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Scanning electron microscopy of the outer and inner surface of the buccal cavity of some Mononchida

Gaetan BORGONIE*+, Rita VAN DRIESSCHE* and August COOMANS*

* *Zoology Institute, University of Gent, K. L. Ledeganckstraat 35, 9000 Gent, Belgium and*
+ *Plant Genetic Systems Nv, Jozef Plateaustraat 22, 9000 Gent, Belgium.*

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Summary – The buccal cavity of six Mononchida was studied using a combination of dissection and extraction to view the inner and outer structures of the buccal cavity with respect to the surrounding tissue. The extraction of the buccal cavities was done by using a solution consisting of ß-mercapto-ethanol and sodium dodecyl sulphate. As these chemicals denature proteins, they allowed elimination of all surrounding tissue of the buccal cavity, making possible detailed study of its surface using Scanning Electron Microscopy. This solution offers the advantage, over the use of the hypochlorite solution, that prolonged incubation does not lead to a loss of fine detail. The extraction of the buccal cavity showed that the outer surface does contain considerable fine detail and some more massive structures previously unreported. The buccal cavity can be divided into two areas : an anterior area with parallel sides and an oblique basal area. This subdivision is arbitrary but it distinguishes between the more or less straight dorsal and two ventrosublateral vertical buccal plates that may carry teeth and the bottom of the buccal cavity, formed by three oblique basal plates, curved towards the centre. The presence of completely detached stoma plates (one dorsal and two ventrosublateral plates) and completely detached basal plates from stoma plates in *Anatonchus tridentatus* distinguishes it from the other Mononchids studied. A possible functional explanation is discussed.

Résumé – ***Étude en microscopie électronique à balayage de la surface interne et externe de la cavité buccale de quelques Mononchides*** – La cavité buccale de six Mononchides est étudiée en utilisant une technique combinant dissection et extraction afin d'observer les structures internes et externes de cette cavité sans léser les tissus l'entourant. L'extraction des cavités buccales est réalisée grâce à une solution de ß-mercapto-éthanol et de dodécyle-sulfate de sodium. Ces produits, dénaturant les protéines, permettent l'élimination des tissus entourant la cavité et, par conséquent, l'observation détaillée de sa surface au microscope électronique à balayage. Cette solution est préférable à une solution d'hypochlorite car une incubation prolongée ne fait pas disparaître les structures fines. L'extraction de la cavité buccale montre que celle-ci comporte quantité de structures fines et quelques structures plus massives, non encore signalées. La cavité buccale peut être divisée en deux zones : une zone antérieure, à parois parallèles, et une zone de base, postérieure, oblique. Cette division est arbitraire mais elle permet la distinction entre *i)* les plaques buccales verticales (une dorsale, deux ventrales) plus ou moins droites et pouvant porter des dents et *ii)* le fond de la cavité buccale, formé par trois plaques basales obliques, courbées vers le centre. La présence chez *Anatonchus tridentatus* de plaques stomatales (une dorsale, deux subventrales), complètement détachées, et de plaques basales complètement détachées des plaques stomatales, distingue cette espèce des autres Mononchides étudiés. Une explication fonctionnelle est discutée.

Key-words : Mononchida, buccal cavity, SEM, nematodes.

The buccal cavity of mononchid nematodes has attracted considerable interest, not only as an important taxonomic characteristic but also as the focus of several studies concerning functional aspects during prey catching (e.g. Jairajpuri & Azmi, 1978). Most studies centre on the stoma morphology and related muscles (e.g. Coomans & Lima, 1965; Grootaert & Wyss, 1978), with some using transmission electron microscopy, or in combination with video techniques (Grootaert & Wyss, 1978). Nevertheless to our knowledge no detailed study has been done of the buccal cavity using Scanning Electron Microscopy (SEM), demonstrating the interior surface (lining the stoma lumen) or the exterior surface (attachment side for the stoma muscles). The present study makes use of dissection of whole nematodes to allow detailed analyses of the interior surface, combined with a novel extraction technique which allowed study of the exterior surface of the buccal cavity.

Material and methods

NEMATODES

Fixed *Prionchulus muscorum* (Dujardin, 1845) Wu & Hoeppli, 1929 and *Prionchulus punctatus* (Cobb, 1917) Andrássy, 1958 were obtained from slides courtesy of Mr. L. Samsoen who had cultured both species some years ago. *Clarkus sheri* (Mulvey, 1967) Jairajpuri, 1970 and *Mylonchulus minor* (Cobb, 1893) Andrássy, 1958 were obtained from samples taken on the Galapagos-archipelago, Isla Fernandina *(C. sheri)* and isla Española

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(M. minor) by one of us (AC) and identified by Mr. P. De Ley. Live *Mononchus aquaticus* Coetzee, 1968 (recovered from a soil sample from a water basin in a greenhouse) and *Anatonchus tridentatus* (de Man, 1876) De Coninck, 1939 (recovered from a soil sample from a lawn) were both from the botanical garden, Gent University.

Dissection

Nematodes collected live and intended for dissection were fixed in 4 % hot formaldehyde subsequently transferred to glycerol. Longitudinal or cross-sections of whole nematodes were made using a scalpel. Glycerol was preferred over water as the higher viscosity of glycerol prevented the nematodes from rolling while being cut. Cut nematodes were picked out, critical point dried and put on a standard SEM specimen stub with one side of the organism put on a glass rod and sputter-coated with gold.

