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

Dyah Rini Indriyanti¹, Suputa² and Siti Nur Jannah¹

¹Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Indonesia
²Studies 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 referred 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 dan imago 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 homogenized 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 at 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 1X TBE 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 transluminator. 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 (Polymerase 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.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>(CO1-F) MtD7</td>
<td>5’ATT AGG AGC HCC HGA YAT AGC ATT 3’</td>
</tr>
<tr>
<td>(CO1-R) MtD9</td>
<td>5’GAG GCA AGA TTA AAA TAT AAA CTT CTG 3’</td>
</tr>
</tbody>
</table>

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

Table 2. Cocktail used for DNA amplification.

<table>
<thead>
<tr>
<th>No.</th>
<th>Material</th>
<th>vol (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5X Kappa buffer</td>
<td>2.5</td>
</tr>
<tr>
<td>2</td>
<td>MgCl2</td>
<td>0.875</td>
</tr>
<tr>
<td>3</td>
<td>dH2O</td>
<td>6.375</td>
</tr>
<tr>
<td>4</td>
<td>dNtp</td>
<td>0.375</td>
</tr>
<tr>
<td>5</td>
<td>DNA Polymerase</td>
<td>0.125</td>
</tr>
<tr>
<td>6</td>
<td>Forward primer</td>
<td>0.625</td>
</tr>
<tr>
<td>7</td>
<td>Reverse primer</td>
<td>0.625</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>12.5</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Steps</th>
<th>Process</th>
<th>Temperature (℃)</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-denaturation</td>
<td>94</td>
<td>3 minutes</td>
</tr>
<tr>
<td>I</td>
<td>Denaturation</td>
<td>94</td>
<td>15 seconds</td>
</tr>
<tr>
<td>II</td>
<td>Annealing</td>
<td>53</td>
<td>15 seconds</td>
</tr>
<tr>
<td>III</td>
<td>Extension</td>
<td>70</td>
<td>1 minute</td>
</tr>
<tr>
<td></td>
<td>Post-extension</td>
<td>72</td>
<td>1 minute</td>
</tr>
</tbody>
</table>

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)
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>
<thead>
<tr>
<th>No.</th>
<th>Species name</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Anastraphaludens</em></td>
<td>AB192462</td>
</tr>
<tr>
<td>2</td>
<td><em>B. caudata</em></td>
<td>GQ458048</td>
</tr>
<tr>
<td>3</td>
<td><em>B. diaphora</em></td>
<td>GQ458043</td>
</tr>
<tr>
<td>4</td>
<td><em>B. papaya</em></td>
<td>DQ917578</td>
</tr>
<tr>
<td>5</td>
<td><em>B. philippinensis</em></td>
<td>DQ995281</td>
</tr>
<tr>
<td>6</td>
<td><em>B. scutellata</em></td>
<td>GQ458046</td>
</tr>
<tr>
<td>7</td>
<td><em>Bactrocerasp</em></td>
<td>Sample</td>
</tr>
<tr>
<td>8</td>
<td><em>B. cucurbitae</em></td>
<td>Sample</td>
</tr>
<tr>
<td>9</td>
<td><em>B. papayae</em></td>
<td>Sample</td>
</tr>
<tr>
<td>10</td>
<td><em>B. cucurbitae_ Prancis</em></td>
<td>JX162208</td>
</tr>
<tr>
<td>11</td>
<td><em>Bactrocera calumniata</em></td>
<td>GQ154808</td>
</tr>
<tr>
<td>12</td>
<td><em>B. cucurbitae</em></td>
<td>DQ116244</td>
</tr>
<tr>
<td>13</td>
<td><em>B. cucurbitae</em></td>
<td>DQ006875.1</td>
</tr>
</tbody>
</table>

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

**Figure-1.** The morphology of *Bactrocera* sp., *B. cucurbitae*, *B. papayae* 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.

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

**Consensus CL**

```
GAATGAATAATATAAGATTTGATTATTACCTCCC
TCTCTTACATTACCTTTTATGAGACGTAGTATAGTGA
AIAACGGAGCTGGTACAGGTTGAACTGTTTATCCT
CCCCTTTCATCAATTATCGCTCATGGTGGAGCCTC
AGTTGATTTAGCTATTTTTTCTCTACATTTAATCTG
GTAACATCAATTATGGGGGCATCAATTTTCATT
ACTACAGTAAATTAATGCCTCAAACAGGAATCA
CATTTGACGCATACCTTTATTCTGAGCTTGA
GTAACAGCTCTTCTTTTACTCTATCTCTACCT
GTGTTAGCCGAGCTATATTACTATACTTTAACAGA
CCGAAATTTAAACACCTCTTCTTGACCACCCGGCTG
GTGGTGGAGACCTATTTTATACCCACATTTATTGT
ATTTCTTTGACACC
```

**Consensus CU**

```
AATAATATAAGATTTTGATTATTACCTCCCCTCTCT
TACATTACTTTTATGAGACGTAGTATAGTGA
AIAACGGAGCTGGTACAGGTTGAACTGTTTATCCT
CCCCTTTCATCAATTATCGCTCATGGTGGAGCCTC
AGTTGATTTAGCTATTTTTTCTCTACATTTAATCTG
GTAACATCAATTATGGGGGCATCAATTTTCATT
ACTACAGTAAATTAATGCCTCAAACAGGAATCA
CATTTGACGCATACCTTTATTCTGAGCTTGA
GTAACAGCTCTTCTTTTACTCTATCTCTACCT
GTGTTAGCCGAGCTATATTACTATACTTTAACAGA
CCGAAATTTAAACACCTCTTCTTGACCACCCGGCTG
GTGGTGGAGACCTATTTTATACCCACATTTATTGT
ATTTCTTTGACACC
```

