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Original Article

Detection of cuticle thickening in pyrethroid-resistant *Aedes aegypti* (Linnaeus) from West Sumatra, Indonesia

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ABSTRACT

Aedes aegypti (Linnaeus) is the primary vector of dengue virus. Prolonged and widespread use of insecticides, particularly pyrethroids for household vector control, has led to increasing insecticide resistance in *Ae. aegypti* populations. Cuticle thickening has been reported as a resistance mechanism, yet evidence for this mechanism in *Ae. aegypti* from Indonesia is still limited. This study aimed to investigate cuticle thickening in pyrethroid-resistant *Ae. aegypti* populations from Gunung Pangilun Village, Padang City, West Sumatra, Indonesia. Mosquito samples were collected using egg traps (ovitraps) and reared until the adult stage. Susceptible and resistant populations were determined by a WHO-based bioassay with impregnated paper 0.025% alpha-cypermethrin, collecting 10 individuals per group, and cuticle thickness was measured at the mid-tibia using scanning electron microscopy (SEM). The results found that cuticle thickness in the resistant group was 1.28-fold thicker than that in the susceptible group. This study suggests the involvement of cuticular penetration resistance, which may reduce the efficacy of contact insecticides such as pyrethroids, as well as provides initial evidence supporting cuticle thickening in pyrethroid-resistant *Ae. aegypti* from Indonesia. These findings emphasize the need to strengthen resistance management strategies, insecticide rotation, and integrated vector control approaches to reduce further increases in resistance.

Key words: alpha-cypermethrin, cuticle, dengue vector, insecticide resistance, penetration

INTRODUCTION

Dengue cases have risen sharply over the past decade, with global reports increasing nearly 29 times between 2000 and 2024 (WHO 2025a). Currently, about 5.6 billion people are at risk of dengue infection (Lim et al. 2025), and *Aedes aegypti* (Linnaeus) is the main vector. Vector control is still the main approach for preventing dengue (Ogunlade et al. 2023), and chemical insecticides are an essential component of these programs (Ranson & Lissenden 2016). However, using insecticides for long periods and on a large scale has put strong pressure on mosquito populations, causing them to develop resistance (Rahayu et al. 2018). There are four main ways mosquitoes become resistant: increased detoxification by metabolic enzymes (metabolic resistance), mutations in target sites that reduce sensitivity (target site resistance), changes in the cuticle that impede insecticide penetration (penetration resistance), and behaviors that help mosquitoes avoid treated surfaces (behavioral resistance) (Panini et al. 2016).

Indonesia provides favorable conditions for the spread of *Ae. aegypti* and is one of the countries with the highest rates of dengue (WHO 2025b). Pyrethroids are the most commonly used insecticides in Indonesia's dengue control programs (Sayono et al. 2016), but resistance to these chemicals has been found in several *Ae. aegypti* populations (Zulfa et al. 2022). Because pyrethroids work by contact, it is important to study how the mosquito cuticle may contribute to penetration resistance. The insect cuticle is the main barrier to the entry of xenobiotics, including insecticides (Balabanidou et al. 2018; Ren et al. 2023), and changes like cuticle thickening can affect how well insecticides get through (WHO 2025b).

Previous studies have reported cuticle thickening associated with insecticide resistance in *Ae. aegypti* (Samal & Kumar 2021; Jacobs et al. 2023). However, to the best of our knowledge, information regarding this mechanism in *Ae. aegypti* populations from Indonesia remains limited. To address this gap, this

study investigated cuticle thickening as a potential penetration resistance mechanism in *Ae. aegypti* population from Padang City, a region with multiple documented cases of insecticide resistance (Amelia-Yap et al. 2019; Hasmiwati & Supargiyono 2018; Hasmiwati et al. 2018; Rahayu & Fatimah 2020; Rahayu et al. 2022). The research was on Gunung Pangilun Village, where *Ae. aegypti* populations have been reported to exhibit resistance to the pyrethroid alpha-cypermethrin (Ardhiah et al. 2017) and the organophosphate temephos (Hasmiwati et al. 2018; Rahayu et al. 2021), as well as harbor mutations in the *Ace-1* gene (Hasmiwati et al. 2018). This study was conducted to provide information on the potential contribution of cuticle thickening to insecticide resistance in this population, which could support dengue vector control strategies in the region.

