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Morphological characteristics of bedbugs (Cimex sp.) from Manado and Sitaro north Sulawesi, Indonesia

[1] Grace Kalangi, [2] Max Tulung, [3] Christin Salaki, [4] Lucia Mandey

[1] Doctoral Students, Departments of Entomology, Graduate Programs University of Sam Ratulangi, Manado, North Sulawesi, Indonesia

[2, 3, 4] Departments of Entomology, Graduate Programs University of Sam Ratulangi, Manado, North Sulawesi, Indonesia

Abstract
Geographically Manado is the capital of North Sulawesi Province, Indonesia, while Sitaro is one of the districts in the province of North Sulawesi directly adjacent to the Philippines. About 50 years stinkbug population decreased dramatically, but an increase in the population of bedbugs in various parts of the world in early 2000. It has carried out research that is focused on the analysis of morphological characteristics Cimex sp. originating from the islands, namely Sitaro district, and from the city of Manado as the capital city of North Sulawesi province. A total of 10 characters morphometry was used to compare bedbugs based on the origin of the sample. Moreover, the structure morphology compared based on qualitative characters such as the color of the body etc. Morphometric characters measured and analyzed using a three-dimensional microscope hirox KH8700 equipped with measurement software. The results showed that Based on morphology, bedbugs from Sitaro have differences with bedbugs from Manado. Differences in morphological characters which is the average length of the body, the intensity of trichomes on the head and thorax, color and size of the abdomen. The results of the factor analysis, three groupings of morphological characters bedbugs from Sitaro and Manado. The results of cluster analysis to form four groupings based on similarities morphometry.

Keywords: bedbugs, morphology, Manado, Sitaro, North Sulawesi

1. Introduction
Bedbugs were used in this study originated from Manado and Sitaro, North Sulawesi, Indonesia. Manado is the capital city of North Sulawesi Province, located on the mainland of Sulawesi island, while Sitaro city is the capital of the District Sitaro, in Saginge Island. The survey results of North Sulawesi Provincial Health Office in 2015 found there was an explosion in the population of bedbugs om Sitaro and Manado. Bedbugs have become a problem for human health since long time ago. Bedbugs are blood-sucking ectoparasites on humans, chickens, bats and also pets (Usinger, 1996) [15]. Until now, still difficult to distinguish from the main host species og bedbugs (Wawrocka and Bartonica, 2014). Two species of bedbugs the best known is Cimex lectuarius Linn. 1758 and Cimex hemipterus also called Indian bedbug because many are found in the slum areas in India and Bangladesh (Ahmed and Begum 1992) [2]. Two major bed bug species feed on human blood; Cimex hemipterus, the tropical bed bug and Cimex lectularius, the common bed bug (Harlan *et al*., 2008). Cimex lectularius Linnaeus, 1758 (Hemiptera: Cimicidae), has been intimately associated with humans for thousands of years (Panagiotakopulu & Buckland 1999; Booth *et al.* 2015). It is an obligate ectoparasite that primarily feeds on human blood in a haematophagic lifestyle, but will readily feed on many bird and mammalian species as well (Usinger 1966) [15, 24, 2, 7, 19, 5].
In 1950, bedbug populations dropped dramatically after the discovery of many synthetic insecticides (Stephen *et al.*, 2012; Porter *et. al*. 2010) [16]. Nevertheless, the early 2000s reported bedbug population explosion in the United States, especially in medium and small businesses and residential areas (Stephen *et. al.* 2012) [16]. The re-emergence of bedbugs, one of the mysteries in Entomology, blood-sucking insects are almost not appear for a period of decades. This may be due to the increase in human migration especially tourism industry and development of insecticides resistance of the insects (Krause-Parello and Sciscione, 2009; Tawatsin *et. al*. 2013) [9, 25]. Manado city is one of the tourist destinations in Indonesia with tourist visits both from within and outside the country. It is likely that the bed bugs were transported on clothes in luggages of travelers (Delaunay and Pharm, 2012) [6]. This human mobility contributes to the spread of lice to the entire world. This indication can be seen among others that bedbugs are found where people come and go as hotels, inns, apartments and dormitories. Bedbugs (including eggs) can be carried inadvertently along with clothing, in suitcases / backpacks, suitcase and others (Ahmad, 2011).Bedbugs in Indonesia until the end of the 1970s many reportedly found living at home, cinemas, hotels, inns and places where human laiinya sleeping and sitting. But in the 1980s, the population of vermin that causes problems for people decreasing very drastic. However, in the 2000s the population of bedbugs in Indonesia, reported increases and becomes a health problem (Natadisastra and Agoes, 2009; CDC, 2014) [13]. Bedbugs may be a vector of disease (Harwood and James, 1979; Sembel 2009). Bedbugs are suspected of transmitting human pathogens among 41 others from bacteria, viruses, Rickettsia, protozoa and worms (Burton, 1963; WHO 2014). Pathogens diseases potentially transmitted by lice is Salmonella sp, Shigella sp, Escherichia coli, Clostridium, Stapilococcus etc. (Sembel, 2009). Bedbugs as vectors of diseases, causes morphological and genetic

