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IDENTIFICATION OF *ANOPHELES* (*NYSSORHYNCHUS*) (DIPTERA: CULICIDAE) OCCURRING IN WESTERN VENEZUELA

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ABSTRACT. Adult females of *Anopheles* (*Nyssorhynchus*) species are difficult to identify in the field using available keys. Entomological studies of vector species and subsequent evaluation of malaria control programs are mainly based on the collection of adult *Anopheles*, hence a reliable means for identification of adult specimens in the field is required. A dichotomous key based on reared adults with associated immature stages was developed to identify field-collected adult females belonging to the subgenus *Nyssorhynchus* from a vivax-malaria endemic area in western Venezuela.

INTRODUCTION

The subgenus *Nyssorhynchus* Blanchard is restricted to the Neotropics (except for *Anopheles albimanus* Wiedemann, which extends into the Nearctic) and contains most of the important vectors of malaria parasites of the region. This subgenus has been found in the past to be taxonomically complicated and has been the subject of recent revision (Faran 1980, Linthicum 1988, Peyton et al. 1992). Faran (1980) subdivided the subgenus *Nyssorhynchus* into two sections, the Albimanus and Argyritarsis sections. These sections are distinguished in the adult primarily by the presence or absence of a basal dark band on hindtarsomere 5 and in the male genitalia by the variously developed, fused ventral claspette. Faran (1980) divided the Albimanus Section into groups, the Albimanus (one species) and Oswaldoi (13 species) groups. He further divided the latter into two subgroups, the Triannulatus (one species) and Oswaldoi (12 species) subgroups.

Previous authors expressed different opinions concerning the most reliable characters for identifying species of the Oswaldoi Subgroup. For instance, Pintos et al. (1968) considered eggs the only means of reliable identification of the females of this group, and, until the present work, all identifications in western Venezuela have been based on eggs. According to Faran (1980), the most reliable characters for species identification are in the morphology of male genitalia and the chaetotaxy of the fourth-instar larva, because the external morphology of the adult female and pupa, particularly in the Oswaldoi Subgroup, is "very similar interspecifically, and usually quite variable intraspecifically."

Identification of species within the Oswaldoi Subgroup from our study area using available keys proved to be very difficult. The supposedly distinctive taxonomic characters were found to be highly variable, and there were many specimens that could not be identified with the keys (Delgado and Rubio-Palis 1992). It was also found that the eggs were highly variable and were not reliable for species identification in the field. In order to determine diagnostic characters that would allow us to identify females in the field, morphometric studies based on associated rearings from field-collected specimens were undertaken.

MATERIALS AND METHODS

The study area and villages have been described previously by Rubio-Palis and Curtis

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Table 1. Mean values and ranges for length of dark band on hindtarsomere 2 divided by length of hindtarsomere 2 in wild-caught and reared females compared with ranges reported by Faran (1980).

Species		n	Mean[1]	SD	Range	Range in Faran (1980)
An. nuneztovari	wild-caught	764	0.270a	0.039	0.11–0.44	0.20–0.32
	reared	162	0.241a	0.095	0.19–0.43	
An. rangeli	wild-caught	31	0.250a	0.036	0.14–0.31	0.24–0.35
	reared	7	0.252a	0.120	0.25–0.38	
An. oswaldoi	wild-caught	43	0.170b	0.034	0.11–0.22	0.12–0.45
	reared	5	0.135b	0.016	0.12–0.19	
An. triannulatus	wild-caught	48	0.391c	0.055	0.20–0.49	0.40–0.70
	reared	96	0.363c	0.036	0.33–0.43	

[1] Means followed by different letters differ at the $P < 0.05$ level of significance.

(1992b), and a detailed description of collection and rearing methods is in Delgado and Rubio-Palis (1992). The determination of diagnostic characters was identical to that previously described. Measurements were made using an American Optical dissecting microscope at 10–60× magnification with an Olympus bifurcated fiber optic light as the source of illumination. A standard white color was established as a reference for determining other colors according to the method of Peyton and Ramalingam (1988). This was accomplished by using 60× magnification to position the light source so that a white surface appeared as white as possible. Among the species collected, the whitest structures were hindtarsomeres 2 and 3. The color of the tarsomeres was compared with that of the pale spots on the wing (E.L. Peyton, personal communication; Wilkerson and Strickman 1990). The morphological terms and abbreviations used follow Harbach and Knight (1980, 1982) and Wilkerson and Peyton (1990).

