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Enterospora canceri n. gen., n. sp., intranuclear within the hepatopancreatocytes of the European edible crab *Cancer pagurus*

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ABSTRACT: Only 1 genus (*Nucleospora*) within 1 family (Enterocytozoonidae) of the Microsporidia contains species that are parasitic within the nuclei of their host cells; to date, all described intranuclear *Nucleospora* spp. parasitise fish. This study describes the first intranuclear microsporidian parasite of an invertebrate, the European edible crab *Cancer pagurus* L. (Decapoda: Cancridae). Infected crabs displayed no obvious external signs, and maximum apparent prevalence of infection within a monthly sample was 3.45%. Infected hepatopancreatic tubules were characterised by varying numbers of hypertrophic and eosinophilic nuclei within epithelial cells. Parasite stages appeared as eosinophilic granular accumulations causing margination of host chromatin. In advanced cases, the tubule epithelia degenerated, with parasites and sloughed epithelial cells appearing in tubule lumens. All life stages of the parasite were observed within host nuclei. Uninucleate meronts were not detected, although binucleate stages were observed. Multinucleate plasmodia (sporogonal plasmodia) contained up to 22 nuclei in section, and late-stage plasmodia contained multiple copies of apparatus resembling the polar filament and anchoring disk, apparently associated with individual plasmodial nuclei. As such, aggregation and early assembly of sporoblast components took place *within* the intact sporogonial plasmodium, a feature unique to the Enterocytozoonidae. Liberation of sporoblasts from plasmodia or the presence of liberated sporoblasts was not observed in this study. However, large numbers of maturing and mature spores (measuring 1.3 ± 0.02 × 0.7 ± 0.01 µm) were frequently observed in direct contact with the host nucleoplasm. Considering the shared features of this parasite with microsporidians of the family Enterocytozoonidae, and the unique presence of this parasite within the nucleoplasm of decapod crustacean hepatopancreatocytes, this parasite (*Enterospora canceri*) is proposed as the type species of a new genus (*Enterospora*) of microsporidian. Molecular taxonomic work is now required, comparing *Enterospora* to *Enterocytozoon* and *Nucleospora*, the 2 other genera within the Enterocytozoonidae.

KEY WORDS: *Cancer pagurus* · Crustacea · *Enterocytozoon* · Enterocytozoonidae · Epithelium · Hepatopancreas · Intranuclear · *Nucleospora* · Nucleoplasm · Nucleus

INTRODUCTION

The Microsporidia are obligate intracellular parasites that infect a wide range of eukaryotic hosts. Host cells are infected when a mature spore discharges its sporoplasm through the polar filament into the host cell cytoplasm. Development generally takes place within the cytoplasm via proliferation (merogony) and formation of mature spores (sporogony) (Lom & Dyková 1992). Infection is deemed detrimental to the host, with complete destruction of the infected cell (and the tissue or organ if infection is extensive). Whilst the majority of Microsporidia are known to infect the cytoplasm of host cells, some undergo merogony and sporogony within the host nucleus. To date, only 1 microsporidian family, the Enterocytozoonidae, contain such species. All intranuclear species within this family infect finfish and fall within the genus *Nucleospora*

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(Modin 1981, Elston et al. 1987, Mullins et al. 1994, Nilsen et al. 1995, Lom & Dyková 2002). The family also contains the genus *Enterocytozoon*, known to infect a range of mammalian and bird hosts. Although these parasites associate intimately with the host cell nuclei, intranuclear infections have not been described to date. Members of the genera *Enterocytozoon* and the fish-infecting *Nucleospora* exhibit significant structural and genetic differences from other microsporidians and are placed amongst the basal groups of the Microsporidia (Lom & Nilsen 2003).

The type species of the genus *Enterocytozoon* (*E. bieneusi*) and family Enterocytozoonidae was described infecting the enterocytes of immunosuppressed human patients with developed AIDS (Desportes et al. 1985, Cali & Owen 1990, Weber et al. 1994). It is now considered as the most prevalent human microsporidian (Mathis et al. 2005). *E. bieneusi* is a widely distributed pathogen in mammals (Fayer et al. 2003, Sulaiman et al. 2003, Santin et al. 2004) and even in birds (Reetz et al. 2002, Haro et al. 2005). As such, its status as a zoonotic infection in humans has been established (Dengjel et al. 2001, Sulaiman et al. 2004, Mathis et al. 2005). In the aquatic environment, *E. bieneusi* has been detected in surface waters (Fournier et al. 2000) and in the filter-feeding zebra mussels *Dreissena polymorpha*. Although mussels were not actively infected, they did harbour viable spores and presumably were able to transmit the parasite via ingestion (Graczyk et al. 2004).

Enterocytozoon salmonis was described by Chilmonczyk et al. (1991) infecting Chinook salmon *Oncorhynchus tshawytscha*. However, Hedrick et al. (1991) had earlier described the same parasite as *Nucleospora salmonis*, this being the type species of a new genus. More recently, confusion in the taxonomy of the 2 genera has been clarified by morphological, developmental and genetic studies of the 2 species (Desportes-Livage et al. 1996, Docker et al. 1997). *N. salmonis* and *E. bieneusi* show 19.8 % genetic divergence in the small subunit (ssu) and large subunit (lsu) genes, thereby showing greater diversity than in any congeneric species examined to date (Docker et al. 1997). With this evidence and the fact that the 2 species show differences in the host and site of infection, *Nucleospora* remains a separate and valid genus from *Enterocytozoon*. Recently, another intranuclear microsporidian has been added to the genus, *N. secunda*, infecting the enterocytes of the fish *Nothobranchius rubripinnis* (Lom & Dyková 2002). Also (based upon morphological and host related features of infections), 2 other species from the lumpfish *Cyclopterus lumpus* (Mullins et al. 1994) and the halibut *Hippoglossus hippoglossus* (Nilsen et al. 1995) are likely members of the *Nucleospora*, since both possess between 8 and 12 turns of the polar filament (compared to 5–6 in *E. bieneusi*) and are genetically similar to *N. salmonis* (Gresoviac et al. 2000, Khattra et al. 2000). Gresoviaco et al. (2000) suggested that a group of related *N. salmonis*-like microsporidians may represent a genetically homogenous population of microsporidians, found principally in salmonid fish and often associated with serious proliferative disorders. Isolates with similar properties may be found in marine fish that exhibit similar disease syndromes (Gresoviac et al. 2000).

