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Biodiversity, ecology, and bionomic aspects of *Anopheles* mosquitoes during the dry season in southern Sumatra, Indonesia

DALILAH DALILAH[1,2,♥], CHAIRIL ANWAR[2], DIN SYAFRUDDIN[3], IRSAN SALEH[4], R. IRPAN PAHLEPI[5]

[1]Science and Biomedical Doctoral Program, Faculty of Medicine, Universitas Sriwijaya. Jl. Dokter Muhammad Ali, Palembang 30114, South Sumatra, Indonesia. Tel./fax.: +62-711-373438, ♥email: dalilah@fk.unsri.ac.id

[2]Department of Parasitology, Faculty of Medicine, Universitas Sriwijaya. Jl. Dokter Muhammad Ali, Palembang 30114, South Sumatra, Indonesia

[3]Department of Parasitology, Faculty of Medicine, Universitas Hasanuddin. Jl. Perintis Kemerdekaan 10, Tamalanrea, Makassar 90245, South Sulawesi, Indonesia

[4]Department of Pharmacology, Faculty of Medicine, Universitas Sriwijaya. Jl. Dokter Muhammad Ali, Palembang 30114, South Sumatra, Indonesia

[5]Loka Laboratorium Kesehatan Masyarakat Baturaja, Kementerian Kesehatan Indonesia, Kemelak Baturaja, Ogan Komering Ulu 32111, South Sumatra, Indonesia

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Abstract. *Dalilah D, Anwar C, Syafruddin D, Saleh I, Pahlepi RI. 2024. Biodiversity, ecology, and bionomic aspects of* Anopheles *mosquitoes during the dry season in southern Sumatra. Biodiversitas 25: 1644-1654.* South Sumatra and Lampung are western parts of Indonesia's provinces that are endemic to malaria. This study aimed to determine *Anopheles* mosquitoes' diversity, ecology, and biting behavior in both provinces. Understanding the diversity, ecology, and bionomy of *Anopheles* supports malaria elimination in endemic areas as well as surveillance of vectors in malaria-eliminated areas. The study was conducted during the dry season from June to July 2023. Mosquitoes were collected from southern Sumatra, Indonesia, i.e. Segara Kembang Village in Ogan Komering Ulu (OKU) District, South Sumatra Province, as well as Gayau and Durian Villages in Pesawaran District, Lampung Province. Adult mosquitoes were caught by human landing, resting, and cattle bait collection, while larvae were collected with dippers in the breeding habitat. A total of 4426 mosquitoes from 8 species of *Anopheles* were identified based on their morphology. The diversity index of *Anopheles* from all sample areas during this season was low (H'<1). *Anopheles barbirostris* s.l. and *An. vagus* found in habitats such as paddy fields and former fish ponds in OKU and Pesawaran District. As for abandoned shrimp ponds in Pesawaran, *An. sundaicus* s.l. and *An. subpictus* s.l. were the dominant ones. Mosquito *An. sundaicus* s.l seems to have a greater tendency to bite humans, whereas *An. subpictus* s.l was generally zoophilic. *An. vagus* was always present in all collection methods in all villages, with the greatest abundance in OKU. The finding indicates *An. vagus* appears to be the most variable in the behavior of all captured mosquitoes; therefore, in the future, it may be able to act as a major vector if transmission is not prevented.

Keywords: *Anopheles*, behavior, biodiversity, bionomic, ecology, malaria

Abbreviations: API: Annual Parasite Incidence; CBC: Cattle Bait Collection; HLC: Human Landing Collection; IB: Indoor Biting; IR: Indoor Resting; MBR: Man Bitting Rate; MHD: Man Hour Density; OB: Outdoor Biting; OKU: Ogan Komering Ulu; OR: Outdoor Resting; RC: Resting Collection

INTRODUCTION

Malaria is a vector-borne disease caused by the *Plasmodium* parasite that lives inside red blood cells. Currently, five types of *Plasmodium* can infect humans, i.e. *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi* (Komaki-Yasuda et al. 2018). *Plasmodium falciparum* provides more severe clinical symptoms than other infections (Komaki-Yasuda et al. 2018). *Plasmodium knowlesi* is a zoonotic malaria in Malaysia and Indonesia, and it's been reported especially in the regions of Aceh Besar, Borneo, Langkat, Sabah, and Sarawak (Sugiarto et al. 2022).

Southeast Asia is the second highest region of malaria cases globally after Africa (World Health Organization 2020), and an estimated eight million cases and 11600 deaths from malaria occur in this region. Indonesia, as a country in the Southeast Asian Region, has a high incidence of malaria. As many as 214 districts and cities in Indonesia are still infected by this disease, which spread from Sumatra to Papua (Kemenkes RI 2021). Based on Situation Report on Malaria Control Program in Indonesia in 2020, six districts were low endemic malaria with Annual Parasitic Incidence (API<1) in South Sumatra Province, including Ogan Komering Ulu (OKU), Ogan Komering Ulu Timur (OKU Timur), Ogan Komering Ulu Selatan (OKU Selatan), Muara Enim, Lahat, and Musi Rawas. In 2023, two districts remain endemic in South Sumatera Provinces, namely Lahat and Muara Enim Regencies, while OKU District have received elimination certificates in June 2023 (South Sumatra Provincial Health Service 2023). In Lampung Province, two districts were still malaria-endemic namely Pesawaran, and Bandar Lampung City. The API number in Pesawaran District was 1.2 with moderate endemicity status (Lampung Provincial Health Service 2023).

The *Anopheles* mosquito transmits malaria, and the genus *Anopheles* currently includes 465 officially named species divided into eight subgenera (Harbach and Kitching 2016). In Indonesia, *Anopheles* mosquitoes are vectors of malaria, filaria, and Japanese Encephalitis (St. Laurent et al. 2017; Garjito et al. 2018; Pratiwi et al. 2019). Moreover, 80 species of *Anopheles* have been identified, and 26 species have been declared malaria vectors: *An. aconitus*, *An. annularis*, *An. balabacensis*, *An. barbumbrosus*, *An. bancrofti*, *An. barbirostris* s.l., *An. farauti*, *An. flavirostris*, *An. karwari*, *An. kochi*, *An.koliensis*, *An. letifer*, *An. leucosphyrus*, *An. ludlowae*, *An. maculatus*, *An. minimus*, *An. nigerrimus*, *An. parangensis*, *An. peditaeniatus*, *An. punctulatus*, *An. sinensis*, *An. subpictus* s.l., *An. sundaicus* s.l., *An. tesselatus*, *An. umbrosus*, and *An. vagus*. Nine species as vectors of filariasis: *An. aconitus*, *An. barbirostris* s.l., *An. subpictus* s.l., *An. bancrofti*, *An. koilensis*, *An. farauti* complex, *An. punctulatus*, *An. nigerrimus*, *An. vagus*, and three species as vectors of Japanese encephalitis: *An. vagus*, *An. annularis*, and *An. kochi* (Garjito et al. 2018; Lempang et al. 2023).

