

Biological characters of fruit flies *Bactrocera umbrosa* (Fabricius) from north sumatera, Indonesia

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Abstract

The present work emphasizes on the biological characters, namely morphometric study of fruit fly *Bactrocera umbrosa* Fabricius that originated from North Sumatera, Indonesia. The fruit flies sampling was carried out at the fruit star crops that located in Namoriam village, Deliserdang district. Fruit flies identification was done by morphology and molecular approaches. The morphological traits that be observed and measured were body length (Y), wing length (X₁), wing width (X₂), hind leg length (X₃) and tibia length (X₄). The morphometric features measuring were done by using software Carl Zeiss Imaging System Axio Vision LE Release 4.8.2 under Stereo Zeiss-Stemi 2000-C microscope. Morphometric traits was analyzed descriptively. Meanwhile, the relationship among body length and other morphological traits be analyzed by stepwise multiple regression with the helping of software IBM SPSS Statistics 22. The research result showed, the fruit flies that be collected from star fruit crops based on both morphology and molecular (mtCOI DNA gene) approaches belong to *Bactrocera umbrosa* Fabricius. The length of mtCOI DNA gene of fruit flies was 688 bp. The mean of body length, wing length, wing width, hind leg length and tibia length of obtained fruit flies *Bactrocera umbrosa* were 7.87, 5.92, 2.78, 5.38 and 1.79 mm, respectively. Due to relationship among body length (Y) toward other morphological traits (X₁-X₄), the tibia length has the highest contribution in determination of body length of fruit flies through linier regression equation model $Y = 5.64 + 1.24X_4$ ($r^2=36.30\%$).

Keywords: morphometric features, *Bactrocera umbrosa*, Deliserdang-North Sumatera

1. Introduction

North Sumatera belong to the center of horticultural crops production in Indonesia, especially fruits and vegetables. In district of Deliserdang, sub district of Pancur Batu, there are some horticultural crops that be planted by the farmer as citrus, guava and star fruit plants [1]. One as important problem that be faced by fruit farmers regarding production increasing is the fruit fly investation on horticultural crops [1, 2].

The fruit fly that infested horticultural crops in Indonesia and cause yield losses mainly belong to *Bactrocera* spp. [2,3,4]. This female fruit flies puncture the soft fruits (near ripening) with help of its ovipositor and lay the eggs under the fruit skin. Furthermore, the maggots which hatch from these eggs bore further into the fruit causing rotting and fruit fall [5]. The fruit flies *Bactrocera* spp. belong to the family Tephritidae of the order Diptera [6].

It has been reported, that fruit fly infestation in one district in North Sumatera has destroyed 17.000 ha citrus crops and therefore cause citrus production became just 20 ton ha⁻¹ prior to 60 ton ha⁻¹ [1].

In order to control and monitoring of insect pest including fruit flies *Bactrocera* spp in horticultural crops, the understanding of their biological characteristics, both based on morphology and molecular approaches are very needed. These knowledges are important due to support the fruit flies management in the field, especially in integrated pest management action.

Studies on fruit flies *Bactrocera* spp that could be found on some crops in Indonesia both based on morphology and molecular markers has been done by some experts [2, 3, 4, 7, 8, 9]. The biodiversity of fruit flies that infested horticultural crops as orange or citrus plantation in highland Karo district

in North Sumatera by a preliminary research has been reported. The research finding showed that there are at least three fruit flies species, namely: *Bactrocera dorsalis*, *Bactrocera umbrosa* and *Bactrocera caudatus* [7].

This research aim is to find out the kind of fruit flies species that infected horticultural crops in Pancurbatu subdistrict in North Sumatera based on its morphology and molecular-mtCOI DNA gene approaches. The other goals of this research is to know the morphometric data of *Bactrocera umbrosa* fruit flies and also in understanding the relationship among morphometric traits of fruit flies toward its body length that until this investigation has never been attempted.

2. Materials and methods

2.1. Study area

Fruit flies sampling was conducted in two hectares star fruit crops in Namo Riam village (N: 03°26'33.7"; E: 098°35'55.1", 114 m above sea level) at Pancur Batu subdistrict, Deliserdang district, in North Sumatera, Indonesia. The fruit flies catching took place in July 2020.

