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A new microsporidium, *Triwangia caridinae* gen. nov., sp. nov. parasitizing fresh water shrimp, *Caridina formosae* (Decapoda: Atyidae) in Taiwan

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ABSTRACT

A new microsporidium was isolated from the endemic, Taiwanese shrimp, *Caridina formosae* (Decapoda, Atyidae) from northern Taiwan. A conspicuous symptom of infection was presence of opaque white xenomas located in the proximity of the alimentary tract, the surface of the hepatopancreas, and the gills. A fully developed xenoma consisted of a hard, thick capsule filled with sporophorous vesicles containing multiple spores. Microsporidia developed synchronously within the same sporophorous vesicle, although the stage of parasite development differed among the vesicles. Fresh spores were pyriform, mononucleated and measured 6.53 × 4.38 μm. The polar filament was anisofilar with 9–11 coils. Phylogenetic analysis based on the small subunit ribosomal DNA sequence showed that the isolate is most similar to the fish microsporidian clade containing the genera *Kabatana*, *Microgemma*, *Potaspora*, *Spraguea*, and *Teramicra*. The highest sequence identity, 80%, was with *Spraguea* spp. Based on pathogenesis, life cycle and phylogenetic analysis, we erect a new genus and species, *Triwangia caridinae* for the novel microsporidium.

1. Introduction

Microsporidia are obligate intracellular eukaryotic parasites reported from nearly all invertebrate phyla. The majority of species are described from arthropod and fish hosts, particularly insects and crustaceans (Wittner and Weiss, 1999). Approximately 43 microsporidian genera from crustaceans have been described (Table 1) and 11 of these genera have been reported from shrimps including, *Agmasoma*, *Ameson*, *Enterocytozoon*, *Inodosporus*, *Myospora*; *Perezia*, *Pleistophora*, *Thelohania*, *Tuzetia*, *Vairimorpha* and *Vavraia* (Table 2).

At least 23 microsporidian species have been described from shrimps. Microsporidia have been reported from about 20 species of marine or estuarian shrimps and eight species of fresh water crayfish (Table 2). The microsporidia *Agmasoma penaei*, *Ameson* sp., *Enterocytozoon hepatopenaei*, *Perezia nelsoni*, *Pleistophora* spp., *Thelohania* spp., and *Tuzetia weidneri* collectively infect at least eight species of penaeid shrimp, a group that contains many species of economic importance including *Penaeus monodon* and *Litopenaeus setiferus* (Table 2). In addition, *Inodosporus spraguei* and *Indosporus octospora* were isolated from *Palaemon* spp. and *Palaemonetes* spp. (Azevedo et al., 2000; Overstreet and Weidner, 1974; Sprague and Couch, 1971), and *Pleistophora crangoni*, *Thelohania giardi* and *Vavraia mediterranica* were recovered from five species of crangonid shrimp (Azevedo, 2001; Breed and Olson, 1977; Krygier and Horton, 1975) (Table 2).

The tiny atyid shrimp, *Caridina formosae*, with an adult body length of 1.5–2.0 cm (Fig. 1), is an endemic species occurring in the streams of northern and western Taiwan (Shy et al., 2001). Shrimps complete their life cycle in the fresh water system and are often reared commercially as live food for aquaculture or are kept as aquarium pets (Hung et al., 1993; Shy et al., 2001). We first observed microsporidian infections in field collected shrimps and noted that symptoms of the disease were obviously different from those of known microsporidioses from marine or other freshwater shrimps. We studied the life cycle, morphology and ultrastructure of this new microsporidian species. We also analyzed the full small subunit ribosomal DNA sequence, compared it with those of other microsporidia in the NCBI public database and performed a phylogenetic analysis. Based on ultrastructural and molecular evidence, we propose that this microsporidium belongs to a new genus closely related to the genus *Spraguea*, a xenoma-forming fish microsporidium.

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Table 1
Microsporidia genera in crustaceans.

