Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Karyotype of Brazilian *Anopheles albitarsis sensu lato* (Diptera:Culicidae)

Míriam Silva Rafael[1], Ivanildo Pereira dos Santos-Junior[2], Wanderli Pedro Tadei[1], Maria Anice Mureb Sallum[3] and Oswaldo Paulo Forattini[3]

[1]Coordenação de Pesquisas em Ciências da Saúde,
Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, AM, Brasil
[2]Programa Integrado de Pós-Graduação em Biologia Tropical e
Recursos Naturais - PPG BTRN/INPA-UFAM, Manaus, AM, Brasil
[3]Departamento de Epidemiologia, Faculdade de Saúde Pública,
Universidade de São Paulo, São Paulo, SP, Brasil
Corresponding author: M.S. Rafael
E-mail: msrafael@inpa.gov.br

Genet. Mol. Res. 4 (4): 684-690 (2005)
Received Sepember 15, 2005
Accepted September 28, 2005
Published November 17, 2005

ABSTRACT. *Anopheles* (*Nyssorhynchus*) *albitarsis sensu lato* is an important malaria vector in Brazil, especially in the Brazilian Amazon region. Chromosome preparations of fourth-instar larvae of *A. albitarsis* from Iranduba and Coari (AM) and Ilha Comprida (SP) were analyzed for karyotype determination and to improve cytogenetic identification of this species. *Anopheles albitarsis* possesses 2n = 6 chromosomes, with two pairs (submetacentric and metacentric) of autosomes and one pair of sex chromosomes, with X-Y dimorphism. The sex pair is homomorphic and acrocentric in females and heteromorphic in males, with a punctiform Y chromosome. Somatic pairing was detected in the prometaphase and metaphase chromosomes of the three *A. albitarsis* populations. Apparently, sex chromosome evolution in the Culicidae does not function as does evolution in the Culicidae, since it occurs in the subfamily Anophelinae, which possesses heteromorphic sex chromosomes and is regarded as primitive, based on several criteria. These karyotype data on the *albitarsis* complex reinforce the hypothesis that sex chromosome evolution in the subfamily Anophelinae is conserved, and the varia-

tion revealed in the mean size of chromosomes in three populations indicates that selective pressure in these populations is occurring only at a genetic level.

Key words: *Anopheles albitarsis* complex, Amazon region, Malaria, Karyotype

INTRODUCTION

Anopheles (*Nyssorhynchus*) *albitarsis* Lynch-Arribálzaga, 1878, which presents a wide-ranging geographical distribution throughout South America, has been cited as an important malaria vector locally in Brazil, especially in the Amazon region (Segura, 1998; Tadei and Dutary, 2000), the origin of 99.7% of these reports (Fundação Nacional de Saúde - FNS/FUNASA, 2000).

Cytogenetic studies for *A. albitarsis* population differentiation were made by Kreutzer et al. (1976). They analyzed samples from Brazil, Venezuela and Colombia and identified three populations based on an inversion on the X polytene chromosomes, which were named B1, B2 and C. Later analyses using isoenzymes (Steiner et al., *op. cit.*, 1982; Narang and Seawright, 1993), hybridization (Klein et al., 1991), morphology and behavior (Rosa-Freitas et al., *op. cit.*, 1990) and RAPD-PCR (Wilkerson et al., 1995a,b) have contributed to the identification of a fourth species; thus, this group is designated as A, B, C, and D. The A species has been described as *A. albitarsis sensu stricto*, the B species has not been described, the C species was named *A. marajoara* Galvão and Damasceno and a fourth species was named *A. deaneorum* Rosa-Freitas. It is known that *A. marajoara* is a significant malaria vector at least in Northeastern Brazil (Conn et al., 2002), but the status of the other species is poorly understood (Cong Li and Wilkerson, 2005). A complication for accurate taxonomy and geographic distribution of *A. albitarsis* samples is the fact that adult females are difficult to distinguish morphologically, and most collections of this group are treated as species complexes (Kreutzer et al., *op .cit.*, 1976; Steiner et al., 1982; Rosa-Freitas et al., 1990).