study of bedbugs important as a basis for tackling population of bedbugs. Genetic variation of bedbugs is high in a population. Study of the specific host and it is relationship with phenotypically plastic and genotific diversity is crucial in understanding the evolution of species diversity and variability of living organisms parasitic strategy. From the literature study conducted, a little studies of morphology of Cimex hemipterus compared Cimex lectularius and Cimex adjunctus which is commonly found in Europe, Australia and the United States. This study aims to conduct a comparative study of the morphology of bedbugs in the islands and on the mainland island of Sulawesi in particular the city of Manado [16, 9, 25, 6, 1, 13].

2. Materials and Methods

2.1 Bed bug Collection

Bed bugs in this study were collected from settlements in Sitaro and Manado City (Fig. 1). Bedbugs have been taken out of the mattress and seat in houses. At each site collected samples of 30 individuals of bedbugs. Bedbugs were preservation in 70 % alcohol and maintained in Laboratory of Biology, State University of Manado, Indonesia.

Fig. 1. Sampling locations of bedbugs

2.2 Study of the morphology of bedbugs

Samples of bedbugs placed on petridish then made observations and measurements using a stereomicroscope hirox KH8700 three dimensions. Morphological characters were observed by (Khan and Rahman, 2012) [10] (Figure 4).

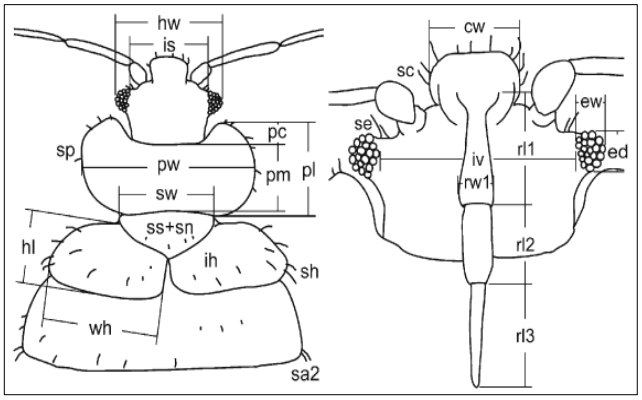

Fig 2

Table

Gambar	Morfometri Kutu busuk (Balvin et. al. 2012).
	Hw = lebar kepala
	Ew = lebar mata
	Eye = diameter mata
	se = pajjang rambut antara mata dan antena
	cw = lebar clypeus
	pw = lebar pronotum
	sw = lebar sculetellum
	hl = panjang hemiltra
	wh = lebar hemiltra

