Composition and abundance of anopheline species according to habitat diversity in Mexico

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Abstract

Objective. To determine the abundance and geographic distribution of the main malaria vectors, which are influenced by habitat characteristics and ecological factors that directly impact adult density and the dynamics of malaria transmission in Mexico. **Materials and methods.** Samples of larvae were collected from 19 states in Mexico. Each larval habitat was characterized *in situ* determining the following parameters: water depth, turbidity, percentage of vegetation cover, amount of detritus, presence of algae, light intensity, type of vegetation, amount of predators, habitat stability, altitude, and hydrologic type. **Results.** A total of 21 687 larvae corresponding to 13 anopheline species were obtained from 149 aquatic habitats. The most abundant species were *Anopheles pseudopunctipennis* (52.91%), *An. albimanus* (39.14%) and *An. franciscanus* (5.29%). The multiple logistic regression analysis showed a negative association between *An. pseudopunctipennis* and water turbidity (β=-1.342; Wald=6.122; p=0.013) and the amount of detritus (β=-2.206; Wald=3.642; p=0.050). While in *An. albimanus,* there was a significant positive association with water turbidity (β=1.344; Wald=4.256; p=0.039), a negative correlation was found with the altitude (β=-3.445; Wald=5.407; p =0.020). The highest mosquito species diversity index was found in Chiapas (Fisher's α=1.20) and the lowest diversity in Chihuahua (Fisher's α=0.26). The greatest richness was found in streams (n=11). **Conclusions.** The two most abundant species were: *An. albimanus* and *An.*

Resumen

Objetivo. Determinar la abundancia y la distribución geográfica de los principales vectores de la malaria, las cuales están influenciadas por las características del hábitat y los factores ecológicos que afectan directamente la densidad de los adultos y la dinámica de la transmisión de la malaria en México. **Material y métodos.** Se obtuvieron muestras de larvas de 19 estados de México. Cada hábitat larvario se caracterizó *in situ* determinando los siguientes parámetros: profundidad del agua, turbidez, porcentaje de cobertura vegetal, cantidad de detritus, presencia de algas, intensidad de luz, tipo de vegetación, cantidad de depredadores, estabilidad del hábitat, altitud y tipo hidrológico. **Resultados.** Se identificaron un total de 21 687 larvas pertenecientes a 13 especies de anofelinos, de 149 hábitats acuáticos. Las tres especies más abundantes fueron *Anopheles pseudopunctipennis* (52.91%), *An. albimanus* (39.14%) y *An. franciscanus* (5.29%). El análisis de regresión logística múltiple mostró una asociación negativa para *An. pseudopunctipennis* y la turbidez del agua (β=-1.342; Wald= 6.122; p=0.013) y la cantidad de detritus (β=-2.206; Wald= 3.642; p=0.050). Para *An. albimanus* se encontró una asociación positiva significativa con la turbidez del agua (β=1.344; Wald= 4.256; p=0.039) y una correlación negativa con la altitud (β=-3.445; Wald=5.407; p=0.020). El índice de diversidad más alto se encontró en Chiapas (α de Fisher=1.20) y la diversidad más baja en Chihuahua (α de Fisher=0.26). La mayor riqueza se encontró en los arroyos

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pseudopunctipennis. Detailed knowledge of the distribution and characteristics of their larval habitats will be useful for the effective implementation of control strategies in Mexico.

Keywords: *Anopheles*; malaria; larval habitat; Mexico

(n=11). **Conclusiones.** Las dos especies más abundantes fueron *An. albimanus* y *An. pseudopunctipennis*. El conocimiento detallado de la distribución y características de sus hábitats larvales será útil para la implementación efectiva de las estrategias de control en México.

Palabras clave: *Anopheles*; malaria; hábitat larvario; México.

Malaria is the most important anopheles-borne disease and causes great mortality worldwide. This disease is endemic to 106 countries, primarily in the tropical and subtropical regions. In 2015, there were approximately 212 million new cases and 429 000 deaths, mostly among children. Currently, half of the world population, or about 3.2 billion people, are at risk of contracting malaria.[1] In Mexico, the number of cases have decreased significantly, from 7 259 in 2000 to 518 in 2016.[2,3] Malaria cases are concentrated in four foci: a) Sinaloa and Nayarit, b) Durango and Chihuahua, c) Campeche and Quintana Roo, and d) Chiapas (Lacandon Forest). The latter is the main focus of transmission in the country, having registered 63.70% of the cases in 2016.[3]

There are 475 species of the genus *Anopheles* in the world, 41 of which have been incriminated for transmitting the *Plasmodium* parasite.[4,5] In Mexico, there are 26 species of *Anopheles*[6,7] distributed along the country, and three were incriminated as the main vectors of malaria: *Anopheles pseudopunctipennis*, *An. albimanus* and *An. vestitipennis*.[8-11] Studies of the larval ecology of anophelines in Mexico date back to the fifties.[6] However, the geographic distribution and abundance of the species have changed due to human activities such as deforestation, increase of croplands and cattle grazing areas, road building, creation of new human settlements, and water control systems, among others.[12,13]

