

Morphologic Character of *Haematopinus Sp.* Nymph and Mature Stadium of Fries Holland Cow from Jember with SEM (Scanning Electron Microscope)

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Abstract: *Haematopinus* is the biggest parasite of insect family of domestic animal having all lifecycles in the female, and can only live some hours out of the female body. This research aims at seeing the ultra-structural difference of nymph stadium with *Haematopinus sp.* of mature insect facing *Fries Holland* (FH) from Jember district based on morphologic identification key of Meleney and Kim. Samples of nymph stadium and mature insect of *Haematopinus sp.* was taken from tail end area, perineum vulvae, area, and around eyes of 5 *Fries Holland* (FH) cows of each infected cow from Jember district. The samples were identified based on morphologic identification key of Meleney and Kim and did *Scanning Electron Microscope* (SEM) of part of *caput* and *abdomen*. Results of morphologic identification and ultra-structure were analyzed descriptively. There are ultra-structural differences of nymph stadium from mature insect, especially ultra-structure of abdomen area. Nymph stadium has not been found in *gonopod* development; while, in mature insect, *gonopod* has developed and can be identified. SEM method can be used to change the morphologic identification, especially in a mature insect. Nymph and mature insect of *Haematopinus sp.* in *Fries Holland* (FH) from Jember district are *Haematopinus quadripertusus* species.

1 INTRODUCTION

Ectoparasites of Haematopinidae frequently parasites in the cow. *Haematopinus euryternus* was reported parasitizing on cows in areas with cold climate, while *Haematopinus quadripertusus* was reported parasitizing on cows in tropical and sub-tropical areas (Scifield *et. al.*, 2012).

In some cases, insect infestation was reported causing a reduction in productivity, and some cases were reported as a disease vector, such as babesiosis, theileriosis, anaplasmosis and others (Norval *et. al.* 1984). The incidence of insect infestation in a great number of cows causes itching, reduction in body weight, irritating, uncomfortable feeling, reduction in dairy water production and reduction in initial harvest product quality (Lasisi *et. al.*, 2010). Hadi and Saviana (2000) suggested that incidence of insect infestation is called as pediculosis and phthiriasis.

Haematopinus sp. has characteristics of 0.5 cm, yellow or grey-brown with a black line of each

margin, having no eye, having three pairs of wide and flat legs (Urquhart *et. al.*, 1987). *Haematopinus sp.* has *spiracle* of dorsal periphery of mesothorax (Noble and Glenn, 1989). Size of the head is elongated with the wider back part than front part and protrusion in the back antenna, the antenna has 5 internodes, and part of the thorax is wide and has *sterna plate* in the lower part (Lapage, 1956).

Mouth area of *Haematopinus sp.* has fine and small proboscis called as *haustellum*, internal part of *haustellum* is completed with small teeth directing to exit functioning to implant in female skin, having 3 prick organs whose shapes are like needles called as *stilet* which may expel to function to absorb blood and inject saliva gland into female body (Hado and Soviana, 2000).

Meleney and Kim (1974) explained key of identification for species of *Haematopinus sp.*, namely, rounding or compact *para tergite* in peripheral area of abdomen with 2 posterior setae, posterior area of abdomen has a non-pointing *gonopod*, *sterna plate* under thorax elongates,

forming an elongating head and having parasite in cows, so that the species directs to *Haematopinus euryternus* species or maybe *Haematopinus quadripertusus*. Female *Haematopinus euryternus* species has body with 2.23 – 3.18 mm in long and male *Haematopinus euryternus* species has body with 1.99 – 2.7 mm in long, having short and round *processus anterolatera*; *sterna plate*, thin abdominal trachea, *subgenital plate median* forming 9 *subtrapezoid*, *tergite* in abdomen with elongating and more protruding *processus anteromedial*, short forehead, and having *subgenital plate* in male insect, completed with 6 anterior setae. Female *Haematopinus quadripertusus* species has 3.42 – 4.75-mm body and male *Haematopinus quadripertusus* species has 3.04 – 3.52-mm body, having elongate *processus anterolateral sterna plate*, long forehead, elongate *processus anterolateral in sterna plate thorax in female insect*, thick abdominal trachea trunks, long and narrow gonopod, wide *subgenital plate median subrectangular*, 9th abdominal *tergite* has short and blunt *processus anteromedial*, and male insect has *subgenital plate* completed with 4 anterior setae.

