

SEM studies on the copulatory apparatus of male *Oesophagostomum columbianum*

C. L. DUGGAL¹, H. KAUR²

¹Department of Zoology, Panjab University, Chandigarh-160014, India; ²Department of Zoology, Punjabi University, Patiala-147002, India

Summary

SEM studies on the cloacal opening of *Oesophagostomum columbianum* indicate that it is located on a raised conical structure called the “genital cone”. The genital cone is provided with a ventral lip and a pair of subdorsal genital appendages. The ventral lip is a triangular structure having a single papilla on it and the genital appendages are covered with wrinkled cuticle with a nerve process projecting to the exterior in center. The bursa is supported by muscular rays which end up as knob-like sessile genital papillae. The inner surface of the bursa is porous. Spicules are two, equal, each provided with an ala which decreases in height distally and end up much prior to the spicular tip.

Key words: nematode; copulation; bursa; spicule; strongylid

Introduction

The structure of copulatory organs has been examined closely in many species of nematodes. The various parts of the copulatory apparatus help in the sex attraction, location and copulation. The sex attraction, predominantly to the female by the male is a conspicuous feature of the nematode mating process (Green, 1980). There are many investigations which relate to the localization of the secretory cells and identification of the chemoreceptors involved, such as in *Aphelenchoides blastophthorus* (Clark & Shepherd, 1977), *Paratylenchus penetrans* (Wen & Chen, 1976) and *Heterodera* spp. (Clark *et al.*, 1973), *Panarellus redivivus* (Duggal, 1978), *Capillaria hepatica* & *Trichuris muris* (Wright, 1978) and *Ascaridia galli* (Duggal & Kaur, 2000).

This paper deals with the scanning electron microscopical studies on the copulatory bursa, genital cone and spicules of *Oesophagostomum columbianum*, which otherwise were not possible to see with light microscopy.

Material and Methods

Adult male specimens of *Oesophagostomum columbianum* were collected from the large intestine of sheep, *Ovis aries*

in 0.89 % saline solution. Specimens were immediately fixed in 2.5 % glutaraldehyde in 0.1M cacodylate buffer pH 7.4 for 4 hrs at 4°C (Keilley *et al.*, 1973); post-fixed in 1 % OsO₄ for 3 – 4 hrs at 4°C. After washing in buffer for 5 – 10 times, specimens were incubated in fresh 1 % saturated filtered thiocarbonylhydrazide (TCH) for 10 min at 25°C. After washing with distilled water 5 – 10 times, specimens were again incubated in 1 % OsO₄ for 30 min and washed in distilled water 5 – 10 times, dehydrated through acetone series to amyl acetate, critical point dried and mounted on stubs, coated with gold and scanned under JEM 1200 Ex JEOL make electron microscope.

Results

The male copulatory apparatus in *Oesophagostomum columbianum* consists of copulatory bursa, spicules and a genital cone (Fig.1). The copulatory bursa has three lobes, one dorsal, two lateral and is supported by eight pairs of bursal rays, 3 pairs in the dorsal lobe and five pairs in the lateral lobes. Out of these, five pairs of bursal rays end on the inner side of bursal margin and the other three pairs end up submarginally on the inner surface of the bursa. SEM studies indicate that the inner margin of the bursa is provided with longitudinal ridges whereas its central part is porous (Fig.4). The spicules are two, equal, measuring 650 – 790 µm in length and are slightly ventrally curved on eversion. Spicules are blunt at their tips and are provided with alae. There is a single ala on each spicule which decreases in height distally and ends prior to the spicular tip. No pore could be seen at the tip (Figs. 2, 3).

SEM studies on the cloacal opening of *O. columbianum* indicate that it is located on a raised, somewhat conical, structure called the “genital cone” (Fig. 5). The genital cone is provided with a ventral lip and a pair of subdorsal genital appendages. The ventral lip is a triangular structure having a single apical papilla on it (Fig. 5). The genital appendages are large, with their surface covered by wrinkled cuticle with a nerve process projecting out of a pore in its center (Fig.6).

