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LOCAL HABITAT CONDITIONS DRIVE MORPHOMETRIC VARIATIONS, BUT NOT SPATIAL PATTERNS OF *Anopheles* MOSQUITOES IN NORTH-CENTRAL, NIGERIA

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ABSTRACT
Morphological traits of species have been used to distinguish variations within and among closely related mosquito species, to understand how phenotypic adaptations can influence malaria transmission dynamics. Here, we investigated the morphometric variations of *Anopheles* larvae collected from diverse aquatic habitats. Mosquito larvae were reared to adulthood under controlled conditions, and external morphological parameters (i.e., antenna, palp, proboscis, thorax, abdomen, wing, femur, tibia, and tarsus) were measured using a calibrated dissecting microscope. Data were analysed using one-way Analysis of Variance (ANOVA) followed by DuncanMultiple Range Test (DMRT), with test of significance at P <0.05. We found significant interspecific differences (P <0.001) in most morphometric traits among mosquito species, with *An. coustani* consistently showed the largest body measurements, while *An. gambiae* showed the smallest dimensions. Despite differences in morphometric traits among mosquito species, we found little evidence to suggest that mosquito species varied in their spatial occurrence dynamics across the two LGAs, indicating an influence of local habitat conditions on certain phenotypic traits of mosquito species, but not their occurrence patterns. In the context of global change, there is an urgent need to continue investigating how local environmental conditions directly or indirectly impact vector-borne insects, considering morphological traits that are key to species' fitness and disease transmission ability.

Keywords: *Anopheles* mosquitoes, Local habitat conditions, Morphometrics, Spatial patterns, Global change

INTRODUCTION
Malaria remains a leading public health challenge in sub-Saharan Africa, especially Nigeria (World Health Organization [WHO], 2024). Vector competence and survival depend not only on species identity but also on phenotypic traits (Gupta *et al*., 2024). Morphometrics provides insights into adaptive variations within and among mosquito species (Martinet *et al*., 2021).However, the role of local habitat conditions in shaping morphometric traits and distribution of *Anopheles* mosquitoes in Nigeria remains underexplored.
The survival, host-seeking behavior and vectorial capacity of *Anopheles* mosquitoes are influenced by morphological traits (Chen *et al*., 2025). Until now, not much is known about how different habitat types and environmental conditions drive these morphometric variations in Nigeria North-Central. Existing knowledge limits prediction about adaptation and persistence of mosquito populations in various ecological environments.
Conventional entomological surveillance often neglects slight phenotypic variations that may affect mosquito survival, dispersal, and vectorial capacity. Through its focus on Awe and Nasarawa Eggon LGAs, this study provides location-specific insights on habitat-driven morphometric variations. The evidence is important for enhancing vector monitoring, formulating ecological-based control strategies and improving predictive models of malaria risk in diverse settings.
To this end, the present study set out to analyse the morphometric variations of Anopheles mosquitoes in Awe and Nasarawa Eggon Local Government Areas in the different habitat types as they relate to phenotypic adaptation with a view to influencing malaria transmission dynamics

MATERIALS AND METHODS
Study Area
The study was conducted in Awe and Nasarawa Eggon LGA of Nasarawa State, Nigeria. The possible risk map of the *Anopheles* breeding habitats is presented in Fig. 1.

