THE EGGS AND FIRST INSTAR LARVAE OF ENCYRTIDAE AND THEIR MORPHOLOGICAL ADAPTATIONS FOR RESPIRATION

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JOHN D. MAPLE

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THE SECRETARY OF THE NAVY
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The President of the United States takes pride in presenting the BRONZE STAR MEDAL posthumously to

LIEUTENANT JOHN DINWIDDIE MAPLE, HOSPITAL CORPS
UNITED STATES NAVAL RESERVE

For service as set forth in the following:

CITATION:

"For meritorious achievement while serving as Member of the First Advance Party of United States Naval Medical Research Unit Number Two in furthering the development and introduction of techniques of aircraft spraying of DDT for the control of disease-transmitting insects in the Southwest Pacific War Area from April 23, 1944 to April 11, 1945. Working tirelessly and with superb efficiency, Lieutenant Maple rendered invaluable service in conducting biological tests under field conditions and in instructing malaria control personnel on Guadalcanal in the use of DDT for mosquito control. In addition, he assisted in the formulation of general plans and procedures to be followed in preparation for our operations against enemy-held Peleliu and Iwo Jima, and at Okinawa, supervised the technical aspects of aircraft-spraying activities during the initial stages of the campaign. Mortally wounded when his plane crashed during an extremely hazardous spraying mission over Okinawa on April 11, Lieutenant Maple, by his outstanding professional ability and sound judgment, had contributed to the saving of many lives and to the successful use of a new method for controlling disease in the future. His conscientious devotion to duty throughout reflects the highest credit upon himself and the United States Naval Service. He gallantly gave his life in the service of his country."

For the President,
(s) JAMES FORRESTAL
Secretary of the Navy

FOREWORD

THIS PUBLICATION is based principally on research conducted by John Maple in the course of his graduate studies in the Division of Beneficial Insect Investigations at Riverside, 1935–1938. The report was revised and expanded by him from time to time, and was submitted in final form as a thesis in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of California in 1940. This final revision was made in Japan, where Maple was engaged in the collection and shipment of beneficial insects to the United States for the U. S. Department of Agriculture. It was then expected that the manuscript would be submitted to the University of California Press for publication in the Entomology series, but the war made this impossible. Before peace came the author met tragic death on Okinawa.

The manuscript represents an outstanding contribution to knowledge of the biology of the Encyrtidae, a family of parasitic insects of great economic significance. The importance of the work is such as to justify its posthumous publication. Slight revisions of certain statements on biologies have been made by Stanley E. Flanders in order to bring them in line with recent discoveries. These are indicated. Also, the bibliography has been brought up to date. Otherwise, except for routine editorial changes, the paper is as the author submitted it.

HARRY S. SMITH
Professor of Biological Control
Entomologist, Experiment Station

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THE EGGS AND FIRST INSTAR LARVAE OF ENCYRTIDAE AND THEIR MORPHOLOGICAL ADAPTATIONS FOR RESPIRATION

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JOHN D. MAPLE

INTRODUCTION

THE FAMILY ENCYRTIDAE is one of the most important families in the order Hymenoptera. Since it is a member of the superfamily Chalcidoidea, it quite naturally possesses peculiar adaptations of great biological interest.

Encyrtids attack many kinds of hosts, the most numerous of which are the scales and mealybugs (Coccidae), a group of great economic importance, particularly in California. One of the functions of the Citrus Experiment Station of the University of California is to search in foreign countries for parasites of coccids and to undertake their introduction, propagation, and liberation. As a result of this work, a number of species of Encyrtidae were available for study, and the quantity of material offered excellent opportunities for research. In addition to the introduced and established species, I discovered a large number of endemic species of Encyrtidae.[1]

This study of the peculiar adaptations of the encyrtid egg and larva was prompted by an observation on the biology of one of the introduced species at the Citrus Experiment Station reported to me by S. E. Flanders. While engaged in the propagation of *Leptomastix dactylopii* How., a parasite of *Pseudococcus citri* (Risso) introduced from South America in 1935, Flanders observed a white elongate area on the deposited egg of the parasite. Since the white or silvery color gradually, yet irregularly, disappeared when the eggs were compressed in certain solutions, he concluded that the silvery appearance was due to air contained in that portion of the egg shell. Searching the literature for a similar phenomenon, Flanders found that F. Silvestri had arrived at the same conclusions with regard to another species of Encyrtidae nearly eighteen years earlier. However, Flanders also noted that the young larvae of *Leptomastix dactylopii* had their spiracles firmly attached to the silvery area. Having observed a similar vital connection between the eggs and young larvae of an *Anagyrus* parasitizing the mealybug *Puto yuccae* (Coq.), I (Maple, 1937) sought other encyrtids for a comparative study.

DEFINITION AND SCOPE OF INVESTIGATIONS

The ovarian egg of Encyrtidae is like a dumbell in shape, consisting of two more or less distinct bodies with a narrow connecting tube. This type of egg, however, is not peculiar to Encyrtidae, for similar shapes can be found in other chalcidoid families and in other superfamilies of Hymenoptera. It has been stated that this shape enables a large egg to pass through a small ovi-

[1] [The new species included in this study were described by Compere (1947) in order that they might be designated by name in this paper. S.E.F.]

positor. In some of the parasitic species the connecting tube may also serve as a stalk for adherence to external surfaces or to internal organs. In fewer species it may remain inserted in the integument of a host insect and serve merely as an anchor.

In the Encyrtidae, however, the structure has a distinctly peculiar significance. In the deposited egg a part of the connecting tube may remain exterior to the host, the remainder passing through the integument to suspend within the host the portion of the egg in which the embryo will develop. The larva upon hatching may remain with its posterior end enclosed by the egg shell. If it is provided with spiracles it may respire air conveyed by the connecting stalk.

Silvestri (1919) showed only that the shells of some encyrtid eggs were structurally modified to contain air after deposition. He ascribed no function to this structure. I believed that the young encyrtid larva, if provided with an open tracheal system, obtained atmospheric air directly from this structure and that the lumen of the stalk which suspends the larva did not serve this purpose in any way. As a corollary it was contended that a larva with caudal spiracles is enabled to respire atmospheric air by no other means. Accordingly this investigation was begun to seek the validity of these contentions.

An investigation thus defined would entail a study of structure and function. It was not possible for me to conduct exhaustive research in either morphology or physiology. Only the gross structure end, to a lesser extent, the internal structure were examined. From the beginning it was apparent that the physiology of respiration was a manifold problem warranting independent investigations and could not well be included in the scope of this study. It was determined to undertake a limited number of simple experiments which would throw some light on the role of the egg shell in respiration. In the main the subject was approached with respect to comparative morphology. Although the Encyrtidae is a large family and a study of the eggs and larvae of all known species would be an almost endless undertaking, I sought to include a representative number of genera from a variety of hosts. Included in this work, therefore, are parasites attacking seven orders of insects, namely, Homoptera, Hemiptera, Neuroptera, Lepidoptera, Coleoptera, Diptera, and Hymenoptera. Emphasis was inevitably placed on species attacking the family Coccidae of the Homoptera because of the abundance of native and introduced species parasitizing that group. For data concerning other hosts, I have drawn largely upon the literature. Species known to be polyembryonic were excluded since these have been abundantly studied and the mode of development is such that the egg shell could not conceivably play any role in larval respiration of atmospheric air.

PROCEDURE AND TECHNIQUE

Acquisition of Material

Material was obtained by insectary propagation and field collection. During the course of this work the University of California Citrus Experiment Station was particularly active in the introduction of scale and mealybug parasites, a large number of which were Encyrtidae. Through the courtesy of Professor Harry S. Smith, in charge of biological control work, adults of certain of the species and host material were made available.

Most of the species were secured from field-collected material. Quantities of any insect known to be attacked by encyrtids were gathered and caged for possible emergence of adult parasites. The variety of parasite species obtained by this means was considerable.

All parasites obtained were fed undiluted honey immediately after emergence. Pending a suitable opportunity for study they were refrigerated at 36° to 40° F. and removed every week to the laboratory for feeding. When stored in this manner they could be kept alive in excellent condition for several weeks.

Examination of Ovarian Eggs

For an investigation of the ovarian eggs the abdomen was severed from the body in Ringer's normal salt solution and slit open along both sides.[2] The ovaries were removed intact. For cursory examination they could be mounted in the dissecting solution.

For detailed study further treatment was necessary. After dissection, most of the fluid was drawn off and a drop of stain solution applied. The only stain at all suitable was a solution of acid fuchsin in lactophenol.[3] A minimum of five minutes was necessary to produce an effective coloration. For mounting, the stain was removed as much as possible and a drop of Hoyer's solution added. The cover glass was carefully pressed so as to spread the ovarioles and straighten the eggs which are often coiled in the ovarioles. The slides were immediately examined, the egg sketched with the aid of a camera lucida, and the dimensions determined.

Mounts prepared in this fashion are not completely satisfactory. The most serious disadvantage is the lack of permanency. After several months, Hoyer's solution tends to crystallize and the stain begins to fade. It is believed that crystallization was avoided by placing slides in a warm oven until the mounting medium was sufficiently hard for ringing the cover slips with shellac. It has not been possible wholly to eliminate the defects of preparation. The technique can also be criticized on the grounds of distortion of the eggs. Pressure applied to the cover glass causes the eggs to expand, and as a result the measurements of eggs of a species differ and the shape does not resemble that of the freshly dissected egg. I believe that exact measurements are of little consequence and that measurements need only indicate relative size. Furthermore, without pressure on the cover glass, detailed examination would have been almost impossible. The fact that the mounted egg is not exactly like the freshly dissected egg is of no importance. The latter is invariably coiled, twisted, and partly collapsed. The mounted egg appears more like the normal deposited egg than the freshly dissected one does and its structure can be more accurately ascertained.

[2] Usually encyrtids contain eggs immediately after emergence, but in several species the females lacked mature ovaries. These were all from single generation scales and had emerged from fully developed hosts at a period when no stages of that host were suitable for attack.

[3] Suggested by the preparation outlined by Langeron (1934). It consists of 50 grams phenol, 50 cc lactic acid, 50 cc distilled water, and ½ gram acid fuchsin.

Securing Oviposition

Since most of the species studied attack Coccidae, the problem of obtaining oviposition was largely concerned with that group. The technique used was dependent upon the type of host and the stage attacked. If the hosts were mealybug-like (motile in nymphal and adult stages) the chances of success were more favorable than if they were scalelike (usually sessile in the later instars). Many of the former can be grown rapidly and in quantity on potato sprouts in the insectary. Female parasites could be placed on the infested sprouts and the hosts removed as parasitized, or a host could be isolated in a capsule for parasitizing. Usually this type of host could be isolated without food for a period sufficient for a study of the early stages and in some specimens for complete development. When very small stages were attacked, it was necessary to supply the food plant. Those species that could not be insectary grown were obtained from the field in suitable stages, parasitized, and isolated in capsules.

Parasites of scale insects posed more of a problem, particularly field-collected species. The females of some did not develop mature ovaries nor could development be induced. Others, in condition to oviposit, emerged at a time when hosts suitable for attack were not available. Some species attack hosts not readily reared, but when this happened the scale hosts could sometimes be kept alive on twigs of the host plant until a study of the early stages was completed. To avoid needless dissection, hosts were marked with a small drop of India ink as they were attacked.

Inevitably there were species that would not ovipost or that showed little interest in the coccid host during the experiments; but with most of them limited oviposition was eventually obtained. In the Encyrtidae mating is fortunately not a prerequisite of oviposition.

In this investigation the only noncoccid hosts of which complete studies of egg and larva were made were two species of Hemiptera. Since these hosts were attacked in the egg stage, the technique employed was relatively simple. They were exposed to parasitization in a vial. The number of female parasites was of no consequence unless they tended to interfere with one another. Once oviposition commenced the egg mass could be removed and the oviposition process observed. As each egg of an egg mass was parasitized, that egg was checked off on a diagram of the group.

It is always advisable to expose many individuals of the host for parasitism. The reasons for this are:

1) Eggs may not be deposited when the host is attacked.

2) There is always the possibility of loss from mortality by accidental or natural causes.

3) Numerous dissections are often necessary in order to confirm an observation.

4) If the species can be propagated it is highly desirable to maintain a stock for possible future work and to secure an adequate series for taxonomic purposes.

Studies of the Deposited Egg

First, the location of the deposited egg was sought by inspection of the host. If the eggs are attached to the integument, that portion which protrudes from the host may sometimes be distinguished, since it looks like a clubbed white seta. If the host has a roughened surface or is covered with wax, examination is tedious. When the entire host was placed in the stain solution used for the ovarian egg study, the stalk readily colored, whereas the host integument remained unchanged. The wax had to be removed from mealybugs to permit the stain to reach any stalk. It was more convenient to dissolve the wax with xylene, but since this solvent penetrates the egg, it was necessary to scrape the wax off carefully.

Upon dissection eggs were often found imbedded in host tissue. The host's integument could be pulled away with the egg attached, but there was less possibility of destruction if the eggs were freed from the tissue and the integument surrounding the stalk was excised.

In those species that did not deposit eggs attached to the host's integument, the egg was not readily found. It was then often necessary to watch the oviposition procedure, to record the exact spot, and to dissect cautiously around the area. Many eggs of this type are easily confused with the body contents of the host and the latter must be slowly examined for any egglike bodies. When these methods failed, the hosts were dissected in aceto-carmine, which stains the host's tissues and not the parasite eggs.

The deposited eggs were examined unmounted in the dissecting fluid and sketched. No measurements were made as they were not believed to have any bearing on the problem.

Studies of the Larva

The chief objective of the larval studies was to determine the type of tracheal system. From experience it was learned that the tracheae, when present, became obscure soon after the larva hatched. This seemed to be caused, in part at least, by the quantity and opaqueness of consumed food. During or immediately after eclosion, feeding begins and the major portion of the larva fills with colored host contents. This obstacle to observation was avoided by securing fully developed embryos. The shell of the egg is transparent and the tracheae appear almost invariably before eclosion. The margin of time for study, however, is very narrow. It was therefore necessary to parasitize a series of hosts and record the exact time of egg deposition in each one of the series. Dissections were then made at intervals until the information was obtained. In the meantime all material was kept at a constant temperature.

At first there were errors in the determination of the proper intervals for observation; however, experience reduced the number of mistakes. The deposited eggs of all species were examined 24 hours after deposition. (None was found to hatch prior to that time.) An examination of the contents of the egg disclosed the degree of embryonic development and the proximity of eclosion. At deposition the egg is white and opaque, and as development proceeds it gradually becomes translucent. This change commences at the periphery of the egg. The opaque portion, which is yolk, gradually becomes smaller but never disappears before eclosion. Eclosion occurs soon after the yolk body occupies one-third or less of the volume of the egg. By this time the stomodeal regions and frequently the tracheae may be apparent. Dissections therefore should be made every few hours thereafter. Embryonic development was usually 12 to 24 hours longer when the eggs were deposited free than when the eggs were attached to the integument. Consequently in examinations of the free eggs, the first interval could be extended to avoid waste of material.

The embryos or larvae were mounted and the higher magnifications of the microscope were used. At first, the different stages were mounted in the dissecting solution, but much skill was required. Too much fluid would float the material from under the cover glass or shift its position while it was being sketched. Examinations had to be rapid, for as the water evaporated the cover glass would descend to flatten the specimen. By use of a simple expedient these difficulties were largely eliminated. Small bits of modeling clay were placed at four or more equidistant places on the circumference of the cover glass, which was then lowered directly over the egg or larva floated in a very small drop of salt solution. While being watched under a binocular microscope, the cover glass was carefully pressed until it just touched the specimen; then, by means of gentle pushing at the sides of the cover glass, the material could be rotated on the longitudinal axis with little danger of rupture, and the egg or larva could be held in any desired position. As the water in the salt solution evaporated, distilled water was added. Observations could be made with ease over an extended period and there was little need for repetition which would diminish a limited stock of material.

Some embryos had not quite reached the development desired. Normal development was resumed for as long as 24 hours, however, when an egg was placed in a culture slide containing normal salt solution. Periodic observations were then easily made and the embryo could be obtained in the desired state of development. Certain phases were examined by these means that could not otherwise have been studied without numerous dissections. It is doubtful, however, if the resulting eclosion was normal; usually the embryo did not hatch even though it was completely developed.

The same technique was used for first instar larvae.

THE EGGS

Definition of Terms

The terms applied by investigators of encyrtid biologies to structures of the egg have been many and sometimes poorly descriptive of the true form or function. So far as possible I have eliminated the less desirable without adding new words or expressions.

The encyrtid egg is shaped like either a dumbbell or Indian club and consequently is called "double-bodied egg." The body, or posterior portion which is deposited first, contains the embryo and is therefore the "egg proper" or "egg" in its narrowest sense. The second body, or anterior portion bears the micropyle. Since in the process of oviposition this body collapses and its contents are forced into the egg proper, the designation "bulb" seems quite applicable. The bulb is joined to the egg proper by a narrow tube called the "neck." Although this structure has been commonly referred to by others as the "pedicel" or "stalk," I reserve these terms for neck and bulb collectively when the egg is in the deposited state, particularly with regard to those eggs that are attached to the integument of the host and partly protrude from it. Investigators have used the terms "stalked" or "pedicelated" eggs with reference to such eggs when deposited. I found this structure to be composed of two parts, the collapsed anterior body of the ovarian egg (bulb) and the connecting tube (neck). To avoid confusion "stalk" and "pedicel" are retained, but applied only to the combined structures.

For the terms pertaining to the egg shell, I have relied on the publications of F. Silvestri, since no other investigator has described and drawn these complicated structures. The reticulations on the egg proper Silvestri called the "band," "aeroscopic plate," and "respiratory plate." Since the reticulations are also found on the neck, I use these terms to designate the entire structure. They may be used almost interchangeably. "Band" indicates this structure when the egg is in the ovary, "aeroscopic plate" suggests its form and "respiratory plate" its function after deposition of the egg. Since its function is still more or less theoretical, the use of "band" or "aeroscopic plate" is preferable.

Comparative Morphology

It is common practice in a comparative study to attempt a classification of the various forms. In this work eggs are segregated primarily as an aid in discussion.

According to structural complexity, eggs may be divided into three groups: unbanded, modified or banded, and intermediate.

UNBANDED TYPE

Ovarian Egg.—The unbanded type of encyrtid egg is the simplest in form. There is no modification of the chorion whatsoever. Yet, like all encyrtid eggs, they are double-bodied; that is, there is a bulb, neck, and egg proper. There is little or no indication, however, of where the bulb ends and the neck begins. For convenience the neck was considered to be only that central portion which is uniform in diameter. The anterior point of increase in width was considered to be the beginning of the bulb.

The bulb is often only slightly wider than the neck, as in *Quaylea whittieri* (fig. 8, *B*) or *Eusemion californicum* (fig. 40), and in no species was it observed to be greater in width than the egg proper. The neck is of variable length. In *Quaylea* it is several times longer than the egg proper and in *Acerophagus pallidus* so short as to be almost nonexistent. The posterior end or egg proper is variable in shape. In many species it is broadest toward the neck, pointed at the apex, and dorso-ventrally more or less symmetrical. The smallest eggs are of this type, those of *Acerophagus* spp. being typical. In other relatively large species the egg proper is convex on the ventral side and only slightly convex or concave on the other. Among these species are found the largest eggs in the family. The eggs of *Cheiloneurus* spp., *Homalotylus* sp., and *Eusemion californicum* are notable examples.

Deposited Egg.—All the evidence at hand indicates that the eggs of the unbanded type are deposited completely free within the host. *Pseudaphycus angelicus* was, however, the only species investigated by the writer. In this the twisted and collapsed bulb is doubled back over the neck and soon melanizes to a light brown color. Other species which deposit their eggs intact are *Cerapterocerus mirabilis* and *Aphidencyrtus aphidivorus* (Silvestri, 1908), *Zarhopalus corvinus* (Clausen, 1924), *Comperiella bifasciata* (Compere and Smith, 1927), *Cheiloneurus noxius* (Le Pelley, 1937), and *Chrysopophagus compressicornis* (Clancy, unpublished).[4] Some of these eggs appear occasionally to be affixed to tissue, but in no species did the neck pass through the host's outer surface to form a projecting stalk.

The neck and bulb usually remain intact though they may be shriveled and collapsed. That the chorion may be unbroken was demonstrated with *Pseudaphycus angelicus.* A parasitized mealybug was dissected immediately after it was attacked. As rapidly as possible the egg was mounted in Hoyer's solution and pressure applied to the cover glass while the slide was examined under a microscope. Some of the contents of the egg proper entered the neck and returned to the bulb, as shown in figure 64, *B*. The egg could not, however, resume the original shape. When the eggs remained in normal salt solution for fifteen minutes or more, compression would burst the egg without reinflating the bulb.

The stalks or pedicels of these eggs eventually break off or disappear, and the shell may then be so weakened or so ruptured that eclosion of the larva is aided.

The eggs of several species, namely, *Cheiloneurus inimicus, Cheiloneurus noxius, Chrysopophagus compressicornis, Eusemion cornigerum, Tetracnemus pretiosus,* and *Zarhopalus corvinus,* have been reported by the various investigators to increase enormously in size after deposition.

BANDED TYPE

Ovarian Egg.—In this type the ovarian egg is more definitely two-bodied than in the unbanded type. The bulb is prominent and often larger than the egg proper. It is usually longer than wide, but sometimes is nearly spherical. The shell is pliable and elastic so that the shape varies in mounted material. The shell is sometimes thickened at the apex and has been said to bear the micropyle.

The neck varies greatly in length. In *Encyrtus fuliginosus* (fig. 30, *A*), it is shorter than either the bulb or the egg proper. In other species, such as *Microterys flavus* (fig. 51, *A*), *Phaenodiscus aeneus* (fig. 63), *Ooencyrtus johnsoni* (fig. 59, *A*), and *Diversinervus elegans,* the neck is two or more times as long as the egg proper. The diameter of the neck is proportionate to the size of the

[4] [Published in 1946. S.E.F.]

other bodies and is usually uniform throughout. In a few species the anterior end of the neck is swollen. This enlargement is correlated with band structure.

The egg proper has a typical shape. The dorsum is always flattened (at most slightly convex) or concave, and the venter invariably convex. The shell is comparatively firm though pliable.

These eggs are distinguished from other encyrtid eggs (and apparently those of all Hymenoptera) by the band or aeroscopic plate. On the neck and egg proper the shell may give the appearance of being sculptured in certain areas. These areas form a stripe down one side of the egg; hence the term "bands." Silvestri (1919) figured and described this structure on the eggs of five species, *Blastothrix sericea, Phaenodiscus aeneus, Encyrtus infidus, Metaphycus melanostomatus,* and *Microterys masii.* Strangely enough, his findings have been completely overlooked. Embleton (1904) probably saw the band on *Encyrtus infelix* because she described a portion of the stalk as having "an appearance of fine papillations or striations on the wall of the tube." Smith and Compere (1920) unquestionably found the same structure when they described a "ventral rib or stay" which extended about two-thirds the length of the egg of *Metaphycus lounsburyi,* giving it rigidity when the egg was inflated.

The band usually occurs on the flat dorsal surface of the egg. With *Encyrtus infelix* and *E. fuliginosus,* however, the curved ventral side bears the band. Even this genus is not uniform in this respect. *Encyrtus infidus,* according to Silvestri, has the dorsal surface banded.

Although the shape, size, extent, and composition of the band vary among species, they are essentially constant for any one species. An examination of the figures will show that no two bands are exactly alike. Most bands extend two-thirds to three-fourths the length of the egg proper. In *Metaphycus melanostomatus* (fig. 48) the band extends only half the length, in *Blastothrix longipennis* it is approximately one-third the length (fig. 13), and in *Encyrtus infelix* and *E. fuliginosus* it is almost the full length of the egg proper. There is much variation in width: it is broad in *Coccidoxenus niloticus* (fig. 21) and narrow in *Metaphycus flammeus* (fig. 66, *A*). The width may be uniform throughout the length (*Metaphycus luteolus,* fig. 47), or irregular (*Erythraphycus argyrocomus,* fig. 35). Some are pointed at the apex; others are rounded. Several species of *Ooencyrtus,* e.g., *O. johnsoni* (fig. 60), have the tip of the band forked.

The bands may be similar in shape and extent but different in composition. Large cells mixed in with smaller ones form a pattern of hexagons with *Coccidoxenus niloticus* (fig. 21) and elongate polygons with *Microterys flavus* (fig. 51). The cells in the band of *Erythraphycus argyrocomus* (fig. 35) are more or less uniform in size. In *Metaphycus howardi* (fig. 44) cell uniformity is broken by a patch of larger cells. The bands of *Ooencyrtus johnsoni* and *O. californicus* are exceptional in that throughout most of the middle area the cells are scattered. Differences are also apparent on the neck of the band. The diameter is usually the same throughout, but in *Anagyrus putonophilus* (fig. 3, *A*), *Coccidoxenus niloticus* (fig. 22, *A* and *B*) and *Pseudleptomastix squam-*

mulata (fig. 66, *B*), the neck is swollen near the bulb and the cells of the band at that point are large. Midway on one side of the neck in *Encyrtus infelix* and *E. fuliginosus*, there is a prominent projection (fig. 31) called the lip, which is actually a part of the band. In *Ooencyrtus johnsoni* (fig. 59, *B*), and *O. californicus*, a portion of the anterior end of the neck has a thickened wall. This is lacking in *O. kuvanae*. The band extends completely around the anterior end of the neck in only a few species—*Ooencyrtus* and a few others. Usually it does not cover more than half. The egg of *Anagyrus putonophilus* (fig. 3, *A*) has a collar or jutting, thickened area on the neck, without cells, the band passing underneath. A similar structure is found on the eggs of the intermediate type.

Banded eggs are by no means rare. Of the 61 species I examined, the eggs of 30 were banded; 14 out of 26 species recorded in the literature apparently have banded eggs. Of the total species in this study half the number have banded eggs. The relative occurrence of the two types in the family is a matter of conjecture but it is certain that banded eggs are far more common than has been heretofore supposed.

Deposited Egg.—In the process of oviposition the portion of the egg in which the embryo will develop, the egg proper, is placed within the host and the neck projects through the derm or shell to the exterior. Embleton was probably the first to observe this peculiarity but Howard and Fiske (1911) more clearly described the host-egg-larva relationship with *Ooencyrtus kuvanae*. At least eight investigators have reported the same phenomenon. Of these, Silvestri (1919) has given by far the most detailed and lucid account.

In connection with the deposited egg of *Phaenodiscus aeneus* one notation of Silvestri is very significant: if the egg, while fresh, is examined in physiological solution or in glycerine, the petiole and the dorsal plate appear black throughout their length because of the presence of air. As noted previously, S. E. Flanders made similar observations fifteen years later with *Leptomastix dactylopii*. I also observed the deposited eggs of a number of species, and all appeared the same. With transmitted light the band on the egg proper and the neck is dull gray or black and, with reflected light, shimmering white or silvery. In some ovarian eggs the bands were not discernible.

Most investigators have believed that the bulb is completely destroyed during oviposition. I find that this is not usually the case. The stalk normally consists of the neck and the remains of the bulb. The portion projecting from the host consists mostly of neck but at its tip is the crumpled bulb. In only one species, *Encyrtus fuliginosus*, has the bulb been observed to be almost invariably missing.

The position of the egg in the host is such that the flatter dorsal side lies outward. Thus, with most species, the band on the egg is directly opposed to the surface of the host. With *Encyrtus infelix* and *E. fuliginosus*, the band is on the convex ventral side and is therefore on the side of the egg farthest from the host's surface.

The stalks of all such species project erect or nearly so. In a few species (e.g., *Coccidoxenus niloticus*) the anterior end of the neck is somewhat curved.

The collapsed bulb may be in a tight wad or extended at an angle to the neck.

In a number of species the deposited egg melanizes. Usually melanization is limited to those portions within the host and in most species observed it was confined to the band. Soon after the egg is deposited, this section may become yellow-brown or gray. The eggs of six species were melanized, namely, *Aenasius maplei*, *Erythraphycus argyrocomus*, *Microterys flavus*, *Microterys saissetiae*, *Ooencyrtus johnsoni*, and *Ooencyrtus californicus*. Clancy [1946] observed the band to become discolored in *Isodromus niger*, and Smith and Compere (1920) have noted that the stalk of the egg of *Metaphycus lounsburyi* darkens. In *Ooencyrtus johnsoni* and *Erythaphycus argyrocomus*, the posterior two-thirds of the egg does not melanize until much later. In the former the band and the anterior third of the shell both discolor. The distal half of the band on the egg of *Microterys* is a lighter yellow-brown than the proximal half.

Melanization of the egg of *Ooencyrtus* spp. is peculiar in that the thickened portion of the neck, apart from the band, becomes an opaque black. Furthermore, this need not occur within the host. The egg blackens when, as occasionally happens, it is misplaced at deposition and all or part lies outside. The cause of this discoloration is unknown. Possibly oxidation would have this effect. There seems to be no correlation with structure. It is noteworthy that the melanization may take place in the ovaries of females that have been dead for several hours. I noted this with *Ooencyrtus johnsoni*, and Clancy informed me that he observed the same to occur with *Isodromus niger*. Frequently, if not always, there is an agglomeration of discolored host material around the stalk immediately below the integument of the host.

INTERMEDIATE TYPE

The eggs of the intermediate type are similar to both unbanded and banded eggs. Such a grouping, though arbitrary, will be shown to be significant.

Ovarian Egg.—The undeposited eggs are constant in shape. The egg proper is more or less flat on one side and convex on the opposite side. The bulb may be smaller or larger than the egg proper. In size and general appearance the ovarian eggs are like many of the unbanded type, but there are modifications of the chorion which would align them more closely with the group exhibiting bands. The degree of semblance is variable.

The eggs least similar to banded eggs are those with "collars"—a thickening of the shell in the region of the neck. This projection is readily colored by acid fuchsin. Its position is usually somewhat nearer the egg proper than the bulb or approximately midway the length of the egg. With *Apoanagyrus californicus* (fig. 11, *B*), *Apoanagyrus* sp. (fig. 9), and *Melanaphycus fumipennis* (fig. 42), the collars project prominently on one side, but with *Clausenia* sp. (fig. 20) the thickening appears to surround the stalk. The collar with *Apoanagyrus californicus* (fig. 11, *B*) is bidentate. The egg of *Isodromus iceryae* was noted by Clancy to have a transparent cone-shaped projection on the neck near the egg proper, within which there appeared to be a smaller but inverted cone. However, the identity of this structure with the so-called "collars" is a matter of conjecture. The possible significance of these neck structures is discussed in connection with the deposited egg.

In two species the eggs have a decided resemblance to the banded type, namely, *Leptomastidea abnormis* (fig. 11, *C* and *D*) and *Ectromatopsis americana* (figs. 27, *A*; 28, *B* and *C*). In the egg of *Leptomastidea* a small aggregation of cells restricted to the neck are as large and distinct as the cells of many true bands. The egg of *Ectromatopsis* perhaps could be classed with the banded type. On the anterior end of the neck there is a small cluster of cells. When the neck is viewed laterally, a row of very minute cells immediately below this cluster can be seen to extend the length of the neck. In the dorsal aspect no band is visible for a short distance below the cluster of cells; but thereafter it can be distinguished on the rest of the neck, though narrow and composed of minute cells. At the anterior end of the egg proper the band widens slightly. A bandlike area, devoid of cells, is discernible on the egg proper. On the anterior end of the neck there is a dentate collar which has a strong affinity for acid fuchsin. Above the collar the neck is bulbous or flask-shaped.

The egg of *Bothriothorax nigripes* has a vague resemblance to banded eggs. A fine line (fig. 17) extends along the neck for nearly its entire length. Though this stained readily, no cells could be found. No structures were visible on the egg proper and the neck is unornamented.

Deposited Egg.—Observations have shown that when the eggs of the intermediate type are deposited, the neck passes through the host's integument to project to the exterior; however, the attachment of the neck to the egg proper is extremely fragile. If dissections are not made immediately after oviposition and with enough precaution, the main body becomes disengaged and floats free. This fact undoubtedly accounts for the mention of free-floating eggs in *Leptomastidea abnormis* by Vierick (1915) and Smith (1917), and may explain similar records with other species which are said to lack a "pedicel" on the egg.

In the intermediate type, the portion of the egg left outside the host is less erect shortly after deposition than in the banded type, and may collapse entirely. If, as in *Melanaphycus fumipennis* (also *Isodromus iceryae*, according to Clancy) several eggs are laid in clusters or close together, the stalks may support each other and remain erect.

The deposited egg of *Ectromatopsis americana* may be more closely associated with the banded type. The attachment is quite firm. The narrow band on the neck has a silvery sheen immediately after deposition, but this sheen, which is considered significant in the light of larval structure and development, is lacking on the egg proper. The integument of the host surrounds the neck of a deposited egg immediately beneath the collar, if such a structure is present. Clancy informed me, however, that he found a similar structure on the egg of *Isodromus iceryae* completely within the host. No function was assigned to it. In either position these structures might serve as a sort of wedge to prevent the egg from slipping. Such a function, however, does not appear to be of any importance in this type of egg.

The bulb, though collapsed, probably remains intact. The experiment of inflating the bulb by crushing freshly deposited eggs was attempted with two species—*Melanaphycus fumipennis* and *Apoanagyrus* sp. Partial inflation was obtained with the former but none with the latter. There was a tangled membranous mass in the stalk of the *Apoanagyrus* which seemed to prevent any material from passing back into the bulb.

One instance of melanization was observed in which the portion of the stalk of the *Melanaphycus* egg lying within the host turned a gray color, visible through the host's integument, and thereby facilitated the location of the eggs.

STRUCTURE IN RELATION TO TAXONOMICAL AND BIOLOGICAL STUDIES

The classification of eggs may have considerable taxonomic significance. There are encyrtid genera in which the eggs of all the species studied belong to the unbanded type, e.g., *Cheiloneurus* and *Acerophagus*, or to the banded type, e.g., *Encyrtus* and *Metaphycus*. Since the intermediate type is indefinite, it is perhaps unlikely that genera exist which have such eggs exclusively. The egg of *Isodromus niger* according to descriptions by Clancy [1946] would belong to the banded type and that of *Isodromus iceryae* to the intermediate type. However, further taxonomic studies may reveal them to belong to two genera.

The egg structure also has some specific value since eggs may differ in type among species of a genus. Differences within the type, more particularly the banded group, are also of value. This is illustrated in my study on *Blastothrix* from lecanine scales. On the basis of adult characters the California encyrtid *Blastothrix longipennis* How. is regarded by such competent authorities as P. H. Timberlake and Harold Compere to be inseparable from *Blastothrix sericea* Dalm., and probably synonymous. I noted, however, that the band on the egg of *B. longipennis* was quite different from that described by Silvestri for *B. sericea*. On the basis of this evidence, as well as of minor differences, it seemed logical to assume that there are two species concerned, though the imagoes are as yet indistinguishable.

Specificity in egg structure may be of value in a study of the parasite fauna of a host. I had not been able to secure laboratory-deposited eggs of *Blastothrix longipennis* and recourse was had to field-parasitized scale. These scales had been heavily attacked by several encyrtids and aphelinids. There was only one egg the structure of which remotely resembled the ovarian egg of the *Blastothrix*, and it was assumed that this must be the deposited egg sought. Subsequently this appears to have been confirmed by rearing. *Blastothrix* emerged and the ovarian eggs of other species reared were the same as those found within the host scale.

Considering the great diversity in band structure, it might be thought that banded eggs are not difficult to distinguish specifically. The eggs of some species, however, are strikingly similar, e.g., *Ooencyrtus johnsoni* and *Ooencyrtus californicus*. The eggs of other species will probably be found to be indistinguishable. Without abundant evidence, the limitations of specificity cannot be defined, but most certainly a study of the egg structure can be of value in a biological investigation.

The presence of the band on the encyrtid egg enabled Flanders (1942) to demonstrate that the eggs of Hymenoptera are absorbed if they are retained in the ovary for lack of suitable oviposition sites. In *Clausenia* sp. (fig. 20) the ring bands, which remain unabsorbed, provide a record of all the eggs that have been produced and not deposited.

Oviposition

The manner in which the egg is deposited varies greatly among species and, to a minor degree, among individuals of a species. The only way in which oviposition in general could be classified would be according to its rate. Rate of oviposition is dependent upon three factors: speed of ovipositor insertion, ultimate destination of the egg, and number of eggs per insertion.

The penetration of the host's surface by the ovipositor is often the most time-consuming operation. For example, several minutes are required for *Ooencyrtus johnsoni* to bore a hole through the shell of the host egg. The shortest period was nine minutes but fourteen minutes or more may elapse. Other parasites require similar periods. Clancy [1946] found that the penetration of the *Chrysopa* integument by *Chrysopophagus compressicornis* was a lengthy process. On the other hand, with many parasites, particularly those attacking soft-bodied coccids, the ovipositor penetrates with one quick thrust.

The final position of the egg, as well as its structure, may influence the rate. The banded and intermediate types require the most time. These, we have seen, are placed with the neck projecting outside the host. The egg-laying procedure follows a definite course. Once the ovipositor is inserted, a distinct pause ensues during which the egg proper is presumably placed in position. The ovipositor is withdrawn, or nearly so, and then another pause occurs. The body of the female vibrates rapidly and fluid can be seen passing down between the stylets; then near the tip the stalk bows outward and the collapsed bulb suddenly springs free. If this process of oviposition is a matter of minutes, most of the time is consumed in placing the egg proper. After that the process is completed in a few seconds. However, with some parasites, for example *Encyrtus fuliginosus* and *Melanaphycus fumipennis*, the entire procedure is so rapid that all the steps are rarely apparent. The latter species is particularly remarkable in that egg-laying is complete in a few seconds. Ordinarily, deposition can be detected by watching the actions of a female, but in these two species it was very seldom possible to be certain of the deposition of an egg. Similarly, eggs of the unbanded type may be laid hurriedly or leisurely. As a general rule, however, oviposition requires only a few moments.

When single eggs are deposited, only a few seconds may be required. Parasites like *Pseudaphycus angelicus*, that may place a number of eggs in one host, take several minutes. A minimum period of five minutes and a maximum of twenty minutes has been noted. As many as twelve eggs may be laid at one insertion of the ovipositor.

Specific and individualistic behavior often prolongs the process. There is a minimum time of egg deposition for every species, but certain individuals may spend hours at the process.

FIRST INSTAR LARVAE

Comparative Morphology

Encyrtid larvae of the first instar are conveniently classified into two groups, those with closed tracheal systems and those with open systems. This classification, unlike that based upon the grouping of the eggs, is on a firm functional basis.

TRACHEAL SYSTEM CLOSED

In this group the tracheae typically make one complete circuit in the larval body from the first to the ninth visible body segment. The lateral tracheal trunks make up most of the circuit, which is completed by connecting commissures at both extremities. The anterior commissure is longer than the posterior one and usually loops forward and above the fore intestine. In diameter it is smaller than the other tracheae in the circuit. Invariably there are cephalic branches at the points of union with the main trunks. The posterior commissure is of the same diameter as the lateral tracheae, or nearly so. It may loop dorsoanteriorly but is most often straight and short. Branches of the lateral tracheae are small and frequently indistinct.

