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A survey of malaria vectors feeding preference, biting site and resting behaviour in the malaria elimination settings of Dembiya District, north-western Ethiopia

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Abstract

Background Despite the progress in scaling vector control interventions in Ethiopia, malaria is still a major health problem in the country. Monitoring of the local vector populations and the effectiveness of vector control strategies is necessary to guide programme decisions to optimize malaria prevention efforts. This study investigated the feeding preference, the biting behaviour and resting behaviours of *Anopheles* mosquitoes in selected localities of Dembiya District.

Methods Adult *Anopheles* mosquitoes were sampled indoors and outdoors from June 2018 to May 2019 using CDC light traps, pyrethrum spray catches, artificial pit shelters, and mouth aspirators at both Guramba Bata and Arebiya study sites. *Anopheles* mosquitoes were identified to the species level. Their blood meal source and *Plasmodium* sporozoite infections were determined using an enzyme-linked immunosorbent assay.

Results *Anopheles* mosquitoes belonging to 11 species were identified from 2,055 collected mosquito specimens. *Anopheles pharoensis* was the predominant species at both the Guramba Bata (46.5%) and Arebiya (46.2%) study sites. The CDC light traps caught the highest number of *Anopheles* mosquitoes in both study sites. In Guramba Bata the density of outdoor host-seeking and resting *Anopheles* mosquitoes were higher than indoors ($P \leq 0.05$). The human blood indexes (HBI) of indoor and outdoor host-seeking *Anopheles arabiensis* were 17.4% and 15.3%, respectively. The entomological inoculation rate (EIR) of outdoor host-seeking *An. arabiensis* was 4.7 infective bites/person/year. Additionally, the outdoor EIR of host-seeking *Anopheles coustani* was 25.7ib/p/year.

Conclusions *Anopheles* mosquitoes in Dembiya district were more likely to seek a host and rest outdoors than indoors. A reevaluation of vector control strategies is needed to ensure Ethiopia remains on the path to malaria elimination. The detection of *Plasmodium* circumsporozoite protein in potential secondary vectors, such as *An. coustani* requires further investigation to substantiate their role in malaria transmission.

Keywords Malaria, *Anopheles arabiensis*, Host-seeking behavior, Resting behavior, Feeding preference

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Background

Globally, an estimated 247 million malaria cases and 619,000 deaths were reported in 2021, of which 96% of cases and 96% of deaths were recorded in the WHO African region [1]. The widespread distribution of different malaria intervention strategies, such as long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) resulted in a substantial reduction in malaria cases and deaths worldwide between 2000 and 2020 [1, 2]. Unfortunately, the development of insecticide-resistant malaria vectors in different parts of the world impedes global malaria control and elimination efforts. Therefore, alternative vector control strategies are required to maintain and consolidate the already achieved successes in malaria reduction [3, 4].

In Ethiopia, malaria is endemic throughout much of the country and 68% of the total population is at risk [5]. In the country, high malaria transmission occurs primarily at an altitude less than 2000 m, although endemic areas with an altitude of > 2000 m have also been reported [5]. The two species of *Plasmodium* parasites, *Plasmodium falciparum* and *Plasmodium vivax*, are responsible for 60% and 40% of the total malaria cases in the country, respectively [6, 7]. *Anopheles arabiensis* is the primary vector of malaria in Ethiopia, whereas other species such as *Anopheles funestus*, *Anopheles pharoensis*, and *Anopheles nili* play a minor role in malaria transmission [8, 9]. Recently, the rapid spread of the highly invasive malaria vector, *Anopheles stephensi*, recorded in Ethiopia, is posing a threat to the countries malaria elimination program [10].

The increased implementation of major malaria intervention strategies, such as artemisinin-based combination therapy, long-lasting insecticidal bed nets (LLINs), and indoor residual spray (IRS) has resulted in a significant decrease in malaria cases and deaths in Ethiopia [9, 11, 12]. When compared to the pre-intervention period (before 2005), the proportion of the population at risk of malaria protected by LLINs increased by 51%, IRS coverage increased by 35%, and active case treatment exceeded 87% during the post-intervention period (2006–2011) [13], . As a result, malaria inpatient cases and deaths in all age groups were reduced by 54% and 68%, respectively, in 2011 compared to the pre-intervention period (2001–2005) [13]. Furthermore, between 2016 and 2019, malaria-caused morbidity and mortality in Ethiopia, decreased by 47% and 58%, respectively [12].

Despite the reduction in overall malaria prevalence, malaria control is challenged by the development of insecticide resistance, a shift in vector species composition, and increasing vector behavioural change [10, 12]. According to recent Ethiopian reports, *An. arabiensis* is resistant to insecticides, such as DDT, permethrin, deltamethrin, and malathion [14–16]. Furthermore, this vector showed an increased tendency to bite and rest outdoors and a shift into early evening biting before people retired to bed [17]. Similarly, the behavioural and molecular resistance of *An. arabiensis* to different classes of insecticides has been reported in various parts of Ethiopia [11, 18].

Dembiya is a malaria-endemic area in Ethiopia with a long history of implementing vector control strategies [19, 20]. Malaria infection in this district was significantly reduced following the increased implementation of malaria intervention strategies [20]. However, a recent study in Dembiya District indicated that malaria is still a public health problem [21]. Limited studies are available on the species composition, ecology, and behaviour of the local malaria vectors in the district. Therefore, this study assessed the species composition, distribution, and behaviour of *Anopheles* mosquitoes in selected localities of Dembiya district. The findings will aid in the development of vector control strategies that take into account the behaviour and ecology of the vectors.

