

Biology and Molecular Characterization of *Leucoptera malifoliella*, the Apple Blotch Leafminer, in India

Rafiya Reyaz

Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir

Twinkle Sinha

Banaras Hindu University

Varun Arya

Banaras Hindu University

Srinivasa N

Banaras Hindu University

Sajad Hussain Mir

drsajadento@skuastkashmir.ac.in

Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir

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Abstract

The apple blotch leafminer, *Leucoptera malifoliella* (Costa) (Lepidoptera: Lyonetiidae), has recently emerged as an important pest of apple (*Malus domestica* Borkh.) in the temperate regions of India. The present study investigated the biology of *L. malifoliella* under controlled laboratory conditions and confirmed its molecular identity using mitochondrial cytochrome c oxidase subunit I (COI) gene sequencing. The species completed its life cycle in 31.49 ± 5.03 days at $25 \pm 2^\circ\text{C}$ and passed through four larval instars. Adult moths were silvery-white with characteristic dark blotches on the forewings, and females exhibited slightly longer longevity than males. Molecular analysis involved amplification and sequencing of a ~ 650 bp fragment of the COI gene. BLAST analysis revealed 99–100% similarity with *L. malifoliella* sequences reported from Europe and Central Asia. Phylogenetic analysis clustered the Indian population firmly within the *L. malifoliella* clade, indicating low intraspecific genetic divergence. The study provides the first integrated account of the biology and molecular characterization of *L. malifoliella* from Indian apple orchards and establishes baseline information essential for future population genetic studies and the development of effective integrated pest management strategies.

INTRODUCTION

Apple (*Malus domestica* Borkh.), a prominent member of Rosaceae family, is one of the most popular and economically vital temperate fruit crops cultivated worldwide. Well recognized for its rich nutritional profile, diverse applications and extended shelf life, apple holds significant commercial value. The domesticated apple is believed to have originated from its wild ancestor *Malus sieversii* with central Asia identified its primary centre of origin (Cornille, 2012). The British had brought apples to India as early as 1865 to the Kullu valley of the Himalayan state of Himachal Pradesh (Javid et al., 2016).

In India, apples are primarily cultivated commercially in Jammu and Kashmir, Himachal Pradesh, and Uttarakhand. The total area under apple cultivation in the country is approximately 313,000 hectares, producing around 2,276,000 metric tons (Anonymous, 2021a). Jammu and Kashmir leads with the highest average yield, contributing over 65 percent of India's total apple production (Shah et al., 2022). Apple cultivation is the cornerstone of Kashmir's fruit industry, accounting for 60 percent of the region's fruit production. The apple industry significantly supports the economy of the Kashmir Valley, contributing about 8 per cent to the Gross State Domestic Product (GSDP) (Islam and Shrivastava, 2017). Among the 20 districts of Jammu and Kashmir, Baramulla ranks first in both cultivation area and production, followed by Shopian, Kulgam, and Anantnag (Altaf et al., 2019). In 2020, the value of apple production in Jammu and Kashmir was approximately Rs.4.23 trillion (Anonymous, 2021b).

In recent years, production of apple in Jammu and Kashmir has been threatened by the invasion of new and destructive pests, among which *Leucoptera malifoliella* (Costa), commonly known as apple blotch leaf miner (ABLM) or the leaf blister moth, a micro lepidopteran pest belonging to family Lyonetiidae has emerged to be a serious concern. The family Lyonetiidae is among the least studied groups of microlepidoptera, comprising about 50 described species worldwide (Baryshnikova, 1996). It was investigated by Buszko (1981) in Poland and in the European part of Soviet Russia by Seskjaeva (1981) that the larvae of Lyonetiids mine beneath the epidermis of leaves and sometimes fruits of the host plants, and some species have considerable economic importance.

Leucoptera malifoliella is recognized as an invasive species and ranks among the most economically significant pests of apple orchards (Baufeld and Freier, 1991). The species is widely distributed across temperate regions of Eurasia, including the whole of Europe, Turkiye, the Caspian region, the Middle East, Central Asia and extending into Northern China (Anonymous, 2022) (Fig. 1a).

