



Original Article

## *Anopheles* species diversity and potential vectors of zoonotic malaria in Central Kalimantan, Indonesia

Diversitas spesies *Anopheles* dan vektor potensial malaria zoonotik di Kalimantan Tengah, Indonesia

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### ABSTRACT

Zoonotic malaria is an emerging public health issue in Indonesia, particularly in regions with significant forest cover and frequent interaction between humans and non-human primates. Information regarding the diversity and behaviour of mosquito vectors in these areas is very limited. This study aims to identify the diversity and bionomics of mosquitoes, particularly *Anopheles* sp., across five ecologically diverse locations in Palangkaraya, Central Kalimantan, to gain an understanding of their potential role in zoonotic malaria transmission. Cross-sectional entomological surveys were conducted during November 2022 (during the rainy season) and October 2024 (at the beginning of the rainy season). Adult mosquitoes were collected using human landing catches (HLC) from 18:00 to 06:00, and larval habitats were surveyed and characterized following the WHO protocol. A total of 1,278 mosquitoes were collected from six genera, with *Anopheles letifer* Sandosham dominating the *Anopheles* group (97%), particularly in swampy secondary forests, such as Nyaru Menteng. This species exhibited peak outdoor biting activity shortly after dusk and late at night, posing a greater risk to those outside or in unscreened homes. Larval surveys conducted at 48 sites revealed *Anopheles* larvae in 27% of habitats, predominantly in swamps (67% positive). Although no significant associations were found with environmental factors, natural and semi-natural water bodies appear key to sustaining vector populations. These findings highlight the ecological flexibility of *An. letifer* and its potential impact on zoonotic malaria transmission in forest-edge communities. Vector control strategies should include integrated methods, such as larval source management and community-based interventions, particularly in peat swamp regions where interactions between humans and non-human primates are frequent.

**Key words:** *Anopheles*, Kalimantan, malaria, mosquito, zoonoses

### ABSTRAK

Malaria zoonotik merupakan penyakit tular vektor yang menjadi salah satu isu kesehatan di Indonesia, khususnya di kawasan hutan yang sering terjadi interaksi antara manusia dan satwa primata. Informasi mengenai keanekaragaman dan perilaku vektor nyamuk di daerah berisiko masih sangat terbatas. Penelitian ini bertujuan untuk mengidentifikasi keragaman dan bionomik nyamuk, khususnya *Anopheles* sp., di lima lokasi dengan kondisi ekologi yang beragam di Palangkaraya, Kalimantan Tengah, guna memahami potensi peranannya dalam penularan malaria

zoonotik. Survei entomologi potong lintang dilakukan pada November 2022 (musim hujan) dan Oktober 2024 (awal musim hujan). Nyamuk dewasa dikoleksi menggunakan metode *human landing catches* (HLC) dari pukul 18:00-06:00, serta dilakukan survei dan karakterisasi habitat larva dengan menggunakan protokol WHO. Terdapat 1.278 nyamuk berhasil dikoleksi dari enam genus, dengan *Anopheles letifer* Sandosham mendominasi kelompok *Anopheles* (97%), terutama di hutan sekunder rawa seperti Nyaru Menteng. Spesies ini menunjukkan aktivitas menggigit di luar ruangan yang mencapai puncaknya sesaat setelah senja dan menjelang akhir malam. Kondisi ini meningkatkan risiko bagi individu yang berada di luar ruangan atau tinggal di rumah tanpa pelindung. Survei larva pada 48 lokasi menunjukkan keberadaan larva *Anopheles* di 27% habitat, terutama pada habitat rawa (67%). Meskipun tidak ditemukan hubungan yang signifikan secara statistik antara keberadaan larva dan faktor lingkungan tertentu, habitat alami dan semi-alami tampaknya berperan penting dalam mendukung populasi vektor. Hasil penelitian menunjukkan fleksibilitas ekologis *An. letifer* dan potensi dampaknya terhadap penularan malaria zoonotik di komunitas pinggir hutan. Strategi pengendalian vektor perlu mencakup pendekatan terpadu seperti pengelolaan habitat dan intervensi berbasis masyarakat, terutama di wilayah rawa gambut yang memiliki tingkat interaksi tinggi antara manusia dan satwa primata.

**Kata kunci:** *Anopheles*, Kalimantan, malaria, nyamuk, zoonosis

## INTRODUCTION

Zoonotic malaria is an emerging public health issue, particularly in the Western part of Indonesia, including Kalimantan, where extensive forested regions create ideal environments for *Anopheles* mosquitoes (Ministry of Health of the Republic of Indonesia 2022). Although zoonotic malaria, primarily caused by *Plasmodium knowlesi*, has been widely reported in Malaysian Borneo, there are fewer recorded cases in Kalimantan (Indonesia part of Borneo) (Sugiarto et al. 2022). This difference prompts inquiries into vector distribution, human exposure, and the effectiveness of surveillance (Thevasagayam & Choon 1979; Wharton et al. 1963). In comparison to Malaysia, where zoonotic malaria accounts for 87.4% of malaria cases, Indonesia reports only 4.4% of malaria infections caused by *P. knowlesi*, the predominant agent of zoonotic malaria in Southeast Asia (WHO 2025). Variations in mosquito ecology and biodiversity might explain this difference. At least five mosquito species are confirmed vectors of zoonotic malaria, and four more are suspected to play a role in its transmission.

