



Field detection, molecular characterization and biology of the tea tortrix, *Homona coffearia* Neitner (Lepidoptera: Tortricidae) on Cocoa (*Theobroma cacao*. L) from India

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Abstract

Cocoa (*Theobroma cacao*. L) is an important commercial crop widely cultivated in humid tropical regions; however, its production faces various constraints including insect pests. The survey conducted in 2022–2023 found significant damage to cocoa nurseries and fields caused by the larvae of *H. coffearia*. The caterpillars web the young leaves, feeds within the sheltered nests and affects the crop canopy. The percent incidence was $27.55 \pm 1.81\%$ in the nursery and $43.77 \pm 3.42\%$ in open fields. A detailed morphological examination and molecular characterization using the mitochondrial cytochrome c oxidase I (COI) gene confirmed the identity of the pest as *H. coffearia*. The study also documented the key biological parameters of *H. coffearia* on cocoa under laboratory conditions. The life cycle from egg to adult was completed in about 49 ± 2.32 days. Female moths laid an average of 106 ± 3.48 eggs, which hatched into larvae that underwent five instars before pupation. Both the larval and pupal stages lasted around 27.69 ± 0.72 and 7.72 ± 0.17 days respectively. To our knowledge, this is the first report of the tea tortrix, *H. coffearia* infesting cocoa in India. As a polyphagous pest, the ability of *H. coffearia* to adapt and feed on new host plants like cocoa poses a significant threat to cocoa production. The findings of this work highlight the need for further research on the population dynamics, damage potential and management strategies for this emerging pest in cocoa ecosystems.

Keywords Cocoa · *Homona coffearia* · Incidence · Life cycle · Molecular characterization

Introduction

Cocoa (*Theobroma cacao* L.: Malvaceae) holds significant economic importance globally. It is native to the tropical rainforests of Central and South America (Ploetz 2007). Cocoa is crucial for the production of chocolates, a multi-billion-dollar industry that impacts livelihood across the world and widely cultivated in humid tropical countries (WCF 2014). Among them, Ivory coast and Ghana are the

two main producers of cacao and are responsible for more than 60% of the global production (ICCO 2022; Lahive et al. 2019). In India, cocoa is cultivated on approximately 1.06 lakh hectares and produces around 28,000 million tonnes annually (DCCD 2023); the production of cocoa is concentrated in southern states of India such as Karnataka, Kerala, Tamil Nadu and Andhra Pradesh (Beg et al. 2017; DCCD 2023). Moreover, cocoa cultivation provides income for millions of smallholder farmers worldwide (Sujatha et al. 2018; Apshara 2019; Voora et al. 2019).

The demand for cocoa beans across the globe has increased over the past 15 years, leading to an expansion of cultivated area and intensification of production (WCF 2014; Tothmihaly et al. 2019). However, there are several production constraints such as insect pests, diseases, nutrition and climatic conditions limiting the productivity of cocoa; of these, insect pests are the major factors affecting cocoa production in India (Thube et al. 2022). Globally, more than 50 insect pests including sap feeders, defoliators, capsules and

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wood borers are known to affect cocoa production (Madhu et al. 2023). More often, insect pests can adapt and feed on new host plants or closely related host species due to changes in climatic conditions for survival and to meet nutritional demands for growth and development (Buxton 2020). In addition, the possibility of the occurrence of new insect pests and diseases on different crops is not unusual in agricultural ecosystems (Laštuvka 2009; Pandian et al. 2024).

