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Field parasitism of *Opisina arenosella* pupae and laboratory development of *Trichospilus pupivorus* on *Galleria mellonella*

HONG-UNG NGUYEN♥, THI-THANH-NGA SON, CHI-HIEU PHAN
School of Agriculture and Aquaculture, Tra Vinh University. Nguyen Thien Thanh Street No. 126, Hoa Thuan Ward, Vinh Long 940000, Viet Nam.
Tel.: +84-294-3855246, Fax.: +84-294-3855217, ♥email: nghongung@tvu.edu.vn

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Abstract. *Nguyen H-U, Son T-T-N, Phan C-H. 2026. Field parasitism of* Opisina arenosella *pupae and laboratory development of* Trichospilus pupivorus *on* Galleria mellonella*. Biodiversitas 27 (3): d270316. https://doi.org/10.13057/biodiv/d270316.* The black-headed caterpillar, *Opisina arenosella* is a major coconut pest in Asia and has caused serious damage in Vietnam's Mekong Delta since its first detection in 2020. Parasitoid wasps play an important role in pest control, among which *Trichospilus pupivorus* is an effective pupal parasitoid of *O. arenosella*. In addition, larvae and pupae of *Galleria mellonella* are widely used as alternative hosts for laboratory rearing of beneficial insects. This study investigated the field occurrence of *T. pupivorus* in coconut orchards of Tra Vinh province and evaluated its development on *G. mellonella* to support laboratory mass rearing. Field surveys were conducted in 15 infested orchards, where a total of 1,500 *O. arenosella* pupae were collected from 150 coconut trees to estimate parasitism rates. Five parasitized pupae from each orchard were reared individually to quantify progeny production per parasitized *O. arenosella* pupa. The results showed that *T. pupivorus* was detected in 86.7% of orchards and 72.7% of sampled trees, while the mean orchard-level parasitism rate was 17.1±7.75%. Mean progeny production was 462.5±91.4 individuals per parasitized *O. arenosella* pupa. Under controlled insectary conditions (27±1°C, 75±1.5% RH, 12L:12D photoperiod), the parasitoid completed development on *G. mellonella* pupae in approximately 16 days, producing 118.3±46.7 individuals per *G. mellonella* pupa with a strongly female-biased sex ratio (>97%). These findings provide baseline quantitative information on the field occurrence and laboratory performance of *T. pupivorus* associated with *O. arenosella* in coconut agroecosystems.

Keywords: Biological control, coconut pest, factitious host, parasitoid wasp, pupal parasitoid

INTRODUCTION

The black-headed caterpillar, *Opisina arenosella* (Lepidoptera: Xyloryctidae), formerly placed in Oecophoridae, is an important pest of coconut palms in tropical Asia. Severe infestations cause extensive leaf mining, leading to complete defoliation, delayed canopy recovery, and in extreme cases, palm mortality, resulting in serious yield losses and long-term landscape degradation (Kumara et al. 2015; Yan et al. 2023). In coconut-based agroecosystems, the persistence and spread of *O. arenosella* are further facilitated by the presence of alternative host plants, including jack fruit (*Artocarpus heterophyllus*) and cashew (*Anacardium occidentale*), which are commonly intercropped with coconut palms (Shameer et al. 2018). Such host plant diversity can sustain pest populations even when coconut foliage is temporarily unsuitable, increasing outbreak risks. In newly invaded regions, where natural enemy communities are still poorly established, damage caused by *O. arenosella* is often more severe than in its native range (Shameer et al. 2018).

Several management approaches have been employed to suppress *O. arenosella*, including pheromone-based monitoring, chemical insecticides, and biological control (Bhanu et al. 2018; Jin et al. 2019; Li et al. 2020; Wu et al. 2024). However, chemical control is difficult to implement in tall coconut canopies and frequently disrupts non-target organisms, including pollinators and natural enemies, thereby reducing overall agroecosystem resilience (Woodcock et al. 2016; Nguyen et al. 2020; Wan et al. 2025). In contrast, biologically based pest management that conserves or enhances natural enemy diversity has become a cornerstone of sustainable agriculture in tropical perennial crops (Perez-Rodriguez et al. 2021). Parasitoid wasps, in particular, play a central role in regulating insect pest populations (Liu et al. 2020; Atashi et al. 2023), and their suppression by indiscriminate pesticide use may trigger secondary pest outbreaks and ecological imbalance (Lu et al. 2023). In response, both exotic and indigenous natural enemies have been considered for biological control within a biodiversity-based pest management framework (Heimpel and Cock 2018; Wyckhuys et al. 2020). Previous studies have identified several parasitoid taxa associated with *O. arenosella*, including species from Trichogrammatidae, Chalcididae, and Eulophidae (Binoy et al. 2024). Among these, *Trichospilus pupivorus* (Hymenoptera: Eulophidae) is a polyphagous gregarious pupal endo-parasitoid that is widely distributed in tropical and subtropical regions and has been recognized as one of the most important natural enemies of *O. arenosella* (Silva et al. 2016).

