Molecular Barcoding and Phylogeny Reconstruction of Rhynchoporus sp in Minahasa North Sulawesi Based Partial Cytochrome Oxidase Sub Unit 1 Gene (CO1)

Sandra Korua^{1*} Jantje Pelealu² Max Tulung² Lucia Mandey² Mokosuli Yermia Semuel³ 1. Doctoral Student, Department of Entomology, Postgraduate Programme, Sam Ratulangi University, Manado, North Sulawesi Indonesia

 Department of Entomology, Postgraduate Programme, Sam Ratulangi University, North Sulawesi Indonesia
 Laboratory of Molecular Biology, Department of Biology, State University of Manado, Tondano, North Sulawesi, Indonesia

Abstract

Molecular phylogeny reconstruction of *Rhynchophorus sp* from *Cocus nucifera* L, *Arenga pinata* and *Metroxylan sagu* was investigated using partial sequences of *Cytochrome c oxidase* subunit I gene (CO1). Three type of *Rhynchophorus sp* according the place of live used this study. *Rhynchophorus sp* from *Cocus nucifera* (AR1), *Metroxylan sagu* (SG1) and *Arenga pinata* (AR1) was analysis the partial CO1 gene. Phylogeny trees was constructed using MEGA 6,0 and Geneous 6,0. From the results of this studied, based on partial CO1 gene, *Rhynchoporus* living at the *Arenga pinata* is *Rhynchoporus palmarum* while *Rhynchoporus* living on Sago palm (*Metoxylan sagu*) and Coconut (*Cocos nucifera*) are *Rynchoporus vulneratus*. The results of this study is the first step of the revision of the uncertain taxonomic status and phylogenetic relationships among the *Rhynchophorus* species as well as other molecular markers.

Keywords: Rhynchophorus sp, CO1, Cocus nucifera, Arenga pinata, Metroxylon sagu Rottb, CO1, phylogeny tree

Intoduction

Based on the previous studies, the morphological characteristics *Rhychoporus sp* in Minahasa. has many variation according to their habitat (Korua, 2015). The number of morphological variation in *Rhychoporus sp* in Minahasa cause problems in the identification of species. Identification based on morphological characteristics may be less accurate in getting the position *Rhychoporus sp* species in Minahasa. *Rhychoporus sp* is an important insect species in Minahasa, North Sulawesi, because it can attack the main agricultural crop (Coconuts). Besides coconut, *Rhychoporus sp* are also found in plants such as Sagu (local name) and Aren (local name). From the results of studies conducted habitat, *Rhychoporus sp* on coconut plants can be moved to and Sagu based on the availability of food (Mokosuli, 2015). It is difficult to identify species *Rhychoporus sp* because of mixing populations increase intraspecies variation. Identification of the *Rynchoporus sp* that live in coconut, Aren and Sagu are important in the effort to control the population. Over population of *Rynchoporus sp* on thats plants had decrease the production. In addition, by knowing the position of the species, it is important to conserve *Rynchoporus sp* as a source of genetic diversity.

Identification of species using mitochondrial DNA gene as a barcode has become a tool for the identification of animal species around the world. Cytochrome oxidase subunit I (COI) mitochondrial gene was established as a bioidentification tool and has been used to study genetic variation in various insect species (Hebert et. al. 2003). Identification of species of insects in Sulawesi using barcode molecular DNA mitochondrial gene CO1 was performed on *Apis dorsata* Binghami and *Apis nigrocincta* Smith (Mokosuli et. al., 2013), Damselfly (Rantung et. al. 2015), Termites subteran (Ngangi et. al. 2015) and Marine insect Gerridae (Warouw et.al. 2016). Lodging in *Rhychoporus sp* identification using CO1 gene has been done on *Rhychoporus sp* living at Sago plant in Sorong and Raja Ampat Islands, Papua (Mokosuli et. al. 2015). In contrast to previous studies that analyze gene CO1 *Rhychoporus sp* that lived at Sago plants alone. The aims of this study was to get top notch and construction species phylogeny *Rhychoporus sp* that lived on plant Coconut, Aren and Sagu in Minahasa, North Sulawesi.

MATERIALS AND METHODS

Sample

Insects collecting used modified method Cheng *et. al.* (2010), by using neuston net. Collection on the fields area randomly. Insects that have been collected will insert in a bottle sample that has been labeled with place and time of data sampling. The bottle was filled with 95% alcohol for identification and preservation.

