

## Species Diversity of Fruit Flies (Diptera: Tephritidae) and Their Associated Parasitoids in Bali Province, Indonesia

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**ABSTRACT:** Fruit flies (Diptera: Tephritidae) are among the most destructive pests of horticultural crops in Bali Province, Indonesia, causing significant losses in fruit quality and yield. Indigenous parasitoids have considerable potential as biological control agents; however, comprehensive information on fruit fly diversity and their associated parasitoids throughout Bali remains limited. This study aimed to identify fruit fly species using morphological and molecular approaches, determine the associated parasitoid species, and evaluate their parasitism rates. The study was conducted from October 2025 to March 2026 across all regencies and the municipality of Bali Province. Fruit samples showing symptoms of fruit fly infestation were collected using purposive sampling and reared under laboratory conditions to obtain adult fruit flies and parasitoids. Morphological identification was carried out at the Integrated Plant Pest and Disease Control Laboratory, Faculty of Agriculture, Universitas Udayana, while molecular identification was performed using mitochondrial cytochrome c oxidase subunit I (COI) DNA barcoding. A total of 5,769 adult fruit flies were obtained, comprising *Bactrocera dorsalis* (3,127 individuals), *B. carambolae* (2,448 individuals), and *B. umbrosa* (194 individuals). Molecular analysis confirmed the identity of all three species through comparison with GenBank reference sequences. Two parasitoid genera belonging to the family Braconidae, *Fopius* sp. and *Opius* sp., were recovered from infested fruits. *Fopius* sp. exhibited the highest overall parasitism rate (11.03%), whereas *Opius* sp. showed a parasitism rate of 1.98%, resulting in a total parasitism rate of 13.01%. These findings provide updated baseline information on the diversity of fruit flies and their associated parasitoids and support the development of location-specific integrated pest management (IPM) strategies for sustainable fruit fly control in Bali.

**KEYWORDS:** *Bactrocera*; Bali; fruit flies; parasitoids; DNA barcoding; parasitism rate.

### I. INTRODUCTION

Indonesia is one of the world's leading tropical countries with highly favorable climatic conditions for horticultural production. Its location along the equator provides abundant sunlight, warm temperatures, and relatively high rainfall throughout the year, creating an ideal environment for cultivating a wide variety of fruit crops. Horticultural commodities play an important role in supporting the national economy through domestic consumption, export opportunities, and income generation for farmers. As consumer demand for high-quality fresh fruits continues to increase, improving both fruit quantity and quality has become a major priority in sustainable agricultural production [11]. However, maintaining fruit quality remains a significant challenge because production is frequently constrained by various insect pests and plant diseases that reduce yield and market value [2].

Among the most destructive insect pests affecting fruit crops are fruit flies (Diptera: Tephritidae), which are widely recognized as major pests of horticultural commodities worldwide. Fruit flies are highly polyphagous insects capable of infesting numerous cultivated and wild host plants, enabling them to survive throughout the year under tropical conditions [9]. Female fruit flies lay their eggs beneath the fruit epidermis using a specialized ovipositor, and the developing larvae feed on the fruit pulp, causing internal damage, premature fruit drop, and rapid fruit decay [14]. Such infestations not only reduce fruit quality and marketability but also limit access to export markets because many fruit fly species are classified as quarantine pests. Consequently, fruit fly infestations have become one of the major biological constraints affecting fruit production in Indonesia [16].

The family Tephritidae comprises approximately 4,000 described species worldwide, with the genus *Bactrocera* representing one of the most economically important groups distributed throughout tropical and subtropical regions [3,19]. Several *Bactrocera* species have been reported as major pests in Indonesia, including *Bactrocera dorsalis*, *B. carambolae*, *B. umbrosa*, *B. albistrigata*, *B. cucurbitae*, and *B. tau* [10]. These species exhibit broad host ranges, high reproductive capacity, and remarkable adaptability to

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different environmental conditions, allowing them to establish persistent populations in fruit-growing areas. Yield losses caused by fruit fly infestation may range from 30% to 100%, depending on host species, fruit maturity, environmental conditions, and pest population density [13]. Economically important fruit crops such as mango, citrus, guava, star fruit, jackfruit, wax apple, avocado, chili pepper, and melon are particularly vulnerable to fruit fly attack [15]. Management of fruit flies has traditionally relied on synthetic insecticides because they provide rapid suppression of pest populations. However, continuous pesticide application has raised concerns regarding insecticide resistance, environmental contamination, pesticide residues on agricultural products, and adverse effects on beneficial organisms. These limitations have encouraged the adoption of environmentally sustainable pest management strategies, particularly integrated pest management (IPM), which combines multiple compatible control methods. Among these approaches, biological control using parasitoids has become one of the most promising alternatives because parasitoids naturally suppress fruit fly populations without causing harmful environmental impacts [19,1]. Parasitoids associated with fruit flies are predominantly members of the family Braconidae, including species of *Fopius*, *Opius*, and *Diachasmimorpha*, which attack immature stages of fruit flies and contribute significantly to natural population regulation [1].

Accurate species identification is fundamental for developing effective pest management strategies because different fruit fly species may differ in host preference, geographic distribution, and susceptibility to biological control agents. Morphological identification remains the standard method for species determination; however, closely related *Bactrocera* species often exhibit overlapping morphological characteristics that complicate accurate identification. Therefore, molecular identification using mitochondrial cytochrome c oxidase subunit I (COI) DNA barcoding has become a reliable complementary approach for confirming species identity and improving taxonomic resolution. The integration of morphological and molecular approaches provides robust information for biodiversity assessment, pest surveillance, and biological control programs. Although several studies have reported fruit fly species and their parasitoids in specific locations of Bali, comprehensive information covering all regencies and cities in the province remains limited. Moreover, studies integrating morphological identification, molecular confirmation using COI DNA barcoding, parasitoid diversity, and parasitism rates across multiple host plants are still scarce. Such information is essential for understanding the distribution of fruit fly species and their natural enemies, thereby supporting the development of effective location-specific integrated pest management programs. Therefore, this study aimed to inventory fruit fly species associated with fruit crops throughout Bali Province using morphological and molecular identification based on the mitochondrial COI gene, identify the parasitoid species associated with fruit flies, and determine their parasitism rates. The results are expected to provide updated baseline information for developing sustainable and environmentally friendly fruit fly management strategies in Bali.