Culture

Attempts were made to culture *A. tridentatus* and *M. aquaticus*. Both nematodes were extracted and after several times, washing in PBS (50 mM $Na2HPO_4$, 140 mM NaCl, pH 7.2) were transferred to NGM agar plates (Brenner, 1974) inoculated four days before with *Panagrolaimus superbus* (courtesy Dr. B. Sohlenius). Additional prey was added twice weekly. All cultures were kept at 22 °C; all stock cultures were kept at 10 °C. Only *M. aquaticus* was cultured successfully, but *A. tridentatus* specimens could be kept alive for several days, which allowed observation of their feeding habits.

Buccal cavity extraction

The buccal cavity was extracted from living animals using 5 ml ß-mercapto-ethanol (Merck, analytic grade) and 2 g sodium dodecyl sulphate (SDS) (BDH Ltd, Poole, England; product 44244) in 100 ml distilled water. The solution was kept in the dark at 4 °C. As both chemicals are poisonous all subsequent steps were carried out in a fume hood. Prior to use the solution was shaken vigorously and 10-15 ml heated to 55-60 °C. Nematodes were cut in half and a few drops of the warm solution were added. Although the solution acts quickly, nematodes were left in the solution (in a closed vessel to prevent evaporation) overnight to allow for maximum dissolution of tissue. To extract the buccal cavity, two methods were used. Either needles were used to tear the cuticle from the buccal cavity, or the cuticle was removed by dissolving it using needles dipped in NaOCl. Because the latter entailed the risk of damaging the buccal cavity itself, physical force was preferred to remove the cuticle. In some instances the buccal cavity, once extracted, was cut longitudinally or a cross-section was made using a scalpel, to allow the interior surface of the buccal cavity to be viewed with as much as possible of the buccal cavity remaining. Because crystals can be formed during drying of the solution, the buccal cavities were transferred to water and then mounted on standard specimen stubs and sputter-coated. Scanning was done using a Jeol JSM-840. Because the extraction solution only acts on non-fixed tissue, *P. punctatus, P. muscorum, C. sheri* and *M. minor* were dissected while, *M. aquaticus* and *A. tridentatus* were both dissected and used for extraction of buccal cavities. Fluorescent staining was done on *M. aquaticus* only.

Nerve staining

Three approaches were used; *i)* FITC staining was done according to Hedgecock *et al.* (1985); 0.8 mg FITC (Sigma) was dissolved in 1 ml PBS and centrifuged to remove undissolved particles. Final concentration of FITC was 0.4 mg/ml; *(ii)* A stock solution of DiO (3, 3'-diotadecycloxacarbocyanine perchlorate) (Bioprobes Inc., Eugene, OR) was made by dissolving 3.0 mg in 0.1 ml DMSO and 0.9 ml ethanol (100 %); the solution was sonicated extensively to break up undissolved particles and the solution was centrifuged to remove any remaining particles; the final concentration DiO used was 200 µg/ml; *(iii)* Diamidino yellow (Sigma) was dissolved in PBS. and applied to the nematodes at final concentration of 2 %. For staining, nematodes cultured on the NGM agar plates were washed off and rinsed three times in ice-cold PBS. Incubation times varied from 2 h to overnight. Because incubation at room temperature caused fluorescent staining of the pharynx and intestine as a result of feeding, making observations of the nerves more difficult.

Results

Incubation with the ß-mercapto-ethanol – SDS mixture effectively eliminated all soft tissue (Fig. 1 A). In addition to the buccal cavity, the body cuticle and the lining of the pharynx also remained intact, necessitating manipulation to prepare the buccal cavity for SEM observations. The length of the incubation period did not affect the resolution of the fine details on the surface of the buccal cavity.

Inner surface

The dorsal plate carries one massive, forward pointing tooth in *P. muscorum, P. punctatus, C. sheri, M. aquaticus* and *M. minor* and a smaller, posteriorly pointing tooth in *A. tridentatus*. The form of the dorsal plate is essentially similar in all species investigated, except for the position of the tooth in *A. tridentatus*. The dorsal plate is straight but at the anterior side the plate is bifurcated with both resulting lobes curving towards the centre. At the frontal side of the buccal cavity and underneath the lips lie twelve small liplets, two above each of the six lobes (Fig. 1 B). The interior surface of these two lobes is marked with fairly deep longitudinal grooves. Just underneath the lobes and above the dorsal tooth the inside surface is almost not smooth although on some photographs fine ribs can be seen running perpendic-

