HYBRIDIZATION EVIDENCE SUPPORTING SEPARATE SPECIES STATUS FOR *ANOPHELES ALBITARSUS* AND *ANOPHELES DEANEORUM* (DIPTERA: CULICIDAE) IN BRAZIL[1, 2]

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Anopheles albitarsus sensu lato Lynch Arribalzaga represents a complex of 3–5 or more species (Kreutzer et al. 1976). According to Linthicum (1988), *An. albitarsus sensu stricto* is restricted to the southern temperate portion of Brazil, Paraguay, Uruguay and northern Argentina, although its true distribution is unknown. The remaining named members of the *An. albitarsus* complex are *An. deaneorum* Rosa-Freitas and *An. marajoara* Galvao and Damasceno (resurrected from synonymy), which has its type locality on the Island of Marajo, Para, Brazil. Preliminary observations indicate that *An. albitarsus* from Costa Marques is morphologically distinct from *An. marajoara*. Therefore, the usage of *An. albitarsus* from Costa Marques in this paper refers to *An. albitarsus sensu lato*.

Anopheles albitarsus is considered a primary vector of malaria in parts of Brazil. Because of the confusion of the taxonomic status of other forms in this complex, their vectorial capacity is questionable (Deane 1988). In Costa Marques, *An. albitarsus* and *An. deaneorum* are sympatric and have similar peaks of biting activity (Klein and Lima 1990).

Anopheles deaneorum, a recently described (Rosa-Freitas 1989) member of the *albitarsus* complex, was colonized (Klein et al. 1990) to provide specimens for extensive biosystematic studies. These studies, along with field efforts, were initiated to determine the taxonomic status, relative abundance, distribution and medical importance of *An. albitarsus* and *An. deaneorum* in Brazil. The hybridization aspects, reported below, were done to determine morphological variation and hybrid viability between the 2 species.

Hybridization experiments were performed at the U.S. Army Medical Research Unit-Brasilia field laboratory in Costa Marques, Rondonia, Brazil, using the colony of *An. deaneorum* and F_1 progeny of *An. albitarsus* (Costa Marques form). Adult *An. deaneorum* were collected on human bait in the city of Costa Marques while specimens of *An. albitarsus* were collected on human bait 7 km outside the city along the highway BR 429 (Klein and Lima 1990). The standard interspecies crosses with parental species as controls were used in the experimental crossing studies. Backcrosses of F_1 hybrids of both sexes to the parental species were done. Artificial mating techniques (Ow Yang et al. 1963) were necessary to maintain *An. deaneorum*. Since *An. albitarsus* rarely inseminated females by these techniques, F_1 progeny from wild collected females were used. The physical location, environmental conditions and the rearing procedures for the colonies were described by Klein et al. (1990). Experimental crosses were made in the same room housing the colony cages, and the test groups were kept in this room under identical laboratory conditions as those of the colonies.

Unmated females were placed in screened cages and provided with bloodmeals on human volunteers. Reciprocal crosses were attempted with engorged females and males of both species. Mated females were placed in screened pint cartons (10–20/carton) and provided a 5% sucrose solution in cotton. On the third day after forced copulation, a wing was removed and females placed in individual oviposition containers as described by Lanzaro et al. (1988). After oviposition, each female was dissected and the spermatheca examined for spermatozoa. Eggs from individual females were counted and placed in petri dishes for hatching. Larvae were counted daily and transferred to rearing pans on day 5, post-oviposition, until pupation. Pupae were removed daily and placed in individual rearing vials. Pupae that died prior to or during

[1] The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense (paragraph 4-3, AR 360-5).

[2] In conducting research described in this report, the investigators adhered to the guidelines of a human use protocol approved by the University of Brasilia and the U.S. Army Surgeon General's Committee for the use of humans as subjects of research.

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Table 1. Results of crossmating of *Anopheles albitarsus* and *Anopheles deaneorum*.

Crosses[1]		No. mated	No. (%) of ovipositions hatched	Mean no. eggs per oviposition	No. (%) of eggs hatched
Male	Female				
A	A	12	2/6 (33)	84	60 (12)
D	D	13	11/13 (85)	114	811 (54)
A	D	29	7/22 (32)	93	255 (12)
D	A	30	9/14 (64)	104	283 (19)
A	A × D	2	0/0 —	—	—
A	D × A	22	2/12 (17)	53	18 (7)
A × D	A	3	0/0 —	—	—
D × A	A	15	0/6 (0)	100	0 (0)
D	A × D	21	0/3 (0)	107	0 (0)
D	D × A	40	3/9 (33)	88	25 (4)
A × D	D	20	0/12 (0)	119	0 (0)
D × A	D	57	0/29 (0)	128	0 (0)

[1] A = *An. albitarsus*, D = *An. deaneorum*, and first letter in hybrid cross = male.

emergence were examined and recorded. Newly emerged F_1 adults were preserved with associated pupal skins, dissected to examine the reproductive organs or used in subsequent crossing studies.

Reciprocal crosses of parent generations produced viable F_1 hybrid progeny. Differences in larval development were not determined in parent crosses because of rearing problems (water source) that resulted in high larval mortality. However, sufficient larvae survived and completed development to permit backcross comparisons.

Results of the crossmating of *An. albitarsus* and *An. deaneorum* are shown in Table 1. In general, *An. deaneorum* males readily inseminate females of *An. deaneorum* (92%), and less frequently inseminate females of *An. albitarsus* (47%). *Anopheles albitarsus* males inseminated females of either *An. albitarsus* or *An. deaneorum* only about one-third of the time (Table 1). In addition, the spermatheca of females mated with *An. albitarsus* males frequently had smaller quantities of spermatozoa when compared with females mated with *An. deaneorum* males.

Hybrid males of *An. deaneorum* × *An. albitarsus* were sterile. Females mated with hybrid males either were not inseminated or their spermatheca (3%, 3/95) contained only a few spermatozoa. Dissection of a sample of the hybrid males (15) showed that the testes were abnormally small (the testes were not observed in dissections of >6 hybrid males) and the vas deferens was incompletely developed, narrow and easily broken during dissection.

Hybrid females appeared to develop normally, produced similar numbers of eggs as the parent crosses and produced progeny. However, the number of eggs hatching was very low for F_1 hybrid females mated with *An. albitarsus* (7%) and *An. deaneorum* (4%) males.

Characteristics of hybrid females were a combination of characters of both species, i.e., the wing scales were white, as in *An. albitarsus* and the dark band on the third hindtarsal segment was generally greater than 60%, similar to *An. deaneorum*. Wing spots were generally larger than those observed in *An. albitarsus*. These character intergrades were not observed in >2,000 wild-collected female mosquitoes, indicating that mating between the 2 species where their distributions are sympatric does not occur or is very rare.

The crossing experiments are evidence that hybrid males are sterile while reproduction is reduced in hybrid females. In addition, field collections indicate that there are natural mating barriers between species where populations are sympatric since hybrid characteristics were not observed.

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