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Biological and Systematic Studies of Developmental Stages in *Aphytis* (Hymenoptera: Aphelinidae)

I. Developmental History of *Aphytis chilensis* Howard

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Paul DeBach, Mike Rose, and David Rosen

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I. Developmental History of *Aphytis chilensis* Howard

Species of *Aphytis* Howard (Hymenoptera: Aphelinidae) are the most effective natural enemies of armored scale insects (Homoptera: Diaspididae). Their classification, based on the morphology of adult wasps, is difficult. The studies herein were intended to explore the possibility of using morphological characteristics of developmental stages in the systematics of this genus.

The developmental stages of *A. chilensis* Howard, the generotype of *Aphytis,* are described. The ovarian egg is double-bodied, whereas the deposited egg is stalked. The larva passes through three instars, each of which differs markedly in shape and size of the mandibles. First-instar larvae have four pairs of spiracles; the second and third instars have eight pairs, *viz.,* one pair in the mesothoracic segment and one in each of the first seven abdominal segments. The cephalic skeleton, respiratory system, and various integumentary structures of the third-instar larva are described. The morphology of the pupa was studied with light and scanning electron microscopy.

At 28 ± 1° C, egg development takes 2 to 3 days, larval development (including the prepupal period) 10 to 12 days, and pupal development 6 to 7 days. Rearings under various constant temperatures gave the theoretical threshold of development as 14.1° C and a thermal constant of 270.2 day-degrees for the completion of development.

II. Larval Criteria in the Systematics of *Aphytis*

Eggs and larvae of six species of *Aphytis,* representing five of the seven species-groups currently recognized in this genus, were

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David Rosen and Avner Eliraz

Biological and Systematic Studies of Developmental Stages in *Aphytis* (Hymenoptera: Aphelinidae)[1]

I. Developmental History of *Aphytis chilensis* Howard

INTRODUCTION

THE GENUS *Aphytis* Howard (Hymenoptera: Aphelinidae) comprises the most effective natural enemies of armored scale insects (Homoptera: Diaspididae). Several of its members have been used successfully in biological control projects against economically important pests in various countries: *Aphytis holoxanthus* DeBach against the Florida red scale, *Chrysomphalus aonidum* (L.); *A. lepidosaphes* Compere against the purple scale, *Lepidosaphes beckii* (Newman); *A. lingnanensis* Compere against the California red scale, *Aonidiella aurantii* (Maskell); *A. melinus* DeBach against both the California red scale and the dictyospermum scale, *Chrysomphalus dictyospermi* (Morgan); *A. paramaculicornis* DeBach and Rosen [formerly recorded as the "Persian strain" of *A. maculicornis* (Masi)] against the olive scale, *Parlatoria oleae* (Colvée); and—most recently—*A. roseni* DeBach and Gordh against the rufous scale, *Selenaspidus articulatus* (Morgan). Numerous other species of *Aphytis* have been instrumental in the natural control of other injurious pests in certain ecosystems without deliberate intervention by man: *A. chrysomphali* (Mercet) on the California red scale; *A. hispanicus* (Mercet) on the chaff scale, *Parlatoria pergandii* Comstock; *A. mytilaspidis* (Le Baron) on the oystershell scale, *Lepidosaphes ulmi* (L.); and *A. proclia* (Walker) on the San Jose scale, *Quadraspidiotus perniciosus* (Comstock), to name but a few (see DeBach, Rosen, and Kennett, 1971; Rosen and DeBach, 1976*a*, 1976*b*; Debach and Rosen, 1976).

The importance of accurate, dependable systematics to biological research and to the success of biological control projects has been amply documented (see Clausen, 1942; Sabrosky, 1955; Schlinger and Doutt, 1964; Compere, 1969; Rosen and DeBach, 1973; Delucchi, Rosen, and Schlinger, 1976; and others). When natural enemies are being sought or transferred from one region to another for biological control, correct identification of both the target pests and their natural enemies is an essential prerequisite for ultimate success. Failure in biological control has often resulted from poor systematics.

Unfortunately, despite the undis-

[1] Accepted for publication September 12, 1977.

puted economic importance of *Aphytis,* systematic knowledge of this large genus has remained unsatisfactory. Current studies (Rosen and DeBach, unpublished data) show that the genus *Aphytis* now comprises some 90 known species, all of which develop as primary ectoparasites of armored scale insects. They are all minute, yellow or grayish wasps, and their identification is generally difficult due to a lack of reliable diagnostic characters. Until recently, misidentification of *Aphytis* species was probably the rule rather than the exception. Many potentially effective natural enemies were not recognized as distinct species, and the inadequate state of the systematics of *Aphytis* had caused serious setbacks in important biological control projects (Rosen and DeBach, 1973). There is an urgent need to discover additional distinguishing characters and to improve the methods to identify the species of this genus.

Recognition of differences between natural enemy species in any given developmental stage may be of great practical importance in biological control projects (Rosen and DeBach, 1973). Field-collected samples of pest species usually contain at least some developmental stages of natural enemies, and it is often difficult to rear them to maturity in the laboratory. Although the developmental stages of numerous natural enemies have been described (see, for instance, Clausen, 1940 and Hagen, 1964), relatively little use has been made of such information in actual identification or classification.

The systematics of the parasitic Hymenoptera, by far the most important group of natural enemies, is based almost entirely on the morphology of adult wasps. Morphological characteristics of developmental stages (mainly larvae and larval exuviae) were sometimes used to identify parasites associated with certain pest species (e.g., Thorpe, 1930; Gerig, 1960; Finlayson, 1960*a*, 1960*b*, 1962, 1963, 1967*b*; Mackauer and Finlayson, 1967; Sadava and Miller, 1967). Attempts to utilize larval morphology in the actual classification of parasitic Hymenoptera have been limited mainly to the Ichneumonoidea (Beirne, 1941; Short, 1952, 1953, 1959; Finlayson, 1967*a*, 1975; Čapek, 1969, 1970; see also Michener, 1953). The developmental stages of the smaller Chalcidoidea offer few diagnostic characters. However, Maple (1967) demonstrated that even the eggs and first-instar larvae of the Encyrtidae may provide useful criteria for identification and classification. A comparative morphological study of egg, larval and pupal characters of the Chalcidoidea might provide new tools for the separation of cryptic species as well as new insights into the higher classification of that difficult but economically important group.

Pupal pigmentation has been used to some extent as a valid taxonomic character in the genus *Aphytis,* and in a few cases has provided a short cut to the separation of closely related species (DeBach, 1959; Traboulsi, 1969; Yasnosh, 1972). The studies reported herein were intended to further explore the possibilities of using morphological characteristics of developmental stages in the systematics of *Aphytis.*

The general life history of *Aphytis* was recently described by Rosen and DeBach (1976*a*) as follows:

> "Armored scale insects are usually free beneath the hard covering scale or shield. The adult *Aphytis* female pierces the shield with her ovipositor, and lays one to several eggs on the body of the scale insect. Protected by the covering scale, the *Aphytis* larvae assume an external feeding position and suck the body fluids of the scale insect. The full-grown larvae then excrete characteristic meconia and pupate underneath the empty scale. The adult parasites usually emerge through a hole that they chew in the covering scale. In addition to the hosts killed by parasitism, numerous scale insects may be killed by predatory host-feeding by the adult *Aphytis* female."

Detailed accounts of developmental history were presented by Imms (1916)

and Griswold (1925) for *Aphytis mytilaspidis,* and by DeBach and Landi (1961) for *A. lepidosaphes.* Descriptions of developmental stages were also presented by Quayle (1910) for *A. chrysomphali* [erroneously recorded as *A. diaspidis* (Howard)], by Parker (1924) for *A. chilensis* Howard [recorded as *A. longiclavae* (Mercet), a synonym], by Taylor (1935) for *A. chrysomphali* (but aparently more than one species was involved in that study), and by Azim (1963*a*, 1963*b*) for several species occurring in Japan. Unfortunately, these descriptions were rather inaccurate in certain important aspects.

MATERIALS AND METHODS

Aphytis chilensis Howard

The species chosen for this study, *A. chilensis,* is the generotype of *Aphytis.* It was described by Howard (1900) from a single female specimen reared from the oleander scale, *Aspidiotus nerii* Bouché [recorded as *A. hederae* (Vallot), a synonym] on ivy in Chile, and has since been recorded as a nearly cosmopolitan parasite of that host as well as of several other species of armored scale insects (Compere, 1955).

