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Isolation and Characterization of Partial Mitochondrial CO1 Gene from Marine Insect Gerridae, Stenobates biroi from Mokupa Beach Manado, North Sulawesi Indonesia

Veibe Warouw[1]* Christina Salaki[2] Remmy E.P Mangindaan[2] Max Tulung[2]
Resway T. D Maramis[2] Mokosuli Yermia Semuel[3]
1.Postgraduate student, Departement of Entomology, Postgraduate Proggrame, Sam Ratulangi University, Manado, Indonesia
2. Departement of Entomology, Postgraduate Proggrame, Sam Ratulangi University, Manado, Indonesia
3. Laboratory Bioactivity and Moleculer Biology, Departement of Biology, State University of Manado

Abstract
Characterization of mitochondrial DNA gene cytochrome oxidase subunit 1 (CO1) of marine insects Gerridae from the Manado beach, North Sulawesi Indonesia after further extracted DNA was amplified by PCR, electrophoresis and sequenced, then the results of CO1 sequences is entered to BLAST program to get the level of homology with sequences from the NCBI gene bank, the results only turned out to have the highest degree of homology of ≤87% with sequences obtained. The results showed that the gene sequences of cytochrome oxidase 1 (CO1) of insects Gerridae sea from the beach Mokupa, North Sulawesi is not the same as other Gerridae marine insects have been recorded and published in the NCBI gene bank.
Keywords: Marine Insect, Gerridae, CO1

INTRODUCTION

The oceans always have held a great fascination to us. Many great voyages were launched to explore the oceans and what lies beyond. A great variety of marine organisms were collected and described during these voyages, but insects appear to have received little attention. Marine habitats can be divided either by salinity or by their position relative to the tidal level (Andersen, 1994; Cheng, 1985; Cheng 1989).

Insects are the dominant group of animals on the earth, they far outnumber all other terrestrial animal, and they occur practically everywhere. Although they are the most abundant animal on the land, insects are relatively rare in marine environments. However, a few thousand insect species belonging to more than 10 family are considered to be marine. The majority of marine insect belong to Coleoptera, Hemiptera, and Diptera, and they can be found in various marine habitats. However, the only insects to live in the open ocean are member of genus Halobates. They belong to Order Hemiptera, family Gerridae. This insect is a tiny organism, measuring only about 0.5 to 1 cm in body length, but they have rather long legs and may have a leg span of 1.5 cm or more, they totally wingless at all stages of their life cycle and are confined to the air-sea interface. Better known as the sea skater or ocean strider because of they ability to walk on the water They are evidently able to avoid UV damage by having cuticles which absorb harmful wavelengths. The exact nature of the UV-absorbing substance is unknown, but it has been shown to contain microsporin-like amino acids surface (Cheng *et. al*. 2001; Andersen 1998; Andersen, 2004).

One method that can be used to trace the phylogenetic relationships of a species that has been accepted universally accuracy is seeing similarities mitochondrial DNA (mtDNA). Much of the research for the study of both animal phylogeny invertebrate and vertebrates using CO I mtDNA genes as markers / genetic barcode. COI gene is a gene that can be used as genetic markers, molecular studies to study the genetic characteristics between species and between individuals (Folmer et al ., 1994).

North Sulawesi save a lot of richness in diversity of marine life that has not been excavated, one of which is data on the presence of insects that live in marine waters of North Sulawesi, especially from Family Gerridae. So it should be a molecular study to get the gene CO1 mtDNA genetic diversity of insects that live in the marine habitat of North Sulawesi and compare with CO1 gene sequences that have been recorded in the NCBI gene bank. Until now, there is no data characteristics insects CO1 gene from North Sulawesi so that the results of this study will provide important information about the genetic characteristics of insects that inhabit the coast of North Sulawesi in particular from the family Gerridae .

MATERIALS AND METHOD

Sampling Methods

Insects collecting used modified method Cheng et. al. (2010), by using neuston net. Collection on the beach and mangrove 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.

