Sequence Analysis of the Second Internal Transcribed Spacer of Ribosomal DNA in *Anopheles oswaldoi* (Diptera: Culicidae)

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J. Med. Entomol. 36(6): 679–684 (1999)

ABSTRACT Sequence divergence in the second internal transcribed spacer (ITS2) of ribosomal DNA was examined for female specimens of *Anopheles oswaldoi* Peryassu from 7 localities in South America. The lengths of ITS2 for all mosquitoes ranged from 348 to 356 nucleotides. After alignment of these sequences, similarity ranged from 87 to 100%. Divergence was within the range of interspecific differences for members of anopheline species complexes. Therefore, specimens were placed into 4 groups that may correspond to at least 4 cryptic species. One is probably related to *An. oswaldoi* sensu stricto and another to *Anopheles konderi* Galvão & Damasceno. The other 2 groups may correspond to species for which morphological identification remains to be clarified. These data provide evidence that *An. oswaldoi* comprise a complex of cryptic species and that DNA identification may help to resolve the taxonomic questions related to this group.

KEY WORDS *Anopheles (Nyssorhynchus) oswaldoi,* cryptic species, ribosomal DNA, second internal transcribed spacer, malaria vector

COMPLEXES OF CLOSELY related species of insects present a challenge to taxonomists, because distinction of members in a complex may explain differences in ecology, vectorial capacity, and response to control measures. The *Anopheles gambiae* complex, for instance, includes both vector and nonvector species of malaria parasites (Coluzzi et al. 1979). The methods used to detect and differentiate cryptic species include mating incompatibility, morphometric analysis, polythene chromosome analysis, isoenzymes electrophoresis (Coluzzi et al. 1979, Collins et al. 1988, Rosa-Freitas et al. 1990, Coetzee et al. 1993, Foley and Bryan 1993), and DNA sequence analysis (Collins et al. 1990; Conn et al. 1993; Paskewitz et al. 1993a, b; De Merida et al. 1995).

Among the analyzed DNA sequences, ribosomal DNA has been used successfully to address taxonomic questions (Collins et al. 1987, 1989; Porter and Collins 1991; Paskewitz et al. 1993a). In insects, rDNA genes are present in tandem repeat arrays separated by intergenic spacers. Each unit contains a conserved transcribed region, within which are the genes for the 18S, 5.8S, and 28S separated by 2 internal transcribed spacers (ITS1 and ITS2) (Beckingham 1982).

Because rDNA spacers evolve at a faster rate than the coding sequences, these regions may distinguish closely related species that otherwise show little genetic divergence (Tautz et al. 1987, Porter and Collins 1991). In mosquitoes, the ITS2 has been used to distinguish groups within closely related species, namely *An. maculipennis* complex (Porter and Collins 1991), *An. gambiae* complex (Collins et al. 1989; Paskewitz et al. 1993a), and *Culex pipiens* complex (Miller et al. 1996) and is becoming a general taxonomic tool to infer phylogenetic relationships (Wesson et al. 1992).

Differences among biting behaviors of *Anopheles oswaldoi* Peryassu captured at several localities have been reported (Rubio-Palis et al. 1992, Delgado and Rubio-Palis 1993). Furthermore, *An. oswaldoi* has been considered an important vector of malaria parasites at certain localities, whereas at others its role is secondary or unimportant (Hayes et al. 1987; Klein et al. 1991a, b; Rubio-Palis et al. 1992; Branquinho et al. 1993, 1996). These data suggest that *An. oswaldoi* may constitute a complex of cryptic species.

Klein et al. (1991a, b) found that in Rondônia, Brazil, only a very low percentage of *An. oswaldoi* females that fed on malaria patients developed salivary gland infections when compared with *An. darlingi.* In addition, *An. oswaldoi* is exophilic and zoophilic, is found more frequently in forest than peridomestic environments, and is consequently regarded as secondary or an unimportant vector in most of its territory (Lourenço-de-Oliveira et al. 1989; Oliveira-Ferreira et al. 1990; Klein et al. 1991a, b; Lourenço-de-Oliveira and Luz 1996).

In contrast, this species has been incriminated as an important malaria vector, associated with *P. vivax* 210 and 247 variant transmission in the Rio Ene Valley, Peru (Hayes et al. 1987, Need et al. 1993) as well as in localities in Venezuela (Rubio-Palis et al. 1992). In the

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