EISSN: 2455-8931 doi: 10.14202/IJOH.2025.147-158 OPEN ACCESS

International Journal of One Health

RESEARCH ARTICLE

Genetic diversity of the *Duffy antigen/receptor for chemokines* gene and its susceptibility to zoonotic malaria in non-human primates and Indonesian populations

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ABSTRACT

Background and Aim: Zoonotic malaria caused by *Plasmodium knowlesi* presents a growing public health challenge in Southeast Asia. Host genetic factors, particularly polymorphisms in the Duffy antigen/receptor for chemokines (DARC), may influence susceptibility to zoonotic transmission. Indonesia's vast ethnic and ecological diversity offers a unique context to explore the genetic interface between human and non-human primate (NHP) hosts and zoonotic malaria. This study aimed to investigate the genetic diversity of the *DARC* gene in sympatric human and NHP populations across Indonesia and its potential role in modulating susceptibility to zoonotic malaria.

Materials and Methods: A cross-sectional survey was conducted in Sabang (Aceh), Palangkaraya (Central Kalimantan), and North Buton (Southeast Sulawesi). Dried blood spots were collected from 68 NHPs and 363 humans. DARC promoter and coding regions were amplified through polymerase chain reaction, sequenced, and analyzed using bioinformatic tools. Phylogenetic analyses and allele-specific comparisons were performed to assess cross-species genetic similarity and regional variation in DARC alleles.

Results: No −46T>C promoter single-nucleotide polymorphism associated with Duffy negativity was found in either humans or NHPs. Three genotypic forms – *FYA, FYB*, and *FYA/FYB* – were observed in human populations, with *FYA* being predominant in Kalimantan and Sulawesi. Notably, individuals with the *FYA* allele in Aceh, a region with high *P. knowlesi* incidence, were less likely to have an infection, suggesting a potential protective role. All NHPs carried the *FY*B* allele. Comparative analyses revealed high DARC sequence homology between humans and NHPs, particularly *Macaca fascicularis* and *Macaca brunnescens*, implicating molecular compatibility in zoonotic transmission dynamics.

Conclusion: This is the first comprehensive study to assess *DARC* gene polymorphisms in both human and NHP populations in Indonesia within the context of zoonotic malaria. The findings underscore the significance of host genetic variation in

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Received: 09-12-2024, **Accepted:** 09-04-2025, **Published Online**: 29-05-2025

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How to cite: Permana DH, Asih PBS, Suryandari DA, Murhandarwati EH, Isfanda I, Rozi IE, Syahrani L, Coutrier FN, Kartapradja H, Afzan H, Hidayah N, and Syafruddin D (2025) Genetic diversity of the *Duffy antigen/receptor for chemokines* gene and its susceptibility to zoonotic malaria in non-human primates and Indonesian populations, Int. J. One Health, 11(1): 147–158.

mediating susceptibility to *P. knowlesi* and highlight regional allele profiles as potential markers for risk stratification. These insights provide a genomic framework to inform surveillance and control strategies in malaria-endemic regions vulnerable to zoonotic transmission.

Keywords: *DARC* gene, genetic polymorphism, host-pathogen interaction, Indonesia, malaria susceptibility, nonhuman primates, *Plasmodium knowlesi*, zoonotic malaria.

INTRODUCTION

Malaria remains a pervasive public health challenge in numerous countries. In 2022 alone, an estimated 249 million cases were reported across 85 malaria-endemic nations, reflecting a 5-million case increase from the previous year [1]. Among the four principal *Plasmodium* species infecting humans – *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium ovale* – evidence suggests evolutionary origins linked to African gorillas and avian hosts [2]. More recently, *Plasmodium knowlesi*, a simian malaria parasite, has garnered attention due to its zoonotic potential. This parasite primarily infects long-tailed macaques (*Macaca fascicularis*), pig-tailed macaques (*Macaca nemestrina*), and ringed langurs (*Presbytis melalophos*) but has also been confirmed as a cause of human malaria [3, 4]. In Indonesia, macaques are predominantly distributed west of the Weber Line, with long-tailed macaques occupying most of the archipelago, excluding Sulawesi, which is home to seven endemic macaque species [5, 6]. Pig-tailed macaques are geographically restricted to Kalimantan and Sumatra [7].

Despite increasing recognition of *P. knowlesi* as an emerging zoonotic threat, the genetic factors underlying host susceptibility remain inadequately characterized, particularly in Indonesia – a region of immense biodiversity and ethnic heterogeneity [8]. Recent epidemiological surveillance has documented approximately 545 zoonotic malaria cases in Aceh, North Sumatra, Jambi, Central Kalimantan, and South Kalimantan [8–10]. Notably, Sabang, Aceh, a locality previously declared malaria-free, reported two clusters of *P. knowlesi* cases in 2014 [10]. In Kalimantan, the first known zoonotic case was documented in 2010, with additional cases reported in subsequent years [9, 11]. In West Kalimantan, at least 11 infections were identified following the mass screening of 1,000 residents [11].

Cross-sectional studies have demonstrated the complexity of transmission dynamics in regions inhabited by non-human primates (NHPs). A mass blood survey of 159 residents in Iboih village (Aceh) and Cikakak (Central Java) revealed no human infections despite a high prevalence of simian malaria in the local primate population [12]. Similarly, a study conducted by Lempang *et al*. [13] in North Buton found no human cases, although *Anopheles* mosquitoes capable of human biting were found to carry *Plasmodium inui*. Historically, malaria has exerted considerable selective pressure on the human genome, shaping genetic adaptations that confer varying degrees of susceptibility and resistance across populations [14]. Genetic factors linked to malaria resistance involve several genetic traits such as sickle cell trait, α-thalassemia, glucose-6-phosphate dehydrogenase deficiency, and polymorphisms in the Duffy antigen receptor for chemokines (*DARC*) gene [15].

The DARC protein is a glycosylated chemokine receptor expressed on erythrocytes and various other tissues, playing a pivotal role in cytokine binding during inflammatory responses [16, 17]. The gene encodes two protein isoforms consisting of 338 and 336 amino acids. Its two principal alleles – *FYA** and *FYB** – encode the Fya and Fyb antigens, which differ by a single amino acid substitution at residue 42, attributed to a single-nucleotide polymorphism (SNP) at position 125 (G→A) [16, 18]. A third allele, *FYO** (Duffy-null), is characterized by a T-to-C substitution at position -46 within the promoter region, which disrupts the TATA box transcription factor binding and leads to the absence of DARC expression on red blood cells [19]. Individuals homozygous for the *FYO** allele are referred to as Duffy negative and exhibit resistance to *P. vivax* infection. This allele is nearly fixed in sub-Saharan African populations, while *FYA** and *FYB** predominate in European and Asian populations, respectively [20].

