Species of *Anopheles* (Diptera: Culicidae) in the larval stage detected in malaria-endemic localities in Western and Northwestern Colombia

Especies de *Anopheles* (Diptera: Culicidae) detectadas en estado larval en localidades endémicas para malaria del occidente y noroccidente Colombiano

Juan José Quintero[*], *Mariano Altamiranda-Saavedra*[*], *Margarita M. Correa*[*†]

Authors' contributions: Juan José Quintero-Montoya processed samples, analyzed and interpreted data, and wrote the manuscript. Mariano Altamiranda-S analyzed data. Margarita M. Correa designed the study, analyzed data, and wrote the manuscript. All authors revised and approved the final version of the manuscript.

ABSTRACT

INTRODUCTION: Larval habitats are important determinants of the *Anopheles* species occurring in a particular malaria endemic area. The aim of this study was to evaluate the anopheline species in the larval stage detected in malaria-endemic localities in Western and Northwestern Colombia.

MATERIALS AND METHODS: *Anopheles* larvae were collected in malaria-endemic localities. Surrounding vegetation and shade at positive larval habitats were registered. In addition, water temperature, pH of the habitats, and their distance from the nearest inhabited place were correlated with the abundance of the species detected.

RESULTS: The Colombian primary vectors *Anopheles nuneztovari, An. darlingi* and *An. albimanus* were the most abundant species found in the larval stage, whereas *An. malefactor* was the least recurrent. There were various types of positive larval habitats, with lakes, jagueys, wetlands, lagoons, and ponds being the most representative. There was a significant correlation between the presence of *An. nuneztovari* larvae and pH and water temperature.

CONCLUSION: This study provides relevant information on environmental and ecological parameters that may determine the presence of vector important species in the larval state; this information represents the basis for the design and application of species-specific vector control interventions.

KEYWORDS: *Anopheles*, larvae, larval habitats, malaria, Colombia.

RESUMEN

INTRODUCCIÓN: Los hábitats larvales son determinantes importantes de la presencia de las especies de *Anopheles*. El objetivo de este estudio fue evaluar las especies anofelinas detectadas en estado larval en zonas endémicas de malaria del occidente y noroccidente de Colombia.

MATERIALES Y MÉTODOS: Se colectaron larvas de *Anopheles* en localidades endémicas para malaria. Para los hábitats larvales positivos se registró la vegetación que rodea a los hábitats y la sombra. Se correlacionaron la temperatura del agua del hábitat, su pH y distancia a la casa más cercana, con la abundancia de las especies detectadas.

RESULTADOS: Las especies encontradas en estado larval en mayor abundancia correspondieron a los vectores principales de Colombia, *Anopheles nuneztovari, An. darlingi* y *An. albimanus* y en baja abundancia, la especie *An. malefactor*. Los tipos de hábitat larvales positivos variaron y los más representativos fueron, lagos, jagüeyes, humedales, lagunas y estanques. Se encontró correlación significativa entre presencia de larvas de *An. nuneztovari* con el pH y la temperatura del agua.

CONCLUSIÓN: Este estudio aporta información relevante sobre algunos parámetros ambientales y ecológicos que pueden determinar la presencia de especies en estado larvario de importancia vectorial, esta información podría constituir la base para el diseño y aplicación de intervenciones de control vectorial especie-específicos.

PALABRAS CLAVE: *Anopheles*, larvas, hábitats larvales, malaria, Colombia.

INTRODUCTION

Malaria is a public health problem in developing countries,[1] with an average of 100.000 annual clinical cases in the last two decades.[2] In 2014, Colombia was the third most endemic country in Latin America which reported 17 % of the total 390,000 malaria cases of the region.[1] In the country, based on epidemiological, entomological records and parasite detection, nine anopheline species are considered vectors of the *Plasmodium* parasites that cause human malaria; three of them are considered of primary importance, *Anopheles* (*Nyssorhynchus*) *albimanus* Wiedemann 1920, *Anopheles* (*Nys.*) *darlingi* Root 1926, and *Anopheles* (*Nys.*) *nuneztovari* Gabaldon 1940, and at least other six species are vectors of local importance.[3-5] Furthermore, *Anopheles triannulatus* and *Anopheles calderoni* were recently detected naturally infected with *Plasmodium* spp.,[6-8] but their epidemiological importance still needs to be further studied.

