REDIA, XCVI, 2013: 69-77

BEATRICE CARLETTI (*) - FRANCESCO PAOLI (*) - NUNZIO ISIDORO (**) - PIO FEDERICO ROVERSI (*)

ULTRASTRUCTURE OF THE ANTERIOR ALIMENTARY TRACT OF *BURSAPHELENCHUS MUCRONATUS* MAMIYA *ET* ENDA (NEMATODA APHELENCHOIDIDAE)

(*) *Consiglio per la ricerca e la sperimentazione in agricoltura - Research Centre for Agrobiology and Pedology, via Lanciola 12/A, 50125 Firenze, Italy;* e-mail: beatrice.carletti@entecra.it
(**) *Dipartimento di Scienze Agrarie, Alimentari ed Ambientali, Università Politecnica delle Marche, via Brecce Bianche 10 – 60131 Ancona*

Carletti B., Paoli F., Isidoro N., Roversi P.F. – Ultrastructure of the anterior alimentary tract of *Bursaphelenchus mucronatus* Mamiya *et* Enda (Nematoda Aphelenchoididae).

In view of the phytosanitary importance of nematodes of the genus *Bursaphelenchus* and the limited information available on their ultrastructure, we carried out a study of the buccal cavity and oesophageal area of *Bursaphelenchus mucronatus* Mamiya *et* Enda, 1979. Like the quarantine species *Bursaphelenchus xylophilus* (Steiner *et* Bührer) Nickle, its development takes place in the resin canals of conifers. The specimens used in our study were taken from a laboratory population collected on *Pinus pinaster* Aiton in meso-thermophilic mixed forests of central Italy (Tuscany) and grown on Petri dishes in a climatic chamber for 20 generations. The ultrastructural features of the mouth opening, anterior intestine, labial sensilla, amphidial canals, stylet and metacorpus were investigated with a TEM. The oesophageal glands are also described. The observed structures are discussed and compared with those described for other nematodes.

KEY WORDS: Aphelenchida, oesophageal gland, nematode ultrastructure, phytoparasitic nematode, nematode buccal cavity.

INTRODUCTION

Species of the genus *Bursaphelenchus* Fuchs and their insect vectors have received increasing attention in recent decades both in Asia and Europe. The epidemic spread of the quarantine species *Bursaphelenchus xylophilus* (Steiner *et* Bührer) Nickle after its accidental introduction into Portugal (MOTA *et al*., 1999) has had dramatic consequences for *Pinus pinaster* Aiton forests in that country (BRAASCH *et al*., 2000; TOMICZEK *et al*., 2003; RODRIGUES, 2008; SOLIMAN *et al*., 2012).

A research program on harmful phytoparasitic nematodes and their vectors has been conducted in Mediterranean forest habitats of Italy to acquire information on the morphology, biology, harmfulness and strategies for the control of indigenous species associated with the decline of host trees. As part of the program, we sampled populations of various *Bursaphelenchus* spp. infesting conifers and broad-leaved trees and set up laboratory colonies (CAROPPO *et al*., 2000; CARLETTI *et al*., 2006; CARLETTI, 2008; FRANCARDI *et al*., 2009; CARLETTI *et al*., 2011). This enabled us to carry out studies on the ultrastructure of these aphelenchoidids, for which only limited information was previously available (ZHANG and ZHANG, 2006). Thus far in fact, the studies on the ultrastructural features of nematodes concerned especially tylenchid phytoparasites. They dealt with the stomatal region of *Ditylenchus dipsaci* Kühn (YUEN, 1968), *Meloidogyne incognita* Kofoid *et* White and *Heterodera glycines* Ichinohe (BALDWIN & HIRSCHMANN, 1976), the stoma, stylet and procorpus of *Tylenchorhynchus dubius* (Bütschli) Filipjev (BYERS and ANDERSON, 1972; ANDERSON and BYERS, 1975), the oesophagus of males of *H. glycines* and *M. incognita* (BALDWIN *et al.,* 1977) and *Pratylenchus penetrans* (Cobb) Filipjev *et* Schuurmans Stekhoven (KISIEL *et al*., 1976). Other studies were conducted on the anterior alimentary tract of the tylenchid *Hexatylus viviparous* Goodey (SHEPHERD and CLARK, 1976) and of the aphelenchids *Aphelenchoides blastophthorus* Franklin (SHEPHERD *et al.,* 1980) and *Aphelenchus avenae* Bastian (RAGSDALE *et al.,* 2008; RAGSDALE *et al.,* 2009; RAGSDALE and BALDWIN, 2010; RAGSDALE *et al.,* 2011).

In the present study, we used a TEM to investigate the ultrastructure of the buccal cavity and oesophageal area of *Bursaphelenchus mucronatus* Mamiya *et* Enda, a species found on European conifers and recently used in place of *B. xylophilus* in research on lumber disinfection techniques in order to update the FAO ISPM-15 standards (EU Project EUPHRESCO “PEKID – Phytosanitary Efficacy of Kiln Drying”).

MATERIALS AND METHODS

Wood samples were collected from trees with obvious signs of decline, with wilting of vast portions of the crown, in pure and mixed *P. pinaster* forests located in the Monfalcone Nature Reserve (Pisa, central Italy). Wood chips were collected with a drill (bit size 10-12 mm) and transported in sealed bags to the Nematology Laboratory of the CRA-ABP in Florence. Live specimens of *B. mucronatus* were extracted with a Baermann funnel followed by the use of trays with a wool paper filter. The collected nematodes were then transferred onto Petri dishes prepared with malt extract agar with 5% of glycerol inoculated with *Botrytis cinerea* Pers. The dishes were stored in a climatic chamber at 9°C (Binder KBWF, Essex, England).

– Received 25 November 2013 Accepted 29 November 2013 Published 6 December 2013

The collected strain was identified by the abbreviation IT16 (w).

Male specimens obtained from the extraction of 10 Petri dishes were used to prepare the mounts and were immersed in 5% glutaraldehyde in 0.1 M phosphate buffer (PB) pH 7.2 to which 5% of sucrose was added. The specimens were then cut in two or more pieces and left overnight in the same fixative. After careful rinsing in PB, the material was post-fixed in 1% OsO4 for 1 h. After rinsing in PB, samples were dehydrated in a graded ethanol series and embedded in a mixture of Epon-Araldite resins. Thin sections obtained with the RCM Power Tome ultramicrotome were stained routinely with uranyl acetate and lead citrate and observed with a Jeol Jem 1011 electron microscope operating at 80 kV.

