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*OF THE INDO-AUSTRALIAN ARCHIPELAGO*

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Vol. 42, pp. 1─67, December 2015



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## CONTENT

**TREUBIA**

**(A JOURNAL ON ZOOLOGY OF THE INDO-AUSTRALIAN ARCHIPELAGO)**

ISSN : 0082 - 6340 Date of issue: DECEMBER 2015
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UDC: 594.1 (594)

Reni Ambarwati

**New record of two mactrid bivalves (Bivalvia: Mactridae) from Indonesia**

TREUBIA, December 2015, Vol. 42, pp. 1–8.

The occurrence of two mactrid bivalves, *Mactra (Mactra) queenslandica* E.A. Smith and *Heterocardia gibbosula* Deshayes, in coastal water of Sidoarjo, East Java, Indonesia is reported here. The two species are examined and illustrated based on the local specimens collected. Previously, the distribution of *M. queenslandica* was reported only from northern – north-east Australia. This finding revealed that the distribution of this bivalve reaches Indonesian waters. Meanwhile, *H. gibbosula* is common in south-east Asian waters, however this is the first record for Indonesian waters. This result indicated that more mactrid bivalves could be discovered in Indonesian waters.

(Reni Ambarwati and Trijoko)

**Key words**: *Heterocardia gibbosula*, *Mactra queenslandica,* Mactridae, Sidoarjo

UDC: 595.78: 577.2 (594.5)

Hari Sutrisno

**Mitochondrial DNA variation of the rice yellow stem borer, *Scirpophaga incertulas* (Lepidoptera: Crambidae) in Java, Indonesia**

TREUBIA, December 2015, Vol. 42, pp. 9–22.

*Scirpophaga incertulas* is an economically important rice pest. A systematic investigation on the biological characteristics of ecological races linked to recent changes of agricultural practices and the environment has been conducted in order to assess genetic variation of *S. incertulas* in Indonesia. A 685bp segment of mitochondrial DNA, COII, was amplified from 42 yellow stem borer samples from five locations in Java (Madiun, Ngawi, Wonogiri, Tasikmalaya, and Indramayu). Six different haplotypes (YSB1, YSB2, YSB3, YSB4, YSB5 and YSB6) were identified in the sequenced yellow stem borer populations, with haplotype YSB2 being dominant.

(Hari Sutrisno)

**Key words**: COII, mitochondrial DNA, *Scirpophaga incertulas*, yellow stem borer

UDC: 595.42: 595.764 (594.59)

Sri Hartini

**Macrochelid mites (Acari: Mesostigmata) associated with dung beetles in Baluran National Park, East Java, Indonesia**

TREUBIA, December 2015, Vol. 42, pp. 23–36.

Eight mite species of the family Macrochelidae (Acari: Mesostigmata) were collected from the body surface of dung beetles in Baluran National Park, East Java, Indonesia. Of these, one species, *Macrocheles subwallacei* sp. nov., is described as new to science. The female of *Macrocheles crispa* (Berlese, 1910) is redescribed and the male is described for the first time. The remaining six species are *Neopodocinum jaspersi* (Oudemans, 1900), *M. dispar* (Berlese, 1910), *M. hallidayi* Walter & Krantz, 1986, *M. entetiensis* Hartini & Takaku, 2005, *M. jabarensis* Hartini & Takaku, 2003 and *M. persimilis* Hartini, Dwibadra & Takaku, 2007.

(Sri Hartini, Dhian Dwibadra, Masahiro Ohara and Gen Takaku)

**Key words**: Baluran, dung beetles, East Java, Indonesia, Macrochelidae

UDC: 574.9: 57.065

Rena Tri Hernawati

**Exploring the dynamics during community assembly through community phylogenetics**

TREUBIA, December 2015, Vol. 42, pp. 37–52.

Species diversity through speciation and accumulate in ecological communities, a process known as community assembly. Relying on both evolutionary mechanisms acting at regional scale and ecological mechanisms acting at local scale, the process of community assembly results from intricate interactions among mechanisms at play across varying spatial and temporal scales. During the last decade, community assembly theory has been reconsidered in the light of evolutionary dynamics of species diversification and ecological dynamics have been formalised in an explicit spatial framework (*i.e*. metacommunity theory). The aims of the present review are: (1) to present the community assembly theory and the main paradigms that have been proposed, (2) to discuss how the metacommunity theory as defined an explicit spatial framework for community ecology, (3) to discuss the potential mechanisms at play during community assembly and their associated predictions, (4) to present new approaches to study community assembly based on phylogenetics approaches and discuss how they have been integrated in empirical studies.