In Vietnam, *O. arenosella* was first detected in 2020 and has since rapidly expanded throughout the Mekong Delta, causing severe damage to coconut orchards (Nguyen et al. 2025). Despite its recognized importance, quantitative information on the field-level contribution of *T. pupivorus* to the regulation of *O. arenosella* in newly invaded coconut-growing areas of Vietnam remains limited. Most available studies have focused on laboratory observations or other geographic regions such as Thailand (Shameer et al. 2018), Malaysia (Mahadi et al. 2019; Mahadi et al. 2020), leaving a critical gap in understanding how this parasitoid is distributed and performs under real orchard conditions in the Mekong Delta. Addressing this gap is essential for establishing baseline information on the occurrence of a key pupal parasitoid associated with *O. arenosella* in coconut agroecosystems. Therefore, the first objective of this study was to quantify the field occurrence of *T. pupivorus* parasitizing *O. arenosella* pupae in coconut orchards in Tra Vinh province.

In addition to field effectiveness, the practical use of parasitoids in biological control programs depends on the availability of reliable mass-rearing systems. However, the suitability of alternative hosts for large-scale production of *T. pupivorus* has not been sufficiently evaluated. The greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae), is widely used as a factitious host for rearing parasitoids and predators because of its large size, high nutritional value, and ease of culture (Kandil et al. 2021). Nevertheless, its capacity to support the complete development and high reproductive output of *T. pupivorus* remains poorly understood. Consequently, the second objective of this study was to assess the developmental performance of *T. pupivorus* on *G. mellonella* pupae under controlled laboratory conditions. Although primarily descriptive, this study provides baseline information from a newly invaded region where quantitative data are limited, thereby serving as a reference for future experimental studies and natural enemy-based pest management programs. Accordingly, the results are presented as descriptive baseline information rather than as direct evidence of pest management effectiveness in coconut orchards.

MATERIALS AND METHODS

Study period and area

The field parasitism rate of *T. pupivorus* on *O. arenosella* was surveyed in 15 coconut orchards in Tra Vinh Province, Vietnam, from December 2024 to March 2025, where damage caused by *O. arenosella* had been recorded. The surveyed orchards were located in major coconut-growing areas of the province and were selected based on the presence of visible infestations of *O. arenosella* and accessibility for sampling. In total, 150 coconut trees were examined across all orchards. The development of *T. pupivorus* on *G. mellonella* pupae was investigated in the insect laboratory of Tra Vinh University from April to November 2025.

Source of the greater wax moth *G. mellonella* and the parasitoid *T. pupivorus*

Trichospilus pupivorus individuals were obtained from *O. arenosella* pupae naturally parasitized in farmers' coconut orchards and identified based on morphological characteristics by an entomologist at Tra Vinh University. *G. mellonella* was sourced from local honeybee farms in Tra Vinh province.

Research procedure

Survey of T. pupivorus *parasitizing* O. arenosella *pupae in coconut orchards*

Field surveys were conducted in Tra Vinh Province, across major coconut-growing districts (Cang Long, Chau Thanh, and Tieu Can) (approximately 9.75-10.05° N, 106.10-106.40° E). A total of 15 coconut orchards (each ≥1,000 m²) were selected based on confirmed presence of *O. arenosella* infestation and farmer consent. The surveyed orchards were predominantly coconut monocultures, based on direct field observations, with little or no reported use of chemical pesticides and no additional interventions specifically targeting *O. arenosella*, as indicated by farmer interviews. The orchards were planted with tall coconut varieties (4-10 years old; 3-8 m canopy height; 7-8 m spacing), corresponding to an estimated planting density of approximately 150-180 trees ha^{-1}, and no systematic intercropping was observed. Infestation by *O. arenosella* was confirmed by visual symptoms on fronds (active leaf mines, frass, and webbing) and by the presence of pupae within mined leaves. The history of pesticide use in the orchards was not specifically documented.