Recent studies on the anopheline larvae ecology were carried out mainly in southeastern Mexico (Chiapas), pointing to *An. pseudopunctipennis* as the main vector in the mountain regions. Their larvae inbreed in puddles along river margins, with a positive association with the presence of filamentous green algae, and the presence of Heteranthera.[14,15] While *An. albimanus* is the main malaria vector in the coastal plain of the Pacific and Atlantic.[6,8,16] In Chiapas, larvae of this species are positively associated to planktonic algae both in the dry and wet seasons, and negatively associated with altitude.[15] During the wet season the larvae can be found in the margins of permanent water bodies, positively associated with floating plants, and during the rainy season they are positively correlated with the presence of emergent plants, particularly Cyperaceae and flood grasses.[15,17] The main vector in the Lacandon Forest (Chiapas) is *An. vestitipennis* and its larval habitat has not been described in Mexico.[6,9] Recently, the larval habitats of *An. darlingi*, the secondary vector in the Lacandon Forest, were studied and characterized.[18] Part of the knowledge obtained in those studies has been the basis for the implementation of successful antilarval measures in Chiapas and Oaxaca, applying the technique of "Elimination of Anopheline Breeding Sites", which consists mainly in removing green algae from rivers through community participation.[19] This strategy reduced the density of *An. pseudopunctipennis* larvae and impacted the adult population in such a way that the numbers of malaria cases were significantly reduced.[20] However, this knowledge cannot be applied equally throughout the Mexican territory because of the high environmental variability caused by its biogeography, because the northern part of the country is located in the Nearctic region, and the southern part, in the Neotropical region.[21] Each biogeographical region has specific climatic, hydrologic and orographic characteristics that are unique to the larval habitats of each anopheline species.[22] It is essential to know the ecology and type of larval habitats of anophelines in order to design control measures of the immature stadia using larvicides, environmental management or the removal of filamentous algae.

Materials and methods

Study area

A descriptive cross-sectional research was carried out in 19 states in Mexico from April to February 2016. The study area was selected based on previous records on Mexican anophelines.[6] The area covers two main biotic regions: Nearctic and Neotropical. Ten states are located in the Nearctic region and nine in the Neotropical region, which include a wide variety of climates, topographic diversity, different land uses and vegetation. The climatic conditions in the Nearctic zone are very varied, but the predominant climates are arid and semi-arid (Koppen BW, BS), with precipitation ranging between 100 and 600 mm, and an annual median temperature ranging between 22°C and 26°C

in some regions, and 18°C and 22°C in the cold and temperate areas. The Neotropical region comprises the states of southern Mexico. The climate is hot and humid with dry and rainy seasons (Koppen Aw) or tropical with year-round rains (Koppen Af), a median annual temperature of 18°C to 22°C, and precipitation ranges between 2 000 and 4 500 mm.[23,24] The search for anopheline larvae was carried out at any available body of water found along the trip both inside and outside urban or rural settlements, by the side of the road, in natural and man-made habitats.

Larvae collection

Each site was visited every two to three months (from February 2012 to April 2016). The larval habitats were sampled along their margin using a 500 mL dipper, according to a standardized method.[17] The water samples were poured in a white 30×30×5 cm tray in order to collect larvae of all stadia (I to IV). The larvae were counted and placed in 1 L plastic containers to be transported to the insectary (*Centro Regional de Investigación en Salud Pública* [CRISP]) for rearing to adulthood. The samples were collected in each habitat for at least 1 hr., between 8:00 and 14:00 hours. Google Earth was utilized in the search for water body images, and the positive sites were georeferenced using a GPS (Garmin eTrex Garmin Ltd., Kansas USA).

Larvae identification and growth

The larvae were raised in the CRISP insectary in a climate-controlled room at 25±1 °C temperature, 70±10% relative humidity and a photoperiod of 12:12 (L: D) h, using a standardized technique.[25] In some cases where the distance did not allow the transportation of biological material to CRISP, an insectary was improvised in the study area under natural conditions of temperature, humidity and photoperiod. All the larvae were raised using water from the same collection site in order to minimize mortality, but taking care of using clean water free of predatory insects.[26] The larvae were fed with finely ground and sterilized commercially available mouse food (Laboratory Rodent Diet 5001, LabDiet, St. Louis, MO). All the adults obtained were mounted and accounted for in a database built for later analysis. Larval densities (LD) were quantified as total number of larvae/the number of dips.[18] All specimens were identified using the Wilkerson and Strickman (1990) keys, based on morphological characteristics.[7]

