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Kudoa hypoepicardialis and associated cardiac lesions in invasive red lionfish *Pterois volitans* in Grenada, West Indies

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ABSTRACT: Invasive red lionfish *Pterois volitans* (Linnaeus, 1758) represent an ongoing ecological threat within temperate and tropical waters. Relatively little is known regarding the overall health of *P. volitans* and their potential for spreading pathogens in non-native regions. Lionfish collected from inshore reefs of Grenada, West Indies, in 2019 and 2021 were identified as *P. volitans* based on cytochrome *c* oxidase subunit 1 barcoding. Gross and microscopic examination of tissues revealed myxozoan plasmodia in the hearts of 24/76 (31.6%) lionfish by histopathology or wet mount cytology. Further histopathologic examination revealed severe granulomatous inflammation and myofiber necrosis associated with developing plasmodia and presporogonic life stages. Fresh myxospores were morphologically and molecularly consistent with *Kudoa hypoepicardialis*, being quadrate in apical view with 4 valves and 4 equal polar capsules. The spore body was 5.1–7.9 (mean: 6.0) µm long, 8.1–9.8 (8.7) µm wide, and 6.9–8.5 (7.7) µm thick. Polar capsules were 2.3–2.7 (2.5) µm long and 0.9–1.6 (1.3) µm wide. 18S small subunit rDNA sequences were 99.81–99.87% similar to sequence data from the original description of the species. Novel 28S large subunit rDNA and elongation factor 2 data, which did not match any previously reported species, were provided. This is the first account of a myxozoan parasite of *P. volitans*, a new host record and locality for *K. hypoepicardialis*, and one of few reports describing pathogen-associated lesions in invasive lionfish.

KEY WORDS: *Kudoa hypoepicardialis* · Lionfish · *Pterois volitans* · Myxozoan · Invasive species

1. INTRODUCTION

Red lionfish *Pterois volitans* are generalist predators native to the Indo-Pacific that have invaded marine environments along the Atlantic seaboard of the USA, the Gulf of Mexico, and the Caribbean Sea (Whitfield et al. 2002). Given a lack of natural predators in their non-native range, high fecundity, rapid growth, and generalist feeding behavior, invasive lionfish can have significant impacts on native species and marine ecosystems (Hare & Whitfield 2003, Albins & Hixon 2008, Morris & Whitfield 2009, Côté et al. 2013).

In their invasive range, parasite diversity and subsequent disease of *P. volitans* are reduced compared to native fishes and less than what is reported in their

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native Indo-Pacific habitat (Sellers et al. 2015). Accounts are largely limited to generalist parasites of endemic fish from areas *P. volitans* have invaded, indicating the acquisition of endemic parasites rather than concomitant invasion by *P. volitans* (see Bullard et al. 2011, Fogg et al. 2016). The role of parasitism, both endemic and non-native, in regulating lionfish invasion is not clear, though some authors have speculated that reduced parasite burden may contribute to the success of this invasive species (Albins & Hixon 2013). Conversely, parasites to which *P. volitans* are not well-adapted may prove more deleterious. While surveys have indicated that *P. volitans* can host diverse parasitic species from multiple phyla (Simmons 2014, Ramos-Ascherl et al. 2015, Fogg et al. 2016, Fails 2017), no myxozoan has been reported from this species to date. Only 1 myxozoan, *Sphaeromyxa zaharoni* Diamant, Whipps & Kent 2004, has been reported from a lionfish species, namely the common lionfish *P. miles* (Bennett, 1758), which was described from the gallbladders of *P. miles* and *Scorpaenopsis barbata* (Rüppell, 1838) collected from the Red Sea (Diamant et al. 2004).

Kudoa Meglitsch, 1947 is a genus of myxozoan parasites of marine and brackish water fishes. *Kudoa* spp. are of considerable economic importance due to deleterious effects on the marketability of aquacultured fishes associated with post-mortem myoliquefaction, which renders filets unmarketable (Yokoyama et al. 2012). Historically, *Kudoa* spp. were delineated into 4 discrete genera (*Pentacapsula*, *Hexacapsula*, *Septemcapsula*, and *Kudoa*) based on the number of myxospore polar capsules and shell valves, in addition to tissue tropism (Lom & Dyková 2006). These genera have since been synonymized with *Kudoa* based on phylogenetic inference using 18S small subunit rDNA (*SSU*) and 28S large subunit rDNA (*LSU*) sequences (Whipps et al. 2004). This revision left *Kudoa* as the only genus in the monotypic family Kudoidae Meglitsch 1960, with subsequent phylogenetic analyses continuing to offer robust statistical support (Wang et al. 2005, Fiala 2006, Li et al. 2020, Woodyard et al. 2020). While the majority of *Kudoa* spp. have been described from somatic muscle (Eiras et al. 2014), 7 species have been formally described from the hearts of marine fishes: *K. aegyptia* Koura, 2000; *K. hypoepicardialis* Blaylock, Bullard, & Whipps 2004; *K. iwatai* Egusa & Shiomitsu, 1983; *K. leptacanthae* Heiniger & Adlard, 2012; *K. pagrusi* Al Quraishy, Koura, Abdel-Baki, Bashtar, El Deed, Al Rasheid & Abdel-Ghaffar, 2008; *K. pericardialis* Nakajima & Egusa, 1978; and *K. shiomitsui* Egusa & Shiomitsu, 1983. Additionally, an unnamed *Kudoa* sp. was recorded from the heart of spotted coral grouper *Plectropomus maculatus* (Bloch, 1790) (Abdel-Ghaffar et al. 2012). Pathology associated with *Kudoa* infections varies by species, ranging from no discernable effects in the case of *K. shiomitsui* (Egusa & Shiomitsu 1983, Zhang et al. 2010) to considerable reduction in cardiac muscle functional area by an unnamed *Kudoa* sp. (Abdel-Ghaffar et al. 2012).

