Received: 01 April 2026 | Revised 20 May 2026 | Accepted 25 May 2026 | Page 26- 40

ORIGINAL RESEARCH

Journal of Research and Review in Science,
Volume 13, Issue 1, 26-40, June 2026
DOI: 10.36108/jrrslasu/6202.31.0130

LARVAE BREEDING HABITAT CHARACTERIZATION AND IDENTIFICATION OF *ANOPHELES* MOSQUITOES IN SELECTED LOCAL GOVERNMENT AREAS IN EKITI STATE, NIGERIA

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Funding information
The authors received no specific funding for this work. This study was self-funded by the authors.

Abstract

Introduction: Malaria remains a major public health challenge in Nigeria despite ongoing control efforts. Understanding the ecological characteristics of mosquito breeding habitats and the molecular composition of malaria vectors is important for improving vector control strategies.

Aims: This study aimed to determine the breeding habitat preferences and physicochemical characteristics of Anopheles mosquito breeding sites and to identify the molecular composition of members of the *Anopheles gambiae complex* in Ado, Oye, and Ikere LGAs of Ekiti State.

Materials and Methods: A cross-sectional study design was employed involving entomological surveys and molecular analyses. Mosquito larvae breeding habitats were characterized using World Health Organization larval habitat characterization forms. Anopheles larvae were collected from nine breeding habitats across the three LGAs and reared to adulthood. Adult mosquitoes were identified morphologically, and sibling species within the *Anopheles gambiae complex* were determined using polymerase chain reaction (PCR). Physicochemical parameters of breeding habitats, including temperature, pH, dissolved oxygen (DO), electrical conductivity (EC), total dissolved solids (TDS), and salinity, were measured. Essential elements such as sodium, calcium, magnesium, copper, iron, phosphate, and nitrogen were also analyzed. Statistical analyses were conducted to assess differences among the LGAs.

Results: All nine breeding habitats identified were temporary water bodies (100%), with 67% characterized by polluted, turbid water and 78% exposed to sunlight. All breeding sites were located near human settlements. Physicochemical parameters recorded across habitats included temperature (25.7–37.8°C), pH (4.0–9.2), dissolved oxygen (16.4–27.4 mg/L), and electrical conductivity (220–554 µS/cm). Significant differences among the LGAs were observed for temperature ($p = 0.02$), pH ($p = 0.03$), and salinity ($p = 0.001$), whereas EC ($p = 0.39$), TDS ($p = 0.05$), and DO ($p = 0.19$) showed no significant differences. Elemental concentrations varied across habitats, including sodium (10.4–71.5 mg/L), calcium (47.4–105.7 mg/L), magnesium (5.20–16.50 mg/L), copper (0.52–2.18 mg/L), iron (0.41–3.25 mg/L), phosphate (7.68–26.32 mg/L), and nitrogen (33.61–96.34 mg/L). Molecular identification showed that *Anopheles coluzzii* constituted 70.2% of sampled mosquitoes, while 29.8% were unidentified. No significant difference in species distribution was observed across the LGAs ($\chi^2 = 1.696$, $p = 0.428$).

Conclusion: Vector management strategies such as molecular surveillance, combinational and continuous resistance monitoring to overcome the malaria endemic challenges are key measures to improving on the efficacy of insecticides in malaria control programs in Ekiti State and other malaria endemic areas.

To Keywords: Larvae, *Anopheles* mosquitoes, morphology, polymerase chain reaction, malaria

All co-authors agreed to have their names listed as authors.

