

## *Paramarteilia canceri* sp. n. (Cercozoa) in the European edible crab (*Cancer pagurus*) with a proposal for the revision of the order Paramyxida Chatton, 1911

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**Abstract:** A new species, *Paramarteilia canceri* sp. n., is described using light and electron microscopy from the edible crab *Cancer pagurus* L. captured from the English Channel. No external symptoms were noted, although infected animals were typically lethargic and unresponsive to external stimuli. Organs of infected animals were shrunken and collapsed compared with apparently healthy individuals. Although the infection was systemic, marked host responses were only noted in the hepatopancreas where the parasite induced a pronounced haemocytic infiltration. Prevalence of infection throughout the study was 1.1%, with a maximum monthly prevalence of 3%. The intracellular parasite was typically 15 µm in length and composed of a primary cell containing up to three secondary cells derived by internal cleavage. Each secondary cell contains two bicellular spores. The parasite is readily differentiated from the other described paramyxean species by a combination of the number of secondary and tertiary cells. In light of this new discovery, a revision of the order Paramyxida Chatton, 1911 is proposed based upon comparison to the original descriptions of this parasite group in various species of invertebrate hosts. The proposed classification is based on the number of cells within the spores (tertiary cells), so that only three genera remain within the order, namely *Marteilia* Grizel, Comps, Bonami, Cousserans, Duthoit et Le Pennec, 1974, *Paramarteilia* Ginsburger-Vogel et Desportes, 1979 and *Paramyxa* Chatton, 1911. Subsequent discrimination of species is based on a combination of the number of secondary cells within the primary cell and the number of tertiary cells within secondary cells. It is proposed that the genus *Marteilioides* Comps, Park et Desportes, 1986 is suppressed and the type species of the genus, *M. chungmuensis* Comps, Park et Desportes, 1986, is transferred to *Marteilia* and that the other representative of the genus, *M. branchialis* Anderson et Lester, 1992, is transferred to *Paramarteilia*. Further, *Paramyxoides* Larsson et Køie, 2005 is considered as a junior synonym of *Paramyxa* and its type and only species, *Paramyxoides nephtys* Larsson et Køie, 2005, is transferred to *Paramyxa*.

**Key words:** *Paramarteilia canceri*, Paramyxia, Paramyxida, Cercozoa, *Paramyxa*, *Paramyxoides*, *Marteilioides*, *Marteilia*, ultrastructure, *Cancer pagurus*

The European edible crab (*Cancer pagurus* L.) supports an important fishery in the United Kingdom, worth approximately 37 million Euros annually. Despite its commercial significance, there is only limited information on the diseases affecting this species (reviewed by Stentiford 2008). Infections with *Hematodinium* sp. giving rise to the condition 'Pink crab disease' (PCD) have been described in stocks in the English Channel (Stentiford et al. 2002). More recently, a new genus and species of microsporean parasite (*Enterospora canceri* Stentiford, Bateman, Longshaw et Feist, 2007) has also been found infecting connective tissues (Stentiford et al. 2007) and a viral infection affecting juvenile crabs has also been reported (Bateman and Stentiford 2008). During a histopathological survey of edible crabs from Guernsey and

English Channel localities close to Weymouth and Portland an unusual infection with a paramyxid parasite was discovered.

The order Paramyxida Chatton, 1911 has been the subject of a number of revisions in particularly being referred to as the phylum Paramyxia Desportes et Perkins, 1990. Following a number of iterations to restructure the protists, the most stable taxonomy proposed is that the order Paramyxida resides within the class Ascetosporia Sprague, 1979 and the phylum Cercozoa Cavalier-Smith, 1998 (Cavalier-Smith and Chao 2003). The order Paramyxida currently contains five genera. Their representatives are metazoan parasites characterised by the production of variable numbers of 'daughter cells' formed by endogeny within a primary cell, leading via a series of further cell di-

visions to the formation of spores (Desportes and Perkins 1990). Members of the genera *Marteilia* Grizel, Comps, Bonami, Cousserans, Duthoit et Le Penne, 1974 and *Marteilioides* Comps, Park et Desportes, 1986 are parasites of marine bivalve molluscs and some species, such as *Marteilia refringens* Grizel, Comps, Bonami, Cousserans, Duthoit et Le Penne, 1974 and *Marteilia sydneyi* Perkins et Wolf, 1976, are important pathogens causing mass mortalities in the European flat oyster (*Ostrea edulis*) and the Sydney rock oyster (*Saccostrea glomerata* [syn. *S. commercialis*]) respectively (Berthe et al. 2004). Both of these species are listed in the World Organisation for Animal Health (Office International des Epizooties (OIE), International Aquatic Animal Health Code (OIE 2006). In addition, *Marteilioides chungmuensis* Comps, Park et Desportes, 1986 is a significant pathogen affecting the ovary of Pacific oyster (*Crassostrea gigas*) (Comps et al. 1986, 1987). Of the remaining genera, *Paramyxa* Chatton, 1911 and *Paramyxoides* Larsson et K oie, 2005 have been described from polychaetes (Desportes 1981, Larsson and K oie 2005) and *Paramarteilia* Ginsburger-Vogel et Desportes, 1979, which contains a single species (*Paramarteilia orchestiae* Ginsburger-Vogel et Desportes, 1979), has been described from the amphipod crustacean *Orchestia gammarellus* (Ginsburger-Vogel and Desportes 1979a,b). This parasite infects the testes and ovary of its host and produces chitinous nodules under the epidermis. Growth and reproduction appear unaffected. However, Ginsburger-Vogel (1991) demonstrated that *P. orchestiae* infections are associated with intersex condition in male *O. gammarellus* and experimentally in two other *Orchestia* species. The genus *Paramarteilia* is currently distinguished from other paramyxans by the presence of two bicellular spores in each secondary cell (sporont), rather than a combination one, two or four bi-, tri- or tetracellular spores in the other genera. Here we report the first description of an infection with a new species, *Paramarteilia canceri* in a decapod crustacean, the European edible crab, and provide a proposal for a simplified taxonomy of the order Paramyxida.

## MATERIALS AND METHODS

Sixteen European edible crabs (*Cancer pagurus*) were captured from the south coast of Guernsey (UK Channel Islands; 49°24'N, 2°32' to 2°43'W) in November 2001. Thirty edible crabs, male and female, were collected each month between December 2003 and November 2004 from the fishery based around Weymouth and Portland, UK using conventional baited parlour pots (50°30'N, 2°27'W). One hundred and sixty female crabs were sampled in July 2007 from the same location. In addition, *C. pagurus* were sampled from the fishery in the South Kimmeridge Bay region of Dorset, United Kingdom (50°36'N, 2°8'W). Dispensation licences from the United Kingdom Marine Fisheries Agency (Council Regulation 850/98) allowed for the sampling of legally undersized (carapace width, CW, <140 mm) and legal-sized (CW >140 mm) crabs, both from the fishery off

South Kimmeridge Bay and from the shoreline directly inshore from the fishery site. One hundred and fifty *C. pagurus* were sampled (60 with CW <100 mm, 50 with CW >100 and <140 mm, and 40 with CW >140 mm).