## II. MATERIALS AND METHODS

### Research Procedure

his study was conducted from October 2025 to March 2026 in fruit production areas throughout Bali Province, Indonesia. Fruit rearing, fruit fly and parasitoid identification were carried out at the Plant Pest Laboratory, Faculty of Agriculture, Udayana University, while molecular analysis and DNA sequencing were conducted at PT Genetika Science, Jakarta. The equipment and materials used included a stereomicroscope, GPS, PCR and electrophoresis equipment, GeneJET Genomic DNA Purification Kit, DreamTaq Green PCR Master Mix, agarose, TBE buffer, 80% ethanol, and other supporting laboratory materials. Field surveys were conducted in nine regencies and one municipality of Bali Province. Fruit samples were collected using a purposive sampling method by selecting fruits showing symptoms of fruit fly infestation, including oviposition punctures, discoloration, fruit decay, or the presence of larvae. The geographic coordinates of each sampling site were recorded using a Global Positioning System (GPS). Each sample was labeled with the sampling location, collection date, and host plant species before being transported to the laboratory for rearing.

Each infested fruit was reared individually in a transparent plastic container containing sand as a pupation medium until adult fruit flies and parasitoids emerged. Adult fruit flies were identified based on morphological characteristics of the head, thorax, abdomen, and wing venation using the identification keys [6,16]. Molecular identification was performed using the mitochondrial cytochrome c oxidase subunit I (COI) gene as a DNA barcode marker. DNA was extracted, amplified by PCR, and sequenced. The obtained sequences were compared with reference sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST), and phylogenetic relationships were reconstructed using the Neighbor-Joining method.

Parasitoids emerging during the rearing process were identified morphologically using the taxonomic guide [8]. The observed variables included the number and species of fruit flies, the number and species of parasitoids, and the parasitism rate. Parasitism was calculated according to [4]. Sequence data were edited using BioEdit version 7.2.5 and analyzed using BLAST and phylogenetic analysis based on the Kimura two-parameter model with 1,000 bootstrap replications in MEGA version 11 [18]. All data on fruit flies, parasitoids, and host plants were analyzed descriptively and presented in tables and figures.

## III. RESULTS AND DISCUSSION

### Fruit Fly Species Associated with Host Plants in Bali

Field surveys conducted in Bali identified three fruit fly species belonging to the genus *Bactrocera*, namely *Bactrocera dorsalis*, *Bactrocera carambolae*, and *Bactrocera umbrosa*. These species were associated with eight fruit host plants, including mango (*Mangifera indica* L.), star fruit (*Averrhoa carambola* L.), guava (*Psidium guajava* L.), chili pepper (*Capsicum annuum* L.), bird's eye chili (*Capsicum frutescens* L.), tomato (*Solanum lycopersicum*), mandarin (*Citrus reticulata* L.), and jackfruit (*Artocarpus heterophyllus* Lam.). *Bactrocera dorsalis* and *B. carambolae* were found on seven host plants, whereas *B. umbrosa* was detected exclusively on jackfruit. The distribution of fruit fly species associated with each host plant is presented in Table I

**Table I. Fruit Fly Species Associated with Host Plants in Bali**

| Family        | Host plant                                          | Fruit fly species                                    |
|---------------|-----------------------------------------------------|------------------------------------------------------|
| Anacardiaceae | <i>Mangifera indica</i> L. (Mango)                  | <i>Bactrocera dorsalis</i> ;<br><i>B. carambolae</i> |
| Oxalidaceae   | <i>Averrhoa carambola</i> L. (Star fruit)           | <i>B. dorsalis</i> ; <i>B. carambolae</i>            |
| Myrtaceae     | <i>Psidium guajava</i> L. (Guava)                   | <i>B. dorsalis</i> ; <i>B. carambolae</i>            |
| Solanaceae    | <i>Capsicum annuum</i> L. (Chili pepper)            | <i>B. dorsalis</i> ; <i>B. carambolae</i>            |
| Solanaceae    | <i>Capsicum frutescens</i> L.<br>(Bird's eye chili) | <i>B. dorsalis</i> ; <i>B. carambolae</i>            |
| Solanaceae    | <i>Solanum lycopersicum</i> (Tomato)                | <i>B. dorsalis</i> ; <i>B. carambolae</i>            |
| Rutaceae      | <i>Citrus reticulata</i> L. (Mandarin)              | <i>B. dorsalis</i> ; <i>B. carambolae</i>            |
| Moraceae      | <i>Artocarpus heterophyllus</i> Lam.<br>(Jackfruit) | <i>Bactrocera umbrosa</i>                            |

The total fruit fly population collected during the study comprised 5,769 adults. *Bactrocera dorsalis* was the dominant species with 3,127 individuals, followed by *B. carambolae* with 2,448 individuals and *B. umbrosa* with 194 individuals. *Bactrocera dorsalis* and *B. carambolae* were distributed across almost all sampling locations in Bali, whereas *B. umbrosa* was restricted to jackfruit-producing areas. The population of each fruit fly species on different host plants and sampling locations is summarized in Table II.

**Table II. Population of Fruit Fly Species Associated with Host Plants in Bali**

| Host plant       | <i>B. umbrosa</i> | <i>B. dorsalis</i> | <i>B. carambolae</i> | Distribution in Bali                                                                  |
|------------------|-------------------|--------------------|----------------------|---------------------------------------------------------------------------------------|
| Mango            | 0                 | 891                | 216                  | Buleleng, Tabanan, Karangasem, Gianyar, Bangli, Badung, Jembrana, Klungkung, Denpasar |
| Star fruit       | 0                 | 340                | 1,387                | Buleleng, Tabanan, Karangasem, Gianyar, Bangli, Badung, Jembrana, Klungkung, Denpasar |
| Guava            | 0                 | 84                 | 199                  | Buleleng, Tabanan, Gianyar, Bangli, Badung, Jembrana, Klungkung, Denpasar             |
| Chili pepper     | 0                 | 416                | 111                  | Buleleng, Tabanan, Karangasem, Gianyar, Bangli, Badung, Jembrana, Klungkung           |
| Bird's eye chili | 0                 | 536                | 177                  | Buleleng, Tabanan, Karangasem, Gianyar, Bangli, Badung, Jembrana, Klungkung, Denpasar |
| Tomato           | 0                 | 445                | 248                  | Buleleng, Tabanan, Karangasem, Gianyar, Bangli, Badung, Jembrana, Klungkung, Denpasar |
| Mandarin         | 0                 | 415                | 110                  | Bangli, Gianyar                                                                       |

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| Host plant   | <i>B. umbrosa</i> | <i>B. dorsalis</i> | <i>B. carambolae</i> | Distribution in Bali                                               |
|--------------|-------------------|--------------------|----------------------|--------------------------------------------------------------------|
| Jackfruit    | 194               | 0                  | 0                    | Buleleng, Karangasem, Gianyar, Bangli, Badung, Jembrana, Klungkung |
| <b>Total</b> | <b>194</b>        | <b>3,127</b>       | <b>2,448</b>         | —                                                                  |

Table II shows the population of fruit fly species associated with different host plants in Bali. *Bactrocera dorsalis* was the most abundant species, with a total of 3,127 adults, followed by *B. carambolae* (2,448 adults) and *B. umbrosa* (194 adults). *Bactrocera dorsalis* and *B. carambolae* were recorded from seven host plants and were widely distributed across Bali, whereas *B. umbrosa* was exclusively associated with jackfruit and was found only in selected regencies. These findings indicate that *B. dorsalis* and *B. carambolae* were the predominant fruit fly species infesting fruit crops in Bali.

