Mem Inst Oswaldo Cruz, Rio de Janeiro, Vol. 90(5):575-582, sep./oct. 1995

The Identification of *Anopheles (Nyssorhynchus) rondoni* (Diptera: Culicidae) in Mato Grosso State, Brazil: An Analysis of Key Character Variability

Richard C Wilkerson/+, Lawrence J Hribar*, Eric G Milstrey, Guillermo Calderon Falero*****

Department of Entomology, Walter Reed Army Institute of Research Washington, DC 20307, USA *Florida Medical Entomology Laboratory, 200 9th St. SE, Vero Beach, FL 32962 **485th Medical Detachment, Fort Polk, LA 71459 ***Pan American Health Organization, Brasília, DF, Brasil

A morphological study was made of a population of Anopheles (Nyssorhynchus) rondoni *(Neiva and Pinto) from northern Mato Grosso, Brazil. This population usually lacked the primary key character of a dark basal band on hindtarsomere 3, i.e., hindtarsomere 3 was all white as in most other members of the subgenus. It was determined that this species can be recognized instead by the presence of a dark spot on the thorax made up of a large dark prescutellar space that is contiguous with a concolorous central area on the scutellum. A secondary character of a dark area on the costa created by the fusion of the humeral dark, presector dark and sector dark proximal spots is also usually reliable. Regression analyses comparing the lengths and ratios of the dark bands on hindtarsomeres 2 to those on 3 describe a straight line relationship. This suggests that the "atypical" population is at one end of a character gradient. We propose that in the subgenus* Nyssorhynchus *individuals that have a long basal band on hindtarsomere 2 are more likely to also have a basal band on hindtarsomere 3. The pupal stage of this species has not been previously described. Reared-associated specimens from this study show that the pupa can be easily differentiated from all other* Nyssorhynchus *by the relatively stout, usually 2 or 3 branched (1-5), setae 1 and 5 on segments IV-VII.*

Key words: *Anopheles (Nyssorhynchus) rondoni* - key characters - identification - pupa

In spite of two recent revisions (Faran 1980, Linthicum 1988), identification of females of *Anopheles (Nyssorhynchus)* species is often difficult. This is because of an incomplete understanding of inter- and intraspecific variability, because of unresolved species complexes and because of lack of study specimens from all parts of species ranges. To identify the highly variable taxa belonging to this subgenus, it is therefore appealing to rely on presumably unambiguous "key" characters. One such character is the presence of a basal dark band on hindtarsomere 3 of *An. (Nys.) rondoni* (Neiva and Pinto). With the exception of *An. (Nys.) nigritarsis* Chagas and other naturally occurring variants (discussed below), this tarsomere is usually entirely white in subgenus *Nyssorhynchus*. We found that this "key" character was uncommon in a population of *An. (Nys.) rondoni* from Peixoto de Azevedo, Mato Grosso State, Brazil, and that this would likely lead to misidentification of these specimens as *An. (Nys.) benarrochi* Gabaldón, Cova Garcia and Lopez.

We offer here an analysis of this and other characters used to distinguish this species and suggest an alternative means for identification. Numerical analysis showed a positive correlation between the extent of basal dark scales on hindtarsomere 2 and those on hindtarsomere 3. This allows us to predict that the occurrence of a dark band on hindtarsomere 3 is more likely in species that have a longer band on hindtarsomere 2. Also, this study provided the first examples of the pupa of this species for future morphological analysis.

MATERIALS AND METHODS

Female *Anopheles* were collected from human bait at Peixoto de Azevedo, Mato Grosso, Brazil on 20 April, 1993 by two of us (E.G.M. and G.C.F.). These females were allowed to feed and then transported to the US Army Research Unit in Rio de Janeiro (USAMRU-B). They were maintained in a humid environment and traumatically induced to lay eggs by removal of a wing after 48-72 hr. Larvae were reared at approximately 25°C. Two or three adults of each sex with associated larval

Partial support for this work was provided by NIH grant AI-31034 to L. Philip Lounibos, Florida Medical Entomology Laboratory, University of Florida, Vero Beach. The views of the authors do not purport to reflect the views of the supporting agencies.

+Corresponding author. Present address: Museum Support Center, Smithsonian Institution, Washington, DC 20560

