MORPHOMETRIC CHARACTERIZATION OF THE MALARIA VECTOR *ANOPHELES NUNEZTOVARI* (DIPTERA: CULICIDAE) FROM WESTERN VENEZUELA

NEREYDA DELGADO AND YASMIN RUBIO-PALIS

División de Investigaciones, Escuela de Malariología y Saneamiento Ambiental, Apartado 2064, Maracay 2101-A, Venezuela

ABSTRACT. Examination of field collected adult females suggested the presence of a morphological variant of *Anopheles nuneztovari* which was provisionally called morphotype II, and which differed from the typical form in the length of the humeral pale spot on the wing. Morphometric studies carried out on field collected adult females and associated rearings showed that *An. nuneztovari* is a highly variable species within the same geographic area. Diagnostic characters for *An. nuneztovari* were compared with those previously reported. *Anopheles trinkae*, a sister species of *An. nuneztovari*, is probably not present in western Venezuela.

INTRODUCTION

Anopheles (*Nyssorhynchus*) *nuneztovari* Gabaldón, 1940 is a Neotropical mosquito that occurs throughout much of the Amazon Basin, and is also found in eastern Panama. It is not known how far it extends westward in the Amazon Basin (Faran 1980).

This species has been incriminated in malaria transmission in Peru (Hayes et al. 1987), Colombia (Elliott 1972, Fajardo and Alzate 1987), Venezuela (Pintos et al. 1968, Rubio-Palis et al. 1992) and Brazil (de Arruda et al. 1986). A similar situation is suspected in Ecuador and Bolivia, although published information is unavailable.

Anopheles nuneztovari was described by Gabaldón (1940) based on distinctive characteristics of the aedeagus and ventral lobes of the claspette of adult males obtained from larvae collected in Cojedes State, Venezuela. The other stages were described by Floch and Abonnenc (1946) and Cova García (1961), and the species was redescribed by Sutil (1976) and Faran (1980).

Early ecological and behavioral observations suggested that *An. nuneztovari* consisted of two distinct allopatric forms. One of these, found in Brazil, Suriname and Ecuador, bites at sunset, is mainly exophagic, and is considered primarily zoophilic (Elliott 1972). The other, found in western Venezuela and northern Colombia, bites around midnight, is primarily endophagic, and is a vector of *Plasmodium vivax* (Grassi and Feletti) (Renjifo and de Zulueta 1952, Elliott 1972). Cytological studies by Kitzmiller et al. (1973) demonstrated the existence of two sibling species of *An. nuneztovari*, one in western Venezuela and northern Colombia and the other in Brazil. These sibling species are separated by an inversion in the right arm of the X chromosome. Steiner et al. (1980) compared isozyme profiles of *An. nuneztovari* from Barinas State, western Venezuela, and from Brokopondo, Suriname. They found high levels of genetic variation in both samples, and suggested that the Est-5 locus may be diagnostic for the two populations. Recently, Conn (1990) studied populations of *An. nuneztovari* from the same locations of the present study and found no significant differences in the chromosome banding pattern compared with the populations of *An. nuneztovari* from Barinas State described by Kitzmiller et al. (1973). However, Conn found that the frequency of inversion 2La had increased significantly in the 16-year interval since the study of Kitzmiller et al., and considered that this could be due to one factor, or a combination of several factors

such as genetic changes within the 2La inversion, environmental changes or within or between year seasonal variations.

Preliminary studies carried out in western Venezuela showed that identification of field collected adult females with available keys proved to be very difficult since the supposedly distinctive taxonomic characters were found to be highly variable, and there were many specimens that could not be identified with the keys. Examination of field collected adult females suggested the presence of a morphological variant of *An. nuneztovari* which was provisionally called morphotype II, and which differed from the typical form in the length of the humeral pale spot on the wing.

In order to determine whether two species or a highly variable one occurs in western Venezuela, morphometric studies were carried out on field collected adult females and associated rearings. Special attention was given to the possibility of finding *An. trinkae* Faran, the adult females of which could be misidentified as *An. nuneztovari* (Faran 1979).

MATERIALS AND METHODS

Specimens were collected monthly between February 1986 and November 1989 in three selected villages in western Venezuela (approximately 7° N 71° W) (Fig. 1). The study area and villages were described by Rubio-Palis and Curtis (1992). This area has an annual rainfall of 3,000–4,000 mm, a mean temperature of 24°C and relative humidity of 83% (Venezuelan Air Force 1989). Altitude ranges from 200 to 400 m. The area is classi-

