



Paradoxium irvingi n.gen. n.sp. (Microsporidia) infecting the musculature of European pink shrimp *Pandalus montagui*

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ABSTRACT

This paper utilises histological, ultrastructure and molecular phylogenetic data to describe a novel genus and species (*Paradoxium irvingi* n.gen., n.sp.) within clade 5 of the phylum Microsporidia. The parasite infects the musculature of the pink shrimp *Pandalus montagui* captured from United Kingdom waters. The novel microsporidium is morphologically and phylogenetically dissimilar to its nearest phylogenetic branch relative *Thelohania butleri* infecting the sister shrimp taxon *Pandalus jordani*. Furthermore, it is morphologically distinct from the type species of the genus *Thelohania*, *Thelohania giardi* infecting European brown shrimp *Crangon crangon*. Since phylogenetic data pertaining to type *T. giardi* is not currently available, our discovery places some doubt on the likelihood that *T. butleri* represents the proposed surrogate for the type taxon. Further it demonstrates potential for significant morphological plasticity in this clade of muscle-infecting microsporidians of crustaceans which contains the genera *Myospora*, *Cucumispora*, *Thelohania*, and now *Paradoxium*. Since it cannot be stated with certainty that *T. butleri* (or other taxa within the clade) represent true close relatives of *T. giardi*, clarity on this issue will only occur with re-discovery and genotyping of type *T. giardi* infecting *C. crangon* from European waters.

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1. Introduction

The Microsporidia are common pathogens of aquatic arthropods (Stentiford et al., 2013a). One of the first microsporidian taxa described was the type species of the genus *Thelohania*, *Thelohania giardi* infecting European brown shrimp (*Crangon crangon*) in the late 1800s (Henneguy and Thélohan, 1892; Hazard, 1976). Although never rediscovered, more recent studies have described a proposed sister taxon, *Thelohania butleri*, an octosporous microsporidium, infecting the musculature of Canadian pink shrimp (*Pandalus jordani*) (Brown and Adamson, 2006). The discovery was notable since it provided the first fully characterised example of a likely 'true' member of the genus *Thelohania* (i.e. those infecting marine hosts) and further, highlighted that the placement in this genus of similarly octosporous (but phylogenetically distant) microsporidians infecting freshwater crayfish and ants, was erroneous (Brown and Adamson, 2006). The genus *Thelohania* is thus recognised as polyphyletic and in need of significant revision.

As part of an effort to rediscover type *T. giardi* infecting *C. crangon* from European waters, we conducted surveillance on a

population of commercially exploited pink shrimp (*Pandalus montagui*), co-habiting with *C. crangon*, in United Kingdom waters. In light of the findings of Brown and Adamson (2006), and based upon previous failure of our laboratory to detect *T. giardi* in *C. crangon* from this population, we aimed to investigate the wider potential for other 'true' members of the genus *Thelohania* to infect marine shrimp. In this paper we utilise histological, ultrastructural and phylogenetic data to describe a novel microsporidium infecting *P. montagui* from United Kingdom waters. The parasite did not display the characteristic morphological features of either *T. giardi* or *T. butleri* and although most closely related to the latter phylogenetically, this similarity was not sufficient to propose that the new taxon is a member of the genus *Thelohania*. Combining morphological and phylogenetic data, we propose that the parasite is the type species of a novel genus of muscle-infecting microsporidian pathogens infecting marine shrimp hosts. Although previous work from our laboratory has focused on the potential for significant morphological plasticity within closely related muscle-infecting Microsporidia (Stentiford et al., 2013b), in this instance, combined evidence proposes that the musculature of pandalid shrimps at least, can be infected by more than one genus of microsporidian parasite. We discuss our findings in relation to the taxonomy of *T. butleri*, and the potential phylogenetic placement of the currently un-studied type taxon, *T. giardi*.

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Fig. 1. External signs of *Paradoxum irvingi* n.gen., n.sp. infection in *Pandalus montagui*. The transparent appearance of uninfected shrimp (bottom) contrasts that of infected shrimp (top) due to the opacity in underlying skeletal musculature. Infected shrimp are lethargic and less able to elicit a tail-flick response. Scale 2 cm.

2. Materials and methods

2.1. Collection of specimens

Pink shrimp (*Pandalus montagui*) were collected in July 2013 during the annual Clean Seas Environmental Monitoring Programme (CSEMP) survey of United Kingdom marine waters. Shrimp were specifically collected from sites within the Wash, North Sea (53.1417N, 0.555W). Upon capture, animals were placed on large trays for visual health assessments. During sorting, abnormal looking animals with loss of translucency and apparently

opaque underlying soft tissues were removed from the catch for processing (Fig. 1). In total, 4 animals presented clinical signs out of approximately 4000 animals (apparent prevalence 0.001%). Representative examples of externally normal shrimp and those displaying signs of tissue opacity were chilled on ice prior to dissection.