DNA identification

DNA identification will be extracted and isolated by using genomic DNA multisource AxyPrep Miniprep Kit, Axygen Biosciences, and the primer CO1, LCO1490 : 5'-GGTCAACAAATCATAAAGATATTGG-3' dan HCO2198 : 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et. al., 1994) gene to be amplified using PCR.

DNA Extraction and Isolation

Insects collected, store frozen in the freezer. Extraction will be preced by entering the insect in ependorf add with 350 µl DH2O then destroy by pestel, sheker for 1 min. DNA isolation will use isolation procedures from Axygen Bioscience, first to wash add 350 µl PBS to ependorf contain insects than add 0.9 µl RNAase to wash the RNA , then homogenized using vortex for 30 sec. 20 µl Proteinase K and 150 µl Buffer will add to destroye protein and than homogenize using vortex for 1 min. Incubation will be conduct for 15 min at 56 ° C using a heater. Add 350 µl of P-D buffer and in homogenize using vortex for 1 min. Centrifuge at 12 000 xg done for 10 min. Supernatant will add to the Miniprep column that already in a 2 ml microfuge tube, then be centrifuge for one min at 12 000 xg. Dischard the filtrate and put the centrifuge Miniprep in new 2 ml microfuge tube. Add 700 µl buffer W1, then centrifuged for 1 min at 12.000 xg, discard the filtrate and add with 700 µl buffer W2, and centrifuged again for 1 min at 12 000 xg. Precipitates will moved to the mini Colom in 1.5 microfuge tube, add 100 µl eluent then centrifuged for 1 min at 12 000 xg after being allowed to stand at room temperature for 1 min.

PCR amplification and Sequensing

DNA will amplification using PCR with primers CO1 (Folmer *et .al*, 1994) (This reference is absent in reference list), 10 µl supernatant will add to ependorf and then immediately add with revers primer 2 µl and forward primers , 1 µl Mg Cl $_{2,}$ 25 µl taqpol2xmastermix, 10 µl DNA template and 11 µl DH_2O. The PCR reaction program consist of 35 cycle of 94^oC redenaturasi conditions for 5 min, denaturation 94^oC for 45 sec, 54^oC for 45 sec annealing, extension 72 ° C for 1 min, final extension 72 ° C for 5 min, the product will visualize by gel electrophoresis. Sequencing results will be read by the BLAST method and compared with gene bank for the species of insects sampled.

RESULT AND DISCUSSION

S.10.M marine insect samples are found in coastal areas Mokupa Beach Manado, with a high population in mangrove areas are covered by *Avicenia* sp. This sample like a quiet area and very little is found in sandy areas. S.10.M samples use a tension of surface water to move. According to Cheng (1976) this insect has a sort of waxy coating on the soles of the feet, making it easier to walk on water. With long legs and a small body (as shown in Figure 1), allows the insect does not sink when walking on water.

