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Fruit Fly Parasitoid on Snake Fruit: Morphological and Molecular Identification, Parasitism Rate and Mass Rearing Method Comparison

Willing Bagariang[1]*, Yuris Tiyanto[1], Ani Widarti[1], Atep Budiman[1], Didah Mahmudah[1], Ranti Riama Pardede[1], Novita Tiarasari[2]

[1]Pest Forecasting Institute, Directorate General of Food Crop, Ministry of Agriculture, Karawang
[2]Biology, Faculty of Mathematics and Science, Diponegoro University
**willingbagariang@gmail.com*

Abstract

Fruit flies (Diptera: Tephritidae) are major pests of horticultural crops reducing fruit quality and limiting export opportunities due to quarantine restrictions in many importing countries. Developing environmentally sustainable pest management strategies is therefore essential to enhance the competitiveness of Indonesian horticultural products. This study aimed to identify fruit fly parasitoid on snake fruits, level of parasitism and to find a feasible mass-rearing method under laboratory conditions. Fruit fly parasitoid was collected from snake fruits in Magelang, Central Java and Sleman, Special Region of Yogyakarta followed by laboratory rearing, morphological and molecular identification and comparison of mass rearing methods using natural host fruits and modified artificial diet. Morphological and molecular identification showed that parasitoid parasitized fruit fly on snake fruits was identified as *Fopius arisanus* (Hymenoptera: Braconidae). *F. arisanus* associating with fruit fly that was morphologically identified as *Bactrocera carambolae* (Diptera: Tephritidae). The average field parasitism rate of *F. arisanus* on fruit fly infesting snake fruits was 27.38%, with sex ratio of males and females were 43.89% and 56.11%). Mass rearing method using natural host fruits (snake fruits) showed successfully parasitizing on fruit fly eggs and resulted sex ratio of males and females were 42.1% and 57.9%, whereas rearing method using the modified artificial host showed no parasitizing on fruit fly eggs and should be further studied and developed. These findings support *F. arisanus* as a potential biological control for fruit fly management programs in Indonesia.

Keywords: *Bactrocera carambolae*, Biological Control, *Fopius arisanus*, Java, Parasitism

Abstrak

Lalat buah (Diptera: Tephritidae) merupakan hama utama pada tanaman hortikultura yang dapat menyebabkan penurunan kualitas dan menghambat peluang ekspor terkait peraturan karantina dari negara tujuan. Pengembangan strategi pengelolaan organisme penggandiggu tumbuhan (OPT) yang ramah lingkungan dan berkelanjutan menjadi hal yang esensial untuk meningkatkan daya saing produk hortikultura di Indonesia. Kajian ini bertujuan untuk mengidentifikasi parasitoid lalat buah yang menyerang buah salak, mengetahui tingkat parasitasi dan mendapatkan metode perbanyakan massal parasitoid lalat buah yang dapat diimplementasikan di laboratorium. Pengumpulan parasitoid lalat buah dilakukan dari buah salak di Magelang, Jawa Tengah dan Sleman, D.I. Yogyakarta yang kemudian dibawa ke laboratorium untuk direaring, diidentifikasi secara morfologi dan molekuler, dan dilakukan perbandingan terhadap metode perbanyakan massal parasitoid menggunakan buah inang alami dengan pakan inang buatan yang dimodifikasi. Hasil identifikasi secara morfologi dan molekuler menunjukkan bahwa parasitoid yang memparasitasi lalat buah pada salak adalah *Fopius arisanus* (Hymenoptera: Braconidae). *F. arisanus* berasosiasi dengan lalat buah pada salak dimana hasil identifikasi secara morfologi adalah spesies *Bactrocera carambolae* (Diptera: Tephritidae). Tingkat parasitasi *F. arisanus* pada lalat buah salak rata-rata 27,38% dengan nisbah kelamin jantan dan betina adalah 43,89% dan 56,11%. Perbanyakan massal *F. arisanus* menggunakan buah salak menunjukkan keberhasilan dalam parasitasi telur lalat buah dengan nisbah kelamin parasitoid jantan dan betina adalah 42,1% dan 57,9%, tetapi metode perbanyakan dengan menggunakan media buatan yang dimodifikasi masih belum menunjukkan keberhasilan untuk memparasitasi telur lalat buah dan diperlukan pengembangan metode lebih lanjut. Hasil ini menunjukkan bahwa *F. arisanus* merupakan agens pengendali hayati yang potensial untuk dikembangkan dalam pengelolaan lalat buah di Indonesia.

Kata kunci: *Bactrocera carambolae*, *Fopius arisanus*, Jawa, Parasitisme, Pengendalian Hayati

Introduction

Fruit flies (Diptera: Tephritidae), particularly members of the tribe Dacini, are among the most destructive pests in horticultural systems worldwide. Their infestation causes significant yield losses, increases production costs, and imposes trade restrictions due to international quarantine regulations (Opoku *et al*., 2025; Yuliadhi *et al*., 2022; Zhao *et al*., 2026). Species such as *Bactrocera carambolae* and *B. dorsalis* exhibit a broad host range, high reproductive capacity, and strong invasive potential, enabling rapid spread and intensifying their economic impact across (Deus *et al*., 2024; Handaru *et al*., 2019; Hidayat *et al*., 2023;

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Wijekoon *et al*., 2025; Zhao *et al*., 2026). Moreover, their oviposition inside fruit tissues limits the effectiveness of insecticide-based control, while increasing the risk of resistance development and negative impacts on non-target organisms (Tarno *et al*., 2025). Prolonged reliance on synthetic insecticides can also suppress natural enemy populations, particularly parasitoids and disrupt agroecosystem balance (Tarno *et al*., 2025; Torres & Bueno, 2018). These limitations have increased the need for more sustainable pest management approaches, particularly those based on biological control using parasitoids.