In contrast, only two species have been recorded in Indonesia. Historical and contemporary entomological research has noted several *Anopheles* species in Kalimantan. Early investigations highlighted *Anopheles roperi* Reid, *Anopheles letifer* Sandosham, and *Anopheles barbirostris* van der Wulp as key vectors (Sugiarto et al. 2022). *Anopheles letifer*, *Anopheles baezai* Gater, and *An. roperi*, which belong to the *Anopheles umbrosus* (Theobald) group, have demonstrated varying levels of competence as malaria vectors for both humans and mouse deer (Wharton et al. 1963).

A comprehensive survey of malaria vectors in South Kalimantan identified *Anopheles leucosphyrus*

Dönitz and *Anopheles balabacensis* Baisas as the dominant species, with sporozoite rates of 1.0% and 1.3%, respectively (Bin Said et al. 2022; Harbach 1987). Research conducted on Sebatik Island in North Kalimantan identified *Anopheles vagus* Dönitz, *Anopheles sundaicus*, (Rodenwaldt) and *Anopheles subpictus* Grassi as the most prevalent species, with both *Anopheles peditaeniatus* (Leicester) and *An. sundaicus* testing positive for *Plasmodium falciparum* (Sugiarto et al. 2017). Additionally, molecular investigations in Kotabaru, South Kalimantan, confirmed the presence of *Plasmodium vivax* in *An. vagus*, *An. peditaeniatus*, and *Anopheles tessellatus* Theobald, thus broadening the list of malaria vectors in the area (Sugiarto et al. 2022b). Deforestation, agricultural land conversion, and urban sprawl have notably changed mosquito habitats in Kalimantan. Research conducted in Sarawak revealed that alterations in land use impact the abundance and behaviour of *Anopheles* species, thereby affecting malaria transmission dynamics (Chang et al. 1997). Similar trends are anticipated in Kalimantan, where extensive oil palm plantations and logging activities develop new breeding sites (Reid & Weitz 1961).

Even with capable vectors, the low incidence of *P. knowlesi* reported in Kalimantan indicates potential underdiagnosis or variations in human-vector contact patterns. Recent molecular studies have identified *P. knowlesi* infections in a limited number of human cases in Central and South Kalimantan; however, standard diagnostic methods often struggle to distinguish *P. knowlesi* from other *Plasmodium* species (Ompusunggu et al. 2015).

This research aims to explore the diversity of mosquitoes, particularly *Anopheles* species, in regions with a high risk of zoonotic malaria transmission in

Central Kalimantan. This study intends to identify vector mosquitoes and their bionomic traits, laying a vital groundwork for effective malaria control strategies. This study strongly believes that precise identification is a critical initial step in effectively managing vector-borne diseases.

METHODS

Study site and location

This cross-sectional study was conducted in Palangkaraya City, the capital of Central Kalimantan, in November 2022 (during the rainy season) and October 2024 (at the beginning of the rainy season). Five study sites were selected to represent the primary ecological and social environments in Central Kalimantan: a forest conservation area, the forest-settlement boundary, an agricultural plantation, an urban residential zone, and a riverside settlement. (see Table 1). These sites reflect the typical ecological conditions of Central Kalimantan, where human-forest interactions take place.

Sample collection

**Human landing catch (HLC).** The entomological surveys included collecting adult mosquitoes and assessing breeding sites. The protocol for mosquito collection was adapted from Russell et al. with several modifications (Russell et al. 2022; WHO 2013). Collection at each sampling station occurred over one night, from 18:00 to 06:00. Each station comprised four houses situated at the forest’s edge or within the forest. Participants took part in the mosquito collection process. Mosquitoes were collected both indoors and outdoors using the HLC method. To reduce collection bias, a set number of houses was used for HLC, and all collectors underwent training before the survey.

Before beginning the HLC, participants were briefed on the study’s objectives and purpose, and those who consented voluntarily signed an informed consent form. All mosquitoes collected during the study were killed using chloroform and preserved in tubes filled with silica gel for future identification.

