Spiracles Morphology of Brown Planthopper (*Nilaparvatalugens*)

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Abstract: Spiracles are the external opening of insect respiratory tract, where the oxygen enters and carbon dioxide is released, further inside part through a tracheal tube has branches and extends throughout the body. The research objective was to determine the morphology of spiracles of brown planthopper including the shape, number and size of the spiracles. The method of observation of the brown planthopper spiracles was Scanning Electron Microscopy (SEM) and it was carried out in Conservation Center Borobudur, Magelang, Central Java. The results showed that spiracles of brown planthopper have ellipse-shaped, where there are two pairs of spiracles in the thorax on mesothorax and metathorax segments, and 6 pairs on segment of the abdomen, from the first to sixth abdominal segments each lays on the left and right lateral side. The smallest size of brown planthopper on spiracles thorax is 239.0μm^2, and the largest is 14 821 μm^2, with the average of 4122.2 ± 4554.7 μm^2 and for abdominal spiracles of broad brown planthopper the smallest and largest sizes are 17.9 μm^2 and 751.0 μm^2 respectively, with mean of 164.18 ± 116.1 μm^2. From the results of morphological observation on spiracles of brown planthopper each segment shows the all spiracle holes are functional or function for gas exchange indicated by hole spiracles that are not rudimentary. It was concluded that the morphology of the brown planthopper spiracles are specific: they have ellipse shape, there are eight pairs of spiracles with varying aperture area. The results of morphological observation showed that all spiracles are functional.

Keywords: Brown planthopper; spiracles; morphology; respiratory; insects.

1. Introduction

Brown planthopper (*Nilaparvatalugens* (Hemiptera: Delphacidae) is the main rice pest and threatens food security in Indonesia. However, since 1970's the significance of this species as a pest increased considerably in Indonesia [1]. The increase in BPH attack occurred in East, Central and West Java during the rainy season of 2009/2010 and in the beginning of the 2010 dry season [2]. Brown planthopper causes 'hopper burn' of rice plants by direct feeding and transmitting the grassy stunt and ragged stunt rice diseases [3]. A common way to control was the application of insecticides. However, this can lead to pest resistance and resurgence [4][5].

Morphological shape of brown planthopper especially its spiracle is not yet available. BPH has a pair of spiracles on each thoracic and abdominal segment. Each spiracle is connected with elastic tubes called trachea [6]. The oxygen enters the spiracles and is transferred into the insect body through a tracheal tube and tracheoles and reach dysfunction cells of the body. The CO_2 coming out of the cells follows the same path through the tracheoles into the trachea and through the holes to the spiracles and go out of the body [7]. Reports also say that the size of the brown planthopper spiracles also has not been studied. Spiracle is a breathing hole outside the insect tracheal tube that leads to the inside part. While trachea is the respiratory system of adult insects, spiracle is the external opening or a hole where the entry of gas is connected with the external environment. Spiracles have cylindrical shaped vessel layered with substance chitin, and located in pairs on each body segment, spiracles were repeated in

every segment of the thorax and abdomen, one on each side. Spiracles have valves that are controlled by the muscles that open and close spiracles which occurs regularly [8].

Spiracle's hole has the size of 50-100 μm. The size of the hole in the thoracic spiracles imago *Chrysomyarufifacies* (Diptera: Calliphoridae) is 50 μm, in larvae *Corticeus* sp. (Coleoptera: Tenebrionidae) is 100 μm, and in migratory locust (Orthoptera: Acrididae) abdominal is 100 μm [9]. Spiracles of grasshopper have ellipse shape with black valve. The shape of spiracles of BPH is unknown.

2. Material & Methodology

2.1. Data

Explanation of how data was collected/generated, explanation of how data was analyzed explanation of methodological problems and their solutions or effects. We need to know how the data was obtained because the method affects the results. Knowing how the data was collected helps the reader evaluate the validity and reliability of your results, and the conclusions you draw from them.