Characterization of larval habitats

Several water bodies were visited, selecting those that were positive and negative for anopheline larvae. Each larval habitat was characterized *in situ* determining the following parameters: water depth, turbidity, plant cover (%), amount of detritus, presence of algae, light intensity, vegetation type, amount of predators, water movement, habitat stability, altitude, and hydrologic type. Water depth was measured using a graduated string with a lead weight, and was categorized in three strata: 0-200 cm, 201-400 cm, and 401-600 cm. Turbidity was determined according to color as "clear or turbid". Plant cover was classified in the following groups: 0-30%, 31-60%, and 61-100%. Detritus, composed of twigs, dry leaves and dead insects, was determined according to presence in the habitat as "low, moderate or high". The algae were characterized as "present or absent". Light intensity was measured according to the amount of vegetation surrounding the habitat and categorized as "sunny, partially sunny, or shaded". Vegetation was categorized as "emergent, floating/submerged, or none". Emerging plants include aquatic vegetation and submerged terrestrial vegetation. Predators were categorized by larval habitat according to abundance: low (≤5 predators), moderate (6 to 10 predators) or abundant (≥11 predators). Water movement was determined as "stagnant, slow, or fast". Habitat stability was considered permanent if the water body persisted throughout the year and temporary if it was only present during part of the year. Altitude was determined by portable GPS as follows: 0-759 meters above the sea level (masl), 760-1 519 masl and 1 520-2 279 masl. The hydrologic type was classified according to the nature of the water body.[18]

Data analysis

A one-way analysis of variance was used (Anova) to compare the absolute larval index (ALI) between hydrologic types, and for species abundance, the results from the identification of the obtained adults were used. A multiple logistic regression analysis was used to determine the association between environmental variables and the occurrence of *An. pseudopunctipennis* and *An. albimanus* larvae. The presence of larvae was categorized as one (1) and the absence of larvae was categorized as zero (0). Data were analyzed using statistical software SPSS 20.0 (SPSS, Inc., Chicago, IL). We determined the species richness with Fisher's α test (number of spe-

cies found in each state and type of habitat), using the software "Species Diversity & Richness version 4".[27,28] Geographic distribution maps of the species were created utilizing the QGIS software Version 2.18.1.

Results

Composition and abundance of anopheline species

In this study, 19 states of the Mexican Republic were examined, selecting 169 larval habitats: 91.94% were natural, and the rest were artificial; 149 tested positive for anopheline larvae, and 20 larval habitats tested negative (figure 1). All water bodies, positive and negative, were sampled throughout the study. Lagoons and fast flowing rivers were the main negative habitats. Of the total positive larval habitats, 21 were located in the Nearctic region and 128 in the Neotropical region. In the Nearctic region of Mexico, five anopheline species were found: *An. pseudopunctipennis, An. albimanus, An. franciscanus, An. punctipennis* and *An. crucians* (figure 2). Of these species, *An. franciscanus* and *An. punctipennis* were specific to this biogeographic region; the rest of the species were found across Mexico. In central Mexico, the transition between the Nearctic and Neotropical regions (the states of Morelos and Puebla), only *An. pseudopunctipennis* was detected, in river margins. Eleven species were found in southern Mexico: *An. pseudopunctipennis, An. albimanus, An. vestitipennis, An. darlingi, An. punctimacula, An. crucians, An. hectoris, An. apicimacula, An. gabaldoni, An. argyritarsis,* and *An. eiseni.* The southern state of Chiapas presented 112 aquatic habitats and 11 mosquito species, in the Lacandon Forest area and the Soconusco area in the coastal plain (figure 2).

A total 21 687 larvae of 13 *Anopheles* species were captured and reared until adulthood with 84.48% of breeding success, i.e. obtaining 18 322 adults. The most abundant species throughout the study was *An. pseudopunctipennis* (52.92%), followed by *An. albimanus* (39.14%) and *An. franciscanus* (5.29%), which comprise 97.35% of the total specimens. The remaining 2.54 % is constituted by the least abundant species: *An. punctipennis* (1.0%), *An. vestitipennis* (0.70%), *An. darlingi* (0.49%), *An. punctimacula* (0.13%), *An. crucians* (0.11%), *An. hectoris* (0.11%), *An. apicimacula* (0.08%), *An. gabaldoni* (0.03%), *An. argyritarsis* (0.01%), and *An. eiseni* (0.01%) (figure 3). *An. pseudopunctipennis* had a significantly higher ALI (±StD) (14.05±21.11), compared to other species (F=1.897;

df=11; *p*< 0.05) (table I). As for the spatial distribution of the main malaria vectors in Mexico, *An. albimanus* was found in 13 states, *An. pseudopunctipennis* in 10 states and *An. vestitipennis* was found only in two states in southern Mexico (figure 4).

