

Ultrastructural characteristics of the caeca of basal polyopisthocotylean monogeneans of the families Chimaericolidae and Hexabothriidae parasitic on cartilaginous fishes

Larisa G. Poddubnaya¹ · Willy Hemmingsen² · Cecile Reed³ · David I. Gibson⁴

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Abstract Ultrastructural differences are shown between the caecal organization in three blood-feeding polyopisthocotylean monogeneans, i.e., the chimaericolid *Chimaericola leptogaster* from the holocephalan *Chimaera monstrosa* and two hexabothriids, *Callorhynchocotyle callorhynchi* from the holocephalan *Callorhynchus capensis* and *Rajonchocotyle emarginata* from the elasmobranch *Amblyraja radiata*. In *C. leptogaster*, digestive cells and connecting syncytium, joined close to the luminal surface by septate junctions, are arranged alternately along the caecal epithelial wall; the nuclear regions of both cell types are sunk below the general level of the caecal epithelium; a concave depression on the apical margin of the digestive cells bears lamellae; and this depression is covered by a lamellate bubble formed by thin projections emanating from the connecting syncytium. The luminal surface of the connecting syncytium is covered with outgrowths terminating in the form of long, narrow processes. In *R. emarginata* and *C. callorhynchi*, the predominant digestive cells are at different stages of development and occur in groups, developing digestive cells bulge into the caecal lumen from the connecting syncytium with contact sites present close to the luminal surface,

and the luminal surface structures of both the connecting syncytium and the digestive cells are short lamellae. In these two hexabothriids, a holocrine (or apocrine) process for the elimination of digestive product is assumed via the detachment of fully differentiated, bulging digestive cells. Free, apparently sloughed digestive cells and residual bodies are present within the caecal lumen, and replacement digestive cells are numerous in the connecting syncytium. In the chimaericolid, free bubbles containing residual bodies and portions of digestive cells filled with degenerating digestive vesicles occur in the caecal lumen along with large amounts of male and female reproductive material. The usefulness of characteristics of the caecal ultrastructure as taxonomic traits at the family level is discussed.

Keywords Ultrastructure · Monogenea · Polyopisthocotylea · Basal taxa · *Chimaericola leptogaster* · *Rajonchocotyle emarginata* · *Callorhynchocotyle callorhynchi* · Caeca · Digestive cells · Taxonomic trait

Introduction

Monogenean parasites of the subclasses Monopisthocotylea and Polyopisthocotylea differ in terms of their diet and the cellular organization of their caecal epithelium. Monopisthocotyleans feed on host epidermal cells and mucus, which are digested by a single type of digestive cell present in the caecal epithelium (Llewellyn 1954; Halton and Jennings 1965; Halton and Stranock 1976; Kearn 2004). Whereas polyopisthocotyleans generally feed on blood, and the caecal epithelium consists of two components, namely digestive cells and a connecting syncytium (Halton et al. 1968; Rohde 1973; Tinsley 1973; Brennan and Ramasamy 1996; Kearn 2004; Allen and Tinsley 1989).

✉ Larisa G. Poddubnaya
poddubny@ibiw.yaroslavl.ru

¹ I. D. Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, 152742 Borok, Yaroslavl Province, Russia

² Department of Arctic and Marine Biology, Faculty of Biosciences, Fisheries and Economics, University of Tromsø, 9037 Tromsø, Norway

³ Department of Biological Sciences, University of Cape Town, Private Bag X3, Rondebosch 7701, Cape Town, South Africa

⁴ Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, UK

The aim of the present study was to examine, for the first time, the ultrastructural organization of the caecal epithelium of three polyopisthocotylean monogenean species belonging to two basal families, the Chimaericolidae and the Hexabothriidae. These species, i.e., the chimaericolid *Chimaericola leptogaster* (Leuckart, 1830) and the hexabothriids *Rajonchocotyle emarginata* (Olsson, 1876) and *Callorhynchocotyle callorhynchi* (Manter, 1955), are all parasitic on the gills of cartilaginous fishes (Holocephali and Elasmobranchii). This account follows our previous ultrastructural studies on basal monogeneans of the subclass Polyopisthocotylea (Poddubnaya et al. 2013, 2014; Poddubnaya and Hemmingsen 2014) and forms part of a search for new ultrastructural characters which might shed light on the evolutionary history of this enigmatic group of parasites.

Materials and methods

For electron microscopy, adult specimens of three polyopisthocotylean monogeneans were recovered from the gills of naturally infected cartilaginous fishes: *C. leptogaster* (Chimaericolidae) from the chimaera (rabbit fish) *Chimaera monstrosa* Linnaeus, 1758 (Holocephali), *R. emarginata* (Hexabothriidae) from the thorny (starry) ray *Amblyraja radiata* (Donovan, 1808) (Elasmobranchii), and *C. callorhynchi* (Hexabothriidae) from the chimaera (Cape elephant fish) *Callorhynchus capensis* Duméril, 1865 (Holocephali). The first two were collected in the Norwegian Sea off Tromsø, Norway, and the latter was from the South-east Atlantic off St Helena Bay on the western coast of South Africa. Live specimens of all three monogenean species were fixed using 3 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 20 days at 5 °C, rinsed four times for 20 min in the same buffer and post-fixed in 1 % osmium tetroxide for 1 h. For ultrathin studies, samples were then dehydrated in a graded series of ethanol and acetone, and embedded in a mixture of Araldite and Epon. Ultrathin sections (70–90 nm in thickness) were stained with uranyl acetate and lead citrate, and examined using a JEOL-JEM-1011 transmission electron microscope operating at 80 kV.

