Validation of the Tropical Sea Cucumber *Holothuria leucospilota* in Jeju Island, Korea: A Potential Candidate for Aquaculture in Warning Oceans

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Background

- Global warming is reshaping marine biogeography, with tropical species expanding poleward into temperate waters. This shift presents both challenges and opportunities for regional aquaculture: while traditional cold-water species face productivity declines, emerging tropical species offer alternative resources adapted to warming conditions.
- *Holothuria leucospilota*, a high-value tropical sea cucumber from the Indo-Pacific, has recently been observed in Korean waters, particularly around Jeju Island. However, variable morphology across populations complicates species identification through morphological analysis alone, requiring molecular confirmation to validate its establishment and potential as a new aquaculture resource.

Research Objective

- To definitively identify *Holothuria leucospilota* from Jeju waters using integrative approach combining morphological and molecular data, providing concrete evidence for climate-driven range expansion of tropical marine species.
- To validate the species identification of *Holothuria leucospilota* in Korean waters and suggest its potential as an aquaculture resource.

Study Area and Sample Collection

- Two specimens were collected from Jeju Island: one from Seongsan tidal pool in January 2024, and another from Seogwipo at 12 m depth in July 2025.



Seongsan and Seogwipo, Jeju Island. *Holothuria leucospilota*.

Morphological Analysis

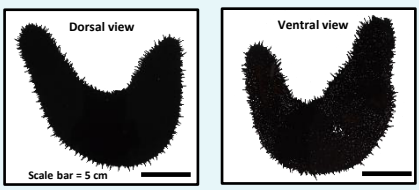
- External morphology observed on live specimens.
- Ossicles extracted from tissue samples through NaOH digestion and ethanol washing were examined using SEM
- Voucher specimens deposited at HNIBR (accession numbers: HNIBRIV19095, HNIBRIV19096)

Molecular Analysis

- **DNA Extraction & Sequencing** Genomic DNA extracted using Qiagen DNeasy kit. Mitochondrial COX1 and 16S rRNA genes amplified by PCR and sequenced.
- **Phylogenetic Analysis** Maximum Likelihood (ML) and Bayesian Inference (BI) analysis with GTR+I+G model.

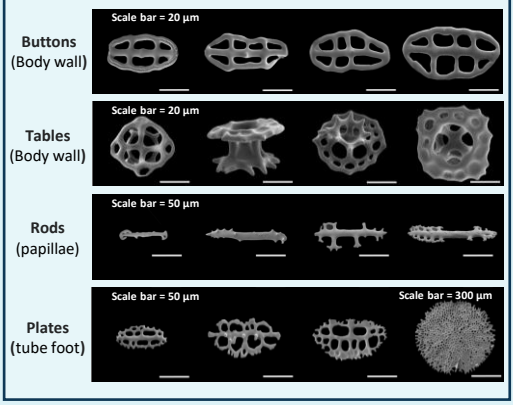
External Morphology

- Specimens displayed uniform black body coloration, elongate cylindrical body shape, leaf-shaped tentacles, and elongated dorsal papillae.



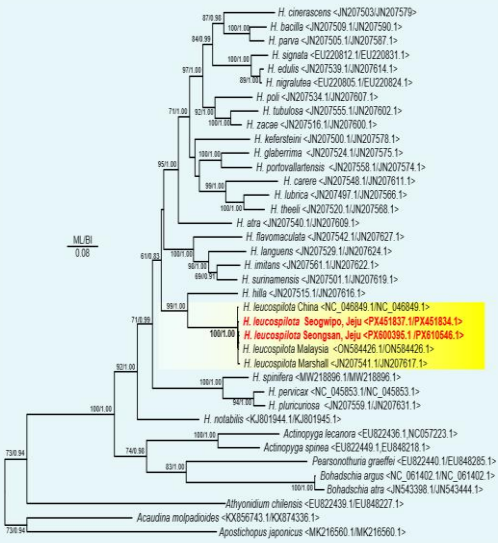
Ossicle Morphology

- Button-shaped and table-shaped ossicles (40-60 μm) observed in body wall. Rod-shaped ossicles (70-150 μm) in dorsal papillae. Prominent end plate (approximately 600 μm diameter) in ventral tube feet. No ossicles in tentacles.



Species Identification Based on COX1 and 16S rRNA

- **Sequence Identity** COI: 97.47–100% with reference sequences 16S rRNA: 96.47–100% with reference sequences
- **Phylogenetic Placement** Phylogenetic analyses (ML and BI) placed both Jeju specimens in a robust monophyletic clade (BS = 100, PP = 1.0) with Indo-Pacific *Holothuria leucospilota* reference sequences, clearly differentiated from



Phylogenetic Tree Based on COX1 and 16S rRNA

Discussion

- This study confirms *Holothuria leucospilota* presence in Korean waters through morphological and molecular analyses. Morphological characteristics are consistent with Indo-Pacific populations, and molecular analysis confirms species identification.
- Winter specimen collection provides evidence for poleward range expansion of this tropical species, suggesting successful establishment in warming waters.
- *Holothuria leucospilota* serves as a biogeographical indicator of climate-driven ecosystem restructuring in Korea. Long-term monitoring is essential to evaluate ecological impacts. This study provides baseline data for future monitoring and potential aquaculture research.