Entomological characterization of malaria in northern Colombia through vector and parasite species identification, and analyses of spatial distribution and infection rates

Camila González1*, Astrid Gisell Molina1, Cielo León1, Nicolás Salcedo1, Silvia Rondón1, Andrea Paz1, Maria Claudia Atencia2, Catalina Tovar3 and Mario Ortiz1

Abstract

Background: Malaria remains a worldwide public health concern and, in Colombia, despite the efforts to stop malaria transmission, the incidence of cases has increased over the last few years. In this context, it is necessary to evaluate vector diversity, infection rates, and spatial distribution, to better understand disease transmission dynamics. This information may contribute to the planning and development of vector control strategies.

Results: A total of 778 Anopheles mosquitoes were collected in fifteen localities of Córdoba from August 2015 to October 2016. Six species were identified and overall, Anopheles albimanus was the most widespread and abundant species (83%). Other species of the Nyssorhynchus subgenus were collected, including Anopheles triannulatus (13%), Anopheles nuneztovari (1%), Anopheles argyritarsis (< 1%) and two species belonging to the Anopheles subgenus: Anopheles pseudopunctipennis (3%) and Anopheles neomaculipalpus (< 1%). Four species were found naturally infected with two Plasmodium species: Anopheles nuneztovari was detected naturally infected with Plasmodium falciparum and Anopheles pseudopunctipennis with Plasmodium vivax, whereas An. albimanus and An. triannulatus were found infected with both parasite species and confirmed by nested PCR.

Conclusions: In general, the obtained results were contrasting with previous studies in terms of the most abundant and widespread collected species, and regarding infection rates, which were higher than those previously reported. A positive relationship between mosquito local abundance at the locality level and human infection at the municipal level was found. Mosquito local abundance and the number of houses with mosquitoes in each village are factors explaining malaria human cases in these villages. The obtained results suggest that other factors related to the apparent variation in malaria eco-epidemiology in northern Colombia, must be identified, to provide health authorities with better decision tools aiming to design control and prevention strategies.

Keywords: Anopheles, DNA Barcode, Malaria, Plasmodium falciparum, Plasmodium vivax

Background

Malaria transmission constitutes one of the greatest public health challenges on a global scale and the World Health Organization (WHO) has established an ambitious plan to work towards the control and elimination of the disease by 2030. The milestones set for 2020 include, among others, the reduction of case incidence and mortality rates by 40% [1]. In Latin America, between 2010 and 2015, there was a reduction of 31% in case incidence and of 37% of the mortality rate. Colombia ranked third after Venezuela (30%) and Brazil (24%) with 10% of the estimated malaria cases in the region.
Although efforts have been directed to meet these goals, in February 2017, the Pan American Health Organization issued an alert [2] due to a significant increase in malaria transmission in the Americas in 2016. Indeed, eight of the 21 endemic countries for disease transmission recorded an increase in the number of cases compared to 2015. Following the same pattern, Colombia reported a reduction of 72% in incidence and 87% in mortality between 2000 and 2014 [3], but in 2015 and 2016 the incidence of cases increased by 37% each year, reaching numbers similar to those found in 2006 [4].

In Colombia, *Plasmodium vivax* was historically reported as the most prevalent parasite species responsible for malaria cases in the country: for instance, in the 2010 outbreak, *P. vivax* represented 71% of the cases and *Plasmodium falciparum* 28% [5]. However, in 2016, the National System of Public Health Vigilance (SIVIGILA by its Spanish acronym) reported 83,356 cases of malaria in Colombia of which 47,497 (57%) were caused by *P. falciparum*, the parasite responsible for cerebral malaria, while 33,055 (40%) corresponded to *P. vivax* and 3.3% to mixed infection [4]. This increase in the number of cases caused by *P. falciparum* was also recorded in other countries, including Venezuela, Ecuador, Peru and Honduras, implying a higher risk of complicated malaria, and indicating failures in the access to treatment and in vector control [2].

According to the WHO [6], vector control is the main strategy to prevent and reduce malaria transmission, mostly through mosquito nets and indoor residual spraying. Entomological surveillance is the first step to make a proper assessment of vector species present in a transmission setting, as well as to establish infestation and infection rates. From this perspective, there is a need for conducting research related to malaria vectors in order to better understand disease transmission and to develop new control strategies [7], especially in the context of the epidemiological alert that is currently being faced [2].

Research on the eco-epidemiology of the disease in Colombia usually is made as a part of outbreak responses or short-term research programmes aiming to reduce the number of cases at a local scale [8–11]. Those studies highlight the entomological surveillance as a useful tool when planning strategies for malaria control.

In 2016, seven out of 32 Colombian departments (Chocó, Nariño, Antioquia, Amazonas, Guainía, Córdoba and Cauca), and the municipality of Buenaventura in Valle del Cauca department, recorded 92.8% of all malaria cases in the country. The department of Córdoba, recorded 1751 cases, and the municipality of Tieralta had the highest proportion of cases (56% of cases), followed by Puerto Libertador (24%), Montelibano (7.5%), Planeta Rica (1.7%) and Montería (1.6%). Córdoba is known for its cattle ranching practices and unplanned agriculture [12]; rice crops are present in this area [13] and *Anopheles pseudopunctipennis* has been found using them as breeding sites [14]. Previous entomological studies conducted in the department reported *Anopheles nuneztovari, Anopheles triannulatus, Anopheles darlingi, Anopheles albimanus* and *An. pseudopunctipennis*, among other species [15–17]. *An. nuneztovari* was the dominant species in those studies, and was considered as the primary malaria vector in the department [17].