The genus, *Homona* consists approximately 30 species and they are distributed widely in Southeast Asia and Australia (Brown 2005). Among them *H. coffearia*, *H. spargatis* and *H. magnanima* Diakonoff are widely known to be economically important (Dugdale et al. 2005). *Homona coffearia* Neitner (1861) (Lepidoptera: Tortricidae) is an important seasonal pest, commonly referred to as the tea tortrix which primarily feeds on tea and coffee (Sharma and Gunasekare 2018). This pest is mainly distributed on the Asian continent including India, China, Japan, Malaysia and Indonesia where it causes considerable economic loss under epidemic conditions (Zeiss and Braber 2001; Hazarika et al. 2009). Being a polyphagous, it feeds on other important hosts including mango (*Mangifera indica* L.), citrus (*Citrus* sp.), guava (*Psidium* sp.), eucalyptus (*Eucalyptus* sp.) soya bean (*Glycine max*), cabbage (*Brassica oleracea*) etc., (Sharma and Gunasekare 2018). The caterpillar webs the leaves at growing points with the silken thread and is eventually filled with leaf fragments and larval frass. The infested plants become weak and stunted under severe conditions. Hitherto, the infestation of cocoa by *H. coffearia* has not been reported from Indian sub-continent. In a first, we have detected and identified this pest through morpho-molecular characterization with life history traits on cocoa.

Materials and methods

Insect collection and maintenance

The survey was conducted during 2022–2023 and infested samples were collected from cocoa fields as well as from saplings in cocoa nurseries at the experimental farm ICAR-Central Plantation Crops Research institute, Regional Station, Vittal, Dakshina Kannada district, Karnataka state, India (12.7704° N, 75.1136° E). The infested leaves were carefully observed based on webbing of leaves using their silken thread at the apical portion of the plants and seedlings. The webbed leaves were collected in insect breeding dish (90 × 40 mm, Himedia, India) and brought to the laboratory. The larvae were separated and transferred to a plastic container (20 × 15 cm) and reared on cocoa leaves until pupation at 27 ± 2 °C with 60–70% relative humidity in the laboratory. The pupae were separated and kept in a small plastic container (72 × 72 × 100 mm, Himedia, India).

Upon emergence, the adults were sexed based on the forewing characteristics (male – presence of distinct oblique bands; female – presence of transverse lines) and released into customized wooden cages (40 × 40 × 40 cm) for mating and egg-laying. Fresh cocoa leaves were provided to facilitate oviposition and cotton bolls moistened with 10% honey solution were used as the adult diet. Adult female laid egg masses on the leaves, they were separated and used for rearing to study the life history traits.

Pest incidence

The caterpillar makes webbing by rolling one leaf over another with the sides twisted together (Fig. 1). Damaged cocoa shoots and leaves from nursery and open fields were collected and examined visually. The nursery area and open fields were divided into six blocks. At least thirty plants were randomly selected and recorded the *H. coffearia* incidence at weekly intervals. The percent plant incidence was calculated using the following formula:

$$\text{Percent plant incidence} = \frac{\text{Number of plants infested by the pest}}{\text{Total number of plants observed}} \times 100$$

Morphological identification and molecular characterization

A detailed morphological description of the suspected insect was conducted at Division of Entomology Laboratory. Larva, pupa and adults were observed under a stereomicroscope (Leica MZ 12.5, Germany) and images were captured with a camera mounted in a system at magnifications from × 20 to × 62.5 with motorized z-axis drive and inbuilt montaging software (Leica MC 170 HD). The collected larval and adult specimens were kept as voucher specimens at ICAR—CPCRI, RS, Vittal insect repository (ID VTL-312). For molecular characterization, the larvae were taken for species level identification using the mitochondrial cytochrome c oxidase I (*COI*) gene. Total insect DNA was extracted from legs and antennae of adults and entire larvae using DNeasy Blood and Tissue Kit® (QIAGEN Strasse 1, Hilden, Germany), following the manufacturer's protocols. Polymerase Chain Reaction (PCR) was performed to amplify the partial *COI* gene using a 50 µL reaction volume containing 5.0 µL of 10 × Taq Buffer A (Genei labs Pvt. Ltd., Bengaluru, India), 2.0 µL each of the forward (LC01490 '5-GGTCAACAAATCATAAAGATA TTGG3') and reverse (HCO2198 '5-TAAACTTCAGGG T GACCAAAAATCA-3') primers (10 pmol/l) (Folmer et al. 1994), 2.0 µl of dNTP mix (2.5 mM each), 1.0 µl Taq DNA polymerase (5U/l), 20 ng of DNA and 36.0 µL sterile distilled water. Thermal cycling (Bio-Rad T100,

