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An ultrastructural study of the cercarial excretory system in *Bucephaloides gracilescens* and *Prosorhynchus squamatus*

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Abstract

The ultrastructure of the flame cells, capillaries, collecting tubes, excretory bladder, excretory atrium, caudal vesicle, lateral caudal ducts and excretory pores of cercariae of *Bucephaloides gracilescens* (Rudolphi, 1819) Hopkins, 1954 and *Prosorhynchus squamatus* Odhner, 1905 (Digenea: Bucephalidae) is described. Both species are essentially similar except for some details. The terminal parts of the protonephridia have all the structural features that are typical of trematodes. The collecting tubes in the cercarial body are composed of cells that are wrapped around the lumen. The main collecting tubes are joined to the excretory bladder syncytium by septate junctions. Features of *P. squamatus* excretory bladder epithelium indicate that it is involved in secretory activity, but this is not the case in *B. gracilescens*. In both species the luminal surface of the excretory bladder epithelium is increased by lamellae, and the basal plasma membrane forms invaginations. In the bladder syncytium of *P. squamatus* both apical lamellae and basal invaginations are more developed and mitochondria are also more numerous. The excretory atrium is lined by a syncytium with nucleated cytons located in the surrounding parenchyma. The atrium lining is not continuous with the body tegument and possesses specific secretory inclusions and a thick glycocalyx. Septate junctions connect the atrium syncytium to the excretory bladder epithelium at its anterior end and to the syncytial excretory epithelium lining the caudal vesicle and the lateral caudal ducts at its posterior. In the excretory pores the caudal duct syncytium is joined to the tegument by septate desmosomes.

Introduction

The only complete descriptions of the fine structure of the excretory system of cercariae have been for *Schistosoma mansoni* (Ebrahimzadeh & Kraft, 1971; Powell, 1973), *Cryptocotyle lingua* (Krupa *et al.*, 1969; Rees, 1977), *Diplostomum pseudospathaceum* (Niewiadomska & Czubaj, 1996) and four species of microphallids (Malkova & Galaktionov, 1989). Some data on this subject are also available for cercariae of *Philophthalmus* sp. (Rohde & Watson, 1992), *Fasciola hepatica* (Bennett & Threadgold, 1973), *Posthodiplostomum minimum* (Powell, 1975), *Ochetosoma aniarum* (Powell, 1972), *Podocotyle staffordi* (Gibson, 1974), *Cercaria stunkardi* (Popiel, 1977), *Tetrapapillotrema concavocorpa* (Kruidenier, 1959) and *Schistosoma japonicum* (Göbel & Pan, 1985). The ultrastructure of the excretory systems of bucephalid cercariae has not been examined. Bucephalids are particularly interesting because they possess a number of structural characteristics that differ from those of other digeneans. The results of an electron microscope investigation of the protonephridial system of

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cercariae of two bucephalid species, *Bucephaloides gracilescens* and *Prosorhynchus squamatus*, are presented.

Materials and methods

Bucephaloides gracilescens and *Prosorhynchus squamatus* cercariae were obtained from naturally infected bivalves *Abra prismatica* (Montagu, 1803) and *Mytilus edulis* L. respectively. Specimens of *A. prismatica* were collected from the depth of 60–80 m off the southern coast of Iceland in September 2000 and those of *M. edulis* from the sublittoral zone of the Onega Bay of the White Sea (Russia) in August 2001. Material intended for transmission electron microscopy was fixed for 7–10 days in 3% or 4% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) containing sucrose (760 μosmol in total). After rinsing in buffer, specimens were post-fixed for 3–4 h at 4°C in 1% osmium tetroxide in 0.1 M cacodylate buffer, rapidly dehydrated in ethanol and acetone and embedded in Epon-Araldite mixture. A series of thin cross- and longitudinal sections were cut on an LKB III ultramicrotome, stained with uranyl acetate and lead citrate and examined with Jeol 1200 and/or Leo 900 electron microscopes at an accelerating voltage of 60 or 80 kV. Living cercariae (*P. squamatus*) and semi-thin sections stained with toluidine blue were studied by light microscopy.

Results

Because of the essential similarity in morphology and ultrastructure of the excretory system of *B. gracilescens* and *P. squamatus* cercariae, a combined description for both species is presented. However, special attention is paid to structural features that differ between the two species.

Light microscopy

Light microscopy demonstrated that anterior and posterior collecting tubules, and their branches (small collecting tubules) that receive capillaries from each group of flame cells, were situated symmetrically on both sides of the cercarial body (fig. 1). They merged into the main collecting tubes just posterior to the pharynx. The main collecting tubes opened distally into the sac-shaped excretory bladder about half way along its length in *B. gracilescens* (fig. 1a) and slightly asymmetrically in the anterior third of the bladder in *P. squamatus* (fig. 1b). The excretory bladder passed posteriorly into a broad median duct which we termed the 'excretory atrium'. In both species the excretory atrium was surrounded by well-developed muscles which were easily seen under the light microscope. The excretory atrium emptied into the small caudal vesicle adjacent to the body–tail junction. The caudal vesicle was more prominent in *P. squamatus*. Both excretory atrium and caudal vesicle could change shape according to the degree of contraction of the surrounding muscles and the tail musculature. At the posterior end, the caudal vesicle was subdivided into a pair of lateral caudal ducts, each opening by an excretory pore on the anterior-lateral surface of the tail stem (fig. 1).