2.2. Histology and electron microscopy

For histological assessment, the hepatopancreas, gills, heart, midgut, gonad and skeletal muscles were dissected from each specimen. Excised samples were placed immediately into Davidson's seawater fixative and fixation was allowed to proceed for 24 h before samples were transferred to 70% industrial methylated spirit for storage prior to processing. Fixed samples were processed to wax in a vacuum infiltration processor using standard protocols. Sections were cut at a thickness of 3–5 µm on a rotary microtome and were mounted onto glass slides before staining with haematoxylin and eosin (HE). Stained sections were analysed by light microscopy (Nikon Eclipse E800) and digital images were taken using the Lucia™ Screen Measurement System (Nikon). For electron microscopy, 2 mm³ blocks of skeletal muscle were fixed in a solution containing 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4), for 2 h at room temperature prior to rinsing in 0.1 M sodium cacodylate buffer (pH 7.4) and post-fixation in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 1 h. Specimens were washed in two changes of 0.1 M sodium cacodylate buffer and dehydrated 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. Semi-thin (0.5–1 µm) sections were stained with Toluidine Blue for viewing with a light microscope to identify suitable target areas. Ultrathin sections (70–90 nm) of

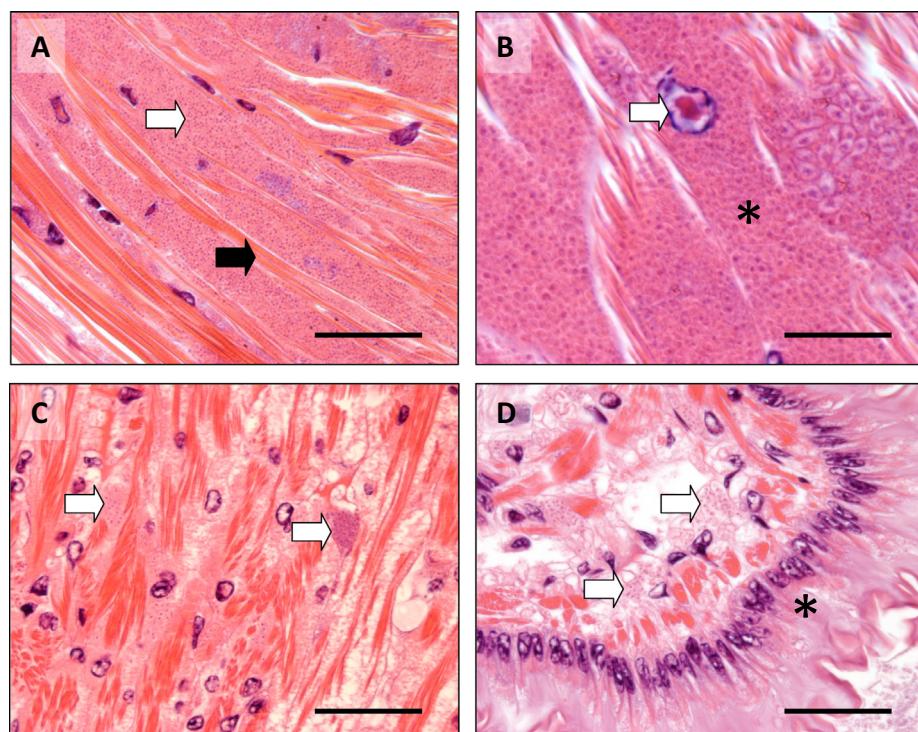


Fig. 2. Histopathology of *Paradoxum irvingi* n.gen., n.sp. infection in the musculature of *Pandalus montagui*. (A) Skeletal muscle. Myofibres (black arrow) are replaced with developmental stages of the parasite (white arrow). Scale 200 µm. (B) Skeletal muscle. Parasite life stages appear to be in direct contact with the sarcoplasm (asterisk). Host cell nuclei often contained a distinctive eosinophilic inclusion (white arrow). Scale 25 µm. (C) Heart myocardium. Discrete parasite cysts replaced heart myofibres (white arrows). Scale 100 µm. (D) Circular and longitudinal muscles surrounding the intestinal epithelium (asterisk) contained discrete parasite cysts (white arrows). Scale 100 µm. All H&E histology.

target areas were mounted on uncoated copper grids and stained with 2% aqueous uranyl acetate and Reynolds' lead citrate. Grids were examined using a JEOL JEM 1400 transmission electron microscope and digital images captured using an AMT XR80 camera and AMT V602 software.

2.3. DNA extraction, PCR, sequencing and phylogenetic analysis

Samples of opaque skeletal muscle corresponding to those regions sampled for histology and electron microscopy were removed and processed for the sequencing of part of the small sub-unit ribosomal RNA (SSU rRNA) gene. Total DNA was extracted from homogenates of four infected pink shrimp. Initial homogenisation was conducted using Lysing Matrix D FastPrep® tubes with 1:10 (w/v) tissue to G2 buffer/Proteinase K (Qiagen, UK) and a Fast Prep cell disrupter (2 min, highest setting). Homogenised samples were incubated for 4 h at 56 °C. Total DNA was extracted using the EZ1 DNA tissue kit and EZ1 Advanced XL BioRobot® (Qiagen, UK) following manufacturer instructions. The partial SSU rRNA gene fragment was amplified using previously published primers Medlin B (Medlin et al., 1988) followed by a nested PCR using MF1/MR1 (Tourtip et al., 2009). All PCR reactions were performed

