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DNA Barcoding and Morphometric of Fruit Fly *Bactrocera dorsalis* Complex (Diptera: Tephritidae*)* from Citrus Crops at Dairi District, North Sumatera, Indonesia

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ABSTRACT

This study aims to identify the species of fruit flies (Diptera: Tephritidae) present on citrus plantation in the Dairi district of North Sumatra. The identification is based on the mtCOI gene and includes an analysis of the fruit fly's morphometric characteristics and the relationship between body length and other morphological features. The fruit flies were captured using Steiner traps as a sampling method. Identification by DNA barcoding approach was carried out by isolation, amplification, sequencing, and blasting steps. The Stereo Zeiss-Stemi 2000-C microscope was used to measure morphological parameters such as body length (Y), wing length (X_1), wing width (X_2), and tibia hind leg length (X_3). The fruit fly species was identified by the research findings as *Bactrocera dorsalis* complex. Its mt COI DNA sequence measured 696-704 bp in length, with T(U), G, C, and A nucleotides present in concentrations of 29.42%, 18.42%, 16.78%, and 35.35%, respectively. The average lengths of the fruit fly's body, wings, wing width, and tibia (n = 30) were 6.73, 5.28, 2.37, and 1.69 mm, in that order. The regression equation between the length of the body and other morphological traits (X_1-X_3) was $Y= 0.37 X_1+ 0.96 X_2 + 2.48$ (R=0.757). The contribution of wing length and wing width to body length was 57.40%.

Key words: DNA barcoding, morphometric, *Bactrocera dorsalis* complex, Dairi district

INTRODUCTION

Dairi, a district in North Sumatra, Indonesia, is known for its orange production. However, the production of oranges has been declining due to attacks by fruit flies on citrus fruit crops. There is a direct correlation between the decline in citrus yield and the rise in fruit flies' infection. Manurung *et al.* (2022 & 2020) reported that 17.000 hectares of orange crops in one district of North Sumatra have suffered damage due to a fruit fly infestation, resulting in a citrus harvest of only 20 tons per hectare. In this instance, the ovipositor organ of the female fruit fly pierces the fragile fruits, allowing the eggs to be laid beneath the fruit's skin. In a few days, a few maggots will emerge derived from the eggs and pierce farther within the fruit, resulting in its eventual rot and decay and eventual fruit fall.

The fruit fly species that attack orange crops and reduce output in North Sumatra, Indonesia, are determined to be members of the *Bactrocera* genus based on the results of investigations into fruit fly infestations. Three fruit fly species *B. dorsalis, B. umbrosa, and B. caudatus* have been successfully identified (Manurung *et al*., 2020a; Sahetapy *et al*., 2019; Martiningsia *et al*., 2017). These fruit flies are *Bactrocera* and they are in the Diptera order's Tephritidae family (Indriyanti *et al*., 2017; Sudiarta *et al*., 2018).

In the field of fruit and vegetable crop infestations, experts have employed a genetic (molecular) approach to support the results of traditional methods, such as identification based on morphology and anatomy. Several studies have used the *mitochondrial cytochrome oxidase subunit I* (mt COI) gene as a genetic indicator in animal classification to identify fruit fly *Bactrocera* species (Manurung *et al*., 2020a; Sahetapy *et al*., 2019; Martiningsia *et al*., 2017; Sharma *et al*., 2018).

Fruit flies can be potential pests for horticulture crops, particularly on citrus plantations. A detailed understanding of their morphology, morphometric, and genetic features is necessary to enable integrated pest management efforts to successfully control and monitor their population. This study aims to identify the morphometric and genetic characteristics of fruit flies that prey on citrus trees in the Dairi area using both classic/traditional taxonomy and molecular (DNA barcoding technique). Additionally, this study also aims to determine the relationship between fruit fly morphometric traits and their body length, which has not been reported before.

MATERIALS AND METHODS

The fruit flies under investigation were collected from a citrus plantation situated in Tanjung Baringin village in Dairi district, North Sumatra, Indonesia (S: 2.757289° 98.438268°164°; N: 2.757230° 98.438279°357°), at an altitude of 1332 meters above sea level. The collection of fruit flies occurred in July

2022.

In a citrus plantation, the fruit flies under inquiry were captured using modified Steiner traps, where cotton was baited with methyl eugenol. After being retrieved, the fruit flies were tagged, kept in alcohol, and sent to the lab for identification and curation. Morphological features were used to determine fly species, which were observed and measured under a stereo microscope in the invertebrate taxonomy laboratory of the Biology Department, Universitas Negeri Medan. The species identification referred to Siwi *et al*. (2006) and Hasinu *et al* (2020). Further, the morphometric trait assessment was carried out according to Manurung *et al*. (2022, 2021). Thirty fruit flies' body length, wing length, wing width, and tibia length were measured and studied utilizing the Carl Zeiss Imaging System Axio Vision LE Release 4.8.2 software under a Stereo Zeiss-Stemi 2000-C microscope. A stepwise multiple regression approach was used with SPSS Statistics v. 23 software to examine the association between the body's length and other morphometric characteristics.