Aphytis chilensis is a distinctive species. It reproduces thelytokously, and males are extremely rare. The female (fig. 1) can be readily recognized by the following characters: eyes coarsely setose; antennal club elongated; first

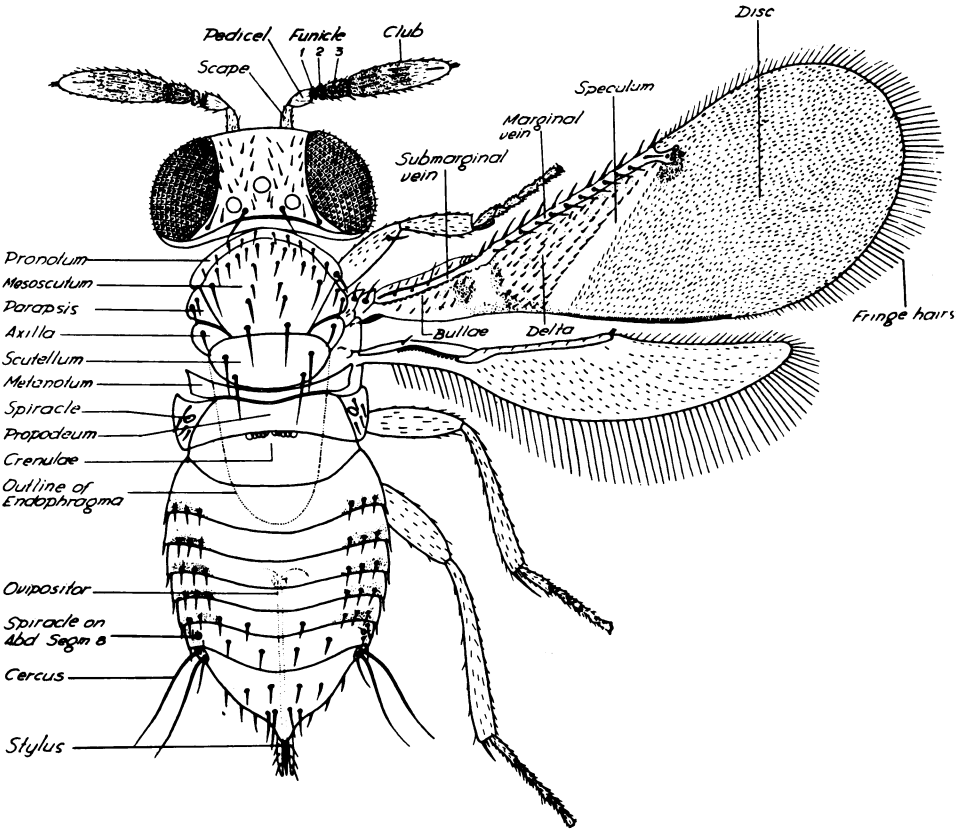


Fig. 1. *Aphytis chilensis* Howard, female (from DeBach, 1964).

funicular segment greatly reduced, considerably smaller than the second segment; mesoscutum usually with 16 to 18 setae; propodeum relatively long, with rounded, distinctly overlapping crenulae; general coloration greyish, rather extensively infuscated, with transverse black bars on the occiput and fuscous stripes on the sides of abdominal tergites. In the rare male, the first two funicular segments are minute, and the club is enormously elongated.

Rearing of the scale insect hosts

Aphytis chilensis was reared on its typical host, the oleander scale, *Aspidiotus nerii* Bouché. The hosts were reared at the Biological Control Institute of the Citrus Marketing Board of Israel, Rehovot, under constant conditions of 24° C and 60 to 65 percent relative humidity. Rearing methods were generally similar to those described by Flanders (1951) for the California red scale, *Aonidiella aurantii* (Maskell). Potatoes and Burpee Butternut (Vine) squash served as convenient plant hosts; the potatoes were irradiated to prevent sprouting. They were infested with the oleander scale by placing them in metal baskets directly beneath baskets containing plant hosts infested with crawler-producing scales. The uniformity and density of infestation was controlled by turning the baskets around at various intervals. The baskets with newly infested potatoes or squash were then placed in closed sleeves to prevent contamination with undesirable organisms.

Rearing of the parasites

A laboratory stock of *A. chilensis* was started from specimens reared from a sample of oleander scale collected on English ivy, *Hedera helix* L., in Jerusalem, Israel. This stock was maintained in 40 × 25 × 20 centimeter plastic cages covered with glass plates on which fine streaks of undiluted honey were placed as food for the adult parasites. Ventilation was provided by large holes drilled in all sides of the cages and covered with cloth.

To achieve some degree of standardization, the developmental stages were obtained from parasite females no more than 24 hours old, that were allowed to oviposit in oleander scale on potatoes for 1 hour under constant conditions of 28 ± 1° C and 70 ± 5 percent relative humidity. This was done by 1) introducing scale-infested potatoes into standard stock cages in which parasite emergence had just begun; 2) introducing young parasite females for 1 hour into 200 centimeter[3] plastic jars containing a single infested potato; or 3) into small oviposition cells attached to an infested potato. The latter consisted of plastic vials, 45 millimeters high and 35 millimeters in diameter, the bottom of which had been removed. The vials were fastened onto the scale-infested host plants with Permagum®[2] and covered with cloth.

The parasitized host material was kept under the same constant conditions. Samples were taken daily, and the various developmental stages of *A. chilensis* were exposed by turning over the covers of parasitized scale insect hosts with a fine forceps under a dissecting microscope. Eggs, larvae and pupae were slide-mounted for study under a phase-contrast microscope. Pupae were also prepared for scanning electron microscopy.

Preparation of slide mounts

Eggs and first-instar larvae were transferred directly into a drop of Hoyer's medium on a glass slide, and covered with a coverglass. Larger larvae and pupae were soaked for several days in a chloral phenol solution (prepared by dissolving 25 parts chloral hydrate with 30 parts phenol) to which a few

[3] A non-toxic, odorless sealing compound distributed by Virginia Chemicals, Inc., West Norfolk, Virginia.

drops of glacial acetic acid were added until a desirable degree of clearing was attained, prior to mounting in Hoyer's medium. Third-instar larvae were first punctured with an entomological micropin to eliminate the opaque gut contents. The slides were dried for about two weeks under room conditions, then were ringed with Zut[3] to prevent their deterioration.

Minute specimens were transferred with micro-pins that had been made into tiny loops and mounted on wooden handles. For soaking, small watch glasses (United States Bureau of Plant Industry model) were most suitable.

Specimens mounted in Hoyer's medium continued to clear for about two days. For best results, such slides were therefore usually studied at least two to three days after mounting.

Addition of small amounts of iodine and potassium iodide to the standard Hoyer's solution tended to improve the resolution besides making it easier to locate the transparent specimens on the slides.

Mounting in Hoyer's medium, with or without previous clearing in chloral phenol, was superior to all other methods of preparation. Staining the specimens with acid fuchsin did not have any advantage for phase-contrast examination, whereas the frequent transfers required by that method resulted in considerable loss or damage to the specimens. Mounting in Canada balsam, which requires transferring the specimens through a series of alcohols, gave similar results and yielded poorer resolution than Hoyer's mounts. Dehydrating agents such as xylol tended to cause severe distortion, especially of punctured larvae.

Temporary mounts were sometimes prepared for special purposes. For instance, cotton blue emphasized the external outline of the egg, whereas immersion in bromo phenol solution was used to observe larval tracheae, these stand out like silvery threads; but the solution penetrates quickly into the respiratory system, so that these mounts should be examined immediately. Live material was often studied in saline solution.

The slide mounts were studied under a Zeiss Photomicroscope II, equipped with phase-contrast objectives and a micrometer eyepiece. All measurements of developmental stages represent averages of 20 specimens or more.

Preparation for scanning electron microscopy

Live pupae were mounted on aluminum stubs with a collodium-amyl acetate solution, and were quick-frozen with liquid nitrogen. They were then coated with gold and studied under a Cambridge Stereoscan S-4 scanning electron microscope.

THE DEVELOPMENTAL STAGES

Hinton (1976) has convincingly argued that the various developmental stages and instars of insects should be defined by apolysis, rather than by ecdysis as is commonly done in the entomological literature. However, he also pointed out that "it has never been suggested by anyone. . . that we should abandon the time-honoured and easy way of describing the life-history of an insect by reference to the ecdyses, which are easier to see than the apolyses." This sound advice has been followed in the present study.

The egg

Upon emergence, the female of *A. chilensis* contains three to five fully-developed eggs. The *ovarian egg* is of the double-bodied type common in the

[3] A slide-ringing compound distributed by Bennett's Paint Products, Salt Lake City, Utah.

Encyrtidae, Miscogasteridae, and some Aphelinidae (Hagen, 1964), comprising the distal egg proper and a proximal bulb connected by a narrower neck. It is transparent white, measuring 289 × 63 microns.

