The *Anopheles albitarsis* complex with the recognition of *Anopheles oryzalimnetes* Wilkerson and Motoki, n. sp. and *Anopheles janconnae* Wilkerson and Sallum, n. sp. (Diptera: Culicidae)

Maysa Tiemi Motoki[1], Richard C Wilkerson[2], Maria Anice Mureb Sallum[1]/+

[1]Departamento de Epidemiologia, Faculdade de Saúde Pública, Universidade de São Paulo, Avenida Dr. Arnaldo 715, 01246-904 São Paulo, SP, Brasil [2]Walter Reed Army Institute of Research, Department of Entomology, Silver Spring, MD, USA

The Anopheles (Nyssorhynchus) albitarsis *complex includes six species:* An. albitarsis, Anopheles oryzalimnetes *Wilkerson and Motoki, n. sp.,* Anopheles marajoara, Anopheles deaneorum, Anopheles janconnae *Wilkerson and Sallum, n. sp. and* An. albitarsis *F. Except for* An. deaneorum, *species of the complex are indistinguishable when only using morphology. The problematic distinction among species of the complex has made study of malaria transmission and ecology of* An. albitarsis s.l. *difficult. Consequently, involvement of species of the* An. albitarsis *complex in human* Plasmodium *transmission is not clear throughout its distribution range. With the aim of clarifying the taxonomy of the above species, with the exception of* An. albitarsis *F, we present comparative morphological and morphometric analyses, morphological redescriptions of three species and description of two new species using individuals from populations in Brazil, Paraguay, Argentina and Venezuela. The study included characters from adult females, males, fourth-instar larvae, pupae and male genitalia of* An. albitarsis, An. marajoara, An. deaneorum *and* An. oryzalimnetes *n. sp. For* An. janconnae *n. sp. only characters of the female, male and male genitalia were analyzed. Fourth-instar larvae, pupae and male genitalia characteristics of all five species are illustrated. Bionomics and distribution data are given based on published literature records.*

Key words: systematics - *Nyssorhynchus* - *Anopheles albitarsis* complex - new species - multivariate analysis

The *Anopheles* (*Nyssorhynchus*) *albitarsis* complex is widely distributed in South America and includes *An. albitarsis* Lynch-Arribálzaga, *Anopheles deaneorum* Rosa-Freitas, *Anopheles marajoara* Galvão and Damasceno, *An. albitarsis* B (Wilkerson et al. 1995c), An. albitarsis E (Lehr et al. 2005) and *An. albitarsis* F (Brochero et al. 2007). Three species are of proven importance as vectors of human malaria parasites in Brazil: *An. deaneorum* [state of Rondônia (RO)] (Klein et al. 1991a, b), *An. marajoara* [state of Amapá (AP)] (Conn et al. 2002) and *An. albitarsis* E [state of Roraima (RR)] (Póvoa et al. 2006).

Indications of the presence of a species complex in *An. albitarsis s.l.* were reported by Kreutzer et al. (1976) and Kitzmiller (1977) who found fixed chromosomal inversions suggesting the presence of two sympatric forms in Southern and Eastern Brazil and a third in Colombia and Venezuela. A similar conclusion was reached by Steiner et al. (1982) who used isozyme analyses of samples collected along a transect from state of São Paulo (SP) to west of Marajó Island to find three genetically distinct populations.

Linthicum (1988) recognized two species in the Albitarsis Complex, *An. marajoara* and *An. albitarsis* and provided characters for their identification. To better characterize the morphological characters of *An. albitarsis s.s.*, Rosa-Freitas and Deane (1989) used specimens collected from the type locality of Baradero, Argentina, to designate a neotype and to redescribe the adult male and female, fourth-instar larva and pupa. Subsequently, Rosa-Freitas (1989) named and described *An. deaneorum* and distinguished it from *An. albitarsis* using morphological characteristics and evidence from allele frequencies of 11 allozyme loci. Morphological distinction of *An. deaneorum* from *An. albitarsis* and *An. marajoara* was based mainly on seta 3-C of the fourth-instar larva (multi-branched in *An. deaneorum*, single or aciculate in the other 2). Later, Narang et al. (1993) employed allozyme markers and Restriction Fragment Length Polymorphisms (RFLP) profiles of the mitochondrial genome to estimate genetic diversity of sympatric populations of *An. deaneorum* and *An. marajoara* from several localities in Brazil.

Wilkerson et al. (1995a, c) used random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) to show that the *An. albitarsis* complex included *An. albitarsis*, *An. deaneorum*, *An. marajoara* and *An. albitarsis* B. Recently, Lehr et al. (2005), based on the

Financial support: FAPESP (05/53973-0 to MAMS), CNPq (472485/2006-7 to MAMS), NIH (AI RO154139 to JE Conn). MTM is a doctorate fellow from Fapesp (2007/07573-5).

+ Corresponding author: masallum@usp.br

The views expressed in this article are those of the authors and do not reflect the official policy or position of the US Department of the Army, the US Department of Defense. This research was performed under a Memorandum of Understanding between the Walter Reed Army Institute of Research and the Smithsonian Institution, with institutional support provided by both organizations.

Received 22 January 2009
Accepted 22 June 2009

results of phylogenetic analyses of the mitochondrial cytochrome c oxidase subunit I (mtDNA COI) gene, proposed a fifth species in the complex, *An. albitarsis* E. In contrast, Li and Wilkerson (2005, 2006) observed consistent and diagnostic, but intragenomically variable differences in the ITS2 of the four species of *An. albitarsis* complex originally recognized with RAPDs, but the existence of *An. albitarsis* E was not corroborated. Using nucleotide sequences of the ITS2 rDNA and *white* gene from putative *An. marajoara* samples collected in Puerto Carreño, Colombia, Brochero et al. (2007) found yet another species, designated *An. albitarsis* F.

The above morphology, isoenzyme and DNA data clearly demonstrated that *An. albitarsis* was a species complex with component species involved in human malaria transmission. It is also clear that accurate species determinations are necessary to evaluate the vector status of each.

Multivariate analyses of measurements of morphological characteristics have been shown to be an effective method for distinguishing morphologically similar species, including medically important insects (Rubio-Palis et al. 1997, Rubio-Palis 1998, Calle et al. 2002). Considering that a primary objective of the present study was to discover means to identify five species of the *An. albitarsis* complex, we used principal component, discriminant and cluster analyses to assess morphological characters of the adult females. Additionally, we describe the adult males and females, fourth-instar larvae and pupae of *An. albitarsis*, *An. marajoara*, *An. deaneorum* and *An. albitarsis* B and adult females and males of *An. albitarsis* E.

MATERIALS AND METHODS

Adult female mosquitoes were collected in several localities in Brazil, Argentina, Paraguay and Venezuela (Table I) to obtain progeny broods of adult males and females with associated pupal and fourth-instar larval exuviae. At least one adult male or female of each progeny was used to identify the species using RAPD-PCR. Terminology for morphological characters follows Harbach and Knight (1980), except we used Belkin (1962) for wing veins and Wilkerson and Peyton (1990) for wing spots. The three life stages of *An. albitarsis* are described in detail; the other species of the complex were compared with *An. albitarsis*, but characteristics found to be identical were not included in their descriptions. For measurements and setal counts of the immatures, 10 slide-mounted specimens of each species were employed, unless otherwise indicated.

Specimens of each species, collected in widely separated localities of their known geographical range, were used in the morphometric analyses. Forty-one variables (Supplementary data) (39 wing spots plus the distance between the eyes and basal dark portion of hindtarsomere 2) were measured in 30 adult females of each species. Measurements were taken with a Wild stereomicroscope connected to a digital micrometric ocular Wild MMS 235® (Heerbrugg, Switzerland).

Statistical analysis - The pattern of pale and dark spots on the wing veins, the distance between the eyes and the dark portion of hindtarsomere 2 were measured in 150 specimens, i.e., 30 specimens each of *An. albitarsis*, *An. albitarsis* B, *An. marajoara*, *An. deaneorum* and *An. albitarsis* E. Because dark and pale wing spots of the costal vein could be present or absent in individual 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 could not be measured and thus scored as missing. Statistical analyses were performed with Minitab 14 for Windows XP and R-2.2.1 for Windows® software program.

Descriptive statistics - Ranges, means and standard deviations of means were calculated for each quantitative variable. Normality and homogeneity tests were carried out to evaluate if data distribution was normal. Because of missing data, five variables that could have compromised the analyses were excluded. Boxplots were employed to display differences between measurements of variables.

Principal components analysis (PCA) - PCA was applied for the complete data set of 36 variables. This is a statistical analysis to reduce the dimensionality of a data set while retaining as much variation in the data as possible (Hugh & Gauch 1982). Principal component analysis was carried out as an intermediate step for understanding other analytical analyses. Because the first 13 principal components retained 80.1% of the variation present in 36-variable data set, they were used to obtain a new data set to carry out the discriminant function analysis.

Variance analysis (ANOVA) - ANOVA was applied to the 36-variable data set to verify if there were differences among the means of all variables ($p \geq 0.05$) of the five species and the equality hypothesis (H: $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5$) was tested. Because it was not possible to reject the equality hypothesis of two variables, these were excluded from discriminant function analysis.

Discriminant function analysis - Discriminant function analysis was applied to determine the possibility of segregation of all five previously determined species. Because of missing variables (Supplementary data), discriminant analyses were conducted in two rounds. Since in the first round missing data were excluded, we used the 23 remaining variables obtained from 126 specimens. In the second round, the first 13 principal component variables obtained in the PCA analysis were used to generate a new set of variables, which were used in the discriminant function analysis. A discriminant model was constructed using Fisher's linear discriminant analysis. Results of this analysis supported homogeneous covariance among five groups using cross validation.

Cluster analysis - Cluster analysis was employed using dissimilarity of variables (level = 41). Distances of each variable were entered for 150 specimens of five species. Based on the transformed data set of PCA, the discriminant analysis was applied to validate the results of the cluster analysis.

RESULTS

Morphometric analysis - Means and standard deviations for 41 variables were obtained from 150 specimens to compare five species of the *An. albitarsis* complex

TABLE I

Material examined of five species of the *Anopheles albitarsis* complex which were identified by random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR)

Country state/province	Locality	Coordinates	Date	Collection	Species (n)
Brazil					
Santa Catarina	Macaranduba	26°35'S 48°58'W	8 Jan 1983	BR010	A(8F)
São Paulo	Ilha Comprida	24°42.8'S 47°31.6'W	9 Feb 1989	BR73	C(7F, 2M, 2MG)
São Paulo	Registro	24°36.8'S 47°53.1'W	26 Jan 1992	BR500, 501	A(9F, 2M, 1MG, 8 Lv, 8 pp), B(14F, 1M, 19Lv, 19Pp)
São Paulo	Ponte Melo Peixoto	22°39.05'S 53°01'W	10 Feb 1992	BR508	A(3M)
Paraná	Santa Helena	24°56'S 54°23'W	30 Jan 1992	BR503	A(4F, 1M), D(2F, 2M, 1MG)
Paraná	Near Guaíra	24°04'S 54°15'S	1 Feb 1992	BR504, 510, 511	A(3F, 1M, 1MG), B(1Lv, 1Pp), D(2F)
Espírito Santo	Águia Branca	18°59'S 40°44'W	20 Jan 1992	BR002	B(1F, 10Lv, 10Pp)
Pará	Ilha de Marajó	1°00'S 49°30'W		BR001	C(8F, 1M, 1MG, 34Lv, 34Pp)
Pará	EMBRAPA	1°27'S 48°29'W	8 Oct 1992	BR008	B(2F, 8Lv, 8Pp)
Pará	Primavera	0°56'S 47°6'W	16 Oct 1992	BR009	B(3F, 16Lv, 16Pp)
Pará	Capanema	1°24'S 47°11'W	Ago 1993	BR403	C(5F, 1Lv, 1Pp), B(15Lv, 25Pp)
Rio de Janeiro	Morro da Panela	22°58'S43°21'W	19 Jan 1993	BR013	B(1F)
Bahia	Itaquara	13°26'S 39°66'W	30 Jan 1993	BR015, 017	A(3Lv, 3Pp), B(1F, 18Lv, 18Pp)
Ceará	Fortaleza	3°43'S 38°30'W	8 Mar 1993	BR018	B(3F, 23Lv, 23Pp)
Ceará	Paraipaba	3°25'S 39°13'W	9 Mar 1993	BR019	B(1F, 27Lv, 27Pp)
Mato Grosso	Peixoto Azevedo	10°23'S 54°54'W	20 Apr 1993	BR020	B(12Lv, 12Pp), C(3F, 8Lv, 8Pp)
Amazonas	Manaus	2°53'S 60°15'W	16 Dec 1993	BR026	C(3F, 2Lv, 2Pp)
Rondônia	Guajará-Mirim	10°50'S 65°20'W	29 July 1992	BR007	D(11F, 32Lv, 32Pp)
Rondônia	Ariquemes	9°56'S 63°04'W		BR700	D(4F)
Rondônia	Costa Marques	12°28'S 64°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(49F, 4M)
Paraguay					
Alto doParaná	Rio Acaray	25°29'S 54°42'W	4 Feb 1992	PA1	A(2M, 1MG)
Alto doParaná	Hernanderias	25°22'S 54°45'W	6 Feb 1992	PA2	B(2F, 1M, 1MG, 7Lv, 6Pp)
Alto doParaná	Near National Airport	not know	8 Feb 1992	PA3	B(3F, 1M, 1 MG)
Argentina					
Buenos Aires	Baradero	33°48'S 59°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)

all specimens were obtained from link-reared offspring (eggs, larvae, pupae and adults) of blood fed females collected in Shannon traps. Species: A: *Anopheles albitarsis*; B: *Anopheles oryzalimnetes* n. sp.; C: *Anopheles marajoara*; D: *Anopheles deaneorum*; E: *Anopheles janconnae* n. sp.