Identification of fruit flies using the DNA barcoding technique based on the mtCOI DNA gene marker was performed to validate the results of the morphological approach (classic/conventional taxonomy). The technique of DNA barcoding, as outlined by Manurung *et al.* (2020a, b&c) involved a series of steps: extraction, amplification, electrophoresis, sequencing, and blasting. The ZR Kit for Miniprep of Tissue and Insect DNA (Zymo Research, D6016) was utilized to extract the fruit fly's genomic DNA from its head and leg tissues. This extraction procedure included preparation, cell lysis, DNA binding, washing, and DNA elution. Through the use of 1% TBE agarose for result analysis, the success of DNA isolation was verified. In the meantime, My Taq Red Mix (Bioline, Catalog No. Bio-25043) was used as the PCR master mix for the thermal cycler's DNA/mt COI gene amplification (Table 1). The forward and reverse primers for mtCOI amplification were LCO-1490 and HCO-2198 (Hasinu *et al*., 2020). Table 2 displays both primers' sequences. Moreover, Table 3 contains the PCR conditions needed to amplify the *cytochrome oxidase I region.*

Table 1. Master mixture for PCR DNA amplification

Component	1 × 25µL
dd H_2O	9.5
MyTaq Red Mix, 2x	12.5
20 µmol / µl LCO 1490 Primer*	1
20 µmol / µl 2198 Primer**	1
DNA Template	1

Table 2. DNA amplification oligonucleotide primers

Primer's name	Sequence
LCO-1490 as forward	5′-GGTCAACAAATCA TAAAGATATTGG-3′
HCO-2198 as reverse	5′-TAAACTTCAGGGTGA CCAAAAAATCA-3′

Table 3. mtCOI PCR amplification conditions

Step	Temperature (°C)	Duration	Cycles
Initial Denaturation	95	1 min	1
Denaturation	95	15 sec	35
Annealing	52	30 sec	
Extension	72	45 sec	
Hold	4	∞	1

Using the Zymoclean Gel DNA Recovery Kit (Zymo Research, D4002), the PCR-amplified product was purified. It was then analyzed through electrophoresis on a 1% TBE agarose gel, which was run at 100 volts for 60 minutes (Wealtec). The purified PCR result was then subjected to bi-directional sequencing at PT Genetika Science Indonesia's genetic facility in Jakarta utilizing an ABI PRISM 3730 XL genetic analyzer. Alignment of the obtained mtCOI gene fragments was accomplished using ClustalW-MEGA XI. The sequence homology was assessed by employing the BLAST program, which is accessible on the National Center for Biotechnology Information (NCBI) website. This analysis involved evaluating sequences to determine their degree of similarity.

RESULTS AND DISCUSSION

In accordance with the findings of the morphology of the fruit flies collected from oranges/citrus crops in the Dairi district, it has been identified that the species is part of *the Bactrocera dorsalis* complex.

The verification of this fruit fly species relied on observable traits present on its thorax, head, legs, wings, and abdomen. In this instance, a distinct dark spot, particularly noticeable within the fruit fly's antennal furrow, may be observed. There is a decrease in the arrangement of bristles (chaetotaxy) on the head and thorax, and characteristics such as anterior supra-alar setae and prescutellar acrostichal setae were noted on the scutum. The prevailing color of the scutum is black, while the lateral part is yellow. On the wings, the costal band merges and overlaps over R2+3, while the subcostal vein sharply bends forward at an

angle close to 90 degrees. There is a obvious black T-shaped mark on the abdominal tergites, and terga III and IV have narrow medial dark parts. The abdomen displays triangular ends of the lateral bands. Additionally, the male tergite shows a row of setae or pecten (Indriyanti et al., 2017; Riastiwi et al., 2021; Siwi et al., 2006).

The findings of the PCR product for fruit fly *Bactrocera* samples 1 and 2 were presented visually through gel electrophoresis in Figure 1. *Bactrocera* was amplified using a single 700 base pair (bp) DNA fragment, whose size varied between 696 and 704 bp, and their corresponding sequences are illustrated in Figure 2. The nucleotide composition (A, G, T (U), and C) in the COI sequence of the Dairi sample was approximately 35.35, 18.42, 29.42, and 16.78%, respectively. This indicates that the concentration of purines was 53.77%, while that of pyrimidines was 46.21%.

