

ABSTRACT

Ulya Agassi. 24020122120043. **Comparative Study of Holothuriidae Identification Techniques Using Morphological, Anatomical, and Molecular Approaches.** Laboratory of Ecology and Biosystematics, Biology Study Program, Department of Biology, Faculty of Science and Mathematics, Diponegoro University. Supervised by Sapto Purnomo Putro and Anggiresti Kinasih.

The identification of sea cucumber is often challenging due to the similarity of interspecific characteristics and morphological variations influenced by environmental factors. This study aimed to identify sea cucumber specimen using morphological, anatomical, and molecular approaches, as well as to evaluate and compare the effectiveness of these identification methods. Morphological identification was conducted through the observation of external body characteristics, while anatomical identification through analysis of ossicle shape and size. Molecular identification was carried out using the cytochrome c oxidase subunit I (COI) gene through polymerase chain reaction (PCR), sequencing, and subsequent analyses including BLAST, genetic distance, phylogenetic reconstruction, conserved region, and haplotype analysis. The result indicated that, based on morphological characteristics, sample UA07 was presumed to belong to the order Aspidochirotida and the genus *Holothuria*. Anatomical analysis revealed ossicle composition and size (dorsal: buttons 69,09 μ m, rods 49,19 μ m, tables 75,80 μ m; ventral: buttons 64,33 μ m, rods 35,09 μ m), supporting classification within the genus *Holothuria* with higher accuracy, while the dominance of button type ossicle and gonad indicated an adult stage. Molecular analysis showed that isolation method used 10% bleach and RNase (P2) produced the best DNA quality with a purity value of $2,30 \pm 0,560$ and the highest concentration (Nanodrop 4,76 ng/ μ L; Quantus 3,53 ng/ μ L), and visualization result indicated that the COIceF/ceR primer pair yielded the most optimal amplification. BLAST result showed that treatment with muscle using an infrared sterilizer (P3) produced the most consistent result. Sequence analysis indicated that sample UA07 kinship to *Holothuria fuscocinerea*. The integration of these three approaches provides a more accurate identification.

Keywords: *DNA barcoding, ossicle, mitochondrial marker*