Cytogenetic studies of mitotic chromosomes are important tools for comprehension of the taxonomy and evolution of these mosquitoes of the *Anopheles* complex, such as *Anopheles dirus* from continental Asia (Baimai, 1984). Chromosomal studies of anophelines have shown a karyotype of 2n = 6 chromosomes (Baimai et al., 1996), which includes two pairs of sex chromosomes, a pair of metacentric and a pair of submetacentric autosomes; this seems to be a conservative characteristic of these mosquitoes (Rao and Rai, 1987; Coluzzi, 1988). This chromosome number has been found in the Brazilian *Anopheles* species, including *A. darlingi*, *A. noroestensis*, *A. argyritarsis*, *A. aquasalis*, *A. nuneztovari* (Schreiber and Guedes, 1959, 1961; Rafael and Tadei, 1998), *A. cruzii* and *A. bellator* (Ramírez, 1989).

We examined metaphase chromosomes of *A. albitarsis* populations, because of the lack of information on karyotypes. This type of data is important for understanding population differentiation and for the development of strategies for human malaria vector control in the Brazilian Amazon.

MATERIAL AND METHODS

Mosquito samples and chromosome preparations

Adult female *A. albitarsis* mosquitoes were collected from Iranduba and Coari in the State of Amazonas, and from Ilha Comprida (24°42.75'S, 47°31.6'W), in the State of São Paulo (Figure 1A,B). Adult females of *A. albitarsis* were collected while they were rest on the houses' wall in Ilha Comprida, São Paulo State, and rest on the houses' wall and feeding on cattle in Iranduba and Coari, Amazonas State. The females were transported to the Insectary of the Laboratory of Malaria Vectors at the Instituto Nacional de Pesquisas da Amazônia, in Manaus, Amazonas. The specimens were identified morphologically (Forattini, 1962; Gorham et al., 1967; Faran and Linthicum, 1981; Consoli and Lourenço de Oliveira, 1994).

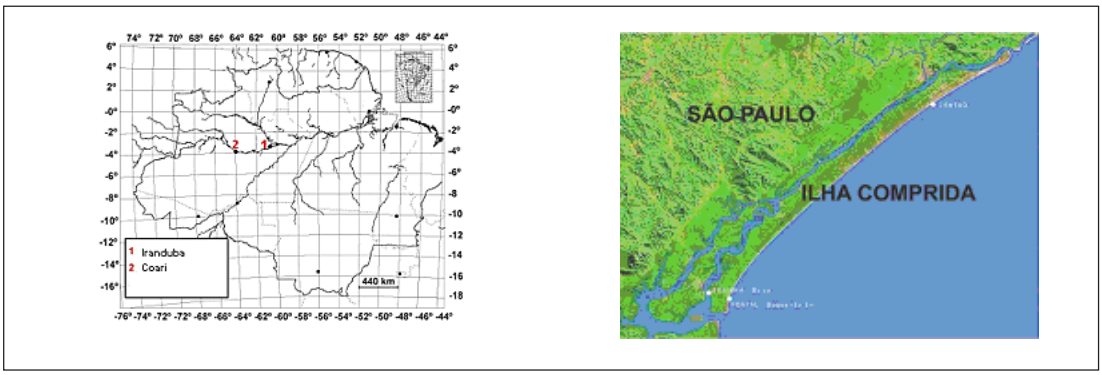

Figure 1. Collection sites. **A.** Central Amazon: Iranduba and Coari, Amazonas. **B.** Ilha Comprida, São Paulo.

Slides of brain ganglia of fourth-instar larvae were prepared, 15 from Ilha Comprida, 18 from Iranduba and 14 from Coari. Brain ganglia were treated with 0.005% colchicine-hypotonic solution, stained with Giemsa (Imai et al., 1988). The chromosomes were photographed and then analyzed under phase contrast, with an optovar 1.25X lens on a Zeiss-Axioplan microscope, photographed with Kodak Imagelink HQ ISO 25 film. The chromosomes were numbered according to the nomenclature proposed by Rai (1963). Arm ratios (AR) and relative size (RS%) of all chromosomes were calculated by the Beçak method (1967) and classified according to Levan et al. (1964).