(Rena Tri Hernawati, Daisy Wowor and Nicolas Hubert)

**Key words**: biogeography, community assembly, dispersal, phylogenetic community structure, speciation

UDC: 595.42 (594.81)

Sri Hartini

**Macrochelid mites (Acari: Mesostigmata) from Kaimana,West Papua, Indonesia, and endemism of macrochelid mite fauna in New Guinea Island**

TREUBIA, December 2015, Vol. 42, pp. 53–67.

As a result of our investigation in Lengguru, Kaimana, West Papua, Indonesia, six species belonging to two genera of macrochelid mites (Acari: Mesostigmata: Macrochelidae) were collected from the body surface of dung beetles (Scarabaeidae). Of these, one is undescribed species *Macrocheles kaimanaensis* sp. nov. *Macrocheles hallidayi* Walter & Krantz, 1986 is newly recorded from Papua and West Papua (Indonesian parts of New Guinea Island). Males of *Holostaspella rosichoni* Hartini & Takaku, 2006 originally described from Papua were recorded for the first time. The other three species were *M. amaliae* Hartini, 2008, *M. dispar* (Berlese, 1910) and *M. waigeoensis* Hartini, 2008, which were previously collected from Raja Ampat, West Papua.

(Sri Hartini and Gen Takaku)

**Key words**: Indonesia, Kaimana, macrochelid mite, West Papua

Treubia 42: 9−22, December 2015

# MITOCHONDRIAL DNA VARIATION OF THE RICE YELLOW STEM BORER, *SCIRPOPHAGA INCERTULAS* (LEPIDOPTERA: CRAMBIDAE) IN JAVA, INDONESIA

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## ABSTRACT

*Scirpophaga incertulas* is an economically important rice pest. A systematic investigation on the biological characteristics of ecological races linked to recent changes of agricultural practices and the environment has been conducted in order to assess genetic variation of *S. incertulas* in Indonesia. A 685bp segment of mitochondrial DNA, COII, was amplified from 42 yellow stem borer samples from five locations in Java (Madiun, Ngawi, Wonogiri, Tasikmalaya, and Indramayu). Six different haplotypes (YSB1, YSB2, YSB3, YSB4, YSB5 and YSB6) were identified in the sequenced yellow stem borer populations, with haplotype YSB2 being dominant.

**Key words**: COII, mitochondrial DNA, *Scirpophaga incertulas*, yellow stem borer

## INTRODUCTION

*Scirpophaga incertulas* (rice yellow stem borer) is an economically important pest of rice throughout South East Asia including Indonesia. The species has caused chronic yield losses that are often estimated at 10% (Zhong *et al*. 2000). The intensity of yellow stem borer epidemics increases with time and outbreaks of stem-borers on rice in northern part of West Java and other parts of Indonesia occur despite the implementation of various control measures. Different rice plant varieties have been planted by farmers with the aim to reduce the populations of rice stem borer, but so far rice borer populations have proved resilient (Amir *et al.* 2004).

Causes of outbreaks are not fully understood. It was thought that this species is a complex species and one of the factors causes failure in the control of this pest may lie in the misidentification of this species and our failure to recognise the rich diversity of this pest in tropical agroecosystems. Indeed, the relationships among host – insect – natural enemies in tropical agroecosystems need to be understood.

In order to get a good level of resistance against the widespread yellow stem borer, researchers conducted transgenic approaches. Resistance to insects has been demonstrated in transgenic plants expressing genes for delta-endotoxins from *Bacillus thuringiensis* (Bt), protease inhibitors, enzymes and plant lectins. The performance of insect resistant GM rice in