2.2. Method

Brown planthopper spiracles brachyptera was observed and recorded using Scanning Electron Microscopy (SEM) JEOL JSM T300 brand [10]. Specimens used were brown planthopper brakhiptera aged 2 days. Brakhiptera colony of brown planthopper was obtained from propagation. The study began with BPH specimen preparation, specimen shooting brown planthopper with SEM, as well as observations of the brown planthopper spiracles. BPH specimen preparation which was as many as 45 heads of brown planthopper were killed with chloroform. A total of 3 heads of the brown plant hopper were placed on the grid with the left lateral position, with a total of 10 grids. A total of 3 heads of the brown plant hopper was placed on the grid with right lateral position, with a total of 5 grids. Total specimens of the brown plant hopper in the left lateral position have more grids (10 grids) and on the left lateral position there are many spiracles that were closed therefore it was more difficult to clearly see the spiracles. Each specimen of BPH on the grid was fused with carbon tape, and placed in a holder. BPH specimen on the grid at the next holder was coated with gold ions using ion sputter fine coat. Fine coat sputter ion was heated for 10 minutes, the grid on the holder was put in a fine coat sputter ion. One activation was only for three units of the grid on the holder. Tool was activated for 15 minutes. BPH specimen that had been coated with gold on a holder was inserted into the specimen stage in the column on the SEM tool, thenit was in-vacuumed until the indicator showed the ready position. One of the brown planthopper objects was seen by adjusting the control panel according to the desired object position. The images of position of spiracles of brown planthopper that was apparent was recorded.

Observations on spiracles on brown planthopper were directed towards the aperture area of spiracles. A total of 30 brown planthoppers were observed from the left lateral position and 15 of them from the right lateral position. The number of specimens of the brown planthopper depends on the already gained three spiracles on each segment which were the clearest. One by one spiracle of specimen of brown planthopper was observed on each segment in the thoracic and abdominal lateral position of left and right. The clearest spiracles of brown planthopper were photographed. The number of brown planthopper spiracles that were photographed on each segment of specimen depends on the presence of spiracles that were in clear visible position. Each picture was stored in file.jpg, labeled, its shape and size were analyzed. Spiracles of broad brown planthopper in file.jpg were measured by semaphore software program. BPH spiracles measured in the aperture area of the brown planthopper. Wide brown planthopper spiracles' sizes were expressed in μm^2. Repetition was done on Deuteronomy 3 units.

3. Results and Discussion

Spiracles of brown planthopper have ellipse-shape, with the smallest and the largest area on the thorax respectively 239.0μm^2 and 14.821μm^2, with mean of 4122.2 ±4,554.7μm^2, the smallest and largest are on abdomen area respectively 17.9μm^2 and 751.0 μm^2, with mean of 164.18 ± 116.1μm^2. The broad spiracles BPH showed that the average area in the thoracic spiracles are approximately 25 times greater than in the abdomen (Table 1).

Table 1. Spiracles of broad brown planthopper

Statistics	Spiracles of brown planthopper	
	Thorax	Abdomen
Number of samples(n)	12	36
The smallest area(μm^2)	239	17.9
The largest area (μm^2)	14,821.00	751
The average area ± s.d (μm^2)	4,122.2 ± 4.554,7	164.18 ± 116.6
t $\alpha_{0,05}$	1.601E-42$^{**)}$	0.077$^{*)}$

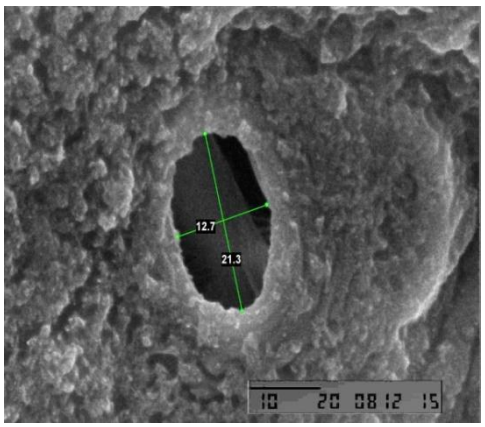
Remarks: $^{**)}$ = very real (P< 0.01), $^{*)}$= real (P< 0.05)

The width of spiracles of brachyptera brown planthopper on the right and left sides of each spinal segment of chest and abdomen seems varied (Table 2 and Figure 1, 2 and 3). Actually, the width of insect's spiracles are equal between the right and left sides because insect's body is symmetrical. The technical use of SEM tools where the right and left side of the brown planthopper individual uses different BPH individual causes a wide variation of spiracles. In addition the tilt position of the specimen on the grid which was not uniform was also influential.