Despite *Nucleospora* and *Enterocytozoon* remaining as separate genera, based on morphological criteria they are more closely related to each other than to any other microsporidian genus. They exhibit nearly all of the distinctive features of this family (polar tube precursors, the formation of electron-dense discs prior to plasmodial division and thickening of the sporogonial plasmalemma). Both genera are polysporous but do not form sporophorous vesicles or pansporoblastic membranes. A major distinguishing feature between the two is that although *E. bieneusi* has been observed in close association with the host nucleus and endoplasmic reticulum, it has never been detected within the nucleoplasm (Desportes-Livage et al. 1996), whereas the developmental stages of the species in the genus *Nucleospora* are found within the nucleoplasm of the infected cell (Hedrick et al. 1991, Mullins et al. 1994, Nilsen et al. 1995).

Microsporidians are among the most frequently observed pathogens known to infect Crustacea (Sprague & Couch 1971, Couch 1983). Of those species described in decapods, the majority infect the skeletal muscle (Sprague 1970, 1977, Sprague & Couch 1971, Johnston et al. 1978, Couch 1983, McGriff & Modi 1983, Herbert 1987, Alderman & Polglase 1988, Lightner 1988, Overstreet 1988, Flegel et al. 1992, Dennis & Munday 1994, Olson et al. 1994, Clotilde-Ba & Toguebaye 1995, Childers et al. 1996, Mori & Salvido 2000, Azevedo 2001), whilst some, including *Pleistophora* spp., can also infect heart muscle and gill, stomach, hepatopancreatic epithelial cells (Overstreet 1973) and ovary (Viosca 1943, Sprague 1970). Few microsporidians have been described infecting the hepatopancreas alone (Azevedo 1987, Anderson et al. 1989). To date, no intranuclear microsporidians have been described infecting decapod (or indeed invertebrate) hosts. However, in a recent molecular phylogenetic study, a microsporidian parasite infecting the parasitic copepod *Lepeophtheirus salmonis* grouped closely with the family Enterocytozoonidae, being most similar to the intranuclear genus *Nucleospora*. This parasite was present throughout the body of the copepod, with xenoma-like cysts forming in the cuticular epidermis but no evidence of intranuclear infection, nor were *Nucleospora*- or *Enterocytozoon*-like features of devel-

opment described (Freeman et al. 2003). *Microsporidium* sp. found infecting freshwater daphnid crustaceans might also be closely related to the genus *Enterocytozoon* though once again, an intranuclear habit has not been demonstrated (Refardt et al. 2002).

This study reports the first case of an intranuclear microsporidian in an invertebrate. The parasite was discovered infecting the nuclei of epithelial cells of the hepatopancreas. The type host species is the decapod crustacean *Cancer pagurus* captured from the English Channel. Only 1 microsporidian parasite has previously been described from this species, infecting the cytoplasm of host myofibres (Vivarès & Azevedo 1988).

MATERIALS AND METHODS

Histology. Thirty European edible crabs *Cancer pagurus*, captured in baited creels from Weymouth Bay (50° 34′ N, 2° 22′ W), were obtained each month between December 2003 and November 2004 as part of a seasonal disease survey. Crabs were placed into filtered, running seawater for 4 h prior to sampling for histology and electron microscopy. Crabs were anaesthetized by chilling on ice for 30 min before removal of heart, gonad, gill, muscle and hepatopancreas. Dissected organs were fixed for 24 h in 10% Davidson's seawater fixative (Hopwood 1996) before transfer to 70% industrial methylated spirit (IMS). Fixed samples were processed to wax in a vacuum infiltration processor using standard protocols. Sections were cut at 3 to 5 µm on a rotary microtome and the resulting tissue sections were mounted onto glass slides before staining with haematoxylin and eosin (H&E). Stained sections were analysed by light microscopy (Eclipse E800, Nikon) and digital images were obtained using the Lucia™ Screen Measurement System (Nikon).

Ultrastructure. Crab tissues excised for histology were also preserved for electron microscopy. Small blocks of tissue (2 mm^3) were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) and 1.75% sodium chloride for 2 h at room temperature. Fixed tissue samples were rinsed in 0.1 M sodium cacodylate buffer with 1.75% sodium chloride (pH 7.4) and post-fixed for 1 h at 4°C in 1% osmium tetroxide, reduced with 1.75% potassium ferrocyanide in 0.1 M sodium cacodylate buffer. Specimens were washed in 3 changes of 0.1 M sodium cacodylate buffer and stained en bloc in 0.5% aqueous uranyl acetate for 1 h. Specimens were embedded in epoxy resin 812 (Agar Scientific-pre-mix kit 812) following dehydration through a graded acetone series. Thick sections were stained with Toluidine Blue for viewing with a light microscope to identify suitable target areas. Ultra thin sections (70 to 90 nm) of these areas mounted on uncoated copper grids and stained with uranyl acetate and Reynolds' lead citrate (Reynolds 1963) were examined using a JEOL 1210 transmission electron microscope. Morphological measurements were made using the on-board calibrated measuring software on the TEM.

RESULTS

Crabs infected with the intranuclear microsporidian displayed no obvious external signs. Maximum apparent prevalence of infection from a single monthly sample was 3.45%, with infected crabs being present in the catch in March (3.45%), April (3.33%), May (3.33%) and June (3.33%). No infected individuals were captured at other times of the year.

Histology

Microscopic changes were characterised by the presence of varying numbers of hypertrophic and eosinophilic nuclei within the epithelial cells of the hepatopancreas (Fig. 1A). Affected cells included the R (reserve)-, F (fibrillar)- and E (embryonic)-cells of the hepatopancreatic tubules, with B (blister)-cells apparently unaffected. Displacement of the basophilic nucleolus and margination of the chromatin occurred. Parasite stages appeared as eosinophilic granular bodies (Fig. 1A,B) and accompanied hypertrophy of affected nuclei. In addition to changes within the nucleus, some epithelial cells contained diffuse cytoplasmic infections (Fig. 1C). In these cases, associated degeneration of the tubule epithelia occurred, with parasites and sloughed epithelial cells present within the tubule lumen (Fig. 1D). In heavily affected specimens, the majority of nuclei contained eosinophilic inclusions and degeneration of the hepatopancreatic tubules occurred. In such cases, cells often appeared to separate from their neighbours (Fig. 1E) and the host elicited an immune response to affected tubules by encapsulating them with mixed populations of hyalinocytes and granulocytes. Necrotic tubules, demarcated with melanin deposition were frequently observed (Fig. 1F). No microsporidian stages were detected in other host organs, either by light microscopy or by electron microscopy.