Lapage (1956) explained lifecycle of *Haematopinus* sp., 1-mm *Haematopinus* egg, elongating shape, white in color, and sometimes brown, eggshell is not hard, female insect lays one or four eggs per day and female insect can expel 24 eggs, eggs will hatch to be nymph (young insect) after 9 – 19 days at 27.5 °C, nymph will come out from egg and grow and molt perfectly and grow into adult.

2 METHODOLOGY

2.1 Location of Research

Samples of nymph and mature stadium of *Haematopinus* sp. Were taken from *Fries Holland* (FH) cows found suffering from pediculosis (ptiriasis_ from Jember district.

The research was conducted in some locations. Morphologic identification of insect was conducted in the Parasitological laboratory, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta. Length scale and picture of insect were measured using Lucida camera in Plant Infest laboratory (Nematological Laboratory), faculty of farming, Gadjah Mada University, Yogyakarta. ultra-structure was identified by *Scanning Electron Microscope* (SEM) in Central Research of Zoology, Institute of Sciences of Indonesia (LIPI), Cibinong.

2.2 Tools and Materials

Tools to take samples included rubber glove, 2-ml *microtube*, pinset, and labeling paper. Tools to identify insect morphology included petri dish, *slide glass*, *cover glass*, measurer (0.05-mm accuracy), Lucida camera, pencil, HVS paper and tracing paper, tools to examine body surface ultra-structure included a *holder*, *stibe*, *vacuum evaporator*, *Ion Coates*, and JEOL JSM-5310LV *Scanning Electron Microscope* (SEM).

Required materials were absolute ethanol, 50% ethanol, 70% ethanol, 85% ethanol, 95% ethanol, 2.5% *glutaraldehyde*, *cocodylate buffer*, 2% *tannin acid*, 1% *osmium tetroxide*, *tertiary butanol*, and aquades.

2.3 Methods

2.3.1 Collection of Samples

Samples of nymph and mature stadium of *Haematopinus* sp. In *Fries Holland* (FH) found suffering from pediculosis (phthiriasis) were taken from the tail area, especially fiber tail end, around perineum vulvae, ear, and surrounding eyes of the cow with 5 mature and nymph insects, using pinset, then inserted into 2-ml *microtube* containing absolute ethanol and labeled. Samples of mature insects and nymph were collected in a separate *microtube*.

2.3.2 Morphologic Identification

Morphologic identification of mature insects and nymph samples included macroscopic and microscopic observations.

Macroscopic observation consisted of identification of body, color, presence or absence of wings, total extremities, and long measures body using measurer.

Microscopic observation consisted of morphological observation of *caput*, *thorax*, *abdomen*, *extremity*, and picture and measure scale were made using a binocular microscope (zooming 4 x 10) completed with Lucida camera drawing the shadow of sample objects using pencil on HVS paper which was then moved to tracing paper.

2.3.3 Identification of Ultra-structure

Identification of ultra-structure with *Scanning Electron Microscope* (SEM) included caput and abdomen. Observation of ultra-structure in caput part included an anterior end to see ultra-structure of mouth type. Observation of ultra-structure in

abdomen part included pleural disk part to see ultra-structure of *para tergite* and *posterior setae* and in the posterior end, part to observe ultra-structure of *gonopod* and *subgenital plate median*.

The process of *Scanning Electron Microscope* (SEM) was conducted in Central Research of Zoology, Institute of Sciences of Indonesia (LIPI), Cibinong. Before the samples were delivered, the samples were fixed in absolute ethanol.