**Larva habitat survey.** The mosquito breeding site survey was conducted in residential areas, focusing on natural breeding habitats and checking for larvae. For each site, key characteristics such as water source type, vegetation cover, and surrounding environmental conditions were recorded, along with GPS coordinates for spatial analysis. Larval collection followed WHO guidelines, using standard dippers (350 ml) for natural habitats and pipettes for small containers, with at least 10 dips per habitat to ensure consistency across sites. To evaluate whether the measured environmental factors were associated with the presence of *Anopheles* spp. larvae, statistical analyses were conducted using the chi-square test. Statistical significance was determined using p-values, with  $p < 0.05$  considered indicative of a significant association.

Molecular analyses

The methodology employed in the extraction of deoxyribonucleic acid (DNA) and the subsequent polymerase chain reaction (PCR) amplification techniques to detect the presence of the parasite *Plasmodium* sp. in *Anopheles* sp. samples collected in October 2024 has been previously documented in the existing literature (Permana et al., 2023). For detecting *Plasmodium* sp., a semi-nested PCR assay targeting the *small subunit ribosomal RNA (ssrRNA)* gene was performed. The first round used primers

Table 1. Geographic and ecological profile of study sites

Location	Coordinates (Lat, Long)	Altitude (m asl)	Habitat type	Vegetation cover	Human settlement density	Land use	Proximity to water source
Nyaru Menteng, Tumbang Tahai	-2.034544°; 113.778110°	0	Secondary forest	Dense	Medium	Mixed farm and forest, wildlife conservation	Swamp
Bukit Tangkiling Natural Park, Banturung	-1.998873°; 113.754273°	0	Secondary forest	Dense	Low	Suburban housing	Artificial pond
Jalan Pariwisata, Banturung	-2.003132°; 113.723928°	0	Plantation	Sparse	Low	Agricultural land	Irrigation ditches
Airport employee official residence of Tjilik Riwut, Panarung	-2.221337°; 113.945340°	0	Residential	Sparse	High	Urban housing	Roadside ditch
Kampung nelayan, Tangkiling	-1.980626°; 113.763446°	0	Riverside residential area	Moderate	High	Suburban housing	River

rplU1 (5'-TCAAAGATTAAGCCATGCAAGTGA-3') and rplU5 (5'-CCTGTTGTTGCCTTAAACTCC-3'), followed by a second PCR with primers rplU1 and rplU4 (5'-TACCCGTCATAGCCATGTTAGGCAATACC-3'). The PCR protocol adhered to the method described by Singh et al., (1999). Amplicons were examined through gel electrophoresis on 1–2% agarose gels. The resulting *ssrRNA* gene sequences were compared against the NCBI nr database using BLAST to verify species identity.

## RESULTS

### Human landing catch (HLC)

A total of 1,278 mosquito specimens from six genera, such as *Anopheles*, *Armigeres*, *Aedes*, *Coquillettidia*, *Culex*, and *Mansonia*, were identified. Of the three *Anopheles* sp. collected, *An. letifer* was the predominant species, and the other species included *An. umbrosus* and *An. tessellatus*. *An. letifer* was mainly collected in Nyaru Menteng, adjacent to the Orangutan Conservation Center. Other genera, such as *Armigeres subalbatus*, (Coquillett) were mainly collected in Nyaru Menteng, with a much smaller amount in Bukit Tangkiling. Genus *Aedes* collected included *Aedes (verrillina)*, *Aedes albopictus* (Skuse), and *Aedes vexans* (Meigen), but the latest species was found in low numbers in various locations. The genus *Coquillettidia* includes *Coquillettidia crassipes* (van der Wulp), which was observed in Nyaru Menteng with limited quantities (Table 2).

Genus *Culex* includes *Culex bitaeniorhynchus* Giles, *Culex gelidus* Theobald, *Culex hutchinsoni* Barraud, *Culex*

*quinquefasciatus* Say, *Culex sitiens* Wiedemann, *Culex tritaeniorhynchus* Giles, and *Culex vishnui* Theobald,, and all were widely distributed. The most prevalent genus collected was *Mansonia*, which includes *Mansonia annulata* Leicester, *Mansonia annulifera* (Theobald), *Mansonia bonneae* Edwards, *Mansonia dives* (Schiner), and *Mansonia indiana* Edwards (Table 2).

This genus was particularly abundant in the official residence of Tjilik Riwt, an airport employee (53%), followed closely by Nyaru Menteng (42%). Human landing catch data indicated that the biting activity of *An. letifer* begins shortly after dusk and reaches a significant peak in 20-21, and it may bite well into the early morning 02-03 (Figure 2). The overall mean human-biting rate (HBR) for *An. letifer*, combining both indoor and outdoor locations, was 5.48.

### Vector incrimination for zoonotic malaria

The PCR amplification of *Anopheles* samples collected in October 2024 through HLC revealed that none were positive for the presence of *Plasmodium* sp. DNA.