RESULTS AND DISCUSSION

During this study, specimens of the following species of subgenus *Nyssorhynchus* were collected: *An. albitarsis* Arribálzaga *s.l.* (318), *An. triannulatus* (Neiva and Pinto) (48), *An. benarrochi* Gabaldón, Cova García and López (1), *An. oswaldoi* (Peryassú) (43), *An. rangeli* Gabaldón, Cova García and López (31), *An. nuneztovari* Gabaldón (764), and *An. strodei* Root (4). From these, the following reared adults were obtained: *An. nuneztovari* (162), *An. oswaldoi* (5), *An. rangeli* (7), and *An. triannulatus* (96).

Anopheles albitarsis s.l. was not included in the morphometric study because it was the only species belonging to the Argyritarsis Section collected in the study area and it could be identified using the key by Faran and Linthicum (1981) (= *An. allopha* Peryassú). Voucher specimens examined by M. G. Rosa-Freitas ruled out the possibility of having *An. deaneorum* Rosa-Freitas, a sister species of *An. albitarsis,* in our study area.

Tables 1–3 show the mean values of the ratio for the characters measured in wild-caught females and associated rearings. In general there was a slight difference between the ranges recorded for wild-caught females and those for associated rearings. Statistical analysis of the data was carried out for the wild-caught females because the sample size was larger.

The mean values for the length of the dark band on hindtarsomere 2 divided by the length of hindtarsomere 2 (TaD/Ta) (Table 1) for *An. nuneztovari* ($\bar{x} = 0.270$) and *An. rangeli* ($\bar{x} = 0.250$) are not significantly different ($P > 0.05$), whereas the means of TaD/Ta for *An. oswaldoi* ($\bar{x} = 0.170$) and *An. triannulatus* ($\bar{x} = 0.391$) were significantly different ($P < 0.05$). This character can be used to separate the latter two species. In *An. nuneztovari,* the range of variation for this character is large, overlapping the range of the other species (Fig. 1A). The mean values are similar to those reported by Faran (1980) ex-

Table 2. Mean values and ranges for length of subcostal pale spot divided by length of sector dark spot in wild-caught and reared females compared with ranges reported by Faran (1980).

Species		n	Mean[1]	SD	Range	Range in Faran (1980)
An. nuneztovari	wild-caught	764	0.375a	0.100	0.10–0.60	0.20–0.55
	reared	162	0.384a	0.117	0.03–0.73	
An. rangeli	wild-caught	31	0.627b	0.107	0.53–0.80	0.45–1.00
	reared	7	0.549b	0.123	0.50–0.80	
An. oswaldoi	wild-caught	43	0.255c	0.110	0.11–0.44	0.10–0.50
	reared	5	0.269c	0.005	0.27–0.31	
An. triannulatus	wild-caught	48	0.153d	0.034	0.07–0.23	0.10–0.20
	reared	96	0.142d	0.014	0.12–0.17	

[1] Means followed by different letters differ at the $P < 0.05$ level of significance.

cept for *An. triannulatus,* which has a range smaller than that reported by Faran (1980) (Table 1). The material examined by Faran (1980) from Venezuela included specimens collected in central and eastern Venezuela. San Carlos, the closest location to our study site, is approximately 400 km away. These locations have different ecological conditions. The same is true for the material examined from Colombia. Further studies are required to clarify this situation.

Mean values of the ratio length of subcostal pale (SCP) spot divided by length of sector dark (SD) are presented in Table 2. In *An. nuneztovari,* this character is highly variable (Fig. 1B). However, the mean values for all four species are significantly different ($P < 0.05$) (Table 2). Differences in ranges allow this character to be used to separate *An. nuneztovari* and *An. rangeli.* In general, the ranges found for these species are within those reported by Faran (1980) except for *An. nuneztovari,* which has a wider range.

Mean values for the ratio length of humeral pale spot (HP) divided by length of prehumeral dark spot (PHD) were significantly large ($P < 0.05$) in *An. rangeli* and *An. oswaldoi* but small in *An. triannulatus* and *An. nuneztovari* (Table 3, Fig. 1C). Cova García and Sutil (1977) and Faran (1980) reported that in *An. nuneztovari* the proportion of HP to PHD was 1:1. However, we found that this character showed a high coeficient of variation (43.8%) (Sokal and Rohlf 1969) in wild-caught females (n = 764), with values ranging between 0.65 and 4.50, and that only 63% of specimens had a proportion of 1:1.

A key was developed based on measurements specified above in about 1,500 specimens, including wild-caught *Anopheles* and associated, reared material. The key to some extent follows that of Faran and Linthicum (1981).

Species identification using this key during a longitudinal study on vector biology and vivax-malaria transmission in the same area

Table 3. Mean values and ranges for length of humeral pale spot divided by length of prehumeral dark spot in wild-caught and reared females compared with ranges reported by Faran (1980).

