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Article

Histopathology of Venerid Clams *Leucoma* (=*Protothaca*) *jedoensis* (Lischke, 1874) Occurring on the South Coast of Korea

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Abstract : Commonly occurring on a shallow subtidal coarse sand bottom in the northwest Pacific region, the Jedo Venus clam *Leucoma* (=*Protothaca*) *jedoensis* (Lischke, 1874) is one of the valuable shellfish resources supporting the local fisheries industry on the south coast of Korea. In this study, we surveyed the pathologic condition of *L. jedoensis* from a shallow subtidal sand flat in Gamakman Bay on the south coast using histology and Ray's fluid thioglycollate medium (RFTM) assay to diagnose infection by the protozoan pathogen *Perkinsus olseni*. In September 2022, a total of 200 clams with shell length (SL) ranging from 29 to 50 mm were obtained from Gamakman Bay, and each clam was subjected to histology and RFTM assay. Histology revealed that all clams collected in September were in the resting stage, indicating that *L. jedoensis* in the study area completed spawning before September. *Perkinsus olseni* and unidentified trematode in the sporocysts stage were observed in histology with a low prevalence of 1.5% and 1%, respectively. Contrary to the histology, the RFTM assay indicated that 4.5% of the Jedo Venus clams examined in this survey were infected with *P. olseni*, with an average infection intensity of 1.1 x 104 cells/g gill, highlighting the presence of *P. olseni* in the area. The histology and RFTM assay suggested that *P. olseni* may not exert a substantial impact on Jedo Venus clam health, compared to Manila clams, the host of *P. olseni* occurring in the south coast exhibiting extremely high levels of *P. olseni* infection intensities and prevalence.

Key words : *Leucoma jedoensis*, *Perkinsus olseni*, trematodes, histopathology, Korea

1. Introduction

The Jedo Venus clam *Leucoma* (=*Protothaca*) *jedoensis*, (Lischke, 1874**)** is a common venerid clam occurring in coarse sand flat from the low intertidal to shallow subtidal along the south coast of Korea, as well as in coastal regions of Japan and China (Okutani 2000; Qi 2004; Park et al. 2006; Park and Yoon 2008). This filter-feeding clam often coexists with Manila clam *Ruditapes philippinarum* (Manila clam) along low-tide shorelines and is considered to be one of the vital shellfish resources on the south coast (Kim et al. 2002; Jung et al. 2004). In general, the color, type, and size of the Jedo Venus clam exhibit variation that depends on environmental parameters, including water depth, temperature, turbidity, nutrient availability, and growth duration (Park and Yoon 2008). Several studies have comprehensively assessed the reproductive cycle, age and growth, and sexual maturation of Jedo Venus clams on the south coast of Korea (Kim et al. 2002, 2003; Jo et al. 2004). According to Park et al. (2006), *L. jedoensis* is infected by the protozoan parasite *Perkinsus olseni*, which has been identified as a major pathogen responsible for the mass mortality of Manila clams and other marine mollusks (Park and Choi 2001; Villalba et al. 2004; Lee et al. 2020, 2021).

Perkinsosis has been officially designated as a notifiable disease by the World Organization for Animal Health (OIE), leading to international measures for its regulation and control (OIE 2021). As determined through DNA sequencing, the genus *Perkinsus* presently encompasses

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seven formally recognized species: *P. marinus*, *P. olseni*, *P. qugwadi*, *P. cheasapeaki*, *P. mediterraneus*, *P. honshuensis*, and *P. beihaiensis* (Villalba et al. 2004; Moss et al. 2008). Within the genus *Perkinsus*, *P. marinus* and *P. olseni* are recognized as the most detrimental species, known for their significant impact. Diseases stemming from infections by these species are collectively referred to as perkinsosis (Villalba et al. 2004). *Perkinsus olseni*, formerly recognized as *P. atlanticus*, is a parasitic organism infecting various marine bivalve species, and it has been documented as a pathogen of Manila clams in regions extending from Europe to Asia, including Korea (Choi and Park 1997; Soudant et al. 2013). Moreover, *P. olseni* has been observed to parasitize additional clam species, specifically Jedo Venus clam *L. jedoensis* and the blood cockle *Anadara kagoshimensis* and *Tegillarca granosa* within the Korean coastal environment (Park et al. 2006; Cho et al. 2022, 2023). However, it is worth noting that its prevalence and infection intensity appear to be of lesser significance in these clam species compared to the impact observed in Manila clams, emphasizing the diversity of host interactions in the context of *P. olseni* infections (Park and Choi 2001; Park et al. 2006; Cho et al. 2022, 2023).