### Larva habitat survey

A total of 48 larva habitats were surveyed, which included ditches, man-made containers, ponds, rain pools, seepages, springs, stream margins, swamps, and water-filled wrecks (Figure 3; Table 3). Ditches represented the most frequently surveyed habitat (15 sites). Of the 48 larva habitat surveys, 13 sites were positive for *Anopheles* larvae (27%) (Table 3), such as swamps (67%), seepages (50%), ponds (40%),

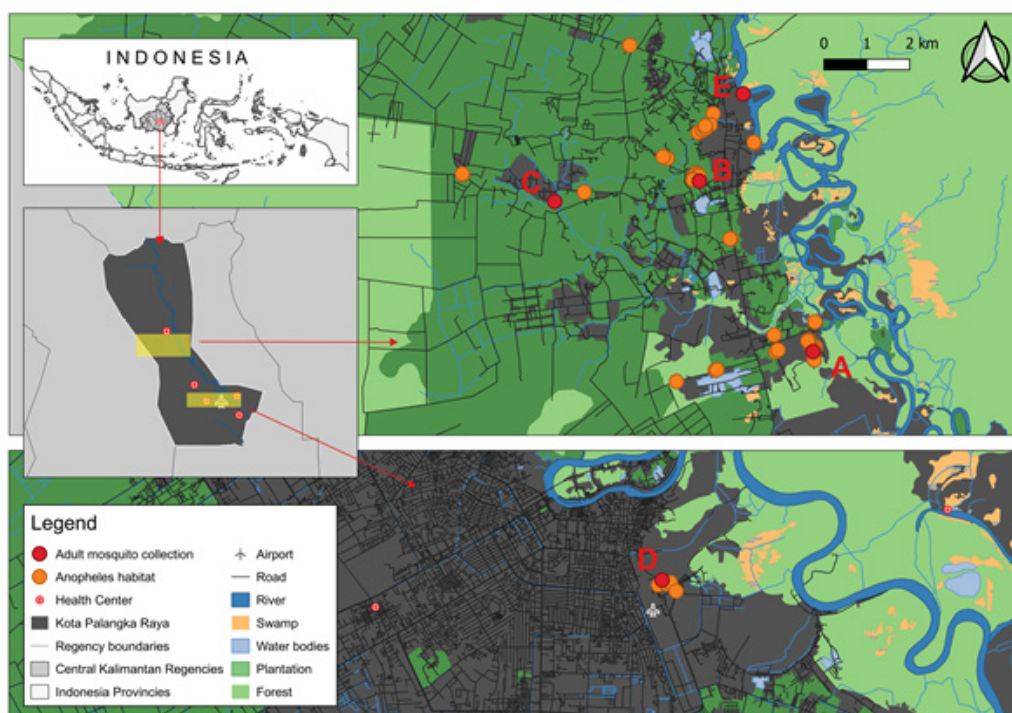


Figure 1. Study site and location (Indonesia Geospatial 2025).



**Table 2.** Composition of mosquito fauna at study locations

Species*	Location					HBR**	95% CI***
	Nyaru Menteng, Tumbang Tahai	Bukit Tangkiling Natural Park, Banturung	Jalan Pariwisata, Banturung	Airport employee official residence of Tjilik Riwut, Pantarung	Kampung nelayan, Tangkiling		
<i>Anopheles letifer</i>	199	11	4	1	4	5.48	4.77–6.25
<i>Anopheles umbrosus</i>	5	0	0	0	0	0.13	0.04–0.29
<i>Anopheles tessellatus</i>	1	0	0	0	0	0.03	0.001–0.14
<i>Armigeres</i> ( <i>Armigeres subalbatus</i> )	87	6	0	0	24	2.93	2.42–3.51
<i>Aedes</i> ( <i>Aedes (verralina)</i> , <i>Aedes albopictus</i> , <i>Aedes vexans</i> )	5	5	1	1	6	0.45	0.27–0.71
<i>Coquillettidia</i> ( <i>Coquillettidia crassipes</i> )	3	0	0	0	0	0.08	0.02–0.22
<i>Culex</i> ( <i>Culex bitaeniorhynchus</i> , <i>Culex gelidus</i> , <i>Culex hutchinsoni</i> , <i>Culex quinquefasciatus</i> , <i>Culex sitiens</i> , <i>Culex tritaeniorhynchus</i> , <i>Culex vishnui</i> )	82	3	40	34	35	4.85	4.19–5.58
<i>Mansonia</i> ( <i>Mansonia annulata</i> , <i>Mansonia annulifera</i> , <i>Mansonia bonneae</i> , <i>Mansonia dives</i> , <i>Mansonia Indiana</i> )	302	15	19	385	0	18.03	16.73–19.39

