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Original Article

Anopheles (Diptera, Culicidae) species diversity and ecological indices in Banjarasri Village, Indonesia

Anis Nurwidayati [1,4] | Hari Purwanto [1] | Raden Roro Upiek Ngesti Wibawaning Astuti [2] | Mujiyono, Mujiyono [3] | Triwibowo Ambar Garjito [4] | Budi Setiadi Daryono [5] |

1 Laboratory of Entomology, Universitas Gadjah Mada, Jl. Teknika Selatan, Sekip Utara, Yogyakarta, 55281, Indonesia. Email: anisnurwidayati@mail.ugm.ac.id

2 Laboratory of Entomology, Faculty of Biology, Universitas Gadjah Mada, Jl. Teknika Selatan, Sekip Utara, Yogyakarta, 55281, Indonesia. Email: upiekastuti@ugm.ac.id

3 Independent Entomologist, Former Staff at Center for Environmental Health Laboratory Salatiga Jl. Hasanudin 123, Salatiga, Central Java, 50721, Indonesia. Email: insulaeflorum@gmail.com

4 Laboratory of Disease Vector and Reservoir, Research Center for Public Health and Nutrition, National Research and Innovation Agency (BRIN), Jl. Hasanudin 123A, Salatiga, Central Java, 50721, Indonesia. Email: triwibowoa@gmail.com

5 Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada, Jl. Teknika Selatan, Sekip Utara Yogyakarta, 55281, Indonesia. Email: bs_daryono@mail.ugm.ac.id

Corresponding author: Hari Purwanto | ✉ hari.purwanto@ugm.ac.id

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ABSTRACT. *Anopheles* mosquitoes are vectors of *Plasmodium* parasites, the causative agents of malaria. This study aimed to investigate the diversity of *Anopheles* mosquitoes in the Menoreh Highland Areas of Banjarasri Village, Kalibawang sub-district, Kulon Progo, Yogyakarta, Java, Indonesia. A total of 667 *Anopheles* mosquitoes were collected from cow and goat farms between 18:00 and 21:00 in August 2024. Morphological identification was performed immediately using a light microscope, and one representative specimen of each species was then examined by polymerase chain reaction (PCR) targeting the mitochondrial *cytochrome c oxidase* subunit I (COI) gene, followed by Sanger sequencing. Six *Anopheles* taxa were identified morphologically and confirmed molecularly, namely *A. vagus* Dönitz, 1902, *A. kochi* Dönitz, 1901, *A. barbirostris* s.l. van der Wulp, 1884, *A. tessellatus* Theobald, 1901, *A. aconitus* Dönitz, 1902, and *A. annularis* van der Wulp, 1884. The specimen identified morphologically as *A. barbirostris* was identified as *A. vanderwulpi* by COI sequence comparison, suggesting possible cryptic diversity within the *A. barbirostris* complex. The dominance index (D) for the total *Anopheles* population was low (0.24), whereas the diversity index (H′) was moderate (1.51), indicating a relatively even species composition in the sampled assemblage. *A. vagus* was the most prevalent species, accounting for 30.58% of the total population. The finding underscores the importance of integrated surveillance and molecular identification in refining targeted, ecosystem-based malaria vector control strategies tailored to local entomological and environmental conditions. Additional research is necessary to explore the environmental factors affecting mosquito distribution, *Plasmodium* infection in local vectors, and their potential resistance to control strategies.

KEYWORDS: COI, Ecological indices, Java Island, Mosquitoes, Vector-borne diseases, Yogyakarta

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INTRODUCTION

Vector-borne diseases make up over 17% of all infectious diseases and result in over 700,000 deaths each year (WHO 2024a). Among the most significant disease vectors, mosquitoes (Diptera: Culicidae) play a crucial role in transmitting various pathogens that cause diseases in humans and livestock. In Indonesia, these insects transmit several disease-causing pathogens through their blood-feeding activities (Franklinos et al. 2019).

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Malaria is a serious disease caused by *Plasmodium* parasites, which are transmitted by female mosquitoes of the genus *Anopheles*. There were an estimated 282 million cases of malaria in 2024, and the estimated number of malaria deaths stood at 610,000 (WHO 2025). The World Health Organization (WHO) recognizes five *Plasmodium* species that infect humans: *P. falciparum, P. malariae, P. vivax, P. ovale* spp., and *P. knowlesi* (WHO 2024b). Despite ongoing control efforts, malaria remains a major public health concern in Indonesia, where its prevalence varies markedly across regions and is not uniformly distributed nationwide (DGDPC 2025). Indonesia ranks second in Southeast Asia in malaria burden, reporting 443,530 cases in 2022, after India (DGDPC 2025). In 2020, Indonesia accounted for approximately 49% of malaria cases in Southeast Asia, with the majority occurring in the eastern provinces of Papua, West Papua, Maluku, and East Nusa Tenggara (DGDPC 2025). Malaria cases in Kulon Progo Regency showed fluctuations between 2013 and 2023. The number of cases decreased from 134 in 2013 to 88 in 2014, then increased to 122 in 2015. Subsequently, cases declined steadily to 95 in 2016, 84 in 2017, 27 in 2018, 19 in 2019, and 7 cases in 2020. However, case numbers rose again to 20 in 2021 and sharply increased to 100 in 2022, before falling to 10 in 2023 (KPRHO 2024).

Indonesia is home to approximately 80 species of *Anopheles* mosquitoes, of which, according to the latest data, 29 have been identified as malaria vectors (Elyazar et al. 2013). Several of them belong to species complexes. Behavioral variations within these species complexes can influence each species' capacity to transmit malaria parasites (Linton et al. 2007; White et al. 2011). Various molecular studies of *Anopheles* mosquitoes have also used the mitochondrial Cytochrome c Oxidase subunit I (COI) gene, including to identify members of the species complex. Twenty-three species of *Anopheles* have been confirmed molecularly in Indonesia using various mitochondrial (COI, COII, Cytb) and nuclear ribosomal (ITS2, D3) markers, and ten of them using the COI gene (Suyono 2015; St. Laurent et al. 2016; Widiarti & Ambar Garjito 2016; Garjito et al. 2019; Davidson et al. 2020; Lempang et al. 2023; Hitipeuw et al. 2025; Nugraheni et al. 2025; Sebayang et al. 2026). In the Menoreh Hills, previous surveys recorded *A. aconitus*, *A. annularis*, *A. balabacensis*, *A. barbirostris*, *A. flavirostris*, *A. kochi*, *A. maculatus*, and *A. vagus* (Barodji et al. 2003; Wathon et al. 2024; Nugraheni et al. 2025), several of which are recognized malaria vectors in Indonesia (Elyazar et al. 2013). However, molecular studies in this area have mostly used the mitochondrial COII gene and focused on the *A. maculatus* complex, and, to our knowledge, the COI gene has not been applied to the local *Anopheles* fauna. The identification of the *A. subpictus* Grassi, 1889 complex in Sri Lanka also used the COI gene (Surendran et al. 2013). Studies on the genetic variation and population structure of *A. subpictus, A. peditaeniatus* Leicester, 1908, and *A. vagus* mosquitoes from five regions in Sri Lanka were also conducted using the COI marker gene (Weeraratne et al. 2018). The COI gene, in particular, has been a cornerstone in studies addressing population structure and systematics of *Anopheles* mosquitoes (Rathnayake et al. 2023).

Understanding the diversity and distribution of *Anopheles* mosquitoes is crucial for effective malaria control in animals and humans (Nugraheni et al. 2025). This study aimed to identify *Anopheles* species in Banjarasri Village, Kalibawang sub-district, using morphological and COI-based molecular identification, and to describe their diversity and dominance indices, as a step toward understanding local malaria transmission dynamics and supporting ecosystem-based control strategies.

