

Use of histopathology, PCR and in situ hybridization methods to detect the parasite



Mikrocytos mackini is the etiological agent of Denman Island disease, which causes significant mortalities in commercially important bivalve species, including the Pacific oyster, *Crassostrea gigas*. A close relative of *M. mackini*, *Mikrocytos* sp., was recently detected in oysters imported into France from Canada. In this study, we examined Pacific oysters from the northern coast of the Yellow Sea, China. Of the one hundred samples examined histologically, a microcell parasite was found in the tissues of four oysters. To identify whether the parasite was *Mikrocytos* sp., DNA was extracted from the oysters and polymerase chain reaction (PCR) amplifications were performed with primers (*Mikrocytos*-F and *Mikrocytos*-R), which yielded the expected 522 bp fragment. DNA sequencing of these products confirmed that they were identical to the corresponding 18S region of *Mikrocytos* sp. (100%) and had close similarity to *M. mackini* (89%). In situ hybridization (ISH) also was performed in this study, and the primer pair MM-like (CCTGTCCTATGTCGGGCAGG) hybridized with the Pacific oyster parasite. This is the first report of *Mikrocytos* sp. in

the Pacific oyster from the coast of China. Although this study suggests a low prevalence of the parasite in China, its potential threat to aquaculture should be considered.

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