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NOTE

An assessment of lesions in bay scallops *Argopecten irradians* attributed to *Perkinsus karlssoni* (Protozoa, Apicomplexa)

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ABSTRACT: Lesions found in bay scallops *Argopecten irradians* imported into Atlantic Canada for culture were attributed to a parasite *Perkinsus karlssoni*. It appears, however, that stages from several organisms were combined to create the life cycle of *P. karlssoni*. The ultrastructure of the agent in lesions of bay scallops is not documented adequately to diagnose affinities to the genus *Perkinsus*. Furthermore, there was discrepancy as to whether *P. karlssoni* enlarged in fluid thioglycollate medium, and the shape and structure of the prezoosporangia as well as the movement and life span of the zoospores were not consistent with the genus *Perkinsus*. *P. karlssoni* cannot be considered as a species of *Perkinsus*.

KEY WORDS: *Perkinsus karlssoni* · Apicomplexa · Parasite · Bay scallop · *Argopecten irradians*

Perkinsus is a genus of protistan parasite which infects and kills marine molluscs. It has been found in more than 30 species of molluscs around the world (Goggin & Lester 1987, Perkins 1988). Four species have been described: *P. marinus* from eastern oysters *Crassostrea virginica* in America (Mackin et al. 1950, Levine 1978); *P. atlanticus* from clams *Ruditapes decussatus* in Portugal (Azevedo 1989); *P. olseni* from abalone *Haliotis rubra* in Australia (Lester & Davis 1981); and *P. karlssoni* from bay scallops *Argopecten irradians* in eastern Canada and adjacent United States (McGladdery et al. 1991, McGladdery et al. 1993). There are significant differences, however, between *P. karlssoni* and the other 3 described species of *Perkinsus*. *P. karlssoni* is used hereafter as a matter of convenience with the recognition that there may be more than 1 organism associated with this name which may later be described outside the genus *Perkinsus*.

Bay scallops *Argopecten irradians* imported into Canada for culture in 1979 and 1980 suffered heavy post-spawning mortalities. McGladdery et al. (1991) described 'swirl' lesions from the bay scallops which they attributed to the parasite *Perkinsus karlssoni*. 'Swirl' lesions were most numerous in post-spawning scallops. Bay scallops, however, usually die after spawning and *P. karlssoni* was not associated with the pathogenesis. The 'swirl' lesion found in *A. irradians* is a generalised response to a foreign agent and is not diagnostic of an infection by parasites in the genus *Perkinsus*. *P. karlssoni* which were healthy when preserved have not been seen under the electron microscope. The organisms identified as *P. karlssoni* in electron micrographs published by Whyte et al. (1994) (Fig. 18a, b) are dead organisms encapsulated by ceroid and do not resemble *Perkinsus*.

In histological sections, the meront of species of *Perkinsus* is distinguished by a large eccentric vacuole which sometimes contains a vacuoplast. A vacuole was rarely observed in *P. karlssoni*, but a dense basophilic body was found next to the nucleus which could be equivalent to the vacuoplast of *P. marinus* (McGladdery et al. 1991). The basophilic body of *P. karlssoni*, however, was not contained in a large eccentric vacuole, which makes this association unlikely. Furthermore, the presence of a basophilic body does not diagnose a *Perkinsus* spp. because the vacuoplast can be either basophilic or eosinophilic in *P. marinus* (Mackin et al. 1950) or slightly eosinophilic (when present) in *P. olseni* (Lester & Davis 1981).

Perkinsus karlssoni differs from described species of *Perkinsus* in other ways. *P. karlssoni* was not recognised by an antibody raised against the meront of *P.*

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marinus that binds to the 3 other described species of *Perkinsus* in histological sections (Dungan & Roberson 1993). The 'swirl' lesion which surrounds *P. karlssoni* does not account for the failure of the immunological test because the antibody recognised *Perkinsus* sp. in similar lesions in clams *Mya arenaria* (C. Dungan pers. comm.). These results suggest that *P. karlssoni* does not belong to the genus *Perkinsus*.

Perkinsus infections are diagnosed by incubating host tissues in fluid thioglycollate medium (FTM) for several days. The parasites enlarge and can be stained with Lugol's iodine after incubation (Ray 1966). Blue-black spheres were found in tissues of *Argopecten irradians* from Canada using this procedure (McGladdery et al. 1991, Whyte et al. 1994). McGladdery et al. (1991) reported that meronts increased from 4.3–23.11 µm to reach 80.0–138.3 µm after incubation in FTM. Whyte et al. (1993a, p. 203) found, in contrast, that *P. karlssoni* did not swell in FTM. The difference in the results of the FTM test suggest that Whyte et al. (1993a, b) isolated a different organism from that described by McGladdery et al. (1991). Indeed, the 'prezoosporangium' isolated from bay scallop tissues that had not been incubated in FTM (Whyte et al. 1993a, Fig. 1b) does not belong to the genus *Perkinsus*. The organism has membranous plates or scales around the cell, multivesicular bodies in the cytoplasm and mitochondrial structure more closely resembling that of the thraustochytrids than that of *Perkinsus* spp. (see Moss 1985).

Meronts of *Perkinsus* spp. enlarge in FTM to form zoosporangia when released into seawater. Cells divide internally to produce thousands of motile zoospores which later escape through a discharge tube or plug on the surface of the zoosporangia. Prezoosporangia of *Perkinsus* spp. do not multiply when released in seawater. In contrast, bay scallop tissues incubated in FTM at 22°C develop aggregations of several hundred large *P. karlssoni* when placed in seawater (McGladdery et al. 1991). Some thraustochytrids appear viable after incubation in FTM (Quick 1972). they could proliferate in seawater and account for this observation.