This study surveyed parasites in Jedo Venus clams using conventional histological techniques and diagnosis of *P. olseni* infection using RFTM assay. Our investigation encompassed a comparative analysis of the infection intensity and prevalence of *P. olseni* within Jedo Venus and Manila clams from similar study sites. Through this comprehensive approach, we aimed to gain insights into the dynamics of *Perkinsus* infections in these bivalve species, shedding light on the potential variations in parasitic prevalence and intensity across different host clams in the surveyed areas.

2. Materials and Methods

Sampling sites and sample collection

The south coast of Korea is characterized by diverse small bays featuring muddy-sand intertidal and subtidal zones, which serve as habitats for various commercially valuable shellfish species, including the Jedo Venus clam. In September 2022, a total of 200 clams, with SL ranging from 29 mm to 50 mm, were collected from the subtidal flat located in Gamakman Bay, Yeosu, on the south coast

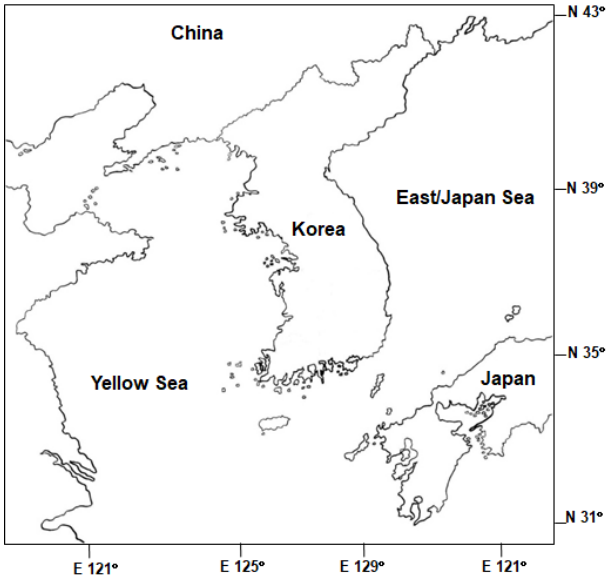

Fig. 1. Map showing the study site. *Leucoma jedoensis* (Jedo Venus clam) was collected from Yeosu, the south coast of Korea

(Fig. 1). Upon collection, the shell length was recorded. Subsequently, the soft body was extracted from the shell, and its wet weight was measured. The condition index (CI) of each clam was then computed as the ratio of the dry tissue weight to the respective dry shell weight (CI = (dry tissue weight/dry shell weight) × 1,000).

Histology

For histology, a 3–5 mm-thick section, including the gills, digestive tubule, gonads, and foot was cut from the middle of the clam body. Subsequently, the tissue section was fixed in Davidson's fixative over 24 hours. Following fixation, the specimens underwent a dehydration process using a progressive series of ethanol. Following dehydration, the tissue specimens underwent paraffin embedding, and sections with a thickness of 5 μm were then prepared from the resulting paraffin blocks. Subsequently, these sections were mounted onto glass slides and underwent a staining procedure, which involved the application of Harris' hematoxylin for primary staining, followed by eosin Y for counterstaining, thus enabling detailed microscopic examination. The clam sections embedded in the histology slides were examined finally under a light microscope, and types of pathogenic organisms were identified.