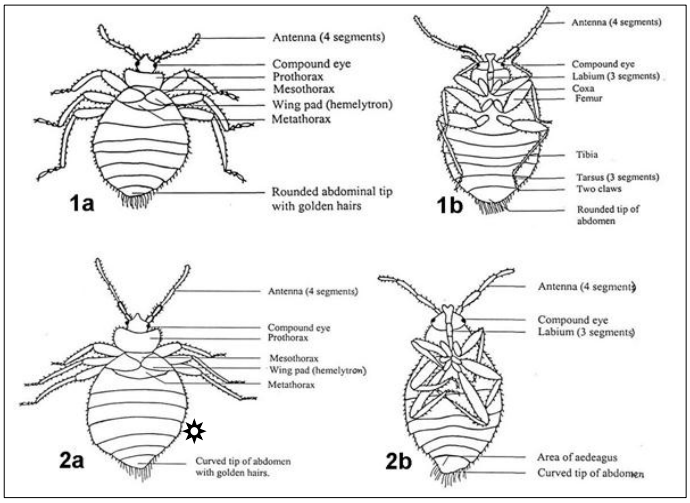

Fig 3

Table

Gambar	Cimex hemipterus betina tampak dorsal (1a), C. Hemipterus betina tampak ventral (1b), C. Hemipterus jantan dewasa tampak dorsal (2a), C. Hemipterus jantan tampak ventral (Khan and Rahman 2012).

3. Results and Discussion

3.1 Description Sampling

Imago of Bedbugs isolated from the bed (mattress), sofa and other places in houses. Bedbugs obtained terpreservasi with alcohol 70% and 98% (Table 2). The treatment of the two types of alcohol for the purpose of analysis of morphology and DNA extraction. Samples for DNA extraction was transferred to the new sample vials after 24 hours of soaking with alcohol. This is done so that the water content in the body decreases Bedbugs bound by alcohol. The water content will affect the concentration and purity of the total DNA extracted from tissue bedbugs.

Bedbugs are preservated by two different types of alcohol water content, showed no difference in the appearance of the surface morphology of the body such as body color, the color of the head, abdomen color, color and color thoracic limbs. Likewise, the structure of the external morphology showed no shrinkage size after seven (7) days terpreservasi with alcohol. In contrast to other insects such as termites, Gerridae, Aedes and Anopheles shows changes in outer structure, especially color and shrinkage in the abdomen after preserved with alcohol during the seven (7) days. It shows the structure of bedbugs eksoskleton compiled by chitin and complex molecules that are polar are more resistant to alcohol is a polar solvent.

Alcohol can draw water in the network so that the organs will appear to shrink for immersion within a certain timeframe (Mokosuli, 2015).

Tabel 3: Location and Number of Samples

S. No	Originally of Samples	Sampling Location	Number of Imago	Samples for analysis morfometry	Preservation
1	Sitaro	1	25	15	Alcohol 70 %
		2	25	15	Alcohol 70 %
		3	25	15	Alcohol 70 %
		4	25	15	Alcohol 90 %
		5	25	15	Alcohol 90 %
		6	25	15	Alcohol 90 %
2	Manado	1	25	15	Alcohol 70 %
		2	25	15	Alcohol 70 %
		3	25	15	Alcohol 70 %
		4	25	15	Alcohol 90 %
		5	25	15	Alcohol 90 %
		6	25	15	Alcohol 90 %
Total			300	180	

3.2 The Morphology of Bedbugs

Bedbugs are obtained from Manado Sitaro and generally have a body dorsal ventral flat oval. Samples that have not suck the blood of humans or other animals, abdomen brownish yellow. While the sample that had sucked the blood of humans or other animals that have a reddish-brown abdomen. As general characteristics C. hemipterus, both samples of Sitaro as well as from the city of Manado has the characteristics of a short head stuck forward, on the head there is one pair of compound eyes, and one pair of antennae. Compound eye shape is oval, black and protruding from the head

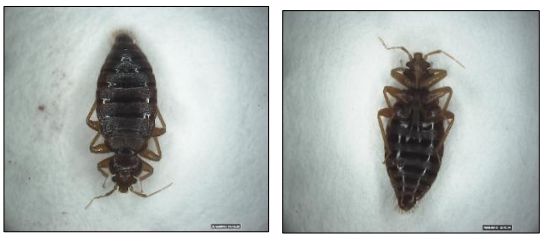

Fig 4: Body Structure Cimex dorsal and ventral views from Sitaro

Antenna consists of four segments, the first segment is shorter than the other segments, the third and fourth segments more slinder, flat and transparent than two initial segments. Fine hairs found in each segment. Samples of Sitaro showing more fine hair on each segment of the antenna compared to samples from Manado. Fine hair is also found in parts of the head except the compound eyes. The intensity of fine hair on the head is found more in bedbugs from Sitaro compared samples from Manado

