Bionomics, seasonal dynamics and insecticide susceptibility of Anopheles mosquitoes in low and high malaria transmission settings of Ethiopia

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Research Article

Keywords: /phrases: Anopheles mosquitoes, Bionomics, Ethiopia, Insecticide resistance Primary Vector, Secondary vectors, Suspected vector

Posted Date: September 8th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3307406/v1

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Abstract

Background

In Ethiopia, malaria is highly seasonal with varying intensity of transmission due to altitudinal and climatic variations. Information on principal, secondary and suspected malaria vector species composition, their seasonal dynamics and/or abundance, bionomics and insecticide susceptibility status at locality levels are important to understand malaria transmission in a particular eco-epidemiological setting in order to design effective vector control strategy.

Methods

Adult female *Anopheles* mosquitoes were collected from Lare district of Gambella Regional State and Jimma Zone of Oromia Regional State, Ethiopia. Mosquitoes were collected using CDC light traps and human landing catches (HLC) for 17 months between June 2017 to October 2020. All the collected *Anopheles* mosquitoes were morphologically identified and properly preserved for further analysis. Larvae and pupae of *Anopheles gambiae* complex, *An. pharoensis* and *An. coustani* were collected from breeding sites and reared to adult under field condition in the field insectary. Bioassays were conducted on non-blood-fed, 3–5 days old adult female mosquitoes emerged from larvae and pupae using discriminating concentrations of WHO insecticide impregnated papers. Randomly selected sub-sample of the morphologically identified *An. coustani* was molecularly identified via Sanger sequencing (COI and COII mitochondrial gene determination). TaqMan qPCR assay was employed to investigate *Plasmodium* parasite infection in *An. pharoensis* and *An. coustani*. Differences in mean *Anopheles* mosquito density between outdoor and indoor catches by HLC were compared using t-test. Mean difference among trapping methods and mosquito density were evaluated using analysis of variance (ANOVA) and Tukey's Post-hoc test. Pearson correlation was used to assess the association between the overall *Anopheles* mosquito density from Lare district with climatic factors such as rain fall and temperature. Duncan post-hoc test was employed to compare mean mortality rate of mosquitoes exposed to different insecticides. Data were analyzed using IBM SPSS statistics for windows v.20.0 and values were considered significant at p < 0.05.

Results

A total of 11,876 *Anopheles* mosquitoes belonging to four species were collected using CDC and HLC methods during the study period. *An. coustani* was by far the most abundant species representing 47.84% (5681/11876) of the total collections and 96.2% of it was sampled using CDC traps. Other *Anopheles* mosquitoes collected were *An. phraoensis* (26.90%, n = 3195), *An. gambiae* complex (16.07%, n = 1909) and *An. funestus* (9.19%, n = 1091). While all the four *Anopheles* species were identified from Lare district, *An. funestus* was not recorded from Jimma zone study sites. The majority (74.22%, n = 8814) of *Anopheles* were collected using CDC light traps (73 trap nights) while the remaining (25.78%, n = 3062) were captured by HLC (288 person-nights). The mean monthly density of female *Anopheles* collected by HLC was 5.17 (95% CI 0.67–9.66) mosquitoes per-person-night while the corresponding mean monthly mosquito density by CDC LT
was 17.37 mosquitoes (95% CI 3.15–31.59) per trap-night. Of all collected *Anopheles* mosquitoes by HLC, the number of *Anopheles* mosquitoes captured outdoor was higher by 1.36 times than the corresponding indoor capture. There was no direct association between *Anopheles* abundance and rain fall and or mean monthly maximum and minimum temperatures in Lare district. *Anopheles pharoensis, An. gambiae s.l* and *An. coustani* complex were susceptible to Primiphose methyl (organophosphate) and Propoxur and bendiocarb (carbamate). However, *Anopheles pharoensis* and *An. gambiae s.l* were resistant to DDT, deltamethrin, permethrin and malathion. On the other hand, *An. coustani* complex were resistant to only DDT. Sub-samples of morphologically identified as *An. coustani* were molecularly confirmed to belong to the *An. coustani*. No *Plasmodium* infection was detected in *An. coustani* and *An. pharoensis*.