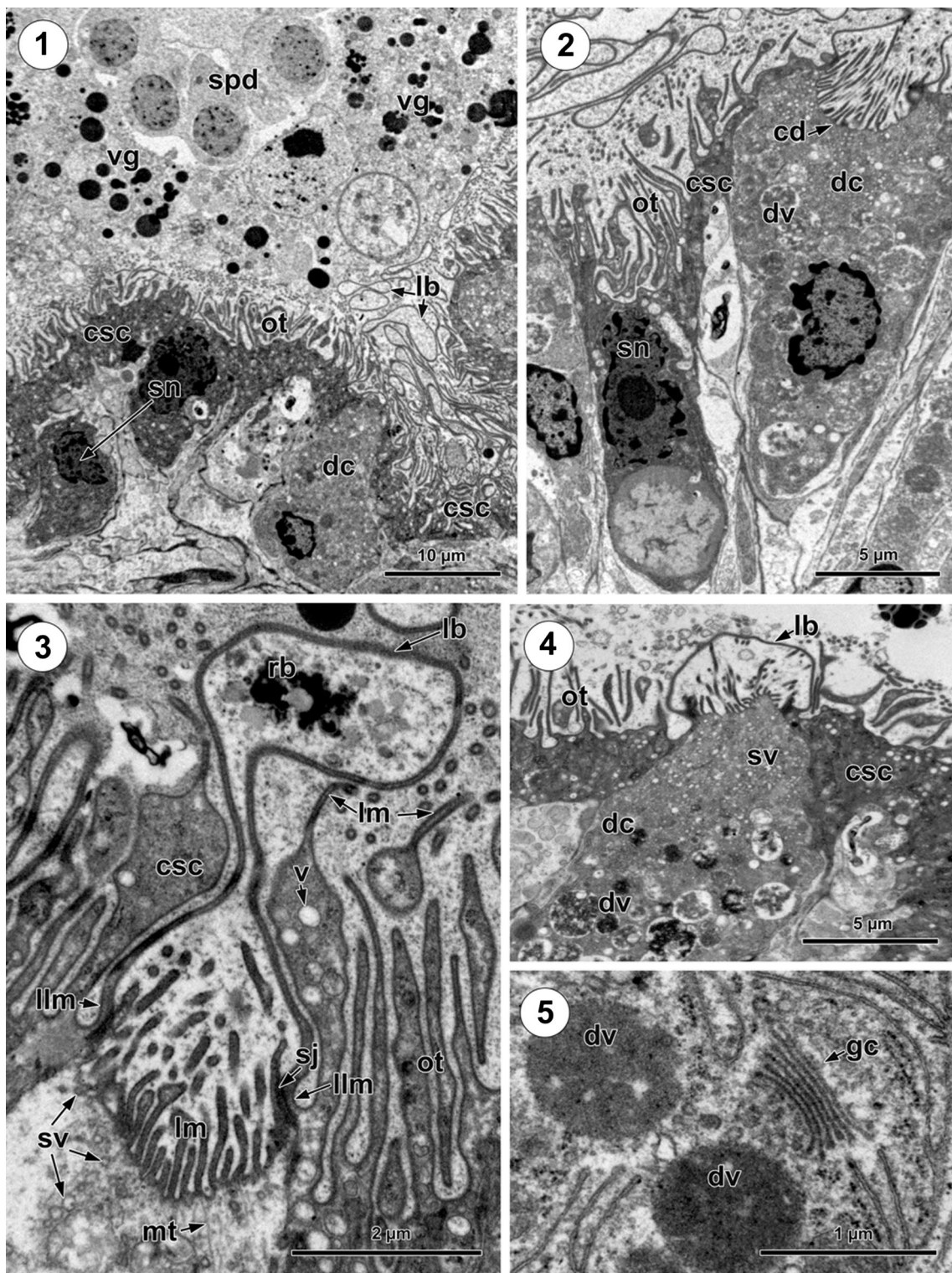
Results on the caecal structure

Chimaericola leptogaster (Chimaericolidae)

The anterior, undivided, region of the intestine of *C. leptogaster* bifurcates at the level of the vaginal pores, forming two caeca which pass posteriorly into the haptor, where they end blindly. The anterior intestine and both caeca bear long, narrow, branching, lateral diverticula.

Fig. 1–5 Ultrastructure of the caeca of *Chimaericola leptogaster*. **1** Section of the caecal epithelium showing the two alternating cell types, differentiated digestive cells and connecting syncytial cytoplasm; note the male and female reproductive material within the lumen. **2** Digestive and connecting syncytial cells of the caecal epithelium; note the concave depression on the luminal surface of the digestive cell. **3** A lamellate bubble covering the luminal surface of the digestive cell; note the adjacent surface lamellae of the connecting syncytial cytoplasm which form the bubble, electron-dense material (residual bodies) within the bubble, septate junctions between the two cell types, lamellae arising from the concave luminal surface of the digestive cell, and numerous luminal surface outgrowths of the connecting syncytium. **4** Apical region of a digestive cell; note the electron-lucent vesicles and the lamellate bubble enveloping the luminal surface of the digestive cell. **5** Organelles in the digestive cell cytoplasm. *Abbreviations:* *cd* concave depression, *csc* connecting syncytium, *dc* fully differentiated digestive cell, *dv* digestive vesicles, *gc* Golgi complex, *lb* lamellate bubble enveloping luminal surface of digestive cell, *lm* lamellae, *llm* lateral lamellae of connecting syncytium, forming lamellate bubble around digestive cell, *mt* microtubules, *ot* outgrowths of luminal surface of connecting syncytium, *rb* residual bodies, *sj* septate junction, *sn* nucleus of connecting syncytium, *spd* spermatids, *sv* small vesicles, *v* vesicles in connecting syncytium, *vg* vitelline granules

The caecal epithelium is formed by two alternating cell types, i.e., digestive and connecting syncytial cells, the perinuclear regions of which are sunk below the general level of the syncytium (Figs. 1, 2, 6, 31). In the digestive cells, the nucleus is always found near the base of these cells (Figs. 1, 2, 31). The apical cytoplasm of the digestive cells contains numerous small (0.1–0.2 µm in diameter), electron-lucent, rounded vesicles, and microtubules directed toward the deeper cell cytoplasm can be observed in this region (Figs. 3, 4). The remaining cell cytoplasm is filled with large, membrane-bound, electron-dense or moderately electron-dense digestive vesicles, the diameter of which varies from 0.3 to 1.9 µm (Figs. 2, 4, 31), and the contents of which may be loosely packed and include light and dark areas (Figs. 4, 5). Golgi complexes, cisternae of granular endoplasmic reticulum (GER) and mitochondria are associated with the digestive vesicles in the perinuclear region of the cell (Figs. 5, 31). Usually, the luminal surface of these cells has a concave depression (Figs. 2, 3, 6, 31) and bears lamellae with a diameter of approximately 0.1 µm and a length up to 3.0 µm (Figs. 2, 3, 31). The lateral surfaces of each digestive cell are surrounded by cytoplasm of the connecting syncytium (Figs. 3, 4, 31). Apically, the plasma membranes of both the connecting syncytium and digestive cells are joined by septate junctions of about 0.5 µm in length (Figs. 3, 31). At the luminal surface of each digestive cell, where it interconnects with the surrounding connecting syncytium, the luminal projections of connective cytoplasm form a lamellate bubble enveloping the top of each digestive cell (Figs. 3, 31). Within these bubbles, electron-dense material (residual bodies) is apparent (Figs. 1, 3). There is no basal lamina supporting the caecal epithelium, and both digestive and connecting syncytial cells are limited by a cell plasma membrane (Figs. 1, 2).



In addition to the fully formed digestive cells, beneath and close to the connecting syncytium, undifferentiated digestive cells are visible throughout the caecal wall (Figs. 7, 9). These cells are characterized by a large nucleus and a thin area of perinuclear cytoplasm.

