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Characterization of microsporidian *Ameson herrnkindi* sp. nov. infecting Caribbean spiny lobsters *Panulirus argus*

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ABSTRACT: The Caribbean spiny lobster *Panulirus argus* supports a large and valuable fishery in the Caribbean Sea. In 2007–2008, a rare microsporidian parasite with spore characteristics typical of the *Ameson* genus was detected in 2 spiny lobsters from southeast Florida (FL). However, the parasite species was not confirmed by molecular analyses. To address this deficiency, reported here are structural and molecular data on single lobsters displaying comparable 'cotton-like' abdominal muscle containing ovoid microsporidian spores found at different locations in FL in 2014 and 2018 and in Saint Kitts and Nevis Islands in 2017. In the lobster from 2014, multiple life stages consistent with an *Ameson*-like monokaryotic microsporidian were detected by transmission electron microscopy. A partial (1228 bp) small subunit (SSU) rRNA gene sequence showed each microsporidia to be identical and positioned it closest phylogenetically to *Ameson pulvis* in a highly supported clade also containing *A. michaelis*, *A. metacarcini*, *A. portunus*, and *Nadelspora canceri*. Using ecological, pathological, ultrastructural, and molecular data, the *P. argus* microsporidian has been assigned to a distinct species: *Ameson herrnkindi*.

KEY WORDS: Crustacea · Disease · Microspora · Parasite

1. INTRODUCTION

The Caribbean spiny lobster *Panulirus argus* is found from North Carolina (USA) to Brazil and throughout the Caribbean basin. It supports important fisheries across much of its range. In Florida (FL), *P. argus* is the most valuable fishery, with the majority landed in the Florida Keys. In the Caribbean region, spiny lobster landings can exceed US $630 M ex-vessel price annually (FAO 2017) and the fishery supports a broader economy in artisanal fishing (e.g. boat builders, fish houses, fishing gear suppliers). Although regulations vary across this region, spiny lobsters are harvested both commercially and recreationally using traps, by hand with a hook or net by divers, or using aggregation devices commonly called 'casitas' (Gutzler et al. 2015).

Despite the size and value of the *P. argus* fishery driving a great deal of research, only a few pathogens and diseases have been reported (Shields 2011). In the mid-1970s, a microsporidian was found in a single female spiny lobster in FL (Bach & Beardsley 1976). Microsporidia comprise a phylum of spore-forming unicellular parasites that inhabit all major

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biomes. They can infect and cause disease in both vertebrate and invertebrate hosts (Canning & Vávra 2000, Smith 2009, Stentiford et al. 2016), and are regarded as one of the most prevalent and pathogenic parasites of many aquatic species — particularly freshwater and marine crustaceans (Sparks 1985, Meyers, 1990, Morado 2011, Stentiford et al. 2013a). However, they are rarely found in lobsters (Stentiford et al. 2010, Shields 2011).

In hosts harboring muscle-infecting species, microsporidia infection most commonly causes the abdomen of crustaceans to appear opaque or white with the underlying muscle tissue having a 'cooked' or 'cotton-like' appearance (Lightner 1988, Edgerton et al. 2002, Morado 2011). Advanced infections typically result in the host becoming lethargic and muscle tissue being replaced by masses of parasite stages that render its meat inedible or blight its esthetics, thus compromising its market value (Stentiford et al. 2016).

In the diseased spiny lobster reported by Bach & Beardsley (1976), abdominal musculature was described as being milky white in color, consistent with advanced microsporidia infection. Histology and transmission electron microscopy (TEM) have more recently been used to demonstrate the presence of microsporidian spores with characteristics of the genus *Ameson* in 2 similarly affected spiny lobsters from the same region (Kiryu et al. 2009). As single spiny lobster specimens displaying cotton-like abdominal muscle caused by advanced microsporidian infection have since been found in Key Largo and near Miami, FL, and in the St Kitts and Nevis islands (SKNI), an opportunity appeared to undertake a molecular analysis to identify the species involved. Reported here are histopathology, spore ultrastructure, and phylogenetic data on a partial (1124 bp) small subunit (SSU) rRNA gene sequence showing each microsporidia to be identical and representing a distinct new species, *Ameson herrnkindi*, related most closely to *A. pulvis*.

