

Article

Detection of *Tenacibaculum* spp. in *Caligus rogercresseyi*: Evidence of Potential Pathogen Carriage in Salmon Aquaculture

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Abstract

Sea lice (*Caligus rogercresseyi*) are major ectoparasites in Chilean salmon aquaculture, causing economic losses and increasing fish susceptibility to secondary infections. Tenacibaculosis has recently emerged as the second leading cause of mortality in farmed salmon. Understanding whether sea lice may harbor or transmit bacterial pathogens is therefore highly relevant. A sea lice survey was conducted at an Atlantic salmon (*Salmo salar*) farm in the Los Lagos Region, Chile. Twenty adult parasites (10 ovigerous females and 10 males) were analyzed using an integrated approach combining immunohistochemistry (IHC) with specific antibodies and conventional PCR with appropriate controls. IHC revealed positive signals for *Tenacibaculum maritimum* in the gastrointestinal tract and cuticle, and for *Tenacibaculum dicentrarchi* in the cuticle and ovigerous sac of *C. rogercresseyi*. A specific PCR test confirmed the presence of both pathogens, yielding amplification products of 1088 bp for *T. maritimum* and 284 bp for *T. dicentrarchi*. This study provides the first dual evidence, by PCR and IHC, of *Tenacibaculum* species in *C. rogercresseyi*. These findings suggest that sea lice may play a role in pathogen dynamics in salmon farming, highlighting the need for further research on their contribution to disease transmission within aquaculture systems.

Keywords: sea lice; *Caligus rogercresseyi*; carrier Tenacibaculosis; salmonids; Chile

1. Introduction

In Chile, Caligidosis caused by the fish ectoparasite *Caligus rogercresseyi* is one of the salmon farming pathologies that generates significant economic and health impacts, with Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*) being the most susceptible cultured species to infestations. In addition to the economic impacts on salmon farming, derived from the cost of preventive and palliative treatments against the parasite, the effects of infestation on fish cause skin damage, chronic stress, and possibly an increased susceptibility to secondary infections [1–3].

Tenacibaculosis is currently considered one of the most important emerging bacterial diseases affecting marine fish and salmon aquaculture worldwide. The disease, caused by several *Tenacibaculum* species, is characterized by severe ulcerative skin lesions, impaired osmoregulation, reduced feeding, and elevated mortalities, particularly in juvenile fish [4,5].



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In Chile, Tenacibaculosis has become the second most important bacterial disease affecting Atlantic salmon production, with mortality associated with this condition increasing from approximately 1% in 2018 to 8.1% in 2025 [6]. Since the implementation of the Passive Surveillance Program (PVP), *T. dicentrarchi* has accounted for more than 70% of confirmed cases, followed by *T. maritimum* [6]. In addition to its growing sanitary impact, the disease remains largely dependent on antimicrobial treatments, as no commercial vaccine is currently registered for its control in Chile [4,6]. Consequently, understanding the epidemiology, transmission routes, and potential biological reservoirs of *Tenacibaculum* spp. has become a priority for improving disease prevention and reducing antibiotic use in salmon aquaculture [6,7].

Although *Tenacibaculum* spp. are recognized as important fish pathogens, their natural reservoirs and transmission pathways remain incompletely understood. These bacteria have been isolated from seawater, sediments, fish mucus, and infected tissues, suggesting multiple environmental and biological reservoirs [4,8]. In addition to waterborne and direct fish-to-fish transmission, marine ectoparasites have been proposed as potential carriers of *Tenacibaculum* spp. Tenacibaculosis itself is characterized by severe ulcerative dermatitis and gill necrosis, conditions that overlap with the damage inflicted by sea lice parasitism and may exacerbate disease outcomes [5,9]. Recent epidemiological evidence from Chile showed that *Caligus rogercresseyi* infestations increase the risk of Tenacibaculosis-associated mortality more than threefold (OR = 3.23) [10]. Furthermore, sea lice and other aquatic invertebrates have been reported to harbor fish pathogens, including *Tenacibaculum* spp. [11,12], although pathogen detection alone does not necessarily demonstrate transmission capability [13–15].

