Species composition, monthly distribution and behaviour of adult Anopheles mosquitoes in areas under elimination setting, Dembia district, Northwestern Ethiopia

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Abstract

Background

Despite of the progress made in scaling-up of the intervention tools in Ethiopia, malaria is still a public health problem in the country. This necessitates a continuous monitoring of the local vector species composition, monthly distribution and behaviour in order to follow up effectiveness vector control strategies in place. Thus, the aim of this study was to investigate species composition, distribution, and behaviour of *Anopheles* mosquitoes in selected localities of Dembia district.

Methods

Anopheles mosquito collection was conducted from June 2018 - May 2019 in selected areas of Dembia district by using Centers for Disease Control and Prevention (CDC) light traps, pyrethrum spray catches (PSCs), artificially constructed pit shelters and mouth aspirators. The sibling species of *An. gambiae s.l* were identified using a polymerase chain reaction (PCR). The blood source and sporozoite infection of *Anopheles* mosquitoes were determined using Enzyme linked immunosorbent assay (ELISA). The data were analyzed using SPSS version 20.

Results

Anopheles mosquitoes belonging to 11 species were identified from 2,055 field collected adult specimens during this study: *An. pharoensis, An. arabiensis*, *An. coustani*, *An. demeilloni*, *An. cinereus, An. funestus, An. ardensis,* and *An. squamosus* were identified from both Guramba Bata and Arebiya study sites while *An. garnhami, An. christyi* and *An. nili* were identified only from Guramba Bata. *Anopheles pharoensis* was the dominant species identified in both Arebiya and Guramba Bata study sites comprising 46.4%, whereas *An. arabiensis* were also comparably dominant in both study sites (38.3%). The density of outdoor host seeking and resting *Anopheles* mosquitoes were higher than indoor host seeking and resting *Anopheles* mosquitoes, however the difference was not statistically significant ($p \geq 0.05$). The human blood indexes (HBI) of indoor and outdoor host seeking *An. arabiensis* were 17.4% and 15.3%, respectively. The overall sporozoite rate of *An. arabiensis*, *An. pharoensis* and *An. coustani* was 0.3%, 0.9% and 5.9% respectively.

Conclusions

Anopheles pharoensis and *An. arabiensis* were the dominant species identified in the study area. *Anopheles* mosquitoes showed an exophagic, exophilic and zoophilic tendency in the study area. Vector control strategies targeting outdoor host seeking and resting *Anopheles* mosquitoes should be sought to achieve the desired malaria control and elimination program in the study area.

Background

Malaria is one of the leading public health problems in Ethiopia. Three quarter of the country's land mass and 68% of the total population is at risk of malaria infection [1, 2]. The two species of *Plasmodium* parasites such as *Plasmodium falciparum* and *P. vivax* are responsible for 60% and 40% of the total malaria cases in Ethiopia, although their relative composition varies across different localities [3, 4, 5]. *Anopheles arabiensis* Patton, is the primary vector of malaria in Ethiopia, whereas other species such as *An. funestus*, *An. pharoensis* and *An. nili* are considered as secondary malaria vectors [6].

In Ethiopia the major malaria intervention strategies are prompt case treatment using artemisinin based combination therapy, prevention and treatment of malaria using intermittent preventive therapy (IPT), and vector control strategies such as long lasting insecticide-treated bed nets (LLINs) and indoor residual spray (IRS) [7]. Long-lasting insecticide treated bed nets (LLINs) reduce malaria transmission by killing or blocking *Anopheles* mosquitoes that attempt to take a blood from human. Whereas IRS kills and reduces longevity of *Anopheles* that rests on insecticide treated surfaces such as walls and other structures [8].

In Ethiopia the scale up distribution malaria intervention strategies was started from 2005 [9, 10]. During post intervention period (2006–2011) the proportion of population at malaria risk protected by LLINs is increased by 51%, IRS coverage increased by 35%, and active case treatment exceeds 87% when compared to pre intervention period (before 2005) [11]. Because of this increased distribution, malaria inpatient cases and death in all age groups were reduced by 54% and 68% respectively in 2011 than pre intervention period (2001–2005) [11].

Despite of reduction in overall malaria prevalence, malaria control is challenged by the development of insecticide resistance, shift in vector species composition and increasing vector behavioural change [12–16]. Recent reports from Ethiopia indicated that *An. arabiensis* was resistant to major class of insecticides, such as DDT, permethrin, deltamethrin, and malathion [17, 13, 18]. Additionally, this vector showed an increased outdoor biting and resting tendency and a shift in biting hour from late in the evening to early evening before people retire to bed has been reported in the country [19].

Entomological indicators including entomological inoculation rate (EIR), vector longevity, feeding preferences, the susceptibility of the vector to the parasites, and biting behaviour of *Anopheles* mosquitoes are important to determine the vectoral capacity of *Anopheles* mosquitoes and malaria transmission intensity in a given area [26–31]. Highly antropophilic *Anopheles* mosquito's species with high EIR, parasite permissibility, and longevity are important vectors of malaria. Hence, it is important to assess the entomological indicators in order to achieve the desired malaria control and elimination strategies in malaria endemic areas.

Dembia is malaria endemic area in Ethiopia with a long history of implementing vector control strategies [20]. The trend of malaria infection in this district has been significantly reduced after the increased implementation of malaria intervention strategies [21]. However, a recent study in Dembia District indicated that malaria is still a public health problem in the District [5, 22]. Limited studies are available on the species composition, ecology, and behaviour of the local malaria vectors in the district. Therefore this study was aimed to assess the species composition, distribution and behaviour of *Anopheles* mosquitoes in selected localities of Dembia district. The result of this study will help to design vector control strategy considering their behaviour and ecology.

Methods

Description of the study area

A longitudinal study on species composition, monthly distribution, behaviour, blood meal source and entomological inoculation rate of *Anopheles* mosquitoes was conducted from June 2018 to May 2019 in the two localities (Guramba Bata and Arebiya) of Dembiya District found in North Gondar administrative zone of Amhara regional state (Figure 1). The district is located at 12°39'59.99" N and 37°09'60.00" E. Kola Diba is the capital city of the district, located 750 km North of Addis Ababa and 35 km southwest of Gondar city. The southern part of the district is bordered by Lake Tana. The district has 45 localities (Kebeles: the lowest administrative unit in Ethiopia) and an estimated population of approximately 271,000, of which 138,000 (50.9%) were male and 133,000 (49.1%) female. The majority of the population (91%) lives in rural areas, with most engaging in farming activities; the remaining 9% live in urban areas. The district has 49,528 rural households with 4.3 mean household sizes [23].

The elevation of Dembiya District is ranging from 1500 to 2600m a.s.l. The agro-ecology of the District is midland (woynadega) with respective mean annual minimum and maximum temperature of 11^0C and 32^0C and the mean annual rainfall ranges from 995 to 1175mm. Information obtained from the district agricultural bureau indicated that the respective proportion of areas considered as plain, mountainous, valleys, and wetland is 87%, 5%, 4.8%, and 3.2%. Out of the total area of the District, 31% is cultivated land, 16% is none cultivable land, 5.6% forest and bush, 12.8% grazing, 8.1% is covered with water, 20.2% swamp and 4.3% is residential areas. The district receives bimodal rainfall, with the short rainy season from March to May and the main rainy season from June to September.