In this context, this study was designed to investigate the entomological scenario of malaria in villages in Córdoba through the study of vector diversity, spatial distribution of vectors and parasites, and infection rates. The study design could be implemented as a tool to monitor vector-borne diseases occurring in domestic settings on a broad geographic scale in tropical regions, where baseline information on the situation of malaria needs to be assessed. In this way, this study can contribute valuable information to better design prevention and control strategies aiming to reduce the burden of the disease.

**Methods**

**Entomological collection**

Fifteen villages within 13 municipalities of the Department of Córdoba, Colombia (09°26′16″ N, 76°30′01″ W), were selected for sampling based on different criteria: their epidemiological importance in terms of vector-borne diseases, accessibility, and security (Fig. 1). Entomological collections within each town were performed in 24 houses, randomly selected to establish potential malaria vector species present in the department. The selection of households was performed using a 25 m × 25 m grid created using ArcMap 10.1 [18] on top of a satellite image of the locality from Google Earth [19] covering all the area with houses. Inside the grid, 24 cells were randomly selected for sampling. For the seven localities with no available satellite images (Villa Lucia-Sahagún, San Juan—Puerto Libertador, Hoja Ancha—San Andrés De Sotavento, Punta Verde—Planeta Rica, Altomirar—Moñitos, Guaimaro abajo—Los Córdobas and Pica Pica—Montelibano) all houses were georeferenced in an exploratory field trip and 24 were randomly selected using R [20].

Houses were numbered from one to 24 and eight houses were sampled each night during three consecutive nights using two CDC light traps; one was located indoors, and the other one, outdoors. In odd numbered houses (e.g. house 1, 3 and 5), a BG Sentinel trap baited with BG-Lure [21] was placed inside. CDC traps were set at 6:00 p.m. and left active for 12 h, and BG Sentinel traps were active for 24 h. The following morning, traps were revised and mosquitoes were sorted for...
During the day, households were examined for breeding sites, and diurnal adult collections were performed using backpack aspirators [22]. Each town was sampled twice between August 2015 and September 2016 (Fig. 1). All male mosquitoes were preserved dry in tubes with silica gel for their accurate taxonomic identification. All female mosquitoes were stored in 70% ethanol to perform molecular analyses for parasite detection. Species identification was performed based mainly on morphological characters of males, although, females, preserved in liquid, were also examined for general morphological characteristics using the keys of González and Carrejal [23], Lane [24], and Forattini [25]. Due to the loss of important taxonomic characters, for 65 specimens we extracted DNA from legs (or abdomen tissue in individuals lacking appendages) and performed species identification using DNA barcoding through amplification of the 658 bp region from the COI gene. The PCR mix consisted of 1 µL of total DNA, 12.5 µL of 2 × GoTaq Green Master Mix (Promega), and 10 µM of the primers LCO1490 and HCO2198 [26]. The cycling conditions were: 1 min at 94 °C; followed by 40 cycles of 40 s at 94 °C, 40 s at 52 °C, 1 min at 72 °C; and a final extension of 5 min at 72 °C. All PCR products were sequenced at the DNA sequencing laboratory UNIANDES using the ABI-3500 Genetic Analyzer (Life Technologies).

Analysis by means of ANOVAS were made to evaluate differences on Anopheles collections between indoor and outdoor traps, and a post hoc test (Tukey test) was performed to assess the effect of locality on mosquito’s abundances.

Parasite detection and species identification
To detect the presence of parasites, DNA was extracted from pools of up to ten female Anopheles mosquitoes from the same species, using ZR Tissue & Insect DNA Miniprep kit (Zymo) following the manufacturer’s manual. Plasmodium sp. detection was performed through nested PCR following previously published methods.

Fig. 1 Study site showing sampled localities and collection dates. Black squares correspond to sampling locations in each locality. Table shows the location and dates for each sampled locality. Coordinates correspond to the 12th house of each locality and sampling dates to the first night of sampling out of three consecutive nights.

| Municipality      | Locality               | Coordinates | Sampling dates |
|-------------------|------------------------|-------------|---------------|
| Loricí            | La Doctrina            | -75.890     | 3/08/15       | 9/06/16       |
|                   | Mata de Caña           | -75.827     | 2/09/15       | 2/09/16       |
| Montería          | El Vidiál              | -75.900     | 2/10/15       | 7/04/16       |
| Sahagún           | Villa Lucía            | -75.382     | 8/10/15       | 10/08/16      |
| Caretía           | Corregimiento Martínez | -75.771     | 16/10/15      | 21/04/16      |
| Planeta Rica      | Punta Verde            | -75.615     | 27/11/15      | 13/07/16      |
| San Andrés de Sotavento | Nueva Unión          | -75.503     | 8/07/15       | 12/10/16      |
|                   | Hoja Ancha             | -75.496     | 8/07/16       | 12/10/16      |
| Molílitos         | Bellacohita            | -76.085     | 19/02/16      | 25/07/16      |
|                   | Altomirar              | -76.071     | 24/02/16      | 21/07/16      |
| Los Córdobas      | Guaimaro Abajo         | -76.364     | 22/09/16      | 9/03/16       |
| Montebello        | Pica Pica Nuevo        | -76.800     | 18/03/16      | 18/08/16      |
| TierraTierra      | Nueva Unión            | -76.179     | 12/05/16      | 8/09/16       |
| Valencia          | San Rafael             | -76.246     | 25/05/16      | 25/08/16      |
| Puerto Libertador | San Juan               | -75.727     | 2/06/16       | 14/09/16      |
[27] using GoTaq Green Master Mix (Promega). All PCR products from second reactions were visualized on an agarose gel, targeting a 205 bp fragment for *P. falciparum* and a 120 bp fragment for *P. vivax*, positive samples were sequenced to confirm species identity. Sequences were cleaned and aligned using CLC Genomics Workbench 3.6.5 Software and compared with publicly available sequences using BLAST. To confirm the identity of the *Anopheles* species present in all the positive pools, 1 µL of the pooled DNA extract was used and the barcode technique was applied as explained before.