Several authors have suggested that fish ectoparasites may participate in the dissemination of pathogens either as mechanical carriers or, in some cases, as biological vectors. However, demonstrating vector competence requires evidence of pathogen acquisition, persistence and successful transmission to a susceptible host [16,17]. While *L. salmonis* has been associated with the transmission of bacterial pathogens such as *Aeromonas salmonicida* [18], salmon alphavirus [19], and infectious salmon anemia virus (ISAV) in Norway [20,21], in Canada *L. salmonis* has been linked to the transmission of bacterial pathogens such as *A. salmonicida*, *T. maritimum*, *Pseudomonas fluorescens*, and *Vibrio* spp. [22]. Recent evidence has also identified the presence of economically important bacterial pathogens such as *T. maritimum*, *T. dicentrarchi* and *Vibrio anguillarum* within sea lice microbiomes, highlighting their potential relevance in the epidemiology of Tenacibaculosis and vibriosis in salmon aquaculture in Ireland [11]. It has also been reported that *L. salmonis* may act as a mechanical vector of infectious hematopoietic necrosis virus (IHNV) [13].

In the case of the fish ectoparasite *C. rogercresseyi*, it has been reported that although it does not act as a vector of *Piscirickettsia salmonis* [14], the concomitant effect of both pathogens is synergistic, increasing the probability of mortality due to Piscirickettsiosis caused by *Piscirickettsia salmonis* in Atlantic salmon farming in Chile [2,23,24]. Moreover, microbiome analysis of *C. rogercresseyi* revealed that the most represented bacterial genome belonged to the fish pathogen *Tenacibaculum ovolyticum* [25]. Additionally, it has been reported that *C. rogercresseyi* has the potential to be a vector of ISAV [26] and other viruses such as the rhabdovirus present in the genome of *C. rogercresseyi*, detected through parasite genome analysis [27]. We hypothesized that *C. rogercresseyi* may harbor *Tenacibaculum* species within distinct anatomical structures, supporting its potential role as a bacterial carrier in salmon aquaculture.

In this study, we report for the first time the molecular detection by conventional PCR and immunohistochemistry (IHC) of the fish pathogens *T. maritimum* and *T. dicentrarchi* in parasites collected from a salmon farming site in the Los Lagos Region, Chile. A sea

lice survey was conducted using both histopathological and molecular methods, where IHC and PCR were applied with specific antibodies and DNA extraction protocols under appropriate controls. This integrated approach provides novel and valuable insights into the interactions between sea lice and bacterial pathogens in aquaculture.

2. Materials and Methods

This study was designed as a preliminary observational survey aimed at detecting the presence of *Tenacibaculum* spp. in *Caligus rogercresseyi* using complementary molecular and histological techniques. The combined use of PCR and immunohistochemistry (IHC) enabled both pathogen detection and anatomical localization within the parasite. Although the sample size was limited, the study was intended as a proof-of-concept investigation to generate baseline evidence for future studies on the epidemiological role of *C. rogercresseyi* in salmon aquaculture.

2.1. Sampling

In January 2021, twenty adult *Caligus rogercresseyi* specimens (10 ovigerous females and 10 males) were randomly collected from five Atlantic salmon (*Salmo salar*) from a rearing site located in the Los Lagos Region, southern Chile. Parasites were recovered during a routine fish health inspection and were carefully removed using tweezers. Each specimen was handled individually to avoid cross contamination. At the time of sampling, fish did not present clinical signs compatible with Tenacibaculosis. Five parasites of each sex were fixed in 10% buffered formalin maintained at room temperature and processed for histological analyses within 48 h of collection. The remaining specimens were preserved individually in RNAlater[®] (Thermo Fisher Scientific, Waltham, MA, USA), transported to the laboratory within 48 h of collection and stored at -20°C until further molecular analyses.