In each orchard, 100 *O. arenosella* pupae were collected from 10 coconut trees (10 pupae per tree). To capture spatial variation in parasitism, the sampled trees were distributed across 5 random sampling points within each orchard, with 2 trees selected at each point. Of these, 4 sampling points were located along the orchard periphery (8 trees in total), and one sampling point was located in the orchard center (2 trees). On each selected tree, pupae of *O. arenosella* were collected from actively infested fronds at the lower and mid-canopy levels (approximately 3-6 m above ground), which are accessible and typically exhibit visible mining symptoms. Fronds were selected based on the presence of fresh leaf mines, frass, and silk webbing, indicating active infestation. For each tree, infested fronds were visually inspected for approximately 10-15 minutes, and pupae were carefully removed from within mined leaflets using forceps. Sampling was conducted during daytime (08:00-16:00 h) under dry weather conditions to ensure consistent visibility and effective sample observation. The same sampling protocol was applied uniformly across all trees and orchards to reduce sampling bias. In total, 1,500 *O. arenosella* pupae were collected and examined, providing a representative estimate of field parasitism at the orchard level.

In addition, five *O. arenosella* pupae showing visible signs of parasitism (e.g., a darkened and hardened cuticle and, in some cases, visible dark-brown oviposition marks on the surface) were randomly selected from each orchard and transported to the laboratory. Each pupa was reared individually in a test tube to determine the number of *T. pupivorus* adults emerging from a single host pupa. Emergence success was defined as the percentage of laboratory-reared pupae yielding at least one adult *T. pupivorus*, whereas pupae producing no adult emergence were recorded as unsuccessful. Pupae that failed to yield adult emergence were subsequently dissected to verify the presence or absence of parasitoid immatures. Parasitism rates of *T. pupivorus* at both orchard and tree levels were determined by dissection of field-collected host pupae (n: 1,500), whereas adult emergence success was calculated based on the number of laboratory-reared pupae yielding adult *T. pupivorus* (n: 75), as summarized Table 1.

The analytical units were defined as follows: i) Orchard-level detection: Each orchard was treated as one unit. An orchard was recorded as positive if at least one parasitized pupa was detected; otherwise, it was recorded as negative. Orchard-level detection was expressed as the percentage of positive orchards among all surveyed orchards. ii) Tree-level detection: Individual coconut trees within orchards were treated as sampling units. A tree was considered positive if at least one parasitized pupa was detected from that tree. Tree-level detection was calculated as the percentage of positive trees among all sampled trees. iii) Orchard-level parasitism rate: To quantify parasitism intensity, 100 *O. arenosella* pupae per orchard were dissected to determine the presence or absence of *T. pupivorus* immatures. The orchard-level parasitism rate was calculated as the percentage of dissected *O. arenosella* pupae found to contain *T. pupivorus* immatures out of the total number of pupae examined.

Orchard-level parasitism rates were summarized across orchards as means ±SD, together with observed ranges. Adult *T. pupivorus* were identified based on diagnostic morphological characters following Ubaidillah (2006) and Silva et al. (2016), including body coloration, antennal structure, head morphology, and wing setation. Specimens were examined under a stereomicroscope and compared with published species descriptions. Species determination was independently verified by an entomologist at Tra Vinh University, Vietnam, to ensure identification accuracy. Representative voucher specimens have been deposited in the Entomology Laboratory, Tra Vinh University, under the collection code TVU-TP-2025-01.

Development of the parasitoid wasp T. pupivorus *on pupae of* G. mellonella

Galleria mellonella was reared on an artificial diet prepared according to the formulation described by Kwadha et al. (2017), consisting of wheat bran, glycerol, and honey. Larvae were maintained at a density of approximately 50-60 individuals per plastic box (25×18×8 cm) with mesh-covered openings for ventilation under controlled insectary conditions (27±1°C, 75±1.5% RH, 12L:12D). Final instar larvae were used to obtain pupae for parasitoid exposure. Larvae were monitored every 6 hours, and pupation time was determined based on the cessation of larval movement. To minimize host size variation, only pupae within a narrow size range were selected, with a body mass of 0.16-0.19 g, length of 17.6-18.4 mm, and width of 4.6-4.7 mm. Pupae were not sexed prior to use because external sexual dimorphism is not readily distinguishable at the pupal stage without invasive manipulation. As pupae were randomly selected within a defined size range, potential sex-related variation in host quality was considered minimal.

