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Biology of *Anagrus incarnatosimilis* and *Anagrus breviphragma* (Hymenoptera: Mymaridae)

Abstract - *Anagrus incarnatosimilis* Soyka and *A. breviphragma* Soyka were obtained from overwintering eggs of *Cicadella viridis* (L.) (Homoptera: Cicadellidae). When reared on *Dicranotropis hamata* (Boheman) or *Muellerianella fairmairei* (Perris) (Homoptera: Delphacidae) both *Anagrus* species are solitary parasitoids; both species are facultatively gregarious parasitoids in *C. viridis* eggs, yielding up to eight progeny per host.
One insemination sufficed to produce fertilized eggs throughout the life of the female, although occasionally a second copulation was observed in *A. breviphragma*. Inseminated *A. breviphragma* females produced 45.2 $\pm$ 22.7 progeny (n = 24); virgin females, only 33.3 $\pm$ 21.6 males (n = 24). Mated and virgin *A. breviphragma* females laid 64 and 62% of their egg complement, respectively, between 6-12 h after their emergence.
Eclosion began 3 days after oviposition and lasted approximately one day at 20 °C. Both species had 2 larval instars. First-instar larvae were immobile and absorbed their food. Second instar larvae were very active appearing 5 days after oviposition. This instar doubled in body size. No difference was detected between mandible sizes of early second instar and the mature larva.
The prepupal and pupal stages lasted 1 and 6-7 days respectively in both species. Changes in coloration permited estimation of pupal age. Adult emergence began after 21 days, but most adults emerged 23-24 days after oviposition.

Riassunto - *Biologia di* Anagrus incarnatosimilis *e* Anagrus breviphragma *(Hymenoptera: Mymaridae)*
Anagrus incarnatosimilis Soyka e *A. breviphragma* Soyka sono stati ottenuti da uova svernanti di *Cicadella viridis* (L.) (Homoptera: Cicadellidae). Quando allevati da *Dicranotropis hamata* (Boheman) o *Muellerianella fairmairei* (Perris) (Homoptera: Delphacidae) entrambe le specie di *Anagrus* sono parassitoidi solitari, mentre sono gregarie facoltative in uova di *C. viridis,* potendosi sviluppare fino a otto individui per ospite.
Un'inseminazione è sufficiente per produrre uova fertilizzate per tutta la vita della femmina, anche se, occasionalmente, un secondo accoppiamento è stato osservato in *A. breviphragma.*
Femmine accoppiate di *A. breviphragma* producono 45,2 $\pm$ 22,7 figli (n = 24);

femmine vergini, solo 33,3 ± 21,6 maschi (n = 24). Femmine accoppiate e vergini di *A. breviphragma* depongono rispettivamente il 64 e 62% del totale delle uova tra le 6 e le 12 ore dopo l'emergenza.
La schiusura inizia 3 giorni dopo l'ovideposizione e dura approssimativamente un giorno a 20 °C. Entrambe le specie hanno due età larvali. Le larve di I età sono immobili e assorbono il cibo attraverso il tegumento. Le larve di II età sono molto attive e compaiono 5 giorni dopo l'ovideposizione. La larva di questo stadio raddoppia la propria taglia durante l'accrescimento, ma nessuna differenza è stata rilevata nella lunghezza delle mandibole della giovane larva di II età rispetto a quelle della larva matura.
Gli stadi di prepupa e pupa durano rispettivamente 1 e 6-7 giorni in entrambe le specie. Le variazioni della colorazione permettono di stabilirne l'età. L'emergenza degli adulti inzia 21 giorni dopo l'ovideposizione, ma la maggior parte degli adulti sfarfalla 23-24 giorni dopo.

Key words: Mymaridae, reproductive potential, oviposition, development, egg parasitoids.

INTRODUCTION

Mymarids are extremely small but their impact on the population dynamics of leafhoppers has fostered major studies (Witsack, 1973; Otake, 1968; 1969). There was a problem regarding the reliability of biological and ethological researches on *Anagrus*, due to the confusing state of its taxonomy untill Chiappini (1989) and Chiappini et al. (in press) reviewed the European and Holarctic species, respectively. Witsack, for example, utilizes the Debauche classification but, from the informations he gives in the text, at least part of what he calls *Anagrus incarnatus* could represent *Anagrus obscurus,* as it came from *Anakelisia fasciata* (Kirschbaum) eggs that correspond to eggs inserted singly along *Carex riparia* Curtis leaf nervures (Chiappini, 1989).

Walker (1979) described *Anagrus mutans* and *A. silwoodensis* and presented biological information on both species. In her review of *Anagrus* European species, Chiappini (1989) synonymyzed *A. mutans* Walker under *incarnatosimilis* Soyka and *A. silwoodensis* Walker under *breviphragma* Soyka. The effects of the hosts on the parasitoids and their oviposition behavior have been described (Moratorio, 1987; 1990). Here, their biology and larval morphology are described and discussed.

MATERIALS AND METHODS

PROVENANCE OF BIOLOGICAL MATERIAL. Stems of *Juncus effusus L.* (Juncaceae) bearing overwintering eggs of the cicadellid *Cicadella viridis* (L.) and the delpha-

cid *Muellerianella fairmairei* (Perris) at Imperial College at Silwood Park, (Ascot, Berkshire, England), and leaves of *Carex riparia* (Cyperaceae), cut at their base and bearing overwintering eggs of *C. viridis* in uncultivated areas along the Po river in Piacenza province Italy, were collected periodically during the winter months, from September to February.

Bundles of about 100 washed stems or leaves were wrapped with wet paper toweling, placed in a closed plastic bag, and stored in the refrigerator at 1-3°C. The toweling was changed biweekly and wetted with a 0.8 g/l solution of methyl P-hydroxybenzoate to reduce mould development.

All *C. viridis* eggs used in this study were obtained from field-collected material.