Ultrastructure

Different life cycle stages of the parasite were observed within the affected epithelial cell nuclei of the hepatopancreatic tubules. In some cases, the

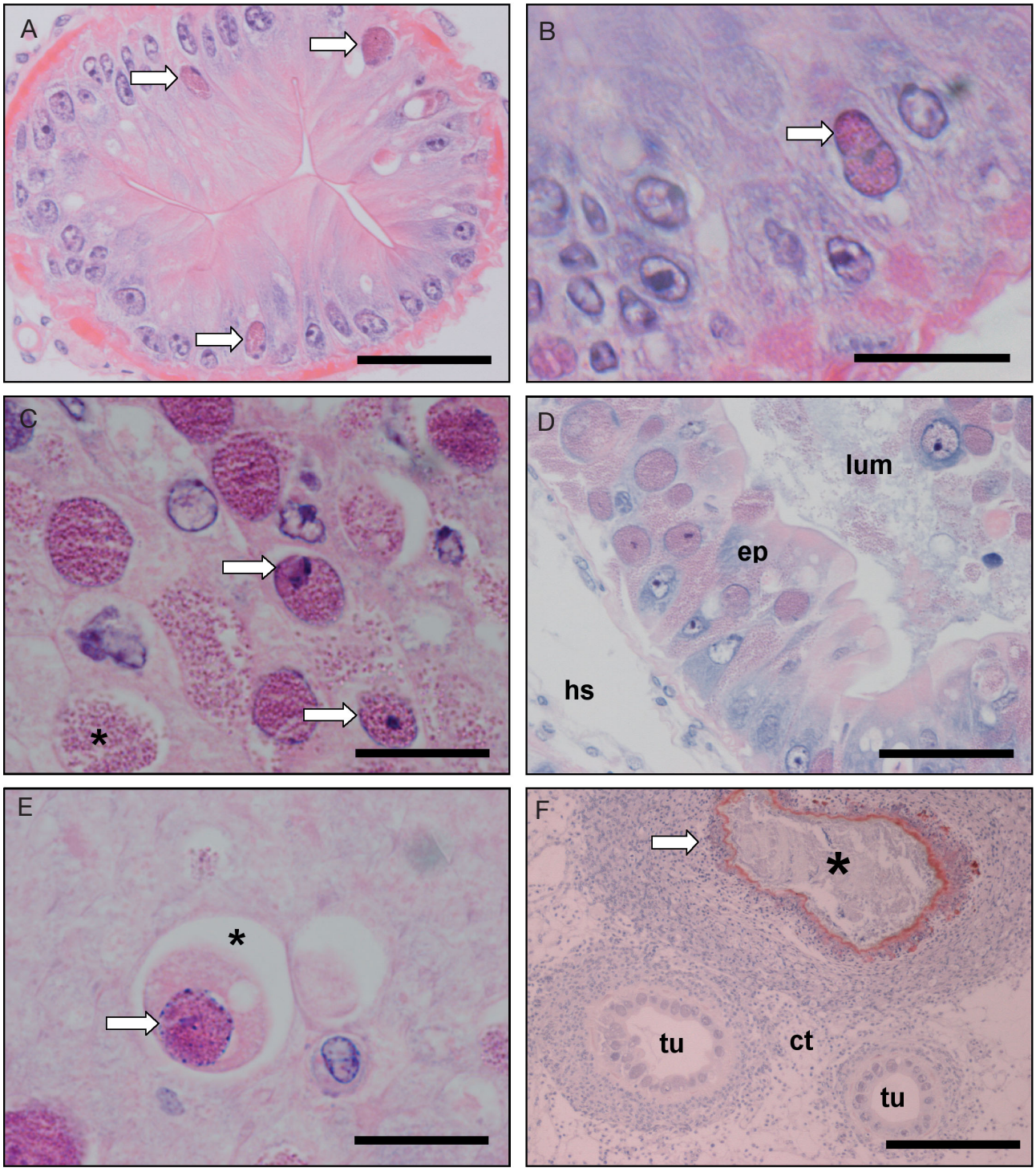

Fig. 1. *Enterospora canceri* infection of hepatopancreatic epithelia of *Cancer pagurus*. Histopathology. (A) Single infected tubule in mild infection, showing hypertrophic eosinophilic nuclei (arrow). (B) Single infected nucleus with granular eosinophilic nucleoplasm and marginalised chromatin (arrow). (C) Advanced disease showing parasites within nuclei (arrows) and liberated spore stages within cytoplasm (asterisks). (D) Advanced disease showing infected nuclei and cytoplasm within tubule epithelia (ep) and masses of liberated spores and cell debris within tubule lumen (lum); parasites or infected host cells were not observed within haemal spaces (hs). (E) Single infected cell showing separation from neighbouring epithelia (asterisk) and advanced nuclear infection (arrow). (F) Advanced infection with encapsulation of degenerated (asterisk and arrow) and relatively intact tubules (tu); connective tissue (ct) is filled with infiltrating haemocytes and is devoid of reserve inclusion cells. All images H&E. Scale bars = (A) 100 µm, (B,C,E) 25 µm, (D) 50 µm, (F) 200 µm

majority of all nuclei were infected. Uninucleate meronts were not detected within infected nuclei, but possible bi-nucleate stages were observed (Fig. 2A). More advanced multinucleate plasmodia (sporogonal plasmodia) contained up to 22 nuclei in section (Fig. 2B,C). Infected host nuclei often contained multiple discrete plasmodia and were hypertrophic, with margination of the remaining host chromatin.

