Vol. 33: 63–66, 1998 | DISEASES OF AQUATIC ORGANISMS Dis Aquat Org | Published May 14

Systemic spironucleosis in sea-farmed Atlantic salmon *Salmo salar*, caused by *Spironucleus barkhanus* transmitted from feral Arctic char *Salvelinus alpinus*?

Erik Sterud[1,*], Tor Atle Mo[2], Trygve T. Poppe[1]

[1]Norwegian College of Veterinary Medicine, PO Box 8146 Dep., N-0033 Oslo, Norway
[2]National Veterinary Institute, PO Box 8156 Dep., Oslo, Norway

ABSTRACT: A hexamitid flagellate was found in the gall bladder and intestine of Arctic char *Salvelinus alpinus* in northern Norway. Scanning and transmission electron microscopy showed this flagellate to be identical to *Spironucleus barkhanus* from grayling *Thymallus thymallus* and farmed Atlantic salmon *Salmo salar*. It is hypothesised that systemic spironucleosis in sea-caged Atlantic salmon was due to transmission of flagellates from feral char to the salmon.

KEY WORDS: *Hexamita* · Flagellate · Diplomonadida · Ultrastructure · Grayling · Aquaculture

INTRODUCTION

Severe systemic hexamitosis was described in sea-caged Atlantic salmon *Salmo salar* L. from 7 locations in Finnmark, Troms and Nordland counties of northern Norway, between 1989 and 1992 (Mo et al. 1990, Brun & Morsund 1992, Poppe et al. 1992, Poppe & Mo 1993). The causative parasite was tentatively identified as *Hexamita* sp. at the light microscopy level, but by scanning and transmission electron microscopy it has been described as *Spironucleus barkhanus* Sterud, Mo & Poppe, 1997. Accordingly, the systemic disease in sea-caged Atlantic salmon should be termed spironucleosis and not hexamitosis.

The type host of *Spironucleus barkhanus* is grayling *Thymallus thymallus* (L.) (Sterud et al. 1997), a non-anadromous freshwater salmonid. Thus, *S. barkhanus* could not have been transmitted from grayling to farmed Atlantic salmon in the sea. Poppe et al. (1992) proposed that the salmon became infected in freshwater, as a majority of the infected farms had received smolts from the same hatchery. Hexamitids were, however, never found in fish from this hatchery, or in feral salmonids in the Adamselv River, Norway, the water supply of the hatchery (Mo & Poppe unpubl.). Furthermore, grayling are not present in this watercourse and could therefore not be the source of infection in farmed Atlantic salmon.

Anadromous Arctic char *Salvelinus alpinus* (L.) were sampled in the estuary of the outlet river from Koifjordvatn Lake, northern Norway by one of the authors (T.T.P.) in 1996. Hexamitid flagellates were found in formalin-fixed gall bladders from these fish. This prompted a closer study to reveal the identity of these hexamitids.

MATERIALS AND METHODS

In September 1996, 15 anadromous Arctic char sized 142 to 1295 g were caught with gill nets in Koifjordvatn Lake in Finnmark county, northern Norway (71° N, 28° E) (Fig. 1). This lake is connected to the ocean via a 4 km river and has a population of Atlantic salmon and both anadromous and resident Arctic char and brown trout *Salmo trutta* L. The fish were killed and shipped cooled to the laboratory. All external and internal

*E-mail: erik.sterud@veths.no

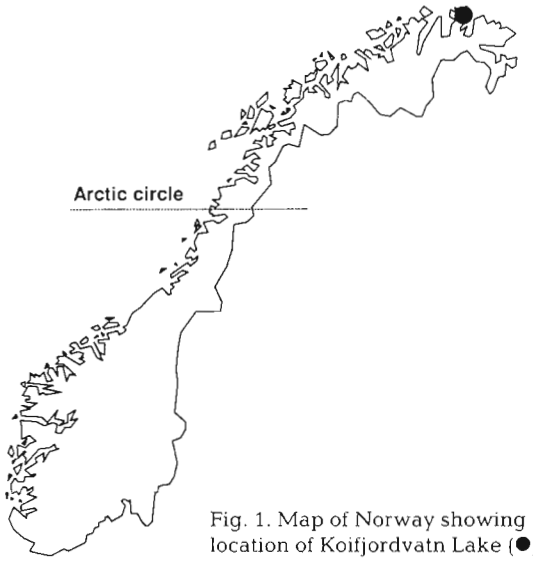


Fig. 1. Map of Norway showing location of Koifjordvatn Lake (●)