Overwintering eggs of *C. viridis* were the principal field source of *A. incarnatosimilis* and *A. breviphragma.*

Overwintering eggs of *M. fairmairei* also yielded a few *A. incarnatosimilis.*

BREEDING. Cultures of *M. fairmairei* and the delphacid *Dicranotropis hamata* (Boheman) were kept at 20 °C (± 1 °C) and 16 h photophase. Muslin-covered cages (50 × 40 × 40 cm) containing 4-5 pots of *Holcus mollis* L. (Graminaceae) were used to rear the leafhoppers. Plastic flower pots (13 cm ∅) containing oat seedlings or *Holcus* grass and covered by a plastic cylinder were used as oviposition chambers. *M. fairmairei* and *D. hamata* oviposited and bred abundantly in *H. mollis.* Oat seedlings were used as host plants for *D. hamata* because the eggs were easier to detect inside the leaf blade. A continuous supply of suitable hosts was secured by changing the seedlings every 3 days and removing the dead adults from the oviposition chambers. Host eggs remained suitable for mass culture of parasitoids for several weeks provided the seedlings were kept in a 5 °C (± 1 °C) cabinet and were continuously watered.

Newly-emerged parasitoids [1] were placed inside breeding cages with an abundance of host eggs left inside the plant tissues in which they were laid. The breeding cages used throughout the study were cotton-stoppered glass vials (9 × 3 cm) containing a 2 cm layer of 1.3% agar and a strip of moist absorbent paper. Methyl P-hydroxybenzoate (0.5 g/l) was added to the agar to prevent mould development. Parasitoid cultures were reared at 20 °C (± 1 °C) unless otherwise stated.

REPRODUCTIVE POTENTIAL. To assess the reproductive potential of *A. breviphragma,* 24 parasitized *C. viridis* eggs, each containing 3 female pupae, were kept

1 Under the stereoscopic microscope, the two specie can be distinguished, when alive, by general body appearance and different coloration: *A. breviphragma,* orange-yellow in colour is comparatively slenderer because of the projecting ovipositor, while *A. incarnatosimilis* is slightly stockier and dark brown in colour.

individually in breeding cages until all 3 females emerged. One was allowed to copulate, and another was kept virgin. The third female was dissected in distilled water using a minuten pin mounted on a wooden handle and the number of eggs present in the ovaries was recorded. Virgin and mated females were then confined individually with 20 *C. viridis* eggs and allowed to oviposit. The *C. viridis* eggs were changed daily until each female died. The females were then dissected and the number of eggs in the ovaries was recorded. The experiment started at 12:00 noon and the host eggs were changed at this time each day. The presence of female pupae showed that the mated females were inseminated.

The fecundity was calculated by adding the number of eggs oviposited by the female (estimated as the number of early second instar larvae present in the host eggs) and the number of eggs left in the ovaries after the female had died (Jackson, 1961).[2]

A paired t-test was performed on these data to asess if the differences were significant ($p < 0.05$).

MORPHOLOGY. Adult specimens were preserved in 70% ethanol or mounted permanently on glass slides either with polyvinyl-lactophenol, Canadian balsam or Faure's liquid.

To study larval morphology and development, several *A. breviphragma* females were allowed to parasitize *C. viridis* eggs for 4 h. Host eggs known to be parasitized, due to the presence of a characteristic black mark on the chorion, were kept at 20 °C (± 1 °C) in Petri dishes on filter paper moistened with physiological solution and 10 were dissected each day after oviposition until the adults emerged.

A binocular dissecting microscope provided with substage illumination and 100× magnification was used throughout the study.

Ovarian eggs, extracted from the female by squashing the abdomen, or deposited ones, obtained by dissection of the host egg, were mounted in Faure's liquid.

Some of the larvae, just after being extracted from the host, were washed in a physiological solution and mounted in Faure's or Hoyer's liquid; others were prepared following three methods modified after Barbosa (1974).

Larvae still in the host were fixed as for paraffin inclusion, then washed many times in alcohol of appropriate concentration and, only at this moment, extracted from the host and mounted with Euparal.

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[2] The fecundity of mymarids has been estimated in several ways. Bakkendorf (1925) squeezed *A. incarnatus* females under a cover slip in water and counted the ovarian eggs expelled from the ruptured metasoma. The same approach was used by Stoner & Surber (1969) to assess the number of eggs in the mymarid *Anaphes ovijentatus* (Crosby & Leonard) with females of various ages. Jackson (1961) estimated the fecundity of *Caraphractus cinctus* as the number of eggs oviposited plus the eggs left in the ovaries. Two females were dissected after oviposition, and 144 eggs were recorded in one, 150 in the other.

Larvae dehydrated by subsequent passages in more concentrated alcohols, dried to the critical point with CO_2 and metallized with gold, were studied under a Hitachi S 2300 scanning electronic microscope.

RESULTS AND DISCUSSION

HOST OVIPOSITION SITES. In *Juncus*, *C. viridis* eggs are inserted through slits into the pith at right angles to the stem surface, leaving a characteristic scar. Occasionally, eggs are deposited just beneath the epidermis and form a distinct swelling. In *Carex*, *C. viridis* oviposits again through slits (in the leaf surface) laying the eggs between the two epidermis and again forming a distinct swelling. A pale, white, waxy substance generally appears around the slit and reveals the oviposition sites. *C. viridis* oviposits in rows of about 10 eggs (range: 4-19), the longitudinal scars varying between 4-12 mm in length.

M. fairmairei lays 5-20 eggs in more compact and shorter rows than those of *C. viridis*, about 1-3 mm in length.

In *Juncus*, careful examination of oviposition scars allows the tips of the eggs to be counted while in *Carex* very often the two edges of the scar collapse closing at least part of it. In this case the eggs are visible by transmitted light.

In *Juncus*, most oviposition sites for both leafhopper species are found within 25 cm of the ground. Sites of *C. viridis* are lower and often covered by the leaf sheath, whereas *M. fairmairei* sites are most common in the upper portion of the stem.

In *Carex*, *C. viridis* ovipositions sites are more frequent in the basal-middle portion of leaves, like in *Juncus*.

ADULT EMERGENCE. Both *Anagrus* species are solitary parasitoids when reared in *D. hamata* or *M. fairmairei* host eggs, and facultatively gregarious in the larger *C. viridis* host eggs, each of which usually yields from 2 to 5 parasitoids (sometimes 1 or 6-8 parasitoids per egg). The adult parasitoid emerges by biting a circular hole in the host's eggshell and the plant material in which the host egg is laid. Parasitoid emergence holes are located at either end of a host egg when development is solitary. When gregarious, the emergence holes can be positioned everywhere and as many as four emergence holes have been found in a *C. viridis* egg in which five *A. breviphragma* had developed.

