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Morphological Re-description of *Cochlochila bullita* (Stål) (Heteroptera: Tingidae), a Potential Pest of *Orthosiphon aristatus* Blume Miq. (Lamiales: Lamiaceae) in Malaysia

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Abstract: One species of lace bug *Cochlochila bullita* Stål (Heteroptera: Tingidae) was found heavily infested *Orthosiphon aristatus* Blume Miq., an important medicinal plant in Malaysia. A morphological re-description of *C. bullita* was done in order to facilitate the identification of this oligophagous insect pest. Five variables, body length and width, antenna length, tibia length and head width were measured from 15 samples from each stage. Among these variables, body length and width were used to construct the ratio for species identification; while body lengths with the other three variables were used to distinguish the nymphs from each developmental stage. The measurements of four traits except the antenna length showed significant differences between the development stages. And thus suggest the body width, tibia length and head width were suitable parameters used to distinguish the nymphal stages. However, the result on the growth factor showed only the sizes of the head followed a more constant growth rate with growth ratios (1.21-1.39) lie between the Dyar's ratio. Body length and width ratio for the adult female and male was 1.51±0.00 and 1.59±0.01, respectively. These data are pertinent for identifying developmental stages and to distinguish the species of the lace bug.

Key words: *Cochlochila bullita*, morphology, morphometrics, *ocimum basilicum, Ocimum tingid*, *Orthosiphon aristatus*

INTRODUCTION

Orthosiphon aristatus is a well known medicinal plant from the family Lamiaceae. The herb is found throughout Southeast Asia and tropical Australia. In Malaysia, this traditional herb is often consumed as an herbal tea and has been used for many centuries for treating ailments, such as promote urinary to prevent or eliminate kidney stones, urinary tract infections and bladder problems (Jaganath and Ng, 2000). Recent scientific findings show that the plant's methanol extracts contain compounds with antioxidant properties (Sahib *et al*., 2009). Moreover, its aqueous extract was noted to be effective in rats for reduce the sugar level in the blood (Sriplang *et al*., 2007) as well as having some diuretic functions (Adam *et al*., 2009). Like any other plants, *O. aristatus* is also prone to insect attacks. One previous study had represented the first record of *C. bullita* on *O. aristatus* in Malaysia (Sajap and Peng 2010). In this study the morphology *C. bullita* was further described in detail to facilitate the identification the insect pest.

MATERIALS AND METHODS

Cultivation of *Orthosiphon aristatus*: *O. aristatus* plants were bought from a local nursery and maintained in the nursery of the faculty of forestry for one month before used for further cultivation. The plants were cultivated by using stem cuttings of about 15-20 cm long. These plants were watered once a day and fertilized once a month. No pesticide was applied on these plants. The plants were examined every 2 days to ensure there was no pest infestation. If infestation did occur the insects were removed manually. Flowers were pruned to delay the process of become woody and promote new growth.

Maintenance of *Cochlochila bullita* colony: The colony was maintained on potted *O. aristatus* plants that were kept in a wooden cage (82×73×56 cm). Six pots were placed in the cage and the plants were watered daily. Ten female and male adults *C. bullita* were collected from the field and released into the cage equipped with the plants. The insects were then allowed to reproduce on the plants inside the cage. Old plants were replaced with new plants and the remaining insects were transferred to the new plants using a soft camel-hair brush.

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Experimental Design: Measurements and morphological description were obtained from 15 nymphs from each instar, adults of both sexes. Eggs were randomly chosen and their colour pattern was described. These eggs were then kept in 70% ethanol for further examination.

Body measurements: Five variables, for nymphs and adults, were measured using a method modified from Horton *et al.* (2008). The specimens were mounted onto points. Measurements were made under a microscope (Leica MZ6) equipped with a camera (Nikon Digital Sight DSFi1). Body lengths and hemelytral widths were taken for adults; while body length head width and pronotal width were taken for all instars. For both nymphs and adults, tibia length was measured on the rear left leg, whereas the antennal measurement was made for the third segment (Fig. 1). The width and length of the egg sac and its opercula were measured. Measurements (Mean±SD error), given in millimetres, were obtained according to Matesco *et al.* (2009). Descriptions for eggs were done followed Livingstone and Yacoob (1987), while for nymphs were referred to Guilbert (2005) and Cheng (1967) while for adults were they described by Broglio *et al.* (2012).

