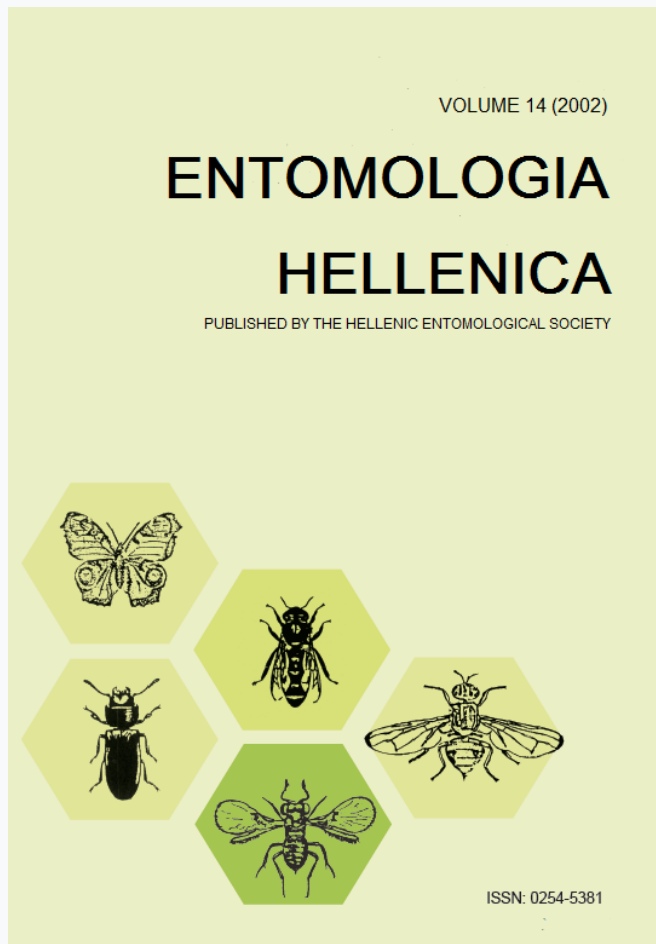


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## Description of the Egg and Nymphal Instars of *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae)

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### ABSTRACT

The egg and the nymphal instars of the predator *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) are described and illustrated. The changes of the egg as observed from outside the plant tissue during incubation and the eclosion process are also described. A key for the separation of the nymphal instars is given based on the relative length of the third and fourth antennal segments and the relative length of wing pads. Changes in the colour of the adult, from its emergence until it has obtained its final colour are also described.

### Introduction

Some species of *Macrolophus* Fieber (Hemiptera: Miridae) are effective biological control agents for pests of outdoor and protected crops such as tomato, pepper-plant and eggplant. *Macrolophus pygmaeus* Rambur was found to be the main biological control agent against aphids in fresh market and processing tomato fields in Boeotia, central Greece (Perdikis and Lykouressis, 1996; Lykouressis et al., 1999-2000). This species is characterized by its polyphagy (Perdikis and Lykouressis, 2000) and its ability to complete development on plant sap in the absence of prey (Perdikis and Lykouressis, 1999). A closely related species, *M. melanotoma* (Costa) (= *M. caliginosus* Wagner) (Carapezza, 1995), has also been effective in controlling whiteflies and aphids on tomato (Malausà et al., 1987; Arzone et al., 1990; Sampson and King, 1996; Tavella et al., 1997).

According to Josifov (1992) these two species are distinguished from each other by the colour of the first antennal segment being uniformly black

in adult *M. pygmaeus* and black but with a white central patch in *M. caliginosus*. The morphology of the adult of *M. pygmaeus* has been given by Wagner (1952), Stichel (1962) and Josifov (1992) but not that of its preimaginal stages. Preimaginal stages have been studied in a number of mirids. Cobben (1968) described the egg and the embryogenesis of *Dicyphus pallidicornis* Fieber (Hemiptera: Miridae) and *Macrolophus nubillus* (Herrich-Schaeffer) whereas Cmoluchowa (1982) gave information on the egg morphology, left paramere and nymphal instars of *M. rubi* Woodroffe (a synonym to *M. costalis* Fieber according to Josifov (1992)).

