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CHARACTER OF CYTOCHROME OXIDASE 1 GENE (CO1) IN MITOCHONDRIAL DNA DAMSELFLY *Agriocnemis femina* FROM LINOW LAKE, TONDANO LAKE AND MOAT LAKE AT NORTH SULAWESI

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ABSTRACT

This study aims to find the character gene cytochrome oxidase subunit 1 (CO1), mitochondrial DNA damselfly (*Agriocnemis femina*) derived from Tondano Lake, Linow Lake and Lake Moat. The research was conducted in several stages, sampling, extraction and purification of total DNA, CO1 gene amplification and sequencing. Sequencing results were analyzed using Geneous Software 5.6.4. Further alignment analysis using NCBI BLAST (www.ncbi.nih.gov). Although the sample morphology Damselfly used for DNA analysis showed differences in the characteristics of phenotypically plastic. CO1 gene sequencing results of the six samples show the sequence of nitrogenous bases Damsefly have identical percentage (similarity), 99 percent or it can be said based gene CO1, six samples are the same species. Similarity 6 Damsefly samples from three different ecosystems that Tondano Lake, Lake and Lake Moat Linow average above 99% with Agriocnemis femina. Some differences morphological characters of each sample from a different lake ecosystems is not sufficient to give effect to CO1 gene mutations in mitochondrial DNA or gene conservation CO1 at Demselfly that live in three lake ecosystems is very high.

Keywords: Character, CO1, Agriocnemis femina, Lake Tondano, Linow Lake, Lake Moat

INTRODUCTION

Sulawesi Island based on zonation of flora and fauna in Indonesia, are on the line Wallacea. In the geological formation Sulawesi Island is not formed from the Asian continent fault or continent of Australia. This condition causes the geographic isolation of the organisms that live on the mainland island of Sulawesi, which from an evolutionary standpoint generate many endemic species or species of animals and plants that are naturally found only in Sulawesi. Biodiversity is an invaluable biological wealth owned by a nation. In regions with high endemitas level, the problem of conservation of genetic resources is a priority.

Research damselfly is still very rare in North Sulawesi. Research conducted by Wakhid *et. al.* 2014, in National Parks Nani Wartabone, Damselfly species that has an abundance of the most commonly found are *Agriocnemis rubescens* 146 individuals (30.10%), family Coenagrionidae. Coenagrionidae overflow was found because of this family is the largest family in the suborder Zygoptera amount and spread evenly across the world (Orr, 2003). Coenagrionidae is one of the Damsefly family where most of the species are found in stagnant water habitat (Kalkman and Orr 2013), so Coenagrionidae can live in various types of habitats both on the aquatic habitat flowing and not flowing. High adaptability of Coenagrionidae, causes a high number of species that are found in various habitats. Thus damselfly research on aquatic ecosystems, especially Lake ecosystem has not been done.

In North Sulawesi, there are three main lake that is Tondano Lake, Lake and Lake Moat Linow. Tondano Lake is the largest lake; along the lake Linow located in Minahasa. While the Moat Lake is the second largest lake located in the South Minahasa regency. Tondano Lake and Lake Linow known geologically formed by volcanic eruptions, known as the volcanic lake. Geographically three

lakes separated by a considerable distance so as not to allow the lake biota naturally migrating between three lakes.

One of the organisms found in all three of those lakes are damselfly. Damselfly are insects belonging to the order Odonata, suborder Zygoptera. The types of Damsefly are usually divided into two major groups, namely dragonfly and damselfly. Damselfly has unique characteristics that make it easily distinguishable from other types of Damsefly, which forms a slender body like a needle and wing upright position at rest.

Damselfly or capung jarum (local name) has a very important ecological role. Population capung jarum may be an indicator of environmental contamination (bio-indicators) of a region. On the condition of waters already polluted, Damsefly life cycle is disrupted and lead to a population decline. Damsefly sustainability needs to be maintained to keep the existence of a life which is mostly water. In addition, damselfly also acts as a biological control agent that is a pest predators. Damselfly played a role as natural enemies to reduce the population of crop pests (Hidayah, 2008). This suggests an important position where damselfly in ecological balance. Damselfly genus Agriochemis is rice crop pest predators. Damselfly prey on other insects such as mosquitoes, gnats, *Orseolia oryzae* (Ganjur), *Nilaparvata lugens, Sogatella furcifera, Nephotetix sp* and other pests in rice (Gangurde, 2007; Erniwati, 2009; Norman, 2005).

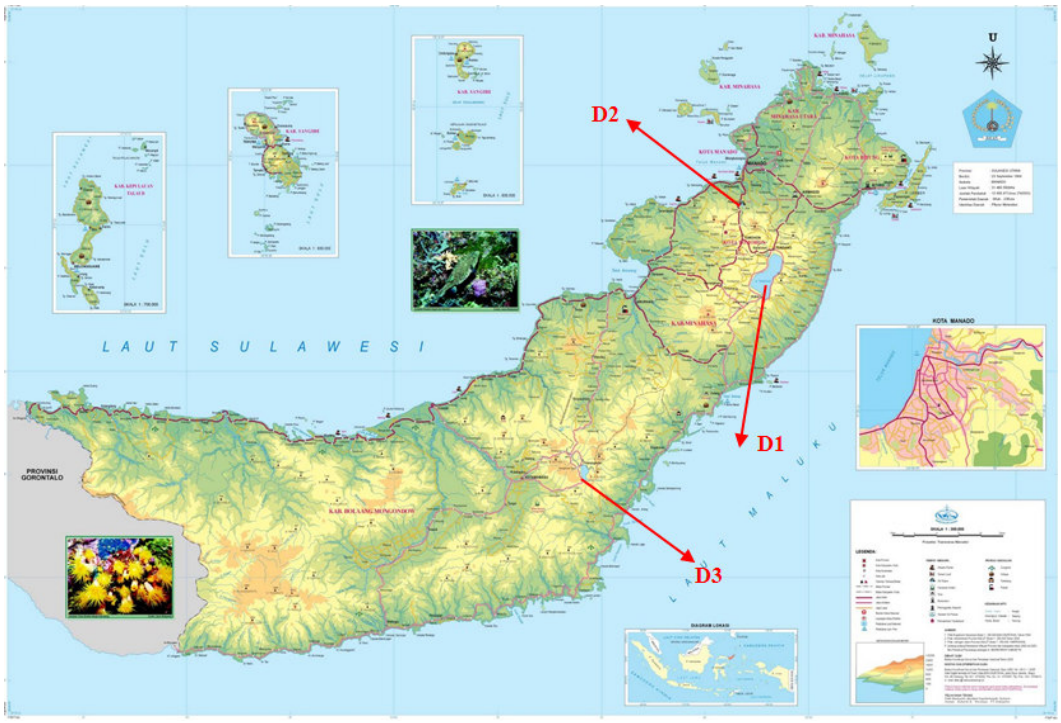
Theoretically geographical isolation can lead to the formation of new species or speciation. Nevertheless damselfly species that live in Lake Tondano, Linow Lake and Lake Moat needs to be studied both in morphology, behavior and molecular identification. Research on Sulawesi damselfly is limited to the study of population abundance, identifying morphological and ecological role (Maramis and Makal 2011, Wakhid *et. al.* 2014). Studies on the molecular level to characterize the molecular character using barcode gene for species identification and ensure its position in the molecular phylogeny has not been done. This study aims to find the character gene cytochrome oxidase subunit 1 (CO1), mitochondrial DNA damselfly (*Agriocnemis femina*) from Tondano Lake, Linow Lake and Moat Lake.

