J. Trop. Plant Pests Dis.
Vol. 26, No. 1, March 2026
Pages: 216–225

ISSN: 1411-7525
E-ISSN: 2461-0399
DOI : 10.23960/j.hptt.126216-225

SHORT COMMUNICATION

First Report of *Atherigona orientalis* (Diptera: Muscidae) infesting C*apsicum annuum* in West Sumatra, Indonesia confirmed by COI barcoding

Nguyễn Phước Sang[1], Novri Nelly[2], Reflinaldon[2], & Hidrayani[2]

Manuscript received: 3 September 2025. Revision accepted: 11 December 2025. Available online: 13 February 2026.

ABSTRACT

Accurate identification of pest species is fundamental to the development of effective integrated pest management (IPM) strategies. This study presents the first molecular identification of the dominant fruit fly species infesting chili (*Capsicum annuum* L.) in West Sumatra, Indonesia, using DNA barcoding of the mitochondrial cytochrome c oxidase subunit I (*COI*) gene. Adult specimens were collected between October 2024 and March 2025, and *COI*-specific primers were used to amplify the genomic DNA extracted from adult tissues. The resulting ~685 bp sequences showed 99.0–99.4% similarity and 96–100% query coverage with reference sequences of *Atherigona orientalis* (e.g., accession codes PQ483146.1, PQ483144.1, EU627707.1) based on BLASTn analysis. Phylogenetic analysis using the Neighbor-Joining method further confirmed species-level identification by clustering the specimens within the *A. orientalis* clade with strong bootstrap support. This study provides the first molecular evidence of *A. orientalis* infestation in chili crops in West Sumatra. The findings offer new insights into the pest status of *A. orientalis* within chili agroecosystems and emphasize the need for targeted pest management strategies. Moreover, these results establish a valuable baseline for future studies on the host range, dispersal patterns, and seasonal dynamics of this emerging pest to support more effective mitigation planning.

Keywords: *Atherigona orientalis, Capsicum annuum* L., DNA barcoding, molecular identification

INTRODUCTION

In recent decades, the production of chili peppers (*Capsicum* spp.), which play a vital role in both global cuisines and agricultural economies, has increased dramatically (Simamora et al., 2021; Aprilia et al., 2023). Worldwide chili production has risen from approximately 5.47 million tonnes in 1970 to 36.97 million tonnes in 2022. Currently, cultivation covers about 2,020,816 ha, yielding an average productivity of 1.83 kg/m^2 (FAOSTAT, 2024a). Indonesia is among the world's leading chili-producing countries. In 2018, it ranked fourth globally with over 2.5 million tonnes of production, increasing to third position by 2022 with nearly 3 million tonnes, following China and Mexico (Sundari et al., 2021; Liu, 2022; FAOSTAT, 2024b). Within Indonesia, West Sumatra is the fifth-largest producer of red chili (Khairat et al., 2024). Among its 19 districts and cities, Agam Regency produced 42,002 tonnes of chili in 2023, followed by Solok (41,168 tonnes), Tanah Datar (22,261 tonnes), and Padang Pariaman (2,006 tonnes), collectively contributing about 50% of the province's total chili output (BPS Statistics Indonesia Sumatera Barat, 2024).

Despite this high production potential, chili cultivation faces serious threats from dipteran pests, particularly fruit-infesting species belonging to the families Tephritidae and Muscidae, which frequently cause significant yield losses. Historically, species of *Bactrocera* have been regarded as the primary culprits (Budiyanti et al., 2019; Hidayat et al., 2023). However, recent reports worldwide have highlighted increasing damage associated with a previously underrecognized species, *Atherigona orientalis* (Schiner, 1868), commonly known as the tomato or pepper fruit fly. This small, highly polyphagous species, often considered saprophagous and associated with decaying organic matter, has demonstrated an ability to infest healthy fruits and even prey upon the larvae of other fruit fly species such as *Dacus* spp. and *Bactrocera* spp. Alarming infestations have been documented in several countries, including Nigeria (Ogbalu & Ebere, 2005), Indonesia (Herawani et al., 2019), Greece (Roditakis et al., 2023), and China (Zhou et al., 2025), where *A.*

Corresponding author:
Nguyễn Phước Sang (npsangdh15bt@gmail.com)

[1]Doctoral Program in Agricultural Science, Faculty of Agriculture, Andalas University, Jl. Dr. Mohammad Hatta, Limau Manis campus in Padang-West Sumatra, Indonesia 25163

[2]Department of Plant Protection, Faculty of Agriculture, Andalas University, Jl. Dr. Mohammad Hatta, Limau Manis campus in Padang-West Sumatra, Indonesia 25163

orientalis has emerged as a serious pest of economically important crops such as pepper. In *Capsicum annuum*, fruit loss during the wet season has been reported to reach up to 73.7% (Ogbalu & Ebere, 2005; Hibbard & Overholt, 2012; Ogbalu, 2014; Herawani et al., 2019; Roditakis et al., 2023). The larvae penetrate fruits at all developmental stages and feed on the ovules, seeds, placenta, and mesocarp, leading to premature fruit drop and increased susceptibility to secondary infections by rot-causing microorganisms (Iheagwam & Nwankiti, 1981; Grzywacz & Pape, 2014; Suh & Kwon, 2016; EPPO, 2025).

