

Research Article

Morphological and Molecular Observation to Confirm the Taxonomic of *Coptocercus biguttatus* (Coleoptera: Cerambycidae) on Cloves in Ambon and Part of Ceram Island

Observasi Morfologi dan Molekuler untuk Konfirmasi Taksonomi Coptocercus biguttatus (Coleoptera: Cerambycidae) pada Tanaman Cengkih di Pulau Ambon dan Sebagian Pulau Seram

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ABSTRACT

This research was conducted to confirm the species of longhorn beetle (*Coptocercus biguttatus*) drilling clove stems in Ambon and part of Ceram Island, Moluccas, which has been noted as an important pest. Aim of this investigation was to characterize the species morphologically, and more detailed with molecular technique via *mtCOI* gene analysis. The longhorn beetle was taken in Ambon and part of Ceram Island, Moluccas and then was established in laboratory with host rearing method on pieces of clove stem. The results showed that *C. biguttatus* attacking clove stems in Ambon and part of Ceram Island, Maluku was closely related and grouped into same cluster with *C. rubripes* and *P. semipunctata* in of New Zealand with 85% homology value. *C. biguttatus* distributed evenly in all clove planting areas in Ambon and part of Ceram Island.

Keywords: Cerambycidae, clove stem borer, *Coptocercus biguttatus*, Mollucas

INTISARI

Penelitian ini dilakukan untuk mengonfirmasi spesies kumbang sungut panjang (*Coptocercus biguttatus*) yang menggerek batang tanaman cengkih di Pulau Ambon dan sebagian dari Pulau Seram, Maluku menggunakan karakter morfologi dan molekuler berbasis gen *mtCOI* untuk sidik kekerabatan. Pengambilan sampel kumbang sungut panjang dilakukan di Pulau Ambon dan sebagian dari Pulau Seram, Maluku dengan cara host rearing pada potongan batang cengkih. Hasil penelitian menunjukkan bahwa *C. biguttatus* di Pulau Ambon dan sebagian dari Pulau Seram, Maluku berkerabat dekat dan berada dalam satu kelompok dengan *C. rubripes* dan *P. semipunctata* di New Zealand dengan nilai homologi 85%. Penyebaran *C. biguttatus* di Pulau Ambon dan sebagian dari Pulau Seram merata di seluruh area pertanaman cengkih.

Kata kunci: Cerambycidae, *Coptocercus biguttatus*, Maluku, penggerek batang cengkih

INTRODUCTION

Longhorn beetle (Coleoptera: Cerambycidae) has been reported as pest on several agricultural commodities (Morillo *et al.*, 2008). Putra *et al.* (2011) stated that family of Cerambycidae was grouped into family causing damage on cacao in Gianyar, Bali. In Central Sulawesi, cacao and coffee were reported to be attacked by pest of Cerambycidae family (Fahri & Sataral, 2015). Larvae of Cerambycidae

damage living tissue, diseased, or died plants, which were generally wooden plants (Amirullah *et al.*, 2014).

Clove belonged into to Family of Myrtaceace and was one of Indonesia original spice crops coming from Maluku Archipelago, in which the most of distributed varieties in several places were Siputih, Sikotok, Zanzibar, and Tunj (Pool *et al.*, 1986). The insect pest attacking clove stem in Ambon Island is believed as stemborer (Boa, 1990), i.e. the longhorn beetle. However, the species of this pest has not been

clearly recognized and determined. This longhorned beetle has been reported attacking the estate crops like cacao (Kalshoven, 1981; Morillo *et al.*, 2008) and coffee (Rhainds *et al.*, 2002), while the prevalence of this insect on clove plants has not been reported yet. According to Kalshoven (1981), stemborers attacking clove plants are determined as *Nothopeus hemipterus*, *N. fasciatipennis*, and *Hexamitodera semivelutina*. Some previous explorations of longhorn beetle found Subfamilies of Prioninae, Lepturinae, Cerambycinae, and Lamiinae in National Park of Halimun Mountain (Makihara *et al.*, 2002); Subfamily of Lamiinae in Bogor Great Garden (Noerdjito, 2010), in Mount of Bromo regions (Makihara *et al.*, 2011), in Lore Lindu National Park (Fahri & Sataral, 2015), and in forest areas of Walat Mountain (Sataral *et al.*, 2015); and Subfamilies of Lamiinae, Cerambycinae, and Prioninae in Mount of Slamet regions (Noerdjito, 2011).

Study on longhorn beetle in Ambon Island has not been previously reported, especially in relation with cloves. This insect attacking cloves in Ambon Island was assumed as exotic species due to the geographical position of Ambon Island which is in eastern side of Wallace line. This research was done to characterize longhorn beetle which was endemic insect on clove in Ambon Island, Maluku. Furthermore, a molecular characterization with mtCO1 gene was done to determine the relationship of this beetle with other longhorned beetle in Ambon Island.

