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Barcode

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Submission date: 07-Jan-2022 08:37AM (UTC+0700)

Submission ID: 1738328918

File name: out.pdf (466.88K)

Word count: 2912

Character count: 15457

Genetic Analysis of White Leafhopper *Cofana spectra* (Distant) from Samosir Island, Indonesia Based on DNA Barcode

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Abstract. White leafhopper *Cofana spectra* is a pest of paddy and could act as a vector of pathogenic viruses on rice. This research aims to find out its genetic data, namely its identification, nucleotide composition and its phylogenetic based on DNA barcode-mtCOI gene. The research methods included leafhopper sampling in rice field, DNA isolation, PCR, electrophoresis, sequencing and blasting. The software Mega X was used to analyze the genetic data. The mtCOI DNA gene sequence obtained was 333 bp in size and blasting result showed that leafhopper belongs to white leafhopper *Cofana spectra* (Distant) species. The dendrogram from the sequence showed that white leafhopper species from Samosir island had the closest relationship with species from Kerala, India. The frequency of nucleotide of T (U), C, A and G of leafhopper were 41.96, 15.47, 28.57 and 13.98%, respectively. Therefore, this mtCOI gene sequence was rich in A/T (70.53%), while G/C was 29.45%. The information obtained from this study stated that the identification of the white leafhopper *C. spectra* based on molecular character using mtCOI DNA gene sequence confirmed the identification result based on the morphological traits and can be used to control and in monitoring the spread of the white leafhopper.

Key words: mt COI, *Cofana spectra*, Samosir-Indonesia, phylogenetic

1. Introduction

White leafhopper *Cofana spectra* is a pest of paddy and could act as a vector of pathogenic viruses on rice. This hopper suck sap from the leaves and results drying of leaf tips leading the leaf flip orange and curl. This species could as a vector of important rice yellow mottle virus (RYMV) that causes yield loss [1,2,3]. Therefore, the hopper has been stated as minor and major pests [2,4]. The occurrence of *Cofana spectra* in Africa, Pacific, Australia, India, Indonesia, Malaysia, Philippines, Sri Lanka and in Taiwan have been reported [5,6]. Furthermore, its presence, morphometric and abundance on Indonesia rice field, especially in Samosir island-Sumatera-Indonesia were reported [3,7].

The success of monitoring and control of *C. spectra* as an important pest and as a vector of virus on paddy field depend on comprehensive comprehension of its biology, especially as about its taxonomy or its identification. The accurate identify of a pest and a vector insect was needed in the management of its distribution in the field.

The identification of leafhopper insect could be done by morphological and molecular approaches. The using of morphological and anatomy approaches regarding insect included leaf and planthoppers



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identification for long time has been done and reported many experts[5,6,7,8], whereas the using of molecular or genetic approach especially throughout DNA barcoding is still new, just since 2000 years[8-11]. In comparing to morphological approach, the using of molecular approach in insect identification has some advantages, i.e. could be used for larval stage, polymorphism form and for female. Meanwhile, morphological approach as a part of conventional or classical taxonomy have some limitations in identification, because it could be used just for adult stage and mainly must be based on the structure of male genitalia.

Identification of leafhopper through DNA barcoding approach was carried out and reported by some experts. For example, in identification of zigzag leafhopper *Maiestas dorsalis*[12-13], green leafhopper *Nephotettix virescens*[14-16], green leafhopper *Nephotettix nigropictus*[17], mango leafhopper, *Amritodus atkinsoni*[18], leafhopper *Thaia subrufa*[19], and white leafhopper *C. spectra* that originated from Kerala, India[2].

This research aim was to implement DNA barcoding-mt COI gene (mitochondrial oxidase sub unit I) to identify the white leafhopper *Cofana spectrata* that was collected from rice field that be found in Samosir island, North Sumatera, Indonesia. This investigation also has objective to study its nucleotide composition and its phylogenetic with related species that until now has never been attempted.

2. Methods

2.1. Study Area

The leafhopper sample was collected on non irrigated and conventional rice cultivation field in Siallagan village (N: 2°40'40"; E: 98°50'05", 898 m above sea level) at Samosir island. The collection of leafhopper was done in May 2019.

2.2. Collecting and Identification of Samples

The leafhopper catching was carried out by using of a sweep net and aspirator[3]. The leafhopper collection was done in the western, eastern and winward sides of the paddy field[20-21]. The collected hoppers were deposited directly in 96% alcohol, labeled, and transported to the laboratory for curation and identification. Identification based on morphological approach was carried out prior to molecular studies. The morphology traits of leafhopper was observed under Olympus SZ 51 stereo binocular microscope in taxonomy laboratory of Biology Department of Universitas Negeri Medan. Regarding identification, obtained morphology characters of leafhopper be consulted on some references[5-6]. The leafhopper samples were stored at - 20°C until the working of the DNA extraction[13,16-17].