Methods

Study area

A longitudinal study on the species composition, monthly distribution, biting, and resting behaviour, blood meal source, and entomological inoculation rate of *Anopheles* mosquitoes was conducted from June 2018 to May 2019 in two localities (Guramba Bata and Arebiya) of Dembiya District in the North Gondar administrative zone of Amhara regional state (Fig. 1). The district is located at 12°39'59.99" N and 37°09'60.00" E. Kola Diba is the administrative town 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 or 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 [22].

The district receives bimodal rainfall, with a short rainy season from March to May and the main rainy season from June to September. Most of the population (91%) lives in rural areas, with most engaged in farming; the remaining 9% live in urban areas. The district has 49,528 rural households with a mean of 4.3 people per household [22]. The elevation of Dembiya District ranges from 1500 to 2600 m above sea level (a.s.l.). The agro-ecology of the district is mid-altitude (Woynadega) with a mean annual minimum and maximum temperature of 11 °C and 32 °C, respectively, and a mean annual rainfall ranging from 995 to 1175 mm [19]. The topographic features considered as plain, mountainous, valleys, and wetland are 87%, 5%, 4.8%, and 3.2%, respectively. Out of the total

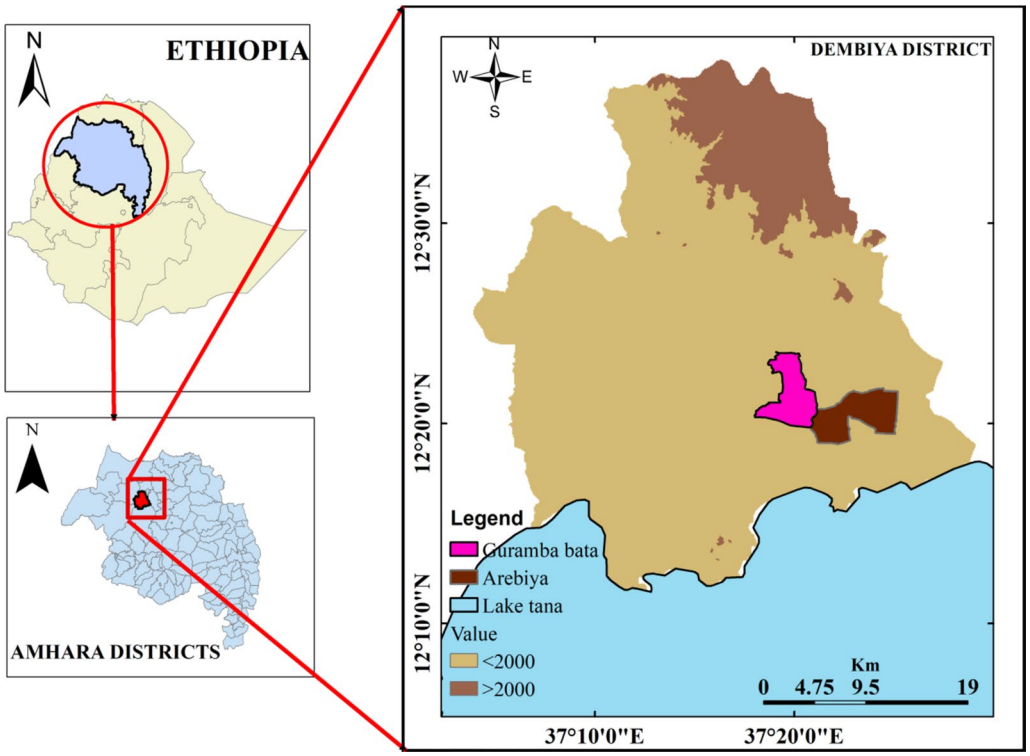

Fig. 1 Map of the study area [23]

area of the district, 31% is cultivated land, 16% is non-cultivable land, 5.6% is forest and bush, 12.8% is grazing, 8.1% is covered with water, 20.2% is swamp, and 4.3% is human settlement areas (unpublished agricultural bureau report and published by Tarekegn et al. [21]).

In Dembia district, Ethiopia, historical vector control efforts primarily involved the use of IRS (Indoor Residual Spraying) with DDT during the 1950s as part of a pilot malaria eradication program. This approach aimed to reduce mosquito populations and malaria transmission. However current vector control activities in Dembia district, Ethiopia, focus on integrated strategies including larviciding, insecticide-treated nets distribution, and community education to combat malaria and other vector-borne diseases [19, 20]. In 2016, about 138,842 LLINs were distributed, and 16 localities were sprayed. However, the burden of malaria remains high, for instance, in over a 46-week interval, 22,166 malaria cases were reported in 2016 in contrast to 10,415 in 2015 [20]. According to the annual performance report of the FMOH 2017, the total malaria cases treated in the health facilities of the district were 1,820,967 [19]. Both *P. falciparum* and *P. vivax* are common *Plasmodium* species in the study area [19–21].

The houses in the study area are largely constructed of mud-plastered timber walls and corrugated iron roofs. The animal shelters are built close to the main house, and some inhabitants keep their domestic animals in their housing during the night. Most people in the two study sites go to bed at 22:00 h [18]. The two study sites selected for this study are characterized by their malaria endemicity and long-term implementation of IRS and LLINs [19–21]. The distance between the two study sites is 9 km.

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 that forms intermittent larval habitats until the end of December. Guramba Bata has one health post and one health center, 1113 households, with 6008 inhabitants (2974 males and 3034 females) 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 a river that serves as a water source during the dry season and flows into Lake Tana. This locality has 1976 households and a total of 8632 inhabitants (4298 males 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 used to study the seasonal distribution, species composition and behaviour of *Anopheles* in two selected localities in Dembiya District. All houses near to potential larval habitats (rivers) were first recorded, and houses between distances of 50 m to 100 m were selected for adult *Anopheles* mosquito collection. The approximate distances between the selected households for *Anopheles* mosquito collection and breeding habitat (river) were between 50 m and 100 m.