Indonesia, recognized as one of the most biologically and ethnically diverse nations globally, comprises over 500 distinct ethnic groups and spans more than 17,000 islands, hosting approximately 25,000 plant species and 200,000 animal species [21].

Despite the increasing recognition of *P. knowlesi* as a significant zoonotic malaria pathogen in Southeast Asia, the molecular and genetic determinants of host susceptibility – particularly in ethnically diverse populations such as those in Indonesia – remain underexplored. The current studies have predominantly focused on epidemiological surveillance, with limited attention to host genetic factors that may influence cross-species transmission. While the DARC has been well established as a critical erythrocyte receptor involved in *P. vivax* invasion, its potential role in mediating susceptibility or resistance to *P. knowlesi* infection remains inadequately characterized. Furth-ermore, the lack of integrative analyses comparing DARC polymorphisms across sympatric human and NHP populations represents a critical knowledge gap in understanding the evolutionary and ecological drivers of zoonotic malaria transmission in Indonesia.

This study aimed to investigate the genetic diversity of the *DARC* gene and its potential association with

susceptibility to *P. knowlesi* infection in both human and sympatric NHP populations in Indonesia. By analyzing DARC promoter and coding region polymorphisms across epidemiologically distinct regions, the study sought to (i) characterize allele distribution patterns in relation to ethnic and geographical background, (ii) assess genetic similarity between humans and NHPs in key ligand-binding domains, and (iii) explore the potential role of specific DARC genotypes in modulating host susceptibility to zoonotic malaria. This integrative genomic approach is intended to provide foundational evidence for refining malaria surveillance strategies in regions vulnerable to simian-to-human transmission.

MATERIALS AND METHODS

Ethical approval

This study received ethical clearance from the Health Research Ethics Committee, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia, for both human participants (approval numbers: 368/UN4.6.4.5.31/PP36/2022 and 9/UN4.6.4.5.31/PP36/2024) and animal subjects (approval numbers: 367/UN4.6.4.5.31/PP36/2022 and 8/UN4.6.4.5.31/PP36/2024). Additional approval was obtained from the Health Research Ethics Committee of the National Research and Innovation Agency, Indonesia (approval number: 090/KE.03/SK/04/2024).

Study period and location

In 2022, sampling was conducted in Aceh (Sabang Municipality) from October 9 to 28 and in Buton (South Sulawesi) from June to August; meanwhile, sampling occurred in Central Kalimantan from November 6 to 18, 2022, and from October 17 to 30, 2024. The samples were processed at the laboratory at Hasanuddin University in Makassar and at the Genomic Laboratory of the National Research and Innovation Agency in Cibinong, Indonesia.

Sample collection

A cross-sectional study was conducted across three ecologically and epidemiologically distinct regions in Indonesia: Sabang (Aceh), Palangkaraya (Central Kalimantan), and North Buton (Southeast Sulawesi). Aceh was selected because it has the second-highest number of *P. knowlesi* cases after North Sumatra. Central Kalimantan and Southeast Sulawesi represented areas with low and undetected incidence, respectively, enabling a comparative analysis of regional variation in host susceptibility.

Human dried blood spot (DBS) samples, including those from confirmed *P. knowlesi* cases, were obtained from the Iboih Health Center in Sabang, Aceh. Archived DBS samples collected between 2020 and 2022 during previous NHP surveillance studies were repurposed for cross-species genetic comparison of DARC polymorphisms [12, 13].

This study employed a design that uniquely targeted human populations co-located with malaria-endemic NHPs, facilitating novel ecological-genetic correlation analyses. All residents aged ≥6 months, irrespective of sex, were eligible for participation. Individuals residing outside the designated study areas were excluded. Following informed consent, capillary blood samples were collected using sterile techniques. Approximately 100–200 µL of capillary blood was transferred into sterile serum tubes, with one to two drops spotted onto Whatman No. 1 filter paper for DBS preparation. Additional samples were used for the preparation of thick and thin blood smears.

Microscopic examination

Microscopic diagnosis was conducted following the World Health Organization protocols [22]. Giemsa-stained blood smears were examined using a compound microscope under 1000× magnification. *Plasmodium* species identification was based on morphological comparison with standard descriptions and images provided in the reference guide Primate Malaria [23]. Species identification was subsequently confirmed using nested polymerase chain reaction (PCR) with species-specific primers as described in previously validated protocols by Raja *et al*. [24] and Lee *et al*. [25].

PCR amplification and sequencing of DARC promoter and coding regions

A novel multi-fragment PCR approach was employed to amplify both the TATA-1 transcription factor binding region of the DARC promoter (−46T>C) and the full DARC coding region. The initial 221-bp promoter region fragment was amplified using primers P38 and P39 [19]. Complete coverage of the *DARC* gene (~1,975 bp) was achieved using a set of three primer pairs (Figure 1 and Table 1).

PCR reactions were performed in a final volume of 25 µL comprising 0.2 µL DNA stock, 12.5 µL MyTaq™ HS Redmix (Bioline, USA), 0.25 µL of 40 µM primers, and 11.8 µL ddH_2O. For promoter region amplification, thermal cycling included initial denaturation at 95°C for 1 min, 35 cycles of 95°C for 15 s (denaturation), 56°C for 15 s (annealing), and 72°C for 15 s (extension), followed by a final extension at 72°C for 7 min. For the

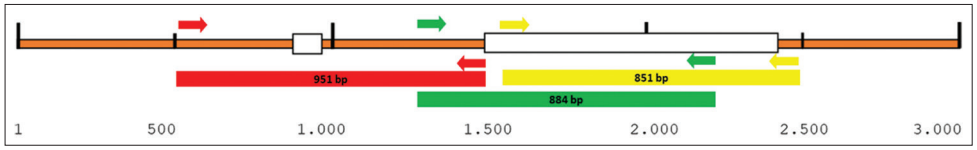

Figure 1: The primer position in the *Duffy antigen/receptor for chemokines* gene.

