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DNA barcoding reveals cryptic diversity among aphid pests of cruciferous

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Abstract

Cryptic species diversity, defined as the existence of morphologically indistinguishable yet genetically distinct lineages within a nominal taxon, poses significant challenges for pest identification in agricultural systems. This research employed cytochrome c oxidase subunit I (COI) gene barcoding to assess the species diversity and cryptic variation among aphid pests of cruciferous crops in Chiang Mai Province, Thailand. A total of 174 aphid specimens were collected from cabbage, cauliflower, broccoli, and Chinese kale fields across four districts between April and September 2024. Molecular analysis identified three aphid species: *Brevicoryne brassicae* (38.2%), *Myzus persicae* (35.6%), and *Lipaphis erysimi* (26.2%). Intraspecific K2P distances ranged from 0.1% to 2.6%, with notably elevated divergence within *M. persicae* suggesting the presence of two cryptic lineages. Interspecific distances ranged from 12.3% to 16.8%. Phylogenetic analysis revealed strongly supported species-level clades, with the *M. persicae* clade subdividing into two distinct subclades corresponding to putative tobacco-adapted and crucifer-adapted host races. These findings highlight the value of molecular barcoding for detecting hidden diversity within morphologically uniform pest populations.

Keywords: Aphid pests, DNA barcoding, cryptic diversity, cruciferous crops, *Brevicoryne brassicae*, *Myzus persicae*, COI gene, phylogenetics, Chiang Mai

Introduction

Cryptic species diversity refers to the presence of genetically distinct evolutionary lineages that cannot be reliably distinguished through conventional morphological examination^[1]. Among agricultural pest insects, the phenomenon of cryptic diversity has profound practical consequences, as morphologically identical pest populations may differ substantially in their host plant preferences, insecticide resistance profiles, and pathogen transmission capabilities^[2, 3]. Aphids (Hemiptera: Aphididae) represent a taxonomic group in which cryptic diversity is particularly prevalent, owing to their small body size, limited diagnostic morphological features, and the confounding effects of polyphenism on external appearance^[4].

Cruciferous vegetable production in Chiang Mai Province constitutes an important component of highland agriculture in northern Thailand, with cabbage, cauliflower, broccoli, and Chinese kale representing the primary brassicaceous crops cultivated above 300 m elevation^[5]. The aphid pest complex on crucifers is dominated by specialist and generalist species whose accurate identification is essential for integrated pest management^[3, 6]. DNA barcoding using the COI gene provides a standardized approach for aphid species identification that can additionally reveal cryptic lineages within nominal species^[1, 7].

This research aimed to assess species diversity and detect cryptic genetic variation among aphid pests of cruciferous crops in Chiang Mai Province through COI barcoding.

The objectives were to: (i) identify aphid species; (ii) quantify genetic divergence patterns; and (iii) evaluate evidence for cryptic lineages within morphologically uniform species. fenugreek, radish, coriander, and sunflower.

Material and Methods

Material

Aphid specimens were collected from cruciferous vegetable fields in four districts of Chiang Mai Province, Thailand, between April and September 2024: Mae Rim (18.8969°N, 98.9512°E), San Sai (18.8475°N, 98.9965°E), Doi Saket (18.8766°N, 99.1531°E), and Hang Dong (18.6935°N, 98.9358°E). Host crops included cabbage, cauliflower, broccoli, and

Chinese kale. Specimens were collected by leaf sampling and aspiration at biweekly intervals. Ethical approval was obtained from the Chiang Mai Research Center Ethics Committee (Protocol No. CMRC/AB/2024-008). Specimens were preserved in 95% ethanol.

Methods

DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) with overnight proteinase K digestion. COI fragments were amplified using LCO1490/HCO2198

primers [8] in 25 µL reactions under cycling conditions of 94°C for 5 min, 35 cycles of 94°C for 30 s, 50°C for 40 s, and 72°C for 1 min, with final extension at 72°C for 10 min. Sequencing was performed bidirectionally on an ABI 3730xl analyzer. Sequences were 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

