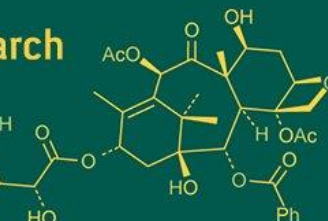
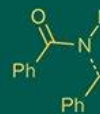
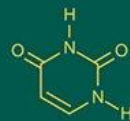


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COI Gene-based molecular taxonomy of scale insect pests in tropical fruit orchards of Selangor, Malaysia

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Abstract

Leading coccidologists have emphasized that molecular approaches are indispensable for resolving the taxonomy of scale insects, a group notorious for morphological conservatism and taxonomic instability. This research employed COI gene barcoding to characterize the molecular taxonomy of scale insect pests in tropical fruit orchards of Selangor, Malaysia. A total of 188 scale insect specimens were collected from durian, rambutan, mangosteen, and starfruit orchards across four districts between March and September 2024. Molecular analysis identified four species: *Aspidiotus destructor* (32.4%), *Parasaissetia oleae* (24.5%), *Coccus hesperidum* (23.4%), and *Unaspis yanonensis* (19.7%). Intraspecific K2P distances ranged from 0.1% to 2.2%, while interspecific distances ranged from 10.5% to 19.8%. Phylogenetic analysis using neighbor-joining methods produced well-resolved species clades with bootstrap values exceeding 96%. These findings contribute the first comprehensive molecular inventory of scale insect pests in Malaysian tropical fruit orchards and provide reference sequences for developing rapid diagnostic tools applicable to quarantine and pest management contexts.

Keywords: Scale insects, DNA barcoding, tropical fruit, COI gene, *Aspidiotus destructor*, molecular taxonomy, Selangor, Coccoidea, pest identification

Introduction

Leading authorities in coccidology have consistently emphasized that molecular approaches are indispensable for achieving stable taxonomic frameworks within the scale insects (Hemiptera: Coccoidea), a group characterized by extreme morphological conservatism, sexual dimorphism, and neotenic body plan reduction that severely limits the utility of traditional diagnostic characters ^[1, 2]. Scale insects rank among the most damaging pest groups in tropical fruit production systems across Southeast Asia, causing direct damage through phloem feeding and indirect losses through honeydew-associated sooty mould development and the transmission of plant pathogens ^[3].

Malaysia is a significant producer of tropical fruits including durian, rambutan, mangosteen, and starfruit, with Selangor serving as a major production and distribution hub ^[4]. The scale insect pest complex on these fruit crops includes armoured scales (Diaspididae), soft scales (Coccidae), and mealybugs (Pseudococcidae), each requiring different management approaches ^[2, 5]. DNA barcoding using the COI gene provides a standardized molecular approach for scale insect identification that overcomes the limitations of morphological taxonomy ^[6, 7].

This research aimed to characterize the molecular taxonomy of scale insect pests in tropical fruit orchards of Selangor using COI barcoding. The objectives were to: (i) identify scale insect species; (ii) quantify genetic divergence; (iii) reconstruct phylogenetic relationships; and (iv) assess the host associations of identified species across different fruit crop systems.

Materials and Methods

Materials

Scale insect specimens were collected from tropical fruit orchards in four districts of Selangor, Malaysia, between March and September 2024: Hulu Langat (3.1073°N, 101.8636°E), Sepang (2.7222°N, 101.7440°E), Kuala Langat (2.8031°N, 101.5100°E), and Sabak Bernam (3.7958°N, 101.0258°E). Orchards of durian (*Durio zibethinus*), rambutan (*Nephelium lappaceum*), mangosteen (*Garcinia mangostana*), and starfruit (*Averrhoa*

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carambola) were surveyed. Specimens were collected by visual inspection and branch sampling at biweekly intervals. Ethical clearance was obtained from the Selangor Institute of Tropical Sciences Ethics Committee (Protocol No. SITS/PP/2024-014). Specimens were preserved in 95% ethanol at -20 °C.

Methods

Genomic DNA was extracted from individual scale insects using the DNeasy Blood and Tissue Kit (Qiagen, Germany) with extended proteinase K digestion and mechanical disruption of the waxy shield. COI fragments were amplified using LCO1490/HCO2198 primers [8]. PCR reactions were conducted in 25 µL volumes under cycling

conditions of 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 50 °C for 45 s, and 72 °C for 1 min, with final extension at 72 °C for 10 min. Products were sequenced bidirectionally, edited in BioEdit v7.2.5, aligned in MEGA 11 [9], and compared against BOLD and GenBank [10]. K2P distances were calculated [11], and neighbor-joining trees constructed with 1,000 bootstrap replicates [12].