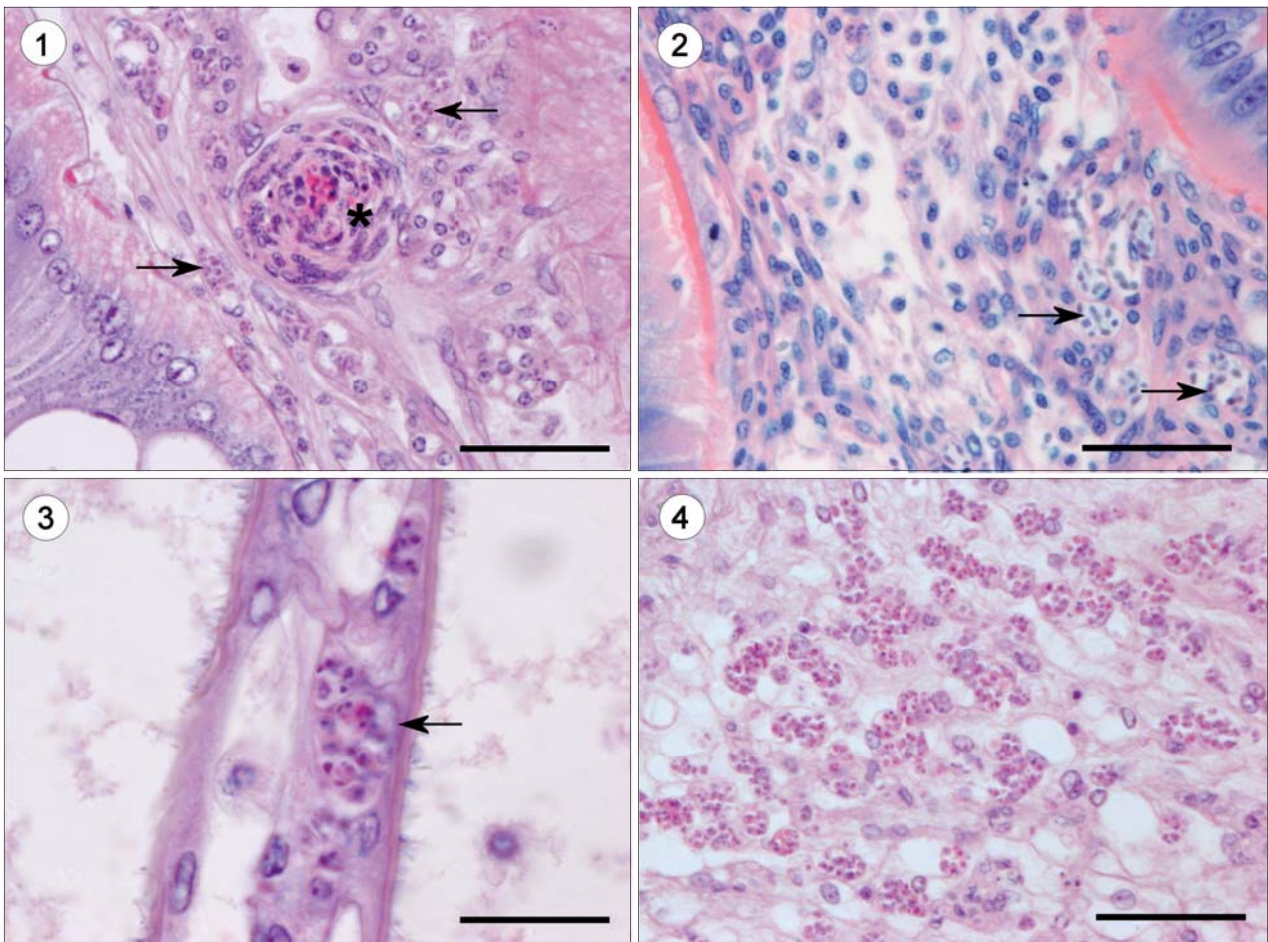
**Histology.** Crabs were anaesthetised by chilling on ice for 30 min before dissection. Hepatopancreas, gill, gonad, heart and body muscle were excised and placed immediately into Davidson's seawater fixative for 24 h, then transferred to 70% industrial methylated spirit. Samples were infiltrated with paraffin under vacuum using standard protocols. Sections were cut at a thickness of 3–5 µm, mounted onto glass slides, and stained with haematoxylin and eosin (H&E). Stained sections were analysed by light microscopy (Nikon Eclipse E800); digital images and measurements were obtained using the Lucia™ Screen Measurement System (Nikon, UK).

**Electron microscopy.** The hepatopancreas and gonad were removed; small blocks of tissue (2 mm<sup>3</sup>) were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 2 h at room temperature. Fixed tissue samples were rinsed in 0.1 M sodium cacodylate buffer (pH 7.4) and post-fixed for 1 h in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer. Specimens were washed in three changes of 0.1 M sodium cacodylate buffer before dehydration through a graded acetone series. Specimens were embedded in Agar 100 epoxy resin (Agar Scientific, Agar 100 pre-mix kit medium) and polymerised overnight at 60 °C in an oven. Semithin (1–2 µm) sections were stained with Toluidine Blue for viewing with a light microscope to identify suitable target areas. Ultrathin sections (70–90 nm) of target areas were mounted on uncoated copper grids and stained with uranyl acetate and Reynolds' lead citrate (Reynolds 1963). Grids were examined using a JEOL JEM 1210 transmission electron microscope and digital images captured using a Gatan Erlangshen ES500W camera and Gatan Digital Micrograph™ software. Stained sections from infected edible crabs were deposited in the Registry of Aquatic Pathology (RAP), Cefas Weymouth and in the collection of the Institute of Parasitology, Academy of Sciences of the Czech Republic,  esk e Bud ejovice.

## RESULTS

### Clinical signs and histopathology

A total of 686 crabs were examined for the presence of the parasite, with maximum prevalence being 3% during January and February 2004. Eight animals were diagnosed as infected with the paramyxan parasite with 1 crab from Guernsey, 5 crabs infected in coastal locations around Weymouth and Portland and 2 infected crabs from South Kimmeridge Bay. Infections occurred in the crabs collected in January, February, October and November 2004 and in July 2007 only. External signs of infection were absent although crabs harbouring the infection were lethargic and unresponsive. Internally, developmental stages of the parasite appeared to be systemic, being detected in several organs and tissues including the hepatopancreas, gill, heart, skeletal muscle, tegmental gland and gonads as well as within congested haemal sinuses. Most parasites were seen in the hepatopancreas associated with an influx of haemocytes and fibroblast-like cells



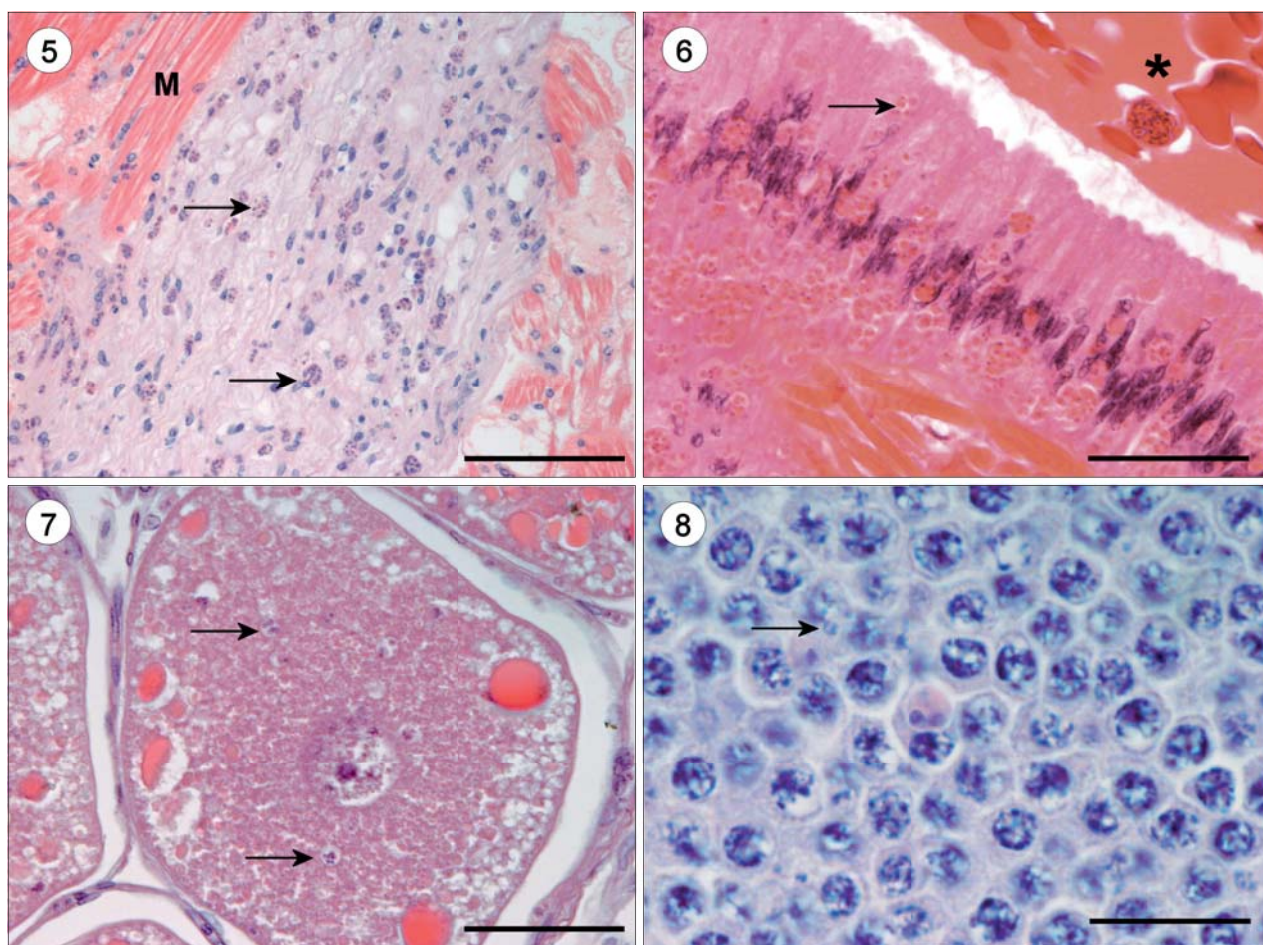
**Figs. 1–4.** Light micrographs of *Cancer pagurus* tissues infected with *Paramarteilia canceri* sp. n. **Fig. 1.** Granulomatous response (asterisk) within haemal sinuses of the hepatopancreas. Parasites are present throughout the tissue (arrows). H&E. **Fig. 2.** Co-infection with a yeast-like organism in the haemal sinuses of the hepatopancreas (arrows). H&E. **Fig. 3.** High power view of parasites infecting the gill epithelium (arrow). H&E. **Fig. 4.** Infected spongy cells in the pericardium of the heart. H&E. Scale bars: Figs. 1, 2, 4 = 50  $\mu$ m; Fig. 3 = 100  $\mu$ m.