MATERIALS AND METHOD

Collection of Specimens.

Sampling was carried out at the damselfly Tondano Lake, Linow Lake located in Minahasa and the Moat Lake, located in the South Minahasa District. Each separated by geography or lakes not connected by a small river streams. Extraction and purification of DNA and amplification of target genes is done at the Laboratory of Biotechnology, Department of Biology, University of Sam Ratulangi; nucleotide sequencing or sequencing the gene CO1 Damsefly performed by service providers sekuensing (First Base, Malaysia). Damselfly samples taken from Lake Tondano, Lake Linow located in Minahasa and the Moat Lake, located in the South Minahasa District (Figure 1). Location lake sampling sites indicated on the map (Figure 2).

Figure 1. Damselfly Tondano Lake, Linow Lake and Moat Lake



For extraction of dsDNA (double strands of DNA) DNA extraction kit total use innuPrep DNA Micro Kit (Analytic Jena, Germany). This kit serves dsDNA total comprises extracting nuclear DNA and mitochondrial DNA with the sample used in this study is a network in the limbs Damsefly. Composition of the kit consists of: genomic digestion buffer, proteinase K, RNase A, genomic lysis / binding buffer, elution buffer genomic, genomic was buffer 1, genomic wash buffer 2, ethanol,

Eppendorf tub and consumables which tips. Primer. CO1 gene primer used was: LCO1490 (5'-GGT CAA CAA ATC TTG ATA ATA AAG G-3 ') and HC02198 (5'-TAA ACT TCA AAA CCA TGA GGG AAT CA-3') was obtained from PT Genecraft Jakarta. Target gene amplification by PCR method. The tools used are mastercyling eppendorf. Visualization of the target gene amplification results using electrophoresis method. As the marker (Marker) consists of a mixture or blue juice and DNA lader. Gel made from a mixture between TAE and agarose. Other tools, among others, refrigerator, freezer, mikropastel, oven, vortex, centrifuge, microwave, UV-Transiluminator and cameras.

Procedure Research

a. DNA extraction and purification

Damsefly life that has been identified, preserved in 95% ethanol. Total DNA extraction (DNA nucleus and mitochondrial DNA) of the specimen damselfly *Agriocnemis femina* performed using DNA Micro Kit (Innurep Analityk Jena, Germany) according to the instructions manufactur with slight modifications. The steps are as follows: In each sample, the specimen leg damselfly put in a 1.5 ml micro tube. Into a tube inserted TLS 200 mL of Lysis Solution and 20 mL Proteinase K, inverted five times and then incubated for 3 hours at a temperature of 55ºC using termoblok and then centrifuged 11,000 rpm for 1 minute. The supernatant is pipetted into a new micro tube and mixed with 200 mL of Binding Solution TBS then divortex. The sample is inserted into the spin filter had been installed reservoir tube and centrifuged as before. The filtrate was discarded and the tube container reassembled. A total of 400 mL of Washing Solution HS inserted and centrifuged as above. The filtrate was discarded and the tube container reassembled. A total of 750 mL of washing solution MS inserted and centrifuged as above. The filtrate was discarded and the tube container reassembled. To dry the filter, empty tube back centrifuged for 2 minutes and then the filtrate is discarded along with the tube container. Spin filter was transferred into a new micro tube, dried for 2 minutes, add in 100 mL of elution buffer, allowed to stand for 2 minutes and then centrifuged. Spin filter was removed and DNA stored at -10ºC.

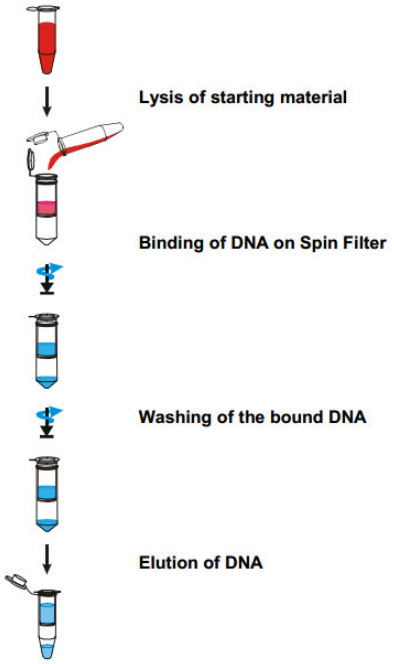


Figure 3. Outline of the extraction process dsDNA Total *Agriocnemis femina*

b. Polymerase Chain Reaction (PCR)

PCR was performed using PCR Master Mix 5X Firepol Ready-to-Load (Solis Biodyne). In each reaction of 50 mL has a 15 pmol of each primer and template DNA. Type of primer pairs that

successfully amplify DNA from mitochondria COI termites are used in accordance Folmer *et al.* (1994), among others LCO1490 (5'-GGT CAA CAA ATC TTG ATA ATA AAG G-3 ') and HC02198 (5'-TAA ACT TCA AAA CCA TGA GGG AAT CA-3'). PCR machine temperature setting is 95ºC for 2 minutes and then resumed 35 cycles of 95ºC for 30 seconds, 50ºC and 72ºC for 40 seconds for 50 seconds. PCR products were separated using 1% agarose gel electrophoresis (in TBE buffer 1x) and observed using UV-Transiluminator. PCR products were sent with primer-primer for sequenced by sequencing service provider (First Base, Malaysia).

c. Sequence Data Processing and Analysis

Chromatograms obtained edited using Geneious software version 5.6 (Drummond *et al.,* 2012). Sequencing results will be compared with Gen Bank using BLAST (Basic Local Alignment Search Tools) NCBI (www.ncbi.com) and BOLDsystems. The phylogenetic tree constructed using Genetic distance Tamura-Nei model of algorithm Neighbor-Joining method with Bootstrap resampling method replication 1000 times.

