

Short Communication: The detection of *Plasmodium* in mosquitoes from Sumba and Sorong districts, Indonesia

MUNIRAH MUNIRAH¹, ISRA WAHID², FIRDAUS HAMID³, SITTI WAHYUNI^{2*}

¹Doctoral Program in Medical Faculty, Universitas Hasanuddin. Jl. Perintis Kemerdekaan 10, Tamalanrea, Makassar 90245, South Sulawesi, Indonesia

²Department of Parasitology Faculty of Medicine, Universitas Hasanuddin. Jl. Perintis Kemerdekaan 10, Tamalanrea, Makassar 90245, South Sulawesi, Indonesia. Tel.: +62-411-586010, Fax.: +62-411-586297, *email: sittiwahyuni@gmail.com

³Department of Microbiology, Faculty of Medical, Universitas Hasanuddin. Jl. Perintis Kemerdekaan 10, Tamalanrea, Makassar 90245, South Sulawesi, Indonesia

Manuscript received: 19 October 2020. Revision accepted: 13 June 2021.

Abstract. Munirah M, Wahid I, Hamid F, Wahyuni S. 2021. Short Communication: The detection of *Plasmodium* in mosquitoes from Sumba and Sorong districts, Indonesia. *Biodiversitas* 22: 2680-2684. Malaria is heading for elimination in 2030, but the disease remains prevalent in Indonesia. Therefore, this study aimed to determine a vector other than *Anopheles* that is potentially responsible for malaria transmission in the country's endemic areas. The mosquitoes were trapped by Kelambu trap, collected using a mouth aspirator, and stored in a tube containing silica gel. They were also examined microscopically following O'Connor identification keys, and Nested PCR was used to detect *Plasmodium* DNA. The number of mosquitoes collected from two areas was 1.336. In Sumba (Gaura village) district, 493 mosquitoes were captured, and the dominant genus was *Anopheles* (58.6%), followed by *Culex* (31%), *Armigeres* (9.9%), *Aedes* (0.2%), and *Lutzia* (0.2%). Among 843 mosquitoes collected from Sorong (Aimas), *Culex* was 98.9%, followed by *Aedes* (0.8%) and *Anopheles* (0.2%). The result of nested PCR found two *An. sundaicus* from Sumba carried *Plasmodium* which belongs to *P. falciparum* and *P. vivax* species (0.14%). Although we did not detect the presence of *Plasmodium* in mosquitoes other than the genus *Anopheles*, in this study, we found *Anopheles* species that have never previously been reported from the area, namely *An. bailey* and *An. barbirostris* from Papua and *An. nivosipes* in West Sumba.

Keyword: *Anopheles*, malaria, nested PCR, *Plasmodium*, vector

INTRODUCTION

Malaria is caused by *Plasmodium* protozoan, and the total cases have reached 228 million globally, with 405,000 deaths in 2018 (WHO 2019). East Nusa Tenggara (Sumba) and West Papua are the areas having the highest prevalence, with 7.04% and 31.29% Annual Parasites Incidence (API), respectively (Indonesian Ministry of Health 2016). Attempts have been made to eliminate the parasite, including mass treatment, breeding site suppression, application of insecticide net (PATH Malaria Centre 2014), and vector surveillance (Indonesian Ministry of Health 2014). However, API's change is still not satisfactory and those methods need expansion to ensure malaria is eliminated.

The high prevalence is inseparable from the existence of *Anopheles*, and the species responsible for spreading malaria is not the same in all areas. In America, the typical vector is *An. freeborni* Aitken, but it is *An. atroparvus van Thiel* in the Middle East and Europe, *An. arabiensis Patton* in Africa and *An. barbirostris van der Wulp* in Asia. In Indonesia, the dominant *Plasmodium* vector differs across all Islands (Sinka et al. 2012), for instance, it is *An. farauti* in Jayapura (Elyazar 2013), *An. balabacensis* in Kalimantan (St. Laurent et al. 2016) and *An. barbirostris* in Sulawesi (Nurdin et al. 2003; Veridiana et al. 2019).

