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KOMUNIKASI SINGKAT

MtCOI DNA sequences from *Sycanus aurantiacus* (Hemiptera: Heteroptera: Reduviidae) provide evidence of a possible new harpactorine species from Bali, Indonesia

Sikuen DNA mtCOI dari *Sycanus aurantiacus* (Hemiptera: Heteroptera: Reduviidae) sebagai bukti kemungkinan spesies harpactorine baru dari Bali, Indonesia

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ABSTRACT

Sycanus aurantiacus Ishikawa & Okajima, found in Bali, was first described in 2007 as a new harpactorine species based on morphological and biological characteristics; however, its genome has not yet been sequenced. In this study, we examine the mitochondrial cytochrome oxidase subunit I (MtCOI) nucleotide sequence of *S. aurantiacus* in order to determine whether it represents a new harpactorine species. A sample from Pancasari, Bali, Indonesia was collected at the same location *S. aurantiacus* was first discovered in 2007. The selected mtCOI gene (650 bp) was successfully amplified using mtCOI primer pairs LCO1490 and HCO2198, and the resulting MtCOI sequence of the *S. aurantiacus* sample was compared with those from other hapactorine species recorded in GenBank. This comparison revealed low genetic similarity between *S. aurantiacus* and most other harpactorine species worldwide, except for the Genus *Sycanus* (JQ888697) from USA whose mtCOI shares approximately 91% similarity with the Pancasari sample. Phylogenetic analysis indicated a close genetic relationship between *Sycanus* from Bali and the Genus *Sycanus* (JQ888697) from the USA. The mtCOI sequence of *S. aurantiacus* had not been recorded previously, and our comparison with existing Sycanus sequences provides support to the understanding that *S. aurantiacus* is indeed its own species.

Key words: mitochondrial cytochrome oxidase I, nucleotide sequence, phylogenetic analysis, predator

ABSTRAK

Sycanus aurantiacus Ishikawa & Okajima pertama kali dilaporkankan pada tahun 2007 sebagai spesies harpactorine baru dari Bali, berdasarkan karakteristik morfologis dan biologis, namun informasi molekuler belum pernah dilaporkan. Tujuan penelitian ini adalah untuk mengetahui sikuen nukleotida mtCOI *S. aurantiacus* (Hemiptera: Heteroptera: Reduviidae) yang merupakan spesies harpactorine baru dari Bali, Indonesia. Sampel dari Pancasari, Bali, Indonesia dikumpulkan dari lokasi yang sama

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ketika *S. aurantiacus* ditemukan pertama kali pada tahun 2007. Gen mtCOI dengan panjang 650 pb berhasil diamplifikasi menggunakan pasangan primer mtCOI LCO1490 dan HCO2198. Urutan nukleotida dari gen mtCOI *S. aurantiacus* dari Bali dibandingkan dengan sekuens lain di GenBank, data menunjukkan *S. aurantiacus* dari Bali memiliki kemiripan yang rendah dengan spesies harpactorine lain di seluruh dunia, kecuali dengan Genus *Sycanus* (JQ888697) dari Amerika Serikat, kesamaan sekitar 91%. Analisis filogenetik menunjukkan bahwa *Sycanus* dari Bali memiliki hubungan genetik yang erat dengan Genus *Sycanus* (JQ888697) dari Amerika Serikat. Namun, urutan mtCOI dari spesies *S. aurantiacus* belum pernah dicatat sebelumnya.

Kata kunci: analisis finogenetik, mitochondrial cytochrome oxidase I, sikuen nukleotida, predator

INTRODUCTION

Members of the insect Subfamily Harpactorinae ("assassin bugs") are natural pest enemies and play a very important ecological role as predators to a large number of other insect genera and species (Putshkov & Putshkov 1985; Maldonado-Capriles 1990). The assassin bug therefore has been utilized for biological pest control in commercial agriculture, such as the *Sycanus dichotomus* Stål which was known to be a predator of the bagworm in oil palm trees (Zulkefli et al. 2004). A new species *Sycanus aurantiacus* Ishikawa & Okajima was discovered in 2004 by researchers conducting a project on "Development of New Bio-Agents for Alternative Farming Systems" conducted by the Tokyo University of Agriculture in collaboration with Udayana University. The new harpactorine species from Bali was first formally described in a publication in 2007, based primarily on morphological characteristics (Ishikawa et al. 2007). *S. aurantiacus* was observed in cabbage plantations across the Bedugul region, including Pancasari (which lies more than 1,000 m above sea level). The Bali assassin bugs were determined to be an important predator of key cabbage pests including lepidopterans and other arthropods. Yuliadhi et al. (2015) continued the biological study of *S. aurantiacus*, assessing whether it would feed on the larvae of *Tenebrio molitor* L. (mealworms commonly used for lab rearing of carnivorous insects). Assassin bug *S. aurantiacus* was successfully reared using the *T. molitor* larvae, which indicates a likelihood that *S. aurantiacus* would be easy to mass produce, since *Tenebrio* larvae are easily procured in bird markets in Bali where they are sold as bird food supplements.

