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NOTE

Microsporidians in penaeid shrimp along the west coast of Madagascar

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ABSTRACT: Three species of penaeid shrimp, *Fenneropenaeus indicus*, *Penaeus monodon* and *P. semisulcatus*, found in trawler catches off the west coast of Madagascar were infected with microsporidian parasites. The infections were evident as muscular lesions with a cottony appearance when abundant. Spore size (2.6 × 1.6 µm) and morphology (ovoid) for the parasites infecting both *F. indicus* and *P. semisulcatus* were not significantly different, suggesting that they might be the same microsporidian species. Spore size (1.4 × 1.1 µm) and morphology (sub-globose to ovoid) in *P. monodon* infections were significantly different from those in the other 2 shrimp species, suggesting that it was a different parasite. The presence of microsporidians in this biogeographical zone means that there is a potential risk of infections of cultured shrimp in farms situated in the vicinity. This must be assessed by increasing current knowledge of the parasites.

KEY WORDS: Microsporidians · Penaeid · *Fenneropenaeus indicus* · *Penaeus monodon* · *Penaeus semisulcatus* · Madagascar

INTRODUCTION

Large quantities of *Fenneropenaeus indicus* (also known as *Penaeus indicus* Milne Edwards 1837) are commercially fished off the west coast of Madagascar. *P. semisulcatus* De Haan 1844 is also found in these catches, but the tonnage is lower and is of limited commercial importance. *P. monodon* (Fabricus 1798) is reared semi-intensively on 5 farms close to these fishing grounds, and wild shrimp are often found associated with farmed livestock.

Observations, made by trawlers in this zone, of shrimp in the catches have indicated the presence of microsporidian-type parasites associated with dispersed dorsal muscular lesions. When these lesions are abundant, infected shrimp muscle takes on a cottony appearance (opaque white) which is visible to the naked eye, therefore enabling them to be eliminated from commercial processing. The presence of these microsporidians is frequently observed in wild shrimp, but only in localized areas (trawler information). The objective of this study was to examine the morphology of microsporidian spores observed in 3 species of shrimp from Madagascar.

MATERIALS AND METHODS

Three economically important penaeid species were examined: *Fenneropenaeus indicus*, *Penaeus monodon* and *P. semisulcatus*. Specimens were selected visually, based on their cottony appearance, as soon as they were caught in professional trawling operations off the west coast of Madagascar, south of Mahajanga.

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Selected specimens were frozen at –18°C on site for later processing. Thus, only frozen samples were delivered to the laboratory, where tissues were taken from parasitised musculature and processed for examination by light microscopy and scanning electron microscopy (SEM; JEOL 6300F). For photonic microscopy, tissues were squashed on slides and stained by the May-Gründwald-Giemsa method (Kit RAL 555). For SEM, small pieces of abdominal muscle were fixed in 4% osmic fixative, dehydrated in an ascending series of ethanol solutions, critical point dried with CO_2 and coated with gold.

Tissue samples from *Penaeus semisulcatus* were also examined under a variable pressure electron microscope (Hitachi S3000N), allowing for simple and rapid preparation. Samples were placed on a filter and observed directly for a simple comparison with results derived from traditional SEM processing. The light microscope was used to detect the tissue location of the spores, but SEM was used to measure spore dimensions.

To compare the dimensions of spores of the 3 species of penaeid shrimp in 2 × 2 comparisons, Student's *t*-test was used for data where distribution was normal, and the Wilcoxon test where it was not (Saporta 1990). Differences were considered significant when $p < 0.05$.

RESULTS

There was diversity in the morphology and size of the microsporidian spores depending upon the penaeid host species. In *Penaeus monodon*, 57 measured spores were of homogeneous size (1.4 × 1.1 µm) and regular oval symmetry (Fig. 1). In *Fenneropenaeus indicus*, 53 measured spores were irregular in size and dimorphic with large (3.79 × 1.93 µm) and small (2.61 × 1.57 µm) forms. The proportion of large spores was 3.8%. They were ovoid with dissymmetic extremities (Fig. 2). In *P. semisulcatus*, 36 measured spores were also dimorphic with large (3.5 × 2.0 µm) and small (2.6 × 1.6 µm) forms. The proportion of large spores was 2.8%. They were oval and irregular and essentially similar in appearance to those from *F. indicus* (Fig. 2). Sporoblasts contained 4 or 8 spores (Fig. 3).

Spores observed through a variable pressure electron microscope (utilisation of a low pressure = 10 Pa) gave a decrease in image definition and an average increase of 0.5 µm compared to SEM observations.

Statistical tests for *Fenneropenaeus indicus* and *Penaeus semisulcatus* were applied only to small

Fig. 2. *Fenneropenaeus indicus.* Microsporidian spores by scanning electron microscopy