Most research on the Colombian anopheline mosquitoes have studied the adults, evaluating aspects such as morphological and molecular taxonomy,[9-12] behavior and vector incrimination,[4,7,13] population structure and phylogenetics.[14-17] However, few studies have been conducted on the ecology of the species, and specifically, on immature stages. One of those studies described the larval habitat of *Anopheles* species detected in the city of Villavicencio, Meta, in Central Colombia.[18] In the study, *Anpheles rangeli* and *An. marajoara,* identified using morphological characters, were the most abundant species in larval stage and fishponds close to the homes presented the greatest species diversity. *Anopheles darlingi* was only found in forested environments, in habitats with clear water and shade. In another study, in the municipality of Cimitarra, Santander, Northeast Colombia, *An. triannulatus* was the most abundant species. Fishing ponds were the most productive larval habitats, characterized by containing permanent, cloudy or clear water, generally fully exposed to the sun and with emergent vegetation.[19]

Several studies in Latin America have evaluated the conditions of the larval habitats used by anopheline mosquitoes, especially those occupied by the important Colombian *Anopheles* vector species, *An. albimanus, An. nuneztovari* and *An. darlingi*. For example, in Boyeros municipality, Cuba, *An. albimanus* was detected in natural lakes and lagoons, but also in artificial larval habitats such as land depressions or holes resulting of runoff processes.[20] While, in Haiti localities, *An. albimanus* occupied larval habitats with clear or turbid, brackish and low or moderate polluted waters.[21] In Colombia, *An. albimanus* immature stages have been detected in brackish ponds, mining excavation holes and fishing ponds.[22] The other important Colombian vector, *An. nuneztovari*, is characterized by its great adaptability to variable larval habitats, particularly

artificial ones.[23] In the State of Acre, Brazil, *An. nuneztovari* was detected along the north margin of the Branco river in irregular excavations associated with manual brick manufacturing;[24] it has also been reported in fresh-water bodies with emergent vegetation or fully exposed to the sun.[25] In Venezuela, specifically in San Rafael, Bolivar State, *An. nuneztovari* used large water bodies, streams and small ponds and mine cavities.[26] In Colombia, in the municipality of Buenaventura, Pacific Coast, *An. nuneztovari* immature stages were detected in fishing ponds associated with emergent vegetation and fully exposed to the sun.[27] Also, in the municipality of Cimitarra, Santander, it was found in fish ponds near urban areas.[19] The Latin-American vector *An. darlingi* is frequently found in shaded and clean waters.[28] In Boa Vista, Roraima State, Brazil, larval habitats were mainly permanent water bodies and shaded patches associated with forest cover;[25] and in the Peruvian Amazon, its larval habitats were forest wells, ponds and streams with partial or full shade and emergent vegetation.[29] In Colombia, in the Bajo Cauca and Magdalena Medio regions, *An. darlingi* immatures were detected in lakes and ponds with floating plants;[22] while, in the municipality of Villavicencio, it was found in rural larval habitats with clean shaded running waters.[18]

The preference of anopheline mosquito for specific water bodies for oviposition is influenced by the physical and chemical factors of the habitat.[30] In addition, *Anopheles* are very sensitive to environmental changes resulting from deforestation or changes in land use;[31] these ecological alterations can cause modifications on physical and biological conditions and availability of suitable larval habitats.[32,33] Further, the evaluation of larval habitat characteristics and immature mosquito populations and productivity, allows predicting the composition and abundance of adult mosquitoes and to generate useful information for vector control.[34,35] Therefore, the aim of this study was to evaluate the anopheline species found in larval stage in malaria endemic localities of northwest and west Colombia, describe the type of larval habitats occupied and correlate their presence with temperature and pH of the water in the habitat. A better understanding of the ecology of the malaria vector species will allow the implementation of targeted control measures for effective control.