Received 5 January 1995
Accepted 12 May 1995

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
JAN 1995		**00-00-1995 to 00-00-1995**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
The Identification of Anopheles (Nyssorhynchus) rondoni (Diptera: Culicidae) in Mato Grosso State, Brazil: An Analysis of Key Character Variability	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Walter Reed Army Institute of Research,Department of Entomology,Washington,DC,20307**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**Same as Report (SAR)**	**8**	

and pupal exuviae (Peixoto F1 females and males) from each of 26 progeny broods and their female parents (Peixoto P1 females) were retained for morphological study. A similar number of larvae and adults of both sexes were frozen for molecular studies. Specimens are deposited in the National Museum of Natural History (NMNH), Smithsonian Institution, Washington, DC.; U.S. Army Medical Research Unit, Rio de Janeiro; Instituto Oswaldo Cruz, Rio de Janeiro; and the Núcleo de Pesquisa Taxonômica e Sistemática em Entomologia Médica, at the Universidade de São Paulo. The Peixoto de Azevedo specimens bear the reference code BR Rio 020 followed by the progeny brood number in parentheses, e.g. BR Rio 020 (19). The following progeny broods are included in this study: (19-21), (25-45), (48) and (53). Other specimens studied are older holdings of the National Museum of Natural History, Smithsonian Institution (NMNH specimens). They are from Corrientes and Jujuy Provinces, Argentina and, São Paulo State and Costa Marques, Rondônia State, Brazil. Except for specimens from the latter locality they were used by Faran (1980) in developing his descriptions and keys.

Measurements were made using an SMZ-10 microscope with a camera lucida and a Summagraphics SummaSketch Model MM1201 using "INPAD" software written by Joseph Russo (Office of Information Management, Smithsonian Institution).

Wing spot names have been converted from the names used by Faran (1980) to the system proposed by Wilkerson and Peyton (1990), abbreviated as follow (Fig. 1): prehumeral dark (PHD); humeral pale (HP); humeral dark (HD); presector pale (PSP); presector dark (PSD); sector pale (SP); sector dark (SD) [the sector dark is divided into two spots, the sector dark proximal (SDP) and sector dark distal (SDD) when an accessory sector pale (ASP) spot is present]; subcostal pale (SCP); and preapical dark (PD). Equivalent names in Faran (1980) are: basal dark = PHD; humeral pale = HP; subbasal dark = HD; subbasal pale = PSP; presectoral dark = PSD; presectoral pale = SP; sectoral dark = SDP; sectoral pale = ASP; subcostal dark = SDD; and preapical dark = PD. Vein nomenclature follows Harbach and Knight (1980). Veins M_{1+2}, M_1, and M_2 correspond to veins M, M_{1+2} and M_{3+4} in Faran (1980)

We observed the presence/absence of the above wing spots and measured their lengths. We also measured the lengths of hindtarsomeres 2 and 3 and lengths of the basal bands on hindtarsomeres 2 and 3. We then calculated ratios of HP to PHD, SCP to SDD, and proportion of hindtarsomeres 2 and 3 dark. All ratios were transformed by $X' = (X + 3/8)^{1/2}$ (Kihlberg et al. 1972) prior to analysis. Possible differences among NMNH specimens, Peixoto P1 females and F1 males and F1 females were assessed by analysis of variance (SAS GLM procedure), and mean separations were performed by the Ryan-Einot-Gabriel-Welsch multiple range test. Differences in frequency of missing wing spots PSP, SP, ASP and dark or light scaling of wing vein M_1 were analyzed by applying the likelihood ratio chi-square (SAS FREQ procedure) to 2 X 4 contingency tables. This test was chosen due to small cell sizes for some of the variables. The number of observations for a given group (e.g., Peixoto P1 females) was not equal for all characters due to damage to specimens, rubbing of wing spots, etc. All statistical analyses were conducted with the aid of the Statistical Analysis System (SAS Institute 1985). Ratios of basal dark bands on hindtarsomeres 2 and 3 were calculated and plotted.

Several morphological characters were used to verify that the 26 progeny broods from Mato Grosso State, Brazil, were *An. (Nys.) rondoni*. The male genitalia of an individual from each brood was examined and found to agree with Faran's (1980) genitalia characters, i.e., the ventral lobes with setae usually on the basal lobule not extending to the base of the apicolateral lobe and the preapical plate very weakly sclerotized and ill-defined. The male genitalia of *An. (Nys.) strodei* Root is quite similar but has the setae of the ventral lobes on the lateral margins usually reaching the base of the apicolateral lobe and the preapical plate is weakly to moderately sclerotized and moderately well-defined. *Anopheles (Nys.) rondoni* is different from *strodei* in two other characters. First, the pale wing scales are white to occasionally white mixed with very pale yellow in *An. (Nys.) strodei* but pale yellow or pale yellow with white on the costal veins in *An. (Nys.) rondoni*. Second, *rondoni* exhibits a distinctive, contrasting, dark prescutellar space, a character not known to us in *strodei*, or any other species of the subgenus.

RESULTS

This study evaluates characters used by Faran (1980) to identify *An. (Nys.) rondoni*. His key characters were: (1) hindtarsomere 3 dark in basal 0.20-0.35; (2) vein C predominantly dark (Fig. 1B), HD, PSD and SDP spots fused into one large spot (i.e. PSP and SP spots missing); and (3) PD of vein M_{1+2} extending uninterrupted onto vein M_1. We evaluated these and other characters to test the likelihood of misidentification of *An. (Nys.) rondoni* which lack a band on hindtarsomere 3.