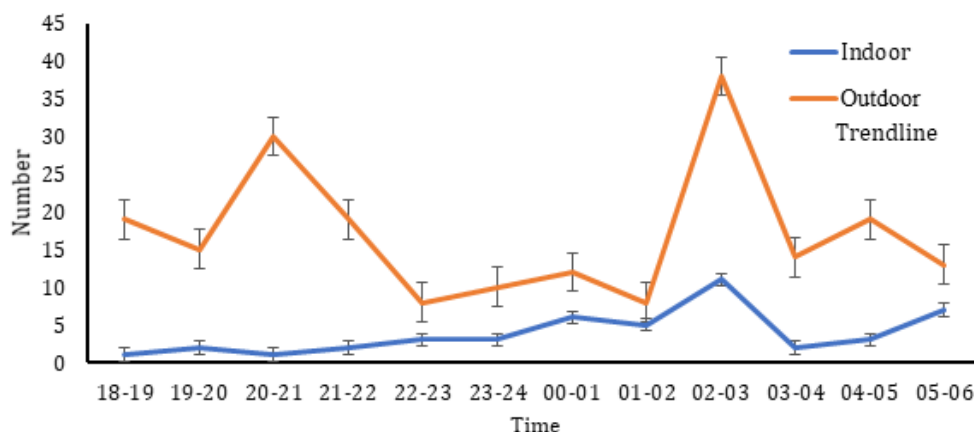
\*: *Anopheles* were identified to species level; other genera were species-identified from a random subset of samples; \*\*: Human biting rate (number of mosquitoes collected per person per night); \*\*\*: 95% coincidence interval, represents the precision of the HBR estimate.

and rain pools (21%). The study investigated various habitat factors affecting the presence of *Anopheles* mosquitoes across different breeding sites (Figure 1; Table 3). No significant statistical correlations were identified between the presence of *Anopheles* and the environmental factors analyzed (p-value > 0.05).

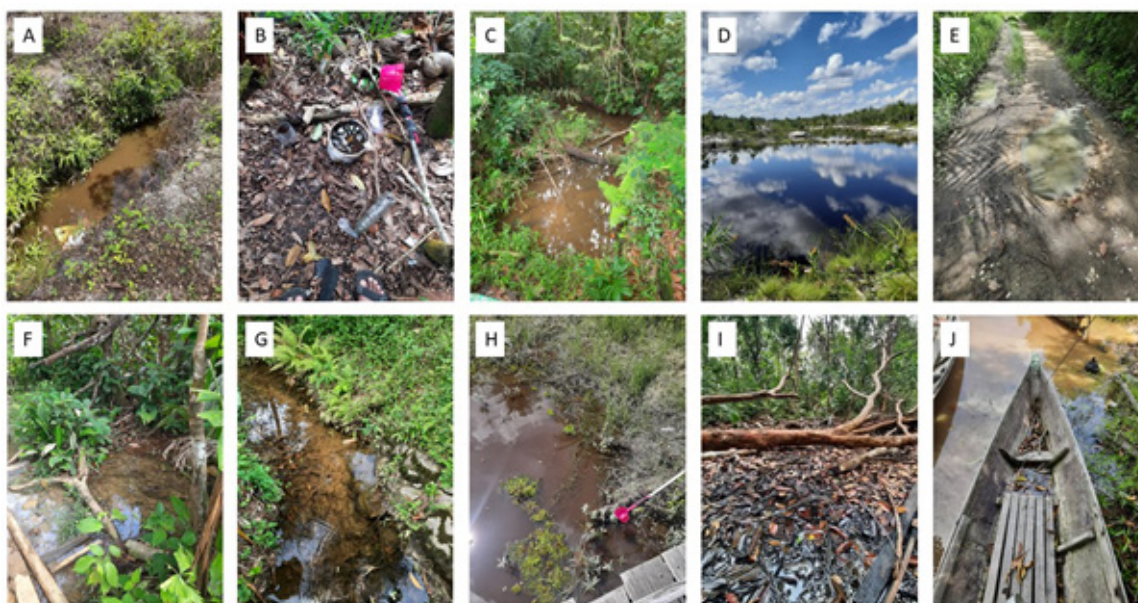
**DISCUSSION**

Biodiversity exerts a considerable influence on human health, particularly in the context of diseases that depend on both reservoir hosts and vectors for transmission. Alterations in biodiversity can influence the risk of disease spread, with this influence being either positive or negative, depending on the nature of the ecological change in question. Ostfeld & Keesing’s “dilution effect” hypothesis posits that greater biodiversity may lead to a reduction in disease transmission by spreading pathogens among a diverse array of host species with differing competency levels. Consequently, the probability of a pathogen being

transmitted back to humans or vectors is reduced due to the presence of less competent or non-amplifying hosts (Ostfeld & Keesing 2011; Ostfeld 2009). This study provides significant insights into the diversity and ecology of mosquito species, particularly *Anopheles*, across diverse ecological sites in Central Kalimantan, which may facilitate zoonotic malaria transmission. The ecological and geographical characteristics of the surveyed areas are indicative of a range of landscape characteristics and patterns of human settlement that could potentially impact the composition and distribution of mosquito species. Despite all study locations being at similar elevations (0 m above sea level), they varied significantly in terms of habitat types, vegetation cover, human settlement density, land use, and distance to water sources. These elements are crucial in mosquito ecology and the dynamics of vector-borne diseases (Ferraguti et al. 2020; Ndoen et al. 2010). In general, the abundance and diversity of mosquitoes are lower in human-altered environments