Results

Of 188 scale insect specimens collected, 163 yielded high-quality COI sequences (86.7% success rate). Four scale insect species representing two families (Diaspididae and Coccidae) were identified through database comparisons.

Table 1: Molecular identification summary of scale insect species from tropical fruit orchards in Selangor, Malaysia

Species	Family	n	Abundance (%)	Intra. Dist. (%)	Host Range
<i>Aspidiotus destructor</i>	Diaspididae	53	32.4	0.1-1.8	Generalist
<i>Parasaissetia oleae</i>	Coccidae	40	24.5	0.2-2.0	Moderate
<i>Coccus hesperidum</i>	Coccidae	38	23.4	0.1-1.5	Moderate
<i>Unaspis yanonensis</i>	Diaspididae	32	19.7	0.3-2.2	Specialist

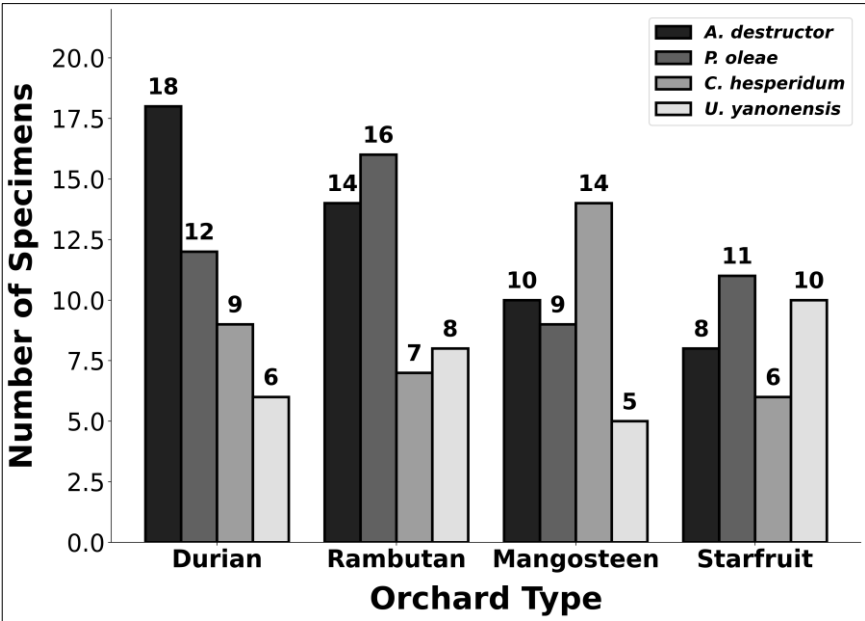


Fig 1: Distribution of scale insect specimens across four tropical fruit orchard types in Selangor, Malaysia

K2P genetic distance analysis revealed clear molecular separation among all four species. Intraspecific distances ranged from 0.1% to 2.2%, while interspecific distances ranged from 10.5% (*A. destructor* vs *U. yanonensis*, both

Diaspididae) to 19.8% (*C. hesperidum* vs *U. yanonensis*). The two diaspidid species formed a well-supported clade separate from the two coccid species, consistent with their family-level classification.

Table 2: Pairwise interspecific K2P genetic distances (%) among four scale insect species

Species	<i>A. destructor</i>	<i>P. oleae</i>	<i>C. hesperidum</i>	<i>U. yanonensis</i>
<i>A. destructor</i>				
<i>P. oleae</i>	16.2			
<i>C. hesperidum</i>	17.5	12.8		
<i>U. yanonensis</i>	10.5	19.8	18.3	

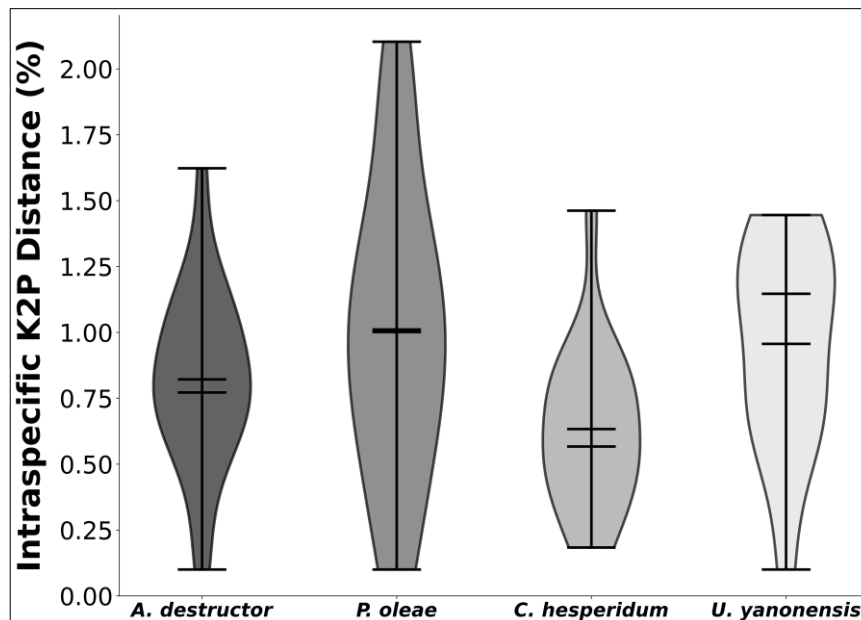


Fig 2: Violin plot of intraspecific K2P distance distributions for four scale insect species from Selangor orchards

