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Molecular identification of house fly, *Musca domestica* L. (Diptera : Muscudae), using mitochondrial DNA partial genes cytochrome oxidase sub unit 1 (CO1) in Manado city

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Abstract
Musca domestica L. becomes a serious problem in tropical country. Its role as a vector of many pathogenic microbes has caused many health problems for humans. A study was conducted to identify house fly in Manado City, using partial gene cytochrome oxidase sub unit 1 (CO1). House fly is obtained from nine different habitats in Manado City. Isolation of DNA were used DNA extraction and purification Kit. Amplification of CO1 gene by PCR method. Sequence analysis using Geneous and MEGA 6.0. The result of this research showed, the sequence of house fly CO1 gene : IBP, IBS, IBT, IKT, IMT and IPB have the highest similarity level with *Musca domestica* cytochrome oxidase subunit I (COI) gene [MG557665.1], while the CO1 gene of IKP and IKT has the highest similarity level with *Musca domestica* ISOLATE CSU 140601CBJI A $ cytochrome oxidase subunit I (COI) gene [KY001857.1]. CO1 gene of IKS showed similarity with *Musca domestica* ISOLATE CSU 140601CBJI A$ cytochrome oxidase subunit I (COI) gene. Intraspecies genetic variation of house flies in Manado city based on partial CO1 gene, are high.

Keywords: *Musca domestica* L., cytochrome oxidase sub unit 1 gene, Manado, Indonesia

Introduction

The house fly (*Musca domestica* L.) is the most frequent house fly species transmitting pathogenic bacteria in humans (Sembel, 2008; Kassiri *et al*. 2012) [8]. House flies can act as vectors of transmission of gastrointestinal diseases, such as cholera, dysentery, typhoid and also carry protozoa, eggs and worm larvae (Santi, 2001; Chandra, 2005). Furthermore, house flies are considered as annoying insects because it is a mechanical vector of several diseases including gastrointestinal infections (Hastutiek, 2007). Transmission of the disease mechanically, i.e. through all parts of the body flies. Disease germs from animal feces, humans and trash can stick to body hair, hairs on legs and probosis. House fly, can spread *Helicobacter pylori, Escherichia coli, Cryptosporidium parvum*, even H5N1 virus (Hastutiek, 2007.

Manado city has 16 working areas of Community Health Center (Loacal Name : Puskesmas). In 2015, the total population of Manado City amounted to 425,633 inhabitants. Diarrhea disease in Manado City 2014 reportedly amounted to 3174 patients; in 2015 increased to 4967 sufferers. For the work area of Puskesmas Minanga, 2015 was 180 patients, Puskesmas Bahu was 260 patients, Puskesmas Ranotana was 284 patients and Puskesmas Wenang was 182 patients. Government General Hospital, Prof. Dr. R.D. Kandou, in 2015 reportedly handles 480 diarrhea sufferers. Diarrhea is one of the 10 largest infectious diseases in North Sulawesi. In many reports, the highest cases of diarrhea in areas with poor sanitation in Manado City (BPS Sulawesi Utara, 2015) [2].

Based on previous research, population of house fly, at various location in Manado city, founded mixed population of flies with other flies species. Morphological studies have found variations in morphological characteristics such as wing length, body length, head structure, compound eye color, limb structure and abdominal structure of houseflies originating from various locations in Manado City (Rotty, 2017). However, morphological characteristics have not been sufficient to distinguish the species of house fly, which exist in Manado city. Answering the problem was a genotypic analysis using mitochondrial DNA of CO1 gene as a molecular barcode used universally for animal identification.

The structure and composition of the genetic information contained in mitochondrial DNA has been extensively researched, can characterize a population, phylogenetic and make it possible to reconstruct evolutionary history (Hebert *et al*. 2003; Lessinger *et al*., 2000; Mokosuli, 2013) [6, 10]. Mitochondrial DNA is maternalistic, so there is no recombination with parental male mitochondrial DNA (Nelson and Cox, 2005; Alberts *et al*. 2005) [15, 1]. In mitochondrial DNA, there is a conservative region that can be used to construct an animal evolutionist relationship (Bruce *et al*. 2006; Hebert *et al*. 2003) [6]. Since, Cytochrome c oxidase subunit 1 (*CO1*) gene is considered as one of the widely used markers in the studies of population genetics and evolution (Hebert *et al*. 2003; Shao *et al*., 2007) [6] because it is among the most conservative protein-coding genes found in the mitochondrial genomes of animals (Bruce *et al*. 2006). The cytochrome oxidase sub unit 1 (CO1) is one of the genes present in the mitochondrial genome and is widely used for

animal molecular identification. The application of a universal CO1 gene for molecular identification of insects in North Sulawesi has been done on *Apis dorsata* Binghami (Mokosuli, 2013) [14], *Aedes* sp. (Kaunang *et al*. 2015), *Anopheles* sp. (Manuahe *et al*. 2016) [12]; (Timah and Mokosuli, 2017) [20], and bed bugs (Kalangi *et al*. 2016) [9], marine gerridae (Warouw *et al*. 2015) [23], frehwater gerridae (Waha *et al*. 2016) [22] and demselfly (Rantung *et al*. 2015).