Leucoptera malifoliella exhibits a broad host range, primarily within the Rosaceae family, with apple (*M. domestica*) as its preferred host. (Beguinot, 2017). Other major hosts include pear (*Pyrus communis*), quince (*Cydonia oblonga*), sweet cherry (*Prunus avium*) and pistachio (*Pistachia vera*) (Anonymous, 2022). The moth is occasionally found on deciduous forests and ornamental shade trees (Tomov and Trenchev, 2001).

Leucoptera malifoliella causes significant damage during its larval stage, by mining the leaves of apple trees. After hatching, the larvae become solitary miners on the upper leaf surface, consuming the mesophyll tissue while leaving the epidermis intact. Infestation is characterized by blotch-like mines that begin as small whitish patches and gradually enlarge, often developing brown necrotic spots. The larval development takes place within these mines, where frass (excrement) accumulates as dark, circular deposits. The damage reduces the leaf surface area, leading to chlorosis, premature leaf fall and in severe cases, extensive defoliation. Under heavy infestation, yield loss can be substantial (Soltani, 2024).

Leucoptera malifoliella has adapted well to Kashmir conditions and has established itself as the major pest in apple growing regions of Kashmir (Fig. 1b). This study represents the first combined biological and molecular characterization of *L. malifoliella* from Indian apple ecosystems. Therefore, it is imperative to study the biology and development of the pest under Kashmir conditions to design suitable management strategies.

Figure 1a. **Distribution of *L. malifoliella* worldwide as per EPPO Global Database, 2024.**

MATERIALS AND METHODS

Field and laboratory investigations on the biology of *L. malifoliella* were conducted during the growing season in the high-density apple orchards of horticulture block of SKUAST K, Wadura, Sopore. Field was thoroughly examined for infested trees and ten trees were selected randomly. From each tree one branch of 15 cm length was taken with each branch containing upto 20 leaves and each leaf containing at least two pupae. Collected branches were reported to the laboratory within one hour. Then these collected branches were placed individually in the beakers with sucrose solution and were placed inside the rearing cages and were monitored for adult emergence. As soon as the adults started emerging, the branches were replaced with fresh uninfested branches to monitor for egg laying and further development. The adults were collected, examined and dimensions were recorded. The samples were preserved in 70 percent ethanol for morphological and molecular confirmation and sex determination. As soon as the mines started appearing, leaves were continuously monitored for larval development through different instars up to the formation of pupae.

Morphometric study of different life stages of *L. malifoliella* was carried out by taking out ten replicates of each stage i.e. egg, larvae of different sizes, prepupa, pupa and adult for linear measurements. In addition to these parameters, colour, shape, size and duration of each stage was also recorded. The study was conducted during June to October 2025.

Molecular Identification:

DNA extraction, PCR and Sequencing.

DNA was extracted from representative specimens using the DNeasy Blood and Tissue Kit, following the manufacturer's protocol. Samples yielding high-quality DNA were used as template DNA for PCR amplification. The Cytochrome Oxidase I (COI) gene was amplified using the primers LC01490 (5'-GGTCAACAAATCATAAAGAATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') as proposed by Folmer et al. (1994). The PCR reaction mixture and thermocycling conditions were followed as per Arya et al. (2024), except for the annealing temperature,

which was set at 47°C. PCR products were separated on a 2% TAE agarose gel stained with ethidium bromide (0.5 µg/mL), and gel images were captured using a Bio-Rad XR + gel documentation system. Samples showing clear PCR amplification were sequenced in the forward direction at Eurofins Pvt. Ltd., Bengaluru, Karnataka.

Molecular Identification, Sequence Submission and Phylogenetic Analysis.