Importantly, *Culex* is the mosquito genus first known as an intermediate host of lymphatic filariasis (Ogoma et al. 2010). Afterward, *Aedes*, *Anopheles*, and *Mansonia* also transmit it (WHO 2013), while malaria cases likely likewise encounter a related situation.

Mutations are changes that occur in the genetic material and it can be passed to heredity (Campbell et al. 2014; Campbell et al. 2015). The mutation allows the parasite to adapt to the host, and it tends to also benefit either or both of them (M'Gonigle 2009). In terms of their interaction, the mutation rate is determined by genome size which is shorter in parasites than the host, therefore causing higher mutation frequency (McDew-White et al. 2019). This condition makes the parasite, known as a pathogenic microorganism, adapt to a new host when exposure occurs continuously (Mackinnon and Marsh 2010). Global warming also plays a role in adding a new host for the parasite (Mills et al. 2010). Stress due to heating causes a mutation in both unicellular or multicellular organisms, which allows them to survive and pass their ability to the next generation for adaptation to the new host environment (Berger et al. 2017). Based on the personal discussion with the head of the communication disease from Sorong Health Office, the survey conducted in one of the district's villages during the malaria outbreak found no *Anopheles* larvae. The theory and case raise the query of whether there is

another genus that potentially carries the *Plasmodium* parasite. This study aims to detect *Plasmodium* as a vector of malaria in female mosquitoes from various genera in malaria-endemic areas.

MATERIALS AND METHODS

Mosquitos collection

Female mosquitoes were collected from the residential area of Gaura Village, South West Sumba District of East Nusa Tenggara, Indonesia (October 2018), and Aimas, Sorong District of West Papua, Indonesia (August 2019). Provincial health offices determined those two areas have the highest API in the districts. Due to the absence of electricity to make a light trap, mosquitoes were collected using Kelambu and the cow available in the area were used as the bait. Kelambu modified from previous studies (Davidson et al. 2019). The mosquito collection in Gaura and Aimas was carried out at 7 different locations, respectively, in one-night collection was done only in one location. Mosquitoes in the Kelambu were collected every hour, from 7 p.m until 1 a.m, using a mouth aspirator. They were placed in 1.5 ml tubes containing silica gel to prevent mold growth and structural damage of the vector, which absorbs moisture to reduce the tube's humidity. Microscopic mosquito identification was performed by following O'Connor's key (O'Connor et al., 1981). Especially for *Anopheles*, the species were validated by PCR using ITS2 primer (Weeraratne et al. 2017), and the product was sent to Genetica Science, Singapore, for sequencing. For *Plasmodium* DNA identification, the head-thorax was separated from the abdomen by a fine needle, and both parts were stored in different tubes.

DNA Extraction

One to five head-thorax parts of mosquitoes from the same species were pooled in a 1.5 ml microtube and macerated using disposable Teflon pestles, while the DNA was extracted using the TIANamp Genomic kit. The head-

thorax was smashed in a 1.5 ml tube, 20 ml proteinase K was added, then vortexed and incubated at 56°C (3 hours). Two hundred µl of buffer GB was added to the tube, mixed thoroughly, vortexed before incubation at 70°C (10 minutes) and then centrifuged. Another 200 µl ethanol (96-100%) was poured into the 1.5 tubes, mixed thoroughly, vortexed, and then centrifuged. The mixture from the 1.5 tubes was moved to the spin column which was then placed onto the collection tube. Five hundred µl of buffer GD was added to the spin column, centrifuged at 12000 rpm (30 seconds), discarded the flow-through, and then placed onto the collection tube. Six hundred µl of buffer PW was added and processed as before and repeated once again, which was centrifuged at 12000 rpm for 2 minutes to dry the membrane completely. The spin-column was put into a 1.5 ml tube and 100 µl of buffer TE was pipetted to the membrane's center. It was incubated at room temperature for 5 minutes and then centrifuged as the previous, followed by storing the purified DNA at -20°C. When the PCR product indicated *Plasmodium* in the head-thorax pool, the corresponding mosquito's DNA from the abdomen pools was extracted separately to determine the number of positive species and then stored at -20° for further analysis.