There are species with tracheal systems which appear incomplete. With *Leptomastidea abnormis* (fig. 36) and *Apoanagyrus californicus* (fig. 7, *B*), only portions of the lateral trunks were discernible. The entire system probably exists, but could not be seen. Two species have been reported to have only partial systems. The larvae of both *Tetracnemus pretiosus* (Clancy, 1934), and *Anarhopus sydneyensis* (Compere and Flanders, 1934) are said to be devoid of trachea at eclosion but later develop a pair of rudimentary trunks (see fig. 67).

The so-called "atracheate" larvae are also placed in this group. I am of the opinion that many species reported to be without tracheae actually have a closed system. I experienced much difficulty in finding tracheae, and it is entirely possible that other investigators overlooked obscure respiratory filaments. Furthermore, a system may be present though not functioning in such a manner as to be distinguishable. To discern tracheae it has been necessary to depend upon the reflection and refraction of light. In direct light tracheae containing air have a silvery sheen and in transmitted light such tubes are black. If air is removed, tracheae disappear. It seems reasonable to suppose that the tracheal system sometimes does not contain air and would not, therefore, be observable by this method. I discovered that by examining fully-developed embryos, a tracheal system could be found; whereas after eclosion it was practically invisible. This method was habitually employed and the only species encountered that could possibly be atracheate in the primary stage was *Pseudaphycus angelicus*. No trace of tracheae could be found before or after eclosion.

Though the larvae of this group have a common type of respiratory system, there is a notable variation in body shape and none can be regarded as typical. In some species the larvae are almost perfectly spherical, e.g., *Pseudaphycus angelicus* (fig. 65), *Metaphycus melanostomatus* (fig. 49), and *M. timberlakei* and *Melanaphycus fumipennis* (fig. 43). Others are broad in the head and thorax, tapering posteriorly, and crescentic in the lateral aspect, e.g., *Ectromatopsis americana* (fig. 29), *Apoanagyrus californicus* (fig. 10), and *Apoanagyrus* sp. (fig. 9). In some the posteriormost segment (or segments) may be greatly attenuated to form a "tail," e.g., *Cheiloneurus* sp. (fig. 19). Larvae of this shape are designated "caudate" or "tailed."

Some spherical larvae may possess an open tracheal system, but larvae that taper posteriorly or are attenuated to form a tail have never been found to have an open system. Larvae of the genus *Encyrtus* may be regarded as exceptional. In these, particularly the later instars, the posterior extremity is prolonged. The tail, however, is bifurcate and bears a spiracle at the tip of each process. Larvae with a single posterior process have no spiracles and the tracheal system terminates anterior to the tail.

Most larvae of this type leave the eggshell completely at eclosion. Only a few remain, entirely or in part, within the eggshell. The larvae of two such species, *Pseudaphycus angelicus* and *Melanaphycus fumipennis,* were noted to be almost completely enclosed by shell. Thus they may remain until body expansion bursts the shell. Clancy (1934) has shown that in *Tetracnemus pretiosus* nearly half the first instar is passed within the enveloping chorion. According to the description of *Anarhopus sydneyensis* by Compere and Flanders (1934), a similar condition prevails in that species.

Some larvae retain the eggshell at their posterior extremities. It more or less caps the tail of those that are caudate in form. *Leptomastidea abnormis* thus retains the eggshell during most of the first stadium. A similar condition is said to prevail in *Carabunia myersi, Cheiloneurus inimicus, Comperiella bifasciata, C. unifasciata,* and *Chrysopophagus compressicornis.* In these species the succeeding cast skins become telescoped with the shell during ensuing stages. In two known records, spherical larvae remained partly enclosed. Clancy's [unpublished manuscript] record of *Isodromus iceryae*, and Silvestri's (1919) of *Metaphycus melanostomatus.* It is worthy of note that in each the egg is attached by the stalk to the integument and the larva is suspended, just as in species with metapneustic tracheal systems. The larva of *Isodromus* is readily dislodged from attachment.

TRACHEAL SYSTEM OPEN

Larvae of this group are unique in Hymenoptera. As in those with closed systems, the tracheae make a complete circuit of the body, but the lateral tracheae extend posteriorly beyond the commissure and bear spiracles. Such larvae are said to have a metapneustic tracheal system as opposed to an apneustic system (lacking spiracles).

The caliber of the tracheae is often greater than in the closed systems and the circuit is always complete. The spiracles are always directly opposed to the aeroscopic plate. The posterior segments are enclosed by eggshell which becomes augmented by the exuviae. Silvestri (1919) called the resultant envelope the "respiratory hood." This hood may become filled with gas and the spiracles may be placed within the bubble instead of flush on the band.

The spiracles may be so firmly attached to the band that larvae cannot be removed intact. The typical position of the larva is in longitudinal suspension relative to the eggshell. The shell which encases the posterior end quite possibly assists in maintaining the spiracles in position. This, however, does not provide a sufficient explanation. Spiracles might be held either by suction or by some other mechanism. In later instars of some species, spiracles appear to be provided with minute finger-like projections. It has been suggested that these function as wedges in the cells of the band; however, no appendages could be detected on the spiracles on newly hatched larvae. Some larvae are easily dislodged; more particularly those that have a spherical body, as for example, *Erythraphycus argyrocomus* and *Isodromus niger.* The shape of these species is such that the shell cannot aid in holding the larva in place.

There are a few exceptions in the structure of the tracheal system and position of the larva as described above. The respiratory arrangement of a metapneustic tracheal system as heretofore observed in first instar larvae has been characterized by two open spiracles at the posterior end. The larva of *Erythraphycus argyrocomus,* however, has four spiracles (fig. 39) and these, instead of being placed crosswise to the aeroscopic plate at the distal end of the egg body, are in a row lengthwise. A single larva of an unidentified parasite, probably *Metaphycus* sp., on soft brown scale (*Coccus hesperidum*), was observed to have two pairs of spiracles.[5]

The position assumed by the larva of two species in relation to the egg shell is of great interest. The larvae of both *Aenasius maplei* (fig. 1) and *Anagyrus putonophilus* (fig. 6) hatch anteroventrally from the egg, so that they lie almost at right angles to the longitudinal axis. The spiracles, consequently, are appressed to the plate near the apex of the egg proper. Such a position may be common with larvae of other species, but has not been heretofore reported.

Most larvae are more or less sausage-shaped. The larvae of *Erythraphycus argyrocomus* and *Isodromus niger* are spherical. Although some taper slightly posteriorly, they are always bluntly rounded. The species of *Encyrtus* have been said to have bifurcate tails, but these are lacking in *Encyrtus fuliginosus* (fig. 30, *D* and *E*), at least in the first instar.

TAXONOMIC SIGNIFICANCE OF OPEN AND CLOSED TYPES OF TRACHEAL SYSTEM

The classification of larvae according to the structure of the tracheal system may have some bearing on taxonomy. Both types, however, are found in the genus *Isodromus.* Since the tracheal system has few variations, and since larval differences may exist in other structures, the type of system is, at most, of secondary importance.

Observations on Embryonic Development

In the discussion of technique it was stated that, in order to secure the newly hatched larva, a series of hosts were parasitized and dissected at intervals. Consequently observations were made on the development of the embryo. One

[5] [Several species of *Metaphycus* including *M. lounsburyi* have recently been found by Flanders to have four spiracles in the first instar. Flanders (1942) states that possession of four spiracles seems to be characteristic of the genus. S.E.F.]

of the phases which will be described in detail here concerns the period between maturing of the embryo and eclosion and has a bearing, direct or indirect, on the problem of larval respiration.

At deposition the egg is filled with a granulated translucent mass which gradually becomes smaller, assuming a position central to the latitudinal axis but nearer the posterior than the anterior end. The embryonic layers form at the periphery of the yolk mass. The yolk is enclosed within the embryonic mid-gut and the entire egg proper is occupied by the embryo. The indentation of the stomadeum indicates that encyrtid embryos develop with their heads in the anterior position and that blastokinesis does not take place. In all of twelve species examined the stomadeal indentations were at the stalk end of the eggs.

Following the acquisition of form, peristaltic movement of the mid-gut commences and the tracheal system appears. The tracheal trunks are the first to become visible, then the commissures and cephalic branches, and finally the lateral branches of the trunks. All those with spiracles have the trunks, commissures, cephalic branches, and most of the secondary trachea filled with gas. The number and situation of the minute secondary tracheae that appear at this time vary among species and the individuals of a species. Only rarely does an entire tracheal system become visible in the mature embryo among those species not destined to have spiracles in the first instar. With many embryos a complete loop and cephalic branches are seen, but no lateral branches. In *Apoanagyrus californicus* only a portion of the trunks are ever visible, and in *Leptomastidea abnormis* tracheae were not observed prior to eclosion. No tracheal system was found in *Pseudaphycus angelicus*, either before or after eclosion.

By the time the embryo is mature and begins to concern itself with eclosion its actions are diverse. Embryos with spiracles which are to lie near the stalk end must reverse their position. This change is accomplished before eclosion. The embryos of four species, namely *Ooencyrtus johnsoni, O. californicus, Microterys flavus*, and *Erythraphycus argyrocomus*, have been found to lie first with the head at the anterior end of the egg and then with the head at the posterior end without rupturing the eggshell. The actual process was observed twice, with different species. The mature embryo of *Microterys* turned without difficulty in a few minutes. The movement was parallel to the band and counterclockwise to the dorsum of the egg. Midway in its rotation it briefly halted. The act with *Erythraphycus* was somewhat different. The larva writhed and squirmed for some time, then finally rotated at right angles to the band with the head farthest removed from the band. Regardless of the method of reversal, it would seem that all embryos rotate that are to lie eventually with the spiracles at the stalk end of the egg. *Aenasius maplei* and *Anagyrus putonophilus*, whose larvae have the spiracles situated at the opposite end, do not alter their position.

One would expect that those lacking spiracles would not reverse within the eggshell. This is believed to be true for the majority of species, but not for all. I found one exception, *Leptomastidea abnormis*. Figure 36 shows a sequence of embryonic stages in which it will be noted that the foregut lies anteriorly in the egg at first but before eclosion assumes the posterior position. The act of rotation was not observed. It is noteworthy that the egg has a "piece of band," that is, a few cells on the stalk, but no band on the egg proper. This may indicate that the egg and larva are transition forms between unbanded eggs and apneustic larvae on the one hand, and banded eggs and metapneustic larvae on the other.

Reversal is not necessarily embryonic movement. Though eclosion has not taken place when it occurs, embryonic development is most certainly complete; the organism therefore may be considered to be in its first larval stage.

Complete eclosion was never observed. With some species the eggs seem to cleave evenly and always along the same line. With others the eggshells seem to have fairly burst so that the shell forms a crumpled mass about the posterior segments of the larva. In *Pseudaphycus angelicus* and *Melanaphycus fumipennis* eclosion appears to be a secondary process. At some period the egg becomes detached from the stalk. The larva is enabled to feed through the resulting aperture and the consequent expansion presumably bursts the enclosing shell. The embryo of a number of species, namely, *Aphidencyrtus aphidivorus, Cheiloneurus noxius, Cheiloneurus inimicus, Chrysopophagus compressicornis, Zarhopalus corvinus,* lies coiled within the shell and eclosion has been said to occur when the embryo unflexes the tail and ruptures the chorion.

Morphology of Banded Eggs

Eggs as stated previously are arbitrarily divided into three types, banded, unbanded, and intermediate, according to the structure of the shell. Unbanded eggs have a thin elastic chorion which is extremely fragile throughout. In the intermediate type the egg proper is more firm and less likely to be damaged during dissections. In general, the eggs of these two groups have a simple unmodified chorion. The banded eggs, however, have more or less complex chorions because of structural modifications which appear to play a role in conveying the air to the developing larvae.

Eggs in Toto

As in the other groups, the shells of the banded eggs are elastic. These eggs, however, can be manipulated considerably without rending the chorion. When the entire egg is compressed, the bulb and the unbanded portions expand, the band remaining unaffected. The neck is flexible though somewhat brittle. The rigidity of the band on the egg proper assists in retaining the shell about the posterior extremity of the newly hatched larva.

In appearance the band is like a section of a cobblestone pavement, a bit of mosaic, or a cross section of a plant root. A change in focus of the microscope may bring about a resemblance to stippling. Silvestri (1919) described the bands as dimpled or reticulated, which would indicate that the areas are merely surface sculpturings of some sort. It appears, however, that the band is a highly modified portion of the eggshell. In the lateral aspect a band is seen to have thickness and to be partitioned at regular intervals into compartments or cells. It is demonstrable in a deposited egg that a band is composed of tiny compartments. In a mounting medium such as glycerine, the fluid displaces the air in the bands so that gradually and irregularly the silvery sheen disappears. The action may halt for a time, then suddenly and rapidly engulf a whole group of "cobblestones." As a result of this observation and because of the similarity in appearance of all bands, the units of which they appear to be composed are called *cells* here.

It has been shown that the size and arrangement of cells form a specific pattern on the egg proper. Variations also occur in the localized thickness of cells. This is frequently noticeable in the anterior region of the neck, as, for example, in *Coccidoxenus niloticus* (fig. 22) and *Anagyrus putonophilus* (fig. 3). Less apparent are thicknesses on the egg itself. In *Coccidoxenus* and *Ooencyrtus johnsoni* cells are slightly deeper at the anterior end. There are a few notably anomalous species. In *Ooencyrtus* the species possess deeper cells at the very apex of the band. The thickness is quite abrupt and very apparent. On the neck of the egg in certain species of *Encyrtus,* namely *fuliginosus* and *infelix,* the band forms a lip which projects on one side. The lip is probably composed entirely of cells (fig. 31) although the arrangement is so complex that their interrelation is difficult to determine.

The upper and lower surface walls of the band are usually of uniform thickness throughout. However, in *Ooencyrtus johnsoni* the cells are beneath a thick wall extending over a considerable area at the anterior end of the neck, as in figures 59, *B* and *C*. Anteriorly and posteriorly the thickening disappears. In a few species there are indications that the outer wall is thin at the anterior end of the neck. Figures 3 and 22 illustrate this appearance in *Anagyrus* and *Coccidoxenus.* In both species the outer wall posteriorly for a short distance is thicker than it is anteriorly. It is quite possible that the cells at the anterior extremity of the neck are open, at least in part, and that the other cells of the band are closed.

Eggs in Section

In an effort to obtain a better picture of the true structure of bands, histological work was undertaken. Eggs of the *Anagyrus* and *Coccidoxenus* were selected, particularly because a section through the large cells at the anterior end might demonstrate whether or not any were open.

Since the egg is so small that manipulation of it is most difficult, the ovaries were sectioned. This would have the additional advantage of providing sections through several eggs at one time. Sections five microns in thickness were found to be preferable. The first preparations were made of ovaries in the normal, coiled position in the belief that the eggs would then be cut at a variety of angles, some of which would certainly supply the necessary information. The result was such a tangle of eggs and ovarian tissue that it was impossible to detect an orderly series. Thereafter, ovaries were uncoiled before being fixed in Bouin's solution.

The results of staining were decidedly unsatisfactory. The first sections were treated with eosin and haemotoxylin. All the structures with the exception of the eggshell would take this stain. If the chorion had become in the least stained, the observations might have been successful. Basic fuchsin, acid fuchsin, borax carmine, and acid fuchsin in lactophenol were tried with appropriate modifications in the procedures. With the exception of the last named, there were no noticeable improvements. Since this one offered some promise, many experiments were undertaken to improve the effect by varying the period in the stain and destaining with alcohol, acetic acid, or water. Not one of the procedures showed much improvement with respect to the staining of the eggshell, and there was no opportunity for further experiments to obtain a reagent specific for eggshell.

Observations were therefore dependent upon the characteristic light refraction of the shell. In sections other than across the band, nothing of the structure could be detected. On the egg proper the band is so thin and the cells are so minute that its nature was not observable. The failure to stain and the minuteness of the cells are not the sole obstacles to observation. In any section the walls of other cells obstruct vision. If each cell had a minute hole in the center on the upper surface, it would not be seen even if the microtome blade should pass directly through one cell; for the surface of the cell situated directly behind would cause the appearance of a solid layer. If sections could be cut as thin as the width of one cell, it might be possible to discern the structure without interference from the walls.

The following data were derived from the only preparations which could be obtained to supplement observations of the whole mounts—those in which the sections intersected the neck at right angles in the areas of larger cells:

Anagyrus putonophilus (fig. 4, *A*.).—The shape of the band on the neck in cross section is like that of a horseshoe. Normally the shape is probably flatter, the ventral nonbanded area becoming extended. The inner wall is slightly thicker than the outer wall. The cells are closely packed together. The size and number are associated with the portion of the neck on which they occur. The number and breadth of cells are the same as that which appears on the surface when viewed in toto. In figure 4 the appearance at several points is diagrammed: *A*, *B*, and *C* are successive views of the first third of the anterior end as shown in figure 3. The definition of cell boundaries is not as clear as indicated. Upper and lower surfaces appear to be slightly thinner in the sections showing the cells farthest anterior but no opening could be detected. The interrelationship of cells could not be determined.

Coccidoxenus niloticus (fig. 23).—No clear sections of the anteriormost portions could be secured because of the curvature. The shape is probably similar to *Anagyrus*. *A* is considered typical of the portion occurring as the posterior third of the anterior end of the neck, as shown in *A* and *B* of figure 22. The size and number of cells depend upon the point of sectioning. The upper wall of each cell is slightly convex. Descending the stalk (*B* and *C*) cells are broader and fewer. On the greater part of the neck there are only four large cells and they appear to be continuous to form tubes down the neck. Just in front of the egg proper (*E*) the cells are more variable in number and size. Posteriorly they then become so small in both dimensions that the structure disappears.

The data given is far too poor for a complete understanding of the composition of bands. There is no clue to the manner of ingress or passage of air. Adjacent cross sections may have a variable number of cells without any suggestion of interconnections. However, because of the general appearance of bands and the behavior of liquids passing within, there is undoubtedly some sort of connecting system.

PHYSIOLOGY OF RESPIRATION

It has been generally believed that endoparasitic larvae without spiracles and those devoid of tracheae obtain their oxygen by way of osmosis, either through the body surface or the intestine. This theory seems adequate for all larvae with closed tracheal systems. Internal parasites that respire atmospheric air are, however, very rare and encyrtids are the only known *Hymenoptera* equipped with posterior spiracles for this purpose. I have confined my studies largely to those encyrtid larvae having metapneustic tracheal systems.

The prevalent explanation of the manner in which the larvae respire is attributable to Howard and Fiske (1911), who held that the stalk of the egg of *Ooencyrtus kuvanae* was hollow and conveyed air to the larva "like a life line attached to a submarine diver." No respiratory system was described. Imms (1918) found that the young larva of *Blastothrix sericea* possessed tracheae with two spiracles situated at the caudal extremity of the body and concluded that it could thereby breathe directly through the egg stalk. The conception that encyrtid larvae with metapneustic tracheal systems respire atmospheric air by way of the hollow egg stalk has persisted up to the present time.

Three observations of Silvestri (1919) on certain species should have altered this concept: (1) the eggshell was very complex in structure; (2) air appeared in the band on the deposited egg from the anterior end of the neck on to the egg proper; (3) the spiracles of the primary larva adhered to that portion of the shell bearing the band. His opinion regarding the manner of respiration was to the effect that the projecting anterior end of the stalk was a sort of porous stopper ("respiratory plug") which permitted the osmotic passage of atmospheric air; the lumen of the petiole or neck functioned like a tracheal tube; the reticulations on the egg proper served as an "aeroscopic membrane" (the exact role played by this structure was not made clear); eggs possessing this structure he called "tracheated eggs." Presumably Silvestri believed that the "plug" provided the means for air to enter the lumen of the neck, which conveyed air to the spiracles of the larva. Consequently his opinion differs from that of other observers only in the manner in which air enters the stalk, the others having maintained that entrance was gained by the open apex of the broken stalk.

My hypothesis, based on observations almost identical with Silvestri's, was that atmospheric air penetrates and pervades the band on the egg but does not enter the lumen of the stalk, the larva obtaining the air directly from the band. To determine the validity of this contention recourse was had chiefly to the dissection of live hosts of many species.

Evidence That the Band Conducts Atmospheric Air

STRUCTURAL CORRELATION BETWEEN THE EGG AND LARVA

A remarkable and significant correlation exists between the structure of an egg's shell and the tracheal system of the larva developing in that egg. The classification of the egg and first stage larva as used herein is based on this correlation.

Larvae hatching from eggs of the unbanded type have, so far as known, a closed respiratory system. An egg of this structure is deposited free or attached to organs but no part is attached to the host's derm or shell. Likewise there are no air-bearing structures. Consequently neither the position nor the structure of the egg provides a means for the passage of atmospheric air.

Larvae from eggs of the intermediate type likewise have a closed respiratory system. An egg of this type is suspended within the host by the neck which passes through the integument. Only in *Ectromatopsis americana* were there air-bearing structures; and in it the air scarcely reached beyond the neck of the egg. The larva of *Ectromatopsis* as with other larvae from eggs of the intermediate type (excepting *Clausenia* and *Bothriothorax*, which were not obtained for study) has an apneustic tracheal system.

In my observations, all larvae with caudal spiracles developed in banded eggs. It may be erroneous, however, to state that only larvae with metapneustic tracheal systems develop in eggs of the banded type. The egg of *Metaphycus melanostomatus* was illustrated by Silvestri as bearing a band (fig. 48) though a description was omitted and the larva (fig. 49) was figured without spiracles. In my opinion additional exceptions may be found to occur. The converse, that larvae with metapneustic tracheal systems hatch only from eggs with bands, appears true although the evidence is scant; however, I observed the correlation with fifteen species. To these can be added four species reported by Silvestri. Other investigators have described or illustrated at least four other species in which such correlation seems very probable.

OCCURRENCE OF AIR WITHIN THE BAND

Air contained in any transparent object immersed in a clear fluid has a typical appearance; it is white or silvery color by reflected light, and gray or black by transmitted light. Banded eggs exhibit this appearance immediately after being deposited. Silvestri was the only one to have stated that air occurs in the band; but of the four banded eggs he described he mentions this fact for only two species, *Encyrtus infidus* and *Phaenodiscus aeneus*. Other investigators probably observed the effect but did not recognize the cause or significance. Smith and Compere (1920) stated that the stalk of the egg of *Metaphycus lounsburyi* became dense white immediately after oviposition. A waxy incrustation was reported to occur on the egg of *Encyrtus infidus* by Clausen (1932).

I examined the deposited eggs of twenty-three species. Of the eighteen that were banded, the color effect appeared only in the band on the neck and egg proper. Nonbanded eggs were entirely translucent. Silvestri stated that in *Phaenodiscus aeneus* air was present the length of the petiole and in the band on the egg proper. Evidently he did not note that air was confined entirely to the band on the petiole; for he, like others, indicated that the lumen of the stalk itself conveyed air.

APEX OF THE STALK

With the exception of Silvestri, writers have stated or implied that air entered by the open apex of the petiole. Silvestri observed the petiole to be capped by the remains of the bulb. I found, in all but one of the species examined, that normally the bulb was simply shriveled and collapsed.

That the bulb may be perfectly intact was demonstrated as described above. Eggs were dissected free from the host as soon as possible after their deposition. They were mounted in Hoyer's solution and very carefully compressed under a cover glass. The procedure was attempted with the eggs of *Encyrtus infelix, Ooencyrtus johnsoni,* and *Anagyrus putonophilus.* With noticeable pressure, material could be forced back into the bulbs of the eggs of *Encyrtus* and *Anagyrus,* which again inflated. Undoubtedly the plug which later appears within the stalk begins to form immediately after deposition of the egg, and it is this that necessitates the undue pressure. Figure 5, *A,* shows a deposited egg of the *Anagyrus* after it has undergone this treatment. No difficulty was encountered in inflating the bulb of the *Ooencyrtus* egg. This effect indicates that, with these species at least, the bulb is not destroyed during oviposition.

Damaged bulbs have been observed, and in *Encyrtus fuliginosus* the bulbs were almost always missing. In any case the bulb may eventually deteriorate so much that the stalk becomes open at the apex. The fact remains, however, that in the great majority of species the stalk is closed at the end.

BLOCKED LUMEN OF STALK

With some eggs a plug occurs in the lumen of the stalk immediately in front of the egg proper. Embleton (1904) reported this "protoplasmic matter" in the ovarian eggs of *Encyrtus infelix* and Thorpe (1936) frequently found the plug in the same species. I have observed plugs in a number of other species. The period of plug formation is variable but never occurs until after deposition of the eggs. It appears in *Anagyrus putonophilus* soon after the egg is deposited, but not in *Aenasius maplei* until after eclosion. *Encyrtus infelix* was extensively investigated but no plug such as was noted by Embleton was found in the neck of the ovarian egg. It appeared, however, soon after deposition.

With the *Encyrtus* (fig. 33), the *Anagyrus* (fig. 5, *B*), *Aenasius maplei* (fig. 1, *B*), and *Leptomastix dactylopii* plugs are black and opaque. With *Metaphycus howardi* (fig. 45) the plug is brown and somewhat transparent. All plugs are solid and do not break up when compressed under a cover glass. Thorpe (1936) thought that the plug in the *Encyrtus* only partly blocked the stalk and could not prevent the passage of air. However, if air penetrated the hollow stalk, the color would change both above and below the plug. In none of the eggs of the above species, or of those eggs of other species without plugs, was the silvery appearance observed within the lumen of the stalk.

The origin of plugs is problematical. Thorpe regarded them as remnants of the apical yolk mass. I believe they may be derived from the vitelline membrane that was contained within the bulb. Though eggs of some species have some sort of crumpled material within the stalk, the deposited eggs of most species lack even this bit of extraneous matter, even though members of the same genus possess plugs. In *Encyrtus fuliginosus* and *Erythaphycus argyrocomus* the stalk appears unobstructed.

LOCATION OF SPIRACLES

Most investigators in discussing the tracheal systems have indicated that the spiracles of the primary larva are at or near the opening of the stalk into the egg proper. In my observation the spiracles appear located on the inner surface of the aeroscopic plate on the egg proper. With a few notable exceptions, they are attached a short distance from the neck. Silvestri stated that in *Phaenodiscus aeneus* and *Encyrtus infidus* the spiracles adhere in a similar position. This appears in the illustration of the former species, but in that of the latter the spiracles are unattached (see figs. 16 and 34). Thorpe has described and figured the same sort of arrangement with *Encyrtus infelix*. Neither Silvestri nor Thorpe ascribed any significance to their findings.

The position of the spiracles in three selected species is unique and offers strong evidence in support of the respiratory function of the band. The larvae of *Anagyrus putonophilus* and *Aenasius maplei* have the spiracles at the end of the egg opposite the stalk; in fact almost at the posterior apex (figs. 6 and 1, respectively). The *Erythaphycus argyrocomus* larva places its four spiracles parallel to the length of the band (fig. 39) and not crosswise as is the usual position.

In all the specimens examined the situation of the spiracles appears so remote from the stalk that it would appear impossible for them to secure air from its lumen.

COURSE OF STAINED OILS

By immersing the entire host in "a thin vegetable oil" which had been strongly stained with a dye "such as Sudan III," Thorpe endeavored to demonstrate that the first instar larva of *Encyrtus infelix* was enabled to utilize atmospheric air by means of the stalk. He states that the colored oil speedily entered the egg stalk and invaded the tracheal system of the larva. I sought to duplicate this experiment and encountered difficulty in securing an oil that not only would penetrate rapidly but also could be stained dark enough to be detected easily in the minute structures. Sundry vegetable oils failed to penetrate and it was evident that a much lighter oil was necessary. Clove oil or a 50:50 combination of xylene and kerosene was finally selected. Neither xylene nor kerosene was suitable by itself. Xylene would not penetrate in less than four minutes and distorted the material by dissolving the fats. Kerosene could not be stained dark enough. The stain selected was Oil Red O (Sudan II), which colored the oils darker than either Sudan III or IV.

This method was used with three species, namely *Ooencyrtus johnsoni, Encyrtus infelix*, and *Anagyrus putonophilus*. The procedure was to immerse the parasitized host from one to five minutes. After immersion the oil was sponged from the surface and the host dissected in normal salt solution. If any oil was observable within a host, that host was discarded; but if none had penetrated, the eggs or larvae were carefully removed intact and observed unmounted. Those in which any stain was observable were then mounted in normal salt solution of glycerine. If the neck and the bulb of the egg were damaged, the specimen was likewise discarded since there was no certainty that the stalk had not been broken prior to or during the immersion and consequently had permitted the oil to enter the lumen of the stalk.

With *Oencyrtus* the brilliant red color was visible within the plate. The band on the egg proper was penetrated to the very end. Owing to the melanization of the band on the petiole, no color was observable there; nor was oil found in the bulb, the lumen of the stalk, or the egg. During one-minute immersions only the cells on the outer margins of the plate on the egg proper were filled. After a longer period the oil entered the center section and the stain in that area was darker. The larval tracheal system also was invaded, for a pink color was seen within the trunks and commissures. In some specimens the silvery appearance remained in the band and the tracheal system even though other specimens within the same individual host had been thoroughly penetrated. For this there seems no plausible explanation.

Similar results were obtained with the other species. In *Encyrtus* no oil entered the bulb. Whether any had entered the lumen of the stalk was not ascertainable because of the abbreviated neck, the slight melanization of that portion within the host, and the minuteness of the band. It was obvious, however, that no oil had penetrated beyond the plug except within the plate and thence into the tracheal system of the larva. In *Anagyrus* the color was seen in the band but not in the tracheae. However, since light was no longer refracted by the tracheae, there was no doubt that the oil had entered.

The experiment was performed with *Anagyrus* larvae free of the host. The larva was placed in a thin film of normal salt solution, a cover glass was supported over the larva by bits of modeling clay, and the cover slip was gently slid and the larva so placed that its entire body and the lower part of the egg shell remained in the solution while the stalk projected free. A minute drop of the stained oil was then placed at the tip of the stalk. The result was the almost immediate elimination of air in all the structures of the egg and larva. Color could be discerned in the band and the posterior section of the tracheal trunks. If any oil had entered the stalk its presence in the lumen would have been obvious.

These results are directly opposed to those obtained by Thorpe.

Means Whereby Atmospheric Air May Enter and Leave the Band

The one factor all theories have had in common is that the hollow stalk served to convey air. Most investigators have maintained that the apex of the stalk was open or became open. It has been shown that with most species the neck is capped by the collapsed bulb and that air cannot, normally at least, freely enter the lumen of the stalk. Even if the chorion at the anterior end were not intact or entrance was gained by osmosis, air could easily be detected. At no time have I observed air within the egg stalk.

All workers, with the exception of Silvestri, have failed to observe the complex structure of the eggshell, which offers a very important clue to another means whereby atmospheric air could be conveyed; careful observations of the deposited egg might have shown that air was contained within this structure. Obviously Silvestri (1919) was remarkably close to a more adequate explanation, but his interpretation of function is unduly complicated and incomplete. Silvestri's theory is essentially that air passed by osmosis to the cavity of the petiole, and the petiole functioned in the manner of a trachea. In my opinion, Silvestri did not realize the role of the shell structure. Although the spiracles of the first instar larvae of two species were said to adhere to the dorsal part of the eggshell, in only one species were the spiracles illustrated as adherent to the plate. If the plate had been considered to be of respiratory significance, the tracheae of all four metapneustic larva would undoubtedly have been drawn or at least explained, as leading to the plates.

Considering the observations and experimental evidence, I believe there is little reason to doubt that the band or plate on the egg provides these larvae with the means to respire atmospheric air. It may well be asked, "How does air get into the plate?" While it is in the ovaries, the egg does not have a silvery sheen in the banded areas; but immediately after its deposition the sheen is usually prominent. If the assumption is correct that this sheen is due to air, the air must have entered at the moment of deposition. On the basis of the information at hand, it is possible to make a few suppositions. In passing down the ovipositor the eggs are greatly compressed. The internal structure of the plate is in the nature of a sponge which is emptied of any fluids by this compression. As the egg is deposited, the "sponge" swells because of the elasticity of the cells. The surface of the plate is impervious to the fluids of the host and therefore a vacuum exists. When the end of the stalk leaves the ovipositor, air rushes in and disperses throughout the structure. This would imply that the cells are open to the atmosphere at the extreme end of the stalk only. It has been pointed out above that there is a suggestion of an opening at this point, but this could not be confirmed by sectioning. I am forced to assume that the structure of the anteriormost portion is such as to permit the entrance of air at that point only. Osmosis may operate as Silvestri suggested, but certainly it does not carry air into the lumen of stalk as Silvestri apparently believed.

The manner in which air is withdrawn from the aeroscopic plate is also obscure. No openings appear through which air could be directly removed. In later instars, some spiracles bore finger-like projections. It is possible that these are glands the secretion from which dissolves away the cell walls permitting the attachment of the spiracles, and thus providing a direct passage for air. No such processes could be found on the spiracles of first instar larvae. I am inclined to believe that air passes solely by osmosis.

Before any definite theory may be advanced concerning the physical phenomena involved in the ingress and mode of exit of atmospheric air, the morphology of the aeroscopic plate obviously must be better understood.

Need of Atmospheric Respiration

The need for atmospheric air has not yet been conclusively demonstrated. If, however, the free flow of air into the stalk were prevented and the parasite died, it should indicate that atmospheric air was necessary for the development of the egg or larva.

To test this hypothesis, nearly mature black scale (*Saissetia oleae*) which had been exposed for two days to the females of *Microterys saissetiae* were selected for two simultaneous experiments. In one, twenty stalks were covered with Vaseline and ten were left undisturbed; in the other, melted paraffin was dropped on twelve stalks and four were retained as checks. In each host there was a maximum of three receiving the treatments and at least one check. At experimental temperatures ranging between 75° and 80° F, eclosion occurred in two days, and dissections were made after four days. The vaselined eggs either failed to hatch or the young larvae died; some unhatched eggs contained little or no air in the band. From the uncovered stalks, normal fourth instar larvae were suspended. The results of the second experiment were identical except that air remained within the bands.

Two outstanding faults in the experiments are recognized. First, the methods alone may have killed the developing parasite; some fluid constituent of Vaseline may have penetrated and killed the egg, thus explaining the absence of air, or the temperature of the paraffin may have been damaging. Second, few data were obtained. Nevertheless, the results indicate that atmospheric air is inducted by way of the stalk and is necessary for the development of the egg and larva of *Microterys saissetiae*.

The variability of the oxygen requirements among encyrtid larvae is scarcely open to question because of the structural variability exhibited by the tracheal system.

[The fact, as stated above, that the development of embryos in eggs attached to the host's integument is more rapid than with embryos in eggs unattached to the host's integument suggests that the rate of development is influenced by additional oxygen obtained through the respiratory band. S.E.F.]

The larva of *Coccidoxenus niloticus* has tubes of large diameter compared with other species, such as *Anicetus annulatus*. Among the apneustic larvae there are those in which only a portion of the tracheae appear before eclosion as opposed to those in which complete loop is visible. *Tetracnemus pretiosus* and *Anarhopus sydneyensis* are said to have "rudimentary" tracheae late in the first period. Then there are primary larvae in which no tracheae can be seen at all.

Metapneustic larvae are in a position to obtain oxygen by the same means as those without spiracles or those lacking tracheae. Osmosis may operate but perhaps not to the same degree, depending upon the character of the parasite integument. The fragility of larvae is noticeably variable. As a rule apneustic larvae are easily damaged and must be carefully handled. The metapneustic larvae of most species can stand rough treatment. Since those with weaker integument may obtain oxygen more easily by osmosis than those that are sturdier, the amount of oxygen taken in by way of the stalk may be in inverse proportion to that obtained by osmosis.

Respiratory needs may be temporarily fulfilled by the host. In dissection of eggs of the squash bug, *Anasa tristis*, several stalks of the eggs of *Ooencyrtus californicus* were noted to have broken off. Instead of there being one parasite egg present, as indicated by the projecting stalk, there were two or three (fig. 58). Very rarely, a stalk became detached so that the larva was floating within the host. It is certain that the attachment had not been broken during dissection, for the larva appeared to be perfectly normal. Sufficient oxygen was no doubt obtained without direct connection with the outside air. Whether this larva would mature or not is a matter of conjecture, for the requirements may be greater in later instars.

This need is also indicated by the fact that the tracheal system of the mature embryo may contain oxygen obtained by osmosis. It was observed repeatedly with embryos possessing spiracles that all or nearly all tracheae, including the fine branches, were discernible without the spiracles' being near the aeroscopic plate. Often the spiracles were at the farthest possible point from the plate.

The band itself could conceivably function as an osmotic surface. A considerable area is exposed to the host's contents and osmosis may occur through that portion of the shell as well as through the parasite integument, and perhaps with greater ease.

Atmospheric air is carried within the eggshell, which is structurally modified to convey it. The lumen of the stalk cannot conceivably play this role and furthermore does not function in this manner, as heretofore supposed. All larvae with open tracheal systems hatch from eggs with air-bearing areas on the shells and place their spiracles against these areas. Although they have the means to breathe air which is outside of the host, many if not most species may be independent of atmospheric air in the early instars.[6]

DESCRIPTION OF EGGS AND FIRST INSTAR LARVAE OF CERTAIN ENCYRTIDS

The following pages discuss the eggs and first instar larvae of all encyrtids, with the exception of the polyembryonic species, which I have observed or which are described in published or unpublished papers. I have drawn freely upon the literature. In many papers, authors' statements or figures indicate that observations were incomplete or inaccurate. The significance of some findings was, in my opinion, overlooked.

Descriptions are largely confined to the egg and the larval respiratory structures. My other observations, though perhaps of biological interest, are inci-

[6] [After Maple had completed his studies on the Encyrtidae, DeBach (1939) demonstrated the existence of an encyrtid which is not endoparasitic. This unique species, *Microterys titiani* Gir., deposits from 1 to 10 eggs *beneath* its host, *Lecanium corni* Bouché. The stalk of a deposited egg comprises about one-half the egg in length. The first instar larva is hymenopteriform and possesses a peripneustic tracheal system. S.E.F.]

dental to the problem. Adequate references to the published records are provided throughout, should further information be desired.

The drawings are largely mine. Originals by Harold Compere and D. W. Clancy were kindly lent to me. Some were copied from other publications, if other authors' observations could thereby be better demonstrated. The sketches of larvae are diagrammatic. Needless detail is left out and only half of the tracheal system is shown in the lateral views, thus confusion should be eliminated without causing undue simplification.

The dimensions given are measurements of single eggs; where an egg is illustrated, its dimension is given.

The arrangement is alphabetical, according to genus.

Acerophagus fasciipennis Timb.