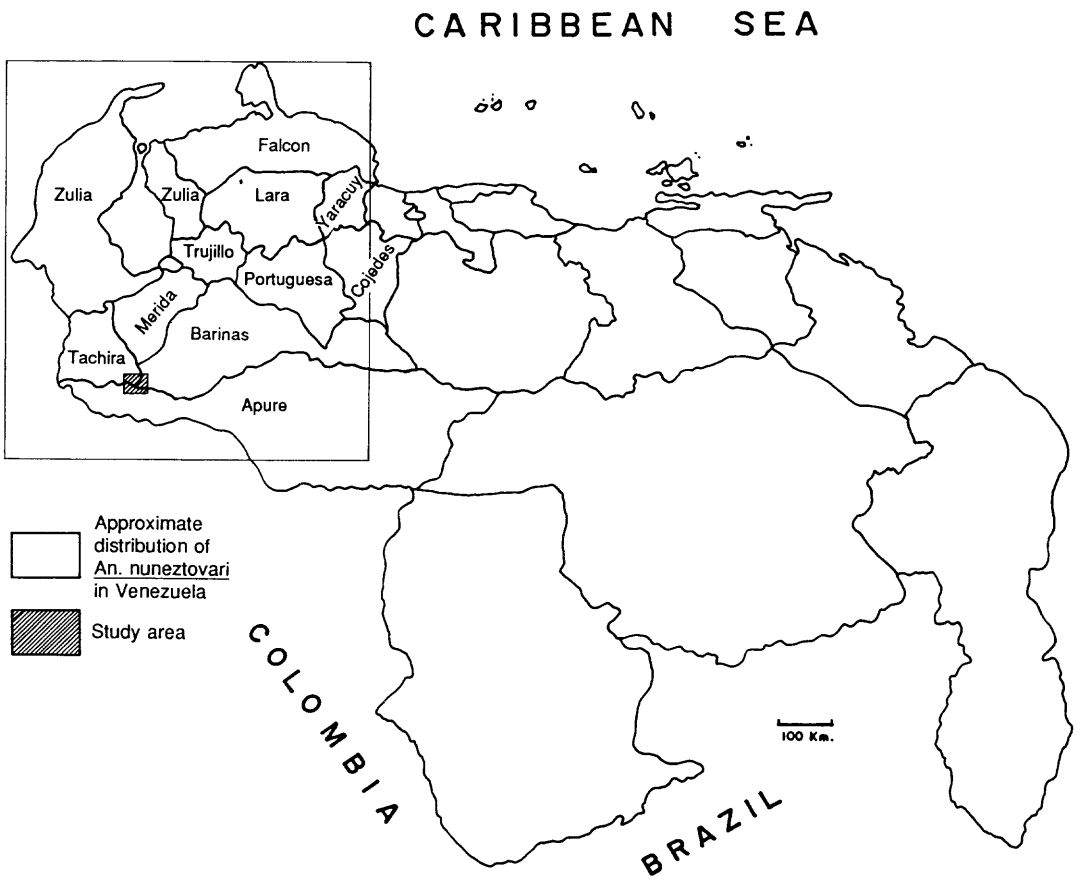

Fig. 1. Location of the study area in western Venezuela. Names of states enclosed by rectangle are those where *Anopheles nuneztovari* has been recorded in Venezuela (Sutil 1976).

fied as wet tropical woodland (Ewel and Madriz 1968).

Anopheles nuneztovari, as well as the other species of the Oswaldoi Subgroup (except *An. albimanus* Wiedemann) have not been colonized and it should be stressed that rearing proved to be very difficult. Only after three years of repeated collection and rearing were progenies obtained in the laboratory in April 1989.

Larvae were collected at the three villages and reared in an insectary in Maracay at 25 ± 2°C. Females collected in the field on human bait were blood fed immediately and transported to the insectary in order to obtain groups of adult males and females with associated larval and pupal exuviae from individual mothers. Larval and pupal exuviae from the offspring of field collected females were preserved in 70% ethanol until mounted on slides following the method of Lane (1974). Adult females were killed with ethyl acetate and mounted on pins. Adult male genitalia were dissected and permanent slides made (John Lane, personal communication). The morphological terms and abbreviations used follow Harbach and Knight (1980, 1982) and Wilkerson and Peyton (1990).

Morphometric analyses were conducted using the following measurements and characters. Larva: 1) length of seta 3-C; 2) distance between setae 2- and 3-C; 3) distance between insertions of seta 2-C; 4) clypeal index (distance between 2- and 3-C on one side divided by distance between insertions of seta 2-C); 5) length of seta 4-C divided by length of seta 3-C; 6) length and number of branches of seta 8-C; 7) length of seta 0-II; 8) length and width of spiracular apparatus (Faran 1980). Pupa: 1) length of pinna divided by the length of meatus; 2) length of seta 9-V divided by length of segment V; 3) length of seta 9-VII divided by length of segment VII; 4) length of seta 9-VIII divided by length of segment VIII (Faran 1980). Male genitalia: 1) ventral claspette length divided by length of sidepiece; 2) width at apex divided by length of claspette; 3) shape of aedeagus and presence of membranous non-serrate leaflets (Faran 1980, Savage 1986). Adult female: 1) length of hindtarsomere 2; 2) length of dark band on hindtarsomere 2; 3) length of sector dark spot (the part of sector dark between the subcostal pale spot and the accessory pale spot); 4) length of subcostal pale spot; 5) length of humeral pale spot, and 6) length of prehumeral dark spot (Faran 1980, Wilkerson and Peyton 1990).