2.2. Collecting, morphologically identification and morphometric features assesment

The fruit flies were collected by using steiner traps that be given with methyl eugenol lure on cotton [1,7]. Fruit flies samples were deposited directly in 96% alcohol, labeled and transported to the laboratory for curation and identification. Fruit flies species identification based on morphology traits was done under stereo binocular microscope SZ 51 in taxonomy laboratory of Biology Department of Universitas Negeri Medan and be consulted on Siwi *et al.* (2006) [6]. Regarding morphometric traits assesment, the method was

based on Manurung *et al.* (2019) [10]. The observation and measurements were carried out under Stereo Zeiss-Stemi 2000-C microscope by using software Carl Zeiss Imaging System Axio Vision LE Release 4.8.2. Morphometric measurement was undertaken on 30 individuals of male fruit flies. Morphometry traits that be measured were body length, wing length or wing expanse, wing width, hind leg length and tibia length. Data analysis included mean and standard error of body length, wing length, wing width, hind leg length and tibia length. Meanwhile, the relationship among body length toward other morphometric traits analyzed by using stepwise multiple regression method with the helping of Software IBM SPSS Statistics 23.

2.3. Molecular Identification

In order to validate the result of identification based on morphology, molecular identification based on DNA barcoding, mtCOI DNA gene marker also was done on obtained fruit flies. The method for this DNA barcoding approach was based on Martiningsia *et al.* (2017) [3], Sudiarta *et al.* (2018) [4], Kunprom & Pramual (2019) [11] and Manurung *et al.* (2020) [12,13,14]. This molecular working consisted of DNA extraction, fragment mt COI amplification, sequencing and blasting into NCBI gene bank. DNA genomic was extracted from the head and leg tissues of fruit flies with ZR Tissue and Insect DNA Mini Prep Kit (Zymo Research, D6016). This extraction included preparing, lysis cell, DNA binding, washing and DNA elution steps. The DNA isolated result was confirmed using 1% TBE agarose. PCR amplification was done by using My Taq Red Mix (Bioline, Bio-25043). The mt COI DNA gene amplification was carried out by using primer LCO-1490 as forward (5'-GGTCAACAAATCA TAAAGATATTGG-3') and HCO-2198 as reverse (5'-TAAACTTCAGGGTGA CCAAAAAATCA-3') [11]. This PCR profile consisted of initial denaturation at temperature of 95°C for 1 min followed by one cycle with denaturation reaction conditions at 95°C for 15 sec, annealing at 52°C for 30 sec, extension at 72°C for 45 sec by 35 cycles and then followed by hold step at 4 °C by one cycle. The purification of PCR product was done by using the Zymoclean Gel DNA Recovery Kit (Zymo Research, D4002). The PCR product was assessed by electrophoresis with 1% TBE agarose. The running agarose was done at 100 volt for 60 min (Wealtec). Furthermore, the purified PCR product was sequenced with Bi-directional Sequencing using an ABI PRISM 3730 XLGenetic Analyzer at genetic lab of PT Genetika Science Indonesia, Jakarta. Furthermore, the obtained mtCOI gen fragments was aligned by using ClustalW-Mega X and sequencing homology was analyzed by using BLAST program at the National Center for Biotechnology Information (NCBI) website. Sequences homology analysis was performed by comparing COI sequence of fruit flies Namoriam. sample with NCBI Gen Bank data base (<https://www.ncbi.nlm.nih.gov>).

3. Results and discussion

3.1. Fruit flies *Bactrocera umbrosa* Fabricius traits based on morphology markers

Based on observation results on morphological characteristics of obtained fruit flies, the fruit flies adults that be collected on star fruits crops in Namoriam village was *Bactrocera umbrosa* Fabricius species. The validation of this fruit flies species was based on its head and thorax features, especially on its wings traits as a key of its morphology marker. On the face there was a dark spot in each furrow. The scutum colour is predominant black, meanwhile its scutellum lateral band is yellow. The presence of three transverse crossband or stripes on fruit flies wing was very clear and confirm. Furthermore, the fruit flies has the abdomen colour orange-brown, meanwhile its legs, especially its femora were pale. All these characteristics pointed out that fruit flies species is *Bactrocera umbrosa* Fabricius [6].

