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Scientific Note

Molecular confirmation of the presence of the South American tomato leafminer, *Phthorimaea absoluta* Meyrick, 1917 (Lepidoptera: Gelechiidae) in Lao PDR

Viengvilay Vorlachith[1], Sengphet Phanthavong[1], Somkhit Sengsay[2], Salah Hafidi[3], Hang Thi Dao[4], Chanthason Vongtharone[1], Bouasa Xaisongkham[1], Kitan Sitthisay[1], George A. C. Beattie[5], Paul Holford[5], Tara Garrard[6], Sally Cowan[7], Lester W. Burgess[8]

[1]Provincial Agriculture and Forestry Office, Pakse, Champasak, Lao PDR. [2]Ministry of Agriculture and Forestry, Vientiane, Lao PDR. [3]Sevea Consulting, Khan Chamkarmorn, Phnom Penh, Cambodia. [4]Plant Protection Research Institute, Ha Noi, Viet Nam. [5]Western Sydney University, Penrith, NSW, Australia. [6]South Australian Research and Development Institute, Urrbrae, SA, Australia. [7]Northern Australian Quarantine Strategy, Cairns, QLD, Australia. [8]University of Sydney, Sydney, NSW, Australia.
Corresponding author: daothihang@hotmail.com

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Abstract. Molecular confirmation of the presence of the tomato leafminer, *Phthorimaea* (*Tuta*) *absoluta* Meyrick, 1917 (Lepidoptera: Gelechiidae), in the Paksong region of the Bolaven Plateau in Champasak province in south-western Lao PDR is reported. The moth, which is native to South America, has invaded many countries in Europe, Africa, and Asia since 2006, and, based on regional records possibly entered Lao PDR between 2014 and 2018. It is a devastating pest of solanaceous crops and threatens tomato production worldwide. Further surveys are required to determine its presence in other regions of Lao PDR and effective management programs must be developed to help farmers minimise losses.

Keywords: Tuta *absoluta*; Morphology, Molecular identification, Tomato, Eggplant.

The South American tomato leafminer (*Phthorimaea* (*Tuta*) *absoluta* Meyrick, 1917 [Lepidoptera: Gelechiidae]), a destructive pest of tomato (*Solanum lycopersicum* L.), potato (*Solanum tuberosum* L.), eggplant (*Solanum melongena* L.), and other solanaceous crops, is native to South America. It damages tomato plants by mining the leaves and boring into apical and flower buds and fruit (Rondon & Gao 2022) can cause crop losses of up to 80-100% (Desneux et al. 2010; Mahlangu et al. 2022) and poses a major threat to tomato production worldwide (Guimapi et al. 2020). Damage to potato, which is less severe than damage caused to tomato, can reduce tuber production. Damage to eggplant is less severe and may not cause significant damage or crop losses (Mahlangu et al. 2022). The leafminer was first recorded as an invasive pest in Europe in Spain in 2006. It was subsequently recorded throughout Southern Europe, in North Africa, and in the Middle East (Desneux et al. 2010; Guillemaud et al. 2015; Rwomushana et al. 2019) and was first recorded in Türkiye in 2009 (Kılıç 2009). It has since been recorded in in Central Asia in Kyrgyzstan (2017) and Tajikistan (2018), in South Asia in India (2014), Bangladesh (2016) and Nepal (2016) (Guimapi et al. 2020), in the Xinjiang Uygur Autonomous Region (2020) at the crossroads of Central and East Asia in China (Zhang et al. 2020), and in Southeast Asia in northern Việt Nam (2019) (Nguyễn & Đào 2019) and in Myanmar (2020) (Yule et al. 2021).

Phthorimaea absoluta was first recorded in Lao PDR near Paksong (15°10'42.60'' N; 106°13'43.68'' E; 1287 m above sea level (ASL)) on the Bolaven Plateau in Champasak Province in the southern region of the country bordering Thailand and Cambodia in December 2018.