eventually resulting in encapsulation (Fig. 1). In a single specimen, a co-infection with a yeast-like organism similar to that described by Stentiford et al. (2003) was detected (Fig. 2). In the gills, thickening of the respiratory epithelium was associated with haemocyte infiltration (Fig. 3). Large numbers of parasites were observed in the pericardial tissues, but haemocyte infiltration and necrosis of host cells were largely absent (Fig. 4). The nerves, including major nerves serving the claw muscles were infected. From histology it was not possible to determine if the parasites were located within nerve cells or were intercellular (Fig. 5). Again, host response was largely absent with little evidence of nerve cell degeneration or necrosis. Particularly heavy infections were found in the vas deferens of a single animal collected in July 2007. Large numbers of parasites were present in the connective tissues supporting the tall columnar epithelium with migration through the epithelium towards the lumen (Fig. 6). Despite the presence of numerous parasites the epithelium

remained intact, suggesting that the parasites were intercellular. However, they were often seen to be intracellular within haemocytes, fixed phagocytes, connective tissue cells, ova and even developing spermatocytes (Figs. 7, 8). Some appeared to be extracellular in affected tissues and were likely to have been released from ruptured host cells.

### Ultrastructure

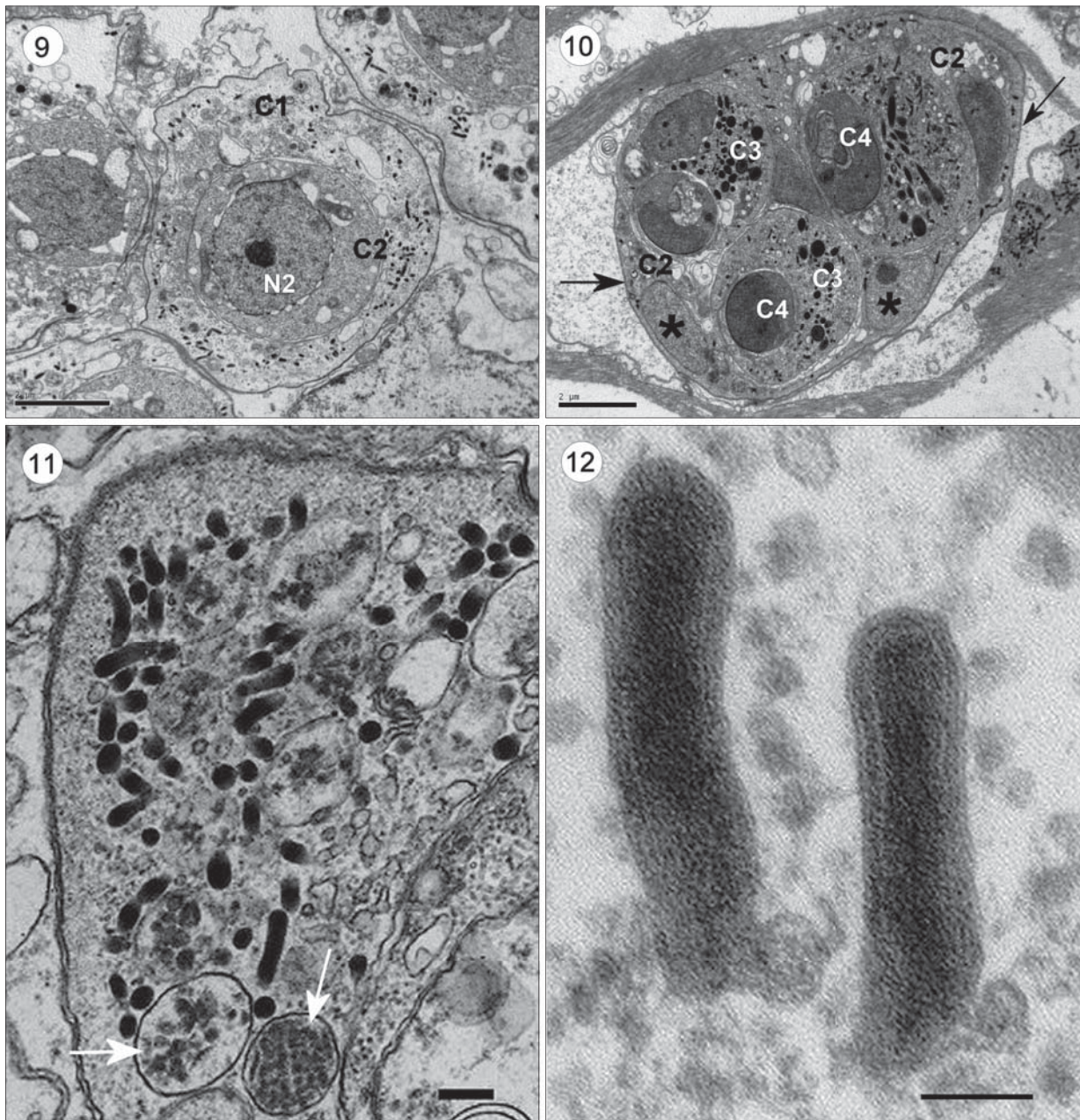
The earliest stage detected consisted of a primary cell containing a single secondary cell intracellularly within epithelial cells (Fig. 9). The primary cell increased in size to accommodate secondary cell division. Although most primary cells examined contained only one nucleus, a single example appeared to contain two nuclei (Fig. 10). The nucleoplasm contained uniformly granular chromatin and a prominent nucleolus observed in a single case (Fig. 10). The plasma membrane of the primary cell was smooth and devoid of pseudopodia-like projections. The primary cell cytoplasm was finely granular with free ribosomes



**Figs. 5–8.** Light micrographs of *Cancer pagurus* tissues infected with *Paramarteilia canceri* sp. n. **Fig. 5.** Multiple parasite stages (arrows) infecting a nerve fibre running through the claw muscle (M). H&E. **Fig. 6.** Multiple parasite stages infecting the epithelium of the vas deferens. Parasites can be seen apparently migrating through the basal and nuclear regions of the epithelium towards the lumen of the duct. Proteinaceous fluid and spermatophores are present in the lumen (asterisk). H&E. **Fig. 7.** Infected early vitellogenic oocyte containing several small parasite stages (arrows). H&E. **Fig. 8.** Infected spermatogonial cell (arrow). H&E. Scale bars: Figs. 5–7 = 100  $\mu$ m; Fig. 8 = 50  $\mu$ m.

and contained numerous electron-dense haplosporosomes. These were cylindrical, bacilliform, usually with a rounded end when mature, measuring  $\sim$ 280 nm in length and 50 nm in width. They were found throughout the cytoplasm but were observed in high concentrations associated with membranous structures and vesicles containing granular material as in multivesicular bodies of other paramyxids and in haplosporidians. These are the sites of haplosporogenesis (Fig. 11). In longitudinal section they consisted of an outer and inner membrane, separating the cortex and medulla (Fig. 12). In maturing primary cell haplosporosomes, one end was empty and the membranes folded, suggesting that these structures may exceed 280 nm in length. The cytoplasm also contained sparse endoplasmic reticulum (ER), mitochondria containing few cristae, small vesicles, membranous whorls and large apparently empty vacuoles (Fig. 13). Up to three secondary cells were present in sections of “mature” parasites (Fig. 13).