Abbreviations adopted in the text and figures

(*a*)	amphids
(*ai*)	anterior intestine
(*bm*)	median bulb membrane
(*c*)	cuticle
(*ca*)	cuticle arm
(*cbz*)	cuticle basal zone
(*ccz*)	cuticle cortical zone
(*cf*)	cephalic framework
(*comu*)	constraining muscle
(*cmz*)	cuticle median zone
(*cs*)	cephalic sensilla
(*d*)	dendrites
(*dg*)	dorsal gland
(*du*)	little duct
(*ec*)	epicuticle
(*er*)	endoplasmic reticulum
(*gc*)	glial cells
(*gn*)	gustatory neuron
(*hy*)	hypodermis
(*ist*)	isthmus
(*m*)	mitochondrion
(*mac*)	marginal cell
(*ml*)	metacorpus lumen
(*mt*)	microtubules
(*mu*)	muscles
(*oij*)	oesophageal-intestinal junction
(*pl*)	procorpus lumen
(*pmu*)	metacorpus pump muscle
(*pr*)	proprioceptor organ
(s)	stylet
(*sc*)	stomatal cuticle
(*sco*)	stylet cone
(*sk*)	stylet knobs
(*sl*)	stylet lumen
(*spm*)	stylet protractor muscle
(*ss*)	stylet shaft
(*tc*)	electron-translucent cuticle layer
(*v*)	valve
(*vg*)	ventral gland
(*vmu*)	radial muscle operating valve

RESULTS

Mouth opening (Figs. I; II, 1, 2 and 4)

In *B. mucronatus*, the mouth opening is hexaradiate. The six labial sensilla are arranged in a circle and their dendritic termination (*d*) can be observed. Also seen are four of the six cephalic sensilla (*cs*) and the two amphids (*a*) surrounding the labial aperture, at the centre of which is the stylet (*s*). Extending from each branch of the central tube is a blade-shaped framework that divides the labial aperture into six sectors, whose border is not uniform. The six areas vary in length from 2.7 µm to 3.5 µm and in maximum width from 1.5 µm to 2.7 µm. Each one consists of glial cells (*gc*) and the basal dendritic termination. The appearance of the stylet protractor muscles (*spm*) can be observed within the cephalic frameworks.

The peripheral part presents a triple stratification of the cuticle: the cuticle basal zone (*cbz*) with a palisade structure, the more electron-dense cuticle median zone (*cmz*) and the cortical layer, in turn composed of an inner layer (*ccz*) and an outer layer (*ec*). Also evident is the hypodermis (*hy*), the underlying tissue that secretes the cuticle.

Stylet, protractor muscle and sensory equipment (Figs. I; II, 3, 5 and 6; Fig. III, 1, 2 and 3)

A reconstruction from serial sections at the level of the buccal region shows that the stylet (*s*) is ca. 15.1 µm long, the stylet shaft (*ss*) is on average 1 µm long and 0.72 µm wide, the stylet cone (*sco*) is ca. 5.1 µm long and tapers toward the tip. The stylet lumen (*sl*) is ca. 0.29 m in diameter and the orifice opens on the ventral surface. The three stylet knobs (*sk*), to which the stylet protractor muscles (*spm*) attach, are small, as in the other species of the genus. The shaft (*ss*) is included in the body tissues while the cone (*sco*) protrudes into the stomatal cavity, which is cuticularized (*sc*), ca. 0.042 µm thick, finely fibrillar and moderately electron-dense.

When the stylet is retracted the cuticle forms an outline around the shaft and cone, with a layer on average 0.08 µm thick.

At the stylet orifice the lumen divides into two small branches. We can then observe what will be the main layer of the stylet, which first appears as a very thin wedge and then progressively widens until the shaft.

Several layers of different thicknesses can be identified in the cone; the main one is crossed by a network of small canals. Around the stylet lumen, we can observe the formation of six pseudocoelomatic cords (*cf*) that separate the dorsal and ventral stylet protractor muscles.

The shaft is completely surrounded by a neuron, which appears to be formed by two branches that merge to form the neural ring. The direct contact between the nervous tissue and the canals of the stylet suggest that this organ is a sensory receptor, presumably of taste (gustatory neuron, *gn*).

Two of the three stylet knobs lead the branches of the gustatory neuron along the stylet shaft. From the third knob emerges the branch of a proprioceptor organ (*pr*), in a dorso-lateral position between the marginal cell (*mac*) of this sector and stylet protractor muscles (*spm*). This is assumed to be a proprioceptor organ (*pr*) that perceives changes in tension of the stylet musculature and associated tissues. The proprioceptor organ is leaf-shaped, tapered at the ends and thickened at the centre, curved to fit the shaft and surrounding tissues, and surrounded by a membrane. It has a maximum length of 1.8 µm and width of 0.55 µm, and it returns to a typically neuronal form at the stylet knobs.

The photographs show the stylet protractor muscles. Three of them (numbers 1, 2 and 3) are larger than the others (4, 5 and 6), branched and attached to the stylet knobs. Most of the energy-producing mitochondria are seen around the musculature and behind. The knobs are not very large, with a maximum diameter of 0.7 µm.

