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A parasitic scuticociliate infection in the Norway lobster (*Nephrops norvegicus*)

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Abstract

A histophagous ciliate infection was discovered in a number of Norway lobsters (*Nephrops norvegicus*) from the Clyde Sea Area, Scotland. Silver-carbonate staining of cultured ciliates revealed an oral apparatus and additional structural features that are morphologically similar to scuticociliates in the genus *Mesanophrys*, which are known to parasitize crustaceans. However, ribosomal DNA sequences (ITS1/5.8S/ITS2) of the ciliate were identical to *Orchitophyra stellarum,* a parasitic scuticociliate of sea stars with a different morphology from *Mesanophrys* spp. and to the ciliate from *N. norvegicus*. Associated pathology included degeneration and necrosis of the myocardial heart muscle, and large numbers of ciliates in the gill filaments.

Keywords: *Nephrops norvegicus*; *Mesanophrys*; Infection; Parasitic ciliate; Orchitophyra

1. Introduction

Systemic infections of parasitic ciliates have been reported from many marine organisms, such as fish (Iglesias et al., 2001; Munday et al., 1997), sea stars (Byrne et al., 1997; Stickle et al., 2001), and bivalve molluscs (Bower and Meyer, 1993; Elston et al., 1999; Karatayev et al., 2002). In contrast, reports of systemic infections of crustacea are relatively rare, but have received recent attention due to their detrimental impact on several ecologically and economically important crustacean species (for review, see Morado and Small, 1995). Systemic ciliate infections have been reported for American lobsters by *Anophryoides haemophilia* (Aiken et al., 1973; Cawthorn et al., 1996), freshwater crayfish by *Tetrahymena pyriformis* (Edgerton et al., 1996), prawns by *Parauronema* sp. (Couch, 1978) and krill by *Collinia* sp. (Gómez-Gutiérrez et al., 2003). In addition, several species of the marine scuticociliate *Mesanophrys* (=*Anophrys*, *Paranophrys*) have been described from a variety of crab hosts (Armstrong et al., 1981; Bang et al., 1972; Cattaneo, 1888; Messick and Small, 1996; Morado and Small, 1994; Poisson, 1930; Sparks et al., 1982), as well as isopods (Hibbits and Sparks, 1983; Wiąckowski et al., 1999).

The occurrence of *Mesanophrys*-like ciliated protozoa in Norway lobsters has previously been observed in association with an infection by parasitic dinoflagellates of the genus *Hematodinium* (Appleton, 1996; Field et al., 1992). However, the ciliates were never examined in detail. In the present study, a systemic parasitic ciliate infection was discovered in two individual Norway lobsters during routine investigations into the seasonal prevalence of *Hematodinium* infection. The first infection was noted in a lobster 2 days after capture, and the second was found in a lobster held in captivity for 14 days. Both hosts were captured from the Clyde Sea Area. These findings provided an opportunity to investigate the morphology, associated histopathology and

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ribosomal DNA (rDNA) sequence data for this parasitic ciliate found in the haemolymph and tissues of *Nephrops norvegicus*.

2. Materials and methods

2.1. Sample collection and infection monitoring

Norway lobsters (*N. norvegicus*) were caught by otter bottom trawl (70-mm mesh size) at locations south of Little Cumbrae Island in the Clyde Sea Area (55.41°N, 4.56°W). Following sterilisation of the cuticle with 70% (v/v) ethanol, haemolymph samples were withdrawn from the base of the fifth pereiopod using a disposable 1-ml syringe and 25-g needle. A drop of haemolymph was smeared onto a poly-L-lysine-coated slide and viewed under light microscopy for the presence of ciliate parasites. Bi-monthly samples of haemolymph from 50 freshly caught lobsters from the same sample location as above from August 2002 until August 2003 were collected and analysed for the presence of ciliates after its initial discovery in the two lobsters in November 2001.

2.2. Histology

Samples of heart, gill, hepatopancreas, and tail muscle tissue were removed from the two infected lobsters. Tissues were fixed in 10% (v/v) formol saline, processed through a standard dehydration in an ethanol series, and embedded in paraffin. Sections of 6 μm thickness obtained from each tissue were stained in haematoxylin and eosin (H&E) prior to examination by light microscopy.

