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Biology and morphometry of *Megaselia halterata*, an important insect pest of mushrooms

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Abstract

This paper aims to provide insights into knowledge on morphology, biology and development of *Megaselia halterata* (Wood), one of the most common insect pests of mushroom houses. We focus on such traits as body length and weight and width of pseudocephalon, and show how these traits differ in subsequent development stages as well as across time.

The development time of a generation, from egg to adult, lasted 16-19 days at 24 °C; for larval stage this time lasted 12-14 days. Mean weight of particular stages ranged from 0.003 mg for eggs up to 0.492 mg for pupae, while mean length from 0.35 mm for eggs to 2.73 mm for 3rd instar larvae. During larval development, mean body weight increased about 48 times and mean body length three times. Measurements of pseudocephalon of larvae showed that between the successive instars it increased approximately 1.4 times. Using the statistical technique inverse prediction, we develop formulae for estimation of larvae development time based on mean body weight and length of larvae found in a sample taken from a mushroom house, on which basis one can decide whether the infection occurred in the mushroom house or during compost production.

Key words: inverse prediction, mushroom, phorid fly, width of pseudocephalon.

Introduction

The family Phoridae (Diptera) comprises more than 200 genera, of which the most numerous is the genus *Megaselia* Rondani, comprising about 45% of all phorid species. Larvae of these flies develop in miscellaneous environments, including decaying organic material and plant tissues; many phorid fly species are parasitoids or parasites of invertebrates and vertebrates, including human (Disney, 1994; 2008).

A large group of phorid flies feed on fungus sporophores, but few species feed on cultivated mushrooms. Throughout the world, *Megaselia halterata* (Wood) (Diptera Phoridae) is a significant pest in mushroom production (e.g., Binns, 1978; Hussey and Hughes, 1964; Czajkowska, 1984; Scheepmaker *et al*., 1997; Jess and Bingham, 2004b; Erler and Polat, 2008; Erler *et al*., 2009a; 2009b). Larvae of this species are obligatory mycetobionts with inconspicuous head and mouthparts adapted to feeding on mycelium (Hussey, 1961a; White, 1985; Rinker and Snetsinger, 1984). This species feeds exclusively on three fungus species, all of which belong to the family *Agaricaceae* (Disney, 1994). By comparison, *Megaselia nigra* (Meigen), another important pest from the family Phoridae, has been found on more than two dozen fungus species belonging to five families (Disney, 1994).

In mushroom houses, *M. halterata* larvae cause serious damage by the consumption of mycelium. In addition, adult forms may be responsible for transmitting spores of mushroom diseases of fungal origin, such as *Verticillum fungicola* (Pruess) Hassebrauk (White, 1981), and mite species that are harmful to mushrooms (Clift and Larson, 1987). These losses in mushroom production caused by *M. halterata* are significant and require control measures. Chemical pesticides are currently the main control method for this pest, although they are not always successful (Keil, 1986; Brewer and Keil, 1989), thus alternative methods are needed. Previous studies have demonstrated that control of phorids can be achieved by applying biopesticides containing the toxin of *Bacillus thuringiensis* Berliner var *israelensis* (Goldberg) (Keil, 1991; Erler *et al*., 2009a), entomopathogenic nematodes (Sheepmaker *et al*., 1997; 1998a; 1998b; Long *et al*., 2000; Jess and Bingham, 2004a; Erler *et al*., 2009a), and predacious mites (Szlendak and Lewandowski, 2000; Jess and Bingham, 2004a). In a recent study by Erler *et al*. (2009b), various plant extracts applied to casing layer gave good results in controlling *M. halterata* populations during mushroom cultivation.

Studying the effectiveness of these organisms/materials in controlling phorid flies requires investigations in strict laboratory conditions and sometimes the knowledge of distinguishing larval instars. Hitherto, however, no efficient criterion for distinguishing larval instars is known; doing that based on development time is imprecise. In addition, having observed *M. halterata* larvae, one may wish to determine their day of development; this may help one, for example, decide whether the infection occurred in mushroom house or in mushroom manufacture, for example during incubation period in which temperature of mushroom substrate is similar to used in our experiments. We do not know any effective method that would be helpful in this.