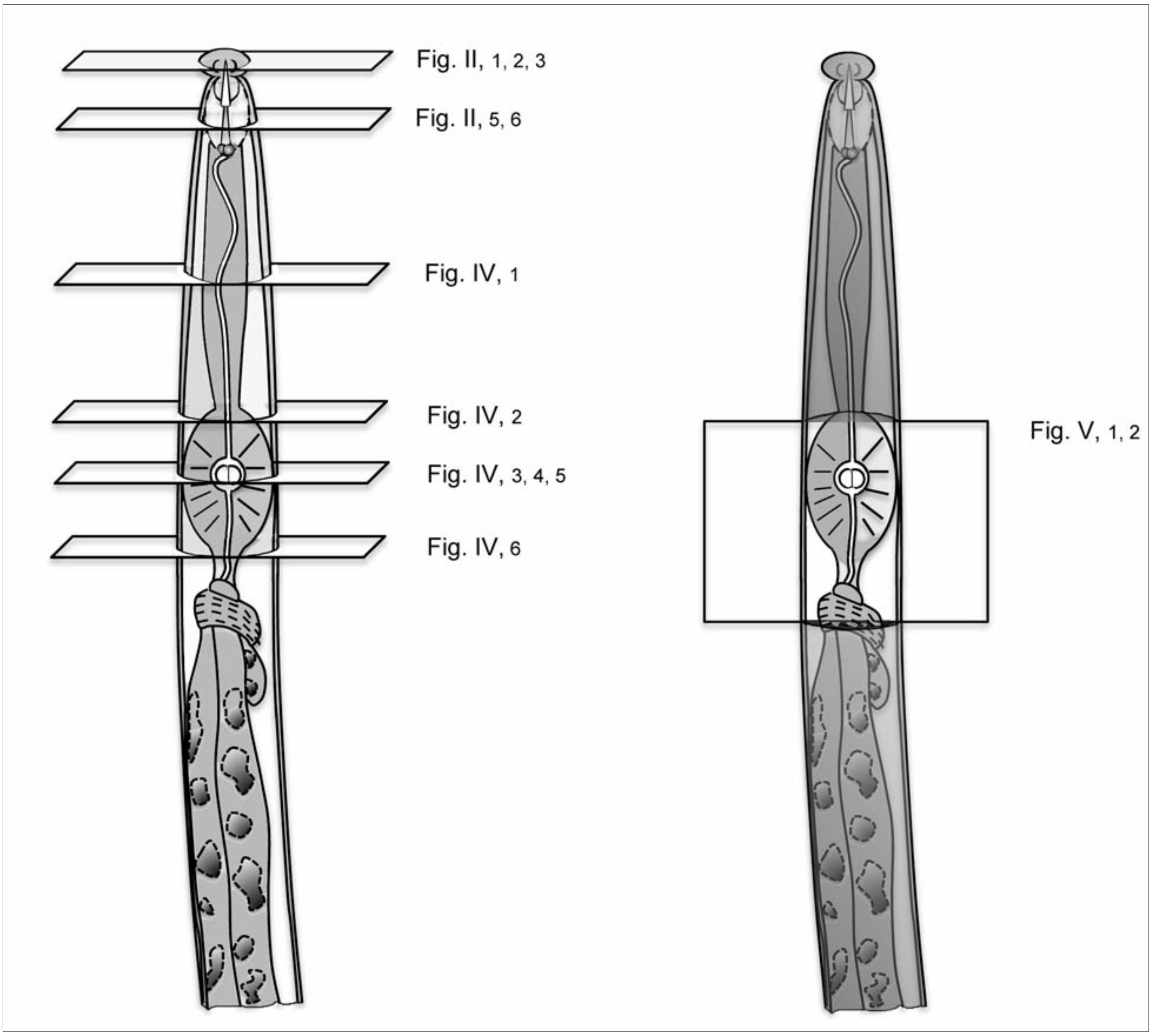

Fig. I – Schematic representation of the anterior region of *Bursaphelenchus mucronatus* showing the levels where sections were made.

PROCORPUS (FIGS. I; IV, 1 AND 2)

The procorpus is ca. 23.3 µm long with a diameter of 2.3 µm and its lumen is often irregular. It is uniform in structure throughout its length and contains no nuclei.

The procorpus lumen (pl) is cylindrical, on average 0.46 µm in diameter, and the cuticle (c) has a mean thickness of 0.14 µm. The layer next to the lumen appears very similar to the corresponding one of the stylet and appears continuous with it. Initially the procorpus occupies a central position in the section, then is situated laterally and finally returns to a central position at the level of the isthmus and median bulb (not shown).

Beneath the stylet the walls of the procorpus occupy the volume of the protractor muscles and sensory organ. There are also muscle bundles of different thickness surrounding the lumen and regulating its opening for the passage of digestive liquids and nutrient solutions (*com*).

The procorpus narrows at the base, where it joins the metacorpus. The median bulb membrane (*bm*) is invaginated slightly around the bulb so that in cross section the base of the procorpus is observed inside the anterior end of the bulb.

METACORPUS (MEDIAN BULB) (FIGS. I; IV, 3, 4, 5 AND 6; FIGS. I; V, 1)

A reconstruction from serial sections at the level of median bulb shows that the typically "aphelencoid" metacorpus is relatively large (occupying most of the width of the body) and has a well-marked outline when observed under the light microscope. On average it is 17.6 µm long and 13.7 µm wide. In the anterior part of the metacorpus there are muscle fibres (*com*) not attached to the lumen but arranged tangentially to form a triangular arrangement around the circular lumen and surrounding marginal cells. This complex arrangement is responsible for the process of aspiration when the muscles contract. Their function is probably maintenance of stability when the powerful pump muscles are acting.

The lumen of the metacorpus (*ml*) changes morphology: initially it has a round section, then becomes triradiate until the valve and remains so even in the terminal part. It presents strong radial oblique muscles (*pmu*) which operate the pump. There are also two other muscle bundles, one operating directly on the valves (*vmu*) and the other forming a ring around the lumen in the anterior part of the bulb.