MATERIALS AND METHODS

Study area. This study was conducted in the malaria-prone Menoreh Hills area, in the Kalibawang sub-district, because this region is part of a potential malaria distribution area. This study used a cross-sectional approach with purposive sampling. The study site was selected based on its status as a malaria-receptive area and the compatibility of its physical environmental characteristics with the ecological requirements and bionomic traits of malaria vectors. Located within the Menoreh Hills, the area is characterized by a distinctive hilly ecosystem that may provide suitable environmental conditions for the presence, development, and dispersal of *Anopheles* mosquitoes. These characteristics make the site relevant for investigating the genetic diversity and population structure of malaria vectors and their potential implications for malaria surveillance and vector control.

Mosquito collection. Sampling was conducted in August 2024 at Banjarasri Village, Kalibawang sub-district, Kulon Progo Regency, Special Province of Yogyakarta, Indonesia (Fig. 1). Three research teams were assigned to collection points: two teams conducted mosquito collection around cow and goat pens (coordinates: 7°42'22.46"S, 110°14'07.6"E; 7°42'25.48"S, 110°14'0.88"E), and one team at a goat shelter (7°42'26.14"S, 110°14'1.27"E). Adult mosquitoes were collected using aspirators near cattle and goat pens between 18:00 and 21:00. Collections were therefore limited to the evening hours of a single night; the resulting sampling bias is discussed in the Discussion. Each team consisted of six collectors, and collections were carried out on a single night. Only adult mosquitoes were collected; no larval collection or breeding-site survey was conducted.

Morphological identification. Captured *Anopheles* mosquitoes were collected in containers, transferred to the laboratory, and subjected to morphological identification under light microscopes immediately, within four hours, using the illustrated guide to female *Anopheles* mosquitoes of Indonesia (O'Connor & Soepanto 1999). All specimens were photographed at a total magnification of 8× (zoom setting 8×, with 10× eyepieces) using a Leica EZ4 stereomicroscope and stacked with Helicon 3D Viewer software. Non-*Anopheles* mosquitoes were excluded from this study because it focused on *Anopheles*, the only genus that transmits human malaria; the diversity indices therefore describe the *Anopheles* assemblage only. Morphological data for *Anopheles* species were evaluated using descriptive methods, systematically summarizing the collected data and comparing them with relevant reference data.

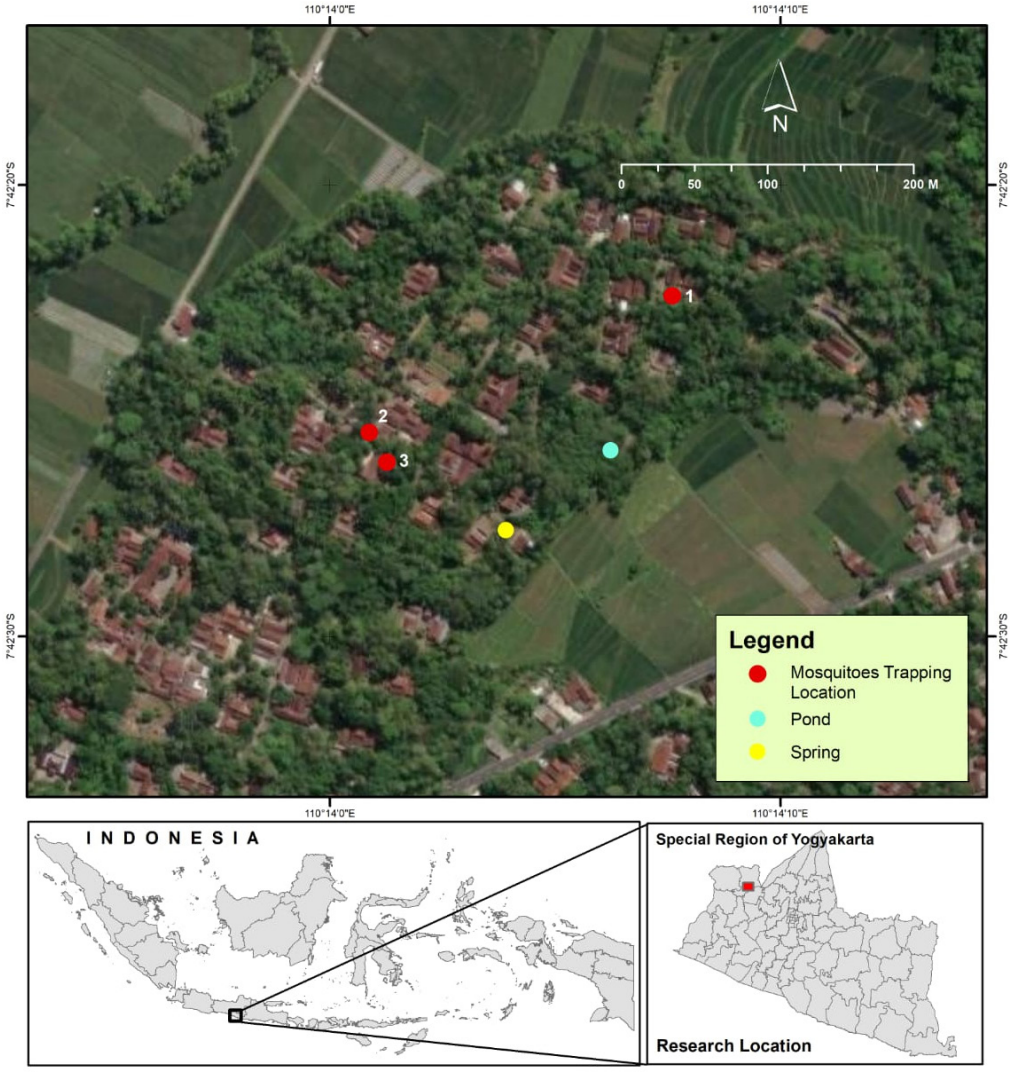


Figure 1. Study area at Banjarasri Village, Kalibawang sub-district, Kulon Progo Regency, Special Province of Yogyakarta, Indonesia.

Ecological Indices analysis

Ecological indices are quantitative measures that describe the structure of a community, such as its species richness, evenness, diversity, and dominance. In this study, two complementary indices were calculated from the number of individuals of each species. The Shannon-Wiener diversity index ($H' = -\Sigma\ p_i \ln p_i$, where p_i is the proportion of individuals belonging to species i) reflects both the number of species and the evenness of their abundances, and is sensitive to rare species (Shannon & Weaver 1949). Simpson's dominance index ($D = \Sigma\ p_i^2$) is the probability that two individuals drawn at random belong to the same species; it is weighted toward the most abundant species (Simpson 1949). The two indices are inversely related: communities with high dominance usually have low diversity. Simpson's Dominance Index (D) was used to assess species dominance, while the Shannon-Wiener Diversity Index (H') was employed to examine species diversity. The H' value was compared with the D value. Diversity index values were low in societies with a high dominance index. According to the diversity index requirements, low diversity is indicated by $H' \leq 1$, moderate diversity by $1 < H' < 3$, and high diversity by $H' \geq 3$ (Michael 1990). The dominance index (D) was determined using Simpson's method and ranged from 0 to 1 (Simpson 1949). A dominance index near 1 implies that a certain species is likely to dominate the population (Odum 1971).

