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# Diversity of mosquitoes and the aquatic insects associated with their oviposition sites along the Pacific coast of Mexico

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## Abstract

**Background:** The abundance, richness and diversity of mosquitoes and aquatic insects associated with their oviposition sites were surveyed along eight states of the Pacific coast of Mexico. Diversity was estimated using the Shannon index ( $H'$ ), similarity measures and cluster analysis.

**Methods:** Oviposition sites were sampled during 2–3 months per year, over a three year period. Field collected larvae and pupae were reared and identified to species following adult emergence. Aquatic insects present at oviposition sites were also collected, counted and identified to species or genus.

**Results:** In total, 15 genera and 74 species of mosquitoes were identified: *Anopheles pseudopunctipennis*, *An. albimanus* and *Aedes aegypti* were the most abundant and widely-distributed species, representing 47% of total mosquito individuals sampled. New species records for certain states are reported. Anopheline diversity was lowest in Sinaloa state ( $H' = 0.54$ ) and highest in Chiapas ( $H' = 1.61$ ) and Michoacán ( $H' = 1.56$ ), whereas culicid diversity was lowest in Michoacán ( $H' = 1.93$ ), Colima ( $H' = 1.95$ ), Sinaloa ( $H' = 1.99$ ) and Jalisco ( $H' = 2.01$ ) and highest in Chiapas ( $H' = 2.66$ ). In total, 10 orders, 57 families, 166 genera and 247 species of aquatic insects were identified in samples. Aquatic insect diversity was highest in Chiapas, Oaxaca and Michoacán ( $H' = 3.60-3.75$ ). Mosquito larval/pupal abundance was not correlated with that of predatory Coleoptera and Hemiptera.

**Conclusion:** This represents the first update on the diversity and geographic distribution of the mosquitoes and aquatic insects of Mexico in over five decades. This information has been cataloged in Mexico's National Biodiversity Information System (SNIB-CONABIO) for public inspection.

## Background

Vector-borne diseases transmitted by mosquitoes of the family Culicidae are responsible for ~1.4 million deaths per year [1] and 17% of all infectious diseases worldwide [2]. The principal pathogens transmitted by these vectors include viruses (dengue, yellow fever, equine encephalitis, etc.), protozoa (e.g., those causing malaria), and nematodes (e.g. those causing filariasis) [3]. Overall, fewer than 150 species of the genera *Anopheles*, *Aedes* and *Culex*, are the indirect cause of morbidity and mortality among humans, more than any other group of organisms [4].

To date, between 18 and 20 genera and 225–247 species of mosquitoes have been reported from Mexico [5-7]. However, only the subfamilies Anophelinae and Culicinae include vector species of medical or veterinary importance, especially those from the genera: *Aedes*, *Anopheles*, *Culex*, *Haemagogus*, *Mansonia*, *Sabethes*, *Psorophora*, and *Coquillettidia* [8,9]. These species oviposit in a wide range of aquatic habitats that also harbor numerous species of aquatic insects and plant species with which they interact. Species interactions are central to the ecology of any habitat, including those of mosquitoes [10]. Identification of the habitats selected as oviposition sites is of clear relevance to mosquito surveillance programs as these habitats are also targeted by mosquito control measures involving habitat elimination or larvicid- ing activities [11].

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Studies on the bionomics of major mosquito vectors in Mexico have been made [12-16], but few studies have addressed their interactions with other organisms, particularly those involving aquatic insects associated with their oviposition sites [17-21]. Overall 13 orders of insects include species with aquatic or semi-aquatic stages, representing >95% of macroinvertebrate species present in aquatic habitats [22,23]. Aquatic insects play key roles in the ecology of aquatic ecosystems and together with other invertebrates, exert an important influence on nutrient cycles and the structure of trophic webs [24]. Apart from their use as biological indicators to evaluate water quality [25], aquatic insects, particularly predatory insects, can play an important role in the biological control of larval and pupal mosquito populations [26].

In this study we describe a comprehensive analysis on the richness, diversity and geographical distribution of mosquitoes in Mexico and the aquatic insects associated with their oviposition sites. The entomological surveys in this study were restricted to the oviposition and immature development sites of mosquitoes because information on the diversity and distribution of endemic vector species is essential to develop vector monitoring and control strategies, which depend on the identity of mosquito species present in each state for effective implementation. This is because public health programs aimed at vector control are decided on a state-by-state basis, depending on state administration budgets and the perceived importance of mosquito control measures and vector borne diseases in each of the 31 states of Mexico. Similarly, this type of baseline information allows detection of changes in the distribution or abundance of species and detection of introduced species of vectors that have extended beyond their natural distribution or biogeographic areas (termed invasive species), and which can cause environmental, economic, and human health impacts.

## Methods

### Study area

The study area comprised eight states of the Pacific coast of Mexico, namely Sinaloa, Nayarit, Jalisco, Colima, Michoacan, Guerrero, Oaxaca and Chiapas (Figure 1). The climatic conditions along the Pacific coast region were predominantly characterized by a warm humid climate, with average annual temperature between 22° and 26°C and an annual rainfall of 1,000 to 2,000 mm [27]. The experimental design consisted of three rounds over the eight study states, one per year for about 55 days, the first was from October 13 to December 6, 2007, the second from September 20 to November 13, 2008 and the third from May 30 to July 23, 2009, giving a total of 165 days of sampling. The selection of sampling sites was based on the reports of type localities for native species of mosquitoes [5,8], and available information on the

geographical distribution of mosquito species of medical and veterinary importance in the country [6,17,28-30]. In each state, temporary and permanent aquatic sites were sampled that represent potential habitats for the development of larval and pupal populations of mosquitoes, such as pools, lakes, streams, rivers, canals, marshes, etc. In addition, visits were made to cemeteries to collect immature stages of mosquitoes in water tanks. The sampling effort varied from 15 to 61 sites per state (Figure 1). Based on area, the most intensively sampled state was Colima with an average of 2.6 samples per 1000 km<sup>2</sup>, the least sampled state was Guerrero with an average of 0.3 samples per 1000 km<sup>2</sup>, whereas the average of all states was 0.6 samples per 1000 km<sup>2</sup>.

