MOLECULAR IDENTIFICATION OF Bactrocera SP. FRUIT FLY FROM MURIA FOREST, CENTRAL JAVA, INDONESIA

by Dyah Indriyanti

VOL. 12, NO. 9, MAY 2017 ISSN 1819-6608
ARPN Journal of Engineering and Applied Sciences

www.arpnjournals.com

MOLECULAR IDENTIFICATION OF *Bactrocera* SP. FRUIT FLY FROM MURIA FOREST, CENTRAL JAVA, INDONESIA

Dyah Rini Indriyanti[1], Suputa[2] and Siti Nur Jannah[1]
[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Indonesia
[2]Studies Program of Pests and Plant Diseases, Faculty of Agriculture, Gadjah Mada University, Indonesia
E-Mail: dyahrini36@gmail.com

ABSTRACT

Bactrocera is one of important fruit flies. There are many *Bactrocera* species attack fruits consumed by human or not. We found *Bactrocera* sp. from forest that attack the fruit that not consumed by human (wild fruit), the species is still doubtful. The morphological identification show that *Bactrocera* sp has most of the same morphological characters with *Bactrocera calumniata* and has the same wings character with the *B. cucurbitae*. The study aimed to confirm the taxonomic status of *Bactrocera* sp using identify the cytochrome oxidase I gene of mitochondrial DNA and its phylogenic. The methods included fresh larvae DNA isolation, polymerase chain reaction (PCR), electrophoresis, and sequencing. Data analysis using BLAST program and MEGA software version 6.06 programs. The results showed that sequence (435 bp) of the *Bactrocera* sp. had highest similarity to *B. cucurbitae* (100%) (GenBankAcc Number DQ006875.1), and 96% homology with *B. calumniata* (96%) (GenBankAcc Number GQ154088.1). The Phylogenetic clearly showed that *Bactrocera* sp have the same common ancestor that came from Switzerland *B. cucurbitae.*

Keyword: cytochrome oxidase I, *Bactrocera* sp, *B. cucurbita*, *B. papayae*.

INTRODUCTION

Bactrocera sp is fruit flies, one of the insect pest that attack fruit. It is one of the most abundant insects in the world. There are around 4000 species of fruit fly in the world and 35% of the total attack soft skinned fruits, including highly economic commercial fruits (Zhang *et al.* (2010). *Bactrocera* sp infects fruits that are consumed by human, for instance apple, guava, papaya, mango, starfruit, soursop, avocado, and cimpedak (CABI, 2007; Indriyanti *et al*., 2014). Muryati (2007) reported that 40% of the total fruit fly population also inhabits and grows in Asteraceae (Compositae) plant. However, some of them also infect fruits that are not consumed by human.

We found *Bactrocera* sp which attack the fruit is not consume by human (wild fruit), in the forest in Kudus, Central Java Indonesia. The fruit is unknown name, included in Cucurbitaceae family and has not been identified. *Bactrocera* sp. that infects the wild fruit shares many similar characteristics with *Bactrocera calumniata*, while its wings resemble *Bactrocera cucurbitae*.

The study aimed to confirm the taxonomic status of *Bactrocera* sp using identify the cytochrome oxidase I gene of mitochondrial DNA and it's phylogenic. Cytochrome oxidase I gene is chosen since it is widely used for DNA barcoding to differentiate many species.

MATERIAL &METHODS

Insect material: The insect material used in this study was larvae and imago of *Bactrocera* sp, that attack wild fruit in Muria forest, Central Java, Indonesia.The morphological identification of *Bactrocera* sp was reffered to Suputa *et al.* (2006). Meanwhile we used *B. cucurbitae* and *B. papayae* samples for comparison were obtained from mass rearing in Laboratory of Basic Entomology, GadjahMada University. These three species of fruit fly were molecularly analyzed in Laboratory of Virology, Gadjah Mada University.

DNA isolation: The fresh larvae danimago was used to extract DNA. The genomic DNA isolation is performed according to genomic DNA mini kit protocol (Geneaid, ISO 9001:2008 QMS) which is carried out as below: thorax tissue of the imago and larva samples were taken using pinset. 30 mg of the samples were weighed and were put into tube. Samples were then grinded using micropestle. During the crushing process, 200 µL of GT Buffer was added into the sample. Grinded samples were then added with 20 µL of Proteinase K and is homogenized using vortex. Samples were then incubated inside tubes at 60°C for 30 minutes. During the incubation, the tube was inverted every 5 minutes.