PCR assay and sequencing

Mosquito DNA was extracted using the Quick-DNA Miniprep Plus Kit-D3024 (Zymo Research Corporation, California, USA) according to the manufacturer's tissue extraction protocol. Purified genomic DNA was then subjected to PCR assays targeting the mitochondrial cytochrome c oxidase subunit 1 (COI) gene region. The genomic DNA was amplified using *LCO1490*(F): 5'-GGTC AACAAATCATAAAGATATTGG-3' and *HCO2198*(R): 5'-TAAACTTCAGGGTGACCAAAAAATCA-3', as described in a previous study (Folmer et al. 1994). Each amplification was carried out in a total reaction volume of 25 µL per reaction, consisting of 12.5 µL of 2× PCR mix GoTaq® green master mix M712 (Promega, Wisconsin, USA), 1 µL of each primer (1 µM), 5 µL of genomic DNA as template, and sterile distilled water to adjust the final volume to 25 µL. The PCR cycle parameters were as follows: an initial denaturation at 94 ˚C for 5 minutes; 5 cycles of denaturation: 94 ˚C for 40 seconds, annealing at 45 ˚C for 1 minute; and extension at 72 ˚C for 1.5 minutes, followed by 30 cycles with denaturation at 94 ˚C for 40 seconds, annealing at 51 ˚C for 1 minute, and extension at 72 ˚C for 1.5 minutes; and a final extension at 72 ˚C for 5 minutes (Lobo et al. 2015). Subsequently, 3 µL of PCR products were electrophoresed on a 1.5% agarose gel stained with SYBR™ Safe (Thermo Fisher Scientific, Massachusetts, USA) and visualized using a UV transilluminator. PCR products were sent to Genetika Science Ltd. (Jakarta) for Sanger sequencing. One representative specimen of each morphologically identified species was amplified and sequenced; intraspecific variation was therefore not assessed.

Phylogenetic analyses

Resulting sequences were processed and edited using Sequencing Analysis software version 5.2 (Applied Biosystems). Multiple sequence alignment was performed using MUSCLE v.5.3 (Edgar 2022). The aligned sequences were further trimmed with trimAl v.1.5 (Capella-Gutiérrez et al. 2009) using the "-automated" setting, which was optimized for Maximum Likelihood (ML) phylogenetic tree reconstruction. ML trees were inferred using MEGA12 under the GTR+G+I substitution model with 1,000 bootstrap replicates, using *Chagasia bonneae* as the outgroup; the best-fit model was selected with ModelFinder (Kalyaanamoorthy et al. 2017). Pairwise genetic distances were calculated using the Kimura 2-parameter (K2P) model (Kimura 1980) with pairwise deletion of gaps in MEGA12 (Kumar et al. 2024) (final dataset of 925 positions). Reference sequences that covered a different region of the COI gene and did not overlap sufficiently with the sequences generated in this study (JX268795, JX268801, and EU797275) were excluded from the analyses, as was KR872407, which was labeled *A. vagus* in GenBank but grouped with *A. albitarsis* in a preliminary tree and was considered a probable misidentification.

RESULTS

Morphological data. *Anopheles* species were primarily identified based on their morphological characters. A total of 667 *Anopheles* mosquitoes were collected and identified from Banjarasri Village, Kalibawang sub-district. *Anopheles vagus*, *A. kochi*, *A. barbirostris* s.l., *A. tessellatus*, *A. aconitus*, and *A. annularis* were the most prevalent species in the malaria-endemic regions of Kalibawang sub-district, located in the Menoreh Highlands (Table 1). Identification followed the illustrated key of O'Connor & Soepanto (1999); the diagnostic characters are shown in Figure 2 and Appendix 1. Subspecies are not used here because they are no longer recognized in mosquito classification (Harbach & Wilkerson 2023).

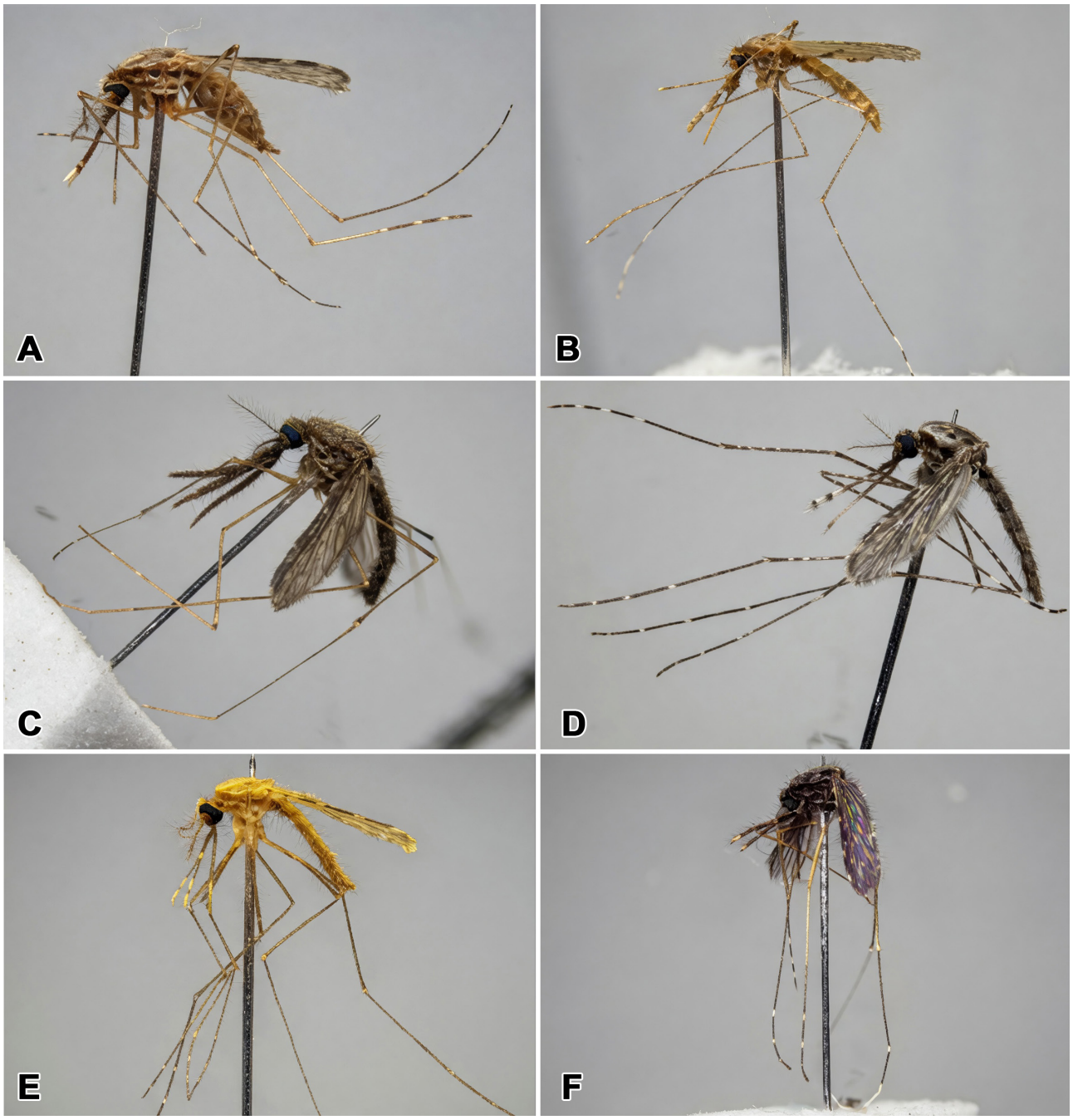


Figure 2. *Anopheles* species documented in Banjarasri Village, Kalibawang sub-district, Indonesia. **A.** *Anopheles vagus* Dönitz, 1902; **B.** *Anopheles kochi* Dönitz, 1901; **C.** *Anopheles tessellatus* Theobald, 1901; **D.** *Anopheles barbirostris* s.l. van der Wulp, 1884; **E.** *Anopheles aconitus* Dönitz, 1902; **F.** *Anopheles annularis* van der Wulp, 1884. Specimens were photographed at 8× magnification; scale bar = 2 mm.