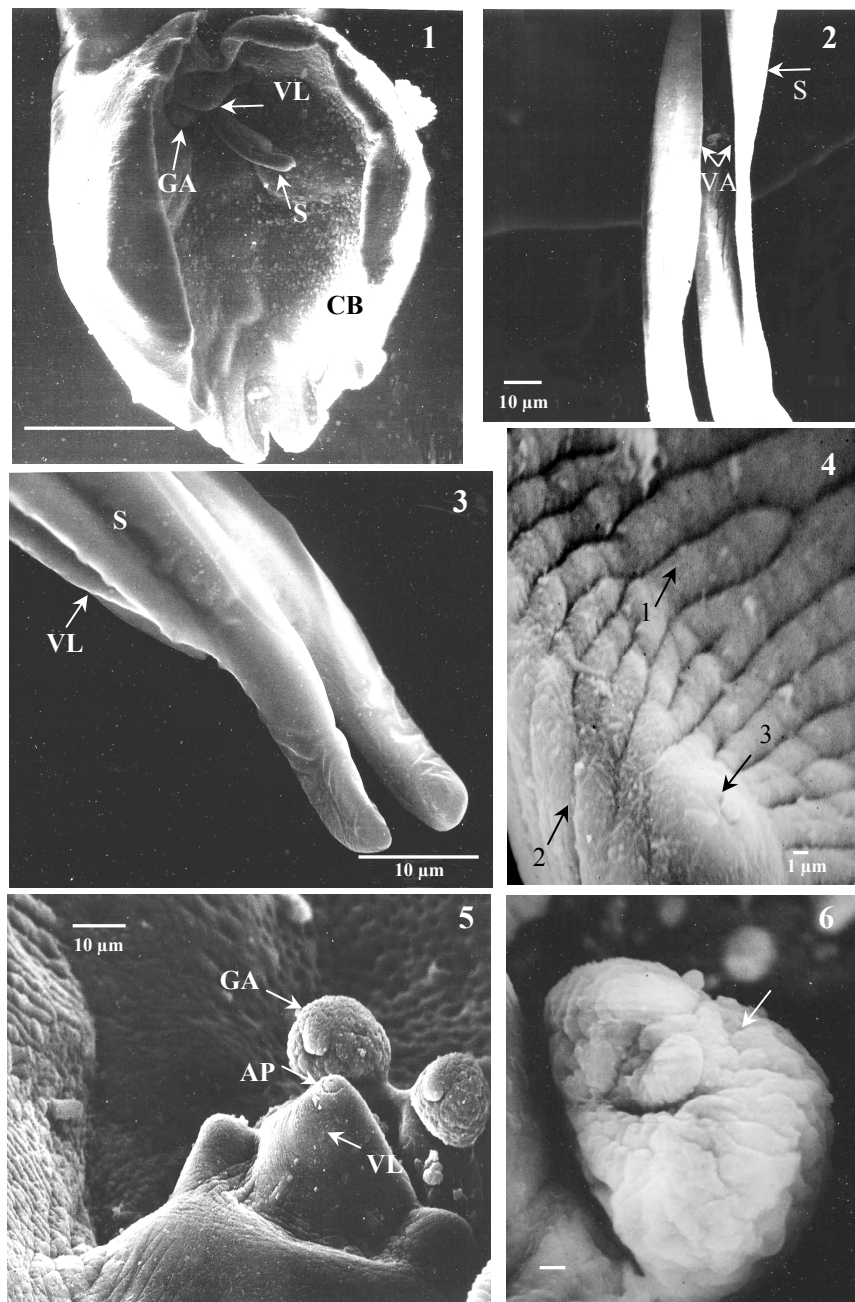


Fig. 1. Copulaotry bursa (CB) of *O. columbianum* showing protruding spicules (S), ventral lip (VL) and genital appendages (GA); Fig. 2. Spicules (S) of *O. columbianum* (ventral view) each showing its single ventral ala (VA); Fig. 3. Spicules (S) of *O. columbianum* (lateral view) each showing its tip and ventral ala (VA); Fig. 4. Inner surface of the copulatory bursa of *O. columbianum* showing central porous area (1) and lateral wrinkled area (2) along with genital papillae (3); Fig. 5. Genital cone of *O. columbianum* showing a triangular ventral lip (VL) with a single apical papilla (AP) and a pair of sub-dorsal genital appendages (GA); Fig. 6. Sub-dorsal genital appendage (3) of *O. columbianum* (magnified) showing its wrinkled surface