DNA Amplification

Polymerase chain reaction (PCR) Thermociclador T100 Biorad was used to detect *Plasmodium* in the mosquitoes. Nested PCR was performed using three pairs of primers, namely rPLU5: 5'-CCTGTTGTTGCCTTAAACTTC-3' and rPLU6: 5'-TTAAAATTGTTGCAGTTAAAACG-3' for *Plasmodium* species in the nested 1. Furthermore, the other two were rVIV1: 5'-CGCTTCTAGCTTAATCCA CATAACTGATAC-3' and rVIV2: 5'-ACTTCCAAGCCG AAGCAAAGAAAGTCCTTA-3' for *Pl. vivax* in nested 2, rFAL1: 5'-TTAAACTGGTTTGGGAAAACCAAATATATT-3' and rFAL2: 5'-ACACAATGAACTCAATCATGACTA CCCGTC-3' for *Pl. falciparum* in nested 2, as previously described (Snounou et al. 1993; Singh et al. 2013).



Figure 1. Map of Indonesia. The sampling areas in this study in: 1. Gaura Village, South West Sumba District of East Nusa Tenggara, Indonesia (October 2018); 2. Aimas, Sorong District of West Papua, Indonesia (August 2019)

The thermal profile of 35 cycles for nested 1 was 94°C denaturation, 55°C annealings, and 72°C extensions, all at 1 minute each. That of nested 2 was 94°C denaturation (30 seconds), 55°C annealings (1 minute), and 72°C extensions (30 seconds). The entire reactions had 12.5 µl volumes and contained 7 µl PCR enzymes (2×Taq plus PCR Mix, Tiangen Biotech), reverse and forward primers at 1 µl each, 2 µl template, with 2.5 µl ddH₂O. The template for Nested 1 was the DNA, while that of 2 was its PCR results which were then run in 2% agarose gel for electrophoresis. After the *Anopheles* species were identified microscopically, their DNA was extracted and amplified by PCR ITS2 primer and then sequenced for confirmations.

RESULTS AND DISCUSSION

Results

A total of 1,336 mosquitoes were trapped using the *Kelambu* in two different islands, where the highest number of females was 843 in Aimas, but 493 in Gaura village. Furthermore, the total species in the samples was 28, 13 species of *Anopheles* and it was the largest species, followed by *Culex* (10), *Armigeres* (2), *Aedes* (2), and *Lutzia* (1).

In Gaura village, the commonest species was found is *Anopheles* (289), dominated by *An. vagus*, followed by *Culex* (153), *Armigeres* (49), *Aedes* (1), and *Lutzia* (1). The *Culex* genus dominated the mosquitoes trapped in the Aimas region, Sorong district (834 out of the 843), and the most numerous species was *C. quinquefasciatus*, while for *Aedes* and *Anopheles*, only 7 and 2 were caught, respectively.

From 285 mosquito pools (each containing 1-5 mosquitoes of the same species), one was positive for *Pl. falciparum*, another for *Pl. vivax*, and both were from the *Anopheles sundaicus* pools. The display of PCR electrophoresis result contained positive *Pl. falciparum* and *Pl. vivax* can be seen in figure 2. From this study, it was found that one species of mosquito that acts as a malaria vector in Gaura Village, namely *An. sundaicus*, while in Aimas, Sorong District, malaria vectors were not found in all of 843 mosquitoes. This study was the first research conducted in malaria-endemic areas. In addition, after the *Anopheles* species sequencing process, we reported the first time that *A. bailey* species was found in Indonesia.