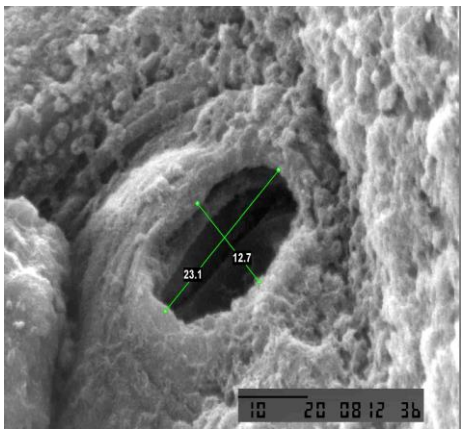
Table 2. The width of spiracles of brachyptera brown planthopper on the thorax and abdomen

Parts of body of brown planthopper	Segment	N (Repetition)	Average size of the brown planthopper spiracles (μm^2)	
			Right	Left
Thorax	2	3	507.3 ± 376.7	1041.6 ± 648.6
	3	3	6133.0 ± 1348.8	8807.0 ± 6236.8
Abdomen	1	3	376.6 ± 325.8	212.0 ± 41.5
	2	3	167.6 ± 30.8	202.0 ± 56.0
	3	3	121.3 ± 84.9	131.6 ± 16.1
	4	3	174.3 ± 71.5	106.4 ± 61.5
	5	3	121.0 ± 4.3	109.8 ± 66.4
	6	3	79.0 ± 54.3	168.3 ± 40.6

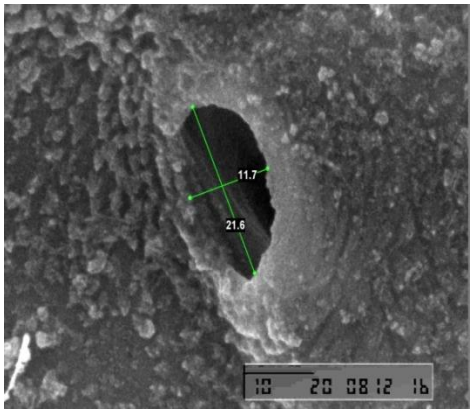
Remarks: shooting by Scanning Electron Microscopy, broad measurement by semaphore program



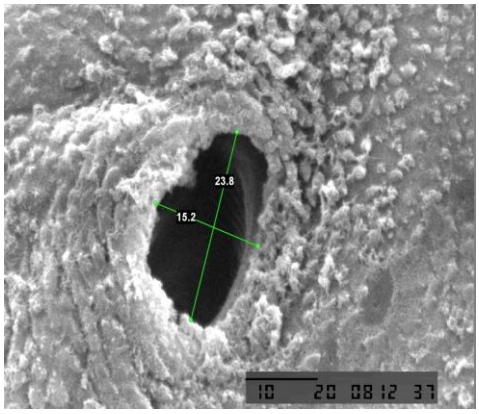
(a) Right abdominal spiracles 1st segment



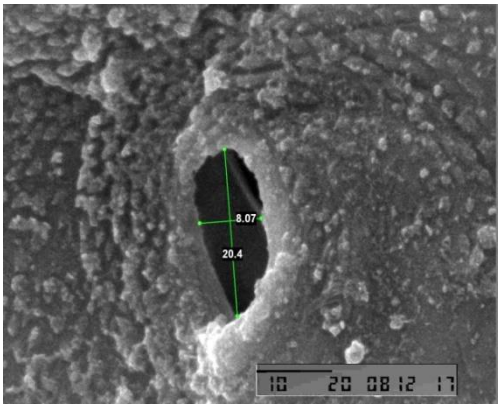
(b) Left abdominal spiracles 1st segment



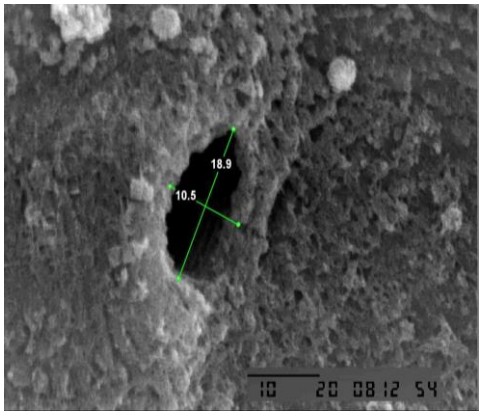
(c) Right abdominal spiracles 2nd segment