Therefore, the aim of this paper was to study the morphological parameters of subsequent developmental stages of *M. halterata*, such as body weight, length and width (for all stages) as well width of cephalic segment (for larval instars).

Materials and methods

Collecting the material and mass rearing

Adult *M. halterata* were collected with aspirators from commercial mushroom houses located near Warsaw, Poland. They were then put in test-glasses (of dimension 10 × 2.7 cm) and transported to a laboratory in a portable refrigerator.

Phorid flies (approximately 100 individuals) were placed in glass isolators covered on the top with fine gauze (about 0.1 mm mesh width). The isolators were then placed on Petri dishes (10.0 cm diameter). A dish (5 cm diameter) filled with fresh compost inoculated by mycelium of *Agaricus bisporus* (Lange) Imbach was put inside the isolator. The Petri dishes with isolators were put into climate chambers (24 ± 0.3 °C, in darkness) for cultivation. Flies were allowed to lay eggs for 24 h. Subsequently, the isolators with phorids were removed, and the dishes were covered with parafilm to maintain humidity in the dishes at a level similar to that of mushroom-growing cellars. To keep the sufficient amount of food for larvae during their development, mushroom grain spawn was added to the dishes.

Development and morphological parameters

Six Petri dishes, prepared according to the above description, were used in the experiment. Every day, until the end of development, 30 individuals were randomly selected from each dish and body parameters and weight were measured. Eggs, larvae and pupae were extracted directly from dishes under stereomicroscope.

Each individual of a particular developmental stage was put into an aluminium cup (diameter 5 mm and height 4 mm) and weighed on the scale Sartorius Supermicromicro (± 0.0001 mg). Prior to weighing, adult flies were caught with an aspirator and euthanized with ethyl acetate. Each emerging adult individual was subjected to the measurements. Placed in separate test-glasses with 70% ethyl acetate for euthanizing and preserving, larvae were measured after the extraction from the liquid. The other stages were measured immediately after weighing.

With the stereoscopic microscope Olympus equipped with a graduated eyepiece (Olympus eyepiece micrometry 10/100 x), the following measurements were taken: length and width of eggs, length of body and width of larvae pseudocephalon, length of body and width of the widest abdomen segment of pupae as well as adult individuals.

The results of the measurements were grouped according to the developmental stage. In the case of larvae, width of cephalic segment was used for this purpose; in addition, the 1st and 2nd instars were distinguished by type of tracheal system. The percentage share of each instar in the subsequent days of development was calculated. Development time (in days) for each instar was estimated based on the percentage share of individuals on the subsequent days of development.

Using mean widths of pseudocephalon of each instar, the coefficient of cephalic segment enlargement in the time of transition into the subsequent larval instar, so-called Brook's ratio, was calculated (Dyar, 1890; Craig, 1975).

Statistical analysis

Body length and weight in the subsequent developmental stages and width of cephalic segment of the subsequent larval instars were compared by means of *t* test; in case of non-equal variances of a particular trait (checked with boxplots and tested with the *F* test), the Welch test was applied.

In order to estimate the prediction functions of day of development based on mean body length and weight, we applied the methodology called the inverse prediction (Sokal and Rohlf, 1995). This involved fitting a linear (or linearized, as was the case in our analyses) model of a dependent variable (mean body weight or length in our case) against a cause variable (day of development in our case), and then transforming the model into an inverse one. With such an inverse model, the cause variables value can be predicted (or rather estimated) based on the known value of a dependent variable. In this study, using the models developed, maturity of larvae found in samples from a mushroom house can be estimated based on their mean body weight and length. Prediction limits can also be determined (Sokal and Rohlf, 1995). Note that since mean body weight and length were determined on different larvae in particular days, we assumed that the measurements of these variables in different days were independent.

