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Case report

First evidence of sea lice (*Caligus fugu*) infestation in captive map pufferfish in Thailand

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Abstract

Sea lice, *Caligus fugu*, belonging to the family Caligidae, cause skin lesions in pufferfish aquaculture. Despite its importance, information on *Caligus spp*. in Thailand is very limited. This study highlights the first evidence of *C. fugu* infestation in map pufferfish, Thailand. A captive map pufferfish from the Phuket Sea region presented with abnormal posture of swimming and breathing. External parasites were observed on the puffer's fins and skin. Based on its morphological characteristics, *C. fugu* was identified and displayed in this study. The map pufferfish was successfully treated with 0.0075% hydrogen peroxide against adult *C. fugu* with negative for parasitic organisms. The presence of *C. fugu* in this study supports the importance of the control of external parasites in aquaculture, especially in aquariums. In addition, we encourage further studies of genetic diversity and also seasonal occurrence of genus *Caligus* in Thailand to fulfill the understanding of the epidemiological factors of this pathogen

Keywords: Aquariums, *Caligus fugu*, External parasites, Map pufferfish, Sea lice

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INTRODUCTION

Sea lice (Caligidae, *Caligus* spp.), also known as copepods, are small parasitic crustaceans that attach themselves to the skin and pose a major problem for fish farms, as they can cause significant harm to the fish and lead to reduced growth and increased mortality which affects the development of marine fish aquaculture (Johnson et al., 2004a; Johnson et al., 2004b; Liu and Bjelland, 2014). *Caligus* spp. live and reproduce on various marine animals, spread by releasing of eggs into seawater. Later, these eggs develop into the infective stages (Johnson and Fast, 2004; Costello, 2006).

Evidence of the widespread occurrence of *Caligus* spp. were reported on farmed captive tuna and salmon in previous studies (Revie et al., 2002; Hayward et al., 2008). In Thailand, the presence of *Caligus* spp. has been identified in marine fish species based on its morphological characteristics. The prevalence of *Caligus* spp. in marine fishes from the Gulf of Thailand were 9.09-46.0% (Purivirojkul and Areechon, 2008; Niyom and Purivirojkul, 2020). Despite the high prevalence in natural marine fish species, epizootic information was limited on *Caligus* spp. in captive marine aquaculture, including aquarium animals.

Caligus fugu (former member of the genus *Pseudocaligus*), host-specific to pufferfish, was first recognized in 1936 (Yamaguti, 1936; Ohtsuka et al., 2009; Venmathi Maran et al., 2011). Previous studies showed the distribution of *C. fugu* mostly in Japan (Ogawa and Inouye, 1997; Ohtsuka et al., 2009; Okawachi et al., 2012). *P. fugu* has been reported to cause skin lesions in tiger puffer (*Takifugu rubripes*) and grass puffer *(T. niphobles*) in Japan (Ohtsuka et al., 2009; Okawachi et al., 2012). The prevalence of *C. fugu* in previous studies were 20 to 35 % in dispersed geographical areas (Okawachi et al., 2012).
This investigation presented the first evidence of sea lice, *C. fugu*, in a captive map pufferfish (*Arothron mappa*) in a Thai aquarium, based on morphological characteristics. The success of hydrogen peroxide (H_2O_2) in treating *C. fugu* was also mentioned in this study. With that, the information in this case report could be useful for external parasite control and animal health care in the aquarium.

HISTORY CLINICAL FINDINGS AND TREATMENT

A map pufferfish (*A. mappa*), with a total length of 30 cm from head to tail, was obtained from fishermen in the Phuket Sea region in November 2021. In terms of environment and feeding, the fish was caught in a 2 cubic meter closed-filter system cabinet with 1 spotted pufferfish (*Dichotomyctere nigroviridis*) and 2 sea urchins (class Echinoidea). Before being brought into the display cabinet, the fish was quarantined for 14 days. The map puffer was provided with 2-3 shrimp and fish fillets per day. However, physical examination and scraping were not performed before being brought into the display cabinet. Fish keepers found by observation that the fish showed no clinical signs and was able to eat, so they then transferred the fish to the display cabinet.

Later, the map pufferfish was displayed in the aquarium for 7 months and started showing clinical signs of gasping, fast breathing, and sinking to the floor of the aquarium. The veterinarian performed a physical examination and found external parasites on the fins and skin of the pufferfish, total body length 3.9 mm (range 3.6 to 4.1 mm), including caudal rami (Figure 1). Skin scraping was conducted to identify the external parasites at the Veterinary Diagnostic Center, Faculty of Veterinary Medicine, Chiang Mai University. The results were positive for *Caligus fugu* infestation using stereomicroscope (Figure 2A) according to their morphological characteristics (Boxshall and El-Rashidy, 2009; Ohtsuka et al., 2009). The morphology of the cephalothoracic shield, thorax, abdomen, and egg strings were observed in female adults and displayed in this study (Figure 2B and 2C). The reduced legs 4 of *C. fugu* were evident (Figure 2C). Water parameters were also investigated in this study and revealed the appropriate range for marine fish (Table 1).

