The fine structure and function of the cephalic appendages of the branchiuran parasite, *Argulus japonicus* Thiele

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CONTENTS

SUMMARY

The fish parasite *Argulus japonicus* Thiele (Crustacea: Branchiura) has recently been introduced into Britain and is now established in the wild. *A. japonicus*, an ectoparasite attaching to, and feeding on, the skin of its host, is a potentially serious pathogen of native freshwater fishes. The anatomy of the attachment and feeding structures is described using light and electron microscopy.

The primary attachment organs are the suckers derived from the maxillules. The extrinsic musculature of the suckers comprises two major muscle groups: the suction muscles which insert on the floor of the sucker and generate suction, and the cup muscles which control the orientation and movement of the sucker as a whole. The inner wall of the sucker cup comprises two hoops of thickened cuticle and provides the rigidity necessary to prevent the sucker from collapsing. These hoops are hinged to allow extra movement of the distal hoop plus its marginal membrane when forming a seal onto the surface of the host. Numerous mucous glands are present in the floor of the sucker.

The elongate mouth tube represents a ventral outgrowth of the head bearing a small labrum and labium distally. The homology of the labium is confirmed by the arrangement of its paired muscles, which originate on the undersurface of the ventral cephalic tendon and pass down through channels in the suboesophageal ganglion. The labrum lacks muscles. The ontogeny of the mouth tube, the adult mandibular musculature and the possible feeding mechanism are described.

The preoral spine lies in the ventral midline of the body anterior to the mouth tube. It consists of a tapering spine carried on a long eversible sheath. When fully retracted the spine and sheath virtually disappear into the body. As the spine retracts the cuticle of the proximal sheath becomes inverted. The epithelium beneath this sheath cuticle is syncytial and is separated from the cuticle-lined, central duct of the spine by a fluid matrix which can be displaced as the spine is retracted. The fluid matrix appears to be secreted by the epithelial cells of the sheath cuticle. Retraction is by means of paired retractor muscles which originate dorsal to the midgut and pass down through the nerve ring. These muscles shorten to about 25% of their maximum length during extreme retraction.

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1. INTRODUCTION

Argulus japonicus Thiele, a serious pathogen of freshwater fishes, has recently been introduced into Britain and is now established in the wild (The Sunday Times, 17 June 1990; Rushton-Mellor 1992). *Argulus* Müller is the only genus of branchiuran found in Britain and was previously represented by two indigenous species *A. foliaceus* (L.) and *A. coregoni* Thorell (Scott & Scott 1913; Fryer 1982). All three species were included by Fryer (1982) in his key to parasitic copepods and branchiurans of British freshwater fishes. *A. japonicus* was included because it is now widespread in continental Europe and Fryer considered its introduction to this country likely. Its distribution in Britain suggests that it was accidentally introduced on Koi carp, *Cyprinus carpio* L., imported to stock ornamental ponds and lakes, and has subsequently escaped into natural waterways.

Argulus species are highly pathogenic. The feeding activity of these parasites causes lesions which are susceptible to secondary, often fatal, bacterial and fungal infections (Chen 1933; Becker 1941; Dogiel *et al.* 1961; Kabata 1970, 1985; Hoffman 1977; Andrews *et al.* 1988). Toxic secretions from the preoral spine gland cause haemorrhaging in the host's skin (Shimura & Inoue 1984), and Kabata (1970) citing Becker (1941) claimed that these secretions caused a severe inflammatory response, especially where many parasites settled close together. Ahne (1985) also noted that the Spring Viraemia disease (infectious dropsy) of Koi carp was transmitted from fish to fish by the parasite's intermittent feeding behaviour.

There have been several substantial accounts of the anatomy and morphology of *Argulus*, including Claus (1875), Leydig (1889), Guberlet (1928), Martin (1932), Meehean (1940), Debaisieux (1953) and Madsen (1964). However, none of these has examined the musculature of the cephalic appendages in sufficient detail to indicate the mode of action of each structure. These accounts also provide conflicting data on important features, such as the function of the preoral spine (Ivanfi 1926; Martin 1932) and the location of the preoral spine glands (Debaisieux 1953; Madsen 1964). This paper examines the structure and function of the attachment and feeding mechanisms of *A. japonicus*. The gross and fine structure of the suckers, raptorial appendages, preoral spine and mouth tube are examined by light and electron microscopy and behavioural observations were made on live *Argulus*.

2. MATERIALS AND METHODS

Live *A. japonicus* were obtained from Koi carp in the ponds of Koi keepers in the South East of England. Observations were made on animals in a drop of water on cavity slides. Cleared, stained exoskeletons were prepared by fixing parasites in 80% alcohol, immersing them in a weak potassium hydroxide solution for 24 h then clearing and staining in indigo-carmine in lactophenol.

Transverse and longitudinal serial (8 μm) sections were made of specimens embedded in paraffin wax and stained by Masson's trichrome and Alcian blue–P.A.S techniques. Some reconstructions from serial sections were made using PC3D version 5.0, a three-dimensional reconstruction computer program published by Jandel Scientific. Specimens examined by transmission electron microscopy were fixed in 3% gluteraldehyde–phosphate buffer for 90 min, washed in buffer, and fixed and stained in 1% osmium tetroxide for 60 min. After washing in buffer, they were stained in 2% aqueous uranyl acetate in the dark for 60 min, dehydrated through a standard acetone series, processed through TAAB resin and acetone solutions, vacuum infiltrated in pure TAAB resin and cured at 65°C for 48 h. Blocks were sectioned at 90 nm intervals. Sections were stained with lead citrate and observed in a Siemens ELMISKOP 101 transmission microscope. The specimen from which most transmission electron micrographs were taken was at an early stage of proecdysis. All the cuticle layers are therefore double, with a thin layer of new cuticle visible beneath the thicker, old cuticle.

Specimens for scanning electron microscopy were fixed and stained in gluteraldehyde and osmium tetroxide, cleaned by washing in a very weak detergent solution for 15 min, sonicated in the same solution for 10 s, dehydrated in a standard acetone series, critical point dried, and sputter-coated with a microlayer of gold–palladium. Observations were made using a Hitachi S800 scanning microscope.

3. ATTACHMENT TO HOST

(*a*) *Hooks*

Three pairs of cephalic appendages, antennules, antennae and maxillae, carry hooks, spines or claws that are used for attachment to the host. The antennule is four-segmented (figure 1*b*). The first segment is triangular and drawn out into a spinous process at the medial angle. The second segment is elongate with anterior and posterior spinous processes, and terminates in a robust, ventrally orientated hook. Adduction which brings the hook on the second segment into contact with the host's surface is brought about by extrinsic muscles originating on the dorsal cephalic wall. The first two segments are heavily sclerotized, unlike the distal two which are thin-walled, slender, bear several terminal setae, and are primarily sensory.

The antenna is five-segmented (figure 1*b*). The rounded coxa is heavily sclerotized and possesses a stout spinous process at the posteromedial angle. No adduction–abduction movement of the coxa is possible and the spinous process probably serves only to prevent the *Argulus* from slipping over the host's surface. The short basis and three slender endopodal segments are weakly sclerotized and lack specialized spines or hooks but possess a small number of terminal setae.

The five-segmented maxilla is well-armed (figure 1*d*). The coxa is large with three robust spinous processes on the posterior margin, anterior to which is a raised area covered with small scales. The basis is

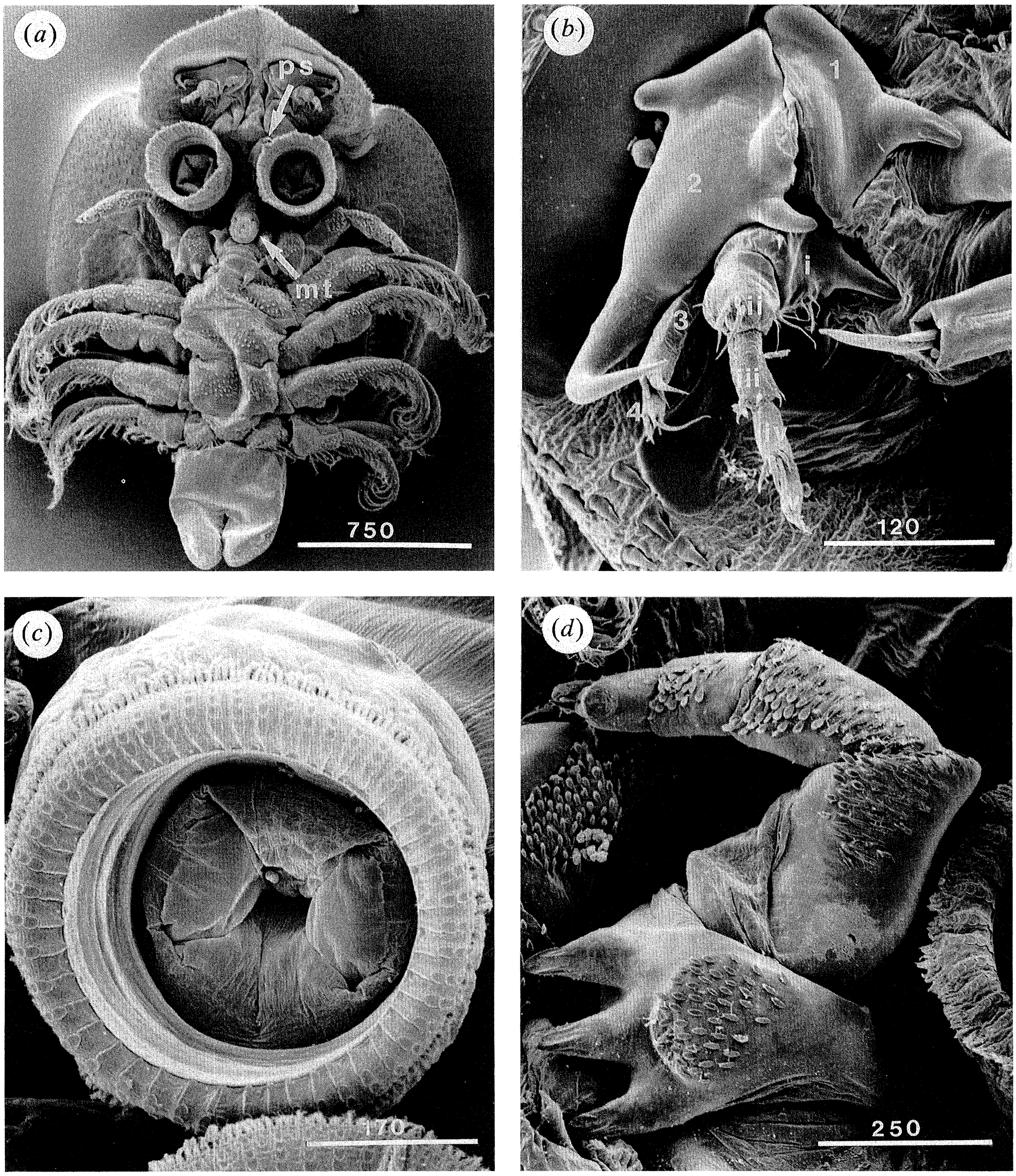

Figure 1. *Argulus japonicus*. (*a*) Male, ventral, showing suckers, preoral spine and mouth tube. (*b*) Antennule and antenna, ventral, with segments of antennule numbered 1 to 4 and those of antenna i to v. (*c*) Sucker, ventral, showing interior of sucker cup and marginal membrane with supporting rods. (*d*) Maxilla, ventral.

trapezoidal with no hooks or spines but possesses a patch of scales distally on the ventral surface. The three-segmented endopod terminates in a small claw and three short spines. The whole limb is raptorial and can be used for grasping the host. The ventral surface of each endopodal segment is ornamented with a patch of scales. These and the processes on the coxa increase friction with the host's surface.

(*b*) ***Suckers***

(i) *Anatomy*

The most important appendages for attachment to the host are the maxillules which are modified as suckers in adult *Argulus* (figure 1*a,c*). Each sucker comprises a wide, cylindrical stalk carrying a distal suction cup with an extensive, flattened rim, the marginal membrane, which is strengthened by radiating, rib-like supporting rods. The supporting rods comprise a series of sclerites, providing increased flexibility. The cuticle of the stalk is relatively thin and typically folded. The walls of the suction cup are lined with cuticle inside and out (figure 2*b*). The outer cuticle is thin, the inner, thicker and sclerotized. The inner wall provides the rigidity necessary to prevent the cup from collapsing. The sclerotized inner wall is divided into proximal and distal hoops separated by a ring of thin cuticle (see inset detail in figure 2*b*). The distal part overlaps the proximal to a small extent. This hinge mechanism allows the distal hoop, plus the marginal membrane which it carries, to move relative to the proximal hoop. The floor of the sucker cup has a thickened plate centrally, surrounded by an area of thin, flexible cuticle. This peripheral zone has abundant underlying mucous cells which stain strongly for acid mucopolysaccharide using the Alcian blue–P.A.S method. Pores from the mucous cells were not observed under scanning electron microscopy (SEM) although the folding of the floor of the sucker cup in fixed specimens may have obscured any pores.