Results

Development cycle of *M. halterata*

The estimated time for development of a generation, from egg to adult, was 16-19 days (table 1; figure 1). The shortest development time was observed for the first larval instar. The overall time from hatching to pupation ranged from 12 to 14 days. Pupal development required 5-8 days, representing almost 50% of the developmental time. Adult emergence was observed after 16 days from hatching and persisted for 8 days for both females and males.

Table 1. Development duration of *M. halterata* stages.

Stage	Time of development [d]
Egg	3 - 5
1st instar	2 - 4
2nd instar	2 - 6
3rd instar	4 - 7
Pupa	5 - 8
Duration of larvae development	8 - 11
Duration of generation development (egg to adult)	16 - 19
Emergence of first adult flies	
Female	17
Male	17
Duration of emergence	
Female	8
Male	8

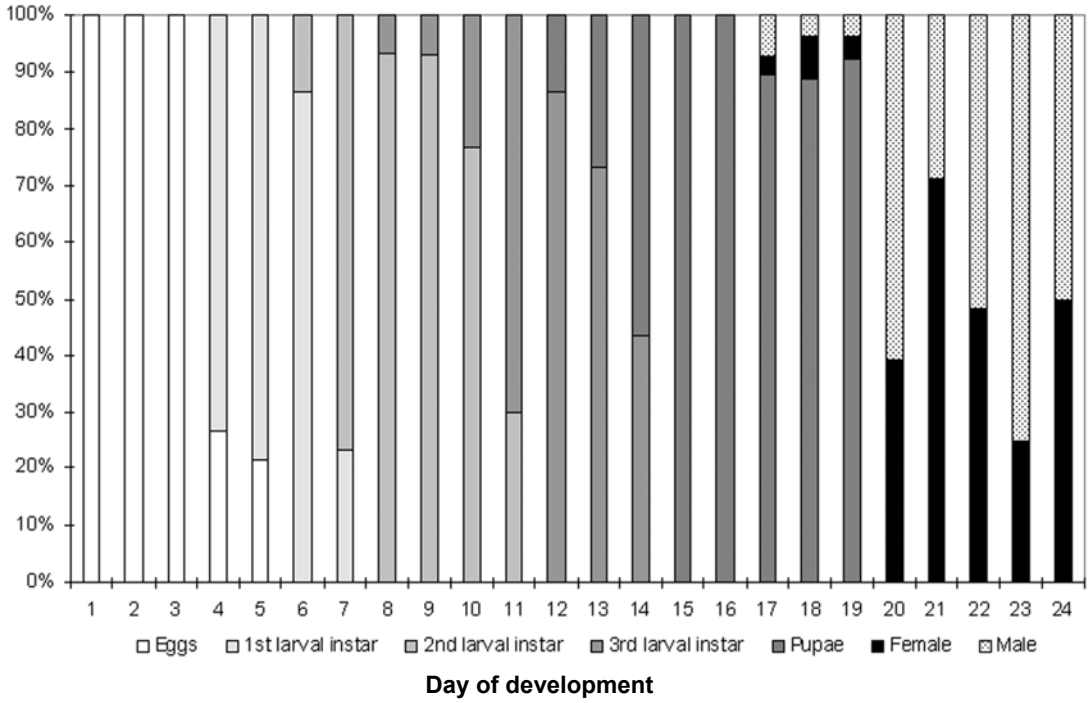

Figure 1. Percentage shares of individuals of particular developmental stages of *M. halterata* in the successive days of development.

During the first three days of *M. halterata* development, only eggs were observed, while in fourth and fifth days, the number of eggs represented 26.7 and 21.4% of the total population, respectively. From fourth to seventh day, first instar individuals were observed. In sixth day, in addition to first instar (86.7%), also second instar (13.3%) larvae were observed. Third instar larvae occurred in eighth day, when the *Petri* dishes contained mainly second instar larvae (93.3%). The latter were observed until eleventh day, although in this day they were not abundant (30%). Predominance of third instar larvae (86.0%) occurred in twelfth day, which was also the day of the first occurrence of pupae. The conclusion of larval development occurred after the fourteenth day, when they represented 43.3% of total life stages, following which, only pupae and adult flies were observed.