Host survey

Information about the total human population living at the two study sites was obtained from the health center. Similarly, the available number of potential hosts such as cattle, goats, dogs, and chickens in the two study sites was collected from the local agricultural offices (unpublished agricultural office report).

Assessment of indoor and outdoor biting

Adult *Anopheles* mosquito collection was carried out for one year, starting from June 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 the 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 [24]. For outdoor host-seeking *Anopheles* mosquito collection, five CDC light traps were installed near animal enclosures in five randomly selected households from each locality. The same houses were used for adult mosquito collection throughout the year.

Assessment of indoor and outdoor resting

Indoor resting *Anopheles* mosquito collections were performed using pyrethrum spray catches (PSCs) from another ten randomly selected houses from each locality, starting from 06:30 to 09:30 h. Before PSC was 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) (active ingredients are Tetramethrin and d-Allethrin). Fifteen minutes after spraying, knocked-down *Anopheles* mosquitoes were collected by using forceps, paper cups, and a torchlight [23]. The collection using PSC was done once a month at each household. In addition, mouth aspirators were used to collect indoor resting mosquitoes, such as on walls, ceilings, underneath household furniture, and on materials hung on the walls (posters, photo frames, and traditional equipment).

An additional five houses from each study site were randomly selected for outdoor resting mosquito collection using artificially constructed pit shelters (constructed in the backyard of each house). The pit shelters were 1.5 m deep and had a 1.2 m x 1.2 m opening. Four cavities with a horizontal depth of 30 cm were dug on each shelter [25]. Mouth aspirators were used to collect resting mosquitoes after covering the mouth with an untreated bed net. The collection was performed twice a month in the morning, from 6:30a.m to 10:00a.m. Mouth aspirators were also used to collect outdoor resting mosquitoes from various possible outdoor mosquito resting sites in each village (ground holes, tree holes, open cattle sheds, and vegetation). The collection was done once a month for 30 min at each possible resting site.

Identification of female *Anopheles* mosquitoes.

Adult *Anopheles* mosquitoes were identified using Gillies and Coetzee's morphological keys [8]. Female *Anopheles* mosquitoes were divided into four categories: unfed, blood-fed, half-gravid, and gravid. Female *Anopheles* mosquitoes were kept in a labeled 1.5 ml Eppendorf tube with cotton wool on top of a silica gel desiccant. All mosquito specimens collected were kept at room temperature (25 °C) for later mosquito processing. Individual specimens of female *An. gambiae sensu lato* (*s.l.*) mosquitoes were identified at the species level using a ribosomal DNA polymerase chain reaction (PCR) including the primers for *Anopheles gambiae sensu stricto* (*s.s.*), *An. arabiensis*, *Anopheles quadriannulatus* and *Anopheles amharicus* [26].

Blood meal analysis

The blood meal source of engorged female *Anopheles* mosquitoes was individually examined using direct ELISA techniques using bovine and human antibodies with little modification [27]. 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 human volunteers and cows blood obtained from abattoirs were used as a positive control.

- ***Plasmodium* parasite detection**.

Circumsporozoite protein (CSP) (*P. falciparum*, *P. vivax* 210, and *P. vivax* 247 CSPs) detection of the parasite within the mosquito gut was performed based on the protocol described in Methods in Anopheles Research

[28]. The plates were read at 405 nm absorbance using an ELISA plate reader. The sample was considered positive if the sample absorbance value was greater than twice the mean absorbance value of the negative samples.

Data analysis

SPSS version 26 (Armonk, NY: IBM Corp) was used for data analysis. Since the response variable was over-dispersed count data with unequal mean and variance, negative binomial regressions with a log link function were used to analyse the effect of locality and site of collection (indoor and outdoor) on the number of female host-seeking and resting *Anopheles* mosquitoes. The result was considered as significant at $P \leq 0.05$. The human blood index (HBI) was estimated as the number of *Anopheles* fed on human blood meal over the total number of *Anopheles* tested for blood meal origin [29]. Similarly, the Bovine Blood Index (BBI) was estimated as the number of *Anopheles* fed on bovine blood meal over the total number of *Anopheles* tested for blood meal origin [29]. A mixed blood meal was included in calculating the human blood index and bovine blood index [30].

The relative feeding preference, or forage ratio (FR), of *Anopheles* was calculated by dividing the percent of blood engorged *Anopheles* that have fed upon either humans or bovines by the percent that fed on either humans or cattle comprise in the area [31].

The sporozoite rate was calculated as the proportion of *Anopheles* positive for either *P. vivax* or *P. falciparum* CSP over the total number of *Anopheles* tested for CSP. The annual entomological inoculation rate (EIR) calculated from mosquitoes collected by CDC light traps using the formula, 1.605 × (no. of CSP positive ELISA results from CDC light traps/no. mosquitoes tested) × (no. of mosquitoes collected from CDC light traps/no. of traps-nights) × 365 days [24, 32].

Results

Species composition and monthly distribution of *Anopheles* mosquitoes.