Malaria transmission is related to the host, agent and environment triangle. *Anopheles* as vectors is closely related to feeding, resting, biting, breeding behaviors, flight range, vector capacity, mortality and reproduction rates, and the resistance of larvae and adult mosquitoes to insecticides (Castro 2017). The diversity of *Anopheles* mosquitoes is a challenge in annihilating malaria transmission. Some *Anopheles* mosquito's environmental and ecology factors influence the growth and development of *Anopheles* mosquitoes and their exposure to humans. Controlling mosquitoes with biting behavior inside the house is certainly different from controlling mosquitoes that have biting behavior outside the house. Likewise, with resting behavior, habitat type, topography, season, and blood feed selection preferences (Pinontoan et al. 2017).

This study aimed to determine *Anopheles* mosquitoes' diversity, ecology, bionomic, and biting behavior during the dry season in June-July in Ogan Komering Ulu (OKU) District in South Sumatra Province and Pesawaran District in Lampung Province. The sampling time was carried out in those months because, according to data from the Padang Cermin Health Center, Pesawaran District, there have been 26 cases of malaria during January-July 2023 originating from Gayau and Durian Villages. Moreover, according to Evaluation Report Program Malaria Lampung Province In 2023, there is an increase in malaria endemicity status from low endemic in 2022 (API<1) to moderate endemic in 2023 (API 1.2) (Lampung Provincial Health Service 2023).

In contrast to Pesawaran District, which experienced an increase in cases for three consecutive years (2020-2023) in OKU District, there was no single case of malaria. The sampling time was carried out in Segara Kembang Village the months after the malaria elimination status was obtained in June 2023 (South Sumatra Provincial Health Service 2023).

The specific strategy has been carried out by the Lampung Provincial Health Office to prevent malaria and to accelerate control with universal coverage such as mass insecticide mosquito net campaigns, Indoor Residual Spraying (IRS) in villages with API > 40‰, and early discovery and appropriate and complete treatment (Lampung Provincial Health Service 2023). However, the incidence of malaria in Pesawaran District is still high. Therefore, this study supports malaria elimination in endemic areas where there was a fairly high increase in cases in the month of sampling (Gayau and Durian Villages) and surveillance of malaria risk factors in areas with malaria elimination status. Identifying the diversity of species, habitats, behaviors of mosquitoes, and their involvement in malaria transmission can provide important information that leads to the planning and implementation of vector control measures and achieving malaria elimination program (Gayau and Durian Villages), and also surveillance of malaria risk factors in areas that have had malaria elimination status (Segara Kembang Village).

MATERIALS AND METHODS

Study area

The study was conducted in southern Sumatra, Indonesia, i.e. Segara Kembang Village (4º18˙43.51" S, 104º3˙28.34" E) in Lengkiti Sub-district, Ogan Komering Ulu (OKU) District, South Sumatra Province; as well as Durian Village (5°37'10.7"S, 105°10'31.7"E) and Gayau Village (5º36˙57.16" S, 105º10˙6.83" E) in Padang Cermin Sub-district, Pesawaran District, Lampung Province from June to July 2023 (Figure 1).

Procedures

The adult mosquito collection was carried out using aspirators with the following methods: Human Landing Collection (HLC), Resting Collection (RC), and Cattle Bait Collection (CBC), inside and outside the house, and near the cattle shed area. The HLC method was carried out from 06.00 pm - 06.00 am. The HLC technique used seven collectors, three inside and three outside the house and; one more person serves as the final collector. In each hour, the collector worked for 45 minutes and rested for 15 minutes. Mosquitoes that land on humans were sucked using an aspirator. In line with the HLC method, The RC method was carried out from 06.00 pm to 06.00 am. The collector caught the resting mosquito inside and outside the house including in piles of animal feed, cage walls, and plants around the cage using an aspirator. The CBC method was carried out from 06.00 p.m. to 00.00 am, using nets stretched horizontally near the shed's wall and around the cattle shed. Mosquitoes would be netted and then were sucked using an aspirator. Due to limitations in mosquitoes collector, the HLC and RC method was only carried out in Gayau Village and Segara Kembang Village, while the CBC method was carried out in all villages. Each mosquito from the human landing and cattle bait catch was put into a paper cup that had been labeled with the time, place, and method type for further morphological identification. The sampling procedure has obtained a certificate of ethical approval, protocol number: 090-2023 from the Research Ethics Committee of Health Medicine Faculty of Medicine Universitas Sriwijaya.