Species		n	Mean[1]	SD	Range	Range in Faran (1980)
An. nuneztovari	wild-caught	764	1.093a	0.221	0.65–4.50	0.70–1.70
	reared	162	1.625a	0.606	0.61–4.00	
An. rangeli	wild-caught	31	2.573b	0.699	2.00–4.50	1.80–3.50
	reared	7	1.941b	0.207	1.80–2.50	
An. oswaldoi	wild-caught	43	2.097c	0.620	1.00–3.50	1.10–3.80
	reared	5	2.133b	0.467	1.67–2.60	
An. triannulatus	wild-caught	48	0.750d	0.386	0.40–1.10	0.50–1.30
	reared	96	0.622c	0.152	0.50–1.00	

[1] Means followed by different letters differ at the $P < 0.05$ level of significance.

was satisfactory (Rubio-Palis 1992a, 1992b; Rubio-Palis and Curtis 1992a, 1992b; Rubio-Palis et al. 1992, 1993). Furthermore, species identification was confirmed by their biting activity (Rubio-Palis 1992b, Rubio-Palis and Curtis 1992b).

KEY TO THE FEMALES OF *ANOPHELES* (*NYSSORHYNCHUS*) FROM WESTERN VENEZUELA

1. Hindtarsomeres 3 and 4 (Ta-III_{3-4}) with pale and dark bands or mostly dark (Fig. 2A) (subgenera *Anopheles, Lophopodomyia, Kerteszia,* and *Stethomyia*)
- Hindtarsomeres 3 and 4 entirely pale (Fig. 2B) (subgenus *Nyssorhynchus*) 2
2. Hindtarsomere 5 (Ta-III_5) entirely white (Fig. 2C) (Argyritarsis Section) 3
- Hindtarsomere 5 with a basal dark band (Fig. 2D) (Albimanus Section) 4
3. Tergum II (Te-II) with dark caudolateral scale tufts (Fig. 3A); hindtarsomere 2 (Ta-III_2) with basal dark band 0.3–0.4 length of tarsomere ..... *braziliensis*
- Tergum II without dark caudolateral scale tufts (Fig. 3B); hindtarsomere 2 with basal dark band 0.5–0.7 length of tarsomere (Fig. 2C) ... *albitarsis*
4. Mesepimeron with a conspicuous anterior patch of pale scales (Fig. 3C); foretarsomere 4 (Ta-I_4) with a pale band on apical 0.40–0.65 (Fig. 3E); hindtarsomere 2 (Ta-III_2) with basal dark band 0.20–0.49 length of tarsomere (Fig. 2E); humeral pale spot (HP) on costa 0.40–1.10 length of prehumeral dark spot (PHD) (Fig. 4A) ..... *triannulatus*
- Mesepimeron without an anterior patch of pale scales (Fig. 3D); foretarsomere 4 predominantly dark (Fig. 3F); hindtarsomere 2 with basal dark band variable; humeral pale spot on costa 0.7–2.5 length of prehumeral dark spot; pale scales on wing white to bright yellow 5
5. Hindtarsomere 2 (Ta-III_2) with basal dark band more than 0.4 length of tarsomere ..... *benarrochi*
- Hindtarsomere 2 with basal dark band less than 0.4 length of tarsomere 6
6. Hindtarsomere 2 (Ta-III_2) with basal dark band 0.11–0.22 length of tarsomere (Fig. 2F); humeral pale spot (HP) 1.0–3.5 length of prehumeral dark spot (PHD); subcostal pale spot (SCP) 0.11–0.44 length of sector dark spot (SD) (Fig. 4B) ..... *oswaldoi*
- Hindtarsomere 2 with basal dark band 0.24–0.35 length of tarsomere (Fig. 2G), or if less, humeral pale spot less than 0.9 length of prehumeral dark spot; subcostal pale spot more than 0.35 length of sector dark spot 7

