

MORPHOMETRIC VARIABILITY OF *ANOPHELES PSEUDOPUNCTIPENNIS*
(DIPTERA: CULICIDAE) FROM DIFFERENT ECOREGIONS
OF ARGENTINA AND BOLIVIA

MARÍA J. DANTUR JURI¹, JONATHAN LIRIA², JUAN C. NAVARRO³, ROBERTO RODRIGUEZ⁴ AND GARY N. FRITZ⁵

¹Instituto Superior de Entomología "Dr. Abraham Willink", Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán, Miguel Lillo 205, T4001MVB, Tucumán, Argentina

²Departamento de Biología, Facyt, Universidad de Carabobo, Valencia edo. Carabobo, C. postal 2005, Venezuela

³Laboratorio de Biología de Vectores, Instituto de Zoología Tropical, Universidad Central de Venezuela, Apdo. 47058 Caracas 1041-A, DC

⁴Ministerio de Salud y Previsión Social, Laboratorio de Entomología Medica, Escuela Técnica de Salud Boliviano Japonesa, Avenida Ancieto Arce No. 440, Cochabamba, Bolivia

⁵Department of Biological Sciences, Eastern Illinois University, 600 Lincoln Ave., Charleston, IL 61920, USA

ABSTRACT

Anopheles pseudopunctipennis is a New World species extending from about 30°S in central Argentina to about 40°N in the USA. It is considered one of the most important malaria vectors in South America and its implication in malaria transmission varies along its distribution. We evaluated the effect of ecoregion and geographical distances on the traits or morphological characters of *An. pseudopunctipennis* females from 15 localities in Argentina and Bolivia using cladistic and morphometric analyses. The cladistic analysis (Maximum Parsimony) demonstrated that the effect of ecoregion characterization in distinguishing between Bolivian and Argentinean populations was stronger than the effect of geographical distances. Morphometric analysis (Discriminant analysis) did not reveal a pattern between Bolivian and Argentinean populations based on geographical distances. Ecoregional characterization of *An. pseudopunctipennis* populations throughout its range, caused us to conclude that studies of morphological traits characteristics are necessary in differentiating between populations. Moreover, molecular studies are required to determine whether geographical distances influence population differentiation in *An. pseudopunctipennis* from Argentina and Bolivia, in the extreme south of South America.

Key Words: *Anopheles pseudopunctipennis*, malaria, cladistics, morphometrics, Argentina, Bolivia

RESUMEN

Anopheles pseudopunctipennis es una especie Neotropical con una amplia distribución geográfica desde los Estados Unidos hasta el centro de Argentina. Es considerado uno de los más importantes vectores de malaria en América del Sur, variando su implicancia en la transmisión de la enfermedad a lo largo de su distribución. En el presente trabajo evaluamos el efecto de las características de las ecoregiones y de las distancias geográficas sobre los rasgos o caracteres morfológicos de las hembras de *An. pseudopunctipennis* de 15 localidades de Argentina y Bolivia utilizando análisis cladísticos y morfométricos. El análisis cladístico (Máxima Parsimonia) demostró que el efecto de las características de las ecoregiones fue mayor que el efecto de las distancias geográficas, diferenciando entre las poblaciones de Bolivia y de Argentina. El análisis morfométrico (Análisis Discriminante) basado en las distancias geográficas fue incapaz de mostrar un patrón entre ambas poblaciones. Concluimos que los estudios sobre los rasgos o características morfológicas como resultado de la caracterización ecoregional de las poblaciones de *An. pseudopunctipennis* son necesarias. Además, se requieren de estudios moleculares para determinar si las distancias geográficas influyen sobre la diferenciación de las poblaciones de *An. pseudopunctipennis* de Argentina y Bolivia, en el extremo sur de América del Sur.

Anopheline mosquitoes transmit the malaria parasites *Plasmodium vivax*, *P. falciparum*, *P. ovale*, *P. malariae* and *P. knowlesi*, which affect

the health of more than 40% people in 90 countries. The World Health Organization estimates malaria incidence at 300-500 million clinical

cases, with 1.5-2.7 million deaths per year (Collins & Paskewitz 1995; Sallum et al. 2000).

Species of *Anopheles* Meigen reported to be important malaria vectors include *An. (Nyssorhynchus) albimanus* Wiedeman, *An. (Nyssorhynchus) aquasalis* Curry, *An. (Nyssorhynchus) darlingi* Root and *An. (Anopheles) pseudopunctipennis* Theobald (Forattini 1962). Some species of the *Nyssorhynchus* subgenus involved in malaria transmission share the same geographical distribution and possess a high degree of morphological similarity (Faran 1980). For example, *An. (Nyssorhynchus) evansae* Brèthes was misidentified as *An. (Nyssorhynchus) benarrochi* Gabaldón, Cova García and López and, furthermore, *An. benarrochi* females were misidentified as *An. (Nyssorhynchus) oswaldoi* Peryassu, confirmed later as *An. benarrochi* species (Quiñones et al. 2001; Calle et al. 2002).

The present study was focused on *An. pseudopunctipennis*, a species with a wide distribution that has distinctive populations defined by allozymes and restriction fragment length polymorphisms (RFLPs) (Estrada-Franco et al. 1993). A more extensive study revealed the existence of three clusters within *An. pseudopunctipennis*: one comprising specimens from the United States, Mexico and Guatemala, a second cluster including samples from Colombia, Ecuador, Peru, Chile and Argentina, and a third cluster comprising Grenada specimens (Manguin et al. 1995). Rueda et al. (2004), describing the neotype of *An. pseudopunctipennis*, suggested the need for morphological, molecular and biochemical studies for a better definition of the species limit.

Within the morphological context, morphometry (Rohlf & Marcus 1993; Adams et al. 2004) appears as an important taxonomic tool for species discrimination and species variations (Dujardin et al. 1997, 1998; Calle et al. 2002; Jaramillo et al. 2002; Monroy et al. 2003; Belen et al. 2004; Lehmann et al. 2005; Yurtas et al. 2005; Feliciangeli et al. 2007). Delgado & Rubio-Palis (1993) examined the morphometric variation of *Anopheles (Nyssorhynchus) nuneztovari* Gabaldón and detected variability within Venezuelan populations. Later, Rubio-Palis (1998, 2000), using measurements of larval and adult characters of *An. darlingi* populations from Venezuela, confirmed the separation of this species from *Anopheles (Nyssorhynchus) marajoara* Galvão and Damasceno, *Anopheles (Nyssorhynchus) braziliensis* (Chagas) and *Anopheles (Nyssorhynchus) argyritarsis* Robineau-Desvoidy, and found biological and morphological variations of this species, suggesting it may represent a species complex. Most of these studies were focused on the molecular or morphological characterization of *Anopheles* species to probe similarity or variability between them or within each,

sharing the same geographical areas, and trying to differentiate these populations and their implications as malaria vectors.

