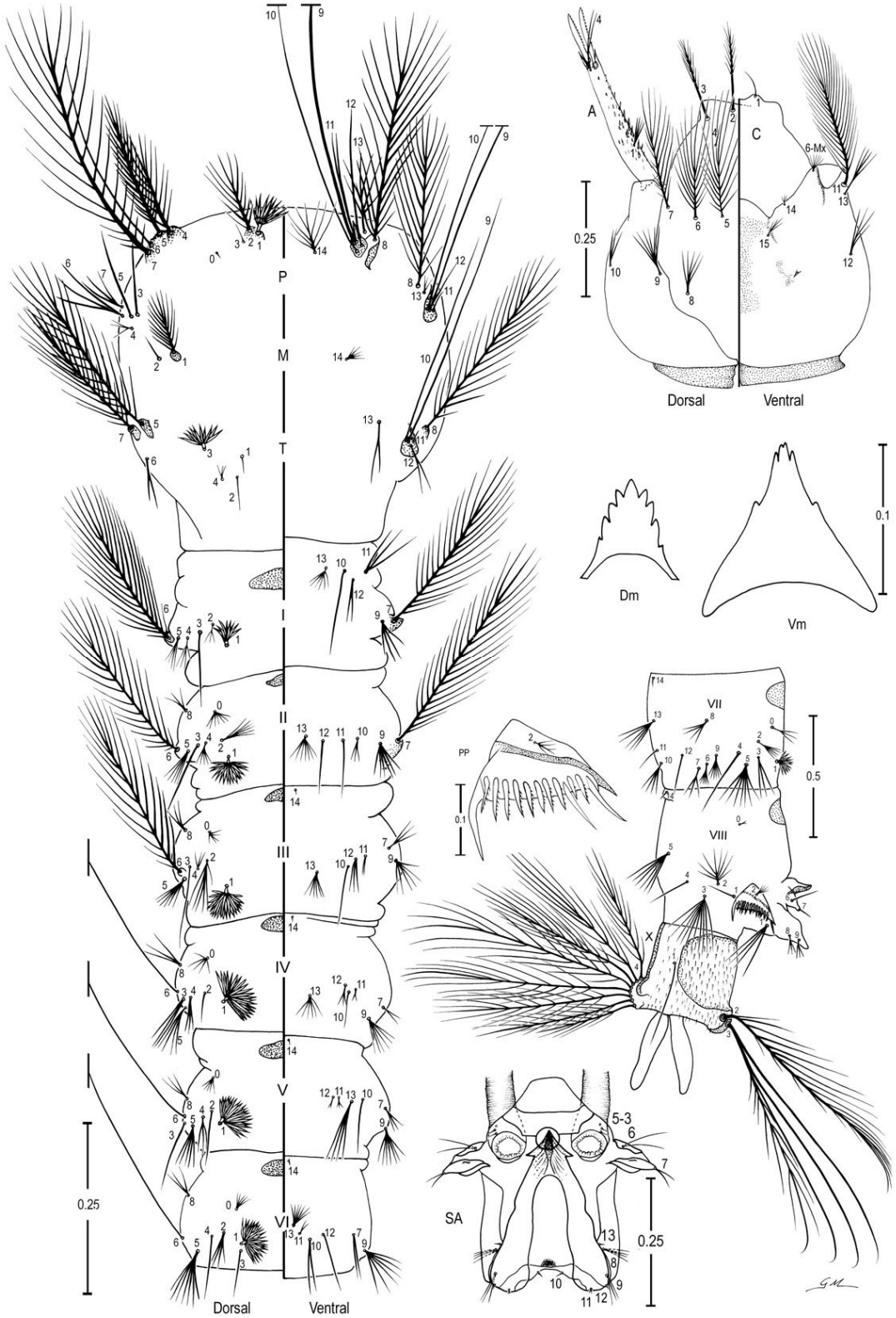

Figure 3. Fourth-instar larva of *Anopheles oswaldoi*. A: antenna; **C**: cranium; **Dm**: dorsomentum; **M**: mesothorax; **P**: prothorax; **PP**: pecten plate; **SA**: spiracular apparatus; **T**: metathorax; **Vm**: ventromentum; **I-VIII**: abdominal segments; **X,** anal lobe. Scales in mm.

Figure 4. A 486 bp ITS2 sequence alignment of *An. oswaldoi* and the next nearest taxon in GenBank, *An. oswaldoi s.l.* from Rondônia, Brazil (AF055069, Marrelli *et al*. 1999b). Only the differing bases are shown. (-) indicates indel events.

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2233333444
             6811155011
             0223545556
OSWALDOI  AA--C--A--
AF055069  TCCCTCGGGC
```

Table 1. Wing spot measurements (in mm) for adult male and female *Anopheles oswaldoi* collected in Jaguaré municipality, Vale do Rio Doce, State of Espírito Santo (ES), and Vale do Ribeira, Mata Atlântica, southern State of São Paulo (SP), Brazil.

Wing spot	Female								Male					
	Range		Mean		SD(±)		n=		Range		Mean	SD(±)	n=	
	(ES)	(SP)	(ES)	(SP)	(ES)	(SP)	(ES)	(SP)	(ES)	(SP)	(ES)	(ES)	(ES)	(SP)
Basal pale + prehumeral pale	0.19-0.25	0.14-0.26	0.22	0.21	0.02	0.04	4	5	0.20-0.21	0.27	0.21	0.01	3	1
Prehumeral dark	0.05-0.11	0.06-0.12	0.08	0.08	0.03	0.03	4	5	0.05-0.08	0.10	0.07	0.02	3	1
Humeral pale	0.21-0.25	0.18-0.27	0.24	0.23	0.02	0.04	4	5	0.24-0.27	0.16	0.25	0.01	3	1
Humeral dark	0.09-0.12	0.06-0.14	0.10	0.12	0.01	0.03	4	5	0.07-0.12	0.37	0.10	0.03	3	1
Presector pale	0.10-0.13	0.11-0.17	0.10	0.12	0.01	0.02	4	5	0.11-0.14	0.11	0.13	0.02	3	1
Presector dark	0.38-0.49	0.36-0.46	0.44	0.39	0.05	0.04	4	3	0.47-0.50	0.09	0.48	0.02	3	1
Sector pale	0.06	0.08-0.28	-	0.15	-	0.11	1	3	0.06-0.07	0.18	0.06	0.00	3	1
Proximal sector pale	0.09	0.09-0.10	-	0.09	-	0.01	1	2	0.05-0.10	0.56	0.08	0.03	3	1
Accessory sector pale	0.10	0.13	-	0.13	-	-	1	2	0.12-0.16	-	0.14	0.02	3	-
Distal sector dark	0.75-0.84	0.71-0.85	0.79	0.78	0.04	0.07	4	5	0.59-0.60	0.40	0.59	0.01	3	1
Subcostal pale	0.13-0.24	0.21-0.36	0.17	0.30	0.05	0.06	4	5	0.30-0.32	0.58	0.31	0.01	3	1
Preapical dark	0.73-0.82	0.69-0.83	0.78	0.75	0.04	0.06	4	5	0.56-0.61	0.58	0.58	0.03	3	1
Preapical pale	0.24-0.27	0.22-0.35	0.25	0.75	0.01	0.06	4	5	0.25-0.28	0.29	0.26	0.01	3	1
Apical dark	0.12-0.13	0.09-0.13	0.12	0.11	0.01	0.01	4	5	0.10-0.11	0.07	0.11	0.00	3	1

Table 2. Number and range (mode) of setal branches of the pupa of *Anopheles oswaldoi*. (n=17, ES; n=3, SP) (n.c.=not counted).

Seta	Cephalothorax	Abdominal Segments									Paddle
No.	CT	I	II	III	IV	V	VI	VII	VIII	IX	P
0	-	-	3 - 8 (5)	4 - 9 (6)	4 - 9 (6)	3 - 7 (4)	3 - 5 (4)	2 - 5 (4)	1 - 2 (1)	-	-
1	1 - 3 (2)	n.c.	5 - 15 (8)	4 - 10 (6)	1	1	1	1	-	1	1
2	1 - 3 (2)	3 - 9 (5)	4 - 12 (5)	4 - 8 (4)	1 - 4 (2)	1 - 4 (2)	1 - 3 (2)	1 - 3 (2)	-	-	1 - 4 (2)
3	2 - 4 (3)	1	1	1	2 - 8 (6)	1 - 4 (3)	1 - 3 (2)	2 - 5 (3)	-	-	-
4	1 - 5 (3)	3 - 7 (5)	1 - 6 (4)	1 - 7 (2)	1 - 4 (1)	1 - 4 (2)	1 - 3 (1)	1 - 3 (2)	1 - 5 (3)	-	-
5	1 - 3 (2)	1 - 4 (2)	2 - 7 (4)	3 - 8 (6)	1 - 4 (2)	1 - 2 (1)	1 - 2 (1)	1	-	-	-
6	1 - 4 (1)	1 - 2 (2)	1 - 4 (2)	1 - 4 (1)	1 - 2 (1)	1 - 2 (1)	1 - 2 (1)	1 - 3 (1)	-	-	-
7	1 - 3 (2)	2 - 5 (3)	2 - 5 (3)	1 - 4 (2)	1 - 5 (2)	1 - 3 (2)	1	1	-	-	-
8	1	-	-	1 - 5 (3)	1 - 4 (2)	1 - 3 (2)	1 - 3 (2)	2 - 5 (3)	-	-	-
9	1 - 3 (2)	1 - 2 (1)	1	1	1	1	1	1	1	-	-
10	1 - 2 (1)	-	1	1 - 3 (1)	1	1	-	1 - 2 (1)	-	-	-
11	2 - 7 (3)	-	-	1 - 4 (1)	1 - 3 (1)	1 - 3 (1)	1 - 2 (1)	1 - 2 (1)	-	-	-
12	1 - 4 (2)	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	1	1	1	1	1	1	-	-

Table 3. Number and range (mode) of setal branches of the fourth-instar larva of *Anopheles oswaldoi* (n=18, ES; n=2, SP) (n. c.=not counted).

	Head	Thorax			Abdominal segments							
No	C	P	M	T	I	II	III	IV	V	VI	VII	VIII	X
0	1	n. c.	-	-	-	5-10 (7)	3-11 (5)	3-8 (5)	3-7 (5)	3-8 (5)	2-5 (3)	2-3 (2)	-
1	1	9-15 (10)	22-35 (27)	1	8-16 (12)	17-27 (24)	20-29 (26)	21-29 (26)	22-31 (27)	21-28 (24)	19-27 (20)	1	1
2	9-15 (12)	11-21 (16)	1-4 (1)	1-2 (1)	1-6 (3)	3-10 (6)	3-8 (6)	1	1-3 (1)	1-5 (4)	3-9 (5)	4-12 (5)	14-19 (17)
3	9-14 (10)	1	1	9-15 (10)	1-3 (1)	1	1-2 (1)	2-4 (3)	1-2 (1)	1	2-3 (3)	4-14 (10)	6-12 (9)
4	2-8 (5)	14-20 (19)	2-5 (3)	1-4 (3)	2-7 (4)	3-8 (5)	2-5 (3)	2-4 (3)	2-4 (3)	1	1-2 (1)	1-5 (1)	8*
5	13-19 (15)	26-37 (29)	1	28-37 (32)	3-4 (3)	4-8 (5)	4-10 (6)	3-9 (4)	3-8 (5)	3-9 (7)	5-10 (7)	4-9 (6)	-
6	10-18 (14)	1	2-3 (2)	1-3 (2)	26-36 (31)	26-39 (34)	20-30 (23)	1	1	1	4-9 (5)	1-S	4-8 (5)
7	13-23 (17)	21-35 (31)	2-4 (3)	24-38 (29)	23-33 (28)	26-35 (28)	1-4 (3)	2-4 (2)	2-4 (3)	2-3 (2)	3-8 (5)	2-S	3-8 (6)
8	4-7 (5)	23-33 (30)	20-29 (23)	25-35 (30)	-	2-4 (3)	2-6 (3)	2-4 (3)	2-4 (3)	2-4 (3)	2-6 (4)	6-S	1-2 (1)
9	3-8 (6)	1	1	1	3-5 (4)	3-9 (6)	5-10 (6)	4-10 (6)	4-9 (7)	4-9 (6)	5-9 (6)	7-S	1
10	1-3 (3)	1	1	1	1	2-5 (3)	1-2 (1)	1-2 (1)	1	1-3 (2)	1-6 (4)	8-S	2-5 (3)
11	n.c.	1-3 (2)	1-2 (1)	n. c.	1-4 (3)	1-2 (1)	1-3 (3)	1-4 (2)	1-3 (3)	1-3 (2)	1-3 (1)	9-S	2-5 (3)
12	2-6 (4)	1	1	1-2 (2)	1-3 (2)	1	1-4 (3)	1-4 (3)	2-3 (2)	1	1	-	-
13	2-6 (4)	3-6 (5)	3-9 (5)	2-3 (2)	4-10 (6)	6-14 (7)	5-11 (10)	4-11 (6)	3-6 (4)	4-11 (8)	3-5 (3)	-	-
14	1-3 (2)	7-11 (8)	6-13 (9)	-	-	-	1	1	1	1	1	1	-
15	2-5 (3)	-	-	-	-	-	-	-	-	-	-	-	-

*8 pairs

Table 4. Distribution of *Anopheles oswaldoi s.l.* in Brazil based on published literature. References in bold implicate *An. oswaldoi s.l.* involved in human malaria transmission in these regions.

State of Brazil	**References**
Acre (AC)	Deane *et al*., 1948; Natal *et al*., 1992; **Branquinho *et al*., 1993**; **Branquinho *et al*., 1996**; Marrelli *et al*., 1998; **Marrelli *et al*., 1999a;** Marrelli *et al*., 1999b; Scarpassa, 2005; Scarpassa & Conn, 2006
Amapá (AP)	Deane *et al.,* 1948; Steiner *et al*., 1982; Marrelli *et al*., 1999b; Póvoa *et al*., 2001
Amazonas (AM)	Deane *et al*., 1948; Arruda *et al*., 1986; **Lourenço-de-Oliveira & Luz, 1996**; Marrelli *et al*., 1999b; Tadei *et al*., 1998; Póvoa *et al*., 2001; Scarpassa, 2005; Scarpassa & Conn, 2006
Espírito Santo (ES)	Peryassú, 1922; Marrelli *et al*., 1999b, herein
Goiás (GO)	Naves *et al*., 1996; Guimarães *et al*., 2004
Maranhão (MA)	Deane *et al.,* 1948; Rebêlo *et al*., 1997
Pará (PA)	Deane *et al*., 1948**; Arruda *et al*., 1986;** Tadei *et al*., 1998; Póvoa *et al*., 2003; **Santos *et al*., 2005**; Silva *et al*., 2006; Scarpassa & Conn, 2006
Paraná (PR)	Lopes & Lozovei, 1995; Lopes *et al*., 2002
Piauí (PI)	Deane *et al*., 1948
Rio de Janiero (RJ)	Peryassú, 1922
Rondônia (RO)	Deane *et al.,* 1948; Tadei *et al*., 1988; Lourenço-de-Oliveira *et al*., 1989; **Oliveira-Ferreira *et al*., 1990**; Klein & Lima, 1990; **Klein *et al*., 1991a; Klein *et al*., 1991b**; Perera *et al*., 1998; Tadei *et al*., 1998; Marrelli *et al*., 1999b; **Marrelli *et al*., 1999a**; Scarpassa, 2005; Scarpassa & Conn, 2006
Roraima (RR)	Deane *et al.,* 1948; Póvoa *et al*., 2006
São Paulo (SP)	**Fonseca & Fonseca, 1942**; Forattini *et al.*, 1993; Guimarães *et al.,* 2000; herein

*The underlined references are cited for vector competence.

4.1 MANUSCRITO 2

“Revision of five species of the *Anopheles albitarsis* complex (Diptera: Culicidae)”

(a ser submetido para publicação na revista Cannadian Entomologist)

Revision of five species of the *Anopheles albitarsis* complex (Diptera: Culicidae)

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Abstract

The *Anopheles albitarsis* Complex includes *An. albitarsis s.s., An. albitarsis* B, *An. marajoara, An. deaneorum* and recently *An. albitarsis* E and *An. albitarsis* F. Except for *An. deaneorum*, the remaining species of the complex are indistinguishable when using only morphology. The problematic distinction among species of the complex have caused some difficult to the knowledge of the adaptative process of *An. albitarsis s.l.* to human environment. Consequently, elucidation of the vector status of members of the *An. albitarsis* complex in the transmission of human *Plasmodium* is not clear in the southeastern of the São Paulo State. With the aim to clarify the taxonomy of the species, morphological redescriptions of three species and description of two species were made using individuals from populations collected in several localities in Brazil, Paraguay, Argentina and Venezuela. Also comparative morphologic and morphometry analysis were made. The study included morphologic characters of all life stages of *An. albitarsis s.s.*, *An. marajoara*, *An. deaneorum* and *An. albitarsis* B using morphological characters of the adult female, male and male genitalia, the fourth-instar larva and pupa, and adult stage of *An. albitarsis* E using morphological characters of the adult female, male and male genitalia. The larva, pupa, and male genitalia are illustrated. Details of bionomics and distribution data are given based on the published literature.

Keywords: *Anopheles albitarsis* complex, morphology, redescription, description, multivariate analysis, *Nyssorhynchus*.

Introduction

Two species of the *An. albitarsis* complex are of great epidemiological importance as competent vectors of human malaria parasites in Brazil, *An. deaneorum* and *An. marajoara* (Klein *et al*., 1991 c, d; Conn *et al*., 2002). Recently, *An. albitarsis* E was considered important in human malaria transmission in Boa Vista, State of Roraima, Brazil (Póvoa *et al*., 2006).

Because of its medical importance, *An. albitarsis s.l.* has been the subject of several epidemiologic, ecologic, morphologic, cytogenetic, biochemistry and molecular studies aimed at facilitating the identification of species as well as the confirmation of the vector status of its representative (Galvão & Damasceno, 1944; Kreutzer *et al.*, 1976; Kitzmiller, 1977; Linthicum, 1988; Rosa-Freitas, 1989; Rosa-Freitas & Deane, 1989; Klein *et al*., 1991a, b; Narang *et al*., 1993; Wilkerson *et al*., 1995a, b; Póvoa *et al*., 2001; Conn *et al*., 2002). Results of these studies were suggestive that *An. albitarsis s.l.* may include at least 4 cryptic species. Based on morphological and biological differences, three subspecies were described, *An. albitarsis limai* (Galvão & Lane, 1937) and *An. albitarsis imperfectus* (Corrêa & Ramos, 1943), transferred to synonymy with *An. albitarsis* and *An. albitarsis domesticus* (Galvão & Damasceno, 1944), synonymyzed under *An. marajoara*.

Cytological studies using polytene chromosome of *An. albitarsis* collected in Brazil, Colombia and Venezuela indicated that at least three chromosomally differentiated populations of this species exist in these areas, two in southern and eastern Brazil, a third in Colombia and Venezuela. These populations are indistinguishable using conventional taxonomic methods, although they are clearly distinct cytologically. These populations could be diferenciated by X chromosome.

Two populations from Brazil are sympatric, population B_1 presents one heterozygous inversion in the X and two in the autosomes, whereas population B_2 differs from B_1 by possessing two inversions in the X and ten in the autosomes; population C from Colombia and Venezuela is closer to B_1, from which differs by three inversions in chromosome 2 and three in chromosome 3 (Kreutzer *et al*., 1976; Kitzmiller, 1977). Following, in 1982, populations of *An. albitarsis* collected from São Paulo to west of Marajó Island were employed in isozyme and chromosome analyses, and thus, were found three genetically distinct populations (Steiner *et al.,* 1982).

Linthicum (1988) considered two taxa within the subgroup *An. albitarsis* and pointed out morphological characters for distinction between *An. marajoara* and *An. albitarsis*. However, separation of those two species continued to be problematic. Rosa-Freitas and Deane (1989) designated the neotype and redescribed the adult male and female, fourth-instar larva and pupa for *An. albitarsis*. Later, Rosa-Freitas (1989) defined *An. deaneorum* based on morphological characters of the egg, adult male and female, fourth-instar larva and pupa. Additionally, Rosa-Freitas used the electrophoretic pattern of 11 enzyme loci to distinguish among populations of *An. albitarsis* from Baradero, Argentina and *An. deaneorum*, showing that they could be distinguished by the allele frequencies. Narang *et al*. (1993) employed the isozymic pattern and Restriction Fragment Length Polymorphisms (RFLP) of the mitochondrial genome to examine genetic diversity among sympatric populations of *An. deaneorum* and *An. marajoara* from several localities in Brazil. Consequently, was identified a complex named *An. marajoara* which included at least two cryptic forms. Finally, Wilkerson *et al*. (1995a, b) used Random Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) based method to confirm the existence of four species within the *An. albitarsis* complex, *An. albitarsis*, *An. deaneorum*, *An. marajoara* and a new species

designated as *An. albitarsis* B (herein). As a result, morphological, biological and genetic diversity observed among populations of *An. albitarsis s.l.* was partially explained by the existence of a complex of cryptic species. Lehr *et al*. (2005) based on the results of phylogenetic analyses of the mitochondrial DNA cytochrome c oxidase subunit I (mtDNA COI) sequences proposed a fifth species in the complex designated *An. albitarsis* E (herein). Recently, Brochero *et al.* (2007) analyzing ITS2 from *An. marajoara* collected in Colombia recognized a new specie designated species F. Merritt *et al.* (2005) corroborated Lehr *et al*.'s result by employing a fragment of the nuclear white gene. Contrastingly, Wilkerson *et al.* (2005) based on partial sequences of two mitochondrial genes (COI and NADH dehydrogenase 4 (ND4), and two ribosomal DNA fragments, nucleotide sequences of the internal transcribed spacer 2 (rDNA ITS2) and D2 expansion of the 28S subunit (D2) using some of the same analyses could not identify species E. Either, Li & Wilkerson (2005) observed intragenomic variability in the ITS2 of four species of *An. albitarsis* complex, and was not possible to identify the existence of *An. albitarsis* E, as hyphothesized in Lehr's *et al*. study.

Marrelli *et al*. (2006) analyzing ITS2 nucleotide sequences deposited in the GenBank verified large intraespecific variation in the ITS2 sequences of *An. marajoara*, *An. deaneorum* and *An. albitarsis*. This pattern of variation could indicate a recent process of speciation within this group, or could represent the misidentification of the specimens that had their DNA extracted and sequenced.

Several studies by Forattini *et al*. (1995, 1996), Forattini & Massad (1998), Santos & Forattini (1999), Santos *et al*. (2002) show well that the problematic distinction among species of the *An. albitarsis* complex is difficulting the knowledgment of the adaptative process of *An. albitarsis s.l.* to human environment. Consequently, elucidation of the vector status of members of the *An. albitarsis* complex in the

transmission of human *Plasmodium* is not clear in the southeastern of the São Paulo State.

Taxonomic problems have been solved by multivariate morphometry; this method has been shown to be useful for the distinction of medically important insects that present problems in their identification (Añez *et al*., 1997; Gebre-Michael & Medhin, 1997; Petrarca *et. al.,* 1998). Morphometry have been used in *Anopheles* of the subgenus *Nyssorhynchus* in Venezuela to separated different species i.e. *An. darlingi, An. marajoara, An. braziliensis* and *An. argyritarsis* (Rubio-Palis *et al*., 1997; Rubio-Palis, 1998), either in Colombia to discrimination of adults females of differences species i.e. *An. rangeli, An. oswaldoi, An. benarrochi*, *An. triannulatus* and *An. nuneztovari* (Calle *et al*., 2002). In this study to help taxonomic problems, principal components analysis, discriminant analysis, and cluster analysis were used.

Morphological characters were employed to search for those characters that define each species. The present study aims: to redescribe all life stages of *An. albitarsis s.s.*, *An. marajoara* and *An. deaneorum,* to describe all life stages of *An. albitarsis* B and adult stage of *An. albitarsis* E to characterize the five species of the *An. albitarsis* complex and also, to define morphological characters to separate all five species of the *An. albitarsis* complex .

Materials and methods

Eggs were collected from females and used to obtain individually reared adults with associated pupal and fourth-instar larval exuviae as reported by Sallum *et al*. (2002). Adult females were collected in several localities in Brazil, Argentina, Paraguay and Venezuela (see list of Material Examined in the Table 1, Figure 1). Most of the

specimens used for the present study were from isofemale progeny broods. At least one adult male or female of each progeny was used to identify the species using RAPD-PCR methods as reported by Wilkerson *et al*. (1995b). After the RAPD-PCR identification, specimens from each taxon collected throughout the species known geographic range were examined to search for characters, which might be consistent to distinguish specimens from several localities. Also, specimens from each progeny were employed for species morphological description, and illustrations.

In addition, distance between eyes and thirty nine wing spots characters were measured in thirty specimens of each species.

Statistical analysis. . Pattern of pale and dark spots on Costa vein was observed in 150 specimens of five species of *An. albitarsis* complex, i.e., 30 specimens of *An. albitarsis* s.s., *An. albitarsis* B, *An. marajoara*, *An. deaneorum* and *An. albitarsis* E. Because dark and pale wing spots of Costa vein could be either present or absent in distinct specimens of a species, the absence of a spot influenced the recognition of flanking spots. Consequently, in the absence of a dark or pale spot, the adjacent spots were not measured, and thus scored as missing variable in the data matrix.

Descriptive statistics. Means and standard deviations were calculated for the measurements, ratios and values of qualitative characters associated with 40 variables. Tests of normality and homogeneity were made.

Principal components analysis (PCA) - was applied for the full data set of 35 variables. This is a statistical ordination technique that objective to obtain a few number of linear combinations from the variables that retain maximum possible information (Hugh & Gauch, 1982).

Variance analysis (ANOVA) - was applied for the full data set of 35 variables to verify if there were differences among the means of all variables ($p \geq 0.05$) of the five species.

Discriminant function analysis - was applied to determine the possibility of segregation of all five already established as distinct species. Because of missing variables (Table 2), 103 out of 150 specimens were employed and of the 40 characters, 27 were used in the discriminant analysis. Additionally, discriminant analysis was employed considering 150 specimens and to solve missing problems, original standardized data were transformed using the ten first principal components. Measures of ten news variables were obtained for 150 specimens. A discriminant model was constructed using discriminant analysis linear of Fisher that supported homogeneous covariance among five groups using cross validation.

Cluster analysis - was employed using dissimilarity of variables (level = 40). Distances of each variable were entered for 150 specimens of five species. Based on the transformed data set of principal components, discriminant analysis was applied to validate cluster analysis.

Statistical analyses were performed with Minitab 14 for Windows XP and R-2.2.1 for Windows® software program. The data matrix was generated in Microsoft Excel software program.

Distance between eyes and thirty nine wing spots characters (Table 2) of adult female were observed and measured (scales in mm) for thirty individuals of each species in a stereomicroscope Wild connected with a digital micrometric ocular Wild.

Twenty slide-mounted immature specimens were used for measurements and setal counts. Measurements were made using AxioVision 3.0.6 SP4 (Carl Zeiss Software

Inc.). Nomenclature adopted for the adults follows Harbach & Knight (1980), for the wing spots Wilkerson & Peyton (1990), and for the wing veins Belkin (1962). In the morphological description, *An. albitarsis s.s*. has all life stages and structures fully described and the other species of the complex are compared to it; characteristics that were identical were not included in the descriptions.

Results

Morphological descriptions

Anopheles albitarsis Lynch Arribalzaga

Anopheles albitarsis Lynch Arribalzaga 1878: 150 (F). Type loc. Baradero, Buenos Aires, Argentina. Senevet 1934: 45 (P*); Pinto 1939: 345 (M*, F*, P*, L* E); Rozeboom 1942 : 238 (E*, tax.); Ross & Roberts 1943: 31 (M*, F*, L*); Cova-Garcia 1946: 31, 83, 118 (E*, L*, F*, M*); Romeo-Viamonte & Castro 1951: 321 (F*); Garcia & Casal 1964 (1965): 6 (P*); Kreutzer *et al*. 1976: 473 (chromosomes); Rosa-Freitas & Deane 1989: 289 (neotype desig.); Wilkerson *et al*. 1995a,b: (RAPD-PCR, tax.); Rozeboom 1942 : 238

Anopheles limai Galvão & Lane 1937: 227 (A, E*, as var.). Type loc. Pinheiros and Butantan, São Paulo, São Paulo, Brazil. Type: Unknown. Lane 1953: 244 (syn.); Belkin *et al*. 1971: 4 (tax.).

Anopheles imperfectus Corrêa & Ramos 1943: 246 (F, as spp). Type loc. Vera Cruz, São Paulo, Brazil (FSP-USP). Lane 1953: 244 (syn.); Belkin *et al*. 1971: 4 (tax.).

Female. Head: integument brown to dark brown. Dorsal vertex and occiput with large, erect, white, truncate, weakly forked scales becoming dark laterally; anteriorly, vertex with a few, small obovate decumbent scales along upper orbital and longer, narrow, white, decumbent scales along interorbital line; ocular setae dark brown to

black. Frontal tuft with long, white setae. Clypeus bare. Pedicel of antenna brown to dark brown with decumbent, white spatulate scales on dorsal surface; flagellomere 1 with semi-erect, white scales on posterior 0.5 of medial surface and at apex of dorsal and lateral surfaces, and a patch of decumbent, flat, broad white scales at base of dorsal surface. Proboscis brown to dark brown, with decumbent scales and short setae; length 2.00-2.14 mm (mean=2.07±0.04) (n=10), 1.30-1.42 length of forefemur (mean=1.36±0.04) (n=10), 0.98-1.12 length of maxillary palpus (mean=1.04±0.04) (n=10). Labella similar in color to labium. Palpus 1.90-2.05 mm (mean=1.99±0.05) (n= 10), 0.88-1.01 length of proboscis (mean=0.96±0.04) (n=10); maxillary palpomere 1 dark-scaled; palpomere 2 mostly dark-scaled with few white scales at apex of lateral and ventral surfaces; palpomere 3 mostly dark-scaled with white scales sparse on lateral surface and at apex of ventral surface; palpomere 4 mostly dark-scaled with white scales on median area of dorsal surface; palpomere 5 white-scaled, scales erect on palpomeres 1 and 2, semi-erect and decumbent on dorsal and lateral surfaces, and erect on ventral surface of palpomere 3, decumbent on palpomere 4 and 5; length palpomere 2 / palpus length 0.22-0.34 (mean=0.28±0.03) (n=10); length palpomere 3 / palpus length 0.30-0.38 (mean=0.35±0.02) (n=10); length palpomere 4 / palpus length 0.17-0.21 (mean=0.19±0.01) (n=10); length palpomere 5 / palpus length 0.11-0.14 (mean=0.13±0.01) (n=10). Thorax: integument pruinose with darker area between dorsocentral area and lateral margin at posterior edge of scutal fossa, at posterior end of dorsocentral area, at posterior edge of scutum, and posteriorly on prescutellar area extending posteriorly onto median scutellar lobe; white spatulate, decumbent scales on acrostichal and dorsocentral areas, scutal fossa, prescutellar area except posteriorly on dark posterior bare area; supraalar and antealar areas with white, spatulate, decumbent scales; elongate, narrow, semi-erect, white, spatulate scales along lateral margin of

antealar area extending posteriorly onto supraalar area; scutum bare anteriorly between acrostichal and dorsocentral areas, posteriorly to scutal fossa, and posteriorly on prescutellar area; anterior promontory with erect piliform, white scales; anterior lateral angle of scutum with erect, broad spatulate white scales dorsally, dark scales ventrally. Scutellum with spatulate, white scales posteriorly, posterior edge with a row of long and few short pale brown setae with golden and reddish reflections. Mesopostnotum bare. Antepronotum with dark setae and a patch of spatulate scales, these scales whitish dorsally, dark ventrally on upper area, remainder of antepronotum without scales, with scattered brown setae. Pleura with small patches of white, spatulate scales on upper mesokatepisternum, posterior border of middle mesokatepisternum, upper region of prealar knob, upper mesepimeron; dark brown setae on upper proepisternum, upper mesokatepisternum and prealar knob, pale yellow setae on middle posterior border of mesokatepisternum, upper mesepimeron and prespiracular area. Wing: length 2.96-4.04 mm (mean=3.35$\pm$0.24) (n=30); veins dark-scaled with spots of white scales on anterior costal, subcostal, R and R_1 veins, pale yellow scales on remainder veins. Halter: scabellum with pale integument; pedicel with pale integument ventrally, dark dorsally with few white scales posteriorly on dorsal surface; capitellum pale-scaled ventrally, dark scales dorsally with a patch of white scales at base. Legs: anterior surface of forecoxa with a patch of spatulate scales mesally, these scales white laterally and dark medially, and a few white, spatulate scales ventrally, posterior surface of forecoxa with a patch of ventrally directed black, spatulate scales, and a patch of white scales at lateral side; outer surface of midcoxa with patches of white, spatulate scales at apex on anterior and posterior surfaces, and patch of white, spatulate, semi-erect scales at base; hindcoxa with patch of semi-erect, spatulate, white scales at apex, and a patch of white, spatulate, appressed scales at base of posterolateral surface. Fore-, mid- and hindtrochanters white

and dark-scaled. Forefemur 1.49-1.56 mm (mean=1.52±0.02) (n=10), 0.70-0.77 length of proboscis (mean=0.73±0.02) (n=10); forefemur with light scales at base forming a pale ring; anterior surface mostly dark brown, often with 2 narrow, pale stripes along margins extending most of length of segment; posterior surface predominantly dark-scaled, with few sparse white scales, apical 0.3 pale-scaled. Midfemur with pale and dark scales at base forming pale and dark rings; anterior surface mostly dark-scaled with a longitudinal white stripe extending from base to near apex; 1,2 patches of white scales near apex; posterior surface entirely pale-scaled. Hindfemur with pale and dark scales at base forming pale and dark bands; dorsal surface mostly dark-scaled with preapical patch of white scales and a small white patch at apex of anterodorsal surface, ventral surface pale-scaled. Tibiae dark-scaled with a narrow, longitudinal line of yellowish scales, posterior surface pale-scaled; all tibiae with white scales at apex. Tarsi with scales varying in color from white to yellowish to dark. Foretarsomeres 1, 2 and 3 predominatly dark-scaled with pale scales at apex; tarsomeres 4 and 5 normally dark-scaled. Midtarsomeres 1 and 2 predominantly dark-scaled with light apical patches of pale scales, tarsomeres 3 and 4 dark-scaled, sometimes with pale scales apically, tarsomere 5 dark-scaled with apical pale scales on dorsal surface. Hindtarsomere 1 dark-scaled with longitudinal stripe of yellowish scales, extending from near base to apex of tarsomere; tarsomere 2 dark-scaled proximally with a basal dark band 0.58-0.73 length of tarsomere (mean=0.65±0.04) (n=10); tarsomeres 3, 4 and 5 white-scaled. Abdomen: tergum I with numerous long, brown setae, without caudolateral tuft of scales; tergum II with numerous brown setae, medially with numerous pale yellow to whitish scales, extending laterally on posterior area, caudolateral tuft always absent on tergum II, sometimes present on tergum III, caudolateral tuft on tergum III with few dark, erect scales extending laterally; tergum IV – VII medially with numerous pale yellow to

whitish scales, with dark scales laterally on posterior margin, caudolateral tufts always present, with large, dark, erect scales extending laterally; tergum VIII with numerous, light yellow scales basally, white scales apically, usually with dark scales posterolaterally, without caudolateral tufts. Sternum I with submedian stripes of white scales; sternum II – VII with numerous dark setae, shorter than those on terga, with longitudinal patches of white scales lateral of midline and with small patches of dark scales posteriorly on midline; sternum VIII with longitudinal patches of white scales lateral of midline and with dark scales on midline.