**Conclusion**

*Anopheles coustani* was the most abundant species in this study. High vector density was recorded following rainy seasons. Relatively higher density of the principal vector, *An. gambiae s.l* was captured using CDC during October-November 2017, June 2017 and September 2018 following the heavy rainy months (June-August) and/or beginning of the long rains (June). There was higher mean mosquito density outdoor than indoor using HLC. Multiple insecticides resistance was observed in both the principal and secondary malaria vector populations in both Lare and Jimma Zone study sites of Ethiopia. Sub-samples of all the morphologically identified *Anopheles coustani* mosquitoes were also confirmed by molecular methods.

**Background**

Though there was a significant (27%) decline in global malaria case incidence between 2000 and 2015, there was a decline only by 2% between 2015 and 2019, indicating a slowing rate of decline in the latter years. Africa, with an estimated 215 million cases in 2019 accounted 94% cases. The World Health Organization [1] has also indicated that there were fewer malaria cases in 2000 (204 million) than in 2019 in the WHO African Region. However, there were an estimated 247 million malaria cases in 2021 globally, increasing malaria cases from 227 million in 2019, with most of the increase coming from countries in the WHO African Region [2]. Similarly, the population living in the WHO African Region increased from about 665 million in 2000 to 1.1 billion in 2019 [1], reflecting the complexity of interpreting changing disease transmission magnitude in a rapidly increasing human population.

Malaria transmission is complicated and exact case prediction looks difficult, necessitating regular surveillance and intervention to curb the disease burden. Due research attention to principal as well as secondary and suspected malaria vectors is of paramount importance to achieve proper designing and control of the disease. Information on principal, secondary and suspected malaria vector fauna, behavior, ecology, dynamics species composition, insecticide susceptibility level, vectorial capacity and competence with the changing environmental conditions such as rain fall, temperature, humidity and other environmental factors over years at locality level are of paramount importance for comprehensive understanding and designing of effective vector control strategy.
Though secondary malaria vectors are considered to play minor roles in malaria transmission [3], their potential vectorial capacity and competence vary according to locality and season [4]. However, studies on the role of secondary and suspected vectors are limited or entirely missing in Ethiopia, possibly due to the extremely low infection rate (IR) or because no infections were reported [5–7].

In Ethiopia, malaria is highly seasonal with varying intensity of transmission due to altitudinal and climatic variations. About 75% of the total area of Ethiopia is malarious [8, 9] and over 60% of the country’s population is estimated to be at risk of the disease [10, 11].

Ethiopia aimed to achieve nationwide malaria elimination by 2030 [12]. However, due to complex and dynamic behavior of both principal and secondary malaria vectors, insecticide resistance and emergence of invasive vector coupled with other anthropogenic factors, it seems difficult to achieve the elimination goal by the country. The role of secondary and/or suspected malaria vectors in malaria transmission in Ethiopia needs to be studied along with the principal malaria vector. There is also paucity of information on the bionomics, seasonal abundance, insecticide susceptibility status, and vectorial capacity of secondary and suspected vectors in Ethiopia.

Identification of malaria vectors is necessary for both basic and operational researches in addition to its importance in designing proper malaria control strategy. Malaria vector species identification using molecular method is very important as many malaria vectors are indistinguishable morphologically [13].

Over 47 Anopheles mosquito species have been documented in Ethiopia [14]. Of which An. arabiensis is the main malaria vector while An. pharoensis, An. funestus and An. nili play a role as secondary vectors [11]. Recently, an invasive malaria vector An. stephensi has also been detected in eastern, north eastern, central, north western and southwestern Ethiopia [15, 16, FMoH unpublished data] and expanding its distribution to other parts of the country. Anopheles coustani is also widely distributed in Ethiopia though its role in transmission was unclear so far in the country [11]. However, An. coustani is considered as secondary malaria vector in other sub-Saharan African countries [3]. Artificial infection experiment using membrane feeding assay showed the Ethiopian populations of An. coustani is susceptible to both Plasmodium falciparum and P. vivax [7].

Secondary malaria vectors are recognized for their importance in malaria transmission, as they may augment or extend the malaria transmission period and potentially sustain malaria transmission after the main indoor resting and indoor biting vectors have been reduced by vector control measures such as indoor residual spraying or long-lasting insecticidal nets (LLINs) [3].

