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The ultrastructure of hypersymbionts on the monogenean *Gyrodactylus salaris* infecting Atlantic salmon *Salmo salar*

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Abstract

There is increasing pressure to develop alternative control strategies against the pathogen *Gyrodactylus salaris,* which has devastated wild Atlantic salmon *Salmo salar* in Norway. Hyperparasitism is one option for biological control and electron microscopy has revealed two ectosymbionts associated with *G. salaris*: unidentified rod-shaped bacteria, and the protist, *Ichthyobodo necator*. No endosymbionts were detected. The flagellate *I. necator* occurred only occasionally on fish suffering costiosis, whereas bacterial infections on the tegument of *G. salaris* were observed throughout the year, but at variable densities. Bacteria were seldom observed attached to fish epidermis, even when individuals of *G. salaris* on the same host were heavily infected. Wounds on salmon epidermis caused by the feeding activity of bacteria-infected *G. salaris* did not appear to be infected with bacteria. On heavily infected gyrodactylids, bacteria were most abundant anteriorly on the cephalic lobes, including the sensory structures, but no damaged tissue was detected by transmission electron microscopy in the region of bacterial adherence. Furthermore, transmission and survival of infected *G. salaris* on wild salmon did not appear to be influenced by the bacterial infection. The lack of structural damage and impact on *G. salaris* biology indicates that these bacteria are not a potential agent for control of gyrodactylosis. However, this may not be the case for all gyrodactylid–bacterial interactions and a review of bacterial infections of platyhelminths is presented.

Introduction

Species of *Gyrodactylus* have an aberrant monogenean life cycle as they are viviparous and embryos retained *in utero* until fully grown enclose their own developing embryo, like a 'Russian-doll' (Cable & Harris, 2002). This unusual microparasitic life history strategy combined with a generation time of only few days (Jansen & Bakke, 1991; Cable *et al.*, 2000) enhances pathogenicity. *Gyrodactylus salaris* is a serious pathogen on wild Atlantic salmon *Salmo salar* L. parr in Norway (e.g. Johnsen *et al.*, 1999). Control measures against *G. salaris* in infected rivers involve migration obstructions, such as closure of fish ladders and application of the biocide rotenone, which has been widely used to exterminate all potential fish hosts, followed by restocking with the original salmon population (Johnsen *et al.*, 1999). Rotenone has been applied to 34 river systems, 15 of which have been declared free from the parasite, ten are still under surveillance for confirmation of parasite-free status, but in nine rivers the parasite has reappeared. This failure rate, which increases with the biological and hydrological river complexity, accelerates the need to develop alternative control measures. Such alternatives include the use of metal ions as selective pesticides against *G. salaris* (see Soleng *et al.* 1999a, 2005; Poléo *et al.*, 2003), but also selective breeding for host resistance (see Bakke & Harris, 1998; Salte & Bentsen, 2004) and biological control by pathogenic infections of *G. salaris.*

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Cone & Odense (1984) suggested that due to the ubiquitous presence of *Gyrodactylus* species on salmonids in North America and the frequent occurrence of bacterial microcolonies on the parasite tegument, ectoparasites may be an important factor in the spontaneously occurring outbreaks of skin-invading bacteria, such as columnaris disease, furunculosis and red sore disease. Wild and hatchery reared salmon are also susceptible to a range of microparasites (Bruno & Poppe, 1996), the impact of which is rarely studied on the population dynamics of wild salmon (Bakke & Harris, 1998), although pathogenic aeromonads have been implicated in salmon epizootics (Snieszko & Bullock, 1968; Brown & Gratzek, 1980). Johnsen (1978) and Heggberget & Johnsen (1982) observed that *G. salaris*-infected salmon showed signs of excessive skin mucus production and cutaneous fungal infections identified as *Saprolegnia*. This led to the commonly cited, but unproven, suggestion that gyrodactylid-induced pathogenicity is caused by secondary bacterial and fungal infections. However, the degree to which ectoparasites, such as *Gyrodactylus* spp., function as vectors for other fish parasites is open to debate. Bacteria, viruses and microsporidians are the most likely candidates for biocontrol of *G. salaris*. Bacteria have been previously observed on the surface of gyrodactylids (Cusack & Cone, 1985; Cone & Cusack, 1988; Cusack *et al.*, 1988), although the nature of the association is not closely explored.

During electron microscopy studies of *G. salaris* on wild populations of Norwegian Atlantic salmon, associations with two epizoans were identified. Their potential for the biocontrol of gyrodactylosis is assessed and a review undertaken of bacterial infections of helminths.

Materials and methods

Parasite and host

Individuals of *Gyrodactylus salaris* were recovered from Atlantic salmon (*Salmo salar*) parr electrofished in the River Lierelva, south east Norway, in 1999 (see table 1). Fish were transported alive to the laboratory and upon arrival anaesthetized with 0.04% chlorobutanol before examination in the same river water under a stereomicroscope with fibre-optic illumination. A sample of *G. salaris* (4–10) individuals was randomly selected from infected fish at each of the four sampling periods and prepared for scanning electron microscopy (SEM) or transmission electron microscopy (TEM). A laboratory population of this strain of *G. salaris* was established from the March sample (see table 1) on hatchery reared salmon parr of the same stock and utilized for subsequent transmission experiments (see below).