Discussion

One perspective regarding malaria transmission is accruing the number of vectors from other species, causing the disease's elimination to become difficult. Therefore this study was conducted to determine the vector of *Plasmodium* in malaria-endemic areas. It was known that mutation occurs in living organisms frequently due to the replication, transcription, and translation process (Lynch 2010; Campbell et al. 2015), and this affects the capability to widen or narrow the symbiosis between mosquito and its borne microbes. Particularly, there is the possibility that *Anopheles* is not the only host for *Plasmodium*. To

ascertain whether a mosquito species is a vector, laboratory experiments were carried out by transfecting it with a certain microbe. However, the host's capacity to accept microorganisms is dependent on the evolution of these microbes (Duffy et al. 2012; Sheppard et al. 2018). The finding of new species that acts as a vector for *Plasmodium* explains why this disease remains prevalent and also creates the opportunity to control the transmission. As best known, identifying mosquito species other than *Anopheles* in malaria-endemic areas in Indonesia has never been done.

Table 1. The mosquito genera and species collected by *Kelambu* trap in housing residents and the total number of pools tested from Sumba and Sorong districts, Indonesia

Area	Genera	Species	No. of mosquitoes collected	No. of pools positive/ no. of pools tested		
Gaura, Sumba	<i>Anopheles</i>	<i>An. vagus*</i>	101	0/21		
		<i>An. sundaicus*</i>	53	2/11		
		<i>An. aconitus*</i>	18	0/5		
		<i>An. kochi</i>	15	0/3		
		<i>An. flavirostris*</i>	6	0/2		
		<i>An. indefinitus</i>	13	0/3		
		<i>An. maculatus*</i>	10	0/2		
		<i>An. minimus*</i>	7	0/2		
		<i>An. annularis*</i>	55	0/13		
		<i>An. nivipes*</i>	1	0/1		
		<i>An. subpictus</i>	10	0/2		
		Sub total	289	2/65		
		Culex	<i>Culex</i>	<i>C. tritaeniorhynchus</i>	36	0/8
				<i>C. fuscocephalus</i>	19	0/4
<i>C. sitiens sitiens</i>	4			0/1		
<i>C. vishnui</i>	8			0/2		
<i>C. gellidus</i>	1			0/1		
<i>C. quinquefasciatus</i>	1			0/1		
<i>C. whitei</i>	1			0/1		
<i>C. hutchinsoni</i>	24			0/5		
<i>C. pseudovishnui</i>	59			0/12		
Sub total	153			0/35		
Armigeres	<i>Armigeres</i>	<i>Arm. kesseli</i>	22	0/5		
		<i>Arm. subalbatus</i>	27	0/6		
		Sub total	49	0/11		
Aedes	<i>Aedes sp</i>	1	0/1			
Lutzia	<i>Lutzia sp</i>	1	0/1			
Aimas, Sorong	<i>Anopheles</i>	<i>An. baileyi *</i>	1	0/1		
		<i>An. barbirostris*</i>	1	0/1		
		Sub total	2	0/2		
Aedes	<i>Aedes</i>	<i>Ae. aegypti</i>	2	0/1		
		<i>Ae. Albopictus</i>	5	0/1		
		Subtotal	7	0/2		
Culex	<i>Culex</i>	<i>C. quinquefasciatus</i>	740	0/148		
		<i>C. bitaeniorhynchus</i>	63	0/13		
		<i>C. tritaeniorhynchus</i>	28	0/6		
		<i>C. gellidus</i>	3	0/1		
		Subtotal	834	0/168		
Totally	1336	Total: 2/285				

Note: **Anopheles* validated by PCR using ITS2 primer (Weeraratne et al. 2017) and sequenced to clarify the species