***P. olseni* Infection Intensity**

Another part of the gill tissue was excised from each clam and placed in 15 ml conical tubes containing 5 ml of Ray's fluid thioglycollate medium (RFTM, Ray 1966), which was fortified with antibiotics, nystatin (200 units/ml) and chloramphenicol (100 ng/ml) to prevent bacterial contamination. Then, the gill tissues underwent a one-week incubation in darkness at room temperature. After the incubation, the gill tissues were digested in 2 M sodium hydroxide (NaOH) at 60°C (Choi et al. 1989). The 2 M NaOH solution was removed by thoroughly rinsing the samples with filtered seawater. Subsequently, the *P. olseni* hypnospores retained in the tubes were resuspended in a phosphate-buffered saline (PBS) solution. The number of *P. olseni* hypnospores in a representative subsample was determined using a hemocytometer. The resulting measure of infection intensity was reported as the count of *P. olseni* cells per gram of gill tissue.

3. Results and Discussion

A total of 200 clams with an average SL of 42.4±3.2 mm were collected and analyzed for histology and RFTM. The clams displayed a mean condition index (CI) of 69. Notably, *L. jedoensis* clams tend to have denser and heavier shells than Manila clams, likely contributing to the lower CI observed in these individuals.

In histology, *P. olseni* trophozoites could be observed in various regions, including the gills, around the digestive tubules, gonads, and the foot (Fig. 2). These *Perkinsus* trophozoites displayed characteristic features, including eccentric vacuole and a signet ring appearance, accompanied by marked hemocyte infiltration (Park et al. 2006). Besides the presence of *P. olseni*, a few clams exhibited larval trematodes and sporocysts containing developing germinal balls within the gonad, as depicted in Fig. 3. The prevalence, the percentage of the infected clams in the total clams examined, of *P. olseni* and the larval trematode was 1.5% and 1%, respectively.

Fig. 4 illustrates the prevalence and mean infection intensities of *P. olseni* in *L. jedoensis* determined using RFTM. Consequently, the infection intensity of *P. olseni* in Jedo Venus clams ranged from 7.0×10^4 to 1.1×10^6, with a mean intensity of 1.1×10^4. A prior study conducted on *L. jedoensis* (=*Protothaca jedoensis*) from Yeosu reported varying infection intensities of *P. olseni*, ranging from 218 to 1.0×10^5 with a mean of 1.1×10^4 in June 2003, and from 356 to 3.1×10^5 with a mean of 0.8×10^4 *Perkinsus* cells per gram of tissue weight in May 2004 (Park et al. 2006). These findings imply that *P. olseni* may not be a prominent protozoan parasite affecting Jedo Venus clams compared to Manila clams (Table 1), which are heavily infected and impacted by *P. olseni* in the context of Korea (Lee et al. 2020, 2021; Yang et al. 2021).

In our current survey, the prevalence of *Perkinsus* determined by RFTM was 4.5%, indicating a relatively low occurrence (Fig. 4). However, in a previous study, the reported prevalence was notably higher, with values of 37% for June 2003 and 53.9% for May 2004 (Park et al. 2006). In our study, *P. olseni* infections were assessed in September, while a previous study conducted the analysis in May and June. Due to this difference in seasons, direct comparisons of infection levels were not feasible. Nevertheless, our findings indicate a decrease in the levels of *P. olseni* infection in Jedo Venus clams compared to the previous study, suggesting a potential temporal variation in the prevalence of this protozoan parasite in the examined clam population. It is noteworthy that, despite these fluctuations, the prevalence of *Perkinsus* in Jedo Venus clams remains considerably lower than that reported for Manila clams on the southern coast of Korea (Table 1). In general, Manila clams demonstrate peak *P. olseni* infection levels, including prevalence and intensity, in September and October during the post-spawning period. Assuming a similar annual infection pattern in Jedo Venus clams, lower infection levels would be anticipated in May and June 2022. However, the observed decrease in *P. olseni* infection in Jedo Venus clams during this period remains unexplained, potentially attributed to environmental factors or changes in the host, such as the development of resistance against *P. olseni*. Further investigation is necessary to elucidate the underlying causes of this observed shift in infection dynamics.