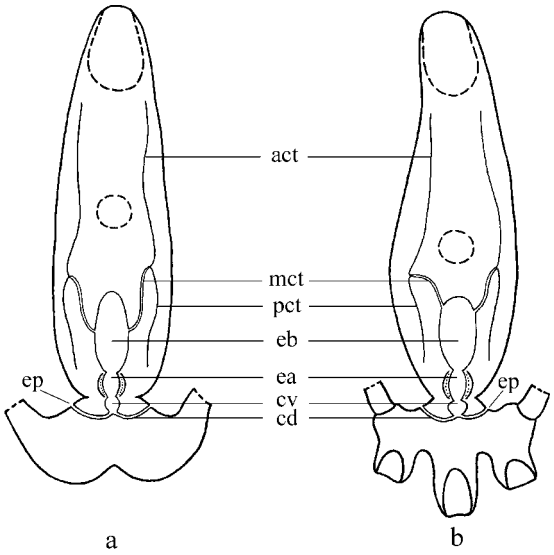

Fig. 1. Schematic drawing of the excretory system of *Bucephaloides gracilescens* (a) and *Prosorhynchus squamatus* (b) cercariae (flame cells, capillaries and small collecting tubules not included).
Key to lettering of figures: act, anterior collecting tubule; acy, cyton of excretory atrium syncytium; as, excretory atrium syncytium; bb, basal bodies; bi, basal invaginations; bl, basal lamina; btj, body–tail junction; c, cilia; cc, cytoplasmic cords; cd, lateral caudal duct; cde, caudal duct epithelium; cm, circular muscles; ct, collecting tubule cell; cv, caudal vesicle; cve, caudal vesicle epithelium; ea, excretory atrium; eb, excretory bladder; ebl, excretory bladder epithelium; el, external leptotriches; ep, excretory pore; er, external ribs; fc, flame cell; gc, Golgi complex; gj, gap junction; gl, glycocalyx; il, internal leptotriches; im, interstitial material; ir, internal ribs; l, lumen; la, lamellae; lm, longitudinal muscles; "m", filtration 'membrane'; mct, main collecting tube; mf, muscle fibres; mt, mitochondria; n, nucleus; nu, nucleolus; pac, parenchymal cell; pc, proximal capillary cell; pct, posterior collecting tubule; r, rootlets; rer, rough endoplasmic reticulum; sia, secretory inclusions of excretory atrium syncytium; sib, secretory inclusions of excretory bladder epithelium; sj, septate junction; t, tegument; ve, vesicle.

Electron microscopy

Terminal part of protonephridium

Each flame bulb comprised a flame cell, bearing a tuft of hexagonally arranged cilia, and part of the proximal capillary cell (figs 2–6). Cytoplasmic processes (ribs) of these two cells interdigitated to form a weir which is thought to function as a filtration apparatus. The internal ribs originated from the flame cell and the external ones were continuous with the proximal capillary cell. Adjacent ribs were interconnected by a filtration 'membrane' consisting of extracellular material. In *B. gracilescens* and *P. squamatus* cercariae the internal and external ribs formed a single row. Both internal and external leptotriches were observed as outgrowths of internal and external ribs respectively (fig. 2). There were only a few of each type of leptotriche in the bucephalid cercariae. The proximal capillary cell was wrapped around itself and joined by septate junction that extended along the weir between two cytoplasmic cords. The tips of these cords were also attached to the flame cell by a

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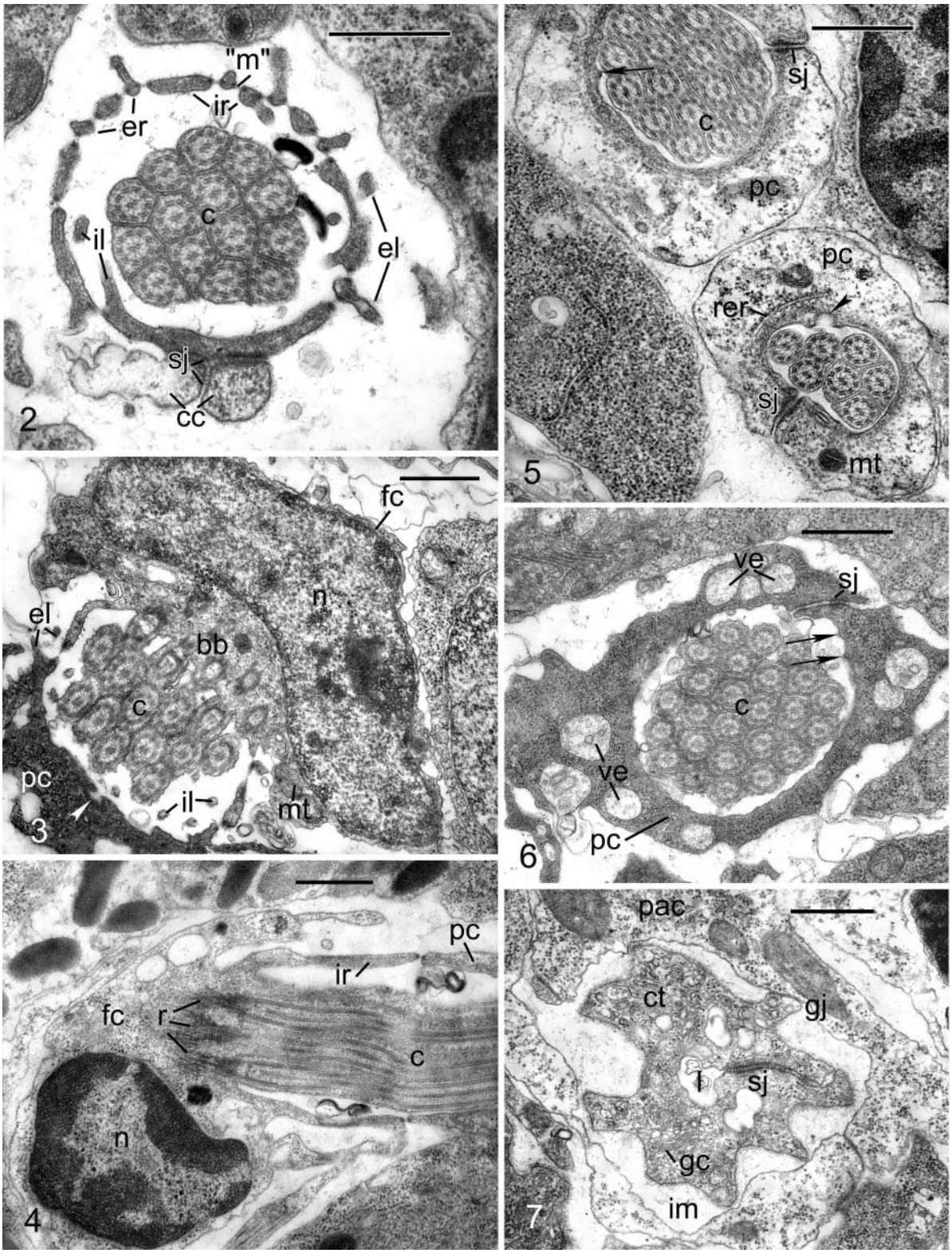

Fig. 2. Cross-section through weir of *Bucephaloides gracilescens* cercaria at the level of junction of cytoplasmic cords to flame cell. Fig. 3. Oblique section through flame bulb of *Prosorhynchus squamatus* cercaria. Fig. 4. Longitudinal section through flame cell of *B. gracilescens* cercaria. Figs 5, 6. Cross-sections through proximal capillaries of *B. gracilescens* (5) and *P. squamatus* (6) cercariae. Note pits (arrowheads) and short ridge-like projections (arrows) on capillary luminal surface. Fig. 7. Collecting tubule of *B. gracilescens* cercaria in cross-section. See fig. 1 for key to labelling. Bars = 0.5 μm.