The alternate arrangement of the two caecal components (digestive and connecting syncytial cells) may include differentiating digestive cells recognizable by a central nucleus and the absence of fully formed digestive vesicles (Fig. 6).

The luminal surface of the connecting syncytial cytoplasm is characterized by the presence of numerous outgrowths which vary in shape and size, and terminate in the form of lamellae (Figs. 1–3, 31). The large nuclei of the connecting syncytium possess prominent nucleoli and perinuclear heterochromatin (Fig. 2). The most numerous inclusions in the electron-dense syncytial cytoplasm of these syncytial cells are small electron-lucent, membrane-bound vesicles along with a few moderately dense and dense vesicles (Figs. 2, 3, 31). These vesicles measure approximately 0.3 μm in diameter.

The lumen of the caecum contains portions of digestive cells that are filled with large digestive vesicles exhibiting degenerative changes, as well as dark large aggregations of residual bodies (Figs. 8, 10). Numerous free luminal bubbles are also visible in the caecal lumen, some of which are empty and others contain an aggregation of residual bodies (Figs. 9, 10). However, most of the luminal volume of the caecum is occupied by male and female reproductive material (nucleated portions of vitelline cells, free vitelline globules and their clusters, lipid droplets, mature spermatozoa at different stages of spermiogenesis and spermatids) (Figs. 1, 8).

Rajonchocotyle emarginata (Hexabothriidae)

The intestine bifurcates close to the pharynx to form two diverticulate caeca, which pass back sublaterally along the body and reunite close to its posterior extremity, from where the resulting single caecum passes into the haptor and extends deep into the haptor appendix.

Two types of cell are present in the caecal epithelium, i.e., digestive and syncytial connecting cells. Digestive cells are dominant and occur in groups, within which these cells differ in their size, shape and stage of development (Figs. 16, 32). Undifferentiated digestive cells, with a large nucleus and a thin region of surrounding perinuclear cytoplasm, are embedded in the connecting syncytial epithelium of the caecal wall, but there are no contact sites at this stage between the membranes of the two types of cell (Figs. 16, 17, 32). Gradually differentiating digestive cells are characterized by the appearance of small, rounded, moderately dense vesicles 0.2–0.5 μm in diameter in an increasing cytoplasmic volume (Fig. 19). These vesicles (digestive vesicles) gradually become larger (about 0.8–1.5 μm in diameter) and darker in the cytoplasm of subsequent stages of digestive cell development (Figs. 18, 32). The developing cells bulge into the caecal lumen, but a thin area of syncytial connecting cytoplasm remains around them (Figs. 18, 19, 32). Between the plasma membrane of these cells and the surrounding connecting syncytium, septate junctions are prominent (Figs. 18, 19, 32). Fully developed digestive cells project deep into the caecal lumen, leaving only their basal region enveloped by an extension of the cytoplasm of the connecting syncytium (Figs. 16, 20, 32). The two cell types are joined close to the luminal surface by septate

Fig. 6–15 Ultrastructure of the caecal luminal contents of *Chimaericola leptogaster* and *Rajonchocotyle emarginata*. **6–10** *C. leptogaster*. **6** Differentiating digestive cell and nucleate region of connecting syncytium. **7** Undifferentiated digestive cells in the basal region of the connecting syncytium. **8** Fragments of digestive cells filled with degenerative digestive vesicles within the caecal lumen; note the isolated dense residual body and free degenerate digestive vesicles. **9** Portion of the lamellate bubble with an aggregation of residual bodies inside the caecal lumen. **10** Fragment of a digestive cell with degenerative digestive vesicles and a portion of a lamellate bubble containing an aggregation of residual bodies within the caecal lumen. **11–15** *R. emarginata*. **11** Digestive cells exhibiting degenerative changes within the caecal lumen; note the residual bodies. **12** Luminal surface of two digestive cells showing short, surface lamellae and small vesicles. **13** Degenerative digestive cell showing digestive vesicles with a dense central material; note this dense material free within the caecal lumen. **14** Fully degenerate digestive cell showing residual bodies. **15** Part of a digestive cell showing short surface lamellae and digestive vacuoles with varying content. **Abbreviations:** *cl* caecal lumen, *csc* connecting syncytial cytoplasm, *dc* fully differentiated digestive cell, *ddc* degenerating digestive cell, *dv* digestive vesicles, *dvm* dense vesicular material, *gdc* gradually differentiating digestive cell, *lb* lamellate bubble enveloping luminal surface of digestive cell, *lm* lamellae, *mc* mature digestive cell, *ot* outgrowths of luminal surface of connecting syncytium, *pdc* portion of digestive cell, *rb* residual bodies, *sn* nucleus of connecting syncytium, *sp* spermatozoa, *sv* small vesicle of digestive cell, *uc* undifferentiated digestive cell