The flagellate *Ichthyobodo necator* was identified according to Lom & Dyková (1992). Isolates of bacteria from *G. salaris* were not identified due to contamination during culturing.

Electron microscopy

For SEM, small fragments of *G. salaris*-infected fins or skin, cut from anaesthetized fishes, were either: (i) placed directly in 100% ethanol at −18°C for 3 h and thereafter kept at 4°C until further processing according to Veltkamp *et al.*'s (1994, 1996) freeze fixation–dehydration method; or (ii) fixed in 2% glutaraldehyde buffered in 0.1 M cacodylate, pH 7.4, at 4°C overnight, rinsed twice in the same buffer for 10 min each at room temperature, and post-fixed in buffered 1% osmium tetroxide for 1 h. Following two 10 min buffer washes, the parasites were dehydrated (70% ethanol for 10 min, 90% for 10 min, 96% for 10 min and finally 4 × 100% for 15 min). Specimens fixed by both methods were CO_2 dried in a Balzer 11–120 critical point drier, mounted on stubs using double-sided carbon tape and sputter-coated with a gold-palladium mixture using a Polaron E5000 SEM coating unit. Specimens were examined in a JEOL JSM-6400 scanning electron microscope. The prevalence and density of bacteria adhered to specimens of *G. salaris* was based on visual screening of electron micrographs.

For TEM, parasites were fixed overnight in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, at 4°C, rinsed twice in buffer at room temperature, and post-fixed in 1% osmium tetroxide with 1.5% potassium ferricyanide for 1 h in the dark. Specimens were rinsed in distilled water 5 times during 10 min and stained with 1.5% uranyl acetate in distilled water for 30 min in the dark. Following ethanol dehydration, the parasites were transferred to propylene oxide for two × 10 min and then infiltrated in Epon 812 resin overnight before embedding in fresh resin. The polymerized blocks were sectioned using a Sorvall ultramicrotome MT5000, stained with lead citrate and examined in a Phillips CM100 transmission electron microscope.

Table 1. Seasonal occurrence of *Gyrodactylus salaris* on Lierelva *Salmo salar* parr in 1999, and associated bacterial infections of *G. salaris*.

	G. salaris	Bacteria sp.	
Season	No. salmon examined (infection %)	No. *G. salaris* examined (infection %)	Density of bacteria sp.
March	20 (100)	10 (100)	+++
May	10 (100)	10 (100)	++
September	12 (100)	10 (100)	+++
December	4 (75)	4 (50)	+

Visual observations: + low, ++ moderate, +++ numerous bacteria.

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Reproduction of bacteria-infected G. salaris *on salmon parr*

An additional eight *G. salaris* infected salmon parr collected in September 1999 were grouped and maintained in a 100 × 100 cm tank (30 cm water level) with circulated de-chlorinated tap water (2 l min^{-1}). The fish were fed unmedicated pellet food (Ewos) under a continuously dim light regime at 9.5°C (range 8.7–10.5°C). After one month, all fish were anaesthetized in 0.04% chlorobutanol and examined under a stereomicroscope with fibre optic illumination to monitor the presence of *G. salaris*.

Transmission of bacteria-infected G. salaris *on salmon parr*

The transmission of bacteria-infected *G. salaris* was monitored following the experimental design of Soleng *et al.* (1999b) using naturally *G. salaris* infected and uninfected salmon parr (from Lierelva DOFA hatchery). Plastic boxes (47 × 37 cm, 10 cm water level) with a wire mesh bottom and perspex lid were divided into two equal compartments and floated in a larger fish tank (100 × 100 cm, 30 cm water level) with circulating water at 12°C (range 11.7–12.5). Individual uninfected salmon ($n = 20$) were placed together with an infected fish with a known number of parasites (range 320–2900) in each compartment. After 24 h, the recipient fish was anaesthetized and the infection level assessed under a stereomicroscope.

For the transmission experiments, random samples of *G. salaris* ($n = 50$) on fish were examined by SEM before and after the trials to check for the continued presence of bacteria. Unfortunately, the lack of fish with no hypersymbiont on *G. salaris* made it impossible to establish control groups to the hyperparasitized worms that could be run at the same time; comparable data is, however, available in Soleng *et al.* (1999b).

Results

Two hypersymbionts were observed on the tegument of *Gyrodactylus salaris*: (i) microcolonies of unidentified bacteria, and (ii) individual flagellates, *Ichthyobodo necator,* firmly attached by a stalk (figs 1–3). No hyperparasites were observed by TEM in the internal tissues, including the gastrodermis and parenchyma, in serial sections of ten worms.