Accurate, timely, and reliable species-level identification of fruit flies is essential for implementing appropriate quarantine and management measures. Although morphological identification remains a fundamental approach, it has notable limitations, particularly in distinguishing cryptic species or when specimens are damaged or immature (White & Elsonharris, 1992). Consequently, several molecular methods have been developed for fruit fly identification, including loop-mediated isothermal amplification (LAMP), real-time polymerase chain reaction (PCR), DNA barcoding, restriction fragment length polymorphism (RFLP), and microfluidic dynamic array techniques (Dhami et al., 2016). Among these, DNA barcoding has become the most widely used method because it can differentiate most species with high precision (Krosch et al., 2020). The mitochondrial cytochrome c oxidase subunit I (*COI*) gene has proven particularly effective due to its universality, variability, and compatibility with global reference databases, offering high interspecific resolution and robust phylogenetic clustering (Hebert et al., 2003; Armstrong & Ball, 2005; Fuentes-López et al., 2020). Additionally, this method provides rapid and reproducible species-level identification that supports quarantine measures, IPM programs, and pest diagnostics systems (Castellanos et al., 2025). Regettably, certain challenges persist, such as the potential amplification of nuclear mitochondrial pseudogenes (numts), reliance on a single locus, and difficulties in resolving species complexes with overlapping intra- and interspecific variation (He et al., 2024). To enhance accuracy, recent recommendations emphasize the use of phylogenetic validation, multilocus approaches, and the continued expansion of curated barcode databases such as GenBank and BOLD (Cheng et al., 2023). Despite these limitations, *COI*-based DNA barcoding remains a fast, affordable, and reliable method for molecular identification, supporting ecological monitoring, biodiversity research, and the development of targeted pest management strategies across diverse agroecosystems (Blacket et al., 2012; Doorenweerd et al., 2020).

Collectively, although Indonesia contributes substantially to global chili production, region-specific entomological threats remain poorly documented. In West Sumatra, *A. orientalis* is widely distributed but is generally regarded as a saprophagous species rather than a direct pest. Consequently, many instances of fruit damage have been broadly attributed to "fruit flies" without species-level verification. Additionally, no official molecular confirmation of this species as a chili pest in the region has been reported. This study, therefore, aims to address this critical knowledge gap by providing the first molecular evidence confirming *A. orientalis* as a major fruit fly pest infesting chili (*C. annuum* L.) in West Sumatra. Through detailed genetic identification and phylogenetic analysis, this research enhances taxonomic clarity, deepens, understanding of regional pest dynamics, and lays the foundation for the development of targeted pest management strategies within West Sumatra's chili agroecosystems.

MATERIALS AND METHODS

Research Site. This study was conducted in major chili-producing areas of West Sumatra Province, Indonesia, including Agam, Tanah Datar, Solok, and Padang Pariaman. These locations were selected because they represent the primary centers of red chili cultivation in the province and encompass contrasting altitudinal ranges and agroecological conditions, which are expected to influence fruit fly abundance and infestation patterns.

Sample Collection and Morphological Pre-identification. Adult fruit fly specimens infesting chili were collected from damaged chili fruits between October 2024 and March 2025 in the major chili-producing regions of Agam (0°17′S, 100°09′E), Tanah Datar (0°27′S, 100°35′E), Solok (0°47′S, 100°39′E), and Padang Pariaman (0°37′S, 100°13′E), in West Sumatra, Indonesia. Sampling was conducted using two approaches: direct hand-picking of adults emerging from infested fruits and baited traps installed in chili fields.

Infested chili fruits were collected using a rectangular diagonal sampling method, including fruits attached to plants and those fallen on the ground. Fruits selected for rearing and molecular analysis were standardized by sizes and maturity stages (ripe or senescing), regardless of attachment status. In addition,

a modified Lynfield trap was installed at the center of each diagonal-rectangle sampling plot, positioned 1.5 m above ground level using wooden stakes for support. Traps were deployed at 07:00 and retrieved at 17:00. Petrogenol 800 L (methyl eugenol) was used as the attractant, cotton wicks were impregnated with 1.5 mL of attractant to ensure full saturation without dripping.

Collected specimens were immediately preserved in 95% ethanol and transported under cold-chain conditions to the Molecular Biology Laboratory, Andalas University, for further processing. Each sample vial was labeled with the collection location, site code, and sampling date.

DNA Extraction. Genomic DNA was extracted individually from adult specimens using the g-SYNCTM DNA Extraction Kit (Geneaid, GS300), following the manufacturer's protocol. The purity and concentration of extracted DNA were assessed using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). A representative sample (G-3962-1; Table 1) exhibited high-purity nucleic acid with an $A_{260/280}$ ratio of 2.21 and a concentration of 15.6 ng/µL, indicating suitability for downstream molecular analysis (Geneaid, 2017).

PCR Amplification. Amplification of the mitochodrial cytochrome c oxidase subunit I (*COI*) gene was performed using the KOD FX Neo PCR Kit (Toyobo, Japan; KFX-201) (Toyobo, 2004). Approximately 700 bp of the target region was amplified using universal primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′).