K. hypoepicardialis was first described from the hearts of 7 fish species in the Gulf of Mexico (Blaylock et al. 2004). While this description provided a robust morphological account of the species, molecular data were limited to *SSU* sequence data. No further accounts of *K. hypoepicardialis* exist in the literature. In subsequent years, *LSU* has become more prominent for delineating *Kudoa* spp., many of which are indistinguishable by *SSU* data alone (Burger & Adlard 2010).

Morphological and molecular characters of isolated myxospores collected from *P. volitans* in Grenada were consistent with *K. hypoepicardialis.* This is the first report of myxozoan parasitism of invasive *P. volitans* and suggests that infection leads to one of the few examples of disease from an endemic primary pathogen in invasive lionfish.

2. MATERIALS AND METHODS

2.1. Sample collection and histological assessment

Sample collection was performed under permit from the Grenada Ministry of Agriculture, Lands, Forestry, and the Environment (Permit 001) and with approval from the St. George's University Institutional Animal Use and Care (IACUC) Committee (18011-R).

Lionfish (n = 76 in total: 37 females, 30 males, 9 unknown) were collected from nearshore waters on the southwest coast of Grenada in 2019 and 2021 (Fig. 1). Fish were collected by scuba divers using spear poles at depths of 3–30 m as part of the regional lionfish culling program. Lionfish were placed in secure containers until completion of the dive after which they were transported on ice to the St. George's University Aquatic Animal Medicine Research Laboratory within 6 h of capture. Each fish was catalogued with its site of origin, total length, weight, and sex (based on gross observation of gonad).

Lionfish collected in January (n = 10) and August (n = 10) 2019 underwent full necropsies, including preservation of representative samples of gill, heart, liver, spleen, kidney, muscle, gastrointestinal tract,

Map Location	Date	Site name (GPS co-ordinates)	Kudoa prev. (%)
1	January 2019	Happy Hill to Black Bay (12°5'42"N, 61°46'19"W)	3/10 (30.0%)
2	August 2019	Happy Hill to Black Bay	2/10 (20.0%)
3	November 2019	Halifax Harbor 12°6'43"N, 61°44'55"W)	9/32 (28.1%)
4	April 17, 2020	Kahonee Reef (12°1'27"N, 61°47'0"W)	0/8 (0%)
5	April 20, 2020	Boss Reef (12°2'3"N, 61°45'55"W)	10/16 (62.5%)

Fig. 1. Site location and names, and prevalence of *Kudoa hypoepicardialis* in lionfish *Pterois volitans* by microscopy in the nearshore waters of Grenada, West Indies

and gonad for histopathology. Tissues were preserved in 10% buffered formalin for >72 h, processed routinely, sectioned at 4 µm thickness, and stained with hematoxylin and eosin. Select slides were further stained with Giemsa and acid-fast stains to examine myxospore morphology. Based on the high prevalence of myxozoans observed in histopathologic sections of heart, this tissue was identified as an organ of interest for future sampling. Hearts were aseptically collected from lionfish in November 2019 (n = 32) and bisected, with one half of each heart preserved for histopathology and the other half placed in 95% ethanol for PCR assay. Hearts from lionfish collected in April 2021 (n = 24) were examined by light microscopy wet mounts to measure spore morphology, and spores were washed from slides into microcentrifuge tubes in 95% ethanol for molecular analysis.

2.2. Morphological characterization of myxospores

Cysts were excised from cardiac tissue by sharp dissection under an Olympus BX53 microscope with phosphate-buffered saline added to slides to produce wet mounts. Where myxospores were detected, photomicrographs were taken with an Olympus DP74 camera attachment and associated cell Sens v. 1.18 imaging software at 1000× magnification. Measurements were documented using ImageJ v. 1.52v (Schneider et al. 2012), calibrated to the photographed scale bar, and morphological comparisons were made with previously reported *Kudoa* spp. collected from hearts.

2.3. Statistical analysis

A Shapiro-Wilk test was used to examine normality and a Brown-Forsythe test was used to examine the equality of group variances, and all data for prevalence and fish weight and length were determined to meet assumptions. A 1-way ANOVA was used to compare the prevalence of myxozoans observed by light microscopy between reef systems and to compare fish weight and length between infected and non-infected fish (GraphPad Prism 8.3.0). Statistical significance was determined when all assumptions were met and when $p \geq 0.05$ within the 1-way ANOVA.

2.4. Molecular characterization

Myxospores used for morphological characterization were rinsed from slides into 1.5 ml microcentrifuge tubes, pelleted by centrifugation (5 min at 10 000 × *g*) for genomic DNA (gDNA) extraction (Qiagen DNeasy Powersoil Kit). In addition, gDNA was isolated from ethanol-preserved cardiac muscle (Qiagen DNeasy Blood and Tissue Kit) to molecularly screen for the presence of immature plasmodia and confirm host species identity. Fragments of the myxozoan 18S *SSU*, 28S *LSU*, and the host cytochrome *c* oxidase subunit 1 mitochondrial gene (*cox*1) were amplified using previously published primers (Table 1). Each PCR consisted of 10 µl of Phusion Green Hot Start II PCR Master Mix, 1 µl of each primer, ~10 ng of DNA template, and nuclease-free water to a total volume of 20 µl. Thermal cycling conditions for myxozoan *SSU* primer pairs consisted of an initial denaturation at 98°C for 3 min, followed by 35 cycles of 98°C for 10 s, 55°C for 30 s, and 72°C for 1 min, followed by a final extension at 72°C for 10 min. Conditions for *LSU* primer pairs were the same as above but with an annealing temperature of 58°C instead of 55°C. Thermal cycling parameters for the FishF1/FishR1 host *cox*1 primer pair were as follows: initial denaturation at

