International Journal of Research in Engineering and Science (IJRES)
ISSN (Online): 2320-9364, ISSN (Print): 2320-9356
www.ijres.org Volume 3 Issue 5 ǁ May 2015 ǁ PP.46-49

DNA Barcode of *a New Species* Insect in Mangrove Ecosystem at Likupang Village, North Minahasa Regency, North Sulawesi Province, Indonesia.

James J.H. Paulus[1], Jantje Pelealu[2], Max Tulung[2] and Grevo Gerung[3]
[1)] Postgraduate student at Sam Ratulangi University, Entomology Program, Manado, North Sulawesi Province, Indonesia. Mob: 081340731840
[2)] Faculty of Agriculture, Sam Ratulangi University, Manado, Indonesia
[3)] Faculty of Fisheries, Sam Ratulangi, Manado, Indonesia

Abstract *– The research area at Mangrove forest in Likupang Village North Minahasa Regency, North Sulawesi Province, Indonesia. The aimed of this research are to document DNA barcode of insect inhabit in mangrove area. Insect were collected with sweep net and directly put in to the alcohol. The insect preparation for PCR (Polymerase Chain Reaction) at Basic Science Laboratory Sam Ratulangi University (MIPA faculty). The primer LCO1490: 5'-ggtcaacaaatcataaagatattgg-3' and HC02198: 5'-taaacttcagggtgaccaaaaaatca-3' at 710-bp. BLAST Analysis and the result barcode has ID :(lcl|Query_113365), 84% identification to species Gergithus iguchii also as the closer (0.20) distance organism, it means this organism as a new species, and has a taxonomy Order : Hemiptera, sub order : Neohemiptera, Super family :Fulgoridea, Family : Issidae.*
Index Term*: Mangrove forest, Insect, DNA Barcode, Taxonomy, New Species Insect*

I. INTRODUCTION

Insect as segmental organism with jointed hard exoskeletone [1]. As a dominant insect on earth and play an important role to the ecosystem [2]. Insect are spread over in several area between land and sea especially in mangrove area. Research about insect their properties, lack and benefit had driven in North Sulawesi [4]. Mangrove area in Likupang village North Minahasa Regency Indonesia recognized has many insect but lack of DNA documentation. Mangrove in Likupang village also play an important role for supply nutrient to the aquatic organism such fish and invertebrate [3]. Likupang of shore also recognized as good area as fishing ground. As a suitable area for eating and laying eggs, mangrove became a target of insect [6][7].

Location observation to the location was in February 2012, to find the insect as residence. The areas are close to the estuary of Likupang River and also 3 kilometer to the Gold Mining company and front of exploration of iron sand at Bangka Island. This area indicated rich of mineral according to Paulus research on (2002) found a soluble metal Cadmium an interstitial water [3]

Diversity of insect is the important as specific branch of biology. Lack document of taxonomy and systematics of insect in North Sulawesi are need to answer with several research especially at the specific area. Managing for sustainable performance of Mangrove ecosystem needed for the ecological function [7]. Spread over the intertidal zone at coastal line, insect live and exploit mangrove as a source of food [10]

II. MATERIALS and METHODS

2.1 Time and Place of Research

This research were conducted between March to October 2012, in Likupang Village district Likupang , North Minahasa Regency, North Sulawesi Provincy (N $0104^0.324'$ E $125^004.001'$). The locations are close to the Estuary of Likupang River as contributors of many materials to the sea. This area some time became as a fisherman shelter for their nets and boat and sometime clear of activity. An intertidal zone as the location of mangrove and have many activity of fisherman. Mangrove tree has ranged diameter bar between 20 – 30 cm. Mangrove Physiology such as salt, heat and light stress, recurrent flooding also influence to the insect [6].

2.2 Research Procedure

2.2.1 Sample collecting

Sweep net and by hand collected this insect in the morning 9 – 12 PM. Insect are hide between leaf protected from enemies and light, also located at the end of branch. Slow down climb to the branch target prevent the insect moving. Sample insect directly put to the alcohol. Sample were preparation and handling in Basic Science Laboratory (MIPA UNSRAT) for DNA purposes [8]

2.3 DNA Analisys

2.2.1. Sample Preparation

Sample insect from field were keep in absolute alcohol. Leg Fragment for 2-3 mm are suitable for DNA extraction [8] . Furthermore sample destruction and pre-filtration using mauve. washing DNA attached through the filter and put on to the eppendorf tube. Added lysis solution SLS (300µl) and Proteinase K (25 µl) using micropipettes. Tube inversion for homogenate the solution contains DNA. Sample incubation by added buffer solution in 55^0C and 45 minutes in thermo block apparatus [9][11].