Each 25 µL PCR reaction contained 4.5 µL nuclease-free water, 12.5 µL PCR Buffer KOD FX Neo, 5 µL dNTPs, 0.75 µL of each primer (10 µM), 1 µL of template DNA, and 0.5 µL KOD FX Neo polymerase. Thermal cycling conditions followed the manufacturer's protocol and standard DNA barcoding recommendationss (Folmer et al., 1994): initial denaturation at 94 ºC for 2 min, followed by 35 cycles of 98 ºC for 10 s, 50 ºC for 30 s, and a final extension at 72 ºC for 45 s.

Gel Electrophoresis and Visualization. PCR amplification success was verified by electrophoresis on a 1% agarose gel prepared in TBE buffer. Electrophoresis was conducted for 25–30 min at 100 V using a horizontal electrophoresis chamber connected to a DC power supply (Green & Sambrook, 2012). Gel were stained with SYBR Safe and visualized under ultraviolet illumination using a GelDoc imaging system (Bio-Rad, USA), following the manufacturer's instructions (Invitrogen, 2007). Successful amplification was indicated by a single distinct band of approximately 700 bp.

Sanger Sequencing and Sequence Assembly. Purified PCR products were sent to PT Genetika Science Indonesia (Tangerang, Indonesia) for bidirectional Sanger sequencing using the capillary electrophoresis. Forward and reverse sequences were assembled into a 685 bp consensus sequence (sample code: G-3962-1) using BioEdit v7.2 software.

BLAST Analysis. Consensus sequences were analyed using the BLASTn algorithm against the NCBI nucleotide database. Sequences showing ≥98% similarity and ≥95% query coverage with reference sequences were considered conspecific matches, confirming species-level identification.

Phylogenetic Analysis. Phylogenetic relationships were inferred using the Neighbor-Joining (NJ) method with Kimura 2-parameter distances via the NCBI BLAST Tree View tool, following approaches reported in previous studies (Marassi, 2011; Chen et al., 2024). Clade robustness was evaluated using 1000 replicates. Reference sequences of *Atherigona orientalis* and closely related Muscidae species were included to validate clustering patterns and confirm species identity.

RESULTS AND DISCUSSION

Morphological Diagnosis of *Atherigona orientalis*. Male and female *Atherigona orientalis* (Figure 1) can be readily distinguished based on a combination of external morphological characters and terminalia. Males are small muscid flies with a grayish-yellow body, darker dorsal markings, and hyaline, elongate wings. The head bears large, closely set reddish compound eyes, a narrow frons, and short, three-segmented antennae with a plumose arista (Folmer et al., 1994; Marassi, 2011; Chen et al., 2024). The thorax

Table 1. DNA quantification results measured using a NanoDrop 2000 spectrophotometer

Name Sample	Code sample	Concentration (ng/µL)	$A_{260/230}$	$A_{260/280}$	Volume (µL)
Chili's Fruit Fly	G-3962-1	15.6	2.21	3.08	40

is gray-brown with faint longitudinal striping, and the yellowish-brown scutellum is armed with strong setae. The slender yellowish-brown legs carry fine bristles, some of which are sexually dimorphic and associated with mating behavior (Green & Sambrook, 2012; CABI, 2021). The abdomen is relatively elongate and posteriorly tapering, terminating in a prominent hypopygium, in which the structure of the surstyli and cerci provides key diagnostic characters for species-level identification (Grzywacz & Pape, 2014; Geneaid, 2017).

Females are generally similar to males in overall coloration and wing morphology; however, they possess a broader frons and more widely separated reddish compound eyes (Roditakis et al., 2023; Chen et al., 2024). The antennae are likewise three-segmented with a plumose arista and show no conspicuous sexual modifications, while the thorax and scutellum retain the gray-brown ground color, faint longitudinal stripes, and well-developed setae characteristic of the species (Marassi, 2011). As in males, the wings are hyaline and elongate with the characteristic *Atherigona* venation, including a distinct R1 vein (Folmer et al., 1994). Female legs are slender and yellowish-brown but lack the specialized courtship-related bristles found in males, and the abdomen is stouter and less abruptly tapering, ending in a well-developed ovipositor adapted for inserting eggs into soft plant tissues. he configuration of the ovipositor and associated sclerites is particularly informative for discriminating females of *A. orientalis* from closely related congeners (Grzywacz & Pape, 2014; Chen et al., 2024; EPPO, 2025).

DNA Extraction, Amplification, and Sequencing. High-quality genomic DNA was successfully extracted from adult fruit fly specimens. The representative sample (G-3962-1) yielded DNA concentration and purity values within acceptable ranges for molecular analyses, with a concentration of 15.6 ng/μL, an $A_{260/280}$ ratio of 2.21, and an $A_{260/230}$ ratio of 3.08, indicating low levels of protein and solvent contamination. These values are consistent with standards recommended for high-fidelity PCR amplification and sequencing workflows (Green & Sambrook, 2012).