trials in China has been quite impressive. From the first insect-resistant genetically modified (IRGM) rice transformation in 1989 in China until October 2009 when the Chinese Ministry of Agriculture issued biosafety certificates for commercial production of two cry1Ab/Ac *Bacillus thuringiensis* (Bt) lines, China has made a great leap forward from basic research of IRGM rice to potential commercialisation of the world's first IRGM rice. Research has been conducted on developing IRGM rice, assessing its environmental and food safety impacts, and evaluating its socioeconomic consequences. Laboratory and field tests have confirmed that these two Bt rice lines can provide effective and economic control of the lepidopteran complex on rice with less risk to the environment than present practices. Commercialising these Bt plants, while developing other GM plants that address the broader complex of insects and other pests, will need to be done within a comprehensive integrated pest management program to ensure the food security of China and the world (Deka & Barthakur 2010, Chen *et al*. 2011) However, the product of this transgenic is still debatable to be applied in the field concerning the impact to the environment, especially in Indonesia. Some potential biological agents may also be developed to control this pest with a minimum risk of ecosystem damage. The most important matter is how to manage the natural enemies to maintain themselves in the environment to keep the pest population low. This strategy can be achieved only when we know the biological characteristic of the pests and their natural enemies.

The species has a wide distribution between the latitude 50.00' N and 34.53 'S. Vietnam is the probable center of origin of *Scirpophaga* because this place is the origin of *Oryza sativa* which is the host plant of many species in this genus. The pattern of distribution and the diversity follow the distribution of rice. It is also possible that the invasion of species from one area into a new area by wind would further increase the diversity of the species as well (Lewvanich 1982). Previous study showed that this species has morphological variations among populations but recent molecular study based on CO I gene sequence proved that *S. incertulas* is valid as a single species based on populations in Java. The genetic distance among five populations is very low, less than 2% (two sequences are defined as a single species when the sequence divergence between them is < 2%) (Amir *et al*. 2004, Sutrisno 2008). However, systematic investigation on biological characteristics of ecological races based on recently changed agricultural practices and environment is still necessary to be conducted in order to assess the knowledge on genetic variations of populations of *S. incertulas* in Indonesia.

CO II is one of the mitochondrial genes that has fast evolutionary rate. This gene has been used to study the relationship among closely-related species. Therefore, much genetic variation can be expected between individuals of the same species. However, studies that analyse the rice yellow stem borer based on mitochondrial DNA in Indonesia are still limited, such as the studies of Sutrisno (2008) and Raffiudin *et al*. (2011)**.**

Furthermore, genetic analysis of the yellow stem borer populations comprehensively sampled from central production rice in Java has not yet been reported. Therefore, a research on mitochondrial DNA analysis of genetic diversity within maternal lines of different populations of yellow stem borer was conducted, in order (1) to generate mtDNA CO II sequences for all the yellow stem borer samples under this study and (2) to provide information on haplotypes and degree of nucleotide sequence diversity of yellow stem borer populations. To conduct the study, the highly variable CO II region of mtDNA on 42 samples of yellow stem borer from five locations in Java was analysed. All results obtained in this study should be useful for control strategies, particularly for yellow stem borer. It is believed that diversity is only secure if diverse conservation strategies are employed.

## MATERIALS AND METHODS

### Samples and study areas

A total of 42 DNA moths were collected from five different locations of rice production centers in Java (Table 1). The samples were collected by using light trap. All samples (abdomen part) were preserved in 96% absolute ethanol and stored at the Bank of Material DNA for Indonesian Fauna, Genetic Laboratory, and the remaining specimens without abdomen were deposited in laboratory of Entomology, Division of Zoology, Research Center for Biology-LIPI. A list of the samples is presented in Table 1.

**Table 1.** List of samples of rice yellow stem borer (*S. incertulas*) from five locations in Java

| No | Locality | Province | Number of samples |
|---|---|---|---|
| 1 | Madiun (M) | East Java | 10 |
| 2 | Ngawi (N) | East Java | 8 |
| 3 | Wonogiri (W) | Central Java | 7 |
| 4 | Tasikmalaya (T) | West Java | 7 |
| 5 | Indramayu (I) | West Java | 10 |
| | Total | | 42 |

All the five sites: Madiun, Ngawi, Wonogiri, Tasikmalaya and Indramayu were presented in Fig. 1 below.


**Figure 1.** Sampling sites: Madiun (1), Ngawi (2), Wonogiri (3), Tasikmalaya (4), Indramayu (5).

## DNA extraction, amplification and sequencing

DNA extraction was conducted by using CTAB method (Sutrisno *et al*. 2006). For PCR amplification and DNA sequencing, we used A-tLEU and B-tLYS primers to amplify the *COII* gene for a total of 685-bp. The amplification of *COII* was conducted as follows: one cycle of denaturation at 94ºC for 10 min, followed by 30 cycles, each consisting of 30 s of denaturation at 94ºC, 60 s of annealing at 47 ºC, and 120 s of extension at 72ºC (Liu & Beckenbach 1992).