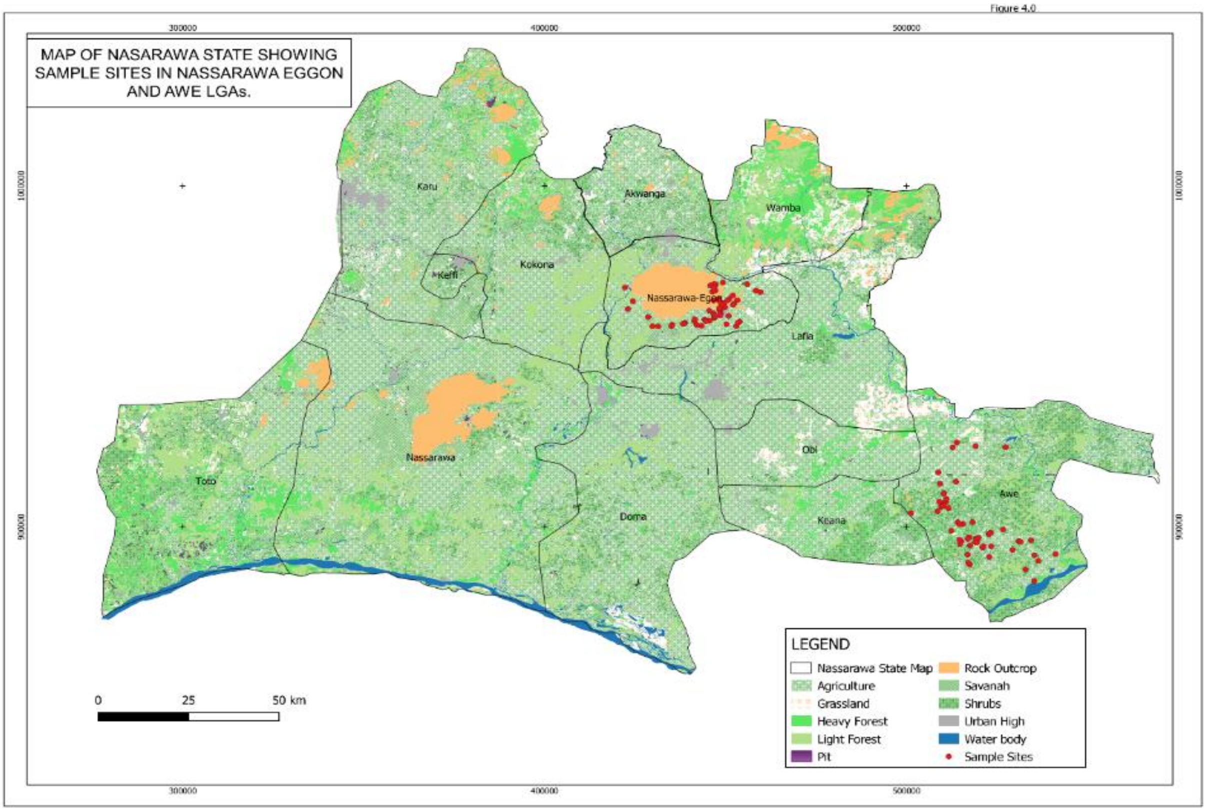

Figure 1: Possible risk map of malaria vectors breeding habitats in Awe and Nasarawa Eggon LGAs, Nasarawa State

Identification of breeding habitats

A survey was conducted to identify potential *Anopheles* mosquito breeding habitats. Coordinates were obtained with the aid of a Garmin GPS navigator (Egwu *et al*., 2018; Luiselli & Pacini, 2025) and a possible risk map was developed from it.

Rearing of *Anopheles* Mosquitoes

The larvae were identified based on their morphological characteristics (Gillies & Coetzee, 1987; Topluoglu *et al*., 2020). Larvae were reared following standard techniques in plastic cages (30 x 25 x 5 cm) and in larval bowls containing water obtained from their respective natural habitats (Lapang *et al*., 2019; Adejoh *et al*., 2025).

Emerged adult *Anopheles* mosquitoes were fed with 10 % sucrose solution (Ngowo *et al*., 2021; Adejoh *et al*., 2025), knocked down in a -20 ^{o}C freezer for 10 min, then further identified under a dissecting microscope using identification keys by Gillies and de Meillon (1968) as well as Coetzee (2020).

Data Analysis

Data analysis was conducted using R statistical software (version 4.4.1) and Minitab software (version 21.1). Descriptive statistics was used to determine the mean values and percentages of larval abundance in the study sites. Chi-square was used to compare abundance of *Anopheles* mosquito larvae between the two LGAs. Differences in mean morphometrics of Anopheles species collected at the study sites were compared using one-way ANOVA, followed by Duncan's Multiple Range Test (DMRT) for post-hoc analysis. The P-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Mean Morphometric Variations in *Anopheles* Mosquitoes across Different Habitat Types and Environmental Conditions in Awe and Nasarawa Eggon LGAs

Table 1 presents the mean morphometric variations among three *Anopheles* species collected from Awe and Nasarawa Eggon LGAs. *Anopheles coustani* was significantly larger (p<0.001) than both *An. gambiae* and *An. funestus* across all morphometric parameters except palp length, where no significant difference was observed (p=0.328). Notably, *An. gambiae* consistently exhibited the smallest body size dimensions, while *An. funestus* showed intermediate values.