**Figure 2.** Biting time of *Anopheles letifer* in Nyaru Menteng.



**Figure 3.** Habitat of *Anopheles*. A: Ditch; B: Man-made container; C: Pond; D: Abandoned mining pond; E: Rain pool; F: Seepage; G: Spring; H: Stream margin; I: Swamp; J: Water-filled wreck.

than in natural habitats. However, it is worth noting that species-specific reactions to habitat changes may vary. While many species of mosquito experience a decline in abundance due to higher levels of urbanization, deforestation, and agricultural expansion, species of significant global health concern often show increased numbers in human-modified environments (Perrin et al. 2022). The Nyaru Menteng and Bukit Tangkiling areas are located within secondary forest regions, distinguished by their dense vegetation and moderate to low levels of human settlement. These regions are linked to natural or semi-natural water sources, such as swamps and artificial ponds, which provide favourable breeding grounds for forest-dwelling *Anopheles* species. The combination of land uses, particularly the juxtaposition of forest and farmland in Nyaru Menteng, suggests a potential intersection between vector habitats and human activities. This raises concerns

about the possibility of zoonotic transmission. This is evidenced by the markedly elevated counts of mosquito species and genera recorded in this area, in comparison to other areas that have been surveyed.

The Jalan Pariwisata and the airport employee residential area represent more anthropogenically modified environments, characterized by sparse vegetation, plantation or urban residential land use, and proximity to artificial water sources such as roadside and irrigation ditches. The sparse vegetation and high human activity levels present in these locations may provide a conducive environment for the proliferation of opportunistic mosquito species that are well adapted to urban or peri-urban settings, including several *Culex* and *Aedes* species. The presence of extensive swamp areas in the residential area surrounding the airport has led to the dominance of *Mansonia* mosquitoes, typically associated with swamps, in the results of mosquito

**Table 3.** Characteristic of the larva habitat at the study sites

Environmental factor	Category	<i>Anopheles</i> positive	Total locations	Positive <i>Anopheles</i> larvae (%)	p-value***
Aquatic predator present*	None	8	27	30	0.902
	Yes	5	21	24	
Breeding site yype	Ditch	4	15	27	0.633
	Man-made container	0	2	0	
	Pond	2	5	40	
	Rain pool	3	14	21	
	Seepage	2	4	50	
	Spring	0	2	0	
	Stream margin	0	2	0	
	Swamp	2	3	67	
	Water-filled wreck	0	1	0	
Water clarity	Clear	11	39	28	0.687
	Turbid	2	9	22	
Water current	Quiet	12	42	29	0.432
	Running	1	6	17	
Water permanency	Permanent	3	8	38	0.721
	Semi-permanent	6	22	27	
	Temporary	4	18	22	
Aquatic vegetation**	No	3	7	43	0.309
	Yes	10	41	24	

\*: fish, aquatic insects (backswimmer, waterbug, dragonfly nymphs), etc; \*\*: water hyacinth, water lettuce, azolla, filamentous algae, moss, water spinach, sedge, etc; \*\*\*: statistical significance (chi-square test),  $p > 0.05$  indicates no significant association between environmental factors and the presence of *Anopheles* larvae.

collections (Apiwathnasorn et al. 2006; Becker et al. 2020).

*Anopheles letifer* has emerged as the predominant *Anopheles* species, representing 97% of all *Anopheles* collected, with the greatest density observed in Nyaru Menteng. This finding corroborates earlier research indicating that *An. letifer* is predominantly found in forested, swamp-forest, and semi-urban areas of Southeast Asia, flourishing in regions with ample breeding habitats and accessible human hosts (Wharton et al. 1963). In Sarawak, Malaysia, the species under discussion typically breeds in dark brown, peaty swamp waters and is often encountered in large quantities in jungle clearings and at the edges of forests. This species is seldom encountered deep within the jungle, where its sibling species, *An. umbrosus*, is

more prevalent (Thevasagayam & Choon 1979). Its proximity to the forest edge heightens the chances of *An. letifer* interacting with local residents, which positions it as a potential malaria vector. This assertion is supported by documented cases of sporozoite rates reaching 0.23% in Sarawak, Malaysia, and 1.01% in Palangkaraya, Indonesia (Chang et al. 1997; Permana et al. 2023). It is evident that several members of the Umbrosus group, including *An. letifer*, *An. collesi*, and *An. roperi*, function as primary vectors for zoonotic malaria (Ali et al. 2023). The presence of *An. letifer* in Central Kalimantan, particularly in regions inhabited by primates such as orangutans, macaques, and *Hylobates*, has led to concerns regarding the potential for zoonotic malaria transmission. Across all five study locations, long-tailed macaques (*Macaca fascicularis*)

were frequently observed in proximity to residential areas, suggesting a likely association with nearby forest habitats. Nevertheless, *An. letifer* was predominantly found only in Nyaru Menteng among the five sites. Three species of *Anopheles* are known to contribute to zoonotic malaria in Kalimantan. The following species are hereby identified: *An. leucosphyrus* Dönitz, *An. balabacensis*, and *An. latens* Sallum & Peyton.

