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Morphology and Molecular Identification of *Bactrocera umbrosa* Collected in North Minahasa, Indonesia

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ABSTRACT

Being one of the most varied and damaging families of fruit flies, the genus Bactrocera is important in ecological, agricultural, and economic contexts. These insects, which are mostly found in tropical and subtropical areas, are infamous for infesting a variety of fruits and vegetables, seriously harming crops, and compromising the world's food security. Because of its possible effects on the local agriculture, *Bactrocera umbrosa* research is especially crucial in North Minahasa. The purpose of this study is to use morphological and molecular identification techniques to characterize the *B. umbrosa* fruit fly in North Minahasa, Indonesia. DNA barcoding of the COI gene was used for molecular identification. Furthermore, the COI gene homology of the discovered *B. umbrosa* was compared to all fly specimens of the same species listed in the NCBI (National Center for Biotechnology Information) GenBank database. According to the morphological identification, HR1 is confirmed as *B. umbrosa*. The molecular identification utilizing the COI gene sequence validated this result. According to the molecular analysis, *B. umbrosa* fly specimens collected in North Minahasa, Indonesia, are distinct from those of their close relatives collected in Papua New Guinea, Palau, Vanuatu, and New Caledonia. The closest relatives of this species, as well as insects in general, exhibit intraspecies variation. The findings of this study serve as the foundation for the necessity of future, more thorough research on fruit flies in Indonesia.

Keywords: *Bactrocera umbrosa*, morphology, molecular identification, North Minahasa

INTRODUCTION

The genus Bactrocera holds significant importance in agriculture, ecology, and economic contexts due to its role as one of the most diverse and destructive groups of fruit flies. Found predominantly in tropical and subtropical regions, these insects are notorious for infesting a wide range of fruits and vegetables, causing severe damage to crops and impacting global food security (1). Many species within the Bactrocera genus, such as the oriental fruit fly (*Bactrocera dorsalis*), are considered major pests and have led to substantial economic losses in the agricultural sector (2). Additionally, the invasive nature of some Bactrocera species poses challenges for international trade, as they necessitate

stringent quarantine and management measures (3). Understanding the biology, behavior, and control strategies of Bactrocera species is crucial for sustainable agricultural practices and the prevention of widespread crop infestations.

Identifying *Bactrocera* species using both morphological and molecular approaches is essential for comprehensive and accurate taxonomic studies. Morphological identification relies on observable physical traits, such as wing patterns, body coloration, and shape of reproductive structures, which have been traditionally used to classify species. This method is cost-effective and can be performed in the field or laboratory, making it an accessible tool for pest management teams (4,5). However, morphological similarities among closely related species and cryptic species complexes can limit its reliability. Molecular approaches, such as DNA barcoding and genome sequencing, complement morphology by providing genetic insights that can distinguish species with overlapping physical traits (6). These techniques are particularly useful for identifying immature stages, such as larvae, or damaged specimens where morphological features are not discernible. Combining morphological and molecular methods enhances the accuracy and efficiency of *Bactrocera* species identification, facilitating better pest control strategies and contributing to the understanding of their evolutionary relationships and ecological roles (7–9).

The study of *Bactrocera umbrosa* is particularly important in North Minahasa due to its potential impact on the region's agriculture. *Bactrocera umbrosa*, commonly known as the lesser jackfruit fruit fly, is a significant pest of tropical fruits, especially jackfruit and other related species (10). These fruits are economically and culturally valuable in North Minahasa, where they serve as staple foods and cash crops for local farmers (11). Understanding the biology, behavior, and distribution of *B. umbrosa* in this region is essential for predicting and mitigating its impact on fruit production (12). Moreover, studying its host preferences, life cycle, and environmental interactions provides insights into effective management strategies, such as implementing targeted control measures and minimizing crop losses. Research on *B. umbrosa* also aids in understanding its role in the local ecosystem, particularly its interactions with native plants and other insect species. By addressing the challenges posed by *B. umbrosa*, researchers can help safeguard the agricultural sustainability and economic well-being of North Minahasa communities.

This research aims to describe the *B. umbrosa* fruit fly in North Minahasa, Indonesia using morphological and molecular identification approaches. Molecular identification was carried out using DNA barcoding of the COI gene. The *B. umbrosa* fruit fly found was then compared for homology of the COI gene with all fly specimens of the same species recorded in the NCBI (National Center for Biotechnology Information) GenBank database.