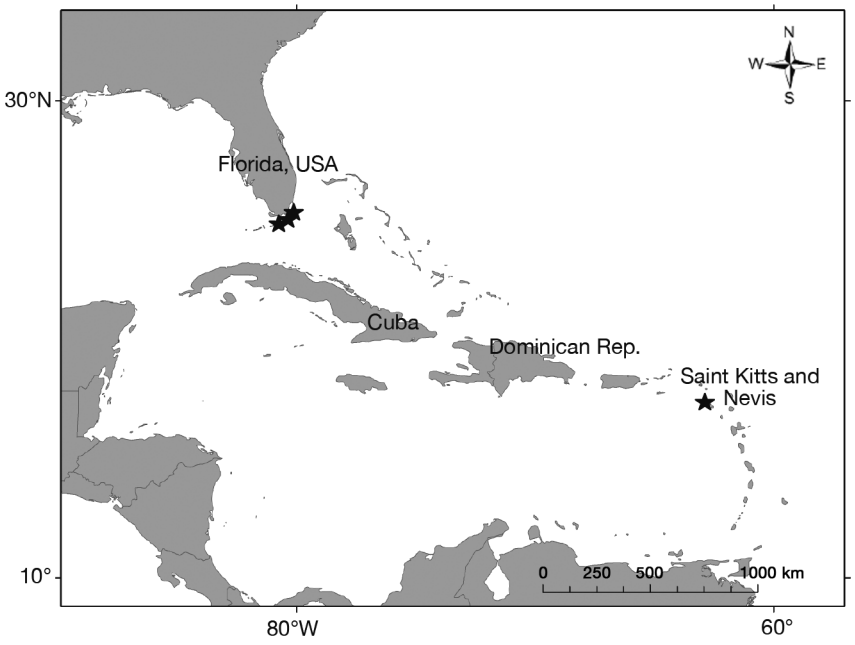

Fig. 1. South Florida, USA, and Caribbean basin showing the Florida and Saint Kitts and Nevis islands locations from which the *Ameson herrnkindi*-infected *Panulirus argus* originated

2. MATERIALS AND METHODS

2.1. Samples

In March 2014, an infected spiny lobster was captured by divers near Grecian Rocks off the coast of Key Largo, FL, USA (Fig. 1). The tail muscle was abnormally white and half of the abdomen was shipped on ice to the Virginia Institute of Marine Science (VIMS). Upon receipt, tissue samples were preserved for histology, TEM, and DNA analysis. In September 2017, another spiny lobster displaying the same abdominal muscle discoloration was identified during a general health screen of lobsters from waters surrounding SKNI. Fresh wet mounts of tissues revealed the presence of high numbers of parasite microspores, and tissues were preserved for histology and DNA analysis. In addition, an archived frozen muscle sample from an earlier case (December 2007, Pompano Beach, FL; Kiryu et al. 2009) and an ethanol-preserved muscle sample from an infected lobster caught from waters off Miami, FL, in April 2018 were also provided to VIMS for DNA analysis.

2.2. Histology

For the spiny lobster processed for histology in 2014 (FL-2014), small pieces of abdominal muscle were fixed in Bouin's solution (Fisher Scientific) for 48 h, rinsed in tap water and transferred to 70% EtOH. Tissue sections were processed using routine histological techniques and

stained with Harris's hematoxylin and eosin (H&E; Humason, 1979). For the spiny lobster processed for histology in 2017 (SKNI-2017), small pieces of abdominal muscle, gills, heart, ovary, antennal gland, gut, hepatopancreas, and eyestalk were excised from and fixed in Davidson's seawater fixative for 48 h. Samples were processed as above and sections were stained with H&E. A subset of sections were also stained with 1% Uvitex 2B (Koch & Pimsler 1987) for 10 min between H&E staining steps to better visualize spores under UV light. Tissue sections were examined using an Olympus BX51 light microscope and images were captured using a DP73 camera and cellSens software (Olympus).

2.3. TEM

To prepare tissues for TEM, small pieces of affected abdominal muscle from one spiny lobster (FL-2014) were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at 4°C for 48 h. Fixed samples were placed in 0.1 M sodium cacodylate buffer pH 7.4 and shipped to the Centre for Environment, Fisheries, and Aquaculture Science (Cefas), Weymouth Laboratory, UK. Fixed tissue samples were further processed as described previously (Small et al. 2014). Samples were embedded in Epoxy Resin Agar 100 (Agar Scientific, Agar 100 pre-mix kit, medium) and polymerized overnight at 60°C. Ultrathin sections (70 to 90 nm) were mounted on uncoated copper grids and stained with uranyl acetate and Reynolds' lead citrate (Reynolds 1963). Grids were examined using a JEOL JEM 1400 transmission electron microscope and digital images captured using an Advanced Microscopy Techniques (AMT) XR80 camera and SAMT V602 software.

2.4. DNA extraction and SSU rRNA gene amplification

DNA was extracted from 4 ethanol-preserved muscle samples. Briefly, tissues were placed in sterile deionized water for 30 min to remove residual ethanol, transferred to a 1.5 ml microcentrifuge tube containing 180 µl ATL buffer and homogenized using a micro pestle (Sigma-Aldrich). DNA was extracted using a DNeasy Blood & Tissue Kit (QIAGEN) following the manufacturer's recommended protocol, and was eluted in 100 µl AE buffer and stored at −20°C. A fragment of the SSU rRNA gene of microsporidia was amplified by PCR using the primers V1 (5′-CAC CAG GTT GAT TCT GCC TGA C-3′) and 1492R (5′-GGT TAC CTT GTT ACG ACT T-3′) (Zhu et al. 1993). PCR reagent concentrations and thermal cycling conditions were as described previously (Small et al. 2014). Reactions were amplified in duplicate for each sample.

2.5. Cloning, sequencing, and phylogenetic analysis

An aliquot (10 µl) of each PCR was analyzed by agarose gel electrophoresis. The remainder of each duplicate PCR was combined for each sample and DNA was purified using a QIAquick PCR Purification Kit (QIAGEN). DNA products from the FL-2007 and FL-2014 lobsters were cloned and sequenced essentially as described previously (Small et al. 2014). Three clones for each sample were bidirectionally sequenced using an ABI 3130 genetic analyzer (Applied Biosystems). DNA products from the SKNI-2017 and FL-2018 lobsters (SKNI and Miami, FL, respectively) were sequenced directly using the V1 and 1492R PCR primers. Aliquots (10 µl) of each reaction were sequenced using the ABI 3130 genetic analyzer. Two forward and reverse direct sequencing reactions were analyzed for each sample.