The sucker musculature dominates cephalic anatomy. Extrinsic muscles originate on the dorsal wall of the cephalon and on the ventral cephalic tendon (figure 2*a*). The most powerful muscles are the suction muscles (figure 2*a*, suc m 1–5) which insert on thickened plates in the floor of the sucker. The typical course of a suction muscle in shown in figure 2. Suction muscle 1 has an elongate origin on the thickened ridge on the dorsal wall of the cephalon. It passes laterally and ventrally through the stalk and

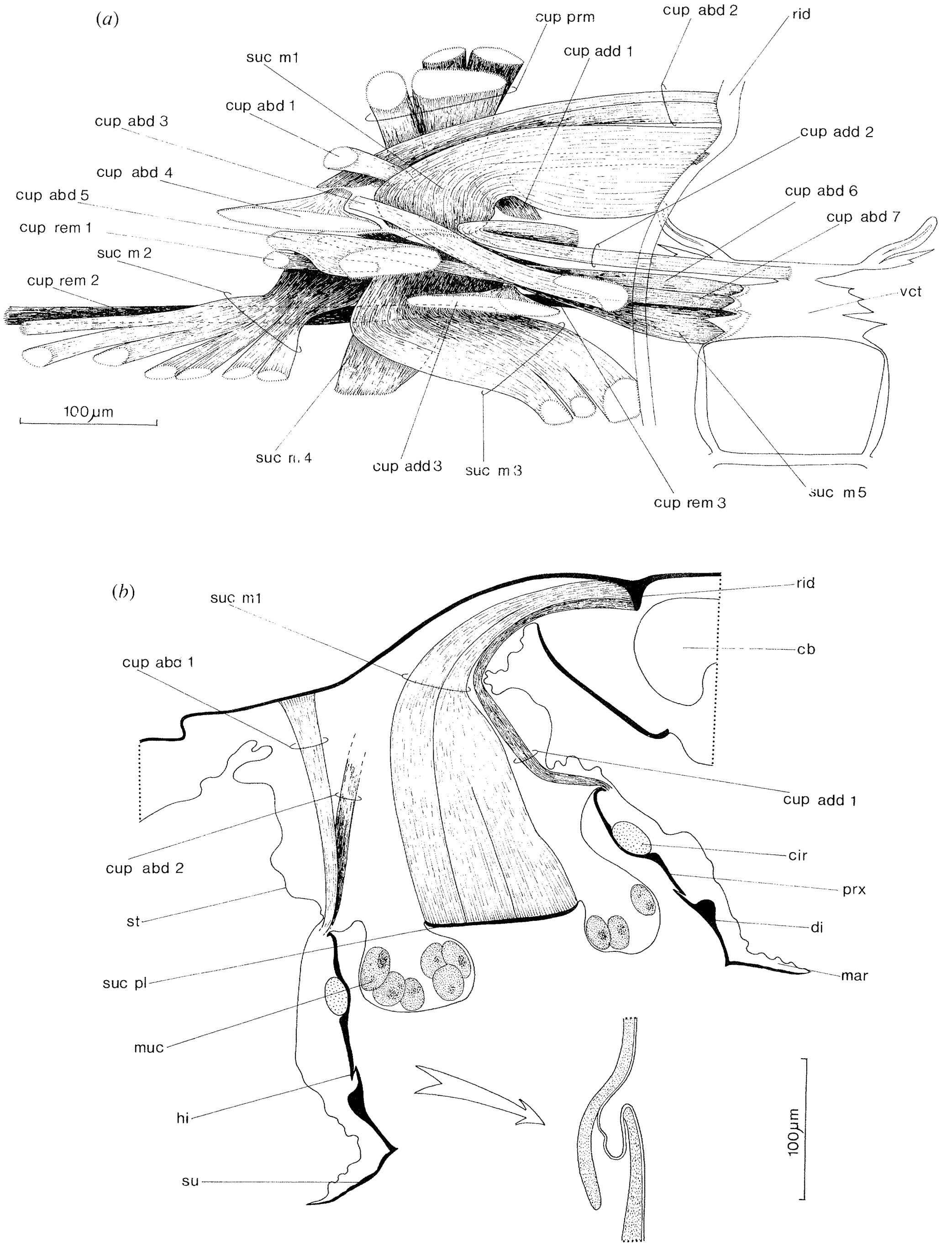

Figure 2. (*a*) Reconstruction of musculature of left maxillulary sucker, dorsal aspect based on serial transverse sections. (*b*) Transverse section through maxillulary sucker stalk and cup showing insertion site of large suction muscle on floor of cup. Thickened cuticle is indicated by solid black. Inset showing detail of hinge mechanism between proximal and distal hoops of thickened cuticle in inner cup wall.

down to its insertion on the central plate, increasing in diameter as it passes ventrally. Large suction muscles 2 and 3 each have a much divided origin on the dorsal wall. Suction muscle 5 originates on the ventral surface of the posterior section of the ventral cephalic tendon.

The second major group of sucker muscles, referred to here as the cup muscles (figure 2*a*), passes down through the stalk to insert around the base of, or in the wall of, the distal sucker cup. Some of these muscles originate on the ventral cephalic tendon but most do so on the dorsal cephalic wall. Those muscles (cup prm) inserting anteriorly tend to swing the cup forwards, those inserting laterally act as abductors (cup abd 1–7), and those inserting posteriorly (cup rem 1–3) and medially (cup add 1–3) swing the cup backwards and medially respectively.

Muscles are present in the wall of the sucker cup. Most are rather diffuse sheets of muscle fibres and appear to act as tensors between inner and outer walls of the cup. A well defined circular muscle (figure 2*b*, cir) extends in a ring around the base of the sucker cup, about in the middle of the proximal sclerotized hoop.

(ii) *Suction mechanism*

Suction is produced by the powerful suction muscles (suc m 1–5) which generate the negative pressure required by raising the floor of sucker cup. Suction is released presumably by relaxation of these muscles. Contraction of the circular muscle (cir) will constrict the base of the cup and also tend to release the suction pressure by reducing the volume of the cup. It may form part of the release mechanism for the sucker. The role of mucus in the attachment process is not confirmed. It probably enhances the seal between the sucker and the substratum.

Movements of the whole sucker are brought about by the cup muscles which insert all round the rim and can move it in any direction. They enable the suckers to be used alternately for a kind of walking locomotion. When the *Argulus* is attached to its host by means of the suckers contraction of all the cup muscles shortens the stalk and pulls the animal down onto the surface of the host. The thin cuticle of the stalk presumably folds in a concertina-like manner as the stalk shortens. The flexible stalk acts as a suspension mechanism changing the angle of the distal cup to match the surface contours of the host. The hinge separating proximal and distal hoops in the cup wall may act as an additional fine tuning mechanism allowing for small adjustments in position of the marginal membrane.

4. FEEDING

(*a*) *The mouth tube*

(i) *Anatomy*

The mouthparts lie between the suckers (figures 1*a* and 3*a*). The posterior part of the feeding apparatus is a proboscis-like mouth tube (mt) which, at rest, is posteriorly directed and lies flat against the ventral body wall. It consists of a long, cylindrical outgrowth of the body, at the distal end of which is the mouth. The mouth is enclosed by an upper labrum and a lower labium. The long mouth tube is given rigidity by the pair of internal mandibular apodemes (mnd ap) which extend virtually from its tip back into the head on either side of the cerebrum, and by a pair of rod-like thickenings, the buccal bars (bu br), in the external wall of the mouth tube (figure 4*c*). Proximally each buccal bar articulates with a medial process off a maxillulary sclerite (mxl scl). This pair of articulations marks the fulcrum about which the entire mouth tube is erected for feeding. The distal end of the buccal bar provides the firm lateral condyle (con) against which the mandible articulates. The oesophagus extends anteriorly from the buccal opening, along the length of the mouth tube before passing dorsally through the nerve ring formed by the cerebrum, circumoesophageal commissures and suboesophageal ganglion. It enters the midgut anteriorly and extends into the lumen of the midgut (figure 5). The lining of the oesophagus just inside the mouth opening is ridged longitudinally and ornamented with fine, inwardly directed spinules (figure 4*c*, spi).

The true labrum is represented by that part of the anterior wall of the mouth tube lying distal to the plane defined by the tips of the buccal bars. It is narrower than the labium and is embraced by it laterally (figure 3*b*). Externally the labrum is generally smooth but possesses three pairs of sensory papillae. The tip of the labrum is incised medially and the margins of this incision are fringed with dentate processes (figure 3*d*). The labrum is strengthened by sclerotizations in the form of a transverse labral bar (figure 4*c*, lab br).

The bulbous labium is much larger than the labrum and its outer surface is ornamented with crescentic rows of tiny spinules. It bears a pair of tubular labial spines (figure 3*d*, lam sp), each of which has an apical pore approximately 1.5 to 2.0 μm in diameter. The labial spines emerge from the medial incision in the labrum. The labium is supplied with a pair of labial muscles (figure 5, lam m) which originate on the ventral surface of the anterior part of the ventral cephalic tendon, pass posteroventrally through channels in the suboesophageal ganglion and insert, via a slender tendon, near the base of the labial spines.

The adult mandibles (figure 5, mnd) are represented only by the coxal gnathobases and are concealed within the buccal cavity except during feeding. Fracturing the mouth tube transversely (figure 3*c*) reveals the mandibles as heavily sclerotized structures entering the buccal cavity from a posterolateral origin. The mandibular gnathobase is armed with four teeth on the outer margin and a row of slender, comb-like spines extending along the inner and outer margins to the tip. The basal articulation of the mandible with the mouth tube is provided with extensive arthrodial membrane all round except where it articulates laterally with the condyle formed by the tip of the buccal bar (figure 4*c*). The mandibular musculature (figure 5) reflects the elongation of the mouth tube. A pair of mandibular adductors (mnd

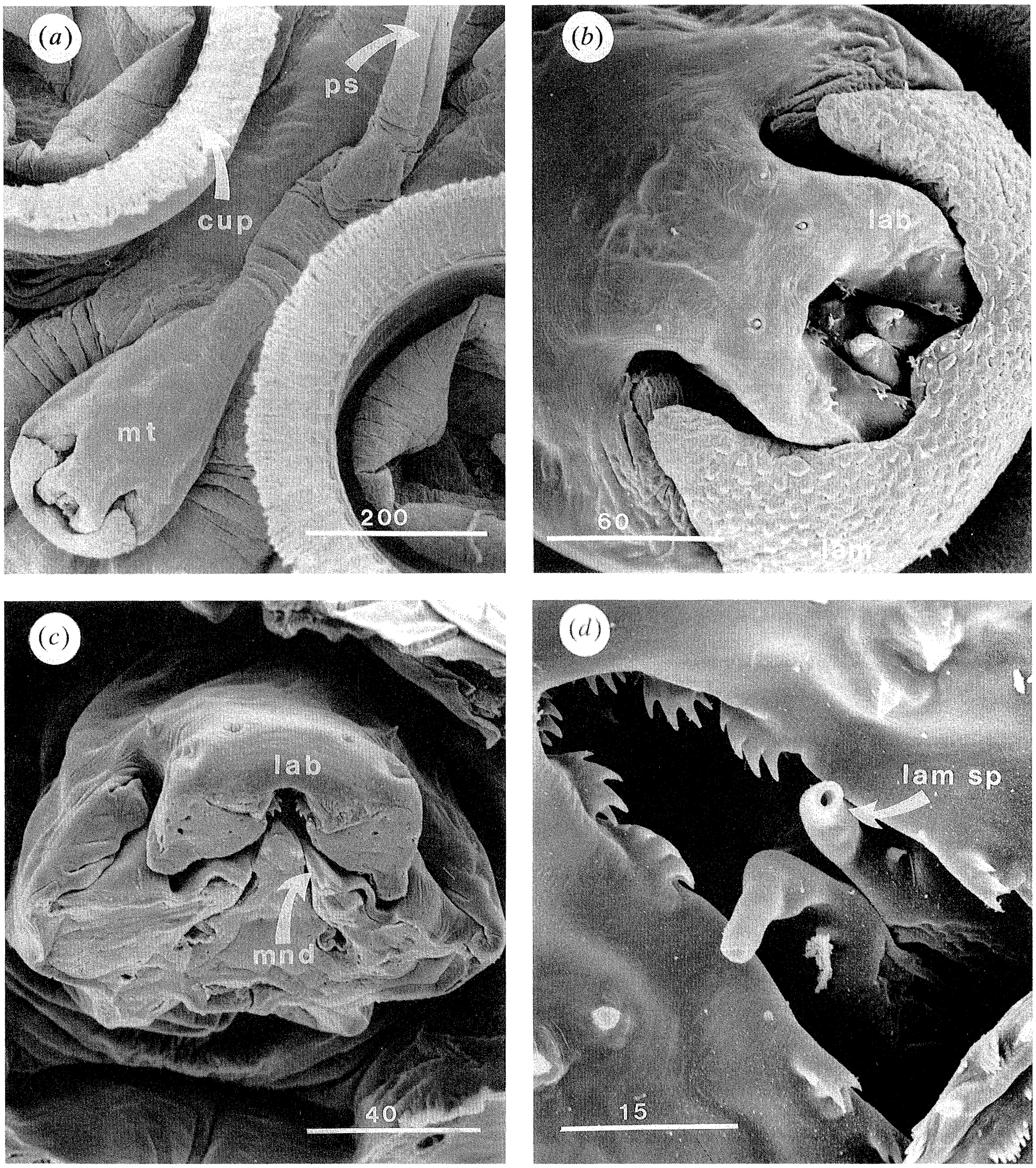

Figure 3. (*a*) Ventral view of oral area, showing mouth tube and base of preoral spine. (*b*) Apex of mouth tube, showing medial labrum largely enclosed by large labium. (*c*) Section through mouth tube from freeze-fractured specimen, showing mandibles entering mouth from sides. (*d*) Detail of tip of mouth tube, showing tubular labial spines and denticulate margin of labrum.