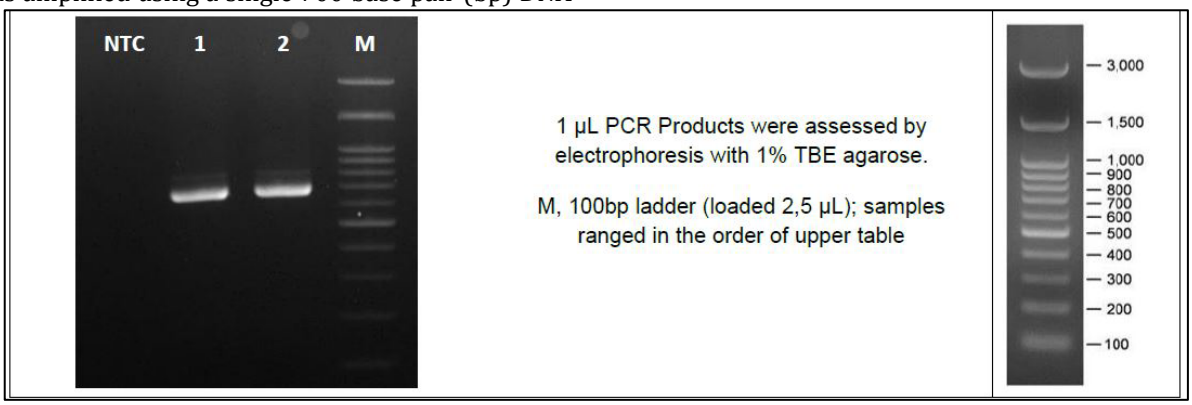

Fig. 1. Gel Electrophoresis Image of PCR Products from Fruit Flies Collected in the Dairi District (Samples 1 and 2), North Sumatra.

Sequence Assembly 704 bp

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1     TAAACTTCCA GGGTGACCAA AAAATCAAAA TAAATGTTGG TAAAGAATAG GATCTCCTCC
61    TCCGGCAGGG TCAAAAAAGG AAGTATTTAA GTTTCGGTCT GTTAGCAGTA TAGTAATAGC
121   CCCTGCTAAA ACTGGTAATG ATAATAAAAG TAATAAAGCT GTTAATACAA CTGCTCAAAC
181   GAATAGAGGT ATTCGATCAA AGGTGATTCC TGTTGATCGT ATATTAATTA CTGTTGTAAT
241   GAAATTTACT GCTCCTAAAA TTGAGGAAAT ACCCGCTAAG TGAAGTGAAA AAATAGCTAG
301   GTCAACTGAA GCTCCTCCGT GTGCAATAAC AGATGATAGG GGTGGGTAAA CTGTTCAACC
361   TGTACCAGCT CCGTTTTCTA CTATACTTCT TACTAATAGT AATGTAAGGG AAGGAGGTAA
421   TAATCAAAAT CTTATATTAT TCATTCGTGG AAATGCTATA TCGGGAGCTC CTAATATTAA
481   AGGAACAAGT CAATTTCCAA ATCCACCAAT TATAATTGGT ATAACTATAA AGAAAATTAT
541   TACGAAAGCA TGAGCTGTTA CAATTACATT ATAAATTTGA TCGTCACCGA TTAAAGCTCC
601   TGGGTGACCG AGTTCAGCTC GGACTAAAAT TCTAAGGGAT GTTCCTACTA TTCCTGCTCA
661   GGCTCCGAAG ATAAAATATA AAGTTCCAAT TATCTTTATG ATTT
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Sequence Assembly 696 bp

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1     AAACTTCCAG GTGACCAAAA AATCAAAATA AATGTTGGTA AAGAATAGGA TCTCCTCCTC
61    CGGCAGGGTC AAAAAAGGAA GTATTTAAGT TTCGGTCTGT TAGTAGTATA GTAATAGCCC
121   CTGCTAAAAC TGGTAATGAT AATAAAAGTA ATAAAGCTGT TAATACAACT GCTCAAACGA
181   ATAGAGGTAT TCGATCAAAG GTGATTCCTG TTGATCGTAT ATTAATTACT GTTGTAATGA
241   AATTTACTGC TCCTAAAATT GAGGAAATAC CCGCTAAGTG AAGTGAAAAA ATAGCTAGGT
301   CAACTGAAGC TCCTCCGTGT GCAATAACAG ATGATAGGGG TGGGTAAACT GTTCAACCTG
361   TACCAGCTCC GTTTTCTACT ATACTTCTTA CTAATAGTAA TGTAAGGGAA GGAGGTAATA
421   ATCAAAATCT TATATTATTC ATTCGTGGAA ATGCTATATC GGGAGCTCCT AATATTAAAG
481   GAACAAGTCA ATTTCCAAAT CCACCAATTA TAATTGGTAT AACTATAAAG AAAATTATTA
541   CGAAAGCATG AGCTGTTACA ATTACATTAT AAATTTGATC GTCACCGATT AAAGCTCCTG
601   GGTGACCGAG TTCAGCTCGG ACTAAAATTC TAAGGGATGT TCCTACTATT CCTGCTCAGG
661   CTCCGAAGAT AAAATATAAA GTTCCAATTA TCTTTA
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Fig. 2. Consensus Sequences of the 696-704 bp Fragment of the mtCOI Gene in Fruit Fly Samples from the Dairi District, North Sumatra.