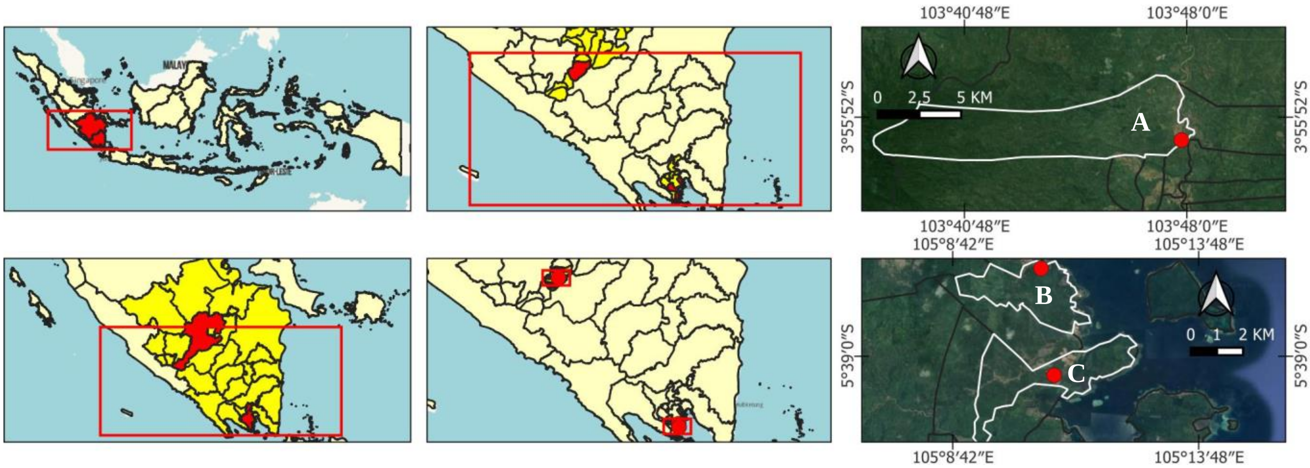


Figure 1. Location of Segara Kembang, Durian, and Gayau Villages. The red dots indicate the sampling sites of *Anopheles* spp.: Segara Kembang (4°18ˈ43.51ˈˈS, 104°3ˈ28.34ˈˈE), Durian (5°37'10.7"S, 105°10'31.7"E); and Gayau (5°36ˈ57.16ˈˈS, 105°10ˈ6.83ˈˈE) Villages

Ecological observations were carried out in the morning after mosquito catching. Habitat observations were alongside the identification of larvae in existing water reservoirs. The larvae collecting was carried out in habitats that were potential breeding sites for *Anopheles*, with a standard dipper of 350 ml. The larvae were taken and reared to adult mosquitoes for further species identification. Mosquito identification uses the *Anopheles* mosquito identification key from O'Connor and Soepanto; Rattanarithikul (O'Connor and Soepanto 2000; Rattanarithikul 2010). Field specimens were placed individually in tubes containing dry silica–els, and cotton swabs immediately after morphological identification. Samples can be used in future studies after being collected and stored at room temperature.

Data analysis

The data were presented in tables, figures, and diagrams regarding the morphology, habitat, and bionomic of *Anopheles* spp. In this study, the species diversity index (H') formulated according to the Shannon-Wiener equation and Simpsons dominance index (C) (Thukral et al. 2019). The density of blood-sucking *Anopheles* mosquitoes per person per night was calculated based on Man Biting Rate (MBR) and Man-Hour Density (MHD) values. The MBR value is obtained from the number of *Anopheles* mosquitoes (certain species) collected per night divided by the number of catchers. Mosquito density (MHD) is the number of mosquitoes that perch per human every hour.

$$H' = -\sum_{i=1}^{S} (P_i \, lon P_i)$$

Where: H': Shannon-Wiener Diversity Index. P_i: fraction of the entire population made up of species i. S: total numbers of species encountered/species richness. ∑ = sum of species 1 to species S

$$C = \sum_{i=1}^{K} (P_i)2$$

Where: C: Simpson's dominance index, Pi: fraction of the entire population made up of species i. K: total numbers of sample size/species richness. ∑ = sum of species 1 to species K.

$$MHD = \frac{\sum \text{Species Mosquitoes Collected}}{\sum \text{Collector X Time Collection (Hours)X Time Collection (Minutes)}}$$

$$MBR = \frac{\sum \textit{Anopheles} \text{ Collected}}{\sum \text{Number of Collectors}}$$

RESULTS AND DISCUSSION

Biodiversity of *Anopheles* spp.

Indonesia has two seasons: the dry season from April to September and the rainy season from October to March. In this study, sampling was carried out from June to July during the dry season in Segara Kembang Village of South Sumatra, Indonesia, and Gayau and Durian Villages of Lampung, Indonesia.

Segara Kembang Village was one of the villages in Lengkiti Sub-district, Ogan Komering Ulu District, South Sumatra Province. The village was in the form of a lowland, with many rubber and oil palm plantations. Temperature and humidity measurements were carried out using a digital hygrometer. During the 12 hours of capture in Segara Kembang Village, the minimum average temperature was measured at 25.15°C and the maximum average temperature at 26.15°C and the relative humidity was 77%.

Padang Cermin Village was one of the sub-districts in Pesawaran District, whose topographic form was coastal, with many paddy fields and shrimp ponds. During the 12 hours of capture in Gayau Village, the minimum average temperature was measured at 27.63 and the maximum average temperature at 29.73 and the relative humidity was 92%, warmer and higher humidity than Segara Kembang.

The number of *Anopheles* mosquitoes caught in this study was 4,426, of which 644 were collected in Segara Kembang Village and 3,782 were collected in Gayau and Durian Villages. The species diversity index (H') in Segara Kembang Village was 0.43, and in Gayau and Durian Villages was 0.35 in calculating the domination index in Segara Kembang Village and Gayau and Durian Villages, the values were obtained as 0.83 and 0.86 respectively.

The HLC and RC methods were used in Segara Kembang and Gayau Village, whereas the CBC method was used in all villages. In Segara Kembang Village, all CBC methods were carried out around the cowshed, yielding 608 mosquitoes. In Gayau and Durian Villages, 2,568 *Anopheles* were collected around the buffalo shed, 1,151 around the cowshed, and 16 around the goat shed (Table 6).

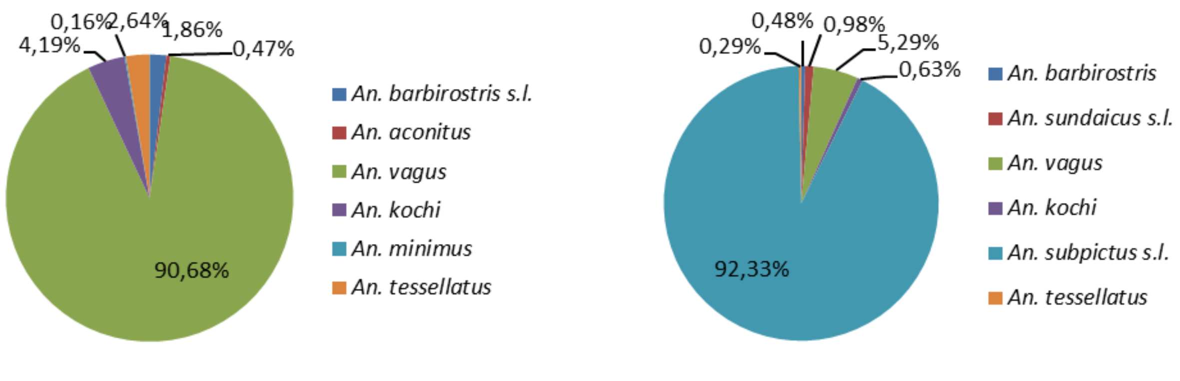
The genus *Anopheles* was distinguished from other genera on palpi and wings. Palpi of *Anopheles* have equal length with proboscis and pale and dark stained wings. The scales on the wings are symmetrical, and the scutellum is one arch (semicircular) with one lobe. They have pale or completely flanged palpi, and their legs are long and slender, with no pulvili (Ministry of Health Republic Indonesia 2000).