In several studies, the separation of nymphal instars in Miridae is based on external features, the most important of which are the relative length of wing pads, the length of antennal segments and the existence of coloured spots or patches on appendages and body. Arrand and McMahon (1974) separated the first from the second instar of *Plagiognathus medicagus* Arrand by the absence of black spots on femora, the relative length of the rostrum and the width of the head across the eyes; the third instar on the presence of wing pads just developed, and finally, the fourth and fifth instars, on the relative length of wing pads and the width of the head across the eyes. In

*Opisthotaenis striata* (Wagner), the nymphs of third, fourth and fifth instar can be separated using, in addition to other characteristics, the relative development of wing pads (Matocq, 1987).

Other distinguishing characteristics are the colour of some body parts, the relative length of thoracic segments, the relative length of antennal segments and the morphology of the area where openings of dorsal scent glands are situated and the wing pads length (Wheeler et al., 1975; El-Dessouki et al., 1976). Dolling (1991) gives a key for separation of Hemipteran nymphs in instars using the length of wing pads for separation between the fourth and fifth and the length of mesonotum and metanotum for separation between the first and second instar nymphs. However, he mentioned that the separation of the first two instars is not easy.

In this study, the morphology of egg and nymphal instars of *M. pygmaeus* are described and illustrated. Also, changes in egg shape and volume in relation to plant surface during incubation period, the eclosion process as well as changes in the colour of some parts of the adult body, are described.

## Materials and Methods

A culture of *M. pygmaeus* originating from adults collected in September 1994 from tomato plants in the area of Boeotia, central Greece, was maintained continuously on eggplant (cv. Bonica) infested with the aphid *Myzus persicae* (Sulzer). The culture was kept in woodframed cages 80 × 80 × 70 cm. These cages were kept in a glasshouse with an average temperature of 22.5±2.5°C and natural lighting. To obtain eggs for morphometric studies, eggplant stems, were brought to the laboratory, carefully dissected under a binocular stereomicroscope, and the eggs gently taken and put on a slide, using minute pins. Observations on changes occurred on egg top visible above plant surface during incubation and eclosion, were made on twenty eggs of less than 24 hours old. Those eggs were kept in the stem where they had been laid under constant conditions at 25°C, 65±5% RH and 16L:8D photoperiod, in a growth cabinet. The eggs were inspected every 24 hours until nymph eclosion. However, during the last hour before eclosion, stems with eggs were transferred in the laboratory and observations were made under a stereomicroscope at a room temperature of approximately 24°C.

To obtain newly emerged nymphs, caged potted eggplants from the glasshouse were placed in

a growth cabinet at 25°C, 65±5% RH and 16L:8D photoperiod. Those young plants, having only 2-3 leaves, had only eggs of *M. pygmaeus*. They were inspected every 24 hours to collect the newly emerged nymphs. Each such nymph was put in a plastic petri dish of 9 cm diameter and 1 cm height, bearing on the cover a hole 3 cm in diameter covered with fine muslin to allow ventilation. The bottom of each petri dish was covered with a water-soaked layer of cotton on which an eggplant leaf or part of it infested with *M. persicae* was put. Nymphs of each instar were collected after each respective moult and at various intervals towards the end of each stadium.

Twenty nymphs of each instar were put in a glass vial filled with lactic acid (80% w/w) and placed in a drying oven at 50°C for 3-4 days for clearing. Subsequently, the nymphs were mounted on slides in Hoyer's fluid and thereafter the slides kept in the drying oven at 50°C for 5 days. Measurements were made on the length and width of the parts seen in the Table 1. The measurements were made using a light microscope micrometer scale.