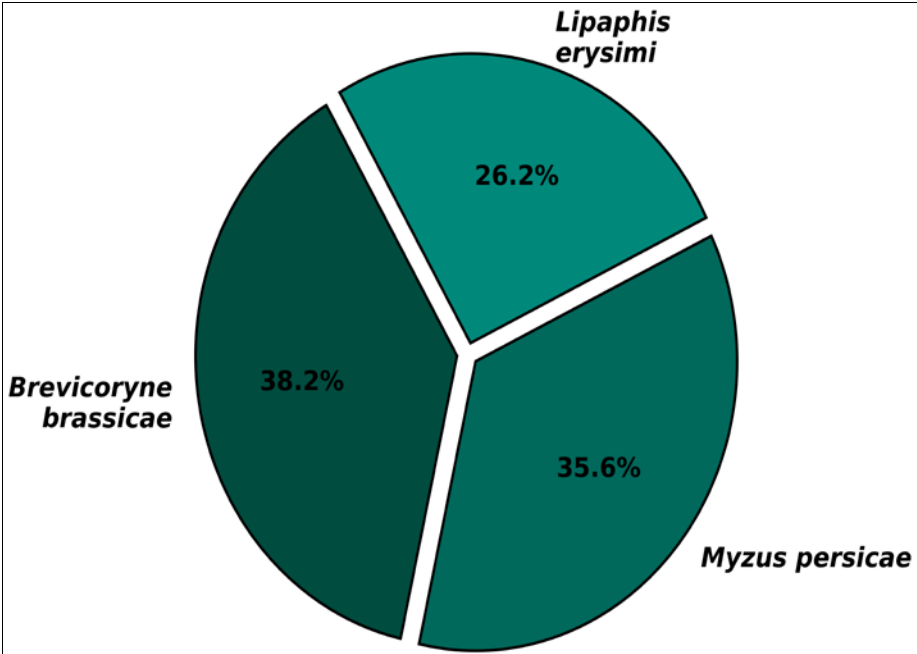


Fig 1: Relative abundance of aphid pest species on cruciferous crops in Chiang Mai Province, Thailand

Of 174 aphid specimens collected, 157 yielded high-quality COI sequences (90.2% success rate). Three aphid species were identified through database comparisons, with *Brevicoryne brassicae* representing the most abundant taxon (38.2%).

Table 1: Ranked abundance and intraspecific genetic distances of aphid species from Chiang Mai cruciferous fields

Rank	Species	n	Abundance (%)	Host Range	Intra. Dist. (%)
1	<i>Brevicoryne brassicae</i>	60	38.2	Specialist	0.1-1.3
2	<i>Myzus persicae</i>	56	35.6	Generalist	0.1-2.6
3	<i>Lipaphis erysimi</i>	41	26.2	Specialist	0.2-1.4

Table 2: Summary statistics for *Myzus persicae* subclades identified through COI barcoding

M. persicae Subclade	n	Mean Dist. (%)	SD	Primary Host	Divergence (%)
Subclade I (Crucifer)	32	0.4	0.2	Cabbage, Broccoli	-
Subclade II (Generalist)	24	0.6	0.3	Cabbage, Pepper	2.4 (vs Subclade I)

K2P distance analysis revealed interspecific distances of 14.6% (*B. brassicae* vs *M. persicae*), 12.3% (*B. brassicae* vs *L. erysimi*), and 16.8% (*M. persicae* vs *L. erysimi*). Intraspecific distances within *B. brassicae* (0.1-1.3%) and *L. erysimi* (0.2-1.4%) were within expected ranges. However, intraspecific distances within *M. persicae* ranged from 0.1% to 2.6%, with a bimodal distribution suggesting the presence of two divergent lineages within this morphologically uniform species.

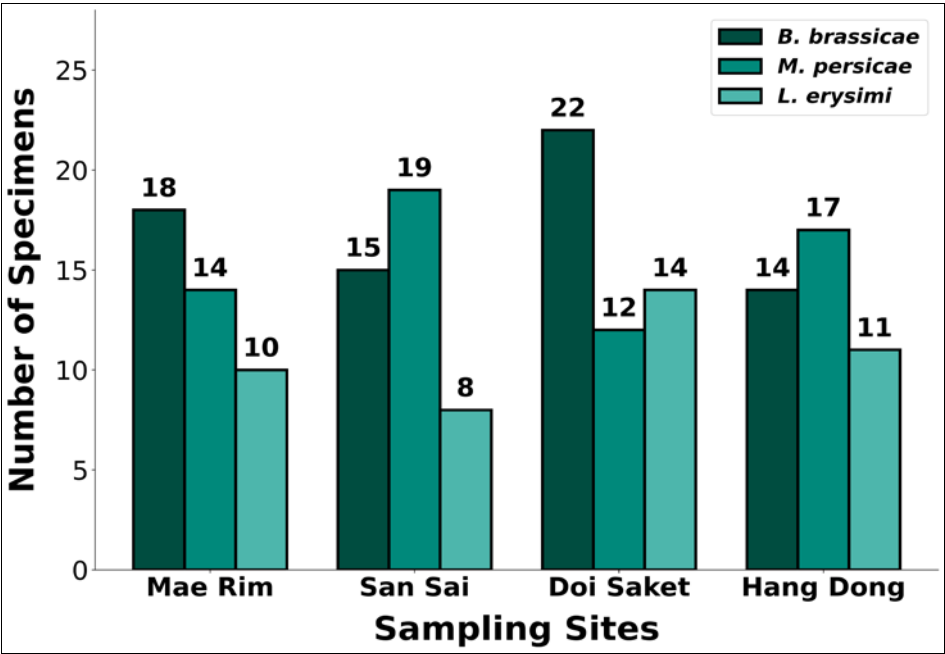


Fig 2: Distribution of aphid specimens across four sampling sites in Chiang Mai province