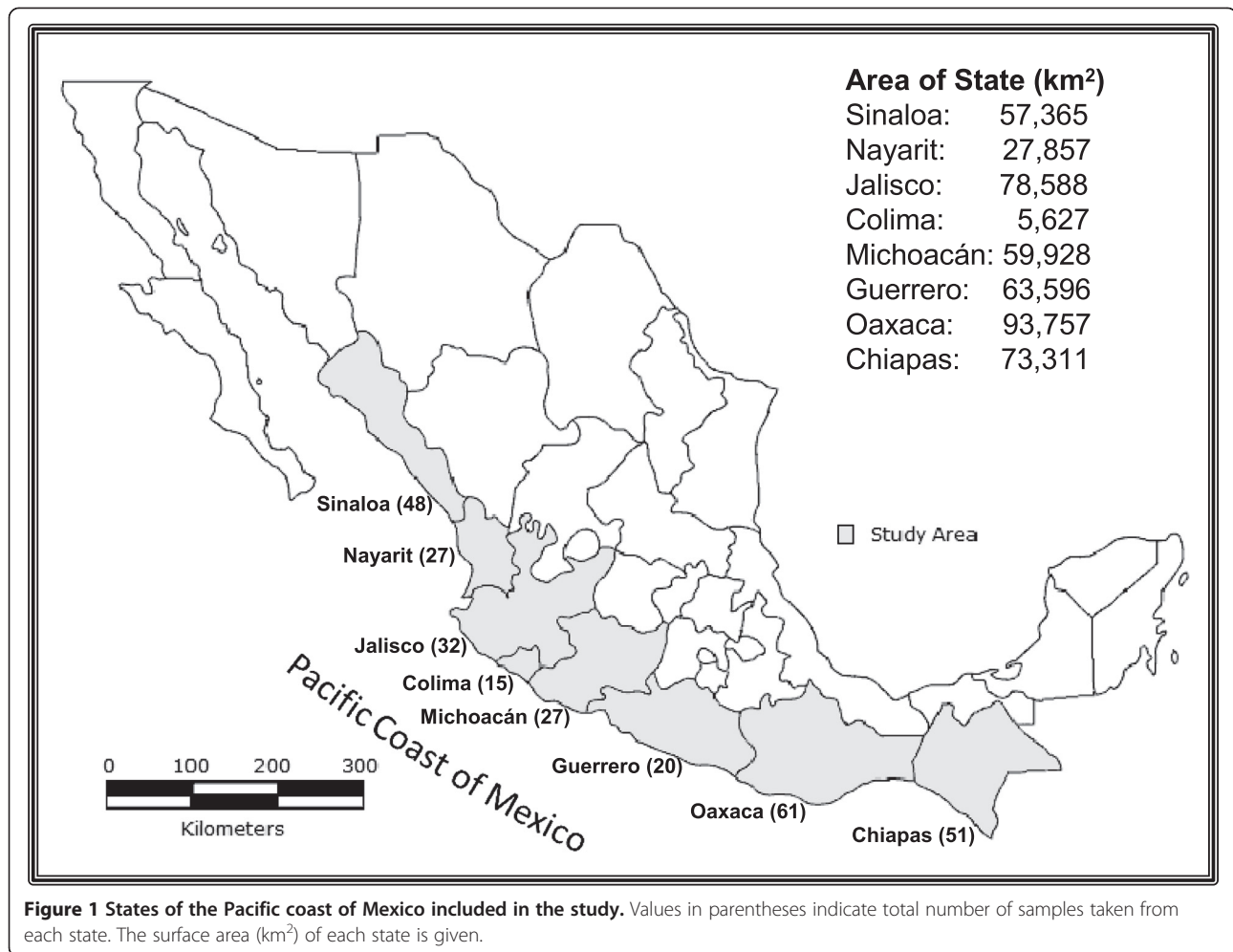
### Sampling methods and identification

Mosquitoes recorded in this study were obtained from field collections, and were compared to information from bibliographic records reported in scientific journals for different states in the study area. Larvae and pupae of mosquitoes were collected by using white enameled dippers, (500 ml capacity), with a flat outer side. All the larvae and pupae from each aquatic site were collected, counted, placed in 8 oz plastic sample bags, transported to the laboratory and then transferred to 1.5 mL plastic vials for adult emergence. Mosquitoes that emerged were placed in perforated plastic vials and were stored in plastic containers with silica gel for transport to the laboratories of the Regional Center for Public Health Research, National Public Health Institute (CRISP-INSP), Tapachula, Chiapas. Adult mosquitoes were identified to species using dichotomous keys [31-33].

Aquatic insects were collected using an aquatic entomological net (24 × 46 cm and mesh size 0.9 mm) that was dragged across the bottom and the surface of the water body at each aquatic site. In the case of lakes, rivers and streams an area of 5 m<sup>2</sup> was sampled for 5 minutes using the traveling kick method [34]. Aquatic insect samples were sorted in white metal trays and were then preserved in 96% ethanol, taken to the laboratory, and identified to genus and species using the appropriate keys [22,35-42]. Reference specimens of all mosquitoes and aquatic insects were deposited in the entomological collection of CRISP-INSP. Field collection records were entered into the CONABIO Biótica information system 4.5 [43], to create a biodiversity database.

### Data analysis

The abundance of aquatic insects recorded in each state were subjected to multivariate analysis of variance (MANOVA) following  $\ln(x + 1)$  transformation to normalize the distribution and eliminate zero values. For each of the eight states,  $\alpha$  diversity of mosquitoes and aquatic insects was estimated by means of the Shannon index ( $H'$ )



[44]. A randomization test was applied to determine significant differences between  $H'$  values using the Species Diversity and Richness III (v. 3.0.2) program [45], which is based on the method described by Solow [46]. The accuracy of index values was estimated by jackknifing, which permitted a reduction in the bias in our estimate of the population value and provided a standard error [47]. Confidence intervals for the statistic were calculated by bootstrap with replacement. Differences amongst samples and the communities of mosquitoes and aquatic insects of all the states ( $\beta$  diversity), were estimated by calculating a quantitative similarity index (Morisita-Horn) and cluster analysis [44,48]. Correlations of the number of mosquitoes sampled against certain orders of aquatic insects order was performed by Spearman rank correlation in Statistica v.7 (StatSoft Inc. Tulsa, OK).

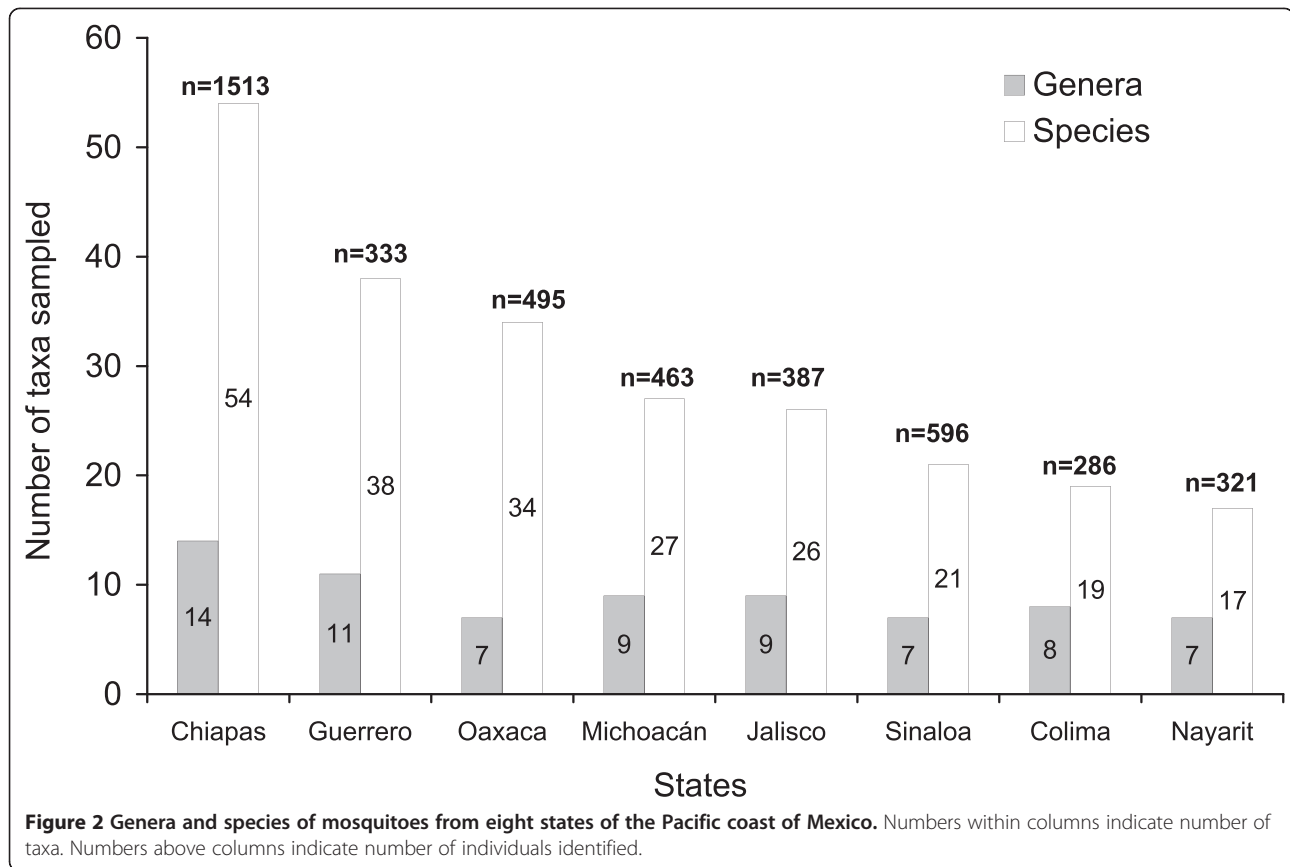
## Results

### Abundance and species richness

Overall, sampling resulted in the identification of mosquitoes representing 15 genera, 74 species and 4394

individuals distributed among the eight states of the Pacific coast of Mexico. Of the mosquitoes identified to species, 23% of individuals were from seasonal rain pools followed by streams (22%), river margins (18%), lakes and lagoons (14%), marshes (10%), irrigation channels (9%), water tanks (2%), dams (1%) and sewers (1%). At the state level, Chiapas had the highest abundance and richness of culicid taxa, with 1513 individuals (Figure 2), distributed among 14 genera and 54 species, followed by Guerrero and Oaxaca with an abundance of 333 and 495 individuals, respectively, and a richness of 11 and 7 genera, and 38 and 34 species, respectively. The states of Michoacán, Jalisco and Sinaloa had intermediate abundance and richness of mosquitoes that fluctuated between 387–596 individuals from 7 to 9 genera and 21 to 27 species. The states with the lowest abundance and richness of taxa were Colima and Nayarit, with abundance and richness values less than half those observed in Chiapas (Figure 2).

Overall, the most abundant mosquito species were *Anopheles pseudopunctipennis*, *Aedes aegypti* and *An.*



*albimanus*, with 747 (17.0%), 664 (15.1%) and 646 (14.7%) individuals sampled, respectively (Additional file 1: Table S1). These species represented 47% of the total mosquitoes collected and were recorded in all eight states of the study area. Additional species that were common and widely distributed in the eight states were *Ochlerotatus taeniorhynchus*, *Culex quinquefasciatus*, *Cx. coronator*, and *Cx. nigripalpus*, which together represented >20% of the specimens collected. In contrast, the distribution of a number of rare species was restricted to a single state. For example, *Ae. albopictus*, *Ae. angustivittatus*, *An. darlingi*, *An. gabaldoni*, *An. neivai*, *An. neomaculipalpus*, *An. vestitipennis*, *Coquillettidia venesuelensis*, and *Co. nigricans* were collected only in Chiapas. Similarly, *Och. infirmatus* was only identified in Guerrero, *Och. hastatus* was collected only in Colima, *Psorophora mathesoni* was restricted to Jalisco, and *Uranotenia orthodoxa* was identified only in samples from Michoacán (Additional file 1: Table S1). Overall, *Anopheles* mosquitoes were the most abundant with 1749 individuals and 19 species, representing almost 40% of the identified mosquitoes, followed by the genus *Culex* with 909 individuals (21%) and 12 species, and *Ochlerotatus* with 618 individuals (14%) and 9 species. The remaining 25% (1118 individuals) included 18 genera and 34 additional species of mosquitoes (Additional file 1: Table S1).