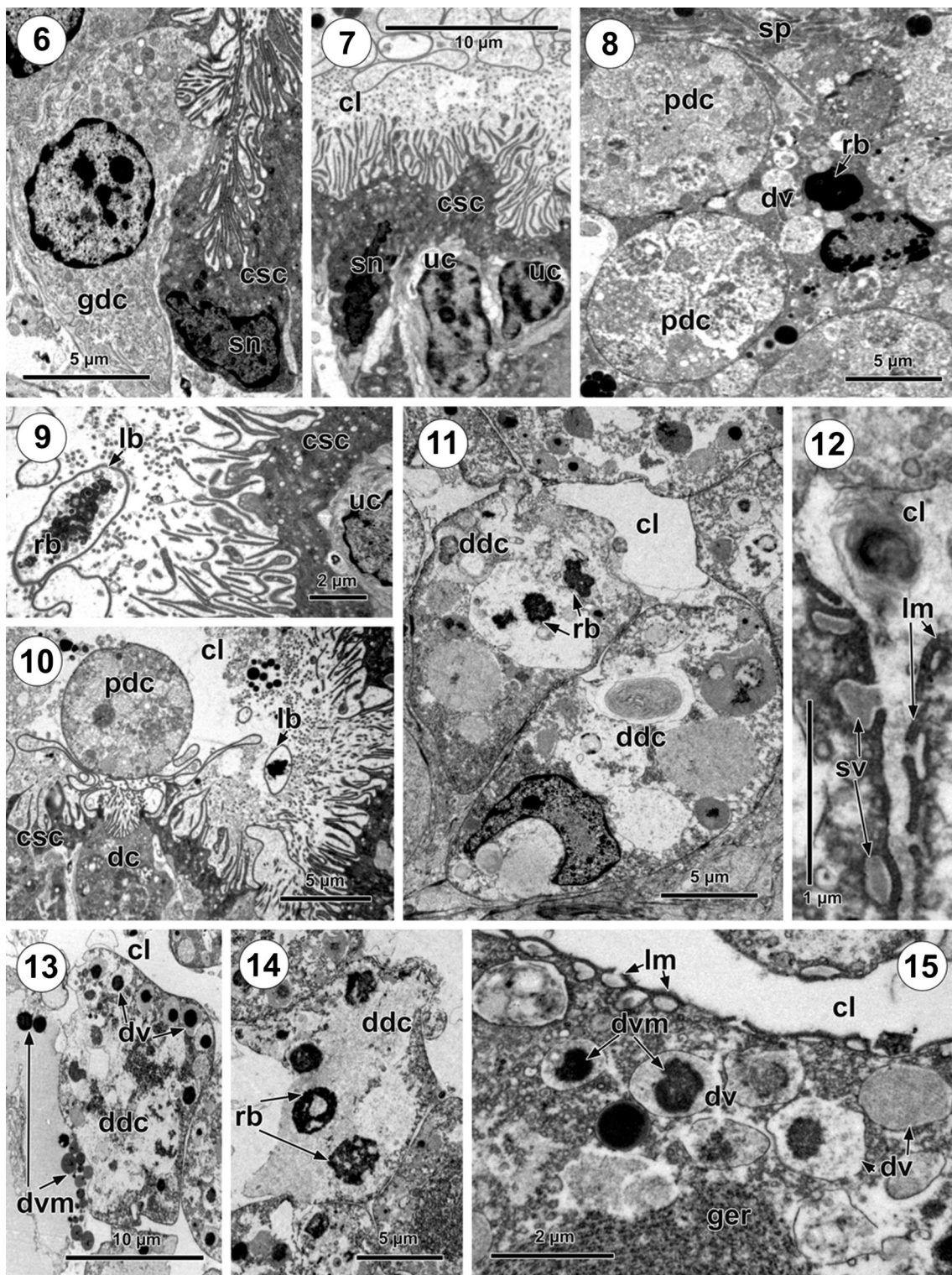
junctions (Figs. 18, 19, 22, 32). The fully developed digestive cells are characterized by a large nucleus which occupies a basal position in the cell cytoplasm and around which occur a large, compact area of GER and Golgi complexes in the form of small dictyosomes with small, adjacent, electron-dense vesicles (Figs. 20, 32). Numerous large, rounded digestive vesicles, varying in content and diameter (1.5–2.5 μm), are evenly distributed in the cytoplasm (Figs. 16, 20, 32). The contents of digestive vesicles may appear almost empty, as the electron-dense material congregates centrally (Figs. 15, 32); smaller digestive vesicles contain a moderately or fully electron-dense, homogeneous content (Figs. 21, 32). At the luminal surface of the digestive cells, small vesicles occur (Figs. 12, 32). Short lamellae, which measure about 0.2 μm in length, arise from the luminal surface of these cells and become aligned parallel to the cell surface (Figs. 12, 15, 32).

The thin layer of connecting syncytium is filled with numerous small, electron-lucent vesicles and contains nuclei that are often located close together in pairs (Figs. 16, 17, 32). Close to the basal membrane of the syncytium, a thin fibrous layer can be observed (Fig. 32).

Within the caecal lumen, free portions of digestive cells exhibit degenerative changes (Figs. 11, 13, 14). A large mass of residual bodies and sloughed digestive cells can be seen in the lumen (Figs. 11, 13, 14).

Callorhynchocotyle callorhynchi (Hexabothriidae)

The intestine of this species bifurcates at about the level of the two vaginal pores to form two lateral caeca and gives off short,



lateral diverticula along its entire length. The caeca reach close to the lateral margins of the body and reunite to form a single caecum in the peduncular region, which passes into, and branches extensively within, the haptor, with a narrow extension reaching into the haptoral appendix.

The structure and arrangement of the two components (digestive cells and connecting syncytium) of the caecal epithelium of this species are rather similar to those of the other hexabothriid species, *R. emarginata*, examined. The digestive cells, arranged in groups, protrude from the caecal wall deeply

into the caecal lumen (Figs. 23, 24). Connecting syncytial cytoplasm overlaps a variable proportion of the lateral margin of each digestive cell, where septate junctions are present at the point of maximum overlap (Figs. 24, 26). Digestive cells contain a large, basal nucleus, and much of their cytoplasmic volume may be occupied by large, rounded, or sometimes oval membrane-bound digestive vesicles (Figs. 24, 26, 30). The diameter of these vesicles varies (1.0–1.7 μm), and they contain a finely granular, moderately dense material with varying amounts of a more dense substance (Figs. 26, 30). Numerous small, electron-lucent vesicles, of 0.3–0.6 μm in diameter, occur close to, and at, the surface of the digestive cells (Figs. 26, 30).

The connecting syncytium consists of occasional nucleated regions in an electron-dense cytoplasm containing many electron-lucent vesicles (Figs. 25, 27). The luminal plasma membrane of both the connecting syncytium and the digestive cells bears the same kind of lamellae. These are approximately 1.0 and 0.6 μm in length on the luminal surface of the connecting syncytium and digestive cells, respectively (Figs. 25, 27).

Isolated portions of digestive cells and entire cells are found scattered free in the caecal lumen (Figs. 23, 24, 28, 29). Some of the fragments exhibit degenerative changes (Fig. 29). Large, dense residual bodies are also visible in the caecal lumen (Fig. 26).

Discussion

Variations in the caecal ultrastructure of members of two basal polyopisthocotylean families

The present study of the ultrastructural organization of the caeca of three monogenean species belonging to basal monogenean families of the subclass Polyopisthocotylea, the Chimaericolidae and Hexabothriidae, demonstrates differences in the ultrastructure and arrangement of the caecal components in members of these two families. It should be noted that, as is the case for most blood-feeding polyopisthocotyleans (Halton et al. 1968; Rhode 1973; Tinsley 1973; Halton 1975, 1997; Allen and Tinsley 1989; Brennan and Ramasamy 1996), in both the chimaericolid *C. leptogaster* and the hexabothriids *R. emarginata* and *C. callorhynchi*, the caecal epithelium is composed of two distinct cell types. These are digestive cells and a connecting syncytium, the latter of which occurs in all regions of the caeca between adjacent digestive cells. However, there are differences between the studied members of these two families.