MATERIALS AND METHODS

The research was conducted from March 2016 until February 2017. The sampling of longhorn beetle was conducted in clove planting areas and endemic area of the stemborer at Ambon and part of Ceram Island, Province of Maluku. Morphological identification was done in Sub Laboratory of Pest Invertebrates, while the molecular one was done in Sub Laboratory of Virology, Department of Crop Protection, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta.

Sampling Procedures

The beetle was collected from the field by chopping wood tissue showing the drilling hole at ± 1.5 m from the soil surface. The chopped tissues were then incubated in closed room and covered with gauze clothes to allow the adult of clove stemborers leave the stems. Pupae obtained from field collection were reared to become adults. The collected beetles were

gathered and transferred into absolute ethanol, and then kept in refrigerator at temperature of -20°C for molecular study as well as morphological study.

Morphological Identification

The characters of insect parts such as size, color, pronotum, elytra, and genitalia were used in this study. Observation on genitalia was carried out by dissecting abdominal apex to obtain genitalia using 0.5% of NaCl solution. Whole observation was performed under Microscope of Leica MZ16, and then was documented with Optilab 2.1 application.

Molecular Identification

DNA extraction from insect parts such as head, thorax, and fore leg (Rusterholz *et al.*, 2015) was conducted following the protocol of Goodwin *et al.* (1994). Those body parts were put into 1.5 ml eppendorf tubes, added with 200 μl of CTAB (CTAB 2%, NaCl 1.4 M, Tris-HCl 100 mM, EDTA 20 mM and Polyvinylpyrrolidone (PVP) 1%, 2-mercaptoethanol), and then grinded using micropastel. The extracted solution was homogenized using vortex for 30 second and was incubated at 65°C for 15 min in waterbath (inversely mixing every 5 min). Afterwards, 200 μl of Chloroform-Isoamyl Alcohol/CIAA (24:1) was added and incubated at ambient temperature for 20–30 minutes prior to centrifugation at 8,000 rpm for 5 minutes. The supernatant was transferred into new 1.5 ml eppendorf tubes, and was followed by adding 250 μl of cool absolute ethanol and 40 μl of Natrium Asetat (NaOAc), and then was mixed gently. The mixed supernatant was incubated for 30 min at temperature of -20°C and then was centrifuged at 11,500 rpm for 15 minutes. The supernatant was removed and 200 μl of 70% cool ethanol was added prior to centrifugation at 11,500 rpm for 15 minutes. The supernatant was then removed again and the pellet was air-dried overnight, diluted into 50 μl of TE 1 \times and homogenized by vortex.

DNA was amplified with universal primers of forward LCO1490 (5'-GGTCAACAAATCATAAA GATATTGG-3') and reverse HCO2198 (5'-TAAA CTTCAGGGTGACCAAAAAATCA-3') amplifying the fragment of mtCO1 gene at approximately 710 bp in size (Folmer *et al.*, 1994) using Automated Thermal Cycler PTC-150 (MJ Research, Massachussets). The 25 μl of PCR reaction consisted of 0.5 μl KOD, 2.5 μl of Buffer, 2.5 μl of dNTPs, 1.5 μl of MgSO_4 , 16 μl of Nuclease-free water, 0.5 μl of forward LCO1490 and reverse HCO2198 primers, as well

as 1 µl of DNA template. Amplification was run with pre-denaturation at 94°C for 2 min, 40 cycles of denaturation at 98°C for 10 s, annealing at 55°C for 20 s, extension at 68°C for 30 s, and final extension at 68°C for 5 min. PCR product was visualized using electrophoresis of 1% agarose gel and observed under Bio-Rad UV Transilluminator 2000 after dipped into 0.1% of ethidium bromide solution for 15 min. As much as 2 µl loading dye plus 5 µl of PCR product was electrophorated at 50 Volt for 50 min with 1 kbp DNA ladder.

Phylogenetic Analysis

The samples were sent to the 1st Base DNA Sequencing for DNA sequencing. Sequenced product was aligned with Bioedit and ClustalOmega programs to obtain consensus of base pair, and then matched to the information of DNA sequence provided in genbank. The percentage of homology for species was generated from BLAST which was available in NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The genetic diversity of alignment product was analyzed with constructing phylogenetic tree through MEGA 7 program (Kumar *et al.*, 2016) using method of Maximum-Likelihood, Kimura 2 parameters and 1000 bootstrap. The phylogenetic tree was built by comparing sampled insects to comparator insects within same genus, and same tribe in sub family, as well as inters sub family in family of Cerambycidae. The outgroup from NCBI was added to improve the prediction of phylogenetic tree. Outgroup was selected based on the closeness of relationship with analyzed insects as well as their significant different with other comparator insects (Dharmayanti, 2011).