2.2. Immunohistochemistry (IHC)

Sea lice samples were fixed in 10% buffered formalin, dehydrated, and embedded in paraffin wax via standard procedures [28]. Each tissue was sectioned at $5\text{ }\mu\text{m}$, and three sections per fish were stained with hematoxylin and eosin (H&E) to describe histopathological alterations. Sections were observed under a Leica DM2000 microscope (Leica Microsystems, Wetzlar, Germany) at different levels of magnification (20). The IHC staining was performed on 3–4 μm sections of paraffin-embedded tissues. This protocol began by blocking the endogenous peroxidase. Then non-specific binding was blocked with 2.5% horse normal serum from the ImmPRESS reagent kit Anti-mouse IgG/Anti-rabbit IgG (Vector Laboratories, Newark, CA, USA) for 20 min. The primary antibodies used in this study (*T. maritimum* isolate Tm-035 from rainbow trout [8] and *T. dicentrarchi* isolate Td-005 obtained from diseased Atlantic salmon during an outbreak in the year 2018) were added at 1:2000 dilution and incubated overnight at 4°C . The next day, the samples were incubated with the ImmPRESS Polymer HRP Anti-Rabbit IgG Reagent (Vector Laboratories, Newark, CA, USA) for 30 min at 20°C . The peroxidase reaction was visualized using the NovaRED kit (Vector Laboratories, CA, USA). Sections were each stained with Harris hematoxylin and Sodium Tetraborate for a few seconds. Then they were examined using Leica DM1000 microscopy coupled with a Leica ICC50HD digital camera (Leica Microsystems GmbH, Wetzlar, Germany) and Motic Easy Scanner (Motic, Xiamen, China) for digitalization of samples.

2.3. Specific PCR Test for Identification of *Tenacibaculum* Species

Extraction of DNA was performed with the E.Z.N.A total DNA KIT (Omega BIOTEK, Norcross, GA, USA), in accordance with the manufacturer's instructions. Negative controls were processed with only phosphate-buffered saline (no *C. rogercresseyi*). Additionally,

DNA obtained from the *T. maritimum*-type strain NCIMB 2154^T and *T. dicentrarchi* NCIMB 15498^T were included as a positive control for each PCR assay. Extracted DNA samples were stored at −20 °C until analysis. All PCR amplifications were performed using a SURECYCLER 880 Thermocycler (Agilent Technologies Inc., Santa Clara, CA, USA) and the GoTaq Green Master Mix Kit (Promega, Madison, WI, USA) according to the manufacturers' instructions. PCR reactions were carried out in a final volume of 25 µL containing 2.5 µL of template DNA (10 ng/µL) and 0.75 µL of each primer (10 µM), resulting in a final primer concentration of 0.3 µM. All samples, including positive and negative controls, were analyzed in duplicate. The GoTaq kit included all of the reagents needed for PCR (buffer, nucleotides, and Taq DNA polymerase) except for the specific primers and DNA template. Sequences of the primer pairs used for each PCR and the expected size of PCR products generated with these primers are presented in Table 1.

Table 1. Primers used in this study.

Bacterial Species	Primers	Sequence 5'–3'	Amplicon Size (bp)	Reference
<i>T. maritimum</i>	MAR1	5'–AATGGCATCGTTTAAA–3'	1088	[29]
	MAR2	5'–CGCTCTCTGTTGCCAGA–3'		
<i>T. dicentrarchi</i>	Tenadi Fw	5'–ATACTGACGCTGAGGGAC–3'	284	[30]
	Tenadi Rv	5'–TGTCCGAAGAAAACCTCTATCTCT–3'		