RESULTS AND DISCUSSION

Larval progeny from mothers identified as *An. nuneztovari* showed no significant difference ($P > 0.05$) between the mean values of the characters analyzed or in their frequency distribution. However, the clypeal index was significantly different between the progeny of the two forms (Table 1), although the frequency distribution of these values was not different. The range for this character is greater than the one described by Faran (1980) for *An. nuneztovari* and *An. trinkae* (Table 1). The other distinctive character studied was the length ratio of setae 4-C/3-C (Table 2). The difference in the values for this character within and between progeny from mothers identified either as *An. nuneztovari* or morphotype II was not significant ($P > 0.05$). Although the range of variation for this character is within the range reported by Faran (1980) for *An. nuneztovari*, the speci-

Table 1. Clypeal index measured in larval progeny of individual mothers identified as typical *An. nuneztovari* and morphotype II compared with those studied by Faran (1980) to distinguish between *An. nuneztovari* and *An. trinkae*.

Form	*N*	Mean*	S.D.	Range	Faran (1980)
An. nuneztovari	68	1.535a	0.342	1.15–2.00	1.0–1.3
Morphotype II	32	1.372b	0.290	1.07–1.75	—
An. trinkae	—	—	—	—	1.25

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

mens from the study site showed a smaller range (0.45–0.50) (Table 2).

The number of branches of setae 8-C and O-II in *An. nuneztovari* (Table 3) coincided with those reported by Faran (1980), while progeny of typical morphotype II mothers had seta 8-C with 2–3 branches like *An. trinkae* (Faran 1979) and seta O-II was either conspicuous as in typical *An. nuneztovari* or inconspicuous as in *An. trinkae*.

The characters of pupae from typical *An. nuneztovari* and morphotype II mothers were not significantly different ($P > 0.05$) (Tables 4,5,6,7). When these values were compared with those reported by Faran (1980) for *An. nuneztovari* and *An. trinkae*, differences were observed. In *An. nuneztovari* from western Venezuela the range of length of pinna divided by length of meatus was 4.2–4.9, being larger than that reported by Faran (1980) (3.5–4.5). The range was even larger for morphotype II (4.2–5.5) (Table 4). The range of length of seta 9-V divided by length of segment V was larger in progeny of morphotype II mothers than in progeny of typical *An. nuneztovari*, although a bit smaller than the reported 0.33 for *An. nuneztovari* (Faran 1980) (Table 5). The range of length of seta

Table 2. Length of seta 4-C divided by the length of seta 3-C in larvae from progeny of individual mothers identified as typical *An. nuneztovari* and morphotype II compared with those studied by Faran (1980) to distinguish between *An. nuneztovari* and *An. trinkae*.

Form	*N*	Mean	S.D.	Range	Faran (1980)
An. nuneztovari	68	0.472	0.097	0.35–0.60	0.3–0.6
Morphotype II	32	0.513	0.127	0.38–0.67	—
An. trinkae	—	—	—	—	0.7–1.0

Table 3. Number of branches for setae 8-C and O-II in larval progeny of individual mothers identified as typical *An. nuneztovari* and morphotype II compared with those studied by Faran (1980) to distinguish between *An. nuneztovari* and *An. trinkae*.

		Number of branches	
Study	Form	Seta 8-C	Seta O-II
Reported here	*An. nuneztovari*	>3	>5, conspicuous
	Morphotype II	2–3	Conspicuous/insconspicuous
Faran (1980)	*An. nuneztovari*	3–5	5–8, conspicuous
	An. trinkae	2–3	1–3, insconspicuous

Table 4. Mean values for the pinna length divided by meatus length measured in pupal progeny of individual mothers identified as typical *An. nuneztovari* and morphotype II compared with those studied by Faran (1980) to distinguish between *An. nuneztovari* and *An. trinkae*.

Form	*N*	Mean	S.D.	Range	Faran (1980)
An. nuneztovari	53	4.5	0.93	4.18–4.85	3.5–4.5
Morphotype II	28	4.8	1.43	4.15–5.51	—
An. trinkae	—	—	—	—	3.6–4.0

Table 5. Mean values for length of seta 9-V divided by length of segment V measured in pupal progeny of individual mothers identified as typical *An. nuneztovari* and morphotype II compared with those studied by Faran (1980) to distinguish between *An. nuneztovari* and *An. trinkae*.