INTRODUCTION

Malaria is a life-threatening disease caused by plasmodia parasites, transmitted to humans through the bites of infected Anopheles mosquitoes (WHO, 2023). Despite global efforts to control and eliminate malaria, it remains a significant public health challenge, particularly in sub-Saharan Africa. Nigeria, being one of the countries with the highest malaria burden, has implemented various strategies to combat the disease (WHO, 2022). Ekiti State, located in the southwestern region of Nigeria, is not exempted from this burden, with malaria contributing to morbidity and mortality, especially among children under five and pregnant women (Adeyanju et al., 2024). Anopheles mosquitoes, belonging to the genus Anopheles, are the primary vectors responsible for transmitting malaria, a devastating disease caused by Plasmodium parasites (Wondji et al., 2012). There are over 460 recognized species of Anopheles mosquitoes, but only about 30-40 of them are capable of transmitting malaria to humans (WHO, 2016). These mosquitoes exhibit a wide range of biological and ecological characteristics, which significantly influence their capacity to spread malaria (WHO, 2016). The *Anopheles gambiae complex* is one of the most efficient malaria vectors in sub-Saharan Africa (Cisse et al., 2015). In Nigeria, the primary Anopheline mosquito species responsible for malaria transmission include members of the *Anopheles gambiae complex* and the *Anopheles funestus* group. The primary members of this complex are *Anopheles gambiae sensu stricto, Anopheles arabiensis,* and *Anopheles coluzzii* (Cisse et al., 2015). *Anopheles gambiae* is highly anthropophilic, preferring to feed on humans, which makes it an effective malaria transmitter (Habibu *et al*., 2017). *Anopheles gambiae sensu stricto (s.s.)* is one of the most efficient malaria vectors in Nigeria (Akpan et al., 2018). This complex includes several morphologically indistinguishable sibling species, each with different behaviors and ecological adaptations. *Anopheles arabiensis*, on the other hand, exhibits more opportunistic feeding behavior, feeding on both humans and animals, while *Anopheles coluzzii* is adapted to breeding in permanent water bodies (Habibu *et al*., 2017). *Anopheles coluzzii* which is previously considered a molecular form of *Anopheles gambiae s.s., Anopheles coluzzii* is now recognized as a distinct species (Ibrahim, 2014). It is adapted to breeding in permanent water bodies and is also an important malaria vector in Nigeria. This research helped collate information, malaria vector control efforts risk becoming ineffective, leading to increased transmission rates and higher disease burden. Understanding the specific Anopheline species present in Nigeria is crucial for designing effective malaria control strategies. Differences in behavior, breeding habitats, and insecticide resistance patterns among these species require tailored approaches to effectively reduce malaria transmission. For instance, the high anthropophilic of Anopheles gambiae s.s. necessitates interventions that target human-mosquito contact, such as ITNs, while the varied feeding habits of Anopheles arabiensis may require integrated vector management strategies that address both human and animal hosts (Shehu et al., 2023). This study aims to address this gap by understudying the mosquito's larvae habitat characterization and ascertaining the mosquito's fauna found in these areas. This essential data will help inform and optimize local and state wide-level malaria vector control programs.

2.0 MATERIAL AND METHODS

2.1 Study Sites

The study was carried out in three selected local government areas in Ekiti State, located in South-West Nigeria. Ekiti State is situated in the tropical region, bounded by longitudes 40^{O} 51" and 50^{O} 451" East of the Greenwich meridian and latitudes 70^{O} 151" and 80^{O} 51" North of the Equator. The state is divided into 16 local government areas (LGAs), comprising 13 rural, 2 peri-urban and 1 urban area. The state comprises of three senatorial districts namely Ekiti Central, Ekiti South, and Ekiti North. The province covers a total land mass of 6,353km2 and a density of 514/km2. The population of the state is approximately 2.4 million people according to the 2006 population census but the 2016 estimates put the number of people in the state at 3.3 million (NBS, 2012). The State capital is in Ado-Ekiti which is both the commercial and political hub of the State (NBS, 2012). The selection of the LGAs was done to include one from each senatorial district and in which one from urban (Ado LGA), peri-urban (Ikere LGA) and rural LGA (Oye, LGA). The study sites were randomly selected within each study location and their respective coordinate were taken accordingly.

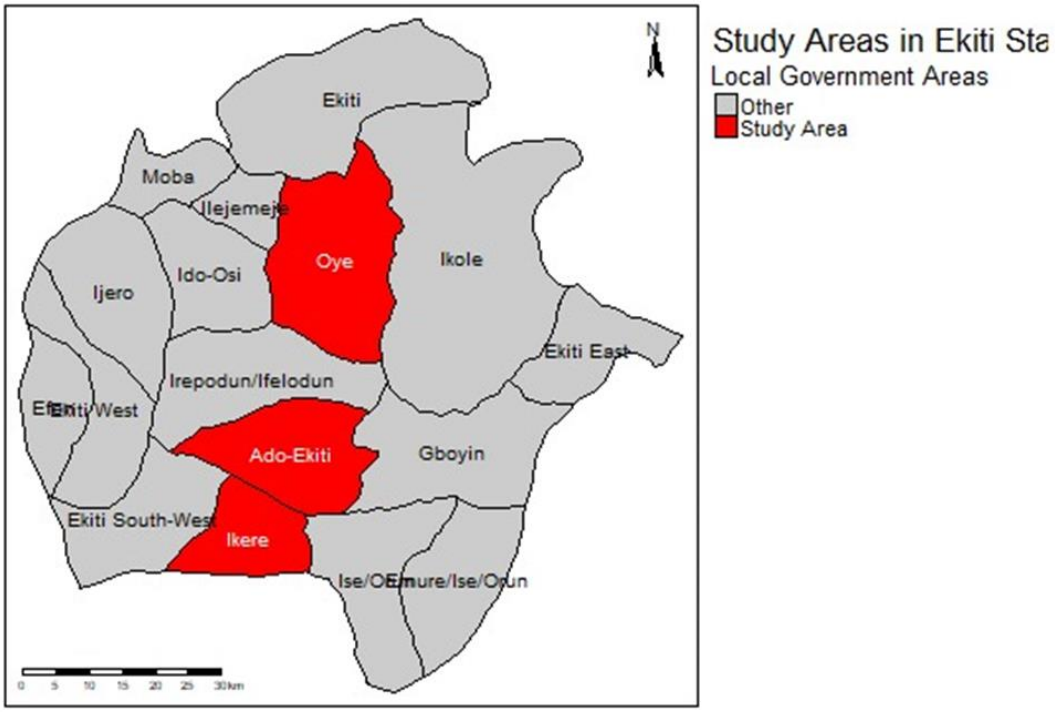

Figure 1: Map of Ekiti state showing the study areas.