Only the ovarian egg of the parasite was examined, and it was extremely simple (fig. 62, *B*). The entire egg is flexible and in no two mounted specimens was the shape the same. In all specimens the neck and bulb were twisted and partly collapsed. The neck is long, narrow, and undifferentiated from the bulb. The length of the two measured was 0.112 mm., and the widest portion was at the distal end. The egg proper measured 0.06 mm. by 0.03 mm. It was widest at the proximal end. In outline it was more nearly angular than rounded. There was no band.

The egg is probably deposited entirely within the host and the newly hatched larva would doubtless not possess a metapneustic tracheal system.

Acerophagus notativentris (Gir.)

The egg of this species was described by Clausen (1924) and appears to be quite similar to that of the preceding species. Eggs that I observed had the outline shape of a teaspoon, the neck and bulb being the handle. The measurements of a typical egg were: length of bulb and neck, 0.128 mm.; widest portion of bulb, 0.017 mm.; length of egg, 0.07 mm.; width of egg, 0.034 mm.

According to Clausen, during the process of oviposition the contents of the smaller body are forced into the egg proper, though the collapsed membrane remains attached; the egg lies free within the host. He did not indicate the type of larval respiratory system. The egg provides no means for securing atmospheric air should the larva possess spiracles.

Acerophagus pallidus Timb.

An extensive study of the parasite was not made although this species is frequently obtained from native mealybugs in southern California, as well as from the introduced species, *Phenacoccus gossypii* Twns. and Ckll.

The ovarian egg (fig. 62, *A*) has no distinctive features and differs in no essential structural detail from the other species of *Acerophagus* discussed. The neck is short and undifferentiated from the bulb, which is long and narrow compared to the remainder of the egg. The measurements were: length of bulb, 0.188 mm.; maximum width of bulb, 0.034 mm.; length of egg, 0.086 mm.; width of egg, 0.06 mm.

The deposited egg and the larva were not examined; nevertheless, there is little doubt that the egg is laid entirely within the host and that the newly hatched larva is without spiracles.

Achrysopophagus modestus Timb.

The early stages of this secondary parasite, when reared on *Zarhopalus corvinus* (Girault) and *Anagyrus subalbicornis* (Girault), appear to be similar to those of *Chrysopophagus* and *Cheiloneurus*. Clausen (1924) found that

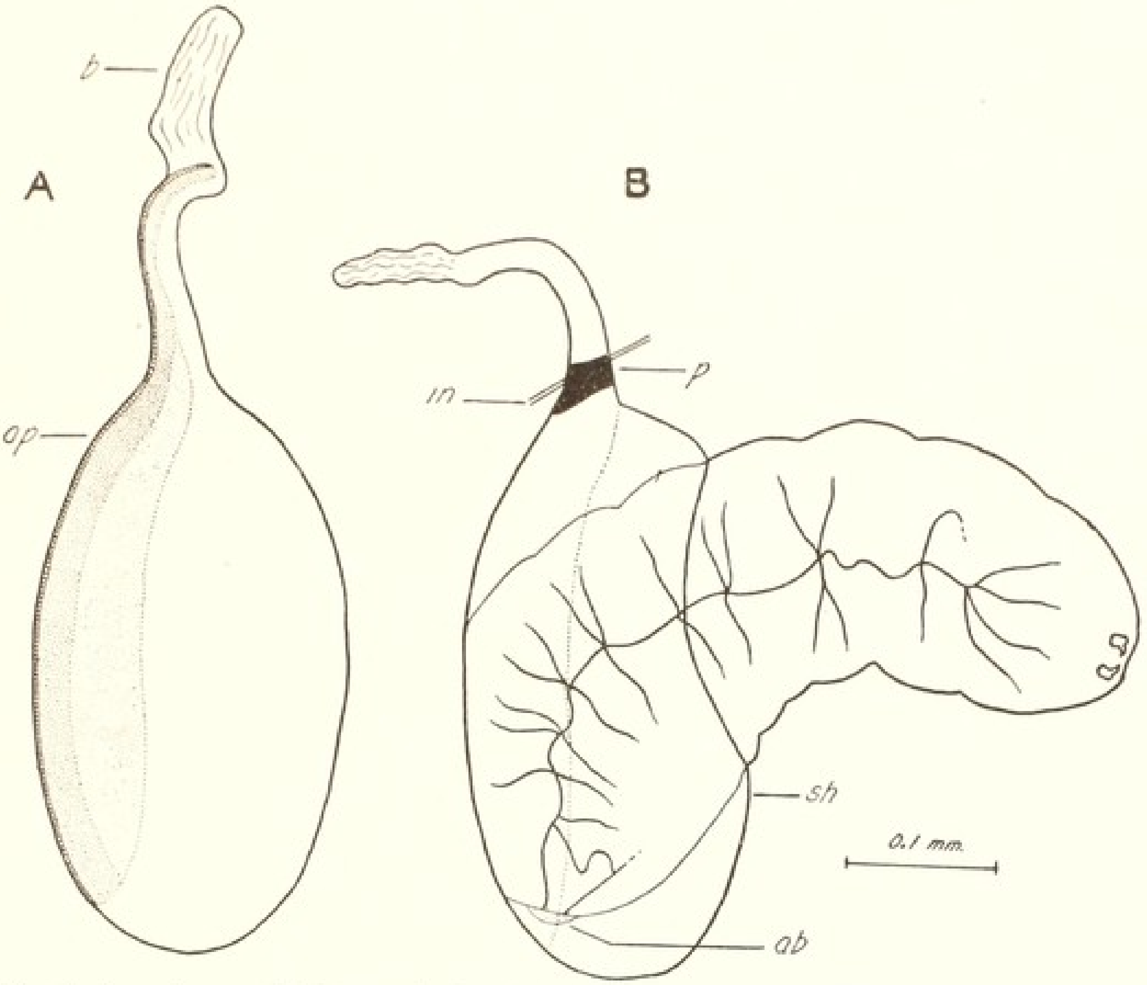


Fig. 1. *Aenasius maplei* Comp. *A*, deposited egg, lateral aspect; *B*, newly hatched larva. The pair of spiracles are centered in the air bubble (*ab*).

the egg floated free within the host's body, the collapsed bulb remaining attached. The larva was noted to be of the tailed type. Although Clausen does not say so, it would necessarily follow that the tracheal system is apneustic.

Aenasius maplei Comp.

An extensive search was conducted for sufficient material with which to study the development of this species. Although its mealybug host, *Puto yuccae* Coq., is abundant, the parasite is exceedingly rare. The following information was secured with the use of a single female.

Deposited egg.—The egg is of the banded type and is therefore attached to the integument of the mealybug. The anterior end of the neck is curved and

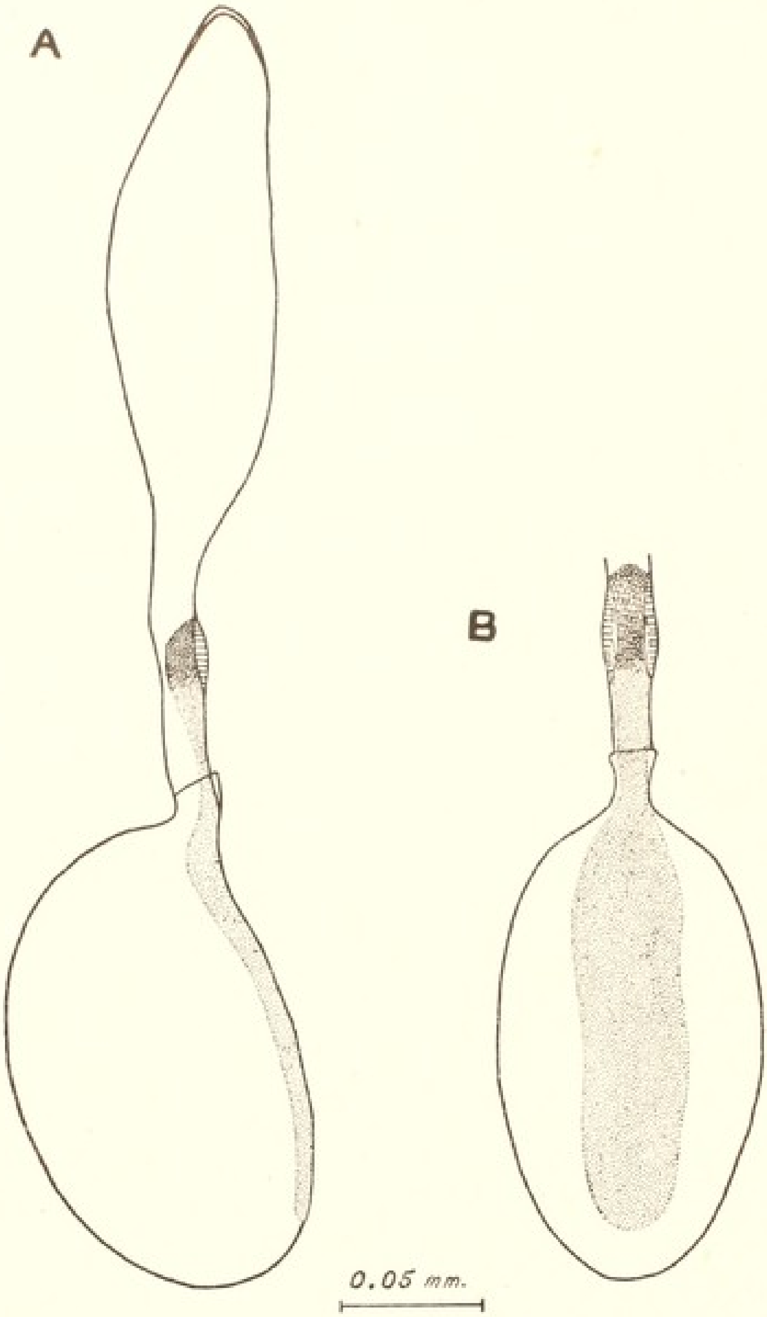


Fig. 2. *Anagyrus putonophilus* Comp. *A*, ovarian egg, lateral view; *B*, neck and egg proper, dorsal view.

this portion, together with the collapsed bulb, projects to the host's exterior. The band on the egg body is wide and long. It is widest near the stalk end and somewhat pointed at the apex. The center longitudinal section is composed of somewhat larger cells than the marginal areas. The band becomes melanized to a light brown prior to eclosion. Figure 1, *A*, shows a deposited egg with host tissue removed.

Larva.—The larva (fig. 1, *B*) is remarkably similar to that of *Anagyrus putonophilus* parasitizing the same host. The completely developed embryo does not reverse its position prior to eclosion but hatches with the head toward the stalk. The single pair of spiracles is placed at the other extreme. In the one specimen studied the spiracles were neither attached to the band nor in opposition to it, but were placed in the center of an air bubble at the bottom of the eggshell. It is highly probable that the larva was dislodged from its normal position by dissection. In later instars the spiracles are situated midway of the band.

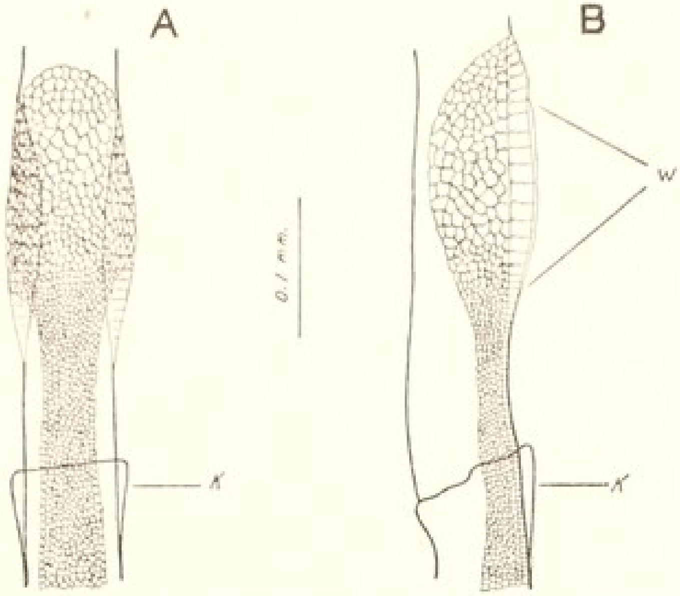


Fig. 3. *Anagyrus putonophilus* Comp. *A*, distal end of the neck of the egg, dorsal view; *B*, lateral view.

The stalk is not plugged in the manner seen in *Anagyrus putonophilus*, but a smaller, solid, and opaque plug forms by the time the second instar is reached. Melanized host material accumulates around the stalk immediately below the integument.

Anagyrus putonophilus Comp.

This encyrtid is the most abundant parasite of the mealybugs *Puto yuccae* Coq. and *P. ambigua* in southern California. The latter host has overlapping generations throughout the year and it was from this host that material was obtained for study at various times over a period of two years.

Ovarian egg.—The ovarian egg (fig. 2) of this *Anagyrus* is of the banded type. The bulb is long and narrow and possesses a slight thickening at the apex. The neck is short, and near the bulb end it is appreciably swollen. At this point the band appears, nearly circumscribing the neck. At the base of the swelling the band narrows to continue down the neck. Detailed views of the distal end of the neck are shown in figure 3.

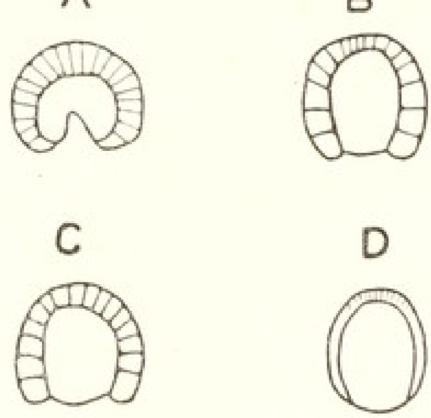


Fig. 4. *Anagyrus putonophilus* Comp. *A*, sections across the anterior of the stalk; *A*, *B*, and *C* in sequence in the area of large cells; *D*, posterior section, semidiagrammatic.

Near the egg proper the neck exhibits a slight projecting lip which is a further modification of the eggshell and not a part of the band. As is true of the other somewhat similar structures, no function has been assigned to this "collar."

The band on the egg body is moderately large and extends nearly to the apex of the egg. The margins are neither straight nor parallel. The measurements were: length of bulb, 0.21 mm.; width of bulb, 0.056 mm.; length of neck, 0.094 mm.; width of neck, 0.017 mm.; length of egg, 0.15 mm.; width of egg, 0.09 mm.; length of band, 0.133 mm.; width of band, 0.034 mm.

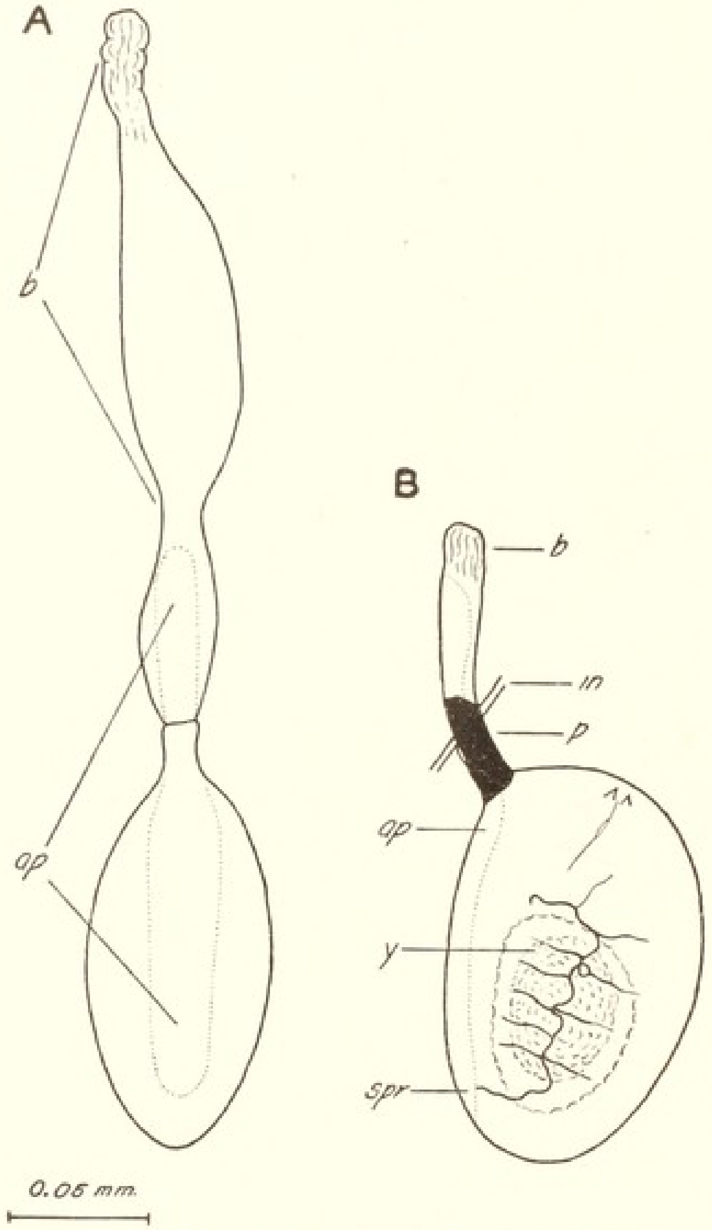


Fig. 5. *Anagyrus putonophilus* Comp. *A*, newly deposited egg after compression, showing the reinflation of the bulb; *B*, deposited egg containing fully developed embryo.

Deposited egg.—The egg is attached to the integument of the mealybug as shown in figure 5, *B*. The portion exterior remains erect. When a newly deposited freshly dissected egg was compressed, the bulb was found to be intact and could be partly reinflated (fig. 5, *A*). Complete expansion to the original size could not be obtained. A few hours after deposition a close-fitting plug forms within the neck, and the contents of the egg body cannot be forced back into the collapsed bulb. This plug becomes opaque, black, and solid before the hatching of the larva takes place. The embryo develops with the head at the stalk end of the egg and hatches from that position.

Larva.—The larva (fig. 6) has a well-developed metapneustic tracheal system. There is a single pair of spiracles which are nearly contiguous, approximately 0.0043 mm. apart; they are attached at the posterior extremity of the band and remain in that position throughout the early larval life.

Anagyrus subalbicornis (Gir.)

Clausen (1924) has stated that the egg of this mealybug parasite differs in no essential respect from that of *Zarhopalus corvinus*. I noted, however, that

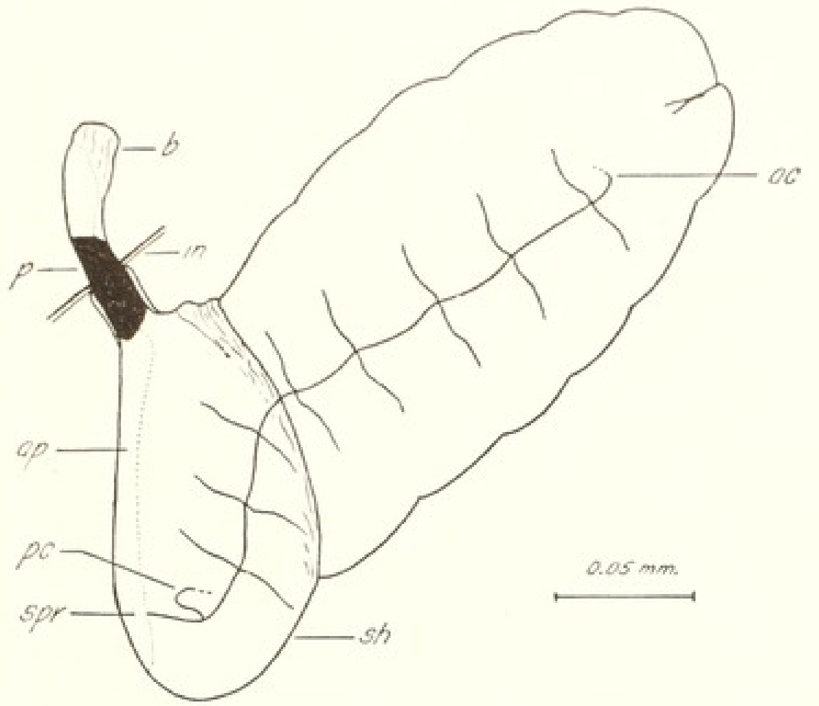


Fig. 6. *Anagyrus putonophilus* Comp. First instar larva. The spiracles are placed at the base of the aeroscopic plate (*ap*).

the egg of *Anagyrus* remains attached to the derm of the host, and the caudal end of the larva remains attached to the stalk up to the last larval stage. The egg of *Zarhopalus*, on the contrary, lay free within the host and no part of the larva remained within the eggshell.

Since the egg is attached to the integument and the larva remains partly enveloped by the eggshell up to the last stage, it may be readily assumed that the egg is banded and the larval tracheal system is metapneustic.

Anarhopus sydneyensis Timb.

Compere and Flanders (1934) found the eggs and larvae to be similar to those of *Tetracnemus pretiosus* (fig. 67); they found the deposited egg to be free in the body fluid of the host mealybug, but suggested that the usual position may be one of attachment to the integument. The minuteness of the eggs made it difficult to determine this point.

For the purposes of this discussion, the larva of *Anarhopus* is assumed to be identical to *Tetracnemus*, i.e., in the first part of the stadia the larva is atracheate but later on it possesses rudimentary tracheae in the thoracic region.

Anicetus annulatus Timb.

Only a limited supply of material of this primary parasite of *Coccus hesperidum* Linn. was available. The egg has a neck slightly longer than the egg proper, which is narrowly elliptical and has a band no wider at any point than the neck. Compere (1924) in describing the egg omitted any reference to a band, but in his drawing of the deposited egg (fig. 7) there is a suggestion of a band.

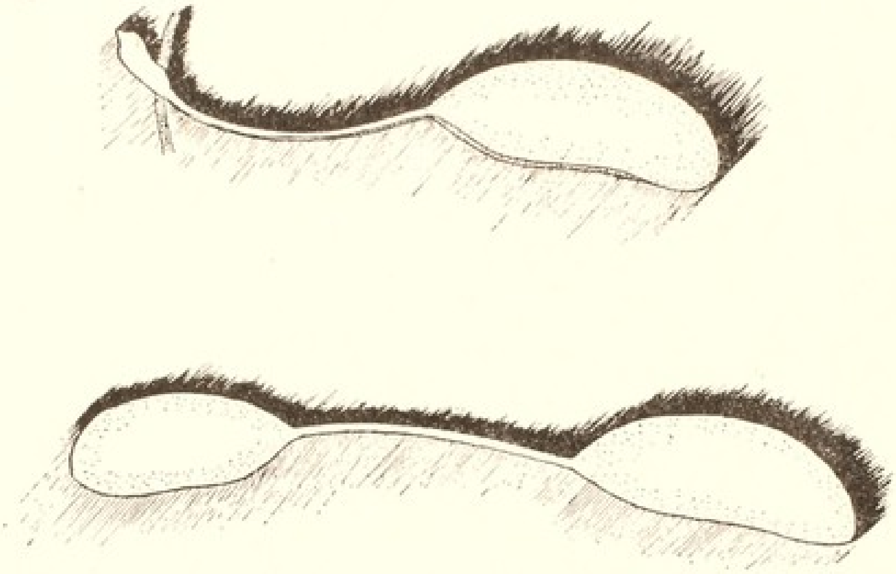


Fig. 7. *Anicetus annulatus* Timb. Upper, newly laid egg with tip of stalk projecting through the integument of the host. Note the bandlike appearance. Lower, egg before oviposition. (Drawing by Compere, 1924.)

The measurements of one egg which I observed were: length of bulb, 0.141 mm.; width of bulb, 0.056 mm.; length of neck, approximately 0.227 mm.; length of egg, 0.184 mm.; width of egg, 0.073 mm.

Compere noted that the deposited egg was suspended by the long stalk from the integument of the host, and surmised that the stalk was probably utilized in respiration of the larva but did not descrbe the tracheal system. My observations were only slightly more adequate. The neck of the deposited egg is so long that it doubles back upon itself. The bulb remains intact though collapsed. The larva develops with the head at the anterior end of the egg and reverses before eclosion, so that the single pair of spiracles lies almost at the very base of the stalk. The tracheae are comparatively minute but the system is complete.

Anisotylus sp.

Only the ovarian egg of this coccinellid parasite was examined. A description is incorporated here as none of the immature stages of any representative of the genus has been reported.

The ovarian egg (fig. 8, *A*) is exceedingly large in comparison with most encyrtid eggs. The bulb is not as long as the egg proper and the neck is shorter than either body. The egg proper is elliptical in shape, without any modification of the chorion. The measurements were: length of bulb, 0.218 mm.; width of bulb, 0.077 mm.; length of neck, 0.103 mm., approximately; width of neck, 0.013 mm.; length of egg, 0.265 mm.; width of egg, 0.141 mm.

Aphidencyrtus aphidivorus (Mayr)

Silvestri (1908) reported this species as a secondary parasite on *Aphidius brassicae* Marsh., a braconid aphid parasite, and as a tertiary parasite on

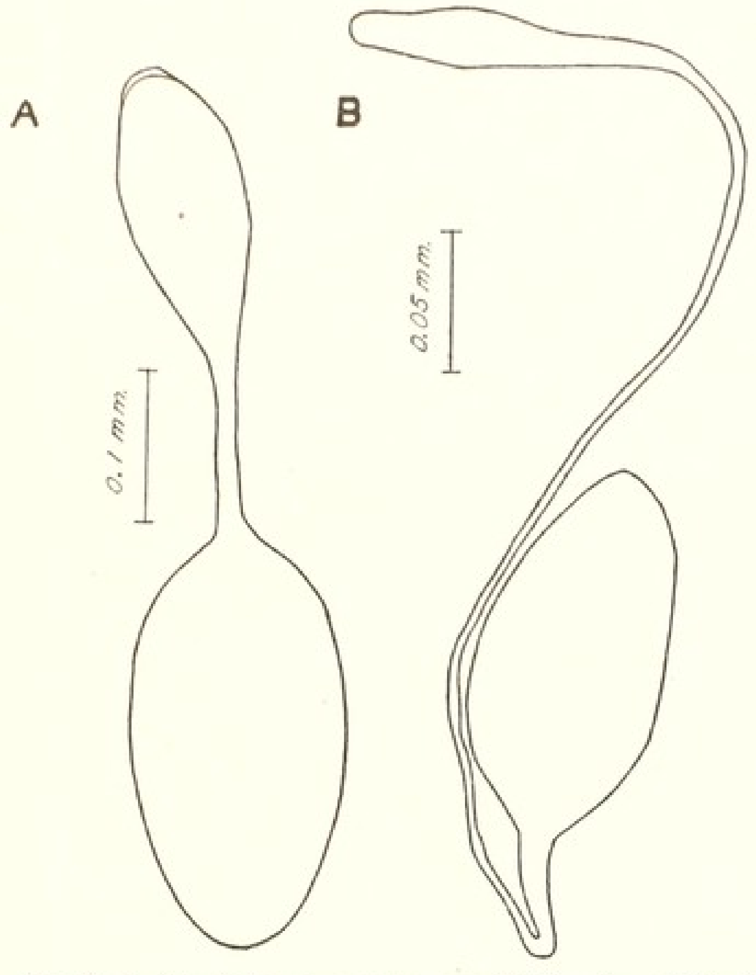


Fig. 8. *A*, *Anisotylus* sp., ovarian egg. *B*, *Quaylea whittieri* (Gir.), ovarian egg, the neck partly collapsed.

Allotria vittrix Westw. var. *infusciata* Kief., a cynipid, and he discussed the various stages of development. Griswold (1929) examined the biology of *Aphidencyrtus inquisitor* Girault, now synonymous with *A. aphidivorus*, and recorded the host *Aphelinus jucundus* Gahan.

Ovarian egg.—According to description, the egg is similar to that of *Acerophagus* species described, without distinctive features.

Deposited egg.—The egg is said to be deposited intact within the host, though the bulb is practically collapsed. The various positions or directions assumed by the "pedicel" and reported by Griswold are of no consequence.

Griswold noted that larva assumed a "curved position with the cephalic and caudal ends folded back toward the middle part of the body."

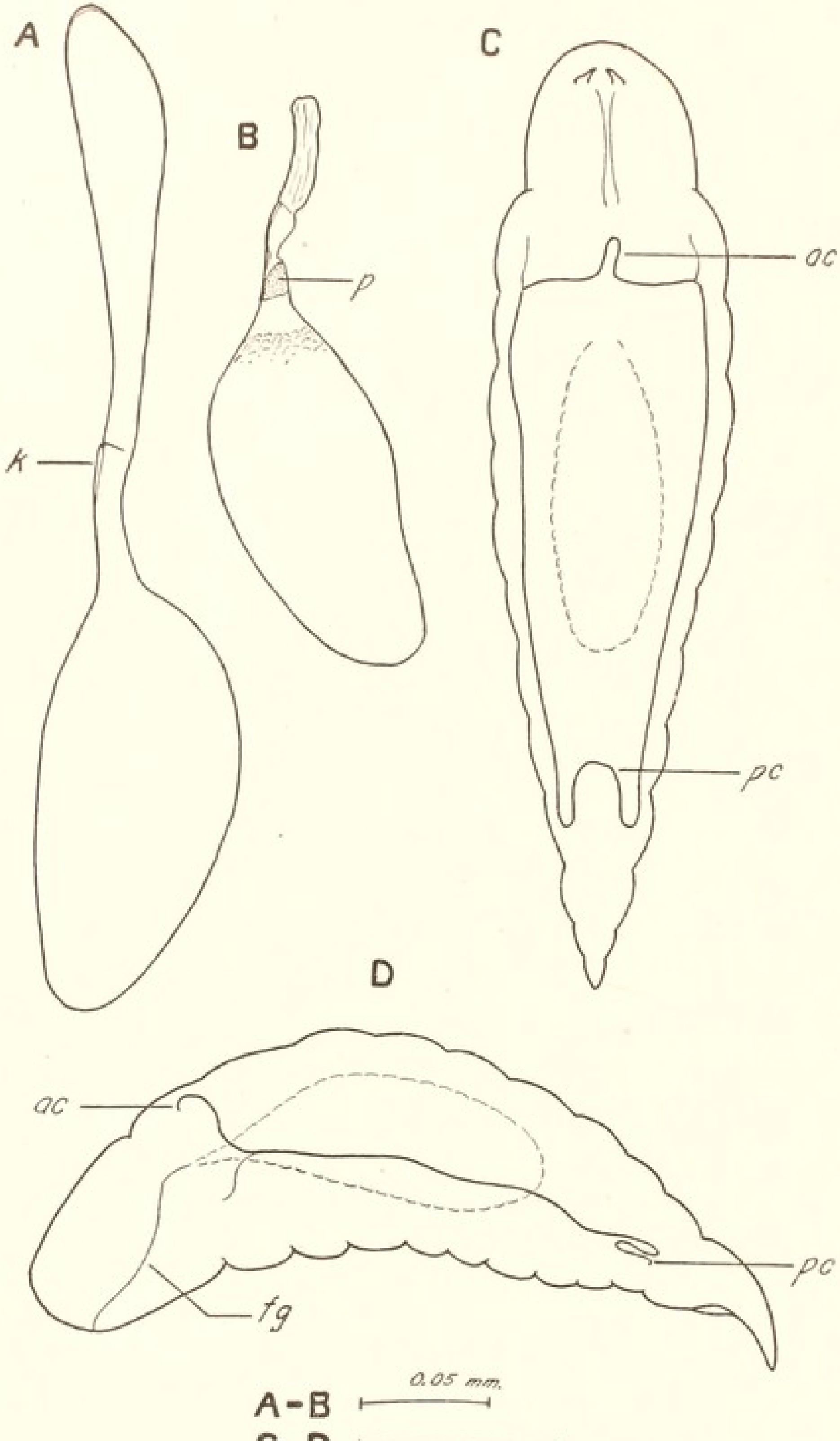


Fig. 9. *Apoanagyrus* sp. *A*, ovarian egg, lateral view; *B*, newly deposited egg; *C*, dorsal view of first instar larva; *D*, lateral aspect of same.

Larva.—The first instar larva has been described as caudate and somewhat crescentic in shape. Although neither Silvestri nor Griswold mentioned the tracheal system, it is apparent from the type of egg and larva that spiracles must be lacking.

Apoanagyrus sp.

Only one female was secured from a native species of mealybug in one locality, and the number of observations was consequently limited.

Ovarian egg.—The structure of the ovarian egg (fig. 9, *A*) is very simple. The neck has a "collar" midway between the bulb and the egg body. The egg proper is only slightly convex on the dorsal side; hence similar to banded eggs. The measurements were: width of bulb, 0.034 mm.; length of bulb and neck to collar, 0.192 mm.; length of neck from collar to egg, 0.051 mm.; length of egg, 0.167 mm.; width of egg, 0.09 mm.

Deposited egg.—The egg (fig. 9, *B*) may be found attached to the integument which surrounds the neck at the collar and lies almost parallel to the surface. The neck becomes constricted immediately below the integument and, if insufficient care is taken with dissections, the main body of the egg is severed from the connection at this point. The remainder of the neck and the bulb extend to the exterior and remain somewhat erect. Forced reinflation of the bulb was prevented by a wad of membranous material at the aperture of the neck.

Larva.—The larva (fig. 9, *C, D*) lies free within the body cavity of the host. Though the abdomen comes to a narrow point, none of the segments is attenuated to form a "tail." Laterally the body is crescentic in shape. The tracheal system is apneustic. The anterior commissure extends in a narrow loop over the foregut; the posterior commissure loops broadly toward the anterior end of the larva. No secondary tracheae could be discerned other than cephalic branches.

Apoanagyrus californicus Comp.

Since this encyrtid attacks many species of native mealybugs in southern California, abundant material could be obtained—a distinct advantage, inasmuch as the parasite was a difficult one with which to work.

Ovarian egg.—This species possesses a much smaller egg (fig. 11, *A*) than the species of *Anagyrus* under investigation. The bulb is quite narrow and the neck is short. The distinguishing feature is the presence of a collar (k). It appears to be a thickening of the chorion which surrounds one half of the neck. Its projecting edge is bidentate. The posterior margin is not sharply demarcated but gradually merges into the remainder of the neck. There is no indication of cells. The structure very readily acquires the color of acid fuchsin in lactophenol. The egg proper is slightly more convex on the ventral side than on the dorsum. The measurements were: length of bulb to collar, 0.128 mm.; maximum width of bulb, 0.017 mm.; length of neck, 0.043 mm.; length of egg, 0.133 mm.; width of egg, 0.064 mm.

Deposited egg.—If the entire host is immersed in the acid fuchsin solution for a few minutes, the protruding neck and collapsed bulb of a newly deposited egg can be readily observed. After several hours the egg is more difficult to

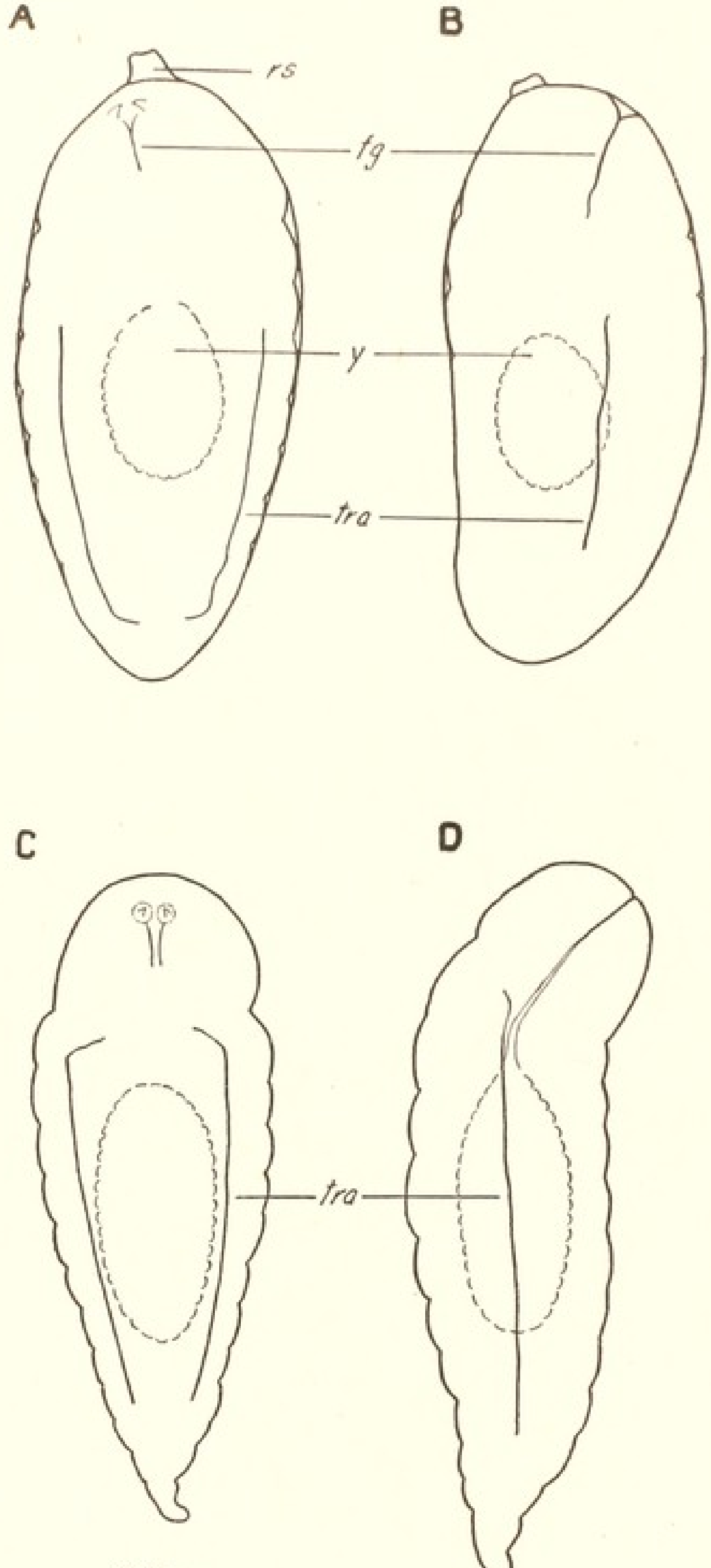


Fig. 10. *Apoanagyrus californicus* Comp. *A*, dorsal aspect of deposited egg containing fully developed embryo; *B*, lateral aspect of the same; *C*, dorsal view of the newly hatched larva; *D*, lateral aspect of the first instar larva.

locate by this method as the exterior portion collapses entirely. Even when the eggs are found and the host is carefully dissected around this point, only rarely is an egg found attached to the integument. The egg proper may sometimes become separated from the neck at oviposition.

