Journal of the American Mosquito Control Association, 19(2):107–114, 2003

MORPHOLOGICAL CHARACTERS OF ADULT *ANOPHELES (NYSSORHYNCHUS) MARAJOARA* IN VENEZUELA

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ABSTRACT. A morphometric study was carried out to find diagnostic characters with which to update taxonomic keys for field identification of *Anopheles (Nyssorhynchus) marajoara* and the 3 other sympatric *Anopheles (Nyssorhynchus)* species (*An. darlingi, An. argyritarsis,* and *An. braziliensis*) that occur in Venezuela. Diagnostic random amplified polymorphic DNA–polymerase chain reaction markers from wild-caught specimens showed that *An. marajoara* was the only species in the *Anopheles albitarsis* complex collected in Venezuela.

KEY WORDS *Anopheles marajoara,* malaria vectors, morphology, random amplified polymorphic DNA–polymerase chain reaction

INTRODUCTION

Anopheles (Nyssorhynchus) marajoara Galvão and Damasceno is a common Central and South American malaria vector. This species was found positive for *Plasmodium vivax* var. 210 circumsporozoite protein in western Venezuela and is considered a secondary vector of malaria (Rubio-Palis et al. 1992). Furthermore, Rubio-Palis (1994) reported that the vectorial capacity of *An. marajoara* during the dry season was similar to that of *An. (Nys.) nuneztovari* Gabaldon, the primary vector in that area. Recently, *An. marajoara* was proven to be the primary malaria vector in the northeastern Brazilian state of Amapá (Conn et al. 2002). Because it is a proven and important vector, our objectives in this study were to investigate morphological characters that could be used to identify *An. marajoara* and related species in Venezuela, and to test for additional members of the *Anopheles albitarsis* complex that might occur in the study area.

At present, good evidence exists for 4 species related to *An. marajoara* with the following names: *An. albitarsis* Lynch-Arribálzaga, *An. deaneorum* Rosa-Freitas, *An. marajoara,* and *An.* species "B" (Wilkerson et al. 1995a, 1995b). *Anopheles marajoara* Galvão and Damasceno (1942) was described from Marajó Island, Brazil, as a species closely related to *An. albitarsis.* However, the authors stressed that all anatomical characters that they used were highly variable in *An. albitarsis* and its near relatives. Galvão (1944) considered *An. marajoara* to be a synonym of *An. albitarsis.* Galvão and Damasceno (1944), based on morphological and behavioral characters, divided *An. albitarsis* into 2 subspecies: a strongly endophilic *An. albitarsis domesticus* and an exophilic *An. albitarsis albitarsis,* based on material from the same locality (Ilha do Marajó, Brazil). According to Faran and Linthicum (1981), material thought by others to be *An. albitarsis* actually represented 2 species: *An. allopha* (Lutz and Peryassú) and *An. albitarsis,* differing in morphology, geographical distribution, and vectorial capacity, with only *An. allopha* able to transmit malaria parasites. However, according to Lourenço-de-Oliveira and Deane (1984), none of the known anopheline species agrees with the original description of *allopha,* which was apparently based on a mixture of species, and they concluded that it should be considered a nomem nudum. Later, Linthicum (1988) considered *An. allopha* a nomem dubium and resurrected *An. marajoara* as the valid species name.

Populations of *An. albitarsis* s.l. from 18 Brazilian states were studied morphologically by Rios et al. (1984). They verified the considerable intrapopulational variability of taxonomically important characters, such as pilosity of the anal lobe of the male genitalia (a character that should differentiate *An. domesticus* from *An. marajoara*), and the percentage of black basally on hindtarsomere 2 (supposedly distinguishing *An. domesticus* from *An. albitarsis*). Following Root (1926) and Davis (1928), they correlated variation in this character with latitude, and found it impossible to separate the 2 species based on criteria used by Galvão and Damasceno (1944).

At present, available evidence based on morphological, behavioral, biological, cytogenetic, biochemical, and molecular studies indicates that *An. albitarsis* is a species complex (Kreutzer et al. 1976; Steiner et al. 1982; Linthicum 1988; Rosa-Freitas 1989; Rosa-Freitas et al. 1990; Klein et al. 1991; Narang et al. 1993; Wilkerson et al. 1995a, 1995b). Furthermore, Wilkerson et al. (1995a, 1995b), who used random amplified polymorphic DNA–polymerase chain reaction (RAPD-PCR), demonstrated that *An. albitarsis* is a complex of at least 4 species: *An. albitarsis* s.s. found in Argentina, Paraguay, and southern Brazil; *An. marajoara* found from Costa Rica to Bolivia; *An. deaneorum* with a distribution from Rondônia and Mato Grosso states in Brazil to northern Argentina; and a 4th species (species B), which has not been formally described, with a wide distribution approximately

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Fig. 1. Collecting sites positive for *Anopheles marajoara* in Venezuela.

between 1° and 25°N and 38° and 54°W in Brazil and Paraguay (Wilkerson et al. 1995b). So far, when using RAPD-PCR, no other species belonging to the *An. albitarsis* complex, except *An. marajoara,* has been detected north of Belém, Brazil, or in Venezuela (Wilkerson et al. 1995b, Conn et al. 2002).

