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Research Article

Distribution and behavior of *Anopheles maculatus* and its potential as a Malaria vector in Indonesia

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ABSTRACT

Background: *Anopheles maculatus* is one of the mosquito species that has been confirmed as a malaria vector in Indonesia. The potential of a mosquito as a vector is influenced by its behavior. Information on the distribution and behavior of *An. maculatus* needs to be carried out to determine the potential for malaria transmission transmitted by the species in an area. The study aimed to obtain information on the distribution, behavior, and potential *of maculatus* as a malaria vector in several provinces in Indonesia.

Method: Mosquito collection was carried out using human-landing collection, animal-baited trap, cattle-bait, light-trap, and resting morning. A survey of mosquitoes was carried out in 29 provinces in Indonesia. Mosquitoes were identified for the species and detected the blood-sucking behavior with an Enzyme-linked immunosorbent assay and the presence of *Plasmodium* using a Polymerase Chain Reaction.

Results: The results showed that *An. maculatus* was found in the Riau Islands, Lampung, Bangka Belitung, West Java, Central Java, East Java, and Central Sulawesi. *Anopheles maculatus* has known to suck the blood of humans and animals with a predominance of animals. *Anopheles maculatus* is also known to suck blood outdoors predominantly. In general, the activity of *An. maculatus* sucking blood begins around 18.00 in the evening. Central Java Province was the province with the highest density of *An. maculatus* mosquitoes, thereby increasing the potential for transmission of malaria cases.

Conclusion: *Anopheles maculatus* was spread in Riau Islands, Lampung, Bangka Belitung, West Java, Central Java, East Java, and Central Sulawesi. This species was known to suck the blood of people and humans, and its blood-sucking activity starts around 18.00 in the evening.

Keywords: *Anopheles maculatus,* Malaria*,* Vector malaria

INTRODUCTION

Anopheles maculatus is a malaria vector species in Indonesia found in some provinces such as Central Java, West Java, DIY, East Java, Bali, Lampung, North Sumatra, West Sumatra, Riau, Jambi, and South Sumatra. [1–4] *Anopheles maculatus* was a malaria vector in Thailand, the Philippines, Malaysia [5,6], Afghanistan, Pakistan, China, and India.[7]

The potential of mosquitoes as malaria vectors can be seen from their behavior. The results of research in Thailand and Purworejo Central Java, Indonesia; *An. maculatus* was known to suck the blood of animals and humans.[5,8] The behavior of sucking mosquito blood significantly affects its potential as a malaria vector. Mosquitoes that have the behavior of sucking human blood have the opportunity to be able to transmit *Plasmodium* from human to human. Another factor that supports mosquitoes in becoming malaria vectors is their long life and resistance to *Plasmodium* entering their body.[9–11]

Studies on the distribution and behavior of *An. maculatus* as a malaria vector need to be carried out considering the importance of this species as a malaria vector in several provinces in Indonesia. Mosquito behavior that influences its potential as a malaria vector is resting behavior and blood-sucking behavior. This was also because there were possible differences in mosquito behavior in locations because each region has different environmental conditions. Based on this background, this study aims to discover the development of *An: maculatus'* distribution behavior and its potential as a malaria vector in Indonesia.

METHOD

The research was conducted in 29 provinces in Indonesia. We selected three districts in each province and six sampling points in each district. Each point represented forest, non-forest, and coastal ecosystems near and far from settlements. Sampling was conducted for five days at each point, so with six points in one district, the sampling duration was 30 days.[2] A list of sampling locations can be found in Table 1. DKI Jakarta Province was an exception because no forest ecosystem was found, so sampling was conducted at locations representing the DKI Jakarta area. The selected locations were Central Jakarta, West Jakarta, South Jakarta, East Jakarta, North Jakarta, and the Thousand Islands.

This study was cross-sectional, and the sample was taken at a particular time. The research sample was *An. maculatus* which was caught using various research methods in all provinces. The study population was mosquitoes caught in all provinces. The research variables were the number of *An. maculatus* responded every hour in each province, the number of *An. maculatus* mosquitoes caught in each method in all provinces, and *Plasmodium sp* positive in *An. maculatus* mosquitoes in each province.

Mosquitoes were caught using the human landing collection method, livestock bait, Animal-baited Trap (ABT), light trap, and morning resting collection. Mosquitoes caught in each method identified their species using a mosquito identification key.[12,13] The time used to catch mosquitoes in each technique was different. A list of fishing times and fishing hours per hour can be seen in Table 2.