The influence of host species on the virulence of *P. olseni* remains uncertain despite reports of differing susceptibility to *P. olseni* among various host clam species (Park et al. 2006). Rodriguez et al. (1994) found that *P. olseni* (=atlanticus) appeared to spread more readily within *Ruditapes philippinarum* compared to *R. decussatus* and *Venerupis pullastra*, as observed six weeks after inoculation with *P. olseni* zoospores. Since the initial report of *P. olseni* in Korea (Choi and Park 1997), our comprehension

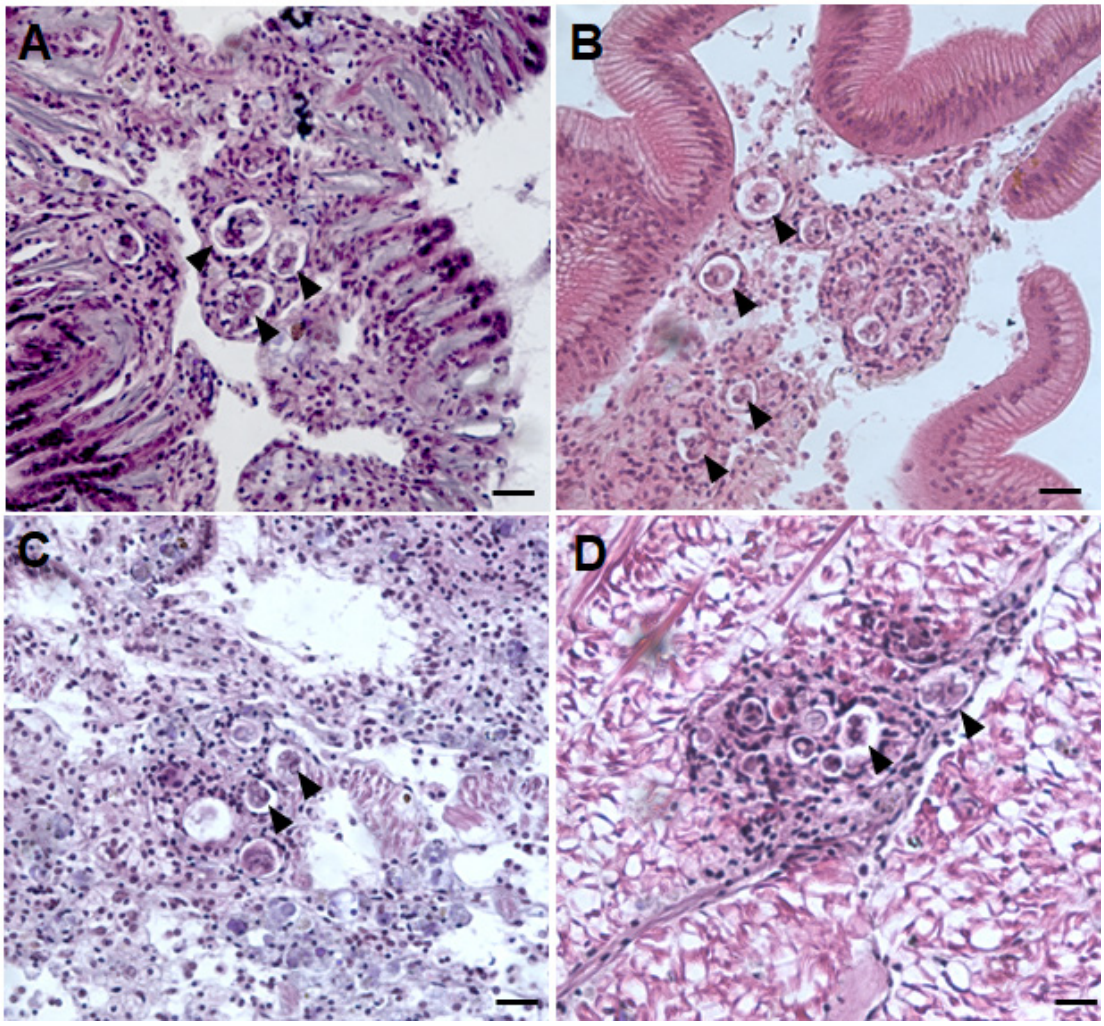

Fig. 2. Histopathological lesions caused by *P. olseni* in the Jedo Venus clam *L. jedoensis* collected from Yeosu, Korea. A large number of trophozoites (arrowheads) are surrounded by infiltrated (asterisks) in the clam gills (a), digestive tubule (b), gonads (c), and foot (d). Scale bar: 20 μm

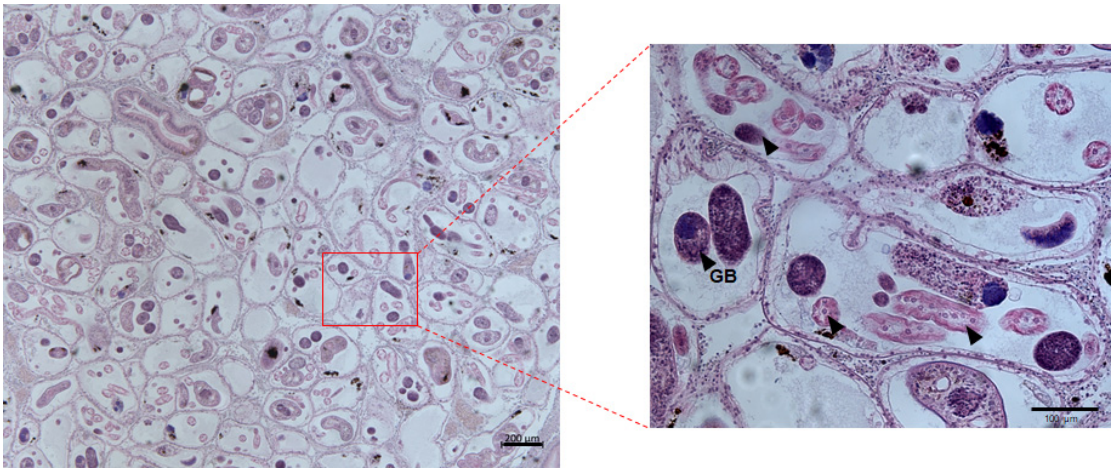

Fig. 3. Heavy infestation by trematode sporocyst containing the cercariae and germinal balls (GB) in the gonad of Jedo Venus clams

of the sublethal effects of *P. olseni* infection and its prevalence in Manila clams has expanded substantially, while limited cases reported *P. olseni* infection and prevalence in other bivalve species. However, Park et al. (2006) previously documented *P. olseni* infection in Jedo Venus clam, and Cho et al. (2022, 2023) reported the first case of *P. olseni* infection in the blood cockle *A. kagoshimensis* and *Tegillarca granosa* on the south coast of Korea. Despite these findings, there remains a notable absence of information concerning the sublethal consequences of *P. olseni* infection in a broader array of bivalves beyond Manila clams in the same geographic vicinity, suggesting that host specificity might play a role in the virulence dynamics of *P. olseni*.