2.2 Larval survey

Larvae sampling was conducted biweekly at specified locations from May to June 2024, during which mosquito larvae were collected. At each sampling visit, habitats were initially inspected for the presence of Anopheles mosquito larvae, and their coordinates were recorded using a GPS device (Garmin 20.0 eTrex). In addition, the specific locations and classifications of breeding sites were documented for each sampled habitat. Larvae were collected from a variety of sources, including puddles, urban areas, ponds, stagnant water, tree hollows, plant leaf axils, clay pots, discarded vehicle tyres, gutters, tyre tracks, and domestic containers.

2.3 Larvae habitat characterizations

The coordinates of each Anopheles mosquito larvae breeding habitat was recorded (**Table 1**). Habitat characteristics were observed and recorded using the WHO characterization form. The environmental features examined included the habitat's exposure to sunlight, water flow, presence of vegetation and algae, and whether the habitat was natural or artificial, as well as its permanence. Observations also included the clarity of the habitat and the present or absent of nearby human settlements. Light intensity was visually classified as either sunlight or shade. The type of substrate was noted, considering the presence of vegetation and algae, and whether the substrate was muddy or clear. Water current was visually assessed and categorized as either flowing or stagnant.

Table 1: The study locations, study sites and their respective coordinates

Study Locations	Study Sites	Latitude (N)	Longitude (E)
Ado-Ekiti	Ado poly	7.6111345	5.2807633
	Housing	7.644760	5.251476
	Opopogboro	7.660506	5.228161
Oye-Ekiti	Fallex	7.509404	5.225441
	Egbe	7.508934	5.227866
	Irare	7.530851	5.221124
Ikere-Ekiti	Idi Oshin	7.497687	5.246650
	Faleye	7°34′22.3	5°12′35.9
	Oke Ikere	7°30′01.6	5°13′39.5

2.4 Larvae collection

Habitat characteristics were recorded using a modified WHO larva habitat characterization form, examining factors like sunlight exposure, water flow, vegetation, algae presence, and proximity to human settlements. Physicochemical parameters such as temperature, pH, dissolved oxygen (DO), and conductivity were measured on-site with hand-held devices. Standard dipper sampling techniques were employed to collect mosquito larvae from all types of larval habitats, including both man - made and natural sources, at each study location throughout the mosquito breeding season, spanning from August 2024 to September 2024. Sampling was done twice a week in each study location. In cases where the use of dippers proved ineffective given the diminutive size of the habitat, collection of mosquito larvae was performed using Pasteur pipettes. Additionally, the entire water from such habitats was collected using a plastic scoop and transferred into small clear plastic bowls. To ensure an adequate number of adult mosquitoes for emergence, a strainer with a mesh size of 0.55mm was utilized to sieve and consolidate all the collected larvae. This pooling process allowed for a higher concentration of larvae in a single container. Subsequently, the bowls containing the larvae were carefully inspected to identify and remove any unwanted organisms or predators present, employing Pasteur pipettes for precise removal. The collected larvae and pupae were gently placed in clean jam containers, which were partially filled with water from their respective breeding sites, to ensure a suitable environment for further observation. Each container was meticulously labeled, indicating the specific time, date, and location of collection to facilitate easy identification. The precise locations of the study sites were determined using Global Positioning System (GPS) technology, allowing for accurate coordinate identification.

2.5 Rearing of the Mosquitoes Larvae to Adult

Mosquito larvae collected were carefully transported in clearly labeled plastic containers to the insectaries at the Entomology unit of the Department of Animal and Environmental Biology, Federal University Oye-Ekiti. There, the mosquito's larvae were raised to adulthood in the entomology unit. Larvae from different breeding sites were kept separately to ensure accurate species identification. In the laboratory, larvae were placed in individual bowls labeled accordingly and maintained under controlled conditions: room

temperature of 27°C and relative humidity of 82%. Adult mosquitoes were fed with yeast and the water in the bowls was regularly changed to prevent mortality from bacteria or algae growth.