Form	*N*	Mean	S.D.	Range	Faran (1980)
An. nuneztovari	53	0.275	0.055	0.20–0.33	0.33
Morphotype II	28	0.294	0.085	0.23–0.35	—
An. trinkae	—	—	—	—	0.50

9-VII divided by the length of segment VII (Table 6) in progeny of typical *An. nuneztovari* and morphotype II mothers was similar to that reported by Faran (1980) for *An. nuneztovari*, which is larger in *An. trinkae*. The length of seta 9-VIII divided by the length of segment VIII (Table 7) showed a similar small range of variation in progeny of *An. nuneztovari* and morphotype II, being a bit smaller than the value reported by Faran (1980) for *An. nuneztovari*.

The male genitalia of progeny from typical *An. nuneztovari* and morphotype II showed characteristics similar to those previously described for *An. nuneztovari* by Gabaldón (1940), Sutil (1976), Faran (1980) and Savage (1986):

1) length of ventral claspette divided by length of sidepiece = 0.40–0.50;
2) width at apex divided by length of claspette = 0.40–0.50;
3) aedeagus rounded at apex and with small, non-serrate, pointed, basolaterally directed leaflets.

Faran (1980) stated that leaflets may be present or absent in *An. nuneztovari*, but were absent in all the specimens that he identified as *An. trinkae*. Nevertheless, Savage (1986) pointed out that leaflets are always present and are diagnostic for *An. nuneztovari*. All the males examined in this study had leaflets on the aedeagus, which indicates that none of them were *An. trinkae*. Table 8 shows the mean values for the length of the dark band on hindtarsomere 2 divided by the length of hindtarsomere 2. It was found that the means for typical *An. nuneztovari* and morphotype II are not significantly different. However, the range of variation is smaller than that reported by Faran (1980) for *An. nuneztovari*. The mean values for the length of the subcostal pale spot divided by the length of the sector dark spot were not significantly different for the two forms (Table 9). Again this range was smaller (0.26–0.50) in *An. nuneztovari* from western Venezuela than the range (0.20–0.55) reported by Faran (1980). Table 10 shows the mean values of the length of the humeral pale spot divided by the length of the prehumeral

Table 6. Mean values for length of seta 9-VII divided by length of segment VII measured in pupal progeny of individual mothers identified as typical *An. nuneztovari* and morphotype II compared with those studied by Faran (1980) to distinguish between *An. nuneztovari* and *An. trinkae*.

Form	*N*	Mean	S.D.	Range	Faran (1980)
An. nuneztovari	53	0.320	0.050	0.27–0.43	0.33
Morphotype II	28	0.312	0.036	0.27–0.38	—
An. trinkae	—	—	—	—	0.50

Table 7. Mean values for length of seta 9-VIII divided by length of segment VIII measured in pupal progeny of individual mothers identified as typical *An. nuneztovari* and morphotype II compared with those studied by Faran (1980) to distinguish between *An. nuneztovari* and *An. trinkae*.

Form	*N*	Mean	S.D.	Range	Faran (1980)
An. nuneztovari	53	0.296	0.023	0.28–0.32	0.33
Morphotype II	28	0.304	0.027	0.28–0.31	—
An. trinkae	—	—	—	—	0.50

Table 8. Mean values for length of dark band on hindtarsomere 2 divided by length of hindtarsomere 2 in wild-caught females identified as typical *An. nuneztovari* and morphotype II compared with the values reported by Faran (1980) to distinguish between *An. nuneztovari* and *An. trinkae*.

Form	*N*	Mean	S.D.	Range	Faran (1980)
An. nuneztovari	485	0.270	0.039	0.23–0.31	0.25–0.32
Morphotype II	235	0.267	0.037	0.23–0.30	—
An. trinkae	—	—	—	—	0.30–0.40

dark spot. This ratio was significantly smaller in typical *An. nuneztovari* than in morphotype II, but within the range reported by Faran (1980) for *An. trinkae.*

Tables 11, 12 and 13, and Figs. 2, 3 and 4 show the comparisons between mothers and progeny of typical *An. nuneztovari* and morphotype II for the mean ratios of the characters analyzed and the frequency distributions of the range of variation of the ratios. The data in Fig. 4 conclusively show that the specimens considered to be morphotype II

Table 9. Mean values for length of subcostal pale spot divided by length of sector dark in wild-caught females identified as typical *An. nuneztovari* and morphotype II compared with the values reported by Faran (1980) to distinguish between *An. nuneztovari* and *An. trinkae.*

Form	*N*	Mean	S.D.	Range	Faran (1980)
An. nuneztovari	485	0.375	0.159	0.26–0.50	0.20–0.55
Morphotype II	235	0.386	0.095	0.29–0.50	—
An. trinkae	—	—	—	—	0.25–0.43

Table 10. Mean values for length of humeral pale spot divided by length of prehumeral dark spot in wild-caught females identified as typical *An. nuneztovari* and morphotype II compared with the values reported by Faran (1980) to distinguish between *An. nuneztovari* and *An. trinkae.*

Form	*N*	Mean*	S.D.	Range	Faran (1980)
An. nuneztovari	485	1.093a	0.221	0.87–1.65	0.70–1.30
Morphotype II	235	2.149b	0.489	1.80–3.00	—
An. trinkae	—	—	—	—	1.30–2.50

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

Table 11. Comparison between mothers and progeny of the mean ratios for the length of dark band on hindtarsomere 2 divided by length of hindtarsomere 2.