In order to study the colour changes in adults, nymphs of the fifth instar from the culture were placed individually in petri dishes following the same procedure as described above. Then, they were kept in a growth cabinet at 25°C, 65±5% RH and 16L:8D photoperiod. The emerged adults, were kept in the same petri dishes and growth cabinet.

## Results and Discussion

### Description of nymphal instars

There are five nymphal instars.

*First instar* (Fig. 1a): Slender, elongate, shining, almost white soon after emergence, but soon becoming yellow except the head, antennae and legs which are pale yellow. The head bears red eyes. The rostrum is pale yellow and directed backwards, with its tip overreaching the third coxae. The abdomen is much longer than wide with clearly distinguished segments. Openings of dorsal scent glands are found at 4-6th abdominal segments and are situated in a yellow-brown area on the abdomen. Average body length 1124 µ and width at the broadest point of abdomen 364 µ (Table 1).

*Second instar* (Fig. 1b): Elongate and shining. Head, thorax and abdomen pale yellow. Antennae pale yellow except for the two first segments which are almost uniformly yellow. Eyes dark red. Rostrum pale yellow reaching third coxae.

TABLE 1. Dimensions in  $\mu$ . and ratios of various body parts of nymphal instars of *Macrolophus pygmaeus* when fed *Myzus persicae* growing on eggplant leaves in petri dishes at 25°C.

Body part		Instar I	Instar II	Instar III	Instar IV	Instar V
Body length	Range	1036-1209	1353-1526	1641-1785	2227-2361	2620-2793
	Mean $\pm$ S.D.	1124 $\pm$ 53	1427 $\pm$ 56	1700 $\pm$ 46	2253 $\pm$ 41	2715 $\pm$ 43
Body width	Range	288-403	407-547	604-720	691-777	842-1036
	Mean $\pm$ S.D.	364 $\pm$ 39	473 $\pm$ 34	666 $\pm$ 31	733 $\pm$ 25	946 $\pm$ 44
Head width across eyes	Range	220-252	279-303	339-371	394-422	417-477
	Mean $\pm$ S.D.	235 $\pm$ 10	290 $\pm$ 6	349 $\pm$ 11	409 $\pm$ 10	443 $\pm$ 16
Length of hind tibiae	Range	390-423	489-633	633-864	1008-1209	1296-1584
	Mean $\pm$ S.D.	404 $\pm$ 9	567 $\pm$ 43	738 $\pm$ 54	1031 $\pm$ 61	1470 $\pm$ 83
Length of antennal segments						
Segment 1	Range	16-23	18-22	23-27	36-41	41-55
	Mean $\pm$ S.D.	18 $\pm$ 1.7	22 $\pm$ 2.2	25 $\pm$ 2.3	37 $\pm$ 1.6	49 $\pm$ 3.7
Segment 2	Range	23-27	32-37	46-55	68-78	110-124
	Mean $\pm$ S.D.	24 $\pm$ 1.4	35 $\pm$ 2	51 $\pm$ 2.7	73 $\pm$ 3.9	113 $\pm$ 4.7
Segment 3	Range	18-27	32-41	46-54	68-82	96-114
	Mean $\pm$ S.D.	24 $\pm$ 2.5	37 $\pm$ 1.8	50 $\pm$ 2.5	76 $\pm$ 3.9	111 $\pm$ 5.8
Segment 4	Range	34-41	41-46	46-55	60-68	68-78
	Mean $\pm$ S.D.	37 $\pm$ 3.6	44 $\pm$ 2	51 $\pm$ 2.8	66 $\pm$ 3.1	75 $\pm$ 3.4
Ratio of length of antennal segments						
I/II	Range	0.7-0.83	0.5-0.71	0.41-0.54	0.47-0.6	0.38-0.48
	Mean	0.78	0.62	0.50	0.51	0.43
II/III	Range	0.83-1.25	0.77-1	0.83-1.09	0.83-1.1	0.96-1.13
	Mean	0.98	0.95	0.99	0.97	1.03
III/IV	Range	0.55-0.75	0.80-0.94	0.95-1.1	1-1.3	1.23-1.66
	Mean	0.64	0.85	1.01	1.15	1.48

Area of dorsal scent glands light brown. Average body length 1427  $\mu$  and maximal width of abdomen 473  $\mu$ .