in a 50 l reaction mix consisting of 1× Green Go Taq buffer, 2.5 mM MgCl₂, 0.25 mM dNTPs, 100 pmol each of the forward and reverse primer, 0.25 units Go Taq Flexi (Promega, UK), and 2.5 l extracted nucleic acid. All amplifications were performed using the following thermal cycler program on a Peltier PTC-225 thermal cycler: 94 °C × 5 min followed by 40 cycles of 95 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min, followed by 72 °C for 10 min, and held at 4 °C. Amplification products were resolved on 2% agarose gels stained with ethidium bromide and visualised using a UV illuminator. Products of the correct size (c. 900 bp) were excised from the gels, purified using the Wizard SV gel and PCR purification system (Promega, UK). Sequence reactions were prepared using the ABI PRISM Big Dye Terminator v3.1 cycle sequencing kit and the primers listed above. PCR was performed using the following thermal cycling program: 94 °C for 30 s followed by 30 cycles of 96 °C for 10 s, 50 °C for 10 s and 60 °C for 4 min, and held at 4 °C. Fluorescently labelled DNA sequences were ethanol precipitated, dried, added to 25 l HDI Formamide, and incubated for 95 °C for 2 min. DNA was sequenced using the ABI 3130xl Avant Genetic analyser (Applied Biosystems, UK). Analysis of the sequences was completed using Sequencher software (Gene Codes Corporation, USA). A 937 bp partial SSU rRNA consensus sequence derived from

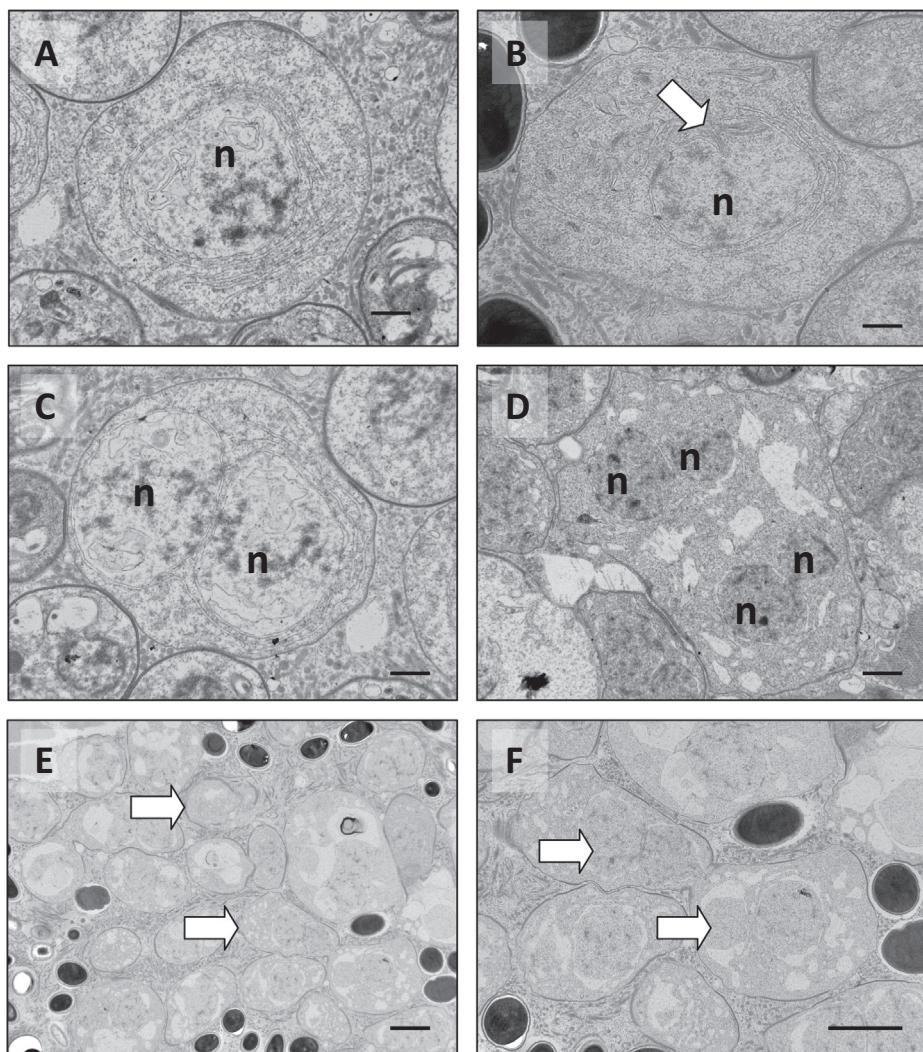


Fig. 3. Merogony and early sporogony of *Paradoxiump* irvingi n.gen., n.sp. infecting musculature of *Pandalus montagui*. (A) Uninucleate (n) meront. Scale 500 nm. (B) Uninucleate (n) meront with visible spindle pole body apparatus at periphery of nucleus (arrow). Scale 500 nm. (C) Bi-nucleate meront (n). Scale 500 nm (D) Apparently tetranucleate (n) meront plasmodium. Scale 500 nm. (E) Plasmotomy leading to formation of early sporonts, with varied nuclear configurations and thickened cell walls (arrows). Scale 2 μm. (F) Early sporonts (product of plasmotomy), with thickened cell walls and some containing diplokaryotic (2n) nuclear sets (arrows). Scale 2 μm. All transmission electron microscopy.