Statistical analysis: Variation in head width, antenna length, tibia length, body length and pronotal width between nymphal developments stages were subjected to one-way ANOVA. Growth ratios were calculated for all the biometric parameters stated above. And linear regressions were carried out to evaluate the pattern of growth between instars.

RESULTS

Morphologies and Morphometrics: Morphological characteristics, body length and pronotal width and hemelytral width for both nymphs and adults for all stages, were described and summarized in Table 1. Body length ($df = 4, 74$, $F = 680.787$, $p<0.0001$) and width ($df = 4, 74$, $F = 1019.890$, $p<0.0001$) between the developmental stage were all significantly different and no overlapped were found between the instars. Besides, adult females of *C. bullita* with body length 2.264±0.019 and body width 1.495±0.013 were also found significantly larger than males that only with 2.051±0.018 and 1.288±0.016, respectively ($t_{28} = 7.954$; $p<0.0001$ and $t_{28} = 9.860$, $p<0.0001$). The results of measurements on all the four traits for both nymphs and adults are shown in Table 2.

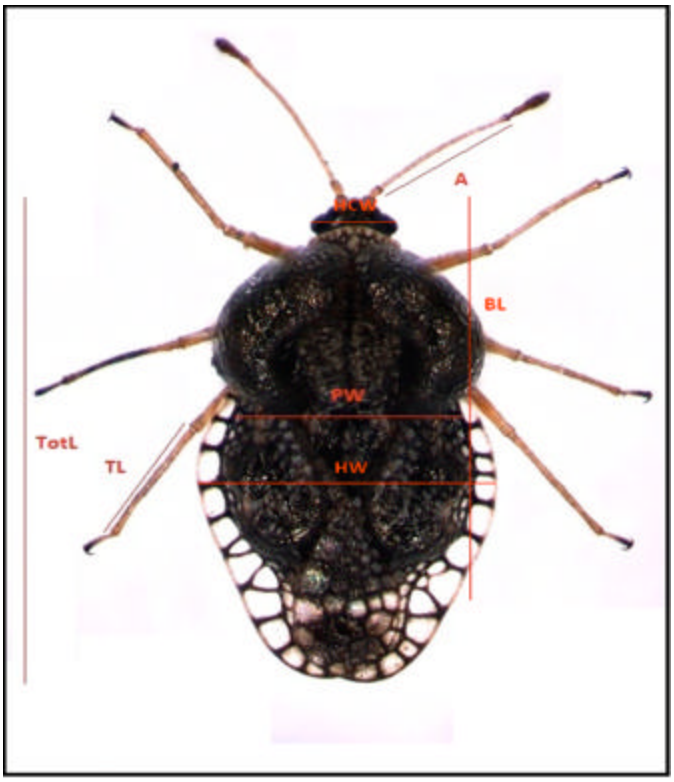

Fig. 1: Four parameters measured on *C. bullita*. TotL, total length, HCW: Head capsule width, HW: Hemelytral width, PW: Pronotal width, A: Length of third antennal segment, TL: Tibial length

Table 1: Morphological characteristics of *C. bullita* stål

Stage	Body length (mm)	Width (mm)	Colour	Description
Egg	0.520±0.002	0.119±0.002	Dark-brown in colour. (chorion is darkly pigmented)	The eggs are oblong and slightly tapered towards the opercula end. Surface of eggs is smooth. The eggs belong to short operculate type
1st instar	0.570±0.010a	0.234±0.004	Pale-brown in colour in the early period and turning into brown colour eventually	Oval shaped body. Without paranotum and wing-buds. Body is relatively smooth with no spine present
2nd instar	0.771±0.013b	0.318±0.004	Dark-brown in the early period and turn into black in colour eventually	Body more rounded in shape compare to 1st instar. Still without paranotum and wing-buds. Body margin bears spines that are especially prominent around the abdomen.
3rd instar	1.033±0.020c	0.409±0.005	Black in colour	Body more rounded in shape compare to that of in 2nd instar. Paranotum developing but wing-buds still absent. Cephalic spines start developed
4th instar	1.416±0.028d	0.536±0.009	Similar with 3rd instar	Paranotum bigger and wider on the thorax compare to that of in 3rd instar. Wing-buds start developed. Cephalic spines become longer
5th instar	1.923±0.026e	0.960±0.016	Similar with 3rd instar	Body shape similar to that the 4th instar except more elongated. Paranotum clearly noticeable. Wing-buds and cephalic spines developed longer and more noticeable
Adult female	2.264±0.019A	1.495±0.013A	Dark-brown coloured pronotum. Transparent wings with brown coloured lacework. Body is in dark-brown colour	A pair of delicate lacy wings with brown swollen part at the discoidal area appeared. The costal margin is relatively curved outward and very slightly concave. The wings are held flat over the body. The end of abdomen with genitalia capsule in V form
Adult	2.051±0.018B	1.288±0.016B		All features are similar to that of adult female except the male end of abdomen with the genitalia capsule in U form (claspers)