The structure of the secondary cell changed during growth. Initially, they appeared as small (3.9  $\mu$ m in diameter) ( $n = 2$ ) cells with a large nucleus occupying most of the cytoplasm (Fig. 14). In all stages the plasma membrane of the secondary cell appeared smooth and devoid of pseudopodia-like projections. The secondary cell nucleus had a smooth envelope with occasional conspicuous nuclear pores, clearly visible where the nuclear membrane and the surrounding cytoplasm were separated. The nucleoplasm was uniformly granular, pale staining with a conspicuous nucleolus. Occasionally, the initial stage of cell division was observed with the centriole associated with an indentation in the nuclear membrane (Fig. 14). The cytoplasm was granular with sparse strands of ER, a single mitochondrion and small vesicles (Fig. 15). A dense granular structure was occasionally adjacent to the nucleus and when observed, the cytoplasm contained microtubules (Fig. 16). Secondary cells appeared devoid of haplosporosomes. As the secondary cell increased to

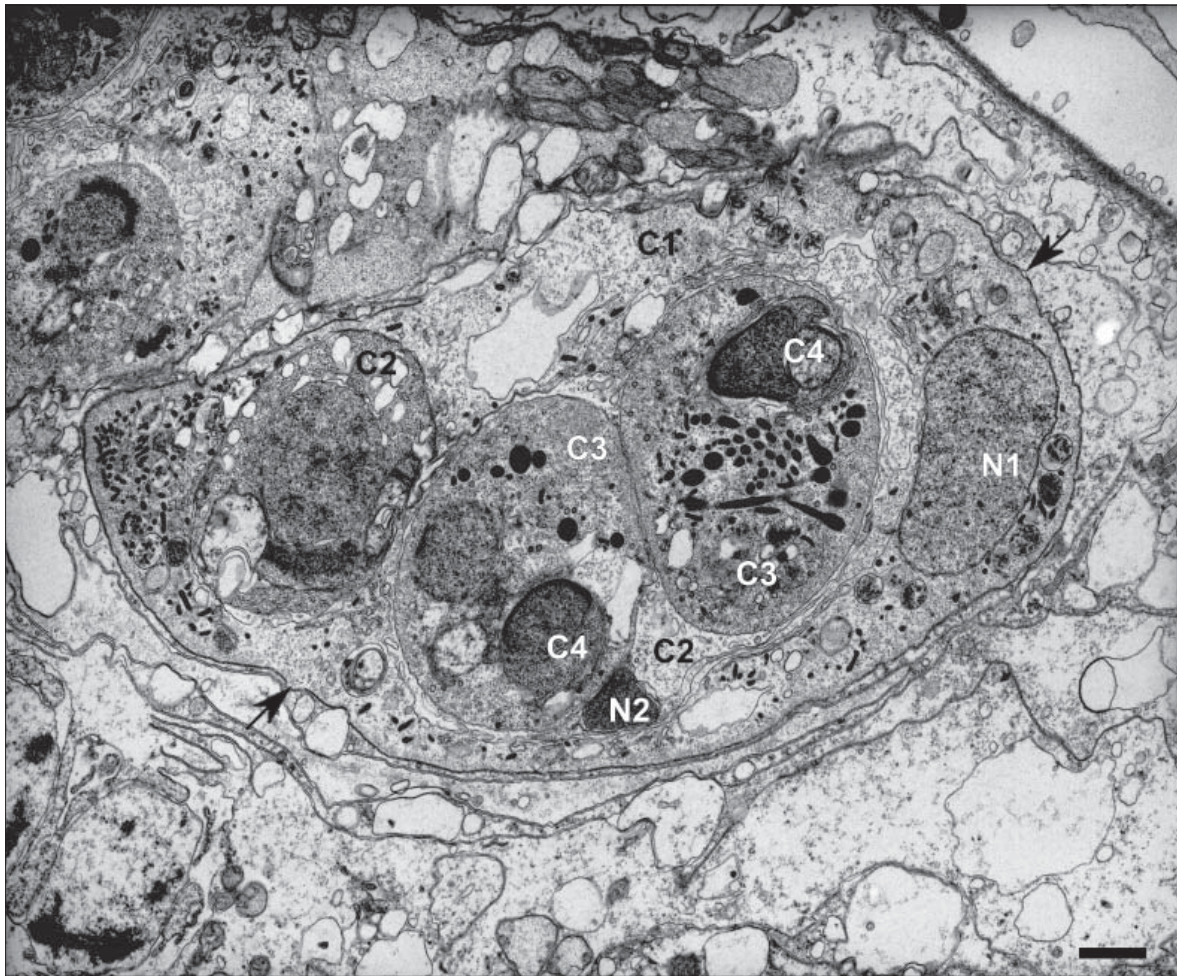


**Figs. 9–12.** Transmission electron micrographs of *Paramarteilia canceri* sp. n. infecting *Cancer pagurus*. **Fig. 9.** Earliest identified stage of *P. canceri* is a primary cell (C1) containing a single secondary cell (C2). The cytoplasm of the primary cell is interspersed with bacilliform haplosporosomes and large vacuoles. The nucleus (N2) of the secondary cell contains a prominent central nucleolus and numerous nuclear pores around the periphery. **Fig. 10.** Developing primary cell (arrow) containing two secondary cells (C2) enclosing tertiary cells (C3) with prominent haplosporosomes and quaternary cells (C4). The primary cell apparently contains two nuclei (\*), one with a prominent nucleolus. **Fig. 11.** High power view of primary cell cytoplasm showing putative haplosporogenesis denoted by the presence of membranous structures and granular-filled vesicles (multivesicular bodies) (arrows) as well as several electron-dense haplosporosomes. **Fig. 12.** Longitudinal section through bacilliform haplosporosomes of the primary cell with outer and inner membranes separating the cortex and medulla. Scale bars: Figs. 9, 10 = 2  $\mu\text{m}$ ; Fig. 11 = 200 nm; Fig. 12 = 60 nm.

a diameter of  $\sim 5.8 \mu\text{m}$  ( $n = 5$ ), it became a pansporoblast (=sporont) within which one or two tertiary cells were produced (Fig. 17). These constrained the nucleus to the periphery of the cell where it appeared triangular with dense chromatin (Figs. 17, 18). It is uncertain whether the nucleus divides although two nuclear sections within

a secondary cell suggested that this may occur (Fig. 18). However, this was observed once, and may have been two profiles of a single attenuated nucleus due to compression by constrained tertiary cells.