In this study, *An. letifer* primarily exhibited outdoor biting behaviour, with activity commencing in the early evening, reaching its apex shortly after dusk, and resuming during the latter part of the night. The trendline demonstrates a marked tendency for elevated average biting rates in outdoor environments, indicating a discernible predilection for nocturnal outdoor biting. This finding is of crucial importance for malaria vector control, particularly in areas where individuals are active outdoors or sleep in unscreened homes at night (Rozi et al. 2024; Rozi et al. 2025). Research conducted in Selangor, Malaysia, revealed that more than 95% of *An. letifer* feeds at night, with peak biting activity occurring between 22:00 and 24:00. A smaller fraction of *An. letifer* collected from Selangor was found during the morning or daytime, primarily in forested regions or in close proximity to human residences (Sugiarto et al. 2022). In this study, mosquito collection was restricted to the hours between 18:00 and 06:00. The presence of mosquitoes during crepuscular periods, such as dusk and dawn, suggests the possibility of enhanced activity extending beyond these times, particularly in the early morning and late afternoon. This atypical daytime biting behaviour may result from low light conditions found in forest habitats, which can resemble twilight and possibly stimulate nocturnal activity patterns even during the day. The HBR is a measurement of the number of bites that a person encounters from a specific species of mosquito. A study of data collected from six different locations revealed that the *Mansonia* species exhibited the highest rate of biting, with an average of 18.03 bites per person per night (WHO 2013). This finding indicates a high level of human exposure to *Mansonia* mosquitoes, suggesting their potential role as a significant nuisance and a possible vector for filariasis in the area (Becker et al. 2020; Alonso et al. 2023). *An. letifer* exhibited the second highest HBR of 5.48, indicating a considerable malaria transmission risk, particularly in regions with high populations of *An. letifer*, such as Nyaru Menteng. In contrast, other *Anopheles* species, including *An. umbrosus* and *An. tessellatus*, exhibited remarkably low HBR values (0.13 and 0.03, respectively), suggesting minimal human-vector interaction. The disparities in HBR among different species and geographical

locations emphasise the importance of localised vector monitoring to inform targeted control initiatives.

The entomological findings were corroborated by breeding site surveys, which revealed the presence of *Anopheles* larvae in 13 out of 48 sites (27%). Despite the absence of statistically significant relationships between specific environmental factors and the presence of *Anopheles* larvae ( $p > 0.05$ ), certain habitat types demonstrated consistent patterns. For instance, swamps and stream edges were identified as the most productive habitats, with each exhibiting a positivity rate of 67%. These environments frequently contain shaded, vegetated areas with stagnant water, which are conducive to *Anopheles* oviposition and larval growth (Adugna et al. 2025). The correlation between swamps and malaria cases has been documented since ancient Greece, a period preceding the understanding of mosquitoes as vectors. At that time, efforts were already underway to drain swamps and marshlands as a malaria risk mitigation strategy (Kousoulis et al. 2013; Encyclopaedia Britannica 2025).

Peat-swamp forests are influenced by a range of spatial and temporal factors, primarily due to the nutrient-poor, highly acidic, and waterlogged nature of their peat soils (Mirmanto 2010; Nishimua et al. 2007). In Central Kalimantan, the terrain comprises a mosaic of peat-swamp forest mixed with tropical heath forest, spanning roughly 4.3 million hectares of wetland, 0.7 million hectares of tidal swamps and 3.6 million hectares of non-tidal swamps (Nishimua et al. 2007; Mulyono 2023; Sukarna & Birawa 2018; Noor et al. 2023). The swamps and the dark brown, peaty water typically found in jungle clearings and at forest margins provide a natural habitat for *An. letifer* and *An. umbrosus*, which are often found in large populations (Thevasagayam & Choon 1979). It is estimated that over 27% of Central Kalimantan is vulnerable to malaria transmission due to the presence of these mosquito species in the peat-swamp areas. Furthermore, human activities in forested areas and settlements within these zones serve to exacerbate the risk.