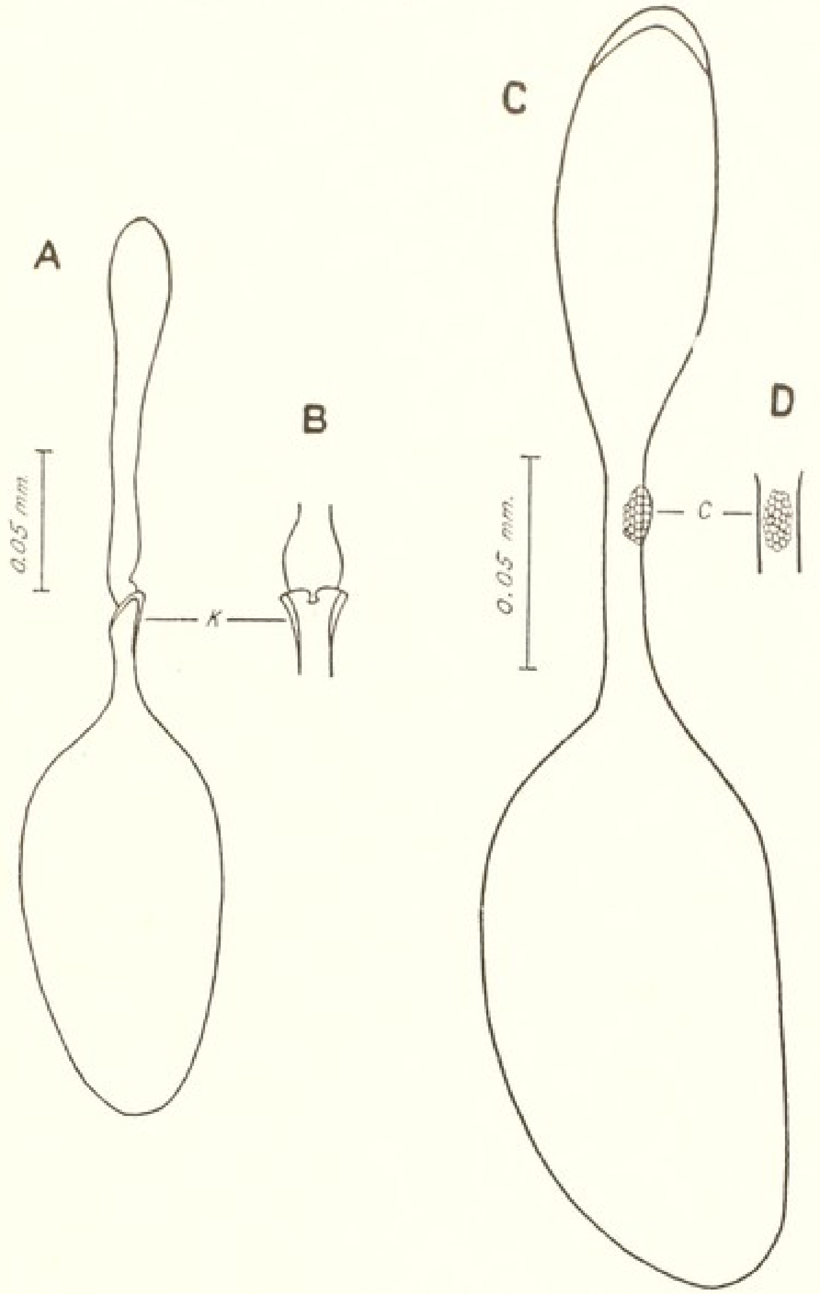


Fig. 11. *A*, *Apoanagyrus californicus* Comp., ovarian egg, lateral view; *B*, dorsal view of neck of ovarian egg; *C*, *Leptomastidea abnormis* (Gir.), lateral view of ovarian egg; *D*, dorsal view of portion of neck of ovarian egg.

Before the embryo is fully developed the egg has become detached from the stalk and floats free when the host is dissected. The embryo develops with the anterior end toward the stump of the broken stalk (fig. 10, *A*, *B*). Eclosion was not observed.

Larva.—The newly hatched larva (fig. 10, *C*, *D*) is wedge-shaped and widest in the thoracic region. The posterior end tapers to a short, hooked tail. No spiracles could be discerned and they probably do not exist because of the lack of any band on the egg and the failure of the larva to remain posteriorly enclosed by the shell after hatching. Lateral tracheal trunks and a portion of the anterior commissure appear but the remainder is not visible. Not many hours after eclosion the larva becomes much elongated and the tracheal system is not apparent. The failure to find tracheae may be ascribed to inadequate technique or to the possibility that the structures ceased to function in the usual manner.

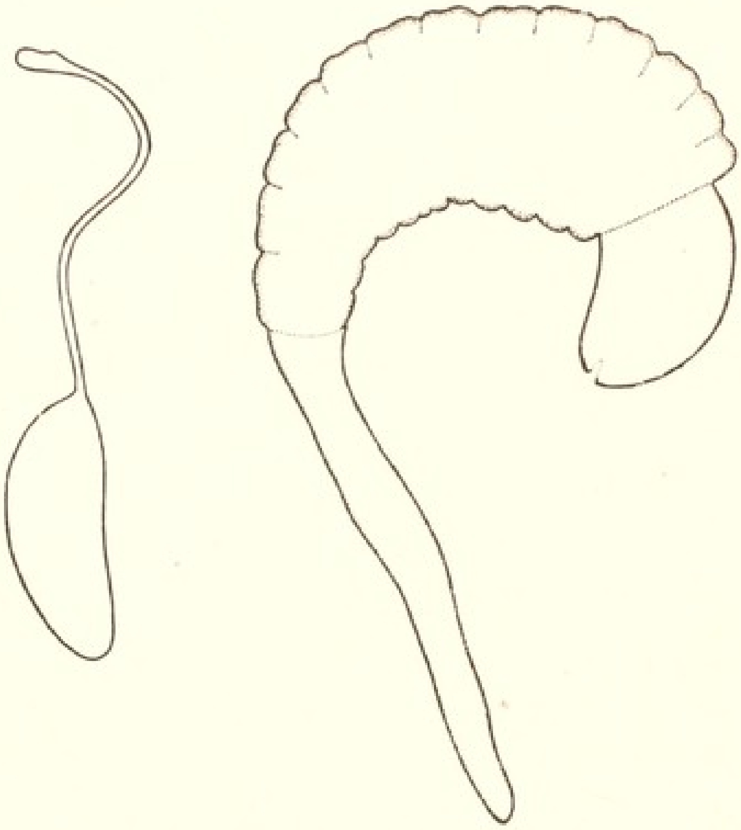


Fig. 12. *Baeoanusia oleae* (Silv.). Newly laid egg and the second instar larvae. From the original drawing by H. Compere. (Compere, 1931*a*.)

In the last embryonic stages only a portion of the tracheae is observable. From the small number of successful dissections, it appeared that only three-fourths of the tracheal trunks and a portion of the posterior commissure assume the silvery sheen.

Baeoanusia minor (Silv.)

Compere (1931) briefly investigated this species but was not able to supply detailed information. The egg and larva are probably similar to those of *Baeoanusia oleae.*

Baeoanusia oleae (Silv.)

It is apparent from the brief description of the developmental stages of this parasite by Compere (1931) that the egg and larva differ in no major respect from other secondary parasites of scale insects. The deposited egg has a long stalk, and in his illustration (see fig. 12), the bulb remains attached though collapsed. The eggs are deposited free within the host, but Compere noted that in his specimens some of the eggs were occasionally too entangled in the internal organs to float out when the host was dissected.

The first-stage larva is not described. From the drawing of the second stage (see fig. 12) it is reasonable to assume that the first instar larva is of similar structure and consequently devoid of spiracles.

Blastothrix longipennis How.

This species is a common enemy of many kinds of lecanine scales in southern California. Although quantities of adults emerged from hosts which had been obtained from widely separated localities, none contained ovarian eggs. A series of females was retained in the laboratory during 1936 and dissected at intervals. On June 15, August 25, and December 26 no indication of developing ovaries was apparent. Similar results were obtained with females found feeding on aphid honeydew in October of that year. Some were dissected at the time of collection and others on December 26; during the interval they were fed weekly on honey and were refrigerated. None contained eggs. Fortunately, however, one female collected in the early summer of 1936 from an infestation of *Lecanium corni* had mature ovaries.

The deposited eggs and the larval stages were observed in field-collected scale. The only evidence that the stages found in the scale and the ovarian eggs from the single female were the same species is the apparent identity in the egg structure. Although such evidence is circumstantial, I believe it to be reliable.

Ovarian egg.—This stage is illustrated in figure 13. The measurements were: length of bulb, 0.1 mm.; width of bulb, 0.047 mm.; length of neck, 0.06 mm.; width of neck, 0.013 mm.; length of egg, 0.175 mm.; width of egg, 0.107 mm.; length of band on egg, 0.06 mm.; width of band on egg, 0.039 mm. The tip of the bulb is very slightly thickened. The neck is of uniform width. The band covers half of the dorsal side for most of the length but widens slightly toward the bulb end. The cells are uniform in size. On the egg body the band differs from those of most other eggs in its nearly circular outline. The cells of the center section are noticeably larger than those of the marginal areas. In the lateral aspect the thickening presented by the band is just visible.

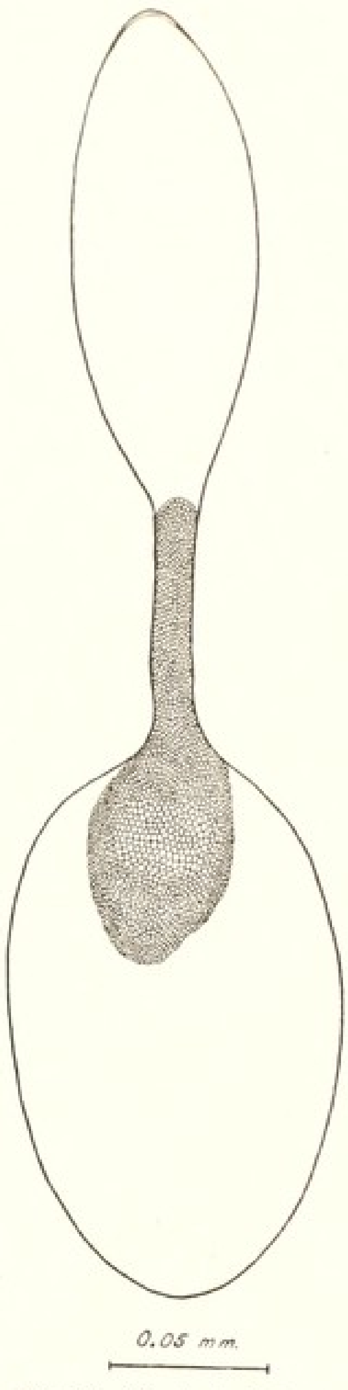


Fig. 13. *Blastothrix longipennis* How. Ovarian egg.

Deposited egg.—As was noted above, deposited eggs were secured only from field-collected material. Three species of host were dissected, and because the eggs found were smilar to the ovarian eggs obtained from the single female, and since females of the same species emerged from these scales, it is assumed that they represent single encyrtid species.

The eggs are laid in medium-sized to small hosts and are inserted dorsolaterally, usually at the posterior end of the scale. In many eggs, especially

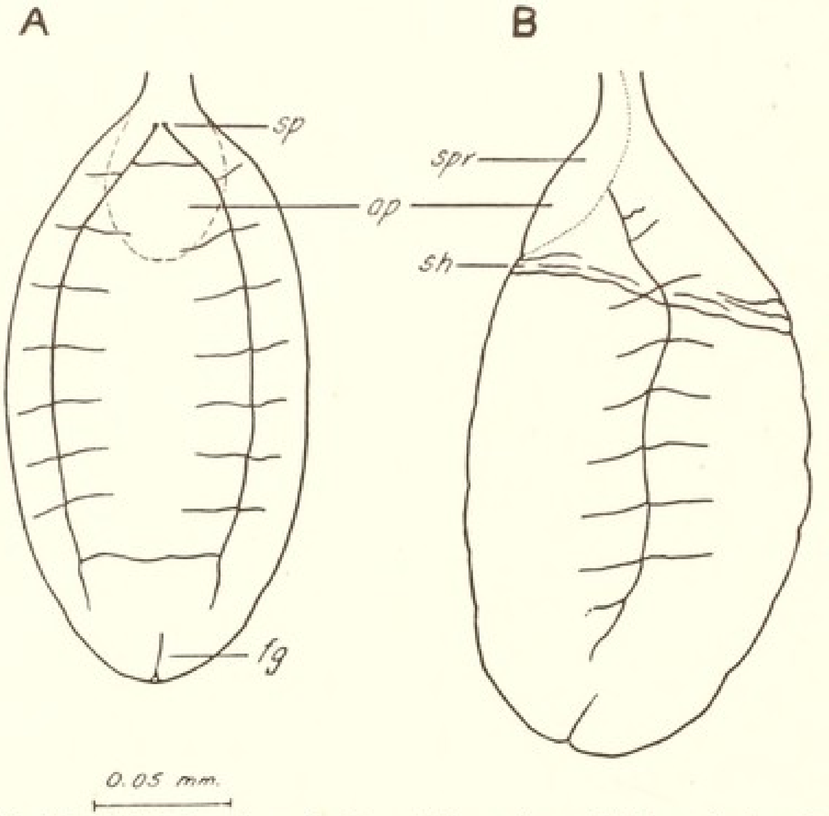


Fig. 14. *Blastothrix longipennis* How. *A*, larva immediately prior to eclosion, dorsal aspect; *B*, newly hatched larva in lateral aspect.

those near the margin of the scale, the silvery band is clearly visible through the integument. The collapsed bulb remains attached to the neck protruding from the host.

One deposited egg was found which clearly showed the embryo to develop with a head at the stalk end of the egg. A later stage of development is shown in figure 14, *A*. The embryo has reversed itself, though eclosion has not occurred. The tracheal system is full of air.

Larva.—After eclosion, the shell, except for the band, lies wrinkled over the posteriormost segments of the larva (fig. 14, *B*). The band holds the remains erect. There are two nearly contiguous spiracles attached to the upper central area of the band on the egg proper. Lateral branches of the tracheae are well developed.

Blastothrix sericea (Dalm.)

Imms (1918) and Silvestri (1919) have given creditable accounts of this scale parasite. Imm's work is notable in that it contains the first description of a metapneustic tracheal system in Hymenoptera.

The intricate structure of the egg was described in detail by Silvestri. Imms gives no account of the ovarian egg nor does he mention any structures which may be compared with those in Silvestri's report, according to which the egg

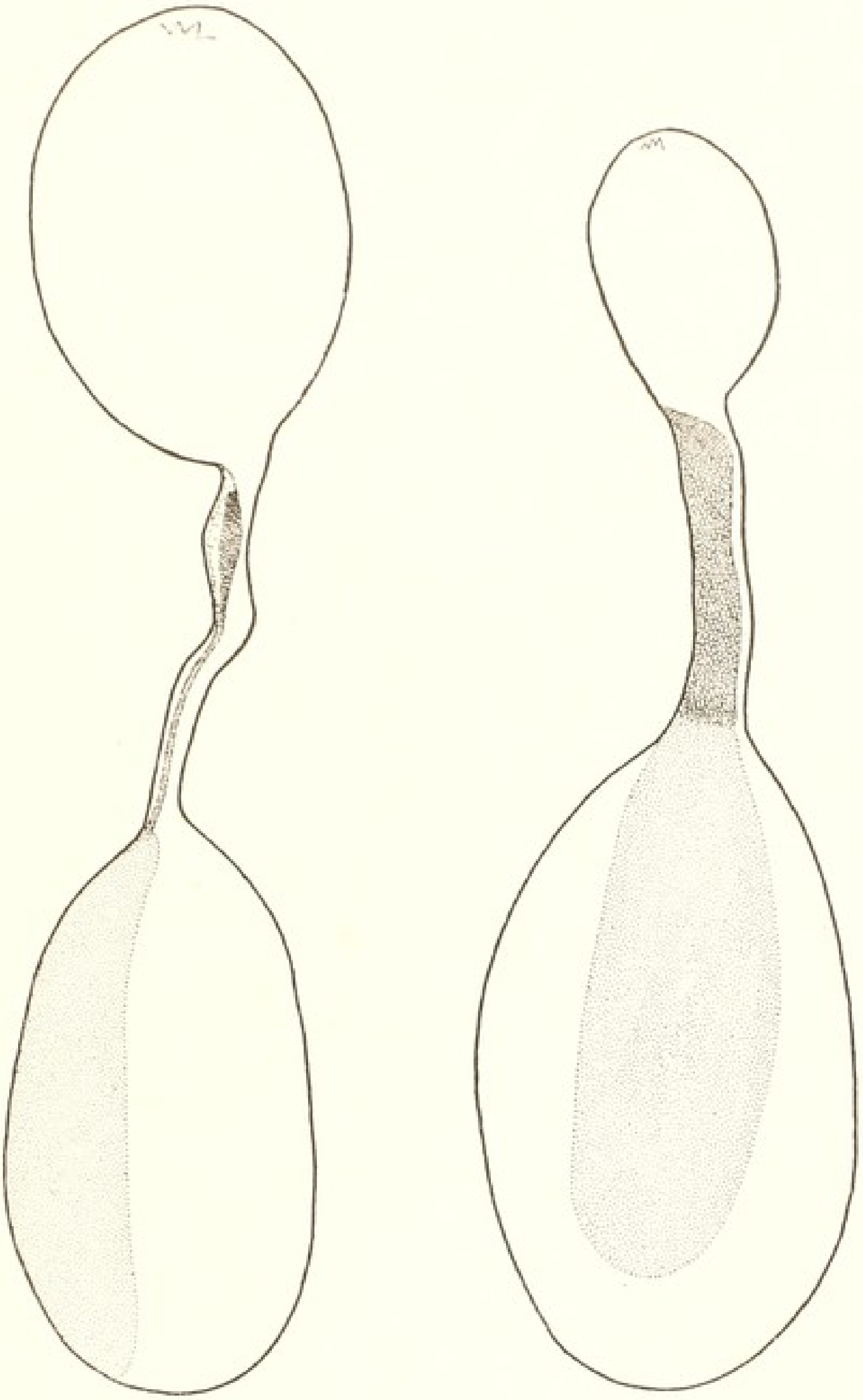


Fig. 15. Left, *Encyrtus infidus* (Rossi) ovarian egg. Right. *Blastothrix sericea* (Dalm.) ovarian egg. (Redrawn from Silvestri, 1919.)

has a prominent aeroscopic plate which extends nearly to the apex of the egg proper. The ovarian egg, redrawn from Silvestri, is shown in figure 15, right.

Imms and Silvestri agree that the respiratory system is metapneustic. Figure 16, right (also from Silvestri), gives a diagrammatic presentation of the first instar larva.

Bothriothorax nigripes How.

This is the only species obtained which is known to attack Diptera; it is supposedly common on syrphid flies in California. Host material was too inadequate to permit definite conclusions to be reached concerning the deposited egg and larva.

The ovarian egg (fig. 17) is singularly marked for the length of the neck with a fine line apparently having no cellular structure. There is indication that the line extends some distance on the egg proper.

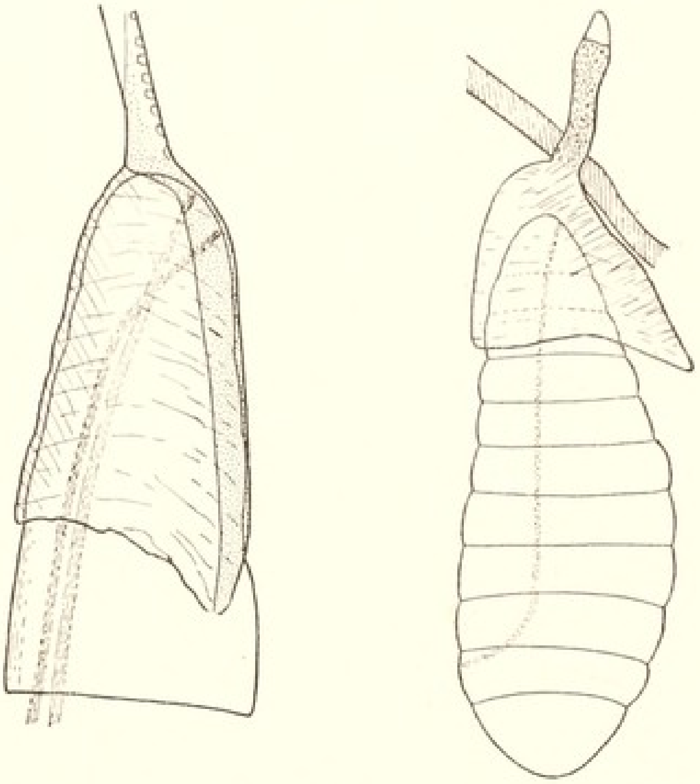


Fig. 16. Left, *Phaenodiscus aeneus* (Dalm.), posterior portion of the larval body enclosed in the remains of the eggshell. The tracheae lead directly to the aeroscopic plate. Right, *Blastothrix sericea* (Dalm.), newly hatched larva suspended from the integument of the host scale. (Redrawn from Silvestri, 1919.)

The developmental stages may be quite similar to that described for *Ectroma* sp.

Carabunia myersi Watrst.

Myers (1930), in discussing this parasite of *Clastoptera* nymphs, omits any description of the ovarian egg.

The deposited egg "was found floating loose among the abdominal organs, but may have become detached during dissection." It has a short pedicel which is about half as long as the egg. The chorion is thin, with no evident sculpturing.

The body of the newly hatched larva is crescentic and tapers sharply to a long tail, which is usually clasped by the remains of the eggshell. The tracheal system is apneustic.

Cerapterocerus mirabilis Westw.

The only investigation of this hyperparasitic species was made by Silvestri (1919) and adequate descriptions were given.

The ovarian egg has a thin nonbanded chorion. The newly deposited egg lies free in the body cavity of the primary host. As development proceeds, the anterior part and the neck remain attached as a membranous appendage to the main egg body.

The first instar larva (fig. 18) has an elongate body with the abdominal segments prolonged into a sharp tail. The tracheal system is apneustic but well developed.

Cheiloneurus inimicus Comp.

The early stages of this hyperparasite of scales have been discussed by Compere (1925). The ovarian egg lacks distinguishing features. In that stage I found its bulb to be long and narrow, slightly wider than the neck and undifferentiated from it. The egg proper is widest at the anterior end and tapers toward the apex. Measurements were: length of bulb and neck, 0.225 mm.; length of egg, 0.18 mm.; width of egg, 0.073 mm.

According to Compere the egg is deposited free within the body of the primary hymenopteron parasite. A pedicel remains attached to the egg and becomes increasingly inconspicuous as the egg increases in size with the growth of the embryo.

The larva is of the tailed type. According to Compere's drawing (fig. 19) the tail represents an attenuation of the 14th visible body segment and is half as long as the remainder of the larva. No information is supplied concerning the tracheal system, which is probably apneustic.

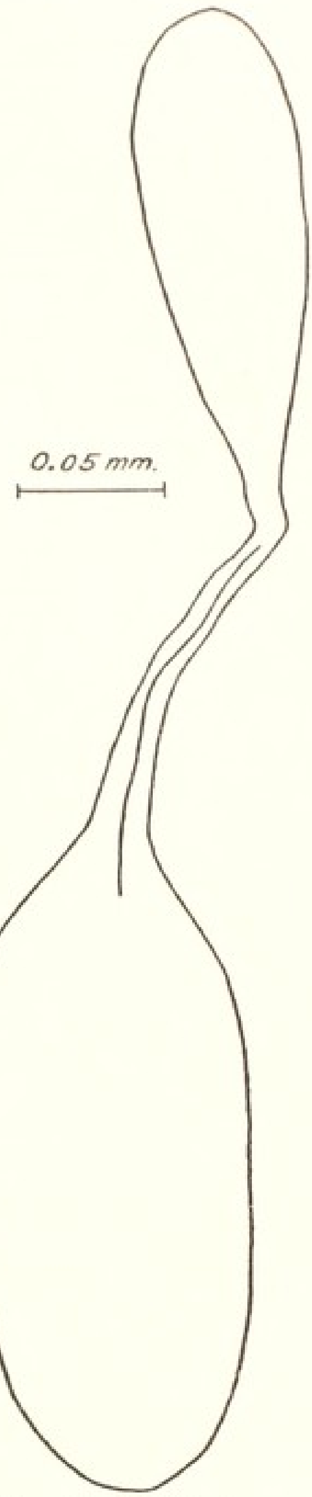


Fig. 17. *Bothriothorax nigripes* How. Ovarian egg. The "line" on the stalk has no cells.

Cheiloneurus noxius Comp.

This species is similar in its habits and bioloby to the aforementioned species and has been discussed in an article by Le Pelley (1937). The ovarian egg has a moderately long neck and the bulb is slightly greater in width. The deposited egg lies free in the body of the host and the collapsed bulb is still attached. Upon eclosion the larva frees itself completely from the egg shell. The larva is of the tailed type common to many apneustic encyrtid larvae. Le Pelley does not mention the tracheal system nor does it appear in the illustrations. By analogy the system is apneustic.

Chrysopophagus compressicornis Ashm.

The only investigation on the biology of any species of *Chrysopophagus* was made by Clancy (unpublished manuscript). *Crysopophagus compressicornis* is a hyperparasite of *Chrysopa californica* Coq. and *Chrysopa majuscula* Banks.

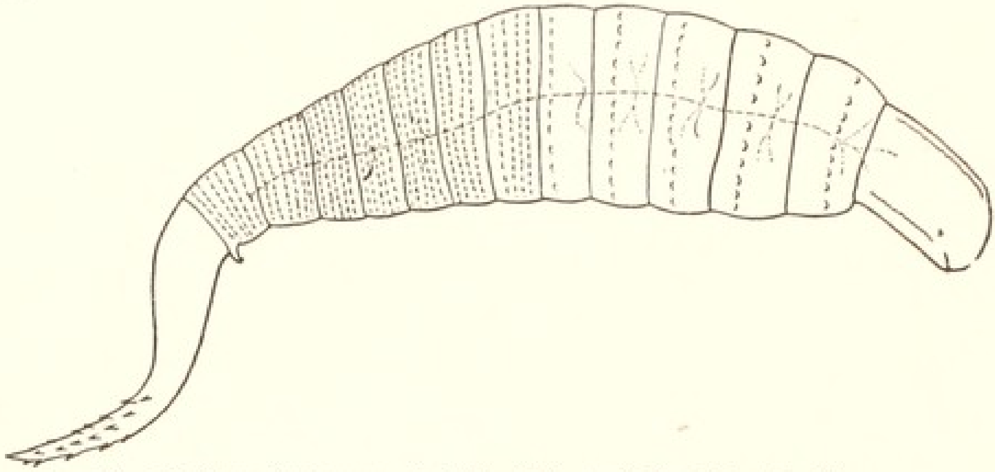


Fig. 18. *Cerapterocerus mirabilis* (Westw.). Newly hatched larva. (Redrawn from Silvestri, 1919.)

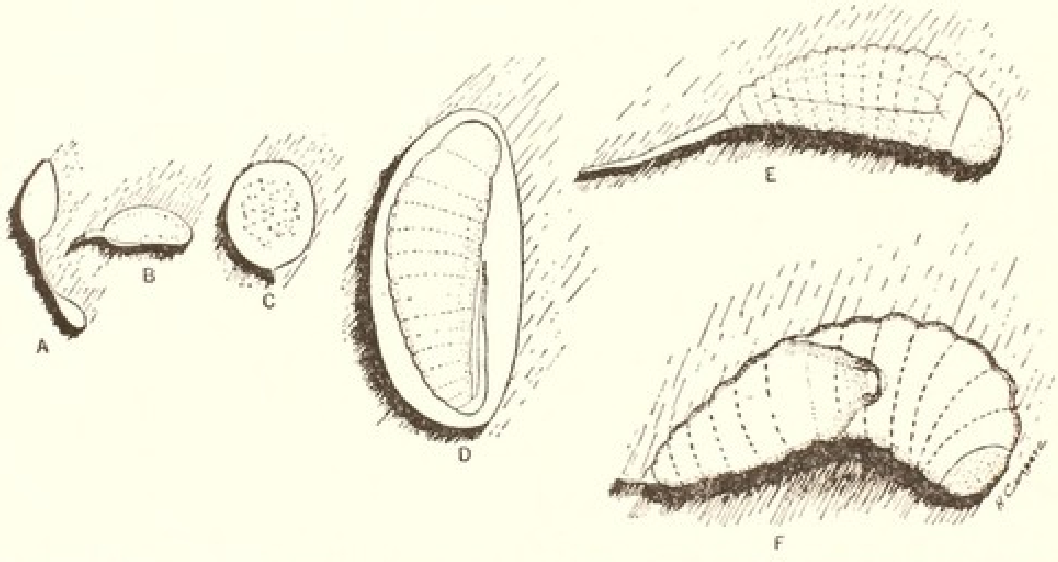


Fig. 19. *Cheiloneurus inimicus* Comp. *A*, ovarian egg; *B*, newly deposited egg; *C*, egg in process of growth; *D*, egg just after hatching, showing larva within; *E*, first stage larva; *F*, partly grown larva with moult skin adhering. From the original drawing by H. Compere. (Compere, 1925.)

The ovarian egg is of the simple double-bodied type. The deposited egg is only slightly different. The bulb is attenuated but intact and together with the neck shrivels as the egg increases in size. The stalk may be anchored occasionally to tissues or fat body but never to the host's integument.

The deposited egg increases to five times the original size. The larva develops with the head at the anterior end and hatches from that position. All but the head and tail is surrounded by a trophamnion, the chorion apparently disappearing.

The larva is of the tailed type and usually somewhat crescentic in shape. The tracheal system is apneustic.

Clausenia sp.

This unidentified species from an unknown host was among the adults that emerged at the University of California Citrus Experiment Station from scale material collected by Harold Compere in South Africa. It was not propagated or released. Since the early stages of species of the genus have not heretofore been described, a description of the egg of this unknown species is given.

The ovarian egg (fig. 20) is nearly identical with the eggs of *Apoanagyrus* spp. The only possible suggestion of a band is a collar on the neck. In all other respects the egg structure is simple. The egg measured as follows: length of egg, 0.146 mm.; width of egg, 0.077 mm.; length of neck from collar, 0.034 mm.; width of neck, 0.012 mm.; length of bulb to collar, 0.299 mm.; maximum width of bulb, 0.034 mm.

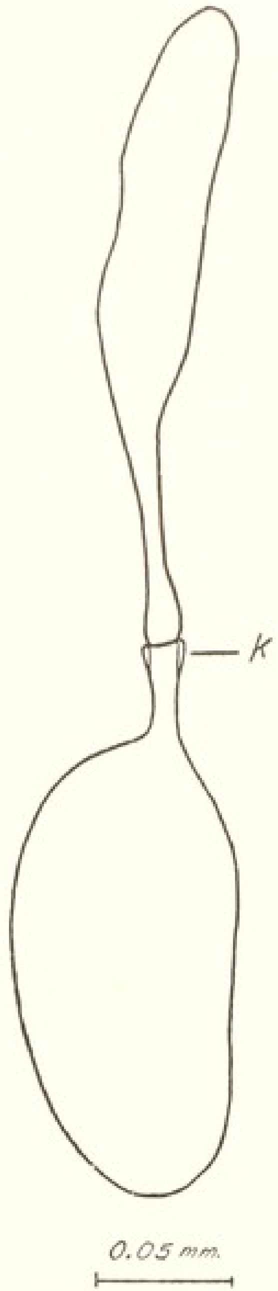


Fig. 20. *Clausenia* sp. Ovarian egg, lateral aspect. Note band which rings neck at *K*.

Coccidoxenus niloticus Comp.

This species was among those introduced by Compere from South Africa. During its propagation material was made available for study.

Ovarian egg.—The ovarian egg (fig. 21, *A*) consists of two large bodies connected by a moderately long neck. The bulb is much larger than the egg body. Within the ovarioles the bulb is distended, whereas the egg proper is not completely filled and the shell is wrinkled. When the ovarian egg is compressed to smooth out the folds, the bulb extends enormously so that its volume must be at least twice that of the main body. The neck is curved toward the bulb and resists all attempts to straighten it. The measurements were: length of bulb, 0.468 mm.; width of bulb, 0.36 mm.; length of neck, 0.288 mm.; width of neck, 0.026 mm.; length of egg, 0.423 mm.; width of egg, 0.19 mm.

Fig. 22, *A* and *B*, gives some indication of the elaborate appearance of the anteriormost portion of the band on the neck. The features immediately noticeable are the greater width of the neck, the grading of cell size in the portion of the band that nearly circumscribes the neck, and the sudden reduction to rows of stipples throughout the remainder of the neck. In the lateral view an abrupt thickening of the margins of the larger cells is seen, which may be significant.

Figure 21, *B*, shows in detail the band on the egg proper. The breadth is in itself distinctive. The cells are mostly very minute, but some are a little larger and are arranged in a definite pattern of polygons.

Deposited egg.—The stalks of the deposited eggs protrude from the dorsum of the scale host in such a manner as to look like little white hooks. The collapsed bulb remains attached (see fig. 24, *A*). The portion of the stalk within the host is curved so that the dorsal surface of the egg is nearly parallel to the integument.

Larva.—The newly hatched larva (fig. 24, B) exhibits no striking departure from the other metapneustic larvae except the larger diameter of the

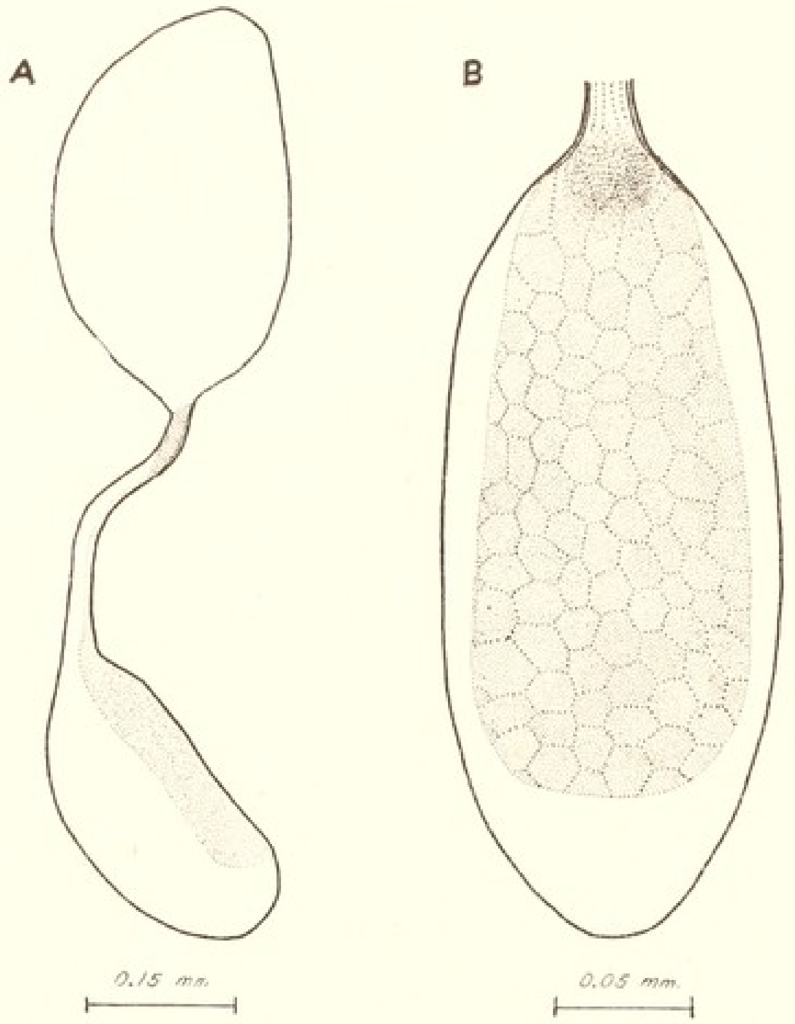


Fig. 21. *Coccidoxenus niloticus* Comp. *A*, ovarian egg, lateral aspect; *B*, dorsal view of egg proper, showing detail of aeroscopic plate. The design is formed by larger cells.

tracheae. The spiracles are placed at the stalk end when the larva is newly hatched, but in the later instars their position is shifted toward the broader portion of the band. All the eggshell collapses except the band.

Comperiella bifasciata How.

This species has been adequately discussed by Compere and Smith (1927). The newly deposited eggs are found free wthin the scale host or loosely attached to its integument. The stalk partly collapses after deposition and apparently serves no useful function. It is apparent from Compere's drawing (his fig. 25, 3) that the embryo develops with its head at the stalk end of the

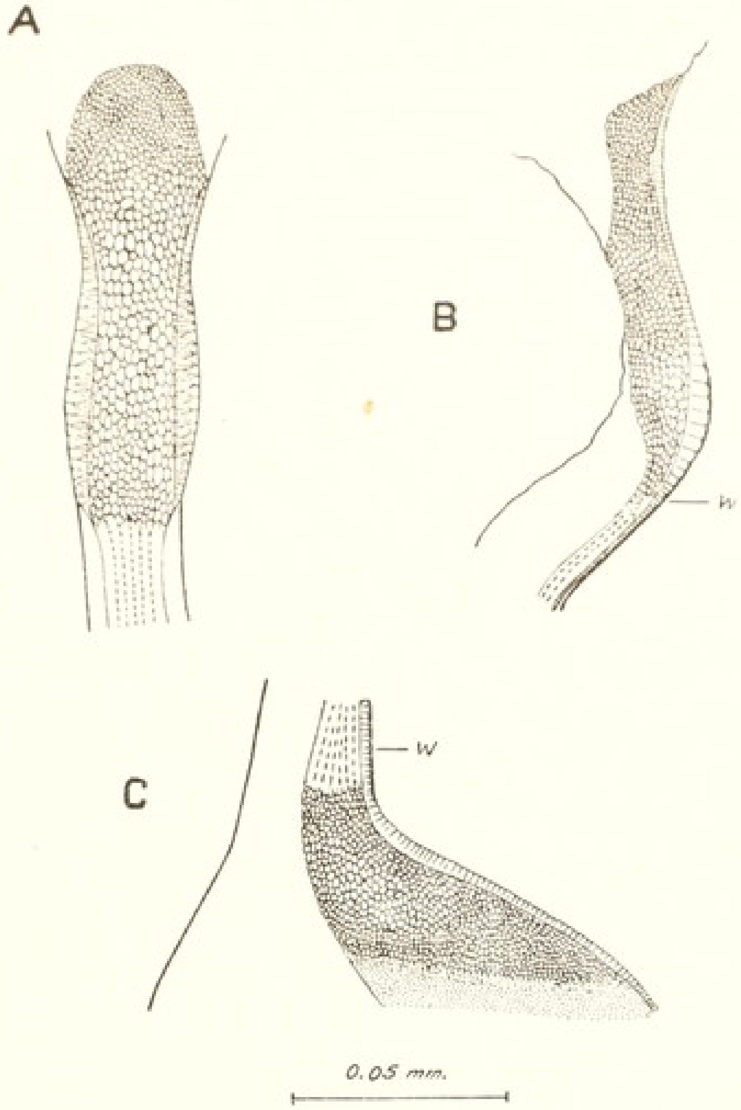
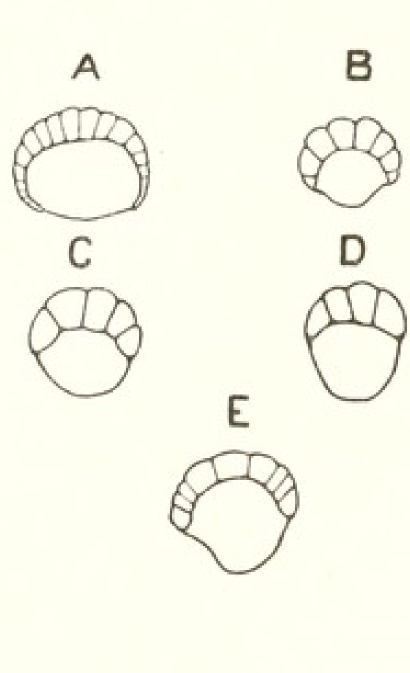


Fig. 22. *Coccidoxenus niloticus* Comp. *A* and *B*, egg, showing two aspects of distal end of neck; *C*, lateral view of distal end of egg proper.