The sequences obtained after sequencing were trimmed to remove stop codons and primer regions, and were subsequently queried in the National Center for Biotechnology Information- Basic Local Alignment Search Tool (NCBI-BLAST) to determine species identity. Query coverage and percentage similarity were also considered during identification. After confirmation, the sequences were submitted to NCBI, and accession numbers were obtained. To further validate the identification, COI sequences of the same species reported from India and other countries (Finland, Canada, Russia, France, and Spain) were retrieved from NCBI, along with sequences of closely related species belonging to the same genus (*L. spartifoliella* and *L. laburnella*). A Neighbor-Joining (NJ) phylogenetic tree was constructed using the Kimura 2-Parameter (K2P) model with 2,000 bootstrap replications, and *Brachyglossina hispanaria* was used as the outgroup. In addition to this, the pairwise genetic distance among the sequences was calculated using the Kimura-2-parameter model through the MEGA XI program (Chandrashekhara et al., 2024)

RESULTS AND DISCUSSION

Egg

The eggs of *L. malifoliella* are translucent, off-whitish in colour, minute, elliptical and flattened, resembling a water droplet on the leaf surface (Fig. 2a). Eggs were laid singly on the leaf surface preferably on the abaxial surface of the leaf. Morphometric measurements revealed that the length of the egg varied from 0.28 to 0.36mm with mean of 0.32 ± 0.03 mm and the breadth of the egg varied from 0.12 to 0.16 mm with mean of 0.13 ± 0.01 mm. The morphometric details of different life stages are presented in Table 1. The results are in close agreement with the findings of Chang (1985) who reported the egg diameter to be 0.3mm.

Duration of the developmental period from egg laying to the hatching of egg was found to be between 4 to 8 days in the warmer months and that of the later generations when the temperature dropped was found to be between 7 to 12 days.

Larva (first, second, third and fourth instar)

The larvae passed through four instars. The freshly emerged larvae were small and translucent greenish white which turned darker near pupation. Head was dorso-ventrally flattened. The thoracic and abdominal segments were prominently rounded giving a monoliform shape to the larva. Thoracic legs were present in all four instars while abdominal prolegs were found to be absent in last instar. Morphometric measurements showed the length of the larvae ranged between 1.50 mm and 2.4mm with the mean of 1.908 ± 0.29 mm (Fig. 2b). The earlier studies on the larval size by Chang (1985), reported the size of fully grown larva as 4mm. The duration of the larval period through all the instars was found to be 6 to 7 days.

Prepupa & Pupa

In this stage, mature larvae of 4th instar emerged from the mines in search of suitable sites for pupation using silken threads, showed reduced activity, discontinued feeding and remained stationary. Prepupa was dark brown in colour with length ranging between 2.4 to 2.7mm with a mean of 2.56 ± 0.11 mm. The prepupa lasted for about 6 to 9 hours.

Pupae were dark brown to black in colour, contained within the spindle-shaped, flattened silken cocoons. The average length and breadth of the pupa was 1.67 ± 0.11 mm and 0.50 ± 0.01 mm respectively. The average duration of pupal period was found to be 3 to 5 days for warmer generations and 7 to 8 days for later generations. The duration of different developmental stages of *L. malifoliella* is summarized in Table 2. The first generation was found to pupate on leaves mainly while the later generations generally pupate on bark crevices and in fruit calyces. (Fig. 2c).

Adult

The freshly emerged adults were inactive with wings clung to their bodies. A few minutes post eclosion, adults spread their wings and started activity. The adult moth was characterised by an overall shiny metallic grey appearance. The apical area of the wing was slightly orange tinged and contained two white dark edged spots and a pale golden spot with black border. Antennae are long and filiform with number of segments between 28 and 32. Adult males possess a tuft of hair on the vertex. Morphological measurements revealed the average body length to be 2.02 ± 0.09 mm and the wing span averaged at 6.928 ± 0.117 mm (Fig. 2d). The morphometric measurements resonate with the findings of Chang (1985).

The adult possessed a typical Lyonetiid shape with lanceolate fringed outer and posterior margins. At rest, the wing appeared pointed and slightly upturned in lateral view. The hind wings were narrow, lance shaped, grey and fringed, and projected beyond the full length of the wing. Similar findings on the hind wing shape were reported by Chang (1985).

The adults lived upto 7 to 10 days, where females live slightly longer than males.

