Developmental Stages of *Lysiphlebus japonicus* Ashmead (Hymenoptera: Aphidiidae), a Bean Aphid Parasitoid[1]

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J. Entomol. Sci. 38(4): 519-524 (October 2003)

Abstract The developmental stages of a solitary parasitoid, *Lysiphlebus japonicus* Ashmead, of the black bean aphid, *Aphis craccivora* Koch, were described by using light and scanning electron microscopy. *Lysiphlebus japonicus* has four larval instars. The first instar is mandibulate, caudate and has sclerotized spines. Supernumerary neonates are eliminated by powerful mandibles of the first hatched larva. The second instar has degenerative mandibles and is covered with small sclerotized bristles on abdominal segments; the cauda is short and blunt. The third instar is mandibulate; its cuticle is smooth and the caudal segment retrogresses to a short salient. The fourth instar is strong and hymenopteriform; their mandibles are smaller than those of the third instar, and various sensory organs are well developed, especially for the mouthpart.

Key Words Developmental stage, Aphidiidae

Lysiphlebus japonicus Ashmead (Hymenoptera: Aphidiidae) is a solitary endoparasitoid that attacks the black bean aphid, *Aphis craccivora* Koch. Its biology and behavior have been largely unstudied although some aspects have been reported (Masaaki 1990). It is still unclear how to distinguish its larval instars, and characteristic descriptions of each instar are lacking.

Only a few species of Aphidiidae have been studied. Little information is available about their larval morphology (Stary 1962, Tremblay 1964, Vander 1971, Couchman and King 1977, Chorney and Mackauer 1979, Cloutier et al. 1981, Chow and Sullivan 1984), and no description has been given to the egg stage. Aphid parasitoids of the Family Aphidiidae have been reported as having 3, 4 or 5 larval instars. Some authors believed that all species in the Aphidiidae have 4 larval instars (Tremblay 1964, Stary 1988), while others stated 3 or 5 larval instars. In this paper, we used light microscopy and scanning electron microscopy to examine morphological differences of the larval instars and distinguished four larval instars in *L. japonicus.*

Materials and Methods

Second-instar black bean aphids, *Aphis craccivora* Koch, of uniform age, were individually exposed to 24-h-old mated females of *L. japonicus.* to avoid superparasitism, each aphid was exposed to only one parasite. Once parasitized, aphids were

[1]Received 21 October 2002; accepted for publication 15 April 2003.
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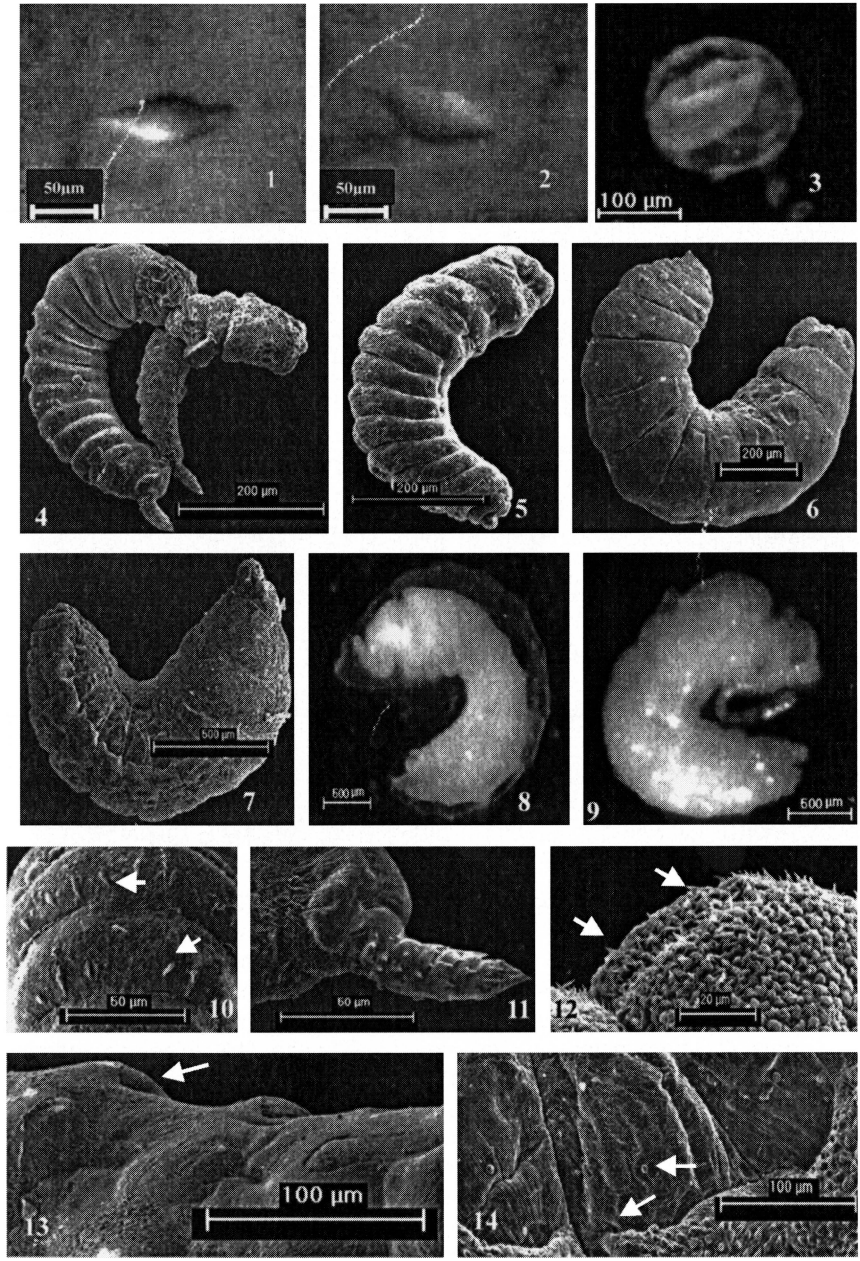

