

RESEARCH

Open Access

# Molecular identification and transmission studies of X-cell parasites from Atlantic cod *Gadus morhua* (Gadiformes: Gadidae) and the northern black flounder *Pseudopleuronectes obscurus* (Pleuronectiformes: Pleuronectidae)

MA Freeman<sup>1,2\*†</sup>, M Eydal<sup>3†</sup>, M Yoshimizu<sup>5</sup>, K Watanabe<sup>6</sup>, AP Shinn<sup>2</sup>, K Miura<sup>7</sup>, K Ogawa<sup>4†</sup>

## Abstract

**Background:** Epidermal pseudotumours from *Hippoglossoides dubius* and *Acanthogobius flavimanus* in Japan and gill lesions in *Limanda limanda* from the UK have been shown to be caused by phylogenetically related protozoan parasites, known collectively as X-cells. However, the phylogenetic position of the X-cell group is not well supported within any of the existing protozoan phyla and they are currently thought to be members of the Alveolata. Ultrastructural features of X-cells in fish pseudotumours are somewhat limited and no typical environmental stages, such as spores or flagellated cells, have been observed. The life cycles for these parasites have not been demonstrated and it remains unknown how transmission to a new host occurs.

In the present study, pseudobranchial pseudotumours from Atlantic cod, *Gadus morhua*, in Iceland and epidermal pseudotumours from the northern black flounder, *Pseudopleuronectes obscurus*, in Japan were used in experimental transmission studies to establish whether direct transmission of the parasite is achievable. In addition, X-cells from Atlantic cod were sequenced to confirm whether they are phylogenetically related to other X-cells and epidermal pseudotumours from the northern black flounder were analysed to establish whether the same parasite is responsible for infecting different flatfish species in Japan.

**Results:** Phylogenetic analyses of small subunit ribosomal DNA (SSU rDNA) sequence data from Atlantic cod X-cells show that they are a related parasite that occupies a basal position to the clade containing other X-cell parasites. The X-cell parasite causing epidermal pseudotumours in *P. obscurus* is the same parasite that causes pseudotumours in *H. dubius*. Direct, fish to fish, transmission of the X-cell parasites used in this study, via oral feeding or injection, was not achieved. Non-amoeboid X-cells are contained within discrete sac-like structures that are loosely attached to epidermal pseudotumours in flatfish; these X-cells are able to tolerate exposure to seawater. A sensitive nested PCR assay was developed for the sub clinical detection of both parasites and to assist in future life cycle studies. PCR revealed that the parasite in *P. obscurus* was detectable in non-pseudotumorous areas of fish that had pseudotumours present in other areas of the body.

**Conclusions:** The inability to successfully transmit both parasites in this study suggests that either host detachment combined with a period of independent development or an alternate host is required to complete the life cycle for X-cell parasites. Phylogenetic analyses of SSU rDNA confirm a monophyletic grouping for all sequenced X-cell parasites, but do not robustly support their placement within any established protist phylum. Analysis of SSU rDNA from X-cells in Japanese flatfish reveals that the same parasite can infect more than one species of fish.

\* Correspondence: mark@um.edu.my

† Contributed equally

<sup>1</sup>Institute of Ocean and Earth Sciences & Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia

Full list of author information is available at the end of the article