The major crops grown in the District includes teff (*Eragrostis tef*), maize (*Zea mays)*, barley (*Hordeum vulgare*), red highland sorghum (*Sorghum bicolor*), and finger millet (*Eleusine coracana*). Besides, legumes and pulses such as chickpeas (*Cicer arietinum*) and cowpeas (*Vigna unguiculata*) are also grown in the district. They also grow some cash crops like pepper (genus *Capsicum*), niger seed (*Guizotia abyssinica*), fenugreek (*Trigonella foenum-graecum*), black cumin (*Nigella sativa*), White cumin (*Cuminum cyminum*), and rice (*Oryza sativa*) with a limited amount of farmlands.

One of the study localities, Guramba Bata ($12^0$21'57.75"N and $37^0$20'25.31" E, altitude 1,795 - 1,820 m.a.s.l.), has a seasonal river "Ahya gedel" or "Nededo" which forms intermittent mosquito breeding water bodies until the end of December. Guramba Bata has one health post and one health center, 1113 households with 6008 inhabitants (2974 male and 3034 female) in 2017/18 (District Health Office report) (Fig. 1).

The second study locality, Arebiya ($12^0$20'26.59"N and $37^0$22'16.04" E) has "Megech" river serve as a water source during a dry season and flows into Lake Tana. This locality has 1976 households and a total of 8632 inhabitants (4298 male and 4384 females) in 2017/18. Arebiya has only one health post (District Health Office report) (Fig. 1).

Study design

A longitudinal study design was implemented to assess the ecology, breeding habitat type and species composition of *Anopheles* in two selected localities of Dembia district. This two study sites were selected based on their high level of malaria endemicity, implementation of IRS and LLINs for long time and accessibility.

Host survey

Human population from the two study sites were obtained from the health center (unpublished). Data about the numbers of potential hosts in the study area including, bovine, cows, goat, dog and chicken were collected from the local agricultural offices.

Indoor and outdoor host seeking mosquito collection

Adult *Anopheles* mosquito collection was carried out for one year starting from Jun 2018 to May 2019. Indoor and outdoor host seeking mosquito collection was performed using Centers for Disease Control and Prevention (CDC) light traps (John W. Hock Ltd, Gainesville, FL., USA). For indoor host seeking *Anopheles* mosquito collection, a total of five CDC light traps were installed near to bed at a height of 1.5 m from 18:00 to 06:00 h in five randomly selected houses from each locality for two consecutive nights per month. For outdoor host seeking *Anopheles* mosquito collection, five CDC light traps were installed near to animal enclosure in five randomly selected households from each locality. The same houses were used for adult mosquito collection through the year.

Indoor and outdoor resting mosquito collection

Indoor resting *Anopheles* mosquito collections were performed using a pyrethrum spray catches (PSCs) from another ten randomly selected houses from each locality starting from 06:30 to 09:30 h. Before PSC is implemented all food items, feeding utensils and small animals were evacuated from houses, and all openings and eaves of windows and doors were sealed. The floors were covered with white sheets before spraying houses with a bygone aerosol (SC. Johnson & Son. Inc, USA). Fifteen minutes after spraying, knocked down *Anopheles* mosquitoes were collected by using forceps, paper cups, and a torch light [24]. In addition mouth aspirators were used to collect indoor resting mosquitoes (such as walls, ceilings, underneath of household furniture, and on materials hung on the walls).

Additional five houses from each village were randomly selected for outdoor resting mosquito collection using artificially constructed pit shelters (constructed in the back yard of each selected house). The pit shelters have a depth of 1.5 m and with an opening of 1.2 m x 1.2 m. In each shelter four cavities with a horizontal depth of 30cm were dug on each side. Mouth aspirators were used to collect resting mosquitoes after covering the mouth with untreated bed net. Collection was done two

times per a month in the morning from 6:30am to 10:00am. Mouth aspirators were also used to collect outdoor resting mosquitoes from various outdoor possible mosquito resting sites in each village (ground holes, tree holes, open cattle sheds and among vegetation). The collection was performed two times per a month for 30 minutes in each possible resting site.

Processing and identification of female *Anopheles* mosquitoes

Identification of all collected adult *Anopheles* mosquitoes in to species level were executed based on morphological key described by Gillies and Coetzee [6]. Female *Anopheles* mosquitoes were further classified as unfed, blood fed, half-gravid and gravid. Morphologically identified *An. gambiae sensu lato* (*s.l*) and female *Anopheles* mosquitoes were kept in a labeled 1.5 ml Eppendorf tube containing silica gel desiccant and cotton wool. All collected mosquito specimens were kept at room temperature (25oC) for later mosquito processing. Sibling species of *An. gambiae s.l* mosquito were identified using a ribosomal DNA polymerase chain reaction (PCR) by including the primers for *An. gambiae s.s.*, *An. arabiensis*, *An. quadriannulatus A* and B [25].

Enzyme-Linked Immunosorbent Assay (ELISA) for Blood meal analysis

Blood meal source of engorged female *Anopheles* mosquitoes were examined using direct ELISA techniques using bovine and human antibodies with little modification [26]. The abdomens of freshly fed female *Anopheles* mosquitoes were grinded in 100µl phosphate buffered saline (PBS), which was further diluted by adding 100µl PBS. A 100µL of prepared samples were added to each well and incubated for 3hr at room temperature. The incubated mixture was washed twice with PBS-tween 20. This was followed by addition of 50µl host specific conjugate of bovine or human diluted 1:2000 (or 1:250 for bovine) in 0.5% boiled casein containing 0.025% Tween 20 to each well and incubated for additional 1hr at room temperature. After 1 h, wells were washed three times with PBS-Tween 20, and 100 µL of ABTS peroxidase substrate was added to each well. Absorbance at 405 nm was determined with an ELISA reader 30 min after the addition of substrate. The result was interpreted as positive if the absorbance value exceeded the mean plus three times the standard deviation of the four negative controls (unfed laboratory colony of *An. arabiensis*). Human blood obtained from humans (volunteer), cows and sheep blood obtained from abattoirs were used as a positive control.

Enzyme-Linked Immunosorbent Assay (ELISA) for *Plasmodium* parasite detection

Circum-sprozoite (CSP) detection of the parasite within mosquito gut was performed based on a protocol developed by Beier *et al* [27]. The head and thorax of *Anopheles* mosquitoes were grinded in labeled 1.5ml centrifuge tube using a pestle by adding a 50µl of grinding buffer. The grinding pestle was washed twice with 100 µl of grinding buffer catching the rinses in the tube containing the mosquitoes triturate until the final volume reached 250µl.