Incubated samples were then added with 200 µL of GBT Buffer and were homogenyzed using vortex for 5 seconds. Samples were then incubated inside a tube at 60°C for 20 minutes. During the incubation, tube was inverted every 5 minutes. During this process, elution buffer (100 µL per sample) was preheated to 60°C. Samples were then centrifuged for 2 minutes at 14,000-16,000 rpm. 1.5 mL of supernatant from the centrifuged samples was then transferred into a new tube. 200 µL of absolute ethanol was added into the supernatant. The supernatant was then shaken vigorously for 10 seconds. A GD column is placed inside a 2 mL collection tube. Sample from 1.5 mL collection tube was then transferred into the GD column. The sample is centrifuged for 2 minutes at 14.000-16.000 rpm. Centrifuged GD column is then transferred into the new 2 mL collection tube. The GD column was then added with 600 µL of wash buffer. GD column was later centrifuged again at 14,000-16,000 rpm for 30 seconds. GD column was transferred into a new 2 mL collection tube. The following GD column was centrifuged at 14,000-16,000 rpm for 3 minutes.GD column was then transferred into 1.5 mL microcentrifuge tube. 100 µL of elution buffer was added to the center of the column matrix. GD column was then incubated at room temperature for 5 minutes and centrifuged for 30 seconds ar 14,000-16,000 rpm. Centrifuged GD column was then discarded, while the tube was stored at -20°C/-40°C.

Electrophoresys

DNA electrophoresys was performed at three samples of *Bactrocera* sp. as follows. Agarose gel was made by dissolving 1.5% of agarose into 1XTBE buffer and was preheated for homogenization. During the process, an electrophoresys container was installed with comb to produce wells. Agarose solution was then transferred into the given electrophoresys container until it solidified (15-20 minutes). Purified DNA of three samples was transferred into the well. The electrophoresys device was then connected with 50 volt of electricity pulse for 45 minutes.

After the electrophoresys process, agarose gel was soaked into EtBr solution for 5 seconds, and then soaked into aquadest for 15 minutes. The agarose gel was then washed to minimize the EtBr contamination. DNA was visualized using UV transiluminator. Results of genomic DNA visualization was estimated according to the genomic DNA band produced from the samples. Isolation gave a distinct result, so the process was followed by DNA amplification using PCR (Poymerase Chain Reaction) Technique.

DNA Amplification

1542 bp band of cytochrome oxidase I (NCBI, 2011) was amplified using a pair of forward primer mtD7 and reverse primer mtD9. Sequence of oligonucleotide primer are listed in Table-1.

Table 1. Oligonucleotide primer for DNA amplification.

Primer name	Sequence
(CO1-F) MtD7	5'ATT AGG AGC HCC HGA YAT AGC ATT 3'
(CO1-R) MtD9	5'GAG GCA AGA TTA AAA TAT AAA CTT CTG 3'

DNA amplification in PCR method used master mix kappa with total cocktail of 12.5 uL in half reaction. The cocktail is listed in Table-2.

Table-2. Cocktail used for DNA amplification.

No.	Material	vol$\frac{1}{2}$ x reaction
1	5x Kappa buffer extract	2.5 ul
2	$MgCl_2$	0.875 ul
3	ddH20	6.375 ul
4	dNtp	0.375 ul
5	DNA Polymerase	0.125 ul
6	Forward primer	0.625 ul
7	Reverse primer	0.625 ul
	Total	12.5 ul

PCR tube that contained DNA and given cocktail solution was then put into thermal cycle machine for 35 cycles in given condition listed in Table-3.

Table-3. Sequence of amplification of cytochrome oxidase I region in mtDNA.

Steps	Process	Temperature (°C)	Duration
	Pre-denaturation	94	3 minutes
I	Denaturation	94	15 seconds
II	Annealing	53	15 seconds
III	Extension	70	1 minute
	Post-extension	72	1 minute

3 µL of samples from PCR product and 2 µL of loading dye were run in 1.5 % of agarose gel to determine the existence and size of amplified DNA.

