GENETIC ANALYSIS AND MOLECULAR PHYLOGENY OF ZIGZAG LEAFHOPPER *MAIESTAS DORSALIS* (MOTSCHULSKY) USING MITOCHONDRIAL COI GENE

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ABSTRACT: The zigzag leafhopper *Maiestas dorsalis* (Hemiptera) is a tungro virus vector that cause damage on rice plants. Genetic analysis of *M. dorsalis* that be isolated from Samosir island, North Sumatra, Indonesia using partial DNA sequence of mitochondrial cytochrome oxidase subunit I (COI) DNA is still limited. This study aims to identify and to find out the genetic data of *M. dorsalis* that be derived from region of its mitochondrial cytochrome oxidase I gene (COI). Analysis was done by four steps, namely: Hopper collection on paddy field by using insect net, DNA extraction by using Zymo Tissue & Insect DNA Mini Preparation, amplification by PCR using My Taq™ HS Red Mix and DNA sequence analysis using ABI PRISM 3730xl Genetic Analyzer. Primer cocktail tRWF-Mlep was used in DNA amplification step. The research result pointed out that COI DNA fragment of *M. dorsalis* has length 521 bp. This COI DNA sequence was dominated by A and T(U) bases with concentration 74.30%. The concentration of T(U), C, A and G nucleotides in the COI sequence were 35.90%, 13.40%, 38.44%, and 12.30%, respectively. Identification of *M. dorsalis* based on this COI DNA sequence confirmed the identification result based on its morphological characters.

KEYWORDS: Auchenorrhyncha, mtCOI, DNA sequence, Samosir

INTRODUCTION

Zigzag leafhopper *Maiestas dorsalis* (Motschulsky) syn. *Recelia dorsalis* is the leafhopper that belongs to family Cicadellidae of order Hemiptera [1]. This is small wedge shaped insect with specific morphology character of forewings with zigzag reddish brown margin [2]. In rice ecosystem, zigzag hopper has economic importance because it can act as virus vector [1,2]. In this case the zigzag species is known as transmitter of tungro, rice dwarf, rice gall dwarf virus disease and the sole vector of orange leaf Mycoplasma-like Organism (MLO) [1,2]. Therefore, the occurrence of that hopper on paddy field direct and indirect could cause damage and the death on paddy plants.

The distribution of *M. dorsalis* in many country has been reported by Faruq *et al*. [3] as in Australia, Bangladesh, Bhutan, China, India, Indonesia, Japan, Kampuchea, Korea, Laos, Malaysia, Pakistan, Philippines, Sri Lanka, Taiwan, Thailand and in Vietnam. The appearance of this hopper in Tapanuli region of North Sumatera-Indonesia especially at Samosir island has been reported by Manurung *et al*. [4] and its abundance was relatively high.

In order to achieve the effective management of pest species damaging crop, the accurate identification is needed [2, 5]. Until now regarding to identification of leaf-and planthoppers, at least there are two approaches that have been used, namely by morphology plus anatomy and molecular or genetic markers [6]. The using of morphometric approach by measuring 10 morphological features for identification of white leafhopper *Cofana spectra* has been done by Manurung *et al*. [7]. Meanwhile, morphometrically identification on *M.dorsalis* based on its body length and fore wing length have been done by Faruq *et al*. [3].

Species identification by using morphology character has been used and familiar for long time, whereas the using of molecular or genetic approach especially throughout DNA analysis is still new, just since 2000 years [8].Genetically analysis, especially through DNA barcoding has become one of the major tools at present that has been used by taxonomic and non taxonomic experts in order to identify animals and plants as well to know their phylogenetic with related species [5,6,9,10,11,12,13,14,15,16,17,18].

The using of genetic approach namely by using DNA barcoding, especially through mitochondrial COI gene in order to identify and to study the nucleotide composition and also the phylogenetic of *M. dorsalis* with its related species that come from Samosir island-North Sumatera-Indonesia until now has never been attempted. Therefore, this investigation has been carried out.

