

## MOLECULAR IDENTIFICATION OF EXOTIC FRUIT FLY *Bactrocera occipitalis* (DIPTERA: TEPHRITIDAE) USING MITOCHONDRIAL CYTOCHROME OXIDASE I (COI) GENE

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### ABSTRACT

Some of fruit flies have been reported as the important pest on fruits and vegetables in the world. Agricultural Quarantine Agency Denpasar reported that there was new coming species (exotic) of fruit flies in Bali in 2014 based on the morphological identification, namely *Bactrocera occipitalis*. However *Bactrocera dorsalis* complex have similar morphological characters and have a less distinctive character for taxonomic identification, therefore it is difficult to identify fruit flies accurately. Based on that phenomena, the accurate identification is needed. One of the more accurate identification techniques is based on molecular identification using DNA-based barcode. To identify fruit flies, DNA-based barcode using mitochondrial cytochrome oxidase I (COI) gene has been conducted. PCR analysis using Fruit Fly MT-COI-F (FFMT-COI-F) 5'-GGAGCATTAATYGGRGAYG-3' as forward primer and HCO 5'-TAAACTTCAGGGTGACCAAAAATCA-3' as reverse primer was successfully amplified around 600 bp of COI gene of fruit flies. Based on similarity of sequence product, the species was identified as *Bactrocera occipitalis* and same result was revealed using morphological identification. Phylogenetic analysis of *B. occipitalis* based on COI genes showed that *B. occipitalis* from Bali were in the same groups with *Bactrocera* species from Tarakan and Philippines. In addition, *Bactrocera occipitalis* as exotic fruit fly is a new report in Bali, Indonesia.

**Keywords:** fruit flies, COI, Molecular identification, Phylogenetic analysis

### INTRODUCTION

Fruit flies (Diptera: Tephritidae) are among the most destructive agricultural pests in the world, eating their way through acres and acres of citrus and other fruits at an alarming rate and forcing food and agriculture agencies to spend millions of dollars in control and management measures. Genus of *Bactrocera* is a group of fruit fly with 450 members of species (Drew and

Hancock, 2000). In addition some of species *Bactrocera* were reported as an importance pest on fruits and vegetables (Allwood *et al.*, 1999). However, until now, the study of fruit flies has been traditionally biased towards applied aspects including the morphological identification, and lack of molecular approach. The traditional method for identification based on morphological characteristic has importance role to

understand species of fruit flies. On the other hand, the morphological identification has some weakness and not completely accurate. A morphological character occasionally is not describing the relationship with the genetic characters and some factors are caused by environmental interaction (Smith *et al.*, 2003; Siwi, 2004). Based on those phenomena the more accurate identification is needed, one of them is molecular identification. Molecular identification has been utilized for improving the accurate information from morphological character, molecular characters more stable compared with morphological character (Hidayat, 2005). DNA barcoding has gradually been verified as an effective tool for identifying species in a wide range of taxonomic groups (Jiang *et al.*, 2014). DNA barcoding using Gen Mitokondrial *Cytochrome Oxidase Subunit I* (MT-CO1) has been utilized for identification of fruit flies. The MT-COI barcode sequences for the diagnosis of fruit flies using 1426 sequences for 73 species of *Bactrocera* distributed worldwide was reported by Jiang *et al.* (2014). In addition, mitochondrial DNA also has been used to describe the genetic variation of animal (Loftus *et al.*, 1994; Suryanto, 2003). As new information, Agricultural Quarantine Agency Denpasar reported that in 2013-2015 there was a new coming species or exotic fruit fly in Bali namely *Bactrocera occipitalis* based

on their morphological characteristics. In this study, DNA barcoding using MT-COI gene was performed to reconfirm both species.