Fig. 23. *Coccidoxenus niloticus* Comp. Sectional views of stalk. *A*, *B*, and *C* in sequence in the area of large cells; *D*, the remainder of the neck; *E*, sections immediately prior to the egg proper. Semidiagrammatic.



egg. At eclosion, instead of issuing from the egg the larva remains with nearly half of the posterior end enclosed. According to whether the egg is attached to the host's integument or free within the host, the larva is secured or free floating. The tracheal system consists of a simple short tracheal trunk without spiracles.

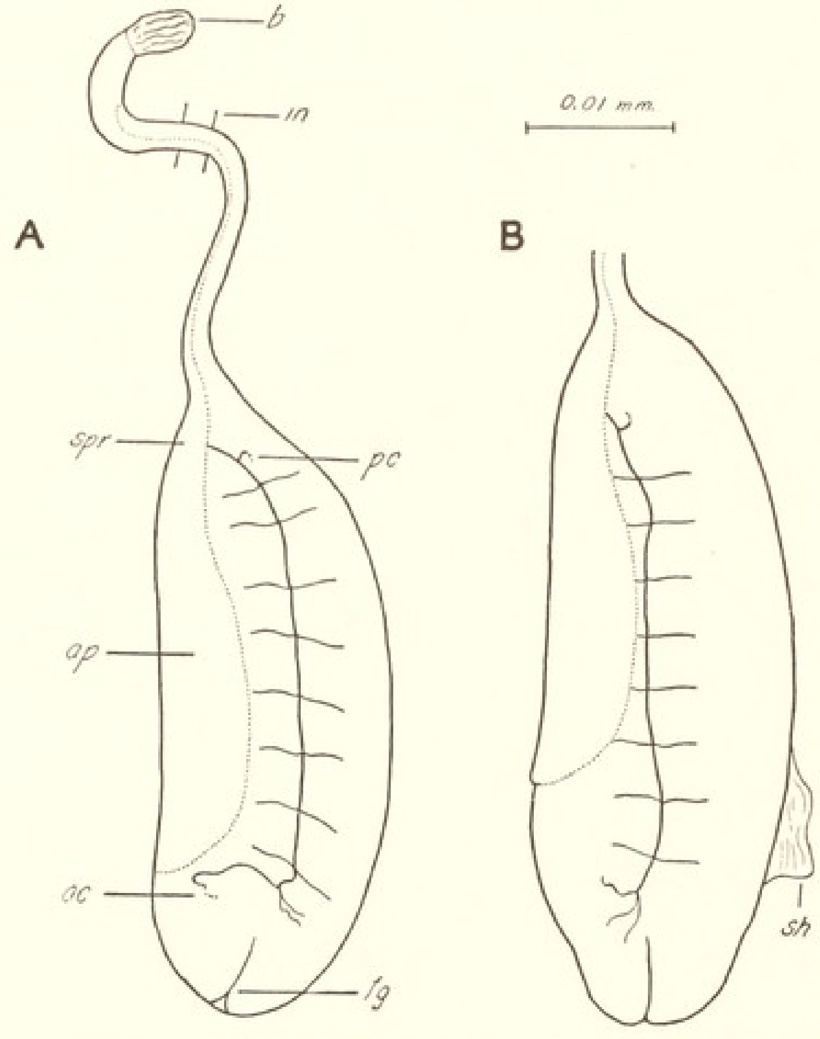


Fig. 24. *Coccidoxenus niloticus* Comp. *A*, deposited egg, showing fully developed embryo; *B*, newly hatched larva almost wholly enclosed by shell.

Comperiella unifasciata Ishii

The deposited egg was noted by Taylor (1935) to be strongly attached to the body wall of the scale host. Since he found "no special apparatus for attachment," he assumed that it "must be effected by some adhesive substance." The stalk probably functions in this respect.

The first instar larva was noted to taper gradually to a point at the posterior end. A tracheal system was not found until the last instar.

Diversinervus elegans Silv.

Compere (1931) stated that the deposited eggs of this scale parasite were of the "aeriferous type" and were noted to be "enclosed in the hind intestines suspended on a long stalk, the end of which is inserted through the anal tissue."

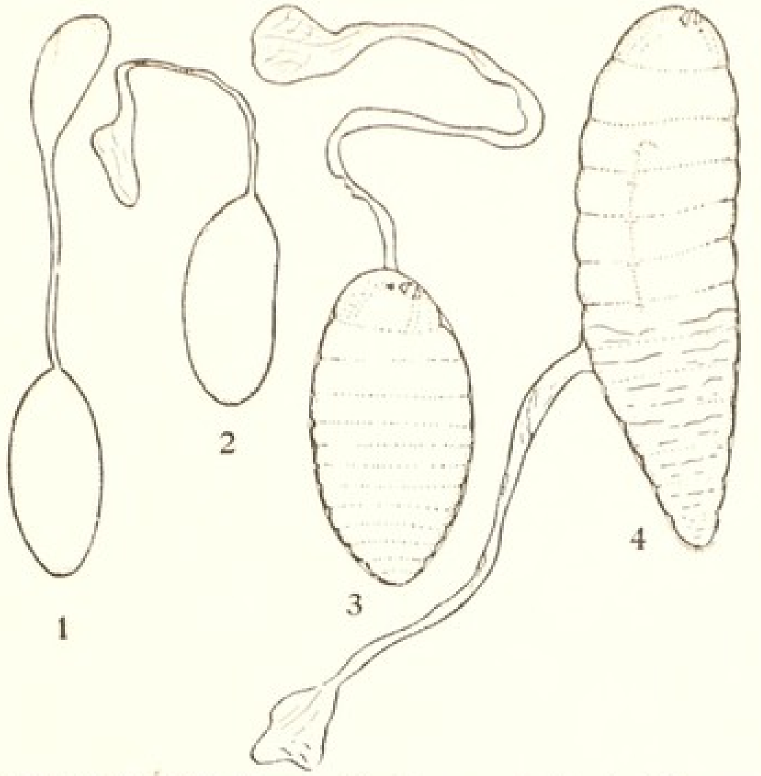


Fig. 25. *Comperiella bifasciata* How. 1, Ovarian egg. 2, Newly laid egg. 3, Egg shortly before hatching. Note position of the embryo. 4, First instar larva. From the original drawing by H. Compere. (Compere and Smith, 1927.)

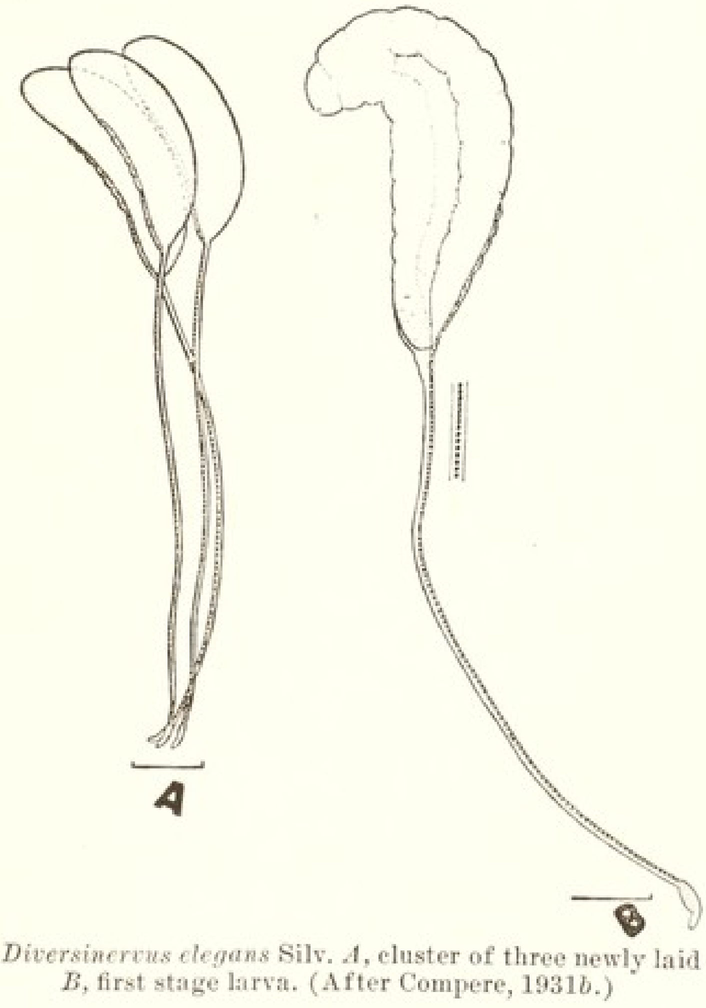


Fig. 26. *Diversinervus elegans* Silv. *A*, cluster of three newly laid eggs; *B*, first stage larva. (After Compere, 1931*b*.)

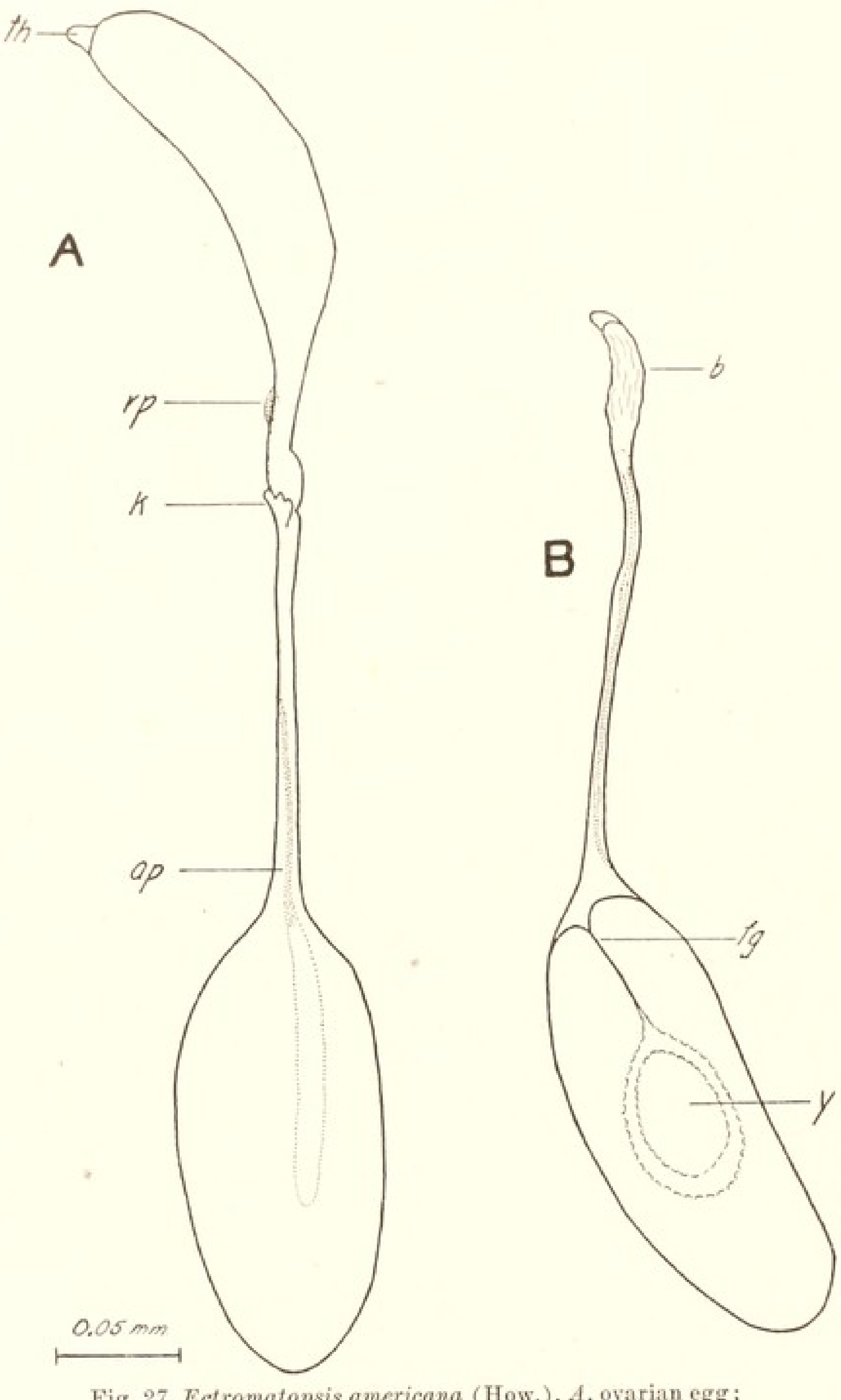


Fig. 27. *Ectromatopsis americana* (How.). *A*, ovarian egg; *B*, deposited egg containing embryo.

That the egg possesses a band is indisputable, as is evident from Compere's illustration (see fig. 26) and the term "aeriferous."

The newly hatched larva was not described, but it would appear from the drawing that the tracheal system is not apneustic but probably has a single pair of spiracles.

Ectromatopsis americana (How.)

This species is a relatively uncommon parasite of native California mealybugs. Although it was propagated with ease the studies of the developmental stages proved difficult.

Ovarian egg.—The ovarian egg was not properly studied. The drawing (fig. 27, *A*) was reconstructed from a series of sketches of a few poor mounts and

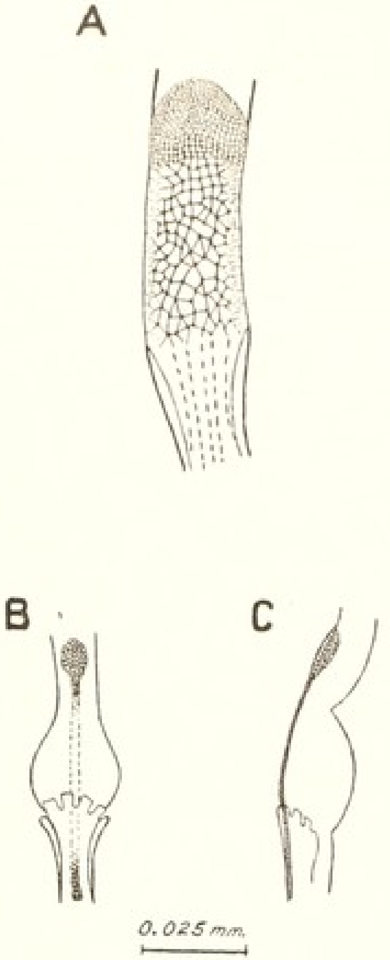


Fig. 28. *A*, *Microterys saissetia* Comp., anterior of neck of egg, dorsal view. *B* and *C*, *Ectromatopsis americana* (How.), two aspects of anterior end of neck.

is therefore not a true camera-lucida drawing. The dimensions can be estimated from the accompanying scale.

The bulb is much longer than it is wide and its apex is thickened into a nipple-like projection. The neck is nearly as long as the egg itself and bears a collar near the bulb. Figure 28, *B* and *C*, shows this region of the neck in detail. The collar surrounds half of the stalk and its projecting margin is dentate. A very minute band extends along the entire length of the neck. A small portion at the bulb end is definitely composed of cells and these may continue as far as the egg proper. On the main body of the egg the band widens slightly and continues for two-thirds its length. The structure is extremely difficult to see even in the best preparations.

Deposited egg.—For oviposition very small mealybugs are selected. Since the egg is relatively large it occupies most of the body of the host and the stalk is doubled back over the egg. The egg is attached to the integument by the stalk. The collapsed bulb remains intact. Even though a band may be observed in the ovarian egg, only a portion has the silvery appearance after deposition. This portion consists of a silvery "string" extending the length of the neck but ending before the body is reached. This condition is correlated with the tracheal structures of the larva.

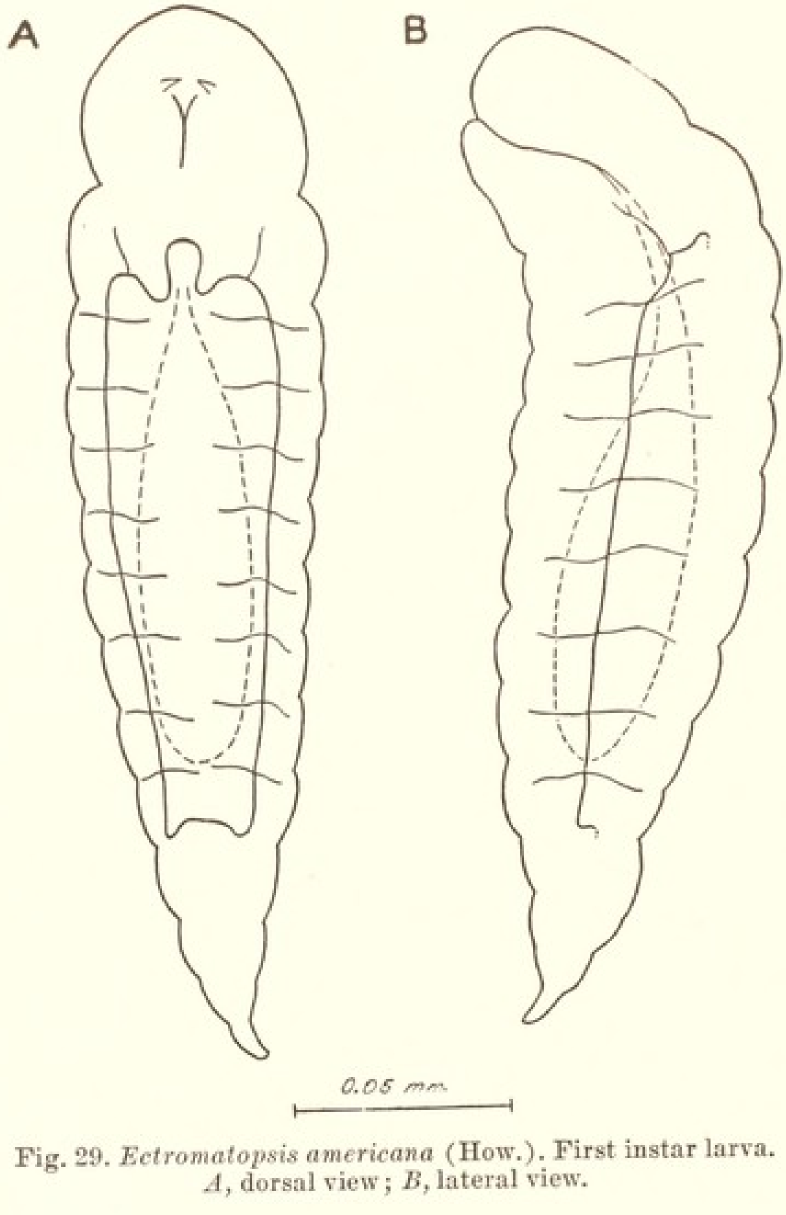


Fig. 29. *Ectromatopsis americana* (How.). First instar larva. *A*, dorsal view; *B*, lateral view.

The embryo develops with its head at the stalk end (fig. 27, *B*). Eclosion was not observed.

Larva.—The newly hatched larva is elongate, laterally somewhat crescentic in shape, with a broad anterior region and a taperng posterior. The tracheal system is closed. The drawings (fig. 29) are reconstructions from camera-lucida sketches.

Encyrtus barbatus Timb.

No description of the egg is available. Ishii (1932) has illustrated and described the young larva, supposedly the first instar. I strongly suspect that the stage is a much later one, possibly the third or fourth. In the drawing the

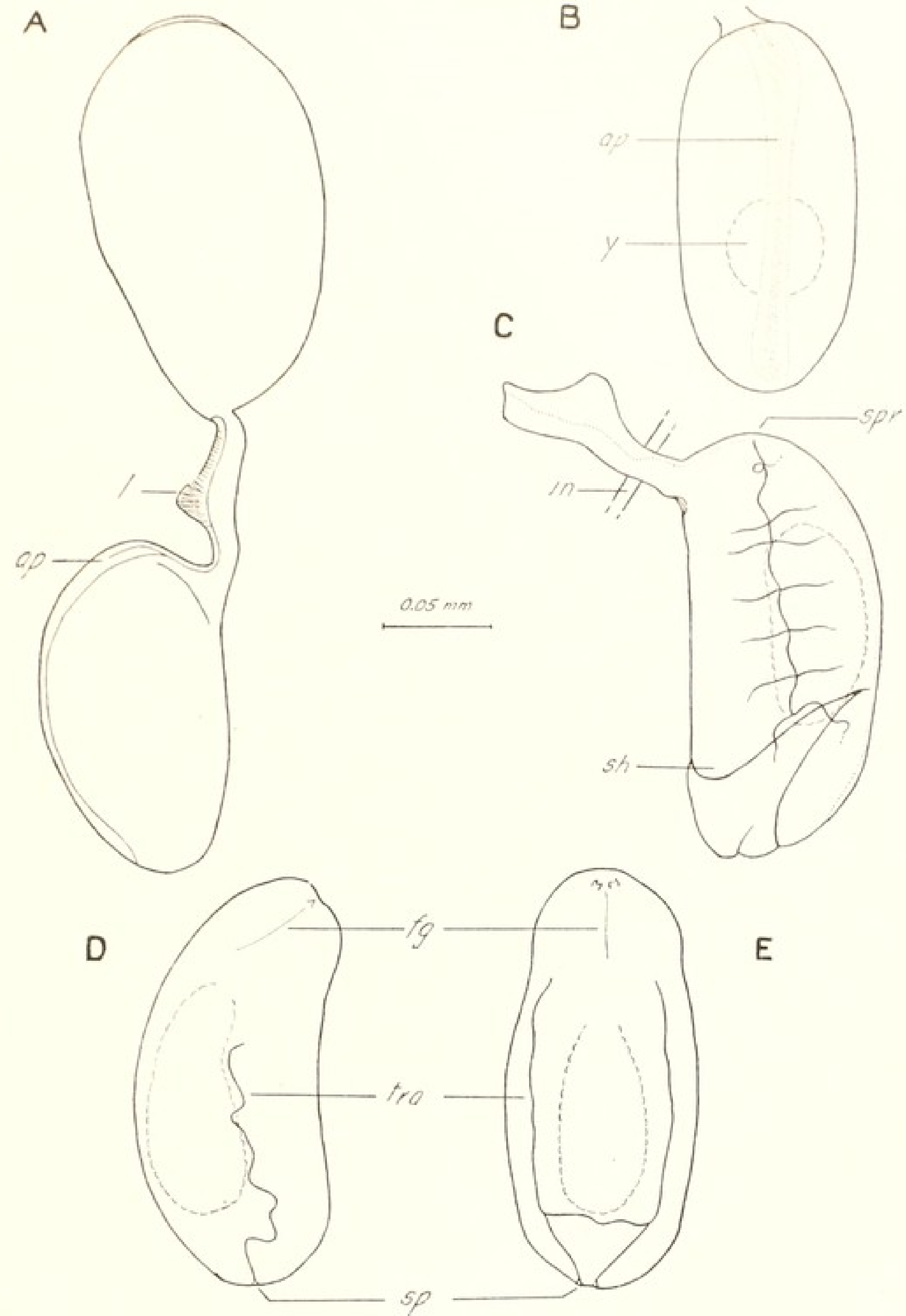


Fig. 30. *Encyrtus fuliginosus* Comp. *A*, ovarian egg in the lateral aspect to show the band on the convex ventral side; *B*, posterior portion of the deposited egg (note position of yolk); *C*, newly hatched larva; *D* and *E*, two views of first instar larva removed from eggshell.

eggshell is extremely small compared with the size of the larva. The long tail with a pair of spiracles at the tip, and the additional spiracles on the thorax and abdomen are characteristic of later instars of *Encyrtus*.

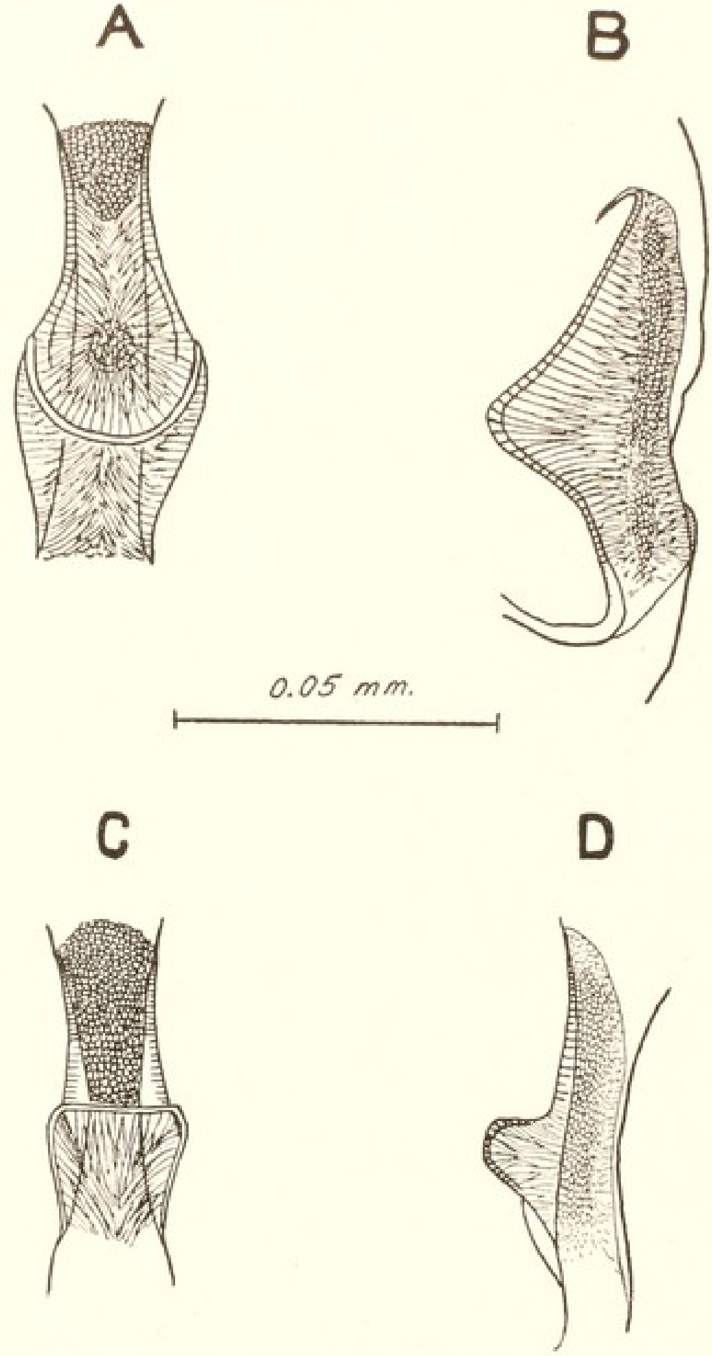


Fig. 31. The lip of two species of *Encyrtus*, dorsal and lateral aspects. *A* and *B*, *Encyrtus fuliginosus* Comp.; *C* and *D*, *Encyrtus infelix* (Embl.).

It does not seem likely that the egg or larva of this scale parasite differs in any important respect from those of the other species of *Encyrtus*.

Encyrtus fuliginosus Comp.

This species is not native to California but was introduced from South Africa by Compere (1940) for use against *Saissetia oleae* Bern. Material for this study was made available during the propagation of the species.

Ovarian egg.—The features common to all the *Encyrtus* eggs that I examined are the comparatively large bulb, the short neck with the projecting lip, the lateral attachment of the neck to the egg body, and the position of the band on the more convex or ventral side. The only portion of the band readily discernible is the prominent lip. The remainder of the band is apparent only after the egg is deposited. The egg of *Encyrtus fuliginosus* may be regarded as typical. Figure 30, *A* shows a lateral view. When freshly dissected the bulb is large and fully rounded, whereas the egg proper is smaller and wrinkled from only partial distension. The peculiar neck structure is illustrated in figure 31, *A* and *B*. The lip is highly complicated and defies comprehensive description. It looks as if it were an agglomeration of interconnecting cells. The drawings were as accurately made as possible and present the appearance as viewed by the usual laboratory microscope.

The measurements were: width of bulb, 0.116 mm.; length of bulb, 0.188 mm.; width of neck, neck, 0.03 mm.; length of neck, 0.068 mm.; width of egg, 0.09 mm.; length of egg, 0.163 mm.

Deposited egg.—The stalk of the egg passes

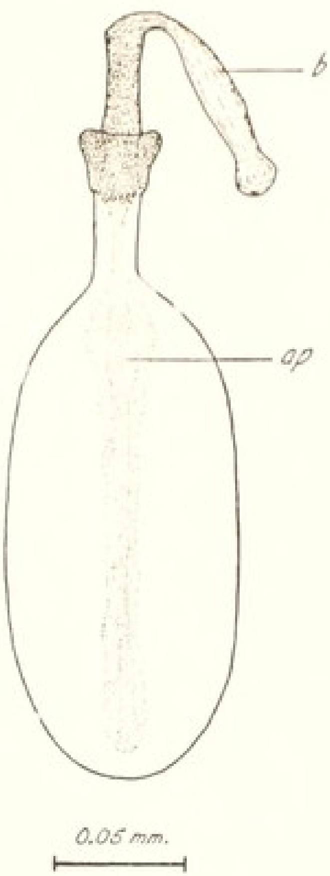


Fig. 32. *Encyrtus infelix* (Embl.). Deposited egg, showing the aeroscopic plate (ap).

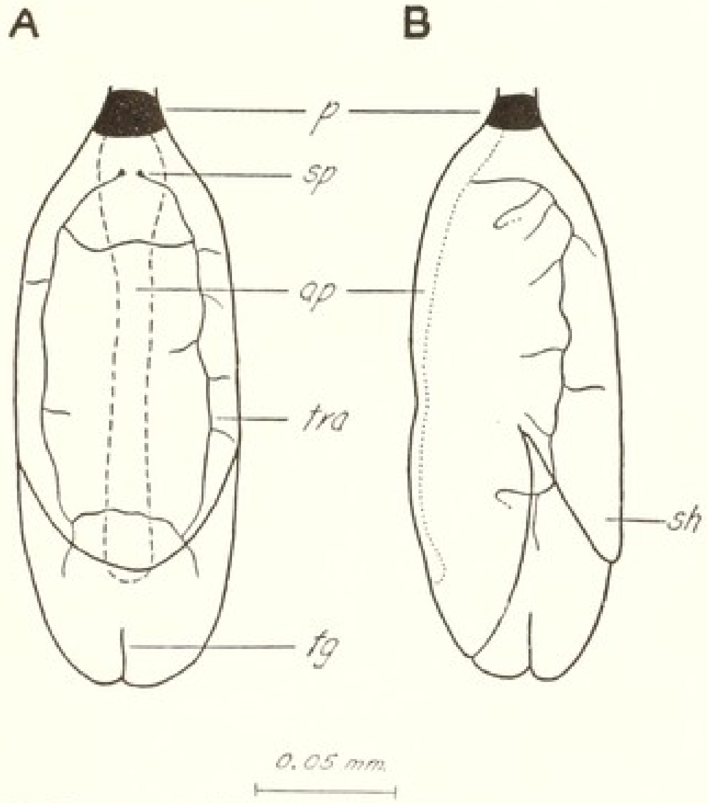


Fig. 33. *Encyrtus infelix* (Embl.). Newly hatched larva. *A*, ventral view; *B*, lateral view.

through the dorsum of the scale. Of those eggs examined the bulb was invariably absent as a complete structure. It was either entirely lacking or a portion remained as a stringy attachment. The long, narrow, silvery band extends nearly to the tip of the egg. Cells appear only as stippling. Those in

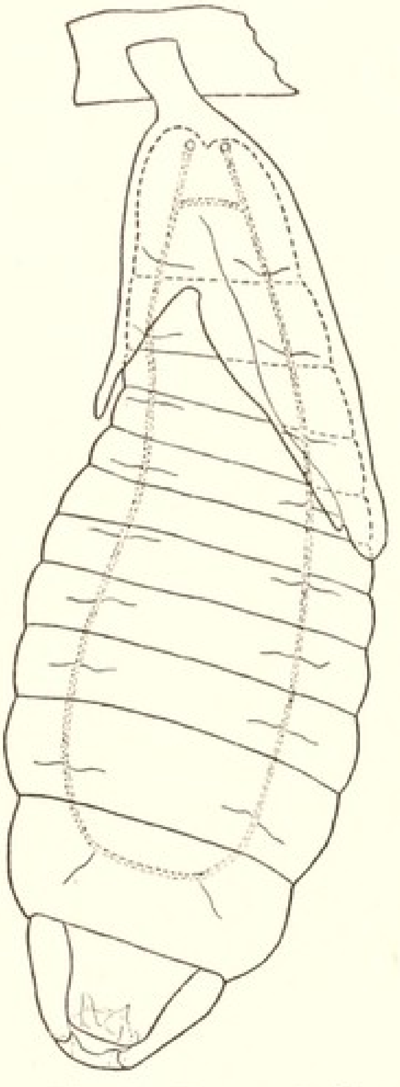


Fig. 34. *Encyrtus infidus* (Rossi). Newly hatched larva encapsulated posteriorly by the shell of the egg, which is suspended from the integument of the host. (Redrawn from Silvestri, 1919.)

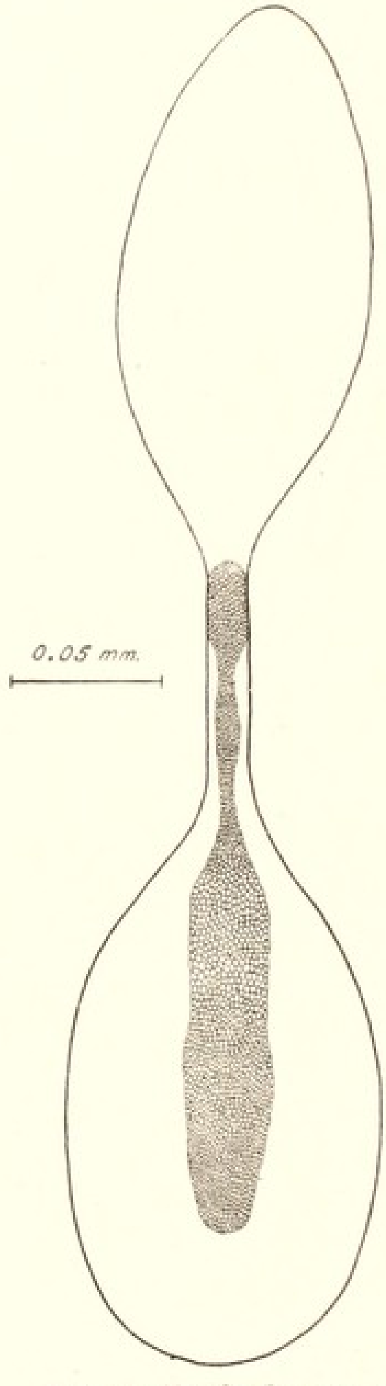


Fig. 35. *Erythaphycus argyrocomus* Comp. Ovarian egg, dorsal view.

the center section appear to be slightly larger. Since the band is on the side opposite that with the deposited eggs of other genera, it is not readily distinguishable through the host's integument. However, after embryonic development has proceeded to the point at which the egg is partly translucent the band may be discernible.

The embryo develops with the head toward the stalk end. This is evident from the position of the yolk as the larva develops (fig. 30, *B*).

Larva.—After eclosion the larva is suspended in the eggshell with all the body except the head enclosed by the chorion (fig. 30, *C*). The two spiracles are placed at the extreme end of the egg at the center of the band. There is no visible plug in the stalk of the eggshell.

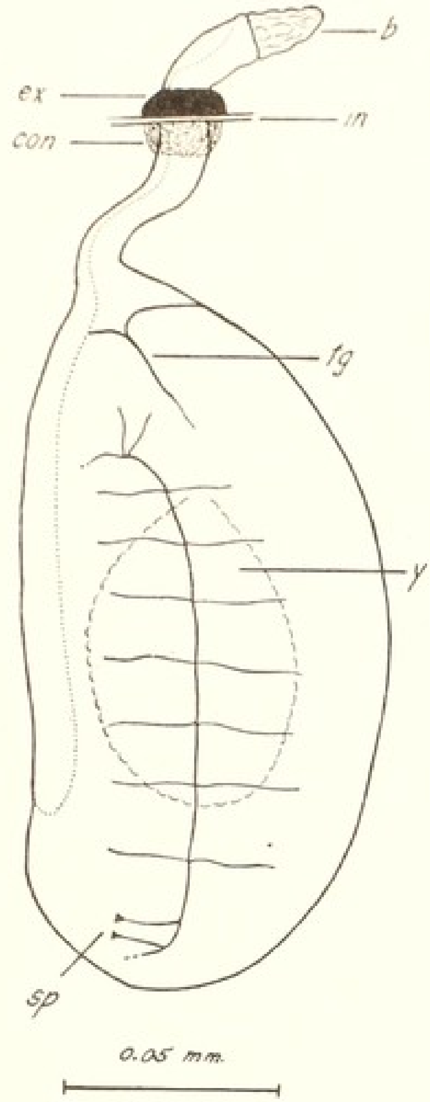


Fig. 36. *Erythaphycus argyrocomus* Comp. Deposited egg containing fully developed embryo.

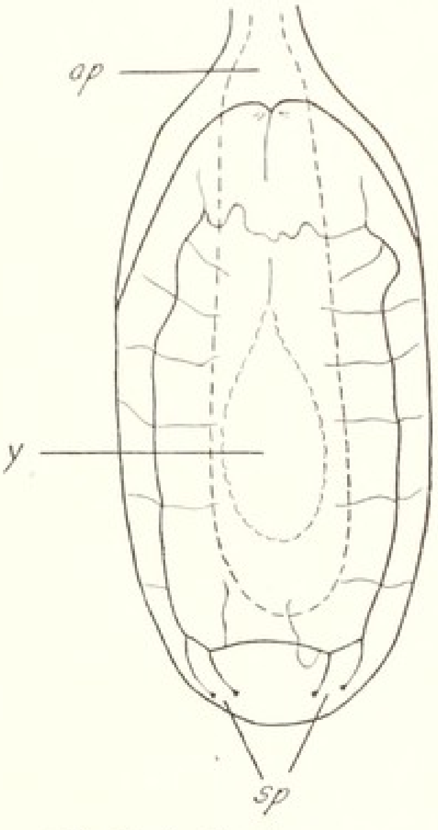


Fig. 37. *Erythaphycus argyrocomus* Comp. Ventral aspect of egg proper, showing tracheal system of the completely developed embryo.