Fig 11: Body Structure *Cimex* dorsal and ventral views from Manado

In the ventral part of the head there is a piercer used to suck the blood of their prey (Figure 17). Found labrum triangular shaped, consisting of three segments labium which reached the bottom protoraks, stylet in mandibel and maxilari sharp, shaped like a knife if observed degan microscope. Mandibuler stylet serves piercing the skin of prey and functional maxillary stylet into the tissue to suck blood. The results of surgery of the head was found on the bottom of the salivary glands. Salivary glands produce blood antipembekuan compound (Khan and Rhaman, 2012) [10].

Fig 12: Cimex female body structure (left) and male (right) ventral view, samples from Sitaro

Thorax consists of three segments. Protoraks greater than mesotoraks and metatoraks. In thoraks, exists Hemeltron or experiencing rudimentasi wing structure (Figure 18). In each there is a pair of thoracic limbs. Bedbugs metatoraks structure of Sitaro greater than metatoraks *Cimex lectuarius*.

Fig 13: Gambar 2 The Thoraks and Abdomen of Bedbugs. Pr (Protoraks), Mst (Mesotoraks), Mt (metatoraks), H (hemeltron/wing pad).

The number of abdominal segments 8, with smooth hair on the dorsal surface of the body. The abdomen of the female is oval while males are more slender. In females fine hair more on the last segment of the abdomen than in males.

Fig 14: Smooth hair on the last segment of the abdomen

Fig 14: The structure of the abdomen, visible smooth hairs

Fig 15: The structure of Thoracic and Head, dorsal and ventral views

Antenna consists of four segments, namely scape (S), pedicel (P), flagellum (f) and distal flagellum (DF). All antenna structures are fine hair as a receptor but on the pedicel fine hair more (Figure 3). Scape shorter and dark brown, while the third and fourth segments transparent in color and thinner. Proboscis consists of two segments. The first segment of the head is longer than the second segment. Proboscis is located right in the ventral part of the head. There are channels for the proboscis to suck blood. The channel also serves in the release of substances generated blood anticoagulation of salivary glands Cimex sp. Compound eyes and black oval located on the left and right side of the head.

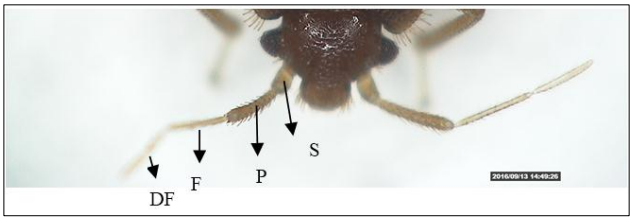

Fig 6: Antenna structure: S (scape), P (pedicel), F (flagellum), DF (distal falgelum)

Fig 17: The structure of the male abdomen (left) and female (right) ventral view

Fig 18: The structure of the female abdomen ventral view

3.4 Factor Analysis of Morphometry Bedbugs from Sitaro and Manado

Based on factor analysis. Formed three components or a factor of 10 morphological characters bedbugs. Eight characters clustered together on the third component.

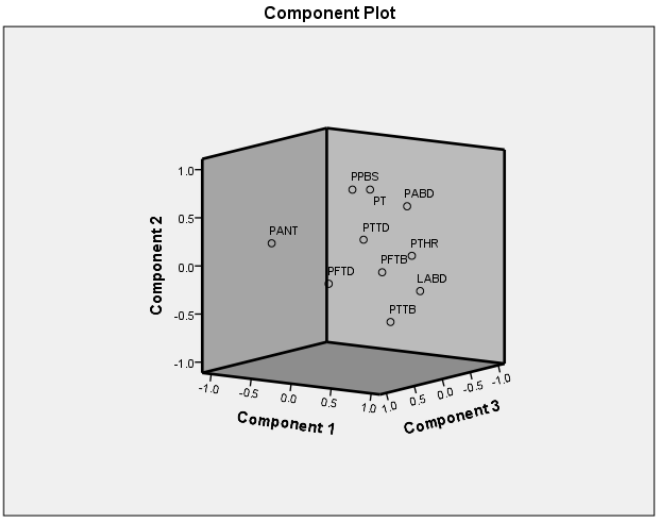

Fig 19: Grouping Characters Morphology On Three Components formed from factor analysis of ten characters morphometry

