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Habitat Preference of *Aedes aegypti* and *Aedes albopictus*: a Case Study on Dengue Endemic Areas of Sumatera, Indonesia

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***Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) are vectors that cause dengue hemorrhagic fever. The cases of this disease have often become an extraordinary event in Bandar Lampung City, Indonesia. Therefore, this study aimed to analyze bioecological factors,** *i.e.* **habitat preference, larvae density, characteristics of the breeding sites, as well as the morphological and molecular identification of *Ae. aegypti* dan *Ae. albopictus* in dengue-endemic areas of Bandar Lampung. Results showed that the larval density of these vectors in three areas was moderate, and *Ae. aegypti* was found dominant. The Maya index (MI) values of *Ae. aegypti* showed moderate and low categories (87 and 13%), whereas those of *Ae. albopictus* were 91 and 9%, respectively. Seven and 11 characters of *Ae. aegypti* and *Ae. albopictus* has been described, respectively. Additionally, their COI gene sequence (size of 725 bp) similarity values ranged from 98–99%. Data from the study can be used in planning a control strategy and identifying the main priority areas for entomological surveys toward disease epidemic control.**

Keywords: bioecological, breeding-sites, density, Maya-index, mosquitoes

INTRODUCTION

Mosquitoes (Diptera: Culicidae) play an essential role in human life. They can be a vector for several pathogens, such as viruses (dengue, chikungunya), parasites (*Plasmodium*), and worms (filaria) (Kraemer *et al*. 2015). *Aedes aegypti* and *Ae. albopictus* have been confirmed to be a vector of the dengue virus, which causes dengue hemorrhagic fever (DHF) and have spread in almost all areas in Indonesia. DHF, one of the country's public health problems, has increasing incidence and sufferers, with expansion in the spread areas. It has become an epidemic in over 100 countries, reaching 400 million cases annually. Furthermore, DHF has spread to 472,597 districts/cities in Indonesia, including Bandar Lampung (Harapan *et al*. 2019).

Occurrences of DHF cases in Bandar Lampung were often extraordinary, reaching more than 1,000 cases in 2020 (Bandar Lampung City Health Office 2019). A high incidence in Bandar Lampung is related to

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increasing human population growth and mobilization as a growth center city in Southern Sumatra, Indonesia. The government of Bandar Lampung is performing various ways to reduce dengue cases (Ministry of Health 2014).

In Indonesia, mosquitoes are controlled by the integrated system, *i.e.* physical and mechanical methods that focus on preventing mosquito bites (Mohiddin *et al.* 2015). The movement of the *Pemberantasan Sarang Nyamuk* (PSN in Bahasa) through the 3M Plus and *Satu Rumah Satu Jumantik* (in Bahasa) programs intervention (latch monitors) still was unable to reduce the cases and the spread in endemic areas (Ministry of Health 2018). Furthermore, biological control can be done using biotic agents such as *Wolbachia*-infected mosquitoes and sterile insect techniques; meanwhile, these methods are still being tested for effectiveness (Lees *et al*. 2015; Nordin *et al*. 2022). Chemical control with insecticides can be applied to resistant pre-adult and adult mosquitoes to suppress their population. However, using insecticides for a long time can result in mosquito resistance (CDC 2018).

Bioecological data on vectors can be used to monitor mosquito populations in the dengue disease control program (Higa 2011) and to identify the priority area (WHO 2011). This study aims to analyze bioecological factors, *i.e.* habitat preference, larvae density, and characteristics of the breeding sites of *Ae. aegypti* and *Ae. albopictus*. The morphological and molecular identification of *Ae. aegypti* and *Ae. albopictus* was also described. The results of this study provide a recommendation for implementing the DHF vector control program.

MATERIALS AND METHODS

Mosquito Larvae Sampling

A sampling of mosquito larvae and pupae was conducted in Bandar Lampung, Indonesia from January–November 2021. Sampling was conducted in 100 houses in the three dengue-endemic districts, *i.e.* Sukarame, Tanjung Seneng, and Kemiling (Figure 1) (Ministry of Health 2014).