add 1) originates on the ventral surface of the ventral cephalic tendon on either side of the midline. These adductors follow a parallel course to the oesophagus, passing through the nerve ring and along the mouth tube. They insert medially on the proximal rim of the mandible. A pair of abductors (mnd abd) originates ventrally on the hollow anterolateral extensions of the ventral cephalic tendon. These also pass through the nerve ring with the oesophagus and finally insert laterally on the rim of the mandible, near its articulation with the distal tip of the buccal bar. The remaining direct mandibular adductors (mnd add 2) originate dorsally on the wall of the cephalon well anterior to the mouth tube. They pass posteroventrally either side of the optic nerves and into the mouth tube. Each comprises two main muscle groups lying just beneath the lateral wall of the mouth tube. These insert posteriorly on the proximal rim of the mandible, via a long tendinous section.

The mandibular apodemes arise at the proximal rim of the mandible. They are hollow for their entire length but have thick walls and are extremely rigid structures. They arise close to the midline but curve laterally and dorsally after a short distance. They

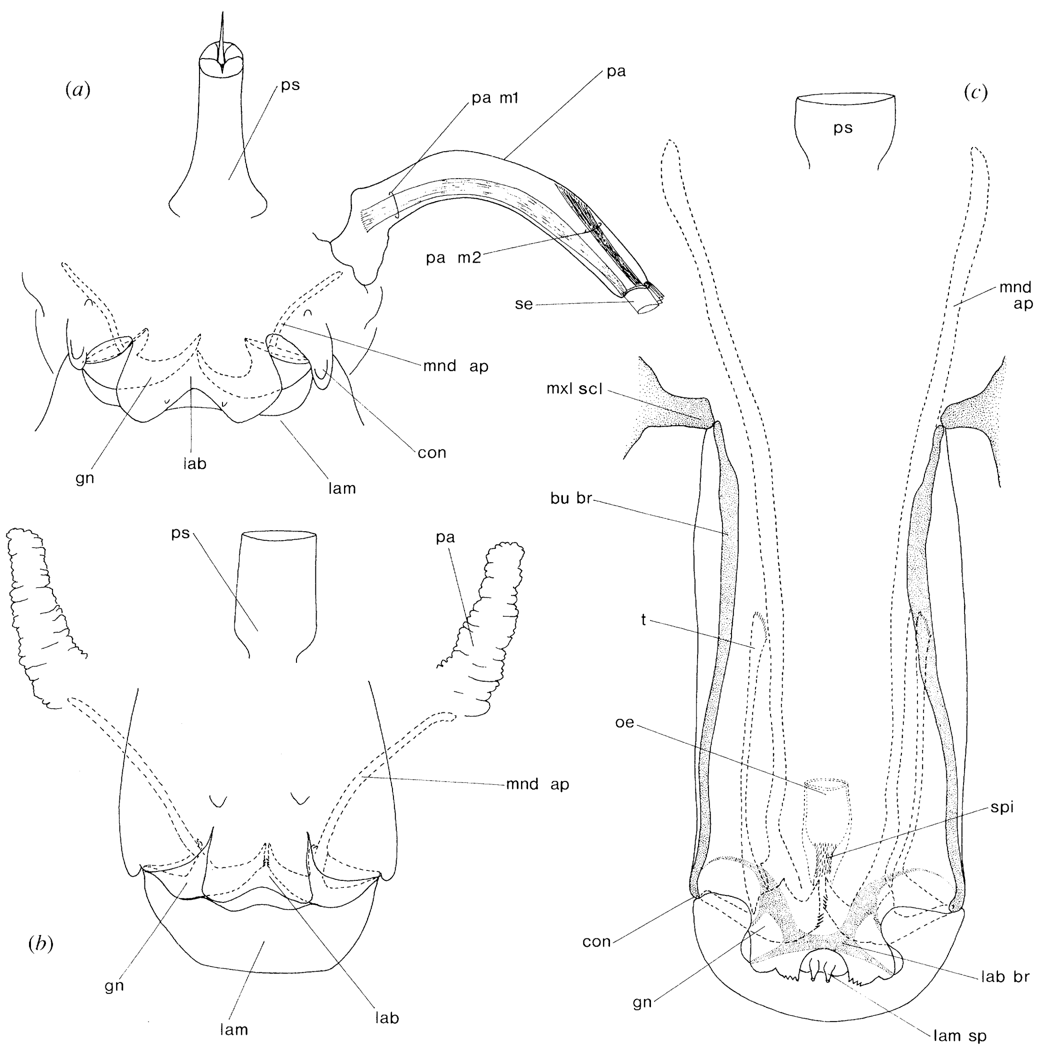

Figure 4. (*a*) Oral region of first stage larva, showing labrum and labium arising from ventral cephalic surface and functional mandibular palp. (*b*) Oral region of second stage larva, showing slight elongation of mouth tube and degenerate state of mandibular palps. (*c*) Mouth tube of adult drawn from KOH digested and cleared specimen, with thickened areas of cuticle stippled, showing elongate mouth tube supported externally by the buccal and labral bars, and internally by the mandibular apodemes.

diverge slightly as they pass anterodorsally up into the head, ending in a rounded tip on either side of the cerebrum. Three pairs of muscles insert on the apodemes. A pair of small erector muscles (figure 5, et m) originates anteriorly on the anterior processes of the ventral cephalic tendon and passes anteriorly to insert posterodorsally near the tip of the apodeme. A larger muscle (figure 5, mnd add 3) originates on the dorsal wall anteriorly and passes posteroventrally to insert on the tip of the apodeme. The third muscle, an abductor, originates lateral to the base of the mouth tube and inserts anteroventrally near the tip of the apodeme.

(ii) *Ontogeny of the mouth tube*

In the first larval stage of *A. japonicus* (figure 4*a*) the mouth tube is extremely short. The labrum and labium both arise directly from the ventral cephalic surface. The lateral margins of the mouth are defined by a pair of spinous processes. The mandibular gnathobases articulate with these condylar processes which are probably rudiments of the buccal bars. The mandibular apodeme is short, directed anterolaterally, and extends almost to the base of the mandibular palp. At this stage the mandible has a palp, a single, laterally directed segment armed with four long setae distally. A pair of intrinsic muscles extends and closes up the array of distal setae on the palp. After the first larval stage the palp degenerates.

Elongation of the mouth tube has begun by the second larval stage. The labrum and labium are carried on a developing ventral outgrowth (figure 4*b*). The lateral margins of the elongated mouth tube are slightly thickened but no well defined buccal bars are yet present. The mandibular apodemes are longer than in the first stage and the palp is now a shrivelled vestige, lacking setae and with atrophied intrinsic musculature.

Subsequent development consists mainly of elongation of the proximal part of the mouth tube and differentiation of laterally located buccal bars. The proximal end of each buccal bar articulates with a

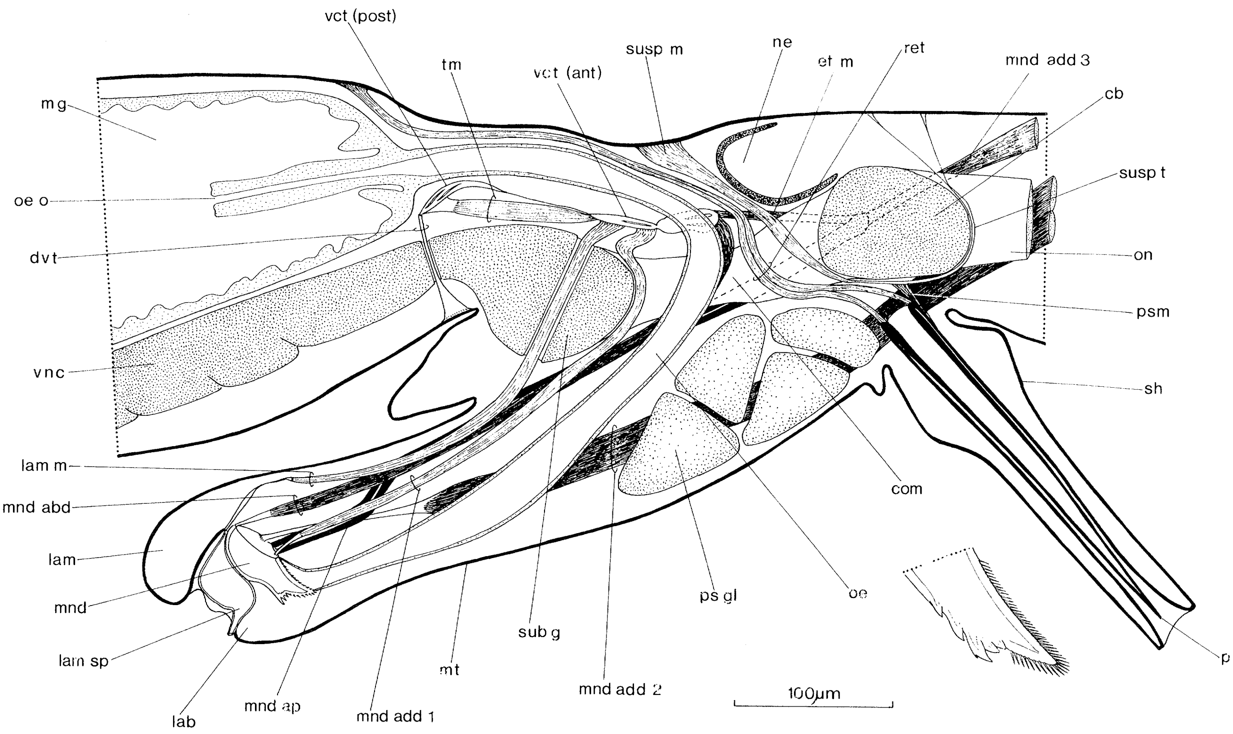

Figure 5. Median internal view of oral region of *A. japonicus*, showing musculature of mouth tube and preoral spine, ventral cephalic tendon system, proboscis glands and other associated organ systems. Inset shows tip of mandible.

sclerite located medial to the maxillulary sucker in the adult. In the first few larval stages the maxillule is a raptorial appendage, rather than a sucker as in the adult, and this particular sclerite is not differentiated.

(*b*) *The preoral spine*

(i) *Anatomy*

Anterior to the mouth tube lies the preoral spine. This consists of an eversible sheath proximally and a tapering distal spine (figure 6*a*,*b*). The boundary between these two parts is well defined in figure 6*a*. In a small adult of 2 mm body length, the length of the fully everted preoral spine plus sheath is about 600 μm. The tip of the spine is swollen and lacks ornamentation. It is 1.5–2.0 μm in diameter (figure 6*d*). The spine is narrowest subterminally. An opening (cd) 0.5–1.0 μm in diameter is located dorsally. This is the orifice of a cuticle-lined duct which has the appearance of a flange on the dorsal side of the spine in its distal third (figure 6*c*). This duct extends the entire length of the spine but is centrally located in proximal two thirds. Located on the ventral surface, opposite the main opening, is a subterminal pore (po) about 0.2 μm in diameter. This pore does not appear to be connected to the dorsal tube and Shimura (1983) suggested that is was possibly the opening of a chemoreceptor. A single axon-like structure, 0.22 μm in diameter, is present in the syncytial epithelial lining of the distal spine (figure 7*b*) and may innervate the chemosensor.