Seasonality in occurrence of hypersymbionts on salmon

Previously, *G. salaris* had been randomly collected from the River Lierelva and routinely prepared for SEM for teaching and research purposes every other year for 10 years, but without observations of hyperparasitism on the parasite's external surfaces. In the present investigation, *G. salaris* was present on salmon parr ($n = 46$) collected throughout the year in Lierelva (table 1). Randomly selected *G. salaris* ($n = 34$) examined by SEM were all positive for bacterial microcolonies, except for two individual worms from the winter sample. *Ichthyobodo necator* was only occasionally observed on *G. salaris* and only on worms from salmon maintained under aquarium conditions for a prolonged period. The intensity of *I. necator* never exceeded one per individual *G. salaris* (fig. 2).

Ultrastructure of bacteria and the tegument of G. salaris

All bacteria were rod-shaped, phenotypically similar but slightly differing in size (1–2 μm × 0.3–0.4 μm), probably representing one species. They occurred all

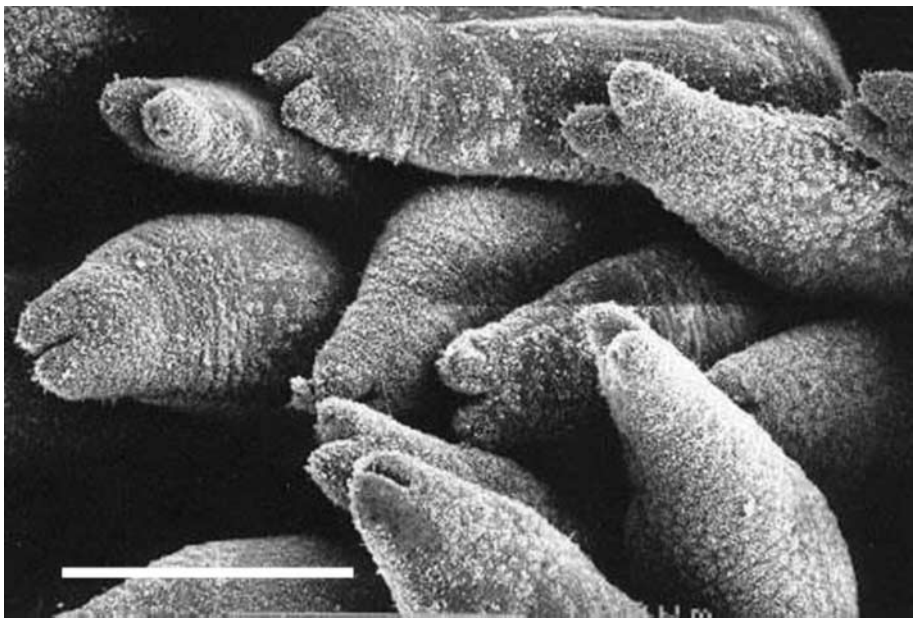

Fig. 1. SEM of bacterial symbionts on the tegument of an infrapopulation of *Gyrodactylus salaris* infecting *Salmo salar* parr from the River Lierelva. Scale bar = 100 μm.

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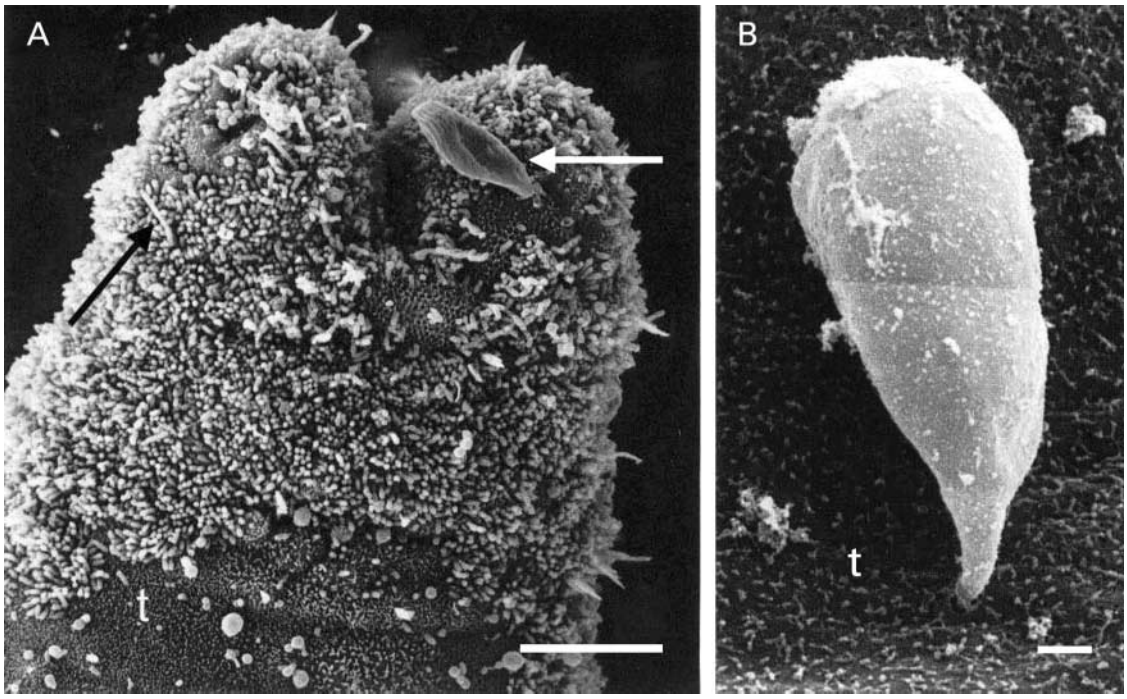