Discussion

The male genital cone is a peculiar structure in strongylids. In *O. columbianum* it is provided with a ventral lip and a pair of dorsolateral genital appendages having a nerve fibre projecting out of a pore on its surface. This suggests a chemosensory function of these organs in addition to possible mechanosensory one, as also suggested by McLaren, 1976;

Dick and Wright, 1974; Clark and Shepherd, 1977; Wagner and Seitz, 1982 in other nematodes. Such possible functions could also be assigned to the pores and papillae on the bursa and its marginal surface. In fact Marchant (1970) has observed 'flaring' of bursa in the presence of females. It is also suggested that the genital cone may be

partly pushed into the female genital pore to facilitate the safe passage of the spermatozoa (Kaur, 1994). However, the muscular rays on the bursa may be helpful in holding the female during copulation.

O. columbianum has two equal spicules, each with a single ala. Such spicules along with their alae form a channel through which the spermatozoa may enter the female reproductive tract thus acting as true intermittent organs as in *Heterodera* spp (Clark *et al.*, 1973), *Hoplolaimus galeatus* (Hogger & Bird, 1974), *Nippostrongylus brasiliensis* (McLaren, 1976); *Pratylenchus penetrans* (Wen & Chen, 1976) and *Panagrellus redivivus* (Duggal, 1978).

Acknowledgements

Financial help rendered by Council of Scientific and Industrial Research (CSIR), New Delhi is duly acknowledged.

Reference

- CLARK, S. A., SHEPHERD, A. M. (1977): Structure of the spicules and caudal sensory equipment in the male of *Ap-helenchoides blastophthorus* (Nematoda: Tylenchida, Ap-helenchina). *Nematologica*, 23: 103 – 111
- CLARK, S. A., SHEPHERD, A. H., KEMPTON, A. (1973): Spicule structure in some *Heterodera* spp. *Nematologica*, 19: 242 – 247
- DICK, T. A., WRIGHT, K. A. (1974): The ultrastructure of the cuticle of the nematode *Syphacia obvelata* (Rudolphi, 1802). III. Cuticle associated with the male reproductive structures. *Can. J. Zool.*, 52: 245 – 256
- DUGGAL, C. L. (1978): Copulatory behaviour of male *Panagrellus redivivus*. *Nematologica*, 24: 257 – 268
- DUGGAL, C. L., KAUR H. (2000): On the functional morphology of the copulatory apparatus of *Ascaridia galli*. *J. Punjab Acad. Sci.*, 2: 161 – 163
- GREEN, C. D. (1980): Nematode sex attractants. *Helminthological Abstracts, Series B*, 49: 327 – 339
- HOGGER, C. H., BIRD, G. W. (1974): Secondary male sex characteristics of *Hoplolaimus galeatus*. *J. Nematol.*, 6: 12 – 16
- KAUR, H. (1994): Studies on the functional morphology, histology and histochemistry of male and female reproductive tracts of some parasitic nematodes. Ph.D. thesis, Dept. of Zoology, Panjab University, Chandigarh
- KEILLEY, R. O., DEKKER, R. A., BLUEMINK, J. G. (1973): Ligand mediated osmium binding: its application in coating biological specimens for SEM. *J. Ultrastruct. Res.*, 45: 254 – 258
- MARCHANT, H. J. (1970). Bursal response in sexually stimulated *Nematospiroides dubius* (Nematoda): *J. Parasit.*, 56: 201 – 202
- MCLAREN, D. J. (1976): Nematode sense organs. In DAWES B. (Ed.): *Advances in parasitology*. 14, Academic press, London and New York, pp. 195 – 265
- WAGNER, G., SEITZ, K. A. (1982): (Structure and function of the male copulatory apparatus of *Pelodera strongyloides* (Nematoda, Rhabditidae): *Zoomorphology*, 97 (1/2): 121 – 132
- WEN, G.Y., CHEN, T. A. (1976): Ultrastructure of the spicules of *Pratylenchus penetrans*. *J. Nematol.*, 8: 69 – 74
- WRIGHT, K. A. (1978): Structure and function of the male copulatory apparatus of the nematodes *Capillaria hepatica* and *Trichuris muris*. *Can. J. Zool.*, 56: 651 – 662

RECEIVED JANUARY 7, 2002

ACCEPTED SEPTEMBER 19, 2005