About at its midlength, the preoral spine is oval in cross section, with a long diameter of 10.35 μm (figure 7*a*). It is hollow and has thick cuticle externally and a thin layer of cuticle lining the central duct. The outer cuticle is laminated but the laminations are asymmetrical, being better developed ventrally. In its outer layer it has dense osmophilic granules (figure 7*b*) which might represent deposits of material that give the cuticle enhanced hardness or strength. Between the clearly laminated layer of the outer cuticle is an homogeneous layer which is lighter staining and is not separated from the cuticle by any boundaries (figure 7*b*). This region is old cuticle (oc) which is being broken down prior to ecdysis and may have lost much of its original structure. Near its proximal end the spine is about 13.8 μm in diameter and there is an extensive epithelial layer containing abundant mitochondria and endoplasmic reticulum beneath the cuticle.

The central duct of the spine is cuticle-lined (figure 7*a*,*b*), the cuticle appearing as a double layer. Near the tip both the outer cuticle and cuticular lining of the central duct are secreted by the thin epithelial layer (figure 7*b*). The epithelium contains vesicles and mitochondria. More proximally, within the sheath, the central duct is bilaterally compressed instead of being oval. A thin epithelial layer surrounds the duct but the only recognisable organelles are elongate mitochondria (figure 8*d*). The dense granular matrix present in the sheath extends a short distance into the spine between the inner and outer epithelia but it is not as homogeneous as in the sheath.

The cuticle of the basal sheath also contains osmophilic granules but it is not as thick and laminated as in the spine. The thin layer of new cuticle is

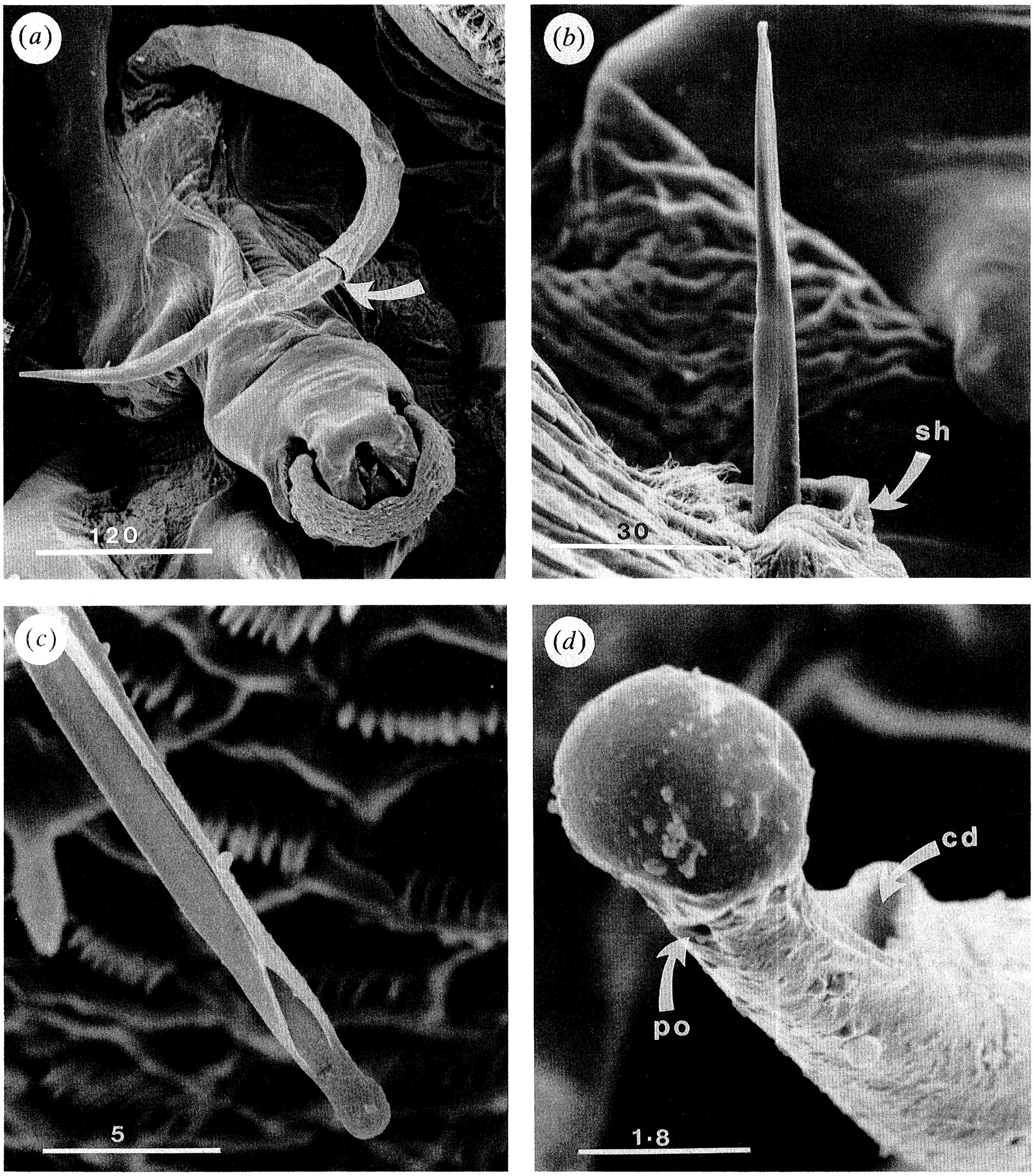

Figure 6. (*a*) Fully everted preoral spine, with groove marking boundary between distal spine and proximal sheath arrowed. (*b*) Tip of partly retracted spine emerging from invaginated sheath. (*c*) Tip of preoral spine, showing large opening of central duct. (*d*) Same, showing opening of central duct and tiny pore of possible chemosensor.

present beneath the old cuticle. The epithelial layer (figures 7*d* and 8*a*) appears to be syncytial and possesses large nuclei and mitochondria. Between the epithelium beneath the outer cuticle and that surrounding the cuticle-lined central duct is a dense matrix which fills most of the space within the retractile sheath. This matrix appears granular in stained transmission electron microscope (TEM) sections. No cellular organelles or cell membranes are present within it.

The epithelium beneath the sheath cuticle and around the lumen of the spine contains large, elongated nuclei with dense nucleoli 1.1 μm in diameter (figure 7*c*,*d*). The nuclear membrane is disrupted by several nuclear pores (figure 7*c*) and the membrane itself is continuous with the rough endoplasmic reticulum. The cytoplasm around these nuclei contains many free and membrane-bound osmophilic ribosomes. The abundance of ribosomes and large nucleoli near to the nuclear membrane, indicates that these cells are involved with protein production. Many vesicles in the epithelial cytoplasm (figure 8*a*) contain a homogeneous grey material which resembles the dense granular matrix. Between the epithelial layer

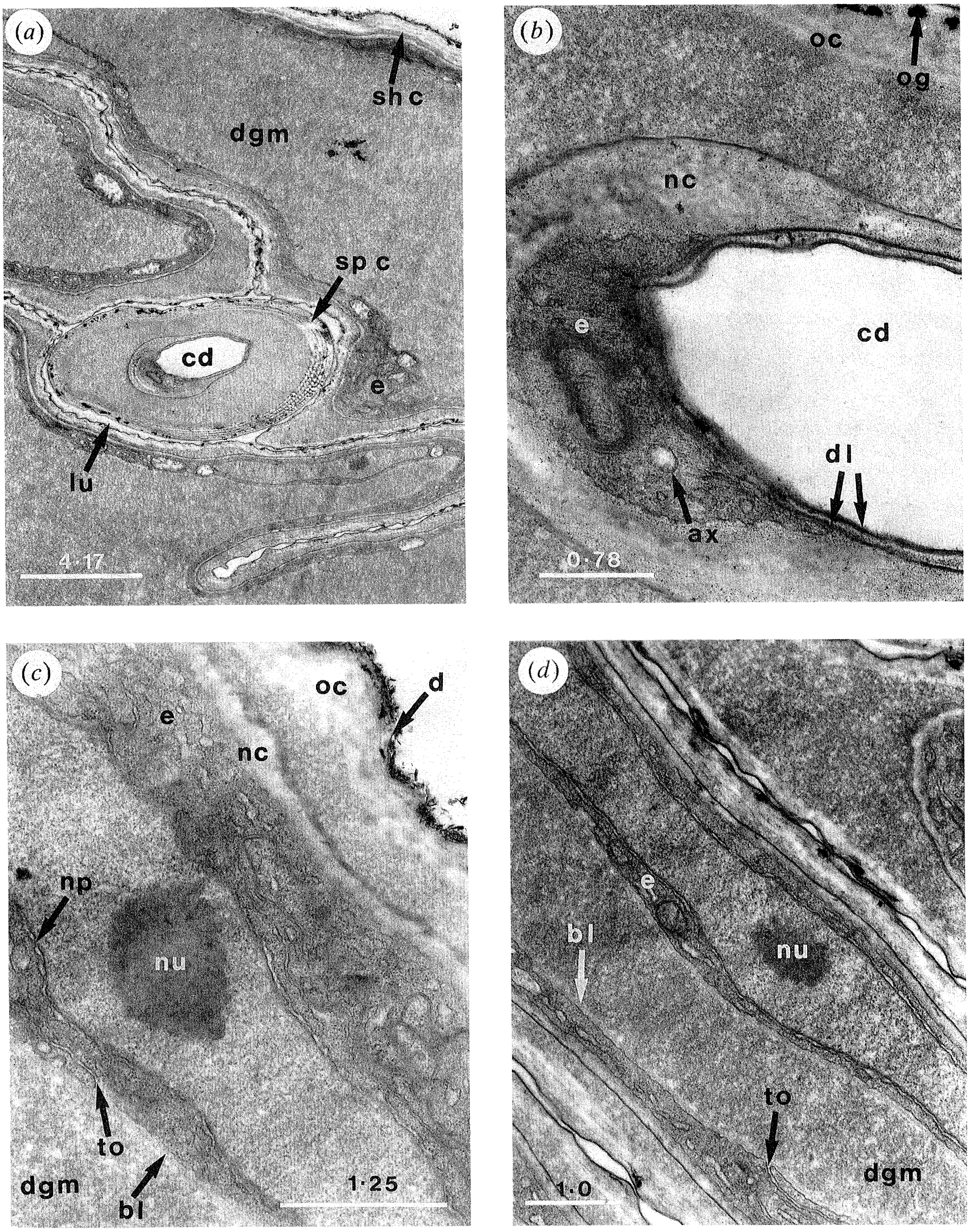

Figure 7. (*a*) Transverse section showing preoral spine within tripartite lumen (lu) of inverted sheath, and separation of outer and folded inner sheath cuticle by dense granular matrix (dgm). (*b*) Detail of (*a*) showing central duct (cd) of preoral spine with its double cuticular lining and an axon-like structure (ax) within its epithelium. (*c*) Section though outer cuticle of sheath, showing old and new cuticle and an elongate nucleus within syncytial epithelium. (*d*) Section through fold in inverted sheath, showing epithelial layers separated by dense granular matrix.

and the granular matrix is a region of slightly lighter staining, fibrillar material. This continuous layer has the appearance of a basal lamina (Bloom & Fawcett 1962; Threadgold 1976; Krstić 1979). The basal lamina is not usually in contact with the epithelial cells but is separated by an amorphous glycocalyx (Threadgold 1976). It underlies the surface of epithelial cells and acts both as a barrier and a structural link between the cell and the stroma (Threadgold 1976). Cytoplasmic tubules open from the epithelial