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DNA Extraction, PCR Amplification and Sequencing

Total genomic DNA was extracted from *Rhychoporus sp* samples using Axygen Bioscience according to the manufacturer's protocol. PCR was performed in a total volume of 25 μ L containing 1 × reaction buffer, 3 mM MgCl2, 0.24 mM dNTPs, 1.4 μ M of each primer LCO1490 : 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198 : 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et. al., 1994), 1U Go Taq Flexi DNA polymerase (Promega Corp.) and 2.5 μ L of DNA (a 100 time dilution of the original DNA). The PCR program was as follows: 94 °C for 5 min, followed by 40 cycles of 94 °C for 1 min, 48 °C for 1 min and 72 °C for 1 min and a final extension at 72 °C for 5 min. PCR products were purified using Wizard SV Gel and PCR Clean-Up System (Promega Corp). Purified PCR products were analyzed by electro-phoresis in 1% agarose gel. The molecular size of the amplified products was estimated using 1 kbp DNA ladder (Biometra). PCR products were sequenced using AB1 PRISM Dye Terminator Cycle Sequencing Ready Reaction System, version 1.1. (Applied Biosystems) in FIRST BASE Singapura

Sequences Analyses and Phylogeny trees reconstructed

Obtained sequences were aligned using MEGA 6.0 and Geneous 6.0 software. Sequences were subjected to Basic Local Alignment Search Tool (BLAST) in order to perform sequence similarity searches (<u>www.ncbi.nih.gov.com</u>). Nucleotide frequencies were calculated using MEGA 6.0 software (Tamura et. al. 2013). The genetic distances (number of nucleotide substitutions per site) among sequences were calculated using the Maximum Composite Likelihood model in Geneous 6.0 software. Phylogenetic trees were reconstructed using two different reconstructed using the Maximum Composite Likelihood methods: (1) neighbor joining (NJ) and (2) maximum parsimony (MP). The NJ tree was reconstructed using the Maximum Composite Likelihood method. Phylogenetic analyses were conducted in MEGA 6.0 software. Bootstrap support values were obtained by 1,000 replications using both methods (Tamura et. al. 2013).

RESULTS AND DISCUSSION

DNA extraction used the tissue on hind legs of *Rhychoporus sp*. The results of PCR partial CO1 gene visualized by electogram of electrophoresis. Accordingly bands that formed showed the high concentration of amplicons partial CO1 gene in all sample (AR1, KL1 and SG1) (Figure 1).



Figure 1. Visualization CO1 gene PCR amplicons by electrophoresis on 1% agarose gel . AR1 (Rynchoporus sp. From palm, coconut and KL1 of SG1 from Sagu).

The sequencing results were interpreted using Geneous 6.0 software. The Molecular weight of partial CO1 gene sequences of KL1, AR1 and SG1 are 658 bp, 620 bp and 658 bp respectively (Gambar 2). Based of the chromatogram were resulted of the sequencing, showed all sequences of partial CO1 gene are good (Figure 2).

