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First SEM-Based Morphological Characterization of *Varroa destructor* in Indonesian *Apis mellifera*: A Descriptive Ultrastructural Study

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ABSTRACT

Varroosis, a parasitic disease caused by *Varroa destructor*, continues to pose a serious threat to global apiculture by undermining honey bee (*Apis mellifera*) health and productivity. The mite not only feeds on the fat body tissue of bees, impairing metabolic and immune functions, but also transmits several pathogenic viruses, including deformed wing virus and acute bee paralysis virus, contributing significantly to colony collapse disorder. While the morphological characteristics of *Varroa* mites have been studied in various countries, there is a notable lack of ultrastructural data from Indonesia. To the best of our knowledge, this is the first study to describe the surface morphology of *Varroa* mites from Indonesian *Apis mellifera* colonies using scanning electron microscopy (SEM). Mite specimens were collected from a commercial apiary in Central Java using the powdered sugar method. Subsequently, they examined under high-resolution SEM at the Integrated Research and Testing Laboratory, Universitas Gadjah Mada. The findings provided detailed ultrastructural descriptions of the gnathosoma, pedipalps, chelicerae, apotele claws, dorsal and ventral shields, ambulacra, and peritreme. Distinct morphological features were documented, including the bidentate cheliceral structure and dual clawed apotele, which are crucial for feeding and host attachment. This descriptive study offers a comprehensive morphological baseline for *Varroa destructor* in Indonesia, serving as a valuable reference for mite identification, diagnostic support, and future comparative acarological research. The insights gained may also inform local surveillance efforts and the development of targeted control strategies within Indonesian apiculture.

Keywords: *Apis mellifera*; *Varroa destructor*; SEM; Indonesia; Morphology.

INTRODUCTION

Honey bees (*Apis mellifera*) are globally recognized as essential pollinators in both natural ecosystems and agricultural landscapes. Their role in maintaining biodiversity and ensuring the productivity of various crops makes them indispensable to food security and ecological sustainability (Goulson et al. 2015). However, in recent decades, honey bee populations have been severely threatened by a complex interplay of factors, including pesticide exposure, habitat degradation, climate change, and pathogenic organisms. Among these, the parasitic mite *Varroa destructor* has emerged as one of the most significant biological threats to apiculture worldwide (Nazzi and Le Conte 2016).

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Varroa destructor, an obligate ectoparasite of honey bees, was originally a parasite of the Eastern honey bee, *Apis cerana*, but successfully shifted to *Apis mellifera* following global apicultural practices and bee trade (Nazzi and Le Conte 2016). This host-switch proved catastrophic for *A. mellifera* colonies, as the Western honey bee lacked co-evolved defenses against the mite. *V. destructor* feeds primarily on the fat body tissue of adult bees and brood, thereby compromising the host's immune system, altering physiological development, and increasing susceptibility to secondary infections and pesticides (Ramsey et al. 2019; Zhu et al. 2022). Furthermore, the mite acts as an efficient vector for numerous bee viruses, most notably the Deformed Wing Virus (DWV), which causes wing deformities, shortened lifespan, and ultimately colony collapse (Martin et al. 2012; Bava et al. 2022).

The global spread of *V. destructor* has prompted extensive research into its biology, ecology, and control. Morphological characterization, especially through high-resolution techniques such as scanning electron microscopy (SEM), has proven crucial in understanding the structural adaptations of the mite that enable parasitism and host specificity (Nazzi and Le Conte 2016). SEM enables detailed visualization of key anatomical structures such as the gnathosoma, pedipalps, chelicerae, ambulacra, and peritreme—features that are often difficult to discern using light microscopy (Ramsey et al. 2019; Zhu et al. 2022). These structures not only facilitate host attachment and feeding but also aid in taxonomic differentiation among *Varroa* species and regional strains (Fanelli and Tizzani 2020; Zaki et al. 2021a).

Despite the importance of morphological studies, most SEM-based research on *V. destructor* has been conducted in Europe, North America, and parts of Asia such as China, Korea, and Egypt (Thapa et al. 2015; Abou-Shaara and Tabikha 2017). In contrast, data from Southeast Asia remain sparse, and to date, no SEM-based morphological study has been published on *Varroa* mites from Indonesia—a country with diverse agroecological zones and rapidly growing apicultural activity. This knowledge gap is particularly concerning, as the structural characteristics of mites can vary based on environmental pressures, host adaptations, and acaricide exposure (Dietemann et al. 2013).

Indonesia, the largest archipelagic nation in the world, possesses a wide range of endemic bee species and imported *Apis mellifera* strains that are increasingly integrated into local beekeeping industries. However, *Varroa* infestations are rarely monitored using advanced diagnostic tools, and little is known about the morphology or potential regional variations of *V. destructor* within Indonesian bee populations. As apiculture expands in regions such as Java, Bali, and Sumatra, a foundational understanding of mite morphology is essential for early detection, surveillance, and tailored control strategies (Hornitzky 2010).

The introduction of *Varroa destructor* into new regions is often silent at first, with symptoms such as reduced brood viability, spotty brood patterns, and shortened adult bee lifespan often attributed to other stressors (Rosenkranz et al. 2010). It is only through long-term monitoring and morphological confirmation that beekeepers and researchers can accurately detect and track infestations. Countries with established SEM-based acarological surveillance programs—such as Germany, the United States, and China—have demonstrated that accurate mite identification can inform resistance management, guide targeted treatment applications, and slow the emergence of acaricide-resistant populations (Rinkevich 2020; Locke et al. 2012). In contrast, the lack of such baseline data in Indonesia places local bee populations at risk, particularly in commercial operations where the movement of colonies increases the spread potential.