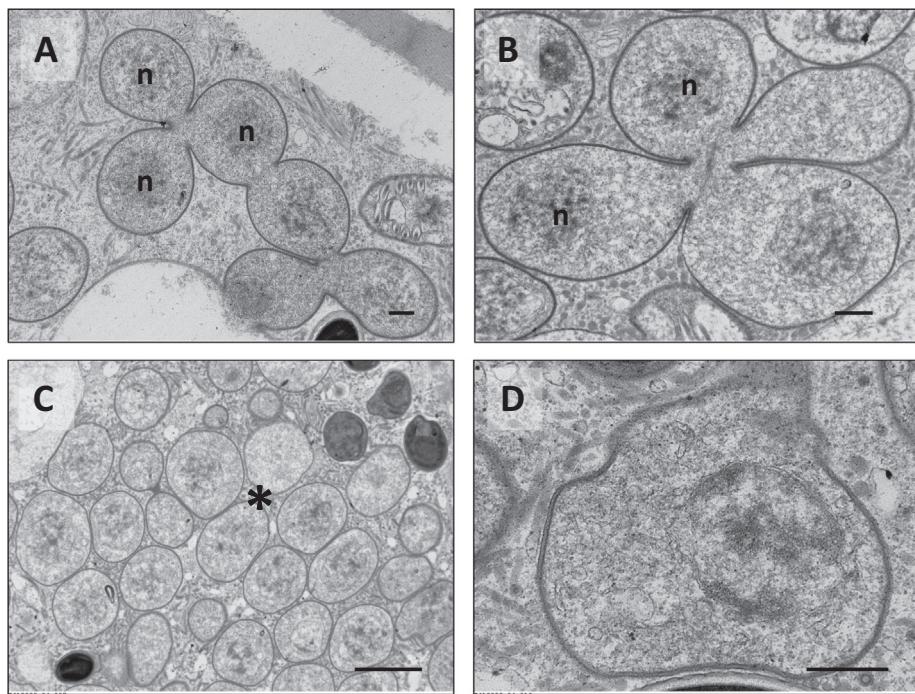


Fig. 4. Early sporogony of *Paradoxium irvingi* n.gen., n.sp. infecting musculature of *Pandalus montagui*. (A, B) Sporogonial plasmodia with differing nuclear configurations undergo budding to form uninucleate (n) sporonts. Scales 500 nm. (C) Closely opposed uninucleate sporonts occur in direct contact with the host sarcoplasm (asterisk). Scale 2 μ m. (D) Uninucleate sporonts contain thickened cell walls but do not contain pre-cursors of the spore extrusion apparatus early after separation from the sporont plasmodium. Scale 500 nm. All transmission electron microscopy.

MF1/MR1 amplicons from the four shrimp samples was placed into a Maximum Likelihood tree containing 40 representative Microsporidia from the five proposed clades of Vossbrinck and Debrunner-Vossbrinck (2005). The phylogenetic analyses was performed with RAxML BlackBox (Stamatakis (2014)) via the Cipres web portal (Miller et al., 2010). Bootstrap values were mapped onto the tree with the highest likelihood value.

3. Results

3.1. Histopathology and ultrastructure of a novel microsporidium

Infected shrimp were lethargic and unable to elicit a normal ‘tail-flick’ response upon stimulation. Histology of shrimp displaying externally-visible clinical signs revealed progressive colonisation of the skeletal musculature with a highly proliferative intracellular pathogen (Fig. 2a). In each case, skeletal muscle fibres and constituent myofibrils of the major abdominal flexor muscles of the abdomen were virtually replaced by masses of parasite life stages (Fig. 2b). Similar pathology was observed in skeletal muscles of the cephalothorax and limbs (data not shown). Remnant muscle cell nuclei often contained a distinctive eosinophilic inclusion (in Fig. 2b). Discrete clusters of parasites were also observed within myofibres of the heart (Fig. 2c) and within longitudinal and circular muscles surrounding the gut (Fig. 2d).

Transmission electron microscopy revealed multiple life stages of a microsporidian parasite within the cytoplasm of host muscle cells. The majority of life stages were apparently unikaryotic and occurred in direct contact with the host cell cytoplasm (i.e. were not contained within an interfacial membrane). Merogony appeared to progress from uninucleate (Fig. 3a and b) to bi-nucleate (Fig. 3c) and tetra-nucleate (Fig. 3d) stages (arranged as two diplokaryons) via nuclear division. Although meront plasmodia containing more than four nuclei were not observed, a single detection of a larger plasmodium, apparently undergoing

plasmotomy to form early sporonts (Fig. 3e and f) suggested that meront plasmodia may readily progress beyond the tetra-nucleate stage. Plasmotomy of multinucleate meronts and thickening of the plasmalemma initiated sporogony, and resulted in an array of configurations of the sporont plasmodium (Fig. 4a and b) which culminated in the production of the uninucleate sporont (Fig. 4c and d). Sporont plasmodia and early uninucleate sporonts did not contain pre-cursors of the spore extrusion apparatus (Fig. 4d). However, in some cases, the sporont cytoplasm contained clusters of distinctive tube-like ‘bristles’ of approximately 25 nm diameter (Fig. 6a and b). Whilst not conclusive, we propose that formation of tubular bristles within the sporont cytoplasm may correlate with eventual presence of externally presenting clusters of bristles at the posterior apex of the sporoblasts and mature spore (Fig. 6c and d). The progression of sporonts to sporoblasts was demarcated by the formation of pre-cursors of the polar filament and anchoring disk, an electron dense posterior vacuole (Fig. 5a and b) and an occasionally observed ‘tuft’ of tubular bristles emerging from the posterior region (Fig. 6c). Increasing electron density and the ordering of cytoplasmic organelles in the late sporoblast (Fig. 5c) preceded maturation of the spore. Oval shaped mature spores (Fig. 5d) had a mean length and width of 1.93 μ m ($\pm 0.2 \mu$ m SE) and 1.07 μ m ($\pm 0.1 \mu$ m SE) ($n = 30$), respectively. They were uninucleate, contained six to eight coils of an isofilar polar filament (which appeared to taper at its terminus and was generally arranged in a single rank, Fig. 5f) and possessed an umbrella-shaped anchoring disc covering the anterior region of the bi-laminar polaroplast (Fig 5e). The spore wall was trilaminar with a thin electron lucent exospore and a thickened, electron lucent endospore overlaying the plasmalemma (Fig. 5g). Although often not observed due to angle of sectioning, the posterior apex of mature spores was decorated with a focal cluster of bristles (Fig. 6d). Since these clusters were also observed occasionally in sporonts and sporoblasts (Fig. 6c), it is assumed that they did not emerge from either the exospore or the endospore layers.