Entomological surveys were performed inside and outside the house from morning to evening (08:00 AM–06:00 PM). Outside, the survey was conducted within a radius

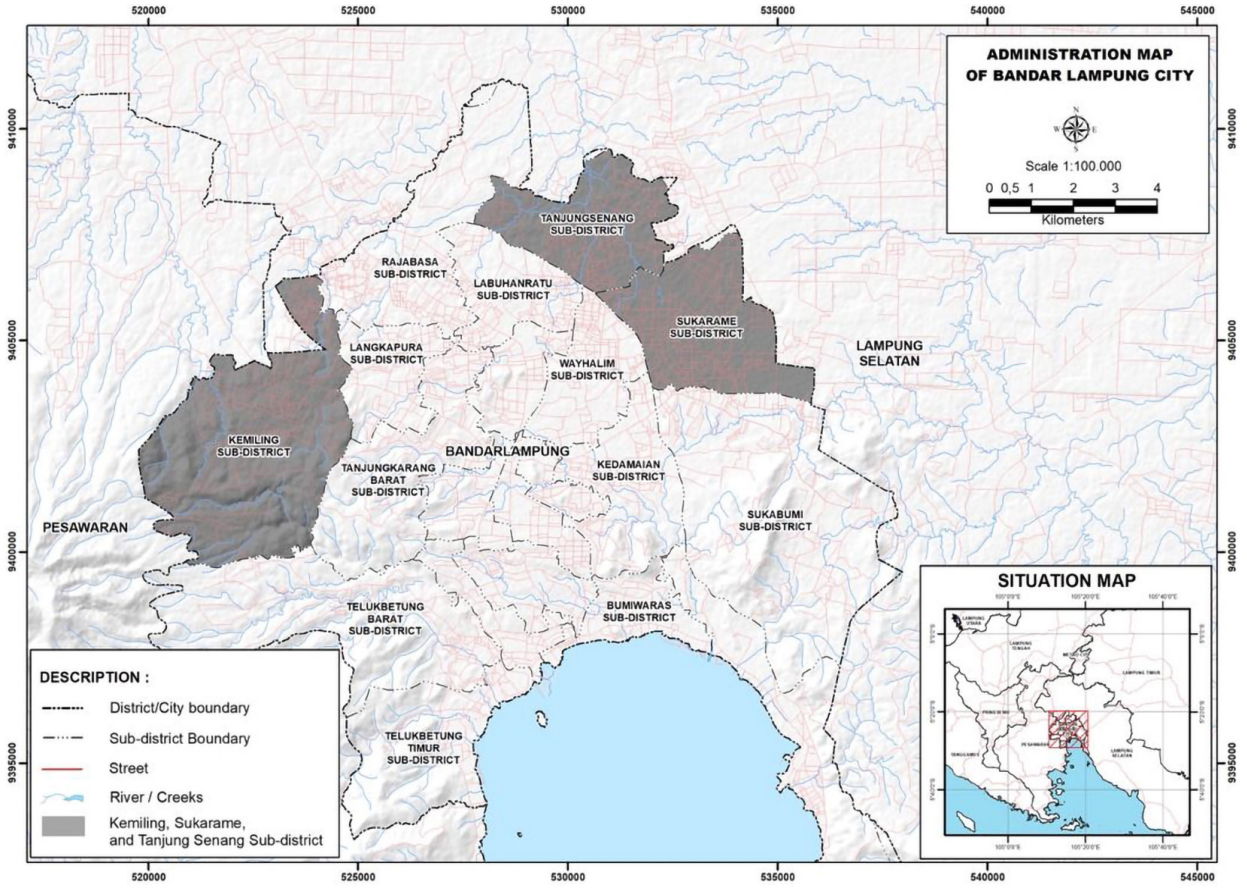


Figure 1. Map of mosquito sampling sites in Bandar Lampung, Sumatera, Indonesia.

of 100 m from the dengue residence as a breeding site using containers and grouped as controllable containers (CC) and disposable containers (DC). The larvae were reared until emerging the imago (adult). The mosquito samples for DNA analysis were preserved in EDTA (ethylenediaminetetraacetic acid). As a control, we used *Ae. aegypti* Liverpool and *Ae. albopictus* Dramaga strains developed by the Laboratory of Health Parasitology and Entomology, School of Veterinary Medicine and Biomedical, IPB University, Bogor, Indonesia.