The means followed by different letters (lowercase) in the column are significantly different at p = 0.05 (One-way ANOVA). The means followed by different letters (uppercase) in the column are significantly different at p = 0.05 (t-test; SPSS)

Table 2: Morphometric traits of *Cochlochila bullita* nymphs and adults (n = 15) (Mean±SD error, mm)

	Measurements (Mean±SE, mm)						
						Adult	
Traits	1st instar	2nd instar	3rd instar	4th instar	5th instar	Female	Male
PW	0.234±0.004^{a}	0.318±0.004^{b}	0.409±0.005^{c}	0.536±0.009^{d}	0.960±0.016^{e}	N/A	N/A
HW	N/A	N/A	N/A	N/A	N/A	1.495±0.013	1.288±0.016
TL	0.104±0.003^{a}	0.162±0.003^{b}	0.260±0.006^{c}	0.372±0.004^{d}	0.599±0.015^{e}	0.825±0.007	0.742±0.009
A	0.090±0.001^{a}	0.112±0.002^{ab}	0.189±0.001^{c}	0.280±0.007^{d}	0.530±0.016^{e}	0.780±0.007	0.674±0.010
HCW	0.181±0.004^{a}	0.252±0.003^{b}	0.311±0.004^{c}	0.375±0.004 ^{d}	0.455±0.003 ^{e}	N/A	N/A

PW: Pronotal width, HCW: Head capsule width, HW: Hemelytral width, TL: Tibia length, A: Antenna. The means followed by different letters in the row are significantly different at p = 0.05 (One-way ANOVA)

Table 3: Linear regression of the morphometric relationship in *C. bullita* nymphs

Variable	Equation	Adjusted R^2	F	Growthfactor
Pronotal width	y = 0.516x-100.094	0.944	0.001	1.28-1.79
Tibia length	y = 0.360x-112.306	0.979	0.003	1.44-1.61
Antenna length	y = 0.324x-129.538	0.945	0.001	1.25-1.89
Head width	y = 0.193x+93.804	0.965	0.002	1.21-1.39

Growth factor, mean measurement of stage (i)/mean measurement of stage (i-1)

The results show that all biometric parameters except the antennal length showed a significant different between the developmental stages Table 2. Tibia length for all instars showed significant difference between the stages as body length and width (df = 4, 74, F = 671.663, p<0.0001). The antennal length of 1st and 2nd instars of *C. bullita* nymph was not significantly different.

Growth patterns of tibia and antenna lengths and body width fitted were well described in exponential curves (Fig. 2a-c), which showed that these traits were grew in a faster rate than from one stage to another; while the growth pattern for head width was almost linear with a more constant growth rate between the instars (Fig. 2d). All the biometric parameters were highly and positively correlated to the body length (x) (Table 3).

Length to width ratio: Length to width ratio on different body part has been widely used as one of the indicator for species classification. In our experiment, body length and width ratios for all five instar stages and adults were showed in Table 4. Ratio were ranged between 2.00±0.01 to 2.64±0.03 for the nymphal stage and 1.51±0.00 and 1.59±0.01 for the adult female and male, respectively. It is believed for every species of insect, this ratio is