MATERIALS AND METHODS

Sampling sites. The localities of study are located in municipalities of two endemic malaria regions of Colombia (Fig.1). They were: El Loro (LOR), in the municipality of Tierralta and Juan José (JJO) in Puerto Libertador, both in the Department of Córdoba, located in the Caribbean natural region.[36] This region is characterized by a wide ecological range, featuring large ecosystems and extensive territorial waters. The localities of San Antonio de Padua (SAP) in the municipality Vigia del Fuerte, Department of Antioquia and Zacarías (ZAC) in Buenaventura, Department of Valle del Cauca are in the Pacific region, with characteristics of humid tropical forest.[36] *Anopheles* larvae were collected by the dipping method.[37] Larvae were counted and separated according to their stage (I, II, III, and IV). Third and fourth instar larvae were preserved in 95% ethanol and transported to the laboratory for their identification and further processing.
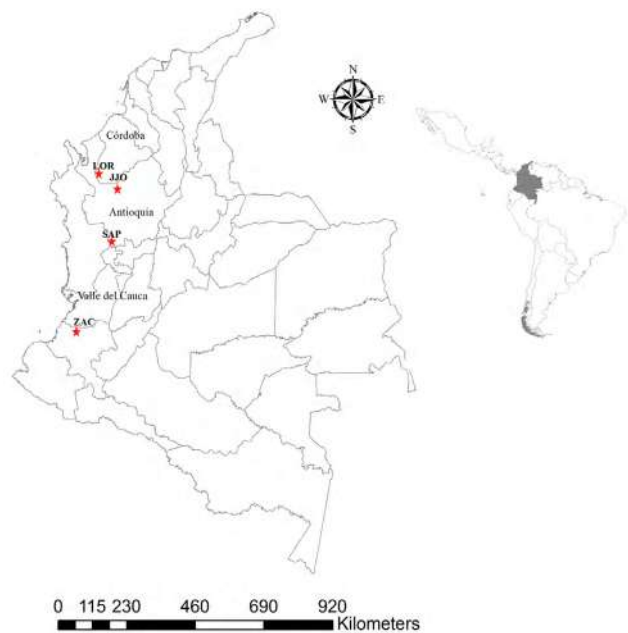

Figure 1. Sampling sites. Localities where *Anopheles* larvae were collected. LOR-Tierralta and JJO-Puerto Libertador in Cordoba Department, SAP-Vigia del Fuerte in Antioquia and ZAC-Buenaventura, in Valle del Cauca.

PARAMETERS DETERMINED FOR LARVAL HABITATS

Water bodies were inspected for the presence of anopheline immature stages and the physicochemical variables water temperature and pH were registered. Other parameters recorded included, permanence of body of water (permanent or temporary), proximity to the nearest house and aspects such as vegetation surrounding the habitats and shade.

MORPHOLOGICAL IDENTIFICATION AND MOLECULAR CONFIRMATION OF SPECIES BY PCR-RFLP-ITS2 AND *COI*

The larvae were identified using a taxonomic dichotomous key.[38] Species assignation was molecularly confirmed using a PCR-RFLP-ITS2[8,9,39] on a representative number of larvae per species, randomly selected based on a sample of a finite population.[40] For *An. malefactor*, species for which a specific PCR-RFLP-ITS2 pattern is not yet defined, a region of the Cytochrome C Oxidase subunit I (*COI*) or barcode was amplified using primers and conditions previously described.[12,15] *COI*-PCR products were sequenced and the obtained sequences were edited using Geneious 6.0.6;[41] the consensus sequences were compared with those available in BOLD platform (http://www.bold-systems.org/) and GenBank, NCBI.

STATISTICAL ANALYSIS

To determine the composition of the *Anopheles* larvae community, total richness (S) was estimated as the total number of species by larval habitat, and also, the absolute abundance or number of larvae per species (*n*). The *t* student test and ANOVA were carried out in GraphPad Prism version 6.04 for Windows, [42] to establish statistically significant differences between species and a larval habitat variable. To establish the relationship between the abundance of the species and the variables: water temperature, pH, and proximity of larval habitats to the nearest house, Spearman correlation coefficient was performed in the program PAST version 2.17.[43] This correlation was not carried out for SAP, Vigía del Fuerte-Antioquia and ZAC, Buenaventura because there were not enough samplings for the analysis.