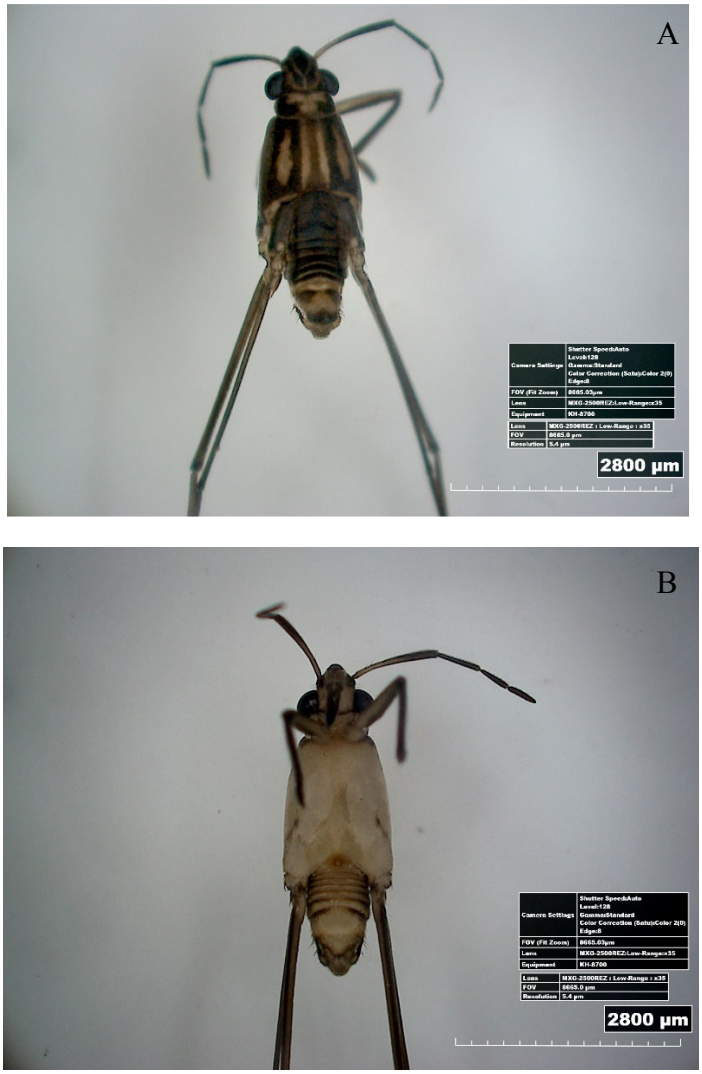


Figure 1. Marine insects S.10.M sample found in coastal areas Mokupa Beach Manado
A. Dorsal view
B. Ventral view

DNA extracted from marine insects S.10.M, that have been preserved in 95% alcohol, isolated using genomic AxyPrep multisource DNA Miniprep Kit, Axygen Biosciences, and by using a primer CO1, LCO1490: 5'-GGTCA AATCATAAAGATATTGG ACA-3 'and HCO2198: 5' –TAAACTTCAGGGTGACC AAAAAATCA-3 ', then amplified using PCR, amplification by PCR results then visualized using electrophoresis with the results as shown in Figure 2.

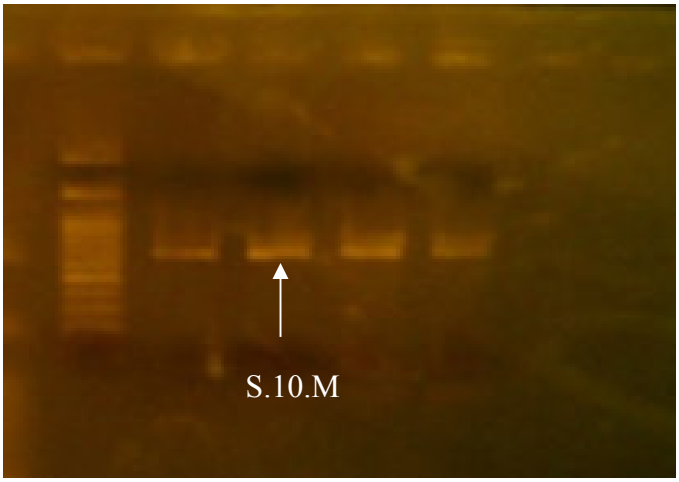


Figure 2. Visualization of mitochondrial DNA from marine insect S.10.M samples were amplified with primers CO1

DNA sequencing results from First Base CO. Malaysia turns marine insect S.10.M samples, after alignment use geneous program (Figure 3) in length as much as 658 bp DNA fragment sequences with a sequence as shown in Figure 4.