Body weight and length of *M. halterata* developmental stages

Subsequent developmental stages of *M. halterata* were quite diverse in terms of body weight (table 2). Although 1st instar larvae had visibly greater mean weight than eggs, the minimal weight of 1st instar larvae was less than that estimated for eggs. During larval development, mean body weight increased about 48 times. Body weight of pupae was the greatest among the de-

Table 2. Body weight (mg) for developmental stages of *M. halterata*.

Stage	*n*	Range	Mean ± SE	SD	SED[1] (df)[2]	*P*-value[3]
Egg	37	0.0019 - 0.0050	0.0033 ± 0.0001	0.0007	-	-
1st instar	64	0.0014 - 0.0292	0.0073 ± 0.0008	0.0061	0.0008 (65.8)	< 0.0001
2nd instar	113	0.0143 - 0.6970	0.1187 ± 0.0101	0.1078	0.0102 (113.3)	< 0.0001
3rd instar	93	0.0595 - 0.7931	0.3523 ± 0.0176	0.1695	0.0203 (149.8)	< 0.0001
Pupa	114	0.2890 - 0.7738	0.4924 ± 0.0100	0.1065	0.0202 (148.3)	< 0.0001
Adult	92	0.1097 - 0.6371	0.2781 ± 0.0105	0.1006	0.0146 (204)	< 0.0001
Body weight of female and male adults						
Female	41	0.1843 - 0.6371	0.3330 ± 0.0162	0.1039	-	-
Male	51	0.1097 - 0.4094	0.2339 ± 0.0102	0.0728	0.0192 (69.2)	< 0.0001

[1] SED: standard error of the difference between the means for body weight of the corresponding stage and that preceding it.

[2] If the degrees of freedom are not integer, they were calculated by Welch approximation, which takes into account different standard deviations of body weight for the two stages compared; standard deviation difference was tested with the *F*-test for 0.05 type I error probability level.

[3] P-value for t-test testing the hypothesis on lack of difference between the mean body weight of two stages; if the variances of body weight were not equal (see point 2), the Welch test was applied.

velopmental stages studied, exceeding those of larvae and adult flies. Males were lighter than females.

From egg to final larval instar, body length increased (table 3); at the final day of larval development, the mean body length of larvae was more than three times greater than that recorded following egg hatch. The pupa however, was visibly shorter than the third instar larva, but quite similar to that of adult flies. Adult females were somewhat longer than males.

Daily increases in weight and length of larvae

A gradual increase in body weight and length was observed (figure 2), the only exception being the 12th day, in which both of them slightly decreased. Mean body weight and length across time were strongly related, the relation being slightly non-linear; a Spearman correlation coefficient was equal 1, showing the perfect monotonic relationship.

The best-fit regression model for body length as affected by day of development was as in the equation (1) with the determination coefficient 97.9%. For body length the best fit model was as in equation (2) with the determination coefficient 95.9%.

Through the inverse prediction, these models can be used for prediction of day of development based on a known value of mean body length or weight of larvae observed in a particular time point. This can be done based on the equation (3) and (4), recalculated from equation (1) and (2).

The 100 (1 – α)% confidence limits [*CL*; Sokal and Rohlf (1995) use the symbol *L*, but to avoid confusion with the symbol of a larval instar, we decided to use *CL* instead] of an estimate of the day of development from either equation (3) or (4), say $\hat{X}_i$, can be determined by the equations (5) and (6), where L_1 and L_2 are lower and upper confidence limits, respectively; $b_{Y \cdot X}$ is the corresponding regression coefficient from equation (1) (0.07222, for body weight) or equation (2) (1.5318, for body length); Y_i is the square root of observed body weight, or body length; $\bar{Y} = 0.3627432$ for body weight and $\bar{Y} = 1.932858$ for body length; D is given by the equations (7) and (8) ($t_{\alpha,9}$ being the value from the t distribution with 9 degrees of freedom and for α level of significance); and H is calculated as in equation (9) and (10).