In the present paper we used cladistics and morphometric analysis to test whether either ecoregional characterization or geographical distance has a greater effect in differentiating *An. pseudopunctipennis* populations found in the Transitional and Yungas ecoregions of Argentina and Convergence ecoregion of Bolivia.

MATERIALS AND METHODS

Sample Area Characterization

Adult mosquitoes were collected in 15 localities, 13 in Argentina and two in Bolivia. In Argentina, Jujuy and Salta localities were from the Yungas ecoregion, while the Tucumán localities were from the transitional area between Yungas and Chaco ecoregions (Cabrera & Willink 1973; Dinerstein et al. 1995). Parque Nacional Carrasco (Cochabamba, Bolivia) is in the convergence of three ecoregions: Cloud Forests (Yungas), Dry Forests (Dry Chaco) and Tropical Amazon Rainforest (The Nature Conservancy 2008). In Taruma (Santa Cruz, Bolivia), the ecoregion is called Transitional Amazon Forest of Chiquitana (IBIF 2006) (Fig. 1).

The Yungas has a large geographical distribution in South America, from Venezuela through northwestern Argentina. It is frequently fragmented by either natural events (flooding rivers) or human activities (agriculture, pastures and wood production). The typical piedmont vegetation consists of two types of vegetation with a north-south orientation in response to temperature gradient: one is the forest of "palo blanco" (*Calycophyllum multiflorum* Griseb. (Castelo) and "palo amarillo" (*Phyllostylon rhamnoides* (J. Poiss.) Taub), and the second is the forest of "tipa" (*Tipuana tipu* (Benth.) Kuntze) and "pacará" (*Enterolobium contortisiliquum* (Vell.) Morong.). Both vegetation types are under anthropic pressure, that in the impoverished south is nearly completely destroyed, and that in the north is more diverse (Prado 1995; Brown et al. 2001).

The Chaco ecoregion extends from southern Bolivia through western Paraguay, southern Brazil and north-central Argentina. It is composed of deciduous xeric forests with grasses, cacti and terrestrial bromeliads, and, also, the savannas and the halophytic steppes (Cabrera & Willink 1973; Cabrera 1976; Dinerstein et al. 1995). In western Chaco province, there is a transitional area (with the Yungas) (Ayarde 1995). The Chaco province is threatened by cattle grazing, seasonal fires and the conversion of natural habitats for agriculture (Dinerstein et al. 1995).

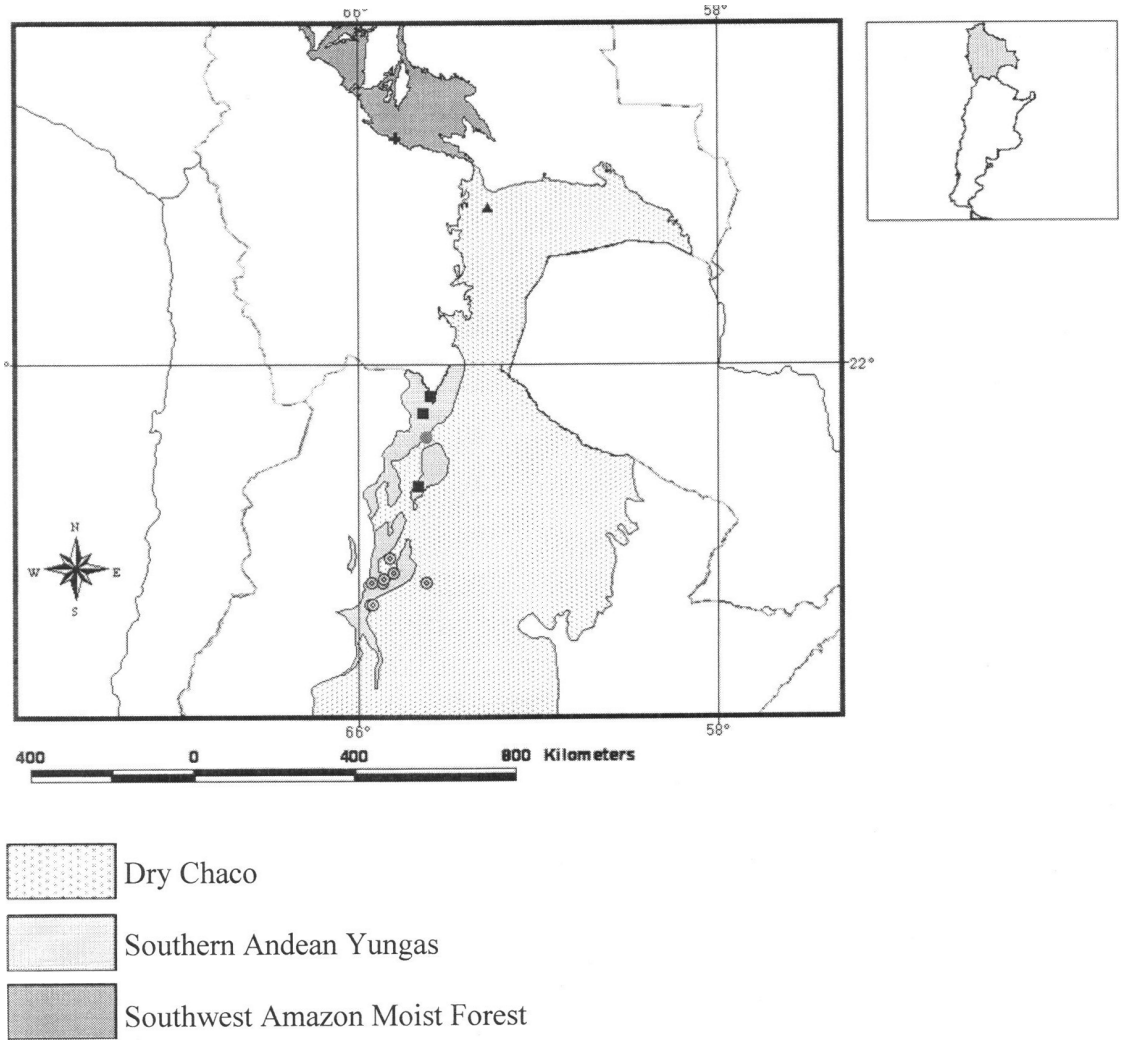


Fig. 1. Localities of *An. pseudopunctipennis* collection in the Argentinean provinces of Salta (■), Tucumán (●) and Jujuy (●) and in the Bolivian provinces of Cochabamba (⊕) and Santa Cruz (▲), within three ecoregions (after Dinerstein et al. 1996).