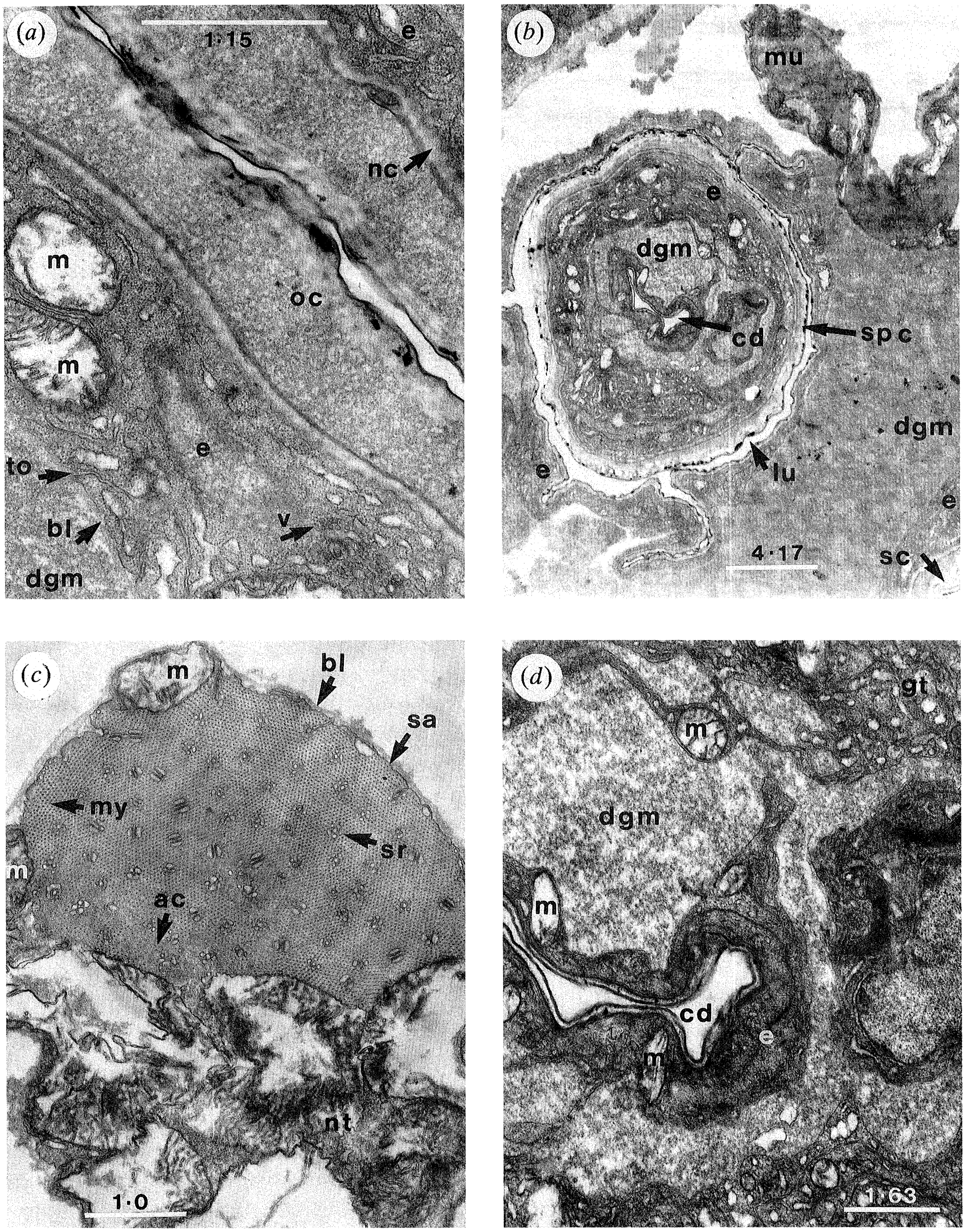

Figure 8. TEM photographs. (*a*) Section through invaginated sheath cuticle, showing syncytial epithelium containing large mitochondria and extensive rough ER which has numerous tubular openings into dense granular matrix. (*b*) Transverse section through retracted spine showing tripartite lumen of inverted sheath and adjacent muscle. (*c*) Detail of preoral spine muscle, showing actin and myosin filaments and extensive sarcoplasmic reticulum. (*d*) Central duct and adjacent glandular tissue in proximal part of preoral spine.

layer into the glycocalyx-basal lamina (figures 7*c*,*d* and 8*a*). Large mitochondria, between 0.5–1.0 μm in diameter, are also present in the epithelial layer.

The central duct passes from the base of the sheath to the spine glands inside the body. It remains hollow but becomes surrounded by numerous mitochondria as it extends into the body. The surrounding epithelial layer contains more vesicles and extensive rough endoplasmic reticulum (ER) (figure 10*c*) and is more conspicuous in the adjacent tissue as the distance between the central duct and the surrounding tissue decreases. The lamellae of the rough ER are typically

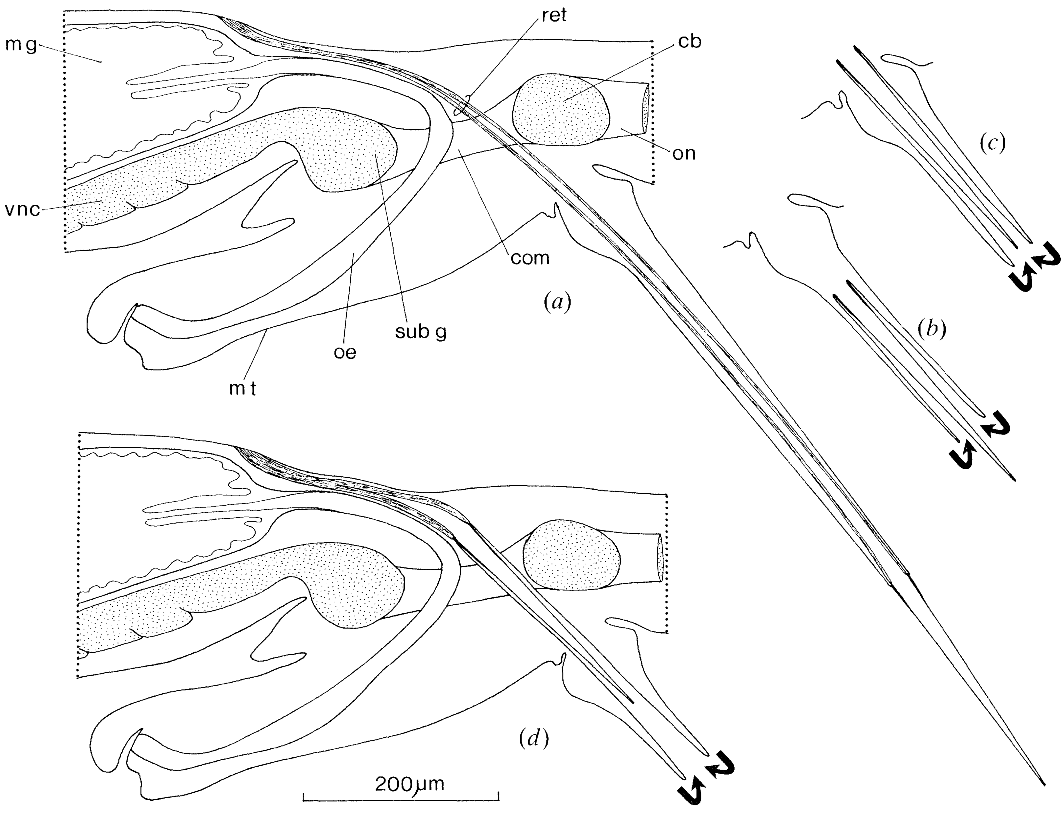

Figure 9. (*a*) Schematic median section showing fully everted preoral spine and its retractor muscles. (*b*) Preoral spine showing inrolling of sheath cuticle as invagination begins. (*c*) As (*b*) but later in process when entire spine withdrawn inside sheath. (*d*) As (*a*) showing fully retracted oral spine withdrawn through nerve ring formed by cerebrum, circumoesophageal commissures and suboesophageal ganglion.

arranged in parallel stacks. Also present in the surrounding tissue are large Golgi bodies (figure 10*d*) with abundant secretory vesicles.

(ii) *Retraction of the preoral spine*

When the spine is retracted most of it is withdrawn into the head but its tip usually protrudes from the sheath. Retraction of the spine into the sheath is a continuous process (figure 9*a–d*). As it is retracted the cuticular wall of the sheath rolls over and is pulled down to form the invagination. The diameter of basal part of the sheath does not increase as the spine is withdrawn telescopically into it. The inverted distal part of the sheath must fold in order to fit within a smaller circumference. It folds into a characteristic tripartite lumen, the branches of which resemble a Manx leg in cross section (figure 7*a*). The proximal extremity of the spine is withdrawn into the body, within the cuticle-lined lumen formed by inversion of the sheath (figure 8*b*). The cuticle-lined, central duct of the spine is compressed. A thin epithelial layer containing mitochondria surrounds the central duct of the spine (figure 5*d*).

Deeper within the body the outer cuticle of the base of the spine is continuous with that of inverted sheath. At this level the lumen formed by the invaginated sheath disappears and the basal rim is produced into two short apodemes each with a closed lumen (figure 10*b*). The spine retractor muscles insert on these apodemes. The central duct, surrounded by soft tissues and enclosed by a basal lamina, extends into the head. Between this basal lamina and that of the surrounding body tissues (figure 10*a*) is a homogeneous dense granular matrix. Endoplasmic tubules open into both basal laminae, and apical vesicles are abundant. The tissue surrounding the basal rim of the spine possesses extensive rough ER in parallel rows, abundant vesicles and free ribosomes in the cytoplasm, all of which are characteristic of active secretory tissue.

Space must also be available within the proximal part of the sheath to accommodate the spine and invaginated distal part of the sheath. This free space is occupied by a dense granular matrix in fixed specimens but this is presumably fluid in life and would be displaced into the body during inversion. It lacks any cellular organelles and membranes that would inhibit this displacement. The fluid matrix is presumably secreted by the epithelial lining of the sheath and the glandular epithelium at the the base of the spine. The secretion is probably released via the tubular openings in the basal lamina. Inversion also involves epithelial layers sliding against each other as the sheath cuticle doubles back on itself. The fluid matrix probably facilitates this process by reducing friction.

The paired retractor muscles (figures 5 and 9) are extremely long, originating on the dorsal body wall, either side of the midline, about at the level where the oesophagus enters the midgut. They pass anteriorly along the dorsal surface of the oesophagus, then

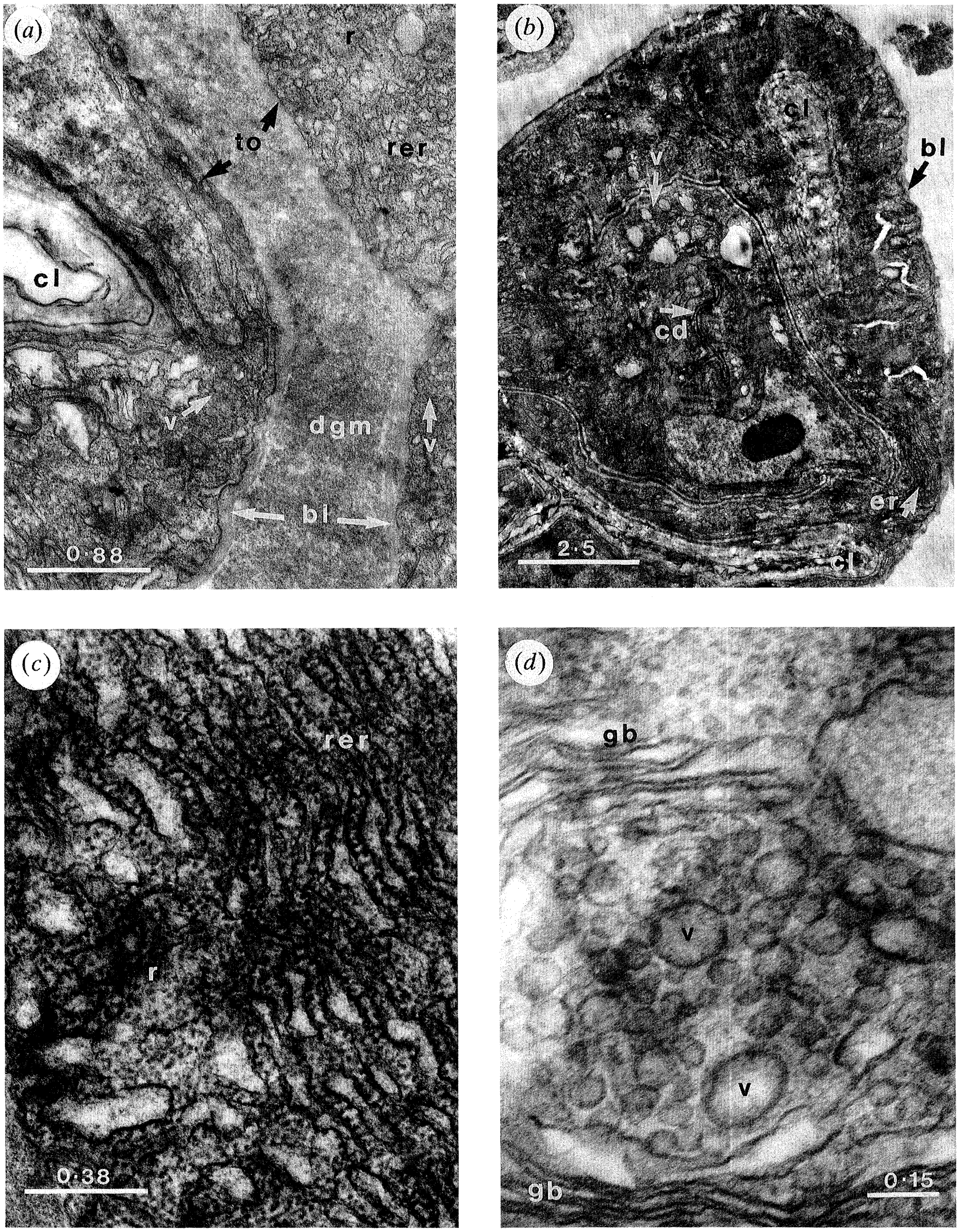

Figure 10. (*a*) Transverse section through base of preoral spine, showing surrounding glandular tissue containing abundant secretory vesicles and rough ER. (*b*) Transverse section through base of spine, showing flattened central duct and short apodemes formed as pair of extensions of proximal rim of spine and adjacent sheath cuticle. (*c*) Glandular tissue around basal rim of spine, showing parallel layers of rough ER. (*d*) Golgi body and associated vesicles from glandular tissue around rim of spine.

anteroventrally through the nerve ring formed by the cerebrum, circumoesophageal commissures and suboesophageal ganglion. When relaxed they pass through the proximal sheath of the extended preoral spine towards their insertion on small apodemes formed at the boundary between the distal spine and proximal sheath (figure 9*a*). Extreme retraction of the preoral spine results in almost the entire spine and much of the sheath being withdrawn into the body, only the most basal part of the largely inverted sheath being visible externally (figure 9*d*). The great length of the retractor muscles, with their posteriorly displaced origin, permits sufficient shortening during contraction for complete inversion. The retractors can shorten to less than 25% of their maximum length (cf. figure 9*a,d*).