Mating period

Mating occurred 24 to 60 hours after adult emergence on the leaves and lasted for about 30 to 50 minutes.

Table 1
Morphometrics of different life stages of *L. malifoliella* on apple. Each data point is the mean of 10 observations

Length			Breadth	
Stage	range	Mean	Range	Mean
Egg	0.28 to 0.36mm	0.32 ± 0.03 mm	0.12 to 0.16 mm	0.13 ± 0.01 mm
Larva	1.50 mm to 2.4mm	1.908 ± 0.29	-	
Prepupa	2.4 to 2.7mm	2.56 ± 0.11 mm.	-	
Pupa	1.5 to 1.79 mm	1.67 ± 0.11 mm	0.49 to 0.50mm	0.50 ± 0.01 mm
Adult	1.9 to 2.10 mm	2.02 ± 0.09 mm	-	

Table 2
Durations of different life stages of *L. malifoliella* on apple.
Each data point is the mean of 10 observations

Stage	Range	Mean
Egg (incubation period) (days)	4 to 8	6.05 ± 1.32
Larva (days)	6 to 7	6.44 ± 0.49
Prepupa(hours)	6 to 9	7.10 ± 1.29
Pupa (days)	3 to 5	3.9 ± 0.70
adult(days)	7 to 10	8 ± 1.23

NATURE OF DAMAGE

The larvae of *L. malifoliella* feed on the leaf tissue by mining through the mesophyll tissue leaving the epidermis intact as soon as the eggs hatch. The larvae form concentric mines which are initially light brown in colour and later turn darker due to the accumulation of larval excreta and frass within the mines. The circular mines can be as small as 1mm to 2cm and more (Fig. 3a). In case of heavy infestation many individual larval mines coalesce, giving blister like appearance and resulting in premature leaf drop thus, reducing the photosynthetic area significantly(Fig. 3b). According to the study of Chang (1985), recurrent defoliation results in weak host trees. Adult moths being mainly sap feeders do not cause any direct damage. In the later generations, pupation occurs on the bark crevices and on fruit calyces (Fig. 3c). Pupation and formation of cocoons in the fruit calyx drastically affects the economic value of the fruit as it renders the fruit unfit for export (Fig. 3d).

Molecular Identification, Phylogenetic tree and Pairwise-Genetic distance.

The COI sequences generated in this study, when queried in NCBI-BLAST, confirmed the species identity as *L. malifoliella*, commonly known as the peach leaf blister moth. The percent similarity and query coverage ranged from 99–100% when compared with samples submitted from other parts of Jammu and Kashmir. Subsequently, the sequences were submitted to NCBI, and the accession numbers PX651152 and PX650288 were obtained. A total of 17 COI sequences belonging to *L. malifoliella* and related taxa were used to construct a Neighbor-joining phylogenetic tree. The analysis revealed that the two sequences generated in this study (PX651152 and PX650288) clustered firmly within the *L. malifoliella* clade. Both sequences grouped closely with previously published Indian isolates from Pulwama, Shopian, Anantnag, Kulgam and Srinagar, forming a distinct and well-supported Kashmir lineage. This indicates high genetic similarity and confirms the identity of the collected specimens as *L. malifoliella*.

Other geographic lineages were clearly resolved: Finland, France, Russia and sequences from Canada formed separate, well-supported clusters, indicating intraspecific divergence related to geographic origin. Sequences belonging to *L. spartifoliella* and *L. laburnella* formed independent branches, each supported with bootstrap values of 100, clearly separating them from *L. malifoliella*. The outgroup (*Brachygossina hispanaria*) rooted the tree correctly, validating the obtained topology. The mean genetic distances between *L. malifoliella* and the related species *L. spartifoliella* (11.7%) and *L. laburnella* (16.5%) indicate high nucleotide divergence, supporting clear species-level separation among these lineages. The genetic distance among all *L. malifoliella* sequences ranged from 0.000 to 0.016 (0–1.6%), indicating very low divergence and confirming that they belong to the same species and represent closely related populations

Declarations

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Author Contribution

SHM conceived and designed the study, supervised the research, interpreted the results, and prepared and finalized the manuscript. RR conducted field sampling, laboratory rearing, life-history observations, morphometric measurements, and contributed to data analysis. TS assisted in molecular experiments, DNA extraction, PCR amplification, and sequence analysis. VA contributed to molecular identification, phylogenetic analysis, and interpretation of genetic data. SN assisted in experimental design, statistical analysis, and manuscript revision. All authors read and approved the final manuscript.

