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

H.J. Small a,*,1, D.M. Neil a, A.C. Taylor a, K. Bateman c, G.H. Coombs b

a Division of Environmental and Evolutionary Biology, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, Scotland, UK
b Division of Infection and Immunity, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, Scotland, UK
c EM Unit, The Centre for Environment, Fisheries and Aquaculture Science (CEFAS) Weymouth Lab, Weymouth, Dorset, DT4 8UB, England, UK

Received 13 June 2005; accepted 15 August 2005

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; Karatyayev 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 Anophrvoides 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; Wiackowski 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...
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; CaCl₂ 2.014 g L⁻¹; MgSO₄ 2.465 g L⁻¹; Na₂SO₄ 0.554 g L⁻¹; and Heps 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 sterilisation 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 x 10⁴ cells ml⁻¹) was added to 0.5 ml formaldehyde, followed by 7.5 ml double distilled H₂O (ddH₂O), and the fixed ciliates were collected immediately by centrifugation at 400g for 5 min. The supernatant was discarded and the remaining cell pellet was resuspended in 10 ml ddH₂O and centrifuged at 400g 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 ddH₂O in a 40-ml beaker: three drops formalin (40% w/v), 5 ml ddH₂O, 20 drops bacteriological peptone solution (5 g bactopeptone dissolved in 100 ml ddH₂O, 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 ddH₂O. 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 100g 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 5nm thick. TEM samples were infiltrated with Epon premix resin 812 and polymerised in an oven overnight at 60°C. Semi-thin sections (1–2μm) were stained with toluidine blue, and ultrathin sections (70–90nm) 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 x 10⁵ cells were centrifuged at 1000g 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
internal transcribed spacer (ITS2) of the ribosomal gene complex were amplified using oligonucleotides previously described to amplify rDNA from scuticociliates (Goggin and Murphy, 2000). The amplification reaction mixtures contained 100 ng genomic DNA, 26.6 mM Tris–HCl, pH 8.8, 13.3 mM KCl, 13.3 mM (NH₄)₂SO₄, 2.6 mM MgSO₄, 2 μg BSA, 0.13% (v/v) Triton X-100, 100 μM dNTPs, 7.5 pmol each primer, 1 U of Pfu DNA polymerase (Promega), and sterile deionised water to a final volume of 15 μL. Reactions were overlaid with 10 μL of mineral oil. Thermal cycling conditions were as follows: denaturation at 94 °C for 1 min; primer annealing at 55 °C for 1 min; chain extension at 72 °C for 5 min; repeated for 30 cycles, with a final cycle incorporating a 7-min extension at 72 °C. Amplification products were separated on 1.5% (w/v) agarose gels, stained with ethidium bromide and viewed under UV illumination; images were obtained using a gel documentation system (Appligene). Each amplification product of approximately 750 bp was excised from the agarose gel and purified using a QIA-quick gel extraction kit (Qiagen). Purified amplification products were ligated into the pGEM-Easy plasmid vector (Promega), and used to transform Escherichia coli (strain JM 109) by heat shock according to the manufacturer’s instructions. Transformed cells were plated onto LB agar [bacto-agar 1.5% w/v in LB medium (NaCl, 10 g L⁻¹; bacto-tryptone, 10 g L⁻¹; yeast extract, and 5 g L⁻¹, pH 7.5)], containing ampicillin (50 μg ml⁻¹), isopropyl-β-D-thiogalactopyranoside (IPTG, 40 μg ml⁻¹) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal, 100 μg ml⁻¹) and grown overnight at 37 °C. Positive transformations were identified by blue/white selection and selected colonies were grown overnight in LB medium containing ampicillin (50 μg ml⁻¹). Recombinant plasmids were purified using a miniprep kit (Qiagen) according to the manufacturer’s instructions. Plasmid DNA concentrations and purity were estimated by measuring the 260/280 optical density ratios. Ligation of correct product was confirmed by restriction enzyme digestion (EcoRI) and analysis of products by agarose gel electrophoresis. Bi-directional sequencing of three independent PCR’s using the ciliate DNA isolate was performed by MWG-AG Biotech (Germany). The consensus sequence was compared to known sequences stored in GenBank using the Basic Local Alignment Search Tool (BLAST) routine (Altschul et al., 1990) available through the National Center for Biotechnology Information (NCBI). Multiple sequence alignments were constructed with similar sequences using the software programs ClustalX 1.81 (Thompson et al., 1994) and BoxShade 3.21 (http://www.ch.embnet.org/software/BOX_form.html).