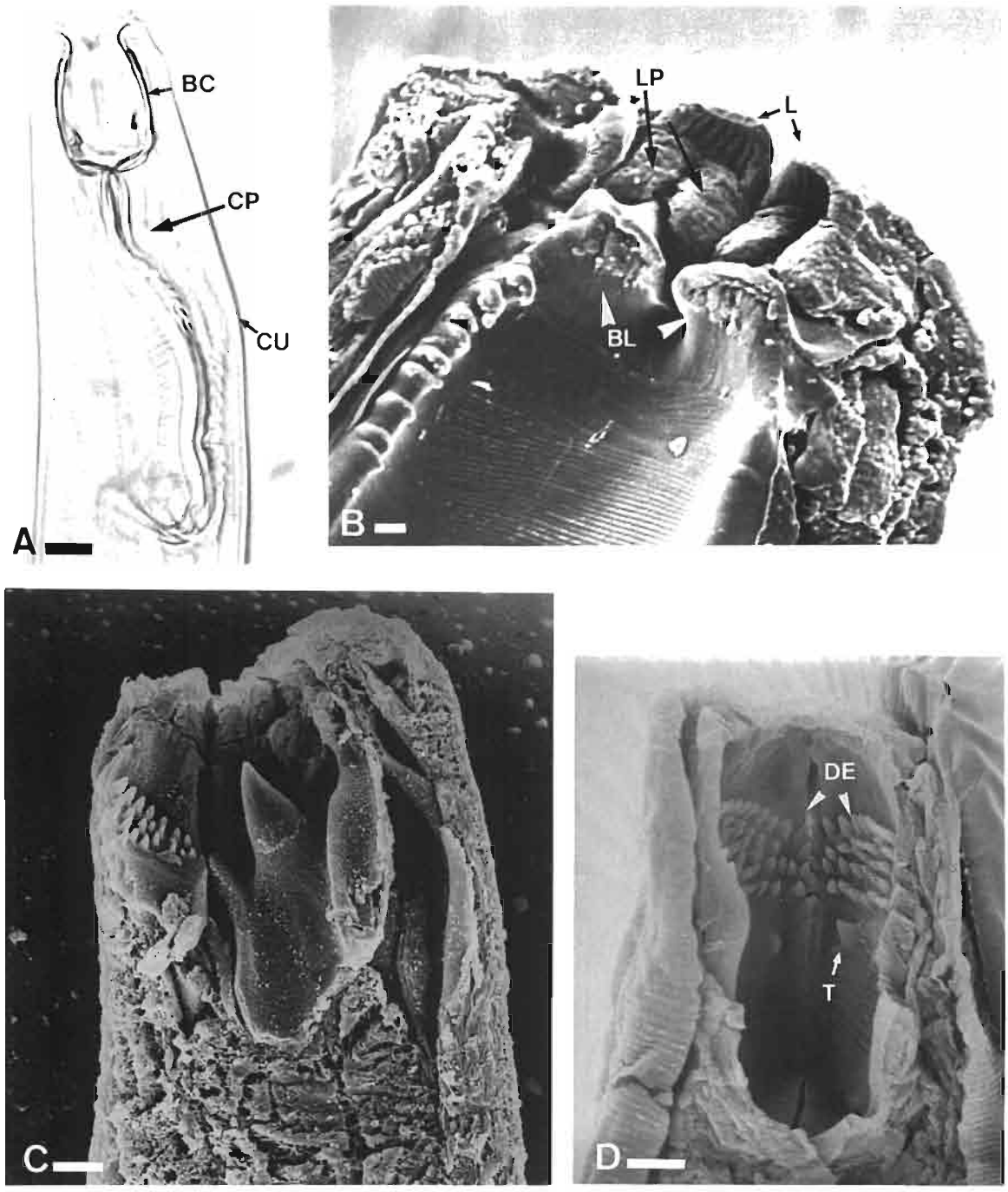

Fig. 1. *A : Light microscope view of the anterior of an* Anatonchus tridentatus *juvenile after incubation with 5 % SDS and 2 % ß-mercapto-ethanol; cuticular lining of the pharynx and body cuticle remain; B : View of one of the ventrosublateral plates of the stoma after dissection. Underlying the lips are the smaller liplets. The interior surface is decorated with fine transverse rings, the bifurcated lobes have longitudinal grooves; C :* Mylonchulus minor *dorsal plate of the stoma and associated tissue; the dorsal tooth sits atop a smooth massive base; D : Subventral teeth and denticles on the ventrosublateral plates of the stoma. (BC = buccal cavity; BL = bifurcated lobes; CP = cuticular lining of the pharynx; CU : body cuticle; DE = denticles; L = lips; LP = liplets; T = subventral tooth.* Bars; A = 10 µm; B = 1 µm; C, D = 5 µm.)

ular to the grooves on the lobes (Fig. 1 B). The dorsal tooth in *M. minor, P. punctatus, P. muscorum* and *C. sheri* and the posteriorly directed tooth in *A. tridentatus* sits atop an elevated, smooth, massive base. The tooth is clearly offset from its base (Fig. 1 C). The remainder of the dorsal plate is straight *(A. tridentatus, P. muscorum, P. punctatus)* or more or less curved *(M. minor,* and *M. aquaticus)*. This area is decorated with fine, well pronounced transverse ribs, visible in light-microscopy in all species.

VENTROSUBLATERAL PLATES

The basic structure of the ventrosublateral plates is essentially the same as the dorsal plate in all species. This is especially evident in *A. tridentatus* where both plates contain a single posteriorly directed tooth. In all other species the interior decoration is different with, instead of a single massive tooth, smaller teeth or denticles differing among species in number, size and position. In *M. minor* there are five to seven rows of small, pyramidal denticles opposite the dorsal tooth, each plate containing between twelve and fifteen in each row (Fig. 1 D). More posterior there are two larger, more flattened subventral teeth, one in each plate with the longitudinal axis pointing away from the centre (Fig. 1 D). At their junction both ventrosublateral plates form a double longitudinal ridge. In *P. punctatus* and *P. muscorum* this ridge bears six to eight small denticles on each side (Fig. 2 A, B). In *C. sheri* no teeth or denticles were observed.

JUNCTIONS

The junctions between the three vertical plates of the buccal cavity differ to a considerable extent in the different species studied. Sometimes the junction is no more than a line, sometimes gaps are present. In some instances the line denoting the junction disappears in the mid region and reappears somewhat posteriorly. The vertical plates join each other at the bottom of the buccal cavity, where the vertical plates – basal plates transition is located (Figs 2 B; 6 A, B).

