Scanning Electron Microscopy of the anterior end of *Hystrichis tricolor* (Nematoda: Dioctophymida)

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Summary. The anterior end of the nematode *Hystrichis tricolor* Dujardin, 1845 is described from observations made using Scanning Electron Microscopy (SEM). Several points made during investigations of the species by Jägerskiöld (1909) and Karmanova (1986) were confirmed. The SEM-investigation revealed a difference in the position of the second ring of cephalic sensilae that is among the anteriormost spines. The nature of two structures tentatively interpreted as amphids remains doubtful. A regular arrangement of cuticular spines on the anterior end could be observed only partially, and this probably does not represent an overlying pattern. The anteriormost spines, for example, are not arranged in any order. Several spines are deformed, detached or broken off. Observation of the detached spines revealed that they consist of a hollow cuticular sheath that is filled by an extension of the epidermis.

Key words: amphids, anterior end, cephalic sense organs, Dioctophymida, Hystrichis, SEM.

The taxon Dioctophymida is one of the least known within Nematoda. It is a heterogenous group of vertebrate parasites, comprising the genera Dioctophyma Collet-Meygret, 1802, Hystrichis Dujardin, 1845, Eustrongylides Jägerskiöld, 1909, Soboliphyme Petrow, 1930 and Mirandonema Kreis, 1945 (Karmanova 1986). Species of the genus Hystrichis, type species H. tricolor Dujardin, 1845, parasitize in the proventriculus of water birds and use oligochaetes as intermediate hosts. Hystrichis is characterized by numerous spines at the anterior end, which are not used for permanent attachment of the nematode (Karmanova 1986). However, they may be used for initial attachment and the boring process to attach the mid-body of the nematode in the proventriculus wall, and thereafter the anterior and posterior ends of the nematode are free in the proventriculus lumen. Most information of H. tricolor was reported by Jägerskiöld (1909) and Karmanova (1986). Two rings of six cephalic papillae and two amphids are present on the anterior end (Karmanova 1986) with the arrangement of cephalic spines being regular and in distinct rings (Jägerskiöld, 1909; Karmanova, 1986). Jägerskiöld (1909) reported the presence of up to 35 circles

with each containing up to 55 spines per circle, whereas Karmanova (1986) reported a chessboard-like pattern of 28 to 34 longitudinal rows each containing 22 to 32 spines. The objective of this study was to re-examine, and more accurately describe, the anterior end of *H. tricolor* using Scanning Electron Microscopy.

MATERIAL AND METHODS

Anterior ends of three specimens of *Hystrichis* tricolor Dujardin, 1845 were made available from the collection of the Institute of Parasitology, Moscow by L.V. Filimonova. The nematodes that had originally been obtained from experimental infections of ducks (*Anas* spp.) near Samtredi, Georgia, were fixed and stored in 70% ethanol, dehydrated in an increasing ethanol series, critical-point dried, and gold-sputtered. Observation was made with a Hitachi S 450 Scanning Electron Microscope.

RESULTS

The anterior end of *Hystrichis tricolor* is broader than the remaining body, forming a terminal bulge with the anterior region of the

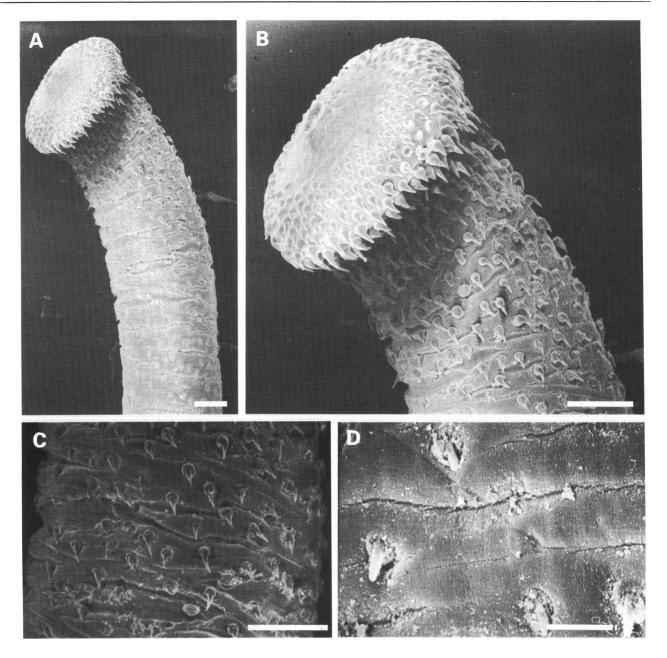


Fig. 1. Hystrichis tricolor: A & B: Anterior end showing bulge with spines and terminal mouth opening; C: Spines in the "neck" region posterior of the bulge; D: Spines at the posteriormost level shown in A. Scale bars: A, B & C - $100 \ \mu m$; D - $20 \ \mu m$.

bulge being almost flat, but with the mouth region being slightly elevated (Fig. 1A & B). A dense cover of body spines begins 50-80 μ m from the mouth opening. The spines are most dense on the bulge but are present with a decreasing density along the entire anterior end of the nematode (Fig. 1A & C). The anterior end posterior to the bulge is characterised by numerous grooves and folds oriented perpendicular to the longitudinal axis of the nematode (Fig. 1A, C & D). Spines are largest at the bulge and posteriorly along the body become progressively shorter and more slender (Fig. 1C & D).