One of the core advantages of SEM in parasitology lies in its ability to reveal microanatomical details that are essential for distinguishing closely related species and subspecies (Walter and Proctor 2013). For the genus *Varroa*, traditional light microscopy techniques are insufficient to resolve fine-scale morphological traits, especially those located on the gnathosoma, ambulacra, or cuticular shields (Nazzi and Le Conte 2016). SEM studies have revealed diagnostic features such as cheliceral shape, the arrangement of dorsal setae, and the structure of the pretarsal claws, all of which contribute to mite identification and understanding of host-mite interactions. These insights are particularly valuable in areas like Indonesia, where local environmental conditions may exert selective pressures on *Varroa* populations, potentially resulting in morphological divergence over time.

Moreover, growing evidence suggests that *Varroa* populations are not homogeneous. Morphological and genetic studies have identified regional differences in mite populations such as the presence of Korean, Japanese, and Chinese haplotypes with varying levels of virulence, reproduction rates, and resistance to acaricides (Nazzi and Le Conte 2016). Thus, it is imperative that countries with emerging apicultural sectors, such as Indonesia, conduct localized SEM studies to identify and document the strains or morphotypes of *Varroa* present in their territories. Such data not only strengthen national biosecurity frameworks but also contribute to global databases used in evolutionary and epidemiological modelling of parasite spread.

The continued reliance on chemical miticides for *Varroa* control has led to the emergence of resistant populations in several regions, including Europe and North America (Nazzi and Le Conte 2016). Resistance mutations often evolve rapidly due to improper use of acaricides, sublethal dosages, or lack of rotation among active compounds. Morphological changes—such as cuticle thickening or modifications in respiratory structures—have been hypothesized as potential resistance mechanisms (Zaki et al. 2021b). SEM-based studies can play a pivotal role in detecting these structural adaptations early, especially in countries such as Indonesia, where chemical treatment protocols are often unregulated and vary widely among smallholder beekeepers.

Additionally, the application of SEM data extends beyond taxonomy and diagnostics. It also provides insights into the functional morphology of mites, helping to elucidate how *Varroa* exploits its host. For example, the specialized ambulacra on the tarsal tips facilitate strong adhesion to host surfaces, while the chelicerae's saw-like edges allow efficient penetration of host tissues. Understanding these structures in high resolution not only adds depth to our knowledge of *Varroa*'s feeding behavior but also opens the door to novel control strategies that could target these anatomical adaptations, such as mechanical removal techniques or behavioral repellents (Zhu et al. 2022).

In Indonesia, research on honey bee health has traditionally focused on pathogens such as *Nosema spp.*, *Tropilaelaps clareae*, or general pesticide impacts (Ratnawati et al. 2020). Very few peer-reviewed publications have addressed the presence or distribution of *Varroa destructor*, let alone examined its morphology at a microscopic level. This gap in the literature presents both a challenge and an opportunity. Establishing the morphological baseline of *Varroa* mites from Indonesian *Apis mellifera* colonies using SEM can serve as a foundation for future taxonomic, genomic, and epidemiological studies. It also offers a critical reference point for identifying potential morphological divergence due to geographic isolation or environmental stressors.

This study represents the first descriptive ultrastructural investigation of *Varroa destructor* collected from *Apis mellifera*colonies in Central Java, Indonesia, using scanning electron microscopy. By detailing the external morphology of the gnathosoma, chelicerae, pedipalps, ventral and dorsal shields, peritreme, and ambulacra, this study aims to provide a morphological baseline for Indonesian *Varroa* populations. The findings are expected to contribute to regional diagnostic capacity, support acarological taxonomic efforts, and offer valuable reference data for future comparative and molecular research. Ultimately, this study bridges a critical gap in Southeast Asian mite morphology and enhances the broader understanding of *Varroa*'s parasitic adaptations in tropical apicultural systems.

MATERIALS AND METHODS

Study area and colony selection

The study was conducted in October–November 2023 at a commercial apiary located in Muntilan, Central Java Province, Indonesia (7°34′S, 110°17′E). This region represents a major honey-producing zone with a tropical monsoonal climate, an average annual temperature of 27°C, and relative humidity ranging from 65–85%. The apiary housed approximately 150 colonies of *Apis mellifera* managed under traditional practices with minimal acaricide use.

For this study, a purposive sampling strategy was adopted. Colonies were selected based on observable clinical signs of varroosis such as deformed wings, irregular brood pattern, and adult bee mortality. Ten colonies showing signs of *Varroa* infection were chosen, and mite collection was performed to ensure the presence of sufficient viable specimens for scanning electron microscopy (SEM) analysis.

Mite collection procedure

Mites were collected using the powdered sugar method, a non-lethal, widely accepted technique for detaching ectoparasitic mites from adult bees (Dietemann et al. 2013). From each selected colony, approximately 300 worker bees were collected from the brood chamber and placed into clean glass jars equipped with a mesh lid.

Approximately two tablespoons (~20g) of commercial powdered sugar were added into each jar, and the contents were gently rolled for 1–2 minutes to ensure uniform coating of bees. The sugar-coated bees were then inverted over a white tray or container and shaken vigorously to dislodge the mites. The dislodged mites were collected with fine brushes and transferred into sterile 1.5mL Eppendorf tubes. To preserve their ultrastructure, the mites were immediately immersed in 2.5% glutaraldehyde buffered with 0.1M sodium cacodylate buffer (pH 7.2) for primary fixation and stored at 4°C until further processing. Care was taken to ensure minimal contamination with debris or bee tissue to preserve morphological detail.