Table 1: List of DARC primers.

Function	Name	Primer sequence (5'-3')	Temperature (°C)	Amplicon size (bp)
Main primer 1	DARC505	AGCACCTCCCTTATC TCTGC	58.87	951
	DARC1455	CCAGAGGAGGCCATCAGAG	58.87	
Main primer 2	DARC1629	CACTGCCCTTCTTCATCCTC	57.96	851
	DARC2479	GGTTGACAGGTGGGAAGAGA	58.94	
Bridge primer	DARC1378	TCCTCTCTGTCCTCCCCTC	58.99	884
	DARC2261	GCAACAGCTTGGACCTCAC	59.05	

DARC=Duffy antigen/receptor for chemokines

DARC coding region, the annealing temperature was adjusted to 60°C, and extension was prolonged to 30 s. The resulting PCR products were electrophoresed on 2% agarose gels at 70 V for 40 min and visualized under ultraviolet illumination.

Bioinformatic analysis and phylogenetics

Sequencing of the amplified DARC promoter and coding regions from both human and NHP samples enabled a comprehensive interspecies comparison of allelic variants. The resulting nucleotide sequences were analyzed to confirm sequence identity and detect polymorphisms. Allele frequency estimation was also conducted to assess population-level variation in DARC alleles.

An integrated bioinformatic pipeline, developed to accommodate cross-species genomic compa-risons, included sequence editing using BioEdit vers-ion 7.2.5 (https://bioedit.software.informer.com/down-load/) [26] and alignment verification through the National Center for Biotechnology Information Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov). Phylogenetic trees were generated in MEGA X software (https://www.megasoftware.net/) using both nucleotide and deduced amino acid sequences to elucidate evolutionary relationships and potential patterns of host-pathogen coevolution [27].

RESULTS

Sample overview

A total of 68 DBS samples from NHPs were analyzed, comprising *M. fascicularis* (n = 33), *M. nemestrina* (n = 3), *Macaca brunnescens* (n = 26), and *Hylobates* species (n = 2). In parallel, 363 human participants were enrolled from three study locations: Sabang (Aceh), Palangkaraya (Central Kalimantan), and North Buton (Southeast Sulawesi) (Figure 2). In addition, ten DBS samples were obtained from *P. knowlesi*-infected patients at the Iboih Primary Health Center, Sabang, Aceh.

Promoter polymorphism and allelic distribution in human samples

Analysis of 141 human DBS samples, including those from confirmed *P. knowlesi* cases, revealed no presence of the −46T>C SNP within the DARC promoter region (Figure 3). Amplification and assembly of three overlapping fragments covering the *DARC* gene produced a contiguous sequence of 1,916 base pairs encoding 336 amino acids (Figure 4).

SNP analysis at position 125 of the *DARC* gene identified three distinct allelic types in the Acehnese population – *FYA*, *FYB*, and the heterozygous *FYA/FYB* – suggesting regional diversity in allele distribution. Notably, the *FYA** allele was exclusively detected in samples from Central Kalimantan and Southeast Sulawesi. Comparative analysis of allele frequencies between infected and uninfected individuals in Aceh suggested a potential association between *FYA** and resistance to *P. knowlesi* infection (Table 2).

DARC gene analysis in NHPs

In contrast to human samples, no −46T>C SNP was detected in any NHP samples. All NHPs exhibited adenine (A) at position 125, corresponding to the *FYB** allele, which results in aspartic acid at codon 42. This uniform presence of the *FYB** allele in NHPs may facilitate *Plasmodium* spp. transmission across species. The observed high degree of amino acid similarity in the DARC region between macaques and humans supports the hypothesis that molecular mimicry contributes to zoonotic compatibility.

M. fascicularis and *M. brunnescens* demonstrated 97% amino acid sequence similarity in the DARC region when compared to humans (Figures 5 and 6), while *Hylobates* species showed slightly lower similarity (93%). Further regional comparison revealed that *M. fascicularis* from Aceh shared greater similarity with conspecifics from Thailand, whereas samples from Kalimantan aligned more closely with those from the Philippines.

Cross-species sequence alignment and evolutionary analysis

Alignment of DARC sequences between humans and NHPs uncovered multiple nucleotide substitutions that resulted in amino acid changes, particularly within the N-terminal 60 residues (Figure 7) – a region critical for *Plasmodium* binding. These substitutions suggest adaptive evolution potentially driven by interspecies transmission pressures.

Phylogenetic analysis further revealed species-specific divergence of the *DARC* gene. Human DARC sequences clustered closely with those of chimpanzees (*Pan troglodytes* and *Pan paniscus*), reflecting shared evolutionary ancestry and providing new insights into host adaptability and zoonotic risk (Figure 8). Samples

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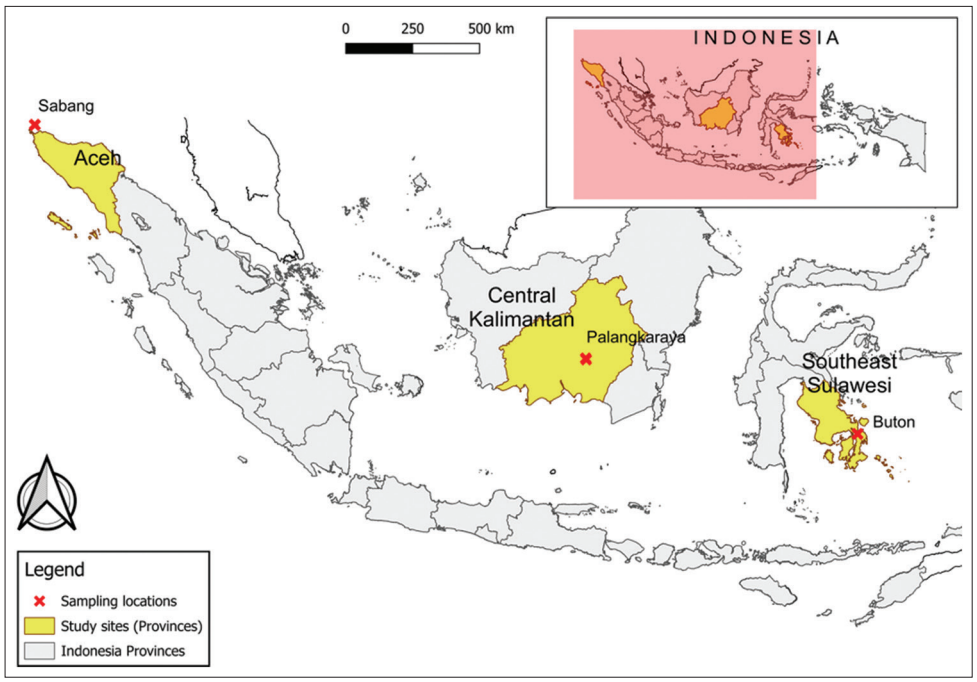

Figure 2: Study site [Source: http://tanahair.indonesia.go.id/portal-web].