BASAL PLATES

The general structure of the basal plates is the same in all species studied. The difference in ornamentation of the vertical plates is not reflected in the basal plates. The ribs on the latter are orientated more fan-like (Fig. 2 C) and they are characterized by the presence of foramina. Each dorsal plate has one, each ventrosublateral plate contains two. In the dorsal wall the foramen is situated near the junction of vertical and basal plate. In the ventrosublateral walls, one is situated near the junction and one inside each basal plate. The foramina lie on the median axis of each sector. This arrangement is present in all species studied here. In *M. minor* some tissue (?) could be seen protruding from the foramina, in other species this was not so and in most cases the foramina were difficult to localize when observed from the inside. The junctions between the vertical buccal and oblique basal plates are clear in *A. tridentatus* (Fig. 2 D) and obscure in the other species studied (Fifs 2 C; 6 A, B).

OUTER SURFACE

As a result of the dissolution of the surrounding tissue and removal of the body cuticle, only the buccal cavity and the cuticular lining of the pharynx remained, the latter firmly attached to the posterior end of the buccal cavity (Fig. 3 A). Attempts to separate both were successful only when considerable physical force was exerted, resulting in some damage to the buccal cavity. At the anterior side of the buccal cavity a similar problem was encountered at the site where the body cuticle is attached to the buccal cavity. Also there it was impossible to remove all cuticle or tear it away without damaging the buccal cavity. The result was that a small piece of body cuticle remained attached to the buccal cavity and in most cases collapsed on the frontal side.

The buccal cavity when viewed from the outside looks like a unit (Fig. 3 A) which can be subdivided into two distinctive areas; the most anterior part resembles a " cap " put on top of a " cask ". This cap smoothly curves inward anteriorly, but forms six protruding solid ridges at the top (Fig. 3), one between each lip. These ridges do not show any ornamentation, although it cannot be excluded that body cuticle covers part of the structure. Just posterior to these ridges the outer surface is covered with rough, longitudinal grooves. More posteriorly the cap is transversely striated (Figs 3 D; 4 A, B, D). Prominent are the three " hinges " which join the buccal plates (Figs 2 B, D; 4 A, B). The " hinges " have two rounded knobs opposite the transversely striated posterior margin of the cap (Figs 4 A; B B). At the level of these knobs, the fine striations of the buccal plates have an irregular pattern (Fig. 4 C). Some make a 180° turn which coincides not only with the rim of the plate as observed in specimens where the hinge was no longer present, but also with the transition between the cap and the remainder of the buccal cavity.

The *en face* view of the extracted buccal cavity shows a starlike pattern with, centrally, the mouth unfortunately partly covered with body cuticle. It is unclear where exactly the cuticle is attached to the buccal cavity. This uncertainty is compounded by the fact that ripping off the cuticle at the anterior end of the buccal cavity also led to the removal of parts of the " cap " (Fig. 5 A).

On the exterior surface the position of the large teeth can be observed. Most of the time a circular or oval opening is present (Fig. 5 B). However, in some of the specimens studied this opening was closed (Fig. 3 B).

Attachment sites of muscles to the outer surface of the buccal cavity were not detected.

