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Short communication

Morphological and molecular characterization of *Pseudocohnilembus longisetus* Thompson, 1965 from farmed black rockfish *Sebastes schlegelii* in Korea

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ABSTRACT

The morphology, infraciliature, silverline system, and the small subunit ribosomal RNA (SSU rRNA) of the little-known marine scuticociliate *Pseudocohnilembus longisetus* Thompson, 1965 from the diseased black rockfish *Sebastes schlegelii* in Korea were studied. This scuticociliate possessed the typical characteristics of the genus *Pseudocohnilembus*, but could be discriminated from *Pseudocohnilembus hargisi*, and *Pseudocohnilembus persalinus* in terms of the body size, shape, the number of somatic kineties and kinetids in somatic kinety 1, and the number/position of contractile vacuole pores. The SSU rRNA gene of *P. longisetus* was sequenced in order to gain a better understanding of appropriate phylogenetic classification. The SSU rRNA was 1754 bp and the sequence was deposited in GenBank under accession number FJ899594. The SSU rRNA gene sequences of *P. longisetus* had an identity of 98.1%, 96.8% and 95.3% with *P. hargisi*, *P. persalinus*, and *Pseudocohnilembus marinus* SSU rRNA sequences, respectively. Our population of *P. longisetus* belonged to the genus *Pseudocohnilembus* and was in an isolated position based on the SSU rRNA gene tree, which was consistent with the conclusions based on the morphological studies. However, further investigation is required to determine the pathogenicity of this species.

1. Introduction

The most important finfish species raised in aquaculture in Korea are the olive flounder *Paralichthys olivaceus* (Temminck and Schlegel, 1846) and Schlegel's black rockfish *Sebastes schlegelii* (Hilgendorf, 1880). They account for more than 70% of the total production of marine finfish aquaculture because they are highly esteemed in Korea for raw consumption in thin slices, and command a good market price for farmers.

Over the past decade, the number of reports on disease outbreaks in marine finfish aquaculture have increased significantly as the industry has prospered. One problem associated with flounder farming is scuticociliatosis (Kim et al., 2004a; Jin et al., 2009; Song et al., 2009). Scuticociliates are one of the serious parasitic threats which produce severe systemic infections through rapid invasion of internal organs, and ultimately induce mass mortalities of the host population (Cheung et al., 1980; Bassleer, 1983). In 1980, nine species of saltwater aquarium fish suffered mortalities due to *Uronema marinum* (Cheung et al., 1980). Cultured striped trumpeter (*Latris lineasta*), and southern bluefin tuna (*Thunnus maccoyii*) suffered from myositis and encephalitis, respectively, due to infection with *Uronema* sp. (Langdon, 1992; Watts et al., 1996). In Korea, *U. marinum* (Jee et al., 2001) and other scuticociliate species such

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Table 1
Primers used in this study.

Primer name	Orientation	Purpose	Primer sequences (5′ → 3′)
SSUF	Forward	Amplification	AACCTGGTTGATCCTGCCAG
SSUR	Reverse	Amplification	GATCYWTCTGCAGGTTCACCTAC
SSU-IF	Forward	Internal sequencing 1	CGGTAATTCCAGCTCCAATAG
T7	Forward	Internal sequencing 2	GATGTGCTGCAAGGCGATTAAGTTGG
SK	Reverse	Internal sequencing 3	CGCTCTAGAACTAGTGGATCCC

Degenerate nucleotides (Y: C/T, W: A/T) are underlined.

as *Philasterides dicentrarchi* (Kim et al., 2004a), *Pseudocohnilembus persalinus* (Kim et al., 2004b), and *Miamiensis avidus* (Jung et al., 2005, 2007; Song et al., 2009) have been reported to be the causative agents of scuticociliatosis in cultured olive flounder. Compared with several scuticociliates isolated from cultured olive flounder, however, little is known about the scuticociliate species screened in the outbreak of scuticociliatosis from farmed black rockfish in Korea.

In this study herein, we first isolated a scuticociliate species, *Pseudocohnilembus longisetus* Thompson, 1965, from diseased black rockfish (*S. schlegelii*) and described its morphological and molecular characteristics to compare with other known scuticociliates.