Early stage plasmodia contained nuclei within a granular cytoplasm and were contained by a simple electron dense membrane that separated plasmodial components from the host cell nucleoplasm (Fig. 2A–C). Further development of plasmodia was marked first by the appearance of multiple, small, spherical, membrane-bound vesicles and later by electron lucent vacuoles, both of which appeared to associate with individual plasmodial nuclei (Fig. 3A,B,E). At this stage, the plasmodial membrane was often observed in close proximity (and potentially in confluence with) the inner nuclear membrane of the host cell (Fig. 3C). Next, multiple copies of apparatus resembling the polar filament and anchoring disk of the mature spore formed within the plasmodial cytoplasm and appeared to associate with individual plasmodial nuclei and small spherical vesicles (Fig. 3D). The electron-dense, isofilar, polar filament precursor lay adjacent to the plasmodial nucleus and even at this early stage of spore development was seen to consist of 4 to 5 turns, capped with a dome-shaped anchoring disk precursor (Fig. 3E,F). Aggregation and early assembly of these presumptive sporoblast components took place *within* the intact sporogonial plasmodium. In several instances, early stage plasmodia and those containing sporoblast precursors were separated from masses of mature spores lying outside the plasmodium and in direct contact with the nucleoplasm of the same host cell (Fig. 4a). In these cases, discrete bounding membranes separated individual plasmodia.

Stages corresponding to the sporoblast were not observed in any of the infected crabs assessed during the current study. Similarly, divisional events that presumably led to the liberation of uninucleate sporoblasts in the host nucleoplasm were also not observed. Only maturing and mature electron dense spore stages were observed. Maturing and mature spores were not contained within sporophorous vesicles but instead lay in direct contact with the host nucleoplasm (Fig. 4B). In many cases, large numbers of spores (several tens to >100) were observed within a given section of host nucleus, suggesting

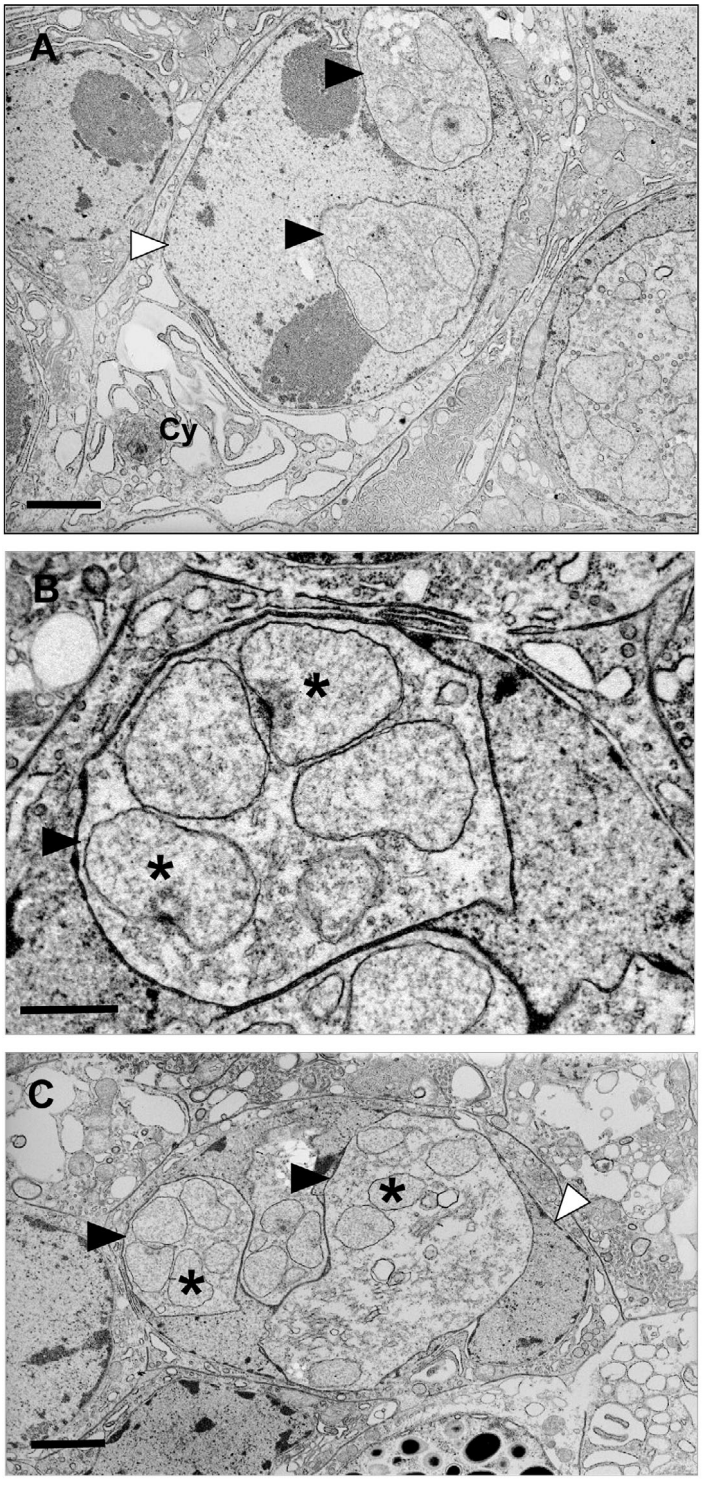

Fig. 2. *Enterospora canceri* in hepatopancreatic epithelia of *Cancer pagurus*. Ultrastructure of early life stages. (A) Early meront stages (black arrowheads) within nucleoplasm of host cell nucleus (white arrowhead); Cy: host cell cytoplasm. (B) Early meront stage (black arrowhead) within nucleoplasm of host cell; parasite nuclei are adjacent (asterisks). (C) Multiple merogonal forms (black arrowheads) within single distended host nucleus (white arrowhead); parasite nuclei shown by asterisks. All scale bars = 1 µm