Due to their importance in the transmission of malaria, anopheline mosquitoes were considered to be of particular interest. In total, 18 species of anophelines were recorded during the development of this study (Table 1), and the state of Chiapas had the highest abundance and richness, with 941 individuals, and 15 species, followed by Michoacán and Oaxaca with 48 and 216 individuals, and 9 and 8 species, respectively. Only 5 species were registered in the states of Guerrero, Jalisco and Nayarit with 170, 70 and 95 individuals, respectively. The states with the least species richness were Colima and Sinaloa with 3 species, and 55 and 154 individuals, respectively (Table 1; Additional file 1: Table S1).

#### Abundance and taxa richness of aquatic insects

A total of 5233 individuals of aquatic insects were collected, identified and assigned to a total of 10 orders, 57 families, 166 genera and 247 species (Table 2). Significant differences were detected in the abundance of species between collections made in different states along the Pacific coast (MANOVA, Pillai's Trace:  $F_{8,240} = 7.683$ ;  $P < 0.0001$ ). The order Coleoptera had the highest abundance of aquatic insects with 1922 individuals and 112 species, followed by the orders Odonata and Hemiptera, with 1128 and 805 individuals, and 64 and 40 species, respectively. Trichoptera, Ephemeroptera, Plecoptera and

**Table 1 Mosquito species recorded from eight states of the Pacific coast of Mexico in previous studies and the present study**

Species	Sinaloa	Nayarit	Jalisco	Colima	Michoacán	Guerrero	Oaxaca	Chiapas
<b>(A) Culicids</b>								
<i>Aedes aegypti</i>	x*	x*	x*	x*	x*	x*	x*	x*
<i>Aedes albopictus</i>								*
<i>Aedes angustivittatus</i>								*
<i>Aedes atropalpus</i>				x	x	x	x	x
<i>Aedes knabi</i>							x	
<i>Aedes quadrivittatus</i>							x	x
<i>Aedes sexlineatus</i>								x
<i>Aedes vexans</i>			x*		x*			
<i>Aedes terreus</i>			x*		x*	‡*	x*	x*
<i>Aedeomyia squamipennis</i>						x‡*		*
<i>Coquillettidia nigricans</i>								*
<i>Coquillettidia venezuelensis</i>								*
<i>Culex apicalis</i>							x	
<i>Culex arizonensis</i>								x
<i>Culex bidens</i>	x							x
<i>Culex bigoti</i>					x	x	x	x
<i>Culex bihaicolus</i>								x
<i>Culex chidesteri</i>						x‡*	*	
<i>Culex conspirator</i>	x*					‡*	x*	x*
<i>Culex corniger</i>	x*					‡*	x*	x*
<i>Culex coronator</i>	x*	*	x*	x*	x*	x‡*	x*	x*
<i>Culex declarator</i>						‡		
<i>Culex derivator</i>								x
<i>Culex educator</i>						‡		
<i>Culex elevator</i>								x
<i>Culex erraticus</i>	x*	*	*	*	*	x‡*	x*	*
<i>Culex herythrorax</i>					x			
<i>Culex inflicus</i>				x				
<i>Culex inpatiens</i>								x
<i>Culex interrogator</i>	x*				x*	*	x*	x*
<i>Culex iolambdis</i>			x*			‡*	x*	x*
<i>Culex maccrackenae</i>							x	x
<i>Culex mutator</i>								x
<i>Culex nigripalpus</i>	x*	x*	x*			x‡*	x*	x*
<i>Culex peccator</i>						‡		
<i>Culex pilosus</i>								x
<i>Culex pinarocampa</i>						x	x	
<i>Culex quinquefasciatus</i>	x*	*	*	x*	*	x‡*	x*	x*
<i>Culex restrictor</i>						*	x*	x*
<i>Culex restuans</i>					x			
<i>Culex salinarius</i>							x	
<i>Culex stigmatosoma</i>	x*	*	x*	*	x*	x*	x*	x*



**Table 1 Mosquito species recorded from eight states of the Pacific coast of Mexico in previous studies and the present study (Continued)**

<i>Psorophora mathesoni</i>			*					
<i>Psorophora varipes</i>			x*	x*		‡*	x*	*
<i>Psorophora virescens</i>		x*		x*		x*	x*	
<i>Sabethes chloropterus</i>					x*			x*
<i>Uranotaenia coatzaacoalcos</i>					x		x	x
<i>Uranotaenia geometrica</i>								x
<i>Uranotaenia lowii</i>	x*	*	*	*		‡*		*
<i>Uranotaenia orthodoxa</i>					*			
<i>Uranotaenia pulcherrima</i>						x		
<i>Uranotaenia socialis</i>	*			*				
<i>Uranotaenia sapphirina</i>	x*			x*	x*	x‡*		x*
<i>Wyeomyia arthro stigma</i>								x
<i>Wyeomyia celaenocephala</i>								x
<i>Wyeomyia jocosa</i>								x
<i>Wyeomyia mitchelli</i>								x*
<i>Wyeomyia personata</i>						‡*		x*
<b>(B) Anophelines</b>								
<i>Anopheles albimanus</i>	x*	x*	x*	x*	x*	x‡*	x*	x*
<i>Anopheles apicimacula</i>					x*			x*
<i>Anopheles argyritarsis</i>		x*			x*		x*	x*
<i>Anopheles aztecus</i>			x*		x*			
<i>Anopheles crucians</i>				*		‡*	*	*
<i>Anopheles darlingi</i>								x*
<i>Anopheles eiseni</i>					x*	x*	x*	x*
<i>Anopheles franciscanus</i>			*				*	*
<i>Anopheles freeborni</i>					*			
<i>Anopheles gabaldoni</i>								x*
<i>Anopheles hectoris</i>								x*
<i>Anopheles neivai</i>								x*
<i>Anopheles neomaculipalpus</i>								x*
<i>Anopheles parapunctipennis</i>							x*	x*
<i>Anopheles pseudopunctipennis</i>	x*	x*	x*	x*	x*	x‡*	x*	x*
<i>Anopheles punctimacula</i>	*	x*			x*	x‡*	x*	x*
<i>Anopheles punctipennis</i>		*	*		x*			
<i>Anopheles vestitipennis</i>								x*
<i>Anopheles xelajuensis</i>							x	
<b>Species records</b>	<b>Sinaloa</b>	<b>Nayarit</b>	<b>Jalisco</b>	<b>Colima</b>	<b>Michoacán</b>	<b>Guerrero</b>	<b>Oaxaca</b>	<b>Chiapas</b>
No. species from previous records (x) [28,29]	22	10	16	13	29	52 ‡	46	67
No. species in present study (*)	21	17	26	19	27	38	34	54
Previous species records confirmed in present study	17	8	15	10	20	36	30	40
Species exclusive to this study	4	9	11	9	7	2	4	14
Total number of species per state	26	19	27	22	36	54	50	81

xIndicates species reported in previous studies [28,29].

\*Indicates species reported in the present study.

‡Double dagger symbol indicates species records from Guerrero state [17].

**Table 2 Aquatic insect species associated with the oviposition sites of mosquitoes and their presence in each of eight states\* along the Pacific coast of Mexico**