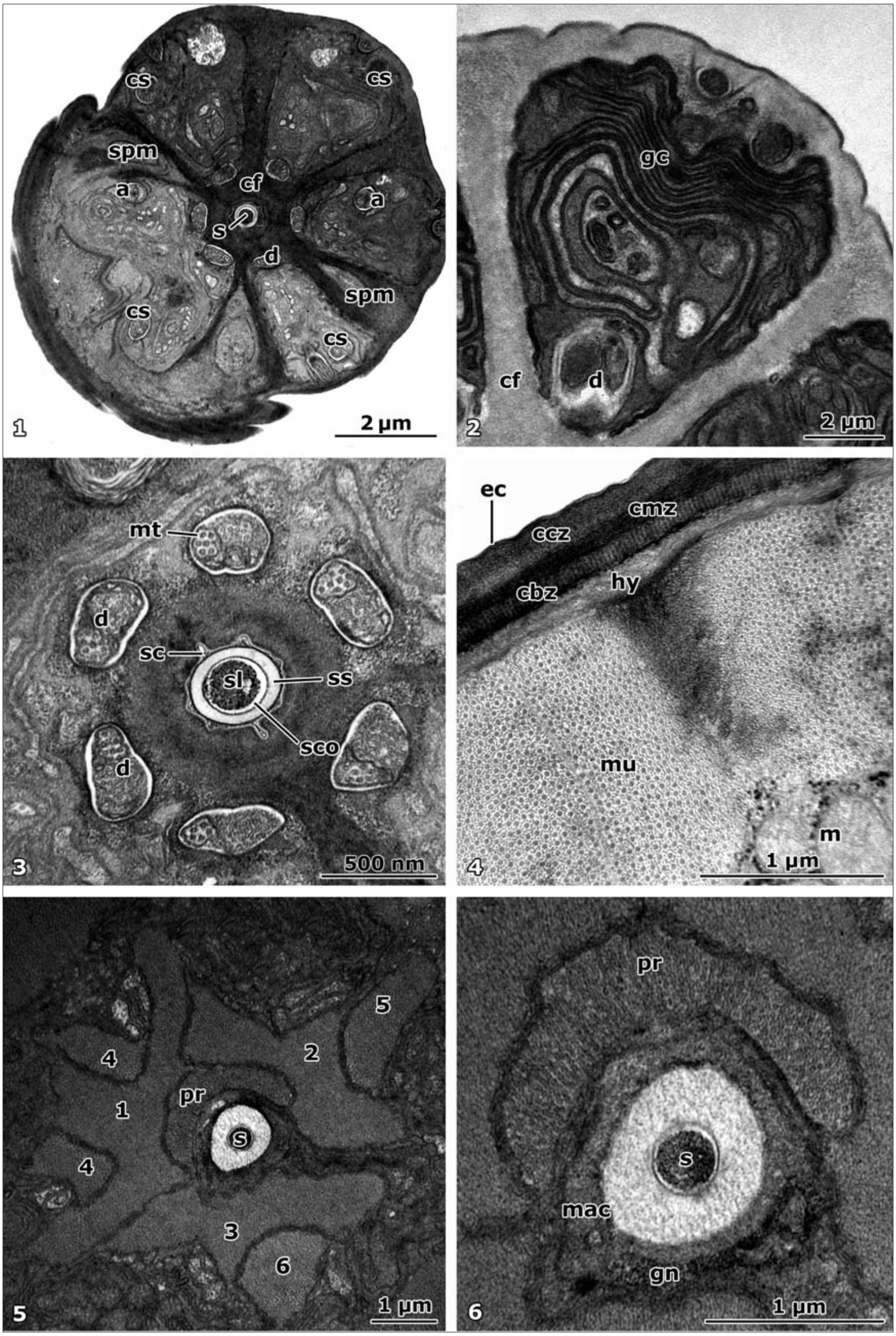

Fig. II – *Bursaphelenchus mucronatus* TEM transverse micrographs. 1. Section of the cephalic framework (*cf*) showing the hexaradiate symmetry (*a*: amphids, *cs*: cephalic sensilli, *spm*: stylet protractor muscle, *s*: stylet, *d*: dendrite). 2. Section of the sextile of the cephalic framework (*cf*) showing the multilamellar dendrite (*d*) and the glial cells (*gc*). 3. Close-up of the dendrites (*d*) surrounding the apical buccal cavity (*mt*: microtubules, *sc*: stomatal cuticle, *sco*: stylet cone; *sl*: stylet lumen; *ss*: stylet shaft). 4. Section of the body wall showing the three-layered cuticle (*ec*: epicuticle, *cmz*: cuticle median zone, *ccz*: cuticle cortical zone, *cbz*: cuticle basal zone), the hypodermis (*hy*), the thick muscles (*mu*) underneath and the mitochondria (*m*). The muscles show the typical nematode arrangement with one filament of myosin (25 to 30 nm in diameter) surrounded by 10 to 15 filaments of actin. 5. Section of the six protractor muscles of the stylet. 6. Inset of the proprioceptor organ (*pr*), the gustatory neuron (*gn*) surrounding the stylet and the marginal cell (*mac*).