Table 1. Primers used in PCR amplification and sequencing of partial 18S small subunit rDNA (*SSU*), partial 28S large subunit rDNA (*LSU*), and partial elongation factor 2 (*EF2*) parasite sequences and partial cytochrome *c* oxidase subunit 1 (*cox*1) mitochondrial host DNA

Primer	Sequence (5′–3′)	Gene target	Reference
Kudoa hypoepicardialis			
ERIB1	ACCTGGTTGATCCTGCCAG	*SSU*	Barta et al. (1997)
ERIB10	CCTCCGCAGGTTCACCTACGG	*SSU*	Barta et al. (1997)
Myxo409F	CAATCCAGACAATGGGAGGT	*SSU*	Rosser et al. (2021)
Myxo1388R	AACTAAGAACGGCCATGCAC	*SSU*	Rosser et al. (2021)
Myxo1F	CTGCCCTATCAACTWGTT	*SSU*	Kent et al. (2000)
NLF160	ACCTCCACTCAGGCAAGATTA	*LSU*	Bartošová et al. (2009)
NLR1694	TCTYAGGAYCGACTNAC	*LSU*	Van der Auwera et al. (1994)
NLR3284	TTCTGACTTAGAGGCGTTCAG	*LSU*	Van der Auwera et al. (1994)
NLR1270	TTCATCCCGCATCGCCAGTTC	*LSU*	Bartošová et al. (2009)
NLF1050	AATCGAACCATCTAGTAGCTGG	*LSU*	Bartošová et al. (2009)
NLR3422	CTCTACYCGTGGTTTCTGTCC	*LSU*	Bartošová et al. (2009)
Kt28S1F	CAAGACTACCTGCTGAAC	*LSU*	Whipps et al. (2004)
28S3R	GAGCACTGGGCAGAAATC	*LSU*	Whipps et al. (2004)
GenMyxoEF2F1	GGWGCKGGKGAAYTRCAYYTNGA	*EF2*	Woodyard et al. (2021)
GenMyxoEF2R1	CCARTGRTCRAACACACAYTGNGGGAA	*EF2*	Woodyard et al. (2021)
EF2IntF2	GATTTRGARGARGATCATGC	*EF2*	Bartošová et al. (2013)
EF2IntR2	CAGTAAAACCRAAAGATTC	*EF2*	Bartošová et al. (2013)
Pterois volitans			
FishF1	TCAACCAACCACAAAGACATTGGCAC	*cox*1	Ward et al. (2005)
FishR1	TAGACTTCTGGGTGGCCAAAGAATCA	*cox*1	Ward et al. (2005)

98°C for 3 min, followed by 35 cycles of 98°C for 30 s, 55°C for 30 s, and 72°C for 20 s, followed by a final extension at 72°C for 10 min.

In addition, myxozoan elongation factor 2 (*EF2*) sequences were obtained using a nested PCR. The primary PCR used the GenMyxoEF2F1/GenMyxoEF2R1 primer set with conditions as above but with a 51°C annealing temperature, while the secondary PCR used the resulting PCR products amplified by the EF2IntF2/EF2IntR2 primer set and a 55°C anneal. All PCR products were visualized by gel electrophoresis in 0.8% agarose gels buffered with sodium-borate and stained with ethidium bromide. Amplicons were excised from gels with sterile scalpel blades, purified (Qiagen Gel Extraction Kit), and commercially sequenced bidirectionally (Eurofins Genomics, Louisville, Kentucky, USA) using the same primers used for amplification. Chromatograms were assembled and trimmed manually in Geneious Prime v. 2022.0.1 bioinformatics software. Matching sequences for each region were searched for in the NCBI nonredundant nucleotide (nr/nt) database (Altschul et al. 1990).

2.5. Sequence homology and phylogenetic analyses

Phylogenetic inference of the evolutionary history of *Kudoa* spp. was assessed by construction of 3 phylogenetic trees: a single-locus *SSU* tree, a concatenated *SSU* and *LSU* tree, and a concatenated *SSU*, *LSU*, and *EF2* tree. Sequence data from the present study were combined with publicly available sequences from GenBank for *Kudoa* spp., excluding all *SSU* and *LSU* sequences <1500 bp. *Unicapsula muscularis* Davis, 1924 was used as an outgroup for both ribosomal data trees. For the *SSU* alignment, sequences were restricted to 1 representative of the 20 most similar species to those from the present study, combined with any from heart-infecting *Kudoa* spp. All *SSU* and *LSU* alignments were made using the MAFFT v. 7.475 multiple sequence alignment algorithm (Katoh & Standley 2013) with the X-INS-i option, with MXSCARNA (Tabei et al. 2008) used for pairwise structural alignment while the *EF2* alignment was made with MAFFT using the default options. Non-overlapping bases were trimmed from the 5′ and 3′ ends of each alignment in Geneious. To ensure that alignment ambiguities did not inflate nodal support in subsequent phylogenetic analyses, signal:noise ratio assessment was carried out on all alignments using Aliscore v. 2.0 (Misof & Misof 2009) with 4*N random pairs compared, a window size of 6, and gaps treated as fifth characters. Finally, alignments were separated into concatenated nucleotide and gap partitions, with gaps coded as binary characters according to the method of Simmons &

Ochoterena (2000) using 2matrix (Salinas & Little 2014). Optimal nucleotide substitution models were determined for each alignment using ModelFinder (Kalyaanamoorthy et al. 2017) as implemented in IQ-TREE 2 (Minh et al. 2020). Bayesian phylogenetic inference was carried out using the MPI version of MrBayes v. 3.2.7 (Ronquist & Huelsenbeck 2003, Altekar et al. 2004) with Markov chain Monte Carlo searches of 2 runs of 4 simultaneous chains with sampling every 100 generations for 1 000 000 generations until convergence was reached, as determined by the standard deviation of split frequencies reaching <0.01. The concatenated *SSU*, *LSU*, and *EF2* analyses was carried out as above but for 2 000 000 generations, with *EF2* data analyzed as codons using the M3 model.

