Biosaintifika 8 (2) (2016) 185-192

Biosaintifika
Journal of Biology & Biology Education

http://journal.unnes.ac.id/nju/index.php/biosaintifika

Characterization of Three Species of Thrips on Weeping Fig, Nutmeg, and Marine Seruni Plants Based on Mtcoi DNA Sequences

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DOI: 10.15294/biosaintifika.v8i2.5448

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History Article

Received 17 March 2016
Approved 17 July 2016
Published 18 September 2016

Keywords:

COI gene; Indonesia; leaf-galls; pest; Phlaeothripidae

Abstract

Thrips are widely reported as pests in vegetable crops. However, the existence of Phlaeothripidae members has a less concern in Indonesia. Phlaeothripidae is the only family of Tubulifera Suborder and some reports suggested that they had potential to be pests in several crops due to their ability to roll up and to make galls on leaves. The first step in pest management attempt is to identify the pest accurately and quickly, so the pest management can be on target and more efficient. One of the identification methods is the molecular identification using DNA barcoding techniques. This study aimed to characterize and to compare species thrips in banyan, nutmeg, and marine seruni based on their molecular characteristics. This research was conducted in Bogor and Kuningan. The process of molecular characterization consisteds of four steps DNA total extraction, amplification by using PCR, COI gene sequence, and data analysis. PCR programme was succesfully to amplified mt-*COI* gene fragment at 710 bp. The length of mt*COI* gene of *Gynaikothrips uzeli, Haplothrips ganglbaueri*, and *Pseudophilothrips ichini* were 704, 686, and 702 bp dominated by A and T bases with nucleotide variation value of 27.8%. This results confirmed that molecular characterization using mt*COI* gene mitochondrial had successfully supported the morphological data.

How to Cite

Kurniawaty, N., Hidayat, P. & Rauf, A. (2016). Characterization of Three Species of Thrips on Banyan, Nutmeg, and Marine Seruni Plants Based on COI Gene. *Biosaintifika: Journal of Biology & Biology Education*, 8(2), 185-192.

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p-ISSN 2085-191X
e-ISSN 2338-7610

INTRODUCTION

Thrips typically feed on and damaged young, growing leaves and immature fruits, colour distortion and stunted growth. Their minute size often 0,5 to 5,0 mm. Among the 6680 thrips species described worldwide only 1% are known as pest species. They are divided into two suborders, i.e. Terebrantia with a blunt or angled body end, and Tubulifera in which the abdomen forms a tube at the end (Boror et al*.,* 1996). There are around 3950 species including Tubulifera Suborder (Mound & Morris, 2007).

The identification to the thrips in Indonesia has been conducted by Sartiami & Mound (2013), as well as Subagyo (2014), but information about the existence of the Phlaeothripidaefamily in Indonesia is very limited. Khalsoven (1981) reported that there was one genus found in association with Graminae (genus of *Haplothrips*). In some countries, the report regarding the members of the Phlaeothripidae family has ever been reported to be predators dan galls former of plants, e.g., *Gynripsikothrips flaviantennus* Multon, *Schedothrips orientalis* Ananthakrishnan, *Crotonothrips dantahasta* (Ramakrishna), *Thilakothrips babuli* Ramakrishna dan *Androthrips flavipes* Schmutz. The members of Tubulifera Suborder that have been reported are *Gynaikothrips uzeli* that attacks banyan trees, *G. ficorum* that attacks bayleaf trees, and *Haplothrips ganglbaueri* that attacks weeds of *Echinochloa crusgalli* (Varadarasan & Ananthakrishnan, 1982). Whereas *Pseudophilothrips ichini* attacks papper plants (Held et al., 2005; Mound et al., 2010).