We recorded *P. absoluta* on tomato and eggplant crops on farms on the Bolaven Plateau during brief surveys for pests on horticultural crops in February 2023 April 2023. The map of the Bolaven Plateau is shown in Fig 1. The plateau is a 4,500 km^2 region ranging from about 1,000-1,350 m ASL (Delang et al. 2013). Vegetable production has an important role in the livelihoods of the local small-holder farms on the Plateau, both for local consumption and for sale elsewhere in the country, including Vientiane. Damage caused by larvae of the moth was observed on leaves, fruits, and stems of infested plants (Fig. 2 A-D).

To confirm the identity of the moth, we collected infested fruits and leaves of tomatoes and eggplants growing in greenhouses at the Lao-China Farm (15°12'03.96'' N; 106°17'33.72'' E; 1294 m ASL), Hoay Set village, Paksong district, in February 2023 and on tomato plants in nearby fields in April 2023. Adult moths were caught with the aid of an insect aspirator. The infested leaves and fruits were transported to a laboratory at the Provincial Agriculture and Forestry Office (PAFO) in Champasak where larvae dissected from the infested leaves and fruits were preserved in 100% ethanol at -20°C. The discoveries were reported to the Plant Protection Center (PPC) Vientiane. Voucher specimens were deposited at the PPC, the PAFO in Champasak, and the Plant Protection Research Institute (PPRI), Hà Nội, Việt Nam. Specimens were also deposited at the Plant Protection Centre (PPC), Vientiane, Lao PDR, and with the Orange Agricultural Institute, NSW Department of Primary Industries, Orange, NSW, Australia.

The preserved adults and larvae were examined under a Leica WILD M3Z stereomicroscope at PPRI. The adult male and female genitalia were mounted permanently on microscope slides and viewed under a Nikon E100 compound microscope (Nikon, Japan). The morphology of larvae, adult moths, and male and female genitalia (Fig. 2 E-I) conformed to published descriptions (Brambila et al. 2010; Desneux et al. 2010; Toševski et al. 2011; Mutamiswa et al. 2017; Sridhar et al. 2014).

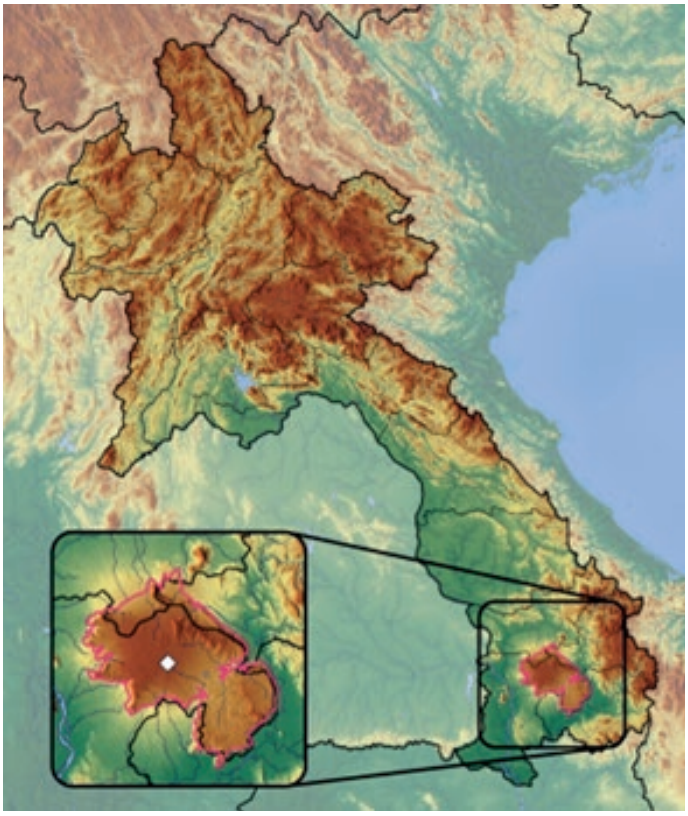

Figure 1. Topographic map of Lao PDR showing location of the Bolaven Plateau in Champasak province bordering Việt Nam to the east, Cambodia to the south, and Thailand to the west. A white diamond added to the map marks the approximate position of the Paksong region where the leafminer has been recorded since 2018. Map: P. Brieux: https://commons.wikimedia.org/wiki/File:Location_of_Bolaven_Plateau_on_Laos_topographic_map.png.