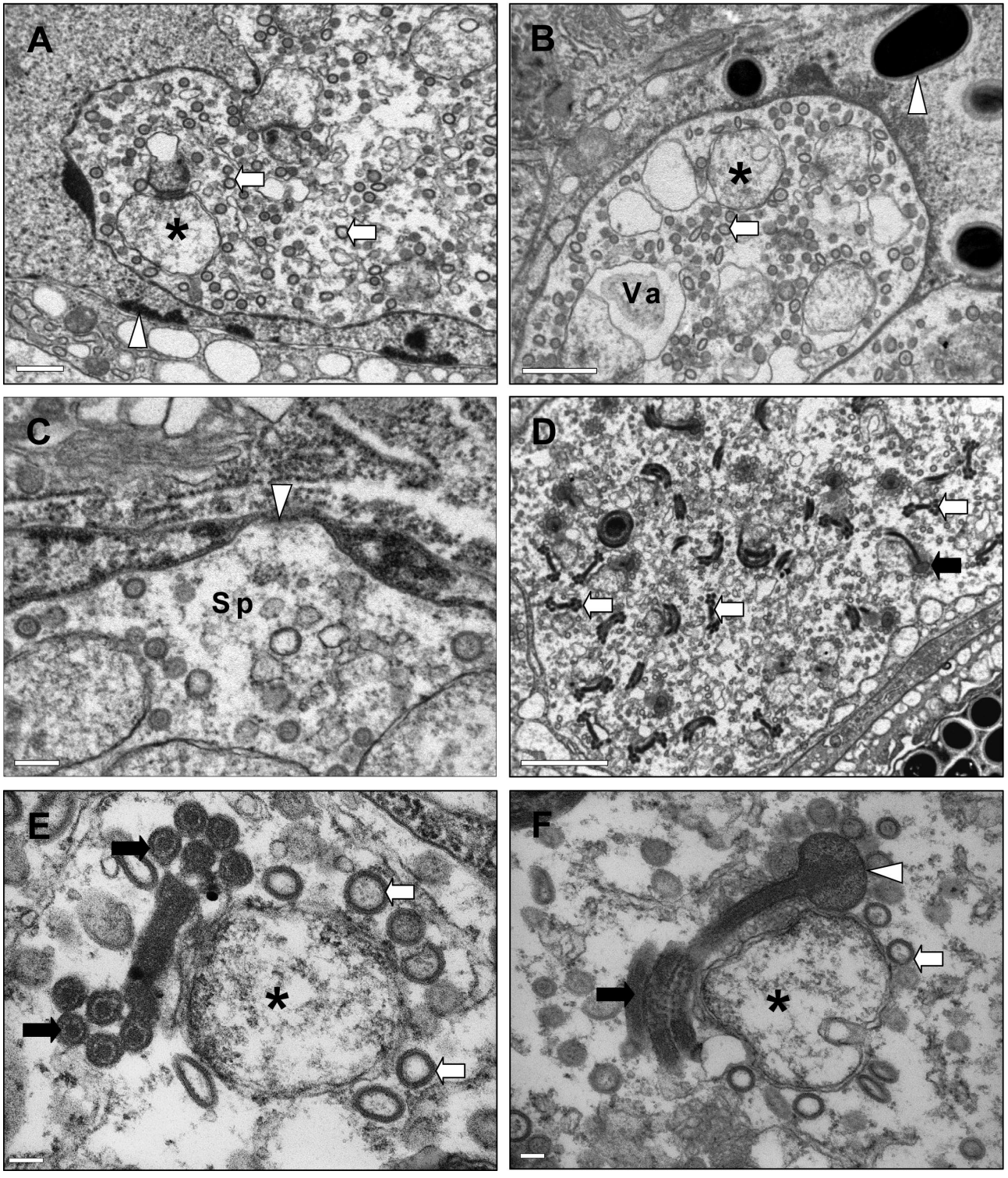

Fig. 3. *Enterospora canceri* in hepatopancreatic epithelia of *Cancer pagurus*. Ultrastructure of sporogonal plasmodial stages. (A) Presumptive early sporogonal plasmodium containing parasite nuclei (asterisk) and small membrane-bound vesicles (arrows); host nuclear border shown by white arrowhead. (B) Early sporogonal plasmodium with nuclei (asterisk), membrane-bound vesicles (arrow) and large electron-lucent vacuoles (Va); mature spores are visible within same host nucleus in direct contact with nucleoplasm (arrowhead). (C) Early sporogonal plasmodium (Sp) in apparent confluence with inner membrane of host nucleus (arrowhead). (D) Later-stage sporogonal plasmodium with precursors of the polar filament (white arrows) and anchoring disk (black arrow) observed in mature spores. (E) Magnification of assembled units of spore precursor, showing parasite nucleus (asterisk) associated with membrane-bound vesicles (white arrows) and a coiled polar filament with 5 turns (black arrows). (F) Spore precursor similar to that in (E), showing parasite nucleus (asterisk), membrane-bound vesicles (white arrow), polar filament (black arrow) and anchoring disk (white arrowhead). Scale bars = (A) 0.5 µm, (B) 1 µm, (C) 0.2 µm, (D) 2 µm, (E,F) 100 nm