RESULTS

ANOPHELES SPECIES AND LARVAL HABITATS

The type of larval habitats detected in the localities included streams, ponds, puddles, wetlands, jagueys, lakes and lagoons. LOR, in the Caribbean Region was the locality presenting greater variety of positive larval habitats (Table 1), followed by JJO- Caribbean Region; while SAP, in the Pacific region, flooded areas, varying from small and temporal to those dedicated to artisanal rice cultivation constituted positive anopheline larval habitats. In ZAC, the Pacific Region, fishing ponds and lagoons were the only positive larval habitats.

A total of 202 third and fourth instar larvae were collected, that after identification and molecular confirmation corresponded to *An. nuneztovari, An. darlingi, An. albimanus* and *An. malefactor*. The larvae collected in LOR corresponded to *An. nuneztovari, An. albimanus* and *An. darlingi. Anopheles nuneztovari* was the dominant species (*n* = 89) and their larvae were found predominantly in lakes (*n* = 35); *An. darlingi* in lagoons (*n* = 13) and in smaller numbers in puddles (*n* = 1); *An. albimanus* were collected only in lakes (*n* = 9) and lagoons (*n* = 3). Lakes, lagoons and puddles were larval habitats where *An. nuneztovari* and *An. darlingi* larvae co-occurred. In JJO, *An. nuneztovari* (*n* = 41) was found predominantly in jagueys (*n* = 23); these are artificial pools of water used in this locality as troughs for animal feed; in SAP, *An. darlingi* (*n* = 7) was collected in flooded areas and dishes used for rice cultivation and *An. malefactor* (*n* = 20) was collected mainly in small bodies of water by the forest. In ZAC, lagoons constituted the the prefered larval habitats for *An. nuneztovari* (*n* = 7) (Table 1).

Comparison of species detected as third and fourth instar larvae to the species collected as adults in these localities (7,12), show that *An. nuneztovari* is detected in both, larval stage and as adult in ZAC, LOR and JJO, and *An. darlingi* in LOR and SAP (Table 2).

Table 1. *Anopheles* species detected in larval stage (third and fourth instar) in localities of west and northwest Colombia

Department/Municipality/ Locality/	El Loro (LOR) Tierralta / Córdoba	Juan José (JJO) / Puerto libertador/Córdoba	San Antonio de Padua (SAP) / Vigía del fuerte/ Antioquia	Zacarías (ZAC)/ Buenaventura Valle del Cauca
Larval habitats	Species name *(n)*	*n*	*n*	*n*
Stream	*An. nuneztovari* (5)	*An. nuneztovari* (5)	---	---
Pond	*An. nuneztovari* (8)	---	---	---
Puddle	*An. nuneztovari* (12) *An. darlingi* (1)	--- ---	--- ---	--- ---
Wetland	*An. nuneztovari* (7)	*An. nuneztovari* (6)	--- ---	--- ---
Jaguey	*An. nuneztovari* (5)	*An. nuneztovari* (23)	---	---
Lake	*An. nuneztovari*(35) *An. darlingi* (6) *An. albimanus* (9)	*An. nuneztovari* (9) --- ---	--- --- ---	*An. nuneztovari* (5) --- ---
Lagoon	*An. nuneztovari* (17) *An. darlingi* (13) *An. albimanus* (3)	*An. nuneztovari* (2) --- ---	--- --- ---	*An. nuneztovari* (7) --- ---
Flooded areas	---	---	*An. malefactor* (20)	---
Flooded areas-dishes for rice cultivation	---	---	*An. darlingi* (7)	---
Total	**121**	45	27	12

Table 2. Abundance of species collected in larval stage (third and fourth instar) and adults at four localities of West and Northwest Colombia