MATERIALS & METHODS

Sampling and Description of Specimen

Fruit fly adults trapped in the field in Steiner traps (modified) were transferred to plastic boxes to be taken to the laboratory. The specimens were taken from this location: 1º28’0”N, 124°55’3” E. Morphological identification of the fruit fly was carried out at the Plant Quarantine Laboratory, North Sulawesi Fish and Plant Animal Quarantine Center. *Bactrocera umbrosa* identification was carried out using a Nikon Nikon Model C-DSD230 microscope. Identification of species based on morphological characters uses the identification key The Australian Handbook for The Identification of Fruit Flies Version 3.1 (13). The identified specimens are then stored in a collection bottle that has been treated with silica gel and lined with tissue.

The morphological characters used are the shape of the facial spot on the face, the

shape of the abdomen, the shape of the terga, the color of the scutum, the shape of the lateral post sutural vittae, the presence or absence of medial post sutural vittae, wing venation, the presence or absence of apical or subapical spots on the legs, the 'T' pattern on the abdomen, and the shape of the anterolateral corner on terga IV. Specific morphological characters are used to refer to the species level. Each morphological character used is also created in the image documentation.

DNA Extraction, Polymerase Chain Reaction (PCR), and Electrophoresis

The specimen of *Bactrocera umbrosa* was placed in a 1.5 ml microtube. Total DNA extraction of the flies used the innuPrep DNA Micro Kit (Analytik Jenna), according to the manufacturer's manual. PCR reactions were carried out using the MyTaq HS Red Mix 2x kit (Bioline) and the total DNA template from the previous step. Each 40 µl PCR reaction had 15 pmol of each primer. The COI gene was amplified using PCR (14), namely with primers LCO1490 (5'-ggt caa caa atc ata aag or ttg g-3') and HC02198 (5'-taa act tca ggg tga cca aaa aat ca-3'). Reaction conditions for the first PCR using both primers: denaturation 95°C (3 minutes) followed by 35 cycles of denaturation 95°C (20 seconds), annealing 50°C (30 seconds), extension 72°C (20 seconds). The components in 40 µl of reaction in a PCR tube are 20 µl of MyTaq HS Red Mix 2x (Bioline), 1.5 µl of each primer, 15 µl of Milli-Q water, and 2 µl of template DNA.

The PCR results were separated using 0.8% agarose gel electrophoresis (in 1x TBE buffer) and observed using a UV-Transilluminator. The PCR product was visualized using a UV-Transilluminator and PCR success was detected by the presence of a single DNA band of 710 bp. Sequencing uses two primers used during PCR. The PCR results and both primers were sent to First Base CO (Malaysia) for sequencing. The results obtained were in the form of a chromatogram containing DNA sequences. DNA editing was carried out using Geneious Prime version 2025.0.3. The length of DNA (without primers) after editing is 658 bp (15,16), displayed in FASTA format. DNA sequences were used for molecular identification using BLAST (Basic Local Alignment Search Tool, https://blast.ncbi.nlm.nih.gov) in the NCBI database.

RESULT AND DISCUSSION

Specimen HR1 was morphologically identified as *Bactrocera umbrosa* (Figure 1). This specimen has a body length of 8.75 mm (Figure 1a). The scutum is black except for the lateral side, the postpronatal lobes and pleuro moto are yellow, there are wide yellow bands that are almost parallel on the lateral side and stop just or slightly behind the intra-alar seta, the scutellum is yellow, the scutum is with lateral postsutural vittae (stripes). The scutum is without vitta or medial line (Figure 1b). The abdomen was yellow-orange and without black T pattern (Figure 1c). Black spot on the face, medium sized, round (Figure 1d). The wings are easily recognized by the appearance of three transverse bands. The wings are 5.2-8.1 mm long. Wings with 3 transverse bands (stripes); along the transverse vein bm-cu; along the transverse veins r-m and dm-cu; crosses apical part of M. With complete costal band, reaches R4+5, and does not widen into a spot at apex. Wings with anal line. Colored cells bc and c. BC cells without extensive layers of microtrichia. C cells with or without extensive layers of microtrichia. Br cells (constricted part) with an extensive layer of microtrichia (Figure 1e). The limbs are pale (Figure 1f).