Hindtarsomeres 2 and 3 (Table I) - Faran (1980) reported that the proportion of basal dark on

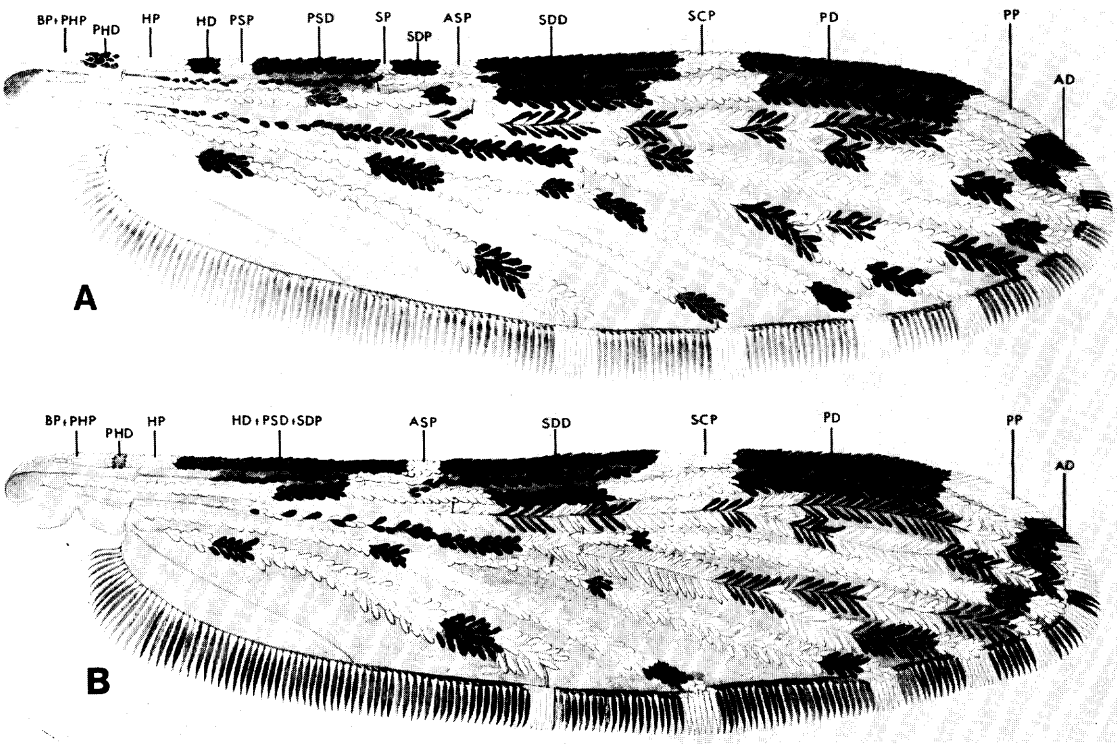

Fig. 1: comparison of the wings of *Anopheles* (*Nys.*) *dunhami* Causey (A) and *An.* (*Nys.*) *rondoni* (B). BP = basal pale; PHP = prehumeral pale; PHD = prehumeral dark; HP = humeral pale; HD = humeral dark; PSP = presector pale; PSD = presector dark; SP = sector pale; SDP = sector dark proximal; ASP = accessory sector pale; SDD = sector dark distal; SCP = subcostal pale; PD = preapical dark; PP = preapical pale; AD = apical dark.

hindtarsomere 2 ranged from 0.65-0.85 and for hindtarsomere 3, 0.20-0.35. Many of the specimens used in Faran's study, and a few additional specimens, were available to us. Among these we found similar proportions; 0.64-0.89 for hindtarsomere 2 and 0.19-0.42 for hindtarsomere 3. All had a basal band on hindtarsomere 3. Specimens from the Peixoto de Azevedo population had less dark on hindtarsomere 2 than the above: F1 females, 0.42-0.77; F1 males, 0.39-0.74; and P1 females 0.47-0.69. The percent having a basal band on hindtarsomere 3 was: females, 29%; males, 27% and P1 females, 19%. For the Peixoto specimens with a band on hindtarsomere 3, the mean was: F1 females, 0.12; F1 males, 0.14; and P1 females, 0.11. F1 females, both with and without the hindtarsomere 3 band, gave rise to progeny in all combinations, both with and without bands. The absence of the band was therefore not correlated with a subset of families, a possible indication of a separate taxon. There were significant differences (Table II) between all Peixoto specimens and NMNH specimens in lengths of dark bands on both hindtarsomeres 2 and 3 and in the relative proportions of dark. Absolute lengths of these tarsomeres differed among progeny, mothers, and NMNH specimens. The basal dark bands on hindtarsomeres 2 and 3 are more similar in size for NMNH specimens than for Peixoto specimens. A regression analysis of the ratios of dark on hindtarsomere 2 compared to dark on hindtarsomere 3 (those with-

TABLE I

Basal dark band on hindtarsomeres 2 and 3, *Anopheles (Nys.) rondoni,* National Museum of Natural History specimens (NMNH) and male and female progeny of wild caught females from Peixoto de Azevedo, Mato Grosso, Brazil