Vector control is one of the pillar strategies implemented in Ethiopia to reduce malaria burden. Therefore, understanding malaria vector species composition, seasonal dynamics and/or abundance, bionomics and insecticide susceptibility status at local levels is among the important information to better understand malaria transmission pattern in a particular setting in order to design optimal vector control strategy aimed at reducing the disease [17]. Determining mosquito density together with other entomological indices such as infection rate and entomological inoculation rate (EIR) are crucial indicators of malaria transmission intensity [18]. Insecticide susceptibility test is also another indicator of insecticide-based vector control effectiveness.
Generating evidence on malaria vector bionomics such as species composition, local spatial and seasonal distribution and dynamics, feeding and resting preference, vectorial capacity and resistance to insecticides are of paramount importance for malaria control [19–21]. Accordingly, investigation of malaria vector species composition, density, insecticide susceptibility status and bionomics across time and space are of significance importance in designing and implementing effective vector control strategy.

Hence, this study aimed at investigating fauna, ecology, behavior, abundance, dynamics and insecticide susceptibility status of *Anopheles* mosquitoes in low malaria transmission Zone of Jimma and high transmission area in Lare district in southwest Ethiopia.

**Methods**

The study was conducted in Jimma zone of Oromia Regional State and Lare district of Gambella Regional State in south western Ethiopia. Jimma is one of the zones of the Oromia region and has an agro-ecological setting of highland (15%), midland (67%) and lowland (18%). The zone is one of the major coffee growing areas of Oromia region, well endowed with natural resources contributing significantly to the national economy of the country. Teff and honey production are another sources of cash after coffee. Jimma Zone also receives good rains, ranging from 1,200–2,800 mm per annum. In most cases, the rainy season extends from February to October. The mean annual temperature approximately ranges between 14°C and 24°C. Gilgel Gibe Hydroelectric Dam (GGHD) is located 260 km south-west of Addis Ababa, is also found in Jimma zone. Jimma town, the capital of the zone is located 354 km southwest of Addis Ababa, Oromia regional state. Jimma lies to the west of the Great Rift Valley at an altitude between 1300 m and 2100 m. It is located between 7°15′ to 8°45′ N and 35°030′ to and 37°030′ E. Jimma town is characterized by warm and humid climate.

Gambella Regional State is one of the eleven (11) Regional States of Ethiopia. The Region is situated between Baro and Akobo rivers. There is also Gambella National Park in the Region, which is a notable landmark, approximately covering 17% of the Region's territory on south of the Baro. Lare is one of woredas (districts) in the Nuer Zone of Gambella Regional State. It is bordered by Anuak Zone on the south and east, on the west by the Baro River which separates it from Jikawo river, and on the north by the Jikawo river which separates it from South Sudan. The terrain in Lare consists of marshes and grasslands; elevations range from 300 to 400 m.a.s.l.

Mosquito samples for this study were collected from Lare district of Gambella and two sites of Jimma zone named Bacho Booree and Asendabo areas of southwest Ethiopia (Fig. 1). Both study sites are known to be malaria endemic and Lare is a high malaria transmission area while the Jimma sites are relatively low to moderate malaria transmission areas. Lare is located southwestern tip of the Ethiopia at about 780 km from the capital, Addis Ababa while Asendabo and Bacho Booree are at 299 km and 352 km respectively, from AA, towards southwest Ethiopia. Bacho Booree is just near to Jimma town, the capital of the zone while Asendabo is 55 km from Jimma town on the way to Addis Ababa. Asendabo is a site that is located in Omo Nada woreda (district). Omo Nada is one of the woredas in the Oromia region of Ethiopia. Part of the Jimma Zone, Omo Nada is bordered on the south by the Gojeb River, which separates it from the Southern Nations, Nationalities and Peoples Region (SNNPR), on the west by Dedo, on the northwest by Kersa, on the north by
Tiro Afeta, on the northeast by Sokoru, and on the east by the Omo River which separates it from the Southern Nations, Nationalities and Peoples’ Region (SNNPR).

**Ethical consideration**

An institutional ethical clearance with Ref. No. IHRPGD/787/2019 was obtained from Institutional Review Board of Institution of Health, Jimma University. Free and informed written consent was obtained from trained volunteers who participated in mosquito collections. Head of families were requested through individual discussions and community meetings, prior to the enrolment of their house in the study. Permission was requested and obtained from inhabitants to perform mosquito collections in and outside their rooms in mosquito collection areas.