### Morphological Identification of Fruit Fly Species

Morphological examination confirmed the presence of three fruit fly species associated with fruit crops in Bali, namely *Bactrocera dorsalis*, *B. carambolae*, and *B. umbrosa*. Species identification was based on diagnostic morphological characters of the head, thorax, abdomen, and wing venation according to the identification keys [6,16]. The morphological characteristics of the three fruit fly species are presented in Figure 1.

*Bactrocera dorsalis* was distinguished by a predominantly black scutum with yellow lateral postsutural vittae, a narrow costal band extending along the wing margin, and a distinct T-shaped abdominal pattern. *Bactrocera carambolae* exhibited a similar general morphology but differed in its wing pattern and abdominal markings. In contrast, *B. umbrosa* was readily distinguished by broad transverse wing bands and the absence of the characteristic T-shaped abdominal pattern. These diagnostic characters enabled reliable identification of the three fruit fly species collected in Bali.

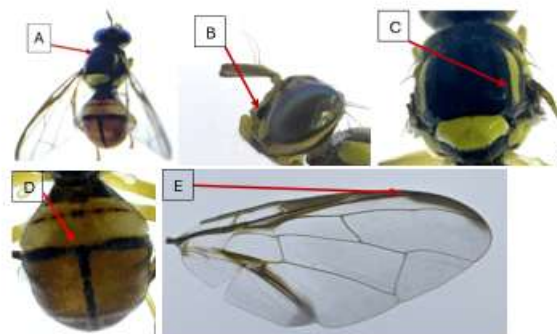


Figure 1. Morphological characteristics of *Bactrocera dorsalis*: (A) adult, (B) head, (C) thorax, (D) abdomen, and (E) wing.

*Bactrocera dorsalis* was distinguished by a predominantly black scutum with yellow lateral postsutural vittae, a narrow costal band extending along the wing margin, and a distinct T-shaped abdominal pattern.

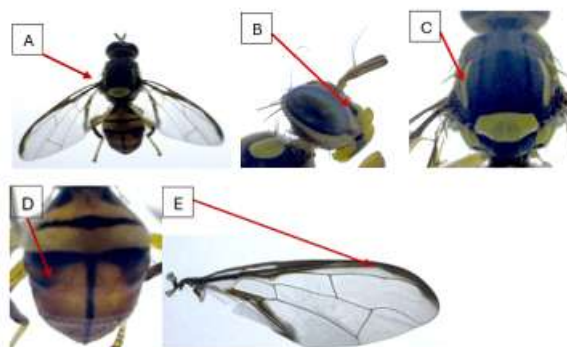


Figure 2. Morphological characteristics of *Bactrocera carambolae* showing the adult (A), lateral view of the head (B), thorax (C), abdomen (D), and wing (E).

*Bactrocera carambolae* exhibited a similar general morphology to *B. dorsalis* but differed in its wing pattern and abdominal markings, allowing reliable separation between the two species.

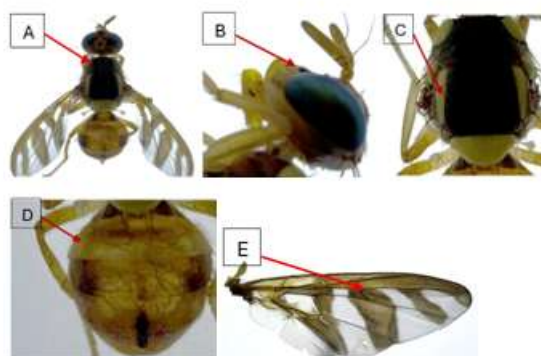


Figure 3. Morphological characteristics of *Bactrocera umbrosa* showing the adult (A), head (B), thorax (C), abdomen (D), and wing (E).

*Bactrocera umbrosa* was readily distinguished by broad transverse wing bands and the absence of the characteristic T-shaped abdominal pattern observed in the other two species.

#### Molecular Identification of *Bactrocera dorsalis*

PCR amplification of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene from *Bactrocera dorsalis* successfully produced a single DNA fragment of approximately 658 bp, indicating successful amplification of the target region (Figure 4). BLAST analysis of the obtained sequence showed 100% identity with several *B. dorsalis* sequences deposited in GenBank, including accessions MG687796.1, OR117773.1, and KF659655.1. The complete BLAST results are presented in Table III.

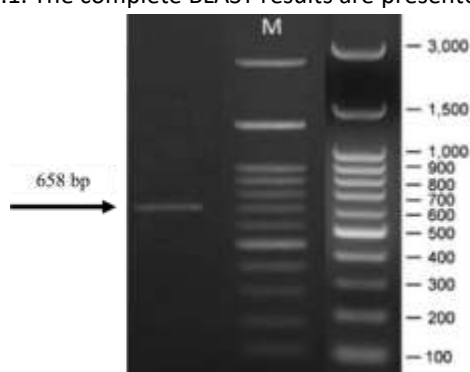


Figure 4. PCR amplification of the COI gene from *Bactrocera dorsalis*. M, DNA marker; 1, *B. dorsalis* isolate; P, positive control; N, negative control. (Sesuaikan label lane dengan foto gel-mu.)