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Data Availability

The data generated during this study, including life-history observations, morphometric measurements, and mitochondrial COI gene sequences, are available from the corresponding author upon reasonable request. The DNA sequences generated in this study have been deposited in the NCBI GenBank database under accession numbers PX651152 and PX650288.

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Figures

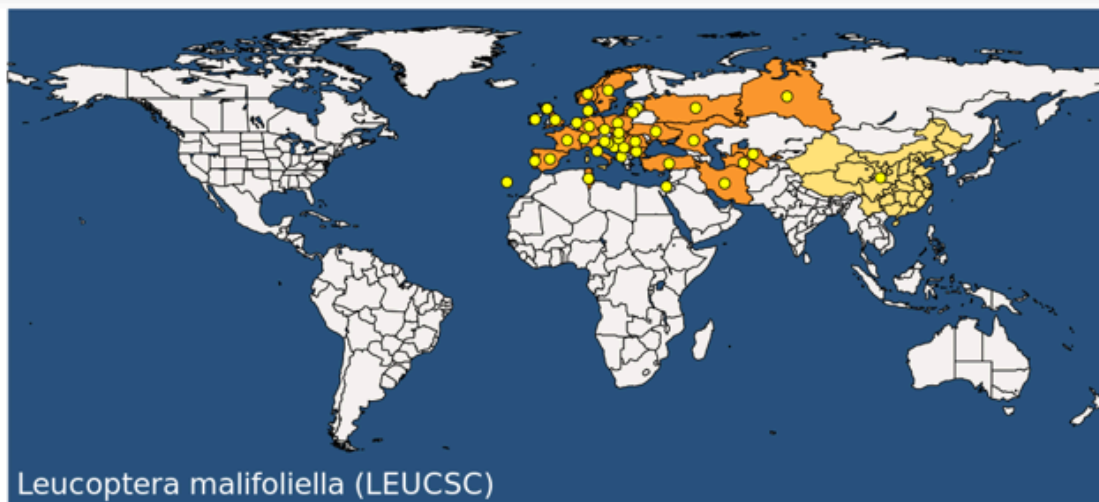


Figure 1a.

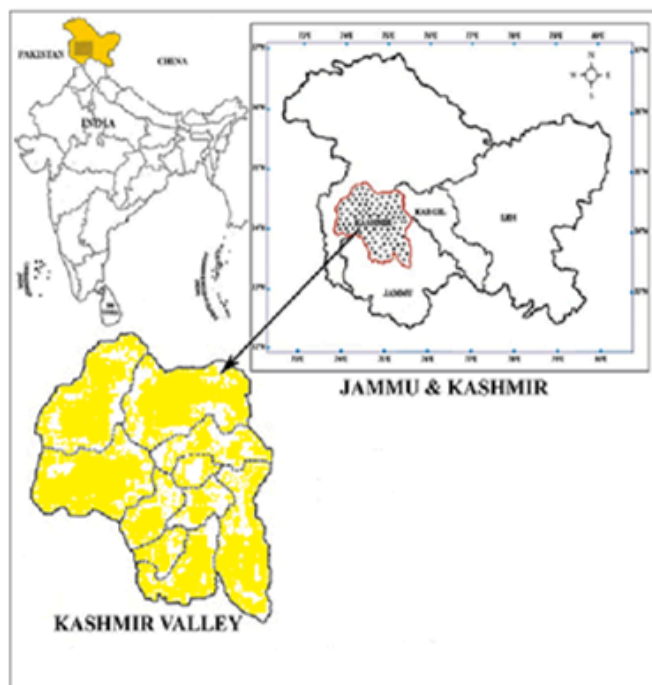


Figure 1b.