During the one-year study period (June 2018-May 2019), 2,055 female *Anopheles* mosquitoes belonging to 11 species were collected from the two sites. Of these, 56.6% ($n=1,164$) were collected from Guramba Bata, and 43.3% ($n=891$) were collected from Arebiya study sites. The difference in number of *Anopheles* collected from the two sites was not statistically significant (OR: 0.985, 95% CI: 0.840–1.156, $P=0.855$). A total of 11 *Anopheles* species (*An. arabiensis*, *An. pharoensis*, *An. coustani*, *Anopheles demeilloni*, *Anopheles garnhami*, *Anopheles christyi*, *Anopheles cinereus*, *An. funestus*, *Anopheles ardensis*, *Anopheles squamosus*, and *An. nili*) were identified from Guramba Bata, and 8 species (*An. arabiensis*, *An. pharoensis*, *An. coustani*, *An. demeilloni*, *An. cinereus*, *An. ardensis*, *An. squamosus* and *An. funestus*) were identified from the Arebiya study site (Table 2). A total of 11 *Anopheles* mosquito species were collected using CDC light traps from both study sites (no. collected = 1402). *Anopheles arabiensis* and *An. pharoensis* were the only two species identified from PSC collections (no. collected = 175), while *An. arabiensis*, *An. pharoensis*, and *An. coustani* were the only species collected from pit shelters (No. collected = 229) (Tables 1, 2).

Anopheles pharoensis was the predominant species identified in Arebiya accounting for 46.2% (412/891) of *Anopheles* collected, and in Guramba Bata, accounting for 46.5% (541/1164) of the *Anopheles* collected. *Anopheles arabiensis* comprised 42.3% (377/891) of *Anopheles* collected at the Arebiya study site and 34.3% (399/1164) at the Guramba Bata study site (Table 2).

The highest density of indoor and outdoor host-seeking *Anopheles* in Arebiya was collected in September (12.2 and 12.8 mosquitoes/CDC trap/night, respectively). The density showed a slow increase starting in May in this study area (Fig. 2a). In Guramba Bata, the highest densities of indoor and outdoor host-seeking *Anopheles* were collected in August (12.2 and 12.8 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.

In Arebiya, the indoor density of host seeking *Anopheles* mosquitoes was lower than the outdoor host seeking density, though the difference was not statistically significant (OR: 0.907, 95% CI: 0.671–1.226, $P \geq 0.05$). The indoor host seeking density of *An. arabiensis* was lower than the outdoor density, but it was not statistically significant (OR: 0.1407, 95% CI: 0.967–2.047, $P \geq 0.05$). Similarly, the difference between the indoor and outdoor host-seeking density of *An. pharoensis* was not significant (OR: 0.935, 95% CI: 0.660–1.325, $P \geq 0.05$).

Table 1 Composition of alternative blood sources in the two study sites, Dembiya District north western Ethiopia

Composition of blood sources	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

Table 2 Species composition and abundance of *Anopheles* mosquito using different adult mosquito collection methods in the two study sites of Dembiya District, north-western 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	0		0		147	12.6
	An. demeilloni	27	3.2	0		0		0		27	2.3
	An. garnhami	1	0.1	0		0		0		1	0.1
	An. christyi	14	1.7	0		0		0		14	1.2
	An. cinereus	5	0.6	0		0		0		5	0.4
	An. funestus	8	0.9	0		0		0		8	0.7
	An. ardensis	12	1.4	0		0		0		12	1
	An. squamosus	9	1.1	0		0		0		9	0.8
	An. nili	1	0.1	0		0		0		1	0.1
	Total	831		127		88	100	118		1164	
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	0		0		2	1.8	75	8.4
	An. cinereus	5	0.9	0		0		0		5	0.6
	An. demeilloni	3	0.5	0		0		0		3	0.3
	An. ardensis	14	2.5	0		0		0		14	1.6
	An. squamosus	2	0.4	0		0		0		2	0.2
	An. funestus	3	0.5	0		0		0		3	0.3
	Total	571		122		87	100	111		891	

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

The indoor host seeking density of *An. coustani* was significantly lower than its outdoor density (OR: 0.373, 95% CI: 0.230–0.605, $P \leq 0.05$). In this district, the density of indoor resting *Anopheles* was lower than outdoor resting *Anopheles* (OR: 0.224, 95% CI: 0.117–1.430, $P \leq 0.05$). The indoor resting density of *An. arabiensis* was lower than its outdoor density (OR: 0.444, 95% CI: 0.253–0.780, $P = 0.0$ $P \leq 0.05$). Similarly, the indoor resting density of *An. pharoensis* was lower than its outdoor density (OR: 0.219, 95% CI: 0.114–0.420, $P \leq 0.05$).

In Guramba Bata, the indoor density of host-seeking *Anopheles* was significantly lower than the outdoor host-seeking density (OR: 0.742, 95% CI: 0.557–0.989, $P \leq 0.05$). The indoor host-seeking density of *An. arabiensis* and *An. pharoensis* were also lower than its outdoor density, but it was not statistically significant (OR: 0.991, 95% CI: 0.691–1.422, $P \geq 0.05$) and (OR: 0.822, 95% CI: 0.596–1.135, $P \geq 0.05$), respectively. The indoor density of host-seeking *An. coustani* was lower than its outdoor host seeking density (OR: 0.369, 95% CI: 0.235–0.579, $P \leq 0.05$). In the district, the indoor resting density of *Anopheles* was lower than the outdoor density (OR: 0.281, 95% CI: 0.470–0.579, $P \leq 0.05$). Similarly, the indoor resting density of *An. arabiensis* and *An. pharoensis* was significantly lower than the outdoor density (OR: 0.402, 95% CI: 0.231–0.699, $P \leq 0.05$) and (OR: 0.131, 95% CI: 0.066–0.258, $P \leq 0.05$), respectively.

Abdominal status of host-seeking and resting *Anopheles* mosquitoes.

Of the total indoor and outdoor host-seeking *Anopheles*, most, 50.5% and 63.9%, respectively, were unfed. Of these, 58.6% of indoor host-seeking and 67.9% of outdoor host-seeking *An. arabiensis* were unfed. Similarly, most of indoor and outdoor host-seeking *An. pharoensis* was unfed (46.8% indoors and 57.4% outdoors,) (Table 3, 4).