Habitat diversity and larval abundance

Twelve hydrologic types were found positive for anopheline larvae in Mexico. The most common hydrologic types were river margins (29.53%), rain puddles (16.77%), ponds (12.75%) and river pools (10.73%). The least frequent were swamps (6.71%), streams (6.71%), ditches (4.02%), pools (4.02%), lagoons (3.35%), gravel pits (2.68%), irrigation canals (2.01%), and drinking troughs (0.67%). The average ALI (±StD) was 14.55 larvae per dip (l/d) (±25.28) with a range of 0 to 153.0 l/d. The greatest ALI averages (±StD) were found in river pools 19.40 l/d (±19.46), ponds 18.99 l/d (±35.75), river margins 18.97 l/d (±31.19), gravel pits 17.65 l/d (±9.13), streams 17.20 l/d (±27.41) and ditches 16.25 l/d (±28.89). No significant differences were found in the absolute larval index (ALI) between hydrologic types (F=0.748; df=11,137; *p*=0.690) (table I).

Larval presence and associated parameters in *An. pseudopunctipennis* and *An. albimanus*

The multiple logistic regression analysis showed a significant negative association between the presence of *An. pseudopunctipennis* and water turbidity (ß=-1.342; 232 Wald=6.122; *p*=0.013) and the amount of detritus (ß=-2.206; Wald=3.642; *p*=0.050) (table II). In contrast *An. albimanus* had a significant positive association with water turbidity (ß=1.344; Wald=4.256; *p*=0.039) and a negative correlation with altitude (ß= −235 3.445; Wald=5.407; *p*=0.020) (table III).

Richness and diversity of anopheline species

The diversity of *Anopheles* species in 19 states exhibited a general Fisher's α value of 1.77. The state with the most species richness was Chiapas (n=11), followed by Coahuila (n=5). In other states, only 1-3 species were detected. The greatest diversity index was found in Chiapas (Fisher's α=1.20), and the lowest diversity index was found in Chihuahua (Fisher's α=0.26) (table IV). The greatest richness was found in streams (n=11), followed by river margins (n=9) and ponds (n=6).

FIGURE 1. STUDY AREA INDICATING THE POSITIVE AND NEGATIVE SAMPLING SITES OF ANOPHELINE LARVAE. FEBRUARY 2010 TO APRIL 2015. MEXICO

Discussion

There were 13 *Anopheles* species identified throughout Mexico in this study. *An. pseudopunctipennis* and *An. albimanus* were the most abundant species and occupied the greatest diversity of habitats, and both are considered the main malaria vectors in Mexico.[8,11] Of the two species mentioned, *An. pseudopunctipennis* exhibited the greatest larval density and was widely distributed throughout the country, in accordance with the published geographic distribution.[29] This species is reported to be the main vector between 200 and 500 masl, and to be associated with river margins with filamentous algae[14] and positively associated with altitude in southern Mexico.[15] In this national study, this species was found in an altitude range that goes from 3 to 2 279

masl, and no association was found with the altitude in the multiple logistic regression analysis. The reason for this lack of association is that this species did not exhibit at the national level a similar pattern to that reported in Tapachula, Chiapas, where populations are abundant at the mountain foothills, at an altitude above 200 masl and up to 500 masl, and is not commonly found in the coastal plain.[14,15] According to the results, we infer that, nationally, the altitude range where this vector may transmit malaria could be greater than the previously reported one of 200 to 500 masl.[3,10] These results agree with the findings of a study in the Yucatán Peninsula, which reports *An. pseudopunctipennis* in a *cenote* (its natural, permanent habitat) at 20 masl.[30] On the other hand, there is a negative association with water turbidity, which indicates that females prefer to lay their eggs

in clear rather than turbid water. This coincides with a previous report from 10 countries, spanning most of the geographic range of the species, whose larvae were found in clear-water breeding sites in 90% of the cases (i.e. in 54 out of 60 larval habitats), but is not consistent with the presence of *An. pseudopunctipennis* in turbid water habitats contaminated with cattle feces in the city of Salinas, Ecuador, and other turbid breeding sites caused by flooding in Monterrey, Mexico.[31] This study is also consistent with various studies about the presence of larval habitats with filamentous algae and their disappearance during the rainy season caused by the strong flow of water.[14,31]