Newly emerged adults of the next generation of *T. pupivorus* were used for morphological and biological observations. A newly emerged female was individually introduced to a newly pupated *G. mellonella* pupa (≤12 hours old) in a ventilated plastic box (6.5×5×4.5 cm) and provided with a 10% honey solution as food. Oviposition timing of *T. pupivorus* was determined by examining host pupae at 1-hour intervals during the first 12 hours following parasitoid exposure. Egg development time was measured as the interval between egg detection and larval hatching. *T. pupivorus* exhibits within-host mating behavior; therefore, newly emerged females were assumed to be mated and were used directly in the experiments without additional mating procedures. Each replicate consisted of a single female exposed to one host pupa, and all individuals were monitored independently. Females were used only once and were not reused at any stage of the experiment. In addition, body size measurements were conducted on randomly selected individuals of each developmental stage (n: 100 per stage), including eggs, early larvae, late larvae, newly formed pupae and adults of *T. pupivorus,* with morphological parameters reported in Table 2 and Table 3. For body size measurements, individuals were randomly selected from parasitized hosts collected throughout the observation period and were measured independently.

After the 12-hour exposure period, host pupae were transferred to clean rearing boxes of the same size. A total of 400 *G. mellonella* pupae were initially exposed to parasitoids. Among these, 300 pupae were confirmed to be parasitized and were used for developmental observations of *T. pupivorus*. For stage determination, parasitized pupae were dissected under a stereomicroscope during a 17-day observation period. In total, 160, 80, and 60 parasitized pupae were observed at the egg, larval, and pupal stages, respectively. Each host pupa was dissected only once and assigned to the developmental stage detected at the time of dissection. The remaining parasitized pupae were reared individually until adult emergence to record developmental duration and progeny production (Table 4).

Progeny production per host pupa was estimated based on the total number of adult offspring emerging from each parasitized host. Offspring sex ratio was determined based on all adult *T. pupivorus* individuals successfully emerging from each parasitized host pupa. All emerged adults were collected and counted immediately after emergence to avoid loss or escape, and sex was determined under a stereomicroscope based on external morphological characters, particularly antennal morphology and body size, following Ubaidillah (2006) and Silva et al. (2016). After completion of adult emergence, each host pupa was dissected under a stereomicroscope to confirm that all parasitoid individuals had exited the host and to verify the absence of dead or unemerged adults remaining inside the host body. Host pupae from which no adult emergence occurred were recorded as unsuccessful parasitism and were excluded from sex ratio calculations. Overall, 400 female *T. pupivorus* and 400 *G. mellonella* pupae were used in the experiment.

Data analysis

Data were summarized using descriptive statistics. Proportional data (detection and parasitism rates) are reported with 95% confidence intervals (CI). To address the heterogeneity among orchards, tree-level detection is reported as a median with an associated range (minimum-maximum). Continuous variables are presented as means±standard deviation (SD). Inferential tests were not applied as the study aimed to establish baseline ecological parameters rather than test experimental hypotheses; consequently, interpretations are limited to the observed descriptive trends.

RESULTS AND DISCUSSION

The parasitism rate of *T. pupivorus* on *O. arenosella* pupae in farmers' coconut orchards

The field survey confirmed the widespread presence of *T. pupivorus* parasitizing *O. arenosella* pupae across the surveyed regions of Tra Vinh Province during the survey period (Table 1). The parasitized pupae were detected in 13 of the 15 surveyed coconut orchards, representing an orchard-level detection rate of 86.7% (95% CI: 62.1-96.3%). At the orchard level, the median percentage of trees containing at least one parasitized pupa was 80.0% with a wide range (20,0-100%) reflecting significant spatial variation.

The orchard-level parasitism rate (based on dissection-confirmed pupae) averaged 17.1±7.75% (95% CI: 13.0-21.2%), with observed values ranging from 0% to 27% per orchard. Among the sub-sample of field-collected pupae showing visible signs of parasitism, the adult emergence success rate was 86.7% (65/75; 95% CI: 77.2-92.7%). From these successful emergences, mean progeny production was 462.5±91.4 individuals per parasitized *O. arenosella* pupa.

Morphological characteristics of *T. pupivorus* reared on pupae of *G. mellonella*

Egg

Under these laboratory conditions, eggs of *T. pupivorus* were elongated and very small when freshly laid, measuring approximately 0.2 mm in length. As embryonic development progressed, the opaque white embryo became clearly visible through the transparent chorion (Figure 1, Table 2).