Materials and Methods

Samples

Adult home fly is obtained by direct capture technique. The location of catching house flies, done in some places in Manado, among others, traditional markets, residential areas and bus terminals. Flies captured, preserved in 70% ethanol. Subsequently used as a sample for DNA analysis. Body parts of flies used as tissue sources for DNA extraction are thorax and legs. This research was conducted in Laboratory Bioactivity and Biology Molecular, Department of Biology, Manado State University. DNA sequencing using ABI PRISM 3730xl sequence Genetic Analyzer engine developed by Applied Biosystems, USA, at First BASE Laboratories Sdn Bhd, Singapore.

Tools and Materials

The tools used in this research were : tissue ruptor (Qiagen), vortex V-1 plus (Biosain), orbitals shaker OS-20 (Biosain), micropipette (eppendorf), mini personal centrifuge Tommy Digital Biology, P-class Nanofotometer, centrifuse 5430R (eppendorf), master cycles pro s (eppendorf), gel documentation system fire reader UVitec, Qiaxel automatic electrophoresis (Qiagen), sequence ABI PRISM 3730xl Genetic Analyzer develop by Applied Biosystems, USA. The materials used are: ethanol p.a. (merck), chloroform p.a. (merck), Genomic DNA Mini KIT (Tissue) Geneaid, 2x MyTaq HS Red Bioline Mix (USA), Qiaxel DNA Screening gel kit and 2 µl tips - 100 µl Qiagen, CO1 Universal primer:
LCO1490: GGTCAACAAATCATAAAGATATTGG
HCO2198: AACTTCAGGGTGACCAAAAAATCA (Folmer *et al*. 1994),

DNA extraction and purification

a. Extraction of house fly DNA

DNA extraction and purification using the Geneaid Mini KIT (Tissue) Genomic DNA procedure. Initial stage before entering on extraction is tissue dissociation consisting of taking 30 mg tissue legs and thorax of house fly, then inserted in vial eppendof 1,5 ml. In the vial, 200 µl of GT Buffer is added. Furthermore, 20 µl Proteinase K was added. The incubation was modified from 30 minutes to 24 hours. The next step follows the Kit protocol. The result of DNA extraction of house fly, then analyzed the concentration and purity by using Implant nanophotometer. DNA purity can be seen with an A260 / A280 ratio of between 1.8 - 2.0 nm. If <1.8 is contaminated with protein and or protein derivate contaminant components that affect DNA molecules, and if> 2.0 means contaminated with RNA (Protocol Kit).

b. Amplification of house fly CO1 gene, by PCR method

The PCR process used 2x MyTaq HS Red Mix Bioline (USA) and CO1 primer is Forward LCO 1490: 5'GGTCAACAAATCATAAAGATATTGG3' and Reverse HCO 2198: 5'TAAACTTCAGGGTGACCAAAAAATCA3'. The PCR component and PCR conditions applied are shown in Table 1 and Table 2.

Table 1: PCR Component

PCR Component	Volume (µL)
2x MyTaq HS Red Mix Bioline	25
Primer Forward	1
Primer Reverse	1
DNA of house fly*	2
ddH_2O	21
Total	50

Table 2: PCR Condition

Cycle	Time (Seconds)	Temperatur (°C)	Phase
35 x	60	94	Denaturasi
	30	50	Annealing
	30	72	Ekstension
	60	72	Final Ekstension

Visualization of PCR products

Amplicons of CO1 gene of house flies, produced at the PCR stage, were visualized using automatic electrophoresis (Qiaxel), by applying a Qiaxel DNA Screning gel (Qiagen) kit. Visualization of PCR results was also performed using conventional electrophoresis.

Sequences analyses and phylogeny trees reconstruction

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 (www.ncbi.nih.gov.com). Nucleotide frequencies were calculated using MEGA 6.0 software (Tamura et. al. 2013) [19]. 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 reconstruction methods: (1) neighbor joining (NJ) and (2) Minimum Evolution (ME). 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) [19].

Results and Discussion

DNA extraction of house flies (Musca sp.) from Manado city

The highest DNA purity of nine house flies samples was 1,75 (sample IKP). While the lowest DNA purity was 1,55 (IKT Samples). In the other hand, the highest DNA concentration was 53.2 µg/ml (IMT sample) while the lowest total DNA concentration was 40.25 µg/ml (IBP sample). DNA purity is not linear, with the DNA concentration of house flies obtained (Figure 1).