(Supplementary data). Both normality and homogeneity tests showed that all variables had a normal distribution. Because multivariate analysis considers only presence data those variables with missing information were excluded, i.e., presector dark, sector pale, accessory sector dark, first dark spot of CuA_1 and second pale spot of CuA_1.

Boxplot - The following characters, i.e., wing length, vein R_{4+5} length and wing 3rd dark spot length of CuA_1, preapical dark, apical dark, sector dark and basal pale plus prehumeral pale spots showed upper median value (Fig. 1A-G), whereas accessory sector pale spot showed lower median value for *An. albitarsis* (Fig. 1H). Generally, specimens of *An. albitarsis* possess a longer wing than the others species of the complex. Wing accessory sector pale spot, 2nd dark spot of CuA_1 and 1st dark spot of R_{4+5} showed upper median value for *An. albitarsis* B (Fig. 1H-J). *An. deaneorum* and *An. albitarsis* E showed the lowest median value of variables, although the subcostal pale spot of *An. deaneorum* (Fig. 1K) and 1st dark spot of CuA_1 and humeral dark of *An. albitarsis* E (Fig. 1L, M) showed upper median value. The subcostal pale spot and sector pale spot of *An. albitarsis* and *An.albitarsis* B are similar (Fig. 1K, N). The 2nd dark spot of CuA_1 of *An. albitarsis* and *An. albitarsis* E are similar (Fig. 1I). For *An. marajoara*, 50% of the lower median value of variables was similar to *An. albitarsis* and *An. albitarsis* B and 50% of the upper median value of variables was similar to *An. deaneorum* and *An. albitarsis* E. The boxplot indicates homogeneity variance of variables among five species of *An. albitarsis* complex.

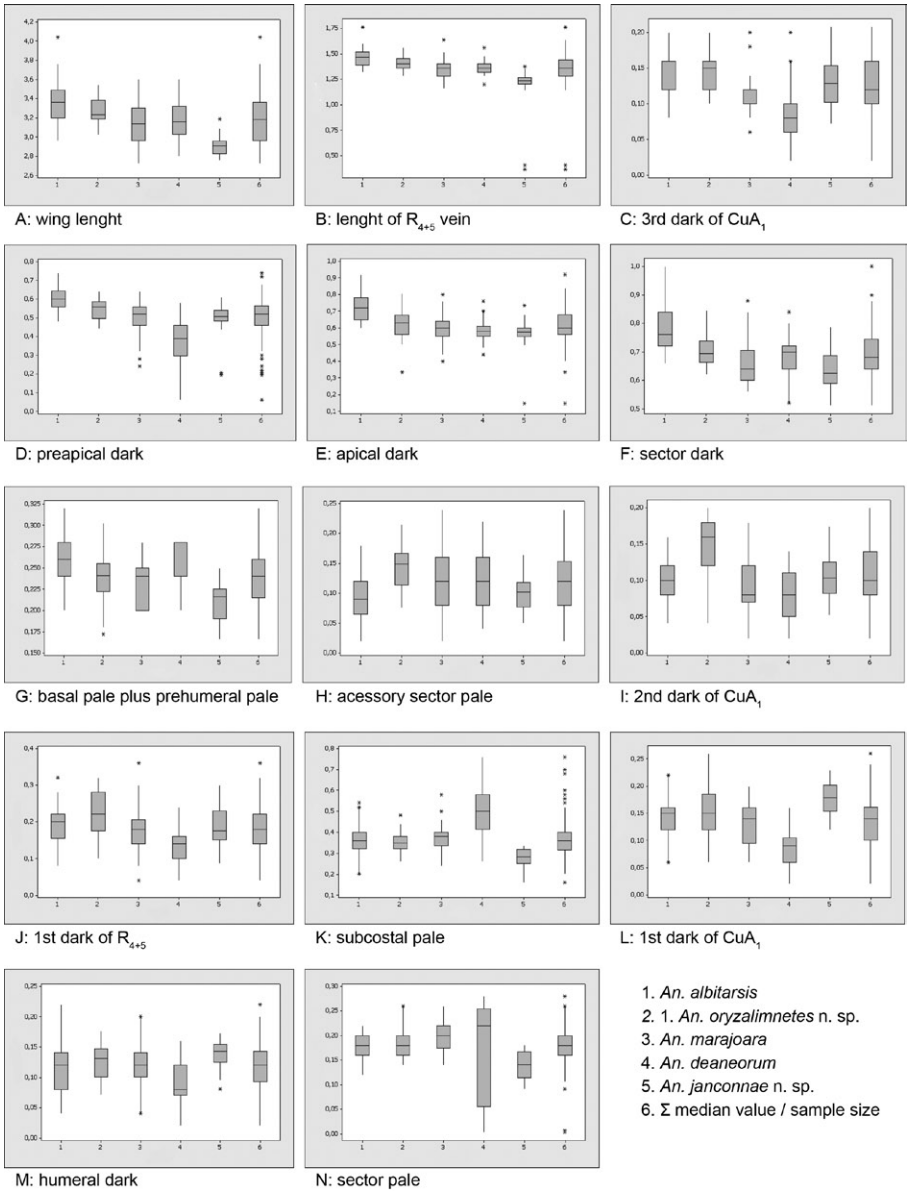

Fig. 1: boxplot of variables of five species of the *Anopheles albitarsis* complex.

PCA - The principal components analysis showed that the first 13 components retained 80.1% of the variation present in the 36-variable data set (Supplementary data).

ANOVA - All variables, except for the distance between the eyes and measurement of the presector pale spot ($p \geq 0.05$), showed mean differences among the five species of the *An. albitarsis* complex. These two variables were therefore excluded from the discriminant analysis (Supplementary data).

Discriminant analysis - Discriminant analyses demonstrated that morphological variables might serve to discriminate the five species of the *An. albitarsis* complex (Supplementary data). In the first round, 96 (76%) of 126 specimens analyzed were correctly identified. In the second round, 112 (75%) of 150 specimens were correctly identified. A high percentage of discrimination was shown in *An. albitarsis* E when compared with the other species (1st round, 100% of 126 specimens; 2nd round, 97% of 150 specimens). The lowest percentage of discrimination was obtained for *An. marajoara* (1st round, 50%; 2nd round, 60%). Moreover, the percentage of correct discrimination for *An. albitarsis*, *An. albitarsis* B and *An. deaneorum* varied from 70-88%.

Cluster analysis - Cluster analysis based on the means of 36 variables revealed morphometric dissimilarities among the clusters. The specimens were clustered in two groups (Supplementary data), one comprising *An. albitarsis* and *An. albitarsis* B and the other formed by *An. deaneorum* and *An. albitarsis* E. Fifty-three percent of the specimens identified by RAPD-PCR as *An. marajoara* clustered in group one, whereas the other 47% clustered in group two. Results of the cluster analysis suggest that *An. albitarsis* and *An. albitarsis* B to be more similar to each other than to any other species of the complex. Similarly, *An. deaneorum* and *An. albitarsis* E clustered together, whereas *An. marajoara* could be differentiated in two distinct groups (Supplementary data). Results of the cluster analysis corroborated the results of the discriminant analysis (Supplementary data). The rate of correct discrimination was 83%.

An. albitarsis Lynch Arribálzaga

An. 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 and Roberts (1943): 31 (M*, F*, L*); Cova-Garcia (1946): 31, 83, 118 (E*, L*, F*, M*); Romeo-Viamonte and Castro (1951): 321 (F*); Garcia and Casal (1964) (1965): 6 (P*); Kreutzer et al. (1976): 473 (chromosomes); Rosa-Freitas and Deane (1989): 289 (M*, F*, P* L* E*, redescription, neotype desig., IOC-RJ); Wilkerson et al. (1995a, c): (RAPD-PCR, tax.). *Anopheles limai* Galvão and Lane (1937a): 227 (A, E*, as var.). Type loc. Pinheiros and Butantan, São Paulo, SP, Brazil. Type: not extant. Lane (1953): 244 (syn.); Belkin et al. (1971): 4 (tax.). *Anopheles imperfectus* Corrêa and Ramos (1943): 246 (F, as ssp). Type loc. Vera Cruz, SP{holotype [Faculdade de Saúde Pública, Universidade de São Paulo (FSP-USP)]}. Lane (1953): 244 (syn.); Belkin et al. (1971): 4 (tax.).

Female: head: integument brown to dark brown. Vertex and occiput with large erect white weakly forked truncate scales becoming dark laterally; anteriorly vertex with a few small obovate decumbent scales along upper orbital line 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 proximal 0.5 of medial surface and at apex of dorsal and lateral surfaces and 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 to labium in color. 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); 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 sparse white scales 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 of palpomere 3, erect on ventral surface of palpomere 3, decumbent on palpomeres 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 areas between dorsocentral areas 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; acrostichal, dorsocentral and prescutellar areas and scutal fossa with white spatulate decumbent scales, but not posteriorly on dark posterior bare area; supraalar and antealar areas with white spatulate decumbent scales; lateral margin of antealar area, extending posteriorly onto supraalar area, with elongate, narrow, semi-erect, white, spatulate scales; 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 row of long and few short pale brown setae with golden and reddish reflections. Mesopostnotum bare. Antepronotum with dark setae and patch of spatulate scales, these scales whitish dorsally, dark ventrally on upper area, remainder of antepronotum without scales but 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 and upper mesepimeron; upper proepisternum, upper

mesokatepisternum and prealar knob with dark brown setae; middle posterior border of mesokatepisternum, upper mesepimeron and prespiracular area with pale yellow setae. Wing: length 2.96-4.04 mm (mean = 3.35 ± 0.24) (n = 30); veins dark-scaled with spots of white scales on anterior area of costa, subcosta, R and R_1, pale yellow scales on remaining 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-scaled dorsally with patch of white scales at base. Legs: anterior surface of forecoxa with patch of spatulate scales mesally, these scales white laterally and dark medially and a few white spatulate scales ventrally, posterior surface of forecoxa with patch of ventrally directed black spatulate scales and patch of white scales laterally; 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 white spatulate scales at apex and patch of white spatulate appressed scales at base of posterolateral surface. Fore, mid and hindtrochanters speckled with white and dark scales. 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 pale ring; anterior surface mostly dark brown, often with two 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 longitudinal white stripe extending from base to near apex; one or two 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 small white patch at apex of anterodorsal surface, ventral surface pale-scaled. Tibiae dark-scaled with 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 predominantly dark-scaled with pale scales at apex; tarsomeres 4 and 5 normally dark-scaled. Midtarsomeres 1 and 2 predominantly dark-scaled with 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 basal dark band 0.52-0.86 length of tarsomere (mean = 0.62 ± 0.08) (n = 30); tarsomeres 3, 4 and 5 white-scaled. Abdomen: tergum I with numerous long brown setae, without caudolateral scale-tufts; tergum II with numerous brown setae, medially with numerous pale yellow to whitish scales extending laterally on posterior area, without caudolateral scale-tufts, sometimes present on tergum III, caudolateral tufts on tergum III with few dark erect scales extending laterally; terga IV-VII with numerous pale yellow to whitish scales medially and 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; sterna II-VII with numerous dark setae, shorter than those on terga and longitudinal patches of white scales lateral of midline and small patches of dark scales posterior 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 semi-erect on basal 0.5 of palpomere 2, decumbent on remainder of palpomere 2 and on palpomeres 3-5; palpomere 2 dark-scaled with pale scales at apex; palpomere 3 with pale scales at base and with pale spot dorsally on medial surface, apex with long setae; palpomere 4 with pale scales on ventral and dorsal surfaces, dark-scaled at apex and base; palpomere 5 with dark setae along ventral surface and pale scales dorsally, dark scales ventrally and basally.

Male genitalia (Fig. 2): segment IX (not shown): sternum rectangular, median posterior border emarginate, anterior border weakly emarginate. Gonocoxite elongate; tergal surface with 3-6 tergomedial setae, one apicolateral seta and one apicomedial seta; dorsomedial rim 0.09-0.12 (mean = 0.1 ± 0.01) (n = 5) length of gonocoxite; tubercle of parabasal seta 0.22-0.41 (mean = 0.32 ± 0.06) (n = 5) length of parabasal seta; accessory setae 0.33-0.41 (mean = 0.36 ± 0.03) (n = 5) length of gonocoxite; internal seta subequal to ventralmost accessory seta. Dorsal claspette with narrow, curved pedicel, rounded at base, apical setae moderately broad, strongly curved mesally, without noticeable basomesal projection. Ventral claspette (in ventral aspect) with apex moderately wide and strongly striated, single, rounded lobe expanded ventrally; preapical plate absent; basal lobules laterally expanded. Gonostylus slender, curved with row of minute setae inserted before gonostylar claw. Aedeagus cylindrical approximately 0.39-0.47 (mean = 0.43 ± 0.03) (n = 4) length of gonocoxite, apex rounded, wider than long, ventromesal subtriangular projections connecting at midline, leaflets absent.