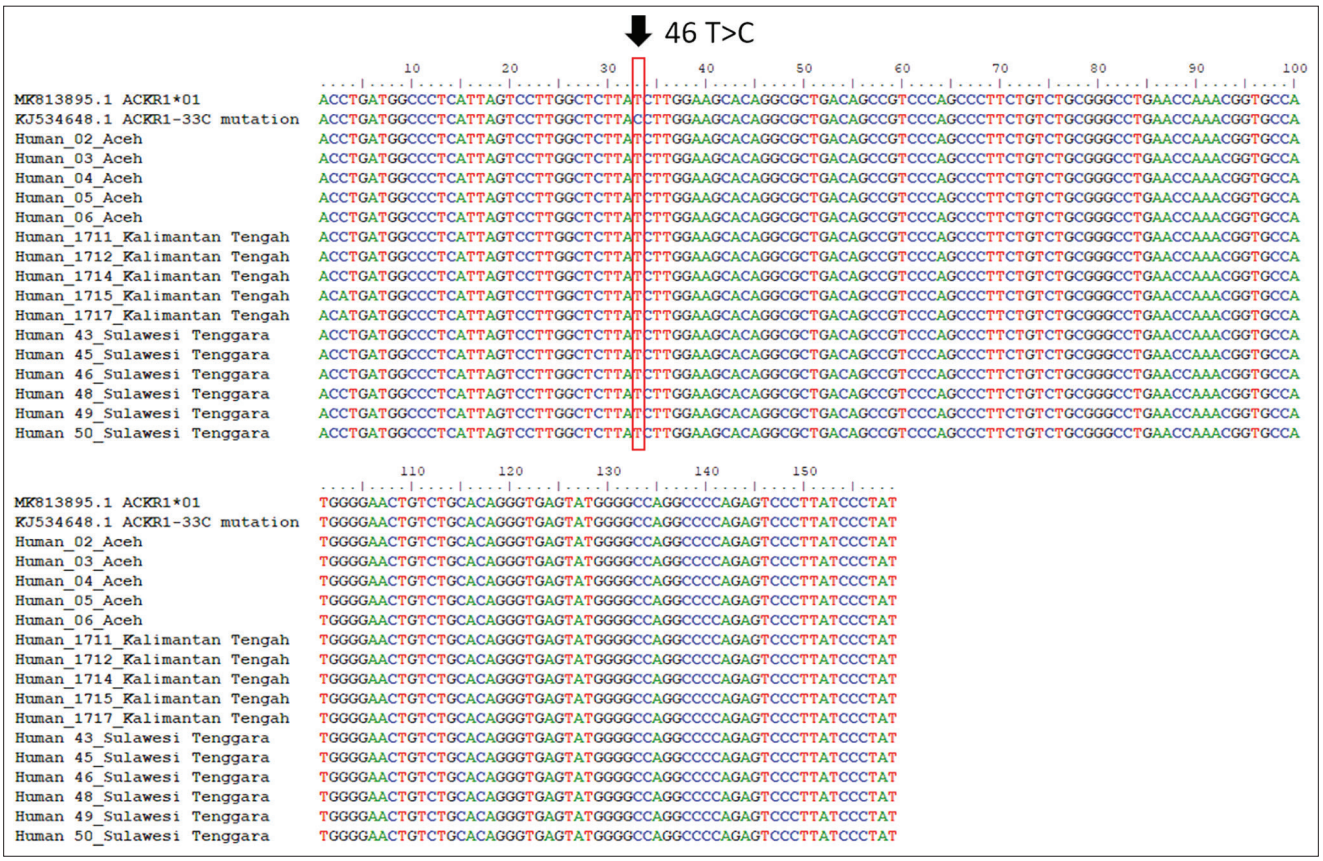

Figure 3: Sequence alignment of the TATA box promoter.

from *M. fascicularis* and *M. brunnescens* formed a monophyletic group with *Macaque* and *Cercocebus atys* (Sooty mangabey), whereas *Hylobates* species grouped with other Hylobatidae members, including *Hylobates moloch*, *Symphalangus syndactylus*, and *Nomascus leucogenys*.

DISCUSSION

Persistence of malaria and the challenge of zoonotic transmission in Indonesia

Malaria continues to pose a significant public health burden in Indonesia, despite the implementation of a national elimination program targeting