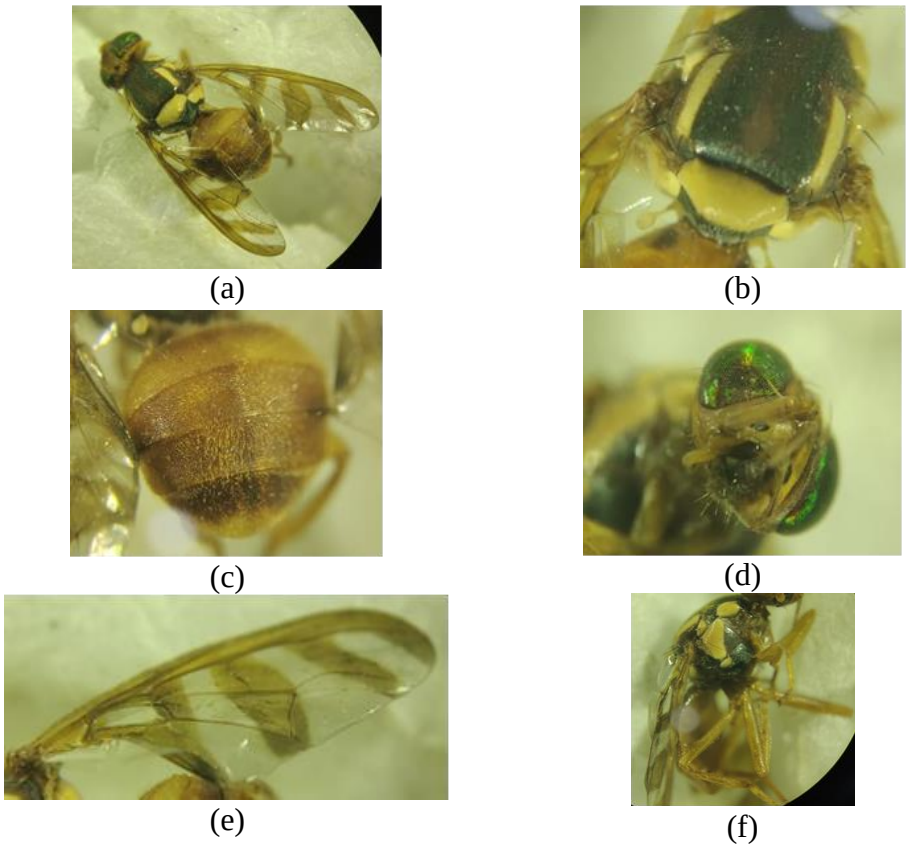


Figure 1. Morphology of HR1 specimen identified as *Bactrocera umbrosa*: (a) body, (b) head, (c) abdomen, (d) face shape, (e) wing, and (f) limb

The success of PCR using this primer was indicated by the presence of a single band 710 bp long, in accordance with the description of Folmer region (14) whose primers were used in the PCR reaction. In the HR1 specimen, there is a single band between the standard bands of 750 bp and 500 bp (Figure 2). This single band is closer to the standard 750 bp band. This confirmed that the predicted PCR product size was 710 bp, which was indeed closer to 750 bp than the 500 bp band. The DNA band of specimen HR1 looks sharp and does not have a smear. This makes it feasible to proceed to the PCR stage.

The results of COI gene sequencing from the PCR product of specimen HR1 are displayed in the chromatogram (Figure 3a). This image consists of two chromatograms read from two opposite directions and then combined to obtain a consensus sequence of 658 bp. This consensus has been trimmed with forward primer and reverse primer sequences. From this chromatogram, clear peaks can be seen, which indicates that the sequencing results produced unambiguous readings. The sequence similarity between the two chromatogram reads confirms this. The 658 bp consensus COI gene DNA sequence was translated into FASTA format (Figure 3b) which will later be used for molecular identification on the NCBI (National Center for Biotechnology Information) website.