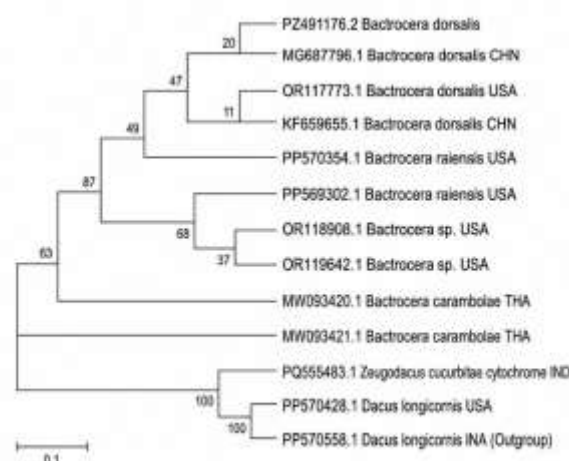
| Description                                                                                          | Identity | Accession  |
|------------------------------------------------------------------------------------------------------|----------|------------|
| <i>Bactrocera dorsalis</i> clone MY_36 cytochrome oxidase subunit I (COI) gene, partial cds          | 100%     | MG687796.1 |
| <i>Bactrocera dorsalis</i> voucher UHIM.ms10057 cytochrome oxidase subunit 1 (COI) gene, partial cds | 100%     | OR117773.1 |
| <i>Bactrocera dorsalis</i> voucher CHN_338 cytochrome c oxidase subunit I (COX1) gene, partial cds   | 100%     | KF659655.1 |
| <i>Bactrocera raiensis</i> USA cytochrome oxidase subunit I (COI) gene, partial cds                  | 100%     | PP569302.1 |
| <i>Bactrocera raiensis</i> USA cytochrome oxidase subunit I (COI) gene, partial cds                  | 100%     | PP570354.1 |
| <i>Bactrocera</i> sp. USA cytochrome oxidase subunit I (COI) gene, partial cds                       | 100%     | OR118908.1 |
| <i>Bactrocera</i> sp. USA cytochrome oxidase subunit I (COI) gene, partial cds                       | 100%     | OR119642.1 |

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| Description                                                                                | Identity | Accession  |
|--------------------------------------------------------------------------------------------|----------|------------|
| <i>Bactrocera carambolae</i> Thailand cytochrome oxidase subunit I (COI) gene, partial cds | 99%      | MW093421.1 |
| <i>Bactrocera carambolae</i> Thailand cytochrome oxidase subunit I (COI) gene, partial cds | 99%      | MW093420.1 |
| <i>Zeugodacus cucurbitae</i> cytochrome oxidase subunit I (COI) gene, partial cds          | 85%      | PQ555483.1 |
| <i>Dacus longicornis</i> cytochrome oxidase subunit I (COI) gene, partial cds              | 83%      | PP570428.1 |
| <i>Dacus longicornis</i> cytochrome oxidase subunit I (COI) gene, partial cds (Outgroup)   | 83%      | PP570558.1 |

Genetic distance analysis revealed that the *B. dorsalis* isolate had a genetic distance of 0.000 from several *B. dorsalis* reference sequences, indicating complete sequence similarity. Higher genetic distances were observed between the isolate and other tephritid species, including *Zeugodacus cucurbitae* and *Dacus longicornis* (**Table IV**). Phylogenetic analysis using the Maximum Likelihood method grouped the isolate within the *B. dorsalis* clade with strong bootstrap support, confirming its taxonomic identity (**Figure 5**).

| No  | Sequence                               | Genetic Distance |       |       |       |       |       |       |       |       |       |       |       |       |
|-----|----------------------------------------|------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|     |                                        | 1                | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    | 13    |
| 1.  | PZ491176_ <i>B.dorsalis</i>            | 0,000            |       |       |       |       |       |       |       |       |       |       |       |       |
| 2.  | MG687796.1_ <i>B. dorsalis</i> _CHN    | 0,000            | 0,000 |       |       |       |       |       |       |       |       |       |       |       |
| 3.  | OR117773.1_ <i>B. dorsalis</i> _USA    | 0,000            | 0,000 | 0,000 |       |       |       |       |       |       |       |       |       |       |
| 4.  | KF659655.1_ <i>B. dorsalis</i> _CHN    | 0,000            | 0,000 | 0,000 | 0,000 |       |       |       |       |       |       |       |       |       |
| 5.  | PP569302.1_ <i>B. raiensis</i> _USA    | 0,002            | 0,002 | 0,002 | 0,002 | 0,000 |       |       |       |       |       |       |       |       |
| 6.  | PP570354.1_ <i>B. raiensis</i> _USA    | 0,002            | 0,002 | 0,002 | 0,002 | 0,004 | 0,000 |       |       |       |       |       |       |       |
| 7.  | OR118908.1_ <i>Bactrocera</i> sp._USA  | 0,002            | 0,002 | 0,002 | 0,002 | 0,000 | 0,004 | 0,000 |       |       |       |       |       |       |
| 8.  | OR119642.1_ <i>Bactrocera</i> sp._USA  | 0,002            | 0,002 | 0,002 | 0,002 | 0,000 | 0,004 | 0,000 | 0,000 |       |       |       |       |       |
| 9.  | MW093421.1_ <i>B. carambolae</i> _THA  | 0,013            | 0,013 | 0,013 | 0,013 | 0,011 | 0,015 | 0,011 | 0,011 | 0,000 |       |       |       |       |
| 10. | MW093420.1_ <i>B. carambolae</i> _THA  | 0,011            | 0,011 | 0,011 | 0,011 | 0,009 | 0,009 | 0,009 | 0,009 | 0,009 | 0,000 |       |       |       |
| 11. | PQ555483.1_ <i>Z. cucurbitae</i> _IND  | 0,206            | 0,206 | 0,206 | 0,206 | 0,209 | 0,203 | 0,209 | 0,209 | 0,202 | 0,202 | 0,000 |       |       |
| 12. | PP570428.1_ <i>D. longicornis</i> _USA | 0,246            | 0,246 | 0,246 | 0,246 | 0,243 | 0,250 | 0,243 | 0,243 | 0,251 | 0,258 | 0,206 | 0,000 |       |
| 13. | PP570558.1_ <i>D. longicornis</i> _JNA | 0,250            | 0,250 | 0,250 | 0,250 | 0,247 | 0,254 | 0,247 | 0,247 | 0,248 | 0,255 | 0,210 | 0,022 | 0,000 |



**Figure 5. Maximum Likelihood phylogenetic tree based on the COI gene showing the relationship between the *Bactrocera dorsalis* isolate from Bali and reference sequences retrieved from GenBank.**

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### Molecular Identification of *Bactrocera carambolae*

PCR amplification of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene from *Bactrocera carambolae* successfully produced a single DNA fragment of approximately 658 bp (Figure 6). BLAST analysis demonstrated that the obtained sequence shared the highest similarity with *B. carambolae* reference sequences deposited in GenBank. The complete BLAST results are presented in Table V.