A total of six species of *Anopheles* mosquitoes were identified from Segara Kembang Village, i.e.: *An. vagus*, *An. barbirostris* s.l., *An. kochi*, *An. aconitus*, *An. minimus*, and *An. tessellatus*. Six species of *Anopheles* mosquitoes are also found in Durian and Gayau Villages. These include *An. sundaicus* s.l., *An. subpictus* s.l., *An vagus*, *An. barbirostris* s.l., *An. kochi*, and *An. tessellatus* (Figure 2 and Figure 3).

Ecology habitat of *Anopheles* spp.

Examining temperature measurement, water pH, and biotic and abiotic component types accompanied this study's habitat type identification. *Anopheles barbirostris* s.l. larvae found in Segara Kembang Village were all in former fish ponds, and no other habitats were found because most of the shallow puddles, such as ditches, wallows, and artificial shelters, shrink in the dry season (Figure 4); one former fish pond found, upon inspection contained no larvae. The water pH is 6, and the water temperature is 27°C; grass was found around the pond's edge and *Eichhornia crassipes* were found on the surface of the pond (Table 1).

In contrast to the larvae in Gayau and Durian Villages, which were more varied in habitat since most of the community's economy relies on agriculture and shrimp farming, they were mostly found easily in ditches, paddy fields, and abandoned shrimp ponds (Figure 4). In these villages, larvae *An. barbirostris* s.l. were found in ditches and puddles, *An. vagus* in the paddy fields, and, as abandoned shrimp ponds, larvae of *An. sundaicus* s.l. and *An. subpictus* s.l. were found. The water pH is 6, and the water temperature ranges from 26-28°C. *Ipomoea aquatica* was found in the ditches, and *Eichhornia crassipes* in the puddles and rice plants (Table 2).



A B

Figure 2. The composition of *Anopheles* from Segara Kembang, Gayau and Durian Villages, southern Sumatra, Indonesia

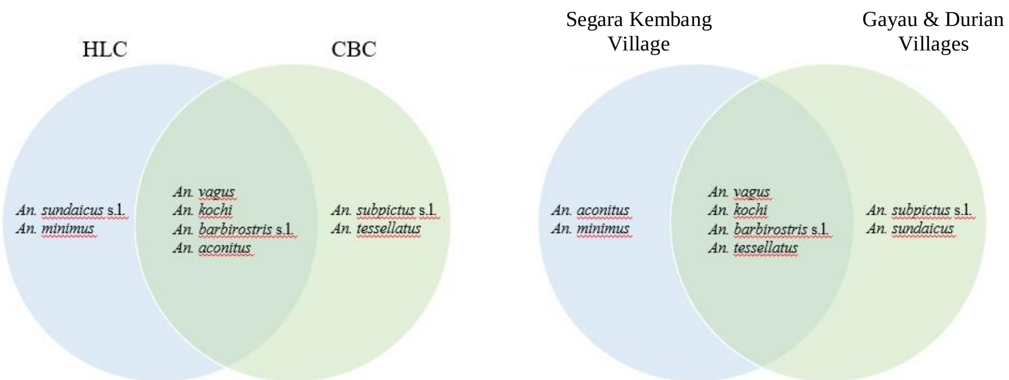


Figure 3. The species composition of mosquitoes from biting behavior and the composition of mosquitoes by region. Intersecting areas indicating mosquitoes were found in the HLC-CBC methods and showing species found in both regions

Table 1. Habitat types identified in Segara Kembang Village, Ogan Komering Ulu District, South Sumatra Province, Indonesia

Type of habitat	Coordinate		Temp. (°C)	pH	Aquatic plants	Larval predator	Species
	Latitude	Longitude					
Former fish pond 1	4.312513	104.058708	27	6.5	Grass, *Eichhornia crassipes*	Fish	*An. barbirostris* s.l.
Former fish pond 2	4.312887	104.05855	27	6.5	Grass	Fish, tad pole	*An. barbirostris* s.l.
Former fish pond 3	4.31223	104.058352	27	6.5	Grass	Fish, tad pole	*An. barbirostris* s.l.
Former fish pond 4	4.312323	104.058258	27	6.5	Grass	Tadpole	-

Table 2. Habitat types identified in Gayau and Durian Villages, Pesawaran District, Lampung Province, Indonesia

Type of habitat	Coordinate		Temp. (°C)	pH	Aquatic plants	Larval predator	Species
	Latitude	Longitude					
Ditches	5.656492	105.180121	26	6	Grass, *Ipomoea aquatica*	Tadpole	*An. barbirostris* s.l.
Puddles	5.656324	105.180379	27	6	Grass, *Eichhornia crassipes*	-	*An. barbirostris* s.l.
Paddy fields	5.648937	105.175106	28	6	Rice plants, grass	Tadpole, fish	*An. vagus*
Abandoned shrimp ponds	5.618415	105.175601	27	6	Grass	-	*An. sundaicus* s.l. *An. subpictus* s.l.

Figure 4. *Anopheles* mosquito habitat in Segara Kembang, Durian and Gayau Villages of southern Sumatra, Indonesia. A. Former fish pond in Segara Kembang Village; B. Paddy fields in Gayau Village; C. Abandoned shrimp ponds Durian Village

Bionomic of *Anopheles* spp.

The collecting carried out in Segara Kembang Village yielded *An. vagus* as the most caught by HLC and RC methods (Table 3). The results of the study in Gayau Village showed that *An. sundaicus* s.l. appeared to have the highest number of mosquitoes caught by the HLC method (Table 4). In contrast to *An. subpictus* s.l., which was very abundant in the Gayau and Durian Villages caught by CBC around cow and buffalo pens (Table 5). The CBC catch in Segara Kembang Village only used one cowshed because no other types of cattle were found. Most of the mosquitoes caught through this method were *An. vagus* (Table 5). In this study, all mosquitoes were caught using outdoor bait, and none were caught using indoor bait. All mosquitoes from three villages were found resting outdoors. *Anopheles vagus* mosquitoes were predominantly captured at 12 hours during Outdoor Biting (OB) and Outdoor Resting (OR) in Segara Kembang Village, whereas *An. kochi* was observed four times throughout the 12-hour mosquito capture period (Figure 5).