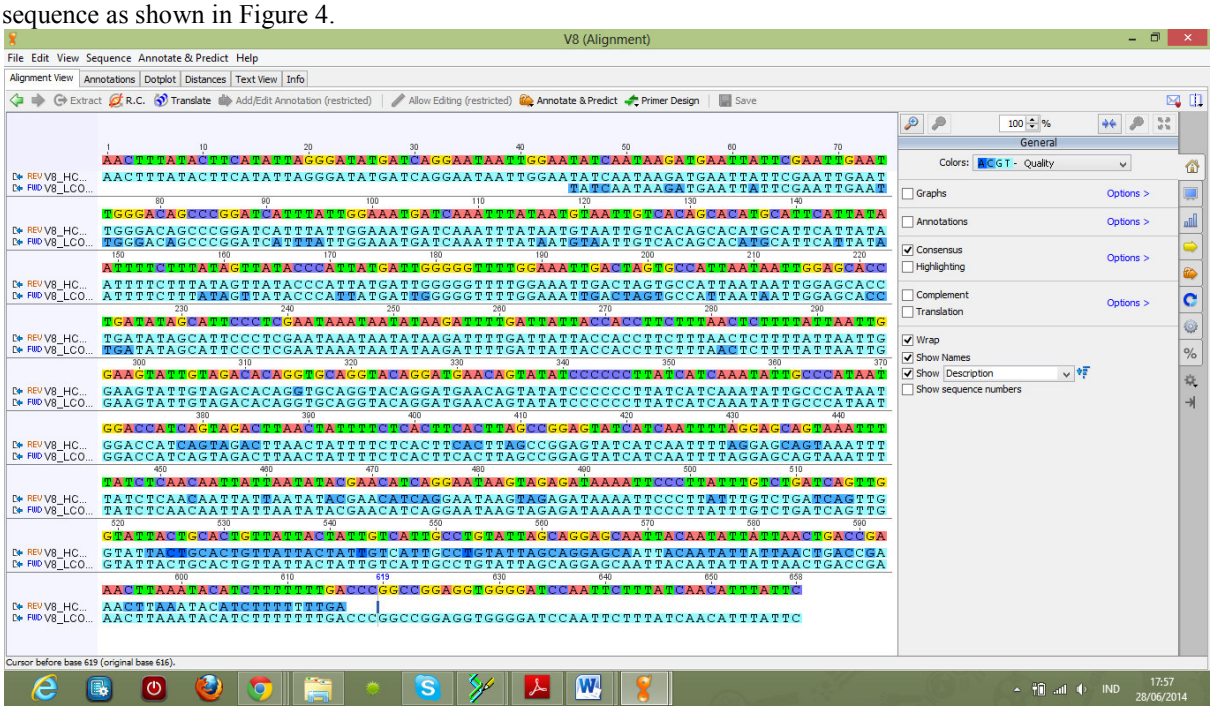


Figure 3. Alignment use geneous program

AACTTTATACTTCATATTAGGGATATGATCAGGAATAATTGG
AATATCAATAAGATGAATTATTCGAATTGAATTGGGACAGCC
CGGATCATTTATTGGAAATGATCAAATTTATAATGTAATTGTC
ACAGCACATGCATTCATTATAATTTTCTTTATAGTTATACCCAT
TATGATTGGGGGTTTTGGAAATTGACTAGTGCCATTAATAAT
TGGAGCACCTGATATAGCATTCCCTCGAATAAATAATATAAG
ATTTTGATTATTACCACCTTCTTTAACTCTTTTATTAATTGGAA
GTATTGTAGACACAGGTGCAGGTACAGGATGAACAGTATAT
CCCCCCTTATCATCAAATATTGCCCATAATGGACCATCAGTAG
ACTTAACTATTTTCTCACTTCACTTAGCCGGAGTATCATCAATT
TTAGGAGCAGTAAATTTTATCTCAACAATTATTAATATACGAA
CATCAGGAATAAGTAGAGATAAAATTCCCTTATTTGTCTGAT
CAGTTGGTATTACTGCACTGTTATTACTATTGTCATTGCCTGT
ATTAGCAGGAGCAATTACAATATTATTAACTGACCGAAACTT
AAATACATCTTTTTTTGACCCGGCCGGAGGTGGGGATCCAAT
TCTTTATCAACATTTATTC

Figure 4. The consensus sequence of the DNA fragment sequences marine insects S.10.M By using primer CO1

Results of DNA fragment sequences of genes cytochrome oxidase 1 (CO1) of marine insects S.10.M samples were analyzed with similar data that has been more publicized in the gene bank. Analysis is conducted alignment analysis that compares CO1 gene sequences from marine insect S.10.M samples obtained by CO1 gene sequences from other organisms that have been recorded in the gene bank. The program used for the analysis is the alignment program BLAST (Basic Local Allignment Search Tools). This program can be accessed through the website of the National Center for Biotechnology Information at the National Library of Medicine in Washington, DC (http: //www.ncbi.nlm.nih .gov / BLAST). BLAST results obtained 58 data from gene banks that have high levels of identic / homology> 87%, as illustrated in Figure 6.