Habitat Characteristics

Habitat characteristics of mosquitoes were assessed using the Maya index (MI) to determine breeding sites. MI is counted based on the breeding risk indicator (BRI) and hygiene risk indicator (HRI). The CC used consists of buckets, flower pots, gutters, drums, wells, baths, bird drinking forks, water tanks, towers, dispensers, and ponds. The DC used consists of bottles, cans, tires, buckets, bamboo, tree holes, puddles, jars, glass, shells, and gallons (Purnama and Baskoro 2012).

Mosquito Identification

The mosquito was identified based on morphological and molecular characteristics, *i.e.* the *cytochrome oxidase I* (*COI*) gene. Morphological identification was conducted using the identification key of *Aedes* (Ministry of Health 2010). The specimens (fourth instar and adult) were photographed by a VHX-200 digital microscope (Keyence®). DNA analysis was conducted in the laboratory of Biotech Center, IPB University, Bogor, Indonesia. The molecular identification was initiated by isolating total DNA using the Dneasy® Blood and Tissue Kit (cat. no. 69504) based on the spin-column protocol (Qiagen 2020), which was modified by adding buffers A and B. The sample was incubated overnight at 56 °C.

The *COI* gene was replicated using the polymerase chain reaction (PCR) technique. The primers used were forward LCO-1490 (5'-TGGTCAACAAATCATAAAGATATTGG-3') and reverse HCO-2198 (3'-TAAACTTCAGGGTGACCAAAAAATCA-5'). The PCR conditions included an initial denaturation step of 94 °C for 3 min, followed by 35 cycles of 94 °C for 45 s, 55 °C for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 6 min. Furthermore, its products were detected by electrophoresis on 1.2% agarose gel using TBE-1x buffer. On-target DNA bands are being sequenced at 1st Base Malaysia, Selangor, Malaysia.

Data Analysis

MI was resulted by calculating the BRI and HRI. BRI value is obtained from the number of CCs in each sample household divided by the average of CC. Meanwhile, the HRI value is obtained from the number of DCs in each sample household divided by the average of DC. The value of MI was determined as high = X > (ì + 1.0 SD), moderate = (ì – 1.0 SD) d" X < +1.0 SD, and low = X < (–1.0 SD), where X is the value of BRI/HRI in each sample, and SD is the standard deviation (Table 1). The mosquitoes' habitat preferences in three endemic areas were analyzed using the principal component analysis (PCA).

The larval density of *Ae. aegypti* and *Ae. albopictus* were assessed based on the entomology index (EI), which consists of the container index (CI), house index (HI), and Bretaeu index (BI). The results of three indices were obtained from the density figure (DF) expressed on a 1–9 scale (Table 2) (WHO 1975).

The molecular data, *i.e.* nucleotide sequences (forward and reverse) were aligned using the Clustal W program, MEGA (Molecular Evolutionary Genetics Analysis) 11.0 (Kumar *et al*. 2016). The *COI* gene sequence was analyzed using BLASTN (Basic Local Alignment Search Tool-nucleotide) on the website https://blast.ncbi.nlm.nih.gov/Blast.cgi to determine the similarity of the samples tested. BLASTN bioinformatics analysis was used to identify species with a high similarity value (>= 97%) between the nucleotides of this study and the GenBank database.

Table 1. Habitat characteristics of mosquitoes assessed by Maya index.