A 50 µlof *P. falciparum*, *P. vivax* 210 and *P. vivax* 247 capture monoclonal antibody (mAb) solution was placed in each well of separate plates assigned for each species. The plates were covered and incubated for 30 minutes at room temperature. The well contents were aspirated and banged on a paper towel five times. Each well were filled with 200 µl blocking buffer (BB) solution and incubated for one hour at room temperature. Well contents were aspirated and banged five times on a paper towel. A 50 µl mosquito sample, a positive control (*P. falciparum*, Pv-210 and Pv-247) and a negative control of unfed *An. arabiensis* from an established colony were added in each respective plate wells. The plates were covered and incubated at room temperature for 2 hours, and well contents were aspirated, banged on a paper towel and washed two times using 200 µl PBS-Tween-20. The well contents were aspirated and banged on a paper towel with each wash.

A 50 µl peroxidase labeled conjugate solutions of *P. falciparum*, Pv-210 and Pv-247 were added to each well to the respective plates and incubated for one hour at room temperature. The plates were washed thrice with 200µl PBS-Tween-20 after the well contents are aspirated and banged on a paper towel. A 100 µl ABTS substrate solution were added in each well and the covered plates were incubated 30 minutes at room temperature. Finally, the plates were read at 405nm absorbance using ELISA plate reader. The sample was considered as positive if the sample absorbance value is above the two times mean absorbance value of negative samples.

Data analysis

The density of *Anopheles* mosquitoes were calculated as a number of female *Anopheles* mosquito/trap/night for each collection method. All dependent variables were checked for normality and $\log_{10}(x+1)$ transformed before subjected to statistical analysis. Student t test was used to compare mean *Anopheles* mosquito density difference between study localities and indoor and outdoor locations. One way analysis of variance (ANOVA) was used to analyze mean density difference between species. Human blood Index (HBI) was estimated as a number of *Anopheles* mosquitoes fed on human blood meal over the total *Anopheles* mosquitoes tested for blood meal origin [28]. Similarly, Bovine blood Index (BBI) was estimated as a number of *Anopheles* mosquitoes fed on bovine blood meal over the total *Anopheles* mosquitoes tested for blood meal origin. Mixed blood meal was included in calculating human blood index and bovine blood index [34]. The relative feeding preference or forage ratio (FR) of *Anopheles* mosquitoes were calculated by dividing the percent of blood engorged *Anopheles* mosquito which have fed up on either humans or bovine to the percent which either human or cattle comprises in the area [29]. If the FR was one (near 1) the host is neither preferable nor avoided by the local vector; If FR was significantly > 1, the host is preferred by the vector and if it was less than 1, the host is not preferable.

The sporozoite rate was calculated as the proportion of *Anopheles* mosquitoes positive for (*P. vivax* or *P. falciparum)* CSPs over the total number of *Anopheles* mosquito tested for CSPs. Annual entomological inoculation rate (EIR) for *Anopheles* mosquito was calculated from mosquito collection by CDC light trap using the formula, 1.605 × (Number of CSP positive ELISA results from CDC light traps/no. mosquitoes tested) × (No. mosquitoes collected from CDC light traps/No. trap-nights) × 365 [30, 31]. All data collected were analyzed using SPSS version 21 (Armonk, NY: IBM Corp).

Results

Availability of *Anopheles* mosquito alternative hosts

Unpublished reports from Dembia district agricultural offices indicated that the district is endowed with a number of cattle's, goats, sheep and chickens which can serve as alternative blood source for *Anopheles* mosquitoes. Accordingly, from the total host population 23.6% were chickens and 19.9% were cattle (Table 1).

Table 1. Composition of alternative blood sources in the two study sites, Dembia District Northwestern Ethiopia.

No	Number	Percentage
Cattle	6,980	19.9
Goat	39	0.1
Sheep	4,334	12.4
Donkey	756	2.2
Chickens	8,275	23.6
Human	14,640	41.8
Total	35,024	100

Species composition and monthly distribution of *Anopheles* mosquitoes

During a one year study period (June 2018-May 2019) a total of 2,055 female *Anopheles* mosquitoes which belongs to 11 species were collected. From which, 56.6% (n= 1,164) were collected from Guramba Bata and 43.3 (n= 891) were collected from Arebiya study areas. The difference in mean number of *Anopheles* mosquitoes between the two study sites were

statistically significant (t_{679} = -1.983, p= 0.048). *An. arabiensis*, *An. pharoensis*, *An. coustani*, *An. demeilloni, An. cinereus, An. ardensis, An. squamosus* and *An. funestus* were identified from Arebiya study site. Whereas, *An. arabiensis*, *An. pharoensis*, *An. coustani*, *An. demeilloni*, *An. garnhami, An. christyi, An. cinereus, An. funestus, An. ardensis, An. squamosus,* and *An. nili* were identified from Guramba bata (Table 2). From which, *An. pharoensis* was the predominant species identified in Arebiya and Guramba Bata study sites, accounted for 46.2% (n=412) and 46.5% (n=541) of the total species. The second dominant species was *An. arabiensis* comprising, 42.3% (n= 377) and 34.3% (n=399) in Arebiya and Guramba study sites respectively (Table 2).

Table 2 Species composition and abundance of *Anopheles* mosquito using different adult mosquito collection methods in the two study sites of Dembia District, Northwestern Ethiopia (June 2010-March 2011).

Study site	Species	CDC Light Trap		Mouth Aspirator		PSC		Pit Shelter		Total	
		no.	%	no.	%	no.	%	no.	%	no.	(%)
Guramba Bata	*An. arabiensis*	227	27.3	38	29.9	70	79.5	64	54.2	399	34.3
	An. pharoensis	381	45.8	88	69.3	18	20.5	54	45.8	541	46.5
	An. coustani	146	17.6	1	0.8	-		-		147	12.6
	An. demeilloni	27	3.2	-		-		-		27	2.3
	An. garnhami	1	0.1	-		-		-		1	0.1
	An. christyi	14	1.7	-		-		-		14	1.2
	An. cinereus	5	0.6	-		-		-		5	0.4
	An. funestus	8	0.9	-		-		-		8	0.7
	An. ardensis	12	1.4	-		-		-		12	1
	An. squamosus	9	1.1	-		-		-		9	0.8
	An. nili	1	0.1	-		-		-		1	0.1
	Total	831	100	127	100	88	100	118	100	1164	100
Arebiya	*An. arabiensis*	207	36.3	45	36.9	63	72.4	62	55.9	377	42.3
	An. pharoensis	264	46.2	77	63.1	24	27.6	47	42.3	412	46.2
	An. coustani	73	12.8	-		-		2	1.8	75	8.4
	An. cinereus	5	0.9	-		-		-		5	0.6
	An. demeilloni	3	0.5	-		-		-		3	0.3
	An. ardensis	14	2.5	-		-		-		14	1.6
	An. squamosus	2	0.4	-		-		-		2	0.2
	An. funestus	3	0.5	-		-		-		3	0.3
	Total	571	100	122	100	87	100	111	100	891	100

CDC: Center for disease control; PSC: *Pyrethrum Spray Catches*

The density of *Anopheles* mosquitoes showed a steady increment starting from June to September in the two study sites however it significantly fails after the end of long rainy season. The highest density of indoor and outdoor host seeking *Anopheles* mosquitoes in Arebiya was recorded in September (12.20 and 12.80 mosquitoes /CDC trap/night, respectively).