Uninucleate tertiary (=sporal) cells were  $< 2.9 \mu\text{m}$  ( $n = 10$ ) in diameter occasionally with pseudopodia-like

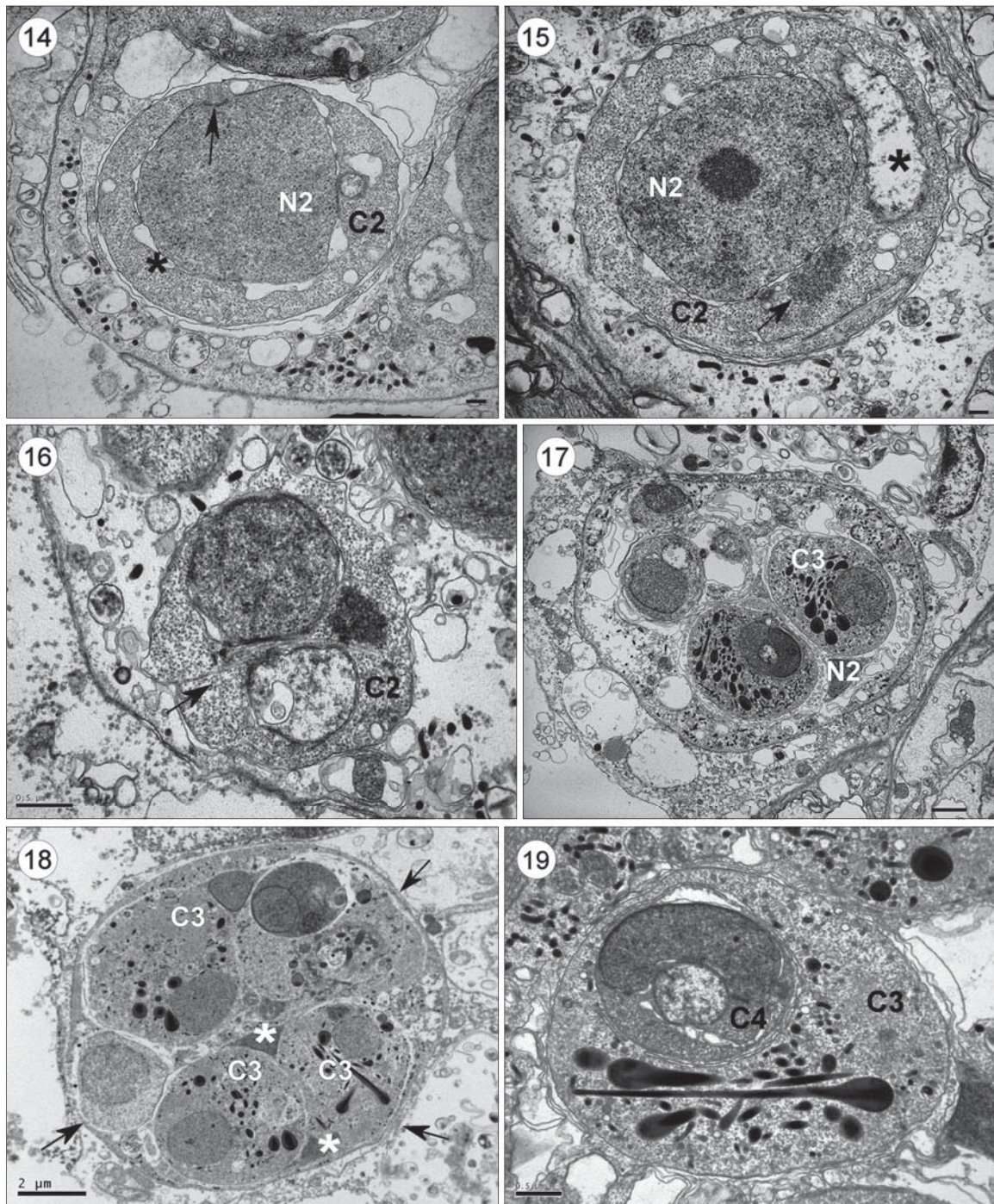


**Fig. 13.** Components of a typical large multicellular stage of *Paramarteilia canceri* sp. n. The primary cell (C1) is delineated from the surrounding tissues by a plasmalemma (arrows) and contains numerous electron-dense bacilliform haplosporosomes in the cytoplasm. Note also the presence of occasional multivesicular bodies and large, apparently empty vacuoles. The primary cell contains a prominent nucleus (N1) and two secondary cells (C2), one of which contains two tertiary cells (C3), each of which in turn enclose a single quaternary cell (C4). One of the secondary cells also contains a typical triangular nucleus (N2). Typically, the tertiary cells contain numerous prominent electron-dense haplosporosomes with bulbous ends. Scale bar = 1  $\mu\text{m}$ .

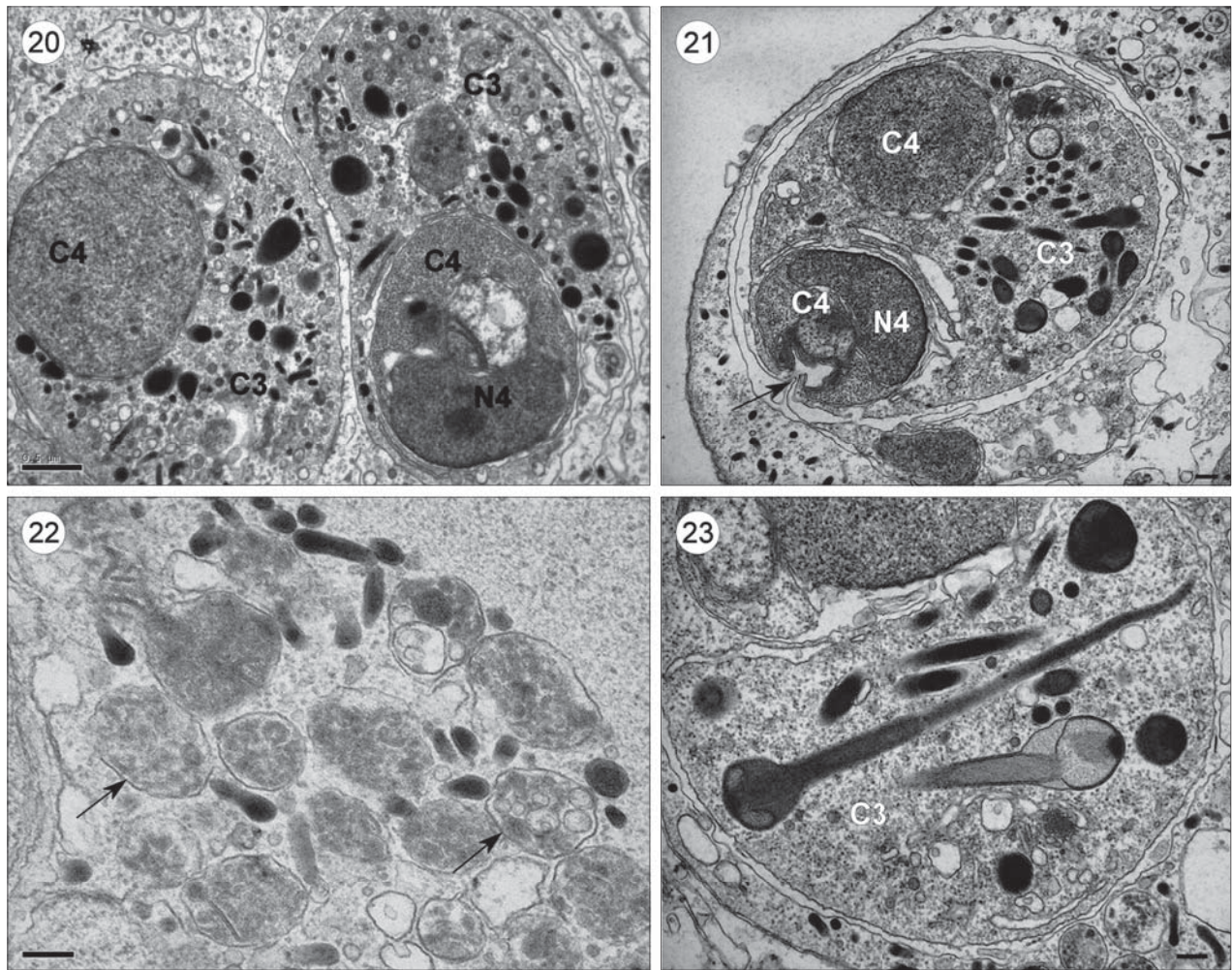
projections from the plasma membrane and were characterised by abundant large haplosporosomes in their cytoplasm (Figs. 19–21). Nuclei were spherical with uniform fine chromatin and many nuclear pores (Fig. 20). Mitochondria were not observed. The cytosol was granular, with ribosomes, vacuoles  $<1 \mu\text{m}$  in diameter and strands of ER surrounding quaternary (=sporoplasm or germinative) cells. Haplosporogenesis was similar to observations on primary cells and included the formation of bacilliform haplosporosomes (Fig. 22). However, haplosporosomes  $<3.5 \mu\text{m}$  long with one end bulb-shaped,  $\sim 500 \text{ nm}$  in diameter and gradually tapering to a final diameter of  $95.6 \text{ nm}$  ( $n = 10$ ), were also noted (Fig. 23). In longitudinal sections of the bulbous region the double membranes seen in the bacilliform forms were evident. At the apex, electron-dense material occurred against the internal membrane (Figs. 23–25), below which fibrillar material was arranged longitudinally along the length of the

haplosporosome and surrounded by the inner membrane (Fig. 25). In transverse sections through the ‘neck’ of the bulbous region, the inner membrane was discontinuous and seen to curl inwards towards the central dense medulla of the haplosporosome (Fig. 25). This was not seen in transverse sections of the narrower parts of the haplosporosome, many of which appeared angular in profile.