Movement within the host egg 24 h before emergence has been recorded for both *Anagrus* species, but never to the extent reported in *Anaphes nipponicus* Kuwayama (Kuwayama, 1935). *Anagrus* adults free their antennae and legs, but their bodies never change position within the host. The newly emerged adults are very active, move rapidly, drink water condensed on the surface of the stems, clean their wings using their hind legs and their antennae with the strigils on their

forelegs. The meconium is shed after emergence. The adults jump backwards when disturbed and feign death, but recover quickly. When about to fly, they raise their wings over their body and spring into the air.

The emergence times of the resultant progeny of seven *A. breviphragma* females that had oviposited in *C. viridis* eggs between 08:00 to 11:00 were recorded and are reported in fig. 1: most parasitoid emergence takes place between 8.00 and 12:00 and no emergence is recorded after 18:00. Males emerge before females.

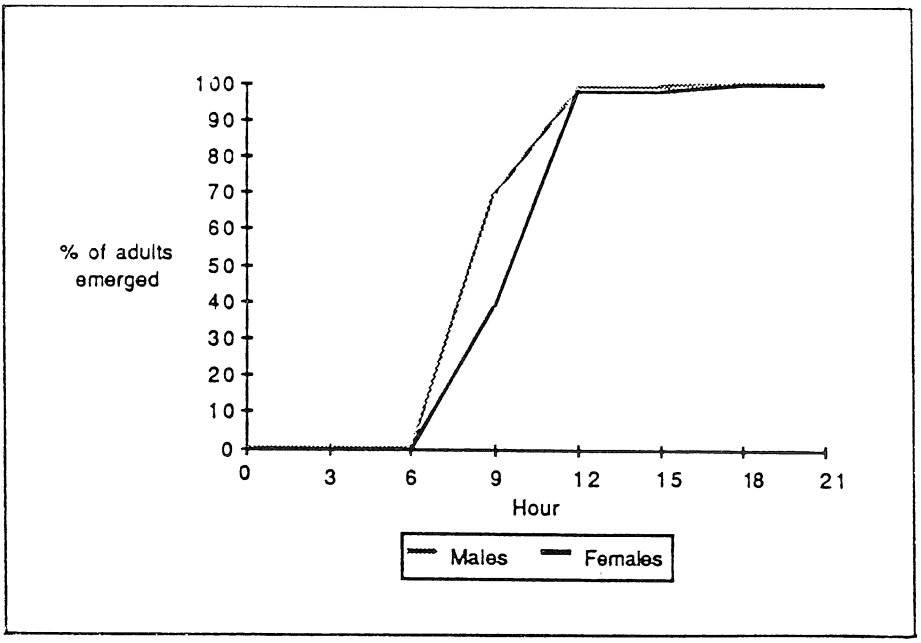


Fig. 1 - Pattern of emergence of *Anagrus breviphragma* adults from *Cicadella viridis* hosts.

COURTSHIP AND COPULATION. *A. incarnatosimilis* and *A. breviphragma* males are protandrous and emerge from *C. viridis* eggs before their female siblings. The first males to emerge remain near the egg from which they emerge, even when only males are present in the egg. Their reaction to movement within the host egg is instantaneous. Several males cluster on an egg from which a female will emerge. One male mates with the female when she emerges, and the other males immediately disband. If another male emerges from an egg, a waiting male occasionally pursues it until recognizing it as a male, whereupon the male in pursuit loses interest.

When a male recognizes a virgin female, he raises his wings perpendicular to the dorsum of his body and moves quickly toward the female. His metasoma

bends forward and the wings vibrate continuosly with rapid, short strokes. A receptive virgin female stops and lifts her hind legs and wings, and the male makes contact with his antennae. The male walks forward with the ventral surface of his metasoma facing upward and clings to the female's wings and body with his forelegs. He then slides beneath the female until his genitalia reach the female's genital opening near the base of the metasoma and copulation occurs. Both wasps remain immobile during copulation. The males of both species of *Anagrus* hold their antennae straight during copulation and antennal stroking does not occur. After an average of 63 + 26 sec (range, 24 – 129 sec, n = 175) in *A. breviphragma* and 96 + 46 sec (range, 40 – 179 sec, n = 20) in *A. incarnatosimilis*, the female walks away.

In the laboratory, several males of *A. incarnatosimilis* and *A. breviphragma* tried to copulate with a female. Once, 14 *A. incarnatosimilis* males attempted to copulate with a female in copula. The males clustered on the copulating pair and all activity ceased instantly when the female disengaged and walked away.

One insemination allows fertilization of eggs throughout the life of a female. Occasionally a second insemination was observed in *A. breviphragma* (5.7%, n = 175) but none in *A. incarnatosimilis*. The second mating in *A. breviphragma* occurred when a female failed to move away quickly from a male and he, or occasionally a different male, succeeded in mounting her again.

Reproductive potential. *A. incarnatosimilis* and *A. breviphragma* are arrhenotokous species. From virgin *A. incarnatosimilis* and *A. breviphragma* females allowed to oviposit, only males are obtained, while all mated females produce both male and female progeny.

The host species in which the female has developed and the number of parasitoids developing in the same host have a marked effect on the fecundity and the progeny produced by the parasitoid (Moratorio, 1987).

The progeny produced by mated and virgin females, the estimated fecundity (calculated by adding also the number of eggs left in the ovaries after the female had died) of mated and virgin females and that of newly emerged dissected females (calculated by counting the eggs in the ovaries after dissection) of *A. breviphragma* are reported in tab. 1.

Paired t-tests show that the differences in progeny size are significant ($p < 0.05$) whereas the estimated fecundities of mated and virgin females do not differ significantly ($p > 0.05$) between each other but do from that of newly emerged females ($p < 0.05$). At the same time the survivorship was the same.

These results lead us to suppose that the total reproductive potential is not completely expressed either in mated or virgin females, but more in the latter than in the former, and that not all the eggs that are laid develop successfully.