organs were examined for parasites with the aid of a dissecting microscope and a light microscope equipped with phase contrast. The gall bladder content of each fish was aseptically collected using a sterile syringe and examined for flagellates. When present, axenic cultures were made using trypticase yeast extract iron serum medium (TYI-S-33) (Keister 1983), as described by Sterud et al. (1997). *In vitro*-cultivated flagellates from exponentially growing cultures were prepared for electron microscopy, as described by Sterud et al. (1997). The ultrastructure of the flagellates were studied using a Cambridge 90 Stereoscan scanning electron microscope (SEM), and a JEOL-100 E transmission electron microscope (TEM).

RESULTS

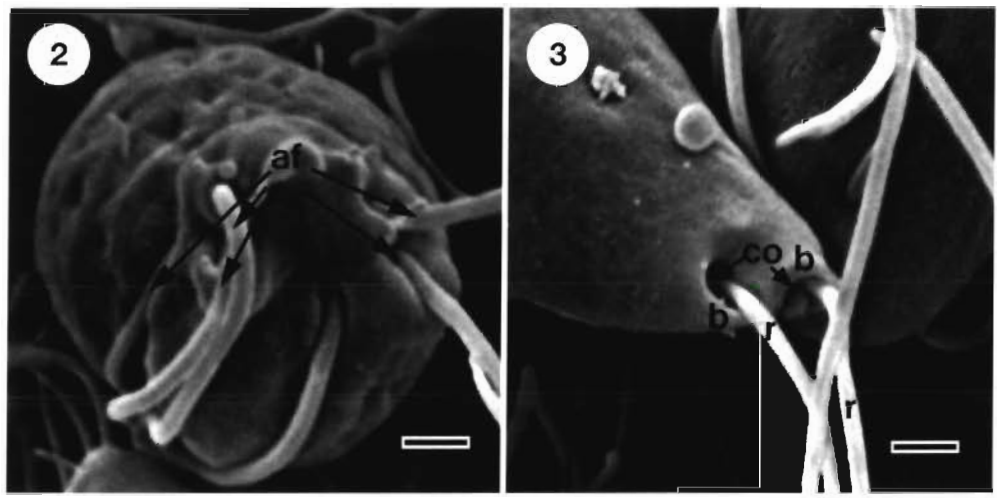
Anadromity of char from Koifjordvatn lake was confirmed by the presence of the marine parasites *Hysterothylacium aduncum* and/or *Anisakis* sp. in all but 1 of the fish (n = 15).

Hexamitid flagellates were found in the gall bladder and intestine of 12 from 15 (80%) of the fish. No pathological changes were associated with the presence of hexamitids.