The partial SSU rRNA gene sequences of the *Panulirus argus* microsporidians were imported into Sequencher (version 5.1), trimmed of vector and primer sequences, and consensus sequences were aligned using MacVector (version 15.1.5). The sequences were deposited in GenBank with Accession numbers MN190182–MN190185. BLAST searches of the NCBI database (www.ncbi.nlm.nih.gov) were performed using MacVector. SSU rRNA sequences from microsporidia with high similarity scores, as well as those from others infecting aquatic hosts were aligned using CLUSTALW in MacVector using the default gap settings for multiple and pairwise alignment. Maximum-likelihood analysis was performed using MEGA6 (Tamura et al. 2013). A partial SSU rRNA sequence from *Kneallhazia solenopsae* (AF031538) was used as an outgroup. The robustness of resulting trees was tested using 1000 bootstrap replicates. The aligned fragment was 1124 bp in length. Genetic distance (uncorrected '*p*') calculations were performed using MEGA6 on an alignment of a trimmed (895 bp) SSU rRNA gene sequence of *Ameson pulvis*, *A. michaelis*, *A. metacarcini*, *A. portunus* and the microsporidia detected in *P. argus*.

3. RESULTS

3.1. Gross presentation, pathology, and ultrastructure

The abdominal musculature of infected spiny lobsters displayed a 'cooked' or 'cotton-like' appearance (Fig. 2). Muscle smears and histological tissue sections revealed the presence of masses of ovoid microsporidian spores (approximately 1–2 µm in dimensions) interspersed between and within muscle fibers (Fig. 3A,B). In the most seriously affected lobster, multifocal coagulative necrosis and hemocyte infiltrations similar to those reported previously (Kiryu et al. 2009) were evident in abdominal muscle (Fig. 3B); however, samples from the lobster collected in 2014 were not fixed until >24 h after death, and thus the coagulative necrosis was possibly due to postmortem degradation. Transverse sections of abdominal muscle bundles varied in infection severity of the microsporidian from light to moderate to heavy (Fig. 3C–E). Uvitex 2B-stained spores were easily visualized in muscle tissues under UV light (Fig. 3D,F) as this stain binds chitin in the spore wall. In the tissues from the SKNI lobster (SKNI-2017), spores were also observed in the retractor muscles of the eyestalk, but not in connective or epithelial tissues of the antennal gland, ovary, gills, gut, hepatopancreas, cuticle, or in cardiac muscle.

Fig. 2. Gross appearance of abdominal muscles of uninfected and *Ameson herrnkindi*-infected *Panulirus argus*. Note the 'cooked' or 'cotton-like' abdominal muscle appearance of the infected lobster (right). Scale bar = 3 cm

Despite tissues from lobster FL-2014 being fixed sub-optimally, TEM revealed multiple microsporidian life stages in direct contact with the cytoplasm of host muscle cells. The earliest stage observed was a uninucleate meront (Fig. 4A), with stages transitioning from a diplokaryotic meront to an early sporont identified by increased vacuolation and thickening of the spore wall (Fig. 4B). Cytoplasmic cisternae, thought to be an early precursor of the polar filament, were observed adjacent to the nucleus. Developing early sporont stages with a short chain-like appearance and thicker electron-dense cell wall were also observed. Early precursors of the spore extrusion apparatus (e.g. polar filament and associated complex) were observed in large electron-lucent vacuoles within both sporonts and early sporoblasts that had undergone cytokinesis (Fig. 4C). Early sporoblasts displayed an increase in the numbers of turns and peripheral arrangement of the polar filament within a single large vacuole (Fig. 4D). Maturing sporoblasts were observed to become increasingly electron-dense with a more ordered peripheral arrangement of the polar filament coils (Fig. 5A,B). Late-stage sporoblasts were elongate, with an electron-dense cytoplasm, and had an early form of a trilaminar wall (Fig. 5C). Fully developed spores were approximately 1.6 × 1.1 µm in size and had 8–9 turns of a polar filament arranged mainly in 1 linear outer rank but occasionally in 2 (Fig. 5D). Spores displayed a laminar polaroplast, electron-dense cytoplasm, anchoring disk, and a trilaminar spore wall comprised of an inner plasmalemma, a thick electron-lucent endospore, and an electron-dense exospore. Host microtubules were observed in close proximity to the developing sporoblast and spore stages. Unlike some *Ameson* species, no exospore ornamentation was observed.