**Mosquito sampling**

Adult female *Anopheles* mosquito collection was conducted from Lare site of Gambella Regional State and two sites of Jimma Zone (Asendabo and Bacho Booree) in Oromia Regional State. The mosquito samples were collected between June 2017 to March 2019 using CDC light traps (BioQuip Model 2836BQ, USA) and human landing catches (HLC). Sample collection was conducted using CDC light traps from eight randomly selected houses, each house being surveyed for two consecutive nights during each of the surveyed months. The survey was carried out for 17 months between June 2017 to October 2020 from eight randomly selected mud constructed with thatched roof animals shades in Lare District of Gambella Regional State. Similarly, eight houses were randomly selected in Jimma Zone of Oromia Regional State, four houses from each of Asendabo and Bacho Booree study sites of the zone. The selected houses from Jimma zone were constructed with wooden walls plastered with mud and thatched roofs or corrugated iron. Lare district of Gambella Regional State was included for mosquito sampling throughout the 17 months of surveying periods while the two study sites of Jimma Zone were considered only over three months (two from Bacho Booree and once from Asendabo) during the study period. The CDC light traps were suspended approximately about 1.5 m above the floor. Traps were switched on at 18:00 PM and switched off at 6:00 AM. Traps were collected from each house every morning at 6:00 AM and mosquitoes were killed using cotton wool treated with chloroform. Mosquito collection was also conducted using human landing catches (HLC) from eight randomly selected houses from Lare and Asendabo for nine months between November 2018 to October 2020. The HLC was performed both indoor and outdoor for two consecutive nights per house during each surveyed month. There were two teams of eight trained collectors who were consented for the HLC operation. Four collectors performed the collection indoor and four outdoor. The HLC was performed between 6:00 PM and 6:00 AM. The period of collection was divided into 6-hour segments; thus, two of the four trained volunteers for indoor for 6-hour collection as well as outdoor collectors were worked for six hours (6:00 PM to mid-night) and sleep six hours alternatively while two of the remaining performed collections from mid-night to 6:00 AM. The team members conducted the collection every night and hence, HLC was operated for two nights per house every month of the study period, making 32 person-nights (16 outdoor and 16 indoor) each month, making 288 person-nights during the 9 months of the study period. The collectors put long-sleeved shirts during the collections to prevent mosquitoes landing and biting on arms [22]. The collectors were seated on stools with their legs exposed from foot to knee to capture mosquitoes using a flashlight and mouth aspirator as soon as they land on the exposed legs before they commence blood-feeding [23, 24]. The
collectors were given chemoprophylaxis (Mefloquine) as per the National malaria diagnosis and treatment guidelines [25].

**Mosquito Identification**

All the collected *Anopheles* mosquitoes using the CDC traps and HLC were sorted by sex and morphologically identified into species using identification keys [26]. The mosquitoes were then individually stored over silica gel in 1.5 µl Eppendorf tubes, labeled and transported to Tropical and Infectious Diseases Research Center (TIDRC) laboratory, Jimma University and stored at -20°C until further processed. Molecular identification of *An. coustani* complex samples was conducted in Greece at the Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology (IMMB, FORTH).

**Insecticide Bioassay**

Mosquito larvae and pupae were collected by dipping from different breeding areas of all the study sites and reared to adult under field condition with mean temperature 28 ± 1 °C, 24.5 °C and mean % relative-humidity of 72 ± 2, 61 ± 1 at Lare and Jimma study sites, respectively. Non blood-fed adult female mosquitoes (3–5 day-old) emerged from larvae and pupae were exposed to insecticide impregnated papers with discriminating concentrations of DDT (4%), deltamethrin (0.05%), malathion (5%), permethrin (0.75%), pirimiphosmethyl (0.25%), propoxur (0.1%), and bendiocarb (0.1%), following WHO insecticide susceptibility test procedure [27]. Insecticide impregnated papers were obtained from the WHO Collaboration Centre, Vector Control Research Unit, School of Biological Sciences, Penang, Malaysia. Batches of 25 mosquitoes in four replicates were exposed to insecticide impregnated papers for 1 h in WHO test tubes for all bioassays and knockdown was recorded at 60 min [27]. Controls in two replicates, each with equal number (25) of mosquitoes were exposed to untreated papers in parallel. After the exposure period, mosquitoes were transferred into holding tubes and provided with 10% sucrose solution soaked into cotton pads. Mortality was recorded 24 h post exposure.

**Genomic DNA extraction from mosquitoes**

Genomic DNA was extracted from samples of 16 randomly selected *An. coustani* complex. The quantity and purity of DNA and total RNA were assessed spectrophotometrically via Nanodrop measurements. The quality of DNA was assessed by 1.0% w/v agarose gel electrophoresis.