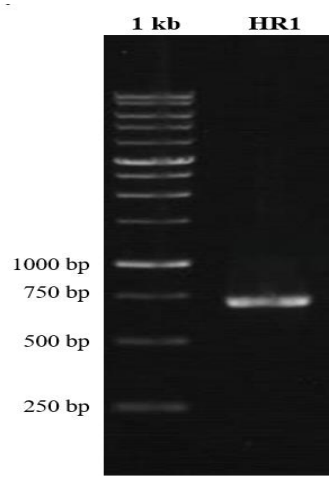
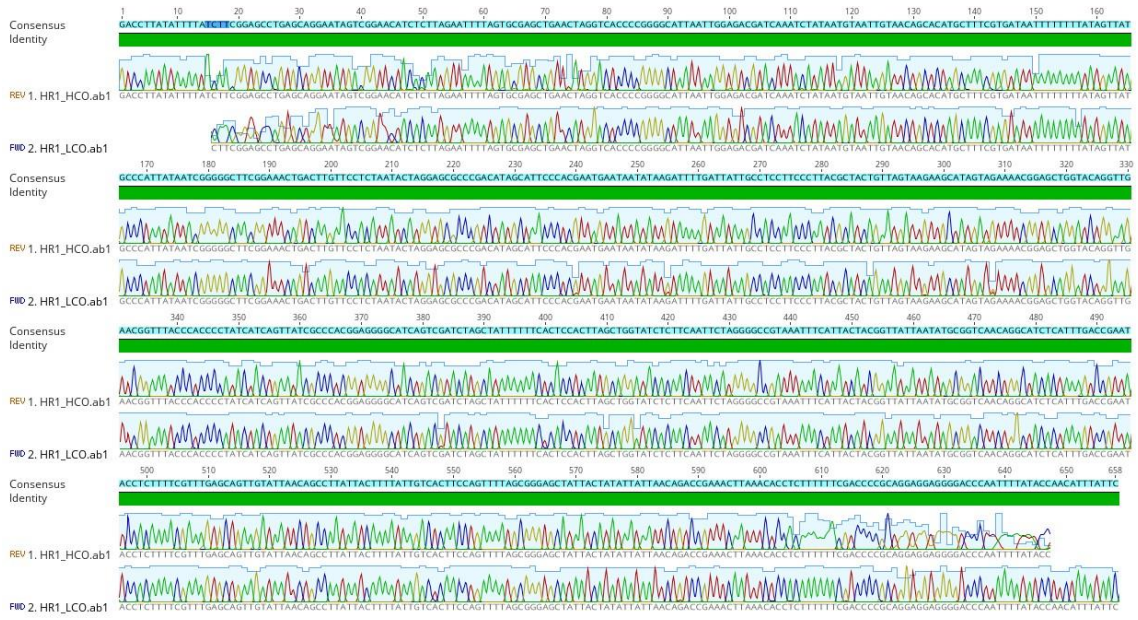


Figure 2. Elektrophoresis gen agarose (0.8%) of PCR product of HR1 specimen



(a)

>HR1
GACCTTATATTTTATCTTCGGAGCCTGAGCAGGAATAGTCGGAACATCTCTTAG
AATTTTAGTGCGAGCTGAACTAGGTCACCCCGGGGCATTAATTGGAGACGATC
AAATCTATAATGTAATTGTAACAGCACATGCTTTCGTGATAATTTTTTTTATAG
TTATGCCCATTATAATCGGGGGCTTCGGAAACTGACTTGTTCCTCTAATACTAG
GAGCGCCCGACATAGCATTCCCACGAATGAATAATATAAGATTTTGATTATTG
CCTCCTTCCCTTACGCTACTGTTAGTAAGAAGCATAGTAGAAAACGGAGCTGG
TACAGGTTGAACGGTTTACCCACCCCTATCATCAGTTATCGCCCACGGAGGGG
CATCAGTCGATCTAGCTATTTTTTCACTCCACTTAGCTGGTATCTCTTCAATTCT
AGGGGCCGTAAATTTCATTACTACGGTTATTAATATGCGGTCAACAGGCATCT
CATTTGACCGAATACCTCTTTTCGTTTGAGCAGTTGTATTAACAGCCTTATTAC
TTTTATTGTCACTTCCAGTTTTAGCGGGAGCTATTACTATATTATTAACAGACC
GAAACTTAAACACCTCTTTTTTCGACCCCGCAGGAGGAGGGGACCCAATTTTA
TACCAACATTTATTC

(b)

Figure 3. Chromatogram (a) and FASTA (b) format of HR1 specimen

Results of homology comparison of DNA sequences in the NCBI database via BLAST, the HR1 specimen sequence has a similarity of 99.70% to *Bactrocera umbrosa*, one of which is the specimen with accession numbers PP568528.1 (17) collected in New Caledonia and PP570048.1 collected in Palau. Similarity of 99.54% to this species also occurs with specimens with accession numbers PP570769.1 (17) from Vanuatu and MK038675.1 (18) from Papua New Guinea. Similarity to this species is also at 99.39% similarity to MK038684.1 and PP569579.1, both from Papua New Guinea, collected by two different research groups, as mentioned previously (Figure 4). These BLAST results have strengthened the results of the morphological identification of *B. umbrosa* fruit flies carried out at the beginning of the research. Through molecular identification, genetic comparisons of this fly species can be made with the sequencing results of other researchers throughout the world. The results of this study show that *B. umbrosa* fly specimens collected in North Minahasa, Indonesia are unique compared to their closest relatives collected in New

