

First report of African malaria vector *Anopheles gambiae* (Diptera: Culicidae) from Ethiopia

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Abstract

Introduction: Reports of different researches conducted in Ethiopia consistently implicated *An. arabiensis* as the primary vector and *An. pharoensis*, *An. funestus* and *An. nili* as secondary vectors. These facts prevailed until today as the vectorial roles of the rest of *Anopheles* are not yet well known due to several limitations. This indicates that there is a need to identify other potential or suspected *Anopheles* species. The aims of this study were to determine *Anopheline* mosquito fauna, abundance and characterize mosquito breeding habitats in selected study sites in Ethiopia.

Materials and methods: A longitudinal study design with purposive and multi-stage sampling technique was conducted in Southern Nations Nationalities Peoples Region of Ethiopia. The study was carried out in Jolie and Gogete study sites from Meskan and Sodo districts, respectively. A total of 4,118 third and fourth instars larvae of *Anopheles* mosquitoes were collected. Moreover, a total of 4,461 indoor adult female *Anopheles* were caught from both study sites. Identifications of *Anopheles* species were done using morphological keys under a compound microscope and polymerase chain reaction test for sibling species detection.

Results: Of the 4,118 samples collected ten *Anopheles* species from Jolie study site and eleven from Gogete study site were identified. Twenty seven percent of the *Anopheles* larvae were collected from marshy breeding site. In both study sites the predominant *Anopheles* species was *An. gambiae* s.l. which is accountable for malaria transmission. Seasonal variations (between dry and wet season) statistically significant differences ($P=0.001$) in Jolie and in Gogete ($P=0.001$) study sites were observed in *Anopheles* larval collection. In Jolie and Gogete study sites *An. gambiae* s.l. was found to be the predominant species making up 64% and 64%, respectively. Statistically significant differences were observed in mean adult *An. gambiae* s.l. ($P=0.002$) and *An. pharoensis* ($P=0.001$) collection among the study sites. Besides, statistically significant ($P=0.001$) variations between wet and dry seasons were observed in the number of adult *An. gambiae* s.l. populations in both study sites. Of 239 *An. gambiae* s.l. analyzed by PCR, 94.1% were identified as *An. arabiensis* and 5.9% were identified as *An. gambiae*.

Conclusion: In the present study *An. gambiae* was reported for the first time in Ethiopia indicating the presence of the highly efficient vector in Ethiopia, thus establishing appropriate control strategy for this species might be necessary. The breeding habitats of *Anopheles* mosquitoes in the study sites particularly in the dry season were due to anthropogenic activities rather than environmental factors that require larval control.

Key words: Malaria vectors, larval habitat, *An. arabiensis*, *Anopheles gambiae*, Ethiopia.

Introduction

Reports of different findings conducted in Ethiopia consistently incriminated that *An. arabiensis* is the primary vector responsible for the transmission of malaria where as *An. pharoensis*, *An. funestus* and *An. nili* are considered as secondary vectors (Abose et al. 1998; Ameneshewa 1995; Gone et al. 2014; Animut et al. 2012; Fettene et al. 2004). Recently, based on strong genetic evidence the *An. gambiae* s.s M and S molecular forms and *An. quadriannulatus* species B are assigned formal names: M form (*An. coluzzii*) S form (*An. gambiae*) and *An. quadriannulatus* species B (*An. amharicus*) (Ethiopia) (Coetzee et al. 2013). As demonstrated in other studies the taxonomy of this complex species, no distinct characters are available for rapid, accurate, morphological identification of the species and thus identifications must be based on the use of molecular methods (Coetzee et al. 2013).

However, the above mentioned are facts until now the vectorial roles of the rest of *Anopheles* were not yet completely known due to several limitations. Most of the currently available entomological information is

emanated only from limited areas where there are easily accessible means thus the information are insufficient (Gebre-Mariam et al. 1988). Besides, communication problem to reach inaccessible areas has hindered the research on malaria and its vectors. For instance, *An. tenobrosus* Donitz was found positive for *P. falciparum* gametocyte in south Ethiopia with infection rates of 15.8% by dissection and 7% by DNA hybridization (Adugna et al. 1998). This indicates that the need to identify other potential *Anopheles* species in the epidemiology of malaria throughout the country is unquestionable. On the other hand, investigating the recent variations in the abundance and dynamics of malaria vector populations is also important (Animut et al. 2012). Since, the abundance and dynamics of *Anopheles* population have significant impact on the intensity of malaria transmission from place to place (Katrijn et al. 2010). For example, a study conducted by Brooker et al. (2004) showed that the existence of variation in the intensity of incidence of malaria within the same area. Pertinently, the density of malaria vectors has shown a

great variation in the same topography even at household level within the same area. For instance, in Ethiopia White et al. (1980) around Gilgil Ghibe River valley reported that a variation in the density of malaria vectors with a result of less than 1 in January-March where as it go greater than 100 in July-October. But, in Gambela areas of Ethiopia, Krafur (1970, 1978) reported that the average hut resting density was less than 30. Meanwhile, a collections made in the 1958 malaria epidemics revealed the average hut resting density were vary from 100 to 150 (Fontaine et al. 1961). However, Indoor Residual Spray (IRS) and LLINs are reliable and effective in a wide range of situations (Noor et al. 2009; MOH 2011; Shargie et al. 2008, 2009). Similarly, the impact of IRS in lowering indoor densities of *An. gambiae* s.l. was reported in study conducted in western foothill of Madagascar (Ratovonjato et al. 2014). Therefore the aim of this study was to identify species composition, abundance and breeding habitat contribution for *Anopheles* mosquitoes in the selected study sites.