The amplification cycles used for denaturation, primer annealing, and extension were carried out according to pathogen-specific PCR protocols [29,30]. For *T. maritimum*, PCR cycling conditions were performed as described by [29], consisting of an initial denaturation at 94 °C for 2 min followed by 35 cycles of 94 °C for 2 min, 45 °C for 1.5 min and 72 °C for 2 min. For *T. dicentrarchi*, PCR cycling conditions were performed as described by [30], consisting of an initial denaturation at 95 °C for 2 min, followed by 35 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 1 min, with a final extension step at 72 °C for 5 min. Amplified products were detected by horizontal 1% (*w/v*) agarose gel electrophoresis for 60 min at 100 V in 1× tris–acetate–EDTA buffer (0.04 M Tris, 0.0001 M EDTA, pH 8.0). The products were visualized using SafeView (NBS Biologicals, Huntingdon, UK), photographed under ultraviolet light, and digitally recorded using a Gel Doc 100 system (Bio-Rad, Berkeley, CA, USA). A 100–3000 bp DNA ladder (AccuRuler 100 bp Plus, Maestrogen Inc., Hsinchu, Taiwan) was used as a molecular size marker.

3. Results

3.1. Immunohistochemical Detection of *Tenacibaculum* in *Caligus rogercresseyi*

Immunohistochemical analysis revealed the presence of *T. maritimum* in distinct anatomical structures of the sea lice *C. rogercresseyi*. Mild to moderate positive signals were observed in the cuticle of both male and female parasites, particularly in superficial regions, indicating that the bacterium can adhere to or colonize external parasite tissues (Figures 1B and 2B).

In the case of immunohistochemistry targeting *T. dicentrarchi*, positive signals were primarily detected in the cuticle of the ectoparasites as well as in the superficial region of the ovigerous sac, where moderate immunoreactivity was consistently observed (Figure 3B).

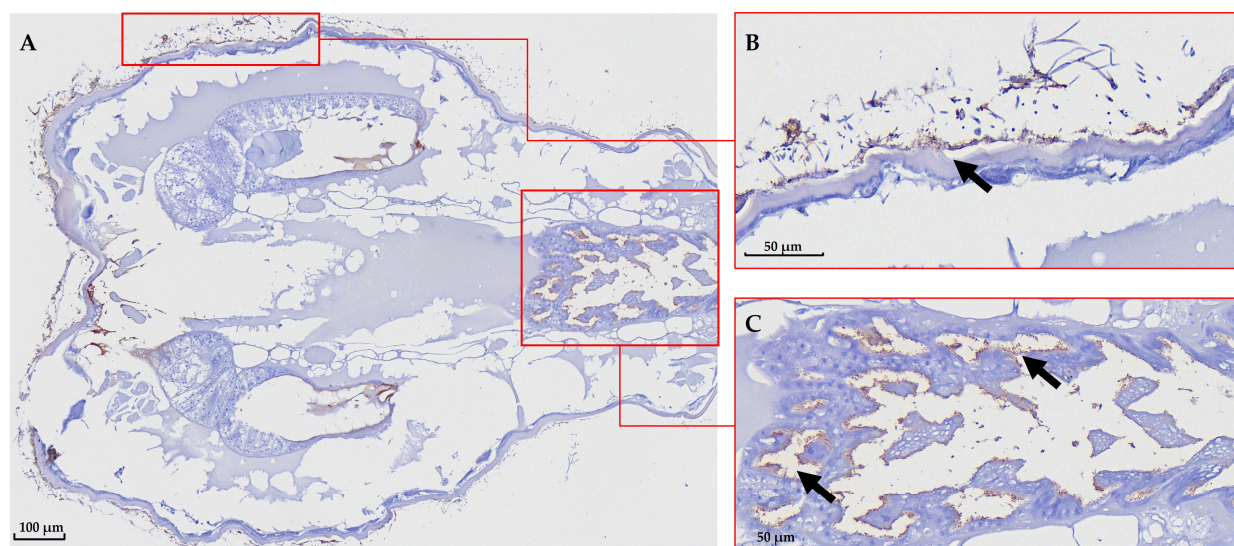


Figure 1. Immunohistochemical detection of *T. maritimum* in Sea Lice (male). (A) Whole sea lice slide, magnification 12 $\times$. (B) Magnification 80 $\times$; a mild positive reaction to *T. maritimum* is observed in the superficial area of the cuticle (arrows). (C) Magnification 80 $\times$; a moderate positive reaction is observed in the gastrointestinal tract. The scale bar for each image is indicated in micrometers (μm) in the lower left corner of the corresponding panel. The arrows indicate positive reactions by IHC.