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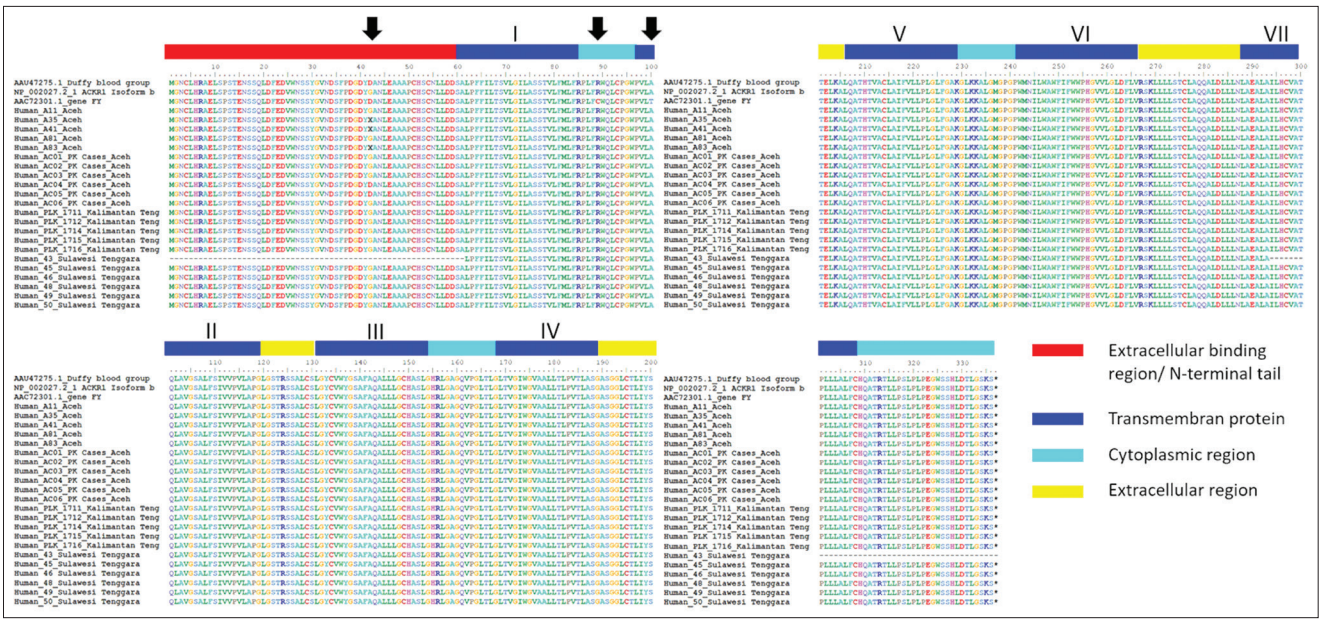

Figure 4: Sequence alignment of amino acid Duffy antigen/receptor for chemokines regions in human samples.

Table 2: DARC profile of human samples.

No.	Sample code	Location	DARC	Length of amino acid	DARC type	TATA promoter (−46)
1	Human_A11_Aceh	Aceh	Complete	336	FY*A	T
2	Human_A35_Aceh	Aceh	Complete	336	FY*A/FY*B	T
3	Human_A41_Aceh	Aceh	Complete	336	FY*A/FY*B	T
4	Human_A81_Aceh	Aceh	Complete	336	FY*A	T
5	Human_A83_Aceh	Aceh	Complete	336	FY*A/FY*B	T
6	Human_AC01_PK Cases_Aceh	Aceh	Complete	336	FY*A	T
7	Human_AC02_PK Cases_Aceh	Aceh	Complete	336	FY*A	T
8	Human_AC03_PK Cases_Aceh	Aceh	Complete	336	FY*A	T
9	Human_AC04_PK Cases_Aceh	Aceh	Complete	336	FY*B	T
10	Human_AC05_PK Cases_Aceh	Aceh	Complete	336	FY*B	T
11	Human_AC06_PK Cases_Aceh	Aceh	Complete	336	FY*A	T
12	Human_43_Kalimantan Tengah	South East Sulawesi	Partial	293	FY*A	T
13	Human_45_Sulawesi Tenggara	South East Sulawesi	Complete	336	FY*A	T
14	Human_46_Sulawesi Tenggara	South East Sulawesi	Complete	336	FY*A	T
15	Human_48_Sulawesi Tenggara	South East Sulawesi	Complete	336	FY*A	T
16	Human_49_Sulawesi Tenggara	South East Sulawesi	Complete	336	FY*A	T
17	Human_50_Sulawesi Tenggara	South East Sulawesi	Complete	336	FY*A	T
18	Human_1711 PLK_Kalimantan Tengah	Central Kalimantan	Complete	336	FY*A	T
19	Human_1712_PLK_Kalimantan Tengah	Central Kalimantan	Complete	336	FY*A	T
20	Human_1714_PLK_Kalimantan Tengah	Central Kalimantan	Complete	336	FY*A	T
21	Human_1715_PLK_Kalimantan Tengah	Central Kalimantan	Complete	336	FY*A	T
22	Human_1716_PLK_Kalimantan Tengah	Central Kalimantan	Complete	336	FY*A	T
23	Human_1717_PLK_Kalimantan Tengah	Central Kalimantan	Complete	336	FY*A	T

DARC=Duffy antigen/receptor for chemokines , TATA box promotor

eradication by 2030. While this program has effectively reduced or eliminated malaria incidence in many regions, the emergence of zoonotic malaria, particularly infections caused by *P. knowlesi* originating from macaques introduces new challenges that may complicate elimination efforts [8, 28]. The presence of competent vectors and NHP hosts across Central and Western Indonesia, coupled with increased human activity in forested areas, heightens the risk of zoonotic malaria transmission [8, 29].

Ethnic diversity and regional variation in genetic susceptibility

The three study sites examined in this investigation represent regions with varied genetic backgrounds relevant to malaria susceptibility. Aceh, which recorded the second-highest number of *P. knowlesi* cases in Indonesia from 2011 to 2021, is inhabited by a div-erse population comprising indigenous Acehnese and individuals of Indian, Persian, Arab, and Turkish descent [30]. This ethnic diversity contrasts with that of Borneo and Southeast Sulawesi, where fewer *P.*

doi: 10.14202/IJOH.2025.147-158

Figure 5: Sequence alignment of amino acid Duffy antigen/receptor for chemokines regions in non-human primates.

knowlesi cases have been reported and population heterogeneity is comparatively limited.

DARC promoter polymorphisms and allelic landscape in Indonesia

Duffy-negative individuals, characterized by the −46T>C SNP in the DARC promoter, exhibit either reduced or absent expression of the DARC receptor on erythrocyte membranes, thereby impeding parasite invasion [19]. The absence of this SNP among both human and NHP samples in the present study suggests that DARC-negativity, which confers resistance to *P. vivax*, does not play a comparable role in *P. knowlesi* susceptibility. The identification of three distinct DARC alleles (*FYA*, *FYB*, and *FYA/FYB*) among Acehnese subjects constitutes a novel finding within the Indonesian genomic landscape, possibly reflecting complex interactions between ancestral lineage and pathogen-driven selective pressures. In contrast, individuals from Central Kalimantan and Southeast Sulawesi predominantly exhibited the *FYB** allele. This divergence is likely attributable to the multi-ethnic composition of the Acehnese population. The absence of the *FYX** allele – previously identified in Brazilian populations and defined by SNPs Arg89Cys and Ala100Thr – further highlights the region-specific nature of DARC evolution in Southeast Asia [31].