| Consensus | 1 GTCAACCA | 10 AATCATAA | 20 agatattggi | 30 AACTCTATACT | 40 TTTATTTTGG | 50 aacttgagca | 60 ggaatag tag | 70 GGAACTTCTCT | 80 Галбалтасті | 90 Cattégageag | 100 Баас табб ал | 110 AATCCAGGATCATTA |
|-----------------------|------------------------|------------------|--------------------------|-----------------------------|----------------------------|---------------------|---------------------------------------|----------------------------|---------------------|--------------------|---------------------|--------------------------|
| Identity | 1 | 10 | 20 | 30 | 40 1 | 50 ' | 60 ' | 70 | 80 ' | 90 ' | 100 | 110 |
| REV 1. AR1_HC | GTCAACCA | | | | | | | | | | | |
| FWD 2. AR1 LC | | | | × | | | | MAN SGAACTTCTCT | MAN AATACT | | | |
| Consensus | 120 Атсббсба | 130 Атбатсала | 140 TCTATAATG | 150 FTATTGTCACA | 160 Agete atgett | 170 TC ATCATAAT | 180 TTTT¹TT AT ? | 190 Agttataccaa | 200 .TTA TÅATTGI | 210 SGGGTTTTGG | 220 AAATTGATTA | 230 AGTCCCCCTTATATT |
| | 120 | 130 | 140 ' | 150 | 160 ' | 170 | 180 | 190 | 200 | 210 | 220 | 230 |
| REV 1. AR1_HC | | | | | AGCTC ATGCTT | | | | | | | |
| FWD 2. AR1_LC | | | | TATTETCACA | GCTC ATSCTT | | | | | GG GG TTTTGG | | MMMMM AGTCCCCCTTATATT |
| Consensus Identity | 240 AGGGGCCC | 250 СТБАТАТА | 260 GCTTTTCCCC | 270 5 57773 à 773 | 280 ACATAAĠATTC | 290 TGGCTTCTTC | 300 ccccctc TT | 310 ГААСТС ТТСТТ | 320 TTA ATAAGA | 330 Agaattstega | 340 AAAAAGGTGC | 350 Aggaac aggetgaa |
| | 240 | 250 | 260 | 270 | 280 | 290 | 300 | 310 | 320 | 330 | 340 | 350 |
| REV 1. AR1_HC | | 216 | GCTTTTCCCC 226 | | | | | | | | | AGGAAC AGGC TGAA |
| FWD 2. AR1_LC | AGGGGCCC | | GCTTTTCCCC | | WMMM CATAAGATTC | TGGCTTCTTC | | | | | | AGGAAC AGGCTGAA |
| Consensus Identity | 360 CAGTTTAC | 370 CCCCCATT | 380 Agcaggaaa | 390 Igtagee cata | 400 Agagg agc ttc | 410 tg tagac tta | 420 GCTATTTT | 430 AGCCTTCATAT | 440 AGC AGGGAT | 450 CTCTTCAATC1 | 460 TTAGGGGCCA | 470 ATTAACTTTATCTCC |
| REV 1. AR1_HC | | 370 | 380 AGCAGGAAA 346 | | | 410 | 420 GCTATTTTT 386 | | AGC AGGGAT | | | |
| FWD 2. AR1_LC | | | AGCAGGAAA | TGTAGCC CATA | MAGAGG AGCTTC | TG TAGACTTA | GCTATTTT7 | WWWW GCCTTCATAT | | | | MMMMMM TTAACTTTATCTCC |
| Consensus Identity | 480 ACAGCTAT | 490 TTAATATAC | 500 Бассалабо | 510 GCATACTTTCC | 520 GACCGACTTT | 530 CTCTATTTAT | 540 CTGAGCC GT1 | 550 FAGAATCACAG | 560 CCC TTCTTC: | 570 rccttctttc? | 580 Асттестя та | 590 TTAGCTGGAGCCAT |
| REV 1. AR1_HC | 480 ACAGCTAT 446 | | 500 GACCAAAGGO 486 | 510 | 520 GACC GAC TTT 486 | | | 550 | 560 526 526 | | | 590 |
| FWD 2. AR1_LC | | | GACCAAAGGO 820 | | GACC GACTTT | | CTGAGCC GTT | | | | CTTCCTGTT | |
| Consensus Identity | TACTATAC 600 | TATTAACT 610 | GACCGAAA T | atcaatacctc | ATTTTTCGAC | CC AGC AGG AG | GAGGGGACCO | AATTC TTTAT | CAACACTTA | TTTGATTT | TTGGTCACC | GGAAGTTTAA |
| REV 1. AR1_HC | | | GAECGAAATS | | ATTTTTCGAC | | GAGGGGAECC | 636 | 646 1 | 3 656 | 66 | 16 676 |
| FWD 2. AR1_LC | | | | | | | GAGGGGACCO | | | | | GGAAGTTTAA |