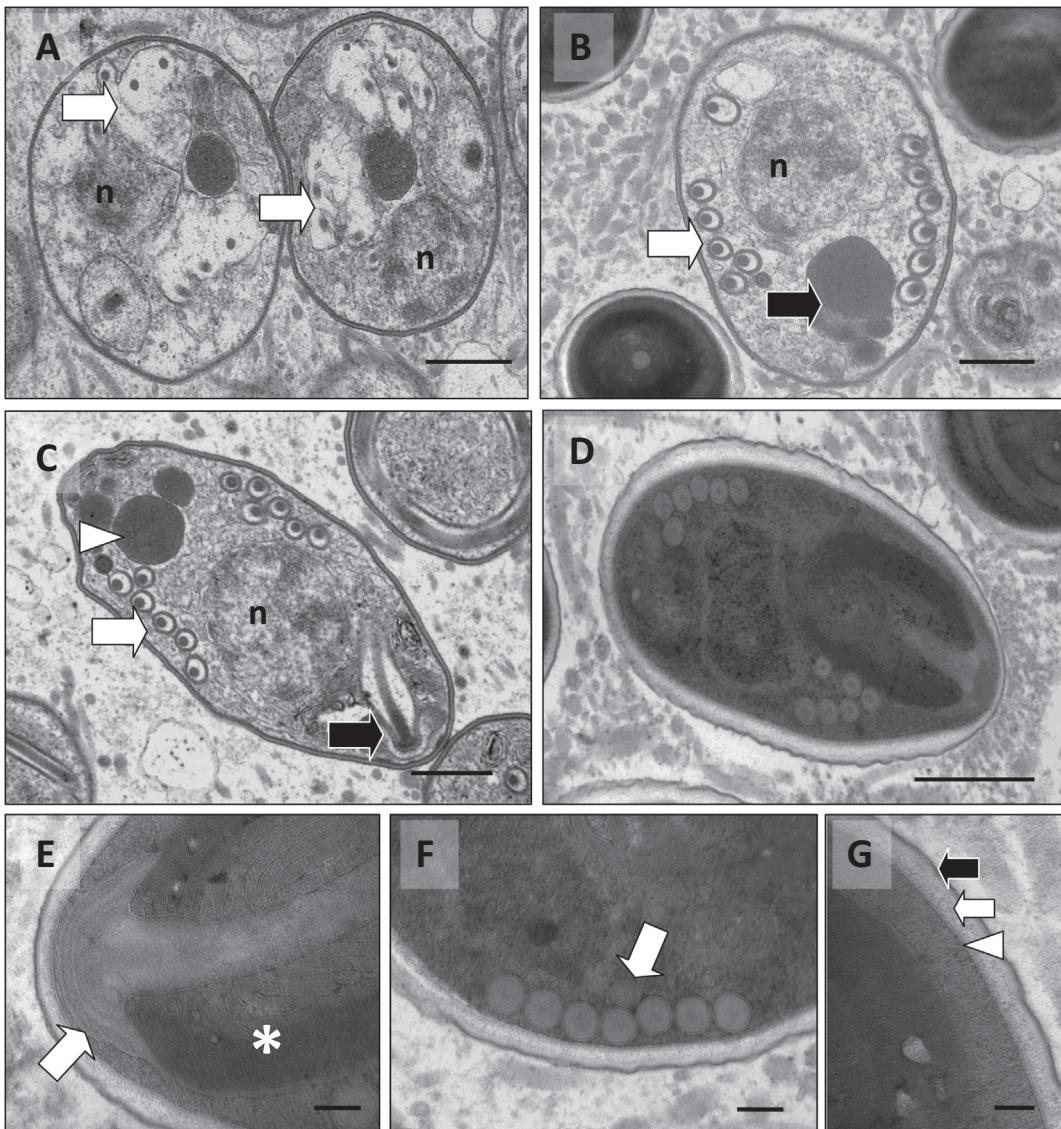


Fig. 5. Sporoblast and spore formation of *Paradoxium irvingi* n.gen., n.sp. infecting the musculature of *Pandalus montagui*. (A) Very early development of polar filament pre-cursors (arrows) within electron lucent vacuoles of two uninucleate (n) sporoblasts (arrows). Scale 500 nm. (B) Early uninucleate (n) sporoblast with peripheral ordering of polar filament (white arrow) and electron dense posterior vacuole (black arrow). Scale 500 nm. (C) Maturing uninucleate (n) sporoblasts with single rank polar filament (white arrow) terminating at an anchoring disk (black arrow) and electron dense posterior vacuole (arrowhead). Scale 500 nm. (D) Mature uninucleate spore. Scale 500 nm. (E) Detail of anchoring disk (arrow) and bilayer polaroplasts (asterisk) in mature spore. Scale 100 nm. (F) Detail of polar filament (here with 7 turns and marginal tapering in the final turn (arrow)). Scale 100 nm. (G) Detail of trilaminar spore wall comprising electron dense exospores (black arrow), electron lucent endospore (white arrow) and plasmalemma (arrow head). Scale 100 nm. All transmission electron microscopy.