Chantarasa-ard et al. (1984) observed that *A. incarnatus* females provided with only water survived for 4 days and produced 37 progeny at 20 °C while in

females fed with honey, the survivorship increased to 13 days at 20 °C, producing an average of 75 parasitized hosts per female. Such an increase in survivorship and parasitization led the authors to suggest that "it appears very likely that the ovigenesis still develop during the adult's lifetime in some females". On the basis of our results we suggest a different explanation: possibly *A. incarnatus* females that lay more eggs and live longer are able to realize a greater proportion of their reproductive potential and do not produce new eggs.

Table 1 - Fecundity, fertility and survivorship of 24 Anagrus breviphragma *females.*

	Estimated fecundity	Product progeny	Survivorship (days)
mated	64.7 + 10.7a	45.2 + 22.7*	2.8 + 0.7 N.S.
virgin	66.6 + 9.4a	33.3 + 21.6	2.8 + 0.8
dissected	71.3 + 7.3b		

Means in column followed by the same letter are not significantly different at the 5% level, Duncan's new multiple range test.
t-test, significance at a = 0.05 (*)

RATE OF OVIPOSITION. In Mymaridae, the females can oviposit as soon as they emerge from their hosts, and if suitable host eggs are available, oviposition begins almost immediately.

Oviposition by several *Anagrus* spp. has been observed 1 h after emergence (Anderson & Paschke, 1969; Jackson, 1966; Mac Gill, 1934; Witsack, 1973), and it has been noted that they are most active during the first 2 days (Mossop, 1929; Raatikainen, 1967). One *A.* sp. nr. *flaveolus* Waterhouse female laid 28 eggs in 24 h but females that lived 7-8 days laid only a few more eggs and ceased reproduction 2-3 days after their emergence (Otake, 1969).

A. breviphragma and *A. incarnatosimilis* oviposit readily before mating.

Mated and virgin *A. breviphragma* females on average lived 2.8 days and laid 32.5 and 20 eggs, respectively, in the first 24 h of their life (72 and 60% of their total progeny, respectively). The daily rate of oviposition of mated and virgin *A. breviphragma* females, expressed as percentages of the total number of eggs laid by all females, and their longevities are reported in fig. 2. During the first 48 h, all mated and virgin females survived and laid 94 and 85% of their eggs, respectively. During the next 24 h, the female population was reduced by almost 50% and few eggs were laid.

DEVELOPMENT OF THE IMMATURE STAGES.

EGG. There is little variation in egg form within the Mymaridae (Clausen, 1940). The body of the egg is ellipsoidal, ovoidal or spindle-shaped. A slender,

tapering peduncule projecting from the caudal end, ranges in length from one-tenth of the egg body in *Anaphes flavipes* (Foerster) (Anderson & Paschke, 1969) to equal its length in *Polynema striaticorne* (Balduf, 1928). The ovarian eggs of several species of *Anagrus* are described by Bakkendorf (1925), Mac Gill (1934), and Witsack (1973). Ganin (1869) reported a very comprehensive study of the egg and larval morphology of a Mymaridae misidentified as *Polynema* sp. and that Henriksen (1922) and Bakkendorf (1925) believed to be an *Anagrus*. The length varies from 0.12 mm in *A. atomus* (MacGill, 1934) to 0.3-0.4 mm in *A. incarnatus* (May, 1971).

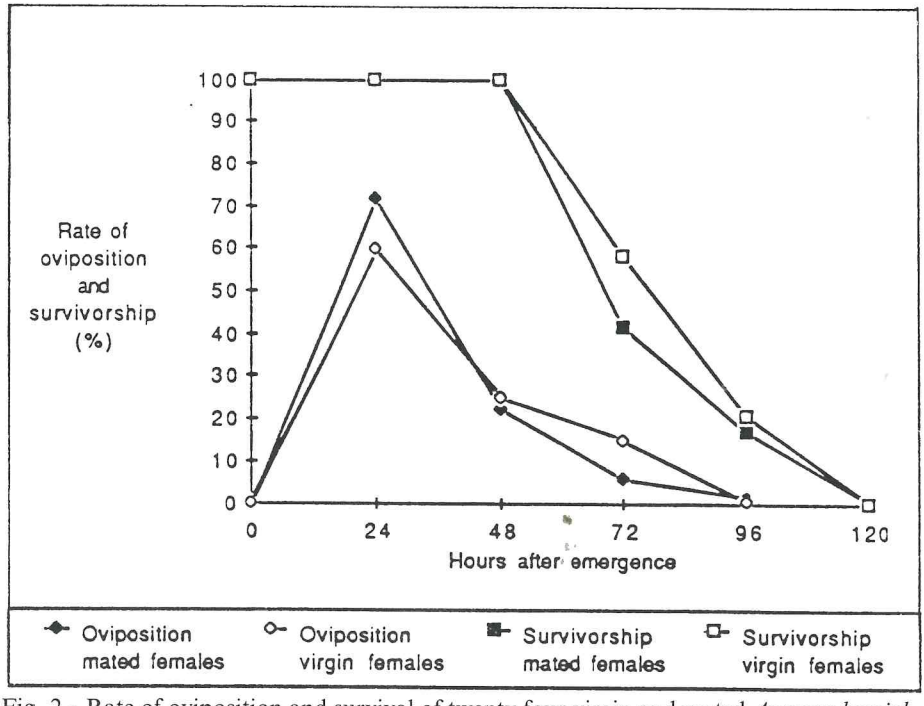
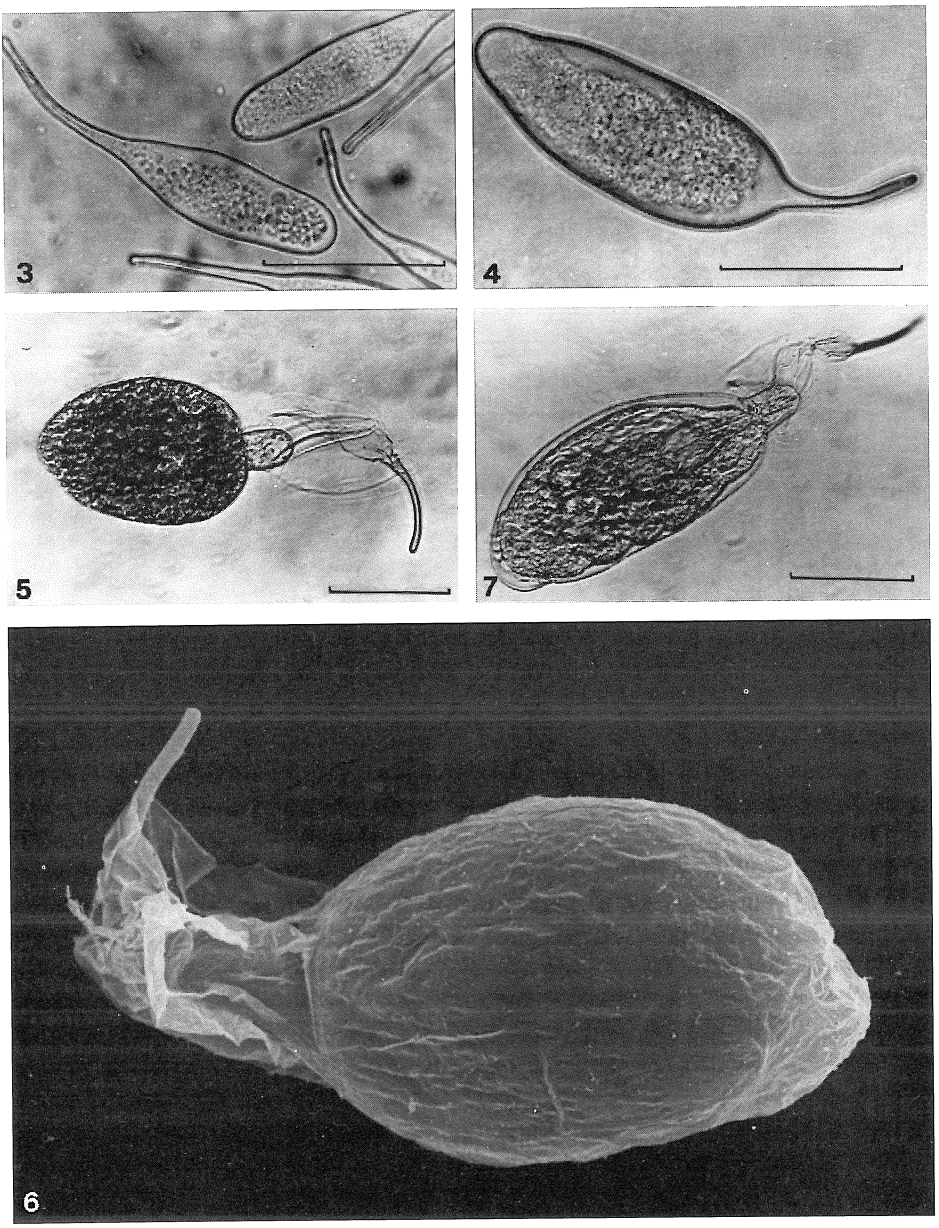


