I. GENERAL INTRODUCTION

1.1. Background

The maritime continent is a distinctive feature of Indonesia, consisting of three biogeographic regions: Sundaland, Wallacea, and the Sahul Shelf. Sulawesi, the largest island in the Wallacea region, was formed by various continental plates and exhibits high biodiversity with unique fauna and flora, including freshwater and diadromous aquatic biota (Michaux 2010, von Rintelen et al. 2012, Von Rintelen et al. 2014). Wallacea is renowned as a natural biogeographic region and center of endemism, where biogeographic units comprise native and endemic species with distinct evolutionary histories (Michaux 2010).

The high biodiversity in Wallacea, especially in relation to native and endemic species, plays a crucial role in the management and conservation of natural resources. This importance becomes even more apparent given the growing threats to these species, including overfishing, habitat destruction, and the invasion of alien species, which are often linked to insufficient taxonomic information, species distribution data, and other factors.

Gobies (Gobiiformes) represent an order with tropical and temperate radiations worldwide, occupying nearly all aquatic habitats and some semi-terrestrial environments (Larson et al. 2014; Keith et al. 2011). These fish are prevalent in the Indo-Pacific and Indo-Malay Archipelago, contributing significantly to fish community diversity in Wallacea, including Sulawesi and the Caribbean island systems, with remarkably high levels of endemism (Keith 2002; Lim et al. 2002; Marquet et al. 1999; Keith 2003).

The highly diverse taxonomic group of Gobiiformes includes marine, freshwater, and diadromous species. However, practical details about their biological cycles and the parameters underlying the evolution of amphidromous gobies remain limited. Although knowledge in this field has grown annually, taxonomic overlaps between earlier and recent reports persist. As a result, taxonomy is often perceived as an academically less relevant discipline (Abdullah 2017). On the contrary, taxonomy holds significant implications for conservation and resource management, particularly in fisheries (Agnarsson & Kuntner 2007).

A case of complex taxonomy in Gobiiformes arises from the genus *Giuris* sp., where all previously described species were synonymized as *G. margaritaceus* (Valenciennes, 1837) approximately 50 years ago (Akihito & Meguro 1974). Discrepancies in species naming, where both *G. margaritaceus* and *G. margaritacea* frequently appear in the literature alongside various other synonyms, add to the confusion (Froese & Pauly 2024). Despite similarities in external morphology, there is a strong suspicion that this taxon should not be regarded as a single species (Kottelat 2013). Recent taxonomic studies combining

classical morphometric and molecular biology approaches have successfully identified and redescribed eight distinct species (Keith et al. 2020; Keith & Mennesson 2020; Ndobe et al. 2023). Similar complexities are observed in other genera within Gobiiformes.

The urgency to identify native species in lake ecosystems is increasing. In three northern Sulawesi lakes, particularly Lake Limboto, Lake Tondano, and Lake Bolano Sau, fish biodiversity data remain limited and are primarily based on classical taxonomic approaches. For example, studies in Lake Limboto (Rauf et al. 2024), Lake Tondano (Sipayung et al. 2023), and Lake Bolano Sau (Makmur et al. 2019) rely on visual identification using morphological characters, without utilizing molecular biology methods such as "DNA barcoding" or environmental DNA (eDNA). This raises concerns about the potential extirpation or even extinction of unrecorded species due to overexploitation or habitat changes caused by human activities and climate change (von Rintelen et al. 2012; Gani et al. 2020; Yanuarita et al. 2020; Bandjolu et al. 2021; Burhanuddin et al. 2022). Accurate species identification can inform conservation status assessments, enabling the design and implementation of management strategies, both in situ and ex situ. This includes domestication efforts, aquaculture development for conservation, and sustainable economic utilization (Hubert et al. 2015; Herjayanto et al. 2019; Serdiati et al. 2020; Ndobe et al. 2023).

DNA barcoding offers significant advantages in identifying species within taxa that are difficult to distinguish using morphology or traditional morphometric methods (Ward et al. 2009). Moreover, phylogenetic approaches, a common method in systematics, allow clearer analysis of interspecies relationships and enhance our understanding of biodiversity through the reconstruction of evolutionary relationships (Astarini et al. 2021). Accurate and comprehensive DNA barcoding also serves as a foundation for biodiversity exploration or monitoring using environmental DNA (eDNA), an increasingly popular method in biodiversity studies (Shaw et al. 2016; Moore et al. 2021; Madduppa et al. 2022).

This study aims to explore the species diversity of Gobiiformes in three major northern Sulawesi lakes (Limboto, Tondano, and Bolano Sau) through an integrative taxonomic approach using DNA barcode analysis and eDNA. The findings are expected to enrich DNA barcode databases in global information sources such as GenBANK, FishBASE, IUCN, and others, as well as contribute DNA sequences from environmental samples to inform biodiversity management and conservation efforts at both local and global scales. This research provides a reference for evaluating the management of native fish with ecosystem-based considerations.