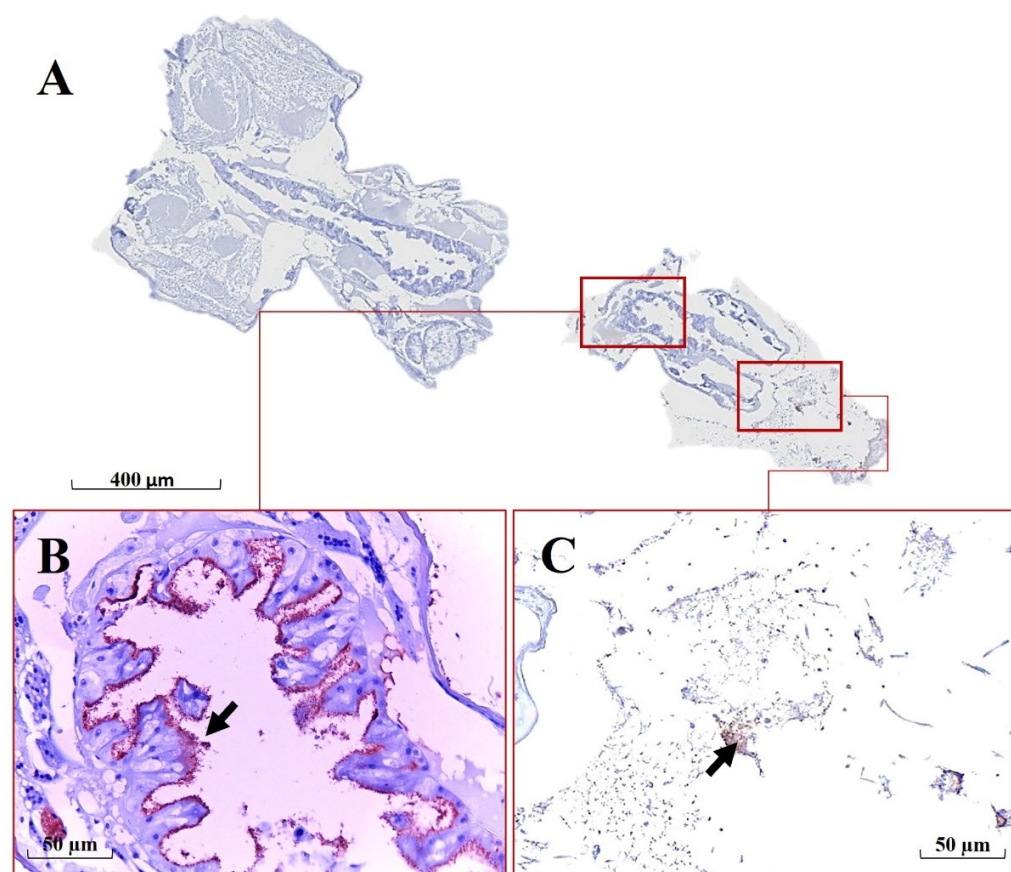


Figure 2. Immunohistochemical detection of *T. maritimum* in Sea Lice (female). (A) Whole sea lice slide, magnification 1.51 $\times$. (B) Magnification 40 $\times$; a positive reaction to *T. maritimum* is observed in the gastrointestinal tract. (C) Magnification 40 $\times$; additional positive signal in the gastrointestinal tract. Scale bars are shown in micrometers (μm) in the lower left or right corner of the corresponding panel. The arrows indicate positive reactions by IHC.