The density showed a slow increment starting from May in this study area (Fig. 2a). In Guramba Bata the highest density of indoor and outdoor host seeking *Anopheles* mosquitoes were recorded in August (12.3 and 13.8 mosquitoes /CDC trap/night respectively) and September (7.2 mosquitoes /CDC trap/night and 15.9 mosquitoes /CDC trap/night respectively) (Fig. 2b).

Host seeking and resting activities of *Anopheles* mosquitoes

Table 3 & 4 shows the indoor and outdoor host seeking and resting density of *Anopheles* mosquitoes in different locations of the study sites. In Arebiya study sites a comparably high mean density of outdoor (4.8 ± 1.8 mosquitoes/trap/night) host seeking *Anopheles* mosquitoes were collected than indoor (4.3 ± 1.7 mosquitoes/trap/night), however the difference was not statistically significant (t_{10}= 0.196, p=0.849) (Table 3). Similarly, insignificant difference was observed between the indoor and outdoor density of host seeking *An. arabiensis* (t_{10}= 0.188, p= 0.855), *An. pharoensis* (t_{10}= -0.121, p= 0.906) and *An. coustani* (t_{10}= -1.224, p= 0.249) in Arebiya study site (Table 3). The density of outdoor resting *An. arabiensis* was higher than indoor resting in this study site, but the difference was not statistically significant (t_{10}= -1.366, p= 0.202). Likewise, the outdoor resting density of *An. pharoensis* was higher than the indoor resting density, though the difference was not statistically significant (t_{10}= -1.614, p= 0.138) (Table 4).

In Guramba Bata study site a relatively higher density of outdoor (8.1 ± 2.6 mosquitoes/trap/night) host seeking *Anopheles* mosquitoes were collected than indoor (5.5 ± 1.7 mosquitoes/trap/night), but the difference was not statistically significant (t_{10}= -0.623, p=0.547) (Table 3). The outdoor host seeking density of *An. arabiensis* in this study site was higher than the indoor density, but it was not statistically significant (t_{10}= -0.855, p= 0.412) (Table 3). The density of indoor and outdoor host seeking *An. pharoensis* was comparably equal (t_{10}= 0.116, p= 0.910) (Table 3). The outdoor density of host seeking *An. coustani* was significantly higher than indoor host seeking density (t_{10}= -2.637, p= 0.025) (Table 3). In Guramba Bata study site the density of outdoor resting *An. arabiensis* was higher than indoor resting, however the difference was not statistically significant (t_{10}= -0.904, p= 0.387) (Table 4). Significantly higher density of outdoor resting than indoor resting *An. pharoensis* was recorded during this study (t_{10}= -2.812, p= 0.018) (Table 4).

Table 3. Host seeking behaviour of Anopheles mosquitoes in selected localities of Dembia District, Northwestern Ethiopia (June 2018-March 2019).

		Collection site and method		
Site	Species	CDC indoor (mean ± se)	CDC outdoor (mean ± se)	*p*. value
Arebiya	*An. arabiensis*	1.8 ± 0.7	1.4 ± 0.4	0.855
	An. pharoensis	2.2 ± 1.0	2.2 ± 0.9	0.906
	An. coustani	0.3 ± 0.1	0.96 ± 0.5	0.249
	Total density	4.3 ± 1.7	4.8 ± 1.8	0.849
Guramba Bata	*An. arabiensis*	1.3 ± 0.494	2.2 ± 0.703	0.412
	An. pharoensis	3 ± 0.997	3.1 ± 1.25	0.910
	An. coustani	0.4 ± 0.3	1.96 ± 0.8	0.025
	Total density	5.5 ± 1.7	8.1 ± 2.6	0.547

Table 4. Resting behaviour of Anopheles mosquitoes in selected localities of Dembia district, Northwestern Ethiopia (June 2018-March 2019).

		Collection site and method		
Study site	Species	PSC (mean ± se)	Pit shelter (mean ± se)	*p*. value
Arebiya	*An. arabiensis*	0.96 ± 0.3	1.93 ± 0.4	0.202
	An. pharoensis	0.4 ± 0.2	1.6 ± 0.6	0.138
	Total	0.73 ± 0.3	1.85 ± 0.7	0.219
Guramba Bata	*An. arabiensis*	1.2 ± 0.3	2 ± 0.7	0.387
	An. pharoensis	0.3 ± 0.2	1.9 ± 0.6	0.018
	Total	0.73 ± 0.3	1.96 ± 0.7	0.241

Abdominal status of host seeking and resting *Anopheles* mosquito

From the total indoor and outdoor host seeking *Anopheles* mosquitoes the majority, 50.5% and 63.9% respectively were unfed. From which, about 58.6% of indoor host seeking and 67.9% of outdoor host seeking *An. arabiensis* were unfed. Similarly, the dominant number of indoor and outdoor host seeking *An. pharoensis* was unfed (46.8% indoor and 57.4% outdoor respectively) (Table 5).

Table 5. Abdominal status of host seeking Anopheles mosquitoes in the study area, Dembia District, Northwestern Ethiopia (June 2018-March 2019).

Species	CDC-LT Indoor					CDC-LT Outdoor				
	Unfed	Freshly Fed	Half Graved	Graved	Total	Unfed	Freshly Fed	Half Graved	Graved	Total
An. arabiensis	130 (58.6)	82 (36.9)	5 (2.3)	5 (2.3)	222	144 (67.9)	52 (24.5)	8 (3.8)	8 (3.8)	212
An. pharoensis	146 (46.8)	125 (40.1)	30 (9.6)	11 (3.5)	312	191 (57.4)	134 (40.2)	7 (2)	1 (0.3)	333
An. coustani	20 (48.8)	18 (43.9)	1 (2.4)	2 (4.9)	41	126 (70.8)	48 (26.9)	4 (2.2)	-	178
An. cinereus	3 (50)	3 (50)	-	-	6	3 (75)	-	1 (25)	-	4
An. demeilloni	2 (12.5)	13 (81.3)	1 (6.3)	-	16	5 (35.7)	8 (57.1)	1 (7.1)	-	14
An. ardensis	-	-	-	-	-	20 (76.9)	6 (23.1)	-	-	26
An. squamosus	-	-	-	-	-	7 (63.6)	4 (36.4)	-	-	11
An. funestus	1 (20)	4 (80)	-	-	5	3 (50)	3 (50)	-	-	6
An. garnhami	1 (100)	-	-	-	1	-	-	-	-	-
An. christyi	3 (75)	1 (25)	-	-	4	9 (90)	1 (10)	-	-	10
An. nili	1 (100)	-	-	-	1	-	-	-	-	-
Total	307(50.5)	246(40.5)	37 (6.1)	18(2.9)	608	508 (63.9)	256(32.2)	21(2.6)	9 (1.1)	794

CDC-LT: CDC Light Trap

From the total indoor and outdoor resting *Anopheles* mosquitoes the dominant groups (53.2% and 68.3% respectively) were freshly fed. More than half of indoor and outdoor resting *An. arabiensis* was also freshly fed. Additionally the majority of indoor and outdoor resting *An. pharoensis* and *An. coustani* were freshly fed (Table 6).

Table 6. Abdominal status of resting Anopheles mosquitoes in the study area, Dembia District, Northwestern Ethiopia (June 2018-March 2019).