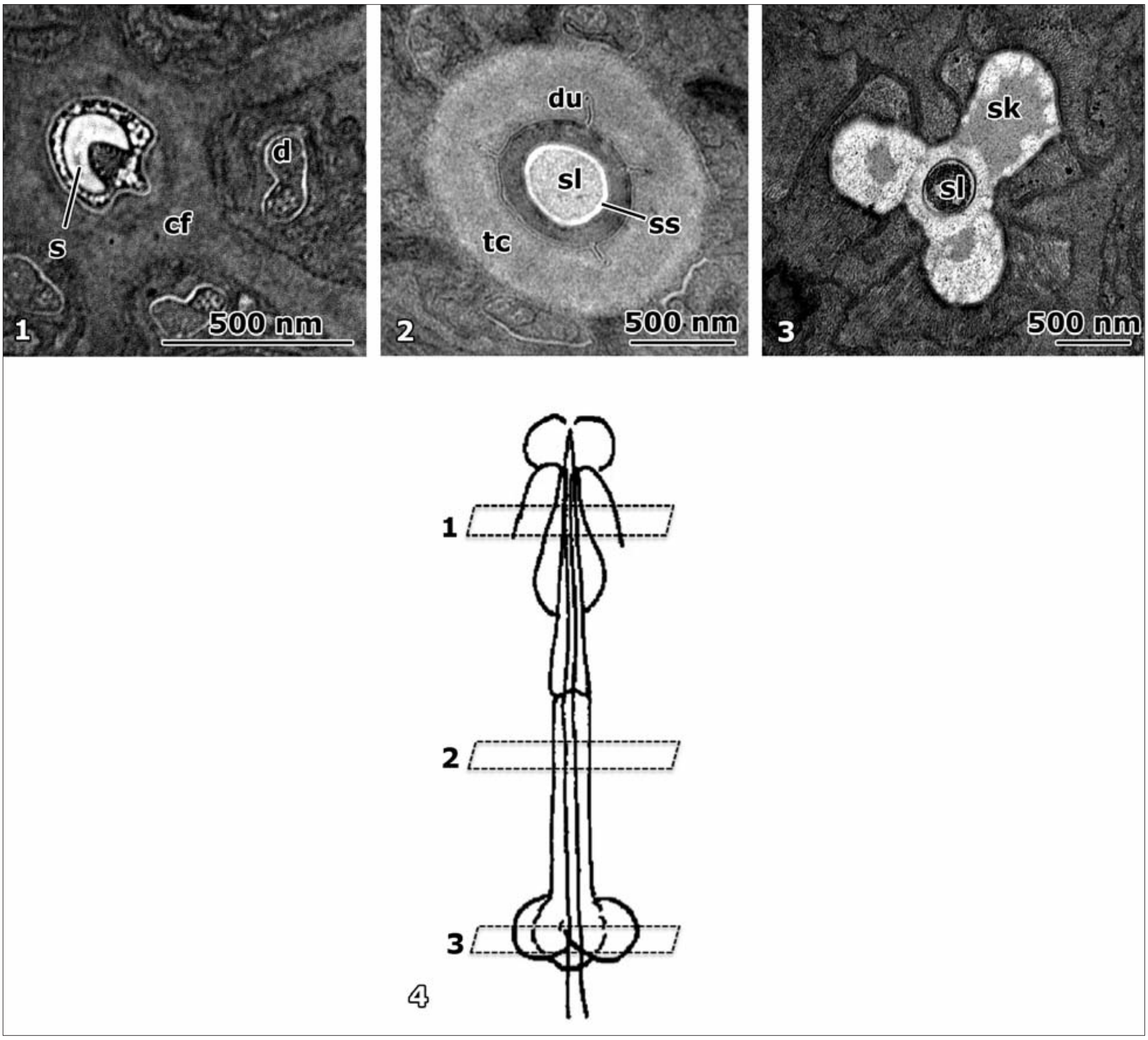

Fig. III – *Bursaphelenchus mucronatus* TEM transverse micrographs of the stylet region. 1. Apical section showing a C-shaped tip of the stylet (*s*) (*d*: dendrite, *cf*: cephalic framework). 2. Middle part of the stylet (*sl*: stylet lumen; *ss*: stylet shaft; *tc*: electron-translucent cuticle layer, *du*: ductile). 3. Basal part of the stylet with the knobs (*sk*). 4. Schematic representation of the stylet showing the three levels to which figs. 1, 2, 3 refer.

The median bulb is delimited by a thick membrane (*bm*) on which are inserted the pump muscles, which exploit the broad surface. The mean thickness of this membrane is 0.08 µm. Most of the nuclei of the bulb are located immediately below this membrane, as are the nuclei of the marginal cells.

The appearance and form of the lining of the oesophageal lumen are diversified and the lining of the procorpus continues in the bulb. The lining of the pump chamber is complex: each of the three arms has an irregular, non-smooth margin and a central spine. The cuticle of the arms is thickened toward the apex and narrows near the lumen of the chamber. This produces a concavity (*ca*) that increases the area of attachment of the muscles in this central region. The thickening is structured, with electron-dense spots on a lighter background.

A distinct translucent area can be observed on both sides of the margin of the spine. The lining is of uniform thickness (ca. 0.038 µm) along each arm. The pump chamber is 0.15 µm wide on average and the radius of each arm measures ca. 1.54 µm from the centre.

The pump mechanism of the metacorpus is operated by a series of muscles (*pmu*) that radiate from the pump chamber toward the periphery of the bulb where they are inserted on the edge of the thick membrane. The myofilaments of these muscles occupy most of the volume of the bulb and can also be observed with a light microscope. The muscles that radiate in front and behind the pump chamber are almost parallel with the lumen, and various muscle cells can be seen, two between each arm of the triradiate lumen. In these sectors there are also six other muscle bundles that operate the valves and are inserted in the small lumen of the chamber (*vmu*).

The dorsal gland duct opens into the metacorpus above the pump chamber, while the two ventral ducts enter the lumen posteriorly.

In the terminal part of the metacorpus, we can see the insertion of the three oesophageal glands, the dorsal (*dg*)