3.2. Phylogeny of a novel microsporidium

Products of the correct size derived from the four infected shrimp samples were identical. The resulting consensus sequence derived from all four samples displayed 91% identity (with 100% coverage) to *T. butleri* (accession number DQ417114) infecting the musculature of sister taxon *P. jordani* and 88% identity (with 93% coverage) to *Myospora metanephrops* (accession number HM140498) infecting the musculature of the marine lobster *Metanephrops challengeri*. When represented in a phylogenetic tree, the novel parasite described here resides in a distinctly branching sub-clade (of muscle-infecting microsporidian parasites of crustacean hosts) within Clade 1 of Vossbrinck and Debrunner-Vossbrinck (2005). Although its closest relative (based upon partial SSU rRNA sequence) was *T. butleri*, SSU rRNA sequence similarity of only 91% (over 100% of the available sequence for *T. butleri*), when

coupled with its distinct ultrastructural characteristics to this and other members of the genus *Thelohania*, led us to propose that the parasite of *P. montagui* is the type species of a novel genus. We hereby name the parasite *Paradoxium irvingi* n.gen., n.sp.

4. Taxonomic description

Phylum Microsporidia (Balbiani, 1882) Class Marinospordia (Vossbrinck and Debrunner-Vossbrinck, 2005). Microsporidian parasites infecting hosts from the marine environment. Clade 5.

4.1. Genus *Paradoxium* n.gen. Stentiford, Ross, Kerr, Bass, Bateman

Majority of observed developmental stages of the parasite unikaryotic. All stages in direct contact with the cytoplasm of host muscle cells. Merogony progresses from uninucleate to bi-nucleate

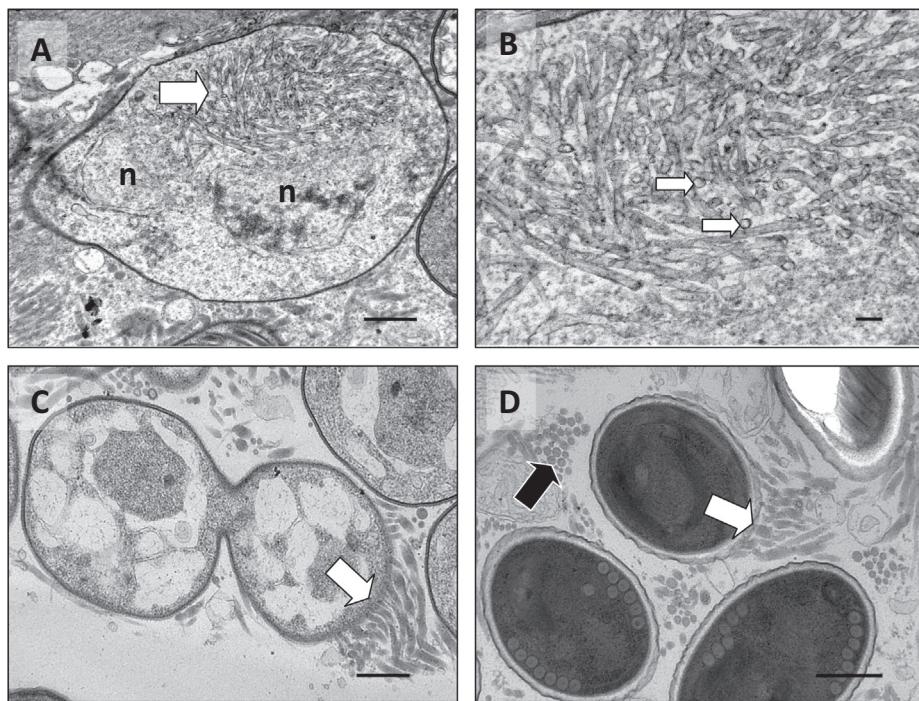


Fig. 6. Bristle formation in *Paradoxiump. irvingi* n.gen., n.sp. infecting the musculature of *Pandalus montagui*. (A) Cluster of tubular bristles (arrow) within the cytoplasm of a binucleate (n) sporont. Scale 500 nm. (B) Detail of bristles showing tubular structure (arrows). Scale 100 nm (C) Appearance of terminal 'tufts' arising from a binucleate sporont wall prior to separation and progression to sporoblast. Scale 500 nm (D) Presence of 'tuft' of tubular bristles emerging from posterior end of mature spore (white arrow). Tufts arising from adjacent spores (out of section) are also indicated (black arrow). Scale 500 nm. All transmission electron microscopy.

