



C.MEGACEPHALA AND C.RUFIFACIES VARIAN IN NORTH SULAWESI

Erwin Kristanto¹, Sonny J.R Kalangi², Johannes Huijbregts³, Dantje T Sembel⁴

¹ Forensic and Medicolegal Department, Faculty of Medicine, University of Sam Ratulangi, Indonesia

² Anatomy and Histology Department, Faculty of Medicine, University of Sam Ratulangi, Indonesia.

³ Naturalis Biodiversity Center, Leiden, Netherlands

⁴ Pest and Plant Diseases Department, Faculty of Agriculture, University of Sam Ratulangi, Indonesia.

ABSTRACT

Background : *C.megacephala* and *C. rufifacies* are the 2 dominant primary necrophagus species in North Sulawesi that in several studies had a different growth rate compared to other studies at other countries and region with similar climate. Aside from temperature and humidity, there are some possibilities that species variation can make this difference possible.

Methods : Research was conducted at Manado, North Sulawesi, using local pig carcass that was put down with potassium cyanide. Sample extraction is done with AxyPrep Multisource Genomic DNA Miniprep Kit modality *C.megacephala* and *C.rufifacies* that were retrieved from the carcass then will be identified and sequenced for identification and analysis.

Results : Differences in the sample confirm the presence of regional genetic variation that can reach 3% of the COI analysis area. This variation shows the findings of a new base sequence with regional variations of North Sulawesi.

BACKGROUND

Proofing is the process of convicting the judge about the truth of the proposition or the arguments presented in a dispute. In Indonesia, to ensure the establishment of truth, justice and legal certainty for a person, the Negatief wettelijk bewijs theorie is adopted. This system states that a judge shall not impose a penalty on a person unless there are at least two valid evidences he / she obtains confidence that a criminal act actually took place and that the defendant was guilty of doing so (Masriani, 2004).

Insects have long been used as a tool for solving legal problems. In China, in 1235 recorded in the book "Washing away from wrongs" by Sung Tz'u depicted the use of flies that come on a sickle, making a farmer claimed to have killed his co-workers with the sickle. (Genard, 2007).

Necrophagus insects have their own value for the purpose of making estimates of death, especially primary necrophagus insects that colonize corpses or animal carcasses. This insect can be used as a benchmark, either through the size of the adult insect, or through the

stages of insect development that can be found in corpses / carcasses.

C. megacephala and *C. rufifacies* are the 2 dominant primary necrophagus species in North Sulawesi. North Sulawesi in bio geographical view is the transition between the oriental and Australian region, this transitional region is rich with endemic species (Kurahashi H, 1997). An understanding of the life cycle, especially the timing of the development of this species, is very important to help make the estimates of death by forensic entomology methods. Estimating the time of death by this method requires the identification of appropriate species and variants, the study of the species flies, and the circumstances surrounding. The authors are interested in investigating the bimolecular identity of primary necrophagus insects, to sharpen estimates of deaths made based on forensic entomology methods.

RESEARCH METHOD

The research was conducted in Malalayang area, Manado city, North Sulawesi in July 2017. The research was conducted after proposing and obtaining ethical clearance study from Medical Research Ethics Commission of Prof.dr.R.D Kandou. Temperature and humidity of the study sites were recorded since the samples were placed until the observations were

completed in the field. Data were compared with data obtained through the local meteorological office. This research was using a local pig weighed 25 kg that was put down by an injection of Potassium cyanide (KCN) and placed in an open field.

Sample Processing

- Sample of mature and immature insects were taken from carcass of experimental animals on the first day until the fifth day. Mature insects were collected with insect net, while immature insects were collected directly from the carcass.
- Carcass was observed and the samples were taken daily.
- Identification initially were made through anatomical morphological method, then supported by bimolecular data, by sequencing mtDNA flies for determination of species of flies. Samples were extracted from adult and adult flies. Extraction, purification, quantification and amplification were done in the biology department of Faculty of Mathematics and Natural Sciences of Sam Ratulangi University of Manado.
- Sample extraction is done with AxyPrep Multisource Genomic DNA Miniprep Kit modality. This