Fig. 2. SEM of hypersymbionts on *Gyrodactylus salaris* infecting *Salmo salar* parr from River Lierelva. A, Single specimen of *Ichthyobodo necator* (white arrow) and numerous bacteria on the tegument (t) of the anterior end of a specimen of *G. salaris,* which also shows the cilium of a sensory receptor (black arrow); B, *I. necator* firmly attached to the tegument (t) of the opisthaptor of *G. salaris*. Scale bars = 10 and 1 μm, respectively.

over the body surface of some *G. salaris* specimens, but with considerable variation in density between worms. Generally, bacteria were less abundant on the central, dorsal side of the body and the opisthaptor, but particularly abundant on the cephalic lobes close to the spike sensilla (fig. 4A). Unlike *I. necator*, there was no evidence that the bacteria penetrated deeply into the tegument of *G. salaris*. Instead, they adhered to the tegument either vertically or horizontally (fig. 4B) with the deepest incursion being ~ one-third the depth of the tegumental surface layer of *G. salaris* (figs 5–6). Bacterial body orientation seemed density-dependent; the higher the density, the more vertical the position and the closer they occurred. Actively dividing bacteria were also detected on the surface of *G. salaris* on several occasions (e.g. fig. 5D).

Bacteria were detected amongst the short microvilli processes (370 × 70 nm), which characterize the apical membrane of the tegumental surface layer of *G. salaris* (fig. 5C,D). Two types of membrane-bound vesicles dominate the surface, namely: (V_1) electron-lucent (150–200 nm diameter) vesicles with fibrous contents, and (V_2) electron-dense (100—200 nm diameter) bodies. The V_1 vesicles were most abundant and frequently observed releasing their contents onto the tegumental surface (figs 5A, 6A), possibly contributing to the mucus-like glycocalyx, which covers the apical membrane. No nuclei, ribosomes, endoplasmic reticulum or Golgi bodies were observed within the surface layer. An internal (basement) unit membrane was attached by hemidesmosomes to the basal lamina complex, which overlies the circular and inner longitudinal myofibres and parenchyma.

The interface between bacteria and *G. salaris* appeared to be mostly a membrane-to-membrane contact (fig. 6A,B) with no connecting structures, such as fimbriae or junctional complexes. Vesicles within the surface layer of *G. salaris* individuals were often observed emptying their contents directly on to the bacterial wall, implying direct contact between the bacteria and vesicle. However, no disrupted syncytium or degenerating cells were detected below the point of bacterial attachment on the gyrodactylid tegument.

Reproduction and transmission of bacteria infected G. salaris

After one month's laboratory maintenance, *G. salaris* infrapopulations ($n = 8$) persisted at a high intensity level despite heavy infections with bacteria as observed by SEM examination of a random selection of parasites from four of the fish at the end of the experiment. As the expected longevity of *G. salaris* infrapopulations at 9.5°C is shorter (Jansen & Bakke, 1991) than 30 days, no obvious evidence of a hyperparasitic-induced reduction in reproduction rate of infected *G. salaris* was observed. Similarly, the transmission of *G. salaris* between live hosts seemed unimpaired by bacterial infections (fig. 7).