3. Results

3.1. Prevalence of ciliates in N. norvegicus

The ciliate infection was originally discovered in two lobsters in November 2001. Bi-monthly sampling and analysis of haemolymph smears from 50 lobsters from August 2002 until August 2003 failed to identify any further lobsters that were infected with ciliates. That is, no ciliate infections were noted in 650 haemolymph samples over the 13 month period.

3.2. Histopathology

Hemocytopenia, a characteristic of crustacean ciliate infections, was indicated by the near absence of haemocytes in haemolymph samples taken from the two infected lobsters. Histologically, the lobsters had extensive systemic infections with ciliates present in all of the tissues examined. The heart had numerous ciliates present in the lumen of the myocardium (Fig. 1) and in the epicardium (Fig. 2).
Myocardial muscle and connective tissues were greatly reduced (when compared with lobsters uninfected by parasites, see Field, 1992). Ciliates were found in large numbers in the haemal spaces of gill filaments, leading to disruption of internal gill structures (Fig. 3). In the hepatopancreatic tissues, ciliates were observed in the haemal spaces between the tubules (Fig. 4). Few haemocytes were present, and those observed formed aggregates, possibly as encapsulation reactions around invading ciliates. The abdominal muscle tissues were largely intact, however ciliates were observed in muscle bundles (Fig. 5). Areas of muscle tissue lysis/consumption were apparent surrounding invading ciliates.

3.3. Morphology and cell structure of the ciliate

The ciliates were uniformly fusiform in shape with a tapering anterior end and a rounded posterior end. Live ciliates were very active and flexible, continually moving forward and stopping to change direction. They have a contractile vacuole (Fig. 6) at the posterior end, and an oral apparatus at the anterior end (Fig. 7). Eleven equally spaced somatic kineties spiralled round the surface of the cell (Fig. 8). Silver impregnated ciliate cells were between

![Fig. 3. Light micrograph of a transverse section of the gills from an infected Norway lobster. Large number of ciliates (arrows) packing gill filaments. Ct, connective tissue. H&E staining. Scale bar = 100 μm.](image)

![Fig. 4. Light micrograph of the hepatopancreas of an infected Norway lobster. Ciliates (arrows) are present in the haemal spaces between the tubules in the hepatopancreas. Hs, haemal space; Tl, tubule lumen. H&E staining. Scale bar = 100 μm.](image)

![Fig. 5. Light micrograph of the abdominal muscle of an infected Norway lobster. Note the areas of apparent tissue lysis surrounding the invading ciliates. H&E staining. Scale bar = 100 μm.](image)

![Fig. 6. Differential interference contrast (DIC) image of a live ciliate from an in vitro culture. Note the contractile vacuole (arrow) and the granular appearance of the cytoplasm. Scale bar = 5 μm.](image)
39 and 48 μm long, and between 12 and 24 μm wide, with one macronucleus and one micronucleus (Table 1). The oral polykinetids (OPK1, OPK2, and OPK3) and oral dikinetids (ODKb and ODKc) of the ciliate were highly ordered ciliary fields positioned at the anterior end between kineties 1 and 11 (Fig. 9). Oral polykinetid 1 was the most anterior of the oral structures, with a mean length of 4.4 μm and lacked fragmentation. Oral polykinetid 2 was intermediate in length (3.5 μm) while oral polykinetid 3 was notably smaller in length (1.9 μm). Oral dikinetid structures were composed of ODKb (7.2 μm) and ODKc, however only ODKb was routinely observed by this staining technique. The ODKb structure originated immediately posterior from OPK2, and proceeded in a curve posteriorly around the cytostome to the ODKc segment. The ODKc segment was rarely stained, consisting of several pairs of kinetosomes aligned in a “Y” configuration at the posterior end of ODKb. Several