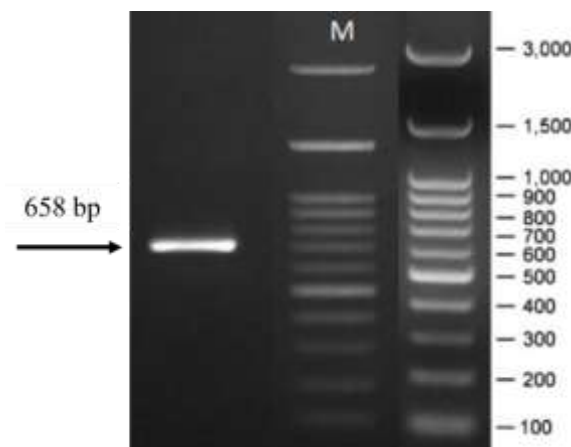


Figure 6. PCR amplification of the COI gene from *Bactrocera carambolae*. M, DNA marker; 1, *B. carambolae* isolate; P, positive control; N, negative control.

| Description                                                                                                           | Identity | Accession  |
|-----------------------------------------------------------------------------------------------------------------------|----------|------------|
| <i>Bactrocera carambolae</i> voucher UHIM.ms10710 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | 100%     | OR119277.1 |
| <i>Bactrocera carambolae</i> voucher UHIM.ms05368 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | 100%     | OR119726.1 |
| <i>Bactrocera dorsalis</i> clone MY_4 cytochrome oxidase subunit I (COI) gene, partial cds                            | 100%     | MG689884.1 |
| <i>Bactrocera dorsalis</i> clone MY_36 cytochrome oxidase subunit I (COI) gene, partial cds                           | 99%      | MG687796.1 |
| <i>Bactrocera dorsalis</i> clone ID_28 cytochrome oxidase subunit I (COI) gene, partial cds                           | 100%     | MG688974.1 |
| <i>Bactrocera raiensis</i> USA cytochrome oxidase subunit I (COI) gene, partial cds                                   | 99%      | PP569302.1 |
| <i>Bactrocera raiensis</i> USA cytochrome oxidase subunit I (COI) gene, partial cds                                   | 98%      | PP570354.1 |
| <i>Bactrocera carambolae</i> Thailand cytochrome oxidase subunit I (COI) gene, partial cds                            | 99%      | MW093421.1 |
| <i>Bactrocera carambolae</i> Thailand cytochrome oxidase subunit I (COI) gene, partial cds                            | 99%      | MW093420.1 |
| <i>Zeugodacus cucurbitae</i> cytochrome oxidase subunit I (COI) gene, partial cds                                     | 85%      | PQ555483.1 |
| <i>Dacus longicornis</i> cytochrome oxidase subunit I (COI) gene, partial cds                                         | 83%      | PP570428.1 |
| <i>Dacus longicornis</i> cytochrome oxidase subunit I (COI) gene, partial cds (Outgroup)                              | 83%      | PP570558.1 |

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Genetic distance analysis revealed minimal genetic divergence between the isolate and *B. carambolae* reference sequences, while larger genetic distances were observed with other species included in the analysis (Table VI). Phylogenetic analysis grouped the isolate within the *B. carambolae* clade with high bootstrap support, confirming its taxonomic identity (Figure 7).

| No  | Sequence                             | Genetic Distance |       |       |       |       |       |       |       |       |       |       |       |       |
|-----|--------------------------------------|------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|     |                                      | 1                | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    | 13    |
| 1.  | Z491175 <i>B. carambolae</i>         | 0,000            |       |       |       |       |       |       |       |       |       |       |       |       |
| 2.  | OR119277.1 <i>B. carambolae</i> USA  | 0,002            | 0,000 |       |       |       |       |       |       |       |       |       |       |       |
| 3.  | OR119726.1 <i>B. carambolae</i> USA  | 0,002            | 0,004 | 0,000 |       |       |       |       |       |       |       |       |       |       |
| 4.  | MG689884.1 <i>B. dorsalis</i> CHN    | 0,002            | 0,000 | 0,004 | 0,000 |       |       |       |       |       |       |       |       |       |
| 5.  | MG687796.1 <i>B. dorsalis</i> CHN    | 0,015            | 0,013 | 0,013 | 0,013 | 0,000 |       |       |       |       |       |       |       |       |
| 6.  | MG688974.1 <i>B. dorsalis</i> CHN    | 0,002            | 0,000 | 0,004 | 0,000 | 0,013 | 0,000 |       |       |       |       |       |       |       |
| 7.  | PP569302.1 <i>B. raiensis</i> USA    | 0,013            | 0,011 | 0,011 | 0,011 | 0,002 | 0,011 | 0,000 |       |       |       |       |       |       |
| 8.  | PP570354.1 <i>B. raiensis</i> USA    | 0,016            | 0,015 | 0,015 | 0,015 | 0,002 | 0,015 | 0,004 | 0,000 |       |       |       |       |       |
| 9.  | MW093421.1 <i>B. carambolae</i> THA  | 0,009            | 0,007 | 0,007 | 0,007 | 0,013 | 0,007 | 0,011 | 0,015 | 0,000 |       |       |       |       |
| 10. | MW093420.1 <i>B. carambolae</i> THA  | 0,011            | 0,009 | 0,009 | 0,009 | 0,011 | 0,009 | 0,009 | 0,009 | 0,009 | 0,000 |       |       |       |
| 11. | PQ555483.1 <i>Z. cucurbitae</i> IND  | 0,205            | 0,208 | 0,202 | 0,208 | 0,212 | 0,208 | 0,216 | 0,209 | 0,208 | 0,208 | 0,000 |       |       |
| 12. | PP570428.1 <i>D. longicornis</i> USA | 0,263            | 0,267 | 0,267 | 0,267 | 0,262 | 0,267 | 0,258 | 0,265 | 0,266 | 0,274 | 0,214 | 0,000 |       |
| 13. | PP570558.1 <i>D. longicornis</i> INA | 0,259            | 0,262 | 0,263 | 0,262 | 0,265 | 0,262 | 0,261 | 0,269 | 0,261 | 0,269 | 0,217 | 0,022 | 0,000 |

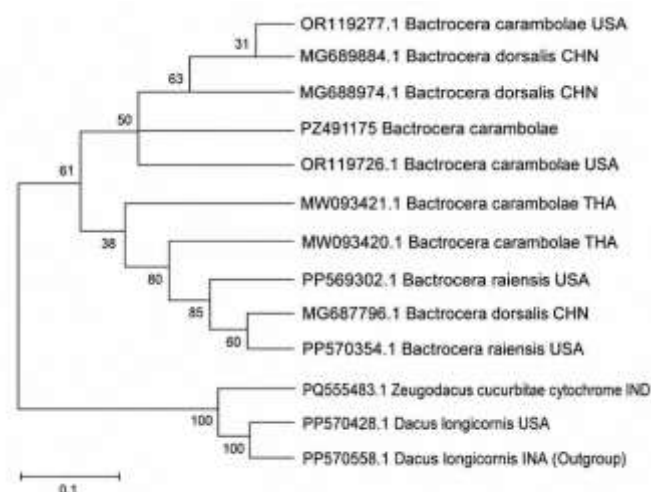


Figure 7. Maximum Likelihood phylogenetic tree based on the COI gene showing the relationship between the *Bactrocera carambolae* isolate from Bali and reference sequences retrieved from GenBank.