On either side of the retracted spine in figure 8*b*, a pair of muscle blocks is present. These muscles originate on a tendinous sheet, located immediately ventral to the cerebrum, which is anchored to the dorsal cephalic wall by suspensory tendons and muscles (figure 5, susp t, susp m). The muscles insert on the broader base of the sheath and are responsible for directing the entire spine from side to side. They display the hexagonal arrangement of a thick (myosin) filament (my) surrounded by six thin (actin) myofilaments (figure 8*c*, ac), regarded as typical for arthropod fast muscle (Fahrenbach 1963, 1967; Briggs 1979). Large mitochondria with a diameter of 0.5–1.0 μm are present around the periphery of the muscle fibre (figure 8*c*) and were not found inside the muscle. In close conjunction with the pair of muscles is a mass of cells resembling arthropodan nervous tissue (Smith 1960; Threadgold 1976). The loosely packed nature of this tissue, the numerous vesicles and abundant membranes, indicate that this could be a neuromuscular junction but this is not certain. The muscle is surrounded by a densely staining sarcolemma (sa) which is, in turn, covered by a basal lamina (bl) which stains light grey (figure 8*c*). Occasionally, the sarcolemma invaginates for a short distance into the block and from such invaginations, numerous tubules arise. These tubules, which are arranged in small bundles of five or six, form a continuous and extensive network throughout the muscle fibre, known as a sarcoplasmic reticulum (Fahrenbach 1963, 1967; Briggs 1979).

(*c*) ***Feeding mechanism***

The only paired appendages involved directly in feeding in an adult *Argulus* are the mandibles. The mandible–mouth tube joint permits mainly adduction–abduction about the single lateral pivot point but limited promotor-remotor rolling movement is also possible. Abduction of the mandible, brought about by the laterally inserting, paired abductor muscles, swings the tip of the gnathobase out into the distal opening between labrum and labium and into contact with the skin of the host. Abduction is also brought about indirectly by the muscles that insert on the mandibular apodemes and originate on the ventral body wall lateral to the base of the mouth tube. Contraction of these muscles must swing the tip of the mandibular gnathobase outwards by pushing the rigid mandibular apodeme down the mouth tube. The mandible is adducted directly by the adductors and indirectly by the muscles (mnd add 3) inserting on the tip of the mandibular apodemes. Adduction dislodges pieces of host tissue that are carried into the mouth opening. Oesophageal peristalsis transports food material down the mouth tube and into the midgut. Adductor muscles 1 and 2 bring about slight promotor–remotor rolling as well as adduction.

Martin (1932) noted that the mouth tube was erected at an angle to the body during feeding. Indeed, it can only come into contact with the host if it is erected. Erection takes place by a forwards swing of the mouth tube about the pair of articulations between the buccal bars and the maxillulary sclerites. A swing of about 45° is sufficient to bring the distal opening into contact with the host's surface and is probably the maximum. Erection is brought about by the short erector muscles originating anteriorly on the ventral cephalic tendon and inserting on the mandibular apodemes. The erector muscles operate at a poor mechanical advantage.

According to Martin (1932) the preoral spine is everted by raising the internal pressure of the blood sinus below it. It is possible that erection of the mouth tube is assisted by the same mechanism. Shimura (1983) noted that pressure applied to the base of the mouth tube caused the mandible to be abducted out through the mouth.

The spine, although slender, is capable of inflicting considerable damage to the host. The lesion produced by *Argulus* is mainly caused by its feeding activity and a large part of the damage occurs within reach of the spine (Kabata 1970). The feeding process involves repeated thrusts of the spine, and the host epithelium exhibits many splits and fissures, caused either by the mechanical action of the spine itself or in combination with its lytic secretions (Becker 1941; Kabata 1970). The subterminal location of the duct opening behind the swollen tip prevents it becoming blocked when thrust into the host. The secretion produced may be lytic or it may have a vasodilatory or anticoagulant function.

The telescopic properties of the spine enable the *Argulus* to penetrate the fish epidermis to various depths during feeding. The role of the spine in feeding is probably to disrupt and fragment the host epithelium. When erected the spine is directed anteroventrally and causes such damage well in front of the mouth tube. The *Argulus* must then move forwards to place the distal opening of its mouth tube in contact with the lesion. This mobile grazing type of feeding behaviour can be observed in captivity but often the parasite is associated with a well delimited lesion and has clearly been feeding in the same place for a period of time.

5. DISCUSSION

The preoral spine is unique to the branchiuran genera *Argulus* and *Dipteropeltis* Calman. The primitive branchiuran genus *Dolops* Audouin and the more specialized *Chonopeltis* Thiele both lack the preoral spine. It appears to be a novel structure, without an obvious homologue in related crustacean groups. Previous accounts have ascribed to the preoral spine the role of a sucking tube, claiming that it was connected to the oesophagus and could suck up host blood and fluids (Ivanfi 1926; Stammer 1959). Martin (1932) and Madsen (1964) however, showed that the spine is not directly connected to the oesophagus and their results are confirmed here. The spine is used for injecting secretion into the skin of the host. The secretion is carried down the central duct and released via the large subterminal pore. Claus (1875), Martin (1932), and Madsen (1964) stated that the site of production of the secretion is large glands located medially, immediately posterior to the base of the spine (figure 5, ps gl). Debaisieux (1953) claimed that the site of preoral spine secretory glands is in the posterolateral lobes of the carapace. Swanepoel & Avenant-Oldewage (1992) also described paired complexes of preoral spine glands located laterally in the carapace of *A. japonicus*, and paired ducts extending through the spine to separate openings at its tip. A single duct, with a single subapical opening, was found in the present study.

The duct carrying the secretion to the spine must accommodate the retraction and eversion movements of the spine. It is cuticle-lined and appears to bend as the spine is retracted.

The internal structure of the preoral spine is highly modified to permit retraction. As the rigid distal spine is withdrawn the cuticle of the proximal sheath is pulled down inside (as indicated by the arrows in figure 9). This can be a continuous process and there are no obviously predefined resting positions though the configuration illustrated in figure 9*b*, with the tip of the spine just protruding out of the sheath, is very typical in fixed specimens. The eversible nature of the spine sheath prohibits any direct connection between the epidermis of the sheath cuticle and any structures passing down the sheath to the distal spine. The cuticle-lined central duct, the nerve to the distal chemosensor and the retractor muscles all remain free in the central fluid matrix as they pass through the sheath.

Martin (1932) noted that the tubular labial spines at the tip of the mouth tube would be the first structures to come into contact with the host's surface. She also postulated that they inject a substance, possibly an irritant, into the host. This appears improbable since their large apical opening would become blocked as soon as it came into contact with the host. Madsen (1964) and Shimura (1983) also advocated a toxic secretory role for the labial spines.

The musculature of the cephalic appendages of *Argulus* has not been previously been studied in sufficient detail to identify sites of origin and insertion. The musculature associated with the mouth tube of *Argulus* can be compared with that of the mouth tube of siphonostomatoid copepods (Boxshall 1990). Until relatively recently branchiurans were classified with the siphonostomatoid copepods on the basis of similarities in their mouth tubes. Superficially the mouth tubes of these two crustacean taxa appear very similar but their musculature is markedly different. In the mouth tube of *Argulus* the labrum is small and lacks labral muscles whereas in siphonostomatoids such as *Lepeophtheirus* Müller, the large labrum has powerful muscles. In the former genus the oesophagus begins almost at the tip of the mouth tube and the longitudinal and circular oesophageal muscles transport food down the mouth tube into the midgut, whereas in siphonostomatoids the oesophagus begins at the base of the mouth tube and the labral muscles are required to move the food down into the oesophagus. In adults of both genera the mandibles are reduced to the gnathobase only, but in *Argulus* the mandibular muscles must pass down the proximal outgrowth that forms most of the mouth tube to the distally located mandibles whereas in *Lepeophtheirus* the extrinsic mandibular muscles pass into the laterally located mandibles and never enter the mouth tube. In both *Argulus* and the siphonostomatoid *Hyalopontius* Sars a pair of long labial muscles originates on the underside of the ventral cephalic tendon and passes down through channels in the suboesophageal ganglion. Labial muscles are absent in *Lepeophtheirus*.

Little is known about the fine structure of branchiuran muscles. The darker staining myosin filaments have a diameter of 160 Å and a spacing of 500 Å, which compares well with the respective values of 150 Å and 480 Å found by Fahrenbach (1963, 1967) for fast fibres in the copepod *Macrocyclops albidus* (Jurine). Fahrenbach (1963) also noted that peripheral mitochondria around muscle bundles were often of similar size to adjacent nuclei, the maximum recorded being 7.0 μm in diameter. Presumably, large mitochondria are able to provide energy rapidly and efficiently for the fast-acting muscle fibres. The arrangement of sarcoplasmic reticulum in *A. japonicus* is different from the dyads (tubules in close association with a cisterna or vesicle) described by Smith (1960), Fahrenbach (1963) and Briggs (1979) for copepod muscles. The preoral spine muscles of *Argulus* appear to possess a completely different kind of sarcoplasmic reticulum from that of copepods. Any functional significance of the groups of five or six tubules remains to be ascertained.

The 75% shortening of the retractor muscles is unusual. Alexander (1968) remarked that few muscles are able to contract to less than 30% of their resting length. A possible explanation is that full eversion of the preoral spine by the hydrostatic eversion mechanism might stretch the retractor muscles beyond their relaxed length. The muscles would store elastic energy during stretching and release it again when eversion pressure was reduced. Such an elastic mechanism could be responsible for part of the overall shortening of the muscles.