**Eco-epidemiological and spatial analyses**

For each sampled town, the minimum infection rate (MIR) for each *Anopheles* species and for all species together was computed, based on the assumption that each positive pool should contain at least one infected mosquito [28]. Using the number of positive houses (i.e. household with at least one mosquito pool infected with *Plasmodium*) a ratio was calculated for each town.

To evaluate if there was a threshold of mosquito abundance that could sustain parasite presence, Pearson correlations between mosquito abundances and the number of positive houses were assessed (after testing for normality of all variables with a Shapiro–Wilk test). Furthermore, multiple regression models were fitted to identify the variables that influence the number of infected inhabitants.

Regarding spatial analyses, to evaluate if the distribution of collected and infected mosquitoes was random in space, Moran’s Index calculations were performed in ArcGIS 10.2. For this, a Global Moran’s I where the null hypothesis is that of random distribution in space, Moran’s Index calculations were performed for each of two feature classes, the first was computed, based on the assumption that each positive pool should contain at least one infected mosquito. The relationship between infection and environment was also evaluated. For this, sampled localities, positive localities for *Anopheles* and localities with mosquitoes infected with *Plasmodium* species were intersected with *An. albimanus* and *A. pseudopunctipennis* were rec- identified by comparing obtained sequences to available sequences on GenBank [31]. DNA barcode analyses allowed for the identification of nine individuals that were not identified using morphology; six individuals corresponded to *An. albimanus* and three to *An. triannulatus*. A total of 35 individuals were found to have been misidentified using morphology (an error of 10.7% in morphological identification). Twenty-six specimens previously identified as *Anopheles benarochi* were rectified, as *An. albimanus* (14) and *An. triannulatus* (12). Also, nine *An. albimanus* were rectified to *An. nuneztovari* (Table 1).

**Parasite detection and species identification**

Of the fifteen sampled localities, nine were positive for two species of *Plasmodium*, *P. vivax* and *P. falciparum*. Two localities were positive for only *P. vivax* (Altomirar and La Doctrina), and two localities only for *P. falciparum* (San Juan and El Vidrial), the remaining five localities were positive for both parasites, and in three of them...
Fig. 2 Proportion of local abundances for the six collected *Anopheles* species in each locality over the total number of *Anopheles* collected.
mixed infections in An. albimanus, pooled from the same house, were detected (Fig. 3).

Mosquitoes collected in 38 households were found to be positive for parasites, 13 exclusively for P. falciparum (34.2%) with 15 positive pools, 23 for P. vivax (57.8%) with 19 positive pools, and three for mixed infection with three positive pools (7.8%). Five DNA samples produced a high number of non-specific PCR products when screened for P. falciparum, in those cases no sequence was obtained and they were therefore designated as ‘failed reactions’. All positive PCR results were confirmed by sequencing [35] (Fig. 3).

Four Anopheles species were found infected with Plasmodium; the positive pools were more commonly An. albimanus (31 positive pools), followed by An. triannulatus (Four positive pools). One pool of An. nuneztovari was found infected with P. falciparum and one pool of An. pseudopunctipennis with P. vivax (Fig. 3) (Additional file 2: Table S2).

**Eco-epidemiological and spatial analyses**

According to our analyses, no positive correlation was found between the local abundances of Anopheles and the number of positive houses ($p = 0.089; p$ value 0.205). Anopheles mosquitoes were present in all sampled towns with one species, An. albimanus, dominating across the whole department and being present in every sampled locality (Fig. 2). The geographic distribution of Anopheles mosquitoes in the municipalities of Córdoba was random in space (Morans I = 0.0054, $z$-score = 0.54, $p$ value = 0.59). Anopheles triannulatus was found in nine localities of eight municipalities and, as was also the case for An. pseudopunctipennis, which was present in four municipalities, was spatially scattered. Anopheles neomaculipalpus was only present in two of the sampled municipalities (Los Córdobas and Valencia), while Anopheles argytarsis was found only in the south of Córdoba in the municipality of Tierra Alta. Interestingly, An. nuneztovari, the most important species in previous studies [36, 37] was only present in Puerto Libertador.

Regarding breeding sites, no Anopheles larvae were found in water sources close to sampled houses.

The number of females per locality was highly variable ranging from one to 159 individuals and no site had all six species coexisting. The most diverse communities had different combinations of four mosquito species maximum. The town of San Juan in the municipality of Puerto Libertador had the highest abundance of mosquitoes ($n = 154$), and a fairly diverse community of four different species: An. albimanus, An. nuneztovari, An. pseudopunctipennis, and An. triannulatus.