An. albimanus was found in the greatest diversity of hydrologic types (11 out of 12), indicating that this species is generalist when it comes to its preference for larval breeding sites.[32] It is the species with the broadest geographic distribution, from Chiapas to Sinaloa on the Pacific side, and from Yucatán to Tamaulipas on the Gulf side, which is consistent with previous reports.[29,32] Larval habitats for *An. albimanus* were characterized as sunny and were positively associated with turbidity, but negatively associated with altitude, which is consistent with reports for southern Chiapas.[15,17] On the Pacific side, there are breeding sites for this species all along the coast up to Sinaloa, but no positive larval habitats were found further north. This habitat limitation may be due to the high temperatures and arid climate persistent in Sonora, which is consistent with previous studies.[32]
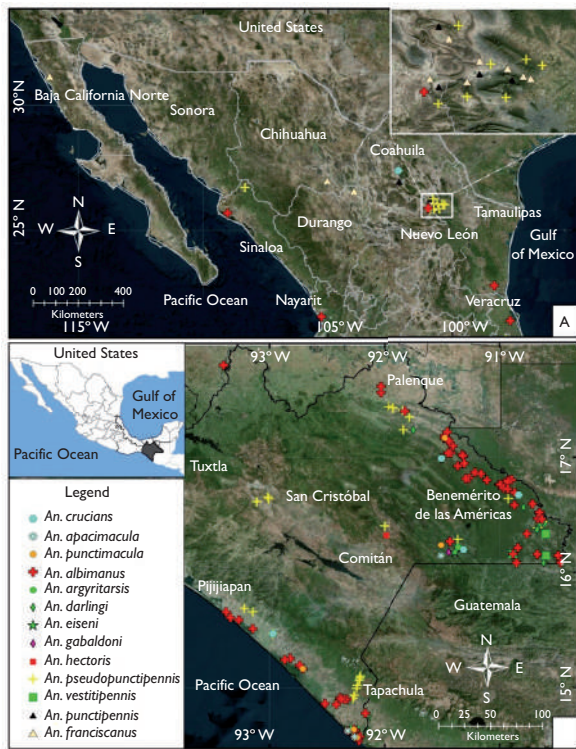

FIGURE 2. SPATIAL DISTRIBUTION OF THE OCCURRENCE POINTS OF *ANOPHELES* SPECIES IN THE NEARCTIC (A) AND NEOTROPICAL (B) AREAS OF MEXICO. FEBRUARY 2010 TO APRIL 2015
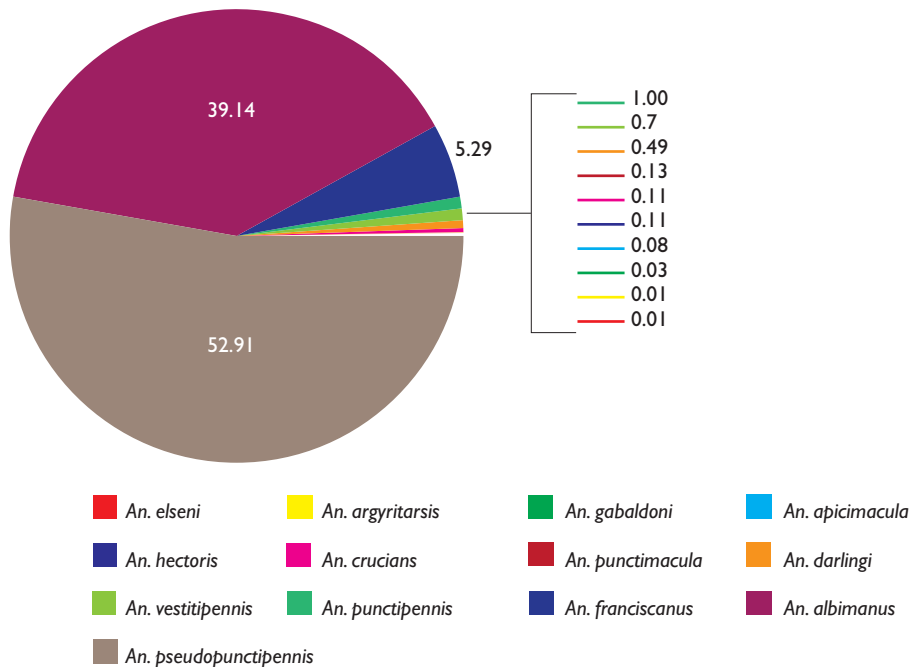

FIGURE 3. PERCENTAGE OF ANOPHELINE SPECIES IN MEXICO. FEBRUARY 2010 TO APRIL 2015

Figure 4. Spatial distribution of the occurrence points of the main malaria vectors in Mexico. a) *An. pseudopunctipennis* b) *An. albimanus*, c) *An. vestitipennis*. February 2010 to April 2015

Another abundant species was *An. franciscanus*, which was found only in northern Mexico, the Nearctic region. The geographic distribution of *An. franciscanus* in this study in northern Mexico apparently is not consistent with a previous report by Vargas and Martínez-Palacios (1956),[6] who reported this species only in northern Baja California. At the time of the study, in 1956, the status of *An. franciscanus* as a species was unclear, a problem that was solved when genetic incompatibility was found to exist between *An. pseudopunctipennis* and *An. franciscanus*, and it was concluded