Locality	Collection date	Larval stages Species	n	Adults Species	n
TAL El Loro (LOR)	November 2008- September 2009	*An. nuneztovari* *An. darlingi* *An. albimanus*	89 20 12	*An. nuneztovari* *An. darlingi* *An. triannulatus*	554 5 3
PLT Juan José (JJO)	July 2009- June 2010	*An. nuneztovari*	45	*An. nuneztovari* *An. darlingi* *An. pseudopunctipennis* *An. punctimacula* *An. argyritarsis*	2143 27 236 1 1
VF San Antonio de Padua (SAP)	April 2009-November 2009	*An. malefactor* *An. darlingi*	20 7	*An. nuneztovari* *An. darlingi* *An. punctimacula*	2 1298 2
BUE Zacarías (ZAC)	April 2009- February 2010	*An. nuneztovari*	9	*An. nuneztovari* *An. neivai*	405 11

Anophles mosquitoes in adult stage are reported in Naranjo *et al.* 2013, Naranjo *et al.* 2014

PARAMETERS DETERMINED FOR LARVAL HABITATS

The features measured were temperature and pH of the water in the larval habitat and the distance to the nearest home; these were correlated with the abundance of *Anopheles* larvae (95% confidence intervals) (Table 3 and 4). *Anopheles nuneztovari* was the dominant species in LOR-Tierralta and their larvae were found predominantly in lakes exposed to the sun with floating vegetation. These habitats were located at an average distance 27.5 m from the nearest inhabited house, the average of water temperatures in the habitats of 31.6 °C and pH 5.5. *Anopheles darlingi* was predominantly found in lagoons that were partially exposed to the sun and surrounded by grass. The average distance to the nearest house was 17.5 m and the water temperature 32.4°C and

25

pH of 5.7. Also, *An. albimanus* larvae were collected in lakes and lagoons exposed to the sun with floating vegetation and surrounded by grass, located to an average of 23.9 m from the nearest inhabited house, with temperatures average of 32.3°C in the body of water and pH of 5.3.

Table 3. Features of larval habitats of *An. nuneztovari, An. darlingi, An. albimanus* and *An. malefactor* in four localities of West and Northwest Colombia

	An. nuneztovari	An. darlingi	An. albimanus	An. malefactor
Larvae (*n*)	143	27	12	20
pH	5.7- 6.1	5.2 – 6.4	4.3 – 5.8	5.6 – 6.3
Water temperature (°C)	30.6 - 32.7	24.5 – 37.0	21.1 – 41.9	14.2 – 39.7
DINMH (m)	16.7 – 41.2	6.2 – 25.0	11.2 – 63.7	100 ≥ 100

DINMH: distance to the nearest inhabited house. 95% confidence intervals.

Table 4. Spearman correlation for water temperature, pH of larval habitats and their distance to the nearest house

Locality	Larval habitat	Especies	Correlation variables – abundance of species					
			Temperature		pH		Distance	
			r	p	r	p	r	p
(LOR)	Wetland	*An. nuneztovari*	-0,81	0,5	**0,81**	**0,25**	0	1
	Wetland	*An. nuneztovari*	**0,83**	**0,33**	0,5	0,5	-0,81	0,25
	Pond	*An. nuneztovari*	-1	0,33	**1**	**0,33**	0	1
	Jaguey	*An. nuneztovari*	0,38	0,66	-0,05	0,66	0	1
	Puddle	*An. nuneztovari*	0,31	1	**0,94**	**0,16**	0	1
	Jaguey	*An .nuneztovari*	-0,31	1	0,4	1	0	0
	Stream	*An. nuneztovari*	0	1	0	1	0	1
	Lake	*An. nuneztovari*	**0,63**	**0,5**	**0,63**	**0,5**	0	1
	Lagoon	*An .nuneztovari*	0,21	0,83	0,21	0,83	0	1
	Lagoon	*An. nuneztovari*	-0,94	0,16	**0,73**	**0,33**	0	1
	Lagoon	*An. nuneztovari*	0,73	0,33	**0,73**	**0,33**	0	1
	Jaguey	*An. nuneztovari*	**0,86**	**0,66**	**0,86**	**0,66**	0	1
	Puddle	*An. darlingi*	-0,81	0,5	0,27	1	0	0
	Lagoon	*An .darlingi*	0,27	1	0,27	1	0	1
	Lagoon	*An. darlingi*	-0,55	1	-0,55	1	0	1
	Lake	*An. darlingi*	**0,54**	**0,75**	-0,54	0,75	0	1
	Lake	*An. albimanus*	0,5	1	-0,55	1	0	1
	Lagoon	*An. albimanus*	-0,54	0,75	-0,54	0,75	0	1
	Lake	*An. albimanus*	-0,54	0,75	-0,54	0,75	0	1
	Lagoon	*An. albimanus*	-0,54	0,77	-0,54	0,75	0	1
(JJO)	Humedal	*An .nuneztovari*	-0,5	1	0	1	0	1
	Arroyo	*An .nuneztovari*	-0,77	0,5	-0,77	0,5	0	1
	Jaguey	*An. nuneztovari*	**0,73**	**0,33**	-0,63	0,5	0	0
	Laguna	*An .nuneztovari*	0	1	**0,86**	**0,66**	0	0
	Laguna	*An. nuneztovari*	**0,77**	**0,5**	0,2	1	0	0