Sequencing

Qualified PCR product was then sequenced. DNA sequencing was performed to determine the nucleotide sequence in cytochrome oxidase I region. The product was sent to Genetica Science Institute in Singapore.

Data analysis

The DNA sequences in ABI file of *Bactrocera* sp., *Bactrocera papaya,* and *Bactrocera cucurbitae*was manually edited using BioEdit v. 7.0.9. Results of sequence editing were analyzed using BLAST (Basic Local Alignment Search Tool) NCBI to indicate the homology from closest species. Phylogeny tree was constructed using neighbor-joining method, where matrix calculation of genetic distance using Kimura-2 model and implemented parameter in pairwise distance calculation using Bootstrap with 1000 times of repetition in MEGA (Molecular Evolutionary Genetics Analysis) software program v. 6.0 (Tamura *et al*., 2013)

ARPN Journal of Engineering and Applied Sciences

www.arpnjournals.com

Sample sequence was compared by several sequences of similar *Bactrocera* sp. collected from GenBank. *Bactrocera* sp. analyzed in this research were obtained from different regions and listed in Table-4.

Table-4. List of analyzed species.

No.	Species name		Origin
1	*Anastraphaludens*	AB192462	America
2	*B.caudata*	GQ458048	Asia
3	*B.diaphora*	GQ458043	Asia
4	*B. papaya*	DQ917578	Asia
5	*B.philippinensis*	DQ995281	Asia
6	*B.scutellata*	GQ458046	Asia
7	*Bactrocerasp**	Sample	Indonesia
8	*B. cucurbitae**	Sample	Indonesia
9	*B. papayae**	Sample	Indonesia
10	*B. cucurbitae_Prancis*	JX162208	France
11	*Bactrocera calumniate*	GQ154808	Asia
12	*B. cucurbitae*	DQ116244	Asia
13	*B.cucurbitae*	DQ006875.1	Switzerland

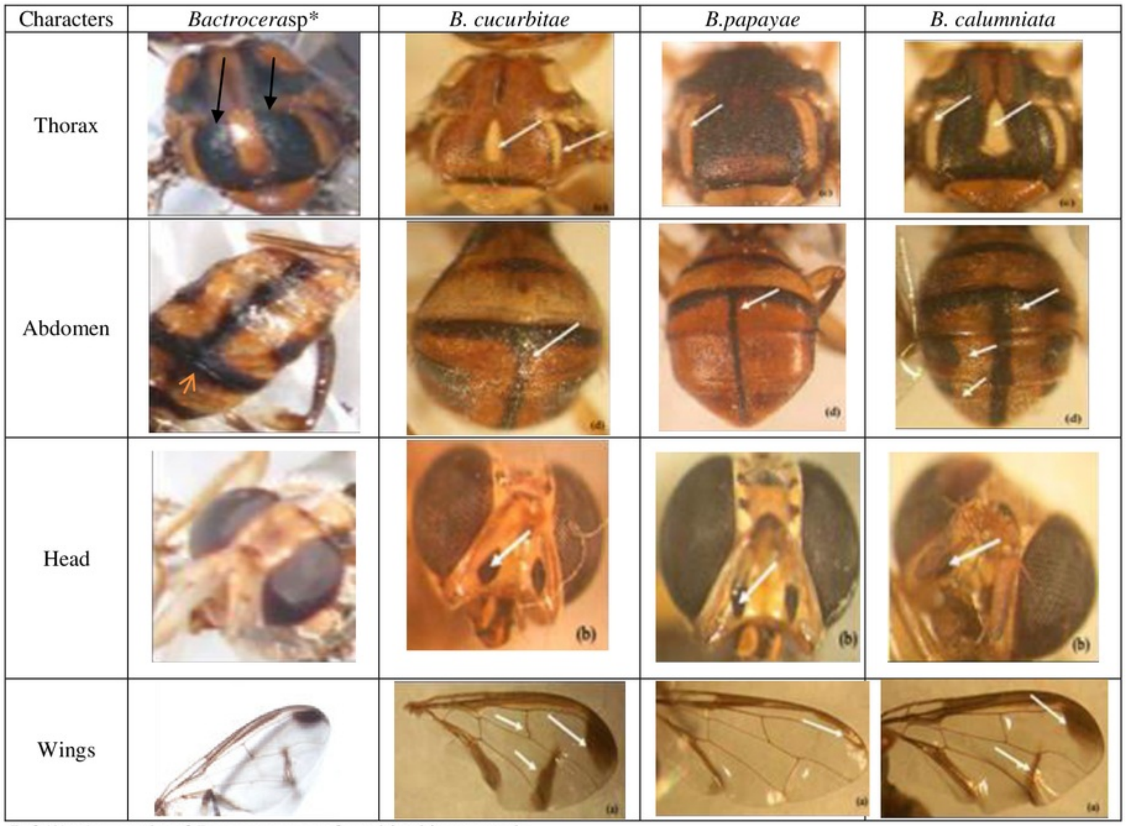
Inf:* = is the sample obtained from the research, while the unmarked species are collection of GenBank.

