# Using 12S in eDNA Studies: A Literature Review

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**Abstract.** Environmental DNA (eDNA) is a very useful tool for the conservation and biomonitoring of natural environments. For being a non-invasive, low-cost and highly efficient method, many biodiversity assessment studies prefer eDNA metabarcoding to traditional methods. Despite of its rising popularity, the choice of marker gene for amplifying genetic material has become a major challenge for researchers employing this technology, as each one has its advantages and disadvantages depending on the environment and the research objectives. This article aims to analyze the 12S gene, which has become a common marker in recent years due to its high specificity in species identification. After a literature review of various studies, it was concluded that the 12S gene is regularly used in aquatic environments to identify fish, and despite its scarcity in databases, it has great potential for future eDNA metabarcoding studies.

**Keywords.** 12S, eDNA metabarcoding, primers, biodiversity assessments

## 1. Introduction

The biodiversity of species on the planet encompasses a myriad of organisms that inhabit a variety of terrestrial and aquatic ecosystems. It is estimated that there are millions of unknow species, many of which have not yet been described by science. The correct identification of these species is crucial for biodiversity conservation, evolutionary studies and the comprehension of ecological interactions [1][2].

In this context, environmental DNA (eDNA) has proved to be a very effective tool for the conservation and biomonitoring of ecosystems. The method consists of collecting DNA from different environments, which can be used to identify species living in the region [3]. Unlike traditional methods, eDNA is less invasive to the habitat and is less time consuming, as it does not involve intensive exploration of the environment and the capture of individuals for sampling, in addition to identifying invasive and rare species more easily [4][5]. The application of eDNA for biomonitoring has two approaches, the species-specific approach, which identifies a single species present in the genetic material collected, and the metabarcoding approach, which identifies multiple species simultaneously using universal primers [6].

One of the challenges for the metabarcoding method is the development of a universal primer for species identification, since this method heavily relies on genetic sequences found in databases such as GenBank and the Barcode of Life Data System (BOLD) [7]. Cytochrome C Oxidase I (COI) has become a primary marker for metabarcoding due to its widespread use in animal barcoding and abundance of sequences in databases [8][9][10]. However, recent research has shown that this gene is unsuitable for the metabarcoding technique due to its non-specific amplification of prokaryotes, hindering its efficiency in identifying species [11][12]. Other genes are therefore used as alternatives or complements to COI in animal identification. The most common of these are the mitochondrial genes 12S and 16S, as well as the ribosomal gene 18S. The advantages of these genes over COI are their more specific amplification of the taxon of interest and the presence of primers with greater universality [13][14].

This article aims to evaluate the efficiency of using alternative genes to COI in metabarcoding eDNA studies. The 12S gene will be analyzed exclusively, as it contains the largest number of primers developed for eDNA amplification, demonstrating its greater use for eDNA metabarcoding studies compared to other genes [15].

## 2. Methods and Materials

Two searches were made in the Web of Science Core Database. The first search used the terms "eDNA metabarcoding" and "12S" and "COI" not "16S" not "18S". In this way, it was possible to find articles using only the 12S gene for species identification, thus excluding research using other eDNA metabarcoding marker genes.

To select the relevant search results for our study, we established the following criteria: articles must have been published in the last five years (2020-Present), be written in English, and assess biodiversity of natural environments through eDNA metabarcoding. To select the relevant search results for our study, we established the following criteria: articles must have been published in the last five years (2020-Present), be written in English, and assess biodiversity of natural environments through eDNA metabarcoding. We excluded any studies using traditional collection methods. The compiled articles underwent a literature review, where we focused on the types of samples collected, the taxa of interest in the research, and the performance of different primers.

# 3. Results and Discussion

A search using only 12S as a marker yielded 39 results, from which 7 articles were selected based on established criteria. Meanwhile, a search using both 12S and COI markers simultaneously resulted in 10 articles, from which 2 were selected. The literature review compiled a total of 9 articles.

## 3.1 Environments

All of the studies collected water, most of which was seawater. Three studies were carried out exclusively in estuaries, of which two collected samples of fresh, brackish and salt water in different regions of the river to determine the distribution of species, given that the difference in salinity in estuaries considerably alters community composition [16]. The study conducted by Thekiso et al. (2023), which preferred to collect only saltwater samples in the estuary, encountered inconsistencies in species identification, as it detected fish species that only inhabit freshwater regions, which occurred due to the transport of eDNA from species exclusive to the upper regions of the river.