Table 1. Species composition, Shannon–Wiener Diversity Index (H′) and Simpson Dominance Index (D) of *Anopheles* in Banjarasri Village, Kalibawang sub-district, Kulon Progo Regency, Yogyakarta.

Mosquito species	Samples	Percentage (%)	Pi	LnPi	H′	D
Anopheles vagus	204	30.58	0.306	-1.185	0.362	0.094
A. kochi	189	28.34	0.283	-1.261	0.357	0.080
A. barbirostris s.l.	122	18.29	0.183	-1.699	0.311	0.033
A. tessellatus	110	16.49	0.165	-1.802	0.297	0.027
A. aconitus	40	6.00	0.060	-2.814	0.169	0.004
A. annularis	2	0.30	0.003	-5.810	0.017	0.00001
Total	667	100			1.514	0.238

Ecological data. The Shannon-Wiener diversity index (H′) for *Anopheles* species in Kalibawang, Kulon Progo was 1.514, indicating moderate species diversity (1<H′<3). Subsequently, the Simpson's dominance index (D) was 0.238, corresponding to low dominance; although *A. vagus* was the most frequently captured species, no single taxon had numerical dominance. Table 1 displays the Shannon–Wiener Diversity Index (H′) and Simpson Dominance Index (D) of *Anopheles* in this study.

Molecular identification. BLAST analyses and comparison with BOLD and GenBank sequences confirmed specimen identification: *Anopheles vagus, A. kochi, A. barbirostris, A. vanderwulpi, A. tessellatus, A. aconitus,* and *A. annularis*. Sequence identity percentages ranged from 97.96% to 100% (query cover 91–97%), and the morphological and molecular identifications were congruent for five of the six taxa (Table 3). The nucleotide sequences were deposited in the GenBank database under the accession numbers PZ342546 (*A. vagus*), PZ336166 (*A. kochi*), PX361355 (*A. barbirostris*), PV960015 (*A. tessellatus*), PZ342548 (*A. aconitus*), and PZ336161 (*A. annularis*). Sequences of one representative specimen per species were deposited; the accession numbers listed in Table 3 refer to the best-matching reference sequences. The morphological identification of five of the six taxa was consistent with the molecular identification. The exception was the specimen identified morphologically as *A. barbirostris* (Seq 31), which gave two different best matches: *A. barbirostris* (OQ108342; 97.96% identity, 95% query cover) and *A. vanderwulpi* (MT394451; 100% identity, 91% query cover; BOLD GBMNC3082-20, 100% similarity). The K2P distance between Seq 31 and *A. vanderwulpi* (MT394451) was 0.00% (identical), compared with 1.54% to *A. barbirostris* (MT450806); the two reference sequences also differed from each other by 1.54%. Distances between Seq 31 and sequences of other *Anopheles* species ranged from 8.65% to 14.78%. In the ML tree (Fig. 3), Seq 31 grouped with *A. barbirostris* (MT450806) and *A. vanderwulpi* (MT394451) in a clade with 99.7% bootstrap support, but the relationships among these three sequences were not resolved (17.6% bootstrap support).

Because the available morphological characters do not allow reliable differentiation among members of the *A. barbirostris* complex, all 122 specimens examined were assigned to *A. barbirostris* s.l. However, only one specimen from this complex was sequenced; therefore, the identities of the remaining specimens remain unresolved. Furthermore, the sequences from all six *Anopheles* mosquitoes collected in this research were utilized to construct a consensus phylogenetic tree using the ML method (Fig. 3).

Table 2. Shannon–Wiener Diversity Index (H′) and Simpson Dominance Index (D) in Banjarasri Village.

Mosquito Diversity	Pi	LnPi	H′	D
Anopheles vagus	0.306	-1.185	0.362	0.094
A. kochi	0.283	-1.261	0.357	0.080
A. barbirostris s.l.	0.183	-1.699	0.311	0.033
A. tessellatus	0.165	-1.802	0.297	0.027
A. aconitus	0.060	-2.814	0.169	0.004
A. annularis	0.003	-5.810	0.017	0.00001
Total			1.514	0.238

Table 3. Molecular identification of *Anopheles* species in Banjarasri Village using the Cytochrome c Oxidase Subunit I (COI) gene.

Sample Code	Fragment length (bp)	Amino acid translation	BLAST		Best-match accession number	BOLD	ID BOLD	Morphological ID	Molecular ID
			Identity (%)	Query Cover (%)	(GenBank)	Identity (%)			
Seq 29	649	208	99.71	96	PQ219136	99.7	GBMNC2687-20.COI-5P	*Anopheles vagus*	*Anopheles vagus*
Seq 33	657	208	99.26	94	MT669940	99.68	GBMNC2682-20	*A. kochi*	*A. kochi*
Seq 31	658	208	97.96	95	OQ108342	-	-	*A. barbirostris* s.l.	*A. barbirostris*
			100	91	MT394451	100	GBMNC3082-20.COI-5P	*A. barbirostris* s.l.	*A. vanderwulpi*
Seq 87	636	176	99.85	97	MT257029	99.84	-	*A. tessellatus*	*A. tessellatus*
Seq 32	657	208	99	97	PQ219149	99.05	GBMNC2682- 20.COI-5P	*A. aconitus*	*A. aconitus*
Seq 30	685	217	99.56	96	OQ102477	-	-	*A. annularis*	*A. annularis*

Note. The accession numbers in this table are those of the best-matching reference sequences. The accession numbers of the sequences generated in this study are PZ342546 (*A. vagus*), PZ336166 (*A. kochi*), PX361355 (*A. barbirostris* s.l.), PV960015 (*A. tessellatus*), PZ342548 (*A. aconitus*), and PZ336161 (*A. annularis*).