RESULT AND DISCUSSIONS

Bactrocera description

Bactrocera sp. found in this research has several similar characters to *B. calumniata*, but it also shares similar wing pattern to *B. cucurbitae*. Those morphological characters are obtained using identification key based on identification guidance of fly fruit (Suputa *et al.*, 2006).

VOL. 12, NO. 9, MAY 2017 ISSN 1819-6608
ARPN Journal of Engineering and Applied Sciences



Inf.(*): A sample of *Bactrocera* sp. found in this research.

Figure-1. The morphology of *Bactrocera* sp, *B. cucurbitae, B. papaye* and *B. calumniate.*

Electrophoresys result of *Bactrocera cucurbitae*, *Bactrocera papaya*, and *Bactrocera* sp. showed visible bands, so it can be used for further analysis of PCR amplification.

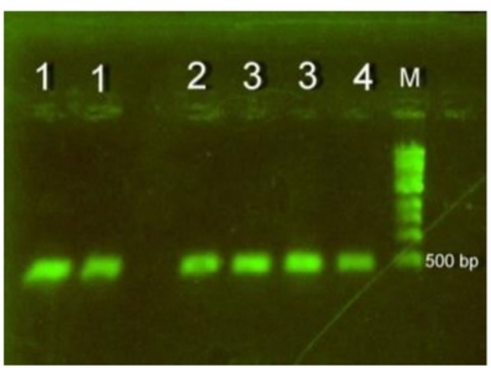


Figure-2. Result of mt DNA amplification of *Bactrocera* sp. in *cox l* region; M=Marker 500 bp, *B. papayae* (No 1),*B. cucurbitae*(No 2),imago of *Bactrocera*sp(No 3),larvae of *Bactrocera* sp. (No 4).

The consensus sequence gene of *Bactrocera cucurbitae* cytochrome oxidase I which attacked wild fruit (CL), *Bactrocera cucurbitae*(CU) and *Bactrocera papayae* (LB).

>Consensus CL
GAATGAATAATATAAGATTTTGATTATTACCTCCC
TCTCTTACATTACTTTTAGTGAGCAGTATAGTAGA
AAACGGAGCTGGTACAGGTTGAACTGTTTATCCT
CCCCTTTCATCAATTATCGCTCATGGTGGAGCCTC
AGTTGATTTAGCTATTTTTTCTCTACATTTAGCTG
GTATTTCATCAATTTTAGGGGCCGTAAATTTCATT
ACTACAGTAATTAATATGCGATCAACAGGAATCA
CATTTGACCGGATACCTTTATTCGTTTGAGCTGTA
GTATTGACAGCTCTTCTTTTACTTCTATCTCTACCT
GTGTTAGCCGGAGCTATTACTATACTTTTAACAGA
CCGAAATTTAAACACCTCTTTCTTCGACCCGGCTG
GTGGTGGAGACCCTATTTTATACCAACATTTATTT
TGATTCTTTGGACACC.