MATERIALS AND METHODS

Study areas

Sampling was conducted from January to October 2020 in Gunung Pangilun Village, Padang, West Sumatra, Indonesia (0°55'00.8" S, 100°21'39.2" E) using purposive sampling. Rearing and specimen preparation prior to scanning electron microscopy (SEM) were performed in the Animal Physiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas. Observations using SEM were conducted at the Biology Research Center, Indonesian Institute of Sciences (LIPI; now integrated into BRIN), Gedung Widya Satwaloka, Cibinong, West Java, Indonesia.

Sampling and rearing

Mosquito eggs were collected using egg traps (ovitraps) placed in ten residential houses, with three ovitraps installed in each house. Ovitraps were made from plastic cups (~9 cm top diameter and ~10 cm height) coated with thick black plastic. A strip of Whatman paper (approximately 6 cm × 10 cm) was placed inside each cup and attached to the inner wall. The cups were filled with tap water to approximately one-quarter of their volume, allowing the paper to remain partially submerged while the upper portion remained exposed to air.

Egg traps were placed in a shady or dimly lit area indoors to minimize the risk of disturbance. The traps were left in place for one week, after which they were collected and replaced with other ovitraps containing fresh Whatman paper and tap water. This procedure was repeated weekly until sufficient egg samples were obtained.

Eggs attached to the Whatman paper were gently brushed using a fine brush into plastic containers (30 cm × 50 cm × 12 cm) filled with tap water to approximately two-thirds of the container volume. The containers were covered with mesh to maintain hygiene and prevent contamination. Larvae of all instars were maintained in the same rearing container. Water was replaced as needed based on visual inspection to maintain cleanliness.

Pupae were transferred to plastic cups (~9 cm top diameter and ~10 cm height) using a pipette and covered with mesh to prevent the escape of emerging adults. Emerging adults were maintained until five days post-emergence prior to use in bioassays. Larvae were fed finely ground commercial chicken feed, while adult mosquitoes were provided with a 10% sucrose solution via cotton pads placed on top of the mesh covering the cups. Rearing was conducted at 27 ± 2 °C and 70–80% relative humidity (RH).

WHO-based bioassay (susceptibility test)

Female *Ae. aegypti* mosquitoes aged 5 days were used in this study. Species identification was performed based on the presence of a lyre-shaped scale pattern on the dorsal thorax, and sex differentiation was determined by antennal morphology (Christophers 1960).

Screening bioassays using a WHO-based bioassay protocol (WHO 2016) were conducted for phenotypic classification of resistant and susceptible mosquitoes using impregnated paper with 0.025% alpha-cypermethrin for exposure. Adult female mosquitoes were tested in batches of up to 20 individuals per exposure, depending on availability. Mosquitoes were introduced into holding tubes lined with untreated paper (12 cm × 15 cm) by aspirator and maintained vertically for 1 h under standard test conditions. The exposure tube containing the insecticide-impregnated paper was then connected to the holding tube, and mosquitoes in the holding tube were transferred to the exposure tube by opening the slide unit and gently blowing until all mosquitoes moved into the exposure tube. After 1 hour of exposure, the mosquitoes were returned to the holding tube by opening the slide unit and were provided with a 10% sucrose solution via cotton pads for a 24-hour recovery period.

After the 24 h post-exposure, surviving mosquitoes were classified as resistant, while those that died were classified as susceptible. No independent biological replicates were performed, and individuals were collected from multiple exposure batches until the required number of samples was obtained.

Classification of susceptible and resistant groups

Ten individuals per group were used for scanning electron microscopy (SEM) analysis. The resistant group (n = 10) consisted of mosquitoes that survived 24 h after insecticide exposure using WHO bioassay, while 10 individuals from the susceptible group consisted of six field-collected mosquitoes that died during the WHO bioassay and four individuals from a laboratory susceptible strain (VCRU-WHO, Universiti Sains Malaysia). The limited number of laboratory strain individuals was due to low emergence success during colony maintenance. All susceptible individuals, both from the field and laboratory strains, were analyzed collectively as the susceptible group in this study.

Mosquito leg preparation for SEM

Mosquitoes were processed immediately after classification. Resistant individuals were euthanized by placing them in a freezer for several minutes prior to dissection. Next, the left middle leg was carefully detached from the mosquito body and gently rubbed with a fingertip to remove scales on the leg surface, particularly along the tibia. The leg was then placed on a glass slide and rinsed with 70% ethanol, and the mid-tibia of *Ae. aegypti* was transversely sectioned using a platinum-coated razor blade (Figure 1). The excised leg segment was transferred into a 1.5 ml microcentrifuge tube containing 70% ethanol and stored until scanning electron microscopy (SEM) analysis. The mid-tibia of the left middle leg was selected to ensure that the same anatomical region was examined consistently across all specimens, thereby providing a consistent basis for comparison among specimens. Previous studies have assessed cuticular thickness or cuticular resistance at different anatomical locations, including the mid-tibia, as in the study of Lilly et al. (2016) in *Cimex lectularius* Linnaeus (Table 2).

