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Article

High Malaria Mosquito Species Diversity, Their Blood Feeding Patterns, Role in Malaria Transmission and The Challenge of Morphological Classification: An Implication for Entomological Surveillance and Monitoring, Southwestern Ethiopia

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Abstract: There are a number of *Anopheles* species playing either primary or secondary roles in malaria transmission. Hence, understanding the species composition, their bionomics, and behaviors are all important in designing and implementing vector control intervention tools. Moreover, accurate identification of different species is vital. This study aimed to assess species composition, sporozoite infection rate, and blood meal origins of malaria mosquitoes in two malaria-endemic villages of Boreda district in Gamo zone, southwest Ethiopia. Thirty houses, 20 for Centers for Disease Control and Prevention (CDC) light traps and 10 for Pyrethrum Spray Catches (PSC), were randomly selected for bimonthly mosquito collection from October 2019 to February 2020. Enzyme-linked-immunosorbent assay (ELISA) test was done to detect the blood meal origins and circumsporozoite proteins (CSPs). The entomological inoculation rate (EIR) was calculated by multiplying the sporozoite and human biting rates from PSCs. *Anopheles gambiae* and *An. funestus* complexes were further identified into species by polymerase chain reaction (PCR). *Anopheles* species with some morphological structures confusing with *An. gambiae* or *An. funestus* complexes were father confirmed by PCR. A total of 15 *Anopheles* species were documented, of which *An. demeilloni* was the dominant one. Only *An. arabiensis* was positive for *P. falciparum* CSP. The overall *P. falciparum* CSP rate of *An. arabiensis* was 0.54%. The overall estimated *P. falciparum* EIR of *An. arabiensis* from PSC was 1.5 infectious bites/person/five months. Of the 145 freshly fed *Anopheles* mosquitoes tested for blood meal source, 57.9% (84/145) had bovine blood meal, 22 (15.2%) had human blood meal origin alone and 24 (16.5%) had mixed blood meal origins of human and bovine. *An. demeilloni* mainly fed on bovine blood (102/126 = 80.9%). Among 420 morphologically classified *An. demeilloni*, 11 (2.6%) were confirmed as *An. lessoni* (one of the *An. funestus* complexes) by PCR. A substantial number of morphologically classified *An. salbaii, An. maculipalpis* and *An. fuscivenosus* were found to be *An. arabiensis* by PCR. Regardless of the high diversity of *Anopheles* mosquitoes, *An. arabiensis* is playing the primary role in malaria transmission. Morphological misclassification of species could be a challenge in malaria mosquito monitoring and surveillance, and hence it should be supported by more sensitive techniques for confirmation.

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Keywords: *Anopheles arabiensis*; Blood meal index; Boreda district; Morphological misclassification

1. Background

Malaria is threating millions of life globally. According to the world malaria report 2020, an estimated 229 million cases of and 409 thousand of deaths occurred worldwide in 2019 [1]. Global malaria case incidence and deaths have been declined between 2015 and 2019. Although there was a reduction in number of malaria cases in 2019 than 2015, no significant progress has been documented [1]. A substantial increase of malaria has been documented in 2020 [2]. Africa accounted for about 94% of global cases. This region is also known by the most notorious malaria vectors [3]. Ethiopia is one of the malaria endemic countries and has contributed a substantial number of malaria cases in 2019. *Plasmodium falciparum* and *Plasmodium vivax* are the two dominant parasites in Ethiopia [2].

In Ethiopia, malaria is affecting millions of people in particular in the fertile lowland areas. Most parts of the country are favorable for malaria mosquitoes and parasite transmission. Malaria prevention and control strategies began in 1950s as pilot projects in Ethiopia [4]. Since then, vector control is a key preventive method for malaria. Currently, insecticide treated bed nets (ITNs) and indoor residual spraying (IRS) are the two main malaria intervention tools [5]. The effectiveness of these interventions, however, varied due to the vector species, and their behavioral responses to intervention tools. There are spatial and temporary variations in feeding and resting behaviors, and the species composition of malaria mosquitoes. For example, the principal malaria vector in a particular area could be a secondary vector in the other and vice versa. These clearly indicate the importance of continuous surveillance and monitoring of malaria mosquitoes in malaria control program [6].