Male. Similar to female except for sexual differences. Maxillary palpus pale and dark-scaled; scales semierect on basal 0.5 of palpomere 2, decumbent on remaining of palpomere 2 and palpomere 3-5; palpomere 2 dark-scaled with pale scales at apex, dorsal surface with a pale spot at 0.5 basal; palpomere 3 with pale scales at base, dorsally with a pale spot on medial surface, long setae at apex; palpomere 4 with pale scales on ventral and dorsal surface, dark-scaled at apex and base; palpomere 5 with dark setae along ventral surface, pale scales on dorsal surface, dark scales on ventral surface and base.

Male g*enitalia* (Fig. 2). Segment VIII not retracted into segment VII**.** Segment IX: sternite medially posterior border weakly emarginated, covered by numerous small spicules. Gonocoxite elongate with numerous spatulate scales; tergal surface with 3-6 setae and subapical surface with 1-2 setae; cuticula with lateral sternal surface with numerous spatulate scales and minute setae in the inner surface; tubercle of parabasal spine 0.22-0.41 (mean=0.32$\pm$0.06) (n=5) length of parabasal spine; dorsomedial rim 0.09–0.12 (mean=0.1$\pm$0.01) (n=5) length of gonocoxite. Spiculose cuticula except below insertion of the accessory spine where there is a nude triangular area; accessory spine 0.33-0.41 (mean=0.36$\pm$0.03) (n=5) length of gonocoxite; internal seta long and

curved; below accessory setae there is the claspette insertion. Dorsal claspette: pedicel narrow, apical leaflets subequal to dorsal claspette. Ventral claspette ventrally apex wide with strongly striated, single, rounded lobe expanded laterally. Basal lobules expanded laterally. Gonostylus slender, curved with a row of minute setae inserted before the gonostylar claw. Phallossome: basal lobule membranous; aedeagus 0.39-0.47 (mean=0.43±0.03) (n=4) length of gonocoxite, aedeagus cylindrical without setae or scales; strongly sclerotized areas in the lateral surfaces; apically rounded at apex.

Pupa (Fig. 2). Position and development of setae as figured; range and modal number of branches in Table 5. All measurements were made in 10 specimens unless otherwise indicated. Cephalothorax: integument and leg cases weakly pigmented; trumpet angusticorn with meatal cleft; pinna moderately pigmented; meatal cleft basally U-shaped. Abdomen: length 2.50-3.11 mm (mean=2.84±0.20); seta 2-I with 4-7 branches; 3-I normally single; 4-I with 5-7 branches; 5-I single to triple, 6-I single or double; 7-I with 2-6 branches; 9-I normally single, sometimes double, as long as 6-I; 0-II-VII moderately developed, 0-II with 3-7 branches, 0-III with 4-7 branches, 0-IV with 2-6 branches, 0-V-VII often 4-branched, 0-VIII normally single; 1-II with 5-10 branches; 1-IV-VII always single, strong, long, extending beyond caudal border of the following segment; 3-IV with 3-7 branches, extending to caudal margin of segment, 3-V with 2-4 branches, extending beyond caudal margin of segment; 5-III with 4-10 branches, 5-IV single to 5-branched, 5-V, VII single, 5-V rarely double, 5-VII single or double; 6-II single or double, 1.12-2.52 length of 7-II (mean=1.76±0.51), 6-III-IV normally double; 7-II with 3-6 branches, 7-III, IV frequently with 5 branches, 7-V with 2-5 branches, 7-VI single, rarely double, 7-VII single; 8-III with 3-5 branches, 8-IV with 2-5 branches, 8-V, VI frequently double; 9-II minute, unpigmented, 9-III short, stout, 1.11-2.50 (mean=1.67±0.45) length of 9-II, 9-IV tick, 1.35-2.82

(mean=2.01±0.49) length of 9-III, 9-V dark, strong, curved, 1.68-3.09 (mean=2.38±0.47) length of 9-IV, 9-VI strong, curved, 1.10-1.64 (mean=1.28±0.16) length of 9-V, 9-VII strong, curved, sharply pointed, 0.98-1.86 (mean=1.42±0.26) length of 9-VI, 9-VIII straight, 0.66-1.10 (mean=0.85±0.15) length of 9-VII, 9-II-IV less than 0.20 length of segment, 9-V-VI less than 0.50 length of segment and 9-VII, VIII about 0.50 length of segment; 10-III with 2-4 branches, 10-IV, V always single; 4-VIII with 2 – 4 branches. Male genital lobe: tick at base with sides sloping toward apex, apex with mamiliform protuberance. Paddle obovate, 1.24-1.47 (mean=1.37±0.06) longer than wide, length 0.70-0.85 mm (mean=0.78±0.05), width 0.49 - 0.62 mm (mean=0.57 mm ± 0.04), refractile index 0.70-0.78 (mean=0.73±0.03), outer margin distal to buttress with very fine, minute spicules, extending around apex; inner margin smooth; seta 2-P often single.

First-instar larva (Fig. 3). The setae present in the first-instar larva are single or double with few exceptions, although, the majority is single. The head of the larva contain the same number of setae found in later instar, and differ significantly only in a few quantity of branchs. The thorax and abdomen, however, are missing setae which are present in the fourth instar. Two ventral setae are absent from abdominal segment I-VII.

Fourth-instar larva (Fig. 4). Position and development of setae as figured; number and modal number of branches in Table 6 Measurements were made in 10 specimens unless otherwise indicated. Head: length 0.62-0.76 mm (mean=0.66 mm±0.06) (n=5), width 0.58-0.69 mm (mean=0.62±0.05) (n=5). Integument weakly pigmented with darker spots along posterior border of dorsal apoteme, on posterior margin of frontal ecdysial line on lateralia, posteriorly on lateral area of lateralia, on ventral lateralia, on anterior area of labiogula and along hypocranial ecdysial line. Mental plate strongly sclerotinized, blackish, median tooth moderately broad, about

twice as wide as adjacent tooth, tapered to point, blunt at apex. Seta 2-C single with tiny, sparse aciculae, 1.14-1.53 length of 3-C (mean=1.35±0.11), distance between bases 3.76-6.59 (mean=4.85±0.77) width base of single seta; 3-C single, 0.65-0.87 length of 2-C (mean=0.74±0.06); clypeal index 0.78-1.79 (mean=1.38±0.26) (distance between bases of 2-C and 3-C on one side/distance between the bases of 2-C); seta 4-C with 1-4 branches, short, extending half way to anterior margin of head, seta 8-C with 2-5 branches, 9-C with 3-5 branches, 10-C triple; 12-C with 4-7 branches; 13-C with 4-7 branches. Collar dark brown, moderately wide dorsolaterally. Antenna: length 0.27-0.35 mm (mean=0.31 mm±0.02), 7.36-8.83 (mean=8.26±0.52) longer than wide, with long and thin spicules on mesal margin, spicules short and sparse on dorsal and ventral surfaces; seta 1-A with 5-11 branches, 0.25-0.34 (mean=0.28±0.03) distance from base. Thorax: seta 1-P with 10-19 leaflets truncate at apex, 2-P with 16-25 branches; 14-P with 5-9 branches, large, branches arising from a short shaft, median branches longer than lateral branches, extending beyond anterior margin of thorax; 1-M plumose, with 24-35 branches; 2-T moderately long, single, extending to caudal margin of thorax, 3-T palmate, with 11-17 narrow leaflets. Abdomen. Integument with minute spicules on ventral surface of segments II-VIII; seta 0-II-VII large, multibranched; seta 1-I with 12-21 leaflets, 1-II-VII with truncate to pointed leaflets; 2-II with 3-4 branches strongly developed, large, 2-III with branches stronger than those of 2-II, 2-IV, V normally single; 2-IV rarely double; 5-I with 3-5 branches, inserted less than its length from lateral margin of abdomen; 5-II with 7-10 branches; 11-I with 3,4 branches; 13-I, III with 3-9 branches, 13-II with 8-15 branches, 13-IV, V with 3-6 branches, 13-IV extending to caudal margin of segment, 13-V extending beyond caudal margin of segment, 13-VI with 7-15 branches. Pecten with 3-5 long, 11-14 short spines, long spines 2.45-2.98 (mean=2.70±0.16) length of short spines; lateral arm of median plate

of spiracular apparatus short to moderately long; seta 8-S with 3-5 branches, 9-S with 5-8 branches. Segment X covered with spicules, spicules stronger on posterior margin; seta 1-X longer than length of saddle; inserted either inside or outside saddle; anal gills longer than saddle.

Distribution. Distribution data presented here are from the published literature records regarding to specimens of *Anopheles albitarsis* that were identified by RAPD-PCR, mtDNA-COI or rDNA-ITS2.

Anopheles albitarsis is found in Argentina, Departments of Misiones, Corrientes and Buenos Aires; Brazil, States of São Paulo, Paraná and Santa Catarina (Wilkerson *et al*., 1995a, Lehr *et al*., 2005, Li & Wilkerson, 2005); Paraguay, State of Alto Paraná (Wilkerson *et al*., 1995a).

Material examined. List of the specimens examined are in Table 1.

Bionomics. Little is known about bionomics of *An. albitarsis s.s.* Kakitani and Forattini (2000) observed that the daily survival rate for *An. albitarsis* (identified as *An. albitarsis* B) is 0.5566+/-0.015 and that the duration of the gonotrophic cycle is 2.046 days in the Ribeira Valley, southeast of the State of São Paulo, Brazil. Lounibos *et al*. (1998) observed in laboratory condition that 22.9% of the females of nulliparous *An. albitarsis* failed to mature eggs after one blood meal. Results of collections using Shannon trap in irrigated rice field area show that there was an increase in *An. albitarsis s.l.* population that could be associated with the development of rice plantation. At the same area, *An. albitarsis s.s.* was attracted to human bait carried out indoor and outdoor at domiciliary environment at sunset and sunrise (Forattini *et al*. 1995).

Medical importance. Because of misidentification of members of the *An. albitarsis* complex, the medical importance of *An. albitarsis* is unknown.

Systematics. Linthicum (1988) pointed out morphological characters to distinguish between *An. marajoara* and *An. albitarsis s.l.*, because only these two species were valid at that time. Lima *et al.* (2004) examined the reproductive isolation between *An. albitarsis s.s.* collected in Massaranduba, State of Santa Catarina, Brazil and *An. deaneorum* collected in Costa Marques, State of Rondônia, Brazil. The results confirmed that *An. albitarsis s.s.* and *An. deaneorum* are distinct species with high degree of post zygotic reproductive isolation. Interestingly, the male hybrids showed low insemination rate, whereas it was higher for the female hybrids. Additionally, *An. albitarsis s.s.* can be differentiated from others species of the *An. albitarsis* complex using RAPD-PCR. Many primers produced potentially diagnostic bands, but *An. albitarsis s.s.* was identified by 3 unique marker bands produced by 3 primers, B16, A01 and C15 (Wilkerson *et al*., 1995 a). Also, *An. albitarsis s.s.* could be identified by partial sequences of the ITS2 and D2 of the 28S both of the ribosomal DNA and fragments of the COI and N4 mitochondrial genes (Wilkerson *et al*., 2005; Li & Wilkerson, 2006) and by an independent marker, the mitochondrial DNA sequences of the cytochrome c oxidase subunit I (COI) gene (Lehr *et al*., 2005).

***Anopheles marajoara* Galvão & Damasceno**

Anopheles marajoara Galvão & Damasceno 1942: 424 (M, F, L); Lane 1953: 244 (syn.); Belkin *et al.* 1971: 4, 46 (type info, tax.); Linthicum 1988: 198 (from syn. with *An. albitarsis*); Quiñones & Suarez 1990: 602-604 (biology); Lounibos & Conn 1991: 57-66 (biology); Narang *et al.* 1993: 97-112 (tax., cryptic species); Rubio-Palis 1995: 482-484 (feeding behavior); Wilkerson *et al.* 1995a: 697-704 (tax., RAPD-PCR).

Anopheles albitarsis domesticus Galvão & Damasceno 1944: 78 (M, F, E*); Correa *et al*. 1950: 280 (biology); Belkin *et al*. 1971: 4 (type info); Linthicum 1988: 198 (syn. under *An. albitarsis*).

Female. Similar to *An. albitarsis s.s*., except for the characters as follow. Proboscis brown to dark brown, with decumbent scales and short setae; length 1.92-2.05 mm (mean=1.97 mm±0.05) (n=5), 1.28-1.41 length of forefemur (mean=1.34±0.05) (n=5), 1.00-1.07 length of maxillary palpus (mean=1.04±0.03) (n = 5). Labella similar in color to labium. Palpus 1.85-1.97 mm (mean=1.91±0.06) (n=5), 0.93-1.00 length of proboscis (mean=0.96±0.02) (n=5); length palpomere 2 / palpus length 0.26-0.32 (mean=0.29±0.02) (n=5); length palpomere 3 / palpus length 0.32-0.37 (mean=0.34±0.02) (n=5); length palpomere 4 / palpus length 0.16-0.21 (mean=0.18±0.01) (n=5); length palpomere 5 / palpus length 0.13-0.15 (mean=0.13±0.01) (n=5). Wing: length 2.72-3.60 mm (mean=3.14 ±0.22) (n=30); veins dark-scaled with spots of white scales on anterior costal, subcostal, R and R_1 veins, pale yellow scales on remainder veins. Forefemur 1.40-1.60 mm (mean=1.49 mm±0.08) (n=5), 0.71-0.78 length of proboscis (mean=0.75±0.03) (n=5); tarsomere 2 dark-scaled proximally with a basal dark band 0.56-0.63 length of tarsomere (mean=0.59±0.03) (n=4).

Male. Similar to *An. albitarsis s.s*.

Male genitalia (Fig. 5). Similar to *An. albitarsis s.s*. except for the characters as follow. Gonocoxite elongate with numerous spatulate scales; tergal surface with 4- setae and subapical surface with 1-2 setae. Tubercle of parabasal spine 0.22-0.25 (mean=0.23±0.04) (n=3) length of parabasal spine; dorsomedial rim 0.10–0.13 (mean=0.11±0.01) (n=3) length of gonocoxite; dorsal accessory setae 0.35-0.42

(mean=0.38±0.03) (n=3) length of gonocoxite; aedeagus 0.41-0.45 (mean=0.42±0.01) (n=3) length of gonocoxite.

Pupa (Fig. 5). Similar to *An. albitarsis s.s.* except for the characters as follow. Position and development of setae as figured; range and modal number of branches in Table 7. All measurements were made in 10 specimens. Abdomen: length 2.43-3.26 mm (mean=2.74 mm±0.26); seta 2-I with 1-5 branches; 3-I single to triple; 4-I with 4-7 branches; 7-I with 1-5 branches; 9-I always single, 0-V-VII often 5-branched, 0-VIII normally single (1-4 branches); 1-II with 5-13 branches; 3-IV with 3-6 branches, 3-V with 2-4 branches; 5-III with 3-11 branches, 5-IV with 2-10 branches, often triple, 5-VI single, double or triple, 5-VII single or double; 6-II single or double, 1.19-2.99 length of 7-II (mean=1.99±0.50), 6-III-V often double; 7-III, V with 3-6 branches, 7-V with 2-4 branches, 7-VI, VII single or double; 8-III with 2-4 branches, 8-IV-VI single to triple; 9-III 1.20-2.59 (mean=1.93±0.51) length of 9-II, 9-IV 1.40-2.70 (mean=1.87±0.42) length of 9-III, dark, 9-V 1.83-3.99 (mean=2.45+0.65) length of 9-IV, 9-VI 0.93-2.18 (mean=1.32±0.34) length of 9-V, 9-VII 1.17-1.79 (mean=1.38±0.20) length of 9-VI, 9-VIII 0.82-1.04 (mean=0.90±0.08) length of 9-VII, 9-V-VI less than 0.5 length of segment, and 9-VII, VIII around 0.5 length of segment; 10-IV, V always single, long; 4-VIII double or triple. Paddle 1.14-1.45 (mean=1.34±0.08) longer than wide, length 0.65-0.79 mm (mean=0.73 mm±0.04), width 0.45-0.66 mm (mean=0.55 mm±0.06), refractile index 0.67-0.75 (mean=0.71±0.03).

Fourth-instar larva (Fig. 6). Similar to *An. albitarsis s.s.* except for the characters as follow. Position and development of setae as figured; number and modal number of branches in Table 8. Measurements were made in 10 specimens unless otherwise indicated. Head: length 0.59-0.75 mm (mean=0.68±0.05), width 0.65-0.82 mm (mean=0.73±0.06) (n=9). Integument with darker spots on central area of dorsal

apoteme along frontal ecdysial line, lateral and ventral areas of lateralia, and labiogula with two pale spots between hypostomal suture and hypocranial ecdysial line. Seta 2-C 1.24-1.55 length of 3-C (mean=1.42±0.09), distance between bases of 2-C 3.91-6.05 (mean=4.92±0.66) width base of single seta; 3-C moderately aciculate, 0.65-0.81 length of 2-C (mean=0.71±0.05); clypeal index 0.97-1.66 (mean=1.17±0.22). Seta 4-C with 2-5 branches; 8-C with 3-7 branches; 9, 10-C often triple; 12-C with 4-7 branches; 13-C with 5-8 branches. Antenna: length 0.27-0.38 mm (mean=0.31 mm±0.03), 7.55-8.91 (mean=8.12±0.39) longer than wide, weakly to strongly pigmented (specimens from São Paulo, Brazil); seta 1-A with 5-11 branches, 0.25-0.34 (mean=0.30±0.03) distance from base. Thorax: seta 1-P palmate, with 12-24 leaflets; leaflets truncate at apex, 2-P with 16-25 branches; 14-P with 5-11 branches; 1-M with 26-37 branches; 3-T with 13-21 leaflets. Abdomen: seta 0-II-VII large, multibranched; 1-I with 14-20 leaflets, 1-II-VII with truncate to pointed leaflets; 2-II with 3, 6 branches, 2-IV, V long, always single; 5-I with 3-6 branches; 5-II with 8-15 branches; 11-I with 3, 4 branches; 13-I, III often 5-branched, 13-IV, V often 4-branched, 13-VI with 9-14 branches. Pecten with 10-14 short spines, long spines 2.53-3.71 (mean=3.04±0.30) length of short spines; lateral arm of median plate of spiracular apparatus short to moderately long; seta 9-S with 6-10 branches. Segment X: seta 1-X inserted either inside or outside saddle.

Distribution. Anopheles marajoara is found in Brazil, in States of São Paulo, Pará, Rondônia, Mato Grosso, Amazonas (Wilkerson *et al*., 1995 a, b; Lehr *et al*., 2005; Li & Wilkerson, 2005), Roraima and Amapá (Conn *et al*., 2002; Li &Wilkerson, 2005); Paraguay, in State of Alto do Paraná (Wilkerson *et al.,* 1995a); Venezuela, Departments of Zulia, Cojedes and Barinas (Wilkerson *et al.,* 1995b; Rubio Palis, 2003; Li & Wilkerson, 2005). *Anopheles marajoara* is the only species of the complex registered in Trinidad (Chadee & Wilkerson, 2006)

Material examined. Specimens examined are in Table 1.

Bionomics. Studies on resting behavior of anopheline females carried out in Colombia, in Departments of Bolivar, Santander, Meta and Caquetá, showed that *An. marajoara* has tendency to rest close to the ground (Quiñones & Suarez, 1990). It is noteworthy that a new phylogenetic species of *An. marajoara* has been hypothesized by Brochero *et al*. (2007), and thus the published literature data for *An. marajoara* in Colombia may refer to both, *An. marajoara* and *An. albitarsis* F. A study conducted in two simian malaria areas, Balbina on the Uatumã River and Samuel on the Jamari River both in Brazilian Amazon, *An. marajoara* and *An. darlingi* were collected in anthropic environment outside the forest and thus the involvement of these species in the transmission of *P. brasilianum* among monkeys and from monkeys to man was demonstrated to be unlike (Lourenço-de-Oliveira & Luz, 1996). Zimmerman *et al*. (2006) determined the human blood indices (HBI) for mosquitoes collected in an area in the Matapi River, State of Amapá, Brazil. As a result, it was observed that *An. darlingi* and *An. marajoara* were the most anthropophilic mosquito species in that area. Additionally, *An. marajoara* showed defined crepuscular biting activity pattern in the State of Amapá, Brazil (Voorham, 2002). Conn *et al*. (2002) conducted entomological surveys in malaria areas of Macapá, State of Amapá, and found that *An. marajoara* was the most frequent anopheline species, replacing *An. darlingi* as the primary vector. Most likely the observed change in mosquito population was a result of deforestation and newly created ground pools for agricultural use that provided adequate larval habitats for *An. marajoara*. In a study carried out in Ilha Comprida, Atlantic Forest domain, southern São Paulo State, Brazil, Kakitani *et al.* (2003) observed that *An. marajoara* activity peaked between 2:00 h and 5:00 h. On the other hand, Chadee (1992) found that *An. marajoara* from Trinidad showed extra-domiciliary and intra-

domiciliary habits, and host-seeking activity started at 17 h and ended at 22 h. They observed differences in the activity peak for *An. marajoara* populations from southern Brazil and Trinidad should be better investigated to examine if they represent distinct populations or species.

*Medical importance***.** *Anopheles marajoara* was thought to be at most a secondary local vector of malaria parasites (reviewed by Linthicum 1988). Recent published data, show that *An. marajoara* seems to be a primary vector of human malaria parasites in Macapá (Conn *et al*., 2002) and Serra do Navio, Amapá State, Brazil (Póvoa *et al*., 2000).

Systematics. *Anopheles marajoara* was described by Galvão and Damasceno (1942) based on characters of the adults male and female and larva. In 1953, Lane synonymized *An. marajoara* with *An. albitarsis s.s*. Later, Linthicum (1988) resurrected *An. marajoara* from synonymy with *An. albitarsis*, and registered several morphological characters to distinguish between *An. marajoara* and *An. albitarsis.* Narang *et al.* (1993) using isozyme data and RFLP profile of the mitochondrial genome of populations from Brazil of *An. deaneorum* collected in Costa Marques, Rondônia State and *An. marajoara* collected in Iguape, São Paulo State and in Ilha do Marajó, Pará State, found evidence that *An. marajoara* included at least three cryptic species. Additionally, Wilkerson *et al*. (1995a,b) employed the RAPD-PCR assay to separate four species of the *An. albitarsis* complex, including *An. marajoara*. It is also possible to separate *An. marajoara* from the other species of the complex utilizing rDNA ITS2 sequence (Wilkerson *et al.,* 2005; Li & Wilkerson, 2005; 2006), a portion of the white gene that contains the fourth intron (Merritt *et al.,* 2005). *Anopheles marajoara* is the only species of the *An. albitarsis* complex that possess the fourth intron. Additionally, Li *et al*. (2005) isolated primers from 40 microsatellite loci and selected 11 polimorphic

scored loci for studying population differentiation in *An. marajoara.* These loci are highly variable and will be useful for studies of instraspecific variation in *An. marajoara*. Recently, Lehr *et al.* (2005), using data derived from the sequences of the cytochrome c oxidase subunit I (COI) gene, hypothesized a fifth phylogenetic species in this complex, designated species E, which was largely misidentified as *An. marajoara* in Boa Vista municipality, Roraima State, Brazil. In contrast, Li and Wilkerson (2005) showed that the polymerase chain reaction technique with a set of species-specific oligonucleotide primers enables the separation of four species of the *A. albitarsis* complex, except species E.

Finally, morphological characters to separate *An. marajoara* from other species of the *An. albitarsis* are problematic and may overlap among the species. In conclusion, discriminating among *An. marajoara* and other species of the *An. albitarsis* complex based on either morphology or molecular markers is still not an easy task (Wilkerson *et al.,* 1995; Lehr *et al*., 2005).

***Anopheles* (*Nyssorhynchus*) *deaneorum* Rosa-Freitas**

Anopheles deaneorum Rosa-Freitas 1989: 535 (M, F*, P, L*, E). Type locality: Palheta, Guajará-Mirim, State of Rondônia, Brazil (IOC). Klein *et al*. 1990: 510-513 (tax., biology); Klein *et al*. 1991b: 301-303 (tax., DNA hybridization); Narang *et al*. 1993: 97-112 (tax., allozyme, RFLP); Wilkerson *et al*. 1995a: 697-704 (tax., RAPD-PCR).

Female. Similar to *An. albitarsis s.s*. except for the characters as follow. Proboscis brown to dark brown, with decumbent scales and short setae; length 1.75-1.92 mm (mean=1.82 mm±0.05) (n=8), 1.28-1.39 length of forefemur (mean=1.33±0.03) (n=8) , 1.06-1.16 length of maxillary palpus (mean=1.10±0.04) (n=8). Labella similar in color

to labium. Palpus 1.57-1.76 mm (mean= 1.67 mm±0.06) (n=8), 0.86-0.95 length of proboscis (mean=0.91±0.04) (n=8); length palpomere 2 / palpus length 0.26-0.31 (mean=0.30±0.02) (n=8); length palpomere 3 / palpus length = 0.28-0.38 (mean=0.33±0.03) (n=8); length palpomere 4 / palpus length = 0.16-0.22 (mean=0.18±0.02) (n=8); length palpomere 5 / palpus length = 0.13-0.16 (mean=0.14±0.01) (n=8). Wing: length 2.80-3.60 mm (mean=3.17±0.19) (n=30); veins dark-scaled with spots of white scales on anterior costal, subcostal, R and R_1 veins, pale yellow scales on remainder veins. Forefemur 1.30-1.43 mm (mean=1.37±0.04) (n=8), 0.72-0.78 length of proboscis (mean=0.75±0.02) (n=8); tarsomere 2 dark-scaled proximally with a basal dark band 0.62-0.70 length of tarsomere (mean=0.67±0.03) (n=8). Abdomen: postero-lateral tuft of scales absent in tergum III.

Male. Similar to *An. albitarsis s.s.* Abdomen: caudolateral tuft always absent on tergum III, caudolateral tuft beginning on tergum IV or V with few dark, erect scales extending laterally.

Male genitalia (Fig. 7). Similar to *An. albitarsis s.s.* except for the characters as follow. Gonocoxite elongate with numerous spatulate scales; tergal surface with 4-5 setae and subapical surface with 1-2 setae. Tubercle of parabasal spine 0.24-0.44 (mean=0.34±0.09) (n=2) length of parabasal spine; dorsomedial rim 0.08–0.10 (mean=0.09±0.01) (n=3) length of gonocoxite; dorsal accessory setae 0.35-0.39 (mean=0.37±0.02) (n=3) length of gonocoxite; aedeagus 0.41-0.46 (mean=0.44±0.02) (n=3) length of gonocoxite.

Pupa (Fig. 7). Similar to *An. albitarsis s.s.* except for the characters as follow. Position and development of setae as figured; range and modal number of branches in Table 9. All measurements were made in 10 specimens. Abdomen: length 2.54-3.04 mm (mean=2.77±0.16); seta 2-I with 4- 7 branches; 3-I single or triple; 7-I with 3-7

branches; 0-II-IV normally 6-branched, 0-V, VI often with 5 branches, 0-VII with 3-5 branches; 1-II with 5-13 branches, 1-IV rarely double; 3-IV with 3-7 branches, 3-V single to 6-branched; 5-III with 4-11 branches, 5-IV with 2-6 branches, 5-V, VII single, 5-VII rarely double; 6-II single or 4-branched, 1.25-2.74 length of 7-II (mean=1.99±0.50), 6-III with 2-5 branches, 6-IV-VI single to triple; 7-II with 4-6 branches, 7-III, IV with 3-7 branches, 7-V normally 3-branched, 7-VI, VII often single; 8-III, IV normally triple, 8-V, VI often double; 9-II minute, unpigmented, 9-III short, stout, 0.91-3.22 (mean=2.22±0.74) length of 9-II, 9-IV 1.51-3.96 (mean=2.44±0.74) length of 9-III, 9-V 1.23–2.07 (mean=2.08±0.43) length of 9-IV, 9-VI 1.11-2.24 (mean=1.35±0.33) length of 9-V, 9-VII, 1.05-1.38 (mean=1.19±0.10) length of 9-VI, 9-VIII 0.82-1.19 (mean=0.97±0.11) length of 9-VII, 9-II-IV less than 0.25 length of segment, 9-V-VI less than 0.50 length of segment, and 9-VII, VIII less than 0.60 length of segment; 10-III with 2-4 branches, 10-IV, V always single, long; 4-VIII with 2–4 branches. Paddle 1.32-1.47 (mean=1.37±0.04) longer than wide, length 0.69-0.83 mm (mean=0.76 mm±0.04), width 0.47-0.63 mm (mean=0.56 mm±0.04), refractile index 0.69-0.78 (mean=0.74±0.03), 2-P often single.