MATERIAL AND METHODS

Study Area

One sampling site at Samosir island on non irrigated rice field has been selected. The sampling site was at Siogung-ogung in Pangururan village (Latitude: N 02°36'41.73''; Longitude: E 098°41'37.34"). Leafhoppers catching were done in June 2019 in conventional rice cultivation field.

Collecting and Identification of Samples

The hopper was captured by using standard sweep net and aspirator [19]. The catching was done in the western, eastern and win ward sides of the paddy field [20, 21]. Hopper samples were deposited in 96% alcohol, labeled, and transported to the laboratory for curation and identification. Morphologically species identification was done under stereo binocular microscope in taxonomy laboratory of Biology Department of Universitas Negeri Medan and be consulted on Wilson &Claridge [1]. The samples were stored at - 20°C until the DNA was extracted.

DNA extraction, Amplification and Sequencing

DNA genomic was extracted with Zymo Tissue and Insect DNA Mini Prep (Zymo Research, D6016). This extraction consisted of preparing, lysis cell, DNA binding, washing and DNA elution steps. The DNA isolated was confirmed using 1% TBE agarose. The amplification of mitochondrial genomic DNA was done with My Taq HS Red Mix (Bioline, Bio-25047). The coctail primer tRWF-Mlep was used to amplify the COI gene in Touch Down PCR condition [12]. This PCR profile consisted of initial denaturation at temperature of 95°C for 3 min followed by 5 cycles with denaturation reaction conditions at 94°C for 40 sec, annealing at 45°C for 40 sec, extension at 72°C for 1 min and then followed by 35 cycles with denaturation reaction conditions at 94°C for 40 sec, annealing at 51°C for 40 sec, extension at 72°C for 1 min and ending with a final phase of extension terminal at 72°C for 1 min. The purification of PCR product was done by using the Zymoclean Gel DNA Recovery Kit (Zymo Research, D4002). The PCR product was assessed by electrophoresis with 1% TBE agarose. The running agarose was done at 100 volt for 60 min (Wealtec). Furthermore, the purified PCR product was sequenced with Bi-directional Sequencing using an ABI PRISM 3730 XLGenetic Analyzer at genetic lab of PT Genetika Science Indonesia, Jakarta.

Alignment and Analyses

Sequences data were aligned using ClustalW. The combination of mtDNA sequence of COI data was analyzed by sequencing homology using BLAST program which can be accessed at the National Center for Biotechnology Information (NCBI) website. Sequences homology analysis was performed by comparing COI sequence of zigzag leafhopper sample with NCBI GenBank Data base. The maximum composite probability estimate of the pattern of nucleotide substitution was based on Tamura-Nei model [22]. Molecular Evolutionary Genetic Analysis (MEGA-X) software program was used for phylogenetic tree construction and evolutionary analyses [23]. The evolutionary history was inferred using the Neighbor-Joining method [24].

RESULTS AND DISCUSSION

Leafhopper sample that has been previously identified based on morphology character as *Maiestas dorsalis* syn. *Recelia dorsalis* [1], its analysis was then continued with molecular marker. In the working with this molecular approach, the mitochondrial cytochrome oxidase I (COI) region of the sample was succesfully amplified using Touch Down PCR with coctailtRWF-Mlep primer. This result confirm the using of that primer in Touch Down PCR condition in the study of leafhopper (Hemiptera: Auchenorrhyncha) taxonomy through DNA barcoding approach [12]. The PCR of COI gene fragment for sample *M. dorsalis* yielded a single product of 521 bp. The length of this COI gene fragment is longer compared to white leafhopper *Cofana spectra* (305 bp) [13] and orange headed leafhopper *Thaia subrufa* (466 bp) [14].