### Molecular Identification of *Bactrocera umbrosa*

PCR amplification of the mitochondrial cytochrome c oxidase subunit I (COI) gene from *Bactrocera umbrosa* successfully generated a single DNA fragment of approximately 658 bp (Figure 8). BLAST analysis showed the highest sequence similarity with *B. umbrosa* reference sequences deposited in GenBank. The complete BLAST results are presented in Table VII.

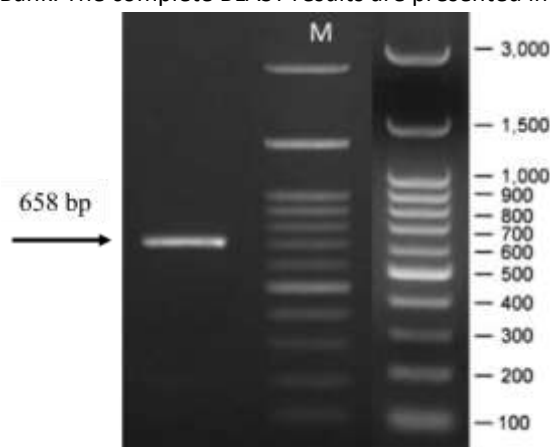


Figure 8. PCR amplification of the COI gene from *Bactrocera umbrosa*. M, DNA marker; 1, *B. umbrosa* isolate; P, positive control; N, negative control.

## Species Diversity of Fruit Flies (Diptera: Tephritidae) and Their Associated Parasitoids in Bali Province, Indonesia

| Description                                                                                             | Identity | Accession  |
|---------------------------------------------------------------------------------------------------------|----------|------------|
| <i>Bactrocera umbrosa</i> voucher CHN_XXX<br>cytochrome c oxidase subunit I (COX1)<br>gene, partial cds | 100%     | KF659817.1 |
| <i>Bactrocera umbrosa</i> voucher CHN_XXX<br>cytochrome c oxidase subunit I (COX1)<br>gene, partial cds | 100%     | KF659813.1 |
| <i>Bactrocera raiensis</i> cytochrome oxidase<br>subunit I (COI) gene, partial cds                      | 89%      | PP569302.1 |
| <i>Bactrocera raiensis</i> cytochrome oxidase<br>subunit I (COI) gene, partial cds                      | 89%      | PP570354.1 |
| <i>Bactrocera</i> sp. cytochrome oxidase<br>subunit I (COI) gene, partial cds                           | 89%      | OR118908.1 |
| <i>Bactrocera</i> sp. cytochrome oxidase<br>subunit I (COI) gene, partial cds                           | 89%      | OR119642.1 |
| <i>Bactrocera carambolae</i> cytochrome<br>oxidase subunit I (COI) gene, partial cds                    | 89%      | MW093421.1 |
| <i>Bactrocera carambolae</i> cytochrome<br>oxidase subunit I (COI) gene, partial cds                    | 89%      | MW093420.1 |
| <i>Zeugodacus cucurbitae</i> cytochrome<br>oxidase subunit I (COI) gene, partial cds                    | 85%      | PQ555483.1 |
| <i>Dacus longicornis</i> cytochrome oxidase<br>subunit I (COI) gene, partial cds                        | 81%      | PP570428.1 |
| <i>Dacus longicornis</i> cytochrome oxidase<br>subunit I (COI) gene, partial cds<br>(Outgroup)          | 81%      | PP570558.1 |

Genetic distance analysis indicated that the isolate had the smallest genetic distance to *B. umbrosa* reference sequences and greater divergence from other tephritid species (**Table VIII**). Phylogenetic analysis placed the isolate within the *B. umbrosa* clade with strong bootstrap support, confirming its species identity (**Figure 9**).

| No | Sequence                                          | Genetic Distance |       |       |       |       |       |       |       |       |       |       |       |
|----|---------------------------------------------------|------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|    |                                                   | 1                | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    |
| 1  | PZ491177_ <i>B. umbrosa</i>                       | 0,000            |       |       |       |       |       |       |       |       |       |       |       |
| 2  | KF659817.1_ <i>B. umbrosa</i> _CHN                | 0,000            | 0,000 |       |       |       |       |       |       |       |       |       |       |
| 3  | KF659813.1_ <i>B. umbrosa</i> _CHN                | 0,000            | 0,000 | 0,000 |       |       |       |       |       |       |       |       |       |
| 4  | PP569302.1_ <i>B. raiensis</i> _USA               | 0,128            | 0,128 | 0,128 | 0,000 |       |       |       |       |       |       |       |       |
| 5  | PP570354.1_ <i>B. raiensis</i> _USA               | 0,128            | 0,128 | 0,128 | 0,000 | 0,000 |       |       |       |       |       |       |       |
| 6  | OR118908.1_ <i>Bactrocera</i> _sp._USA            | 0,128            | 0,128 | 0,128 | 0,000 | 0,000 | 0,000 |       |       |       |       |       |       |
| 7  | OR119642.1_ <i>Bactrocera</i> _sp._USA            | 0,128            | 0,128 | 0,128 | 0,000 | 0,000 | 0,000 | 0,000 |       |       |       |       |       |
| 8  | MW093421.1_ <i>B. carambolae</i> _THA             | 0,128            | 0,128 | 0,128 | 0,011 | 0,011 | 0,011 | 0,011 | 0,000 |       |       |       |       |
| 9  | MW093420.1_ <i>B. carambolae</i> _THA             | 0,128            | 0,128 | 0,128 | 0,008 | 0,008 | 0,008 | 0,008 | 0,008 | 0,000 |       |       |       |
| 10 | PQ555483.1_ <i>Z. cucurbitae</i> _IND             | 0,196            | 0,196 | 0,196 | 0,169 | 0,169 | 0,169 | 0,169 | 0,169 | 0,169 | 0,000 |       |       |
| 11 | PP570428.1_ <i>D. longicornis</i> _USA            | 0,262            | 0,262 | 0,262 | 0,233 | 0,233 | 0,233 | 0,233 | 0,242 | 0,242 | 0,197 | 0,000 |       |
| 12 | PP570558.1_ <i>D. longicornis</i> _JNA (Outgroup) | 0,273            | 0,273 | 0,273 | 0,243 | 0,243 | 0,243 | 0,243 | 0,243 | 0,243 | 0,202 | 0,020 | 0,000 |