3.2. Molecular phylogeny

The partial (1154 bp) SSU rRNA gene sequence from the FL (FL-2007, FL-2014, and FL-2018) and SKNI-2017 lobsters was identical. BLAST analysis indicated a relationship with other *Ameson* spp. infecting marine crustaceans (Table 1). Phylogenetic analysis positioned the *Panulirus argus* microsporidian closest to *A. pulvis*, and within a highly supported (bootstrap support = 100) clade containing *A. michaelis*, *A. portunus*, *A. metacarcini*, and *Nadelspora canceri*. (Fig. 6). Genetic distance values of a trimmed 895 bp SSU rRNA gene sequence showed the *P. argus* microsporidian (0.012–0.047) to be similarly

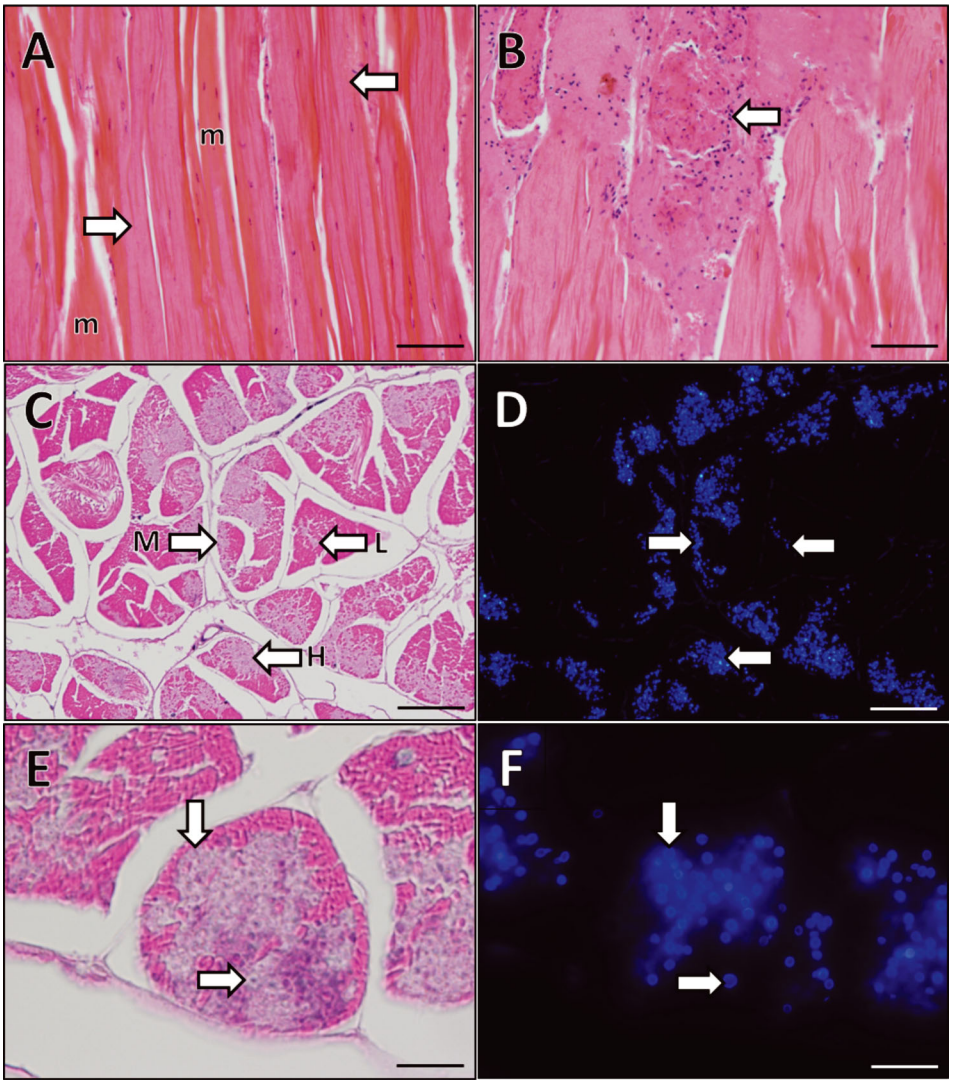

Fig. 3. Histopathology seen in *Panulirus argus* abdominal muscle infected with *Ameson herrnkindi* sp. nov. Longitudinal abdominal muscle sections showing (A) masses of parasite spores (light pink, arrows) interspersed among muscle fibers (dark pink, m) and (B) localized areas of coagulative necrosis (arrow) and postmortem artefacts. Transverse abdominal muscle sections showing (C) muscle bundles with light (L), moderate (M), and heavy (H) infections (arrows) and (D) viewed using UV light and DAPI filter to show numerous spores (blue, arrows) localized throughout the muscle bundles. Transverse section of heavily infected abdominal muscle showing (E) parasites occupying almost the entire muscle bundle (arrows) and (F) viewed using UV light and DAPI filter to see stained cell walls of individual spores (blue, arrows). Scale bars = (A,B) 100 µm, (C,D) 50 µm, (E,F) 10 µm

distant from the other *Ameson* species assessed as any of these species were to each other (0.014–0.072; Table 2).

3.3. Taxonomic summary

Type species: *Ameson herrnkindi* sp. nov.
Description: Parasite stages infecting the abdominal muscle and eyestalk retractor muscle of a tropical to subtropical marine crustacean. Monokaryotic ovoid spores measuring approximately 1.6 × 1.1 µm in tissue fixed for electron microscopy. Mature spores contained 8–9 turns of a polar filament with the majority forming one linear outer rank and the remainder oriented towards the center of the spore. Observed stages suggest a life cycle that progresses from a uninucleate meront to a diplokaryotic meront, chain-like quadranucleate sporonts, with cytokinesis to produce individual sporoblasts which develop into microspores.
Type host: *Panulirus argus* (Latreille 1804)
Type locality: Atlantic coast of FL, USA (multiple locations), and SKNI.
Site of infection: Striated muscle tissues.
Etymology: The species is named in honor of Dr. William F. Herrnkind, a specialist in the ecology and behavior of *P. argus*.
Type material: Syntype specimens of stained histological sections have been deposited with the Registry of Aquatic Pathology at the Cefas Weymouth Laboratory, UK (www.cefas.co.uk/cefas-data-hub/registry-of-aquatic-pathology/). The partial SSU rRNA gene sequences obtained from *A. herrnkindi* have been deposited in GenBank with Accession numbers MN190182-MN190185.