Fig. 1 Infestation of *H. coffearia* on cocoa shoots (a), larva feeding on cocoa leaf (b) and it undergo pupation inside the leaf nest (c)



Hercules, CA, USA) consisted of an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 53 °C for 1 min, extension at 72 °C for 1 min and a final extension at 72 °C for 5 min. The amplified products were verified by electrophoresis on an agarose gel (1.2%) (Sambrook and Russell 2001). PCR amplified products were outsourced for Sanger's sequencing (Biokart India Pvt. Ltd., Bengaluru, India).

The sequenced results were aligned using BioEdit (Biological Sequence Alignment Editor —Tom Hall, <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>) and each sequence was BLAST searched in the Barcode of Life Data (BOLD) database (<http://www.boldsystems.org/>) for a species match. The obtained sequences were deposited in NCBI database. Furthermore, phylogenetic analysis was performed using MEGA software (t) for both larvae and adult sequences.

Biological parameters of *H. coffearia* on cocoa

Five egg masses (~200 eggs) were randomly collected from the reared colony and kept in the insect breeding dishes (90 × 40 mm, Himedia, India). One-day old newly hatched first instar larvae were transferred to a plastic container (30 × 45 cm) using a fine brush (Camlin No. 1).

Cephalic capsule length was measured to determine the development process of *H. coffearia* larvae using Leica microscope (Leica MZ 12.5, Germany). Fresh cocoa leaves were provided until pupation and larval development period was recorded. Pupae were separated and maintained in a small plastic container. A pair of adults were released into the rearing cage provided with cocoa leaves for oviposition and cotton balls dipped in 10% honey solution as moth diet. Egg masses were collected, fecundity and the longevity of adults were recorded during their life span. The insects were reared for three consecutive generations in the laboratory.

Data analysis

Insect morphological features were observed under a stereomicroscope (Leica MZ 12.5, Germany). The data on percent incidence, developmental period (egg, larva, pupa and adult) and the fecundity were analysed using One-way analysis of variance (ANOVA) at $\alpha=0.05$ level. The percent data were arcsine transformed to normalize the variance before being analysed in SPSS software (ver 20, IBM, NY, USA). The *COI* sequence data from molecular analysis were compared with existing sequences in the GeneBank for confirmation.

Results

Morpho-molecular characterization

The larval and adult characteristics of *H. coffearia* were identified as described by Sharma and Gunasekare 2018 and CABI 2022. The first instar larvae are tiny, pale yellow with a shiny black colored head and gradually become greenish in the final instar; whereas, the pupae were initially greenish and turned reddish brown as they matured (Fig. 2). The adult moths are small with a wing span of 20–25 mm. The moth is brownish yellow and has oblique bands (♂) and transverse lines on the forewings (♀); however, the hind wings are yellow and devoid of distinct markings (Fig. 3). The consensus sequence was used in BLASTN (Basic Local Alignment Search Tool) search to find matching sequences (Altschul et al. 1990). The sequence was matched with 15 *Homona* spp. viz., EPNG10649-18, PMANK168-06, WILFC002-19, EPNG531-09, LEVIE1901-13, LNAUX307-18, LEVIE295-12, LNAUX312-18, LNAUX301-18, LNAUX311-18, LNAUX302-18, CATS087-10, GBMNE63078-22, LNAUV747-17. The results showed that the *COI* sequence and phylogenetic tree matched the *H. coffearia* (100% identity) sequence LNAUX304-18 (Fig. 4); hence, the molecular data confirmed that the present specimen as *H. coffearia* and the same sequence was deposited in NCBI (GenBank PP483867). The phylogenetic tree was constructed using MEGA software.

