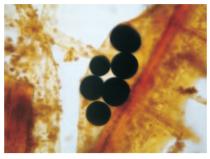
## First report of an OIE listed protozoan parasite, Perkinsus olseni, from Indian pearl oyster population

 $\bigwedge \bigwedge$  orld 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.

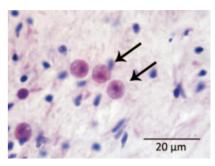
For confirming the RFTM results and the generic level identity of the patho-



Perkinsus Hypnospores in mantle

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



Trophozoites of Perkinsus sp.

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)

AF441209 Perkinsus olseni

AF441211 Perkinsus olseni AF441213 Perkinsus olseni AF441215 Perkinsus olseni AF441207 Perkinsus olseni AY435092 Perkinsus olseni

AY820757 Perkinsus olseni



gen, 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 I-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)

PfTt3 Perkinsus olseni VP05 Perkinsus olseni - PSU07698 Perkinsus sp. EF204082 Perkinsus olseni EF204083 Perkinsus otseni EF204086 Perkinsus olseni oyster samples examined, indicated its AF522321 Perkinsus sp. HS01 Perkinsus olseni presence in the entire geographical area. WO5 Perkinsus olseni The decline of the natural Pearl oyster POU07701 Perkinsus olseni beds at Tuticorin during the past few PSU07699 Perkinsus sp. decades was thought to be caused by 99 F AY487834 Perkinsus mediterraneus various reasons including over AY487835 Perkinsus mediterraneus DO516696 Perkinsus hanshuensis exploitation and pollution. - DQ516697 Perkinsus honshuensis Preliminary investigations 64 DQ516698 Perkinsus honshuensis reflects a possibility that L DQ516699 Perkinsus honshuensis Perkinsosis could be one of 48 AY295180 Perkinsus marinus the major reasons for the gg AY295188 Perkinsus marinus decline of the pearl oyster EU068080 Perkinsus beihalensis EF204015 Perkinsus beihaiensis EF204068 Perkinsus beiheiensis – EU068095 Perkinsus beihaiensis AY876302 Perkinsus che sapeaki - AF 091541 Perkinsus sp. - AF151528 Perkinsus quqwadi Phylogenetic relationships of Perkinsus olseni using 20 maximum parsimony analysis