Indicator	BRI 1 (low)	BRI 2 (moderate)	BRI 3 (high)
HRI 1 (low)	Low	Low	Moderate
HRI 2 (moderate)	Low	Moderate	High
HRI 3 (high)	Moderate	High	High

[HRI] hygiene risk indicator; [BRI] breeding risk indicator

Table 2. Density of figure (DF) of entomology index.

Density figure	House index (HI)	Container index (CI)	Breauteu index (BI)
1	1–3	1–2	1–4
2	4–7	3–5	5–9
3	8–17	6–9	10–19
4	18–28	10–14	20–34
5	29–37	15–20	35–49
6	38–49	21–27	50–74
7	50–59	28–31	75–99
8	60–76	32–40	100–199
9	77–	41–	200–

RESULTS

The habitat characteristics of *Ae. aegypti* and *Ae. albopictus* grouped based on CC and DC was shown in Figure 2. Observations of breeding habitat from 300 houses indicated that *Ae. aegypti* was found in CC and dominated in Sukarame (651 containers, 84 CC and 2 DC were positive larvae). Meanwhile, population of *Ae. albopictus* was found in DC and dominated in the Kemiling (639 containers, 8 CC and 16 DC were positive larvae) (Table 3 and 4). Results of PCA showed the cumulative percentage of principal component 1 (PC1) and principal component 2 (PC2) had exceeded the minimum percentage, which can explain the variability of the data (> 75%) in the three sample areas (Figure 2). These results described that the *Ae. aegypti* population occupies more containers inside the house, whereas *Ae. albopictus* occupies more containers outside the house.

Analysis of larval breeding sites showed BRI value in three endemic regions for *Ae. aegypti* was the moderate and high categories (87 and 13%), whereas *Ae. albopictus* was 93.7 and 6.3%, respectively. The habitat analysis based on the HRI of *Ae. aegypti* revealed moderate and high categories (87 and 13%), whereas *Ae. albopictus* had moderate and high categories (91 and 9%, respectively). The MI value of *Ae. aegypti* showed moderate and high categories (87 and 13%), whereas those of *Ae. albopictus* were 91 and 9%, respectively (Figure 5).

The density of *Ae. aegypti* and *Ae. albopictus* in the three endemic areas showed moderate value. In *Ae. aegypti*, HI and BI were highest (36 and 86%) in Sukarame, whereas the highest CI (8.07%) was found in Tanjung Seneng. The highest HI of *Ae. albopictus* (16%) was found in Sukarame, whereas CI and BI were the highest (8 and 49%) in Kemiling (Table 5).

Larvae of *Ae. aegypti* has combs along the anal segment with 5–19 teeth, arranged irregularly in one or two rows, and the teeth have deep and clear grooves). On the other hand, larvae of *Ae. albopictus* has teeth without clear indentations such as tassels (Figure 3) (Ministry of Health 2010). The different characteristics of two