Parque Nacional Carrasco near Cochabamba, Bolivia lies in the Sub-Andean range. This park has vegetation typical of the semihumid Puna, the perennial wet to semi-wet forest, the Amazonic Sub-Andean forest, the pre-Andean forest, the inter-Andean dry forest and the Tucumani-Bolivian subtropical mountainous forest, also known as Yungas. The main problems in this area are deforestation to allow cultivation, commercialization of tree species of economic value, and highway construction (boliviaenlared.com 2005-2006).

At Tarumá (Santa Cruz Department in eastern Bolivia), the climate in the region largely de-

termines the predominant vegetation known as the Transitional Amazon Forest of Chiquitana (IBIF 2006). This is a characteristic forest with Amazonian humid weather alternating with Chaco dry weather. Thus two well-marked seasons exist, a dry and a wet one. During the wet season (Apr-Nov) most of the rainfall occurs, and during the dry season (May-Jul) rainfall decreases considerably. In recent years the environment has changed and the weather has become drier. The forest is mostly dry tropical, with some interspersed savanna. To the north is the humid part of the forest and the savannas. The pastures present the most important problem because of soil degradation, but

recently sustainable agriculture has started to be used in an effort to try to conserve the environment (IBIF 2006).

Specimen sources

In Argentina, the adult specimens were collected as described by Dantur et al. (2003, 2005, 2009). In Bolivia, specimens were collected individually as larvae and reared to adults. They were subsequently sacrificed and identified using the taxonomic key of Wilkerson & Strickman (1990). To carry out the morphometric measurements (in mm), the specimens were mounted on entomological pins, labeled, and examined with a stereoscopic microscope.

One hundred and seventy-three *An. pseudopunctipennis* female specimens were analyzed: 24 from Salta (localities: Parque Nacional El Rey, El Oculito and Aguas Blancas), 41 from Jujuy (INTA Yuto and Lagunita Yuto), 51 from Tucumán (Iltico, Dique El Molino, Quebrada de Lules, Potrero Las Tablas, El Cadillal, Arroyo Molle Yaco, Arroyo Hornillo and La Sala) and 57 from Bolivia (Parque Nacional Carrasco and Taruma) (Table 1). The following characters were measured: length of proboscis and length of pal-

pomeres 1-5, pale and dark scale spots on the costal vein. For wing spots, we adopted the nomenclature proposed by Wilkerson & Peyton (1990): length of the basal pale (BP), sectoral dark (SD), subcostal pale (SCP), preapical dark (PD), preapical pale (PP).

Data analysis

Cladistics. Parsimony analysis was conducted with the software TNT 1.0 (Goloboff et al. 2005), which allows the use of continuous characters to obtain a phylogenetic hypothesis. Once the confidence intervals were calculated ($CI = \text{Mean} \pm Z_{\alpha/2} \cdot S/\sqrt{N}$) with $\alpha = 0.05$ (Norman & Streiner 1996) for each locality of *An. pseudopunctipennis* (Table 2), the algorithm proposed by Goloboff et al. (2006) was used, where the morphometric characters were treated as additive, and the ranges were optimized directly on the most parsimonious cladograms. The neotype re-description of *An. pseudopunctipennis* was used (Rueda et al. 2004) as the outgroup and for rooting cladograms. Finally, the exact algorithm of implicit enumeration for the search of the most parsimonious solution was followed.

Morphometrics: The logarithmic transformation ($\text{Log}_{10}(X + 1)$) was carried out on the complete

TABLE 1. *ANOPHELES PSEUDOPUNCTIPENNIS* COLLECTION LOCALITIES IN ARGENTINA AND BOLIVIA.

Localities	Dates	Geographical coordinates	N ¹	Collectors ²
Argentina				
Tucumán Province				
Iltico	13/12/2000	27°20'05"S 65°38'55"W	10	LMA
El Molino	14/12/1999	27°19'48"S 65°42'W	10	LMA
Quebrada de Lules	12/12/1998	26°50'S 65°40'W	1	LMA
Potrero Las Tablas	19/04/1999	26°51'S 65°27'W	10	LMA and MJDJ
El Cadillal	08/04/2000	26°36'36"S 65°12'W	4	LMA, MJDJ and GM
Arroyo Molle Yaco	30/04/1999	26°17'00"S 65°16'45"W	3	LMA
Arroyo Hornillos	22/04/1999	26°13'S 65°25'48"W	5	LMA
La Sala	29/04/1999	26°45'S 65 °23'W	10	LMA and MJDJ
Salta Province				
Parque Nacional El Rey	22/02/2000	24°42'S 64°37'48"W	4	LMA
El Oculito	05/01/2002	23°06'S 51°48'W	10	MJDJ, NV and EL
Aguas Blancas	07/01/2002	22°43'48"S 64°21'36"W	10	MJDJ, NV and EL
Jujuy Province				
INTA Yuto	26/10/2005	23°37'60"S 64°28'W	30	MJDJ, NV and EL
Lagunita Yuto	26/10/2005	23°38'22"S 64°27'11'W	11	MJDJ, NV and EL
Bolivia				
Cochabamba Department				
Parque Nacional Carrasco	03/01/1995	16°58'60"S 65°7'60"W	31	GNF and RR
Santa Cruz Department				
Taruma	09/12/1991	18°33'S 63°4'60"W	26	GNF and JEC

¹N: number of specimens.

²LMA: Lucrecia Mónica Augier, MJDJ: Maria Julia Dantur Juri, GM: Gustavo Molina, NV: Neri Vianconi, EL: Enrique Laci, GNF: Gary Fritz, RR: Roberto Rodríguez and JEC: Jan E. Conn.

TABLE 2. MORPHOMETRICS CONFIDENCE INTERVALS (95% CI) FOR MEASUREMENTS OF PALPOMERES AND BASAL PALE OF AN. PSEUDOPUCTIPENNIS COLLECTED FROM LOCALITIES IN ARGENTINA AND BOLIVIA.