RESULTS AND DISCUSSION

Extraction and Purification of dsDNA Total

Total dsDNA extracted using leg damselfly obtained total DNA concentration and purity is 54.3 ug / ml and 1.85. Purity total DNA showed high levels of dsDNA free of protein, RNA and other contaminants that affects the target DNA amplification stage. Distribution of DNA purity is good at 1.8 to 2.0. The concentration of total DNA obtained showed the magnitude of dsDNA both nuclear DNA and mitochondrial DNA is successfully extracted using a kit. The higher the concentration of total DNA means a lot of stock available DNA that is used as a templete to process the target gene amplification by PCR.

CO1 gene amplification

The concentration and purity of total DNA obtained in the extraction stage, showing the effectiveness or absence of DNA extraction kits are used to extract the total DNA leg damselfly. CO1 gene amplification of six samples from the lake Tondano Damsefly namely (MM and TM), Lake Linow (LH and LM) and Lake Moat (MM and TH) qualitatively show good results amplicon evidenced by the band (band) formed on the visualization of results PCR with electrophoresis is good, consistent or not smears (Figure 4).

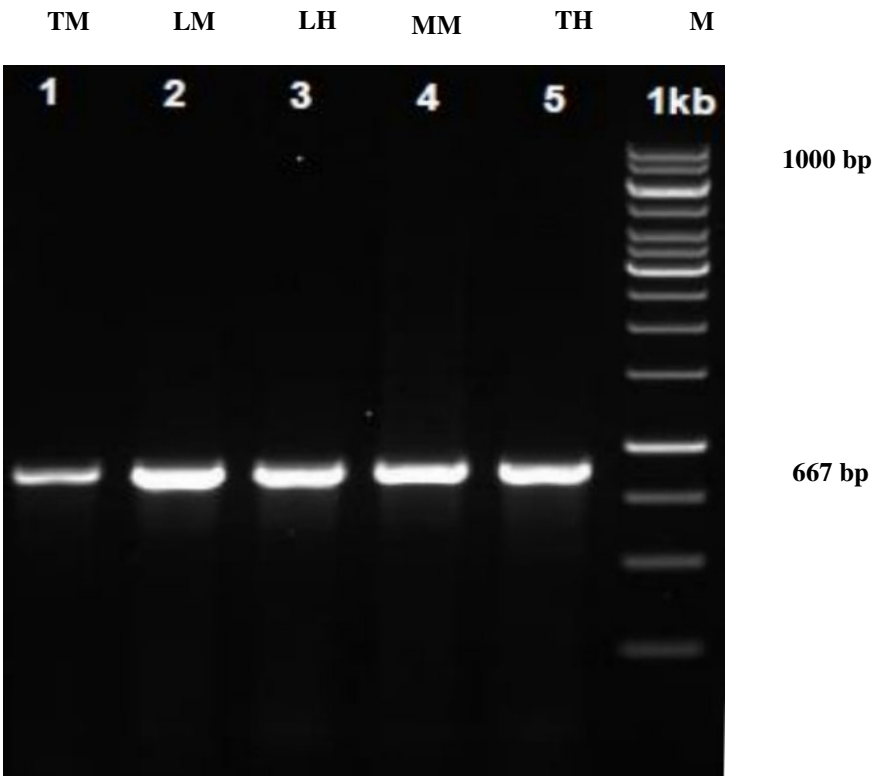


Figure 4. Electrogram profile Damsefly CO1 gene amplification product with the primer LCO1490 and HC0219: marker (M) 100 nt (1 kb) with a sample TM (1), LM (2), LH (3), MM (4) and TH (5).

Sequencing

Tracking the nucleotide sequences of DNA amplification using PCR amplicon result of gene CO1 mitochondrial DNA Damsefly from Lake Tondano, Lake Linow and Lake Moat. Length of nucleotide bases of each sample was analyzed with Geneous 5.6.4 are shown in Table 1. The sequence CO1 of Damsefly that comes from Lake Tondano, Lake Moat and Lake Linow after combined sequences LCO and HCO sequences of each sample shows the distribution of 712 bp to 719 bp (Table 1).

Table 1. Length of mitochondrial DNA COI gene sequencing results Damsefly samples from three lakes
in North Sulawesi.

No	Sample code	Location	Nucleotide length (bp)
1	MH	Tondano Lake	712 bp 633
2	TM	Tondano Lake	718 bp 637
3	LM	Linow Lake	712 bp
4	LH	Linow Lake	715 bp
5	MM	Moat Lake	717 bp
6	TH	Moat Lake	719 bp

The sixth sample shows the sequence of nitrogenous bases Damsefly have identical percentage (similarity), 99 percent or it can be said based gene CO1, six samples are the same species. BLAST results showed that six samples, have the same nucleotide sequence which is identical to *Agriocnemis femina*. Results of pairwise alignments with multiple align method using Geneious 5.6.4,

obtained nucleotide sequences in FASTA format each sample Damsefly from 3 lakes in North Sulawesi are:

CO1 sequences MH:
GGTCAACAAATCATAAAGATATTGGAACTCTCTACTTAATATTTGGAGCATGGGCAGGTA
TAGTTGGAACTGCCTTAAGTATATTAATTCGGGTAGAACTAGGACAACCAGGATCACTAA
TTGGTGATGATCAAATTTATAATGTAGTAGTAACTGCACACGCTTTTGTAATAATTTTTTT
TATAGTAATACCAATTATAATTGGGGGATTTGGAAACTGACTGGTTCCTTTAATGTTAGGC
GCACCAGATATAGCCTTCCCACGGCTTAATAACATGAGATTTTGATTATTACCCCCTTCAC
TAACATTATTACTGGCAAGTAGTTTAGTAGAAAGAGGAGCGGGTACTGGATGAACGGTCT
ATCCTCCTTTAGCGGGAGCTATTGCTCATGCAGGGGGATCAGTAGATCTTACTATTTTTTC
ACTTCATTTGGCTGGAGTCTCATCAATTTTAGGTGCAATCAATTTTATTACTACAACTATT
AATATAAAATCACCCGGAATAAAAATAGAACAGTTACCTCTATTTGTATGAGCAGTAGTA
ATTACTGCTGTATTATTATTGTTATCTTTACCTGTATTAGCAGGTGCAATTACTATACTATT
AACAGATCGTAATATCAATACATCATTTTTTGATCCGGCAGGGGGAGGAGACCCAATTTT
ATATCAACACCTATTTTGATTTTTTGGTCACCCTGAAAGTTTAA