In *C. leptogaster*, (a) the two cell components are arranged alternately along the caecal epithelial wall, i.e., digestive cells are separated by a nucleate area of connecting syncytium; (b)

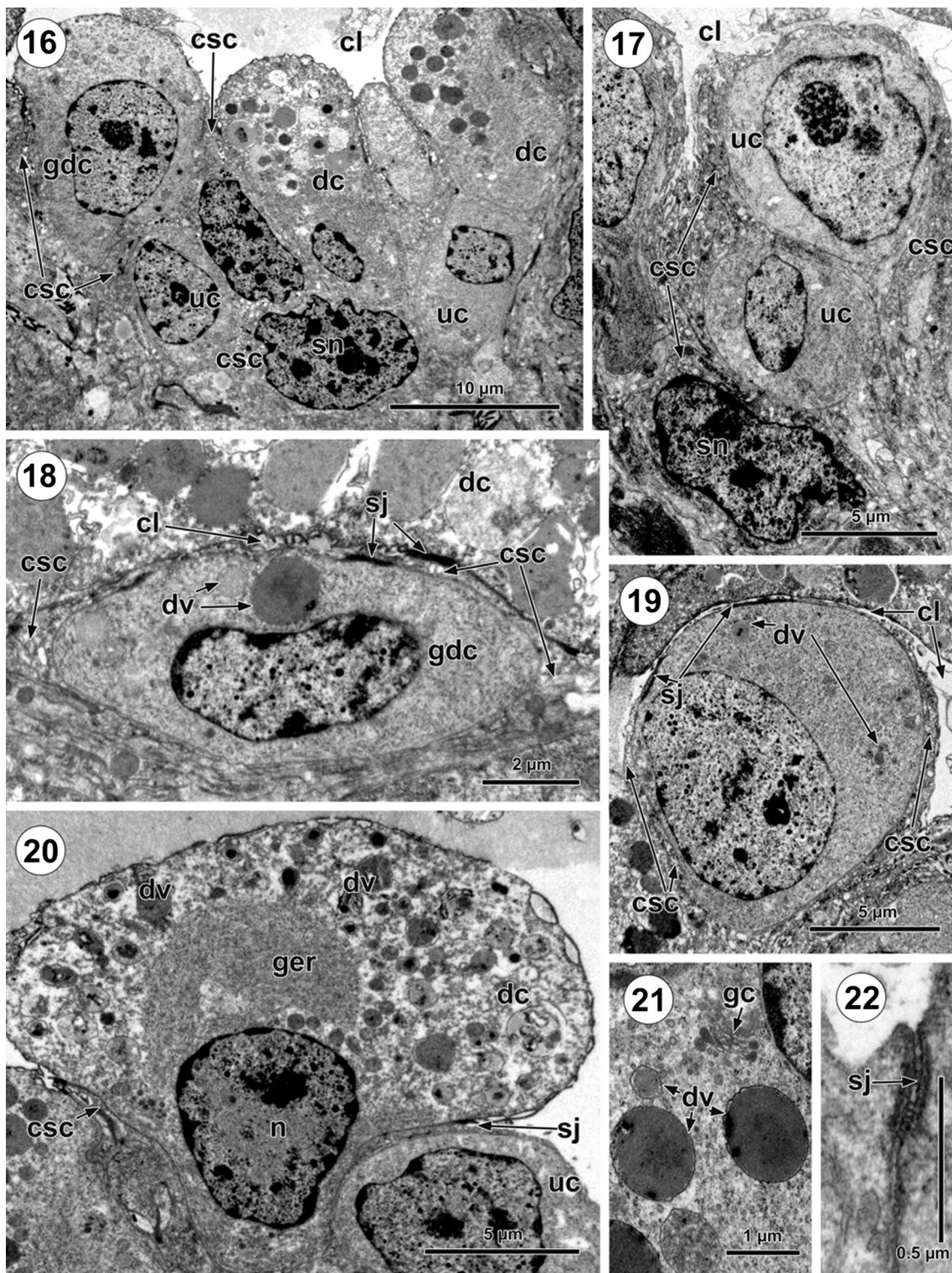
Fig. 16–22 Ultrastructure of the caecal epithelium of *Rajonchocotyle emarginata*. **16** Section of a caecum showing a group of digestive cells at different stages of development and two neighbouring nuclei of the connecting syncytium. **17** Undifferentiated digestive cells within the connecting syncytium; note a nucleus of the connecting syncytial cytoplasm. **18** Gradually differentiating digestive cell, most of which is within the connecting syncytium; note the septate junctions between the thin layer of connecting syncytium and both the fully differentiated and gradually differentiated digestive cells. **19** Gradually differentiated digestive cell protruding into the caecal lumen; note septate junctions between the thin connecting syncytial cytoplasm and this cell. **20** Fully differentiated digestive cell; note the GER, digestive vesicles and septate junctions close to the luminal surface. **21** Section through digestive cell cytoplasm showing a Golgi dictyosome and digestive vesicles. **22** Typical septate junction between the two types of caecal cells. *Abbreviations:* *cl* caecal lumen, *csc* connecting syncytial cytoplasm, *dc* fully differentiated digestive cell, *dv* digestive cell vesicles, *gc* Golgi complex, *gdc* gradually differentiated digestive cell, *ger* granular endoplasmic reticulum, *n* nucleus of digestive cell, *sj* septate junction, *sn* nucleus of connecting syncytium, *uc* undifferentiated digestive cell

the nuclear regions of both cell types are sunk below the general level of the caecal epithelium; (c) a concave depression on the luminal surface of every digestive cell bears a dense concentration of lamellae; (d) this depression is covered by a bubble formed by a thin luminal projection emanating from the syncytial connecting cytoplasm surrounding the lateral sides of each digestive cell; and (e) the general surface structures arising from the luminal surface of the connecting syncytium are outgrowths terminating in the form of long, narrow processes.

In the hexabothriids *R. emarginata* and *C. callorhynchi*, (a) the predominant digestive cells occur in groups; (b) they bulge into the caecal lumen; and (c) the luminal surface structures of both the connecting syncytium and the digestive cells bear short lamellae. Moreover, in *R. emarginata*, every group of digestive cells included cells at different stages of development.

In the chimaericolid and hexabothriids studied, the digestive cells and connecting syncytium are joined close to the luminal surface by septate junctions. The presence of contact sites between these two cellular components in other polyopisthocotylean monogeneans has been shown for the diclidophorid *Diclidophora merlangi* by Halton et al. (1968) and for polystomatids of the genera *Polystomoides*, *Neopolystoma* and *Concinocotyla* by Rohde (1973) and Watson and Whittington (1996). It is, however, worth noting that in the thoracocotylid *Pricea multae* (see Brennan and Ramasamy 1996) and the microcotylid *Atriaseter heterodus* (see Santos et al. 1998), no such contact sites were reported.