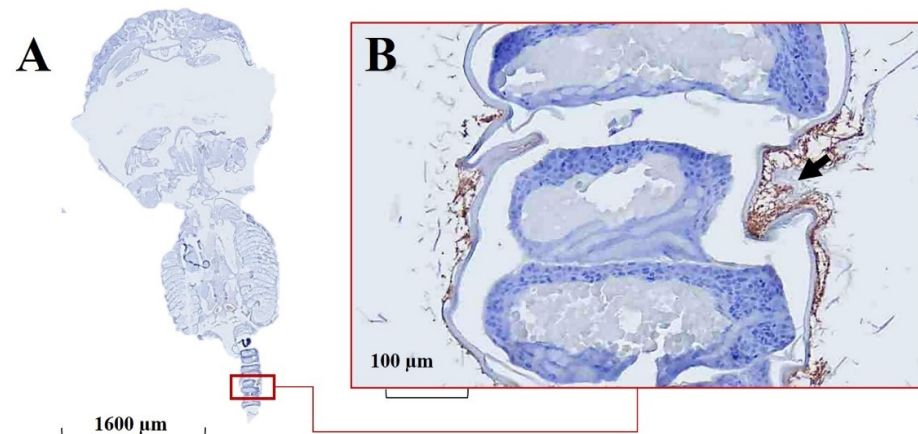


Figure 3. Immunohistochemical detection of *T. dicentrarchi* in Sea Lice (female). (A) Whole sea lice slide, magnification 1×. (B) Magnification 20×; a moderate positive reaction to *T. dicentrarchi* is observed in the superficial region of the ovigerous sac. The scale bar for each image is indicated in micrometers (µm) in the lower left corner of the corresponding panel. The arrows indicate positive reactions by IHC.

3.2. Specific PCR-Based Detection of *Tenacibaculum* Species in *Caligus rogercresseyi*

Conventional PCR assays were performed separately on the 10 parasite samples obtained (five males and five ovigerous females), using the species-specific primers described in Table 1 for the detection of *T. maritimum* and *T. dicentrarchi*. Five of the ten samples analyzed (5/10) tested positive for *T. maritimum* which amplified the 1088 bp amplification fragment (Figure 4A). In the case of *T. dicentrarchi*, seven of the ten samples analyzed (7/10) tested positive for this pathogen, which amplified the 284 bp amplification fragment. Positive controls were consistent with the amplicon sizes generated by the corresponding reference strains used in this study.