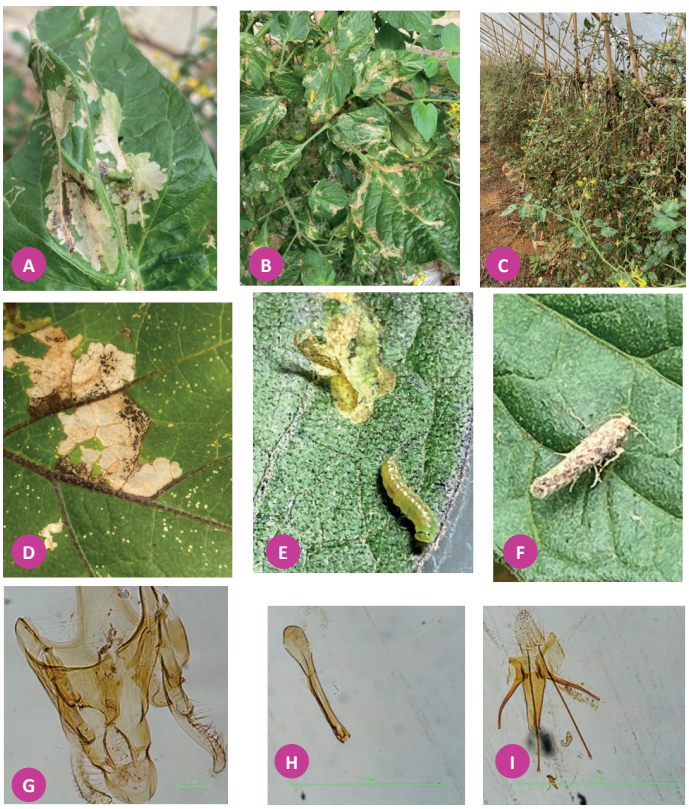

Figure 2. South American tomato leafminer, *Phthorimaea absoluta* Meyrick 1917: (A) & (B) larval feeding damage on tomato leaves, (C) damaged tomato plants, (D) feeding damage on eggplant leaf, (E) larva on tomato leaf, (F) adult, (G) male genitalia, (H) phallus, (I) female genitalia.

One adult moth from tomato plant, two larvae from infested tomato leaves, and one larva from an infested eggplant leaf were used, separately, for DNA extraction, amplification, and sequencing at PPRI. DNA was extracted using a QIAamp DNA Micro Kit (QIAGEN Pty Ltd., Valencia, California). The DNA extraction was conducted following the procedure provided by the manufacturer.

Part of the cytochrome oxidase subunit I (*COI*) gene was amplified using the primer pair: LCO1490 GGTCAACAAATCATAAAGATATTGG and HCO2198 TAAACTTCAGGGTGACCAAAAAATCA (Folmer et al. 1994); the annealing temperature was 50 °C. The amplicons were purified using either a Wizard® Genomic DNA Purification Kit (Promega) or EXoSAP-IT (Exonuclease I [New England Biolabs® Inc., Ipswich, Massachusetts, U.S.A.)-shrimp alkaline phosphatase (Promega)]. Purified amplicons were sequenced by First BASE Laboratories, Taman Serdang Perdana, Selangor, Malaysia. The forward and reverse DNA sequences were assembled, edited, aligned and subjected to phylogenetic analysis as per Dao et al. (2020). The forward and reverse DNA sequences of both genes were assembled, edited and aligned using ChromasPro Version 1.7.1 (2015, Technelysium Pty Ltd, South Brisbane, Australia). Phylogenetic and molecular evolutionary analyses were conducted using MEGA (Version 6, Tamura et al. 2013).

Sequences of the *COI* gene of the three larvae and one adult from which DNA was extracted were successfully amplified. The DNA sequences comprised 685 bp. Alignment of the results and phylogenetic analysis show that the four sequences were identical, and also identical with Genbank accessions of *P. absoluta* from various locations worldwide (Fig. 3).

