Phenotypical Morphometry Variation of Aedes Aegyti in Manado

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Abstract- **Aedes aegypti (Linne, 1762) is the main vector of arboviruses, namely yellow fever, dengue and Chikungnya. In North Sulawesi it has found variation in morphology, ecology, behavior and vector competence. Vector control is still the best way to prevent dengue fever. Understanding the morphological characteristics that become characters in the phenotypic mosquito is very important in the efforts of controlling and classification. This study aims to find morphological variation of Aedes aegypti in the city of Manado. This research applies descriptive research techniques with direct experiments in the laboratory. The morphologic character of mosquito samples from selected locations were then measured by using a stereomicroscope KH8700 completed with cameras, measurement software of morphological characters and statistical programs. The morphometry data were then analyzed with multivariate and factor analysis by using IBM SPSS 20. The results showed that of the twelve morphological characters were analyzed, it was found that Aedes spp from 4 areas in the city of Manado has undergone phenotypical characters variations (morphology), characterized by the formation of five clusters or groups based on the degree of similarity in morphometry. There are three groupings of morphological characters which are variance factor of 1: (5.941 / 12) x 100% = 49.508%, variance factor of 2: 1.798 / 12) x 100% = 14.98%, and variance 3 factors: (1,184 / 12 x 100% = 9.86%.**

Index Terms**— variation, morphometry, Aedes aegypti, Manado**

I. Introduction

Aedes is a mosquito kind normally found in the tropics. Its name is derived from the Greek word *Aedes*, which means "not nice", because this mosquito spreads dangerous diseases, such as dengue fever and yellow fever. Aedes albopictus sp. is a common species in Asia. His legs are black and white stripes. Aides sp. also known as the spreads of dengue and yellow fever. Aedes aegypti sp. and Aedes albopictus sp. are found in the tropics and sub-tropics. In Asia it is on the first rank in terms of the number of patients with Dengue Fever in each year. Meanwhile, from 1968 to 2009, the World Health Organization (WHO) noted that Indonesia as the country with the highest number of cases on Dengue Fever in Southeast Asia. Of the total number of such cases, about 95% cases occured in children below 15 years.

Aedes aegypti breeds in water reservoirs, such as bathtub, jars, drums, flower vases, and used items that can be container of rain water in urban and suburban areas. Aedes albopictus, likewise, but they are usually more widely available outside of houses. Aedes aegypti mosquitoes prefer to bite on sheltered areas, such as around the house. Biting activity reaches the top during the light intensity change. They can bite all day and the highest is before sunset. Short flight range is 50-100 meters, except in the wind. Mosquitoes will rest in dark and cool places after they suck blood, until the blood absorption for egg development completed. Mosquitoes will find a runny place to put and lay their eggs, and then the mosquitoes will begin to look for blood again for the next spawning cycle.

From January to August this year it occurred 324 cases, with 11 of them ended the children death. The area with the highest number of cases, namely Districts of Malalayang, Wanea, and Tikala (Depkes Manado, tribunsnews.com). In East Bolaang Mongondow, along the current year local health department has recorded 23 cases, two of whom died. They were residents of the Bai village and citizens of Kotabunan. The highest number of cases occurred between May and July, in Nuangan District as the highest number of dengue cases territory.

Aedes spp is the main vector of dengue virus result in dengue fever. Several studies have found a bionomic change of dengue mosquitoes in some areas. Changes in mosquito behavior can affect the mosquitoes morphology, The changes are caused by the habitat shift, climate change, and some efforts that eradicate mosquitoes with insecticide that stimulates mosquito resistance reactions.

Morphological characteristics or phenotype is the expression of genes that are affected by environment. Individual species pphenotype resulted from genotype (genes) and environment influence these organisms. Substantial phenotypic variation in a population is due to differences in genotype. Variations derived from DNA mutation, migration between populations (gene flow), and changes in the composition of genes through recombination in sexual reproduction. Although there are variations that occur continuously through these processes, most of the species genome is identical in all individuals of that species. (1) Genetic variation is the variation due to genetic changes (mainly mutations) and passed on to offspring through the cell nucleus in the gametes. (2) Environmental variation is the variation caused by environmental changes, while the genetic material remains and is not inherited. Small changes in genotype can lead to dramatic changes in the phenotype. Changes in gene frequency in a population can occur rapidly when there is human intervention. Several factors that can change gene frequencies in a population are mutation, migration between populations, genetic drift and selection. The influences of these four factors determine the genes frequency and population characteristics, and then randomly passed on to the next generation.