The mitochondrial *COI* gene was reliably amplified, producing an approximately 700 bp fragment (Figure 2), which corresponds to the commonly used DNA barcode region for insects

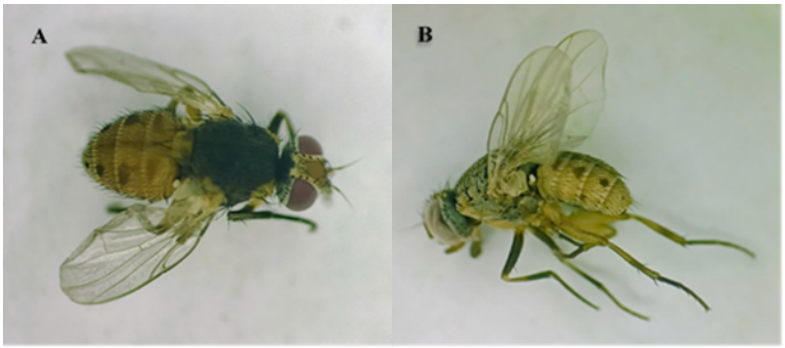

Figure 1. Adult pepper fruit fly *Atherigona orientalis* (Diptera: Muscidae). A. Male; B. Female.

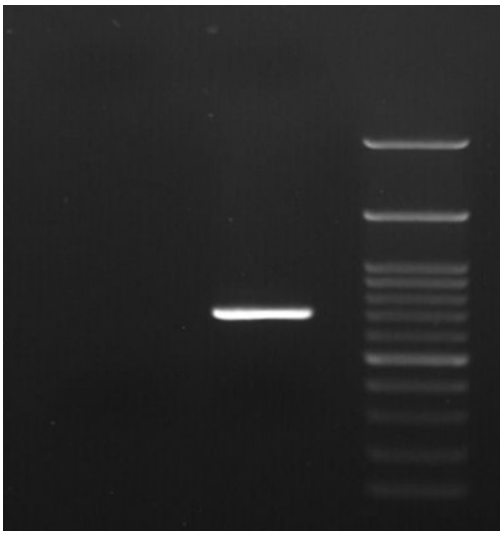

Figure 2. PCR amplification of pepper fruit fly *COI* primers from specimens collected in West Sumatra.

(Folmer et al., 1994; Hebert et al., 2003). The absence of amplification in negative controls further confirms that laboratory procedures effectively prevented cross-contamination, an essential requirement for accurate species authentication in Diptera (Collins & Cruickshank, 2013).

Clean, bidirectional chromatograms enabled the assembly of a high-quality 685 bp consensus sequence (Figure 3), exceeding the minimum threshold recommended for reliable species-level identification and phylogenetic analysis (Hebert & Gregory, 2005; Ratnasingham & Hebert, 2007). Similar sequencing success has been reported in studies identifying *Atherigona* spp. and other Muscidae using mitochondrial markers (Singh & Achint, 2017), indicating that the protocol employed in this study is robust and reproducible. The resulting *COI* sequence will be submitted to GenBank, contributing to the global molecular reference library for Muscidae and supporting future taxonomic, ecological, and biosecurity research.

BLAST Analysis and Species Confirmation. BLASTn analysis of the consensus *COI* sequence against the NCBI nucleotide database produced E-values of 0.0, indicating extremely high-confidence homologous matches (Figure 4). The query sequence shared 99.0–99.4% identity and 96–100% query coverage with multiple authenticated *Atherigona orientalis* accessions, including PQ483146.1, PQ483144.1, and EU627707.1 (Table 2). These levels of similarity fall well within the accepted thresholds for species-level

```
1       TAAAGATATT GGAACCCTTT ATTTTATTTT CGGAGCTTGA TCTGGAATAG TAGGAACTTC
61      TTTAAGTATT TTAATTCGAG CAGAATTAGG TCATCCAGGA GCTTTAATTG GAGATGATCA
121     AATTTATAAC GTAATTGTTA CTGCTCATGC TTTTATTATA ATTTTTTTTA TAGTAATACC
181     AATTATAATT GGAGGATTTG GAAATTGATT AGTGCCTTTA ATATTAGGAG CTCCTGATAT
241     AGCCTTTCCT CGAATAAATA ATATAAGATT TTGACTTCTT CCCCCTGCTT TAACACTTTT
301     ATTAGTTAGT AGTATAGTTG AAAACGGAGC TGGGACTGGA TGAACAGTTT ACCCACCTTT
361     ATCTTCTAAT ATTGCTCATG GAGGAGCTTC AGTAGATTTA GCAATTTTTT CTTTACATTT
421     AGCTGGAATT TCTTCAATTT TAGGGGCAGT AAATTTTATT ACAACAGTTA TTAATATACG
481     ATCAACAGGA ATTACATTTG ATCGAATGCC TTTATTTGTA TGATCAGTAG TAATTACTGC
541     CTTATTACTT TTATTATCTT TACCAGTATT AGCAGGAGCT ATTACTATAT TACTAACAGA
601     TCGAAATTTA AATACTTCAT TTTTTGATCC AGCTGGTGGT GGAGATCCTA TTCTTTATCA
661     ACATTTATTT TGATTTATTT GGTCA
```

Figure 3. Sequence assembly results of the amplified *COI* fragment (685 bp).