RESULTS AND DISCUSSIONS

Morphological Identification

Morphological identification confirmed the species as *Coptocercus biguttatus*. However, it had variations in body length. For example, Hiroshi and Noerdjito (2004) found that the body length of *C. biguttatus* reached 19.1 mm. Anterior head was characterized with short smooth hair and smaller than pronotum, while lateral head did not bear such hair (Figure 1c). The site bearing antenna on head was pronouncing. No V or Y pattern was found on frons. Frons shape was downward. Apical maxila palpi and labial palpi broadened (Figure 1d).

Type of antenna was phylliform with 11 segments in which the pedicel of second segment was smaller

than others (Figure 1e). The length of antenna exceeded the body length, with short smooth hair on whole antenna, while the longer hair was appeared in the lateral antenna which damped towards posterior. Spine was seen on 3rd to 7th segments of antenna. The 3rd segment of antenna was longer than scape of 4th and 5th segments (Figure 1a and 1b).

There were five pronouncing tubercles on pronotum in which two tubercles on anterior pronotum were similar big circle; two bigger tubercles on posterior pronotum were circle; and one oval tubercle which was the biggest amongst others (Figure 1e). No spine was arose on lateral pronotum. Pronotum was characterized with very dense and smooth white yellowish hair, excluded tubercle area on central pronotum towards anterior near to head which was distinguished by long disperse hair. The length of male pronotum was 2.5–4.8 mm in range with breadth of 1.9–3.5 mm in range; while the female one was 2.7–4.7 mm in length and 1.9–3.2 mm in breadth. Scutellum was semi-circle with similar characteristic hair on pronotum (Figure 1e).

The dense, shoot, smooth and irregular punctures on metasternum were also found (Figure 1f). Abdomen consisting of 5 segments which was characterized by smooth hair. Apical abdomen on male was indicated with rounded-semi circle apex, while there was additional truncated-like segment (not rounded-semi circle) on female which was distinguished by dense smooth hair (Figure 1g and 1h).

Elytra was brown reddish, while head and pronotum were darker than elytra. However, whole body of this beetle was red brownish. Elytra was almost three times longer than pronotum. The length of male elytra was 5.2–13.1 mm in range, while female was about 5.7–13.4 mm in range. A pair of white spot closed to suture was found on center of elytron in which its size was getting bigger on lateral elytron. Meanwhile such white yellowish spot was also generated on basal of elytron and was getting bigger on lateral elytron. White oval spot was characterized on apical of elytron. A very dense puncture was appeared on elytra in which the size was bigger than metasternum and the position was deeper on basal to center of elytra. However, puncture was getting sparse on center of elytra to epical, the size was smaller and the position was shallower. One hair was appeared on each central puncture. The growing hair on humerus region of puncture was curved and longer than that on other region. Epical elytron was not