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septate desmosome (fig. 2). The bean-shaped nucleus of each flame cell was situated laterally to the cilia tuft (fig. 4). The thin layer of perikaryon cytoplasm contained mitochondria, free ribosomes, electron-lucent vacuoles, basal bodies and striated rootlets (figs 3, 4). The maximum number of cilia observed was 16 and 26 in *B. gracilescens* and *P. squamatus* cercariae, respectively. Indentations, possibly representing endo- or exocytotic vesicles, and short ridge-like projections were visible on the luminal surface of the proximal capillary (figs 3, 5). The cytoplasm of the capillary cell contained mitochondria, free ribosomes and sparse rough endoplasmic reticulum (RER). In *P. squamatus* the capillary cell also included large vesicles with heterogeneous contents of moderate electron density (fig. 6).

Collecting tubes

Small collecting tubules, anterior and posterior collecting tubules and main collecting tubes shared the same basic morphology (figs 7–9). Like the proximal capillaries, they were composed of large flattened cells wrapped around the lumen. Adjacent cells were connected by septate junctions (fig. 9). Again, as in the proximal capillaries, the luminal surface possessed ridge-like projections, the frequency and length of which slightly increased in larger ducts. The basal plasma membranes of tube cells were usually surrounded by a thick layer of interstitial material except at junctional complexes (gap junctions) with the parenchymal cells (fig. 7). The cytoplasm contained free ribosomes, sparse RER, mitochondria and occasional Golgi complexes (figs 7, 8). In *B. gracilescens* there were also small membrane-bound vesicles filled with electron-dense material, electron-lucent vesicles and coated vesicles (fig. 8). In *P. squamatus* large vesicles, similar to those in proximal capillaries, were most frequently observed (fig. 9). The main collecting tubes were connected to the excretory bladder epithelium by ring-shaped septate junctions (figs 10, 11).

Excretory bladder

The bladder wall consisted of a syncytial epithelium which was externally supported by a fibrous basal lamina and thin muscle fibres sunk into a thick layer of interstitial material (figs 13, 14). Large nuclei contained bulky heterochromatin aggregates and distinct nucleoli. In *B. gracilescens* the nuclei were usually located in enlarged portions of the thin syncytial layer (fig. 13). In *P. squamatus* the perikarya were always well detached in a form of large outgrowths, protruding deeply into the bladder lumen (figs 14, 19). The luminal surface in both species was increased by lamellae which branched, anastomosed and rejoined the surface to form loops. In *P. squamatus* the lamellae displayed better development and occupied the bulk of the bladder lumen. Both in *B. gracilescens* and *P. squamatus* the basal plasma membrane of the bladder syncytium was extended into broad infoldings and deep narrow invaginations with slightly swollen tips (figs 12–15). Small electron-lucent vesicles were occasionally observed near these tips (fig. 16). Basal invaginations were also more numerous in the excretory bladder of *P. squamatus* (fig. 15). The basal plasma membrane of the bladder epithelium, like that of the collecting tubes, formed junctional complexes (gap junctions) with parenchymal cells (fig. 13). The electron-dense cytoplasm of the bladder lining contained free ribosomes, mitochondria, RER and Golgi complexes. The two latter organelles were more abundant in the secretory active bladder epithelium of *P. squamatus*. Secretory inclusions observed in the excretory bladder of this species were membrane-bound and contained fine granular material of moderate electron density (fig. 14). The shape and size of the inclusions varied widely. Large inclusions appeared to arise by the fusion of small ones. Secretory inclusions were often seen close to the apical plasma membrane, but no clear morphological evidence was available to suggest that they discharged their contents into the bladder lumen. No secretory inclusions were present in the bladder wall of *B. gracilescens*. Mitochondria were also more numerous in the bladder epithelium of *P. squamatus*, especially close to the basal invaginations (fig. 15). They were much larger than those in *B. gracilescens* and had better developed cristae. The mitochondrial matrix contained small electron-dense granules that were absent in *B. gracilescens*. The excretory bladder epithelium was joined at its posterior end to the lining of the excretory atrium by a septate junction (figs 17, 18).

Excretory atrium

The excretory atrium was lined by a syncytial epithelium, which was structurally similar to that of the tegument. The anucleate layer of cytoplasm possessed a greatly folded luminal surface and contained numerous secretory bodies (figs 18–20), which were probably responsible for a characteristic thick glycocalyx. In both species secretory bodies were similarly small round or rod-shaped inclusions with a moderate or high electron density. (No similar structures were observed in the body tegument.) The excretory atrium lining was connected by thin cytoplasmic connections to nucleated cytons lying beneath the muscle bundles near the posterior end of the excretory bladder (figs 17, 18). These cytons possessed features to suggest that they undergo degeneration. The cytoplasm of the cytons was deeply penetrated by invaginations of the outer plasma membrane, and only a few cytoplasmic structures were observed. Underlying the atrium lining was a thin basal lamina, outside which were well-developed layers of circular and longitudinal muscles respectively (figs 17, 18, 20). In *B. gracilescens* the excretory atrium was enlarged to form a funnel in the region of the body–tail junction so that its lining abutted with the tegument (fig. 21). In the same part of the body of *P. squamatus*, thick outgrowths of the atrium lining and the body tegument extended towards each other in a dorso-ventral direction (figs 19, 22). Several specimens of each species were examined in a series of thin sections, but no connections between the body tegument and the atrium lining were observed.