Braconid parasitoids within the subfamily Opiinae are recognized as key natural enemies of fruit flies, with genera such as *Fopius*, *Diachasmimorpha*, and *Opius* commonly associated with *Bactrocera* species in tropical agroecosystems (Garcia *et al*., 2020; Tarno *et al*., 2025). However, their effectiveness is highly variable across ecological contexts, with reported parasitism rates ranging from low to more than 50%, depending on host species, environmental conditions, and host plant characteristics (Amini *et al*., 2024; Chen *et al*., 2018; Haran *et al*., 2019). Among these, *Fopius arisanus* is of particular interest due to its ability to parasitize the egg and the first larval stage, allowing suppression of host populations and reducing the occurrence of fruit damage (Aryuwandari *et al*., 2020; Moquet *et al*., 2023). Due to *F. arisanus* develops in the whole stage of its host and it is also known as egg-pupal parasitoid (Rousse *et al*., 2005). Its successful introduction into Hawaii, USA further demonstrated its high reproductive capacity and stable parasitism performance under tropical conditions (Groth *et al*., 2016), highlighting its potential as a strategic agent in fruit fly management programs. In Indonesia, *F. arisanus* has been reported in association with fruit fly infestations (Muhlison *et al*., 2026; Koswanudin, *et al*. 2025). Although *F. arisanus* has been reported in association with fruit fly infestations, its practical application in biological control and integrated information combining morphological and molecular identification, and parasitism performance are still scarce in Indonesia.

Mass rearing of *F. arisanus* using artificial host substrate reported successfully to control fruit fly population in, Hawaii, USA (Manaokis *et al.* 2011) and Afrika (Vergas *et al.* 2016). However, developing parasitoid as biological control agent for fruit fly was still limited in Southeast Asia including Indonesia. The mass production method and the availability of host are essential aspects for a stable mass rearing of parasitoids. Laboratory of Vapor Heat Treatment, Pest Forecasting Institute, Ministry of Agriculture has been maintaining mass rearing of some fruit fly species under laboratory conditions for many years. However, utilization of parasitoids as biological control agent for fruit fly and mass rearing of parasitoids remains limited including in controlling fruit fly infestation in snake fruit orchards. Therefore, this study aimed to identify parasitoids associated with fruit flies infesting snake fruits, to study its parasitism rate and to compare two mass rearing methods i.e. using natural host fruits and modified artificial diet that has been successfully implemented for mass rearing of *F. arisanus* (Manaokis *et al.* 2011; Vergas *et al.* 2016).

Materials and Methods

Parasitoid collection

Parasitoid collection was carried out in the the area of snake fruit orchards at Wonokerto Village, Turi District, Sleman, Yogyakarta Special Region (-7,6262S, +110,3831E, 580 m asl) on May 2025 and Kaliurang Village, Srumbung District, Magelang, Central Java (-7,6065S, +110,3652E, 611 m asl) on August 2025. The variety of snake fruits collected from Srumbung, Magelang was Salak Nglumut and the variety of snake fruit collected from Turi, Sleman was Salak Pondoh. The maturty of snake fruit collected was 60-70%. To get a sufficient amount of snake fruits infested by fruit fly and availability of infested snake fruits by fruit fly in orchads, the collection of snake fruit took place at the packing house around the orchard. Around 20-30 kg per of snake fruits showing symptoms of fruit fly infestation were collected from each location. The snake fruits were placed in the fruit containers and transported to Vapor Heat Treatment Laboratory, Pest Forecasting Institute.

Morphological Identification

Fruit flies and parasitoids emerging from infested snake fruits were identified based on morphological characteristics. Specimens were observed using an Olympus SZX7 stereo microscope equipped with an Olympus DP23 digital camera. Fruit flies were identified following The Australian Handbook for the Identification of Fruit Flies (Plant Health Australia, 2018), while parasitoids were identified using Hymenoptera of the World: An Identification Guide to Families (Goulet & Huber, 1993) and Opiine parasitoids (Hymenoptera: Braconidae) of tropical fruit flies (Diptera: Tephritidae) of the Australian and South Pacific region (Carmichael *et al*., 2005).

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Molecular Identification

Molecular identification was performed through DNA extraction, amplification, visualization, and sequencing of the mitochondrial cytochrome c oxidase subunit I (COI) gene. Genomic DNA was extracted from parasitoids emerging from snake fruits using the DNeasy® Blood and Tissue Kit (Qiagen) following the manufacturer's instructions (Zida *et al*., 2022).

PCR amplification was carried out using using primers: LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTGG -3') and HCO 2198 (5'-TAA ACT TCA GGGTGA CCA AAA AAT CA-3') with a product length of 650 bp. PCR reaction used Cytiva Lifescience™ illustra™ PuReTaq Ready-To-Go™ PCR Beads in a total reaction volume of 25 µL, consisting of 20.5 µL sterile ddH_2O, 1.25 µL of each primer (10 pmol), and 2 µL of DNA template. For sequencing purposes, the reaction volume was increased to 50 µL. PCR conditions included an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 51°C for 30 s, and extension at 72°C for 60 s, with a final extension at 72°C for 10 min and a holding temperature at 4°C. PCR products were visualized using 2% agarose gel electrophoresis prepared in 1× TAE buffer. Electrophoresis was conducted at 94 V for 45 min, followed by staining with SYBR Green and visualization under UV transillumination to confirm the expected fragment size (~650 bp).