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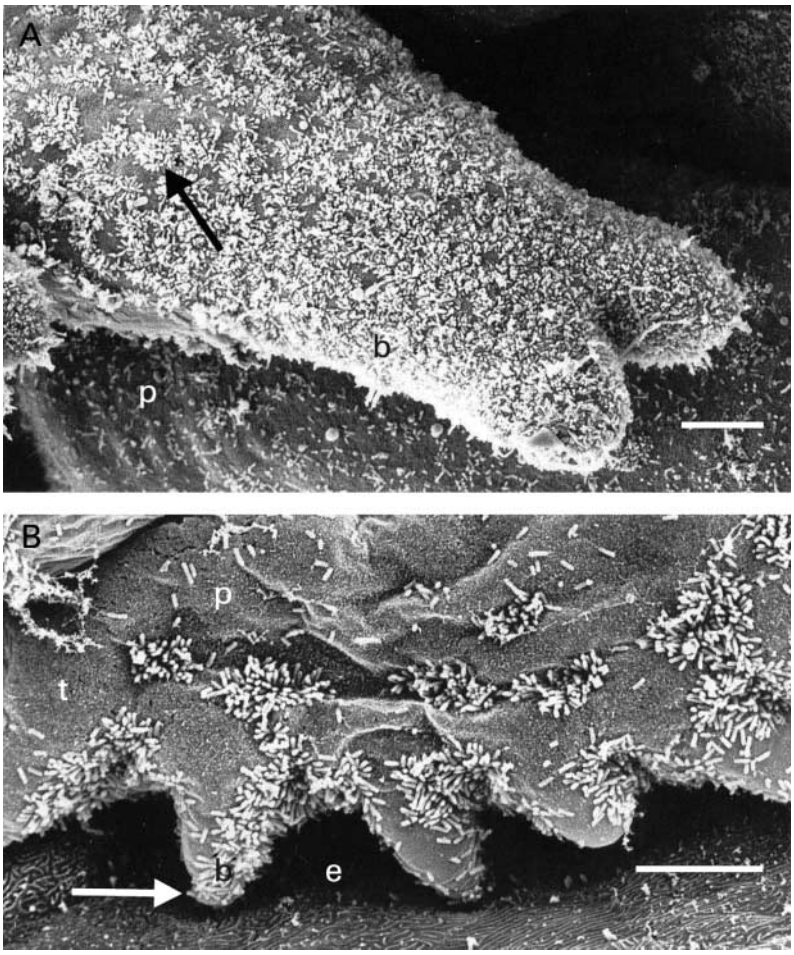

Fig. 3. SEM of bacteria (b) on *Gyrodactylus salaris*. A, The anterior body of a specimen of *G. salaris* heavily colonized by bacteria, but with a patchy distribution (black arrow), overlying a less heavily infected parasite (p). B, Higher magnification of the bacterial population on the dorsal margin of the parasite's (p) opisthaptor. High densities of bacteria even around the marginal hook regions (white arrow). Small areas of parasite tegument (t) and fish epidermis (e) devoid of bacteria. Scale bars = 10 μm.

Pathogenesis of G. salaris *infection*

Feeding wounds caused by *G. salaris* were observed frequently on the host's epidermis, but no gross histopathology was associated with these lesions. Bacteria were seldom observed on the salmon epidermis, including areas damaged by *G. salaris* during feeding and attachment.

Discussion

Two hypersymbionts were found associated with *Gyrodactylus salaris, Ichthyobodo necator* and unidentified bacteria. *Ichthyobodo necator* is an ectoparasitic pathogenic flagellate, which commonly infects the epidermis of freshwater fish (Lom & Dyková, 1992). Most hyperparasites described from helminths are protists (Dollfus, 1946; Canning, 1975), but bacterial infections are also common (table 2) and even hyperinfections of helminth parasites have been recorded (Rego & Gibson, 1989). Reports of ectohyperparasites of monogeneans are rare, whereas endohyperparasites associated with the gastrodermis, possibly ingested with food, are the most frequently observed (Morris & Halton, 1975). There are no previous reports of endohyperparasites within gyrodactylids as far as is known, but ectohypersymbiontic bacteria have been described in association with three other *Gyrodactylus* species (Cone & Odense, 1984; Cusack & Cone, 1985; Cone & Cusack, 1988; Cusack *et al.*, 1988; see table 2). Amongst these only Cusack *et al.* (1988) were able to identify the bacteria on *G. colemanensis*, which consisted of multiple infections with *Pseudomonas* sp. and two Vibrionaceae spp. Slightly more success has been met with the identification of bacteria from other helminths (see table 3). Neither viruses nor microsporidians have been described from gyrodactylids, but they do occur in other monogeneans (e.g. Cable & Tinsley, 1992).

There are no previous hyperparasitic reports of *I. necator* and only occasionally is this parasite observed attached to the tegument of *G. salaris*. The number of *I. necator* never exceeded one per infected *G. salaris*, even during heavy epizootics of costiosis on salmon parr. Clearly, *G. salaris* is