In Africa it has been found variation in the morphology, ecology, and behavior and vector competence. Even Aedes aegypti has been divided into Aedes aegypti (Aaa) that is specific domestic (living) live in life settlements area / anthropophilic and cosmotropical distribution with lighter / brighter body color/morphology, while Aedes aegypti formosus (Aaf) which is endemic to Africa is darker and its habitat is farther from the domestic (Paupy et. al. 2010). Rondonuwu reported that there has been a shift in habitat between Aedes albopitus that inhabit marginal areas near the forest area towards domestic / urban in the Manado city.

One common method used to determine the phenotypical variation of species populations is a morphometric analysis. Morphometry can be defined as the method described morphologic characters through measurement, calculation or scoring (Bookstein and Strauss, 1982). Morphometry can be applied to determine the particular species kinship, the various species differentiation, and variations in the species and to identify a species. Some morphometric studies have been done, such as morphometry of Apis dorsata Binghami and Apis nigrocincta Smith (Mokosuli, 2013), morphometric variation of Papilio polythes L (Makhzuni et.al, 2013), and morphometric analysis of Anopheles subpictus (Chillar, 2014).

Based on the literature study conducted by the authors, there is only a few published research reports on morphological variation of Aedes spp. Morphological variation study needs to be done, because with the increase in dengue cases, the efforts to eradicate mosquitoes as vectors intensified. Generally the mosquito eradication uses insecticide. Insects are animals that have a high degree of genetic adaptation to various conditions or have a high adaptive capability. Routine insecticide treatments will bring Aedes variants resistant to insecticides, such as Anopheless spp which has high resistance levels to organoclorin and organophosphates insecticides in Indonesia today. Resistance is formed due to the mosquito's physiological reactions to adapt or survive. Resistance is also influenced by genetic factors or genetic modification as a mosquitoes adaptation form. Genetic modification will have implications on mosquitoes' phenotypes or morphology. Until now there has been no research study report of the morphological variation of Aedes albopictus in Manado. This study aims to find out morphological variation of Aedes spp in Manado City. This study will then be equipped with barcode genetic studies using mitochondrial DNA CO1 gene.

II. MATERIALS AND METHODS

Research Location and Time

Mosquito samples obtained from several locations in Manado the city, namely Malalayang, Winangun, Kairagi, and Tuminting. The sample areas can be clustered based on the height of the sea surface. Each region was taken 50 adult mosquitoes without classifying males or females.

Tools and Materials

Tools used were pyrex glassware, three-dimensional stereo microscope of hirox KH-8700 models and stereo microscope of Carl Zeizz "STEMI DV 4" stereomicroscope. Both microscopes are equipped with CPUs, monitors, cameras, and measurement software of bee organs. The material used consists of 70% alcohol, chloroform p.a., cotton, and distilled water. Adult mosquito samples were obtained in domestic area.

Research procedures

a. Sampling Mosquitoes

Alive mosquitoes terminated with 70% alcohol. Mosquito samples from each location is stored in the sample bottle with 70% alcohol and placed in the sample box to maintain the temperature of 200-250C. Mosquitoes further sorted and only mosquitoes that have complete body parts (not defective / broken) during an arrest or preparation used for morphological analysis.

b. Morphometry analysis

The mosquitoes morphological analysis used 3-dimensional robotic stereo Mikroscope of KH700 hirox completed with morphological measurement software, camera, and statistical programs. Morphological characters were measured according to the Andrew and Bar method (2013); Rueda, (2008), namely the head, antenna, proboscis, Torax, wings, legs and abdomen structures (Figure 1).