>Consensus CU
AATAATATAAGATTTTGATTATTACCTCCCTCTCT
TACATTACTTTTAGTGAGCAGTATAGTAGAAAAC
GGAGCTGGTACAGGTTGAACTGTTTACCCTCCCCT

VOL. 12, NO. 9, MAY 2017 ISSN 1819-6608
ARPN Journal of Engineering and Applied Sciences

www.arpnjournals.com

TTCATCAATTATCGCTCATGGTGGAGCCTCAGTTG
ATTTAGCTATTTTTTCTCTACATTTAGCTGGTATTT
CATCAATTTTAGGGGCTGTAAATTTCATTACTACA
GTAATTAATATACGATCAACAGGAATTACATTTG
ACCGAATACCTTTATTCGTTTGAGCTGTAGTATTA
ACAGCTCTTCTTTTACTTCTATCTCTCCCAGTATTA
GCTGGAGCTATTACTATACTTTTAACAGACCGAA
ACTTAAATACATCTTTCTTCGACCCAGCTGGTGGT
GGAGATCCTATTTTATACCAACACTTATTTTGATT
CTTTGGAC

>Consensus LB
AATAATATAAGATTTTGATTATTACCTCCTTCCCT
TACATTACTATTAGTAAGAAGTATAGTAGAAAAC
GGAGCTGGTACAGGTTGAACAGTTTACCCACCCC
TATCATCTGTTATTGCACACGGAGGAGCTTCAGTT
GACCTAGCTATTTTTTCACTTCACTTAGCGGGTAT
TTCCTCAATTTTAGGAGCAGTAAATTTCATTACAA
CAGTAATTAATATACGATCGACAGGAATCACCTT
TGATCGAATACCTTTATTCGTTTGAGCAGTTGTAT
TAACAGCTTTATTACTTTTATTATCATTACCAGTT
TTAGCAGGGGCTATTACTATATTACTAACAGACC
GAAACTTAAATACTTCCTTTTTTGACCCTGCCGGA
GGAGGAGATCCTATTCTTTACCAACATTTATTTTG
ATTCTTTGGAC

In this study, cytochrome oxidase I gene of *Bactrocera* sp. is obtained in size of 430 bp, *B. cucurbitae* and *B. papayae* in size of 427 bp that encodes 140 types of amino acid with one variation of amino acid in subgenus *Bactrocera* sp. According to result of PCR amplification, *cox l* fragment of *Bactrocera* sp., *Bactrocera cucurbitae*, and *Bactrocera papayae* is well amplified using a pair of forward primer mtD7 and reverse primer mtD9. According to Yuwono (2006), factors that affects the PCR amplification are DNA purity from extraction process, reactant compositions, and proper PCR condition, especially in annealing process (primer attachment). Annealing process requires optimum temperature to ensure the primer specifically attaches in both end of DNA template (melting temperature). Result of sequence analysis using Mega software 6.0 showed percentage of base content in *cox l* region of *Bactrocera* sp., as shown in Table-5.

Table-5. Nucleotide base contents in *Bactrocera* sp. from Mega software 6.0 program.

No.	Species name	T	A	G	C	G+C	A+T
1	*B. cucurbita_Swiss*	36.6	28.8	16.0	18.6	34.6	65.4
2	*Bactrocera*sp*	37.2	25.7	16.6	20.5	37.0	63.0
3	*B. cucurbita**	37.9	26.9	15.2	19.9	35.1	64.9
4	*B. papayae**	35.8	29.0	15.7	19.4	35.1	64.9
5	*B. caudata*	31.0	35.5	19.7	13.9	33.6	66.4
6	*B. diaphora*	29.8	35.1	20.5	14.6	35.1	64.9
7	*B. papayae*	34.3	39.2	10.2	16.2	26.5	73.5
8	*B. philippinensis*	34.4	39.2	10.2	16.1	26.4	73.6
9	*B. scutellata*	29.7	34.7	20.7	14.9	35.6	64.4
10	*B. calumniata*	36.3	28.6	16.9	18.2	35.1	64.9
11	*B. cucurbitae_Ind*	36.7	27.8	16.6	18.9	35.5	64.5
12	*B. cucurbita_Perancis*	34.4	28.3	17.9	19.4	37.3	62.7
13	*Anastrephaludens*	36.3	34.2	12.9	16.6	29.5	70.5

Inf: * are samples in this research

A-T base content is higher than G-C base content in mtDNA of *Bactrocera* sp. This result shows the same pattern as reported by Zhang *et al*. (2010), Muraji& Nakahara (2002), Muraji& Nakahara (2001), Jamnongkluk*et al*. (2003). This is caused by A-T bond, which is a noncoding region that has further evolution rate compared to coding region. Beside that,G-C bond is more stable since it has three hydrogen bonds, compared to A-T bond that only has two hydrogen bonds.