	Range Mean	STD	Legs/ individuals	%With band
Hindtarsomere 2				
NMNH females	0.64-0.890.77	0.07	30/27	100%
Peixoto P1 females	0.47-0.690.57	0.05	24/24	100%
Peixoto F1 females	0.42-0.770.59	0.66	97/49	100%
Peixoto F1 males	0.39-0.740.59	0.07	92/47	100%
Hindtarsomere 3				
NMNH females	0.19-0.420.29	0.05	30/26	100%
Peixoto P1 females	0.0-0.190.11[a]	0.05[a]	24/24	19%
Peixoto F1 females	0.0-0.220.12[a]	0.06[a]	96/49	29%
Peixoto F1 males	0.0-0.270.14[a]	0.07[a]	92/47	27%

[a]: of those with a band

TABLE II

Morphological characters of museum specimens, wild-caught mothers, and female progeny of *Anopheles (Nys.) rondoni*. Means ± 1 standard error within rows followed by the same letter are not different (Ryan-Einot-Gabriel-Welsch multiple range test)

	Peixoto F1 females	Peixoto F1 males	Peixoto P1 females	NMNH females	F value	df	P
[1]HT 3 dark (mm)	0.021 ± 0.005^{b}	0.023 ± 0.004^{b}	0.015 ± 0.007^{b}	0.216 ± 0.010^{a}	198.25	3, 238	0.0001
HT 2 dark (mm)	0.511 ± 0.006^{b}	0.498 ± 0.006^{b}	0.522 ± 0.013^{b}	0.811 ± 0.026^{a}	145.03	3, 239	0.0001
[2]Ratio HT 3 dark	0.116 ± 0.009^{b}	0.140 ± 0.011^{b}	0.112 ± 0.026^{b}	0.291 ± 0.010^{a}	63.22	3, 85	0.0001
Ratio HT II dark	0.594 ± 0.009^{b}	0.594 ± 0.007^{b}	0.569 ± 0.011^{b}	0.768 ± 0.018^{a}	62.85	3, 239	0.0001
HT 3 length (mm)	0.603 ± 0.005^{c}	0.600 ± 0.004^{c}	0.640 ± 0.013^{b}	0.737 ± 0.013^{a}	53.28	3, 238	0.0001
HT 2 length (mm)	0.861 ± 0.006^{c}	0.838 ± 0.005^{c}	0.919 ± 0.014^{b}	1.050 ± 0.019^{a}	93.65	3, 239	0.0001
PHD (mm)	0.039 ± 0.003^{b}	0.034 ± 0.003^{b}	0.065 ± 0.005^{a}	0.036 ± 0.006^{b}	3.31	3, 215	0.021
SCP (mm)	0.187 ± 0.005^{a}	0.151 ± 0.003^{b}	$0.173 \pm 0.009^{a,b}$	0.145 ± 0.014^{b}	8.53	3, 238	0.0001
DSD (mm)	$0.642 \pm 0.006^{b,c}$	0.625 ± 0.006^{c}	0.679 ± 0.009^{b}	0.754 ± 0.035^{a}	15.20	3, 202	0.0001

1: includes those without a band on hindtarsomere 3
2: includes only those with a band on hindtarsomere 3

TABLE III

Frequency tabulation of presence or absence of wing spots; presector pale (PSP), sector pale (SP), accessory sector pale (ASP) and preapical dark spot of vein M_{1+2} with or without pale scales at base of M_1

	PSP		SP		ASP		M_1	
	Present	Absent	Present	Absent	Present	Absent	Pale scaled	All dark
NMNH females	6(20%)	24(80%)	0	30(100%)	8(26.7%)	22(73.3%)	8(27.6%)	21(72.4%)
Peixoto P1 females	0	20(100%)	1(5%)	19(95%)	21(100%)	0	5(41.7%)	7(58.3%)
Peixoto F1 females	13(13.3%)	85(86.7%)	1(1.1%)	97(98.9%)	92(93.9%)	6(6.1%)	45(47.9%)	49(52.1%)
Peixoto F1 males	4(4.3%)	90(95.7%)	0	94(100%)	91(96.8%)	3(3.2%)	54(62.1%)	33(37.9%)

out a band not included) (Fig. 2) of all groups resulted in a straight line described by the formula: Y = -0.46 + 0.93X. Regression analysis of the actual measurements of basal dark also resulted in a straight line described by the formula Y = -0.19 + 0.50X (not shown). Both suggest a continuous character gradient.

Vein C (Table III) - Faran (1980) described vein C as predominantly dark because of the fusion of the subbasal, presectoral and sectoral dark spots (= HD, PSD and SDP spots), i.e., the PSP and SP spots were missing.