For phylogenetic analysis of fish host sequence data, *cox*1 sequences from the present study, along with *Pterois* spp. sequences ≥500 bp obtained from GenBank, were aligned using MAFFT. Sequences <95% similar to the majority of conspecific sequences were excluded. The tree was constructed by Bayesian inference in MrBayes v. 3.2.7. Analysis was run until standard deviation of split frequencies reached <0.01 at 3 400 000 generations with a cold chain temperature of 0.01. *Dendrochirus zebra* (Cuvier, 1829) sequences were included as an outgroup. All trees and matrices were formatted and annotated using Figtree v. 1.4.4 and Adobe Illustrator v. 23.0.4.

3. RESULTS

3.1. Host identification

Lionfish weighed 310.1 ± 230.3 g (mean ± SD) and were 25.3 ± 7.1 cm in length. The *cox*1 sequences from hosts in the present study were 99.83–100% similar to each other and 99.23–100% identical to previously published *Pterois volitans* sequences. All protein sequences were identical. This degree of intraspecific variation at *cox*1 is consistent with previous reports of intraspecific variation for fish species (Ward et al. 2005). Bayesian phylogenetic analysis grouped the *P. volitans* sequence data from the present study within a well-supported (posterior probability of 1) clade of previously published *P. volitans* sequences (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/d149p097_supp.pdf).

3.2. Histological assessment

Histopathological examination of 13 infected cardiac tissues revealed mild to marked expansion of the subepicardial tissue to the compact layer of the atrium and ventricle, with clusters of up to 75 intact (Fig. 2a) or necrotic plasmodia up to 250 µm in diameter. Intact plasmodia were lined by thin hyalin walls and were filled with myriad pyriform to quadrate myxospores approximately 5 µm in diameter (Fig. 2b). In 9/13 (69.2%) infected fish, plasmodia were necrotic and spores were replaced by abundant eosinophilic and basophilic cellular and acellular debris admixed with degenerate spores (Fig. 2c). The level of inflammation surrounding intact and degenerate plasmodia varied. There was often encapsulation by epitheloid macrophages with mild to moder-

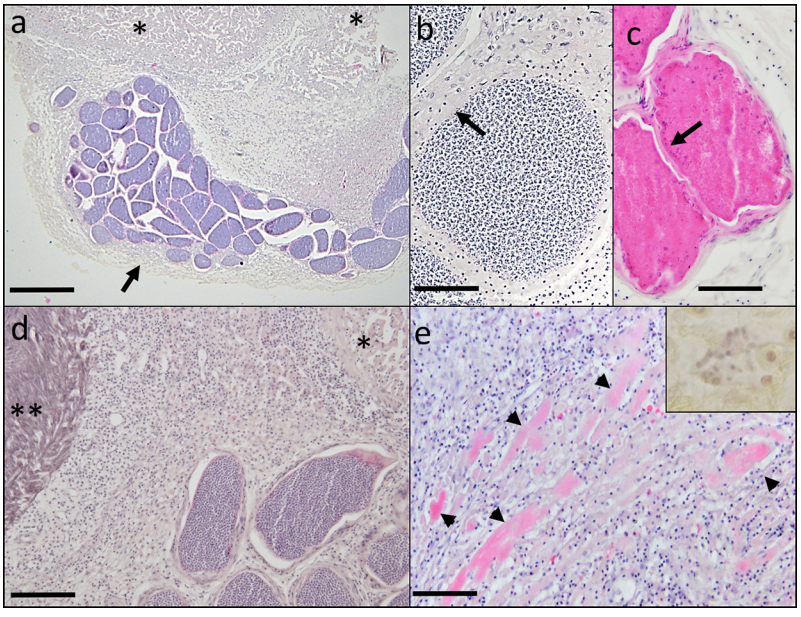

Fig. 2. Histopathology of lionfish *Pterois miles* cardiac tissue infected with *Kudoa hypoepicardialis*. (a) Large clusters of plasmodia expanding the space between the ventral myocardium (*) and the epicardium (arrow) (scale bar = 500 µm). (b) Plasmodia were filled with numerous myxozoan spores or were (c) replaced by eosinophilic acellular necrotic material and separated by thin hyaline membranes (arrows) (both scale bars = 100 µm). (d) Plasmodia were associated with granulomatous inflammation that expanded the space between the ductus arteriosus (*) and the myocardium (**) (scale bar = 50 µm). (e) In few fish, granulomatous inflammation diffusely effaced the heart and was associated with myofiber necrosis (arrowheads) (scale bar = 50 µm) and 1–2 µm in diameter round organisms that were weakly positive with Lillie-Twort stain (inset)

ate infiltration of lymphocytes, plasma cells, and additional macrophages that expanded the epicardium (Fig. 2d). The most pronounced inflammation surrounded what were interpreted to be presporogonic myxozoan stages free within the parenchyma or within developing plasmodia. In these instances, sheets of granulomatous inflammation, discrete granulomas, and necrotic debris extended deep into the myocardium. In 2 fish, inflammation diffusely effaced the ventricular myocardium and was associated with myofiber necrosis characterized by pale to hypereosinophilic fragmented myofibers that lacked cross striations (Fig. 2e). Within granulomas were organisms 1–2 µm in diameter that were weakly positive with Lillie-Twort and Giemsa staining (Fig. 2e). Organisms free within the myocardium parenchyma were consistent with those associated with developing plasmodia.