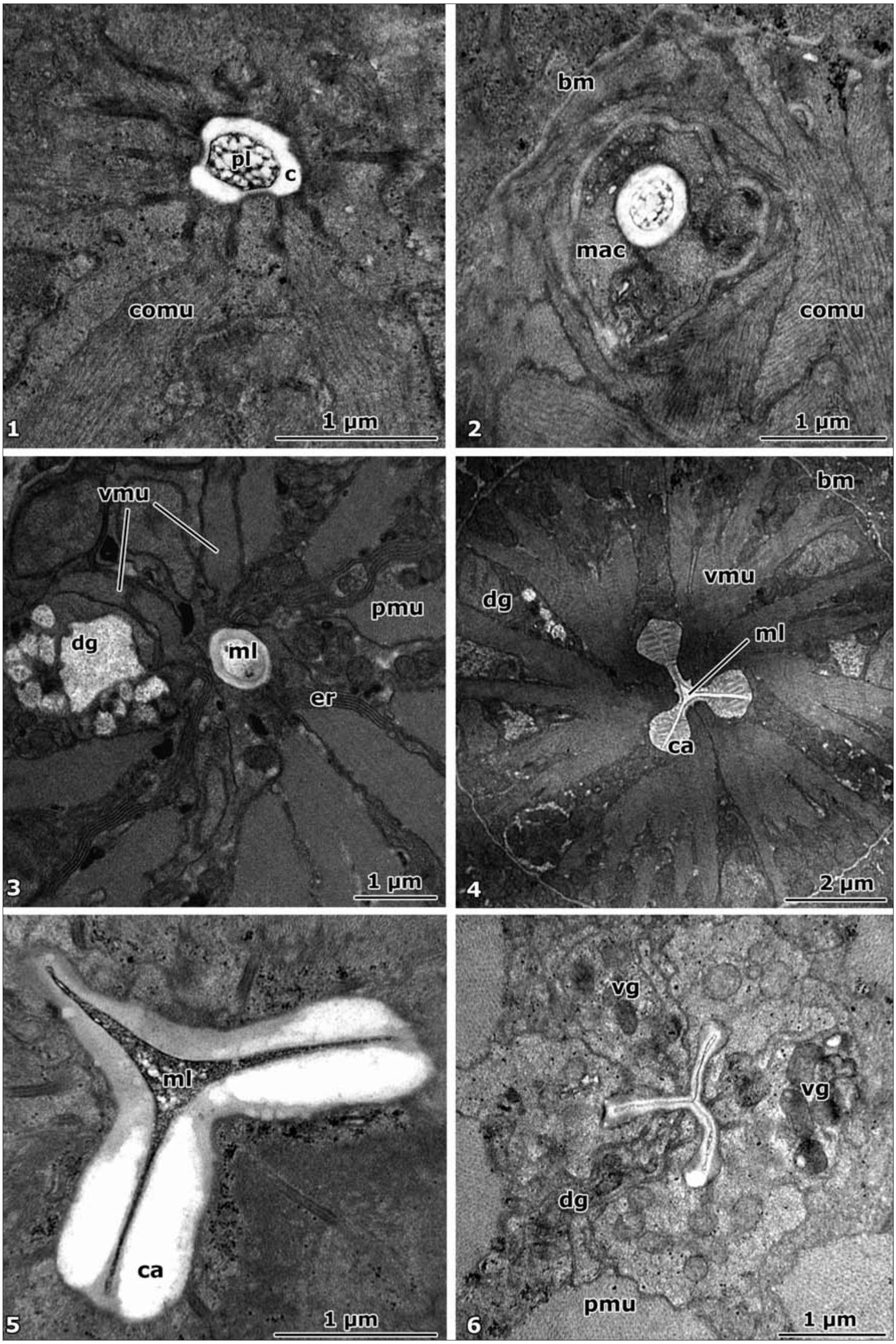

Fig. IV – *Bursaphelenchus mucronatus* TEM transverse section. 1. Basal part of the procorpus (*pl*: procorpus lumen, *c*: cuticle, *comu*: constraining muscle). 2. Apical part of the metacorpus (*bm*: mediam bulb membrane, *mac*: marginal cell). 3. Mid-apical part of the metacorpus showing the dorsal gland (*dg*) just before its opening into the metacorpus lumen (*ml*) (*pmu*: metacorpus pump muscle, *vmu*: radial muscle operating valve, *er*: endoplasmic reticulum). 4. Middle part of the metacorpus showing the triradiate valve in the centre of the bulb (*ca*: cuticle arm). 5. Basal part of the metacorpus showing the ductules of the glands that open into the metacorpus lumen. 6. Basal part of the metacorpus showing the insertion of the glands into the metacorpus, beside the valve (*vg*: ventral gland).

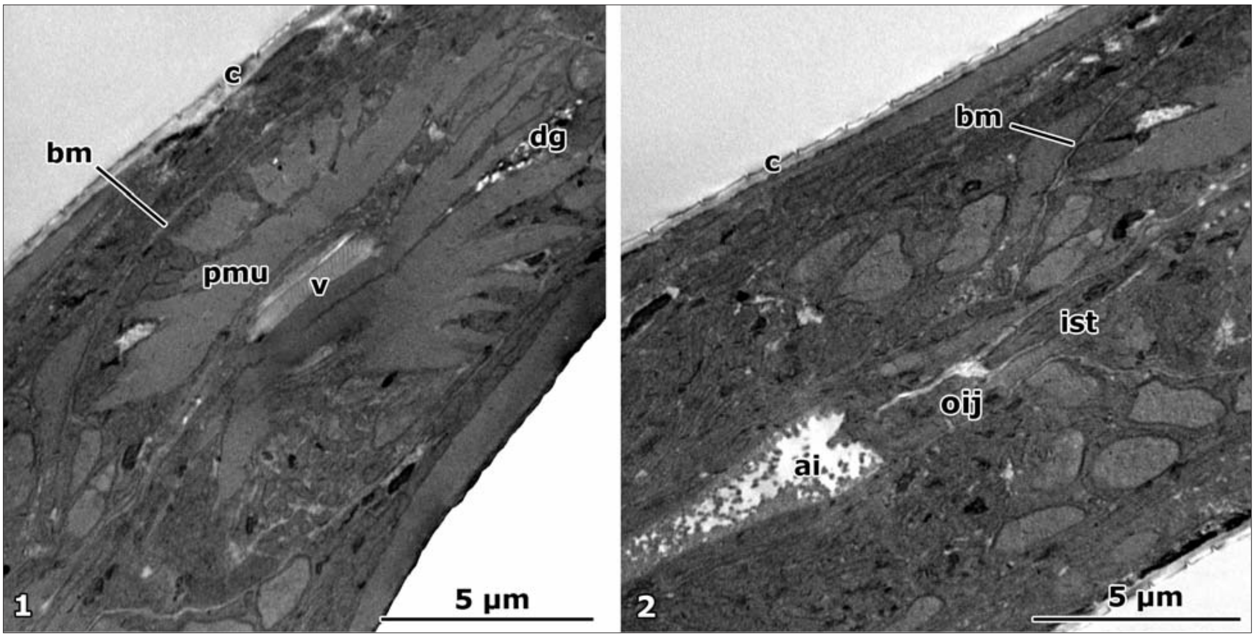

Fig. V – *Bursaphelenchus mucronatus* TEM longitudinal micrographs. 1. Section throughout the metacorpus (*c*: cuticle, *bm*: median bulb membrane, *pmu*: metacorpus pump muscle, *v*: valve, *dg*: dorsal gland). 2. Section of the metacorpus and the anterior intestinal region (*ai*: anterior intestine, *ist*: isthmus, *oij*: oesophageal-intestinal junction).

and the two subventral (*vg*), and in particular the ducts (*d*) that transport the enzyme solutions produced by the glands into the metacorpus lumen.

ISTHMUS (FIGS. I; V, 2)

This region cannot be observed with the light microscope because it is located inside the base of the median bulb. The isthmus is very short and composed entirely of extensions of cells whose nuclei are situated inside the median bulb. In the isthmus the lumen remains triradiate but the diameter is smaller (mean 0.015 µm), as is the cuticle (mean 0.076 m). The valve of the isthmus is operated by six radial muscle filaments plus circular and longitudinal filaments.