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Table 1. Sampling location of *An. maculatus* in several provinces in Indonesia

Number	Provinsi	City/County Name
1	Papua	Biak, Merauke , Sarmi
2	Central Java	Pati, Pekalongan, Purworejo
3	South Sumatra	Banyuasin, Lahat, Organ Komeling Ilir (OKI)
4	Central Sulawesi	Parigi Moutong, Tojo Una Una, Toli-toli
5	Aceh	Aceh Timur, Aceh Barat, Pidie
6	West Sumatra	Pesisir Selatan, Padang Pariaman, Pasaman Barat
7	Lampung	Tanggamus, Lampung Selatan, Pasawaran
8	Bangka Belitung	Bangka, Belitung, Bangka Tengah
9	West Java	Garut, Subang, Pangandaran
10	East Java	Malang, Banyuwangi, Pasuruan
11	Banten	Pandeglang. Lebak, Serang
12	Nusa Tenggara Barat	Lombok Barat, Bima, Lombok Utara
13	Nusa Tenggara Timur	Belu. Ende, Sumba Tengah
14	West Kalimantan	Sambas, Ketapang, Kayong Utara
15	South Kalimantan	Barito Kuala, Kota Baru, Tanah Laut
16	North Sulawesi	Minahasa, Kota Manado, Kota Belitung
17	Southeast Sulawesi	Muna, Konawe, Bombana
18	Maluku	Maluku Tenggara Barat, Maluku Tenggara, Kepulauan Aru
19	North Maluku	Halmahera Tengah, Halmahera Selatan, Pulau Morotai
20	Riau	Bengkalis, Dumai, Kepulauan Meranti
21	Jambi	Bungo, Sarolangun, Tanjung Jabung Barat
22	Special Region of Yogyakarta	Kulon Progo, Gunung Kidul, Bantul
23	Bali	Jembrana, Badung, Karangasem
24	Central Kalimantan	Gunungmas, Pulang Pisau, Murung Raya
25	South Sulawesi	Pangkajene Kepulauan, Bulukumba, Luwu Timur
26	West Papua	Raja Ampat, Manokwari, Fak-fak
27	Riau Islands	Kota Batam, Bintan, Lingga
28	DKI Jakarta	Central Jakarta, West Jakarta, South Jakarta, East Jakarta, North Jakarta, the Thousand Islands.
29	North Kalimantan	Bulungan, Nunukan, Tarakan

Table 2. The duration and catchment method of *Anopheles maculatus*

Method	Catch time	Length of catchment
Indoor human bait	6 am - 6 pm	40 minute
Outdoor human bait	6 am - 6 pm	40 minute
Animal baited trap	6 am - 6 pm	15 minute
Cattle bait	6 am - 6 pm	15 minute
Morning resting	6 pm - 9 pm	3 hour

Anopheles maculatus found in all capture methods was prepared by separating the head and thorax from the abdomen and then analyzing its Plasmodium's presence using PCR. Primers used in detecting Plasmodium were rPLU1 (5' - TCA AAG ATT AAG CCA TGC AAG TGA –

3'), rPLU5 (5' - CCT GTT GTT GCC TTA AAC TCC – 3'), rPLU3 (5' - TTT TTA TAA TAA GGA TAA CTA CTA CGG AAA AGC TGT – 3'), rPLU4 (5' - TAC CCG TCA TAG CCA TGT TAG GCC AAT ACC – 3') [14,15] The blood-fed or half-gravid *An maculatus* mosquitoes will be tested their blood feed using the direct-ELISA method.

RESULTS

Based on the results of the study showed that *An. maculatus* was not found in all provinces in Indonesia. The results of the survey in 29 provinces in Indonesia, *An. maculatus* was distributed in Riau Islands, Lampung, Bangka Belitung, West Java, Central Java, East Java, and Central Sulawesi (Figure 1). In all seven provinces, 141 *An. maculatus* mosquitoes were collected. The highest density of mosquitoes *An. maculatus* was found in Central Java Province.

