Phylogenetic relationships among Indonesian wood borer pests, xyleutes, and its allied genera (Lepidoptera: Cossidae)

Y S Fitriana¹, Darmawan¹, S Y Wiyati² and Sutrisno¹*

¹Division of Zoology, Research Center for Biology, the Indonesian Institute of Sciences, Jalan Raya Bogor Km 46, Cibinong 16911, Indonesia
²Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Jalan Agatis Dramaga Bogor, 16680, Indonesia

*Corresponding author: sutrisnohari@yahoo.com

Abstract. Xyleutes and its allied genera are two of the major wood borer pests in South East Asia, especially in Indonesia. The systematics of this group is still vague since all these genera were mostly described based on superficial external characters only. Therefore, the validity of each genus within this group needs to be assessed based on more comprehensive data. To assess the monophyly of each genus, and to elucidate the relationship of the Indonesian Xyleutes and its allied genera, we analyzed 28 species from 11 genera based on nucleotide sequence variation across a 579-bp region in the CO I gene. The results showed that ML tree-building methods recovered almost all sections recognized based on morphological characters. Some genera such as Morpheis, Chalcidica, and Rapdalus are valid genera and their relationship within each genus well resolved. On the other hand, genera Trimoslemos, Zeuzera, and Xyleutes seem to be paraphyletic groups. Those genera, indeed, need revision based on materials not only from Indonesia but also from other countries. The current phylogeny proposed here is necessary to be evaluated by involving conserved genes to get better resolution for the relation among all genera.

Keywords: CO I gene, maximum likelihood, parsimony

1. Introduction

Xyleutes and its closely related genera such as Bergaris, Chalcidica, Rapdalus, Trismelasmos, Duomitus, Zeuzera, Hermophyllon, and Rugigegat (hereafter refer to Xyleutes and its allied genera) are two of the most important wood borers pests in South East Asia, especially in Indonesia [1]. Most larvae are borers in commercial trees [1]. In Indonesia, they bore several trees such as Callicarpa, Clerodendrum, Gmelina, Tectona (Verbenaceae), Erythrina, Sesbania (Leguminosae), Coffea spp., and Avocado (Persea gratissima) [1, 2].

The systematics of this group is vague, especially on the validity of each genus and their phylogenetic relationships among them [1]. Systematic studies of this group have never been conducted based on Indonesian fauna except for Roepke [1, 3] and Holloway [4] who studied based on Malayan, New Guinean, and Bornean fauna. In their study, they included several species from both the African and Asian continents to subtropics Australasia into Xyleutes even though they realized that this genus was probably a paraphyletic (table 1) [1, 3]. Even the latter stated that Zeuzera is not the sister group of Xyleutes but it might be a section within of Xyleutes group [4]. Moreover, Zeuzera was
divided into two clades with Z. borneana at the basal clade based on CO I gen [5]. He also suggested that a modern revision of this genus based on the world fauna is necessary.

Table 1. Treatment of the member of Xyleutes by Roepke [1,3], Holloway [4] and Schoorl [6]

| No. | Author               | Roepke [1,3]       | Holloway [4]       | Schoorl [6]       |
|-----|----------------------|--------------------|--------------------|-------------------|
| 1   | X. strix             | Xyleutes strix     | Xyleutes strix     |                   |
| 2   | X. persona           | X. persona         | X. persona         |                   |
| 3   |                      |                    | X. keyensis        |                   |
| 4   | X. mineus            | X. mineus          | Chalcidica mineus  |                   |
| 5   | X. cinerosa          | Trismelasmos cinerosa |                 |                   |
| 6   | X. jordani           | X. jordani         |                   |                   |
| 7   | X. papuana           | T. papuana         |                   |                   |
| 8   | X. tectoria          | T. tectorius       |                   |                   |
| 9   | X. elegans           | T. elegans         |                   |                   |
| 10  | X. albicans          | T. albicans        |                   |                   |
| 11  | X. kalisi            | T. kalisi          |                   |                   |
| 12  | X. major             | T. major           |                   |                   |
| 13  | X. distyograpta      | T. distyograpta    |                   |                   |
| 14  | X. maculatus         | X. maculatus       | T. maculatus       |                   |
| 15  | X. malayica          | Berggaris malayica |                   |                   |
| 16  | X. lutescens         | B. lutescens       |                   |                   |
| 17  | X. pardicolor        | Rapdalus pardicolor|                   |                   |
| 18  | X. anceps            | Hermophyllon anceps|                   |                   |
| 19  | X. ceramica          | Duomitus ceramica  |                   |                   |
| 20  | X. quarlesi          | Panau quarlesi     |                   |                   |

Schoorl [6] made a tremendous progress in the revision and reconstruction of the phylogenetic relationship within subfamily Zeuzerinae (including Xyleutes and its closely-related genera) based on morphological characters. He divided a large and heterogeneous Xyleutes into several small genera such as Bergaris, Chalcidica, Rapdalus, etc. (table 1). In his study, genus Xyleutes itself was defined based on several synapomorphies. In his study, genus Xyleutes is restricted to three species: X. strix, X. persona and X. keyensis. Moreover, he proposed the phylogenetic relationships within subfamily Zeuzerinae including Xyleutes and its closely related genera through his cladogram (figure 1). However, his hand phylogenetic hypothesis is still necessary to be evaluated by using a computer program involving molecular data.