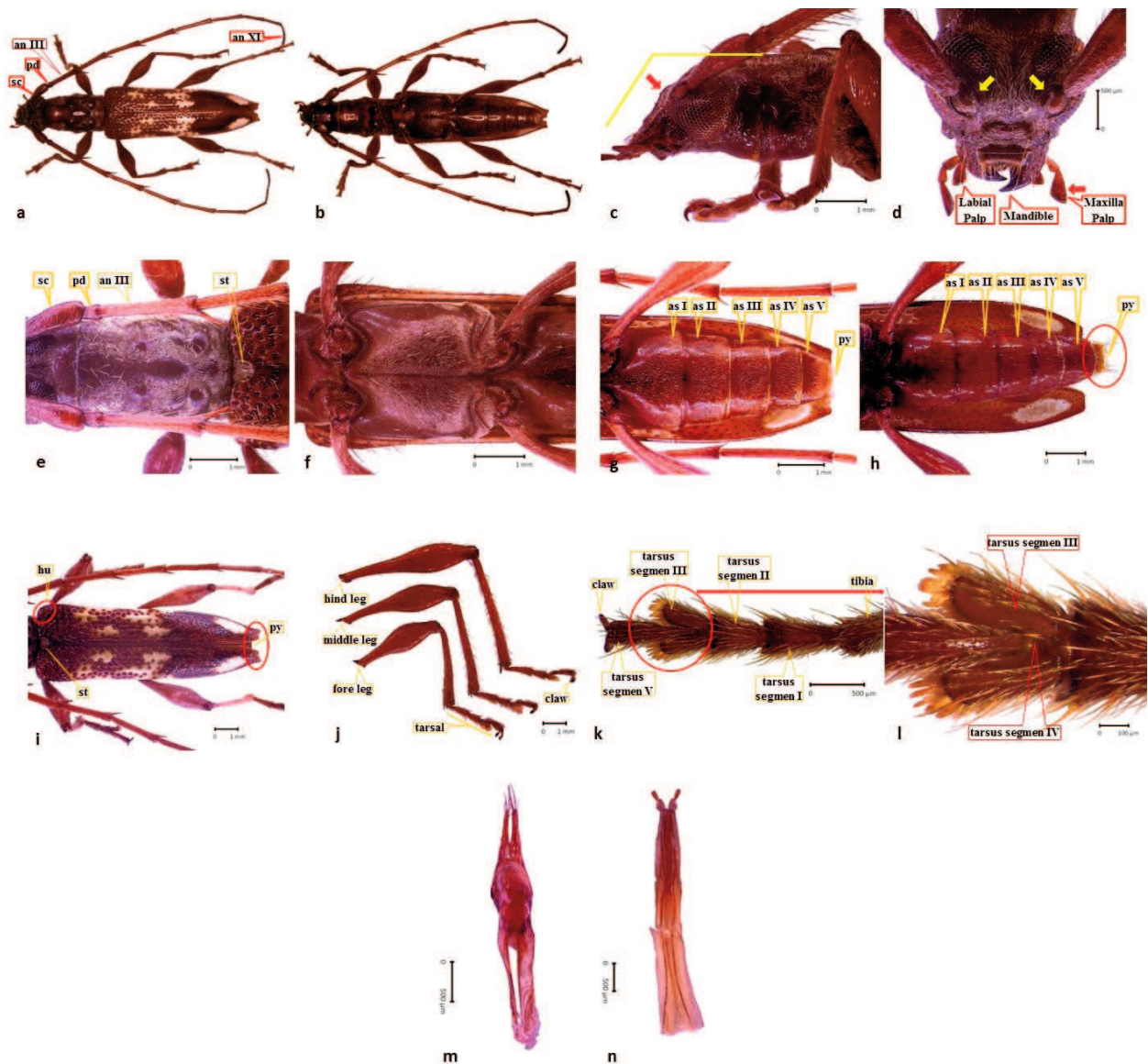


Figure 1. Adult of *Coptocercus biguttatus*; dorsal view (a), ventral view (b), Scape (sc), pedicel (pd), Antennomere III (An III), Antennomere XI (An XI), lateral view (c), anterior view (d), Pronotum and antenna (e), Metasternum (f), scape (sc), pedicel (pd), Antennomere III (An III), scutellum (st), ventral view of male abdomen (g), ventral view of female abdomen (h), abdominal sternite I (as I), abdominal sternite II (as II), abdominal sternite III (as III), abdominal sternite IV (as IV), abdominal sternite V (as V), pygidium (py), Elytra (i), scutellum (st), humerus (hu), pygidium (py), leg (j), Tarsal (k), Tarsus segment IV (l), male genitalia (m), female genitalia (n)

semi-circle, but truncated-like and the generated spine was very small and short (Figure 1i).

The legs were distinguished by sparse hair on femur region, and dense hair on tibial region and tarsus. Two identical long spurs were also found on tibial region. Formula of tarsus was 5-5-5 (Figure 1j), but it seemed to be 4-4-4, due to the 4th segment of tarsus was hidden and the size was also shorter than other segments (Figure 1k and 1l). The 3rd segment was characterized by heavy peg. Wang (1998) elaborated that the important characters for Cerambycidae, Subfamily of Cerambycinae, tribe of Phoracanthini

could be visualized from several parts of their body such as head, antenna, thorax, elytra, and abdomen.

Molecular Identification

Molecular identification of *C. biguttatus* was performed in 4 stages, namely extraction of its DNA total, amplification of mtCOI gene using universal primers of LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'), electrophoresis in order to recognize the quality of PCR product, and sequencing to figure out the arrangement of nucleotide in PCR product. The primers of LCO1490 and

HCO2198 were universal which amplified the fragment of mtCO1 gene at approximately 710 bp in size (Folmer *et al.*, 1994). This was complied with the visualization of PCR product of mtCO1 gene amplification using the above primers (Figure 2). The result of sequencing found nucleotide of approximately 648 bp in size (Figure 4). This was in line with Sharma and Kobayashi (2014) who stated that the use of LCO1490 and HCO2198 primers against various family, genus and species could generate the variation in nucleotide size of amplified mtCO1 gene fragment. The sequencing was conducted to figure out the nucleotide arrangement of PCR product in order to recognize the relationship of sampled insect in built diagram of phylogenetic tree. *C. biguttatus* was not listed yet in NCBI database, so that its relationship was compared to Order, Sub-family, Tribe, and Genus of similar insect from several places in the world which had been recorded in NCBI database.