Incidence of *H. coffearia* on cocoa

A survey of Vittal's cocoa fields and surrounding nursery areas revealed considerable damage inflicted by *H. coffearia*. The damage was characterized by the webbing of apical leaves along with shoots. The larvae construct the leaf nests by webbing young foliage and feeding within shelters. The cocoa trees/saplings in flush are most susceptible since larvae prefer to feed on young plant parts and cause proportionate damage to unexpanded young leaves. Caterpillar frass was largely visible on all damaged parts of the plant due to continuous feeding. It completes five instars in the larval stage and undergo pupation inside the webbing. The incidence of *H. coffearia* recorded in the nursery and open fields were 27.55 ± 1.81 and $43.77 \pm 3.42\%$, respectively (Fig. 5).

Biology of *H. coffearia* on cocoa

The developmental and reproductive parameters of *H. coffearia* on cocoa are listed in Table 1. The adult moth laid pale, flattened, yellow/orange eggs in masses on the upper surface of the leaves with a mean fecundity of 106.4 ± 3.48 eggs/female. Upon hatching, the first instar larvae feed on leaves from the margin and start webbing with silken thread from the second instar. It undergoes five larval instars. The developmental process of larvae was identified based on cephalic capsule length and these were 0.03 ± 0.01 ,

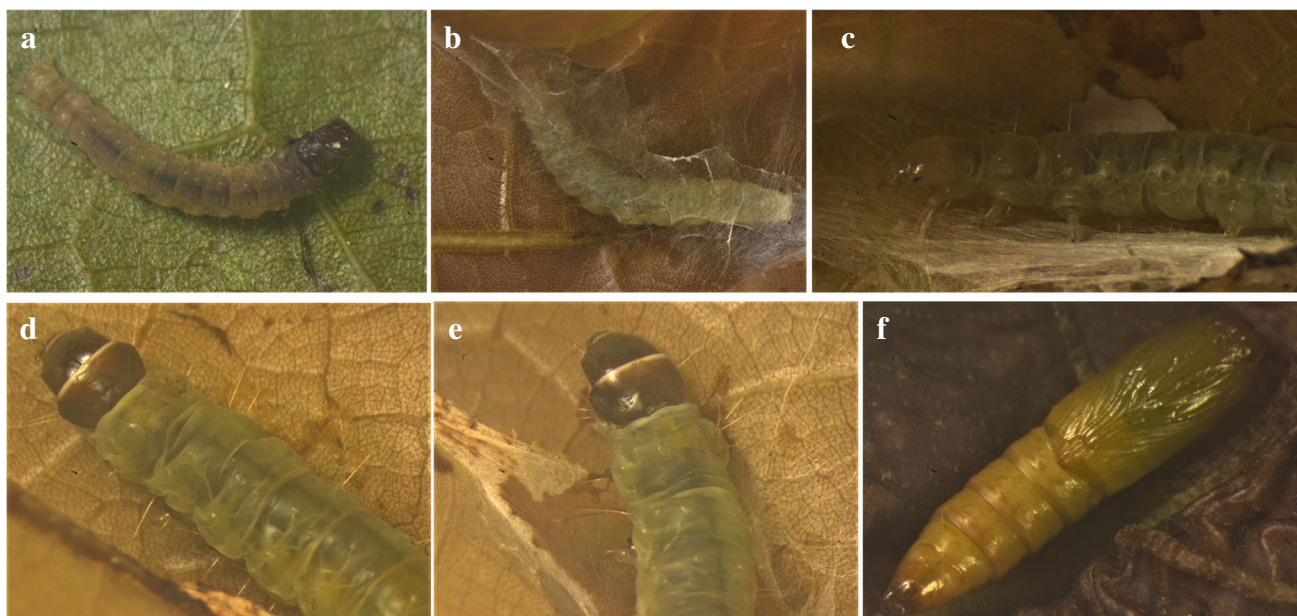


Fig. 2 The developmental process of *H. coffearia*. **a** 1st instar **(b)** 2nd instar, **(c)** 3rd instar **(d)** 4th instar **(e)** 5th instar **(f)** pupa