**Molecular identification of mosquitoes**

Species identification of *An. coustani* complex was conducted via direct Sanger sequencing of mitochondrial genes. The mitochondrial genes COI and COII were sequenced in *An. coustani* samples after cleaning-up the PCR products [28, 29]. The detailed protocol and primers used is presented in Table 1.

**Detection of Plasmodium infection**

The heads and thoraces of 266 (144 *An. coustani* and 122 *An. pharoensis*) mosquito samples were dissected and analyzed with TaqMan qPCR assay developed by [30] to detect *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae* as per protocol and primers described in Table 1.
Table 1  
<table>
<thead>
<tr>
<th>Assay</th>
<th>Primers/Probes 5'-3'</th>
<th>Reaction Protocol</th>
<th>Thermal Protocol</th>
<th>PCR product</th>
</tr>
</thead>
<tbody>
<tr>
<td>COI (Species ID)</td>
<td>F: GGA GGA TTT GGA AAT TGA TTA GTT CC</td>
<td>Kapa Taq</td>
<td>95°C/3min x 1 cycle</td>
<td>700 bp</td>
</tr>
<tr>
<td></td>
<td>R: GCT AAT CAT CTA AAA ATT TTA ATT CC</td>
<td>[2.5 mM MgCl2]</td>
<td>(95°C/30sec, 55°C/30sec, 72°C/45sec) x 35 cycles; 72°C/10min x 1 cycle</td>
<td>[run 10 uL, keep the rest for sequencing]</td>
</tr>
<tr>
<td>COII (Species ID)</td>
<td>F: TCT AAT ATG GGA GAT TAG TGC</td>
<td>Kapa Taq</td>
<td>95°C/3min x 1 cycle</td>
<td>730 bp</td>
</tr>
<tr>
<td></td>
<td>R: ACT TGC TTT CAG TCA TCT AAT G</td>
<td>[2.5 mM MgCl2]</td>
<td>(95°C/30sec, 55°C/30sec, 72°C/45sec) x 35 cycles; 72°C/10min x 1 cycle</td>
<td>[run 10 uL, keep the rest for sequencing]</td>
</tr>
<tr>
<td>Plasmodium detection</td>
<td>F: GCTTAGTTACGATATAGGAGTAGGTTG</td>
<td>The TaqMan reactions were performed in a 10 uL final volume containing 1x TaqMan universal master mix (Applied Biosystems), 800 nM of each primer and 300 nM of each probe.</td>
<td>Cycling conditions were: 10 min at 95°C, 45 cycles of 15 s at 92°C and 1 min at 60°C.</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>R: GAAAATCTAGAGATTTACCTCTGACA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P: Falcip+ (FAM-TCTGAATACGAATGTC-GMB)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P: OVM+ (HEX-CTGAATACAGATGCC-GMB')</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data analysis

Mosquito abundance and species of *Anopheles* mosquitoes in different collection methods and sites were expressed with descriptive statistics using percentages. The monthly average density of *Anopheles* mosquitoes using CDC and HLC were expressed as the total number of female *Anopheles* mosquitoes collected per month divided by overall total monthly catches for each sampling method during the specific collection month. Mean *Anopheles* mosquitoes Density (D) = (number of females of each mosquito species ÷ number of houses) ÷ number of nights. Differences in mean density of *Anopheles* mosquitoes between outdoor and indoor catches using HLC were compared using t-test. Differences among the sampling methods (HLC and CDC and mosquito species were evaluated by analysis of variance (ANOVA) and Tukey’s Post-hoc test. Correlation analysis was used to highlight association between the overall seasonal *Anopheles* mosquito density with rain fall and temperature using one-way ANOVA. Duncan multiple comparison test was
employed to compare mean mortality rate of mosquitoes exposed to different insecticides. Data were first log transformed for normality and analyzed using IBM SPSS statistics for windows v.20.0 (IBM Corp, Armonk, NY, USA). In all tests, values were considered significant at p < 0.05.

Results

Species composition and bionomics of Anopheles mosquitoes

A total of 11,876 Anopheles mosquitoes belonging to four species were collected using the two sampling methods during the study period. Anopheles coustani was by far the most abundant representing 47.84% (5681/11876) in the collections conducted between October 2017 and September 2020 from the study sites of Ethiopia (Table 2). The other Anopheles mosquitoes were An. phraeensis (26.90%, n = 3195), An. gambiae complex (16.07%, n = 1909) and An. funestus (9.19%, n = 1091). While all the four species were identified from Lare district, An. funestus was not recorded from both study sites of Jimma zone. Of the 11,876 total Anopheles mosquitoes collected the majority (74.22%, n = 8809) were collected using CDC light traps (73 trap nights) while the remaining (25.78%, n = 3062) were captured by HLC (288 person-nights).