The larva is firmly attached to the shell and no attempts to remove it succeeded. After hosts containing young parasite larvae had been immersed in stain-saturated kerosene-xylene mixture for other purposes, the attachment was less firm and the larvae could occasionally be removed intact. The newly hatched larva is shaped like a sausage (fig. 30, *D* and *E*). The two spiracles are flush with the rounded posterior end, as opposed to later instars which have their posterior extremities prolonged into a forked tail with a single spiracles on each process.

Lateral tracheal branches are barely distinguishable. Their number and distribution vary.

Encyrtus infelix (Embl.)

This parasite of hemispherical scale, *Saissetia hemispherica* (Targ.), was the first encyrtid species of which the biology was investigated to any great extent. In her monumental work Embleton (1904) describes a portion of a structure which in this paper is referred to as an aeroscopic plate or band, a peculiarity of the encyrtid egg which until recently has been largely overlooked by other workers.

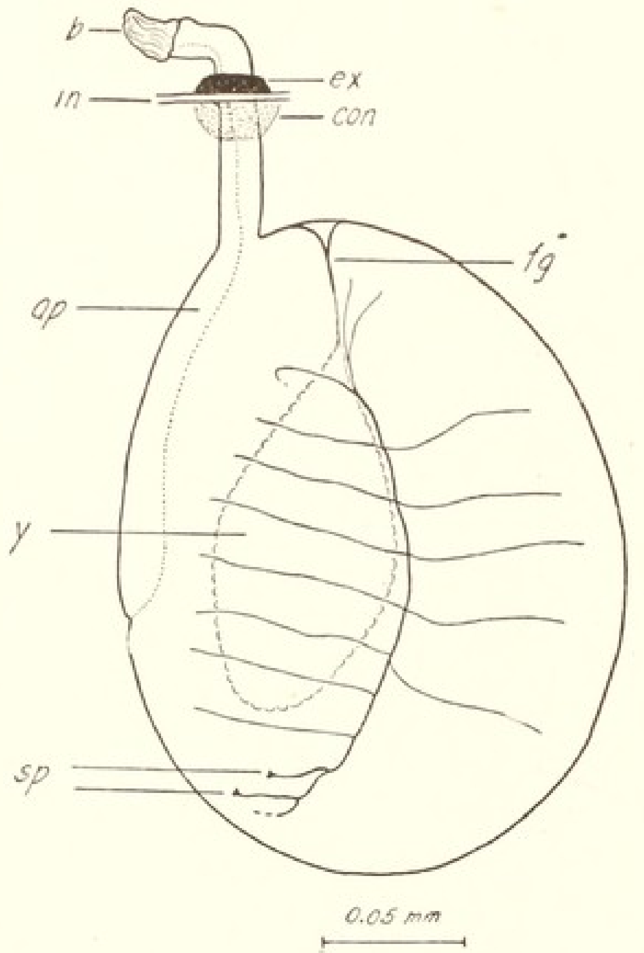


Fig. 38. *Erythraphycus argyrocomus* Comp. Deposited egg immediately prior to eclosion.

Ovarian egg.—The undeposited egg is almost identical with that of *Encyrtus fuliginosus.* The bulb is nearly spherical and as large as, or larger than, the main body of the egg. Embleton (1904) and Thorpe (1936) stated that the bulb disappears with the old ovarian eggs. I could not confirm this; on the contrary, the bulb was found to be fully distended. The egg proper was somewhat collapsed and the chorion was wrinkled.

The neck has a prominent lip which differs from that of *Encyrtus fuliginosus* in its more acute angle of projection from the neck, but in structure is fully as complicated. Cells of the band are apparent here as elsewhere on the neck. No doubt Embleton (1904) saw the band at this point, for fine papillations or striations were noted on the wall of the tube. The band on the egg proper is either not visible or, on those rare occasions when it stains, indistinct.

The dimensions of an ovarian egg were: length of bulb, 0.12 mm.; width of bulb, 0.103 mm.; length of egg, 0.223 mm.; width of egg, 0.107 mm.; length of neck 0.107 mm.

Deposited egg.—The neck of the deposited egg passes through the scale's integument; contrary to the statements of Embleton and Thorpe, the bulb neither disappears nor breaks off but as a rule is merely collapsed and remains attached (fig. 32). The band on the main body of the egg becomes visible because of the silvery effect of the contained air. With small, nearly transparent hosts the band may be observed through the integument. The "conspicuous finely shagreened band" which Thorpe (1936) noticed on the empty chorion after eclosion was undoubtedly the aeroscopic plate.

The band extends posteriorly to a point just short of the apex of the egg. The width is slightly irregular, but the widest points are at the anterior end. All along the central section there is an area of somewhat larger cells.

Soon after deposition a plug forms within the stalk near the egg proper becoming solid black. Thorpe and Embleton stated that this substance appears when the eggs are in the last stages of development within the ovaries. Thorpe considered the plug to be the remains of the "apical yolk mass."

Larva.—None of the larval stages described by Embleton was of the first stage. Thorpe has stated that the respiratory system of the first instar is metapneustic and that "the tenth post-cephalic segment of the body is prolonged in the form of two processes, each bearing a spiracle at the tip." I tend to question this observation even though my own was incomplete; my questioning is based on my own findings with *Encyrtus fuliginosus* and the figures presented by Thorpe. The first stage larva of *Encyrtus fuliginosus* is bluntly rounded at the caudal end. The processes on which the spiracles are to be borne appear in the next stadium. The head capsule of the larva illustrated by Thorpe (1936) is too large for the primary stage in comparison with those I have observed.

A single pair of spiracles is attached to the center of the band at its widest point near the stalk fig. 33, *A* and *B*). Thorpe observed the processes of later stages to be similarly fastened. The tracheal system is well developed, but many of the lateral branches of the tracheal trunks are not filled with air until some time after eclosion.

Encyrtus infidus (Rossi)

The life history of this scale parasite has been investigated by Silvestri (1919) and Clausen (1932). Although Clausen noted differences in the form and habit of the various stages and considered it probable that the species studied by Silvestri was not identical with that occurring in Japan, I do not consider the differences in the egg and first instar larva significant enough to warrant separate discussion.

Silvestri noted that the egg had a large and prominent band on the dorsum (fig. 15, left). At the distal end of the neck the band was brick-red, thickened, and spongy in appearance. The egg as described and drawn by Silvestri is quite different in several respects from the eggs I investigated in either

Encyrtus fuliginosus or *Encyrtus infelix*, in which the bands were found on the more convex (ventral) side of the egg, and the distal end of the necks was much more intricate in structure and had a projecting lip.

According to Silvestri, air is contained in the band when the egg is deposited. Undoubtedly Clausen observed the same phenomenon. He wrote: "on the dorsal side is what appears to be a waxy incrustation which extends from the base of the stalk to three-fourths the length of the main body. It is also found upon the stalk for its entire length, and in both the egg body and the stalk it covers approximately one-fourth the circumference."

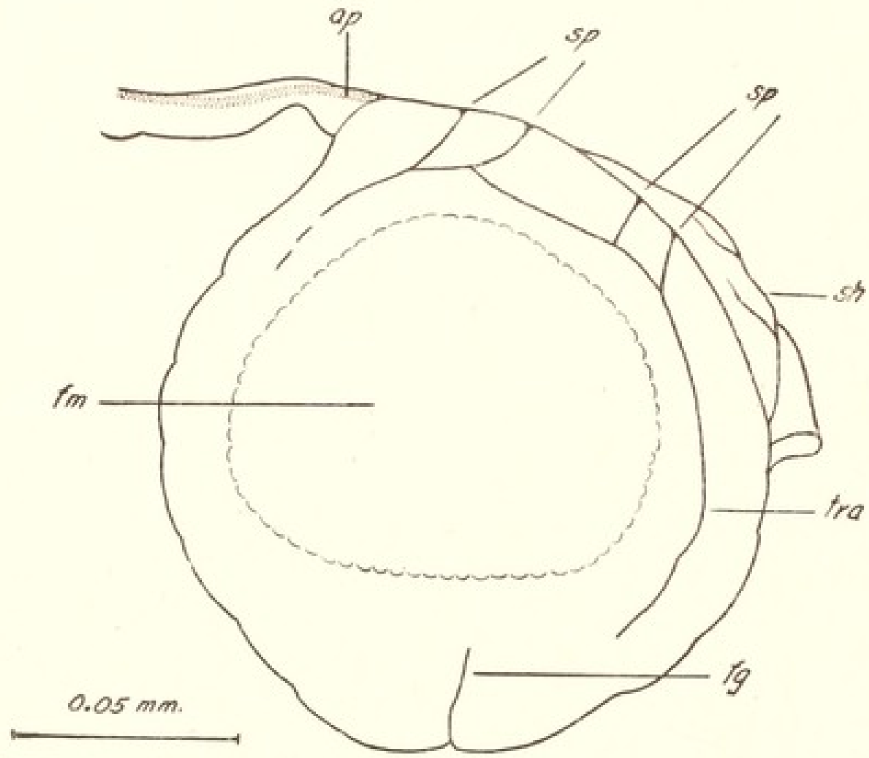


Fig. 39. *Erythraphycus argyrocomus* Comp. First instar larva shortly after eclosion. A large portion of the tracheal system has become invisible.

Silvestri (1919) and Clausen (1924) agree that after eclosion the newly hatched lerva remains partly encapsulated by the chorion of the egg (fig. 34). Silvestri observed that caudad the body is slightly bilobed and that to the posterior part of each lobe is applied a spiracle which adheres to the dorsal portion of the eggshell.

Encyrtus sasakii Ishii

Only the ovarian egg is described and illustrated by Ishii (1932). The thickness of the neck in the figure presented suggests a structure similar to that found on the eggs of other species of *Encyrtus*. If this proves to be the case, the early stages of this species will not be found to differ in any major respect from those of other species previously described.

Erythraphycus argyrocomus Comp.

The adult of this species is similar in general appearance to *Metaphycus howardi* and both attack the same host. This was the commoner species and material could be obtained at almost any time during the investigation.

Ovarian egg.—The only feature of the egg (fig. 35) worthy of note is the slight difference of the band; at no point does it surround the neck completely, and the width varies. On the egg proper the width is also variable and the cells are quite apparent, those at the anterior end being somewhat larger. The dimensions were: length of bulb, 0.188 mm.; width of bulb, 0.09 mm.; length of stalk, 0.103 mm.; length of egg, 0.18 mm.; width of egg, 0.111 mm.; length of band 0.137 mm.; width of band 0.034 mm.

Deposited egg.—The deposited egg (fig. 36) can be readily located by the presence of a ring of blackened material that has accumulated around the protruding stalk. (A similar ring of brownish material forms within the host.) The projecting portions of the egg do not extend vertically but are bent at an angle of 45° or more. The collapsed bulb remains attached. No plug forms within the stalk. The band eventually becomes slightly melanized from the point where the stalk enters the host to one-quarter of the length of the egg. The embryo develops with the head at the stalk end and apparently hatches from that position.

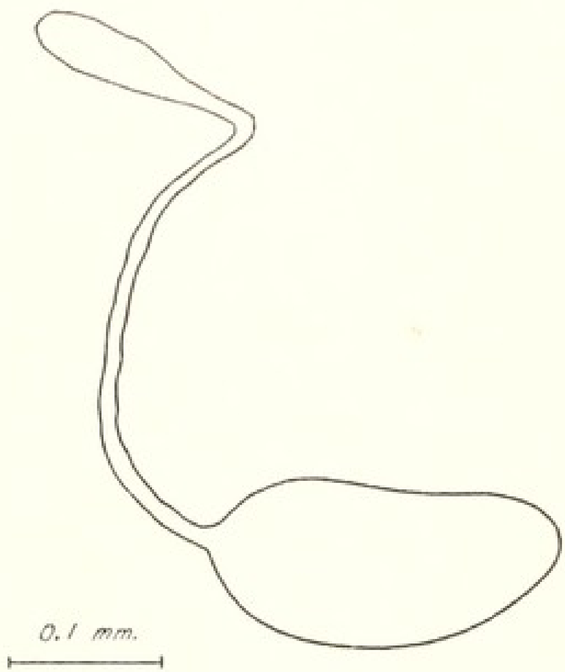


Fig. 40. *Eusemion californicum* Comp. Ovarian egg. The neck is usually more irregular in diameter and somewhat collapsed.

The first larva (fig. 39) is unusual. Instead of the usual simple pair of spiracles it possesses two pairs. It lies at right angles to the band and the four spiracles are placed along the longitudinal axis. In one larva the spiracles were in the center of the band. In other larvae they had shifted so that only one of the pairs was thus situated. Since the spiracles are not firmly attached, dissection probably caused them to alter their position. The band portion of the shell remains extended and straight whereas the remainder lies confusedly about the posterior section of the larva. At this stage of development the tracheal system is difficult to discern and little more than the spiracles and connecting tracheae are seen.

The tracheal system is readily discernible in the embryonic stages immediately prior to eclosion. Figures 36, 37, and 38 of unhatched eggs show the respiratory structures, which are filled with gas, yet the spiracles are not yet in contact with the band. Four spiracles, commissures, lateral tracheae, and secondary tracheae are clearly discernible. The larva of this species had two tracheal branches arising from the posterior commissure between the inner pair of spiracles.

Eusemion californicum Comp.

The ovarian egg (fig. 40) of this scale hyperparasite is large compared with those of many encyrtids. The bulb is not sharply differentiated from the neck and is not more than three times as broad. The length of the neck is nearly twice that of the egg. The egg proper is convex ventrally and somewhat concave dorsally. The measurements were: length of bulb, approximately 0.158 mm.; width of bulb, 0.038 mm.; length of neck, approximately 0.321 mm.; length of egg, 0.217 mm.; width of egg, 0.111 mm.

Compere (1925) observed that the deposited egg floated free within the body of the host. The long stalk, "the greater part of which was shriveled and functionless," remained attached. The larva lay free in the fluid contents of the primary larva. When newly hatched it had a tail appendage about one-third as long as the remainder of the larva. From Compere's drawing of the first instar larva it is apparent that the tracheal system is apneustic.

Eusemion cornigerum (Walker)

The egg of this secondary parasite was found by Timberlake (1913) to be deposited free within the body of the larva or pupa of its host. It was described as decidedly minute with only a short pedicel which is functionless after maturation and finally shrivels away. The development is apparently similar to that of *Cheiloneurus* and related secondary parasites in that the egg increases in size so that the newly hatched larva is many times the size of the freshly deposited egg.

The first instar larva has a conspicuous tail. No mention of the tracheal system was made by Timberlake, but it is undoubtedly apneustic.

Habrolepis dalmani (Westw.)

Gourlay (1935) described the larva as "attached to the body wall by a chitinized breathing tube." Although the eggs and larvae are not discussed in detail, it may be assumed that the structure of the stages follows the same pattern as with other encyrtids utilizing a "breathing tube." Another species also attacking scales, *Habrolepis rouxi*, is known to have a banded egg.

Isodromus iceryae How.

Clancy (1946) has given an extensive account of this parasite which attacks larvae of *Chrysopa*. The ovarian egg has no band. On the distal end of the neck Clancy noted a transparent colorless cone-shaped projection, somewhat similar in structure to collars on other eggs. Clancy was not able to assign any function to this structure.

Upon deposition a portion of the neck and the collapsed bulb are left extending beyond the surface of the host.

The newly hatched spherical larva remains weakly attached to the old eggshell by its posterior segments. In this instar spiracles are lacking.

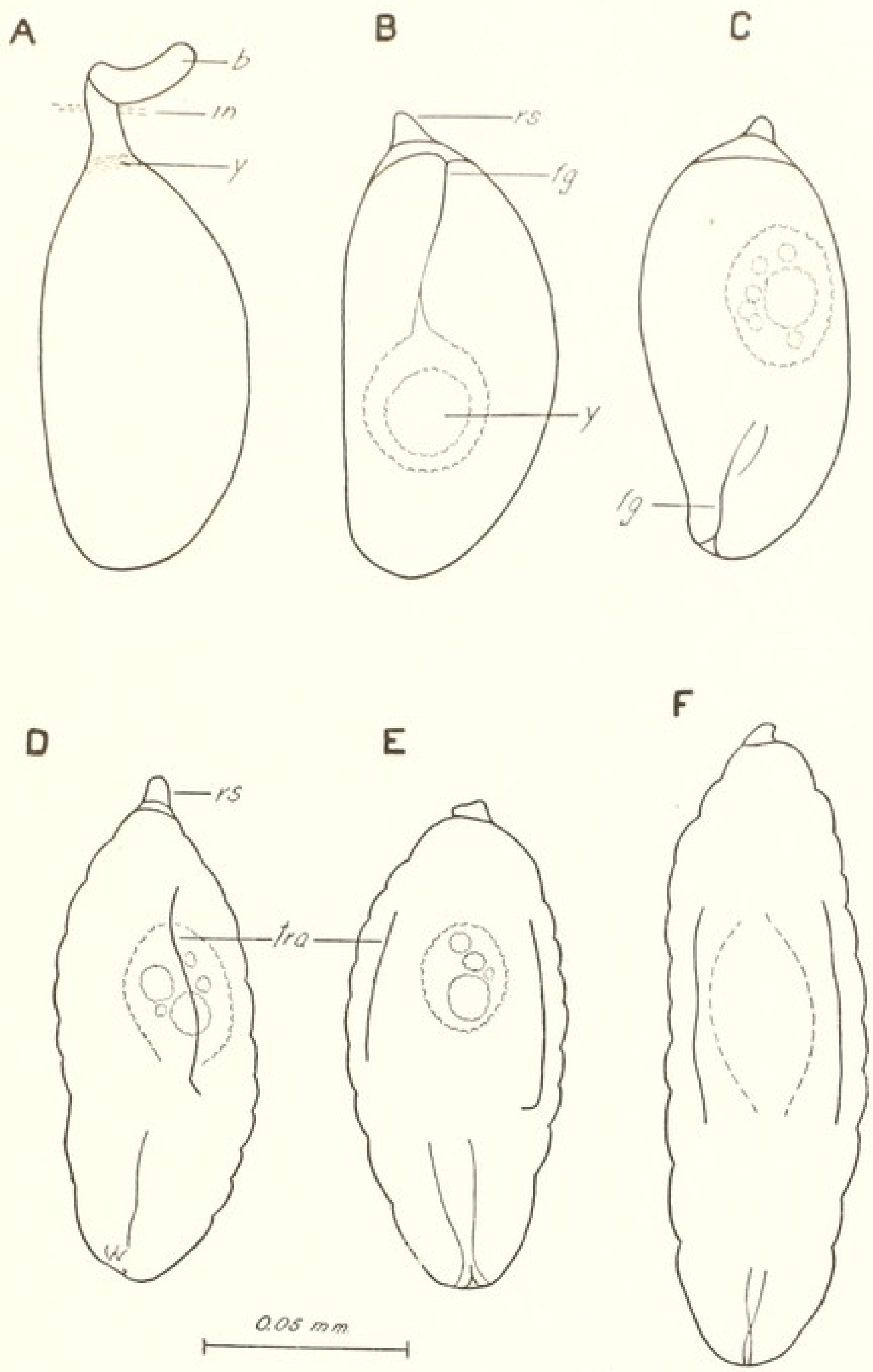


Fig. 41. *Leptomastidea abnormis* (Gir.). *A*, newly deposited egg; *B*, egg which has now become detached from the host's integument, showing developing embryo; *C*, same showing embryo in reserve position; *D, E,* and *F*, larva in progressive stages of growth, eclosion as yet incomplete.

Isodromus niger Ashm.

This parasite of *Chrysopa* larvae has also been thoroughly investigated by Clancy (1946) and was found to differ remarkably in the type of egg and larva from the other species of the genus on congeneric hosts. The ovarian egg has a well-defined band. In the deposited state the collapsed bulb and a portion of the neck project from the host's integument. The nearly spherical first stage larva remains attached, its posterior segments contained within the eggshell and the single pair of spiracles in close opposition to the band.

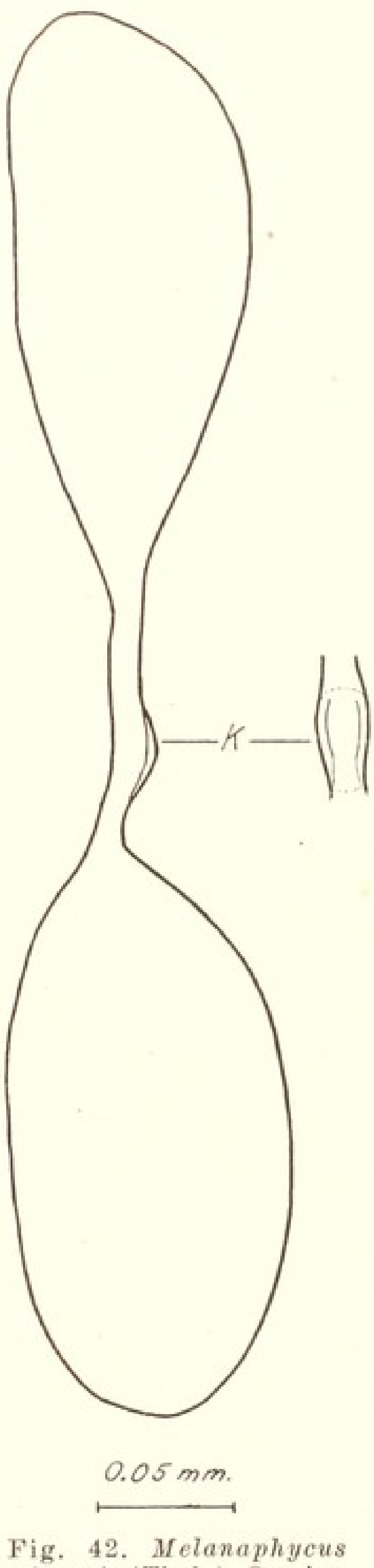


Fig. 42. *Melanaphycus fumipennis* (Timb.). Ovarian egg and dorsal view of neck.

Leptomastidea abnormis (Gir.)

Ovarian egg.—The ovarian egg (fig. 11) of this primary parasite of young citrus mealybugs (*Pseudococcus citri* Risso) is a little different in general shape from others in the Encyrtidae. With higher magnification and with the aid of stains it can be seen that the egg of this species differs in an important feature. On the neck just below the bulb (fig. 11, *D*) there is a cluster of approximately two dozen cells similar to those found in aeroscopic plates. This small elliptical group is the only indication of a band at any point on the egg.

The size of the egg is comparatively small. The measurements were: length of bulb, 0.12 mm.; width of bulb, 0.039 mm.; length of neck, 0.064 mm.; length of egg, 0.145 mm.; width of egg, 0.073 mm.

Deposited egg.—The egg in the deposited state (fig. 41, *A*) is also peculiar. Rather than being laid free in the body it is attached to the integument of the mealybug with the collapsed bulb and a portion of the neck protruding. The protruding stalk may be observed if the oviposition is watched or subsequently through staining. Shortly after deposition the protruding part is collapsed onto the sufrace of the host, and with the aid of stains it is barely noticeable as a small flat spot. If dissections are not made with enough caution immediately after oviposition, the main body of the egg becomes disengaged and floats free in the body contents of the host.

The embryo first lies with the head at the anterior end of the egg (fig. 41, *B*) but at completion of embryonic development the reverse position is assumed (*C*). Eclosion was not observed.

Larva.—The larva increases in size while still partly enclosed within the shell, for the stump of the detached stalk remains at the posterior end. (Fig. 41, *D*, *E*, *F*.) The chorion is eventually shed and the posterior segments become attenuated into a short tail.

The tracheal system which is usually most apparent in other species prior to eclosion and shortly thereafter is scarcely detectable. At no time were more than three-fourths of the tracheal trunks visible and neither commissures nor tracheal branches could be seen. This may be due in part to the minuteness of the tracheae.

Melanaphycus fumipennis (Timb.)

Although this species attacks the same host as the two previous encyrtids, the structure of the early stages is entirely dissimilar.

Ovarian egg.—The ovarian egg (fig. 42) is extremely simple. Its sole distinctive feature is a slight swelling in the lower regions of the neck. The measurements were: length of bulb, 0.197 mm.; width of bulb, 0.077 mm.; length of neck, 0.086 mm.; length of eggs, 0.197 mm.; width of egg, 0.098 mm.

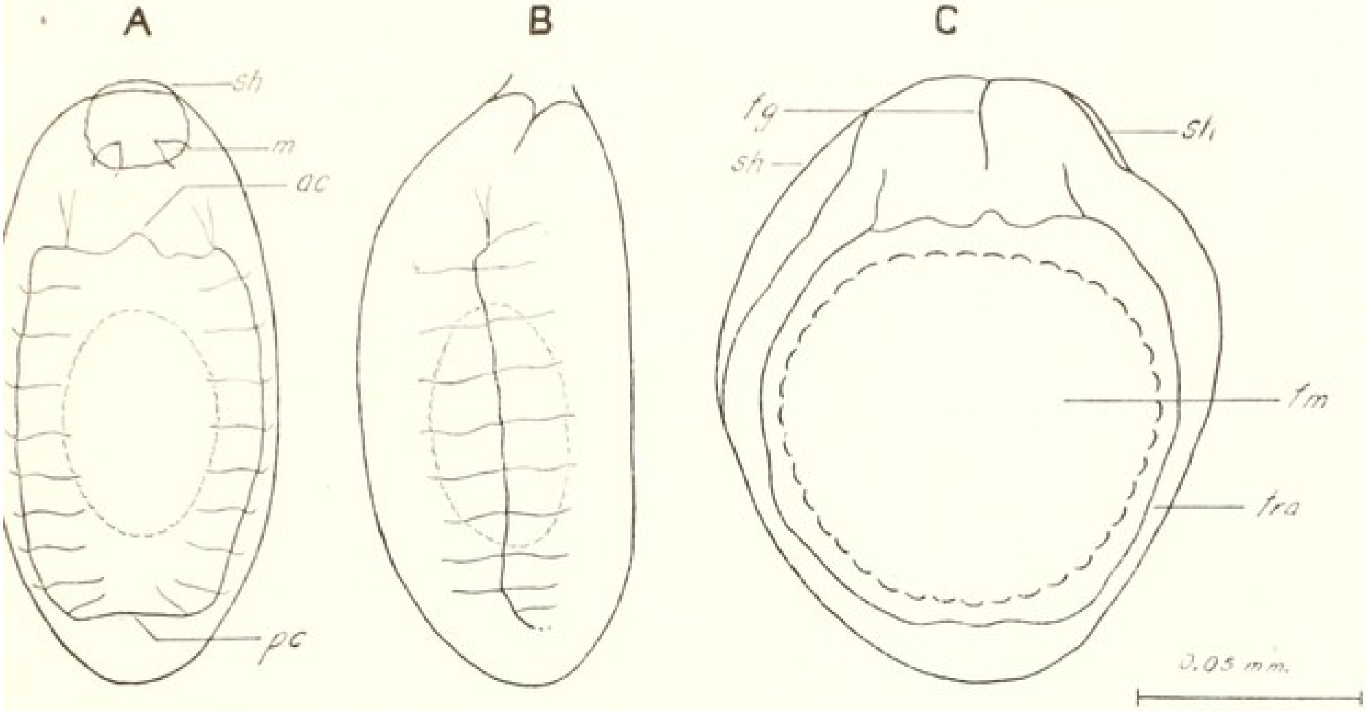


Fig. 43. *Melanaphycus fumipennis* (Timb.). *A* and *B*, two aspects of the mature embryo; *C*, larva. Apparently all the body but the head is enclosed by shell.

The deposited egg is attached to the integument of the host, this was ascertained only after repeated careful dissections of small hosts. The bulb and neck do not project as much as do those of banded eggs, but are more or less flattened to the integument. If several eggs are deposited in a group, which frequently happens, the stalks are more erect. A few hours after deposition that portion of the stalk within the host melanizes to a gray color which is visible through the host's derm.

The embryo develops with the head toward the stalk. The larva apparently hatches from this position, for larvae that had obviously fed retained the shell on most of the body (fig. 43, *C*). It is believed that the larval contortions break the connection with the stalk and the larva emerges by enlarging this rupture.

Larva.—The newly hatched larva floats freely within the host and when a host is dissected the parasite is seldom found near the stalk of the egg. At this state (fig. 43, *C*) it is almost a perfect sphere without the barest suggestion of segmentation and several times the larva appeared to be enclosed by shell.

The tracheae are very difficult to discern because of their minute size and the abundance of globules of food material. To trace them it was necessary to obtain eggs just prior to eclosion.

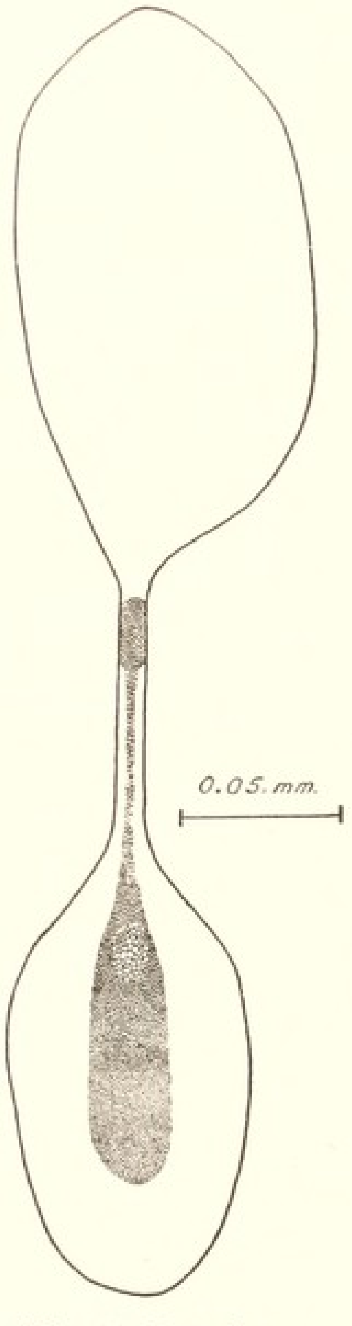


Fig. 44. *Metaphycus howardi* [Ckll.]. Dorsal view of ovarian egg.

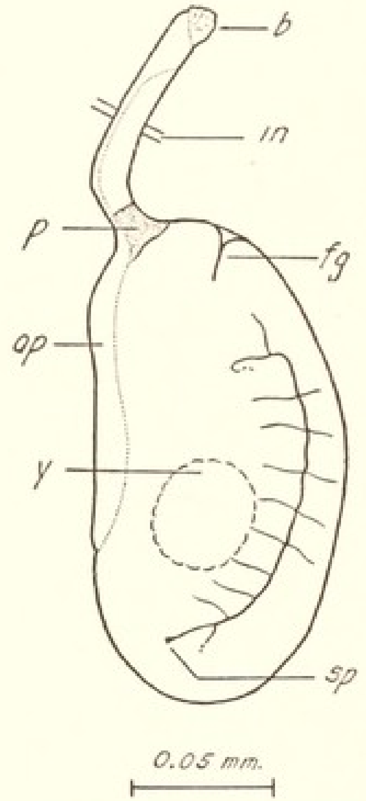


Fig. 45. *Metaphycus howardi* [Ckll.]. Deposited egg containing completely developed embryo.

Figure 43 (*A* and *B*) shows two views of unhatched eggs containing fully developed embryos. The tracheal system has no spiracles but the tracheae, commissures, and lateral branches are well developed.

Metaphycus alberti (How.)

References to this species were discovered among the unpublished notes of Harold Compere, who kindly lent them to me. No description is given of the eggs or larvae, but drawings of the egg indicate very definitely that a band is present. According to these sketches the structure is similar to that found among other species of *Metaphycus*. The following dimensions were noted: length of bulb, 0.09 mm.; length of neck, 0.04 mm.; length of egg, 0.12 mm.

Metaphycus flammeus Comp.

One female of this species was obtained from *Lecanium quercitronis*. No host material in a suitable stage could be secured for studies of the development.

Although this is a close relative of *Metaphycus howardi*, its ovarian egg differs in structure. It is classed with the banded type (fig. 66, *A*). The band near the bulb end covers less than half the neck and immediately narrows to

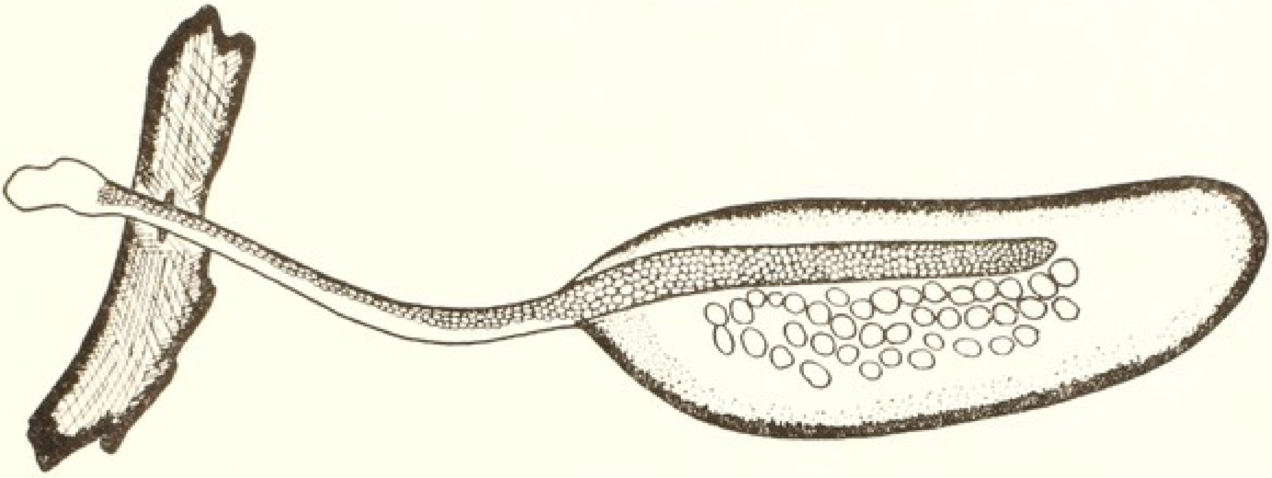


Fig. 46. *Metaphycus lounsburyi* (How.). Deposited egg. Note the distinct aeroscopic plate. (Redrawn from Smith and Compere, 1928.)

extend the length of the neck. On the egg proper the band widens somewhat, but is scarcely wider than the diameter of the neck. Cells are just barely distinguishable.

The measurements were: length of bulb, 0.137 mm.; width of bulb, 0.06 mm.; length of neck, 0.09 mm.; width of neck, 0.009 mm.; length of egg, 0.158 mm.; width of egg, 0.077 mm.

Metaphycus howardi [Ckll.]

This species was reared near Riverside, California, from material of an unknown species of *Eriococcus*. A few females were obtained for study from this same locality over a period of more than a year.

Ovarian egg.—The ovarian egg (fig. 44) differs in minor details from other banded eggs. The bulb is larger than the egg proper. The neck is not as long as the main body of the egg. The band toward the bulb end completely surrounds the neck. The remainder of the band on the neck is only three cells wide. On the egg proper the band widens and increases in width, although less abruptly, to its apex, a point about two-thirds the length of the egg. Most of the cells are very minute except in one central locality near the anterior end. The measurements were: length of bulb, 0.193 mm.; width of bulb, 0.094 mm.; length of neck, 0.086 mm.; length of egg, 0.137 mm.; width of egg, 0.081 mm.; length of band on egg, 0.103 mm.; width (maximum), 0.03 mm.

Deposited egg.—The deposited egg (fig. 45) is found attached to the integument of the host and is continuous with the projecting stalk. Before the larva has hatched, a yellowish brown plug has formed in the lumen of the stalk.

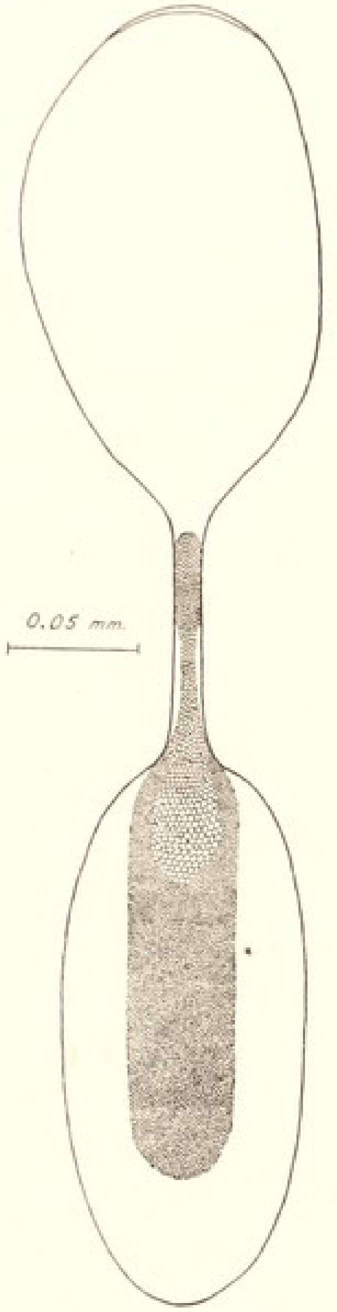


Fig. 47. *Metaphycus luteolus* (Timb.). Ovarian egg, dorsal view.

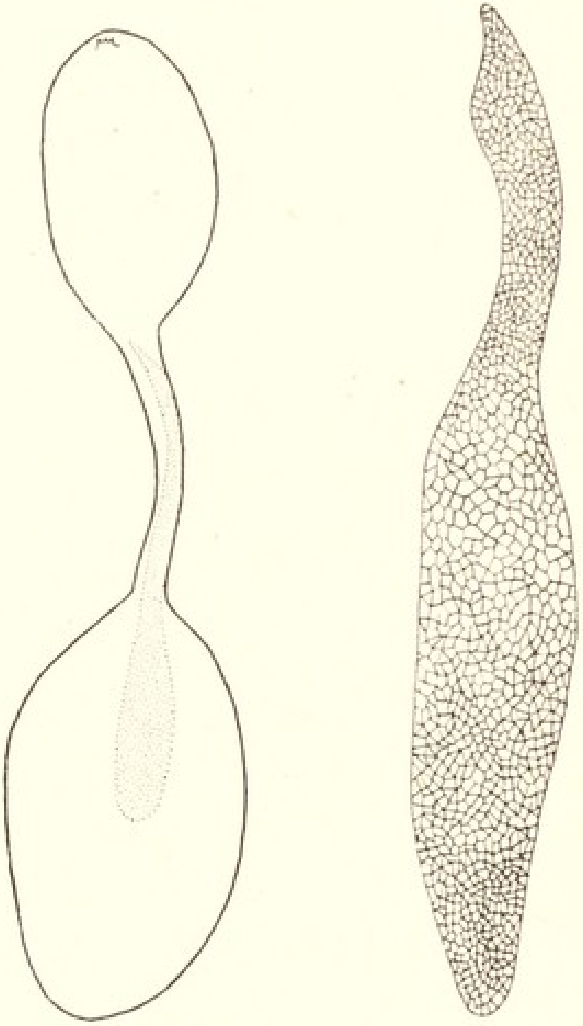


Fig. 48. *Metaphycus melanostomatus* (Timb.). Left, ovarian egg. Right, detail of the aeroscopic plate of the ovarian egg. (Redrawn from Silvestri, 1919.)