(d) Left abdominal spiracles 2nd segment



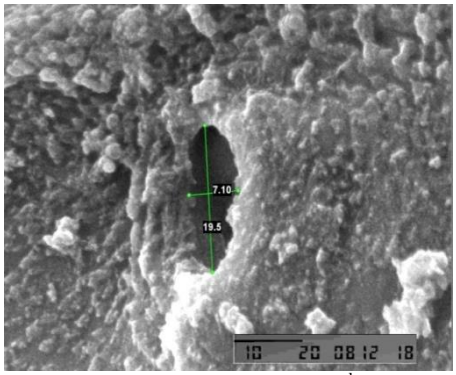
(e) Right abdominal spiracles 3rd segments



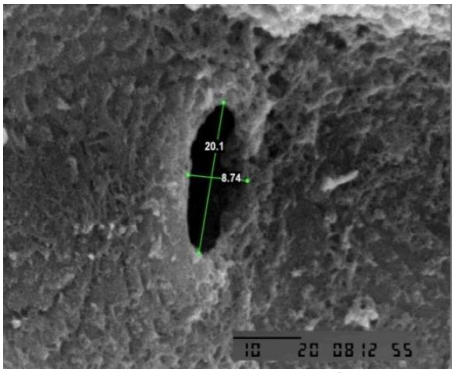
(f) Left abdominal spiracles 3rd segment

Figure 1. Spiracles brachyptera BPH at right and left side of the 1st, 2nd, and 3rd section (Scanning Electron Microscopy)

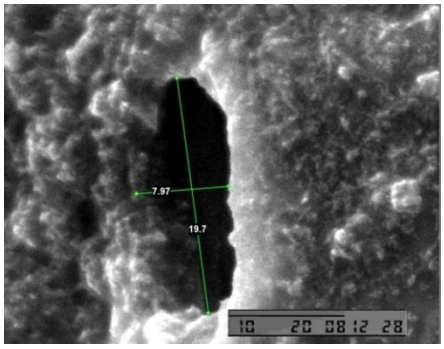
BPH has a pair of spiracles on each segment on the right and left side thorax and abdomen. Spiracles are the external opening point of oxygen (O_2) entry and the exit of carbon dioxide (CO_2) or the place where gas exchanges with the environment. Each spiracle is connected to the air channels which is elastic called the trachea [6]. Oxygen entering the spiracles are transferred continuously through a small channel namely trachea which diameter is approximately 2 µm. Finer branched channel is called trakheol, its branches reach into every cell in the body. CO_2 comes out of the cell which follows the same path which is through the trakheol channel into the trachea to the spiracles hole and out of the body [7][8].



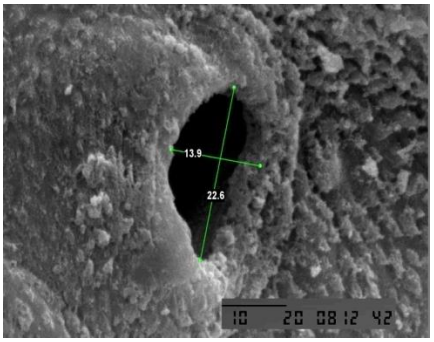
(a) Right abdominal spiracles 4th segment



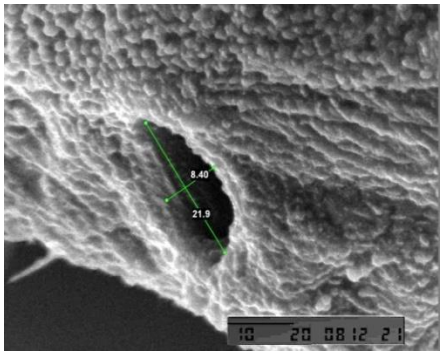
(b) Left abdominal spiracles 4th segment



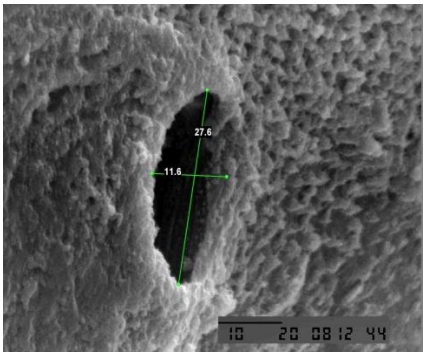
(c) Right abdominal spiracles 5th segment