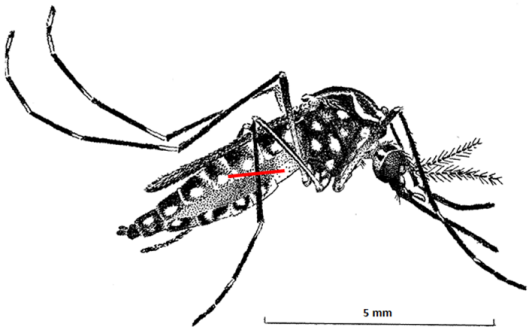

Figure 1. Schematic illustration of the transverse cut location on the tibia of the left middle leg of *Aedes aegypti* used for cuticle thickness analysis. The thick red line indicates the sectioning site. Adapted from Christophers (1960).

Scanning electron microscopy (SEM)

Sample preparation for SEM was performed using a modified protocol based on Goldstein et al. (1992). Specimens were immersed in cacodylate buffer for approximately 2 h, followed by ultrasonic agitation for 5 min. Then, samples were fixed in 2.5% glutaraldehyde for 24 h and were immersed in 2% tannic acid for 6 h, followed by rinsing in cacodylate buffer for 5 min, repeated four times. Dehydration was performed using a graded ethanol series: 50% ethanol for 5 min (four times), followed by 70%, 85%, and 95% ethanol for 20 min each at room temperature. Samples were subsequently immersed in absolute ethanol for 10 min, repeated twice. Specimens were then frozen in tert-butanol for 10 min (twice), stored in a freezer, and dried using a vacuum dryer until completely dry.

Specimens were mounted and sputter-coated with gold at 5–8 mA for 5 min using an ion coater (Eiko IB-2), followed by vacuum treatment for 15 min. Observations were performed using a JSM-5000LV SEM. SEM imaging was conducted at an accelerating voltage of 3.0 kV, working distance of 11.7–13.2 mm, and under high-vacuum conditions. Images used for measurements were acquired at magnifications ranging from 1000× to 3000×.

Data analysis

Cuticle thickness was measured from SEM micrographs at the mid-tibia using ImageJ software (version 1.52p). Measurements were not performed under blinded conditions. However, identical measurement criteria were applied to all specimens to reduce potential measurement bias. Cuticle thickness was determined by delineating the outer and inner boundaries of the mosquito leg cuticle. Regions that do not have clearly defined boundaries, such as areas affected by structural damage, debris, or cuticle derivatives, were excluded from the measurement. This approach is similar to that used by Wood et al. (2010) for SEM-based measurement of leg cuticle thickness in *Anopheles funestus* Giles. Because different magnifications were used, each micrograph was independently calibrated using its corresponding scale bar prior to measurement.

For each mosquito, 25 measurement points were recorded. The mean cuticle thickness per mosquito was calculated as the average of these measurements and used for statistical analysis. The individual mosquito was treated as the unit of analysis. Data normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene's tests, respectively. Differences in mean cuticle thickness between resistant and susceptible groups were evaluated using an

independent-samples t-test. Statistical analyses were performed using R software (version 4.5.2) (R Core Team 2025).

RESULTS

The detailed records of the total number of individuals tested and classified in each bioassay were not retained, since the WHO bioassay was conducted as part of a phenotypic selection procedure to obtain individuals representing both susceptible and resistant phenotypes for subsequent cuticle thickness analysis. The distribution of cuticle thickness measurements across 25 points is shown in Figure 2. The mean cuticle thickness of the alpha-cypermethrin-resistant *Ae. aegypti* population was significantly higher (1.28-fold) than that of the susceptible population (Table 1; Figure **3**). For comparison, four susceptible individuals of the laboratory strain had a mean cuticle thickness of 1.949 µm, whereas six susceptible individuals from the field had a mean cuticle thickness of 1.885 µm. Thus, the descriptive difference between the laboratory-strain and field-susceptible groups was small.