Genus	Crustacean host		Reference
Triwangia	Shrimp	*Caridina formosae*	This study
Agmasoma	Shrimp	*Farfantepenaeus duorarum*; *Fenneropenaeus* spp. (2); *Litopenaeus setiferus*; *Penaeus monodon*	Sprague and Couch (1971), Kelly (1979) and Pasharawipas and Flegel (1994)
Ameson	Crab	*Callinectes sapidus*	Zhu et al. (1993)
	Crayfish	*Austropotamobius pallipes*	Edgerton et al. (2002)
	Shrimp	*Penaeus monodon*	Anderson and Nash (1989)
Abelspora	Crab	*Carcinus maenas*	Azevedo (1987)
Agglomerata	Copepod	*Acanthocyclops vernalis*	Bronnvall and Larsson (2001)
	Cladoceran	*Sida crystallina*	Larsson and Yan (1988)
Amblyospora	Copepod[a]	*Acanthocyclops* spp.(2); *Cyclops strenuus*; *Diacyclops bicuspidatus*; *Mesocyclops annulatus*; *Paracyclops fimbriatus fimbriatus*	Micieli et al. (2000a,b) and Vossbrinck et al. (2004)
Baculea	Water flea	*Daphina pulex*	Loubès and Akbarieh (1978)
Berwaldia	Water flea	*Daphnia pulex*	Larsson (1981)
Binucleata	Water flea	*Daphnia magna Straus*	Refardt et al. (2008)
Cougourdella	Copepod	*Megacyclops viridis*	Larsson (1989)
Cucumispora	Amphipod	*Dikerogammarus villosus*	Ovcharenko et al. (2010)
Desmozoon	Copepod	*Lepeophtheirus salmonis*	Freeman and Sommerville (2009)
Dictyocoela	Amphipod	*Echinogammarus berilloni*; *Gammarus* spp. (3); *Orchestia* spp. (2); *Talorchestia deshayesei*	Hogg et al. (2002), Terry et al. (2004) and Krebes et al. (2010)
Hepatospora	Crab	*Eriocheir sinensis*	Stentiford et al. (2011) and Wang and Chen (2007)
Enterocytozoon	Shrimp	*Penaeus monodon*	Tourtip et al. (2009)
Enterosora	Crab	*Cancer pagurus*; *Eupagurus bernhardus*	Stentiford et al. (2007) and Stentiford and Bateman (2007)
Facilispora	Copepod	*Lepeophtheirus salmonis*	Jones et al. (2012)
Flabelliforma	Water flea	*Daphnia. magna*	Larsson et al. (1998)
Glugoides	Water flea	*Daphnia* spp. (2)	Larsson et al. (1996)
Gurleya	Water flea	*Atyephira* spp. (2); *Daphnia* spp. (2); *Macrocyclops albidus*; *Moina rectirostris*	Doflein (1898), Jirovec (1942), Sprague and Couch (1971), Green (1974), Voronin (1996), Friedrich et al. (1996)
Gurleyides	Water flea	*Ceriodaphnia reticulata*	Voronin (1986)
Holobispora	Copepod	*Thermocyclops oithonides*	Issi (1986)
Hyalinocysta	Copepod[a]	*Orthocyclops modestus*	Andreadis and Vossbrinck (2002)
Inodosporus	Shrimp	*Palaemon* spp. (2); *Palaemonetes* spp. (2)	Codreanu (1966), Sprague and Couch (1971), Overstreet and Weidner (1974) and Azevedo et al. (2000)
Lanatospora	Copepod	*Macrocyclops albidus*	Voronin (1986)
Larssonia	Water flea	*Daphnia* spp. (2)	Vidtman and Sokolova (1994), Bengtsson and Ebert (1998)
Marssoniella	Copepod	*Cyclops* spp. (2)	Vossbrinck et al. (2004) and Vávra et al. (2005)
Microsporidium	Water flea	*Daphnia pulex*	Refardt et al. (2002)
Mrazekia	Copepod	*Macrocyclops albidus*	Issi et al. (2010)
	Isopod	*Asellus aquaticus*	Leger and Hesse (1916)
Myospora	Lobster	*Metanephrops challengeri*	Stentiford et al. (2010)
Nadelspora	Crab	*Cancer* spp. (2)	Olson et al. (1994), Childers et al. (1996)
Nelliemelba	Copepod	*Boeckella triarticulata*	Milner and Mayer (1982)
Norlevinea	Water flea	*Daphnia longispina*	Vávra (1984)
Nosema	Amphipod	*Gammarus* spp. (2)	Terry et al. (1999) and Haine et al. (2004)
	Crab	*Carcinus maenas*; *Callinectes sapidus*; *Pachygrapsus marmoratus*	Leger and Duboscq (1909) and Sprague and Couch (1971)
	Ostracod	*Stenocypris major*	Diarra and Toguebaye (1996)
Ordospora	Water flea	*Daphnia magna*	Larsson et al. (1997)
Ormieresia	Crab	*Carcinus mediteraneus*	Vivarès et al. (1977)
Paranucleospora	Copepod	*Lepeophtheirus salmonis*	Nylund et al. (2010)
Perezia	Crab	*Carcinus maenas*	Sprague and Couch (1971)
	Shrimp	*Farfantepenaeus aztecus*; *Litopenaeus setiferus*	Sprague (1950), Sprague and Vernick (1969) and Canning et al. (2002)
Pleistophora	Amphipod	*Gammarus duebeni celticus*	Terry et al. (2003)
	Crab	*Callinectes sapidus*	Sprague and Couch (1971)
	Crayfish	*Cambarellus puer*	Sprague (1966) and Sprague and Couch (1971)
	Shrimp	*Atyephira sp.*; *Branchinella thailandensis*; *Crangon* spp. (4) *Farfantepenaeus* spp. (2); *Litopenaeus setiferus*; *Palaemonetes pugio*	Kudo (1924), Baxter and Rigdon (1970), Sprague and Couch (1971), Streets and Sprague (1974), Krygier and Horton (1975), Breed and Olson (1977), Kelly (1979) and Purivirojkul and Khidprasert (2009)
Thelohania	Crab	*Carcinus maenas*; *Eupagurus bernhardus*; *Petrolisthes armatus*	Sprague and Couch (1971)
	Crayfish	*Astacus* spp. (3); *Cambarellus* spp. (2); *Cherax destructor*	Henneguy (1892), Sprague (1950), Sogandares-Bernal (1962), Sprague and Couch (1971) and Moodie et al. (2003a,b)
	Shrimp	*Crangon crangon*; *Farfantepenaeus* spp. (3); *Palaemonetes variens*; *Pandalus jordani*; *Penaeus semisulcatus*	Sprague and Couch (1971), Vernick et al. (1977), Johnston et al. (1978) and Kelly (1979)
Tuzetia	Copepod	*Boeckella triarticulata*; *Cyclops albidus*	Kudo (1921) and Milner and Mayer (1982)
	Shrimp	*Farfantepenaeus aztecus*; *Litopenaeus setiferus*	Canning et al. (2002)
Vairimorpha	Crayfish	*Cherax destructor*	Moodie et al. (2003c)
Vavraia	Shrimp	*Crangon crangon*	Azevedo (2001)

Note: Figure in brackets refers to number of species belong to the genus.
[a] Intermediate host.