The eggs are usually laid on the dorsal aspect of the body of the scale insect host, near the margin. During oviposition, the bulb collapses and its contents are forced into the egg proper, which is the first part to travel through the ovipositor (Hagen, 1964). The resulting *deposited egg* (figs. 2–6) is stalked. It is milky white, 189 microns long (176 to 224) and 85 microns wide (76 to 104), with a smooth, semitransparent chorion that appears somewhat thicker at the distal end (i.e., opposite the stalk end). There apparently is a distinct border separating the egg proper from the stalk. However, when some pressure is applied to a newly deposited egg (e.g., by manipulating the cover slip), some of the contents of the egg proper may flow back into the collapsed bulb (see fig. 3).

Incubation takes two to three days. During early blastoderm formation (fig. 3), the developing embryo appears as a small group of cells at the proximal one-third of the egg. By the third day, the embryo nearly fills the lumen of the egg, but still encompasses a mass of undigested yolk (fig. 5). The head capsule of the fully formed embryo (fig. 6) is always directed toward the stalk end of the egg. Prior to hatching, the embryo performs frequent pendulum-like movements to the right and left of the longitudinal axis of the egg, accompanied by vigorous movements of the mandibles, which usually move one at a time.

Stalked eggs are common among the parasitic Hymenoptera. In the Aphelinidae, this egg type has been recorded in the genera *Aphytis, Marietta,* and *Centrodora,* which are closely related, and also in *Aspidiotiphagus* (Hagen, 1964).

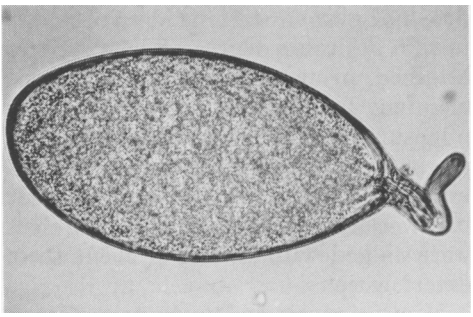


Fig. 2. Newly deposited egg.

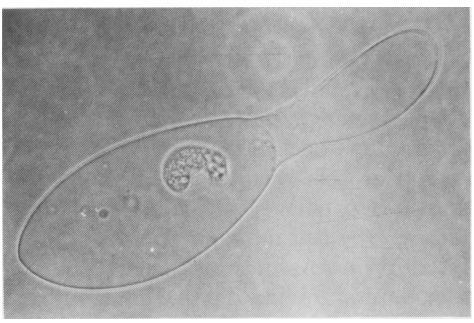


Fig. 3. Early blastoderm formation (the pressure of the cover slip has forced some of the contents of the egg proper back into the bulb).

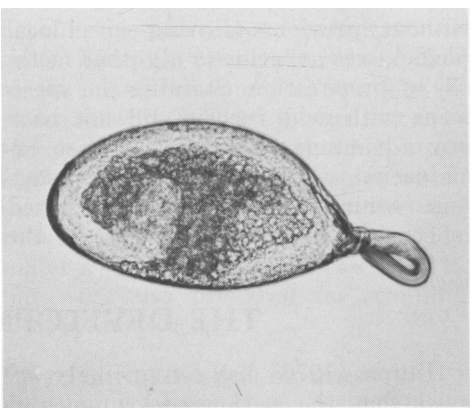


Fig. 4. Advanced blastoderm formation.

The larva

It is often difficult to determine the number of larval instars in minute parasitic Hymenoptera. Shape of the mandibles is usually the best landmark, but the number of spiracles may also be of value. Ectoparasitic Chalcidoidea may

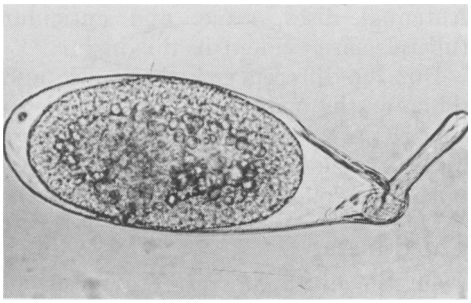


Fig. 5. Egg with double-layered embryo.

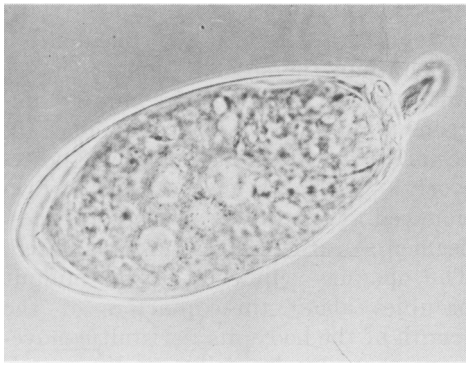


Fig. 6. Egg with fully-formed embryo; note head capsule facing stalk end.

have four or five pairs of spiracles in the first instar, up to nine pairs in subsequent instars (Hagen, 1964).

All aphelinid genera apparently have three larval instars (Nikol'skaya and Yasnosh, 1966), and *A. chilensis* is no exception. The three instars differ markedly in the shape and size of their mandibles (fig. 7).

First instar

Hatching (fig. 8) starts on the third day after oviposition. The newly hatched larva is ovoid, measuring 128 × 83 microns. Undigested yolk is still evident at this stage as an amorphous yellow mass. When fully developed in three days, the first-instar larva is 168 microns long (128 to 250) and 91 microns wide (83 to 137). Segmentation is rarely visible in slide mounts, but in some specimens the head and 12 body segments are evident.

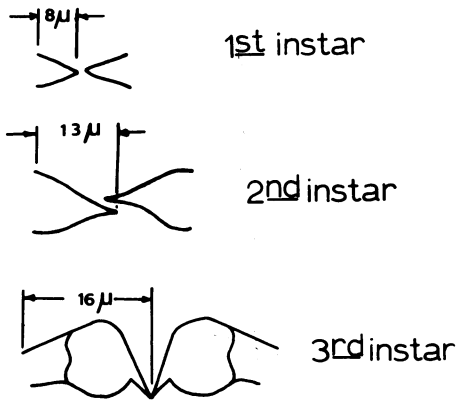


Fig. 7. Mandibles of the three larval instars.

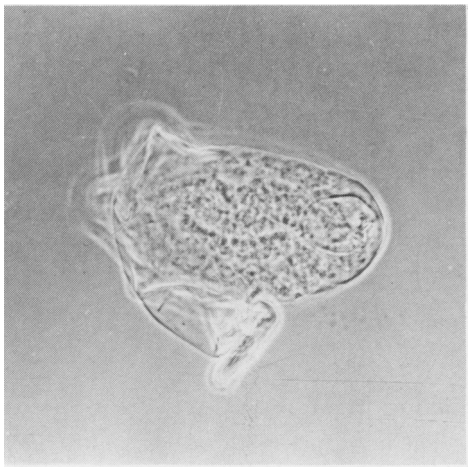


Fig. 8. First-instar larva hatching from egg.

The first-instar larva of *A. chilensis* possesses four pairs of spiracles: one pair in the mesothoracic segment and one in each of the first three abdominal segments (fig. 9). The spiracles are 2 microns in diameter. The cephalic skeleton is readily visible (see fig. 8); the mandibles (fig. 7) are minute, triangular, 8 microns long. Antennal discs, setae, or any other cuticular formations could not be detected in this instar.

Parker (1924) was evidently incorrect in stating that the first-instar larva of *A. chilensis*, like subsequent instars, possesses eight pairs of open spiracles.

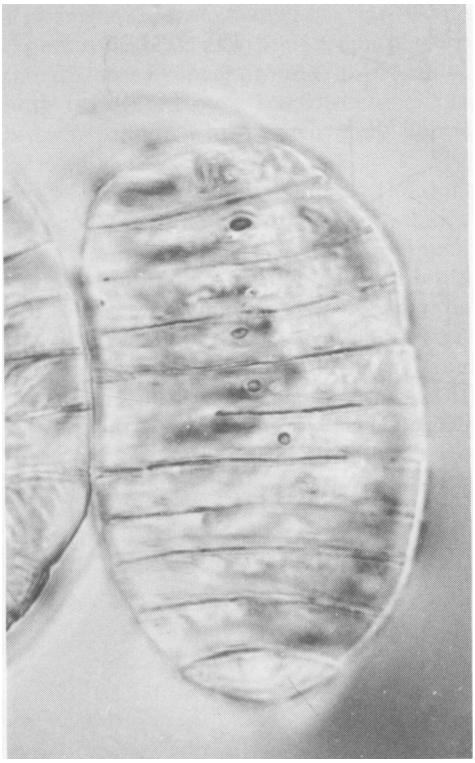


Fig. 9. First-instar larva, showing four open spiracles.