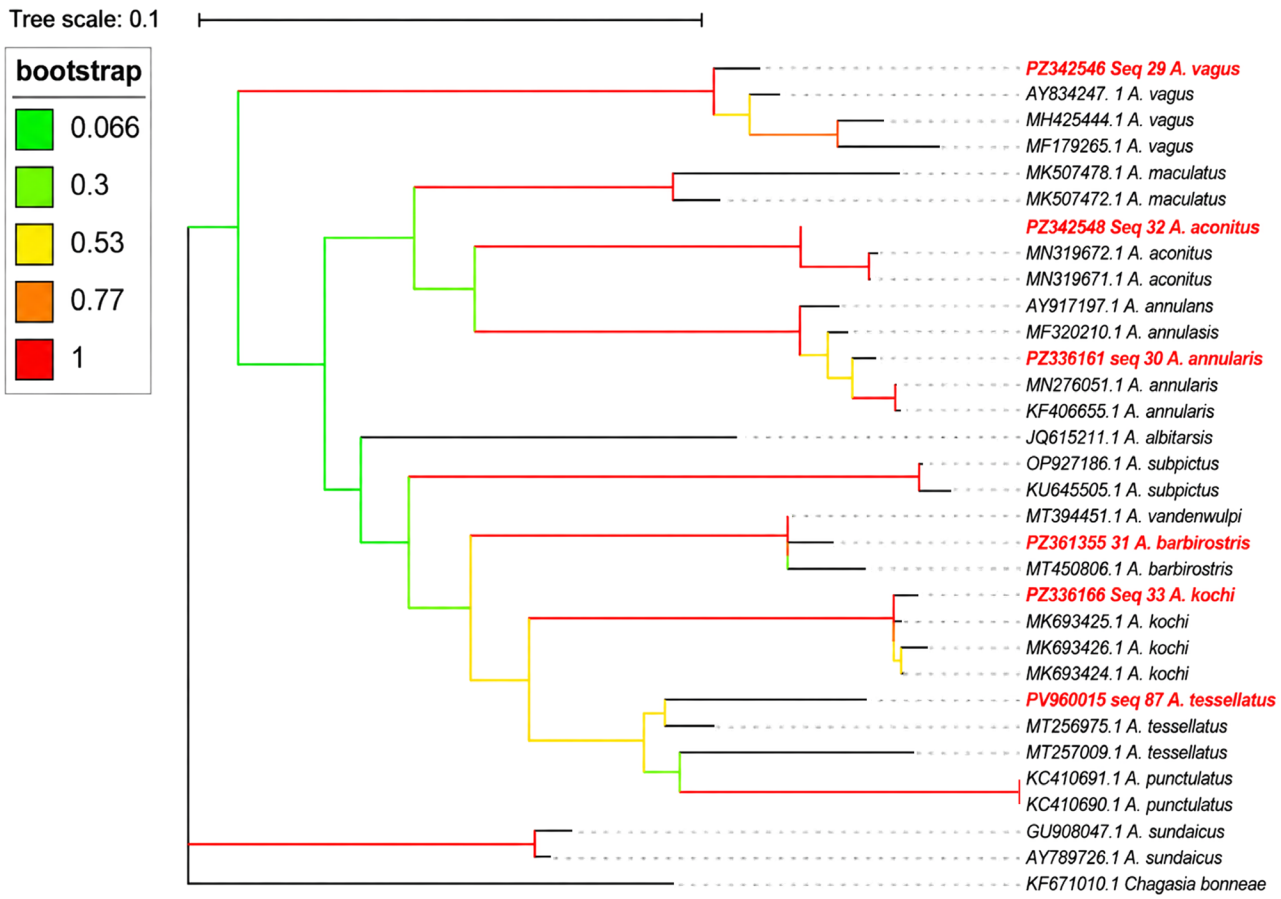


Figure 3. Maximum-likelihood phylogenetic tree based on mitochondrial cytochrome c oxidase subunit I (COI) sequences of *Anopheles* mosquitoes collected from Banjarasri, Kulon Progo, Yogyakarta, Indonesia, and reference sequences retrieved from GenBank. Bootstrap support values (proportions of 1,000 replicates) are indicated by branch colors, as shown in the legend. Specimens collected from Banjarasri are highlighted in red. The scale bar represents the number of nucleotide substitutions per site. *Chagasia bonneae* served as the outgroup.

DISCUSSION

In total, six *Anopheles* taxa were identified morphologically in Banjarasri Village, Kalibawang sub-district, Kulon Progo Regency, Yogyakarta, and all six were confirmed by COI sequencing; the specimen identified morphologically as *A. barbirostris* was identified as *A. vanderwulpi* by its COI sequence. The assemblage showed moderate diversity (H' = 1.51) and low dominance (D = 0.24). These findings highlight the presence of multiple competent malaria vector species and a relatively even species composition within the sampled assemblage.

Research in Kokap, a nearby location within the Menoreh Hills area, has recorded eight *Anopheles* species (*A. aconitus, A. annularis, A. barbirostris, A. balabacensis, A. flavirostris* Ludlow, 1914*, A. maculatus, A. kochi,* and *A. vagus*), reflecting temporal and spatial variability in the local assemblage (Barodji et al. 2003). A morphological study in 2023 reported seven species in Kalibawang and Samigaluh, also in the Menoreh Hills area, including *A. vagus, A. maculatus, A. kochi, A. barbirostris, A. balabacencis* Baisas, 1936, *A. aconitus,* and *A. annularis* (Wathon et al. 2024). The study used morphological and molecular methods, using the mitochondrial Cytochrome c Oxidase II (COII) gene in the Menoreh Hills area, and found four species: *Anopheles maculatus* Theobald, 1901, *A. barbirostris*, *A. annularis*, and *A. vagus* (Nugraheni et al. 2025). This study identified six species of *Anopheles* in Kalibawang sub-district, i.e., *A. kochi, A. tessellatus, A. barbirostris* group, *A. vagus, A. annularis,* and *A. aconitus*, similar to the number detected in prior local surveys. Apart from morphological identification, this study included molecular data using the COI gene to support identification. Unlike the earlier surveys, *A. maculatus*, *A. balabacensis*, and *A. flavirostris* were not collected here, whereas *A. tessellatus* was not listed in the surveys cited above. These differences may reflect differences in sampling methods, season, and collection time (see the limitations below).

The Shannon-Wiener Diversity Index (H') of 1.514 observed for *Anopheles* species on location indicates a moderate level of species diversity, and the dominance index (D) of 0.238 indicates that no single species dominated the sampled assemblage. A mixed assemblage of this kind can complicate malaria control efforts because several species with different vector competence co-occur. Mosquito population characteristics are critical for determining a region's susceptibility to the spread of mosquito-borne diseases. When assessing the risk of pathogen transmission by mosquitoes, it is important to account for both human behavior and environmental conditions (Nugraheni et al. 2025).

Environmental observations during mosquito capture revealed optimal conditions for mosquito proliferation: bright weather, with air temperatures ranging from 25.7 ˚C to 26.7 ˚C and humidity levels ranging from 70–77%. Several prior studies have reported that the temperature and humidity in the Kulon Progo region ranged from 24 ˚C to 27 ˚C and from 90% to 92%, respectively (Handayani & Darwin 2006). Each *Anopheles* species that works as a malaria vector in each location differs physiologically and ecologically (Garjito et al. 2004). The temperature recorded during sampling was within the range reported previously for Kulon Progo, whereas the humidity was lower than the previously reported values. These measurements were taken during sampling only and indicate the microclimatic conditions at that time.

This study revealed different results between the morphological and molecular identification of one of our specimens. The specimen that was morphologically identified as *A. barbirostris* came out as *A. vanderwulpi* Townson & Harbach, 2013, by its COI sequence in BOLD. This supports recent work showing that the *A. barbirostris* complex in Indonesia is a meta-species group comprising at least eight populations, each with unique ecological profiles and potential vector capacities (Satoto et al. 2025). Precision in species identification, especially through COI barcoding, is critical: cryptic sibling species can differ significantly in their capacity for malaria transmission. Molecular analysis showed concordance between morphological classification and COI gene-based identification for five of the six taxa, corroborating previous findings on the utility and accuracy of DNA barcoding in Southeast Asian *Anopheles* taxa. However, the 1.54% distance between the two reference sequences and the low bootstrap support within the clade mean that the species-level assignment of Seq 31 within the complex remains tentative. This finding is consistent with a study that found *A. barbirostris* s.l. in Indonesia to be a more complex metapopulation consisting of at least eight populations, including *A. barbirostris* s.s. in

Java and Kalimantan, *A. vanderwulpi* in Java and Sumatra, and *A. saeungae* Taai & Harbach, 2015 in Sumatra, as well as the first report of *A. wejchoochotei* Taai & Harbach in North Sulawesi and a unique *barbirostris* population in Magelang, Central Java. The accurate identification of the target populations is vital to better understand the geographical distribution and relative involvement in disease transmission of each member of the *A. barbirostris* complex (Satoto et al. 2025).