From the total indoor and outdoor resting *Anopheles* mosquitoes, most (53.2% and 68.3%, respectively) were freshly fed. More than half of indoor and outdoor resting *An. arabiensis* was also fresh-fed. Additionally, most of indoor and outdoor resting *An. pharoensis* and *An. coustani* were freshly fed (Table 3).

- **Blood meal sources and host preference of *Anopheles*.**

The size of the human population was 14,640 (41.8%), which is three times higher than the size of cattle and chickens in the two study sites (Table 1). During this

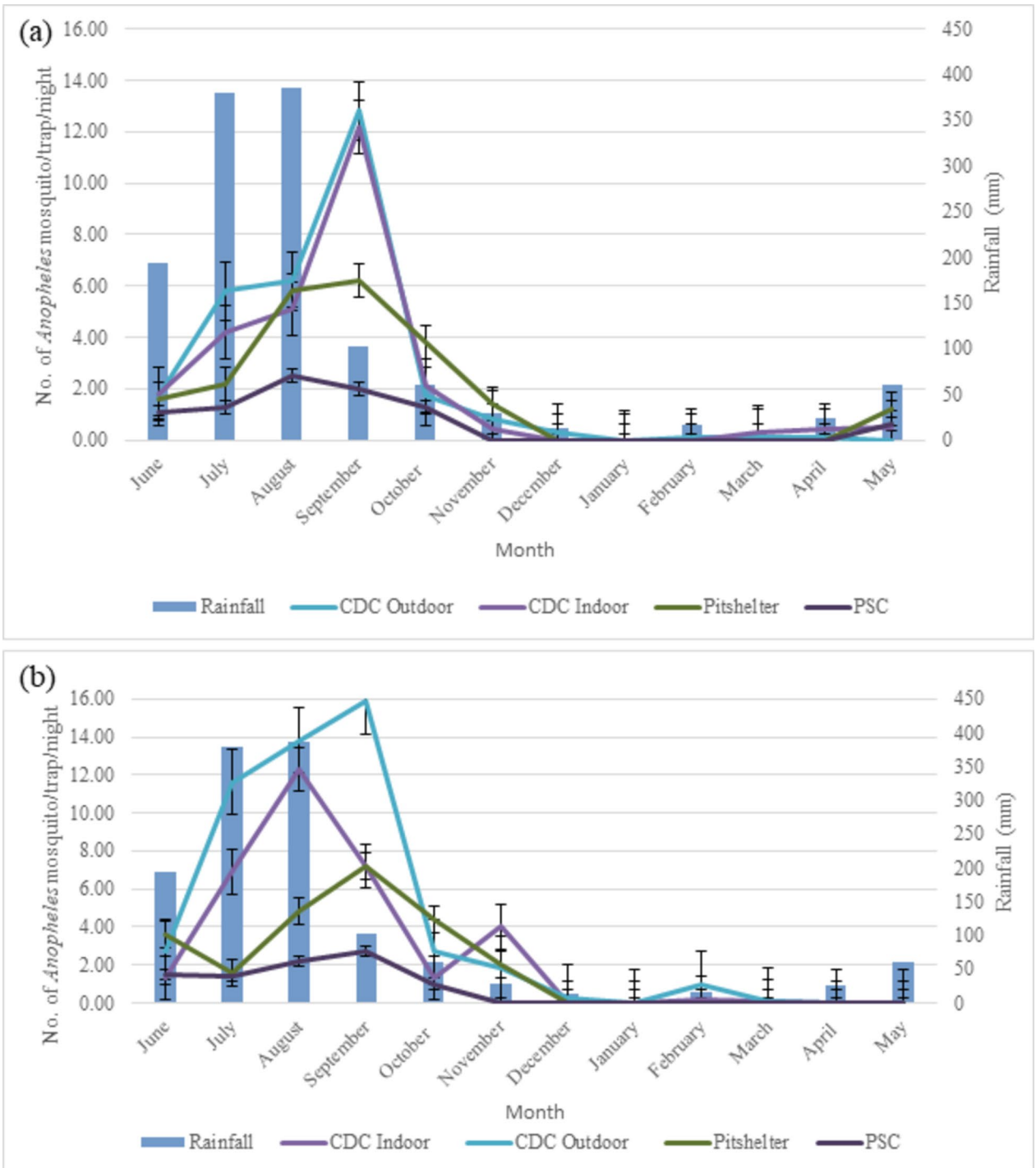

Fig. 2 Monthly distribution of *Anopheles* mosquitoes in Arebiya (**a**) and Guramba Bata (**b**) study sites (June 2010-March 2011)

study, 522 *Anopheles* were tested for blood meal source analysis using a direct ELISA. From these, 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 (Tables 5 and 6). However, *An. arabiensis* collected by indoor and outdoor CDC light traps had human blood indices of 17.4%, and 15.3%, respectively and bovine blood indices of 50% and 20.3%, respectively (Table 5).

Table 3 Abdominal status of host seeking *Anopheles* mosquitoes in the study area, Dembiya District, north-western Ethiopia (June 2018-March 2019)

Species	CDC-LT Indoor					CDC-LT Outdoor				
	Unfed	Freshly Fed	Half Gravid	Gravid	Total	Unfed	Freshly Fed	Half Gravid	Gravid	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)	0	178
An. cinereus	3 (50)	3 (50)	-	0	6	3 (75)	-	1 (25)	0	4
An. demeilloni	2 (12.5)	13 (81.3)	1 (6.3)	0	16	5 (35.7)	8 (57.1)	1 (7.1)	0	14
An. ardensis	0	0	0	0	0	20 (76.9)	6 (23.1)	0	0	26
An. squamosus	0	0	0	0	0	7 (63.6)	4 (36.4)	0	0	11
An. funestus	1 (20)	4 (80)	0	0	5	3 (50)	3 (50)	0	0	6
An. garnhami	1 (100)		0	0	1	0	0	0	0	0
An. christyi	3 (75)	1 (25)	0	0	4	9 (90)	1 (10)	0	0	10
An. nili	1 (100)		0		0 1	0	0	0		0
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