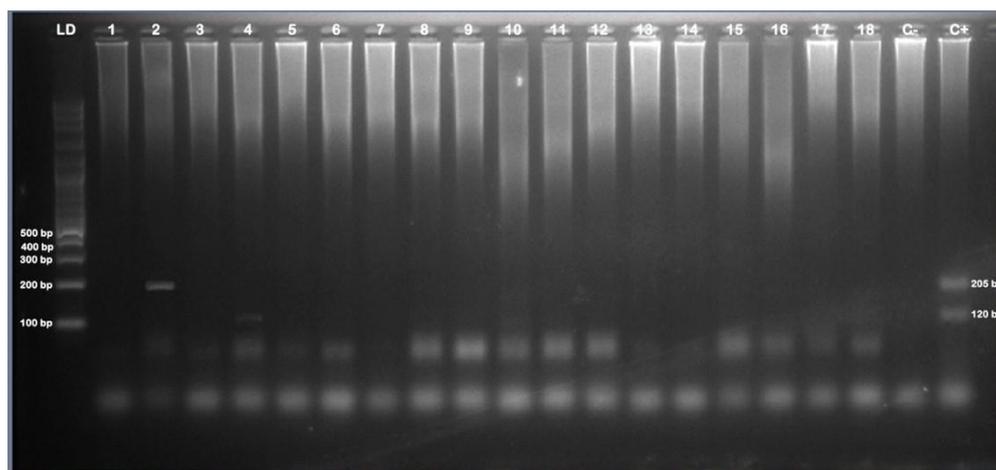


Figure 2. The result was based on electrophoresis of nested PCR mosquito samples. (LD: DNA ladder, C-: control negative, C+: control positive, 2: *An. sundaicus* for positive *Plasmodium falciparum* and 4: *An. sundaicus* for positive *P. vivax*). Also, there were 120 bp (base pairs) for positive *Plasmodium vivax* and 205 bp for positive *P. falciparum*

This study found that *An. sundaicus* was the vector of malaria in Gaura. As we know that *An. sundaicus* is an important vector for spreading malaria in Indonesia (Elyazar et al. 2013; Sugiarto et al. 2016). It has a habit of biting at dusk and dawn (Chakim and Pumpaibool 2019) and is generally found in coastal areas (Surendran et al. 2010). Furthermore, it sucks blood from humans (anthropophilic) and animals (Arifianto 2015), which allows them to breed quickly. In the last decades, *An. sundaicus* has been reported as resistant to several insecticides (Silva et al. 2014; Raghavendra et al. 2017), which causes this species to survive longer. The contribution of mosquitoes to malaria transmission is influenced by environmental factors, blood-feeding, vector competence, season, and others. Overall, no vector other than *Anopheles* was detected as a *Plasmodium* host in this study. However, we also found differences between Gaura and Aimas (24 and 8 species, respectively). Environmental characteristics between the two villages seem responsible for those conditions. Compared to Aimas, people living in Gaura are more diverse because some live in Inland, coastal areas, and hills.

The Abundance of *An. vagus* found in Gaura village requires investigation into its possible contribution to malaria transmission in this village, considering *An. vagus* has been reported as a malaria vector in the South Sumatra region (Budiyanto et al. 2017). In this study *An. vagus* was captured outdoor. This allows the species to be protected from vector control programs such as Indoor Residual Spraying (IRS). Testing of *Anopheles*, *Culex*, *Armigeres*, *Aedes* vectors caught in malaria-endemic areas for the presence of *Plasmodium* may be helpful to confirm their role in malaria transmission.

Apart from COI, ITS2 primers are often used to differentiate *Anopheles* species because of their excellent accuracy. In this study, *An. nivipes* was found in West Sumba while *An. baileyi* and *An. barbirostris* were found in West Papua. From reports on the distribution and bionomics of *Anopheles* as a malaria vector, there are no reports of this species in that area. *Anopheles nivipes* has

been reported in Sumatera while *An. baileyi* in Thailand (Somboon et al. 2020). Furthermore, *An. barbirostris* is an essential vector for the spread of malaria in the country. However, this species has never been reported in West Papua, but only in Kalimantan (Indriyati et al. 2017), Sumatra (Yulidar 2017; Yahya et al. 2020), Java (Ndoen et al. 2010; Hastuti 2011) and Sulawesi (Pinontoan et al. 2017).

The detection of *Plasmodium* in mosquitoes was performed by placing 1-5 head-thorax from the same species in one pool for DNA analysis that allowed more amplification. Once it was detected, each abdomen of the vectors was molecularly examined to determine the number of species carrying parasites in the pool. This method reduces time and budget consumption, especially in a developing country where malaria is usually endemic.