3.2. Fruit flies *Bactrocera umbrosa* Fabricius traits based on mtCOI gene markers

The research result due to mtCOI gene amplification of fruit flies showed that obtained mtCOI sequence has length 688 bp. This fragment has sequence as followed:

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1 GGTGACCAA AAATCAGAAT AAATGTTGGT ATAAATTTGG GGTCCCCCCC TCCTGCGGGG
61 TCGAAAAAAG AGGTGTTTAA GTTTCGGTCT GTTAATAATA TGGAATAGC TCCCGCTAAA
121 ACTGGAAGTG ATATAAAAAG TAATAAGGCT GTTAATACAA CTGCTCAAC GAAAAGAGGT
181 ATTCGGTCAA ATGAGATGCC TGTTGACCCG ATATTAATAA CTGTAGTAA GAAATTTACG
241 GCCCCTAGAA TTGAAGAGAT ACCAGCTAAG TGGAGTAAA AAATAGCTAG ATCGACTGAT
301 GCTCCTCCGT GGGCGATAAC TGATGATAGG GGTGGGTAAA CCGTTCAACC TGTACCAGCT
361 CCGTTTTCTA CTATGCTTCT TACTAACAGT AGCGTAAGGG AAGGAGGCAA TAATCAAAT
421 CTTATATTAT TCATTCTGGG GAATGCTATG TCGGTGCTC CTAGTATTAG AGGAACAAGC
481 CAGTTTTCCGA AGCCCCGAT TATAATGGGC ATACTATAA AAAAAATTAT CACGAAGCA
541 TGGCTGTGTA CAATTACATT ATAGATTGTA TCGTCTCCGA TTAATGCCCC GGGGTGACCT
601 AGTTCAGCTC GCACTAAAAT TCTAAGAGAT GTCCCGACTA TTCCTGCTCA GGCTCCGAAG
661 ATAAATATA AGGTCCAAT ATCTTTAT
    
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Fig 1

The blasting result of above mtCOI gene fragment on NCBI gen bank pointed out that Namoriam fragment has similarity until 99.85% with the mtCOI sequence of fruit flies *Bactrocera umbrosa* that originated from Yunnan, China (gen bank accession number KJ7539330.1 & KJ7539331.1) and Songkla, Thailand (gen bank accession number KF659825.1) (Table 1).

This finding confirmed, the fruit flies that originated from star fruit crops at Namoriam village in North Sumatera, Indonesia belong to *Bactrocera umbrosa* Fabricius species. This investigation result furthermore stated that the using of molecular approach that be based on DNA barcoding, especially through mtCOI DNA gene has corroborated the morphological identification and also become a valuable tool in animal taxonomy [12, 13, 14]. There was about 99.85% the homologous among North Sumatera, Indonesia *Bactrocera umbrosa* isolated and Yunnan, China and also Songkala, Thailand isolated.

It meant there was low genetic variation among the fruit flies in three countries, Therefore, geographical distance and also ecological differences between three countries may be don't have significant contribution on the mtCOI DNA gene variation creating toward the three populations.

It could also stated that three fruit flies populations (Indonesia, Thailand and China) probably have the same common ancestor.

Table 1: The Result of BLASTN analysis on Namoriam fruit flies sample

Species	Accession	Query Cover	Percent Identity
<i>Bactrocera umbrosa</i> COI	KJ753931.1	99%	99.85%
<i>Bactrocera umbrosa</i> COI	KJ753930.1	98%	99.85%
<i>Bactrocera umbrosa</i> COI	KF659825.1	95%	99.85%
<i>Bactrocera umbrosa</i> COI	DQ006868.1	99%	99.42%
<i>Bactrocera umbrosa</i> COI	DQ006867.1	99%	99.27%

3.3. Morphometric traits of *Bactrocera umbrosa* Fabricius

The research result due to five morphometric features of fruit flies *Bactrocera umbrosa* Fabricius originated from Ramoniam village in North Sumatera, Indonesia were displayed in Table 2.