The process of *Scanning Electron Microscope* (SEM) included process of specimen preparation consisting of 5 stages: first, the samples were washed by soaking in *coccodylate buffer* (2 hours), then the samples were agitated in *Ultrasonic cleaner* (5 minutes); second, the samples were prefixed by inserting the samples in 2.5% *glutaraldehyde* (2-3 hours); third, the samples were fixed by using 2% *tannin acid* (6 hours), then washed by *coccodylate buffer* (5 minutes), the process of washing was conducted repeatedly until 4 times, then processed by second washing by 1% *osmium tetroxide* (one hour), third washing by aquades (15 minutes); fourth, dehydration process was conducted by soaking the samples in stratified ethanol of 5% ethanol (5 minutes), this process was continued by using 70% ethanol (20 minutes), 85% (20 minutes), and 95% (20 minutes) and absolute ethanol (10 minutes) conducted two times at room temperature; fifth, drying process by soaking the samples in tertiary butanol (10 minutes), this process was conducted until 2 times, then frozen by Freezed Drier until dry. The process of *Scanning Electron Microscope* (SEM), the samples which had been covered by copper with Ion Coates (15 minutes) and observed by *Scanning Electron Microscope* (SEM) of JEOL JSM-5310LV.

3 RESULTS AND DISCUSSION

Fries Holland (FH) from Jember found suffering from pediculosis (phthiriasis) had nymph stadium and mature insect of *Haematopinidae* family. The ectoparasites infestation area covered areas of the hairy tail end, around the ear, and around perineum vulvae, especially in the lower part. In the area, insect eggs binding to hair were also found.

3.1 Morphologic Identification

Nymph stadium of *Haematopinus* sp. In *Fries Holland* (FH) from Jember was found black, body divided into 3 parts (*caput*, *thorax*, and *abdomen*), dorso-ventral flat body shape, having no wings,

having 3 pairs of legs (*proleg*, *mesoleg*, and *metaleg*) where each leg is divided into 4 internodes (*coxa*, *femur*, *tibia*, *tarsus*) with 1 claw in each leg (Figure 1). The mature insect of *Haematopinus* sp. In *Fries Holland* (FH) from Jember was black-grey in color, a body is divided into 3 parts (*caput*, *thorax*, and *abdomen*), dorso-ventral flat body shape. Having no wings, having 3 pairs of legs (*proleg*, *mesoleg*, and *metaleg*) where each leg is divided into 4 internodes (*coxa*, *femur*, *tibia*, *tarsus*) with 1 claw in each leg (Figure 2). Body lengths of nymph samples of *Haematopinus* sp. In *Fries Holland* (FH) from Jember are 3.10 mm, 2.60 mm, 2.90 mm, 2.75 mm, and 2.95 mm (Table 1), or averagely body length of nymph samples is 2.86 mm. Body lengths of mature insect samples of *Haematopinus* sp. In *Fries Holland* (FH) from Jember are 4.70 mm, 4.60 mm, 4.20 mm, 4.15 mm, and 4.20 mm (Table 2), or averagely body length of mature insect is 4.37 mm.

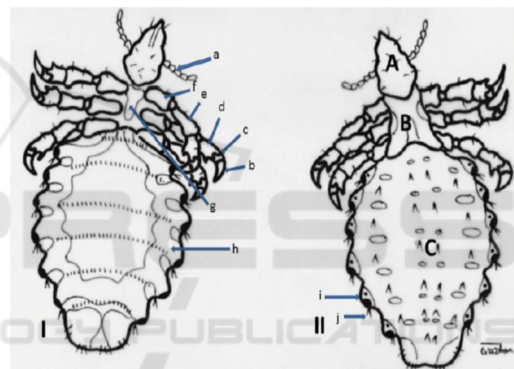


Figure 1. Nymph of *Haematopinus* sp. Of *Fries Holland* (FH) from Jember: I. Ventral (a. antenna, b. claw, c. tarsus, d. tibia, e. femur, f. coxa, g. sterna plate, h. abdominal tracheae trunks, i. gonopod); II. Dorsal (A. caput, B. thorax, C. abdomen, i. para tergite, j. setae). Scale bar: 0.222 mm.