2.3. DNA extraction, Amplification and Sequencing

The DNA genomic of leafhopper was extracted from the tissue of one individual. The DNA extraction was done by using Zymo Tissue and Insect DNA Mini Prep (Zymo Research, D6016). The steps in this extraction consisted of preparing, lysis cell, DNA binding, washing and DNA elution. The amplification of mitochondrial genomic DNA was carried out with My Taq HS Red Mix (Bioline, Bio-25047) in Touch Down PCR condition and with primer COI KJ186109 [1]. The forward of that primer was 5'-CACCTGATATAGCTTTCCCCCG-3', meanwhile its reverse is 5'-AGCTCCTGCTAA²³CAGGTAAAG-3'). The 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 52°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 of mtCOI gene was assessed by electrophoresis with 1% TBE agarose. The running agarose was done at 100 volt for 60 min (Wealtec). Furthermore, the purified of mtCOI PCR product

was sequenced with Bi-directional Sequencing using an ABI PRISM 3730 XL Genetic Analyzer at genetic laboratory of PT Genetika Science Indonesia, Jakarta.

2.4. Alignment and Analyses

The obtained sequence data was aligned by using Clustal W-Mega X and consensus was taken for the analysis. The combination of mtCOI DNA gene sequence 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 white leafhopper Samosir sample with NCBI Gen Bank data base. The maximum composite probability estimate of the pattern of nucleotide substitution was based on Tamura-Neimodel [22,23,24]. Molecular Evolutionary Genetic Analysis (MEGA-X) software was used for phylogenetic tree construction and evolutionary analyses[25]. The evolutionary history was inferred using the Neighbor-Joining method[26,27].

3. Result and Discussion

Leafhopper sample that identify based on morphology characters prior to molecular traits was *Cofana spectra* (Distant)[5,6].The amplification of mitochondrial cytochrome oxidase I (COI) DNA gene of white leafhopper *C. spectraby* using primer COI KJ 186109 in Touch Down PCR conditiontook place succesfully.This result confirmed the succes of Sreejith & Sebastian (2014)[1]regarding mtCOI amplification of white leafhopper *C. spectrathat* originated from Kerala, India. The succes of mtCOI DNA gene amplification for some leafhopper species by using specific primer have been reported many experts[13,14,15,16,17,19].

The length of mtCOI DNA gene of *C. spectra* Samosir sample was 333 bp. This mtCOI gene fragment product was longer compared to the same species that originated from Kerala, India (305 bp)[1].The length of mtCOI gene of *C. spectra* Samosir isolated was shortest compared to other leafhopper species that originated from Samosir island.The mtCOI gene fragment product for *Maiestas dorsalis* was521 bp[13], for *Nephotettix virescens* 677-681 bp[16] and for *Nephotettix nigropictus* 510 and 523 bp[17].

Table 1.The Result of BLASTN analysis on Sample S1.

Species	Accession	Query Cover	Percent Identity
<i>Cicadellidae sp.</i> COI	GU013583.1	98 %	86.85%
<i>Cofana spectra</i> COI	KJ186109.1	82%	99.27%
<i>Cuerna cuesta</i> COI	KR567267.1	97%	86.81%
<i>Cuerna nielsoni</i> COI	KF919702.1	97%	86.81%
<i>Cuerna septentrionalis</i> COI	KR582355.1	98%	86.85%
<i>Cuerna sp.</i> COI	KR564500.1	97%	86.81%
<i>Cuerna sp.</i> COI	MF832746.1	97%	86.81%
<i>Cuerna sp.</i> COI	MF835609.1	97%	86.81%
<i>Cuerna sp.</i> COI	MF838072.1	97%	86.81%
<i>Hemiptera sp.</i> COI	KT879865.1	98%	98.47%

Furthermore, the blasting of Samosir white leafhopper mtCOI DNA gene toward data NCBI gen bank showed that the fragment was similar with *Cofana spectra* species from India (gen bank accession number KJ186109.1) (Table 1). This blasting finding confirmed the result of *C. spectra* identification based on morphological features. Therefore, the using of molecular approach that be based on DNA barcoding, especially through mtCOI DNA gene has corroborated the morphological identification and also become a valuable tool in animal taxonomy[13,16,17].

There was about 99.27% the homologous between Samosir *C. spectra* isolated and Kerala, India isolated. It meant there was just small differences (about 0.73%) between the both leafhopper populations. This research result, furthermore, stated that there was low genetic variation between Kerala and Samosir leafhopper populations and therefore, geographical distance and also ecological differences between two countries may be don't have significant contribution on the gene variation creating toward the both populations. It could also stated that both of white leafhopper populations (Kerala, India and Samosir, Indonesia) probably were originated from a single ancestor.