- kit was chosen for its ability to extract genetic material from various sources. This kit utilizes a column purification technique capable of extracting total cell DNA which is nuclear DNA and mitochondrial DNA (Allesandrini F, 2008).
- PCR of approximately 1270 bp of the amplified COI gene, were done using primers for *Chrysomya* flies: (Harvey ML, 2008)
 - o C1-J-1718f (5' – GGAGGATTTGGAAATT GATTAGTTCC)
 - o TL2-N-3014r (5' – TCCAATGCACTAATCT GCCATATTA)
 - o TL2-N-3014MODr (5' – TCCATTGCACTAATCTT GCCATATTA)
 - Primers were constructed and processed by PT.Genetika Science Indonesia, appropriate temperature analysis for PCR cycle assisted with Oligo Analyzer software from Integrated DNA Technologies (IDTDNA, 2017). The use of two reverse primers was performed to obtain more specific results in both dominant primary fly species in the study.

- The analysis is done by sequencing COI bar coding which is commonly used for identification of flies.

RESULT

Temperature and humidity at the study sites both indicated by BMKG data and direct measurement data showed relatively stable temperature and humidity, with daily average temperature of 250C-270C, and humidity 56% -72%. (BMKG, 2017)

Calliphoridae are the first insects to lay eggs on the carcass which make them very important tool in calculating post mortem interval. *C.megacephala* and *C.rufifacies* were abundantly found in both mature and immature form. Adult *Sarcophagidae* and *Musca domestica* were also found in small numbers, but no maggots or pupae were found on research site. *Ophyra* sp. both mature and immature were also found in this study.

The "sequencing" of cytochrome oxidase (COI) genes in *Chrysomya megacephala* samples in this study were processed with Sequence Scanner software version 1.0 of Applied

Biosystems and gave the following results:

ACGAATAAATAATATAAG
TTTCTGACTTTTACCTCCTGC
ATTAAC TTTATTATTAGTAAG
TAGTATAGTAGAAAATGGAG
CTGGAACAGGATGAACTGTT
TACCCACCTTTATCTTCTAAT
ATTGCTCATGGAGGAGCATC
AGTTGATTTAGCTATTTTCTC
TTTACACTTAGCAGGAATTT
CTTCAATTTTAGGAGCTGTA
AATTTTATTACAAC TGTAAATT
AATATACGATCTACAGGAAT
TACATTTGATCGAATACCTTT
ATTTGTATGATCTGTAGTTAT
TACTGCTCTATTATTATTATT
ATCTTTACCAGTATTAGCTG
GAGCTATTACTATATTATTA
ACTGACCGAAATCTAAATAC
TTCATTCTTTGATCCAGCAGG
AGGAGGAGATCCTATTTTAT
ACCAACATTTATTTTGATTCT
TTGGACATCCTGAAGTTTAT
ATTTTAATTTTACCTGGATTC
GGAATAATTTCTCATATTATT
AGTCAAGAATCAGGAAAGA
AGGAAACTTTCGGATCTTTA
GGAATGATTTATGCTATACT
AGCTATTGGTCTATTAGGAT
TTATTGTATGAGCTCACCAC
ATGTTTACTGTTGGAATAGA
CGTAGACACACGAGCTTATT
TCACTTCAGCTACAATAATT

ATTGCTGTACCAACTGGAAT
TAAGATTTTCAGTTGATTAG
CAACTCTTTACGGAACACAA
TTAAATTATTCTCCAGCTACT
TTATGAGCTTTAGGATTTGTA
TTTTTATTTACTGTAGGAGGA
TTAACTGGAGTTGTTTTAGCT
AATTCATCAATTGACATTATT
TTACATGATACATATTATGT
AGTAGCTCACTTTCATTATGT
TCTATCAATGGGAGCTGTAT
TTGCTATTATAGCAGGATTT
GTTCAATTGATTCCCTCTATTT
ACTGGATTAACTTTAAATAG
CAAGTTATTAAGAGTCAAT
TTGCTATTATATTTATCGGAG
TAAATTTAACATTCTTCCCTC
AACATTTCTTAGGATTAGCA
GGTATACCTCGACGATACTC
AGACTATCCAGACGCTTACA
CAGCTTGAAATGTAATTTCT
ACAATTGGTTCAACAATTTCT
ATTATTAGGAATTTTATTCTT
CTTTTTCATTATTTGAGAAAG
TTTAGTATCTCAACGACGAG
TTTTATTCCCTGTTCAACTAA
ATTCATCAATTGAATGATTA
CAAATACTCCACCAGCTGA
ACACAG