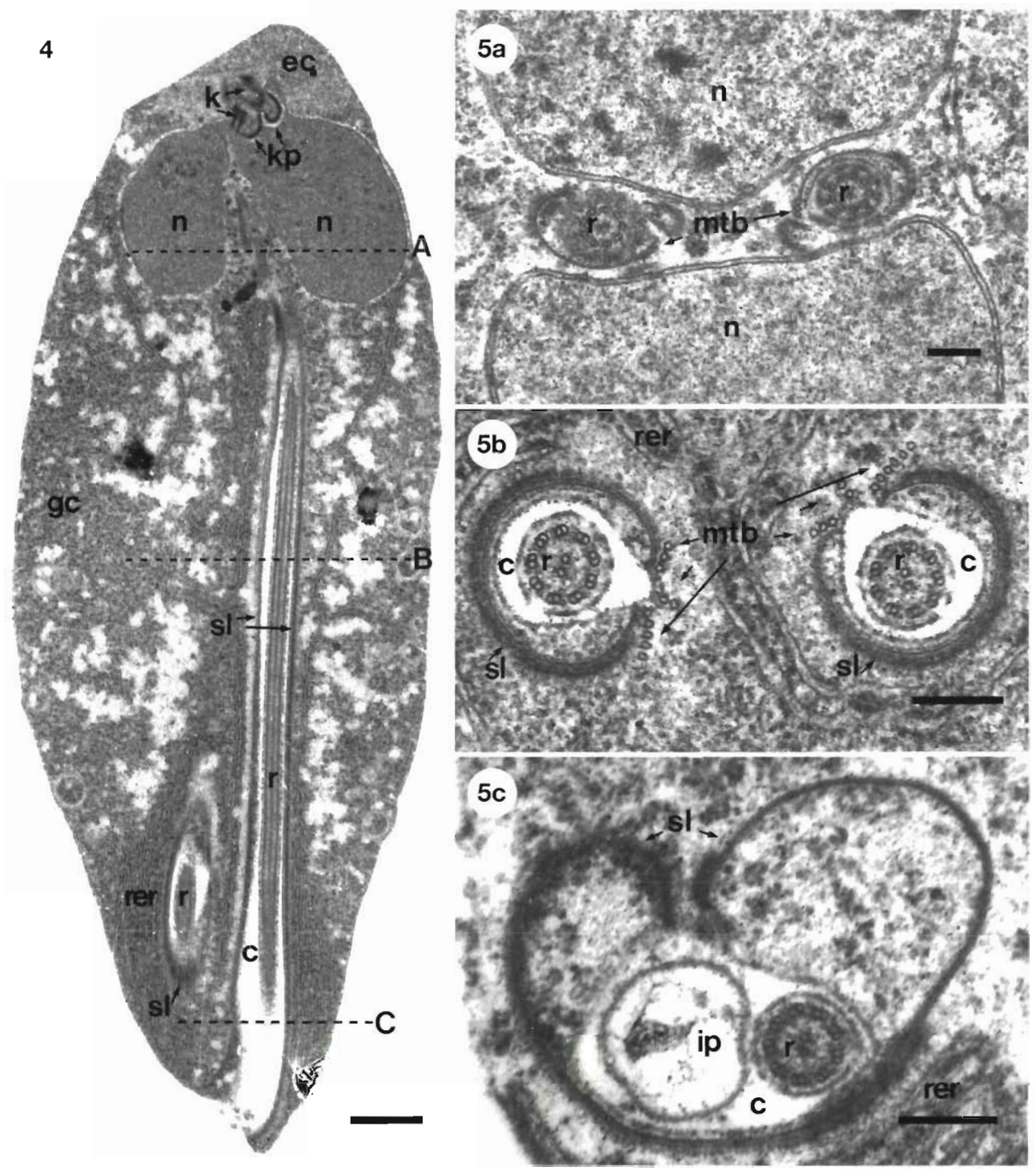
Electron microscopical observations

SEM. Axenically cultivated flagellates had a typical hexamitid appearance, possessing 6 anterior and 3 posterior flagella (Figs. 2 & 3). The cytostome openings, where the posterior flagella emerged, were both halfway surrounded by a crescent-shaped ridge (barkhan), orientated in opposite directions on the 2 openings (Fig. 3). Other surface adornments could not be seen.

TEM. Longitudinal sections revealed 2 sac-like nuclei, closely apposed at their anterior ends, in the anterior end of the organisms (Fig. 4). From each of the 2 kinetosomal complexes at the anterior end of the nuclei, 1 recurrent flagellum ran posteriorly between the nuclei (Figs. 4 & 5A). Each recurrent flagellum was inside a cytostome partially surrounded by a striated lamina (Figs. 4 & 5B, C). In the posterior end the striated laminae widened and were characteristically heart-shaped (Fig. 5C). Three microtubular bands, arranged in a distinctive spoke pattern, accompanied each recurrent flagellum and were located in



Figs. 2 & 3. *Spironucleus barkhanus*. Scanning electron micrographs of axenically cultivated flagellates from Arctic char *Salvelinus alpinus*. af: anterior flagellum; b: barkhan; co: cytostome opening; r: recurrent flagellum. Scale bars = 1 µm. Fig. 2. Anterior view showing 6 anterior flagella. Fig. 3. Posterior end of a flagellate, showing 2 recurrent flagella emerging from terminal cytostome openings. Note the crescent-shaped ridges (barkhans) partially surrounding each opening