CO1 sequences TM
TGGTCAACCAAATCATAAAGATATTGGAACTCTCTACTTAATATTTGGAGCATGGGCAGG
TATAGTTGGAACTGCCTTAAGTATATTAATTCGGGTAGAACTAGGACAACCAGGATCACT
AATTGGTGATGATCAAATTTATAATGTAGTAGTAACTGCACACGCTTTTGTAATAATTTTT
TTTATAGTAATACCAATTATAATTGGGGGATTTGGAAACTGACTGGTTCCTTTAATGTTAG
GCGCACCAGATATAGCCTTCCCACGGCTTAATAACATGAGATTTTGATTATTACCCCCTTC
ACTAACATTATTACTGGCAAGTAGTTTAGTAGAAAGAGGAGCGGGTACTGGATGAACGG
TCTATCCTCCTTTAGCGGGAGCTATTGCTCATGCAGGGGGATCAGTAGATCTTACTATTTT
TTCACTTCATTTGGCTGGAGTTTCATCAATTTTAGGTGCAATCAATTTTATTACTACAACT
ATTAATATAAAATCACCCGGAATAAAAATAGAACAGTTACCTCTATTTGTATGAGCAGTA
GTAATTACTGCTGTATTATTATTGTTATCTTTACCTGTATTAGCAGGTGCAATTACTATACT
ATTAACAGATCGTAATATCAATACATCATTTTTTGATCCGGCAGGGGGAGGAGACCCAAT
TTTATATCAACACCTATTTTGATTTTTTGGTCCCCTGGAAGTTTAAA

CO1 sequences MM
NNTCAACCNNNNATAAAGATATTGGAACTCTCTACTTAATATTTGGAGCATGGGCAGGTA
TAGTTGGAACTGCCTTAAGTATATTAATTCGGGTAGAACTAGGACAACCAGGATCACTAA
TTGGTGATGATCAAATTTATAATGTAGTAGTAACTGCACACGCTTTTGTAATAATTTTTTT
TATAGTAATACCAATTATAATTGGGGGATTTGGAAACTGACTGGTTCCTTTAATGTTAGGC
GCACCAGATATAGCCTTCCCACGGCTTAATAACATGAGATTTTGATTATTACCCCCTTCAC
TAACATTATTACTGGCAAGTAGTTTAGTAGAAAGAGGAGCGGGTACTGGATGAACGGTCT
ATCCTCCTTTAGCGGGAGCTATTGCTCATGCAGGGGGATCAGTAGATCTTACTATTTTTTC
ACTTCATTTGGCTGGAGTTTCATCAATTTTAGGTGCAATCAATTTTATTACTACAACTATT
AATATAAAATCACCCGGAATAAAAATAGAACAGTTACCTCTATTTGTATGAGCAGTAGTA
ATTACTGCTGTATTATTATTGTTATCTTTACCTGTATTAGCAGGTGCAATTACTATACTATT
AACAGATCGTAATATCAATACATCATTTTTTGATCCGGCAGGGGGAGGAGACCCAATTTT
ATATCAACACCTATTTTGATTTTTTGGTCACCCTGAAAGTTTAAA

CO1 sequences LH
ATAATTTTCTTTATAGTTATACCAATCATAATTGGAGGATTCGGAAATTGATTAGTACCAC
TAATATTAGGAGCACCAGATATAGCTTTTCCTCGATTAAATAATATAAGATTCTGATTATT
ACCCCCATCATTAACGCTACTTTTAGCAAGAAGTTTAGTAGAAAGAGGTGCGGGAACTGG
GTGAACTGTATACCCGCCTTTAGCAGGTGTAATTGCCCATGCCGGGGCATCAGTAGATTT
AACTATTTTCTCCTTACACTTGGCAGGAGTATCTTCAATTCTAGGAGCAATCAATTTTATT
ACAACTACTATTAATATAAAGTCGCCAGGAATAAATATAGATCAAATACCATTATTTGTA
TGAGCTGTAGTTATCACGGCAGTATTATTATTACTATCTCTACCTGTCCTAGCAGGTGCAA

TCACAATATTATTAACAGACCGTAACATTAATACATCTTTTTTTGATCCAGCAGGAGGAG
GAGATCCAATTTTATATCAACACCTATTTTGATTTTTTGGTCACCTGGAAAGTTTAAA

CO1 sequences LM
TTTGTTCAACCAAATCTAAAGATATTGGAACTCTCTACTTAATATTTGGAGCATGGGCAG
GTATAGTTGGAACTGCCTTAAGTATATTAATTCGGGTAGAACTAGGACAACCAGGATCAC
TAATTGGTGATGATCAAATTTATAATGTAGTAGTAACTGCACACGCTTTTGTAATAATTTT
TTTTATAGTAATACCAATTATAATTGGGGGATTTGGAAACTGACTGGTTCCTTTAATGTTA
GGCGCACCAGATATAGCCTTCCCACGGCTTAATAACATGAGATTTTGATTATTACCCCCTT
CACTAACATTATTACTGGCAAGTAGTTTAGTAGAAAGAGGAGCGGGTACTGGATGAACG
GTCTATCCTCCTTTAGCGGGAGCTATTGCTCATGCAGGGGGATCAGTAGATCTTACTATTT
TTTCACTTCATTTGGCTGGAGTTTCATCAATTTTAGGTGCAATCAATTTTATTACTACAACT
ATTAATATAAAATCACCCGGAATAAAAATAGAACAGTTACCTCTATTTGTATGAGCAGTA
GTAATTACTGCTGTATTATTATTGTTATCTTTACCTGTATTAGCAGGTGCAATTACTATACT
ATTAACAGATCGTAATATCAATACATCATTTTTTGATCCGGCAGGGGGAGGAGACCCAAT
TTTATATCAACACCTATTTTGATTTTTTGGTCCCCTTGAAAGTTTAAAA