Additionally, their minute size and cryptic behaviour make them difficult to detect either in the field on in fresh vegetation trasported throught international trade. Consequently, many species have now spread from the original natural habitats an host to favourable new environments, including valuables crops (Brunner et al., 2002). In the identification of thrips, it is also important to understand their biology and to empower integrated pest management strategies. The identification of thrips has been based on external morphology (appearance) (Mehle & Trdan, 2012). Illustrated keys have a major advantage in that they graphically display the characters of interest.

However, there are many obstacles in the use of morphological identification key. Identification on thrips species by morphological examination is restricted to adult specimens, because there are no adequate key for identification for eegs, larvae or pupae (ISPM, 2010). Morphological appearance can vary in many ways within species, such as in colour and size (Mehle & Trdan, 2012). When identifying adult thrips species using traditional, printed dichotomous keys, a series of questions must be answered in a fixed order, and this sequential approach can limit identification if an early character is not identifiable (Mehle & Trdan, 2012). Some identification keys have limited morphological characters and number of pictures (Brunner et al*.,* 2002; Mound & Morris, 2007). In most cases, identification keys needed additional characteristics that can be used to identify the thrips more rapidly and correctly.

Thrips identification of the members of Phlaeothripidae family based on the molecular characteristics had never been conducted in Indonesia since the members were considered non-pests. Molecular assays can be applied to all life stages including the immature stages for which morphological identification to species is not possible (Ubaidillah & Sutrisno, 2009).This research aimed to identify and to compare molecular characteristics of three thrips species found in banyan, nutmeg, and marine seruni plants through nucleotides sequences of *COI* gene. The objectives were in the form of data of nucleotides sequences of *COI* gene which can be used as the additional characteristics to complete the morphological data in the process of thrips identification.

METHODS

This research was conducted from September 2014 to August 2015 in Bogor and Kuningan. Molecular identification was conducted in Plant Virology laboratory, Plant Protection Department, Faculty of Agriculture, Bogor Agricultural University Indonesia.

Sample Collection

The sample collection was carried directly by picking up the thrips on the flowers and plants of banyan, nutmeg, and marine seruni plants that had symptoms caused by the thrips attack. Then, the samples were sorted based on its morphological characteristics and was inserted to the eppendorf tube containing 96% alcohol. The samples were then inserted to the clips plastic with description. The location of sampling was marked by using Global Positioning System (GPS) to get the coordinate.

Slide preparation which has been modified based on Mound & Kibby (1998) method. Imago thrips was removed from the collecting fluid into clean 96% alcohol and attempt to open the wings and straighten the antennae using micro-

pins. Place a drop of hoyers solution on the object glass with a diameter of 13 mm and place a thrips into this drop, ventral side uppermost, and gently lower a slide onto the drop. Invert the slide as soon as the solution has spread sufficiently. Place immediately into an oven at 35-40°C. Then leave for a few hours before attempting to study. When the mountant was dried, ring with nail varnish and labelled appropriately. Identification of trips conducted directly under a stereo microscope OLYMPUS CX21FSI with Dino-eye camera AM4232. Identification used key identification of Mound and Kibby (1998), Sartiami & Mound (2013) & Subagyo (2014).

Molecular Characterization

DNA Extraction

Total genomic DNA was extracted from single thrips using the slightly modified protocol by Goodwin et al. (1994). Briefly, individual thrips imago were placed in eppendorf tube 1.5 mL, then it was added the CTAB bufer extraction 100 µL (CTAB 2%, NaCl 1.4 M, Tris-HCI 100 mM, EDTA 20 mM, and PVP 1% [-40ºC]), 1 µL of protainase K. Plastic grinders were used to crush the insect, an the tube were incubated at 65 ºC for 3 minutes. The tube was added CI 100 µL with 24:1 ratio. It were vortexed for 3 minutes, and were centrifuged at 10 000 rpm for 15 minutes. The supernatant that had been formed was taken of 60 µL, and moved into new tube. Then, added sodium acetate 3 M (pH 5.2) 6 µL and 44 µL isopropyl. The homogenate was store -20ºC for 24 hours. After the preparation, the tube were centrifuged at 10.000 rpm for 10 minutes and the supernatant were disposed. The pellets was washed by using 100 µL of 80% ethanol and was centrifuged at 8000 rpm for 5 minutes. Final step, the supernatant was disposed and the pellets was dried for less than 1 hour and then resuspent using TE buffer 20 µL for subsequent PCR amplification.