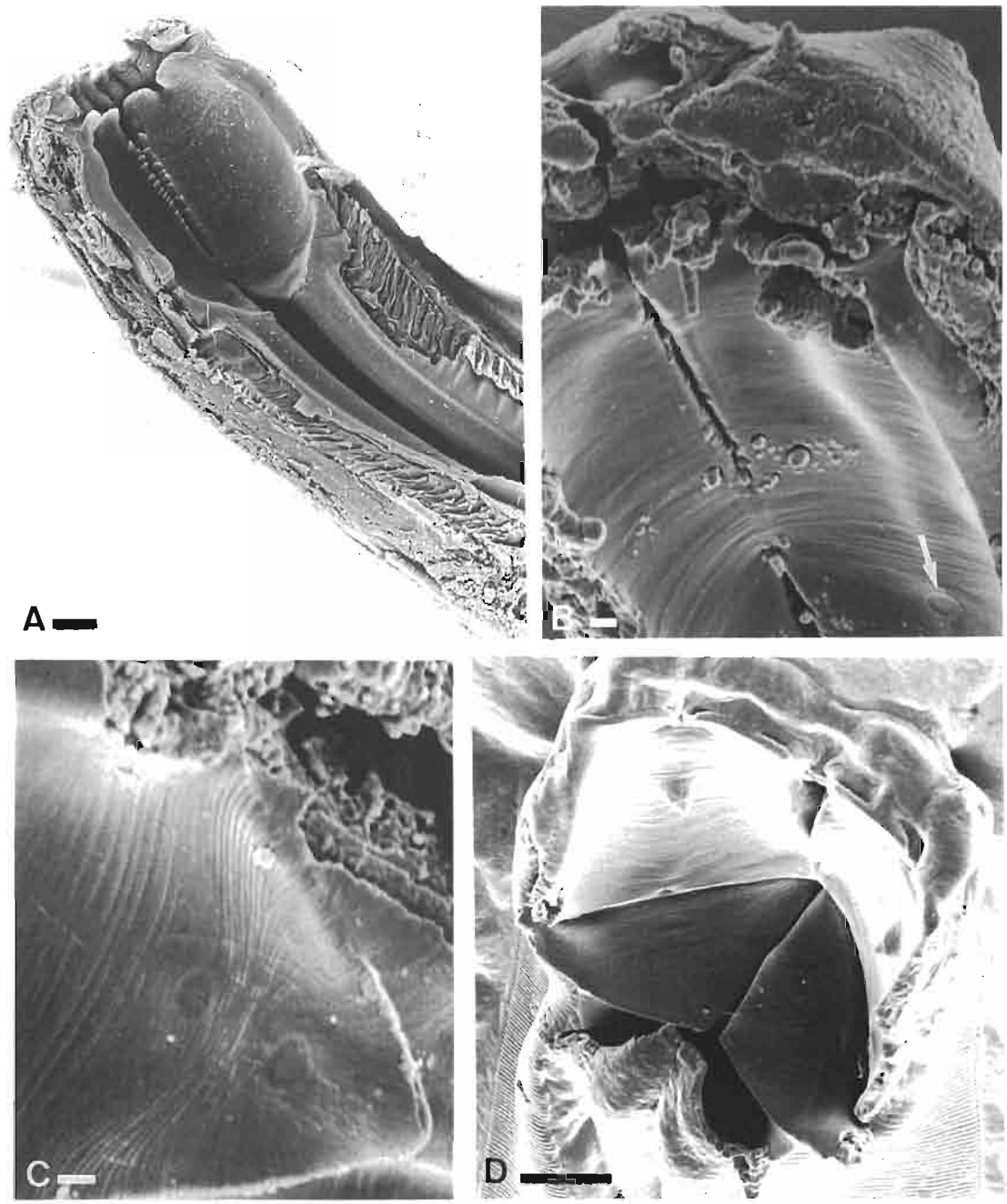

Fig. 2. *A :* Prionchulus muscorum *stoma showing dissected ventrosublateral plates carrying denticles at their junction; B :* P. muscorum : *stoma plates not continuously separated. Foramen observed at lower right as a slight depression (arrow); C :* Prionchulus punctatus *detail of ventrosublateral foramina (Note that transverse rings on the basal plates fanning out from the centre of the plate; no distinct junction present between vertical buccal and basal plates); D :* Anatonchus tridentatus : *in contrast to the previous image, the junction vertical-basal plates is obvious; the most anterior foramen is positioned at the base of the vertical plates, whereas in C it is at the junction.* (Bars : A, D = 5 µm; B, C = 1 µm.)

