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The first instar larva of *Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Chalcidoidea: Pteromalidae) with a pair of appendages on the labrum and closed spiracles

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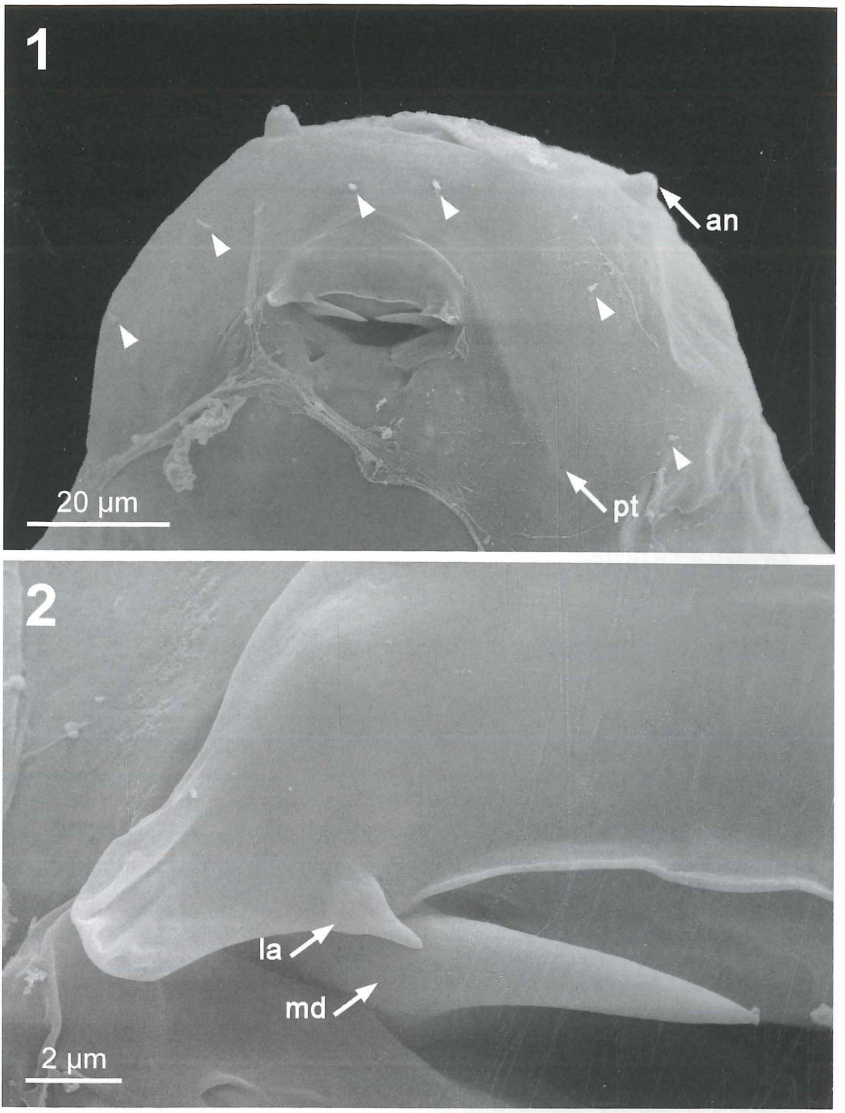
(With 5 figures)

Abstract

There are only a few investigations into the morphology of chalcidoid larvae. Papers on the morphology of the larvae of Pteromalidae most often discuss structures visible with a light microscope or external structures on the surface of last instar larvae shown on SEM photographs. Morphological studies on the four larval stages of the ectoparasitic wasp *Nasonia vitripennis* (Walker, 1836) revealed structures which were unknown until now: the first instar larva shows a pair of appendages on the labrum and we have evidence that the four pairs of spiracles are closed.