Larva

Under these laboratory conditions, larvae of *T. pupivorus* were initially small and opaque white, measuring approximately 0.4 mm in length shortly after hatching, and increased markedly in size during development. At the final larval stage, they reached a maximum length of about 1.4 mm. At this stage, the entire body appeared ivory yellow, and the head, thorax, and abdomen were clearly distinguishable under a stereomicroscope (Figure 2, Table 2).

Table 1. Parasitism metrics of *Trichospilus pupivorus* on *Opisina arenosella* pupae in coconut orchards (Tra Vinh, Vietnam)

Survey parameter	Units	Sample size	Result (Mean±SD)	95% Confidence interval
Orchard-level detection	(%)	15 orchards	86.7	62.1-96.3
Tree-level detection	(%)	150 trees	80.0 (20.0±100)*	N/A
Parasitized pupae rate (dissection-confirmed)	(%)	1,500 pupae	17.1±7.75	13.0-21.2
Adult emergence success	(%)	75 pupae**	86.7	77.2-92.7
Progeny per parasitized pupa	individuals	65 pupae	462.5±91.4	440.1-484.9

Note: SD for tree-level detection represents variance across the 15 surveyed orchards. *Proportions and CIs calculated based on total samples per category. **Sub-sample selected based on visible signs of parasitism. A total of 65 pupae successfully yielded adult *Trichospilus pupivorus* and were included in the calculation of progeny per parasitized pupa

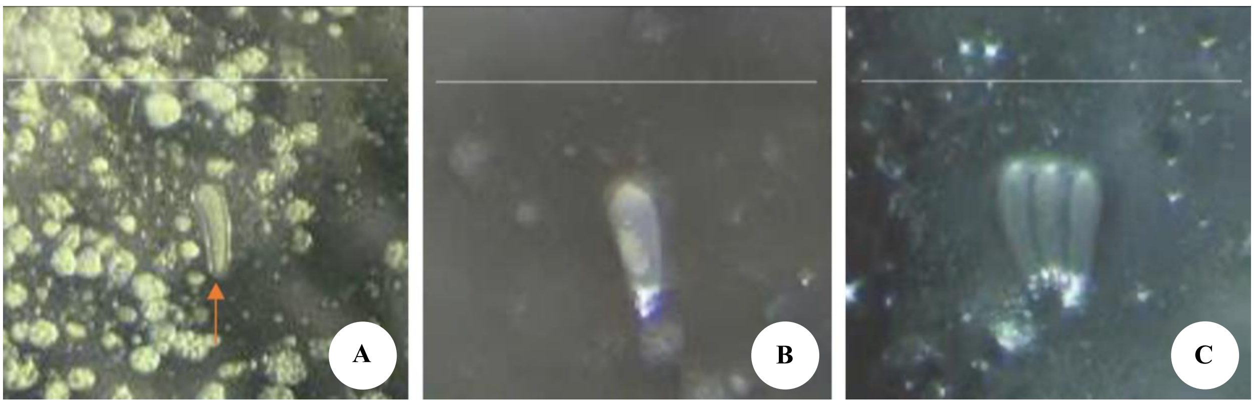

Figure 1. Egg morphology of *Trichospilus pupivorus*. A. Newly laid egg, B. 1-day-old egg, C. 2-days-old egg. Scale bar: 0.5 mm

Pupa

Pupae of *T. pupivorus* were naked and initially milky white, gradually changing to ivory white and then brown as development progressed under these laboratory conditions. Eye color shifted from light red to dark red, and the head and legs became dark yellow at later stages. At the final pupal stage, the entire body turned dark brown, with the abdomen appearing blackish brown. The average pupal length and width were approximately 1.4 mm and 0.5 mm, respectively (Figure 2, Table 2).

Adult

Adult *T. pupivorus* were covered with sparse, stiff setae on the head and body. Males were light brown, with an almost rectangular head and a thorax occupying most of the body, whereas females were generally larger and darker brown, with an almost round head. Females averaged about 1.5 mm in body length and had a wider wingspan (approximately 2.5 mm) than males, which averaged about 1.0 mm in body length and a wingspan of about 1.9 mm. In both sexes, the thorax was oval (Figure 3, Table 3). Adult *T. pupivorus* exhibited clear sexual dimorphism in head shape and abdomen morphology. Females had a darker, almost rounded head with a convex frons, whereas males had a lighter, rectangular head. Antennae were short and slender, with female antennae darker and longer than those of males. Wings were membranous and transparent, with forewings bearing a distinct dark vein and sparse setae, while hindwings were smaller, paler, and fringed with hairs along the margins. Sexual dimorphism was also evident in the abdomen, which was larger, rounder, and darker in females, but smaller and lighter in males under these laboratory conditions.