Phylogenetic analysis produced a well-resolved topology with all four species forming strongly supported monophyletic clades (bootstrap>96%). The two armoured scale species (*A. destructor* and *U. yanonensis*) grouped together as a sister clade to the soft scale pair (*P. oleae* and *C. hesperidum*), reflecting the established systematic division between Diaspididae and Coccidae within the Coccoidea. Host association analysis revealed that *A. destructor* was the most generalist species, occurring on all four fruit crop types, while *U. yanonensis* showed the strongest host preference for starfruit orchards.

Discussion

The molecular identification of four scale insect species in Selangor tropical fruit orchards establishes the first comprehensive barcode-based inventory for this pest group in Malaysian fruit production systems. The dominance of *A. destructor* (32.4%) is consistent with its globally recognized status as one of the most polyphagous armoured scale species, with recorded host plants spanning over 100 families [1, 3].

The relatively lower sequencing success rate (86.7%) compared to softer-bodied insect groups reflects the challenges of DNA extraction from scale insects with heavily sclerotized or waxy body coverings [2, 6]. The diaspidid species, which possess rigid armoured covers, showed lower individual extraction success than the soft-bodied coccid species, suggesting that optimized mechanical disruption protocols should be developed for routine molecular diagnostics of armoured scales.

The interspecific distances (10.5-19.8%) substantially exceed the 3% species delimitation threshold, confirming unambiguous molecular separation [5, 7]. The phylogenetic topology is consistent with current molecular systematic treatments of the Coccoidea, reinforcing the reliability of COI barcoding for species-level identification within this taxonomically challenging group [1, 2].

The host association patterns identified have practical implications for orchard-specific pest management. The generalist nature of *A. destructor* suggests that control programmes in mixed-fruit production landscapes must account for potential cross-infestation between adjacent

orchards, while the apparent host specialization of *U. yanonensis* on starfruit may allow more targeted interventions in starfruit monocultures [3, 4].

Quarantine Diagnostics

Several of the identified scale insect species are regulated quarantine pests in international fruit trade. The COI reference sequences generated provide a foundation for developing molecular diagnostic protocols applicable at Malaysian phytosanitary inspection facilities, enabling rapid and unambiguous identification of intercepted specimens on export fruit consignments [2, 5].

Biological Control Integration

Accurate molecular identification of scale insect species is prerequisite for selecting appropriate parasitoid wasps and predatory insects for biological control programmes. The species-level resolution achieved through COI barcoding enables targeted deployment of natural enemies with demonstrated host specificity against individual scale insect taxa in Malaysian fruit orchards [3, 4]. The parasitoid wasp *Aphytis lingnanensis*, for instance, demonstrates high efficacy against diaspidid scales but is ineffective against coccid species, underscoring the importance of molecular identification for biological control decision-making.

Economic Impact Assessment

The host association data generated in this research provide a foundation for estimating species-specific economic impacts across different fruit crop systems in Selangor. The generalist feeding behavior of *A. destructor*, occurring across all four surveyed fruit types, suggests that economic impact assessments should account for cross-crop damage potential in mixed orchard landscapes. Conversely, the apparent specialization of *U. yanonensis* on starfruit allows for more precise damage attribution and targeted cost-benefit analyses of species-specific control interventions in starfruit production systems [1, 3, 4].

Conclusion

Scale insect pests represent a persistent challenge for tropical fruit production in Selangor, Malaysia, yet

molecular characterization of this pest complex has been notably absent from the regional literature until the present research.

Four species spanning two families were identified through COI barcoding, with clear genetic boundaries confirmed by interspecific distances exceeding 10.5%. Phylogenetic analysis produced family-level groupings consistent with established systematic frameworks, and host association patterns revealed species-specific orchard preferences.

It is recommended that the molecular reference library established in this research be incorporated into Malaysian quarantine inspection protocols and integrated pest management decision-support systems for tropical fruit production.

Future research should expand molecular surveys to additional fruit-producing states across Peninsular Malaysia and Borneo, incorporate nuclear markers for population-level analyses, and evaluate the efficacy of barcode-based rapid diagnostic assays under field inspection conditions.

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