Entomological information like vector species composition, their role in parasite transmission, feeding, and resting behaviors is very important. These data provide information about the intensity of malaria transmission and help in designing and implementing an appropriate intervention [6]. Moreover, information collected through entomological surveillance assist in understanding the effectiveness of malaria vector control tools and the spatial and temporal changes in vector species [7]. An accurate identification of the malaria mosquitoes also is crucial in vector control program. In this regard, morphological identification plays a key role in immediate documentation of the most important malaria vectors and implementing effective control tools [8]. There are however challenges in areas where several species are cohabiting and if there are species with some morphological similarity. In such areas misclassification could be a common phenomenon, and it could lead to incorrect decision in vector control program.

We used PSC and CDC light traps techniques to investigate the *Anopheles* mosquito species composition, blood feeding behavior patterns and their role in malaria transmission in two villages of Boreda district of Gamo zone, southwest Ethiopia. Molecular identification of *An. gambiae* and *An. funestus* complexes was done. Some species with morphological features

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confusing with *An. gambiae* or *An. funestus* complexes were confirmed by PCR using the complexes primers. This was done to understand the challenge related with morphological misclassification in entomological monitoring and surveillance.

2. Materials and methods

2.1. Description of the study area

This study was carried in the Kodo-Awsato Menuka and Zefine-Menuka *Kebeles* (villages: the lower administrative level) of Boreda district in Gamo zone (Figure 1). Boreda district is found in Gamo zone of South Nations Nationalities and Peoples' Region state (SNNPRs). It is found at the distance of 94 km from Arba Minch town, the capital of Gamo. It is bordered with Wolaita zone at the north, Mirab Abaya district at the south-east, Chencha district at the south-west and Kucha district at the west side of its location. Boreda district has 31villages. Using the 2007 CSA census, the district has an estimated population of 67,960. Of which 34,460 are men and 33,500 women. The district has both highland and lowland areas. The lowlands are malaria endemic. Kodo-Awsato Menuka and Zefine-Menuka villages are among the lowland villages of the district that located at about 16 km distance from the Zefine town, the central town of the district. There are about 270 households in Kodo-Awsato Menuka village and 390 in Zefine-Menuka village. The estimated population of Kodo-Awsato Menuka village is 1830 and Zefine-Menuka village is 2680.

Agriculture is the basic economic activity for the inhabitants. Malaria is one of the major health problems affecting the health of the people. This study was done in localities with high malaria transmission to generate entomological data which provide information on the local disease transmission pattern and dynamics. Though local evidences are crucial for decision making to improve the disease control strategy, there is no such study in the Boreda district.