Caudal vesicle and caudal ducts

The small caudal vesicle and lateral caudal ducts of *B. gracilescens* were composed of a thin syncytial excretory epithelium with thick, short cytoplasmic projections on the luminal surface (figs 21, 23, 24, 25). This epithelium

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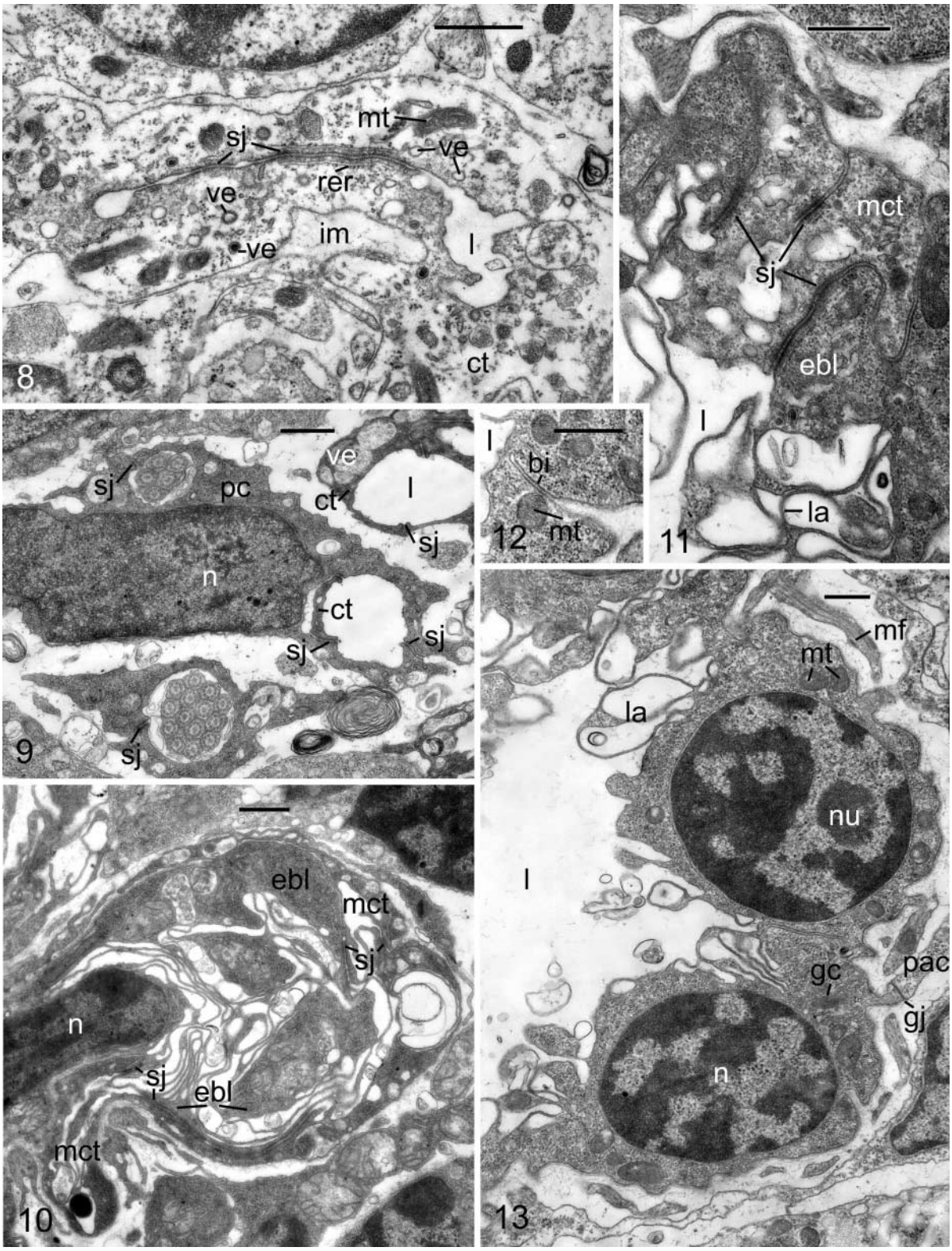

Fig. 8. Collecting tubule of *Bucephaloides gracilescens* cercaria showing various types of vesicles in tubule cell cytoplasm. Fig. 9. Junction of proximal capillary cell to collecting tubule cell in cercaria of *Prosorhynchus squamatus*. Figs 10, 11. Points of opening of main collecting tubes into excretory bladder in *P. squamatus* (10) and *B. gracilescens* (11) cercariae. Figs 12, 13. Excretory bladder epithelium of *B. gracilescens* cercaria. See fig. 1 for key to labelling. Bars = 0.5 μm.

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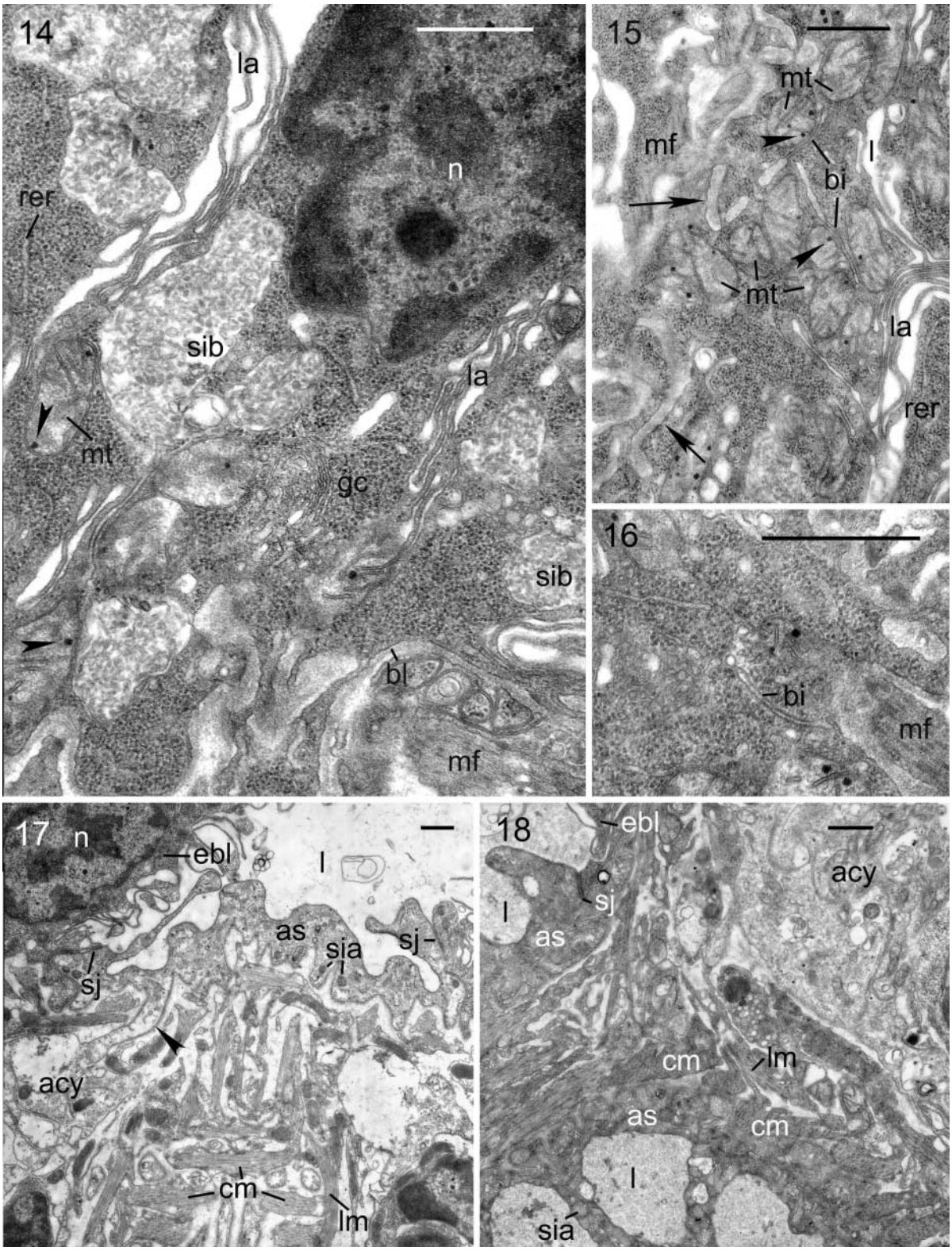