	Palpomere 1		Palpomere 2		Palpomere 3		Palpomere 4		Palpomere 5		Basal Pale	
Parque Nacional El Rey	0.178	0.277	0.335	0.562	0.688	0.905	0.275	0.382	0.228	0.290	0.063	—
El Oculito	0.245	0.255	0.445	0.495	0.740	0.837	0.379	0.430	0.244	0.267	0.033	—
Aguas Blancas	0.255	0.281	0.414	0.475	0.730	0.823	0.349	0.414	0.242	0.274	0.106	—
Potrero Las Tablas	0.148	0.206	0.518	0.616	0.822	0.936	0.381	0.473	0.226	0.319	0.090	0.151
El Molino	0.122	0.146	0.513	0.599	0.808	0.906	0.394	0.480	0.238	0.273	0.022	—
Iltico	0.129	0.159	0.457	0.549	0.751	0.877	0.358	0.440	0.232	0.304	0.018	—
Arroyo Molle Yaco	0.127	0.193	0.372	0.504	0.680	0.887	0.355	0.454	0.237	0.303	0.000	—
El Cadillal	0.030	0.147	0.146	0.726	0.155	0.907	0.075	0.506	0.053	0.276	0.000	—
Quebrada de Lules	0.101	—	0.506	—	0.758	—	0.379	—	0.253	—	0.000	—
Arroyo Hornillo	0.126	—	0.435	0.596	0.658	0.859	0.305	0.494	0.189	0.327	0.000	—
La Sala	0.123	0.150	0.516	0.612	0.850	0.955	0.400	0.469	0.233	0.293	0.000	—
INTA Yuto	0.126	0.139	0.508	0.560	0.869	0.889	0.430	0.453	0.258	0.293	0.091	0.120
Lagunita Yuto	0.103	0.127	0.485	0.530	0.815	0.876	0.400	0.436	0.259	0.283	0.071	0.094
Parque Nacional Carrasco	0.099	0.116	0.451	0.498	0.680	0.730	0.348	0.376	0.208	0.231	0.093	0.107
Taruma	0.100	0.109	0.439	0.480	0.658	0.705	0.335	0.364	0.204	0.222	0.092	0.102
Grenada	—	—	0.200	0.330	0.320	0.410	0.150	0.220	0.080	0.130	—	—

TABLE 2. MORPHOMETRICS CONFIDENCE INTERVALS (95% CI) FOR MEASUREMENTS OF PALE AND DARK SCALE SPOTS ON THE COSTAL VEIN OF *AN. PSEUDOPUNCTIPENNIS* POPULATIONS SAMPLED AT LOCALITIES IN ARGENTINA AND BOLIVIA.

	Sector Dark		Subcostal Pale		Pre-apical Dark		Pre-apical Pale		Proboscis Length	
Parque Nacional El Rey	2.238	2.640	0.332	0.578	0.711	0.919	0.233	0.323	2.119	2.670
El Oculito	2.155	2.496	0.344	0.419	0.805	0.934	0.283	0.344	2.046	2.246
Agua Blancas	2.350	2.538	0.351	0.478	0.728	0.971	0.289	0.353	1.965	2.195
Potrero Las Tablas	2.629	3.156	0.325	0.416	0.922	1.101	0.254	0.313	2.303	2.533
El Molino	2.463	2.718	0.374	0.425	0.873	0.992	0.267	0.355	2.317	2.471
Iltico	2.243	2.625	0.342	0.467	0.804	0.975	0.272	0.344	2.165	2.420
Arroyo Molle Yaco	1.736	2.308	0.302	0.389	0.549	0.940	0.203	0.269	1.990	2.273
El Cadillal	1.822	2.725	0.289	0.443	0.610	1.038	0.241	0.290	0.526	2.710
Quebrada de Lules	2.401	—	0.506	—	0.708	—	0.303	—	2.249	—
Arroyo Hornillo	2.407	2.768	0.339	0.450	0.837	1.064	0.208	0.267	2.292	2.450
La Sala	2.544	2.840	0.334	0.441	0.785	1.010	0.238	0.318	2.352	2.607
INTA Yuto	2.662	2.759	0.384	0.425	0.992	1.042	0.262	0.284	2.305	2.369
Lagunita Yuto	2.543	2.719	0.388	0.430	0.929	1.010	0.250	0.301	2.181	2.277
Parque Nacional Carrasco	2.175	2.274	0.387	0.421	0.787	0.832	0.256	0.282	1.905	1.994
Taruma	2.066	2.246	0.371	0.413	0.744	0.824	0.246	0.277	1.825	1.958
Grenada	—	—	0.070	0.100	0.190	0.250	0.050	0.100	0.970	1.130

data set to minimize intrapopulation variation due to static allometry. Subsequently, the size effects were corrected following Klingenberg (1996) based on the common model of linear growth (Common Principal Components Analysis or CPCA). The CPC scores were considered as an estimate of within-group variation and were used as variables in a canonical discriminant analysis (CDA) except for CPC1, which is an estimate of the common allometric pattern (Klingenberg 1996; Dujardin 2000). The variables were introduced in the PAD 0.81 program (Dujardin 2006) to carry out a Discriminant Analysis (DA). The results were graphically represented in a scatter plot using the first two canonical discriminant functions as axes. To examine the morphological similarity between the populations, the Mahalanobis distances obtained in CDA and the Cluster Analysis with Unweighted Pair Group Method Analysis (UPGMA) NEIGHBOR 3.6 (Felsenstein 2004) were used, and the resulting dendrogram was visualized in TREEVIEW 1.6.6 (Page 2001). To test for significant differences among populations, size variables were analyzed with the Kruskal-Wallis test ($\alpha = 0.05$).

RESULTS

In the cladistic analysis six equally parsimonious trees were obtained of 4,608 steps in length, with consistency and retention indices of 0.91 and 0.82, respectively. The strict consensus of these trees (Fig. 2) depicts the Bolivian populations (Tarumá and Parque Nacional Carrasco) as more basal (ancestral), sister of the node that includes all the Argentinean populations (Salta, Jujuy and Tucumán) consisting of a polytomy that includes 7/8 of the Tucumán localities (Arroyo Mole Yaco, El Cadillal, Quebrada de Lules, El Molino, Iltico, Arroyo Hornillo and La Sala), a clade that includes a mixture of Jujuy (INTA Yuto and Lagunita Yuto) and Tucumán (Potrero Las Tablas) localities and finally a clade that contains the Salta localities (El Oculito, Parque Nacional El Rey and Aguas Blancas).



Fig. 2. Strict consensus tree of *An. pseudopunctipennis* from localities in Argentina (Salta, Jujuy and Tucumán) and Bolivia.