One PCR product was sent to First BASE Laboratories (Malaysia) for sequencing. The obtained sequences were analyzed using Bio Edit program ver. 7.2.6 for windows (Hall, 1999). Forward and reverse chromatograms were manually inspected, and low-quality bases at both ends of the sequences were trimmed prior to sequence assembly. Sequence quality was evaluated based on chromatogram readability and the absence of ambiguous nucleotide calls. After editing and quality control, a consensus sequence of 619 bp was obtained. The sequences then submitted to Basic Local Alignment Search Tool (BLAST) against the GenBank database (NCBI) to confirm possible species identity.

Phylogenetic analysis was conducted using MEGA version 10. Reference sequences were selected based on taxonomic relevance, sequence completeness, and the availability of high-quality COI barcode sequences. A Neighbor-Joining (NJ) phylogenetic tree was constructed using the Kimura 2-parameter (K2P) evolutionary-distance model (Gaduaa & Kareem, 2023). Gaps and missing data were treated using the pairwise deletion option. Branch support was assessed through bootstrap analysis with 1,000 replicates. The phylogenetic tree was rooted using *F. vandenboschi* and *Opius* species as the outgroup obtained from NCBI.

Field Parasitism Rate, Sex Ratio, and Longevity of Parasitoids

The snake fruits collected from field showing symptoms of being attacked by fruit flies with sign of the fruit rots were evaluated in the laboratory to calculate the field parasitism rate, sex ratio and adult longevity. The amount of snake fruits used for parasitism rate were 30 snake fruits from Magelang and 30 snake fruits from Sleman. Each snake fruit was individually placed in a plastic container (13 cm in length x 13 cm in width and 10 cm in height) containing rice husk as a pupation medium. Parasitoids emerging from snake fruit were provided with cotton soaked in water and a 10% honey solution as a food source. Observations were conducted daily to record the number of emerging fruit flies and parasitoids, determine the sex ratio, and parasitoid's longevity. The parasitism rate was calculated using the following formula (Tarno *et al*., 2025; Vayssires *et al*., 2012)

$$Parasitism\ rate: = \frac{\sum Parasitized\ host}{(\sum Parasitized\ host + \sum Unparasitized\ host)}\ x\ 100\%$$

The observation of adult longevity was conducted on snake fruits collected from Magelang with 30 snake fruits indicating fruit fly infestation. Because of number of parasitoids emerging from each snake fruit can more than one adult, we considered the day of adult emerging, number of adults emerging each day and the day of dead.

Comparison of mass rearing method for parasitoid

The comparison of mass rearing methods for fruit fly parasitoids was conducted to identify an effective and sustainable approach for parasitoid production. Two rearing methods were tested: modified artificial host diet following the method described by Manoukis *et al*., (2011) and Vergas *et al*. 2016 against the use of snake fruit as natural host substrate. Parasitoids used in this experiment were obtained from the first generation emerging from infested snake fruits collected in the field.

Oviposition methods used in modified artificial diets are as follows: fruit fly eggs collected and put on petri dish covered with filter paper then exposed to *F. arisanus* for 21-24 hours. The eggs on agar then were transferred to larval diet of fruit fly and then transferred to pupation box (36 cm in length x 36 cm in width x 36 cm in height) containing sawdust as a pupation medium after 5 days. However, oviposition methods using natural oviposition are as follows: snake fruits were inserted into fruit fly cage

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containing ±2500 flies, then exposed to *F. arisanus* for 21-24 hours and transferred to pupation box.

Larvae that developed into pupae were separated from sawdust and transferred into plastic emergence boxes covered with a 12-mesh wire lid. This system enabled the separation of emerging insects, with fruit flies remaining inside the box, while parasitoids exited through the mesh and moved into the rearing cage (45 cm in length x 45 cm in width x 90 cm in height). Adult parasitoids were provided with cotton soaked in water and a 10% honey solution as a food source. The observation of each method was conducted daily until the emergence of parasitoid.

Results and Discussion

Morphological and Molecular Identification

Parasitoids emerging from infested snake fruits were morphologically identified as *F. arisanus* (Hymenoptera: Braconidae). *F. arisanus* identified showed a pterostigma on fore wing, metasoma is black, apex of ovipositor lacking dorsal ridges and distinctly constricted subapically, frons and vertex rugose-punctate, tergum-2 densely striate, notauli crenulate, filiform antennae exceeding body length, body was dominantly black and hind tibia dorso-posteriorly without basal carina. This morphological character corresponds closely with published description of *F. arisanus* reported by Adnyana *et al*., (2019) and Carmichael *et al*., (2005), supporting the reliability of taxonomic identification. Based on morphometric measurements the body length of *F. arisanus* is 3.86 mm on average, head width is 1.12 mm on average, wing length is 3.47 mm on average, and the antennae length is 5.04 mm on average (**Table 1**). *F. arisanus* has antennae length that is longer than body length. The morphometric comparison between male and female *F. arisanus* showed not significantly different (P>0,05) in body length, wing length and head width, however its antennae length showed significantly (P<0,05) between male and female *F. arisanus* (**Figure 3**). This is consistent with the findings of King *et al*. (2018) who reported that male antennae are longer than female, which function in mate searching and pheromone detection. Males also tend to emerge earlier than females, which increases mating opportunities shortly after female emergence.