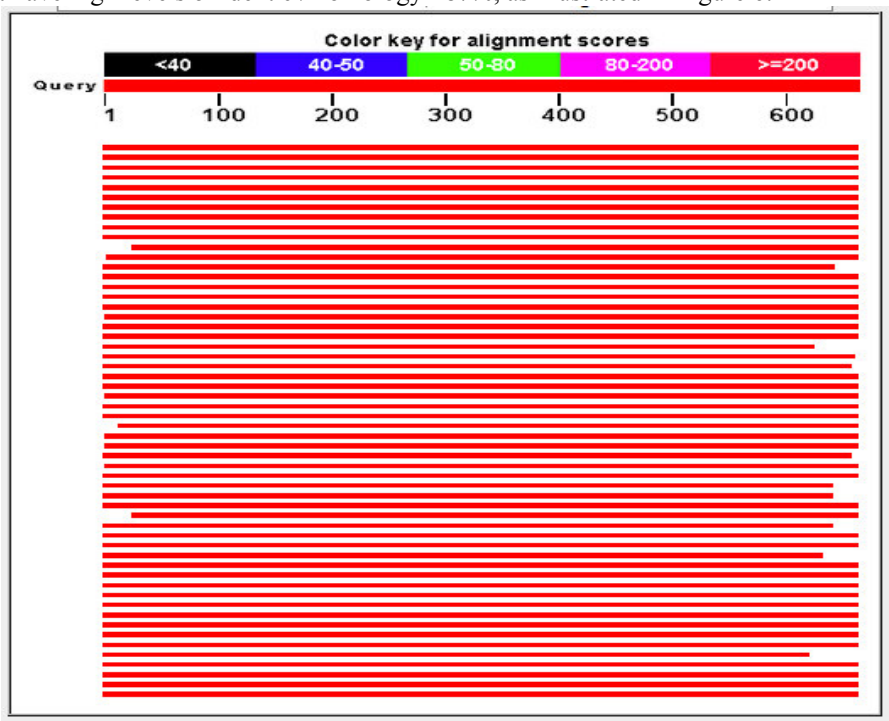
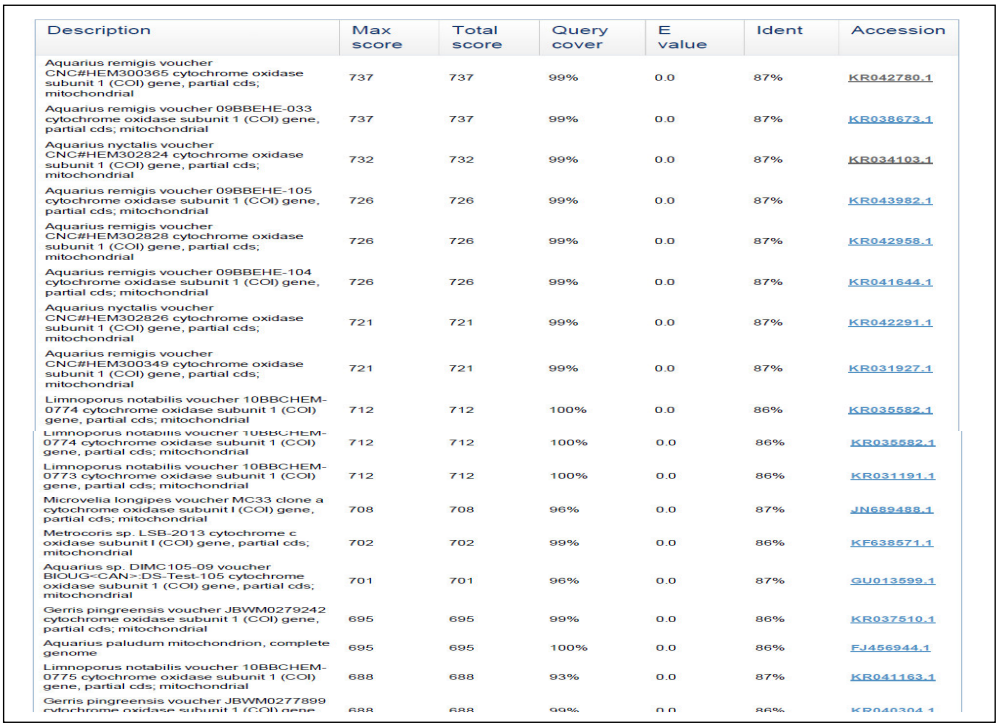