Anopheles is the most suitable host for *Plasmodium*. However, the larvae's absence found in the survey during the malaria outbreak in Sorong district requires an explanation of whether the samples taken were less representative. Also, this is potentially due to other reasons, such as the existence of alternative vectors. The number of mosquitoes collected in this study indirectly affects the results. The more samples in detection, the better it will be in the future. Also, the inability to carry the nitrogen tank to the plane caused ten days collection time in each area to prevent mosquitoes' damage because they were placed at room temperature only.

ACKNOWLEDGEMENTS

We thank the local community for allowing their cows used as baits for mosquitoes and the safety provided during the collection at night. We also thank Nirwana Nur from Hasanuddin University Entomology Laboratory for the assistants rendered in detecting mosquito species and Handayani Halik from Hasanuddin University Medical Research Center (HUM-RC) for her assistance in molecular works. This study was funded by the Ministry of Research and Technology of the Republic of Indonesia.

REFERENCES

- Arifianto RP. 2015. Studi Bionomik Nyamuk *Anopheles sundaicus* Rodenwaldt di Desa Bangsring Kecamatan Wongsorejo Kabupaten Banyuwangi. [Essay]. Jember University, Jember. [Indonesian].
- Berger D, Stanberg J, Grieshop K, Allibert IM. 2017. Temperature effects on life-history trade-offs, germline maintenance and mutation rate under simulated climate warming. *Proc Biol Sci* 284: 1-10. DOI: 10.1098/rspb.2017.1721.
- Budiyanto A, Ambarita LP, Salim M. 2017. Konfirmasi *Anopheles sinensis* dan *Anopheles vagus* sebagai vektor malaria di Kabupaten Muara Enim Provinsi Sumatera Selatan. *Aspirator* 9: 51-60. DOI: 10.22435/aspirator.v9i2.5998.51-60. [Indonesian]
- Campbell IM, Yuan B, Robberecht C, Pfundt R, Szafranski P, McEntagart ME, Nagamani SC, Erez A, Bartnik M, Wiśniowiecka-Kowalnik B, Plunkett KS, Pursley AN, Kang SH, Bi W, Lalani SR, Bacino CA, Vast M, Marks K, Patton M, Olofsson P, Patel A, Veltman JA, Cheung SW, Shaw CA, Vissers LE, Vermeesch JR, Lupski JR, Stankiewicz P. 2014. Parental somatic mosaicism is underrecognized and influences recurrence risk of genomic disorders. *Am J Hum Genet* 95 (2): 173-82. DOI: 10.1016/j.ajhg.2014.07.003.
- Campbell IM, Shaw CA, Stankiewicz P, Lupski JR. 2015. Somatic mosaicism: Implications for disease and transmission genetics. *Trends Genet* 31 (7): 382-392. DOI: 10.1016/j.tig.2015.03.013.
- Chakim I, Pampaibool T. 2019. The diversity of *Anopheles* blood feeding patterns suggests different malaria protection strategies in different localities. *F1000Res* 8: 1217. DOI: 10.12688/f1000research.19341.4.
- Davidson JR, Wahid I, Sudirman R, Makuru V, Hasan H, Arfah AM, Nur N, Hidayat MY, Hendershot AL, Xiao H, Yu X, Asih PBS, Din Syafruddin D, Neil F, Lobo NF. 2019. Comparative field evaluation of kelambu traps, barrier screens and barrier screens with eaves for longitudinal surveillance of adult *Anopheles* mosquitoes in Sulawesi, Indonesia. *Parasites Vectors* 12 (1): 1-13. DOI: 10.1186/s13071-019-3649-7.
- Duffy MA, Ochs JH, Penczykowski RM, Civitello DJ, Klausmeier CA, Hall SR. 2012. Ecological context influences epidemic size and parasite-driven evolution. *Sci* 335: 1636-1638. DOI: 10.1126/science.1215429.