Introduction

There are few papers on the larval morphology of Chalcidoidea because of the small size and the most often unknown biology of the species. A first full-scale investigation into different stages of the larvae was published by Parker (1924) also referring to nine species of Pteromalidae. Additional descriptions of larvae of other pteromalids were published by Richardson (1913), Kearns (1931), Kashef (1954), Cutler (1955), Huiza *et al.* (1985) and by Thomazini *et al.* (2000). In this paper we describe morphological peculiarities of the first instar larva of *Nasonia vitripennis* (Walker, 1836).



Figs 1-2. *Nasonia vitripennis* (Walker), head of first instar larva: **1** - antenna (*an*); position of posterior tentorial pit, *pt* (sensillae are indicated by arrowheads); **2** - labral appendage (*la*), mandible (*md*).

Material and methods

The parasitoid *Nasonia vitripennis* (Walker, 1836) was reared in the laboratory on pupae of *Calliphora vomitoria* (Linnaeus, 1758) (Diptera). The external structures of the head and spiracles were examined with the scanning electron microscope

(SEM) LEO 1525. Larvae were fixed using Bouin's method, dehydrated with acetone, fixed again in amyl acetate and dried using the critical point method. The objects were sputtered with gold and the pictures taken by SEM. The tracheal system was studied by light microscope, using specimens prepared after Janzon (1984).

Results

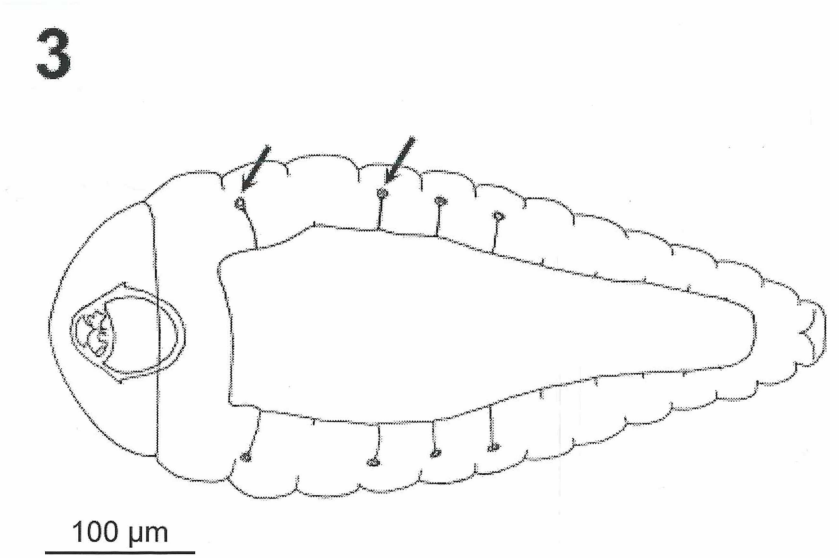
The first instar larva of *N. vitripennis* has a weakly sclerotized head capsule with antennae, six short sensillae and posterior tentorial pits (Fig. 1). In the mouth region, the sickle-shaped mandibles and wartlike, shortened palpae of the maxillae and labium can be seen. Besides these structures, the first instar larva has a pair of acute appendages on the labrum (Fig. 2). The three following larval instars do not have these labral appendages.