Statistical analysis indicated that the data were normally distributed based on the Shapiro–Wilk test for both the susceptible (W = 0.906, p = 0.253) and resistant (W = 0.929, p = 0.440) groups. Homogeneity of variance was confirmed by Levene's test (F = 0.021, p = 0.888). An independent-samples t-test revealed a significant difference in tibial cuticle thickness between groups (t(18) = −2.354, p = 0.030), with a mean difference of −0.545 (95% CI: −1.031 to −0.059). The effect size was large (Hedges' g = 1.01).

DISCUSSION

This study indicates an association between pyrethroid resistance and increased cuticle thickness in the *Ae. aegypti* population from Gunung Pangilun. Similar observations have been reported in pyrethroid-resistant insects across multiple species, life stages, and body regions, where resistant individuals exhibited thicker cuticles than susceptible ones, with differences of up to 2.3-fold (Table 2). In addition, Table 2 shows that cuticle thickening associated with insecticide resistance has been reported not only in adult insects but also in immature developmental stages, including larvae and nymphs. This pattern suggests that differences in cuticle thickness associated with resistance are already present even before adulthood.

As contact insecticides, pyrethroids must penetrate the insect cuticle before reaching their neural targets, where the cuticle serves as the primary physical interface regulating insecticide entry (Ren et al. 2023). Therefore,

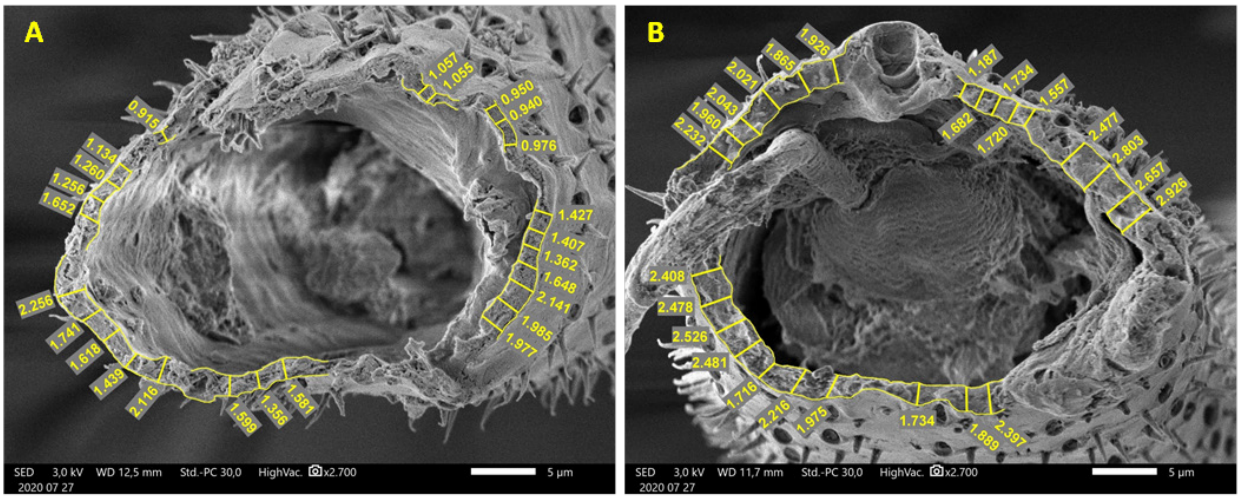

Figure 2. Scanning electron micrographs of transverse sections of the mid-tibia of *Aedes aegypti* showing cuticle thickness measurements in susceptible (A) and alpha-cypermethrin–resistant (B) individuals. Each micrograph corresponds to one individual mosquito. A total of 25 cuticle thickness measurements (µm) were obtained per individual. Magnification: ×2700. Scale bar = 5 µm.

Table 1. Mean cuticle thickness (± SD) and 95% confidence intervals of susceptible and alpha-cypermethrin-resistant *Aedes aegypti* populations

Group	n	Mean ± SD (µm)	95% CI
Susceptible	10	1.91 ± 0.49	1.56–2.26
Resistant	10	2.45 ± 0.54	2.07–2.84

Note: Effect size calculated as Hedges' g = 1.01.