Table1: Study sites, location and elevation of selected data collection sites in Southern Nation Nationality People Region (SNNPR), May 2013 to April 2017

Zone	Districts/Wereda	Study site	Latitude/Longitude	Elevation
Gurage	Meskan	Jolie	N 08° 13.217'; E 038 28. 268'	1842 m
	Sodo	Gogete	N 08° 10.371'; E 038° 30. 449'	1846 m

Study design: longitudinal study design using purposive and multi-stage sampling technique Southern Nations Nationalities and Peoples region (SNNPR), Gurage Zone as well as the two districts were purposively selected to identify species composition, abundance and breeding habitat contribution in the study sites. This study used two study sites and it was done by random selection from the list of malarious *Kebeles* (localities) in the district health office. Thus, study was carried out in Jolie and Gogete study sites from Meskan and Sodo districts, respectively.

Sampling procedures

Larva collection, species identification and investigation of their habitat: Immature stage collections were carried out to supplement the data of adult collection to identify species complex and determine the principal *Anopheles* species accountable for malaria transmission in the study areas (WHO 2013). The collections of *Anopheles* larvae were conducted in a variety of aquatic habitats of the study areas once per month by two collectors using dippers, pipettes and containers from temporary and permanent breeding sites (ponds, pools of rain water, water from broken pipes, River pocket, stagnant water in irrigation canals and marsh) in the study areas and the number of larvae collected from each larval habitats were compared to identify the major contributor of *Anopheles* mosquitoes breeding. The collected *Anopheles* larvae were killed in hot water (about 50°C) and preserved in small vials containing 70% ethyl

Materials and methods

Description of the Study sites: The study sites were Jolie and Gogete study sites (Kebeles = the grass root level of government administrative structure) around Butajira in Meskan and Sodo District, respectively, in Gurage Zone, which is found within Southern Nation Nationalities People Region (SNNPR) of Ethiopia. Jolie & Gogete are found below at an average altitude of 1850 meters above sea level at a distance of 120 Km south of Addis Ababa (Table1).

In both study sites there are few houses with corrugated iron sheet roof but dominantly houses with thatched roof. The inhabitants are engaged mainly in cultivating maize in wet season and during dry season vegetables such as onion, pepper, tomato, potato and cabbage by using irrigation. As in most parts of Ethiopia, the areas have two major seasons; the rainy season starts in June and lasts in mid-September, which then followed by the short rain in March and April. The study has been conducted from May 2013 to April 2017.

alcohol (WHO 2013). All the specimens collected from a particular breeding place deposited in a labeled bottle or vial. The label were written in pencil on a piece of paper and dropped into the specimen bottle and transported to Addis Ababa (EPHI or AAU laboratory) for identification. The preserved specimens were soaked for 12 hours in watch glasses containing distilled water. Each larva is then mounted on a glass slide separately in a drop of gum chloral mountant and covered with a piece of cover slip. Each cover slip measuring 22x22 mm is cut into four parts and one piece is used for a single specimen. The gum chloral mountant is prepared in the laboratory as described in Gordon and Lavoipierre (1969). The constituents are 25 ml distilled water, 160 gm crystal chloral hydrate, 15gm gum Arabic, 10 gm glucose syrup and 5ml glacial acetic acid which are then mixed in the above order in a water bath at a temperature of 80°C. At all times the mountant is kept in a dark and well-screwed bottle. The identifications of species on immature made by using third and fourth instars larvae based on Verrone (1962a) and Gillies and Coetzee (1987) under a compound microscope.

Adult Anopheles collections, identification and density determination: Indoor female *Anopheles* mosquito collection using mouth suction aspirators, CDC- light traps and pyrethrum spray collection methods were carried out once every month for a total of 26 months in households of the study sites (WHO 1992). The collected female *Anopheles* mosquitoes were sorted, counted, categorized as unfed, fed, half

gravid and gravid, finally placed in a separate labeled paper cups thus all collected specimens transported to Addis Ababa (EPHI or AAU laboratory) for species identification. The procedure of collection by the three methods described below (Samples collected from the study sites were stored in Entomology laboratory of EPHI with voucher number NATESA 312).

Pyrethrum spray collection: The pyrethrum spray collection method as described by WHO (2013) was employed to collect indoor resting adult *Anopheles* mosquitoes. Pyrethrum spray collections of indoor resting mosquitoes were carried out once every month early in the morning beginning 6:00 am to 10:00 am. After permission is granted from the head of household or inhabitants, all human occupants, animals, domestic utensils, exposed food and water were voluntarily moved out of the dwellings prior to the spraying all household items that cannot be moved out were covered with plastic material. Then, the entire floor were covered with white cloth sheets cut to 1.2mx2.20m, and doors and windows were closed, openings and eaves also properly covered and all entire parts of the rooms were sprayed with aerosol (containing pyrethrum) after spraying the dwelling were left closed for 20-30 minutes to produce a knockdown effect.

Mouth suction aspirator collection: Mouth suction aspirator collections of *Anopheles* mosquitoes were carried out on a different day once every month, early in the morning beginning 6:00 am to 10:00 am. Mouth suction aspirator and flash light were used to collect indoor resting mosquitoes from walls, under roofs, hanging clothes, household utensils and dark corners.

