International Journal for Parasitology 45 (2015) 1–16

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Morphology and phylogeny of *Agmasoma penaei* (Microsporidia) from the type host, *Litopenaeus setiferus*, and the type locality, Louisiana, USA ☆

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ARTICLE INFO

Article history:
Received 3 June 2014
Received in revised form 15 July 2014
Accepted 16 July 2014
Available online 18 October 2014

Keywords:
Microsporidia
Decapoda
Agmasoma penaei
Litopenaeus setiferus
Louisiana
Electron microscopy
Molecular taxonomy

ABSTRACT

Since June 2012, samples of wild caught white shrimp, *Litopenaeus setiferus,* from the Gulf of Mexico, Plaquemines and Jefferson Parishes (Louisiana, USA) with clinical signs of microsporidiosis have been delivered to the Louisiana Aquatic Diagnostic Laboratory for identification. Infection was limited predominantly to female gonads and was caused by a microsporidium producing roundish pansporoblasts with eight spores (3.6 × 2.1 μm) and an anisofilar (2–3 + 4–6) polar filament. These features allowed identification of the microsporidium as *Agmasoma penaei* Sprague, 1950. *Agmasoma penaei* is known as a microsporidium with world-wide distribution, causing devastating epizootic disease among wild and cultured shrimps. This paper provides molecular and morphological characterisation of *A. penaei* from the type host and type locality. Comparison of the novel ssrDNA sequence of *A. penaei* from Louisiana, USA with that of *A. penaei* from Thailand revealed 95% similarity, which suggests these geographical isolates are two different species. The *A. penaei* sequences did not show significant homology to any other examined taxon. Phylogenetic reconstructions using the ssrDNA and alpha- and beta-tubulin sequences supported its affiliation with the Clade IV Terresporidia sensu Vossbrink 2005, and its association with parasites of fresh and salt water crustaceans of the genera *Artemia*, *Daphnia* and *Cyclops*.

1. Introduction

Microsporidiosis is the most common and harmful disease of decapods (phylum Arthropoda: class Crustacea: order Decapoda) caused by eukaryotic microbes (Overstreet, 1973; Kelly, 1979; Johnson, 1995; Morado, 2011). Microsporidial infections caused by more than 20 species belonging to 17 genera have been reported from a variety of decapod species belonging to the family Penaeidae, suborder Dendrobranchiata, as well as four infraorders of the suborder Pleocyemata, namely, Caridea, Astacidea, Brachiura and Anomura (Canning et al., 2002; Stentiford et al., 2013b, 2014). Microsporidiosis in decapods is often linked with reduced host fecundity, elevated susceptibility to predators and to other diseases, and sensitivity to unfavorable environmental conditions (Hutton et al., 1959).

Since June 2012, samples of wild *Litopenaeus setiferus* (Latin names of penaeids from this point forward adhere to taxonomy according to Perez Farfante (1997)) from Plaquemines and Jefferson Parishes (Louisiana (LA), USA) fisheries with clinical signs of microsporidiosis (white tumour-like growths within and below the carapace) were delivered to the Louisiana Aquatic Diagnostic Laboratory on four occasions for further examination. The shrimp were collected by commercial trawlers working in the areas of Bay Jimmy and Barataria Bay. Light (LM) and electron microscopy (EM) examination indicated the infection was caused by a microsporidium producing octets of spores in sub-persistent roundish sporophorous vesicles (SVs). The microsporidium was readily identified as *Agmasoma penaei* based on morphological characters, geographical locality, host species and tissue tropism. This species, formerly known as *Thelohania penaei* Sprague 1950, was later transferred from the genus *Thelohania* to a newly erected

☆ *Note:* Nucleotide sequence data reported in this paper are available in the GenBank database under accession numbers KF549987, PRJNA206557, KJ579182, KJ579183.

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http://dx.doi.org/10.1016/j.ijpara.2014.07.013
0020-7519/

monotypic genus, *Agmasoma,* by Hazard and Oldacre in 1976 in their revision of the genus *Thelohania* (Sprague, 1950; Hazard and Oldacre, 1975).

Agmasoma penaei has been known to cause "cotton disease" in White Atlantic shrimp, *L. setiferus*, since 1920 (Viosca, 1945; Miglarese and Shealy, 1974). It has been recorded in eight species of penaeid shrimp (Table 1), and has been shown to adversely affect commercial shrimp fisheries and shrimp aquaculture worldwide (Sprague and Cough, 1971; Sprague, 1977; Kelly, 1979; Flegel et al., 1992; Clotilde-Ba and Toguebaye, 1994, 2001; Vidal-Martínez et al., 2002; Toubiana et al., 2004; Laisutisan et al., 2009).

In the Gulf of Mexico, the most severe epizootic of microsporidiosis among white shrimp *L. setiferus* due to *A. penaei* was recorded in 1929. It resulted in an infection prevalence of 90%, mass mortality, loss of 99% of egg production and an unprofitable fishery industry for several years (Gunter, 1967; Muncy, 1984). Except for epizootic peaks, the infection rate of this parasite in wild populations of white shrimp in the Gulf of Mexico normally does not exceed 1% (Lightner, 1996). During the last 2 years, the reports of microsporidian infections in areas of the Gulf of Mexico have increased, particularly in locations adjacent to the Deepwater Horizon oil spill (J. Hawke, unpublished observations and conversations with local marine biologists and agents)).