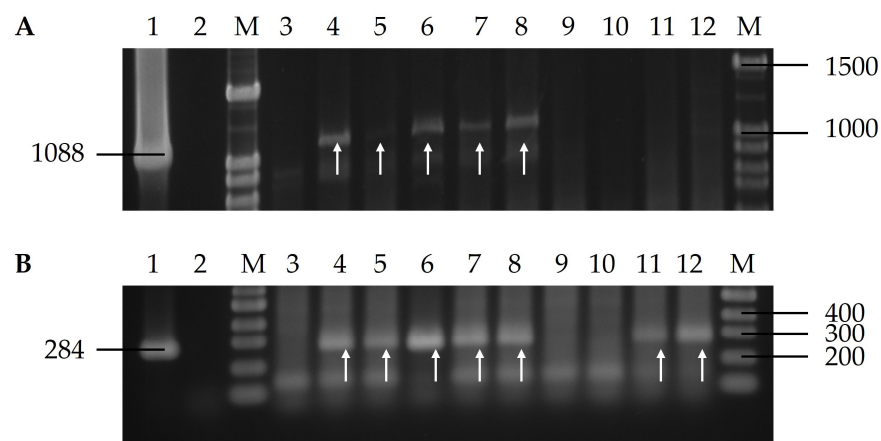


Figure 4. Detection of *Tenacibaculum* spp. in *Caligus rogercresseyi* by conventional PCR. (A) Amplification of the 1088 bp fragment specific for *T. maritimum* using the MAR1/MAR2 primer set. C+ (Lane 1): positive control (*T. maritimum* NCIMB 2154^T); C− (Lane 2): negative control (no DNA template); M: molecular weight marker. Lanes 3–12 correspond to individual *C. rogercresseyi* samples. Arrows indicate positive amplification products. Numbers on the right indicate molecular marker sizes (bp), and the number on the left indicates the expected amplicon size (1088 bp). (B) Amplification of the 284 bp fragment specific for *T. dicentrarchi* using the Tenadi Fw/Rv primer set. C+ (Lane 1): positive control (*T. dicentrarchi* NCIMB 15498^T); C− (Lane 2): negative control (no DNA template); M: molecular weight marker. Lanes 3–12 correspond to individual *C. rogercresseyi* samples. Arrows indicate positive amplification products. Numbers on the right indicate molecular marker sizes (bp), and the number on the left indicates the expected amplicon size (284 bp). The arrows indicate positive identification by PCR.

4. Discussion

Ectoparasites are increasingly recognized as important drivers in the dissemination of infectious diseases in aquaculture. The presence of sea lice such as *C. rogercesseyi* may contribute to the spread of emerging pathogens, particularly species of the genus *Tenacibaculum* (*T. maritimum*, *T. dicentrarchi*, *T. finnmakense*), which are globally relevant fish pathogens associated with ulcerative skin disorders in salmonids and other species of economic importance [4,17]. In Chile, the primary impacts of *C. rogercesseyi* infestations on salmon are immunosuppression and mechanical damage to the skin, producing extensive petechial hemorrhages. These lesions provide an entry point for opportunistic bacteria and increase host susceptibility to *P. salmonis*, the major bacterial pathogen affecting salmon farming in Chile [2].

The skin microbiota of Atlantic salmon plays a central role in shaping the bacterial community associated with *C. rogercesseyi* [31]. As the parasite feeds on host mucus and epidermal tissues, it acquires not only cellular debris but also bacteria inhabiting the skin surface [31,32]. Opportunistic genera such as *Polaribacter*, *Photobacterium*, and *Tenacibaculum* have been detected in both the salmon skin and the parasite, indicating direct microbial transfer [32]. Similar interactions have been described in other fish–parasite systems, where ectoparasites were shown to modulate host-associated microbial communities, further evidencing the role of parasites as key drivers of microbiota composition and dynamics [33,34]. However, high-throughput sequencing often resolves bacterial identity only to the genus level; thus, *Tenacibaculum* was identified as a dominant genus in previous studies, but species-level resolution was not possible [31]. In contrast, the present study provides direct evidence of *T. maritimum* and *T. dicentrarchi* in *C. rogercesseyi*, confirmed through species-specific PCR and immunohistochemistry. These findings also validate the reliability of the molecular assays employed, providing robust confirmation of the presence of both *Tenacibaculum* species in the analyzed *C. rogercesseyi* specimens.

Histological analysis further demonstrated the tissue-specific localization of *T. maritimum* within the parasite. Mild to moderate immunohistochemical signals were observed in the superficial cuticle, whereas consistent moderate positive reactions were detected in several regions of the gastrointestinal tract of both male and female lice. Similarly, *T. dicentrarchi* was detected in the cuticle and superficial ovigerous sac, suggesting that the bacterium may adhere to or colonize parasite surfaces that are in direct contact with the host's mucus and skin. This localization may favor environmental persistence, while its presence in the ovigerous sac raises the possibility that bacterial cells are associated with reproductive structures, potentially contributing to horizontal and, possibly, vertical transmission pathways. The detection of both pathogens in distinct anatomical structures indicates that they are not restricted to external contamination but may also persist within the parasite, supporting the hypothesis that *C. rogercesseyi* may share a similar ecological role to *L. salmonis* as a potential carrier of bacterial pathogens [15,35]. Similar observations have been reported in other ectoparasite systems. Ticks are well-recognized carriers and vectors of a wide range of bacterial, viral, and protozoan pathogens, and interactions among pathogens, microbiota, and arthropod hosts have been shown to influence transmission success [15]. Likewise, aquatic ectoparasites such as *L. salmonis* have been reported to harbor several putative fish pathogens, including bacterial and viral agents, while other marine invertebrates, such as jellyfish, have been implicated in the transport of fish pathogens [11,12,22]. However, the detection of microorganisms within an ectoparasite does not necessarily demonstrate vector competence, as successful pathogen transmission requires experimental evidence of acquisition, persistence, and transfer to a susceptible host. Previous studies on *L. salmonis* and *C. rogercesseyi* have highlighted the importance of distinguishing pathogen carriage from true vectorial capacity [13,14]. At least for *T. maritimum*, these localization patterns are