that these were two different species.[33] The literature also reported *An. pseudopunctipennis* var Willardi in Sonora, Chihuahua, Coahuila and San Luis Potosí, but this variety disappeared and was integrated to the species *An. franciscanus*.[34,35] Finally the results of the present study are consistent with those studies regarding the geographic distribution reported for northern México.[6] In this study, this species was not found in Tamaulipas, as was previously reported,[36] but was found for the first time in Nuevo León and Durango. The lack of reports for this species in Nuevo León could be derived from the lack of taxonomic studies in this geographic area, or possibly because of a range expansion derived from anthropogenic changes or climate change.[12,13] Apparently *An. franciscanus* has little importance as a malaria vector today or in the past;[34] however, it is necessary to deepen taxonomical and binomial analyses in the states of Sinaloa, Nayarit, Durango and Chihuahua, where malaria cases have been reported.[3] The vector *An. vestitipennis* was found only in southern Mexico, in the Neotropical region, where it is one of the main malaria vectors in the Lacandon Forest.[9] Larval habitats for this species in the Lacandon Forest were found in ponds, ditches and rain puddles. The most abundant larval habitat were ditches, which are caused by man-made changes in orography, because road building avoids natural river paths, allowing the formation of water bodies that are attractive for this species. The same pattern coincides in a report from Colombia, where road building favored the development of several species of *Anopheles*.[32] Further studies are required about the incrimination of vectors in the regions of Soconusco, Chiapas, and Catemaco, Veracruz, where this species was found and its role as a malaria vector is unknown. This study describes for the first time the characteristics of the larval habitats of this species in the Lacandon Forest area in Chiapas.

The greatest diversity of anopheline species was found in the Lacandon Forest area in Chiapas, with 11 species and the highest Fisher's α diversity value: 1.20. These results are consistent with studies in other regions of the world, where it has been confirmed that tropical zones have the greatest biodiversity of insect species.[37] It must be noted that one of the main historical references of abundance and distribution of Mexican anophelines (published in 1956),[6] does not mention any anopheline species for the Lacandon Forest in Chiapas. At that time in the 1950s, despite there being populated areas in the Lacandon Forest, there were few taxonomic works,[38-40] mainly due to the difficult access to these areas.[41] At present, despite the Lacandon Forest being the most important malaria focus in Mexico,[3] very little has been written about vectors.[9] This is the first report that exposes the high anopheline biodiversity for this

Table I
Average adult density (±StD) of anophelines obtained by hydrologic type, in 19 States of Mexico. February/2010 to April/2015

Hydrologic type	n	Ap	Aa	Af	Apu	Av	Ad	Apc	Ac	Ah	Api	Ag	Aar	Ae
River margin	56	18.35±27.43	9.65±16.80	4.83±0.28	5.00	0	0.01±0.008	0.55±0.07	0.55±0.63	2.00	0.30±0.28	0.50	0.10	0.10
Rain puddle	25	1.20±0.90	6.87±17.65	0	0	2.10	0.07±0.03	0.10	0	0	0	0	0	0
Pond	19	23.62±28.53	11.91±16.60	0	0	0.65±0.77	0.10±0.06	0.50	0.10	0	0	0	0	0
River pool	16	16.93±14.81	4.80±5.18	2.00	5.00	0	0	0	0	0	0	0	0	0
Swamp	10	0.53±0.58	5.76±2.51	0	0	0	0	0	0.40	0	0.80	0	0	0
Stream	10	10.46±11.65	3.93±3.61	2.30±1.85	2.76±0.49	1.00	0.18±0.15	0	0.40	0	0	0	0	0
Ditch	6	0.87±1.03	3.25±1.06	70.00	0	2.33±1.75	0.25±0.17	0	0	0	0	0	0	0
Ground pool	6	1.70±0.70	5.44±3.88	0	0	0	0.21±0.08	0	0	0	0	0	0	0
Lagoon	14	0	2.85±3.04	0	0	0	0	0	0	0	0	0	0	0
Gravel pit	4	14.25±6.35	2.95±2.41	0	0	0	0	0	0	0	0	0	0	0
Irrigation canal	3	0	11.6±16.60	0	0	0.75±0.35	0	0.60	0	0	0	0	0	0
Drinking trough	1	0	0	1.20	0	0	0	0	0	0	0	0	0	0
Total	149	14.05±21.11	7.54±13.45	9.69±21.26	3.66±1.27	1.43±1.23	0.47±0.36	0.46±0.20	0.40±0.36	2.00	0.46±0.35	0.50	0.10	0.10

Ap=An. pseudopunctipennis; Aa=An. albimanus, Af=An. franciscanus, Apu=An. punctipennis, Av=An. vestitipennis, Ad=An. darlingi, Apc=An. punctimacula, Ac= An. crucians, Ah=An. hectoris, Api=An. apicimacula, Ag=An. gabaldoni, Aar=An. argyritarsis, Ae=An. eiseni.