The prezoosporangia described by McGladdery et al. (1991) differ from those of *Perkinsus* species in other ways. Successive bipartitioning of the protoplast of the cell as described for *Perkinsus* spp. (Perkins & Menzel 1966) was not observed in *P. karlssoni*. Furthermore, the discharge tube of the 'zoosporangium' of *P. karlssoni* was filled with cytoplasm which is unlike other *Perkinsus* spp. (Perkins & Menzel 1966, Lester & Davis 1981, Azevedo et al. 1990). The 'zoosporangia' described by McGladdery et al. (1991) may be equivalent to the hyphal form of *P. marinus* found occasionally in FTM (Ray & Chandler 1955, Quick 1972) and culture media (La Peyre et al. 1993). Whether this hyphal form is a phase of the life cycle in *P. marinus* or a contaminant is not clear. Cells with hyphal-like outgrowths, however, are neither the usual nor the dominant form of the parasite and failure to observe the typical prezoosporangia of *Perkinsus* casts doubt on the diagnosis of McGladdery et al. (1991).

Perkinsus spp. release zoospores from the prezoosporangia (developed in FTM) after several days in seawater. The zoospores are biflagellate with filamentous mastigonemes on one side of the anterior flagellum. Whyte et al. (1993a) found that the most efficient method to collect 'zoosporangia' and zoospores included the maceration of the scallop tissue followed by incubation in seawater at 26°C for 3 d. We attempted to isolate zoospores of *P. karlssoni* using the same procedure. We placed tissues directly into sterile artificial seawater and biflagellates appeared in our cultures after 4 d. DNA was extracted from these organisms and the small subunit ribosomal DNA (SSU rDNA) amplified by polymerase chain reaction (PCR) (Goggin & Barker 1993). The product was sequenced using automatic sequencing (Applied Biosystems, Inc.) and the data compared to those held in computer databases. The sequence (1680 bp) was most similar to the SSU rDNA data from *Cafeteria roenbergensis* (EMBL accession number L27633; Leipe et al. 1994) with differences in 5 positions (2 additional, 2 different, 1 missing). Fourteen ambiguous positions in the SSU rDNA data from *C. roenbergensis* were resolved by our work. These molecular analyses indicate that the biflagellates found in our seawater cultures were members of the genus *Cafeteria* and were most likely *C. roenbergensis*.

Cafeteria roenbergensis is a bicosoecid flagellate reported from waters near Denmark, Australia and Hawaii (Fenchel & Patterson 1988). The cell body of *C. roenbergensis* measures 4–6 × 4–4.5 µm, which is similar in size to that of *Perkinsus marinus* (4–6 × 2–3 µm) and *P. karlssoni* (5.8 × 3.2 µm). *C. roenbergensis* is a heterokont and has a bilateral array of tubular mastigonemes on the anterior flagellum (5 to 8 µm) and a trailing posterior flagellum (5 to 8 µm) which lacks mastigonemes. The zoospores of *P. marinus* have an anterior flagellum with filamentous mastigonemes (10 to 18 µm) and a posterior, naked flagellum (6 to 10 µm) (Perkins & Menzel 1966). The zoospores of *P. karlssoni* are also biflagellate (McGladdery et al. 1991), with a posterior flagellum (2.7 to 5.4 µm) of similar length and an anterior flagellum (11.9 to 17.3 µm) that is longer than that of *C. roenbergensis*. It is possible that Whyte et al. (1993a) confused *C. roenbergensis* with *P. karlssoni* zoospores under the light microscope, particularly as they had not incubated tissues in FTM.

There are other differences between the zoospores of *Perkinsus karlssoni* and those of other species of *Perkinsus*. The behaviour of the zoospores of *P. karlssoni*, as described by McGladdery et al. (1991), is not typical of *Perkinsus* spp. The zoospores of *P. marinus* swim with a jerky motion (Perkins & Menzel 1966), do not adhere usually to the substrate and live for several hours (F. O. Perkins pers. comm.). Zoospores of *P. karlssoni*, in contrast, remained close and adhered weakly to the bottom and were viable for up to 9 wk at 4°C (McGladdery et al. 1991). Interestingly, *Cafeteria roenbergensis* can swim but usually adhere to the substrate by the posterior flagellum. The zoospores of *P. karlssoni* were also unlike those of other species of *Perkinsus* (Perkins 1988, 1991) because they lacked an apical complex, mastigonemes on the anterior flagellum and a large cylindrical inclusion in each kinetosome lumen (McGladdery et al. 1991).

Apparently, the life cycle of *Perkinsus karlssoni* comprises several unrelated organisms. It is not likely that the differences observed between *P. karlssoni* and *Perkinsus* species result from 'ten years of transmission via hatchery manipulated spawning' as postulated by McGladdery et al. (1991). The 'prezoosporangia' and 'zoospores' attributed to *P. karlssoni* do not belong to the genus *Perkinsus*. Perhaps the lesions in bay scallops are caused by a *Perkinsus* sp. and most parasites die. The hepantotype of *P. karlssoni* deposited in the Canadian Museum of Nature (CMNP1990-0027) has 'swirl' lesions and is infected by a coccidian as described by Whyte et al. (1994). Whyte et al. (1994), however, reported no apparent response of the bay scallop to the coccidian. Whether the coccidian causes bay scallops to produce 'swirl' lesions under some conditions is unknown. The agent that causes the bay scallop to produce granulomas needs further investigation to determine its taxonomic affinities. It is not certain that it is a species of *Perkinsus* and we do not recognise *P. karlssoni* as a species of *Perkinsus*.

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