DNA Amplification

The Polymerase Chain Reaction (PCR) was used to amplified 710 bp of *COI* gene. All PCRs were performed in 25µL reaction with Perkin Elmer 480 Thermocycler (Applied Biosystem,US). The PCR contained *Go Tag Green Master Mix* 12.5 µL, 9.5 µL ddH2O, forward primer 1µL, reverse primer 1µL, and DNA template 1 µL. A portion of mt*COI* was amplified using the universal primers LCO1490(3'-GGTCAACAAATCATAAAGATATTGG-5') and HCO2198(5'TAAACTTCAGGGTG

ACCAAAAAATCA-3') (Folmer et al.,1994). Thermocycling was 5 minutes at 94ºC, an then with 35 cycles of 1 minute at 94ºC, 35 seconds at 52ºC, 1 minute 30 seconds 72ºC and 7 minutes at 72ºC. Visualization of DNA fragment as the result of electrophoresis amplification used agarose gel 1% in Tris-borate (TBE) buffer 0.5X with voltage of 50 volt for 50 minutes. PCR product were electrophoresis in 2% agarose gels. Gels were stained ethidium bromide for 15 minutes, visualized and photographed under UV light.

Data Analysis

Direct sequenced at Genetica Science. Sequence were trimmed to aligned manually in BioEdit ver.7.0.9. then analyzed by using BLAST programme. Nucleotide matrix formation was conducted with Clustal x (1.83) software, while the phylogenetic tree from morfological data constructed by NTSys21, and sequence data constructed by Molecular Evolutionary Genetic Analisis (MEGA 6) software with UPGMA methode and bootstrap for 1000 replicates

RESULT AND DISCUSSION

Based on the results of this research, the thrips found in banyan, nutmeg, and marine seruni plants were the members of the Phlaeothripidae family. The morphological identification showed that the three samples belong to different species. They were *Gynaikothrips uzeli* (Zimmerman), *Haplothrips ganglbaueri* (Schmutz), and *Pseudophilothrips ichini* (Table 1).

G. uzeli sample had morphological characteristics such as: an antenna consisting of 8 segments, shape of the end of its stylet is curve and narrow, cheeks without setae, the pronotum usually with less than 5 paors of well-developed setae, forewings without venation with paralel sides, tergites with only 2 pairs of wing-retaining

Table 1. Host plants and thrips species of the Phlaeothripidae found in research location.

Species	Host Plants	Location (Village/ Subdistrict/ Regency)	Coordinate
G. uzeli	Banyan	Cisantana/ Cigugur/ Kuningan	102°26'42.21''E;6°56'37.71''S
H. ganglbaueri	Marine Seruni	Sukajadi/ Tamansari/ Bogor	106°43'38.47''E;6°38'16.29''S
P. ichini	Nutmeg	Ciapus/ Ciomas/ Bogor	106°44'21.88''E;6°37'32.48''S

setae. *H. ganglbaueri* had antenna consisting 8 segments, forewings constricted medially, matanotum with weakly reticulates sculpture, antennal segment IV with 4 sense cone. *P. ichini* has usually with 5 pairs of well-developed setae and sculpture usually more or less striate or in distinc. forewings without venation with paralel sides, tergites with only 2 pairs of wing-retaining setae.