Bases that were printed in red ink are overlapping data from samples with primary forward and reversed primers. Both data at the same point appear to be mutually confirm. Analysis from

PT.Genetika Science Indonesia and animal identification (COI) from Bold Systems confirmed that the specimen was *Chrysomya megacephala*, with 99.83% certainty value (Bold systems, 2017).

The results of cytochrome oxidase sequencing (COI) in *Chrysomya rufifacies* samples in the study were processed with Sequence Scanner software version 1.0 of Applied Biosystems and gave the following results:

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GTTTTGACTTTTACCTCCTG
CATTAACCTTACTATTAGTAA
GTAGTATAGTAGAAAATGGA
GCTGGAACAGGATGAACTGT
TTATCCACCTTTATCATCTAA
TATGGCACATGGTGGAACCTG
CAATTGATTTAGCTATTTTTT
CTTTACACTTAGCTGGAATTT
CATCAATTTTAGGAGCCGTA
AATTTTATTACAACCTGTTATT
AATATACGATCTACAGGAAT
TACATTTGATCGAATACCTTT
ATTTGTATGATCTGTAGTTAT
TACTGCTCTTCTTTTATTATT
ATCATTACCAGTATTAGCAG
GTGCAATTACTATATTATTA
ACTGATCGAAATTTAAATAC
TTCATTCTTTGATCCAGCAGG
AGGGGGAGACCCTATTTTAT
ATCAACACTTATTTTGATTCT
TTGGTCATCCAGAAGTTTAT
ATTTAAATTTTACCTGGATTC
GGAATAATTTCTCATATTATT
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AGTCAAGAATCAGGAAAAA
AGGAAACCTTTGGATCTTTA
GGAATAATTTATGCAATATT
AGCTATTGGATTATTAGGAT
TTATTGTATGAGCTCATCAC
ATATTCACTGTAGGAATGGA
TGTAGATACTCGAGCATATT
TCACTTCAGCTACAATAATC
ATTGCTGTACCAACTGGAAT
TAAAATTTTTAGTTGATTAGC
AACTCTTTATGGAACTCAAT
TAAATTAATTCTCCAGCTACTT
TATGAGCCTTAGGATTTGTA
TTCTTATTTACTGTAGGAGG
ATTAACCTGGAGTAGTATTAG
CTAATTCATCTATTGATATTA
TTTTACATGACACATACTAT
GTAGTAGCTCACTTCCATTAT
GTTCTTTCAATAGGAGCTGT
ATTTGCTATTATAGCAGGAT
TTGTACATTGATTCCCATTAT
TACTGGATTAACCTTTAAAT
AATAAAATACTAAAAAGTCA
ATTTGCTATTATATTTATTGG
AGTAAATTTAACATTCTTCCC
TCAACATTTTTTAGGACTAG
CTGGTATACCTCGACGATAC
TCAGACTATCCAGATGCTTA
TACAGCATGAAATGTTATTT
CA
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Bases that were printed in red ink are overlapping data from samples with primary forward and reversed primers. Both data at the same point appear to be

mutually confirm. Analysis of PT.Genetika Science Indonesia and animal identification (COI) from Bold Systems confirmed that the specimen was *Chrysomya rufifacies*, with 100% certainty value (Bold systems, 2017).

