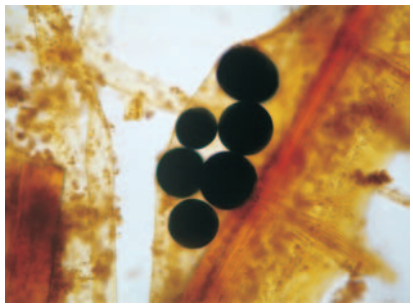
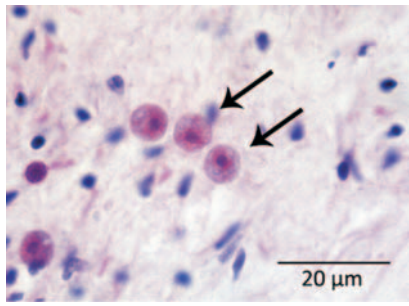
First report of an OIE listed protozoan parasite, *Perkinsus olseni*, from Indian pearl oyster population

World over, there have been many reports on the mass mortalities in natural and farmed bivalves and protozoan parasites of the genus *Perkinsus* have been incriminated as a cause of mass mortalities in various bivalves. So far, incidence of Perkinsosis/ other OIE notified pathogens in mollusks has not been reported from the Indian subcontinent (NACA 2009).

Pearl oysters from the traditional oyster beds at Tuticorin were examined using OIE approved diagnostic techniques. Ray's Fluid Thioglycollate Medium (RFTM) assay of the *P. fucata* tissues showed enlarged blue-black hypnospores characteristic of *Perkinsus* sp. with a prevalence of 100%. Histopathological examination of the gill and mantle tissues revealed the presence of the trophozoites of *P. olseni* in the connective tissues.

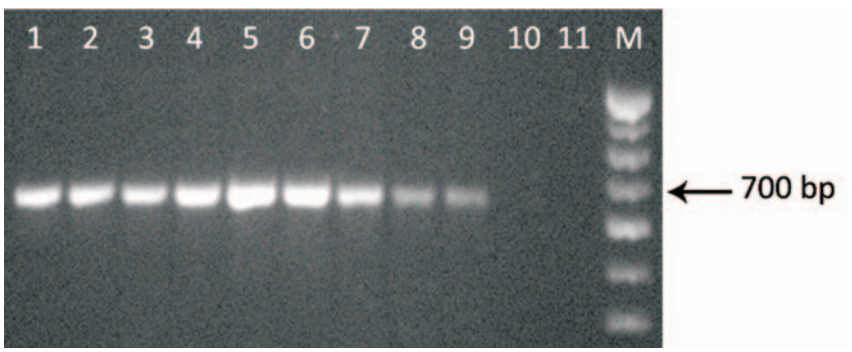


Perkinsus Hypnospores in mantle



Trophozoites of Perkinsus sp.

For confirming the RFTM results and the generic level identity of the pathogen, the samples were further screened using Perkinsus genus-specific ITS-85 and ITS-750 primers. All the samples were positive for PCR and specific amplicons of *Perkinsus* sp. was obtained (ca. 700 bp), confirming the presence of *Perkinsus* sp. in *P. fucata.*



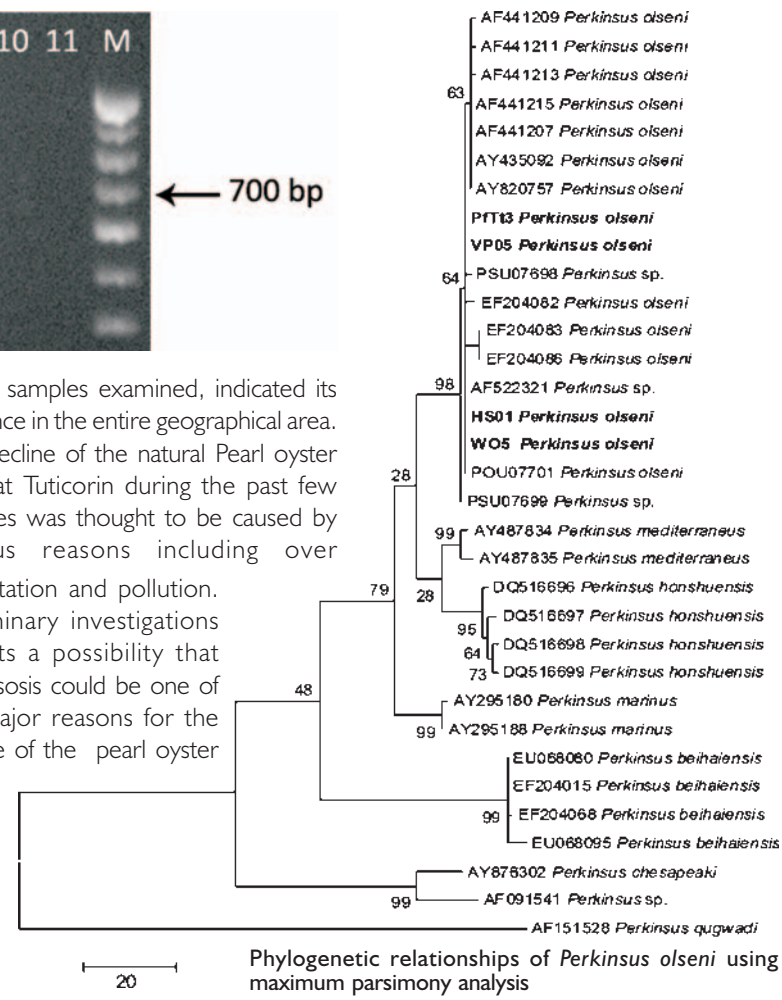
Agarose gel electrophoresis of the amplified products of the PCR using *Perkinsus* genus specific ITS 85 & ITS 750 primers (703 bp); lanes 1-3, DNAs of oysters from Tuticorin; 4-5, DNAs of oysters from Vellapatti; 6-7, DNAs of oysters from Kayalpatanam; 8-9, DNAs of Hatchery reared oysters from Tuticorin; 10, negative tissue control; 11, negative control and M, molecular size marker (100 bp ladder)

The amplified PCR products were sequenced and phylogenetic analysis done. The pairwise genetic distance between the present isolate and other members of the *P. olseni* group studied, was very low, confirming the taxonomic identity of the parasite as *Perkinsus olseni.* The maximum parsimony and neighbor joining analysis of the nucleotide sequences of the ITS region of the parasite further confirmed its identity as *Perkinsus olseni.*

Presence of *Perkinsus* sp. in all the oyster samples examined, indicated its presence in the entire geographical area. The decline of the natural Pearl oyster beds at Tuticorin during the past few decades was thought to be caused by various reasons including over exploitation and pollution. Preliminary investigations reflects a possibility that Perkinsosis could be one of the major reasons for the decline of the pearl oyster beds at Tuticorin over a period of time.

This forms the first report on the existence of a *Perkinus olseni.*, a protozoan parasite in *P. fucata* from the southeast coast of India and is also the first report of an OIE listed pathogen from the Indian sub-continent and south Asia. The host range and epizootiology of the parasite in Indian waters remains to be determined and more studies are required.

(Marine Biotechnology Division)



Phylogenetic relationships of *Perkinsus olseni* using maximum parsimony analysis