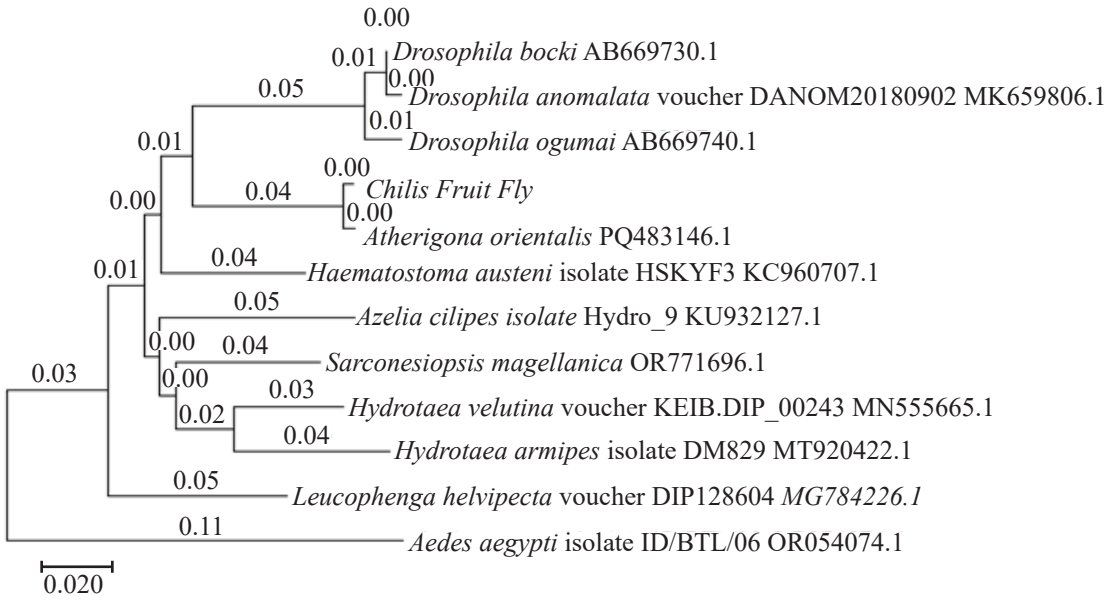

Figure 4. Neighbor-Joining phylogenetic tree based on mitochondrial COI sequences showing the position of the pepper fruit fly *Atherigona orientalis* (specimen G 3962 1, "chili fruit fly") relative to selected Diptera reference sequences from NCBI. The tree was inferred using the Kimura 2-parameter model with 1000 bootstrap replicates. Numbers at the nodes indicate bootstrap support values, and the scale bar represents 0.02 substitutions per site.

Table 2. Summary of BLASTn database matches of *COI* sequences

No.	Species name	Accession number	Query coverage (%)	Identity (%)	E-value
1	*Atherigona orientalis*	PQ483146.1	99	99.41	0.0
2	*Atherigona orientalis*	PQ483144.1	99	99.41	0.0
3	*Atherigona orientalis*	EU627707.1	100	99.12	0.0

resolution in insects using *COI* barcoding, which typically requires >97–98% identity for reliable identification (Hebert et al., 2003; Ratnasingham & Hebert, 2007). Comparable high-identity matches have been reported in previous molecular studies on Muscidae, supporting the diagnostic robustness of *COI* for distinguishing *A. orientalis* from congeners and other sympatric dipteran pests (Chan et al., 2014; Singh & Achint, 2017). The present results therefore confirm that *COI* barcoding remains a highly dependable tool for rapid and accurate species confirmation in operational pest management and biosecurity programs.

Phylogenetic reconstruction using the Neighbor-Joining method further validated the BLAST-based identification, with the G-3962-1 sequence clustering tightly within the *A. orientalis* clade and receiving strong bootstrap support (>98%). This pattern of tight clustering is consistent with the low intraspecific *COI* divergence commonly reported for *A. orientalis* populations across Asia and Africa (Achint & Singh, 2021). The phylogenetic placement also indicated geographic independence, suggesting minimal regional structuring and reinforcing the species' genetic stability across its distribution. Collectively, the combination of high BLAST identity, strong phylogenetic support, and minimal intra-clade divergence confirms the precise molecular identification of *A. orientalis* and aligns with the broader literature emphasizing the reliability of *COI*-based diagnostics in Muscidae taxonomy and pest surveillance (Collins & Cruickshank, 2013).

Relevance in Pest Dynamics and Regional Context. This study provides the first molecular evidence confirming *A. orientalis* infestation in chili (*C. annuum*) crops in West Sumatra, Indonesia, which represents an important finding for regional agricultural entomology. In Bogor, Java, *A. orientalis* has been recorded emerging from chili fruits, occasionally at higher frequencies than the common tephritid pest *Bactrocera dorsalis*, suggesting its potential role as a primary pest in that region (Herawani et al., 2018). Globally, *A. orientalis* is recognized as a pantropical species commonly associated with decaying organic matter; however, it has become a significant pest of tomatoes and peppers in regions such as Egypt, India, Nigeria, and Iraq (Jackson, 2022).

Of particular note, *A. orientalis* has been observed infesting pepper fruits independently, without the presence of tephritid larvae, as reported in greenhouse pepper production systems in Crete, Greece (Roditakis et al., 2023). This behavior underscores the species' capacity to function as a stand-alone pest rather than merely a secondary invader. The findings from West Sumatra therefore align with global trends, indicating a possible behavioral shift in *A. orientalis* from a saprophagous habit to an active phytophagous pest exploiting healthy chili fruits.