Regardless of the primers used and the taxa of interest, studies that collected samples from estuaries with varying salinities identified a greater number of species and genera than those that collected only one type of sample. This is expected as estuaries are nutrient-rich areas due to the influx of nutrients from rivers and tides, making them attractive for the reproduction and feeding of various species [17]. However, it is important to consider that other factors can also enhance the efficiency of species detection, such as the volume of water collected and the eDNA extraction methods [18].

## 3.2 Taxons

Among all the articles, five taxa of interest were found: fish, mammals, birds, reptiles and amphibians (Table 1). Even in studies where 12S was used as a universal primer for vertebrates [19], fish were consistently identified with greater success. The greater efficiency of 12S in identifying fish explains why most studies focus exclusively on identifying this taxon. However, the study conducted by Thekiso et al. 2023 identified terrestrial mammals and microorganisms with the fish-specific primer MiFish [20], the author mentions that this is a phenomenon that has already occurred in other studies.

## 3.3 Primers

The articles revealed a wide variety of universal 12S gene primers, each targeting different taxa. The primers were specific to the following taxa: vertebrates (Vert01), fish (MiFish), teleosts (Teleo02), chondrichthyans (Chon01) and elasmobranchs (Elas02). Except for the study by Zainal Abidin et al. (2022), MiFish was mostly used in single-marker studies, while the others were used in multi-marker studies. The efficiency of the two approaches in faunal surveys was similar when considering the specificity of fish identification. Therefore, we recommend using the MiFish primer when metabarcoding ichthyofauna, as it has similar results to multi-marker techniques, making it a more cost-effective and time-efficient option.

Both studies that utilized COI support the notion that this gene is not appropriate for metabarcoding. Zainal Abidin et al.'s (2022) study was more effective in detecting fish using MiFish than COI, while Ip et al.'s (2021) study, which employed three sets of COI primers (two universal for fish and one specific for sharks), was able to identify a larger number of fish with Vert01 (Tabela 1).

## 3.4 Limitations

The main issue with eDNA metabarcoding is its reliance on databases for species identification. To ensure high accuracy in assessing biodiversity, the species must be present in a genetic database. The 12S gene, in particular, has significant gaps in genetic reference databases, particularly for species from tropical regions [21][22]. This dependence has hindered the compiled studies from identifying all the species present in the collected genetic material, only achieving identification at the genus or family level.

# 4. Conclusion

This literature review demonstrates that the 12S gene is a suitable marker for eDNA metabarcoding studies. It is very efficient at identifying vertebrates, especially fish, which explains why all the studies carried out faunal surveys in bodies of water. Among the primers used, MiFish was found to be the most accurate in identifying species, outperforming methods that use several primers simultaneously. However, the lack of sequences in reference databases seriously hampers studies using the 12S gene.

| Author | Primers | Environment | Taxons studied | Species |
|---|---|---|---|---|
| Sani et al (2021) | Mifish | Sea | Fish | 58 species<br>41 genera |
| Thekiso et al (2023) | Mifish | Estuary | Fish | 22 genera<br>16 species |
| Lozano Mojica et al (2021) | Vert01 | Sea, estuary, lake | Fish, birds, amphibians, mammals, reptiles | 99 species<br>97 genera |
| Zainal Abidin et al (2022) | COI, Mifish | Estuary | Fish | 178 species<br>127 genera |
| Alam et al (2020) | Mifish | Estuary | Fish | 73 species<br>57 genera |
| Polanco et al (2021) | Teleo01, Chon01, Vert01 | Estuary, sea | Fish, birds, amphibians, mammals, reptiles | 79 species<br>134 genera |
| Jensen et al (2023) | Elas02, Teleo2 | Sea | Fish, birds, mammals | 81 species<br>72 genera |
| Ip et al (2021) | COI, Vert01 | Sea | Fish | 16 species<br>12 genera |
| Sales et al (2021) | Mifish | River | Fish | 34 species<br>28 genera |

**Tab 1:** Articles compiled during the study.

Therefore, an expansion of 12S sequences in genetic databases is necessary if advances are to be made in the area of eDNA metabarcoding.

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