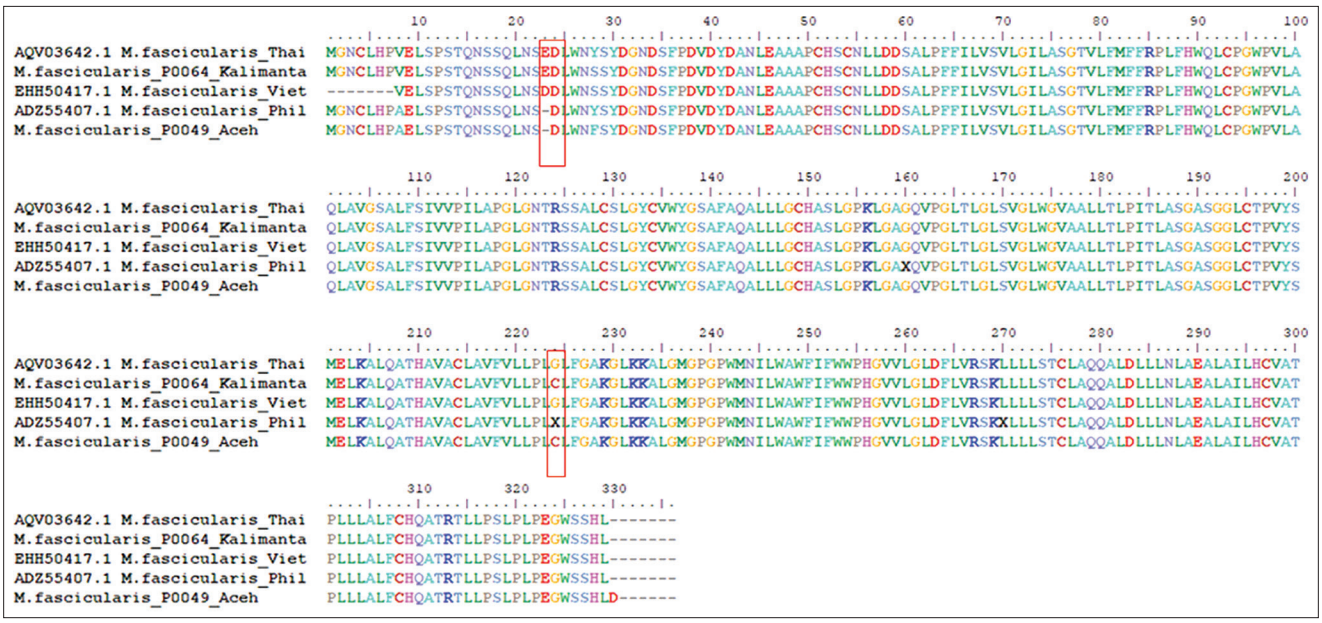

Figure 6: Sequence alignment of amino acid macaque samples.

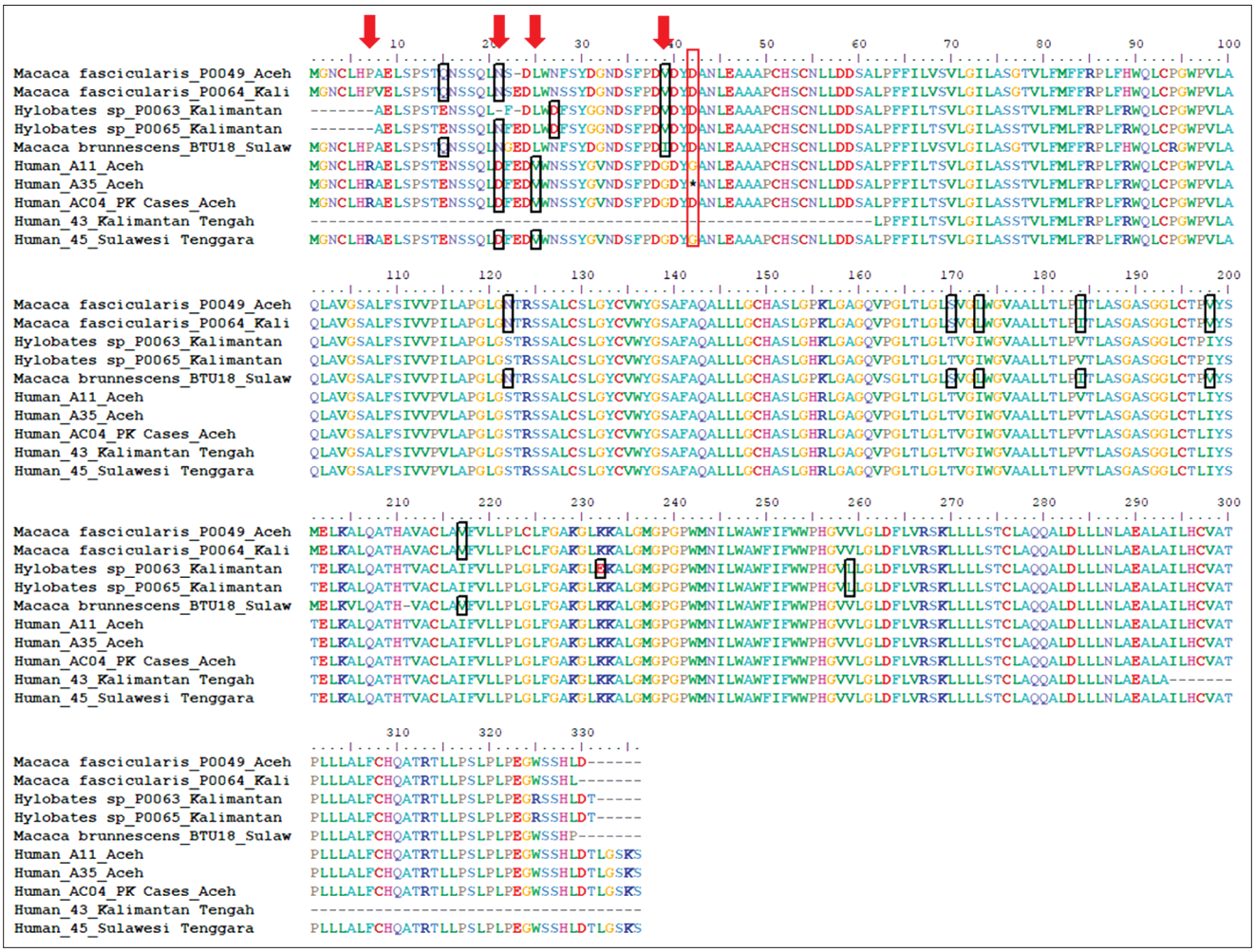

Figure 7: Sequence alignment of amino–non-human primate samples.

