Microsporidian infection of the hepatopancreatic epithelial cells of Chinese mitten crab *Eriocheir sinensis* from the River Thames, UK

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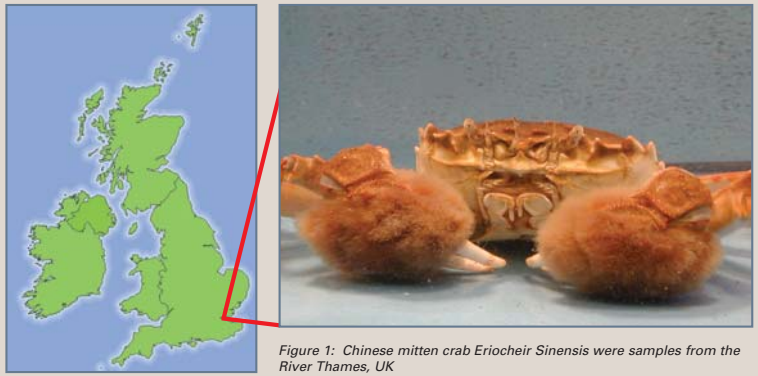
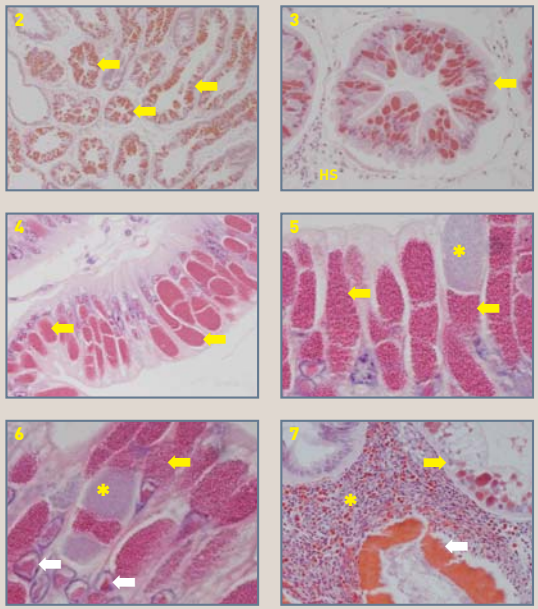


Figure 1: Chinese mitten crab Eriocheir Sinensis were samples from the River Thames, UK

The Chinese mitten crab *Eriocheir Sinensis* is an alien species to UK waters originating from the Far East. The invasive species is thought to have been introduced into Western Europe via ship ballast water and has spread throughout the region, becoming established within the River Thames, UK from the 1970's onwards. Mitten crabs were collected monthly from the Thames estuary over a 12-month period and examined for the presence of pathogens and parasites.



Figures 2-7: Hepatopancreas of microsporidian-infected Chinese mitten crab.
(2) Infected HP tubules. Microsporidian packets within HP epithelial cells (arrows);
(3) Single infected tubule (arrow) with normal haemal sinus (HS) surrounding;
(4) Multiple packets of Microsporidian parasites in epithelial cells (arrows);
(5) Two types of packet are observed – a eosinophilic (pink) type containing mature spores (arrows) and a basophilic (mauve) type containing parasite plasmodial stages (asterisk);
(6) Similar to Figure 4 with spore packets (yellow arrows) and plasmodial stages (asterisk). Note multiple nuclei within individual HP cells and the presence of eosinophilic inclusions in the nucleoplasm (white arrows);
(7) Host response to a degenerate tubule (white arrow). Necrotic region surrounded by masses of hyaline cells and granulocytes (asterisk). An infected HP tubule appears not to elicit such a response (yellow arrow). All images H&E.