3.3. Prevalence and intensity

Myxozoan plasmodia were observed in the hearts of 24/76 (31.6%) lionfish by histopathology (14/52, 26.9%) or tissue wet mounts (10/24, 41.7%) (Fig. 1). There was a significantly higher prevalence of infection in fish collected from Boss Reef in 2021 compared to Happy Hill/Black Bay in 2019 ($p = 0.02$) and Kahonee Reef in 2021 ($p = 0.01$) (Fig. 1). We found no significant differences in fish length or weight between infected and non-infected lionfish.

3.4. Myxospore morphology

Measurements are reported as range (mean ± SD, with SD only indicated when data are reported from ≥30 measurements). Spores were quadrate in apical view, spheroid in lateral view, consistent with the genus *Kudoa* (Fig. 3). Fresh myxospores were morphologically consistent with *K. hypoepicardialis* (Table 2), being 5.1–7.9 (6.0) µm long, 8.1–9.8 (8.7 ± 0.4) µm wide, and 6.9–8.5 (7.7 ± 0.5) µm thick. There were 4 equal valves with sutural lines not being prominent. The 4 polar capsules were pyriform and equal in dimensions, being 2.3–2.7 (2.5 ± 0.1) µm long and 0.9–1.6 (1.3 ± 0.2) µm wide, each with 1 coil.

Ethanol-preserved spores were slightly smaller than fresh spores, with bodies 4.5–6.1 (5.2) µm long, 6.6–7.7 (7.3) µm wide, and 5.1–6.6 (5.9) µm thick. Polar capsules were 2.2–2.7 (2.5) µm long and 1.0–1.4 (1.2) µm wide. Polar tubules were not observed in the ethanol-preserved spores.

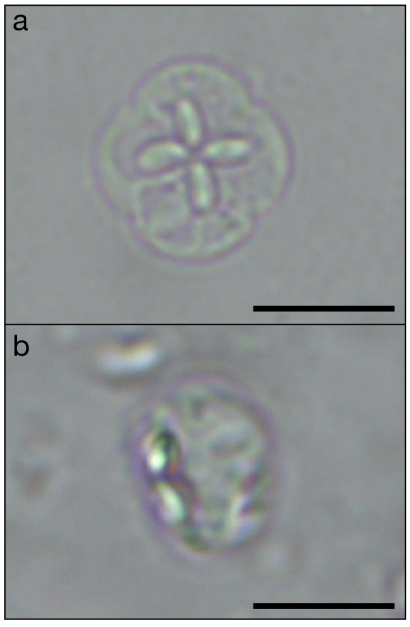

Fig. 3. Fresh myxospores of *Kudoa hypoepicardialis* in (a) apical and (b) lateral view. Scale bars = 10 µm

Of the hosts previously reported for *K. hypoepicardialis*, myxospores in the present study were most similar to those described from *Pogonias cromis* (Linnaeus, 1766). However, those from *P. volitans* differed by having slightly shorter polar capsules (2.2–2.7 µm as opposed to 2.8–3.7 µm). (Blaylock et al. 2004). Most ranges also overlapped with those of *K. pericardialis* from *Seriola quinqueradiata* Temminck & Schlegel, 1845, although *K. hypoepicardialis* were wider (6.6–7.3 µm compared to 4.5–5 µm) (Nakajima & Egusa 1978).

3.5. Taxonomy

Family: Kudoidae Meglitsch, 1960
Genus: *Kudoa* Meglitsch, 1947
Species: *Kudoa hypoepicardialis* Blaylock, Bullard & Whipps 2004
Host: *Pterois volitans*
Site of infection: Pericardium
Localities: Happy Hill, Black Bay, Halifax Harbor, Kahonee Reef, and Boss Reef, Grenada, West Indies
Prevalence: 24/77 (31.2%) *P. volitans*; 14/52 (26.9%) examined histologically, 10/25 (40%) examined by wet mounts.
Deposited material: Photomicrographs are deposited as vouchers at the Harold W. Manter Laboratory of Parasitology, University of Nebraska-Lincoln, Lincoln,

Table 2. Length × width × thickness for *Kudoa hypoepicardialis* ex *Pterois volitans* compared with prior accounts of *Kudoa* spp. from hearts of fish hosts. Measurements are given in micrometers rounded to the nearest 0.1 µm. Data from the present study are indicated in **bold**. nr: measurements not reported in the referenced study