Form		*N*	Mean	S.D.	Range
An. nuneztovari	Mothers	434	0.267	0.037	0.22–0.35
	Progeny	99	0.263	0.042	0.22–0.35
Morphotype II	Mothers	204	0.265	0.032	0.25–0.35
	Progeny	63	0.278	0.048	0.25–0.34

Table 12. Comparison between mothers and progeny of the mean ratios for the length of subcostal pale spot divided by length of sector dark spot.

Form		*N*	Mean	S.D.	Range
An. nuneztovari	Mothers	434	0.355	0.097	0.15–0.50
	Progeny	99	0.385	0.101	0.20–0.50
Morphotype II	Mothers	204	0.377	0.093	0.22–0.50
	Progeny	63	0.418	0.122	0.24–0.50

Table 13. Comparison between mothers and progeny of the mean ratios for length of humeral pale spot divided by length of prehumeral dark spot.

Form		*N*	Mean	S.D.	Range
An. nuneztovari	Mothers	434	1.050*	0.242	0.87–1.50
	Progeny	99	1.526*	0.573	0.80–2.50
Morphotype II	Mothers	204	2.153*	0.486	1.90–2.75
	Progeny	63	1.816*	0.662	1.00–2.50

* $P < 0.05$.

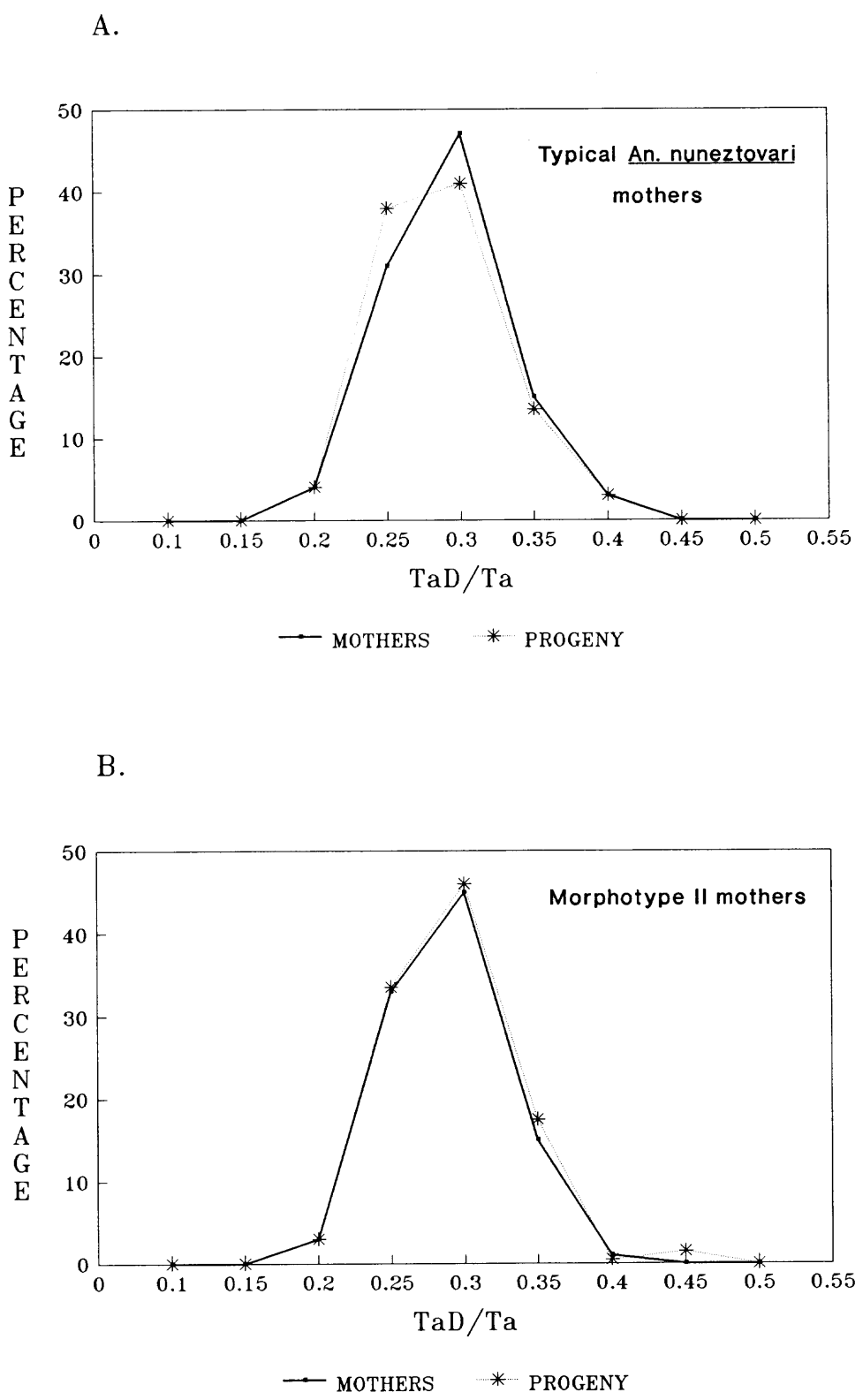

Fig. 2. Frequency distribution for the length of the dark band on hindtarsomere 2 (TaD) divided by the length of hindtarsomere 2 (Ta). A, Typical *Anopheles nuneztovari* mothers. B, Morphotype II mothers.

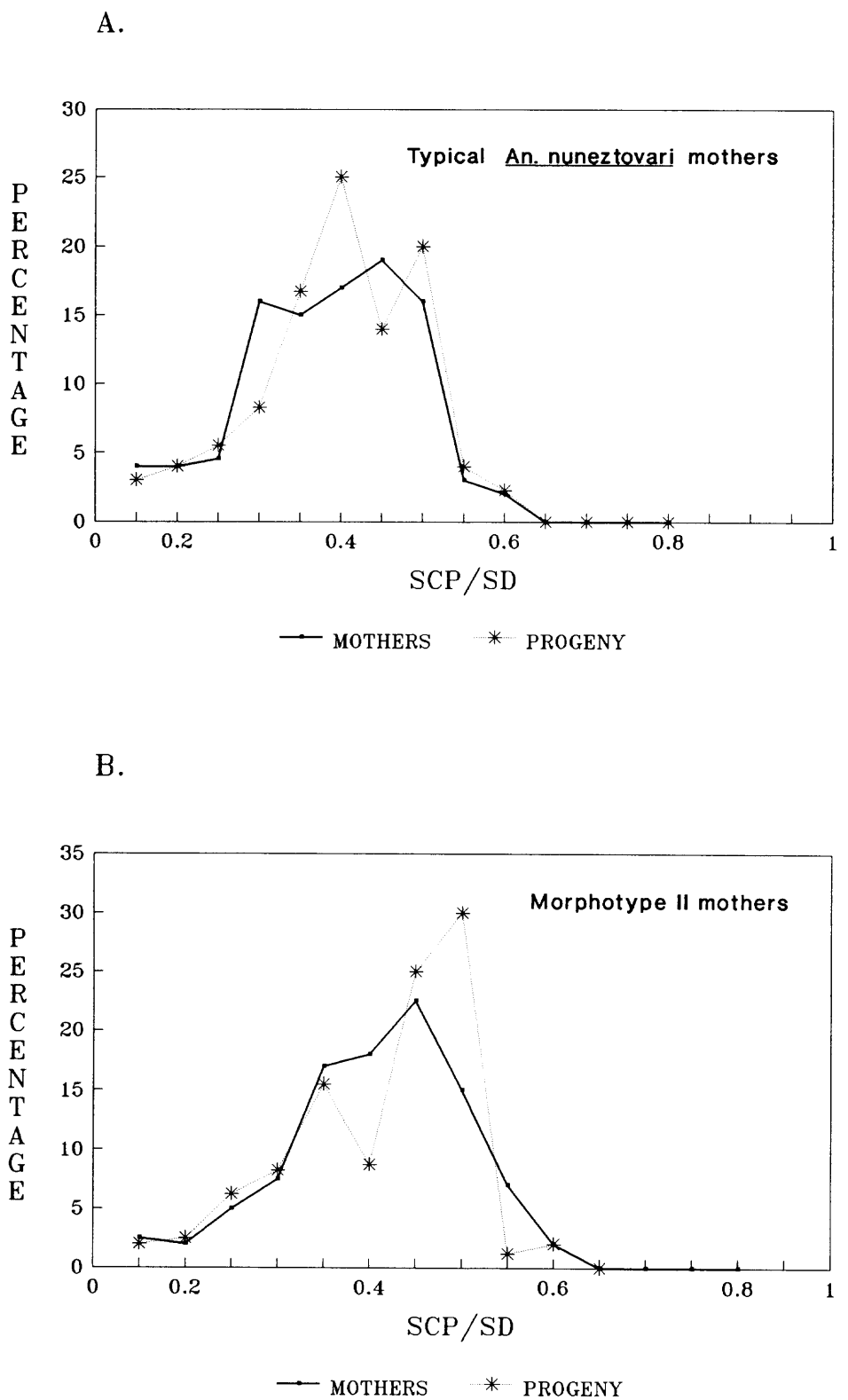

Fig. 3. Frequency distribution for the length of the subcostal pale spot (SCP) divided by the length of the sector dark spot (SD). A, Typical *Anopheles nuneztovari* mothers. B, Morphotype II mothers.