2.2.2 DNA qualitative and quantitative test

1% Agarose gel, boiled in 100 ml 1xTAE buffer, added 2 µl maestro safe prestained. Putting down in to gel tray up to gel condition for around 30 minutes. 4 µl DNA as isolation result added with 1 µl loading buffer. Putting down 5 µl mixture to the well of agarose. Putting down 2 µl 100bp ladder DNA marker as qualitative indicator as result of DNA amplification. electrophoresis gel agarose for 20 minutes up to fluorescent colour present. The other ways for quantitative test are using spectrophotometer [8][9][11][12][13].

2.2.3 PCR (Polymerase Chain Reaction)

Working in dead –air PCR Box, use glove usually to avoid of contamination and clean laboratory. Usually using negative and positive in each reaction. Stock primer as stok added with 90 µl sterile ddH_2O up to 10 pmol/ µl, keep in -20^0C. Using Generix PCR Master Mix as solution.

Two times centrifuge tube for safe mixture. Sample tube putting down in to the PCR apparatus and adjust the temperature and time.

2.2.4 Electrophoresis test

PCR product was preparation through agarose gel filtration for analytical qualitative evaluation. Several well made by attached a well maker apparatus and ready for DNA mixture solution test. Pipetting 9 µl as PCR solution result and mix with 1 µl sample loading buffer at parafilm. Put the sample DNA mixture to the agarose well and put 4 µl 100 bp ladder DNA as a weight molecule marker. The fluorescent colour in agarose gel indicate the presenting of DNA, under monitor in UV trans-illumina apparatus.

III. III. RESULT AND DISCUSSION

DNA sequencing were editing use geneieus software and analyzed with BOLD gen bank connecting on line [5]. According to the DNA sequence result the new species insect sample are below :

NNANNTTTTTTTTTTGGGTATCTGATCAGGACTAATTGGAATAATAATAAGAATAATTATCCGAA
CCGAATTATCACAACCAGGTTCATTAATTAAAAATGACCAAATTTACAATTCAATTGTTACAGCAC
ACGCATTCATCATGATTTTCTTTATAGTTATACCAATTATAATTGGAGGTTTCGGAAACTGACTTGT
ACCAATAATAATTGGTGCACCAGATATAGCATTTCCACGAATAAATAATATAAGATTTTGGCTATT
ACCAACATCCTTATCACTTCTTCTATCAAGAGCACTAACTGGATCAGGATCCGGAACAGGATGAAC
TGTATACCCGCCCTTATCTGGTCAAGAAGCTCACGCAGGACCCTCAGTAGATCTAACAATTTTTTC
ACTTCATAGTGCAGGAATTAGATCCATTATAGGAGCCATTAACTTTATATCTACAATTTTCAATATA
CGAACAACAGGAATAAATATAGAAAAAACACCATTATTCTGCTGATCAGTATTAATCACTGCAATT
CTACTATTAGTTTCATTACCCGTCCTTGCAGGAGCAATTACTATACTTATTATAGACCGAAACTTTA
ATACATCATTCTTTGATCCTTCAGGAGGAGGTGATCCTATTCTTTACCAACACTTATTTTGATTTTTT
GGTCACCCTGNAAGTTTAAAN

Figure 01. DNA Sequence

DNA Sequencing composition were analyze with BOLD systems were connecting on line systems and have an ID : (lcl|Query_113365) as new species [5]. The Taxonomy are : *Order : Hemiptera, sub order : Neohemiptera, Superfamily :Fulgoridea, Family : Issidae.*

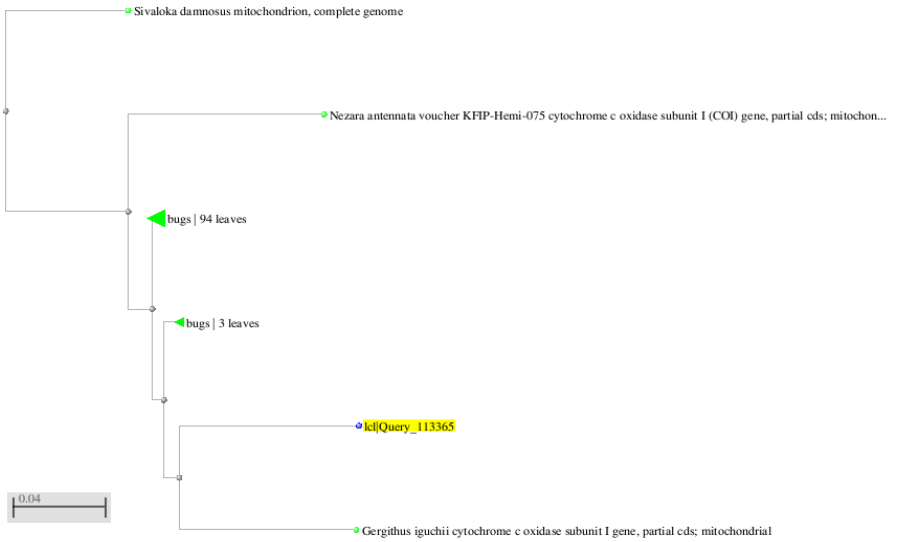

Figure 02 : Tree diagram of *:lcl|Query_113365*

A new Species as *:lcl|Query_113365,* is clearly different with the other species of insect and close to the species Gerghitus igucii and have 84% identification.