Species	Distribution	Species	Distribution	Species	Distribution	Species	Distribution	Species	Distribution
<i>Abedus ovatus</i>	Ch, G, O	<i>Crenitis</i> sp.	C, M	<i>Hesperagrion heterodoxum</i>	O	<i>Macrelmis</i> sp.	Ch	<i>Phyllogomphoides</i> sp.	Ch
<i>Ablabesmyia</i> sp.	C	<i>Cryphocricos</i> sp.	Ch	<i>Hesperocorixia vulgaris</i>	Ch	<i>Macronychus</i> sp.	Ch	<i>Phyllogomphoides suasus</i>	Ch, G, N
<i>Acilius</i> sp.	J, M, N	<i>Curicta howardi</i>	O	<i>Hetaerina cruentata</i>	Ch, G, N, O	<i>Macrothemis inacuta</i>	G, N, O	<i>Platyvelia</i> sp.	Ch
<i>Acneus</i> sp.	Ch	<i>Curicta</i> sp.	J, S	<i>Hetaerina vulnerata</i>	J, N, S	<i>Macrothemis pseudimitans</i>	Ch, G, N, O, S	<i>Potamyia flava</i>	Ch
<i>Rhionaeschna multicolor</i>	J, M	<i>Cybister</i> sp.	C, G, J, M, N, O, S	<i>Hetarina americana</i>	G, J, N, O	<i>Macrothemis ultima</i>	J, S	<i>Progomphus clendoni</i>	Ch
<i>Agabinus</i> sp.	J	<i>Cylloepus</i> sp.	Ch	<i>Heteragrion tricellulare</i>	Ch, O	<i>Macrovelia hornii</i>	Ch	<i>Progomphus</i> sp.	Ch
<i>Agabus</i> sp.	Ch	<i>Cyphon</i> sp.	J, N, S	<i>Heterelmis obesa</i>	Ch, O	<i>Macrovelia</i> sp.	M	<i>Psephenus</i> sp.	Ch
<i>Ambrysus mormon</i>	C, Ch, J, S	<i>Derallus rudis</i>	C, Ch, O, S	<i>Hydaticus</i> sp.	C, Ch, J, M, S	<i>Megadytes</i> sp.	J, M	<i>Pseudoleon superbus</i>	J, M, S.
<i>Ambrysus</i> sp.	C, G, M, N, O, S	<i>Derovatellus</i> sp.	S	<i>Hydraena</i> sp.	J	<i>Mesovelia mulsanti</i>	C, N	<i>Ranatra</i> sp.	C, Ch, G, J, M, N, O, S
<i>Anacaena suturalis</i>	C, J, M, S	<i>Desmopachria</i> sp.	C, J, M, N	<i>Hydrobiomorpha casta</i>	J, M, O, S	<i>Mesovelia</i> sp.	Ch, C, J, M	<i>Rhagovelia</i> sp.	C, Ch, O
<i>Anacroneuria</i> sp.	Ch	<i>Desmopachria striola</i>	S	<i>Hydrocanthus oblongus</i>	J, N	<i>Metrobates</i> sp.	Ch	<i>Rhantus calidus</i>	O
<i>Anax amazili</i>	C, Ch, N, S	<i>Dicranopselaphus</i> sp.	Ch	<i>Hydrocanthus</i> sp.	C, G, J, M, N, O, S	<i>Micrathyria aequalis</i>	Ch, N, J	<i>Rhantus gutticollis</i>	O, S
<i>Anax junius</i>	J, M	<i>Diglotta</i> sp.	Ch	<i>Hydrochara</i> sp.	Ch	<i>Micrathyria hagenii</i>	C, M	<i>Rhantus</i> sp.	O, S
<i>Antocha</i> sp.	Ch	<i>Dineutus ciliatus</i>	G, O	<i>Hydrochus</i> sp.	C, J, M, N, S	<i>Microcylloepus inaequalis</i>	Ch, G	<i>Rhionaeschna psilus</i>	M
<i>Apteraliplus</i> sp.	C, S	<i>Dineutus discolor</i>	Ch	<i>Hydroisotoma</i> sp.	Ch	<i>Microvelia beameri</i>	Ch	<i>Rhionaeschna</i> sp.	M, S
<i>Aquarius</i> sp.	J	<i>Dixella</i> sp.	Ch	<i>Hydrometra australis</i>	Ch	<i>Microvelia</i> sp.	C, Ch, J, M, N, O	<i>Scirtes</i> sp.	J
<i>Archilestes</i> sp.	M	<i>Dryops</i> sp.	Ch	<i>Hydrometra</i> sp.	C, Ch, N	<i>Neocylloepus</i> sp.	Ch, M	<i>Simulium</i> sp.	Ch, M
<i>Argia anceps</i>	N	<i>Dubiraphia</i> sp.	Ch	<i>Hydrophilus insularis</i>	O	<i>Neoperla</i> sp.	C, Ch, G, J, M, N, S	<i>Steinovelia stagnalis</i>	Ch
<i>Argia fissa</i>	M, N, S	<i>Dytiscus</i> sp.	J, M	<i>Hydrophilus smaragdinus</i>	O	<i>Nerthra mexicana</i>	Ch, O	<i>Stenelmis</i> sp.	Ch
<i>Argia oculata</i>	C, Ch, G, N, O	<i>Enallagma</i> sp.	C, J	<i>Hydrophilus</i> sp.	G, S	<i>Nerthra</i> sp.	C, O	<i>Stenus</i> sp.	Ch
<i>Argia oenea</i>	Ch, G, O	<i>Enochrus blatchleyi</i>	O	<i>Hydrophilus triangularis</i>	Ch	<i>Notomicrus</i> sp.	C	<i>Stratiomys</i> sp.	J, S



**Table 2 Aquatic insect species associated with the oviposition sites of mosquitoes and their presence in each of eight states\* along the Pacific coast of Mexico (Continued)**

<i>Argia pulla</i>	C, Ch, G, M, N, O, S	<i>Enochrus mexicanus</i>	C, Ch, J, M, N, O, S	<i>Hydropsyche betteni</i>	Ch	<i>Notonecta</i> sp.	G, J, M, O, S	<i>Suphis</i> sp.	J, M, S
<i>Atopsyche</i> sp.	Ch	<i>Enochrus ochraceus</i>	O	<i>Hydropsyche</i> sp.	Ch, M	<i>Ochterus</i> sp.	Ch	<i>Suphisellus lineatus</i>	C, N, S
<i>Baetis</i> sp.	Ch	<i>Enochrus pseudochraceus</i>	M	<i>Hydroscapha</i> sp.	S	<i>Optioservus</i> sp.	Ch	<i>Suphisellus</i> sp.	J, M, N, O
<i>Baetodes</i> sp.	Ch	<i>Enochrus pygmaeus</i>	C, G, J, M, O, S	<i>Ischnura capreolus</i>	C, Ch, N	<i>Ordobrevia</i> sp.	Ch, M	<i>Sympetrum illotum</i>	M, S
<i>Barbaetis</i> sp.	Ch	<i>Erpetogomphus elaps</i>	Ch, G, N, O	<i>Ischnura demorsa</i>	C, J, M, N, O, S	<i>Orthemis ferruginea</i>	J, M, S	<i>Tabanus</i> sp.	M
<i>Belostoma</i> sp.	C, Ch, J, S	<i>Erpetogomphus eutainia</i>	Ch, G, O	<i>Ischnura hastata</i>	Ch, G, M, N, O, S	<i>Orthemis</i> sp.	M	<i>Telebasis salva</i>	C, J, M, N, S
<i>Berosus arneti</i>	S	<i>Erpetogomphus</i> sp.	Ch	<i>Ischnura ramburii</i>	C, G, J, M, N, O, S	<i>Pachydiplax longipennis</i>	C	<i>Telebasis</i> sp.	C, N, S
<i>Berosus exiguus</i>	O	<i>Erythemis attala</i>	C, M, N, O	<i>Ischnura</i> sp.	C, Ch, J, M, N, S			<i>Tenagobia</i> sp.	M
<i>Berosus infuscatus</i>	O, S	<i>Erythemis plebeja</i>	C, Ch, G, J, N, O, S	<i>Isonychia</i> sp.	Ch	<i>Pachydrus princeps</i>	G, M	<i>Thermonectus basillaris</i>	J, O, S
<i>Berosus mexicanus</i>	C, G, J, M, N, O, S	<i>Erythemis</i> sp.	M, N, S	<i>Laccobius</i> sp.	M, N	<i>Pachydrus</i> sp.	M	<i>Thermonectus marmoratus</i>	G, O, S
<i>Berosus sayi</i>	M	<i>Erythemis vesiculosa</i>	C, G, N, O	<i>Laccodytes</i> sp.	Ch, C, M, S	<i>Palaemnema desiderata</i>	Ch, O	<i>Thermonectus ornatocollis</i>	S
<i>Bibiocephala grandis</i>	Ch	<i>Erythrodiplax</i> sp.	C, M	<i>Laccophilus fasciatus</i>	Ch, C, G, J, M, N, O, S	<i>Pantala flavescens</i>	C, Ch, G, N, S	<i>Thermonectus</i> sp.	Ch, C, J, O, S
<i>Bidessonotus</i> sp.	C	<i>Erythrodiplax umbrata</i>	C, G, J, N, O	<i>Laccophilus hyalinus</i>	M	<i>Pantala hymenaea</i>	J, S	<i>Tramea abdominalis</i>	C, J
<i>Brachydeutera</i> sp.	S	<i>Gelastocoris oculatus</i>	J, N	<i>Laccophilus maculosus</i>	G, J, O, S	<i>Pantala</i> sp.	Ch, S	<i>Trepobates pictus</i>	N
<i>Brechmorhoga mendax</i>	N	<i>Gelastocoris</i> sp.	M, N, S	<i>Laccophilus pictus</i>	Ch, G, J, O, S	<i>Paracloeodes</i> sp.	Ch	<i>Trepobates</i> sp.	Ch, C, J, N
<i>Brechmorhoga praecox</i>	Ch, G, N, O	<i>Gerris</i> sp.	Ch	<i>Laccophilus</i> sp.	C, Ch, J, M, S	<i>Paracymus armatus</i>	Ch	<i>Triacanthagyna</i> sp.	M
<i>Brechmorhoga vivax</i>	Ch	<i>Gonielmis</i> sp.	Ch	<i>Laccophilus undatus</i>	C	<i>Paracymus confusus</i>	O	<i>Trichocorixa</i> sp.	O, S
<i>Bryothinusa</i> sp.	Ch	<i>Graphoderus</i> sp.	J	<i>Lara</i> sp.	S	<i>Paracymus regularis</i>	C, Ch, M, N, O, S	<i>Tricorythodes</i> sp.	Ch
<i>Buenoa</i> sp.	C, Ch, G, J, M, N, O, S	<i>Graptocorixa</i> sp.	J, S	<i>Leptobasis vacillans</i>	M	<i>Paradelphomyia</i> sp.	Ch	<i>Trochopus</i> sp.	Ch
<i>Caloparyphus greylockensis</i>	Ch	<i>Gyrinus parvus</i>	O	<i>Leptohyphes</i> sp.	Ch	<i>Paraleptophlebia</i> sp.	Ch	<i>Tropisternus blatchleyi</i>	S