The association between fruit fly and *F. arisanus* across sampling locations figured host–parasitoid relationship within snake fruit orchard in sampling area (Magelang and Sleman). Fruit flies and their associated parasitoids from snake fruit in Sleman (DI Yogyakarta) and Magelang (Central Java) showed a consistent species composition across locations. Fruit flies emerging from *snake fruit* were identified as *B. carambolae* (Diptera: Tephritidae) based on morphological keys provided in The Australian Handbook for the Identification of Fruit Flies (Plant Health Australia, 2018). This identification was supported by several diagnostic characters, including costal band slightly overlapping R2+3 and expanding around R4+5, may appear fishhook like, rectangular shaped band on anterolateral corners tergum IV, terga III-V with a wraparound T pattern, black scutum and broad parallel sided lateral vittae enclosing intra alar setae (**Figure 4**).

Morphological identification indicated that the parasitoid associated with fruit flies infesting snake fruit was *F. arisanus*. However, species within the subfamily Opiinae exhibit considerable morphological similarity, which can limit the reliability of identification based solely on external characters. This is particularly relevant in regions where closely related species, such as *Fopius vandenboschi*, are also present. To minimize the risk of misidentification and ensure accurate ecological interpretation, molecular confirmation was conducted using the mitochondrial cytochrome c oxidase subunit I (COI) gene marker.

Molecular analysis indicated that the parasitoid associated with snake fruit belongs to *F. arisanus*. The result of BLAST showed 100% query coverage and 94.83% similarity with *F. arisanus* sequences in GenBank. It was further supported by the phylogenetic tree (**Figure 5**), which showed that *F. arisanus* collected from snake fruits clustered within the *F. arisanus* clade together with reference sequences KY689074.1, KY689081.1, KY689077.1, and JN640568.1. The isolate was most closely associated with *F. arisanus* sequence JN640568.1, with a bootstrap value of 94%, whereas the broader *F. arisanus* clade received bootstrap support of up to 99%. In contrast, *Opius* sp. and *F. vandenboschi* formed distinct lineages outside the *F. arisanus* cluster. Together with the morphological characteristics observed, these results support the identification of the parasitoid as *F. arisanus*.

Phylogenetic analysis showed that the obtained sequences consistently clustered with reference sequences of *F. arisanus* from the GenBank database, supported by high bootstrap values (99–100%). The sequences were clearly separated from those of *Opius* sp. and *F. vandenboschi*, indicating clear species-level differentiation. The strong bootstrap support (>90%) confirms the robustness of the inferred phylogenetic relationships and provides reliable evidence for species identification. These results validate morphological identification and confirm that the parasitoid associated with snake fruit in this study is *F. arisanus*. Notably, the local isolate did not form a distinct clade separate from reference sequences, suggesting a high degree of genetic

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similarity with previously reported populations. This pattern indicated low nucleotide variation and reflected a close evolutionary population (Sujaya *et al*., 2016). Such genetic similarity may be associated with population connectivity or a relatively stable genetic structure across regions. However, further analysis using additional molecular markers is required to better resolve population-level variation and genetic diversity within *F. arisanus*.

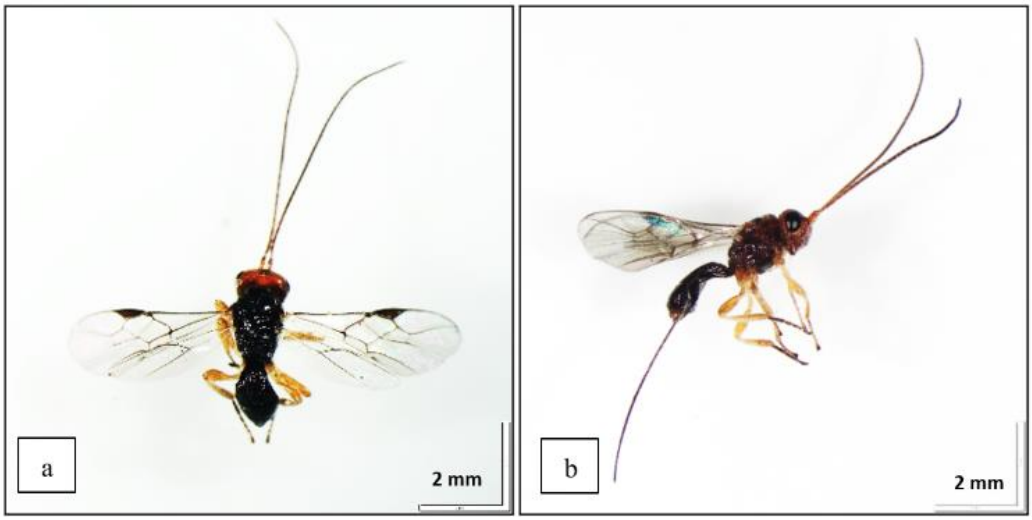

Figure 1. Morphological characteristics of *F. arisanus* (Hymenoptera: Braconidae) Male (a) and Female (b)