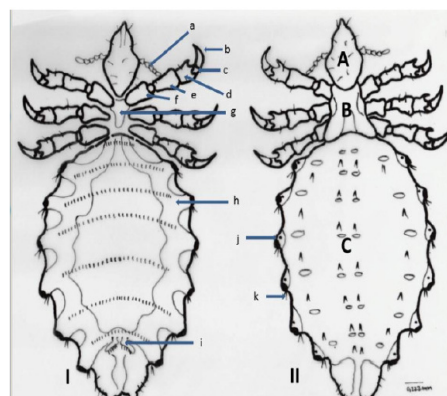


Figure 2. The mature insect of *Haematopinus* sp. Of *Fries Holland* (FH) from Jember: I. Ventral (a. antenna, b. claw, c. tarsus, d. tibia, e. femur, f. coxa, g. sterna plate, h.

abdominal tracheae trunks, i. gonopod); II. Dorsal (A. Caput, B, Thorax, C. Abdomen, j. para tergite, k. setae). Scale Bar: 0.222.

Table 1. Samples of the nymph of *Fries Holland* (FH) from Jember.

Identification	Result
Color	: Black
Body Shape	: Dorsoventral flat
Body parts	: 3 parts (caput, thorax, abdomen)
Caput	
▪ Shape	: Narrow and pointing (smaller than thorax part)
▪ Mouth type	: Pricking and absorbing
▪ Total antennas	: 2 pairs
▪ Antenna internodes	: 5 internodes
▪ Eye	: Absent
Thorax	
▪ Shape	: No segment
▪ Total extremities	: 3 pairs: Proleg, Mesoleg, Metaleg
▪ Extremity internodes	: 4 ruas: Coxae, Femur, Tibia, Tarsus
▪ Claw	: Single
▪ Wing	: No wing
Abdomen	
▪ Shape	: Segmental
▪ Paratergite	: Rounding, having pleural disks with 2 pairs of setae in angular part
▪ Respiratory tract	: Abdominal tracheae tract is seen clearly
▪ Reproduction tool	: Unobservable
Body length size	
▪ Nymph 1	: 3.10 mm
▪ Nymph 2	: 2.60 mm
▪ Nymph 3	: 2.90 mm
▪ Nymph 4	: 2.75 mm
▪ Nymph 5	: 2.95 mm

Table 2. Samples of mature insect of *Fries Holland* (FH) from Jember

Identification	Result
Color	: Black-grey
Body Shape	: Dorsoventral flat
Body parts	: 3 parts (caput, thorax, abdomen)
Caput	
▪ Shape	: Narrow and pointing (smaller than thorax part)
▪ Mouth type	: Pricking and absorbing
▪ Total Antenna	: 2 pairs
▪ Internodes	: 5 internodes
▪ Eye	: Absent
Thorax	
▪ Shape	: No segment
▪ Total extremities	: 3 pairs: Proleg, Mesoleg, Metaleg
▪ Internodes	: 4: Coxae, Femur, Tibia, Tarsus
▪ Claw	: Single
▪ Wing	: No wing
Abdomen	
▪ Shape	: Segmental
▪ Paratergite	: Rounding, having 5 setae hairs in angular part
▪ Respiratory tract	: Abdominal tracheae tract is seen clearly
▪ Reproduction tool	: Long and narrow Gonopod
Body length size	
▪ Insect 1	: 4.70 mm
▪ Insect 2	: 4.60 mm
▪ Insect 3	: 4.20 mm
▪ Insect 4	: 4.15 mm
▪ Insect 5	: 4.20 mm