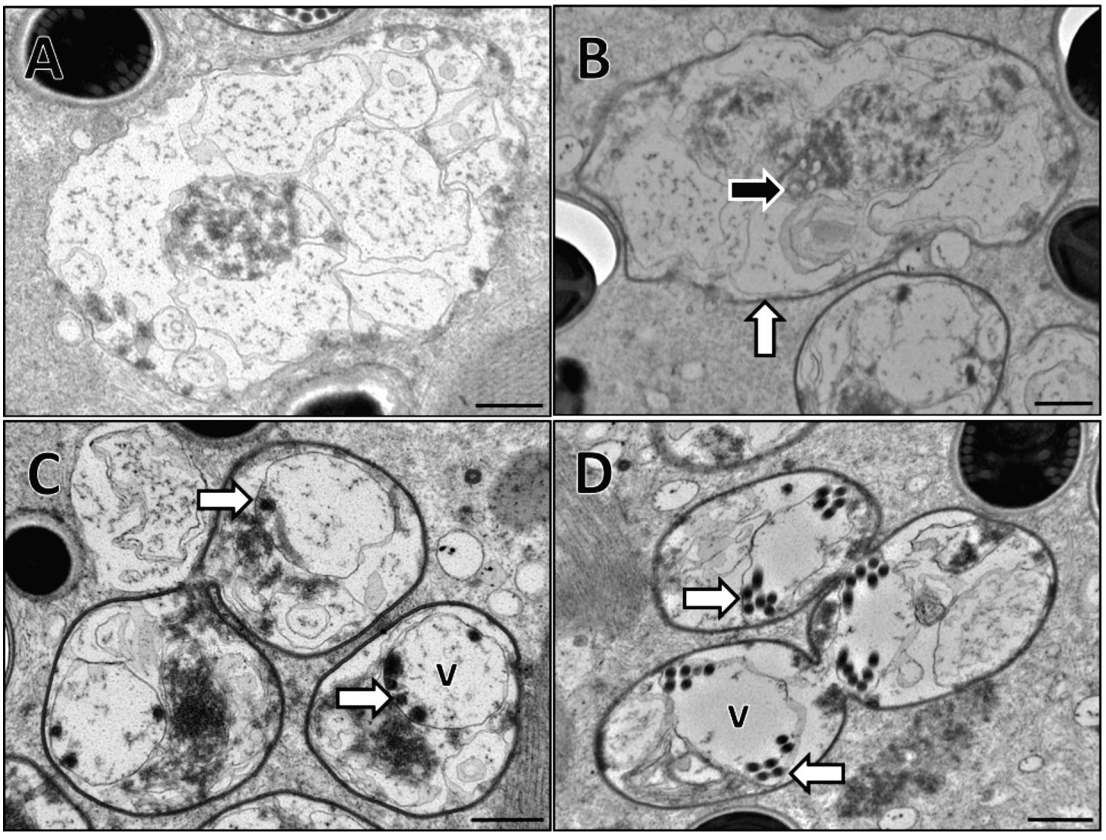

Fig. 4. Transmission electron microscopy showing *Ameson herrnkindi* meronts and early sporogony in abdominal muscle cells of *Panulirus argus*. (A) uninucleate meront; (B) diplokaryotic sporont with relatively electron-dense membrane (white arrow) and cytoplasmic cisternae (black arrow); (C) initial phase of cytokinesis of sporonts and a single early sporoblast, with spore extrusion precursors, such as a nascent polar tube (arrows), visible within a large electron-lucent vacuole (v); (D) early sporoblasts displaying an increased number of turns and a more peripheral arrangement of the polar filament (arrows) within a single large vacuole (v). Scale bars = 500 nm

4. DISCUSSION

Described here are ecological, histological, structural, and molecular data identifying a new microsporidian species, *Ameson herrnkindi*, of spiny lobsters *Panulirus argus* inhabiting FL and SKNI. Histology and TEM showed that the parasite infected skeletal muscle and eyestalk retractor muscle cells but not cardiac muscle or myoepithelial cells and possessed developing and mature spore morphologies similar to *Ameson* spp. found in other decapod crustaceans. Phylogenetic analysis of a partial SSU rRNA gene sequence aligned *A. herrnkindi* most closely to *A. pulvis* in a clade also containing *A. michaelis*, *A. metacarcini*, *A. portunus*, and *Nadelspora canceri*, species that, to date, have only been detected in portunid and cancrid crabs.