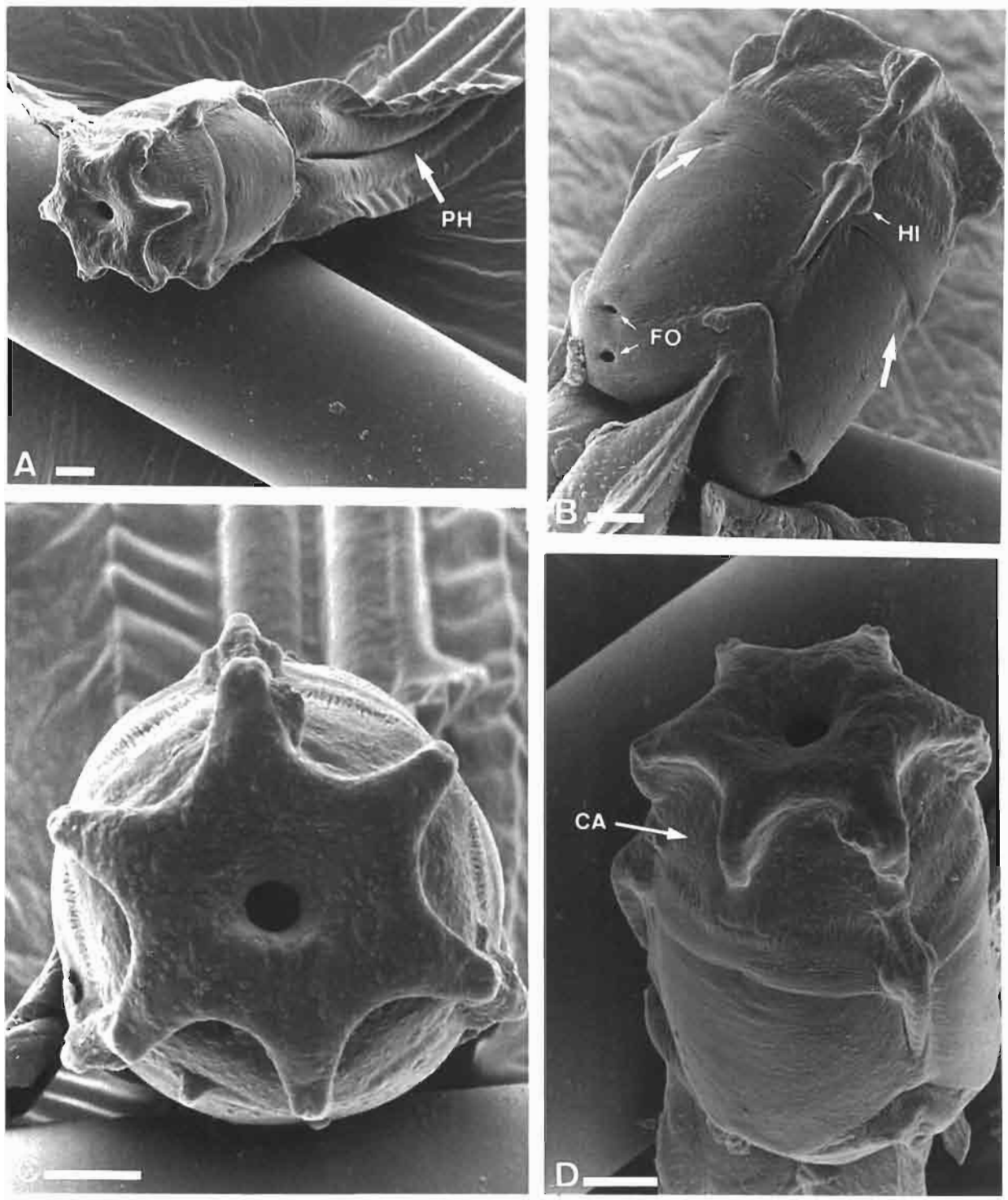

Fig. 3. *A :* Anatonchus tridentatus, *general view of buccal cavity and cuticular lining of pharynx after dissolution of soft tissue and removal of body cuticle; the most anterior cuticle probably collapsed on the buccal cavity; B : Detail at level of the junction between ventrosublateral and dorsal plate; the position of the teeth is visible, although closed (arrow); C : en face view; the six ridges and the three hinges are prominent; body cuticle probably covers most of the mouth; D : Position of the hinge; appearing more clearly is the " cap ".* (CA = cap; FO = foramina; HI = hinge; PH = pharynx. Bars 5 μm.)

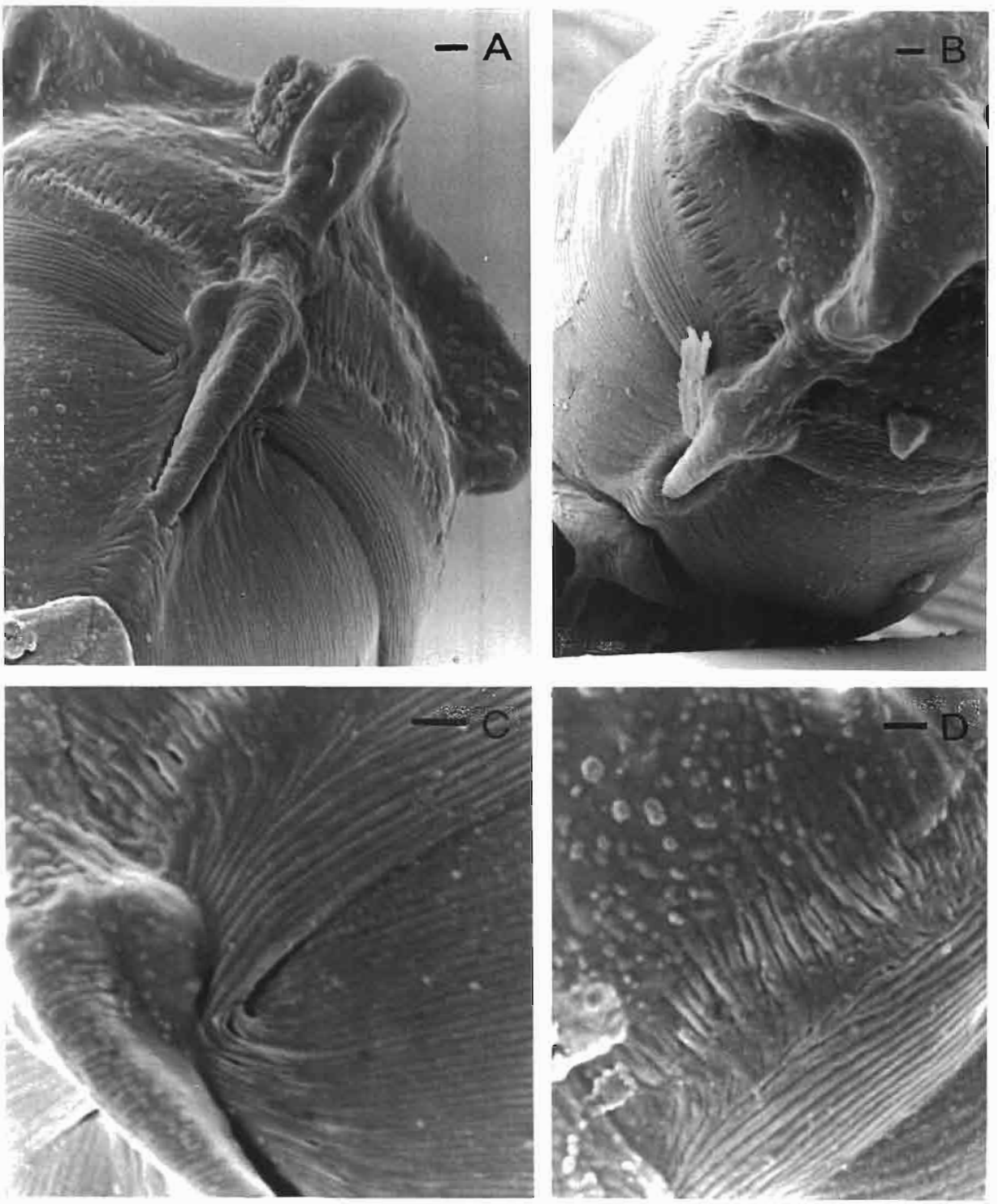

Fig. 4. Anatonchus tridentatus *exterior surface of buccal cavity. Detail of hinge structure. A : Posteriorly; B : Anteriorly; C : Detail of the 180° turn of the striations at the level of the two rounded knobs; D : Detail of grooves and striations on the "cap".* (Bars = 1 µm.)