Interestingly, mosquito abundances were related to sampling locality ($p$ value two-way anova = 0.0025). This effect however, was limited to the localities of San Juan in the municipality of Puerto Libertador and San Rafael in the municipality of Valencia, with the first town having 3.16 times (S.E = 0.85) more mosquitoes per night per house than the latter.

Sampling was performed in areas classified in either of five categories according to the CORINE classification: urban areas, transitory cultures, grasses, agricultural areas, and continental water areas. Mosquito presence was registered in all sampling areas (background). Distribution of infected mosquitoes was geographically scattered in Córdoba (Morans I = −0.04, $z$-score 0.0013, $p$ value = 0.99) although presence of infected mosquitoes was disproportionately higher in areas covered by grasses (mainly used for cattle) in relation to the overall grass coverage in the sampling areas. For other land cover categories, the presence of infected mosquitoes was either proportional to the sampling background or lower.

In terms of seasonality, the number of collections varied during the seasons and more species were collected during the rainy season (six) than during the dry season (three), however, only five localities could be sampled in both seasons due to logistic difficulties. Based on these localities, the average number of mosquitoes collected in each season for each species was higher in the rainy season for An. albimanus (8.6 in rainy season, 8.2 in dry season), An. triannulatus (6.2 in rainy season, 1 in dry season) and An. pseudopunctipennis (1.6 in rainy season, 1 in the dry season). The remaining species An. argyritarsis, An. neomaculipalpus and An. nuneztovari, were only collected in the rainy season.

**Discussion**

Overall, the obtained results are contrasting with previous entomological studies performed in Córdoba between the years 2008 and 2014, which reported An. nuneztovari as the most abundant species; in this study it accounted only for 1.3% of collected specimens and was only found in one locality and only in the rainy season [15–17]. The most abundant and widespread species

---

**Table 1** Reference sequences used for comparison of DNA barcodes generated in this study, including species name, GenBank number, % identity threshold used for identifications and sources of the reference sequences

| Species              | GenBank sequence | % identity (%) | Origin |
|----------------------|------------------|----------------|--------|
| A. pseudopunctipennis| KC354819.1        | 98             | [32]   |
| A. neomaculipalpus   | JX05125.1         | 98             | [33]   |
| A. albimanus          | KC354823.1        | 99             | [32]   |
| A. triannulatus      | JX05112.1         | 98             | [33]   |
| A. nuneztovari       | KU865556.1        | 99             | [34]   |
Fig. 3 Distribution of infected mosquitoes and *Plasmodium* species found in each locality; positive municipalities are represented by simple hatch and negative localities by the black square symbol.
was *An. albimanus*, found in every sampled locality and both seasons. This species is of epidemiological importance since it is considered as a primary vector in the low coastal regions of the Caribbean and Pacific coasts in Colombia [17], and our results contribute the first records of this species in two localities, Los Cordobas and Puerto Libertador. The second most abundant species, *An. triannulatus*, was originally described as zoophilic, and is widely distributed in Colombia [23]. Although it has been incriminated as local or regional vector in neighboring countries, its incrimination in Colombia has not been determined yet, but Gutierrez et al. suggested that this species could be acting as secondary malaria vector due to the high infection rates it exhibits [30, 36, 38, 39]. Rosero et al. [39] reported this species in northern Colombia, collected in Córdoba only in the rainy season of 2010, and occurring, as found in this study, in sympatry with primary malaria vectors such as *An. albimanus*, *An. nuneztovari* and *An. darlingi* and they detected *An. triannulatus* infected both with *P. vivax* and *P. falciparum*. An effect of rain seasonality on species composition was found in the present study; since community composition and abundances are related to the sampling season, generating a complete survey requires multi-season sampling. Furthermore, caution must be taken when comparing studies performed at different seasons. For example, the study of Rosero et al. found that in Córdoba, *An. nuneztovari* was the dominant species during the rainy season in August and November 2009 and June 2010 whereas the dominant species in the dry season of February 2010 was *An. pseudopunctipennis*.

*Anopheles pseudopunctipennis* was found infected with *P. vivax*; this species was previously collected in the south of Córdoba but always in lower abundances compared to those found in this study [16, 17]; this species is here reported for the first time in the northern part of the department, in the municipality of Moñitos. This scenario could be reflecting the effect of the sampling methods, using exclusively traps for insect collection, but might be providing valuable information that should be deepened regarding changes in the ecology of disease transmission related to the current changes in malaria epidemiology recorded in the Americas [2].

For instance, the proportion of *P. falciparum* detected when compared to previous studies seems to be increasing, although it is still lower than *P. vivax* (Table 2). Both species were detected sympatrically, occurring in the same localities, and even three pools of *An. albimanus* were detected with mixed infection. However, mixed infections in mosquitoes seem not to be critical from the epidemiological perspective, since mixed infections in humans seem to be the result of multiple inoculations (Table 2) [40].