The fully developed embryo has been noted with its head near the stalk. Eclosion was not observed, but the midgut of one unhatched larva contained food materials, therefore showing that the larva had fed before eclosion and had not reversed. Quite possibly the larva perforates the shell with the mandibles, feeds, increases in size, and eventually bursts the shell.

Larva.—Difficulty in keeping parasitized hosts alive prevented observations on the larva, but it is apparent from the camera-lucida drawings of the completely developed embryo (fig. 45) that a single pair of spiracles is to be found in the first instar larva.

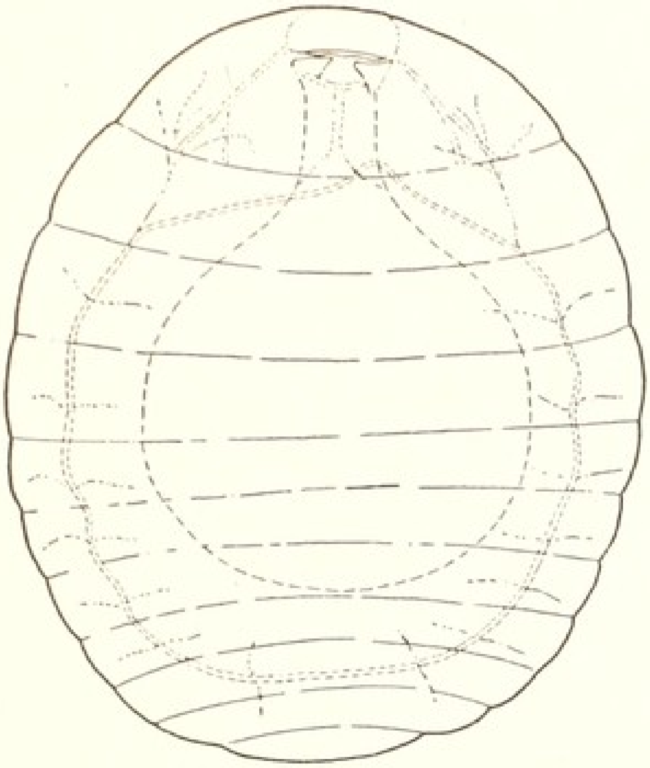


Fig. 49. *Metaphycus melanostomatus* (Timb.). First instar larva. (Redrawn from Silvestri, 1919.)

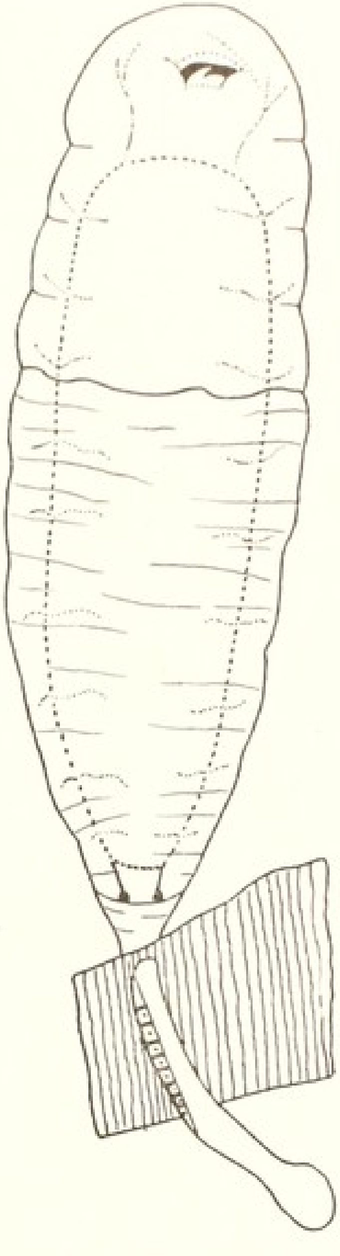


Fig. 50. *Microterys ferrugineus* (Nees). First stage larva. The figured neck indicates that a band is found with this species. (Redrawn from Parker and Thompson, 1925.)

Metaphycus lounsburyi (How.)

The biology and habits of this primary parasite of *Saissetia oleae* (Bern.) have been described in detail by Smith and Compere (1920, 1928). I observed the ovarian egg only.

Ovarian egg.—The ovarian egg is similar to that of *Metaphycus luteolus,* but the band on the egg proper is somewhat narrower and more wedge-shaped, being widest toward the neck. The cells are of moderate size and uniform except at the distal end where they are slightly larger. The large cells are not

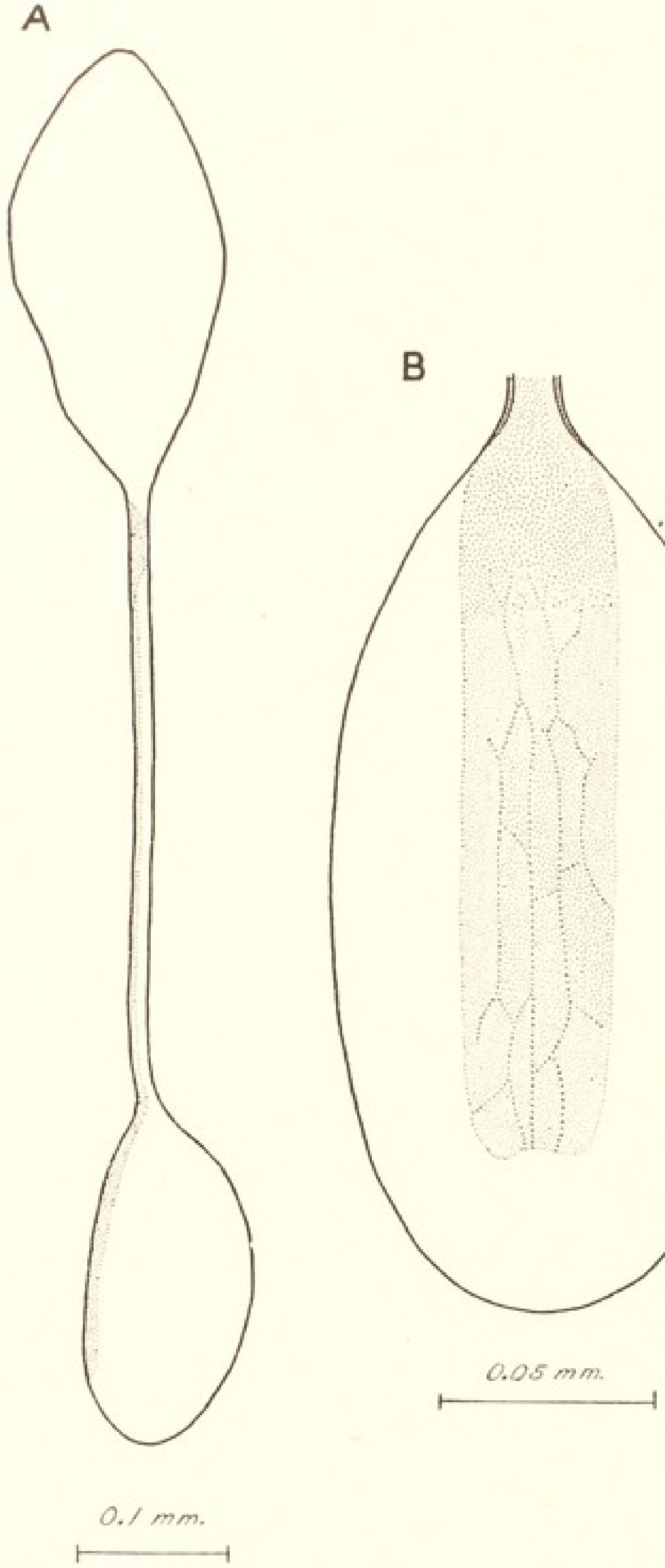


Fig. 51. *Microterys flavus* (How.). *A*, ovarian egg; *B*, dorsal aspect of egg proper, showing detail of aeroscopic plate. Larger cells from pattern.

in a demarcated area as in *M. luteolus* eggs, but gradually become mixed with the more minute cells in the sourrounding regions. Measurements were: length of bulb, 0.201 mm. width of bulb, 0.09 mm.; length of neck, 0.128 mm.; width of neck, 0.013 mm.; length of egg body, 0.218 mm.; width of egg body, 0.094 mm.; length of band on egg body, 0.154 mm.

Deposited egg.—Smith and Compere (1928) found the newly deposited egg to be stalked. Beyond a doubt the band was observed but was not recognized as such. They described a "ventral rib or stay" which extended about two-thirds the length of the egg body and which became dense white immediately after deposition. The drawing (see fig. 46) of the deposited egg shows this "ventral rib" clearly.

Larva.—The first instar larva was described by Smith and Compere.[7] Upon hatching it remains with the posterior five of the thirteen body segments enclosed by the eggshell. The tracheal system is metapneustic.

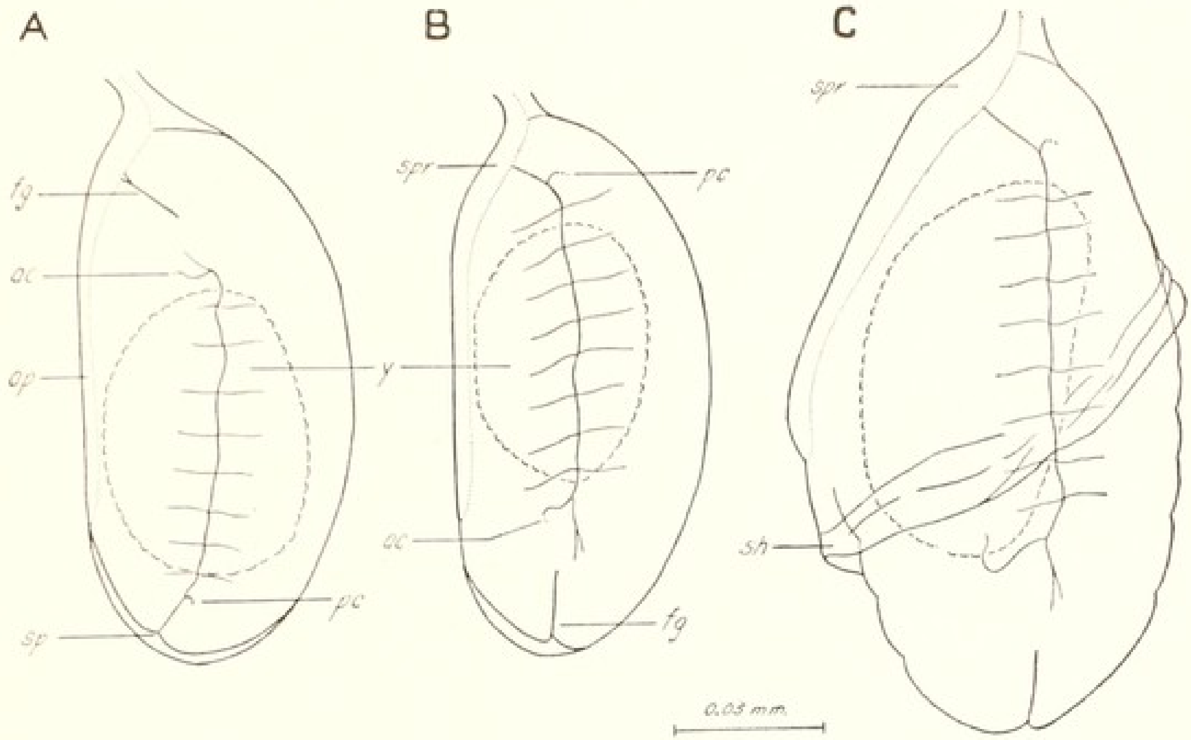


Fig. 52. *Microterys flavus* (How.). *A*, fully developed embryo prior to reversal; *B*, the same embryo after reversal, the eggshell intact; *C*, first instar larva immediately after eclosion.

Metaphycus luteolus (Timb.)

The ovarian egg (fig. 47) is very large compared with the size of the female and only a few eggs are matured at any one time. The egg is banded. Near the bulb the band extends nearly around the neck but more abruptly recedes until near the anterior end of the egg proper, where it commences to broaden. On the egg proper it is uniform in width. Most of the cells are minute, but at the end toward the neck there is a differentiated area of much larger cells. From a lateral aspect these cells appear noticeably thicker than those of the remainder of the band. The measurements were: length of bulb, 0.188 mm.; width of bulb, 0.103 mm.; length of neck, 0.086 mm.; length of egg, 0.18 mm.; width of egg, 0.093 mm.; length of band on egg proper, 0.137 mm.; width of band, 0.043 mm.

[7] The figure of the first instar larva in the earlier paper is probably actually the second instar as evidenced by the comparative size of the larva and eggshell, as well as the greater diameter and degree of development of the tracheae.

Neither deposited eggs nor larvae were examined. Smith and Compere (1928) report the egg to be suspended by the stalk from the dorsum of the host scale, but do not describe the larva. The larva will probably be found to have a metapneustic tracheal system in the first instar.

Metaphycus melanostomatus (Timb.)

Both Imms (1918) and Silvestri (1919) have investigated the development of this European species, which is parasitic on scale insects. Silvestri described the various stages as those of *Aphycus punctipes* (Dalm.) and considered *Metaphycus melanostomatus* as synonymous. Imms also synonymized the former with the latter. According to P. H. Timberlake, these are distinct species and both Silvestri and Imms undoubtedly dealt with *Metaphycus melanostomatus* and not *Aphycus punctipes*.

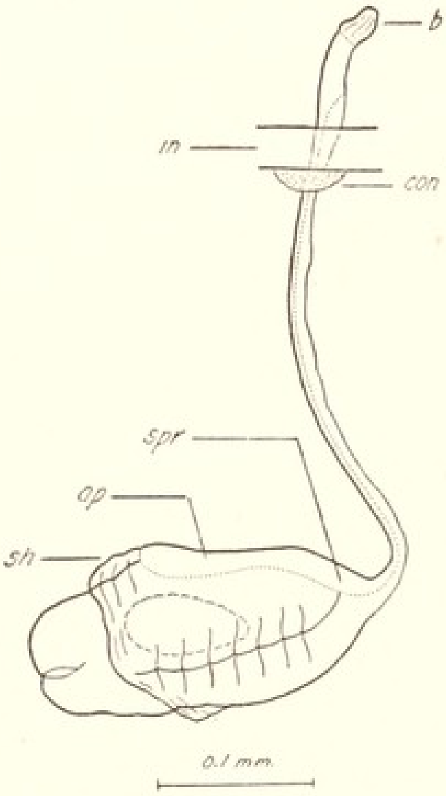


Fig. 53. *Microterys flavus* (How.). Newly hatched larva suspended by the remains of the eggshell attached to the integument of the host. The neck, with the exception of the part bearing the band, is collapsed.

According to Silvestri the ovarian egg is similar in form to that of *Blastothrix sericea* only somewhat smaller. No respiratory band is described, yet the figures and legend indicate that a distinct band was observed (fig. 48).

Silvestri found the deposited egg to be attached to the integument with the petiole projecting to the exterior of the host. Imms, on the other hand, reported the egg to lie free in the body cavity and to be devoid of a petiole. It is of some significance that Silvestri also found a band on the egg of *Blastothrix sericea*, whereas Imms failed to record one on the egg of supposedly the same species.

In the main, both workers agree regarding the structural details of the newly hatched larva: the tracheal system being simple and apneustic, consisting only of two main tracheal trunks, anterior and posterior commissures, and a single cephalic branch on either side (fig. 49). But Silvestri reported the newly hatched larva as being posteriorly encapsulated by the eggshell which was suspended from the host's integument. Imms records no such relationship.

If Silvestri's evidence is accepted, the larva of this species constitutes the single exception to the statement that with species of encyrtids having banded eggs the early larval stages have metapneustic tracheal systems. In view of the confusion in the taxonomy and the variations of the findings of the two investigators, further examination seems warranted.

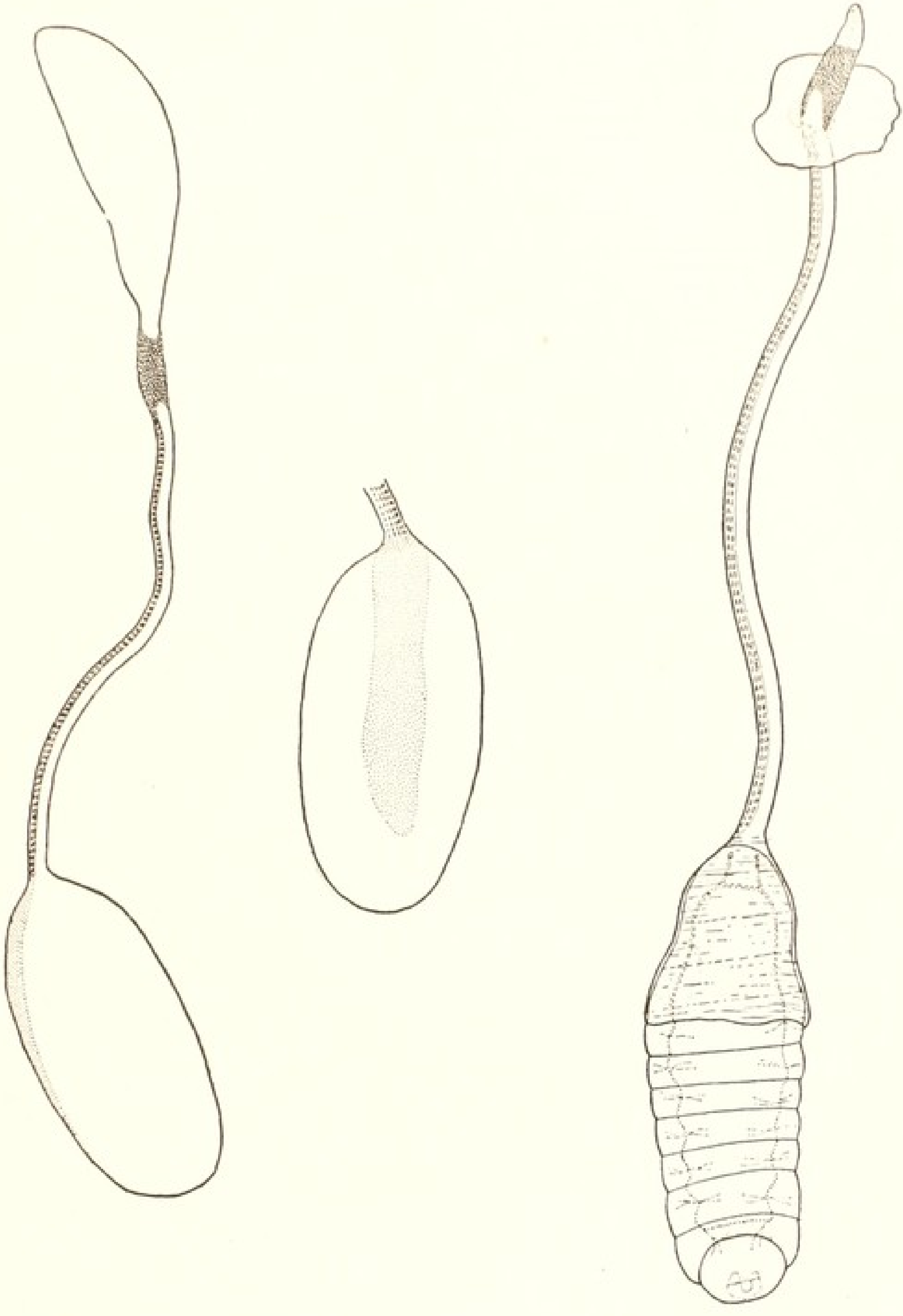


Fig. 54. *Microterys masii* Silv. Left, ovarian egg. Center, posterior portion of the same. Right, newly hatched larva suspended by the petiole attached to the host's integument. (Redrawn from Silvestri, 1919.)

Metaphycus timberlakei (Ishii)

The type of egg is not apparent from the descriptions of Ishii (1932), nor does he state whether the deposited egg is attached or free. The larva figured is nearly identical in shape and structure with the first instar larva of *Metaphycus melanostomatus.* The body is nearly as wide and long. The tracheae form a complete loop with one cephalic branch on either side. Spiracles are evidently lacking.

Microterys clauseni Comp.

An illustration by Ishii (1932) of the deposited eggs shows that it is of the banded type. In this drawing the stalks of the eggs are covered with a minute network which no doubt represents the cells of a band.

Microterys ferrugineus (Nees)

In addition to summarizing the available information on the egg and larva of encyrtids, Parker (1924), in his well-known work on the postembryonic forms of Chalcids, described the larva of this scale parasite.

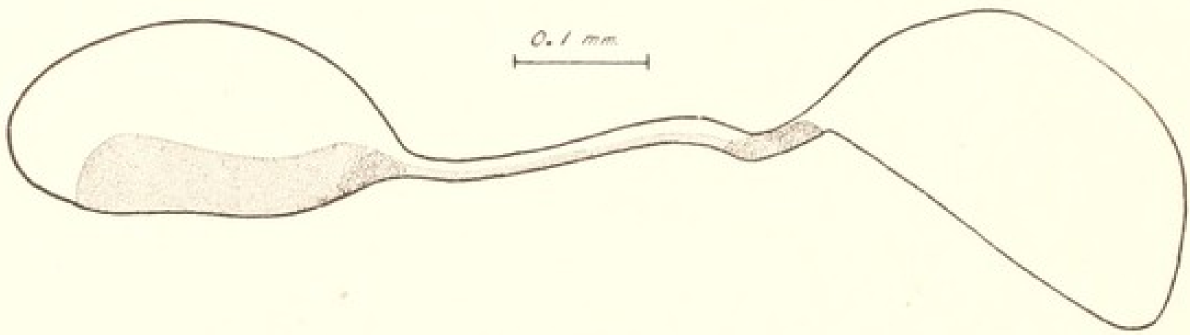


Fig. 55. *Microterys saissetiae* Comp. Ovarian egg, lateral view.

The newly hatched larva remains with the posterior part of the abdomen sheathed by the eggshell which is attached by the pedicel to the integument of the host. The tracheal system is metapneustic. No band structure was described or drawn. In a later paper by Parker and Thompson (1925) there was presented a figure of the sheathed larva which suggests that a band was observed (see fig. 50).

Microterys flavus (How.)

Timberlake (1913) has made observations on this, a parasite of soft brown scale (*Coccus hesperidum* Linn.). I was able to confirm and enlarge his report on the egg and larva.

Ovarian egg.—The ovarian egg (fig. 51, *A*) is distinctive in that its neck is more than twice as long as the main body. Timberlake did not observe the prominent band. Most of the cells are minute but larger cells form a network presenting an appearance similar to that shown in figure 48, *B*. The measurements were as follows: length of bulb, 0.3 mm.; width of bulb, 0.15 mm.; length of neck, 0.428 mm.; width of neck, 0.013 mm.; length of egg, 0.227 mm.; width of egg, 0.107 mm.

Deposited egg.—The egg is attached to the integument and the collapsed bulb and a portion of the neck protrude. The band is silvery throughout and immediately apparent after deposition. The stalk is curved so that the main body lies parallel to the host's integument. Just below the integument there is an agglomeration of melanized host material. The stalk also becomes melanized by the time the larva hatches. The distal half becomes yellow and proximal half brown.

The embryo develops with the head at the stalk end (fig. 52, *A*). (One embryo reversed its position prior to eclosion.) Immediately following this phase of development the embryo proceeds to expand and squirm until the eggshell is finally split to wrinkle backward over the body.

Larva.—Timberlake did not describe the tracheal system of the first instar larva. The existance of an open system was implied in the statement that the egg stalk was strictly homologous and similar to that of *Ooencyrtus kuvanae*, the function of which had been described as respiratory.

Figures 52, *C*, and 53 show the newly hatched larva. The tracheal system does not deviate from the general plan of metapneustic encyrtid larva. The posterior commissure is short and straight, whereas the anterior one is more than twice as long and arches over the foregut. Lateral branches are readily distinguishable. The spiracles are placed on the band so that each lies at a point one-third the distance from the margin of the band.

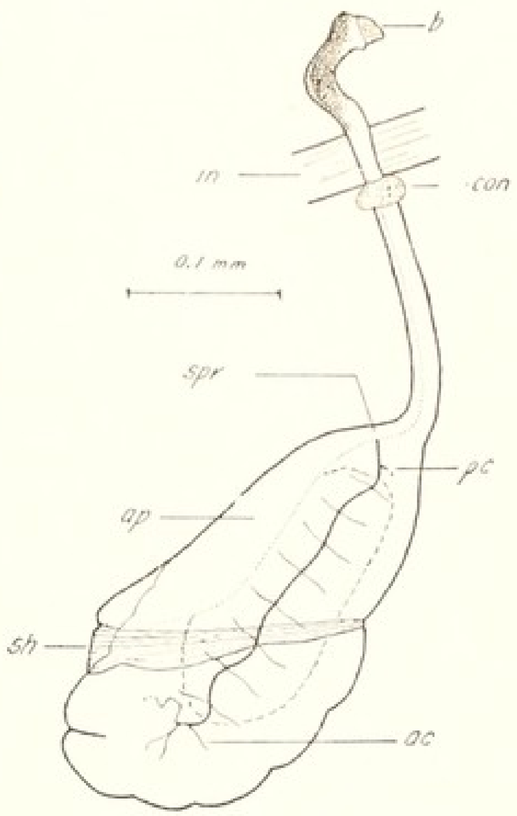


Fig. 56. *Microterys saissetiae* Comp. Newly hatched larva suspended by the eggshell attached to the integument of the host.

Microterys masii Silv.

Silvestri (1919) stated that the egg of this species is similar to that of *Phaenodiscus aeneus*, but had a shorter neck. The stage is not described in detail. From his drawings it is apparent that a band is present (see fig. 54).

The deposited egg and first instar larva are also similar to those of *Phaenodiscus.* Although the respiratory system of the larva is metapneustic, in Silvestri's drawings of the newly hatched larva the spiracles do not appear to have any relation to the band.

Microterys saissetiae Comp.

This parasite of black scale (*Saissetia oleae* Bern.) was introduced into California by Harold Compere. A limited amount of material for study was made available during its propagation.

Ovarian egg.—The egg of this species (fig. 55) resembles in certain respects the eggs of other *Microterys* and that of *Coccidoxenus niloticus.* It resembles the former in the length of the neck which is slightly longer than the main body of the egg. The band has the general shape and structure found in *Coccidoxenus.* The anterior of the neck is similarly ornamented, consisting of a network of four-sided or three-sided cells with slight thicknesses at the corners, as illustrated in the drawing (fig. 28, *A*). Unlike *Coccidoxenus,* there is no pattern on the band of the egg proper made by larger cells. The measurements were approximately as follows: width of bulb, 0.16 mm.; length of bulb, 0.3 mm.; length of neck, 0.32 mm.; width of egg, 0.14 mm.; length of egg, 0.31 mm.; width of band (maximum) 0.11 mm.

Deposited egg.—The egg in the deposited state is of the stalked type with the anterior extremity of the neck and the collapsed bulb protruding from the host's integument. The projecting neck is rigidly curved so that a definite hook is formed. The stalk soon becomes melanized throughout its length with the exception of the exterior portion. A black mass of host material congregates around the stalk immediately below the integument. The embryo evidently

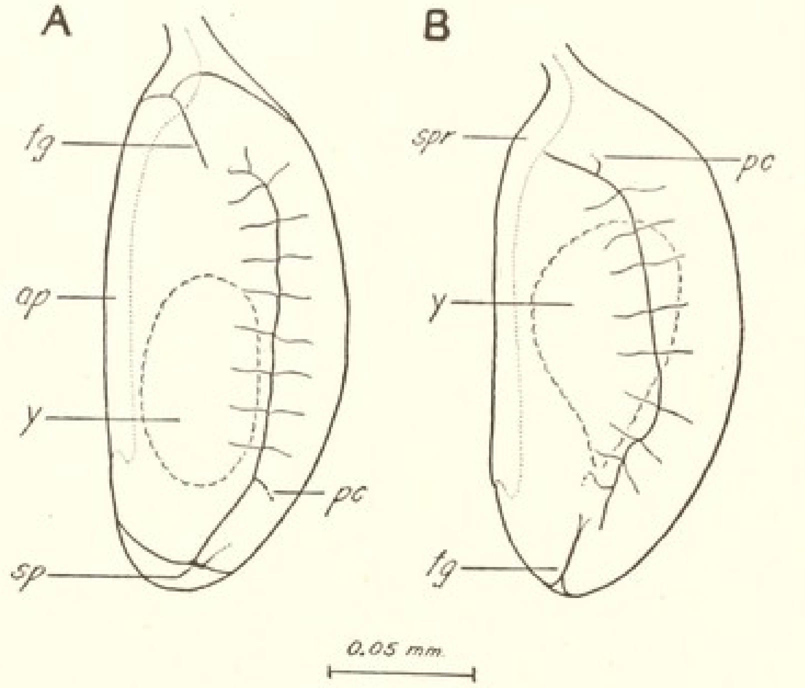


Fig. 57. *Ooencyrtus californicus* Gir. *A*, mature embryo prior to reversal; *B*, after reversal.

develops with the anterior end near the stalk, although this was observed only once. Lack of material prevented additional observations.

Larva.—The larva (fig. 56) differs in no particular detail from the newly hatched larva of *Coccidoxenus.* The single pair of spiracles is located near the stalk but, unlike *Coccidoxenus,* retains the same general position in the later instars.

Microterys speciosus Ishii

Ishii (1932) has given an extensive account of this encyrtid. The ovarian egg appears to be similar to that of most *Microterys* in that the neck is long and slender. There is no indication in the description or drawings of the presence of a band although the function of the pedicel was described as respiratory. In a later publication Ishii (1932) remarked that the chorion is minutely reticulated, which may indicate the presence of a band. The deposited egg is attached to the integument of the host scale and a portion of it protrudes. After eclosion the larva remains with the caudal five segments enclosed by the eggshell. The tracheal system is metapneustic.

Ooencyrtus californicus Gir.

Material for study was secured from an infestation of *Anasa tristis* De Geer at Riverside, California.

The undeposited egg is remarkably similar to that of *Ooencyrtus johnsoni*. The structure of the band on the neck is identical with *O. johnsoni*, but the division at the apex of the band on the egg body is not so prominent, and the band is slightly wider relative to the size of the egg. The dimensions were: length of bulb, 0.188 mm.; width of bulb, 0.068 mm.; lenkth of neck, approximately 0.377 mm.; length of egg, 0.180 mm.; width of egg, 0.094 mm.; length of band on egg, 0.150 mm.

The deposited egg lacks distinguishing features. The development parallels that described for *Ooencyrtus johnsoni*. Figure 57 shows an unhatched larva prior to reversal (*A*) and after reversal (*B*). The larval (fig. 58) respiratory systems are alike.

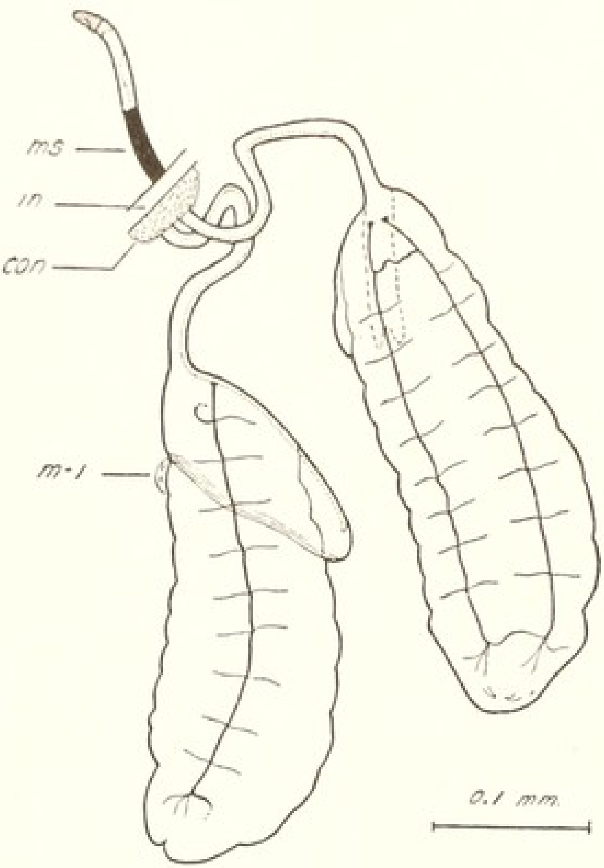


Fig. 58. *Ooencyrtus californicus* Gir. First instar larvae attached to the shell of the host. (From the exterior of the host it would have appeared that only a single egg had been deposited.)

Ooencyrtus johnsoni (How.)

The eggs of the harlequin cabbage bug, *Murgantia histrionica* (Hahn), are commonly attacked by this encyrtid in southern California.

Ovarian egg.—The ovarian egg (fig. 59, *A*) is quite large and its neck is half again as long as the main body of the egg. The bulb is smaller than the egg proper and its apex is slightly thickened. Measurements were: length of bulb, 0.158 mm.; width of bulb, 0.064 mm.; length of neck, approximately 0.3 mm.; width of neck, 0.013 mm.; length of egg, 0.18 mm.; width of egg, approximately 0.09 mm.

A distinct band is present which, in certain respects, differs from those of all encyrtid eggs but those of another species of *Ooencyrtus*. The most striking variation is in the appearance of the band on the main body (fig. 60). The marginal areas are composed of minute cells. At the center of the anterior end the cells are large and contiguous, then abruptly become scattered throughout the central section. The band is irregular in breadth and at the apex is divided into two short prongs composed of thick cells. The length measures 0.15 mm. and the greatest width is approximately 0.03 mm.

An additional structure, either nonexistent or obscure in most eggs, is noted at the apex of the neck (fig. 59, *B* and *C*). At that point the cells completely surround the neck, and as the band narrows the cells underlie a thickened wall for nearly 0.1 mm. The wall may cover the rest of the neck and the upper part of the main body. Frequently it appeared that the band was situated under a thin layer at the junction of the neck with the egg, although this may be a microscope effect.

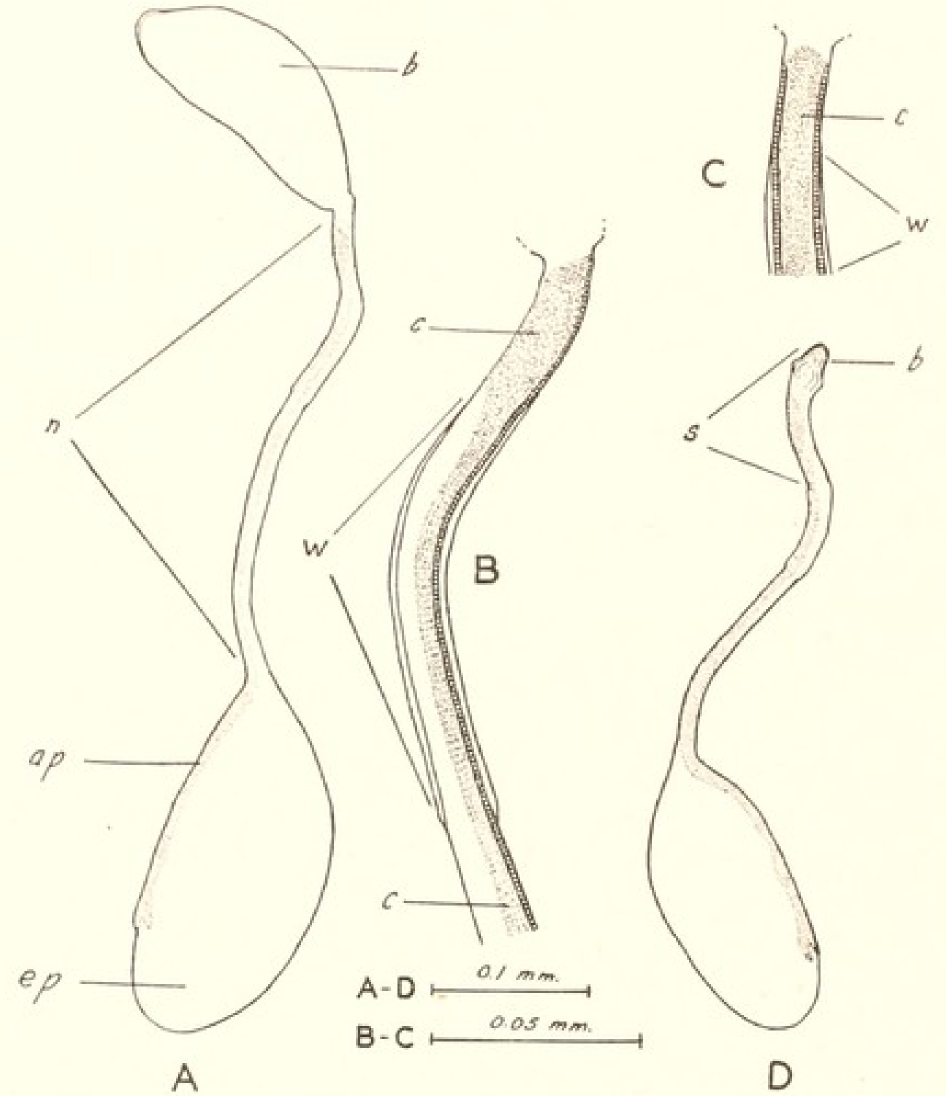


Fig. 59. *Ooencyrtus johnsoni* (How.). *A*, ovarian egg. *B*, lateral aspect of anterior section of the neck. The band passes beneath a thickened wall. *C*, dorsal dorsal view of the bulb end. *D*, deposited egg.

Deposited egg.— In the process of oviposition the contents of the bulb are forced into the egg proper and the remains of the bulb are left attached to the protruding neck, as shown in figure 59, *D*). The thickened end fits over the neck like a cap.

Usually the chorion of the host egg surrounds that portion of the neck which has thickened walls. Eggs may be deposited singly or in groups of two or three.

Melanization commences soon after deposition. The thickened portion of the neck becomes blackened even though all or part lies outside the host. Throughout the neck and upper part of the egg body the band becomes brown.

The embryo first lies with its head at the anterior end, as shown in figure 61, *A* (p. 108), and later reverses itself (*B* and *C*).

Larva.—At eclosion the eggshell ruptures and wrinkles about the posterior of the larva, the band remaining fairly straight and unbroken; two spiracles are affixed to it near the stalk. Lateral tracheal branches are very minute and difficult to discern. Figure 61, *D*, shows a larva that has just hatched. The larva remains attached in a similar manner during most of its existence.