Second instar

The larva passes into the seond instar five to six days after oviposition. Unfortunately, larval exuviae could not be detected in *A. chilensis* prior to pupation. However, the second instar, lasting two to three days, differs markedly from the first in having eight pairs of functional spiracles: one pair in the mesothoracic segment and one in each of the first seven abdominal segments.

The second-instar larva is 262 microns long (190 to 308) and 227 microns wide (175 to 284). Segmentation is much more pronounced in this instar, with the head and 13 body segments clearly evident, and the cephalic skeleton also clearly visible (fig. 10). The mandibles (figs. 7, 10) are longer and more acutely pointed than in the first instar, averaging 13 microns in length. Antennal discs, setae, and cuticular tubercles are evident in this instar.

But for differences in body size and shape of the mandibles, the second instar is nearly identical with the third instar, which is described in greater detail in the following paragraph.

Third instar

On the ninth day after oviposition, the larva enters the third and final instar that lasts five to six days (including the prepupal stage). The third instar larva (fig. 11) is considerably larger than the second, elongated, 804 microns long (760 to 840) and 609 microns wide (550 to 720), rounded anteriorly and somewhat narrower posteriorly. Segmentation is quite pronounced, with the head and 13 body segments rather clearly demarcated. The opaque, yellow or brown midgut occupies about three-quarters of the length of the body; its peristaltic movements are clearly visible in live specimens.

The *cephalic skeleton* (figs. 12, 13) is rather simple, the epistomal, pleurostomal, hypostomal and tentorial sclerites forming a continuous ring. The mandibles (figs. 7, 12, 14) are larger than in

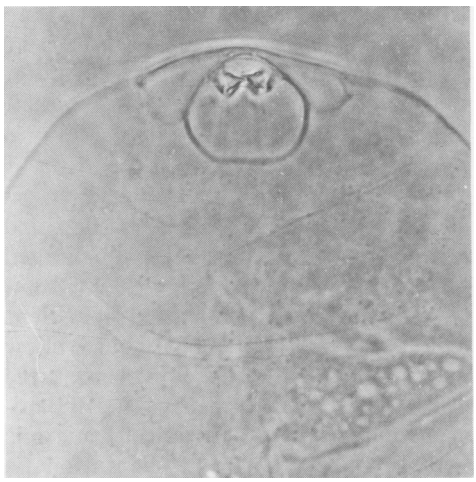


Fig. 10. Anterior portion of second-instar larva, showing the cephalic skeleton and mandibles.

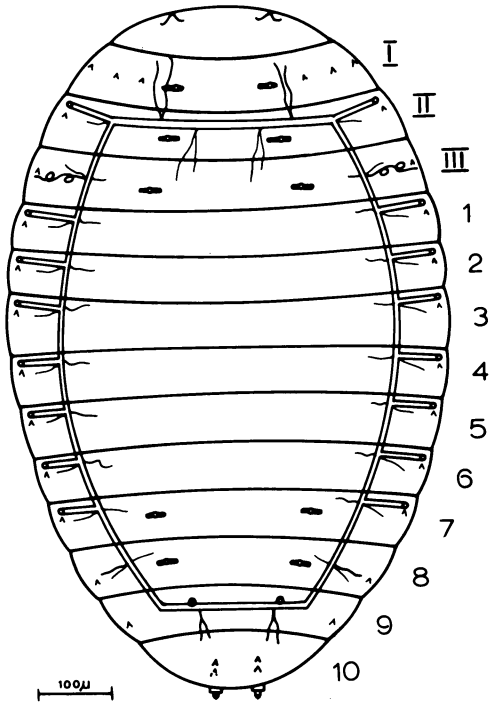


Fig. 11. Third-instar larva, showing the respiratory system and some integumentary formations (semidiagrammatic).

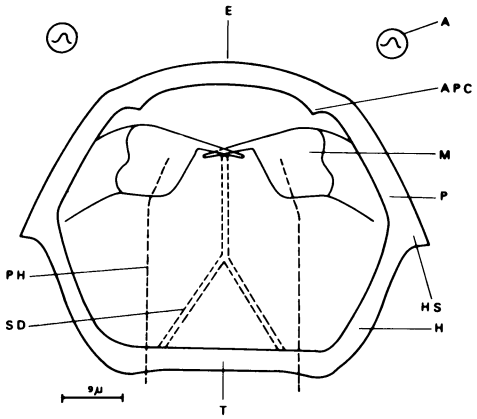


Fig. 12. Cephalic skeleton of third-instar larva. A = antenna; APC = anterior pleurostomal process; E = epistoma; H = hypostoma; HS = hypostomal spur; M = mandible; P = pleurostoma; PH = pharynx; SD = salivary duct; T = tentorium.

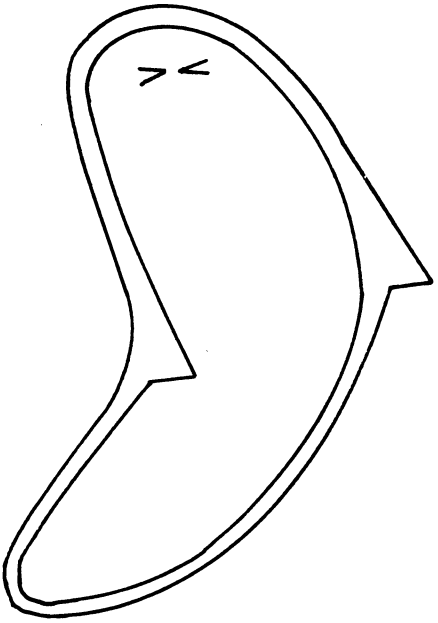


Fig. 13. Cephalic skeleton of third-instar larva, lateral view.

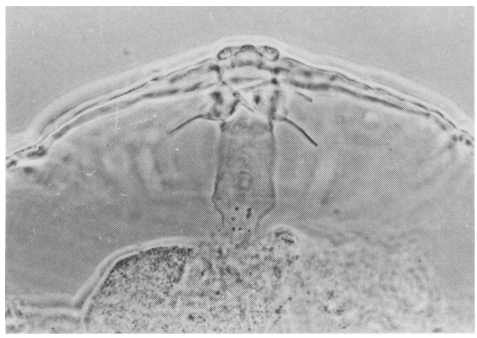


Fig. 14. Anterior portion of third-instar larva, showing mandibles and pharynx.

the preceding instar, with a distinct, pointed denticle. The pharynx and salivary duct appear to open between the mandibles. The one-segmented antennae are represented by minute discs (fig. 12).

The *tracheal system* (figs. 11, 15 to 18) consists of a pair of longitudinal trunks passing laterad of the midgut, connected by transverse commissures in the mesothorax and in the ninth abdominal segment, thus forming a complete

ring. Ten short trunks extend dorsolaterally from the longitudinal trunk on each side of the body, eight of them leading to open spiracles: one pair in the mesothorax and one in each of the first seven abdominal segments. The lateral trunks in the metathorax and in the eighth abdominal segment appear to be more slender and are sometimes twisted, forming tiny loops (figs. 11, 15). They are slightly thickened apically, but do not end in functional spiracles. Two additional branches depart from the longitudinal trunk at the junction of each of the 10 lateral trunks (figs. 11, 16 and 17): one branch is directed ventro-laterally toward the integument, the other toward the midgut and

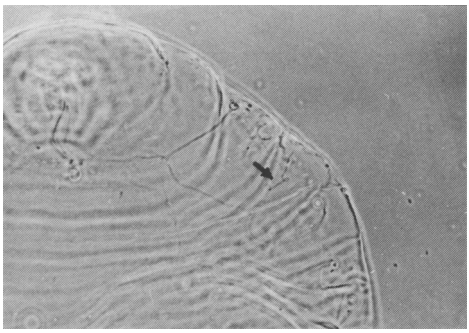


Fig. 15. Anterior portion of third-instar larva, showing spiracle in mesothoracic segment and looped lateral tracheal trunk in metathoracic segment (indicated by arrow).

branching profusely around it. The anterior transverse commissure sends out a pair of dichotomous branches toward the head, and another pair, mesad of the latter, toward the midgut. The posterior transverse commissure (fig. 18) sends a similar pair of dichotomous branches toward the caudal end of the body.