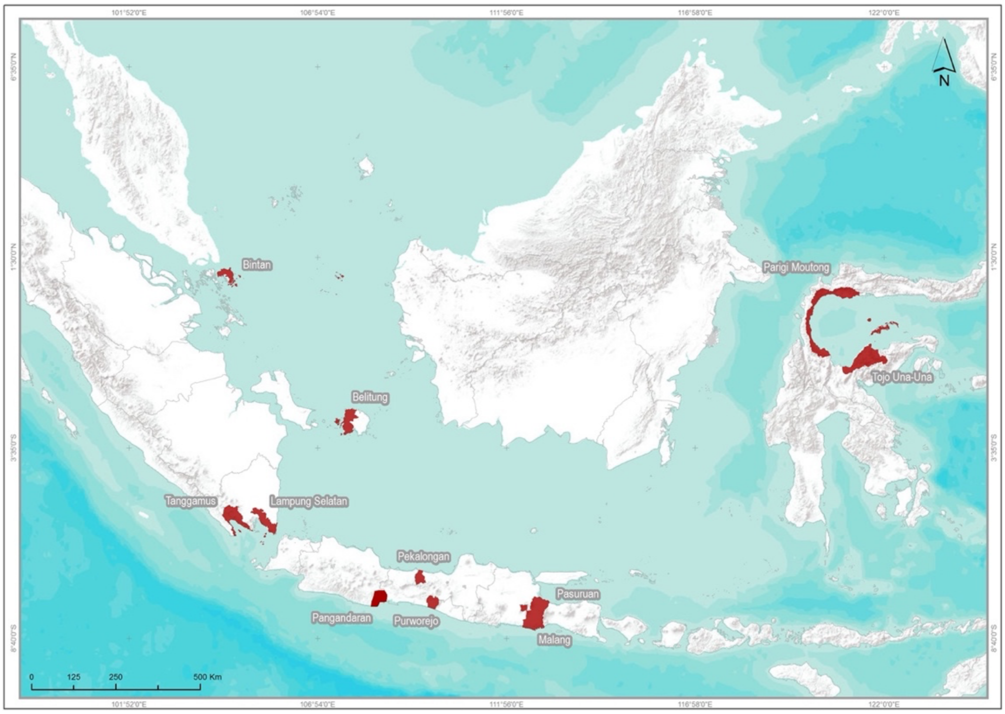

Figure 1. Distribution *An. maculatus* in Indonesia

Anopheles maculatus collection in each province showed different fluctuations and densities. Based on the behavior of sucking blood, *An. maculatus* suck blood starting at 6:00 p.m. in all provinces. However, the *An. maculatus* activity of sucking blood generally does not occur all night. *An. maculatus in* Central Java Province was found sucking blood throughout the night with peak density at 21.00-22.00. Fluctuations in mosquito density based on blood-sucking time in each province showed in Figure 2.

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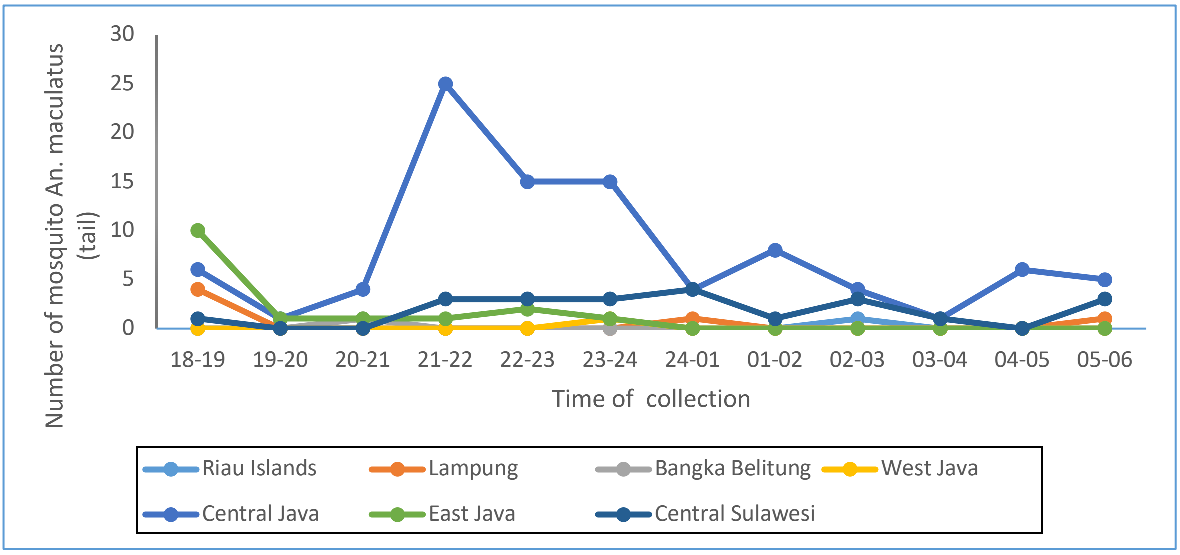

Figure 2. Density fluctuation *An. maculatus* based on blood-sucking time in Indonesia.