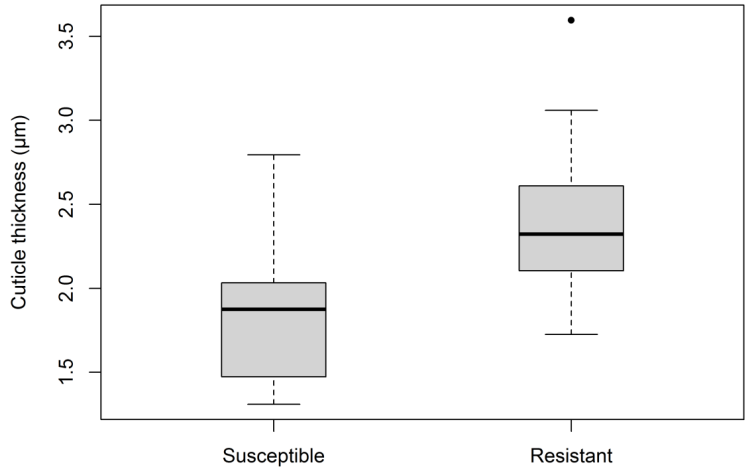

Figure 3. Boxplot of cuticle thickness (µm) in susceptible and alpha-cypermethrin-resistant *Aedes aegypti*. Boxes represent the 25th and 75th percentiles, and the horizontal line within each box indicates the median.

Table 2. Reported cuticle thickening associated with insecticide resistance across insect species

Species	Life stage	n	Point of section	Cuticle thickness (µm)		-fold thicker	References
				Susceptible	Resistant		
Aedes aegypti	Larva (fourth instar)	10	First abdominal segment	2.89 ± 0.24	5.69 ± 0.43	1.97	Samal & Kumar 2020
Anopheles gambiae	Adult (3–5 days old)	25	Leg–femur (apical region)	1.87 ± 0.30	2.13 ± 0.32	1.13	Balabanidou et al. 2016
	Adult (3–5 days old)	20	Leg–femur (apical region)	1.53 ± 0.11	1.85 ± 0.21	1.20	Yahouédo et al. 2017
	Adult (3–5 days old)	25	Leg–tarsus (right after the two claws)	0.86 ± 0.03	1.32 ± 0.05	1.52	Balabanidou et al. 2019
Anopheles funestus	Adult (10 days old)	10	Left middle leg–tarsomere 1	2.00 ± 0.32	2.21 ± 0.15	1.10	Wood et al. 2010
Anopheles sinensis	Adult (3–5 days old)	8	Leg–tarsus	1.95 ± 0.44	2.62 ± 0.53	1.34	Li et al. 2025
Bactrocera cucurbitae	Adult (10 days old)	10	Middle leg–tarsus	8.24 ± 0.25	9.79 ± 0.10	1.18	Verma et al. 2019
Bactrocera dorsalis	Larva (third instar)	10	Thorax	19.36 ± 0.82	25.96 ± 1.00	1.34	Lin et al. 2012
Spodoptera litura	Larva (third instar)	nr*	Pronotum	nr	nr	1.70–2.30**	Wang et al. 2025
Cimex lectularius	Adult (9 days old)	10	Middle leg–tibia	8.73 ± 0.18	10.13 ± 0.15	1.16	Lilly et al. 2016
Triatoma infestans	Nymph (fourth instar)	9	Second tergite	17.80 ± 5.40	32.10 ± 5.90	1.80	Pedrini et al. 2009

*: not reported; **: For three strains (InRS, FInRS(CS), and FInRS(XT)).

increased cuticle thickness likely acts as a kinetic barrier that slows pyrethroid penetration, effectively extending the time available for detoxification systems to metabolize and neutralize the toxic compound (Pedrini et al. 2009; Balabanidou et al. 2018). Rather than acting alone, penetration resistance may function as a co-resistance mechanism that enhances the effectiveness of other resistance mechanisms, including elevated activity of detoxification enzymes (esterases, cytochrome P450 monooxygenases, and glutathione S-transferases) and target site mutations in voltage-gated sodium channels (VGSCs) (Liu 2015), as well as behavioral avoidance responses that reduce effective exposure time (Yang et al. 2019; Sukkanon et al. 2020).

Comparative bioassay studies further support the functional role of the cuticle as a barrier. The increased susceptibility observed in injection assays, where insecticides are injected directly into the hemocoel,

bypassing the cuticle, compared with topical applications that must pass through the cuticle, suggests the cuticle's involvement in limiting insecticide penetration (Wood et al. 2010; Meng et al. 2023; Wang et al. 2025). These findings strengthen the interpretation that cuticular modifications contribute to reduced insecticide uptake.

The insect cuticle is structurally composed of two main, compositionally distinct layers: the epicuticle (outer layer) and the procuticle (inner layer). The epicuticle contains lipoproteins, fatty acids, and a waxy layer enriched with cuticular hydrocarbons (CHCs), while the procuticle is predominantly composed of chitin and structural proteins (Gillot 2005; Balabanidou et al. 2016). Cuticular thickening can occur in one or both layers (Jacobs et al. 2023; Li et al. 2025), suggesting multiple structural pathways that may delay insecticide uptake.