CO1 sequences TH
TTCCCCCCAATTAAAAGATATTGGAACTCTCTACTTAATATTTGGAGCATGGGCAGGTAT
AGTTGGAACTGCCTTAAGTATATTAATTCGGGTAGAACTAGGACAACCAGGATCACTAAT
TGGTGATGATCAAATTTATAATGTAGTAGTAACTGCACACGCTTTTGTAATAATTTTTTTT
ATAGTAATACCAATTATAATTGGGGGATTTGGAAACTGACTGGTTCCTTTAATGTTAGGC
GCACCAGATATAGCCTTCCCACGGCTTAATAACATGAGATTTTGATTATTACCCCCTTCAC
TAACATTATTACTGGCAAGTAGTTTAGTAGAAAGAGGAGCGGGTACTGGATGAACGGTCT
ATCCTCCTTTAGCGGGAGCTATTGCTCATGCAGGGGGATCAGTAGATCTTACTATTTTTTC
ACTTCATTTGGCTGGAGTTTCATCAATTTTAGGTGCAATCAATTTTATTACTACAACTATT
AATATAAAATCACCCGGAATAAAAATAGAACAGTTACCTCTATTTGTATGAGCAGTAGTA
ATTACTGCTGTATTATTATTGTTATCTTTACCTGTATTAGCAGGTGCAATTACTATACTATT
AACAGATCGTAATATCAATACATCATTTTTTGATCCGGCAGGGGGAGGAGACCCAATTTT
ATATCAACACCTATTTTGATTTTTTGGCCCTGGAAAAATTTAAAAA

After the merger between sequences Reverse (LCO.ab1) and Forward (HCO.ab1) of each sample, the obtained variation nucleotides long. Analysis of the composition of the constituent base sequences of each sample shows, the content of nitrogen base AT greater than GC on CO1 all samples Damsefly. MH sample nucleotide sequences, TM, MM, LH, LM 100% identical while samples TH 99.8% identical. A combination of 6 samples showed 82.6% Identics sites with identical pairs 93.9% (Table 2).

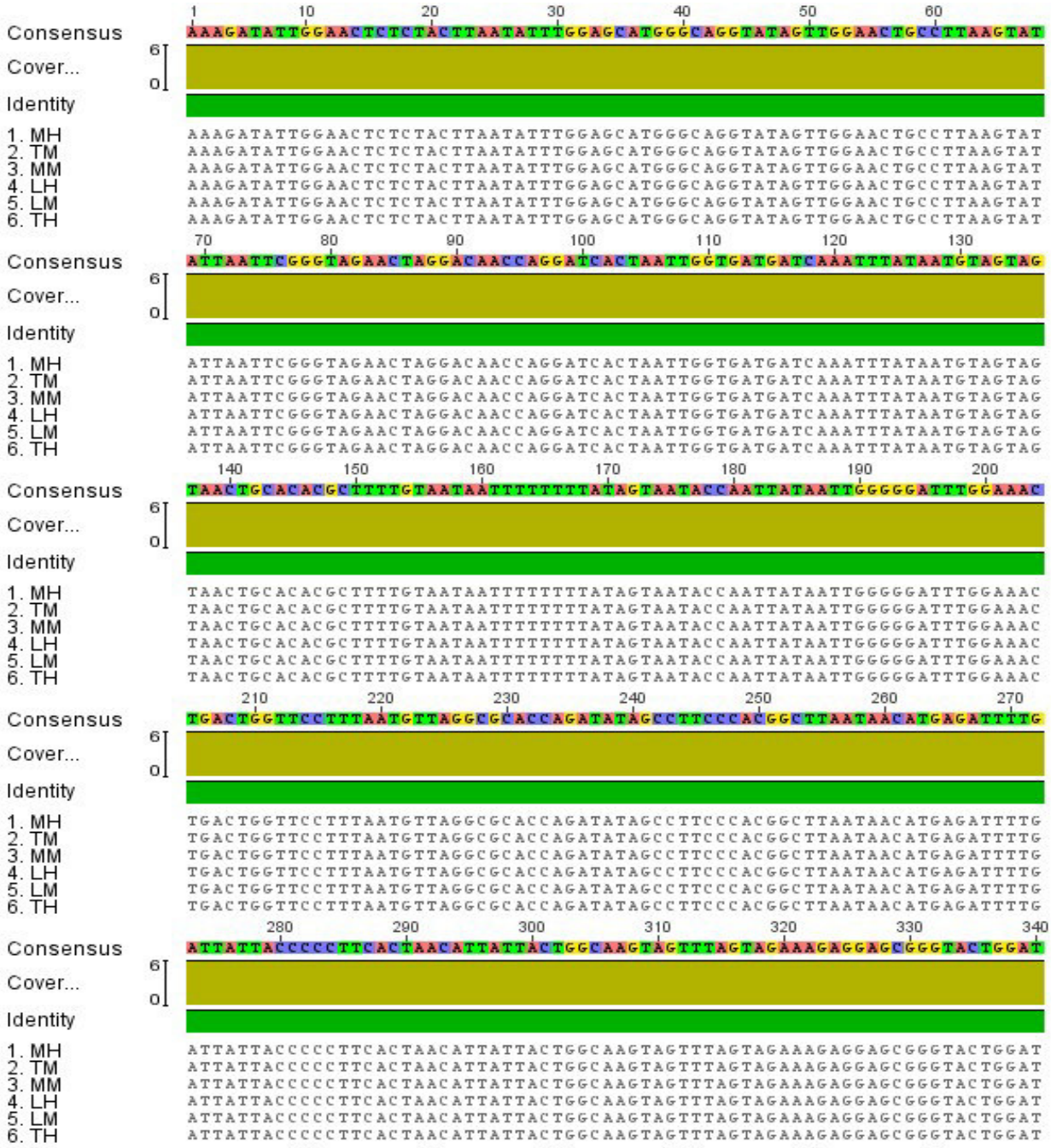
Table 2. Characteristics of Six Sample CO1 gene sequences Damsefly