**Table 2 Aquatic insect species associated with the oviposition sites of mosquitoes and their presence in each of eight states\* along the Pacific coast of Mexico (Continued)**

<i>Camelobaetidius</i> sp.	Ch	<i>Haliplus</i> sp.	J, M	<i>Leptonema</i> sp.	Ch	<i>Pelocoris</i> sp.	C, Ch, G, J, M, N, O, S	<i>Tropisternus collaris</i>	C, G, J, M, N, O, S
<i>Celina</i> sp.	J	<i>Hebrus sobrinus</i>	Ch	<i>Lestes alacer</i>	M, O	<i>Pelonomus obscurus</i>	O	<i>Tropisternus lateralis</i>	J, M, O, S
<i>Ceratopsyche</i> sp.	Ch	<i>Helichus</i> sp.	Ch, S	<i>Lestes tenuatus</i>	G, J, M, N, O	<i>Pelonomus</i> sp.	C	<i>Tropisternus mixtus</i>	M, N
<i>Chaetarthria</i> sp.	M, S	<i>Helobata</i> sp.	Ch, S	<i>Lestes tikalus</i>	Ch, C, O	<i>Peltodytes dietrichi</i>	S	<i>Tropisternus paredesi</i>	S
<i>Chimarra</i> sp.	Ch	<i>Helochares normatus</i>	C, G, S	<i>Leucotrichia</i> sp.	Ch	<i>Peltodytes muticus</i>	M	<i>Tropisternus</i> sp.	C, G, J, M, N, O, S
<i>Copelatus caelatipennis</i>	O	<i>Helochares sallaei</i>	S	<i>Libellula foliata</i>	Ch	<i>Peltodytes</i> sp.	S	<i>Uvarus</i> sp.	C, M, S
<i>Copelatus</i> sp.	J, M	<i>Helophorus</i> sp.	J	<i>Liodessus fuscatus</i>	G, O, S	<i>Perissolestes</i> sp.	M	<i>Zaitzevia</i> sp.	Ch
<i>Corisella decolor</i>	M	<i>Hemerodromia</i> sp.	M	<i>Liodessus</i> sp.	C, J, M, N, S	<i>Perithemis</i> sp.	S		
<i>Corydalus cornutus</i>	C, Ch, M	<i>Henochrus</i> sp.	Ch	<i>Lipogomphus</i> sp.	Ch, C, J	<i>Petrophila confusalis</i>	Ch		

\*C = Colima, Ch = Chiapas, G = Guerrero, J = Jalisco, M = Michoacán, N = Nayarit, O = Oaxaca, S = Sinaloa.

Diptera (not including mosquitoes) presented an intermediate abundance with 523, 331, 283 and 141 individuals, and 8, 9, 2 and 10 species, respectively. The least represented groups in the collections were Collembola with only one individual and one species, and Lepidoptera and Megaloptera, with 30 and 69 individuals respectively, each represented by a single species (Figure 3A and 3B). Chiapas state had the highest taxa richness, with 116 species distributed in 100 genera, 46 families and 10 orders (Figure 4), followed by the states of Sinaloa, Michoacan and Oaxaca, with between 86 and 71 species. The states with the lowest species richness were Jalisco, Colima, Nayarit and Guerrero with between 68 and 41 species collected during the sampling program (Figure 4).

#### Anopheline diversity

Shannon index diversity values differed between states ( $\delta = 0.39-1.06$ ;  $P < 0.001$ ), and allowed states to be classified into four groups (Figure 5A). The highest diversity values were observed in collections made in the states of Chiapas and Michoacán ( $H' = 1.61$  and  $1.56$ , respectively). Collections made in the states of Oaxaca and Jalisco generated index values of  $1.21$  and  $1.10$ , respectively. A third group of states comprised the states of Nayarit, Colima and Guerrero with diversity values of  $0.80$ ,  $0.72$  and  $0.69$ , respectively. Finally, the state of Sinaloa had the lowest  $H'$  value at  $0.54$ , which did not differ significantly from the value calculated for the state of Colima. Jackknifing indicated that diversity index values were underestimated by  $4.14\%$  for *Anopheles* spp. (Table 3). Diversity index findings were consistent with the cluster analysis, which also discriminated four groups depending on their species abundance, with the only difference being that in the analysis of diversity, Sinaloa clustered in a group that comprised Guerrero, Nayarit and Colima (Figure 6A).

#### Culicid diversity

Significant differences were detected between culicid diversity values from the eight states. Culicid diversity values fell into one of three groups: first, Chiapas state with a diversity index value of  $2.66$  that was significantly higher than the values of the seven remaining states. The second group comprised the states of Oaxaca ( $H' = 2.37$ ), Guerrero ( $H' = 2.30$ ) and Nayarit ( $H' = 2.22$ ) with similar diversity values. The third group comprised Jalisco ( $H' = 2.01$ ), Sinaloa ( $H' = 1.99$ ), Colima ( $H' = 1.95$ ) and Michoacán ( $H' = 1.93$ ) with the lowest diversity values (Figure 5B). Cluster analysis broadly supported these findings, the main difference being that Chiapas clustered with Oaxaca and Guerrero, rather than forming a separate group (Figure 6B). Jackknifing indicated that culicid diversity index values were underestimated by  $4.57\%$  (Table 3).

#### Aquatic insect diversity

Diversity analyses on aquatic insect samples (Figure 5C), indicated that Chiapas had the highest Shannon index value ( $3.75$ ), followed by the states of Oaxaca ( $3.63$ ), Michoacan ( $3.60$ ). Intermediate  $H'$  values were estimated for the states of Sinaloa, Guerrero, Jalisco and Nayarit that varied between  $3.34$  and  $3.54$ . The lowest index value was calculated for the state of Colima ( $3.22$ ), which was significantly lower than all other values, except that calculated for the state of Nayarit. Jackknifing indicated that  $H'$  values were underestimated by  $3.34\%$  for aquatic insects (Table 3). Cluster analysis supported the existence of three diversity groups consisting of Chiapas alone (Figure 6C), the second group consisting of Oaxaca, Michoacan and Sinaloa and the third group consisting of Guerrero, Jalisco, Nayarit and Colima. Minor differences in the placement of states within a particular group were observed, but the overall patterns were in agreement with the calculated diversity index values.

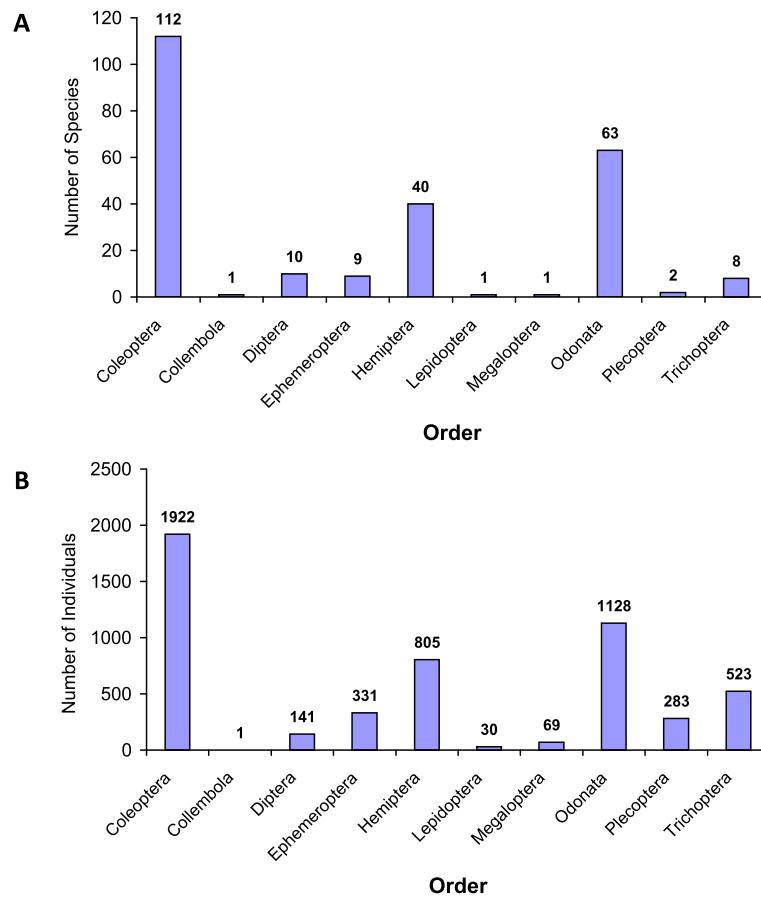
#### Similarity analyses and correlations

Morisita-Horn similarity index values for comparison of species composition of mosquitoes among the states of Chiapas-Oaxaca or Chiapas-Guerrero were estimated at  $70\%$  or at  $91\%$  in the comparison of the states of Oaxaca-Guerrero (Table 4). Similar results were observed in the cluster analysis, in which culicids from these three states clustered in a single group (Figure 6B). Similarity in species composition of mosquitoes among the states of Jalisco, Colima and Sinaloa were close to  $70\%$ , whereas comparison of the mosquito composition of Michoacan and Jalisco was extremely high at  $92\%$  (Table 4). These findings were consistent with those of the cluster and diversity analyses that included the states of Jalisco, Colima, Sinaloa and Michoacán within a group according to their species composition or diversity (Figures 5A and 6A).