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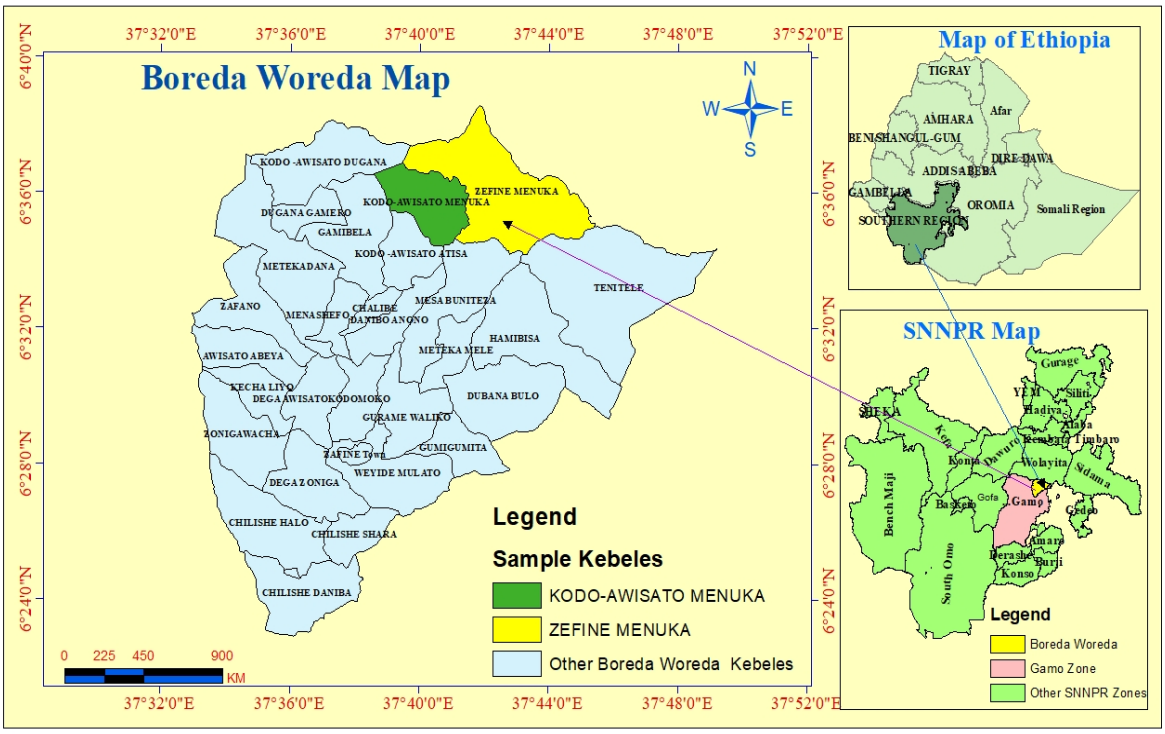

Figure 1: The map of the study area

2.2. Study design and mosquitoes sampling

An entomological sampling was done for five months from October 2019 to February 2020 using two entomological sampling techniques such as pyrethrum spray and CDC light traps.

2.2.1. Pyrethrum spray collection

PSC was employed to sample indoor resting adult *Anopheles* mosquitoes in selected households. Five houses were selected randomly from each study village for indoor resting mosquito collection. The selected households were visited twice monthly. Verbal consent was obtained from each household head before starting the mosquito sample collection.

Indoor resting collection was done in the morning from 6: 00 am to 8:00am. Roach killer aerosol was used for spraying the houses to knockdown indoor resting mosquitoes. The openings roofs and eaves were closed by a piece of cloth to prevent mosquitoes from escaping and the food items in the room were removed and heavy food storages were closed carefully before spraying the houses. Then, the floor and furniture were covered with a white sheet and the spray taken place by two persons one at the outside and the other inside the house starting at one side and moving the house circle. The house kept closed for 10 minutes and the knocked-down mosquitoes were collected from the white sheet. The mosquitoes then transferred to vials by using forceps and preserved with silica gel before traveling to Arba Minch University Medical Entomology Laboratory for further analysis. Morphological identification of female *Anopheles* mosquitoes was done using

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a standard key [9], and their abdominal stage was identified under a microscope.

2.2.2. CDC light traps collection

For CDC light trap collection, 10 households were selected randomly from each study village. Totally twenty households were randomly taken for CDC light traps collection. The CDC light traps were installed 45 cm above the feet of a person slept under the insecticide treated but several times washed mosquito net. The traps were hanged at 7:00 pm and collected at 7:00am in the next morning. All other people in the trapping room were instructed to sleep under the insecticide nets. The trap bags were tightened to prevent mosquitoes from escaping the bags. The mosquitoes then transferred to vials and preserved with silica gel. Female *Anopheles* mosquitoes were morphologically identified into species, and their abdominal stage was determined under a microscope. Then, the specimens were transported to Arba Minch University Medical Entomology Laboratory for further analysis.

2.3. Mosquito identification

The standard morphological key was used to identify female *Anopheles* mosquito species [9]. The sibling species of *An. gambiae* [10] and *An. funestus* [11] were further identified by PCR technique in Arba Minch University Medical Entomology laboratory. *Anopheles* species with some confusing morphological structures with *An. gambiae* or *An. funestus* were screened by PCR using the primers of *An. gambiae* and *An. funestus* complexes, respectively. *Anopheles gambiae* complex, *An. salbaii*, *An. stephensi* like, *An. dancalicus*, *An. maculipalpis* and *An. fuscivenosus* were tested for *An. gambiae* complexes, whereas *An. demeilloni* and *An. funestus* group were tested for *An. funestus* complexes. Legs and wings were used for molecular identification of the *Anopheles* species.