Fourth-instar larva (Fig. 8). Similar to *An. albitarsis s.s.* except for the characters as follow. Position and development of setae as figured; number and modal number of branches in Table 10. Measurements were made in 10 specimens unless otherwise indicated. Head: length 0.65-0.73 mm (mean=0.71±0.03) (n=6), width 0.61-0.66 mm (mean=0.65 mm±0.02) (n=6). Integument weakly pigmented with darker spots along posterior border of dorsal apoteme, on posterior margin of frontal ecdysial line on lateralia, on ventral lateralia, labiogula darkened along hypocranial ecdysial line and hypostomal suture. Seta 2-C 1.21-1.41 length of 3-C (mean=1.28±0.07), seta 2-C far from mate of opposite side, distance between bases 4.10-7.06 (mean=5.55±0.83) width

base of single seta; 3-C branched, 0.71-0.83 length of 2-C (mean=0.78±0.04); clypeal index 0.76-1.29 (mean=1.02±0.14). Seta 4-C single to 6-branched; 8-C with 3-8 branches; 9-C with 3-6 branches; 10-C with 2-4 branches; 12-C with 4-7 branches; 13-C with 4-8 branches. Antenna: length 0.28-0.34 mm (mean=0.31 mm±0.02), 7.38-8.78 (mean=8.21±0.52) longer than wide; seta 1-A with 4-11 branches, 0.26-0.34 (mean=0.30±0.03) distance from base; seta 2-A with minute spicules on mesal margin. Thorax: leaflets point to truncate at apex, 2-P with 14-26 branches; 14-P with 5-12 branches; 1-M with 26-38 branches; 3-T with 14-22 leaflets. Abdomen: seta 0-II-VII multibranched; 1-I with 13-22 leaflets, 1-II-VII with truncate to pointed leaflets; 2-II with 3-8 branches, 2-IV, V single, long; 5-I with 4-8 branches; 5-II with 10-16 branches; 13-I, III often 5-branched, 13-II with 6-14 branches, 13-IV with 3-5 branches, 13-VI with 8-16 branches. Pecten with 9-13 short spines, long spines 2.36-3.24 (mean=2.68±0.28) length of short spines; seta 8-S with 4-8 branches, 9-S with 4-10 branches. Segment X: seta 1-X longer than length of saddle, inserted either inside or outside saddle.

Distribution. Anopheles deaneorum is found in Brazil, in Paraná and Rondônia States; Argentina in Corrientes State (Wilkerson *et al*., 1995b; Li & Wilkerson, 2005). According M.A.M. Sallum (personal communication, 2007), *An. deaneorum* is found in Acrelândia, Acre States, Brazil.

Material examined. Specimens examined are in Table 1.

*Bionomics***.** Klein *et al.* (1991a) compared the biting behavior of *Anopheles* mosquitoes in Costa Marques, Rondônia. Consequently, it was observed that *An. deaneorum* and *An. darlingi* are more anthropophilic than the other anophelines, also both species were more frequent inside the houses than the remaining *Anopheles* species. *Anopheles deaneorum* was also collected on human bait outside and inside

houses (6% and 9% of anophelines captured, respectively) in Costa Marques, Rondônia State (Klein & Lima, 1990). Using deltamethrin-impregnated mosquito nets, *Anopheles* density decreased in the intradomicile. Although the decrease in anophelines frequency indoors, *An. deaneorum* accounted for 35.3% of mosquitoes collected in Costa Marques (Santos *et al.*, 1999).

Medical importance. *Anopheles deaneorum* is considered to be a potential vector of human malaria parasites in Rondônia State in western Brazil (Klein *et al*., 1991b,d). Klein *et al*. (1991c) comparing the susceptibility of five anopheline mosquitoes to *Plasmodium falciparum* in the State of Rondônia, Brazil, demonstrated the oocyst positive rate and the mean number of oocysts in *An. deaneorum* to be similar to those found for *An. darlingi*. The salivary gland sporozoite infection rate was similar to that found for *An. mediopunctatus s.l.* when compared to *An. darlingi*. However, the susceptibility level to *P. falciparum* was lower for *An. deaneorum* when compared to *An. darlingi* (Klein *et al.* 1991d). Regarding to *P. vivax*, the percentage of salivary gland infection for *An. deaneorum* was similar to that found for *An. darlingi*. Using specific monoclonal antibodies direct against the repeats of the circumsporozoite protein of *P. falciparum*, *P. vivax*, *P. vivax* VK247 and *P. malariae*, it was found that the infection rates for *An. deaneorum* were 2.76% for *P. falciparum*, 0.55% for *P. vivax* and 0.82% for *P. vivax* VK 247 (Branquinho *et al*., 1993). Also, *P. vivax*-like parasites were reported from 2 out of 168 examined individuals of *An. deaneorum* tested using serological tests (Marrelli *et al*., 1998).

Systematics. Rosa-Freitas (1989) described and named *An. deaneorum* on the basis of morphological characters of eggs, adult male and female, larval and pupal stages. Specimens employed in the description were from Costa Marques, Rondônia State, Brazil. According to the author, *An. deaneorum* can be distinguished from *An.*

albitarsis s.s. and *An. marajoara* by possessing posterolateral scales tuft beginning in the abdominal terga IV or V, while in *An. albitarsis s.s*. and *An. marajoara* the posterolateral tufts of scales are beginning in terga III. Additionally, the fourth-instar larvae of *An. deaneorum* possesses the seta 3-C branched, whereas in others species of Albitarsis complex the seta 3-C is single with sparse, minute aciculae. Klein *et al*. (1990) maintained colonies of *An. deaneorum* and *An. albitarsis s.s*. using artificial mating. Consequently, it was possible to observe both morphological and behavioral differences between *An. albitarsis s.s*. and *An. deaneorum*. Later, aiming to determine morphological variation and hybrid viability between both species, Klein *et al*. (1991b) employed hybridization experiments and showed that hybrid males were sterile and in hybrid females the reproduction was reduced. Narang *et al*. 1993 using allozyme and RFLP identified *An. deaneorum* and *An. marajoara* in sympatric in Costa Marques, Rondônia State. Following, Wilkerson *et al*. (1995a) corroborated the hypothesis that *An. deaneorum* is a valid species within the *An. albitarsis* complex and designated it as *An. albitarsis* D.

***Anopheles (Nyssorhynchus) albitarsis* B**

Anopheles albitarsis species B of Wilkerson *et al* 1995a: 699; of Wilkerson *et al*. 1995b: 721.

Anopheles albitarsis A of Forattini *et al*. 1995: 21; of Forattini *et al*. 1996: 299; of Kakitani & Forattini 2000: 33.

Female. Similar to *An. albitarsis s.s*. except for the characters as follow. Proboscis brown to dark brown, with decumbent scales and short setae, length 1.98-2.25 mm (mean=2.07±0.10) (n=6), 1.28-1.44 length of forefemur (mean=1.40±0.04) (n=6), 1.02-1.14 length of maxillary palpus (mean=1.06±0.04) (n=6). Labella similar in color to

labium. Palpus 1.90-2.00 mm (mean=1.96±0.03) (n=6), 0.88-0.98 length of proboscis (mean=0.95±0.04) (n=6); length palpomere 2 / palpus length 0.27-0.30 (mean=0.29±0.01) (n=6); length palpomere 3 / palpus length 0.32-0.37 (mean=0.35±0.02) (n=6); length palpomere 4 / palpus length 0.16-0.20 (mean=0.18±0.02) (n=6); length palpomere 5 / palpus length 0.12-0.15 (mean=0.13±0.01) (n=6). Wing: length 3.02-3.54 mm (mean=3.27±0.14) (n=30); veins dark-scaled with spots of white scales on anterior costal, subcostal, R and R_1 veins, pale yellow scales on remainder veins. Forefemur 1.43-1.57 mm (mean=1.48±0.05) (n=6), 0.69-0.75 length of proboscis (mean=0.72±0.02) (n=6); tarsomere 2 dark-scaled proximally with a basal dark band 0.42-0.49 length of tarsomere (mean=0.45±0.03) (n=6).

Male. Similar to *An. albitarsis s.s.*

Male genitalia (Fig. 9). Similar to *An. albitarsis s.s.* except for the characters as follow. Gonocoxite elongate with numerous spatulate scales; tergal surface with 4-5 setae and subapical surface with 2-3 setae. Tubercle of parabasal spine 0.33-0.35 (mean=0.34±0.01) (n=2) length of parabasal spine; dorsomedial rim 0.09–0.11 (mean=0.10±0.01) (n=3) length of gonocoxite; dorsal accessory setae 0.33-0.41 (mean=0.37±0.04) (n=3) length of gonocoxite; aedeagus 0.35-0.43 (mean=0.39±0.04) (n=3) length of gonocoxite.

Pupa (Fig. 9). Similar to *An. albitarsis s.s.* except for the characters as follow. Position and development of setae as figured; range and modal number of branches in Table 11. All measurements were made in 10 specimens unless otherwise indicated. Abdomen: length 2.48-3.19 mm (mean=2.69±0.21); seta 2-I with 2-5 branches; 4-I frequently with 6 branches (5-9 branches); 5-I normally double, 6-I most often single, both 5,6-I long; 7-I with 2-5 branches, shorter than 6-I; 0-III with 4-8 branches, 0-IV

normally 4-branched, 0-V with 3-6 branches, 0-VI, VII frequently 4-branched; 1-II with 5-12 branches; 3-IV 3-5-branched, extending to caudal margin of segment, 3-V with 2-5 branches, extending beyond caudal margin of segment; 5-III with 5-10 branches, shorter than length of following segment, 5-IV with 3-7 branches, 5-V single, double or triple, 5-VI single or double, 5-VII always single; 6-II single, 1.22 - 3.0 length of 7-II (mean = 2.22±0.50) (n = 8), 6-III normally 3-branched, 6-IV with 1-4 branches; 7-II with 2-6 branches, 7-III, IV frequently with 5 branches, 7-VI with 1-3 branches; 8-III with 3-6 branches, 8-IV with 1-4 branches; 9-III 1.42-3.90 (mean=2.11±0.70) length of 9-II, 9-IV 1.04-2.13 (mean=1.67±0.33) length of 9-III, 9-V 1.67-2.93 (mean=2.19±0.42) length of 9-IV, 9-VI 1.14-1.80 (mean=1.37±0.21) length of 9-V, 9-VII 1.01-1.67 (mean=1.25±0.17) length of 9-VI, 9-VIII 0.78-1.08 (mean=0.90±0.08) length of 9-VII, 9-II-IV less than 0.20 length of segment, 9-V-VIII 0.32, 0.40, 0.51, and 0.44 length of segment, respectively; 10-III normally triple, 10-IV single or double, long, 10-V single, long; 4-VIII 3-4-branched. Paddle 1.35-1.51 (mean=1.42±0.06) longer than wide, length 0.71-0.87 mm (mean=0.77±0.05), width 0.49-0.64 mm (mean=0.54±0.04), refractile index 0.70-0.77 (mean=0.75±0.02); seta 1-P stronger than 2-P, 2-P often double.

Fourth-instar larva (Fig. 10). Similar to *An. albitarsis s.s.* except for the characters as follow. Position and development of setae as figured; number and modal number of branches in Table 12. Measurements were made in 10 specimens unless otherwise indicated. Head: length 0.68-0.73 mm (mean=0.71±0.02), width 0.60-0.64 mm (mean=0.62±0.01) (n=9). Integument weakly pigmented with darker spots posteriorly on dorsal apoteme and on lateralia; labiogula darkened along hypostomal suture and hypocranial ecdysial line. Seta 2-C 1.17-1.50 length of 3-C (mean=1.31±0.11), distance between bases 4.50-5.0 (mean=4.75±0.26) width base of

single seta; 3-C 0.67-0.86 length of 2-C (mean=0.77±0.06); clypeal index 1.10-1.44 (mean=1.23±0.12); 8,9-C with 3-5 branches; 10-C with 2-5 branches; 12-C with 3-7 branches; 13-C with 3-9 branches. Antenna: length 0.31-0.34 mm (mean=0.32±0.01) enlarged toward base, 7.56-9.14 (mean=8.12±0.44) longer than wide; seta 1-A with 5-10 branches, 0.25-0.35 (mean=0.30±0.04) distance from base. Thorax: setae 1,2-P sometimes sharing a common tubercle; 1-P palmate, with 12-20 leaflets, leaflets pointed at apex, 2-P with 16-24 branches; 14-P with 6-10 branches, large; 1-M strongly plumose, with 25-33 branches; 3-T palmate, with 12-19 moderately long, narrow leaflets. Abdomen: seta 0-II-VII large, often with 5, 6 branches; 1-I-VII palmate, 1-I with 13-20 leaflets, 1-II-VII with dark, moderately narrow, pointed leaflets; 2-II with 3, 4 branches, 2-IV single, 2-V long, single or double; 5-I with 4-6 branches, 5-II with 8-14 branches; 11-I triple, large; 13-I, III often triple, 13-II with 8-13 branches, 13-IV with 3, 4 branches. Pecten with 4, 5 long, 10-16 short spines, long spines 2.30-2.88 (mean = 2.53 ± 0.17) length of short spines; lateral arm of median plate of spiracular apparatus short to moderately long, direct dorsolaterally; seta 8, 9-S with 3-6 and 5-9 branches, respectively. Segment X: seta 1-X inserted inside saddle.

Distribution. Anopheles albitarsis B seems to be a widely distributed species in Brazil and Paraguay. In Brazil is found in States of São Paulo, Pará, Paraná, Espírito Santo, Mato Grosso, Bahia, Ceará and Rio de Janeiro (Wilkerson *et al*., 1995a, b; Lehr *et al.,* 2005). In Paraguay, *An. albitarsis* B was found in Hernanderias and near National Airport (Wilkerson *et al.*, 1995a).

Material examined. The specimens examined are in table 1.

*Bionomics***.** Because *An. albitarsis* B has been largely misidentified with *An. albitarsis*, *An. deaneorum* and *An. marajoara*, little is known about its bionomics and medical importance. Kakitani and Forattini (2000) identified *An. albitarsis* B as *An.*

albitarsis A, and thus, it was demonstrated that the daily survival rate was 0.5339+/-0.047 and the duration of the gonotrophic cycle was 1.99 days. Also, *An. albitarsis* B (misidentified as *An. albitarsis* A) was attracted to human bait and it is frequent in rice field and in the dewelling environment, in the Ribeira Valley, southeast of the São Paulo State, Brazil. In this region *An. albitarsis* and *An. albitarsis* B are sympatric. Furthermore, *An. albitarsis* B showed unimodal sunset crepuscular rhythms (Forattini *et al*., 1996). In another study carried out in the same region of the Ribeira Valley, Forattini *et al*. (1995) observed a concentration of *An. albitarsis* A and *An. albitarsis* B in rice field environment. *Anopheles albitarsis* B was also collected at sunset and sunrise on human bait performed indoors and outdoors in a rice plantation environment.

Medical importance. Nothing is known about medical importance of *An. albitarsis* B.

Systematics. Wilkerson *et al.* (1995a, b) used the RAPD-PCR method to differentiate species of the *An. albitarsis* complex. As a result, it was possible to recognize an unnamed species, which was designated as *Anopheles albitarsis* B. *An. albitarsis s.s.* and *An. albitarsis* B can be distinguished by the combination of the extent of the basal dark portion of hindtarsomere 2 and the length of the wing presectoral dark spot (PSD). *An. albitarsis s.s.* has 50% or less basal dark, while *An. albitarsis* B has more than 50% basal dark; PSD is absent or short (0.09 length o f wing) in *An. albitarsis s.s.*, whereas PSD is longer (0.05 – 0.14 length of wing) in *An. albitarsis* B. It is noteworthy to point out that Forattini *et al.* (1995, 1996) and Kakitani and Forattini (2000) considered *An. albitarsis* B to be *An. albitarsis s.s.* while *An. albitarsis* A was *An. albitarsis* B.

Anopheles (Nyssorhynchus) albitarsis E

Anopheles albitarsis specie E of Lehr *et al.* 2005: 908-917.

Female. Similar to *An. albitarsis s.s.* except for the characters as follow. Proboscis brown to dark brown, with decumbent scales and short setae, length 1.68-1.97 mm (mean=1.78±0.10) (n = 10), 1.27-1.43 length of forefemur (mean=1.35±0.06) (n=10), 1.01-1.12 length of maxillary palpus (mean=1.06±0.03) (n = 10). Labella similar in color to labium. Palpus 1.62-1.83 mm (mean=1.69 mm±0.07) (n=10), 0.89-0.99 length of proboscis (mean=0.94±0.03) (n = 10); length palpomere 2 / palpus length 0.25-0.30 (mean=0.28±0.01) (n=10); length palpomere 3 / palpus length 0.33-0.39 (mean=0.36±0.02) (n=10); length palpomere 4 / palpus length 0.16-0.21 (mean=0.18±0.01) (n=10); length palpomere 5 / palpus length 0.12-0.15 (mean=0.14±0.01) (n=10). Wing: length 2.76-3.19 mm (mean=2.91±0.10) (n=30); veins dark-scaled with spots of white scales on anterior costal, subcostal, R and R_1 veins, pale yellow scales on remainder veins. Forefemur 1.26-1.40 mm (mean=1.33±0.05) (n=10), 0.70-0.78 length of proboscis (mean=0.73±0.02) (n=10); tarsomere 2 dark-scaled proximally with a basal dark band 0.38-0.51 length of tarsomere (mean=0.45±0.05) (n=10).

Male. Similar to *An. albitarsis s.s.*

Male genitalia (Fig.11). Similar to *An. albitarsis s.s.* except for the characters as follow. Gonocoxite elongate with numerous spatulate scales; tergal surface with 4-5 setae and subapical surface with 1-2 setae. Tubercle of parabasal spine 0.22-0.35 (mean=0.28±0.05) (n=3) length of parabasal spine; dorsomedial rim 0.11–0.12 (mean=0.12±0.01) (n=2) length of gonocoxite; dorsal accessory setae 0.36-0.45 (mean=0.39±0.03) (n=4) length of gonocoxite; aedeagus 0.43-0.47 (mean=0.45±0.01) (n=4) length of gonocoxite.

Distribution. An. albitarsis E occurs in northern Brazil and Venezuela (Lehr *et al.*, 2005).

Material examined. Specimens examined are in table 1.

Bionomics. Póvoa *et al.* (2006) observed that *An. albitarsis* E was the most abundance species collected in Boa Vista, Roraima State, Brazil. In this region this species is anthropophilic and the activity biting of *An. albitarsis* E peaks was predominantly before midnight as observed for *An. marajoara* in Western Venezuela (Póvoa *et al*., 2006; Rubio-Palis & Curtis, 1992).

Medical importance. Based on ELISA, *An. albitarsis* E was found infected by *P. vivax* VK210 (25 specimens), *P.vivax* VK247 (2 specimens), *P. falciparum* (10 specimens) and *P. malariae* (9 specimen). Consequently, the species was confirmed as an important vector of human plasmodium in savannah habitat around Boa Vista, Roraima State, Brazil (Silva-Vasconcelos *et al*. 2002; Póvoa *et al*., 2006).

Systematics. Lehr, *et al*. (2005) based on the results of genetic and phylogenetic analyses of the mtDNA COI sequences suggested the existence of an unnamed phylogenetic species in the *An. albitarsis* complex. Contrasting, Wilkerson *et al*. (2005) and Li & Wilkerson (2006) did not find evidences from partial sequences of the ITS2 and D2 of the 28S domain both of the ribosomal DNA, and from fragments of the COI and N4 mitochondrial genes to corroborate Lehrs *et al*. (2005) hypothesis for *An. albitarsis* E.

Morphological variation

Means and standard deviations for 40 variables from 150 specimen of the *An. albitarsis* complex were obtained for comparisons (Table 2). Both tests of normality and homogeneity showed that all variables had a normal distribution. The variables

presector dark, sector pale, accessory sector dark, first dark spot of CuA_1 and second pale spot of CuA_1 were excluded from PCA analysis. These variables contained a large number of missing (Figure 12).

Principal components analysis

The principal components analysis showed that the ten first components explained 80.1% of the overall variance (Table 11). For a large number of components none variable contains coefficient with low value, thus it was not possible to reduce the number of variables.

Variance analysis

Variance analysis (ANOVA) indicated that all variables were showed differences between mean of all variables, except distance between eyes and presector pale that no show differences ($p \geq 0.05$) between the five species. Thus these two variables were excluded for the discriminate analysis (Table 12).

Discriminant analysis

Results of discriminant analysis with 150 specimens and 35 variables, or 103 specimens and 27 variables were the same. The morphometric multivariate techniques (discriminant analysis) demonstrated that some combinations of morphological variables may serve to discriminate the five species of the Albitarsis Complex (Tables 13, 14). Seventy four out of 103 (72%) and 114 out of 150 (68%) specimens analyzed were correct discriminated. High percent of discrimination was showed in *An. albitarsis* E (96% and 93%) when compared with the other species. The lowest percentage of discrimination was obtained for *An. marajoara* (50% and 40%). However the percent correct classification for the *An. albitarsis s.s.*, *An. albitarsis* B and *An. deaneorum* varied from 62% to 77%.

Cluster analysis

The cluster analysis that was based on the means of 35 variables resulted in morphometric dissimilarities among the clusters. The specimens were clustered in two groups (Table 15), one comprising of *An. albitarsis s.s.* and *An. albitarsis* B, and the other formed by *An. deaneorum* and *An. albitarsis* E. Fifty percent of the specimens identified by RAPD-PCR as *An. marajoara* were clustered in group one, the other 50% clustered in group two. Results of cluster analysis demonstrated that *An. albitarsis s.s.* and *An. albitarsis* B are more similar to each other than to the other species of the Albitarsis Complex. Similarly, *An. deaneorum* and *An. albitarsis* E were clustered together, whereas *An. marajoara* could be differentiated in two distinct groups (Table 15).

Results of the cluster analysis of these two groups separate corroborated the results of the discriminant analysis (Table 16), the rate of identification error was low. In conclusion, results of the cluster analysis suggest that the specimens collected in states of São Paulo, Pará, Mato Grosso and Amazonas from Brazil, and Zulia from Venezuela, which have an identical RAPD-PCR pattern, may belong to two distinct species.

In spite of being polymorphic, the pattern of pale and dark spots is considered to be an important taxonomic tool for species separation within the genus *Anopheles* (Faran, 1980; Hribar & Lawrence, 1995). Faran (1980) used the range and ratio of the length of several spots of the Costa vein in the adult identification key for species of the Albimanus section of *Nyssorhynchus*. In addition, Hribar & Lawrence (1995) used the length of wing Costal spots to separate populations of *An. nuneztovari* from Brazil and Venezuela.

Multivariate analysis of morphometric characters can be employed to separate sibling species of *Anopheles* (*Nyssorhynchus*). Similarly, this method of analysis was shown to be a useful tool to separate other medically important species of insects.

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References

Añez, N., Valenta, D.T., Cazorla, D., Quicke, D.J., Feliciangeli, M.D. 1997. Multivariate analysis to discriminate species of pheblotomine sand flies (Diptera: Psychodidae): *Lutzomyia towsendi* , *L. spinicrassa* and *L. youngi.* J Med Entomol, 34: 312-316.

Belkin, J. N., Schick, R.X., Heinemann, S.J. 1971. Mosquito studies (Diptera, Culicidae). XXV. Mosquitoes originally described from Brazil. Contr Am Entomol Inst, 7(5): 1-64.

Branquinho, M. S., Lagos, C.B., Rocha, R.M., Natal, D., Barata, J.M., Cochrane, A.H., Nardin, E., Nussenzweig , R.S., Kloetzel, J.K. 1993. Anophelines in the state of Acre, Brazil, infected with *Plasmodium falciparum*, *P. vivax*, the variant *P. vivax* VK247 and *P. malariae*. Trans R Soc Trop Med Hyg, 87(4): 391-394.

Brochero, H.H.L., Li, C., Wilkerson, R.C. 2007. A newly recognized species in the *Anopheles* (*Nyssorhynchus*) Albitarsis complex (Diptera: Culicidae) from Puerto Carreño, Colombia. Am J Trop Med Hyg, 76(6): 1113-1117.

Calle, D.A., Quiñones, M.L., Erazo, H.F., Jaramillo, J.O. 2002. Morphometric discrimination of females of five species of *Anopheles* of the subgenus *Nyssorhynchus* from Southern and Northwest Colômbia. Mem Inst Oswaldo Cruz, 97(8): 1191-1195.

Chadee, D.D. 1992. Indoor and outdoor host-seeking rhythms of *Anopheles albitarsis* (Diptera: Culicidae) in Trindad, West Indies. J Med Entomol, 29: 567-569.

Chadee, D.D., Wilkerson, R.C. 2006. Ecology of the malaria vector, A*nopheles* (*Nyssorhynchus) marajoara* Galvão and Damasceno in Trindad, West Indies. J Am Mosq Control Assoc, 22(1): 22-28.

Conn, J.E., Wilkerson, R.C., Segura, M.N., de Souza. R.T., Schlichting. C.D., Wirtz. R.A., Povoa. M.M. 2002. Emergence of a new neotropical malaria vector facilitated by human migration and changes in land use. Am J Trop Med Hyg, 66(1): 18-22.

Corrêa, R. R., Ramos, A.S. 1943. Descrição de uma nova sub-especie de anofelino do sub-genero *Nyssorhynchus* Blanchard, 1902 (Diptera, Culicidae). Rev Paul Med, 22: 246-248.

Corrêa, R. R., Lima, F.O., Coda, D.1950. Observations on the flight and longevity in nature of *Anopheles albitarsis domesticus*. J Nat Malar Soc, 9: 280-284.

Cova-Garcia, P. 1946. Notas sobre los anofelinos de Venezuela y su identifcacion. Caud Amar Conf Sanit Panamer, 12: 208 pp.

Faran, M.E. (1980) Mosquito studies (Diptera: Culicidae) XXXIV. A revision of the Albimanus Section of the subgenus *Nyssorhynchus* of *Anopheles*. Cont Amer Entomol Inst, 15(7):1-215.

Forattini, O.P., Kakitani, I., Massad, E., Marucci, D. 1995. Studies on mosquitoes (Diptera: Culicidae) and anthropic environment. 7-behaviour of adults *Nyssorhynchus* anophelines with special reference to *Anopheles albitarsis s.l.* in south-eastern Brazil. Rev Saude Publica, 29(1): 20-6.

Forattini, O.P., Kakitani, I., Massad, E., Marucci, D. 1996. Studies on mosquitoes (Diptera:Culicidae) and anthropic environment. 12-host-seeking behavior of

Anopheles albitarsis s.l. in south-eastern Brazil. Rev Saude Publica, 30(4): 299-303.

Forattini, O.P., Massad, E. 1998. Culicidae vectors and anthropic changes in a southern Brazil natural ecosystem. Ecosystem Health, 4: 9-19.

Galvão, A. L. A., Lane, J. 1937. Nota sobre os *Nyssorhynchus* de S. Paulo. VII. Estudo sobre as variedades deste grupo com a descrição de *Anopheles* (*Nyssorhynchus*) *albitarsis* Arrib., 1878 var. *limai* n. var. Ann Fac Med, 13: 211-238.

Galvão, A. L. A., Damasceno, R. G.. 1942. Sobre um novo anofelino da Ilha de Marajó, *Anopheles* (*N.*) *marajoara* n. sp. Ann Paulist Med Cir, 44: 424-427.

Galvão, A. L. A., Damasceno, R.G. 1944. Observações sobre anofelinos do complexo *albitarsis* (Diptera Culicidae). Ann Fac Med S. Paulo, 20: 73-87.

Garcia, M., Casal, O.H. 1964 (1965). Descripcion de tres pupas de mosquitos neotropicales (Diptera, Culicidae). Revta Soc Entomol Argent, 27: 5-10.

Gelbre-Michael, T., Medhin G. 1997. Morphometric separation of female of *Phelebotomus* (*Phlebotomus*) *dudoscqui* and *P.*(*P.*) *bergetroti* (Diptera: Psycodiae). J Med Entomol, 34: 383-386.

Harbach, R. E., Knight, K. L. 1980. Taxonomists'glossary of mosquito anatomy. Plexus, Marlton, N. J.

Hribar, L.J. 1995. Costal wing spot variation within and among progeny of single female *Anopheles nuneztovari* (Diptera: Culicidae). Mosq Syst, 27(1): 1-15.

Hugh, G., Gauch, J.R. Multivariate in community ecology. New Cork, Cambridge, 1982.

Kakitani, I., Forattini, O.P. 2000. Parity and ovarian development of *Anopheles albitarsis* l.s. in irrigated agroecosystem field. Rev Saude Publica 34(1): 33-38.

Kakitani, I., Ueno, H.M., Forattini, O.P. 2003. Parity and wind impacto n the frequency of *Anopheles marajoara* in Brazil. Rev Saúde Pública, 37(3): 280-284.

Kitzmiller, J.B. 1977. Chromosomal differences among species of *Anopheles* mosquitoes. Mosq Syst, 9(2): 112-122.

Klein, T. A., Lima, J.B.1990. Seasonal distribution and biting patterns of *Anopheles* mosquitoes in Costa Marques, Rondônia, Brazil. J Am Mosq Control Assoc, 6(4): 700-707.

Klein, T. A., Lima, J.B., Tang, A.T. 1990. Colonization and maintenance of *Anopheles deaneorum* in Brazil. J Am Mosq Control Assoc, 6(3): 510-3.

Klein, T. A., Lima, J.B., Tang, A.T. 1991a. Biting behavior of *Anopheles* mosquitoes in Costa Marques, Rondônia, Brazil. Rev Soc Bras Med Trop, 24(1): 13-20.

Klein, T. A., Lima, J.B., Tang, A.T. 1991b. Hybridization evidence supporting separate species status for *Anopheles albitarsus* and *Anopheles deaneorum* (Diptera: Culicidae) in Brazil. J Am. Mosq Control Assoc, 7(2): 301-303.

Klein, T. A., Lima, J. B., Tada, M. S. 1991c. Comparative susceptibility of anopheline mosquitoes to *Plasmodium* falciparum in Rondonia, Brazil. Am J Trop Med Hyg, 44(6): 598-603

Klein, T. A., Lima, J.B., Tada, M.S., Miller, R. 1991d. Comparative susceptibility of anopheline mosquitoes in Rondônia, Brazil to infection by *Plasmodium vivax*. Am J Trop Med Hyg, 45(4): 463-470.

Kreutzer, R. D., Kitzmiller, J.B., Rabbani, M.G. 1976. Cytogenetically distinguishable sympatric and allopatric populations of the mosquito *Anopheles albitarsis*. Acta Amazônica, 6(4): 473-481.

Lane, J. 1953. Neotropical Culicidae. 1112 pp., illus. Sao Paulo, Brazil.

Lehr, M.A., Kilpatrick, C.W., Wilkerson, R.C., Conn, J.E. 2005. Cryptic species in the *Anopheles (Nyssorhynchus) albitarsis* (Diptera: Culicidae) complex: incongruence between Random Amplified Polymorphic DNA-Polymerase chain reaction identification and analysis of mitochondrial DNA *COI* gene sequences. Ann Entomol Soc Am, 98(6): 908-917.

Li, C., Wilkerson, R.C. 2005. Identification of *Anopheles (Nyssorhynchus) albitarsis* complex species (Diptera: Culicidae) using rDNA internal transcribed spacer 2-basepolymerase chain reaction primers. Mem Inst Oswaldo Cruz, 100: 495-500.

Li, C., Wilkerson, R.C., Fonseca, D.M. 2005. Isolation of polymorphic microsatellite markers from the malaria vector *Anopheles marajoara* (Diptera: Culicidae). Mol Ecol Notes, 5: 65-67.

Li, C., Wilkerson, R.C. 2006. Intragenomic rDNA ITS2 variation in the Neotropical *Anopheles* (*Nyssorhynchus*) *albitarsis* complex (Diptera: Culicidae). J Heredity, 98(1): 51-59.

Lima, J.B.P., Valle, D., Peixoto, A.A. 2004. Adaptation of a South american malaria vector to laboratory colonization suggests master-male evolution for mating ability. BMC Evol Biol, 4: 1-6.

Linthicum, K.J. 1988. A revision of the Argyritarsis Section of the subgenus *Nyssorhynchus* of *Anopheles* (Diptera: Culicidae). Mosq Syst, 20: 98-271.

Lounibos, L.P., Lima, D.C., Lourenco-de-Oliveira, R., Escher, R.L., Nishimura, N. 1998. Egg maturation in Neotropical malaria vectors: one blood meal is usually enough. J Vector Ecol, 23(2): 195-201.

Lounibos, L.P., Conn, J.E. 1991. Fecundity, parity, and adult feeding relationships among *Nyssorhynchus* malaria vectors from Venezuela. Mem Inst Oswaldo Cruz, 86(1): 57-66.

Lourenço-de-Oliveira, R., Luz, S.L. 1996. Simian malaria at two sites in the Brazilian Amazon- II. Vertical distribution and frequency of anopheline species inside and outside the forest. Mem Inst Oswaldo Cruz, 91(6): 687-94.