2.6 Sample Preservation

All the Anopheles mosquitoes that were subjected to insecticides susceptibility test were identified morphologically and preserved in labeled Eppendorf tubes containing silica gel desiccant. Samples were kept in the laboratory at a temperature of 27±2°C.

2.7 Morphological Identification of Anopheles mosquitoes

Morphological identification was conducted using a stereomicroscope to identify the species group of Anopheles mosquitoes. Morphological keys from Gillies and Coetzee (Gillies and Coetzee, 1987) and (Coetzee and Koekemoer, 2013). were utilized as references during the identification process. These keys provided detailed information and characteristics necessary for distinguishing between different species and determining the sex of the mosquitoes accurately. This allows the percentage mortality by species group and species siblings to be properly calculated (WHO 2013b).

2.8 Molecular Identification of Sibling Species within the Anopheles gambiae complex

Molecular identification of sibling species within the *Anopheles gambiae complex* was conducted at the Molecular Entomology and Vector Control Research Laboratory of the Nigerian Institute of Medical Research (NIMR) in Yaba, Lagos State. In order to identify the sibling species, PCR amplification of DNA extracted from the legs and wings of *Anopheles gambiae s.s* specimens collected during the study was performed.

2.9 Data analysis

The results were presented as percentages and the environmental characteristics of habitat were assessed using a chi-square test. The distribution of Anopheles species groups, identified through both morphological and molecular methods, was also evaluated using a chi-square test. Differences in knockdown time and other relevant sample collections were assessed using the Wilcoxon Signed Ranks Test in SPSS version 27. Data were initially entered in Microsoft Excel and subsequently imported into RStudio for analysis. Descriptive statistics, including mean and standard deviation (SD), were calculated to summarize the physio-chemical parameters and essential elements of the locations using the group by function from the duly package. Analysis of Variance (ANOVA) was conducted to determine significant differences in means at a 95% confidence level, followed by Tukey's HSD post-hoc test to identify specific group differences. Mean values with similar superscripts across the columns indicate no significant difference.

3.0 RESULTS AND DISCUSSION

3.1 Characterization of larvae breeding habitats for Anopheles mosquito across the study locations

Table 2 shows the physio-parameters of waters measured across the sampled sites in the three Local Government Areas (LGAs) of Ado, Oye, and Ikere. The water bodies across Ado-Ekiti, Oye-Ekiti, and Ikere-Ekiti showed distinct characteristics based on their origin, permanency, and environmental features. Water bodies in Ado-Ekiti (Ado Poly, Housing, and Opopogboro) and Ikere-Ekiti (Idi-Osi, Oke, and Faleye) were primarily natural, while Oye-Ekiti (Faleex and Egbe) included manmade sources, except for Irare, which was natural. All the water bodies were temporary, with specific types varying from ponds and ditches to gutters and tire tracks. Most water currents were stagnant, except for slow-flowing water at Egbe in Oye-Ekiti. Turbidity varied significantly; Ado-Ekiti had a mix of polluted and clear water, while Ikere-Ekiti showed instances of dark and clear water, and Oye-Ekiti included polluted, clear, and turbid conditions. Substrate

types were predominantly muddy across locations, with some clear substrates at Faleye. Settlements were present near all water bodies, and sunlight was the dominant light source, except for Idi-Osi in Ikere-Ekiti, which had no light, and Faleye, which had partial sunlight. Vegetation was present in some areas, particularly in Ado Poly, Housing, Egbe, Oke, and Faleye, while others lacked vegetation. Algae were common in many locations, with exceptions at Egbe, Irare, and Idi-Osi, which showed no algae presence. These variations highlight the diversity in water body characteristics across the study areas.

3.2 Physio-chemical parameters larvae breeding habitats for Anopheles mosquito across the study locations

Table 3 shows the physio-chemical parameters of waters measured across the sampled sites in the three Local Government Areas (LGAs) of Ado, Oye, and Ikere. For temperature, Ado recorded a mean of 26.2 ± 1.37°C, which was significantly lower than Oye's 34.6 ± 2.94°C, but overlapped with Ikere's intermediate value of 29.3 ± 3.26°C ($p = 0.02$). The pH in Ado (8.24 ± 0.99) was significantly higher than Oye (5.07 ± 1.11) and Ikere (6.67 ± 1.16) ($p = 0.03$). Salinity showed the lowest mean in Ado (0.35 ± 0.40 ppm), followed by Oye (1.46 ± 0.24 ppm), while Ikere recorded the highest value (2.99 ± 0.72 ppm) ($p = 0.001$). Electrical conductivity (EC) did not vary significantly across locations, with means of 451 ± 137 µs/m in Ado, 329 ± 45.4 µs/m in Oye, and 337 ± 129 µs/m in Ikere ($p = 0.39$). Similarly, total dissolved solids (TDS) in Ado (350 ± 35.7 mg/l), Oye (235 ± 37.5 mg/l), and Ikere (388 ± 88.4 mg/l) showed partial overlap, with Ikere slightly distinct ($p = 0.05$). Dissolved oxygen (DO) exhibited no significant variation, with mean values of 23.7 ± 3.20 mg/l, 18.9 ± 1.99 mg/l, and 20.1 ± 3.47 mg/l for Ado, Oye, and Ikere, respectively ($p = 0.19$). Overall, temperature ($p = 0.02$), pH ($p = 0.03$), and salinity ($p = 0.001$) showed statistically significant differences among the LGAs, while EC ($p = 0.39$), TDS ($p = 0.05$), and DO ($p = 0.19$) did not.