*Third instar* (Fig. 1c): Elongate and shining. Head pale yellow, thorax green and abdomen pale green. Eyes dark red. Antennae pale yellow except for the two first segments which are almost uniformly yellow. Rostrum pale yellow, its tip reaches third coxae. Legs pale yellow, almost colourless. Wing pads start to be seen as short rounded projections of meso- and metathoracic segments. Dorsal scent glands are surrounded by a dusty yellow area on the abdomen. Average body length 1700  $\mu$  and maximal width of abdomen 666  $\mu$ .

*Fourth instar* (Fig. 1d): Pyriform with the abdomen relatively broader than in earlier stages. Head colourless in the middle and pale green at the sides. Thorax uniformly green, and the abdomen pale green with shiny yellow reflections. First and second antennal segments yellow at their posterior ends, the rest of antennae pale yellow. Rostrum pale green, reaching the second coxae. First and second femora pale green, third femora slightly darker. Wing pads reaching almost the middle of second abdominal segment. Dorsal scent glands in a light green area in the middle of abdomen. Average body length 2253  $\mu$  and width of the widest point of abdomen 733  $\mu$ .

*Fifth instar* (Fig. 1e): Pyriform. The colour of the head opalescent at the middle, pale green at the sides and yellow at the front between the base of antennae. Thorax and abdomen green or dark green. The eyes dark red, very well developed. First antennal segment yellow, the remaining segments pale yellow. Rostrum pale green, reaching second coxae. Fore and middle femora pale green, hind femora darker. Tibiae pale green. The wing pads very well developed reaching the fourth abdominal segment. They are green with yellow reflections at their sides and at their posterior margins. Dorsal scent glands in a light green area on the abdomen. Average body length 2715  $\mu$ . and width of the broadest point of abdomen 946  $\mu$ .

The dimensions of the body and appendages of each nymphal instar of *M. pygmaeus* and ratios are shown in Table 1. Although, practically it is not easy to distinguish the nymphal instars using the width of the head or the length of the hind tibiae, those characters do not overlap except for the width of the head between fourth and fifth instars.

The ratio of the length of the third to the fourth antennal segment can be used for separation between first and second nymphal instars as it is statistically different ( $F = 145.92$ , d.f. = 1,40,  $P < 0.001$ ) and there is no overlapping (Table 1). Also, the body length and shape can also be taken into