Pupa (Fig. 2): position and development of setae as figured; range and modal number of branches in Table II. All measurements were made on 10 specimens unless otherwise indicated. Cephalothorax: integument and legs weakly pigmented; trumpet angusticorn with meatal cleft; pinna moderately pigmented; meatal cleft U-shaped basally. Abdomen: length 2.50-3.11 mm (mean = 2.84 ± 0.20); seta 3-I usually single, 5-I single to triple, 6-I single or double, 9-I usually single, sometimes double, as long as 6-I; 0-II-VII moderately developed, 6-II single or double, 1.12-2.52 length of 7-II (mean = 1.76 ± 0.51), 9-II min, unpigmented, 9-II-IV less than 0.20 length of segment; 6-III, IV normally double, 9-III short, stout, 1.11-2.50 (mean = 1.67 ± 0.45) length of 9-II; 1-IV-VII always single, strong, long, extending beyond caudal border of following segment, 3-IV with branches extending

TABLE II

Range (mode) of setal branches of the pupa of *Anopheles albitarsis*

Seta		Abdominal segments									
n	Cephalothorax	I	II	III	IV	V	VI	VII	VIII	IX	Paddle
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)	nc	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)	2-4 (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	-	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	1	-	-

nc: not counted.

beyond caudal margin of segment, 9-IV thick, 1.35-2.82 (mean = 2.01 ± 0.49) length of 9-III, 10-IV, V always single; 0-V-VII often 4-branched, 3-V with branches extending well beyond caudal margin of segment, 8-V, VI frequently double, 9-V dark, strong, curved, 1.68-3.09 (mean = 2.38 ± 0.47) length of 9-IV, 9-V, VI less than 0.50 length of segment; 7-VI usually single, rarely double, 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; seta 10-IV absent; 9-VII, VIII about 0.50 length of segment; 0-VIII normally single, 9-VIII straight, 0.66-1.10 (mean = 0.85 ± 0.15) length of 9-VII. Male genital lobe: thick at base with sides tapering toward apex, apex with mammiliform 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 including apex with very fine, minute spicules, inner margin smooth; seta 2-Pa usually single.

Fourth-instar larva (Fig. 3): position and development of setae as figured; range and modal number of branches in Table III. Measurements were made on 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 apotome, on posterior margin of frontal ecdysial line on lateralia, posteriorly on lateral area of lateralia, ventrally on lateralia, on anterior area of labiogula and along hypocranial ecdysial line. Dorsomentum strongly sclerotized, blackish, median tooth moderately broad, tapered to point and blunt at apex, about twice as wide as adjacent tooth. 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 thin spicules on mesal margin, spicules short and sparse on dorsal and ventral surfaces, seta 1-A, 0.25-0.34 (mean = 0.28 ± 0.03) distance from base. Seta 2-C single with minute, 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 of 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 branched, short, extending 0.5 distance to anterior margin of head, 10-C triple. Collar dark brown, moderately wide dorsolaterally. Thorax: seta 1-P with apically truncate leaflets, 14-P with long branches arising from short shaft, median branches longer than lateral branches, extending beyond anterior margin of thorax; 1-M plumose; 2-T moderately long, single, extending beyond caudal margin of thorax, 3-T palmate, with narrow leaflets. Abdomen: integument with minute spicules on ventral surface of segments II-VIII; seta 5-I with branches arising from short stem less than length of seta from lateral margin of abdomen; 0-II-VII large, multibranched, 1-II-VII with variable truncate to pointed leaflets, 2-II, III large with strongly developed branches; 2-IV rarely double,13-IV extending to caudal margin of segment; 2-V normally single, 13-V extending beyond caudal margin of segment. 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 arms of median plate of spiracular apparatus short to moderately long. Segment X covered with spicules, those on posterior margin stronger, seta 1-X longer than length of saddle, inserted on or off saddle, anal papillae longer than saddle.

TABLE III

Range (mode) of setal branches of the fourth-instar larva of *Anopheles albitarsis*

Seta		Thorax			Abdominal segments								
n	Head	P	M	T	I	II	III	IV	V	VI	VII	VIII	X
0	-	1	-	-	-	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	11-17 (14)	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)	3-5 (4)	4-8 (6)	4-7 (5)	2-4 (3)	2-4 (3)	2-4 (3)	1	1	1	8[a]
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	nc	1-3 (2)	1, 2 (1)	1, 2 (2)	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)	-	-	-	-	-	-	-	-	-	-	-	-

a: pairs of setae; M: mesothorax; nc: not counted; P: prothorax; T: metathorax.

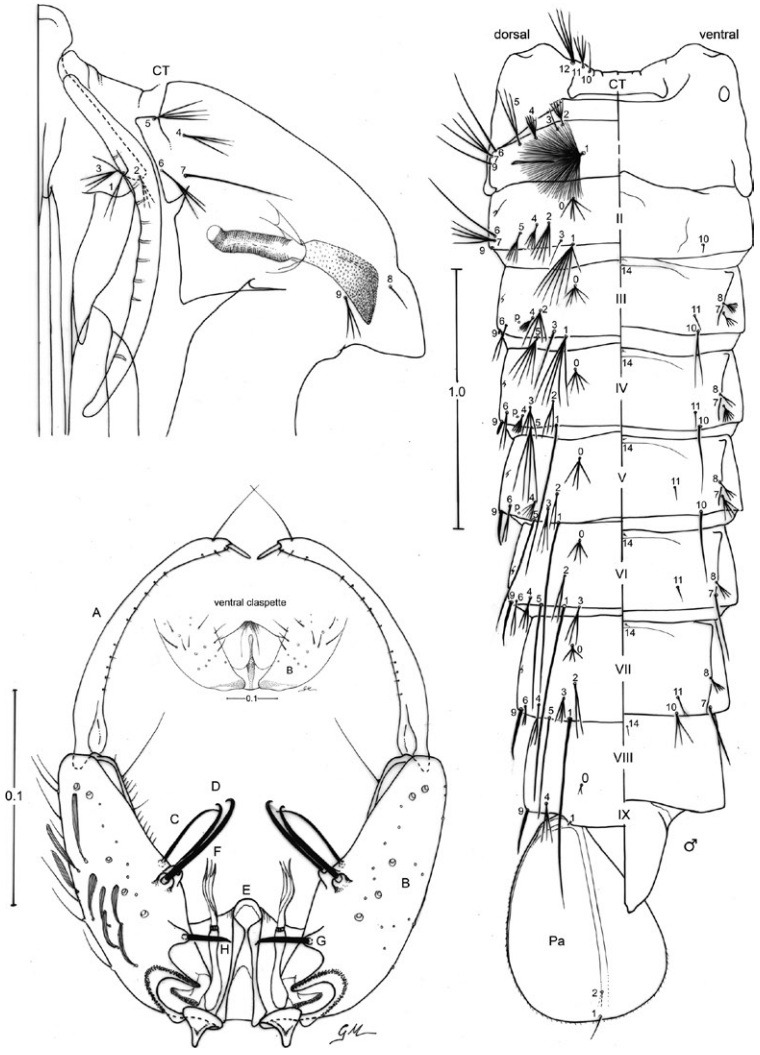

Fig. 2: pupa and male genitalia of *Anopheles albitarsis*. Pupa: CT: cephalothorax; p: puncture; Pa: paddle; I-IX: abdominal segments. Male genitalia: A: gonostylus; B: gonocoxite; C: internal seta; D: accessory setae; E: aedeagus; F: dorsal claspette; G: tubercle of parabasal spine; H: ventral claspette.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

Distribution - Distribution data presented here are from the published literature records referring to specimens of *An. albitarsis* identified by RAPD-PCR, mtDNA-COI or rDNA-ITS2. *An. albitarsis* is found in Argentina, Provinces of Misiones, Corrientes and Buenos Aires; Brazil, states of Santa Catarina, Paraná (PR) and SP (Wilkerson et al. 1995c, Lehr et al. 2005, Li & Wilkerson 2005); and Paraguay, state of Alto Paraná (Wilkerson et al. 1995c).

Material examined - Table I.

Type data - *An. imperfectus*, holotype, Vera Cruz, SP (GR Ramalho coll. (1939), Corrêa and Ramos (1943), det., pinned adult female in moderately good condition, inside a glass vial, collection number E-1265, FSP-USP, SP.

Bionomics - Kakitani and Forattini (2000) observed that the daily survival rate for *An. albitarsis* (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, in the southeastern part of SP. Lounibos et al. (1998) observed that under laboratory conditions 22.9% of nulliparous females of *An. albitarsis* failed to mature eggs after one blood meal.

An. albitarsis seems to be adapted to human environments. Results of collections carried out using Shannon traps in irrigated rice fields showed that there was an increase in both *An. albitarsis A and An. albitarsis B* densities that could be associated with the development of rice farming. At the same area, *An. albitarsis* fed on humans indoors and outdoors, at sunset and sunrise, in a domestic environment (Forattini et al. 1995).

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

Molecular characterization - *An. albitarsis* can be recognized by a RAPD-PCR profile produced using Operon primers B16, A01 and C15 (Wilkerson et al. 1995a, c). However, this method can be unreliable as an

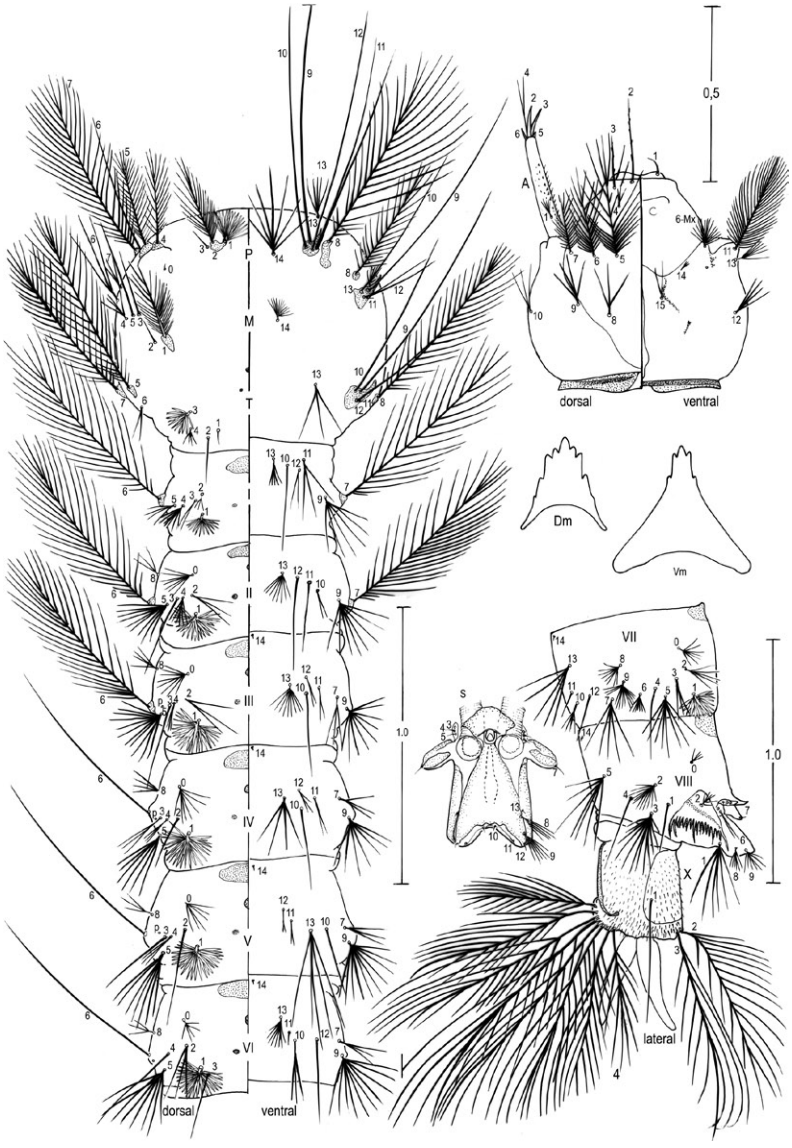

Fig. 3: fourth-instar larva of *Anopheles albitarsis*. A: antenna; C: cranium; Dm: dorsomentum; M: mesothorax; P: prothorax; p: puncture; S: spiracular apparatus; T: metathorax; Vm: ventromentum; I-VIII, X: abdominal segments. Scales in mm.

identification tool because results might differ depending on the DNA extraction protocol, reagents and type of PCR thermocycler (Li & Wilkerson 2005). A preferable method of identification is the use of a combination of species-specific primers based on rDNA ITS2 sequence developed by Li and Wilkerson (2005). Lehr et al. (2005) and Wilkerson et al. (2005) corroborated the monophyly of *An. albitarsis* when using COI mtDNA sequence data and combined COI, ND4 mtDNA, ITS2, 28S rDNA sequence data, respectively.

Anopheles marajoara Galvão and Damasceno

Anopheles marajoara Galvão and Damasceno (1942): 424 (M, F, L). Type: holotype, adult male with associated fourth instar larval and pupal exuviae (FSP-USP). Type loc. Santa Maria Farm, 400 m distant from Camará River, between Cachoeira do Arari and Salvaterra municipalities, Ilha de Marajó, Pará (PA), Brazil. Lane (1953): 244 (syn.); Belkin et al. (1971): 4,46 (type info, tax.); Linthicum (1988): 198 (from syn. with *An. albitarsis*); Quiñones and Suarez (1990): 602-604 (biology); Lounibos and Conn (1991): 57-66 (biology); Narang et al. (1993): 97-112 (tax., cryptic species); Rubio-Palis (1995): 482-484 (feeding behavior); Wilkerson et al. (1995c): 697-704 (tax., RAPD-PCR); Chadee and Wilkerson (2006): 22-28 (ecology, feeding behavior); Senise et al. (2006): 453-457 (type info, exclusion of the male genitalia slide associated with the holotype from the type material). *An. albitarsis domesticus* Galvão and Damasceno (1944): 78 (M, F, E*). Type loc. Cachoeira do Arari, Ilha de Marajó, PA. Correa et al. (1950): 280 (biology); Belkin et al. (1971): 4 (type info); Rios et al. (1984): 461 (tax.); Linthicum (1988): 198 (syn. with *An. marajoara*).

TABLE IV

Range (mode) of setal branches of the pupa of *Anopheles marajoara*

Seta		Abdominal segments									
n	Cephalothorax	I	II	III	IV	V	VI	VII	VIII	IX	Paddle
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)	nc	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	1	-	-

nc: not counted.