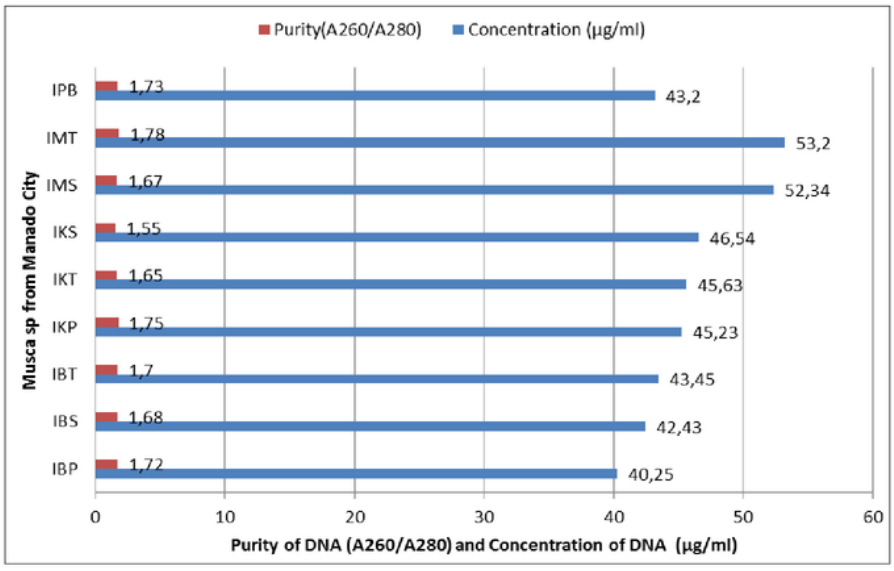

Fig 1: Concentration and Purity dsDNA of house fly from Manado City

Based on the concentration and purity of the extracted DNA, it showed that the Genomic DNA Mini KIT (Tissue) Geneaid, which is used to extract house fly DNA, has effective in extracting total DNA from legs and thorax flies. The difficulty in extracting insect DNA, compared with other animal samples is the number of complex biomolecule contaminants from the exoskeleton. Common contaminants found in insect DNA extraction are chitin, complex proteins and peptides from exoskeleton. This contaminant may decrease the effectiveness of buffers and proteinase enzymes in the kit (Timah and Mokosuli, 2017; Manuahe *et al*. 2015; Mokosuli, 2013) [20, 14]. In this research, modification of protocol kit is done by the destruction of thorax and legs, using tissue ruptor and tissue immersion time with protenase K according to protocol kit, 30 minute has modified to 24 hours. This modification proved to increase the concentration and purity of the house fly DNA extraction results. The total DNA concentration distribution based on the Kit protocol used was 30 µg / ml up to 70 µg / ml. Thus the total DNA concentration obtained in this study is quite good. While total DNA purity is at the distribution of 1,7 - 2,0 (A260 / A280). Total DNA purity of the results of this study is still quite good. However, the mitochondrial DNA content present in extracted DNA is known after amplification of the target gene, using a universal CO 1 primer.

PCR and Visualization of Amplikon Gen CO1 Home Flies (Musca sp.) From Manado

Extracted DNA, amplified by PCR method. Of the 4 stages of PCR, the annealing is the most important stage, so temperature and time modification greatly affect the optimization of CO1 gene amplification. In this study, modified annealing temperatures are proven to produce amplicons as targeted. Visualization of amplicons content of CO1 gene is done by electrophoresis technique. Electrophoresis condition was 0.8% agarose gel, the number of ladder DNA that is applied to each well 0.2 µg with the volume of samples per well 1 µl. The band on the electrogram shows the amplicon content of the flies CO1 gene successfully amplified by the PCR method. The PCR results show that sample amplicons are 6. (IKP), 7. (IKT), 8. (IKS), 9. (IMT), 10. (IPB). was formed optimally while sample 3. (IBS), 4. (IBP), the band is very thin (Figure 2).

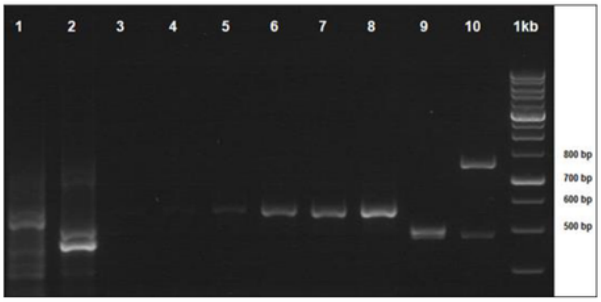

Fig 2: Visualization of CO1 gene amplicons, house flies from Manado City. Description :1. (control) 2. (IBT), 3. (IBS), 4. (IBP), 5. (IMS), 6. (IKP), 7.(IKT), 8. (IKS), 9. (IMT), 10. (IPB).

Sequensing

Output sequencing from First BASE Singapore, read with Geneous 10.1. and MEGA 6.0. Based on the chromatogram of sequenced results, the sequencing showed well. This is evidenced by chromatogram bands representing different types of nucleotides perfectly or not coincident (Appendix 1.). After the contig analysis, the length of the CO1 gene sequence of flies from Manado was between 558bp - 691 bp and HQ (68.5% - 92.8%). Characteristics of the CO1 fly gene sequence from Manado were then shown in Table 2. The sequence of CO1 gene lies at length 600 - 700 bp (Herbert, 2003). Thus, the nine sequences of the fly fly CO1 gene from Manado were on the long-range CO1 gene, according to the characteristics of the CO1 gene as molecular barcodes for animal identification.