Lynch Arribalzaga, F. 1878. Descripcion de tres nuevos Culicidae de Buenos Aires. Nat Argent, 1:149-152. Madr, 25: 313-330.

Marrelli, M.T., Branquinho, M.S., Hoffmann, E.H., Taipe-Lagos, C.B., Natal, D., Kloetzel, J.K. 1998. Correlation between positive serology for *Plasmodium vivax*-like/*Plasmodium simiovale* malaria parasites in the human and anopheline populations in the State of Acre, Brazil. Trans R Soc Trop Med Hyg, 92(2): 149-151.

Marrelli, M.T., Sallum, M.A.M., Marinotti, O. 2006. The second internal transcribed spacer of nuclear ribosomal DNA as a tool for Latin American anopheline taxonomy – A critical review. Mem Inst Oswaldo Cruz, 101(8): 817-832.

Merritt, T.J.S., Young, C.R., Vogt, R.G., Wilkerson, R.C., Quattro, J.M. 2005. Intron retention identifies a malaria vector within the Anopheles (Nyssorhynchus) albitarsis complex (Diptera: Culicidae). Mol Phylog Evol, 35: 719-724.

Narang, S.K., Klein, T.A., Perera, O.P., Lima, J.B., Tang, A.T. 1993. Genetic evidence for the existence of cryptic species in the *Anopheles albitarsis*

complex in Brazil: allozymes and mitochondrial DNA restriction fragment length polymorphisms. Biochem Genet, 31(1-2): 97-112.

Petrarca, V., Sabatelli, G., Toure, Y.T., Dideco, M.A. 1998. Morphometric multivariate analysis in *Anopheles gambiae* s.s. (Diptera: Culicidae). J Med Entomol, 35: 16-25.

Pinto, C. 1939. Disseminação da malária pela aviação; biologia do *Anopheles gambiae* e outros anofelineos do Brasil. Mem Inst Oswaldo Cruz, 34: 293-294.

Póvoa, M.M., Wirtz, R.A., Lacerda, R.N.L., Miles, M.A. Warhurst, D. 2001. Malaria vectors in the municipality of Serra do Navio, State of Amapá, Amazon Region, Brazil. Mem Inst Oswaldo Cruz, 96(2): 179-184.

Póvoa, M.M., Souza de, R.T.L., Lacerda, R.N.L., Rosa, E.S., Galiza, D., Souza de, J.R., Wirtz, R.A., Schilichting, C.D., Conn, J.E. 2006. The importance of *Anopheles albitarsis* E and *An. darlingi* in human malaria transmission in Boa Vista, State of Roraima, Brazil. Mem Inst Oswaldo Cruz, 101(2): 163-168.

Quiñones, M.L., Suarez, M.F. 1990. Indoor resting heights of some anophelines in Colombia. J Am Mosq Control Assoc, 6(4): 602-604.

Romeo-Viamonte, J. M., Castro, M. 1951. Estudio de la morfologia de la armadura faringea de algunos anofilinos. (Dipt. Culic.). Revta San Hig Publ Madr, 25: 313-330.

Rosa-Freitas, M.G. 1989. *Anopheles* (*Nyssorhynchus*) *deaneorum*: a new species in the albitarsis complex (Diptera: Culicidae). Mem Inst Oswaldo Cruz, 84: 535-543.

Rosa-Freitas, M.G., Deane, L.M.. 1989. The neotype of *Anopheles albitarsis* (Diptera: Culicidae). Mem Inst Oswaldo Cruz, 84: 289-302.

Ross, E.S., Roberts, H.R. 1943. Mosquito atlas. Part I. The Nearctic *Anopheles*, important malaria vectors of the Americas and *Aedes aegypti*, *Culex quinquefasciatus*. 44 pp., illus.

Rozeboom, L.E. 1942. Subspecific variations among Neotropical *Anopheles* mosquitoes, and their importance in the transmission of malaria. Am J trop Med, 22: 235-255.

Rubio-Palis, Y., Curtis, C.F. 1992. Biting and resting behaviour of anophelines in western Venezuela and implications for control of malaria transmission. Med Vet Entomol, 6: 325-334.

Rubio-Palis, Y. 1995. Differential attraction of Venezuelan Anophelines to human collectors. J Am. Mosq Control Assoc, 11(4): 482-484.

Rubio-Palis, Y., Manguin, S., Ayesta, C., Guzman H., Arcia, J.M., Gonzalez, J.Jr., Perez, E. 1997. Revisión taxonômica de los anofelinos vectores de malaria em el sur de Venezuela. Bol Dir Malariol San Amb, 37: 35-48.

Rubio-Palis, Y. 1998. Caracterización morfométrica de poblaciones de *Anopheles (Nyssorhynchus) darling*i del sur de Venezuela. Bol Entomol Venez, 13: 141-172.

Rubio-Palis, Y., Wilkerson, R.C., Guzman, H. 2003. Morphological characters of adult *Anopheles (Nyssorhynchus) marajoara* in Venezuela. J Am Mosq Control Assoc, 19: 107-114.

Sallum, M.A.M., Schultz, T.R., Foster, P.G., Aronstein, K., Wirtz, R.A., Wilkerson, R.C. 2002. Phylogeny of Anophelinae (Diptera: Culicidae) based on nuclear ribosomal and mitochondrial DNA sequences. Syst Entomol, 27: 361-382.

Santos, R.L., Forattini, O.P., Burattini, M.N. 2002. Laboratory and field observations on duration of gonotrophic cycle of *Anopheles albitarsis* s.l. (Diptera: Culicidae) in southeastern Brazil. J Med Entomol, 39(6):926-930.

Santos, R.L., Forattini, O.P. 1999. Marking-release-recapture methods for determining the size of the natural population of *Anopheles albitarsis l.s* (Diptera:Culicidae). Rev Saude Publica, 33(3): 309-313.

Santos, J.B., dos Santos, F., Macedo, V. 1999. Variation of *Anopheles* density with deltamethrin-impregnated mosquito nets in an endemic malaria area of the Brazilian Amazon. Cad Saude Publica, 15(2): 281-292.

Senevet, G. 1934. Contribution a l'etude des nymphes d'anophelines. (4e Memoire). Arch Inst Pasteur Alger, 12: 29-76.

Silva-Vasconcelos, A., Kato, M.Y., Mourao, E.N., de Souza R.T., Lacerda, R.N., Sibajev, A., Tsouris, P., Povoa, M.M., Momen H., Rosa-Freitas, M.G. 2002. Biting índices, host-seeking activity and natural infection rates of anopheline species in Boa Vista, Roraima, Brazil from 1996 to 1998. Mem Inst Oswaldo Cruz, 97: 151-161.

Steiner, W.W.M., Narang, S., Kitzmiller, J.B., Swofford, D.L. 1982. Genetics diversity and evolution in neotropical *Anopheles* (Subgenus *Nyssorhynchus*). In Steiner, W.W.M., Tabachnick, W.J., Rai, K.S., Narang, S.K. [eds.], Recent Developments in the Genetics of Insects Disease Vectors, Stipes, Champaign, IL, pp. 523-550.

Voorham, J. 2002. Intra-population plasticity of *Anopheles darlingi*'s (Diptera, Culicidae) biting activity patterns in the state of Amapá, Brazil. Rev Saude Publica, 36(1): 75-80.

Wilkerson, R. C., Peyton, E.L. 1990. Standardized nomenclature for costal wing spots of the genus *Anopheles* and other spotted-wing mosquitoes (Diptera: Culicidae). J Med Entomol, 27: 208-224.