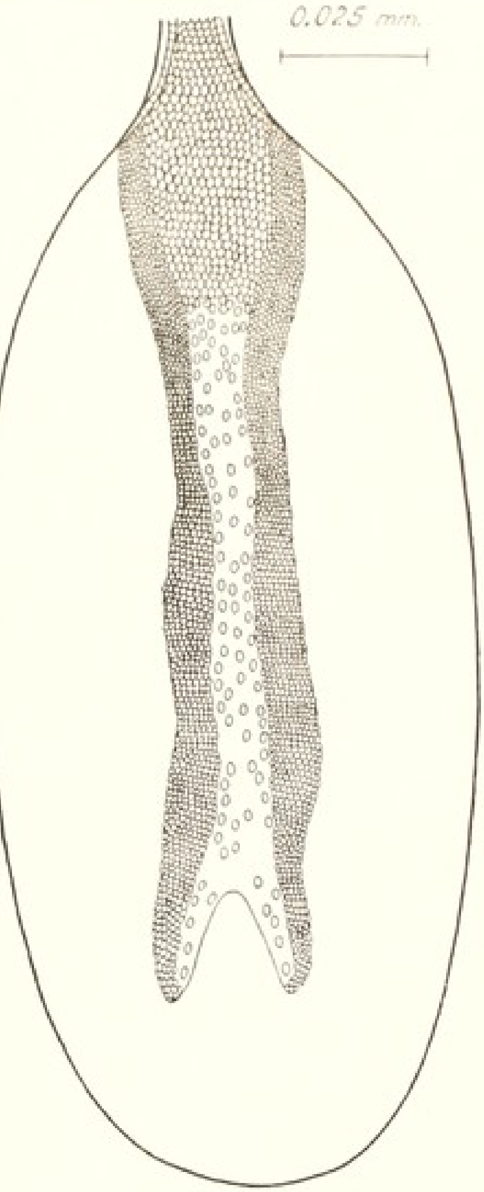


Fig. 60. *Ooencyrtus johnsoni* (How.). Dorsal aspect of egg proper, showing detail of band.

Ooencyrtus kuvanae (How.)

The investigation of this species reported by Howard and Fiske (1911) has been the standard reference in many subsequent articles on encyrtid biologies. Theirs was the first report of the so-called "stalked eggs." However, they erred in interpreting the function of the stalk and their error has frequently appeared in subsequent writings. By means of preserved parasitized host material and live adult parasites kindly supplied by R. C. Brown of the U. S. Department of Agriculture, Forest Entomological Laboratory at New Haven, Conn., I have been able to correct and amplify some of the description by Howard and Fiske.

Ovarian egg.—The ovarian egg resembles that of other species of *Ooencyrtus* described here, having the same general shape but a different structure. Near the bulb the neck is broader; the increased breadth continues for a short distance, thence the neck becomes uniformly narrow. It is possible that this bulge may serve to retain a portion of the egg protruding from the host.

The band on the egg proper differs as well. The general shape—broad at the distal end, narrower toward the center, then broad again—is the same. But the cells are small except for an ovoid area occupying most of the anterior end. They are sparse only near the center of the slightly bifurcate apex. The cells here are no thicker than others. Those on the neck do not appear to have thickened walls. The cells surround the neck immediately above the bulge and some of these are slightly larger than the others.

Deposited eggs.—In all the preserved material examined the end of the stalk protruded from the host's eggshell. Where the host embyro was completely formed, the egg was found within the body cavity in the same manner as described and drawn by Howard and Fiske. The portion of the egg protruding from the host egg consists of the anterior portion of the neck and the col-

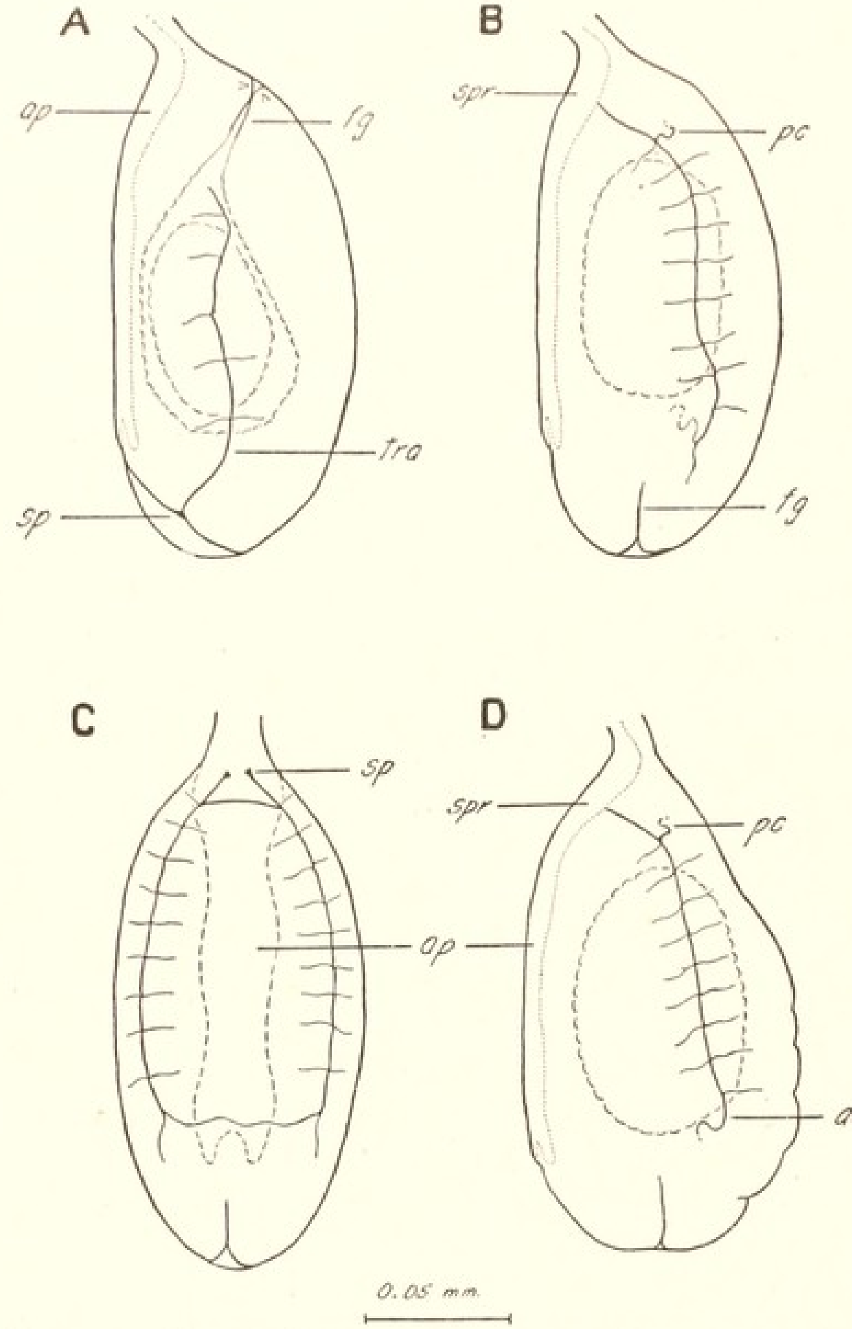


Fig. 61. *Ooencyrtus johnsoni* (How.). *A*, mature embryo prior to rotation; *B* and *C*, two aspects of embryo subsequent to reversal; *D*, newly hatched larva.

lapsed bulb. Even though the material had been preserved in Bouin's fixative, the silvery sheen resulting from the presence of air within the band was frequently observable.

Larva.—Satisfactory material was unfortunately not obtainable for observations of the larva. As with others of this type, the larva remains with the posterior end enclosed by the eggshell. Although *Oöencyrtus kuvanae* has been repeatedly referred to as remarkable in that the larva respires through a tube, the existance of spiracles was not demonstrated until recently, when

Parker (1933) noted two open spiracles at the point of attachment to the eggshell. An observation by Howard and Fiske appears open to question. They report the stalk to increase in thickness as the larva grows. Since the egg stalk consists solely of shell it is not conceivable that it is capable of growth of itself. Among those which I observed, none increased in thickness. The melanization of the stalk as development proceeds does cause the stalk to appear more prominent and it is therefore quite possible that it created an illusion of greater thickness.

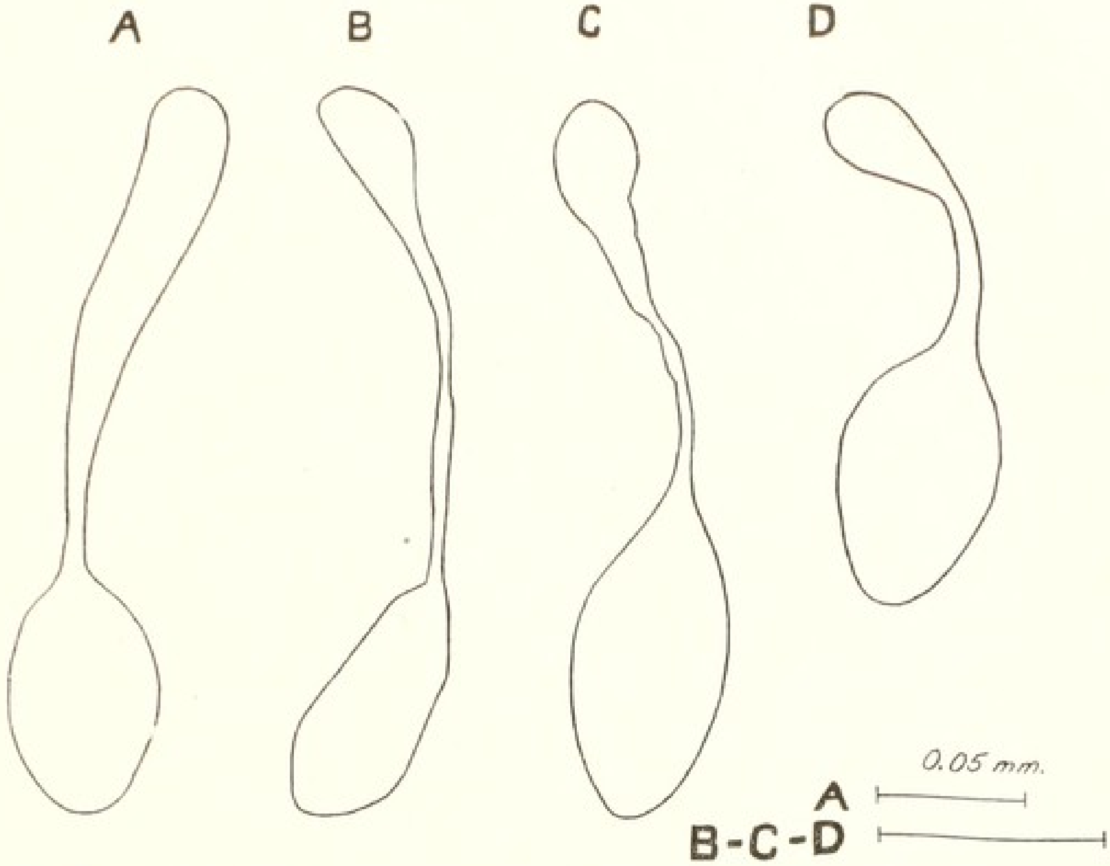


Fig. 62. Ovarian eggs of: *A, Acerophagus pallidus* Timb.; *B, Acerophagus fasciipennis* Timb.; *C, Stemmatosteres apterus* Timb.; *D, Paralitomastix pyralidis* (Ashm.).

Paralitomastix pyralidis (Ashm.)

Although no investigation of the eggs and larvae of polyembryonic encyrtids was made in this work, the ovarian egg of this species was examined for purposes of comparison.

The egg (fig. 62, *D*) is extremely simple, small, and its shell is unmodified. The bulb is short and only slightly wider than the neck. The egg proper is elliptical, the anterior end being the broader. The apex is somewhat more pointed than in other eggs. The measurements were: length of bulb and neck, 0.072 mm.; maximum width of bulb and neck, 0.017 mm.; length of egg, 0.06 mm.; width of egg, 0.034 mm.

It is evident from the literature on polyembryonic species (see especially Silvestri, 1908, *et seq.*) that the egg is deposited entirely within the host and that the larvae that develop do not possess spiracles in the primary stages.

Phaenodiscus aeneus (Dalm.)

Silvestri (1919) has given a detailed description of the developmental stages which is well supported by drawings, three of which are duplicated in figures 63 and 16. The neck of the egg is nearly four times as long as the main body. The band on the egg is minutely "dimpled" and extends from the bulb nearly to the apex of the egg. At the bulb end the neck is slightly larger and at this point the band extends completely around. This portion remains exterior to the host and was termed by Silvestri the "respiratory plug." The egg is deposited with the anterior body and a part of the connecting tube remaining outside the host integument as in other stalked eggs. The newly hatched larva retains the last few abdominal segments within the eggshell. The single pair of posterior spiracles adheres to the aeroscopic plate.

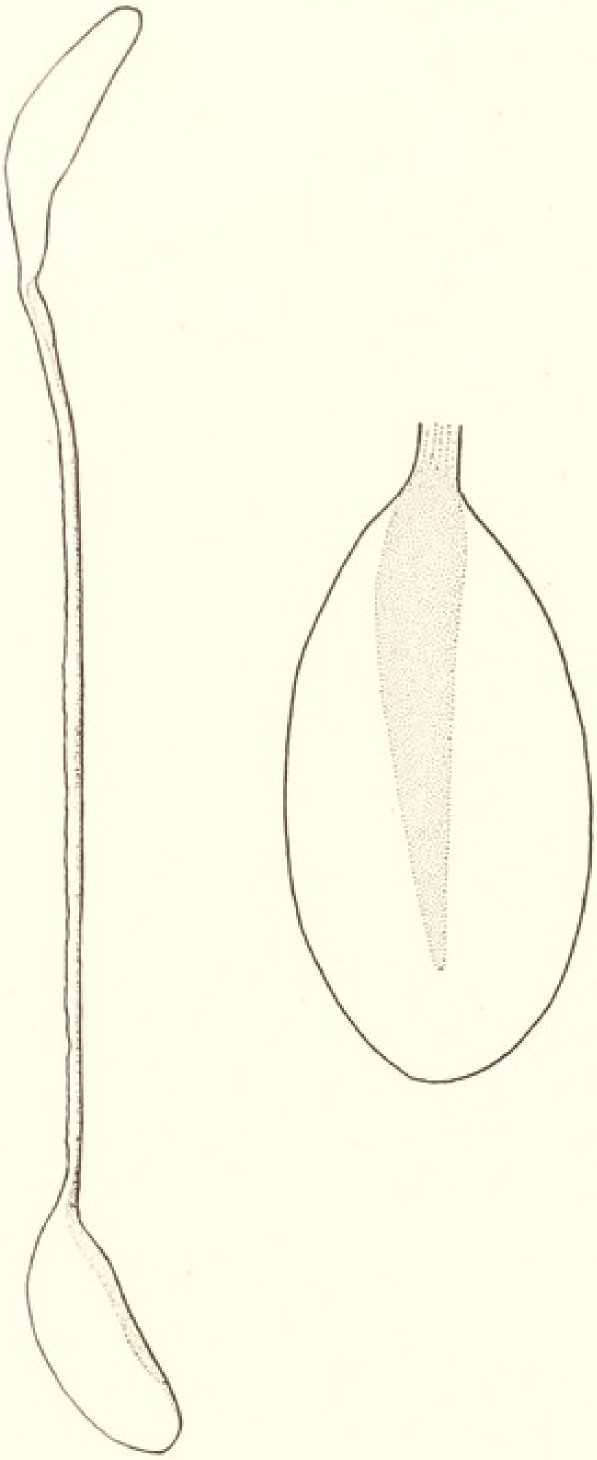


Fig. 63. *Phaenodiscus aeneus* (Dalm.). Left, ovarian egg. Right, posterior portion of same. (Redrawn from Silvestri, 1919.)

Pseudaphycus angelicus (How.)

This is a parasite of the Mexican mealybug, *Phenacoccus gossypii* Twns. and Ckll., and several other mealybug species in southern California.

Ovarian egg.—The undeposited egg (fig. 64, *A*) is extremely simple in structure, lacking any suggestion of a band or any modification of the chorion. The bulb is long and narrow and not sharply differentiated from the neck. The egg when compressed is convex on the ventral side and nearly straight on the dorsal. The measurements were: length of bulb and neck, 0.312 mm., approximately; maximum width of bulb, 0.051 mm.; length of egg, 0.175 mm.; width of egg, 0.098 mm.

Deposited egg.—The deposited egg (fig. 65, *A*) lies entirely within the host. A portion of the neck and the collapsed bulb are doubled back over the basal portion of the neck and become melanized soon after deposition of the egg. In some specimens a crumpled mass of material was observed at the proximal end of the neck.

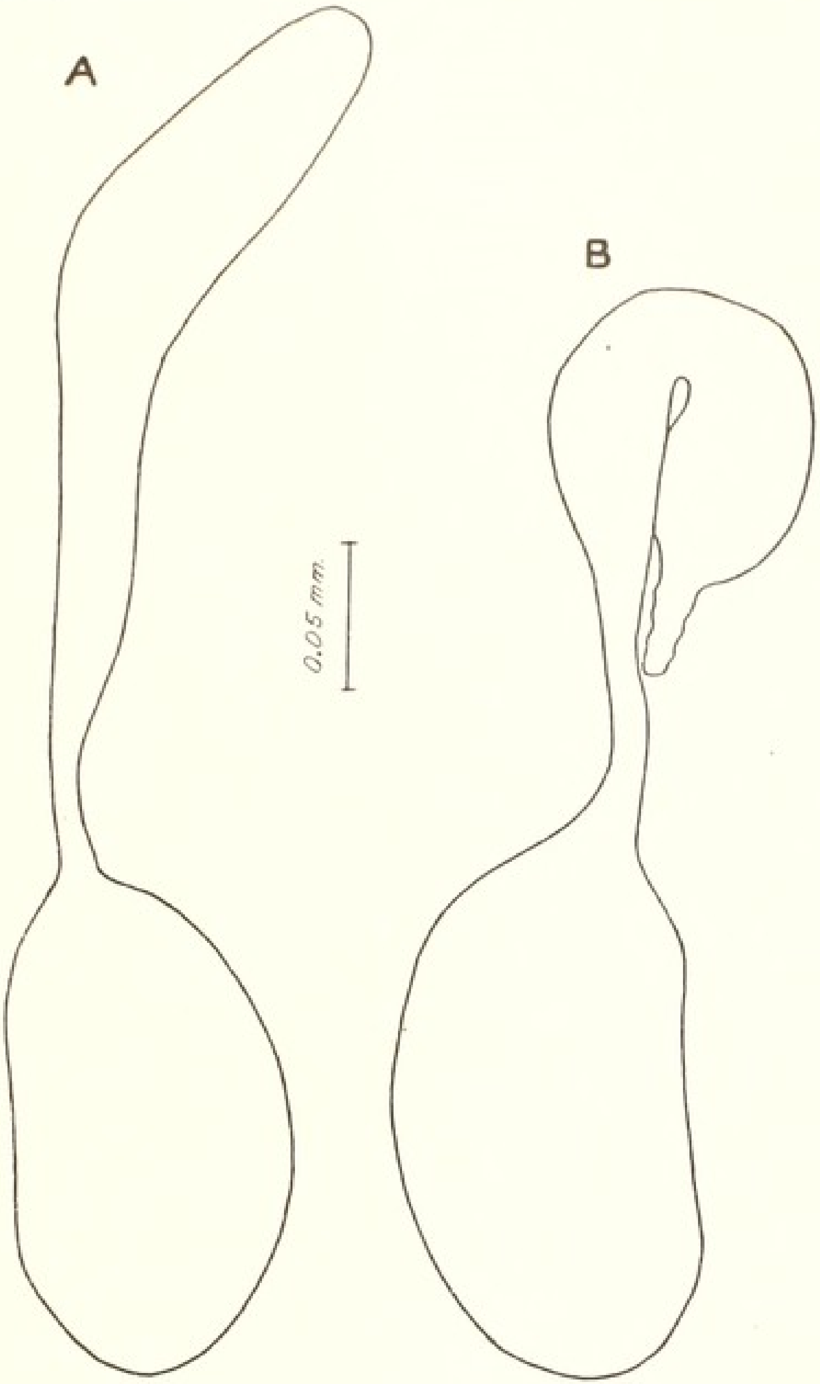


Fig. 64. *Pseudaphycus angelicus* (How.). *A*, ovarian egg; *B*, compressed newly deposited egg, showing reinflation of bulb.

Larva.—No trace of a tracheal system could be seen in either the completely developed embryo or the first instar larva. Usually the tracheal structures are most easily discernible in the embryo. Figure 65 shows a fully developed embryo, *A*, and a first instar larva, *B*. The larva had obviously fed, yet the small circle around the mandibular region suggests that most of the larval body is still enclosed by the shell. Although tracheae may be present and extremely minute, spiracles are undoubtedly absent.

Pseudleptomastix squammulata Gir.

This encyrtid is the parasite most frequently obtained in southern California from *Amonostherium lichtensioides* Ckll., a coccid common on *Artemisia californica*. Lack of suitable material prevented satisfactory observations of the larva. The same species was found to attack *Pseudococcus maritimus* by Clausen (1924) who was successful in investigating all stages.

The ovarian egg is banded (fig. 66, *B*) and in general resembles most other eggs of the type. At the bulb end of the neck the band is broad and composed

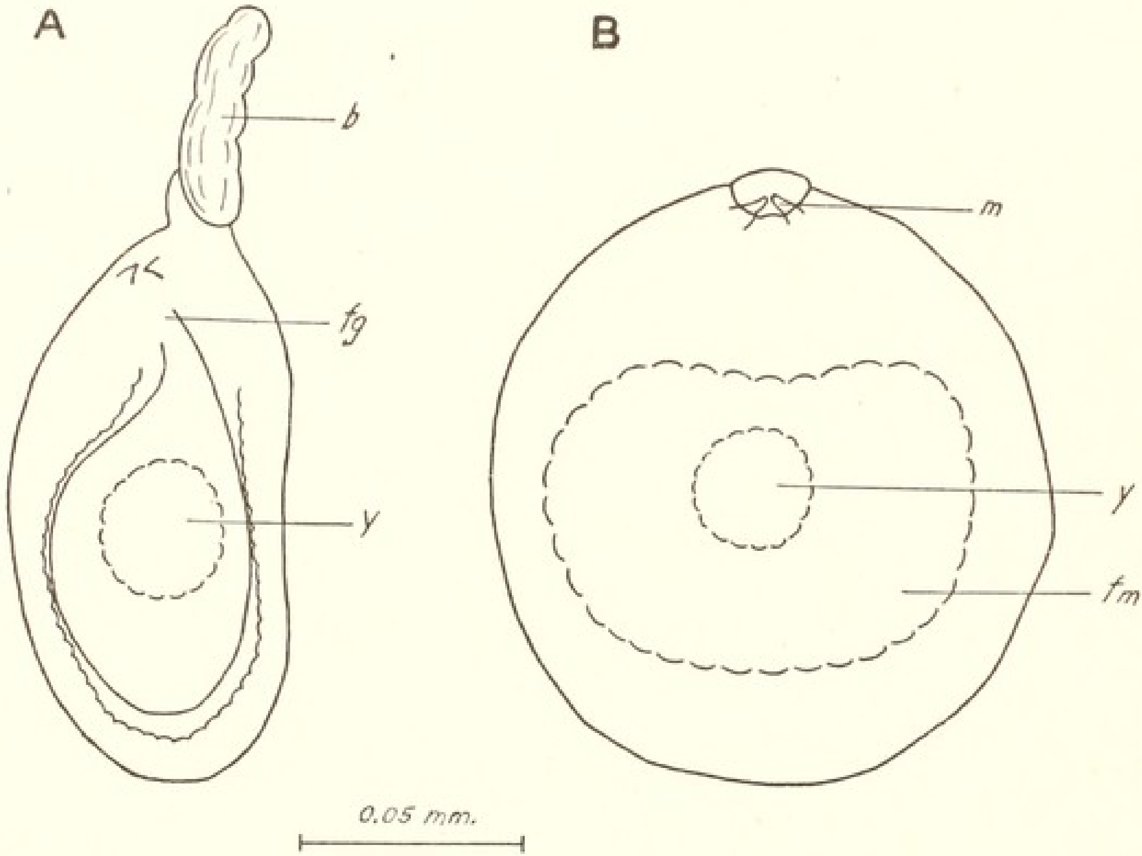


Fig. 65. *Pseudaphycus angelicus* (How.). *A*, deposited egg containing fully developed embryo; *B*, first instar larva. Almost the entire body is enveloped in shell.

of prominent cells arranged in much the same pattern as in *Anagyrus putonophilus;* it then abruptly narrows to a breadth of two cells, and on the main body of the egg broadens again until it is slightly wider than the neck although nevertheless narrow compared with the band on most eggs. Clausen does not indicate that he saw a band.

The measurements were: width of bulb, 0.042 mm.; length of bulb, 0.163 mm.; width of egg, 0.06 mm.; length of egg, 0.146 mm.; length of neck, 0.107 mm.

On *Amonostherium* the eggs are deposited singly or in groups of two and three protruding from the same aperture. The swollen portion of the neck protrudes from the host's integument. According to Clausen, single eggs are laid in *Pseudococcus maritimus* in the same manner.

I was not able to observe the larva; however, Clausen noted that the young larva remains attached at its anal end to the stalk, "the function of which has been described as respiratory." The type of tracheal system was not specified, but it is probably metapneustic.

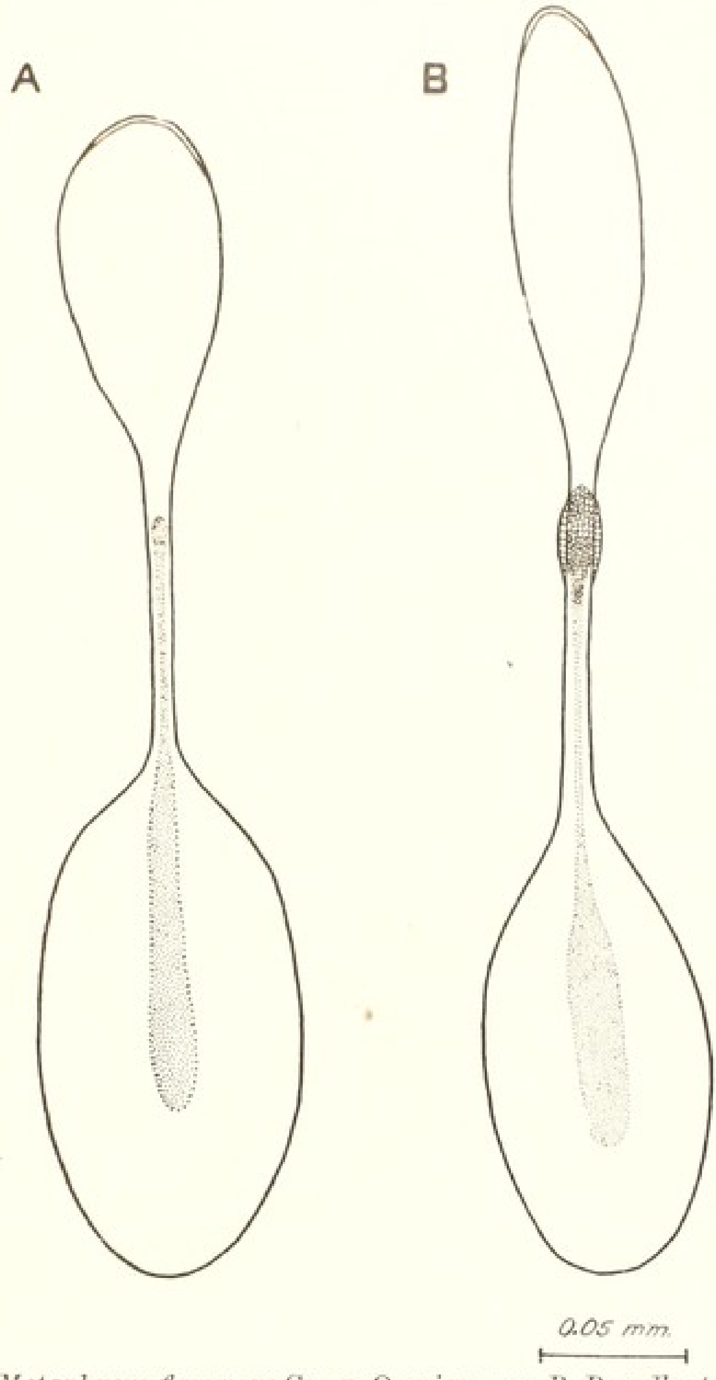


Fig. 66. *A, Metaphycus flammeus* Comp. Ovarian egg. *B, Pseudleptomastix squammulata* Gir., ovarian egg.

Quaylea whittieri (Gir.)

This common, introduced species is hyperparasitic on many of the parasites of scale insects introduced into California. An extensive study was not made.[8]

The undeposited egg (fig. 8, *B*) may be said to resemble that of *Cheiloneurus*. The similar features are simplicity in structure, long neck, and small undifferentiated bulb. The neck is obviously fragile and becomes readily dis-

[8] [The first instar larva of *Quaylea whittieri* is atracheate. It molts while enclosed in its embryonic membrane (trophamnion). S.E.F.]

torted in mounting procedures. In the prepared mounts it is never regular in outline but is partly collapsed. The bulb is slightly wider than the neck, is short, and has a truncated apex. The measurements were: length of bulb, 0.107 mm.; width of bulb, 0.021 mm.; length of neck, approximately 0.47 mm.; length of egg, 0.133 mm.; width of egg, 0.073 mm.

Spaniopterus crucifer Gahan

Taylor (1935) was able to obtain very little information on this scale parasite. Deposited eggs were not found. Ovarian eggs and young larvae were said to be similar to those of *Comperiella unifasciata*. The first instar larvae are probably apneustic or possibly atracheate.

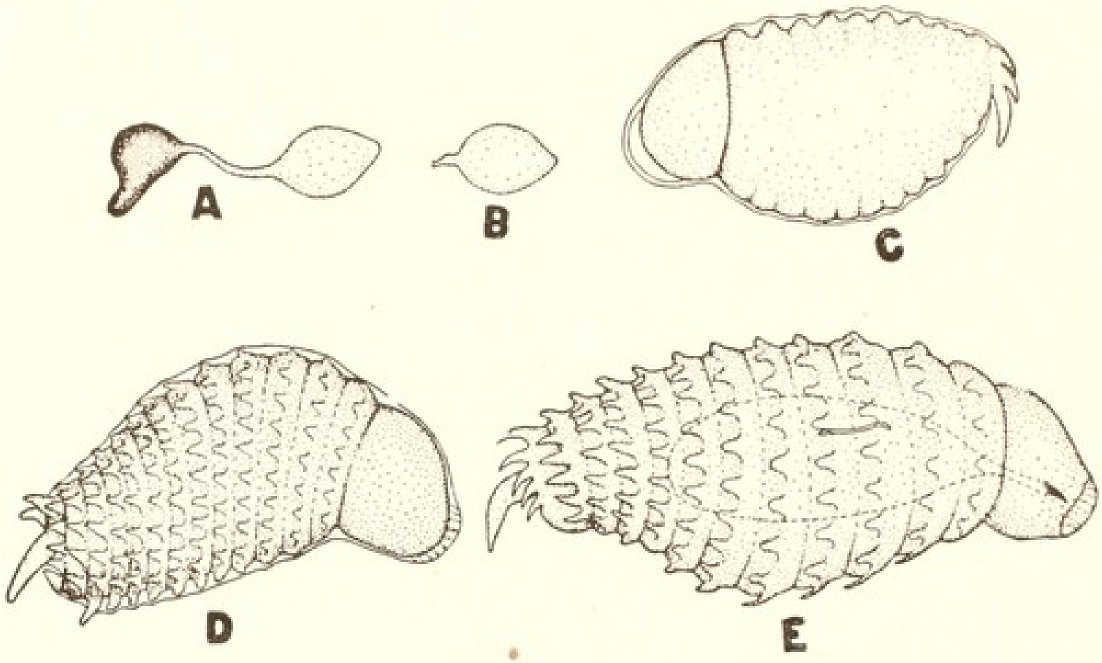


Fig. 67. *Tetracnemus pretiosus* Timb. *A*, ovarian egg; *B*, newly laid egg; *C*, first instar larva on fourth day within chorion; *D*, on fifth day; and *E*, on seventh day. (After Clancy, 1934.)

Stemmatosteres apterus Timb.

This minute wingless species was secured from a single infestation of an unknown species of *Phaenacoccus* near Fillmore, California. Deposited egg and newly hatched larva were not examined.

The ovarian egg (fig. 62, *C*) is similar to those of *Acerophagus* spp., the simplest type of egg. The measurements were: length of bulb and neck, 0.12 mm.; maximum width of bulb, 0.021 mm.; length of egg, 0.09 mm.; maximum width, 0.042 mm.

From the appearance of the ovarian egg it is assumed that the deposited egg lies free within the host and the larva has no spiracles.

Tetracnemus pretiosus Timb.

This primary parasite of mealybugs was discussed by Compere and Smith (1932) and in more detail by Clancy (1934). Only a few points need to be repeated here. Immature stages are shown in figure 67.

The ovarian egg is double-bodied with a smooth surface throughout. The deposited egg lies free in the body fluids of the host and gradually increases in size with the development of the embryo. The first instar larva does not hatch immediately but remains enveloped by the chorion for nearly half of the stadium. During that time it is said to be atracheate. After eclosion the only suggestion of a respiratory system is a tiny pair of tracheal trunks. This condition has been found with only one other encyrtid, *Anarhopus sydneyensis* Timb.

Zarhopalus corvinus (Gir.)

This primary parasite of mealybugs has an unmodified ovarian egg. The bulb is almost spherical in outline. There is no thickening of the apex. The neck is notably short and the structure of the chorion differs in no way from that of the rest of the egg. The main body is as wide as the bulb but one-third again as long. The shape is more or less oval, regardless of aspect. The eggs of a single female differed slightly in size and shape. The average measurements were approximately as follows: length of bulb, 0.15 mm.; width of bulb, 0.1 mm.; length of neck, 0.021 mm.; length of egg, 0.22 mm.; width of egg, 0.098 mm.

All stages were investigated by Clausen (1924). The deposited egg was noted to be free-floating within the body of the mealybug host. The bulb part of the ovarian egg was collapsed after deposition and was occasionally observable as a small irregular protuberance. The larva was of the tailed type, i.e., the last segment was prolonged into an appendage which was bent at an angle of 30 degrees to the axis of the body. The tracheal system appeared to be apneustic.

Miscellaneous

A number of species could not be adequately investigated but sufficient evidence was secured concerning them to indicate the probable type of egg and tracheal system in each. The following species probably have banded eggs and metapneustic larvae:

Chalcaspis phenacocci (Ashm.)
Encyrtus californicus (Gir.)
Habrolepis rouxi Comp.
Leptomastix dactylopii How.
Metaphycus eruptor (How.)
Metaphycus fuscipennis (How.)
Metaphycus lecanii (How.)
Metaphycus similis (Timb.)
Metaphycus spp.
Ooencyrtus sp.

The following species are likely to have unbanded eggs and apneustic larvae:

Bothriencyrtus sp.
Cheiloneurus lineascapus Gahan
Chrysoplatycerus ferrisi Timb.
Chrysoplatycerus splendens How.
Chrysopophagus amplicornis (Gahan)
Cirrhencyrtus ehrhorni (Timb.)
Diversinervus smithi[9] Comp.
Homalotylus spp.

[9] [*Diversinervus smithi* deposits unbanded eggs entirely within the central nerve ganglion of the black scale, *Saissetia oleae*. As many as 25 eggs may be deposited in one host. The first instar larvae is unique in possessing a pair of large, thin-walled cephalic vesicles. It lacks a tail. The first larval molt is inhibited until the host is gravid. S.E.F.]

SUMMARY

The Encyrtidae are a large family of minute parasitic Hymenoptera that most frequently attack such insects as mealybugs and scales. Nearly all the species of this family pass the entire developmental period within the host. The structure and behavior of certain primary larvae of the Encyrtidae are correlated with a respiratory modification of the eggshell, the aeroscopic plate, or band.

Correlating factors are: (1) the presence of air within the aeroscopic plate, (2) the position of the larval spiracles with respect to the aeroscopic plate, (3) the closed lumen of the egg stalk, (4) the course of stained oils in the aeroscopic plate and larval tracheae, and (5) the association between banded and unbanded eggs and open and closed tracheal systems.

The ovarian egg consists of two ovoid bodies connected by a narrow tube. One body serves as a reservoir for the contents of the other as the egg passes down the ovipositor. The entire contents of the egg remain in the posterior body when oviposition is completed.

On the basis of structure the eggs are of three types; unbanded, banded, and intermediate. An unbanded egg has a smooth shell, with no modifications, and is always placed wholly within or without the host. A banded egg has a shell of intricate structure. One side of the connecting neck and an elongate area on the egg proper appear to have a cell-like composition. This band or plate differs among species but is constant within each species. In the process of deposition the banded egg is placed so that the neck projects from the host's body. The band invariably has the appearance of containing air. Intermediate eggs lack very complex shells but possess thickenings or modifications which, in varying degrees, are suggestive of bands. These are also placed so that the neck extends from the body. The morphological differences exhibited by the three types of eggs are of value in generic and specific differentiations.

The larvae of the primary stages may be placed in two categories on the basis of the respiratory structures—those with closed systems of tracheae and those with tracheal systems bearing two, rarely four, caudal spiracles. Larvae that hatch from eggs of the intermediate and unbanded types are apneustic, and usually lie completely free within the hosts. Metapneustic larvae hatch from banded eggs only and remain partly enclosed posteriorly by shell so that the spiracles are in position to gain contact with the air-bearing structures on the egg proper.

Larvae, with or without spiracles, may obtain oxygen from the blood of the host by osmosis. It has heretofore been assumed that those with spiracles utilized atmospheric air passing through the lumen of the neck of their eggshells. Evidence is given which indicates that this is not correct and that the band structures in the wall of the neck provide means whereby atmospheric air is directly conveyed to the larvae. The supply of oxygen obtained by this means is necessary for the complete development of metapneustic larvae.

The respiratory adaptations of sixty-seven species of Encyrtidae are discussed in some detail. Twenty species are mentioned briefly.

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SYMBOLS USED IN FIGURES

ab = air bubble
ac = anterior commissure
ap = band or aeroscopic plate
b = bulb
c = cells
con = conglomeration of host material
ep = egg proper
ex = exterior conglomeration of host material (melanized)
fg = foregut
fm = food material
in = integument of host
k = collar
l = lip
m = mandibles
ms = melanized stalk
pc = posterior commissure
n = neck (also stalk)
p = plug in lumen of stalk
rp = "respiratory plug" of Silvestri
rs = remnant of neck
s = portion extending exterior to host
sh = persistent shell of parasite egg
sp = spiracles
spr = spiraclar region
tra = tracheae
w = thickened wall
y = yolk