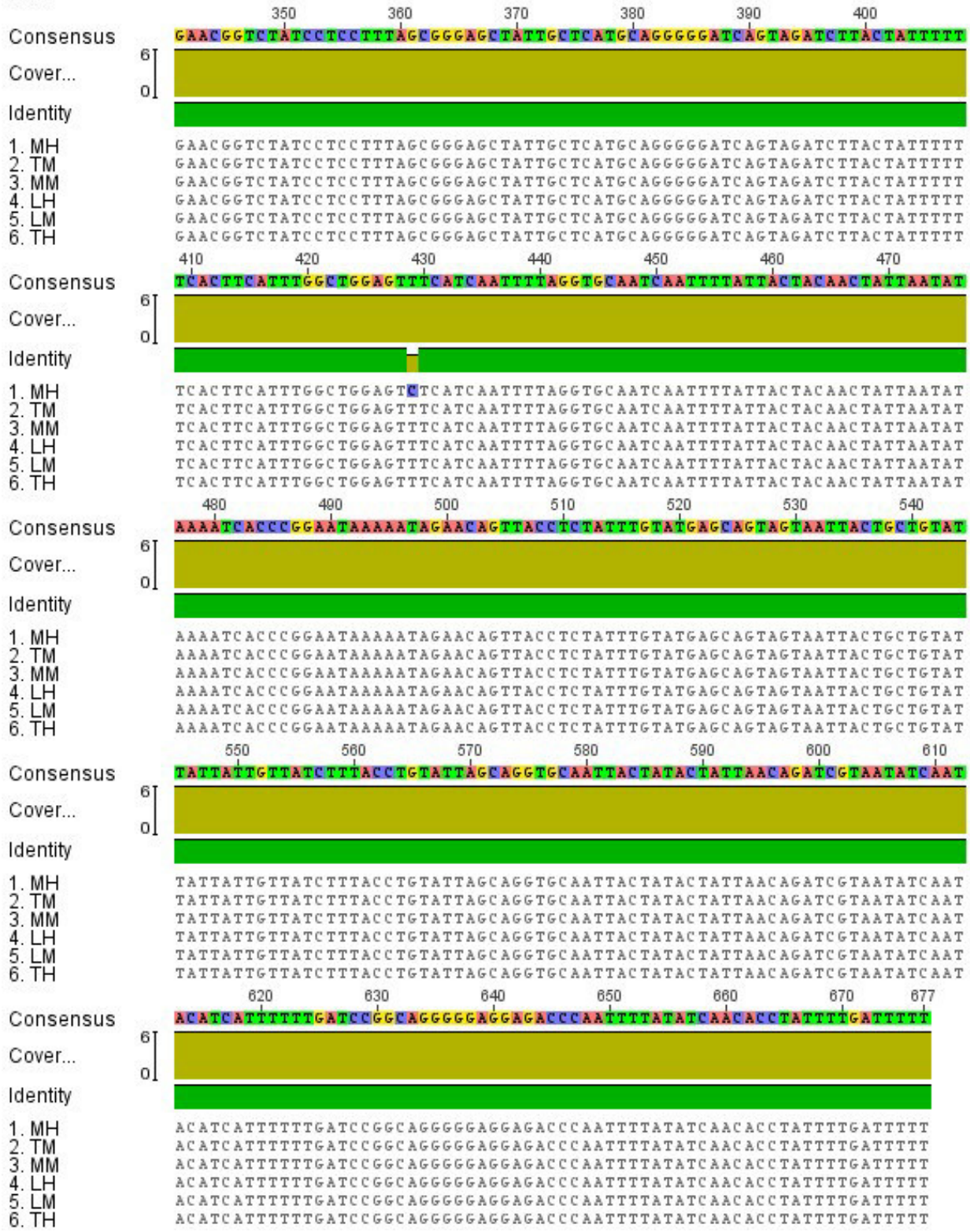
Characteristics	MH		TM		MM		LH		LM		TH	
	Freq	%	Freq	%	Freq	%	Freq	%	Freq	%	Freq	%
Komposisi Basa N												
A	392	30.6	371	30.3	391	30.6	416	32.3	390	30.5	385	30.5
C	213	16.6	202	16.5	211	16.5	220	17	211	16.5	210	16.6
G	237	18.5	223	18.2	235	18.4	225	17.4	233	18.2	230	18.2
T	440	34.3	428	35	441	34.5	430	33.3	445	34.8	439	34.7
GC	450	35.1	425	34.7	446	34.7	445	34.5	444	34.7	440	34.8
N	0	0	0	0	6	0.5	0	0	0	0	0	0

	MH	TM	MM	LH	LM	TH
Length sequences (nt)	712	718	712	715	717	719
Site identical	570 (100%)	506 (100%)	572 (100%)	576 (100%)	562 (100%)	545 (99.8 %)
Combined 6 sequences	A 1.315 (30.7%)		Sequens lenght : 720			
	C 709 (16.5%)		Identical site 593 (82,6 %)			
	G 788 (18.4%)		Percents of identical pairs : 93.9 %			
	T 1.470 (34.3)					
	GC 1.497 (34%)					
	N 6 (0.1 %)					

Description: TH = Damsefly original green color Tondano, Tondano TM = Damsefly origin in red
LM = origin Linow red Damsefly, Damsefly origin Linow LH = green,
MM = Damsefly origin Moat red color, and origin Moat MH = Damsefly green

Table 3. Alignment (Alignment) COI sequences of samples





Analysis of sequence homology or alignment between Damsefly (677 bp), indicating similarity or similar sequence similarity with the closest to the NCBI gene bank which can be said to be identical (Figure 3 and Table 3). Mutations found only in MH sequences that is at the base to 429 wherein the nitrogenous bases in the consensus sequence is thymine (T), while the MH sequence is cytosine (C). Another sample sequences that MM, LH, LM and TH in the same position by consensus.

Comparison of sequence similarity indicates the degree of similarity of 100% in all samples from both Damselfly from Tondano lake, Linow Lake and Moat lake. Compared with the CO1 gene sequence data NCBI gene bank refers Damsefly obtained in 3 different locations of the lake is *Agriocnemis femina* (Table 4).

Table 4. Matrix comparison Similarity Between sequences

Sample	MH	TM	MM	LH	LM	TH
MH		99,9%	99,9%	99,9%	99,9%	99,9%
TM	99,9%		100%	100%	100%	100%
MM	99,9%	100%		100%	100%	100%
LH	99,9%	100%	100%		100%	100%
LM	99,9%	100%	100%	100%		100%
TH	99,9%	100%	100%	100%	100%	

Description: TH = Damsefly original green color Tondano, Tondano TM = Damsefly origin in red
LM = origin Linow red Damsefly, Damsefly origin Linow LH = green,
MM = Damsefly origin Moat red color, and origin Moat MH = Damsefly green

Construction phylogeny using Tamura-Nei model of Neighbor Joining method with bootstrap 1000x. A total of 15 gene sequences BLAST results are used to construct phylogenetic trees. *Musca domestica* used as outgrup. Phylogenetic tree constructed to form two branches (branch) that is out group branches and ingroup branch. In the six nodes which form ingroup fifth Damsefly samples forming the same node / parallel with *Agriocnemis femina* or it can be said fifth species do not differ genetically, especially in gene CO1 mitochondrial DNA with *Agriocnemis femina* (Figure 4). Similarity 6 Damsefly samples from three different ecosystems that Tondano Lake, Linow Lake and Moat Lake average above 99%, *Agriocnemis femina* similarity between samples as well as over 99% (Table 5).