Figure 5. Distribution of 100 Blast Hits on the query sequences of marine insects S.10.M By using primer CO1



Description	Max score	Total score	Query cover	E value	Ident	Accession
Aquarius remigis voucher CNC#HEM300365 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	737	737	99%	0.0	87%	KR042780.1
Aquarius remigis voucher 09BBEHE-033 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	737	737	99%	0.0	87%	KR038673.1
Aquarius nyctalis voucher CNC#HEM302824 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	732	732	99%	0.0	87%	KR034103.1
Aquarius remigis voucher 09BBEHE-105 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	726	726	99%	0.0	87%	KR043982.1
Aquarius remigis voucher CNC#HEM302828 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	726	726	99%	0.0	87%	KR042958.1
Aquarius remigis voucher 09BBEHE-104 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	726	726	99%	0.0	87%	KR041644.1
Aquarius nyctalis voucher CNC#HEM302826 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	721	721	99%	0.0	87%	KR042291.1
Aquarius remigis voucher CNC#HEM300349 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	721	721	99%	0.0	87%	KR031927.1
Limnoporus notabilis voucher 10BBCHEM-0774 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	712	712	100%	0.0	86%	KR035582.1
Limnoporus notabilis voucher 10BBCHEM-0774 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	712	712	100%	0.0	86%	KR035582.1
Limnoporus notabilis voucher 10BBCHEM-0773 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	712	712	100%	0.0	86%	KR031191.1
Microvelia longipes voucher MC33 clone a cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	708	708	96%	0.0	87%	JN689488.1
Metrocoris sp. LSB-2013 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	702	702	99%	0.0	86%	KF638571.1
Aquarius sp. DIMC105-09 voucher BIOUG<CAN>:DS-Test-105 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	701	701	96%	0.0	87%	GU013599.1
Gerris pingreensis voucher JBWM0279242 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	695	695	99%	0.0	86%	KR037510.1
Aquarius paludum mitochondrion, complete genome	695	695	100%	0.0	86%	FJ456944.1
Limnoporus notabilis voucher 10BBCHEM-0775 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	688	688	93%	0.0	87%	KR041163.1
Gerris pingreensis voucher JBWM0277899 cytochrome oxidase subunit 1 (COI) gene	688	688	99%	0.0	86%	KR040304.1

Figure 6. The level of similarity CO1 gene sequences (658 bp) from the marine insects S.10.M with the data in the gene bank NCBI (www.ncbi.nlm.nih.gov).

For further analysis only focused on organisms with the level of homology / indentic ≥ 87%. Results of the analysis with the highest level of 87% identical in some marine insects of the family Gerridae recorded in the NCBI gene bank is *Aquarius remigis, Aquarius nyctalis* and *Mycrovelia longipes,* while *Limnoporus notabilis, Metrocoris sp., Gerris pinreensis, Gerris argenticolis* only had identical rates were 86% and *Gerris Incognitus* have identical levels as much as 85%. The phylogenetic tree use neighbor joining tree method of marine insects S.10.M samples found in Mokupa beach Manado with some insects based on data in the NCBI gene bank depicted in Figure 7.