Figure 1

a. Distribution of *L. malifoliella* worldwide as per EPPO Global Database, 2024.

b. Distribution of *L. malifoliella* in India, Jammu and Kashmir

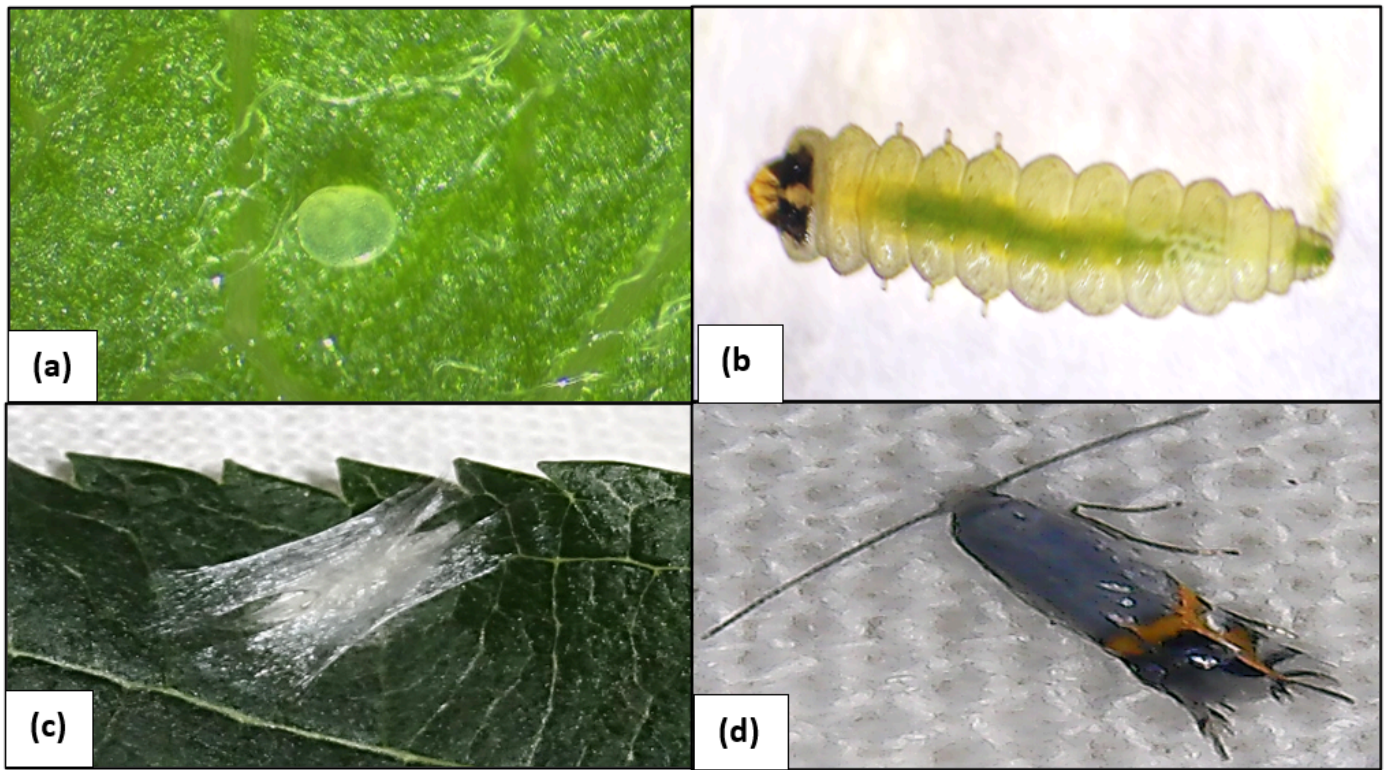


Figure 2