Figs 14–16. Excretory bladder epithelium of cercaria of *Prosorhynchus squamatus*. Arrows indicate broad infoldings of basal plasma membrane and arrowheads – electron-dense granules in mitochondria. Figs 17–18. Junction of excretory bladder epithelium to excretory atrium syncytium in *Bucephaloides gracilescens* (17) and *P. squamatus* (18) cercariae. Note cytoplasmic connection (arrowhead) between atrium lining and cyton. See fig. 1 for key to labelling. Bars = 0.5 μm.

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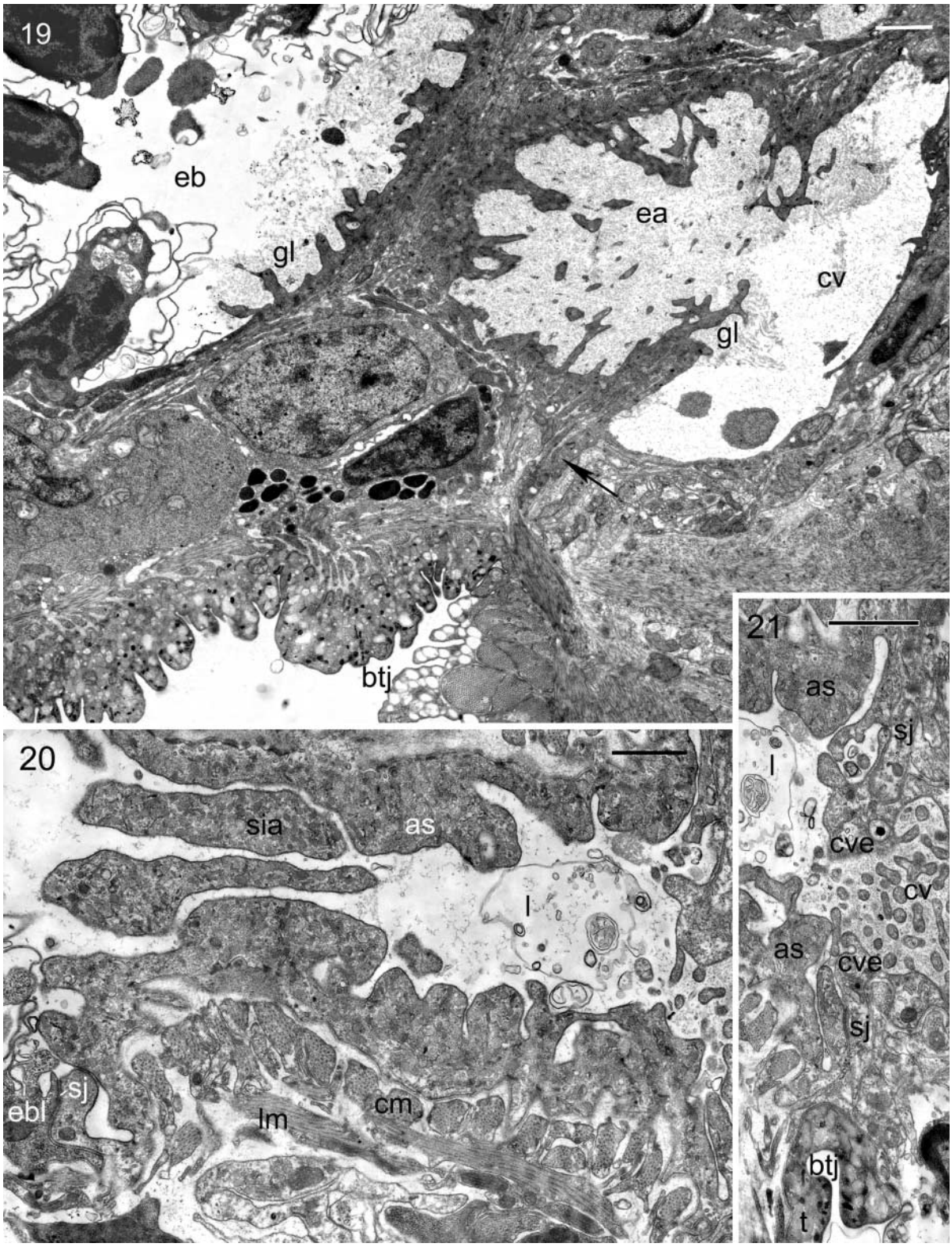

Fig. 19. Sagittal section through excretory bladder, excretory atrium and caudal vesicle of *Prosorhynchus squamatus* cercaria. Note outgrowth of atrium lining (arrow) extended towards body surface. Fig. 20. Excretory atrium of *Bucephaloides gracilescens* cercaria. Fig. 21. Junction of atrium lining to excretory epithelium of caudal vesicle in *B. gracilescens* cercaria. Note funnel-like dilation of atrium in the region of body-tail junction. See fig. 1 for key to labelling. Bars = 1 μm.