Due in part to the infrequency of spiny lobsters with 'cotton-like' abdominal muscle being found and/or noted to authorities and the *A. herrnkindi* samples available for analysis coming for different sources, tissues from only a single spiny lobster from FL (FL-2014) were available for TEM. Despite tissues from this lobster being fixed postmortem, uninucleate meronts, diplokaryotic meronts (likely formed by the division of the uninucleate meront), and both binucleate and chain-like tetranucleate sporonts were observed, the former more commonly, suggesting that the parasite life cycle might involve both Developmental Pathways I (uninucleate meront elongation and division to form chain-like sporonts) and II (diplokaryotic meront division followed by cytokinesis to form binucleate sporonts; Wang et al. 2017). Alternatively, the observation of binucleate sporont structures might be from preseparation of a third sporoblast from a chain-like form or sectioning artefact. Precursors of the polar filament were clearly observed within electron-lucent vacuoles in uninucleate, binucleate, and chain-like sporonts prior to them maturing and separating to form mature spores.

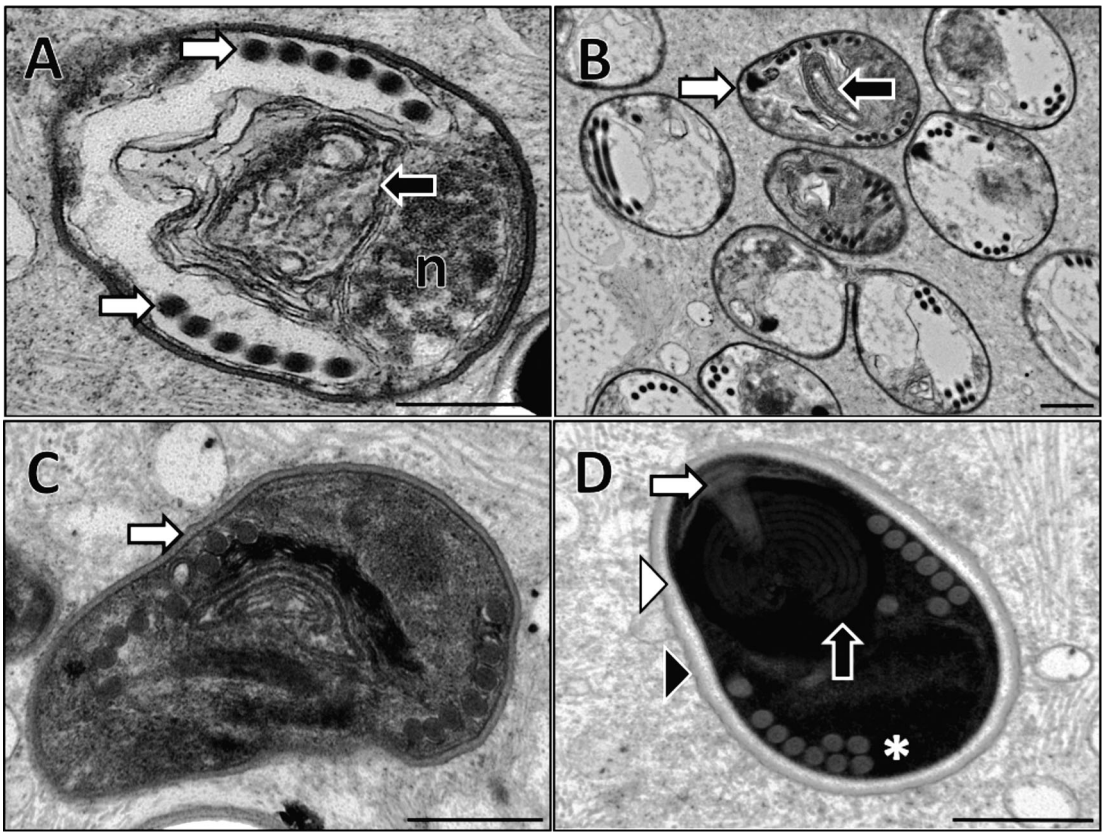

Fig. 5. Transmission electron microscopy of *Ameson herrnkindi* late-stage sporogony in abdominal muscles of *Panulirus argus*. (A) Maturing sporoblast displaying a peripheral arrangement of polar filament coils (white arrows), a single nucleus (n), and nascent polaroplast (black arrow); (B) late sporoblast (white arrow) displaying an electron-dense cytoplasm and a developing polaroplast (black arrow); (C) immature spore with an electron-dense cytoplasm and early formation of a trilaminar wall (arrow); (D) mature spore displaying approximately 8–9 turns of the polar filament (asterisk), laminar polaroplast (black arrow), electron-dense cytoplasm, anchoring disk (white arrow) and trilaminar spore wall comprised of an inner plasmalemma, a thick electron-lucent endospore (white arrow head), and thin electron-dense exospore (black arrow head). Scale bars = 500 nm

Collectively, the morphological characteristics of the microsporidian life stages observed were similar to those of other *Ameson* species (Sprague 1965, Weidner 1970, Vivarès & Sprague 1979, Stentiford et al. 2013b, Small et al. 2014, Wang et al. 2017).

A. herrnkindi sporogony progressed in a manner similar to other *Ameson* species (Sprague 1965, Vivarès & Azevedo 1988, Stentiford et al. 2013b, Small et al. 2014, Wang et al. 2017). In liberated sporoblasts, the spore extrusion apparatus was contained within a single vacuole and observed to migrate to peripheral positions within mature spores. As sporoblasts matured they became increasingly electron-dense with a developing laminar polaroplast and trilaminar spore wall. Mature *A. herrnkindi* spores did not possess microvilli (hair-like protrusions) that have been described on spores of other *Ameson* species (Sprague et al. 1968, Vivarès & Azevedo 1988, Stentiford et al. 2013b, Small et al. 2014, Wang et al. 2017). However, this might be a postmortem artefact (due to delayed fixation), as Kiryu et al. (2009) showed that the same parasite possessed microvilli extending from the surface of the exospore.