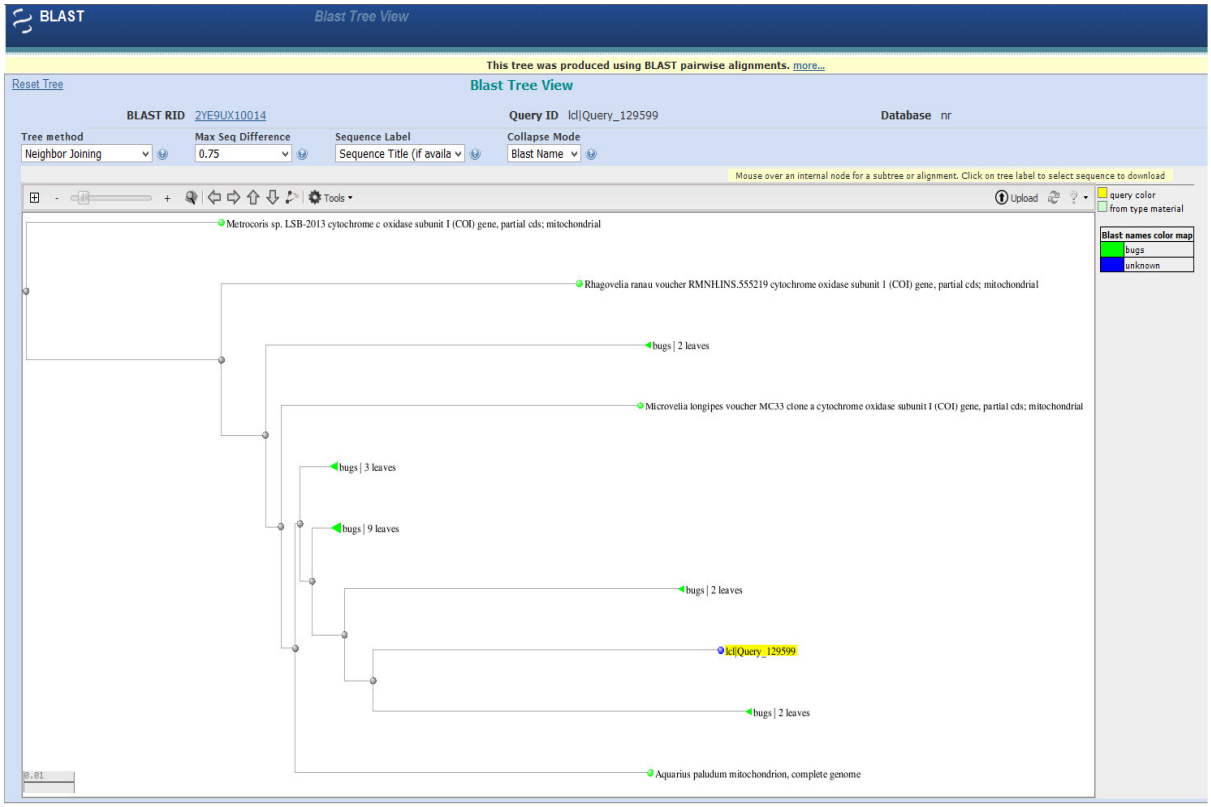


Figure 7. CO1 gene phylogenetic tree of insects marine waters Mokupa Beach compared with data from NCBI with the closest similarity level and the comparator (outsider).

CONCLUSIONS

Marine insect S.10.M samples inhabit coastal areas Mokupa Beach Manado, was found in a quiet area of mangrove. Based on the analysis of gene sequences BLAST cytochrome oxidase 1 (CO1) marine insects S.10.M samples originating from Mokupa Beach Manado different with gene sequences of cytochrome oxidase 1 (CO1) northwest of family Gerridae insects that have been recorded and published in the NCBI gene bank.

There are still many unidentified marine insects and has not been recorded in the NCBI gene bank, so that research on marine insects that live in marine waters of North Sulawesi needs to be done .

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