RESULTS

Fifty-seven metaphase preparations from Ilha Comprida, 59 from Iranduba and 38 from Coari were photographed and analyzed. The *A. albitarsis* preparations showed a chromosome number of 2n = 6, with acrocentric X and Y punctiform chromosomes, metacentric (pair II) and submetacentric (pair III) autosomes (Figures 2 and 3). Only heteromorphic (XY) and homomorphic (XX) chromosomes in the males and females were observed in the sex pair in the three *A. albitarsis* populations. The chromosomes were named sex pair (I) and autosome pairs (II and III), according to Rai (1963). Another characteristic detected in the chromosomes of the three populations was somatic pairing during metaphase (Figure 2C).

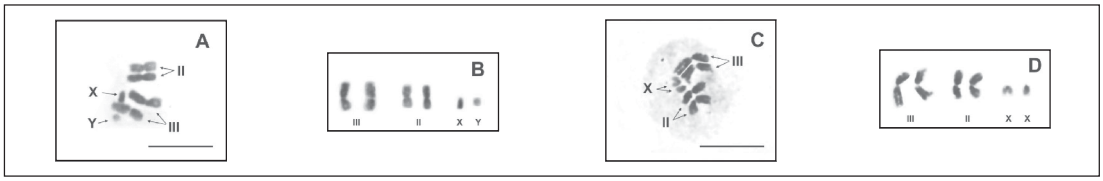

Figure 2. Karyotype of *Anopheles albitarsis* from Ilha Comprida. **A.** Male metaphase neuroblast cells. **B.** Karyotype. **C.** Female metaphase neuroblast cells. **D.** Karyotype. Scale: 10 µm.

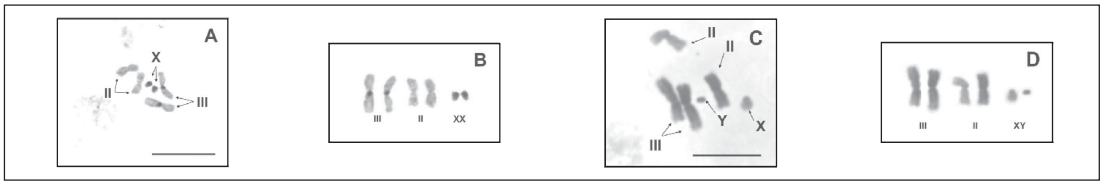

Figure 3. Karyotype of *Anopheles albitarsis*. **A.** Female metaphase neuroblast cells from Iranduba - AM. **B.** Karyotype. **C.** Male metaphase neuroblast cells from Coari - AM. **D.** Karyotype. Scale: 10 µm.

Morphometric sex chromosome and autosome measurements were obtained from brain ganglium cells from 10 metaphase samples of progeny of *A. albitarsis* from the three sites; the mean autosome anopheline sizes differed (Tables 1, 2 and 3). The mean lengths and mean arm ratios were higher for the three chromosome pairs in the Coari population (Table 3) than in the Ilha Comprida and Iranduba populations (Tables 1 and 2). The mean size of the X chromosome varied, the smallest being observed in Ilha Comprida (Table 1). The relative size of the Y chromosome was calculated as a proportion of the mean size of the X chromosome. The relative sizes for autosomes and X chromosomes were calculated according to the haploid genome size.

Table 1. Chromosome measurements (mean ± standard deviation) of 10 individuals of *Anopheles albitarsis* from Ilha Comprida, SP, according to Beçak (1967) and Levan et al. (1964).

Chromosomes	Average length (µm)	Average arm ratio	Relative length (%)	Classification
X	1.62 ± 0.41	-	13.4 ± 2.59	Acrocentric
Y	1.31 ± 0.22	-	10.65 ± 1.71	Punctiform
II	4.38 ± 1.02	1.09 ± 0.03	38.57 ± 2.23	Metacentric
III	5.41 ± 1.02	1.35 ± 0.06	48.02 ± 2.19	Submetacentric

Total length of the haploid genome: 11.41 ± 2.45.

Table 2. Chromosome measurements (mean ± standard deviation) of 10 individuals of *Anopheles albitarsis* from Iranduba, AM, according to Beçak (1967) and Levan et al. (1964).

Chromosomes	Average length (µm)	Average arm ratio	Relative length (%)	Classification
X	1.93 ± 0.3	-	13.1 ± 1.28	Acrocentric
Y	1.4 ± 0.28	-	10.34 ± 3.1	Punctiform
II	5.29 ± 1.11	1.05 ± 0.04	38.99 ± 1.84	Metacentric
III	6.5 ± 1.34	1.18 ± 0.07	47.82 ± 2.74	Submetacentric

Total length of the haploid genome: 13.73 ± 2.75.