Malaria transmission in the Department during the time of our study, as recorded by the SIVIGILA in 2015–2016, included 3195 cases of malaria, with Tierralta contributing 58%, Puerto Libertador 20% and Montelíbano 6%. One case of mixed infection from Puerto Libertador was detected, while *P. falciparum* caused 11 cases (in Tierralta, Puerto Libertador, Canalete and Montelíbano). Transmission areas where human cases were reported were also areas where collected *Anopheles* were infected with parasites. In Puerto Libertador, *P. falciparum* was detected in one pool of *An. nuneztovari*, while in Montelíbano and Tierralta *An. albimanus* was found infected with *P. falciparum* and *P. vivax*. However, the locality of Los Cordobas which in this study exhibited the highest number of infected pools with both parasite species, did not record human cases. This situation could be indicating that population is acting as an asymptomatic reservoir, so asymptomatic infections must be

---

**Table 2** Results of infection by locality showing the minimum infection rate (MIR), calculated as a ratio, considering one positive mosquito per pool, proportion of positive houses, and parasite species detected

| Village                  | Total *Anopheles* screened | Infected pools | MIR (ratio) | Proportion of positive houses | Total over 24 sampled houses | Positive for *P. vivax* | Positive for *P. falciparum* | Positive for mixed infection |
|-------------------------|---------------------------|---------------|-------------|------------------------------|------------------------------|------------------------|--------------------------|-------------------------------|
| Alto Mirar              | 9                         | 1             | 0.11        | 0.04                         | 0.04                         | 0                      | 0                        |                               |
| El Vidrial              | 12                        | 2             | 0.17        | 0.08                         | 0.08                         | 0                      | 0                        |                               |
| Guaimaro abajo         | 100                       | 12            | 0.12        | 0.63                         | 0.42                         | 0.17                   | 0.04                     |                               |
| La Doctrina            | 90                        | 1             | 0.01        | 0.04                         | 0.04                         | 0                      | 0                        |                               |
| Mata de Caña           | 32                        | 3             | 0.09        | 0.13                         | 0.04                         | 0.08                   | 0                        |                               |
| Nueva Unión            | 76                        | 8             | 0.11        | 0.33                         | 0.25                         | 0.04                   | 0.04                     |                               |
| Pica pica nuevo        | 108                       | 5             | 0.05        | 0.13                         | 0.08                         | 0.04                   | 0                        |                               |
| San Juan               | 198                       | 1             | 0.01        | 0.04                         | 0                            | 0.04                   | 0                        |                               |
| Villa Lucia            | 15                        | 4             | 0.27        | 0.17                         | 0.05                         | 0.1                    | 0.04                     |                               |

*Note:* Table 2 provides a summary of the minimum infection rate (MIR) for each village, calculated as a ratio considering one positive mosquito per pool, along with the proportion of positive houses and the parasite species detected.
taken into account, in the same way that symptomatic infections, involve circulating gametocytes which can infect mosquitoes even at low densities, contributing to parasite transmission [41].

In general, MIRs found in this study were much higher than those found, for example, by Ahumada et al. [8]; this could be the result of our preservation method (absolute ethanol), which has shown to be more efficient than silica gel, as high concentrations of ethanol can denature proteins which degrade DNA [42]. In addition, the method standardized by Rath et al. [43] was performed for Plasmodium spp. identification by pools of up to 10 individuals, which can increase the detection capacity compared to individual extraction. Also, the methodology by Snounou et al. [27] was followed, and sequencing was carried out in order to confirm the amplified products; in spite of the high numbers, the detected infection rates in the present study are reliable. An important comment on the detected infection rates, is that whole mosquitoes were used to perform parasite detection, and for the incrimination of malaria vectors it is advisable to use only head and thorax, to demonstrate the species ability to be infectious [43, 44]. In this way, it cannot be assumed that infected mosquitoes could subsequently transmit malaria parasites and act as vectors.

In the present study, six Anopheles species were collected in Córdoba, all of them occurring in human settlements in urban localities, and were equally collected inside and outside houses. This number of species and the number of collected specimens is small when taking into account that 47 species of Anopheles are recorded in the country [43], 20 species are known to occur in the Department [23] from the 47 species of Anopheles recorded in the country [45], and that previous studies have collected thousands of Anopheles using human-landing catches [11, 36]. Regarding collection numbers and species composition, this could be affected by the use of light traps or traps artificially baited. Human-landing catches have been the most commonly used method in malaria studies [15, 16, 36, 46, 47], however, it has been controversial due to ethical considerations, since it threatens the subject acting as bait [48]. For the purpose of large-scale sampling and comparative studies, the use of traps is advised. Qiu et al. [49] performed a study using Mosquito Magnet® traps with the addition of CO₂ to a blend of ammonia + l-lactic acid + 3-methyl-butanolic acid as synthetic odors which considerably increased the capture of anthropophilic Anophelines as well as other mosquito species; additionally, Mosquito Magnet® trap has an advantage over the CDC light trap and the BG-Sentinel® as it can function for weeks without batteries or propane replacement. However, an optimum performance trap for Anopheles collection that does not compromise the quality of the samples is still required. Based on previous studies [50, 51], we expected the BG sentinel traps to be the more efficient, however, CDC light trap, performed better, possibly due to the low efficacy of the attractants used in the BG sentinel traps [49].

Regarding species identification, all the samples subjected to barcode analyses exhibited 98–99% sequence identity to published sequences, 98% for An. triannulatus and An. neomaculipalpus [33], 98% for An. pseudopunctipennis [52] and 99% for An. albimanus, and 99% for An. nuneztovari [34]. Concordance between molecular and morphological identification can be used as an indication of reliability of the overall results, and it can help in species identification of species complex, such as An. triannulatus and An. nuneztovari.