and tetra-nucleate (two diplokaryons) plasmodia via nuclear division. Production of plasmodia with more than four nuclei likely (though not observed). Plasmotomy of multinucleate meronts and thickening of the plasmalemma initiates sporogony, culminating in production of uninucleate sporonts. Early sporonts do not contain pre-cursors of the spore extrusion apparatus but sometimes contain clusters of tube-like bristles within their cytoplasm. Sporoblasts demarcated by formation of polar filament and anchoring disk precursors, electron dense posterior vacuole and occasionally, a 'tuft' of tubular bristles in the posterior region. Oval shaped mature spores uninucleate with mean length 1.93 µm (± 0.2 µm SE) and width 1.07 µm (± 0.1 µm SE) ($n = 30$). Spores contain six to eight coils of an isofilar polar filament (with extreme terminal tapering and arranged in a single or double rank) and umbrella-shaped anchoring disc covering the anterior region of bi-laminar polaroplast. Spore wall trilaminar with thin electron lucent exospore and thickened, electron lucent endospore overlaying plasmalemma. Posterior apex of mature spore decorated with cluster of bristles (not observed in all spores due to focal anchoring of the bristles to posterior apex). Distinctive partial SSU rRNA gene sequence from closest branching neighbours *Cucumispora* spp. and *T. butleri*.

4.2. Type species: *Paradoxiump. irvingi* n.sp. Stentiford, Ross, Kerr, Bass, Bateman

Description as for genus *Paradoxiump*. Unikaryotic, oval shaped mature spores with mean length 1.93 µm (± 0.2 µm SE) and width 1.07 µm (± 0.1 µm SE) ($n = 30$). Spore contains six to eight coils of an isofilar polar filament. Posterior apex of mature spores decorated with cluster of bristles (not observed in all spores due to focal anchoring of the bristles to posterior apex). Distinctive partial SSU rRNA gene sequence from closest branching neighbours *Cucumispora* spp. and *T. butleri*.

4.3. Type host: Pink shrimp (*Pandalus montagui*) (Decapoda: Pandalidae)

Site of infection: Infection located in skeletal musculature, heart myofibres and longitudinal and circular muscles surrounding the gut.

4.4. Type locality: The Wash, North Sea (53.1417 N, 0.555 W)

Etymology: The generic epithet *Paradoxiump* is derived from the relative placement of the parasite described herein to its closest phylogenetic relative *T. butleri* (infecting the sister taxon *P. jordani*). It notes that despite this relative placement, phylogenetic and morphological similarity to *T. butleri* is low. Further, the parasite described herein infects a host which co-habits with the type host (*C. crangon*) for infection with type *Thelohania* (*T. giardi*), although again, it does not resemble it morphologically. Finally, since no phylogenetic information is available for *T. giardi*, we are only able to compare the novel parasite herein with *T. butleri* (a previously proposed surrogate for *T. giardi*, Brown and Adamson, 2006). Given its dissimilarity to *T. butleri*, the parasite described herein thus creates a taxonomic paradox which justifies erection of a novel genus to contain it. The specific epithet '*irvingi*' is an honorary designation relating to Dr. Stephen Irving, retired Director of the Aquatic Health and Hygiene Division at the Centre for Environment, Fisheries and Aquaculture Science (Cefas). It marks his significant contributions to aquatic animal health research over his career at Cefas (See Fig. 7).

Type material: Histological sections and TEM resin blocks from the UK specimens are deposited in the Registry of Aquatic Pathology (RAP) at the Cefas Weymouth Laboratory, UK. *P. irvingi* SSU rRNA gene sequences from samples collected in the United Kingdom have been deposited in Gen-Bank (accession numbers to be assigned).