Caledonia, Palau, Vanuatu and Papua New Guinea. The intraspecies variation that occurs in this species is a normal thing that also occurs in its closest relatives (19–21) as well as insects in general (22–25). For this reason, the results of this research are the basis for the need for more extensive research in the future on fruit flies in Indonesia. Research should use a larger number of specimens and study the genus Bactrocera in general. Future research could also be carried out by constructing a phylogeny tree to see the relationship between species in this genus in Indonesia.

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Bactrocera umbrosa voucher UHIM.ms11594 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Bactrocera umbr…	1205	1205	100%	0.0	99.70%	1493	PP568528.1
Bactrocera umbrosa voucher UHIM.ms11625 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Bactrocera umbr…	1205	1205	100%	0.0	99.70%	1493	PP569468.1
Bactrocera umbrosa voucher UHIM.ms11410 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Bactrocera umbr…	1205	1205	100%	0.0	99.70%	1493	PP570048.1
Bactrocera umbrosa isolate 7 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	Bactrocera umbr…	1205	1205	100%	0.0	99.70%	658	MK038680.1
Bactrocera umbrosa voucher UHIM.ms11122 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Bactrocera umbr…	1205	1205	100%	0.0	99.70%	1493	PP568314.1
Bactrocera umbrosa voucher UHIM.ms11577 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Bactrocera umbr…	1205	1205	100%	0.0	99.70%	1464	PP569226.1
Bactrocera umbrosa voucher UHIM.ms11120 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Bactrocera umbr…	1205	1205	100%	0.0	99.70%	1493	PP568461.1
Bactrocera umbrosa voucher UHIM.ms11959 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Bactrocera umbr…	1199	1199	100%	0.0	99.54%	1493	PP570769.1
Bactrocera umbrosa voucher UHIM.ms11957 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Bactrocera umbr…	1199	1199	100%	0.0	99.54%	1493	PP571475.1
Bactrocera umbrosa voucher UHIM.ms11119 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Bactrocera umbr…	1199	1199	100%	0.0	99.54%	1493	PP568472.1
Bactrocera umbrosa isolate 2 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	Bactrocera umbr…	1199	1199	100%	0.0	99.54%	658	MK038675.1
Bactrocera umbrosa voucher UHIM.ms04469 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Bactrocera umbr…	1199	1199	100%	0.0	99.54%	1493	PP568974.1
Bactrocera umbrosa voucher UHIM.ms12001 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Bactrocera umbr…	1199	1199	100%	0.0	99.54%	1493	PP568501.1
Bactrocera umbrosa voucher UHIM.ms11960 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Bactrocera umbr…	1199	1199	100%	0.0	99.54%	1493	PP571447.1
Bactrocera umbrosa voucher UHIM.ms11958 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Bactrocera umbr…	1199	1199	100%	0.0	99.54%	1493	PP569352.1
Bactrocera umbrosa isolate 11 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	Bactrocera umbr…	1194	1194	100%	0.0	99.39%	658	MK038684.1
Bactrocera umbrosa voucher UHIM.ms11626 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Bactrocera umbr…	1194	1194	100%	0.0	99.39%	1493	PP569579.1
Bactrocera umbrosa isolate 4 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	Bactrocera umbr…	1188	1188	100%	0.0	99.24%	658	MK038677.1
Bactrocera umbrosa isolate 8 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	Bactrocera umbr…	1188	1188	100%	0.0	99.24%	658	MK038681.1

Figure 4. BLAST result of HR1 specimen, confirm identification as *Bactrocera umbrosa*

CONCLUSION

The morphological identification suggests that HR1 identify as *B. umbrosa*. This result confirmed by the molecular identification using COI gene sequence. The molecular identification results of this study show that *B. umbrosa* fruit fly specimens collected in North Minahasa, Indonesia are unique compared to their closest relatives collected in New Caledonia, Palau, Vanuatu and Papua New Guinea. The intraspecies variation that occurs in this species also occurs in its closest relatives and insects in general.

Declaration by Authors

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Conflict of Interest: The authors declare no conflict of interest.

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