1.2. Problem Formulation

The research problems are formulated as follows:

1. How can historical data on Gobiiformes and ichthyofaunal communities in Lake Limboto be evaluated?
2. What are the recent records of Gobiiformes presence in the northern Sulawesi lakes (Limboto, Bolano Sau, and Tondano)?
3. What are the morphomeristics and phylogenetics of *Giuris* sp. in the northern Sulawesi lakes (Limboto, Bolano Sau, and Tondano)?
4. How can faunal biodiversity be explored using metabarcoding of environmental DNA in Lake Limboto, Gorontalo Province?

1.3. Research Objectives

The objectives of this research are as follows:

1. Evaluating historical data on Gobiiformes and ichthyofaunal communities in the northern Sulawesi lakes (Lake Limboto, Lake Bolano Sau, and Lake Tondano).
2. Review the latest records of Gobiiformes presence in the northern Sulawesi lakes (Lake Limboto, Lake Bolano Sau, and Lake Tondano).
3. Mapping the morphomeristics and phylogenetics of *Giuris* sp. in the northern Sulawesi lakes (Lake Limboto, Lake Bolano Sau, and Lake Tondano).
4. Exploring faunal biodiversity through metabarcoding of environmental DNA in Lake Limboto, Gorontalo Province

1.4 Research Framework

The conceptual framework and research stages can be seen in Figure 1.

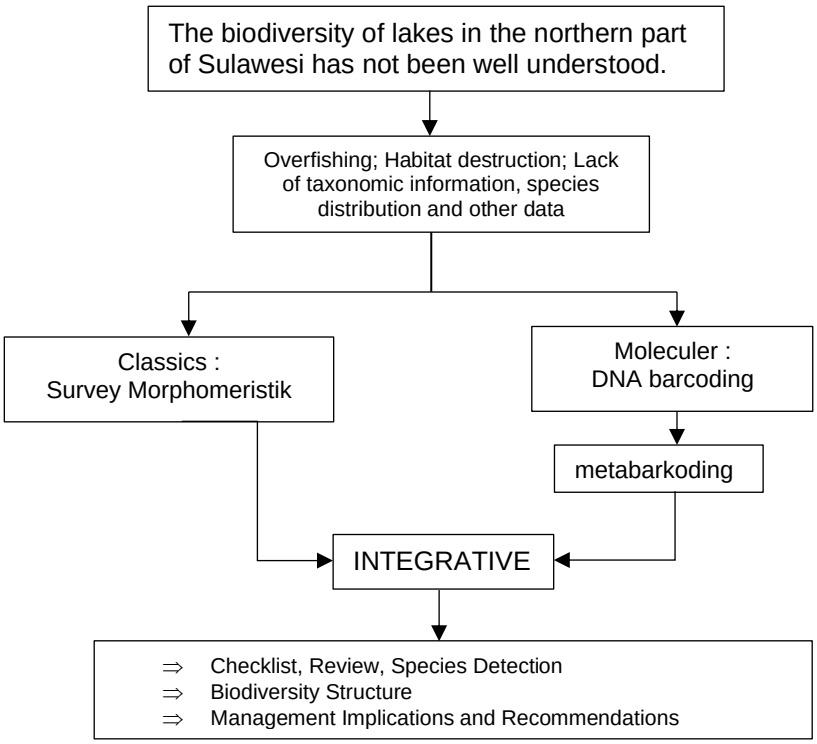

Figure 1. Framework Research

1.5 Research Limitations

This research is limited to the *Gobiiformes* group valued for their organoleptic qualities, specifically *Giuris* sp. and *Glossogobius* sp., in the northern Sulawesi lakes, particularly Lake Limboto in Gorontalo Province. Comparative locations for specific parameters include two geographically interconnected lakes: Lake Tondano in North Sulawesi and Lake Bolano Sau in Central Sulawesi.

1.6 Novelty

The novelty of this research lies in the following aspects::

1. A checklist of ichthyofauna in Lake Limboto
2. DNA barcoding of specimens to complement distribution data of the genus *Giuris* (*Gobiiformes*; *Eleotridae*)
3. Enrichment of DNA barcodes in the NCBI GenBank for new locations with validated voucher specimens and identities
4. Species clarification of three *Gobiiformes* genera in the three northern Sulawesi lakes
5. The first exploration of ichthyofaunal biodiversity using metabarcoding of environmental DNA in the waters of Lake Limboto, Sulawesi, Wallacea

1.7 Publication

1. Fisheries and Aquatic Science, Q3 Journal (Accepted on 22 Nov 2025 with manuscript ID fas-2024-0152
2. Aquatic Conservation Marine and Freshwater Ecosystem, Q1 Journal (On Process)
3. Revista de Biologi Tropical, Q2 Journal (Under Review with ID submission 63075)
4. Belgian Journal of zoology, Q2 Journal (On Process)