Wilkerson, R.C., T.J. Parsons, T.A. Klein, T.V. Gaffigan, E. Bergo, J. Consolim. 1995a. Diagnosis by random amplified polymorphic DNA polymerase chain reaction of four cryptic species related to *Anopheles* (*Nyssorhynchus*) *albitarsis* (Diptera: Culicidae) from Paraguay, Argentina, and Brazil. J Med Entomol, 32(5): 697-704.

Wilkerson, R.C., T.V. Gaffigan, J.B. Lima. 1995b. Identification of species related to *Anopheles* (*Nyssorhynchus*) *albitarsis* by random amplified polymorphic DNA-polymerase chain reaction (Diptera: Culicidae). Mem Inst Oswaldo Cruz, 90(6): 721-32.

Wilkerson, R.C., Foster, P.G., Li, C., Sallum, M.A.M. 2005. Molecular phylogeny of Neotropical *Anopheles (Nyssorhynchus) albitarsis* species complex (Diptera: Culicidae). Ann Ent Soc Amer, 98(6): 918-925.

Zimmerman, R.H., Galardo, A.K.R., Lounibos, L.P., Arruda, M., Wirtz, R. 2006. Bloodmeal hosts of *Anopheles* species (Diptera: Culicidae) in a malaria-endemic area of the Brazilian Amazon. J Med Entomol, 43(5): 947-956.

Figure legends

Figure 1. Known distribution sites of five species of the *An. albitarsis* complex

Figure 2. Pupa and male genitalia of *Anopheles albitarsis s.s.* Pupa. **CT**: cephalothorax; **P**: paddle; **I-IX**: abdominal segments. **Male genitalia.** **A**: gonostylus; **B**: dorsal view of gonocoxite; **C**: internal seta; **D**: accessory seta; **E**: aedeagus; **F**: dorsal claspette; **G**: tubercle of parabasal spine; **H**: ventral claspette. **Ventral claspette**. Ventral view of gonocoxite**.** Scales in mm.

Figure 3. First-instar larva of *Anopheles albitarsis s.s.* **A**: antenna; **C**: cranium; **Dm**: dorsomentrum; **M**: mesothorax; **P**: prothorax; **SA**: spiracular aparatus; **T**: metathorax; **Vm**: ventromentum; **I-VIII**: abdominal segments; **X**: anal lobe. Scales in mm.

Figure 4. Fourth-instar larva of *Anopheles albitarsis.* **A**: antenna; **C**: cranium; **Dm**: dorsomentrum; **M**: mesothorax; **P**: prothorax; **SA**: spiracular aparatus; **T**: metathorax; **Vm**: ventromentum; **I-VIII**: abdominal segments; **X**: anal lobe. Scales in mm.

Figure 5. Pupa and male genitalia of *Anopheles marajoara*. Pupa. **CT**: cephalothorax; **P**: paddle; **I-IX**: abdominal segments. **Male genitalia.** **A**: gonostylus; **B**: dorsal view of gonocoxite; **C**: internal seta; **D**: accessory seta; **E**:

aedeagus; **F**: dorsal claspette; **G**: tubercle of parabasal spine; **H**: ventral claspette. **Ventral claspette**. Ventral view of gonocoxite**.** Scales in mm.

Figure 6. Fourth-instar larva of *Anopheles marajoara.* **A**: antenna; **C**: cranium; **Dm**: dorsomentrum; **M**: mesothorax; **P**: prothorax; **SA**: spiracular aparatus; **T**: metathorax; **Vm**: ventromentum; **I-VIII**: abdominal se segments; **X**: anal lobe. Scales in mm.

Figure 7. Pupa and male genitalia and of *Anopheles deaneorum*. Pupa. **CT**: cephalothorax; **P**: paddle; **I-IX**: abdominal segments. **Male genitalia.** **A**: gonostylus; **B**: dorsal of gonocoxite; **C**: internal seta; **D**: accessory seta; **E**: aedeagus; **F**: dorsal claspette; **G**: tubercle of parabasal spine; **H**: ventral claspette. **Ventral claspette**. Ventral view of gonocoxite**.** Scales in mm.

Figure 8. Fourth-instar larva of *Anopheles deaneorum*. **A**: antenna; **C**: cranium; **Dm**: dorsomentrum; **M**: mesothorax; **P**: prothorax; **SA**: spiracular aparatus; **T**: metathorax; **Vm**: ventromentum; **I-VIII**: abdominal segments; **X**: anal lobe. Scales in mm.

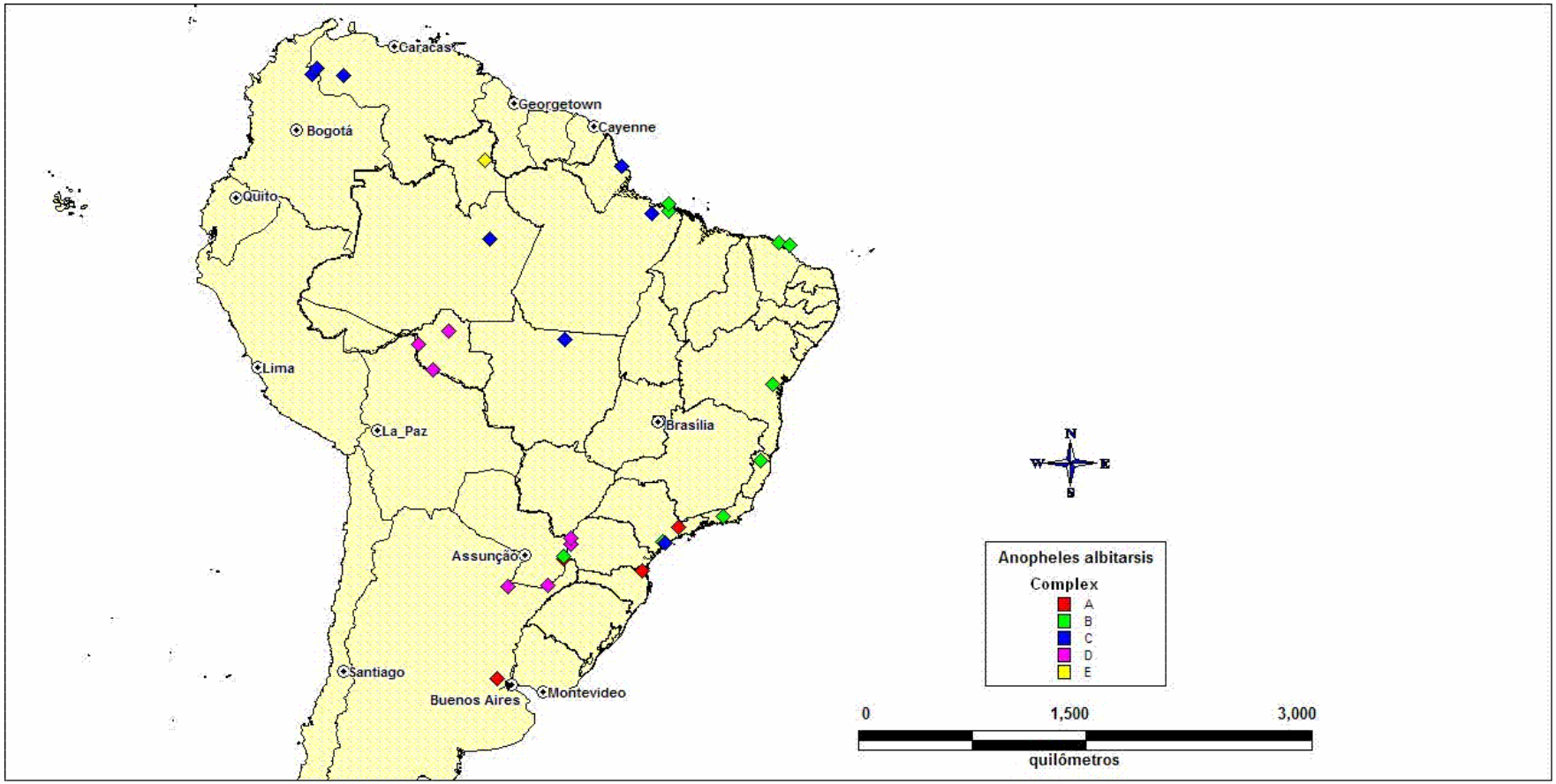
Figure 9. Pupa and male genitalia of *Anopheles albitarsis* B. Pupa. **CT**: cephalothorax; **P**: paddle; **I-IX**: abdominal segments. **Male genitalia.** **A**: gonostylus; **B**: dorsal view of gonocoxite; **C**: internal seta; **D**: accessory seta; **E**: aedeagus; **F**: dorsal claspette; **G**: tubercle of parabasal spine; **H**: ventral claspette. **Ventral claspette**. Ventral view of gonocoxite**.** Scales in mm.

Figure 10. Fourth-instar larva of *Anopheles albitarsis* B. **A**: antenna; **C**: cranium; **Dm**: dorsomentrum; **M**: mesothorax; **P**: prothorax; **SA**: spiracular aparatus; **T**: metathorax; **Vm**: ventromentum; **I-VIII**: abdominal segments; **X**: anal lobe. Scales in mm.

Figure 11. Male genitalia and ventral claspette of *Anopheles albitarsis* E. Male genitalia. **A**: dorsal view of gonostylus; **B**: gonocoxite; **C**: internal seta; **D**: accessory seta; **E**: aedeagus; **F**: dorsal claspette; **G**: tubercle of parabasal spine; **H**: ventral claspette. **Ventral claspette**. Ventral view of gonocoxite**.** Scales in mm.

Figure 12. Missing data of 30 characters measured from 150 specimens of *An.* albitarsis complex.

Figure 1. Known distribution sites of five species of the *An. albitarsis* complex.



A - *An. albitarsis s.s.*; B - *An. albitarsis* B; C - *An.marajoara*; D - *An. deaneorum*; E - *An. albitarsis* E.

Fig. 2

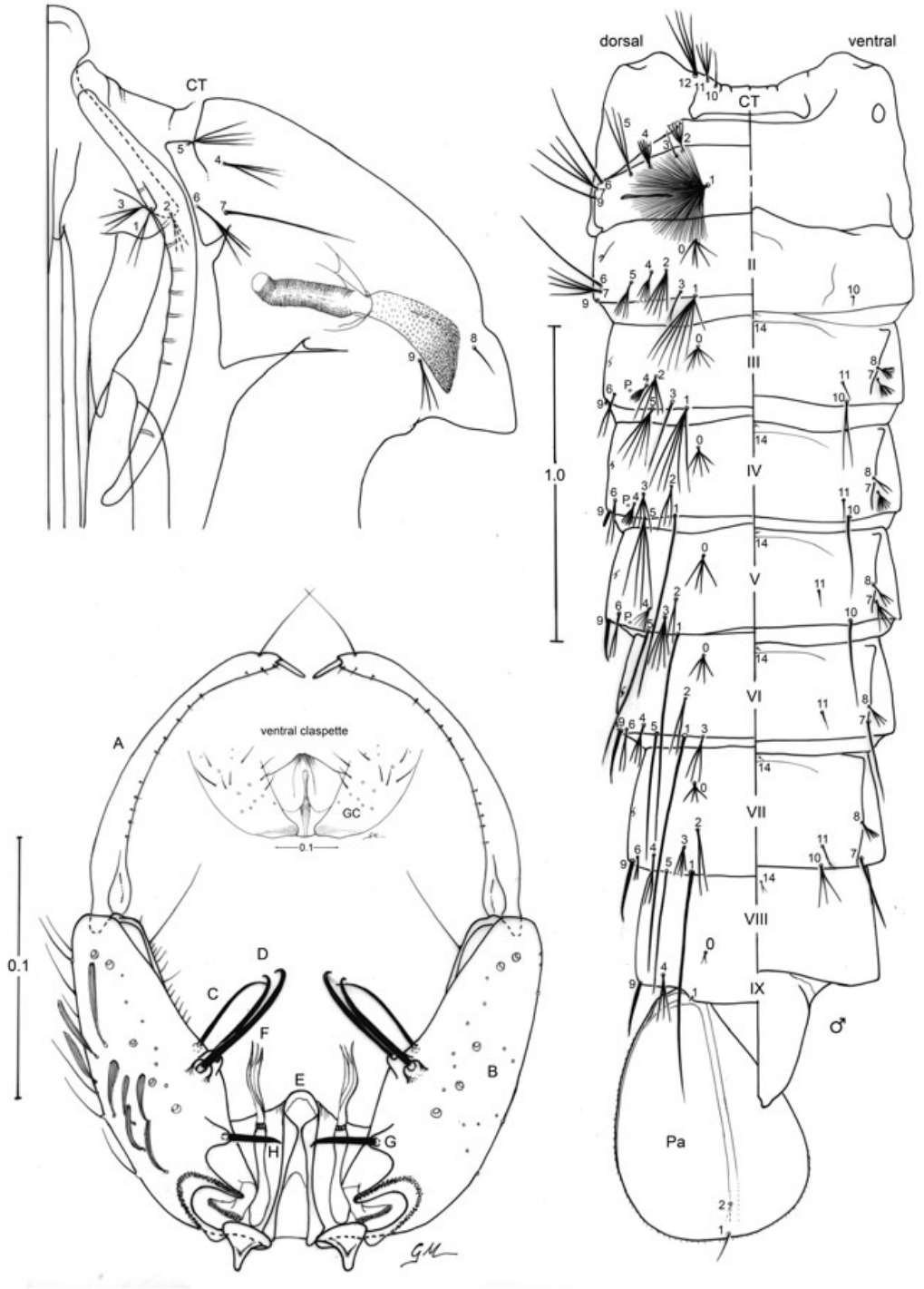

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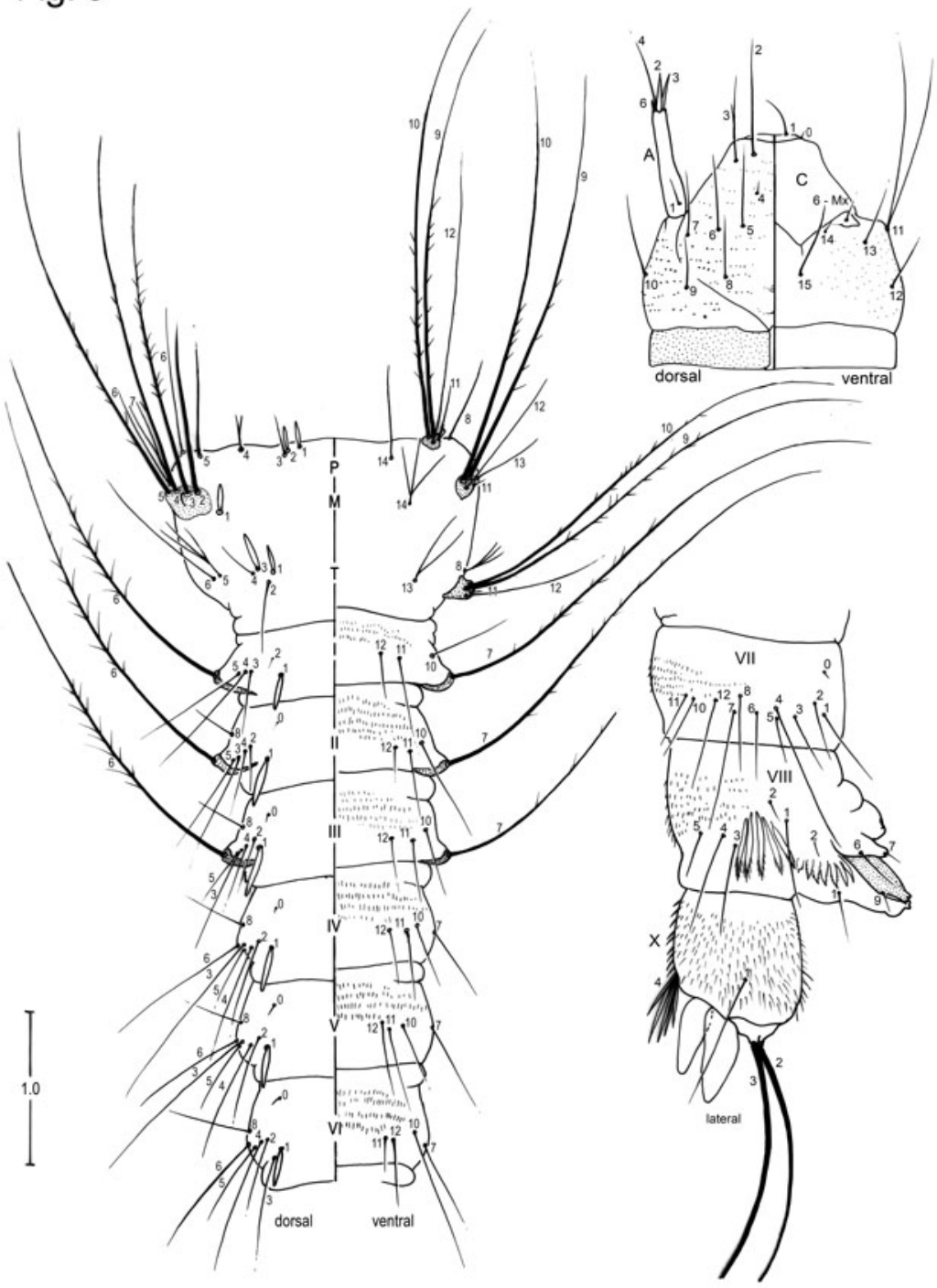
Fig. 3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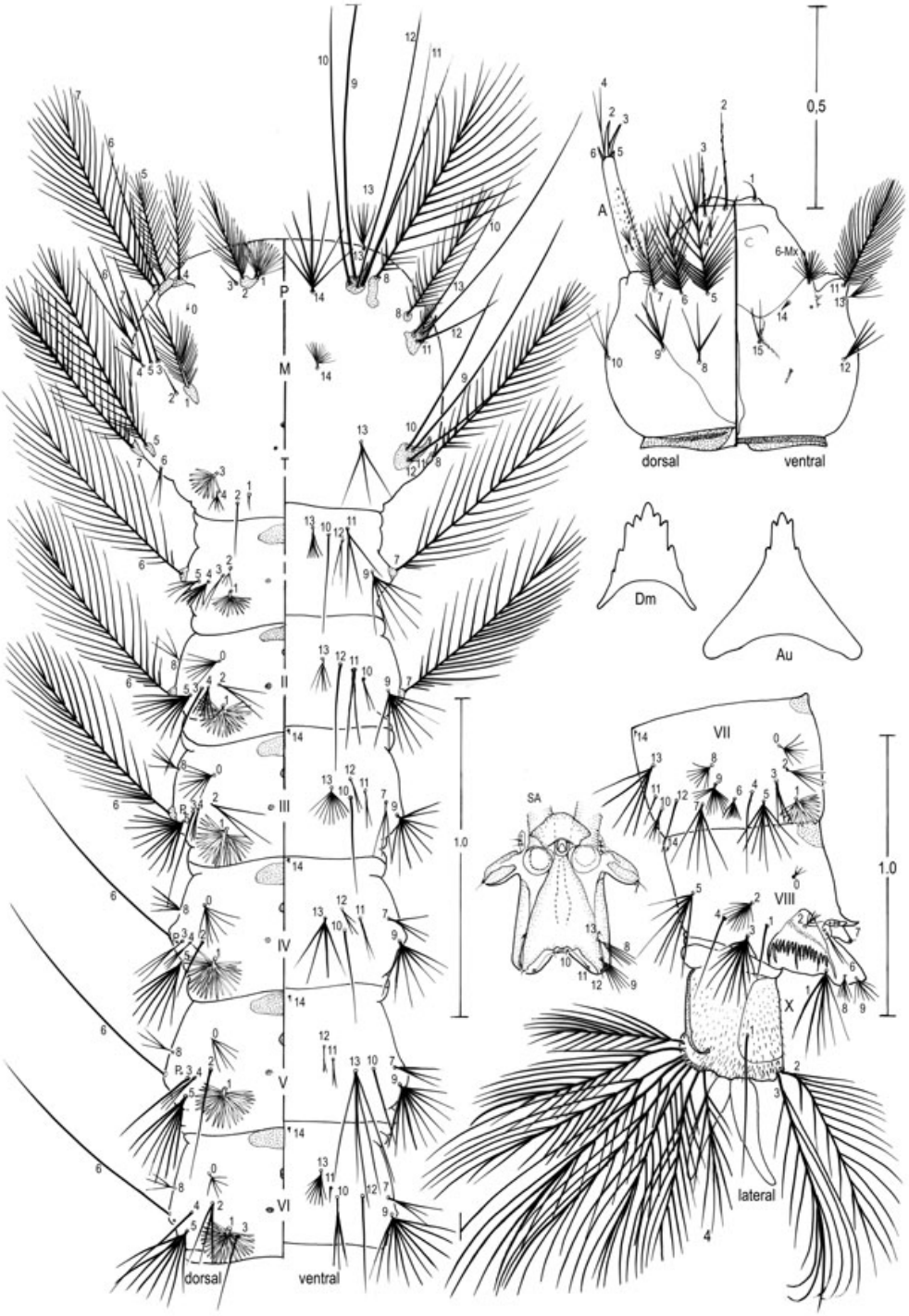
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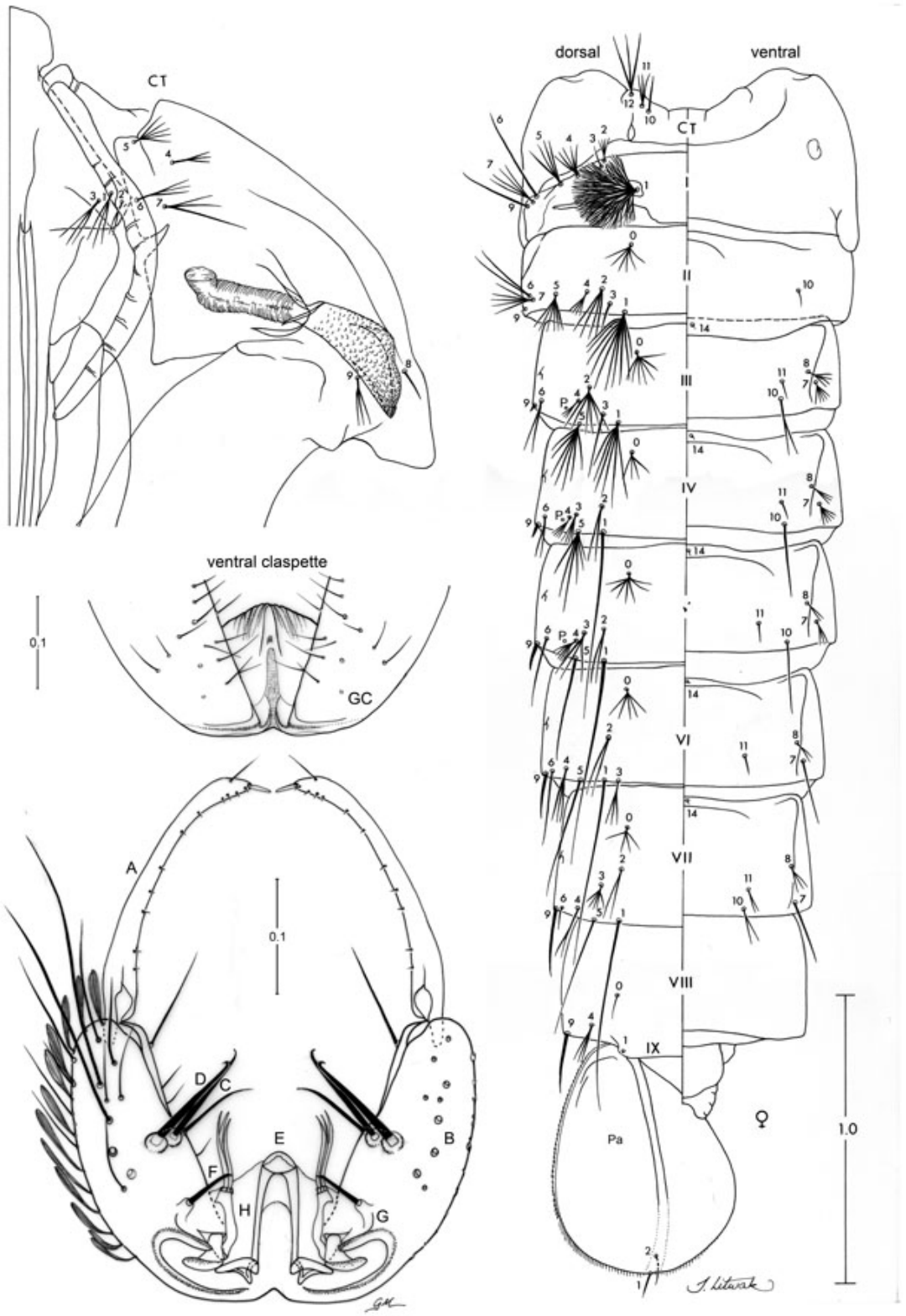
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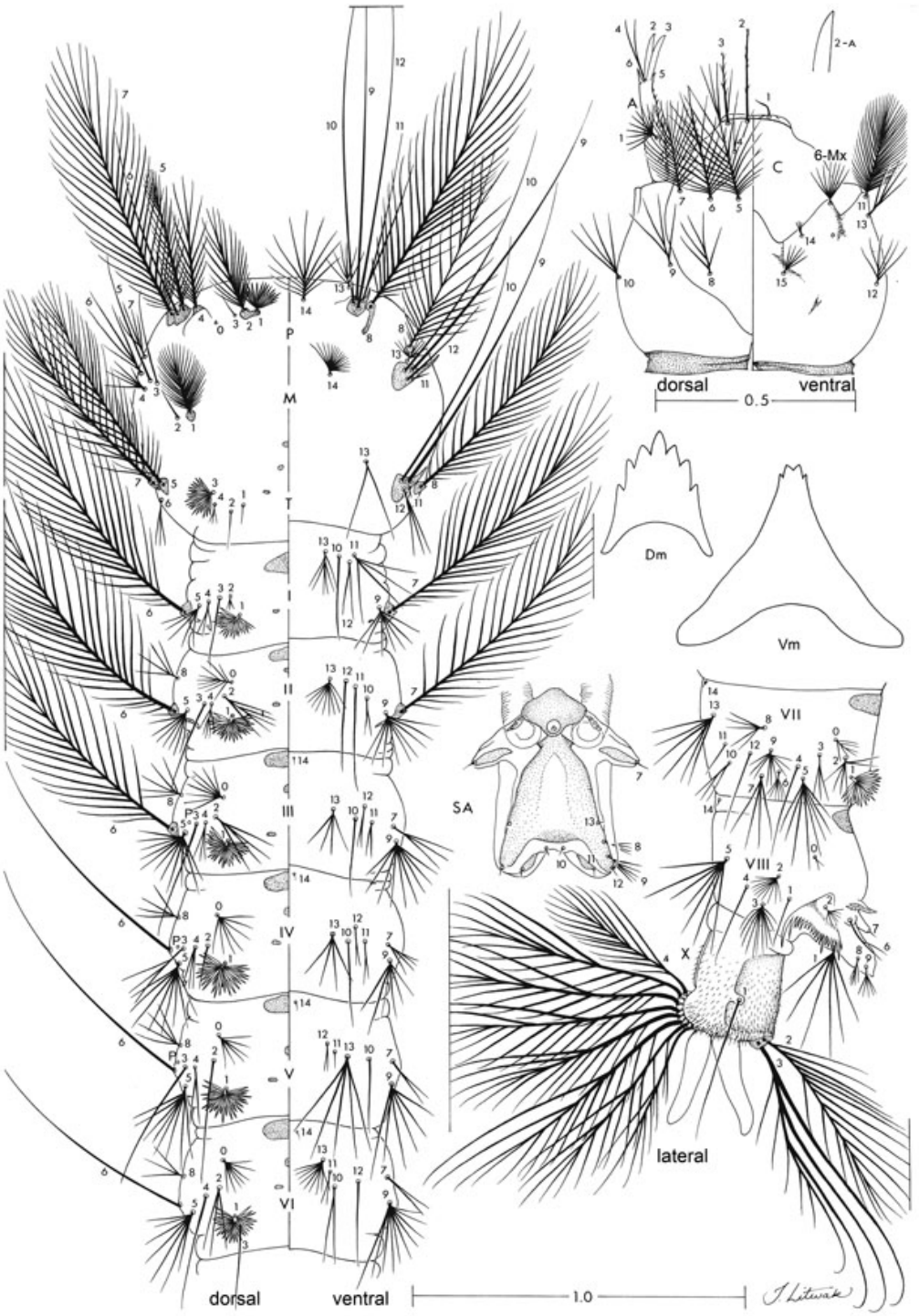
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Fig. 7

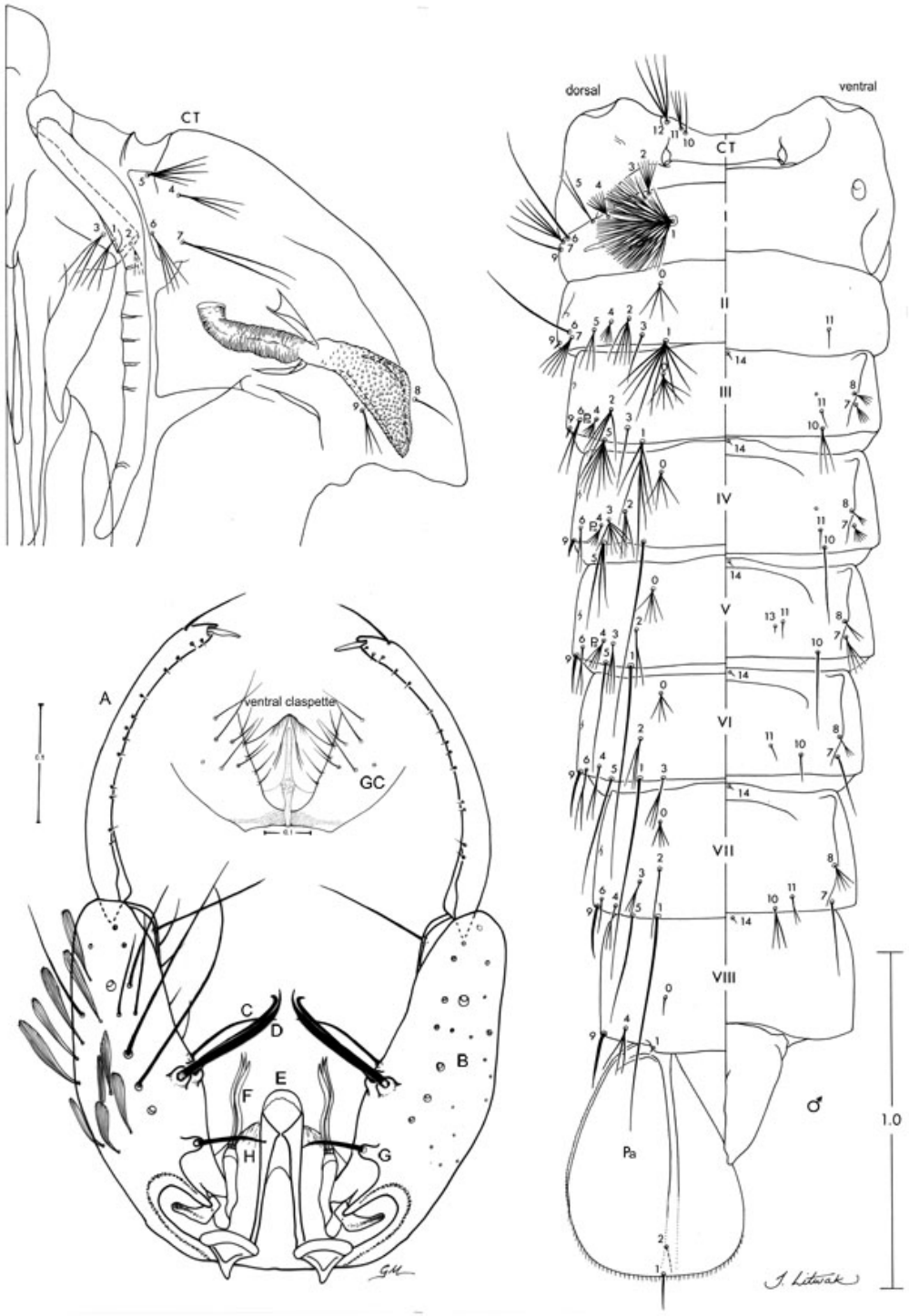
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Fig. 8

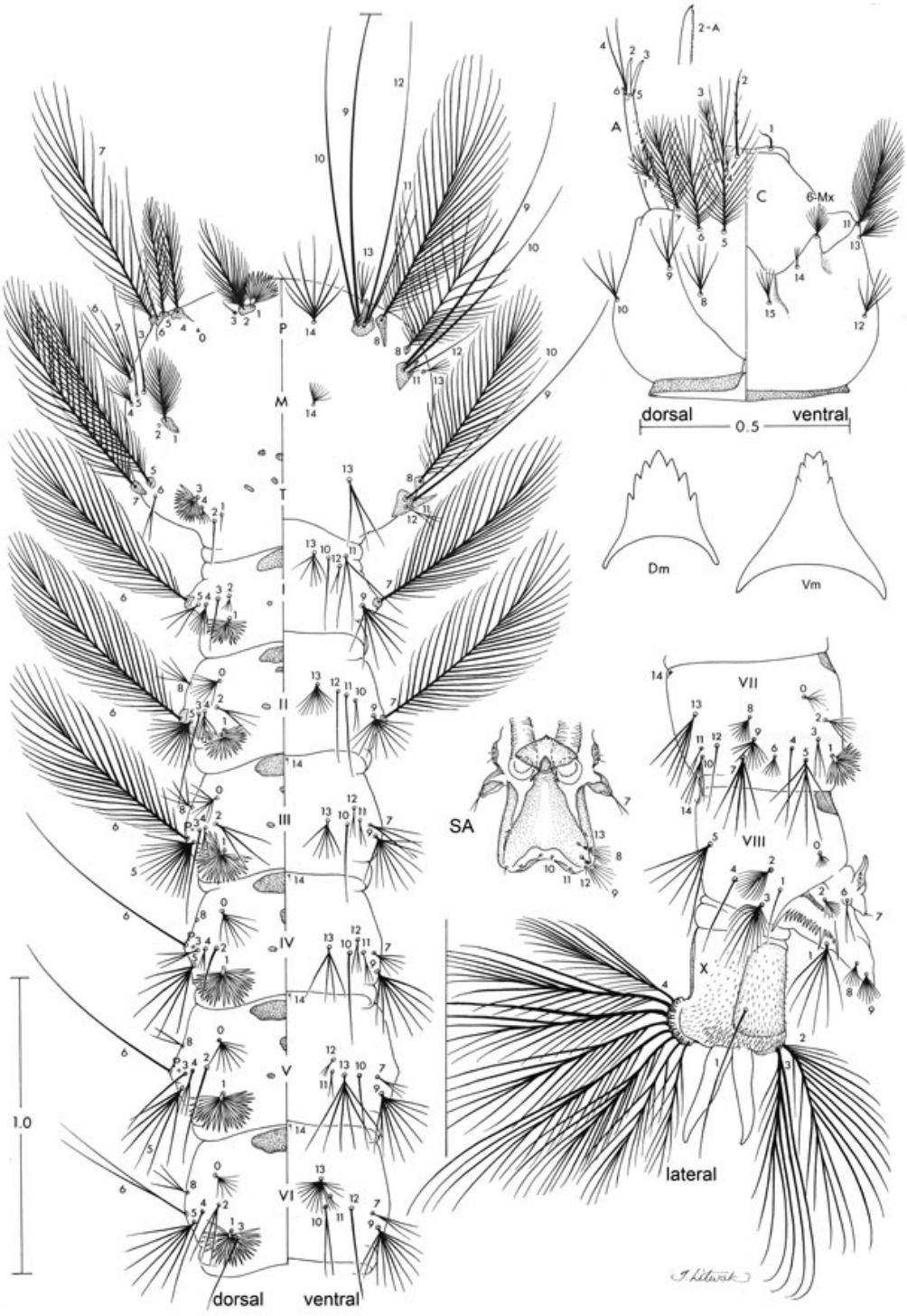
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Fig. 9

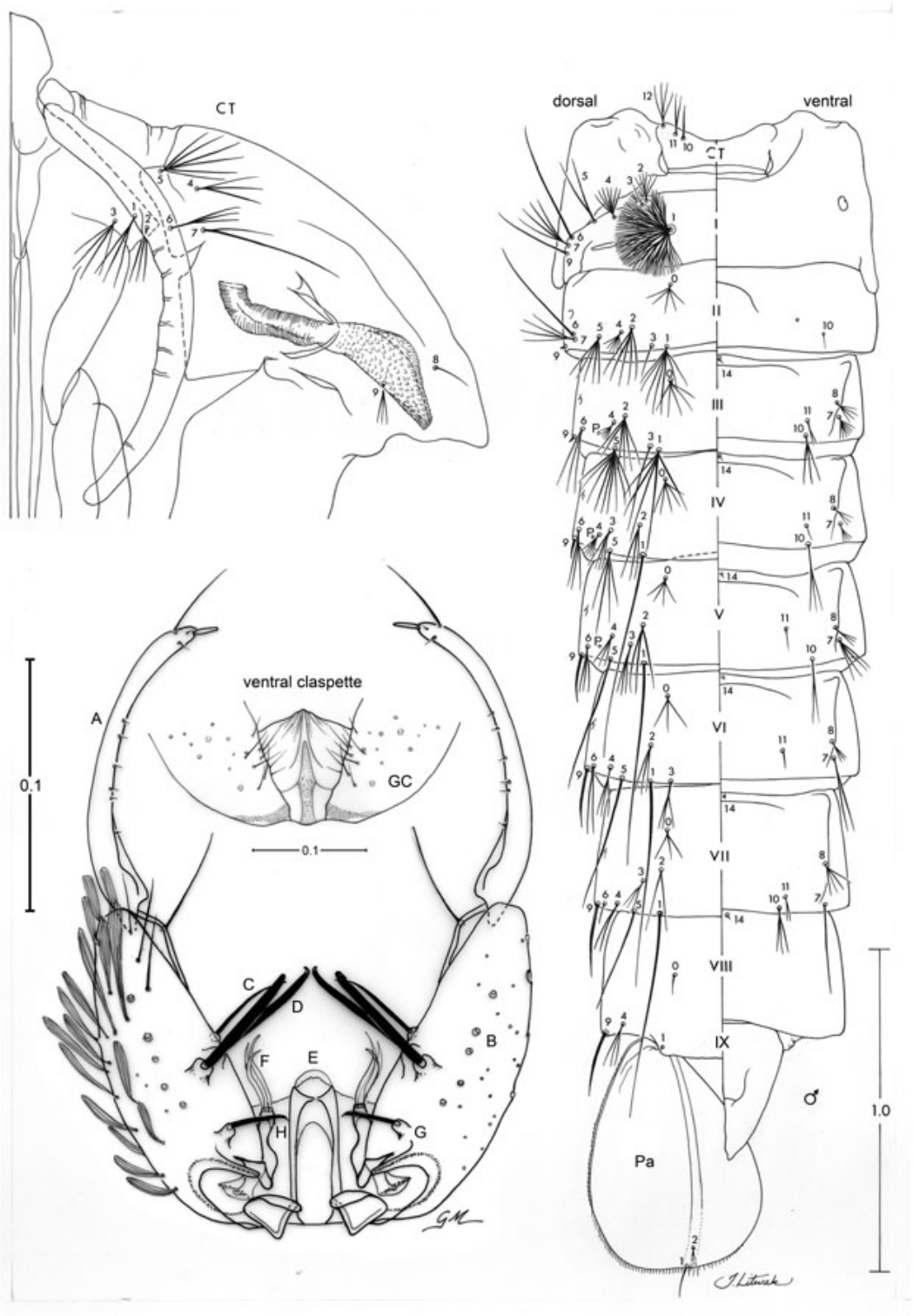

Fig. 10

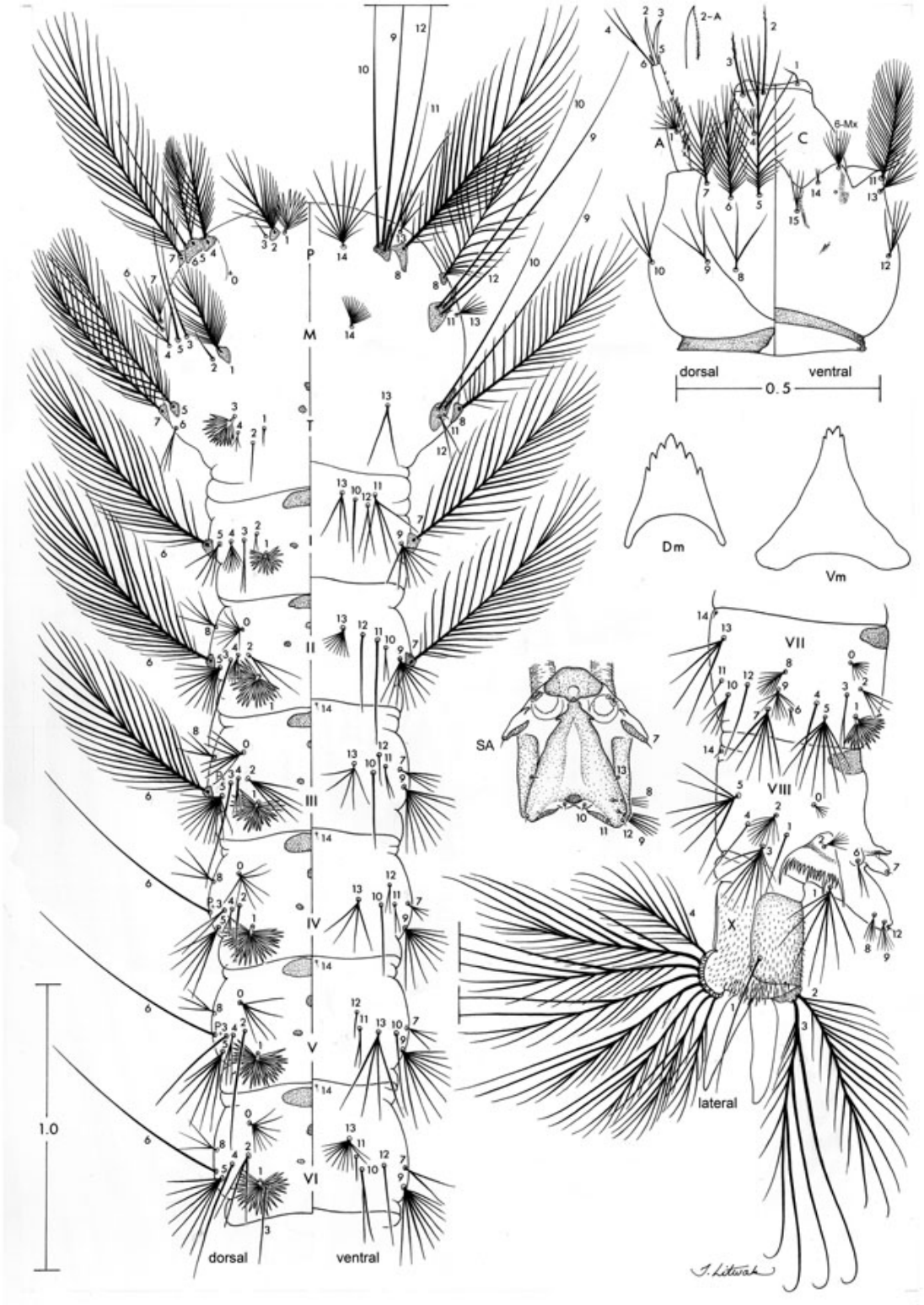
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Fig. 11

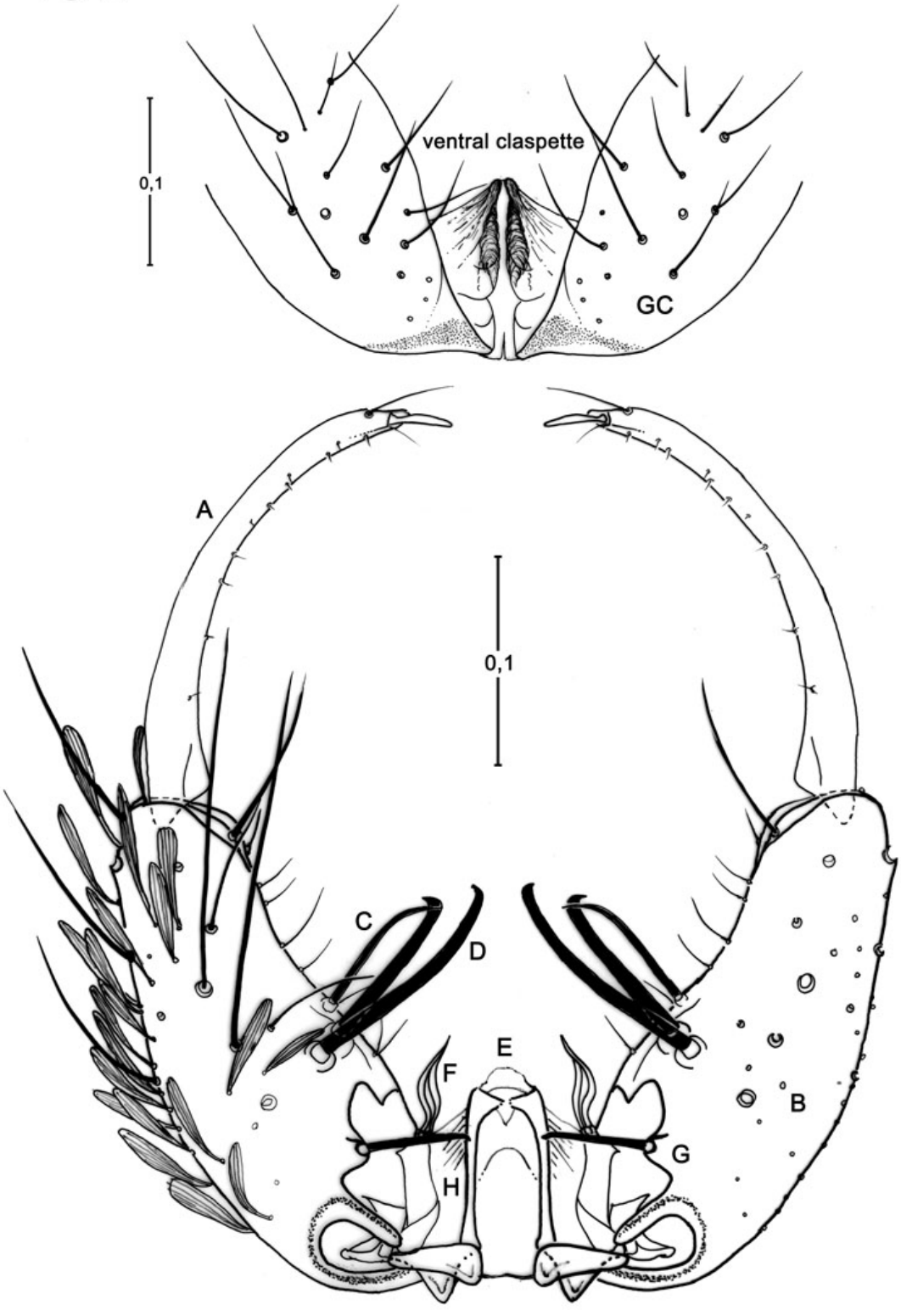

Figure 12. Missing data of 40 characters measured from 150 specimens of *An. albitarsis* complex.

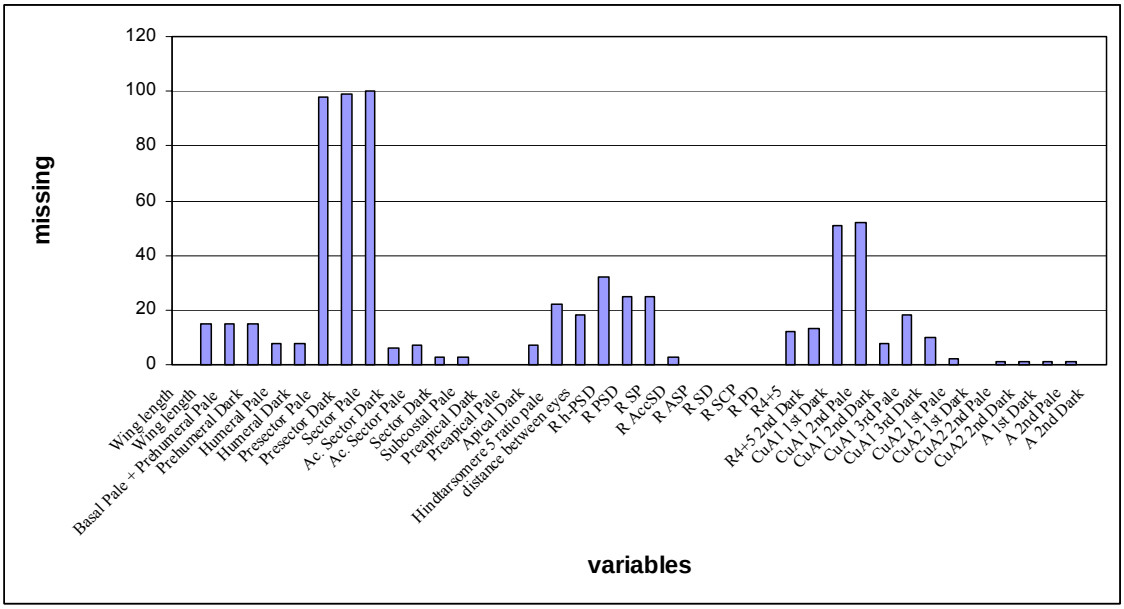

Table 1. Material examined of several localities of five species of *An. albitarsis* complex.