Female: similar to *An. albitarsis* except as follows. 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 area of costa, subcosta and R and R_1, pale yellow scales on remaining 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); hindtarsomere 2 dark-scaled proximally with a basal dark band 0.40-0.63 length of tarsomere (mean = 0.49 ± 0.06) (n = 30).

Male: similar to *An. albitarsis*.

Male genitalia (Fig. 4): similar to *An. albitarsis* except for the characters as follow. Tubercle of parabasal seta 0.22-0.25 (mean = 0.23 ± 0.04) (n = 3) length of parabasal seta; 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. 4): similar to *An. albitarsis* except as follows. Position and development of setae as figured; range and modal number of branches in Table IV. All measurements were made on 10 specimens. Abdomen: length 2.43-3.26 mm (mean = 2.74 mm ± 0.26); 3-I single to triple, 9-I always single; 6-II single or double, 1.19-2.99 length of 7-II (mean = 1.99 ± 0.50); 6-III-V usually double, 9-III 1.20-2.59 (mean = 1.93 ± 0.51) length of 9-II; 5-IV usually triple, 8-IV-VI single to triple, 9-IV 1.40-2.70 (mean = 1.87 ± 0.42) length of 9-III, 10-IV, V always single, long; 0-V-VII often 5-branched, 9-V 1.83-3.99 (mean = 2.45 ± 0.65) length of 9-IV, 9-V, VI less than 0.5 length of segment; 5-VI with 1-3 branches, 7-VI, VII single or double, 9-VI 0.93-2.18 (mean = 1.32 ± 0.34) length of 9-V; 5-VII single, 9-VII 1.17-1.79 (mean = 1.38 ± 0.20) length of 9-VI, 9-VII,VIII about 0.5 length of segment; 0-VIII normally single (1-4 branches), 9-VIII 0.82-1.04 (mean = 0.90 ± 0.08) length of 9-VII, 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. 5): similar to *An. albitarsis* except as follows. Position and development of setae as figured; range and modal number of branches in Table V. Measurements were made on 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 apotome, along frontal ecdysial line and on lateral and ventral areas of lateralia, labiogula with two pale

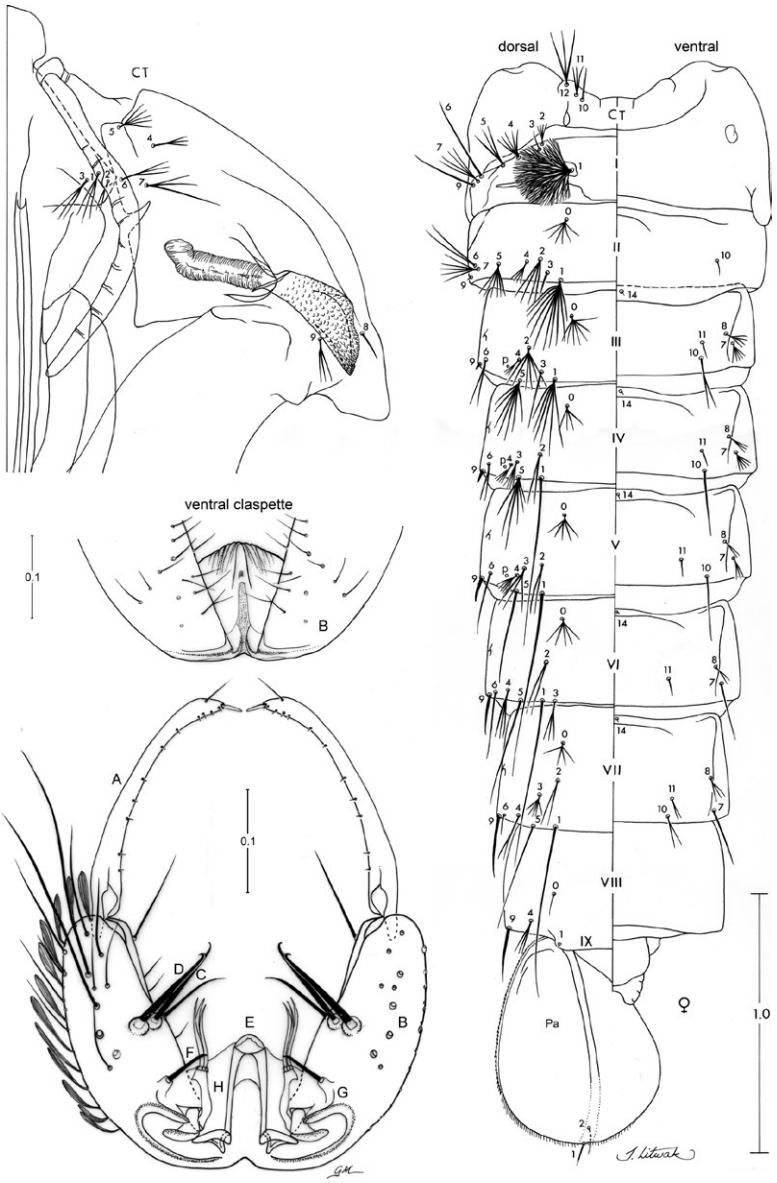

Fig. 4: pupa and male genitalia of *Anopheles marajoara*. Pupa: CT: cephalothorax; p: puncture; Pa: paddle; I-IX: abdominal segments. Male genitalia: A: gonostylus; B: gonocoxite; C: internal seta; D: accessory setae; E: aedeagus; F: dorsal claspette; G: tubercle of parabasal spine; H: ventral claspette (ventral view). Scales in mm.

spots between hypostomal suture and hypocranial ecdysial line. 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 SP); seta 1-A with branches 0.25-0.34 (mean = 0.30 ± 0.03) distance from base. 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), 9, 10-C often triple. Thorax: seta 1-P palmate, with leaflets truncate at apex. Abdomen: seta 13-I, III often 5-branched; 0-II-VII large, multibranched, 1-II-VII with truncate to pointed leaflets; 2-IV single or double, 2-V long, always single, 13-IV, V often 4-branched. Pecten with 4-5 long, 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. Segment X: seta 1-X inserted on or off saddle.

Distribution - *An. marajoara* is found in Brazil, states of Mato Grosso (MT), Amazonas (AM), SP, PA and RO (Wilkerson et al. 1995a, c, Lehr et al. 2005, Li & Wilkerson 2005) and AP (Conn et al. 2002, Li & Wilkerson 2005); Paraguay, state of Alto do Paraná (Wilkerson et al. 1995c). Records from RR, and Departments of Zulia, Cojedes and Barinas, Venezuela (Wilkerson et

TABLE V

Range (mode) of setal branches of the fourth-instar larva of *Anopheles marajoara*

Seta		Thorax			Abdominal segments								
n	Head	P	M	T	I	II	III	IV	V	VI	VII	VIII	X
0	-	1	-	-	-	7-12 (10)	6-12 (9)	5-8 (6)	5-10 (7)	5-10 (7)	4-8 (6)	3-6 (4)	-
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)	9-15 (10)	15-21 (17)
3	1	1	1	13-21 (17)	1	1	1	3	1	1 ,2 (1)	2-4 (3)	8-13 (13)	8-15 (9)
4	2-5 (3)	20-24 (21)	2-6 (4)	2-4 (3)	3-8 (5)	4-7 (5)	2-5 (3)	3 ,4 (3)	2-4 (3)	1	1	1	6-8 (8)[a]
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)	4-8 (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	nc	1 ,2 (2)	1 ,2 (1)	1	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	nc	5-11 (7)	9-15 (12)	-	-	-	1	1	1	1	1	1	-
15	3-8 (7)	-	-	-	-	-	-	-	-	-	-	-	-

a: pairs of setae; M: mesothorax; nc: not counted; P: prothorax; T: metathorax.

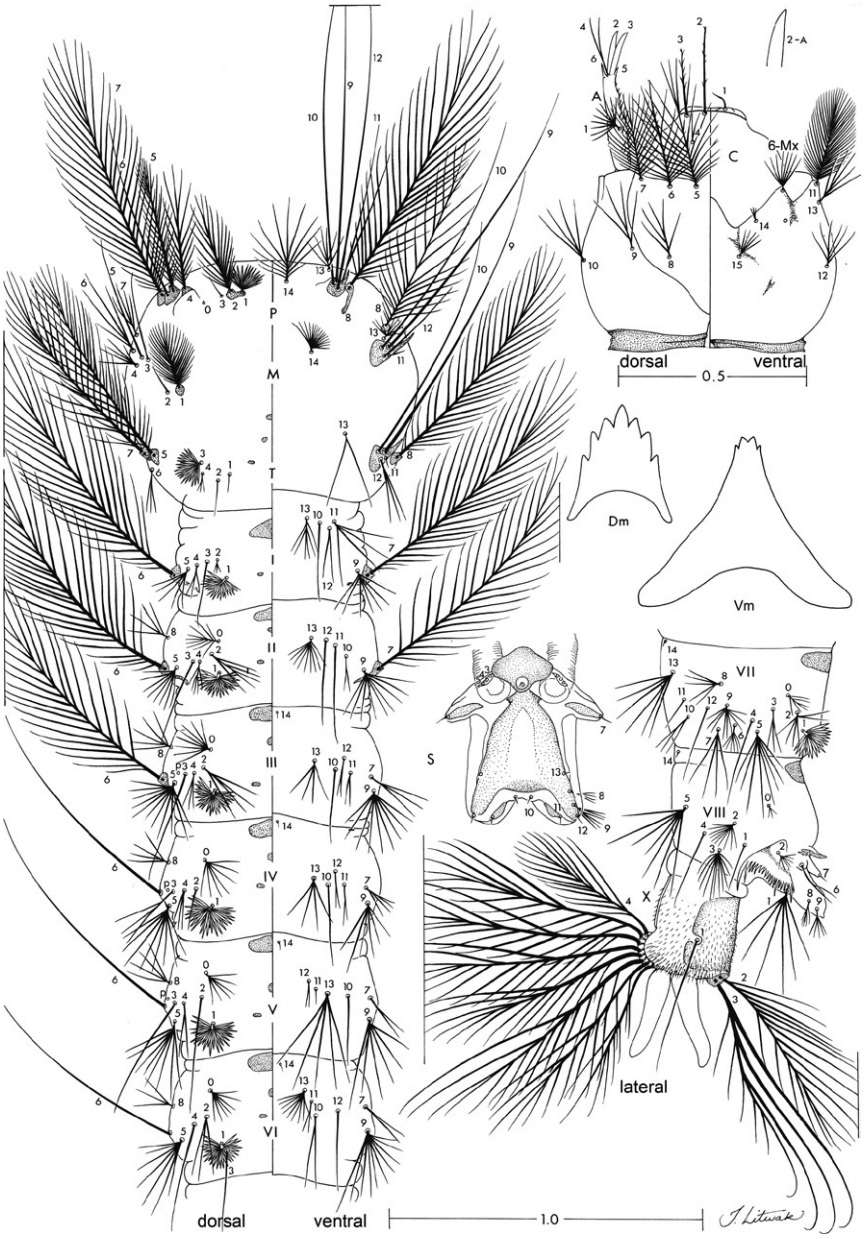

Fig. 5: fourth-instar larva of *Anopheles marajoara*. A: antenna; C: cranium; Dm: dorsomentum; M: mesothorax; P: prothorax; p: puncture; S: spiracular apparatus; T: metathorax; Vm: ventromentum; I-VIII, X: abdominal se segments. Scales in mm.

al. 1995a, Rubio Palis 2003, Li & Wilkerson 2005) may refer to *Anopheles janconnae* n. sp *An. marajoara* is so far the only species of the complex found in Trinidad (Chadee & Wilkerson 2006).

Material examined - Table I.

Bionomics - Since a new species related to *An. marajoara*, *An. albitarsis* F, has been recognized in Colombia by Brochero et al. (2007), published literature records in Colombia and neighboring countries may refer to *An. marajoara, An. albitarsis* F or *An. janconnae* n. sp. Until the distributions of these species are better known caution should be exercised when interpreting the literature referring to *An. marajoara*.

Conn et al. (2002) conducted entomological surveys in malaria areas of Macapá, AP, and found *An. marajoara* frequently infected, replacing *Anopheles darlingi* as the primary vector. It is hypothesized that the observed change in mosquito population densities was caused by deforestation for agriculture that resulted in newly created ground pools favoring *An. marajoara* larvae. In a study carried out in Ilha Comprida, Atlantic Forest domain, southern SP, Kakitani et al. (2003) observed that the biting activity of *An. marajoara* peaked between 2-5 h. In contrast, Chadee (1992) found that *An. marajoara* from Trinidad showed extra-domiciliary and intra-domiciliary biting behavior, with host-seeking activity starting at 17 h and ending at 22 h. They recom-

mended, because of the differences in the peak activity for *An. marajoara* from Southern Brazil and Trinidad, that these populations should be more closely investigated to determine if they represent distinct species.

Medical importance - Historically, *An. marajoara* was thought to be at most a secondary local vector of malarial parasites (Linthicum 1988). However, recent published data show that *An. marajoara* is a primary vector of human malarial parasites in Macapá (Conn et al. 2002) and Serra do Navio, AP (Póvoa et al. 2001).