Scanning electron microscopy preparation

The collected *Varroa* mites were processed for scanning electron microscopy (SEM) at the Integrated Laboratory for Research and Testing (LPPT), Universitas Gadjah Mada, following established preparation protocols for ultrastructural analysis of mites (Thapa et al. 2015). Specimens were initially fixed in 2.5% glutaraldehyde prepared in 0.1 M sodium cacodylate buffer (pH 7.2) and kept at 4°C to preserve the external morphology. After fixation, samples were rinsed three times in the same buffer to remove residual aldehyde and prepare tissues for post-fixation. A secondary fixation step was performed using 1% osmium tetroxide at room temperature for two hours to enhance membrane contrast and improve electron density (Delfinado-Baker and Aggarwal, 1987). The mites were then dehydrated through a graded ethanol series, progressing from 30% to 100%, with each step lasting 10 minutes to ensure thorough dehydration. To minimize distortion caused by surface tension during drying, the samples were processed using critical point drying with liquid CO_2, a method widely recognized for preserving fine external structures in soft-bodied arthropods (Walter and Proctor 2013). Once dried, the mites were mounted onto aluminum SEM stubs using double-sided carbon adhesive tape. Specimens were positioned to expose multiple views including the dorsal, ventral, and lateral body surfaces. All mounted samples were coated with a 20nm layer of gold-palladium using a sputter coater, rendering them conductive for SEM analysis and preventing surface charging under the electron beam (Abou-Shaara and Tabikha 2017; Zaki et al. 2021b).

SEM observation and imaging

Morphological examination of the mites was carried out using a JEOL JSM-6510LA scanning electron microscope operated at 10 kV. This instrument provides high-resolution imaging suitable for revealing detailed surface architecture of ectoparasitic mites (Ramsey et al. 2019). Imaging was performed at variable magnifications ranging from 150× to 2000×, allowing observation of both the overall body morphology and microstructural features of the gnathosoma, pedipalps, chelicerae, apotele claws, ventral and dorsal shields, legs, ambulacra, and peritreme.

Digital image acquisition and measurement were performed using the integrated SEM software, and representative views were selected to illustrate taxonomically and functionally important structures. These views provided a comprehensive morphological record suitable for descriptive comparison with previously documented *Varroa* populations from other geographic regions (Thapa et al. 2015; Fanelli and Tizzani 2020).

Special care was taken during image selection to avoid artifacts and ensure clarity of features relevant to mite identification and functional morphology.

Ethical considerations

This study did not involve vertebrate animals or procedures requiring institutional ethics approval. The bee colonies sampled were part of a commercial apiary and were handled using non-lethal standard procedures. Collection of *Varroa* mites did not result in significant harm or mortality to the bee populations. Permission to access the apiary and collect samples was granted by the farm owner. Sample collection and laboratory processing complied with biosafety guidelines outlined by the Faculty of Veterinary Medicine, Universitas Gadjah Mada, and the Indonesian Quarantine Agency. This study focused exclusively on the external morphology of *Varroa destructor* from a single geographic location (Central Java). No genetic or morphometric analyses were conducted, and no assessment of acaricide exposure history or resistance was included. Despite these limitations, this is the first SEM-based descriptive report of *Varroa destructor* morphology in Indonesian *Apis mellifera*, contributing essential baseline data for future taxonomic, ecological, and molecular studies.

Descriptive Analysis

This study adopted a descriptive morphological approach without morphometric or statistical comparison. Terminologies for anatomical features followed standard acarological references (Walter and Proctor 2013; Thapa et al. 2015). Descriptions emphasized shape, surface features, presence and arrangement of setae, articulations, and diagnostic traits observed under SEM. Comparative references from literature on *Varroa* mites in other geographic regions (e.g., Egypt, Korea, China, and Europe) were used to identify shared and potentially unique features in the Indonesian specimens (Abou-Shaara and Tabikha 2017; Fanelli and Tizzani 2020; Zaki et al. 2021b).

RESULTS

Scanning electron microscopy (SEM) observation of *Varroa destructor* collected from *Apis mellifera* colonies in Central Java revealed detailed external morphological features across multiple anatomical regions. The images provided high-resolution visualizations of the gnathosoma, ventral and dorsal shields, ambulacra, peritreme, and associated structures. All observations were based on intact, adult female mites, which are the predominant parasitic stage found in honey bee colonies.

The gnathosoma of *V. destructor* was clearly visible in an anteroventral position and was composed of two pedipalps and a pair of chelicerae, forming the primary feeding apparatus (Fig. 1A). The chelicerae appeared bidentate, saw blade-like, and were shaped like short, wide knives with a slightly curved profile. They were positioned ventrally and partially covered by the pedipalps. The distal ends of the chelicerae bore two pairs of hypostomal setae and a trough-like groove—the preoral trough—presumed to assist in feeding by directing liquefied host tissue toward the mouth (Fig. 1B).

The pedipalps were segmented into four distinct parts, with the final segment terminating in an apotele claw (Fig. 1C). Setae were distributed along the segments, appearing in two distinct sizes: large setae with a thick, spine-like appearance resembling sensilla chaetica and smaller, thinner setae resembling trichodea sensilla. The arrangement of setae was conserved across all observed specimens. The apotele claw (Fig. 1D) showed a dual-branch structure, with one longer, pointed, and slightly curved claw, and a shorter, straighter counterpart with a blunt end. These claws are presumed to function in integument penetration and attachment to host surfaces.