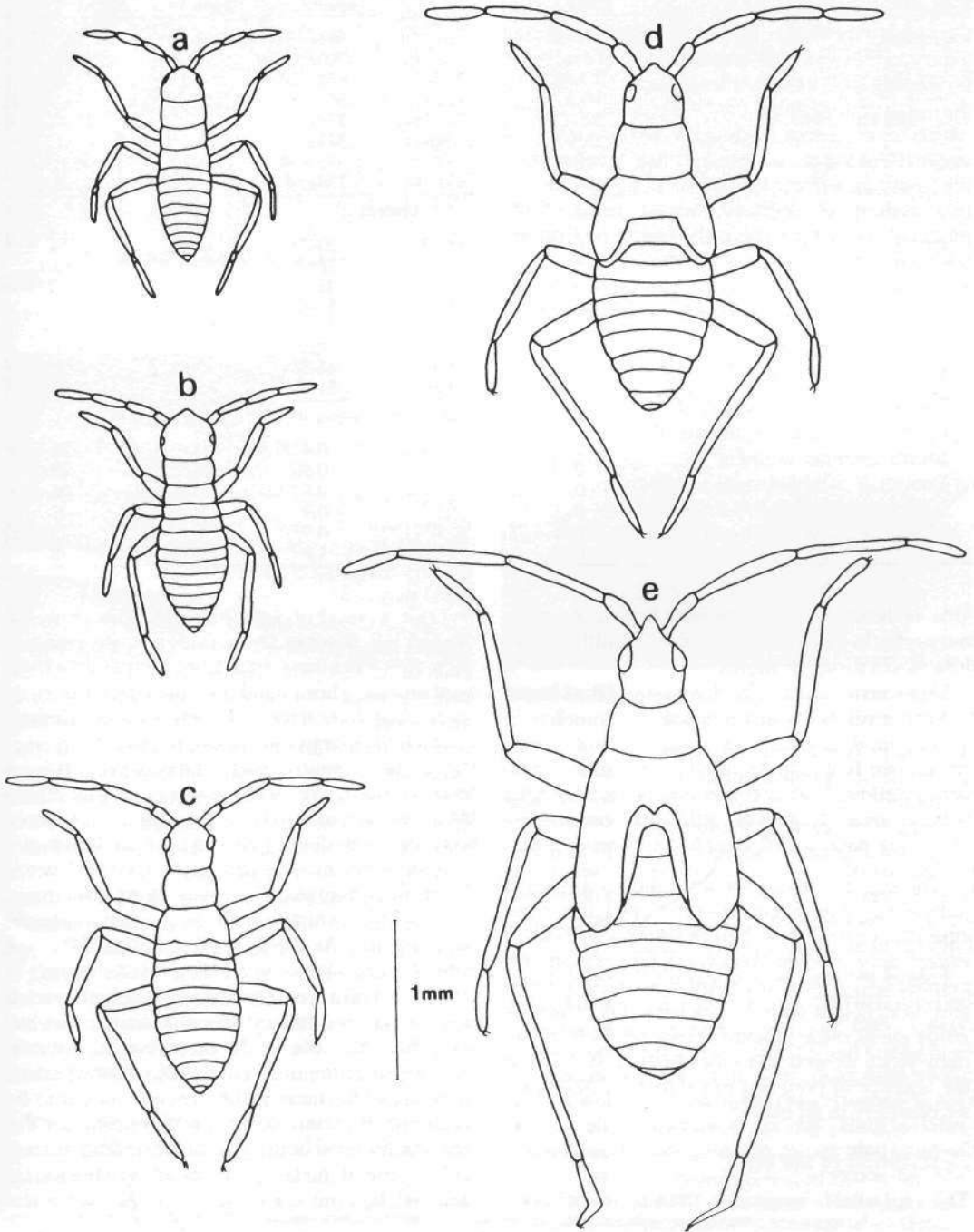


FIG. 1. Nymph of the first (a), second (b), third (c), fourth (d) and fifth (e) instar of *Macrolophus pygmaeus*.

consideration for easier separation of those two nymphal instars whereas the width of the head across eyes and the length of the hind tibiae could also be used for their separation as they do not overlap (Table 1). The identification of the third, fourth and fifth nymphal instars can be based on the relative length of the wing pads, a feature which is very easy to follow. In the third nymphal instar the wing pads are very little developed, in the fourth they reach almost the middle of the second abdominal segment, whilst in the fifth nymphal instar they reach the fourth or fifth abdominal segment.