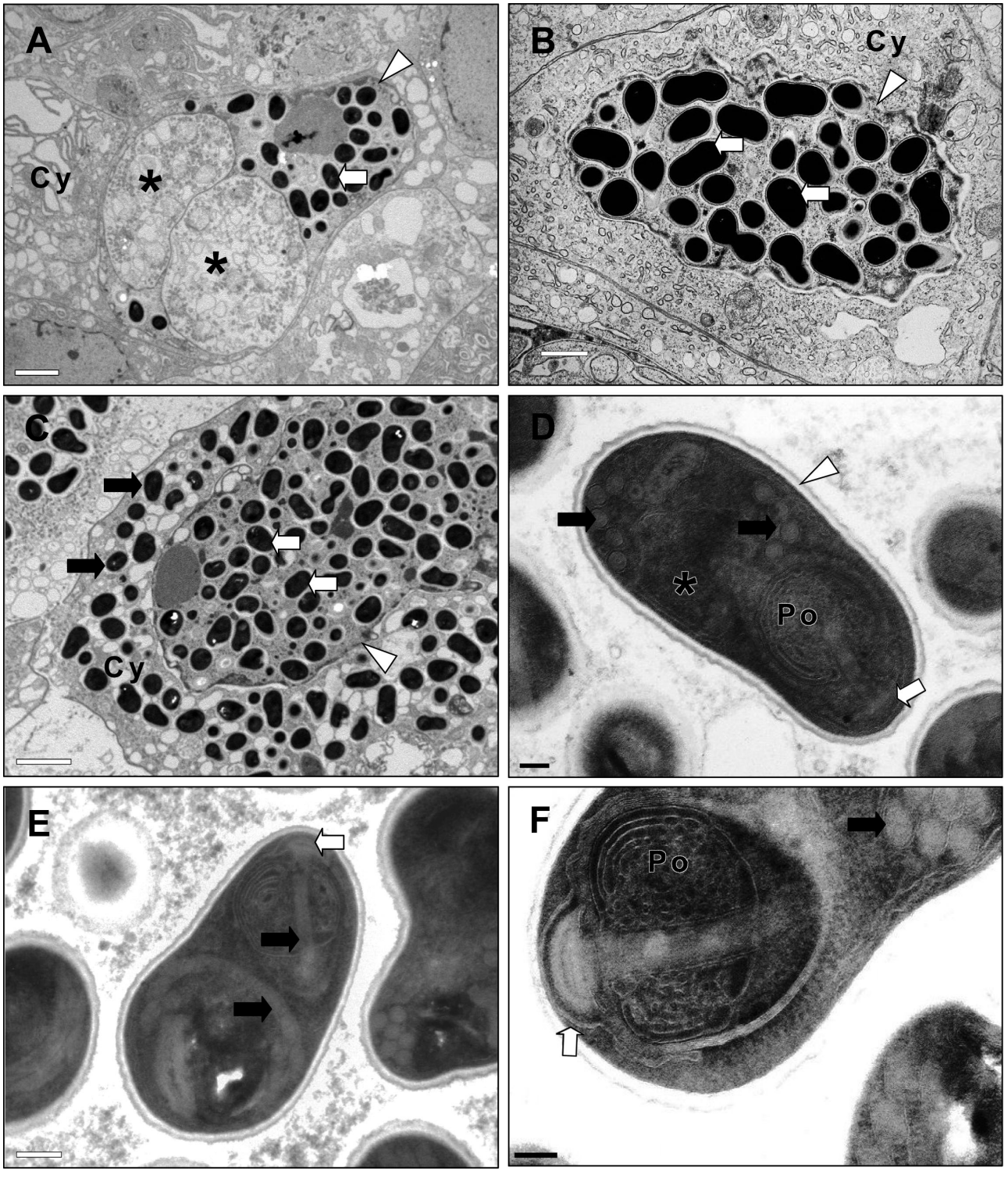

Fig. 4. *Enterospora canceri* in hepatopancreatic epithelia of *Cancer pagurus*. Ultrastructure of spore development. (A) Multiple parasite life cycle stages in single infected hypertrophic host nucleus (arrowhead), showing 2 large sporogonal plasmodia (asterisks) adjacent to mature spores lying in direct contact with host nucleoplasm (arrow); Cy: host cell cytoplasm. (B) Hypertrophic host nucleus containing multiple mature spores (arrows); nuclear margins are distended by spores (white arrowhead); Cy: host cell cytoplasm. (C) Advanced infection manifested as mature spore stages within nucleoplasm (white arrows) and cytoplasm (Cy, black arrows) of host cells; white arrowhead: nuclear border. (D) Mature spore with trilaminar wall (arrowhead), polar filament with 4 to 5 coils (black arrows), nucleus (asterisk), polaroplast (Po) and anchoring disk (white arrow). (E) Mature spore with same features as that in (D), showing different orientation of polar filament (black arrows) and associated anchoring disk (white arrow). (F) Detail of anchoring disk (white arrow), polaroplast (Po) and polar filament (black arrow). Scale bars = (A,C) 2 µm, (B) 1 µm, (D,F) 100 nm, (E) 0.2 µm

that at least several hundred can be present within the nucleus as a whole. Host chromatin was restricted to the outer margins of the nucleus. In host cells showing advanced infections, maturing and mature spores were present within the nucleoplasm and within the cytoplasm of the same cell (Fig. 4C). Spores measured 1.3 ± 0.02 × 0.7 ± 0.01 µm (mean n = 30) and were contained within a trilaminate wall consisting of a cell membrane (5.45 ± 0.19 nm), an endospore (16.9 ± 0.51 nm) and an exospore (14.8 ± 0.65 nm) (Fig. 4D,E). An anchoring disk apparatus was clearly visible within maturing and mature spores (Fig. 4F). Degenerate epithelial cells containing partially developed and presumably defective spore stages were occasionally observed attached to the basement membrane of infected tubules and also detached within the lumen of infected hepatopancreatic tubules. Such cells contained effete plasmodial stages in otherwise intact host cell nuclei (Fig. 5A) and enlarged lysosomes (Fig. 5B). Effete spore stages (Fig. 5C) were also observed within the lumens of infected hepatopancreatic tubules (Fig. 5D). Table 1 provides the main pathological and morphological features used to differentiate the proposed new genus from the other microsporidian genera of the family Enterocytozoonidae.

DISCUSSION

This study describes the developmental stages of a microsporidian parasite of the European edible crab *Cancer pagurus*, a commercially exploited species of decapod crustacean captured from the English Channel, UK. The parasite is the first reported example of an intranuclear microsporidian in an invertebrate. The parasite was discovered in crabs captured between

