

MEGACEPHALA AND C.RUFIFACIES VARIAN IN NORTH SULAWESI

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C.MEGACEPHALA AND C.RUFIFACIES VARIAN IN NORTH SULAWESI

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ABSTRACT

Background : *C.megacephala* and *C. ruffacies* 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.ruffacies* 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.

A. 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. ruffiacies* 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.

B. 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' – GGAGGATTTGGAAATTGATTAGTTCC)
 - o TL2-N-3014r (5' – TCCAATGCACTAATCTGCCATATTA)
 - o TL2-N-3014MODr (5' – TCCATTGCACTAATCTTGCCATATTA)
- 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.

C. 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:

```
ACGAATAAATAATATAAGTTTCTGACTTTTACCTCCTGCATTAACCTTATTATTAGTAAGTAGTATAGT
AGAAAATGGAGCTGGAACAGGATGAACTGTTTACCCACCTTATCTTCTAATATTGCTCATGGAGGAGC
ATCAGTTGATTTAGCTATTTTCTTTACACTTAGCAGGAATTTCTTCAATTTTAGGAGCTGTAAATTTTAT
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TCAACTGTAATTAATATACGATCTACAGGAATTACATTTGATCGAATACCTTTATTTGTATGATCTGTAG
TTATTACTGCTCTATTATTATTATTATCTTTACCAGTATTAGCTGGAGCTATTACTATATTATTAAGTACC
GAAATCTAAATACTTCATTCTTTGATCCAGCAGGAGGAGGAGATCCTATTTTATACCAACATTTATTTTGA
TTC⁴TTGGACATCCTGAAGTTTATATTTAATTTTACCTGGATTCGGAATAATTTCTCATATTATTAGTCAA
GAATCAGGAAAGAAGGAAACTTTCGGATCTTTAGGAATGATTTATGCTATACTAGCTATTGGTCTATTAG
GATTTATTGTATGAGCTCACCACATGTTTACTGTTGGAATAGACGTAGACACACGAGCTTATTTCACTTC
AGCTACAATAATTATTGCTGTACCAACTGGAATTAAGATTTTCAGTTGATTAGCAACTCTTTACGGAACA
CAATTAATTATTCTCCAGCTACTTTATGAGCTTTAGGATTTGTATTTTATTTACTGTAGGAGGATTAAC
TGGAGTTGTTTTAGCTAATTCATCAATTGACATTATTTTACATGATACATATTATGTAGTAGCTCACTTTC
ATTATGTTCTATCAATGGGAGCTGTATTTGCTATTATAGCAGGATTTGTTCA⁴TTGATTCCCTCTATTACT
GGATTAAC⁴TTAAATAGCAAGTTATTAAGAGTCAATTTGCTATTATATTATCGGAGTAAATTTAACATT
CTCCCTCAACATTTCTTAGGATTAGCAGGTATACCTCGACGATACTCAGACTATCCAGACGCTTACACA
GCTTGAAATGTAATTCTACAATTGGTTCAACAATTTCAATTATTAGGAATTTTATTCTTCTTTTTCATTATT
GAGAAAGTTTAGTATCTCAACGACGAGTTTATTCCCTGTTCAACTAAATTCATCAATTGAATGATTACAA
AATACTCCACCAGCTGAACACAG

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:

GTTTTGACTTTTACCTCCTGCATTAAC⁴TTTACTATTAGTAAGTAGTATAGTAGAAAATGGAGCTGGAAC
AGGATGAACTGTTTATCCACCTTTATCATCTAATATGGCACATGGTGGAACTGCAATTGATTTAGCTATTT
TTCTTTACACTTAGCTGGAATTTCAATTTTAGGAGCCGTAATTTTATTACAAC⁴GTTATTAATATAC
GATCTACAGGAATTACATTTGATCGAATACCTTTATTTGTATGATCTGTAGTTATTACTGCTCTTCTTTTAT

TATTATCATTACCAGTATTAGCAGGTGCAATTACTATATTATTAAGTATCGAAATTTAAATACTTCATTCT
TTGATCCAGCAGGAGGGGGAGACCCTATTTTATATCAACACTTATTTTGATTCTTTGGTCATCCAGAAGT
TTATATTTAATTTTACCTGGATTCCGAATAATTTCTCATATTATTAGTCAAGAATCAGGAAAAAAGGAA
ACCTTTGGATCTTTAGGAATAATTTATGCAATATTAGCTATTGGATTATTAGGATTTATTGTATGAGCTCA
TCACATATTCAGTGTAGGAATGGATGTAGATACTCGAGCATATTTCACTTCAGCTACAATAATCATTGCT
GTACCAACTGGAATTAATAATTTTATGTTGATTAGCAACTCTTTATGGAAGTCAATTAATAATTCTCCAGC
TACTTTATGAGCCTTAGGATTTGTATTCTTATTACTGTAGGAGGATTAAGTGGAGTAGTATTAGCTAATT
CATCTATTGATATTATTTACATGACACATACTATGTAGTAGCTCACTTCCATTATGTTCTTTCAATAGGA
GCTGTATTTGCTATTATAGCAGGATTTGTACATTGATTCCATTATTTACTGGATTAAGTAAATAATAA
AATACTAAAAAGTCAATTTGCTATTATATTATTGGAGTAAATTAACATTCTTCCCTCAACATTTTTTAGG
ACTAGCTGGTATACCTCGACGATACTCAGACTATCCAGATGCTTATACAGCATGAAATGTTATTCA

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 ruffifacies*, with 100% certainty value (Bold systems, 2017).