The anterior end of each of the three specimens investigated differed slightly in some details. In one specimen (Fig. 2B), there was a ring-like groove between the mouth opening and the beginning of the spines, whereas in the two other specimens this area was completely smooth (Fig. 2A & C). The mouth opening is clearly

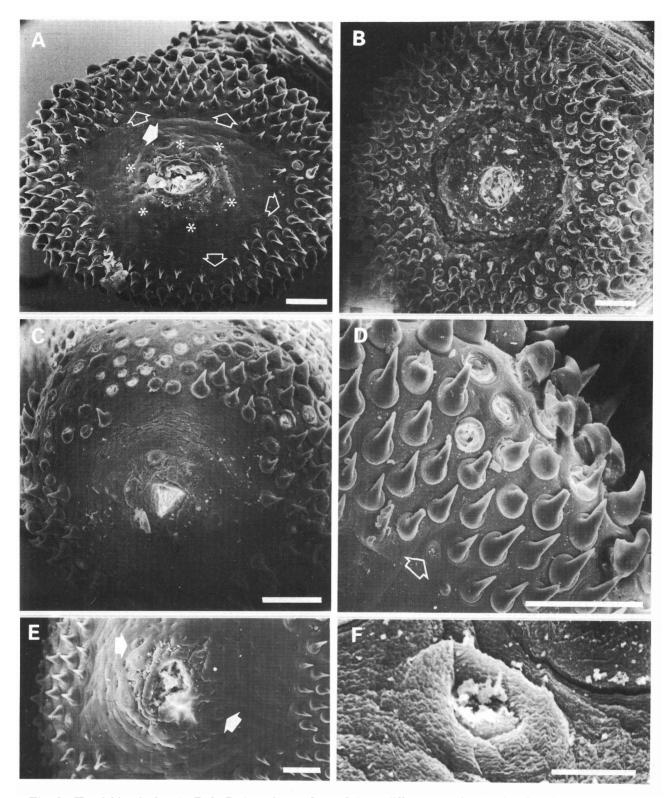


Fig. 2. Hystrichis tricolor. A, B & C: Anterior surface of three different specimens showing the mouth opening, cephalic papillae of the first ring (asterisks), second ring (arrows) (only four positions clearly recognizable), and probable amphid (solid arrow); D, E & F: Higher magnification of specimen in A showing D: Anteriormost spines with pocket-like cephalic sense organ of second ring (arrow), E: Cephalic sensillae of first ring with probable amphids (arrowed); F: Pocket-like cephalic sense organ of the second ring. Scale bars: A to E - 50 μ m; F - 5 μ m.

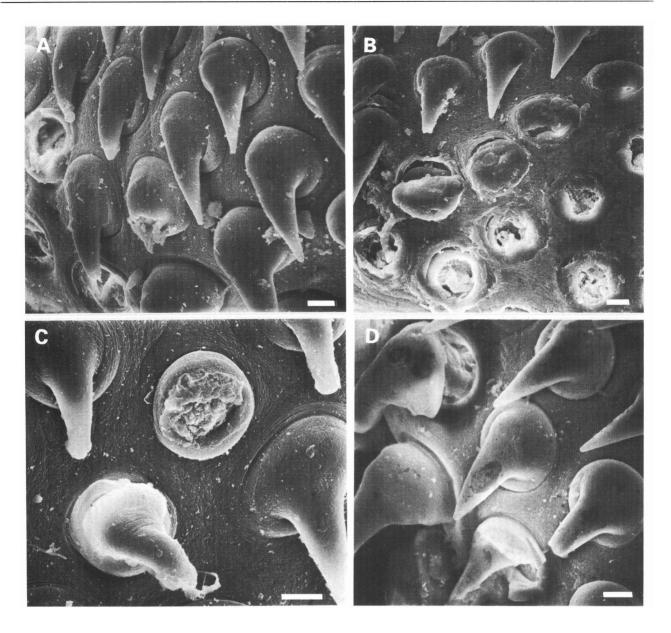


Fig. 3. Hystrichis tricolor. A: Spines with deformed tip; B: Detached and broken spines; C: Deformed spines; D: Partially detached spine. Scale bar - $5 \mu m$.