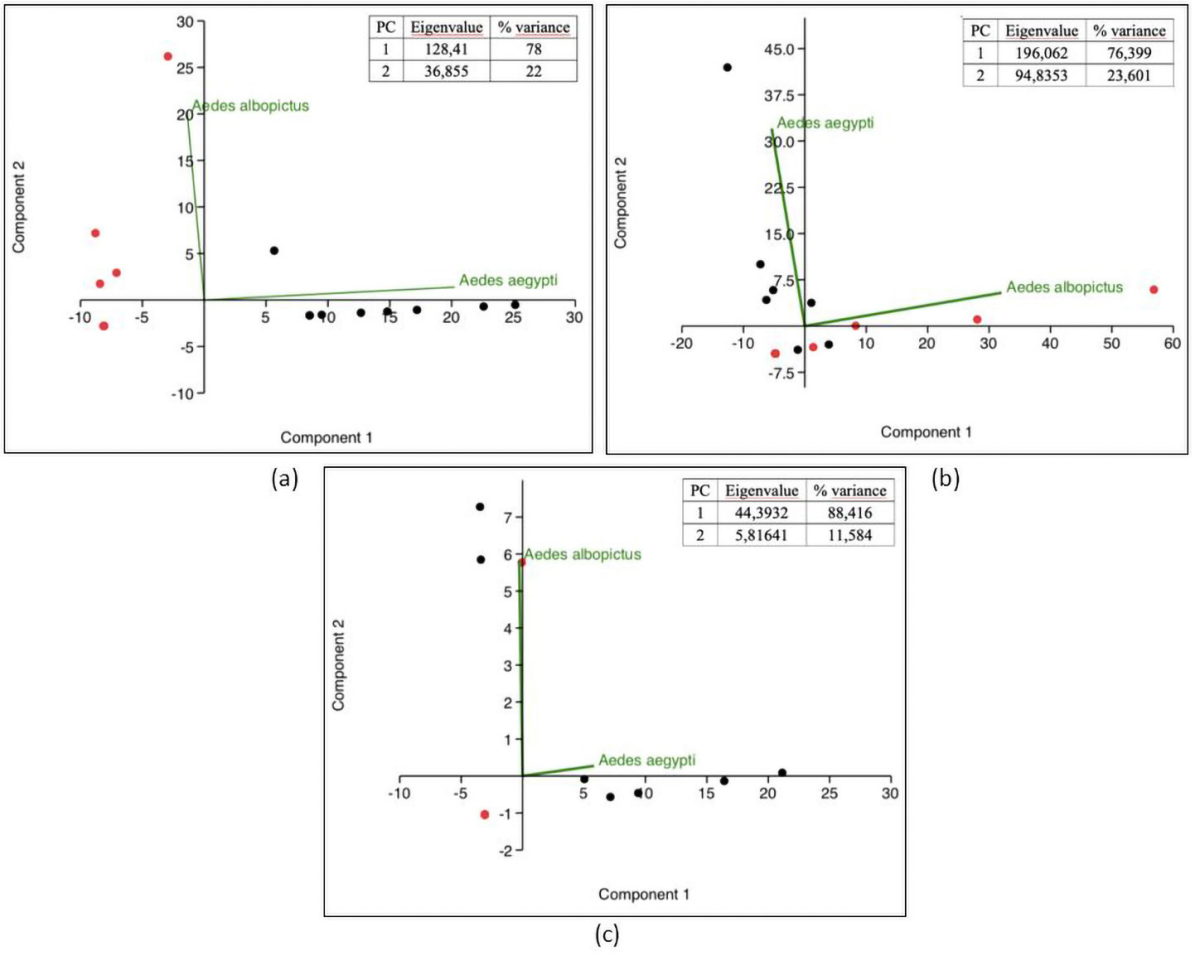


Figure 2. Principal component analysis of habitat characteristics of *Aedes aegypti* and *Aedes albopictus* grouped as controllable container (CC, black dot) and disposable container (DC, red dot): [a] Sukarame, [b] Kemiling, and [c] Tanjung Seneng.

Table 3. The percentage of sampled household based on HRI, BRI, and MI categories for *Aedes aegypti.*

Area	Total houses	Total containers	CC positive for larvae	DC positive for larvae	BRI			HRI			MI		
					L	M	H	L	M	H	L	M	H
Sukarame	100	651	84	2	0	88	12	0	88	12	0	88	12
Kemiling	100	639	49	1	0	86	14	0	86	14	0	86	14
Tj. Seneng	100	601	49	0	0	87	13	0	87	13	0	87	13
Total	300	1891	182	3	0	261	39	0	261	39	0	261	39

[CC] controllable container, [DC] disposable container, [BRI] breeding risk index, [HRI] hygiene risk index, [MI] Maya index, [L] low, [M] moderate, and [H] high

Table 4. The percentage of sampled household based on HRI, BRI, and MI categories for *Aedes albopictus*.