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was connected to the atrium lining by septate junction at the level of the body–tail junction (fig. 21). The syncytium cytoplasm contained mitochondria and vesicles, similar to those in collecting tubes (fig. 23). One or two nuclei were observed in the distal part of the caudal ducts (fig. 24). The anterior portion of the caudal vesicle in *P. squamatus* was formed by a prolongation of the atrium lining, and the excretory syncytium formed only the posterior part of the excretory vesicle and the lateral caudal ducts (figs 22, 26). As in *B. gracilescens*, the boundary between these epithelia was marked by a septate desmosome. The excretory syncytium of the caudal ducts of *P. squamatus* was highly folded and lacked nuclei. In both species the lining of the lateral caudal ducts was connected to the tegument by septate junctions close to the excretory openings (figs 25, 27).

Discussion

The general morphology of the excretory system of *B. gracilescens* and *P. squamatus* cercariae was studied previously by Matthews (1973, 1974) using light microscopy. The present study makes a further contribution by describing, in both species, the muscular excretory atrium positioned posteriorly to the excretory bladder and the caudal vesicle in front of the bifurcation of the lateral caudal ducts. These structures have not been reported for other bucephalid cercariae. However, Woodhead (1929) noted in cercariae of *Rhipidocotyle papillosum* (syn. *Bucephalus papillosus*) 'the well-developed muscles' around the outlet of the excretory bladder which very likely correspond to the musculature of the excretory atrium. In addition, Kniskern (1952; fig. 14, 15, p. 327), in his drawings of mature cercariae of *R. septpapillata*, showed a pronounced dilation of the caudal excretory duct at the point of its bifurcation that resembled the caudal vesicle of *B. gracilescens* and *P. squamatus* cercariae. These observations together with the present results, derived from three bucephalid genera, suggest that both the excretory atrium and the caudal vesicle are common features of bucephalid cercariae as a whole. An examination of several series of cross- and sagittal sections of *B. gracilescens* and *P. squamatus* mature cercariae has not revealed a continuation of the caudal ducts into the furcae as previously mentioned by Matthews (1973, 1974).

Studies carried out over the last thirty years have shown remarkably uniform organization of the terminal parts of protonephridia in all Trematoda and Monogenea examined. The origin of the internal ribs of the weir from the flame cell and the origin of the external ribs of the weir from the proximal capillary cell are characteristic features as well as the presence of a septate junction in the proximal capillary, which passes along the weir between two cytoplasmatic cords (Rohde & Watson, 1992; Rohde, 2001). The flame bulbs of *B. gracilescens* and *P. squamatus* cercariae have the same basic morphology. The characteristics that differentiate them, such as a small number of cilia in the tuft, a single-row arrangement of internal and external ribs, and less well developed internal and external leptotriches, are most likely age-specific. Niewiadomska & Czubaj (1996, 2000) showed that changes took place in the ultrastructure of the terminal part of protonephridia in the course of post-cercarial development in *Diplostomum pseudospathaceum*. According to their data, the number of cilia in the flame increased from 30 in cercariae to 50 in metacercariae, and a cup-shaped fibrous structure and internal leptotrichs, absent in cercariae, were formed in metacercariae (Niewiadomska & Czubaj, 2000, figs 2, 3, 5, p. 309).

The fact that the collecting tubes have a cellular structure and the lumen is extracellular have been observed in most digeneans examined to date. In some, however, the distal part of collecting ducts (adjacent to the excretory bladder) appeared to be syncytial, as reported for *Fasciola hepatica* (Bennett & Threadgold, 1973; Bennett, 1977), *Brachylaimus aequans* (Soboleva *et al.*, 1988), *Paramphistomum epiclitum*, *Fischoederius elongatus* (Mattison *et al.*, 1992) and *Diplostomum pseudospathaceum* (Niewiadomska & Czubaj, 2000). In these cases, no septate junctions between the lining of the collecting ducts and the excretory bladder epithelium were observed. Powell (1975) paid particular attention to the absence of such desmosomes in cercariae of *Posthodiplostomum minimum*. On the other hand, septate desmosomes connecting main collecting tubes to the excretory bladder syncytium were repeatedly recorded for trematodes with collecting tubes of uniform structural plan throughout their length. Besides the bucephalids examined, the following features of collecting tubes were shown in *Ochetosoma aniarum* (Powell, 1972), *Cryptocotyle lingua* (Rees, 1977), *Paragonimus ohirai* (Orido, 1987), and four microphallid species (Malkova & Galaktionov, 1989).

In common with most trematode cercariae examined (Krupa *et al.*, 1969; Powell, 1972, 1975; Gibson, 1974; Rees, 1977; Malkova & Galaktionov, 1989), the excretory bladder of *B. gracilescens* and *P. squamatus* cercariae is lined by a nucleated syncytium. These bucephalid larvae have shown that members of one trematode family may differ notably in the functional morphology of the bladder epithelium. For example, in contrast to most cercariae studied (Krupa *et al.*, 1969; Powell, 1972, 1975; Gibson, 1974; Popiel, 1977; Rees, 1977; Malkova & Galaktionov, 1989), the excretory bladder of *B. gracilescens* larvae displays no secretory features. The secretory activity of the excretory bladder lining of *P. squamatus* cercariae is obvious as indicated by numerous secretory inclusions, the exact role of which remains obscure. In previous studies, it has been proposed that the secretory products of the cercarial bladder may serve to detoxify metabolic wastes at the metacercarial stage (Krupa *et al.*, 1969) or to participate in metacercarial cyst formation (Leong & Howell, 1971; Malkova & Galaktionov, 1989). However, no firm evidence for these assertions has been presented. The apical lamellae and basal invaginations are characteristic features of trematode excretory epithelium and are believed to be concerned with its absorptive capacity. The greater level of development of these structures in *P. squamatus* cercariae apparently indicates more intensive transport of fluids through the bladder lining of this species in comparison with that in *B. gracilescens* cercariae. The larger number of mitochondria in the bladder epithelium of *P. squamatus*, presumably providing energy for these activities, is indirect evidence