There were significantly different percentages of Peixoto F1 females, F1 males, P1 females, and NMNH specimens lacking the PSP ($X^2 = 12.178$, df = 3, $P < 0.014$) and ASP ($X^2 = 79.018$, df = 3, $P < 0.0001$) wing spots. No differences in frequencies of missing wing spots among female

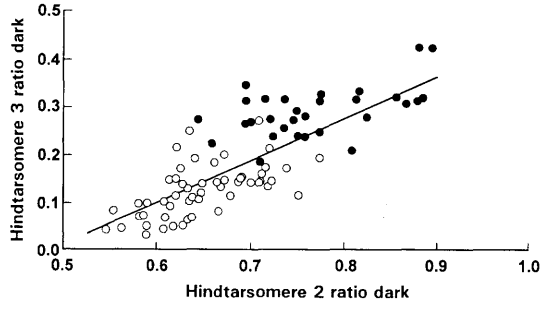

Fig. 2: comparison of the proportion of basal dark scales on hindtarsomere 2 to the proportion of basal dark scales on hindtarsomere 3 of *Anopheles* (*Nys.*) *rondoni*. Regression line is described by the formula Y = -0.46 + 0.93X. Open circles are specimens from Peixoto de Azevedo, Mato Grosso, Brazil; closed circles are specimens primarily from northern Argentina, in text referred to as NMNH specimens. Specimens which lacked a basal band on hindtarsomere 3 are not included.

TABLE IV

Presence and/or absence of selected pale costal wing spots in *Anopheles (Nys.) rondoni* from specimens in National Museum of Natural History, Smithsonian Institution (NMNH) and from Peixoto de Azevedo, Mato Grosso, Brazil

PSP	SP	ASP	NMNH females	Peixoto F1females	Peixoto P1 mothers	Peixoto F1 males
0	0	0	19(63.3%)[3]	6(6.1%)	0	3(3.2%)[1]
0	0	1	5(17.7%)	78(79.6%)[7]	19(95%)	87(92.6%)[5]
0	1	1	0	1(1)	1(5%)[1]	0
1	0	0	3(10%)[1]	0	0	0
1	0	1	3(10%)	13(13.3%)	0	4(4.3%)
0	1	0	0	0	0	0
1	1	1	0	0	0	0
1	1	0	0	0	0	0
Wings			30	98	20	94
Individuals			26	49	20	47

0 = absent; 1 = present; number (percent) [no. also lacking subcostal pale spot]; PSP = presector pale spot; SP = sector pale spot; ASP = accessory sector pale spot

progeny, male progeny, mothers, and museum specimens were found for the SP spot ($X^2 = 5.329$, df = 3, $P < 0.149$).

Of the eight possible combinations of these three pale spots (Table IV) the majority of wings from Peixoto de Azevedo (85.7% of F1 females, 95.8% of F1 males and 95% of P1 females) exhibited an absence of the PSP and SP spots (classes 000 and 001); most specimens, but not all, therefore agreeing with Faran (1980). Among the NMNH specimens, 81% agreed with Faran (1980) (63.3% were missing all three spots, 17.7% had the ASP), the remainder did not agree. Few Peixoto specimens (6.1% of females and 2.1% of males) had all three pale spots missing. Some individuals, in addition to missing most or all of the above pale spots, also lacked the subcostal pale (SCP), thus giving the costa of the wing an even darker appearance. Overall, the NMNH specimens were therefore darker on this part of the wing than the Peixoto population.

The occurrence of pale spots appears to be non-random since several classes of spots were rare or did not occur. For instance, PSP and SP absent, ASP present (001) is the most common class for Peixoto specimens but PSP absent, SP present, ASP absent (010) was not represented, even though both classes have only one of the spots missing.

The ratio of the HP to the PHD spots and the ratio of the SCP to the SDD spots (Table V) were also calculated because of their usefulness in distinguishing other Oswaldoi Subgroup species. The PHD spot was missing or relatively short in comparison to the HP in *An. (Nys.) rondoni*, which is consistent with Faran (1980); however, the ranges of both the NMNH specimens and Peixoto specimens for the ratio HP/PHD, 1.95 - 10.48 and 1.04 - 14.2 respectively, exceed that given by Faran (1980) (2.0-3.3). The SCP is relatively short or missing, also consistent with Faran's (1980) figure of *rondoni*. With only one exception, the SCP is less than 0.5 the size of the DSD. A specimen with a SCP that is >0.5 of the DSD could be interpreted as being *An. (Nys.) rangeli* Gabaldón, Cova Garcia and Lopez.

No statistically significant differences between groups were found for the following variables: ratio of HP and PHD spots ($F = 1.08$; df = 3, 154; P

TABLE V

Ratios of the prehumeral dark spot to the humeral pale spot (PHD/HP) and subcostal pale spot to the distal sector dark spot (SCP/DSD) in *Anopheles (Nys.) rondoni* from specimens in the National Museum of Natural History, Smithsonian Institution (NMNH) and from Peixoto de Azevedo, Mato Grosso, Brazil (020)

	Range	Mean	STD	No. wings
HP/PHD				
NMNH females	1.95-10.48	3.94	1.87	19
Peixoto P1 females	1.78-5.76	3.06	1.16	8
Peixoto F1 females	1.42-10.24	4.11	1.79	70
Peixoto F1 males	1.04-14.2	4.35	2.32	61
SCP/SDD				
NMNH females	0.16-0.33	0.25	0.06	8
Peixoto P1 females	0.14-.35	0.26	0.06	21
Peixoto F1 females	0.09-.52	0.30	0.07	91
Peixoto F1 males	0.09-0.47	0.26	0.08	86

> 0.3557); ratio of SCP and SDD wing spots (F = 4.55; df = 3, 202; P > 0.05); and length of HP wing spot (F = 0.70; df = 3, 154; P > 0.5563).