The study aims to test the validity of all genera within Xyleutes and its allied genera and to elucidate the phylogenetic relationship of the Indonesian species based on CO I gene sequences. Due to the limitation on available materials for this study, however, we focused on the validity of the relationships of Xyleutes and its allied genera only (Section 2a part 1 and Section 2a part 2 in Schoorl’s Cladogram).

2. Materials and methods

2.1. Material sources
A total of 28 species of Xyleutes and its allied genera were used. Among them, 19 species were collected from different localities in Indonesia and deposited in Museum Zoologicum Bogoriense, Research Center For Biologi LIPI. While the other nine species and two species outgroup Cossinae data were taken from the GenBank. All the species involved in this study were presented in table 2.
2.2. Protocol DNA analysis
A non-destructive method to avoid damage of the whole specimen was used to extract the DNA and then continued using a spin column following steps on QIAGEN animal tissue protocol kit [7, 8].

Figure 1. A. Hand Cladogram Section 2a part 1 and B. Hand Cladogram Section 2a part 2 [6].
The pair of primer of COI gene used were barcode primer for Lepidoptera: LepF1 and LepR1. The protocol of PCR amplification, PCR purification, Sequencing and alignment following the previous studies on DNA barcode for Lepidoptera [7-13].

2.3. Phylogeny reconstruction
MEGA version 5.10 was used to reconstruct Maximum Likelihood (ML) tree [14]. PAUP* version 4.0b.10 was used to build Maximum Parsimony (MP) tree [15]. The robustness of a particular clade in all the tree-building methods was assessed by using the bootstrap test with 1000 replications.

3. Results

3.1. DNA sequence
Sequences of 28 species of Xyleutes and its allied genera and two species outgroup (E. acontucha and E. affinis) were aligned (579-bp). The base no. 96 ATTATAATTTTT TT 109 and no. 411 ATTATAATACG 421 were the most conserved, there was no any substitution. GenBank accession numbers for all species were presented in table 2.

| No  | Species                  | Voucher Specimens | GenBank Accession Number |
|-----|--------------------------|-------------------|--------------------------|
| 1   | Xyleutes strix           | MZB.Lepi. 144     | AB983482                 |
| 2   | X. persona              | MZB.Lepi. 145     | AB983483                 |
| 3   | Duomitus ceramic         | MZB.Lepi. 146     | AB983484                 |
| 4   | Hermophyllon anceps     | MZB.Lepi. 147     | AB983485                 |
| 5   | Chalcidica mineus mineus| MZB.Lepi. 150     | AB983490                 |
| 6   | C. mineus hyphinoe      | MZB.Lepi. 151     | AB983491                 |
| 7   | Rapdalus pardicolor     | MZB.Lepi. 152     | AB983492                 |
| 8   | Panau quarlesi          | MZB.Lepi. 158     | AB983493                 |
| 9   | Bergaris malayica       | MZB.Lepi. 160     | AB983489                 |
| 10  | Trismelasmos maculatus  | MZB.Lepi. 148     | AB983486                 |
| 11  | T. Donovan              | MZB.Lepi. 157     | AB983487                 |
| 12  | T. distyograpta         | MZB.Lepi. 159     | AB983488                 |
| 13  | T. tectorius            | ANICM421-10       | HQ952073                 |
| 14  | Trismelasmos sp         | ANIC M390-10      | HQ952045                 |
| 15  | Skeletophyllon tempestua| ANIC M418-10      | HQ952070                 |
| 16  | Zeuzera coffeae         | MZB.Lepi. 121     | AB935215                 |
| 17  | Z. conferta             | MZB.Lepi. 122     | AB935216                 |
| 18  | Z. indica1              | MZB.Lepi. 123     | AB935217                 |
| 19  | Z. borneana             | MZB.Lepi. 124     | AB935218                 |
| 20  | Z. lineata              | MZB.Lepi. 125     | AB935219                 |
| 21  | Z. caudata              | MZB.Lepi. 126     | AB935220                 |
| 22  | Z. indica 2             | MZB.Lepi. 128     | AB935221                 |
| 23  | Z. aeglopsila           | ANICM448-10       | HQ952094                 |
| 24  | Z. pyrina               | GWORE2017-09      | HM393503                 |
| 25  | Z. quieta               | ANICM452-10       | HQ952098                 |
| 26  | Morpheis xylotribus     | ANICM448-10       | JQ 551745                |
| 27  | M. pyracmon             | BLPCCH 730-08     | JQ 564029                |
| 28  | M. ramose               | BLPAC796-07       | JQ571058                 |
| 29  | E. acontucha            | AYT-04-0833-06    | LTOL866-08               |
| 30  | E affinis               | RMNH.INS.13731    | LEPKA214-08              |
Table 3 shows the contribution of each codon position of the CO I gene to the phylogenetic information. The highest phylogenetic informative site was found in the third codon position (25.56%), while the lowest was found in the second codon position (6.7%). The total number of phylogenetic informative site of CO I gene was high (43.86%).