### Table 1

<table>
<thead>
<tr>
<th>Character</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body length</td>
<td>48.2</td>
<td>4.6</td>
<td>39.2</td>
<td>57.9</td>
<td>47</td>
</tr>
<tr>
<td>Body width</td>
<td>17.5</td>
<td>2.9</td>
<td>12.5</td>
<td>24.5</td>
<td>47</td>
</tr>
<tr>
<td>No. somatic kineties</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>47</td>
</tr>
<tr>
<td>Micronucleus length</td>
<td>2.6</td>
<td>0.4</td>
<td>1.5</td>
<td>3.5</td>
<td>47</td>
</tr>
<tr>
<td>Micronucleus width</td>
<td>1.9</td>
<td>0.4</td>
<td>1.3</td>
<td>2.9</td>
<td>47</td>
</tr>
<tr>
<td>Macronucleus length</td>
<td>10.6</td>
<td>0.4</td>
<td>8.0</td>
<td>15.4</td>
<td>47</td>
</tr>
<tr>
<td>Macronucleus width</td>
<td>8.6</td>
<td>1.2</td>
<td>6.1</td>
<td>11.4</td>
<td>47</td>
</tr>
<tr>
<td>OPK1 length</td>
<td>4.4</td>
<td>0.5</td>
<td>3.0</td>
<td>5.5</td>
<td>41</td>
</tr>
<tr>
<td>OPK2 length</td>
<td>3.5</td>
<td>0.3</td>
<td>2.8</td>
<td>4.2</td>
<td>41</td>
</tr>
<tr>
<td>OPK3 length</td>
<td>1.9</td>
<td>0.2</td>
<td>1.3</td>
<td>2.3</td>
<td>41</td>
</tr>
<tr>
<td>ODKb length</td>
<td>7.2</td>
<td>1.2</td>
<td>5.2</td>
<td>10.7</td>
<td>41</td>
</tr>
<tr>
<td>Distance from OPK1-OPK3</td>
<td>10.3</td>
<td>0.9</td>
<td>8.0</td>
<td>11.9</td>
<td>41</td>
</tr>
<tr>
<td>Distance from OPK1-OPK2</td>
<td>8.1</td>
<td>0.8</td>
<td>6.3</td>
<td>9.9</td>
<td>41</td>
</tr>
<tr>
<td>Distance from OPK2-OPK3</td>
<td>5.6</td>
<td>0.4</td>
<td>4.5</td>
<td>6.8</td>
<td>41</td>
</tr>
<tr>
<td>Distance anterior end-OPK3</td>
<td>12.5</td>
<td>1.4</td>
<td>8.4</td>
<td>15.6</td>
<td>41</td>
</tr>
<tr>
<td>Distance anterior end-ODK</td>
<td>16.1</td>
<td>1.7</td>
<td>13.3</td>
<td>19.4</td>
<td>41</td>
</tr>
</tbody>
</table>

All dimensions are in μm. OPK, oral polykinetid; ODK, oral dikinetid.

Fig. 7. Scanning electron micrograph of a ciliate from an in vitro culture. Note the oral apparatus (arrow) at the anterior end. Scale bar = 5 μm.

Fig. 8. Pyridine-silver-carbonate impregnated ciliate from an in vitro culture. Anterior end of the ciliate illustrates 11 somatic kineties on the outer surface of the cell. Arrow, oral polykinetid structures. Scale bar = 10 μm.

Fig. 9. Pyridine-silver-carbonate impregnated ciliate from an in vitro culture showing the oral apparatus. M, macronucleus; m, micronucleus; OPK1, first oral polykinetid; OPK2, second oral polykinetid; OPK3, third oral polykinetid; ODKb, oral dikinetid b; and ODKc, oral dikinetid c. Scale bar = 10 μm.
mitochondrial structures were observed between the somatic kineties surrounding the ciliate cell (Fig. 10). Many vesicles were observed within the cytoplasm of the cells (Fig. 11), and an irregular distribution of possible mucocysts was also observed.

3.4. Ribosomal DNA sequence comparison

The three independent PCR, cloning and sequencing steps resulted in identical rDNA sequences. This sequence was deposited in GenBank (Accession No. DQ087397). BLAST searches of GenBank indicated that the ciliate consensus sequence was identical to Orchitophrya stellarum (GenBank Accession Nos. AF107773, AF107774, AF107775, and AF107776), and very similar to Mesanophrys pugettensis (AF107777) and M. chesapeakensis (AF107778). Multiple sequence alignments revealed that the ITS1, 5.8S and ITS2 rDNA sequences of the ciliate under study were identical to four isolates of O. stellarum. Both M. pugettensis and M. chesapeakensis were also identical to each other over the ITS1, 5.8S and ITS2 regions. Because of this, O. stellarum (AF107773) and M. pugettensis (AF107777) were used in the multiple alignment generated to represent the identical sequences (Fig. 12). The rDNA sequence descriptions by Goggin and Murphy (2000) allowed the ITS1 (140 bp), 5.8S (119 bp), and ITS2 (236 bp) domain lengths to be calculated for the ciliate. The consensus sequence of the ciliate from N. nephrops (GenBank Accession No DQ087397) differed by 9.2% in the ITS1 (8 transversions, 2 transitions, 2 additions, and 1 deletion) and 5.0% in the ITS2 (3 transversions, 6 transitions, and 3 additions) from that of the M. pugettensis. The 5.8S gene was completely conserved in all sequences between species of ciliates.