Preapical dark spot (PD) of vein M - Faran (1980) noted that *An. (Nys.) rondoni* differed from other species in the Oswaldoi Group by having no pale spot on M_{1+2} and M_1 where M_{1+2} branches into M_1 and M_2. We found this to be partially true (Table III). If this character is strictly interpreted then there should not be pale scales on M_1 where it branches from M_{1+2}. We found pale scales on the posterior portion of M_1 in a large proportion of our sample. However, we never noted pale scales on the anterior portion of this vein. A survey of other *Nyssorhynchus* in the collection of the NMNH showed that the pale spot on both the anterior and posterior portions of the vein usually to be present; but specimens similar to *rondoni* with dark scales on the anterior part of the wing were found in *An. (Nys.) strodei* of the Albimanus Section, and *An. (Nys.) marajoara* Galvão and Damasceno and *An. (Nys.) braziliensis* (Chagas) of the Argyritarsis Section. A significant frequency of a darker M_1 (all dark vs dark anterior with pale scales on posterior portion of the vein) was found in the museum specimens compared to Peixoto mothers and progeny (X^2 = 11.383, df = 3, P < 0.01).

Prescutellar space - In Faran's (1980) discussion and in the original description (Neiva & Pinto 1922) it is noted that the presence of a very dark brown to black, subtriangular, prescutellar space distinguishes this species. This dark area is contiguous with a concolorous central area on the scutellum, and together form a conspicuous spot (Fig. 3). We found this character to be present in all *An. (Nys.) rondoni* examined and absent in other *Nyssorhynchus*. In addition, the central dark area on the scutellum lacks scales, a condition not known to us in other *Nyssorhynchus*.

Pupal characters - The pupa of *An. (Nys.) rondoni* has not been described and the larva is poorly known. Material studied here includes associated pupal and larval exuviae which will allow for future morphological descriptions. The pupa is quite distinct from all other known *Nyssorhynchus* and can be recognized by the relatively stout, usually 2 or 3 (1-5) branched setae 1 and 5 on segments IV-VII.

DISCUSSION

The discovery of a population of *An. (Nys.) rondoni* not exhibiting the primary "key" character of a dark basal band on hindtarsomere 3 requires a reevaluation of characters used to recognize this species. If a specimen of *rondoni* has a basal band on hindtarsomere 3 it can be easily identified, unless one of the variants of other Albimanus Section species, that also have this band, are encountered at the same time (see below).

The two other characters used by Faran (1980)

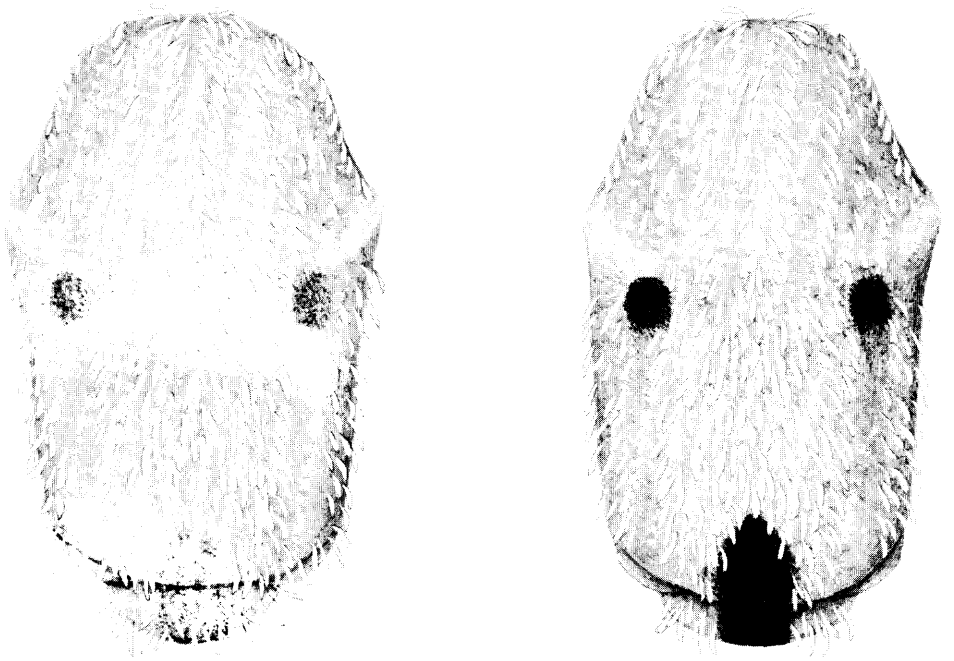

Fig. 3: scutum and scutellum of *Anopheles (Nys.) strodei* (A) and *An. (Nys.) rondoni* (B). Note the darker and larger prescutellar space and the lack of median scales on the scutellum in the latter.