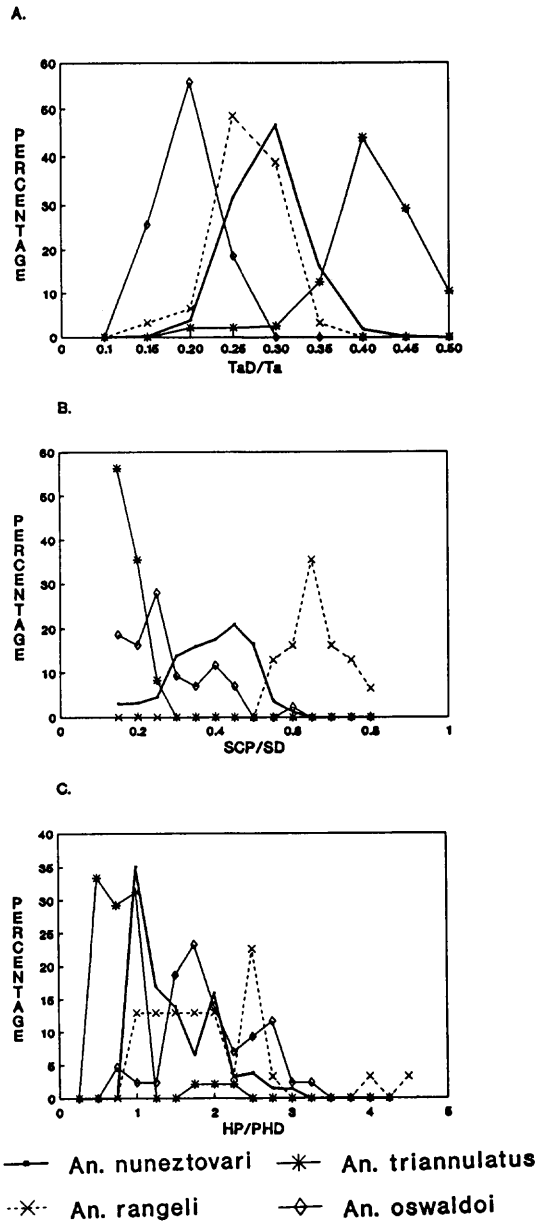

Fig. 1. Frequency distributions. A, The length of the dark band on hindtarsomere 2 (TaD) divided by the length of hindtarsomere 2 (Ta); B, the length of the subcostal pale spot (SCP) divided by the length of the sector dark spot (SD); C, the length of the humeral pale spot (HP) divided by the length of the prehumeral dark spot.

7. Subcostal pale spot (SCP) more than 0.50 (0.53–0.80) length of sector dark spot (SD); humeral pale spot (HP) more than 1.8 length of prehumeral dark spot (PHD) (Fig. 4C) .. *rangeli*
- Subcostal pale spot less than 0.50 length of sector dark spot (Fig. 4D); humeral pale spot