The PCR products were purified using QiaquickPCR purification Kit (Qiagen. USA). Sequencing was performed using ABI PRISM Dye Terminator Cycle Sequencing Ready reaction kit (Perkin-Elmer) on ABI PRISM model 310 Genetic analyser (PE Applied Biosystems). The sequence was aligned using BioEdit sequence alignment Editor (Hall 1999).

## Data analysis

The base frequency's option in PAUP* version 4.0b.10 for Windows was used to evaluate the base composition of each sequence (Swofford 2001). DnaSPv5 was used to analyse the polymorphic sites (Librado & Rozas 2009). Molecular Evolutionary Genetic

Analysis (MEGA) version 5.10 was used for phylogenetic and analysis of the molecular evolution (Tamura *et al*. 2011). Neighbour Joining method was used to reconstruct the phylogeny tree and the statistical confidence of this method was evaluated using bootstrap test with 100 replicates. For analysis the sequence divergence, K2P distance (Kimura 1980) was selected.

## RESULTS

A total of 42 PCR products from the samples of yellow stem borer was successfully sequenced, with a total length of about 685 base pairs. The results showed that CO II gene sequence of *S. incertulas* was A+Trich. Table 2 shows the proportion of A-C-G-T of CO II gene, and its bias (*C*). The bias was calculated following Irwin *et al*. (1991)

$$C = \left(\tfrac{2}{3}\right)\sum_{i=1}^{4}\left|c_i - 0.25\right|$$

where $c_i$ is base frequency *i*. The results showed that the base composition was A+T biased (*C*: 0.0228) with the average of A+T contents was 71.75%.

**Table 2.** Proportion of each nucleotide and the bias in CO II gene sequence of *S. incertulas*

| Nucleotide | Codon position | | | Mean |
|---|---|---|---|---|
| | 1st | 2nd | 3rd | |
| A | 0.30131 | 0.37521 | 0.37030 | 0.34894 |
| C | 0.17904 | 0.17544 | 0.17345 | 0.17598 |
| G | 0.11790 | 0.18181 | 0.01963 | 0.10645 |
| T | 0.40175 | 0.26750 | 0.43661 | 0.36862 |
| AT Bias | | | | 0.02280 |

There was no evidence of insertion and deletion. Aligned sequences (42 sequences) have been submitted to the Genbank with accession numbers presented in the Table 3.

The study also showed that genetic variations among five populations of *S. incertulas* in Java fall into six haplotypes: YSB1, YSB2, YSB3, YSB4, YSB5 and YSB6. All haplotypes and their distribution found in this study are presented in Table 4.