CDC-Light traps collection: CDC light traps collections were used to collect *Anopheles* mosquitoes once every month; CDC light traps were positioned inside and outside of selected houses from 06:30 pm to 6:00 am at night to collect *Anopheles* mosquito. To identify the species of *Anopheles* mosquito two methods were employed. One of the methods is morphologically based on keys under a stereo dissecting microscope with a magnification power of 20x (Verrone 1962b; Gillies and Coetzee 1987), the second is by using polymerase chain reaction (Scott et al. 1993).

Molecular identification of adult *An. gambiae* complex using Polymerase Chain Reaction (PCR) DNA extraction, amplification and gel electrophoresis procedures: Almost 10% of indoor collected and morphologically identified as *An. gambiae* s.l were selected randomly and identified to sibling species by polymerase chain reaction (PCR), using an adapted version developed by Scott et al. (1993) based on species specific single nucleotide polymorphism (SNPs) in the intergenic spacer region

(IGS) following a minor modification by Wilkins et al. (2006) incorporating intentional mismatch primer (IMP) to increase specificity was used for the allele amplification. Genomic DNA was extracted from legs and/or abdomen of individual mosquitoes according to the method described by Collins et al. (1987). The *Anopheles gambiae* s.l. legs and/or abdomen were grinded in 100µl grinding buffer solution (0.2M sucrose, 0.5% SDS, 0.1 M tris-HCL pH 7.5, 0.1 NaCl, 0.05M EDTA pH 9.1) using electric motor pestle with a sterile blue loop on centrifuge tubes until all parts remain homogeneous. The products of the grinded solution were heated at 65°C for 30 minutes. The DNA were precipitated by adding 18µl of 5M ice-cold Potassium acetate (KAC) and incubated in a container filled with ice for 30-60 minutes, next the solutions were centrifuged at maximum speed of 13, 200 revolutions per minute at room temperature for 20 minutes. The DNA supernatant were gently transferred (let alone transferring the precipitate) to a new labeled centrifuge tubes followed by adding 200µl of 100% ethyl alcohol, after that the solution mixed by shaking inverting the centrifuge tube and incubated inside a negative 20°C deep-refrigerator for overnight. In the next day the DNA were precipitated by centrifuging at maximum speed of 13, 200 revolutions per minute for 30 minutes at 4°C and then the supernatants were gently discarded without disturbing the pellets. Then, 200µl of 70% ethyl alcohol added and re-centrifuged for 10 minutes at 13, 200 revolutions per minute. Finally, the 70% alcohol was disposed slowly without disturbing the pellets; the DNA sediments were allowed to dry for 30 minutes at room temperature. Lastly, the DNA pellets were dissolved in 100µl sterilized water with gentle tapping of the tube to allow the DNA to re-suspend for amplification process.

Four species-specific and a universal primer were used in DNA coping process, species-specific primer for *An. arabiensis* (AR-3T-R, GTG TTA AGT GTC CTT CTC CGT C); for *An. gambiae*, (GA-3T-R, GCT TAC TGG TTT GT CGG CAT GT); for *An. quadrimaculatus*, (QD-3T-R, (GCA TGT CCA CCA ACG TAA ATC C); for *An. merus/melas* (ME-3T-R, CAA CCC ACT CCC TTG ACG ATG) and one universal primer sequence (IMP-UN-F, GCT GCG AGT TGT AGA GAT GCG)). Mopti and Savanna rDNA were analyzed using PCR amplified rDNA by the methods of Fanello et al. (2002) and Favia et al. (1997) and furthermore by DNA sequencing. Targeting rDNA intergenic spacer (IGS) for the *Anopheles gambiae* complex species were amplified in a multiplex reaction as illustrated by Wilkins et al. (2006). PCR reaction was carried out using TaqDNA polymerase (AccuStart II PCR Supermix) and the manufacturer's (Quanta Biosciences) recommended buffer at 1×concentration was used for all reactions. PCR reactions buffer consisted of a 25pmol (3.25 DI

ornuclease free water, 6.25 AccuStart II PCR Supermix, 0.5 IMP-UN-F and 0.5µl of each primer) concentration and 0.5µl of DNA in a final 12µl reaction mix. The plate covers were fasten tightly and spine down in microcentrifuge at maximum speed for two to three minutes and then amplified.

The final reaction mix, thermal cycling for all analyses was performed in a Bio-Rad iCycler®, PCR cycling consisted of melting at PCR conditions rn 1: 95°C for 4' followed by 34 cycles of: 95°C for 30"; 60°C for 30" and 72°C for 30" and a final elongation step at 72°C for 5'. Agarose gel was prepared on the bases described by Wilkins et al. (2006) protocol. The prepared agarose gel was placed in to electrophoresis box and 1µl of molecular weight ladder was loaded into the first and the last lane of the agarose gel, then 1µl of PCR amplified DNAs (the experimental and control) were loaded into the rest wells of the agarose gel.

The electrophoresis box positive and negative ion electrodes properly plugged to the gel electrophoresis machines and subsequently allowed to run at a voltage of 90, 400amper for 90 minutes. DNA fragments of *An. gambiae* complex species were visualized by using Benchtop UV Transilluminator machine. MultiDoc-It™ Imaging System-Masterflex computer software was used to identify DNA fragments and capture photo of the DNA bands. The gel electrophoresis (DNA fragment) results were interpreted by using bands of the markers on the first and the last lane of the gel.