Table 4 contains days of development estimated by these two models for the data from our experiment along with the 95% confidence limits for the estimates.

Equation 1-10.

$$\sqrt{\text{mean body weight}} = -0.2872 + 0.07222 \cdot \text{day of development} \quad \textbf{(1)}$$

$$\text{mean body length} = -2.586 + 1.5318 \cdot \sqrt{\text{day of development}} \quad \textbf{(2)}$$

$$\text{estimate of day of development} = \frac{\sqrt{\text{mean body weight}} + 0.2872}{0.07222} \quad \textbf{(3)}$$

$$\text{estimate of day of development} = \left(\frac{\text{mean body length} + 0.2586}{1.5318} \right)^2 \quad \textbf{(4)}$$

$$CL_1 = 9 + \frac{b_{Y \cdot X}(Y_i - \bar{Y})}{D} - H \text{ , } CL_2 = 9 + \frac{b_{Y \cdot X}(Y_i - \bar{Y})}{D} + H \text{ (for mean weight)} \quad \textbf{(5)}$$

$$CL_1 = \left[9 + \frac{b_{Y \cdot X}(Y_i - \bar{Y})}{D} - H \right]^2 \text{, } CL_2 = \left[9 + \frac{b_{Y \cdot X}(Y_i - \bar{Y})}{D} + H \right]^2 \text{ (for mean length)} \quad \textbf{(6)}$$

$$D = 0.005215168 - t_{\alpha,9} 0.00001254908 \text{ (for body weight)} \quad \textbf{(7)}$$

$$D = 2.346475 - t_{\alpha,9} 0.01119450 \text{ (for body length)} \quad \textbf{(8)}$$

$$H = \frac{t_{\alpha,9}}{D} \sqrt{0.001380399 \left(\frac{12}{11} D + \frac{(Y_i - 0.3627432)^2}{1001} \right)} \text{ (for body weight)} \quad \textbf{(9)}$$

$$H = \frac{t_{\alpha,9}}{D} \sqrt{0.03663737 \left(\frac{12}{11} D + \frac{(Y_i - 1.932858)^2}{1001} \right)} \text{ (for body length)} \quad \textbf{(10)}$$

Table 3. Body length (mm) for developmental stages of *M. halterata*.

Stage	*n*	Range	Mean ± SE	SD	SED[1] (df)[2]	*P*-value[3]
Egg	37	0.257-0.414	0.351 ± 0.0046	0.028	-	-
1st instar	72	0.324-1.242	0.820 ± 0.0275	0.220	0.0279 (66.5)	< 0.0001
2nd instar	113	0.914-3.760	1.962 ± 0.0577	0.614	0.0640 (154.2)	< 0.0001
3rd instar	93	1.450-3.800	2.730 ± 0.0533	0.514	0.0786 (203.9)	< 0.0001
Pupa	114	1.370-2.650	1.990 ± 0.0151	0.162	0.0554 (106.9)	< 0.0001
Adult	92	1.440-2.400	1.877 ± 0.0213	0.204	0.0261 (171.4)	< 0.0001
Body weight of female and male adults						
Female	41	1.500-2.400	2.000 ± 0.0290	0.186	-	-
Male	51	1.440-2.125	1.780 ± 0.0224	0.160	0.0360 (90)	< 0.0001

[1] SED: standard error of the difference between the means for body length of the corresponding stage and that preceding it.

[2] If the degrees of freedom are not integer, they were calculated by Welch approximation, which takes into account different standard deviations of body length for the two stages compared; standard deviation difference was tested with the *F*-test for 0.05 type I error probability level.