Table II
Multiple logistic regression analysis of the larval abundance and environmental parameters for *Anopheles pseudopunctipennis* in Mexico. February 2010 to April 2015

Variables	Coefficient (ß)	S.E.	Wald	df	Sig	Exp(ß)	CI95% Exp(ß)	
							Lower limit	Upper limit
Interception	1.126	2.557	0.194	1	0.660			
Water depth								
0-200	0			0				
201-400	-1.156	0.000		1		0.315	0.315	0.315
401-800	0			0				
Turbidity								
Clear	-1.342	0.542	6.122	1	0.013	0.261	0.090	0.757
Turbid	0			0				
Coverage %								
0-30	-0.564	0.808	0.487	1	0.485	0.569	0.117	2.771
31-60	-0.592	0.945	0.393	1	0.531	0.553	0.087	3.525
61-100	0			0				
Detritus								
Low	-1.148	0.692	2.756	1	0.097	0.317	0.082	1.230
Moderate	0			0				
High	-2.206	1.156	3.642	1	0.050	0.110	0.011	1.061
Algae								
Absent	0.656	0.675	0.943	1	0.331	1.927		
Present	0			0				
Light intensity								
Sunny	-0.694	0.892	0.605	1	0.437	0.500		
Sun/shade	-1.320	0.976	1.829	1	0.176	0.267		
Shade	0			0				
Vegetation								
Emergent	-0.007	1.362	0.000	1	0.996	0.993	0.069	14.325
Floating/submerged	0.385	1.396	0.076	1	0.783	1.469	0.095	22.675
None	0.119	1.471	0.007	1	0.935	1.127	0.063	20.137
Predators								
Low	0.452	0.539	0.705	1	0.401	1.572	0.547	4.517
Moderate	0			0				
Abundant	-1.121	1.315	0.727	1	0.394	0.326	0.025	4.291
Water movement								
Stagnant	0			0				
Slow	0			0				
Fast	0.052	1.165	0.002	1	0.964	1.053	0.107	10.344
Habitat stability								
Permanent	0.417	0.706	0.348	1	0.555	1.517	0.380	6.054
Temporary	0			0				

(continuation)

Altitude								
0 -759	1.264	1.018	1.541	1	0.214	3.540	0.481	26.056
760 -1 519	0							
1 520 -2 279	0.926	1.558	0.353	1	0.552	2.524	0.119	53.487
Hydrologic type								
Ditch	-1.439	1.488	0.935	1	0.333	0.237	0.013	4.381
Drinking trough	20.230	0.000		1		6.104E-5	6.104E-5	6.104E-5
Gravel pit	-19.127	7 132.062	0.000	1	0.998	4.934E-9	0.000	
Pools	-1.223	1.488	0.675	1	0.411	0.294	0.016	5.439
Irrigation canal	18.154	8 502.676	0.000	1	0.998	76 627 495.02	0.000	
Lagoon	18.715	6 306.399	0.000	1	0.998	134 229 308.46	0.000	
Pond	0.663	1.232	0.290	1	0.590	1.941	0.174	21.703
Rain puddle	1.471	1.299	1.282	1	0.257	4.355	0.341	55.594
River margins	-0.752	1.031	0.532	1	0.466	0.472	0.063	3.555
River pools	-1.395	1.242	1.262	1	0.261	0.248	0.022	2.826
Creeks	-0.408	1.304	0.098	1	0.755	0.665	0.052	8.572
Swamps	0			0				

Sig: significant; df: degrees of freedom

Table III

MULTIPLE LOGISTIC REGRESSION ANALYSIS OF LARVAL ABUNDANCE AND ENVIRONMENTAL PARAMETERS FOR *ANOPHELES ALBIMANUS* IN MEXICO. FEBRUARY 2010 TO APRIL 2015

Variables	Coefficient ß	S.E.	Wald	df	Sig	Exp(ß)	CI95% Exp(ß)	
							Lower limit	Upper limit
Interception	1.317	2.580	0.261	1	0.610			
Water depth								
0-200	0.054	0.797	0.005	1	0.946	1.056	0.221	5.040
201-400	0			0				
401-600	0			0				
Turbidity								
Clear	1.344	0.652	4.256	1	0.039	3.836	1.069	13.760
Turbid	0			0				
Coverage %								
0-30	-0.005	0.922	0.000	1	0.995	0.995	0.163	6.058
31-60	-0.703	1.229	0.327	1	0.568	0.495	0.045	5.509
61-100	0			0				
Detritus								
Low	1.528	0.829	3.394	1	0.065	4.609	0.907	23.421

(continues…)

(continuation)