Functional insights into DARC genotypes and malaria resistance

Evidence suggests that DARC genotypes confer varying degrees of resistance to malaria infection. Duffy-negative individuals (−46C) with *FYA/FYB* and *FYB/FYB* genotypes exhibit reduced susceptibility to *P. vivax*, whereas genotypes *FYA/FYB* and *FYA/FYA* have been associated with increased risk. In addition, genotypes *FYB/FYX* and *FYA/FYX* have been linked to lower parasitemia [31]. Among the *P. knowlesi* cases from

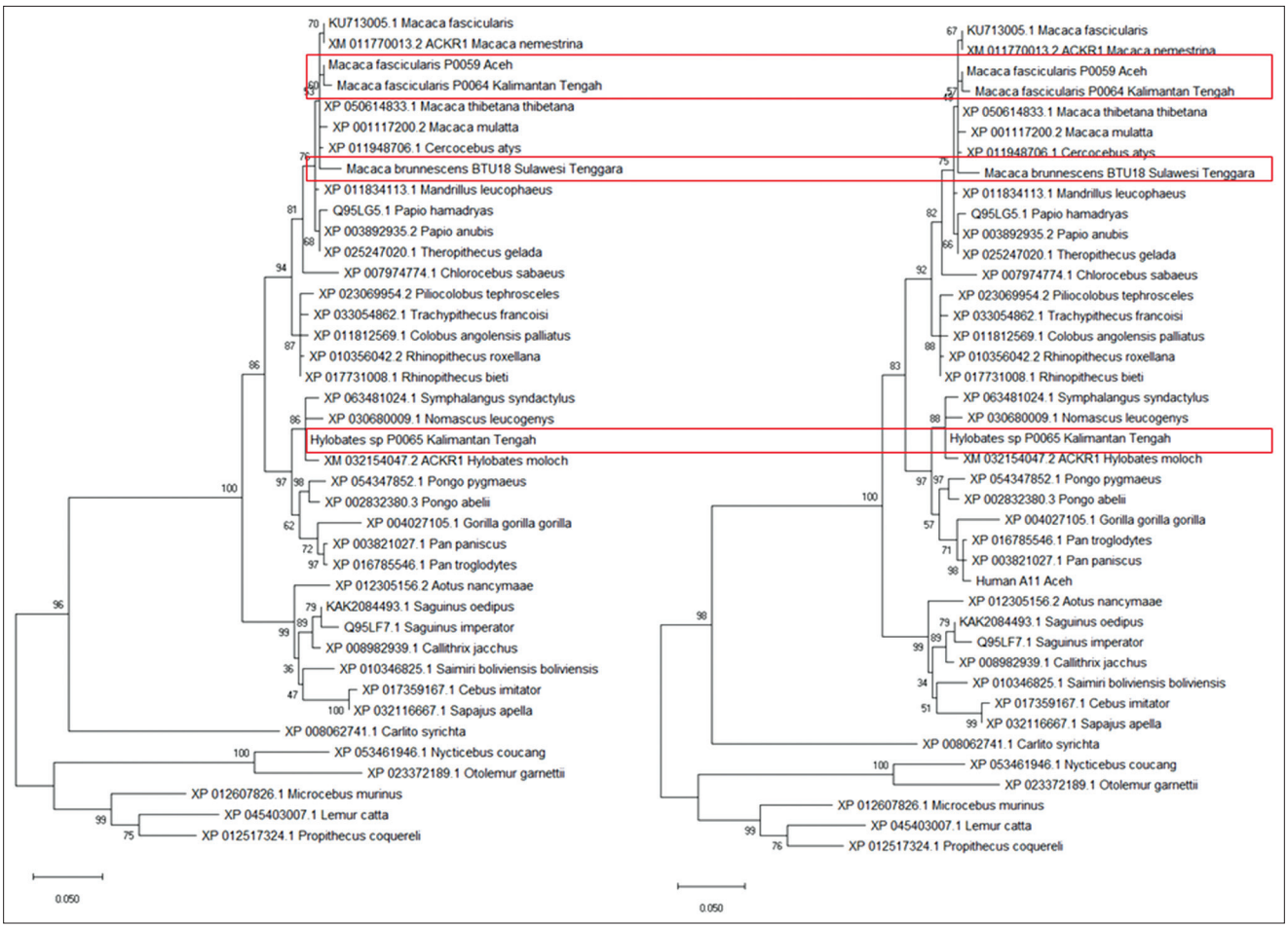

Figure 8: Phylogenetic tree of non-human primates and humans for DARC.

Aceh, only *FYA** and *FYB** genotypes were detected, with no individuals harboring the heterozygous *FYA/FYB* genotype. A comparative analysis revealed an overrepresentation of the FY*A** allele among non-cases, suggesting a potential protective role against *P. knowlesi* infection. These findings imply that *FYA** may serve as a genetic marker of resistance. However, given the differing mechanisms by which *P. vivax* and *P. knowlesi* utilize Duffy antigens for erythrocyte invasion, further investigation is warranted to elucidate the functional consequences of these interactions. This study contributes to the existing literature by proposing a novel role for *FYA** in modulating *P. knowlesi* invasion, distinct from its function in *P. vivax* resistance [32, 33].

Molecular mechanisms of reduced parasite binding

Unlike the *FYB** allele, *FYA** significantly reduces the binding affinity of *P. vivax* Duffy binding protein (PvDBP) to erythrocyte surfaces, thereby lowering the risk of clinical vivax malaria. Erythrocytes expressing *FYA** demonstrate half the PvDBP binding efficiency of *FYB** erythrocytes and exhibit greater antibody-mediated inhibition of PvDBP attachment. In studies conducted in the Brazilian Amazon, individuals with the $Fya^{+}b^{-}$ phenotype exhibited a 30%–80% reduced risk of clinical vivax malaria [34]. The findings of the present study suggest that the Acehnese population may be more susceptible to both *P. vivax* and *P. knowlesi* than populations from Central Kalimantan and Southeast Sulawesi, which may partly explain the higher burden of zoonotic malaria in Aceh. To validate these observations, future research should incorporate larger sample sizes of *P. knowlesi* cases from both Aceh and other regions.