In Venezuela, *An. marajoara* was 1st collected (as *An. albitarsis*) by Root in 1927 (Hill 1928). Cova-García (1951) reported *An. albitarsis* from 17 of the 22 Venezuelan states. At present, the key by Cova-García and Sutil (1977) is widely used for the identification of anophelines in Venezuela and other South American countries. It includes *An. albitarsisis* s.l., which is separated from the similar *An. braziliensis* (Chagas) by the absence of lateral scale tufts on abdominal segment II. The objectives of this study were to establish the presence of *An. marajoara* in Venezuela and suggest diagnostic characters that can be used to update taxonomic keys for field identification. To evaluate the diagnostic power of the characters identified among the populations of *An. marajoara,* the other 3 sympatric species belonging to the Argyritarsis Section of the subgenus *Nyssorhynchus* (Linthicum 1988) (*An. argyritarsis* Robineau-Desvoidy, *An. darlingi* Root, and *An. braziliensis*) were included in the analysis. To reinforce the RAPD-PCR identification of *An. marajoara,* we compared our RAPD profiles to profiles of 2 of the remaining 3 species in the complex that have a more northern South American range.

MATERIALS AND METHODS

Mosquito collections: From 1989 to 1999 we collected samples of *An. marajoara* from 6 states in Venezuela (Fig. 1 and Table 1) which represent a large portion of its geographic range in this country. Mosquitoes were collected on human bait and the methodology followed was similar to that described for *An. darlingi* in Rubio-Palis et al. (1997)

Table 1. Geographic location of Venezuelan populations of *Anopheles marajoara* that were analyzed.

State	Locality	Longitude, latitude
Apure	Capanaparo	6°48′N, 69°24′W
Barinas	Jabillos	7°33′N, 71°33′W
Bolívar	Corobal	7°48′N, 65°42′W
Bolívar	Caicara	7°49′N, 66°12′W
Bolívar	Aguas Nieves	7°30′N, 65°40′W
Bolívar	La Yuca	7°51′N, 65°40′W
Bolívar	Aripao	7°20′N, 65°10′W
Bolívar	San Francisco	7°5′N, 63°37′W
Delta Amacuro	Valle Nuevo	8°27′N, 62°30′W
Delta Amacuro	Piacoa	8°40′N, 62°10′W
Guárico	Finca Burgos	8°58′N, 67°25′W
Táchira	Caño Lindo	7°33′N, 71°50′W