Table 01. Distance Table

	PTH8_HCO....	Gergithus ig...	Falcophantis...	Scolops viridis	Catonidia em...	Oliarus polyp...	Bilbilicallia sp
PTH8_HCO.ab1 (reversed)		0.20	0.20	0.19	0.22	0.23	0.20
Gergithus iguchii	0.20		0.21	0.22	0.20	0.24	0.18
Falcophantis westcotti	0.20	0.21		0.19	0.23	0.22	0.21
Scolops viridis	0.19	0.22	0.19		0.21	0.19	0.20
Catonidia emeiensis	0.22	0.20	0.23	0.21		0.25	0.24
Oliarus polyphemus	0.23	0.24	0.22	0.19	0.25		0.23
Bilbilicallia sp	0.20	0.18	0.21	0.20	0.24	0.23	

Distance of the related species base on the BOLD gen bank are 0.18 to 0.25. The species *Gergithus igucii , Falcophantis westcotti* and *Scolops viridis* have the closer position to the new species.

IV. CONCLUSION

The organism as a new species insect (84 % Identification) barcode ID : *lcl|Query_113365* and have the taxonomy : *Order : Hemiptera, sub order : Neohemiptera, Superfamily :Fulgoridea, Family : Issidae.* The Distance is ranged 0.18 to 0.25.

ACKNOWLEDGEMENT

Our thanks to Prof Ellen Kumaat as a rector of Sam Ratulangi University, Prof Lucia Mandey as a director of post graduate program, Prof Jantje Pelealu as a dean of agriculture faculty and Prof Grevo Gerung as a dean of Marine Science and Fisheries faculty that support of this research.

REFERENCES

[1] Chapman R.F., 1998. The Insect, Structure and Function. 4th edition. Cambridge University press. United Kingdom. pp770
[2] Triplehorn C.A., and Johnson N., 2005. Study of Insect. 7Th edition. United States of America. Thomson Brook/Cole. Pp 864.
[3] Paulus J. 2002. A Study of Environmental Chemicals in Mangrovee Area (*Emphasis on Cadmium, Nitrate and Phosphate*). *Thesis*. Post Graduate Program. Sam Ratulangi University, Manado. 42 pp.
[4] Rondonuwu F.B., Maramis R.T.D., Tulung M., Pelealu J., 2014. Natural Chili (Capsicum L.) Cultivation to Mitigate Chili Leaf Curl Complex in North Sulawesi, Indonesia. *International Journal of Research and Science (IJRES). Vol 2 Issue 9*.p 01-08.
[5] Anonymous 2015 : NCBI.nlm.nih.gov. (BOLD data) National Center for Biotechnology Information. *On line :5 January 2015.*
[6] Medina. 1999. Mangrove Physiology: The Challenge of Salt, Heat, and Light stress under reccurent flooding. P 109 – 126. In A. Yanez-Arancibia y A.L. Lara-Dominguez (edt). Ecosystemas de Manglar en America Tropical. Instituto de Ecologia A.C. Mexico. UICN/ORMA, Costa Rica. NOAA/NMFS Silver Spring MD USA 380 p.
[7] Anonymous 2009. Environmental Impact Assesment-Mangrove Restoration Plan Framework Point Lisas Port, Trinidad. Worley Parsons Infrastructure and Environmental Limited.20p
[8] Jonathan B. 2014. DNA Barcoding sebagai perangkat Riset. FMIPA Unsrat. Materi Workshop.11p.
[9] Sugiaman D. 2014. Teknologi PCR dan Aplikasinya. PT Sentra Biosains Dinamika. Jakarta. Indonesia

[10] Nieves-Rivera A.M., Tattar T.A., and Williams E.H Jr. 2002. Sooty Mould-Planthopper Association On Leaves Of the Black Mangrove Avicevia Germinans (L.) Stearn In Southwestern Puerto Rico. Arboricultural Journal. Vol. 26. Pp. 141 – 155.

[11] Balke M and Schmidt S. 2012. Indonesian-German Network for Teaching, Training and Research Collaboration (IGN-TTRC). Training of Trainers and Students. Module II : DNA Barcoding. Zoologische Staatssammlung Munich. DAAD. Entomology Zoologische Staatssammlung Munchen. Uviversitas Brawijaya. Germany 32 p.

[12] Folmer O., Black M.,Hoeh W., Lutz R, and Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology 3(5). Pp. 294 – 299.*

[13] Ivanova V. N., deWaard J.R., Hajibabaei M., Hebert P.D.N., 2015. Protocols for High-volume DNA Barcode Analysis. Biodiversity Institute of Ontario, University of Guelph, Guelph, Ontariio, Canada, NIG 2WI.