Country, State/Province	Locality	Coordinates	Date	Collection no.	Species (n)
BRAZIL					
Santa Catarina	Macaranduba	26°35'S48°58'W	8 Jan 1983	BR010	A(8F)
São Paulo	Ilha Comprida	24°42.8'S47°31.6'W	9 Feb 1989	BR73	C(7F, 2M, 2MG)
São Paulo	Registro	24°36.8'S47°53.1'W	26 Jan. 1992	BR500, 501	A(9F, 2M, 1MG, 8 Lv, 8 pp), B(15F, 2M, 1MG, 190Lv, 202Pp)
São Paulo	Ponte Melo Peixoto	22°39.05'S 53°01'W	10 Feb 1992	BR508	A(3M)
Paraná	Santa Helena	24°56'S54°23'W	30 Jan 1992	BR503	A(4F, 1M), D(2F, 2M, 1MG)
Paraná	Near Guaíra	24°04'S54°15'S	1 Feb 1992	BR504, 510, 511	A(3F, 1M, 1MG), B(1Lv, 1Pp), D(2F)
Espírito Santo	Águia Branca	18°59'S40°44'W	20 Jan 1992	BR002	B(1F, 10Lv, 10Pp)
Pará	Ilha de Marajó	1°00'S49°30'W		BR001	C(8F, 1M, 1MG, 34Lv, 34Pp)
Pará	EMBRAPA	1°27'S48°29'W	8 Oct 1992	BR008	B(2F, 8Lv, 8Pp)
Pará	Primavera	0°56'S47°6'W	16 Oct 1992	BR009	B(3F, 16Lv, 16Pp)
Pará	Capanema	1°24'S47°11'W	Ag 1993	BR403	C(5F, 1Lv, 1Pp), B(16Lv, 26Pp)
Rio de Janeiro	Morro da Panela	22°58'S43°21'W	19 Jan 1993	BR013	B(1F)
Bahia	Itaquara	13°26'S39°66'W	30 Jan 1993	BR015, 017	A(3Lv, 3Pp), B(1F, 18Lv, 18Pp)
Ceará	Fortaleza	3°43'S38°30'W	8 March 1993	BR018	B(3F, 23Lv, 23Pp)
Ceará	Paraipaba	3°25'S39°13'W	9 March 1993	BR019	B(1F, 28Lv, 28Pp)
Mato Grosso	Peixoto Azevedo	10°23'S54°54'W	20 April 1993	BR020	B(12Lv, 12Pp), C(3F, 8Lv, 8Pp)
Amazonas	Manaus	2°53'S60°15'W	16 Dec 1993	BR026	C(3F, 2Lv, 2Pp)
Rondônia	Guajará-Mirim	10°50'S65°20'W	29 July 1992	BR007	D(11F, 32Lv, 32Pp)
Rondônia	Ariquemes	9°56'S63°04'W		BR700	D(4F)
Rondônia	Costa Marques	12°28'S64°16'W	28 Mar 1992	BR645	D(2Lv, 2Pp)
Roraima	Boa Vista	2°49'S60°40'W		BR11, 17, 19, 23, 24, 25, 26, 27, 31, 34, 36	E(30F)
PARAGUAY					
Alto doParaná	Rio Acaray	25°29'S 54°42'W	4 Feb 1992	PA1	A(2M, 1MG)
Alto doParaná	Hernanderias	25°22'S54°45'W	6 Feb 1992	PA2	B(5F, 2M, 1MG, 7Lv, 6Pp)
Alto doParaná	Near National Airport	Not know	8 Feb 1992	PA3	B(3F, 2M, 1 MG)
ARGENTINA					
Buenos Aires	Baradero	33°48'S59°30'W	6 Feb 1992	AR7	A(8F, 9Lv, 8Pp)
Corrientes	Laguna Brava	27°28'S 58°50'W	31 Jan 1992	AR3	A(2F, 1M, 1MG, 1Lv, 1pp), D(3F, 2M, 1MG, 2Lv, 2Pp)
Misiones	Posadas	27°23'S 55°53'W	30 Jan 1992	AR1	A(2F, 2M, 1MG), D(1Lv)
VENEZUELA					
Zulia	Rio Socuavo			SOC191, 209, 212, 217	C(4F)

Table 2. Length of morphological characters of five species of the *An. albitarsis* complex

Variables	*Anopheles albitarsis s.s.*			*Anopheles albitarsis B*			*Anopheles marajoara*			*Anopheles deaneorum*			*Anopheles albitarsis* E		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
Wing length	3.35	0.24	30	3.27	0.14	30	3.14	0.22	30	3.17	0.19	30	2.91	0.10	30
Basal pale+prehumeral pale	0.26	0.03	24	0.24	0.03	29	0.23	0.03	29	0.25	0.02	24	0.21	0.02	29
Prehumeral dark	0.21	0.04	24	0.08	0.02	29	0.07	0.02	29	0.05	0.01	24	0.08	0.01	29
Humeral pale	0.08	0.03	27	0.08	0.03	29	0.19	0.03	29	0.21	0.02	24	0.16	0.02	29
Humeral dark	0.09	0.04	28	0.13	0.03	29	0.12	0.04	30	0.09	0.03	29	0.14	0.02	27
Presector pale	0.18	0.06	29	0.11	0.12	29	0.09	0.03	30	0.10	0.03	29	0.08	0.02	27
Presector dark	0.72	0.08	29	0.29	0.03	**10**	0.34	0.04	**11**	0.32	0.06	**18**	0.31	0.08	**10**
Sector Pale	0.72	0.08	29	0.05	0.01	**10**	0.05	0.01	**11**	0.05	0.02	**17**	0.06	0.01	**09**
Accessory sector dark	0.53	0.05	29	0.11	0.04	**10**	0.10	0.03	**11**	0.07	0.02	**17**	0.11	0.02	**08**
Acessory sector pale	0.10	0.04	29	0.14	0.03	29	0.12	0.05	30	0.12	0.05	29	0.10	0.04	28
Sector dark	0.18	0.08	29	0.70	0.07	29	0.67	0.08	30	0.69	0.07	29	0.64	0.06	28
Subcostal pale	0.14	0.05	30	0.23	0.04	30	0.23	0.07	30	0.24	0.05	30	0.17	0.05	28
Preapical dark	0.46	0.08	30	0.62	0.09	30	0.60	0.09	30	0.58	0.08	30	0.58	0.05	28
Preapical pale	0.36	0.05	30	0.26	0.06	30	0.24	0.05	30	0.26	0.03	30	0.20	0.03	30
Apical dark	0.60	0.04	30	0.13	0.03	30	0.11	0.04	30	0.09	0.13	30	0.14	0.02	30
Distance between eyes	0.19	0.09	30	0.11	0.09	30	0.11	0.10	30	0.11	0.14	30	0.11	0.06	23
R h-PSD	0.10	0.03	30	0.50	0.03	30	0.49	0.06	25	0.51	0.14	18	0.46	0.04	26
R PSD	0.11	0.04	17	0.09	0.04	30	0.09	0.04	25	0.07	0.04	18	0.12	0.04	30
R SP	0.10	0.04	28	0.18	0.03	30	0.20	0.03	22	0.18	0.11	08	0.14	0.03	29
R AccSD	0.54	0.07	28	0.19	0.04	30	0.11	0.05	26	0.04	0.03	10	0.16	0.04	29
R ASP	0.14	0.04	30	0.21	0.02	30	0.26	0.03	26	0.21	0.11	10	0.22	0.04	29
R SD	0.74	0.07	30	0.42	0.05	30	0.36	0.24	29	0.33	0.07	30	0.36	0.04	30
R SCP	0.19	0.05	30	0.35	0.05	30	0.38	0.06	30	0.51	0.11	30	0.29	0.04	30
R PD	0.16	0.03	30	0.54	0.06	30	0.50	0.10	30	0.38	0.11	30	0.51	0.04	30
R4+5	0.14	0.10	30	1.41	0.07	30	1.35	0.10	30	1.37	0.08	30	1.24	0.05	30
R4+5 1st dark	0.26	0.05	30	0.22	0.06	30	0.18	0.06	30	0.14	0.04	30	0.18	0.05	30
R4+5 1st pale	0.86	0.09	30	0.76	0.08	30	0.77	0.08	26	0.85	0.08	22	0.70	0.07	30
R4+5 2nd dark	0.10	0.03	30	0.11	0.03	30	0.08	0.03	26	0.06	0.02	21	0.12	0.03	30
CuA1 1st dark	0.11	0.04	**17**	0.11	0.04	28	0.10	0.05	**22**	0.05	0.03	**11**	0.10	0.04	**21**
CuA1 2nd pale	0.14	0.03	**16**	0.14	0.03	28	0.15	004	**22**	0.13	0.07	**11**	0.12	003	**21**
CuA1 2nd dark	0.10	0.04	28	0.14	0.04	30	0.09	0.04	29	0.08	0.04	25	0.11	0.03	30
CuA1 3rd pale	0.54	0.07	28	0.47	0.05	30	0.56	0.23	26	0.58	0.10	19	0.42	0.04	30
CuA1 3rd dark	0.14	0.04	30	0.15	0.03	30	0.12	0.03	27	0.09	0.04	23	0.13	0.04	30
CuA2 1st pale	0.74	0.07	30	0.72	0.05	30	0.68	0.06	30	0.73	0.06	29	0.63	0.04	29

CuA2 1st dark	0.19	0.05	30	0.17	0.05	30	0.18	0.07	30	0.13	0.04	30	0.18	0.04	30
CuA2 2nd pale	0.86	0.08	30	0.82	0.06	30	0.82	0.06	30	0.83	0.06	29	0.69	0.04	30
CuA2 2nd dark	0.16	0.03	30	0.16	0.03	30	0.13	0.04	30	0.11	0.03	29	0.18	0.03	30
A 1st dark	0.14	0.04	30	0.15	0.05	30	0.13	0.04	30	0.09	0.03	30	0.18	0.03	29
A 2nd pale	0.64	0.10	30	0.67	0.08	30	0.65	0.07	30	0.68	0.09	30	0.53	0.04	29
A 2nd dark	0.26	0.05	30	0.24	0.05	30	0.22	0.05	30	0.17	0.07	30	0.26	0.05	30

* The underlined numbers refer for missing datas

Table 3. Number and range (mode) of setal branches of the pupa of *Anopheles albitarsis s.s.*

Seta	Cephalothorax	Abdominal Segments									Paddle
No.	CT	I	II	III	IV	V	VI	VII	VIII	IX	P
0	-	-	3 - 7 (5)	4 - 7 (7)	2 - 6 (5)	3 - 6 (4)	3 - 5 (4)	3 - 6 (4)	1 - 2 (1)	-	-
1	2 - 4 (3)	n.c.	5 - 10 (8)	3 - 10 (6)	1	1	1	1	-	2 - 6 (3)	1
2	2 - 3 (3)	4 - 7 (5)	3 - 8 (5)	3 - 7 (4)	1 - 3 (2)	1 - 3 (2)	1 - 3 (1)	1 - 2 (1)	-	-	1 - 3 (1)
3	2 - 4 (3)	1 - 2 (1)	1	1	3 - 7 (5)	2 - 4 (3)	1 - 3 (3)	2 - 5 (4)	-	-	-
4	2 - 4 (3)	5 - 7 (5)	4 - 7 (5)	3 - 6 (5)	3 - 6 (4)	3 - 5 (4)	2 - 4 (3)	1 - 3 (2)	2 - 24(3)	-	-
5	2 - 5 (4)	1 - 3 (2)	3 - 7 (5)	4 - 10 (6)	1 - 5 (3)	1 - 2 (1)	1	1	-	-	-
6	1 - 4 (3)	1 - 2 (1)	1 - 2 (1)	1 - 4 (2)	1 - 3 (2)	1 - 2 (2)	1 - 2 (2)	1 - 2 (1)	-	-	-
7	1 - 3 (2)	2 - 6 (3)	3 - 6 (5)	4 - 6 (5)	2 - 6 (5)	2 - 5 (4)	1 - 2 (1)	1	-	-	-
8	1	-	-	3 - 5 (4)	2 - 5 (3)	2 - 3 (2)	1 - 3 (2)	2 - 5 (4)	-	-	-
9	2 - 4 (3)	1 - 2 (1)	1	1	1	1	1	1	1	-	-
10	1	-	-	2 - 4 (3)	1	1	-	2 - 3 (2)	-	-	-
11	2 - 5 (4)	-	-	1	1	1	1	1 - 2 (1)	-	-	-
12	1 - 5 (3)	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	1	1	1	1	1	-	-

n.c. = not counted

Table 4. Number and range (mode) of setal branches of the fourth-instar larva of *Anopheles albitarsis s.s.*

Seta	Head	Thorax			Abdominal segments								
No	C	P	M	T	I	II	III	IV	V	VI	VII	VIII	X
0	0	nc	-	-	-	6 - 11 (9)	6 - 10 (7)	5 - 8 (7)	6 - 9 (8)	5 - 8 (6)	4 - 8 (5)	4 - 6 (5)	-
1	1	10 - 19 (15)	24 - 35 (33)	1 - 4 (1)	12 - 21 (15)	22 - 30 (22)	22 - 31 (27)	19 - 32 (28)	21 - 30 (28)	23 - 32 (25)	22 - 27 (23)	1	1
2	1	16 - 25 (21)	1 - 4 (1)	1	3 - 5 (3)	3 - 4 (3)	3	1 - 2 (1)	1	3	4 - 8 (5)	8 - 13 (10)	15 - 22 (17)
3	1	1	1	3 - 5 (4)	1 - 2 (1)	1	1	2 - 3 (3)	1 - 3 (1)	1	2 - 3 (3)	7 - 13 (11)	8 - 10 (9)
4	1 - 4 (2)	13 - 23 (21)	3 - 5 (4)	11 - 17 (14)	4 - 8 (6)	4 - 7 (5)	2 - 4 (3)	2 - 4 (3)	2 - 4 (3)	1	1	1	8
5	15 - 28 (20)	21 - 38 (27)	1	28 - 43 (32)	3 - 5 (4)	7 - 10 (9)	7 - 12 (9)	4 - 6 (5)	4 - 7 (5)	4 - 8 (6)	5 - 8 (7)	5 - 7 (6)	-
6	14 - 25 (19)	1	3 - 4 (3)	2 - 3 (2)	25 - 36 (31)	26 - 40 (31)	23 - 32 (25)	1	1	1	5 - 9 (6)	1-S	4 - 7 (6)
7	18 - 26 (23)	30 - 42 (38)	2 - 4 (3)	26 - 36 (26)	25 - 33 (29)	27 - 35 (31)	3 - 4 (3)	3 - 5 (4)	2 - 4 (3)	2 - 3 (2)	4 - 6 (5)	2-S	5 - 9 (6)
8	2 - 5 (4)	30 - 38 (36)	22 - 29 (27)	27 - 40 (34)	-	3 - 5 (4)	3 - 5 (5)	2 - 7 (3)	2 - 5 (3)	3 - 4 (3)	5 - 10 (8)	6-S	1 - 2 (2)
9	3 - 5 (3)	1	1	1	5 - 8 (6)	7 - 12 (8)	6 - 10 (9)	6 - 9 (7)	6 - 10 (9)	7 - 10 (9)	7 - 13 (9)	7-S	1 - 2 (1)
10	3	1	1	1	1	2 - 3 (2)	1	1	1	2 - 3 (2)	3 - 6 (5)	8-S	3 - 5 (3)
11	0	1 - 3 (2)	1 - 2 (1)	nc	3 - 4 (3)	1	2 - 3 (2)	1 - 3 (2)	1 - 3 (2)	1 - 2 (2)	1 - 3 (1)	9-S	5 - 8 (6)
12	4 - 7 (5)	1	1	2 - 3 (2)	1 - 3 (2)	1	2 - 4 (3)	2 - 5 (3)	2 - 4 (2)	1	1 - 2 (1)	-	-
13	4 - 7 (5)	3 - 5 (4)	5 - 9 (7)	2	3 - 9 (6)	8 - 15 (11)	3 - 9 (7)	3 - 6 (3)	3 - 6 (4)	7 - 15 (12)	3 - 6 (4)	-	-
14	4 - 7 (4)	5 - 9 (7)	8 - 15 (10)	-	-	-	1	1	1	1	1	1 - 3 (1)	-
15	4 - 7 (6)	-	-	-	-	-	-	-	-	-	-	-	-

Table 5. Number and range (mode) of setal branches of the pupa of *Anopheles marajoara*.

Seta	Cephalothorax	Abdominal Segments									Paddle
No.	CT	I	II	III	IV	V	VI	VII	VIII	IX	P
0	-	-	3 - 7 (5)	4 - 8 (5)	3 - 5 (4)	3 - 6 (5)	3 - 6 (5)	3 - 7 (5)	1 - 4 (1)	-	-
1	2 - 4 (3)	n.c.	5 - 10 (8)	3 - 11 (8)	1	1	1	1	-	2 - 5 (3)	1
2	2 - 5 (3)	1 - 5 (4)	3 - 8 (5)	3 - 6 (5)	1 - 4 (2)	1 - 3 (3)	1 - 3 (2)	1 - 3 (1)	-	-	1 - 3 (2)
3	2 - 4 (3)	1 - 3 (1)	1	1 - 3 (1)	3 - 6 (4)	2 - 4 (3)	1 - 4 (2)	2 - 5 (3)	-	-	-
4	2 - 5 (3)	4 - 7 (6)	4 - 7 (5)	3 - 7 (4)	3 - 5 (4)	3 - 5 (3)	1 - 4 (2)	1 - 4 (2)	2 - 3 (3)	-	-
5	3 - 7 (3)	1 - 3 (2)	3 - 7 (5)	3 - 11 (5)	2 - 10 (3)	1 - 3 (2)	1 - 3 (1)	1	-	-	-
6	2 - 3 (2)	1 - 2 (1)	1 - 2 (1)	1 - 32 (2)	1 - 3 (2)	1 - 2 (2)	1 - 2 (1)	1 - 3 (1)	-	-	-
7	2 - 4 (3)	1 - 5 (4)	3 - 6 (5)	3 - 6 (3)	3 - 6 (4)	2 - 4 (3)	1 - 2 (1)	1 - 2 (1)	-	-	-
8	1	-	-	2 - 4 (4)	1 - 3 (3)	1 - 3 (2)	1 - 3 (2)	2 - 7 (3)	-	-	-
9	1 - 3 (2)	1	1	1	1	1	1	1	1	-	-
10	1	-	-	1 - 4 (3)	1	1	-	1 - 3 (2)	-	-	-
11	2 - 4 (4)	-	-	1 - 2 (1)	1 - 2 (1)	1	1	1 - 2 (1)	-	-	-
12	2 - 6 (3)	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	1	1	1	1	1	-	-

n.c. = not counted

Table 6. Number and range (mode) of setal branches of the fourth-instar larva of *Anopheles marajoara*.

Seta	Head	Thorax			Abdominal segments								
No	C	P	M	T	I	II	III	IV	V	VI	VII	VIII	X
0	0	nc	-	-	-	7 - 12 (10)	6 - 12 (9)	5 - 8 (6)	5 - 10 (7)	5 - 10 (7)	4 - 8 (6)	4 - 6 (5)	-
1	1	12 - 18 (17)	26 - 37 (33)	1	14 - 20 (16)	24 - 32 (27)	22 - 32 (26)	22 - 28 (26)	19 - 30 (29)	19 - 30 (25)	17 - 25 (24)	1	1
2	1	16 - 25 (21)	1 - 2 (1)	1	2 - 4 (3)	3 - 6 (5)	3 - 5 (3)	1 - 2 (1)	1	2 - 3 (3)	3 - 8 (6)	8 - 13 (10)	15 - 21 (17)
3	1	1	1	2 - 4 (3)	1	1	1	3	1	1 - 2 (1)	2 - 4 (3)	7 - 13 (11)	8 - 15 (9)
4	2 - 5 (3)	20 - 24 (21)	2 - 6 (4)	13 - 21 (17)	3 - 8 (5)	4 - 7 (5)	2 - 5 (3)	3 - 4 (3)	2 - 4 (3)	1	1	1	6 - 8 (8)
5	18 - 29 (24)	23 - 35 (28)	1	33 - 41 (34)	3 - 6 (5)	8 - 15 (12)	8 - 14 (13)	4 - 8 (7)	6 - 9 (7)	6 - 10 (7)	6 - 10 (7)	5 - 7 (6)	-
6	16 - 23 (20)	1	2 - 4 (3)	1 - 3 (2)	28 - 35 (32)	30 - 39 (36)	27 - 39 (29)	1	1	1	4 - 8 (6)	1-S	4 - 7 (6)
7	18 - 33 (23)	31 - 46 (38)	2 - 5 (4)	28 - 37 (35)	26 - 36 (32)	29 - 38 (34)	3 - 5 (3)	3 - 5 (4)	2 - 4 (3)	2 - 3 (2)	3 - 6 (4)	2-S	5 - 10 (7)
8	3 - 7 (5)	33 - 45 (36)	22 - 30 (26)	28 - 42 (35)	-	3 - 5 (4)	4 - 6 (5)	3 - 6 (4)	3 - 5 (4)	3 - 4 (3)	5 - 8 (7)	6-S	1 - 3 (2)
9	2 - 5 (3)	1	1	1	5 - 9 (7)	6 - 9 (8)	7 - 10 (10)	6 - 10 (9)	6 - 10 (7)	6 - 11 (9)	8 - 12 (9)	7-S	1 - 2 (1)
10	2 - 5 (3)	1	1	1	1	2 - 3 (2)	1	1	1	2 - 3 (2)	3 - 5 (4)	8-S	3 - 6 (4)
11	0	1 - 2 (2)	1 - 2 (1)	nc	3 - 4 (3)	1	2 - 4 (2)	2 - 3 (3)	1	1 - 3 (2)	1 - 3 (2)	9-S	6 - 10 (7)
12	4 - 7 (5)	1	1	2 - 3 (2)	2 - 3 (2)	1	2 - 4 (2)	2 - 4 (2)	1 - 3 (2)	1	1	-	-
13	5 - 8 (6)	5 - 7 (6)	6 - 10 (9)	2 - 3 (2)	4 - 6 (5)	8 - 13 (10)	4 - 7 (5)	3 - 6 (4)	3 - 5 (4)	9 - 14 (10)	3 - 6 (4)	-	-
14	0	5 - 11 (7)	9 - 15 (12)	-	-	-	1	1	1	1	1	1 - 3 (1)	-
15	3 - 8 (7)	-	-	-	-	-	-	-	-	-	-	-	-

Table 7. Number and range (mode) of setal branches of the pupa of *Anopheles deaneorum*.

Seta	Cephalothorax	Abdominal Segments									Paddle
No.	CT	I	II	III	IV	V	VI	VII	VIII	IX	P
0	-	-	4 - 7 (6)	4 - 8 (6)	4 - 8 (6)	3 - 6 (5)	4 - 6 (5)	3 - 5 (4)	1 - 2 (1)	-	-
1	2 - 4 (3)	n.c.	5 - 13 (8)	4 - 10 (6)	1 - 2 (1)	1	1	1	-	2 - 3 (2)	1
2	2 - 5 (2)	4 - 7 (5)	3 - 7 (5)	3 - 6 (4)	1 - 3 (2)	1 - 3 (2)	1 - 3 (1)	1 - 2 (1)	-	-	1 - 3 (2)
3	2 - 5 (3)	1 - 3 (1)	1	1 - 3 (1)	3 - 7 (6)	1 - 6 (3)	2 - 4 (3)	3 - 5 (3)	-	-	-
4	2 - 4 (3)	4 - 8 (6)	4 - 8 (6)	3 - 7 (4)	3 - 6 (5)	3 - 4 (3)	2 - 4 (3)	2 - 3 (3)	2 - 4 (3)	-	-
5	2 - 6 (3)	1 - 5 (2)	2 - 7 (4)	4 - 11 (7)	2 - 6 (5)	1 - 4 (1)	1 - 4 (1)	1 - 2 (1)	-	-	-
6	2 - 4 (3)	1 - 3 (1)	1 - 4 (1)	1 - 5 (3)	1 - 3 (2)	1 - 3 (2)	1 - 3 (1)	1	-	-	-
7	2 - 3 (2)	3 - 7 (4)	4 - 6 (5)	3 - 7 (3)	3 - 7 (4)	2 - 4 (3)	1 - 2 (1)	1 - 3 (1)	-	-	-
8	1	-	-	2 - 5 (3)	2 - 4 (3)	2 - 3 (2)	2 - 4 (2)	3 - 6 (4)	-	-	-
9	2 - 5 (3)	1	1	1	1	1	1	1	1	-	-
10	1 - 2 (1)	-	-	2 - 4 (3)	1	1	-	2 - 4 (2)	-	-	-
11	2 - 5 (4)	-	-	1	1	1	1	1 - 2 (2)	-	-	-
12	2 - 5 (3)	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	1	1	1	1	1	-	-

n.c. = not counted

Table 8. Number and range (mode) of setal branches of the fourth-instar larva of *Anopheles deaneorum*.

Seta	Head	Thorax			Abdominal segments								
No	C	P	M	T	I	II	III	IV	V	VI	VII	VIII	X
0	0	nc	-	-	-	6 - 11 (8)	6 - 9 (6)	4 - 8 (7)	5 - 9 (6)	4 - 8 (5)	3 - 6 (4)	4 - 6 (5)	-
1	1	12 - 20 (16)	26 - 38 (32)	1	13 - 22 (18)	19 - 29 (23)	21 - 28 (26)	22 - 31 (24)	20 - 30 (24)	20 - 28 (24)	20 - 26 (22)	1	1
2	1	14 - 25 (16)	1 - 2 (1)	1	3 - 5 (4)	3 - 8 (3)	2 - 5 (3)	1	1	3 - 5 (3)	4 - 7 (6)	8 - 13 (10)	16 - 19 (18)
3	1	1	1	3 - 6 (3)	1	1 - 2 (1)	1	2 - 3 (3)	1	1	3	7 - 13 (11)	8 - 12 (8)
4	1 - 6 (2)	14 - 26 (20)	3 - 7 (4)	14 - 22 (16)	3 - 6 (5)	3 - 6 (4)	2 - 3 (3)	2 - 4 (3)	2 - 3 (3)	1	1	1	8
5	21 - 27 (22)	16 - 35 (16)	1	35 - 44 (35)	4 - 8 (5)	10 - 16 (13)	10 - 18 (14)	5 - 8 (5)	5 - 9 (6)	5 - 10 (7)	6 - 10 (8)	5 - 7 (6)	-
6	18 - 29 (21)	1	2 - 5 (3)	2 - 4 (2)	29 - 36 (34)	30 - 40 (36)	27 - 37 (30)	1	1	1	4 - 8 (6)	1-S	5 - 7 (6)
7	21 - 32 (25)	35 - 42 (35)	3 - 5 (4)	25 - 38 (29)	29 - 38 (34)	31 - 39 (35)	2 - 5 (3)	3 - 44 (3)	2 - 3 (3)	1 - 3 (2)	3 - 9 (5)	2-S	5 - 9 (7)
8	3 - 8 (6)	31 - 38 (36)	24 - 31 (27)	33 - 43 (39)	-	2 - 5 (3)	3 - 4 (4)	3 - 5 (4)	3 - 4 (3)	2 - 4 (3)	5 - 9 (7)	6-S	1 - 2 (2)
9	3 - 6 (4)	1	1	1	5 - 9 (7)	6 - 14 (9)	6 - 12 (10)	5 - 11 (9)	6 - 9 (9)	8 - 12 (10)	6 - 12 (9)	7-S	1 - 2 (1)
10	2 - 4 (3)	1	1	1	1	2 - 4 (3)	1	1	1	2 - 3 (2)	3 - 6 (4)	8-S	4 - 8 (6)
11	0	2 - 3 (2)	1 - 2 (2)	nc	3 - 4 (3)	1	2 - 3 (2)	1 - 3 (2)	1 - 2 (2)	2 - 3 (2)	2 - 3 (2)	9-S	4 - 11 (7)
12	4 - 7 (5)	1	1	2 - 3 (2)	2	1	2 - 4 (2)	2 - 3 (2)	1 - 3 (2)	1	1 - 2 (1)	-	-
13	4 - 9 (6)	6 - 12 (9)	6 - 10 (8)	2 - 3 (2)	4 - 8 (5)	6 - 14 (11)	3 - 10 (5)	3 - 5 (4)	5 - 8 (5)	8 - 16 (10)	4 - 6 (5)	-	-
14	3	3 - 5 (5)	9 - 19 (11)	-	-	-	1	1	1	1	1	1 - 3 (1)	-
15	3 - 11 (4)	-	-	-	-	-	-	-	-	-	-	-	-

Table 9. Number and range (mode) of setal branches of the pupa of *Anopheles albitarsis* B.

Seta	Cephalothorax	Abdominal Segments									Paddle
No.	CT	I	II	III	IV	V	VI	VII	VIII	IX	P
0	-	-	4 - 7 (5)	4 - 8 (6)	3 - 7 (4)	3 - 6 (5)	2 - 6 (4)	1 - 5 (4)	1 - 4 (2)	-	-
1	1 - 3 (3)	n.c.	7 - 12 (8)	4 - 10 (6)	1	1	1	1	-	2 - 3 (3)	1
2	2 - 4 (3)	2 - 5 (4)	4 - 6 (5)	3 - 7 (5)	1 - 4 (3)	2 - 4 (3)	2 - 3 (2)	1 - 3 (1)	-	-	1 - 3 (2)
3	2 - 4 (3)	1 - 2 (1)	1	1 - 2 (1)	3 - 5 (5)	2 - 5 (3)	1 - 4 (2)	3 - 5 (4)	-	-	-
4	1 - 4 (3)	5 - 9 (6)	4 - 8 (5)	3 - 7 (4)	2 - 6 (4)	2 - 6 (3)	2 - 3 (3)	1 - 5 (2)	3 - 4 (3)	-	-
5	2 - 7 (4)	1 - 4 (2)	4 - 7 (5)	5 - 10 (8)	3 - 5 (4)	1 - 3 (1)	1 - 2 (1)	1	-	-	-
6	1 - 5 (3)	1 - 2 (1)	1	2 - 4 (3)	1 - 4 (3)	1 - 3 (2)	1 - 3 (2)	1 - 4 (1)	-	-	-
7	1 - 3 (2)	2 - 5 (3)	2 - 6 (4)	2 - 6 (5)	2 - 6 (5)	2 - 5 (3)	1 - 3 (1)	1	-	-	-
8	1	-	-	3 - 6 (4)	1 - 4 (3)	1 - 3 (2)	1 - 3 (2)	3 - 5 (4)	-	-	-
9	2 - 5 (3)	1 - 2 (1)	1	1	1	1	1	1	1	-	-
10	1	-	-	2 - 4 (3)	1 - 2 (1)	1	0	1 - 3 (3)	-	-	-
11	2 - 4 (3)	-	-	1 - 3 (1)	1 - 2 (1)	1 - 2 (1)	1 - 2 (1)	1 - 2 (1)	-	-	-
12	2 - 6 (3)	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	0	0	0	0	0	-	-

n.c. = not counted

Table 10. Number and range (mode) of setal branches of the fourth-instar larva of *Anopheles albitarsis* B.

Seta	Head	Thorax			Abdominal segments								
No	C	P	M	T	I	II	III	IV	V	VI	VII	VIII	X
0		1	-	-	-	6 - 10 (6)	5 - 10 (6)	5 - 8 (5)	5 - 8 (6)	5 - 7 (6)	3 - 8 (5)	3 - 7 (4)	-
1	1	12 - 20 (16)	25 - 33 (29)	1	13 - 20 (15)	20 - 27 (24)	21 - 29 (25)	21 - 31 (25)	20 - 31 (26)	22 - 28 (22)	20 - 25 (21)	1	1
2	1	16 - 24 (20)	1	1	3 - 4 (3)	3 - 4 (3)	3 - 4 (3)	1	1 - 2 (1)	2 - 3 (3)	4 - 8 (5)	8 - 13 (11)	12 - 24 (19)
3	1	1	1	12 - 19 (15)	1	1	1	2 - 3 (3)	1	1	2 - 3 (3)	6 - 12 (10)	6 - 11 (10)
4	1 - 4 (2)	17 - 27 (21)	1	3 - 4 (3)	5 - 9 (6)	4 - 8 (5)	2 - 4 (3)	2 - 4 (3)	2 - 3 (3)	1	1	1	6,5 - 8 (8)
5	19 - 27 (23)	21 - 35 (28)	3 - 6 (4)	24 - 40 (35)	4 - 6 (4)	8 - 14 (9)	9 - 15 (11)	5 - 8 (5)	5 - 8 (6)	6 - 9 (6)	7 - 10 (9)	4 - 6 (5)	-
6	17 - 25 (19)	1	2 - 4 (3)	1 - 3 (2)	26 - 36 (32)	27 - 38 (33)	19 - 34 (26)	1	1	1	5 - 9 (6)	1-S	5 - 8 (6)
7	20 - 31 (25)	30 - 43 (38)	2 - 4 (3)	24 - 35 (31)	27 - 39 (29)	23 - 39 (33)	3 - 4 (3)	3 - 7 (4)	2 - 4 (3)	2 - 3 (2)	4 - 6 (5)	2-S	4 - 10 (6)
8	3 - 5 (3)	30 - 41 (35)	20 - 32 (22)	29 - 41 (36)	-	3 - 5 (3)	3 - 6 (4)	2 - 5 (3)	3 - 4 (3)	2 - 4 (3)	6 - 9 (6)	6-S	1 - 3 (2)
9	3 - 5 (3)	1	1	1	5 - 7 (6)	6 - 9 (8)	5 - 11 (7)	6 - 9 (8)	7 - 11 (9)	7 - 11 (10)	8 - 13 (10)	7-S	1 - 2 (1)
10	2 - 5 (3)	1	1	1	1	1 - 2 (1)	1	1	1	1 - 3 (2)	3 - 5 (5)	8-S	3 - 6 (4)
11	45 - 58 (45)	2 - 3 (2)	1 - 3 (2)	1 - 3 (1)	3	2 - 3 (3)	2 - 3 (2)	1 - 3 (2)	1 - 2 (2)	1 - 2 (2)	1 - 2 (2)	9-S	5 - 9 (7)
12	3 - 7 (5)	1	1 - 2 (1)	2 - 5 (2)	1 - 2 (2)	1	2 - 3 (2)	2 - 4 (2)	1 - 2 (2)	1	1	-	-
13	3 - 9 (6)	3 - 6 (5)	5 - 9 (7)	2 - 3 (2)	3 - 5 (3)	8 - 13 (9)	3 - 6 (3)	3 - 4 (3)	3 - 6 (4)	8 - 13 (11)	3 - 5 (4)	-	-
14	0	6 - 10 (7)	8 - 13 (11)	-	-	-	1	1	1	1	1	1	-
15	4 - 8 (6)	-	-	-	-	-	-	-	-	-	-	-	-

Table 11. Principal component analysis (contribution of 35 variables to the first 10 principal components) calculated from 150 specimens of five species of the *An. albitarsis* complex.

Variable	Cp 1	Cp 2	Cp 3	Cp 4	Cp 5	Cp 6	Cp 7	Cp 8	Cp 9	Cp 10
Wing length	-0,25	-0,24	-0,04	-0,04	0,12	0,00	0,03	0,06	0,01	-0,02
Basal pale+prehumeral pale	-0,20	-0,12	0,10	-0,06	0,01	-0,01	0,13	-0,10	-0,08	0,26
Prehumeral dark	0,16	-0,07	-0,29	0,17	0,31	-0,10	-0,17	0,21	-0,12	0,10
Humeral pale	-0,24	0,00	0,06	-0,16	-0,19	0,04	0,23	-0,29	0,21	-0,23
Humeral dark	0,15	-0,16	0,05	0,08	0,25	0,12	-0,25	0,15	-0,05	0,21
Presector pale	-0,04	-0,04	-0,13	-0,18	0,00	-0,34	0,27	0,60	0,31	-0,13
Acessory sector pale	-0,04	0,09	-0,34	0,07	0,07	-0,14	0,24	-0,14	-0,29	-0,43
Sector dark	-0,16	-0,27	0,06	0,01	0,07	0,14	-0,12	-0,01	0,11	0,15
Subcostal pale	-0,14	0,17	-0,29	-0,17	0,14	0,10	-0,14	0,02	0,03	-0,02
Preapical dark	-0,08	-0,28	0,24	0,04	-0,07	-0,11	0,10	0,14	-0,13	-0,05
Preapical pale	-0,18	0,05	-0,23	-0,04	0,28	0,03	0,02	-0,31	0,00	0,04
Apical dark	0,08	-0,24	0,03	0,00	-0,08	-0,26	-0,15	0,16	-0,11	0,02
Distance between eyes	-0,06	-0,07	-0,07	0,38	0,10	-0,39	0,02	-0,38	0,01	0,18
R h-PSD	-0,19	-0,12	-0,09	0,14	-0,23	-0,17	-0,33	-0,07	-0,05	-0,17
R PSD	0,09	-0,08	-0,01	0,35	0,20	-0,02	0,30	-0,05	0,51	0,16
R SP	-0,17	-0,01	-0,30	0,20	-0,35	0,19	-0,04	0,17	-0,11	0,00
R AccSD	0,07	-0,21	-0,11	-0,17	0,25	-0,30	-0,22	-0,09	-0,05	-0,29
R ASP	-0,10	0,01	-0,15	0,48	-0,36	0,00	-0,23	0,07	0,15	0,00
R SD	-0,03	-0,30	-0,03	0,01	-0,03	0,20	0,07	-0,07	-0,05	0,01
R SCP	-0,25	0,16	-0,09	-0,09	0,08	-0,04	-0,10	0,10	0,17	0,09
R PD	0,07	-0,29	0,17	0,01	-0,12	0,03	0,13	-0,01	-0,20	-0,19
R4+5	-0,24	-0,25	-0,03	-0,05	0,08	0,03	0,02	0,08	-0,02	0,00
R4+5 1st dark	0,11	-0,20	-0,20	-0,10	0,03	0,39	0,08	-0,01	0,08	-0,02
R4+5 1st pale	-0,28	-0,08	0,21	0,09	0,11	-0,11	0,00	0,03	-0,06	-0,01
R4+5 2nd dark	0,14	-0,15	-0,11	-0,13	-0,22	-0,27	-0,04	-0,16	0,39	-0,03
CuA1 2nd dark	0,07	-0,17	-0,39	-0,10	-0,03	0,14	0,05	-0,03	0,12	0,04
CuA1 3 rd pale	-0,25	-0,02	0,23	0,05	0,12	0,06	-0,03	0,01	0,17	-0,12
CuA1 3rd dark	0,09	-0,19	-0,22	-0,11	-0,26	-0,02	0,18	-0,01	-0,12	0,29
CuA2 1st pale	-0,24	-0,12	-0,08	-0,22	-0,09	-0,06	-0,17	-0,06	0,01	0,20
CuA2 1st dark	0,09	-0,19	0,02	0,34	0,12	0,26	0,14	0,06	0,04	-0,35
CuA2 2nd pale	-0,29	-0,11	-0,04	-0,03	0,10	0,09	-0,04	0,03	0,06	0,02
CuA2 2nd dark	0,14	-0,20	-0,05	-0,12	-0,10	-0,12	0,21	-0,14	-0,12	0,22
A 1st dark	0,18	-0,20	-0,04	0,00	0,13	-0,04	-0,03	-0,12	0,02	-0,07
A 2nd pale	-0,28	-0,06	-0,16	0,08	0,12	-0,04	0,15	0,11	-0,11	-0,04
A 2nd dark	0,14	-0,18	0,05	-0,17	-0,04	0,12	-0,38	-0,12	0,28	-0,26

Table 12. Analysis of variance of 35 variables from 150 specimens of five species of the *An. albitarsis* complex.

Variables	p value
Wing length	0.000
Basal pale+prehumeral pale	0.000
Prehumeral dark	0.000
Humeral pale	0.000
Humeral dark	0.000
***Presector pale**	0.160
Acessory sector pale	0.000
Sector dark	0.000
Subcostal pale	0.000
Preapical dark	0.000
Preapical pale	0.000
Apical dark	0.000
***Distance between eyes**	0.317
R h-PSD	0.001
R PSD	0.001
R SP	0.000
R AccSD	0.000
R ASP	0.000
R SD	0.000
R SCP	0.000
R PD	0.000
R4+5	0.000
R4+5 1st dark	0.000
R4+5 1st pale	0.000
R4+5 2nd dark	0.000
CuA1 2nd dark	0.000
CuA1 3 rd pale	0.000
CuA1 3rd dark	0.000
CuA2 1st pale	0.000
CuA2 1st dark	0.000
CuA2 2nd pale	0.000
CuA2 2nd dark	0.000
A 1st dark	0.000
A 2nd pale	0.000
A 2nd dark	0.000

***** The underlined variables are excluded

Table 13. Discriminant analysis of 27 variables from 103 specimens of five species of *An. albitarsis* complex.