## MATERIALS AND METHODS

### Fruit fly Trapp

The method of trapping of fruit fly was conducted base on protocol of survey of pests and diseases by Agricultural Quarantine Agency. Trapping area was designed all regencies around Bali using (*Trapping*) tipe Steiner (*Steiner Trap*) with *Cue lure* and *Methyl eugenol* attractant. The collecting of fruit fly was conducted every week to collect sample for morphological and molecular identification. Sample *B. occipitalis* Tarakan was collected from Laboratory of Agricultural Quarantine Agency Tarakan.

### Morphological identification

The morphological identification was conducted as the first method to recognize the species of fruit fly. However, the method requires expert examination and may require additional supporting evidence such as the molecular diagnosis or host association records. Morfological identification was conducted based on identification key on book guide "*Fruit Flies of Indonesia : Their Identification, Pest Status and Pest Menegement*" by Griffith University, Brisbane, Australia and *The Australian Handbook for the Identification of Fruit Flies* (Plant Health Australia, 2011). Key features

used for the morphological diagnosis of adult fruit flies include: wing morphology and infuscation, presence or absence of various setae, and relative setal size. (Note: Chaetotaxy, the practice, of setal taxonomy, is not as important in this group as some others.), overall colour and colour patterning, as well as presence, shape and color of thoracic vittae, a vitta is a band or stripe of colour (Plant Health Australia, 2011).

### **Molecular Identification**

#### **DNA extraction and MT-COI gen amplification**

Qiagen DNeasy® (Qiagen, Hilden, Germany) Blood and Tissue kit was used to extract the DNA of fruit flies. The result of DNA extraction was amplified using PCR with general primers MT-CO1 Fruit Fly MT-CO1-F (FFMT-CO1-F)5'-GGA GCA TTA ATY GGR GAY G-3' as forward primer and HCO 5'-TAA ACT TCA GGG TGA CCA AAA ATC A-3' as reverse primer (Plant Health Australia, 2011). PCR amplification was performed in a 25 µl final reaction volume consisting of master mix 12,5 µl (1x BSA, 10x Buffer, dNTP's, MgCl<sub>2</sub>, dan NEB Taq), 1 µl LCO1490-F, 1 µl HCO-R, DNA template 3 µl, dan *Nuclease free water* 7,5 µl., was conducted in *Thermal cycler*. The PCR thermal cycle program consisted of initial denaturation at 94 °C for 2 min, followed by 40 cycles of denaturation at 94

°C for 30 s, annealing at 52 °C for 30 s and extension at 72 °C for 1 min with final extension at 72 °C for 7 min and finally holding at 4 °C until analyzed. (Plant Health Australia, 2011).

#### **Electrophoresis, sequencing and phylogeny analysis**

The PCR products with 1 µl loading dye were analyzed by gel electrophoresis on 2 % agarose gel at 70 V for 120 min in 1× TAE Buffer. DNA amplification product was visualized and captured by UV light using UV transilluminator at 302 nm wavelengths. The DNA target of both of fruit flies is *around* 600 bp (Delomen *et al.*, 2013, Pramudi *et al.*, 2013).

PCR product of fruit flies was analyzed in First Base Laboratory, Malaysia for DNA sequencing., The sequence of nucleotide was used to search similarity in *GeneBank* with Program *Basic Local Alignment Tool* (BLAST) (NCBI 2014) on site of *the European Bioinformatic Institute* (EBI) ([www.ebi.ac.uk](http://www.ebi.ac.uk)). The homolog sequence of nucleotide of fruit flies was aligned to understand the similarity score using program ClustalW. Data from the sequencing of MT-COI DNA of fruit flies were analyzed using BioEdit 7.0.1 to determine the consensus sequences based on the conservative sequences and MEGA (Molecular Evolutionary Genetics Analysis)

6: 06 program to construct phylogeny trees (Tamura *et al.*, 2013).

After some treatments, all of the samples were identified. From the morphological key of new species or exotic species in Bali, the species was identified as *Bactrocera occipitalis*. *Bactrocera occipitalis* from Tarakan was use as control positive (Fig. 1).

## RESULTS AND DISCUSSION

The collected samples of fruit fly from trap were calculated in laboratory of Agricultural Quarantine Agency Denpasar.