Area	Total houses	Total container	CC positive for larvae	DC positive for larvae	BRI			HRI			MI		
					L	M	H	L	M	H	L	M	H
Sukarame	100	651	1	11	0	100	0	0	92	8	0	92	8
Kemiling	100	639	8	16	0	86	14	0	86	14	0	86	14
Tj. Seneng	100	601	4	2	0	95	5	0	95	5	0	95	5
Total	300	1891	13	29	0	281	19	0	273	27	0	273	27

[CC] controllable container, [DC] disposable container, [BRI] breeding risk index, [HRI] hygiene risk index, [MI] Maya index, [L] low, [M] moderate, and [H] high

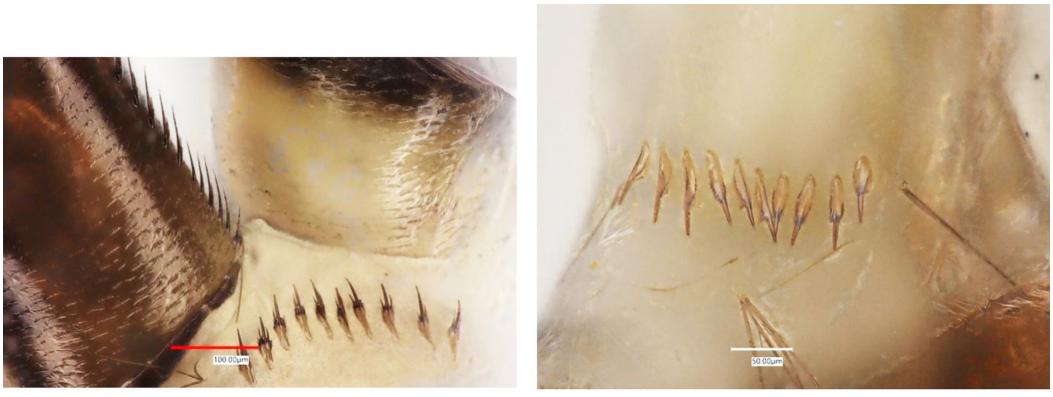
Table 5. Larval density of *Aedes aegypti* and *Aedes albopictus*.

Larval species	Index	Sukarame	Kemiling	Tanjung Seneng
Ae.aegypti	HI	36	25	26
	CI	1.6	7.03	8.07
	BI	86	45	24
	DF	5 (moderate)	4 (moderate)	4 (moderate)
Ae.albopictus	HI	16	14	5
	CI	0.93	8.07	0.98
	BI	12	49	6
	DF	3 (moderate)	4 (moderate)	2 (moderate)

[HI] house index, [CI] container index, [BI] Breteau index, and [DF] density figure

species of mosquito are found in the mesonotum. *Aedes aegypti* has a scale pattern, a pair of curved lines (lyre shape) at the edges, and submedian white lines in the middle. Meanwhile, *Ae. albopictus* has a collection of broad white scales above the base of the wings between the supra alar scales (Figure 4) (Ministry of Health 2010; Chan *et al.* 2014).

The *COI* gene of *Ae. aegypti* and *Ae. albopictus* sized about 725 bp. The *COI* gene sequence of *Ae. aegypti* and *Ae. albopictus* had similarity values ranging from 98–99% (Tables 6 and 7). The similarity showed that the morphological identification was in line with molecular data. The cover query value in *Ae. aegypti* and *Ae. albopictus* ranged from 98–100% with an e-value close to zero, indicating a high sequence accuracy.



a b

Figure 3. The morphological characteristics of abdominal 8thsegment larval: [a] *Aedes aegypti* has comb teeth with deep and clear grooves; [b] *Aedes albopictus* has comb teeth without clear indentations.