Results of observation for nymph samples and mature insect) Table 1 and Table 2) macroscopically and microscopically indicate that there is no morphologic difference of nymph from mature insect, except nymph samples are seen black than the mature

insect. Key of morphologic identification of *Haematopinus* sp., according to Meleney and Kim (1974) is that abdomen has round and compact para tergite with 2 posterior setae, and gonopod is not pointing in posterior part. *Haematopinus quadripertusus* has body lengths of 3.42 – 4.75 mm (female) and 3.04 – 3.52 mm (male), having thick abdominal tracheae trunks, long and narrow gonopod. *Haematopinus euryternus* species has body length sizes of 2.23 – 3.18 mm in female and 1.99 – 2.7 mm in male, thin abdominal trachea, short and compact gonopod.

Based on the key of identification, samples of insect are from *Haematopinus quadripertusus* species.

3.2 Identification of Ultra-structure

Ultra-structure of the anterior end of nymph samples and mature insect (Figure 3 and Figure 4) is seen as pointing shape of the suction tube in *haustellum* with similar function to proboscis, *labrum* (lip), and 4 pairs of setae. Organ of suction tube in *haustellum* belongs to an insect with suction tube functioning to suck blood from hospes as main food insect and flexible to pull and expel according to need. *Haustellum* is an organ with similar function to mouth of higher level animals. Whitlock (1960) explained that *Haematopinus* sp. Is different from other insects with blood suction tubes because they have no long proboscis pricked into the female body. Hadi and Soviana (2000) indicated that *Haematopinus* sp. Has mouth consisting of the fine and small proboscis (*haustellum*). The internal part of *haustellum* is completed with small teeth directing to exit to implant into the female skin, having 3 organs of the suction tube to suck blood and inject saliva gland into a female body.

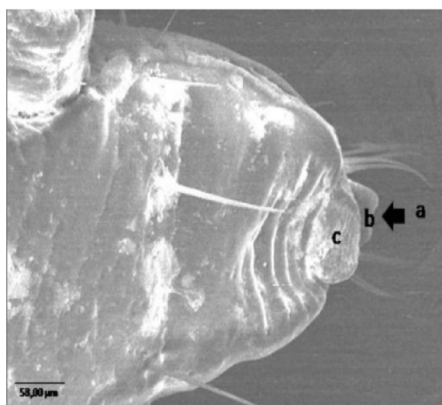


Figure 3. Ultra-structure of the ventral anterior end of *Haematopinus* sp. Nymph of *Fries Holland* (FH) from Jember: a. suction tube, b. Labrum, c. Setae. Scale bar: 58.00 μm .

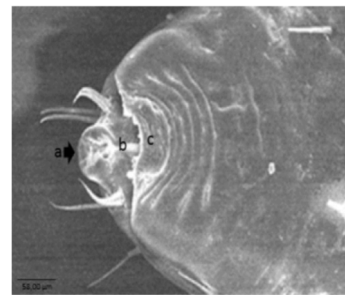


Figure 4. Ultra-structure of the ventral anterior end of mature insect of *Haematopinus* sp. Of *Fries Holland* (FH) from Jember: a. suction tube, b. Labrum, c. Setae. Scale Bar: 58.00 μm .

Ultra-structure of abdomen area of pleural disk part of nymph samples and mature insect of *Haematopinus* sp. (Figure 5 and Figure 6) indicates that *para tergite* is rounded with *spiraculum* in end part and has 2 setae hairs in posterior part in the lateral area of the abdomen. In ventral part, mature female insect samples have elongated and narrowing gonopod shape and posterior part of gonopod has subgenital plate median in subrectangular shape with hollow in the central part (Figure 8), different from the picture of ultra-structure in nymph samples unseen for gonopod shapes (Figure 7). According to Meleney and Kim (1974), *Haematopinus quadripertusus* has long and narrow gonopod and *subgenital plate median* of subrectangular shape.