An. gambiae s.l. sibling species were identified by comparing the DNA band size with already known molecular weight ladder bands. DNA fragments having 463bp were determined to be *An. gambiae* Giles/*An. colluzzi* Coetzee and Wilkerson, 528bp *An. melas* Theobald/*An. merus* Donitz, 636bp *An.*

quadrianulatus Theobald/ *An. amharicus* Hunt, Wilkerson and Coetzee and 387bp *An. arabiensis* Patton. The bp numbering is as previously designated [Genbank: AY787486].

Data analysis: The collected data were computerized using Epi Info 7 and analyzed using Stata/SE 11.0, percentage, mean, proportion and 95% CI was constructed to help in making inference towards the target population, for significance differences ($p < 0.05$) two-sample t test with equal variance and two-samples t test of proportion were used. The proposal was presented and reviewed by Addis Ababa University and Ethiopian Public Health Institute (EPHI) of Scientific and Ethical Review Office (SERO). In addition verbal and signed consent were obtained from the study participants for permission to carry out indoor adult female *Anopheles* mosquito collections using mouth suction aspirators, CDC- light traps and space spray in their houses.

Results

Species composition, monthly variation and breeding habitats preference of *Anopheles* larvae: A total of 4638 mosquito larvae were collected from different types of breeding sites, of which 4253 were *Anopheles* mosquitoes and the rest 385 were *Culex* larvae. About 135 of the larvae of *Anopheles* could not be identified because of bad preparation and mechanical damage, thus 4118 third and fourth instar larva were identified. The majority 27.51% of *Anopheles* mosquito larvae were collected from marshy breeding site which served as a major breeding habitat during the dry season, followed by irrigation canals 24.50%, rain pools 20.45%, river pockets 17.63% and ponds 9.91% in the study areas (Figure 1).

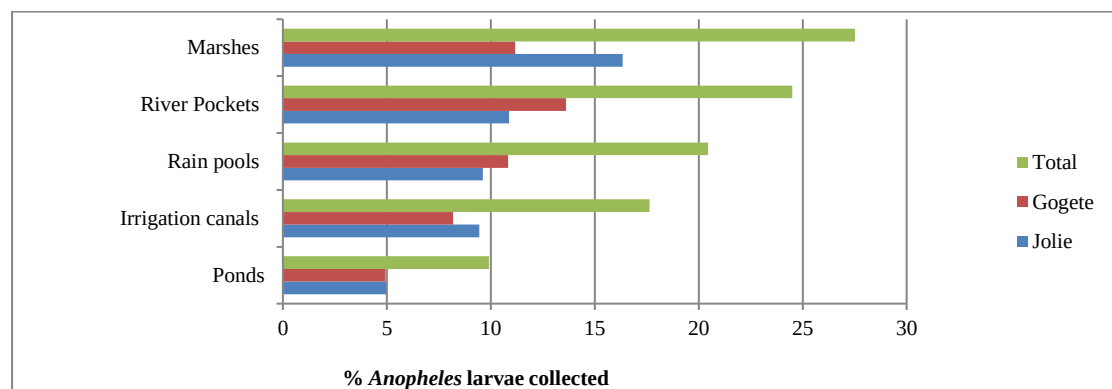
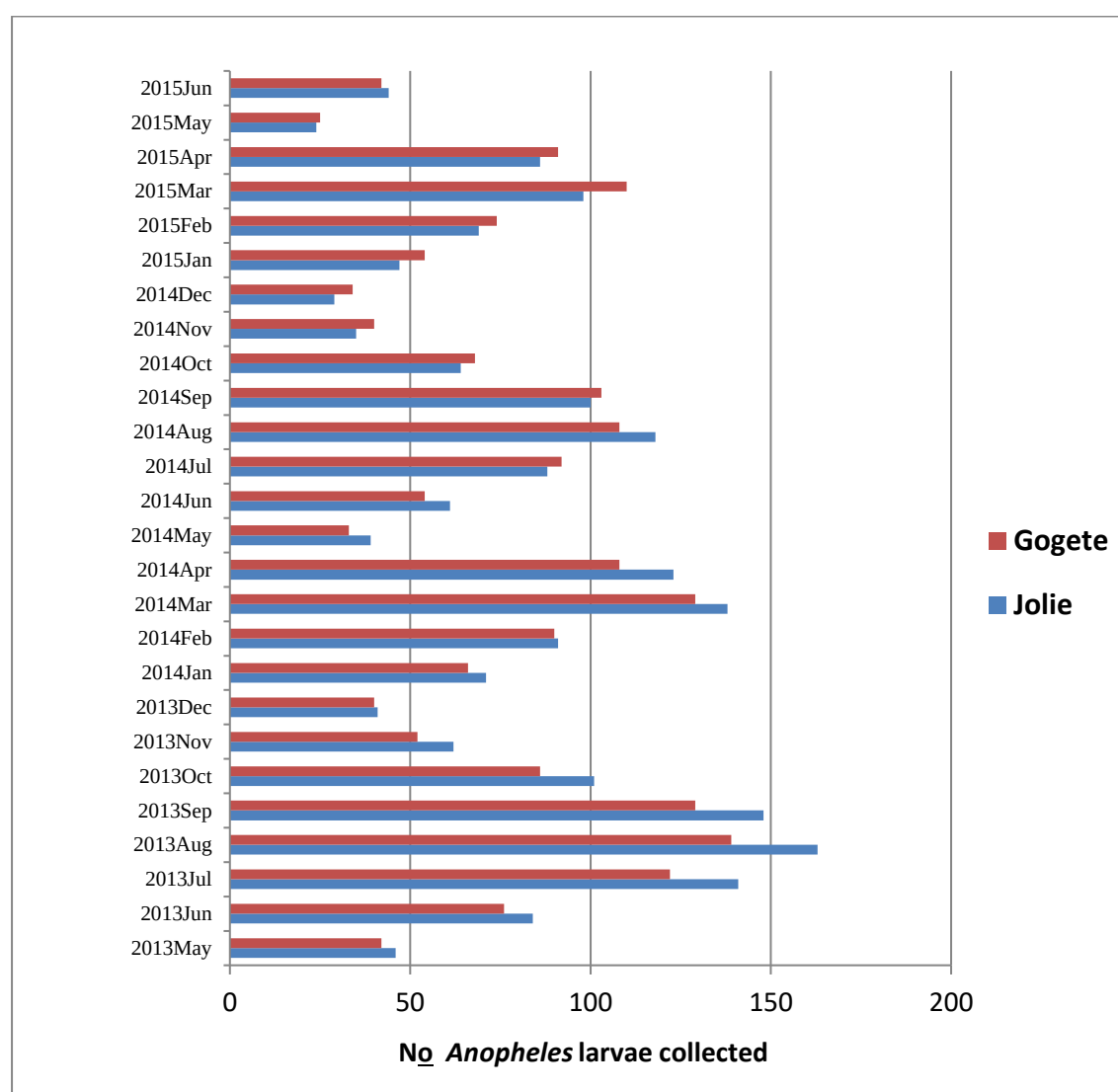