Table 2: Characteristics of CO1 gene house fly sequences, from Manado City

No	Sample	Lenght of Sequens (bp)	MW dsDNA (kDa)	HQ (%)	Nucleotide Composition				
					A	C	G	T	% GC
1	IBP	682	421,29	92,7	259 (38%)	111(16,3%)	108 (15,8%)	204(29,9%)	219(32,1%)
2	IBS	558	344,69	68,5	160(28,7%)	82(14,7%)	90(16,1%)	214(38,4%)	171(31,5%)
3	IBT	695	429,32	84,3	195(28,1%)	109(15,7%)	117(16,8%)	274(39,4%)	226(32,5%)
4	IKP	691	426,85	92,8	204(29,5%)	113(16,4%)	114(16,5%)	260(37,6%)	227(32,9%)
5	IKT	694	428,71	91,2	201(29,0%0	110(15,9%)	119(17,1%)	264(38,0%)	229(33,0%)
6	IKS	690	426,23	92,8	200(29,9%)	108(15,7%)	112(16,2%0	270(39,1%)	220(31,9%)
7	IMS	686	423,76	92,3	201(29,3%)	109(15,9%)	111(16,2%)	265(38,6%)	220(32,1%)
8	IMT	683	421,91	93,1	258(37,8%)	111(16,3%)	108(15,8%)	207(30,2%)	219(32,1%)
9	IPB	682	421,28	92,7	259(38%0	111(16,3%)	109(15,0%)	203(29,8%)	220(32,2%)

Alignment analysis with the NCBI BLAST Method (https://blast.ncbi.nlm.nih.gov/Blast.cgi)

The consensus area of the house fly CO1 gene sequence from Manado City, each used for alignment analysis with the BLAST method on the NCBI website. The BLAST results indicate that nine CO1 gene sequences of flies in Manado City have the highest percentage of similarities with the three *Musca domestica* CO1 gene sequences that have been recorded in the gene bank NCBI. The sequence of CO1 gene IBP, IBS, IBT, IKT, IMT and IPB have the highest similarity level with *Musca domestica* cytochrome oxidase subunit I (COI) gene [MG557665.1], while the CO1 IKP gene sequence has the highest similarity level with *Musca domestica* ISOLATE CSU 140601CBJI A $ cytochrome oxidase subunit I (COI) gene [KY001857.1] (table 3). The nucleotide difference between flies from nine sites in Manado is shown in Table 4. The nucleotide difference is indicated by the dot, on the output of the alignment analysis.

Table 3: Similarity level of house fly in Manado City, based on alignment analysis on NCBI website

No	Samples	Percentage Similarity	Similarity Species	Assesion Number	Author and Country Origins
1	IBP	99%	Musca domestica cytochrome oxidase subunit I (COI) gene	MG557665.1	Aslam,A.F.M., Rain,F.F. and Howlader,A.J. Submitted (22-NOV-2017) Zoology, DNA Barcoding Laboratory, Department of Zoology, Jahangirnagar University, Dhaka, Savar 1342, Bangladesh
2	IBS	99%	Musca domestica cytochrome oxidase subunit I (COI) gene	MG557665.1	Aslam,A.F.M., Rain,F.F. and Howlader,A.J. Submitted (22-NOV-2017) Zoology, DNA Barcoding Laboratory, Department of Zoology, Jahangirnagar University, Dhaka, Savar 1342, Banglades
3	IBT	99%	Musca domestica cytochrome oxidase subunit I (COI) gene	MG557665.1	Aslam,A.F.M., Rain,F.F. and Howlader,A.J. Submitted (22-NOV-2017) Zoology, DNA Barcoding Laboratory, Department of Zoology, Jahangirnagar University, Dhaka, Savar 1342, Bangladesh
4	IKP	99%	Musca domestica cytochrome oxidase subunit I (COI) gene	MG557665.1	Alkhedir,H., Mashaly,A.M.A. and Karlovsky,P. Submitted (22-JAN-2016) Agricultural Entomology and Molecular Phytopathology and Mycotoxin Research, Georg-August-University Goettingen,Grisebachstrasse 6, Goettingen 37077, Germany
5	IKT	99%	Musca domestica cytochrome oxidase subunit I (COI) gene	MG557665.1	Alkhedir,H., Mashaly,A.M.A. and Karlovsky,P. Submitted (22-JAN-2016) Agricultural Entomology and Molecular Phytopathology and Mycotoxin Research, Georg-August-University Goettingen,Grisebachstrasse 6, Goettingen 37077, Germany
6	IKS	99%	Musca domestica ISOLATE CSU 140601CBJI A$ cytochrome oxidase subunit I (COI) gene	KY001857.1	Guo,Y.-D., Cai,J.-F. and Ren,L.-P. Submitted (17-OCT-2016) Department of Forensic Medicine, Central South University, Tongzipo Road No. 172, Changsha, Hunan 410013, China
7	IMS	99%	Musca domestica cytochrome oxidase subunit I (COI) gene	MG557665.1	Aslam,A.F.M., Rain,F.F. and Howlader,A.J. Submitted (22-NOV-2017) Zoology, DNA Barcoding Laboratory, Department of Zoology, Jahangirnagar University, Dhaka, Savar 1342, Bangladesh
8	IMT	99%	Musca domestica cytochrome oxidase subunit I (COI) gene	MG557665.1	Aslam,A.F.M., Rain,F.F. and Howlader,A.J. Submitted (22-NOV-2017) Zoology, DNA Barcoding Laboratory, Department of Zoology, Jahangirnagar University, Dhaka, Savar 1342, Bangladesh
9	IPB	99%	Musca domestica cytochrome oxidase subunit I (COI) gene	MG557665.1	Submitted (22-NOV-2017) Zoology, DNA Barcoding Laboratory, Department of Zoology, Jahangirnagar University, Dhaka, Savar 1342, Bangladesh