Figure 2a . Nigrogen base sequence of the gene CO1 AR1 read used Geneous Program 6.0

| Consensus Identity | 1 10 Ттббтсааса | AAT <mark>CA</mark> TAA. | 20 AGATATTGO | 30 FAACCC TATA1 | 40 | 50 GGTACCTGAG(| 60 CAGGAATAGCI | 70 AGGTACCTCAT | 80 TAAGTATAT | 90 ГААТТСБАБС | 100 TGAATTAGG3 | 110 AAGACC TGGATCTT |
|-----------------------|----------------------------|-----------------------------|-------------------|-----------------------------|--------------------|--------------------|--------------------|---------------------------------------|--------------------|----------------------|-------------------|----------------------------|
| | 1 19 | | 20 | 30 | 40 | 50 | 60 ' | 70 | 80 | 90 | 100 | 110 |
| REV 1 KL 1 HC | AMA | MMM | | MMW | | | MMM | | | | | WWWWW |
| in the line | | | | | 1 | 10 ' | 20 ' | 30 | 40 | 50 | 60 | |
| FWD 2. KL1 LC | | | | | ATTTT | Dichar | | AGGTACCTCAT | M.MM | | | MAGACC TEGATCTT |
| Consensus | 120 1 ТААТТGGTGA | 30 16 atc a aa | 140 TTTATAATO | 150 5 TAATTGTTA C | 160 AGC TCATGC | 170 FTTTATTATA | 180 ATTTTTTTCA | 190 tagttatacct | 200 ATCATAATT | 210 GGAGGATTC G | 220 GAAATTGATT | 230 ragttccactaata |
| Identity | 120 1 | 30 1 | 140 1 | 150 1 | 160 | 170 | 180 1 | 190 | 200 | 210 | 220 | 230 |
| | MMM | MWW | to ANW | Mmm | MMM | mm | www | www | MMM | mm | Mww | wwwww |
| REV 1. KL1_HC | TAATTGGTGA | TGATCA AA | 100 | 110 | AGC TCATGC | 130 | 140 | 150 | 160 | ITO | GAAATTGATT | 190 |
| | MM | MMM | Mww | WW | WWW | WWW | WWW | MMMM | hmm | WWW | MMM | MMMMM |
| FWD 2. KL1_LC | 240 | ТGАТСААА 250 стсатата | 260 | 270 | 280 | 290 | 300 | 310 | 320 | 330 | GAAATTGATT 340 | 350 |
| Identity | 240 | 250 | 260 | 270 | 280 | 290 | 300 | 310 | 320 | 330 | 340 | 350 |
| | ALAAAAAA | Annan 1 Ma | think | MAAAAAAA | 1. AAA AAAAAAA | MANANAN | Manhan | 10000 | AAAAAAAA | In MANAAAA | AMMAAAA | MANA A MANA |
| REV 1. KL1_HC | CTTGGAGCAC | CTGATATA 210 | GCCTTTCC 220 | 230 | ATA TAAGC T 240 | TTTGATTACT 250 | rectecttet | TTAACCCTCTT 270 | ATTAATAAG. 280 | AGAATTGTT 290 | GAAAAAG GAG | SCTGGC ACAGGATG 310 |
| | A.M.M.M.M. | TATA ANA | MMM | MAAAAAA | MAAAAA | MAMAAA | MANA | MMMMM | MMM. | MMMM | MMM | Manadan |
| FWD 2. KL1_LC | CTTGGAGC AC | CTGATATA 370 | GCCTTTCC | CGATTAAATA 390 | ATATAAGCT | TTTGATTACT | A20 | TTAACCCTCTT 430 | ATTAATAAG. 440 | AGAATTGTT 450 | GAAAAAGGAG 460 | CTGGCACAGGATG 470 |
| Consensus Identity | AACTGTTT AT | CCÁCCC TT. | AGC AGC TAI | ATGTEGÉCEAT | AGAGGÁGCC | IC TGTTGATC : | FAGC TAT TTT | TAGTE TŤEATA | TAGCAĠGTA | TTCTTCTAT | TCTAGGÁGCI | FATTAA TİTCATCT |
| | 360 | 1.1. | 380 | 390 | | 410 | 420 | 430 | 440 | | | 410 |
| REV 1. KL1_HC | AACTGTTTAT 320 | CCACCC TT | AGC AGC TAR | ATGTCGCCCAT | | CTGTTGATC | TAGC TAT TTT | TAGTC TTCATA | | TTCTTCTAT | TCTAGGAGCT 420 | |
| | XA. A. AAAA | 1. 11. | | 11. 11. | M.M.M | A.A.A.A.A | . MAXAA MA | 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 | | MM.M.A. | M. M.M.M | 10000 Man 100 |
| FWD 2. KL1_LC | ACTGTTTAT | CCACCCTT. | AGC AGC TA | TGTCGCCCAT | AGAGGAGCC | TC TGTTGATC | TAGCTATTTT | TAGTC TTCATA | TAGCAGGTA | TTCTTCTAT | TCTAGGAGCT | ATTAATTCATCT |
| Consensus Identity | CTACAGCTAT | TAATATAC | GACCCTCAG | GTATATTT | TGACCGAAT | ATCCTTATT | STCTGAGCTG | таасааттаст | GC TATTTA | TATTATTAT | CTTTACC TG | TTCTAG CTGGAGCA |
| | 480 | 490 | 500 | 510 | 520 | 530 | 540 | 550 | 560 | 570 | 580 | 590 UL CJ |
| REV 1. KL1_HC | CTACAGCTAT | | GACCETCA | WWWW GTATATTTT | TGACCGAAT | WWWW ATCCTTATTT | GTCTGAGCTG | MMMMM TAACAATTACT | GC TATTTA | | CTTTAC TGT | MMMMM TTC TAG CTG GAGCA |
| 1851C-1 | 440 | 450 | 460 | 470 | 480 | 490 | 500 | 510 | 520 | 530 | 540 | 550 |
| FWD 2. KL1_LC | CTACAGETAT | | GACCCTCAC | GTATA TTTT | TGACCGAAT | | GTCTGAGCTG | | GC TATTTA | MWWW I TATTATTA T | CTTTACC TGT | TETAG C TGGAGE A |
| Consensus | 600 ATCACTATAT | 610 ТАТТА́АСА | 620 GATCGA 441 | 630 ГАТТАА ТАСТИ | 640 CATTTTTG | 650 ATCCTGCTGG | 660 Aggaggagati | 670 CCCATTCTTA | 680 TC AGC ATTT | 690 TTTTGATTT | 700 TTTGGTCGCC | 709 |
| laoning | 800 T. A | 610 | 620 | 630 | 640 | 650 A | 660 1 | 670 | | | | |
| | WWW | MM | WWW | WWW | land | town | 0.000 | 2003 | | | | |
| T. KLI_HG | 560 | 570 | 580 | 590 | 600 | 610 | 620 | 630 | 640 | 650 | 660 | 669 1 |
| FWD 2, KL1 LC | | | GATCGAAA | | CATTTTG | | | | MMMoto | MMM TTTTGATTT | TTTGGTCGC | ANT ANA |

Figure 2b . Nigrogen base sequence of the gene CO1 KL1 read used Geneous Program 6.0