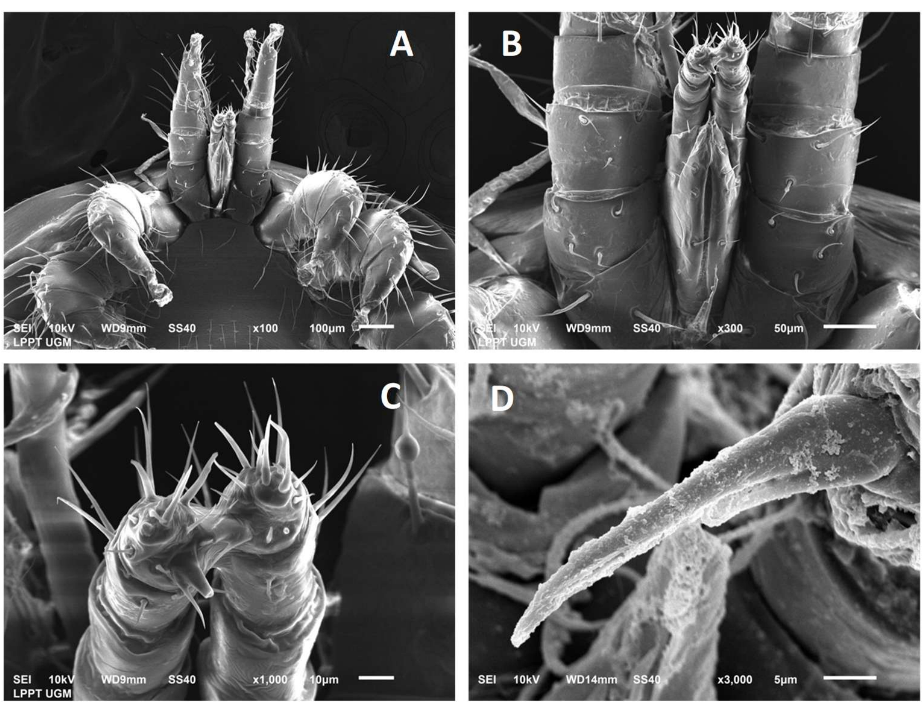

Fig. 1: Scanning electron micrographs of the gnathosoma and associated appendages of *Varroa destructor* female mite. (A) Ventral view showing the full gnathosoma, including pedipalps (pp) and chelicerae (ch). (B) Close-up of chelicerae exhibiting paired bidentate distal ends (bd). (C) Four-segmented pedipalps bearing multiple long and short setae. (D) Apical segment of pedipalp showing dual-clawed apotele (ac) with curved major claw and blunt accessory claw.

The ventral view of the mite revealed a well-defined shield complex consisting of sternal, genital, anal, endopodal, and metapodal shields (Fig. 2A). The sternal shield was located anteriorly and bordered the genital shield posteriorly. The genital shield was oval in shape and covered with fine, evenly spaced setae. This shield extended posteriorly toward the anal shield, indicating the contiguous nature of the ventral structures.

The endopodal shields were located laterally to the coxae of legs III and IV. They exhibited a Y-shaped depression at the junction with the sternal and metapodal shields. These shields bore 13–16 setae and were slightly granulated in surface texture. The metapodal shields appeared as wide, triangular plates situated at the lateral posterior region of the mite, supporting numerous long and short setae. The anal shield, triangular in shape, was positioned posteriorly and included an anal valve (Fig. 2B). The anal opening was surrounded by three prominent setae: a terminal septae, a post-anal seta, and a lateral seta. The anal valve was encircled by a distinct sclerotized ring, the anal sclerite, marking the boundary of the posterior margin.

All four pairs of legs were well-articulated and emerged from the ventrolateral aspect of the mite body. Each leg was composed of seven segments: coxa, trochanter, femur, genu, tibia, metatarsus, and tarsus. The pretarsal structure of the tarsus terminated in an ambulacrum (Fig. 2C), which was a circular, sucker-like structure with flexible membranes. The ambulacra were flanked by the basal stalk, which was sclerotized and shaped like an inverted cone. Ambulacra are believed to aid in surface adhesion, allowing the mite to move securely on the body of the host bee or within the brood cell environment. SEM imaging also revealed the presence of fine ridges and micropores on the surface of the ambulacra, features that may enhance grip and anchoring.

The peritreme was observed laterally between legs III and IV, appearing as a tubular channel connected to a small sac-like structure (Fig. 2D). The base of the peritreme was bulb-shaped and housed the stigmata or spiracular opening. Internally, micropapillae were observed lining the inner lumen of the peritreme, likely playing a role in regulating gas exchange or filtering environmental particles. The structure of the peritreme was consistent with descriptions of plastron-like adaptations in mites exposed to high-humidity microenvironments.

SEM imaging of the dorsal surface showed a well-sclerotized dorsal shield (Fig. 3A), oval to ellipsoidal in shape, with a reddish-brown appearance under electron reflectance. The shield was densely populated with long, tapering setae arranged in both central and marginal rows. The surface of the shield displayed shallow longitudinal striations interspersed with small pits, a pattern thought to enhance flexibility while maintaining structural rigidity. The idiosoma was flattened dorsoventrally, a feature typical of phoretic ectoparasites that facilitates movement through the narrow spaces of brood cells.