4. Discussion

The morphological characteristics of live and silver-stained specimens of the ciliate found infecting Norway lobsters are consistent with those of the genus Mesanophrys (Small and Lynn, 1985), and in particular M. carcini (Grolière and Leglise, 1977). However, rDNA sequence information suggests a close affinity with O. stellarum, a parasitic scuticociliate of sea stars of the family Asteriidae (Cépède, 1907). This discrepancy is difficult to explain, but may be due to phenotypic plasticity in scuticociliates as a whole. Given the morphological similarity with Mesanophrys spp., but the DNA sequences of O. stellarum, we hesitate to place the ciliate from the Norway lobster into either genus.
Ribosomal DNA sequences from the ciliate infecting Norway lobsters were identical to _O. stellarum_ over the ITS1, 5.8S and ITS2 regions. _Orchitophyra stellarum_ is a scuticociliate parasite castrator of male sea stars of the family Asteriidae from the North Atlantic and Pacific (Byrne et al., 1997; Cépède, 1907; Claereboudt and Bouland, 1994; Leighton et al., 1991). It was first described by Cépède (1907) infecting the sea star _Asteracanthion rubens_ from France. The ciliate was very rare and only three out of the several thousand sea stars sampled were infected. The ciliate has a pointed anterior and a rounded posterior, with a granular structure of the cytoplasm and one macronucleus and one micronucleus. Its length is between 35 and 65 μm, and it has a width of 12–26 μm. Its oral structures are at the anterior end and consist of 3 oral polykinetids and an oral dikinetid. The ciliate infecting Norway lobsters appears morphologically similar to this description, but importantly, _O. stellarum_ is reported to have between 18 and 20 somatic kineties (Cépède, 1907; Claereboudt and Bouland, 1994; Stickle et al., 2001), whereas that from the Norway lobster has only 11 somatic kineties.

Differences in the ITS regions between strains and species of ciliates have been previously observed. Diggles and Adlard (1997) reported that four wild isolates of the parasitic ciliate of fish, _Cryptocaryon irritans_, differed by up to 4% in ITS1. Additionally, two species of _Tetrahymena_ differed by 3% in the ITS1 and 10% in the ITS2 (Engberg et al., 1990). ITS1 sequences from _C. irritans_ have also been observed to change rapidly in laboratory cultures, differing by as much as 5.9% over the period of 1 year from initial isolation (Diggles and Adlard, 1997). However, Goggin and Murphy (2000) noted that _M. chesapeakensis_ isolated from the blue crab, _Callinectes sapidus_ from the Atlantic ocean, and _M. pugettensis_ from the Dungeness crab, _Cancer magister_ from the Pacific ocean had no differences in both ITS and the 5.8S ribosomal regions; yet, both ciliates had been held in culture for 18 months, suggesting that the observed differences with the ciliate from the Norway lobster may not be due to prolonged culture.

Comparison of mean length and width characteristics of the ciliate from _N. norvegicus_ with other species of _Mesanophrys_ that have been stained with silver impregnation techniques...
(Messick and Small, 1996; Morado and Small, 1994; Small and Lynn, 1985; Wiąckowski et al., 1999), indicate that all have similar structural features and are closely related. However, these ciliates were stained with a number of different silver impregnation techniques (i.e., Protargol) and as such no conclusive comparison can be made from the body measurements from the different species. Furthermore, nutritional status of the ciliate and cell fixation techniques can affect morphometric measurement (H. Small, personal observation).