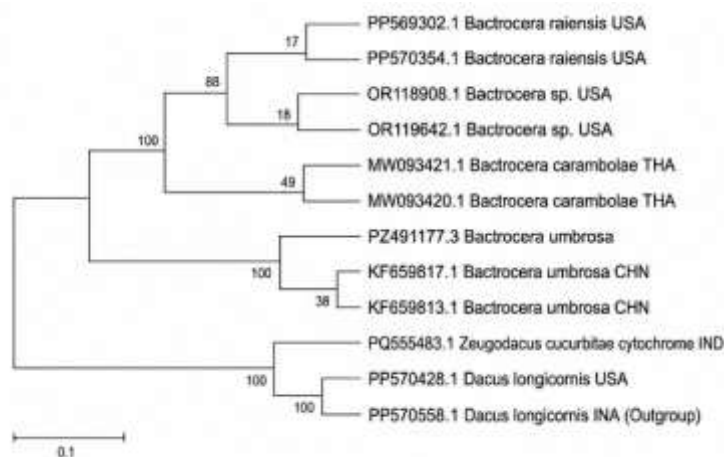


Figure 9. Maximum Likelihood phylogenetic tree based on the COI gene showing the relationship between the *Bactrocera umbrosa* isolate from Bali and reference sequences retrieved from GenBank.

### Parasitoid Species Associated with Fruit Flies

Field observations revealed two parasitoid species associated with fruit flies infesting fruit crops in Bali, namely *Fopius* sp. and *Opius* sp., both belonging to the family Braconidae (Hymenoptera) (Figure 10).

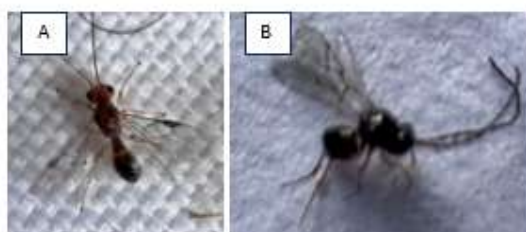


Figure 10. Adult parasitoids associated with fruit flies collected from fruit crops in Bali: (A) *Fopius* sp. and (B) *Opius* sp.

*Fopius* sp. was characterized by a body length of approximately 2.6–4.7 mm, with females generally larger than males. The adults possessed a dark brown to black body, an orange-brown head with a distinct median black stripe, yellowish-brown antennae, a long ovipositor, and light brown legs. *Opius* sp. was smaller (<2 mm) and exhibited a dark brown to black body, a reddish-brown head, yellow antennal scape with a brown flagellum, and light brown legs with dark tarsi. These morphological characteristics enabled differentiation of the two parasitoid genera collected during the study.

### Parasitism Rate

The parasitism rates of parasitoids associated with fruit flies on different host plants are presented in Table IX.

| Host Plant         | Number of Adult Fruit Flies | Number of Adult Parasitoids | Parasitism Rate (%) |                   | Total Parasitism (%) |
|--------------------|-----------------------------|-----------------------------|---------------------|-------------------|----------------------|
|                    |                             |                             | <i>Opius</i> sp.    | <i>Fopius</i> sp. |                      |
| Mango              | 1107                        | 48                          |                     | 4.16              | 4.16                 |
| Jackfruit          | 194                         |                             |                     |                   |                      |
| Starfruit          | 1727                        | 103                         | 1.98                | 3.79              | 5.77                 |
| Guava              | 283                         | 9                           |                     | 3.08              | 3.08                 |
| Large Chili Pepper | 527                         |                             |                     |                   |                      |
| Small Chili Pepper | 713                         |                             |                     |                   |                      |
| Tomato             | 693                         |                             |                     |                   |                      |
| Orange             | 525                         |                             |                     |                   |                      |
| Total              | 5769                        | 160                         | 1.98                | 11.03             | 13.01                |

## Species Diversity of Fruit Flies (Diptera: Tephritidae) and Their Associated Parasitoids in Bali Province, Indonesia

**Table IX.** Parasitism rates of fruit fly parasitoids associated with different host plants in Bali.

The highest parasitism rate was recorded on star fruit (5.77%), followed by mango (4.16%) and guava (3.08%). *Fopius* sp. was recovered from mango, star fruit, and guava, whereas *Opius* sp. was detected only on star fruit, with a parasitism rate of 1.98%. No parasitoids were recovered from jackfruit, large chili pepper, bird's eye chili, tomato, or mandarin. Overall, *Fopius* sp. exhibited a higher total parasitism rate (11.03%) than *Opius* sp. (1.98%), resulting in an overall parasitism rate of 13.01%.

### DISCUSSION

Three fruit fly species, namely *Bactrocera dorsalis*, *B. carambolae*, and *B. umbrosa*, were identified from fruit crops in Bali. Among these, *B. dorsalis* was the dominant species, occurring on seven host plants and exhibiting the highest population abundance. The predominance of *B. dorsalis* is consistent with its well-known polyphagous behavior, allowing this species to utilize a wide range of cultivated fruit crops and adapt to diverse agroecosystems. Previous studies have also reported that *B. dorsalis* is one of the most widespread and economically important fruit fly species in Southeast Asia because of its broad host range, high reproductive capacity, and strong dispersal ability [5,7]. In contrast, *B. umbrosa* was detected exclusively on jackfruit, supporting previous reports that this species has a relatively narrow host range, primarily associated with species of the genus *Artocarpus* [12].

Host plant characteristics are also likely to contribute to the observed distribution of fruit fly species. Female fruit flies select oviposition sites based on physical and chemical characteristics of fruits, including peel thickness, fruit firmness, color, and volatile compounds released during ripening. Host plants that produce fruits continuously throughout the year may provide more favorable conditions for oviposition and larval development, resulting in higher fruit fly populations. Consequently, differences in host availability and fruit characteristics may explain the predominance of *B. dorsalis* and *B. carambolae* across multiple host plants, whereas *B. umbrosa* remained restricted to jackfruit [7,17].

The consistency between morphological identification and molecular identification further confirmed the taxonomic status of the three fruit fly species collected in Bali. Morphological identification based on diagnostic characteristics of the head, thorax, abdomen, and wing venation was supported by DNA barcoding using the mitochondrial cytochrome c oxidase subunit I (COI) gene. BLAST analysis showed high sequence similarity (>98%) between the representative isolates and corresponding reference sequences deposited in GenBank, while phylogenetic analysis grouped each isolate within its respective species clade with strong bootstrap support. These findings demonstrate that the integration of morphological and molecular approaches provides a reliable method for distinguishing closely related *Bactrocera* species and supports accurate species identification for biodiversity assessment, pest surveillance, and quarantine programs.