Variations in the ultrastructural organization of the caeca of members of different families of the polyopisthocotylean order Mazocraeidea have also been observed (Halton et al. 1968; Brennan and Ramasamy



1996; Santos et al. 1998). For example, in the diclidophorid *D. merlangi*, the digestive cells have been illustrated as alternating with the connecting syncytium within the caecal wall (Halton et al. 1968), whereas, in

the thoracocotylid *P. multae*, the digestive cells occur in groups and may project deep into the lumen (Brennan and Ramasamy 1996). Other variations in the caecal epithelium are evident in the non-blood-feeding polystomatids of

the genera *Polystomoides*, *Polystomoidella*, *Neopolystoma* and *Concinnocotyla* from chelonians (Rohde 1973; Allen and Tinsley 1989; Watson and Whittington 1996), and in the blood-feeding genus *Protopolystoma* from a species of *Xenopus*, which are characterized by the luminal surface of the connecting syncytium being smooth and the presence of lamellae on the luminal surface of the digestive cells (Tinsley 1973).

Ultrastructure of the digestive cells of polyopisthocotyleans

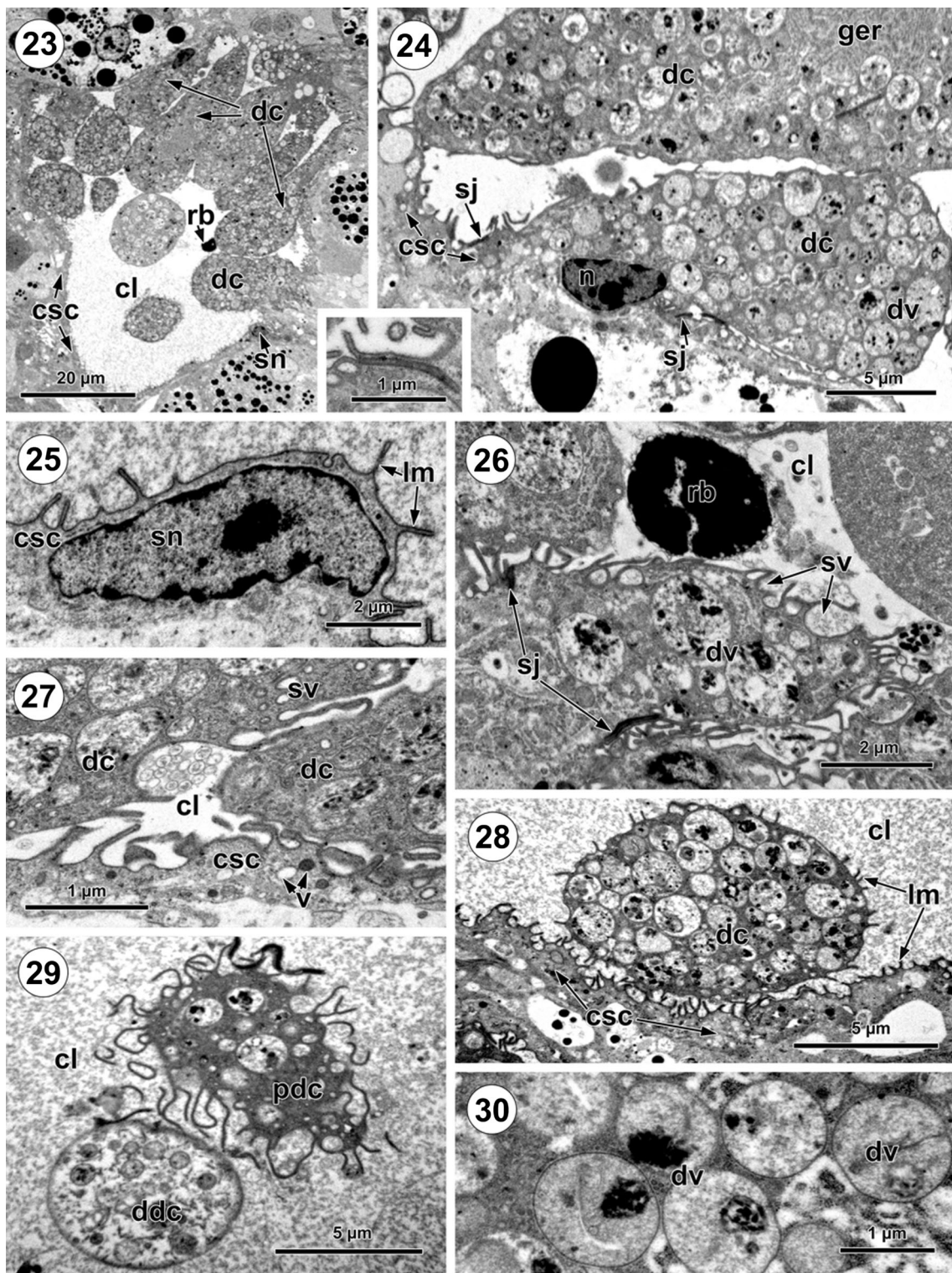
In the chimaericolid and hexabothriids studied, the basal third of the digestive cells is occupied by the nucleus and a complex of perinuclear organelles, i.e., GER, Golgi complexes, and mitochondria, similar to the arrangement reported for other polyopisthocotyleans (Halton 1975; Bogitsh 1993). As indicated by Halton (1975), in the apical region of the digestive cells close to the lumen, small clear vesicles are the predominant organelles. This is also found in *C. leptogaster*, where the apical region forms a distinct area filled with small, clear vesicles. Nevertheless, in the hexabothriids studied and in members of the order Mazocraeidea (Halton et al. 1968; Brennan and Ramasamy 1996; Santos et al. 1998), at the cell surface, the small vesicles are distributed among large digestive vesicles which represent the dominant inclusions in the major part of the digestive cell volume of all polyopisthocotyleans (Halton 1975). According to Halton (1975, 1997), the digestive cells of blood-feeding monogeneans function in the uptake and intracellular digestion of host-blood protein (haemoglobin). He was of the opinion that the nucleate region of the cell, with its associated organelles, is involved in the synthesis of digestive enzymes. The small clear vesicles are pinocytotic in nature in *D. merlangi* (see Halton et al. 1968, 1975), *P. multae* (see Brennan and Ramasamy 1996), and *Atriaseter heterodus* (see Santos et al. 1998), suggesting that the endocytosis of host macromolecules occurs. These small pinocytotic vesicles are responsible for the transmission of absorbed haemoglobin for intracellular digestion by enzymes of GER-Golgi origin (Halton 1975). In the chimaericolid studied, the apical area of the cell, with its mass of small vesicles, contains microtubules which extend from the cell surface deep into the body of the cell. The presence of microtubules in the apical region of digestive cells has also been mentioned for the polystomatid *Concinnocotyla australensis* by Watson and Whittington (1996), where the authors have suggested that microtubules form conductive pathways for pinocytotic vesicles. Intracellular digestion subsequently leads to an accumulation of haematin within large membrane-bound vesicles, and Halton (1975, 1997) considered that the digestion of haemoglobin and the elimination of any residual matter indicates that the digestive cells have elements of a lysosomal system, such that the coated vesicles of