The mean morphometric variations among *Anopheles* species collected specifically from Awe LGA. *Anopheles coustani* was significantly larger (p<0.001) than both *An. gambiae* and *An. funestus* across all eight significant morphometric parameters, consistently recording the highest mean values (Table 2). Conversely, *An. gambiae* exhibited the smallest body dimensions, while *An. funestus* showed intermediate values, with palp length being the only parameter that did not differ significantly among species (p=0.326).

Table 3 summarizes the mean morphometric measurements for antenna, palp, proboscis, thorax, abdomen, wing, femur, tibia, and tarsus among three

Anopheles species collected from Nasarawa Eggon LGA. One-way ANOVA revealed highly significant differences (p<0.001) for all parameters except palp length (p=0.289).

Table 1: Mean morphometric variations in *Anopheles* mosquitoes across different habitat types and environmental conditions in Awe and Nasarawa Eggon LGAs

***Anopheles* species**	**Morphometric Parameters (mm)**								
	Antenna	**Palp**	**Proboscis**	**Thorax**	**Abdomen**	**Wing**	**Femur**	**Tibia**	**Tarsus**
An. gambiae	1.05±0.11[c]	0.62±0.08[a]	1.468±0.16[c]	0.78±0.08[c]	1.14±0.12[c]	1.34±0.15[c]	0.97±0.11[c]	0.97±0.10[c]	2.65±0.29[c]
An. funestus	1.61±0.12[b]	2.86±1.93[a]	2.22±0.16[b]	1.19±0.08[b]	1.80±0.13[b]	2.09±0.15[b]	1.51±0.11[b]	1.49±0.10[b]	4.02±0.28[b]
An. coustani	3.05±0.045[a]	3.41±1.11[a]	3.64±0.04[a]	1.92±0.02[a]	2.97±0.03[a]	3.43±0.04[a]	2.48±0.03[a]	2.45±0.03[a]	6.51±0.08[a]
df	2	2	2	2	2	2	2	2	2
f	190.716	1.117	139.920	139.354	147.137	146.313	146.636	142.068	128.491
P value	<0.001*	0.328	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

Mean ± Standard Error (M±S.E.), *= Significant at p<0.05
NB: Values within the same column with no common superscript differed significantly according to Duncan Multiple Range Test (DMRT)

Table 2: Mean morphometric variations in *Anopheles* mosquitoes across different habitat types and environmental conditions in Awe LGA

***Anopheles* species**	**Morphometric Parameters (mm)**								
	Antenna	**Palp**	**Proboscis**	**Thorax**	**Abdomen**	**Wing**	**Femur**	**Tibia**	**Tarsus**
An. gambiae	1.21±1.39[c]	0.73±0.93[a]	1.73±1.94[c]	0.93±1.01[c]	1.36±1.54[c]	1.59±1.79[c]	1.16±1.29[c]	1.15±1.29[c]	3.10±3.49[c]
An. funestus	1.73±1.41[b]	1.07±1.01[a]	2.38±1.90[b]	1.26±0.98[b]	1.91±1.54[b]	2.20±1.76[b]	1.59±1.27[b]	1.58±1.26[b]	4.23±3.39[b]
An. coustani	3.02±0.83[a]	3.28±19.35[a]	3.67±0.69[a]	1.93±0.37[a]	2.99±0.62[a]	3.47±0.66[a]	2.51±0.47[a]	2.45±0.48[a]	6.54±1.47[a]
df	2	2	2	2	2	2	2	2	2
F	88.20	1.13	65.39	64.47	70.02	71.03	71.00	67.12	60.98
P Value	<0.000	0.326	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000

M±S.E., *= Significant at p<0.05
NB: Values within the same column with no common superscript differed significantly according to Duncan Multiple Range Test (DMRT)