[3] *P*-value for *t*-test testing the hypothesis on lack of difference between the mean body length of two stages; if the variances of body weight were not equal (see point 2), the Welch test was applied.

Table 4. Actual and estimated days of development based on the models for body weight and length. CL_1 and CL_2 stand for lower and upper confidence limits, respectively.

Day	Estimation based on larva body weight			Estimation based on larva body length		
	Estimate	95% CL_1	95% CL_2	Estimate	95% CL_1	95% CL_2
4	4.7	3.5	6.0	4.4	3.3	5.8
5	4.8	3.6	6.0	4.5	3.3	5.8
6	5.5	4.3	6.7	5.5	4.2	7.0
7	6.6	5.4	7.8	6.4	5.0	8.0
8	8.0	6.8	9.2	8.3	6.7	10
9	8.9	7.7	10.1	9.8	8.0	11.7
10	10.6	9.4	11.8	11.2	9.3	13.3
11	11.5	10.3	12.7	11.7	9.7	13.8
12	11.2	10.0	12.5	11.3	9.4	13.4
13	12.8	11.6	14.0	12.1	10.1	14.2
14	14.4	13.2	15.6	13.9	11.8	16.2

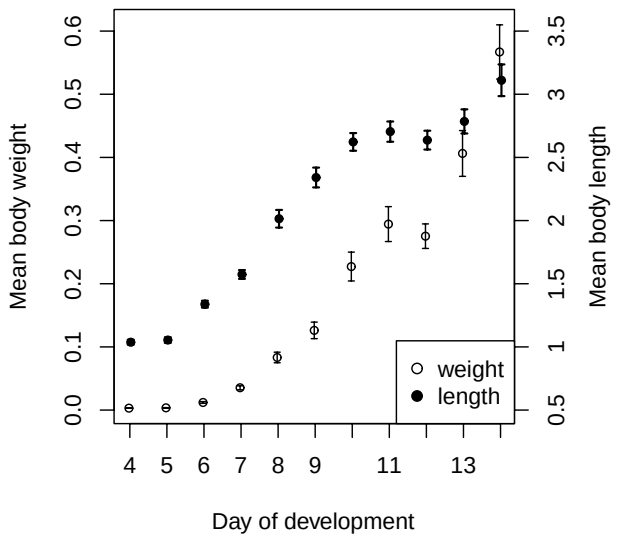

Figure 2. Increases in body weight and length of *M. halterata* larvae. Error bars represent the standard error of mean.

Width of pseudocephalon of *M. halterata* larval instars

Three larval instars were characterized by different mean width of pseudocephalon (table 5). Brook's ratio, describing the transition of cephalic segment width between the larval instars, was equal 1.58 and 1.30. The frequency polygon of cephalic segment width (figure 3) shows that this trait may be used to distinguish the three instars. As stated in material and methods, 1st and 2nd instars were additionally distinguished by arrangement of spiracles on the larval body, which method enables the perfect distinction between the two stages. However, because this does not work for distinguishing between 2nd and 3rd instars, these instars were distinguished only based on the width of cephalic segment of the instar; based on figure 3 we decided that the border between the stages be 0.0875.

Discussion

Development time of *M. halterata* generation from laying eggs up to emergence of adult flies lasted 16-19 days in the laboratory conditions with 24 °C. In Hussey

Table 5. Width of pseudocephalon for three larval instars of *M. halterata*.

Stage	*n*	Range	Mean ± SE	SD	*SED*[1] *(df)*[2]	P-*value*[3]	*Brook's ratio*
L1	72	0.0400-0.0575	0.0488 ± 0.0006	0.0050	–	–	–
L2	113	0.0600-0.0880	0.0771 ± 0.0007	0.0072	0.0009 (181.6)	< 0.0001	1.58
L3	93	0.0900-0.1350	0.0999 ± 0.0010	0.0093	0.0012 (171.0)	< 0.0001	1.30

[1] SED: standard error of the difference between the means for body length of the corresponding stage and that preceding it.