- Elyazar IR, Sinka ME, Gething PW, Tarmidzi SN, Surya A, Kusriastuti R, Winarno, Baird JK, Hay SI, Bangs MJ. 2013. The distribution and bionomics of *Anopheles* malaria vector mosquitoes in Indonesia. *Adv Parasitol* 83: 173-266. DOI: 10.1016/B978-0-12-407705-8.00003-3.
- Indriyati L, Sembiring WSR, Rosanji A. 2017. Keanekaragaman *Anopheles* Spp di Daerah Endemis Malaria Desa Siayuh (Trans) Kabupaten Kotabaru Provinsi Kalimantan Selatan. *ASPIRATOR* 9: 11-20. DOI: 10.22435/aspirator.v9i1.4979.11-20. [Indonesian]
- Ministry of Health of the Republic of Indonesia. 2014. Pusat Data dan Informasi Kementerian Kesehatan RI. Infodatin Kemenkes, Jakarta. [Indonesian]
- Ministry of Health of the Republic of Indonesia. 2016. Malaria. Infodatin Kemenkes, Jakarta. [Indonesian]
- Lynch M. 2010. Evolution of the mutation rate. *Trends Genet* 26 (8): 345-352. DOI: 10.1016/j.tig.2010.05.003.
- McDew-White M, Li X, Nkhoma SC, Nair S, Cheeseman I, Anderson TJC. 2019. Mode and tempo of microsatellite length change in a malaria parasite mutation accumulation experiment. *Genome Biol Evol* 11 (7): 1971-1985. DOI: 10.1093/gbe/evz140.
- Mackinnon MJ, Marsh K. 2010. The selection landscape of malaria parasites. *Sci* 328: 866-871. DOI: 10.1126/science.1185410.
- M'Gonigle LK, Shen JJ, Otto SP. 2009. Mutating away from your enemies: The evolution of mutation rate in a host-parasite system. *Theor Popul Biol* 75 (4): 301-311. DOI: 10.1016/j.tpb.2009.03.003.
- Mills JN, Gage KL, Khan AS. 2010. Potential influence of climate change on vector-borne and zoonotic diseases: A review and proposed research plan. *Environ Health Perspect* 118 (11): 1507-1514. DOI: 10.1289/ehp.0901389.
- Ndoen E, Wild C, Dale P, Sipe N, Dale M. 2010. Relationships between anopheline mosquitoes and topography in West Timor and Java, Indonesia. *Malar J* 9: 242. DOI: 10.1186/1475-2875-9-242.
- Nurdin A, Syafruddin D, Wahid I, Noor NN, Sunahara T, Mogi M. 2003. Malaria and *Anopheles spp* in the villages of Salubarana and Kadaila, Mamuju District, South Sulawesi Province, Indonesia. *Med J Indones* 12 (4): 252-258. DOI: 10.13181/mji.v12i4.119
- Ogoma SB, Lweitojira DW, Ngonyani H, Furer B, Russell TL, Mukabana WR, Killeen GF, Moore SJ. 2010. Screening mosquito house entry points as a potential method for integrated control of endophytic filariasis, arbovirus and malaria vectors. *PLoS Negl Trop Dis* 4 (8): e773. DOI: 10.1371/journal.pntd.0000773.
- PATH Malaria Center of Excellence. 2014. Transformative Tools for Malaria Elimination. Available from Center for Strategic & International Studies, Washington DC.
- Pinontoan OR, Supadmanaba IGP, Manuaba IBA, Sukrama IDM, Manuaba IBP. 2017. Local diversity and biting pattern of *Anopheles* species in Southern Minahasa. *Interdiscip Perspect Infect Dis* 2017: 1-6. DOI: 10.1155/2017/6313016.
- Raghavendra K, Velamuri PS, Verma V, Elamathi N, Barik TK, Bhatt RM, Dash AP. 2017. Temporo-spatial distribution of insecticide-resistance in Indian malaria vectors in the last quarter-century: Need for regular resistance monitoring and management. *J Vector Borne Dis* 54 (2): 111-130.