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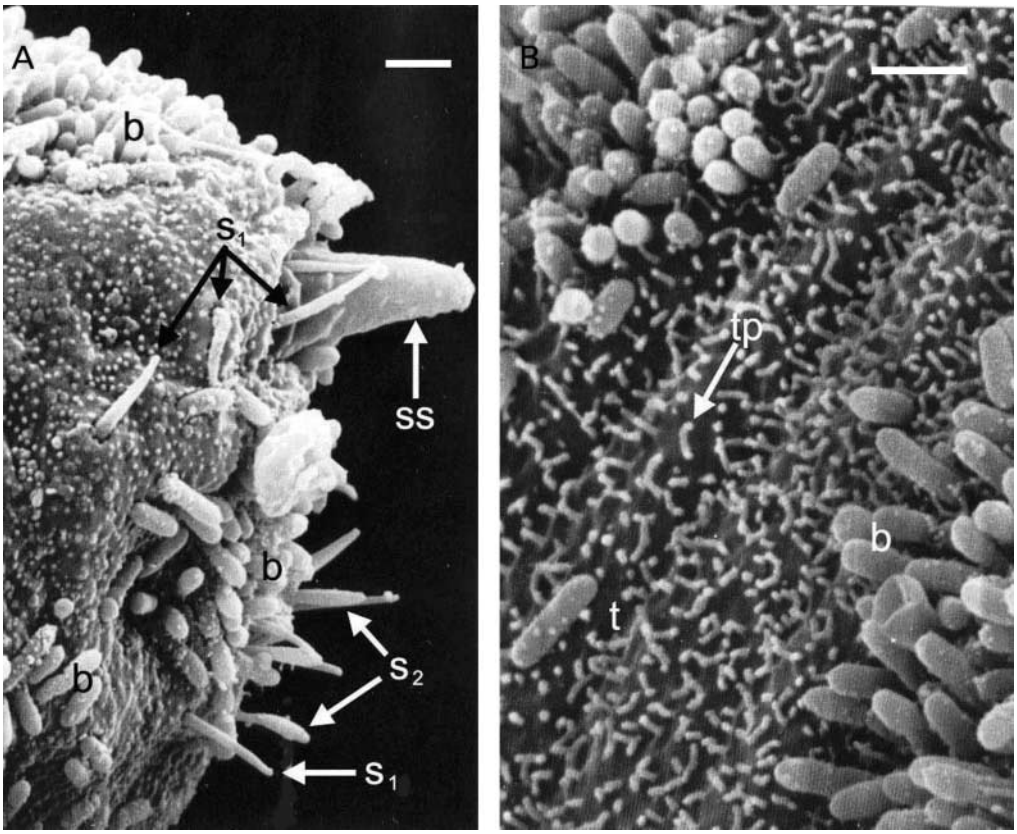

Fig. 4. SEM of bacteria (b) attached to the tegument of *Gyrodactylus salaris*. A, Bacteria on the cephalic lobe of *G. salaris* equipped with sensory structures, including the spike sensilla (SS) and two different forms of uniciliate receptors (S_1, tapering sensilla and S_2, club sensilla). B, The tegument (t) of *G. salaris* with horizontally and vertically orientated bacteria (b) amongst the tegumental projections (tp). Scale bars = 1 μm.

not a preferred substrate for *I. necator*. In contrast, microcolonies of bacteria adhering to the tegument of *G. salaris* were observed from salmon collected throughout the year indicating that this is a common symbiotic association in the River Lierelva. Some worms fixed directly from the field were covered by bacteria indicating that this hypersymbiont could not have arisen through laboratory contamination. Even when large, actively proliferating microcolonies of bacteria colonized the surface of *G. salaris* in situ as observed by SEM, bacteria were neither observed on the epidermis of adjacent salmon nor on the attachment wounds caused by *G. salaris*. Therefore, *G. salaris* is unlikely to be a vector for the transmission of these bacteria to fish. It is possible that the apparent difference in substrate preference of the *G. salaris* bacterium arose from its dislodgement from the fish epidermis due to increased mucus production or the effect of fixation and subsequent processing, but this is unlikely. Similarly, Cone & Odense (1984) reported little evidence of bacterial involvement in the feeding wounds on *S. gairdneri* (= *O. mykiss*) caused by *G. salmonis*. There are no obvious indications of any fish pathology due to the present bacteria, but a comprehensive assessment of pathogenicity requires species identification.

How bacteria adhere so tenaciously to the surface of *G. salaris* is unknown. Following the division of vertically attached bacteria, the distal bacterium is probably dislodged from the host. Except when dividing, no extracellular connections between the bacteria were observed. Therefore, horizontally adhered, dividing bacteria probably represent the main basis for growth of this hypersymbiont on *G. salaris*. Cusack *et al.* (1988) noted that bacteria on gyrodactylids were associated with a polysaccharide-like matrix. However, neither junctional complexes, nor secretions or filaments (see Morris, 1973) were detected between the bacteria and gyrodactylids in the current study. However, the lack of such connecting structures is common for many extracellular protozoan parasites and their hosts (Vickerman, 1972).

The high intensity of bacteria on the surface of *G. salaris*, which themselves have a relatively short life span (Jansen & Bakke, 1991), indicates a high proliferation rate and transmission success. The transmission of bacteria-infected *G. salaris* between live salmon parr suggests a horizontal spread of the bacteria amongst *G. salaris* infected salmon and that the transfer of *G. salaris* is not impaired by bacteria covering the cephalic lobes. The latter are equipped with a battery of putative chemo- and tango-receptors (Lyons 1969a,b; Kritsky, 1978), and probably play a key role in host–parasite interactions during primary attachment (e.g. Whittington *et al.*, 2000). In addition,