Most tertiary cells contained a single quaternary cell within their cytoplasm. This was usually spherical but occasionally ovoid and  $<1.9 \mu\text{m}$  ( $n = 5$ ) in diameter (Figs. 26–28). The cytoplasm contained many free ribosomes and a discrete dense accumulation of granular material (ribosomes?) similar to those in secondary cells and, a sparse ER. The nucleus was reniform in cross-section and contained a dense granular nucleoplasm with a single nucleolus (Fig. 26). It was peripheral against the plasmalemma where the close apposition of the nuclear and cell membranes gave the appearance of a markedly extended



**Figs. 14–19.** Transmission electron micrographs of *Paramarteilia canceri* sp. n. infecting *Cancer pagurus*. **Fig. 14.** A secondary cell (C2) undergoing division. The large secondary cell nucleus (N2) occupies the majority of the cytoplasm and the nuclear membrane is interspersed with numerous nuclear pores visible as conspicuous separation of the nuclear membrane and the surrounding cytoplasm (\*). A centriole is visible in close proximity to the secondary cell nucleus (arrow). **Fig. 15.** Developing secondary cell (C2) containing a single mitochondrion (\*) and numerous small vesicles. Occasionally, a dense granular structure (arrow) was noted in the secondary cell cytoplasm associated with the nucleus which may represent ribosomes. The nucleus (N2) contains a prominent nucleolus. **Fig. 16.** Further development and internal cleavage of the secondary cell (C2) with microtubules present in the cytoplasm (arrow). **Fig. 17.** Early pansporoblast (sporont) containing secondary cells and tertiary cells (C3) with typical electron-dense bulbous haplosporosomes. The nucleus of the secondary cell (N2) is constrained to the periphery of the secondary cell and assumes a triangular appearance in cross-section (see Fig. 13). **Fig. 18.** Further divisions within the secondary cells leads to production of two tertiary cells per secondary cell. Three secondary cells are visible in section (arrows). Note the two triangular profiles of the C2 nucleus (\*). **Fig. 19.** Details of tertiary cell (C3) containing numerous electron-dense haplosporosomes and a single quaternary cell (C4). Scale bars: Figs. 14, 15, 17 = 200 nm; Figs. 16, 19 = 0.5 μm; Fig. 18 = 2 μm.



**Figs. 20–23.** Transmission electron micrographs of *Paramarteilia canceri* sp. n. infecting *Cancer pagurus*. **Fig. 20.** Tertiary cells (C3) with typical haplosporosomes of variable size and shape in the cytoplasm. Quaternary cells (C4) are clearly visible within the tertiary cells and contain a prominent nucleus (N4). **Fig. 21.** Tertiary cell (C3) with two quaternary cells (C4), one of which contains a typical reniform nucleus (N4). Note the apparent ingestion of plasma membranes by one of the quaternary cell (arrow). **Fig. 22.** Haplosporogenesis in the tertiary cell involves granular-filled vesicles (multivesicular bodies) (arrows) and the production of bacilliform haplosporosomes. **Fig. 23.** Numerous bulb-ended haplosporosomes in the cytoplasm of the tertiary cell (C3) at different stages of development. Note the apparent presence of a cortex and medulla in some of the haplosporosomes. Scale bars: Fig. 20 = 0.5  $\mu\text{m}$ ; Figs. 21–23 = 200 nm.

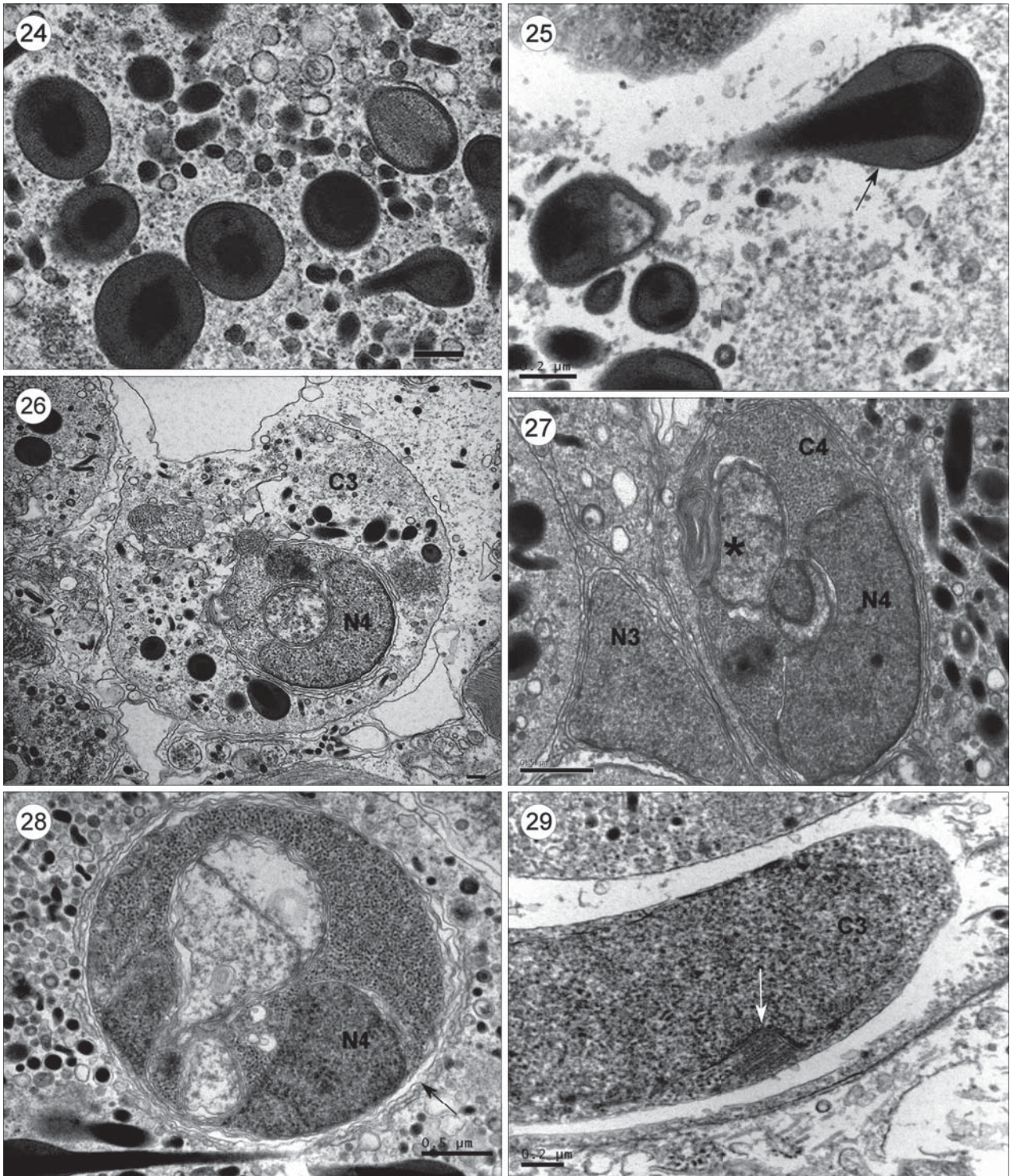
nuclear pore (Figs. 23, 26). A mitochondrion with inconspicuous cristae was located in a cup-shaped depression in the nucleus (Fig. 27). Haplosporosomes were absent from the cytoplasm of the quaternary cell. The presence of a centriole close to the nucleus (Fig. 27) suggested that quaternary cells were capable of division. Apparent ingestion of plasma membranes by a quaternary cell (Fig. 21) also suggested a mechanism for release into the primary cell and subsequent division (Fig. 29).