Table 3. Chromosome measurements (mean ± standard deviation) of 10 individuals of *Anopheles albitarsis* from Coari, AM, according to Beçak (1967) and Levan et al. (1964).

Chromosomes	Average length (µm)	Average arm ratio	Relative length (%)	Classification
X	1.94 ± 0.42	-	11.1 ± 0.62	Acrocentric
Y	1.05 ± 0.13	-	10.65 ± 1.7	Punctiform
II	6.43 ± 1.75	1.09 ± 0.03	40.17 ± 1.62	Metacentric
III	7.78 ± 2.01	1.28 ± 0.04	48.73 ± 1.24	Submetacentric

Total length of the haploid genome: 16.15 ± 4.18.

The mean lengths (in micrometers) of the autosomal and sex chromosomes of the *A. albitarsis* samples from Ilha Comprida, Iranduba and Coari were compared by analysis of variance (ANOVA) and the *t*-test. There were no significant differences among the three populations.

DISCUSSION

Chromosomal karyotypes have been reported for some 20 genera and more than 300 species in the Culicidae (Rai 1963; White 1980; Rai and Black, 1999). The diploid chromosome number of all species examined has remained at six, despite the ancient origin of the Culicidae family and the incorporation of extensive chromosomal repatterning (Kumar and Rai, 1990; Mori et al., 1998). The only exception is *Chagasia bathana* (2n = 8), subfamily Anophelinae, which possesses three autosome pairs and a heteromorphic pair of sex chromosomes (Kreutzer, 1978); all other anopheline species possess two pairs of generally metacentric chromosomes of unequal size and one pair of heteromorphic sex chromosomes, X and Y (Kitzmiller, 1963; White, 1980).

Morphological data were obtained on the karyotypes (2n = 6); they had acrocentric X and Y punctiform chromosomes, metacentric (pair II) and submetacentric (pair III) autosomes. These characteristics have also been reported for *A. darlingi*, *A. aquasalis* (Frizzi and Ricciardi, 1955), *A. noroestensis*, *A. argyritarsis* (Schreiber and Guedes, 1959, 1961) from Minas Gerais, Brazil, *A.* (*Kerstezia*) *cruzii* from southeastern Brazilian populations (São Paulo) by Ramírez (1989) and *A. darlingi* from Macapá (Amapá) and Manaus (Amazonas) by Rafael and Tadei (1998).

As found in the males of *A. albitarsis*, punctiform sex (Y) chromosomes have been reported from other Brazilian *Anopheles* species: *A. quadrimaculatus*, *A. aquasalis* (Frizzi and Ricciardi, 1955), *A. noroestensis*, *A. argyritarsis* (Schreiber and Guedes, 1959, 1961), *A. cruzii* (Ramírez, 1989), *A. darlingi* and *A. nuneztovari* (Rafael and Tadei, 1998). The prometaphase and metaphase of the *A. albitarsis* samples that we studied showed somatic pairing. Traut et al. (1990) also reported somatic pairing to be a common phenomenon in *Diptera*. The same pattern is known for Culicidae chromosomes, such as *A. cruzii* (Ramírez and Dessen, 1994), *A. darlingi* and *A. nuneztovari* (Rafael and Tadei, 1998).

The size of *Anopheles* mitotic chromosomes varies (Rai and Craig, 1961; Kitzmiller, 1963; Rafael and Tadei, 1998). We found no significant size differences between the X chromo-

somes of the *A. albitarsis* populations compared to those of *A. darlingi* (Rafael and Tadei, 1998) and *A. cruzii* (Ramírez, 1989). The largest mean size of the three chromosome pairs of *A. albitarsis*, was found in the Coari population. The Ilha Comprida population had the smallest X chromosomes. However, the differences in chromosome size among these populations were not significant.

Significant chromosome size variation has not occurred during the evolution of the *A. albitarsis* complex, in contrast to *Chagasia bathana* species, an Anopheline subfamily with 2n = 8 chromosomes. Based on the variation we found in the mean chromosome size in the three populations of the *albitarsis* complex, selective pressure in these populations is apparently occurring only at a genetic level.