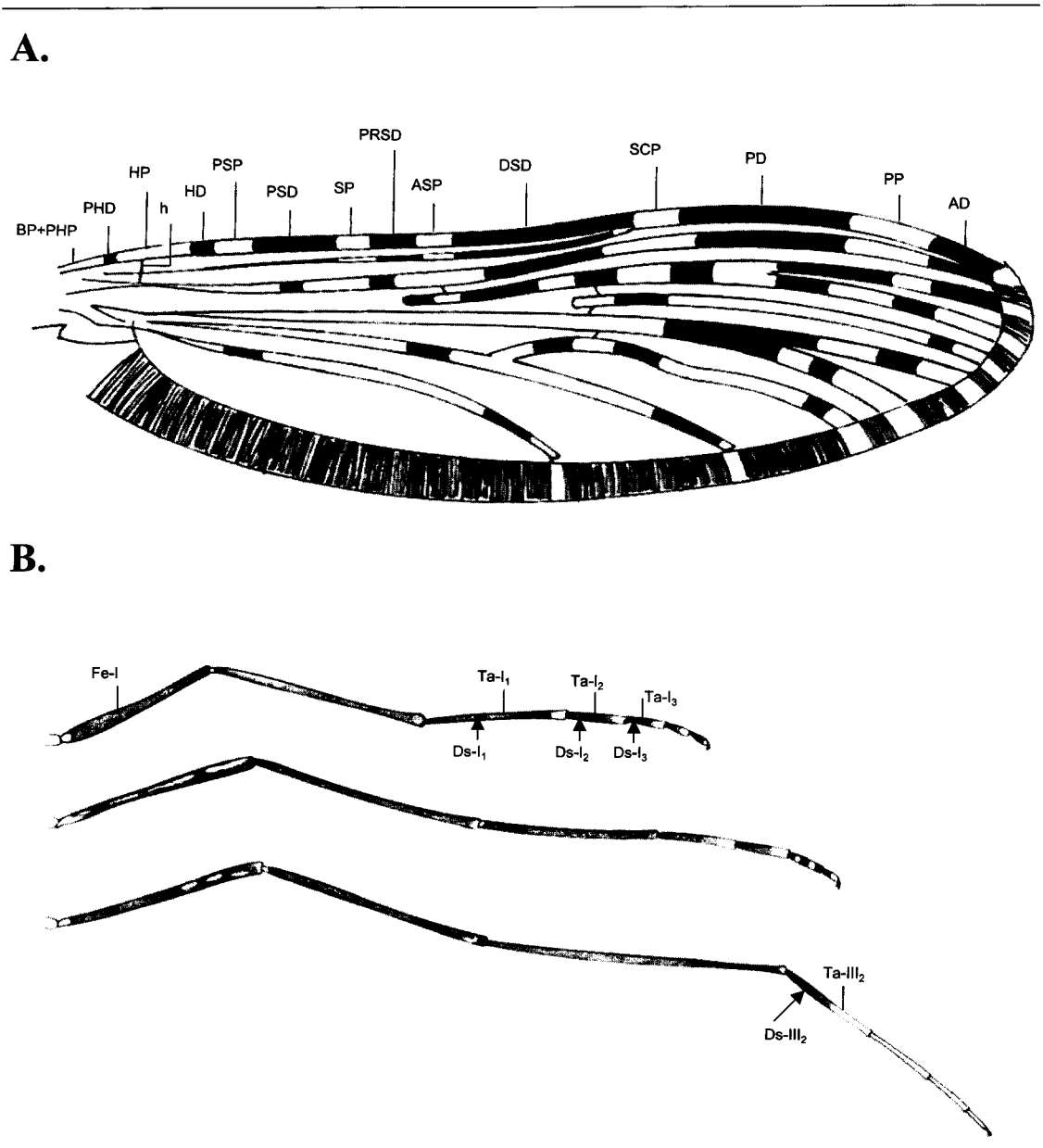

Fig. 2. Wing and leg characters of *Anopheles marajoara*. (A) Dark and pale spots measured on the costa. BP+PHP, basal pale + prehumeral pale; PHD, prehumeral dark; h, humeral crossvein; HP, humeral pale; HD, humeral dark; PSP, presector pale; PSD, presector dark; SP, sector pale; PRSD, proximal sector dark; ASP, accessory sector pale; DSD, distal sector dark; SCP, subcostal pale; PD, preapical dark; PP, preapical pale; AD, apical dark. (B) Legs showing characters measured. Fe-I, forefemur; Ta-I_1, foretarsomere 1; Ds-I_1, dark spot on foretarsomere 1; Ta-I_2, foretarsomere 2; Ds-I_2, dark spot on foretarsomere 2; Ta-I_3, foretarsomere 3; Ds-I_3 = dark spot on foretarsomere 3; Ta-III_2, hindtarsomere 2; Ds-III_2, dark spot on hindtarsomere 2.

and Rubio-Palis (1998). Samples consisted of both wild-caught adults and individuals from isofemale progeny broods.

Morphometric study: Selected wing, leg, and head characters were measured on 195 females from 12 Venezuelan localities (Fig. 1 and Table 1) by using an Olympus dissecting microscope at 40× magnification, with an eyepiece micrometer (100 divisions = 1 mm). Head characters measured were the lengths of maxillary palpus, palpomeres 2–5, proboscis, and antenna. Wing length was measured from base to tip excluding fringe. Costal wing dark and pale spots were measured and divided by the wing length (Fig. 2A and Table 4). Leg characters

Table 2. Ratios (mean ± SD) of male genitalia characters observed in 5 populations of *Anopheles marajoara* in Venezuela (n = 50) compared to those reported by Linthicum (1988).

Proportion[1]	Mean ± SD	*An. marajoara* (Linthicum 1988)	*An. albitarsis* (Linthicum 1988)
lGc/wGc	2.54 ± 0.32	2.5–2.7	3.0
lAsSV/lAsSD	0.78 ± 0.10	0.75–0.80	0.70
lInS/lAsSV	0.95 ± 1.17	InS < AsSV	InS < AsSV
lGs/lb	7.16 ± 0.91	—	—
lGc/lb	0.69 ± 0.18	0.44–0.70	0.60
lCl/lGc	0.33 ± 0.05	0.35	0.35
lPH/lGc	0.49 ± 0.04	0.50	0.45–0.50
lPH/lCl	1.52 ± 0.38	1.60	1.40
lAe/wAe	1.09 ± 0.25	—	—

[1] l, length; w, width; Gc, gonocoxite; AsSV, accessory ventral seta; AsSD, accessory dorsal seta; InS, internal seta; Gs, gonostylus, b, seta b; Cl, claspette; PH, phallosome; Ae, aedeagus.

measured were the length of foretasomeres 1–3, the length of hindtarsome 2, and the lengths of the dark bands on foretarsomeres 1–3 and hindtarsome 2 (Fig. 2B and Table 5). Male genitalia were dissected and mounted in Canada balsam. The characters measured were length and width of the gonocoxite; lengths of the parabasal seta, parabasal lobule, apodeme of the gonocoxite, accessory ventral seta, accessory dorsal seta, internal seta, gonostylus, gonostylar claw, seta b, claspette, phallosome, and aedeagus; and width of the aedeagus.