The NCBI BLAST database result pointed out that partial COI gene sequence of *M. dorsalis* population isolated from Samosir showed 98.65% similarity with *M. dorsalis* (KX786285.1) from Guilin, Guangxi province, China (Table 1). This finding stated that identification with molecular marker has corroborated the morphological identification and also become a valuable tool in animal taxonomy [6,10,25,26]. Regarding into the high similarity of COI gene sequences of *M. dorsalis* leafhopper between Indonesia (Samosir island) and China populations, this research result revealed that genetic variation between the both leafhopper populations were very low and therefore geographical distance and ecological differences between two countries may be don't have significantly contribution on the creating of their gene variation. This research finding stated also that the both populations have the same ancestor.

Species	Accession number cover	Query	Percent identity
Maiestas dorsalis mitochondrion complete genom	KX786285.1	100%	98.65%
Cicadellidae sp. 4AY-2013 mitochondrial partial COI	HF968654.1	62%	98.47%
Cicadellidae sp. 7 AY-2013 mitochondrial partial COI	HF968658.1	63%	98.18%
Cicadellidae sp. 1 AY-2013 mitochondrial Partial COI	HF968651.1	63%	98.17%
Cicadellidae sp. 5AY-2013 mitochondrial partial COI	HF968655.1	64%	97.89%
Recilia dorsalis isolate RD3 cytochrome oxidase sub unit I (COI)	KU324165.1	66%	96.50%
Recilia dorsalis cytochrome oxidase sub unit I COI	KU258183.1	66%	96.25%
Recilia dorsalis isolate RD2 cytochrome oxidase Sub unit I (COI)	KU324164.1	66%	95.97%

Table 1. Result of BLASTN Analysis on Sample P5

The composition of nucleotide of *M. dorsalis* sample showed clear bias to nucleotide AT at concentration 74.30%. The occurring of nucleotide AT bias on *M. dorsalis* is in line with leafhopper *Nephotettix virescens, C. spectra* and *T. subrufa* [5,13,14]. The composition of T(U), C, A and G nucleotides in the COI sequence were 35.90%, 13.40%, 38.44% and 12.30%, respectively (Table 2). The maximum composite probability estimate of the pattern of nucleotide substitution is presented in Table 3. In this case, each entry is the probability of substitution (r) from one base (row) to another base (column). Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics. This substitution pattern and approximate rate were based on the 2 parameter model Kimura [22].

Species (Gen Bank Accession number)	Nucleotide Composition (%)				Total
	T(U)	C	A	G	
*Cicadellidae sp.1*AY-2013 COI (HF968651.1)	37.6	15.4	33.0	13.9	675
*Cicadellidae sp.4*AY-2013 COI (HF968654.1)	37.5	15.8	32.7	14.0	670
*Cicadellidae sp.5*AY-2013 COI (HF968655.1)	37.8	15.4	32.9	13.9	677
Cicadellidae sp.7 AY-2013 COI (HF968658.1)	37.3	15.5	32.8	14.3	676
Japanushyalinus-whole genom (KY129954.1)	33.9	13.7	42.7	9.7	15364
Maiestasdorsalis-whole genom (KX786285.1)	34.3	12.3	44.4	8.9	15352
Reciliadorsalis COI (KU258183.1)	37.7	15.1	33.7	13.5	689
Receliadorsalis isolate RD2 COI (KU324164.1)	37.9	15.1	33.5	13.5	689
Receliadorsalis isolate RD3 COI (KU324165.1)	37.9	15.0	33.5	13.6	686
Receliadorsalis isolate RD4 COI (KU324166.1)	37.9	14.9	33.4	13.8	680
Receliadorsalis voucher COI ZYJ70 (MF716885.1)	37.7	15.3	33.3	13.7	658
Sample P5	35.9	13.4	38.44	12.3	521
Average	34.7	13.4	41.8	10.1	

Table 2. The length and percentage of nucleotide composition of the COI sequence of *M. dorsalis*sample from Samosir island (sample P5) and related species

From\To	A	T	C	G
A	-	*7.23*	*2.79*	**2.75**
T	*8.69*	-	**12.30**	*2.10*
C	**8.69**	*31.90*	-	*2.10*
G	*11.37*	**7.23**	*2.79*	-

Table 3. Maximum composite probability estimate of the pattern of nucleotide substitution

The evolutionary divergence of *M. dorsalis* within Cicadellidae family is given in the Table 4. A 1.35% difference is observed between Indonesia (Samosir) population when compared to *M. dorsalis* from China. Meanwhile, the highest variation (15.85%) was with *Japananus hyalinus* also from China [27].