Fig. 2 - Rate of oviposition and survival of twenty four virgin and mated *Anagrus breviphragma* females.

The ovarian eggs of both *A. incarnatosimilis* and *A. breviphragma* are stalked (fig. 3) with the body elongate-ovoidal to ovoidal. There is no difference in the average length in the two species (tab. 2) but *A. incarnatosimilis* eggs are significantly wider than *A. breviphragma*. Unfortunately, the width of the ovarian eggs is subject to considerable measurement error and therefore not a reliable criterion for distinguishing these species. Ten hours after the death of the female, the ovarian eggs change in volume.



Figs. 3-7 - *Anagrus breviphragma* Soyka. Ovarian egg (fig. 3). Egg dissected from host 48 h after laying (fig. 4). Newly hatched first instar larvae (fig. 5). The same under Scanning Electron Microscope (fig. 6). First instar larvae, approximately 24 h before moulting (fig. 7).

Only one egg was found in each ovariole and it was oriented with the pedicel directed toward the posterior end of the female's body. The elastic chorion of the egg facilitates its passage through the ovipositor, and once inside the host egg, the *Anagrus* egg quickly increases in size to double its width.[3]

The oviposition can take place in host eggs of different ages and therefore the parasitoid egg can be situated floating in the egg contents or inside the almost complete host embryo. We found parasitoid eggs in the abdomen of embryos completely extracted from the egg chorion. In any case the parasitoid egg cannot be detected in the host egg unless this is dissected.

Table 2 - Size of the developmental stages of Anagrus incarnatosimilis *and* A. breviphragma *bred in* Cicadella viridis *hosts.*

	length (mm)	width (mm)
A. breviphragma		
ovarian egg	0.185 + 0.014 (20)	0.019 + 0.0051 (20)
first instar	0.33 + 0.02 (11)	0.12 + 0.01 (11)
early second	0.51 + 0.03 (6)	0.13 + 0.01 (6)
late second	0.97 + 0.05 (11)	0.24 + 0.01 (11)
male pupa	0.96 + 0.05 (4)	
female pupa	1.03 + 0.06 (4)	
A. incarnatosimilis		
ovarian egg	0.185 + 0.017 (25)	0.027 + 0.0047 (25)

Mean + SD (Number of observations)

During the second day after oviposition, the eggs are considerably swollen, but no increase in length is observed (fig. 4). Hatching starts 3 days after oviposition and most have hatched by the fourth day (tab. 3). At eclosion, the eggshell of the swollen egg splits longitudinally, starting from the anterior pole. The cast eggshell remains attached to the posterior end of the first-instar larva (fig. 5).

FIRST INSTAR LARVA. Bakkendorf (1933) first ascribed *Anagrus* to the mymarid group, with the first instar larva "bag shaped with clumsy cephalic and caudal parts", that afterwards Clausen (1940) called "sacciform".

Ganin (1869) described the larval stages of a *Polynema*, (*Anagrus, teste* Henriksen, 1922), and illustrated the development of the first instar. Henriksen (1922) observed that the first instar of *Anagrus brocheri* Schultz had the cephalic end se-

[3] Oviposition alters the shape of the egg, Satterthwait (1931) reported a reduction in size in *Anaphoidea calendra* Gahan. Anderson & Paschke (1969) reported *Anaphes flavipes* eggs narrowed and lengthened when oviposited and *Caraphractus cinctus* eggs remained distorted 10 h after oviposition (Jackson, 1969).