in his key to separate *An. (Nys.) rondoni* from other members of the Oswaldoi Group are likewise not constant. In his key one could be led to an identification of *An. (Nys.) aquasalis* Curry or *An. (Nys.) benarrochi* with the following combination of characters: hindtarsomere 3 pale, PSP or SP present, M_1 with pale scales, base of hindtarsomere 2 0.3-0.6 dark, and SCP less than 0.5 length of the SDD. *Anopheles (Nys.) aquasalis* can be eliminated on the basis of various characters found in the description, and because it is not known to be sympatric with *An. (Nys.) rondoni*. *Anopheles (Nys.) benarrochi*, though, is similar in many ways and occurs with *rondoni*. We suggest that the best character for recognition of adults of *rondoni* therefore is the large, dark, prescutellar space and contiguous dark area, lacking scales, on the scutellum (Fig. 3). The secondary key character of a dark area on vein C resulting from the fusion of the HD, PSD and SDP was also usually in agreement with Faran (1980).

The case of *An. (Nys.) rondoni* individuals lacking a basal dark band on hindtarsomere 3 is similar to instances of variability in other *Nyssorhynchus* species which, instead, gain bands on hindtarsomere 3 and sometimes 4. Varieties, species or variants have been recognized in the following: *An. (Nys.) nigritarsis* (a valid species with diagnostic characters of basal bands on hindtarsomeres 3 and 4); *An. (Nys.) albimanus* Wiedemann (vars. *bisignatus* Hoffman and *trisignatus* Hoffmann) [Hoffmann 1938, Rozeboom 1963, Faran 1980]; *An. (Nys.) aquasalis* vars. *guarauno* Anduze and *delta* Anduze and the synonym *deltaorinoquensis* Cova Garcia, Pulido F. and Amanista M.) [Anduze 1948, Faran 1980]; *An. (Nys.) strodei* (variant) [Rachou and Ferraz 1951]; *An. (Nys.) triannulatus* (Neiva and Pinto) (synonym *Cellia cuyabensis* [Neiva and Pinto]) [Pinto 1938, Galvão and Lane 1941]; *An. (Nys.) darlingi* Root (variant) [Harbach et al. 1993]; and *An. (Nys.) albitarsis* (synonym *imperfectus* Correa and Ramos) [Correa and Ramos 1943, Galvão 1943]. In all of the above, the band on hindtarsomere 2 is relatively long compared to other *Nyssorhynchus* species. They are as follow (after Faran 1980 and Linthicum 1988): *An. (Nys.) strodei*, 0.30 - 0.50; *An. (Nys.) darlingi*, 0.35 - 0.55; *An. (Nys.) albitarsis*, 0.60 - 0.90; *An. (Nys.) albimanus*, 0.40 - 0.80; *An. (Nys.) triannulatus*, 0.40 - 0.70; and *An. (Nys.) aquasalis*, 0.30 - 0.60. One might predict that the extra band on hindtarsomere 3 will be found in other species with a relatively long hindtarsomere 2 band, e.g. *An. (Nys.) galvaoi* Causey, Deane and Deane, *An. (Nys.) benarrochi*, *An. (Nys.) pictipennis* (Philippi) or *An. (Nys.) deaneorum* Rosa-Freitas. Regression analysis of the ratios of dark on hindtarsomere 2 vs hindtaromere 3, and regression analysis of the actual measurements, supports the hypothesis that it is more likely that there will be a dark basal band on hindtarsomere 3 if a larger proportion of hindtarsomere 2 is dark (Fig. 2).

The statistical comparisons between Peixoto de Azevedo and NMNH specimens show some significant differences, i.e., incidence of PSP and ASP spots, dark scales on M_1, and the lengths and proportions of the basal dark on hindtarsomeres 2 and 3. The differences in these morphological characters could be evidence of separate species, but they probably simply represent seasonal or geographic variation. Any attempt to establish separate species status for the Peixoto specimens must await further collections, since the Peixoto and NMNH collections are almost at opposite ends of the known distribution of the species. The differences in tarsomere banding may be an expression of some pressure that is either seasonal or geographic (Le Sueur & Sharp 1991, Kitthawee et al. 1992, Le Sueur et al. 1992) which contribute to darkening on the wing as well.