Final-instar larvae of most aculeate Hymenoptera have 10 pairs of open spiracles, whereas those of most parasitic Hymenoptera usually have nine pairs (Hagen, 1964). In *A. chilensis*, only eight pairs are present, the lateral tracheal trunks in the metathorax and

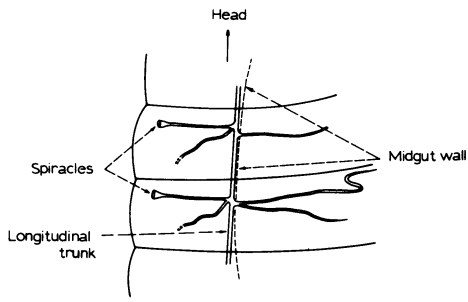


Fig. 16. Main tracheal branches of third-instar larva (semidiagrammatic).

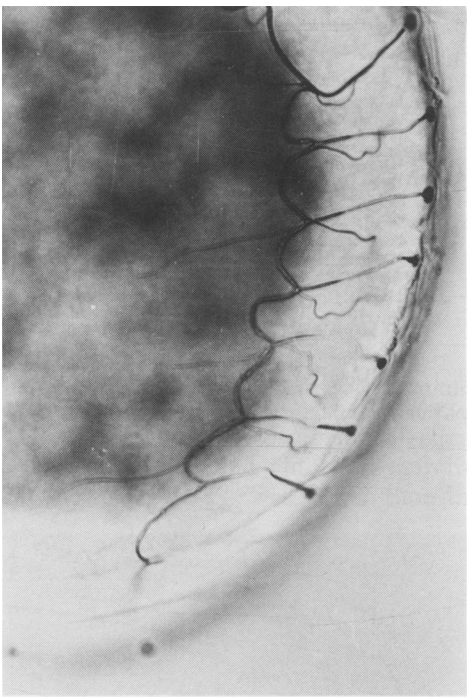


Fig. 17. Tracheal system of third-instar larva, showing main branches.

in the eighth abdominal segment apparently representing vestigial spiracles.

The *spiracles* (fig. 19) are slightly sunk in the integument. Their structure is rather simple, consisting of a spherical atrium followed by a series of chambers leading to the trachea. A closing mechanism could not be detected. Both the thoracic and abdominal spiracles of the third-instar larva are five microns

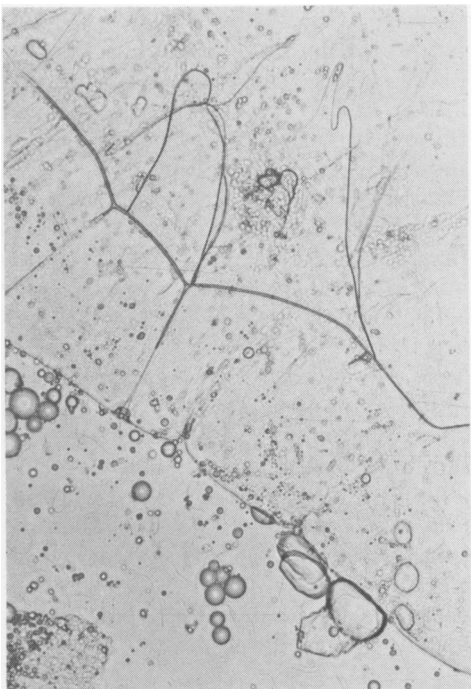


Fig. 18. Tracheal system of third-instar larva, showing dichotomous branches arising from posterior transverse commissure.

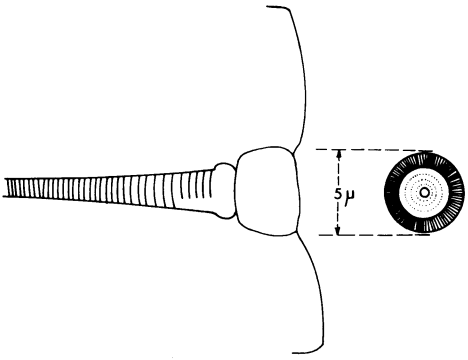
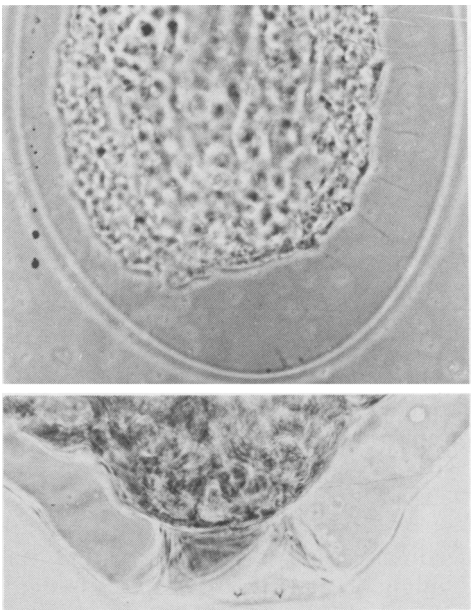


Fig. 19. Spiracle of third-instar larva.

in diameter, but the former appear more distinct.

The *integument* of the intact larva appears to be entirely smooth, whereas in the punctured larva, a strigose sculpture is usually evident. Minute cuticular tubercles are present on all segments: five pairs on the head between the mouthparts and antennal discs (some of these may, in fact, be minute pores rather than tubercles, but their exceedingly small size precludes a closer study of their nature with present equipment), three in a dorsal transverse row on each side of the prothorax, and one pair of dorsolateral tubercles on each of the two posterior thoracic segments and first nine abdominal segments, adjacent to a spiracle if present. Two pairs of submedian tubercles—one dorsal and one ventral—are present on the 10th abdominal segment (see fig. 11).

A pair of minute, three-segmented sensoria, 5 microns long, are present at the caudal end of the 10th abdominal segment one on each side of the anus (figs. 11, 20, 21).



Figs. 20 & 21. Caudal sensoria of third-instar larva.

A pair of transverse, "bacilliform" rod formations, each containing a "spore-like" discoid structure, is present dorsally on each of the three thoracic segments, and on the seventh, eighth, and ninth abdominal segments. Apparently sclerotized, their form is quite variable, with the discoid structure positioned on either side of

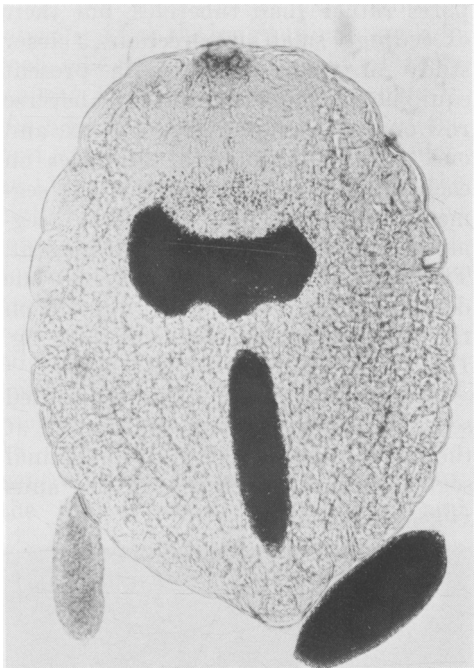


Fig. 22. Third-instar larva expelling meconia.

the center of the rod; on the ninth abdominal segment, often only the discoid structures are present (fig. 11). The arrangement of these peculiar formations is rather constant, but their function is unknown.

On the 11th or 12th day after oviposition, the third-instar larva enters the *prepupal stage*. Inasmuch as in the parasitic Hymenoptera this stage is not marked by apolysis and ecdysis, it cannot be considered a distinct instar. All feeding ceases at this point, and the hindgut becomes linked with the midgut (Hagen, 1964). The larva turns on its back, with its ventral aspect facing the covering scale of the host, and excretes fecal material in the form of 7 to 15 distinctive, cylindrical, dark brown meconia. It then enters a resting period, during which rapid metamorphosis takes place.

The prepupal stage lasts about two days. The prepupa is milky white, and its caudal end is more distinctly pointed than in earlier larval stages (fig. 22). Larval structures, including the cephalic skeleton, the tracheal system, and all cuticular formations are still present throughout this stage. The developing pupal organs are readily visible in cleared, slide-mounted specimens.

Differentiation of pupal structures first becomes evident with the appearance of a transverse row of rectangular cells along the intersegmental line separating the head from the prothorax. This is followed by development of the pupal antennal cases. Next, the pupal mouthpart and the leg and wing cases develop simultaneously. The leg and wing cases are directed toward the midline of the prepupa (fig. 23), and the constriction between the pupal head and thorax is now apparent. The abdomen appears to be the last part of the body to become differentiated.