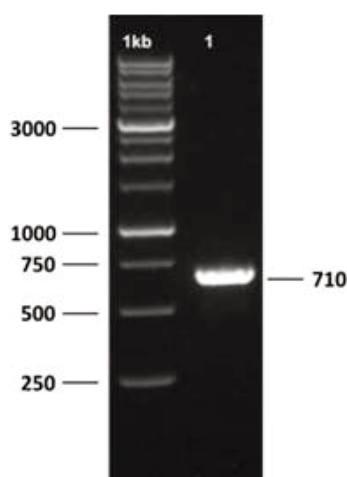


Figure 2. Electrophoresis of PCR product from amplification of mtCO1 gene of *Coptocercus biguttatus* by using primer of LCO1490-HCO2198 with 1kb marker

Sequence analysis was performed using Basic Local Alignment Search Tool (BLAST) program on National Center of Biotechnology Information (NCBI) website. BLAST result indicated that *C. biguttatus* which was found in Ambon and part of Ceram Island, Maluku was identical with *Coptocercus rubripes* (Accession Number in GenBank of KC593312) and *Phoracantha semipunctata* (Accession Number in GenBank of KC593339). That result was then utilized to perform multiple alignment analysis with Bioedit program and analysis of homology with MEGA 7 program to visualize the comparison between samples and comparator. Phylogenetic analysis was demonstrated using MEGA 7 program to build phylogenetic tree. The results of multiple alignment and homology analyses expressed that samples of tested clove stem-borer, *C. biguttatus*, was identical with wood borer beetles from New Zealand i.e. *C. rubripes* and *P. semipunctata*. Sequences of those comparator insects were obtained online from GenBank.

Phylogenetic tree showed that *C. biguttatus* in Ambon and part of Ceram Island, Maluku was closely related and was grouped into same cluster with *C. rubripes* and *P. semipunctata* in New Zealand (Figure 3) with value of homology about 85% (Table 1). *C. biguttatus* was categorized into similar cluster to *C. rubripes* since these insects belonged into genus of *Coptocercus*. Morphological features of both species had some similarities such as length and color of body. However, they were different in some features. For example, *C. rubripes* had two spines on apical elytra; the elytron was characterized with yellow brownish lengthening stripe on the central prior to basal; the lateral pronotum possessed spherical tubercle; spine was also appeared on lateral pronotum; and the body was red blackish so that it was darker than *C. biguttatus* (Sopow *et al.*, 2015). Meanwhile, it was closely related to *P. semipunctata*

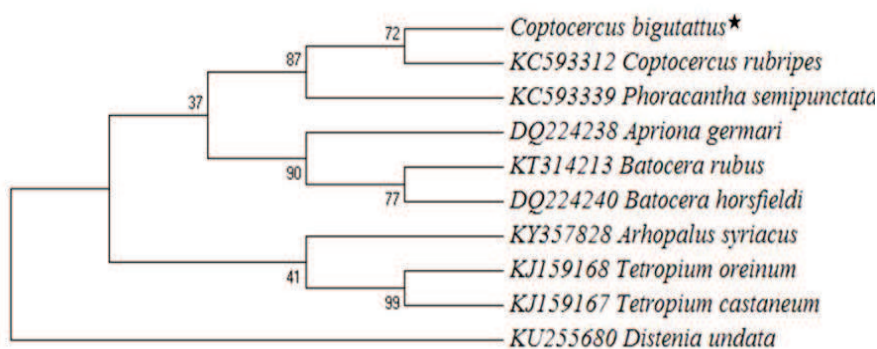


Figure 3. Phylogenetic tree of *Coptocercus biguttatus* (*) from Ambon and part of Ceram Island with comparator species from GenBank database

CLUSTAL O(1.2.4) multiple sekuen alignment

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KU255680_Distenia_undata      CTAGCAACATTTTCATGGAACACAAATTAATTTTAGT-----CCTGCA-ACTTTATGA
KJ159168_Tetropium_oreinum    CCTGATATATCATTTCCTCGTCTAAATAATTTAAGATTTTGATTCTACCCCATCATT
KJ159167_Tetropium_castaneum  CCTGATATATCATTCCCTCGACTAAACAATTTAAGATTTTGTTCTACCTCCGTCATTA
KC593312_Coptocercus_rubripes CCAGATATAGCATTCCCACGAATAAACAATATAAGATTCTGACTACTGCCCCGTCATTA
Coptocercus_biguttatus        CCAGACATGGCATTTCACGAATAAACAATATAAGATTCTGACTACTGCCCCCTCATT
KC593339_Phoracantha_semipunctata CCGATATAGCATTTCACGAATAAATAATATAAGATTTTGACTCTGCCTCCCTCATT
DQ224240_Batocera_horsfieldi  -----AATGAATAATATAAGATTTTGATTATTACCCCATCATT
DQ224238_Apriona_germari      -----AATAAATAATATAAGATTTTGATTGTACCCCATCATT
KT314213_Batocera_rubus      CCTGATATAGCTTTCCCGAATAAATAATATAAGATTTTGATTATTACCCCATCATT
KY357828_Arhopalus_syriacus   CCAGACATAGCTTTCCCGAATAAATAATATAAGATTTTGACTTTTACCCCTTCTCT
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KU255680_Distenia_undata      GCTCTGGGATTTGTATTTT-----TTTACTGTAGGAGGATTGACCGGAGTAGTCTT
KJ159168_Tetropium_oreinum    ATTCTTCTAATTATAGGAATAATTGTTGAAAAAGGAGCAGGAACCTGTTGAACAGTTTAT
KJ159167_Tetropium_castaneum  ATTCTTTTAAATTATAGGAATAATTGTTGAAAAAGGAGCAGGAACCTGTTGAACAGTTTAT
KC593312_Coptocercus_rubripes ACACCTTAAATTATGAGAAGAATTGTAGAAAATGGTGACGGAACCGGATGAACAGCTTAC
Coptocercus_biguttatus        ACACCTCTTATTATAAGAAGAATTGTAGAAAATGGTGACGGAACCGGATGAACAGTTTAT
KC593339_Phoracantha_semipunctata ACTCTCTAATTATAAGAAGAATTGTTGAAAACGGAGCAGGAACAGGATGAACCTGTTTAT
DQ224240_Batocera_horsfieldi  ACTTTATTAAATTATAAGAAGAATCGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTAC
DQ224238_Apriona_germari      ACTTTATTAAATTATAAGAAGAATTGTAGAAAATGGCGCAGGAACCTGATGAACCTGTTTAC
KT314213_Batocera_rubus      ACTTTATTAAATTATAAGTAGAATCGTAGAAAACGGTGACGGAACAGGTTGAACAGCTTAT
KY357828_Arhopalus_syriacus   ACTCTTTTAAATTATAAGAAGAATTGTTGAAAATGGAGCAGGACAGGATGAACAGCTTAC
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                                *  * * * * *