**Figure-2.** Result of mt DNA amplification of *Bactrocera* sp. in *cox* I 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).
TTCTCAATATCGCTCATGGTGGAGCCTCAGTTGATTATTAGCTATTTTTTCTCTACATTTAGCTGGTATTT
CATCAATTTTAGGGGCTGTAAATTTCATTACTACA
GTAATTAATATACGATCAACAGGAATTACATTTGACCGAATACCTTTATTCGTTTGAGCTGTAGTATTA
ACAGCTCTTCTTTTACTTCTATCTCTCCCAGTATTA
GCTGGAGCTATTACTATACTTTTAACAGACCGAA
ACTTAAATACATCTTTCTTCGACCCAGCTGGTGGT
GGAGATCCTATTATACCAACACTTATTTTGATTCTTGGAC
>Consensus LB
AATAATATAAGATTTTGATTATTACCTCCTTCCCTTACATTACTATTAGTAAGAAGTATAGTAGAAAAC
GGAGGAGCTGGTACAGGTTGAACAGTTTACCCACCCCTATCATCTGTATTGCACACGGAGGAGCTTCAGTT
GACCTACTATTATTTCATCTCCTAGGGGTATTTCTCTCAATTTTAGGGGCTGTAAATTTCATTACTACA
GCTGGAGCTATTACTATACTTTTAACAGACCGAA
ACTTAAATACATCTTTCTTCGACCCAGCTGGTGGT
GGAGATCCTATTATACCAACACTTATTTTGATTCTTGGAC

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.

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

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

<table>
<thead>
<tr>
<th>Characters</th>
<th>Bactrocera sp</th>
<th>Bactrocera sp</th>
<th>B. cucurbitae</th>
<th>B. papayae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homology</td>
<td>96%</td>
<td>96%</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Gaps</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Acc.Number</td>
<td>DQ006875.1</td>
<td>GQ154088.1</td>
<td>DQ116244.1</td>
<td>FJ903487.1</td>
</tr>
<tr>
<td>Homology of nucleotide to</td>
<td>284-718 bp</td>
<td>240-658 bp</td>
<td>189-613 bp</td>
<td>260-686 bp</td>
</tr>
<tr>
<td>E-value</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

According to the result from BLAST analysis, obtained sequence from Bactrocera sp. shared 96% homology in its cox I 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 fragment 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.
Molecular analysis is combining genes. This method has correlation between morphological characters and morphological identification. Best method to analyze the using cytochrome oxidase I gene gave different result with sp. This research also informs that molecular identification the same ancestors with Indonesian or Oriental and its ancestors. Study of evolution on evolution and evolutional corelation between offsprings of Bactrocera cucurbitae cucurbitae cytochrome oxidase I gene from observed elaborated in this research included sequence analysis of highly important to control the species. The information cytochrome oxidase I gene. Such occurrence was also reported by Dolemon between (2013), stating that offspring species from crossbreeding cucurbitae between and molecular data is allegedly caused by crossbreeding mismatched result between morphological identification to natural selection and genetic drift (Smith, 2002). Accumulated difference between subgenus that happen due geographical distance. Furthermore, this will reduce mismatched result between morphological identification and were included into one subgenus had relatively close genetic distance. This proximity is allegedly caused by crossbreeding and its ancestors. Study of evolution on of evolution and evolutional correlation between offsprings which shares the same ancestors with European Bactrocera 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.

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

<table>
<thead>
<tr>
<th>No</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.019</td>
<td>0.019</td>
<td>0.018</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.026</td>
<td>0.026</td>
<td>0.025</td>
<td>0.025</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025</td>
<td>0.016</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.019</td>
<td>0.019</td>
<td>0.019</td>
<td>0.003</td>
<td>0.025</td>
<td>0.025</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.019</td>
<td>0.019</td>
<td>0.018</td>
<td>0.005</td>
<td>0.025</td>
<td>0.025</td>
<td>0.004</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025</td>
<td>0.017</td>
<td>0.004</td>
<td>0.025</td>
<td>0.025</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.010</td>
<td>0.010</td>
<td>0.003</td>
<td>0.019</td>
<td>0.025</td>
<td>0.025</td>
<td>0.019</td>
<td>0.018</td>
<td>0.025</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>0.010</td>
<td>0.010</td>
<td>0.018</td>
<td>0.025</td>
<td>0.025</td>
<td>0.019</td>
<td>0.018</td>
<td>0.025</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.007</td>
<td>0.007</td>
<td>0.011</td>
<td>0.019</td>
<td>0.026</td>
<td>0.025</td>
<td>0.019</td>
<td>0.019</td>
<td>0.025</td>
<td>0.011</td>
<td>0.111</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>0.026</td>
<td>0.026</td>
<td>0.026</td>
<td>0.026</td>
<td>0.026</td>
<td>0.026</td>
<td>0.026</td>
<td>0.026</td>
<td>0.026</td>
<td>0.026</td>
<td>0.026</td>
<td>0.026</td>
<td>0.026</td>
</tr>
</tbody>
</table>

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 evolutionary correlation 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


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.