Table 2. The percentage of nucleotide composition of the COI sequence of *C. spectra* sample from Samosir island (sample S₁) and related species.

Species (accession number)	T(U) (%)	C (%)	A (%)	G (%)
<i>Cicadellidae sp.</i> (GU013583.1)	39.97	13.86	31.26	14.89
<i>Cofana spectra</i> (KJ186109.1)	41.45	14.54	29.45	14.54
<i>Cuerna cueta</i> (KR567267.1)	40.90	13.53	30.63	14.93
<i>Cuerna nielsoni</i> (KF919702.1)	41.18	13.67	30.39	14.74
<i>Cuerna septentrionalis</i> (KR582355.1)	41.03	13.82	30.54	14.58
<i>Cuerna sp.</i> (KR564500.1)	40.63	13.80	30.79	14.76
<i>Cuerna sp.</i> (MF832746.1)	41.58	13.05	30.41	14.94
<i>Cuerna sp.</i> (MF835609.1)	41.77	13.15	30.75	14.30
<i>Cuerna sp.</i> (MF838072.1)	41.29	13.10	30.70	14.90
<i>Hemiptera sp</i> (KT879865.1)	42.70	13.22	29.63	14.43
Sample S₁ (Samosir)	41.96	15.47	28.57	13.98

The nucleotide composition of *C. spectra* Samosir sample showed clear bias to nucleotide AT (70.53%), whereas for CG was 29.45%. The finding of a higher AT content in *C. spectra* also be found in Kerala as be reported by Sreejith & Sebastian (2014)[1]. The occurring of nucleotide AT bias in *C. spectra* leafhopper is in line with the study result in leafhopper *Maiestas dorsalis*[13], *Nephotettix nigropictus*[17], *Nephotettix virescens*[14,16] and *Thaia subrufa* [19]. The composition of T (U), C, A and G nucleotides in the COI sequence of Samosir *C. spectra* isolated was 41.96%, 15.47%, 28.57% and 13.98%, respectively (Table 2). This finding showed that thymine/uracil percentage was highest than three others nucleotides and be followed by adenine, cytosine and guanine. The highest of thymine/uracil bases among nucleotides that be found in mtCOI DNA gene sequence of hopper has been reported of many experts [1,13,14,16,17,19].

The maximum composite probability estimate of the pattern of nucleotide substitution in mt COI gene sequence of white leafhopper *C. spectra* 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[22,23]. This result stated that probability of the highest transitional substitutions occurred between C nucleotide and T(U) nucleotide (45.15), meanwhile, the highest transversional substitutions took place between A nucleotide and T(U) and between G and T(U) (0.75) (Table 3).

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

	A	T/U	C	G
A	-	0.75	0.25	11.84
T/U	0.55	-	15.28	0.27
C	0.55	45.15	-	0.27
G	24.08	0.75	0.25	-

Phylogenetic tree among *C. spectra* isolated from Samosir(Sample S₁) with other leafhopper that belong to Cicadellidae family member is displayed in Figure 1. This finding confirmed again that COI DNA gene mitochondria could elucidate the molecular evolution and phylogenetic relationship of leafhopper. The result of this phylogenetic tree pointed out that *C. spectra* isolated from Samosir island-Indonesia is the nearest relative of *C. spectra* from Kerala, India, therefore the both white leafhopper populations probably have the evolutionary similarity. In this case, some experted have stated that the distantly connected species will show less than 90% within the same sequence, whereas the closely connected species shows more than 90% similarity[1,13,16,17,29].

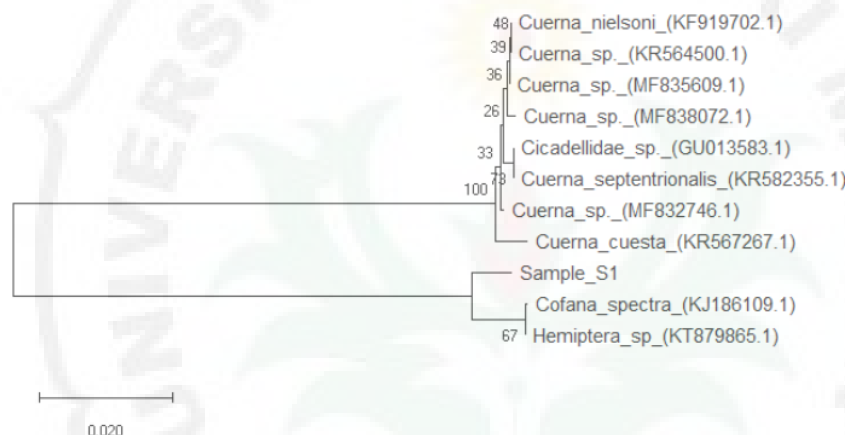


Figure 1. Phylogenetic tree-Neighbor Joining of *C. spectra* from Samosir (Sample S₁) based on fragment mitochondria COI DNA gene sequence with related species.