The presence of mosquito larvae was not significantly correlated with the prevalence of aquatic insect predators of the orders Coleoptera (Spearman's  $r_s = -0.056$ ,  $N = 180$ ), or Hemiptera (Spearman's  $r_s = -0.141$ ,  $N = 80$ ). Additional correlations were not performed as the low numbers of independent points reduced the validity of the correlation, e.g., as was the case for Odonata.

#### Discussion

The diversity of mosquito species was determined for each of eight states along  $2,500$  km of the Pacific coast of Mexico. This represents the most complete mosquito survey of this region to date and markedly expands the information provided by the only previous detailed studies on the mosquito fauna of Mexico performed in the 1950's [28,29]. A large and taxonomically diverse group of aquatic insects associated with mosquito oviposition sites was also identified.

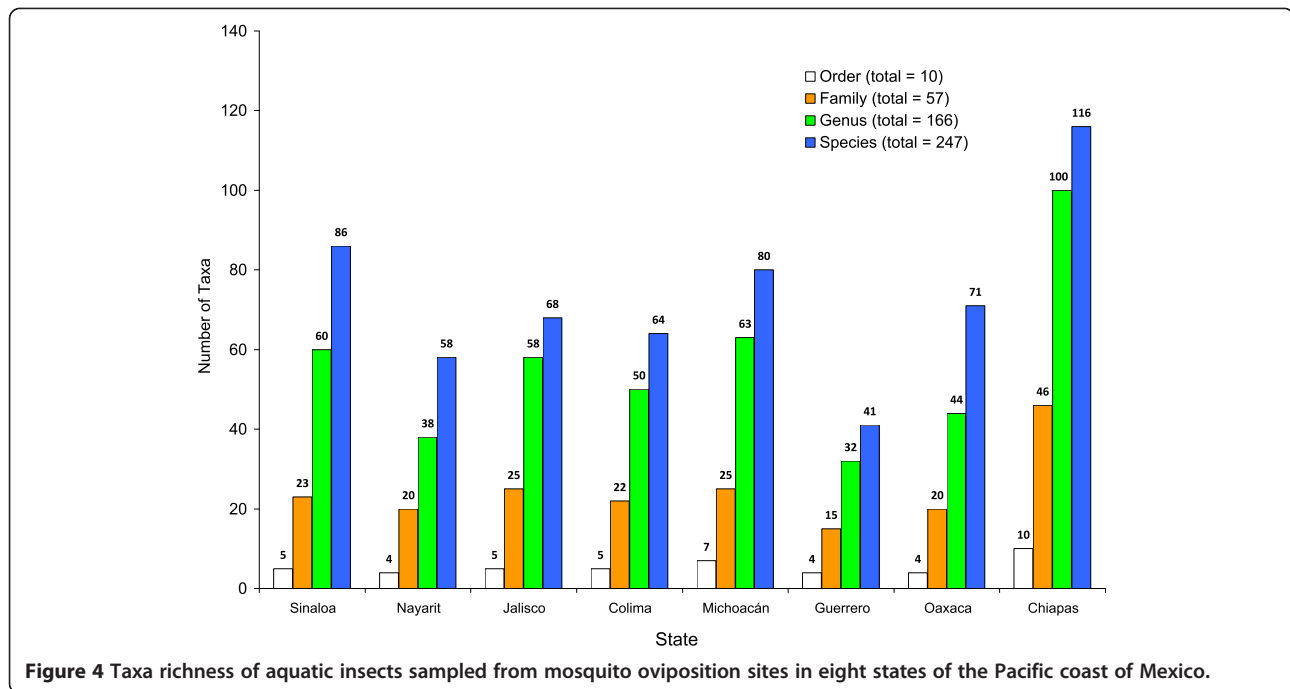


**Figure 3** Numbers of (A) species and (B) individuals identified from 10 orders of aquatic insects sampled from mosquito oviposition sites in eight states of the Pacific coast of Mexico.

The selection of sampling sites within each state was based on established information available on the type of ecosystem and habitat used by anophelines and culicids as likely oviposition sites. Decisions on which habitats to sample, and in which period of the year, doubtless affected the likelihood of identifying particular species. This is because the oviposition site preferences of each mosquito species reflect a characteristic and habitat-specific suite of biotic and abiotic factors that favor the development and survival of their offspring [9]. However, such targeted sampling regimes are an inevitable consequence of finite financial and human resources available for faunistic studies of this kind.

Globally, the family Culicidae comprises 3,525 species distributed among 111 genera [49]. Mosquito diversity tends to be highest in tropical habitats [4]. In the present study a total of 15 genera and 74 species were identified, which represents ~2% of global species. Within Mexico, the recorded mosquito fauna consists of between 15 and 16 genera and between 217 and 239 species [5,6], although the genera *Chagasia*, *Orthopodomyia*, *Shannoniana*, and *Toxorhynchites* were excluded from these

reports because these genera have no species of medical importance. The mosquito species reported in the present study represented 30 - 34% of the total Mexican mosquito fauna. Surveys on the mosquito fauna of Mexico are remarkably sparse. A total of 52 species from 11 genera have been recorded for the state of Guerrero State [17,28,29], in addition to species from other genera, such as *Toxorhynchites* species, that were not considered in the present study. This compared with 38 species from 10 genera recorded in the present study. In the remaining seven states 17–54 species were identified from 7–14 genera with the highest taxa richness in the state of Chiapas and the lowest in Nayarit. Overall, the present study extended the number of species records by just two species in Guerrero state and by up to 14 species in Chiapas state (Table 1). Similarly, the percentage of recorded species was increased by just 4% in Guerrero (2 additional species/52 previously reported species), but was almost doubled for Colima (9 new records/10 reported species), underlining the value of the present study to understanding the distribution of vector species in this region. Surveys have also been conducted in other states of Mexico, such as



Veracruz [7], Yucatán [50-52], and Quintana Roo [53] but their geographical location, ~1000 km from the Pacific coast of Mexico, and marked differences in the type of ecosystems present in the Atlantic and Caribbean coasts compared to the Pacific coast, means that these studies are of limited relevance to the present findings.