Table 2: Morphometry of *Bactrocera umbrosa* originated from Nomoriam village in North Sumatera, Indonesia

No	Morphological traits	Mean ± SE (n=30) (mm)	Range (mm)
1	Body length-BL (Y)	7.87 ± 0.04	7.4-8.4
2	Wing length-WL (X ₁)	5.92 ± 0.03	5.5-6.4
3	Wing width-WW (X ₂)	2.78 ± 0.04	2.2-3.2
4	Hind Leg length-HL (X ₃)	5.38 ± 0.02	5.1-5.6
5	Tibia length-TL(X ₄)	1.79 ± 0.02	1.6-2.1

The mean of body length of *Bactrocera umbrosa* that could be found on star fruits was 7.87 mm, with the minimum and maximum length were 7.4 mm and 8.4 mm. In comparison to body length of *Bactrocera dorsalis* Hendel male adults that could be found in mango as reported by Sharma and Gupta (2018) [5], the finding of *Bactrocera umbrosa* body length in this research was shorter, whereas compare with *Bactrocera dorsalis* that infested guava and peach was longer [5]. The result of this investigation also showed, the body length of *Bactrocera umbrosa* originated from Namoriam village was longer compared with *Bactrocera zonata* that infested mango, guava and peach in Himachal Pradesh, India [5]. Regarding wing length or wing expanse, research finding pointed out that *Bactrocera umbrosa* originated Namoriam village has expanse 5.90 mm with range 5.5-6.4 mm. This length was very shorter compared to *Bactrocera dorsalis* and *Bactrocera zonata* species, in this case, the wing length of both fruit flies were around 12.00 mm [5,15]. However, as compared to *B. caudatus* (4.4 – 6.5 mm), the wing expanse of *B. umbrosa* in this research was longer. The wing width mean of obtained fruit flies *Bactrocera umbrosa* was 2.78 mm, with wing width minimum 2.2 mm and width maximum 3.2 mm. This wing width was broader compared to male insect white leafhopper *Cofana spectra* that has wing width average 1.35 mm [10]. The research finding pointed out, that the hind leg length average of *Bactrocera umbrosa* originated from Pancur Batu subdistrict in North Sumatera was 5.38 mm with range 5.1-5.6 mm. This leg length was shorter in comparison to leg length of leafhopper *Cofana spectra* (6.89 mm) [10]. The tibia length mean of obtained fruit flies *Bactrocera umbrosa* was 1.79 mm with minimum value 1.6 mm and maximum value 2.1 mm.

3.3. The relationship among body length toward other morphometric traits

The relationship among body length (Y) of fruit flies

Bactrocera umbrosa Fabricius with other its morphological traits (X₁ to X₄) in regression equation are presented in Table 3. Based on data in Table 3, it was found that there were three morphological traits that contributed on the determination of body length (Y) of fruit flies, namely: tibia length (X₄), wing length (X₁) and hind leg length (X₃). The morphological traits that have highest contribution on the prediction or determination of fruit flies body length was tibia length, X₄ (Y =5.64 + 1.24X₄, r²= 36.30%) (Table 3). In contrast to this research finding, abdomen and head length variables was two morphometric feature that has highest contribution in prediction of male body length of insect *Cofana spectra*, whereas wing length just in determination of *Cofana spectra* female body length [10]. Meanwhile, compared to fruit flies *Bactrocera dorsalis* Hendel, the morphometry trait that has the highest contribution regarding body length determination was wing length with determinat coefficient about 52% [16].

Table 3: Regression equation and determinat coefficient of some morphological traits in determination of fruit flies *B. umbrosa* body length

No	Regression equation	Determinant coefficient (R ²)
1	Y = 5.64 + 1.24X ₄	36.30%
2	Y = 3.96 + 0.29X ₁ + 1.21X ₄	41.70%
3	Y = 1.50 + 0.30X ₁ + 0.60X ₃ + 0.72X ₄	46.50%
4	Y = 1.54 + 0.30X ₁ + 0.007X ₂ + 0.59X ₃ + 0.73X ₄	46.51%

4. Conclusion

The fruit flies that originated from star fruit crops at Namoriam village in North Sumatera, Indonesia both based on morphology and mtCOI DNA gene markers belong to *Bactrocera umbrosa* Fabricius. The length of its mtCOI DNA gene was 688 bp. The mean of body length, wing length, wing width, hind leg length and tibia length were 7.87, 5.92, 2.78, 5.38 and 1.79 mm, respectively. The tibia length is the trait that has highest contribution (36.30%) due to body length determination of *Bactrocera umbrosa*.