The potential use of *S. aurantiacus* for biological pest control requires further research including documentation of unique molecular identifiers. However, molecular information including mitochondrial sequences have not been obtained for *S. aurantiacus*. To pinpoint these identifying molecular sequences, we used DNA barcoding methods for insect mtCOI (Barrett & Hebert 2005; Wilson 2010; Hashemi et al. 2017). DNA barcoding is useful not only for insect genomes but for other animals including birds (Hebert et al. 2004; Hwang et al. 2018), and fishes (Mabragana et al. 2011). The general DNA primers for insect identification include the COI mitochondria primer pairs of LCO1490 and HCO2198 (Folmer et al. 1994). This study was conducted in order to document the mtCOI nucleotide sequence of *S. aurantiacus*, a newly described harpactorine species from Bali, Indonesia.

MATERIALS AND METHOD

Sample collection

Samples were collected from the Bedugul area of Bali, Indonesia, from the same location where *S. aurantiacus* was first observed in 2007. Bedugul is located at an altitude of 1,200 m above sea level, and experiences average relative humidity of greater than 80% and average annual temperatures of 18,7 °C (Figure 1 and 2).

Extraction of total DNA

Complete nuclear DNA was extracted from a single insect using a modified CTAB method (Doyle & Doyle 1987). A preserved single insect was frozen with liquid nitrogen and finely ground using mortar and pestle, then transferred to a microtube (2.1 ml). Five hundred microliters of buffer extracting solution (EDTA-20 mM, Tris-HCl, pH 8-100 mM, NaCl-1,4 M, CTAB-2%,

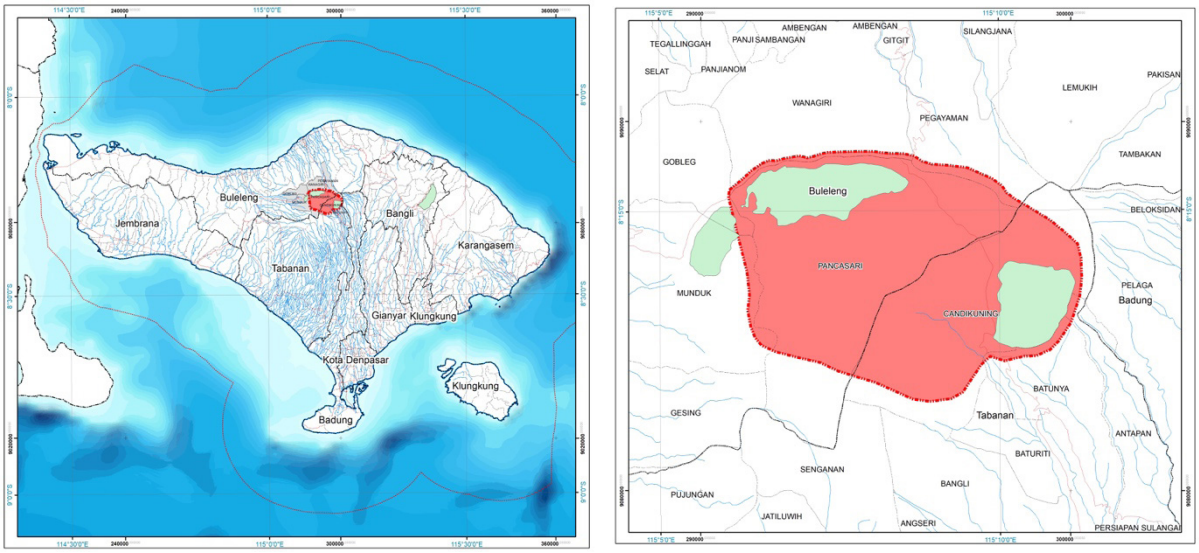

Figure 1. The area of *Sycanus aurantiacus* sampling on Bali island, Indonesia (Bedugul region in red).