Biological characteristics of the parasitoid wasp *T. pupivorus* reared on pupae of *G. mellonella*

Under controlled laboratory conditions, *T. pupivorus* successfully completed its life cycle on *G. mellonella* pupae in 16.1±0.27 days (95% CI: 16.0-16.2 days). The mean progeny production per host was 118.3±46.7 individuals (95% CI: 105.4-131.2 individuals), demonstrating high reproductive output on this alternative host. The offspring sex ratio was found to be exceptionally high at 97.1±1.34% (95% CI: 96.7-97.5%), confirming a strong female bias. These findings, including high survival, successful development completion, substantial progeny production, and a female-biased sex ratio, support the suitability of *G. mellonella* for the mass rearing of this parasitoid (Table 4).

Table 2. Size (mean±SD) of developmental stages of *Trichospilus pupivorus* reared on *Galleria mellonella* pupae under laboratory conditions

Developmental stage	Sample size	Body length (mm)	Body width (mm)
Egg	100	0.2±0.01	0.1±0.01
Newly hatched larva	100	0.4±0.07	0.1±0.02
Late-stage larva	100	1.4±0.08	0.4±0.04
Pupa	100	1.4±0.13	0.5±0.05

Note: Measurements were conducted using a calibrated ocular micrometer under a stereomicroscope (Meiji EMZ-8TR trinocular microscope). Values are presented as mean±SD; n indicates the number of individuals measured

Table 3. Morphometric characteristics (mean±SD, n: 30) of adult *Trichospilus pupivorus* reared on *Galleria mellonella* pupae

Developmental stage	Sample size	Wingspan (mm)	Body length (mm)	Body width (mm)	Antennae length (mm)
Female adults	100	2.5±0.14	1.5±0.13	0.4±0.04	0.4±0.03
Male adults	100	1.9±0.17	1.0±0.12	0.3±0.01	0.3±0.02

Note: Measurements were conducted using a calibrated ocular micrometer under a stereomicroscope (Meiji EMZ-8TR trinocular microscope). Values are presented as mean±SD; n indicates the number of individuals measured

Table 4. Development and reproductive parameters of *Trichospilus pupivorus* reared on *Galleria mellonella* pupae

Biological parameter	Units	Sample size (n)	Mean±SD	Range	95% Confidence Interval
Egg duration	hours	160 parasitized pupae	43.2±8.39	36.0-60.0	38.0-48.4
Larval duration	days	80 parasitized pupae	6.10±0.74	5.00-7.00	5.64-6.56
Pupal duration	days	60 parasitized pupae	6.44±0.39	6.00-7.00	6.20-6.68
Pre-oviposition period	hours	50 individuals	4.03±1.02	3.00-6.00	3.75-4.31
Oviposition period	hours	50 individuals	18.5±1.66	16.0-20.0	18.0-19.0
Life cycle	days	50 individuals	16.1±0.27	15.0-17.0	16.0-16.2
Female longevity	days	50 individuals	5.71±1.77	3.00-7.00	5.22-6.20
Male longevity	days	50 individuals	2.68±0.75	2.00-4.00	2.47-2.89
Progeny per host pupa	individuals	50 parasitized pupae	118.3±46.7	65.0-208	105.4-131.2
Offspring sex ratio	(%)	50 parasitized pupae	97.1±1.34	94.5-98.8	96.7-97.5

Note: Sample size (n) refers to the number of parasitized host pupae; 95% Confidence Intervals were calculated to provide distributional context for baseline life-history traits