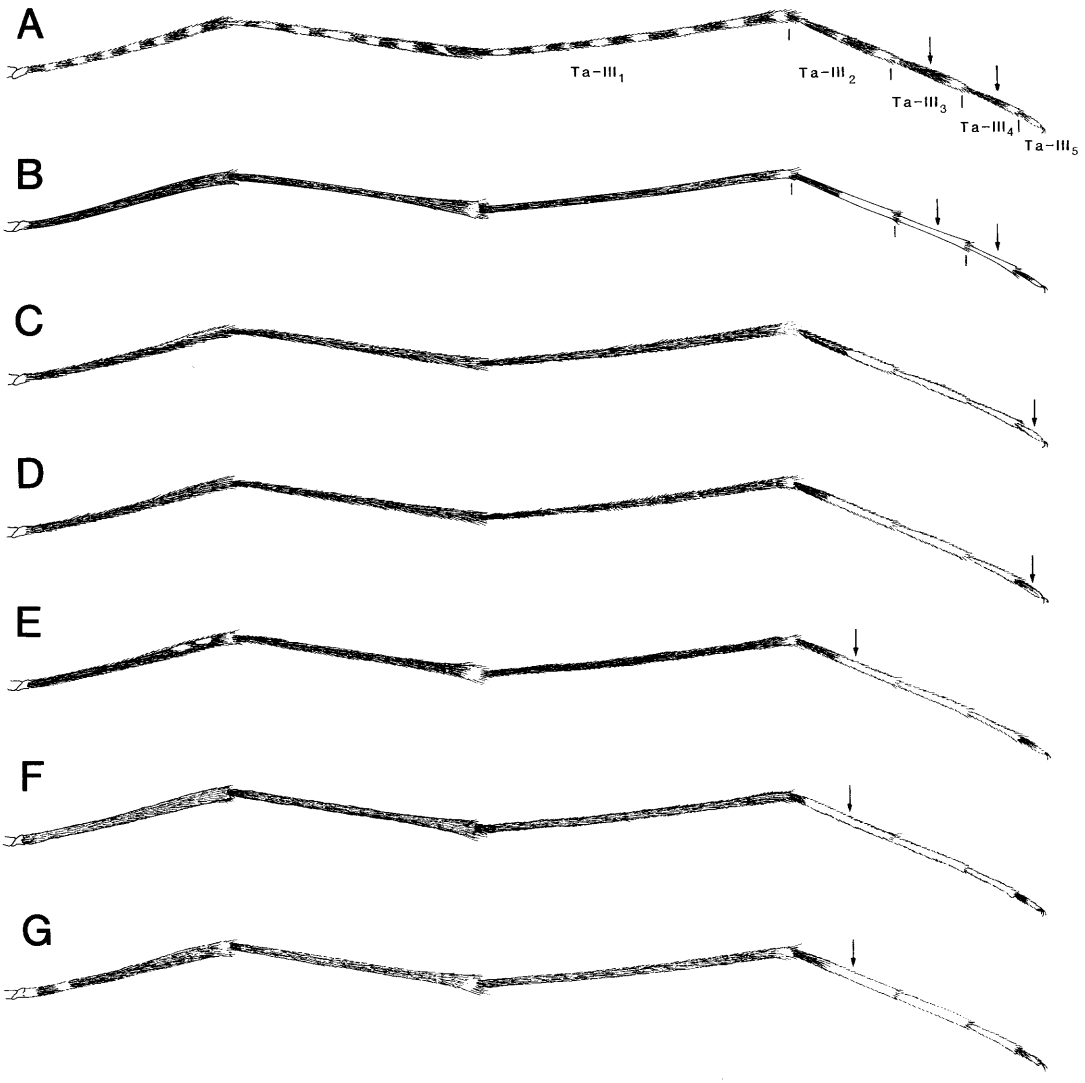

Fig. 2. A, Subgenus *Anopheles,* hindtarsomeres 3 and 4 with pale and dark band; B, subgenus *Nyssorhynchus,* hindtarsomeres 3 and 4 entirely pale; C, Argyritarsis Section, hindtarsomere 5 entirely white; D, Albimanus Section, hindtarsomere 5 with a basal dark band; E, *An. triannulatus,* note hindtarsomere 2; F, *An. oswaldoi,* basal dark spot of hindtarsomere 2 0.11–0.22 length of tarsomere; G, *An. nuneztovari,* basal dark spot of hindtarsomere 2 variable (0.24–0.35 length of tarsomere).