The morphological terms and abbreviations used follow Harbach and Knight (1980), Wilkerson and Peyton (1990), and Rubio-Palis (1998). The program Statistica (Statsoft 1993) was used for statistical analysis among populations with $n > 10$. An analysis of variance (ANOVA) was used to determine the significance of the estimated ratios among the Venezuelan populations of *An. marajoara* and among the other sympatric species of the Argyritarsis Section.

Molecular study: A subset of 60 individuals, randomly chosen from wild-caught collections made at Finca Burgos, Guárico State, on March 1999, was used to confirm the identity of putative *An. marajoara.* The DNA was extracted and RAPD bands were amplified by using operon primers A01, C16, C19, and D01 as described in Wilkerson et al. (1995a, 1995b). For comparison, positive controls consisting of single individuals from progeny broods used in Wilkerson et al. (1995a, 1995b) (species "B" progeny brood BR 019(11), Ceará, Parapaíba, Brazil; *An. marajoara* progeny brood BR/R 001(45), Ilha de Marajó, Brazil; and *An. deaneorum* progeny brood AR 2(4), Corrientes Province, Argentina) were amplified. *An. albitarsis* was not included in this comparison because it has a southern distribution and has only been found in southern Brazil, Paraguay, and northern Argentina.

RESULTS

Male genitalia

A total of 50 genitalia from the populations of Corobal, Caicara, Aguas Nieves, La Yuca, and Aripao were dissected and measured. Results were compared to those reported by Linthicum (1988) for *An. marajoara* and *An. albitarsis* (Table 2). In general, the ranges of variation for the characters measured are within the ranges reported by Linthicum (1988) for *An. marajoara,* except for the ratio of the length of the gonocoxite divided by length of seta b, which showed a wider range of variation for the Venezuelan populations. The ratio of the length of the gonocoxite divided by the width of the gonocoxite for *An. marajoara* from Venezuela was smaller than the reported value for *An. albitarsis* sensu Linthicum. These values could not be compared with published measurements of *An. albitarsis* s.s. because Rosa-Freitas and Deane (1989) did not report morphometric values.

Adult females

Head: Table 3 shows the range of variation for the ratios estimated on the head for *An. marajoara, An. darlingi, An. argyritarsis,* and *An. braziliensis.* The ANOVA of the ratios analyzed among the specimens of *An. marajoara* were not significantly different ($P > 0.05$). The ratio of the palpus to the length of the proboscis is 0.78–1.17 and antenna mean length is 0.59 times the length of the proboscis. Linthicum (1988) reported the palpus to be 0.95 times the length of the proboscis and the antenna to be 0.7 times the length of the proboscis. For head characters, no significant diagnostic differences ($P > 0.05$) were found among the ratios estimated for *An. marajoara, An. darlingi, An. argyritarsis,* and *An. braziliensis.*

Wing: Table 4 shows the range of variation for the ratios of wing spots on the costa of *An. marajoara* from 12 localities (n = 195), *An. darlingi* from 8 localities (n = 204), *An. argyritarsis* from 1 locality (n = 20), and *An. braziliensis* from 2 localities (n = 55). The ANOVA among Venezuelan populations of *An. marajoara* showed that the ratio differences were not significant ($P > 0.05$). Regarding the dark and pale spot pattern on the costa, we found only 2 patterns similar to those

Table 3. Range and analyses of variance of the ratios of head characters (maxillary palpus, proboscis, and antenna) measured in females of *Anopheles marajoara* from 10 localities (n = 68), *An. darlingi* from 8 localities (n = 138), *An. argyritarsis* from 1 locality (n = 20), and *An. braziliensis* from 2 localities (n = 35) from Venezuela.

Variable[1]	*An. marajoara*	*An. darlingi*	*An. argyritarsis*	*An. braziliensis*	F-value	*P*
$MPlp_2/MPlp$	0.32–0.33	0.27–0.82	0.32–0.36	0.25–0.52	1.13	0.342
$MPlp_3/Mplp$	0.30–0.37	0.27–0.81	0.33–0.36	0.28–0.50	0.44	0.777
$MPlp_4/MPlp$	0.16–0.23	0.11–0.38	0.15–0.20	0.07–0.23	0.75	0.559
$MPlp_5/MPlp$	0.06–0.13	0.05–0.14	0.10–0.13	0.07–0.22	0.49	0.739
MPlp/P	0.78–1.17	0.75–1.15	0.95–1.02	0.63–1.02	1.07	0.374
Ant/P	0.45–0.77	0.29–0.98	0.67–0.81	0.48–0.78	0.26	0.90

[1] MPlp, length of maxillary palpus; $MPlp_{2-5}$, lengths of palpomeres 2–5; P, length of proboscis; Ant, length of antenna.

described for *An. darlingi*: type I and type VI (Rubio-Palis et al. 1997, Rubio-Palis 1998, Manguin et al. 1999), with type VI (sector pale absent) being more frequent (72%). The ANOVAs of the estimated ratios of the 4 sympatric species were significantly different ($P < 0.01$), resulting in valid diagnostic characters to differentiate these species, except for the ratios distal sector dark : wing length and preapical dark : wing length.