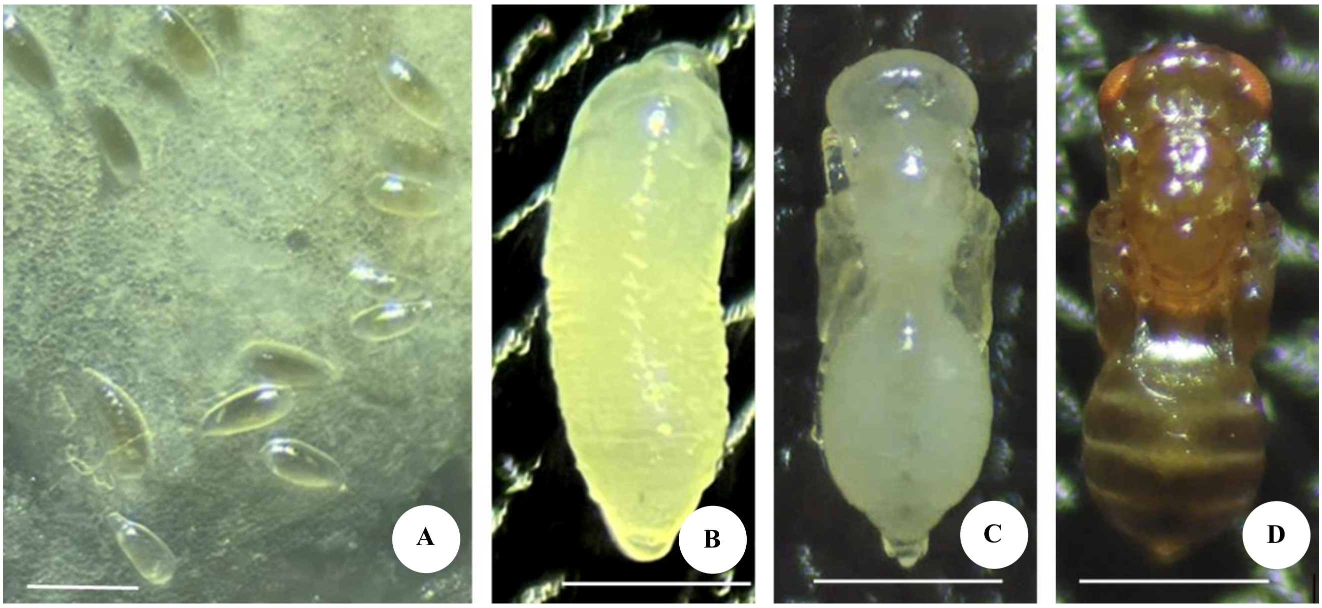

Figure 2. Morphology of *Trichospilus pupivorus*. A. Newly hatched larva, B. 5-days-old larva, C. Newly pupa, D. 6-days-old pupa. Scale bar: 0.5 mm

Figure 3. Adult morphology of *Trichospilus pupivorus*. A. Female and male adults, B. Female genitalia, C. Male genitalia. Scale bar: 1 mm

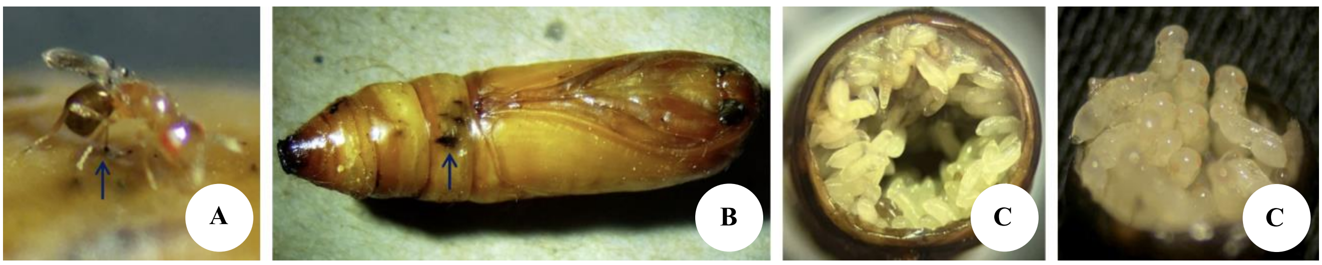

Figure 4. Parasitism process of *Trichospilus pupivorus* on *Galleria mellonella* pupae. A. Female inserting ovipositor into host pupa, B. Parasitized pupa, C and D. Larvae and pupae developing inside the host

Discussion

The widespread occurrence of *T. pupivorus* in farmers' coconut orchards in Tra Vinh province during the survey period suggests that this parasitoid is established in local agroecosystems and may contribute to the natural regulation of *O. arenosella* populations. Surveys conducted in 2023 recorded an average parasitism rate of 26.7±11.9% on *O. arenosella* pupae (Nguyen et al. 2023), higher than the mean parasitism observed in the present study. These differences may reflect sampling season, orchard management, infestation intensity, and methodology. These findings suggest that *T. pupivorus* is an active and potentially important natural enemy in coconut production systems of southern Vietnam. The present results are broadly consistent with earlier reports describing *T. pupivorus* as a commonly recorded pupal parasitoid of *O. arenosella* in Southeast Asia. Parasitism of *O. arenosella* pupae by *T. pupivorus* has been documented in Malaysia and Thailand (Lu et al. 2023), and high parasitism rates have also been reported in Vietnam (Le et al. 2023). Furthermore, *T. pupivorus* is widely distributed and parasitizes several lepidopteran pests (Mahadi et al. 2019; Le et al. 2023).