Alterations in the epicuticle (the outer layer of the cuticle), particularly increases in cuticular hydrocarbons, have been reported in resistant insects. Pyrethroid-resistant populations have been shown to possess higher levels of CHCs, a major component of the epicuticle (Balabanidou et al. 2016; Simma et al. 2019; Yahouédo et al. 2017). Increased CHC content can form a chemical barrier that reduces the diffusion of lipophilic compounds across the insect surface (Koganemaru 2015). A thicker epicuticle has been observed in *Anopheles gambiae* Giles (Balabanidou et al. 2016), where increased CHC levels have been correlated with reduced pyrethroid uptake (Yahouédo et al. 2017). Those findings suggest that both structural thickening and enrichment of cuticular hydrocarbons may contribute to penetration resistance.

Penetration resistance also involves transcriptional changes in cuticle-related genes. Structural proteins belonging to the CPR, CPAP, and CPF families, as well as genes involved in chitin synthesis such as chitin synthase (CHS), have been shown to contribute to both cuticle thickening and structural modifications (Fang et al. 2015; Huang et al. 2018; Balabanidou et al. 2019; Guo et al. 2019; Wang et al. 2025; Li et al. 2025; Xu et al. 2022; Sun et al. 2017). Upregulation of the *CYP4G16* gene, which is involved in hydrocarbon biosynthesis and directly linked to increased cuticular hydrocarbon production, has also been reported (Balabanidou et al. 2016; Yahouédo et al. 2017; Balabanidou et al. 2019). Additionally, other studies have also found overexpression of ABC transporters, which facilitate the redistribution of cuticle components, thus supporting cuticle reinforcement (Matowo et al. 2014; Xu et al. 2023; Kefi et al. 2023). Together, these transcriptional changes in multiple cuticle-related genes indicate that cuticle thickening represents a regulated physiological adaptation contributing to resistance.

Furthermore, functional studies also provide direct evidence for the role of cuticle structure in resistance. Silencing of cuticle protein genes such as *BgCPLCP1* has been shown to reduce cuticle thickness and restore susceptibility to β-cypermethrin in *Blattella germanica* (Linnaeus) (Cai et al. 2024). Several additional mechanisms affecting cuticle physical properties have also been linked to resistance-related traits. Alterations in pigmentation pathways, such as increased *YELLOW-E* gene expression, have been associated with increased cuticle tanning, which may indirectly contribute to penetration resistance through changes in cuticle rigidity and permeability (Simma et al. 2019). Furthermore, a denser chitin layer in the endocuticle has been observed in resistant *Bactrocera dorsalis* (Hendel) strains compared with susceptible strains, suggesting increased cuticle compaction (Lin et al. 2012). These findings are further supported by the overexpression of cuticle-related genes in leg regions, which are major sites of insecticide contact (Huang et al. 2018; Xu et al. 2020; Kefi et al. 2023; Li et al. 2025).

Taken together, previous studies have shown that several cuticle-related modifications may contribute to reduced insecticide penetration. The thicker cuticles in the resistant population found in this study support the possibility that structural changes in the cuticle play a role in penetration resistance.

However, this study is limited by its relatively small sample size, which reduces statistical generalizability. Additionally, the inclusion of field-collected mosquitoes in the susceptible group may contribute to biological variability compared with fully standardized laboratory strains. Further studies incorporating larger sample sizes, standardized susceptible reference strains, and complementary analyses such as molecular and biochemical assays are needed to validate and extend our findings.

Collectively, this study provides preliminary evidence of cuticular involvement in pyrethroid-resistant *Ae. aegypti* from Gunung Pangilun Village, Padang. Our findings provide the basis for further comprehensive investigations to elucidate the resistance mechanisms and assess their implications for dengue vector control strategies.

CONCLUSION

This study provides evidence suggesting cuticle thickening as a potential resistance mechanism in pyrethroid-resistant *Ae. aegypti* from Gunung Pangilun Village, Padang, West Sumatra. To the best of our

knowledge, this is the first report describing cuticle thickening associated with pyrethroid resistance in *Ae. aegypti* in Indonesia. These findings contribute to the understanding of insecticide resistance mechanisms and may support the development of more effective resistance management strategies for controlling this primary dengue vector.

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