Kudoa sp.	Host(s)	Myxospore	Polar capsule	Reference
K. aegyptia (unknown)	*Rhabdosargus haffara*	9.1–11.4 × 7.1–8.5 × 3.2–5.9	3.2–5.9 × nr	Koura (2000) as cited by Al Quraishy et al. (2008)
K. hypoepicardialis (fresh)	*Nomeus gronovii*	6.5 × 9.3–11.2 × 7.4–9.3	2.8 × 0.9–1.4	Blaylock et al. (2004)
	Lutjanus campechanus	5.6–7.4 × 8.4–9.3 × 6.5–8.4	2.8–3.7 × 0.9	
	Pomatomus saltatrix	5.6–6.5 × 7.4–9.3 × 6.5–8.4	1.9–3.7 × 0.9–1.8	
	Caranx crysos	5.6–6.5 × 8.4–9.3 × 5.6–7.4	2.9–3.7 × 0.9–1.9	
	Pogonias cromis	4.7–6.5 × 6.5–8.4 × 5.6–6.5	2.8–3.7 × .09–1.9	
	Lobotes surinamensis	4.7–6.5 × 7.4–9.3 × 5.6–8.4	2.8–3.7 × 0.9–1.4	
	Hyporthodus nigritus	4.7–6.5 × 7.4–10.2 × 6.5–8.4	2.8 × 0.9	
***K. hypoepicardialis* (fresh)**	***Pterois volitans***	**5.1–7.9 × 8.1–9.8 × 6.9–8.5**	**2.3–2.7 × 0.9–1.6**	**Present study**
***K. hypoepicardialis* (ethanol)**	***Pterois volitans***	**4.5–6.1 × 6.6–7.3 × 5.1–6.6**	**2.2–2.7 × 1.0–1.4**	**Present study**
K. iwatai (fresh)	*Pagrus major*	7.2–8.4 × 9–11.0 × 9–11.0	4–5.5 × 1.8–2.4	Diamant et al. (2005)
	Sparus aurata	8.3–9.4 × 9.5–10.8 × 9–9.7	4.3–5.2 × 2.2–2.8	
K. leptacanthae (frozen)	*Zoramia leptacantha*	4.7–7.2 × 7.2–9.6 × 6.0–8.0	2.2–3.6 × 1.0–1.9	Heiniger & Adlard (2012)
	Z. viridiventer	5.1–7.1 × 6.9–9.0 × 5.9–7.8	1.6–2.9 × 1.0–1.9	
K. pagrusi (fresh)	*P. pagrus*	6.5–8.6 × 5.8–7.2 × nr	2.6–4.2 × 1.0–1.8	Al Quraishy et al. (2008)
K. pericardialis (fresh)	*Seriola quinqueradiata*	4–4.2 × 4.5–5 × 6–7	2.4–3 × 1–1.5	Nakajima & Egusa (1978)
K. shiomitsui (fresh)	*Takifugu rubripes*	5.6–6.8 × 8.13–9.58 × 6.7–7.7	2.4–2.9 × 1–1.3	Egusa & Shiomitsu (1983)
Kudoa sp. (fresh)	*Plectropomus maculatus*	4.7–6.8 × 4.66.5 × nr	1.3–3.5 × 1.1–2.2	Abdel-Ghaffar et al. (2012)

Nebraska (HWML 216820). Partial 18S *SSU*, 28S *LSU*, and *EF2* sequences from *K. hypoepicardialis* are deposited in GenBank (accession numbers: OK076896–OK076897, OK077754–OK077756, and OK086043). Partial *cox*1 mitochondrial gene sequences from *P. volitans* are also deposited in GenBank (OK076983–OK076988).

3.6. Molecular characterization and phylogenetic analyses

Intraspecific *SSU* sequence similarity was 99.81% (3/1581 bp difference) while at *LSU* similarity was 99.84–99.92% (1–2/1242 bp). SSU sequences of *K. hypoepicardialis* from *P. volitans* were 99.81–99.87% similar to the *SSU* sequence from the original description by Blaylock et al. (2004) in a 1581 bp single species alignment. In an *SSU* alignment comparing those from the present study with other *Kudoa* spp., they were 99.66–99.83% similar to *K. hypoepicardialis* from Blaylock et al. (2004) and >95% similar to the other heart-infecting kudoids: *K. shiomitsui*, *K. leptacanthae*, and *K. pagrusi* (Table 3).

Single-locus *SSU* (Fig. 4) and concatenated *SSU* and *LSU* phylogenies (Fig. S2) all placed *K. hypoepicardialis* in a robustly supported clade with other heart-infecting *Kudoa* species. In the analysis incorporating *SSU*, *LSU*, and *EF2* data, *K. hypoepicardialis* formed a clade with *K. crumena* Iversen & van Meter, 1967, with *K. dianae* Dyková, Fajer Avila & Fiala, 2002 basal to these (Fig. S3).

4. DISCUSSION

Appropriate identification of fish host species has long been considered of critical importance to the identification and establishment of myxozoan species (Lom & Arthur 1989, Molnár 1994). However, this is problematic in the case of the invasive lionfishes *Pterois* spp., whose taxonomy is contentious and in a current state of flux (Wilcox et al. 2018, Burford Reiskind et al. 2019). While unraveling the genetic diversity and invasion history of *Pterois* spp. is

Table 3. Sequence similarity (%) between 18S small subunit rDNA (*SSU*), 28S large subunit rDNA (*LSU*), and elongation factor 2 (*EF2*) sequences for *Kudoa hypoepicardialis* ex *Pterois volitans*, other heart-infecting *Kudoa* species for which sequence data were available, and the 10 most similar sequences at each locus. Sequences are sorted from most to least similar at *SSU*. The *SSU* alignment had 606 sites, with 576 bases and 30 gaps in the consensus sequence. The *LSU* alignment had 942 sites, with 867 bp and 75 gaps in the consensus sequence. The *EF2* alignment had no gaps and consisted of 622 bases. Dashes indicate lack of available sequence data for comparison. Taxonomic authorities are only included for species that are not mentioned in the main text