(a) Egg (b) Larva (c) Pupa (d) Adult of *L. malifoliella*

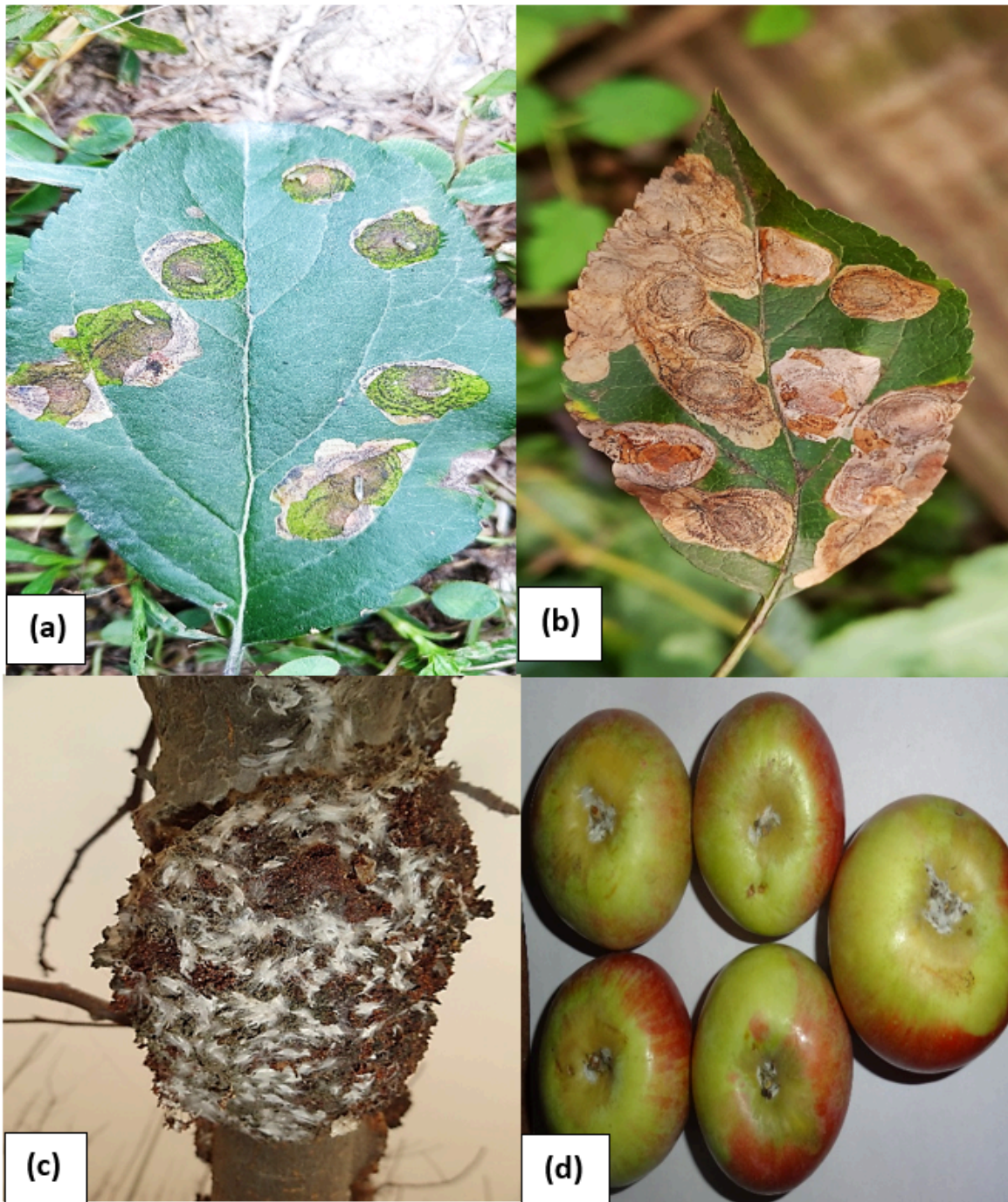


Figure 3

a) Concentric mines with larvae within b) coalesced larval mines giving blister like appearance c) pupation in bark crevices d) pupation in fruit calyces