Amplification and Alignment *COI* gene

The diagnostic PCR succesfull distinguished the three *COI* of thrips. Specimens with *COI* gene produced a single PCR product of ± 710 bp (Figure 1). The second band from *G. uzeli* (banyan tree), third band from *H. ganglbaueri* (marine seruni/ *Widelia biflora*) and fourth band from *P. ichini* (nutmeg tree). This study succeed to identified thrips species by using *COI* gene. Based on the results there were three thrips sequences in which each sample had different lenght in nucleotide array. It was happened bacause the three samples from different taxonomy level in genus. As described Karimi et al. (2010) based on *COI* gene sequences, also successfully distinguished some of thrips species of four different genus. Furthermore *COI* gene sequence possible identify unknown specimens by comparing their *COI* sequence and has been used for identification purposes in projects known as species barcoding. Basically, biodiversity and polymorphism can be seen from DNA sequences of certain fragments of an organism genome (Suryanto, 2001).

Sequence analysis is a technique considered as the best technique to see biodiversity in a group of organism. Basically, biodiversity and polymorphism can be seen from DNA sequences of certain fragments of an organism genome (Suryanto, 2001). The characteristics of DNA nucleotide of *COI* gene *G. uzeli* was 704 pb, but with the content of the lowest A base (Adenine) and T base (Thymine) of 70,60% followed by *P. ichini* 702 pb sequences with AT 73.03% composition. The height of AT composition was a special characteristic of nucleotide array of mt*COI* gene on insects. A and T base have weak hydrogen bonds so that easy to changed (Liu & Beckenbench, 1992). Base on the results of nucelotide array of BLAST of *G. uzeli* in Indonesia has similarity value of similarity of 95% with the same species from China, *H. ganglbaueri* found has 92% with *H.*

Figure 1. Morphological character, (a) G. uzeli, (b) H. ganglbaueri, (c) P.

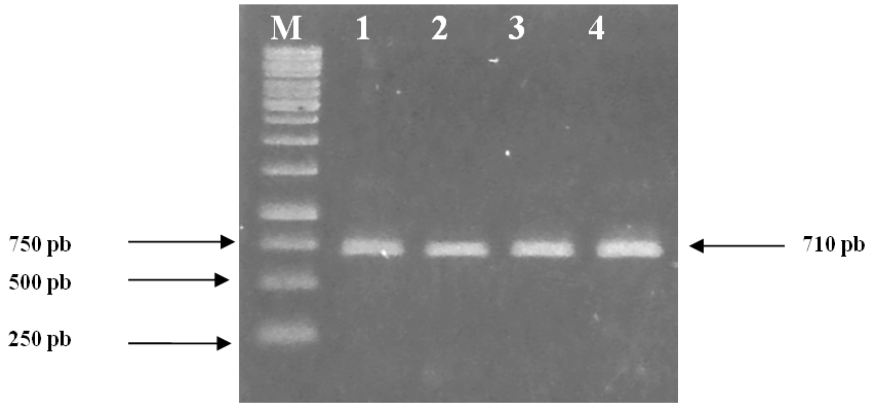

Figure 2. Results of DNA visualization of COI gene fragment of three thrips species by using universal primer (M) Marker 1 kb (Thermo Scientific, US), (1) Positive control (*G. uzeli*), (2) *G. uzeli,* (3) *H. ganglbaueri*, and (4) *P. ichini.*

Table 2. DNA BLAST of *COI* gene on three thrips species by using BLAST-N programme

Description	Origin	Max Score	Total Score	Query cover (%)	E value	Ident (%)	Accession
G. uzeli	China	950	950	91	0.0	93	JN181200
H. ganglbaueri	Australia	830	830	87	0.0	92	EF468730
P. ichini	Canada	628	628	90	0.0	84	KJ087886

ganglbaueri from Australia, *P. ichini* has 84% with *P. ichini* from Canada. It is so since the three samples took place in different taxonomy level (Table 2). Karimi *et al.* (2010) suggest that based on *COI* gene sequences, it had successfully distinguished some of thrips species of four different genus.