The possibility of using DNA barcodes to confirm species identification is strongly recommended [53, 54]. Although these analyses can be expensive to perform in developing countries, they allow optimizing through molecular biology, the information gathered from valuable field samples such as species identification, infection and blood source analyses, critical steps to perform vector species incrimination. From our experience, it is also important to establish adequate preservation procedures for the collected samples. Insect samples should be transported frozen [55] as an alternative to ethanol since insects kept in ethanol solution suffer the loss of important characters used in their morphological identification, such as legs, scales and setae [56].

Entomological collections were made in villages, surrounded by agricultural matrices and disturbed ecosystems, so deforestation and land use changes can influence vector species composition and diversity. It is known that agriculture has an impact on the establishment of transmission cycles of vector-borne diseases, particularly affecting the availability of breeding sites; sampling localities coincided mostly with grasses and agricultural settings (both permanent and transitory) with positive sites being disproportionately associated with grasses [57]. With the obtained results, looking for breeding sites could provide an important contribution to be able to relate species occurrences and land coverage, especially in changing scenarios.

Lastly, no correlation was found between rain seasonality and species abundances or composition. The adult abundance of An. albimanus was, with few exceptions, directly related to high precipitation [58] and nearly disappeared during the dry season [59].

**Conclusions**

The most abundant and widespread species found in our study was An. albimanus, a result contrasting with previous studies; it is important to establish whether this difference is due to bias in the type of traps used, or if
indeed there is a variation in species composition and distribution possibly due to climate changes. Further studies should also assess the role of *An. triannulatus* in malaria transmission since its role has not been established yet but has been found infected.

The infection rates found in mosquitoes using molecular analysis, and subsequent sequencing are higher than those reported in previous studies. The use of DNA bar-coding as a tool for support taxonomic identification is advised, when inadequate storage of mosquitoes impedes morphological identification.

### Additional files

**Additional file 1.** *Anopheles* database including capture sites, geographic location and gender.

**Additional file 2.** Localities where positive pools were detected showing the total number of *Anopheles* screened, positive pools, parasite species, and minimum infection rate.

### Authors’ contributions

CG designed the study, CG, NS, AP analysed and interpreted data, AGM, NS and MO carried out morphological identification, CL performed DNA extraction and Barcoding, CG, NS, AP, MCA, CT conducted entomological sampling. CG, AGM, MO, CL, NS, AP, CT drafted and edited the manuscript. All authors read and approved the final manuscript.

### Author details

1. Centro de Investigaciones en Microbiología y Parasitología Tropical, CIMPAT, Departamento de Ciencias Biológicas, Universidad de los Andes, Cra. 1 No 18A-12, Bogotá, Colombia. 2 Facultad de Ciencias de la Salud, Universidad del Sinú, Cra 1w No 38-153, Montería, Colombia. 3 Grupo de Enfermedades tropicales y Resistencia Bacteriana, Facultad de Ciencias de la Salud, Universidad del Sinú, Cra 1w No 38-153, Montería, Colombia.

### Acknowledgements

Authors wish to thank Mailyn González and Instituto Alexander von Humboldt for their support with DNA barcode procedures, J. Bedoya, E. Martínez, E. Monterrosa and M. Carvajal for fieldwork activities. G.P. Randazzo provided valuable help with figure formatting. We thank Universidad de los Andes and Universidad del Sinú for supporting this research. The valuable contribution of the editor and reviewers to improve the manuscript is greatly appreciated.

### Competing interests

The authors declare that they have no competing interests.

### Availability of data and materials

Supporting data are included as an additional file entitled Additional file 2, containing the information on *Anopheles* species collected, the collection locality, gender and the trap used.

### Consent for publication

Not applicable.

### Ethics approval and consent to participate

Consent was obtained by the household owners for mosquito traps to be placed in their property after socialization of the project through the local health authorities.

### Funding

This project was funded by General System of Royalties Contract Number 754-2013, Colombia, and Government of Córdoba.

### Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 5 July 2017 Accepted: 19 October 2017 Published online: 27 October 2017

### References

1. WHO. World Malaria Report 2016. Geneva, World Health Organization. 2016. http://apps.who.int/iris/bitstream/10665/252038/1/9789241511711-eng.pdf?ua=1. Accessed 18 June 2017.

2. PAHO. Alerta Epidemiológica. Aumento de casos de malaria. Pan American Health Organization. 2017. http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=38148&lang=es. Accessed 18 June 2017.

3. WHO. World Malaria Report 2015. Geneva, World Health Organization. 2015. http://apps.who.int/iris/bitstream/10665/200018/1/9789241565158_eng.pdf. Accessed 18 June 2017.

4. SIVIGILA. Boletín Epidemiológico Semanal. Semana epidemiológica número 52 de 2016. 2016. http://www.sis.gov.co/boletin-epidemiologico/boletinesepede/2016/boletin_epidemiologico_semana52.pdf. Accessed 18 June 2017.

5. Chaparro P, Padilla J, Vallejo A, Herrera S. Charactarization of a malaria outbreak in Colombia in 2010. Malar J. 2013;12:330.

6. WHO. Fact Sheet: World Malaria Report 2016. Geneva, World Health Organization. 2016. http://www.who.int/malaria/media/world-malaria-report-2016/en/. Accessed 18 June 2017.

7. da Silva A, Pinto KS, Leite IS, das Virgens GR, dos Santos TM, Falqueto CB. Ecology of Anopheline Mosquitoes (Diptera: Culicidae) in the Central Atlantic forest biodiversity corridor, Southeastern Brazil. J Med Entomol. 2013;50:24–30.