**Table 3.** Specimens of *S. incertulas* selected for molecular study and their Genbank accession numbers

| No | Sample specimen | Collectors | Acc. Number of Genbank |
|---|---|---|---|
| 1 | *S. incertulas* N1 | Sutrisno, H, Darmawan, Ubaidillah | AB930216 |
| 2 | *S. incertulas* N2 | Sutrisno, H, Darmawan, Ubaidillah | AB930217 |
| 3 | *S. incertulas* N4 | Sutrisno, H, Darmawan, Ubaidillah | AB930218 |
| 4 | *S. incertulas* N6 | Sutrisno, H, Darmawan, Ubaidillah | AB930219 |
| 5 | *S. incertulas* N7 | Sutrisno, H, Darmawan, Ubaidillah | AB930220 |
| 6 | *S. incertulas* N8 | Sutrisno, H, Darmawan, Ubaidillah | AB930221 |
| 7 | *S. incertulas* N9 | Sutrisno, H, Darmawan, Ubaidillah | AB930222 |
| 8 | *S. incertulas* N10 | Sutrisno, H, Darmawan, Ubaidillah | AB930223 |
| 9 | *S. incertulas* M11 | Sutrisno, H, Darmawan, Ubaidillah | AB930224 |
| 10 | *S. incertulas* M12 | Sutrisno, H, Darmawan, Ubaidillah | AB930225 |
| 11 | *S. incertulas* M13 | Sutrisno, H, Darmawan, Ubaidillah | AB930226 |
| 12 | *S. incertulas* M14 | Sutrisno, H, Darmawan, Ubaidillah | AB930227 |
| 13 | *S. incertulas* M15 | Sutrisno, H, Darmawan, Ubaidillah | AB930228 |
| 14 | *S. incertulas* M16 | Sutrisno, H, Darmawan, Ubaidillah | AB930229 |
| 15 | *S. incertulas* M17 | Sutrisno, H, Darmawan, Ubaidillah | AB930230 |
| 16 | *S. incertulas* M18 | Sutrisno, H, Darmawan, Ubaidillah | AB930231 |
| 17 | *S. incertulas* M19 | Sutrisno, H, Darmawan, Ubaidillah | AB930232 |
| 18 | *S. incertulas* M20 | Sutrisno, H, Darmawan, Ubaidillah | AB930233 |
| 19 | *S. incertulas* W31 | Ubaidillah R, Darmawan, Sarino | AB930234 |
| 20 | *S. incertulas* W32 | Ubaidillah R, Darmawan, Sarino | AB930235 |
| 21 | *S. incertulas* W33 | Ubaidillah R, Darmawan, Sarino | AB930236 |
| 22 | *S. incertulas* W34 | Ubaidillah R, Darmawan, Sarino | AB930237 |
| 23 | *S. incertulas* W35 | Ubaidillah R, Darmawan, Sarino | AB930238 |
| 24 | *S. incertulas* W36 | Ubaidillah R, Darmawan, Sarino | AB930239 |
| 25 | *S. incertulas* W37 | Ubaidillah R, Darmawan, Sarino | AB930240 |
| 26 | *S. incertulas* T22 | Sutrisno, H & Darmawan | AB930241 |
| 27 | *S. incertulas* T24 | Sutrisno, H & Darmawan | AB930242 |
| 28 | *S. incertulas* T25 | Sutrisno, H & Darmawan | AB930243 |
| 29 | *S. incertulas* T26 | Sutrisno, H & Darmawan | AB930244 |
| 30 | *S. incertulas* T27 | Sutrisno, H & Darmawan | AB930245 |
| 31 | *S. incertulas* T29 | Sutrisno, H & Darmawan | AB930246 |
| 32 | *S. incertulas* T30 | Sutrisno, H & Darmawan | AB930247 |
| 33 | *S. incertulas* I41 | Ubaidillah R, Darmawan, Sarino | AB930248 |
| 34 | *S. incertulas* I42 | Ubaidillah R, Darmawan, Sarino | AB930249 |
| 35 | *S. incertulas* I43 | Ubaidillah R, Darmawan, Sarino | AB930250 |
| 36 | *S. incertulas* I44 | Ubaidillah R, Darmawan, Sarino | AB930251 |
| 37 | *S. incertulas* I45 | Ubaidillah R, Darmawan, Sarino | AB930252 |
| 38 | *S. incertulas* I46 | Ubaidillah R, Darmawan, Sarino | AB930253 |
| 39 | *S. incertulas* I47 | Ubaidillah R, Darmawan, Sarino | AB930254 |
| 40 | *S. incertulas* I48 | Ubaidillah R, Darmawan, Sarino | AB930255 |
| 41 | *S. incertulas* I49 | Ubaidillah R, Darmawan, Sarino | AB930256 |
| 42 | *S. incertulas* I50 | Ubaidillah R, Darmawan, Sarino | AB930257 |

**Table 4.** Haplotype distribution of 42 sequences of mitochondrial DNA CO II

| No | Haplotype | Individual with same haplotype | Number of haplotype | % | Sites and Substitution |
|---|---|---|---|---|---|
| 1 | YSB1 | N1, N6, N8, N9<br>M14, M15, M16, M17, M19<br>T25, T29<br>W31, W33, W34, W37 | 15 | 35.7 | |
| 2 | YSB2 | N2, N4, N7, N10<br>M11, M12, M13, M18, M20<br>T24, T26, T27, T30<br>W32<br>I41, I43, I46, I47, I49, I50 | 20 | 47.6 | No. 8 = A → C<br>No. 395 = G → T<br>No. 569 = G → A |
| 3 | YSB3 | T22<br>I42, I48 | 3 | 7.14 | No.506 = G → A |
| 4 | YSB4 | W35, W36 | 2 | 4.76 | No. 5 = A → G<br>No. 500 = A → G |
| 5 | YSB5 | I44 | 1 | 2.38 | No. 8 = A → C |
| 6 | YSB6 | I45 | 1 | 2.38 | No.395 = G → T<br>No.564 = G → A |
| | | | 42 | 100 | |