Table 4 Abdominal status of resting *Anopheles* mosquitoes in the study area, Dembiya District, north-western 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)	0	8 (3.2)
	Freshly Fed	93 (55.7)	41 (48.2)	0	134 (53.2)
	Half Gravid	46 (27.5)	28 (32.9)	0	74 (29.4)
	Gravid	22 (13.2)	14 (16.5)	0	36 (14.3)
Total		167	85	0	252
Outdoor (Pit shelter and Mouth Aspirator)	Unfed	4 (2.3)	0	0	4 (0.99)
	Freshly Fed	113 (64.6)	158 (70.9)	3 (100)	274 (68.3)
	Half Gravid	40 (2.3)	49 (21.97)	0	89 (22.2)
	Gravid	18 (10.3)	16 (7.2)	0	34 (8.5)
Total		175	223	3	401

PSC: Pyrethrum Spray Catches

The bovine blood index of resting *Anopheles* was higher than the human blood index (Table 6). The human blood index of mosquitoes collected by pit shelters, indoor mouth aspirators, outdoor mouth aspirators, and PSC collected *An. arabiensis* were 7.3%, 0%, 12.5%, and 8.3%, respectively. The bovine blood index of mosquitoes collected by pit shelter, indoor mouth aspirator, outdoor mouth aspirator, and PSC collected *An. arabiensis* were 41.5%, 27.3%, 62.5%, and 37.5%, respectively (Table 6).

The bovine blood index of pit shelter, indoor mouth aspirator, outdoor mouth aspirator, and PSC collected *An. pharoensis* were 50%, 0%, 50%, and 0%, respectively (Table 5). However, none of the indoor and outdoor resting *An. pharoensis* analysed for blood meal were positive for human blood (Table 6).

- **Foraging ratio of *Anopheles* mosquitoes**.

Anopheles arabiensis showed a 6 times stronger preference for bovine blood than human blood. The preference of *An. pharoensis* and *An. funestus* for bovine blood was 15 and 3 times higher than that of human blood, respectively. In this study, the bovine blood preference of *An. coustani* was 9 times higher than that in human blood (Table 7).

Table 5 Blood meal sources of host seeking *Anopheles* mosquitoes in the study area, Dembiya District, north-western Ethiopia. (values in parenthesis are percentages)

Species	CDC Indoor					CDC Outdoor				
	No.	HBI (%)	BBI (%)	MB (%)	Un (%)	No.	HBI (%)	BBI (%)	MB (%)	Un (%)
An. arabiensis	46	0.17(17.4)	0.5(50)	0.04(4.3)	0.3(32.6)	59	0.15(15.3)	0.2(20.3)	0.03(3.4)	0.67(67.8)
An. pharoensis	152	0.1(10.5)	0.6(60.5)	0.07(7.9)	0.36(36.8)	110	0.1(10)	0.55(55.5)	0.05(5.5)	0.4(40)
An. coustani	15	0.06(6.7)	0.6(60)	0.06(6.7)	0.4(40)	34	0.14(14.7)	0.55(55.9)	0.11(11.8)	0.41(41.2)
An. cinereus	5	-	0.2(20)	0	0.8(80)	1	0	0	0	1
An. demeilloni	6	0.16(16.7)	0.16(16.7)	0	0.66(66.7)	13	0	0.38(38.5)	0	0.61(61.5)
An. funestus	3	0.33(33.3)	0.33(33.3)	0	0.33(33.3)	4	0	0.5(50)	0.25(25)	0.5(50)
An. chrysti	1	0	0	0	1	2	0.5(50)	0.5(50)	0.5(50)	0.5(50)
An. ardensis	0	0	0	0	0	5	0	0	0	5
An. sqaumosus	0	0	0	0	0	3	0	0.66(66.7)	0.33(33.3)	0.33(0.3)
Total	228	0.11(11.8)	0.56(56.6)	0.06(6.6)	0.38(38.2)	231	0.12(12.1)	0.44(44.2)	0.06(6.5)	0.5(50)

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

Table 6 Blood meal sources of resting *Anopheles* mosquitoes in the study area, Dembiya District, north-western Ethiopia

Collection method	Location	Species	No. analyzed	HBI (%)	BBI (%)	MB (%)	Un (%)
Pit shelter	Outdoor	*An. arabiensis*	41	0.07(7.3)	0.41(41.5)	0.049(4.9)	0.56(56.1)
		An. pharoensis	2	0	0.5(50)	0	0.5(50)
Mouth Aspirator	Indoor	*An. arabiensis*	11	0	0.27(27.3)	0	0.72(72.7)
		An. pharoensis	2	0	0	0	2
	Outdoor	*An. arabiensis*	8	0.12(12.5)	0.6(62.5)	0	0.25(25)
		An. pharoensis	2	0	0.5(50)	0	0.5(50)
		An. coustani	1	0	0	0	1
Pyrethrum Spray catches	Indoor	*An. arabiensis*	24	0.08(8.3)	0.37(37.5)	0	0.54(54.2)
		An. pharoensis	2	0	0	0	2
	13.	Total	93	0.06(6.5)	0.38(38.7)	0.02(2.2)	0.56(56.9)

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

Table 7 Foraging ratio of *Anopheles* mosquitoes in the study area, Dembiya District, north- western 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: Percent human blood; %HP: Percent human in population; %BB: Percent bovine blood; %BP: Percent bovine in population; Human Forage ratio (FR) = %HB/ %HP; Bovine Forage ratio (FR) = %BB/ %BP

Proportion of mosquitoes infected with malaria parasites

A total of 792 female *Anopheles* mosquitoes belonging to nine species, *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 circumsporozoite protein (CSP) in their salivary gland (presence of *P. falciparum*, *P. vivax* 210, and *P. vivax* 247 CSPs). From the species analysed for CSP, nine specimens (*An. arabiensis* ($n=1$), *An. coustani* ($n=4$), *An. pharoensis* ($n=3$) and *An. squamosus* ($n=1$) collected using the CDC light trap were positive for CSP (Table 8).