[2] If the degrees of freedom are not integer, they were calculated by Welch approximation, which takes into account different standard deviations of body length for the two stages compared; standard deviation difference was tested with the *F*-test for 0.05 type I error probability level.

[3] *P*-value for t-test testing the hypothesis on lack of difference between the mean body length of two stages; if the variances of body weight were not equal (see point 2), the Welch test was applied.

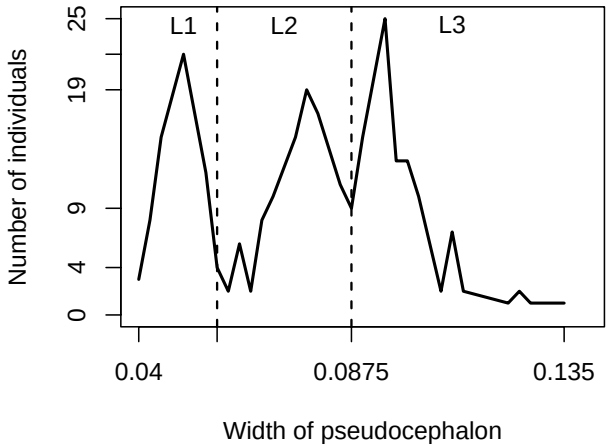

Figure 3. Frequency polygon of width of cephalic segment of larval instars of *M. halterata*.

(1961b), van Zaayen (1983), White (1983) (all cited after Disney, 1994) and Hussey *et al*. (1969) experiments, in the some temperature this time was shorter and ranged from 13 to 15 days. However, even longer development time was noted for the laboratory culture of *M. halterata* reared on commercial mycelium of *A. bisporus* in 25 °C (Smith *et al*., 2006); in that case, emergence of the filial generation of adult flies began after 21 days. These differences, especially in the maximum number of days of generation development, among the present studies and those in Hussey (1961b), Hussey *et al*. (1969), van Zaayen (1983) and White (1983), could be attributed to longer development of larval stages.

The egg development time (3-5 days) was longer than that given by other authors (Hussey, 1961b). The pupa development was also slightly longer. In Hussey (1961b) experiment it was 7.5 days while in ours 8-11 days (table 3). Emergence of adult flies, both male and female, started in the 17th day of development and lasted for 8 days.

Larvae of *M. halterata*, the species belonging to higher Brachycera, have an acephalic head, most of which is retracted into the thorax. Consequently, instead of head capsule width, Hussey (1961a) used the width of cephaloskeleton to determine larval instars, the measurements of which he made from microscope slide preparations. Due to long time required for preparation of the slides, this method seems of little importance in case of necessity of quick determination of a larval stage. It is much simpler to make the measurements of pseudocephalon based on water slides. Head skeleton is invisible then, but its width seems equivalent to width of pseudocephalon, which is shown by the comparison of our results with those by Hussey (1961a); see table 6.

Slight differences in width of pseudocephalon we observed and width of cephalic skeleton obtained by Hussey (1961a) may come from different accuracy of the methods of measurements. Quite high values of the Crosby growth rule ratio were determined in both experiments: 21.5 in ours and 21.7 in Hussey (table 6). Such high values of the ratio, greater than 10%, might suggest that one larval instar was omitted (Craig, 1975). However, De Moor (1982) showed that in the case of larvae with bright head and thus indistinct body edge, such a difference in Brook's ratio between the subsequent instars might come from measurement errors.

The measured widths of cephalic segment of larval instars of *M. halterata* let us draw a frequency polygon for this trait (figure 3); such a polygon may help one to determine the subsequent fly larval instars (e.g., De Moor, 1982). Precision of this procedure was checked by the arrangement of spiracles on the larval body for first two instars, which showed that in the case of these two stages the frequency polygon slightly underestimated the boundary width of cephalic segment. Note that such a polygon is used to determine a number of larval instars (e.g., De Moor, 1982), but according to Dyar's rule (Dyar, 1890) the determination of intervals of size of sclerotized structures enables one to determine the instars with good precision (note that Dyar's rule is equivalent to Brook's rule; see Crosby, 1973).