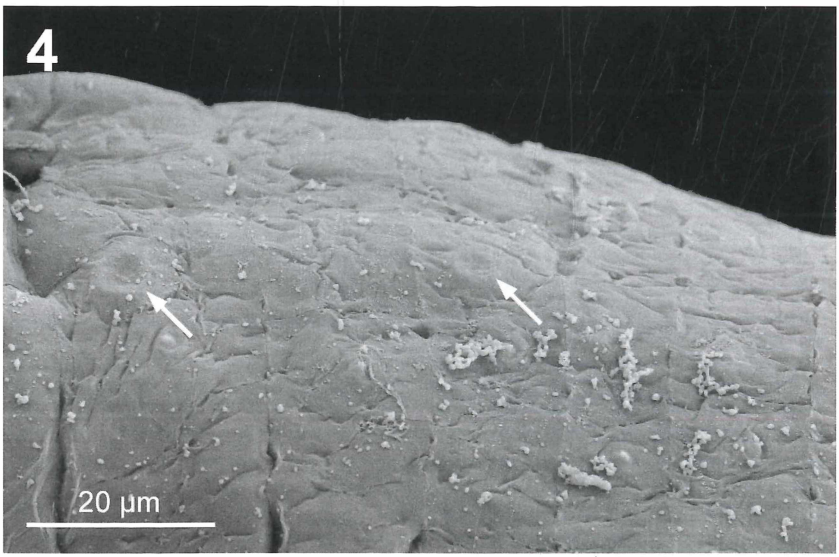
The tracheal system of the first instar larva consists of two lateral trunks which are connected by a commissure in body segments I and XI (Fig. 3). In segments II-XI lateral branches are attached to the trunks. The branches in segments III and VII-XI are short and do not reach the integument. Under the light microscope specimens appear to have normal spiracles at the branches in segments II and IV-VI. However no spiracles on the integument of the first instar larva are present in SEM photographs. Only in some specimens which have lost the normal turgor during preparation, the place and form of the spiracles are indicated by very shallow impressions (Fig. 4). The second instar larva has ten pairs of open spiracles in body segments II-XI. The four spiracles on segments II and IV-VI which were closed in the first instar larva are larger than the newly developed spiracles (Fig. 5). All spiracles except the last pair on segment XI which remains small, have a similar size in the following two larval instars.

Discussion

The pair of labral appendages of the first instar larva of *N. vitripennis* has not been described previously, and is also unknown for other species of Chalcidoidea. Papers on the larval morphology of chalcids mostly examine later stages, while first instar larvae are much less investigated. SEM pictures of pteromalid first instar larvae have been published more recently, but reveal no details in the region of the oral aperture (Rodríguez-Leyva *et al.* 2000; Thomazini *et al.* 2000).

The labral appendages cannot be assigned to structures on the head of hymenopteran larvae that were previously described. We find it necessary to refer to these structures because till now it was not possible to find a pair of appendages on the insect labrum which could be homologous to extremities. The labral appendages are possibly sensory organs of the first instar larva of *N. vitripennis*. The apocritan first instar larva can sometimes be distinguished from the successive larval instars by morphological modifications and a particular mode of life. The planidium of Perilampidae, Eucharitidae and other parasitoids is highly modified as an adaptation to the necessity of locating its host (Tripp 1961, Heraty and Darling 1984, Darling 1999). Mobility is an advantage for the first instar larvae of solitary parasitoids because it enables them to find and kill com-





Figs 3-4. *Nasonia vitripennis* (Walker), first instar larva: **3** - tracheal system; **4** - closed spiracles of body segments II and IV (the corresponding spiracles are indicated by arrows in both figures).

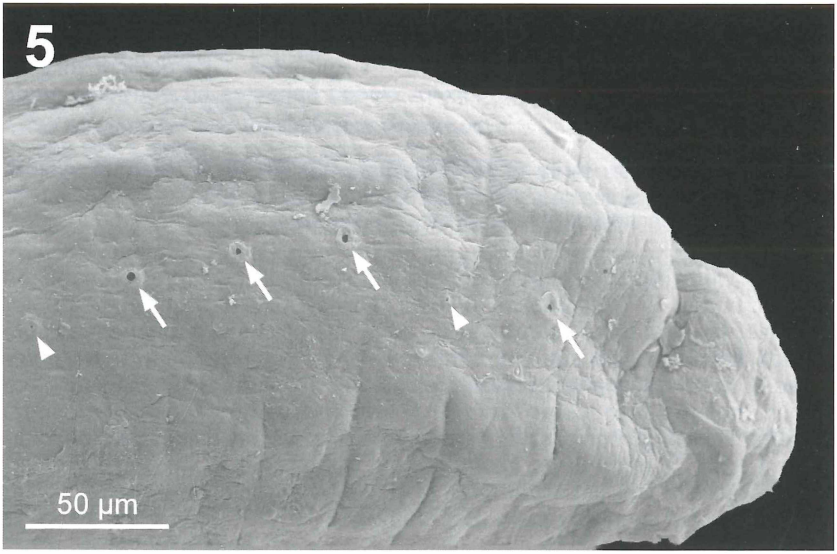


Fig. 5. *Nasonia vitripennis* (Walker), second instar larva: head and the first seven body segments, lateral view (large spiracles are indicated by arrows, small spiracles by arrowheads).