At the anterior dorsal region (Fig. 3B), the bases of the legs were clearly visible emerging from coxal sockets. Setae in this area were noticeably shorter than those on the posterior dorsal surface. Each leg bore distinct tactile and mechanosensory setae, especially on the coxae and femur, which were long, needle-like, and unbranched. These setae likely function in spatial orientation and host surface detection during movement. Marginal setae along the dorsolateral surface (Fig. 3C) were shorter and more curved than central dorsal setae. Their distribution along the periphery of the shield may aid the mite in interlocking with the fine hairs of the bee’s body, particularly during the phoretic phase. These observations support earlier hypotheses that setae on the dorsal shield contribute not only to mechanical protection but also to host adhesion.

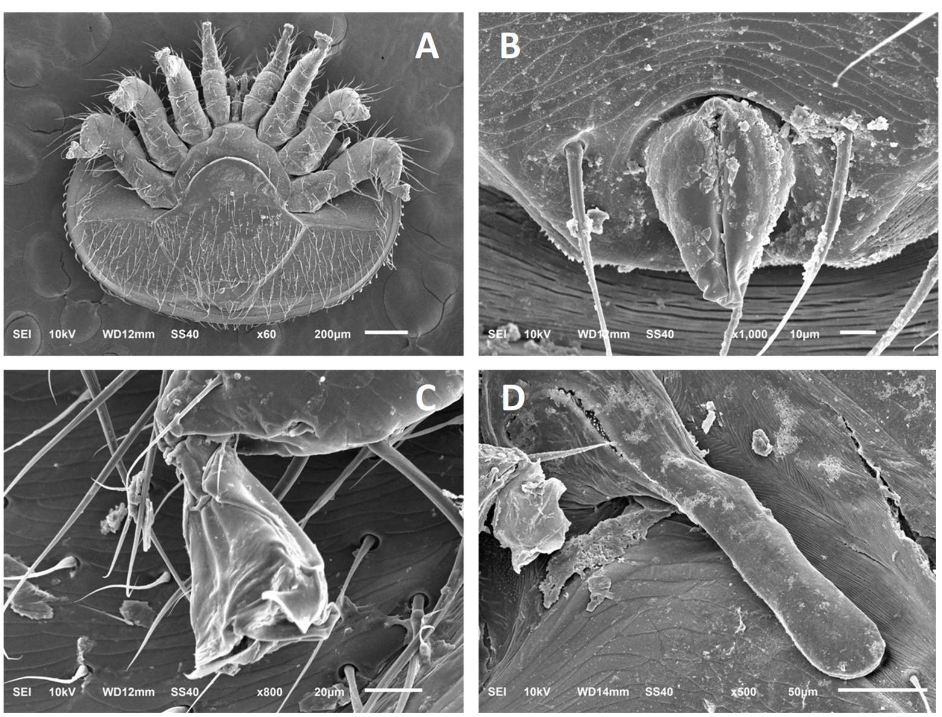

Fig. 2: Ventral morphological features of *Varroa destructor*. (A) Overview of ventral shields, including sternal (ss), genital (gs), endopodal (es), metapodal (ms), and anal shields (as). (B) Close-up of triangular anal shield with visible anal valve (av) and three surrounding setae (as). (C) Terminal end of leg IV showing ambulacrum (am) with suction-cup-like pad and sclerotized basal stalk. (D) Peritreme (pt) located between legs III and IV, exhibiting a bulbous base and elongated tubular channel.

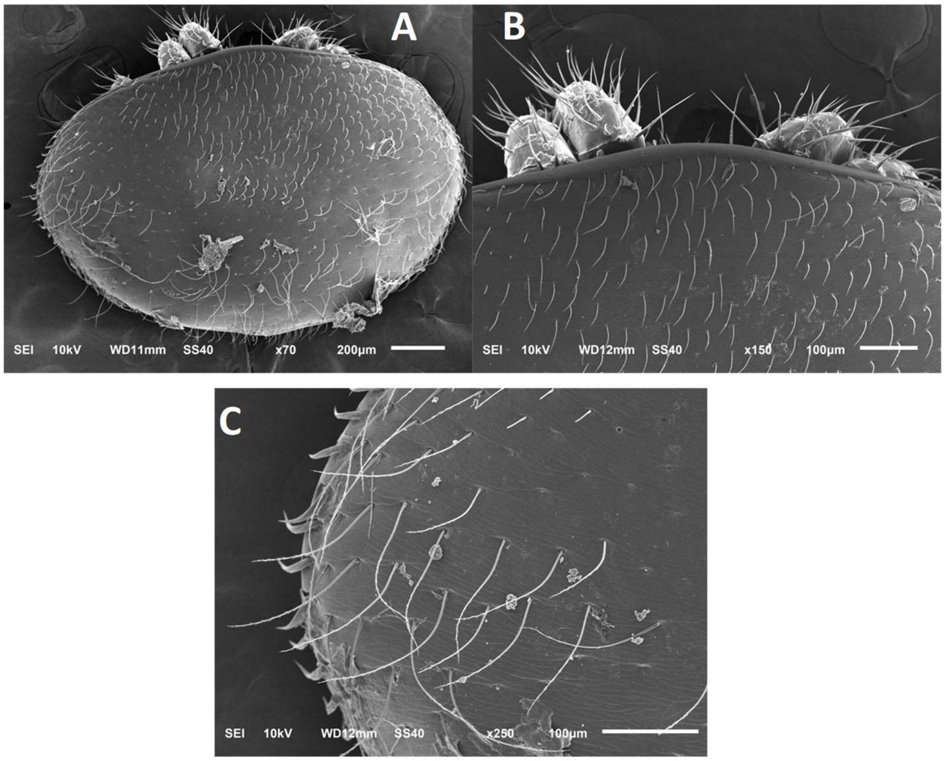

Fig. 3: Dorsal and dorso-lateral surface structures of *Varroa destructor*. (A) Entire dorsal shield (ds) with longitudinal ridges and centrally distributed dorsal setae (ds). (B) Anterolateral view showing base of legs and associated coxal regions with tactile setae. (C) Dorso-lateral edge of the idiosoma displaying curved marginal setae aligned along the periphery.