No	Species name with GenBankAcession No.	Divergence (%)
1	*Maiestas dorsalis* China-KX7862851	1.35
2	*Cicadellidaesp* 4 AY-2013-HF9686541	1.53
3	*Cicadellidaesp*7 AY-2013-HF9686581	1.82
4	*Cicadellidaesp 1* AY-2013-HF9686511	1.83
5	*Cicadellidaesp 5* AY-2013-HF9686551	2.11
6	*Recelia dorsalis*V ZYJ 70- MF7168851	2.47
7	*Recelia dorsalis* RD4-KU3241661	3.25
8	*Recelia dorsalis* RD3-KU3241651	3.50
9	*Recelia dorsalis*-KU2581831	3.75
10	*Recelia dorsalis* RD 2-KU3241641	4.03
11	*Japananus hyalinus* China-K1299541Y	15.85

Table 4. Percentage of evolutionary divergence of *M. dorsalis* from Samosir with related species

Molecular phylogenetic tree among *M. dorsalis* isolated from Samosir with other leafhoppers that belongs to member of Cicadellidae family is displayed in Figure 1. This result confirmed that COI gene mitochondria could elucidate the molecular evolution and phylogenetic relationship of leafhopper. The result of phylogenetic tree showed that *M. dorsalis* isolated from Samosir island-Indonesia is the nearest relative of *M. dorsalis* from China and therefore could be stated that the both leafhopper populations have the evolutionary similarity. In this case, the closely connected species shows more than 90% similarity within the standardized DNA sequence whereas distantly connected species will show less than 90% within the same sequence [13].

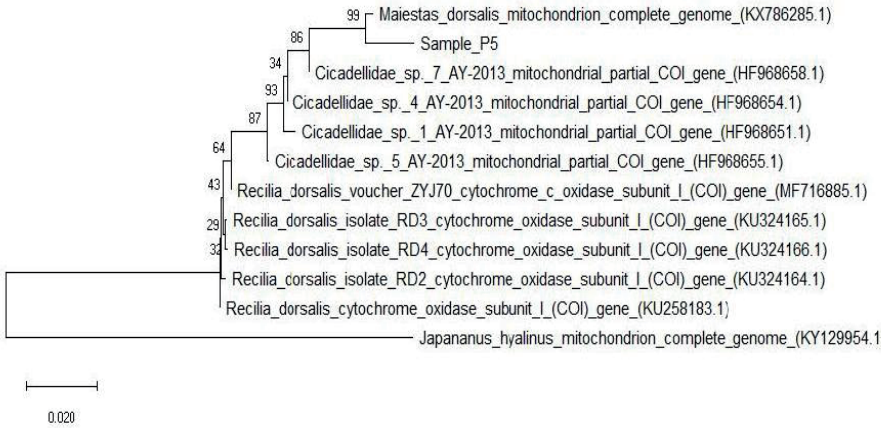

Figure 1. Phylogenetic Tree-Neighbor Joining of *M. dorsalis* from Samosir (Sample P5) based on DNA fragment mitochondria COI sequence with related species

CONCLUSION

Identification of zigzag leafhopper *M. dorsalis* sample from island Samosir-Indonesia based on DNA barcoding marker could confirm the result of identification based on morphology characters. The leafhopper has the length of nucleotide 521 bp and bias on nucleotide AT with the concentration 74.30%). The composition of T (U), C, A and G nucleotides were 35.9%, 13.40%, 38.44% and 12.30%, respectively.

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