3.5 Cluster Analysis of Bedbugs morphology from Sitaro and Manado

Dendogram formed based on cluster analysis, showed grouping morphological characters based on common characteristics of morphometry bedbugs from Sitaro and Manado. Dendogram show that morphometric PTHR, PTTB, PANT, PPBS, PFTB, PFTD AND PTTD of Bedbugs from Sitaro and from Manado to form a one cluster. Thus, have similarities in terms of the size of the morphology (morphological characters). The degree of similarity is high based on the average distance in the group. PABD and LABD formed cluster, means having the degree of similarity morphometry. PPBS and PABD form clusters but average lower morphometric similarities. PPBS, PABD and PT form clusters with distances.

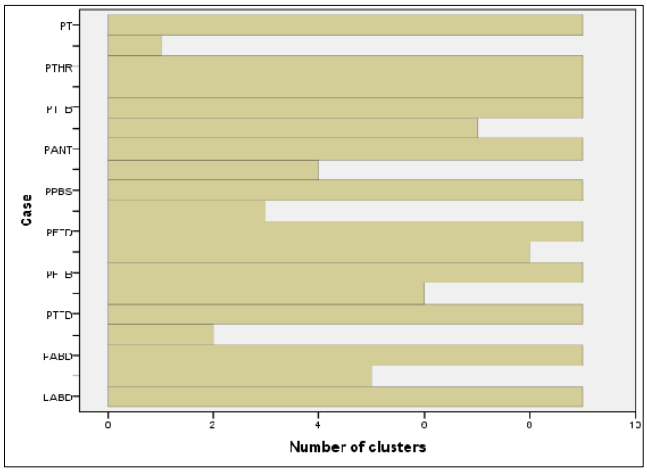

Fig 20

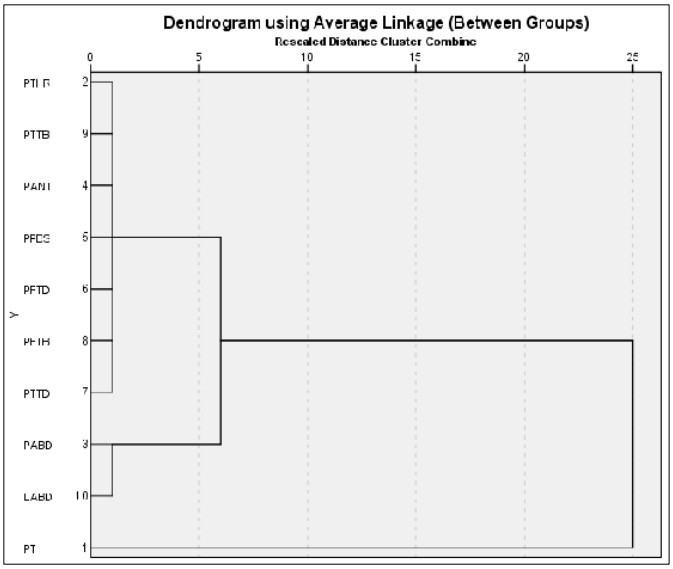

Fig 21: Cluster formed based of ten morphometry of *Cimex sp* from Manado and Sitaro

4. Conclusion

1. Based on morphology, bedbugs from Sitaro have differences with bedbugs from Manado. Differences in morphological characters which is the average length of the body, the intensity of trichomes on the head and thorax, color and size of the abdomen.
2. The results of the factor analysis, three groupings of morphological characters bedbugs from Sitaro and Manado. The results of cluster analysis to form four groupings based on similarities morphometry.

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