Relatively high number of principal vector, An. gambiae s.l. was captured using CDC light traps during Oct.-Nov. and June of 2017 as well as in Sept. of 2018 after the rainy months in the area and/or early rainy month (June).

An. pharoensis, which is the secondary malaria vector in Ethiopia was captured in relatively large number during October (2017), June (2017 & 2018) and September (2018), making about 19.27% (1558/8084) of total mosquito vectors captured using CDC light traps in Lare from animal shades. Of 5681 An. coustani complex collected during the study period, 5465 (96.2%) captured using CDC light traps, of which 4892 (86.11%) were from Lare District. On the other hand, the capture of An funestus was relatively smaller (9.19%) from animal shades as depicted in Table 2. Moreover, An. funestus appeared relatively in large number during dry seasons through November-December-January after the peak period of the principal malaria vector in Ethiopia though the result was not consistent in different years of the study period.
Table 2
Anopheles mosquito species composition and abundance by collection method and site, southwest Ethiopia

<table>
<thead>
<tr>
<th>Anopheles species</th>
<th>Site</th>
<th>CDC</th>
<th>HLC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>An. coustani</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lare</td>
<td>4892</td>
<td>86.11</td>
<td>211</td>
<td>3.71</td>
</tr>
<tr>
<td>Jimma</td>
<td>573</td>
<td>10.09</td>
<td>5</td>
<td>0.09</td>
</tr>
<tr>
<td>Total</td>
<td>5465</td>
<td>96.20</td>
<td>216</td>
<td>3.80</td>
</tr>
<tr>
<td>An. pharoensis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lare</td>
<td>1558</td>
<td>48.76</td>
<td>1524</td>
<td>47.70</td>
</tr>
<tr>
<td>Jimma</td>
<td>113</td>
<td>3.54</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>1671</td>
<td>52.30</td>
<td>1524</td>
<td>47.70</td>
</tr>
<tr>
<td>An. gambiae s.l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lare</td>
<td>1218</td>
<td>63.80</td>
<td>554</td>
<td>29.02</td>
</tr>
<tr>
<td>Jimma</td>
<td>44</td>
<td>2.30</td>
<td>93</td>
<td>4.87</td>
</tr>
<tr>
<td>Total</td>
<td>1262</td>
<td>66.11</td>
<td>647</td>
<td>33.89</td>
</tr>
<tr>
<td>An. funestus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lare</td>
<td>416</td>
<td>38.13</td>
<td>675</td>
<td>61.87</td>
</tr>
<tr>
<td>Jimma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>416</td>
<td>38.13</td>
<td>675</td>
<td>61.87</td>
</tr>
<tr>
<td>Total</td>
<td>8814</td>
<td>74.22</td>
<td>3062</td>
<td>25.78</td>
</tr>
</tbody>
</table>

Of the 3062 Anopheles mosquitoes sampled using HLC, 57.54% (n = 1762) were captured outdoor while the remaining 42.46% (n = 1300) were from indoor. However, there was no significant difference between mean mosquito density caught outdoor and indoor (df = 19, p > 0.05). The largest HLC captured anopheles mosquitos in this study were An. pharoensis (n = 1524), followed by An. funestus (n = 675) while the least number of HLC captured Anopheles mosquitoes were An. coustani (n = 216).

The mean Anopheles mosquito catches per trap night and per person-night is presented in Fig. 2 for each species. The mean monthly density of female Anopheles collected by HLC was 5.17 (95% CI 0.67–9.66) mosquitoes per man per night while the corresponding value of CDC per trap night was 17.37 (95% CI 3.15–31.59).

Weather stations recorded temperature and rainfall data from Lare district throughout the study period except lack of rain fall data during July 2020-September 2020 where rain fall data was not recorded. Overall, there were an increasing number of Anopheles specimens caught during the rainy season (except for An. funestus) as compared to the dry season—with the most significant increase being immediately after the rainy seasons (Fig. 3A). However, statistical analysis using a Pearson correlation test demonstrates that mean monthly rainfall (MMRF) did not show a significant correlation with the mosquito density in Lare (r = 0.140, p = 0.591). Mean monthly temperature ranged between lows of 21.40°C and highs of 39.65°C fluctuations during the study period in Lare District. Statistical analyses (Pearson) demonstrated there was no strong correlation
between mean montly minimum temperature, MMINT \( r = 0.147, p = 0.573 \) as well as mean monthly maximum temperature, MMAXT \( r = 0.353, p = 0.165 \) and mean of *Anopheles* mosquitodensity in Lare District (Fig. 3B).