Across all observed specimens, no gross morphological abnormalities or asymmetries were noted. The external anatomy of *V. destructor* appeared remarkably conserved in structure, indicating a high degree of phenotypic stability among the population sampled in Central Java. No damage attributable to acaricides, desiccation, or physical trauma was detected. The preservation quality achieved through critical point drying and gold sputter coating enabled clear visualization of minute structures without structural collapse or imaging artifacts.

DISCUSSION

This study provides the first scanning electron microscopy (SEM)-based description of *Varroa destructor* mites collected from *Apis mellifera* colonies in Indonesia. The external morphology of female mites was described in detail, highlighting the structural complexity of anatomical regions such as the gnathosoma, pedipalps, chelicerae, ventral and dorsal shields, ambulacra, and peritreme. These features, essential for the mite's parasitic lifestyle, were found to be consistent in form across all examined specimens. This study addresses a significant knowledge gap in Southeast Asian acarological research and provides critical baseline data that may serve as a foundation for diagnostic, taxonomic, and control-focused studies in Indonesia and the surrounding region (Fanelli and Tizzani 2020; Zaki et al. 2021a).

The gnathosoma plays a vital role in host interaction and feeding. SEM imaging in this study revealed a bidentate cheliceral structure with two sharp, blade-like projections and an accompanying preoral trough. These features are functionally adapted to pierce the host's integument and facilitate the extraction of nutrients. The chelicerae, positioned ventrally, appear structurally suited for repetitive penetration and anchoring within soft tissues. The presence of two pairs of hypostomal setae likely assists in feeding control and fluid uptake, as similarly interpreted in mite feeding mechanics (Zaki et al. 2021a).

The pedipalps, segmented into four distinct parts and equipped with both large and small setae, were observed terminating in a dual-clawed apotele. The asymmetry in claw morphology—one long and curved, the other short and blunt—suggests a division of function, with the long claw aiding in penetration and the short claw assisting in anchoring. The identification of these traits supports the hypothesis that *V. destructor* is evolutionarily specialized for stable attachment during phoretic and reproductive phases within the bee colony (Thapa et al. 2015; Abou-Shaara and Tabikha 2017). While similar structures have been reported in other regions, this study confirms their presence and uniformity in Indonesian populations.

The ventral shields form the basis of *Varroa*'s protective and locomotive support system. The genital and sternal shields were found to be tightly apposed, suggesting compact internal organization and a robust cuticular exoskeleton. The endopodal and metapodal shields displayed clear setal coverage, with variation in setae length and density, characteristics useful in taxonomic distinction among mite species and populations. The anal shield, triangular in shape, displayed an anal valve with three key setae around the anal opening. This configuration, consistently found in all specimens, appears to be a reliable diagnostic marker for identifying female mites (Zaki et al. 2021a).

Observation of the ambulacra at the end of each leg provided insight into *V. destructor*'s unique mode of locomotion. The structure of the ambulacrum as a flexible, suction-like pad, combined with the sclerotized basal stalk, supports strong adhesion to smooth and irregular host surfaces. This adaptation is critical for stability during movement on adult bees, wax surfaces, and within brood cells. SEM images revealed micropores and surface texturing within the ambulacra that may play a role in fine-tuned grip or environmental sensing (Thapa et al. 2015).

Another significant finding was the peritreme, a spiracle-associated structure located between legs III and IV. The bulb-shaped base and tubular channel were clearly visible in SEM, along with micropapillae lining the internal walls. The structure of the peritreme in these Indonesian specimens matches descriptions of respiratory adaptations seen in *Varroa*populations from other humid, subtropical environments. This respiratory configuration may help the mite maintain gas exchange efficiency in the hypoxic conditions found within capped brood cells, where carbon dioxide levels are often elevated (Zaki et al. 2021a).

The dorsal shield, observed in Fig. 3A–3C, was oval, densely sclerotized, and featured longitudinal striations and centrally positioned long setae. These features likely contribute to both passive defense and biomechanical resilience. The arrangement and morphology of the dorsal setae also support interlocking with bee cuticular hairs during the mite's phoretic stage, enhancing host adherence. In addition, the marginal setae were curved and shorter than central setae, forming a periphery that may act as a sensory or stabilizing interface between the mite and host (Zaki et al. 2021b).

The uniformity of the anatomical structures observed across all samples in this study suggests a conserved morphology among Indonesian *V. destructor* populations. No apparent deformities, structural anomalies, or variations in setal pattern were detected, despite all specimens originating from separate colonies. This homogeneity may reflect the recent introduction or genetic bottlenecking of *Varroa* populations in Indonesia, though this remains to be confirmed by molecular analysis (Dietemann et al. 2013).