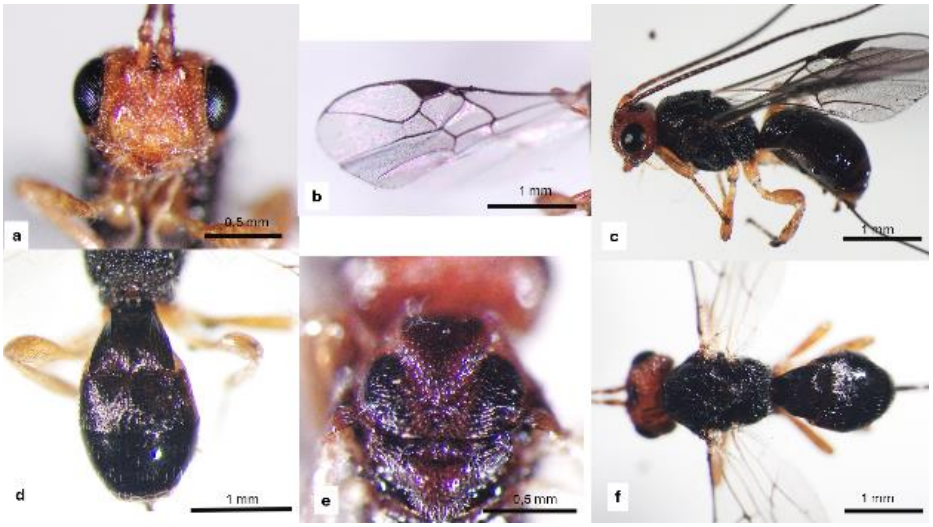

Figure 2. Morphology of *F. arisanus*: head (a); forewing (b); lateral view of body (c); metasoma (d); mesosoma (e); dorsal view of body

Table 1. Measurement of body length, head width, wing length and antennae length of *F. arisanus* collected from snake fruit

Character	Size (mm)		
	Mean	±	SE
Body length	3.86	±	0.05
Head width	1.12	±	0.01
Wing length	3.47	±	0.04
Antennae length	5.04	±	0.07

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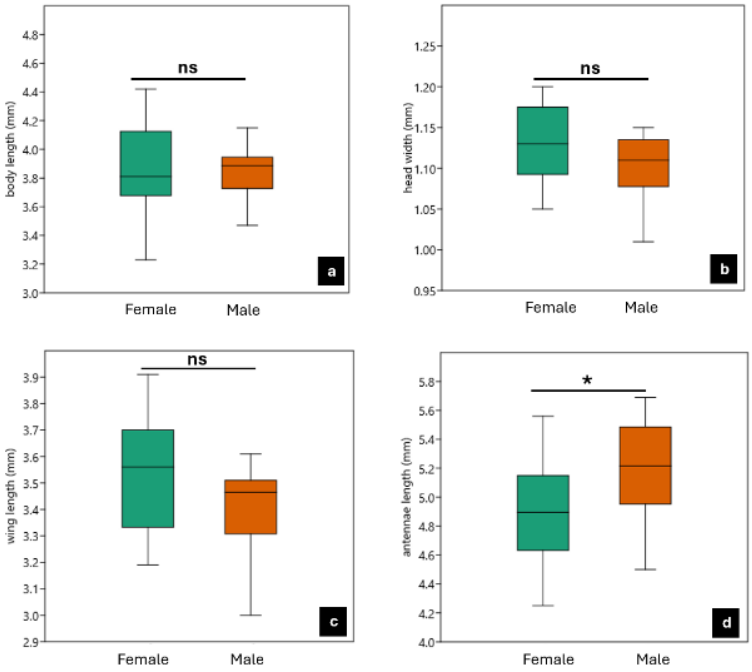

Figure 3. Comparison of body length (a), head width (b), wing length (c) and antennae length (d) of female and male *F. arisanus* collected from snake fruit (ns: not significantly different, $P > 0,05$; *: significantly different, $P < 0,05$)

Figure 4. Morphological characteristics of *B. carambolae*: thorax, parallel yellow vittae enclosing i.a setae (a); wing, costal band slightly overlapping R2+3 and expanding around R4+5 (b); abdomen, rectangular shaped band on antero lateral corners tergum I V, terga III-V with a wraparound T pattern (c)

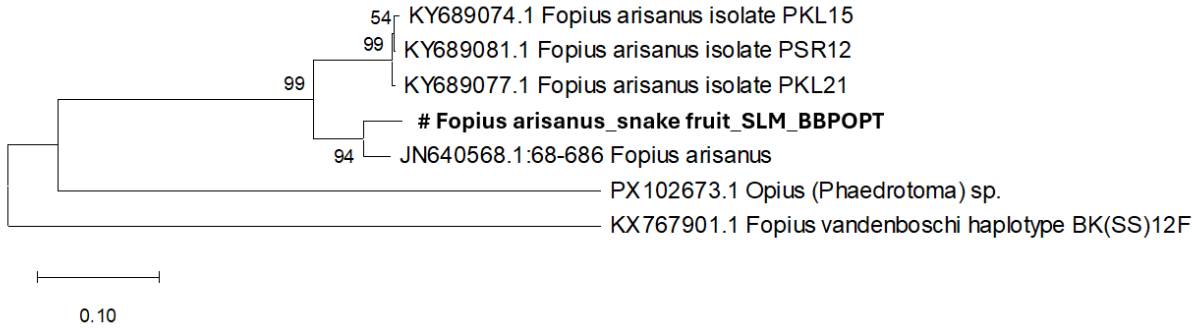

Figure 5. Neighbor-Joining (NJ) phylogenetic tree based on COI sequence analysis using the Kimura 2-parameter (K2P) evolutionary-distance model. Some *F. arisanus* sequences were also included, such as KY689074.1, KY689081.1, KY689077.1 and JN640568.1. Sequence of Opius sp. (PX10267.3) and *Fopius vandenboschi* (K767901.1) were used as out group. **#:** *F. arisanus* collected from snake fruit.