Fig. 23–30 Caecal ultrastructure of *Callorhynchocotyle callorhynchi*. **23** Section of a caecum showing the digestive cells projecting into the caecal lumen. **24** Two digestive cells, the lower of which retains its septate junctions with the connecting syncytium and the other (above) which has lost them. Insert (bottom left). Septate junction between the two cell types. **25** Nucleus of the connecting syncytium. **26** Region of a digestive cell with digestive vesicles retaining links with the connecting syncytium via septate junctions; note the small discharging vesicles and a large, dense inclusion of a dark residual body within the caecal lumen. **27** Region of the connecting syncytium showing small, electron-lucent and dense vesicles in its cytoplasm; note the discharging of small vesicles at the surface of the digestive cells. **28** Section of a digestive cell in the caecal lumen losing its link with the connecting syncytium; note the same kind of lamellae on the surfaces of the connecting syncytium and the digestive cell. **29** Two portions of digestive cells within the caecal lumen, one of them showing degenerative changes in its digestive vesicles. **30** Digestive vesicles in the fully differentiated digestive cell cytoplasm. *Abbreviations:* *cl* caecal lumen, *csc* connecting syncytial cytoplasm, *dc* digestive cell, *ddc* degenerate digestive cell, *dv* digestive cell vesicles, *ger* granular endoplasmic reticulum, *lm* lamellae, *pd* portion of digestive cell, *sj* septate junction, *rb* residual bodies, *sn* nucleus of connecting syncytium, *sv* small vesicle of digestive cell *dv* vesicles in connecting syncytium

the digestive cells are heterophagosomes or pre-lysosomes and the haematin-rich residues, in the form of large, membrane-bound vesicles, are released into the caecal lumen.

Holocrine (apocrine) process for the elimination of digestive products

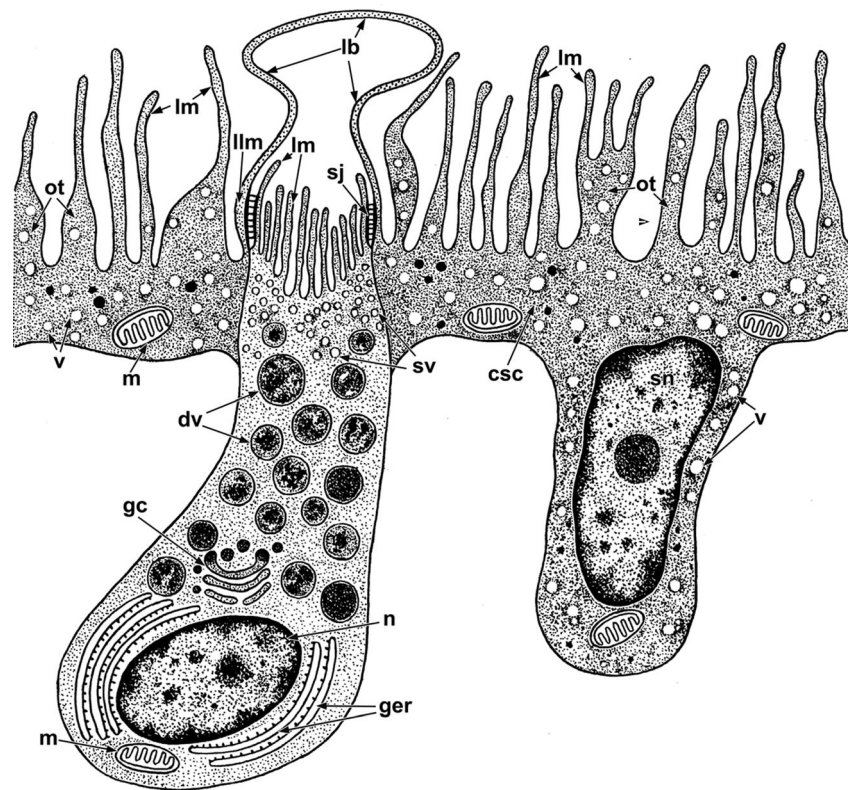
Different mechanisms involved in the elimination of accumulated haematin from the digestive cells have been observed in polyopisthocotyleans. In the studied hexabothriids, *C. callorhynchi* and *R. emarginata*, a holocrine (or apocrine) process is apparent. As part of this process, whole digestive cells appear free in the caecal lumen and exhibit degenerative changes. Indeed, large masses of residual, electron-dense bodies were observed free in the lumen along with the apparent sloughing of these cells, suggesting that such cells represent a terminal phase in the digestive cycle. In relation to this, it is worth noting that using spectroscopical and histochemical methods in eight polyopisthocotylean species, including a member of the hexabothriid genus *Hexabothrium*, Llewellyn (1954) demonstrated the presence of pigment granules and free pigment cells in the caecal lumen. Similarly, a case of the holocrine and apocrine-like elimination of indigestible residues was observed in the polystomatids *Polystomoides malayi* and *P. renschi* by Rohde (1973). Furthermore, free fragments of digestive cells have been reported from the caecal lumen of an unidentified species of *Polystomoides* (by Allen and Tinsley 1989), the thoracocotylid *P. multae* (by Brennan and Ramasamy 1996) and the polystomatid *Neopolystoma spratti* (by Watson and Whittington 1996, who referred to it as “the soups” of partially digested material). Despite these apparent observations, the abovementioned authors all supported a view of Halton (1975) that the shedding



and renewal of digestive cells does not occur and that cellular “defecation” involves only exocytosis. Nevertheless, the monopisthocotylean *Euzetrema knoepffleri*, an endoparasite of amphibians which has a partly haematophagous diet,

extrudes haematin in an “apocrine-like” fashion, during which cellular disintegration and shedding are an important part of a synchronous digestive cycle and serve to eliminate haematin from the caecal epithelium (Fournier 1978). It should be noted