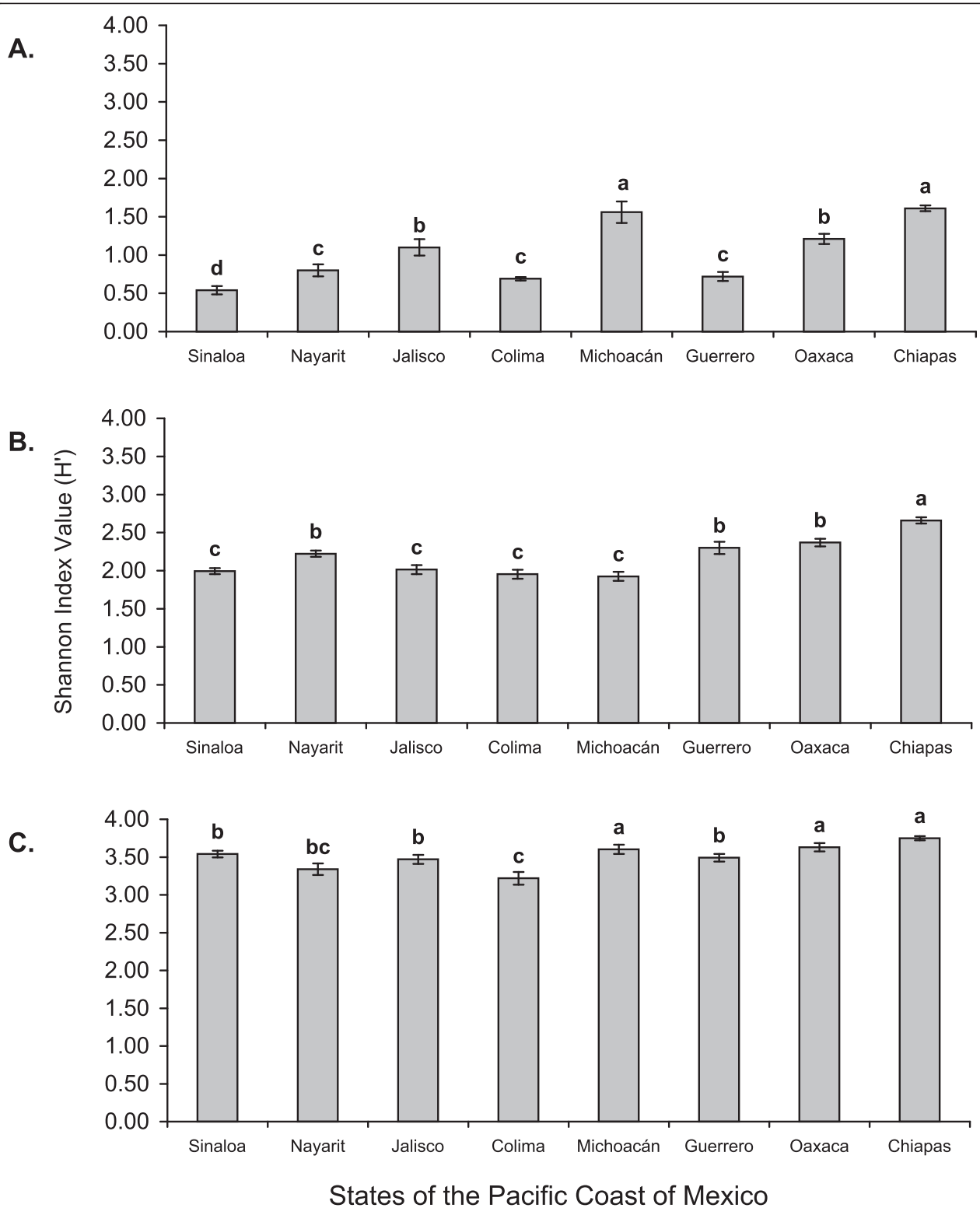
The diversity index values of mosquitoes in the eight states included in this study ranged from 1.93 for the state of Michoacan to 2.66 for the state of Chiapas. There are no previous systematic surveys of mosquitoes in the Pacific coast region, with the exception of the studies performed in the 1950's [28,29], and one study in Guerrero in the 1970's [17]. The high mosquito diversity present in the Pacific coast region is likely due to a combination of the overlap between Nearctic and Neotropical fauna and the great diversity of ecosystems present in this region, which is largely responsible for the status of Mexico as one of the world's megadiverse countries. In this respect, the states of Chiapas and Oaxaca represent the states with the highest diversity in Mexico, followed by the states of Veracruz, Guerrero and Michoacán [54].

Globally, 465 species of *Anopheles* have been recognized, of which approximately 70 species have the capacity to transmit human malaria parasites [9,55], and 41 are considered to be dominant vector species, capable of transmitting malaria at a level of major concern to public health [56]. Approximately one third of the population of Mexico lives in areas prone to malaria transmission [57]. Between 26 and 28 species of *Anopheles* have been reported in Mexico [5,6,58], but only two of these are considered as being of major public health concern: *An.*

*pseudopunctipennis* and *An. albimanus* are the principal vectors of *P. vivax* in mountain foothills and coastal areas, respectively [13,59].

A total of 18 species of anophelines were recorded during this study, in addition to one previous species record (*Anopheles xelajuensis*) [28]. The state of Chiapas had the highest anopheline species richness (15) and the highest diversity value (1.61), followed by the states of Michoacan (9 species) and Oaxaca (9 species). Our study expands the anopheline fauna of seven of the eight states studied (Table 1). Specifically, *An. crucians* is a new species record for Chiapas, Oaxaca and Colima states; *An. franciscanus* is a new record for Chiapas, Oaxaca and Jalisco states; *An. freeborni* is a new record for Michoacán state; *An. punctimacula* is a new record for Sinaloa state, and *An. punctipennis* is a new record for Jalisco and Nayarit states. No new anopheline records were obtained for the state of Guerrero [17].

The mosquitoes *Ae. aegypti* and *Ae. albopictus* are vectors of dengue in tropical and subtropical regions throughout the world and represent a major public health concern [60]. However, despite its dramatic global expansion in the last three decades, *Ae. albopictus* is believed to have a lesser role in dengue virus transmission compared to *Ae. aegypti*, due to differences in host preference and vector competence [61]. Of these, only *Ae. aegypti* was present in all eight states of our study area, whereas the exotic invasive species *Ae. albopictus* was only present in Chiapas. In Mexico, *Ae. albopictus* was first recorded in 1988 in the northeastern state of Tamaulipas [62,63], but was also reported in the



**Figure 5** Shannon index ( $H'$ ) values for (A) anophelines, (B) culicids and (C) aquatic insects from samples taken in eight states along the Pacific coast of Mexico. Columns labeled with different letters differ significantly for comparisons of States within each graph ( $P < 0.001$  pairwise randomization test).

**Table 3 Values of total diversity estimated by jackknife for culicid, anopheline and aquatic insects sampled in eight states of the Pacific coast of Mexico**

Source	Number of analyzed samples**	Values of $H'$	Pseudovalues $\phi$	Jackknifing ( $\pm$ SE)	Confidence limits (95%)*	Error (%)
Culicids	All (8)	2.87				
	(2,3,4,5,6,7,8)	2.72	2.81			
	(1,3,4,5,6,7,8)	2.88	1.06			
	(1,2,4,5,6,7,8)	2.88	1.48			
	(1,2,3,5,6,7,8)	2.89	1.48	3.00 $\pm$ 0.15	2.65 - 3.35	4.57
	(1,2,3,4,6,7,8)	2.88	1.48			
	(1,2,3,4,5,7,8)	2.84	1.69			
	(1,2,3,4,5,6,8)	2.82	1.27			
	(1,2,3,4,5,6,7)	2.90	1.06			
All (8)	1.48					
Anophelines	(2,3,4,5,6,7,8)	1.29	3.92			
	(1,3,4,5,6,7,8)	1.54	2.80			
	(1,2,4,5,6,7,8)	1.48	2.80			
	(1,2,3,5,6,7,8)	1.48	2.73	1.54 $\pm$ 0.20	1.07 - 2.01	4.14
	(1,2,3,4,6,7,8)	1.48	2.80			
	(1,2,3,4,5,7,8)	1.45	3.08			
	(1,2,3,4,5,6,8)	1.51	3.22			
	(1,2,3,4,5,6,7)	1.54	2.66			
Aquatic insects	All (8)	4.46				
	(2,3,4,5,6,7,8)	4.44	4.60			
	(1,3,4,5,6,7,8)	4.45	4.53			
	(1,2,4,5,6,7,8)	4.45	4.53			
	(1,2,3,5,6,7,8)	4.45	4.53	4.61 $\pm$ 0.04	4.52 - 4.70	3.34
	(1,2,3,4,6,7,8)	4.42	4.74			
	(1,2,3,4,5,7,8)	4.46	4.46			
	(1,2,3,4,5,6,8)	4.41	4.81			
	(1,2,3,4,5,6,7)	4.43	4.67			

\*Confidence intervals were calculated by bootstrap.

\*\*The numbers 1 to 8 represent each of the states in the following order, 1. Chiapas, 2. Guerrero, 3. Oaxaca, 4. Colima, 5. Jalisco, 6. Michoacán, 7. Nayarit, 8. Sinaloa.

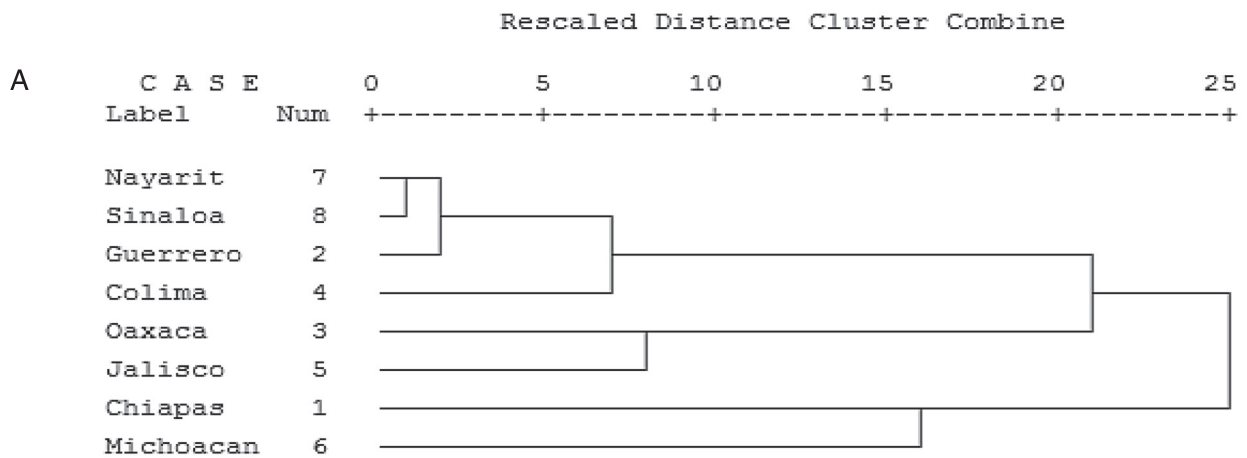
southern state of Chiapas in 2002 [64], and recently in the central state of Morelos [65] and the Gulf coast states of Yucatán [66], and Veracruz [67]. Given the presence of *Ae. albopictus* in Chiapas, its introduction, establishment, and spread to the other states of the Pacific coast is an issue of considerable concern. As invasive mosquitoes have the potential for biotic impacts on native species, ecosystems, and on human and animal health [68], an invasive mosquito that replaces a resident species via competition may alter disease transmission and amplify the importance of vector borne disease in affected areas [69].