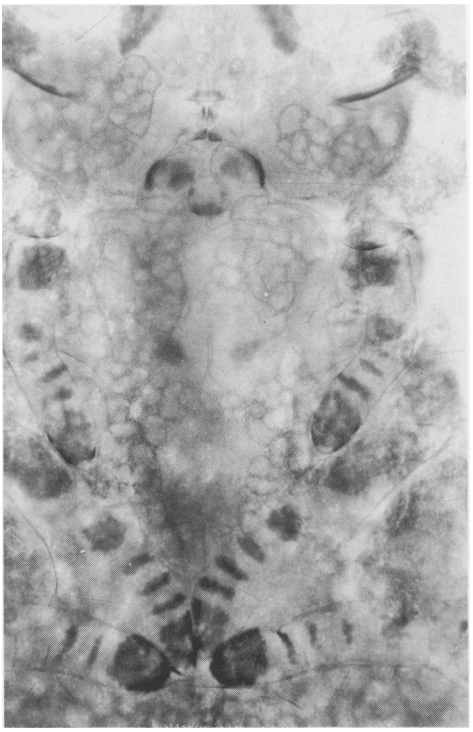


Fig. 23. Prepupa, showing partial pigmentation of pupal appendages; the leg and wing cases are directed toward the midline.

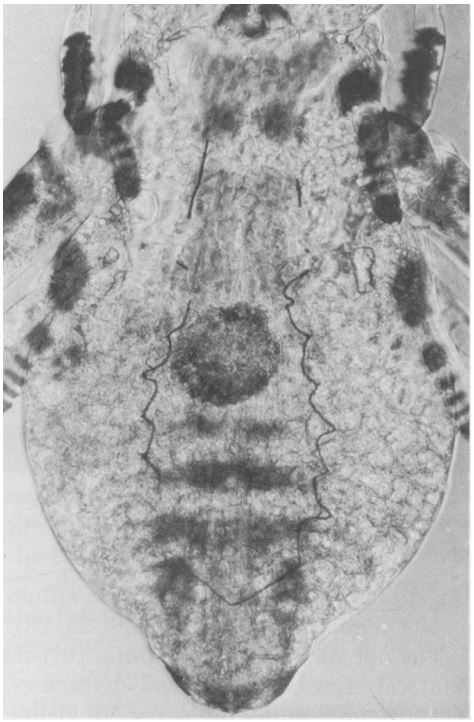


Fig. 24. Newly formed pupa; the appendages are still only partly pigmented, but have migrated to the sides of the body.

Pigmentation of pupal organs progresses in the following order: first the inner margins of the eyes (adjacent to the frontovertex), then the bases and tips of antennal cases, the tips of mouthpart, leg and wing cases, and finally the tip of the abdomen. Pigment then spreads gradually from the tip to the base of each organ, except for the leg cases where pigmentation of the tip is followed by the appearance of three distinct bands and a basal blotch (figs. 23, 24).

The pupa

Pupation, or the ecdysis marking the onset of the six- to seven-day pupal stage, usually occurs on the 14th day after oviposition, when partial pigmentation has been attained. During the molting process, the leg and wing cases migrate to the sides of the body (fig. 24). The young pupa soon becomes uniformly black, except for the venter of the abdomen which remains partly greyish yellow. The crumpled larval exuvium often adheres to the tip of the abdomen.

As in other Hymenoptera, the pupa of *A. chilensis* is exarate. It is 975 microns long (830 to 1052) and 442 microns wide (380 to 510); the head is 330 microns wide. The pupa is distinctly flattened dorsoventrally, only up to 250 microns thick. It invariably lies on its back, with its ventral aspect (and mouthparts) facing the covering scale of the host.

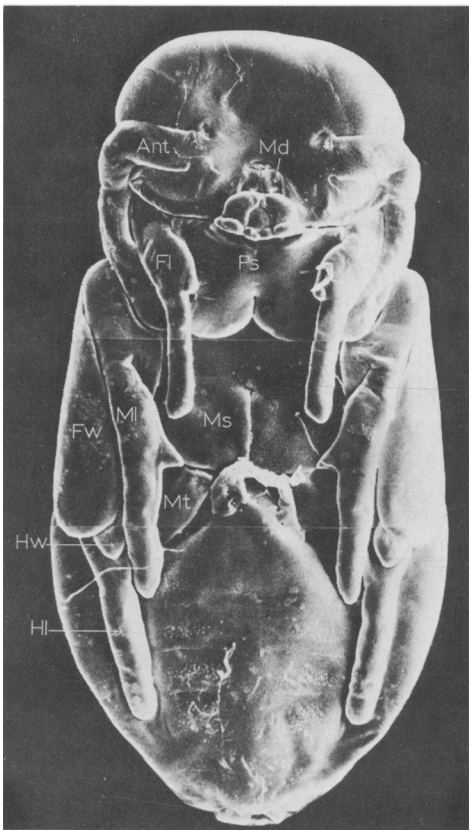


Fig. 25. Female pupa, ventral view (a composite scanning electron micrograph). Ant = antennal case; Fl = foreleg case; Fw = fore wing case; Hl = hind leg case; HW= hind wing case; Md = mandible; Ml = middle leg case; Ms = mesosternum; Mt = metasternum; Ps = prosternum.

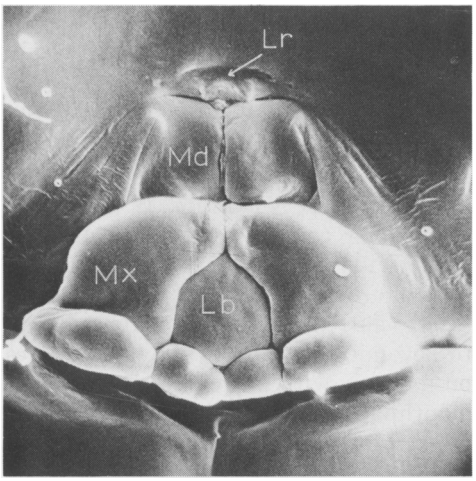


Fig. 26. Pupa: mouthpart cases (scanning electron micrograph). Lb = labium; Lr = labrum; Md = mandible; Mx = maxilla.

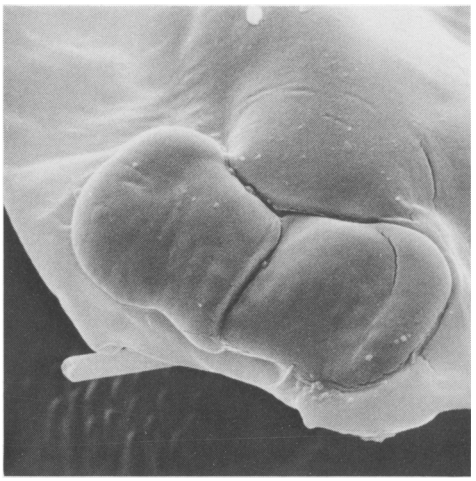


Fig. 27. Female pupa: rectangular plates near tip of abdomen (scanning electron micrograph).

On the ventral aspect of the pupa (fig. 25), the geniculate antennal cases (Ant) are present on each side of the head. Between their bases are the mouthpart cases (figs. 25, 26): the labrum (Lr) is small and transverse, and immediately below it are the quadrate mandibles (Md); the pupal maxillae (Mx) are two-segmented, but the labium (Lb) is composed of three parts. The prosternum (Ps) and mesosternum (Ms) are large, bilobed plates, whereas the metasternum (Mt) is represented by two triangular plates. Six sternal plates are evident in the abdomen. The cases of the forelegs (Fl) are situated mesad and caudad of the antennal cases; they are followed by the cases of the middle legs (Ml) and hind legs (Hl). A triangular process on the middle leg case marks the site of the developing mid-tibial spur. The fore wing cases (Fw) are situated mesad of the middle legs, with the tips of the hind wing cases (Hw) protruding from underneath their caudal edge. In the female pupa, two minute subrectangular plates are present ventrally near the tip of the abdomen (fig. 27), but are absent in the rare male pupa. The integument of the pupa is generally smooth except for sublateral patches of minute spines on the abdominal sternites (figs. 25, 28).

The dorsal aspect of the pupa reveals relatively few morphological characters. The antennal and wing cases are visible also from this side. The dorsum of the thorax appears to be undivided, whereas the abdominal region consists of 10 poorly defined tergites, each marked by a pair of sublateral patches of minute spines. Three pairs of spira-

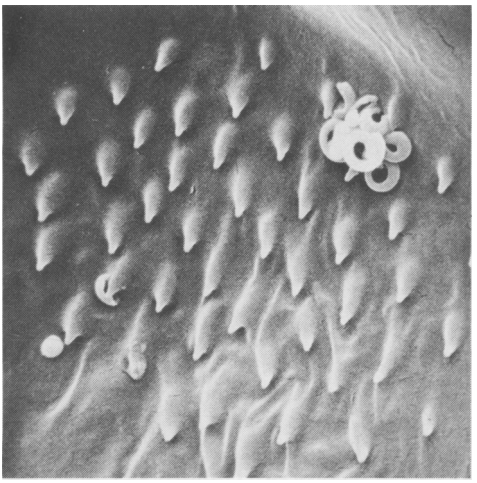


Fig. 28. Patch of spines on abdominal sternite of pupa (scanning electron micrograph); the rings are wax particles from the scale insect host.