| Table 3. The contribution of each codon position to the phylogenetic information |
|----------------------------------|--------|--------|--------|--------|
|                                  | 1st codon | 2nd codon | 3rd codon | Total  |
| Constant (%)                    | 113 (19.5) | 140 (24.17) | 23 (3.97) | 276 (47.83) |
| Uninformative (%)               | 13 (2.2)   | 8 (1.3)    | 26 (4.49) | 47 (8.1)    |
| Informative (%)                 | 67 (11.57) | 39 (6.7)   | 148 (25.56) | 254 (43.86) |

Table 4 shows the proportion of each base on each codon position and its bias that was estimated based on bias calculation [16]. The results show that CO I was low A+T biased (C: 0.00016) with the A+T content was 69.9%.

| Table 4. Proportion of each base on each codon and its bias |
|----------------------------------------------------------|
|                                  | 1st codon | 2nd codon | 3rd codon | Mean    |
| A                                | 0.3366    | 0.1480    | 0.4828    | 0.30428 |
| C                                | 0.1622    | 0.2664    | 0.0621    | 0.16364 |
| G                                | 0.2446    | 0.1548    | 0.0099    | 0.13650 |
| T                                | 0.2564    | 0.4306    | 0.4996    | 0.39557 |
| A + T Bias                       |           |           |           | 0.00016 |

There was very low interspecific variation in the base composition in CO I for the total nucleotides and there was no significant difference in the frequency of bases between taxa based on the chi-square test (Chi-square = 19.969755 (df=81), P = 0.99999666).

3.2. Maximum parsimony (MP)
Among all the set data, which have been evaluated by using the MP tree building method, no consensus tree resulted in robust topology (table 5).
Therefore, only the most reliable strict consensus of the 4 MP trees resulted from weighting character 2:1 (transversion: transition) is presented in figure 2. Almost all the sections recognized by Schoorl [6] are rediscovered (Section 2 part 2, section 2a part 1 and section 2a part 2). However, the relationship among those sections is unresolved. In the section 2a part 1, genus Morpheis is split from the rest members of section 2a part 1 (Xyleutes, Rapdalus, Panau, Hermophyllon, and Chalcidica). The result also showed that genus Zeuzera (part members of section 2a part 2) is divided into two distinctive clades with Z. borneana is excluded from them.

| Table 5. Results of maximum parsimonious trees based on different data set |
|------------------------|---------------|--------------|-------------|---------------|--------|
| Data set               | Parsimonious | MP Tree     | Tree Length | CI            | RI     | Robustness |
| All codon              | 254           | 84           | 1117        | 0.3742        | 0.5196 | Worst      |
| 1st position only      | 67            | 3            | 225         | 0.4222        | 0.6524 | Worst      |
| 2nd position only      | 39            | 10200        | 66          | 0.8030        | 0.9078 | Worst      |
| 3rd position only      | 148           | 15           | 798         | 0.3388        | 0.4383 | Worst      |
| 1st + 3rd position     | 215           | 30           | 1041        | 0.3506        | 0.4855 | Worst      |
| 3rd + 2nd position     | 187           | 21           | 876         | 0.3687        | 0.4884 | Worst      |
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| 1st + 2nd position | 106 | 15 | 298 | 0.4966 | 0.7087 | Worst |
| TI:TV = 1:2       | 255 | 4  | 1677| 0.3566 | 0.5343 | Moderate |
| TI:TV = 1:3       | 253 | 6  | 2160| 0.3602 | 0.5484 | Low |

Figure 2. A strict consensus tree of the four MP trees based on weighting 2:1 (Transversion: Transition) (Bootstrap support are shown at the nodes).