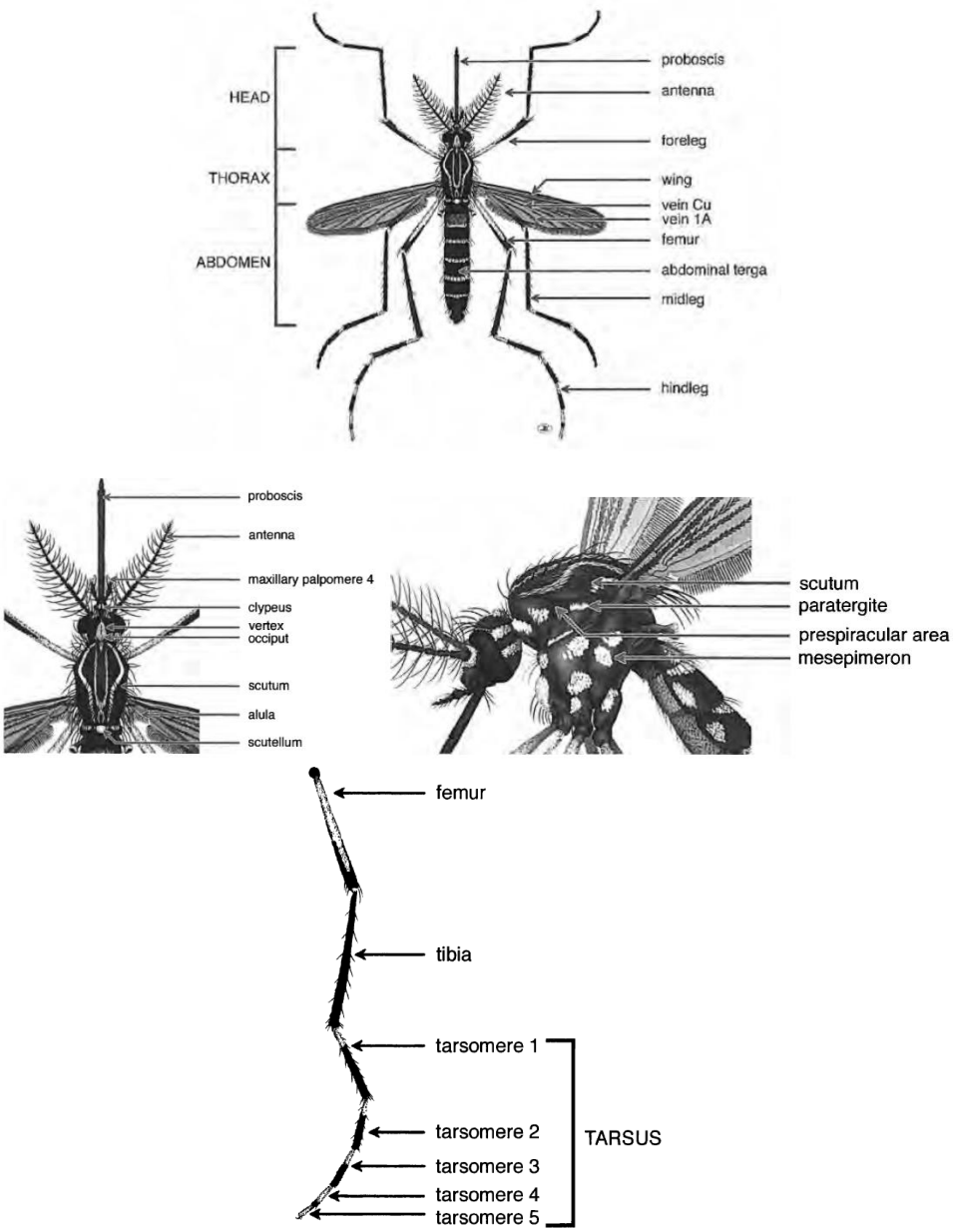

Fig. 1. Morphology Parameters used for morphometric analysis (Rueda LM. 2008)

c. Data Analysis Techniques

Measuring the length and width of the morphological characters used the software on the microscope. Measurement result data of morphological characters analyzed by Cluster and factorial used IBM SPSS 20.