| Consensus Identity | 1 10 2 ТТТБСТС ААС <mark>АААТС</mark> АТААЛ | 0 30 Agatattggaacccta: | 40 TATTTTATTTTT TATTTTTT | 50 ggtac ctgage. | 60 70 аф датарс ар фта | 80 .cc tc atta ag tat. | 90 ATTA ÅTTCGAGCT | 100 геалттасса | 110 AGACC TGGATCT |
|-----------------------|--|--------------------------------|--|---------------------------|--|------------------------------------|--------------------------|---------------------|----------------------|
| , | | 0 30 | 40 ' | 50 | 60 70 | 80 | 90 ' | 100 | 110 |
| REU 1 801 HCO ab1 | MMMM | www.www.ww | <u>www.</u> | MMMM | MMMMM | MMMM | wwww | MMM | MMMM |
| New 1. 301_HCO.ab1 | TILEVIC ANCAMPLE ATAM | WATATIOWACCCIA. | 1 4 | 14 | 23 32 | 42 | 52 | 62 | |
| 500 2 801 L CO ab1 | | | M? | De War | A Anna ho | Whomas | MMM | WWW | MAM MM |
| Consensus | 120 130 | 140 150 ГТТАТААТСТА АТТСТ | 160 TACAGCTCATGC | 170 TTTTATTATAA | 180 190 TTTTTTCATAGTT | 200 ATACCTATCATAA | 210 TTGG AGGATTCG | 220 AAATTG ATT | 230 AGTTC CACTAAT |
| Identity | 120 130 | 140 150 | 160 | 170 | 180 190 | 200 | 210 | 220 | 230 |
| | 11111111111111111111111111111111111111 | MAAAM. MAAAAAA | A. A | MAAAAAAAAA | ANANA TAAAAAA | MaMaaMaaMa | MMMM | MANAAA | ANATATAAAAAA |
| REV 1. SG1_HCO.ab1 | TTAATTGGTGATGATCAAAT 82 92 | TTT ATAATGTA ATTGT 102 112 | TACAGCTCATGC 122 | TTTTATTATAA 132 | TT TT | ATACCTATCATAA 162 | TTGG AGGATTCG C | AAATTGATT 182 | AGTTCCACTAAT 192 |
| | the att hallow | MAAAAA LAANK | M. timesta | | M. M. M. M. M. | AAAAAAAAAA | MATTATA | MARAMAAA | MANAMA HAMAN |
| FWD 2. SG1_LCO.ab1 | TTAATTGGTGATGATCARAT 240 250 | 260 270 | TACAGCTCATGC | TTTTATTATAA 290 | TTTTTTTCATAGTT 300 31 | ATACCTATCATAA 0 320 | TTGGAGGATTCGC 330 | SAAATTGATT | AGTTC CACTAAT |
| Consensus Identity | ACTTGGA GCACCTGA TATAG | GCC TTTCCACG ATTAA | атаататаадст | TTTGÅTTACTT | CC TCCTTCTTTAAC | сс теттатта ата. | AGAAGAATTGTT | БАЛАЛА GAG | CTGGC ACAGGAT |
| | 240 250 | 260 270 | 280 | 290 | 300 31 | 0 320 | 330 | 340 | 350 |
| REV 1. SG1 HCO.ab1 | | WWWWWW | | MMMM TTTGATTACTT | WWWWW | MMMMM CC TC TT ATTA ATA | MMMM AGAAGAATTGTT | | CTGGC AC AGGAT |
| 0.110.050 | | 222 232 | 242 | 252 | 262 27 | 2 282 | 292 | 302 | 312 |
| FWD 2. SG1 LCO.ab1 | | CC TTCCACG ATTAA | | TTTGATTACTT | MMMMMM CETECTTCTTTAAC | MMMMMM CC TCTTATTAATA | AGAA GAATTGTT | | TGGC AC AGGAT |
| Consensus | 360 370 GAÁCTGTTTATCCACCCTT | 380 390 AGC AGCTAATG TCGCC | 400 CATAGAGGAGCC | 410 тстбт тбатст. | 420 4 AGCTATTTTTAGTC | 30 440 ††¢ atatage ågg * | 450 FATTTCTTCTATT | 480 CTAGGAGCT | 470 ATTAA TTTCATC |
| Identity | 360 370 | 380 390 | 400 | 410 | 420 4 | 30 440 | 450 | 460 1 | 470 |
| | MMMMM | MMMMM | MMMM | MMM | MMMMM | MMMM | www | WWW | mmm |
| REV 1. SG1_HCO.ab1 | GAACTGTTTATCCACCCTT 322 332 | GCAGCTAATGTCGCC 342 352 | CATAGAGGAGCC | TCTGTTGATCT. 372 | AGCTATTTTTAGTC 382 3 | TTCATATAGCAGG 92 402 | 412 | 422 | ATTAA TTTCATC 432 |
| | MMMMM | handhan | MMMM | MMM | MMMMM | mmm | www | MMM | mmm |
| FWD 2. SG1_LCO.ab1 | GAACTGTTTATCCACCCTT 480 490 | AGC AGCTAATG TCGCCI 500 510 | CATAGAGGAGCC | TCTGTTGATCT. 530 | AGCTATTTTTAGTC 540 | ТТСАТАТАGСАGG 550 560 | 570 | 580 | ATTAA TTTCATC |
| Identity | 490 400 | 500 510 | FTCTGACCGAAT. | ATCCTTATTTG | 540 | 550 580 | 570 | SOD | TCTAGCTGGAGC |
| | | | | | | | | | |
| REV 1. SG1_HCO.ab1 | | | TTCTGACCGAAT. | ATECTTATTTG | TC TGAGCTG TAACA | ATTACTGCTATT | | TTTACC TGT | TC TAGCTGGAGC |
| | ALLAN A. MANANAMANA | M. AAAA | MA. MANJAN | AAAAA AAAAA | | AAAA | | LALAN L | 14141.1/14 |
| FWD 2. SG1_LCO.ab1 | TCTACAGCTATTAATATAC | WWWWWWWWW FACCCTCAGGTATATT | TTCTGACCGAAT. | ATCCTTATTTG | TC TGAGC TG TAACA | WVVVW MVVW | VWY WWWW FATTATTATTAT | WWWWW TTTACC TGT | TCTAGCTGGAGC |
| Consensus Identity | BUU BIU AATCACTATATTATTAACAG | 620 630 GATEGAAATATTAATA | CTACATTTTTTG. | ATCCTGCTGGA | 660 GGAGGAGATCCCAT | 670 680 TC TTTATCAG CAT | TATTTGATTT | TTGGTC ACC | CTGGA AGTTT |
| | | 620 630 | | 650 | 662 | | | | |
| REU 1 801 HCO ab1 | MMMMM | MMMMM | WM 20 | Alanda | 20000 | | | | |
| 1.001_HC0.ap1 | 562 572 | 582 592 | 2 602 | 612 | 622 | 632 642 | . 652 | 662 | 674 |
| FWD 2 SG1 LCO ab1 | | MMMMMM | | ATCETECTEC | MMMMMM | | | WWW | CTEEAAETT |
| 2.001_L00.db1 | | | | | | | | | |