3.3. Maximum likelihood (ML)
The phylogenetic relationship was reconstructed by using the Maximum Likelihood tree building method based on the Hasegawa-Kishino-Yano model [17]. The best tree with the highest log likelihood value (-5550.1514) is presented in figure 3. The analysis involved 28 nucleotide sequence ingroups and 2 nucleotide sequence outgroups. All the data set were included in the analysis. There were a total of 579 bp in the final dataset. The topology resulting from ML tree was shown to be almost similar to those found in a strict consensus of 4 MP trees. The slight differences were shown in the related species within the genus *Xyleutes* and *Morpheis*.

4. Discussion
The results showed that sequence of CO I genes from 28 species of *Xyleutes* and its allied genera was a high A+T content. It is not surprisingly since this phenomenon also has occurred in other Lepidoptera as previously reported by many authors. The topology resulted in a strict consensus of the four MP trees.
based on weighting 2:1 (Transversion: Transition) and ML tree based on all transition and transversion in this study recovered almost all sections within Zeuzerinae that those found in the previous study (Section 2 part 2, Section 2a part 1 and Section 2a part 2) [6]. The position of section 2 part 2 agreed with the previous study except for genus Bergaris. It was possibly due to Bergaris being represented by a single taxon only, thus making the position uncertain in this study. Involving four other species of Bergaris is necessary to get the most reliable relationship with other genera in a future study. Moreover, the relationships among the three closely related genera within this section 2 part 2: Trismelasmos, Duomitus, and Skeletophyllon were well resolved except for the position of species T. distyograpta (the identity of this species is still need to be re-examined). Duomitus branched off first and then was followed by Skeletophyllon and Trismelasmos. In the previous study, the relationships among those three genera were unresolved (figure 2) [6].

![Figure 3. Maximum Likelihood tree based on all data set (Bootstrap value are shown at the nodes).](image)

The results of our study also agreed with the previous study based on external morphology that within section 2a part 1, genus Morpheis branched off first and was then followed by the rest members. However, the relationships among genera within this section disagreed with the previous study [6]. Possibly this was caused by some genera such as Alophonotus and Rugigegat no being
represented in the analysis due to no available materials for this study. The other reason is that some genera were represented by a single taxon (Panau and Hermophyllum) in the analysis.

The findings of this study showed that genera Chalcidica, Trimelasmos, Rapdalus, Hermophyllum, Bergaris, and Duomitus were split from Xyleutes and each of them shown to be a good monophyletic group (at least based on ML tree). These findings reject the treatment of some Indonesian species under genus Xyleutes by Roepke [1, 3] and supported the school's treatment (table 1).

The other most significant finding from this study was in the position of section 2a part 2, in which Z. borneana was excluded from the two distinctive clades of Zeuzera and positioned at the basal clade. Moreover, the relationship among species within the clade (indica + (aeglopsila + caudata)) consistently had high bootstrap supports at any tree-building methods (> 91%). On the other hand, the relationship species within clade (pyrina + (queita + coffeae) + (conferta + lineata)) had less bootstrap support. Holloway [18] also stated that this genus consists of distinctive species with transverse black striate on pale grey to whitish ground. Indeed, a comprehensive revision of this genus is necessary to be conducted by including more representative taxa to justify do they are enough to be split into several taxa whether at a subgeneric or a generic level based on comprehensive data.

This study showed that this gene was able to resolve the relationships of a certain closely-related species such as clade Trimelasmos, Chalcidica, Morpheis, and Xyleutes. Previous studies showed that this gen was very useful when combined with Cytochrome b or CO II to elucidate the relationships within genus Morpho and within genus Papilio [19, 20]. Moreover, combination between CO I and EF-1a was useful to elucidate the phylogenetic relationships of Ectoedemia s. str. (Lepidoptera: Nepticulidae) [21].

The low bootstrap value on any tree-building methods is possibly due to there are many conflicts among data sets and also a lack of sampling materials (ideally all species of each genus included in the analysis). By increasing the number of sample species/genera in the analysis and including the data sets of conserved gene that having a slow evolutionary rate will result a better resolution [22, 23].

5. Conclusion

The phylogenetic analysis of 28 species from 11 genera based on mitochondrial CO I gene was able to recover almost all sections in the previous study even though the relationships among those sections was not well resolved. The CO I was also able to resolve the relationship among close-related species within some genera of Xyleutes and its allied genera. Moreover, the COI was able also to show that Morpheis, Chalcidica, Xyleutes, Trimelasmos, and Rapdalus were good monophyletic groups. This study is still premature to conclude that this result is final hypothesis on the relationship of Xyleutes and allied genera. More other species/genera and other more conserved genes such as wingless and α elongation factor nuclear genes are necessary being inculed in the next study to test the validity of the relationships proposed here.

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