Regional contrasts and the role of *FY*X* and other factors

Comparative studies from Brazil and Malaysia examining populations with and without the *FYX** allele have revealed notable differences in infection rates. Regions with a predominance of the *FYA** allele report higher incidence of *P. knowlesi*, suggesting that additional host-parasite dynamics may influence infection risk. Factors such as antibody cross-reactivity, parasite genetic diversity, and region-specific selective pressures may contribute to these disparities [32, 33, 35, 36]. Of the approximately 30 *Plasmodium* species known to naturally infect primates, nearly one-quarter are capable of infecting humans [37].

Sequence homology in DARC and zoonotic transmission risk

A comparative analysis of DARC amino acid sequences between human subjects and *M. fascicularis* identified four motifs that differ within the first 60 residues, particularly positions 8–42, a domain critical

for *Plasmodium* binding [38]. The high degree of sequence similarity in this region may facilitate zoonotic transmission, offering a novel conceptual framework for evaluating transmission risk based on homology within invasion domains.

Phylogenetic context and evolutionary divergence

Phylogenetic analyses revealed that the human *DARC* gene clusters closely with those of chimpanzees, gorillas, and orangutans, consistent with hominid evolutionary relationships as inferred from genomic studies [38–40]. The observed phylogenetic tree topology aligns with prior investigations, underscoring the evolutionary conservation and species-specific divergence of the *DARC* gene [17]. These findings suggest that interspecies differences in DARC polymorphisms may reflect the broader patterns of primate evolution and have important implications for host susceptibility to zoonotic malaria.

CONCLUSION

This study provides the first comprehensive genomic investigation of the DARC polymorphisms in both human and sympatric NHP populations across distinct malaria-endemic and non-endemic regions in Indonesia. The absence of the −46T>C SNP in both humans and NHPs indicates that Duffy-negativity, which confers resistance to *P. vivax*, is not a key factor in susceptibility to *P. knowlesi* in these populations. Genotypic profiling revealed that *FYA**, *FYB**, and *FYA*/*FYB* alleles are present in Aceh, while only *FYA** was found in Central Kalimantan and Southeast Sula-wesi. Importantly, *FYA** was overrepresented in unin-fected individuals in Aceh, suggesting a potential protective role against *P. knowlesi*. High DARC sequence homology between humans and macaques, particularly *M. fascicularis* and *M. brunnescens*, highlights a molecular basis for zoonotic compatibility.

These findings have important practical impli-cations for malaria surveillance and control strategies. The identification of regional DARC allele patterns provides a genomic marker that could support risk stratification for zoonotic malaria. Moreover, under-standing the host genetic architecture underlying *P. knowlesi* susceptibility could inform the development of targeted interventions in populations at heightened risk of zoonotic spillover.

The strengths of this study include its integrative cross-species design, which enables novel comparisons between human and NHP DARC sequences, and its regionally stratified sampling across diverse ecological zones. The use of archived and newly collected samples further supports a robust temporal and spatial genetic comparison.

However, limitations include the relatively small number of *P. knowlesi*-infected human cases analyzed, which constrain the statistical power to confirm genotype-phenotype associations. In addition, while DARC was the focal gene of interest, other erythrocyte receptors and immunological factors likely contribute to susceptibility and were not assessed in this study.

Future research should expand sample sizes and geographic coverage to validate the association between *FYA** and resistance to *P. knowlesi*. Longitudinal studies incorporating vector behavior, parasite genotyping, and host immune profiling are essential to delineate the complex interplay driving zoonotic transmission. Furthermore, exploration of functional assays to assess DARC-parasite binding efficiency across genotypes will be critical to establishing mechanistic insights.

In conclusion, this study provides foundational genomic evidence for the role of DARC polymorphisms in shaping host susceptibility to zoonotic malaria in Indonesia. It underscores the value of incorporating host genetics into malaria elimination frameworks, particularly in regions where simian malaria is an emer-ging threat.

DATA AVAILABILITY

All relevant data are included in the manuscript.

AUTHORS' CONTRIBUTIONS

DS, PBSA, DHP, and DAS: Conceptualized the study. DS, PBSA, and DHP: Drafted the manuscript. DHP, PBSA, DAS, EHM, II, IER, LS, FNC, HK, HA, NH, and DS: Investigated, formal analysis, and contributed to the data curation and design methodology. All authors contributed to this study. All authors have read and approved the final manuscript.

ACKNOWLEDGMENTS

We gratefully thank Lembaga Penelitian dan Pengabdian Kepada Masyarakat of Hasanuddin University, Direktorat Pengabdian dan Pemberdayaan Masyarakat of Indonesia University, Penelitian dan Pengabdian Kepada Masyarakat of Gadjah Mada University, the Eijkman Research Center for Molecular Biology, National Research and Innovation Agency Indonesia (BRIN), and Lembaga Pengelola Dana Pendidikan (LPDP) - Indonesia Endowment Fund for Education Agency for their continued support and encouragement. We thank the Malaria and Vector Resistance Laboratory, Hasanuddin University Medical Research Center, and Genomic Laboratory Facilities, BRIN. The authors thank the Public Health Officer of Aceh Province, Central Kalimantan, and Southeast Sulawesi; Amna Yusra and Anastasia Febiola for their assistance in mass blood sampling in Iboih, Sabang Aceh; and Meyby Lempang Eka Putri for her assistance in sampling in North Buton, Southeast Sulawesi. These activities were part of the doctoral studies of the DHP at the University of Indonesia and the DBR (Degree by Research Programme) at the BRIN. Sample collection and analysis from North Buton, Southeast Sulawesi, and Iboih, Sabang Aceh were supported by funding from Riset Kolaborasi Indonesia 2024; the Ministry of

Education, Culture, Research, and Technology, Republic of Indonesia; and NHP's sample collection and analysis in Central Kalimantan were supported by RIIM-Ekspedisi from BRIN and LPDP. Hasanuddin University, Makassar, supported the article processing charge. The funders played no role in the study design or collection and interpretation of data.

COMPETING INTERESTS

The authors declare that they have no competing interests.

PUBLISHER'S NOTE

Veterinary World (Publisher of the International Journal of One Health) remains neutral with regard to jurisdictional claims in published maps and institutional affiliation.

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