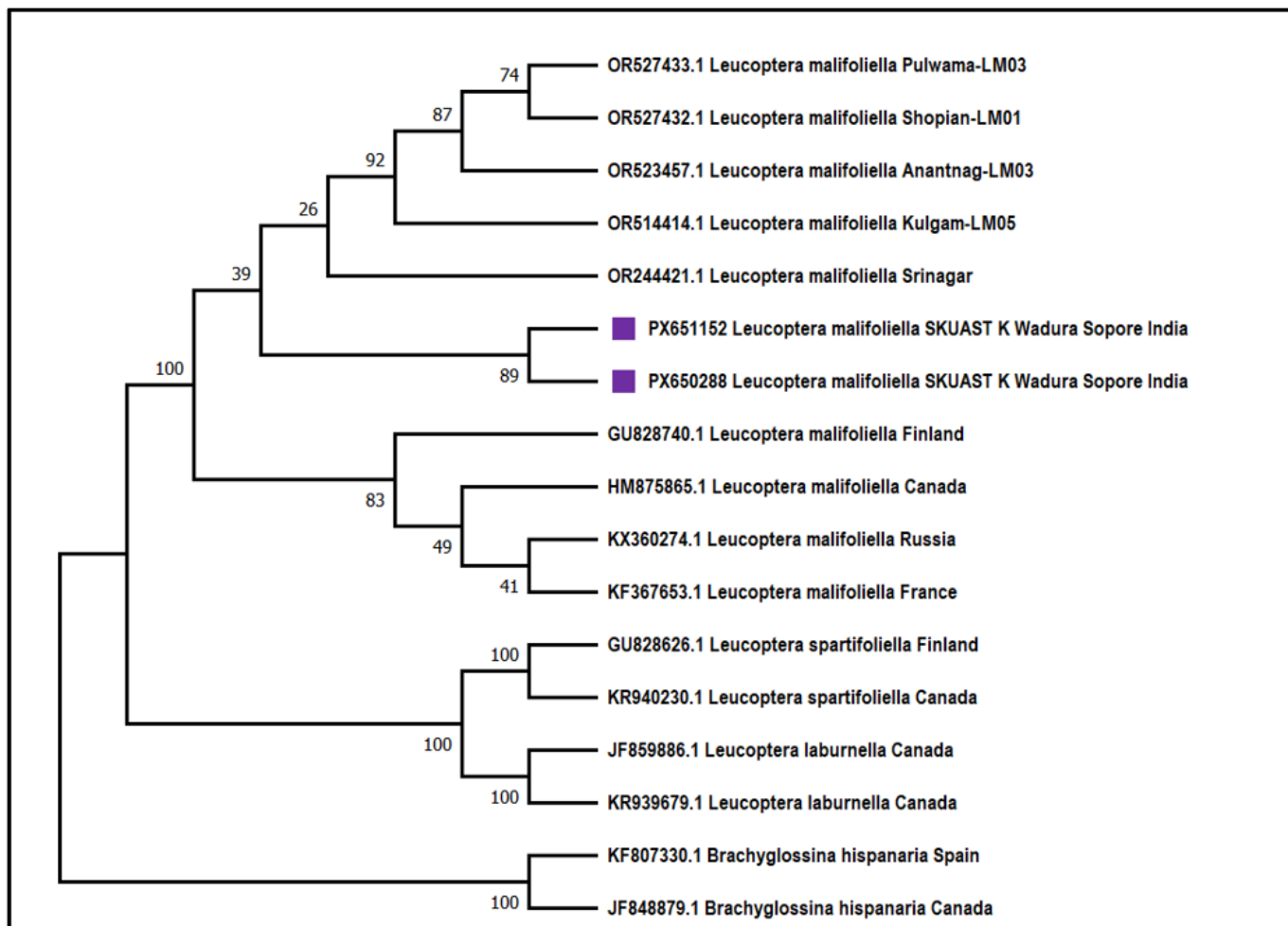


Figure 4

Figure caption: The evolutionary Neighbor-Joining phylogenetic tree of *L. malifoliella* with related taxa. The boxes with purple-coloured samples are result of present study.