The node that separates Argentinean and Bolivian populations is supported by the following synapomorphies: length of palpomere 1 (character 0: 0.109-0.116 \rightarrow 0.127), length of palpomere 5 (character 4: 0.208-0.222 \rightarrow 0.237-0.253), length of the basal pale (character 5: 0.092-0.093 \rightarrow 0.018-0.033) and proboscis length (character 10: 1.905-1.958 \rightarrow 1.990-2.195). The Salta clade (El Oculito+Parque Nacional El Rey+Aguas Blancas) is characterized by the length of palpomere 1 (character 0: 0.127 \rightarrow 0.245-0.255), and Parque Nacional El Rey and Aguas Blancas clustered together by the length of the basal pale spot (character 5: 0.033 \rightarrow 0.063). The clade comprising Potrero Las Tablas, INTA Yuto and Lagunita Yuto populations is supported by the length of the basal pale spot (character 5: 0.022-0.033 \rightarrow 0.090-0.091). Populations of *An. pseudopunctipennis* in some localities can be defined by autapomorphies: Aguas Blancas by the length of the basal pale spot (character 5: 0.063 \rightarrow 0.106), Potrero Las Tablas by the length of palpomere 1 (character 0: 0.127 \rightarrow 0.0148-0.206), Iltico by the length of palpomere 1 (character 0: 0.127 \rightarrow 0.129-0.159), Quebrada de Lules by the length of sector dark spot (character 7: 0.388-0.443 \rightarrow 0.506) and Lagunita Yuto by proboscis length (character 10: 2.303-0.2305 \rightarrow 2.181-2.277).

In contrast, the DA factorial map, starting from the first nine conformation components (representing 98% of the variation of the conformation), showed partial separation of the Salta population and overlap between Tucumán and Jujuy, Cochabamba and Santa Cruz populations (Fig. 3A). The reclassification of the five populations was as follows: Salta 23/24 (95%) and Jujuy 35/41 (85%) and Cochabamba 27/31 (87%), and it was reduced in Tucumán 38/51 (74%) and Santa Cruz 17/26 (65%).

The cluster analysis UPGMA (Fig. 3B) shows the differentiation of Salta from the other four. Tucumán population differs from the cluster formed by Cochabamba, Santa Cruz and Jujuy. The size variable (Fig. 3C) showed significant differences ($p < 0.05$), from the smallest to the largest, for three groups of populations: Cochabamba, < Salta, Tucumán and Santa Cruz < Jujuy.

DISCUSSION

The geographical distribution of *An. pseudopunctipennis* is a sympatric with other species, such as *An. abimanus* and *An. argyritarsis*; but each of these species occupies a somewhat different typical environment.

As reported by Patz et al. (2000) and Alencar et al. (2009), it is known that environmental changes will modify vector-borne disease transmission patterns. Accordingly in Argentina, Burgos et al. (1994) reported that malaria and *An. pseudopunctipennis* distributions acquire different patterns when moving from the foothills to

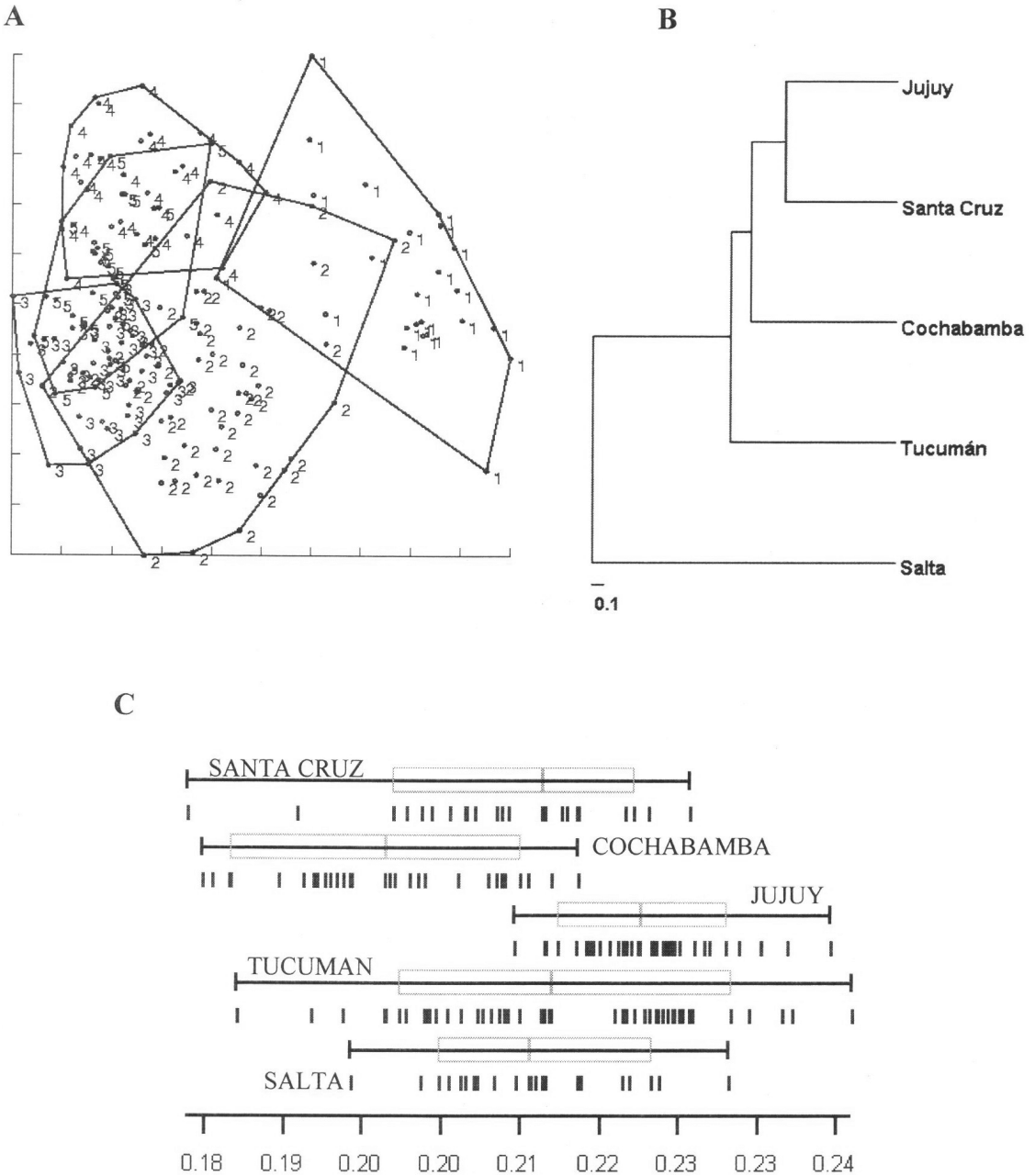


Fig. 3. A) Factorial map generated by Discriminant Analysis for the conformational variables of *An. pseudopunctipennis* in populations of Salta (1: $n = 24$), Tucumán (2: $n = 51$), Jujuy (3: $n = 41$), Cochabamba (4: $n = 31$) and Santa Cruz (5: $n = 26$). B) Dendrogram of the UPGMA from Mahalanobis distance in populations of *An. pseudopunctipennis* from Bolivia (Cochabamba and Santa Cruz) and Argentina (Jujuy, Tucuman and Salta). C) Isometric size derived from the PCA.