BASAL BULB AND OESOPHAGEAL GLANDS

The basal bulb can be considered a thin elastic sac containing the dorsal and subventral glands, with a non-uniformly thick membrane. The three oesophageal glands, the dorsal (*dg*), extended forward, and the two subventral (*vg*), are syncytial, bulbiform and multinucleate. The morphology of the oesophageal glands varies during nematode development. In the juvenile stages, the subventral glands present a large number of secretory granules, while the dorsal gland shows lesser development. In the adult stage, the subventral glands are smaller, while the dorsal gland reaches a mean length of 67 µm and contains various secretory granules.

Posterior to the median bulb is the intestine with short bulbous microvilli lining the lumen. The oesophageal-intestinal junction (*oij*) is observed between the oesophagus and intestine.

DISCUSSION

Thus far ultrastructural studies of nematode pests have dealt mainly with species in the tylenchids, while only limited information is available for aphelenchid species. Our findings on the ultrastructure of *B. mucronatus* agree with those of ZHANG and ZHANG (2006) and expand on their preliminary data. In *B. mucronatus* the stylet protractor muscles are arranged around the lumen with three main bundles that branch and three smaller bundles arranged within these branches. The lumen of the stylet has an almost circular shape throughout its length and does not change in size. The stylet shaft is surrounded by the proprioceptor organ, marginal cells and a gustatory neuron. The proprioceptor organ identified in *B. mucronatus* also appears in other aphelenchid genera and a similar organ, involving the odontophore, has been described by ROBERTSON (1976) for nematodes of the family Longidoridae.

Our TEM observations revealed differences with respect to the tylenchid nematodes. In *B. mucronatus* the oesophageal pump presents a very marked radial musculature inside the median bulb, whereas in the tylenchids the musculature of the triradiate chamber consists of thinner myofilaments and the median bulb membrane is not as thick (YUEN, 1968; KISIEL *et al.*, 1976; BALDWIN *et al.*, 1977). Moreover, in *B. mucronatus* the so-called “junction cells” (SHEPHERD *et al.,* 1980) are evident in the arms of the bulb chamber, while below the median bulb membrane it is possible to observe a high number of mitochondria that satisfy the energy demands of the median bulb musculature, similar to what was reported by ANDERSON and BYERS (1975) in *Tylenchorhynchus dubius*.

Studies on some tylenchid species have shown that muscles and filaments in the isthmus can have different functions: increasing the thickness of the wall (KISIEL *et al.*, 1976), facilitating the flow of oesophageal gland secretions (BALDWIN *et al.*, 1977) or controlling the passage of food. In *B. mucronatus* there is a radial musculature in the isthmus that probably controls the opening of the lumen to regulate the passage of enzymatic substances and nutrient solutions, as occurs in *Aphelenchoides blastophthorus* (SHEPHERD *et al.*, 1980).

The dorsal gland in *B. mucronatus* extends forward until it overlaps the anterior end of the intestine, and the two subventral glands are of equal size. Sections of the central part of the median bulb also showed that the dorsal gland becomes flattened laterally to reach the lumen of the bulb, opening between the bundles of the radial musculature. The glands also contain abundant secretory granules evenly distributed in the lumen. *Bursaphelenchus* species living on conifers develop in the resin canals, a rather inhospitable environment since resin is the main means of defence for these trees. As suggested for other nematodes whose larvae are exposed to different environments or must cross tissue barriers during their migration (LEE, 1968; BIRD, 1969; JENKINS, 1971; VEGNI TALLURI *et al*., 1986), the oesophageal glands may also secrete products to facilitate movement in the host's resin canals. Further studies will be carried out to define the composition of the secretory granules.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Laura Ambrogioni and Dr. Natsumi Kanzaki for her assistance, suggestions and constructive comments.

RIASSUNTO

ULTRASTRUTTURA DEL TRATTO ALIMENTARE ANTERIORE DI *BURSAPHELENCHUS MUCRONATUS* MAMIYA *ET* ENDA (NEMATODA: APHELENCHOIDIDAE)

In considerazione dell'importanza fitosanitaria di nematodi appartenenti al genere *Bursaphelenchus* e alle limitate informazioni disponibili sulla loro ultrastruttura, abbiamo condotto uno studio sulla cavità orale e sull'area esofagea di *Bursaphelenchus mucronatus* Mamiya et Enda, 1979. Come la specie da quarantena *Bursaphelenchus xylophilus* (Steiner et Bührer) Nickle, il suo sviluppo avviene nei canali resiniferi di conifere. Gli esemplari utilizzati nel nostro studio sono stati presi da una popolazione di laboratorio raccolta su *Pinus pinaster* Aiton in foreste miste meso-termofile dell'Italia centrale (Toscana) e allevata su piastre Petri, in una camera climatizzata per 20 generazioni. Sono state studiate con il TEM le caratteristiche ultrastrutturali dell'apertura boccale, dell'intestino anteriore, dei sensilli labiali, dei canali anfidiali, dello stiletto e del metacorpo. Sono state descritte anche le ghiandole esofagee. Le strutture osservate sono discusse e confrontate con quelle descritte per altri nematodi.

REFERENCES

ANDERSON R.V., BYERS J.R., 1975 – *Ultrastructure of the oesophageal procorpus in the plant parasitic nematode,* Tylenchorhynchus dubius*, and functional aspects in relation to feeding.* - Canadian Journal of Zoology, 53: 1581-1595.

BALDWIN J.G., HIRSCHMANN H., 1976 – *Comparative fine structure of the stomatal region of males of* Meloidogyne incognita *and* Heterodera glycines. - Journal of Nematology, 8 (1): 1-17.

BALDWIN J.G., HIRSCHMANN H., TRIANTAPHYLLOU A.C., 1977 – *Comparative fine structure of the esophagus of males of* Heterodera glycines *and* Meloidogyne incognita*.* - Nematologica, 23: 239-252.

BIRD A.F., 1969 – *Changes associated with parasitism in nematodes. V. Ultrastructure of the stylet exudation and dorsal esophageal gland contents of female* Meloidogyne javanica. - The Journal of Parasitology, 55(2): 337-345.

BRAASCH H., BURGERMEISTER W., HARMEY M.A., MICHALOPOULOS-SKARMOUTSOS H., TOMICZEK C., CAROPPO S., 2000 – *Pest risk analysis of pinewood nematode related* Bursaphelenchus *species in view of South European pine wilt and wood imports from Asia.* - Final Report of UE Research Project Fair CT 95-0083.