Legs: The ranges of variation of ratios for *An. marajoara, An. darlingi, An. argyritarsis,* and *An. braziliensis* are shown in Table 5. Analyses of variance of the ratios among Venezuelan populations of *An. marajoara* were not significantly different ($P > 0.05$). The percentage of dark scaling on hindtarsomere 2 of *An. marajoara* recorded in this study is smaller (30–62%) than that reported by Linthicum (1988) (40–90%). Significant differences ($P < 0.0001$) were observed for all the ratios of legs among *An. marajoara, An. darlingi, An. argyritarsis,* and *An. braziliensis* (Table 5), resulting in valid diagnostic characters to distinguish these 4 species. Linthicum (1988) only considered the ratio length of dark spot on hindtarsomere 2 : length of hindtarsomere 2 as diagnostic to separate these 4 species, which is still valid.

Molecular study

All 60 specimens amplified with primers A01, C16, C19, and D01 produced diagnostic bands for *An. albitarsis* species "C" (*An. marajoara*) as described in Wilkerson et al. (1995a, 1995b). These are (primer and size of fragment): A01, 1.22 and 0.85 kilobase pairs (kbp); C16, 0.993 kbp; C19, 1.172 and 0.88 kbp; and D01, 0.55 kbp. Occurrence of these correlated bands confirms that all specimens in this subset are *An. marajoara.* As an example, Fig. 3 shows the diagnostic bands produced by primer C19.

DISCUSSION

The present study reports the morphological analysis of adult females (12 populations) and male genitalia (5 populations) of *An. marajoara.* The

Table 4. Range and analysis of variance of the ratio of the length of each spot on costa divided by the length of wing of females of *Anopheles marajoara* from 12 localities (n = 195), *An. darlingi* from 8 localities (n = 204), *An. argyritarsis* from 1 locality (n = 20), and *An. braziliensis* from 2 localities (n = 55) from Venezuela.

Variable[1]	*An. marajoara*	*An. darlingi*	*An. argyritarsis*	*An. braziliensis*	F-value	*P*[2]
BP+PHP/L	0.04–0.12	0.02–0.06	0.04–0.11	0.05–0.11	415.39	0.0000001*
PHD/L	0.02–0.04	0.06–0.12	0.01–0.05	0.01–0.04	1141.74	0.0000001*
HP/L	0.04–0.08	0.007–0.05	0.03–0.06	0.02–0.07	230.09	0.0000001*
HD/L	0.02–0.05	0.02–0.23	0.02–0.22	0.02–0.22	6.58	0.000241*
PSP/L	0.02–0.05	0.00–0.04	0.00–0.05	0.00–0.05	47.06	0.0000001*
PSD/L	0.07–0.17	0.00–0.39	0.00–0.18	0.00–0.18	5.37	0.001243*
SP/L	0.00–0.04	0.00–0.07	0.00–0.03	0.00–0.02	10.02	0.000002*
PRSD/L	0.00–0.04	0.00–0.31	0.00–0.24	0.00–0.08	5.59	0.000927*
ASP/L	0.02–0.06	0.00–0.05	0.00–0.04	0.01–0.06	31.81	0.0000001*
DSD/L	0.13–0.26	0.00–0.58	0.00–0.23	0.14–0.25	1.57	0.194016
SCP/L	0.02–0.10	0.00–0.06	0.03–0.06	0.009–0.09	60.02	0.0000001*
PD/L	0.13–0.25	0.00–0.26	0.17–0.23	0.12–0.24	0.55	0.643969
PP/L	0.06–0.10	0.009–0.06	0.04–0.24	0.02–0.08	62.15	0.0000001*
AD/L	0.02–0.06	0.03–0.08	0.04–0.10	0.03–0.06	41.15	0.0000001*
PHD/HP	0.22–1.00	1.37–15.0	0.33–1.50	0.25–1.66	92.85	0.0000001*

[1] L, wing length; BP+PHP, basal pale + prehumeral pale; PHD, prehumeral dark; HP, humeral pale; HD, humeral dark; PSP, presector pale; PSD, presector dark; SP, sector pale, PRSD, proximal sector dark; ASP, accessory sector pale; DSD, distal sector dark; SCP, subcostal pale; PD, preapical dark; PP, preapical pale; AD, apical dark; PHD/HP: prehumeral dark/humeral pale (key diagnostic character).

[2] *, Significantly different ($P < 0.01$).

Table 5. Range and analysis of variance of the ratios of characters measured on the legs of females of *Anopheles marajoara* from 12 localities (n = 195), *An. darlingi* from 8 localities (n = 204), *An. argyritarsis* from 1 locality (n = 20), and *An. braziliensis* from 2 localities (n = 55) from Venezuela.