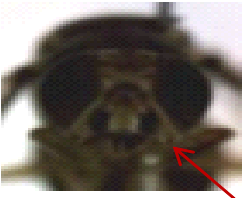






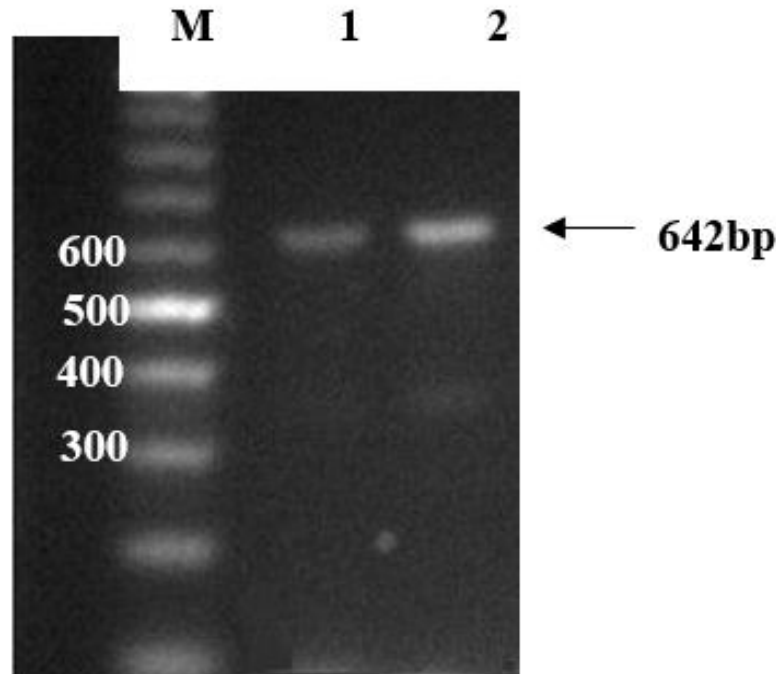
Charateristic	<i>B. occipitalis</i> Bali	<i>B. occipitalis</i> Tarakan
Caput		
Thorax		
Wing		
Abdomen		

Fig. 1. morphological characteristic of *b. Occipitalis*. (a,b) Caput or head with large oval of spot on face, (c,d) black scutellum except of posterior and around prescutellar setae with brown colour, yellow stripe of scutum, (e,f) costal band overlapping R2+3, pointed of cubital streak (g,h) abdominal terga III-V orange-brown witha narrow to medium width medial longitudinal dark fuscous to black band over all three terga and lateral dark

### DNA amplification

*Mitochondrial cytochrome Oxidase I* gene (MT-COI) of both fruit flies were successfully amplified by PCR using forward primer MT-CO1-F(FFMT-CO1-F)

5'-GGAGCATTAATYGGRGAYG-3' and reverse primer: HCO 5'-TAAACTTCAGGGTGACCAAAAATCA-3', around 600 bp (Fig. 2). The PCR products were purified and used to sequence analysis.



**Fig. 2. PCR Product of amplification of MT-COI Gen of fruit flies.** Lane M : marker 100 bp (Fermentas); 1. MT-CO1 gen of *Bactrocera occipitalis* Bali; 2. MT-CO1 gen of *Bactrocera occipitalis* Tarakan

### Sequence homology

To understand the species and homology of fruit flies from Bali with other fruit flies, the nucleotide sequence of MT-COI gene was analyzed using software BLAST in Genebank. The homology

sequence of fruit fly with morphological character looks like *B. occipitalis*. The alignment data indicated MT-COI gen of *B. occipitalis* Bali has high similarity with MT-COI gen *B.occipitalis* from Filipina with homology 95,2 % (Table 1).