Table 5 Matrix of similarity between the samples of various species of Damsefly with NCBI gene bank based on the sequence of nucleotides 677 CO1 Tamura-Nei model Neighbor Joining method

	Coeliccia did...	Musca dome...	Ischnura het...	Ischnura asi...	Azuragrion b...	Chromagrion...	Agriocnemis ...	Anax imperator	Agriocnemis ...	LH	LM	TH	TM	MH	MM
Coeliccia didyma		80.5%	84.1%	83.7%	83.7%	84.3%	82.9%	83.7%	85.7%	85.7%	85.7%	85.7%	85.7%	85.5%	85.7%
Musca domestica	80.5%		78.3%	80.1%	77.9%	77.5%	75.3%	81.7%	78.3%	78.5%	78.5%	78.6%	78.5%	78.3%	78.5%
Ischnura heterosticta	84.1%	78.3%		87.1%	86.7%	85.9%	83.3%	82.7%	85.1%	84.7%	84.7%	84.7%	84.7%	84.7%	84.7%
Ischnura asiatica	83.7%	80.1%	87.1%		85.7%	83.5%	82.9%	82.9%	84.3%	84.5%	84.5%	84.5%	84.5%	84.5%	84.5%
Azuragrion buchholzi	83.7%	77.9%	86.7%	85.7%		84.3%	83.5%	84.3%	86.3%	86.1%	86.1%	85.9%	86.1%	86.1%	86.1%
Chromagrion conditum	84.3%	77.5%	85.9%	83.5%	84.3%		83.9%	84.1%	84.9%	85.1%	85.1%	85.1%	85.1%	85.1%	85.1%
Agriocnemis forcipata	82.9%	75.3%	83.3%	82.9%	83.5%	83.9%		80.3%	86.1%	86.5%	86.5%	86.3%	86.5%	86.3%	86.5%
Anax imperator	83.7%	81.7%	82.7%	82.9%	84.3%	84.1%	80.3%		83.9%	83.9%	83.9%	83.7%	83.9%	83.9%	83.9%
Agriocnemis femina	85.7%	78.3%	85.1%	84.3%	86.3%	84.9%	86.1%	83.9%		99.2%	99.2%	99.1%	99.2%	99.0%	99.2%
LH	85.7%	78.5%	84.7%	84.5%	86.1%	85.1%	86.5%	83.9%	99.2%		100%	99.9%	100%	99.8%	100%
LM	85.7%	78.5%	84.7%	84.5%	86.1%	85.1%	86.5%	83.9%	99.2%	100%		99.9%	100%	99.8%	100%
TH	85.7%	78.6%	84.7%	84.5%	85.9%	85.1%	86.3%	83.7%	99.1%	99.9%	99.9%		99.9%	99.7%	99.9%
TM	85.7%	78.5%	84.7%	84.5%	86.1%	85.1%	86.5%	83.9%	99.2%	100%	100%	99.9%		99.8%	100%
MH	85.5%	78.3%	84.7%	84.5%	86.1%	85.1%	86.3%	83.9%	99.0%	99.8%	99.8%	99.7%	99.8%		99.8%
MM	85.7%	78.5%	84.7%	84.5%	86.1%	85.1%	86.5%	83.9%	99.2%	100%	100%	99.9%	100%	99.8%	

TH = Damsefly original green color Tondano, Tondano TM = Damsefly native red, LM = Damsefly origin Linow red, LH = Damsefly origin Linow green, MM = Damsefly origin Moat red color, and origin Moat MH = Damsefly green