Seepages and ponds were found to harbour relatively high numbers of *Anopheles* larvae. This finding suggests that semi-permanent water bodies, characterised by moderate sunlight exposure and abundant vegetation, can function as vital larval habitats. It is noteworthy that artificial water-filled structures such as ditches and discarded containers (including water-filled wrecks) exhibited a less consistent correlation with larval presence, despite their recognised capacity to support mosquito growth in other contexts. The variation in larval presence among different habitat



types emphasises the necessity of performing site-specific habitat evaluations instead of relying solely on broad classifications. Nevertheless, trends indicate that specific types of breeding sites, water conditions, and vegetation presence might impact mosquito distribution. Further research employing larger sample sizes could yield more comprehensive insights into the ecological determinants of *Anopheles* breeding in the study area.

The absence of statistically significant associations in this research could be attributed to the limited sample size and narrow temporal range. Previous research indicates that the presence of *Anopheles* larvae is affected by water quality and various physical, chemical, and biological traits of breeding sites (Hinne et al. 2021). Sunlight exposure notably impacts larval density, with a strong positive correlation between sunlight and larval abundance (Tsegaye et al. 2023). Additionally, water depth, transparency, and aquatic vegetation are key habitat characteristics that influence the occurrence and abundance of *Anopheles* larvae (Teklu et al. 2010). However, this study's limitations—such as a small number of larval habitats, single cross-sectional surveys, and broad environmental categories without detailed physicochemical data—may have hindered the identification of relationships with environmental factors.

It is imperative to comprehend the biting behavior of malaria vectors, encompassing the optimal times and preferred locations for biting, as well as their larval habitats, to formulate effective disease control strategies (Esayas et al. 2024; Elyazar et al. 2013; Manguin et al. 2008; Subbarao et al. 2019). The exophagic tendency indicates that traditional indoor control is less effective.

These two primary malaria prevention methods—bed nets and IRS—offer protection mainly when people are resting or sleeping indoors. As a result, outdoor mosquito bites that cause transmission continue to be a concern. Several potential methods could address outdoor transmission, including insecticide-treated hammocks and clothing, spatial and topical repellents, and barrier screens, all designed to minimize human-mosquito interactions. This study emphasizes the importance of implementing Integrated Vector Management (IVM) strategies customized to Central Kalimantan's ecological and social conditions. Since *An. letifer* breeds mainly close to human settlements and exhibits exophagic biting behavior, traditional methods like IRS and LLINs are less effective. Consequently, localized IVM strategies are essential, such as larval source control in semi-permanent swamp areas,

community-led environmental modifications and water management, and outdoor protection measures. (Burton et al. 2025; Rozi et al. 2024). Primary health centers can significantly contribute to health promotion by raising awareness and encouraging behavioral changes to reduce outdoor mosquito exposure at night (Duana et al. 2021; Groepe et al. 2013; Onyinyechi et al. 2023). This includes educational campaigns that promote protective clothing, repellents, and insecticide-treated hammocks for those who are active outdoors after sunset.

Additionally, conducting targeted vector surveillance in forest-edge settlements, where humans and non-human primates interact, is crucial for mitigating the risk of zoonotic malaria transmission (Permana et al. 2023). These approaches, executed within a One Health framework, are well-adapted to the ecological context of peat swamp areas and respect local socio-cultural practices. This study provides important insights into the diversity and biting behavior of *Anopheles* mosquitoes in Central Kalimantan, but some aspects should be interpreted with caution.

The entomological collections were limited to two cross-sectional periods, which did not allow for a full assessment of seasonal variation. The number of larval habitats surveyed was also relatively small, and environmental variables were described broadly rather than through detailed physicochemical measurements. Additionally, the five study sites may not cover all ecological settings across Central Kalimantan. These points suggest that future longitudinal surveys covering multiple seasons, a larger number of larval habitats, and a broader ecological range will be useful to build on and extend the current findings.

## CONCLUSION

This study emphasises the substantial biodiversity and ecological adaptations exhibited by mosquito species, notably *An. letifer*, in Palangkaraya, Central Kalimantan, underscoring the potential for zoonotic malaria transmission. The predominance of *An. letifer* in semi-natural areas such as Nyaru Menteng, in conjunction with its outdoor biting behaviour and propensity for swampy, peaty habitats, underscores its potential role as a malaria vector in communities situated in proximity to forest edges. The close proximity of human settlements to forested areas inhabited by non-human primates further elevates the risk of spillover transmission. These findings underscore the necessity of integrating ecological and entomological data when assessing the risk of vector-borne diseases in biodiverse and changing landscapes.

## ETHICAL APPROVAL

The Ethics Committee for Health Research at the Faculty of Medicine, Hasanuddin University in Makassar, Indonesia, approved the study protocol (Protocol number: UH22060274, No. 371/UN4.6.4.5.31/PP36/2022, and extended No. 30/UN4.6.4.5.31/PP36/2024), and Research Ethics Committee - National Research and Innovation Agency No: 090/KE.03/SK/04/2024.

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