3.3 Concentration of essential minerals across larvae breeding habitats for Anopheles mosquito across the study locations

Table 4 shows the concentration of essential elements across larvae breeding habitats for Anopheles mosquitoes varied significantly among the three LGAs studied. In Ado LGA, sodium (Na) concentrations ranged from 25.2 to 28.9 mg/l, with a mean of 26.6 ± 1.60 mg/l, while calcium (Ca) ranged from 47.4 to 58.3 mg/l, with a mean of 52.4 ± 4.83 mg/l. Magnesium (Mg) levels averaged 10.3 ± 0.81 mg/l, copper (Cu) 0.86 ± 0.06 mg/l, iron (Fe) 0.71 ± 0.16 mg/l, total nitrogen (T.N) 42.3 ± 4.45 mg/l, and total phosphate (T. Phosphate) 10.7 ± 2.19 mg/l. In Oye LGA, sodium concentrations were higher, with a mean of 63.4 ± 8.77 mg/l, calcium averaged 95.9 ± 7.78 mg/l, magnesium 14.3 ± 1.84 mg/l, copper 1.51 ± 0.57 mg/l, iron 2.57 ± 0.54 mg/l, total nitrogen 79.0 ± 19.0 mg/l, and total phosphate 21.5 ± 4.24 mg/l. In Ikere LGA, sodium concentrations ranged widely, with a mean of 28.0 ± 22.3 mg/l, calcium averaged 60.2 ± 28.8 mg/l, magnesium 9.74 ± 3.80 mg/l, copper 0.813 ± 0.24 mg/l, iron 0.74 ± 0.35 mg/l, total nitrogen 45.0 ± 12.5 mg/l, and total phosphate 9.82 ± 1.74 mg/l. Statistical analysis showed significant differences in the mean concentrations of sodium ($P = 0.000$), calcium ($P = 0.001$), magnesium ($P = 0.012$), copper ($P = 0.005$), iron ($P = 0.000$), total nitrogen ($P = 0.001$), and total phosphate ($P = 0.000$) in Oye LGA, compared to the other LGAs.

Table 2: Physical parameters of larvae breeding habitats for *Anopheles* mosquito in the study areas.

	Ado-Ekiti			Oye-Ekiti			Ikere-Ekiti		
	Ado Poly	**Housing**	**Opopogboro**	**Faleex**	**Egbe**	**Irare**	**Idi-Osi**	**Oke**	**Faleye**
Origin of water body	Natural	Natural	Natural	Manmade	Manmade	Natural	Manmade	Natural	Natural
Permanency	Temporary	Temporary	Temporary	Temporary	Temporary	Temporary	Temporary	Temporary	Temporary
Specific water body	Pond	Pond	Ditches	Gutter	Pound	Ditch	Tire track	Pond	Pond
Water current	Stagnant	Stagnant	Stagnant	Stagnant	Slow flowing	Stagnant	Stagnant	Stagnant	Stagnant
Turbidity	Polluted	Polluted	Clear	Polluted	Clear	Turbid	Clear	Polluted	Dark
Substrate type	Mud	Mud	Mud	Mud	Mud	Mud	Mud	Mud	Clear
Presence of settlement	Present	Present	Present	Present	Present	Present	Present	Present	Present
Light intensity	Sunlight	Sunlight	Sunlight	Sunlight	Sunlight	Sunlight	None	Sunlight	Partial
Presence of vegetation	Yes	Yes	No	No	Yes	No	No	Yes	Yes
Algae present	Yes	Yes	Yes	Yes	No	No	No	Yes	Yes

Table 3: Physio-chemical parameters of larvae breeding habitats for *Anopheles* mosquito in the study areas.