2.4. CSPs detection

Female *Anopheles* mosquitoes were processed for CSPs detection in the Arba Minch University Medical Entomological laboratory. The heads and thoraces of all the collected female *Anopheles* mosquitoes were used for detection of CSPs of *P. falciparum* and *P. vivax*_210 malaria parasites using Enzyme-Linked Immuno-Sorbent Assay (ELISA) [12]. Heads and thoraces were grinded in 50 µl blocking buffer (BB) by using plastic grinder. Then 100 µl BB was added twice to bring the final volume to 250 µl per mosquito. BB was removed from plates after 1 hr and 50 µl of each homogenized mosquito was added per plate and *P. falciparum* and *P. vivax*_210 positive sample and laboratory-colony of *An. arabiensis* were used as negative controls, respectively. After 2 hr incubation, plates were washed twice with PBS-Tween 20. Then Horseradish peroxidase (HRP)-conjugated monoclonal antibody was added to each plate and after one hour incubation, plates were washed 3 times with PBS-Tween 20. Finally, 100 µl of peroxidase substrate was added per well and incubated for 30 min. The plates were observed visually for green color and also their optical density determined at 414 nm in the microplate ELISA reader. Samples with green color and with optical

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density values of greater than two times the average optical density of the negative controls were considered as sporozoite positive.

2.5. Blood-meal origin assay

Freshly fed *Anopheles* mosquitoes from CDC light traps and PSC were examined for blood-meal origins determination with human and bovine antibodies by ELISA technique [13] in different microtitre plate wells. The abdomen of *Anopheles* mosquitoes was homogenized in 50 µl phosphate buffered saline (PBS) solution (pH 7.4), and diluted to a volume of 200 µl by PBS. Then, 50µlof sample was added to each well in a 96-well micro titre plate, and incubated overnight at room temperature. PBS containing a Tween-20solution was used to wash twice each well. Then, 50µlhost-specific conjugate (anti-human IgG and anti-bovine IgG) was added in each well of separate 96-microtitre plates and incubated for one hr. The wells were then washed three times by PBS-Tween-20 solution and finally 100µlof peroxidase was added to each well, and after30 minutes the absorbance of 405 nm was recorded with in ELISA plate reader (MRX Microplate Reader, Dynex Technologies, 20151-1683, USA). Human and bovine blood meals were used as a positive control, and unfed laboratory-reared *An. arabiensis* were used as a negative control. To record results as positive, the absorbance value should exceed the mean plus three times the standard deviation of four negative controls.

2.6. Data analysis and interpretation

The data are analyzed and categorized according to the similarities and differences of items and on the base of methods involved in the collection processes. All the data were entered to version 20 and analyzed using it. The entomological inoculation rate and sporozoite rate were calculated. Sporozote rate is the fraction of vector mosquitoes with *Plasmodium* sporozoite protein in their salivary glands. The EIR is calculated as the product of the human biting rate and the sporozoite rate [14]. The SR and human biting rate (HBR) were determined for PSC catches. For PSC based EIR, the standard EIR was calculated as (number of sporozoite positive ELISAs/number of mosquitoes tested) x Human biting rate (HBR) (which is number of freshly fed mosquitoes per total number of occupants in the collection room). Chi-square test was used to compare the blood meal sources of the mosquitoes.

2.7. Ethical approval

All the ethical considerations were recognized. Household heads were consented before carrying out mosquito collection. Household heads, and village and district authorities were informed about the objectives of the study.

3. Results

3.1. Anopheles species composition

A total of 680 *Anopheles* mosquitoes were collected by CDC light traps (550: 80.9%) and PSC (130; 19.1%). *Anopheles demeilloni* was predominantly collected species (61.7%) followed by *An. arabiensis* (28.8%). Thirteen

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Anopheles species were morphologically identified from CDC light traps collections, and ten were documented from PSC (Table 1).

Table 1. Morphologically identified *Anopheles* mosquitoes species collected by CDC light traps and PSC from villages in Boreda district, southwest Ethiopia (October 2019-February 2020).