the central region of the country where climatic conditions are more favorable for the development of both. Curto et al. (2003) disagreed with these authors, and asserted that *An. pseudopunctipennis* and malaria distribution would change

in relation to modifications in the climatic conditions and eventually would be restricted to the extreme northwest of the country.

Environmental changes will directly affect vector populations by causing modification within

each population of traits including morphological characters that evolve under ecological pressure (Rundle & Nosil 2005; Alencar et al. 2009). Different studies have concluded that morphometry is useful because it is focused on morphological traits and modifications in them, and is a good tool for the discrimination of vector species as well as for studies of intraspecific population variability (Dujardin et al. 1997, 1998; Rubio-Palis 1998; Calle et al. 2002; Yurtas et al. 2005). Just as in *Haemagogus capricornii* Lutz and *Haemagogus janthinomys* Dyar populations in Brazil, the molecular and morphological diversity of *An. pseudopunctipennis* populations can be expected to evolve under different types of ecological pressure (ecoregional characteristics) (McKinnon et al. 2004; Alencar et al. 2009).

There are several works dealing with *An. pseudopunctipennis* populations in America. By analyses of specimens from various localities, Estrada-Franco et al. (1993) and Manguin et al. (1995) recognized the existence of three *An. pseudopunctipennis* population groups: one from the southern USA, Mexico and Guatemala, another from South America through Central America including Belize, and a third on Grenada Island. Previous to the present study, there had been no examination of external morphological characters of *An. pseudopunctipennis* populations in relation to ecoregional characteristics. This is the first report that deals with the morphological traits of specimens that have been affected by the ecological conditions that characterize each ecoregion.

In view of the risk of malaria re-emergence in the extreme northwest of Argentina, where until now only a few cases were reported, the behavior of this species becomes very important. Because there is active transmission of the disease in certain areas of Bolivia near Argentina, the need to differentiate between the various *An. pseudopunctipennis* populations in relation to transmission patterns is critical. The cladistic analyses described here accurately differentiated between Argentinean and Bolivian populations by proboscis and palpomere lengths. Similarly, Manguin et al. (1999) found that proboscis length and the forefemur differentiated populations of *An. darlingi*.

Morphometric analyses did not reveal any population differentiation of *An. pseudopunctipennis* based on geographical distances. Bolivian and Tucumán populations should be the most differentiated, but the results of the Discriminant Analysis showed that Salta populations have the least overlap. The UPGMA separated the Salta group of specimens from the remaining four population groups, and PCA detected the greatest difference between Jujuy and Cochabamba.

We conclude that the methods used were useful for the analysis of *An. Pseudopunctipennis* populations, allowing us (1) to differentiate be-

tween two *An. pseudopunctipennis* populations (Bolivian and Argentinean), and (2) to discover that the characteristics of the ecoregion, i.e., the local environment, cause such differentiation between populations to occur. Finally, it is necessary to consider the effect of the changing global weather and in particular, the consequent increase in average global temperatures and accumulated rainfall (Magnuson 2001; Moreno 2006; Alencar et al. 2009) that will affect not only malaria transmission patterns, but also cause the vector populations involved to evolve further (McMichael 2001; Alencar et al. 2009). For this reason it is necessary to acquire further knowledge about the possible existence of *An. pseudopunctipennis* populations in the extreme south of South America.

ACKNOWLEDGMENTS

The authors thank Nery Vianconi and Enrique Laci, Technicians of the National Coordination of Vectors Control, Ministry of Health of the Argentina, for their field assistance. This work was supported by Grants (PICT 01-04347; PICT 02- 12605) from Agencia Nacional de Promoción Científica y Tecnológica (FONCYT), Consejo Nacional de Investigaciones Científicas Técnicas (CONICET), Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT) and Coordinación Nacional de Control de Vectores (Ministerio de Salud de la Nación). The samples from Bolivia were collected by G. N. Fritz and R. Rodriguez, supported in part by the United States National Institutes of Health, Grant R01 AI 31034.

REFERENCES CITED

- ADAMS, D. C., ROLHF, F. J., AND SLICE, D. E. 2004. Geometric morphometrics: Ten years of progress following the 'revolution'. *Ital. J. Zool.* 71: 5-16.
- ALENCAR, J., RODRÍGUEZ-FERNÁNDEZ, J., DÉGALLIER, N., BRISOLA MARCONDES, C., MARTINS COSTA, J., AND GUIMARÃES, A. E. 2009. Multivariate Discrimination between two cryptic *Haemagogus* species associated with the transmission of Yellow Fever Virus in the Americas. *J. Am. Mosquito Control Assoc.* 25: 18-24.
- AYARDE, H. 1995. Estructura de un sector de selva pedemontana, Reserva Fiscal Parque La Florida, Tucumán, Argentina, pp. 69-78 *In* A. D. Brown and H. R. Grau [eds.], *Investigación, Conservación y Desarrollo en las selvas subtropicales de montaña*, Laboratorio de Investigaciones Ecológicas de las Yungas, Universidad Nacional de Tucumán, Tucumán, Argentina.
- BELEN, A., ALTEN, B., AND AYTEKIN, A. M. 2004. Altitudinal variation in morphometric and molecular characteristics of *Phlebotomus papatasi* populations. *Med. Vet. Entomol.* 18: 343-350.
- BOLIVIAENLARED.COM. 2005-2006. *Biología: Ecología: Áreas protegidas: Parque Carrasco*. <http://www.boliviaenlared.com/html/parque-carrasco.html> [Accessed 20 May 2008]
- BROWN, A. D., GRAU, H. R., MALIZIA, L., AND GRAU, A. 2001. *Los Bosques Nublados de la Argentina*, pp.