Figure 1: Breeding habitats and percentage of *Anopheles* larvae collected in the study areas (May 2013 to June 2015)

Table 2: Species composition and percentage of *Anopheles* mosquitoes identified from larval collections in the study areas Ethiopia (May 2013 to June 2015)

Species	# Mosquitoes larvae collected n(%)		Total n (%)
	Jolie	Gogete	
<i>An. gambiae s.l.</i>	763 (36.1)	719 (35.8)	1482 (36.0%)
<i>An. cinereus</i>	509 (24.1)	479 (23.9)	988 (24.0%)
<i>An. christyi</i>	488 (23.1)	459 (22.9)	947 (23.0%)
<i>An. pharoensis</i>	149 (7.1)	135 (6.7)	284 (7.0%)
<i>An. demeilloni</i>	113 (5.4)	101 (5.0)	214 (5.2%)
<i>An. garnhami</i>	37 (1.8)	29 (1.4)	66 (1.6%)
<i>An. longipalpis</i>	28 (1.3)	21 (1.1)	49 (1.2%)
<i>An. marshali</i>	20 (0.9)	13 (0.6)	33 (0.8%)
<i>An. pretoriensis</i>	2 (0.1)	2 (0.1)	4 (0.1%)
<i>An. sergentii</i>	2 (0.1)	2 (0.1)	4 (0.1%)
<i>An. squamosus</i>	0 (0)	47 (2.4)	47 (1.1%)
Total	2111 (100)	2007 (100)	4118 (100)

**Figure 2: *Anopheles* larvae collected in different months in Jolie and Gogete study sites (May 2013 to June 2015)**

Identification of *Anopheles* larvae: A total of 2111 and 2007 *Anopheles* mosquito larvae were collected from Jolie and Gogete study sites, respectively. In

Jolie study site ten *Anopheles* mosquito species were identified, *An. gambiae s.l.* was the predominant species accounted for 36.1% (n=763) followed by *An.*

cinereus 24.1% (n=509), *An. christyi* 23.1% (n=488) and *An. pharoensis* 7.1% (n=149) the rest were collected in small amount. In Gogete study site eleven *Anopheles* mosquito species were identified, similarly *An. gambiae* s.l. was also the predominant species accounted for 35.8% (n=719), followed by *An. cinereus* 23.9% (n=479), *An. christyi* 22.9% (n=459) and *An. pharoensis* 6.7% (n=135) (Table 2).

Monthly dynamics of *Anopheles* larvae in study sites:

Statistically significant differences in Jolie (P=0.001) and in Gogete (P=0.001) study sites (seasonal variations) in *Anopheles* larval collection were observed. The highest mean 129.25 collection were done in the months of July, August, September and March in Jolie study site and in similar months the highest mean 121.5 collection were done in Gogete. The peaks of *Anopheles* mosquitoes were collected in major and minor rainy seasons (Figure 2).

Indoor *Anopheles* mosquito collection: A total of 4461 indoor adult female *Anopheles* were caught in both study sites, of which 31.1% (n=1386) from Jolie and 68.9% (3075) from Gogete. Of 1386 female *Anopheles* mosquitoes caught from Jolie study site *An. gambiae* s.l. was the predominant species accounted for 63.9%, followed by *An. pharoensis* 19% (Figure 3). In Gogete study site, of 3075 of female *Anopheles* caught *An. gambiae* s.l. was also the predominant species accounted for 64%, followed by *An. pharoensis* 19.1% (Figure 3). Statistically significant differences were observed in both *An. gambiae* s.l. (P=0.002) and *An. pharoensis* (P=0.001) collection among the study sites, i.e. higher number of *An. gambiae* s.l. and *An. pharoensis* were collected in Gogete than the Jolie study site.