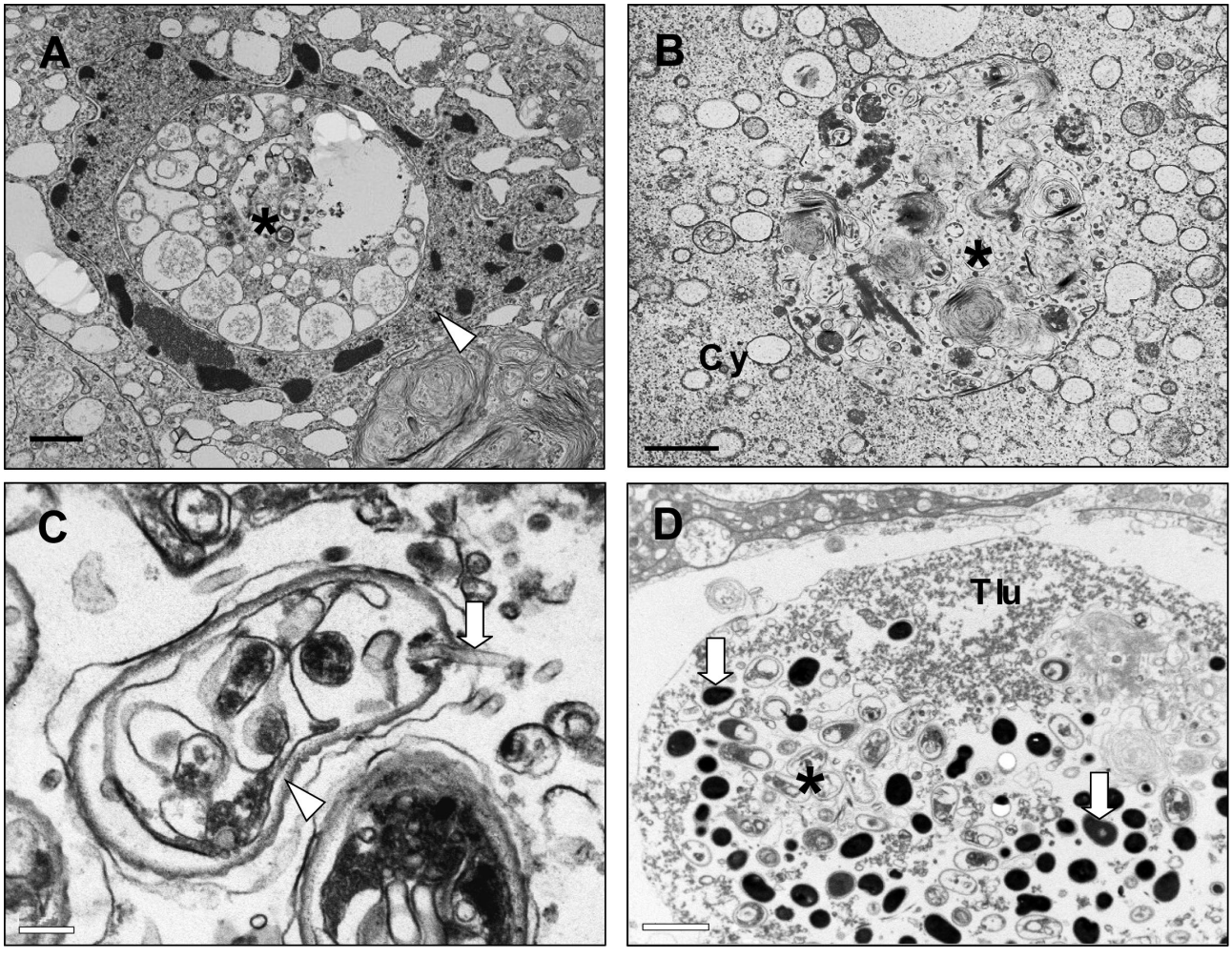

Fig. 5. *Enterospora canceri*-infected *Cancer pagurus*. Ultrastructure of effete parasite stages and host cell pathology. (A) Effete sporogonal plasmodium (asterisk) within host cell nucleus (arrowhead). (B) Lysosome (asterisk) within cytoplasm (Cy) of infected host cell. (C) Effete spore stage (arrowhead) with everted polar filament (arrow). (D) Liberated mature spores (arrows), effete spores (asterisk) and cell debris within lumen of a hepatopancreatic tubule (Tlu). Scale bars = (A,B) 1 µm, (C) 0.2 µm, (D) 2 µm

Table 1. Enterocytozoonidae. Comparison of parasite and host-related features between genera of the family

Feature	*Enterocytozoon*	*Nucleospora*	*Enterospora*
Host cell type	Gut epithelium (mammal)	Blood cell lineage or epithelium (fish)	Hepatopancreas epithelium (invertebrate)
Cellular location	Cytoplasm	Nucleoplasm	Nucleoplasm
Earliest developmental stage observed	Uninucleate meront	Uninucleate meront	Binucleate meront
Extrusion apparatus assembly site	Sporogonic syncitium	Sporogonic syncitium	Sporogonic syncitium
Multiple stages in same host cell or nucleus	Yes	?	Yes
Origination site of anchor disc	Invagination of parasite nucleus	Invagination of parasite nucleus	Close association with parasite nucleus
Approximate mature spore size	1.5 × 0.5 µm	2 × 1 µm	1.3 × 0.7 µm
Number of spores per nucleus section	Not applicable	2–8	>20
Number of turns of polar filament	4–5	8–12	4–5

March and June 2004 and was not observed in crabs sampled at other times of the year. Maximum apparent prevalence at the Weymouth Bay sampling site was 3.45%. Since all crabs sampled were destined for the live commercial market, all were larger than the minimum landing size (MLS) of 140 mm carapace width. No information on infection prevalence is available for crabs below the MLS or from sites outside Weymouth Bay.

A large number of microsporidian parasites have been described infecting decapod crustaceans (see Sprague & Couch 1971, Sprague 1977, Couch 1983), with several known to cause mortality and economic impact in cultured and wild populations. The microsporidian parasite described in the current study shares key ultrastructural features with those already described within the family Enterocytozoonidae. The formation of spore organelles (extrusion apparatus such as the polar filament and anchoring disk) prior to fission of the sporogonic plasmodium into uninucleate sporoblasts and spores is perhaps most characteristic. In all other microsporidian families, the spore organelles only become visible following separation of the sporoblasts from the plasmodium. It is appropriate therefore that the microsporidian parasite from *Cancer pagurus* is placed within this family of microsporidians. Since *Nucleospora* is the only genus within this family (and indeed within the Microsporidia) with an intranuclear habit, it is appropriate for us to make direct comparison between the microsporidian from *C. pagurus* with the species already described within this genus from finfish.

The development cycle of the parasite from *Cancer pagurus* appears to occur completely within the nucleoplasm of the hepatopancreatic epithelial cells, with development of meronts, sporogonal plasmodia and eventually mature spores. While sporogonal nuclei are separated from the host cell nucleoplasm by a simple sporogonal membrane, mature spores lie in direct contact with the host nucleoplasm and are not contained within a bounding membrane or vesicle. In the latter stages of infection, the nucleus appears to degenerate, thereby liberating mature spores to the cytoplasm. Eventually, death of the host cell leads to sloughing of infected epithelial cells and release of spores into the lumen of the hepatopancreatic tubules. Presumably, the parasite exits the host via the faeces. Infection of the host midgut or hindgut epithelial cells (or of any other organ) was not observed during the current study, suggesting that these parasites are specialised inhabitants of the hepatopancreatic epithelia.