variable, 0.7–4.5 length of prehumeral dark spot; if length of subcostal pale spot is more than 0.50, then humeral pale spot is less than 1.8 length of prehumeral dark spot 8

8. Humeral pale spot (HP) variable, 0.7–4.5 length of prehumeral dark spot (PHD) (Fig. 5A,B,C); humeral crossvein (h) may or may not touch apex of prehumeral dark spot (PHD); pale spots on wing variable in color, cream to bright yellow ..... *nuneztovari*

– Humeral pale spot more than 2.5 length of prehumeral dark spot; humeral crossvein does not touch apex of prehumeral dark spot; pale spots on wing white ..... *strodei*

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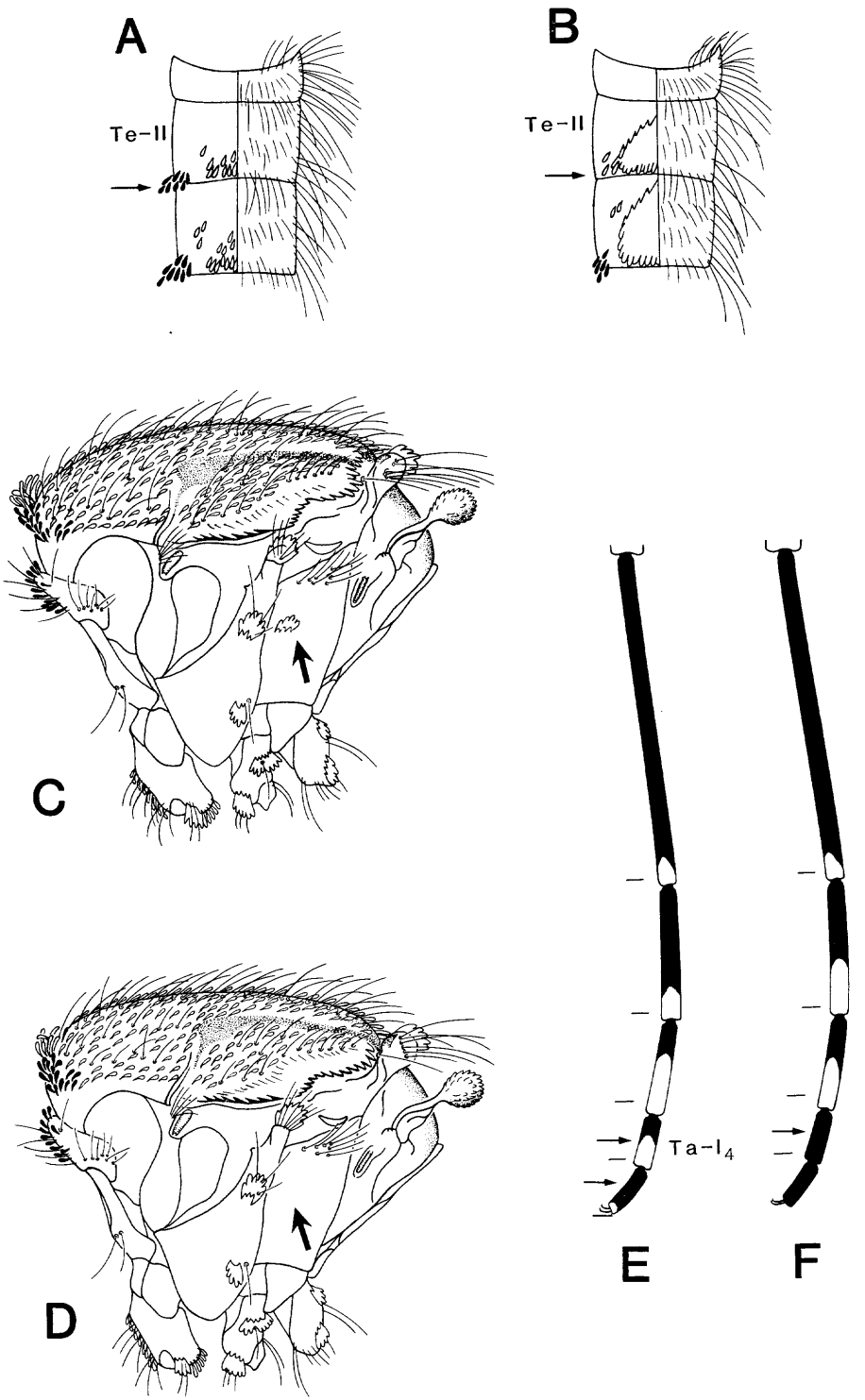