KU255680_Distenia_undata      GCCAATTCATCAATTGATATTATCTTACACGATACTTATTATGTAGTCGCCCATTTTCAT
KJ159168_Tetropium_oreinum    CCCCCCTTTTCAGCAAAATATTGCCCATAGAGGATCCTCAGTAGACTTAACATTTTATAGC
KJ159167_Tetropium_castaneum  CCACCCTTATCAGCAAAATATTGCCCATAGGGGATCATCAGTAGATTAACTATTTTATAGT
KC593312_Coptocercus_rubripes CCCCCATTATCAGCCAACATCGCACATAGAGGATCCTCTGTTGACCTTGAACATTCACAGC
Coptocercus_biguttatus        CCCCCCTATCCGCCAACGTTGCACACAGGGGATCCTCTGTTGATCTAGCCATTTTATAGG
KC593339_Phoracantha_semipunctata CCTCTTTATCTGCTAAATGTGTCACATAGAGGTTTCTCTGTTGATTAGCAATTTTCACT
DQ224240_Batocera_horsfieldi  CCCCCCTTAGCTGCAAAATATTGCCACAGTGGTCTCTGTTGACTTAGCCATTTTATAGC
DQ224238_Apriona_germari      CCCCCCTTAGCTGCTAAATGTAGCCCATAGAGGTTTCTCTGATAGTTTAGCAATTTTATAGT
KT314213_Batocera_rubus      CCCCCACTAGCTGCAAAATATTGCCCATAGCGGTTCTCTGATAGTTTAGCTATTTTATAGA
KY357828_Arhopalus_syriacus   CCTCTCTTTTCTATCTAATATCGCCCATAGAGGAGCTTCTGATAGTTTGGCTATTTTATAGT
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KU255680_Distenia_undata      TACGTTTTATCAATTAGGAGCTGATTTTGCTATTATAGCAGGGATAATTCAATGATTTCCA
KJ159168_Tetropium_oreinum    C-----TACATTTAGCAGGAATTTTCATCAATTTTAAAGTGCTATCAATTTTATTACAACA
KJ159167_Tetropium_castaneum  T-----TACATTTAGCAGGAATTTTCATCCATTTTAAAGTGCTATCAATTTTATTACAACA
KC593312_Coptocercus_rubripes C-----TCCACCTGGCAGGAATTTCTTCGATTCTAGGAGCAGTAAATTTTATTCCACA
Coptocercus_biguttatus        C-----TACATTTAGCAGGAATTTCTCTCAATTTCTAGGAGCAGTAAATTTTATTCCACA
KC593339_Phoracantha_semipunctata C-----TTCACCTCGCCGGAATCTCATCAATTTCTAGGAGCAGTAAATTTTATTCTACT
DQ224240_Batocera_horsfieldi  T-----TACACTTAGCTGGAATTTCTCTTATTTCTAGGCGCAGCTAAATTTTATTACTACT
DQ224238_Apriona_germari      C-----TTCATTTAGCTGGAATTTCTTCAATTTCTGGTGCGAGTAAATTTTATTACTACC
KT314213_Batocera_rubus      C-----TCCATTTAGCAGGAATTTCTATCTATTCTAGGAGCTGTAATTTTATTACCACA
KY357828_Arhopalus_syriacus   T-----TACATTTAGCGGAATTTCTTCAATTTTAGGGGCTGTAATTTTATTACAACA
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                                *  * * * * *