Molecular characterization - *An. marajoara* can be recognized by RAPD-PCR banding profiles produced by Operon primers D01, A01, C19 and C16 (Wilkerson et al. 1995a, c). Similarly, Li and Wilkerson (2005) demonstrated that *An. marajoara* can be distinguished from other species of the complex by species specific ITS2 sequence. Lehr et al. (2005), using COI mtDNA sequence data, found at least two species under the name *An. marajoara* among specimens that were first identified by the RAPD-PCR profile and ITS2 sequence. Consequently, using the results of phylogenetic analyses, Lehr et al. (2005) hypothesized a fifth species, *An. albitarsis* E, which had been identified as *An. marajoara*. Wilkerson et al. (2005) using ND4, COI, ITS2 and 18S sequence data recovered a nonexclusive clade consisting of individuals of *An. marajoara* and *An. deaneorum*. Lack of exclusivity of sequences of *An. marajoara* and *An. deaneorum* was considered suggestive of ancestral introgression or perhaps a recent speciation event. Merritt et al. (2005) used coding sequence of the *white* gene including its fourth intron, to examine relationships among five species of the *An. albitarsis* complex. Results of sequence analyses showed the presence of the fourth intron in *An. marajoara*, whereas it is absent in the other species of the complex. Phylogenetic placement of *An. marajoara* within the complex was not entirely resolved because Wilkerson et al. (2005) found high support for a close relationship between *An. marajoara* and *An. deaneorum*, while Merritt et al. (2005) found support for *An. marajoara* sister to *An. albitarsis* B.

Anopheles (Nyssorhynchus) deaneorum Rosa-Freitas

An. deaneorum Rosa-Freitas (1989): 535 (M, F*, P, L*, E). Type: holotype, adult female with associated fourth instar larval and pupal exuviae (IOC-RJ). Type locality: Palheta, Guajará-Mirim, RO. Klein et al. (1990): 510-513 (tax., biology); Klein et al. (1991c): 301-303 (tax., DNA hybridization); Narang et al. (1993): 97-112 (tax., allozyme, RFLP); Wilkerson et al. (1995c): 697-704 (tax., RAPD-PCR).

Female: similar to *An. albitarsis* except as follows. 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. Maxillary 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

TABLE VI

Range (mode) of setal branches of the pupa of *Anopheles deaneorum*

Seta		Abdominal segments									
n	Cephalothorax	I	II	III	IV	V	VI	VII	VIII	IX	Paddle
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)	nc	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)	-	1	2-4 (3)	1	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	1	-	-

nc: not counted.

TABLE VII

Range (mode) of setal branches of the fourth-instar larva of *Anopheles deaneorum*

Seta		Thorax			Abdominal segments								
n	Head	P	M	T	I	II	III	IV	V	VI	VII	VIII	X
0	-	1	-	-	-	6-11 (8)	6-9 (6)	4-8 (7)	5-9 (6)	4-8 (5)	3-6 (4)	3-6 (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)	10-14 (10)	16-19 (18)
3	nc	1	1	14-22 (16)	1	1, 2 (1)	1	2, 3 (3)	1	1	3	6-17 (12)	8-12 (8)
4	1-6 (2)	14-26 (20)	3-7 (4)	3-6 (3)	3-6 (5)	3-6 (4)	2, 3 (3)	2-4 (3)	2, 3 (3)	1	1	1	8[a]
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)	5-11 (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)	3-4 (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	nc	2, 3 (2)	1, 2 (2)	1	3, 4 (3)	1	2, 3 (2)	1-3 (2)	1, 2 (2)	2, 3 (2)	2, 3 (2)	9-S	4-10 (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	4-8 (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	-
15	3-7 (4)	-	-	-	-	-	-	-	-	-	-	-	-

a: pairs of setae; M: mesothorax; nc: not counted; P: prothorax; T: metathorax.

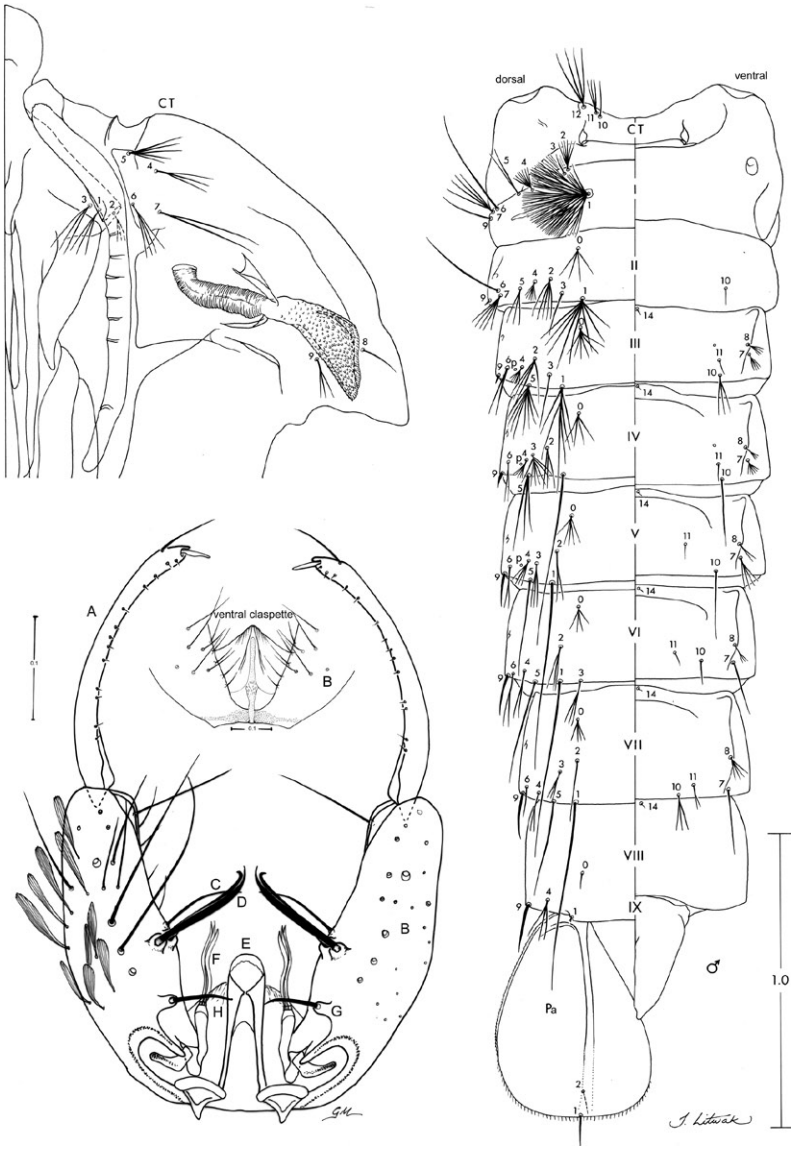

Fig. 6: pupa and male genitalia and of *Anopheles deaneorum*. Pupa: CT: cephalothorax; p: puncture; Pa: paddle; I-IX: abdominal segments. Male genitalia: A: gonostylus; B: gonocoxite; C: internal seta; D: accessory setae; E: aedeagus; F: dorsal claspette; G: tubercle of parabasal spine; H: ventral claspette. Scales in mm.

with spots of white scales on anterior area of costa, subcosta and R and R_1, pale yellow scales on remainder of 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); hindtarsomere 2 dark-scaled proximally with basal dark band 0.51-0.90 length of tarsomere (mean = 0.67 ± 0.08) (n = 30). Abdomen: posterolateral tufts of scales absent from tergum III.

Male: similar to *An. albitarsis*. Abdomen: caudolateral tufts always absent from tergum III, tergum IV with few dark, erect caudolateral scales, terga V-VII with caudolateral scale tufts.

Male genitalia (Fig. 6): similar to *An. albitarsis* except as follows. 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. 6): similar to *An. albitarsis* except as follows. Position and development of setae as figured; range and modal number of branches in Table VI. All measurements were made on 10 specimens. Abdomen: length 2.54-3.04 mm (mean = 2.77 ± 0.16); 3-I usually single; 0-II-IV normally 6-branched, 6-II 1-4 branched, 1.25-2.74 length of 7-II (mean = 1.99 ± 0.50), 9-II min, unpigmented, 9-II-IV less than 0.25 length of segment; 8-III, IV normally triple, 9-III short, stout, 0.91-3.22 (mean = 2.22 ± 0.74) length of 9-II; 1-IV rarely double, 6-IV-VI single to triple, 9-IV 1.51-3.96 (mean = 2.44 ± 0.74) length

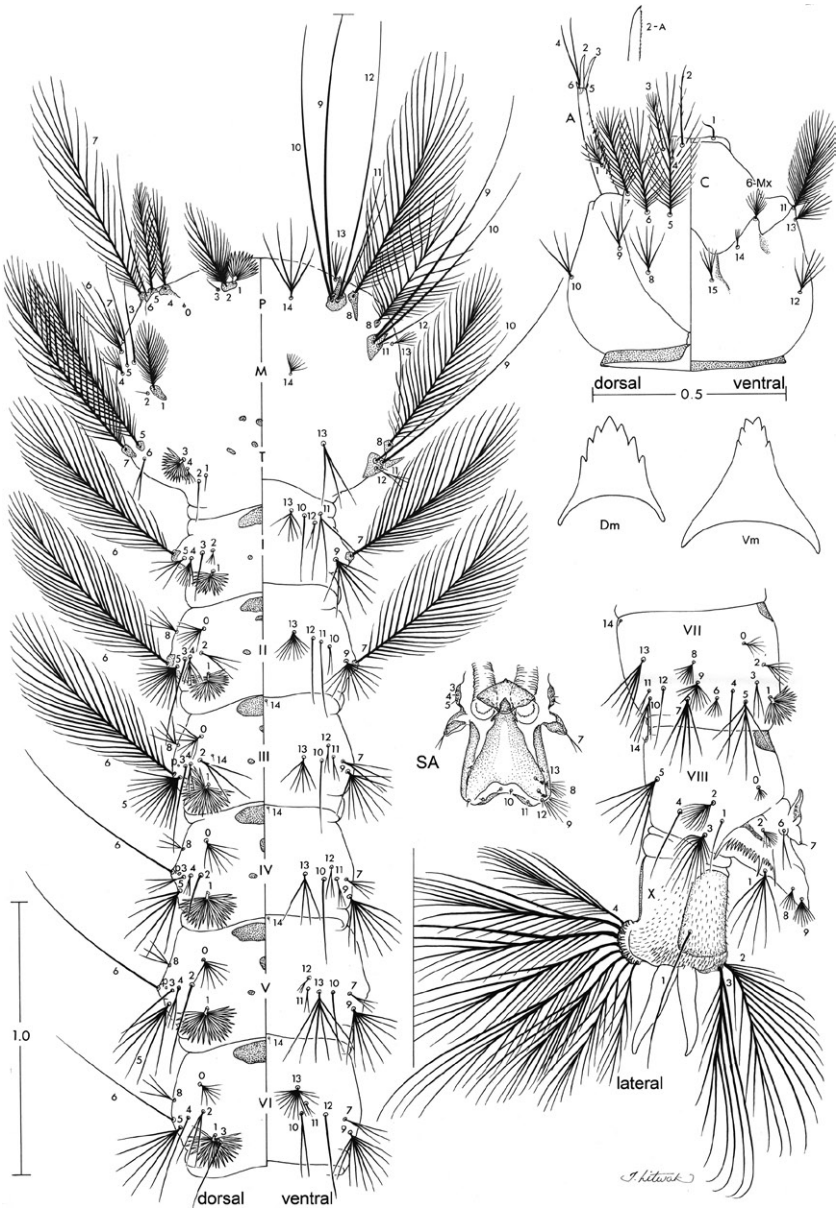

Fig. 7: fourth-instar larva of *Anopheles deaneorum*. A: antenna; C: cranium; Dm: dorsomentum; M: mesothorax; P: prothorax; p: puncture; S: spiracular apparatus; T: metathorax; Vm: ventromentum; I-VIII, X: abdominal segments. Scales in mm.

of 9-III, 10-IV, V always single, long; 0-V,VI usually with five branches, 3-V single to 6-branched, 5-V, VI with 1-4 branches; 5-VII single or double, rarely double, 7-V normally 3-branched, 8-V,VI often double, 9-V 1.23-2.07 (mean = 2.08 ± 0.43) length of 9-IV, 9-V, VI less than 0.50 length of segment; 7-VI,VII often single, 9-VI 1.11-2.24 (mean = 1.35 ± 0.33) length of 9-V; 5-VII rarely double, 9-VII, VIII less than 0.60 length of segment, 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. 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-Pa usually double.

Fourth-instar larva (Fig. 7): similar to *An. albitarsis* except as follows. Position and development of setae as figured; range and modal number of branches in Table VII. Measurements were made on 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 ± 0.02) (n = 6). Integument weakly pigmented with darker spots along posterior border of dorsal apotome, on posterior margin of frontal ecdysial line, on lateralia and on ventral lateralia, labiogula darkened along hypocranial ecdysial line and hypostomal suture. 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 insertion 0.26-0.34 (mean = 0.30 ± 0.03) distance from base, seta 2-A

with minute spicules on mesal margin. Seta 2-C 1.21-1.41 length of 3-C (mean = 1.28 ± 0.07), seta 2-C relatively 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 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. Thorax: leaflets of 1-P pointed to truncate at apex. Abdomen: seta 13-I, III often 5-branched; 0-II-VII multibranched, 1-II-VII with truncate to pointed leaflets; 2-IV, V single, long. Pecten with 3-5 long, 9-13 short spines, long spines 2.36-3.24 (mean = 2.68 ± 0.28) length of short spines. Segment X: seta 1-X longer than length of saddle, inserted on or off saddle.

Distribution - *An. deaneorum* is found in the Province of Corrientes, Argentina (Wilkerson et al. 1995a, Li & Wilkerson 2005). In Brazil, the species was recorded in PR and RO (Wilkerson et al. 1995a, Li & Wilkerson 2005) and Acrelândia municipality, state of Acre (MAM Sallum 2008, personal communication).

Material examined - Table I.

Bionomics - Klein et al. (1991d) compared the biting behavior of *Anopheles* mosquitoes in Costa Marques, RO, and found that *An. deaneorum* and *An. darlingi* are more anthropophilic than other anophelines at that site. Also, both species were more frequent inside houses than other *Anopheles* species. *An. deaneorum* was also collected on human bait outside and inside houses (6% and 9% of anophelines captured, respectively) in Costa Marques, RO (Klein & Lima 1990).

