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Note

Studies on microsporidian infection in *Parapenaeopsis stylifera*

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ABSTRACT

Parapenaeopsis stylifera, infected with microsporidians showed a distinct whitish discolouration in their abdominal musculature. Histopathological and histochemical studies of the infected tissues revealed the presence of microsporidian spores in different stages of development and accumulation of iron respectively.

Most of the protozoan parasites are opportunistic pathogens, which cause diseases only when the animal is under stress (Soni, 1986; Johnson, 1990; Sindermann, 1990). Cotton shrimp or milk shrimp disease usually occurs in wild and pond cultured prawns and is caused by microsporidians (Johnson, 1990). Incidences of this disease have been reported in penaeid prawns in different parts of the world (Overstreet, 1973; Owens and Glazebrook, 1988) and in the natural populations of pandalid shrimps namely *Pandalus borealis* from the northwest Atlantic and *P. jordani* from the northeast Pacific (Olsen and Lannan, 1984; Parsons and Khan, 1986). In India, cotton shrimp disease is reported in the natural populations of *Penaeus indicus, P. semisulcatus, Metapenaeus affinis, M. monoceros* and *M. brevicornis* (Soni, 1986). The present study reports the disease in *Parapenaeopsis stylifera* for the first time from India.

Microsporidia are intracellular parasites. Both the sexes of prawns are affected by this. The important species of microsporidians reported from the affected prawns, by histopathological investigations are *Thelohania penaei, T. semisulcata, T. duorara, Nosemia nelsoni* and *Pleistophora* sp. (Lightner, 1988; Soni, 1986). The diseased organisms exhibit symptoms such as opaque, white areas in the abdominal muscle and gonad, brick-red feed line, reddish turned hepatopancreas, inactive swimming, tumor-like swelling of the overlying cuticle due to infection of subcutis, and they often may have dark blue or blackish discolouration due to the expansion of cuticular chromatophores (Lightner, 1983). Experimental transmission studies suggested the involvement of a conditioning intermediate host for transmission (Lightner, 1983). A specific DNA probe was developed by Pasharawipas and Flegel (1994) to identify the intermediate host of a microsporidian parasite of *Penaeus merguiensis.*

The present study was carried out in *Parapenaeopsis stylifera,* which exhibited symptoms of cotton shrimp disease. Two diseased specimens were

obtained from Tuticorin Fishing Harbour, Tamil Nadu, during the southwest monsoon period (June, 1996). They were 55 and 60 mm long from the tip of the rostrum to the tip of the telson and their abdominal musculature clearly exhibited opaque, whitish discolouration.

The abdominal muscles, taken from live animals were kept in normal saline, cut into small pieces of 4 to 5 mm size and fixed in Davidson's fixative (George Clark, 1981) for 24 hours and transferred to 70% ethanol. After the completion of the dehydration process (80, 90 and 100% ethanol for one hour each), the tissues were cleared by immersing in alcohol-chloroform (1:1) mixture for 1-2 hours. Then, two changes were given in absolute chloroform for half an hour each and was left in saturated chloroform-wax mixture overnight. The tissues were processed for routine histology and the deprafinised sections (6-7 u) were stained in haematoxylin-eosin (Culling *etal.,* 1985). The sections were cleared in xylene, mounted in DPX and observed under a microscope. Photomicrographs were taken with a Koehler illumination, automatic exposure unit and colour temperature metering. Histochemically, the presence of iron in the deparaffinised sections was revealed by Bathophenanthroline method, after Hukill and Putt (Pearse, 1985).

Out of the two specimens, one was heavily infected and a large number of spores were observed in the muscular bundles (Fig. 1 and 2). Muscle fibres were found separated from one another and spores in different developmental stages were seen. These were very clear in the longitudinal sections (Fig. 2). In some muscle fibres, the spores were present along the periphery, whereas in others, they were in the central region. They were mostly oval in appearance.

Fig. 1. Cross section of the abdominal muscle of *P. stylifera* infected with microsporidian parasite. Arrows indicate the spores of the parasite. H & E. x 200.

In the advanced stages of infection, the entire muscle fibres were seen occupied by the spores, replacing the nuclei and sarcoplasm (Fig. 2). The endomysium alone was retained. As the pathogens multiplied further, this covering also was ruptured and the spores were liberated outside the muscle fibre. In some regions, the muscle fibres were found severely damaged. Host response to the infection is least developed, as the pathogen did not elicit any significant inflammatory response in the host.

Muscle fibres around the microsporidian spores stained reddish with Bathophenanthroline method, which indicated the presence of iron in the

tissues. The spores coloured dark blue and the non-infected tissues appeared almost transparent. The reddish tinge was high in areas of severe muscle necrosis.

Fig. 2. Longitudinal section of the abdominal muscle of *P. stylifera* infected with microsporidian parasite. Arrows indicate the spores of the parasite. H & E. x 200.

Although detailed studies of cotton shrimp disease have been done in countries outside India (Lightner, 1988; Sindermann, 1990), in India very little work has been done on decapod microsporidiosis, except the studies of Soni (1986) on taxonomy, pathogenecity and histopathology of microsporidians in *P. semisulcatus.* In the present study, the infected organisms viz., *P. stylifera* were obtained from the capture sector at Tuticorin. In India, this disease has been reported in the natural population of *P. indicus, P. semisulcatus, M. affinis, M. monoceros* and *M. brevicornis* (Soni, 1986). The present study gives the first report of *P. stylifera* being infected by cotton shrimp disease in India.

Different organs of prawns such as body muscle, gonads, midgut and hepatopancreas are found to be affected by microsporidians (Lightner, 1983). In the present case, the main target of infection was body muscle, as evidenced by the histological changes observed in them. Breed and Olsen (1977), also reported muscle tissue as the main site of microsporidian infection in *Crangon* sp.

Presence of spores in the muscle fibres and in the inter-muscular spaces, and the eventual replacement of the affected muscle fibres by masses of spores were the noted features in the present study. Lightner (1985) and Soni (1986) also reported spores, released by the rupture of muscle in the inter-muscular spaces. This causes auto-infection.

Histochemical studies revealed the presence of iron, in the necrotised regions. Ferrous ions present in the necrotised area might have been reacted with Bathophenanthroline which resulted in the production of a reddish tinge. Such an observation has not been reported in the microsporidian infected muscles elsewhere.

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