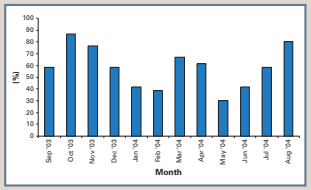
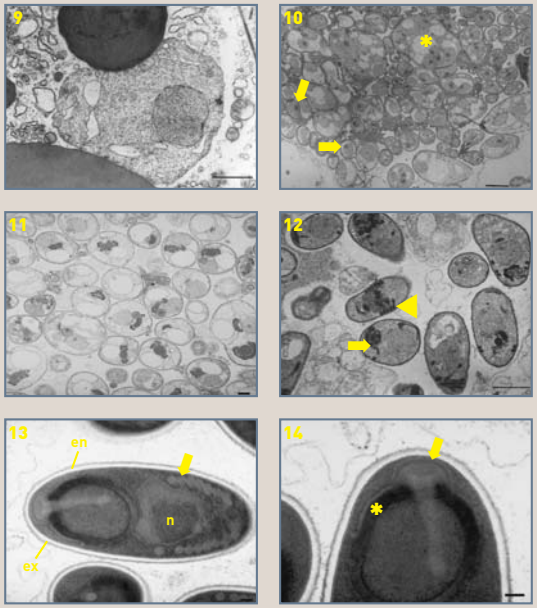


Figure 8: Prevalence of infection over 12 month study

Histology revealed a microsporidian parasite infecting the hepatopancreas. Microsporidia are obligate intracellular parasites and are the most common parasites in Crustacea causing severe pathology in some host species. Most infect muscle with few described species infecting the hepatopancreas. White focal patches were observed on the hepatopancreas though infected crabs showed no external symptoms of disease. Infection was highly prevalent (Figure 8) peaking in late autumn and early winter.



Figures 9-14: Transmission Electron Micrographs
(9) Meront within the cytoplasm of an epithelial cell of a hepatopancreatic tubule. Nuclei can be seen within the cell. Scale bar = 1µm;
(10) Meronts (*) and sporonts (arrow) developing within a sporophorous vesicle. Meronts are multinucleate large cells, sporonts are uninucleate and starting to develop organelles. Scale bar = 2µm;
(11) Sporonts developing within sporophorous vesicle. Nuclei can be seen along with endoplasmic reticulum and vacuoles. Scale bar = 500nm;
(12) Maturing sporoblasts within a sporophorous vesicle. Nuclei can be seen as can endoplasmic reticulum, polar filament is forming (arrow) and aligning along the side of the sporoblast (arrow head). Scale bar = 1µm;
(13) Mature spore clearly showing the isofilar polar filament, 7-8 turns (arrow). Anchoring disc and polaroplast is present. Spore wall shows two layers the electron lucent endospore (en) and the electron dense exospore (ex). The nucleus (n) can be seen centrally located. Scale bar = 100nm;
(14) Anchoring disc (arrow) and polaroplast (*) within a mature spore. Scale bar = 100nm.

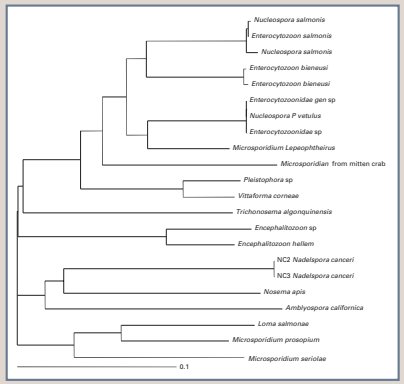


Figure 15: Clustal similarity tree showing the relationship between the microsporidian in mitten crab to those observed in a range of terrestrial and aquatic hosts based on partial (260bp) 18S rRNA gene sequences. PCR primers were based on an alignment of the published sequences for microsporidium and the amplified product was sequenced using the ABI PRISM™ dye terminator cycle sequencing system (Applied Biosystems, Warrington, UK) and analysed on a 3100 genetic analyser. Multiple alignment and the similarity tree were generated using the Multiple Alignment package (MAGI) at the Human Genome Mapping Project Resource Centre (HGMP-RC), Hinxton, UK and TreeView.

Here we report on a microsporidian infection of Chinese mitten crabs (*Eriocheir sinensis*) from the River Thames, UK. The parasite has not been reported from native populations of *E. sinensis* in Asia suggesting that this is a relatively recent emergent pathogen in this species in UK waters. Molecular phylogeny revealed a <84% and a <82% nucleotide identity with the microsporidian species *Microsporidium lepeophtheirus* and *Nucleospora salmonis* respectively. This together with the morphological and histological data suggests that this organism represents the first of a new species of a novel genus of Microsporidia. The significance of this pathogen as a mortality factor and therefore population regulator in mitten crabs from the River Thames has not been investigated.

Acknowledgements

The authors acknowledge the support of the London Port Health Authority for funding this work.