Table 4: Analysis of alignment, the CO1 gene of house flies in Manado

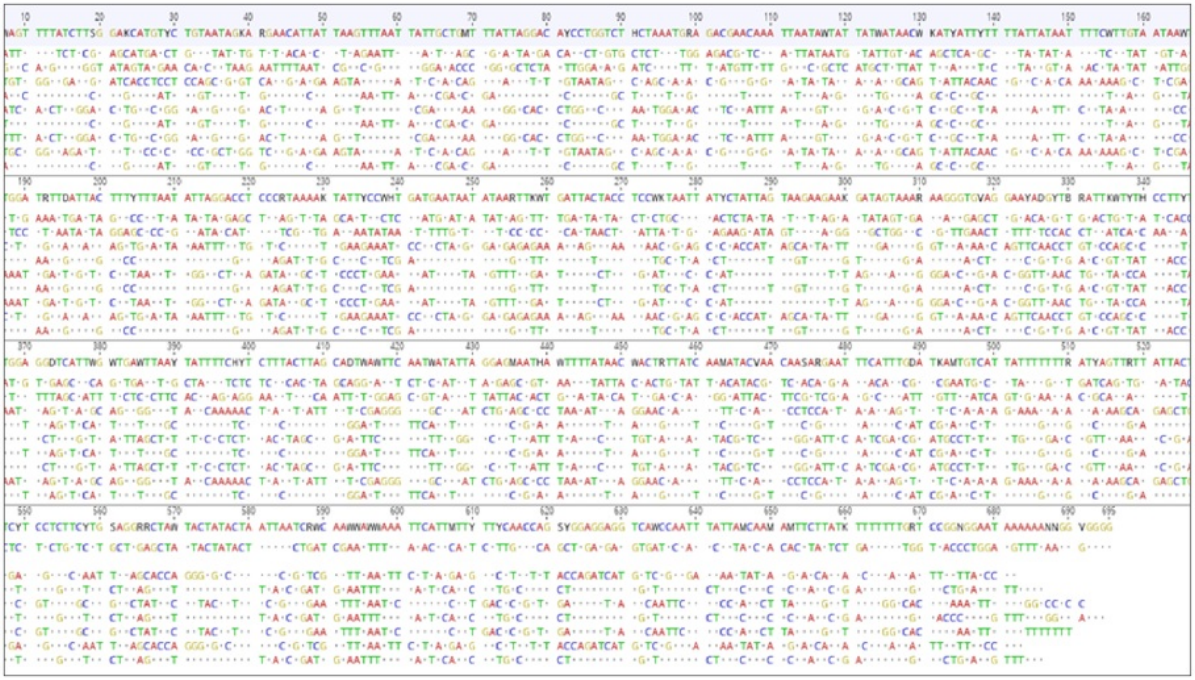

Table 5: Genetic distance of house flies from Manado and similarity sequence of BLAST result on NCBI webs

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1																										
2	4.96																									
3	4.92	0.05																								
4	4.42	3.09	3.31																							
5	3.46	3.67	4.47	1.73																						
6	4.40	3.01	3.21	0.01	1.73																					
7	3.40	3.68	3.74	1.75	0.01	1.75																				
8	3.44	3.64	3.70	1.68	0.01	1.73	0.01																			
9	4.20	4.78	4.79	1.98	2.88	1.99	3.02	3.05																		
10	3.17	3.86	3.95	2.99	2.00	2.92	2.08	2.16	1.84																	
11	2.95	4.55	3.72	3.57	3.44	3.57	3.53	3.52	4.33	3.48																
12	4.98	1.56	1.53	3.50	4.56	3.53	4.53	4.54	4.72	3.32	2.89															
13	2.28	3.03	3.30	2.61	4.06	2.58	4.20	4.15	4.35	3.39	2.67	2.86														
14	2.28	3.03	3.30	2.61	4.06	2.58	4.20	4.15	4.35	3.39	2.67	2.86	0.00													
15	2.28	3.03	3.30	2.61	4.06	2.58	4.20	4.15	4.35	3.39	2.67	2.86	0.00	0.00												
16	2.28	3.03	3.30	2.61	4.06	2.58	4.20	4.15	4.35	3.39	2.67	2.86	0.00	0.00	0.00											
17	2.28	3.03	3.30	2.61	4.06	2.58	4.20	4.15	4.35	3.39	2.67	2.86	0.00	0.00	0.00	0.00										
18	2.28	3.03	3.30	2.61	4.06	2.58	4.20	4.15	4.35	3.39	2.67	2.86	0.00	0.00	0.00	0.00	0.00									
19	2.28	3.03	3.30	2.61	4.06	2.58	4.20	4.15	4.35	3.39	2.67	2.86	0.00	0.00	0.00	0.00	0.00	0.00								
20	2.28	3.03	3.30	2.61	4.06	2.58	4.20	4.15	4.35	3.39	2.67	2.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00							
21	4.71	2.14	2.48	4.52	4.44	4.56	4.50	4.53	4.69	4.71	4.72	1.82	4.45	4.45	4.45	4.45	4.45	4.45	4.45	4.45	4.45					
22	2.28	3.03	3.30	2.61	4.06	2.58	4.20	4.15	4.35	3.39	2.67	2.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.45				
23	3.36	2.96	3.06	2.05	2.18	2.00	2.20	2.15	2.96	1.78	3.62	3.15	3.38	3.38	3.38	3.38	3.38	3.38	3.38	3.38	3.38	4.74	3.38			
24	4.98	1.56	1.53	3.50	4.56	3.53	4.53	4.54	4.72	3.32	2.89	0.00	2.86	2.86	2.86	2.86	2.86	2.86	2.86	2.86	2.86	1.82	2.86	3.15		
25	4.99	3.10	3.17	4.29	3.22	4.22	3.18	3.17	4.56	4.44	4.63	3.13	2.90	2.90	2.90	2.90	2.90	2.90	2.90	2.90	2.90	2.08	2.90	3.42	3.13	
26	4.99	3.10	3.17	4.29	3.22	4.22	3.18	3.17	4.56	4.44	4.63	3.13	2.90	2.90	2.90	2.90	2.90	2.90	2.90	2.90	2.90	2.08	2.90	3.42	3.13	0.00
27	3.71	3.27	3.17	2.45	2.47	2.43	2.52	2.36	2.25	2.52	4.69	2.89	4.56	4.56	4.56	4.56	4.56	4.56	4.56	4.56	4.56	4.56	3.62	4.56	2.22	2.89	4.72