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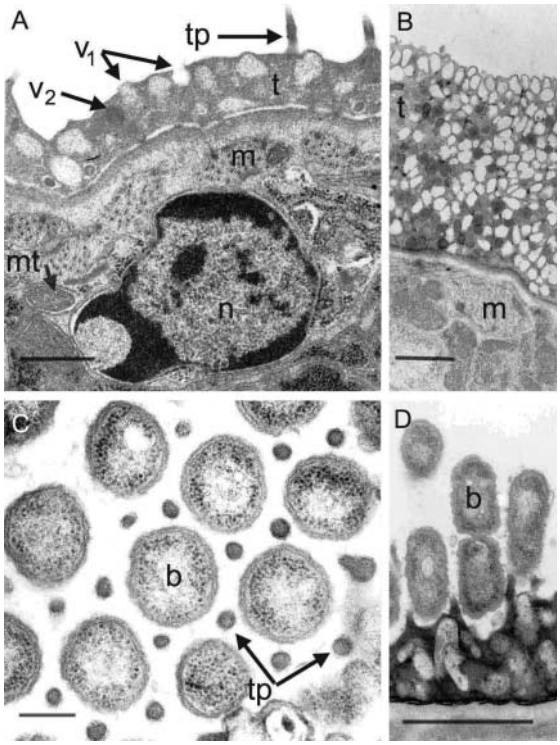

Fig. 5. TEM of the tegument of *Gyrodactylus salaris*. A, B, Surface layer (t) with two different types of vesicle (V_1 and V_2) releasing their contents between the tegumental projections/microvilli (tp), underlying muscle (m) blocks separate this surface layer from putative subtegumental cell containing nucleus (n) and mitochondrion (mt). C, Tangential and D, perpendicular sections of bacteria attached to the tegument of *G. salaris* to show the hexagonal distribution of tegumental projections (tp) around the bacteria (b) and the tegumental depression under the dividing bacterium. Scale bars A and D = 500 nm; B = 1 μm; C = 200 nm.

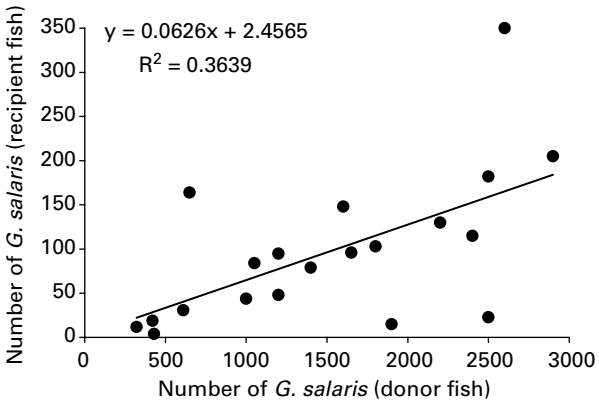

Fig. 7. Transmission rate of *Gyrodactylus salaris* between two individually constrained *Salmo salar* parr after 24 h of exposure; the number of *G. salaris* on the recipient parr is shown as a function of the initial number of bacterial-infected *G. salaris* on the donor parr.

there was no evidence of bacterial-induced alterations in the reproduction or survival of *G. salaris* on salmon. However, due to the lack of experimental controls (no salmon with only non-hyperparasitized parasites), the influence of bacteria on the growth rate of *G. salaris* cannot be excluded, particularly as Lierelva *G. salaris* demonstrate relatively low infection levels compared with other populations of *G. salaris* from other Norwegian rivers (Jansen & Bakke, 1993a,b).

The primary route of bacterial infection of *G. salaris* is unknown, but in addition to infection via water, the vertical transmission of bacteria may occur from mother to offspring during birth. As regards pathology of the bacterium towards *G. salaris*, the numerous tegumental

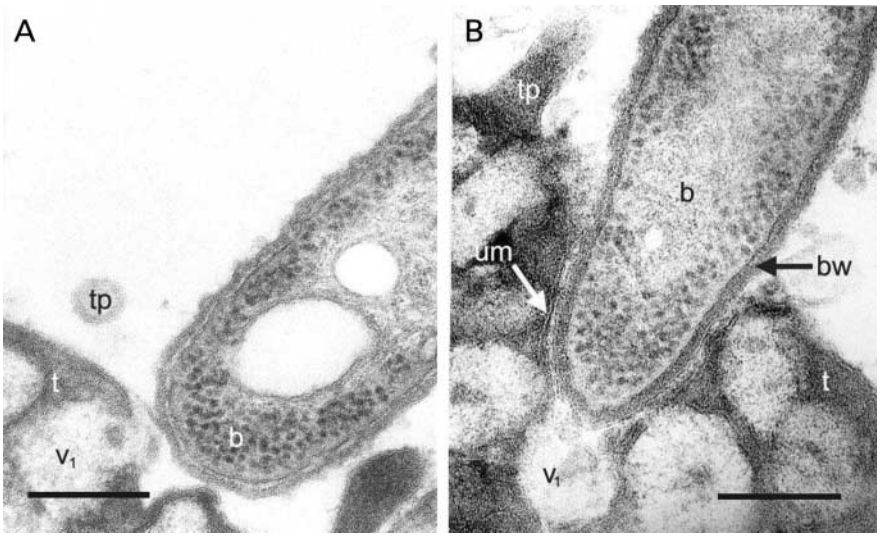

Fig. 6. TEM of bacteria (b) associated with the tegument (t) of *Gyrodactylus salaris*. A, Tegumental projections (tp) and tegumental vesicles (V_1) emptying their contents in close proximity to the bacteria. B, The intimate contact between the unit membrane (um) of the tegument of *G. salaris* and the bacterial wall (bw), and the deep depression in the tegument of *G. salaris* probably caused by a bacterium. Scale bars = 200 nm.