The sporozoite rate of *Anopheles* mosquitoes collected using different methods is indicated in Table 8. The overall sporozoite rate of *An. arabiensis* was 0.3%, and the respective sporozoite rates of indoor and outdoor CDC collected *An. arabiensis* was 0 and 0.9%. The sporozoite

Table 8 Sporozoite rate of *Anopheles* mosquitoes in the study area, Dembiya District, north- western Ethiopia

Species	Type of CSPs	Indoor			Outdoor		
		LT	PSC	MA	LT	PS	MA
An. arabiensis	No. tested	89	37	20	108	63	18
	No. of Pv (210) + ve (%)	0	0	0	1(0.9)	0	0
	No. of Pv (247) + ve (%)	0	0	0	0	0	0
An. pharoensis	No. tested	182	4	4	127	7	8
	No. of Pv (210) + ve (%)	1(0.5)	0	0	0	0	0
	No. of Pv (247) + ve (%)	2(1.1)	0	0	0	0	0
An. coustani	No. tested	15	0	0	50	1	2
	No. of Pv (210) + ve (%)	1(6.7)	0	0	3(6)	0	0
	No. of Pv (247) + ve (%)	0	0	0	0	0	0
An. squamosus	No. tested	0	0	0	4	0	0
	No. of Pv (210) + ve (%)	0	0	0	0	0	0
	No. of Pv (247) + ve (%)	0	0	0	1(25)	0	0
Total	No. tested	286	41	24	289	71	28
	No. of Pv (%)	4 (1.4)	0	0	5 (1.7)	0	0

LT: Light trap; PS: Pit shelter; MA: Mouth aspirator; PSC: Pyrethrum spray catch; Pv: *Plasmodium vivax*; CSPs: Circum-sporozoite proteins; +ve: Positive

Table 9 Annual entomological inoculation rate of *Anopheles* mosquitoes in the study area, Dembiya District, north-western Ethiopia

Species	Variables	Indoor CDC	20.	Outdoor CDC	21.
An. arabiensis	SR (95% CI)	0	22.	0.9 (0–2.7)	23.
	EIR	0	25.	4.7	26.
An. pharoensis	SR (95% CI)	1.6 (0–3.49)	27.	0	28.
	EIR	12.1	30.	0	31.
An. coustani	SR (95% CI)	6.7 (0–19.29)	32.	6 (0–12.58)	
	EIR	6.9	33.	25.7	34.
An. squamosus	SR (95% CI)	0	35.	25 (0–67.44)	36.
	EIR	0	38.	7.2	39.
Overall	SR (95% CI)	1.4 (0.04–2.76)	40.	1.7 (0.23–3.23)	41.
	EIR	20.8	43.	32.67	44.

SR: Sporozoite Rates; EIR: Entomological Inoculation Rate

rate of overall indoor and outdoor host-seeking *An. pharoensis* was 0.9%, 1.6%, and 0%, respectively. The sporozoite rate of indoor and outdoor CDC collected *An. coustani* was 6.7 and 6%, respectively (Table 8).

Entomological inoculation rate (EIR) of *Anopheles* mosquitoes

The estimated annual EIR of *Anopheles* mosquitoes collected using CDC light traps in the selected locality of Dembiya District is presented in Table 9. The outdoor *P. vivax* EIR of *An. arabiensis* was 4.7 infective bites/person/year (ib/p/year). The indoor *P. vivax* EIR of *An. pharoensis* was 12.1 ib/p/year. Indoor and outdoor *P. vivax* EIRs *An. coustani* was 6.9 and 25.7 ib/p/year, respectively (Table 8). Additionally, the outdoor *P. vivax* EIR of *An. squamosus* was 7.2 ib/p/year (Table 9).

Discussion

This study investigated the species composition, monthly distribution, and behaviour of *Anopheles* mosquitoes in selected localities of Dembiya District, north-western Ethiopia. The results of this study showed that *An. pharoensis* was the most abundant species in the two malaria endemic localities of Dembiya District. Concurrent with this study, *An. pharoensis* was the predominant species in the irrigated village of central Ethiopia during the dry season [33]. The high density of *An. pharoensis* could be associated with the presence of cattle near the households [34] or the less endophilic and endophagic behaviour of *An. pharoensis* which makes them less susceptible to indoor vector control strategies [30]. In addition, the presence of suitable larval habitats for *An. pharoensis* near human dwellings could also be the reason for its high density. *Anopheles arabiensis* was the second abundant vector identified during this study. Similar to this study, *An. arabiensis* was the second most common *Anopheles* species in south-central Ethiopia [18, 35, 36].

The density difference between indoor and outdoor host-seeking *An. arabiensis* was not statistically significant in the two study localities of Dembiya District. Likewise, a study conducted in Kenya found not statistically significant difference between the indoor and outdoor densities of host-seeking *An. gambiae s.l* [30]. In contrast, a lower outdoor host-seeking density of *An. arabiensis* has been reported in different parts of Ethiopia [37] and Kenya [38]. However, the outdoor

density of host-seeking *An. gambiae s.s.* and *Anopheles melas* were high after the intensification of indoor vector control strategies in Equatorial Guinea [39]. This increased the outdoor host-seeking density of *An. arabiensis* could be due to the exito-repellency effect of LLINs and IRS [17, 40]. The presence of other alternative hosts, such as outside cattle, could also contribute to the exophilic tendency of *An. arabiensis* [41, 42].