Implications for Integrated Pest Management (IPM). The molecular confirmation of *A. orientalis* (Figure 4) as a chili pest has critical implications for IPM programs in Indonesia. Current IPM strategies for chili cultivation—such as mass trapping, protein bait sprays, and cultural control practices—are primarily designed to target tephritid fruit flies. However, *A. orientalis* may respond differently to these attractants and control methods. For instance, calyx morphology has been shown to influence oviposition behavior; pepper varieties with open or grooved calyces tend to be more susceptible to infestation (EPPO, 2024).

In regions like Greece, the emergence of *A. orientalis* as a new pest has necessitated modifications to existing IPM protocols, as conventional measures targeting other insect groups (Roditakis et al., 2023). Similar adjustments may be required in Indonesia to manage this species effectively. Additionally, preventive cultural practices—such as avoiding the use of chicken manure as a breeding substrate—are recommended to minimize population buildup and reduce infestation risk (EPPO, 2024).

CONCLUSION

The study provides the first molecular evidence confirming *Atherigona orientalis* infestation of chili (*Capsicum annuum* L.) in West Sumatra, Indonesia. Species-level identification was validated through *COI* DNA barcoding, with BLAST and phylogenetic analyses showing more than 99% sequence similarity to reference *A. orientalis* sequences. These findings

clarify previous uncertainties regarding the species responsible for fruit fly infestations in the region and highlight the need to revise existing integrated pest management (IPM) strategies, as this muscid fly may not respond effectively to conventional control approaches designed for tephritid fruit flies. Establishing this molecular baseline provides a foundation for future research on the ecology, distribution, and population dynamics of *A. orientalis,* ultimately supporting the development of more targeted and sustainable management strategies to reduce yield losses and ensure the long-term resilience of chili production systems.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Entomology Laboratory, Department of Plant Protection, Faculty of Agriculture, Universitas Andalas, for providing facilities and technical support during the course of this research. The authors also extend their sincere appreciation to the doctoral dissertation assessment committee for their valuable scientific contributions and constructive evaluations that improved the quality of this study. In addition, the authors thank the chili farmers for granting access to their cultivation areas for field sampling.

FUNDING

This research was fully supported by the corresponding author's personal funds. No external financial support, grants, or institutional funding were received for the completion of this study or for article processing charges.

AUTHORS' CONTRIBUTIONS

NPS, NN, R, and H conceptalized the research idea and designed the study. NPS conducted field sampling, performed the literature review, carried out molecular analyses, and performed data analysis and interpretation. NPS also prepared the manuscript and managed submission to the journal.

COMPETING INTEREST

The authors declare that they have no competing interests. All costs associated with this study were borne by the corresponding author.

REFERENCES

Achint R & Singh D. 2021. Application of *COI* gene for identification of some economically and forensically important muscid flies of India (Diptera: Muscidae). *Int. J. Trop. Insect Sci.* 41(4): 3023–3029. https://doi.org/10.1007/s42690-021-00494-8

Aprilia SE, Susilawati, & Hermawan A. 2023. Assesing the impact of pruning and planting media on floating red chili morphology and yield. *Biovalentia.* 9(2): 97–102.

Armstrong KF & Ball SL. 2005. DNA barcodes for biosecurity: Invasive species identification. *Philos. Trans. R. Soc. Lond. B Biol. Sci*. 360(1462): 1813–1823. https://doi.org/10.1098/rstb.2005.1713

BPS-Statistics Indonesia Sumatera Barat. 2024. *Produksi Tanaman Sayuran Menurut Kabupaten/Kota dan Jenis Tanaman di Provinsi Sumatera Barat [Vegetable Crop Production by Regency/City and Crop Type in West Sumatra Province]*. https://sumbar.bps.go.id/id/statistics-table/3/ZUhFd1JtZzJWVVpqWTJsV05XTllhVmhRSzFoNFFUMDkjMw==/produksi-tanaman-sayuran-menurut-kabupaten-kota-dan-jenis-tanaman-di-provinsi-sumatera-barat.html?year=2023. Accessed 15 July 2025.

Blacket MJ, Semeraro L, & Malipatil MB. 2012. Barcoding Queensland fruit flies (*Bactrocera tryoni*): Impediments and improvements. *Mol. Ecol. Resour.* 12(3): 428–436. https://doi.org/10.1111/j.1755-0998.2012.03124.x

Budiyanti S, Hidrayani, & Hamid H. 2019. Species diversity of fruit flies (Diptera: Tepthritidae) and their damage on vegetables in Padang, West Sumatera, Indonesia. *Asian J. Agric. & Biol.* Special Issue: 224–230.

CABI. 2021. *Atherigona orientalis (pepper fruit fly)*. https://doi.org/10.1079/cabicompendium.7731

Castellanos NL, Yan J, Gunawardana DN, Mccarthy B, George S, & Li D. 2025. DNA barcodes for fruit fly species from Pacific Islands and development of multiplex real-time PCR assay for *Bactrocera facialis*, *B. passiflorae*, *B. kirki* and *B. distincta*. *Appl. Sci.* 15(16): 8889. https://doi.org/10.3390/app15168889

Chan A, Chiang LP, Hapuarachchi HC, Tan CH, Pang SC, Lee R, Lee KS, Ng LC, & Lam-Phua SG. 2014. DNA barcoding: Complementing morphological identification of mosquito species in Singapore. *Parasites Vectors*. 7(1): 569. https://doi.org/10.1186/s13071-014-0569-4