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Table 2. Bacterial hypersymbionts of monogeneans previously reported from the literature.

	Monogeneans			
Bacteria	Species	Site infected	Host species	References
Unidentified	*Gyrodactylus salmonis*	Body surface, opisthaptor	*Salmo gairdneri*	Cone & Odense (1984)
Unidentified (slender and stout shaped)	*G. avalonia*	Body surface, dorsal surface of opisthaptor	*Gasterosteus wheatlandi*	Cusack & Cone (1985)
Unidentified	*Zeuxapta seriolae*	Pharyngeal cells, parenchymal cells	–	Rohde (1986)
Pseudomonas sp. Vibrionaceae spp.	*G. colemanensis*	Body surface	*S. gairdneri* (= *Oncorhynchus mykiss*)	Cusack *et al.* (1988)
Unidentified	*G. colemanensis* *G. salmonis*	Body surface	*Salvelinus fontinalis* *Salmo gairdneri* *S. salar*	Cone & Cusack (1988)
Unidentified	*Diclidophora merlangi*	Gut lumen	*Merlangius merlangus*	Morris & Halton (1975)
Prokaryotes	*Cichlidogyrus halli typicus*	Hamulus glands	*Tilapia* spp.	El-Naggar & Kearn (1989)
Unidentified	*Pseudodiplorchis americanus*	Gut lumen, digestive cells parenchyma, gland cells vitelline ducts, uterine wall	*Scaphiopus couchii*	Cable & Tinsley (1992)

vesicles of gyrodactylids may serve to counteract any adverse effects caused by hyperparasites. The mucopolysaccharide-containing vesicles appear to be produced throughout life (Lyons, 1970) and may provide protection against a range of environmental hazards, including pathogens. However, no data exist at present as to whether the rate of vesicle production is influenced by such infections. In view of the complexity of the natural microfauna of fish (Crouse-Eisnor *et al.*, 1985 and references), further research is required to assess multi-species interactions. Hypersymbionts that are not parasitic to their host (i.e. the primary parasite) could be pathogenic to their parasite's host and therefore actual pathogenicity of the primary parasite could be misinterpreted if multi-species interactions are not fully considered. In the case of gyrodactylids, pathogenicity is often attributed to secondary infections, but with little supporting evidence.

To conclude, whereas *I. necator*, an ectoparasite of salmon, only rarely attaches to *G. salaris*, the converse is true for unidentified ectohypersymbiontic bacteria. The latter preferentially infect *G. salaris* and rarely attach to the salmon epidermis. However, the bacteria have no obvious detrimental effect on the survival, reproduction

Table 3. Bacterial hypersymbionts of turbellarians and digeneans previously reported from the literature.

	Flatworms				
Bacteria	Class	Species	Site infected	Host species	References
Unidentified	Turbellaria	*Meara stichopi* *Meara* sp. *Nemertoderma westbladi*	Epidermis	Holothuria	Lundin (1998)
Rickettsia sp.	Turbellaria	*Temnocephala novaezealandiae*	Testes	–	Williams (1991)
Spirillum-like *Rickettsia* sp.	Turbellaria	*Troglocaridicola mrazeki*	Intestinal cavity	*Troglocaris anophthalmus*	Williams (1992)
Unidentified	Digenea	*Megalodiscus temperatus*	Tegument	*Rana pipiens*	Morris (1973)
Salmonella typhimurium	Digenea	*Schistosoma* spp.	Gut	Man	Lo Verde *et al.* (1980)
Salmonella sp.	Digenea	*Schistosoma japonicum*	Gut	Man	Tuazon *et al.* (1985)
Achromobacter sp. *Edwardsiella tarda* *Enterobacter agglomerans* and unidentified spp.	Digenea	*Clinostomum marginatum*	Mouth	*Ardea herodias*	Aho *et al.* (1991)
Unidentified	Digenea	*Culaeatrema inconstans*	Tegument	*Culaea inconstants*	Lasee & Sutherland (1993)
Campylobacter jejuni *C. coli*	Digenea	*Schistosoma mansoni*	Tegument	Man	Lindblom & Nilsson (1994)
Spirochaeta, Nano-bacteria, Eubacteria and unidentified sp.	Digenea	*Gyliauchen nahanensis*	Tegument	*Siganus* spp.	Hughes-Stamm *et al.* (1999)

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or transmission of *G. salaris* and therefore neither microbe is a likely candidate for biocontrol of gyrodactylosis.

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