III. RESULTS AND DISCUSSION

Grouping Morphometry Characters

To determine the grouping of morfometerical characters used multivariate analysis technique that is cluster analysis with IBM SPSS 20. Cluster analysis application traditionally aimed to explore and establish a classification / taxonomy empirically. Since its ability to partition, the cluster analysis can be applied widely. Cluster analysis aims to classify objects or characters, so that each object or character that has the closest similarity to the other is in the same cluster. Clusters that are formed have a high internal homogeneity and high external heterogeneity. Cluster analysis is used to process the insect morphometry data with classifying species aim based on similar morphometry or classifying certain morphometric characters based on morphometry similarity (Mokosuli, 2013; Seenivasagan et. Al., 2009; Chhilar, 2014). A total of 11 clusters that were formed on the cluster analysis of morphometric characters of Aedes spp obtained in 4 areas in Manado city on 12 Aedes' morphological characters measured (Figure 2).

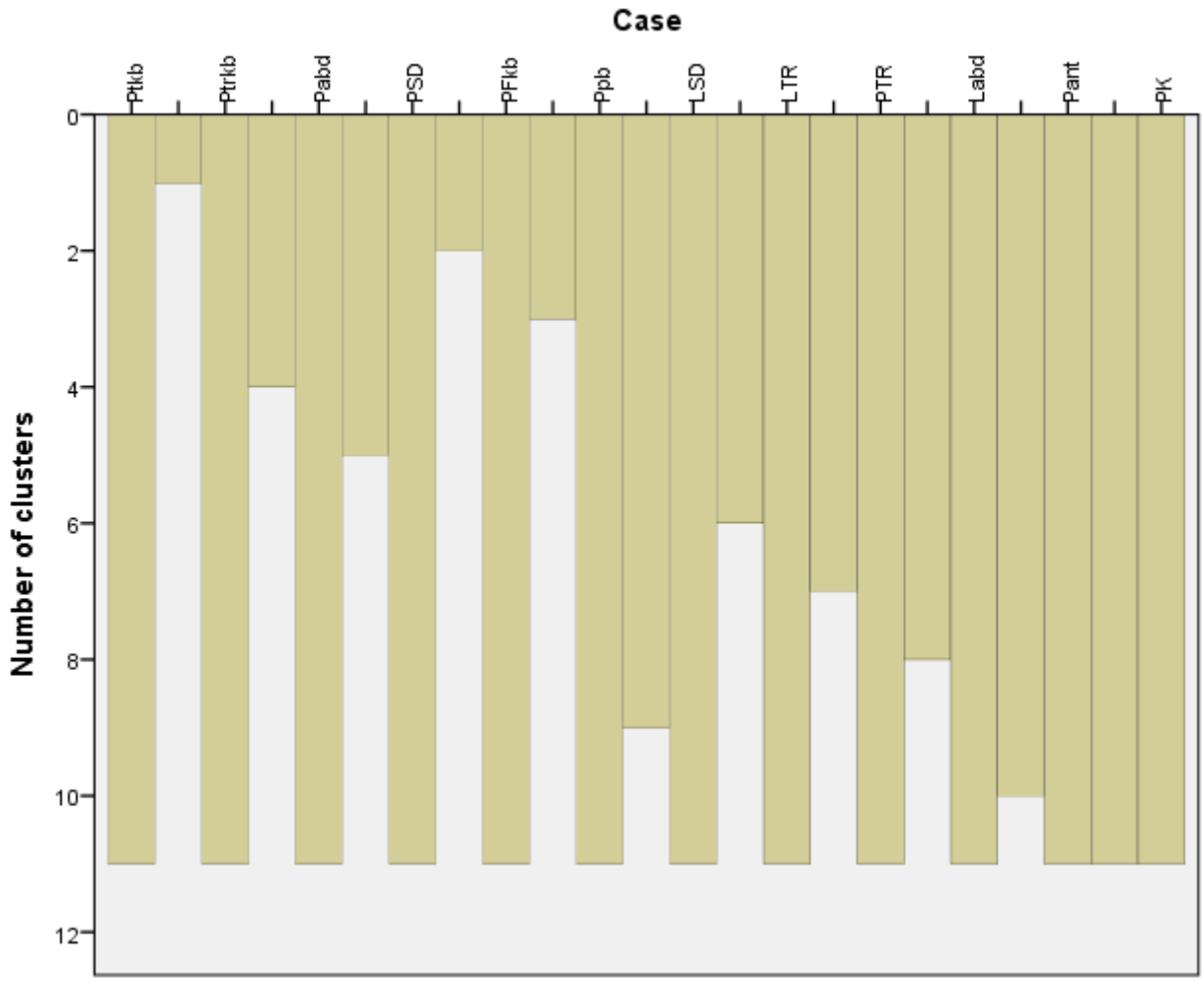

Fig. 2. Morphological Character Cluster of Aedes spp in Manado

Clusters that are formed subsequently manifested in a dendogram to determine the grouping of properties / morphological characters based on the similarity degree. In dendogram analysis results obtained morphological characters of the head length (PK), the antenna length (Pant), the abdomen width (Labd), tarsus length (PTR), the tarsus width (LTR), the front wing width (LSD) and a long proboscis (ppb) of 4 samples regions to form one cluster (the first cluster). Therefore, it has very close similarity level of mosquitoes morphology from 4 regions on these characters. LTR character has similarities with the character of the rear leg femur length (PFkb) with ecludian distance 5, thus forming the cluster itself (second cluster). LTR and PFkb character has similarities with of the abdomen length (Pabd) character with ecludian distance 18, thus forming a third cluster. Character of the front wing length (PSD) has a very close resemblance to the character of the rear leg tarsus length (Ptkb), thus forming a fourth cluster. The last formed cluster is LTR characters, PFkb, and Pabd with farthest ecludian distance 25 (Figure 3).