DISCUSSION

Research conducted on tropical rain forest subtype areas has many advantages over research on other climates and other climate sub-types. Less substantial temperature changes and other relatively constant climatic factors, leading to studies that involve factors of temperature change and other climatic factors are easier over time, without bias due to changes in these factors.(Kreitlow KLT, 2010)

From the 11 Diptera that were shown in the literature existed in Sulawesi region, only *C.megacephala* and *C. ruffifacies* seems to be dominant in this study (Kurahashi H, 1987). Dominant *Chrysomya* species that were found in this research are the same as those reported in other parts of tropical Asia and South China (Heo CC, 2008). Decomposition stage in these study in South China and Malaysia had much longer period compared that were found in studies in Indonesia. (Wangko S, 2015).Immature *Sarcophagidae* that were reported in many forensic cases in other countries, cannot be found in this study. Apparently

Sarcophagidae cannot be used as PMI indicator in Sulawesi (Byrd JH, 2010).

Similarity analysis of the COI base sequence across the samples shows that the Ch sample. *megacephala* and Ch. *The ruffifacies* obtained from this study, none of which have a 100% resemblance compared to other samples in the Genbank and Bold systems database (GenBank, 2017). Differences in the sample confirm the presence of regional genetic variation that can reach 3% of the COI analysis area. This variation shows the findings of a new base sequence with regional variations of North Sulawesi.

Comparison of sample data with available data in the Genbank database requires adjustment of sample data in the form of cutting of sequenced samples according to fragments available in Genbank. As a visualization of the software process, samples of sample data *Ch. megacephala* to compare with the existing data in Genbank.

“*Data Accession number*” EU418535,
Sidney Australia

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1   ACTTTTACCT CCTGCATTAA
    CTTTATTATT AGTAAGTAGT
    ATAGTAGAAA ATGGGGCTGG
61  AACAGGATGA ACTGTTTACC
    CACCTTTATC TTCTAATATT
    GCTCATGGAG GAGCATCAGT
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121 TGATTTAGCT ATTTTCTCTT
TACACTTAGC AGGAATTTCT
TCAATTTTAG GAGCTGTAAA
181 TTTTATTACA ACTGTAATTA
ATATACGATC TACAGGAATT
ACATTTGATC GAATACCTTT
241 ATTTGTATGA TCTGTAGTTA
TTRACTGCTCT ATTATTATTA
TTATCTTTAC CAGTATTAGC
301 TGGAGCTATT ACTATATTAT
TAACTGACCG AAATCTAAAT
ACTTCATTCT TTGATCCAGC
361 AGGAGGAGGA GATCCTATTT
TATATCAACA TTTATTTTGA
TTCTTTGGAC ATCCTGAAGT
421 TTATATTTTA ATTTTACCTG
GATTCGGAAT AATTTCTCAT
ATTATTAGTC AAGAATCAGG
481 AAAAAAGGAA ACTTTCGGAT
CTTTAGGAAT GATTTATGCT
ATACTAGCTA TTGGTCTATT
541 AGGATTTATT GTATGAGCTC
ACCACATGTT TACTGTTGGA
ATAGACGTAG ACACACGAGC
601 TTATTTCACT TCAGCTACAA
TAATTATTGC TGTACCAACT
GGAATTAAGA TTTTCAGTTG
661 ATTAGCAACT CTTTACGGAA
CACAAATAAA TTATTCTCCA
GCTACTTTAT GAGCTTTAGG
721 ATTTGTATTT TTATTTACTG
TAGGAGGATT AACTGGAGTT
GTTTTAGCTA ATTCATCAAT

781 TGACATTATT TTACATGATA
CATATTATGT AGTAGCTCAC
TTCCATTATG TTCTATCAAT
841 GGGAGCTGTA TTTGCTATTA
TAGCAGGATT TGTTCAATTGA
TTCCCTCTAT TTRACTGGATT
901 AACTTTAAAT AGCAAGTTAT
TAAAGAGTCA ATTTGCTATT
ATATTTATCG GAGTAAATTT
961 AACATTCTTC CCTCAACATT
TCTTAGGATT AGCAGGTATA
CCTCGACGAT ACTCAGACTA
1021 TCCAGACGCT TACACAGCTT
GAAATGTAAT TTCTACAATT
GGTTCAACAA TTTCAATTATT
1081 AGGAATTTTA TTCTTCTTTT
TCATTATTTG AGAAAGTTTA
GTATCTCAAC GACGAGTTTT
1141 ATTCCCTGTT CAACTAAATT
CATCAAT