The *Anopheles sundaicus* s.l. mosquito was predominantly captured at Gayau Village during a 12-hour period through Outdoor Biting (OB) and Outdoor Resting (OR) (Table 6 and Figure 6). *Anopheles vagus* had the highest MHD value (0.33) in Segara Kembang Village (Table 6 and Figure 7), while *An. sundaicus* s.l. had the highest MHD value (1.25) in Gayau Village (Table 6).

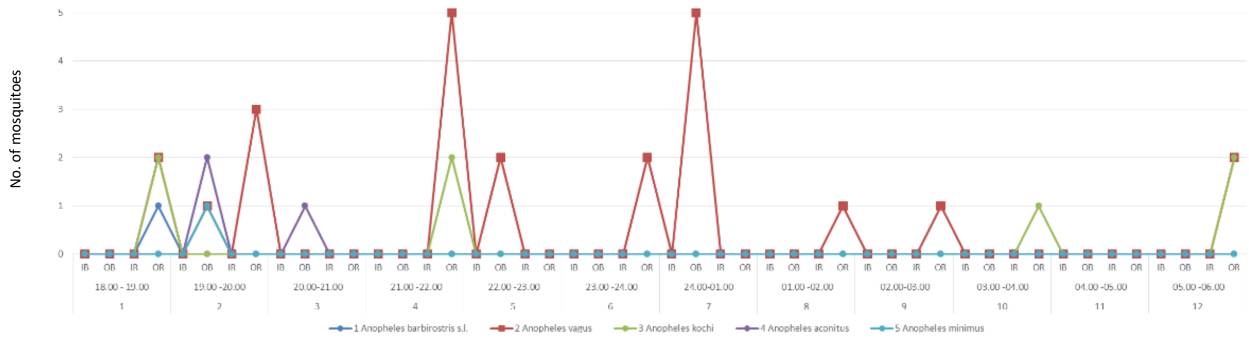


Figure 5. Total *Anopheles* spp. mosquitoes caught with HLC and RC methods per hour for 12 hours in Segara Kembang Village, Ogan Komering Ulu District, South Sumatra Province, Indonesia

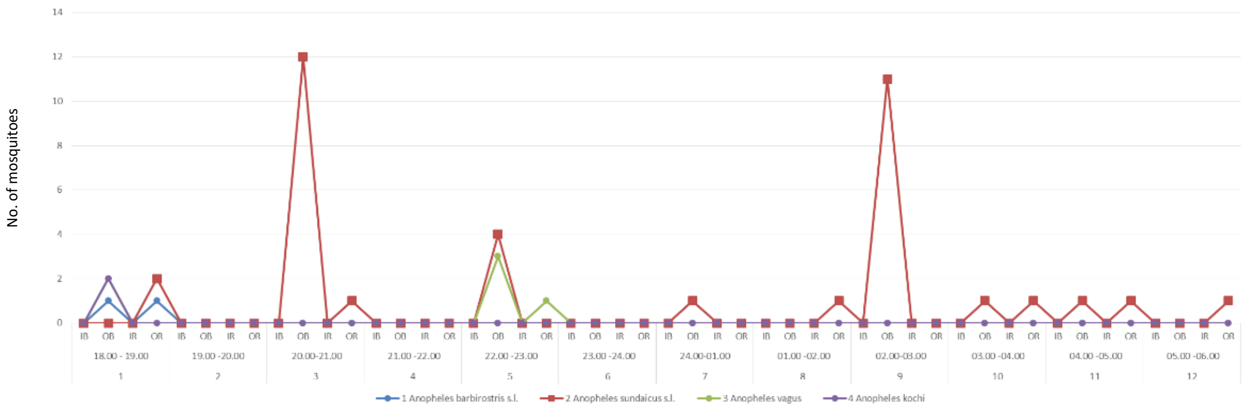


Figure 6. Total *Anopheles* spp. mosquitoes caught with HLC and RC methods per hour for 12 hours in Gayau Village, Pesawaran District, Lampung Province, Indonesia

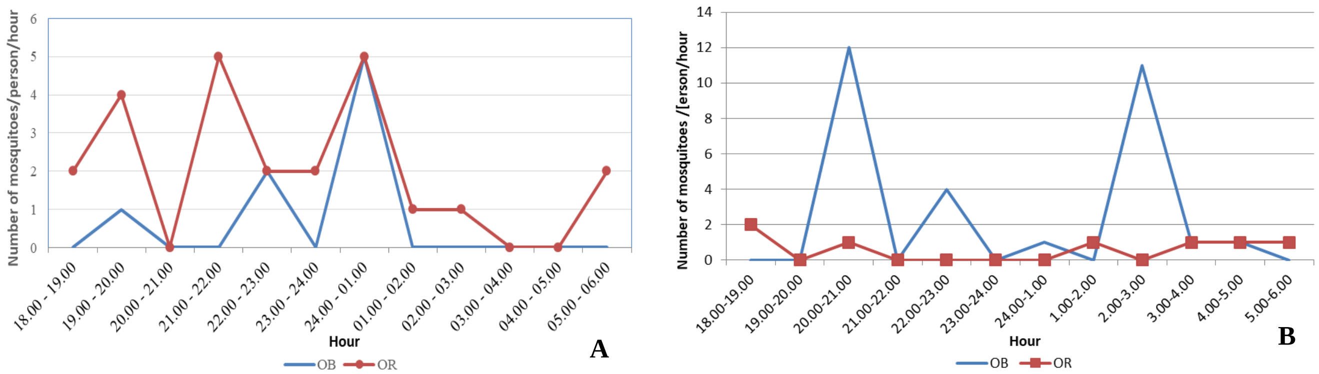


Figure 7. A. *An. vagus*' pattern of biting humans per hour from Segara Kembang Village, Ogan Komering Ulu District, South Sumatra Province; and B. *An. sundaicus* s.l. pattern of biting humans per hour from Gayau Village, Pesawaran District, Lampung Province, Indonesia