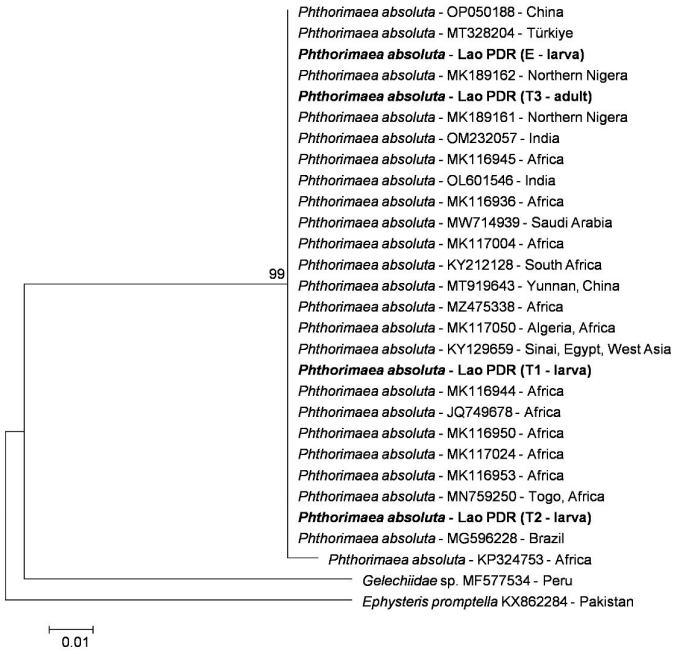

Figure 3. Tree with the highest log likelihood (-1219.4) derived from maximum likelihood analysis of the COI region of accessions (emboldened) of *Phthorimaea absoluta* on tomato (T1 - larva, T2 - larva, T3 - adult) and eggplant (E - larva) in Lao PDR. The Tamura-Nei model (Tamura & Nei 1993) with a discrete gamma distribution was used to model evolutionary rate differences among sites (6 categories (+G, parameter = 0.3172)). The percentages of trees from 1000 bootstrap replications in which the associated taxa clustered together are shown next to the branches. The tree was rooted using accessions of *Ephyteris promptella* (Staudinger 1859) (KX862284) and Gelechiidae sp. (MF577534) [Lepidoptera: Gelechiidae] as the outgroups. The accessions from this study are emboldened.

How and when *P. absoluta* invaded Lao PDR has not been determined, but our records and those from Central, South, East and Southeast Asia since 2017, as detailed in the introduction, suggest it was introduced to Lao PDR before 2018 and after 2014.

Guimapi et al. (2020) attributed incursions and spread of the pinworm in Africa and Asia to international crop trade, movement of people, and poor biosecurity. Farmers and local extension officers have been informed of the presence of *P. absoluta* in Lao PDR and the threat it poses to the vegetable industry on the Bolaven Plateau. Surveys are being undertaken to determine its presence in other regions of the

country. Insecticide application is used to control this pest in Lao PDR, which may face a challenge as *P. absoluta* can develop the resistance to insecticides (Guedes et al. 2019). In our study sites, the commonly used chemical pesticides were cyromazine, abamectin and chlorpyrifos. Management programs are being developed to help farmers minimise losses.

Because this pest has recently invaded the region, limited studies on the control of *P. absoluta* in the neighbouring countries. However, as *P. absoluta* is an important pest globally, its control has been thoroughly studied. The control approaches include resistant varieties, mass trapping, biological control, agronomic and cultural control, and chemical control (Soares et al. 2019; Maluf et al. 2010; Berxolli & Shahini 2017; Desneux et al. 2022). Biological controls using a wide range of beneficial organisms including predatory mirid (Hemiptera: Miridae), egg parasitoids, entomopathogenic fungi, and bacteria have been documented (Calvo et al. 2012; Desneux et al. 2022). An early release of *Nesidiocoris* (*Cyrtopeltis*) *tenuis* (Reuter 1895) (Heteroptera: Miridae) could provide a good control of *P. absoluta*. on tomato in the United States (Calvo et al. 2012). Therefore, the future strategy for management should be explored in Lao DPR based on the successful control programs in other parts of the world.

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Authors' Contributions

VV: Investigation, resources; SP: Investigation, resources; SS: Investigation; SH: Investigation; HTD: Investigation, Writing - original draft; CV: Investigation; BX: Investigation; KS: Investigation; GACB: Writing - review & editing; PH: Writing - review & editing; TG: Investigation; SC: Investigation, resources; LWB: Supervision.

Conflict of Interest Statement

Authors have no conflict of interests.

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