Base on the results of nucelotide array of BLAST of *G. uzeli* from Indonesia, it had the similarity percentage of 93% with the same species from China. *H. ganglbaueri* found in marine seruni (*Widelia biflora*) showed the similarity percentage of 92% with *H. ganglbaueri* species from Australia. Then, samples of *P. ichini* nutmeg tree indicated the similarity percentage of 84% with *P. ichini* from Canada (Table 2).

The results of DNA sequences alignment of *COI* gene of the three samples (Table 3) showed that the three species were taxonomically separated. It can be seen from the nucleotide array of mt*COI* gene that had been successfully aligned in which there were differences in some points of nucleotide base sequences of each genus. The number of *converse* nucleotide of the three species was 622 pb and the nucleotide variation was 27.8% or 173 pb. That was the variation of the array of each sample. When there was variation of array occured in a nucleotide alignment with the old ones, it would show the mutation, including insertion, deletion, or rearrangement of genetic materials (Dharmayanti, 2011; Hoy, 2003).

Genetic Distance and Phylogenetic Tree Based on Gene Sequence

Based on the mt*COI* gene sequences of three species, it can be analyzed genetic distance and phylogenetic construction. High value of genetic distance indicates that they are closely related. *G. uzeli* has genetic disctance value 6.2% with the same species from China. *H. ganglbaueri* 8.9% with the same species from Australia, while *P. ichini* 15.8% with *P. ichini* species from Canada (Table 4).

G. uzeli and *H. ganglbaueri* species showed hight similarity levels with haplotype in GenBank as showed low genetic distance value. It was quite different from *P. ichini* in which the similarity levels with haplotype in GenBank was low. However, according to Li et al. (2009), genetic distance within insect species was recieved with genetic distance value 0.05-0.23 or homology value 73-95%. It can be seen from *Caligula japonica* (Lepidoptera: Saturniidae) (Li et al., 2009), Diadegma (Hymenoptera: Ichneumonidae) (Wagener et al, 2006), and mosquitos (Cywinska et al., 2006). Ubaidillah & Sutrisno (2009), the presence of the differences of nucleotide sequence array occur due to the mutation. It can cause the change of nucleotide base which then change encoded amino acid (Hawkes et al., 2004). Our analyses clearly indicate that genetic differentiation happen because geographic isolation and genetic drift. According to Templeton (1998) genetic variation is distributed spatially within a species geographical range. Resricted gene flow and historical events can be interwined, and cladistical analyses can constructed their temporal juxta position, thereby yielding great insight into both the evolutionary history an population structure of the species.

Phylogenetics tree based on *COI* gene (Figure 3) showed that there were two main groups of thrips, *P. ichini* and *G. uzeli.* Claodgram construction was based on the DNA sequences of mt*COI* gene demonstrating the separated classification with bootsrap value between *in-group* sequences (*G. uzeli, H. ganglbaueri, P. ichini*) and *out-group* sequence (*Ceratothripoides brunneus*). The first group was *P. ichini* with *H. ganglbaueri* as sister-grup with the bootstrap value of 84%. The second group was *G. uzeli,* from Indonesia classifying with *G. uzeli* from China with the *bootsrap* value of 100%.

This study investigated the utility of *COI* for identifying thrips species. As demonstrated in this work, there is a relationship between phylogeny and origin evolution of thrips species. We interpret our analyses of congruence phylogenetics tree as suggesting that the different data sets provide complementary information that is not contradictory. Crespi et al. (1998) result showed adult morphology data was not significant from sequences of *COI* gene so that can support the results of morphological identification of thrips species classification from Tubulifera (Phlaeothripidae). Karimi et al. (2010) also used *COI* gene to identified some of thrips species, the result showed there was closely relations of evolu-