Species determination is later processed using BLAST analysis, which compares the sequence of *Bactrocera* sp., *Bactrocera cucurbitae*, and *Bactrocera papayae* with given database in GenBank. Result of BLAST analysis in Table-6.

VOL. 12, NO. 9, MAY 2017 ISSN 1819-6608
ARPN Journal of Engineering and Applied Sciences

www.arpnjournals.com

Table-6. Result of BLAST analysis of DNA sequence from cytochrome oxidase I gene.

	Cytochrome oxidase I gene			
	***Bactrocera* sp**	***Bactrocera* sp**	***B.cucurbitae***	***B. papayae***
Characters	CL	CL	CU	LB
Homology	100	96%	100	100
Gaps	0%	0%	0%	0%
Acc.Number	DQ006875.1	GQ154088.1	DQ116244.1	FJ903487.1
Homology ofnucleotide to	284-718 bp	240-658 bp	189-613 bp	260-686 bp
E-value	0.0	0.0	0.0	0.0

According to the result from BLAST analysis, obtained sequence from *Bactrocera* sp. shared 96% homology in its *cox l* region with given gene from GenBank with Acc Number of GQ154088.1(*Bactrocera* sp.) This homology value that was less than 99% indicated that the observed species was different from known *Bactrocera* sp. This showed a mismatch from morphological identification result that assumed the observed *Bactrocera* sp. was *Bactrocera calumniata*. Further BLAST analysis was performed for observed *Bactrocera* sp. sample and *Bactrocera cucurbitae* from GenBank collection with Acc Number of DQ006875.1. This analysis showed that both samples shared 100% homology. It showed that the observed *Bactrocera* sp. was similar to *B. cucurbitae*.

According to *neighbor-joining* analysis (NJ) (Saitou & Nei, 1987) with 1000x bootstrap repetition (Felsenstein, 1985), a phylogeny construction was obtained (Nei & Kumar, 2000) from *Bactrocera* sp. sample that infected wild fruits compared to *Bactrocera cucurbitae* and *Bactrocera papayae* from GenBank collection (Zhang *et al*., 2010).

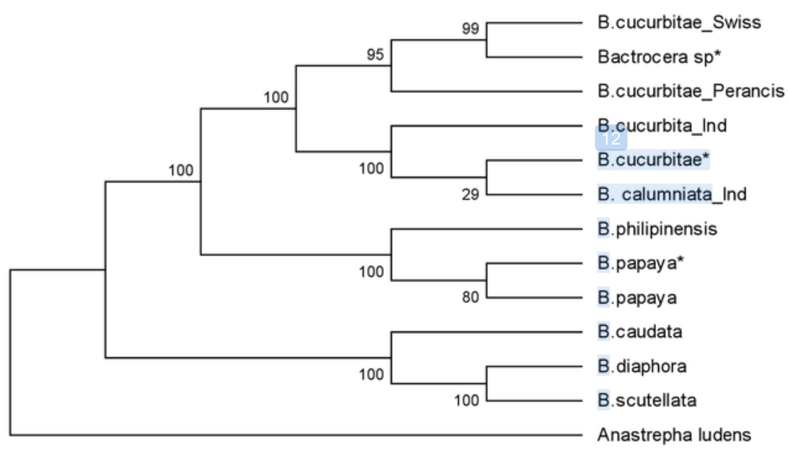


Figure-3. The Cladogram of fragmen *cox I Bactrocera* sp.
*) a sample of the research; *Bactrocera* sp* a sample that attack wild fruit

Result of genetical correlation proximity using Pairwise Distance Calculation (Tamura *et al*., 2013)from analyzed *Bactrocera* sp. is shown in Table-7.

ARPN Journal of Engineering and Applied Sciences

Table-7. Pairwise distance calculation result of *Bactrocera* sp.