Mature spores (1.6 × 1.1 µm) were most similar in size to *A. michaelis* found in *Callinectes sapidus* (1.6 × 1.2 µm; Sprague 1965, Sprague et al. 1968) and *A. portunus* found in *Portunus trituberculatus* in China (1.4 × 1.2 µm; Wang et al. 2017) (Table 1). Kiryu et al. (2009) found that mature spores of what is likely *A. herrnkindi* ranged from 1.2 to 1.6 µm in length and 0.8 to 1.4 µm in width, similar to the spore dimensions of *A. pulvis*, *A. michaelis*, and *A. portunus*. Due to the size variations in mature spores formed by these species, spore size alone cannot be used for definitive species identification.

Although microsporidia have traditionally been classified based on their nuclear configurations, life cycle, and ultrastructural features, more recent ap-

Table 1. Ecological and morphological data on known described *Ameson* spp. that infect skeletal muscle of marine decapod crustaceans. TEM: transmission electron microscopy; PFCs: polar filament coils

Name	Location	Host(s)	Spore size (µm, TEM)	No. of PFCs	Reference(s)
A. pulvis	Near Montpellier and Arcachon, France	*Carcinus maenas*, *C. mediterraneus*	1.3 × 1.0	8	Perez (1905), Vivarès & Sprague (1979)
	South coast England, UK	*C. maenas*	1.2 × 1.0[a]	8–9	Stentiford et al. (2013b)
A. michaelis	Patuxent River and Chesapeake Bay, USA	*Callinectes sapidus*	1.6 × 1.2	11	Sprague (1965, 1970), Sprague et al. (1968)
A. atlanticum	Northern Brittany, France	*Cancer pagurus*	1.9 × 1.5	12	Vivarès & Azevedo (1988)
A. metacarcini	British Columbia, Canada	*Metacarcinus magister*	1.8 × 1.4	9–12	Small et al. (2014)
A. portunus	Jiangsu Province, China	*Portunus trituberculatus*	1.4 × 1.0	8–9	Wang et al. (2017)
Ameson sp.	Moreton Bay, Australia	*P. armatus*	1.3 × 1.0	6–8	Shields & Wood (1991), Sumpton (1994), J. D. Shields (unpubl. data)
Ameson sp.	Florida, USA	*Panulirus argus*	1.4 × 1.0	7–8	Kiryu et al. (2009)
Ameson sp.	Sea of Okhotsk, Russia	*Paralithodes camtschaticus*, *P. platypus*	1.6 × 1.2	8–10	Ryazanova & Eliseikina (2010)

[a]Only ovoid spore dimensions are given

proaches have included their pathology (site of infection), ecology (host and geographic location), morphology (ultrastructure, life cycle), and gene sequences (Vossbrinck & Debrunner-Vossbrinck 2005, Stentiford et al. 2010, 2013b, Small et al. 2014, Wang et al. 2017). The combined ecological, pathological, and morphological features of the spiny lobster microsporidian placed it within the genus *Ameson*. However, its presence in *P. argus* in subtropical (Pompano Beach) and tropical waters of FL and the Caribbean (SKNI) differentiates *A. herrnkindi* from other *Ameson* species detected to date in portunid and cancrid crabs from temperate waters (see Table 1), and host specificity tends to be very high in this taxon. Phylogenetic analysis clearly placed *A. herrnkindi* within the genus *Ameson*, most closely related to *A. pulvis* detected in *Carcinus maenas* crabs in Europe, and within a highly supported clade containing *A. metacarcini*, *A. michaelis*, *A. portunus*, and *Nadelspora canceri* (all infecting crabs from the Americas and Asia).

The presence of *N. canceri*, which forms elongated needle-like spores, within the *Ameson* spp. clade has been discussed previously (Stentiford et al. 2013b, Small et al. 2014). Although there is potential for synonymy between these morphologically divergent genera (Stentiford et al. 2013b), no needle-like spores were observed in tissues, including cardiac muscle, in the single *P. argus* examined. Spore morphological plasticity of this and other *Ameson* spp. thus remain to be investigated.

A. herrnkindi appears to be a rare parasite of *P. argus*, with only 6 infected individuals formally reported to date (Bach & Beardsley 1976, Kiryu et al. 2009). This is not unusual, as microsporidia of wild crustaceans are usually found at relatively low prevalence (Shields et al. 2015). However, aquaculture can result in a substantially higher prevalence (Wang et al. 2017). Here, and in the study of Kiryu et al. (2009), the lobsters examined from FL were caught by recreational fishers and only identified as abnormal (discolored abdominal muscle tissue) once the abdomen was removed from the cephalothorax in a practice termed 'wringing'. As recreational fishers may well discard and not report abnormally colored tails, light infections, which may be the norm in crustaceans, would largely go unnoticed. Likewise, the infected lobster from SKNI was only identified as abnormal during necropsy. Lobsters harvested commercially are required to be landed whole in FL and are desired whole by consumers in the Caribbean and in the global live-lobster market. As such, it is likely that the majority of infections go undetected or unreported. However, the parasite has not been observed in sur-