Three geographical isolates parasitising three different penaeid hosts from different geographic locations, were examined ultrastructurally. These are: the Louisiana isolate from Atlantic white shrimp, *L. setiferus* (Hazard and Oldacre, 1975), the Senegal isolate from the Southern pink shrimp, *Farfantepenaus notialis* (Clotilde-Ba and Toguebaye, 1994), and the Thailand isolate from Pacific white shrimp, *Litopenaeus vannamei* (Laisutisan et al., 2009). All three isolates displayed a similar pattern of octosporous sporogony, pyriform shape of spores and spore ultrastructure with conspicuous anisofilar polar filaments. Prior to this study, data on fine morphology of the Louisiana isolate was limited to two EM images (Hazard and Oldacre, 1975). The Senegal and Thailand isolates of *A. penaei* have been studied more thoroughly, however the ultrastructure of the organism has not been described sufficiently. Data on pathogenicity, tissue tropism, transmission and sexuality are scarce and controversial among these three and other geographical isolates of *A. penaei* (Table 1). Only one *A. penaei* isolate parasitising cultured *Fenneropenaeus merguiensis* and *Penaeus monodon* in Thailand has been characterised using molecular tools and a fragment of its ssrDNA sequence is available via GenBank (Pasharawipas and Flegel, 1994; Pasharawipas et al., 1994).

The major goal of this paper is to provide morphological and molecular characterisation of *A. penaei* from the type host and type locality, which would serve as an important reference for identification of geographical isolates of this microsporidium. We also comparatively evaluate the fine morphology of three geographical isolates of *A. penaei*, assess relatedness of *A. penaei* from Louisiana to other microsporidia parasitising decapods using ssrDNA-based phylogenetic analysis, and present molecular and phylogenetic comparisons of *A. penaei* genes for ssrRNA and alpha- and beta-tubulins with other microsporidian orthologues available in GenBank.

2. Materials and methods

2.1. Materials

White shrimp, *L. setiferus* Linnaeus 1767, were caught by commercial trawling in the Gulf of Mexico in the bays and offshore from Plaquemines and Jefferson Parishes, Louisiana, USA. An unusually high number of shrimp harbouring macroscopic whitish lesions and tumour-like growths on the carapaces and abdomens were noticed by shrimpers. Thirty shrimps with these clinical signs were delivered to the Louisiana Aquatic Diagnostic Laboratory (Louisiana State University (LSU) School of Veterinary Medicine (SVM), Baton Rouge, LA, USA) from May 2012 to November 2013. In all of the cases, shrimp were caught alive, kept on ice and delivered within 6–24 h after being caught. All delivered shrimp were females, with sizes ranging from 100 to 120 mm for those caught in May, and 130 to 180 mm for those caught later in the year. The material examined in this study included 18 shrimp; two sampled on 16 May 2012 (case LADL12-047); two on 22 May

Table 1
Records of occurrence of *Agmasoma penaei* among penaeid shrimps.

Shrimp species	Locality	Wild/ cultured	Tissues	Techniques	References
Atlantic white shrimp *Litopenaeus setiferus* (Type host)	Gulf of Mexico (LA, MI,FL coast); type locality: around Grand Isle, LA	Wild	Gonads	LM, TEM, SEM, ssrDNA	Sprague (1950) Hazard and Oldacre (1975) This paper
Pacific white shrimp *Litopenaeus vannamei*	Thailand, Indian Ocean	Cultured	Abdominal muscles, hepatopancreas	LEM, TEM	Laisutisan et al. (2009)
Indian prawn *Fenneropenaeus indicus*	Republic of South Africa shore: South Atlantic/Indian Ocean	Wild	Gonads	LM	Sprague and Cough (1971) Sprague (1977)
Banana shrimp *Fenneropenaeus merguiensis*	Thailand, Indian Ocean	Wild Cultured	No data	LM, ssrDNA	Pasharawipas and Flegel (1994) Pasharawipas et al. (1994)
Pink shrimp *Farfantepenaeus duorarum*	Atlantic coast of South Florida	Wild	Muscles, hepatopancreas, gonads	LM	Kelly (1979)
Pink shrimp *Farfantepenaeus notialis*	West African coast of South Atlantic (Senegal)	Wild	Gonads, hepatopancreas, heart, intestine, nervous system, muscle; xenomas	LM, TEM	Clotilde-Ba and Toguebaye (1994)
Black tiger shrimp *Penaeus monodon*	West African coast of South Atlantic (Senegal)	Cultured	Muscles	LM	Clotilde-Ba and Toguebaye (2001)
	West coast of Madagascar	Wild	No data	SEM	Toubiana et al. (2004)
	Thailand, Indian Ocean	Cultured	No data	LM, ssrDNA	Pasharawipas and Flegel (1994), Pasharawipas et al. (1994)
Green tiger prawn *Penaeus semisulcatus*	Thailand, Indian Ocean	Wild	No data	SEM	Toubiana et al. (2004)

LA, Louisiana, USA; MI, Mississippi, USA; FL, Florida, USA; LM, light microscopy; TEM, transmission electron microscopy; SEM, scanning electron microscopy.