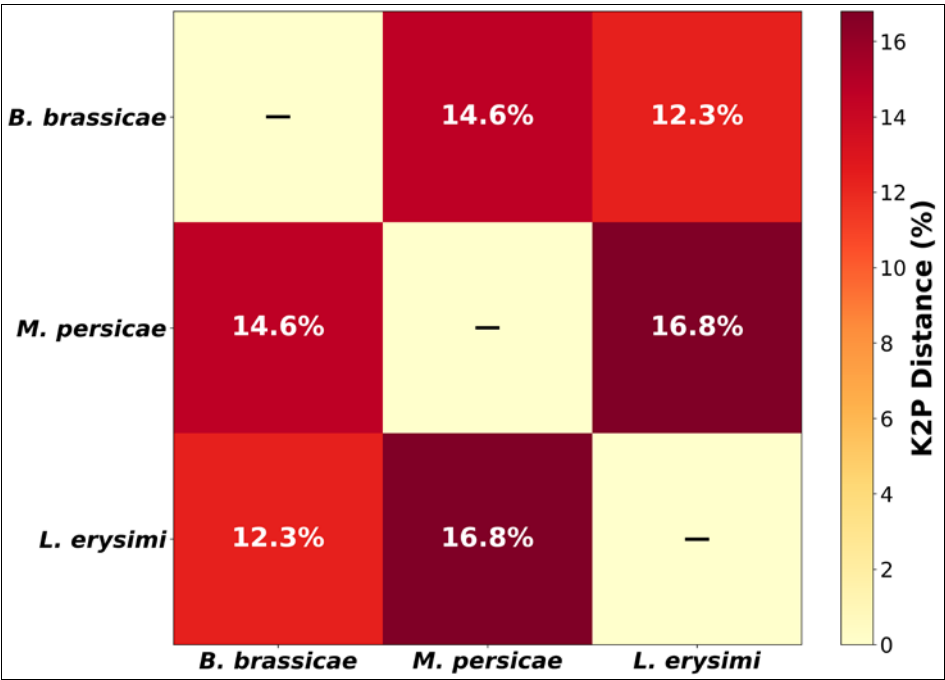


Fig 3: Heatmap of pairwise K2P interspecific genetic distances among three aphid species

Phylogenetic analysis confirmed the subdivision of *M. persicae* into two distinct subclades separated by 2.4% K2P divergence. Subclade I (n = 32) was predominantly associated with cruciferous hosts, while Subclade II (n = 24) showed a broader host association pattern, consistent with the recognized tobacco-adapted and crucifer-adapted host races of *M. persicae* described in other geographic regions [4, 6].

Discussion

This research provides the first molecular characterization of the aphid pest complex on cruciferous crops in Chiang Mai Province, revealing three species with evidence of cryptic lineage divergence within *M. persicae*. The detection of two genetically distinct *M. persicae*

subclades is consistent with host race differentiation documented globally in this species [4, 6]. The 2.4% COI divergence between subclades approaches species delimitation thresholds applied in some aphid taxa [1, 3]. The specialist species *B. brassicae* and *L. erysimi* exhibited lower intraspecific divergence, consistent with their restricted host ranges. Interspecific distances (12.3-16.8%) confirm unambiguous molecular separation [2, 7]. The practical implications extend to resistance management, as tobacco-adapted *M. persicae* populations carry distinct resistance allele profiles [4].

Virus transmission implications

The cryptic lineage structure within *M. persicae* has relevance for understanding differential virus transmission.

M. persicae transmits over 100 plant viruses, and host race identity may influence vector competence for specific viral pathogens affecting cruciferous crops in northern Thailand [3, 6].

Methodological refinements

The 90.2% sequencing success rate, while adequate, was lower than rates achieved in other insect groups in this research programme. Aphid specimens are prone to DNA degradation due to their soft body structure. Future collections should prioritize immediate ethanol preservation and cold chain maintenance to improve sequencing yields [8, 10].

Conclusion

Aphid pests of cruciferous crops in Chiang Mai Province represent a constant threat to highland vegetable production, yet molecular characterization of this pest complex has been lacking until the present research.

Three species were identified through COI barcoding, with the notable finding of cryptic lineage divergence within *M. persicae* corresponding to putative host-adapted races. Clear interspecific distances confirmed reliable species boundaries for all three taxa.

It is recommended that pest monitoring programmes in northern Thailand incorporate molecular diagnostics to distinguish *M. persicae* host races, enabling targeted resistance management and virus transmission risk assessment.

Future research should employ multi-locus approaches and whole-genome sequencing to fully resolve the extent of reproductive isolation between *M. persicae* host races in Thai cruciferous production systems and determine whether host race identity influences vector competence for regionally important plant viruses.

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