All of the three species of *Anopheles* mosquitoes tested against multiple WHO insecticides were found to be susceptible to baseline level concentrations of two classes of three insecticides (Fig. 4). Accordingly, *Anopheles pharoensis*, *An. gambiae* s.l and *An. coustani* complex were susceptible to Organophosphate (Primiphose methyl) and Carbametes (Propoxur and Bendiocarp) as per WHO-specified thresholds for resistance determination [31]. However, *Anopheles pharoensis* and *An. gambiae* s.l mosquitoes were phenotypically resistant to three classes of insecticides at baseline level: DDT, deltamethrin and permethrin and malathion. On the other hand, *An. coustani* complex was phenotypically resistant to only DDT.

**Mosquito molecular Identification and Detection of parasite infection**

All the sub-samples that were morphologically categorized as *An. coustani* complex were confirmed to belong to the *An. coustani* species. None of the 266 samples of *Anopheles* mosquitoes analyzed with TaqMan qPCR were found positive for the infective (sporozoite) stage *Plasmodium* detection (Fig. 5).

**Discussions**

Malaria vector control is one of the key components of malaria control strategy. In 2017, the Ministry of Health (MOH) of Federal Democratic Republic of Ethiopia developed national malaria elimination roadmap and set the goal to eliminate malaria by 2030 [12]. However, the development of insecticide resistance by malaria vectors, vector behaviour change as well as emergence and spread of exotic invasive vector *An. stephensi* seem to make the elimination goal unlikely. In the current study, three species of *Anopheles* mosquitoes: *An. gambiae* s.l, *An. pharoensis* and *An. coustani*, showed resistance and/or possible resistance to melathion, deltamethrin, permethrin, perimethrin and DDT and they were susceptible to bendiocarb, pirimiphosmethyl and propoxur as per. Study from Tanzania [32] reported that *An. funestus* and *An. arabiensis* were resistant to the pyrethroids (permethrin and deltamethrin) and organochloride (DDT), but susceptible to the organophosphate (pirimiphosmethyl). The study also confirmed susceptibility of these species to carbamates (bendiocarb). Such resistance might be due to selection pressure as a result of increased ITN coverage [33]. Although the overall density of *An. coustani* was higher than the other *Anopheles* mosquito species in the current study, the role of this vector in malaria transmission has not yet been well investigated and documented as it is considered as suspected vector in Ethiopia. The higher density of *An. coustani* in this study contrasts with previous report from Ethiopia and Tanzania where *An. arabiensis* was the highest [5, 34] and from Kenya [33] in which *An. arabiensis* and *An. funestus* density was higher. The differences could be attributed to different factors like study site and setting difference where marshy type environment is found in the nearby of Lare study site. Moreover, most of the *An. coustani* were collected from animal shelters in the current study using CDC light traps suggesting, their zoophilic behaviour.

Human landing catch (HLC) is the standard and reliable method to study vector bionomics, as this technique is focused on host-seeking mosquitoes which may represent the most relevant proportion of the mosquito
population for disease transmission [35, 36] and hence enables to assess human exposure to *Anopheles* biting [37]. In the present study, largest proportion of mosquito species captured using HLC was *An. pharoensis*, followed by *An. funestus* while the smallest proportion of *Anopheles* mosquito species captured using HLC was *An. coustani*. The majority of *Anopheles* mosquitoes were collected outdoor than indoor using HLC.

A higher proportion of *Anopheles* mosquitoes was collection from outdoor compared to indoor using HLC in the current study is in line with other studies [33, 38, 39] where predominantly indoor transmission is replaced by greatly lowered residual transmission while a greater proportion of residual transmission occurred outdoors as a result of LLINs and IRS that target endophilic, endophagic and anthropophilic vectors. A study in 2020 on ownership and use of long-lasting insecticidal nets in Ethiopia confirmed that Gambella Regional State was the highest (87.9%) in net utilization in the country [40]. This could be attributed to the higher number of malaria vectors collected outdoors in Larae district of the region. However, this study did not confirm whether such local vector populations show such feeding and resting behaviour or it is in response to high coverage and prolonged utilization of IRS and ITNs in the area.