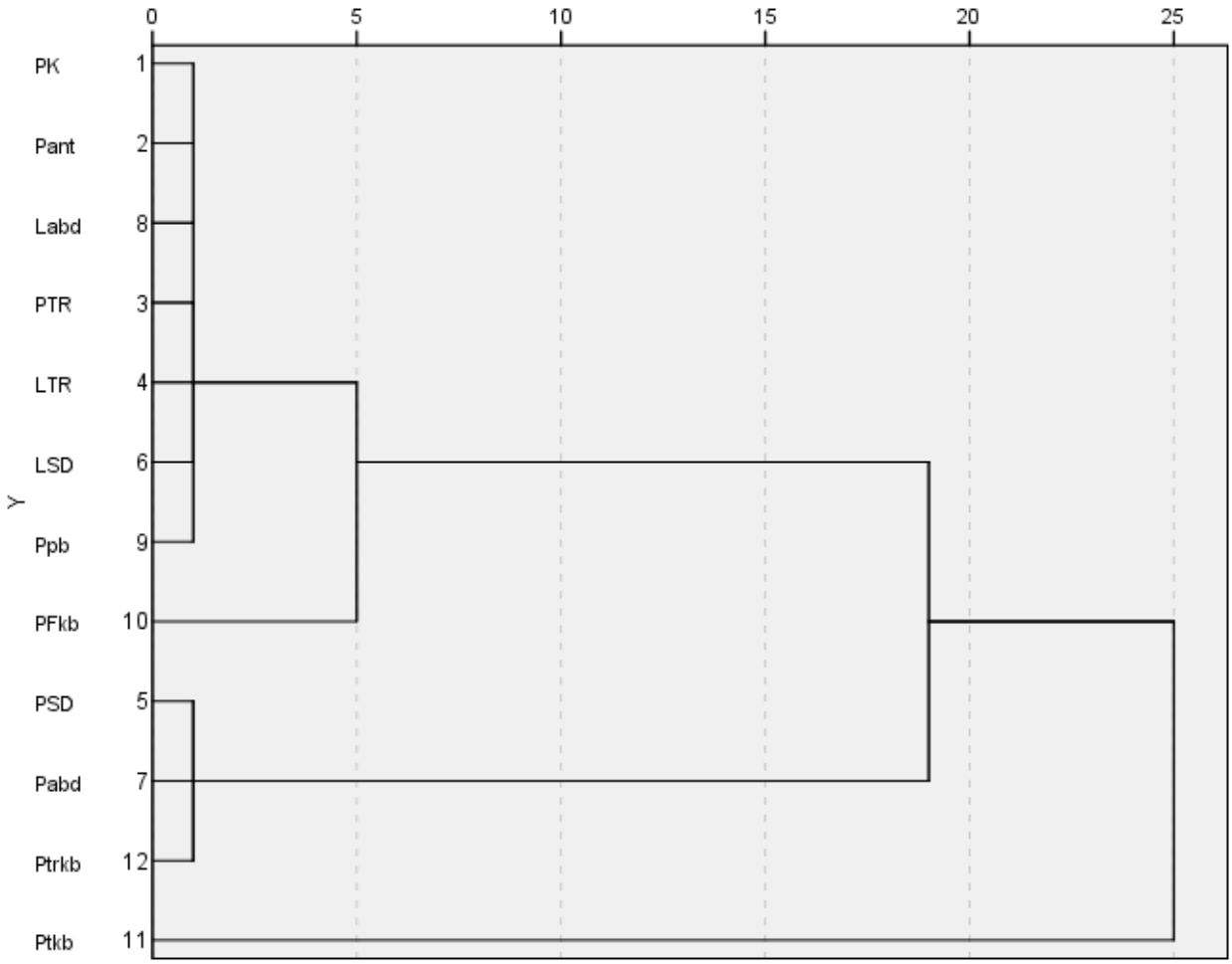

Fig. 3. Grouping Dendogram of Morphology Character on Aedes obtained from 4 regions in Manado city.

Relationships among Morphology Characters

To obtain the relationship form of morphometric variables used factor analysis. Factor analysis is a statistical analysis technique that aims to explain the relationship structure between the variables observed by raising a number of factors or components or latent variables less than a number of variable origins. The main purpose of factor analysis is to explain the relationships structure among many variables in the form factor or latent or formations variables.

Factor analysis with PCA method used IBM SPSS 20. There are three factors (components) with the eigenvalues values of 5.941; 1.798; and 1,184 (Appendix 1). Eigenvalues values indicate the variable numbers that become factor members. Accordingly, PCA analysis morphometry of Aedes bee ... on factor of one consisting of 5.941 variables, factor of two consisting of 1.798 variables, and factor of three consisting of 1,184 variables. Of the 12 components (variables) were included in the factors analysis that each