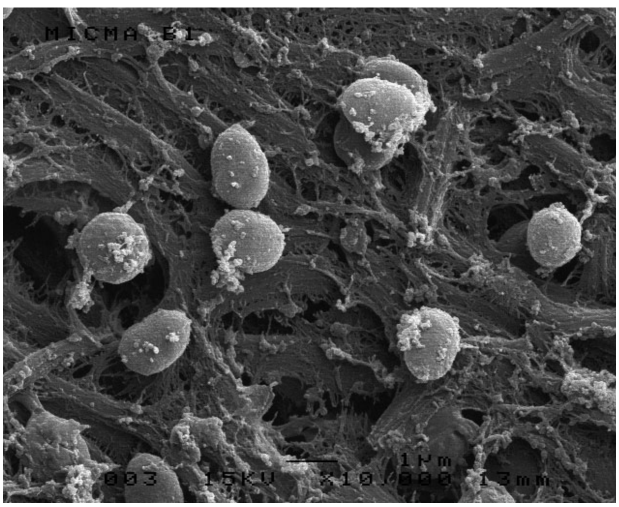

Fig. 1. *Penaeus monodon.* Microsporidian spores by scanning electron microscopy

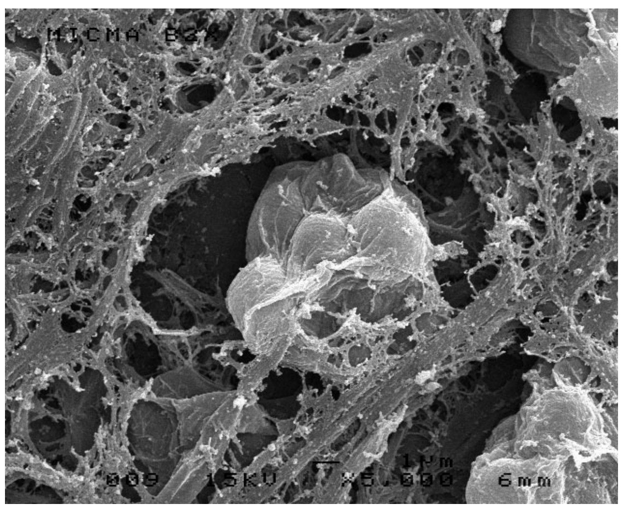

Fig. 3. *Penaeus semisulcatus.* Sporoblast by scanning electron microscopy

spores because the proportion of large spores was statistically too small. The length and width of spores from *P. monodon* and width of spores from *F. indicus* did not follow a normal distribution, while the length and width of spores from *P. semisulcatus* and lengths of spores from *F. indicus* did. There were significant differences between spores found in *P. monodon* and in *F. indicus* or *P. semisulcatus* ($p < 0.0001$ in all comparisons), but there were no significant differences between spores found in *F. indicus* and *P. semisulcatus* ($p = 0.89$).

DISCUSSION

Lightner (1996) listed 11 species of economically important penaeid shrimp in the world that are infected with microsporidian parasites belonging to the genera *Ameson*, *Agmasoma* and *Pleistophora*. With the exception of *Pleistophora penaei*, *Agmasoma duorara*, *A. penaei* and *Ameson nelsoni* described in the Gulf of Mexico (Lightner 1996), the majority of these species have not been identified.

Microsporidian parasites of penaeid shrimp have been reported from numerous biogeographic regions. *Agmasoma penaei* appears to be a parasite of *Penaeus monodon* and *Fenneropenaeus merguiensis* (also known as *P. merguiensis*) from farms in Thailand (Flegel et al. 1992), and *P. notialis* and *P. monodon* in Senegal (Clotilde-Ba & Toguebaye 1994, 2000). Microsporidians of the genus *Thelohania* are parasites of *F. merguiensis* in tropical Australia (Owens & Hall-Mendelin 1990), of *Pandalus jordani* in the United States (Olson & Lannan 1984), and of *P. semisulcatus* in Mandapam in India (Thomas 1976). The genus *Pleistophora* has been reported as parasitising *Pandalus jordani* in the United States (Olson & Lannan 1984), *Litopenaeus stylirostris* (also known as *Penaeus stylirostris*) in Mexico (Alarcon-Gonzales 1990), and *Crangon franciscorum*, *C. nigricauda* and *C. stylirostris* in Yaquina Bay in Oregon (Breed & Olson 1977). Other species of microsporidians have been observed: *Ameson nelsoni* parasitising *Parapenaeus longirostris* in the Mediterranean (Campillo & Comps 1977, Loubes et al. 1977), *Inodosporus* sp. in *Palaemon serratus* (Decapode) along the Atlantic coast of France (Azevedo 1991), and other non-identified microsporidian forms parasitic on *F. indicus* from the south of India (Ramasamy & Pandian 1985), *Pandalus borealis* along the coast of Labrador (Parsons & Khan 1986) and *P. monodon* in Malaysia (Anderson et al. 1989). A new species, *Tuzetia weidneri*, has also been recently described as a parasite on *Litopenaeus setiferus* and *Farfantepenaeus aztecus* (Canning et al. 2002).

Fresh *Agmasoma penaei* (Sprague 1950) spores from *Penaeus monodon* in Senegal (Clotilde-Ba & Toguebaye 2000) were described as measuring 3.9×2.1 µm, which is larger than the spores we found in *P. monodon* along the west coast of Madagascar. However, we could not compare our results with those in other studies because they used fresh samples, whereas all samples examined for the present study were frozen. The fact that samples were frozen may explain why the length and width of some of the spores did not follow a normal distribution.

Use of the variable pressure electron microscope (VPEM) possibly increased spore dimensions due to dilatation under low pressure, and in the absence of fixation; however, a comparative study of results using standard SEM and VPEM scales could be used to correct for artefact size differences. Image quality could also be improved by decreasing pressure up to the charging-effect limit. The method shows potential, but sample preparation must be refined before VPEM results can be used for concrete comparative morphology studies.

Microsporidians can seriously impact fish farms, such as those events which have occurred with seabream in farms on the Languedoc coast of France (Mathieu-Daude et al. 1992). The microsporidians in shrimp from the west coast of Madagascar therefore require further study of their taxonomy, epizootiology and distribution, to accurately assess their potential impact on both wild penaeid populations, as well as the pathogenic risk to shrimp farms located nearby.

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