The results of mosquito catching by various methods show that, generally, *An. maculatus* dominant was found to suck blood in animals (96.67%), and only a small percentage were found to suck people's blood outside the home (3.33%). The distribution and percentage of *An. maculatus* behavior in sucking blood in each province can be seen in Table 3.

Table 3. Blood-sucking behavior of *Anopheles maculatus* in various provinces in Indonesia

Province name	The number of mosquitoes by the method				
	ABT	CB	IHB	OHB	MR
Riau Islands	**0**	**0**	**0**	**1**	0
Lampung	0	0	0	1	0
Bangka Belitung	0	0	0	1	0
West Java	0	0	0	1	0
Central Java	68	26	0	0	0
East Java	1	0	0	0	0
Central Sulawesi	21	0	0	0	0
Total	90	26	0	4	0
The total base of blood-sucking behavior	116		4		
Percentage of mosquitoes sucking animal blood.	96.67				
Percentage of mosquitoes sucking human blood.			3.33		

Information: ABT: Animal-baited trap; CB: Cattle-bait; IHB: Indoor Human-bait; OHB: Outdoor human-bait; MR: Morning resting

DISCUSSION

Our study shows that *An. maculatus* was found in some provinces in Indonesia, for example, in the Riau Islands, Lampung, Bangka Belitung, West Java, Central Java, East Java, and Central Sulawesi. Based on previous studies, *An. maculatus* was known as a malaria vector in the provinces of Central Java, West Java, DIY, East Java, Bali, Lampung, North Sumatra, West Sumatra, Riau, Jambi, and South Sumatra.[2] The previous study also mentioned that *An. maculatus* be a malaria vector in Laos with *An. rampae, An. sawadwongporni, An.*

pseudowillmori, An. dravidicus, An. minimus, An. aconitus, An. pampanai, An. harrisoni, An. dirus, An. baimaii, An. nemophilou,[16] Peninsula Malaysia, along with *An. cracens.*[17] In addition, *An. maculatus* was also known as the primary malaria vector in Morong, Bataan, Philippines, along with *An. flavirostris.*[18]

The discovery of *An. maculatus* in several provinces in Indonesia shows the potential for transmission and an increase in cases of malaria transmitted by *An. maculatus*. East Java Province was a malaria-receptive area because *An. maculatus* was found, previously confirmed as a malaria vector despite receiving a malaria elimination certificate. Meanwhile, Riau, Lampung, Bangka Belitung, West Java, Central Java, and Sulawesi provinces can transmit malaria because the provinces were still found in malaria-endemic areas.[19]

The potential of *An. maculatus* as a malaria vector can be seen from its blood-sucking and resting behavior. Based on the results of studies showing that *An. maculatus* was known to be dominantly found to suck the blood of livestock, but it was also found to suck human blood. Although it was not dominantly found to suck human blood, contact with humans allows malaria transmission in the local area. This was because most of the regions found by *An. maculatus* were malaria-endemic areas.[19] Studies on China's Hainan Island also show *An. maculatus* was found to suck human and animal blood. *Anopheles maculatus* in the area was known to have blood-sucking behavior several times during one gonotrophic cycle. This condition increases the possibility of malaria transmission.[20] Results of research in Bangladesh *An. maculatus* was also known to suck human and animal blood.[21] Study in Laos *An. maculatus* was the dominant species whose behavior was sucking animal blood (98%).[16] Study in Pu Teuy Village, Sai Yok Region, Kanchanaburi Province, west-central Thailand, and Morong, Bataan Pilipina *An. maculatus* was also found to be predominantly sucking the blood of livestock compared to human blood.[05,18] Study in Vietnam *An. maculatus* tends to be zoophilic and predominantly sucks the blood of buffaloes and cows.[22]

The potential of *An. maculatus* as a vector was also influenced by its ability to survive when Plasmodium enters and develops in its body. Although the results showed no discovery of Plasmodium in the *An. maculatus* examination, considering that the species has been confirmed as a malaria vector, the potential for malaria transmission transmitted by the species can occur again if in the environment there are still cases of malaria. Results of studies in Thailand and Myanmar *An. maculatus* was known to be positive for Plasmodium with a sporozoite index of 1.1%. *Anopheles maculatus* was known to be the dominant species in the area alongside *An. sawadwongporni*.[23] Studies in Nepal also show *An. maculatus* was known to be sporozoite positive with *An. fluviatile* during the spring and found in the vicinity of cages in the forests of Churia Hills and during the summer in the village without spraying.[24] Study in northern Central Vietnam. *An. maculatus* with *An. sinensis, An. aconitus, An. harrisoni, An. sawadwongporni, An. peditaeniatus*, and *An. philippinensis* found protein circumsporozoite.[22]

