Biology and morphology of immature stages of *Centrodora scolypopae* (Hymenoptera: Aphelinidae)

P. J. GERARD
Department of Biological Sciences, University of Waikato, Private Bag, Hamilton, New Zealand.*

ABSTRACT

The egg, larval instars and pupae of *Centrodora scolypopae* (Valentine) are described. All 3 instars show a reduction of morphological differentation when compared to other Aphelinidae. The 1st instar retains the egg chorion and lacks visible mouthparts, segmentation and tracheal system. The 3rd instar has 4 pairs of spiracles only.

Keywords: *Centrodora scolypopae*, morphology, immature stages, egg, larvae, pupa.

INTRODUCTION

Most aphelinids are primary parasites of the sternorrhynchous Homoptera (Aphidoidea, Aleyrodoidea & Coccoidea) and much is known about the biology of the genera *Coccophagus, Encarsia* and *Aphytis* which are of considerable economic importance for biological control (Viggiani 1984). The genus *Centrodora* differs from most other aphelinid genera in that its members are specialised egg parasites. Apart from *C. scolypopae* and *C. coocata*, which is a parasite of a cicadellid forestry pest of wattle trees (Annecke & Insley 1972), the genus has not been of much importance for biological control and consequently little is known of the biology and development of the immature stages.

The genus *Centrodora* consists of 29 known species (Hayat 1974, 1981). They are minute, yellow or pale coloured parasites. Definite host records are known for 16 species only (Hayat 1974). All are parasitic on eggs (often in woody tissue) of orthopterous and homopterous insects, with the exception of *C. speciosissima* which parasitises pupae of dipterous, chalcid and proctotrupid insects.

Centrodora scolypopae was first found in *Scolypopa australis* (Homoptera: Ricaniidae) eggs in the Bay of Islands, New Zealand in 1962 (Cumber 1966). It was identified as a new species and the adult external morphology described by Valentine (1966). Cumber (1966) observed several biological features of *C. scolypopae*. Host eggs nourished 1-4 individuals although 1 of each sex was most common. The sex ratio at emergence favoured females, 2:1. Parasitised eggs were readily distinguished by a general darkening, a transverse dark band and a dark spot at the point of ovipositor insertion. A period of arrested development was observed that synchronised adult emergence with the new season's passion-vine hopper eggs.

This paper describes the main biological and morphological features of the egg, larval instars and pupa of *C. scolypopae*.

MATERIALS AND METHOD

Centrodora scolypopae parasitises overwintering *S. australis* eggs that are laid beneath the surface of woody twigs and stems of a wide range of plant hosts. Parasite adults were readily obtained throughout most of the year by rearing them from plant material bearing numerous host eggs collected from a gully near Hamilton. The most convenient method was to place the parasitised oviposition material in "growth" jars at a temperature of 20-25°C with 16 hours of light per day. Each jar consisted of a 1000 ml "Agee" jar containing water in the base with the oviposition material held above on a gauze mat

* Present address: Ruakura Agricultural Research Centre, Ministry of Agriculture and Fisheries, Private Bag, Hamilton, New Zealand.

supported by plastic pipe (see Fig. 1). The jars were closed with an "Agee" seal and ring. Each seal had a hole punched in the centre in which a rubber bung was fitted. Eggs were inspected at weekly intervals to determine the approximate time of initiation of pupation. When adults were about to emerge, the solid bung was replaced with a hollow one pushed up through from the inside. A test-tube could then be fitted to the bung in an inverted upright position (Fig. 1). The jar was placed in a box and covered so only the test tube protruded and was exposed to light. *Centrodora* adults show strong positive phototaxis and negative geotaxis and so they collected readily in the test tube end. When test tubes contained adults, they were removed from the growth jars daily and replaced with fresh tubes.

Centrodora scolypopae females mated and oviposited readily when in test tubes. Fresh plant material containing *Scolypopa* eggs was collected from a home garden in Hamilton where *C. scolypopae* numbers were low. Raspberry was the main host plant used because almost no parasitism was found on it naturally, possibly because annual pruning prevented any carryover from previous generations. Also host eggs were relatively free from disease and mites, so parasites could be reared with few losses.