Collection methods	Status	*An. arabiensis*	*An. pharoensis*	*An. coustani*	Total
Indoor (PSC and Mouth Aspirator)	Unfed	6 (3.6)	2 (2.4)	-	8 (3.2)
	Freshly Fed	93 (55.7)	41 (48.2)	-	134 (53.2)
	Half Graved	46 (27.5)	28 (32.9)	-	74 (29.4)
	Graved	22 (13.2)	14 (16.5)	-	36 (14.3)
Total		167	85	-	252
Outdoor (Pit shelter and Mouth Aspirator)	Unfed	4 (2.3)	0	-	4 (0.99)
	Freshly Fed	113 (64.6)	158 (70.9)	3 (100)	274 (68.3)
	Half Graved	40 (2.3)	49 (21.97)	-	89 (22.2)
	Graved	18 (10.3)	16 (7.2)	-	34 (8.5)
Total		175	223	3	401

PSC: *Pyrethrum Spray Catches*

Blood meal sources and host preference of *Anopheles* mosquitoes

A total of 552 *Anopheles* mosquitoes were subjected to blood meal source analysis using a direct ELISA. The result indicated that, of the total tested *Anopheles* mosquitoes 5.3% (n=29), 42.5% (n=235), 5.8% (n=32) and 46.4% (n=256) had a blood meal origin of human, bovine, mixed and unknown, respectively (Table 7 & 8).

Anopheles arabiensis collected using indoor and outdoor CDC light trap had a low human blood index (17.4%, and 15.3%, respectively) (Table 7). On the other hand, *An. arabiensis* collected using indoor and outdoor CDC light trap had a relatively high bovine blood index (50% and 20.3%, respectively) (Table 7). Similarly, *An. pharoensis* collected using indoor and outdoor CDC light trap exhibited a high bovine blood index (60.5% and 55.5%, respectively) than human blood index (10.5% and 10%, respectively) (Table 7).

Table 7 Blood meal sources of host seeking Anopheles mosquitoes in the study area, Dembia District, Northwestern Ethiopia. (Values in parenthesis are percentages)

Species	CDC Indoor					CDC Outdoor				
	No	HBI (%)	BBI (%)	MB (%)	Un (%)	No	HBI (%)	BBI (%)	MB (%)	Un (%)
An. arabiensis	46	0.2 (17.4)	0.5 (50)	0.04 (4.3)	0.3 (32.6)	59	0.2 (15.3)	0.2 (20.3)	0.03 (3.4)	0.7 (67.8)
An. pharoensis	152	0.1 (10.5)	0.6 (60.5)	0.1(7.9)	0.4 (36.8)	110	0.1 (10)	0.6 (55.5)	0.1 (5.5)	0.4 (40)
An. coustani	15	0.1(6.7)	0.6 (60)	0.1 (6.7)	0.4 (40)	34	0.1 (14.7)	0.6 (55.9)	0.1 (11.8)	0.4 (41.2)
An. cinereus	5	-	0.2 (20)	-	0.8 (80)	1	-	-	-	1
An. demeilloni	6	0.2 (16.7)	0.2 (16.7)	-	0.7 (66.7)	13	-	0.4 (38.5)	-	0.6 (61.5)
An. funestus	3	0.3 (33.3)	0.3 (33.3)	-	0.3 (33.3)	4	-	0.5 (50)	0.3 (25)	0.5 (50)
An. chrysti	1	-	-	-	1	2	0.5 (50)	0.5 (50)	0.5 (50)	0.5 (50)
An. ardensis	-	-	-	-	-	5	-	-	-	5
An. sqaumosus	-	-	-	-	-	3	-	0.7 (66.7)	0.3 (33.3)	1 (0.3)
Total	228	0.1 (11.8)	0.6 (56.6)	0.1 (6.6)	0.4 (38.2)	231	0.1 (12.1)	0.4 (44.2)	0.1 (6.5)	0.5 (50)

HBI: Human blood index; BBI: Bovine blood index, Un: Unknown; MB: Mixed Blood

Resting *Anopheles* mosquitoes collected using pit shelters, indoor mouth aspirator, outdoor mouth aspirator and PSC had a higher bovine blood index than human blood index (Table 8). The human blood index of *An. arabiensis* collected using pit shelters, indoor mouth aspirator, outdoor mouth aspirator and PSC were 7.3%, 0%, 12.5% and 8.3%, respectively. Whereas, the bovine blood index of *An. arabiensis* collected using pit shelter, indoor mouth aspirator, outdoor mouth aspirator and PSC was 41.5%, 27.3%, 62.5% and 37.5%, respectively (Table 8).

The bovine blood index of *An. pharoensis* collected using pit shelters, indoor mouth aspirator, outdoor mouth aspirator and PSC were 50%, 0%, 50% and 0%, respectively (Table 8). However, none of indoor and outdoor resting *An. pharoensis* analyzed for blood meal were positive for a human blood (Table 8).

Table 8. Blood meal sources of resting Anopheles mosquitoes in the study area, Dembiya District, Northwestern Ethiopia.

Collection method	Location	Species	No. analyzed	HBI (%)	BBI (%)	MB (%)	Un (%)
Pit shelter	Outdoor	*An. arabiensis*	41	0.1 (7.3)	0.4 (41.5)	0.05 (4.9)	0.6 (56.1)
		An. pharoensis	2	-	0.5 (50)	-	0.1 (50)
Mouth Aspirator	Indoor	*An. arabiensis*	11	-	0.3 (27.3)	-	0.7 (72.7)
		An. pharoensis	2	-	-	-	2
	Outdoor	*An. arabiensis*	8	0.1 (12.5)	0.6 (62.5)	-	0.3 (25)
		An. pharoensis	2	-	0.5 (50)	-	0.5 (50)
		An. coustani	1	-	-	-	1
Pyrethrum	Indoor	*An. arabiensis*	24	0.1 (8.3)	0.4 (37.5)	-	0.5 (54.2)
Spray catches		*An. pharoensis*	2	-	-	-	2
		Total	93	0.1 (6.5)	0.4 (38.7)	0.02 (2.2)	0.6 (56.9)

HBI: Human Blood Index, BBI: Bovine Blood Index; MB: Mixed Blood, Un: Unknown

Foraging ratio of *Anopheles* mosquitoes

The result indicated that *An. arabiensis*, *An. pharoensis*, *An. coustani, An. funestus* shows a strong relative feeding preference of bovine blood over a human blood. *An. arabiensis* showed a 6 times strong preference of bovine blood than human blood. The relative bovine feeding preference of *An. pharoensis* and *An. funestus* was 15 and 3 times higher than the human blood respectively. In this study a bovine blood preference of *An. coustani* was 9 times higher than human blood (Table 9).

Table 9. Foraging ratio of Anopheles mosquitoes in the study area, Dembia District, Northwest Ethiopia.