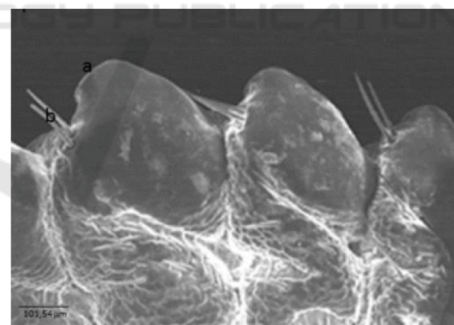


Figure 5. Ultra-structure of pleural (ventral) disk of *Haematopinus* sp. Nymph of *Fries Holland* (FH) from Jember: a. *spiraculum*, b. Setae. Scale bar: 101.54 μm .

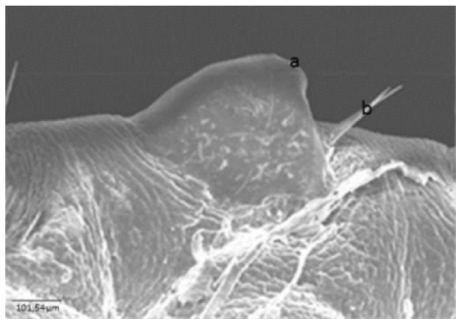


Figure 6. Ultra-structure of pleural (ventral) disk of *Haematopinus* sp. A mature insect of *Fries Holland* (FH) from Jember: a. spiraculum, b. Setae. Scale bar: 101.54 μm.

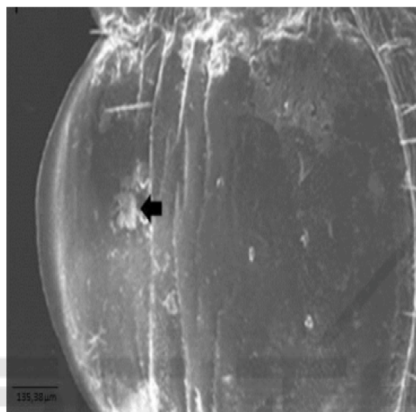


Figure 7. Ultra-structure of ventro posterior nymph of *Haematopinus* sp. Of *Fries Holland* (FH) from Jember. Gonopod shape is not seen (arrow). Scale bar: 135.38 μm.

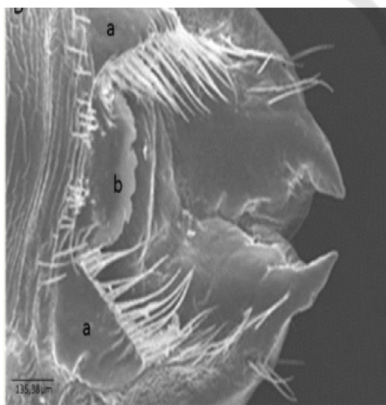


Figure 8. Ultra-structure of ventro posterior mature insect of *Haematopinus* sp. Of *Fries Holland* (FH) from Jember. Gonopod shape is not seen (arrow). Scale bar: 135.38 μm.

Based on identification key of Meleny and Kim (1974), specially strengthened by the picture of ultra-structure of mature insect sample consisting of a suction tube, para tergite rounding with 2 setae hairs in posterior part, long and narrow gonopod,

subrectangular *subgenital plate median*, then the sample was identified as *Haematopinus quadripertusus* species. Identification of insect using *Scanning Electron Microscope* (SEM) can be used to strengthen morphologic identification of insect, especially in the mature stadium. In nymph, especially in the posterior end, it cannot be a reference of identification because gonopod did not have developed, but the shape of pleural disk part could be observed.

4 CONCLUSIONS AND RECOMMENDATIONS

Scanning Electron Microscope (SEM) method can be used to strengthen morphologic identification of insect of *Haematopinus* sp. In mature stadium. Samples of nymph and mature insect of *Fries Holland* (FH) from Jember is *Haematopinus quadripertusus* species.

Recommendations

Research of identification of species needs to add molecular methods to see the *phylogenetic tree* of researched species.

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