## DISCUSSION

Of the five currently recognised genera within the order Paramyxida, the parasite currently described from *Cancer pagurus* most closely fits the description of *Paramarteilia*

as defined by Ginsburger-Vogel and Desportes (1979b). Only two other paramyxids containing bicellular spores have been described, the type species of the genus *Paramarteilia*, *P. orchestiae*, and *Marteilioides branchialis*. The latter species only contains a single bicellular spore and can readily be discriminated from the current species on that basis. Additionally, *M. branchialis* contains between 2 and 6 (rarely 12) secondary cells within the primary cell. *Paramarteilia orchestiae* however contains two tertiary cells in common with the current species but a greater number of secondary cells (up to 10), compared with a maximum of 3 cells found in the *C. pagurus* parasite. The secondary cell cytoplasm of the current species has few ribosomes, compared with the cytoplasm of sec-





**Figs. 24–29.** Transmission electron micrographs of *Paramarteilia canceri* sp. n. infecting *Cancer pagurus*. **Fig. 24.** Transverse sections through the bulbous end of large haplosporosomes of the tertiary cell with a noticeable core of electron-dense material present in most. Various-sized vesicles are also present in the cytoplasm. **Fig. 25.** Cross-section of haplosporosomes of the tertiary cell. Note presence of inner and outer membrane, although the inner membrane appears to be discontinuous in the apex of the haplosporosome (arrow). **Fig. 26.** Tertiary cell (C3) containing typically spherical quaternary cell with a reniform nucleus (N4). Limited cell contents are present in the cytoplasm of the quaternary cell. **Fig. 27.** Ovoid quaternary cell (C4) with nucleus (N4) and mitochondrion with inconspicuous cristae (\*). Typically triangular nucleus (N3) of the tertiary cell is apparent. **Fig. 28.** Developing quaternary cell with dense granular cytoplasm (ribosomes), peripheral nucleus (N4) and surrounded by strands of endoplasmic reticulum (arrow). **Fig. 29.** Microtubules (arrow) present in the cytoplasm of a dividing tertiary cell. Scale bars: Figs. 24–26, 29 = 200 nm; Fig. 27, 28 = 0.5  $\mu$ m.

ondary cell of *P. orchestiae*, which is rich in ribosomes. Occasionally, pseudopodia-like projections from the plasma membrane of the tertiary cells were apparent, which was not noted in *P. orchestiae*. Also, the current species is approximately half the size of *P. orchestiae*. Given the difference in the number of secondary cells, differences in cellular contents and overall size we propose the erection of a new species of *Paramarteilia*, namely *P. canceri* sp. n.

Whilst most members of the Paramyxida occur in specific tissues, the systemic nature of the current parasite suggests a number of routes exist within the host which may exacerbate the pathology and increase mortality rates through multiple organ failure. This may explain the low prevalence of infection observed as heavily infected individuals may readily succumb to infection. Low prevalence may also indicate a geographical range limit for *P. canceri* since other paramyxids come from areas with higher ambient water temperatures. However, additional surveys for paramyxid parasites and *P. canceri* in particular are required to confirm this. Unlike *P. orchestiae* infections of male *Orchestia gammarellus* (see Ginsburger-Vogel and Desportes 1979a), there was no evidence of intersex in male or female *C. pagurus* associated with the presence of the parasite.

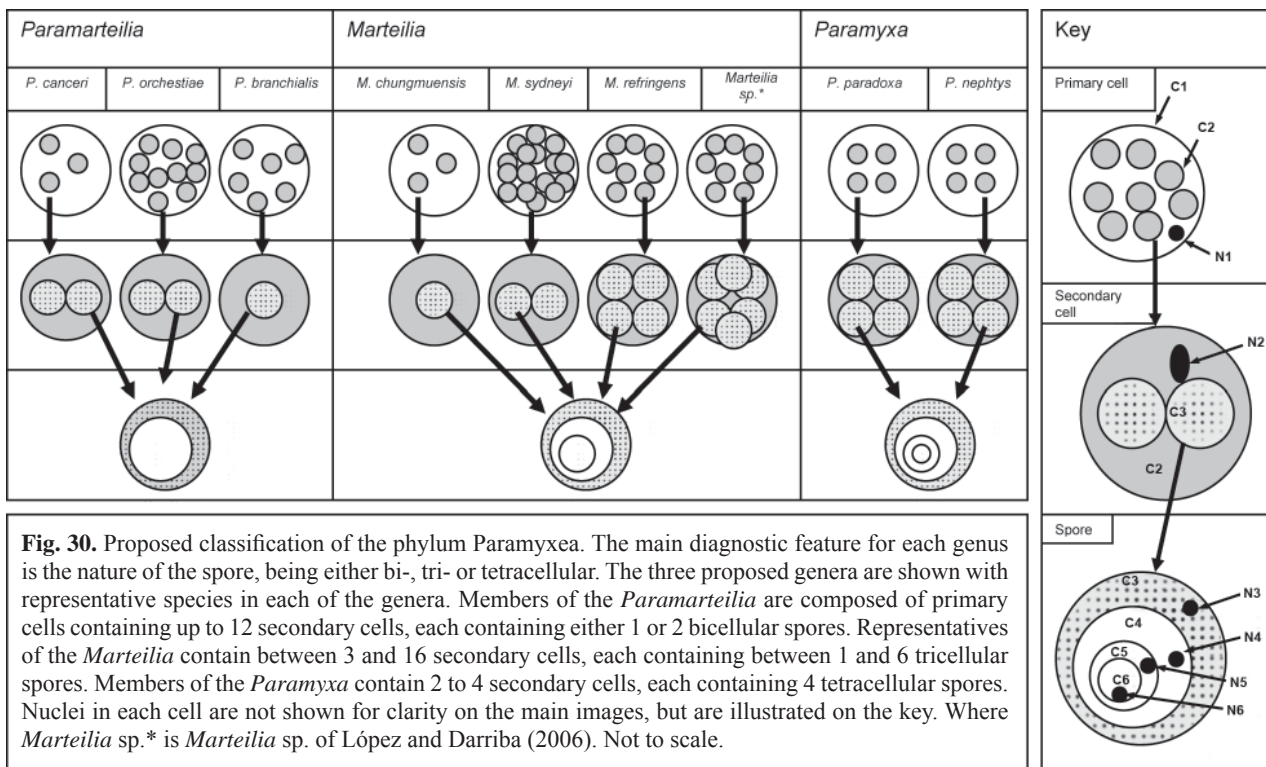
Current criteria for discriminating genera and species within the order Paramyxida are based on different diagnostic features at the light and ultrastructural level. The first paramyxid to be described, *Paramyxa paradoxa* Chatton, 1911 from polychaetes, was originally described using light microscopic features and was discriminated from its congeners much later using ultrastructural features (Desportes 1981). The genus *Marteilia*, accommodating parasites of bivalve molluscs and copepods (Audemard et al. 2002), was erected by Grizel et al. (1974), and discriminated from *Paramyxa* mainly on light microscopy features. The next genus in the order to be described was *Paramarteilia* by Ginsburger-Vogel and Desportes (1979b) who used a combination of host (amphipods), the number of secondary cells and the nature of the spore to discriminate this new genus from *Marteilia* and *Paramyxa*. *Marteilioides*, infecting bivalve molluscs was discriminated from others in the order by only a single tricellular spore in the secondary cell (Comps et al. 1986). However, Anderson and Lester (1992) in describing *Marteilioides branchialis*, amended the genus definition to include parasites that possessed single tricellular or bicellular spores in the secondary cell. Most recently, a fifth genus in the order, *Paramyxoides*, was described from polychaetes by Larsson and Køie (2005) who suggested that whilst morphologically similar to *Paramyxa*, the ornamentation of the spore (tertiary cell) and the presence of “haplosporosomes” in two of the four spore cells readily discriminated this parasite from the others in the order.

In order to try to resolve some of the long-standing issues about just two species within the genus *Marteilia*, Longshaw et al. (2001) used light and ultrastructural features to show that *Marteilia* species in oysters and mussels could not be readily discriminated. They suggested that the morphological criteria used within the phylum Paramyxia (and thus the order Paramyxida) needed to be re-examined, particularly the use of haplosporosome morphology, which was shown to be variable. Longshaw et al. (2001) proposed that *Marteilia maurini* Comps, Pichot et Papagianni, 1982 was a junior synonym of *M. refringens*, which was later confirmed using molecular methods (Berthe et al. 2000), suggesting that mussels and oysters contained different strains of the same parasite.