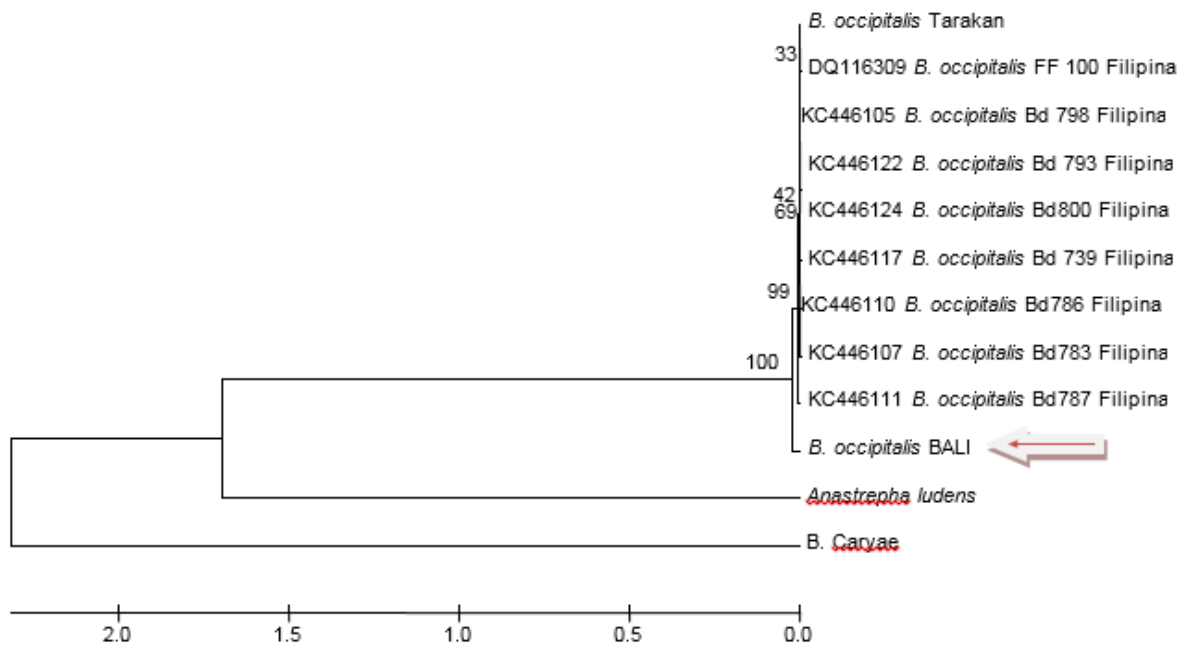
**Table 1.** The homology (%) sequence of of MT-COI gen of *B. occipitalis* with other species *Bactrocera* in *GenBank*. [1] *B. occipitalis* from Bali; [2] *B. occipitalis* from Tarakan; [3] *B. occipitalis* KC446122 Bd793 from Filipina; [4] *B. occipitalis* KC446124 Bd 800 from Filipina; [5] *B. occipitalis* KC446117 Bd 739 from Filipina; [6] *B. occipitalis* KC446107 Bd 783 from Filipina; [7] *B. occipitalis* DQ116309 Isolat FF 1000 from i Filipina; [8] *B. occipitalis* KC446110 Bd786 from Filipina; [9] *B. occipitalis* KC446111 Bd787 from Filipina; [10] *B. occipitalis* KC446105 Voucher Bd739 from Filipina; [11] *Anasthrepa lugens* HM538364 as outgroup; [12] *B. caryae* AY941232 as outgroup

	1	2	3	4	5	6	7	8	9	10	11	12
[1]	ID											
[2]	95	ID										
[3]	95	99,6	ID									
[4]	95,2	99,8	99,8	ID								
[5]	95,2	99,8	99,4	99,6	ID							
[6]	95	99,6	99,2	99,4	99,4	ID						
[7]	94,6	99,6	99,2	99,4	99,4	99,2	ID					
[8]	95	100	99,6	99,8	99,8	99,6	99,6	ID				
[9]	94,8	99,8	99,4	99,6	99,6	99,8	99,4	99,8	ID			
[10]	94,5	99,4	99	99,2	99,2	99,4	99	99,4	99,6	ID		
[11]	45,8	46,8	46,6	46,8	46,8	46,8	46,5	46,8	46,6	46,3	ID	
[12]	37	37,4	37,4	37,4	37,4	37,4	37,2	37,4	37,4	37,4	34,3	ID