BYERS J.R., ANDERSON R.V., 1972 – *Ultrastructural morphology of the body wall, stoma, and stomatostyle of the nematode,* Tylenchorhynchus dubius *(Bütschli, 1873) Filipjev, 1936.* - Canadian Journal of Zoology, 50: 457-465.

CARLETTI B., IRDANI T., COSI E., BRANDSTETTER M., PENNACCHIO F., ROVERSI P.F., AMBROGIONI L., 2006 – *First record of* Bursaphelenchus fraudulentus *Rühm (Goodey) (Nematoda Aphelenchoididae) in Italy.* - Redia, 88 (2005): 27-35.

CARLETTI B., 2008 – Bursaphelenchus *species with their natural vectors in Italy: distribution and essential diagnostic features.* - Redia, 91: 111-117.

CARLETTI B., COTRONEO A., ROVERSI P.F., 2011 – *Inoculation experiments of* Bursaphelenchus eremus *Rühm (Goodey) (Nematoda Parasitaphelenchidae) on* Quercus robur *L.* - Redia, 94: 159-162.

CAROPPO S., CAVALLI M., CONIGLIO D., AMBROGIONI L., 2000 – *Pathogenicity studies with various* Bursaphelenchus *populations on conifer seedlings under controlled and open air conditions.* - Redia, 83: 61-75.

FRANCARDI V., DE SILVA J., PENNACCHIO F., ROVERSI P.F., 2009 – *Pine volatiles and terpenoid compounds attractive to European xylophagous species, vectors of* Bursaphelenchus *spp. nematodes.* - Phytoparasitica, 37: 295-302.

JENKINS D.C., 1971 – *The ultrastructure of the pharynx of some developing larvae of* Ascaris suum*.* - Zeitschrift für Parasitenkunde, 37: 255-266.

KISIEL M.J., HIMMELHOCH S., ZUCKERMAN B.M., 1976 – *Fine structure of the esophagus of* Pratylenchus penetrans*.* - Journal of Nematology, 8: 218-228.

LEE D.L., 1968 – *The ultrastructure of the alimentary tract of the skin-penetrating larva of* Nippostrongylus brasiliensis *(Nematoda).* - Journal of Zoology, 154: 9-18.

MOTA M., BRAASH H., BRAVO M.A., PENAS A.C., BURGERMEISTER W., METGE K., SOUSA E., 1999 – *First report of* Bursaphelenchus xylophilus *in Portugal and Europe.* - Nematology, 1: 727-734.

NICKLE W.R., 1970 – *A taxonomic review of the genera of the Aphelenchoidea (Fuchs, 1937) Thorne, 1949 (Nematoda: Tylenchida).* - Journal of Nematology, 2: 375-392.

RAGSDALE E.J., BALDWIN J.K., 2010 – *Reconciling phylogenetic incongruence to articulate homology and phenotypic evolution: a case study from Nematoda.* - Proceedings of the Royal Society of London B, 277: 1299-1307.

RAGSDALE E.J., CRUM J., ELLISMAN M.H., BALDWIN J.G., 2008 – *Three-dimensional reconstruction of the stomatostylet and anterior epidermis in the nematode* Aphelenchus avenae *(Nematoda: Aphelenchidae) with implications for the evolution of plant parasitism.* - Journal of Morphology, 269: 1181-1196.

RAGSDALE E.J., NGO P.T., CRUM J., ELLISMAN M.H., BALDWIN J.G., 2009 – *Comparative, three-dimensional anterior sensory reconstruction of* Aphelenchus avenae *(Nematoda: Tylenchomorpha).* - Journal of Comparative Neurology, 517: 616-632.

Ragsdale E.J., Ngo P.T., Crum J., Ellisman M.H., Baldwin J.G., 2011 – *Reconstruction of the pharyngeal corpus of* Aphelenchus avenae *(Nematoda: Tylenchomorpha), with implications for phylogenetic congruence.* - Zoological Journal of the Linnean Society, 161: 1-30.

Robertson W.M., 1976 – *A possible gustatory organ associated with the odontophore in* Longidorus leptocephalus *and* Xiphinema diversicaudatutm. - Nematologica, 21: 443-448.

Rodrigues J., 2008 – *National eradication programme for pinewood nematode. In*: Vieira P, Mota M (eds) Pine wilt disease: a worldwide threat to forest ecosystems. Springer, Dordrecht, pp 5-14.

Shepherd A.M., Clark S.A., 1976 – *Structure of the anterior alimentary tract of the passively feeding nematode* Hexatylus viviparous *(Neotylenchidae: Tylenchida, Aphelenchina).* - Nematologica, 22: 332-342.

Shepherd A.M., Clark S.A., Hooper D.J., 1980 – *Structure of the anterior alimentary tract of* Aphelenchoides blastophthorus *(Nematoda: Tylenchida).* - Nematologica, 26: 313-357.

Soliman T., Mourits M.C.M., van der Werf W., Hengevald G.M., Robinet C., 2012 – *Framework for modelling economic impacts of invasive species, applied to pine wood nematode in Europe.* - Plos One, 9(7): 1-12.

Tomiczek C., Braasch H., Burgermeister W., Metge K., Hoyer U., Brandstetter M., 2003 – *Identification of* Bursaphelenchus *spp. isolated from Chinese packaging wood imported to Austria.* - Nematology, 5: 573-581.

Vegni talluri M., Paggi L., Orecchia P., Dallai R., 1986 – *Fine structure of buccal cavity and esophagus in* Toxocara canis *(Nematoda, Ascaridae) infective larvae.* - Journal of Ultrastructure and Molecular Structure Research, 97: 144-157.

Yuen P.H., 1968 – *Electron microscopical studies in* Ditylenchus dipsaci *(Kühn). II. Stomatal region.* - Nematologica, 14: 385-394.

Zhang C., Zhang L., 2006 – *Ultrastructural observation on esophageal glands and their secretory granules in* Bursaphelenchus xylophilus *and* Bursaphelenchus mucronatus *by TEM.* - Journal of Beijing Forestry University, 28 (3): 119-122.