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Field Parasitism Rate, Sex Ratio, and Longevity of The Parasitoid

Observation of snake fruit samples infested by fruit fly showed that 58.5% had the fruit fly parasitoid, *F. arisanus* and 41.5% had no F. *arisanus* (**Figure 6**). This data indicated the established relationship between *F. arisanus* and its host in sampling area. The average fruit fly found of each infested snake fruit was 17.6 individuals per fruit, whereas the average *F. arisanus* found of each snake fruit was 6.66 individuals per fruit and the overall parasitism rate of *F. arisanus* was 27.38% (**Table 2**). This level of parasitism suggested that *F. arisanus* functions primarily as a population regulator rather than a complete suppressor of fruit fly populations. While a parasitism rate of 27.38% represents a meaningful contribution to population reduction, it is unlikely to provide full control in the absence of complementary management strategies. Moquet *et al*. (2023) reported that parasitism by *F. arisanus* is strongly influenced by tri-trophic interactions among parasitoids, *Bactrocera* hosts, and host plants.

In this study, parasitism rate of *F. arisanus* was higher in Magelang (32.56%) than in Sleman (21.58%), indicating local variation in host–parasitoid dynamics. These differences are likely driven by ecological factors such as host density, habitat conditions, and the availability of nutritional resources, all of which influence host-searching efficiency and parasitism success. Together, these findings highlight that the effectiveness of *F. arisanus* is context-dependent and closely linked to environmental conditions. The parasitoid population exhibited a female-biased sex ratio, with 56.11% females and 43.88% males. A female-biased sex ratio is advantageous for biological control, as only females contribute directly to host suppression through oviposition and the previous studies reported that parasitism success is closely associated with the proportion of female parasitoids in the population (Muliani & Srimurni, 2022) In haplodiploid systems such as Braconidae, sex allocation is often influenced by host quality and environmental conditions that favor the production of female offspring (Godfray, 1994). Adult longevity of *F. arisanus* ranged from 6 to 20 days, with a mean lifespan of approximately 12 days (**Figure 7**). Longevity is a key determinant of parasitoid performance, as it directly influences reproductive output and host exploitation capacity. Previous studies have shown that longer lifespan enhances reproductive potential (Groth *et al*., 2016), while the persistence of adult parasitoids under favorable conditions is critical for maintaining effective biological control in the field (Harris *et al*., 2022). Overall, the result showed the moderate parasitism rates, a female-biased sex ratio, and a relatively long adult lifespan indicated that the local population of *F. arisanus* collected from snake fruits has potential as a biological control agent. However, its impact is likely to be regulatory rather than suppressive, suggesting that to achieve the optimal control outcomes would require integration with other pest management strategies.

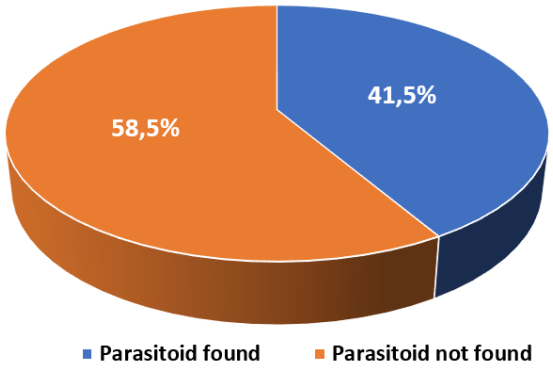

Figure 6. Proportion of snake fruits containing parasitoids collected from Magelang, Cetral Java and Sleman, Special Region of Yogyakarta (n=53)

Table 2. Parasitism rate and sex ratio of *F. arisanus* collected from snake fruits in Sleman and Magelang

Location	Fruit Fly (individual/fruit)	Parasitoid (individual/fruit)	Parasitism Rate (%)	Sex Ratio Male: Female (%)
	mean ± SE	mean ± SE	mean ± SE	
Sleman, DI Yogyakarta	21.52 ± 2.82	7.20 ± 2.10	21.58 ± 5.74	38.85 : 61.14
Magelang, Jawa Tengah	14.11 ± 2.47	6.18 ± 1.70	32.56 ± 6.36	49.37 : 50.62
Total	17.60 ± 1.92	6.66 ± 1.33	27.38 ± 4.34	43.88 : 56.11

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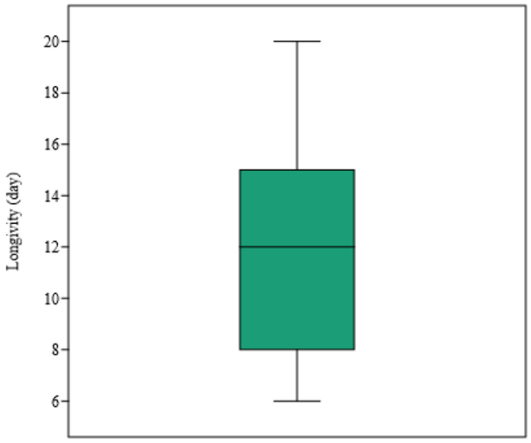

Figure 7. Boxplot of adult *F. arisanus* longevity emerging from snake fruits under laboratory conditions (n=30)