Figure 2c. Nigrogen base sequence of the gene CO1 SG1 read used Geneous Program 6.0

BLAST analysis results AR1 CO1 gene sequences showed the highest degree of similarity with *Rynchophorus vulneratus* accesion number [LN 612634.1][(Table 1). On the other hand partial CO1 gene sequences of KL1 and SG1 respectively showed the highest degree of similarity with *Rynchophorus cruentatus* accesion number [AY131113.1] (Table 2 and Table 3).

Table 1 The percentage of sequence similarity AR1 CO1 gene sequences compared with the top ten recorded in the NCBI gene bank (www.ncbi.nih.gov/blast.com)

| Description | Max score | Total score | Query cover | E value | Ident | Accession |
|--|--------------|-------------|----------------|------------|-------|------------|
| Rhynchophorus vulneratus mitochondrial partial COI gene for cytochrome oxidase subunit 1, isolate RV01A01 | 1024 | 1024 | 94% | 0.0 | 98% | LN612634.1 |
| Rhynchophorus vulneratus mitochondrial partial COI gene for cytochrome oxidase subunit 1, isolate RV01A02 | 1013 | 1013 | 96% | 0.0 | 97% | LN612635.1 |
| Rhynchophorus vulneratus mitochondrial partial COI gene for cytochrome oxidase subunit 1, isolate RV01A03 | 1000 | 1000 | 92% | 0.0 | 98% | LN612636.1 |
| Rhynchophorus vulneratus isolate RED1111 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | 664 | 664 | 61% | 0.0 | 98% | KF311631.1 |
| Rhynchophorus vulneratus isolate RED939 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | 664 | 664 | 61% | 0.0 | 98% | KF311629.1 |
| Rhynchophorus vulneratus isolate RED253 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | 664 | 664 | 61% | 0.0 | 98% | KF311567.1 |
| Rhynchophorus vulneratus isolate RED1144 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | 658 | 658 | 61% | 0.0 | 98% | KF311633.1 |
| Rhynchophorus vulneratus isolate RED935 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | 658 | 658 | 61% | 0.0 | 98% | KF311628.1 |
| Rhynchophorus vulneratus isolate RED843 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | 658 | 658 | 61% | 0.0 | 98% | KF311621.1 |
| Rhynchophorus vulneratus isolate RED823 cytochrome c oxidase subunit I (COI) gene, partial eds: mitochondrial | 658 | 658 | 61% | 0.0 | 98% | KF311617.1 |

Tabel 2. The percentage of sequence similarity KL1 CO1 gene sequences compared with the top ten recorded in the NCBI gene bank (www.ncbi.nih.gov/blast.com)

| Description | Max score | Total score | Query cover | E value | Ident | Accession |
|--|--------------|----------------|----------------|------------|-------|------------|
| Rhynchophorus cruentatus cytochrome oxidase subunit I (COI) gene, partial ods; mitochondrial gene for mitochondrial product | 693 | 693 | 99% | 0.0 | 86% | AY131113.1 |
| Dinoptera collaris voucher GBOL_Col_FK_6727 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | 669 | 669 | 99% | 0.0 | 85% | KM445325.1 |
| Dinoptera collaris voucher BFB_Col_FK_9033 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | 664 | 664 | 99% | 0.0 | 85% | KM439730.1 |
| Nosodendron fasciculare voucher BFB_Col_FK_8752 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | 662 | 662 | 100% | 0.0 | 85% | KM452239.1 |
| Myrmechixenus vaporariorum voucher ZMUO <fin>000424 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial</fin> | 662 | 662 | 100% | 0.0 | 85% | KJ961927.1 |
| Pterostichus cristatus voucher GBOL_CoL_FK_5123 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | 658 | 658 | 100% | 0.0 | 85% | KM444764.1 |
| Protapion ruficrus voucher GBOL_CoL_FK_3091 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | 658 | 658 | 99% | 0.0 | 85% | KM443977.1 |
| Pterostichus cristatus voucher GBOL_CoL_FK_4257 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | 654 | 654 | 100% | 0.0 | 85% | KM440434.1 |
| Protapion ruficrus voucher BFB_Col_FK_9071 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | 652 | 652 | 99% | 0.0 | 85% | KM445949.1 |
| Tachinidae gen. tachJanzen01 sp. Janzen01 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial | 652 | 652 | 99% | 0.0 | 85% | JQ576361.1 |