Variable[1]	*An. marajoara*	*An. darlingi*	*An. argyritarsis*	*An. braziliensis*	F-value	*P*
$Ds\text{-}I_1/Ta\text{-}I_1$	0.76–0.91	0.81–0.95	0.87–0.91	0.37–0.91	52.87	0.00001*[2]
$Ds\text{-}I_2/Ta\text{-}I_2$	0.27–0.77	0.54–0.86	0.45–0.76	0.25–0.85	132.15	0.00001*
$Ds\text{-}I_3/Ta\text{-}I_3$	0.17–0.73	0.25–0.80	0.41–0.78	0.10–0.83	16.90	0.00001*
$Ds\text{-}III_2/Ta\text{-}III_2$	0.30–0.62	0.23–0.71	0.14–0.82	0.20–0.65	30.37	0.00001*

[1] $Ds\text{-}I_1$, length of dark spot on the foretarsomere 1; $Ta\text{-}I_1$, length of foretarsomere 1; $Ds\text{-}I_2$, length of dark spot on foretarsomere 2; $Ta\text{-}I_2$, length of foretarsomere 2; $Ds\text{-}I_3$, length of dark spot on foretarsomere 3; $Ta\text{-}I_3$, length of foretarsomere 3; $Ds\text{-}III_2$, length of dark spot on hindtarsomere 2; $Ta\text{-}III_2$, length of hindtarsomere 2.

[2] *, Significantly different ($P < 0.001$).

characters analyzed were not significantly different ($P > 0.05$) among populations throughout the range of distribution of this species in Venezuela, making this species more homogeneous morphologically, compared with the results obtained for *An. darlingi* (Rubio-Palis 1998). The ratios in the male genitalia were, in general, within the range of variation reported by Linthicum (1988) for *An. marajoara.* The head characters analyzed were not found to be reliable in separating *An. marajoara* from the other species of the Argyritarsis Section in Venezuela.

Adult females of *An. marajoara* can be identified by having foretarsomere 2 dark in basal 17–73%, hindtarsomere 2 dark in basal 30–62%, range of ratios of wing spots prehumeral dark : humeral pale (PHD/HP) 0.22–1.00, basal pale + prehumeral pale : wing length 0.04–0.12, prehumeral dark : wing length 0.02–0.04, presector pale : wing length