variable has variance 1, the total variance is 12 x 1 = 12. In accordance with the number of factors that are formed and the amount of variance of each variable that is known, it can further explained that each factor as well as overall form factor: Variance factor of 1: (5.941 / 12) x 100% = 49.508%, Variance factor of 2: 1.798 / 12) x 100% = 14.98%, and Variance factor of 3: (1,184 / 12 x 100% = 9.86%. This means that 49.50% of the factor variance variability of one can be explained by this factor, and respectively 14.98% and 9.86% can be explained by a factor of two and a factor of three. Accordingly, these three factors can explain 73.95% of the variability of 12 morphological characters of Aedes spp.

Component Matrix[a]

	Component		
	1	2	3
PK	-.096	.373	.732
Pant	.619	.616	-.031
PTR	.673	.441	-.091
LTR	.824	.006	-.016
PSD	.883	-.202	.026
LSD	.564	-.535	.056
Pabd	.958	-.116	-.097
Labd	.296	-.764	.303
Ppb	.864	-.246	.000
PFkb	.833	.150	.143
Ptkb	.321	.124	.671
Ptrkb	.878	.249	-.252

Extraction Method: Principal Component Analysis.

a. 3 components extracted.

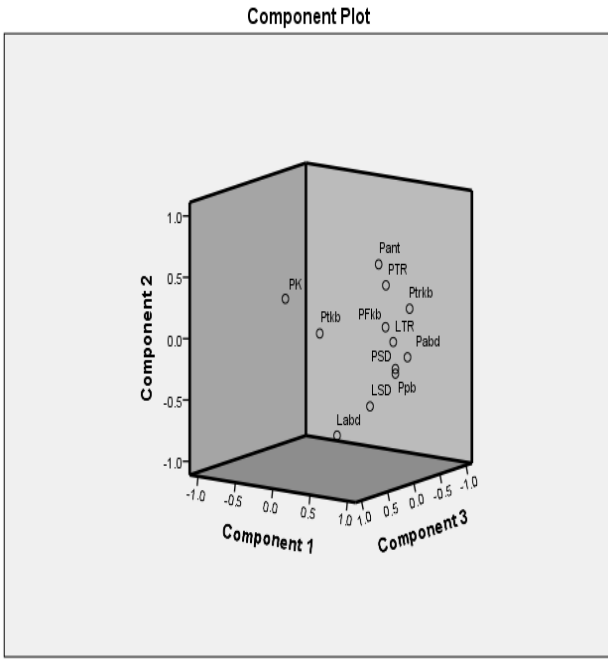

Image Grouping variables from three factors formed.