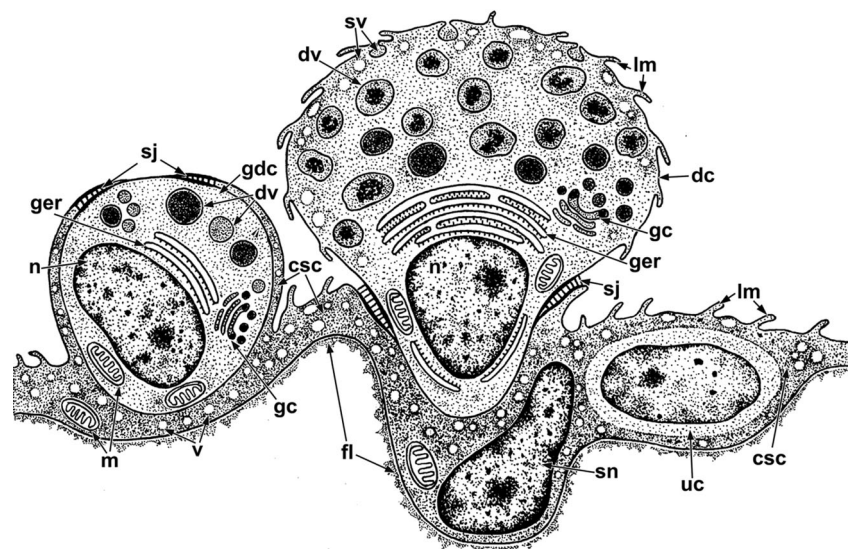
Fig. 31 Diagrammatic illustration of the caecal epithelial cell arrangement in the chimaericolid *Chimaericola leptogaster*. Abbreviations: *csc* connecting syncytial cytoplasm, *dv* digestive cell vesicles, *gc* Golgi complex, *ger* granular endoplasmic reticulum, *lb* lamellate bubble enveloping luminal surface of digestive cell, *lm* lamellae, *llm* lateral lamellae of connecting syncytium which form laminate bubble, *m* mitochondrion, *ot* lamellate outgrowths of luminal surface of connecting syncytium, *sj* septate junction, *sn* nucleus of connecting syncytium, *sv* small vesicles of digestive cells, *v* vesicles in connecting syncytium



that in *E. knoepffleri*, replacement digestive cells are formed from undifferentiated cells situated against the basal membrane of the caecum (Fournier 1978). With respect to the occurrence of apocrine and holocrine-like processes involved in the elimination of digestive products in the hexabothriids *R. emarginata* and *C. callorhynchi*, the gradual differentiation

of digestive cells from undifferentiated cells localized within the connecting syncytium was observed in the former. The presence of undifferentiated cells, possibly representing embryonic digestive cells, has been reported by Tinsley (1973) in the polystomatid *Protopolystoma xenopi* and by Brennan and Ramasamy (1996) in the thoracocotylid *P. multae*. Despite the

Fig. 32 Diagrammatic illustration of the caecal epithelial cell arrangement in the hexabothriid *Rajonchocotyle emarginata*. Abbreviations: *csc* connecting syncytial cytoplasm, *dc* fully differentiated digestive cell, *dv* digestive cell vesicles, *fl* fibrous layer, *gc* Golgi complex, *gdc* partly differentiated digestive cell, *ger* granular endoplasmic reticulum, *lm* lamellae, *m* mitochondrion, *sj* septate junction, *sv* small vesicles of digestive cells, *v* vesicles in connecting syncytium, *uc* undifferentiated digestive cell



fact that a holocrine-like process takes place in the two hexabothriids studied, we did not observe any degradation of the caecal epithelium. It is clear that, when there is a renewal of digestive cells, these cells eventually lose contact with the connecting syncytium due to the fact that fully differentiated digestive cells bulge into the caecal lumen, with a subsequent detachment and release into the caecal lumen.

Elimination of digestive products in chimaericolids

Firstly, it is worth noting the unusual contents of the caeca in the chimaericolid *C. leptogaster*, the lumens of which contain large amounts of both male and female reproductive material (spermatids, spermatozoa, vitelline cells and free vitelline material) along the entire length of the caeca, including the haptoral region. The presence of small amounts of vitelline material and spermatozoa within the caecal lumen has previously been reported for other polyopisthocotyleans (Rohde and Ebrahimzadeh 1969; Kearn 2004; Rohde 1973; Brennan and Ramasamy 1996). Such reproductive material is released into the caecal lumen via the genito-intestinal canal, the presence of which is a discriminatory character of the Polyopisthocotylea (Euzet and Combes 2003) and which is generally considered to be an outlet for apparently superfluous or expended reproductive material that is to be degraded and recycled (Smith and Halton 1983). In the case of *C. leptogaster*, there is also a connection between each vagina and the intestine (Poddubnaya et al. 2013), which may account for the apparently greater amount of reproductive material within its caecal lumens.

The eliminated digestive products in *C. leptogaster* are mixed with reproductive material within the caecal lumen, i.e., the fragments of digestive cells filled with degenerative digestive vesicles and the aggregations of residual bodies. In this case, we can assume the likely presence of an apocrine elimination of digestive products. Nevertheless, in view of an unusual trait of chimaericolid digestive cells, i.e., their luminal surface being isolated from the caecal lumen by an enveloping lamellate bubble, within which the residual bodies are concentrated, we suggest that there is another kind of digestive product elimination in chimaericolids. The apparent occurrence of free bubbles containing residual bodies inside the caecal lumen indicates their separation from the epithelial surface. By means of this separation, residual bodies and digestive cell material surrounded by the bubble are discharged into the caecal lumen. The presence of undifferentiated and gradually differentiating digestive cells indicates a permanent process of renovation of these cells in chimaericolids. We suggest that the bubble, which envelopes the fully formed digestive cell (Fig. 31), is homologous with the thin layer of connecting syncytium enveloping the developing digestive cell in the hexabothriids (Fig. 32).

Final comments

In the present paper, we have investigated the characteristics of the caecal cytoarchitecture of two basal groups of blood-feeding monogeneans of the subclass Polyopisthocotylea, the Chimaericolidae and Hexabothriidae, the members of which are ectoparasites on the gills of relictual groups of cartilaginous fishes, holocephalans and elasmobranchs. The study has revealed differences in the ultrastructure and arrangement of the caecal components between members of these two families, which we consider represent characteristics of these groups and are not dependent on the host or habitation of the parasites. Chimaericolids are only associated with holocephalans, relictual deep-water forms, whereas hexabothriids are parasites of elasmobranchs and holocephalans. Despite their different host types, both of the studied hexabothriids, *C. callorhynchi* from a holocephalan and *R. emarginata* from an elasmobranch, possess the same caecal cytoarchitecture, whereas the chimaericolid, *C. leptogaster* from a holocephalan, is characterized by a different kind. Variations in the ultrastructural organization of the caeca of members of different families of the polyopisthocotylean order Mazocraeidea have also been observed (Halton et al. 1968; Brennan and Ramasamy 1996; Santos et al. 1998). At the present time, the evidence suggests that, at higher taxonomic levels of the Polyopisthocotylea, i.e., at the family level, we can consider that characteristics of the caecal ultrastructure may represent useful taxonomic traits. Such important biological characters are likely to have more phylogenetic relevance than some of the morphological features based on attachment which are currently used to classify these groups. Nevertheless, studies on the caecal ultrastructure of this blood-feeding group are few and scattered, and further investigations are needed to confirm this.

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