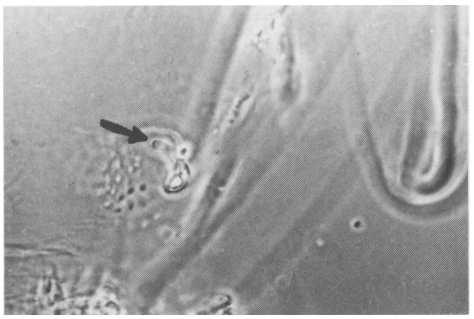


Fig. 29. Pupa: posterior abdominal spiracle, showing blind branch of trachea (indicated by arrow).

cles are present: one concealed on the pronotum, one on the propodeum and one on the eighth abdominal tergite. In the posterior spiracle (figs. 29, 30), the trachea forms a short, blind branch, in addition to the trunk that leads to the tracheal system of the pupa. The opening itself is covered by a protective cuticular operculum.

The eyes of the pupa gradually turn from pale cream color to red to greyish green, thus providing an indication of the age of the pupa. The pupal stage lasts six to seven days, or about one-third of the total developmental period of *A. chilensis*.

Emergence of the adult

Eclosion occurs underneath the covering scale of the host. The pupal skin is broken into three parts. The cephalic exuvium, including the antennal cases, and the thoracic exuvium, including the leg and wing cases, are strongly sclerotized and usually recognizable after emergence, in contrast, the abdominal exuvium is considerably thinner and much more fragile, and is often torn and fragmented.

Emergence from the host is usually effected by chewing an exit hole through the covering scale. Like the pupa, the emerging wasp lies on its back, with its ventral aspect—and mouthparts—facing the covering scale. It gnaws a small opening in the scale, then extends one mandible through it and proceeds to chew, in scissor-like fashion, a circular or somewhat oval hole. From time to time, the wasp stops gnawing and retracts its head, presumably to discard the pieces of cut material. In later stages, it tries to push its head through the hole. As soon as the exit hole is large enough, the wasp rotates its head through it, first one eye and then the other. It then slowly extricates itself, until the forelegs become free and help pull the rest of the body out. The entire process, from the onset of gnawing to complete emergence, takes about 30 minutes. If more than one wasp developed on a single host, they all invariably emerge through one exit hole.

The newly emerged wasp rests for several minutes, then proceeds to groom its body. Grooming is usually performed in clockwise order, from front to rear. First, the right foreleg is used to clean the eyes, the head, and the right antenna. The middle leg then cleans the foreleg. The hind leg cleans the middle leg, and subsequently grooms the abdomen and wings. The wasp then rubs both hind legs and proceeds to groom the other side of the body. The grooming process is repeated several times before the wasp finally moves away.

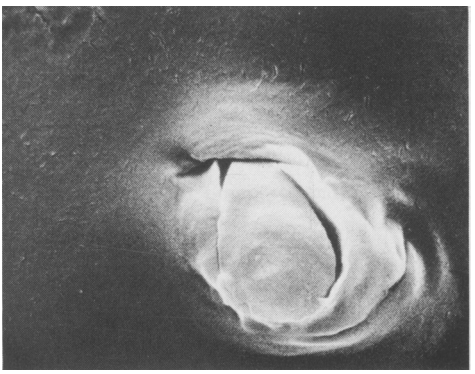


Fig. 30. Pupa: posterior abdominal spiracle, showing protective operculum (scanning electron micrograph).

EFFECT OF TEMPERATURE ON THE DURATION OF DEVELOPMENT

Small host plants, about equally infested with oleander scale, were placed singly in 500-centimeter[3] glass jars. Several scores of *A. chilensis* females were introduced into each jar, and were allowed to oviposit in the scale insects for 1 hour at 28 ± 1° C and 70 ± 5 percent relative humidity. The host plants were then removed, cleaned thoroughly with a flow of CO_2, and transferred into clean, silk-covered jars that were placed in incubators at constant temperatures of 19°, 24°, 28°, and 32° C. Two host plants were placed at each temperature, and parasite emergence was recorded daily. The results are presented in table 1.

The equilateral hyperbola equation, assuming a thermal constant that is a product of developmental time and temperature, is a convenient way of expressing the effects of temperature on the duration of development of invertebrates (Bodenheimer, 1926, 1958). The hyperbola equation for *A. chilensis* was calculated using the reciprocal of the duration of development at the various temperatures and calculating the regression line by the least squares method. The parameters of the equilateral hyperbola were obtained from the straight line equation.

TABLE 1
DURATION OF DEVELOPMENT OF *APHYTIS CHILENSIS* AT VARIOUS CONSTANT TEMPERATURES

Temperature (°C)	Duration of development (days)		Number of emergent parasites
	Average	Range	
19	48.8	45–61	144
24	25.5	24–30	113
28	18.6	17–21	66
32	–	–	–

Fig. 31 gives the regression line and corresponding equilateral hyperbola,

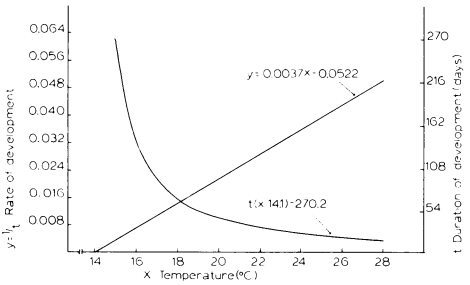


Fig. 31. Effect of temperature on the duration and rate of development of *Aphytis chilensis*.

calculated for *A. chilensis* on the basis of our experimental rearing data. The equation of the regression line is: $y = 0.0037x - 0.00522$, and the equation of the hyperbola is: $t\ (x - 14.1) = 270.2$. In other words, the theoretical threshold of development of *A. chilensis* is 14.1° C, and a thermal constant of 270.2 day-degrees is required for the completion of development.

Development of *A. chilensis* appears to be considerably slower, and the developmental threshold higher, than in several other members of the genus *Aphytis*. For instance, at 28° C, development of *A. chilensis* averaged 18.6 days, compared to 16 days in *A. hispanicus* (Mercet) (Gerson, 1968) and 12 days in *A. coheni* DeBach (Avidov *et al.*, 1970). The theoretical threshold of development was calculated as 12.9° C for *A. hispanicus* (Gerson, 1968), 12° C for *A. coheni* (Avidov *et al.*, 1970), 11° C for *A. melinus* DeBach and only 8.5° C for *A. chrysomphali* (Mercet) (Abdelrahman, 1974). It appears, therefore, that under similar conditions *A. chilensis* will have fewer annual generations than any of these species.

ACKNOWLEDGMENTS

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LITERATURE CITED

ABDELRAHMAN, J.
1974. Growth, development and innate capacity for increase in *Aphytis chrysomphali* Mercet and *A. melinus* DeBach, parasites of California red scale, *Aonidiella aurantii* (Mask.), in relation to temperature. Austral. Jour. Zool. 22:213–30.

AVIDOV, Z., M. BALSHIN, and U. GERSON
1970. Studies on *Aphytis coheni*, a parasite of the California red scale, *Aonidiella aurantii*, in Israel. Entomophaga 15:191–207.

AZIM, A.
1963*a*. *Aphytis cylindratus* Compere (Hymenoptera, Aphelinidae), an effective parasite of *Pseudaonidia duplex* Cockerell. Mushi 37:53–63.
1963*b*. Systematic and biological studies on the genus *Aphytis* Howard (Hymenoptera, Aphelinidae) of Japan. Part 2. Biology and mass production. Jour. Fac. Agric. Kyushu Univ. 12:291–321.

BEIRNE, B. P.
1941. A consideration of the cephalic structures and spiracles of the final instar larvae of the Ichneumonidae (Hym.). Trans. Soc. Brit. Ent. 7:123–190.

BODENHEIMER, F. S.
1926. Über die Voraussage der Generationenzahl von Insekten. II. Die Bedeutung des Klimas für die landwirtschaftliche Entomologie. Z. Angew. Ent. 12:91–122.
1958. Animal ecology today. Monogr. Biol. VI, Junk, den Haag, 276 pp.

ČAPEK, M.
1969. An attempt at a natural classification of the family Braconidae based on various unconventional characters (Hymenoptera). Proc. Ent. Soc. Wash. 71:304–12.
1970. A new classification of the Braconidae (Hymenoptera) based on the cephalic structures of the final instar larva and biological evidence. Can. Ent. 102:846–75.