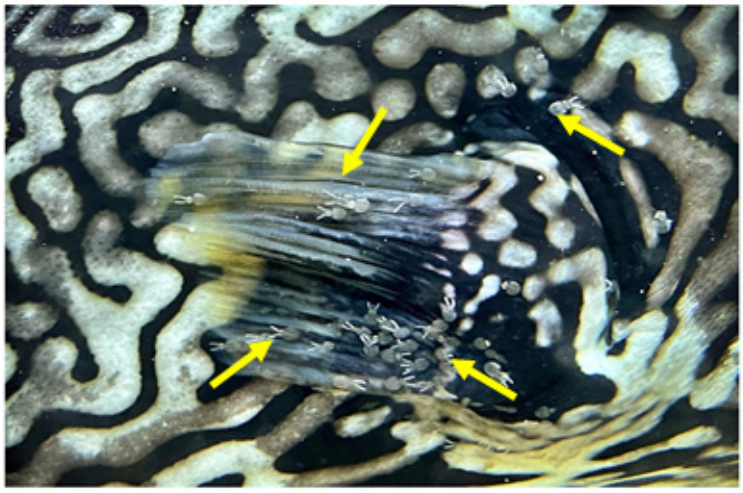

Figure 1 Physical examination revealed the evidence of external parasites (arrows) on the map puffer's fins and skin.

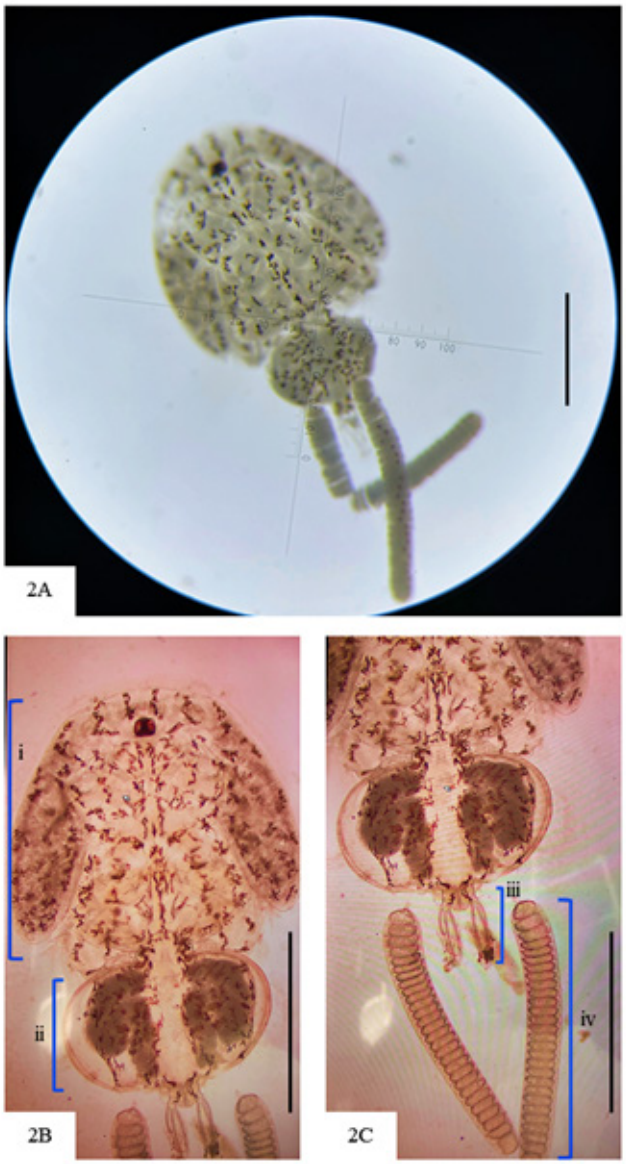

Figure 1 Morphological characteristics of *C. fugu* were observed under stereomicroscope in this study. (A) stereomicroscopic examination showed the total length of habitus (dorsal view) of an adult female *C. fugu*, (B) cephalothorax (i), abdomen (ii) and (C) legs (iii) and egg strings (iv) of the C. fugu. Scale bars (black line): 1 mm.

Table 1 The results of water parameters in the display cabinet with the map puffer in the aquarium on the day that *Caligus* spp. were found. The results were compared with the suitable values (Stoskopf, 1993).