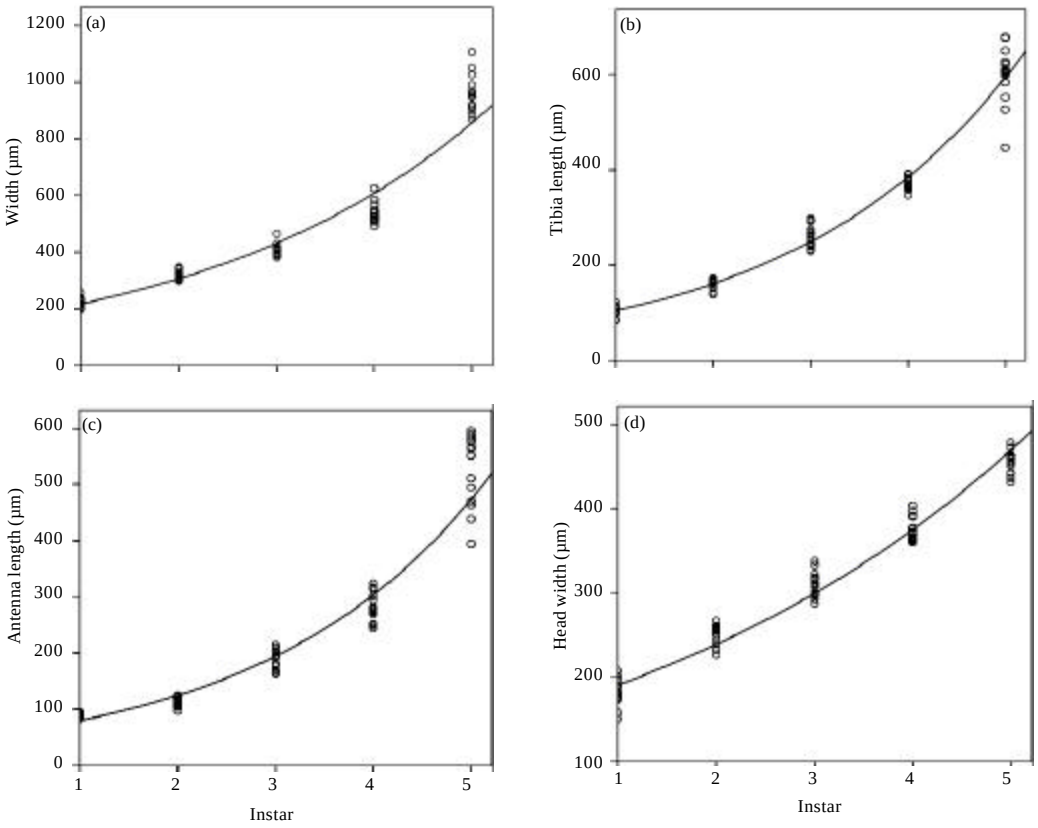

Fig. 2(a-d): Growth patterns of four biometric parameters of *C. bullita* in relation to the development stage. (a) Width, (b) Tibia, (c) Antenna and (d) Head

Table 4: Length to width ratio of *Cochlochila bullita* nymphs and adults (n = 15) (Mean±SD error, mm)

	L/wratio (Mean±SE, mm)
Nymphs	
1st instar	2.55±0.05
2nd instar	2.42±0.02
3rd instar	2.53±0.04
4th instar	2.64±0.03
5th instar	2.00±0.01
Adults	
Female	1.51±0.00
Male	1.59±0.01

L/w: Length to width

essentially the same and thus the result showed here might be useful for this tiny bug identification in the future.

DISCUSSION

Regression analyses were used in many literatures to distinguish the stages of instars. These results also describe the relationship between the stages or length of the nymphs and other biometric parameters (Linares *et al*., 2010). The results of this study corroborated with previous results done on other Hemiptera. The biometric parameters, the body width, tibia length, antenna length and also the head width of *C. bullita* were highly correlated with the developmental stages and the increase of body length of the nymphs.

The sizes of the head width, tibia length and body width of the nymphs are equally good criteria for distinguishing developmental stages of the lace bug. All these parameters are significantly different between the stages of nymphal development. However, among the growth factor, only the head width growth ratio (1.21-1.39) lied between the Dyar's ratio of 1.2-1.4. The results on the growth ratios are close to the values reported in Hemiptera, Gerridae (Klingenberg and Zimmermann, 1992) and concur with the hypothesis proposed by Cole (1980) who believed that the other parameters are higher is due to allometry, where the relative growths were affected by the energy used and in this case it was believed lace bug allocated more energy to the locomotors' structures. Thus, it is evident that the head width was the most reliable criterion for distinguishing different developmental stages of *C. bullita* nymphs.

Body length and width ratio measured for each instars and adults. This figure were believed to be a reference to distinguish this species of lace bug from e morphologically alike relative species such as *C. conchata*, *Moanthia humuli* and *Dictyla convergens*. The ratio was taken because it is believed for every species of insect, that ratio is essentially the same.

CONCLUSION

In conclusion, the general morphological descriptions, morphometrics to distinguish the instar stages and the oviposition patterns of *C. bullita* are fundamental for the recognition, identification and monitoring of this species and last but not least it is important for further studies and for control of this insect pest. As the morphometrics and scaling relationships are highly integrated with genetical, development, physiology, functional and environmental, further study on the reasons that bring up these scaling patterns will be an interesting task.

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