Note: N= Ngawi, M= Madiun, T= Tasikmalaya, W= Wonogiri, I= Indramayu

The results also showed that there were transition and transversion substitutions from the YSB1 into YSB2, YSB3, YSB4, YSB5, and YSB6. Haplotype YSB2 was derived from YSB1 through twice transversions and a single transition. The transversion from the A to C at the base no. 8, transversion from G to T at the base no. 395, and transition from G to A at the base no. 569. YSB3 was derived from YSB1 through transition at the base no. 506 from G to A. YSB4 was derived from YSB1 through twice transition substitutions from A to G at the base no. 5 and 500. YSB5 was derived from YSB1 through a single transversion at the base no. 8 from A to C. YSB6 was derived from YSB1 by twice substitution through transversion at based 395 from G to T and transition at the base no. 564 from G to A.

The haplotype diversity and nucleotide diversity were analysed for each region (N= Ngawi, M= Madiun, W= Wonogiri, T= Tasikmalaya and I= Indramayu), and for the whole population. The results of the genetic diversity are presented in Table 5. Based on the entire data set, the nucleotide diversity in Java was 0.00269. While among populations in Java, Indramayu was the lowest (0.00139) and Wonogiri was the highest (0.00264).The highest

haplotype diversity was found in yellow stem borer from Tasikmalaya and Wonogiri regions (0. 667), while the lowest was found in population from Madiun (0.533). Based on the whole sequences, haplotype diversity in Java was 0.657, slightly lower than those found in Tasikmalaya and Wonogiri.

**Table 5.** Genetic diversity (haplotype & nucleotide diversity) of yellow stem borer in Java

| Population* | Haplotype diversity (Hd) | Nucleotide diversity (Nd) | Number Haplotype | Haplotype** |
|---|---|---|---|---|
| Ngawi/ N (8) | 0,571±0,094 | 0,00250±0,00041 | 2 | YSB1 (0.095), YSB2 (0.095) |
| Madiun/ M (10) | 0,533±0,095 | 0,00234±0,00041 | 2 | YSB1 (0.11), YSB2 (0.090) |
| Wonogiri/ W(7) | 0,667±0,160 | 0,00264±0,00090 | 3 | YSB1 (0.095), YSB2 (0.023), YSB4 (0.047) |
| Tasikmalaya/ T (7) | 0,667±0,160 | 0,00250±0,00075 | 3 | YSB1 (0.047), YSB2 (0.095), YSB3 (0.023) |
| Indramayu/ I (10) | 0,644±0,152 | 0,00139±0,00049 | 4 | YSB2 (0.14), YSB3 (0.047), YSB5 (0.023), YSB6 (0.023) |
| Java (42) | 0,657±0.046 | 0,00269±0,00022 | 6 | YSB1 (0.38), YSB2 (0.45), YSB3 (0.07), YSB4 (0.047), YSB5 (0.023), YSB6 (0.023) |

Note: Hd: Haplotype diversity (expressed as average±1SE)
Nd: Nucleotide diversity (expressed as average±1SE)
* The number of individuals sampled are shown within parentheses
**The frequencies of the haplotypes are shown within parentheses