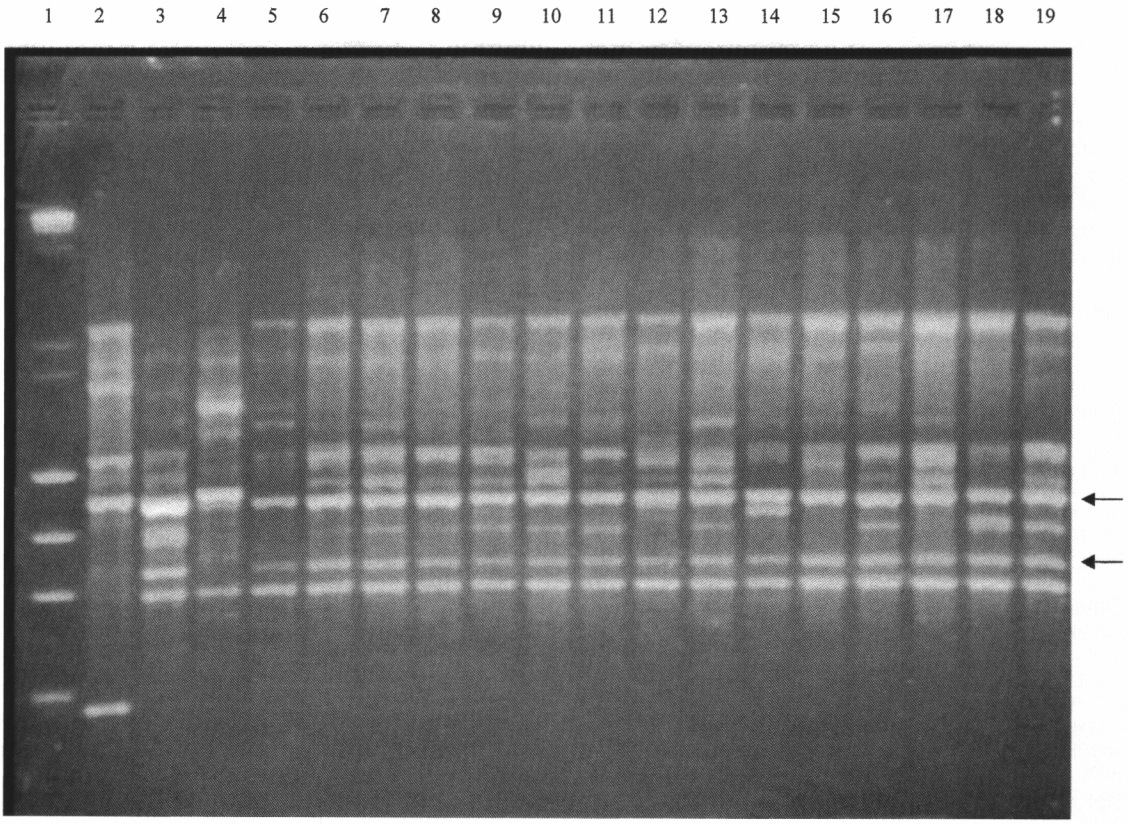

Fig. 3. Random amplified polymorphic DNA–polymerase chain reaction with operon primer C19. Gel demonstrates identification of *An. albitarsis* complex mosquitoes from Guárico State, Venezuela. The 1st and last lanes are a size standard produced by mixing lambda DNA digested with *Hind*III and Phix 174 DNA digested with *Hae*III. Lane 2 is species "B" from Ceará, Paraíba, Brazil; lane 3 is *Anopheles marajoara* from Ilha de Marajó, Brazil; and lane 4 is *An. deaneorum* from Corrientes Province, Argentina. Lanes 5–19 are confirmed as *An. marajoara* based on the occurrence of 2 diagnostic markers (arrows).

0.02–0.05, distal sector dark: wing length 0.13–0.26, and apical dark: wing length 0.02–0.06. The PHD/HP ratio represents a key character to separate *An. marajoara* from *An. braziliensis, An. darlingi,* and *An. argyritarsis.* The sector pale spot may be present or absent. Two wing patterns were observed: type I and type VI (sector pale [SP] absent), with the latter more frequent (72%). It is interesting to point out that none of these patterns are similar to the 5 patterns described by Rosa-Freitas and Deane (1989) for *An. albitarsis* s.s., although it appears that their type "a" corresponds to our type VI, but they misidentified the accessory sector pale (ASP) as SP because the ASP is the spot of pale scales associated with crossvein r_1-r_s (Wilkerson and Peyton 1990). Rosa-Freitas and Deane (1989) described 5 different patterns for *An. albitarsis* s.s. where the most frequent was when PHD was absent. None of these patterns are similar to the 8 patterns previously described for *An. darlingi* (Rubio-Palis et al. 1997, Rubio-Palis 1998, Manguin et al. 1999). The characters analyzed on wing costa and legs in populations of *An. marajoara, An. darlingi, An. argyritarsis,* and *An. braziliensis* proved to be diagnostic, confirmed by the Euclidean distances among these species estimated by cluster analysis (Rubio-Palis 1998). The diagnostic ratios identified in the present study will be useful to update keys for the identification of Venezuelan anophelines.

Amplification by RAPD-PCR of previously determined diagnostic bands for *An. marajoara* in unidentified wild-caught *An. albitarsis* s.l. further confirms this as a reliable method for species identification in this group of sibling species. So far, *An. marajoara* is the only species of the *An. albitarsis* complex to be found in Venezuela.

ACKNOWLEDGMENTS

Thanks to all those persons involved in mosquito collections. Special thanks are due to Alfredo Gutiérrez for helping with the statistical analysis. Drawings of the wing and legs were made by Belkys Pérez and Joaquín Salcedo, respectively. The study was financed by CONICIT-MPS-RPIV-1130032-9 and PCEE-I-001-97.

REFERENCES CITED

Conn JE, Wilkerson RC, Nazare O, Segura M, de Souza RTL, Schlichting CD, Wirtz RA, Póvoa MM. 2002. Emergence of a new Neotropical malaria vector facilitated by human migration and changes in land use. *Am J Trop Med Hyg* 66:18–22.

Cova-García P. 1951. *Distribución geográfica y datos bionómicos de los anofelinos de Venezuela* Publicaciones de la División de Malariología 10. Ministerio de Sanidad y Asistencia Social. Caracas, Venezuela: Imprenta Nacional.

Cova-García P, Sutil E. 1977. *Claves gráficas para la clasificación de anofelinos de Venezuela.* Maracay, Venezuela: Publ Div End Rurales, Dir Malariol San Amb MSAS.

Davis NC. 1928. A consideration of variability in the *Nyssorhynchus* group of the genus *Anopheles. Am J Hyg* 8:539–563.

Faran ME, Linthicum KJ. 1981. A handbook of the Amazonian species of *Anopheles* (*Nyssorhynchus*) (Diptera: Culicidae). *Mosq Syst* 13:1–81.

Galvão ALA. 1944. Chaves para a determinação das espécies do subgênero *Nyssorhynchus* do Brasil. *Arq Hig Saude Publica* 8:141–153.

Galvão ALA, Damasceno RG. 1942. Sobre un novo anofelino da Ilha de Marajó, *Anopheles* (*Nyssorhynchus*) *marajoara. Folia Clin Biol São Paulo* 14:60–66.

Galvão ALA, Damasceno RG. 1944. Observações sobre anofelinos do complexo *albitarsis* (Diptera, Culicidae). *Ann Sao Paulo Univ Fac Med* 20:73–87.