ACKNOWLEDGMENTS

The authors thank the Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM)/PIPT, and the Ministério da Ciência e Tecnologia (MCT) (grant PPI 3680 and the project PIATAM/CTPETRO) for financial support.

REFERENCES

Baimai V (1984). Review of the current situation regarding malaria vector species complexes and intraspecific variations in the following geographical area: South East Asia. In: Malaria vector complexes and intraspecific variations: relevance for malaria control and orientation for future research (Orni E and Muir D, eds.). UNDP/WORLD BANK/WHO, Bangkok, Thailand, pp. 35-47.

Baimai V, Treesucon A and Kijchalao U (1996). Heterochromatin variation in chromosome X in a natural population of *Anopheles willmori* (Diptera, Culicidae) of Thailand. *Genetica* 97: 235-239.

Beçak ML (1967). Cariótipos e evolução cromossômica em Amphibia - Anura. Doctoral thesis, Faculdade de Medicina de Ribeirão Preto, USP, Ribeirão Preto, SP, Brazil.

Coluzzi M (1988). Anopheline mosquitoes: genetic methods for species differentiation. In: Malaria. Principles and practice of malariology (Wernsdorfer WH and McGregors S, eds.). Churchill Livingstone, Great Britain, UK, pp. 411-430.

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

Conn JE, Wilkerson RC, Segura MNO, de Souza RL et al. (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.

Consoli R and Lourenço de Oliveira R (1994). Principais mosquitos de importância sanitária no Brasil. Ed. Fiocruz, Rio de Janeiro, RJ, Brazil.

Faran ME and Linthicum KJ (1981). A handbook of the Amazonian species of *Anopheles* (*Nyssorhynchus*) (Diptera: Culicidae). *Mosq. Syst.* 13: 1-91.

Forattini OP (1962). Entomologia médica. Faculdade de Higiene e Saúde Pública da USP, Editora da USP, São Paulo, SP, Brazil.

Frizzi G and Ricciardi I (1955). Introduzione allo studio citogenetico della fauna anofelica del Brasile. *Rev. Bras. Malariol. Doenças Trop.* 7: 399-407.

FUNASA - Fundação Nacional de Saúde (2000). http://www.funasa.gov.br. Accessed by Google.com.br - September 12, 2005.

Gorham JR, Stojanovich CJ and Scott HG (1967). Chave ilustrada para los mosquitos anofelinos de Sudamerica Oriental. Department of Health, Education and Welfare, Public Health Service, Washington, DC, USA, p. 64.

Imai HT, Taylor RW, Crosdland WJ and Crozier RH (1988). Modes of spontaneous chromosomal mutation and karyotype evolution in ants with reference to the minimum interaction hypothesis. *Jap. J. Genet.* 63: 159-185.

Kitzmiller JB (1963). Mosquito cytogenetics. A review of the literature, 1953-62. *Bull. WHO* 29: 345-355.

Klein TA, Lima JB and Toda-Tang A (1991). Hybridization evidence supporting separate species status for

