

ABSTRACT

LARASATI INTAN ARYADINI. 24020122130094. Analysis of Freshwater Fish Species Diversity at Padangbulia Waterfall, Bali Using an eDNA Under the guidance of Karyadi Baskoro and Ni Kadek Dita Cahyani.

Bali Island is part of the Sundaland biodiversity hotspot, which remains underexplored at the molecular level, particularly regarding freshwater fish in lotic habitats such as Padangbulia Waterfall. This study aims to identify freshwater fish species diversity using an environmental DNA (eDNA) approach based on Oxford Nanopore Technology (ONT) and to analyze their ecological, economic, and conservation functions. Water samples were collected from three replicate points, followed by filtration (Pall filter paper 0.45 μm), DNA extraction (Zymo Quick-DNA™ Fecal/Soil Microbe Miniprep Kit), amplification (MiFish 12S-U primer), sequencing (ONT MinION), and bioinformatics analysis including basecalling (Dorado), quality filtering (NanoFilt), clustering (VSEARCH, OTU 90%), taxonomy assignment (BLASTN, query coverage 90%), and visualization (R Studio). Diversity was analyzed using Shannon-Wiener and Simpson indices with the R package VEGAN. Sequencing yielded 1.200.950 reads. After filtering, nine freshwater fish species were identified, consisting of three native Indonesian species (*Barbodes binotatus*, *Cyclocheilichthys apogon*, *Sicyopterus microcephalus*), three introduced species (*Systemus sarana*, *Systemus orphoides*, *Poecilia reticulata*), and three non-native species indicated as false positives due to database limitations. The highest diversity was found at site DBP012717 ($H' = 5.016$; $1-D = 0.987$). The five valid fish species play ecological roles as predator and one as prey, have economic value as ornamental fish, and are classified as Least Concern in conservation status. The ONT-based eDNA approach proved effective but remains constrained by the limited reference databases for local species.

Keywords: *eDNA metabarcoding, freshwater fish, Bali, Oxford Nanopore Technology, MiFish 12S.*