triangular in one specimen (Fig. 2C), a triangular shape is present in another specimen (Fig. 2A), but this is not visible in the third specimen (Fig 2B). One ring with 6 inconspicuous papillae is present around the mouth opening (Fig. 2A-C & E), and these papillae are on a slight elevation and possess a small tubercle on top. In one specimen, six pocket-like head sensory organs were observed between the anteriormost spines (Fig. 2A & D) consisting of a pore surrounded by a small bulge (Fig. 2F). Two circular structures were observed close to the papillae surrounding the mouth opening (Fig. 2A, C & E). These could be distinguished from the surrounding cuticle because they were slightly bulging (Fig. 2C), or because they were surrounded by a fine circular groove (Fig. 2 E). Due to their paired appearance these structures might represent amphids.

The spines are not arranged in order, and defined rings were not observed. A chessboardlike pattern is present in several areas, but it is not possible to follow this pattern in larger areas. This is best reflected by the anteriormost spines, which do not occur in any pattern and are arranged more or less irregularly (Fig. 2A-C).

The spines closest to the mouth opening are

small circular structures with a central knob-like elevation (Fig. 2D). They are slightly, but clearly, set off from the surrounding cuticle. In more mature spines, the central element is enlarged and forms a pointed hook that is curved in the posterior direction (Fig. 2D). The base of the spine has a maximum diameter of about 16 μ m. The largest spines are about 25 μ m long.

Several spines are deformed, detached, or broken off (Figs. 2C, D & 3A-D). In deformed spines, either the tip is not pointed or the spine is not bent in a posterior direction (Fig. 3A, C & D). When spines detach, they lose contact with their basal plate. Lost spines leave circular holes (Fig. 3B & C). The spines probably are not solid cuticular structures, because in spines that are partially detached it appears that tissue runs through the opening into the spine (Fig. 3D).

DISCUSSION

In general, the observations by Jägerskiöld (1909) and Karmanova (1986) were confirmed. However, contrasting results were obtained in the interpretation of cephalic sense organs, and in the arrangement of the pattern of the spines. The first ring of cephalic papillae is situated close to the mouth opening, as reported by Karmanova (1986), and they have a base and an apical tubercle. According to Karmanova (1986) the second ring of papillae is present at the level of the third ring of spines, but in SEM, six pocket-like structures were observed at the level of the anteriormost spines. These are interpreted as representing the second ring of cephalic papillae. Also, Karmanova (1986) reported two large sucker-like amphids close to the first ring of papillae. In the present study rounded compartments were confirmed close to the first ring of papillae, but no openings were observed. The interpretation of these compartments as representing amphids is based on their position, rather than their structure.

The arrangement of spines is interpreted as not reflecting any order such as a chessboard pattern. The density of spines creates a regular pattern in some regions, but it was not possible to trace rings or longitudinal rows, particularly with the anteriormost spines. The origin of the deformation of spines could not be determined, but possibly occurs as a result of abrasion during the life of the nematodes, or as a result of handling of the fixed specimens. However, detached spines revealed the hollow nature of the spines, which include projections from the epidermis.

The anterior end is probably capable of some movement along the longitudinal axis, as indicated by the different appearances of the circumoral surface that may be due to differently retracted or protracted stages. However, it 'seems unlikely that larger areas of the anterior end can be inverted, such as occurs with the introverts of priapulids, kinorhynchs or loriciferans. Such introverts are assumed for a group comprising the Priapulida, Kinorhyncha, Loricifera and Nematomorpha (e.g. Malakhov & Adrianov, 1995), or for a group additionally including Nematoda (Nielsen, 1995). However, this latter hypothesis is based on structures present in the enoplid nematode Kinonchulus, that are not present in Hystrichis.

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Schmidt-Rhaesa A. Сканирующая электронная микроскопия переднего конца *Hystrichis tricolor* (Nematoda: Dioctophymida).

Резюме. Приводится описание морфологии головного конца *Hystrichis tricolor* Dujardin, 1845, основанное на данных сканирующей электронной микроскопии (СЭМ). Подтверждены ранее сообщенные Егершельдом (1909) и Кармановой (1986) данные по морфологии этого вида. При помощи СЭМ было выявлено расположение второго круга головных сенсилл среди передних шипов вооружения головного конца. Истинная природа двух образований, трактуемых до сих пор как амфиды, остается неясной. Лишь частично подтвердились данные о правильности расположения шипов на головном конце, так как их упорядоченное шахматное расположение наблюдается лишь на части головного конца. Так, например, самые передние шипы располагаются беспорядочно. Некоторые шипы на исследованных образцах были деформированы, оторваны или сломаны. Изучение мест прикрепления оторванных шипов показало, что они состоят из полого кутикулярного покрова, заполненного выступом эпидермиса.