The ciliate from the Norway lobster had a single macronucleus, a single micronucleus, and 11 somatic kineties, similar to ciliates within the genus Mesanophysys. Its OPK1 and OPK2 are of approximate equal length to each other, with OPK1 slightly larger (4.4, 3.3 μm, respectively, Table 7.1). OPK3 is the shortest oral structure (1.9 μm) in length, but it is also the widest. The biometric oral polykinetids measured are slightly smaller than those of M. pugettensis (as pyridine-silver-stained specimens) in which OPK1 measured 4.8 μm, OPK2 measured 3.9 μm, and OPK3 measured 1.5 μm (Morado and Small, 1994). In the early stomatogenesis of M. pugettensis, a fourth transitional ciliary field occurs, suggesting that the first oral polykinetid (OPK1) is composed of two functionally related segments. As a result, M. pugettensis was described as a new species (Morado and Small, 1994). Wiąckowski et al. (1999) also observed OPK1 segmentation in Mesanophysys sp. from the isopod Saduria entomon, but interpreted it as a general character linked with stomatogenesis, suggesting that it should not be used as a specific character. Because the silver carbonate staining technique was employed during this study, and protargol staining was not attempted, the fine detail of the kinetosomal structure of the oral apparatus was not observed.

Relatively, few ciliate infections of wild crustacean populations have been documented (Cattaneo, 1888; Gómez-Gutiérrez et al., 2003; Lavallée et al., 2001; Morado et al., 1999; Poisson, 1930). Of these, very small numbers of infected individuals are generally the rule, with Anophryoides haemophila being the possible exception (Sherburne and Beane, 1991). Poisson (1930) found only 0.2% (7 of 3000) of shore crabs Carcinus maenas, with histophagous ciliates, while Lavallée et al. (2001) recorded a prevalence of 0.39% for A. haemophila in the American lobster Homarus americanus. However, Hibbits and Sparks (1983) reported a higher prevalence of 14% (5/37) for Paranophysys infection of the isopod Gnorimosphaeroma oregonensis. A Paranophysys-like ciliate had previously been observed in aquaria-hold, Hematodiniun-infected Norway lobsters (Field et al., 1992; Field and Appleton, 1996) and on only one other occasion was it identified from a freshly caught lobster (Appleton, 1996). The present study failed to identify any further infected Norway lobsters within wild stocks, indicating that the affected individuals probably came from a localised short-term infection event or were compromised in some way.

The available data suggest that ciliates of the genus Mesanophysys are considered to be facultative histophages, with infection of crustaceans being opportunistic in nature. The route of entry for ciliates into lobsters is not known but Morado et al. (1999) suggested that shell condition and size are important factors for M. pugettensis infection of Dungeness crabs (C. magister). Unfortunately, the condition of the infected Norway lobsters was not noted at the time of ciliate infection diagnosis. Experimental infection studies are needed to establish the route of infection.

Extensive penetration into and destruction of host tissues are common observations in infections by Mesanophysys (Armstrong et al., 1981; Hibbits and Sparks, 1983; Messick and Small, 1996; Sparks et al., 1982), Tetrahydromena (Edgerton et al., 1996), and Collinia (Gómez-Gutiérrez et al., 2003) infections of crustaceans. The ciliate from Norway lobsters was found in several tissues causing extensive damage, particularly in the myocardium and epicardium of the heart, and in the gills. In the heart, the normal architecture of myocardial muscle had completely degenerated and tissue necrosis was apparent. In the tail muscle, areas of tissue lysis were also apparent surrounding the invading ciliates. Armstrong et al. (1981) suggested that extracellular lysosomes provide chemical means for disrupting tissues and cells during Mesanophysys infection of Dungeness crabs. The ciliate from the Norway lobster had several structural features resembling mucocysts, and the contents of which may be released by exocytosis. The ciliate under investigation was found to secrete a number of proteolytic enzymes (Small et al., in press) and these are thought to facilitate tissue degradation and nutrient uptake by the ciliates.

In summary, the ciliate parasite of the Norway lobster is morphologically very similar to members of the genus Mesanophysys, but rDNA sequences suggest a puzzling affinity with O. stellarum. One explanation is that there may be different rDNA sequences in the macro and micronucleus of the ciliate resulting in preferential amplification; however identical rDNA sequences (from one independent PCR reactions utilising a high fidelity DNA polymerase) obtained in this study and by Goggin and Murphy (2000) indicate that this is probably not the case. The data therefore suggest that the number of somatic kineties that a ciliate possesses may not be a robust morphological feature when identifying closely related scuticociliate species. Sequencing of the ITS regions of all nominal Mesanophysys and O. stellarum species in conjunction with silver impregnation staining techniques will be essential in order to resolve this issue.

Acknowledgments

H.J.S. was the recipient of a Natural Environment Research Council Studentship (NER/S/A/2000/03368). We thank Dr. Genoveva Esteban for help with the silver carbonate staining technique. Dr. Jeffrey Shields critiqued the manuscript.
References