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6. References

1. Manurung B, Prastowo P, Tarigan EE. Daily activity pattern and population dynamic of fruit fly *Bactrocera dorsalis* complex on citrus plantation at highland Karo district, North Sumatera. J.HPT.Tropika. 2012; 12(2):103-110.
2. Sahetapy B, Uluputty MR, Naibu La. Identifikasi Lalat Buah (*Bactrocera spp.*) Asal Tanaman Cabai (*Capsicum annuum L.*) dan Belimbing (*Averrhoa carambola L.*) Di Kecamatan Salahutu Kabupaten Maluku Tengah. Jurnal Agrikultura 2019; 30(2):63-74.
3. Martiningsia D, Wijaya IN, Sudiarta IP.

- Karakteristik molekular dan filogeni lalat buah *Bactrocera occipitalis* (Diptera: Tephritidae) dari Tarakan berdasarkan sekuen nukleotida gen COI. *J. Agric. Sci. and Biotechnol.* 2017; 6(1):18-26.
4. Sudiarta IP, Martiningsia D, Wijaya IN. Molecular identification of exotic fruit fly *Bactrocera occipitalis* (Diptera: Tephritidae) using Mitochondrial Cytochrome Oxidase I (COI) gene. *International Journal Biosciences and Bio technology.* 2018; 6(1):34-43.
 5. Sharma I, Gupta D. Morphometry of *Bactrocera dorsalis* and *B. zonata* on mango (*Mangifera indica*), guava (*Psidium guajava*) and peach (*Prunus persica*). *Journal of Entomology and Zoology Studies.* 2018; 6(4):395-397.
 6. Siwi SS, Hidayat P, Suputa. *Taksonomi dan Bioekologi Lalat Buah Penting di Indonesia (Diptera: Tephritidae)*. BB Biogen & Dept. Agriculture, Fisheries & Forestry Australia, Bogor, 2006.
 7. Manurung B, Gusmita E. Provosional studies of fruit flies ecology at orange plantation in Karo district. *Jurnal Sains Indonesia.* 2005; 29(4):135-139.
 8. Nismah, Susilo FX. Keanekaragaman dan kelimpahan lalat buah (Diptera: Tephritidae) pada beberapa sistem penggunaan lahan di Bukit Rigis, Sumberjaya, Lampung Barat. *J. HPT Tropika.* 2008; 8(2):82-89.
 9. Indriyanti, Suputa D, Jannah SN. Molecular identification of *Bactrocera* sp. fruit fly from Muria forest, Central Java, Indonesia. *ARPN Journal of Engineering and Applied Sciences.* 2017; 12:2954-2961.
 10. Manurung B, Hasairin A, Daulae AH, Silaban P, Hasibuan N. Morphometric studies on white leafhopper (*Cofana spectra*) from rice fields in Tapanuli region North Sumatera-Indonesia. *Journal of Entomology and Zoology Studies.* 2019; 7(1):701-705
 11. Kunprom C, Pramual P. DNA barcoding of fruit flies (Diptera: Tephritidae) in Thailand: ambiguity, misidentification and cryptic diversity. Mitochondrial DNA part A, 2019. <https://doi.org/10.1080/24701394.2019.1693550>.
 12. Manurung B, Hasairin A, Daulae AH. Genetic analysis and molecular phylogeny of rice green leafhopper *Nephotettix nigropictus* (Stal) based on the mitochondrial COI DNA gene. *Annals of Biology.* 2020; 36(2):181-185.
 13. Manurung B, Hasairin A, Daulae AH. Genetic analysis and molecular phylogeny of zigzag leafhopper *Maiestas dorsalis* (Motschulsky) using mitochondrial COI gene. *IOP Conf.Series: Earth and Environmental Sciences*, 457 012021, 2020b. Doi:10'1088/1755-1315/457/1/012021.
 14. Manurung B, Hasairin A, Daulae AH. Molecular analysis of rice green leafhopper, *Nephotettix virescens* (Distant) from Samosir Island-Indonesia using mitochondrial COI gene. *J. ent. Res.* 2020c; 44(2):183-188.
 15. Singh S, Sharma DR. Biology and morphometry of *Bactrocera dorsalis* and *Bactrocera zonata* on different fruit crops. *Indian Journal of Agricultural Sciences.* 2013; 83(12):1423-25.
 16. Manurung B, Hasairin A, Prastowo P. Morphometric and genetic analysis of fruit fly *Bactrocera dorsalis* complex on horticultural crops in North Sumatera-Indonesia. *Book of Abstract The 4th International Conference on Community Research and Service Engagements (IC2RSE)-Virtual Conference*, Medan, Indonesia. 2020, 141.