Fig. 4. Aedes sp sample from Manado

Fig. 5. Samples of Aedes albopictus from Airmadidi

Fig. 6. Comparison of the Aedes Head from Malalayang and Kairagi

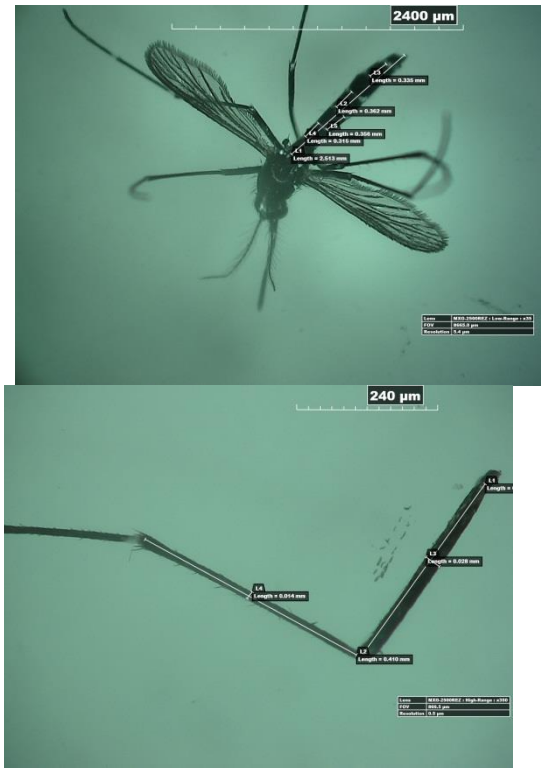

Fig. 7. Characters Morphometry of Aedes Morphology

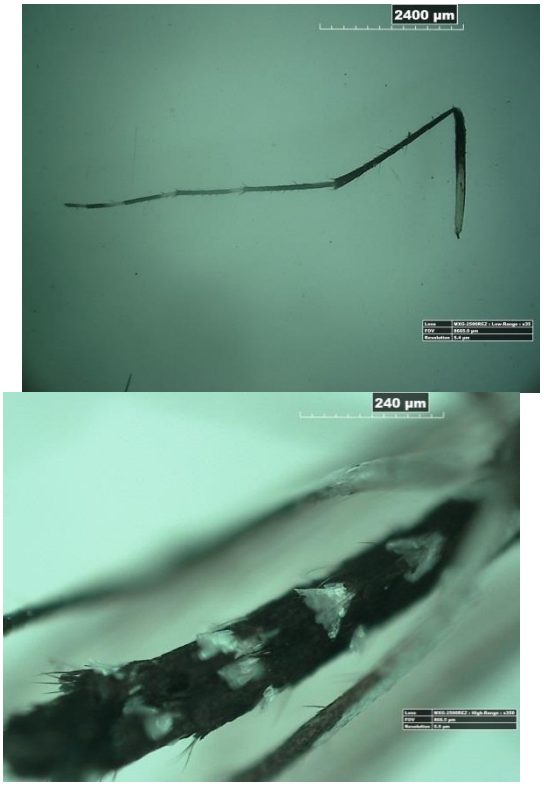

Fig. 8. Front legs and abdomen of Aedes sp from Kairagi

Morphological variation of Aedes sp in an area widely studied io different areas of endemic dengue. Morphological variation in tarsomer character was found in Aedes simpsoni in Uganda, Kenya, and North Africa. Midtarsomer 1 and 2 have a longer band or strip than the male and female adult mosquito on anthropophilic and non-anthropophilic population in Kenya and Uganda. Besides, on tarsomer also be found in scutal character variation, middle leg claws (Lutwama and Mukwaya, 1994). Paupy et. al. (2010) found variations in the pale scale character of Aedes aegypti abdominal in Niakhar, Senegal. This variation depends on the season and nesting places of mosquito larvae. Aedes has the ability to perform a striking morphological variation, although it is still located in the same living area (biotypes). Aedes in Manado has shown some variations in morphological characters. Variations in morphology (phenotypical) should be followed up in the genetic analysis using specific DNA gene as a barcode or standard to see the genetic variation rate in the mosquitoes that live in the same biotype. Morphological modifications in the adaptation response to pressure changes of the environment, climate, habitat, and defense against insecticides is a common response to the species, in the class of insects that are known to have high adaptability. Morphological variations of Aedes spp become an important study, because Aedes ecological role as a dengue virus vector causes hemorrhagic fever and viral chikugunya. Morphological modifications based on the evolution theory have two possibilities, namely progressive or retrogressive. In this study, morphological modifications actually lead to the progressive evolution or the species member formation in a more stable population. In another study conducted by the author, the dengue cases prevalence in Manado city has increased, therefore morphological modifications do no direct mosquito population member to the inability to survive or adapt (retrogressive).

IV. CONCLUSION