#### Key for separation of the nymphal instars

- |                                                                                                                                                                                               |               |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|
| 1. Wing pads absent. . . . .                                                                                                                                                                  | 2             |
| Wing pads present . . . . .                                                                                                                                                                   | 3             |
| 2. Third antennal segment 0.64<br>(0.55-0.75) times the length of<br>fourth antennal segment* . . . . .                                                                                       | First instar  |
| Length of third antennal segment<br>0.85 times (0.80-0.94) the length<br>of fourth antennal segment* . . . . .                                                                                | Second instar |
| 3. Wing pads look like short, curved<br>projections on the back, lateral<br>sides of meso- and metathoracic<br>notum. Third antennal segment<br>almost as long as fourth<br>segment . . . . . | Third instar  |
| Wing pads easily distinguished . . . . .                                                                                                                                                      | 4             |
| 4. Wing pads reaching almost the<br>middle of second abdominal<br>notum . . . . .                                                                                                             | Fourth instar |
| Wing pads reaching the fourth<br>or fifth abdominal notum . . . . .                                                                                                                           | Fifth instar  |

The key was tested for separation to instars of individuals of *M. pygmaeus* developed on tomato, eggplant, cucumber and green beans with *Trialeurodes vaporariorum* Westwood, on eggplant, cucumber and melon with *Aphis gossypii* Glover, and on eggplant with *T. vaporariorum*, *Macrosiphum euphorbiae* (Thomas) and *Tetranychus urticae* Koch as well as in the above host plants in the absence of prey and found that it can be used successfully in all cases.

#### Description of the egg

The egg of *M. pygmaeus* ( $844 \mu \times 285 \mu$ ) is whitish opalescent, elongate and slightly curved

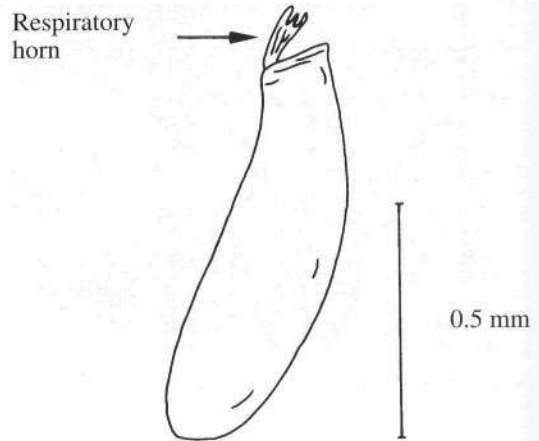


FIG. 2. Egg outline of *Macrolophus pygmaeus*.

(Figs. 2, 3). A neck like ring is formed at its top, while it is rounded at its posterior end (Figs. 2, 3). At the operculum of the egg protrudes a shining, slender, almost transparent respiratory horn which forms about 45° angle with operculum (Fig. 4). This horn consists of a basal part of which 2 equally long finger like projections protrude. The basal part is 3 times as long as the branches (Fig. 2). Quite often, a shorter branch appears in the middle between these two branches. Each branch ends in an aeropyle. The surface of the egg, except the neck, is reticulate consisting mainly of hexagonal cells. The walls of these cells are relatively wide and has many small spots. Such spots occur also within the cells, but at a lower density than on the walls of the hexagonal cells. These spots, if seen under high magnification (400X), look like cells with polygonal shapes in which a small spot is centrally situated.

Eggs of other mirid species have a restricted neck, a rim of different width and an operculum which is usually above the plant tissue (Cobben, 1968). As described by Cobben (1968), the egg of *Campyloneura virgula* (Herrich-Schaeffer) has two long respiratory horns bearing several branches. The egg of *D. pallidicornis* does not bear respiratory horns. On its whole surface there is hexagonal print of follicular cells similar to the print found in this study for *M. pygmaeus*. The respiratory horn of the egg in *M. nubillus* is similar in general shape to that in *M. pygmaeus*, but has only two branches. The egg of *M. rubi* is also of similar shape to that of *M. pygmaeus*, but it

\* See Table 1 for ratios of other antennal segments.



FIG. 3. Egg of *Macrolophus pygmaeus* within the plant tissue.



FIG. 4. Egg of *Macrolophus pygmaeus* as seen above plant tissue soon (one day) after oviposition.



FIG. 5. Egg of *Macrolophus pygmaeus* as seen above plant tissue about seven days after oviposition at 25°C.