4. Conclusion

The white leafhopper that could be found on rice field in Samosir island, Sumatera-Indonesia based on morphological and molecular characters, DNA barcoding-mtCOI gene belong to species *Cofana spectra* Distant. The length of its mt COI DNA gene was about 333bp and bias on nucleotide AT with concentration about 70.53%, whereas its CG content was just 29.45%. The composition of T (U), C, A and G nucleotides of *C. spectra* Samosir isolated were 41.96%, 15.47%, 28.57% and 13.98%, respectively.

References

- [1] Sreejith, K & C.D. Sebastian. 2014. *IJANS*3(2):135-140.
- [2] Mitra, S., Rupa, H., Niladri, H., and Abhijit, M. 2014. *Int.J.Trop.Insect Sci.*34 (1): 14-21.
- [3] Manurung, B., A.Hasairin., A H Daulae., P S Silaban & N Hasibuan. 2019. *J.Entomo..Zool.*7(1): 701-705.
- [4] Mitra, S., Rupa, H., Niladri, H., and Abhijit M. 2014. *INS*6(1): 33-42.
- [5] Wilson, M.R. dan M.F. Claridge. 1991. *Handbook for the identification of leafhoppers and planthoppers of rice*. Wallingford-UK: CAB International.
- [6] Meshram NM, Ramamurthy VV. 2014.). *AEMNP*54(1):57-64.
- [7] Manurung, B., Prastowo, P., Daulae, A.H. 2017. *IJSR*6 (9): 1412-1417.
- [8] Gopurenko, G., M.Fletcher.,H.Loecker & A. Mitchell. 2013. *Rec West Aust Mus*83: 253-285.

- [9] Hebert, PD & TR Gregory. 2005. *Syst Bio.* **54**(5):852-859.
- [10] Ottit, R., Maw, E & Hebert, PDN. 2014. *Plos One* **9** (7): 1-10.
- [11] h, W.B., C.T Yang & C.F. Hui. 2005. *Zoolstud.* **44**(4):519-535.
- [12] Du, Y., C.Zhang, C.H.Dietrich., Y.Zhang & W.Dai. 2017. *Sci Rep.* **7**:14197.
- [13] Manurung, B., A.Hasairin & A.H.Daulae. 2020. *IOP Conf. Ser.: Earth Environ. Sci.* **457**012021.
- [14] Sreejith, K & C.D. Sebastian. 2015. *Indian J Sci Technol.* **8** (1): 61-64.
- [15] Yuniawati, R., R. Raffiudin & I.M.Samudra. 2019. *TLSR* **30** (3): 95-110.
- [16] Manurung, B., A.Hasairin & A.H.Daulae. 2020. *J. ent. Res.* **44** (2): 183-188.
- [17] Manurung, B., A.Hasairin & A.H.Daulae. 2020. *Ann Biol.* **36** (2): 181-185.
- [18] Choudhary, J.S., C.S. Prabhakar., N. Naaz., M. Lemtur., B. Das., S. Kumar & B.P. Bhatt. 2019. *Mitochondrial DNA BResour.* **4** (1): 261-264.
- [19] eejith, K & C.D. Sebastian. 2015. *J.Entomol.Zool.* **3**(3): 135- 139.
- [20] Manurung, B., Witsack, W., Mehner, S., Gruentzig, M & Fuchs, E. 2004. *Virus Res.* **100** (1): 109-113.
- [21] Manurung, B., Witsack, W., Mehner, S., Gruentzig, M & Fuchs, E. 2005. *JPlant Dis Prot.* **112** (5): 497-507.
- [22] imura M. 1980. *J.Mol.Evol.* **16**:111-120.
- [23] Tamura K. and Nei **20** 1993. *Mol.Biol.Evol.* **10**:512-526.
- [13] Tamura K., Nei M., and Kumar S. 2004. *PNAS USA.* **101**:11030-11035.
- [25] Kumar S., Stecher G., Li M., Knyaz C., and Tamura K. 2018. *Mo.Biol.Evol.* **35**:1547-1549.
- [26] senstein J. 1985. *Evol.* **39**:783-791.
- [27] Saitou N. and Nei M. 1987. *Mol.Biol.Evol.* **4**:406-425.
- [28] Asokan, R., N.K.K Kumar., A.Verghese. 2007. *Curr.Sci.* **93**(12): 1668-1669.
- [29] Gurney, T., Elbel, R., Ratnapradipa, D and R Brossard., S.J.Karcher, Eds. 2000. *Proc.of 21st Work/Confe.of ABLE21*: 63-79.

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