LGA	Sampling sites	Temp (ºC)	pH	EC (µs/m)	TDS	DO (mg/l)	Salinity (ppm)
Ado	Ado-Poly	27.4	8.3	554	381	27.4	0.25
	Housing	26.5	9.2	295	311	21.8	0.8
	Opopogboro	24.7	7.22	503	358	21.9	0.01
	Mean ± SD	26.2 ± 1.37^{a}	8.24 ± 0.99^{a}	451 ± 137^{a}	350 ± 35.7^{a}	23.7 ± 3.20^{a}	0.35 ± 0.40^{a}
Oye	Faleex	37.8	6.21	319	237	17.7	1.64
	Egbe	34.1	4	290	197	17.8	1.54
	Irare	32	5	379	272	21.2	1.19
	Mean ± SD	34.6 ± 2.94^{b}	5.07 ± 1.11^{b}	329 ± 45.4^{a}	235 ± 37.5^{a}	18.9 ± 1.99^{a}	1.46 ± 0.24^{ac}
Ikere	Idi-Oshi	25.7	5.9	475	485	20.5	3.64
	Okekere	32.1	8	315	367	23.3	2.21
	Faleye	30	6.1	220	312	16.4	3.11
	Mean ± SD	29.3 ± 3.26^{ab}	6.67 ± 1.16^{ab}	337 ± 129^{a}	388 ± 88.4^{ab}	20.1 ± 3.47^{a}	2.99 ± 0.72^{b}
	p-value	0.02	0.03	0.39	0.05	0.19	0.001

Table 4: Concentration of essential elements across larvae breeding habitats for *Anopheles* mosquitoes

LGA	Sampling sites	Na (mg/l)	Ca (mg/l)	Mg (mg/l)	Cu(mg/l)	Fe (mg/l)	T.N (mg/l)	T. PHOSPHATE (mg/l)
Ado	Ado Poly (1)	28.3	47.4	11.308	0.782	0.523	44.345	13.516
	Ado Poly (2)	28.9	47.8	11.316	0.778	0.52	44.36	13.509
	Housing (1)	25.8	51.3	9.859	0.876	0.729	36.614	9.274
	Housing (2)	25.4	51.6	9.862	0.882	0.735	36.622	9.278
	Opopogboro (1)	25.2	58.2	9.618	0.912	0.862	45.916	9.249
	Opopogboro (2)	25.8	58.3	9.624	0.917	0.87	45.91	9.253
	Mean ± SD	26.6 ± 1.60[a]	52.4± 4.83[a]	10.3± 0.81[a]	0.86 ± 0.06[a]	0.71 ± 0.16[a]	42.3 ± 4.45[a]	10.7 ± 2.19[a]
Oye	Faleex	52.8	88.5	12.428	0.926	2.077	55.262	21.307
	Faleex	52.3	88.9	12.433	0.932	2.07	55.256	21.314
	Egbe (1)	66.3	93.2	13.906	1.434	2.387	85.528	16.845
	Egbe (2)	66.2	93.6	13.914	1.428	2.374	85.534	16.842
	Irare (1)	71.6	105.4	16.502	2.178	3.246	96.343	26.311
	Irare (2)	71.5	105.7	16.497	2.19	3.244	96.338	26.316
	Mean ± SD	63.4 ± 8.77[b]	95.9 ± 7.78[b]	14.3 ± 1.84[b]	1.51 ± 0.57[b]	2.57 ± 0.54[b]	79.0 ± 19.0[b]	21.5 ± 4.24[b]

Ikere	Idi-Oshi (1)	10.8	55.8	8.968	0.528	0.624	40.786	10.313
	Idi-Oshi (1)	10.4	55.5	8.972	0.522	0.629	40.792	10.32
	Okekere (1)	16.9	30.3	5.917	0.873	0.415	33.608	7.674
	Okekere (1)	16.5	30.6	5.93	0.879	0.411	33.612	7.681
	Faleye (1)	56.7	94.4	14.315	1.036	1.169	60.59	11.465
	Faleye (1)	56.5	94.3	14.322	1.041	1.16	60.593	11.459
	Mean ± SD	28.0 ± 22.3[a]	60.2 ± 28.8[a]	9.74 ± 3.80[a]	0.813 ± 0.24[a]	0.74 ± 0.35[a]	45.0 ± 12.5[a]	9.82 ± 1.74[a]
	p-value	0.000	0.001	0.012	0.005	0.000	0.001	0.000

3.4 Molecular characterization of Anopheles species identified across the study LGAs

Table 5 shows the molecular characterization of Anopheles species across the study LGAs revealed variations in species distribution. In Ado LGA, 16 Anopheles mosquitoes were examined, with 11 (68.8%) identified as *An. coluzzii* and 5 (31.2%) remaining unidentified. In Ikere LGA, 15 mosquitoes were examined, of which 9 (60%) were *An. coluzzii* and 6 (40%) were unidentified. Oye LGA had the highest proportion of *An. coluzzii*, with 13 (81.3%) of the 16 mosquitoes examined, leaving 3 (18.7%) unidentified. (Figures 1-3). Overall, out of the 47 mosquitoes examined across all LGAs, 33 (70.2%) were identified as *An. coluzzii*, while 14 (29.8%) were unidentified. Statistical analysis showed no significant difference in the distribution of *An. coluzzii* and unidentified species across the LGAs (Chi-square = 1.696, p = 0.428). This indicates a relatively consistent pattern of species identification across the study locations.