2.3. Ciliate culture

The culture medium consisted of 10% (v/v) heat-inactivated foetal calf serum (HI-FCS) in autoclaved, balanced, *N. norvegicus* saline (Appleton and Vickerman, 1998, containing NaCl, 27.99 g L^{-1}; KCl 0.95 g L^{-1}; $CaCl_2$ 2.014 g L^{-1}; $MgSO_4$ 2.465 g L^{-1}; Na_2SO_4 0.554 g L^{-1}; and Hepes 1.92 g L^{-1}) adjusted to pH 7.8, with penicillin G (10 U ml^{-1}) and streptomycin (10 μg ml^{-1}) added to inhibit bacterial contamination. Following sterilization of the cuticle with 70% (v/v) ethanol, haemolymph samples were withdrawn aseptically from the base of the fifth pereiopod using a 1-ml disposable syringe and 25-g needle. The parasites were isolated in 3.5-cm well-plates with 0.2 ml infected haemolymph added to 5 ml filter sterilised (0.2 μm) culture medium in each well. Cultures were incubated at 8 °C.

2.4. Pyridine silver carbonate staining

Ciliates cultured in *N. norvegicus* saline supplemented with 10% (v/v) FCS and antibiotics were used for ammonical silver carbonate staining as described by Fernández-Galiano (1994) with slight modifications. Briefly, 2 ml ciliate culture (5×10^4 cells ml^{-1}) was added to 0.5 ml formaldehyde, followed by 7.5 ml double distilled H_2O (ddH_2O), and the fixed ciliates were collected immediately by centrifugation at 400*g* for 5 min. The supernatant was discarded and the remaining cell pellet was resuspended in 10 ml ddH_2O and centrifuged at 400*g* for 5 min. This washing step was repeated four times to remove salts from the culture medium. The following were added in strict order to the fixed ciliates in 0.5 ml ddH_2O in a 40-ml beaker: three drops formalin (40% w/v), 5 ml ddH_2O, 20 drops bacteriological peptone solution (5 g bactopeptone dissolved in 100 ml ddH_2O, with the addition of 25 drops formalin (40% w/v)), 10 drops pyridine, 2 ml ammoniacal silver carbonate solution (see Fernández-Galiano, 1994), and 10 ml ddH_2O. The suspension was mixed and the beaker was placed in a water bath at 65 °C for approximately 15 min until the solution darkened to a brown black colour. The impregnated ciliate cell suspension was then centrifuged at 100*g* for 2 min, the supernatant was removed and the residual ciliate suspension was placed on slides and viewed under a microscope. Morphometric measurements of stained ciliates were taken using the computer package NIH Image (Scion).

2.5. Electron microscopy

Ciliate cells were fixed in 2.5% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) and 1.75% (w/v) sodium chloride for 2 h at room temperature. Fixed ciliates were washed in 0.1 M sodium cacodylate buffer (pH 7.4) before being post-fixed in 1% (w/v) osmium tetroxide in sodium cacodylate buffer (pH 7.4) for 1 h. Samples were rinsed in buffer and then dehydrated through a graded acetone series. SEM samples were critical point dried and sputter coated in a layer of gold approximately 5 nm thick. TEM samples were infiltrated with Epon premix resin 812 and polymerised in an oven overnight at 60 °C. Semi-thin sections (1–2 μm) were stained with toluidine blue, and ultrathin sections (70–90 nm) were collected on copper grids and stained using uranyl acetate and Reynolds' lead citrate. Preparations were examined using a JEOL 1210 transmission electron microscope and a JEOL 5200 scanning electron microscope.

2.6. Ribosomal DNA amplification and sequencing

Ciliate genomic DNA was extracted from an in vitro culture according to standard procedures (Sambrook et al., 1989). Briefly, 1×10^5 cells were centrifuged at 1000*g* for 4 min at 4 °C. The resulting cell pellet was resuspended in 250 μl extraction buffer (50 mM Tris, 5 mM EDTA, and 100 mM NaCl, pH 8), 100 μl of 10% (w/v) SDS and proteinase-K (0.28 ng μl^{-1}) and incubated at 56 °C for 18–24 h. DNA was purified by a single step standard phenol/chloroform (1:1) extraction, precipitated in 550 μl of 100% ethanol using 20 μl of 5 M NaCl, and resuspended in 100 μl sterile deionised water. DNA concentrations and purity were estimated by measuring the 260/280 optical density ratios using a spectrophotometer and adjusted accordingly. The first internal transcribed spacer (ITS1), 5.8S gene and second