Table 3. *Anopheles* mosquito abundance within HLC, RC, and CBC methods in Segara Kembang Village, Ogan Komering Ulu District, South Sumatra Province, Indonesia

Segara Kembang Village												
Species	**HLC**				**RC**				**CBC**		**H'**	**C**
	IB	**OB**	**Total (n)**	**%**	**IR**	**OR**	**Total (n)**	**%**	**Total (n)**	**%**		
An. barbirostris s.l.	0	0	0	0.0	0	1	1	4.2	11	1.8	0.43	0.83
An. aconitus	0	3	3	25.0	0	0	0	0.0	0	0.0		
An. vagus	0	8	8	66.7	0	16	16	66.7	560	92.1		
An. kochi	0	0	0	0.0	0	7	7	29.1	20	3.3		
An. minimus	0	1	1	8.3	0	0	0	0.0	0	0.0		
An. tessellatus	0	0	0	0.0	0	0	0	0.0	17	2.8		
Total	0	12	12	100	0	24	24	100	608	100		

Table 4. *Anopheles* mosquito abundance within HLC and RC method in Gayau Village, and CBC methods in Gayau and Durian Village, Pesawaran District, Lampung Province, Indonesia

Species	Gayau Village								Gayau and Durian Villages		H'	C
	HLC				RC				CBC			
	IB	OB	Total (n)	%	IR	OR	Total (n)	%	Total (n)	%		
An. barbirostris s.l.	0	1	1	2.6	0	1	1	11.1	16	0.4	0.35	0.86
An. sundaicus s.l.	0	30	30	78.9	0	7	7	77.8	0	0.0		
An. vagus	0	5	5	13.2	0	1	1	11.1	194	5.2		
An. kochi	0	2	2	5.3	0	0	0	0.0	22	0.6		
An. subpictus s.l.	0	0	0	0.0	0	0	0	0.0	3,492	93.5		
An. tessellatus	0	0	0	0.0	0	0	0	0.0	11	0.3		
Total	0	38	38	100	0	9	9	100	3,735	100		

Note: n: Number of mosquitoes; IB: Indoor biting; OB: Outdoor biting; IR: Indoor resting; OR: Outdoor resting; H': Diversity Index; C: Dominance Index

Table 5. Total mosquito distribution using the CBC method in Segara Kembang Village, Ogan Komering Ulu District, South Sumatra Province and Gayau and Durian Villages, Pesawaran District, Lampung Province, Indonesia

Species	Buffalo shed (n)	%	Cowshed (n)	%	Goat shed (n)	%	Total (n)	%
Segara Kembang Village								
An. barbirostris s.l.	0	0	11	2	0	0	11	2
An. kochi	0	0	20	3	0	0	20	3
An. tessellatus	0	0	17	3	0	0	17	3
An. vagus	0	0	560	92	0	0	560	92
Total	0	0	608	100	0	0	608	100
Gayau and Durian Villages								
An. barbirostris s.l.	7	0.3	5	0.4	4	25.0	16	0.4
An. kochi	10	0.4	9	0.8	3	18.8	22	0.6
An. subpictus s.l.	2,467	96.1	1,023	88.9	2	12.4	3,492	93.5
An. tessellatus	6	0.2	5	0.4	0	0.0	11	0.3
An. vagus	78	3.0	109	9.5	7	43.8	194	5.2
Total	2,568	100	1,151	100	16	100	3,735	100

Table 6. *Anopheles'* MHD per species in Segara Kembang Village, Ogan Komering Ulu District, South Sumatra Province, and Gayau Village, Pesawaran District, Lampung Province, Indonesia

Species	Total (n)	MHD
Segara Kembang Village		
An. vagus	8	0.33
An. aconitus	3	0.13
An. minimus	1	0.04
Gayau Village		
An. barbirostris s.l.	1	0.04
An. sundaicus s.l.	30	1.25
An.vagus	5	0.21
An. kochi	2	0.08

Discussion

Biodiversity and morphology investigation of Anopheles *spp.*

In this investigation, eight *Anopheles* mosquito species were caught, exhibiting differences in diversity between the three villages. *Anopheles sundaicus* s.l. and *An. subpictus* s.l. typically found in Pasawaran District; and *Anopheles vagus*, *An. kochi*, *An. barbirostris* s.l., and *An. tesselatus* found in both regencies (Figure 6). The diversity index for *Anopheles* species in all sample sites was low (0.43 and 0.35 <1). In contrast with the dominance index result, both areas had a high dominance value (0.83 and 0.86 > 0.75). The lower value of diversity indicated the dominance of certain species of *Anopheles* mosquitoes. In this study, certain *Anopheles* mosquitoes were dominant in different collection methods, such as *An. sundaicus* s.l., which was mostly caught with HLC in Gayau and Durian Villages and *An. vagus* and *An. subpictus* s.l. were dominant in the CBC method in Segara Kembang Village, and Gayau and Durian Villages respectively.

Anopheles sundaicus s.l., *An. subpictus* s.l., *An. vagus* mosquitoes belong to the *Pyretophorus series* subgenus *Cellia* (Harbach and Kitching 2016). Morphologically, *An. subpictus* s.l, *An. sundaicus* s.l, and *An. vagus* has a palpi with three or more pale bracelets, but each palpi character has its distinctive feature. The apical pale band of *An. sundaicus* s.l. is longer than *An. subpictus* s.l.; the difference also lies in the presence or absence of spots on the legs (Sindhania et al. 2020). The *An. vagus* is characterized by the presence of a pale bracelet at the proboscis end; the length of the apical pale band ranges from 3 to 5 times longer than the dark subapical band, and several variations of palpi and wings are found in this mosquito (Kaur 2016). *An. barbirostris* s.l. has dark palpi with very hairy palpomers 1 and 2 and a tuft of black scales on the abdomen of the sternum VII (Taai and Harbach 2015). *Anopheles kochi* has a characteristic of at least 4 pale bracelets on the palpi. In sternit II to VII of the abdomen, there are brushes of dark scales and pale bracelets on the tarsi of the wide hind legs (Harbach and Kitching 2016). *Anopheles tessellatus* has distinctive characteristics: at least one-half of the apical proboscis has a pale scale; the abdomen does not have a dark brush of scales as in *Anopheles kochi*; scales on the scutum and narrow abdomen; the whole halter is scaly white (Gunathilaka and Nayana 2017). *An. aconitus* mosquitoes are characterized by half of the pale proboscis on the dorsum and venter. In contrast, the proboscis in *An. minimus* is entirely dark or partially pale on the ventral side. Costa wings of *Anopheles aconitus* have four spots of pale color, the edges of the hind wings usually have pale fringe points on the veins. The wings are also covered with scales that are dark to pale. In the *An. minimus* costa, there is a pale spot of the humeral part.