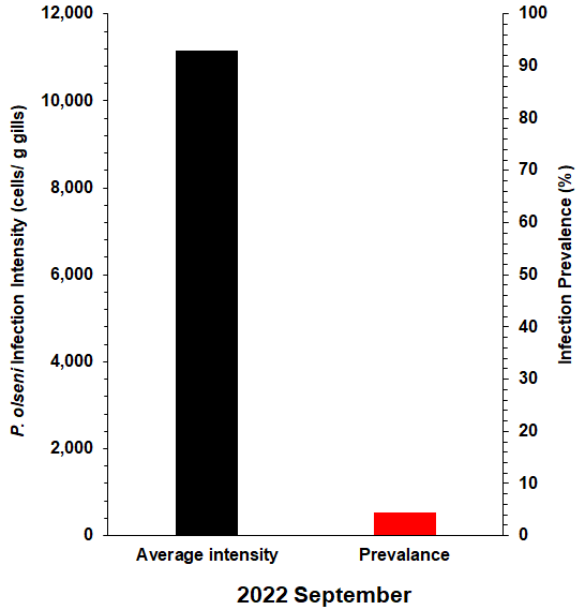

Fig. 4. Mean *P. olseni* infection intensity and prevalence in Jedo Venus clam in Yeosu, Korea

In this study, we observed trematode sporocysts completely occupying the gonad of the Jedo Venus clam. While such infections by trematode sporocysts have been documented in Manila clams residing in fine sediments within shallow to intertidal zones across temperate regions globally, the Northwest Pacific exhibits explicitly at least three morphologically identified species of sporocysts, including *Cercaria tapidis*, *C. pectinata*, and *Parvatrema duboisi* (Ngo and Choi 2004; Park et al. 2008; Le et al. 2015; Jung et al. 2021; Cho et al. 2022). These sporocysts,

Table 1. A summary of *Perkinsus* infection intensity and prevalence in bivalves along the southern coast of Korea. (NA-Not available)

Location on the South Coast	Host	Sampling Period	*P. olseni* Infection Intensity	Prevalence (%)	Reference
Yeosu	*Leucoma jedoensis*	September	1.1×10^4	4.5	This study
Yeosu	*Leucoma jedoensis*	May	0.8×104	53.9	Park et al. 2006
Yeosu	*Leucoma jedoensis*	June	1.1×10^4	37	
Kangjin	*Anadara kagoshimensis*	September	$1.2 \times 10^5 \pm 2.5 \times 10^5$	13.5	Cho et al. 2022
Yeoja	*Tegillarca granosa*	October	NA	1.4	Cho et al. 2023
Yeoja	*Tegillarca granosa*	November	NA	1.1	
Kangjin	*Ruditapes philippinarum*	June	$6.9 \times 10^5 \pm 1.1 \times 10^6$	100	
Wando	*Ruditapes philippinarum*	June	$1.2 \times 10^5 \pm 1.5 \times 10^4$	84	
Changheong	*Ruditapes philippinarum*	November	$1.1 \times 10^4 \pm 1.0 \times 10^4$	95	
Yeosu	*Ruditapes philippinarum*	October	$5.1 \times 10^5 \pm 5.1 \times 10^5$	100	
Mokpo	*Ruditapes philippinarum*	June	$7.1 \times 10^5 \pm 3.4 \times 10^5$	100	Park and Choi 2001
Tongyeong	*Ruditapes philippinarum*	March	$4.7 \times 10^5 \pm 1.4 \times 10^5$	66	
Geoje	*Ruditapes philippinarum*	March	$8.7 \times 10^5 \pm 8.4 \times 10^5$	93	
Sachon	*Ruditapes philippinarum*	March	$2.5 \times 10^5 \pm 2.5 \times 10^5$	93	
Jinhae	*Ruditapes philippinarum*	March	$6.4 \times 10^4 \pm 8.0 \times 10^4$	86	
Wando	*Ruditapes philippinarum*	May	$3.6 \times 10^6 \pm 2.6 \times 10^5$	NA	Kang et al. 2015
Geoje	*Ruditapes philippinarum*	September	$1.3 \times 10^6 \pm 1.2 \times 10^6$	100	Lee et al. 2020

varying in number and size, contribute to the deterioration of host tissues, particularly in the gonad, a phenomenon recognized as parasitic castration (Baudoin 1975). Although previous studies have reported trematode infections and their consequences on various commercial marine bivalves, the specific species responsible for the observed sporocyst occupation in Jedo Venus clams remains unidentified in this study. Future research efforts are imperative for a detailed understanding of these sporocysts and their potential impacts on the host.

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