Medical importance - *An. deaneorum* is considered to be a potential vector of human malarial parasites in RO (Klein et al. 1991b, c). Klein et al. (1991a) compared the susceptibility of five anopheline mosquitoes to *Plasmodium falciparum* in RO, and demonstrated that the oocyst positive rate and the mean number of oocysts in *An. deaneorum* are similar to those found in sympatric *An. darlingi*. The salivary gland sporozoite rate was similar to that found in *Anopheles mediopunctatus s.l.* when compared to *An. darlingi*. However, the susceptibility to *P. falciparum* was lower for *An. deaneorum* when compared to *An. darlingi* (Klein et al. 1991b). For *Plasmodium vivax*, the salivary gland infection rate in *An. deaneorum* was similar to that found in *An. darlingi*. In *An. deaneorum*, monoclonal antibodies for circumsporozoite proteins gave the following positive results: *P. falciparum*, 2.76%; *P. vivax*, 0.55%; *P. vivax* VK247, 0.82%; and *Plasmodium malariae*, 0.0% (Branquinho et al. 1993). Also, *P. vivax*-like parasites were reported from two out of 168 *An. deaneorum* tested using serological tests (Marrelli et al. 1998).

Molecular and morphological characterization - *An. deaneorum* is the only species of the *An. albitarsis* complex that can be recognized morphologically by the distinctly branched fourth-instar larval seta 3-C, which is single and simple or aciculate in *An. albitarsis*, *An. marajoara* and *Anopheles oryzalimnetes* n. sp. Rosa-Freitas (1989) suggested that the absence of posterolateral scales on the third abdominal segment of the adult female could be employed to distinguish *An. deaneorum*. However, this character is variable and the scales are easily lost when specimens are not adequately preserved. *An. deaneorum* can be recognized by diagnostic bands of the RAPD-PCR profile produced using Operon primers D01, C15, B11, A12 and A08 (Wilkerson et al. 1995a, c). In addition, Li and Wilkerson (2005) demonstrated that *An. deaneorum* could be distinguished from other species of the complex by species-specific ITS2 sequence. Lehr et al. (2005), using COI mtDNA sequence data, found that *An. deaneorum* clustered together in a strongly or moderately supported monophyletic lineage in a clade that includes *An. marajoara*. Consequently, Lehr et al. (2005) hypothesized that, either *An. marajoara* could consist of at least two phylogenetic species or *An. deaneorum* and *An. marajoara* were not valid species. Similar results were obtained by Wilkerson et al. (2005) in phylogenetic analyses employing the position 3 partition of the COI and ND4 mtDNA sequences.

Anopheles (Nyssorhynchus) oryzalimnetes Wilkerson and Motoki, n. sp.

An. albitarsis species B Wilkerson et al. (1995c): 699; Wilkerson et al. (1995a): 721. *An. albitarsis* A of Forattini et al. (1995): 21; Forattini et al. (1996): 299; Kakitani and Forattini (2000): 33.

Etymology - the name *oryzalimnetes* is a combination of *oryza*, Greek for rice and *limnetes*, Greek for inhabiting or born in a lake or marsh. This mosquito species was first found in a rice plantation.

Female: similar to *An. albitarsis* except as follows. 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 area of costa, subcosta, R and R_1, pale yellow scales on remaining 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); hindtarsomere 2 dark-scaled proximally with basal dark band 0.29-0.52 length of tarsomere (mean = 0.43 ± 0.06) (n = 30).

Male: similar to *An. albitarsis*.

Male genitalia (Fig. 8): similar to *An. albitarsis* except as follow. 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.

TABLE VIII

Range (mode) of setal branches of the pupa of *Anopheles oryzalimnetes* n. sp.

Seta		Abdominal segments									
n	Cephalothorax	I	II	III	IV	V	VI	VII	VIII	IX	Paddle
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)	nc	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	-	1	2-4 (3)	1 ,2 (1)	1	-	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	-	-	-	1	1	1	1	1	1	-	-

nc: not counted.

Pupa (Fig. 8): similar to *An. albitarsis* except as follows. Position and development of setae as figured; range and modal number of branches in Table VIII. All measurements were made on 10 specimens unless otherwise indicated. Abdomen: length 2.48-3.19 mm (mean = 2.69 ± 0.21); 5-I usually double, 6-I usually single, both 5,6-I long, 7-I with branches shorter than 6-I; 6-II single, 1.22-3.0 length of 7-II (mean = 2.22 ± 0.50) (n = 8), 9-II-IV less than 0.20 length of segment; 5-III with branches shorter than length of following segment, 9-III 1.42-3.90 (mean = 2.11 ± 0.70) length of 9-II; 10-III usually triple; 0-IV normally 4-branched, 9-IV 1.04-2.13 (mean = 1.67 ± 0.33) length of 9-III, 10-IV single or double, long; 3-V with branches extending beyond caudal margin of segment, 9-V 1.67-2.93 (mean = 2.19 ± 0.42) length of 9-IV, 9-V-VIII 0.32, 0.40, 0.51 and 0.44 length of segment, respectively, 10-V single, long; 0-VI,VII frequently 4-branched, 5-VI single or double, 9-VI, 1.14-1.80 (mean = 1.37 ± 0.21) length of 9-V; 5-VII always single, 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. 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-Pa stronger than 2-Pa, 2-Pa often double.

Fourth-instar larva (Fig. 9): similar to *An. albitarsis* except as follows. Position and development of setae as figured; range and modal number of branches in Table IX. Measurements were made on 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 apotome and on lateralia; labiogula darkened along hypostomal suture and hypocranial ecdysial line. 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 0.25-0.35 (mean = 0.30 ± 0.04) distance from base. 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). Thorax: setae 1, 2-P sometimes sharing a common tubercle, 1-P palmate with large pointed leaflets; 1-M strongly plumose; 3-T palmate with moderately long narrow leaflets. Abdomen: seta 1-I-VII palmate, 11-I triple, large, 13-I, III often triple; 0-II-VII large, 1-II-VII with dark, moderately narrow pointed leaflets; 2-IV single, 2-V long, single or double. Pecten with four or five 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, directed dorsolaterally. Segment X: seta 1-X inserted inside saddle.

Distribution - *An. oryzalimnetes* n. sp. seems to be a widely distributed species in Brazil and Paraguay. In Brazil it is found in the states of Espírito Santo, SP, PA, PR, MT, Bahia, Ceará and Rio de Janeiro (RJ) (Wilkerson et al. 1995a, c, Lehr et al. 2005). In Paraguay, *An. oryzalimnetes* n. sp was found in Hernanderias and near the National Airport (Wilkerson et al. 1995c).

Material examined - Table I.

Type data - All individuals from the type series of this species and *An. janconnae* n. sp were obtained from link-reared offspring of females collected in a Shannon trap and later given a blood meal in the laboratory. The accession codes are as follows: the first two capi-

TABLE IX
Range (mode) of branches for setae of the fourth-instar larva of *Anopheles oryzalimnetes* n. sp.

Seta		Thorax			Abdominal segments								
n	Head	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)	3-6 (4)	3 ,4 (3)	5-9 (6)	4-8 (5)	2-4 (3)	2-4 (3)	2 ,3 (3)	1	1	1	6-8 (8)[a]
5	19-27 (23)	21-35 (28)	1	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	nc	6-10 (7)	8-13 (11)	-	-	-	1	1	1	1	1	1	-
15	4-8 (6)	-	-	-	-	-	-	-	-	-	-	-	-

a: pairs of setae; M: mesothorax; nc: not counted; P: prothorax; T: metathorax.

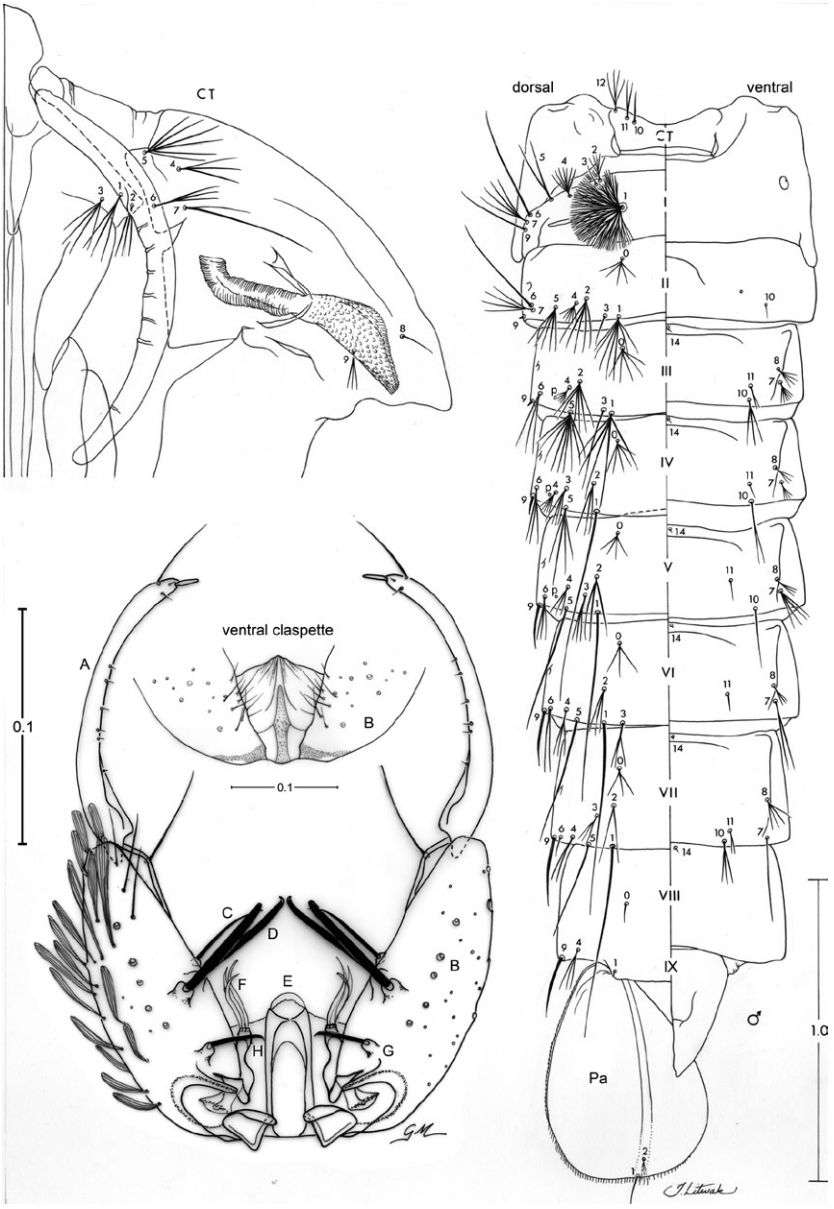

Fig. 8: pupa and male genitalia of *Anopheles oryzalimnetes* n. sp. Pupa: CT: cephalothorax; p: puncture, Pa: paddle; I-IX: abdominal segments. Male genitalia: A: gonostylus; B: gonocoxite; C: internal seta; D: accessory setae; E: aedeagus; F: dorsal claspette; G: tubercle of parabasal spine; H: ventral claspette. Scales in mm.

tal letters are the country code, followed by the collection number, the number between brackets specifies the field collected female and the last number denotes the specimen number. Specimen numbers > 100 indicate the adult is linked with a pupal exuviae, whereas numbers < 99 means indicate that the adult has associated larval and pupal exuviae. *Holotype*: adult male with associated with larval and pupal exuviae mounted on microscope slide: Brazil, SP, registro municipality, Centro de Desenvolvimento Agrícola do Vale do Ribeira (CEDAVAL) (24º36.8'S 47º53.1'W), 26 January 1992, Wilkerson and Klein coll., WRBU Acc. 1527, BR500(45)-9, deposited in the Entomological Collection of FSP-USP, accession E-12935. *Allotype*: adult female with associated larval and pupal exuviae mounted in microscope slide, with the same collection data of the holotype and from the same progeny, WRBU Acc. 1527, BR500(45)-12, deposited in the Entomological Collection of the National Museum of Natural History (NMNH), Smithsonian Institution. *Paratypes*: two hundred twenty-six adults male and female, collection code WRBU Acc. 1527, as follows: BR500(1), 11 adults male and female with associated larval and pupal exuviae mounted on microscope slides; BR500(18), eight adults with associated larval and pupal exuviae mounted on microscope slides; BR500(2), nine adults with associated larval and pupal exuviae mounted on microscope slides; BR500(35), 12 adults with associated larval and pupal exuviae mounted on microscope slides; BR500(37),