Such behaviour of outdoor biting and/or resting behavior of the vectors in the study area could pose challenge to the anticipated malaria elimination goal as the use of ITNs and IRS are still the major vector control strategies currently used in the study areas and in Ethiopia in general. In another study, similar exophagic behaviour of *Anopheles* vectors may be a challenge to control using indoor-based vector control interventions (IRS and LLINs). Hence, this calls for developing and implementing new or alternative innovative vector control tools that targets outdoor feeding and resting malaria vectors. It is common that typically only very few (about 1%) mosquitoes may be infected with infective stage parasites (sporozoite) parasites, indicating that very large mosquito samples should be collected and tested to accurately quantify the mosquito infection rates [41]. Furthermore, the fact that none of the 266 (144 *An. coustani*, and 122 *An. pharoensis*) samples to be positive for infective stage parasite does not conclusively confirm the absence of parasite as the occyst stage was not investigated in the current study, which may later develop to sporozoite from occyst-infected mosquitoes. Therefore, locality and time-specific estimates of mosquito infection rates considering sizable proportion of vector population need to be regularly assessed for better understanding of infection status and effective control of the disease.

Though there was an overall increase in mosquito abundance during rainy seasons in our current study from Lare site, there was no direct association between rainfall and mosquito abundance. This could be due to destruction of mosquito larval habitats by floods and water currents created by heavy rainfall resulting in reduced adult mosquito abundance. In another study, a positive association between relative humidity with mosquito abundance with no significance correlation with temperature and rainfall was reported from Balngladesh [21]. In another study, presence of weak correlation between mosquito abundance and some climatic factors including rainfall and temperature has also been reported [42]. In contrast, [43] stated that there was no any correlation between rainfall and *Anopheles* mosquitoes in a study conducted in Malaysia-Thailand border.

**Conclusions**
An. coustani was the most abundant in both Jimma and Gambella study sites. The fact that most of the Anophles mosquitoes showed exophagic behaviour coupled with occurrence of An. funestus after the malaria peak season (dry season) and phenotypic resistance to multiple insecticides may jeopardize the commonly used IRS & ITNs vector control strategies, necessitating regular malaria vector monitoring and development of innovative vector control tools. Although there was an overall increase in the number of Anopheles specimens caught during the rainy seasons, as compared to the dry season—with the most significant increase being immediately after the rainy season, rainfall and temperature did show significant correlations with mosquito abundance. Additional information on mosquito fauna and abundance in relation to meteorological factors in a particular locality is essential for the development of efficient vector control interventions.

Declarations

Acknowledgments

This work received financial support from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No. 731060 (INFRAVEC2). The authors are grateful to Professor John Vontas (Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Crete, Greece) for allowing his laboratory during mosquito molecular analysis. The authors are thankful to Dr. Konstantinos Mavridis for critically reviewing the manuscript.

Authors' contributions

DY and DW conceived and designed the study. DW performed the field and laboratory experiments, analyzed both field and laboratory data and drafted the manuscript. DY critically reviewed the manuscript. The authors read and approved the final manuscript.

Funding

This work received financial support from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No. 731060 (INFRAVEC2).

Availability of data and materials

The data used are available from the corresponding author upon request.

Ethics approval and consent to participate

This study received ethical approval (Ref. No. IHRPGD/787/2019) from Review Board of Institution of Health, Jimma University. Free and informed written consent was obtained from trained volunteers who participated in mosquito collections. Head of families were requested through individual discussions and community meetings, prior to the enrolment of their house in the study. Permission was requested and obtained from inhabitants to perform mosquito collections in and outside their rooms in mosquito collection areas.

Consent for publication
Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**References**


**Figures**

**Figure 1**

Map of study sites
Figure 2

Mean *Anopheles* mosquito density per person-night and per trap-night, southwestern Ethiopia

Figure 3

Seasonal abundance of *Anopheles* mosquitoes and mean monthly rain fall (MMRF) (A), mean monthly maximum (MMAXT) and minimum (MMINT) temperature (B) between June 2017 to September 2020, im
Lare, Ethiopia

**Key:** In months labelled with asterisk (*), mosquitoes were collected using HLC and calculated as mean *Anopheles* density per person-night are those

**Figure 4**

Bioassays of *Anopheles* mosquito species to different insecticides in Lare and Jimma sites, Ethiopia.

**Key:** L = Lare, J = Jimma
Figure 5

Detection of *Plasmodium* in *An. coustani* and *An. pharoensis* samples