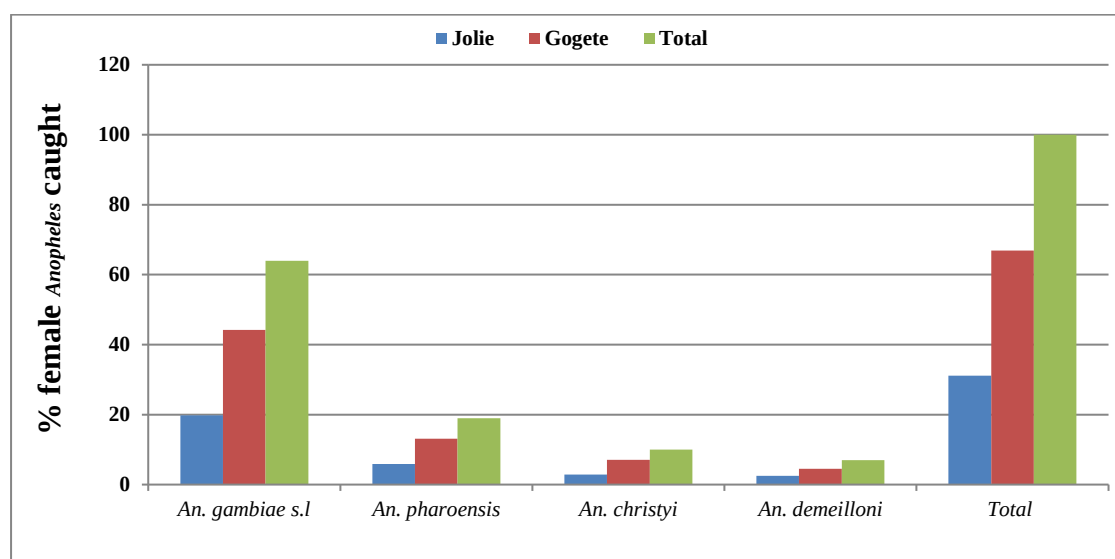


Figure 3: Percentages of adult *Anopheles* mosquito species collected indoors using different collection methods in the two study sites (May 2013 to June 2015)

There was significant difference (P=0.001) in mean percentage in *An. gambiae* s.l. between wet and dry seasons. In both Jolie and Gogete study sites, i.e. higher number of *An. gambiae* s.l. collected during wet season than dry season presented in Figure 4.

Molecular identification of Adult *Anopheles gambiae* complex:

About 272 indoors collected and

morphologically identified as *An. gambiae* s.l. were subjected for molecular identification by PCR, of which due to technical problem the result of 33 specimens were rejected. Thus our result showed that, of the total (n=239) *An. gambiae* s.l. (Figure 5) tested 94.1% were identified as *An. arabiensis* and 5.9% as *An. gambiae* (Table 3).

Table 3: The number and percentages of *Anopheles arabiensis* and *Anopheles gambiae* identified by polymerase chain reaction (PCR) from Jolie and Gogete study sites

Species	Jolie n (%)	Gogete n (%)	Total n (%)
<i>An. arabiensis</i>	117 (48.9)	108 (45.2)	225 (94.1)
<i>An. gambiae</i>	4 (1.7)	10 (4.2)	14 (5.9)
			239 (100)