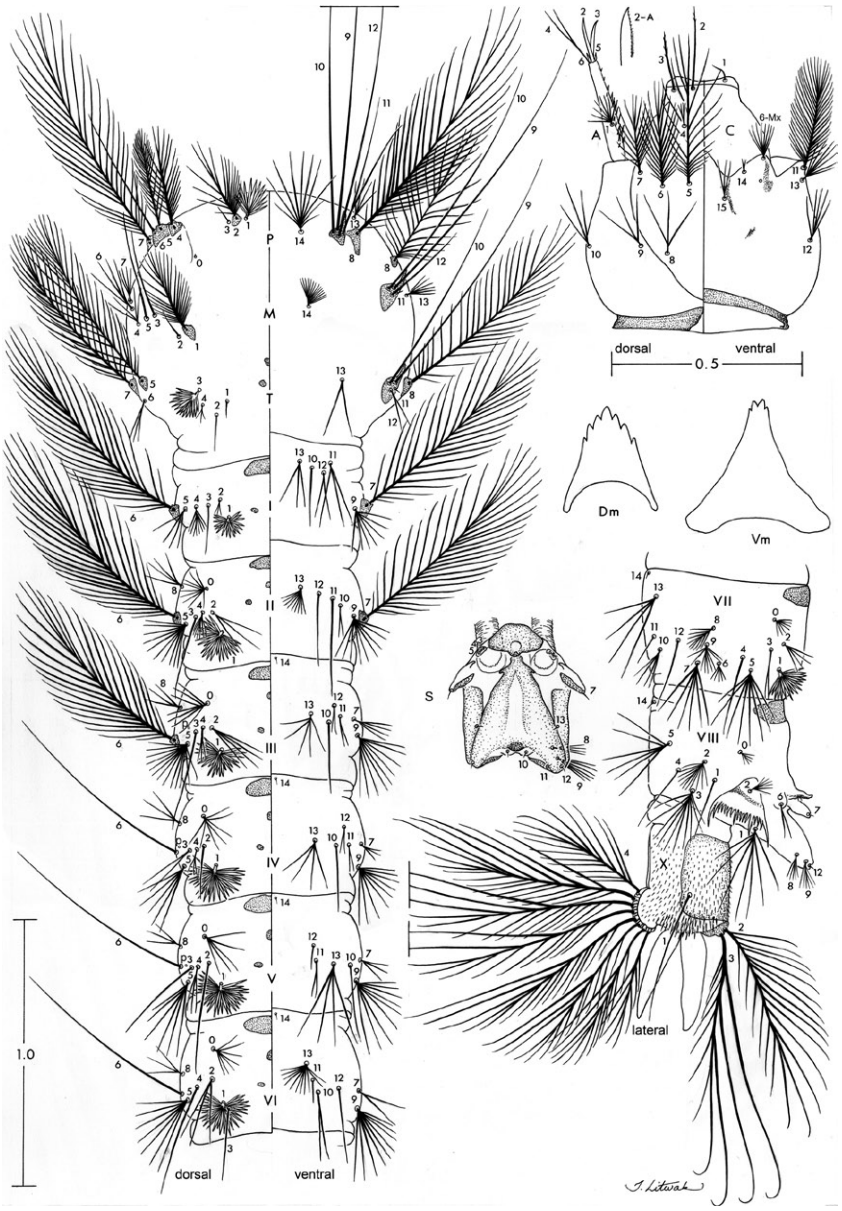

Fig. 9: fourth-instar larva of *Anopheles oryzalimnetes* n. sp. A: antenna; C: cranium; Dm: dorsomentum; M: mesothorax; P: prothorax; p: puncture; S: spiracular apparatus; T: metathorax; Vm: ventromentum; I-VIII, X: abdominal segments. Scales in mm.

17 adults with associated larval and pupal exuviae mounted on microscope slides; BR500(4), 12 adults with associated larval and pupal exuviae mounted on microscope slides; BR500(44), 19 adults with associated larval and pupal exuviae mounted on microscope slides; BR500(45), 10 adults with associated larval and pupal exuviae mounted on microscope slides, six with pupal exuviae, four not associated with immature; BR500(46), 11 adults with associated larval and pupal exuviae mounted on microscope slides, 62 with pupal axuviae, four not associated with immature; BR500(5), 10 adults with associated LE and Pe mounted on microscope slides; BR500(6), eight adults with associated LE and Pe mounted on microscope slides; BR500(7), 11 adults with associated LE and Pe mounted on microscope slides; BR500(8), eight adults with associated LE and Pe mounted on microscope slides; BR500(9), 10 adults with associated LE and Pe mounted on microscope slides; BR500(1), eight adults with associated LE and Pe mounted on microscope slides; BR501(2), nine adults with associated LE and Pe mounted on microscope slides; BR501(34), nine adults with associated Le and Pe mounted on microscope slides, three with Pe, five not associated with immature; BR501(4), nine adults with associated LE and Pe mounted on microscope slides; BR501(5), nine adults with associated LE and Pe mounted on microscope slides. Paratypes are deposited in the Entomological Collection of the NMNH, FSP-USP and Natural History Museum, London, UK.

Bionomics - Because *An. oryzalimnetes* n. sp has been largely misidentified as *An. albitarsis*, *An. deaneorum* or *An. marajoara*, little is known about its bionomics and medical importance. Kakitani and Forattini (2000) (as *An. albitarsis* A) demonstrated that its daily survival rate was 0.5339 ± 0.047 and the duration of the gonotrophic cycle was 1.99 days. Also, *An. oryzalimnetes* n. sp. (as *An. albitarsis* A) was attracted to human bait and was common in rice fields and in surrounding dwellings in the Ribeira Valley, southeast of SP, Brazil. In the Ribeira Valley, *An. albitarsis* and *An. oryzalimnetes* n. sp. are sympatric. Also, in Paraguay *An. albitarsis, An. oryzalemnetes* and *An. deaneorum* were found to be sympatric (Wilkerson et al. 1995c). Furtermore, *An. oryzalimnetes* n. sp 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* and *An. oryzalimnetes* n. sp. in rice field environments. *An. oryzalimnetes* n. sp. was also collected at sunset and sunrise on human bait captures performed indoors and outdoors in a rice plantation environment.

Medical importance - Nothing is known about medical importance of *An. oryzalimnetes* n. sp.

Molecular characterization - *An. oryzalimnetes* can be recognized by species-specific RAPD-PCR bands (Wilkerson et al. 1995a, c) generated with Operon primers C07, B02 and C15. Additionally, Li and Wilkerson (2005) developed species-specific ITS2 primers to separate *An. oryzalimnetes* from other species of the *An. albitarsis* complex. Lehr et al. (2005) and Wilkerson et al. (2005) using distinct molecular markers corroborated the monophyly of *An. oryzalimnetes*. Also, Merrit et al. (2005) and Brochero et al. (2007) using the *white* gene sequence data corroborated *An. oryzalimnetes* to be distinct from the other species of the *An. albitarsis* complex.

Anopheles (Nyssorhynchus) janconnae Wilkerson and Sallum, n. sp.

An. albitarsis species E of Lehr et al. 2005: 908-917 (tax., mtDNA); Póvoa et al. 2006: 163-168 (biology).

Etymology - we are pleased to honor Jan Conn, collaborator, respected scientist, poet and friend, for her outstanding contributions to the advancement of medical entomology, especially on population genetics and phylogeography of species of *Anopheles* (*Nyssorhynchus*).

Female: similar to *An. albitarsis* except as follows. 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 ± 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 area of costa, subcosta, R and R_1, pale yellow scales on remaining 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); hindtarsomere 2 dark-scaled proximally with basal dark band 0.28-0.51 length of tarsomere (mean = 0.39 ± 0.06) (n = 30).

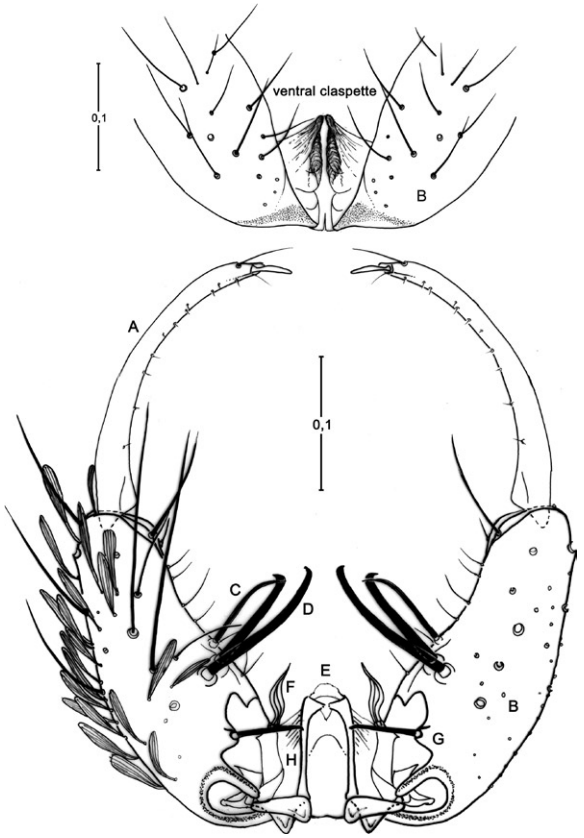

Fig. 10: male genitalia of *Anopheles janconnae* n. sp. A: gonostylus; B: gonocoxite; C: internal seta; D: accessory setae; E: aedeagus; F: dorsal claspette; G: tubercle of parabasal spine; H: ventral claspette. Scales in mm.

Male: similar to *An. albitarsis*.

Male genitalia (Fig. 10): similar to *An. albitarsis* except as follows. 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. janconnae* n. sp is known from Northern Brazil and Venezuela (Lehr et al. 2005).

Material examined - Table I.

Type data - Holotype: adult male with dissected male genitalia on microscope slide. Brazil, RR, Boa Vista municipality, (2º49'S 60º40'W), 2002, Wilker-

son coll., WRBU BR24(9)-100, deposited in the FSP-USP collection, under Acc. E-12936. Paratypes: 1 MG (WRBU Acc. 1734, BR24(5)-100, FSP Acc. E-12946); 7 F (WRBU Acc. 1734, BR17-10, BR17-32, BR17-40, BR17-20, BR24(9)-101, BR11-107, BR17-1, deposited in the FSP-USP under the Acc. E-12947, E-12948, E-12949, E-12950, E-12951, E-12952, E-12953); 42 F (WRBU Acc. 1734, BR11-13, BR11-14, BR11-15, BR11-106, BR11-108, BR17-18, BR17-19, BR17-28, BR17-39, BR17-42, BR19-3, BR19-4, BR19-6, BR19-10, BR19-11, BR19-14, BR19-24, BR19-25, BR19-26, BR24(4)-1, BR24(9)-1, BR25-11, BR25-12, BR25-16, BR25-18, BR25-22, BR25-24, BR26-1, BR26-7, BR26-9, BR27-1, BR27-5, BR27-108, BR27-109, BR30-1, BR30-5, BR30-7, BR30-8, BR31-3, BR31-7, BR34-3, BR36- 103, deposited in NMNH, USA), 1M (WRBU Acc. 1734, BR23(4)-1, deposited in NMNH, 2MG (WRBU Acc. 1734, BR24(8)-100, BR24(9)-2, deposited in NMNH, same collection data as holotype.

Bionomics - Póvoa et al. (2006) observed that *Anopheles janconnae* n. sp. was the most abundant species collected in Boa Vista, RR. In this region this species is anthropophilic with peak biting activity usually occurring before midnight as was observed for *An. marajoara* in Western Venezuela (Rubio-Palis & Curtis 1992).

Medical importance - *An. janconnae* n. sp is an important malaria vector at least in the savannah biome around Boa Vista, RR. Specimens of *An. janconnae* were found infected with *P. falciparum*, *P. vivax* VK 210, *P. vivax* VK247 and *P. malariae* (Póvoa et al. 2006).

Molecular characterization - Genetic and phylogenetic evidence support the existence of *An. janconae* in Northern Brazil and Venezuela. Kreutzer et al. (1976) found three chromosomally differentiated populations with the "C" form found in Venezuela and Colombia. Also, it is possible that the "iii" group designated by Rosa-Freitas et al. (1990), based on allozyme data, correspond to *An. janconnae* n. sp. Support for *An. janconnae* n. sp. as a distinct species were also found in a phylogenetic analysis of the complete COI gene by Lehr et al. (2005). However, Wilkerson et al. (1995a, c) using RAPD-PCR profiles and Li & Wilkerson (2005) using ITS2 sequence found no evidence to support a distinct species within the *An. albitarsis* complex.

DISCUSSION

To name the two species described here we considered the possibility that a name currently in synonymy might already exist for these species. In synonymy with *An. marajoara* is *An. domesticus* Galvão and Damasceno, which was originally described as a subspecies of *An. albitarsis,* but later shown by Rios et al. (1984) to be indistinguishable from their concept of *An. albitarsis*. This nominal species was later synonymized with *An. marajoara* by Linthicum (1988). Given that there is no extant type and that its type locality is the same as for *An. marajoara,* we agree with Linthicum's (1988) assessment. Another name associated with *An. marajoara* is *Anopheles allopha* Peryassú. Because it has proven impossible to characterize and because the existing syntypes are actually *Anopheles. argyritarsis*, it was considered to be a *nomen nudum* by Lourenço-de-Oliveira and Deane (1984) and a *nomen dubium* by Linthicum (1988). *An. imperfectus* was described by Corrêa and Ramos (1943) as a variety of *An. albitarsis*, based on an adult female collected in municipality of Vera Cruz, SP. Accordingly, *An. imperfectus* was considered to be morphologically similar to *An. albitarsis* from which it could be distinguished by having a dark ring on the basal 0.3 of hindtarsomere 3 and no SC pale spot the costa. Lane (1953) transferred *An. imperfectus* to the synonymy of *An. albitarsis*, which was later accepted by Linthicum (1988). We examined the holotype and confirmed both the presence of a dark ring on the basal 0.3 of hindtarsomere 3 and the absence of a SC pale spot on the costa. Additionally, hindtarsomere 2 has dark scales on the basal 0.8 and pale scales on the apical 0.2. By comparing the holotype of *An. imperfectus* with species of *An. albitarsis* complex included in this study, we conclude that *An. imperfectus* should be remain in the synonymy with *An. albitarsis* since it has hindtarsomere 2 dark-scaled in the basal 0.8. Among members of the *An. albitarsis* complex, only *An. deaneorum* and *An. albitarsis* have hindtarsomere 2 dark in the basal 0.51-0.90 and 0.52-0.86, respectively. However, in *An. deaneorum*, hindtarsomere 1 has a conspicuous ring of white scales distally, whereas in *An. albitarsis*, hindtarsomere 1 is entirely dark. Similar to *An. albitarsis*, *An. imperfectus* has hindtarsomere 1 dark at apex. The presence of dark scales on the basal 0.3 of hindtarsomere 3 of the holotype of *An. imperfectus* may represent a variant of *An. albitarsis*. Similar variability also has been documented in *An. darlingi* (Harbach et al. 1993). Furthermore, Wilkerson et al. (1995b) demonstrated that in *Anopheles rondoni* the basal dark ring on hindtarsomere 3 may be present or absent, additionally it was proposed that in the subgenus *Nyssorhynchus*, individuals with a long dark band on hindtarsomere 2 were more likely to have a basal dark band on hindtarsomere 3.