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APPENDIX 1. LIST OF ABBREVIATIONS USED IN FIGURES

abd	abductor
ac	actin filament
add	adductor
ant	anterior
ap	apodeme
ax	axon
bl	basal lamina
br	bar
bu	buccal
cb	cerebrum
cd	central duct
cir	circular sucker muscle
cl	closed lumen
com	circumoesophageal commissures
con	condyle
cup	sucker cup
d	debris
dgm	dense granular matter
di	distal hoop of sucker
dl	double cuticular lining
dvt	dorsoventral tendon
e	epithelium
er	endoplasmic reticulum
et m	erector muscle
gb	Golgi body
gn	mandibular gnathobase
gt	glandular tissue
hi	hinge
lab	labrum
lam	labium
lam sp	labial spine
lam m	labial muscle
lu	lumen
m	mitochondria
mar	marginal membrane
mg	midgut
mnd	mandible
mt	mouth tube
mu	muscle
muc	mucous cells
mxl	maxillulary
my	myosin filament
nc	new cuticle
ne	nauplius eye
np	nuclear pore
nt	nerve tissue
nu	nucleolus
oc	old cuticle
oe	oesophagus
oe o	oesophageal opening
og	osmophilic granule
on	optic nerve
pa	mandibular palp
pa m	palp setal fan muscles
po	pore
post	posterior
prm	promotor muscle
prx	proximal hoop of sucker
ps	preoral spine
psm	preoral spine muscle
ps gl	proboscis gland
r	ribosomes
rem	remotor muscle
rer	rough endoplasmic reticulum
ret	retractor
rid	dorsal ridge
sa	sarcolemma
scl	sclerite
se	setae
sh c	sheath cuticle
sh	sheath
sp c	spine cuticle
spi	spinules
sr	sarcoplasmic reticulum
st	stalk of sucker
su	supporting rod
subg	suboesophageal ganglion
suc	sucker
suc m	sucker muscles
suc pl	sucker plate
susp m	suspensor muscle
susp t	suspensor tendon
t	tendon
tm	tensor muscle
to	tubular opening
v	vesicle
vct	ventral cephalic tendon
vnc	ventral nerve cord

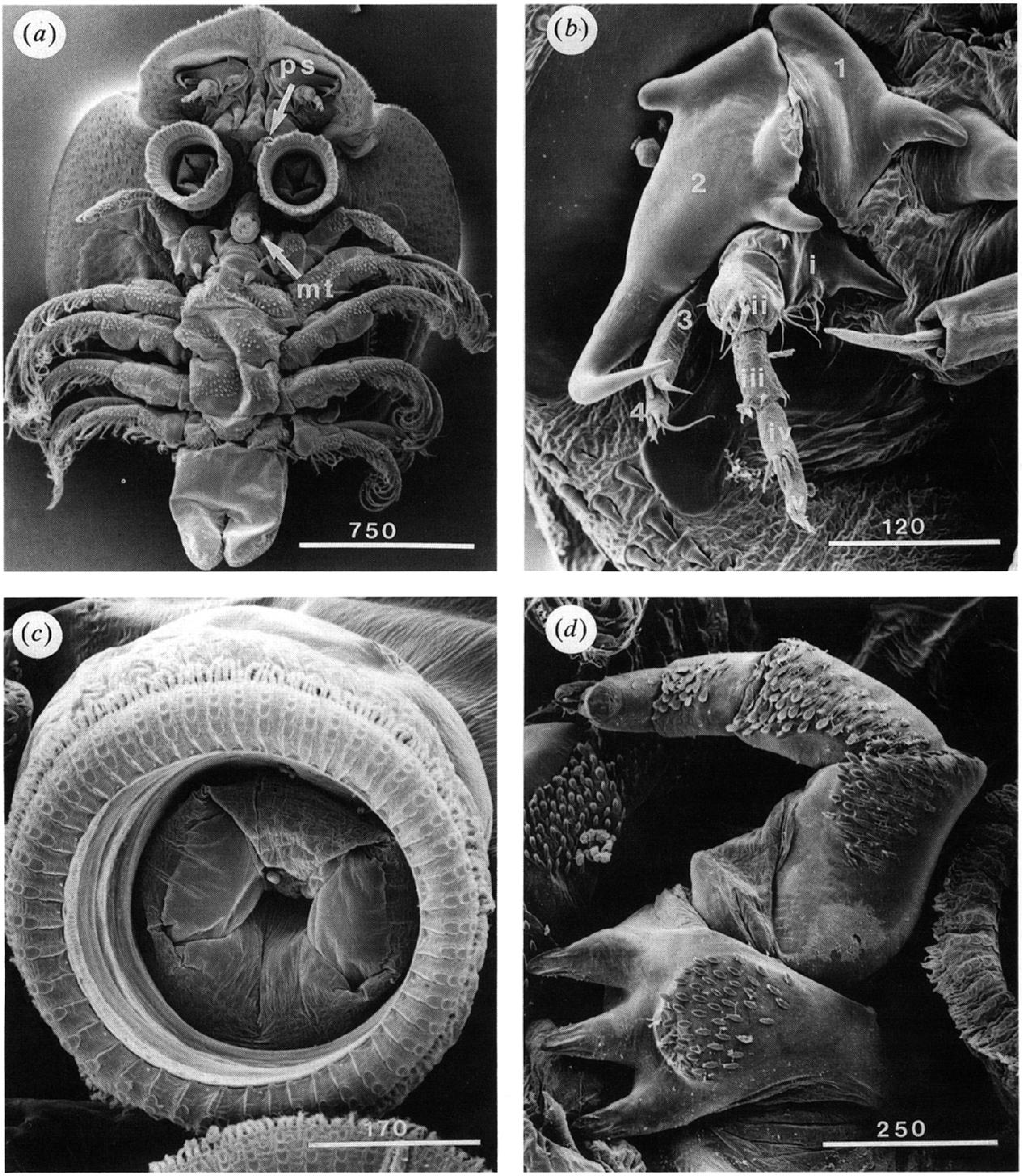

Figure 1. *Argulus japonicus*. (*a*) Male, ventral, showing suckers, preoral spine and mouth tube. (*b*) Antennule and antenna, ventral, with segments of antennule numbered 1 to 4 and those of antenna i to v. (*c*) Sucker, ventral, showing interior of sucker cup and marginal membrane with supporting rods. (*d*) Maxilla, ventral.

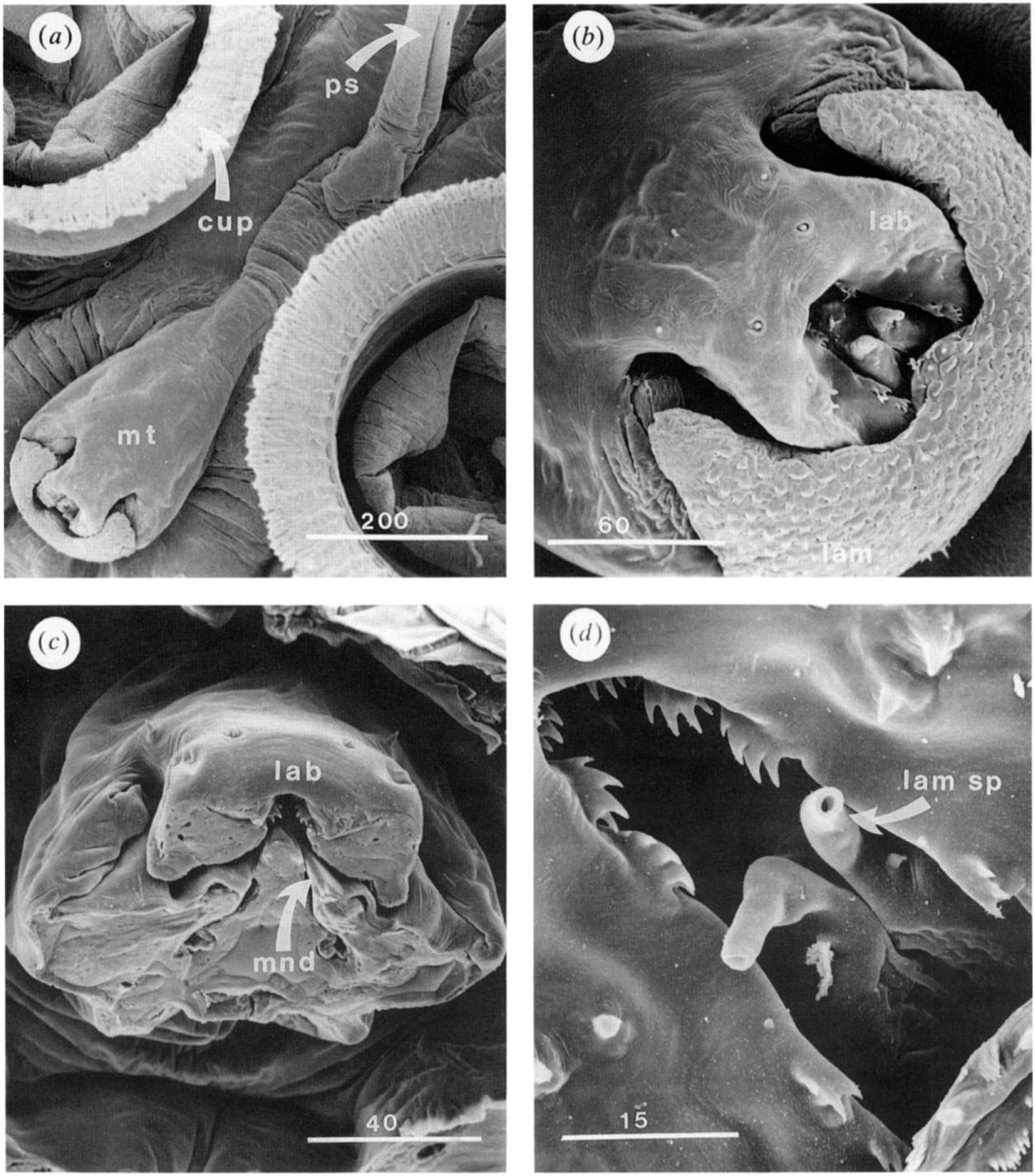

Figure 3. (*a*) Ventral view of oral area, showing mouth tube and base of preoral spine. (*b*) Apex of mouth tube, showing medial labrum largely enclosed by large labium. (*c*) Section through mouth tube from freeze-fractured specimen, showing mandibles entering mouth from sides. (*d*) Detail of tip of mouth tube, showing tubular labial spines and denticulate margin of labrum.

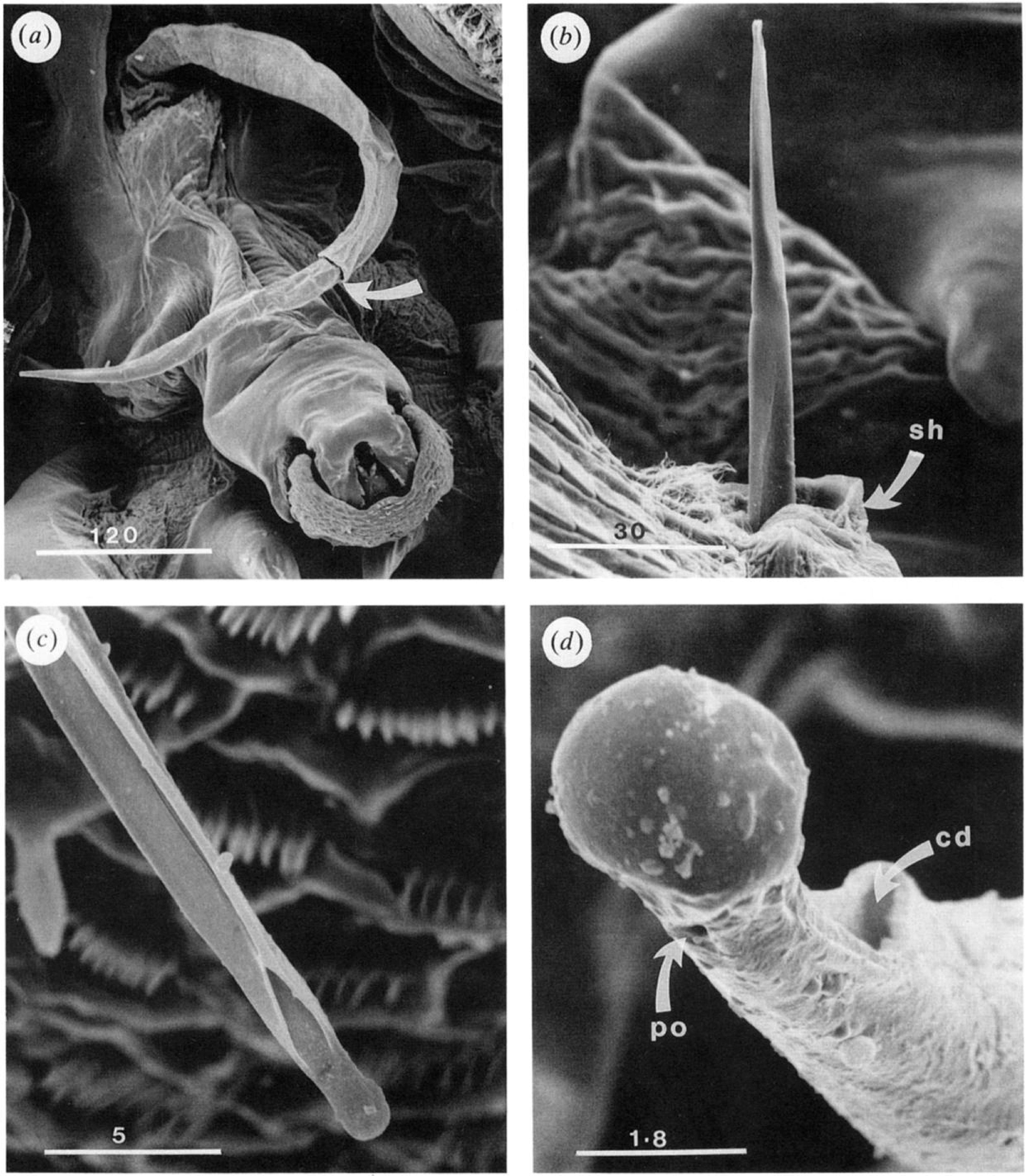

Figure 6. (*a*) Fully everted preoral spine, with groove marking boundary between distal spine and proximal sheath arrowed. (*b*) Tip of partly retracted spine emerging from invaginated sheath. (*c*) Tip of preoral spine, showing large opening of central duct. (*d*) Same, showing opening of central duct and tiny pore of possible chemosensor.