The taxa richness and diversity of aquatic insects associated with breeding sites of mosquitoes in this study was high, with a total of 10 orders, 57 families, 166

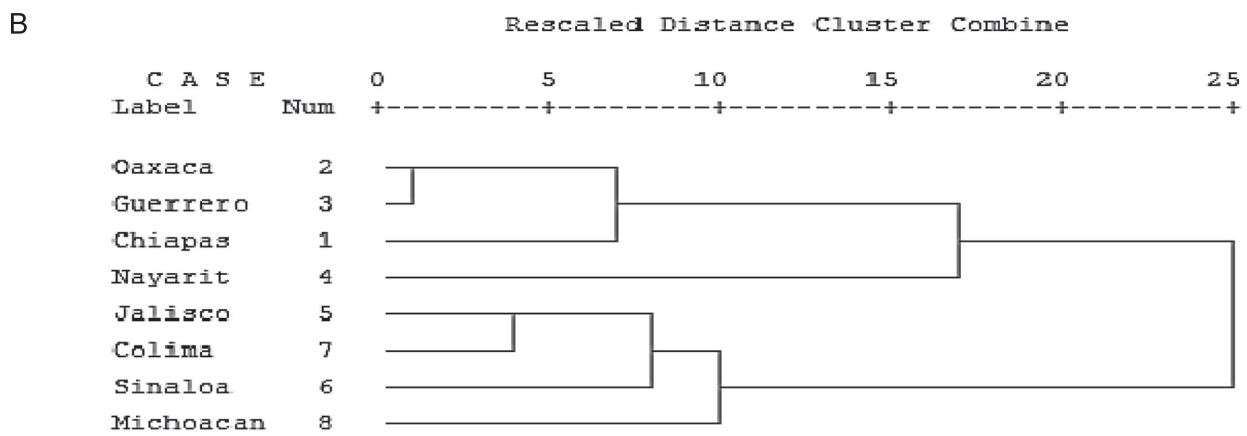
genera and 247 species, with Shannon index values between 3.22 and 3.75, depending on the state in question. The orders of greatest abundance and species richness were Coleoptera, Odonata and Hemiptera that represented 87% of the total species sampled.

In northern Mexico, 39 genera, 27 families, and 7 orders of aquatic insects were reported in association with oviposition sites of *An. pseudopunctipennis* [18]. Similarly, 52 genera, 19 families, and 3 orders of aquatic insects were reported in association with *An. albimanus* oviposition sites in southern Chiapas, of which Coleoptera was the most abundant and diverse order [19]. In comparison, another study in southern Chiapas reported 90 genera, 40 families and 10 orders of aquatic insects associated with oviposition sites of *An. pseudopunctipennis*. In

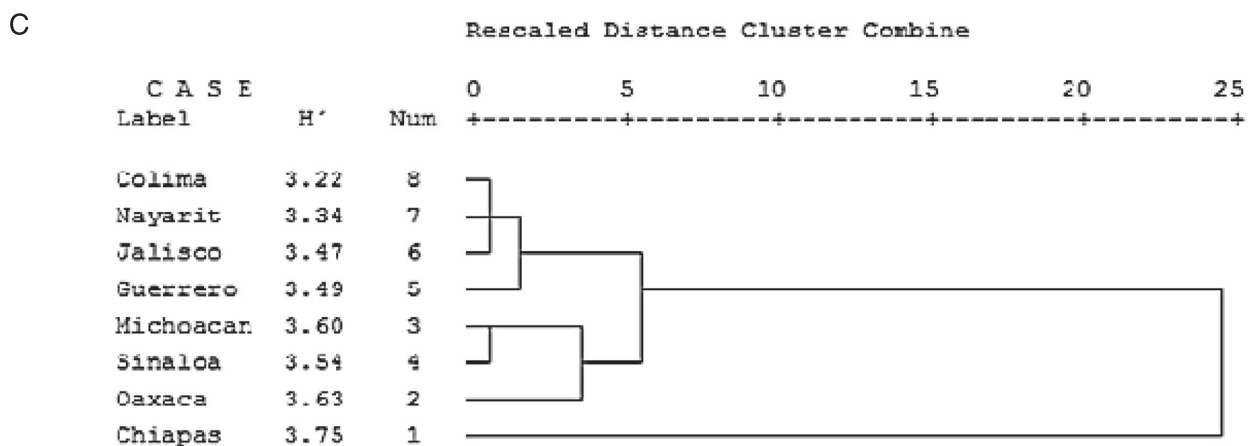
Dendrogram using Ward Method



Dendrogram using Ward Method



Dendrogram using Centroid Method



**Figure 6** Dendrogram generated by cluster analysis using Ward and Centroid methods for (A) anophelines, (B) culicids and (C) aquatic insects. Samples were obtained from eight states along the Pacific coast of Mexico.



**Table 4 Similarity values for species composition of mosquitoes from eight states of the Pacific coast of Mexico, using Morisita Horn index**

	Chiapas	Oaxaca	Guerrero	Nayarit	Jalisco	Sinaloa	Colima	Michoacán
Chiapas	1.00	0.71	0.73	0.51	0.27	0.44	0.43	0.20
Oaxaca	0.71	1.00	0.91	0.53	0.48	0.52	0.50	0.32
Guerrero	0.73	0.91	1.00	0.60	0.43	0.55	0.56	0.27
Nayarit	0.51	0.53	0.60	1.00	0.26	0.51	0.27	0.16
Jalisco	0.27	0.48	0.43	0.26	1.00	0.46	0.68	0.91
Sinaloa	0.44	0.52	0.55	0.51	0.46	1.00	0.69	0.36
Colima	0.43	0.50	0.56	0.27	0.68	0.69	1.00	0.57
Michoacán	0.20	0.32	0.27	0.16	0.91	0.36	0.57	1.00

this case Shannon index values fluctuated between 2.4 and 3.2 [21]. However, these previous studies were focused on the oviposition sites of single species of mosquitoes, and restricted to a single state, which contrasts with our effort to evaluate the diversity of aquatic insects associated with immature mosquitoes along the Pacific coast of Mexico. Comparable studies in temperate regions have reported markedly lower diversity of aquatic insects, which was ascribed to the harsh environmental conditions and instability of the habitat studied [11].

The presence of mosquito larvae was not significantly correlated with the abundance of aquatic insect predators of the orders Coleoptera and Hemiptera. A negative tendency was observed in the correlation between mosquito abundance and predator abundance, but in neither case was this significant, probably due to the large number of observations of single individuals in the dataset used. Previous studies have highlighted the importance of these orders, since many members of these orders are known to prey on mosquito larvae [26,70]. In addition to regulating mosquito populations by direct predation, a number of these predators can influence adult mosquito oviposition decisions or affect the rate of development of immature stages [10,71-73]. There is renewed interest in employing natural enemies for the control of mosquito larvae to complement existing vector control measures. In this respect, due to their numerical and functional responses, naturally occurring predators can be a significant density dependent mortality factor in the regulation of mosquito populations [74,75]. However, apart from some species of fish and bacteria-based biological insecticides, the adoption of biological vector control in most countries remains extremely limited.

## Conclusion

This study represents the first systematic update to the inventory and distribution of mosquitoes in Mexico in over five decades. The majority of the individuals reported were catalogued in databases of mosquitoes and aquatic insects in Mexico's National Biodiversity Information System

(SNIB-CONABIO), and are available for public inspection. We believe this represents a valuable contribution to recording the diversity and geographic distribution of the mosquitoes and aquatic insects in this region that is affected by major vector borne diseases, particularly dengue and malaria. Numerous new species records for different states along the Pacific coast are reported. Considerably greater sampling effort would be required to yield realistic estimates of total mosquito species richness of the country, particularly for the Mexican Culicidae inventory, given the great diversity of ecosystems present in this megadiverse country.

## Additional file

**Additional file 1: Table S1.** Numbers of field-collected mosquitoes that were reared in the laboratory and identified to species following adult emergence. Columns indicate numbers of each species in samples taken from eight states of the Pacific coast of Mexico.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

MCM obtained funding via a competitive proposal. MCM, JGB, CFM, AU, AOB, MM performed field studies. JGB, MCM, CFM, AU, MM performed mosquito rearing. AOB identified mosquitoes. JGB, HQM, RNG identified aquatic insects. JGB, TW performed statistical analyses. JGB, CFM, TW wrote the manuscript. All authors read and approved the final version of the manuscript.

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