consistent with a surface-associated lifestyle: its moderately hydrophobic cells rapidly form viable biofilms that may act as transient reservoirs in aquaculture environments, providing a plausible mechanism for persistence on the louse cuticle and other parasite-associated interfaces [36].

Analogous situations have been documented in other host–parasite systems. For instance, the hydromedusa *Phialella quadrata* was reported to act as a reservoir and vector of *T. maritimum*, causing gill disease in Atlantic salmon in Scotland [12]. Similarly, *L. salmonis* in the Northern Hemisphere has been associated with bacterial pathogens such as *A. salmonicida*, *T. maritimum*, *T. dicentrarchi*, *P. fluorescens*, *V. anguillarum* and *Vibrio* spp., as well as viral pathogens including salmonid alphavirus and infectious salmon anemia virus [11,21,26,27]. Experimental studies have also demonstrated that *L. salmonis* can act as a mechanical rather than biological vector of IHNV, transmitting the virus only after feeding on infected donor fish [13].

Taken together, these findings reinforce the hypothesis that *C. rogercresseyi* may share a similar ecological status with *L. salmonis* as a potential carrier of bacterial pathogens. Our study is the first to demonstrate the presence of *T. maritimum* and *T. dicentrarchi* in *C. rogercresseyi* through combined molecular and histological approaches. While the evidence confirms their presence within the parasite, further experimental work is needed to establish whether sea lice actively transmit Tenacibaculosis among salmon within cages or across sites under specific environmental conditions. Considering the persistence of Sea Lice in Chilean salmon farming since the early stages of the industry [37,38], and its potential to harbor pathogens already documented in related ectoparasites, our findings highlight the urgent need for targeted studies to clarify the epidemiological role of *C. rogercresseyi* in disease dynamics.

5. Conclusions

This work constitutes the first report showing the presence of *Tenacibaculum* species in *Caligus rogercresseyi* through the combined use of immunohistochemistry and PCR. While PCR confirmed the presence of *T. maritimum* and *T. dicentrarchi*, immunohistochemistry provided evidence of their anatomical localization within the parasite. The results demonstrate that *C. rogercresseyi* can harbor both pathogens, supporting the potential role of *C. rogercresseyi* as a carrier of *Tenacibaculum* spp. in salmon aquaculture. These findings expand current knowledge regarding potential pathogen reservoirs in farming systems; however, they should be interpreted as evidence of pathogen carriage rather than transmission, since no evidence of vector competence or pathogen transmission was generated in the present study. Experimental studies are required to determine whether *C. rogercresseyi* can act as a mechanical or biological vector of *Tenacibaculum* spp.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. Access to the data may be subject to restrictions imposed by collaborating companies to protect confidential and commercially sensitive information.

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Conflicts of Interest: Authors Pedro Ilardi, Eugenia Jerez, Sara Valdes, Harold Oliva were employed by the company Investigación y Desarrollo Farmacología en Acuicultura Veterinaria FAV S.A. Author Carlos Sandoval was employed by the company Research & Development Veterinary Histopathology Center SpA. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ISAV	Infectious Salmon Anemia Virus
IHN	Infectious Hematopoietic Necrosis Virus
IHC	Immunohistochemistry
NCIMB	National Collection of Industrial, Food and Marine Bacteria
PCR	Polymerase Chain Reaction

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