	Collection methods			
Species	CDC	PSC	Total	%
An. demeilloni	355	65	420	61.7
An. gambiae complex	148	48	196	28.8
An. coustani	8	1	9	1.3
An. cinereus	3	1	4	0.6
An. dancalicus	1	0	1	0.14
An. funestus group	4	0	4	0.6
An. fuscivenosus	3	0	3	0.4
An. maculipalpis	4	1	5	0.7
An. salbaii	4	2	6	0.9
An. stephensi like	7	2	9	1.3
An. pretoriensis	7	2	9	1.3
An. rhodesiensis	1	0	1	0.14
An. tenebrosus	2	0	2	0.3
An. garnhami	0	4	4	0.6
An. longipalpis	0	1	1	0.14
Unidentified	3	3	6	0.9
	550	130	680	

3.2. Molecular identification of Anopheles species

A total of 644 *Anopheles* mosquitoes were tested for either *An. gambiae* or *An. funestus* complexes based on their morphological similarity (Table 2). There was morphological misclassification of *Anopheles* species. Among the 420 morphologically classified *An. demeilloni* and tested for *An. funestus* complex, 11 (2.6%) were *An. lessoni,* the species of *An. funestus* complex. Of the six morphologically identified *An. salbaii* tested for *An. gambiae* complexes, four were *An. arabiensis*. Also, *An. arabiensis* were morphologically misclassified as *An. maculipalpis* and *An. fuscivenosus*. None of the nine morphologically identified *An. stephensi* like species tested for *An. gambiae* complexes was positive. We preserved the DNA samples for further analysis like sequencing to identify the correct species.

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Of the 196 *An. gambiae* complex tested for sibling species, 183 (93.4%) were amplified for *An. arabiensis.* The rest were not amplified. *Anopheles lessoni* was the only species identified from *An. funestus* complexes.

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Table 2: Molecular identification of *Anopheles* mosquitoes collected from Boreda district of Gamo zone, southwest Ethiopia (October 2019-February 2020)

	PCR tested for *An. gambiae* complex			PCR tested for *An. funestus* complex			
Morphologically identified spp.	#Tested	#PCR positive	PCR identified spp.	Morphologically identified spp.	#tested	#PCR positive	PCR identified spp.
An. gambiae	196	183 (93.4%)	*An. arabiensis*	*An. demeilloni*	420	11 (2.6%)	*An. lessoni*
An. salbaii	6	4 (66.7%)	*An. arabiensis*	*An. funestus*	4	4 (100%)	*An. lessoni*
An. stephensi like	9	0	None				
An. dancalicus	1	0	None				
An. maculipalpis	5	2 (40%)	*An. arabiensis*				
An. fuscivenosus	3	1(33.3%)	*An. arabiensis*				
Total	220	190			424	15	

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3.3. Plasmodium CSP rates

A total of 680 female *Anopheles* mosquitoes (186 *An. arabiensis* and 494 other species) were tested for CSPs. Of which, a single *An. arabiensis* was found to be positive for *P. falciparum* CSPs. The overall *P. falciparum* CSP rate of *An. arabiensis* was 0.54% (1/186: 95% CI: 0.01-2.9). The CSP rate of *An. arabiensis* for PSC was 4.7% (1/21: 95% CI: 0.1-23.8).The overall CSP rate of *Anopheles* mosquito was 0.15% (1/680: 95% CI: 0.003-0.81).

3.4. Entomological inoculation rate

The EIR is obtained from the product of human biting rate (HBR) (which is number of freshly fed mosquitoes per total number of occupants in the room for PSC and the sporozoite rate (SR). The *P. falciparum* EIR of *An. arabiensis* was 1.5 ib/p/five months.

3.5. Blood meal sources

A total of 145 fresh fed *Anopheles* mosquitoes, 124 from CDC and 21 from PSC, comprising *An. demeilloni, An. arabiensis, An. lessoni* and *An. stephensi* were tested using ELISA for detection of their blood meal origins. Among the *Anopheles* mosquitoes tested for blood meal origins, 84 (57.9%; 95% CI: 49.5-66.1) had bovine blood origins. Only 22 (15.2%; 95% CI: 9.8-22.1) them fed on human blood meal alone, and about 24 (16.5%; 95% CI: 10.9-23.9) had mixed blood meal origins of both human and bovine (Table 3).