Fig. 1. Continued on next page.

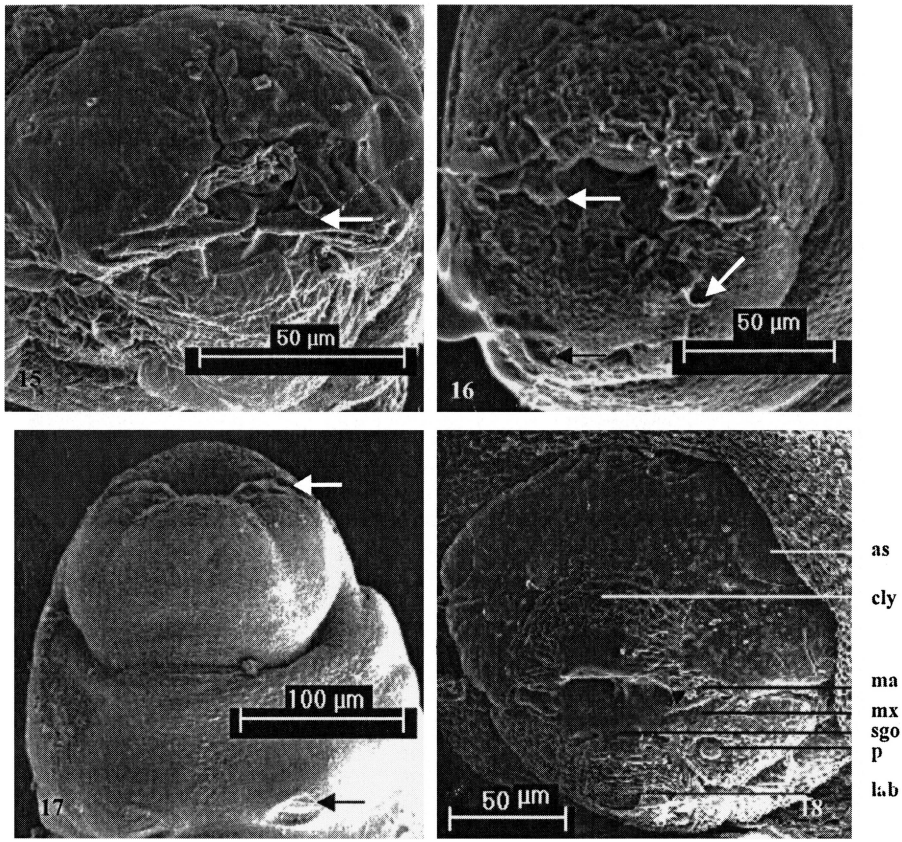

Fig. 1. Scanning electron micrographs of *L. japanicus:* (1)-(9), lateral view from egg to pupa; (10)-(14), body features of different larval instars; (15)-(18), head of different larval instars. (1) egg removed from female parasitoid. (2) egg 6 to 12 h after oviposition. (3) embryo. (4) the previously-hatched first instar killed other larvae with its powerful mandibles. (5) second instar larva. (6) third instar larva. (7) fourth instar larva. (8) prepupa. (9) pupa. (10) spines on abdominal segment of first instar larva. (11) caudal segment of first instar larva. (12) spines on abdominal segment of second instar larva. (13) pseudopods of second instar larva. (14) valve and wart-like protuberances of 4th instar larva. (15) head of first instar larva (arrow denotes the mandibles). (16) head of second instar larva (arrows denote the mandibles, wart-like receptors and beard-like receptors respectively). (17) mandibles and pseudopods of third instar larva. (18) head of fourth instar larva. Abbreviations: as, antennal socket; cly, clypeus; ma, mandible; mx, maxilla; sgo, silk-grand orifice; p, palpus; lab, labial sclerite.

placed on young horsebean seedlings and held at 25 ± 1°C, 50 to 70% RH with a 16L:8D photoperiod. Parasitized aphids were dissected at 6-h intervals after oviposition in order to ascertain the duration and morphology of the egg and larvae.

For each timepoint determination, 15 specimens of *L. japonicus* larvae were observed by scanning electron microscopy (SEM). Parasitized larvae were dissected from aphids directly in 0.1 M Sorenson's phosphate buffer containing 2.5% glutaraldehyde (pH 7.0). Larvae were fixed in glutaraldehyde solution at 4°C for 18 h, washed in phosphate buffer for 3 h and then transferred into a 1% osmium tetroxide solution for 1 h. Dehydration was performed by passing specimens through a series of alcohol washes and the alcohol removed using the critical point method. Specimens were coated with 250Å layer of gold and examined using a Philips XL30 scanning electron microscope.

Results and Discussion

Following oviposition into a suitable host, *L. japonicus* undergoes three molts. The parasitoid larvae complete embryonic stage and four larval stages inside the host aphid. The last instar kills the aphid and becomes a "mummy," in which pupation occurs.