8. Jiménez I, Conn J, Brochero H. Malaria vectors in San José del Guaviare, Orinoquia, Colombia. J Am Mosq Control Assoc. 2014;30:91–8.

9. Jiménez I, Conn J, Brochero H. Preliminary biological studies on larvae and adult Anopheles mosquitoes (Diptera: Culicidae) in Miraflores, a malaria endemic locality in Guaviare Department, Amazonian Colombia. J Med Entomol. 2014;51:1002–9.

10. Conde M, Pareja R, Orjuela L, Ahumada M, Durán S, Jara J, et al. Larval habitat characteristics of the main malaria vectors in the most endemic regions of Colombia: potential for larval control. Malar J. 2015;14:476.

11. Ahumada M, Orjuela L, Pareja P, Conde M, Cabarcas D, Cubillos E, et al. Spatial distributions of Anopheline species in relation to malaria incidence at 70 localities in the highly endemic Northwest and South Pacific coast regions of Colombia. Malar J. 2016;15:407.

12. Pineda-Guererro A, González-Mayá P, Pérez-Torres J. Conservation value of forest fragments for medium-sized canivores in a silvopastoral system in Colombia. Mammalia. 2014;79:115–9.

13. FEDEARROZ. Mapa Seccionales. 2017. http://www.fedearroz.com.co/new/mapa.php. Accessed 18 June 2017.

14. Olano V, Brochero H, Sáenz R, Quiñones M, Molina J. Mapas preliminares de la distribución de especies de Anophelines vectores de malaria en Colombia. Biomedica. 2001;21:402–8.

15. Parra-Henao E, Alarcón G. Observaciones sobre la bionomía de Anophelines spp. (Diptera: Culicidae) en el municipio Valencia, departamento Córdo-oba, Colombia. Bol Malarial y Sal Amb. 2008;48:95–8.

16. Narango-Diaz N, Rosero DA, Rua-Uribe G, Luckhart S, Correa MM. Abundance, behavior and entomological inoculation rates of anthropophilic anophelines from a primary Colombian malaria endemic area. Parasites Vectors. 2013;6:61.

17. Schiemann DJ, Quiñones ML, Hankel T. Anthropophilic Anopheles spe-cies composition and malaria in Tierradentro, Córdoba, Colombia. Mem Inst Oswaldo Cruz. 2014;109:384–7.

18. ESRI. ArcMap. Redlands, USA. 2012. http://www.esri.com/.

19. Google Inc. Google Earth version 7.1.8.3036. Mountain View. 2009. https://earth.google.com/.
20. R Core Team. R: a language and environment for statistical computing. 2015. https://www.r-project.org/.

21. Irish S, Chandra F, NGuessan R. Comparison of octenol- and BG Lure -baited biogents sentinel traps and an encephalitis virus surveillance trap in Portland, OR. J Am Mosq Control Assoc. 2008;24:393–7.

22. Vázquez-Prokopec G, Galvin W, Kelly R, Kitson U. A new, cost-effective, battery-powered aspirator for adult mosquito collections. J Med Entomol. 2009;46:1256–9.

23. González R, Carrejo N. Introducción al estudio taxonómico de Anopheles de Colombia: claves y notas de distribución. 2nd ed. Cali: Programa editorial Universidad del Valle; 2009.

24. Lane J. Neotropical Culicidae: Tribe Culicina, Deinocerites, Uranotaenia, Mansonia, Orthopodomyia, Aedeomyia, Aedes, Psorophora, Haemagogus, Tribe Sabethini, Triarchopsopon, Wyeomyia, Phenomyia, Limatus and Sabethes. São Paulo: Universidad de São Paulo; 1953.

25. Forattini O. Entomología Médica. Culicini: Culex, Aedes and Psorophora. São Paulo: Universidade de São Paulo; 1965.

26. Bolster O, Black M, Hoeh W, Lust M, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biotechnol. 1994;2:294–9.

27. Snounou K, Vrijakosoglou, Zhu S, Jarra XP, Pinheiro W, do Rosario L, et al. High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. Mol Biochem Parasitol. 1993;61:15–20.

28. Bustamante DM, Loid CC. Sources of error in the estimation of mosquito infection rates used to assess risk of arbovirus transmission. Am J Trop Med Hyg. 2010;82:1172–84.

29. IDEAM. Leyenda Nacional de Coberturas de la Tierra. Metodología CORINE Land Cover adaptada para Colombia Escala 1:100.000. 2010. http://siatco.com/document_library/get_file?file_id=564269ad2bde4e1e-a561-fc16b8037522&groupId=762. Accessed 18 June 2017.

30. Guzmán D, Ruíz J, Cadena M. Regionalización de Colombia degún la estimación de la precipitación mensual, a través de Analisis de Componentes Principales (ACP). 2014.

31. GenBank. https://www.ncbi.nlm.nih.gov. Accessed 21 June 2017.

32. Arevalo-Herrera M, Quiliones ML, Guerra C, Céspedes N, Giron S, Arevalo-Herrera M, Quiñones ML. DNA barcoding: complementing morphological identification of mosquito species in Singapore. Parasites Vectors. 2014;7:569.

33. Rath A, Prusty MR, Barik SK, Das M, Tripathy HK, Hazra RK. Development, standardization and validation of molecular techniques for malaria vector species identification, trophic preferences, and detection of Plasmodium falciparum. J Vector Borne Dis. 2017;54:25–34.