Fig. 3 *H. coffearia* moth (a), forewings with oblique bands in male (b) and transverse lines in female (c)

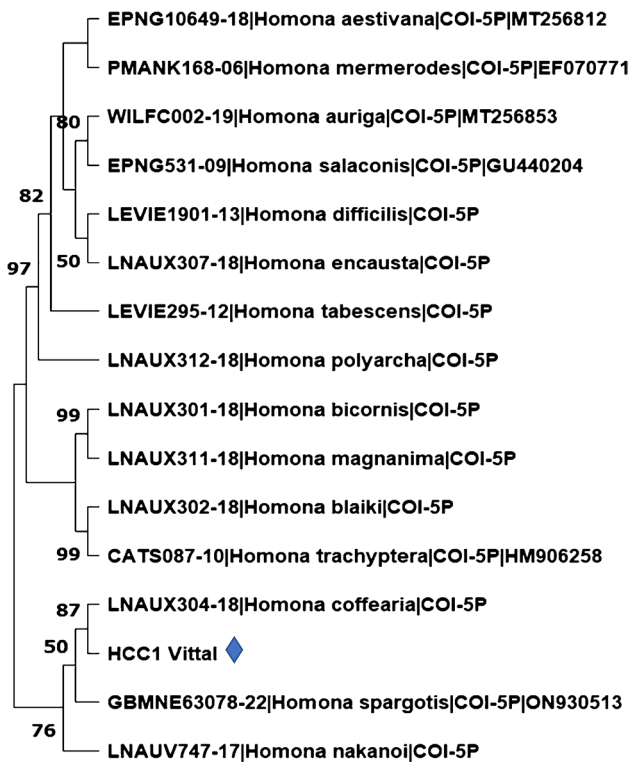
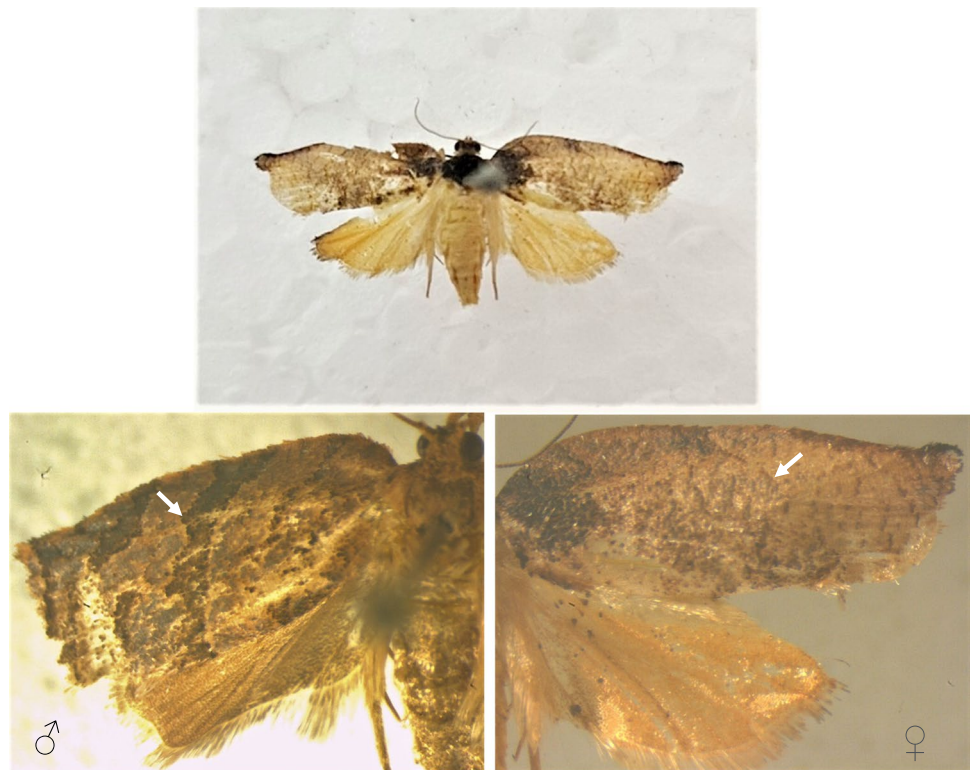