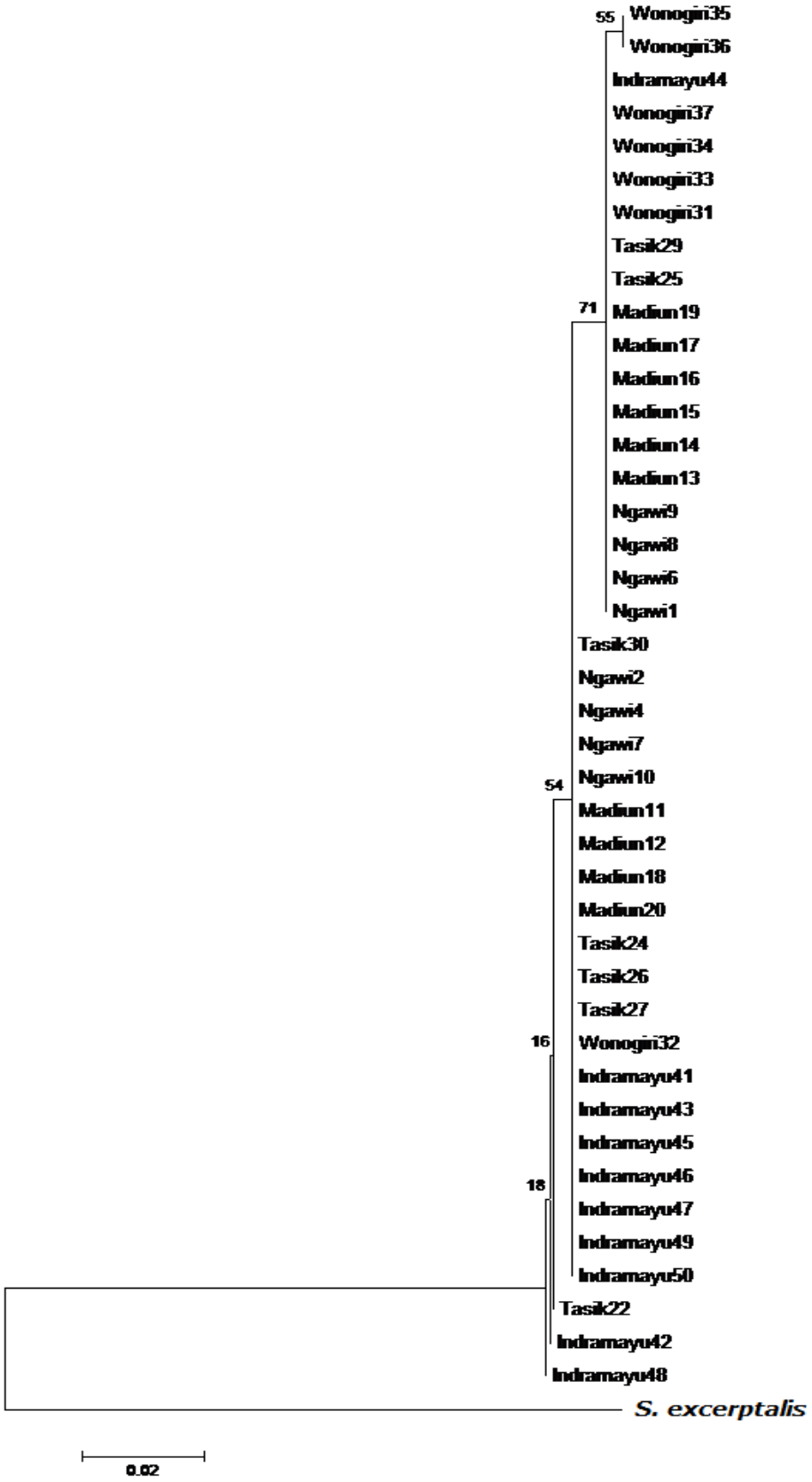


**Figure 2.** Neighbor Joining Tree based on Kimura two-parameter model with a bar scale to indicate the substitution/site.

The result of genetic distance calculations showed that genetic distances within regions/populations ranged from 0.001911 – 0.004693 (Ngawi = 0.002503, Madiun = 0.004693, Tasikmalaya = 0.002242, Wonogiri = 0.003129, and Indramayu = 0.00191) and the genetic distance among populations is 0.002038. Thus, both genetic distance within populations and among the different populations of yellow stem borer were very low. The relationship among populations of *S. incertulas* was reconstructed by using Neighbor-Joining method based on genetic distance of Kimura two-parameter model substitution (Fig. 2).