The Cytochrome c Oxidase subunit I (COI) gene is a powerful tool for the molecular identification of *Anopheles* mosquitoes, primarily due to its high-resolution capability in distinguishing morphologically similar cryptic species complexes, which often differ critically in their vector competence for malaria. This utility is clearly demonstrated in research on the *A. barbirostris* group. A study by Paredes-Esquivel et al. successfully used COI barcoding to delineate several cryptic species within this complex, revealing distinct genetic lineages that were previously unrecognizable through traditional morphology (Paredes-Esquivel et al. 2009). This genetic identification allowed researchers to accurately map the distribution and investigate the differing roles of each sibling species in malaria transmission, thereby providing essential data for refining and targeting vector control strategies in the region. Additionally, comprehensive reference libraries, such as the Barcode of Life Data Systems (BOLD), which contain thousands of curated COI sequences, facilitate the rapid and accurate identification of field-collected specimens. This utility was demonstrated by Kumar et al., who confirmed its reliability in distinguishing Indian mosquito species. The combination of conserved priming sites, which enable universal amplification, a clear distinction between intra- and interspecific genetic distances, and a robust phylogenetic signal establishes COI as an essential, standardized tool for taxonomy, phylogeography, and vector surveillance (Kumar et al. 2007). Several molecular studies have previously been conducted in the Menoreh Hills area of Kulon Progo; however, their research objectives and molecular markers differed from those employed in the present study. Most molecular investigations in the Menoreh Hills area have focused on the analysis of the *Anopheles maculatus* species complex. In addition, previous studies on *Anopheles* fauna diversity in the Menoreh Hills area primarily utilized the mitochondrial COII gene. Therefore, the present study provides a novel contribution by applying the mitochondrial COI gene for the molecular identification of *Anopheles* mosquitoes in this area. Furthermore, our findings indicate the potential occurrence of sibling species within the *Anopheles barbirostris* complex in Kulon Progo, suggesting the presence of previously unrecognized cryptic diversity. Notably, the study was conducted in the southernmost part of the Menoreh Hills, a mountainous landscape that extends across the provinces of Central Java and the Special Region of Yogyakarta and constitutes the largest malaria enclave on Java Island. Consequently, these findings provide valuable baseline information for malaria vector surveillance and contribute to evidence-based malaria control strategies, particularly within the Menoreh Hills landscape where accurate vector identification is essential for effective vector management and malaria elimination efforts.

The *Anopheles* species identified in this study vary in their epidemiological importance for malaria transmission. Among them, *A. aconitus* and members of the *A. barbirostris* complex, including *A. vanderwulpi*, are recognized as important malaria vectors in Indonesia and Southeast Asia, whereas *A. vagus*, despite being the most abundant species, is generally regarded as a secondary vector with documented involvement in local malaria transmission. *Anopheles tessellatus* and *A. annularis* have also been implicated as secondary vectors in specific ecological settings, while *A. kochi* is considered a minor vector due to its predominantly zoophilic behavior (Elyazar et al. 2013). These findings indicate that the coexistence of several confirmed and potential malaria vectors in Banjarasri may sustain local transmission risk and highlight the importance of continuous vector surveillance, supported by molecular identification, to guide targeted malaria control strategies. Species-level identification of the local vectors in Banjarasri requires further studies.

This study has several limitations. Mosquitoes were collected only between 18:00 and 21:00, on a single night in August 2024, at three sites near livestock pens, using aspirators. This design is likely to favor species that are active early in the evening and attracted to or resting near livestock, and may under-represent species that are active later at night or that do not feed on livestock. The species composition and the diversity (H') and dominance (D) indices therefore describe the sampled assemblage and should not be generalized to the whole *Anopheles* community or to human-biting

populations. Non-*Anopheles* mosquitoes were not counted, so the indices do not describe overall Culicidae diversity. Larvae and breeding sites were not surveyed, and only one specimen per species was sequenced, so intraspecific variation was not assessed. Species reported in earlier surveys in the Menoreh Hills, such as *A. maculatus* and *A. balabacensis*, were not collected, which may reflect these limitations. Further studies are needed to support locally tailored vector control in Banjarasri and the wider Menoreh Hills. These include (*i*) detection of *Plasmodium* in field-caught *Anopheles* by PCR or circumsporozoite ELISA to incriminate the local vectors; (*ii*) all-night and multi-season collections using both animal- and human-baited methods; (iii) larval and breeding-site surveys; (*iv*) blood-meal analysis to determine host preference; (*v*) insecticide susceptibility testing; and (*vi*) sequencing of more *A. barbirostris* s.l. specimens to determine which members of the complex are present.

In this study, COI-based identification indicated the presence of *A. vanderwulpi* within the *A. barbirostris* complex, underscoring the value of molecular tools for resolving species complexes and informing vector surveillance. This study deepens the understanding of vector ecology in Banjarasri, Kalibawang, Kulon Progo, and provides an evidence base for developing precision malaria control programs tailored to local entomological and environmental conditions.

AUTHOR CONTRIBUTIONS

The authors confirm their contributions to the paper as follows: A. Nurwidayati: Writing – original draft, Methodology, Investigation, Data Curation, Visualization. H. Purwanto: Conceptualization, Methodology, Supervision, Validation, Writing – review & editing. R.R.U.N.W. Astuti: Conceptualization, Methodology, Supervision, Writing – review & editing. Mujiyono: Investigation, Data Curation. T. Ambar Garjito: Methodology, Investigation, Validation, Writing – review & editing. B.S. Daryono: Supervision, Validation. The authors read and approved the final version of the manuscript.

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AVAILABILITY OF DATA AND MATERIAL

The specimens listed in this study are deposited in Laboratory of Entomology, Faculty of Biology, Universitas Gadjah Mada, and are available from the curator upon request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study only included arthropod material, and all required ethical guidelines for the treatment and use of animals were strictly adhered to in accordance with international, national, and institutional regulations. No human participants were involved in any studies conducted by the authors for this article.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

GENERATIVE AI STATEMENT

AI tools (ChatGPT) were used for minor image editing, including the removal of scratches and adjustment of lighting.

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REFERENCES

Barodji, Damar, T.B., Boesri, H., Sudini, S. (2003) Vector bionomics and malaria situation in Kokap District, Kulonprogo Regency, Yogyakarta. *Indonesian Journal of Health Ecology*, 2, 209–216.

Capella-Gutiérrez, S., Silla-Martínez, J.M. & Gabaldón, T. (2009) trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25, 1972–1973. https://doi.org/10.1093/bioinformatics/btp348

Davidson, J.R., Wahid, I., Sudirman, R., Small, S.T., Hendershot, A.L., Baskin, R.N., Burton, T.A., Makuru, V., Xiao, H., Yu, X., Troth, E.V., Olivieri, D., Lizarraga, S., Hasan, H., Arfah, A., Yusuf, M., Nur, N., Syafruddin, D., Asih, P. & Lobo, N.F. (2020) Molecular analysis reveals a high diversity of *Anopheles* species in Karama, West Sulawesi, Indonesia. *Parasites & Vectors*, 13 (1), 379. https://doi.org/10.1186/s13071-020-04252-6

DGDPC (2025) Directorate General of Disease Prevention and Control – Latest Situation Report on the Progress of the Malaria Control Program in Indonesia in 2024. Jakarta. Available from: https://malaria.kemkes.go.id/sites/default/files/2025-05/Factsheet%202024.pdf [Accessed November 16, 2026]

Edgar, R.C. (2022) Muscle5: High-accuracy alignment ensembles enable unbiased assessments of sequence homology and phylogeny. *Nature Communications,* 13, 6968. https://doi.org/10.1038/s41467-022-34630-w

Elyazar, I.R., Sinka, M.E., Gething, P.W., Tarmidzi, S.N., Surya, A., Kusriastuti, R., Winarno, B., J.K., Hay, S.I. & Bangs, M.J. (2013) The distribution and bionomics of *Anopheles* malaria vector mosquitoes in Indonesia. *Advances in Parasitology,* 83:173-266. https://doi.org/10.1016/B978-0-12-407705-8.00003-3

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3 (5), 294–299.