	Kudoa hypoepicardialis ex *Pterois volitans*		
	SSU	*LSU*	*EF2*
K. hypoepicardialis	99.66–99.83	–	–
K. shiomitsui	95.06–95.23	90.45–90.57	–
K. leptacanthae	95.23–95.40	–	–
K. pagrusi	95.23–94.40	–	–
K. amamiensis	91.84–92.86	–	–
K. barracudai Abdel-Baki, Al-Quraishy, Omar & Mansour, 2016	92.69–92.86	–	–
K. kenti Burger & Adlard, 2010	92.86–93.03	–	–
K. scomberi Li, Sato, Tanaka, Ohnishi, Kamata & Sugita-Konishi, 2013	91.08–91.25	–	–
K. thunni Matsukane, Sato, Tanaka, Kamata & Sugita-Konishi, 2011	90.82–91.08	–	–
K. crumena	90.24–90.40	86.20	84.57
K. trachuri Matsukane, Sato, Tanaka, Kamata & Sugita-Konishi, 2011	89.33–89.50	–	–
K. musculoliquefaciens (Matsumoto, 1954)	88.07–87.90	86.21–86.32	–
K. ogawai Yokoyama, Yanagida & Shirakashi, 2012	87.73–87.90	82.39–82.72	–
K. scomberomori Adlard, Bryant, Whipps & Kent, 2005	87.63–87.80	–	–
K. iwatai	87.20–87.54	84.49–84.61	–
K. lateolabracis Yokoyama, Whipps, Kent, Mizuno & Kawakami, 2004	87.71–87.88	–	–
K. bora (Fujita, 1930)	87.20–87.37	85.04–85.15	–
K. lutjanus Wang, Huang, Tsai, Cheng, Tsai, Chen, Chen, Chiu, Liaw, Chang & Chen, 2005	87.20–87.37	84.61–84.72	–
K. thyrsites (Gilchrist, 1923)	87.03–87.71	80.59–83.20	–
K. mirabilis Naidenova & Gaevskaya, 1991	86.69–86.86	–	–

outside the scope of this work, sequence homology and phylogenetic analyses indicate that fish from the present study were *P. volitans*.

This account is one of the first to describe a pathogen-associated disease in invasive lionfish and represents the only myxozoan reported from *P. volitans*. Further, this is only the second account of myxozoan parasitism of the genus *Pterois* besides *Sphaeromyxa zaharoni*. Myxospores recovered from *P. volitans* were morphologically and molecularly consistent with *Kudoa hypoepicardialis.* It should be noted that some minor disagreement exists between myxospore measurements of *K. hypoepicardialis* from different hosts in the original description. While most ranges overlap, the spore width of *K. hypoepicardialis* from *Nomeus gronovii* (Gmelin, 1789) (9.3–11.2 µm) does not overlap with that reported from *Pogonias cromis* (6.5–8.4 µm). Additionally, the spore width of *K. hypoepicardialis* from *N. gronovii* (6.5 µm) does not agree with that from *Lobotes surinamensis* (Bloch, 1790) (4.7–5.6 µm). It is entirely possible that these differences reflect morphological variability within *K. hypoepicardialis*. Given the broad host ranges reported for some *Kudoa* spp., the previously accepted utility of *SSU* for delineating myxozoan species and *SSU* sequence homology among host species reported in the original description, these minor differences would not have been sufficient to suspect these were distinct species when *K. hypoepicardialis* was described. Unfortunately, no *LSU* data are presently available for *K. hypoepicardialis* for molecular comparison. *LSU* was first used for myxozoans by Whipps et al. (2004), and there are no accounts of *K. hypoepicardialis* subsequent to the original description by Blaylock et al. (2004). *LSU* data are increasingly used for their greater inter- and intraspecific variability, especially between kudoids (Whipps & Kent 2006) (Table 3) and their utility in concatenated phylogenetic analysis (Bartošová et al. 2009, 2013). More recent descriptions of *Kudoa* spp. suggest insufficient resolution of *Kudoa* to species level using the *SSU* marker alone, with morphologically distinct species showing ≥99% sequence simi-