Correlations were carried out only for LOR and JJO in Cordoba Department, localities that presented adequate abundances of larvae for the analysis.

In JJO-Puerto Libertador, *An. nuneztovari* was found predominantly in jagueys partially exposed to the sun, with floating vegetation and located at an average distance of 31.5 m from the nearest inhabited house. Their water temperature averaging 31°C and pH 6.5 (Table 2). In SAP, *An. darlingi* was found only in flooded areas, partially exposed to the sun, with plant material on its surface, at an average distance of 24 m from the nearest inhabited house, water temperature of 26°C and pH of 6.0. In this locality, *An. malefactor* was also collected in small (< 2 m²) temporal larval habitats by the forest with water temperatures averaging 27.0°C, pH of 5.7 and at distances higher than 100 m from the nearest inhabited house (Table 3).

In ZAC-Buenaventura, lakes constituted the larval habitats for *An. nuneztovari*; in general, they were partially exposed to the sun and their surface was covered with floating vegetation and located at an average distance of 35 m to the nearest inhabited house; the water temperature averaging 31.4°C and pH 6.7 (Table 3).

The *t* student test with a 95% confidence interval and ANOVA test showed significant differences ($t = 2.546$ df $= 41$, $f = 0.0148$), ($F = 2.895$ $p = 0.0445$) for larval habitats of *An. malefactor* with respect to the variable distance to the nearest inhabited house. Spearman correlation (Table 4), indicated that in LOR, the presence of *An. nuneztovari* in wetlands, lakes, lagoons and jagueys, is correlated with pH values of 5.7 - 6.1 and water temperatures of 30.6-32.7°C; but there was no significant correlation with the distance to the nearest inhabited home. In JJO, presence of *An. nuneztovari* was correlated with water temperatures of 28.4 - 35.3°C in jagueys and lakes and with pH 5.9 - 7.2. There was no significant correlation for *An. darlingi* or *An. albimanus* with any of the variables evaluated.

DISCUSSION

Knowledge of larvae ecology of *Anopheles* mosquitoes is important to implement effective malaria vector control interventions, specifically those directed to the immature stages.[44] This study provides information on aspects of anopheline larval ecology in malaria endemic localities of Antioquia, Córdoba and

Valle del Cauca Departments. In the localities, the three main vector species of Colombia, *An. albimanus*, *An. nuneztovari* and *An. darlingi*, were collected in larval stage (third and fourth instar), as well as, *An. malefactor*. *Anopheles nuneztovari* was the larval species more frequently collected in a variety of larval habitats, found in the localities of Córdoba and Valle del Cauca, but it was not detected in SAP, Antioquia, where *An. malefactor* was the dominant species followed by *An. darlingi*. *Anopheles nuneztovari* has great adaptability and colonize artificial breeding sites in environments disturbed by human activities.[40] In this study, its larvae were detected in both, sunlit and shaded habitats, as previously reported,[25] and in those localities more impacted by antrophic activities.