During this study, the HBI of indoor host-seeking *Anopheles* mosquitoes was comparable with the outdoor HBI. The BBI index of indoor host-seeking *Anopheles* mosquitoes was higher than the BBI index of outdoor host-seeking *Anopheles* mosquitoes. In Kenya, the indoor bovine blood index (BBI) (71.8%) of *An. arabiensis* was significantly higher than outdoors BBI (41.3%), whereas the indoor and outdoor human blood index (HBI) did not differ significantly between the two populations [43]. This high bovine blood index and mixed feeding behaviour of indoor collected *Anopheles* mosquitoes could be due to interrupted feeding, a response to increased vector control strategies, and the location of cattle close to human dwellings or cattle sharing human houses [44]. Hence, treating livestock with insecticides and constructing separate cattle sheds may reduce malaria transmission in settings such as this.

A high proportion of the *Anopheles* mosquito's blood meal source was unidentified during this study. Similarly, a study in southwestern Ethiopia indicated that the blood meal source of a large proportion of *An. arabiensis* was unidentified [36]. The result could be associated with the limited number of antibodies used during this study, which is not enough to identify other available blood sources in the area. Furthermore, the low sensitivity of ELISA to distinguish blood meal origins from different species may result in an overestimation of the unknown blood meal source [45]. Therefore, it is crucial to use a variety of antibodies for the ELISA test or a highly accurate technique such as PCR to identify the blood meal sources of *Anopheles* mosquitoes.

The results of this study indicated that *An. arabiensis* showed a strong zoophilic tendency. Similarly, *An. arabiensis* was more zoophilic in southwest Ethiopia [41]. Additionally, an equal proportion of *An. arabiensis* that have fed on humans and bovines has been reported from south-central Ethiopia [46]. In contrast, *An. arabiensis* showed a strong anthropophilic nature in Konso District, southern Ethiopia [47], and east, south, and west Ethiopia [48]. Indoor and outdoors collected *An. pharoensis* had a more zoophilic tendency, in agreement with previous works from south-central Ethiopia [46]. The indoor BBI of *An. pharoensis* was higher than the outdoor BBI, possibly because cattle share a people's house during the night.

Studies have indicated that for vector control to reduce malaria prevalence, the EIR should be less than 1 ib/p/year [49]. In this study, the overall outdoor EIR of *An. arabiensis* was 4.7 *P.vivax* ib/p/year, suggesting a possibility of high outdoor malaria transmission in the study area. Therefore, additional vector control strategies are necessary to avert outdoor malaria transmission. Similar or higher EIRs for *An. arabiensis* have been reported from southwest Ethiopia (5.3 infection bites/person/eight months) [50], south-central Ethiopia (33 and 14.5 *P. vivax* ib/p/year year one and two respectively) [46], and southwestern Ethiopia [51]. The variation could be due to the difference in the number of *Anopheles* mosquitoes tested for CSP and the level of malaria endemicity.

In addition, in this study *An. coustani*, *An. pharoensis* and *An. squamosus* were positive for *Plasmodium* circumsporozoite protein. This result suggests that these vectors could play a role in maintaining malaria transmission when the density of primary malaria vectors has been suppressed with indoor-based vector control strategies, such as LLINs and IRS. The EIR of *An. pharoensis* collected from indoor CDC light traps was 12.1 ib/p/year. This result is higher when compared with the EIR of *An. pharoensis* in south-central Ethiopia (0 and 2.3 *P. vivax* ib/p/year for years one and two, respectively) [46].

Interestingly, the EIRs of indoor and outdoor CDC light traps collected *An. coustani* were 6.9 and 25.7 ib/p/year, respectively, regardless of their zoophagic behaviour. Previous studies also detected a *Plasmodium* CSP and a comparably high EIR in *An. coustani* in Ethiopia [52] and Kenya [53]. Similarly, PCR-based circum-sporozoite detection in Madagascar revealed that *An. coustani* was mainly responsible for malaria transmission with a high EIR (61.2 ib/p/year), compared with principal malaria vectors such as *An. arabiensis* and *An. funestus* [54]. The high EIR of *An. coustani* could be due to a false positivity in ELISA results, which led to an overestimation of EIR in zoophagic *Anopheles* mosquitoes [55]. Therefore, it is necessary to conduct a further investigation into the vectoral role of *An. coustani*, *An. pharoensis*, and *An. squamosus* using PCR.

Conclusions

Anopheles arabiensis and *An. pharoensis* were the predominant vector species identified at the two study sites. No significant differences were observed between the indoor and outdoor densities of host-seeking and resting *Anopheles* mosquitoes. *Anopheles arabiensis*, *An. pharoensis*, *An. coustani, and An. squamosus* showed a strong zoophilic tendency. A *P. vivax* circumsporozoite protein was detected from specimens of *An. arabiensis*, *An. pharoensis*, *An. coustani*, and *An. squamosus*. The annual outdoor EIR of *An. arabiensis* was high, indicating that outdoor malaria

transmission is a potential challenge to malaria control in this area. The detection of *P. vivax* CSP in specimens of *An. pharoensis*, *An. coustani*, and *An. squamosus* suggests their role as malaria vectors in the two study areas.

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

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Author 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 the first draft, and YW, HT, SD, and YN revised the manuscript. All authors read and approved the final manuscript.

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

Declarations

Ethics approval and consent to participate
Ethical clearance was obtained from Addis Ababa University by the 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 household and other study participants.

Consent for publication
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Competing interests
The authors declare no competing interests.

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