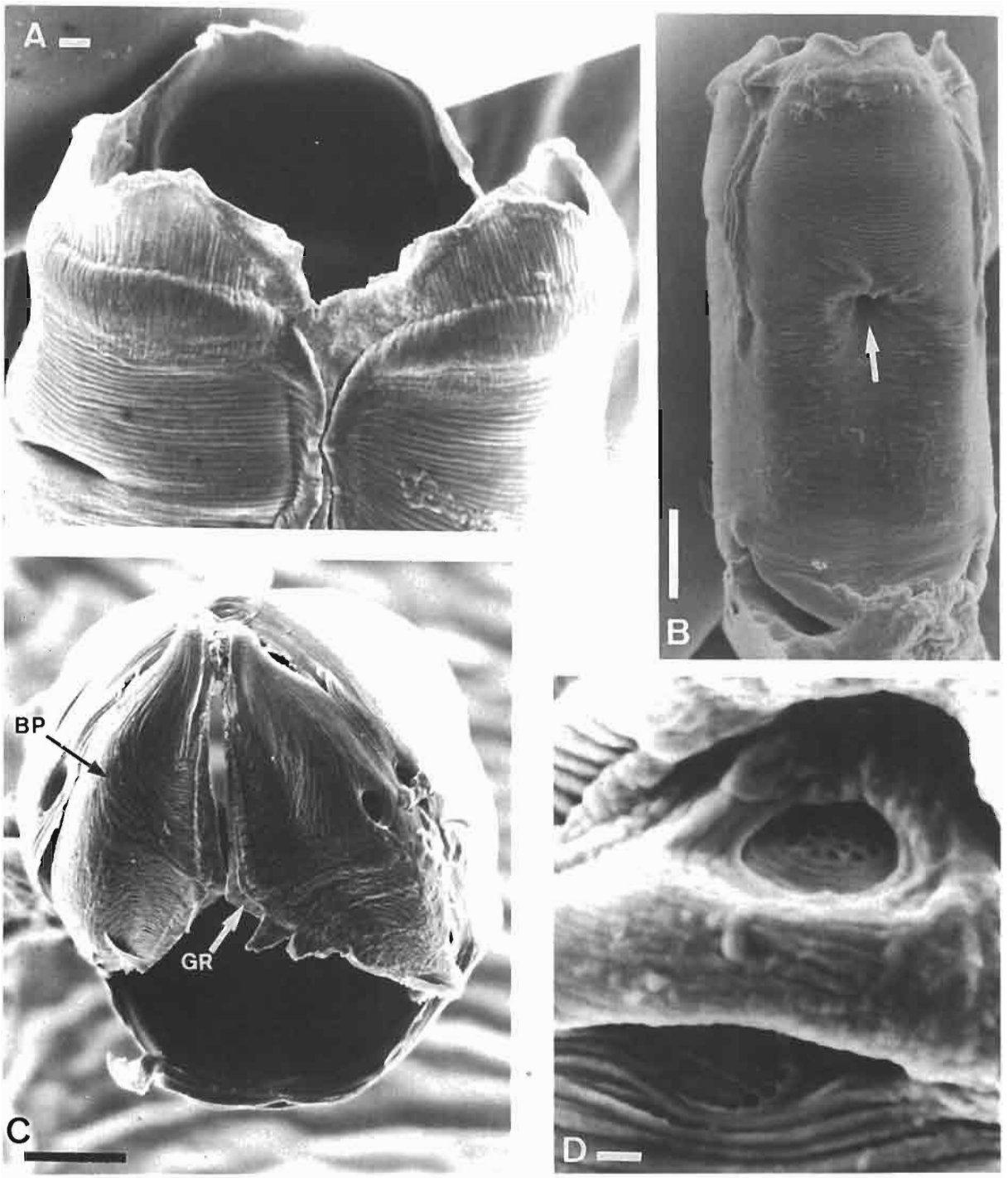

Fig. 5. Mononchus aquaticus. *A : View of the anterior part of the buccal cavity after forceful removal of the body cuticle; part of the bifurcated lobes and all of the more anteriorly positioned structures have gone; B : At the position of the dorsal tooth a distinct opening (arrow) is visible; C :* Anatonchus tridentatus. *Posterior view of extracted buccal cavity showing groove-like closing structure of basal plates; D : Detail of the honeycomb structure of the foramina. (BP = basal plate; GR = groove.* Bars : A = 1 μm, B, C = 5 μm, D = 0.5 μm.)