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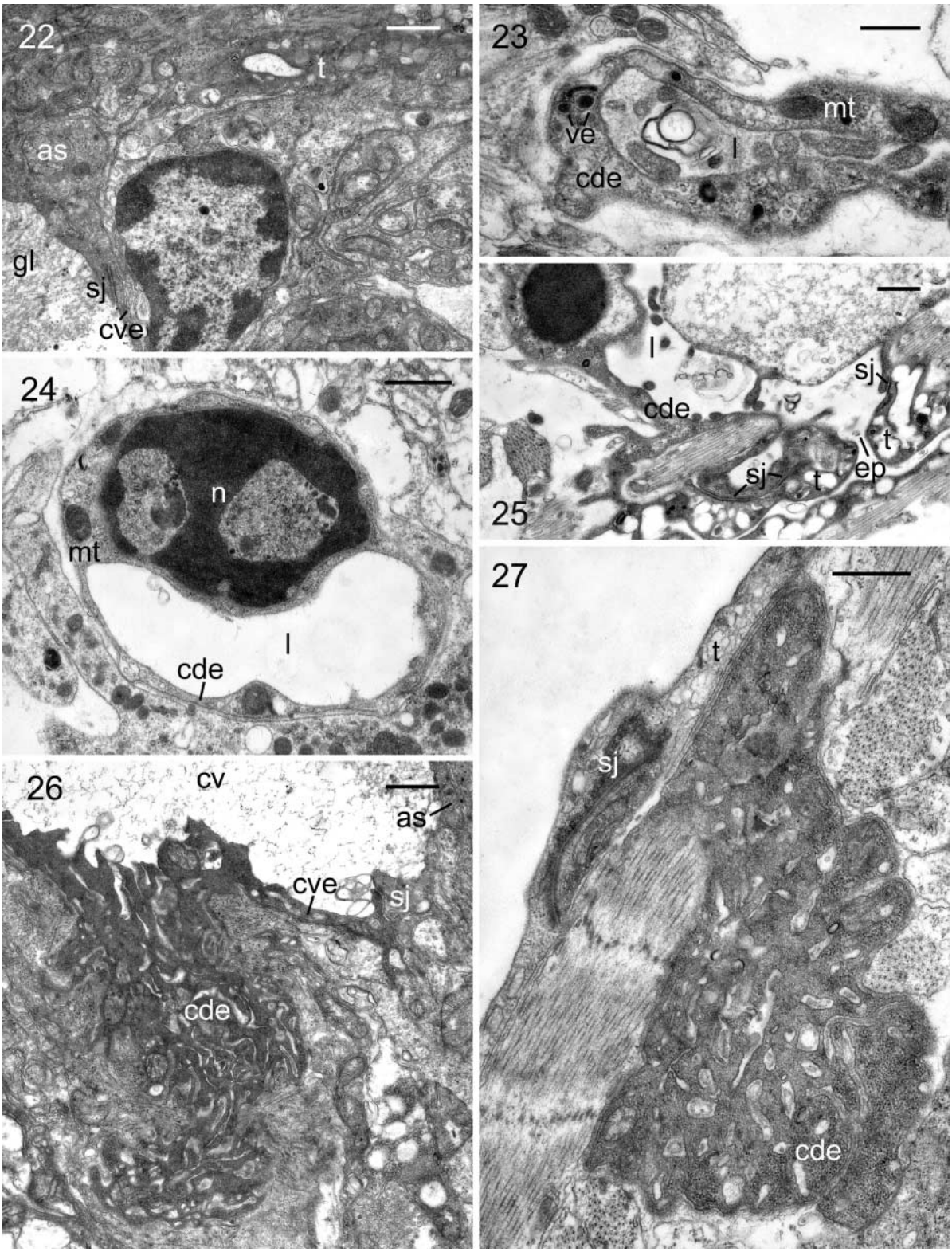

Fig. 22. Anterior part of caudal vesicle of *Prosorhynchus squamatus* cercaria. Note prolongation of atrium lining, its junction to excretory epithelium of caudal vesicle and outgrowth of body tegument (arrow) extended towards atrium lining. Figs 23, 24. Lateral caudal ducts of *Bucephaloides gracilescens* cercaria; (Fig. 25) Excretory pore of *B. gracilescens* cercaria. Fig. 26. Posterior part of caudal vesicle and proximal portion of lateral caudal duct of *P. squamatus* cercaria. Fig. 27. Distal portion of lateral caudal duct of *P. squamatus* cercaria. Note septate desmosome connecting excretory epithelium to tegument. See fig. 1 for key to labelling. Bars = 0.5 μm.

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for this assumption. Some correlation was noted between the functional activity of the excretory bladder epithelium and its differentiation into perikarya regions and cytoplasmic layer. On morphological evidence, both the secretory and absorptive potential of the bladder lining of *P. squamatus* was greater than that of *B. gracilescens*. Accordingly, the perikarya regions in the excretory bladder of *P. squamatus* were more distinctly detached and extended into its lumen. The tendency towards isolation of perikarya in the bladder epithelium of cercariae has been observed in some other trematodes, however, it was organized in a different way. For *Ochetosoma aniarum* (Powell, 1972) and members of the family Microphallidae (Malkova & Galaktionov, 1989) the nuclei and their associated cytoplasm were reported to be submerged into the surrounding parenchyma.

The ultrastructure of the excretory atrium in both species studied is relatively similar (fig. 28). Its location, just in front of the body–tail junction in close proximity to the outer tegument, indicates convincingly that the excretory atrium is a primordial definitive excretory pore. The formation of this pore is only completed after bucephalid cercariae penetrate into the second intermediate host. An analogous