Table 3 - Hatching of Anagrus breviphragma *eggs at 20 °C.*

days after oviposition	*Anagrus* eggs recovered (n)	early first instar larva (n)	*C. viridis* eggs dissected (n)
1	161	0	48
2	240	0	105
3	19	51	23
4	1	20	9

parated from the body by a constriction. Dumbleton (1934) illustrated a young first-stage larva of *Anagrus armatus* Ashmead and reported that it did not present any structure and was incapable of movement.

Subsequent authors, in spite of the fact that these two previous ones had interpreted and described the first instar of *Anagrus* well, confused the first stage larvae with the eggs.

Mac Gill (1934) dissected an *Erythroneura* egg parasitized by *A. atomus* and found a specimen that she believed to be an egg of *Anagrus*. According to her description, the stalk of the egg seemed to have completely disappeared and the specimen had an oval translucent body. Although she did not publish an illustration, her description fits a first-instar larva better than an egg. Furthermore, her description of the first and the late instar larva both corresponded to that of the second instar.

Descriptions of first stage larvae (May, 1971; Whalley, 1969) and newly moulted larvae (Tay, 1972) all corresponded to early second instars. The presence of the mandibles and cuticular outgrowths in their illustrations are characteristic of the second instar. The illustrations of an *Anagrus* egg at hatching in Whalley (1969) and Witsak (1973) undoubtedly depict first instars with the eggshell attached to the basal portion.

A precise description and illustrations of first instar larvae of *Anagrus optabilis* are given by Sahad (1984).

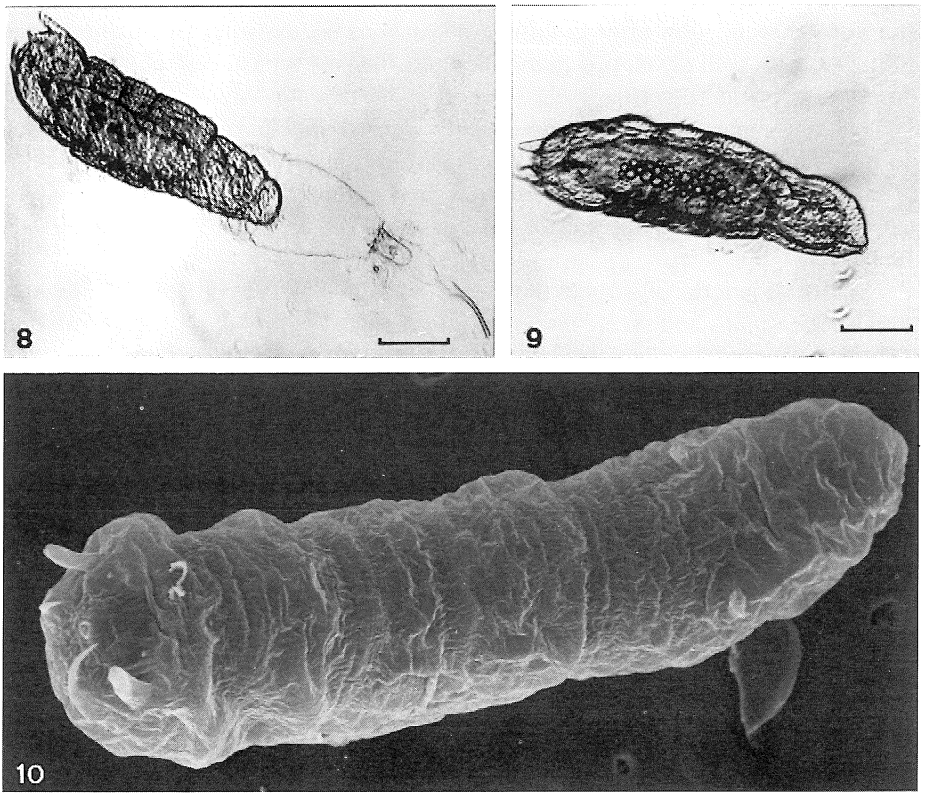
The young *A. breviphragma* first-instar larva (figg. 5-7) is oblong, with a basal constriction. The segmentation shows clearly, but the cephalic portion remains undifferentiated.

As development proceeds, the cephalic end shows a constriction which became more evident toward the end of the stage; at the same time, the future second-instar larva can be seen through the integument of the late first-instar larva: its cephalic portion is completely developed, showing mandibles and cephalic outgrowths. A few hours later, the second instar moults, leaving behind the remains of the first instar exuviae with the chorion still attached (fig. 8).

At 20 °C, first-instar larvae of *A. breviphragma* are found in dissected hosts 3-6 days after oviposition (tab. 3).

Several *A. incarnatosimilis* and *A. breviphragma* first-instar larvae were observed, but no movement was detected. Food material is taken by absorption. At the end of this instar, the stomodeum is differentiated and filled with liquid, but shows no trace of yolk spheres. Measurement of 5-day-old larvae of *A. breviphragma* are recorded in tab. 2.

The transparent and motionless first instar larva of *A. breviphragma* cannot be seen unless the host is dissected.



Figs. 8-10 - *Anagrus breviphragma* Soyka. Hatching second instar larvae, leaving behind the remains of the first instar larvae with the eggshell still attached to it (fig. 8). Second instar larvae 3 h after fig. 8 (fig. 9). The same under Scanning Electron Microscope (fig. 10).

SECOND INSTAR LARVA. The second-instar larva in *Anagrus* was first described by Ganin (1869), who coined the term "histriobdella-like" to describe these cylindrical larvae divided into six segments by constrictions.

Histriobdellid larva have been associated only with the sacciform first-instar larvae (Clausen, 1940). Histriobdellid larva are reported for various species of *Anagrus* (Mac Gill, 1934; Otake, 1968; Sahad, 1984; Whalley, 1969 and Witsack, 1973).

The head bears the mandibles and a pair of large, conical or cylindrical, fleshy processes while the last segment bears a pair of large 'ear-shaped' organs (*sensu* Ganin, 1869) of unknown function (fig. 9).

In both *Anagrus incarnatosimilis* and *breviphragma*, the very active second instar can be seen, soon after moulting, as early as 5 days after oviposition. It is colorless, with well developed mandibles and the two typical cephalic and abdominal outgrowths (fig. 10).