FIG. 6. Emerging nymph of *Macrolophus pygmaeus*.

bears two respiratory horns, unequal in length (Cmoluchowa, 1982); the longer one is almost identical in shape to the respiratory horn of *M. pygmaeus*. Constant et al. (1994) illustrated the shape of the egg of *M. caliginosus* which resembles that of *M. pygmaeus*. The egg of *Psallus ambiguus* (Fallén) is not provided with respiratory horns (Niemczyk, 1967). The eggs of four species of the genus *Nabis* (Hemiptera: Nabidae) have a more or less similar shape to that of *M. pygmaeus*, and have a sculpture of hexagonal cells, however, they do not bear respiratory horns (Chiappini and Reguzzi, 1998).

#### Sites of oviposition

*M. pygmaeus* prefers to oviposit in a relatively hard substrate like the stem of the plant but it also may oviposit at a lower rate in the petiole or in the main vein of the leaf. Tissue hardness has been demonstrated by Constant et al. (1996) to play an important role in the selection of oviposition sites of *M. caliginosus*. The female of *M. pygmaeus*

lays its eggs individually in the plant tissue. The long axis of the laid egg is almost parallel to the longitudinal axis of the stem and at the site it is inserted no swelling of plant tissue is noticeable.

#### Eclosion

In the newly laid-eggs only the operculum and the respiratory horn can be seen above the plant tissue. During incubation the yolk plug is gradually protruded above the plant surface. This projection takes the shape of a filled sac and has a white-opalescent colour. On the 4th, 7th and 10th day of incubation at 25°C this projection reaches approximately 20, 40 and 65% (Fig. 5) of the height of the horn, respectively. On the 11th day, when eclosion takes place, it reaches 75%. Approximately, one hour before eclosion this projection consists of two parts of different colour; the upper part (serosal cuticle) is whitish whilst the lower one (embryonic cuticle) is yellow. Gradually the colour of the whole projection becomes yellow because of the upward movement of the embry-

onic cuticle.

Twenty three to twenty five minutes before eclosion, due to the upward movement of the nymph, at the bottom of the projection of the egg, and just under the plant surface, the eyes of the emerging nymph are visible as narrow red vertical lines. Twenty minutes before eclosion the eyes reach the horn height. Then, insertion of air in the form of elongate bubbles in the body starts. These bubbles move downwards to the ventral part of the nymph's body. After a minute the eyes obtain their final shape. In the following five minutes there is no air insertion, while the body of the emerged nymph is about twice as high as the horn. In the following five minutes air-bubbles, which resemble small balls, are deposited one above the other, under the level of the eyes. Nine minutes before eclosion, the height of the emerging nymph is 4 times higher than that of the horn.

A minute later, the segments of antennae and rostrum can be distinguished, but only the first and the second segments of them are free of the body. At this point the last abdominal segments of the emerging nymph are visible above the plant tissue (Fig. 6). Air-bubbles still move downwards but this time, they move through the dorsal side of the body. Five minutes before eclosion, antennae and rostrum are completely free at the front of the body and two minutes before eclosion the legs also become free. During the last minute of this process almost the entire nymphal body has emerged. In an effort to free itself the nymph bends and stretches its body repeatedly backwards-forwards. Finally, its legs touch the plant and, immediately, it starts walking. At the site of the eggshell the remains of serosal and embryonic cuticles exceed the horn. The knowledge of the rate of development of that projection of the egg above the plant tissue during the incubation period is very helpful in estimating the time of eclosion and also in knowing whether incubation proceeds normally.

The incubation and eclosion procedure described above is similar to that in other species of Miridae. During the late stages of the incubation period of the eggs of *M. pygmaeus* the yolk plug protrudes above the plant surface as in the case of *Notostira erratica* L. (Johnson, 1934). Also, Constant et al. (1994) pointed out that the length and the width of the egg of *M. caliginosus* increased during the incubation period.