Franklinos, L.H.V., Jones, K.E., Redding, D.W. & Abubakar, I. (2019) The effect of global change on mosquito-borne disease. *The Lancet Infectious Diseases*, 19 (9), e302–e312. https://doi.org/10.1016/S1473-3099(19)30161-6

Garjito, T.A., Jastal, Wijaya, Y., Lili, Chadijah, S., Erlan, A., Rosmini, Samarang, Udin, Y. & Labatjo, Y. (2004) Bioecological study of *Anopheles* mosquitoes in the east coast region of Parigi-Moutong Regency, Central Sulawesi. *Buletin Penelitian Kesehatan*, 32, 49–62.

Garjito, T.A., Widiastuti, U., Mujiyono, M., Prihatin, M.T., Widiarti, W., Setyaningsih, R., Alfiah, S., Widartono, B.S., Syafruddin, D. & Satoto, T.B. (2019) Genetic homogeneity of *Anopheles maculatus* in Indonesia and origin of a novel species present in Central Java. *Parasites & Vectors,* 12, 351. https://doi.org/10.1186/s13071-019-3598-1

Handayani, F.D. & Darwin, A. (2006) Malaria vector resting habitat in the endemic area of Kokap District, Kulon Progo Regency, Special Region of Yogyakarta Province. *Indonesian Journal of Health Ecology*, 5 (2), 438–446.

Harbach, R.E. & Wilkerson, R.C. (2023) The insupportable validity of mosquito subspecies (Diptera: Culicidae) and their exclusion from culicid classification. *Zootaxa*, 5303 (1), 1–184.

Hitipeuw, D., Nuranindita, R., Martini, M., Prasetyo, A.S., Han, J.-H., Jun, H., Jeon, B.Y., Garjito, T.A., Rohmadi, R. & Muh, F. (2025) Genetic diversity and phylogeographic structure of *Anopheles kochi*, *Anopheles maculatus*, and *Anopheles vagus*: ITS2-based analysis of highland transboundary populations in the Menoreh Hills, Java, Indonesia. P*arasites, Hosts and Diseases*, 63(3), 228–242. https://doi.org/10.3347/PHD.25026

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A. & Jermiin, L.S. (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods*, 14 (6), 587–589. https://doi.org/10.1038/nmeth.4285

Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111–120. https://doi.org/10.1007/BF01731581

KPRHO (2024) Kulon Progo Regency Health Office–Malaria case report in Kulon Progo. Yogyakarta. 146 pp.

Kumar, N.P., Rajavel, A.R., Natarajan, R. & Jambulingam, P. (2007) DNA barcodes can distinguish species of Indian mosquitoes (Diptera: Culicidae). *Journal of Medical Entomology*, 44 (1), 1–7. https://doi.org/10.1093/jmedent/41.5.01

Kumar, S., Stecher, G., Suleski, M., Sanderford, M., Sharma, S. & Tamura, K. (2024) MEGA12: Molecular evolutionary genetic analysis version 12 for adaptive and green computing. *Molecular Biology and Evolution*, 41, msae263. https://doi.org/10.1093/molbev/msae263

Lempang, M.E.P., Permana, D.H., Asih, P.B.S., Wangsamuda, S., Dewayanti, F.K., Rozi, I.E., Syahrani, L., Setiadi, W., Malaka, R., Muslimin, L. & Syafruddin, D. (2023) Diversity of *Anopheles* species and zoonotic malaria vector of the Buton Utara Wildlife Sanctuary, Southeast Sulawesi, Indonesia. *Malaria Journal,* 22, 221. https://doi.org/10.1186/s12936-023-04647-7

Linton, Y.M., Smith, L., Koliopoulos, G., Zounos, A.K., Samanidou-Voyadjoglou, A., Patsoula, E. & Harbach, R.E. (2007) The *Anopheles* (*Anopheles*) *maculipennis* complex (Diptera: Culicidae) in Greece. *Journal of Natural History*, 41 (41–44), 2683–2699. https://doi.org/10.1080/00222930701403255

Lobo, N.F., St. Laurent, B., Sikaala, C.H., Hamainza, B., Chanda, J., Chinula, D., Krishnankutty, S.M., Mueller, J.D., Deason, N.A., Hoang, Q.T., Boldt, H.L., Thumloup, J., Stevenson, J., Seyoum, A. & Collins, F.H. (2015) Unexpected diversity of *Anopheles* species in Eastern Zambia: Implications for evaluating vector behavior and interventions using molecular tools. *Scientific Reports*, 5. https://doi.org/10.1038/srep17952

Michael, P. (1990) *Ecological Methods for Field and Laboratory Investigations*. Tata McGraw- Hill Publishing Company Limited, New Delhi. 404 pp.

Nugraheni, Y.R., Awaludin, A., Primatika, R.A., Kartiko, B.P., Dewi, D.A., Ariyadi, B., Arnuphapprasert, A., Nurcahyo, W. & Prastowo, J. (2025) Molecular identification of *Anopheles* mosquitoes in goat farms of Menoreh Highland, Kulon Progo, Yogyakarta. *Veterinary Parasitology: Regional Studies and Reports,* 61, 101268. https://doi.org/10.1016/j.vprsr.2025.101268

O'Connor, C. & Soepanto, A. (1999) *Illustrated key to adult Anopheles mosquitoes in Indonesia*. Directorate General of Infectious Disease Prevention and Residential Environmental Health, Ministry of Health, Indonesia, Jakarta. 39 pp.

Odum, E. (1971) *Fundamentals of Ecology*. 3rd ed. W.B. Saunders, Philadelphia. 574 pp.

Paredes-Esquivel, C., Donnelly, M.J., Harbach, R.E. & Townson, H. (2009) A molecular phylogeny of mosquitoes in the *Anopheles barbirostris* Subgroup reveals cryptic species: Implications for identification of disease vectors. *Molecular Phylogenetics and Evolution*, 50 (1), 141–151. https://doi.org/10.1016/j.ympev.2008.10.011

Rathnayake, R.A.S., Wedage, W.M.M., Muthukumarana, L.S. & De Silva, B.G.D.N.K. (2023) Genetic diversity, phylogenetic and phylogeographic analysis of *Anopheles culicifacies* species complex using ITS2 and COI sequences. *PLoS One*, 18 (8), e0290178. https://doi.org/10.1371/journal.pone.0290178

Satoto, T.B.T., Garjito, T.A., Shinta, S., Landi, S., Frutos, R. & Manguin, S. (2025) *Anopheles barbirostris* in Indonesia: a more complex metapopulation than expected. *PLoS One,* 20 (11), e0321707. https://doi.org/10.1371/journal.pone.0321707

Sebayang, B.F., van de Straat, B., Kurniawan, A., Haq, A.M., Garjito, T.A., Saeung, M., Manguin, S., Lubis, I.N.D., Grigg, M.J., Russell, T.L. & Burkot, T.R. (2026) First molecular confirmations of *Anopheles dirus* and *Anopheles scanloni* in Indonesia, with DNA of zoonotic, enzootic and human malarias detected in *An. dirus*. *Scientific Reports*, 16, 11572. https://doi.org/10.1038/s41598-026-42478-z

Shannon, C.E. & Weaver, W. (1949) *The Mathematical Theory of Communication*. University of Illinois Press, Urbana, 117 pp.