The mandibles are dark brown, slightly curved and sharply acuminate. They do not seem retractable like in other parasitoids and probably do not have a lot of movable capacity as also hypothesized by Dumbleton (1934). They are situated just below the cephalic end of the body, almost on the dorsal surface (figg. 10-11).

Mandible lengths of early (6 days old) and full size (10 days old) second instar *A. incarnatosimilis* and *A. breviphragma* are reported in tab. 4; a t-test showed no difference in mandible lengths of early and full size second instar larvae. However, mandibles of *A. incarnatosimilis* are twice the length of those of *A. breviphragma*, a difference that is highly significant ($t = 21.48$, D.F. $= 24$).

Table 4 - Mandible size (mm) in Anagrus incarnatosimilis *and* A. breviphragma *larva bred in* Cicadella viridis *hosts.*

	A.incarnatosimilis	*A. breviphragma*
early second instar	0.056 + 0.004 (6)	0.027 + 0.003 (6)
mature larvae	0.054 + 0.004 (7)	0.027 + 0.003 (7)

Mean + SD (Number of observations)

Both *A. incarnatosimilis* and *A. breviphragma* larvae have cephalic and abdominal outgrowths.

The cephalic outgrowths are borne on the top of the cephalic end and are called "antennae" by Ganin (1869) and Henriksen (1922), who specify that they are movable; "first pair of legs" by Bakkendorf (1934); and "labial processes" by Whalley (1969). Their interpretation is difficult because it can be based only on their position as they do not show any structure or sensillum that could lead to any more precise conclusion. Their shape changes in different species: more slender and pointed in *A. breviphragma* larvae, more rounded in *A. incarnatosimilis*. The relative size of the outgrowths decreases as the larva grows.

Between them a circular opening represents the mouth as already stated also

by Dumbleton (1934) and drawn by Bakkendorf (1934) for *A. armatus* and *A. incarnatus* respectively. Below, another tinier hole is the exit of the salivary glands duct (fig. 12) as demonstrated by Bakkendorf (1934). At its sides two sensilla are present (fig. 13).

Specular pair of little lumps are situated at mouth opening sides, in the ventral surface of second segment, laterally and caudally to the cephalic outgrowths and behind the mandibles on the dorsal surface (fig. 11).

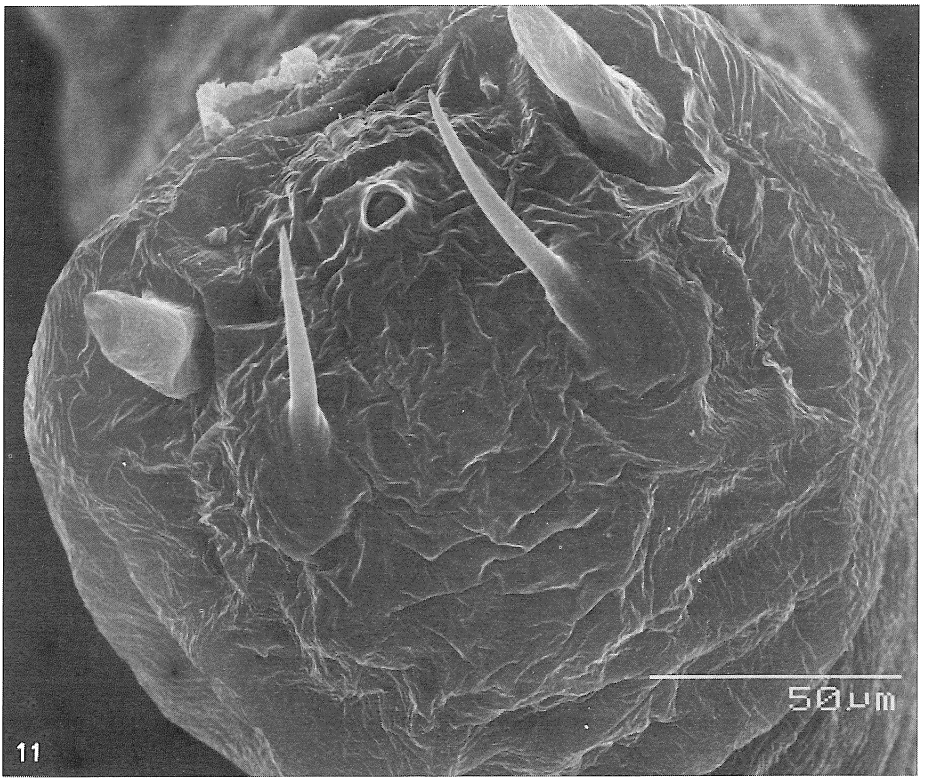
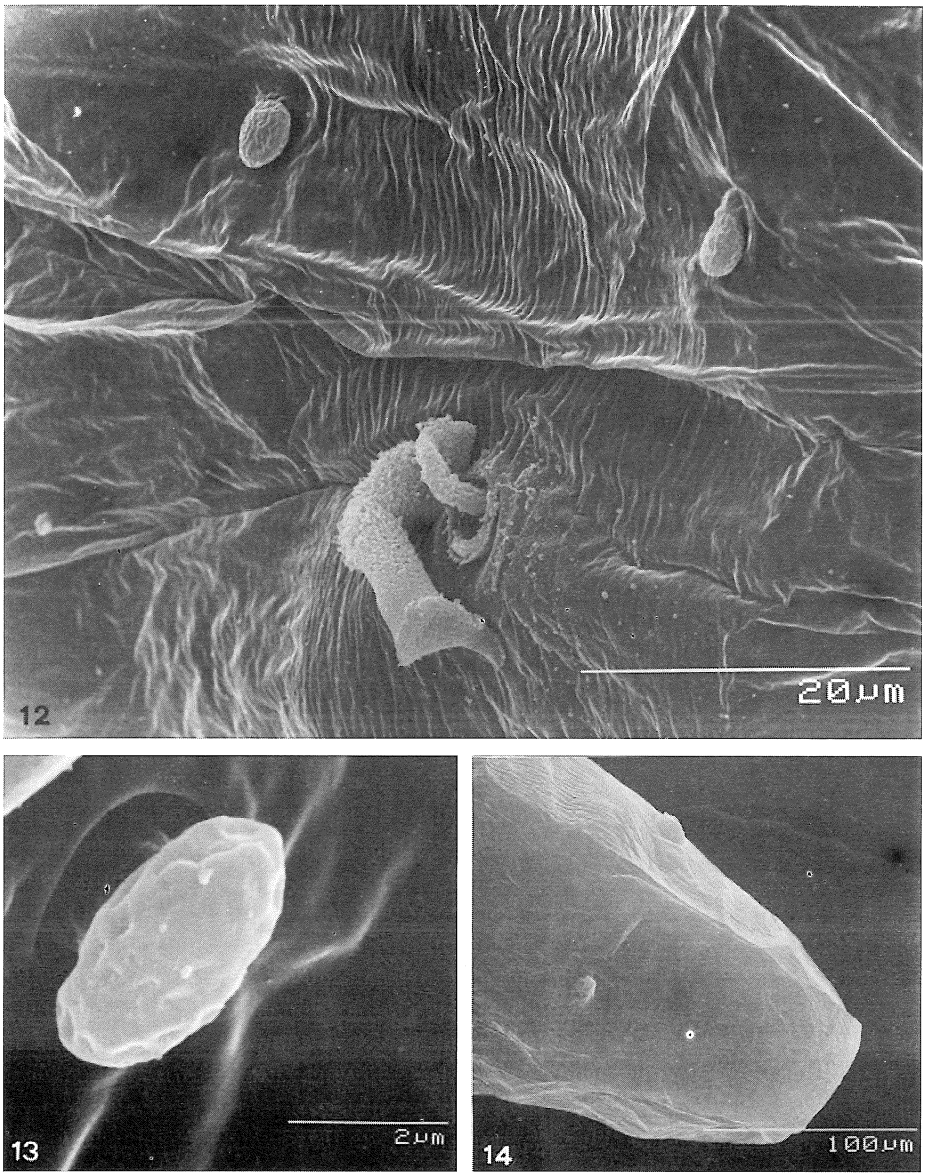