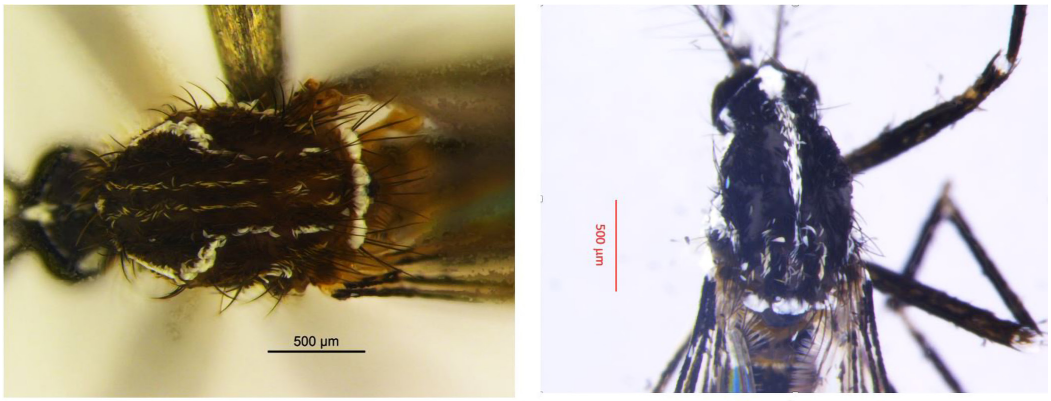


Figure 4. The morphological characteristics of adults mesonotum: [a] *Ae. aegypti* has a scale pattern with a pair of curved lines; [b] *Ae. albopictus* has a collection of broad white scales above the base of the wings.

Table 6. Identification of *Aedes aegypti* based on BLASTN in NCBI.

Area	Sample code	Query cover (%)	E-value	Similarities	Species	Accession no.
Sukarame	SA11	99	0.0	98.74	*Aedes aegypti*	KY022526.1
	SA21	100	0.0	99.30	*Aedes aegypti*	KY022526.1
Kemiling	KA12	98	0.0	99.26	*Aedes aegypti*	KY022526.1
	KA22	100	0.0	99.30	*Aedes aegypti*	KY022526.1
Tanjung Seneng	TA13	99	0.0	98.31	*Aedes aegypti*	KY022526.1
	TA23	98	0.0	99.26	*Aedes aegypti*	KY022526.1
Control	C1	99	0.0	98.59	*Aedes aegypti*	MK300222.1
	C2	99	0.0	98.31	*Aedes aegypti*	MK300222.1

Table 7. Identification of *Aedes albopicus* based on BLASTN in NCBI.

Area	Sample code	Query cover (%)	E-value	Similarities	Species	Accession no.
Sukarame	SB11	99	0.0	98.74	*Aedes albopictus*	KY022526.1
	SB21	100	0.0	99.30	*Aedes albopictus*	KY022526.1
Kemiling	KB12	98	0.0	99.26	*Aedes albopictus*	KY022526.1
	KB22	100	0.0	99.30	*Aedes albopictus*	KY022526.1
Tanjung Seneng	TB13	99	0.0	98.31	*Aedes albopictus*	KY022526.1
	TB23	98	0.0	99.26	*Aedes albopictus*	KY022526.1
Control	D1	99	0.0	98.59	*Aedes albopictus*	MK300222.1
	D2	99	0.0	98.31	*Aedes albopictus*	MK300222.1

DISCUSSION

DHF is a vector-borne disease and a public health concern in Indonesia. This country is suitable for vector mosquito breeding due to its tropical nature. Furthermore, Bandar Lampung, located in the south of Sumatra connected to Java, is a strategic area with high human mobilization and population growth. These factors provide breeding habitats for dengue vectors. *Ae. aegypti* is more common in human habitation, whereas *Ae. albopictus* is more found in suburban areas, specifically in settlements close to secondary vegetation (Azmi and Saad 2010).