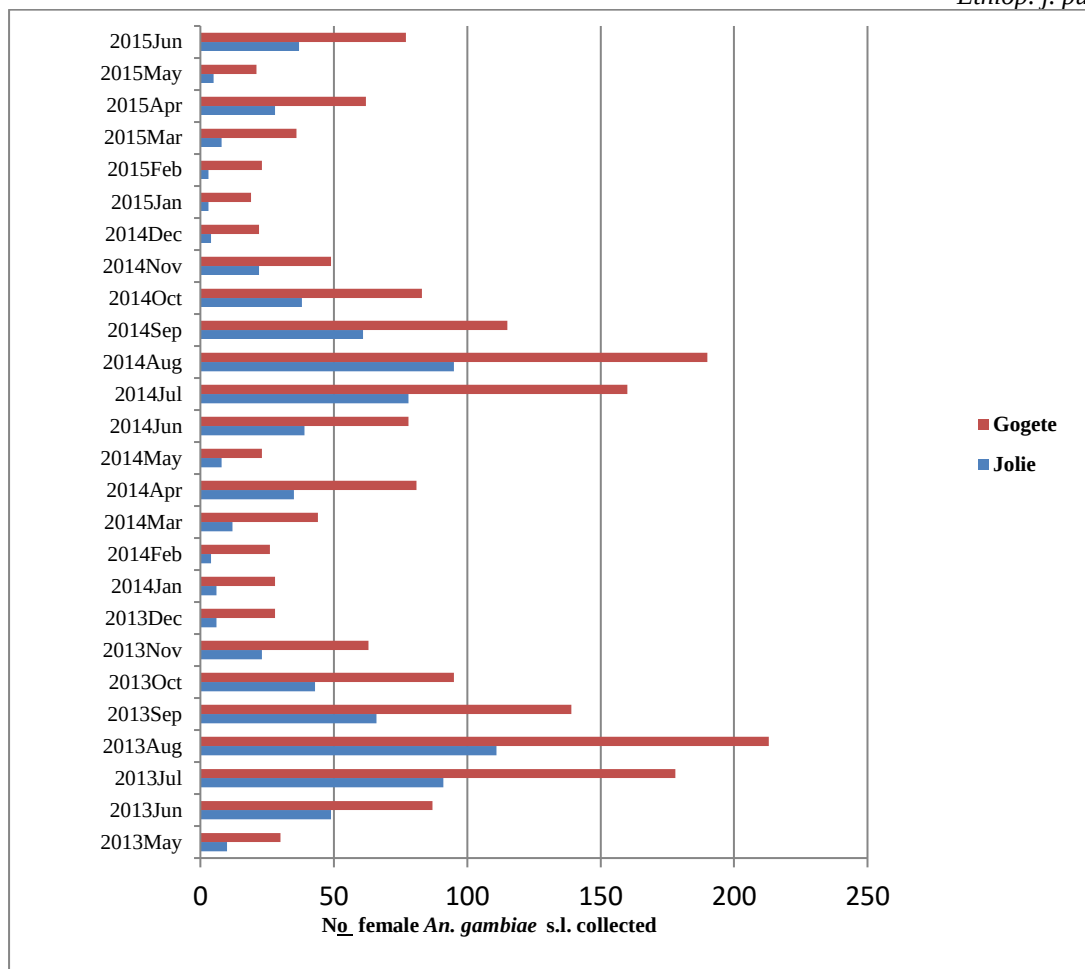


Figure 4: Monthly indoor collections of *Anopheles gambiae* s.l. in Jolie and Gogete study sites (May 2013 to June 2015)

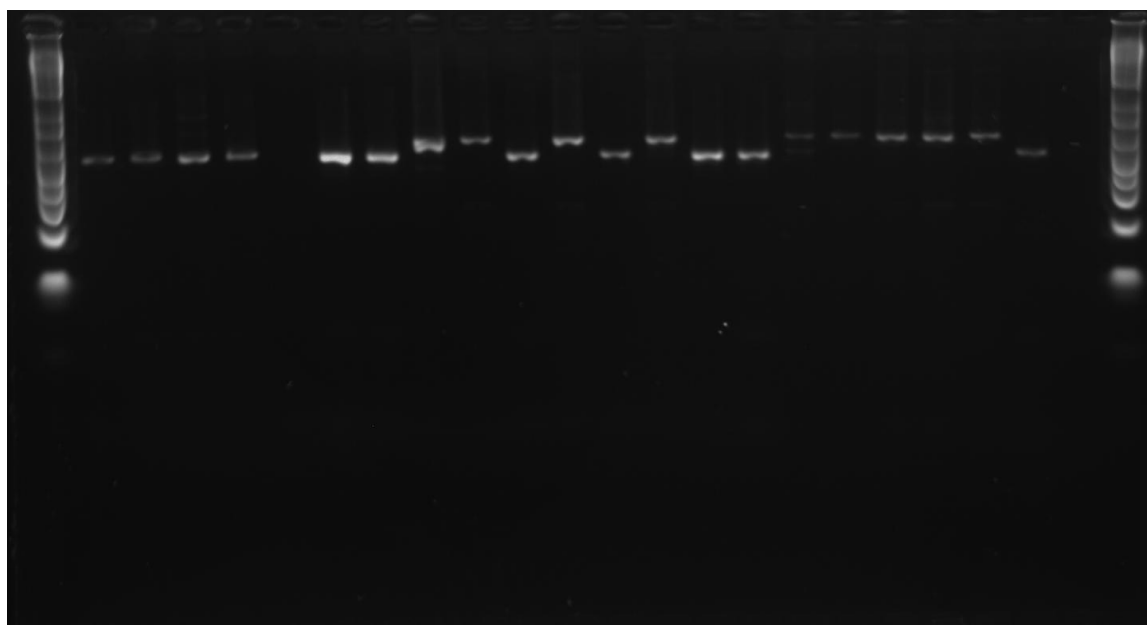


Figure 5: Image of PCR result of Gel electrophoresis of *Anopheles gambiae* s.l. species identification comparing products run on a 2% agarose gel red. Lanes 1 and 24 1kb ladder, lanes 2-5, 6 not amplified, lanes 7, 8, 11, 13, 15 and 16 were *An. arabiensis* and lanes 9, 10, 12, 14, and 17-20 were *An. gambiae* and lanes 21-23 control.

Discussion

This study showed the presence of *Anopheles* larvae throughout the study period. The persistence of *Anopheles* mosquitoes throughout the year in Jolie and Gogete study sites were mainly associated with irrigation practices and river pocket. In addition, the existence of permanent vector breeding habitats created by poor management of water supported the survival of vector species throughout the year. Ten *Anopheles* species in Jolie and eleven in Gogete study sites were identified during data collection period. In both localities the predominant *Anopheles* species was *An. gambiae* s.l. which is the main vector in the country (Abose et al. 1998; Amenshewa 1995; Animut et al. 2012; Gona et al. 2014). *An. cinereus* and *An. christyi* are the second predominant species in the study areas. Similar to the present study *An. cinereus* and *An. christyi* have been reported from the neighboring villages at about the same altitude (Animut et al. 2012). The occurrence of high number of *An. cinereus* and *An. christyi* in this study might be the presence of marsh habitat in the area which is ideal habitat for these two species (Woyessa et al. 2004; Tesfaye et al. 2011; Animut et al. 2012). Substantial numbers of *An. pharoensis* were collected in the present study similar to the study conducted by (Animut et al. 2012) in the neighboring village.

Irrigation activities in the study area might be associated with the existence of *An. pharoensis* throughout the year, similar to the study conducted in the central rift valley of Ethiopia (Kibret et al. 2010). However, higher numbers of *Anopheles* mosquito larvae were collected during the wet seasons both in major and minor rainy seasons. The occurrence of higher number of *Anopheles* larvae in this study during the wet season is not consistent with the findings of the study conducted in neighboring villages by Animut et al. (2012) where more larval collections were recorded during the dry season. Frequent larval sampling during the wet season might be the reason for encountering larval positives rain pools in the present study compared to a study conducted by Animut et al. (2012). Local meteorological and other environmental factors might explain for the dominance of *Anopheles* larvae during the wet season.