Description

1. (IBT), 2. (IBS), 3. (IBP), 4. (IMS), 5. (IKP), 6.(IKT), 7. (IKS), 8. (IMT), 9. (IPB), 10. Musca domestica [KY001857.1], 11 Musca domestica [KY001856.1], 12. Musca domestica [KY001855.1], 13. Musca domestica [KY001854.1], 14. Musca domestica [KT272839.1], 15. Musca domestica [KT272838.1], 16. Musca domestica [KT272837.1], 17. Musca domestica [KT272836.1], 18. Musca domestica [KT272834.1], 19. Musca domestica [KT272833.1], 20. Musca domestica [KT272831.1], 21. Musca domestica [KT272830.1], 22. Musca domestica [KT272829.1], 23. Musca domestica [KR921687.1], 24. Musca domestica [KT444442.1], 25. Musca domestica [KP713680.1], 26. Musca domestica [KJ496775.1], 27. Musca domestica [JX861432.1], 28. Musca domestica [JX861431.1], 29. Musca domestica [KF562113.1],

Subtitution Matrix

The analysis of the substitution matrix, nine gene sequences of house flyflies from Manado with BLAST sequenced

sequences, was performed using the MEGA 6.0 program, using the Maximum Likelihood model. Adenine nucleotide frequency (32.8%), Timin (38.30%), Cytosin (14.24% and Guanin (14.58%)) The average transition substitution was printed with bold numbers while the average transversion substitution was printed with italics in table 4.

Table 6: Substitution matrix with maximum likelihood model

	A	T/U	C	G
A	-	11.72	4.36	3.63
T/U	10.07	-	7.31	4.46
C	10.07	19.67	-	4.46
G	8.18	11.72	4.36	-

Discussion

Modified Genomic DNA Mini KIT (Tissue) Geneaid, can optimize the concentration and purity DNA of house flies. Insect DNA extraction has its own difficultie, compared with mammals (Mege and Mokosuli, 2017) [13]. This is caused, among others, by exoskeleton in adult insects. The exosketon is often mixed in the tissue to be extracted because the insect tissue is in the exoskeleton. This affects the purity and concentration of targeted DNA. Because this study uses genes present in mitochondrial DNA, mitochondrial DNA must be extracted and purified optimally, in order to become templete in the process of CO1 gene amplification. On the other hand, the use of thoracic and legs, in which there were many muscle tissues, has successfully isolated mitochondrial DNA on insects optimally. Extraction and purification of insect DNA using legs and thorax have been performed on *Aedes* sp. (Timah and Mokosuli, 2017; *Anopheles* sp. (Manuahe *et al.* 2016) [12], *Droshophila* sp. (Sumampouw and Mokosuli, 2017) [17] and bed bugs (Kalangi et. 2017) [9], *Apis dorsata* Binghami (Mokosuli *et al.* 2013). Thus thorax and legs were best used for the isolation of mitochondrial DNA in insects.

The results of the alignment analysis with the BLAST method on the NCBI site showed that house fly in Manado City had the closest similarity, with 3 species of house fly in three different countries (Table 3). This reinforces, that the variation intraspecies, house fly in Manado City is high. Alignment between the CO1 gene sequence of flies in Manado City, has found many polymorphic sites or sites where nucleotide differences occur (Table 4). Furthermore, genetic distance analysis also showed differences in genetic distance between house flies from nine sites in Manado City had more than one (Table 5). This showed the proportion of nucleotides of the CO1 gene, the nine house flies in Manado City have shown high genetic variation. Previous morphological analysis has also found morphological variations, especially in the thorax, legs and body length. Although morphology in general still shows the characteristics of house fly (Figure 3). Variations of CO1 gene, commonly found in insects. *Aedes* sp. in North Sulawesi, which lives in different habitat characteristics, has shown a high variation of CO1 gene (Timah and Mokosuli, 2016) [12].