Species	%HB	%HP	Human FR	%BB	%BP	Bovine FR
An. arabiensis	12.3	41.8	0.3	37.6	19.9	1.9
An. pharoensis	10.0	41.8	0.2	57.4	19.9	2.9
An. coustani	12	41.8	0.3	56	19.9	2.8
An. funestus	28.6	41.8	0.7	42.9	19.9	2.2

% HB: Human blood; %HP: Percent human in population; Human Forage ratio (FR) = %HB/ %HP; Bovine Forage ratio (FR) = %BB/ %BP

Sporozoite rate of *Anopheles* mosquitoes

A total of 792 female *Anopheles* mosquitoes belongs to nine species such as *An. arabiensis* (n=335), *An. pharoensis* (n=332), *An. coustani* (n=68), *An. ardensis* (n=10), *An. cinereus* (n=11), *An. demilloni* (n=21), *An. funestus* (n=7), *An. squamosus* (n=4) and *An. christyi* (n=4) were tested for the presence of circum-sporozoite protein (CSP) in their salivary gland (presence of *P. falciparum*, *P. vivax* 210, and *P. vivax* 247 CSPs). From the species analyzed for CSP, 9 specimens (*An. arabiensis* (n=1)*, An. coustani* (n=4), *An. pharoensis* (n=3) and *An. squamosus* (n=1) collected using CDC light trap were positive for CSP. From the total CSP, *An. coustani* (n= 4), *An. pharoensis* (n= 1) and *An. arabiensis* (n= 1) were positive for *P. vivax 210.* In addition, *An. pharoensis* (n= 2) and *An. squamosus* (n= 1) were positive for *P. vivax* 247*.* None of the species analyzed were found to be positive for *P. falciparum* CSP (Table 10).

The sporozoite rate of *Anopheles* mosquito collected using different method is indicated in Table 10. The overall sporozoite rate of *An. arabiensis* was 0.3% and the respective sporozoite rate of indoor and outdoor CDC collected *An. arabiensis* was 0 and 0.9%. The respective sporozoite rate of overall, indoor and outdoor host seeking *An. pharoensis* was 0.9%, 1.6%, and 0%. The sporozoite rate of indoor and outdoor CDC collected *An. coustani* was 6.7 and 6% respectively. The overall sporozoite rate of *An. coustani* was 5.9%. The sporozoite rate of indoor and outdoor CDC collected *An. squamosus* was 0 and 25% respectively (Table 10).

Table 10. Sporozoite rate of Anopheles mosquitoes in the study area, Dembia District, Northwestern Ethiopia.

Species	Type of CSPs	Indoor			Outdoor		
		LT	PSC	MA	LT	PS	MA
An. arabiensis	No of tested	89	37	20	108	63	18
	No of Pv (210) +ve (%)	-	-	-	1(0.9)	-	-
	No of Pv (247) +ve (%)	-	-	-	-	-	-
An. pharoensis	No of tested	182	4	4	127	7	8
	No of Pv (210) +ve (%)	1(0.5)	-	-	-	-	-
	No of Pv (247) +ve (%)	2(1.1)	-	-	-	-	-
An. coustani	No of tested	15	-	-	50	1	2
	No of Pv (210) +ve (%)	1(6.7)	-	-	3(6)	-	-
	No of Pv (247) +ve (%)	-	-	-	-	-	-
An. squamosus	No of tested	-	-	-	4	-	-
	No of Pv (210) +ve (%)	-	-	-	-	-	-
	No of Pv (247) +ve (%)	-	-	-	1(25)	-	-
Total	No of tested	286	41	24	289	71	28
	No of Pv (%)	4 (1.4)	-	-	5 (1.7)	-	-

LT: Light trap; PS: Pit shelter; MA: Mouth aspirator; PSC: Pyrethrum spray catch; Pv: *Plasmodium vivax*.

Entomological inoculation rate (EIR) of *Anopheles* mosquitoes

The estimated annual entomological inoculation rate (EIR) of *Anopheles* mosquitoes collected using CDC light trap is selected localities of Dembia district is indicated in Table 11. The annual *P. vivax* EIR of *An. arabiensis* collected from outdoor CDC light trap was 4.7 infective bites/person/year (ib/p/year). The annual *P. vivax* EIR of *An. pharoensis* collected from indoor was 12.1 ib/p/year. The annual *P. vivax* EIRs of indoor and outdoor CDC collected *An.coustani* were 6.9 and 25.7 ib/p/year respectively. Outdoor CDC collected *An. squamosus* had annual *P. vivax* EIR of 7.2 ib/p/year.

Table 11. Annual entomological inoculation rate of Anopheles mosquitoes in the study area, Dembia district, Northwestern Ethiopia.

Species	Variables	Indoor CDC	Outdoor CDC
An. arabiensis	SR	0	0.9
	EIR	0	4.7
An. pharoensis	SR	1.6	0
	EIR	12.1	0
An. coustani	SR	6.7	6
	PvEIR	6.9	25.7
An. squamosus	SR	0	25
	Annual EIR	0	7.2
Overall	SR	1.4	1.7
	Annual EIR	20.8	32.67

Discussion

The result of this study showed that *An. pharoensis*, the secondary malaria vector in Ethiopia was the dominant species in the study areas. This species outnumbered the primary and dominant malaria vector, *An. arabiensis*. Similarly, *An. pharoensis* was the predominant species in irrigated village of central Ethiopia during the dry season [32]. The presence of cattle in close proximity to the households may contribute for high density of more zoophilic vectors such *An. pharoensis* [33]. In addition, the less endophilic and endophagic behaviour of *An. pharoensis*, could make them less susceptible to indoor based control strategies [34]. Moreover, the presence suitable breeding habitats of *An. pharoensis* near to human dwelling could also be the reason for its high density in the study areas. *An. arabiensis* was the second dominant vector identified during this study. Similarly, this vector was reported as a second dominant vector in south central Ethiopia [35]. Though, *An. arabiensis* was the second dominant vector identified, its percentage composition was comparable with studies from different part of Ethiopia [36, 37].

The overall density of *Anopheles* mosquitoes was higher outdoor than indoor, which is in line with studies from southwestern Ethiopia [36], central Ethiopia [19] and south central Ethiopia [35] where indoor vector control strategies are implemented. Additionally, the outdoor density of host seeking *An. gambiae s.s* and *An. melas* was high following initiation of vector control strategies in Equatorial Guinea [38]. This increased outdoor vector density could be attributed with the long term use of vector control strategies (LLINs and IRS) which induce exophagic and exophilic tendency [16, 19] or it could be associated with the availability of cattle's outdoor [16, 39].

An. arabiensis showed a high endophagic tendency, with high indoor host seeking density than outdoor density. This is in agreement with study conducted in Kenya [34]and southwestern Ethiopia [40]. However, the exophagic tendency of *An. arabiensis* in this study was higher when compared to the study conducted in Kenya in a village with low level of LLINs coverage [41]. This study also showed that the exophilic tendency of *An. arabiensis* was significantly higher than its endophilic tendency. Similarly, high outdoor resting tendency of *An. arabiensis* was reported in western Kenya [34]. The result may be associated with, insecticide induced avoidance of the vector contact with insecticide treated surfaces and rapid exit from house [15].