During our study both temporary and permanent breeding habitats were dominantly occupied by *An. gambiae* s.l. throughout the wet seasons. Alike, the study conducted in Butajira area near to the present study sites conducted by Animut et al. (2012) *An. gambiae* s.l. was the predominant species in every types of habitat during the wet season. The monthly variation in the number of *An. gambiae* s.l. in the present study might be due to climatic factors such as the amount of rain, temperature and relative humidity.

The breeding habitats of *An. gambiae* s.l. in the study localities particularly during the dry season are due to human activities rather than environmental causes. That is, the water accumulated from overflow of irrigation canals, interrupted marshy areas and River pockets remain the most important breeding sites created by human activities. Similarly, studies conducted in Butajira area and western Kenya, reported that marshes, irrigation canals and river pockets were served as breeding habitat for *An. gambiae* s.l. during months of low precipitation (Animut et al. 2012; Kenea et al. 2011; Imbahale et al. 2011; Kweka et al. 2015).

The result of adult *Anopheles* collection revealed the presence of five species throughout the study period. *An. gambiae* s.l., *An. christyi*, *An. pharoensis*, *An. demellion* and *An. cinereus* were collected as an adult stage whereas *An. gambiae*, *An. longipalpis*, *An. squamosus*, *An. marshali*, *An. pretoriensis* and *An. sergentii* were only collected as larvae. The reason for this discrepancy might be the resting behaviour of *Anopheles* mosquitoes and the lack of efficient adult collection methods. This is supported by the higher number of *An. christyi*, *An. demellion* and *An. cinereus* in larval collection compared to lower indoor adult collection in the present study, the reason might be CDC-light trap may not capture enough mosquitoes outdoor in absence of host close to collection area since some species of *Anopheles* mosquitoes need CO₂ as cues in addition to light (WHO 2013).

Of the five species identified in adult collections *An. gambiae* s.l. was also found in greater number, this species was collected indoor more frequently than the other. Looking at the evidences from the present data no other malaria vector is important than *An. gambiae* s.l. in both study areas. In agreement with other studies, this species is incriminated as the principal vector of malaria in Ethiopia (Abose et al. 1998; Amenshewa 1995; Animut et al. 2012; Kibret et al. 2010) and elsewhere in East, West and South Africa (Abose et al. 1998; Amenshewa 1995; Kenea et al. 2011; Imbahale et al. 2011; Kweka et al. 2015). The presence of this species in the study area during the dry months is an indication for the uninterrupted malaria transmission throughout the year. However, *An. pharoensis* was found in both study sites therefore this species can be considered as a secondary vector of malaria in the two study areas as in other parts of Ethiopia (Animut et al. 2012; Kibret et al. 2010).

In this study, seasonal and local variations in indoor mosquito collection were observed. For instance, higher indoor collections were observed in both study sites during the wet season. Similarly, the presence of higher indoor numbers of *An. gambiae* s.l. during the

wet seasons were observed in a studies conducted in Ethiopia and Kenya (Massebo et al. 2013; Gari et al. 2016; Amek et al. 2012) during the wet season. The possible explanation of higher *An. gambiae* s.l. during the wet season in the present study and other studies conducted in different parts of Ethiopia could be linked with the abundance of small temporary rain pools which is a suitable larval habitat especially for *An. gambiae* s.l. (Munga et al. 2013; Kweka et al. 2012). On the contrary, higher number of *An. gambiae* s.l. was reported during the dry season in a previous study conducted near to the present study area (Animut et al. 2013). The number of *An. gambiae* s.l. and *An. pharoensis* collected during the study period were greater in Gogete than Jolie study site. Similarly, Katrijn et al. (2010) indicated the abundance and dynamics of *Anopheles* population have significant impact on the intensity of malaria transmission from place to place. Besides, a study conducted by Brooker et al. (2004) showed that the existence of variation in the incidence of malaria within the same area. Pertinently, the density of malaria vectors has shown a great variation in the same topography and even at household level within the same area. In Ethiopia White et al. (1980) around Gilgil Ghibe River valley reported seasonal variation in the density of malaria vectors. But, in Gambela areas of Ethiopia, Krafur (1970; 1978) reported that the mean mosquito resting density was less than 30 mosquitoes/house. Meanwhile, a collections made in the 1958 malaria epidemics revealed the mean mosquito density vary from 100 to 150 mosquito/house (Fontaine et al. 1961).

In the present study *An. gambiae* was reported for the first time in Ethiopia indicating the presence of more efficient vector of malaria in the *An. gambiae* complex in addition to *An. arabiensis*. Previous and recent studies conducted in different part of Ethiopia reported that *An. arabiensis* and *An. amharicus* are the only two member species of the *gambiae* complex identified in Ethiopia (Coetzee et al. 2013; Animut et al. 2012). The absence of *An. gambiae* in the previous study conducted in different parts of Ethiopia might be the lack of sensitivity and specificity of molecular assays coupled with technical and technological short comings.

Conclusion

The breeding habitats of *Anopheles* mosquitoes in the study sites particularly in the dry season were due to human activities rather than environmental factors that require larval control. In the present study *An. gambiae* was reported for the first time in Ethiopia indicating the presence of an efficient vector in Ethiopia, thus establishing appropriate control strategy for this species might be necessary.

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