The morphological characteristics observed in this study are broadly consistent with previous descriptions (Ubaidillah 2006; Silva et al. 2016), suggesting a conserved developmental pattern across populations and host species. Minor variation in the size and appearance of immature stages likely reflects differences in host quality and rearing conditions, indicating developmental plasticity. However, comparative morphological data across habitats and host species remain limited and warrant further investigation. The successful development of *T. pupivorus* on pupae of *G. mellonella* demonstrates that this species can utilize alternative hosts under laboratory conditions. Developmental durations of the egg, larval, and pupal stages were generally consistent with previous reports, although moderate variation among studies was observed. Similar influences of environmental and host-related factors on parasitoid development and progeny production have been widely documented (Li et al. 2015; Štefková et al. 2017; Yi et al. 2019).

A pronounced female-biased sex ratio was recorded in this study. Female bias is a common trait among gregarious parasitoids and is generally associated with adaptive reproductive strategies that maximize population growth and host exploitation efficiency. Consistent with earlier studies, adult females lived longer than males (Mahadi et al. 2020; Le et al. 2023), reflecting sex-specific life-history strategies related to oviposition and host utilization. Previous studies have shown that adult *T. pupivorus* exhibits host exploration and parasitism behaviors (Mahadi et al. 2019). Parasitoid wasps can detect hosts using chemical cues, and neural receptors located in the ovipositor may contribute to host recognition (Ruschioni et al. 2015). Sexual dimorphism, particularly in antennal length, likely enhances host detection and mating efficiency in females (Silva et al. 2016). The relatively short developmental period reported across studies, including on *O. arenosella* pupae (Le et al. 2023), further highlights the high reproductive potential of this gregarious parasitoid.

This study was descriptive and conducted during a specific survey period and under one set of controlled laboratory conditions. Therefore, field parasitism rates represent observations within a limited seasonal window, and laboratory biological parameters reflect performance under specific environmental conditions. Seasonal variation and environmental sensitivity may influence these parameters. Moreover, parasitoids reared on factitious hosts can vary in parasitism efficiency and flight ability, as demonstrated for *Trichogramma japonicum* reared on different host eggs (Gowda et al. 2021). Fitness-related traits such as developmental time, adult size, and reproductive output may also vary depending on the host used (Shi et al. 2020). In some cases, reproductive performance and behavioral traits of parasitoids reared on factitious hosts may not correspond to those reared on natural hosts, potentially affecting host acceptance and post-release effectiveness (Malabusini et al. 2023). These findings highlight the need for caution when extrapolating laboratory results to field conditions.

Despite limitations, the consistent field occurrence of *T. pupivorus*, together with its successful development and female-biased progeny on *G. mellonella*, suggests strong potential for augmentative biological control programs targeting *O. arenosella* in coconut orchards. Rapid development, efficient host-searching behavior, and high reproductive output represent ecological traits that may enhance the capacity of this parasitoid to influence host populations in agroecosystems. Future studies should evaluate seasonal dynamics, compare alternative hosts for mass rearing, assess environmental effects on biological performance, and examine the influence of orchard management practices on parasitism under field conditions to support the practical implementation of biological control strategies.

In conclusion, this study demonstrates that *T. pupivorus* was widely detected in coconut orchards of Tra Vinh province during the survey period (December 2024-March 2025), with orchard-level detection reaching 86.7% and a dissection-based parasitism rate of 17.1% across 1,500 examined pupae. These findings indicate a consistent host-parasitoid association during the survey period. Under controlled laboratory conditions (27±1°C, 75±1.5% RH), *T. pupivorus* successfully completed development on *G. mellonella* pupae, exhibiting high emergence success (86.7%), substantial progeny production (mean 462.5 individuals per parasitized pupa), and a strongly female-biased sex ratio. These biological traits suggest that *G. mellonella* pupae may serve as a suitable alternative host for laboratory rearing. Given that the study was conducted within a single seasonal window and under one experimental regime, further multi-season field assessments and environmental sensitivity studies are required. Nevertheless, the field parasitism data and laboratory performance parameters reported here provide baseline information for future research and biodiversity-based pest management discussions involving *T. pupivorus*.

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