Egg. Initially elongate measuring 118.4 ± 16.4 and 35.3 ± 0.4 μm in length and width respectively [Fig. 1(1)]. Compared with the egg dissected from the ovary of female parasitoids, two poles of the egg dissected from the aphid 6 to 12 h after oviposition are more blunt [Fig. 1(2)]. With the development of the embryo, the egg gradually becomes spherical. The embryo appears as a pale streak within the egg envelope [Fig. 1(3)]. At the end of the embryonic stage, the size and shape of the embryo are similar to those of first instars.

Because the eggs of Aphidiidae are very small, it is difficult to locate in the aphid hemocoel by conventional light microscopy. There are only a few reports describing the location of Aphidiidae eggs after oviposition. Griffiths (1960) succeeded in finding the egg of the parasitoid, *Monoctonus crepidis* Marshall, in slide-mounted hosts, but its location was not clear enough due to the inferior quality of the photographs. Calvert and Bosch (1972) found the eggs of *Monoctonus paulensi* (Ashmead) located in the fused thoracic-abdominal ganglia and entirely or partially embedded within the nerve tissue. Our observation showed that the eggs of *L. japonicus* were oviposited at the thorax or between the thorax and the abdomen. Some of them were dissociated in the host hemocoel, while others adhered to the aphid embryos or were embedded within the fat body in the thoracico-abdominal segment.

First instar. Newly-hatched first instars are found in the aphid hemocoel. Initially, the first instar is approximately 385.6 μm long by 96.4 μm wide. After full development, the larval size is approximately 684.4 μm long and 142.4 μm wide. The first instar consists of a head, 3 thoracic and 10 abdominal segments. The head is large and triangular. It possesses a distinct mouth opening, inside which are two sickle-shaped mandibles, moderately curved, measuring 38.64 μm in length [Fig. 1(4)]. The first-hatched first instar searches and kills other larvae by its powerful mandibles [Fig. 1(5)]. Abdominal segments 1 to 9 are armed each with a transverse row of about 8 to 11 sclerotized spines, posteriorly oriented, approximately 5.0 to 12.5 μm in length. However, there are no spines on the thoracic segments [Fig. 1(6)]. The abdomen ends in a sclerotized caudal segment which is covered with spicules. Irregularly arranged short spines locate on the surface of spicules [Fig. 1(7)].