Table 3: Mean morphometric variations in *Anopheles* mosquitoes across different habitat types and environmental conditions in Nasarawa Eggon LGA

***Anopheles* species**	**Morphometric Parameters (mm)**								
	Antenna	**Palp**	**Proboscis**	**Thorax**	**Abdomen**	**Wing**	**Femur**	**Tibia**	**Tarsus**
An. gambiae	0.86±1.34[c]	0.49±0.87[a]	1.15±1.78[c]	0.62±0.94[c]	0.91±1.41[c]	1.06±1.65[c]	0.77±1.19[c]	0.77±1.19[c]	2.09±3.23[c]
An. funestus	1.49±1.42[b]	0.79±0.92[a]	2.06±1.91[b]	1.11±1.01[b]	1.68±1.54[b]	1.97±1.79[b]	1.42±1.30[b]	1.40±1.29[b]	3.82±3.49[b]
An. coustani	3.08±0.85[a]	3.61±22.52[a]	3.59±0.89[a]	1.91±0.44[a]	2.94±0.77[a]	3.38±0.84[a]	2.44±0.60[a]	2.43±0.61[a]	6.47±1.68[a]
df	2	2	2	2	2	2	2	2	2
f	102.38	1.25	73.33	73.92	75.81	73.87	74.27	73.88	68.17
P value	<0.000	0.289	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000

M±S.E., *= Significant at p<0.05
NB: Values within the same column with no common superscript differed significantly according to Duncan Multiple Range Test (DMRT)

The analysis showed some level of difference in some morphological characters between the *Anopheles* species from Awe and Nasarawa Eggon LGAs. In particular, the measurements of the antenna, proboscis, thorax, abdomen, wings, femur, tibia and tarsus were found to be significantly different while the length of the palps did not show significant difference. Results indicate that the environmental and ecological factors unique to each LGA strongly impact *Anopheles* mosquitoes' (Agyekum *et al.*, 2021) physical development. For instance, differences in antenna and proboscis lengths indicate regional adaptations for feeding behaviour and host availability (Zhou *et al.*, 2022). According to research by Arora *et al.* (2023), it might be easier for *Anopheles* mosquitoes with longer mouths to draw blood from a variety of hosts present in each habitat. Additionally, the change in size of the thorax and abdomen may reflect adaptations to different modes of flight and reproduction which may assist survival and reproduction in the differing environmental conditions of Awe and Nasarawa Eggon. The changes in size and shape of the wing as well as the size of the legs (femur, tibia and tarsus) also point to the fact that due to local habitat conditions, the characteristics of the mosquitoes are also changing. Changing the size or shape of wings may facilitate more efficient flight and dispersal while changing the legs' morphology may impact the ability of the mosquitoes to manoeuvre through the type of vegetation or substrate available in each LGA (Chen *et al.*, 2025). These morphological features are directly linked to the ecological dynamics of the mosquitoes as well as their success in their habitats. The similarity between the palp lengths indicates that this trait is stable across diverse environments. Palps probably play a sensory and host location role of which has resulted

their evolutionary stability. The observation that palp length is similar across different habitats indicates that this must be a strong evolutionary constraint that the mosquitoes cannot do without.
According to the findings of this study, the environmental factors could have an impact on the morphology of an insect. Studies by Agyekyum *et al*. (2021) noted that the physical environment, such as increased temperature and humidity, could determine the size and shape of mosquitoes, giving context to our findings. Avramov *et al*. (2023) also mentioned that habitat-specific conditions like water quality and vegetation influence mosquito morphology. The findings of the current study were similar. It means have local environmental effects on the physical characteristics of an *Anopheles* population.

CONCLUSION AND RECOMMENDATIONS

Nasarawa Eggon and Awe LGAs differ significantly in their morphological characteristics. The findings showed significant variations in the body parts of the mosquito antenna, proboscis, thorax, abdomen, wing, femur, tibia, and tarsus. However, the length of the palps did not show statistically significant differences. In line with this, molecular screening of the *Anopheles gambiae* complex should be extended to both study sites in order to distinguish the sibling species and corroborate the observed morphometric variations.

Conflict of interest: The authors declare no conflicts of interest.

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