- 623-659 In M. Kappelle and A. D. Brown [eds.], *Bosques Nublados de Latinoamérica*, Editorial INBio, Costa Rica.
- BURGOS, J. J., CURTO DE CASAS, S. I., CARCAVALLO, R. U., AND GALINDEZ-GIRÓN, G. I. 1994. Global climate change influence in the distribution of some pathogenic complexes (malaria and chagas disease) in Argentina. *Entomología y Vectores* 1: 69-78.
- CABRERA, A. L. 1976. Regiones fitogeográficas argentinas. *Enciclopedia Argentina de Agricultura y Jardinería*, Vol. II, ACME, Buenos Aires, Argentina, 85 pp.
- CABRERA, A. L., AND WILLINK, A. 1973. *Biogeografía de América Latina*. Monografía 13, Serie de Biología, OEA, Washington, D.C.
- CALLE, D. A., QUINÓNEZ, M., ERAZO, H., AND JARAMILLO, N. 2002. Morphometric discrimination of females of five species of *Anopheles* of the Subgenus *Nyssorhynchus* from Southern and Northwest Colombia. *Memórias do Instituto Oswaldo Cruz* 97: 1191-1195.
- COLLINS, F. H., AND PASKEWITZ, S. M. 1995. Malaria: Current and future prospects for control. *Annu. Rev. Entomol.* 40: 195-219.
- CURTO, S. I., CARBAJO, A. E., AND BOFFI, R. 2003. Aplicación de Sistemas de Información Geográfica en Epidemiología. Caso de estudio: Malaria en la Argentina (1902-2000). GAEA, Sociedad Argentina de Estudios Geográficos, 2003: 239-248.
- DANTUR JURI, M. J., ZAIDENBERG, M., AND ALMIRÓN, W. R. 2003. Fluctuación estacional de *Anopheles (Anopheles) pseudopunctipennis* (Diptera: Culicidae) en un área palúdica de Salta, Argentina. *Entomología y Vectores* 10: 457-468.
- DANTUR JURI, M. J., ZAIDENBERG, M., AND ALMIRÓN, W. R. 2005. Distribución espacial de *Anopheles pseudopunctipennis* en las Yungas de Salta, Argentina. *Revista de Saúde Pública* 39: 565-70.
- DANTUR JURI, M. J., ZAIDENBERG, M., CLAPS, G. L., SANTANA, M., AND ALMIRÓN, W. R. 2009. Malaria transmission in two localities in north-western Argentina. *Malaria J.* 8: 18.
- DELGADO, N., AND RUBIO-PALIS, Y. 1993. Identification of *Anopheles (Nyssorhynchus)* (Diptera: Culicidae) occurring in Western Venezuela. *Mosquito Systematics* 25: 222-230.
- DINERSTEIN, E., OLSON, D. M., GRAHAM, D. J., WEBSTER, A. L., PRIMM, S. A., BOOKINBER, M. P., AND LEDDEC, G. 1995. Una evaluación del estado de conservación de las ecoregiones terrestres de América Latina y el Caribe. World Bank, Washington, D.C.
- DUJARDIN, J. P. 2000. Introducción a la Morfometría, con Énfasis en Triatominae y Phlebotominae. Institut de Recherches pour le Développement (IRD), Montpellier - France. <http://www.mpl.ird.fr/morphometrics/> [Accessed 15 March 2008]
- DUJARDIN, J. P. 2006. PAD (Análisis Discriminante y Permutaciones) versión 0.81. Institut de Recherches pour le Développement (IRD), Montpellier - France. <http://www.mpl.ird.fr/morphometrics/> [Accessed 15 March 2008]
- DUJARDIN, J. P., BERMUDEZ, H., CASINI, C., SCHOFIELD, C. J., AND TIBAYRENC, M. 1997. Metric differences between sylvatic and domestic *Triatoma infestans* (Hemiptera: Reduviidae) in Bolivia. *J. Med. Entomol.* 34: 544-551.
- DUJARDIN, J. P., FORGUES, G., TORREZ, M., MARTINEZ, E., CORDOBA, C., AND GIANELLA, A. 1998. Morphometrics of domestic *Panstrongylus rufotuberculatus* in Bolivia. *Ann. Trop. Med. Parasit.* 92: 219-228.
- ESTRADA-FRANCO, J. G., LANZARO, G. C., MA, M. C., WALKER-ABBEY, A., ROMANS, P., GALVAN-SANCHEZ, C., CÉSPEDES, J. L., VARGAS-SAGARNAGA, R., LAUGHINGHOUSE, A., COLUMBUS, I., AND GWADZ, R. W. 1993. Characterization of *Anopheles pseudopunctipennis sensu lato* from three countries of Neotropical America from variation in allozymes and ribosomal DNA. *Am. J. Trop. Med. Hyg.* 49: 735-745.
- FARAN, M. 1980. Mosquito Studies (Diptera: Culicidae) XXXIV. A revision of the Albimanus Section of the subgenus *Nyssorhynchus* of *Anopheles*. *Contributions of the American Entomol. Institute* 15: 1-214.
- FELICIANGELI M. D., SANCHEZ-MARTIN, M., MARRERO, R., DAVIES, C., AND DUJARDIN, J. P. 2007. Morphometric evidence for a possible role of *Rhodnius prolixus* from palm trees in house re-infestation in the State of Barinas (Venezuela). *Acta Tropica* 101: 169-177.
- FELSENSTEIN, J. 2004. NEIGHBOR-Neighbor-Joining and UPGMA methods. <http://evolution.gs.washington.edu/phylip/> [Accessed 15 March 2008]
- FORATTINI, O. P. 1962. *Entomologia Médica*. Vol. 1. Parte general, Diptera Anophelini. Faculdade de Higiene y Saude Pública, São Paulo. 662 pp.
- GOLOBOFF, P. A., FARRIS, J. S., AND NIXON, K. 2005. Tree Analysis Using New Technology (TNT). Versión 1.0. <http://www.zmuc.dk/public/phylogeny/tnt> [Accessed 15 March 2008]
- GOLOBOFF, P. A., MATTONI, C. I., AND QUINTEROS, A. S. 2006. Continuous characters analyzed as such. *Cladistics* 22: 1-13.
- INSTITUTO BOLIVIANO DE INVESTIGACIONES FORESTALES (IBIF). 2006. Estaciones de campo: La Chonta. http://www.ibifbolivia.org.bo/ESP/estaciones_de_campo/la_chonta.htm [Accessed 20 May 2008]
- JARAMILLO, N., CASTILLO, D., AND WOLFF, M. 2002. Geometric morphometric differences between *Panstrongylus geniculatus* from field and laboratory. *Memórias do Instituto Oswaldo Cruz* 97: 667-673.
- KLINGENBERG, C. P. 1996. Multivariate allometry, pp. 23-49 In L. F. Marcus, M. Corti, A. Loy, G. J. Naylor and D. E. Slice [eds.], *Advances in Morphometrics*. Proc. NATO-ASI on Morphometrics. Plenum Pres, New York.
- LEHMANN, P., ORDOÑEZ, R., OJEDA-BARANDA, R., MENDEZ DE LIRA, J., HIDALGO-SOSA, L., MONROY, C., AND RANSEY, J. M. 2005. Morphometric analysis of *Triatoma dimidiata* populations (Reduviidae: Triatominae) from Mexico and Northern Guatemala. *Memórias do Instituto Oswaldo Cruz* 100: 477-482.
- MAGNUSON, J. 2001. 150-year global ice record reveals major warming trend. *Inter-Am. Inst. Glob. Change Res.* 24: 22-25.
- MANGUIN, S., ROBERTS, D., PEYTON, E. L., FERNÁNDEZ-SALAS, I., BARRETO, M., FERNÁNDEZ-LOAYZA, R., SPINOLA, R. E., MARTINEZ-GRANAOU, R., AND RODRIGUEZ, M. H. 1995. Biochemical systematic and population genetic structure of *Anopheles pseudopunctipennis*, vector of malaria in Central and South America. *Am. J. Trop. Med. Hyg.* 53: 362-377.
- MANGUIN, S., WILKERSON R. C., CONN, J. E. RUBIO-PALIS, Y., DANOFF-BURG, J., AND ROBERTS, D. 1999. Population structure of the primary malaria vector in South America, *Anopheles darlingi*, using isoenzyme, random amplified polymorphic DNA, internal transcribed spacer 2, and morphologic markers. *Am. J. Trop. Med. Hyg.* 60: 364-376.