Second instar. Several obvious morphological changes differentiate first and second instars. The head is flattened anteriorly and more round than first instar. Both a pair of wart-like receptors and a pair of beard-like receptors can be seen beneath the mouth. The mandibles evidently degenerate, which are about 16.7 μm long [Fig. 1(8)]. A pair of pseudopods are present on ventro-surface of each 3 thoracic segments [Fig. 1(9)]. The dorso-lateral surface of the 1 to 9 abdominal segments are covered with small sclerotized bristles, about 3 to 5 μm long. These small bristles are not regularly oriented [Fig. 1(10)]. The short and large spines on the caudal segment observed in first instars are absent [Fig. 1(11)].

Third instar. The shape is similar to the second instar, but the mandibles are developed, about 50.0 μm long. A pair of pseudopods are present on ventro-surface of each 3 thoracic segments [Fig. 1(12)]. The cuticle is smooth. The caudal segment retrogresses to a short salient [Fig. 1(13)].

Fourth instar. the fourth instar is strong and hymenopteriform, and various sensory organs are well developed. The larva always appears yellow, apparently due to fat accumulation. The mouth deflects posteriorly, with heavily sclerotized mandibles, triangular in shape, about 35.5 μm long, less than the third instar. Two pairs of wart-like receptors can be observed beneath the mouth. Maxilla, clypeus, silk-gland orifice and labial sclerite are evident [Fig. 1(14)]. The cuticle is densely covered with short knob- or wart-like protuberances, which extend from dorso-surface to ventro-surface on first thoracic segment and to lateral surface on second, third thoracic segments [Fig. 1(15)]. A pair of valves are observed on lateral-surface of each abdominal segments of 2 to 7 and a pair of strumae are present on ventro-surface of each abdominal segments of 1 to 7 [Fig. 1(16)].

Prepupa. At the end of the fourth instar, the host becomes a mummy. The parasitoid larva bites a hole on the mummy abdominal surface and spins threads to make the mummy stick to the host plant. Metabolic waste produced during growth accumulates in the gut. The fourth instar expels the waste from body and enters prepupal stage. Prepupae shorten and swell. The shape of the larva can be seen clearly through transparent cuticle in the early time [Fig. 1(17)]. Subsequently, the segment between the thorax and abdomen begins to plunge. Antennae, legs and wing buds differentiate gradually, so do the reproductive organs. At the end of the prepupal stage, the larval red eyes are visible.

Pupa. The pupa possesses the adult shape, consisting of head, thorax and abdomen. Antennae, wings, legs and eyes are all fully developed [Fig. 1(18)].

Summary. Our study using scanning electron microscopy reveals that there are four larval instars in *L. japonicus*. It agrees with the results described with *Aphidius smithi* Sharma (Chorney and Mackauer 1979). Some parasites of Aphidiidae have been reported to have 3 or 5 larval instars. For example, 5 larval instars were reported in *Aphidius ervi* Haliday (Stary 1962) and *Aphidius pisivorus* Smith (Vander 1971) and 3 larval instars in *Trioxys utilis* Muesebeck (Schlinger and Hall 1961) and *Monoctonus paulensi* Ashmead (Calvert and Van der Bosch 1972). This variability is probably due to the different interpretation of the morphological features by these authors. In fact, in *L. japonicus,* second and third instars are so similar under light microscopy that they are hardly distinguished, and can be only identified from the small sclerotized bristles and the mandibles by using SEM. According to the literature and our observation, the sickle-shaped mandibles and the abdominal spines or thorns seem to be characteristic of the first instar to Aphidiidae, although the abdominal spines may

differ in shape, location and number in some other species (Schlinger and Hall 1961, Vander 1971).

Acknowledgment

This study was supported in part by the president special funding of Chinese Academy of Sciences.

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