Another criterion for determination of larval instars might be body weight. In our study, *M. halterata* mean body weight ranged form 0.0073 to 0.3523 mg, and during the development time it increased over 48 times. A much higher increase was observed between 1st and 2nd instars (16 times) than between 2nd and 3rd ones (3 times). Unfortunately, despite the significant differences in mean body weight, this trait seems little helpful in discrimination between the larval instars owing to a noticeable overlap of body weights of adjacent instars. The same conclusion applies to body length as a criterion for determination of larval instars.

Table 6. Comparison of width of pseudocephalon and cephaloskeleton of *M. halterata* larvae.

	Width of pseudocephalon (present investigations)		Width of cephaloskeleton (Hussey, 1961a)	
	Width [μm]	Brook's ratio	Width [μm]	Brook's ratio
L1	50		50	
L2	72	1.58	84	1.68
L3	93	1.30	116	1.38
Crosby's growth role ratio		21.5%		21.7%

One of our main goals was to determine formulae for estimation of day of development of larvae based on their mean body weight and length. The models we have estimated fit very well to our data, and it seems that these two traits can be efficiently used to estimate the developmental time of larvae. Nonetheless, without additional studies we cannot say anything about the quality of the formulae for different experimental conditions. Hence at this stage we think that these formulae should work well for the conditions similar to those we had in other study, although even this should be double checked.

Body weight of particular developmental stages has seldom been reported for flies. Such reports are available for *Lycoriella ingenua* (Dufour) from the Sciaridae family (Berg, 2000; Lewandowski *et al*.; 2004). Range of weight of all the stages of this fly was from 0.0015 mg (for eggs) to 2.13 mg (for the last larval instar) (Lewandowski *et al*., 2004). Interestingly, mean weight of eggs as well as of first three larval instars was similar for *M. halterata* and *L. ingenua*, but body weight at the end of the larval development was five times smaller for *M. halterata* than for *L. ingenua*. Note, however, that the latter has four larval instars while the former three. That mean egg weight was similar for both species may be surprising because females of *M. halterata* are almost two times lighter than females of *L. ingenua*. Perhaps it is for exactly this reason that the fertility of these species differ: phorid females lay about 50 eggs (Hussey, 1959) while sciarid females even more than 150 (Hussey and Gurney, 1968).

Besides larvae, the morphological measurements were made also for other developmental stages. Some of them slightly differed from those reported in literature. Length and width of eggs we observed were slightly smaller than those given by Hussey (1961a). Length of adult flies slightly differed from that given by Fletcher *et al*. (1986), in whose studies it was about 2-3 mm; the authors, however, did not give any other detailed data on the measurements (like body length mean and variation), hence it is difficult to compare our results with theirs. It is possible that all these differences, at least to some extent, can be attributed to the geographical diversity of the corresponding phorid fly populations.

Conclusions

Studies on morphology and biology of insects can provide much valuable information, which can be used in further, more detailed investigations. Unfortunately, such information about *M. halterata* is sparse even though it is one of the most important mushroom pests, frequently occurring in mushroom houses. We believe that our paper adds some important facts to the current knowledge on this species.

The results presented in this paper enable one to determine efficient criteria of determination of larval instars of *M. halterata*, and provide insights into morphology and biology of all developmental stages of this species. We have shown that larval instars can be quite precisely identified based on width of cephalic segment as well as the development time of particular stages, the latter in the case of laboratory conditions. We have also proposed the inverse-prediction formulae for estimation of the development day of larvae based on mean body weight and length of larvae found in a mushroom house. These formulae should be checked for various growing conditions to test the formulae's stability across them; if they are unstable and strongly depend on the conditions, such models should be applied only for the conditions they were fit for.

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