Fig. 4 Phylogenetic tree constructed by neighbor joining method inferred from *COI* sequences showing relationships among different species of *Homona* (Tamura-Nei substitution model and nearest neighbour- interchange search options with 1000 bootstrap replicates were used). The tree was constructed using MEGA version 11

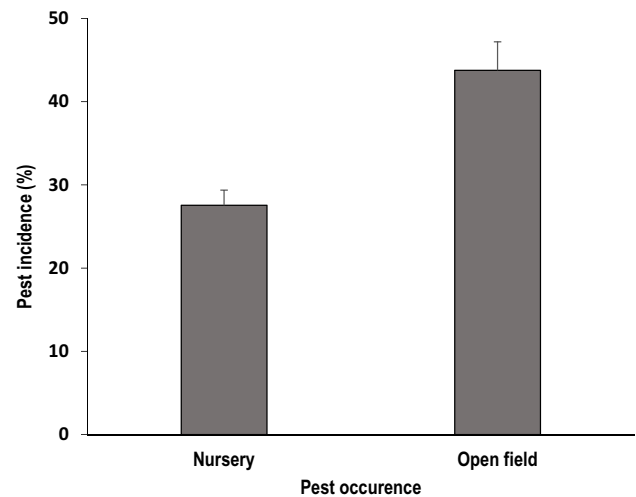


Fig. 5 Percent Incidence of *H. coffearia* on nursery and open fields of Cocoa

0.14 ± 0.10, 0.56 ± 0.18, 1.10 ± 0.38 and 2.27 ± 0.44 mm in first, second, third, fourth and fifth instars respectively. Pupation occurs inside the nest with a mean duration of 27.69 ± 0.72 and 7.72 ± 0.17 days, respectively. However, the longevity of male adults (8.56 ± 1.21) were relatively lower than that of females (10.12 ± 1.38) and the total life cycle of *H. coffearia* on cocoa was 49.14 ± 2.32 days.

Table 1 Biological parameters of *H. coffearia* on cocoa

Parameters	Duration (days)
Egg incubation ($n=200$)	4.40 ± 0.13
Larval period	
I Instar ($n=185$)	3.68 ± 0.14
II instar ($n=177$)	4.76 ± 0.16
III Instar ($n=174$)	5.84 ± 0.15
IV Instar ($n=172$)	6.24 ± 0.12
V Instar ($n=172$)	7.16 ± 0.15
Pupal period ($n=154$)	7.72 ± 0.17
Adult characteristics	
Fecundity ($n=57$)	106.4 ± 3.48 (eggs/female)
Pre-ovipositional period	3.28 ± 0.45
Ovipositional period	5.84 ± 0.61
Longevity	
Male ($n=50$)	8.56 ± 1.21
Female ($n=50$)	10.12 ± 1.38
Total life cycle	49.14 ± 2.32

n – Number of individuals used for the study. Developmental duration was analyzed using One-way ANOVA at $\alpha=0.05$ level

Discussion

Under the climate change scenarios, new pest incidences are becoming increasingly prevalent and pose a major threat to both natural and agroecosystems (Chakravarthy et al. 2016; Madhu et al. 2023). Rising temperatures, altered precipitation patterns, and shifting ecological dynamics are creating favorable conditions for pests to thrive in the regions where they were previously uncommon or absent (Prabhulinga et al. 2017; Nagaraju et al. 2024). Hence, a detailed description of a new pest and its association with the host is necessary to sensitize stakeholders and formulate adaptive strategies. To the best of our knowledge, this is the first scientific report, detailing the presence of *H. coffearia* on cocoa from India.