The outdoor host seeking density *An. pharoensis* was higher than indoor density in agreement with previous works in Ethiopia [19]. However, appreciably high density of indoor host seeking *An. pharoensis* was recorded when compared with study conducted in south central Ethiopia [35] and Kenya [34, 42]. This endophilic tendency of *An. pharoensis* could be associated the presence of cattle shelter in a close proximity to human residence or they share humans house during the

night. An experimental study conducted in southwest Ethiopia proved that *An. pharoensis* was more prevalent indoor when a calf was present either inside, or adjacent to a tent relative to a tent without a calf present [33].

Blood meal source preference of *Anopheles* mosquitoes determines their malaria transmission efficiency. The HBI of host seeking *Anopheles* mosquitoes collected indoor was comparable with the outdoor HBI, while a significantly higher BBI was recorded from indoor than outdoor collected host seeking *Anopheles* mosquitoes. Consistently, the bovine blood index (BBI) for *An. arabiensis* was significantly higher for populations collected indoors (71.8%), than populations collected outdoors (41.3%), but the human blood index (HBI) did not differ significantly between the two populations in Kenya [42]. High indoor BBI of *Anopheles* mosquitoes could be associated with their response to increased vector control strategies or with the location of cattle in a close proximity to a people house or cattle share peoples house, so that mosquitoes bite indoor [43]. Hence, treating livestock with insecticides and constructing a separate shade from human house is important to control zoophilic malaria vectors.

The relative feeding preference result of this study indicated a strong zoophilic tendency of *An. arabiensis*. Similarly, zoophilic tendency of *An. arabiensis* was reported from south central Ethiopia [44] and similar proportion of *An. arabiensis* that fed on human and bovine were reported from south central Ethiopia [45]. Differently, the anthropophilic nature of this vector was reported from Konso District southern Ethiopia [46]. Previous works before the scale up of vector control strategies from east, south, and west Ethiopia also indicated that *An. arabiensis* were more anthropophilic in nature [47]. Even though, the vector show a zoophilic tendency in this study, appreciably high HBI of *An. arabiensis* was recorded from both indoor and outdoor collected vector specimens suggesting the opportunistic behaviour of this vector.

An. pharoensis collected from indoor and outdoor has had a strong zoophilic tendency in agreement with previous works from south central Ethiopia [45]. Because cattle share a peoples house during the night the indoor BBI of *An. pharoensis* was higher than the outdoor. In addition, a meaningful number of HBI of *An. pharoensis* were recorded indicated that *An. pharoensis* have opportunistic feeding behaviour.

The sporozoite rate of *An. arabiensis* was low (0.3% for *P. vivax* and 0% for *P. falciparum*) as compared to 0.46 *P. vivax* and 0.46 *P. falciparum* from southwest Ethiopia [48] and 1.7% *P. vivax* and 0.2% *P. falciparum* from south central Ethiopia [45]. In this study a lower annual EIR (*P. vivax*) of *An. arabiensis* (4.7 infective bites/person/year (ib/p/year)) was recorded when compared with a study from southwest Ethiopia (5.3 infection bites/person/eight months) [48]. None of *Anopheles* mosquitoes tested for CSP was positive for *P. falciparum*, the rationale behind may be the number of *Anopheles* tested for CSP was limited in this study.

In addition *An. coustani, An. pharoensis* and *An. squamosus* were positive for CSP. The annual EIR of *An. pharoensis* collected from indoor was 12.1ib/p/year. The annual EIRs of indoor and outdoor CDC collected *An.coustani* were 6.9 and 25.7 ib/p/year respectively. Comparably, the EIRs of *An. coustani* in Kenya was 23.9 ib/p/year [49]. Thus, it is important to give attention about outdoor malaria transmission role of *An.coustani* and *An. pharoensis*.

Conclusions

During this study a total of 2,055 *Anopheles* specimens were collected from Arebiya and Guramba Bata study sites, Dembia District, Northwestern Ethiopia. From these specimens 11 species of *Anopheles* mosquitoes such as *An. pharoensis, An. arabiensis*, *An. coustani*, *An. demeilloni*, *An. cinereus, An. funestus, An. ardensis*, and *An. squamosus* were identified from the two study sites. Whereas, *An. garnhami, An. christyi* and *An. nili* were identified only from Guramba Bata study site. *Anopheles arabiensis* and *An. pharoensis* were the dominant vector species identified in the two study sites. The monthly distribution of *Anopheles* mosquitoes showed a steady increase from June to September. A relatively high indoor density of host-seeking and resting *Anopheles* mosquitoes were collected than indoor density of host seeking and resting *Anopheles* mosquitoes. *Anopheles arabiensis*, *An. pharoensis, An. coustani*, and *An. squamosus* showed a strong zoophilic tendency. The annual entomological inoculation rate of *An. coustani*, *An. squamosus* and *An. pharoensis* was higher than the annual

entomological inoculation rate of *An. arabiensis*. Further study about the evaluation of the vectoral role of *An. pharoensis*, *An. coustani*, and *An. squamosus* in the study area and behavioural study using a human landing catch is recommended. Stakeholders should give attention to designing and implementing vector control strategies that target outdoor resting and host-seeking *Anopheles* mosquitoes to prevent outdoor malaria transmission.

Abbreviations

LLINs
Long lasting insecticide treated bed nets
IRS
Indoor residual spray
CDC
Center for disease control
PSC
Pyrethrum spray catch
PCR
Polymerase chain reaction
PBS
Phosphate buffered saline
ELISA
Enzyme- linked immunosorbent assay
CSP
Circum-sporozoite protein
mAb
Monoclonal antibody
BB
blocking buffer
HBI
Human blood index
BBI
Bovine blood index
FR
Forage ratio
EIR
Entomological inoculation rate.

Declarations

Ethics approval and consent to participate

Ethical clearance was obtained from Addis Ababa University, institutional ethical review board of the College of Natural and Computational Sciences (Ref No CNSDO/692/10/2018). Written consent was obtained from the head of the house hold and other study participants before sampling.

Consent for publication

Not applicable

Availability of data and materials

The data sets supporting the conclusions of this article are provided in the manuscript.

Competing Interest

The authors declare that there is no conflict of interest.

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Authors' contributions

MT, HT, YW and SD designed the study.HT, YW and SD supervised and MT and YN conducted the experiments. MT conducted the statistical analyses. MT developed first draft, HT, YW, SD and YN revised the manuscript. All authors read and approved the final manuscript.