peting larvae on the host. We observed that first instar larvae of the gregarious species *N. vitripennis* were more active on the host than later instars who remain almost immobile. The development of additional sensory organs in the first instar larva may be associated with the higher mobility.

The tracheal system of chalcidoid larvae was described early in the last century (Richardson 1913, Haviland 1922, Parker 1924). It seemed certain that the tracheal system of the ectoparasitic first instar larvae of the chalcidoid families Leucospidae, Eurytomidae, Pteromalidae, Torymidae, Eupelmidae, Aphelinidae, Eulophidae and Elasmidae have four pairs of open spiracles in body segments II and IV-VI (Parker and Thompson 1925, Rosen & Eliraz 1978). Under the light microscope, the tracheal system of the first instar larva of *N. vitripennis* seems to correspond to these bibliographical references. The spiracles can be found in the 2nd thoracic segment and in the first three abdominal segments (Fig. 3).

Our SEM examinations revealed no spiracles on the surface of the cuticle, in contrast to those discussed above. The specimens, whose bodies collapsed after fixation and critical point drying, show clearly that the spiracles of the first instar larvae are closed. In these preparations the closed spiracles become apparent underneath the cuticle (Fig. 4). Also in In the SEM picture of the first instar larva of *Catolaccus hunteri* (Crawford, 1908) (Pteromalidae) no spiracles can be detected (see Rodríguez-Leyva 2000). It has to be checked if the tracheal system is actually closed in all species which have been examined in former investigations. This con-

cerns all ectoparasitic first instar larvae of the quoted families of Chalcidoidea. Heraty & Darling (1984) describe a ground plan of the chalcidoid larva and assume four pairs of functional spiracles. Character 9 in their paper probably has to be changed to "closed spiracles on body segments II and IV-VI".

The second instar larva of *N. vitripennis* has an open tracheal system with 10 pairs of spiracles. The four pairs of spiracles which were predeposited in the first instar larva are particularly large (Fig. 5). The remaining spiracles become enlarged in the later instars. Closed tracheal systems were described for endoparasitic larvae living in the hemolymph of their hosts. In *Stenomalina micans* (Olivier, 1813) (Pteromalidae) for example, the first three endoparasitic larval instars have a tracheal system with all spiracles missing, while the following two larval instars are ectoparasitic and have an open tracheal system (Kearns 1931). We have shown that the first instar larva of *N. vitripennis* has a closed tracheal system. This is in contrast to its ectoparasitic life style, but can be connected with its embryonic development. While still in the egg, the tracheal system of the first instar larva of *N. vitripennis* is filled with gas, which is produced by secretory cells (Bull 1982).

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Zusammenfassung

Die Frühstadien der Chalcidoidea sind morphologisch äußerst unzureichend untersucht. Dies gilt auch für die Pteromalidae, bei denen in morphologischen Arbeiten zur Larvalentwicklung entweder nur lichtmikroskopisch erkennbare Strukturen beschrieben werden oder mit REM-Bildern die äußeren Strukturen von Altlarven dargestellt werden. Die morphologische Untersuchung der vier Larvenstadien der ectoparasitischen Erzwespe *Nasonia vitripennis* (Walker, 1836) erbrachte bisher unbekannte morphologische Strukturen: Die Erstlarve besitzt paarige Anhänge am Labrum, und wir konnten nachweisen, dass die vier Stigmenpaare des Tracheensystems verschlossen sind.