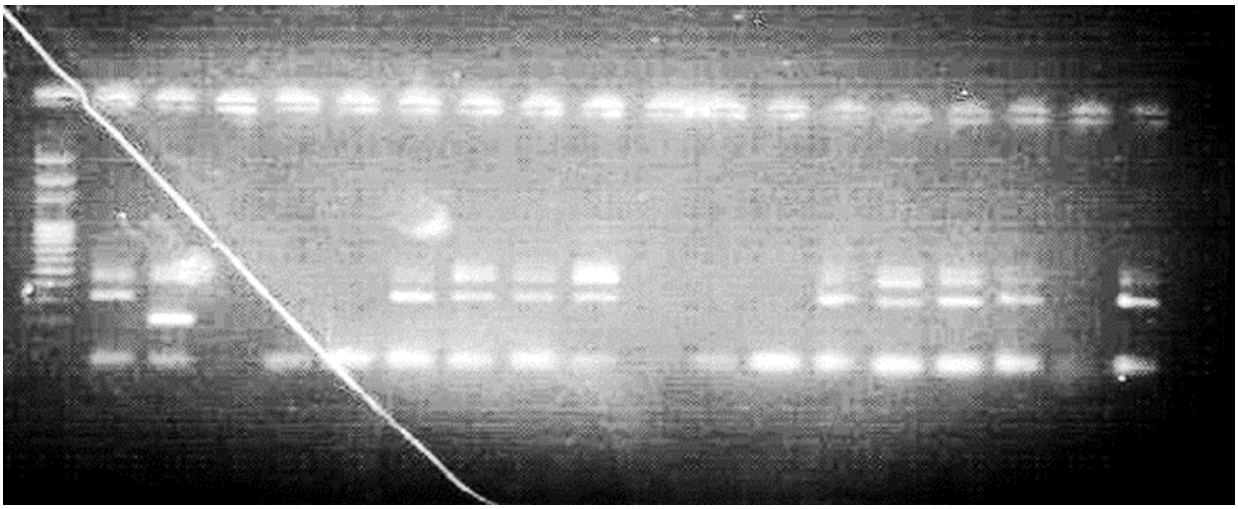

Figure 2: Electrophoretic gel of molecular identification of members of *An. gambiae s.l* showing the amplification of 463 and 333 bp for *An. Coluzzi* in Ikere LGA

Key: Lane 1 and 19: 100 bp DNA ladder,
Lane 1: 100 bp DNA ladder
Lane 2: Positive control for *Anopheles coluzzii* (463 and 333 bp)
Lane 3: Positive control for *Anopheles gambiae* (463 and 221 bp)
Lane 4: Negative control
Lane 7,8,9,10,14,15,16,17 and 19 are amplified are indicates *Anopheles coluzzii*
Lane 5,6,11,12,13 and 18 were unamplified.

Table 5: Molecular characterization of Anopheles species identified across the study LGAs

LGA	Total examined	*Anopheles*	*An. coluzzii*	Unidentified	Chi-square	p-value
Ado	16		11 (68.8)	5 (31.2)	1.696	0.428
Ikere	15		9 (60)	6 (40)		
Oye	16		13 (81.3)	3 (18.7)		
Total	47		33 (70.2)	14 (29.8)		

4.0 DISCUSSION

Studies on the habitats of Anopheles mosquitoes in Ado, Oye and Ikere LGAs of Ekiti State showed that temporary water sources are the preferred ones for larval development. This is because the habitats of these water bodies are stagnant, and they are suitable for the ecological requirements of Anopheles mosquitoes, as supported by an abundant literature of evidence (Adejanya et al., 2024). Water sources differed considerably, from natural ponds and streams to anthropogenic habitats like gutters, containers that humans had discarded, and tire tracks. The Anopheles larvae presence in different environments is similar to findings reported by (Ranson and Lissenden, 2016), stressing the need for environmental management to break their breeding cycles. Temperature, pH, and salinity were all found to have important roles in larval development at the three studied LGAs. For example, in Ado, high larval densities were supported by cooler temperatures (26.2°C) and a slightly alkaline pH (8.24). Conversely, Oye's warmer temperatures (34.6°C) and lower pH (5.07) severely curtailed larval growth. Similar correlations between water chemistry and mosquito population dynamics in West Africa have also been reported by (Hernández, 2020), whose findings resonate with the ones of this study. Polluted habitats around human settlements were also found in previous research study to worsen the risks of malaria transmission (Ranson and Lissenden, 2016). Anopheles larvae develop optimally in organic rich stagnant water bodies of poor sanitation conditions. The abundance of mineral nutrients and organic pollutants in larval habitats enhances the survival and adaptability of Anopheles larvae, particularly in urban environments. Studies have shown that members of the Anopheles gambiae complex can thrive in polluted water bodies characterized by high levels of dissolved solids, heavy metals, and organic matter (Awolola et al., 2007; Azrag and Mohammed, 2018). Similar findings have been reported in Nigeria and Senegal, where anthropogenic activities create nutrient-rich breeding sites that promote larval development and complicate malaria control efforts (Sabtiu et al.,2025; Ndiaye et al., 2024). Furthermore, *Anopheles coluzzii* has been consistently identified as the predominant sibling species across multiple African settings, including Nigeria and Ghana (Klinkenberg et al., 2008; Ezeike et al., 2026; Sabtiu et al.,2025). *Anopheles coluzzii* is well adapted to urban and peri-urban habitats and is often predominant in such environments due to its tolerance to polluted and organically enriched breeding sites (Awolola et al., 2007; Omotayo et al., 2022). This species has been reported as the most common member of the *Anopheles gambiae complex* in parts of southwestern Nigeria, particularly in urban settings such as Lagos (Omotayo et al., 2022), which aligns with this observation.

