Analysis of ITS2 DNA Sequences from Brazilian *Anopheles darlingi* (Diptera: Culicidae)

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ABSTRACT Specimens of *Anopheles darlingi* Root, the major vector of malaria in Brazil, were collected from several states in Brazil: Sao Paulo (Dourado), Bahia (Itabela), Rondônia (Porto Velho), Roraima (Boa Vista), and Acre (Plácido de Castro). Sequence divergence in the 2nd internal transcribed spacer (ITS2) was examined. The ITS2 sequences of mosquitoes captured in the Amazon region (Porto Velho, Boa Vista and Plácido de Castro) and in the northeast of Brazil (Itabela) were almost identical; however, a 4–5% sequence divergence was observed in the ITS2 of mosquitoes captured in the southeast (Dourado). Further analysis is needed to determine if these differences indicate that Dourado population may be a separate species.

KEY WORDS *Anopheles darlingi*, second internal transcribed spacer, ribosomal DNA, polymorphism

MALARIA REMAINS A serious health problem in the Amazon Basin as a result of massive immigration by miners and by colonization of the forest close to the rivers where *Anopheles darlingi* Root mosquitoes are abundant (Walsh et al.1993, Tadei et al. 1998).

Anopheles darlingi is the primary malaria vector in Brazil (Forattini 1962, Deane 1988, Lourenço-de-Oliveira et al. 1989), and its geographic distribution extends from southern Mexico to northen Argentina (Faran and Linthicum 1981). Previous studies on the biology of this vector indicated that geographically distinct populations present different patterns of biting activity (Elliott 1972, Forattini 1987, Rosa-Freitas et al. 1992, Charlwood 1996). Rosa-Freitas et al. (1992) pointed out that this polymorphic behavior might not be a useful taxonomic character, because it is affected by seasonal variation and local environmental conditions.

Kreutzer et al. (1972) and Tadei et al. (1982) found that Amazonian populations of *An. darlingi* showed elevated polymorphism with a high frequency of inversion heterozygotes in salivary gland polytene chromosomes, whereas mosquitoes from Dourado, Sao Paulo State, were less polymorphic and appeared to show fixation of certain chromosomic arrangements.

Using cuticular hydrocarbons and isoenzymatic patterns, Sibajev-Freitas et al. (1995) distinguished *An. darlingi* captured in Dourado from those captured in Costa Marques, indicating intraspecific variation. Differences were not observed by analysis of mitochondrial DNA restriction sites.

The spacers of rDNA evolve at a faster rate than the coding sequences and the 2nd internal transcribed spacer (ITS2) has been used as a tool that distinguishes among some mosquito sibling species (Porter and Collins 1991, Paskewitz et al. 1993, Cornel et al. 1996). The purpose of our study was to compare ITS2 sequences obtained from *An. darlingi* specimens from several states of Brazil.

Materials and Methods

Mosquitoes. Adult females of *An. darlingi* species were collected during 1994–1998 at human bait in the states of Sao Paulo (Dourado, 22°06′ S, 48°19′ W), Rondônia (Porto Velho, 8°49′ S, 63°54′ W), Acre (Plácido de Castro, 10°20′ S, 67°11′ W), Roraima (Boa Vista, 2°49′ N, 60°1′ W), and Bahia (Itabela, 16°34′ S, 39°24′ W) (Fig. 1). Mosquitoes were identified to species with morphological keys (Faran and Linthicum 1981, Linthicum 1988) and stored at room temperature in isopropanol until genomic DNA extraction.

Cloning and Sequencing. Genomic DNA was extracted according to Moreira-Ferro et al. (1998) with some modifications. Individual mosquitoes were homogenized in 50 μl of lysis buffer (100 mM Tris-HCl, pH 7.5; 100 mM NaCl; 100 mM EDTA; 1% SDS; 1 mg/ml proteinase K) and incubated for 1 h at 60°C. Genomic DNA was extracted with phenol (2×), phenol/chloroform (1×), chloroform/isoamylic (1×) and precipitated by cold ethanol overnight. After centrifugation (12,000 × *g* for 5 min), the pellet was resuspended in 1 ml of TE (10 mM Tris, 1 mM EDTA) containing 10 mg/ml RNase. The DNA again was precipitated by addition of 20% (vol:vol) of sodium acetate 3 M and 1 ml of isopropanol. The suspension was kept for 15 min at room temperature and centrifuged (12,000 × *g* for 30 min).

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