KU255680_Distenia_undata      TTATT-----TACAGGATTAACCTTTAAATAATAATCTTCTTAAAGTTCAATTT
KJ159168_Tetropium_oreinum    ATTATAAATATACGGCTAAAGGTATAACCTTAGATCAAATACCTTT---ATTTGTATGA
KJ159167_Tetropium_castaneum  ATTATAAATATACGACCTAAAGGTATAACCTTAGATCAAATACCGCT---ATTTGTATGA
KC593312_Coptocercus_rubripes GTAATTAATATACGACCAACCGGAATAACTCCTGAACGAATACCTTT---ATTTGTTTGA
Coptocercus_biguttatus        GTAATTAATATGCGACCTGTAGGAATAACCCAGAACGAATAACATT---ATTTGTTTGA
KC593339_Phoracantha_semipunctata GTAATTAATATACGAACAGAAGGAATAACCCAGAACGAATACCTTT---ATTTGTTTGA
DQ224240_Batocera_horsfieldi  GTAATTAATATACGACCTCAGGAATAACTATAGATCGTCTTCCCT---ATTTGTATGA
DQ224238_Apriona_germari      GCTGTAGTTATTA---CAGCAATCTCT-----
KT314213_Batocera_rubus      GCAGTAGTTATTA---CTGCCATTCTTTTACTTTTATCCCTGCCTGTTCTAGCAGGAGCA
KY357828_Arhopalus_syriacus   GCTGTGTTCAAATTA---CTGCAATCTTACTTTTATTAATCACTACAGATTAGCTGGAGGA
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                                *  * * * * *

KU255680_Distenia_undata      GCAAGAATATTTATAGGAGTAAATTTAATCTTCTTCCACAACATTTTATAGGATTAAGA
KJ159168_Tetropium_oreinum    GCAGTAATAATCA---CTACAATTTTATTACTTATCTCTTACCTGTTCTAGCTGGTGCT
KJ159167_Tetropium_castaneum  GCAGTAATAATCA---CCACAATTTTATTACTTATCTCTTACCTGTTCTAGCTGGTGCT
KC593312_Coptocercus_rubripes GCTGTAGTTATCA---CAGCCATTCTATTATTGCTCTCACTCCAGTTCTAGCAGGAGCA
Coptocercus_biguttatus        GCTGTAGTTATTA---CAGCAATCTCT-----
KC593339_Phoracantha_semipunctata GCAGTAGTTATTA---CTGCCATTCTTTTACTTTTATCCCTGCCTGTTCTAGCAGGAGCA
DQ224240_Batocera_horsfieldi  GCTGTGTTCAAATTA---CTGCAATCTTACTTTTATTAATCACTACAGATTAGCTGGAGGA
DQ224238_Apriona_germari      GCCGTAAGTAAGTAA---CAGCTATTCTTTTATTACTTTCTCTTCTGTTTATAGCTGGAGCA
KT314213_Batocera_rubus      GCAGTAAGTAAGTAA---CTGCAATCTTCTTTTACTATCTCTTCCAGTTTATAGCTGGAGGT
KY357828_Arhopalus_syriacus   GCGGTAGTAAGTAA---CTGCTATTTTACTTTTATCTTACTCTTACTGTTTATAGCCGGTGCA
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Figure 4. Multiple Sequence Alignment of Nucleotide on *Coptocercus biguttatus* and comparator species from GenBank database

Table 1. Percentage of homology from nucleotide base of *Coptocercus biguttatus* and comparator species from GenBank database

No. Species	1	2	3	4	5	6	7	8	9	10
1 <i>Coptocercus biguttatus</i>	ID									
2 <i>Coptocercus rubripes</i>	85%	ID								
3 <i>Phoracantha semipunctata</i>	85%	85%	ID							
4 <i>Batocera rubus</i>	81%	78%	82%	ID						
5 <i>Batocera horsfieldi</i>	80%	77%	77%	87%	ID					
6 <i>Apriona germari</i>	78%	79%	81%	86%	85%	ID				
7 <i>Arhopalus syriacus</i>	81%	79%	81%	83%	81%	82%	ID			
8 <i>Tetropium oreinum</i>	77%	76%	78%	80%	79%	80%	81%	ID		
9 <i>Tetropium castaneum</i>	76%	75%	77%	78%	79%	79%	81%	93%	ID	
10 <i>Distenia undata</i>	45%	47%	51%	50%	48%	45%	45%	51%	49%	ID