Fig. 11 - *Anagrus breviphragma* Soyka. Mandibles and cephalic processes.

Abdominal 'ear-like' outgrowths are situated at three quarter distance from the cephalic end, a little behind the caudal one. Their structure is clearly evident in fig. 14. They are more developed in *A. incarnatosimilis* than in *A. breviphragma* larvae and therefore are detectable at low magnification only in the former species.

No spiracles could be detected on body surface.

The longer mandibles of *A. incarnatosimilis* larvae and their more developed abdominal outgrowths distinguish these species.



Figs. 12-14 - *Anagrus incarnatosimilis* Soyka. Exit of salivary glands duct. (fig. 12). Sensillum at sides of the exit of salivary glands duct (fig. 13).
Anagrus breviphragma Soyka. Abdominal 'ear-like' outgrowths (fig. 14).

The second instar larva soon begins to feed: the yolk spheres of the host egg are sucked inward by contraction and expansion of the esophagus. By the 6th day, the gut of the larva is distended with ingested food.

Measurements of 6-day-old larvae of *A. breviphragma* are recorded in tab. 2 (early second).

Both *A. breviphragma* and *A. incarnatosimilis* 7-day-old larvae show small, white spherical bodies or aggregations that can be noticed scattered over the stomodeum. These are "urate cells" defined by Snodgrass (1935) as "cells of the fat body that become charged with urate crystals".[4] Their size increases as the larvae grow. They are moved in all directions with the help of the churning movements of the gut and its contents. Their movements are enhanced by the slow, whirling movements of the larvae.

The larvae turn orange on their 8th day of life. Their coloration grows in intensity as the larva develops, becoming reddish-orange by the 11th day, and red when the larva reaches the prepupal stage on the 12th day.

The early second instar (5-9 days old) remains very active and churns the contents of the host eggs. Whalley (1969) suggested that this movement makes more food available to the larva by keeping the host-egg contents in continuous circulation. This is particularly true if the oviposition has taken place in an egg with an already formed embryo: in this case the parasitoid larva has to destroy the embryo tissues and not only to mix the host-egg contents.

Mature second instar larvae (10-11 days old) are less active and roll their bodies over very slowly. All movement ceases by the 12th day, when the larva develops into the prepupa. The second larval stadium thus lasts about 7 days.

There is a two-fold increase in the size of the second instar larva during this stage: measurements of neonate second instars (6 days old) and mature (10 days old) *A. breviphragma* larvae are reported in tab. 2.

PREPUPA. The prepupal stage lasts 1 day during which constrictions develop defining the head, thorax and metasoma of the future pupa. The urate cells now become free in the gut and coalesce to form an opaque mass which occupies part of the thorax and abdomen. This stage ends with the pupa shedding the larval exuvium. The moult lasts about half an hour and is observable through the host egg corion paying attention to the position of the mandibles relative to the body: they can be seen leaving the apical position and, passing through the body, pass over the caudal end. The larval mandibles and exuviae remain situated at the caudal end of the newly formed pupa.

PUPA. The pupal stadium lasts 6-7 days. Several changes in coloration occur

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[4] Such aggregations were reported by Ganin (1869). Berlese (1909) and Bakkendorf (1934) called them "cellule uriche" and "symbiotic cells" respectively.

which permit estimation of the age of the pupa. Jackson (1961) showed that the ages of *Caraphractus cinctus* pupa could be assessed by their coloration. By the 13th day following oviposition, the newly formed pupa shows all the aggregations contained in the metasoma. The compound eyes of the pharate imago and the ocelli are pale brown and orange red, respectively. By the 14th day, the compound eyes and ocelli darken, the latter become brown, and by the end of the 15th day, the mandibles are dark brown. The body of the pupa is dark red to brown just before adult emergence. The appendages are fully formed by the 14th day. The mandibles and the apex of the ovipositor are brown by the 17th day, when all the appendages can be seen through the chorion of the host. One day before emergence, (i.e. the 20th day), the pupa is dark brown with black patches on the dorsum, mesosoma and abdomen. The pupa is exarate, with the pupal appendages free. The antennae and legs move and change position frequently but the body does not change its place. Emergence occurs as early as the 21st day, but most adults emerge 23-24 days after oviposition. The female pupa is slightly longer than the male due to the projection of the ovipositor (tab. 2).

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