Because *Centrodora* adults usually died within 24 hours when not fed, a fine streak of honey was usually placed on each test tube along with 1-2 host egg batches per female before the tubes were closed with a bung. Oviposition usually commenced within minutes and the host material was left with the adults till the following day when it was replaced with fresh material. Most oviposition occurred in the first 24 hr.

The parasitised host material was then put into uncovered labelled glass vials, placed in a "growth" jar (Fig. 1) and incubated at 20 or 25°C. Desiccation was thus avoided although fungi, enhanced by the high humidity, were a minor problem. The above procedure was repeated many times so that at the inspection times, a range of parasites of known age were available.

The developmental stages of *C. scolypopae* were ascertained with the use of a stereo microscope by dissecting out the parasitised eggs from the woody oviposition material. The parasitised eggs were immersed in 70% ethanol which cleared the host egg chorion and made the parasite easily visible in most cases. Closer examination of the parasite was obtained by dissecting the host egg in insect Ringer's solution. Live material was often mounted in Ringer's solution for study under higher magnification. Permanent slide

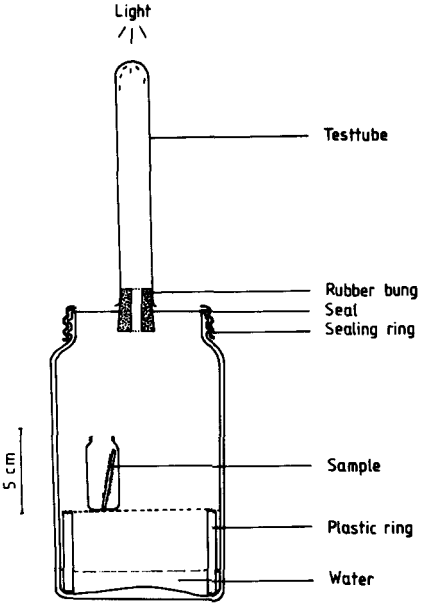


Fig. 1: Apparatus used in the rearing and collection of *C. scolypopae*.

mounts were prepared by putting eggs and first instar larvae directly into a drop of Hoyer's medium on a glass slide and covering with a coverslip. Larger larvae were punctured with an entomological pin first to rid them of the opaque gut content.

DESCRIPTION OF IMMATURE STAGES

Eggs

The deposited *C. scolypopae* egg is about 270μ long and 50μ wide. It has a transparent, unsculptured chorion and tapers towards the cephalic end forming a stalk, separated from the egg proper by a distinct border (Fig. 2a). Incubation takes 9 days at 20°C and there is no change in size during this time. The yolk of the host egg becomes clear during incubation of the parasite egg, which sinks to the lowest portion of the host egg, indicating the breakdown of yolk structure by parasite enzymes. It is possible that these come from the parasite female during oviposition as the clearing still occurs when parasite eggs fail to hatch (e.g. at 10°C) and in the very rare host egg bearing a parasite oviposition scar but containing no parasite.

Larvae

Centrodora scolypopae has 3 larval instars, as is apparently the case in all aphelinid genera (Nikol'skaya & Jasnosh 1966). The 3 instars differ in shape of the mandible and in amount of morphological differentation.

i. *First instar*

The newly hatched larvae is ovoid, measuring 150-175μ in length. No segmentation or mouthparts are visible. The gut is visible as a light yellow barrel shape in the centre of the larva. The anterior of the larva remains within the egg chorion (Fig. 2b). No tracheal system or spiracles were observed.

ii. *Second instar*

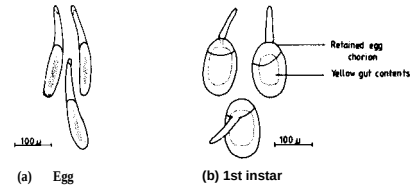
The second instar, measuring $266 \pm 10\mu$ in length (S.E. = 10, n = 10), shows slight differentation. The barely discernable cephalic skeleton has transparent triangular mandibles (Fig. 3). The thoracic and abdominal segments are faintly visible. No tracheal system or spiracles were observed. The 1st instar exuviae and egg chorion remain loosely attached to the last segments (Fig. 2c). Male and female larvae within a single host egg can be distinguished by their size, the male being smaller than the female.

iii. *Third instar*

This is the last instar. It has visible segmentation, with the head and 13 body segments clearly demarcated. The gut is opaque white and occupies most of the body cavity. Initially the body segments taper sharply but the larvae quickly become oval in shape when viewed dorsally. The ventral surface is flat or slightly concave, the dorsal surface convex. Maximum size of the female is determined by the *S. australis* egg dimensions ($845 \pm 5\mu$ long $400 \pm 4\mu$ wide, n = 50). Males average $385 \pm 35\mu$ long and $181 \pm 31\mu$ wide (n = 10).