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Figures

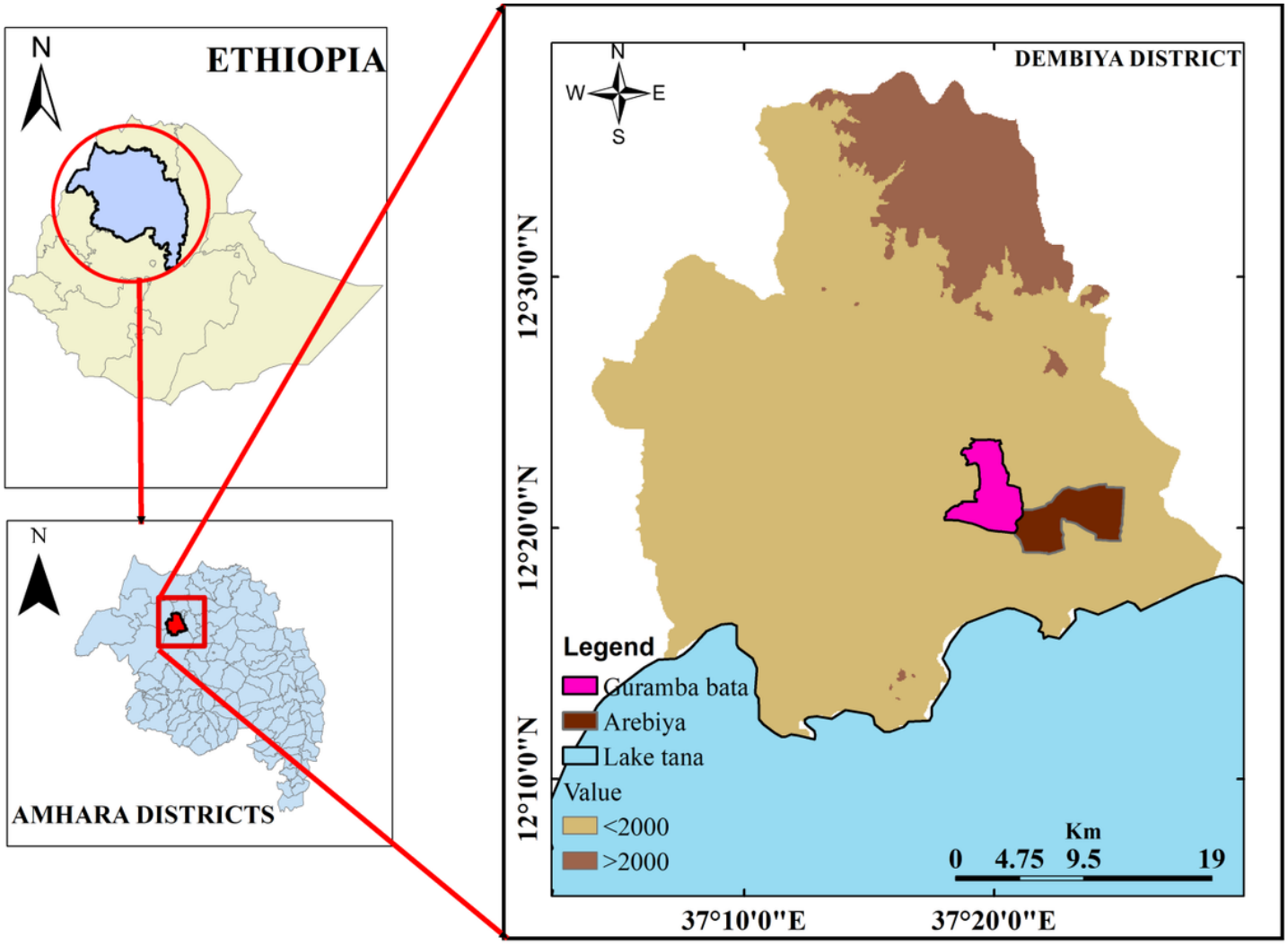

Figure 1

Map of the study area [22].

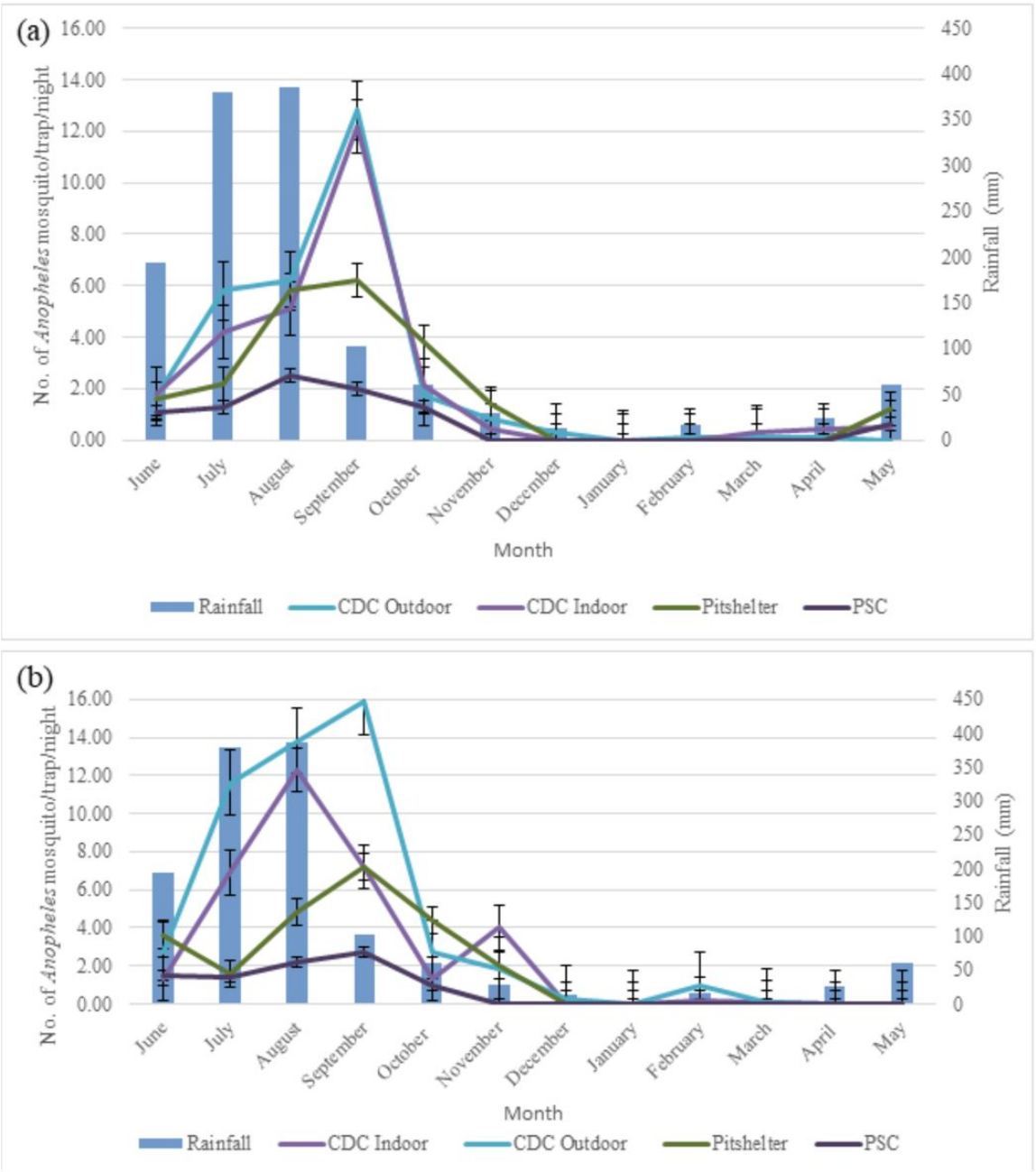

Figure 2

Monthly distribution of Anopheles mosquitoes in Arebiya (a) and Guramba Bata (b) study sites (June 2010-March 2011).