Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Labyrinthula zosterae* (Labyrinthulomycetes) as the Causal Pathogen of Wasting Disease in the Seagrass *Zostera marina* in Korea

Soon Jeong Lee,¹ Jun Bo Shim,² and Sang-Rae Lee^{3,†}

¹ Mokpo Regional Office, National Fishery Products Quality Management Service (NFQS), 130 Tongil-daero, Mokpo, Jeollanam-do, 58746, Korea

² Fisheries Seed and Breeding Research Institute, National Institute of Fisheries Science, Mokpo 58746, Korea

³ Marine Research Institute, Pusan National University, Busan 46241, Korea

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Zostera marina L. plants have been seriously impacted by wasting disease along the Atlantic coasts of North America and Europe since the 1930s (Muehlstein 1989). Sudden declines in the population sizes of *Z. marina* affect primary and secondary producers of different trophic levels in blue carbon ecosystems (Gleason et al. 2013). Muehlstein et al. (1991) first identified *Labyrinthula zosterae* (Labyrinthulomycetes) as the pathogen causing wasting disease in *Z. marina*. However, there have been no reports of wasting disease pathogens affecting seagrass in Korea. In this study, we collected leaves of *Z. marina* showing symptoms of wasting disease in the southern region of South Korea (Dongdaeman, Namhae, Gyeongnam Province) during field monitoring (from April to September 2013). The pathogen of wasting disease, *L. zosterae*, has been isolated from the infected leaves of *Z. marina* and established as a culture strain. Samples of *Z. marina* and *L. zosterae* were deposited at the National Fishery Products Quality Management Service (Republic of Korea). Microscopic examination of the infected leaf tissues

revealed fusiform or spindle-shaped vegetative *Labyrinthula* cells (4 to 5 × 15 to 20 µm). These were similar in size and shape to those previously described for *Labyrinthula* species. The fusiform cells were cultured in 1% serum seawater agar medium, and they formed colonies and showed gliding motility along a network of hyaline slime filaments. To validate the pathogenicity, reinoculation tests by *L. zosterae* were performed with the isolated strains in accordance with Koch's postulates. Healthy leaves of *Z. marina* collected from the field were used in the reinoculation tests and were cultured at 15°C under white fluorescent irradiation of approximately 20 µmol·photons·m⁻²·s⁻¹ and a 12-h/12-h light/dark cycle. *L. zosterae* reisolated from artificially infected leaves of *Z. marina* was confirmed by DNA sequence similarity analysis. Total genomic DNA from the infected leaf cells and the culture strains was extracted using the QIAamp DNA Stool Mini Kit (Qiagen, Germany). Internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA were determined to identify *Labyrinthula* species. *L. zosterae*-specific primers (Lz2forward [5'-CTAAGACTAAACGAGGCGAAAGCCTAC-3'] and Lz2reverse [5'-AGGTTTACAAAACACACTCGTCCACA-3']) in Bergmann et al. [2011]) were used to confirm the infection of *L. zosterae* in the leaves from the field samples and the reinoculation test samples. Next, PCR products were cloned using a pLUG-Prime TA-cloning Vector (iNtRON Biotechnology, Korea) and commercially sequenced (SolGent, Korea). The ITS sequence of Korean *L. zosterae* (accession no. MW357748) showed high sequence similarity (99.3 to 100%) with that of *L. zosterae* deposited in GenBank (National Center for Biotechnology Information) from BLAST searches. These findings confirm that this is the first report of *L. zosterae* as the causal pathogen of wasting disease in *Z. marina* in Korea.

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[†]Indicates the corresponding author.
S.-R. Lee; antithamnion@gmail.com