References

Bull, A. L., 1982: Stages of living embryos in the jewel wasp *Mormoniella* (*Nasonia*) *vitripennis* (Walker) (Hymenoptera: Pteromalidae). - Int. J. Insect Morphol. Embryol., **11** (1): 1-23. Elmsford.

Cutler, J. R., 1955: The morphology of the head of the final instar larva of *Nasonia vitripennis* Walker (Hymenoptera: Chalcidoidea). - Proc. R. Entomol. Soc. Lond., (A), **30** (4-6): 73-81. London.

Darling, D. C., 1999: Life history and immature stages of *Steffanolampus salicetum* (Hymenoptera: Chalcidoidea: Perilampidae). - Proc. Entomol. Soc. Ont., **130**: 3-14. Sault Ste. Marie.

Haviland, M. D., 1922: On the post-embryonic development of certain chalcids, hyperparasites of aphides, with remarks on the bionomics of hymenopterous parasites in general. - Quart. J. Microsc. Sci., **66** (2): 321-338. London.

Heraty, J. M. & Darling, D. C., 1984: Comparative morphology of the planidial larvae of Eucharitidae and Perilampidae (Hymenoptera: Chalcidoidea). - Syst. Ent., **9**: 309-328. London.

Huiza, I. R., Sánchez, U. & Palacios, M., 1985: Biología y comportamiento de *Dibrachys cavus* (Hym.: Pteromalidae) en el Perú. - Revista Peruana Entomol., **28**: 13-17. Lima.

Janzon, L.-Å., 1984: *Tephritis conura* (Loew) (Diptera: Tephritidae) and its parasitoids (Hymenoptera: Pteromalidae, Eurytomidae, Eulophidae) in Sweden. - Ent. Scand., **15**: 411-418. Metala.

Kashef, A., 1954: Morphologie et anatomie de la larve de *Lariophagus distinguendus* Först. (Hym. Pteromalidae). - Bull. Soc. Entomol. Fr., **59**: 93-96. Paris.

Kearns, H. G. H., 1931: The larval and pupal anatomy of *Stenomalus micans* Ol. (Pteromalidae), a chalcid endoparasite of the gout-fly of barley (*Chlorops taeniopus* Meig.), with some details of the life history of the summer generation. - Parasitology, **23** (3): 380-395. Cambridge.

Parker, H. L., 1924: Recherches sur les formes post-embryonnaires des Chalcidiens. - Ann. Soc. Entomol. Fr., **93**: 261-379. Paris.

Parker, H. L. and Thompson, W. R., 1925: Notes on the larvae of the Chalcidoidea. - Ann. Entom. Soc. Am., **18** (3): 384-395. College Park, Md.

Richardson, C. H., 1913: Studies on the habits and development of a hymenopterous parasite, *Spalangia muscidarum* Richardson. - J. Morphol., **24**: 513-557. Philadelphia.

Rodríguez-Leyva, E., Leyva, J. L., Gómez, V., Bárcenas, N. M. & Elzen, G. W., 2000: Biology of *Catolaccus hunteri* (Hymenoptera: Pteromalidae), a parasitoid of pepper weevil and boll weevil (Coleoptera: Curculionidae). - Ann. Entom. Soc. Am., **93** (4): 862-868. College Park, Md.

Rosen, D. & Eliraz, A., 1978: Biological and systematic studies of developmental stages in *Aphytis* (Hymenoptera: Aphelinidae) I. Developmental history of *Aphytis chilensis* Howard. - Hilgardia, **46** (3): 77-95. Berkeley.

Thomazini, M. J., Lino Neto, J., Costa, V. A. & Berti Filho, E., 2000: Caraçterizacão morfológica das fases imaturas e tempo de desenvolvimento de *Muscidifurax uniraptor* Kogan & Legner (Hymenoptera: Pteromalidae). - An. Soc. Entomol. Bras., **29** (4): 691-696. Itabuna.

Tripp, H. A., 1961: The biology of a hyperparasite, *Euceros frigidus* Cress. (Ichneumonidae) and description of the planidial stage. - Can. Ent., **93**: 40-58. Ottawa.

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