Tabel 3. The percentage of sequence similarity SG1 CO1 gene sequences compared with the top ten recorded in the NCBI gene bank (www.ncbi.nih.gov/blast.com)

| Description | Max score | Total score | Query cover | E value | Ident | Accession |
|---|--------------|-------------|----------------|------------|-------|------------|
| Rhynchophorus cruentatus cytochrome oxidase subunit I (COI) gene, partial ods; mitochondrial gene for mitochondrial product | 693 | 693 | 99% | 0.0 | 86% | AY131113.1 |
| Dinoptera collaris voucher GBOL_Col_FK_6727 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | 669 | 669 | 99% | 0.0 | 85% | KM445325.1 |
| Dinoptera collaris voucher BFB_Col_FK_9633 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | 664 | 664 | 99% | 0.0 | 85% | KM439730.1 |
| Nosodendron fasciculare voucher BFB_Col_FK_8752 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | 662 | 662 | 100% | 0.0 | 85% | KM452239.1 |
| Myrmechixenus vaporariorum voucher ZMUO <fin>:000424 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial</fin> | 662 | 662 | 100% | 0.0 | 85% | KJ961927.1 |
| Pterostichus cristatus voucher GBOL_Col_FK_5123 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | 658 | 658 | 100% | 0.0 | 85% | KM444764.1 |
| Protapion ruficrus voucher GBOL_Col_FK_3091 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | 658 | 658 | 99% | 0.0 | 85% | KM443977.1 |
| Pterostichus cristatus voucher GBOL_Col_FK_4257 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | 654 | 654 | 100% | 0.0 | 85% | KM440434.1 |
| Protapion ruficrus voucher BFB_Col_FK_9871 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | 652 | 652 | 99% | 0.0 | 85% | KM445949.1 |
| Tachinidae gen. tachJanzen01 sp. Janzen01 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial | 652 | 652 | 99% | 0.0 | 85% | JQ576361.1 |

Analysis of Sequences partial CO1 gene

Analysis of transition and transversion (R = 0.72) with a model Maximum likelihood used MEGA 6,0; obtained frequency of nucleotides A = 25 %, T = 25 %, C = 25 % and 25 % Guanine. The forms of nucleotide substitution are shown in Table 4.

Table 4. Nucleotides substitution form of partial CO1 gene of AR1, KL1 and SG1

| tes substitution form of partial COT gene of ART, RET and SOT | | | | | | | | | | |
|---|---------|---------|---------|---------|--|--|--|--|--|--|
| From\to | А | Т | С | G | | | | | | |
| А | - | 7.2727 | 7.2727 | 10.4546 | | | | | | |
| Т | 7.2727 | - | 10.4546 | 7.2727 | | | | | | |
| С | 7.2727 | 10.4546 | - | 7.2727 | | | | | | |
| G | 10.4546 | 7.2727 | 7.2727 | - | | | | | | |

According to table 5, the similarity of *Rhynchoporus sp.* showed that KL1 had 19,5 % different with SG1 and AR1 respectively. SG1 and AR1 based of the genetic distance analysis are the same species (Table 5). Table 5. Genetic distances among nucleotide sequences from *Rhynchophorus* spp. based on the

| | pairwise analysis of CO1 gene. sequences. | | | | | | | | | | | |
|-----|---|------|------|------|------|------|------|------|------|------|-----|--|
| No. | Sample | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1 | AR1 | 100 | | | | | | | | | | |
| 2 | KL1 | 80.5 | 100 | | | | | | | | | |
| 3 | SG1 | 80.5 | 100 | 100 | | | | | | | | |
| 4 | Nosodendron fasciculare | 78.6 | 84.7 | 84.7 | 100 | | | | | | | |
| 5 | Dinoptera collaris | 79.5 | 84.7 | 84.7 | 85.4 | 100 | | | | | | |
| 6 | Rhynchoporus palmarum | 82.2 | 81.8 | 81.8 | 81.7 | 80.8 | 100 | | | | | |
| 7 | Rhynchoporus bilineatus | 91.5 | 81.2 | 81.2 | 78.4 | 76.2 | 83.7 | 100 | | | | |
| 8 | Rhynchoporus vulneratus 1 | 97.7 | 78.4 | 78.4 | 75.7 | 74.7 | 82.1 | 92.4 | 100 | | | |
| 9 | Rhynchoporus vulneratus 2 | 98 | 80.7 | 80.7 | 78.5 | 79.2 | 82.3 | 92.2 | 98 | 100 | | |
| 10 | Rhynchoporus cruentatus | 79.7 | 81.2 | 81.2 | 76.5 | 78.3 | 85 | 85.1 | 85.3 | 84.9 | 100 | |