Table 3 Variation of DNA nucleotide array of *COI* gene of thrips

Species	Nucleotide position																													
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
H.ganglbaueri	A	T	T	A	A	T	T	T	T	T	G	G	A	A	A	T	A	T	C	T	T	T	C	T	T	T	C	C	T	A	G
*H.ganglbaueri*_Aus_EF468730	.	.	.	.	.	C	.	.	.	.	A	T	.	.	.	A	.	.	.	.	A	.	T	C	.	C	.	T	.	.	.
G.useli	G	C	.	.	.	.	A	.	C	A	A	C	T	G	T	G	.	C	.	A	A	C	T	C	A	A	T	T	C	.	C
*G.useli*_Chn_JN81200	.	.	.	.	.	.	A	.	C	.	A	T	T	G	T	G	.	.	.	A	A	C	T	.	.	A	T	T	.	.	.
P. ichini	.	A	.	T	.	.	A	.	.	.	A	T	T	.	T	.	.	.	.	.	.	.	T	.	.	A	T	.	C	T	.
*P.ichini*_Knd_KJ087886	.	.	A	T	T	.	A	A	.	.	A	T	T	.	.	A	C	.	A	A	A	A	T	.	A	.	T	T	.	T	.

Species	Nucleotide position																													
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
H.ganglbaueri	A	T	G	A	A	A	G	A	A	C	A	T	G	A	T	G	T	T	C	T	G	G	T	A	T	G	A	T	T	T	T
*H.ganglbaueri*_Aus_EF468730	.	A	.	.	.	G	A	.	.	.	.	.	.	.	.	T	.	.	T	.	A	.	.	T	.	A	T	.	A	.	.
G.useli	.	A	A	T	G	.	A	T	G	.	T	.	.	T	.	A	.	C	A	.	T	A	G	.	A	.	T	G	.	A	C
*G.useli*_Chn_JN81200	.	A	A	T	G	.	A	T	G	.	T	.	.	T	.	A	.	C	A	.	T	A	.	.	A	.	T	.	.	A	C
P. ichini	.	A	A	C	.	.	A	G	G	.	.	.	A	.	A	A	A	G	T	C	T	A	.	T	G	A	T	.	.	.	C
*P.ichini*_Knd_KJ087886	G	.	.	T	.	.	A	T	.	T	T	C	A	.	.	A	A	C	A	.	T	A	A	.	A	.	.	.	.	.	A

Species	Nucleotide position																													
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
H.ganglbaueri	G	C	T	G	A	A	G	T	T	A	G	T	T	A	A	A	T	T	A	T	T	G	C	A	T	G	A	T	T	A	T
*H.ganglbaueri*_Aus_EF468730	.	T	.	.	.	.	.	.	.	G	.	.	A	G	.	T	C	.	T	C	.	A	.	.	.	.	T	.	.	.	.
G.useli	C	G	A	T	.	T	A	.	A	T	T	G	.	.	T	T	G	C	.	C	.	A	T	T	A	A	.	A	.	T	C
*G.useli*_Chn_JN81200	C	G	G	.	.	T	A	.	.	T	T	.	.	.	T	T	.	.	.	.	.	A	T	T	A	A	.	A	.	T	C
P. ichini	C	T	A	A	T	T	A	A	.	.	C	.	C	T	.	T	.	.	T	.	C	A	G	G	.	.	.	.	G	G	.
*P.ichini*_Knd_KJ087886	C	G	A	.	.	.	A	.	.	.	A	.	.	.	C	T	.	.	T	C	A	A	A	.	.	T	T	A	.	.	C

Species	Nucleotide position							
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
H.ganglbaueri	A	A	T	A	T	A	A	A
*H.ganglbaueri*_Aus_EF468730	T	.	.	T	.	.	.	G
G.useli	.	.	.	.	C	.	.	G
*G.useli*_Chn_JN81200	.	.	.	.	C	.	G	.
P. ichini	T	G	.	.	.	G	.	.
*P.ichini*_Knd_KJ087886	.	.	A	.	.	G	.	.

