Molecular detection of protozoan parasite infections in marine bivalves in Korean waters

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Backgrounds and Objective

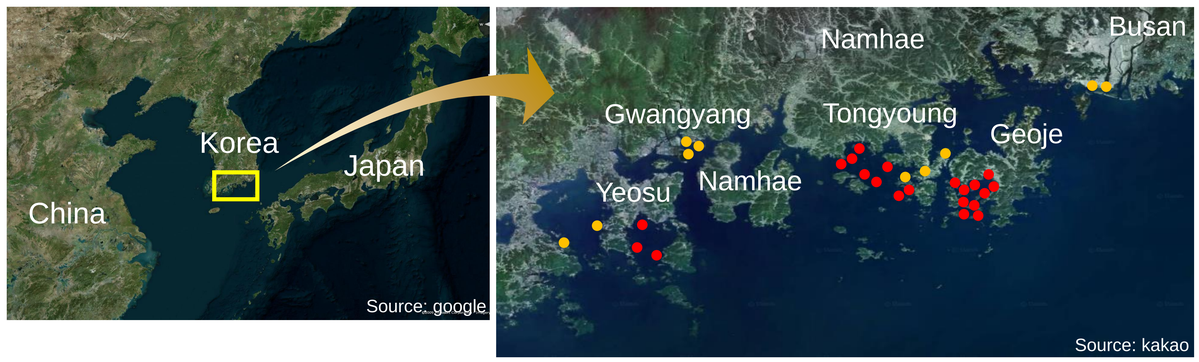
- Several species of protozoan parasites, including the members in the genus *Perkinsus*, *Marteilia*, and *Bonamia* are listed and regulated by the world organization for animal health (OIE), as they often cause mass mortalities of the host organisms.
- In Korea, small bays on the south coast are used as major culture grounds of the Pacific oysters (*Crassostrea gigas*), where approximately 150,000 MTs of oysters are produced annually.

Suspended long-line culture system in oyster grow-out fields and harvesting oysters using an automatic oyster-string processor

- The Pacific oysters on the south coast of Korea are known to be infected by *Marteillioides chungmuensis*, while infection by other OIE-listed protozoan parasites is unknown.
- In this study, possible infections by *Bonamia ostreae*, *B. exitiosa*, *Marteilia refringens*, *Perkinsus marinus*, and *P. olseni* in the Pacific oysters from the south coast of Korea.

Materials and Methods

- For the analysis, commercially raised (N=1,260) and wild (N=600) Pacific oysters were collected from 31 sites on the south coast in spring (pre-spawning season) and fall (post-spawning season) 2020.



- For the analysis, commercially raised (N=1,500) and wild (N=360) Pacific oysters were collected from 31 sites on the south coast in spring (pre-spawning season) and fall (post-spawning season) 2020.
- The OIE-listed five protozoan parasites in the oysters was examined using PCR with the species-specific primers recommended by the OIE.

Species	Primers	Length (bp)	Reference
B. ostreae	F: CATTTAATTGGTCGGGCCGC	300 bp	Cochennec *et al*. (2002)
	R: CTGATCGTCTTCGATCCCCC		
	F: CAATGGTGCGTTCAACGAT	352 bp	Engelsma *et al*. (2010)
	R: GGGTTCGCGGTTGAATTTTA		
B. exitiosa	F: CATTTAATTGGTCGGGCCGC	304 bp	Cochennec *et al*. (2000)
	R: CTGATCGTCTTCGATCCCCC		
M. refringens	F: CCGCACACGTTCTTCACTCC	1,090 bp	Le Roux *et al*. (2001)
	R: CTCGCGAGTTTCGACAGACG		
P. marinus	F: TTTTGYTWGAGWGTTGCGAGATG	509 bp	Audemard *et al*. (2004)
	R: CGAGTTTGCGAGTACCTCKAGAG		
P. olseni	F: CCGCTTTGTTTGGATCCC	490 bp	Kang *et al*. (2017)
	R: ACATCAGGCCTTCTAATGATG		

Results and Conclusion

- A total of 1,860 oysters examined in this study, *B. ostrea*, *B. exitiosa*, *M. refringens*, and *P. marinus* were not detected from any individual oyster.
- *Perkinsus olseni* was detected from 5 individuals out of 1,860 oysters collected from intertidal areas, and quantification by q-PCR indicated that the infection intensities were below 100 cells.
- The PCR also indicated that none of the oysters collected from subtidal long-lines were positive for *P. olseni*.

		Bonamiosis		Marteiliosis	Perkinsosis	
		B. ostreae	*B. exitiosa*	*M. refringens*	*P. marinus*	*P. olseni*
Commercial culture area	Spring	(-) 0/630	(-) 0/630	(-) 0/630	(-) 0/630	(-) 0/630
	Fall	(-) 0/630	(-) 0/630	(-) 0/630	(-) 0/630	(-) 0/630
Intertidal area	Spring	(-) 0/300	(-) 0/300	(-) 0/300	(-) 0/300	(-) 0/300
	Fall	(-) 0/300	(-) 0/300	(-) 0/300	(-) 0/300	(+) 5/300

- It is yet to be clear to define the detection of *P. olseni* in the oyster as infection.
- Further study is needed to confirm the *P. olseni* infection using histology.

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