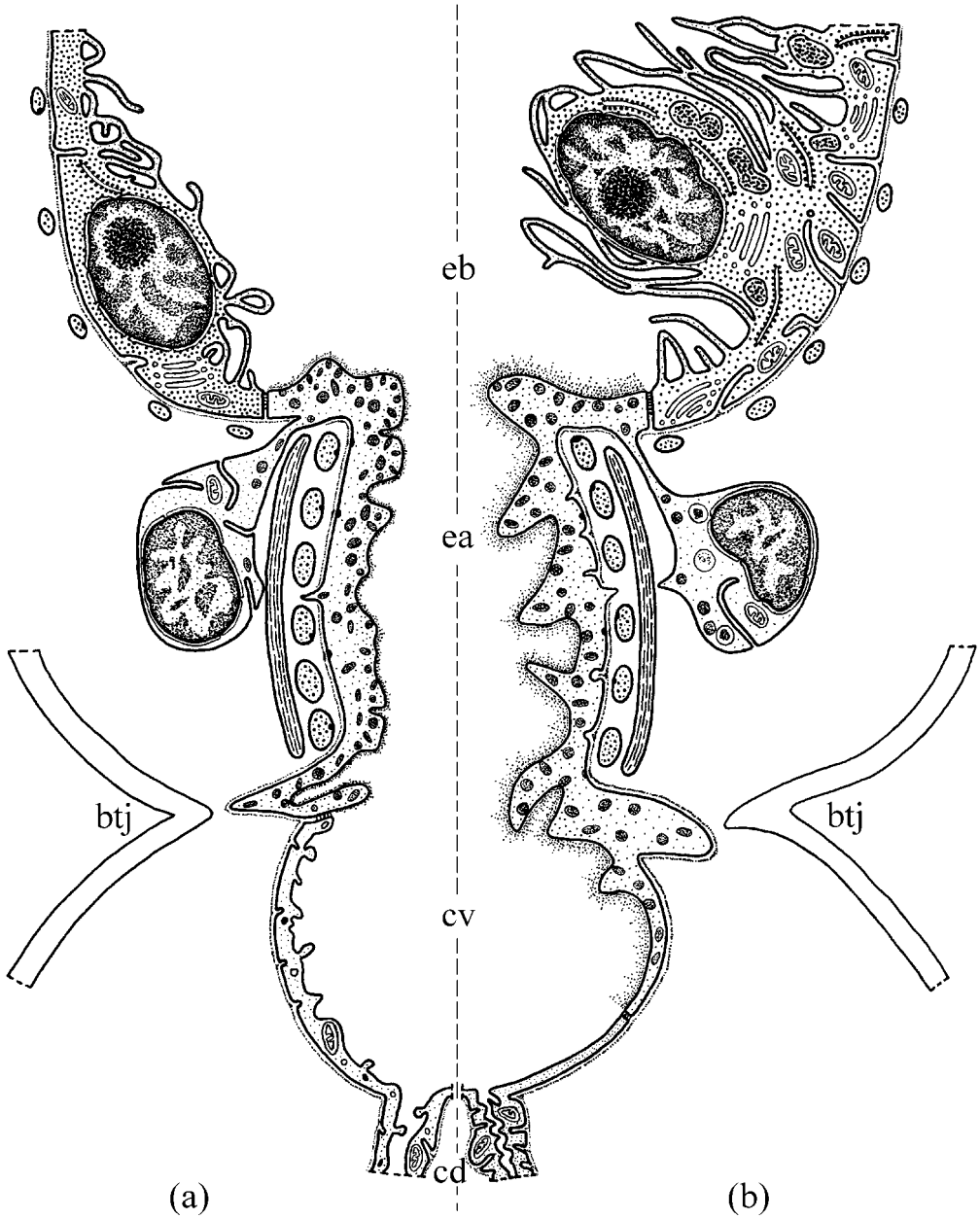

Fig. 28. The distal part of the excretory system of *Bucephaloides gracilescens* (a) and *Prosorhynchus squamatus* (b) cercariae. Lateral view. See fig. 1 for key to labelling.

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structure, lined by tegument and similarly situated near the body–tail junction, was described by Powell (1973, 1975) in *Schistosoma mansoni* and *Posthodiplostomum minimum* cercariae. In these species, unlike the bucephalids, the atrium lining which was connected by a thick cytoplasmatic ligament to the body tegument, was similar to the tegumental ultrastructure. These features of the excretory atrium of *S. mansoni* led Powell (1973) to conclude that this was possibly formed by an 'inpocketing' of the body wall. Rees (1977) later demonstrated that the definitive excretory pore of *Cryptocotyle lingua* cercariae was formed in a similar way. The lack of continuity between the atrium lining with the body tegument and differences in their fine structure in *B. gracilescens* and *P. squamatus* cercariae suggest that, in this case, the excretory atrium is formed independently of the body wall. Preliminary observations on the development of the excretory system in *B. gracilescens* and *P. squamatus* cercariae also support this contention.

The principle difference in the fine structure of the caudal part of the excretory system of *B. gracilescens* and *P. squamatus* cercariae is the relative extent that the atrium syncytium acts as a lining of the caudal vesicle (fig. 28). The ultrastructure of caudal excretory ducts in mature cercariae has also been observed in *S. mansoni* (Ebrahimzadeh & Kraft, 1971; Powell, 1973) and *Diplostomum pseudospathaceum* (Niewiadomska & Czubaj, 1996). In all species examined, including bucephalids, no septate junctions have been found in the walls of the caudal ducts (Powell, 1973; Niewiadomska & Czubaj, 1996; this study). This indicates that these ducts are organized as a syncytium rather than an intracellular arrangement as suggested by some authors (Ebrahimzadeh & Kraft, 1971; Powell, 1973). Bucephalid larvae, unlike cercariae of *S. mansoni* and *D. pseudospathaceum*, have secondarily formed excretory pores (Matthews, 1973, 1974). Their fine structure, however, displays no essential differences from descriptions of the primary excretory pores of *S. mansoni* (Ebrahimzadeh & Kraft, 1971; Powell, 1973) and *D. pseudospathaceum* (Niewiadomska & Czubaj, 1996).

The analysis presented here demonstrates the lack of knowledge concerning the ultrastructure of the excretory system of cercariae belonging to different taxonomic groups. This makes these data difficult to use for comparative morphological-functional analysis and phylogenetic studies. Certain similarities in the structure of the excretory system of bucephalid cercariae and that described earlier for furcocercariae of Strigeidida and Schistosomatida are noteworthy. These include the presence of a pronounced excretory atrium (a provisional definitive excretory pore in bucephalids and strigeidids), as well as the similar structure of the caudal ducts. It should be borne in mind, however, that the excretory atrium in furcocercaria of Strigeidida and Schistosomatida probably originates from the body tegument, whereas in bucephalid larvae it is formed as a separate structure. It would therefore be incorrect to adopt the presence of an excretory atrium in cercariae as a feature for supporting a possible relationship between bucephalids and strigeidid–schistosomatid phylogenetic clade digeneans.

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