In this study, we found that *Ae. aegypti* prefer an indoor habitat, whereas the *Ae. albopictus* in outdoor habitat. The pre-adult stage of a mosquito requires water to complete its life cycle. Genus *Aedes* oviposited their eggs on the edge of the container where there was standing water. *Ae.*

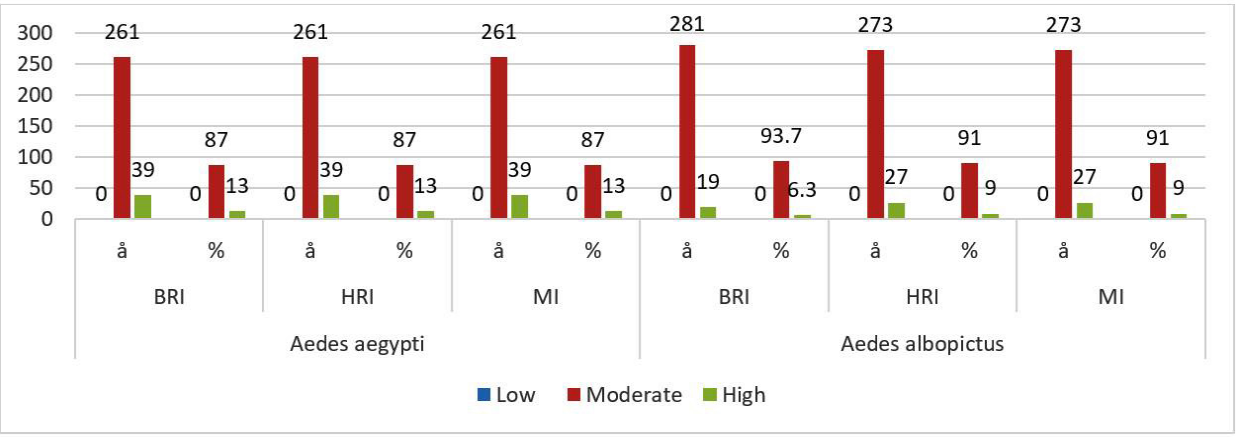


Figure 5. Percentage of sampled household based on HRI, BRI, and MI categories in *Aedes aegypti* and *Aedes albopictus*; [BRI] breeding risk index, [HRI] hygiene risk index, and [MI] Maya index.

aegypti prefers clean water in various containers inside the house or near human habitation, whereas *Ae. albopictus* is often found in artificial or natural containers outside the house that contain organic waste (Chareonviriyaphap *et al*. 2003; Dom *et al*. 2013). *Ae. albopictus* is commonly found in settlements such as Kemiling, with some plant species found such as rubber, cassava, and secondary vegetation. Meanwhile, *Ae. aegypti* observed in Sukarame and Tanjung Seneng as an urban housing area.

Dengue fever control is conducted to reduce the mosquito vector population. It is essential to observe the breeding habitat of vectors by surveying the sites (Fadilla *et al.* 2015). In this study, the MI was advantageous for identifying areas at risk of dengue transmission. The MI depends on the hygiene risk index (HRI) and potential mosquito breeding habitats (BRI). The analysis was performed to identify a potential breeding habitat for *Aedes* sp. Furthermore, the moderate value of BRI and HRI showed that the three regions as mosquito breeding sites and have poor environmental sanitation (Purnama and Baskoro 2012; Banerjee *et al.* 2013).

The MI value corresponds with the average larval density because many containers are breeding sites in the three sampled areas. It indicates that environmental hygiene needs to be considered to reduce mosquito habitats. It implies they have significant risks as DHF transmission areas (Dhewantara and Dinata 2015). The factors causing the expansion of the endemic regions in Indonesia are very complex. In addition to population growth, environmental factors such as temperature, rainfall, and humidity also affect the population of mosquitoes. Therefore, control strategies must be conducted dynamically with attention to priority areas of disease spread and the bioecological factors of mosquito vectors (Brown *et al.* 2014).

CONCLUSION

Results of habitat characterization in the dengue endemic areas of Sumatera showed *Ae. Aegypti* prefers controllable containers, whereas *Ae. albopictus* prefers disposable containers. Based on the MI value of two species of *Aedes* in three study areas indicated a moderate category of dengue transmission. Based on morphological and molecular identification, the larvae observed were *Ae. aegypti* and *Ae. albopictus*, respectively.

CONFLICT OF INTEREST

The authors declared that there are no potential conflicts of interest concerning the research, authorship, and publication of this article.

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