(d) Left abdominal spiracles 5th segment



(e) Right abdominal spiracles 6th segment



(f) Left abdominal spiracles 6th segment

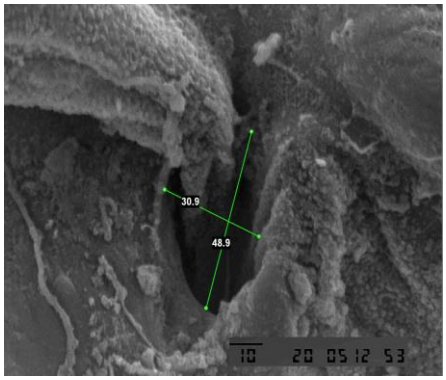
Figure 2. Spiracles of brachyptera BPH on abdomen right and left sides at the 4th, 5th, and 6th sections (Scanning Electron Microscopy)

From the results of spiracles images both on the thorax and abdomen, it appears that all of the brown planthopper spiracles are not rudimentary. This means all spiracles function or are functional, which can come in through spiracles O_2 and CO_2 can exit through spiracles. The total area of six spiracle's holes of pests namely *Hydroscapha* sp. (Coleoptera; Psephenidae), excluding areas under conditions of spiracles open, was 1-2x106μ^2/mg. While the shape of spiracles of *Sphaerius* sp. (Coleoptera; Psephenidae) is crooked, making it more difficult to calculate the surface area of the spiracles holes [11].

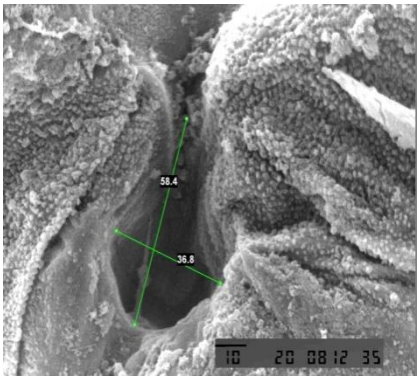
BPH has a pair of spiracles on each segment of the body, along the sides at the thorax and abdomen. Spiracles are the external hole where O_2 enters and carbon dioxide is released or where gas exchanges with the environment. Each spiracle is connected with an air tube which is elastic or trachea [6]. Oxygen entering the spiracles are transferred continuously through small tubes namely the trachea. Smaller branching tubes are called trakheol. The branch reaches into every cell in the body. Carbon dioxide is emitted from the cells following the same path which is through trakheol tube, into the trachea to the spiracles hole and out of the body [7].

4. Conclusion

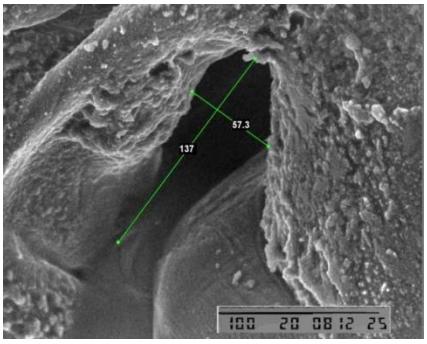
Ellipse-shaped spiracles brown planthopper, with a mean area of spiracles at the thorax 4122.2 ± 4,554.7μm^2 and abdomen are with a mean of 164.18 ±116.1μm^2, therefore the width of thoracic spiracles are 25 times larger than abdominal spiracles. Spiracles of brown planthopper have eight pairs, two pairs are on the thorax, and 6 pairs are on the abdomen. All spiracles both on the thorax and abdomen were functional.



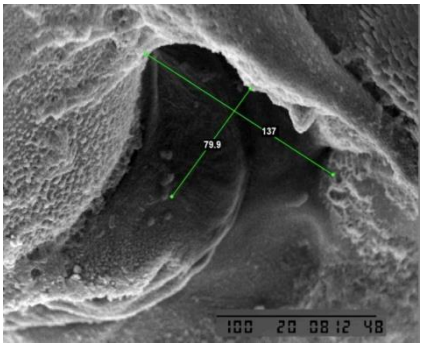
(a) Spiracles segment mesothorax right side



(b) Spiracles segment mesothorax left side



(c) Spiracles segment metathorax right side



(d) Spiracles segment metathorax left side

Figure 3. Spiracles of brachyptera brown planthopperon metathorax and mesothorax right and left sides (Scanning Electron Microscopy)

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