CLAUSEN, C. P.
1940. Entomophagous insects. McGraw-Hill, New York and London, 688 pp.
1942. The relation of taxonomy to biological control. Jour. Econ. Ent. 35:744–48.

COMPERE, H.
1955. A systematic study of the genus *Aphytis* Howard (Hymenoptera, Aphelinidae) with descriptions of new species. Univ. Calif. Publ. Ent. 10:271–319.
1969. The role of systematics in biological control: A backward look. Israel Jour. Ent. 4:5–10.

DEBACH, P.
1959. New species and strains of *Aphytis* (Hymenoptera, Eulophidae) parasitic on the California red scale, *Aonidiella aurantii* (Mask.), in the Orient. Ann. Ent. Soc. Amer. 52:354–62.
1964. Some species of *Aphytis* Howard (Hymenoptera: Aphelinidae) in Greece. Ann. Inst. Phytopathol. Benaki, N.S. 7:5–18.

DEBACH, P., and J. LANDI
1961. The introduced purple scale parasite, *Aphytis lepidosaphes* Compere, and a method of integrating chemical with biological control. Hilgardia 31:459–97.

DEBACH, P., and D. ROSEN
1976. Armoured scale insects. Chapter 6 *in:* Studies in Biological Control (V. L. Delucchi, editor). Int. Biol. Prog. Vol. 9, Cambridge University Press, pp. 139–78.

DEBACH, P., D. ROSEN, and C. E. KENNETT
1971. Biological control of coccids by introduced natural enemies. Chapter 7 *in:* Biological Control (C. B. Huffaker, editor). Plenum Press, New York and London, pp. 165–94.

DELUCCHI, V., D. ROSEN, and E. I. SCHLINGER
1976. Relationship of systematics to biological control. Chapter 4 *in:* Theory and Practice of Biological Control (C. B. Huffaker and P. S. Messenger, editors). Academic Press, New York and London, pp. 81–91.

FINLAYSON, T.
1960*a*. Taxonomy of cocoons and puparia, and their contents, of Canadian parasites of *Neodiprion sertifer* (Geoff.) (Hymenoptera: Diprionidae). Can. Ent. **92**:20–47.
1960*b*. Taxonomy of cocoons and puparia, and their contents, of Canadian parasites of *Diprion hercyniae* (Htg.) (Hymenoptera: Diprionidae). Can. Ent. **92**:922–41.
1962. Taxonomy of cocoons and puparia, and their contents, of Canadian parasites of *Diprion similis* (Htg.) (Hymenoptera: Diprionidae). Can. Ent. **94**:271–82.
1963. Taxonomy of cocoons and puparia, and their contents, of Canadian parasites of some native Diprionidae (Hymenoptera). Can. Ent. **94**:475–507.
1967*a*. A classification of the subfamily Pimplinae (Hymenoptera: Ichneumonidae) based on final-instar larval characteristics. Can. Ent. **99**:1–8.
1967*b*. Taxonomy of final-instar larvae of the hymenopterous and dipterous parasites of *Acrobasis* spp. (Lepidoptera, Phycitidae) in the Ottawa region. Can. Ent. **99**:1233–71.
1975. The cephalic structures and spiracles of final-instar larvae of the subfamily campopleginae, tribe Campoplegini (Hymenoptera: Ichneumonidae). Ent. Soc. Can. Mem. **94**, 137 pp.

FLANDERS, S. E.
1951. Mass culture of the California red scale and its golden chalcid parasites. Hilgardia **21**:1–42.

GERIG, L.
1960. Zur Morphologie der Larvenstadien einiger parasitischer Hymenopteren des Grauen Lärchenwicklers (*Zeiraphera griseana* Hübner). Z. Angew. Ent. **46**:121–77.

GERSON, U.
1968. The comparative biologies of two hymenopterous parasites of the chaff scale, *Parlatoria pergandii*. Entomophaga **13**:163–73.

GRISWOLD, G. H.
1925. A study of the oyster-shell scale, *Lepidosaphes ulmi* (L.), and one of its parasites, *Aphelinus mytilaspidis* Le B. Part II. Biology of a parasite of the oyster-shell scale. Cornell Univ. Agr. Exp. Sta. Mem. **93**:57–67.

HAGEN, K. S.
1964. Developmental stages of parasites. Chapter 7 *in:* Biological Control of Insect Pests and Weeds (P. DeBach, editor). Chapman and Hall, London, pp. 168–246.

HINTON, H. E.
1976. Notes on neglected phases in metamorphosis, and a reply to J. M. Whitten. Ann. Ent. Soc. Amer. **69**:560–66.

HOWARD, L. O.
1900. A new genus of Aphelininae from Chile. Can. Ent. **32**:167–68.

IMMS, A. D.
1916. Observations on the insect parasites of some Coccidae. I.—On *Aphelinus mytilaspidis* Le Baron, a chalcid parasite of the mussel scale (*Lepidosaphes ulmi* L.). Quart. Jour. Microsc. Sci., N.S. **61**:217–74, pl. 19–20.

MACKAUER, M., and T. FINLAYSON
1967. The hymenopterous parasites (Hymenoptera: Aphediidae et Aphelinidae) of the pea aphid in Eastern North America. Can. Ent. **99**:1051–82.

MAPLE, J. D.
1947. The eggs and first instar larvae of Encyrtidae and their morphological adaptations for respiration. Univ. Calif. Publ. Ent. **8**:VIII+25–122.

MICHENER, C. D.
1953. Comparative morphological and systematic studies of bee larvae with a key to the families of hymenopterous larvae. Univ. Kansas Sci. Bull. **35**:987–1102.

NIKOL'SKAYA, M. N., and V. A. YASNOSH
1966. Aphelinids of the European part of the U.S.S.R. and the Caucasus. Opred. Faun. SSSR 91. Nauka, Moscow and Leningrad, 296 pp. (In Russian).

PARKER, H. L.
1924. Recherches sur les formes post-embryonnaires des chalcidiens. Ann. Soc. Ent. Fr. **93**:261–379, pl. 2–39.

QUAYLE, H. J.
1910. *Aphelinus diaspidis* Howard. Jour. Econ. Ent. **3**:398–401.

ROSEN, D., and P. DEBACH
1973. Systematics, morphology and biological control. Entomophaga **18**:215–22.
1976*a*. Biosystematic studies on the species of *Aphytis* (Hymenoptera, Aphelinidae). Mushi **49**:1–17.
1976*b*. Diaspididae. *In:* Introduced Parasites and Predators of Arthropod Pests and Weeds: A World Review (C. P. Clausen, editor), pp. 78–128. Agr. Handbook 480, U.S.D.A.
SABROSKY, C. W.
1955. The interrelations of biological control and taxonomy. Jour. Econ. Ent. **48**:710–14.
SADAVA, D., and C. D. F. MILLER
1967. Taxonomy of last-instar larval remains of parasites reared from *Spilonota ocellana*. Can. Ent. **99**:436–42.
SCHLINGER, E. J., and R. L. DOUTT
1964. Systematics in relation to biological control. Chapter 8 *in:* Biological Control of Insect Pests and Weeds (P. DeBach, editor). Chapman and Hall, London, pp. 247–80.
SHORT, J. R. T.
1952. The morphology of the head of larval Hymenoptera with special reference to the head of the Ichneumonoidea, including a classification of the final instar larvae of the Braconidae. Trans. R. Ent. Soc. London **103**:27–84.
1953. A grouping by larval characters of some species of the genus *Apanteles* (Hymenoptera: Braconidae). Bull. Ent. Res. **44**:327–32.
1959. A description and classification of the final instar larvae of the Ichneumonidae (Insecta, Hymenoptera). Proc. U.S. Nat. Mus. **110**:391–511.
TAYLOR, T. H. C.
1935. The campaign against *Aspidiotus destructor*, Sign., in Fiji. Bull. Ent. Res. **26**:1–102.
THORPE, W. H.
1930. Observations on the parasites of the pine-shoot moth, *Rhyacionia buoliana*, Schiff. Bull. Ent. Res. **21**:387–412.
TRABOULSI, R.
1969. Contribution à l'étude des *Aphytis* Howard du Liban (Hym. Chalcidoidea, Aphelinidae). Ann. Soc. Ent. Fr. (N.S.) **5**:5–72.
YASNOSH, V. A.
1972. On the biosystematic characteristics of species of the genus *Aphytis* Howard (Chalcidoidea, Aphelinidae)—parasites of scale insects in the U.S.S.R. Ent. Obozr. **51**:240–53. (In Russian). (English transl.: Ent. Rev. **51**:146–52.)