An. limai was described as a variety of *An. albitarsis* by Galvão and Lane (1937a). Validation of *An. limai* was based on the eggs and adult female characteristics; however, no type was designated. In the Entomological Collection of FSP-USP there are three microscope slides with the midguts of females of *An. albitarsis* collected in Pinheiros and Butantan district, municipality of SP. These slides represent specimens employed by Galvão and Lane (1937b) in experimental malaria infection tests. There are no other remnants in the FSP-USP collection of these females or of the eggs used to characterize *An. albitarsis* var. *limai*. Considering that the only specimens available were not used for the validation of *An. limai*, we consider that no types exist to represent this species. Galvão and Lane (1937a) reported that females of *An. limai* could be distinguished from specimens of *An. albitarsis* of Root (1926) in having hindtarsomere 2 with a more extensive basal dark ring. The hindtarsomere illustrated by Root (1926; Pl. II) is dark in more than basal the 0.5. In considering that Galvão and Lane (1937a) described the basal dark area of hindtarsomere 2

of *An. limai* as more extensive than that of *An. albitarsis* shown by Root (1926), we concluded that *An. limai* was correctly transferred to synonymy with *An. albitarsis*. Furthermore, Linthicum (1988) examined individuals from the type locality of *An. limai* and concluded that this nominal species is conspecific with *An. albitarsis*. For the reasons mentioned above, *An. limai* and *An. imperfectus* are retained in synonymy with *An. albitarsis.* Since the species we treat here are distinct from known nominal species, they are described as *An. oryzalimnetes* n. sp. and *An. janconnae* n. sp.

Multivariate analysis of morphometric characters has been employed as a useful tool for separating sibling species of *Anopheles* (*Nyssorhynchus*). For example, Calle et al. (2002) discriminated adult females of *Anopheles rangeli, Anopheles oswaldoi, Anopheles benarrochi*, *Anopheles triannulatus* and *Anopheles nuneztovari* from Colombia. Similarly, Rubio-Palis et al. (1997) and Rubio-Palis (1998) separated adult females of *An. darlingi, An. marajoara, Anopheles braziliensis* and *An. argyritarsis* in Venezuela.

Despite of being polymorphic, the pattern of pale and dark wing spots is often employed for *Anopheles* species identification (Faran 1980, Linthicum 1988, Hribar 1995). Faran (1980) and Linthicum (1988) used the range and ratio of the length of several pale and dark wing spots to identify species of the Albimanus and Argyritarsis Sections of *Anopheles* (*Nyssorhynchus*), respectively. Later, Hribar (1995) used the length of the costal wing spots to separate individuals of *An. nuneztovari* from Brazil and Venezuela. To examine the utility of the wing spots to separate individuals of *An. nuneztovari* from other morphologically similar species, Ramos et al. (2008) examined individuals from three localities in Colombia to evaluate variation in the ratio of several wing spots used in adult female identification keys and found that 5% of the specimens could be misidentified as other species of the subgenus *Nyssorhynchus.*

Results of the statistical analyses carried out in the present study suggest that the specimens of *An. marajoara* collected in SP, PA, MT and AM, Brazil, and Zulia Department of Venezuela, which have identical RAPD-PCR profiles, belong to two distinct species. These results validate the hypothesis of Lehr et al. (2005) regarding the existence of an unidentified species, *An. albitarsis* E (= *An. janconnae*), in areas of savannah in RR and in Venezuela.

Merritt et al. (2005) found a reliable method based on a fragment of the nuclear *white* gene to distinguish *An. marajoara* from the other members of the *An. albitarsis* complex. A pronounced difference in size of the nuclear *white* locus fragment was due to the absence of intron IV in all species of the *An. albitarsis* complex, except *An. marajoara*. Consequently, Merrit et al. (2005) observed that a simple PCR of the *white* gene could separate *An. marajoara* by the visualization of the PCR product in a gel stained with ethidium bromide. However, when analyzing the same *white* gene fragment for a larger sample size, which included specimens from Colombia and also *An. janconnae* n. sp. (as *An. albitarsis* E), Brochero et al. (2007) observed that *An. janconnae* n. sp. (also as *An. albitarsis* E) clustered together with *An. marajoara* with strong bootstrap support. It is interesting that *An. janconnae* n. sp. also possess intron IV. Consequently, the presence of *white* gene intron IV separates *An. marajoara* and *An. janconnae* n. sp. from the other species of the *An. albitarsis* complex.

An. deaneorum is distinguished by the development of larval 3-C seta, which is branched in *An. deaneorum* (Rosa-Freitas 1989) and single in the other species. Furthermore, results of the statistical analyses carried out in the present study show that based on the ratio of the length of the basal dark portion of hindtarsomere 2 and hindtarsomere 2 length it is possible to distinguish two major groups. One group is formed by *An. albitarsis* (mean = 0.62) and *An. deaneorum* (mean = 0.67). In this group the ratio varied from 0.51-0.90. In contrast, in a second group composed of *An. oryzalimnetes* (mean = 0.42), *An. marajoara* (mean = 0.48) and *An. janconnae* (mean = 0.39), the ratio varied from 0.28-0.63. There is an overlap of this character, however, when using the extreme values it is possible to separate species into two groups with confidence. Additionally, some wing spots are useful for separating species of the *An. albitarsis* complex. For example, the length of the accessory sector pale spot can distinguish *An. albitarsis* and *An. oryzalimnetes* n. sp. For *An. albitarsis,* the accessory sector pale spot showed a higher mean value, whereas for *An. oryzalimnetes* n. sp. it showed a lower mean value (Fig. 1H). The mean value of the length of the subcostal pale spot is high for *An. deaneorum*. Consequently, it can be used to separate *An. deaneorum* from the others species (Fig. 1K). *An. janconnae* can be separated using both the 1st dark spot of vein CuA_1 and the humeral dark spot of the costa (Figs 1L, 1M). The mean values of these characters are higher in *An. janconnae* n.sp. than in the other species of the complex.

ACKNOWLEDGEMENTS

To two reviewers, for their great contribution to the improvment of the text, to Eduardo S Bergo, SUCEN, SP, and JB Lima, Fiocruz, RJ, for helping with mosquito collections in Brazil.

REFERENCES

Belkin JN 1962. *The mosquitoes of the South Pacific (Diptera, Culicidae)*, Vol. 1, University of California Press, Berkeley and Los Angeles, 608 pp.

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

Branquinho MS, Lagos CB, Rocha RM, Natal D, Barata JM, Cochrane AH, Nardin E, Nussenzweig R, Kloetzel JK 1993. Anophelines in the state of Acre, Brazil, infected with *Plasmodium falciparum*, *P. vivax*, the variant *P. vivax* VK247 and *P. malariae*. *Trans Royal Soc Trop Med Hyg 87*: 391-394.

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

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

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

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

Conn JE, Wilkerson RC, Segura NO, Souza RTL, Schlichting CD, Wirtz RA, Povoa MM 2002. Emergence of a new neotropical malaria vector facilitated by human migration and changes in land use. *Am J Trop Med Hyg 66*: 18-22.

Corrêa RR, Lima FO, Coda D1950. Observations on the flight and longevity in nature of *Anopheles albitarsis domesticus*. *J Nat Malar Soc 9*: 280-284.

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

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

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

Forattini OP, 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*: 20-26.

Forattini OP, 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*: 99-303.

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

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

Galvão ALA, Lane J 1937a. 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 ALA, Lane J 1937b. Notas sobre os "*Nyssorhynchus*" de São Paulo, IV. Sobre a infecção experimental do *Anopheles albitarsis* e *An. strodei* da cidade de S. Paulo pelo *Plasmodium vivax*. *Folia Clin et Biol 3*: 65-69.

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

Harbach RE, Knight KL 1980. *Taxonomists'glossary of mosquito anatomy*, Plexus Publishing, Marlton, 415 pp.

Harbach RE, Roberts DR, Manguin S 1993. Variation in the hindtarsal markings of *Anopheles darlingi* (Diptera: Culicidae) in Belize. *Mosq Syst 25*: 192-197.

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

Hugh G, Gauch JR 1982. *Multivariate in community ecology*, Cambridge University Press, Cambridge, 298 pp.

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

Kakitani I, Ueno HM, Forattini OP 2003. Parity and wind impacto n the frequency of *Anopheles marajoara* in Brazil. *Rev Saude Publica 37*: 280-284.

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

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

Klein TA, Lima JB, Tada MS 1991a. Comparative susceptibility of anopheline mosquitoes to *Plasmodium falciparum* in Rondonia, Brazil. *Am J Trop Med Hyg 44*: 598-603.

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

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

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

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

Kreutzer RD, Kitzmiller JB, Rabbani MG 1976. Cytogenetically distinguishable sympatric and allopatric populations of the mosquito *Anopheles albitarsis*. *Acta Amazônica 6*: 473-481.

Lane J 1953. *Neotropical Culicidae*, Universidade de São Paulo, São Paulo, 1112 pp.

Lehr MA, Kilpatrick CW, Wilkerson RC, Conn JE 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*: 908-917.

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

Linthicum KJ 1988. A revision of the Argyritarsis section of the subgenus *Nyssorhynchus* of *Anopheles* (Diptera: Culicidae). *Mosq Syst 20*: 98-271.

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

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

Lourenço-de-Oliveira R, Deane LM 1984. What is *Anopheles allopha* (Lutz & Peryassu, 1921) (Diptera: Culicidae). *Mem Inst Oswaldo Cruz 79*: 509-510.

Lynch Arribalzaga F 1878. Descripcion de tres nuevos Culicidae de Buenos Aires. *Nat Argent 1*: 149-152.

Marrelli MT, Branquinho MS, Hoffmann EH, Taipe-Lagos CB, Natal D, Kloetzel JK 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*: 149-151.

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

Narang SK, Klein TA, Perera OP, Lima JB, Tang AT 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*: 97-112.

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 MM, Souza de RTL, Lacerda RNL, Rosa ES, Galiza D, Souza de JR, Wirtz RA, Schilichting CD, Conn JE 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*: 163-168.

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

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

Ramos MF, Obando RG, Suárez MF, López D, Wilkerson RC, Sallum MAM 2008. Morphological analysis of three populations of *Anopheles* (*Nyssorhynchus*) *nuneztovari* Gabáldon (Diptera: Culicidae) from Colômbia. *Mem Inst Oswaldo Cruz 103*: 85-92.

Rios RI, Nascimento LZ, de Oliveira AC 1984. Complexo *Anopheles (Nyssorhynchus) albitarsis*: impossibilidade de separá-lo em duas subespécies, *A. albitarsis albitarsis* e *A. albitarsis domesticus* (Diptera: Culicidae). *Rev Brasil Bio 44*: 461-465.

Romeo-Viamonte JM, Castro M 1951. Estudio de la morfologia de la armadura faringea de alguns anofilinos. (Díptera: Culicidae). *Rev San Hig Publ Madr 25*: 313-330.

Root FM 1926. Studies on Brazilian mosquitoes. I. The anophelines of the Nyssorhynchus Group. *Amer J Hyg 6*: 684-717.

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

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

Rosa-Freitas MG, Deane LM, Momen H 1990. A morphological, behavioural and isoenzymatic study in *Anopheles albitarsis* from 10 populations. *Mem Inst Oswaldo Cruz 85*: 275-289.

Ross ES, Roberts HR 1943. *Mosquito atlas. Part I. The Nearctic Anopheles, important malaria vectors of the Americas and Aedes aegypti*, *Culex quinquefasciatus*. The American Entomological Society, the Academy of Natural Science, Philadelphia, 44 pp.

Rozeboom LE 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 1995. Differential attraction of Venezuelan Anophelines to human collectors. *J Am Mosq Control Assoc 11*: 482-484.

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, Curtis CF 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, Manguin S, Ayesta C, Guzman H, Arcia JM, Gonzalez JJr, 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, Wilkerson RC, Guzman H 2003. Morphological characters of adult *Anopheles* (*Nyssorhynchus*) *marajoara* in Venezuela. *J Am Mosq Control Assoc 19*: 107-114.

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

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

Steiner WWM, Narang S, Kitzmiller JB, Swofford DL 1982. Genetics diversity and evolution in Neotropical Anopheles *(Subgenus Nyssorhynchus).* In WWM Steiner, WJ Tabachnick, KS Rai, SK Narang (eds.), *Recent developments in the genetics of insects disease vectors*, Stipes, Champaign, p. 523-550.

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

Wilkerson RC, Gaffigan TV, Lima JB 1995a. Identification of species related to *Anopheles* (*Nyssorhynchus*) *albitarsis* by random amplified polymorphic DNA-polymerase chain reaction (Diptera: Culicidae). *Mem Inst Oswaldo Cruz 90*: 721-32.

Wilkerson RC, Hribar LJ, Milstrey EG, Falero GC 1995b. The identification of *Anopheles* (*Nyssorhynchus*) *rondoni* (Diptera: Culicidae) in Mato Grosso state, Brazil: an analysis of key character variability. *Mem Inst Oswaldo Cruz 90*: 575-582.

Wilkerson RC, Parsons TJ, Klein TA, Gaffigan TV, Bergo E, Consolim J 1995c. 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*: 697-704.

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