Anopheles sundaicus and *An. subpictus* s.l. were specifically found in Gayau and Durian Villages. Both mosquitos were typically found in coastal and less on the hilly ground of freshwater habitats (Sindhania et al. 2020; Syafruddin et al. 2020). From the HLC method conducted in Gayau Village, *An. sundaicus* s.l. was large in number and found using outdoor biting methods. Mosquito *An. subpictus* s.l. was very abundant, and more than 90% of these mosquitoes were caught using the CBC method. *Anopheles sundaicus* s.l. was the most commonly caught by the HLC method on outdoor bait.

The abundance of *An. vagus* mosquitoes were discovered in Segara Kembang Village, with 66.7% captured using HLC and RC methods and 92.1% using CBC. Compared to all mosquitoes caught by the HLC method, these were also found to be the most numerous in outdoor biting. *Anopheles aconitus* and *An. minimus* were only captured in the Segara Kembang Village region during this study. *Anopheles minimus* is usually found in valley areas and forested hills (Dev and Manguin 2016). *Anopheles aconitus* and *An. vagus* were widespread in the landscape of Indonesia. This mosquito can be found in practically any type of topography, from coastal to hilly and mountainous (Anwar et al. 2015).

In this study, the diversity of *Anopheles* spp. in Segara Kembang Village slightly differed from previous studies in other villages in Ogan Komering Ulu District. In a previous study conducted by Yahya et al. 2020 in Padang Bindu Village, Ogan Komering Ulu, South Sumatra Province, five species of *Anopheles* were found using the CBC method: *An. tesselatus*, *An. vagus*, *An. barbirostris* s.l., *An. kochi*, and *An. nigerrimus* (Yahya et al. 2020).

The diversity of mosquitoes found in Gayau and Durian Village, Pesawaran District was fewer when compared to previous studies. This study found six species of *Anopheles* mosquitoes were found whereas, in a previous study in Pesawaran District, sixteen species of *Anopheles* mosquitoes were found by various catching methods (Ritawati and Supranelfy 2018). This difference in results could be due to the number of villages included in the study and the length of the field sampling period. In this study, only two villages were studied in the span of two sampling in two months during the dry season. In comparison, previous research was conducted in eleven villages during years of field sampling (Ritawati and Supranelfy 2018). This is likely related to the abundance and presence of mosquito species associated with the seasons (Agyekum et al. 2021) and sampling frequency. The possibility of certain species according to certain habitat characteristics must be considered; hence, habitat limitations in the dry or rainy season will affect mosquitoes' type and abundance (Amaechi et al. 2018; Ondiba et al. 2019). Another study also revealed that species abundance and diversity influenced climate, season and habitats, and depended on the species' peculiarities, topography (Anwar et al. 2015), and host-pathogen interaction (Roiz et al. 2014). So, diversity and abundance apparently depend on various multifactorial factors affecting the specific *Anopheles* species.

Ecology habitat of Anopheles *spp.*

The results of this study were similar to previous studies, showing that each *Anopheles* mosquito had different habitat preferences and was influenced by the environmental conditions around the habitat, both biotic and abiotic. Biotic components include all living things, such as plants and animals, while abiotic components include temperature, pH, humidity, and salinity (McLaughlin et al. 2019). *Anopheles sundaicus* s.l. and *An. subpictus* s.l. are mostly found along the coast of western to central Indonesia, and there is less fresh water in hilly areas. Brackish water as breeding sites are found in estuaries and swamps that are closed in connection with the sea, which are suitable breeding places for *An. sundaicus* s.l. and *An. subpictus* s.l. (Jude et al. 2014; Nixon et al. 2014). In this study, *An. sundaicus'* s.l. and *An. subpictus'* s.l. were found in abandoned shrimp ponds near the estuary to sea water.

The result identified that larvae of *Anopheles barbirostris* s.l. were found in ditches, rice fields, and puddles in former fish ponds. *Anopheles barbirostris* s.l. in previous studies can also be found in all levels of altitude, mountainous, hilly areas, rice fields, puddles, wallow, used ponds, ditches and other puddles with pH 4-7.5 (Bashar et al. 2016). In contrast to *An. barbirsotris* s.l., *An. kochi* was more commonly found in coastal areas than hilly areas. *Anopheles kochi* is a major important vector of malaria in Sumatra, Sulawesi, and Maluku (St. Laurent et al. 2017). The larvae of *An. kochi* were found in buffalo puddles, and wallows near cowsheds, lakes, and old ponds (Yahya et al. 2020).

In this study, larvae of *An. vagus* were found in former fish ponds and rice fields. This larva can be found in almost all topographic conditions and habitat forms, such as small wallow on beaches, springs, edges of rice fields, lagoons, swamps in muddy ponds, in animal footprints or in brackish water, and various artificial containers, such as old tires, drums and boats (Yahya et al. 2020). Slightly different from *An. vagus*, the habitat of *An. tessellatus* was usually in shady ponds in forests, or ponds shaded by bamboo plantations or rubber trees, swamps, and freshwater fish ponds (Chua et al. 2019).

In this study, only deep water reservoirs, such as ponds and irrigated rice fields could be found as potential breeding sites for *Anopheles* mosquitoes. The few mosquito breeding sites found may be influenced by the seasons, where shallow breeding grounds, such as puddles and trenches, dry out in the dry season.

The temperature and pH of the water examined in the habitat of *Anopheles* mosquitoes in Segara Kembang Village were lower than Gayau and Durian Village, but both were still within the optimal temperature and pH range for breeding *Anopheles* mosquitoes. Biotic factors such as aquatic plants and grasses around the habitat may increase the breeding of mosquito larvae (Ma et al. 2016). However, other studies revealed that larval habitat was affected by pH, temperature and the presence of biotic predators and was not related to other biotic factors such as vegetation and aquatic plants (McLaughlin et al. 2019). In this study, the density of larvae was not calculated, but in the area that contained larvae, plant vegetation such as grass and hyacinths were also found with or without predators. There seem to be more biotic and abiotic factors besides those studied that are influential in larval breeding and their density.