Genetic variation in partial CO1 gene was supported by the results of previous studies, the morphometric analysis of KL1, AR1 dan SG1 were found some differences in morphometric characters among other forms of pronotum and color strip on the tip of the antenna on Rhynchoporus sp. thatch beige and Rhynchoporus sp. the black palm (Korua et al. 2015). Polimorfisme can occur in a population when more than one morphological variations at the same location and time (Ford, 1965, Abad et. Al. 2014). In case of random mating and every individual has the potential to mate, then morphological changes can take place in a population (Abad et. Al. 2014). Research conducted at Rhynchoporus Rhynchoporus ferrugineus Oliver and Rhynchoporus schach in the central and southern Philippines found that polymorhism were major factor morphology modifications of the Rhynchoporus sp. Morphological modifications are also found in Rhynchoporus phonicis in Cameroon (Abad et. al., 2014: Tambe et. al. 2013).

In previous studies conducted by the author had founded morphological modifications of imago Rhynchoporus sp. which livet on a palm tree, palm and sago palm in Minahasa, North Sulawesi Province (Korua, 2015). The results of this study imply that *Rhynchoporus sp.* has a high ability to adapt in their environment.

Reconstruction of Phylogenetic tree based partial COI gene.

Nucleotide sequences were used to construct this tree. Construction phylogeny performed using three models namely Maximum Likelihood (ML), Neighbor-Joining (NJ) and Minimum Evolution (ME) (Tamura et. al., 2013, Kimura, 1980) (Figure 3). Three models are used to determine whether there differences in phylogenetic relationships AR1, KL1 and SG1 when the tree phylogeny constructed with different models.



(c)

Figure 3. Comparison of Construction Phylogeny CO1 sequences AR1, KL1 and CO1 CO1 SG1 with three types of models (a) ME, (b) ML and (c) NJ

Reconstruction of the phylogeny tree was also performed using BLAST sequence results at www.ncbi.nih.gov/blast.com. Phylogeny tree constructed using the Neighbor Joining method using Geneous 6.0 program. KL1 and SG1 form one node but still be monophyletic with Rhynchoporus palmarum and Rhynchoporus cruentatus, But the phylogeny tree had formed, showed KL1 and SG1 closer relationship with Rhynchoporus palmarum. Different from KL1 and SG1, partial CO1 gene of AR1 had formed node with Rynchophorus

vulneratus (Figure 4).



0.04

Figure 4. Tree phylogeny Rhynchoporus sp. (KL1 = Coconut, SG1 = Rumbia 1, AR1 = Aren).

CONCLUSIONS

From the results of the present study, based on partial CO1 gene, note that *Rhynchoporus* living at the *Arenga pinata* is *Rhynchoporus palmarum* while living on Sago palm (*Metoxylan sagu*) and Coconut (*Cocos nucifera*) are *Rynchoporus* vulneratus.

REFERENCES

- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*. 3, 294-297.
- Hebert PDN, Cywinska A, Ball SL, deWaard JR. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society* London B 270 (2003) 313-321.
- K. Tamura, M. Nei, S. Kumar, Prospects for inferring very large phylogenies by using the neighbor-joining method, *Proceedings of the National Academy of Sciences* (USA) 101 (2004) 11030-11035.
- K. Tamura, J. Dudley, M. Nei, S. Kumar, MEGA4:molecular evolutionary genetics analysis (MEGA)software version 4.0., *Molecular Biology and Evolution* 24(2007) 1596-1599.
- Mokosuli YS. 2013. Karakter Morfologi, Sumber Pakan dan Bioaktivitas farmakologis Racun lebah madu endemic Sulawesi Apis dorsata Binghami dan Apis nigrocincta Smith (Hymenoptera : Apidae). [Disertasi]. Program Pascasarjana Universitas Sam Ratulangi.
- Mokosuli YS, Worang RL, Paskhalina, Dimara A. 2016. Konstruksi Filogeni *Rhynchophorus spp*.Dari Tanaman Sagu di Sorong dan Kepulauan Raja Ampat Papu. Laporan Penelitian Penerapan Ipteks. Lembaga Penbelitian Universitas Negeri Manado.

- Rantung R, Rondonuwu ST, Tulung M, Mantiri FR, Mokosuli YS. 2015. Character of cytochrome oxidase 1 gene (CO1) in mitochondrial DNA Damselfly *Agriocnemis femina* from linow lake, tondano lake and moat lake at north Sulawesi. *Advances in Life Science and Technology* Vol. (38) : 40-53
- Tamura K, Stecher G, Peterson P, Filipski A and Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular. Biology and Evolution. 30(12):2725–2729 doi:10.1093/molbev/mst197
- Warouw V, Salaki C, Mangindaan REP, Tulung M, Maramis RTD, Mokosuli YS. 2016. Isolation and Characterization of Partial Mitochondrial CO1 Gene from Marine Insect Gerridae, Stenobates biroi from Mokupa Beach Manado, North Sulawesi Indonesia *Journal of Biology, Agriculture and Healthcare* Vol.6, No.6, 2016