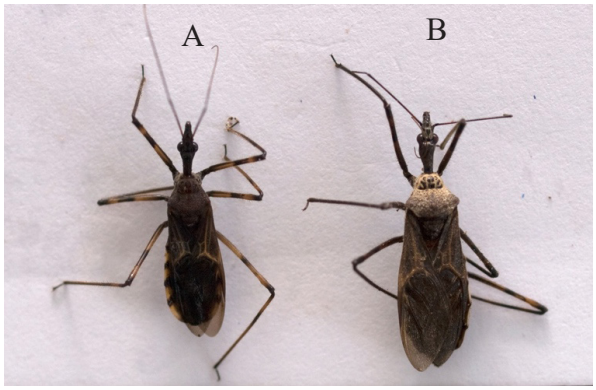

Figure 2. Imago of *Sycanus aurantiacus.* A: male and B: female.

and Mercaptoethanol-0.2%) was added to the microtube and the tube was held in incubation at 65 °C for 60 minutes with occasional mixing by gently inverting the tube. After incubation, an equal volume of phenol:chloroform:isoamyl alcohol (ratio 25:24:1) was added and the tube was inverted several times to mix the liquids before subjecting it to centrifugation at 12,000 rpm for 15 minutes. The resulting upper phase was transferred to a new microtube to which was added sodium acetate (1/10x volume) and cold isopropanol (2/3x volume) mixture. This was incubated overnight at -20 °C to precipitate the DNA. After incubation, the tube was centrifuged at 12,000 rpm for 10 minutes and the supernatant was removed. The pellet containing total DNA was washed with 70% ethanol and centrifuged at 8,000 rpm for 5 minutes. Pellet DNA was air-dried and resuspended in TE buffer solution (1x) and stored at -80 °C for further use.

Polymerase chain reaction (PCR)

Amplification was conducted using back-to-back primers LCO1490 (5'-GGTCAACA AATCATAAAGATATTGG-3') and HC02198 (5'-TAAACTTCAGGGTGACC AAAAAATCA-3') targeting an MtCOI fragment of ±650 bp (Folmer et al. 1994). The DNA was amplified in a GeneAmp PCR system 9,700 machine follows: 5 min at 94 ºC for pre-heating, followed by 30 cycles of denaturation (60s at 94 ºC), annealing (35s at 52 ºC), and extension (90s at 72 ºC), with final extension of 7 min at 72 ºC. Amplicons were then visualized on 1% agarose gel using electrophoresis in TBE 0,5x buffer.

Nucleotides sequence and phylogenetic analysis

The PCR products were sent for sequencing to 1st BASE Laboratories (Malaysia). Sequence contigs were assembled using CLC sequence viewer 7,5, then aligned with sequence isolates from GenBank using Bioedit 7.2.5 to analyze the sequence homologies.

Phylogenetic analysis of *S. aurantiacus* was conducted by comparing the sample sequences to others sequences found in GenBank. A phylogenetic tree of *S. aurantiacus* was constructed using the programs ClustalX, Bio Edit 7.2.5, and MEGA 6.12.

RESULTS AND DISCUSSION

Sequence similarity and phylogeny

The purified complete DNA/genome was used as a template for PCR amplification. The 650 bp mtCOI gene from the collected genome was successfully amplified using paired primers LCO1490 and HC02198, and the PCR product was sequenced for analysis. The resulting mtCOI nucleotide sequence of was compared against other mtCOI nucleotide sequences in the GenBank database, with results indicating high similarity (91%) between mtCOI from *S. aurantiacus* (LC490205_Sycanus_aurantiacus_Bali2 and LC490206_Sycanus_aurantiacus_Bali3) and that of *Sycanus* sp. from USA (JQ888697_Sycanus_sp_USA), and low similarity with sequences of other Reduviidae in its database.

Phylogenetic analysis showed that *S. aurantiacus* (LC490205_Sycanus_aurantiacus_Bali2 and LC490206_Sycanus_aurantiacus_Bali3) branches out from the same node as—and shares an ancestor with--- *Sycanus* sp. from USA (JQ888697_Sycanus_sp_USA). *S. aurantiacus* can be grouped with *Gminatus*, *Agriosphodrus*, *Margasus*, and *Sinea*, with *Castolus* representing an outgroup (Figure 3). Based on similarity of MtCOI sequences, and the resulting derived phylogeny, these genera belong to Family Reduviidae and Subfamily Harpactorinae. While they all share the same family and subfamily, *S. aurantiacus* has greater similarity and a close genetic relationship with *Sycanus* sp. from USA. The data from USA JQ888697 was recorded as belonging to the Sycanus genus, however a species name was not completely reported (Table 1). The