Bionomic of Anopheles *spp.: bitting behavior*

Anopheles vagus was caught in all methods (HLC, RC, and CBC) in all study sites, and it seems to be in greater abundance in Segara Kembang Village, both with HLC and CBC methods. With habitat heterogeneity and diverse blood feed, *An. vagus* with its abundant population seemed to have the ability to adapt to environmental changes. This should be considered as one of the factors that can increase the transmission of malaria transmission through *An. vagus.*

External interactions in humans, livestock, and vectors have a major influence on the tendency of hungry mosquitoes to adjust their biting behavior. The existence of several blood feed options (humans, primates, and other animals), the use of protection such as mosquito nets, or the use of insecticides can change the behavior of mosquitoes that were previously zoophilic can become slightly anthropophilic, or vice versa. When the preferred blood feed becomes scarce, mosquitoes seek alternative available blood feed (Pinontoan et al. 2017; Gueye et al. 2023).

The study in Gayau Village showed that *An. sundaicus* s.l. appeared to have the highest number of mosquitoes caught by the HLC method with the highest MHD (Table 8).. In contrast, *An. subpictus* s.l. was particularly abundant caught by CBC Method. From three collection methods conducted in Segara Kembang Village, the dominant mosquitoes collected was *An. vagus* with the highest MHD (Table 6). The collection by CBC method in Segara Kembang village only used cowshed because no other types of livestock were found. The most netted mosquito through this method was *An. vagus*; in this study, no mosquito was caught using indoor bait.

Female *Anopheles* mosquitos suck nutrient-rich blood to produce eggs and mature (Santos et al. 2019; Harrison et al. 2021). Host blood is selected based on congenital genetics, abundance and environmental factors such as heterogeneity attractive host, and resting area are also important in host selection (Santos et al. 2019; Bashar et al. 2020). In the results of this study, the mosquito that dominated the HLC method in Gayau Village was *An. sundaicus* s.l. while in collecting around livestock sheds was *An. subpictus* s.l. It is following its bionomic nature, *An. sundaicus* s.l. and *An. barbirostris* s.l. are generally anthropophilic and sometimes also zoophilic, while *An. subpictus* s.l., *An. tesellatus*, *An. kochi*, and *An. vagus* are more zoophilic (St. Laurent et al. 2017; Bourke et al. 2021). However, it differs from the results in Segara Kembang Village; in this study, *An. vagus* and *An. kochi* mosquitoes were also found in the HLC method, and both were biting outdoors. Although in this study, no blood feed analysis was carried out, either immunoassay or molecular, the HLC and CBC methods can determine the behavior of these mosquitoes, which can choose several host blood options when there are several choices available in the environment (Jeyaprakasam et al. 2022).

Anopheles subpictus s.l. was not caught in this study using the HLC method at Gayau Village, but it was widespread in the CBC method, particularly in buffalo shed, proving that these mosquitoes are often zoophilic. However, earlier research has shown that *An. subpictus* s.l. enjoyed human blood (Jude et al. 2014; Singh et al. 2014).

Human biting patterns of Anopheles *spp.*

HLC was performed indoors and outdoors to assess bite times and overall mosquito activity at night. In this study, *Anopheles* was found biting outdoor in the house. From this study, *An. vagus*' peak biting time was at 09.00-10.00 pm and the second peak was at 12.00-01.00 am (Figure 7). According to a previous study, the peak biting time of these mosquitoes was between 08.00-09.00 pm (Pinontoan et al. 2017), but other research also found that *An. vagus* biting peaked between 10:00 to 11:00 pm (Rahmawati et al. 2014). It seems that the peak biting time for *An. vagus* varies in each study site. Fully engorged females *An. vagus* can be found resting outdoors (Rahmawati et al. 2014); however, a recent study has found *An. vagus* to be the predominant anthropophilic and resting indoors (Al-Amin et al. 2023). *Anopheles vagus* was known as a secondary vector in malaria, but with many detections of *P. falciparum* and *P. vivax* sporozoites in South Sumatra, Lampung, East Nusa Tenggara, and South Kalimantan from these mosquitoes (Alam et al. 2017; St. Laurent et al. 2017), and the tendency to favor humans as blood feed, therefore it has the potential to become the main vector of malaria.

Anopheles sundaicus s.l. was the dominating species that bite humans because the MHD and MBR values were higher than those of other species in Gayau Village. Although some other studies have shown these mosquitoes can also be endophagic, female mosquitoes are generally anthropophilic and exhibit external feeding tendencies (exophagic) (Rahim et al. 2019). Peak biting activity occurred between 08.00 pm and 03:00-04:00 in the morning (Figure 6). Fully engorged females can rest indoors or outdoors (Sugiarto et al. 2016; Rahim et al. 2019). *An. sundaicus* s.l. is considered as a major vector in western Indonesia, including Southern Sumatra, Kalimantan and central Indonesia (Sugiarto et al. 2016; Syafruddin et al. 2020; Munirah et al. 2021).

In conclusion, the diversity and dominance indices are parameters for assessing diversity in a community. The *Anopheles* diversity index in both regencies was low. The dominance of certain types of *Anopheles* mosquitoes (*An. sundaicus* s.l., *An. subpictus* s.l., were dominant in Pesawaran District, and *An. vagus* was dominant in Ogan Komering Ulu District) can be influenced by seasons, topography and habitat. Coastal and brackish water were the dominant areas in Pesawaran District, and they were excellent habitats for *An. sundaicus* s.l. and *An. subpictus* s.l, while the former freshwater fishponds found in Ogan Komering Ulu District were the most habitats for *An. vagus*. Different bionomy was found in mosquitoes predominantly from these two regencies. High-density, dominant human-biting behavior and adequate habitats in Pesawaran District make *An. sundaicus* s.l. the main vector of malaria in Lampung, so the population and transmission must be controlled. *Anopheles vagus* from two districts tends to choose humans when the unavailable livestock as the main blood feed makes interactions between the two more intense. Therefore, these mosquitoes will have a strong opportunity in the future to become the main vector of malaria. In-depth surveillance and deepening research on these vectors' diversity, ecology, bionomic, molecular, and susceptibility to insecticides are needed to prevent disease transmission in these two districts.

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