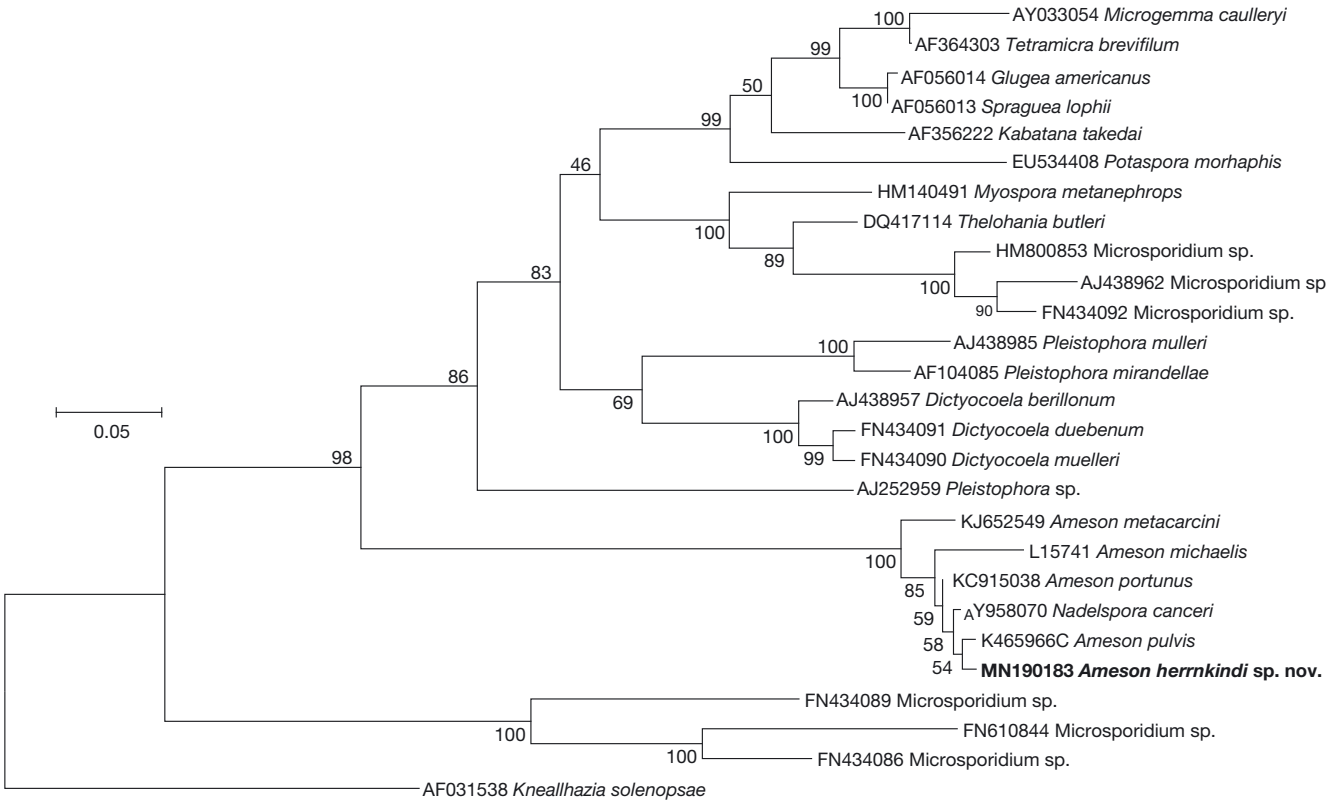

Fig. 6. Maximum-likelihood tree of a CLUSTALW multiple alignment of a 1124 bp small subunit (SSU) rRNA gene sequence of *Ameson herrnkindi* sp. nov. (in **bold**) and other aquatic microsporidia. *Kneallhazia solenopsae* was used as the outgroup. Numbers at nodes represent bootstrap support values for each clade. Sequences were analyzed using the Tamura-Nei model of evolution in the MEGA6 software

Table 2. Genetic distance (uncorrected '*p*') values between 895 bp SSU rRNA sequences from the microsporidian infecting *P. argus* (in **bold**) and other formallydescribed species of *Ameson*

Species (GenBank accession no.)	Table ID	1	2	3	4	5
A. herrnkindi sp. nov. (MN190183)	1	–				
A. pulvis (KC465966)	2	**0.012**	–			
A. michaelis (L15741)	3	**0.047**	0.051	–		
A. portunus (KC915038)	4	**0.014**	0.014	0.040	–	
A. metacarcini (KJ652546)	5	**0.047**	0.050	0.072	0.043	–

veys of several hundred juvenile (<40 mm carapace length) spiny lobsters for *Panulirus argus* virus 1 (PaV1) from the Florida Keys and Caribbean (Shields & Behringer 2004, Behringer et al. 2012, Moss et al. 2013). Other than as reported here, the distribution of *A. herrnkindi* remains unknown. Five of 6 reported cases are from lobsters inhabiting locations along the Atlantic coast of FL and suggest that *A. herrnkindi* is localized to FL, but the case from SKNI, ~2000 km from FL, suggests a Caribbean-wide distribution.

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