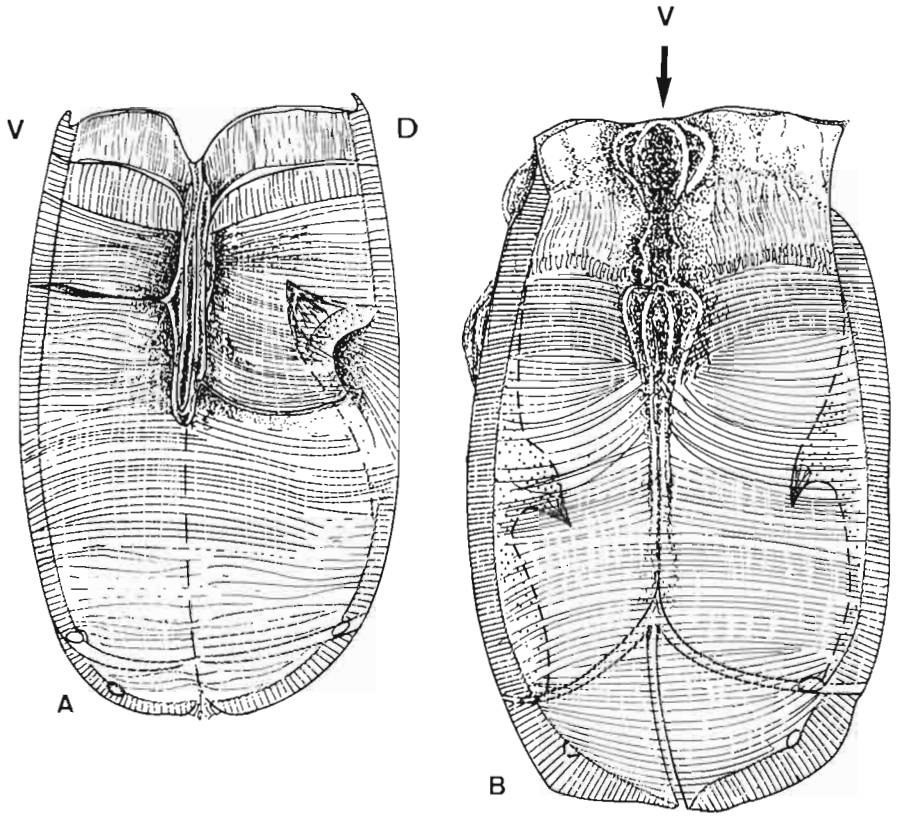

Fig. 6. *A : Buccal capsule of* Mononchus aquaticus *in left lateral view. (V = ventral; D = dorsal); B : Buccal capsule of* Anatonchus tridentatus *in ventral view. (V = ventral).*

THE BASAL PLATES

The striations of the outer surface of the basal plates are more pronounced than on the interior surface and can be considered as a continuation of the rings present on the vertical wall of the buccal cavity. The attachment of the cuticular lining of the pharynx to the buccal cavity is extensive. The three radii of the pharyngeal lumen reach the base of the buccal cavity, thereby covering the junctions between the basal plates (Fig. 3 B). At least in *A. tridentatus* it was observed that the rims of the basal plates form a groove allowing adjoining plates to fit in (Fig. 5 C). Also in *A. tridentatus* the corners of the plates are all linked to one another.

The foramina are prominent features which are clearly seen as holes in the plates. The fine structure of the foramina was the same for both species studied *(A. tridentatus* and *M. aquaticus).* Observed from the outside, the foramen appears circular or oval. Inside the opening, the surface forms a depression where it exhibits honeycomb-like perforations (Fig. 5 D).

Attempts were made to stain putative nerves associated with the foramina using three different fluorescent stains. Diamidino yellow produced very little fluorescent staining and was therefore discarded. The two other stains, DiO and FITC, did stain nerves associated with amphids (data not shown) but nothing could be discerned at the level of the foramina.

Discussion

Using SEM in combination with dissection and extraction of the buccal cavity of several mononchid nematodes, it has been possible to study the outer and inner surfaces. The technique employed for the extraction has the advantage that " hard " structures within the nematodes remain unaffected as evidenced by the fine detail visible on the outer surface.

The " hinges " described above are in fact the shallow radii of the buccal cavity (compare Fig. 5 B with Fig. 3 E in Coomans & Lima, 1965) seen from the outside.

Siddiqi (1983) attributed a chemoreceptory function to the foramina (called geusids) but without supportive evidence. Grootaert and Wyss (1978) using TEM, mentioned that in *M. aquaticus* nerves were observed " in the region " although no direct link between these and the foramina was evident. SEM offered no additional information about the structures associated with or the function (chemoreception, proprioreception) of the foramina. Attempts to use fluorescent stains to determine any nerve endings associated with the foramina

failed. Probably because the foramina are positioned deep into the buccal cavity and staining at 5 °C, to block feeding, prevented stain reaching the foramina.

The junction between the vertical and the oblique basal plates of the buccal cavity is another point of interest. The difference between the junction of *A. tridentatus* and the other species studied reflects the basic difference between the two groups of mononchs. From the structure of the junctions it can be deducted that *A. tridentatus* has more flexible basal plates. Although the initial phases of feeding is the same in both species, there is a considerable difference in the way the prey is treated. *M. aquaticus* pierces its prey open and then sucks on the (outflowing) contents. *A. tridentatus*, however, swallows its entire prey in several powerful sucking movements, a feeding habit rarely observed in *M. aquaticus* and only when the prey is small. If swallowing whole prey is the standard mode of feeding, as it seems to be in *A. tridentatus*, then the structure of the buccal cavity and position of the teeth might be given a functional explanation. Posteriorly directed teeth might be more suited to hold the prey in the buccal cavity between two sucking cycles. The distinct junctions between vertical and oblique plates might allow more flexibility needed for swallowing a larger prey. Both adjustments would not be necessary if feeding require ripping the prey open (with a large, anteriorly directed tooth) and sucking on the soft contents. Observations reported in the literature lend support to this interpretation. In his review of prey of predatory nematodes, Small (1987) pointed to the wide range of prey identified in *A. tridentatus* species and proposed that this difference is due to the larger size of *A. tridentatus* allowing not only a wider range of prey but also complete ingestion. Coomans and Lima (1965) reported on the gut content of *A. amiciae*, mentioning the presence of juvenile *Mononchus*, juvenile and adult tylenchs and dorylaims and the odontostyle of a *Xiphinema*. Arpin and Kilbertus (1981) did an extensive ultrastructural study of the gut contents of several Mononchida (e.g. *M. minor*) and reported that only parts of nematodes could be found. Similar observations were reported for *M. aquaticus* by Grootaert and Maertens (1976).

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