Groups	1	2	3	4	5
(1) *An. albitarsis s.s.*	13	1	1	2	1
(2) *An. Albitarsis* B	2	19	5	0	0
(3) *An. marajoara*	0	6	11	3	0
(4) *An. deaneorum*	1	0	3	8	0
(5) *An. albitarsis* E	1	1	2	0	23
Total	17	27	22	13	24
Correct classification	13	19	11	8	23
Cross validation	0,77	0,70	0,50	0,62	0,96
Analyzed specimens	103				
Correct classification	74				
Cross validation	0.72				

Table 14. Discriminant analysis of 35 variables from 150 specimens of five species of *An. albitarsis* complex.

Groups	1	2	3	4	5
(1) *An. albitarsis s.s.*	21	2	4	3	0
(2) *An. albitarsis* B	1	21	6	0	1
(3) *An. marajoara*	3	4	12	6	1
(4) *An. deaneorum*	1	2	7	20	0
(5) *An. albitarsis* E	4	1	1	1	28
Total	30	30	30	30	30
Correct classification	21	21	12	20	28
Cross validation	0,70	0,70	0,40	0,67	0,93
Analyzed specimens	1150				
Correct classification	102				
Cross validation	0,68				

Table 15. Cluster analysis of 35 variables from 150 specimens of five species of *An. albitarsis* complex.

Species	group 1	group 2
An.albitarsis s.s.	25	5
An.albitarsis B	26	4
An. marajoara	15	15
An. deaneorum	3	17
An.albitarsis E	2	28

Table 16. Discriminant analysis of two groups clustered by Cluster analysis.

Groups	1	2
1	49	6
2	8	40
Total	57	46
Correct classification	49	40
Cross validation	0.86	0.87
Analyzed specimens	103	
Correct classification	89	
Cross validation	0,86	

4.3 MANUSCRITO 3

“Notes on the holotype of *Anopheles marajoara* Galvão & Damasceno (Diptera: Culicidae)”

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Notes on the holotype of *Anopheles marajoara* Galvão & Damasceno (Diptera, Culicidae)

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ABSTRACT. Notes on the holotype of *Anopheles marajoara* Galvão & Damasceno, 1942 (Diptera, Culicidae). During studies on the dynamics of malaria transmission in Marajó Island, State of Pará, Brazil, Galvão & Damasceno (1942) collected a single specimen of a new species that they named *Anopheles* (*Nyssorhynchus*) *marajoara* Galvão & Damasceno, 1942. Now, examining genitalia slide associated to the holotype, we observed that the ventral claspette of the male genitalia is distinct from those of all other species of the Argyritarsis Section and consequently from members of the complex *Anopheles albitarsis* Lynch Arribalzaga, 1878. The male genitalia of the slide belong to a specimen of *Anopheles aquasalis* Curry, 1932, nevertheless, it was originally labeled as *Anopheles marajoara*. To solve this problem, we are setting aside the male genitalia slide associated with the holotype of *Anopheles marajoara* and excluding it from the type material. Illustrations of the male genitalia and adult male are included.

KEYWORDS. *Anopheles albitarsis* Complex; Culicidae; holotype; *Nyssorhynchus*.

RESUMO. Sobre o holótipo de *Anopheles marajoara* Galvão & Damasceno, 1942 (Diptera, Culicidae). Durante estudos sobre a dinâmica de transmissão da malária na Ilha de Marajó, Estado do Pará, Brasil, Galvão & Damasceno (1942) coletaram um espécime de nova espécie de anofelíneo, que foi denominada *Anopheles* (*Nyssorhynchus*) *marajoara* Galvão & Damasceno, 1942. Ao examinarmos a lâmina com a genitália, que acompanha o holótipo, observamos que o claspete ventral da genitália masculina difere daqueles apresentados pelas espécies da Seção Argyritarsis e, conseqüentemente, de membros do Complexo *Anopheles abitarsis* Lynch Arribalzaga, 1878. Consideramos que a genitália masculina que foi montada na lâmina associada ao holótipo pertence a um espécime de *Anopheles aquasalis* Curry, 1932, embora o adulto e as exúvias de larva e da pupa sejam de *Anopheles marajoara*. Com o intuito de resolver este problema , nós excluímos a lâmina com a genitália de macho do material tipo de *Anopheles marajoara*. A título de elucidação, foram feitas ilustrações da genitália masculina em questão, bem como do adulto de *An. marajoara*.

PALAVRAS-CHAVE. Complexo *Anopheles albitarsis*; Culicidae, holótipo; *Nyssorhynchus*.

Anopheles subgenus *Nyssorhynchus* is a monophyletic Neotropical group (Sallum et al. 2000, 2002) that currently contains 32 named species (Harbach 2004). The most important vectors of human malaria parasites in Central and South America are members of the subgenus and include *An. darlingi* Root, 1926, *An. albimanus* Wiedemann, 1820 and *An. marajoara* Galvão & Damasceno, 1942 (Conn et al. 2002). *Anopheles marajoara* belongs to a group of morphologically similar species, the Albitarsis Complex, which also contains *An. albitarsis* Lynch-Arribálzaga, 1878, *An. deaneorum* Rosa-Freitas, 1989 and *An. albitarsis* "B" of Wilkerson et al. (1995). The *An. albitarsis* Complex species are largely indistinguishable morphologically with nearly all characters in all stages either identical or overlapping. *Anopheles albitarsis* s. l. was suspected to contain more than one species by various authors based on epidemiological, behavioral, morphological, cytological, and biochemical evidences (Galvão and Damasceno 1944; Kreutzer et al. 1976; Linthicum 1988; Rosa-Freitas and Deane 1989; Rosa-Freitas et al. 1990; Narang et al. 1993). Wilkerson et al. (1995) using random amplified polymorphic DNA (RAPD-PCR) demonstrated the existence of the above four species. Recently, Lehr et al. (2005) hypothesized a fifth species (*An. albitarsis* E) in northern Brazil and Venezuela related to *An. marajoara* based on complete sequence of the cytochrome c oxidase I gene (COI).

Anopheles marajoara was demonstrated to be involved in the dynamics of the transmission of human malaria in the Amazon Region. Population studies using molecular markers for species identification showed a significant increase in infected adults female of *An. marajoara* by *Plasmodium* Marchiafava & Celli, 1885, in Macapá, State of Amapá, Brazil. Consequently, the importance of *An. marajoara* in the dynamics of the malaria transmission in Macapá is currently higher than that of *An. darlingi*. More important, the change in the forest environment caused by a continuous, unplanned urbanization and human migration are creating ecological conditions that are adequate for survivorship and dispersion of *An. marajoara* (Conn et al 2002). Also, *An. deaneorum* was infected by *P. falciparum* and *P. vivax* (Klein *et al.* 1991) in the laboratory.

During studies on the dynamics of malaria transmission in Cachoeira do Arari (01º00'36''S 48º57'36''W), Marajó Island, State of Pará, Brazil, Galvão and Damasceno (1942) collected immatures of *An. albitarsis*, *An. aquasalis* Curry, 1932 (identified as *Anopheles tarsimaculatus* var. *aquasalis* Curry,

1932) and a single larva of a new species named *An.* (*Nyssorhynchus*) *marajoara*. The collection site was situated in Santa Maria Farm, 400 meters distant from Camará River that is the natural limit between Cachoeira do Arari and Salvaterra municipalities and runs into the Marajó Basin. In the breeding habitat, the water was turbid, polluted, standing, with fishes and immatures of several insect species.

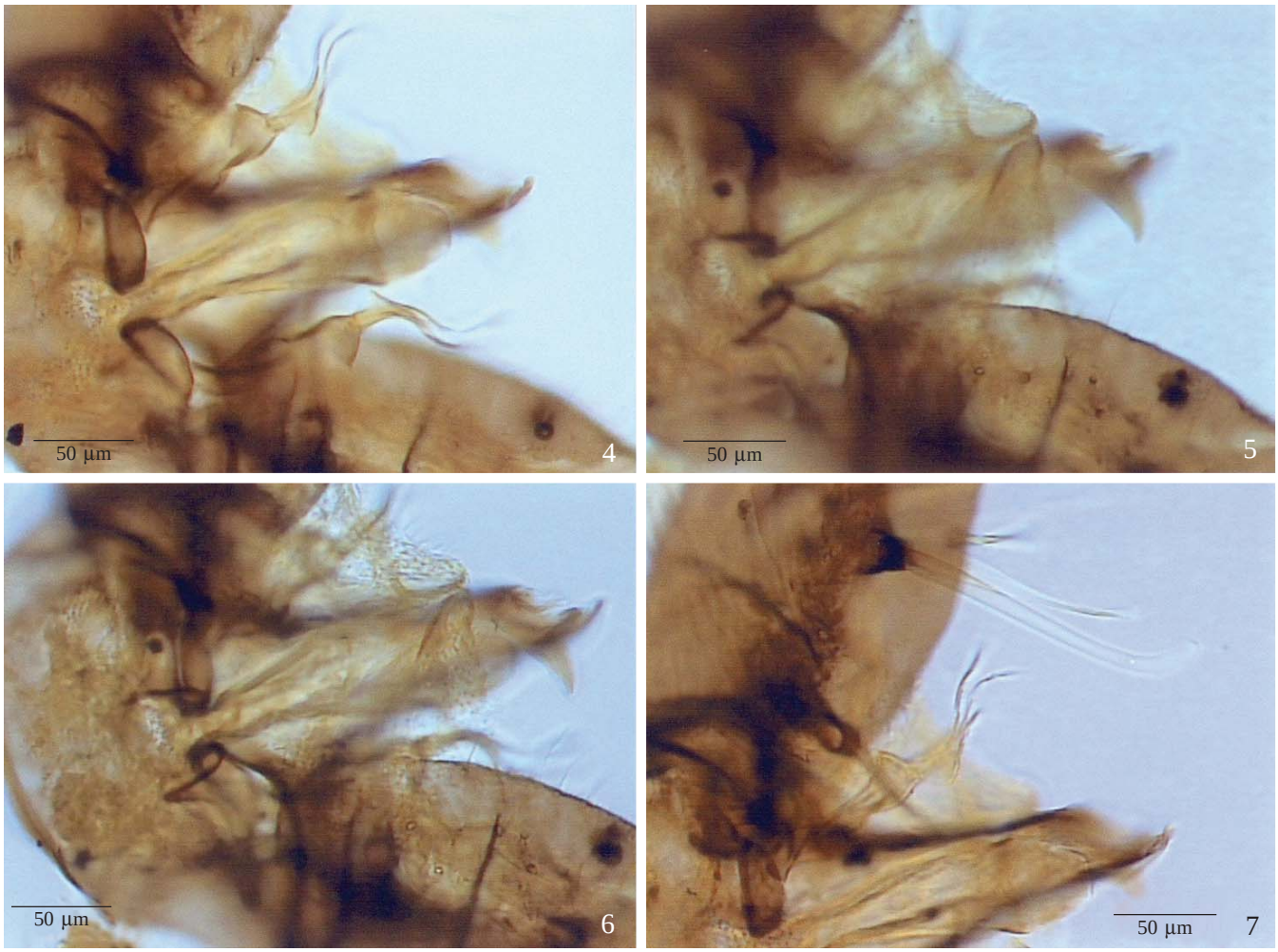
Galvão & Damasceno (1942) named and defined *Anopheles marajoara* based on morphological characters of the fourth-instar larva, pupa and adult male, including those of the male genitalia, and designated the holotype, which was deposited in the Entomological Collection of the Parasitology Department of Faculdade de Medicina, Universidade de São Paulo (FMUSP). The accession number in the collection was FMUSP 619. The FMUSP Entomological Collection was transferred to Faculdade de Saúde Pública, Universidade de São Paulo (FSP-USP), in the early 1970s. Consequently, the holotype of *An. marajoara* was deposited in the FSP-USP where it has the accession number E-2120.

Galvão & Damasceno (1944) compared specimens of *An. albitarsis* collected in the Marajó Island with samples from the State of São Paulo, Brazil, and from Tucuman, north Argentina, thus found morphological and ethological differences between the south and north populations. Although the egg of the specimens of the Marajó Island population was morphologically similar to that of São Paulo population, they differed in the exocorion that in the former population was somewhat a mosaic. Also, the adults of the Marajó Island were smaller when compared to samples from São Paulo. *Anopheles albitarsis* samples collected in Tucuman were morphologically more similar to those of São Paulo than to those of Marajó Island. Additionally, Tucuman samples were exophilic and exophagic, whereas those from Marajó Island were collected in the intradomiciliary environment. Because of those differences, the Marajó Island population was considered to be a subspecies named *Anopheles albitarsis domesticus* Galvão & Damasceno, 1944. Galvão & Lane (1937) described *Anopheles albitarsis limai* Galvão & Lane, 1937 using samples obtained in Pinheiros and Butantan Districts in São Paulo municipality, and Corrêa & Ramos (1943) named *Anopheles albitarsis imperfectus* Corrêa & Ramos, 1943 based on specimens from Vera Cruz, both municipalities of State of São Paulo, Brazil. Lane (1953) considered *An. albitarsis limai*, *An. albitarsis imperfectus* and *An. marajoara* to be morphological variants of *An. albitarsis*, which were transferred to the synonymy of *An. albitarsis albitarsis*.

Belkin et al. (1971) restricted the type locality of *An. marajoara* to the limits of Cachoeira do Arari, and accepted the synonymy of this species with *An. albitarsis*. Additionally, they considered that there was a type-series "Type: males, females, larvae" (page 4) for *An. marajoara*. However, in the present study, we are considering the type series as an invalid designation for the following fact. Galvão & Damasceno (1942) declared that the holotype of *An. marajoara* was an adult male, which was deposited in FMUSP. The adult male is associated with the fourth-instar larval and pupal exuviae and male genitalia.

Figs. 1-3. *Anopheles marajoara* holotype: 1, Lateral view, habitus. 2, Abdominal sternite I, showing detail of submedial stripes of white scales. 3, Hindtarsomeres, showing detail of the white-scaled hindtarsomere 5.

Linthicum (1988) studied the species of the *Argyritarsis* Section and resurrected *An. marajoara* from the synonymy of *An. albitarsis*. Additionally, Linthicum transferred *An. albitarsis domesticus* to the synonymy of *An. marajoara*. Interestingly, in his revision, Linthicum illustrated the male genitalia of a specimen of *An. marajoara* collected in the Canal Zone, Panama. However, we observed that in the illustration (Figure 14, page 266), both the ventral claspette and the apex of the aedeagus differ from those of *An. albitarsis* (Figure 12, page 264) and



Figs. 4-7. *Anopheles marajoara* holotype, male genitalia: 4, Dorsal view of the aedeagus. 5, Dorsal view of the ventral claspette. 6, Ventral view of the ventral claspette. 7, Dorsal view, showing detail of the accessory setae of the gonocoxite and dorsal claspette.

Anopheles deaneorum Rosa-Freitas, 1989. Both species, *An. albitarsis* and *An. deaneorum* show nearly identical ventral claspette and aedeagus. Consequently, we are considering that the morphological characters described for *An. marajoara* by Linthicum (1988), specially those in Figure 14 should be analyzed carefully because it is possible that they are of an undescribed species of the *An. albitarsis* Complex.

Guimarães (1997) considered "Cachoeira do Colombia" (page 26) to be the type locality of *An. marajoara*. We believe that this information in incorrect because there is none locality named Cachoeira do Colombia in Marajó Island. It is possible that Guimarães was mentioning Cachoeira municipality. Cachoeira do Arari was previously designated as both Cachoeira and Arari (Governo do Pará, 2005). Colombia is the country where *An. marajoara* was also reported in addition to Guyana, French Guyana, Bolivia and Brazil.

While examining the male genitalia slide associated with the holotype of *An. marajoara*, we observed that the ventral claspette is distinct from those of all other species of the Argyritarsis Section and consequently from members of the *An. albitarsis* Complex. The adult male holotype of *An. marajoara* is in good condition (Figs. 1-7), as well as the associated larval and pupal exuviae, which are both mounted on a microscope slide that has two labels with inscription "nº 619, nota 812-1, D.H. 384, Marajó, Pará, Alfaia col. 1941" and " *Anopheles* (*N.*) *marajoara* n. sp. Galvão et Damasceno det. 1941". The male genitalia (Figs. 4-7) was dissected and mounted on a microscope slide separate from the immatures and brings two labels with inscription " no 819, nota 821-1, D. H. 383, Marajó, Pará, Alfaia col. 1941" and "*Anopheles* (*N.*) *marajoara* n. sp. Galvão et Damasceno det. 1941". The adult male is mounted on the apex of a little white triangle on an entomological pin, and brings two labels with inscription "*Anopheles* (*N.*) *marajoara* n. sp. Galvão & Damasceno 1942" and "435-12". Additionally, two labels were added when the holotype was transferred from FMUSP to FSP-USP collection, one label with the accession number E-2120 and the other is a red holotype label.

By examining the adult holotype (Figs. 1-7), we observed that it can be easily recognized as a species of the *An. albitarsis*

Complex of the Argyritarsis Section by having the sternum I with a submedial stripe of white scales (Fig. 2); the hindtarsomere V entirely covered with white scales (Fig. 3); and caudolateral scale tufts absent on abdominal segment II. Regarding to the fouth-instar larval and pupal exuviae associated with the adult male, we can also recognize both as members of the *An. albitarsis* Complex. The pupa by having seta 9-V short, less than or equal to 3.0 length of 9-IV; seta 3-C triple; and pinna of trumpet moderately long. The fourth-instar larva shares the following characters with other members of the *An. albitarsis* Complex: seta 1-P always palmate with lanceolate branches; 1,2-P inserted on a common sclerotized tubercle; setae 3-T and 1-I palmate, with well-developed lanceolate branches; seta 2-C widely spaced; and pecten with median teeth mostly subequal. In contrast, the male genitalia associated with the holotype of *An. marajoara* can be recognized as of a specimen of the Albimanus Section. Obviously, it is the same individual described and illustrated by Galvão & Damasceno (1942). Arguing in favor of our hypothesis there are the following facts: according to Linthicum (1988) members of the Argyritarsis Section can be distinguished from those of the Albimanus Section, except *Anopheles albimanus*, by the lack of spicules on the ventral claspette and the preapical plate and the refringent structure are at most weakly developed and are often absent. Additionally, members of the Albitarsis Subgroup are distinct from those of the Albimanus Section by possessing the aedeagus without subapical leaflets, apex of ventral claspette somewhat rounded, with a moderately shallow median sulcus, dorsal claspette narrow, apical setae without a well-developed basomesal projection, and preapical plate absent. The male genitalia associated with the holotype of *An. marajoara* (Figs. 4-7) can be easily identified as *An. aquasalis* by having the following characters, listed in Faran (1980): ventral claspette spiculate (Figs. 5, 6); setae along basal margin of basal lobule of ventral claspette moderately short, about equal to or slightly longer than width of aedeagus, setae usually not reflexed; and preapical plate moderately small, circular to oval and weakly sclerotized (Figs. 5, 6). In agreement with our hypothesis that the male genitalia is not derived from the adult male designated as holotype, it is important to say that Galvão & Damasceno (1942) collected specimens of *An. aquasalis* (as *An. tarsimaculatus*) and *An. albitarsis* in association with *An. marajoara*. It is possible that either during the preparation process or by labeling mistake, the male genitalia of an *An. aquasalis* specimen had been labeled as the male genitalia of the holotype of *An. marajoara*. Unfortunately, except for the holotype of *An. marajoara* there is no other specimen either of *An. aquasalis* or *An. albitarsis* collected in Ilha do Marajó by Galvão & Damasceno (1942) that has been deposited in the FSP-USP collection. Also, in favor of our hypothesis, new recent collections carried out in the type locality in Marajó Island allowed us to examine adults male and female associated with immature stages and male genitalia. All male genitalia which we have examined are distinct from that associated with the holotype of *An. marajoara*, but similar to those of members of the *An. albitarsis* Complex. The International Code of Zoological Nomenclature (1999), article 73.1.5 says "if a subsequent author finds that a holotype which consists of a set of components (e.g. disarticulated body parts) is not derived from an individual animal, the extraneous components may, by appropriate citation, be excluded from the holotype." Consequently, with the International Code of Zoological Nomenclature endorsement and considering the epidemiological importance of *An. marajoara*, the slide with male genitalia associated with the holotype of *An. marajoara* was set aside and excluded from the type material, because it belongs actually to another species as evidences pointed out.

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REFERENCES

Belkin, J. N.; R. X. Schick & S. J. Heinemann. 1971. Mosquito Studies (Diptera, Culicidae). XXV. Mosquitos originally described from Brazil. **Contribution of American Entomological Institute** (Ann Arbor) 7: 1–64.

Conn, J. E.; R. C. Wilkerson; M. N. Segura; R. T. de Souza; C. D. Schlichting; R. A. Wirtz & M. M. Povoa. 2002. Emergence of a new Neotropical malaria vector facilitated by human migration and changes in land use. **American Journal of Tropical Medicine and Hygiene 66**: 18–22.

Corrêa, R. R. & A. S. Ramos. 1943. Descrição de uma nova subespécie de anofelino do subgênero *Nyssorhynchus* Blanchard 1902 (Diptera, Culicidae). **Revista Paulista Medica 22**: 246–248.

Faran, M. E. 1980. Mosquito Studies (Diptera, Culicidae). XXXIV. A revision of the *Albimanus* Section of the subgenus *Nyssorhynchus* of *Anopheles*. **Contribution of American Entomological Institute** (Ann Arbor) 15: 75–88.

Galvão, A. L. A. & R. G. Damasceno. 1942. Sobre um novo anofelino da Ilha de Marajó, *Anopheles* (*Nys.*) *marajoara* n. sp. **Folia Clinica Biologica 14**: 60–66.

Galvão, A. L. A. & R. G. Damasceno. 1944. Observações sobre os anofelinos do complexo *Albitarsis* (Diptera, Culicidae). **Annais da Faculdade de Medicina 20**: 73–87.

Galvão, A. L. A. & J. Lane. 1937. Nota sobre os *Nyssorhynchus* de São Paulo. VII. Estudo sobre as variedades deste grupo com a descrição de *Anopheles* (*Nyssorhynchus*) *albitarsis* Arrib. 1878 var. *limai* n. var. **Annais da Faculdade de Medicina de São Paulo 13**: 227.

Governo do Pará. 2005. http://pa.gov.br/conhecaopara/cachoeiradoarari.asp (accessed in 15th August 2005).

Guimarães, J. H. 1997. **Systematic Database of Diptera of the Americas South of the United States (Family Culicidae)**. São Paulo, Brasil, Plêiade/ Fapesp, 286 p.

Harbach, R. E. 2004. The classification of genus *Anopheles* (Diptera: Culicidae): a working hypothesis of phylogenetic relationships. **Bulletin of Entomological Research 94**: 537–553.

ICZN. 1999. **International Code of Zoological Nomenclature**, 4th edn. The International Trust for Zoological Nomenclature, NHM, London, 306 p + xxix.

Klein, T. A.; J. B. Lima; M. S. Tada & R. Mille. 1991. Comparative susceptibility of anopheline mosquitoes in Rondonia, Brazil to infection by *Plasmodium vivax*. **American Journal of Tropical Medicine and Hygiene 45**: 463–470.

Kreutzer, R. D.; J. B. Kitzmiller & M. G. Rabbani. 1976. Cytogenetically distinguishable sympatric and allopatric populations of the mosquito *Anopheles albitarsis*. **Acta Amazonica 6**: 473–481.

Lane, J. 1953. **Neotropical Culicidae**. São Paulo, Brasil, Universidade de São Paulo, 548 p.

Lehr, M. A.; C. W. Kilpatrick; R. C. Wilkerson & J. E. Conn. 2005. Cryptic species in the *Anopheles* (*Nyssorhynchus*) *albitarsis* (Diptera: Culicidae) Complex: Incongruence between random amplified polymorphic DNA-polymerase chain reaction identification and analysis of mitochondrial DNA COI gene sequences. **Annals of the Entomological Society of America 98**: 908–917.

Linthicum, K. J. 1988. A revision of the Argyritarsis Section of the subgenus *Nyssorhynchus* of *Anopheles* (Diptera: Culicidae). **Mosquito Systematic 20**: 98–271.

Narang, S. K.; T. A. Klein; O. P. Perera & A. T. Tang. 1993. Genetic evidence for the existence of cryptic species in the *Anopheles albitarsis* complex in Brazil: allozymes and mitochondrial DNA restriction fragment length polymorphisms. Biochemical Genetics 31: 97–112.

Rosa-Freitas, M. G. 1989. *Anopheles* (*Nyssorhynchus*) *deaneorum*: a new species in the Albitarsis Complex (Diptera: Culicidae). **Memórias do Instituto Oswaldo Cruz 84**: 535–543.

Rosa-Freitas, M. G.; L. M. Deane & H. Momen. 1990. A morphological isoenzymatic and behavioral study of ten populations of *Anopheles albitarsis* Lynch-Arribalzaga 1878 (Diptera: Culicidae) including from the type locality, Baradero, Argentina. **Memórias do Instituto Oswaldo Cruz 85**: 275–290.

Sallum, M. A. M; T. R. Schultz & R. C. Wilkerson. 2000. Phylogeny of Anophelinae (Diptera, Culicidae) based on morphological characters. **Annals of the Entomological Society of America 93**: 747–775.

Sallum, M. A. M.; T. R. Schultz; P. G. Foster; K. Aronstein; R. A. Wirtz & R. C. Wilkerson. 2002. Phylogeny of Anophelinae (Diptera: Culicidae) based on mitochondrial and nuclear ribosomal DNA sequences. **Systematic Entomology 27**: 361–382.

Wilkerson, R. C.; T. J. Parsons; T. A. Klein; T. V. Gaffigan; E. Bergo & J. Consolim. 1995. Diagnosis by random amplified polymorphic DNA polymerase chain reaction of four cryptic species related to *Anopheles* (*Nyssorhynchus*) *albitarsis* (Diptera: Culicidae) from Paraguay, Argentina, and Brazil. **Journal of Medical Entomology 32**: 697–704.

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5 CONSIDERAÇÕES FINAIS

Em relação ao complexo *An. oswaldoi,* o estudo se restringiu a um dos membros deste complexo, a espécie *An. oswaldoi s.s..* Esta espécie foi caracterizada morfologicamente com a redescrição da larva de quarto estadio, da pupa, dos adultos fêmea e macho e da genitália masculina. Designou-se o lectótipo de *An. oswaldoi s.s*. que se encontra depositado no Museu Nacional do Rio de Janeiro. Paralelamente, fez-se a caracterização molecular da espécie com a utilização de seqüência da região ITS2 do DNA ribossômico. Estas seqüências poderão auxiliar a identificação da espécie, bem como na diferenciação dos membros do complexo, incluindo a possibilidade da descoberta de espécies novas. Espera-se que com a definição da sequência do marcador molecular ITS2 de *An. oswaldoi s.s.*, os trabalhos futuros sejam beneficiados com o acesso às seqüências que estão depositadas no banco de genes de domínio público denominado Genbank (números de acesso EF457228 – EF457239). Vale assinalar que *An. oswaldoi* pode ser confundida com *An. konderi* e *An. ininii*. Portanto, a adequada caracterização da espécie será de enorme importância para estudos sistemáticos, ecológicos e epidemiológicos de avaliação da competência e capacidade vetoras.

Apesar das dificuldades para a identificação das espécies do subgênero *Nyssorhynchus* e, principalmente, para a separação das espécies, foi possível separar cinco táxons do complexo *An. albitarsis* através da análise morfométrica de caracteres da asa. Paralelamente, verificou-se a hipótese dos espécimes de *An. marajoara* estudados caracterizarem morfologicamente duas espécies distintas. Ao mesmo tempo, foi feita a descrição de *An. albitarsis* B e *An. albitarsis* E e a redescrição de *An. albitarsis s.s., An. marajoara e An. deaneorum*. Os resultados das análises estatísticas dos caracteres morfológicos obtidos para 30 indivíduos das cinco espécies demonstraram ser possível a separação morfológica das mesmas. Acresce considerar que verificou-se evidência de que os indivíduos identificados por RAPD-PCR como sendo de *An. marajoara* possam pertencer a dois táxons distintos.

Outra contribuição dos estudos apresentados nesta monografia foi a invalidação da lâmina com a genitália masculina que acompanha o holótipo de *An. marajoara*. Pelo fato da lâmina com a genitália masculina pertencer a um espécime de *Anopheles aquasalis,* embora o adulto e as exúvias da larva e da pupa sejam de *An. marajoara*, ela foi excluída da série tipo. Dessa maneira, foi possível contribuir para a estabilidade da nomenclatura de *An. marajoara*.

REFERÊNCIAS

Añez N, Valenta DT, Cazorla D, Quicke DJ. Feliciangeli MD. Multivariate analysis to discriminate species of pheblotomine sand flies (Diptera: Psychodidae): *Lutzomyia towsendi* , *L. spinicrassa* and *L. youngi.* J Med Entomol. 1997;34: 312-316.

Arruda M de, Carvalho MB, Nussenzweig RS, Maracic M, Ferreira AW, Cochrane AH. Potential vectors of malaria and their different susceptibility to *Plasmodium falciparum and Plasmodium vivax* in northern Brazil identified by immunoassay. Am J Trop Med Hyg.1986;35:873-881.

Belkin JN. The mosquitoes of the South Pacific (Diptera, Culicidae). Vol. 1. University of California Press, Berkeley and Los Angeles,USA; 1962, 620pp.

Belkin JN, Schick RX, Heinemann SJ. Mosquito Studies (Diptera: Culicidae). XXV. Mosquitoes originally described from Brazil. Cont Am Entomol Inst. 1971;7:1-64.

Berti J, Zimmerman R, Amarista J. Spatial and temporal distribution of Anopheline larvae in two malarious areas in Sucre State, Venezuela. Mem Inst Oswaldo Cruz. 1993;88:353-362.

Bonne C. Variability of *Anopheles tarsimaculata* Goeldi (Diptera: Culicidae). Insecutor Inscitiae Menstruus. 1923;11:127-128.

Bonne C. Remarques sur les *Cellia* sud-americaines. Bull Soc Pathol Exot (Paris). 1924;17:132-137.

Branquinho MS, Lagos CBT, Rocha MR, Natal D, Barata JMS, Cochrane AH, Nardin E, Nusserzweig RS, Kloetzel JK. Anophelines in the State of Acre, Brazil, infected with *Plasmodium falciparum, P. vivax*, the variant *P. vivax* VK247 and *P. malariae*. Trans R Soc Trop Med Hyg, 1993;87:391-394.

Branquinho MS, Araújo MS, Natal D, Marrelli MT, Rocha MR, Taveira FAL, Kloetzel JK. *Anopheles oswaldoi* a potential malaria vector in Acre, Brazil. Trans R Soc Trop Med Hyg. 1996; 90:233.

Brochero HHL, Li C, Wilkerso RC. A newly recognized species in the *Anopheles* (*Nyssorhynchus*) Albitarsis complex (Diptera: Culicidae) from Puerto Carreño, Colombia. Am J Trop Med Hyg. 2007;76(6):1113-1117.

Calle DA, Quiñones ML, Erazo HF, Jaramillo JO. Morphometric discrimination of females of five species of *Anopheles* of the subgenus *Nyssorhynchus* from Southern and Northwest Colômbia. Mem Inst Oswaldo Cruz. 2002;97(8):1191-1195.

Causey OR, Deane LM, Deane MP. Studies on Brazilian anophelines from the northeast and Amazon regions. II. An illustrated key by male genitalia characteristics for the identification of thirty-four species of Anophelini, with a note on dissection technique. Amer J Hyg, Monograph Series. 1946;18:21-31.

Chadee DD. Indoor and outdoor host-seeking rhythms of *Anopheles albitarsis* (Diptera: Culicidae) in Trindad, West Indies. J Med Entomol. 1992;29:567-569.

Chadee DD, Beier JC. Diel oviposition and fecundity of *Anopheles oswaldoi* in Trinidad, West Indies. J Am Mosq Control Assoc. 1996:402-406.