since these insects were categorized into the same of Tribe of Phoracanthini. Their morphological properties were also similar. Some differences among them were characterized by the elytra of *P. semipunctata* which was dominated by brown with white yellowish stripe on central region to suture. The stripe and a pair of spines were found on apical elytra, and spine was also generated on lateral pronotum (Wang, 1998; Sopow *et al.*, 2015). *C. biguttatus*, *C. rubripes*, and *P. semipunctata* were classified into same Subfamily of Cerambycinae, the larger Subfamily in Family of Cerambycidae. In addition, the tested insect was also compared to insects of other Subfamilies such as Lamiinae and Spondylidinae, in order to more completely figured out the relationship with *C. biguttatus*. Comparator insects of Lamiinae Subfamily were *Apriona germari*, *Batocera rubus*, and *Batocera horsfieldi* from Tribe of Batocerini; while those of Spondylidinae Subfamily were *Arhopalus syriacus*, *Tetropium oreinum*, and *Tetropium castaneum*, which were belonged into Tribe of Asemini. The outgroup was added to achieve more trustable information of sequence. Dharmayanti (2011) suggested that outgroup should have related sequences with samples and comparator with a few distinguisher. The selected outgroup was *Distenia undata* of Family Disteniidae, Subfamily Disteniinae, and Tribe Disteniini. This outgroup was chosen as it was also grouped into same Superfamily with sampled and comparator insects, i.e. Cerambycoidea.

Distribution of *C. biguttatus*

This observation showed that invasion of *C. biguttatus* on clove plant in Ambon and part of Ceram Island was not affected by altitude as shown by fact that *C. biguttatus* could survive in host rearing system at low places (15 m asl) as well as at high places (864 m asl) (Table 2). This observation also confirmed that *C.*

biguttatus was also found on Ambon and part of Ceram Islands, which was differed with previous report by Gressitt (1951); Wang *et al.* (1996); Brockerhoff & Bain (2000) who mentioned that this species was only distributed in Papua New Guinea, Australia, and New Zealand. In addition, other species of *Coptocercus* such as *C. sumatranus* was found in Java and Sumatra; *C. quatuordecimsignatus* is found in the Philippines; and *C. q. celebensis* is found on the island of Sulawesi (Miroshnikov, 2016).

C. biguttatus was obtained from all host rearing of clove stem. This fact indicated that *C. biguttatus* was distributed evenly through all clove planting areas in Ambon and part of Ceram Island (Figure 5). All collected samples found on main stem of clove plants were belonged into Family of Myrtaceae. This was complied with Wang (1998) who stated that longhorn beetle especially from Tribe of Phoracanthini was associated with Families of Myrtaceae, Leguminosae and Rutaceae, some conifers, genera Eucalyptus, Acacia and citrus. Dry and wet seasons in Ambon and part of Ceram Island did not influence the invasion of *C. biguttatus* on clove plant. Investigation by Dhahri *et al.* (2016) reported that infestation of Phoracanthini Tribe (*P. semipunctata* and *P. recurva*) on eucalyptus plant was not affected by season. Such condition was caused by the biological feature of stemborer larvae which was active within clove stem, so that it was not directly influenced by season. In their three reports, Hanks *et al.* (1991, 1993, and 1999) found that stemborers lived in plant stem by penetrating the bark, destroying cambium and phloem tissues for their survival, since the tissues contained high nutrient. Larvae could stay in plant stem with diameter of 5 cm. So, it is important for doing further investigation to figure out the content of clove plants that supports the survival of *C. biguttatus*.

Table 2. Distribution areas of *Coptocercus biguttatus* in Ambon and part of Ceram Island, Maluku

Regency	District	Village	Latitude		Altitude (m asl)
Western Seram	Kairatu	Kairatu	128,40284	-3,39683	44
		Seruawan	128,44175	-3,42741	111
		Kamarian	128,46828	-3,42839	406
		Tihulale	128,49502	-3,43591	125
		Rumahkay	128,53731	-3,40275	864
Central Maluku	Salahutu	Liang	128,33806	-3,50514	30
		Waai	128,30879	-3,54769	155
		Tulehu	128,30927	-3,57576	35
		Suli	128,30625	-3,60143	62
	West Leihitu	Hatu	128,05694	-3,68997	167
		Liliboi	128,03759	-3,69836	539
		Allang	128,01095	-3,73556	98
		Wakasihi	127,98102	-3,74108	421
	Leihitu	Seith	128,03487	-3,61492	266
		Hila	128,10605	-3,58685	15
		Mamala	128,21407	-3,54055	87
		Morella	128,22626	-3,5194	110
Kota Ambon	Teluk Ambon Baguala	Passo	128,25577	-3,59784	255
		Lateri	128,24606	-3,64102	44
	Sirimau	Ema	128,24585	-3,70866	540
	Nusaniwe	Amahusu	128,14882	-3,74103	427

Figure 5. Distribution map of *Coptocercus biguttatus* on cloves planting areas in Ambon and part of Ceram Island, Maluku

CONCLUSION

Both morphological and molecular identification are confirmed that *C. biguttatus* in Ambon and part of Ceram Island, Maluku was closely related and belonged into the same cluster with *C. rubripes* and *P. semipunctata* in New Zealand.

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