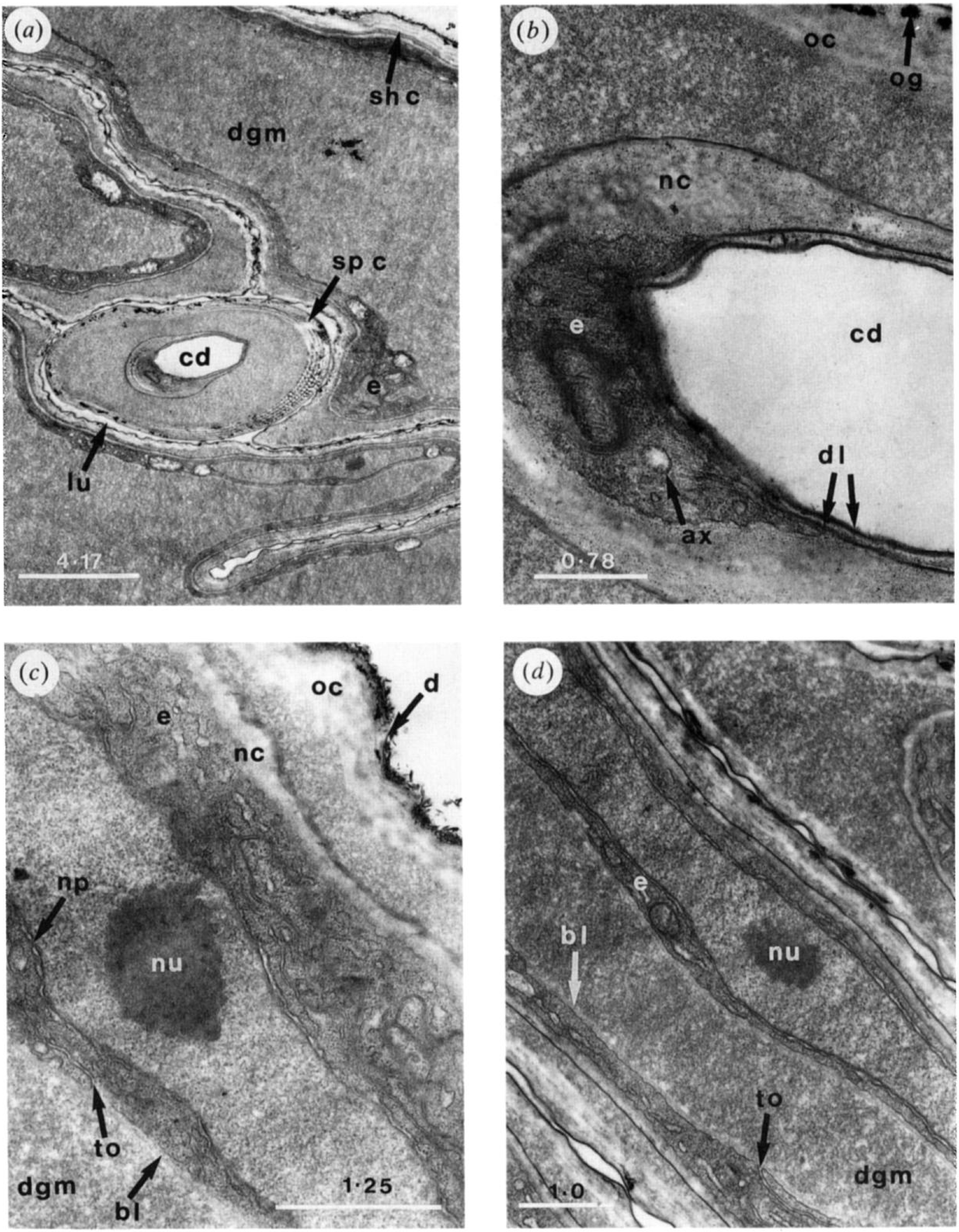

Figure 7. (*a*) Transverse section showing preoral spine within tripartite lumen (lu) of inverted sheath, and separation of outer and folded inner sheath cuticle by dense granular matrix (dgm). (*b*) Detail of (*a*) showing central duct (cd) of preoral spine with its double cuticular lining and an axon-like structure (ax) within its epithelium. (*c*) Section though outer cuticle of sheath, showing old and new cuticle and an elongate nucleus within syncytial epithelium. (*d*) Section through fold in inverted sheath, showing epithelial layers separated by dense granular matrix.

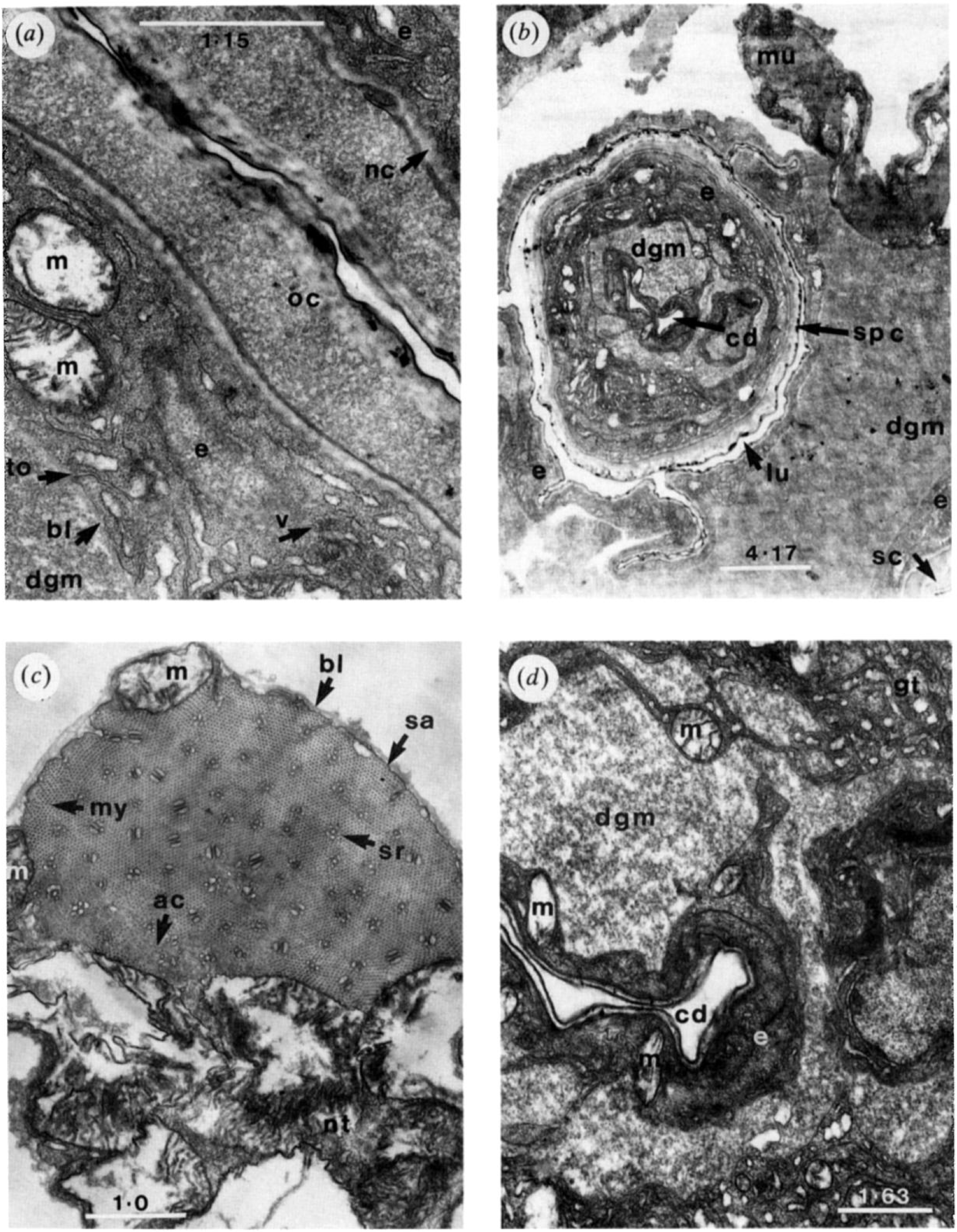

Figure 8. TEM photographs. (*a*) Section through invaginated sheath cuticle, showing syncytial epithelium containing large mitochondria and extensive rough ER which has numerous tubular openings into dense granular matrix. (*b*) Transverse section through retracted spine showing tripartite lumen of inverted sheath and adjacent muscle. (*c*) Detail of preoral spine muscle, showing actin and myosin filaments and extensive sarcoplasmic reticulum. (*d*) Central duct and adjacent glandular tissue in proximal part of preoral spine.

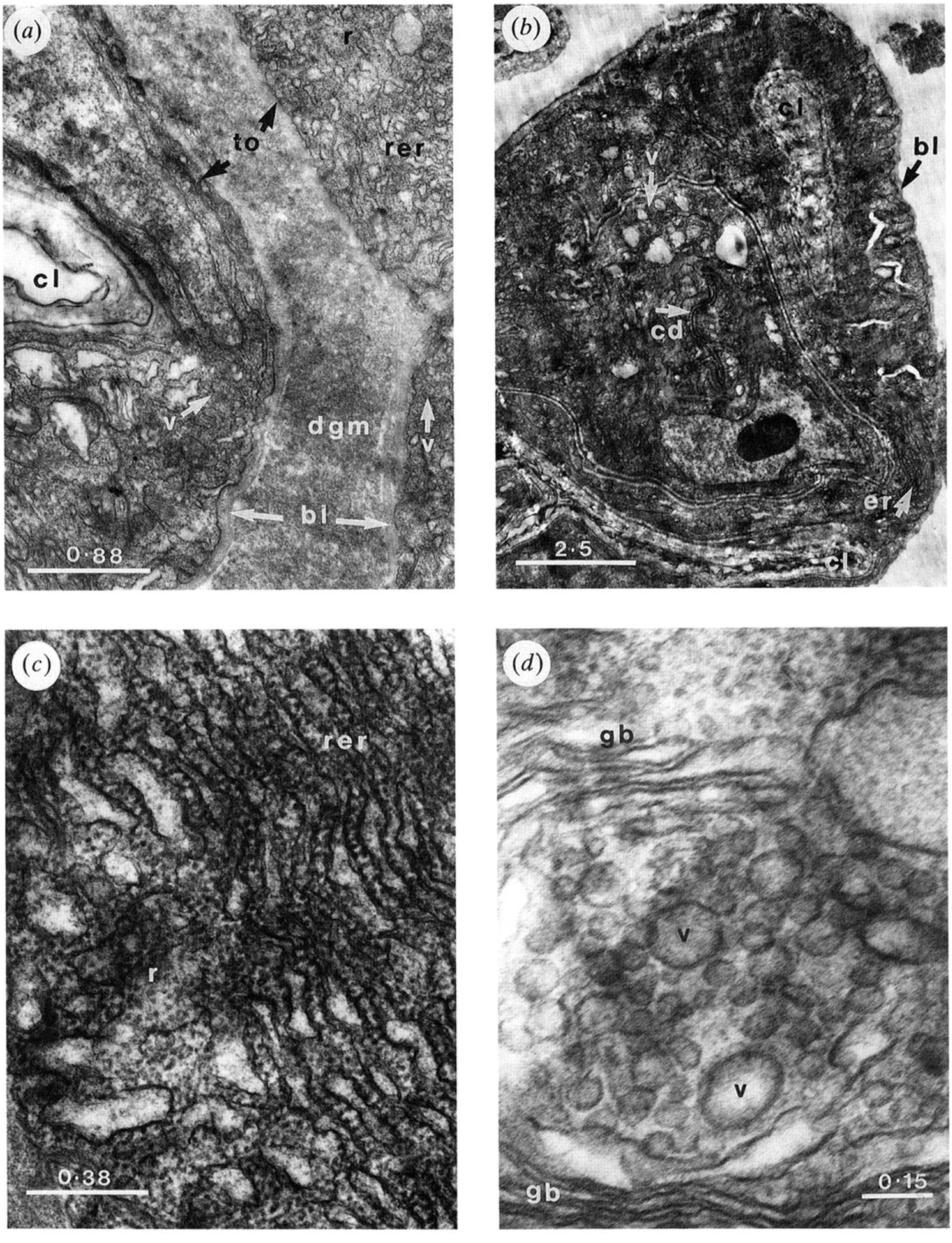

Figure 10. (*a*) Transverse section through base of preoral spine, showing surrounding glandular tissue containing abundant secretory vesicles and rough ER. (*b*) Transverse section through base of spine, showing flattened central duct and short apodemes formed as pair of extensions of proximal rim of spine and adjacent sheath cuticle. (*c*) Glandular tissue around basal rim of spine, showing parallel layers of rough ER. (*d*) Golgi body and associated vesicles from glandular tissue around rim of spine.