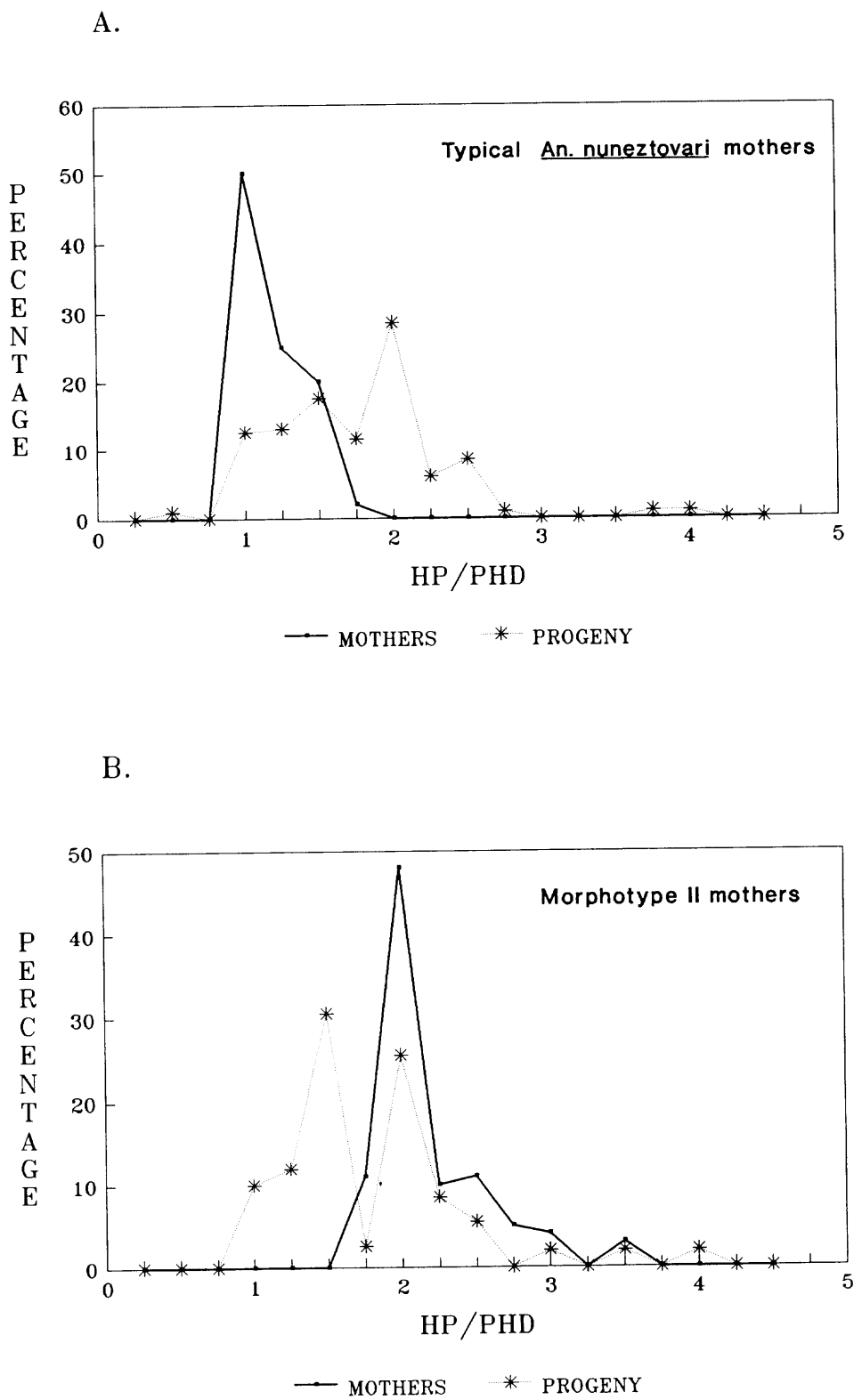

Fig. 4. Frequency distribution for the length of the humeral pale spot (HP) divided by the length of the prehumeral dark spot. A, Typical *Anopheles nuneztovari* mothers. B, Morphotype II mothers.

actually belong to the species *An. nuneztovari*: typical *An. nuneztovari* mothers give rise to progeny of typical *An. nuneztovari* as well as morphotype II, and morphotype II mothers give rise to progeny of typical *An. nuneztovari* and morphotype II. The difference between typical *An. nuneztovari* and morphotype II shown in Fig. 4 can be considered as a polymorphism within one species because the distribution of HP/PHD ratio shows definite bimodality and not unimodality.

The present study shows that *An. nuneztovari* is a highly variable species. The results reported in the present study suggest that *An. trinkae* is probably not present in western Venezuela.