If keys for the identification of *Nyssorhynchus* species rely only on leg characters, it would be possible to assign "nontypical" specimens to the wrong subgenus, i.e. Argyritarsis Section species with basal bands on hindtarsomeres 2-5 could be mistaken for subgenus *Kerteszia*. We do not believe this to be a problem, however, since numerous other characters exist to separate *Nyssorhynchus* from *Kerteszia* (Peyton et al. 1992). Linthicum (1988) uses the acrotstichal and dorsocentral areas having numerous scales to separate *Nyssorhynchus*, and Wilkerson and Strickman (1990) use scutal markings and wing spots.

This report of a common, easily misidentified variant of what formerly was considered to be a readily identifiable species, exemplifies problems of identification of species in the subgenus *Nyssorhynchus*. Relatively little is known about inter- and intraspecific variation in *Nyssorhynchus*, or how to identify the numerous cryptic species. Without an accurate understanding of these taxa, results of epidemiological, ecological and control studies can be easily confused. This is a clear illustration of the need for further extensive collection and analysis of the species in the subgenus *Nyssorhynchus*, whose member species are responsible for the majority of malaria parasite transmission in the Neotropics.

ACKNOWLEDGMENTS

To José Bento Lima, for laboratory assistance in Brazil. To Taina R Litwak for the preparation of Figs 1 and 3, Bonnie K Pattok for preparing Fig. 2 and Jayson I Glick, E L Peyton and Terry A Klein for their helpful reviews of the manuscript.

REFERENCES

Anduze PJ 1948. Notas entomologicas I. Dos variedades nuevas de *A. aquasalis*. *Bol Med* (Caracas) *1*: 17-19.

Correa RR, Ramos AS 1943. Descrição de uma nova sub-espécie de anofelino do sub-genero *Nyssorhynchus* Blanchard, 1902 (Diptera, Culicidae). *Rev Paulista Med 22*: 246-248.

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

Galvão ALA 1943. Chaves para a determinação das espécies do subgenero *Nyssorhynchus* do Brasil. *Arq Hig Saúde Pública 8*: 141-162.

Galvão ALA, Lane J 1941. Observações sôbre alguns anofelinos de Salobra, Mato Grosso (Diptera, Culicidae). *Rev Biol Hyg 11*: 10-18.

Harbach RE, Knight KL 1980. *Taxonomists' glossary of mosquito anatomy*. Plexus, Marlton, N.J.

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

Hoffmann CC 1938. La formación de razas en los *Anopheles* mexicanos. II. *Anopheles albimanus* y sus variedades en la Republica Mexicana. *An Inst Biol Univ Nac Auton Mex 9*: 167-180.

Kihlbeg JK, Herson JH, Schultz WE 1972. Square root transformation revisited. *J Roy Statist Soc Ser C Appl Statist 21*: 76-81.

Kitthawee S, Edman JD, Upatham ES 1992. *Anopheles dirus* size and fecundity: relationship to larval density and protein accumulation. *Southeast Asian J Trop Med Public Health 23*: 128-131.

Le Sueur D, Sharp BL 1991. Temperature-dependent variation in *Anopheles merus* larval head capsule width and adult wing length: implications for adult taxonomy. *Med Vet Entomol 5*: 55-62.

Le Sueur D, Sharp BL, Appleton CC 1992. Dark-scaled areas on adult *Anopheles* mosquitoes are selectively affected by temperature-related size variation. *Med Vet Entomol 6:* 396-398.

Linthicum KJ 1988. A revision of the Argyritarsis Section of the subgenus *Nyssorhynchus* of *Anopheles* (Diptera: Culicidae). *Mosq Syst 20*: 99-271.

Neiva A, Pinto C 1922. Contribuição para o conhecimento das anophelinas do estado de Matto Grosso com a descripção de uma nova espécie. *Braz - Med 36:* 321-322.

Peyton EL, Wilkerson RC, Harbach RE 1992. Comparative analysis of the subgenera *Kerteszia* and *Nyssorhynchus* of *Anopheles* (Diptera: Culicidae). *Mosq Syst 24*: 51-69.

Pinto C 1938. Disseminação da malaria pela aviação; biologia de *Anopheles gambiae* e outros anofelinos do Brasil. *Mem Inst Oswaldo Cruz 34*: 293-430.

Rachou RG, Ferraz DM 1951. Contribuição ao conhecimento da distribuição geografica dos anofelinos no Brasil: estado de Santa Catarina. *Rev Bras Malariol Doencas Trop 3*: 540-554.

Rozeboom LE 1963. Mutant forms of *Anopheles albimanus* Wiedemann (Diptera: Culicidae). *Proc Entomol Soc Wash 65*: 110-114.

SAS Institute. Inc 1985. *SAS user's guide: statistics*. Ver. 5th edition. Cary, NC.

Wilkerson RC, Strickman D 1990. Illustrated key to the female anopheline mosquitoes of Central America and Mexico. *J Am Mosq Control Assoc 6*: 7-34.

Wilkerson RC, Peyton EL 1990. Standardized nomenclature for the costal wing spots of the genus *Anopheles* and other spotted-wing mosquitoes (Diptera: Culicidae). *J Med Entomol 27*: 207-224.