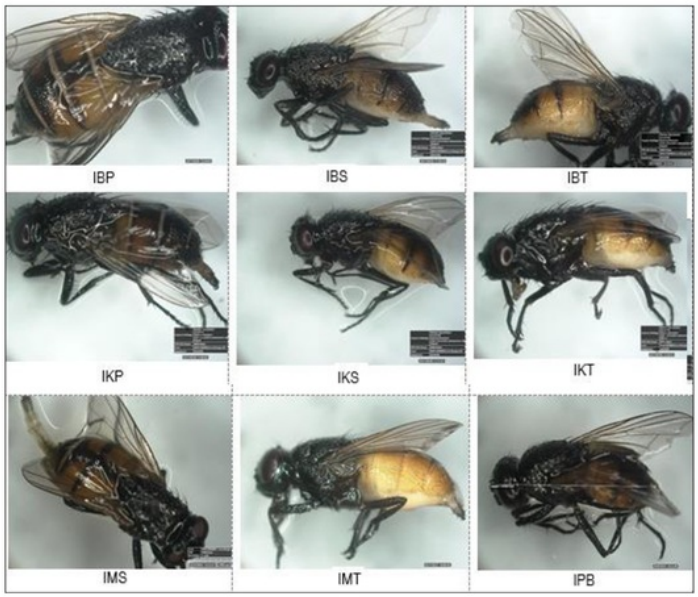

Fig 3: House fly from different habitat in Manado City

The reconstruction of phylogeny trees was carried out using two models. Neither Neighbor Joining nor Minimum Evolution models was showed the same phylogenetic tree topography. The phylogeny tree was built with 500x bootsrap. The phylogeny tree was built, consisting of 2 monophyletic clades. IBS, IBP and ITS were on the first monophyletic clade, however IBT, IKP, IKS, IMT, IPB and IKT were in other monophyletic clades. Thus the species of house fly in Manado City has varied based on the evolutionary relationship.

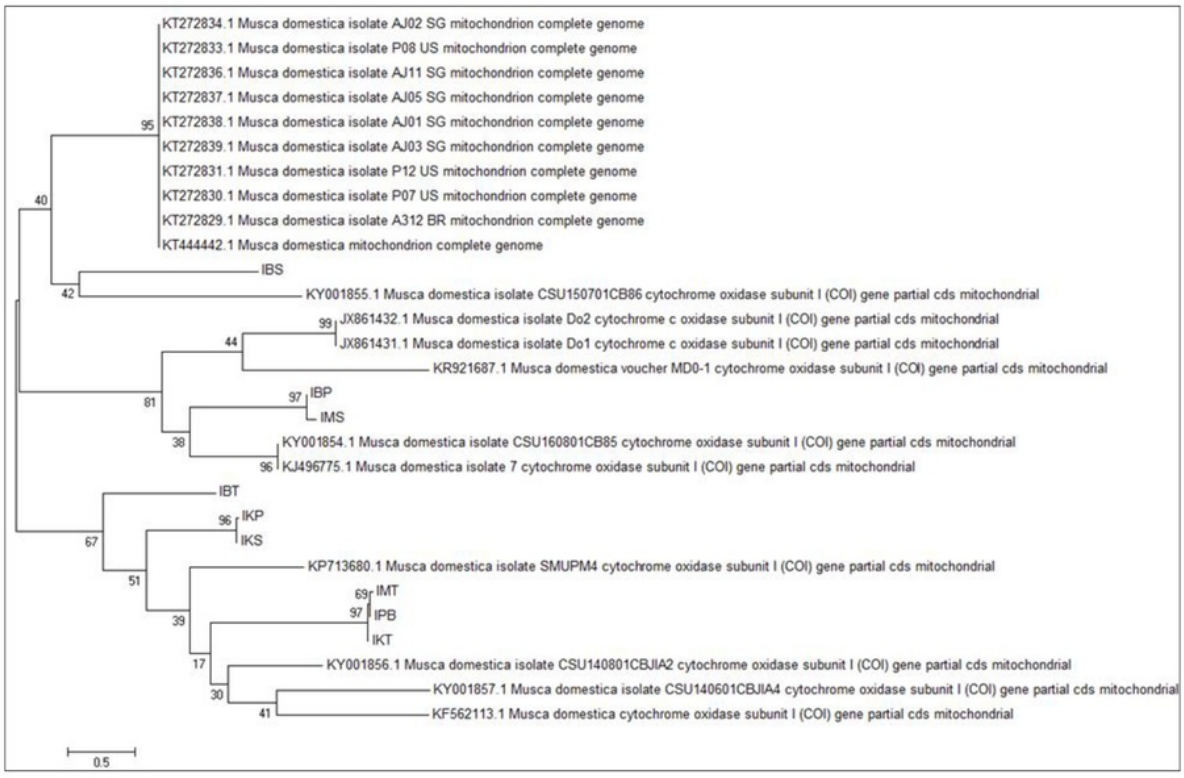

Fig 4: Reconstruction of the house fly phylogeny tree in Manado, Neighbor Joining model (Bootsrap 500x).