Comparison of Mass-Rearing Methods for F. arisanus

The comparison of mass-rearing methods revealed a clear contrast between modified artificial host substrate and natural host fruits (**Table 3**). Mass rearing method using natural host fruits (snake fruits) was successfully implemented under laboratory conditions. This study resulted the consistent sex ratio of female that was higher than male (female was 57.9 % and male was 42.1 %). In contrast, the use of modified artificial host substrate by placing fruit fly eggs on agar exposed to *F. arisanus* resulted in unsuccessful parasitism. However, Manaokis *et. al* (2011) reported that *F. arisanus* can oviposited the fruit fly eggs on agar. The successful oviposition of *F. arisanus* might depend on volatile compounds produced by fruit tissues infested with fruit fly eggs, while artificial agar does not produce these volatile cues. Pérez *et al*., (2013) reported that female *F. arisanus* were attracted to fruits containing fruit fly eggs rather than eggs alone. In addition, *F. arisanus* can discriminate against host eggs through olfactory cues. Therefore, agar-based media may be recognized as non-host substrates because they lack specific chemical signals associated with fruit fly oviposition in fruit tissues. We suggested that method using non host substrate or artificial medium should be further studied to find the methods that support the *F. arisanus* to parasitize the fruit fly eggs as reported by Manoukis *et al*. (2011). The effectivity of a parasitoid in locating its host is largely determined by its ability to search for the host. This host-searching behavior can be divided into three steps: host habitat location, host location, and host acceptance. During the host selection, the parasitoid utilizes cues involving visual, vibrations, and chemical cues to locate its host (Fatouros, *et al.* 2008). Therefore, further development methods should investigate the use of chemical compounds such as the addition of host fruit juice to attract parasitoids in locating their hosts. The use of oviposition-induced host plant cues indicating the presence of the host could be a solution in host searching of *F. arisanus*.

The successful method using natural host (snake fruits) indicated that reproductive success is strongly influenced by ecological cues associated with the natural host environment. Ecologically, egg-pupal parasitoids such as *F. arisanus* operate within a tri-trophic system, where host location and acceptance are mediated by a combination of visual, mechanical, and chemical cues. Volatile compounds released from infested fruits or induced during fruit fly oviposition act as kairomones that guide host-searching behavior and stimulate oviposition. Previous studies have demonstrated that female *F. arisanus* respond selectively to such oviposition-induced volatiles, resulting in increased parasitism success (Cai *et al*., 2020; Moquet *et al*., 2023). This strong dependence on natural cues is consistent with previous findings that the effectiveness of *F. arisanus* is closely linked to habitat conditions that provide both chemical signals and nutritional resources for adult parasitoid (Harris *et al*., 2022). Therefore, the success of mass rearing systems depends not only on host availability but also on their ability to mimic the ecological complexity of natural host environments.

To support the development of a practical mass rearing of *F. arisanus* using natural host, we suggested following a sequential process. The first step is the fresh snake fruits were chosen and punctured with a needle before being exposed to *B. carambolae* to allow oviposition. We conducted 5 punctures per fruit to make it easier for the fruit fly to lay eggs inside the snake fruits. Approximately 1 kg of snake fruits placed in a cage containing ±2,500 adult fruit flies, then oviposition of fruit flies on snake fruits were allowed for about 30 minutes. We

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did not suggest exposing snake fruits for more than 30 minutes to avoid the fruit flies laying more eggs inside the snake fruits. Snake fruits containing fruit fly eggs were subsequently transferred into a parasitoid cage and exposed to *F. arisanus* for 21-24 hours under laboratory conditions (26–28 °C). Then, snake fruits were moved to pupation box containing sawdust to support larval development and pupation. After 7 days or the last stage of larva, the skins of snake fruit were peeled to make it easier for larvae to become pupae. When the larvae became pupae, the pupae were sieved and transferred to a screening box covered with 12-mesh wire lids. The screening box is used to separate *F. arisanus* and fruit flies. The screening box was placed in the parasitoid cage. Adult parasitoids were provided with a food source consisting of cotton soaked in water, a 10% honey solution, and a mixture of autolyzed yeast and sugar (1:3). The colonies of *F. arisanus* were maintained in an air-conditioned room at 26–28 °C.

These findings indicated that mass-rearing based on natural host substrates remain the most reliable approach for maintaining parasitoid propagation despite its limitations such as the availability of fresh fruits. Developing a standardized mass rearing method for *F. arisanus* remains a major challenge in Indonesia. The selection of oviposition methods, artificial diet, good mass propagation techniques for fruit fly parasitoids and appropriate augmentation techniques need to be taken into consideration in the development of fruit fly parasitoids. Parasitoid of fruit fly, *F. arisanus* was reported successfully reared in small scale without using fruits in Hawaii USA (Manoukis *et al*., 2011) and introduced and reared in Benin and Sinegal, Africa to control oriental fruit fly (Vargas *et al*., 2016). This is an opportunity to start mass rearing for *F. arisanus* in Indonesia. Recent studies in Indonesia showed successful fruit fly parasitoid conservation using augmentation devices as an environmentally friendly sanitation method for controlling fruit fly on chili (Muhlison *et al*., 2026). This augmentation technique can be implemented for controlling fruit fly on fruits. Another aspect that is taken into consideration for a stable mass rearing parasitoids is the availability of host (Mastrangelo *et al*., 2021). Laboratory of Vapor Heat Treatment, Pest Forecasting Institute, Ministry of Agriculture has been maintaining mass rearing of four species of fruit fly such as *B. dorsalis, B. carambolae, B. albistrigata and Zeugodacus cucurbitae* for many years. This is an advantage to develop *F. arisanus* as a biological control agent. These results were a preliminary study, the improvement of oviposition methods, diet formulation and field performance study should be supported to be developed to bring biological control implemented in fruit fly management in Indonesia.