Fig. 3. A,B, Abdomens, dorsal views, of *An. braziliensis* (A) and *An. albitarsis* (B); C,D, thoraces, lateral views, showing mesepimeron with a conspicuous anterior patch of scales (C) and without an anterior patch of scales (D); E,F, foretarsi, (E) foretarsomere 4 with a light band on apical 0.40–0.65 and (F) foretarsomere 4 dark (drawings from Faran and Linthicum 1981).

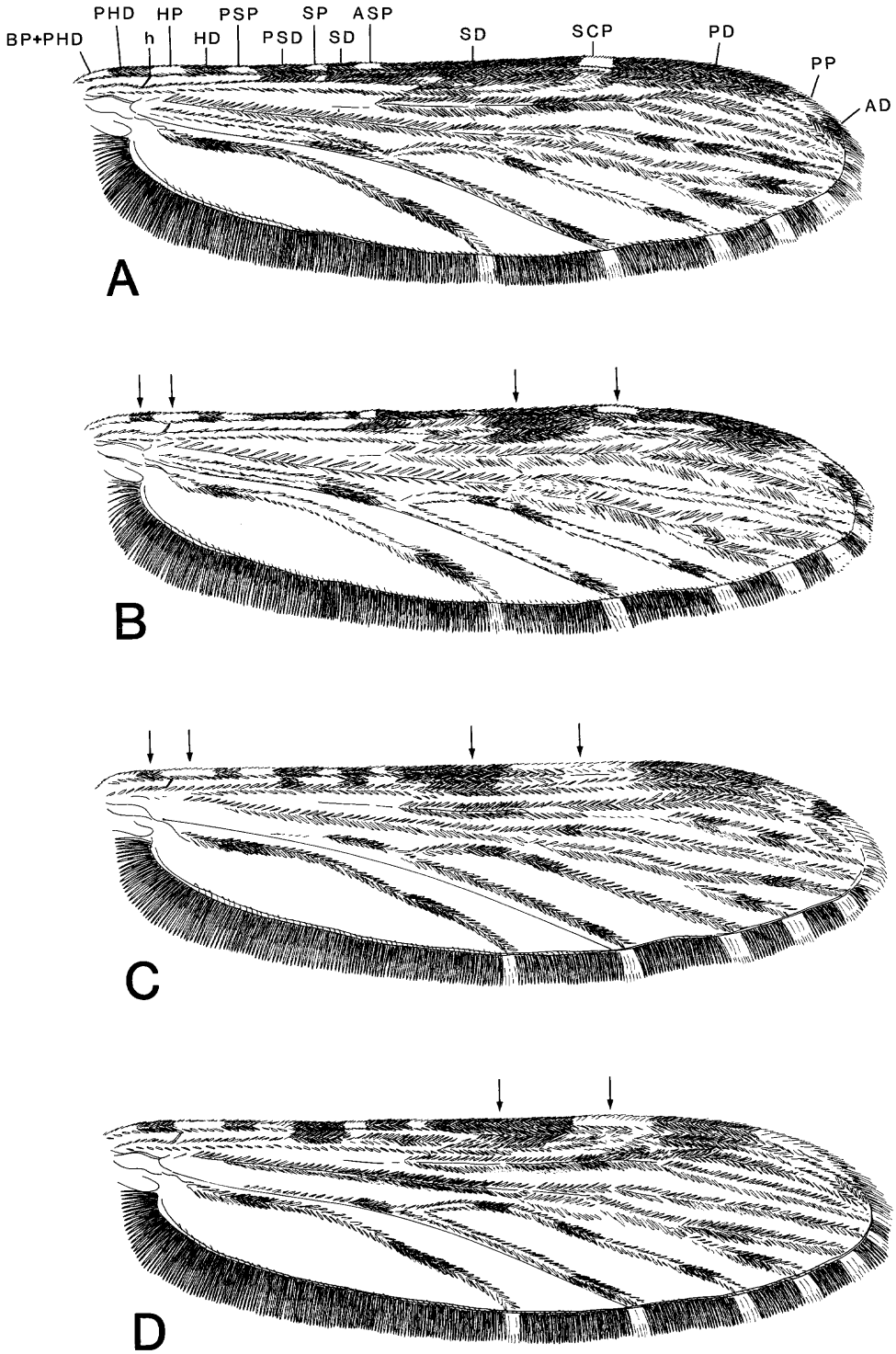

Fig. 4. Wings. A, *An. triannulatus*; B, *An. oswaldoi*; C, *An. rangeli* with subcostal pale spot more than 0.50 (0.53–0.80) length of sector dark spot; D, *An. nuneztovari* with subcostal pale spot less than 0.50 length of sector dark spot.