Simpson, E.H. (1949) Measurement of diversity. *Nature*, 163, 688. https://doi.org/10.1038/163688a0

St. Laurent, B., Supratman, S., Asih, P.B.S., Bretz, D., Mueller, J., Miller, H.C., Baharuddin, A., Shinta, Surya, A., Ngai, M., Laihad, F., Syafruddin, D., Hawley, W.A., Collins, F.H. & Lobo, N.F. (2016) Behaviour and molecular identification of *Anopheles* malaria vectors in Jayapura district, Papua province, Indonesia. *Malaria Journal,* 15 (1), 1–8. https://doi.org/10.1186/s12936-016-1234-5

Surendran, S.N., Sarma, D.K., Jude, P.J., Kemppainen, P., Kanthakumaran, N., Gajapathy, K., Peiris, L.B.S., Ramasamy, R. & Walton, C. (2013) Molecular characterization and identification of members of the *Anopheles subpictus* complex in Sri Lanka. *Malaria Journal*, 12, 304. https://doi.org/10.1186/1475-2875-12-304

Suyono, I.J. (2015) *Kajian Spesies Sibling Anggota Anopheles punctulatus Group (Diptera: Culicidae) di Kabupaten Jayapura*. Unpublished Ph.D. Dissertation. Faculty of Biology, Universitas Gadjah Mada. 130 pp.

Wathon, S., Senjarini, K., Masruroh, B., Oktarianti, R. & Rehmann, H. (2024) Relative frequency (composition) of *Anopheles* sp. mosquitoes as malaria vectors in Kulon Progo District, Special Region of Yogyakarta. *Journal of Biological Research and Its Application*, 6 (1), 20–33. https://doi.org/10.26740/jrba.v6n1.p20-33

Weeraratne, T.C., Surendran, S.N., Walton, C. & Karunaratne, S.H.P.P. (2018) Genetic diversity and population structure of malaria vector mosquitoes *Anopheles subpictus*, *Anopheles peditaeniatus*, and *Anopheles vagus* in five districts of Sri Lanka. *Malaria Journal*. 17 (1), 271, 1–8. https://doi.org/10.1186/s12936-018-2419-x

White, B.J., Collins, F.H. & Besansky, N.J. (2011) Evolution of *Anopheles gambiae* in relation to humans and malaria. *Annual Review of Ecology, Evolution, and Systematics*, 42, 111–132. https://doi.org/10.1146/annurev-ecolsys-102710-145028

Widiarti, W., Ambar Garjito, T. (2016) Genetic diversity of *Anopheles balabacensis*, Baisas in various regions of Indonesia based on ITS2 ribosomal DNA gene sequences. *Buletin Penelitian Kesehatan*, 44, 1–12.

WHO (2024a) World Health Organization: Vector-borne diseases Fact sheet, 26 September 2024. Available from: https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases [Accessed November 16, 2026]

WHO (2024b) World Health Organization: Malaria - Overview. Available from: https://www.who.int/health-topics/malaria [Accessed July 3, 2026]

WHO (2025) World Health Organization: World malaria report 2025. Geneva. Available from: https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2025 [Accessed November 2, 2026]

تنوع گونه‌ای پشه‌های جنس *Anopheles* (Diptera, Culicidae) و شاخص‌های بوم‌شناختی در بانجاراسری، اندونزی

آنیس نور ویدایاتی [۱،۴]، هاری پوروانتو [۱]، رادن رورو اوپیک نگستی ویباوانینگ آستوتی [۲]، موجیونو موجیونو [۳]، تریویبوو امبار گارجیتو [۴]، بودی ستیادی داریونو [۵]

۱ آزمایشگاه حشره‌شناسی، دانشگاه گاجاه مادا، یوگیاکارتا، اندونزی.
۲ بخش انگل‌شناسی، آزمایشگاه حشره‌شناسی، دانشکده زیست‌شناسی، دانشگاه گاجاه مادا، یوگیاکارتا، اندونزی.
۳ حشره‌شناس مستقل؛ کارمند پیشینِ مرکز آزمایشگاه سلامت محیطی سالاتیگا، جاوه مرکزی، اندونزی.
۴ آزمایشگاه ناقلان و مخازن بیماری، مرکز پژوهشی سلامت عمومی و تغذیه، آژانس ملی پژوهش و نوآوری (برین)، سالاتیگا، اندونزی.
۵ آزمایشگاه ژنتیک و اصلاح نژاد، دانشکده زیست‌شناسی، دانشگاه گاجاه مادا، یوگیاکارتا، اندونزی.

نویسندهٔ مسئول: هاری پوروانتو | hari.purwanto@ugm.ac.id

ویراستار علمی
علیرضا سازمند

دریافت: ۲۶ خرداد ۱۴۰۵
ویرایش: ۲۸ مهر ۱۴۰۵
پذیرش: ۲۲ آبان ۱۴۰۵
انتشار: .....

چکیـده: پشه‌های *Anopheles* ناقل انگل‌های *Plasmodium*، عامل بیماری مالاریا، هستند. این مطالعه با هدف بررسی تنوع پشه‌های جنس *Anopheles* در مناطق مرتفع منِوره در روستای بانجاراسری، بخش کالیباوانگ، شهر کالون پروگو، یوگیاکارتا، جاوه، اندونزی، انجام شد. در مجموع، ۶۶۷ نمونه از پشه‌های جنس *Anopheles* در ماه اوت ۲۰۲۴، از ساعت ۱۸:۰۰ تا ۲۱:۰۰، از محل نگهداری دام (گاو و بز) جمع‌آوری شدند. شناسایی ریخت‌شناختی بلافاصله و با استفاده از میکروسکوپ نوری انجام گرفت. سپس از هر گونه، یک نمونه نماینده با واکنش زنجیره‌ای پلیمراز (PCR) و با هدف تکثیر ژن میتوکندریایی زیرواحد یک سیتوکروم c اکسیداز (COI) بررسی شد و در ادامه، توالی‌یابی به روش سانجر انجام گرفت. شش آرایه از جنس *Anopheles* شامل: *A. vagus* Dönitz, 1902، *A. kochi* Dönitz, 1901، *A. barbirostris* s.l. van der Wulp, 1884، *A. tessellatus* Theobald, 1901، *A. aconitus* Dönitz, 1902 و *A. annularis* van der Wulp, 1884 از نظر ریخت‌شناختی شناسایی و با روش مولکولی تأیید شدند. نمونه‌ای که بر اساس ویژگی‌های ریخت‌شناختی *A. barbirostris* شناسایی شده بود، بر پایه مقایسه توالی COI به‌عنوان *A. vanderwulpi* تعیین هویت شد؛ این یافته می‌تواند نشان‌دهنده تنوع پنهان احتمالی در مجموعه گونه‌ای *A. barbirostris* باشد. شاخص غالبیت (D) برای کل جمعیت *Anopheles* پایین (۰/۲۴) و شاخص تنوع (H′) متوسط (۱/۵۱) بود که بیانگر ترکیب نسبتاً یکنواخت گونه‌ها در مجموعه نمونه‌برداری‌شده است. آرایهٔ *A. vagus* فراوان‌ترین گونه بود و ۳۰/۵۸ درصد از کل جمعیت را تشکیل می‌داد. این یافته بر اهمیت پایش یکپارچه و شناسایی مولکولی برای بهبود راهبردهای هدفمند و مبتنی بر بوم‌سازگان در کنترل ناقلان مالاریا، متناسب با شرایط محلی حشره‌شناختی و محیطی، تأکید می‌کند. برای بررسی عوامل محیطی مؤثر بر پراکنش پشه‌ها، عفونت ناقلان محلی به *Plasmodium* و مقاومت احتمالی آن‌ها در برابر راهبردهای کنترل، پژوهش‌های بیشتری لازم است.

واژگـان کلیدی: سیتوکروم اکسیداز ۱، شاخص‌های بوم‌شناختی، جزیره جاوه، پشه‌ها، بیماری‌های ناقل‌زاد، یوگیاکارتا