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Table 3. Comparison of mass-rearing methods for *F. arisanus* under laboratory conditions.

Medium	Host (Fruit Fly Species)	Oviposition Observation	Emerging Insects		Remarks
			Fruit Fly	Parasitoid	
Modified artificial host diet (fruit fly eggs on agar + artificial larval diet)	*B. carambolae*	Parasitoids did not parasitize the fruit fly eggs on agar	Yes	No	Further study is required to determine methods of attracting parasitoids to parasitize fruit fly eggs on agar reported by Manaoukis *et al*. 2011 and Vergas *et al*. 2016
Natural host fruit (snake fruit)	*B. carambolae*	Parasitoids successfully parasitized the fruit fly eggs inside snake fruits	Yes	Yes	This method can be used for the mass rearing of *F. arisanus* under laboratory conditions

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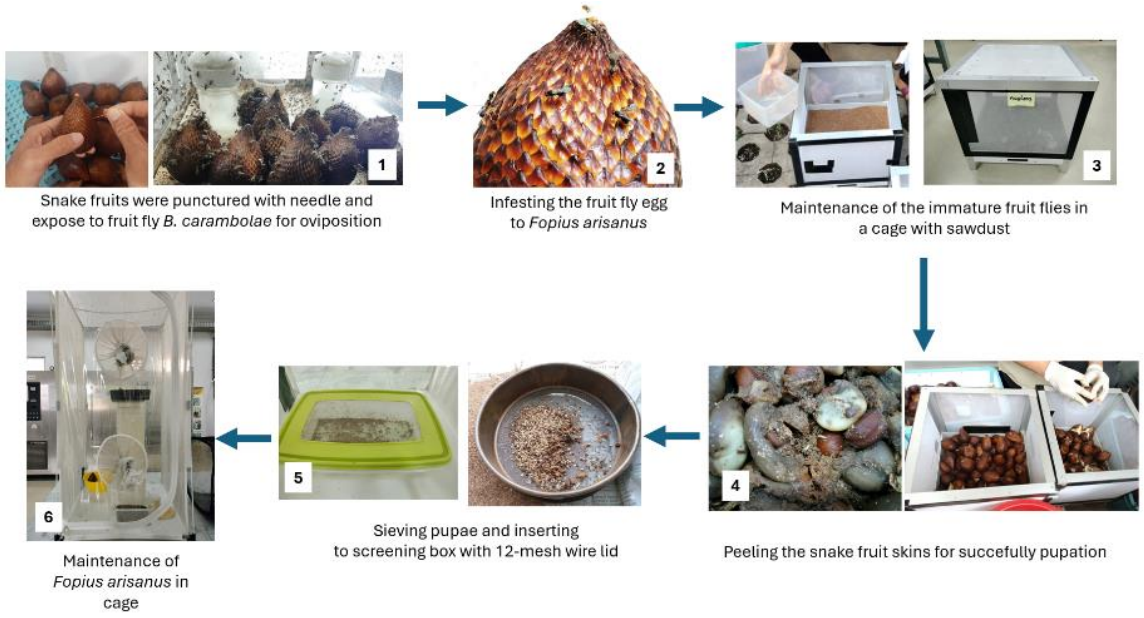

Figure 8. Mass rearing of *F. arisanus* using snake fruits under laboratory condition

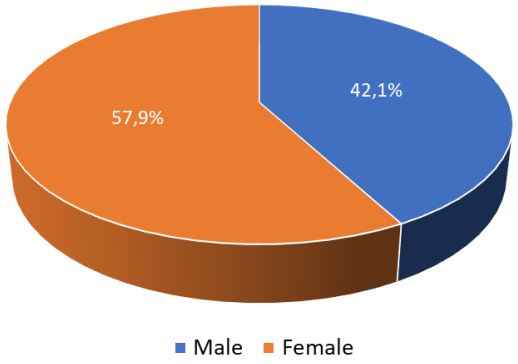

Figure 9. Sex ratio of *F. arisanus* parasitoids reared on snake fruits under laboratory conditions (n=76)

Conclusion

This study showed that parasitoid parasitized fruit fly on snake fruits was identified as *F. arisanus* based on morphological and molecular identification. *F. arisanus* had a role as parasitoid of *B. carambolae* infesting snake fruits in Magelang, Central Java and Sleman, Special Region of Yogyakarta. *F. arisanus* collected from snake fruits showed field parasitism rate of 27.38% on average and sex ratio of males were 43.89% and females were 56.11%. Mass rearing of *F. arisanus* using natural host fruits (snake fruits) showed sex ratio of males were 42.1% and females were and 57.9%. Natural oviposition using snake fruits resulted in parasitizing the fruit fly eggs. However, artificial oviposition using fruit fly eggs placed on agar resulted in no parasitizing. The mass rearing method using natural host fruits (snake fruits) can be used for parasitoid propagation under laboratory conditions, despite its limitations such as the availability of host fruits. This study was preliminary study therefore we suggested that mass rearing method for *F. arisanus* should be developed to produce a high parasitoid population, a stability of mass rearing and augmentation method on field as well. Further development of a standardized mass rearing method for *F. arisanus* is very essential to support large-scale program for fruit fly controlling in Indonesia.

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