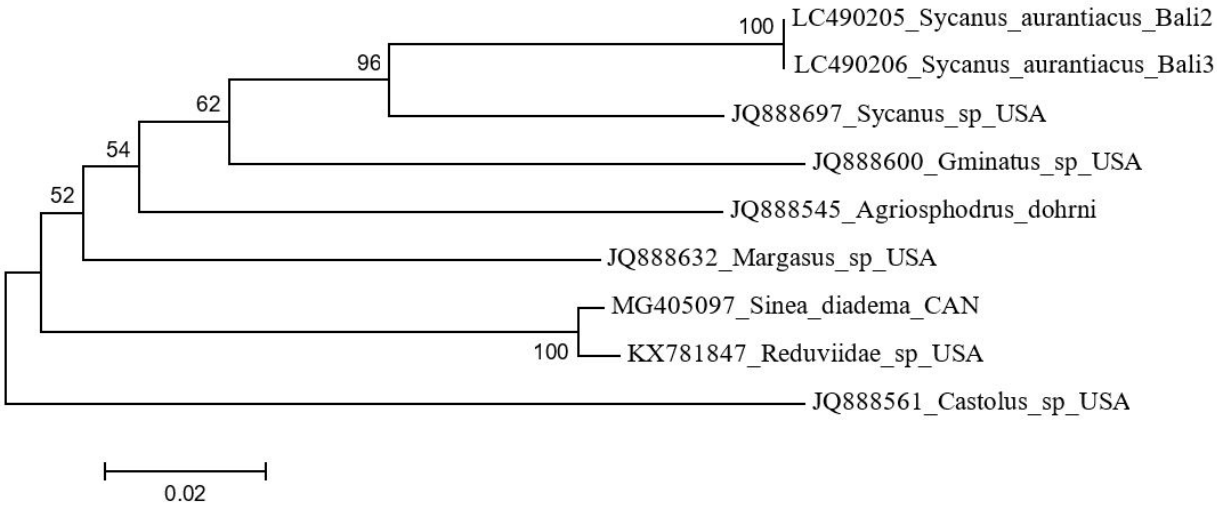

Figure 3. Phylogenetic tree derived from mtCOI sequences for *Sycanus aurantiacus* using Mega 6.06 (Algorithm Neighbor Joining with 1,000 bootstraps replicates).

Table 1. Identity matrix of *Sycanus aurantiacus*

Sequnce	1	2	3	4	5	6	7	8	9
1. LC490205_Sycanus_aurantiacus_Bali2	ID	100%	91%	83%	85%	86%	86%	86%	86%
2. LC490206_Sycanus_aurantiacus_Bali3	100%	ID	91%	83%	85%	86%	86%	86%	86%
3. JQ888697_Sycanus_sp_USA	91%	91%	ID	83%	86%	87%	85%	86%	85%
4. JQ888561_Castolus_sp_USA	83%	83%	83%	ID	84%	82%	85%	82%	84%
5. JQ888632_Margasus_sp_USA	85%	85%	86%	84%	ID	87%	86%	85%	86%
6. JQ888600_Gminatus_sp_USA	86%	86%	87%	82%	87%	ID	84%	84%	85%
7.MG405097_Sinea_diadema_CAN	86%	86%	85%	85%	86%	84%	ID	85%	99%
8. JQ888545_Agriosphodrus_dohrni_USA	86%	86%	86%	82%	85%	84%	85%	ID	85%
9. KX781847_Reduviidae_sp_USA	86%	86%	85%	84%	86%	85%	99%	85%	ID

data indicated the mtCOI sequence of harpactorine species from Bali (*S. aurantiacus*) is first recorded in GeneBank.

S. aurantiacus was found (in 2019) in a farmer's plastic house in the Bedugul region of Bali, in an area of especially abundant moss in Pancasari Village. The observation differed somewhat that described previously by Ishikawa et al. (2007) and Yuliadhi et al. (2015) who reported that the species was an important predator of some key lepidopteran cabbage pests. However, now the species was being found in abundance in farmer's plastic house containing various crops. Many arthropods can be found in plastic walls of farmer's plastic house commonly used in Indonesia are highly suitable for pest predator species. The greenhouse habitat and density of arthropod pests as prey are conducive to natural pest enemies (Koprivnikar et al. 2017). The plastic house made by farmers in Bedugul does not completely prevent insects from entering the plastic house. This is because the plastic house is made in a traditional way, the size of the mesh holes is too large, the walls are rarely cleaned, etc. The environmental conditions make insects comfortable living in the plastic house like their natural habitat. Those phenomena indicated the plastic house in Bedugul is still not fully modern, and plastic house ecosystems is semi-complex. Suitable environmental conditions are of key importance to attract pest predators. Many agroecosystems are unfavorable environments for natural enemies due to high levels of disturbance; therefore habitat management is necessary to maintain the populations of natural enemies (Landis et al. 2000). In addition, the Bedugul area is a center of horticulture in Bali, located at a high elevation 1,000–1,500 m above sea level. The average annual relative humidity exceeds 80%. with average annual temperatures of 18,7–22,5 °C and annual average rainfall of 2,315–2,392 mm (Statistics Indonesia 2017). These climatic factors may play an important role in supporting *S. aurantiacus* abundance in the area. Abiotic factors may directly influence community structure by influencing biotic interactions; and abiotic factors can affect the predator-prey relationship (Anderson et al. 2001).

CONCLUSIONS

The mtCOI gene of *S. aurantiacus* from Bali Indonesia was sequenced for the first time, and discovered to share a high level of similarity and close genetic relationship with *Sycanus* sp. from USA.

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