4.1 LIMITATIONS OF THE STUDY AND RECOMMENDATIONS FOR FURTHER STUDIES

This paper presents useful insights into the ecological and molecular features of Anopheles mosquitoes in selected LGAs of Ekiti State; yet, some limitations should be addressed. The study was limited to three LGAs and a brief sampling period, which may not reflect the complete regional and seasonal variety of mosquito populations and breeding habitats. The extremely restricted sample size for molecular analysis and the failure to identify 29.8% of specimens suggest limitations in the PCR technology utilised and the possible presence of cryptic species. In addition, the study focused only on the Anopheles gambiae complex, omitting other significant vectors such as *Anopheles funestus*. Detailed examination of pesticide resistance was also not included, and crucial climatic and socio-ecological issues such as rainfall and land use were not addressed.

Future studies should therefore increase geographical coverage and utilise longitudinal methodologies to capture seasonal fluctuations. The identification of species and the discovery of cryptic diversity will be enhanced by increasing sample size and utilising contemporary molecular tools such as next-generation sequencing. Additional vector species and thorough pesticide resistance profiling should be included in future studies. A more comprehensive understanding of the variables affecting mosquito reproduction and malaria transmission will also result from the integration of environmental and socio-ecological aspects.

4.2 CONCLUSION

The enumeration of mosquitoes across the three Local Government Areas (LGAs) in Ekiti State, South-West Nigeria, facilitated insights into the ecology and molecular composition of Anopheles mosquitoes. Based on the analysis, the following key conclusions were drawn: Anopheles mosquitoes were predominantly found in breeding sites characterized by Stagnant water, Sunlight exposure, Organic material, Proximity to settlements and Temporary water sources. Strong correlations were observed between larval density and physio-chemical parameters, supporting their importance in habitat suitability.

Morphological Identification showed that *An. gambiae complex* and *An. coluzzii* were the predominant species.
Molecular analysis confirmed *An. coluzzii* as the major sibling species across all LGAs. This research contributes important knowledge on malaria vector ecology and species dynamics in Ekiti State, Nigeria, and it is critical for malaria control strategies. It determined the main malaria vector species, primarily of the *Anopheles gambiae complex*, such as *An. coluzzii*, and the need to identify the species correctly to be able to target resources in an efficient way.

ACKNOWLEDGEMENTS

This study was carried out at the Department of Animal and Environmental Biology, Federal University OyeEkiti, Nigeria. We express our gratitude to the reviewers and editors from the Federal College of Education, Ilawe-Ekiti, and Lagos State University for their invaluable suggestions. We also thank Dr. Oyeniyi T.A. and Mrs. Izekor T.R., Laboratory Scientists at the Nigeria Institute of Medical Research (NIMR), for their technical assistance with the molecular aspects of the research. Gratitude is expressed to field and laboratory workers, together with local officials and community members in the designated Local Government Areas of Ekiti State for their collaboration.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

Victor Gboyega: Study design, field and lab investigation, original draft, funding acquisition; Adejumoke Ajayi: Supported the literature search and writing of the paper; Abiodun Adams: Drafting and Editing Manuscript, data analysis and interpretation; Hillary Okoh: Conceptualization and Supervision, Lab and field supervision. All authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

CONSENT

Informed consent was obtained from local community members within the study area, including a designated contact person, prior to the commencement of field activities.

ETHICAL APPROVAL

Research ethics committee approval was obtained from Faculty of Science Ethics committee, Federal University Oye, Ekiti State. In each visit to the study area, an informed consent was gotten from the local people within the vicinity and a contact within the study area was also carried along to show spots with the desired characteristics.

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