Parameter	Result	Suitable values*
pH	8.08	7.5 - 8.9
Temperature	28.30 °C	24-29 °C
Salinity	33 ppt	29 – 35 ppt
Chlorine	0	0
NH_3	0.0153	< 0.4
Nitrate	0.008	< 0.01

The map pufferfish in this case was treated against *C. fugu* infestation by separated bathing in 0.0075% H_2O_2 for 20 minutes. Increment of the frequency of water changes, and also removal of the sand from the enclosure to break the vicious circle of this parasite were actuated. Patient follow-up and parasitic investigation were performed on day 1 and 7 post-treatment. On physical examination, the fish showed normal signs with a normal gait swimming and a normal appetite. The treatment was considered accomplished when the external appearance of the patient was negative for parasitic organisms.

DISCUSSION

Caligidae or sea lice have not been widely reported as being pathogenic to farmed fish, however, they can cause damage to the host's skin and fins in severe infestations and lead to death in some cases (Johnson et al., 2004; Ohtsuka et al., 2009; Liu and Bjelland, 2014). In terms of aquaculture, sea lice have several documented records on the salmonid farming industry (Jackson et al., 1997; Costello, 2009; Torrissen et al., 2013). Despite the importance and numerous information of *Caligus* spp. in aquaculture farms, parasitic evidence in fish aquariums is limited. In Thailand, *Caligus* spp. has been reported in natural marine fishes from the Gulf of Thailand (Purivirojkul and Areechon, 2008; Niyom and Purivirojkul, 2020). This report highlighted the evidence of *C. fugu* in captive map puffer, which was caught in the Phuket Sea of Thailand, and characterized the parasites by the morphological observation under stereomicroscope. *C. fugu* was specified by a reduced leg 4 (Yamaguti, 1936; Ohtsuka et al., 2009; Venmathi Maran et al., 2011; Hayes et al., 2021). In addition, previous studies suggested that *C. fugu* seems to disappear from the Grass puffer (*T. niphobles*) in cold seasons (Venmathi Maran et al., 2011; Okawachi et al., 2012). Considering the water parameters in this case report (Table 1), there was a possibility of the high-temperature phenomenon to *C. fugu* incidence on hosts. However, the spotted pufferfish that entered the display cabinet at the same time did not show clinical signs, and no external parasites were detected. Therefore, it is difficult to conclude that the case of map pufferfish in this study was infested by *C. fugu* outside or inside the aquarium.

C. fugu has been reported as a sea lice specie that is host-specific and causes skin lesions in the pufferfish (Ohtsuka et al., 2009; Freeman and Ogawa, 2010). This report provided evidence of *C. fugu* in a map pufferfish obtained

from nature to be captive with clinical signs. However, other dimensions including complete blood count and blood chemistry investigations, or host immune response were beyond our investigations.

Sea lice are primarily controlled by the use of chemicals, which are applied to the fish in the form of bath treatments or feed additives (Grave et al., 2004; Bravo et al., 2010; Marín et al., 2015). H_2O_2 causes paralysis and detaches the lice to the water surface (Bruno and Raynard, 1994; Treasurer et al., 2000). Likewise, this study showed a good response to the treatment of H_2O_2 against *C. fugu*. However, a previous study described that H_2O_2 was good at controlling adult *Caligus*, but had poor effects over the chalimus stages (Johnson and Margolis, 1993). Apart from chemical control, other risk factors in the fish environment should be controlled including water quality, and removing the sand in the cabinet. In addition, paying attention to external parasites and abnormalities in captive fish and other animals in the aquarium is necessary for animal welfare.

CONCLUSIONS

Data from this current case report highlights the identification of *C. fugu* infestation by its morphological characteristics in captive aquarium fish, map pufferfish, in Thailand. This report also mentioned the success of using chemical treatment, H_2O_2, against adult *C. fugu* infestation. The evidence of *C. fugu* in this study supports the importance of external parasite control in aquarium fish and other animals to prevent parasitic diseases. Apart from the characterization, further studies regarding *C. fugu* are needed including the genetic characteristics and diversity of *C. fugu* and/or seasonal occurrence of *C. fugu* infestation in Thailand for the full understanding of the epidemiological factors of the pathogen for disease control.

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AUTHOR CONTRIBUTIONS

Conceptualization, BN, SP; Clinical methodology, SB, PJ, AB, PK, TP; Laboratory methodology, SC, DS, BN; investigation, SB, PJ, AB, PK, TP, data interpretation, BN; writing-original draft preparation, BN; editing and approval of final draft, BN, SP. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare no competing interests.

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