Anopheles albimanus immatures were only found in LOR, Tierralta, Cordoba Department, using lakes and lagoons as larval habitats. A greater variety of habitats have been describe for this species, such as lakes, marshes, man-made depressions to store rainwater, animal footprints and brackish ponds.[20,22] Therefore, the limited positive habitats for *An. albimanus* in LOR may be influenced by the type of land uses in this locality, mainly timber extraction, crop production and livestock.[13] Furthermore, in Colombia this species is predominantly distributed in coastal areas[3] and LOR is an inland locality.

Larvae of *An. darlingi* were detected in SAP, Antioquia, in flooded areas and dishes for artisanal rice cultivation, and in LOR, Cordoba, in puddles, lakes and lagoons, partially exposed to the sun and surrounded by grass. These results agree with reports for this species that indicate its preference in larval habitats with clear water, shade and vegetation, for instances, lagoons, forested streams and river margins covered with vegetation.[29] But, *An. darlingi* has also been registered in habitats with direct sunlight and in habitats with water temperatures of 20-28°C and pH 6.5-7.3;[45] there are even reports of *An. darlingi* larvae in waters with temperature as high as 40°C.[46] These values are similar to those reported in this study in the range of 24.51 – 37.05°C. According to the mean values of water temperature of larval habitats, *An. malefactor* was the species with the wider range of tolerance (14.29-39.71 °C). However, the higher water temperatures were found in larval habitats of *An. albimanus*. One advantage of high water temperatures in the larval

habitats is that those are detrimental to the presence of any other aquatic arthropods, including predators, which consequently increases the survivorship of anopheline larvae.[47]

In this study, the mean pH values of the larval habitats were neutral or slightly acidic. There was a positive correlation between pH and the presence of *An. nuneztovari* larvae in LOR and JJO, in Cordoba; the importance of the pH in the waters of larval habitats has been evidenced by reports that indicate that a neutral or marginally basic pH favor anopheline larvae development.[48]

Regarding the variable distance of the larval habitats to the nearest inhabited house, the positive larval habitats for the tree primary Colombian vectors were relatively close to the houses; particularly, *An. darlingi* habitats were at distances as short as six meters from the nearest inhabited house. Of interest, in SAP, Antioquia, *An. darlingi* larvae were detected in flooded areas and dishes used for artisanal rice cultivation, relatively close to houses. In a mosquito survey conducted in this locality in 2010, this species was detected infected with *P. falciparum*,[7] which suggests the need to direct interventions to control immatures in these larval habitats. The flooded areas were *An. malefactor* was established in SAP, were at distances greater than 100 m from the nearest inhabited house. Although, *An. malefactor* has not yet been implicated in transmission, it was the most abundant species registered in larval habitats in this locality. This species has also been registered in Puerto Nare, Department of Antioquia.[38] There is little knowledge on the biology of *An. malefactor* and it would be of interest understanding the specific factors that contributed to its abundance as immature stage in SAP, Vigia del Fuerte municipality, in Antioquia.

The occurrence of *An. nuneztovari* and *An. darlingi* in the same larval habitats, lakes and lagoons, in LOR, could be attributed to similarity of conditions in those habitats; as demonstrated, water temperature and pH values were not significantly different. The dominance of *An. nuneztovari* larvae in ZAC, LOR and JJO is attributed to the evidenced anthropogenic impacts, such as deforestation. This species is characterized by its adaptability to disturbed environments.[23,49]

In summary, this work reports on the presence of *An. nuneztovari*, *An. darlingi*, *An. albimanus* and

An. malefactor in larval stage (third and fourth instar) in malaria endemic localities of west and northwest Colombian. These data helps to update the inventory of anopheline species previously carried out in the Departments of Antioquia, Córdoba and Valle del Cauca.[4,7,13] In addition, the information is important for the implementation of effective vector control interventions aimed at immature stages.

Competing interests

The authors declare no to have competing interests.

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