### Phylogeny tree and Genetic distance

Phylogeny analysis with *unweighted pair group* method (UPMGA) 100x *bootstrap* (Brinkman, F & D. Leipe 2001; Dharmayanti, 2011; Holil, 2004; Tamura *et al.*, 2013) was used to construct phylogenetic tree of *Bactrocera occipitalis* Bali (Fig. 3).

Phylogenetic tree showed that the *B. occipitalis* Bali is in the same clade with *B. occipitalis* Tarakan and *B. occipitalis* Philippines. The data also showed that *B. occipitalis* Bali is same *monophyly* group with *B. occipitalis* Tarakan and *B. occipitalis* Filipina (Fig. 3).



**Fig. 3. Phylogeny tree of *B. occipitalis* and other *Bactrocera* from other countries base on MT-CO1 gen**

Genetic distance of MT-COI gen of *B. occipitalis* Bali with *B. occipitalis* Tarakan and Filipina around 0,043-0,052. The closer genetic distance of *B. occipitalis* Bali is with *B. occipitalis* KC446124 Voucher Bd800 Philippines and *B. occipitalis* KC446117 Voucher Bd793 Philippines which was 0,043 (Table 2).

The distribution of *B. occipitalis* is in The Philippines, Eastern Malaysia (Sabah), Brunei, as well as Indonesia (Kalimantan, West Java, dan Sumatera) (Drew and Romig, 2010). The main of host plants of *B. occipitalis* is *Mangifera indica*, *Psidium*

*guajava*, jeruk *Citrus* sp, *Achras zapota* and *Averrhoa carambola* (Clarke *et al.*, 2005; Delomen *et al.*, 2013; Yu *et al.*, 2005; Allwood *et al.*, 1999; Pujiastuti *et al.*, 2009).

**Table 2.** Matrix of genetic distance of *B. occipitalis* Bali and other *Bactrocera* from other countries base on MT-CO1 gen. [1] *B. occipitalis* Bali [2]; *B. occipitalis* KC446117 Voucher Bd793 Filipina; [3] *B. occipitalis* KC446122 Filipina Voucher Bd 798; [4] *B. occipitalis* KC446124 Voucher Bd800 Filipina; [5] *B. occipitalis* KC446107 Vouher Bd783 Filipina; [6] *B. occipitalis* Tarakan; [7] *B. occipitalis* KC446105 Voucher Bd739 Filipina; [8] *B.occipitalis* DQ 116309 Filipina; [9] *B. occipitalis* KC446110; [10] *B. occipitalis* KC446111; [11] *Anasthrepa lugens* HM538364; [12] *B. caryae* AY941232

	1	2	3	4	5	6	7	8	9	10	11	12
[1]												
[2]	0,043											
[3]	0,043	0,004										
[4]	0,043	0,004	0,000									
[5]	0,046	0,006	0,006	0,006								
[6]	0,046	0,002	0,002	0,002	0,004							
[7]	0,046	0,002	0,002	0,002	0,004	0,000						
[8]	0,046	0,002	0,002	0,002	0,004	0,000	0,000					
[9]	0,048	0,004	0,004	0,004	0,002	0,002	0,002	0,002				
[10]	0,052	0,008	0,008	0,008	0,006	0,006	0,006	0,006	0,004			
[11]	3,436	3,386	3,386	3,386	3,386	3,386	3,386	3,386	3,388	3,393		
[12]	4,761	4,589	4,589	4,589	4,589	4,589	4,589	4,589	4,589	4,589	4,929	

## CONCLUSIONS

Based on morphological and molecular identification the *Bactrocera occipitalis* was found as a new coming species (exotic) of fruit fly in Bali.

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