Harbach RE, Knight KL. 1980. *Taxonomists' glossary of mosquito anatomy* Marøn, NJ: Plexus Publishing, Inc.

Hill RB. 1928. El Paludismo en Venezuela. *Gac Med de Caracas* 35:353–359.

Klein TA, Lima JB, Toda Tang A. 1991. Hybridization evidence supporting separate species status for *Anopheles albitarsis* and *Anopheles deaneorum* (Diptera: Culicidae) in Brazil. *J Am Mosq Control Assoc* 7:301–303.

Kreutzer RD, Kitzmiller JB, Rabbani MG. 1976. Cytogenetically distinguishable sympatric and allopatric populations of the mosquito *Anopheles albitarsis. Acta Amazonica* 3:473–482.

Linthicum KJ. 1988. A revision of the Argyritarsis Section of the subgenus *Nyssorhynchus* of *Anopheles* (Diptera: Culicidae). *Mosq Syst* 25:101–271.

Lourenço-de-Oliveira R, Deane LM. 1984. What is *Anopheles allopha*? *Mem Inst Oswaldo Cruz* 79:509–510.

Manguin S, Wilkerson RC, Conn J, Rubio-Palis Y, Danoff-Burg JA, Roberts DR. 1999. Population structure of the primary malaria vector in South America, *Anopheles darlingi,* using isozyme, RAPD, ITS2 and morphological markers. *Am J Trop Med Hyg* 60:364–376.

Narang SK, Klein TA, Perera OP, Lima JB, Tang AT. 1993. Genetic evidence for the existence of cryptic species in the *Anopheles albitarsis* complex in Brazil: allozymes and mitochondrial DNA restriction fragment length polymorphisms. *Biochem Genet* 31:97–112.

Rios RJ, Nascimento LP, Oliveira AC. 1984. Complexo *Anopheles albitarsis*: imposibilidade de separá-lo em 2 subespécies *Anopheles albitarsis albitarsis* e *Anopheles albitarsis domesticus. Rev Bras Biol* 44:461–465.

Root FM. 1926. Studies on Brazilian mosquitoes. I. The anophelines of the *Nyssorhynchus* group. *Am J Hyg* 6: 684–717.

Rosa-Freitas MG. 1989. *Anopheles* (*Nyssorhynchus*) *deaneorum,* a new species in the Albitarsis Complex. *Mem Inst Oswaldo Cruz* 84:535–543.

Rosa-Freitas MG, Deane LM. 1989. The neotype of *Anopheles albitarsis* (Diptera: Culicidae). *Mem Inst Oswaldo Cruz* 84:289–302.

Rosa-Freitas MG, Deane LM, Momen H. 1990. A morphological, isoenzymatic and behavioural study of ten populations of *Anopheles* (*Nyssorhynchus*) *albitarsis* Lynch-Arribalzaga, 1878 (Diptera: Culicidae) including from type-locality-Baradero, Argentina. *Mem Inst Oswaldo Cruz* 85:275–289.

Rubio-Palis Y. 1994. Variation of the vectorial capacity of some anophelines in western Venezuela. *Am J Trop Med Hyg* 50:420–424.

Rubio-Palis Y. 1998. Caracterización morfométrica de poblaciones del vector de malaria *Anopheles* (*Nyssorhynchus*) *darlingi* Root (Diptera: Culicidae) en Venezuela. *Bol Entomol Venez* 13:141–172.

Rubio-Palis Y, Manguin S, Ayesta C, Guzmán H, Arcia JM, González J, Pérez E. 1997. Revisión taxonómica de los anofelinos vectores de malaria en el sur de Venezuela. *Bol Dir Malariol San Amb* 37:35–48.

Rubio-Palis Y, Wirtz RA, Curtis CF. 1992. Malaria entomological inoculation rates in western Venezuela. *Acta Trop* 52:167–174.

Statsoft. 1993. *Statistica for Windows 4.0* Tulsa, OK: Statsoft Inc.

Steiner WWM, Narang S, Kitzmiller JB, Swofford DL. 1982. Genetic divergence and evolution in Neotropical *Anopheles* (subgenus *Nyssorhynchus*). In: Steiner WWM, Tabachnick WJ, Rai KS, Narang S, eds. *Recent developments in the genetics of insect disease vectors* Champaign, IL: Stipes Publishing. p 523–550.

Wilkerson RC, Gaffigan TV, Lima JB. 1995a. Identification of species related to *Anopheles* (*Nyssorhynchus*) *albitarsis* by random amplified polymorphic DNA–polymerase chain reaction (Diptera: Culicidae). *Mem Inst Oswaldo Cruz* 90:721–732.

Wilkerson RC, Parsons TJ, Klein TA, Gaffigan TV, Bergo E, Consoli J. 1995b. Diagnosis by random amplified polymorphic DNA polymerase chain reaction of four cryptic species related to *Anopheles* (*Nyssorhynchus*) *albitarsis* (Diptera: Culicidae) from Paraguay, Argentina and Brazil. *J Med Entomol* 32:697–704.

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