Figs. 4 & 5. *Spironucleus barkhanus*. Transmission electron micrographs of axenically cultivated flagellates from Arctic char *Salvelinus alpinus*. c: cytostome; ec: electrolucent cytoplasm; gc: granular cytoplasm; ip: ingested particle; k: kinetosome, kp: kinetosomal pocket; mtb: microtubular bands, n. nucleus, r: recurrent flagellum; rer· rough endoplasmatic reticulum; sl: striated lamina. Fig. 4. Longitudinal section of an organism Dashed lines mark the approximate position of the transverse sections in Fig. 5A to C. Scale bar = 1 µm. Fig 5. (A) Transverse section through the nuclei to show the recurrent flagella passing between the nuclei. The striated lamina and 1 microtubular band along each flagellum are visible. Scale bar = 0.2 µm. (B) Transverse section from the mid portion of a cell showing the distinctive spoke pattern of the microtubular bands accompanying the recurrent flagella. Scale bar = 0 2 µm (C) Transverse section of a recurrent flagellum in the posterior end Note the heart-shaped striated lamina and a particle in the cytostome Scale bar = 0 2 µm

a groove created by the incomplete closure of the striated lamina (Fig. 5B). The maximum number of microtubules observed in these 3 bands were 6 + 2 + 3 (Fig. 5B).

DISCUSSION

Among the ultrastructural characteristics of the hexamitid flagellate found in the intestine and gall bladder of the Arctic char were the crescent-shaped ridges facing opposite directions around the cytostome openings in the posterior end, the spoke-like pattern of the 3 microtubular bands accompanying the recurrent flagella and the heart-shape of the striated laminae in posterior transverse sections. These features are as described for *Spironucleus barkhanus* (Sterud et al. 1997), and the present flagellates were assigned to this species. One minor difference was found between *S. barkhanus* from char and the previous isolates from grayling and salmon. The maximum number of microtubules observed in the 3 microtubular bands accompanying each recurrent flagellum were 6 + 2 + 3 in the char isolate and 5 + 2 + 3 in the other two. As the microtubular bands taper posteriorly, the present observation could have been from a more anterior section than those from the salmon and grayling isolates, or the observed difference may be due to natural variation. The importance of using SEM and TEM to identify fish hexamitids should be stressed. Species-specific characteristics can be seen by electron microscopy only.

This is the first report of Arctic char as a host species for *Spironucleus barkhanus*, and it is also the first report of this parasite in feral anadromous salmonids. The present study does not show that the char harboured the hexamitid while at sea. However, we find this most likely as the parasite was demonstrated in char caught in brackish water, as mentioned in the 'Introduction'. Kent et al. (1992) showed that systemic infection in farmed chinook salmon *Oncorhynchus tshawytscha* Walbaum, 1792 with an unknown hexamitid flagellate was transferred to previously uninfected fish during cohabitation in salt water. Poppe & Mo (1993) observed one case of systemic spironucleosis where farmed salmon were probably infected via processing water from a slaughtering facility close to the fish farm. This facility had recently slaughtered salmon with systemic spironucleosis from another farm, indicating that transmission could occur in the sea. A transmission of flagellates from feral char to sea-caged salmon therefore seems possible, as it is well known that fish farms often attract wild fish, which utilise surplus food. This hypothesis is not incompatible with that of Poppe et al. (1992) and Poppe & Mo (1993) suggesting that farmed salmon were infected as juveniles in freshwater.

The present study suggests a potential source of and route for the systemic infection caused by *Spironucleus barkhanus* in Atlantic salmon in northern Norway. Noteworthy is the high incidence (80%) of symptomless carriage of the flagellate in *Salvelinus alpinus*. The factors triggering a systemic spread of the flagellate within the salmon remain unknown. The case of systemic hexamitid infection reported from chinook salmon in British Columbia, Canada (Kent et al. 1992), occurred in the same period as the Norwegian cases. Other cases from salmonids are not known. Possibly, the same factor or a combination of factors caused similar changes in parasites and/or hosts resulting in systemic spironuclosis in both areas. Systemic spironucleosis has subsequently disappeared in both Norway and Canada (authors' unpubl. data, Kent pers. comm.). However, as long as the factors triggering systemic spironucleosis remain unknown, *S. barkhanus* is still a potential threat to salmonid farming.

Acknowledgements. Thanks to Harry Pedersen and members of Gamvik fishing and hunting club association for providing fish from Koifjordvatn Lake. Thanks also to Duncan Colquhoun for help with the English language.

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Editorial responsibility: Wolfgang Körting, Hannover, Germany

Submitted: November 10, 1997; Accepted: March 9, 1998
Proofs received from author(s): April 29, 1998