34. Stevenson J, Norris D. Implicating cryptic and novel anophelines as malaria vectors in Africa. Insects. 2017;8:e1.

35. Aycock MR, Correa MM, Zarate E, Padron C. Determination of species anofelines in a localidad endémica de malaria en el departamento de Córdoba, noroeste de Colombia. Entomotropica. 2016;31:294–301.

36. Kilama W. Health research ethics in malaria vector trials in Africa. Malar J. 2010;9(Suppl 3):S3.

37. Yasuoka J, Levins R. Impact of deforestation and agricultural development on Anopheles darlingi and Sabethes. São Paulo: Universidade de São Paulo; 1953.

38. Rivero J, Zoghbi N, Rubio-Palis Y, Urdaneta L, Herrera F. DNA degradation and Sabethes. São Paulo: Universidade de São Paulo; 1953.

39. Rosero D, Naranjo-Diaz N, Alvarez N, Cienfuegos AV, Torres C, Luckhart S, et al. PCR-based detection of Plasmodium species in Anopheles darlingi (Diptera, Culicidae) in Suriname and the relation with its biting behavior. J Med Entomol. 2011;48:1039–46.

40. Qiu Y, Smallegange R, Cotte E, Lamy AP, Petraglia F, Vargas G, et al. Attractiveness of MM-X traps baited with human or synthetic odor to mosquitoes (Diptera: Culicidae) in the Gambia. J Med Entomol. 2007;44:970–83.

41. Pombi M, Guellbeowo G, Calletta M, Sagnon NF, Petracca V. Evaluation of a protocol for remote identification of mosquito vector species reveals BG-Sentinel trap as an efficient tool for Anopheles gambiense outdoor collection in Burkina Faso. Malar J. 2015;14:161.

42. Li J, Guo Y, Li Z, Zhou X, Menard S, Sun F, et al. Seasonal variation of Plasmodium species composition across a climate gradient in the Amazon Basin. Am J Trop Med Hyg. 2007;46:669–74.

43. Vazquez-Prokopec G, Galvin W, Kelly R, Kitson U. A new, cost-effective, battery-powered aspirator for adult mosquito collections. J Med Entomol. 2009;46:1256–9.

44. Stevenson J, Norris D. Implicating cryptic and novel anophelines as malaria vectors in Africa. Insects. 2017;8:e1.

45. González R, Carrejo N. Introducción al estudio taxonómico de Anopheles de Colombia: claves y notas de distribución. Cali: Programa editorial Universidad del Valle; 2007.

46. Álvarez N, Rosero DA, Gómez GF, Corea MM. Detección de mosquitos Anopheles infectados naturalmente con Plasmodium spp. en Puerto Libertador, Córdoba, Colombia. Hechos Microb. 2011;2:27–35.

47. Aycock MR, Corea MM, Zarate E, Padron C. Determinación de especies anofelinas en una localidad endémica de malaria en el departamento de Córdoba, noroeste de Colombia. Entomotropica. 2016;31:294–301.

48. Kilama W. Health research ethics in malaria vector trials in Africa. Malar J. 2010;9(Suppl 3):S3.

49. Qiu Y, Smallegange R, Cotte E, Lamy AP, Petraglia F, Vargas G, et al. Attractiveness of MM-X traps baited with human or synthetic odor to mosquitoes (Diptera: Culicidae) in the Gambia. J Med Entomol. 2011;48:1039–46.

50. Pombi M, Guellbeowo G, Calletta M, Sagnon NF, Petracca V. Evaluation of a protocol for remote identification of mosquito vector species reveals BG-Sentinel trap as an efficient tool for Anopheles gambiense outdoor collection in Burkina Faso. Malar J. 2015;14:161.

51. Chan A, Chiang L, Hapuarachchi HC, Tan C, Pang S, Lee R, et al. DNA barcoding: complementing morphological identification of mosquito species in Singapore. Parasites Vectors. 2014;7:569.

52. Batovska J, Blacket MJ, Brown K, Lynch SE. Molecular identification of mosquitoes (Diptera: Culicidae) in southeastern Australia. Ecol Evol. 2016;6:3001–11.

53. Rivero J, Zogbi N, Rubio-Palis Y, Urdaneta L, Herrera F. DNA degradation and Sabethes. São Paulo: Universidade de São Paulo; 1953.

54. Rivero J, Zogbi N, Rubio-Palis Y, Urdaneta L, Herrera F. DNA degradation and Sabethes. São Paulo: Universidade de São Paulo; 1953.

55. Rivero J, Zogbi N, Rubio-Palis Y, Urdaneta L, Herrera F. DNA degradation and Sabethes. São Paulo: Universidade de São Paulo; 1953.

56. Rivero J, Zogbi N, Rubio-Palis Y, Urdaneta L, Herrera F. DNA degradation and Sabethes. São Paulo: Universidade de São Paulo; 1953.

57. Yasuoka J, Levins R. Impact of deforestation and agricultural development on Plasmodium ecology and malaria epidemiology. Am J Trop Med Hyg. 2007;46:650–60.

58. Frederiksen C. Biometrics and control of Anopheles albimanus. Washington, D.C.: Pan American Health Organization; 1993.

59. Delgado LL, Rodriguez LR, Almeida TE, Marro TO, Basalto TP. Observaciones entomológicas en un brote de paludismo durante la etapa de vigilancia intensiva en Albian, Camaguey. Rev Cuba Med Trop. 2002;54:118–26.