No	1	2	3	4	5	6	7	8	9	10	11	12	13
1													
2	0												
3	0.01	0.01											
4	0.019	0.019	0.018										
5	0.026	0.026	0.025	0.025									
6	0.025	0.025	0.025	0.025	0.016								
7	0.019	0.019	0.019	0.003	0.025	0.025							
8	0.019	0.019	0.018	0.005	0.025	0.025	0.004						
9	0.025	0.025	0.025	0.025	0.017	0.004	0.025	0.025					
10	0.010	0.010	0.003	0.019	0.025	0.025	0.019	0.018	0.025				
11	0.010	0.010	0	0.018	0.025	0.025	0.019	0.018	0.025	0.003			
12	0.007	0.007	0.011	0.019	0.026	0.025	0.019	0.019	0.025	0.011	0.011		
13	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	

Inf: *B. cucurbitae*_Swiss (1), *Bactrocera* sp* (No 2), *B. cucurbitae** (No 3), *B. papayae** (No 4), *B. caudata* (No 5), *B. diaphora* (No 6), *B. Papayae* (No7), *B. philipinensis* (No 8), *B. scutellata**(No 9), *Bactrocera* sp (No 11),*B. cucurbitae_Reonion* (No 12),*Anastraphaludens* (No 13).

According to pairwise distance calculation result, *Bactrocera* sp. samples that originated from same region and were included into one subgenus had relatively close genetic distance. This proximity is allegedly caused by non random mating and gene flow due to close geographical distance. Furthermore, this will reduce accumulated difference between subgenus that happen due to natural selection and genetic drift (Smith, 2002). Mismatched result between morphological identification and molecular data is allegedly caused by crossbreeding between *Bactrocera calumniata* with *Bactrocera cucurbitae* that shares the same subgenus, *Zeugodacus*. Such occurrence was also reported by Dolemon *et al* (2013), stating that offspring species from crossbreeding between *B. occipitalis*and *B. philipinensis* used cytochrome oxidase I gene.

Molecular analysis on observed *Bactrocera* sp. could reveal the real species identity to discover its history of evolution and evolutional corelation between offsprings and its ancestors. Study of evolution on *Bactrocera* sp. is highly important to control the species. The information elaborated in this research included sequence analysis of cytochrome oxidase I gene from observed *Bactrocera cucurbitae* which shares the same ancestors with European *Bactrocera cucurbitae*. Meanwhile, *B. cucurbitae* that infect momordica and *B. papayae* that infect zalacca share the same ancestors with Indonesian or Oriental *Bactrocera* sp. This research also informs that molecular identification using cytochrome oxidase I gene gave different result with morphological identification. Best method to analyze the correlation between morphological characters and molecular analysis is combining genes. This method has already proven by Zhang *et al*. (2010) that used *Cox I* gene and 16S rDNA, also by Muraji& Nakahara (2008) that used *Cox I* and *Cox II* gene.

CONCLUSIONS

Sequence result from cytochrome oxidase I gene of *Bactrocera* sp. that infected wild fruits was identified as *B. cucurbitae*. From this sequencing process, a nucleotide base chain in size of 435 bp was obtained. Phylogenetic tree construction in *Bactrocera* sp clearly showed that *Bactrocera* sp have the same common ancestor that came from Switzerland *B. cucurbitae.*

REFERENCES

[CABI] Center in Agricultural and Biological Institute. 2007. *Crop Protection Compendium (CD-ROM) Wallingford*: CAB International 2 CD-ROM.

Dolemon MLC, Mendioro MS, Diaz MGQ. 2013. Morphometric Analysis and DNA Barcoding of Fruit Flies *Bactroceraoccipitalis(Bezzi)* and *B. philippinensis* Drew and Hancock (Diptera: Tephritidae) from Cavite and Davao del Norte. Philippine Journal of Science. 142(1): 69-76.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution. 39:783-791.

Indriyanti D.R., Y.N. Isnaeni and B. Priyono. 2014. Identifikasi dan Kelimpahan Lalat Buah Bactrocera pada Berbagai Buah Terserang. Biosaintifika: Journal ofBiology and Biology Education. 6(1): 39-45.doi:http://dx.doi.org/10.15294/.v6i1.2933.

Jamnongluk W, Baimai V, Kittayapong P. 2003. Molecular Phylogeny of Tephritid Fruit Flies in The *Bactrocera* tau Complex Using the Mitochondrial Co1 Sequences. Genome.46:112-118.

Muraji M and Nakahara S. 2001. Phylogenetic relationships among fruit flies, Bactrocera (Diptera: Tephritidae), based on the mitochondrial rDNA sequences. Insect Mol. Biol. 10: 549-559.