Anopheles albitarsus and *Anopheles deaneorum* (Diptera: Culicidae) in Brazil. *J. Am. Mosq. Control Assoc.* 7: 301-303.
Kreutzer RD (1978). A mosquito with eight chromosomes: *Chagasia bathana* Dyar. *Mosq. News* 38: 554-558.
Kreutzer RD, Kitzmiller JB and Rabbani MG (1976). Cytogenetically distinguishable sympatric and allopatric populations of the mosquito *Anopheles albitarsis*. *Acta Amazonica* 6: 473-481.
Kumar A and Rai KS (1990). Chromosomal localization and copy number of 18S + 28S ribosomal RNA genes in evolutionarily diverse mosquitoes (Diptera, Culicidae). *Hereditas* 113: 277-289.
Levan A, Fredaga K and Sandberg AA (1964). Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201-220.
Mori A, Severson DW and Christensen BM (1999). Comparative linkage maps for the mosquitoes, *Culex pipiens* and *Aedes aegypti*, based on common RFLP loci. *J. Hered.* 90: 160-164.
Narang SK and Seawright JA (1993). *In situ* hybridization mapping of histone genes in *Anopheles albimanus*. *J. Am. Mosq. Control Assoc.* 9: 147-149.
Rafael MS and Tadei WP (1998). Metaphase karyotypes of *Anopheles* (*Nyssorhynchus*) *darlingi* Root and *A.* (*N.*) *nuneztovari* Gabaldón (Diptera; Culicidae). *Genet. Mol. Biol.* 21: 351-354.
Rai KS (1963). A comparative study of mosquito karyotypes. *Ann. Entomol. Soc. Am.* 56: 160-170.
Rai KS and Craig GB (1961). A study of karyotypes of some mosquitoes. *Genetics* 46: 891 (Abstract).
Rai KS and Black WC (1999). Mosquito genomes: structure, organization and evolution. *Adv. Genet.* 41: 1-33.
Ramírez CCL (1989). Estudo cromossômico em uma população de *Anopheles* (*Kerteszia*) *cruzii* Dyar & Knab, 1909. M.Sc. thesis, Inst. de Biociências, USP, São Paulo, SP, Brazil.
Ramírez CCL and Dessen EMB (1994). Cytogenetic analysis of natural population of *Anopheles cruzii*. *Rev. Bras. Genet.* 17: 41-46.
Rao PN and Rai KS (1987). Comparative karyotypes and chromosomal evolution in some genera of *Nematocerous* (Diptera, Nematocera) families. *Ann. Entomol. Soc. Am.* 80: 321-332.
Rosa-Freitas MG, Deane LM and Momen H (1990). A morphological, isoenzymatic and behavioural study of ten populations of *Anopheles* (*Nyssorhynchus*) *albitarsis* Lynch-Arribálzaga 1878 (Diptera: Culicidae) including from type-locality - Baradero, Argentina. *Mem. Inst. Oswaldo Cruz* 85: 275-289.
Schreiber G and Guedes AS (1959). Estudo comparativo do cromosoma X em algumas espécies de *Anopheles* do subgenus *Nyssorhynchus* (Diptera, Culicidae). *Cienc. Cult.* 11: 128-129.
Schreiber G and Guedes AS (1961). Cytological aspects of the taxonomy of anophelines (subgenus *Nyssorhynchus*). *Bull. WHO* 24: 657-658.
Segura MNO (1998). Estudo do *Anopheles* (*Nys*) *darlingi Root*, 1926 e *Anopheles* (*Nys*) *albitarsis Arribalzága*, 1878 (Diptera:Culicidae) como vetores de malária numa mesma área de transmissão, e caracterização de espécies do complexo *albitarsis*. M.Sc. thesis, Universidade Federal do Pará, Belém, PA, Brazil.
Steiner WWM, Narang S, Kitzmiller JB and Swofford DL (1982). Genetic divergence and evolution in Neotropical *Anopheles* (subgenus *Nyssorhynchus*). In: Recent developments in the genetics of insect disease vectors. Stipes Publishing L.L.C, Champaign, IL, USA, pp. 523-551.
Tadei WP and Dutary TB (2000). Malaria vectors in the Brazilian Amazon: Anopheles of the subgenus *Nyssorhynchus*. *Rev. Inst. Med. Trop. São Paulo* 42: 87-94.
Traut W, Khuong NT and Schneider S (1990). Karyotypes of *Megaselia scalaris* (Diptera) wild-type and translocation strains. *Genetica* 83: 77-84.
White GB (1980). Academic and applied aspects of mosquito cytogenetics. In: Insect cytogenetics (Blackman RL, Hewitt GM and Ashburner M, eds.). Blackwell Scientific Publications, London, England, pp. 245-274.
Wilkerson RC, Parsons TJ, Klein TA, Gaffigan TV et al. (1995a). Diagnosis by random amplified polymorphic DNA polymerase chain reaction of four cryptic species related to *Anopheles* (*Nyssorhynchus*) *albitarsis* (Diptera: Culicidae) from Paraguay, Argentina, and Brazil. *J. Med. Entomol.* 32: 697-704.
Wilkerson RC, Gaffigan TV and Lima JB (1995b). Identification of species related to *Anopheles* (*Nyssorhynchus*) *albitarsis* by random amplified polymorphic DNA-polymerase chain reaction (Diptera: Culicidae). *Mem. Inst. Oswaldo Cruz* 90: 721-732.