The affinity for host epithelial cells shown by the crab microsporidian is similar to that reported for the genus *Enterocytozoon*, with *E. bieneusi* predominantly infecting the enterocytes of the human duodenum and ileum (Desportes et al. 1985). Members of the genus *Nucleospora* may also infect the epithelial cells of the urinary tubules and the mesangial cells of the glomerulus but are predominantly found within the haemopoeitic cell lineages. The two other prospective members of the genus *Nucleospora*, from lumpfish and Atlantic halibut, also infect the lymphocytic cells of the renal interstitium (and associated organs such as the spleen) and have caused mortalities in aquaculture settings (Mullins et al. 1994, Nilsen et al. 1995). Recently, another intranuclear microsporidian has been added to the genus, *N. secunda*, infecting the enterocytes of the killifish *Nothobranchius rubripinnis* (Lom & Dyková 2002).

While it is apparent that the crab microsporidian clearly resembles *Nucleospora* based upon its intranuclear habit and also, more generally, the Enterocytozoonidae based upon the formation of spore organelles prior to plasmodial fission and features of the mature spore, several features of the crab parasite justify the formation of a new genus within the Enterocytozoonidae. A comparison of pathological and morphological features of the crab microsporidian with those from microsporidian genera of the family Enterocytozoonidae is given in Table 1. Significantly, the number

of turns of the polar filament in the mature spore is similar in the crab parasite to that of *Enterocytozoon bieneusi* (4 to 5 turns). In contrast, *N. salmonis* possesses 8 to 12 polar filament turns. The spore size of the crab microsporidian is also similar to that of *E. bieneusi* (1.3 × 0.7 µm and 1.5 × 0.5 µm, respectively). The mature spore size in *N. salmonis* is given as 2 × 1 µm. Finally, while potentially a function of differing nuclear size in respective hosts (Desportes-Livage et al. 1996), the number of spores present per section of nucleus appears to be significantly greater in the crab microsporidian than in *N. salmonis* (2 to 8 and over 20, respectively). *E. bieneusi* does not exist within the host nucleus. Taken together, these features when allied to information on the taxonomic distinctness of the respective host organisms (mammals for *Enterocytozoon*, finfish for *Nucleospora* and Crustacea for the parasite described herein), justify the establishment of the crab parasite as the type species of a new genus within the Enterocytozoonidae. Since the morphological features of the crab parasite most closely resemble members of the genus *Enterocytozoon*, we propose the erection of a new genus and species to accommodate the parasite described here, namely *Enterospora canceri*.

The material analysed in the current study was collected as part of a disease survey of the English Channel *Cancer pagurus* fishery. As such, material was not collected and preserved for molecular analysis. Recent studies of microsporidian taxonomy based upon analysis of regions of the parasite genome have placed members of the Enterocytozoonidae in a basal position amongst the microsporidia (Lom & Nielsen 2003). The discovery of an intranuclear species infecting a marine crustacean, with developmental similarities to the human pathogen *Enterocytozoon bieneusi* and to the genus *Nucleospora* appears remarkable. To this end, it has been speculated that a considerably long evolutionary time appears to have passed since members of the Enterocytozoonidae (*Nucleospora* and *Enterocytozoon*) shared a common ancestor with the other fish-infecting microsporidians (Lom & Nilsen 2003). With the apparent discovery of *Enterospora* as a new genera residing in this family and infecting decapod crustaceans, the possibility for linking the apparently disparate Enterocytozoonidae to other microsporidian families may now be feasible. Further work should attempt to gather appropriate infected material from *C. pagurus* and to investigate the taxonomy of other hepatopancreatic microsporidians from crustaceans. That a currently undescribed species, with developmental features of the Enterocytozoonidae and infecting the nucleoplasm of hepatopancreatic cells has recently been discovered from the hermit crab *Eupagurus bernhardus* (Stentiford & Bateman 2007, this issue) supports this view.

The significance of this parasite as a mortality driver in *Cancer pagurus* stocks has not been assessed. While the apparent prevalence of infection in the Weymouth Bay stock appears to be low, no information is available on infection prevalence in other European stocks or for juvenile crabs that fall below the legal minimum landing size. The discovery of a new genus of parasite in this host highlights the relative dearth of baseline data available for disease agents of decapod crustaceans and points towards this host group as a significant resource for researchers of parasite taxonomy and pathology. Furthermore, the description of these pathogens in important commercial hosts is fundamental for future consideration of factors affecting stock health and sustainability.

TAXONOMIC SUMMARY

Phylum Microsporidia (Balbiani, 1882)
Class Microsporea (Levine & Corliss, 1963)
Order Microsporidia
Suborder Apansporoblastina
Family Enterocytozoonidae

Genus *Enterospora* n. gen.

Definition. Merogonic and sporogonic stages occur within the nuclei of epithelial cells of the hepatopancreatic tubules. Asynchronous merogonic development. Meronts bound by a single membrane-bound vesicle within the nuclei of hepatopancreatic epithelial cells. One to several vesicles may be present within a single nucleus. Meront cytoplasm contains granular substance and sparse endoplasmic reticulum. Sporogenesis occurs within intact plasmodia by aggregation of the sporoblast components. Mature spores appear in direct contact with the host nucleoplasm.

Enterospora canceri n. sp.

Specific diagnosis. Spores ovoid, measuring 1.3 ± 0.02 µm × 0.7 ± 0.01 µm in fixed tissue for electron microscopy. Sporogenesis occurs within intact plasmodia by aggregation of the sporoblast components with a single plasmodial nucleus. Polar tube with 4 to 5 turns visible in sporoblast precursors and in maturing and mature spores.

Type host. *Cancer pagurus* L.

Type locality. Weymouth Bay, English Channel, United Kingdom (50° 34′ N, 2° 22′ W).

Site of infection. Nucleus of the epithelial cells of the hepatopancreas.

Etymology. The generic epithet reflects the site of infection of the spore (*Entero-* from the greek 'enteros' meaning 'within') and *spora* meaning spore. The specific epithet relates to generic names of the type host.

Type material. Syntype slides of histological sections stained with H&E have been deposited in the Registry of Aquatic Pathology (RAP) held at the CEFAS Weymouth Laboratory. Wax blocks containing infected material have also been also been deposited in the Natural History Museum, London (awaiting accession number).

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