As Indonesia continues to expand its apicultural industry, particularly with the adoption of *A. mellifera* colonies for commercial honey production, understanding the local characteristics of *Varroa* mites becomes increasingly important. These morphological findings lay the groundwork for future comparative studies aimed at evaluating intraspecific variation, strain identification, and regional adaptation of *V. destructor*. They also provide essential reference points for microscopic diagnostics in clinical and quarantine settings, especially where traditional light microscopy fails to capture fine morphological distinctions (Dietemann et al. 2013; Hornitzky 2010).

The detailed external morphology of *Varroa destructor* observed in Indonesian specimens aligns with key structural features reported in several international SEM studies, reinforcing the global consistency of this species' anatomical adaptations. Nonetheless, slight variations in surface patterning, shield shape, and setal distribution have been noted across regions, suggesting potential population-specific differences. Comparative studies from Korea (Thapa et al. 2015), Egypt and Libya (Zaki et al. 2021b) provide important context for assessing whether environmental or host-specific factors influence morphological expression.

The chelicerae and gnathosoma morphology observed in this study are consistent with *Varroa*'s function as a piercing–sucking ectoparasite. The bidentate cheliceral tips and preoral trough work synergistically to penetrate and extract host tissues, particularly the fat body, which has recently been confirmed as the primary feeding target rather than hemolymph (Ramsey et al. 2019). The structural rigidity and knife-like shape of the chelicerae likely facilitate tissue slicing, while the pedipalps may provide stability during feeding. These features have been described with similar clarity in SEM studies from Chinese and Thai mite populations (Fanelli and Tizzani 2020), suggesting their conservation across strains.

The dual-clawed apotele observed in this study demonstrates mechanical specialization for host attachment. As mites navigate the smooth, hairy surfaces of adult bees and the confined spaces of brood cells, effective anchorage is critical. The longer curved claw likely penetrates host integument or grooves between cuticular plates, while the shorter blunt claw may function to stabilize the body during feeding or locomotion. This dual-claw configuration, also described in Korean and Egyptian populations (Thapa et al. 2015; Abou-Shaara and Tabikha 2017), is an important trait that can be used to confirm species-level identification under SEM.

The ventral shields provide not only protective but also structural support to the mite's reproductive and digestive systems. The compact overlap between the sternal and genital shields observed in Indonesian specimens may reflect efficient spatial organization within the idiosoma. The presence of specific setal arrangements, especially on the endopodal and metapodal shields, offers valuable taxonomic markers. In Egypt, for example, Zaki et al. (2021a) reported population-specific differences in the length and positioning of these setae when compared to Libyan specimens. While the Indonesian mites in this study exhibited uniform shield morphology, further sampling from other regions in the archipelago could reveal subtle phenotypic plasticity.

The triangular anal shield and its associated anal valve, observed in all specimens, reinforce its taxonomic value. The consistent presence of three surrounding setae—post-anal, lateral, and terminal—has been repeatedly cited in literature as a defining characteristic of adult female *V. destructor* (Dietemann et al. 2013). These setae may also serve sensory roles or help deflect fecal output away from the mite's ventral surface during brood cell infestation.

One of the most functionally critical structures observed in this study is the ambulacrum. The SEM resolution allowed detailed visualization of the pad-like ambulacra and their basal stalks. These structures support both phoretic attachment and locomotion on uneven bee surfaces. The cuticular flexibility of the ambulacral pads and their micropapillae may enhance friction or suction, enabling the mite to remain attached even during host grooming. In Korea, Thapa et al. (2015) noted minor variations in ambulacral surface texture, suggesting this structure may show population-level adaptation to different host grooming behaviors or hive microclimates.

The peritreme, associated with the mite's respiratory system, is also of adaptive significance. In high-CO_2 brood environments, the peritreme functions to modulate gas exchange and may also serve as a barrier to fluid or pathogen ingress (Dietemann et al. 2013). The peritreme's location between the third and fourth pair of legs, and its bulb-shaped base with micropapillae, are features that were clearly observed in this study and closely resemble those described in European and Southeast Asian populations. Given the importance of respiration in the mite's survival within sealed brood cells, the conserved nature of this structure supports its critical physiological role.

From a diagnostic and surveillance perspective, the anatomical features confirmed in this study offer practical markers for field identification and laboratory classification. In contexts where molecular methods are inaccessible, SEM provides a valuable alternative for species confirmation. While SEM equipment may not be routinely available in all regions of Indonesia, collaborative efforts with universities and research institutes can support targeted acarological investigations, especially in provinces with expanding commercial beekeeping industries.

Importantly, this SEM-based baseline serves as a reference for monitoring morphological changes over time, especially in response to acaricide pressure. In regions where *V. destructor* has developed resistance to amitraz, fluvalinate, or coumaphos, SEM studies have reported changes in cuticle thickness, sensory organ exposure, or leg articulation (Rinkevich 2020). Although such adaptations were not detected in this study, future comparisons with mites from colonies under chemical treatment regimens in other parts of Indonesia could reveal early signs of structural adaptation or resistance development.

The implications of these findings extend beyond taxonomy and diagnostics. The conservation of key morphological structures across global *V. destructor* populations, including the Indonesian cohort, underscores the evolutionary success of this species. Its ability to adapt to various climates, host subspecies, and hive environments is facilitated in part by the robustness of its anatomy. Documenting these features regionally can inform control strategies, such as the development of mechanical removal devices or mite-resistant bee lines that exploit vulnerabilities in mite attachment or reproduction.