As in *M. pygmaeus*, air bubbles can be seen through a slit of the embryonic cuticle to pass beneath the labrum and to accumulate in the thorax

of the nymph of *N. erratica* (Johnson, 1934). The eclosion process of *M. pygmaeus* resembles that of *P. ambiguus* as described by Niemczyk (1967). In *M. pygmaeus* the serosal and embryonic cuticles protrude during the incubation period above the plant surface at a similar height to that observed in the case of *P. ambiguus*. The movements of the nymph of *M. pygmaeus* in its effort to move up and finally to become free, also follow the same succession as in *P. ambiguus*. In *M. pygmaeus* the serosal and embryonic cuticles are pulled up beyond the egg surface much higher than in the case of *D. pallidicornis* (Cobben, 1968). When the head of *D. pallidicornis* nymph goes through the constricted neck of the egg the eyes look like lines obtaining their final shape later. After eclosion, the serosal and embryonic cuticles form a kind of sleeve above the empty egg shell as in the case of *M. pygmaeus*.

#### *Development of colour in the adult*

The final colour of the adult *M. pygmaeus* is pale green to dark green except for the first antennal segment. The longitudinal stripe behind the eye and the spot at the apex of the clavus on the hemielytra near the membrane, are black and the posterior area of the membrane is also blackish. These coloured body parts do not obtain their final colour immediately after adult emergence. Soon after emergence the colour of the adult is wholly whitish and the eyes bright red. Four hours after emergence at 25°C, the first antennal segment and the stripe behind the eye are very faint black but they still look yellow. The spot on the dorsum is also faint black and the membrane is greyish, mainly at its posterior area. Seven hours after emergence, the first antennal segment is darker than earlier and almost black at its base, the posterior area of the membrane and the stripe behind the eye are still faint black, but well distinguished and darker than earlier. The eyes look dark red and the spot on the dorsum has already become black. Nine hours after emergence, the first antennal segment becomes darker but it is not yet wholly black and the stripe behind the eye becomes blackish. Gradually, the colour of these parts becomes darker and darker and, finally, about 30 hours after emergence the adult has obtained its final colour.

The knowledge of the rate of colour development in adult of *M. pygmaeus* is essential in studies on the systematics of *Macrolophus* species, because the colour of the first antennal segment and the stripe behind the eye are used as distinc-



tive characters for species separation (Josifov, 1992).

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KEY WORDS: Egg, Hemiptera, *Macrolophus pygmaeus*, Miridae, morphology, nymphal stages, predator

## Περιγραφή του Ωού και των Νυμφικών Σταδίων του *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae)

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### ΠΕΡΙΛΗΨΗ

Περιγράφεται η εξωτερική μορφολογία του ωού και των νυμφικών σταδίων του αρπακτικού *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae). Περιγράφονται, επίσης, οι αλλαγές που συμβαίνουν στο τμήμα του ωού όπως διακρίνεται έξω και πάνω από τη φυτική επιφάνεια, κατά τη διάρκεια της επώασης του καθώς και η διαδικασία εκκόλλαιψης. Παρατίθεται κλείδα για το διαχωρισμό των νυμφικών σταδίων του αρπακτικού. Το πρώτο και το δεύτερο νυμφικό στάδιο διακρίνονται μεταξύ τους χρησιμοποιώντας τη σχέση μήκους μεταξύ του 3ου και 4ου άρθρου της κεραίας. Τα υπόλοιπα νυμφικά στάδια διακρίνονται μεταξύ τους από το μήκος των περοθηκών. Τέλος, περιγράφονται οι αλλαγές του χρώματος του ενήλικου από την εμφάνισή του έως την απόκτηση του τελικού χρωματισμού του. Η δυνατότητα αξιοποίησης αυτών των αποτελεσμάτων σε μελέτες βιολογίας και συστηματικής του εντόμου αυτού επισημαίνονται στο κείμενο.