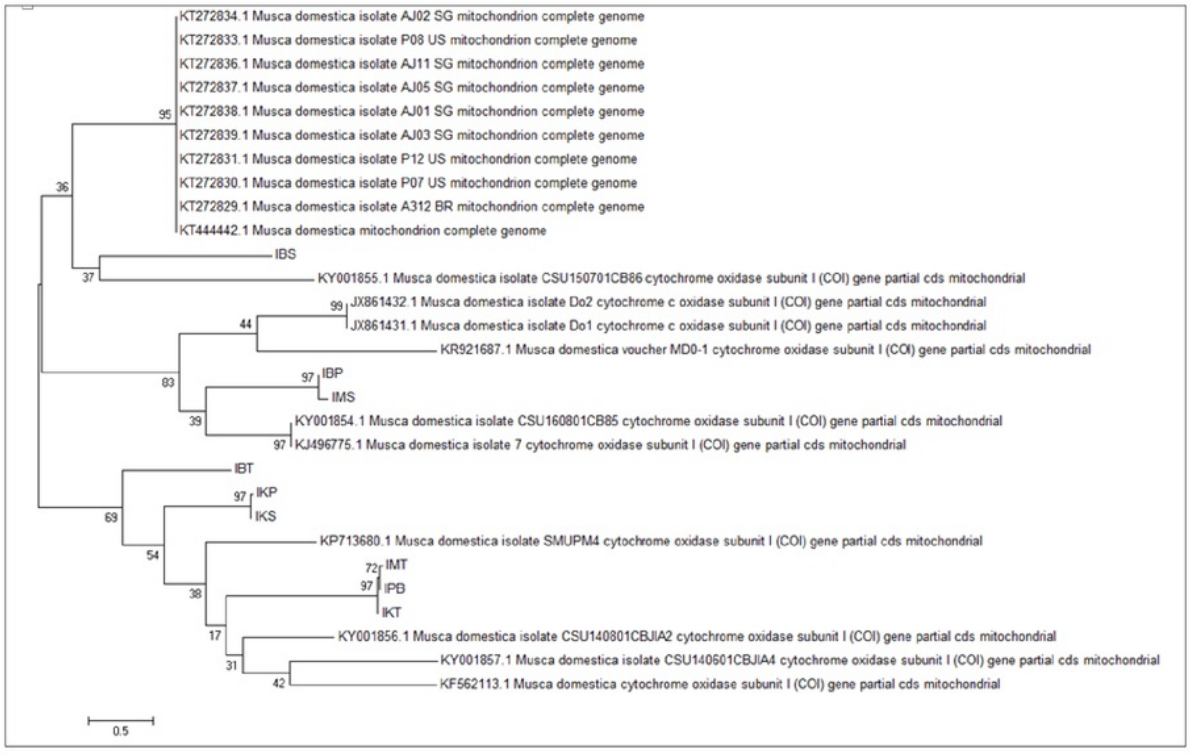

Fig 5: Reconstruction of the house fly phylogeny tree in Manado, Minimum Evolution model (Bootsrap 500x).

Reconstruction of phylogeny trees based on partial gene CO1, house fly from Manado obtained two monophyletic clade. The first clade consists of IKT, IPB, IMS (one node) and IBT, IBS (one node). While the second clade consists of IPB and IMT (one node). IKP and IKS form their own nodes. From the phylogenetic tree formed on the CO1 gene, it was found that the genetic variation of flies in manado based on the CO1 gene was high. Based on the phylogeny tree formed, then house fly IKP and IKS,the oldest by evolution (Figure 6).

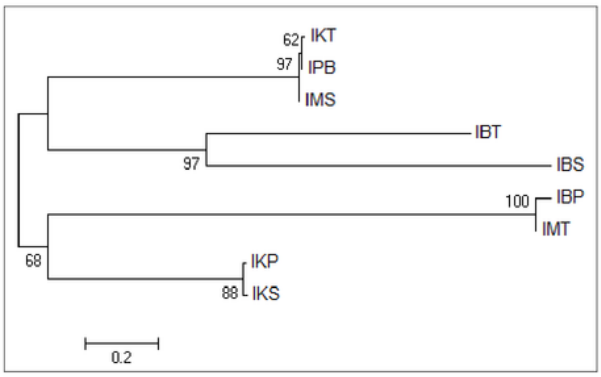

Fig 6A

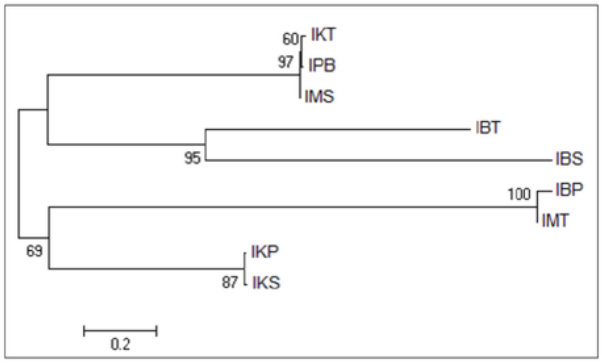

Fig 6B

Fig 6: Reconstruction of phylogeny trees, house flies in Manado originating from nine locations. (a). Model Neighbor Joining (Bootsrap 500x). (b). Model Minimum Evolution (Bootsrap 500x).

Conclusion

The sequence of CO1 gene IBP, IBS, IBT, IKT, IMT and IPB have the highest similarity level with *Musca domestica* cytochrome oxidase subunit I (COI) gene [MG557665.1], while the CO1 gene of IKP and IKT has the highest similarity level with *Musca domestica* ISOLATE CSU 140601CBJI A $ cytochrome oxidase subunit I (COI) gene [KY001857.1]. CO1 of IKS showed similarity with *Musca domestica* ISOLATE CSU 140601CBJI A$ cytochrome oxidase subunit I (COI) gene. Genetic variation intraspecies, house fly in Manado city based on CO1 gene, was high.

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