The occurrence of two braconid parasitoid genera, *Fopius* sp. and *Opius* sp., indicates the presence of indigenous natural enemies that may contribute to the biological control of fruit fly populations in Bali. *Fopius* sp. exhibited a higher overall parasitism rate than *Opius* sp., suggesting that this genus may play a more important role in regulating fruit fly populations under local environmental conditions. Differences in parasitism rates among host plants may be associated with variations in fruit morphology, host density, fruiting period, and parasitoid foraging behavior. The highest parasitism rate observed on star fruit may reflect greater accessibility of immature fruit fly stages to parasitoids, whereas the absence of parasitoids on chili pepper, bird's eye chili, tomato, mandarin, and jackfruit may indicate lower parasitoid preference or unsuitable ecological conditions for successful parasitism. Similar observations have been reported in previous studies, which demonstrated that parasitoid abundance and parasitism rates are strongly influenced by host availability and habitat characteristics [1,19]. The integration of host plant surveys, morphological identification, DNA barcoding, and parasitoid assessment provides comprehensive baseline information on fruit fly diversity and their associated natural enemies in Bali Province. These findings contribute to a better understanding of fruit fly ecology and highlight the potential use of indigenous parasitoids as environmentally friendly biological control agents within integrated pest management (IPM) programs.

### IV. CONCLUSION

Three fruit fly species, namely *Bactrocera dorsalis*, *B. carambolae*, and *B. umbrosa*, were identified from fruit crops in Bali. Among these, *B. dorsalis* was the predominant species, followed by *B. carambolae*, whereas *B. umbrosa* was exclusively associated with jackfruit. Morphological identification was consistent with molecular identification using the mitochondrial COI gene, with all representative isolates showing high sequence similarity (>98%) to reference sequences deposited in GenBank and clustering with their respective species in phylogenetic analysis. Two braconid parasitoids, *Fopius* sp. and *Opius* sp., were associated with fruit flies, with *Fopius* sp. exhibiting a higher overall parasitism rate (11.03%) than *Opius* sp. (1.98%). These findings provide valuable baseline information for fruit fly identification and highlight the potential contribution of indigenous parasitoids to integrated and sustainable fruit fly management in Bali.

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## REFERENCES

- 1) Adnyana, I. W. D., Darmiati, N. N., & Widaningsih, D. 2019. *Asosiasi Lalat Buah (Bactrocera spp.) (Diptera: Tephritidae) dan Parasitoidnya pada Tanaman Jambu Biji Kristal (Psidium guajava L.) yang Dibudidayakan di Bali*. Agrotrop: Journal on Agriculture Science, 9(2).
- 2) Angreini, N., Rahim, M., & Salam, I. 2021. *Analisis Pengembangan Komoditas Unggulan Sub Sektor Hortikultura Di Kabupaten Konawe*. Jurnal Perencanaan Wilayah, 6(1), 46.
- 3) Apriyadi, R., Saputra, H. M., Sintia, S., & Andini, D. E. 2021. *The Diversity of Fruit Fly (Diptera: Tephritidae) on Combination of Attractant and Different Trap Height in Cucumber Field (Cucumis sativus L.)*. IOP Conference Series: Earth and Environmental Science, 486, 1–12.
- 4) Buchori, D., Meilin, A., Hidayat, P., & Sahari, B. 2010. *Species Distribution of Trichogramma and Trichogrammatoidea Genus in Java*. J. ISSAAS, 16(1), 83–96.
- 5) Clarke, A. R., et al. 2005. *Biology and Management of Bactrocera dorsalis (Hendel) (Diptera: Tephritidae): A Review of Current Knowledge*. Australian Journal of Entomology.
- 6) Drew, R. A. I. 2011. *The Australian Handbook for the Identification of Fruit Flies*. Plant Health Australia.
- 7) Drew, R. A. I., & Romig, M. C. 2013. *Tropical Fruit Flies of South East Asia*. Brisbane: Griffith University.
- 8) Goulet, H., & Huber, J. T. 1993. *Hymenoptera of the World: An Identification Guide to Families*. Agriculture Canada Publication.
- 9) Hasinu, J. V., Patty, J. A., & Tuhumury, G. N. C. 2020. *Morphological Identification and Population of Fruit Fly (Bactrocera sp.) in Chili Fields*. Journal HPT Tropika, 20(2), 123–129.
- 10) Hudiwaku, S., Himawan, T., & Rizali, A. 2022. *Keanekaragaman, Komposisi Spesies dan Kunci Identifikasi Lalat Buah (Diptera: Tephritidae: Dacinae) di Pulau Lombok*. Jurnal Entomologi Indonesia, 19, 111–126.
- 11) Lihang, A., Sasinggala, M., & Butar-Butar, R. R. 2022. *Identifikasi Keanekaragaman Tanaman Hortikultura di Kecamatan Modinding Kabupaten Minahasa Selatan*. Jurnal Biologi Makassar, 7(2), 44–50.
- 12) Manwan, S. W., & Nurjanani. 2017. *Identifikasi dan Karakteristik Morfologi Lalat Buah di Kabupaten Soppeng*. J. Agrotan.
- 13) Maung, L. K., et al. 2019. *Diversity and Abundance of Fruit Flies (Family: Tephritidae) in Myanmar's Tropical Region*. Journal of Entomology and Zoology Studies, 7(4), 574–579.
- 14) Nugnes, F., Russo, E., Viggiani, G., & Bernardo, U. 2018. *First Record of the Invasive Fruit Fly Belonging to the Bactrocera dorsalis Complex (Diptera: Tephritidae) in Europe*. Insects, 9, 182.
- 15) Sari, D. E., Sunarti, Nilawati, Mutmainna, I., & Yustia, D. 2020. *Identifikasi Hama Lalat Buah (Diptera: Tephritidae) Pada Beberapa Tanaman Hortikultura*. Jurnal Agrominasia, 5(1), 1–9.
- 16) Siwi, S. S., Hidayat, P., & Suputa. 2006. *Taksonomi dan Bioekologi Lalat Buah Penting di Indonesia (Diptera: Tephritidae)*.
- 17) Sulaeha, Endang S. R., & Purwatiningsih, S. 2018. *Studi Lalat Buah Zeugodacus cucurbitae (Diptera: Tephritidae) dengan Perhatian Utama pada Deteksi Senyawa Kairomon dari Tanaman Inang*. Institut Pertanian Bogor.
- 18) Tamura, K., Stecher, G., & Kumar, S. 2021. *MEGA11: Molecular Evolutionary Genetics Analysis Version 11*. Molecular Biology and Evolution, 38(7), 3022–3027.
- 19) Vargas, R. I., Piñero, J. C., & Leblanc, L. 2015. *A Review of Bactrocera Fruit Flies (Diptera: Tephritidae) and the Integration of Biopesticides with Other Biological Approaches for Their Management*. Insects, 6, 297–318.



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