When compared with the sample, it
will look the following variations:

1
ACTTTTACCTCCTGCATTA
ACTTTATTATTAGTAAGTAGTAT
AGTAGAAAATGGAGCTGG
61
AACAGGATGAACTGTTTACCC
ACCTTTATCTTCTAATATTGCTCATG
GAGGAGCATCAGT
121
TGATTTAGCTATTTTCTCTTTA

CACTTAGCAGGAATTTCTTCAATTTT	661	ATTAGCAACTCTTTACGGAAC
AGGAGCTGTAAA		ACAATTAATTAATTCTCCAGCTACTT
181		TATGAGCTTTAGG
TTTTATTACAACCTGTAATTAA	721	ATTTGTATTTTTATTTACTGTA
TATACGATCTACAGGAATTACATTT		GGAGGATTAACCTGGAGTTGTTTTAG
GATCGAATACCTTT		CTAATTCATCAAT
241	781	TGACATTATTTTACATGATAC
ATTTGTATGATCTGTAGTTAT		ATATTATGTAGTAGCTCACTTTCATT
TACTGCTCTATTATTATTATTATCTT		ATGTTCTATCAAT
TACCAGTATTAGC	841	GGGAGCTGTATTTGCTATTAT
301		AGCAGGATTTGTTCAATTGATTCCTC
TGGAGCTATTACTIONTATATTATT		TATTTACTGGATT
AACTGACCGAAATCTAAATACTTCA	901	AACTTTAAATAGCAAGTTATT
TTCTTTGATCCAGC		AAAGAGTCAATTTGCTATTATATT
361		ATCGGAGTAAATTT
AGGAGGAGGAGATCCTATTTT	961	AACATTCTTCCCTCAACATTT
ATACCAACATTTATTTTGATTCTTTG		CTTAGGATTAGCAGGTATACCTCGA
GACATCCTGAAGT		CGATACTCAGACTA
421	1021	TCCAGACGCTTACACAGCTTG
TTATATTTTAATTTTACCTGG		AAATGTAATTTCTACAATTGGTTCA
ATTCGGAATAATTTCTCATATTATTA		ACAATTTCAATTATT
GTCAAGAATCAGG	1081	AGGAATTTTATTCTTCTTTTTT
481		ATTATTTGAGAAAGTTTAGTATCTC
AAAGAAGGAAACTTTCGGAT		AACGACGAGTTTT
CTTTAGGAATGATTTATGCTATACTA		
GCTATTGGTCTATT		
541		
AGGATTTATTGTATGAGCTCA		
CCACATGTTTACTGTTGGAATAGAC		
GTAGACACACGAGC		
601		
TTATTTCACTTCAGCTACAAT		
AATTATTGCTGTACCAACTGGAATT		
AAGATTTTCAGTTG		

ATTCCCTGTTCAACTAAATTC
ATCAAT

The comparison of the two basic sequences in the data bank shows the transition at a particular loci, which reinforces that the two samples being compared are from different sample sources. This comparison is a simplified visualization of the comparison process with the software, which performs a comparison analysis of the basic sequence data in each database in Genbank. Molecular biological data samples *Ch. megacephala* and *Ch. rufifacies* in North Sulawesi in particular and Indonesia have generally not been recorded in Genbank and Bold systems, and are new findings useful for the identification of forensic insects, as well as related analyzes.

CONCLUSION

This study confirms that the dominant primary necrophage species in North Sulawesi are *C. megacephala* and *C. rufifacies*, but the two species found in North Sulawesi have variants that are different from those in other regions, both in Indonesia and in the world.

These findings help explain the differences in development time of *C. megacephala* and *C. rufifacies* found in studies in North Sulawesi with the findings of development time found in other

countries. The study of the timing of the development of these two species needs to be deeper to make the standard time of development of *C. megacephala* and *C. rufifacies* variants present in North Sulawesi. The standards obtained will be helpful in sharpening the estimated time of death made based on the timing of the development of these two species.

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Conflict of interest

The author affirms no conflict of interest in this study.

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