Anopheles demeilloni predominantly fed on the bovine blood meal origins, also showed statistical significance (Table 4). The BBI of *An. demeilloni* including the mixed blood meal origins was 80.9 (95% CI: 73.0-87.4), while the HBI including mixed blood meals was 28.6% (95% CI: 20.9-37.3). *Anopheles arabiensis* had blood meal sources of both human and bovine, though most of them had human blood origins than bovine, which was also statistically significant (Table 4). The HBI of *An. arabiensis* was 64.3% (95% CI: 35.1-87.2), while the BBI was 14.3% (95% CI: 1.8-42.8).

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Table 3: Blood meal origins of freshly fed *Anopheles* mosquitoes collected from Boreda district of Gamo zone, southwest Ethiopia southwest Ethiopia (October 2019-February 2020).

	CDC					PSC				
Anopheles species	# tested	Human	Bovine	Mixed	# not identified	# tested	Human	Bovine	Mixed	# not identified
An. demeilloni	109	10	70	21	8	17	3	9	2	3
An. arabiensis	10	6	2	0	2	4	3	0	0	1
An. lessoni	4	0	3	0	1	0	0	0	0	0
An. stephensi like	1	0	0	1	0	0	0	0	0	0
Total	124	16	75	22	11	21	6	9	2	4

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Table 4: Comparison of human (including mixed) and bovine blood meal origins (including mixed) of freshly fed *An. demeilloni* and *An. arabiensis* collected from Boreda district of Gamo zone, southwest Ethiopia southwest Ethiopia (October 2019-February 2020).

		$\chi 2$ value	p-value (2-tail)	Odds ratio (95% CI)
# *An. demeilloni* tested	126			
Human	36			ref
Bovine	102	57.0	<0.0001	6.7 (4.1-11.4)
Negative	11			
#*An. arabiensis* tested	15			
Human	11	7.9	0.002	12.5 (2.3-104.7)
Bovine	2			ref
Negative	4			

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4. Discussion

About 15 *Anopheles* species were morphologically identified in the study Boreda district of southwest Ethiopia. Though *An. demeilloni* was the dominant species, only *An. arabiensis* was found to be positive for *P. falciparum* CSPs. *Anopheles demeilloni* predominantly fed on the bovine blood meal origins. Unlike *An. demeilloni*, *An. arabiensis* had predominantly human blood meal origin than bovine. Morphological misclassification of *An. leesoni* (species of *An. funestus* complex) to *An. demeilloni*, and *An. arabiensis* (the *An. gambiae* complex) to *An. salbaii*, *An. dancalicus*, and *An. multicinctus* was documented.

There was high species diversity of malaria mosquitoes in the study district. Unlike many other studies in the region [15–17], *An. demeilloni* was the dominant species in the current study villages. Though dominance of *An. arabiensis* varied, its principal role in malaria is the same as documented in many other studies. ITNs and IRS have brought change in species composition and dynamics of malaria mosquitoes in many malaria endemic countries [18]; little or no change has been documented against *An. arabiensis*. After long implementation of vector control tools, *An. arabiensis* has maintained its position as a primary vector of malaria in many countries [18]. Controlling *An. arabiensis* using the existing vector control interventions could have little impact on malaria transmission and future malaria elimination plan, and hence, it could demand more intervention tools to target the species in different fronts in addition to the existing once [19]. Moreover, the predominance of *An. demeilloni* and the observed human-vector contact could require attention for further investigation to understand the role of this species.

From the total tested *Anopheles* mosquitoes for CSPs, *An. arabiensis* was found to be positive for *P. falciparum* CSP. The overall *P. falciparum* CSP rate of *An. arabiensis* was comparable with CSP rate from Sille village in 2006 [15] and Chano Mille in 2013 [17]. But, there is methodological difference, in current study entomological sampling was done by CDC light traps and PSC, while Taye et al. [15] done by HLC. The collection method employed by Massebo et al. [17] was similar with the current study. The findings of the current and previous studies have shown the primary role of *An. arabiensis* in malaria transmission in the country [16,17,20]. On the other hand, it is important to understand variation in CSP rate of *An. arabiensis* from place to place and the method of collection [20].