Chen HP, Chan FT, Shiao SF, & Chiu MC. 2024. New record of Carnidae (Diptera) from Taiwan and potential challenges in DNA barcode amplification due to pseudogene. *Biodivers. Data J.* 12: e137532. https://doi.org/10.3897/BDJ.12.e137532

Cheng Z, Li Q, Deng J, Liu Q, & Huang X. 2023. The devil is in the details: Problems in DNA barcoding practices indicated by systematic evaluation of insect barcodes. *Front. Ecol. Evol.* 11: 1149839. https://doi.org/10.3389/fevo.2023.1149839

Collins RA & Cruickshank RH. 2013. The seven deadly sins of DNA barcoding. *Mol. Ecol. Resour.* 13(6): 969–975. https://doi.org/10.1111/1755-0998.12046

Dhami MK, Gunawardana DN, Voice D, & Kumarasinghe L. 2016. A real-time PCR toolbox for accurate identification of invasive fruit fly species. *J. Appl. Entomol.* 140(7): 536–552. https://doi.org/10.1111/jen.12286

Doorenweerd C, Jose MS, Leblanc L, Barr N, Geib S, Chung AYC, Dupuis J, Ekayanti A, Fiegalan ER, Hemachandra KS, Hossain MA, Huang CL, Hsu YF, Morris KY, Mustapeng AM, Niogret J, Pham T H, Sirisena UGAI, & Rubinoff D. 2020. DNA barcodes and reliable molecular identifications in a diverse group of invasive pests: Lessons from Bactrocera fruit flies on variation across the *COI* gene, introgression, and standardization. *BioRxiv (Peer review)*. https://doi.org/10.1101/2020.11.23.394510

EPPO. 2024. *EPPO Alert List – Atherigona orientalis (Diptera: Muscidae)*. https://www.eppo.int/ACTIVITIES/plant_quarantine/alert_list_insects/atherigona_orientalis. Accessed 15 July 2025.

EPPO. 2025. *Atherigona orientalis.* https://gd.eppo.int/taxon/ATHEOR/distribution. Accessed 15 July 2025

FAOSTAT. 2024a. *Chillies and peppers, dry (Capsicum spp., Pimenta spp.), production in the world.* https://data.un.org/Data.aspx?d=FAO&f=itemCode%3A689. Accessed 15 July 2025.

FAOSTAT. 2024b. *Chilies and peppers, dry (Capsicum spp., Pimenta spp.), raw. Crops and livestock products*. Food and Agriculture Organization. Elsevier. Rome, Italy. Accessed 10 July 2025.

Folmer O, Black M, Hoeh W, Lutz R, & Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3(5): 294–299. https://pubmed.ncbi.nlm.nih.gov/7881515/

Fuentes-López A, Ruiz C, Galián J, & Romera E. 2020. Molecular identification of forensically important fly species in Spain using *COI* barcodes. *Sci. Justice.* 60(3): 293–302. https://doi.org/10.1016/j.scijus.2019.12.003

Geneaid. 2017. *gSYNCTM DNA Extraction Kit*. http://www.geneaid.com/sites/default/files/GS15_0.pdf. Accessed 10 August 2025.

Green MR & Sambrook J. 2012. *Molecular cloning: A laboratory manual (4th ed.)*. https://rnajournal.cshlp.org/content/18/7/local/advertising.pdf. Accessed 20 August 2025.

Grzywacz A & Pape T. 2014. Larval morphology of *Atherigona orientalis* (Schiner) (Diptera: Muscidae) —A species of sanitary and forensic importance. *Acta Trop.* 137: 174–184. https://doi.org/10.1016/j.actatropica.2014.05.018

He R, Wang S, Li Q, Wang Z, Mei Y, & Li F. 2024. Phylogenomic analysis and molecular identification of true fruit flies. *Front. Genet.* 15: 1414074. https://doi.org/10.3389/fgene.2024.1414074

Hebert PDN, Cywinska A, Ball SL, & DeWaard JR. 2003. Biological identifications through DNA barcodes. *Proc. Biol. Sci.* 270(1512): 313–321. https://doi.org/10.1098/rspb.2002.2218

Hebert PDN & Gregory TR. 2005. The promise of DNA barcoding for taxonomy. *Syst. Biol*. 54(5): 852–859. https://doi.org/10.1080/10635150500354886

Herawani F, Rauf A, & Santoso S. 2018. Status Serangan dan Biologi Lalat *Atherigona orientalis* (Schiner) (Diptera: Muscidae) pada Buah Cabai [Attack Status and Biology of the

Fly *Atherigona orientalis* (Schiner) (Diptera: Muscidae) on Chili Pepper Fruit]. *Thesis*. IPB University. Bogor. http://repository.ipb.ac.id/handle/123456789/95040

Herawani F, Rauf A, & Santoso S. 2019. Status of infestation and biology of pepper fruit fly, *Atherigona Orientalis* (Schiner) (Diptera: Muscidae). *J. Trop. Plant Pests Dis.* 19(1): 64–73. https://doi.org/10.23960/j.hptt.11964-73

Hibbard KL & Overholt WA. 2012. Pepper fruit fly *Atherigona orientalis* (Schiner) (Insecta: Diptera: Muscidae): EENY-539 IN948, 9 2012. *Edis.* 2012(10): 2–5. https://doi.org/10.32473/edis-in948-2012