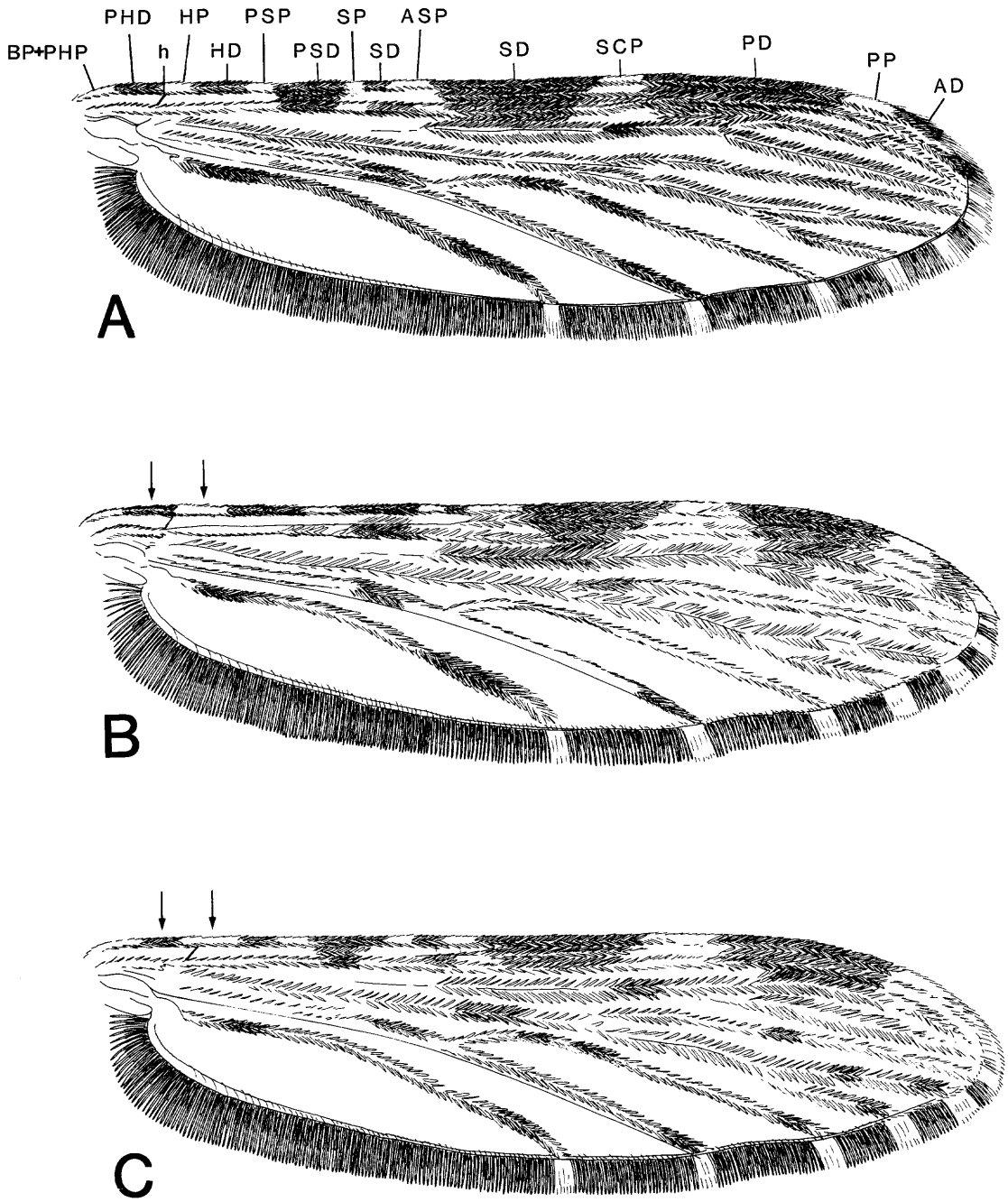

Fig. 5. Wings of *An. nuneztovari* showing the range of variation in the length of the humeral pale spot.

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REFERENCES CITED

Cova García, P. and E. Sutil. 1977. Claves gráficas para la clasificación de anofelinos de Venezuela. Publicación de la División de Endemias Rurales, Dirección de Malariología y Saneamiento Ambiental, Ministerio de Sanidad y Asistencia Social, Maracay, Venezuela.

Delgado, N. and Y. Rubio-Palis. 1992. Morphometric characterization of the malaria vector *Anopheles nuneztovari* (Diptera: Culicidae) from western Venezuela. Mosq. Syst. 24:231–241.

Faran, M.E. 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(7):1–214.

Faran, M.E. and K.J. Linthicum. 1981. A handbook of the Amazonian species of *Anopheles* (*Nyssorhynchus*) (Diptera: Culicidae). Mosq. Syst. 13:1–81.

Harbach, R.E. and K.L. Knight. 1980. Taxonomists' glossary of mosquito anatomy. Plexus Publishing, Inc., Marlton, NJ.

Harbach, R.E. and K.L. Knight. 1982. Corrections and additions to *Taxonomists' glossary of mosquito anatomy*. Mosq. Syst. (1981) 13:201–217.

Linthicum, K.J. 1988. A revision of the Argyritarsis Section of the subgenus *Nyssorhynchus* of *Anopheles* (Diptera: Culicidae). Mosq. Syst. 20:101–271.

Peyton, E.L. and S. Ramalingam. 1988. *Anopheles* (*Cellia*) *nemophilous*, a new species of the Leucosphyrus Group from peninsular Malaysia and Thailand (Diptera: Culicidae). Mosq. Syst. 20:272–299.

Peyton, E.L., R.C. Wilkerson and R.E. Harbach. 1992. Comparative analysis of the subgenera *Kerteszia* and *Nyssorhynchus* of *Anopheles* (Diptera: Culicidae). Mosq. Syst. 24:51–69.

Pintos, P., H. Sabril and V. López. 1968. Esporozoitos en *Anopheles* (*N.*) *nuñeztovari* en área de malaria refractaria. Bol. Dir. Malariol. Saneamiento Ambiental 8:375–381.

Rubio-Palis, Y. 1992a. Influence of moonlight on light trap catches of the malaria vector *Anopheles nuneztovari* (Diptera: Culicidae) in Venezuela. J. Am. Mosq. Control Assoc. 8:178–180.

Rubio-Palis, Y. 1992b. Abundancia y actividad hematofágica de *Anopheles rangeli, An. strodei* y *An. neomaculipalpus* en el occidente de Venezuela. Bol. Dir. Malariol. Saneamiento Ambiental 32:59–67.

Rubio-Palis, Y. and C.F. Curtis. 1992a. Evaluation of different methods of catching anopheline mosquitoes in western Venezuela. J. Am. Mosq. Control Assoc. 8:261–267.

Rubio-Palis, Y. and C.F. Curtis. 1992b. Biting and resting behaviour of anophelines in western Venezuela and implications for control of malaria transmission. Med. Vet. Entomol. 6:325–334.

Rubio-Palis, Y., R.A. Wirtz and C.F. Curtis. 1992. Malaria entomological inoculation rates in western Venezuela. Acta Trop. 52: 167–174.

Rubio-Palis, Y., R.A. Wirtz and C.F. Curtis. 1993. Feeding patterns of anophelines collected in a vivax-malaria endemic area in western Venezuela. Bull. Ent. Res. (in press).

Sokal, R.R. and F.J. Rohlf. 1969. Biometry. W.H. Freeman and Company, San Francisco.

Wilkerson, R.C. and E.L. Peyton. 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.

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