Chadee DD, Wilkerson RC. Ecology of the malaria vector, A*nopheles* (*Nyssorhynchus*) *marajoara* Galvão and Damasceno in Trindad, West Indies. J Am Mosq Control Assoc. 2006;22(1):22-28.

Collins FH, Paskewitz SM. A review of the use of ribosomal DNA (rDNA) to differentiate among cryptic *Anopheles* species. Inst Mol Biol. 1996;5:1-9

Conn JE, Wilkerson RC, Segura NO, Souza RTL, Schlichting CD, Wirtz RA, Povoa MM. Emergence of a new Neotropical malaria vector facilited by human migration and changes in land use. Am J Trop Med Hyg. 2002;66 (1):18–22.

Conn JE, Cockburn AF, Mitchell SE. Population differentiation of the malaria vectors *Anopheles* using mitochondrial DNA. J Hered. 1993;84:248-253.

Consoli R, Lourenço-de-Oliveira R. Principais Mosquitos de Importância Sanitária no Brasil*.* Rio de Janeiro: Fiocruz; 1994.

Corrêa, RR, Ramos, AS. Descrição de uma nova subespécie de anofelino do subgênero *Nyssorhynchus* Blanchard, 1902 (Diptera, Culicidae). Rev Paul Med. 1943;22:246-248.

Corrêa R R, Lima FO, Coda D. Observations on the flight and longevity in nature of *Anopheles albitarsis domesticus*. J Nat Malar Soc. 1950;9:280-284.

Cova-Garcia P. Notas sobre los anofelinos de Venezuela y su identificacion. Caud Amar Conf Sanit Panamer. 1946;12:1-208

Curry DP. Some observations on the *Nyssorhynchus* group of the *Anopheles* (Culicidae) of Panama. Am J Hyg. 1932;15:566-572.

Deane LM, Causey OR, Deane MP. Distribuição e biologia dos anofelinos. Rev Serv Saúde Pública.1948;1,874 – 875.

Dyar HG. The mosquitoes of Panamá (Diptera: Culicidae). Insecutor Inscitiae Menstruus.1923;11:167-186.

Faran ME. Mosquito studies (Diptera: Culicidae) XXXIV. A revision of the Albimanus Section of the subgenus *Nyssorhynchus* of *Anopheles*. Cont Am Entomol Inst.1980;15:1-215.

Faran ME, Linthicum KJ. A handbook of the Amazonian species of *Anopheles* (*Nyssorhynchus*) (Diptera: Culicidae). Mosq Syst. 1981;13:1-81.

Faria FS, Leoncini O. Evolution of ribosomal genes in the family Culicidae. Rev Brazilian Genet. 1988;11:275-285.

Flores-Mendoza C, Fernandez R, Escobedo-Vargas KS, Vela-Perez Q, Schoeler GB.) Natural *Plasmodium* infections in *Anopheles darlingi* and

Anopheles benarrochi (Diptera: Culicidae) from eastern Peru. J Med Entomol. 2004a;41:489-494.

Flores-Mendoza C, Peyton EL, Wilkerson RC, Lourenço-de-Oliveira R. *Anopheles (Nyssorhynchus) konderi* Galvão and Damasceno: Neotype designation and resurrection from synonymy with *Anopheles (Nyssorhynchus) oswaldoi* (Peryassú) (Diptera: Culicidae). Proc Entomol Soc Washington. 2004b;106:118-132.

Fonseca JAB da, Fonseca F da. Transmissão da malária humana por anofelíneos da série tarsimaculatus. Mem Inst Butantan. 1942;16:93-124.

Forattini OP. Culicidologia médica. São Paulo: EDUSP, 2002. 2 v.

Forattini OP, Kakitani I, Massad E, Marucci D. Studies on mosquitoes (Diptera: Culicidae) and anthropic environment. 2 – Immature stages research at a rice irrigation system location in South-Eastern Brazil. Rev Saúde Pública. 1993; 27:227-236.

Forattini OP, Kakitani I, Massad E, Marucci D. Studies on mosquitoes (Diptera: Culicidae) and anthropic environment. 7 – Behaviour of adults *Nyssorhynchus* anophelines with special reference to *Anopheles albitarsis s.l.* in South Eastern Brazil. Rev Saúde Pública. 1995;29(1): 20-26.

Forattini OP, Kakitani I, Massad E, Marucci D. Studies on mosquitoes (Diptera:Culicidae) and anthropic environment. 12- Host-seeking behaviour of *Anopheles albitarsis s.l.* in South-Eastern Brazil. Rev Saúde Pública. 1996;30:299-303.

Forattini OP, Massad E. Culicidae vectors and anthropic changes in a southern Brazil natural ecosystem. Ecosystem Health. 1998;4:9-19.

Galvão, ALA, Lane, J. Nota sobre os *Nyssorhynchus* de S. Paulo. VII. Estudo sobre as variedades deste grupo com a descrição de *Anopheles* (*Nyssorhynchus*) *albitarsis* Arrib., 1878 var. *limai* n. var. Ann Fac Med. 1937;13: 211-238.

Galvão, ALA, Damasceno, RG. Sobre um novo anofelino da Ilha de Marajó, *An.* (*Nys.*) *marajoara* n. sp. Folia Clínica. 1942;14:60-66.

Galvão, ALA, Damasceno, RG. Observações sobre anofelinos do complexo *albitarsis* (Diptera Culicidae). Ann Fac Med S. Paulo. 1944;20:73-87.

Garcia M, Casal OH. Descripcion de tres pupas de mosquitos neotropicales (Diptera, Culicidae). Revta Soc Entomol Argent. 1964 (1965);27:5-10.

Gelbre-Michael T, Medhin G. Morphometric separation of female of *Phelebotomus* (*Phlebotomus*) *dudoscqui* and *P.*(*P.*) *bergetroti* (Diptera: Psycodiae). J Med Entomol. 1997;34:383-386.

Grillet ME. Factors associated with distribution of *Anopheles aquasalis* and *An. oswaldoi* (Diptera: Culicidae) in a malarious area, northeastern Venezuela. J Med Entomol. 2000;37:231-238.

Guimarães AE, Mello RP, Lopes CM, Gentile C. Ecology of mosquitoes (Diptera: Culicidae) in areas of Serra do Mar State Park, State of São Paulo, Brazil. II- habitat distribution. Mem Inst Oswaldo Cruz. 2000;95: 17-28.

Guimarães AE, Gentile C, Alencar J, Lopes CM, Mello RP. Ecology of anophelinae (Diptera: Culicidae), malaria vectors around the Serra da Mesa Reservoir, State of Goiás, Brazil. 1- Frequency and climatic factors. Cad Saúde Pública. 2004;20:291-302.

Harbach RE, Knight K L. Taxonomists' glossary of mosquito anatomy. Plexos: Marlton, N. J.; 1980.

Harbach RE, Kitching IJ. Phylogeny and classification of the Culicidae (Diptera). Syst Entomol. 1998;23:327-370.

Harbach RE. The classification of genus *Anopheles* (Diptera, Culicidae): a working hyphotesis of phylogenetic relationships. Bull Entomol Res. 2004:537-553.

Harbach RE, Kitching LJ. Reconsideration of anopheline mosquito phylogeny (Diptera: Culicidae: Anophelinae) based on morphological data. Syst Biodiversity. 2005;3:345-374.

Harbach RE, Rattanarithikul R, Harrisson BA. *Baimaia*, a new subgenus for *Anopheles kyondawensis* Abraham, a unique crabhole-breeding anopheline in Southeastern Asia. Proc Entomol Wash. 2005;107:750-761.

Hayes J, Calderon G, Falcon R, Zambrano V. Newly incriminated anopheline vectors of human malaria parasites in Junin Department, Peru. J Am Mosq Control Assoc. 1987;3:418-422.

Hribar LJ. Costal wing spot variation within and among progeny of single female *Anopheles nuneztovari* (Diptera: Culicidae). Mosq Syst. 1995;27(1):1-15.

Hugh G, Gauch JR. Multivariate analysis in community ecology. New York: Cambridge; 1982.

Kakitani I, Forattini OP. Parity and ovarian development of *Anopheles albitarsis l.s.* in irrigated agroecosystem field. Rev Saúde Pública. 2000;34(1):33-38.

Kakitani I, Ueno HM, Forattini OP. Parity and wind impacto n the frequency of *Anopheles marajoara* in Brazil. Rev Saúde Pública. 2003;37(3):280-284.

Kitzmiller JB. Chromosomal differences among species of *Anopheles* mosquitoes. Mosq Syst. 1977;9(2):112-122.

Klein TA, Lima JB. Seasonal distribution and biting patterns of *Anopheles* mosquitoes in Costa Marques, Rondônia, Brazil. J Am Mosq Control Assoc. 1990;6:700-707.

Klein TA, Lima JB, Tang AT. Colonization and maintenance of *Anopheles deaneorum* in Brazil. J Am Mosq Control Assoc. 1990;6(3):510-513.

Klein TA, Lima JB, Tang AT. Biting behavior of *Anopheles* mosquitoes in Costa Marques, Rondônia, Brazil. Rev Soc Bras Med Trop. 1991a;24(1):13-20.

Klein T A, Lima JB, Tang AT. Hybridization evidence supporting separate species status for *Anopheles albitarsis* and *Anopheles deaneorum* (Diptera: Culicidae) in Brazil. J Am. Mosq Control Assoc. 1991b;7(2):301-303.

Klein TA, Lima JBP, Tada M.S. Comparative susceptibility of anopheline mosquitoes to *Plasmodium falciparum* in Rondônia, Brazil. Am J Trop Med Hyg. 1991c;44:598-603.

Klein TA, Lima JBP, Tada MS, Miller R. Comparative susceptibility of anopheline mosquitoes in Rondônia, Brazil to infection by *Plasmodium vivax*. Am J Trop Med Hyg. 1991d;45(4):463-470.

Kreutzer R D, Kitzmiller JB, Rabbani MG. Cytogenetically distinguishable sympatric and allopatric populations of the mosquito *Anopheles albitarsis*. Acta Amazônica; 1976;6(4): 473-481.

Krzywinski J, Wilkerson RC, Besanski NJ. Evolution of mitochondrial and ribosomal gene sequence in Anophelinae (Diptera:Culicidae): implications for phylogeny reconstruction. Mol Phylog Evol. 2001a;18:479-487.

Krzywinski J., Wilkerson RC, Besanski NJ. Toward understanding Anophelinae (Diptera: Culicidae) phylogeny insights from nuclear single copy genes and the weight of evidence. Syst Biol. 2001b;50:540-556.

Kumar S, Tamura K, Nei M. MEGA 3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefings in Bioinformatics. 2004;5:150-163.

Lane J. Neotropical Culicidae. São Paulo: Universidade de São Paulo; 1953. v. 2.

Lane RP. Multivariate discrimination between *Lutzomyia welcomei*, a vector of mucocutaneous leishmaniasis, and *Lu. complexus* (Diptera: Phlebotominae). Ann Trop Med Parasitol. 1985;79:469-472.

Laubach HE, Validium L, Bonilla JA, Agar A, Cummings R, Mitchell C, Cuadrado RR, Palmer CJ. Identification of *Anopheles aquasalis* as a possible vector of malaria in Guyana, South America. West Indian Med J. 2001;50:319-321.

Lehr MA, Kilpatrick CW, Wilkerson RC, Conn JE. Cryptic species in the *Anopheles* (*Nyssorhynchus*) *albitarsis* (Diptera: Culicidae) complex: incongruence between Random Amplified Polymorphic DNA-Polymerase chain reaction identification and analysis of mitochondrial DNA *COI* gene sequences. Ann Entomol Soc Am. 2005;98(6):908-917.

Li C, Wilkerson RC. Identification of *Anopheles* (*Nyssorhynchus*) *albitarsis* complex species (Diptera: Culicidae) using rDNA internal transcribed spacer 2-basepolymerase chain reaction primers. Mem Inst Oswaldo Cruz. 2005;100:495-500.

Li C, Wilkerson RC, Fonseca DM. Isolation of polymorphic microsatellite markers from the malaria vector *Anopheles marajoara* (Diptera: Culicidae). Mol Ecol Notes. 2005;5:65-67.

Li C, Wilkerson RC. Intragenomic rDNA ITS2 variation in the Neotropical *Anopheles* (*Nyssorhynchus*) *albitarsis* complex (Diptera: Culicidae). J Heredity, 2006;98(1): 51-59.

Lima AC. Sobre algumas anophelinas encontradas no Brasil. Mem Inst Oswaldo Cruz. 1928;3:91-113.

Lima JBP, Valle D, Peixoto AA. Adaptation of a South american malaria vector to laboratory colonization suggests master-male evolution for mating ability. BMC Evol Biol. 2004;4:1-6.

Linthicum KJ. A revision of the Argyritarsis Section of the subgenus *Nyssorhynchus* of *Anopheles* (Diptera: Culicidae). Mosq Syst. 1988;9271.

Lopes J, Lozovei AL. Ecologia de mosquitos (Diptera: Culicidae) em criadouros naturais e artificiais de área rural do Norte do Estado do Paraná, Brasil. I – Coletas ao longo do leito de ribeirão. Rev Saúde Pública. 1995;29:183-191.

Lopes J, Zequi JAC, Nunes V, Oliveira O, Neto BPO, Rodrigues W. Immature Culicidae (Diptera) collected from the Igapó Lake located in the urban area of Londrina, Paraná, Brazil. Brazilian Arch Biol Technol. 2002;45:465-471.

Lounibos LP, Conn JE. Fecundity, parity, and adult feeding relationships among *Nyssorhynchus* malaria vectors from Venezuela. Mem Inst Oswaldo Cruz. 1991;86(1):57-66.

Lounibos LP, Duzak D, Linley JR. Comparative egg morphology of six species of the Albimanus Section of *Anopheles* (*Nyssorhynchus*) (Diptera: Culicidae). Entomol Soc Am. 1997;34:136-155.

Lounibos LP, Wilkerson RC, Conn JE, Hribar LJ, Fritz GN, Danoff-Burg JA. Morphological, molecular and chromosomal discrimination of cryptic *Anopheles* (*Nyssorhynchus*) (Diptera: Culicidae) from South America. J Med Entomol.1998a;35:830-838.

Lounibos LP, Lima DC, Lourenço-de-Oliveira, Escher RL, Nishimura N. Egg maturation in Neotropical malaria vectors: one blood meal is usually enough. J Vector Ecol. 1998b;23:195-201.

Lounibos LP, Conn JE. Malaria vector heterogeneity in South America. Am Entomol. 2000;46:238-249.

Lourenço-de-Oliveira R, Guimarães AE, Arlé M, Silva TF, Castro MG, Motta MA, Deane LM. Anophelines species, some of their habits and relation to malaria endemic areas of Rondônia State, Amazon Region of Brazil. Mem Inst Oswaldo Cruz. 1989;84:501-514.

Lourenço-de-Oliveira R, Luz SLB. Simian malaria at two sites in the Brazilian Amazon – II. Vertical distribution and frequency of Anopheline species inside and outside the forest. Mem Inst Oswaldo Cruz. 1996;91:687-694.

Lynch Arribalzaga, F. Descripcion de tres nuevos Culicidae de Buenos Aires. Nat Argent. 1878;1:149-152. Madr. 1878;25:313-330.

Maldonado V, Finol HJ, Navarro JC*.* *Anopheles aquasalis* eggs from two Venezuelan localities compared by scanning electron microscopy. Mem Inst Oswaldo Cruz. 1997;92(4):487-491.

Marrelli MT, Branquinho MS, Hoffmann,EHE, Taipe-Lagos CB, Natal D, Kloetzel JK. Correlation between positive serology for *Plasmodium vivax*-like/*Plamodium simiovale* malaria parasites in the human and anopheline populations in the State of Acre, Brazil. Trans R Soc Trop Med Hyg. 1998;92:149-151.

Marrelli MT, Honório NA, Flores-Mendoza C, Lourenço-de-Oliveira R, Marinotti O, Kloetzel JK. Comparative susceptibility of two members of the *Anopheles oswaldoi* complex, *An. oswaldoi* and *An. konderi*, to infection by *Plasmodium vivax*. Trans R Soc Trop Med Hyg. 1999a;93:381-384.

Marrelli MT, Malafronte RS, Flores-Mendoza C, Lourenço- de- Oliveira R, Kloetzel JK, Marinotti O. Sequence analysis of the second internal transcribed spacer of ribosomal DNA in *Anopheles oswaldoi* (Diptera: Culicidae). J Med Entomol. 1999b;36(6):679-684.

Marrelli MT, Sallum MAM, Marinotti O.The second internal transcribed spacer of nuclear ribosomal DNA as a tool for Latin American anopheline taxonomy – A critical review. Mem Inst Oswaldo Cruz*.* 2006;101:817-832

Marucci D. Estudo exocorial de *Anopheles (Nyssorhynchus) albitarsis s.l*. com autilização de microscópio eletrônico de varredura e sistema de análise de Imagens. [dissertação de mestrado]. São Paulo: Faculdade de Saúde Pública da USP; 1995.

Mayr E. Principles of systematic zoology. New York: McGraw-Hill; 1969.

Merritt TJS, Young CR, Vogt RG, Wilkerson RC, Quattro JM. Intron retention identifies a malaria vector within the Anopheles (Nyssorhynchus) albitarsis complex (Diptera: Culicidae). Mol Phylog Evol. 2005;35:719-724.

Miller BR, Crabtree MB, Savage HM. Phylogenetic relationship of the Culicomorpha inferred from 18S and 5.8S ribosomal DNA sequences (Diptera: Nematocera). Insect Mol Biol. 1997;6(2):105-114.

Munstermann LE, Conn JE Systematics of mosquito disease vectors (Diptera: Culicidae): impact of molecular biology and cladistic analysis. Ann Rev Entomol 1997;36(6):679-684.

Narang SK, Klein TA, Perera OP, Lima JB, Tang AT. Genetic evidence for the existence of cryptic species in the *Anopheles albitarsis* complex in Brazil: allozymes and mitochondrial DNA restriction fragment length polymorphisms. Biochem Genet. 1993;31(1-2):97-112.

Natal D, Barata JMS, Taipe-Lagos CB, Rocha RM. Nota sobre culicídeos (Diptera: Culicidae) da bacia do rio Purus, Acre, Amazônia (Brasil). Rev Saúde Pública. 1992;26:129-131.

Naves HAM, Carvalho MESD, Carneiro E, Sales KP. Espécies de Culicidae de Goiânia - Goiás - Brasil. Rev Patol Trop. 1996;25:31-42.

Oliveira-Ferreira J, Lourenço-de-Oliveira R, Teva A & Deane LM. Natural malaria infections in anophelines in Rondônia State, Brazilian Amazon. Am J Trop Med Hyg. 1990;43:6-10.

Perera OP, Cockburn AF, Mitchell SE, Conn J, Seawright JA. Species-specific repeat units in the intergenic spacer of the ribosomal RNA cistron of *Anopheles aquasalis* Curry. Amer J Trop Med Hyg. 1998;59:673-678.

Peryassú A. Duas novas espécies de mosquitos do Brasil. A folha médica. 1922;3:179.

Petrarca V, Sabatelli G, Toure Y, Dideco MA. Morphometric multivariate analysis in *Anopheles gambiae s.s*. (Diptera: Culicidae). J Med Entomol. 1998;35:16-25.

Peyton EL, Roberts DR, Pinheiro FP, Vargas R., Balderama F. Mosquito collections from a remote unstudied area of southeastern Bolivia. Mosq Syst. 1983;15:61-89.

Peyton EL, Wilkerson RC, Harabch RE. Comparative analysis of subgenera *Kerteszia* and *Nyssorhynchus* of *Anopheles* (Diptera: Culicidae). Mosq Syst. 1992;24(1):51-69.

Pinto C. Disseminação da malária pela aviação; biologia do *Anopheles gambiae* e outros anofelineos do Brasil. Mem Inst Oswaldo Cruz. 1939;34:293-294.

Póvoa MM, Machado RL, Segura MN, Vianna GM, Vasconcelos AS, Conn JF. Infectivity of malaria vector mosquitoes: correlation of positivity between ELISA and PCR-ELISA tests. Trans R Soc Med Hyg. 2000;94:106-107.

Póvoa MM, Wirtz RA, Lacerda RNL, Miles MA, Warhurst D. Malaria vectors in Municipality of Serra do Navio, State of Amapá, Amazon Region, Brazil. Mem Inst Oswaldo Cruz. 2001;96:179-184.

Póvoa MM, Conn JE, Schlichting CD, Amaral JC, Segura MN, Silva AN da, Santos C C dos, Lacerda RN, Souza RT de, Galiza, D, Santa Rosa EP, Wirtz RA. Malaria vectors, epidemiology, and the re-emergence of *Anopheles darlingi* in Belém, Pará, Brazil. J Med Entomol. 2003;40:379-386.

Póvoa MM, Souza RTL, Lacerda RNL, Rosa ES, Galiza D, Souza, JR, Wirtz RA, Schlichting CD, Conn JE. The importance of *Anopheles albitarsis* E and *Anopheles darlingi* in human malaria transmission in Boa Vista, State of Roraima, Brazil. Mem Inst Oswaldo Cruz. 2006;101:163-168.

Quiñones ML, Suarez MF. Indoor resting heights of some anophelines in Colombia. J Am Mosq Control Assoc. 1990;6(4):602-604.

Quiñones ML, Ruiz F, Calle DA, Harbach RE, Erazo HF, Linton YM Incrimination of *Anopheles* (*Nyssorhynchus*) *rangeli* and *An.* (*Nys.) oswaldoi* as natural vectors of *Plasmodium vivax* in Southern Colombia. Mem Inst Oswaldo Cruz. 2006;101;617-623.:

Rambajanl, I. An annotated checklist of the *Anopheles* of Guyana, South America. Mosq Syst. 1987;19:146-161.

Rebêlo JMM, Silva AR, Ferreira LA, Vieira JA. *Anopheles* (Culicidae, Anophelinae) e a malária em Buricutu – Santa Luzia, Pré-Amazônia Maranhense. Rev Soc Bras Med Trop. 1997;30: 107-111.

Reigada SMB. Diagnóstico em análise discriminante. [dissertação de mestrado]. São Paulo: Instituto de Matemática e Estatística da USP; 2004.

Reinert JF, Harbach RE, Kitching IJ. Phylogeny and classification of Aedini (Diptera: Culicidae) based on morphological characters of all life stages. Zool J Linn Soc. 2004;142:289-368.

Romeo-Viamonte J M, Castro M. Estudio de la morfologia de la armadura faringea de algunos anofilinos. (Dipt. Culic.). Revta Sanit Hig Publ Madr. 1951;25:313-330.

Root FM. Further notes on the male genitalia of American *Anopheles*. Am J Hyg. 1924;4:456-465.

Rosa-Freitas MG. *Anopheles* (*Nyssorhynchus*) *deaneorum*: a new species in the *Albitarsis* complex (Diptera: Culicidae). Mem Inst Oswaldo Cruz. 1989;84:535-543.

Rosa-Freitas MG, Deane LM. The neotype of *Anopheles albitarsis* (Diptera: Culicidae). Mem Inst Oswaldo Cruz. 1989;84:535-543.

Rosa-Freitas MG, Lourenço-de-Oliveira R, Carvalho-Pinto CJ, Flores-Mendoza C, Silva-do-Nascimento TF. Anophelinae species complexes in Brazil. Current knowledge of those related to malaria transmission. Mem Inst Oswaldo Cruz. 1998;93:651-655.

Ross ES, Roberts HR. Mosquito atlas. Part I. The Nearctic *Anopheles*, important malaria vectors of the Americas and *Aedes aegypti*, *Culex quinquefasciatus*. 1943, 44 pp., illus.

Rozeboom LE. Subspecific variations among Neotropical *Anopheles* mosquitoes, and their importance in the transmission of malaria. Am J Trop Med. 1942;22:235-255.

Rubio-Palis Y, Curtis CF. Biting and resting behaviour of anophelines in western Venezuela and implications for control of malaria transmission. Med Vet Entomol. 1992;6:325-334.

Rubio-Palis Y, Curtis CF, Gonzáles C, Wirtz RA. Host choice of anopheline mosquitoes in a malaria endemic area of Western Venezuela. Med Vet Entomol. 1994;8:275-280.

Rubio-Palis Y. Differential attraction of Venezuelan Anophelines to human collectors. J Am Mosq Control Assoc. 1995;11(4):482-484.

Rubio-Palis Y, Manguin S, Ayesta C, Guzman H, Arcia JM, Gonzalez JJr, Perez E. Revisión taxonômica de los anofelinos vectores de malaria em el sur de Venezuela. Bol Dir Malariol San Amb. 1997;37:35-48.

Rubio-Palis Y. Caracterización morfométrica de poblaciones de *Anopheles (Nyssorhynchus) darling*i del sur de Venezuela. Bol Entomol Venez. 1998;13:141-172.

Rubio-Palis Y, Wilkerson RC, Guzman H. Morphological characters of adult *Anopheles* (*Nyssorhynchus*) *marajoara* in Venezuela. J Am Mosq Control Assoc. 2003;19:107-114.

Ruiz F, Quiñones ML, Calle DA, Erazo HF, Alzate JF, Linton YM. Molecular differentiation of *Anopheles* (*Nyssorhynchus*) *benarrochi* and *Anopheles (Nyssorhynchus) oswaldoi* from Southern Colombia. Mem Inst Oswaldo Cruz. 2005;100:155-160.

Sallum MAM, Schultz TR, Wilkerson RC. Phylogeny of Anophelinae based on morphological characters. Ann Entomol Soc of Am. 2000;93:745-775.

Sallum MAM, Schultz TR, Foster PG, Aronstein K, Wirtz RA, Wilkerson RC. Phylogeny of Anophelinae (Diptera: Culicidae) based on nuclear ribosomal and mitochondrial DNA sequences. Syst Entomol. 2002;27:361-382.

San Sebastián, M., Jativa, R. & Goicolea, I. Epidemiology of malaria in the Amazon basin of Ecuador. Revta Panam Salud Pública. 2000;7:24-28.

Santos RLC. Medida da capacidade vetorial de *Anopheles albitarsis* e *Anopheles* (*Kerteszia*) da região Sudeste do Brasil. [tese de doutorado]. São Paulo: Faculdade de Saúde Pública da USP; 2001.

Santos RLC, Forattini OP. Método da marcação - soltura – recaptura para determinar o tamanho de população natural de *Anopheles albitarsis s.l.* (Diptera: Culicidae) Rev Saúde Pública. 1999;33:309-313.

Santos JB dos, Santos F, Macedo V. Variation of *Anopheles* density with deltamethrin-impregnated mosquito nets in an endemic malaria area of the Brazilian Amazon. Cad Saúde Pública. 1999;15(2):281-292.

Santos RL, Forattini OP, Burattini MN. Laboratory and field observations on duration of gonotrophic cycle of *Anopheles albitarsis* s.l. (Diptera: Culicidae) in southeastern Brazil. J Med Entomol. 2002;39(6):926-930.

Santos RLC, Sucupira IMC, Lacerda RNL, Fayal AS, Póvoa MM. Inquérito entomológico e infectividade durante epidemia de malária no município de Anajás, Estado do Pará. Rev Bras Med Trop. 2005;38:202-204.

Scarpassa VM. Isozyme similarity in *Anopheles oswaldoi sensu lato* (Diptera; Culicidae) from the Amazon region. J Med Entomol. 2005;42:319-326.

Scarpassa VM, Conn JE. Molecular differentiation in natural populations of *Anopheles oswaldoi* sensu lato (Diptera: Culicidae) from the Brazilian

Amazon, using sequences of the COI gene from mitochondrial DNA. Gen Mol Res. 2006;5:493-502.

Senevet G. Contribution a l'etude des nymphes d'anophelines. (4e Memoire). Arch Inst Pasteur Alger. 1934;12:29-76.

Senevet G, Abonnenc E. Quelques anophelines de la Guyane Francaise. Arch Inst Pasteur Alger. 1938;16:486-512.

Senise LV, Motoki MT, Sallum MAM. Notes on the holotype of *Anopheles marajoara* Galvão & Damasceno (Diptera: Culicidae). Rev Bras Entomol. 2006;50(4):453-457.

Sierra DM, Vélez ID, Linton YM. Malaria vector *Anopheles* (*Nyssorhynchus*) *nuneztovari* comprises one genetic species in Colombia based on homogeneity of nuclear ITS2 rDNA. J Med Entomol. 2004;41(3):302-307.

Silva ANM, Fraiha-Neto H, Santos CCB dos, Segura MNO, Amaral JCOF, Gorayeb IS, Lacerda RNL, Sucupira IMC, Pimentel LN, Conn JE, Póvoa MM. Fauna anofélica da cidade de Belém, Pará, Brasil: dados atuais e retrospectivos. Cad Saúde Pública. 2006;22:1575-1585.

Silva-Vasconcelos A, Kato MY, Mourao EN, Souza RT de, Lacerda RN, Sibajev A, Tsouris P, Povoa M.M., Momen H, Rosa-Freitas MG. Biting índices, host-seeking activity and natural infection rates of anopheline species in Boa Vista, Roraima, Brazil from 1996 to 1998. Mem Inst Oswaldo Cruz. 2002;97:151-161.

Simmons, J.S. Malaria in Panama. São Paulo; 1979 [acesso em jan 2007]. Disponível em http:// www.books.google.com

Steiner WWM, Narang S, Kitzmiller JB, Swofford DL. Genetics diversity and evolution in neotropical *Anopheles* (Subgenus *Nyssorhynchus*). In Steiner WWM, Tabachnick WJ, Rai KS, Narang SK. Recent Developments in the Genetics of Insects Disease Vectors. Stipes: Champaign IL; 1982; p. 523-550.

Tadei WP, Santos JMM, Costa, WLS & Scarpassa VM. Biologia de anofelinos amazônicos. XII. Ocorrência de espécies de *Anopheles*, dinâmica na transmissão e controle da malária na zona urbana de Ariquemes (Rondônia I). Rev Inst Med Trop São Paulo. 1988;30:221-251.

Tadei WP, Thatcher BD, Santos JMM, Scarpassa VM, Rodrigues IB, Rafael M. Ecological observations on Anopheline vectors of malaria in the Brazilian Amazon. Am J Trop Med Hyg. 1998;59:325-335.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins, DG. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 1997;24: 4876-4882.

Voorham J. Intra-population plasticity of *Anopheles darlingi*'s (Diptera, Culicidae) biting activity patterns in the state of Amapá, Brazil. Rev Saúde Pública. 2002;36(1): 75-80.

Wilkerson RC, Peyton EL. Standardized nomenclature for the costal wing spots of the genus *Anopheles* and other spotted-wing mosquitoes (Diptera: Culicidae). J Med Entomol. 1990;27**:**207-224**.**

Wilkerson RC, Gaffigan TV, Bento-Lima J. Identification of species related to *Anopheles (Nyssorhynchus) albitarsis* by random amplified polymorphic DNA- polymerase chain reaction (Diptera: Culicidae) Mem Inst Oswaldo Cruz. 1995a;90:721-732.

Wilkerson RC, Parsons TJ, Klein TA, Gaffigan TV, Bergo E, Consolim J. Diagnosis by random amplified polymorphic DNA polymerase chain reaction of four cryptic species related to *Anopheles* (*Nyssorhynchus*) *albitarsis* (Diptera: Culicidae) from Paraguay, Argentina and Brazil. J Med Entomol. 1995b;32:697-704.

Wilkerson RC, Foster PG, Li C, Sallum MAM. Molecular phylogeny of Neotropical *Anopheles* (*Nyssorhynchus*) *albitarsis* species complex (Diptera: Culicidae). Ann Ent Soc Amer. 2005;98(6):918-925.

Wood DM, Borkent A. Phylogeny and classification of Nematocera. In: Mcalpine JF Wood DV. Manual of Neartic Diptera. Otawa: Res Branch Agric Can; 1989. v.3, p. 1333-1370.

Zavortink TJ. Classical taxonomy of mosquitoes – a memorial to John N. Belkin. J Amer Mosq Control Assoc. 1990;6:593-599.

Zimmerman RH, Galardo AKR, Lounibos LP, Arruda M, Wirtz R. Bloodmeal hosts of *Anopheles* species (Diptera: Culicidae) in a malaria-endemic area of the Brazilian Amazon. J Med Entomol. 2006;43(5):947-956.