ACKNOWLEDGMENTS

We are grateful to Ramón Alvarado, Jorge Anson, Ramiro Briceño, Flaymir Silva, Luis Bautista, José Casadiego, Miguel, Juan, Dency Ortiz, Yadira Rangel and Antonio Guerra, who participated in the field activities. Special thanks are due to Robert Zimmerman for his advice and to E.L. Peyton for checking our species identifications and his most valuable discussions. We are grateful to Ralph Harbach and E.L. Peyton for their comments and review of the manuscript. We are most grateful to PAHO for sponsoring and coordinating technical and administrative resources. Financial support was provided by the U.S. National Academy of Sciences/National Research Council (MVR-VE-1-87-81) by means of a grant from the U.S. Agency for International Development.

REFERENCES CITED

Conn, J. 1990. A genetic study of the malaria vector *Anopheles nuneztovari* from western Venezuela. J. Am. Mosq. Control Assoc. 6:400–405.

Cova García, P. 1961. Notas sobre los anofelinos de Venezuela y su identificación. Editorial Grafos, Segunda Edición, Caracas.

de Arruda, M., M.B. Carvalho, R.S. Nussenzweig, M. Maracic, A.W. Ferreira and A.H. Cochrane. 1986. Potential vectors of malaria and their different susceptibility to *Plasmodium falciparum* and *Plasmodium vivax* in northern Brazil identified by immunoassay. Am. J. Trop. Med. Hyg. 35:873–881.

Elliott, R. 1972. The influence of vector behavior on malaria transmission. Am. J. Trop. Med. Hyg. 21:755–763.

Ewel, J.J. and A. Madriz. 1968. Zonas de vida de Venezuela. Ministerio de Agricultura y Cría, Caracas.

Fajardo, P. and A. Alzate. 1987. *Anopheles nuneztovari* como vector de malaria en el Bajo Calima, Buenaventura. Colombia Méd. 18:14–18.

Faran, M.E. 1979. *Anopheles (Nyssorhynchus) trinkae*, a new species in the Albimanus Section (Diptera: Culicidae). Mosq. Syst. 11:26–39.

Faran, M.E. 1980. Mosquito Studies (Diptera, Culicidae) XXXIV. A revision of the Albimanus Section of the subgenus *Nyssorrynchus* of *Anopheles*. Contrib. Am. Entomol. Inst. (Ann Arbor) 15(7):1–214.

Floch, H. and E. Abonnenc. 1946. Sur *A. nuñez-tovari* et *A. pessoai* en Guyane Française. Table d'identification des *Nyssorhynchus* guyanais. Arch. Inst. Pasteur Guyane Territ. Inini Publ. 126:1–5.

Gabaldón, A. 1940. Estudios sobre anofelinos. Serie I. 1. Descripción de *Anopheles (Nyssorhynchus) nuñez-tovari* n. sp. y consideraciones sobre una sub-división del grupo *Nyssorhynchus* (Diptera, Culicidae). Publ. Div. Malariol. 5:3–7.

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

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

Hayes, J., G. Calderón, R. Falcón and V. Zambrano. 1987. Newly incriminated *Anopheles* vectors of human malaria parasites in Junin Department, Peru. J. Am. Mosq. Control Assoc. 33:418–422.

Kitzmiller, J.B., R.D. Kreutzer and E. Tallaf-

erro. 1973. Chromosomal differences in populations of *Anopheles nuñeztovari*. Bull. WHO 48:435–455.

Lane, J. 1974. The preservation and mounting of insects of medical importance. WHO mimeographed document, WHO/VBC/74.502.

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

Renjifo, S. and J. de Zulueta. 1952. Five years' observations of rural malaria in eastern Colombia. Am. J. Trop. Med. Hyg. 1:598–611.

Rubio-Palis, Y. and C.F. Curtis. 1992. Biting and resting behaviour of anophelines in western Venezuela and implications for control. Med. Vet. Entomol. 8:(in press).

Rubio-Palis, Y., R.A. Wirtz and C.F. Curtis. (1992). Malaria entomological inoculation rate in western Venezuela. Acta Trop. (submitted).

Savage, H.M. 1986. Identification and location of the holotype and paratypes of *Anopheles* (*Nyssorhynchus*) *nuneztovari* Gabaldón (Diptera: Culicidae). Mosq. Syst. 18:279–283.

Steiner, W.W.M., J.B. Kitzmiller and D.L. Osterbur. 1980. Gene differences in chromosome races of *Anopheles nuneztovari*. Mosq. Syst. 43:287–290.

Sutil, E. 1976. Redescripción de la especie *Anopheles* (*Nyssorhynchus*) *nuñez-tovari* Gabaldón, 1940, y su distribución geográfica en Venezuela. Bol. Dir. Malariol. Saneamiento Ambiental 16:33–45.

Venezuelan Air Force. 1989. Datos climatológicos. Base aérea Santo Domingo del Táchira.

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.