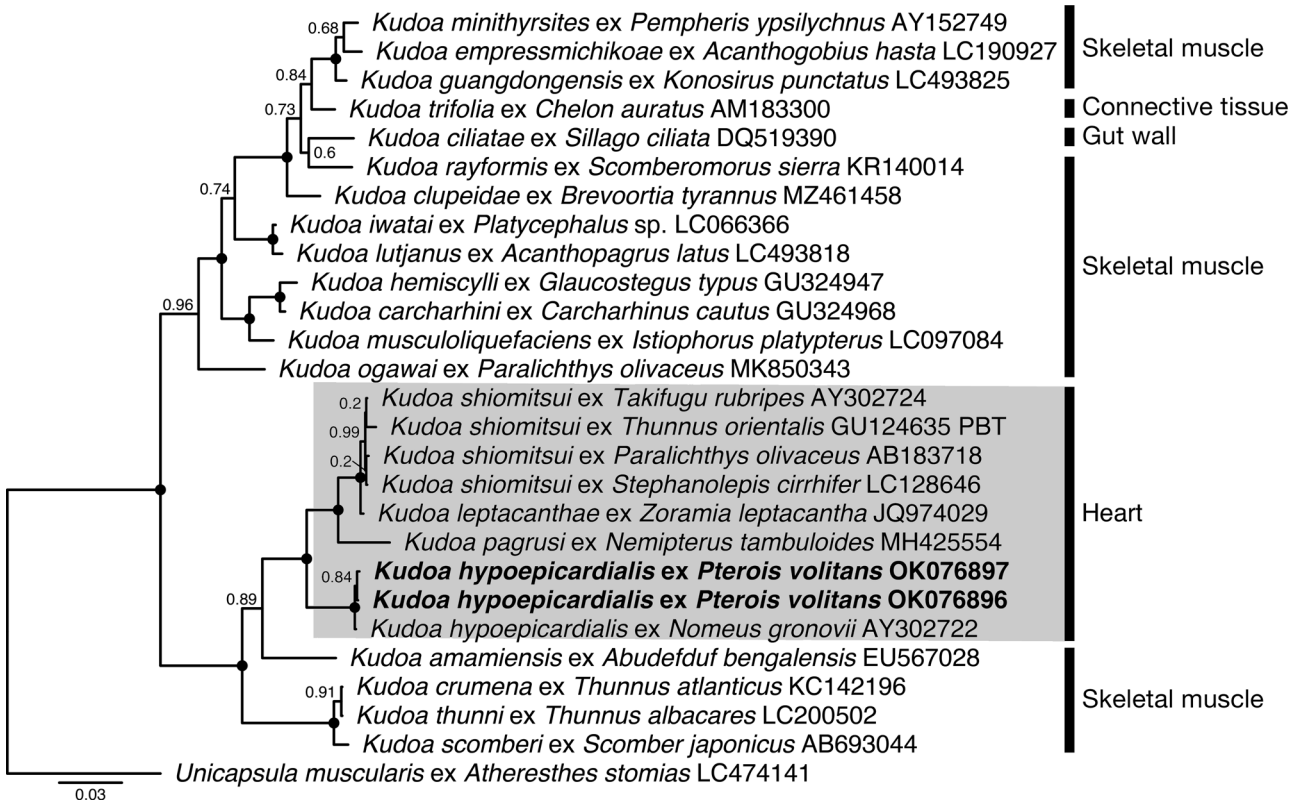

Fig. 4. Single locus 18S small subunit rDNA (*SSU*) phylogenetic tree constructed in MrBayes. The alignment consisted of 1423 sites, with 1367 bp and 56 gaps in the consensus sequence. For Bayesian inference, the nucleotide partition used the GTR+F+I+ Γ_4 nucleotide substitution model while the gap partition was treated as restriction site data with ascertainment bias correction. Nodes are labeled with myxozoan species ex host species followed by GenBank accession number. Values adjacent to nodes indicate posterior probabilities. Dots indicate 100% nodal support. Data from the present study are indicated in **bold**. Heart-infecting *Kudoa* spp. are highlighted in grey. Scale bar represents average number of substitutions per site

larity (Özer et al. 2018, Li et al. 2020). Reexamination of previously described hosts of *K. hypoepicardialis* using more resolute molecular markers may be warranted.

In the concatenated *SSU*, *LSU*, and *EF2* phylogenetic analysis, the 2 other *Kudoa* spp. for which data are available grouped with *K. hypoepicardialis* with robust nodal support. Given that *EF2* has minimal phylogenetic signal relative to *SSU* and *LSU* (Fiala et al. 2015, Woodyard et al. 2022), as well as the dearth of available *EF2* data for *Kudoa* spp., inference from this analysis is limited. However, these data may have utility in future phylogenetic analyses or life cycle studies. *LSU* and *EF2* sequence data will aid in future identification of *K. hypoepicardialis* and closely related *Kudoa* spp., especially in instances where *SSU* data are ambiguous.

The presence of *K. hypoepicardialis* in *P. volitans* is consistent with prior accounts in which only endemic, generalist parasites have been reported (Bullard et al. 2011, Ramos-Ascherl et al. 2015, Fogg et al. 2016). The broad host range of these parasites, including *K. hypoepicardialis* (Blaylock et al. 2004), suggests that invasive lionfish may spread pathogens to native fishes. Overall, *K. hypoepicardialis* prevalence in lionfish was 32%. Depending on the technique used to observe plasmodia, it may have ranged up to 40%. This is substantially higher than prevalence levels described by Blaylock et al. (2004), which ranged from <1 to 16% in fish species within the northern Gulf of Mexico. This prevalence in lionfish could suggest a relatively high susceptibility and possibly that lionfish, which were first identified in Grenada in 2011 (C. Finney pers. obs.), may remain immunologically naïve to *K. hypoepicardialis*. It is also possible that this prevalence is typical for reef fish in Grenada, and further epidemiologic studies are warranted to examine prevalence in local species that share common habitats with lionfish.

Myocarditis and myonecrosis were pronounced in a number of lionfish infected with *K. hypoepicardialis*, although it is difficult to assess morbidity and mor-

tality without controlled laboratory studies. Inflammatory infiltrates and muscle fiber necrosis appeared more severe in lesions associated with developmental stages of the parasite. Pathology associated with heart-infecting *Kudoa* spp. appears to vary between species. *K. pagrusi* infection has been characterized by encapsulation of plasmodia, resulting in a reduction in functional area of the heart muscle (Abdel-Ghaffar et al. 2009). Conversely, *K. shiomitsui* infections have been observed without notable harmful effects on the host (Egusa & Shiomitsu 1983, Zhang et al. 2010). In their description of *K. hypoepicardialis*, Blaylock et al. (2004) noted marked inflammation surrounding lesions associated with its developing plasmodia. However, these authors also noted a lack of tissue architecture disruption.

5. CONCLUSION

Host *cox*1 sequence data confirmed *Pterois volitans* as the host for *Kudoa hypoepicardialis* using morphological data from fresh myxospores as well as sequence homology from host and parasite sequence data. The original description is supplemented with *LSU* and *EF2* sequence data. Plasmodia of *K. hypoepicardialis* were associated with granulomatous inflammation of the pericardium and in some cases myofiber necrosis. This is the first account of a myxozoan parasite of *P. volitans* and a novel host and locality for *K. hypoepicardialis*.

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