Muraji M and Nakahara S. 2002. Discrimination among pest species of Bactrocera (Diptera: Tephritidae) based on PCR-RFLP of the mitochondrial DNA. Applied Entomology and Zoology. 37: 437-446.

Muraji M & Nakahara S. 2008. Phylogenetic Analyses of Bactrocera Fruit Flies (Diptera: Tephritidae) Based on Nucleotide Sequences of the Mitochondrial COI and COII Genes. Res. Bull. Pl. Prot. 44: 1-12.

Muryati, Hasyim A, Kogel de WJ. 2007. Distribusi spesies lalat buah di Sumatera Barat dan Riau. J. Hort. 17 (1):61-68.

NCBI. 2010.http: nucleotide.ncbi.nlm.nih-gov. [3 September 2014].

Nei M and Kumar S. 2000.Molecular Evolution and Phylogenetics. Oxford University Press, New York, USA.

Saitou N andNei M. 1987. The neighbor-joining method: A new method forreconstructing phylogenetic trees. Molecular Biology and Evolution. 4:406-425.

Smith, P.T, Mcpheron, B.A., Kambhampati, S. 2002. Phylogenetic analysis of mitochondrial DNA supports the monophyly of Dacinifruit flies (Diptera: Tephritidae). *Ann. Ent.* Soc. Am. 95(6): 658-664.

Suputa, Cahyati, Kustaryati A, Railan M, Issusilaningtyas, Taufiq A. 2006. Pedoman Identifikasi Lalat Buah(Diptera: Tephritidae). Yogyakarta: UGM.

Tamura K, Stecher G, Peterson D, Filipski A, and Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution.30: 2725-2729.

Yuwono T. 2006. Teori Dan Aplikasi Polymerase Chain Reaction. Yogyakarta: C.V Andi Offset.

Zhang B, Liu YH, Wu WX, Wang Z. 2010. Molecular Phylogeny of *Bactrocera species* (Diptera: Tephritidae:Dacini) Inferred from Mitochondrial Sequences of 16s Rdna and Coisequences. Florida Entomologist. 93(3): 369-377.

MOLECULAR IDENTIFICATION OF Bactrocera SP. FRUIT FLY FROM MURIA FOREST, CENTRAL JAVA, INDONESIA

ORIGINALITY REPORT

15%	12%	5%	10%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Al-Nahrain University Student Paper	6%
2	journal.unnes.ac.id Internet Source	2%
3	Submitted to University of Babylon Student Paper	1%
4	Submitted to Universiti Putra Malaysia Student Paper	1%
5	aut.researchgateway.ac.nz Internet Source	1%
6	R. Asokan. "Molecular differences in the mitochondrial cytochrome oxidase I (mtCOI) gene and development of a species-specific marker for onion thrips, Thrips tabaci Lindeman, and melon thrips, T. palmi Karny (Thysanoptera: Thripidae), vectors of tospoviruses (Bunyaviridae)", Bulletin of Entomological Research, 10/2007 Publication	1%

7	Submitted to iGroup Student Paper	1%
8	Submitted to University of Newcastle upon Tyne Student Paper	<1%
9	Submitted to Universiti Tunku Abdul Rahman Student Paper	<1%
10	Khoa, Nguyễn Đắc, Nguyễn Đặng Ngọc Giàu, and Trần Quốc Tuấn. "Effects of Serratia nematodiphila CT-78 on rice bacterial leaf blight caused by Xanthomonas oryzae pv. oryzae", Biological Control, 2016. Publication	<1%
11	Hoi-Sen Yong, Sze-Looi Song, Phaik-Eem Lim, Kok-Gan Chan, Wan-Loo Chow, Praphathip Eamsobhana. "Complete mitochondrial genome of Bactrocera arecae (Insecta: Tephritidae) by next-generation sequencing and molecular phylogeny of Dacini tribe", Scientific Reports, 2015 Publication	<1%
12	media.neliti.com Internet Source	<1%
13	synapse.koreamed.org Internet Source	<1%

14	www.translational-medicine.com Internet Source	<1%
15	hub.hku.hk Internet Source	<1%
16	brage.bibsys.no Internet Source	<1%

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		