The results of the blasting on the NCBI GenBank database revealed that the mtCOI gene fragment (sequence) from the Dairi district exhibits a high degree of similarity (up to 99.15%) with the mtCOI sequences of *Bactrocera dorsalis* fruit flies originating from Vietnam (MG689384.1), Thailand (MG689320.1) and Laos (MG689153.1, MG689146.1, MG689103.1) (Table 4). This finding confirms that the Dairi fruit fly belongs to the *Bactrocera dorsalis* species. The findings of this research demonstrate that the application of the DNA barcoding technique, particularly utilizing the mtCOI DNA gene marker,

supports the traditional taxonomy grounded in morphological traits (Manurung *et al*., 2020a; Sahetapy *et al*., 2019; Martiningsia *et al*., 2017; Sharma *et al*., 2018). The homology finding, indicating approximately 99.15% similarity among Indonesia and Vietnam, Thailand, and Laos, implies that ecological differences and physical distance between these nations may not have a substantial effect on the variance in the mtCOI DNA gene between these two populations. Consequently, it can be inferred that both fruit fly populations (Indonesia, Vietnam, Thailand, and Laos) likely share a common ancestor.

Table 4. BLASTN Analysis Results for Dairi Fruit Fly Samples

Species	Accession number	Query cover	Percent identity
Bactrocera dorsalis COI	MG689384.1	100%	99.15%
Bactrocera dorsalis COI	MG689320.1	100%	99.15%
Bactrocera dorsalis COI	MG689153.1	100%	99.15%
Bactrocera dorsalis COI	MG689146.1	100%	99.15%
Bactrocera dorsalis COI	MG689103.1	100%	99.15%

The findings regarding the morphometric characteristics of *Bactrocera dorsalis* fruit flies from the Dairi area are detailed in Table 5. The average length measurements of the fruit fly's body, wings, wing width, and tibia were 6.73, 5.28, 2.37, and 1.69 mm, in that order. When compared to the body length of *Bactrocera dorsalis* from India, as reported by Sharma and Gupta (2018) [6], the size of Dairi fly appeared diminutive and shorter, whereas compare with *Bactrocera dorsalis* originating from Karo and Simalungun areas in North Sumatra, as reported by Manurung *et al.* (2022a&b), it was also found to be diminutive and shorter. Meanwhile, in comparison to the size of *Bactrocera umbrosa* from Namoriam village, Deli Serdang area, North Sumatra (Manurung *et al*., 2020a), the *Bactrocera dorsalis* acquired during the investigation had a shorter or diminutive.

Table 5. Morphometric Traits of *Bactrocera dorsalis* in the Dairi District, North Sumatra, Indonesia

No	Morphological traits	Mean ± SE (n=30) (mm)	Range (mm)
1	Body length-BL (Y)	6.73 ± 0.07	5.4-7.4
2	Wing length-WL (X_1)	5.28 ± 0.09	4.5-6.3
3	Wing width-WW (X_2)	2.37 ± 0.03	2.0-2.6
5	Tibia length-TL(X_3)	1.69 ± 0.02	1.5-2.0

The regression equation illustrating the relationship between body length (Y) and other morphometric features (X_1 to X_3) of *B.dorsalis* fruit fly is shown in Table 6. The correlation between body length (Y) and wing measurements (X_1 and X_2) is represented by the equation $Y=2.48+0.37X_1+0.96X_2$. Multiple regression analysis indicates that two morphometric traits significantly contribute to the determination of the fruit fly's body length (Y), namely wing length (X_1) and wing width (X_2). This discovery aligns with the study conducted by Manurung *et al* (2022) on *B.dorsalis* morphometric that originated from the Karo area (Manurung *et al*., 2022b). The dominant determinant in this study was wing length (X_1) with a contribution of 48.40%, and when combined with wing width (X_2), the contribution increased to 57.40%. In contrast, the investigation findings for the fruit fly *Bactrocera umbrosa*, differed significantly. Manurung *et al*. (2020a) indicated that the length of the tibia was the morphometric characteristic contributing significantly to influence the body length of *B. umbrosa*, accounting for 56.80% of the variance.

Table 6. Regression Equation and Determinant Coefficient for Morphometric Traits in Predicting Body Length of *Bactrocera dorsalis* in Dairi Fruit Flies

No	Regression equation	Determinant coefficient (R^2)
1	$Y = 3.82 + 0.55X_1$	48.40%
2	$Y = 2.48 + 0.37X_1 + 0.96X_2$	57.40%

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