Moderate	0			0				
High	1.421	1.287	1.219	1	0.270	4.140	0.333	51.534
Algae								
Absent	-0.544	0.835	0.424	1	0.515	0.581	0.113	2.985
Present	0			0				
Light intensity								
Sunny	0.225	0.937	0.058	1	0.810	1.252	0.199	7.865
Sun/shade	0.843	0.966	0.762	1	0.383	2.324	0.350	15.422
Shade	0			0				
Vegetation								
Emergent	0.234	0.694	0.114	1	0.736	1.264	0.324	4.923
Floating/submerged	0			0				
None	0.651	0.944	0.475	1	0.491	1.917	0.301	12.204
Predators								
Low	-0.157	0.637	0.061	1	0.805	0.854	0.245	2.980
Moderate	0			0				
Abundant	-16.759	7 018.994	.0000	1	0.998	5.27E8	0.000	
Water movement								
Stagnant	0			0				
Slow	0			0				
Fast	1.886	1.335	1.996	1	0.158	6.594	0.482	90.252
Habitat stability								
Permanent	-1.456	0.828	3.092	1	0.079	0.233	0.046	1.182
Temporary	0			0				
Altitude								
0 - 759	-3.445	1.486	5.407	1	0.020	0.032	0.002	0.581
760 - 1 519	0			0				
1 520 - 2 279	15.470	0.000		1		5 230 658.306	5 230 658.306	5 230 658.306
Hydrologic type								
Ditch	1.531	1.993	0.590	1	0.443	4.621	0.093	229.885
Drinking trough	34.453	0.000		1		9.180E-14	9.180E-14	9.180E-14
Gravel pit	-19.824	7 540.604	0.000	1	0.998	2.457E-9	0.000	
Pools	0.969	1.935	0.251	1	0.617	2.635	0.059	116.950
Irrigation canal	-17.419	9 242.478	0.000	1	0.998	2.722E-8	0.000	
Lagoon	2.077	1.674	1.540	1	0.215	7.984	0.300	212.396
Pond	-1.091	1.623	0.452	1	0.501	0.336	0.014	8.079
Rain puddle	-3.157	1.936	2.660	1	0.103	0.043	0.001	1.891
River margins	0.808	1.380	0.343	1	0.558	2.244	0.150	33.555

(continues…)

(continuation)

River pools	0.791	1.505	0.276	1	0.599	2.205	0.115	42.124
Creeks	-0.282	1.711	0.027	1	0.869	0.754	0.026	21.568
Swamps	0			0				

Sig: significant; df: degrees of freedom

Table IV
Ecologic diversity of anophelines in 19 states in Mexico. February 2010 to April 2015

Geographic region/State	Number of habitats	Number of larvae	Species richness	Fisher's α
Nearctic region				
B. California	1	10	1	0.71
Sinaloa	2	1 160	2	0.38
Nayarit	1	25	1	0.50
Chihuahua	1	750	1	0.26
Coahuila	3	959	5	1.00
Durango	1	12	1	0.65
Nuevo León	6	4 245	3	0.42
Tamaulipas	1	60	2	0.65
Puebla	4	285	1	0.33
Morelos	1	65	1	0.39
Neotropical region				
Jalisco	1	55	1	0.50
Colima	1	32	1	0.51
Guerrero	1	56	1	0.41
Oaxaca	3	987	2	0.38
Chiapas	112	11 667	11	1.20
Veracruz	6	816	3	0.59
Tabasco	1	88	1	0.50
Campeche	1	65	1	0.41
Quintana Roo	2	350	2	0.45
Total	149	21 687	13	1.77

area. The high incidence of malaria cases in this region could be accounted for by the high diversity of species, as was reported in Colombia, where there was a positive correlation between richness of anopheline species and risk of transmission.[42,43] Another phenomenon that may cause the high incidence of malaria could be the movement of people between communities for work or business, or the emigration of people from endemic areas who are traveling north or to the United States, which results in elevated transmission risk for *P. vivax* and *P. falciparum* in this area.[3,44]

The second region with the greatest species diversity, with a Fisher's α diversity index of 1.0, is the state of Coahuila, in northeastern Mexico, where we found five species: *An. crucians*, *An. franciscanus*, *An. punctipennis*, *An. pseudopunctipennis* and *An. albimanus*. In this area, the climate is extreme and semi-desertic (Koppen *BS*). This area in northeastern Mexico is free of malaria despite the high species diversity. The explanation is complex and may be attributable to environmental factors such as extreme climate with very hot summers that reach up to 45 °C and cold winters, as low as -8 °C, most human

dwellings are made of concrete and use screens in the windows, compared to Oaxaca, Chiapas and Tabasco in southeastern Mexico, where most houses in the rural areas are made of plant matter or wood with palm roofs.[23] On the other hand, vector characteristics such as abundance, seasonal distribution, life expectancy, degree of antropophilia and vector competition may influence the malaria transmission cycle.[45,46]

The success of focalized control is based on community involvement or the search and treatment of sick people and environmental management through elimination of anopheline breeding sites (the ECA, "*Eliminación de Criaderos de Anofelinos*").[3,19] This control strategy had a resounding success in Oaxaca in the nineties and may be used as a model to be implemented in other areas with active transmission or during new disease outbreaks. In order to ensure the elimination of malaria in Mexico, it is necessary to eliminate or manage those larval habitats at risk in or near communities, applying antilarval measures and based on the knowledge about larval ecology in every specific region and for every anopheline species.[46]

Funding

Acknowledgements

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