The cephalic skeleton is typical of the chalcidoids with little differentation, the mandibles being the main discernible parts. The epistomal, pleurostomal, hypostomal and tentorial sclerites form a continuous ring. The mandibles are smooth, tapering and gently curved (Fig. 3). The pharynx opens between the mandibles. The 1-segmented antennae are disc-like structures in the middle of circular antennal sockets. There are 4 circular structures, with a possible sensory function, and 2 cuticular protruberences on either side of the mandibles (Plate 1).

Pairs of spiracles are found on the mesothoracic and 2nd, 3rd, and 7th abdominal segments (Fig. 4). The internal respiratory system is simple, consisting of the anterior transverse commissure, and a loop formed by the posterior transverse commissure and 2 longitudinal trunks. From the latter were 5 pairs of lateral branches, 4 to the spiracles and a pair of incomplete branches in the 6th abdominal segment (Fig. 4).



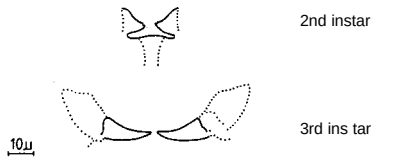


Fig. 3: Mandibles of *C. scolypopae* larvae.

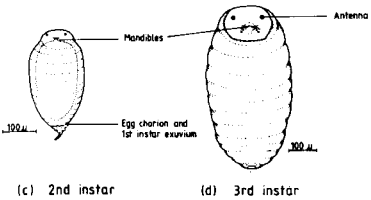


Fig. 2: Egg and larval stages of *C. scolypopae.*

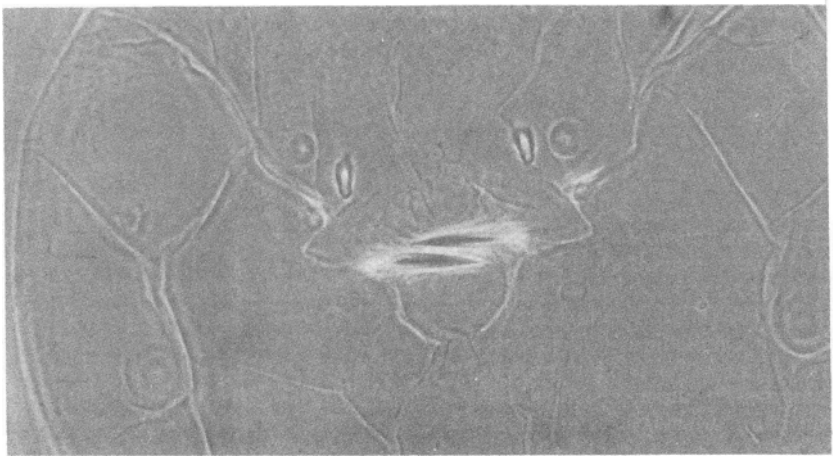


Plate 1: Antennae and mouthparts of 3rd instar larva (Differential interference contrast. mag 1250x).

IV. *Pre pupae*

The prepupal stage of the 3rd instar can be divided into 2 phases similar to those described by Hagen (1964). The eonymphal phase begins when the 3rd instar ceases to feed. The male, if present, assumes a position at the base, furthest away from the micropyle. A dark grey bar, visible from the outside, appears at the ventral surface. This is in a double layered membrane, which is part of the *Scolypopa* egg as the puncture made by the C. *scolypopae* female is easily visible in it, often with grey pigmentation surrounding it also. The appearance of the bar is also accompanied with an increase in host egg size. Host eggs containing mature parasite larvae average 845 ± 5µ (n = 50) while barred eggs average 933 ± 6µ (n = 82) in length. The larval cuticle becomes more transparent and the gut shrinks as the content becomes more dense. The larvae overwinter in this stage which lasts 8-9 months in the Hamilton area. However at a constant temperature of 20°C or above and with a long day light regime, (ie in the absence of diapause), this phase takes about 7 days.