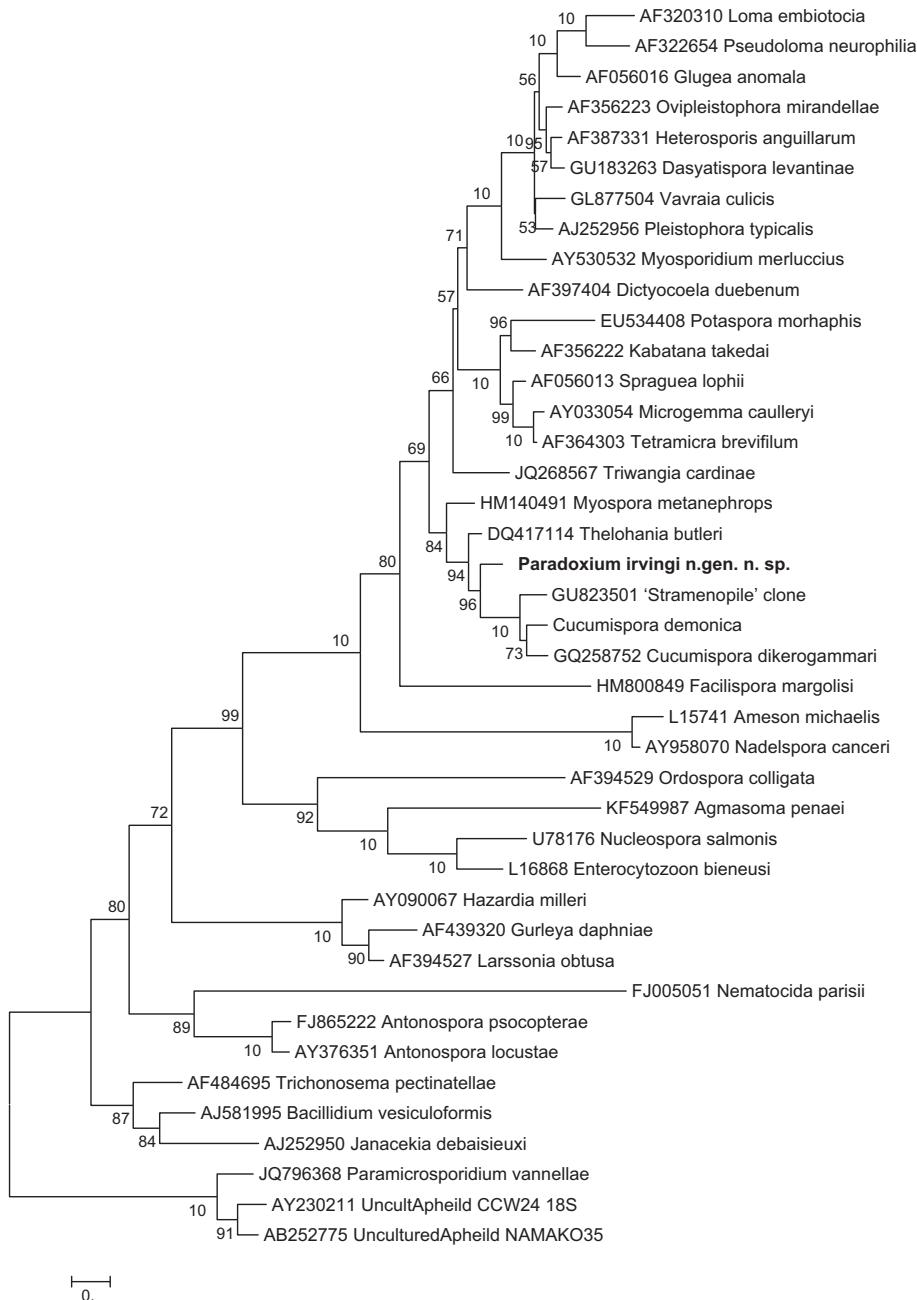


Fig. 7. Maximum likelihood tree containing *Paradoxium irvingi* n.gen., n.sp. (this study) and other microsporidian SSU sequences from GenBank. Representative sequences from each clade 1–5 (Vossbrinck and Debrunner-Vossbrinck, 2005) are included. Bootstrap value of greater than 70% are shown. The scale bar represents substitutions per nucleotide site.

5. Discussion

In this study we describe the type species of a novel genus of microsporidian parasite infecting the marine pandalid shrimp, *P. montagui*, from European waters. The parasite is herein named as *P. irvingi* n.gen., n.sp. based upon host ecology, histological and ultrastructural characteristics, and on sequencing of a partial fragment of the SSU rRNA gene of the parasite. The novel microsporidium is morphologically and phylogenetically dissimilar to its nearest phylogenetic branch relative *T. butleri* (Brown and Adamson, 2006). Furthermore, it is morphologically distinct from the type species of the genus *Thelohania*, *T. giardi* infecting European brown shrimp *C. crangon* (Henneguy and Thélohan, 1892; Hazard, 1976). It also clusters with three other muscle-infecting microsporidians from crustacean hosts

(*M. metanephrops* in lobsters and two *Cucumispora* sp. in gammarid amphipods). It is worthy to note that despite this clustering, described life cycle characters of these genera do not include development within a sporophorous vesicle typical of *Thelohania* (Stentiford et al., 2010; Ovcharenko et al., 2010; Bojko et al., 2015), providing further evidence for potentially significant morphological plasticity in closely related microsporidian parasites infecting the musculature of arthropods (Stentiford et al., 2013b). Alternatively, currently undiscovered life-cycle variants (e.g. in other hosts) may exist. Taken together with the fact that *P. irvingi* groups with two other pathogens which develop in direct contact with the host cytoplasm (*M. metanephrops*, *C. dikerogammari*) and one that does not (*T. butleri*), and that phylogenetic information pertaining to the type taxon *T. giardi* is not currently available, it is conceivable that octosporous development (itself a

pleiomorphic trait within the phylum) is impractical for defining members of the genus *Thelohania*. To this end, it cannot be stated with certainty that *T. butleri* represents a true close relative of *T. giardia*, as proposed in Brown and Adamson (2006). Clarity on this issue will only occur with re-discovery and genotyping of *T. giardi* infecting the type host from European waters.

We observed the formation of distinct tubular bristles within the sporont cytoplasm of *P. irvingi*. Further, it appears that these bristles later form a tuft which emerges from the posterior pole of the sporont and is maintained during sporoblast and spore maturation. Ornamentation has been observed in numerous described in numerous microsporidian taxa including *Ameson*, *Anncaliia*, *Areospora*, *Hirsutosporos*, *Janecekia*, *Larssonella*, *Ringueletium*, *Tabanispora* and *Trichocystosporea* (see Stentiford et al., 2014 for context). In other genera including *Tuzetia*, *Trichotuzetia* and *Nudispora*, similar fibrils have been shown to form during sporogony and are apparently inherited by the mature spore; much like that observed in the current study (in Vávra and Lukeš, 2013). These authors have proposed that spore ornamentation is a relatively common trait in microsporidians from aquatic hosts and may aid transmission by increasing spore buoyancy whilst in the water column. Further, it may be a necessity where multi-trophic transmission is required for completion of the parasite life cycle. At present, we know nothing about the transmission dynamics of *P. irvingi* and as such, alternative hosts, and alternative life cycle characters cannot be ruled out.

Acknowledgments

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