- Sheppard SK, Guttman DS, Fitzgerald JR. 2018. Population genomics of bacterial host adaptation. *Nat Rev Genet* 19 (9): 549-565. DOI: 10.1038/s41576-018-0032-z.
- Singh B, Daneshvar C. 2013. Human infections and detection of *Plasmodium knowlesi*. *Clin Microbiol Rev* 26: 165-184. DOI: 10.1128/CMR.00079-12
- Sinka ME, Bangs MJ, Manguin A, Rubio-Palis Y, Chareonviriyaphap T, Coetzee M, Mbogo CM, Hemingway J, Patil AP, Temperley WH, Gething PW, Kabaria CW, Burkot TR, Harbach RE, Hay SI. 2012. A global map of dominant malaria vector. *Parasites Vectors* 5: 69.
- Silva AP, Santos JM, Martins AJ. 2014. Mutations in the voltage-gated sodium channel gene of anophelines and their association with resistance to pyrethroids - a review. *Parasites Vectors* 7: 450. DOI: 10.1186/1756-3305-7-450.
- Snounou, G., Viriyakosol, S., Zhu, X. P., Jarra, W., Pinheiro, L., Do Rosario, V. E., et al. (1993) High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. *Mol. Biochem. Parasitol.* 61, 315-320. DOI: 10.1016/0166-6851(93)90077-b
- Somboon P, Phanitchakun T, Namgay R, Wangdi T, Pemo D, Harbach RE. 2020. Molecular and morphological evidence of sibling species in *Anopheles baileyi* Edwards (Diptera: Culicidae) in Bhutan and Thailand. *Acta Trop* 209: 105549. DOI: 10.1016/j.actatropica.2020.105549.
- St. Laurent B, Supratman S, Asih PBS, Bretz D, Mueller J, Miller HC, Baharuddin A, Shinta, Surya A, Ngai M, Laihah F, Syafruddin D, Hawley WA, Collins FH, Lobo NF. 2016. Behaviour and molecular identification of *Anopheles* malaria vectors in Jayapura district, Papua province, Indonesia. *Malar J* 15: 192. DOI: 10.1186/s12936-016-1234-5.
- Sugiarto, Hadi KU, Soviana S, Hakim L. 2016. Confirmation of *Anopheles pedtaeniatus* and *Anopheles sundaicus* as Malaria Vectors (Diptera: Culicidae) in Sungai Nyamuk Village, Sebatik Island North Kalimantan, Indonesia Using an Enzyme-Linked Immunosorbent Assay. *J Med Entomol* 53 (6): 1422-1424. DOI: 10.1093/jme/tjw100.
- Surendran SN, Singh OP, Jude PJ, Ramasamy R. 2010. Genetic evidence for malaria vectors of the *Anopheles sundaicus* complex in Sri Lanka with morphological characteristics attributed to *Anopheles subpictus* species B. *Malar J* 9: 343. DOI: 10.1186/1475-2875-9-343.
- Veridiana NN, Chadajah S, Srikandi Y, Octaviani O. 2019. Konfirmasi vektor malaria dengan elisa di daerah Mendui, Kec. Bungku Tengah, Kab. Morowali, Sulawesi Tengah. *Jurnal Vektor Penyakit* 3 (1): 26-31. [Indonesian]
- Weeraratne TC, Surendran SN, Reimer LJ, Wondji CS, Perera MDB, Walton C, Karunaratne SHPP. 2017. Molecular characterization of Anopheline (Diptera: Culicidae) mosquitoes from eight geographical locations of Sri Lanka. *Malar J* 16: 1-14. DOI: 10.1186/s12936-017-1876-y.
- World Health Organization (WHO). 2013. Global Program to Eliminate Lymphatic Filariasis. WHO, Italy.
- World health organization (WHO). 2019. Malaria. (accessed October 18, 2019). <https://www.who.int/news-room/fact-sheets/detail/malaria>.
- Yahya, Haryanto D, Pahlepi RI, Budiyanto A. 2020. Keanekaragaman jenis nyamuk *Anopheles* di sembilan kabupaten (tahap pre-eliminasi malaria) di Provinsi Sumatera Selatan. *Vektora* 12: 41-52. [Indonesian]
- Yulidar. 2017. Survei nyamuk *Anopheles* yang diduga berpotensi sebagai vektor malaria di Kabupaten Aceh Besar. *Jurnal Biologi Edukasi* 9 (1): 1-5. [Indonesian]