Table 4. Genetic distance of DNA sequences of *COI* gene on three thrips species

Species	Origin	Accession	1	2	3	4	5	6
G. uzeli	Indonesia	-	ID					
G. uzeli	China	JN181200	0.062	ID				
H. ganglbaueri	Indonesia	-	0.239	0.182	ID			
H. ganglbaueri	Australia	EF468730	0.247	0.205	0.089	ID		
P. ichini	Indonesia	-	0.355	0.292	0.266	0.303	ID	
P. ichini	Canada	KJ087886	0.210	0.155	0.140	0.175	0.158	ID

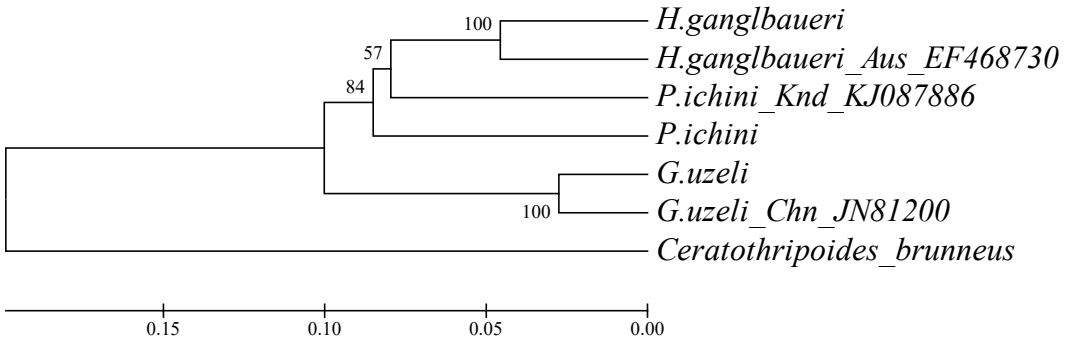

Figure 3. Thrips cladogram based on DNA sequnce nuclotide fragment of *COI* gene by using *UPGMA* method

tion of each thrips species. The phylogeny construction with out-group showed that there was a clear separation among the species. Sequence of *COI* gene considered as a very great genetic marker in encoding DNA. The data of DNA sequence array of *COI* gene is also the completing data of biological and morphological data. The phylogenetic tree formed in here was based on the results of a research by Karimi et al. (2010) that used DNA of mt*COI* gene to identify some of thrips species in which there was familiarity relations of evolution of each thrips species. The cladogram construction with out-group showed that there was a clear separation among three species.

Molecular identification by using *COI* gene had successfully identified the three thrips species because the gene were conserve (Herbert et al., 2003). Karimi et al. (2010) added that DNA of *COI* gene is considered as a very great genetic marker in encoding DNA. The data of DNA sequence array of *COI* gene is also the completing data of biological data and morphological characteristics on insects. Beside the DNA nucleotide array of mt*COI* gene, the morphological characteristics can also be used in supporting the results of identification using nucleotide characteristics of *COI* gene. Crespi et al. (1998) stated that the data from imago morphological characteristics was not different from the identification using DNA sequences of *COI* gene that can support the results of morphological identification of thrips species classification from Phlaeothripidae.

CONCLUSION

Gynaikothrips uzeli (Zimmerman), *Haplothrips ganglbaueri* (Schmutz), and *Pseudophilothrips ichini* was found in weeping fig (*Ficus benjamina*), nutmeg (*Myristica fragrans*), and marine seruni (*Wedelia biflora*) plants. The characteristics of mtCOI of *G. uzeli*, *H. ganglbaueri*, and *P. ichini* were 704, 686, and 702 bp dominated by Adenine and Thymine base with nucleotide variation value of 27.8%. Sequences data of mtCOI DNA can be used for thrips identification.

ACKNOWLEDGMENT

This research was well supported by BPOTN research grant programme number 319/IT3.41.2/L2/SPK/2014.

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