In addition, the consistent morphology found in this study lays the groundwork for integrating SEM data with future genetic, transcriptomic, or proteomic investigations. As regional strain differentiation is increasingly explored through molecular markers (Dietemann et al. 2013), the correlation of morphotypes with haplotypes could enhance surveillance and modeling efforts. The alignment of ultrastructural traits with genetic lineages may also support the identification of novel *Varroa* variants or confirm the introduction of foreign strains through colony trade or migration.

The findings of this study carry significant implications for Indonesia's beekeeping sector, which is increasingly adopting *Apis mellifera* colonies for honey production, pollination, and smallholder income generation. Despite the economic value of beekeeping, surveillance of parasitic threats such as *Varroa destructor* has been limited. This research marks the first application of scanning electron microscopy to characterize *Varroa* morphology from Indonesian bee colonies, providing a vital baseline for future disease management, epidemiological monitoring, and acarological research in the region (Dietemann et al. 2013; Hornitzky 2010).

Given Indonesia's vast geographic and ecological diversity, this foundational morphological study invites the next phase of research—comparative SEM analysis across the archipelago's varied climates and bee stocks. Provinces such as East Java, Bali, and North Sumatra, each with differing beekeeping intensities and genetic variability in bee populations, could yield insights into regional strain differences or adaptation patterns in *Varroa* mites. Furthermore, the increasing use of *A. cerana*, *A. dorsata*, and stingless bee species (*Trigona spp.*) opens opportunities to examine host-specific mite morphological adaptations, if any.

The detailed characterization of features such as the gnathosoma, ambulacra, and peritreme also provides a framework for developing targeted mechanical or behavioral interventions. For example, the cheliceral size and claw architecture observed in this study could guide the design of physical mite removal tools or mesh-based exclusion devices tailored to disrupt feeding or attachment (Rosenkranz et al. 2010). Likewise, understanding the positioning of ambulacral suckers may support the development of hive materials or treatments that interfere with mite locomotion.

This study also reinforces the potential for SEM imaging to be applied in acaricide monitoring programs. Though no morphological alterations suggestive of chemical resistance were detected in the present specimens, previous research has shown that chronic exposure to miticides can lead to changes in cuticle thickness, sensory organ regression, and reduced claw grip (Rinkevich 2020). Establishing a baseline before large-scale chemical use becomes widespread in Indonesia is therefore essential. Routine SEM assessments could serve as early warning tools for structural changes correlated with resistance, especially when integrated with bioassay and molecular data.

Another relevant consideration is the role of morphology in species and haplotype identification. While morphologically consistent with global *V. destructor* descriptions, future studies should attempt to pair SEM imaging with mitochondrial DNA sequencing or microsatellite profiling. This integrated approach would help validate whether the Indonesian population belongs to the Korea-derived haplotype, as seen in Thailand and Vietnam (Dietemann et al. 2013), or whether it contains unique regional lineages introduced via apicultural trade.

While this study offers valuable insights, it also has several limitations. First, the sample size was limited to colonies in Central Java, potentially underrepresenting morphological diversity within the broader Indonesian *Varroa* population. Second, due to the descriptive focus of this work, morphometric and statistical analyses were not conducted. While intentional, this limits the study's ability to quantify variation among individuals or detect subtle inter-regional distinctions. Third, although SEM provides unparalleled surface resolution, it cannot detect internal physiological or histological changes associated with aging, reproduction, or chemical stress. These areas could be explored using complementary imaging techniques such as transmission electron microscopy (TEM) or confocal laser scanning microscopy

(CLSM) in future investigations.

Despite these constraints, this research makes a vital contribution to the limited body of acarological work in Indonesia. It establishes morphological reference images and terminology that future researchers can use for comparative, ecological, and control-based studies. It also demonstrates the feasibility of SEM for arthropod parasitology in Indonesian laboratories and advocates for broader collaboration among entomologists, veterinary parasitologists, and apicultural extension agencies.

Going forward, several research directions are recommended. A nationwide SEM survey of *Varroa* mites, combined with morphometric and genetic profiling, would provide a comprehensive understanding of population structure, distribution, and evolutionary relationships. Investigation of *Varroa*'s interactions with different bee species in Indonesia—particularly native or less commonly studied hosts—could yield critical insights into host specificity and potential reservoirs. In addition, longitudinal studies monitoring mite morphology in colonies exposed to chemical and non-chemical control methods would be invaluable for resistance detection and integrated pest management.

Finally, greater attention should be directed toward linking morphology with functional outcomes. Observing mite locomotion, feeding, and reproduction in real-time or semi-natural conditions, coupled with SEM documentation of corresponding anatomical regions, would deepen our understanding of how structural adaptations influence behavior. Such insights are essential for designing more sustainable and species-specific control strategies, particularly in tropical ecosystems like Indonesia where conventional temperate beekeeping models may not apply directly.

Conclusion

This study presents the first scanning electron microscopy (SEM)-based morphological characterization of *Varroa destructor* mites from *Apis mellifera* colonies in Indonesia. The detailed examination revealed conserved anatomical features such as the gnathosoma, pedipalps, chelicerae, ambulacra, peritreme, and dorsal and ventral shields—consistent with *V. destructor* described globally. These observations provide essential baseline data for regional diagnostics, surveillance, and potential future assessments of morphological adaptation, strain variation, or acaricide resistance. The uniformity of traits across specimens suggests limited intraspecific variation within the sampled population, though broader geographic sampling is needed. This work contributes to closing the acarological knowledge gap in Southeast Asia and underscores the value of SEM as a tool in honeybee health and integrated parasite management.

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