Of the twelve morphological characters analyzed, it has been found that Aedes spp from 4 areas in Manado city has undergone phenotypical character variation (morphology). It is characterized by five clusters or groups formation based on the morphometry similarity degree. There are three groupings of morphological characters, namely factor variation of 1: (5.941 / 12) x 100% = 49.508%, factor variation of 2: 1.798 / 12) x 100% = 14.98%, factor variation of 3: (1,184 / 12 x 100% = 9.86%.

REFERENCES

[1] Bookstein, F. L. and R. E. Strauss. 1982. The Truss: Body Form Reconstruction in Morphometrics. Systematic Zoology3: 113-115.

[2] Chhilar JS. 2014. Morphometric Analysis of Taxonomic Characters of Malaria Vector Mosquito Anopheles (Cellia) subpictus Grassi (Diptera: Culicidae). Journal of Entomology and Zoology Studies 2014; 2 (3): 32-38

[3] Fanani AF. Novarino W. dan Tjong DH. 2012. Variasi Morfologi Arachnothera longirostra (Passeriformes, Nectariniidae) (Latham, 1790).(J. Bio. UA.) 1(1) – September 2012 : 78-85

[4] Lutwama JJ and Mukwaya LG, 1994. Variation in morphological characters of adults of the Aedes (stegomyza) szmpsonz complex from Uganda, Kenya, and South Africa (Diptera: Culicidae). Mosquito Systematics, 26(3): 145-157, 1994

[5] Makhzuni M. Syaifullah) dan Dahelmi. 2013. Variasi Morfometri Papilio polytes L. (Lepidoptera: Papilionidae) di Beberapa Lokasi di Sumatera Barat. Jurnal Biologi Universitas Andalas (J. Bio. UA.) 2(1) – Maret 2013 : 50-56 (ISSN : 2303-2162)

[6] Mokosuli YS. 2013. Karakter Morfologi, Sumber Pakan dan Bioaktivitas Farmakologis Racun Lebah Madu Endemik Sulawesi Apis dorsata Binghami dan Apis nigrocincta Smith (Hymenoptera : Apidae). [Disertasi]. Program Pascasarjana Universitas Sam Ratulangi Manado.

[7] Paupy C,, Brengues CC, Ndiath, O, Toty C, Herve JP, Simard, F.Morphological and genetic variability within Aedes aegypti in Niakhar, Senegal. Infection, Genetics and Evolution 10 (2010) 473–480

[8] Rueda LM. 2008. Pictorial Keys for the Identifications of Mosquitoes (Diptera: Culicidae) Associated with Dengue Virus Transmission. WalteR reed Biosystematics Departement of Entomology Walter Reed Institute Research. http://wrbu.si.edu

[9] Seenivasagan T, Sharma KR, Shrivastava A, Parashar BD, Pant SC&Prakash, S. 2009. Surface morphology and morphometric analysis of sensilla of Asiantiger mosquito, Aedes albopictus (Skuse): an SEM investigation. J Vector Borne Dis46, June 2009, pp. 125–135.