D. 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 ruffacies 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 GTCATGGAG GAGCATCAGT
121    TGATTTAGCT ATTTTCTCTT TACTTAGC AGGAATTCT TCAATTTAG GAGCTGTAAA
181    TTTTATTACA ACTGTAATTA ATATACGATC TACAGGAATT ACATTTGATC GAATACCTTT
241    ATTTGTATGA TCTGTAGTTA TTAGTCTCT 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 AACTAGCTA TTGGTCTATT
541    AGGATTTATT GTATGAGCTC ACCACATGTT TACTGTTGGA ATAGACGTAG ACACACGAGC
601    TTATTTCACT TCAGCTACAA TAATTATTGC TGTACCAACT GGAATTAAGA TTTTCAGTTG
661    ATTAGCAACT CTTTACGGAA CACAATTAAT TTATTCTCCA GCTACTTTAT GAGCTTTAGG
721    3 ATTTGTATTT TTATTTACTG TAGGAGGATT AACTGGAGTT GTTTLAGCTA ATTCATCAAT
781    3 TGACATTATT TTACATGATA CATATTATGT AGTAGCTCAC TTCCATTATG TTCTATCAAT
841    3 GGGAGCTGTA TTTGCTATTA TAGCAGGATT TGTTCAATGA TTCCTCTAT TTAGTGGATT
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901 ³ AACTTTAAAT AGCAAGTTAT TAAAGAGTCA ATTTGCTATT ATATTTATCG GAGTAAATTT
961 ³ AACATTCTTC CCTCAACATT TCTTAGGATT AGCAGGTATA CCTCGACGAT ACTCAGACTA
1021 TCCAGACGCT TACACAGCTT GAAATGTAAT TTCTACAATT GGTTCAACAA TTTCAATTATT
1081 AGGAATTTTA TTCTTCTTTT TCATTATTTG AGAAAGTTTA GTATCTCAAC GACGAGTTTT
1141 ATCCCTGTT CAACTAAATT CATCAAT

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

1 ACTTTTACCTCCTGCATTAACCTTTATTATTAGTAAGTAGTATAGTAGAAAATGGAGCTGG
61 AACAGGATGAACTGTTTACCCACCTTTATCTTCTAATATTGCTCATGGAGGAGCATCAGT
121 TGATTTAGCTATTTTCTCTTTACACTTAGCAGGAATTTCTTCAATTTTAGGAGCTGTAAA
181 TTTTATTACAACCTGTAATTAATATACGATCTACAGGAATTACATTTGATCGAATACCTTT
241 ATTTGATGATCTGTAGTTACTGCTCTATTATTATTATTATCTTTACCAGTATTAGC
301 TGGAGCTATTACTATATTATTAACCTGACCGAAATCTAAATACTTCATTCTTTGATCCAGC
361 AGGAGGAGGAGATCCTATTTTATACCAACATTTATTTGATTCTTTGGACATCCTGAAGT
421 TTATATTTAATTTTACCTGGATTCCGGAATAATTTCTCATATTATTAGTCAAGAATCAGG
481 AAAGAAGGAACTTTTCGGATCTTTAGGAATGATTTATGCTATACTAGCTATTGGTCTATT
541 AGGATTTATTGTATGAGCTCACCACATGTTTACTGTTGGAATAGACGTAGACACACGAGC
601 TTATTTCACTTCAGCTACAATAATTATTGCTGTACCAACTGGAATTAAGATTTTCAGTTG
661 ATTAGCAACTCTTTACGGAACACAATTAATTTCTCCAGCTACTTTATGAGCTTTAGG
721 ATTTGTATTTTATTTACTGTAGGAGGATTAACCTGGAGTTGTTTTAGCTAATTCATCAAT
781 TGACATTATTTTACATGATACATATTATGTAGTAGCTCACTTTTATTATGTTCTATCAAT
841 GGGAGCTGTATTTGCTATTATAGCAGGATTTGTTTATTGATTCCCTCTATTTACTGGATT
901 AACTTTAAATAGCAAGTTATTAAGAGTCAATTTGCTATTATTTATCGGAGTAAATTT
961 AACATTCTTCCCTCAACATTTCTTAGGATTAGCAGGTATACCTCGACGATACTCAGACTA

1021 TCCAGACGCTTACACAGCTTGAAATGTAATTTCTACAATTGGTTCAACAATTCATTATT
1081 AGGAATTTTATTCTTCTTTTCATTATTTGAGAAAGTTTAGTATCTCAACGACGAGTTTT
1141 ATCCCTGTTCAACTAAATTCATCAAT

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.

E. 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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Differences of plasma histamine level between acute coronary syndrome and stable coronary artery disease patients
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Endothelin-1 plasma level gives a tendency toward increasing rate of in-hospital major adverse cardiovascular outcomes in ST-elevation myocardial infarction undergoing fibrinolysis

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QT interval prolongation after non-ST elevation myocardial infarction in type 2 diabetic compared with nondiabetic patients

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