To date, molecular information has been obtained for *Marteilia sydneyi*, *M. refringens*, *Marteilioides branchialis* and *M. chungmuensis*, reflecting the relative importance of these pathogens in bivalve aquaculture. The molecular data confirm that these species are related, fit well within the phylum and validate the use of morphological characteristics to discriminate the four species within the phylum (Berthe et al. 2000). The 18S rDNA sequence data for *Marteilioides chungmuensis* and *Marteilioides* sp. (Kleeman et al. 2002 and Itoh et al. 2003, respectively), showed that these species fall within the genus *Marteilia*, although these authors did not propose taxonomic revision.

During the current study it became apparent that a review and revision of the genera within the order was necessary in light of new knowledge since the original descriptions. The use of different diagnostic criteria has created confusion in the order. For example, *Marteilioides chungmuensis* and *M. branchialis* were grouped in the same genus on the basis of a single secondary cell, which is bicellular in *M. branchialis* but tricellular in *M. chungmuensis*. However, *Marteilia* spp. are grouped together on the basis of possession of a variable number of tricellular spores. Uncertainty in taxonomic criteria has caused species to remain undescribed or poorly described (Comps 1976, 1985, Moyer et al. 1993, Norton et al. 1993, López and Darriba 2006, Virvilis and Angelidis 2006). Age-related variability in spore wall morphology and the utilisation of different host taxa by these parasites further restricts the number of suitable characters to discriminate genera and species (Longshaw et al. 2001).

Size and shape of haplosporosomes have also been used to discriminate between species and genera. Whilst these are useful, they should not be used in isolation from other morphological criteria as they can be pleomorphic, varying markedly within a species and overlapping in size range between species. In addition, it is important to use correct terminology when describing the different organelles in paramyxids. Larsson and Køie (2005) suggested that the inclusion bodies in cell 5 of *Paramyxoides* spores were haplosporosomes, despite lacking a medul-



la, typical of haplosporosomes. They may be haplosporosome-like bodies similar to those in a haplosporidian parasite of *Carcinus maenas* (see Stentiford et al. 2004). Similar structures to those in cell 5 of *Paramyxoides nephtys* reported by Larsson and Køie (2005) are referred to as “rod-like structures” in cell 4 of *Marteilia* spp. (Comps 1985, Norton et al. 1993, Longshaw et al. 2001, López and Darriba 2006) and reported by Perkins (1976) as vesicles within the middle sporoplasm cell (C4). Therefore these structures, used by Larsson and Køie (2005) to distinguish the genus *Paramyxoides*, are probably taxonomically invalid.

In attempting to resolve taxonomic confusion within the order, we considered the number of cells in each genus, since the principal distinguishing characteristics at the genus level include the numbers of spores per secondary cell, the number of cells per spore and the shape of the spores (Larsson and Køie 2005). Initially, the number of cells in the spore distinguishes three genera, those with bicellular spores, those with tricellular spores and then finally those with tetracellular spores (Fig. 30). Further subdivisions to species within these genera then appear to be related initially to the number of spores within the secondary cells and then the number of secondary cells within the primary cell. Whilst reducing the number of genera, this appears to stabilise the order and will facilitate the description of new species within the group. Differences in spore morphology, including spore appendages, may then further discriminate species. The additional use of molecular techniques may elucidate cryptic species and parasite

strains within the same species. This is particularly important given the recent reporting of *Marteilia refringens* in razor clams by López-Flores et al. (2008), which may be conspecific with the *Marteilia* sp. reported by López and Darriba (2006) which contained up to 6 spores, rather than the expected 4 in *M. refringens*.

We propose suppressing the genus *Marteilioides* and transferring the type species *M. chungmuensis* to *Marteilia*, whilst moving *M. branchialis* to *Paramarteilia* to take account of the number of cells present in the spore. Similarly, we propose suppressing the genus *Paramyxoides* since there are insufficient grounds to justify the erection of that genus, particularly given that the organelles reported as haplosporosomes in cell 5 of *Paramyxoides* are not unique to the genus, the variability existing with the spore morphology and the lack of difference in the number of cells present in the primary, secondary and tertiary cells compared with *Paramyxa*. The type and only species of the genus, *P. nephtys*, should be transferred to *Paramyxa*.

## REVISED CLASSIFICATION OF PARAMYXIDA

### Order Paramyxida Chatton, 1911

Sporulation results from endodyogeny within an amoeboid stem cell that germinates from spores in tissues of invertebrate marine animals. Development characterised by production of offspring cells that remain inside the parent cell. Spores consist of several cells enclosed inside each other.

### *Paramyxa* Chatton, 1911

Sporogony by endogenous budding. Amoeboid vegetative stages (primary cell) internally cleave to produce 2 or 4 secondary cells. Sporulation: each secondary cell (sporonts) cleaves to produce 4 tertiary cells (spores) which contain three additional cells so that spores are tetracellular.

Type species: *Paramyxa paradoxa* Chatton, 1911.

Other species: *P. nephtys* (Larsson et K oe, 2005) comb. n. (syn. *Paramyxoidea nephtys*).

### *Marteilia* Grizel, Comps, Bonami, Cosserans, Duthoit et Le Pennec, 1974

Sporogony by endogenous budding. Amoeboid vegetative stages (primary cell) internally cleave to produce secondary cells. Sporulation: each secondary cell (sporonts) cleaves internally to produce a variable number of tertiary cells (spores) which contain a further two cells so that spores are tricellular.

Type species: *Marteilia refringens* Grizel, Comps, Bonami, Cosserans, Duthoit et Le Pennec, 1974.

Other species: *M. sydneyi* Perkins et Wolf, 1976; *M. christenseni* Comps, 1983; *M. chungmuensis* (Comps, Park et Desportes, 1986) comb. n. (syn. *Marteilioides chungmuensis*).

### *Paramarteilia* Ginsburger-Vogel et Desportes, 1979

Sporogony by endogenous budding. Amoeboid vegetative stages (primary cell) internally cleave to produce secondary cells. Sporulation: each secondary cell (sporonts) cleaves to produce a variable number of tertiary cells (spores) which contain a single cell so that spores are bicellular.

Type species: *Paramarteilia orchestiae* Ginsburger-Vogel et Desportes, 1979.

Other species: *P. branchialis* (Anderson et Lester, 1992) comb. n. (syn. *Marteilioides branchialis*); *P. canceri* sp. n.

### Taxonomic summary

Phylum Cercozoa Cavalier-Smith, 1998

Order Paramyxida Chatton, 1911

Genus *Paramarteilia* Ginsburger-Vogel et Desportes, 1979

### *Paramarteilia canceri* sp. n.

**Specific diagnosis.** Primary cell amoeboid and uninucleate containing haplosporosomes in cytoplasm. Early primary cell usually spherical, becoming ovoid as parasite increase in size, measuring up to 15 µm along longest axis. Secondary cells formed by internal cleavage contain few ribosomes, a single mitochondrion, are devoid of haplosporosomes and measure approximately 4–6 µm. Up to three secondary cells present, containing 1–2 tertiary cells per secondary cell. Tertiary cells spherical measuring approximately 3 µm in diameter and contain a single quaternary cell measuring approximately 2 µm. Haplosporosomes present in cytoplasm of tertiary cells elongate with bulb-like projection at one end.

Type host: *Cancer pagurus* L. (Crustacea, Decapoda).

Type locality: English Channel, off Portland, Dorset (50°30'N, 2°27'W).

Site of infection: Systemic throughout animal.

Type material: Syntype slides of histological sections stained with H&E, wax blocks, EM blocks and uranyl acetate-stained grids, deposited in the Registry of Aquatic Pathology (RAP) held at the Cefas Weymouth Laboratory (Acc. Nos. 884). H&E-stained syntype slides deposited in the collection of the Institute of Parasitology, Academy of Sciences of the Czech Republic,  esk e Bud ejovice (Acc. No. IP ProtColl 7).

Etymology: The specific epithet refers to the generic name of the host.

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