## DISCUSSION

The results of our study showed that CO II genes from 42 populations of *S. incertulas* A+T biased. It is consistent with mitochondrial genomes of other insects previously reported by many authors (reviewed by Simon *et al*. 1994). In other genera of Lepidoptera, high A+T contents have been found in *CO II* of *Glyphodes* (Sutrisno *et al*. 2006) which ranged from 62% to74%. The average of A+T proportion in the present study (71.7%) was comparable with those found in other genera of Lepidoptera. In addition, A+T rich was also found in 16S mitochondrial gene of *S. incertulas* and *S. excerptalis* as has been reported by Raffiudin *et al*. (2011) and Lange *et al*. (2004). They reported that the A+T content of *S. incertulas* from several population in West Java and *S. excerptalis* (sugar cane stem borer) from Papua New Guinea were 65% and 79.1%. The A+T rich content was also found in mitochondrial *CO I* gene of *S. incertulas* as has been reported in the previous study (Sutrisno 2008). In other genera of Lepidoptera, high A+T contents have been found also in *CO I* of *Helicoverpa*, *Glyphodes, Mythimna, Aganiinae* (Kranthi *et al*. 2006, Sutrisno *et al*. 2006, Sutrisno 2011, 2012) which ranged from 62 % to 74 %. In addition, the bias in base compositions was found to be the greatest at the third-base position. This is perhaps because first- and second-codon positions were more constrained by the amino acid composition of the encoded protein (van Dorp 2004, Zhang *et al*. 2011).

The number of haplotype found in this study was higher than those found in the population of this species from West Java and other provinces based on 16S mitochondrial and CO I genes (Sutrisno 2008, Raffiudin *et al*. 2011). Across COI genes sequences of 10 populations in Java, three haplotypes were recorded. While among all 16S mitochondrial gene sequences in West Java, three haplotypes from Bogor, Karawang, Cirebon and

Indramayu samples were recorded. The first 16S mitochondrial gene haplotype was the common haplotype found from three locations (Karawang, Indramayu and Cirebon). However, haplotype diversity found in this study was slightly lower than those found in population of *Heliothis armigera* in North and North East of Brazil based on data CO I and CO II genes (Mastrangelo *et al*. 2014).

The result studies showed that the transition was higher than transversion. It is slightly different from those found in the population of this species in West Java based on 16S (Raffiudin *et al*. 2011). This result was higher than those found in the previous study based on COI and16S (not only transition but also deletion was found in the population of this species in West Java). Deletion event is very rare in the study of population even interspecies based on mitochondria CO I and CO II genes in Lepidoptera (Sutrisno *et al*. 2006, Sutrisno 2012).

The sequence divergence of CO II gene within population was relatively low (0.22). However, this result was higher than those found in the previous study based on COI and16S (Sutrisno 2008, Raffiudin *et al*. 2011). I have showed the sequence divergence within 10 populations of *S. incertulas* (Alas Purwo National Park - East Java, Baluran National Park - East Java, Magetan - East Java, Wates – Yogyakarta, Sleman - Yogyakarta, Baturraden - Central Java, Wonosobo - Central Java, Garut - West Java, Gunung Halimun-Salak National Park - West Java, Ciamis - West Java) was very low (0.0001). Raffiudin *et al*. (2011) showed that intraspecific genetic distance among population of this species from West Java (Bogor, Karawang, Indramayu and Cirebon) based on 16S mitochondrial gene was 0.003. All of this finding indicated that the evolution rate of CO II gene was higher than 16S and CO I genes within *S. incertulas* as has been reported also in other studies on different genus (Sutrisno *et al*. 2006, Roe & Sperling 2007). In addition, study on genetic variation among populations of this species collected from 28 hotspot locations in India using the randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) that has been conducted by Kumar *et al*. (2001) also produced almost the same results. In their study, 32 primers were used and 354 amplification products were observed. No RAPD-PCR bands diagnostic to the pest population from any specific region were identified. Cluster analysis using UPGMA showed that, with the exception of the pest population from Pattambi, all of the populations cluster as one group with genetic distance values in the range of 6–22%, suggesting that gene

flow between populations is independent of geographic distance and appears to be unrestricted. This study also indicates that all populations cluster as one group in NJ tree with low genetic distance values (in the range 0.19 – 0.46%). Moreover, genetic diversity of the species *S. incertulas* from five population in Java was also low. It is similar with those found from three population of *H. armigera* in Brazil based on CO I and CO II genes (Mastrangelo *et al*. 2014).

## CONCLUSIONS

Based on the analysis of mitochondrial DNA CO II sequence from 42 samples of yellow stem borer, it was concluded that yellow stem borer within populations and among populations in Java exhibit only limited or very low genetic diversity. This study provided a first definition of mtDNA diversity based on CO II gene sequence. YSB1, YSB2, YSB3, YSB4, YSB5, YSB6 were found in the rice yellow stem borer in Java. The distance between haplotypes of yellow stem borer population was also low. Moreover, additional samples may be needed to obtain significant conclusions.

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