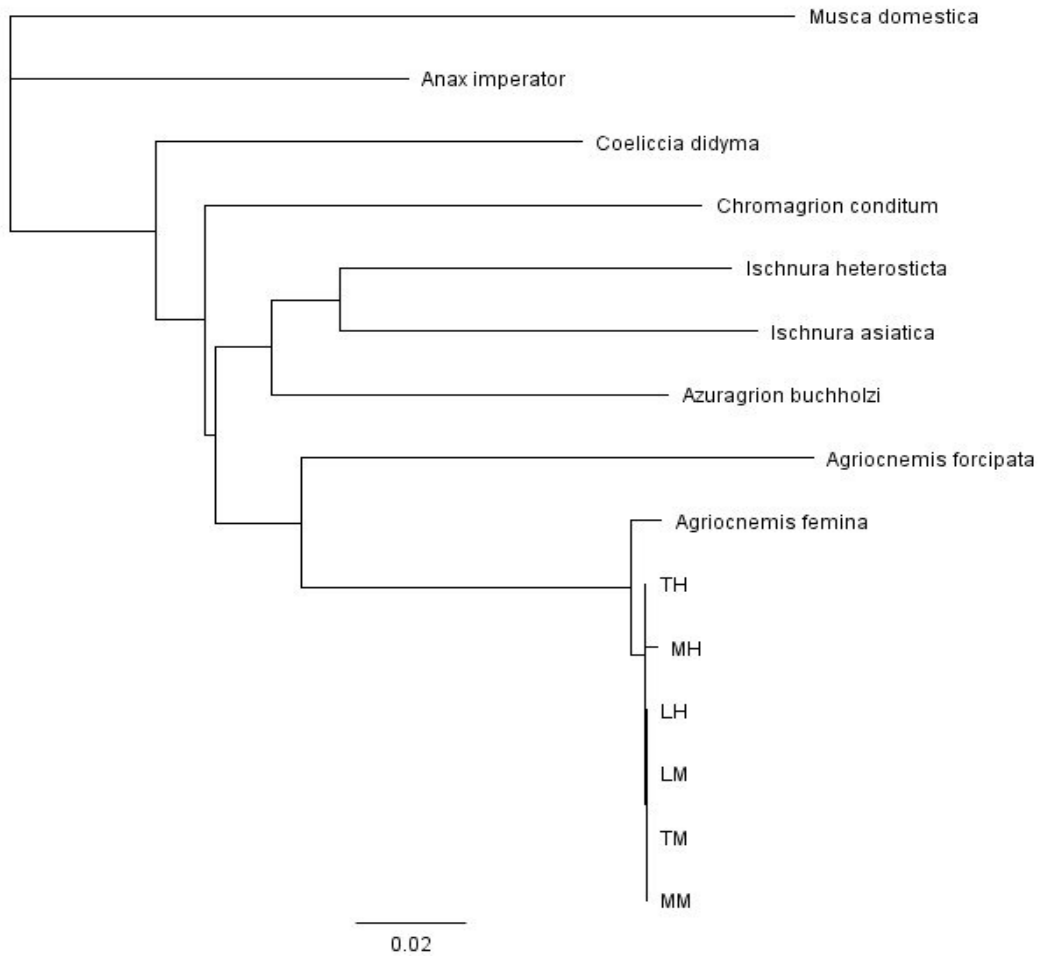


Figure 5 Construction of phylogenetic tree Damsefly five samples from three major lake in North Sulawesi is Lake Tondano (TH, MH), Lake Linow (LH and LM) and Lake Moat (TM and MM) using methods neighbor Joining compared with the CO1 gene sequence alignment recorded in the NCBI gene bank for Damsefly.

Discussion

Fifth Damsefly samples from different habitats and ecosystems refer to the same species that is *Agriocnemis femina*. In the sixth phylogeny tree of the Damsefly samples form a monophyletic clade or derived from a common ancestor. Not found strong indications of the formation of a new species of the Damsefly sixth based gene CO1 mitochondrial DNA. Gene CO1 mitochondrial DNA have been used widely and internationally accepted as a marker or a species known as genetic barcode. CO1 application in insects has been successfully proven its accuracy as a differentiator interspecies on Lepidoptera (Herbert *et. al.* 2003; Hajibabaei *et al.* 2005), beetles (Funk *et al.* 1995), some insect pests (Toda & Murai 2007) moth *Hamona mermerodes* (Hulrc *et al.* 2007), mosquitoes (Cywinska *et al.* 2006). COI sequence is used to examine the co-evolution of insect herbivore with its host plant (Rivera *et al.,* 2009).

This study was able to prove the existence of the phenomenon of gene expression that is interesting because phenotypically plastic morphological characters Damsefly in different colors of red and green, different habitats and ecosystems; geographically separated or isolated so it is not possible recombination events between populations of the three lakes but do not show a striking variation in nucleotide sequences CO1 gene as a genetic barcode. Results of this study demonstrate that geographic isolation, and isolation of habitat, reproductive isolation does not always lead to a species increases the genetic variability. Genetic variation can be induced by the environment in which a spcies life. Tondano Lake, Linow Lake and Moat Lake has very different characteristics. Linow lake lake known as a very high contents of sulfur, Lake Tondano as the largest lake in North Sulawesi with

high agricultural activities have experienced a lot of pollution and Moat Lake relatively less polluted. Environmental conditions that exist have not been enough to trigger mutations in the gene CO1 Damsefly that can lead to high variations in the sequence of bases CO1 gene in each sample Damsefly. According to the Law of Hardy Weinberg genetic variation of a species will be relatively constant if there is no mutation, recombination, migration and natural selection. Seeing the results of the sequence order of nitrogenous bases sixth Damsefly samples were analyzed, the processes that lead to genetic variation Damsefly maximum not occur.

In some insect species amino acids in the reaction center of the cytochrome oxidase I highly conserved, while certain areas have a very high variation. This is why CO1 very useful in the study of animal evolution. Most of the mitochondrial DNA control region rich in A + T bases, more than 85% composed by both these bases, even in Drosophila melanogaster was found 96% A + T (Zhang and Hewitt, 1997). The phenomenon of cryptic species found in this study. Cryptic species is two or more separate species classified in the same species have a similar morphology. Many phylogenetic studies, filogeografi and population genetics are not currently paying attention to genetic divergenitas but cryptic morphology (Pfeninger and Schwenk, 2007).

CONCLUSIONS

From the results of this study concluded: CO1 sequences of Damsefly that comes from Lake Tondano, Moat Lake and Lake Linow after combined sequences LCO and HCO sequences of each sample shows the distribution of 712 bp to 719 bp. 2. The six samples Damsefly shows the base sequence of nitrogen have identical percentage (similarity), 99 percent or it can be said based gene CO1, six samples are the same species. BLAST results showed that six samples, have the same nucleotide sequence which is identical to Agriocnemis femina. 3. Similarity 6 Damsefly samples from three different ecosystems that Tondano Lake, Lake and Lake Moat Linow average above 99% Agriocnemis femina similarity between samples as well as above 99%. 4. Perbebedaan some morphological characters of each sample from a different lake ecosystems is not sufficient to give effect to CO1 gene mutations in mitochondrial DNA or gene conservation CO1 at Damsefly that live in three ecosystems of the lake is very high.

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. Mol. Mar. Biol. Biotechnol. 3, 294-297.

AJ Drummond, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, R Moir, Stones-Havas S, S Sturrock, Thierer T, Wilson A (2012) Geneious v5.6. New Zealand.

Erniwati. 2009. Diversity and Distribution of Insects in Island Regions - Small Island Karimunjawa National Park. News journal Biology 9 (4): 349-358.

Maramis RTD and Makal HVG. 2011. Species diversity and abundance of the population of aquatic insects as biological indicators of water contamination in das in Langowan. Eugenia Volume 17 No. August 2, 2011

Gangurde S. 2007. Aboveground arthropod pest and predator diversity in Irrigated rice (Oryza sativa L.) production system of the philippnes. Journal of tropical agriculture 45 (1-2): 1-8.

Pfenninger M and K. Schwenk 2007. Cryptic animal species are homogeneously distributed Among taxa and biogeographical regions. BMC Evolutionary Biology. 7: 121 doi: 10.1186 / 1471-2148-7-121

Wakhid2), Roni Koneri1) *, Trina Tallei1), Pience V. Maabuat1 2014. Population Abundance Damsefly Needle (Zygoptera) in Region Nani Wartabone Park, North Sulawesi. (Population Abundance of Damselfly (Zygoptera) in Nani Wartabone National Park, North Sulawesi). Bioslogos Journal, August 2014, VOL. 4 No. 2, pp 41-47

Zhang DE and Hewitt GM. 1997. Insect control mithochondrial region: A review of its structure evolution and usefulness in evolutionary studies. Biochemical Systematics and Ecology, vol. 25. No. 2 pp: 99-20.