Hidayat, Adilah NB, Maryana N, & Suputa. 2023. Review of species, host plants, and distribution of fruit flies (Diptera: Tephritidae) in Indonesia. *IOP Conf. Ser.: Earth Environ. Sci.* 1208(1): 012018. https://doi.org/10.1088/1755-1315/1208/1/012018

Iheagwam EU & Nwankiti OC. 1981. Dipterous insect pests of pepper, *Capsicum* spp., in the eastern states of Nigeria. *Revue de Zoologie Africaine*. 94(4): 936–939. https://www.cabidigitallibrary.org/doi/full/10.5555/19810583781

Invitrogen. 2007. *SYBR Safe DNA Gel Stain: User Manual. Invitrogen, Thermo Fisher Scientific.* https://documents.thermofisher.com/TFS-Assets/LSG/manuals/sybr_safe_dna_gel_stain_man.pdf. Accessed 15 August 2025.

Jackson G. 2022. *Pepper Fruit Fly.* https://apps.lucidcentral.org/pppw_v12/text/web_mini/entities/pepper_fruit_fly_416.htm. Accessed 20 July 2025.

Khairat R, Hakimi R, Syahni R, Syarfi IW, & Andalas U. 2024. Farming analysis of red chili in Solok regency. *Jurnal Pertanian Agros.* 26(1): 4920–4928.

Krosch MN, Strutt F, Blacket MJ, Batovska J, Starkie M, Clarke AR, Cameron SL, & Schutze MK. 2020. Development of internal *COI* primers to improve and extend barcoding of fruit flies (Diptera: Tephritidae: Dacini). *Insect Sci.* 27(1): 143–158. https://doi.org/10.1111/1744-7917.12612

Liu Z. 2022. *The Green Development of Special Agricultural Product Chili in China.* https://openknowledge.fao.org/server/api/core/bitstreams/42a2fede-db3d-4f6f-9822-b7661f5401f7/content. Accessed 30 July 2025.

Marassi FM. 2011. *Mycobacterium tuberculosis* Rv0899 defines a family of membrane proteins widespread in nitrogen-fixing bacteria. *Proteins*. 79(10): 2946–2955. https://doi.org/10.1002/prot.23151

Ogbalu OK & Ebere N. 2005. Oviposition deterrent effect of extracts of neem against *Atherigona orientalis* (Schiner) on pepper (*Capsicum annum*) and tomato (*Lycopersicon esculentum*) fruits in Nigeria. *Indian J. Agric. Res.* 39(1): 18–24.

Ogbalu OK. 2014. Assessment of pepper fruits loss in backyard gardens by *Atherigona orientalis* [Schiner] in the Niger Delta. *Niger Delta Biol.* 13(1): 20–29.

Ratnasingham S & Hebert PDN. 2007. Bold : The barcode of life data system. *Mol. Ecol. Notes.* 7(3): 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Roditakis E, Kremi K, Mylona K, Georgousis V, Avtzis DN, & Simoglou KB. 2023. First report of the pepper fruit fly *Atherigona orientalis* (Schiner 1968) (Diptera: Muscidae) infesting commercial pepper crops in Greece. *Insects.* 14(4): 393. https://doi.org/10.3390/insects14040393

Singh D & Achint R. 2017. Molecular identification of some Indian Muscid flies (Diptera: Muscidae) based on mitochondrial gene *COII*. *BioRxiv (Peer review)*. https://doi.org/10.1101/208314

Simamora BSM, Zulfatri, & Armani. 2021. Aplikasi biochar sekam padi dan bakteri pelarut fosfat *Paenibacillus* sp. pada tanaman cabai merah (*Capsicum annum* L) di medium ultisol [The application of rice husk and phosphate solubilizing bacteria *Paenibacillus* sp. on the red chili plants (*Capsicum annum* L) in Ultisol Medium. *Jurnal Dinamika Pertanian*. XXXVII(3): 209–218. https://doi.org/10.25299/dp.2021.vol37(3).8929

Suh SJ & Kwon YJ. 2016. First finding of a quarantine pest, *Atherigona* (Acritochaeta) orientalis Schiner (Diptera: Muscidae), in Korea. *Entomol. Res.* 46(3): 185–189. https://doi.org/10.1111/1748-5967.12161

Sundari MT, Darsono, Sutrisno J, & Antriyandarti E. 2021. Analysis of chili farming in Indonesia.

IOP Conf. Ser.: Earth Environ. Sci. 905: 012046. https://doi.org/10.1088/1755-1315/905/1/012046

Toyobo. 2004. *KOD FX Neo*. https://www.toyobo-global.com/sites/default/static_root/products/lifescience/support/manual/KFX-201.pdf. Accessed 15 August 2025.

White IM & Elsonharris MM. 1992. *Fruit Flies of Economic Significance: Their Identification and Bionomics*. CAB International, Wallingford, UK.

Zhou Z, Luo Y, Qin J, Wang X, Ning S, He J, & Zhou Q. 2025. Occurrence, biological characteristics, and annual dynamics of *Atherigona orientalis* (Schiner 1968) (Diptera : Muscidae) in China. *Insects.* 16(9): 931. https://doi.org/10.3390/insects16090931