2. Materials and methods

2.1. Ciliate isolation and in vitro culture

Scuticociliates were isolated from diseased black rockfish *S. schlegelii* obtained from a local fish farm in Jeju, Korea, in August 2005. Wet preparation of gill, skeletal muscle, skin, fin and brain tissue showing typical symptoms of infection (ulceration and hemorrhage of skeletal muscle) were examined for the presence of ciliates. The infected tissues were washed 3 times with sterilized seawater (containing 1% yeast extract and 1% proteose peptone) and cultivated at 15 °C for 7 days. After 7 days in culture, ciliates were cloned using the limited dilution method with some modifications (Goding, 1993). Briefly, a series of dilutions was made from the original culture until one ciliate remained in each well of a 96-well tissue-culture plate containing MillportS (NaCl 1.5 g, $MgCl_2 \cdot 6H_2O$ 0.25 g, KCl 0.04 g, $CaSO_4$ 0.012 g, as described by Provasoli et al., 1957) and 0.1% brain heart infusion broth medium. The cloned ciliate was sub-cultured and maintained in the same medium in a petri-dish at 15 °C.

2.2. Staining and microscopic characteristics

Cloned ciliates were wet-mounted and observed in order to record vital characteristics and motility under a differential-interference-contrast (DIC) microscope. The wet Chatton–Lwoff silver nitrate impregnation method (Foissner, 1991) and the silver carbonate impregnation method (Ma et al., 2003) were used to reveal the infraciliature and silverline system. Impregnated ciliates were examined by light microscopy and measured using an ocular micrometer and image-analyzing software (Image-Pro Plus 3.0, USA).

2.3. Nuclear DNA extraction, PCR amplification of SSU rRNA and sequence analysis

Cultured ciliates were collected by centrifugation at 1000 × *g* for 10 min and washed with sterilized seawater. Genomic DNA was extracted using the QIAmp DNA Mini kit (Qiagen, Germany), and the concentration of total genomic DNA was measured on a SmartSpec™ Plus Spectrophotometer (Bio-Rad, USA). Based on SSU rRNA sequences of scuticociliates in GenBank (Accession number: *U. marinum* Z22881, *Uronema elegans* AY103190, *Thyrophylax vorax* AY541686), SSUF and SSUR were designed to amplify the SSU rRNA sequences of the ciliate (Table 1). PCR reactions were performed in a 50 μl PCR reaction mixture containing 20 pmol of each primer, 2.5 U of Ex Taq polymerase (Takara, Japan) and 50 ng of genomic DNA. In a Takara PCR Thermal cycler (Takara, Japan), the reaction was run for 30 cycles at 95 °C for 30 s, 55 °C for 35 s, and 72 °C for 2 min with a pre-denaturation step at 95 °C for 5 min. The amplified products were analysed by electrophoresis on a 1% agarose gel. PCR products were cut from the gel and extracted using the Accuprep™ Gel purification kit (Bioneer, Korea). The purified product was ligated into pBluescript II SK(-) and used to transform *Escherichia coli* DH10b (Stratagene, USA). Recombinant plasmid was prepared by the alkaline lysis method using the Accuprep™ Plasmid Extraction kit (Bioneer, Korea). The complete sequences of SSU rRNA were confirmed by sequencing with internal primer SSU-IF, universal primers SK and T7 on an ABI 377 DNA sequencer (Applied Biosystems, USA) (Table 1).

2.4. Phylogenetic analyses

The sequences were aligned using ClustalW 1.80 (Thompson et al., 1994). Alignments were analysed with the MEGA 4.0 program to produce neighbor-joining (NJ) trees using the Kimura 2-parameter model (Saitou and Nei, 1987). The confidence estimate in the NJ trees was obtained based on bootstrap generation of 1000 replicates. The nucleotide sequences used to align the newly sequenced SSU rRNA gene in this paper were obtained from the GenBank/EMBL databases (Fig. 2).

3. Results

3.1. Morphological characteristics of P. longisetus

The ciliate body was generally of a slender shape with an elongated bluntly tapered anterior, and narrowly rounded posterior end (Fig. 1A). Under optimal nutritional conditions, the ciliate had an oval shape with