- MCKINNON, J. S., MORI, S., BLACKMAN, B. K., DAVID, L., KINGSLEY, D. M., JAMIESON, L., CHOU J., AND SCHLUTER, D. 2004. Evidence for ecology's role in speciation. *Nature* 429: 294-298.
- MCMICHAEL, A. J. 2001. Global environmental change as "risk factor": can epidemiology cope? *Am. J. Public Health* 91: 1172-1174.
- MONROY, C. BUSTAMANTE, D. M., RODAS, A., ROSALES, MEJIA, M., AND TABARU, Y. 2003. Geographic distribution and morphometric differentiation of *Triatoma nitida* Usinger 1939 (Hemiptera: Reduviidae: Triatominae) in Guatemala. *Memórias do Instituto Oswaldo Cruz* 98: 37-43.
- MORENO, A. R. 2006. Climate change and human health in Latin America: drivers, effects, and policies. *Region. Environ. Change* 6: 157-164.
- NORMAN, G. R., AND STRINER, D. L. 1996. *Bioestadística*. Rubes Editorial Harcourt SL, ed. Madrid: Harcourt Brace de España, S.A., pp. 150-162.
- PAGE, R. 2001. TreeView—Tree drawing software for Apple Macintosh and Microsoft Windows. <http://taxonomy.zoology.gla.ac.uk/rod/rod.html> [Accessed 20 March 2008].
- PATZ, J. A., GRACZYK, T. K., GELLER, N., AND VITTOR, A. Y. 2000. Effects of environmental change on emerging parasitic diseases. *Int. J. Parasitol.* 30: 1395-1405.
- PRADO, D. E. 1995. Selva pedemontana: contexto regional y lista florística de un ecosistema en peligro, pp. 19-52 *In* A. D. Brown and H. R. Grau [eds.], *Investigación, Conservación y Desarrollo en las selvas subtropicales de montaña*, Laboratorio de Investigaciones Ecológicas de las Yungas, Universidad Nacional de Tucumán, Tucumán, Argentina.
- QUINONES M. L., HARBACH R. E., CALLE, D. A., RUIZ, F., ERAZO, H. F., AND LINTON, Y. M. 2001. Variante morfológica de hembras de *Anopheles benarrochi* (Diptera: Culicidae) en Putumayo, Colombia. *Biomédica* 21: 351-359.
- ROLHF, F. J., AND MARCUS, L. F. 1993. A revolution in morphometrics. *Trends Ecol. Evol.* 8: 129-132.
- RUBIO-PALIS, Y. 1998. Caracterización morfológica de poblaciones de *Anopheles (Nyssorhynchus) darlingi* del sur de Venezuela. *Boletín Entomología Venezolana* 13: 141-172.
- RUBIO-PALIS, Y. 2000. *Anopheles (Nyssorhynchus)* de Venezuela: Taxonomía, bionomía, ecología e importancia médica. Escuela de Malariología y Saneamiento Ambiental "Dr. Arnoldo Gabaldon" y el Proyecto Control de Enfermedades Endémicas. Maracay, Venezuela. 120pp.
- RUEDA, L. M., PEYTON, E. L., AND MANGUIN, S. 2004. *Anopheles (Anopheles) pseudopunctipennis* Theobald (Diptera: Culicidae): Neotype designation and description. *J. Med. Entomol.* 41: 12-22.
- RUNDLE, H. D., AND NOSIL, P. 2005. Ecological speciation. *Ecol. Letters* 8: 336-352.
- SALLUM, M. A. M., SHULTZ, T. R., AND WILKERSON, R. C. 2000. Phylogeny of Anophelinae (Diptera: Culicidae) based on morphological characters. *Ann. Entomol. Soc. Am.* 93: 745-775.
- THE NATURE CONSERVANCY. 2008. Bolivia: Places we protect: Amboro and Carrasco National Park. <http://www.nature.org/wherewework/southamerica/bolivia/work/art10696.html> [Accessed 20 May 2008]
- WILKERSON, R. C., AND PEYTON, E. L. 1990. Standardized nomenclature for costal wing spots of the genus *Anopheles* and other spotted-wing mosquitoes (Diptera: Culicidae). *J. Med. Entomol.* 27: 207-224.
- WILKERSON, R. C., AND STRICKMAN, D. 1990. Illustrated key to the female anopheline mosquitoes of Central America and Mexico. *J. Amer. Mosquito Control Assoc.* 6: 7-34.
- YURTAS, H., ALTEN, B., AND AYTEKIN, A. M. 2005. Variability in natural populations of *Anopheles sacharovi* (Diptera: Culicidae) from southeast Anatolia, revealed by morphometric and allozymic analysis. *J. Vector Ecol.* 30: 206-212.