The EIR of *An. arbiensis* was calculated from PSC collection. Accordingly, the *P. falciparum* EIR of *An. arabiensis* was 1.5ib/p/five months and this indicated that the existence of active malaria transmission. Few studies have been attempted to estimate the EIR in Ethiopia followed similar mosquito sampling technique [17, 21]. Hence, it would be possible to compare and contrast the current result with other studies. This result is comparable with study in south-central Ethiopia [21] and southwest rift valley [17] and it implies that this species is playing a key role in malaria transmission in the region. The future malaria control program might focus on expanding the control toolbox to address the challenge related with *An. arabiensis* [22]. This species has an opportunistic and flexible feeding and resting behaviors which could challenge the future control program [23].

Blood meal origins test result of current study showed that most of *An. demeilloni* displayed the tendency to feed on bovine blood meal source, while *An. arabiensis* showed slight tendency to feed on human blood meal sources. Similar study confirmed the tendency of *An. demeilloni* to bovine blood meal source [24]. In the current study, *An. arabiensis* exhibited a tendency to feed on human blood than bovine blood and this is a bit different from other studies that documented bovine blood feeding tendency of *An. arabiensis* [24, 25]. Several studies have indicated opportunistic feeding behavior of *An. arabiensis*, it is mainly determined by the accessibility of the hosts [26, 27].

There was morphological misclassification of *Anopheles* mosquitoes. Species with some morphologically similar structures with *An. gambiae* or *An. funestus* complexes were molecularly identified. Among the *An. salbaii*, *An. dancalicus*, *An. maculipalpis* and *An. fuscivenosus* morphologically identified, a substantial number were *An. arabiensis*.

Similarly, a substantial number of *An. lessoni* (member of *An. funestus* complex) morphologically identified as *An. demeilloni.* This implies that morphological identification alone may result in wrong speciation of *Anopheles* mosquitoes due to the morphological similarity of some species and this in turn might affect malaria control and elimination program. It might contribute for wrong documentation of *Anopheles* species that also leads to wrong designing of intervention strategy. This might contribute for failure of vector control. Though the molecular technique is expensive and time consuming than a morphological technique, the correct identification of the species using molecular technique might benefit the control program [28]. Morphological identification of the species might be more difficult if specimens have lost important external features [8]. Moreover, the skill of the entomology personnel affects the morphological identification of the species. Therefore, it is vital to do molecular assays in conjunction with morphological identification to minimize the problem related with misidentification.

5. Conclusions and recommendations

The current study revealed the existence of the varieties of *Anopheles* species identified by morphological and molecular methods. The dominant *Anopheles* species was *An. demeilloni* followed by *An. arabiensis. Anopheles arabiensis* was the only vector of malaria in the area which found to be positive for *P. falciparum* CSPs. According to the current blood meal sources analysis, most of the tested *Anopheles* mosquitoes displayed the tendency to feed on bovine blood meal origin. Morphological misidentification of the *Anopheles* species displaying some confusing morphological similarity with *An. gambiae* and *An. funestus* complexes was documented and corrected by a molecular test. Using molecular method is important for correct identification of *Anopheles* species and it could help to apply the appropriate vector intervention in malaria control.

Supplementary Materials: none

Author Contributions: Conceptualization: A.A., N.E., F.M.; Methodology: A.A., N.E., F.M.; Validation: N.E., FM.; Formal analysis: A.A., F.M.; Investigation: A.A.; Writing-original draft: F.M.; Writing-review and editing: A.A., N.E., F.M.; Funding acquisition: F.M.

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Informed consent statement: This study was reviewed and ethically approved. We obtained consent from household heads before starting mosquito collection.

Data availability statement: All available data are included in this manuscript

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Conflicts of interest: All authors declared that there is no conflict of interest. The funder has no role in this study design, data collection and other activities.

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