The incidence, damage potential and life cycle of *H. coffearia* on cocoa was systematically studied in the present study. The genus *Homona* consists of 30 species, among them *H. coffearia* is considered an important polyphagous pest and was reported to feed more than 20 species of hosts including fruit crops and legumes (Brown 2005). The nature of the feeding behavior and damage caused by *H. coffearia* was very similar to that of other crops (Das and Rahman 2023). Recently, the incidence of this pest was also reported in jute (*Corchorus olitorius*) from West Bengal, India (Babu et al. 2020). This pest mainly infests new flushes at the apical portion; due to the ovipositional behaviour of female moth. The moth lays eggs in masses ranging from 38 to 53 near growing tips and thus facilitates the larva to web the leaves using its silken thread and nibble it internally; as a result, the appearance of numerous shot holes in the infested region.

The larva can move to the adjacent shoots as it progresses and make webbings, which ultimately affect the crop canopy. Studies from cotton reported that pink bollworm, *Pectinophora gossypiella* prefers to lay eggs on young leaves of the crop canopy based on the feeding behavior of larvae (Madhu and Mohan 2021; Shah et al. 2023). However, the yield potential in cocoa is largely influenced by the dry matter stored in the leaves. To support this, a study from Ghana reported that leaves served as a source of nutrition in cocoa and thus significantly influenced the overall growth and development of cocoa pods (Goudsmit et al. 2023); besides, nutrition also plays a key role in the development of cephalic capsule (Madhu et al. 2021).

The molecular data has been widely utilized for deciphering insect taxonomy and gaining reliability for species identification (Chesters 2020; Shivakumara et al. 2024). The mitochondrial cytochrome c oxidase subunit I (*COI*) sequencing through DNA barcoding of our collected sample was searched in NCBI website with 15 *Homona* spp.; however, the sampled species was linked to *H. coffearia* population from Sri Lanka (LNAUX304-18) with 100% similarity. In addition, the characteristic host damage and morphological identity of the pest was similar to *H. coffearia* reported on tea and jute, respectively (Hazarika et al. 2009; Babu et al. 2020). It suggests that this pest is expanding its host range and geographic distribution. Besides, most farmers in India who are into cocoa production rely on saplings from various organizations/institutes; hence, effective sanitary and phytosanitary measures are necessary to prevent further distribution of this pest. Although the life cycle of *H. coffearia* on cocoa (49.14 ± 2.32) was very similar to that of other hosts (approximately 8 weeks); the female moths lived longer than males (Sharma and Gunasekare 2018; Das and Rahman 2023). However, six parasitoids have been reported to parasitize *H. coffearia* including *Brachymeria lasus* Walker, *Telenomus* sp. Haliday, *Apophua* sp, *Macrocentrus homonae* Nix., *Phytodeitus spinipes* (Cam.), and *Palexoris solennis* (Hazarika et al. 2001; Das et al. 2010); but, we did not record any parasitized larva under field conditions. Further, integrated approaches are necessary for effective management of this pest. Emamectin benzoate possessed high insecticidal activity against *H. coffearia* in tea plantations (Das and Rahman 2023); employing biocontrol and pheromone tactics could also limit the spread and distribution of *H. coffearia* (Kochansky et al. 1978; Walgama 2015).

Conclusion

In this study, the identification of *H. coffearia* has been conclusively established based on the nature of the feeding, host plant damage symptoms, morphological features and molecular characterization. Early detection and characterization of

new pests is crucial for developing effective control measures and safeguarding crop yields. In this regard, we suggest employing collective approaches including chemical measures (contact insecticides) against larval stages and sex pheromones (1-dodecanol, 1-dodecyl acetate, and (*E*)-9-dodecen-1-yl acetate) for trapping adult moths in the cocoa ecosystem.

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Data Availability All the data relevant to the present study are included in the article.

Declarations

Conflict of interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this work.

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