Based on the study of *An. maculatus* blood-sucking activity starting at 18:00 in all provinces, with different fluctuations. In general, blood-sucking activities do not occur throughout the night. In contrast to *An. maculatus* in Central Java Province, it was found that blood-sucking activity occurred throughout the night. This will enlarge contact with humans allowing the transmission of malaria. The results of a study in the Philippines *An. maculatus* found that the peak of density occurred at 22.00-00.00. The opportunity of *An. maculatus* as a malaria vector was also influenced by its density. Central Java had the highest density of mosquitoes compared to other provinces. High density has the potential to increase the occurrence of contact with humans, thereby increasing the chances of malaria transmission. The results of the research in Purworejo, Central Java, *An. maculatus* was found to be dominant compared to other species. Some of the species found together with *An. maculatus* include *An. balabacensis* (19.76%), *An. vagus* (11.74%), *An. kochi* (5.23%), *An. barbirostris* (3.53%), *An. aconitus* (3.40%), *An. minimus* (1.28%), *An. flavirostris* (1.00%), *An. annularis* (0.04%), *An. tesselatus* (0.01%), and *An. koliensis* (0.01%).[8] Based on studies in Thailand, *An. maculatus* has a high density in the highlands, and the highest density occurs in the rainy season.[25] A survey in Bataan Pilipina *An. maculatus* revealed that peak density occurs at the end of the dry season.[18]

Based on studies showing that *An. maculatus*, in general, was found sucking blood outdoors. This behavior causes the potential for malaria transmission transmitted by *An. maculatus* tends to occur outdoors. Studies in malaria-endemic areas, umphang valley, Tak Province, Western Thailand *An. maculatus* was also found predominantly found outdoors.[26] Studies in sub-Saharan Africa have the potential for malaria transmission to occur outdoors; this was because malaria vectors were found outdoors. The discovery of mosquito blood-sucking activities outdoors can be caused by vector control in the house with insecticidal mosquito nets and indoor residual spraying (IRS).[27] Studies on Guinea's Bioko Island show *An. gambie* was previously found indoors after prolonged application of vector control in the home with insecticidal mosquito nets caused the mosquito to adapt and change its behavior of sucking blood outdoors.[28] Studies in Sub-Saharan Africa show the application of insecticidal mosquito nets and the IRS can reduce cases and deaths caused by malaria. However, the application of vector control in the home impacts changes in vector behavior and vector types that play a role in malaria transmission. This will affect the control methods that should be carried out.[27] Studies in Uganda of vector control applications with the IRS and insecticidicide mosquito nets, in addition to decreasing vector populations and the average number of sporozoites, also led to changes in the type of vectors that act as malaria vectors. The mosquito that worked as a malaria vector before the application of vector control in the house was *An. gambiae*. In contrast, the one that acts as a vector after applying vector control was *An. arabiensis*. The species was widely found outdoors.[29] Due to changes in vector behavior, vector control was also carried out indoors and outdoors.[30]

Another factor that affects *An. maculatus* can play the role of a vector was the breeding places. In general, *An. maculatus* was found in ponds with clear, muddy water containing aquatic plants with an environmental situation of shading. *An. maculatus* was also found around rivers with slow water flow.[5,6] The potential of *An. maculatus* is a malaria vector because apolipoprotein D and cathepsin D proteins were found in the salivary glands in this species.[7]

CONCLUSION

Anopheles maculatus was distributed in the Riau islands, Lampung, Bangka Belitung, West Java, Central Java, East Java, and Central Sulawesi. *Anopheles maculatus* predominantly sucks the blood of livestock and partially sucks the blood of people, with most of its activity occurring outdoors. *Anopheles maculatus* generally sucks blood starting around 18:00, and blood-sucking action typically does not happen overnight. Central Java Province had the highest density of mosquitoes and blood-sucking activities at night. *Anopheles maculatus* was a potential malaria vector in Central Java compared to several other provinces.

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Declarations

Authors' contribution

RS was the main contributor responsible for a technical research team, supervising the research process, analyzing data, compiling research articles, and improving the results of research article revisions. SP was the main contributor to data analysis and the technical research team. MTP is responsible for the technical team of research and data analysis. FDA, ASP, and SN as technical research teams. SA, LS, and ES as a technological research and management tennis team. TWAG as a technical research team and research article reviewer. JC was an auxiliary contributor in charge of creating a distribution map of *An. maculatus*

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Conflict of interest

There was no conflict of interest in this research.

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