The 2nd or pronymphal phase of the prepupal stage is marked by the larvae assuming a position with the head closest to the host egg micropyle (and surface) and the appearance of faecal material, in the form of 4-6 orange meconial pellets which lie neatly alongside the abdomen. Differentiation of the pupal structures is rapid.

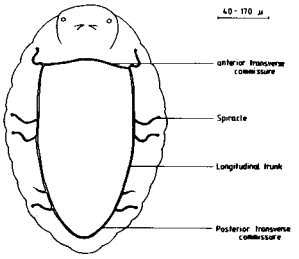


Fig. 4: Respiratory system of 3rd instar *C. scolypopae* larvae.

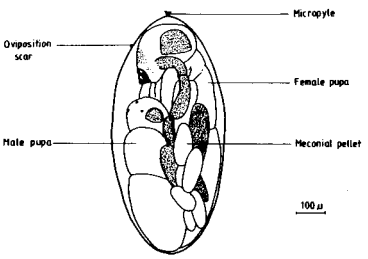


Fig. 5: Male and female *C. scolypopae* pupae within host egg.

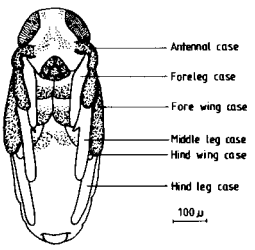


Fig. 6: Female *C. Scolypopae* pupa, ventral view.

Pupa

The pupa is exarate. The female averages 858 ± 73μ long and the male 579 ± 19μ. The female lies on the ventral side of the host egg with her head near the micropyle facing upwards. The male, if present, lies facing the female at the base of the egg (Fig. 5). The ventral aspect of the pupa is depicted in Fig. 6. Antennal cases are present on each side of the head. Below these are the mouthpart cases; the labrum is absent, the mandibles triangular, the pupal maxillae have 2 segments and the labium has 3. The prosternum and mesosternum are large bilobed plates but the metasternum is formed by 2 separate triangular plates. The forelegs are above and adjacent to the antennal cases posterior to which are the cases of the mid and hind legs. A triangular process on the mid leg cases marks the site of the prospective mid-tibial spur. The forewing cases and tip of the hind wing cases can be seen to the side between the mid and hind leg case. The antennal cases, mouthparts and leg and wing cases become pigmented a dark grey. The eyes change from colourless to grey, then to dark red.

Eclosion occurs within the host egg. The pupal integument splits and moves to the lateral sides of the parasite. The adults do not emerge immediately but remain within the host egg for several days. The female then chews around the uppermost portion of the egg and pushes through the frass formed by the host when ovipositing. The male, if present, follows through the same exist hole.

DISCUSSION

The deposited *C. scolypopae* egg is identical in shape and size to the ovarian egg. This is unlike many Encyrtidae and some Aphelinidae, such as *Aphytis chilensis*, which have a double-bodied ovarian egg and a deposited egg with a narrow stalk (Rosen & Eliraz 1978).

Stalked eggs are common in the parasitic Hymenoptera. In the Aphelinidae they occur in the genera *Aphytis, Marietta* and *Centrodora*, which are closely related, and also in *Aspidiotiphagus* (Hagen 1964). They are often associated with Hymenoptera with long ovipositors, the extension permitting the egg to be compressed and stretched while passing down the ovipositor (Hagen 1964).

The lack of morphological structures in the first instar of *C. scolypopae* is marked when compared to other aphelinids, such as *Aphytis chilensis* (Howard), which has 4 pairs of spiracles and a readily visible cephalic skeleton (Rosen & Eliraz 1978). The most noticeable feature, the retention of the egg chorion, also occurs in the Encyrtidae where it is thought to play a role in respiration (Maple 1947). Families that are predominantly egg parasites (Mymaridae, Trichogrammitidae and Scelonidae) have 1st instar larvae that lack spiracles (i.e. are apneustic) and the mymarid and trichogrammatid larvae (the latter being closely related to Aphelinidae) never develop a tracheal system (Fisher 1971). Mature chalcidoidea larvae usually have 9 open spiracles. The lack of morphological differentiation particularly in the 1st instar, the absence of spiracles in the first 2 instars, and the reduction in the number of spiracles and simplification of the associated respiratory system in the mature *C. scolypopae* larvae all indicate a specialised adaptation to existence as an egg parasite.

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