diversity

MDPI

Article

Building a DNA Reference for Madagascar's Marine Fishes: Expanding the COI Barcode Library and Establishing the First 12S Dataset for eDNA Monitoring

Jean Jubrice Anissa Volanandiana [1,2,*], Dominique Ponton [3], Eliot Ruiz [2], Andriamahazosoa Elisé Marcel Fiadanamiarinjato [1], Fabien Rieuvilleneuve [2], Daniel Raberinary [1], Adeline Collet [4], Faustinato Behivoke [1], Henitsoa Jaonalison [5], Sandra Ranaivomanana [1], Marc Leopold [6], Roddy Michel Randriatsara [1], Jovial Mbony [1], Jamal Mahafina [1], Aaron Hartmann [7], Gildas Todinanahary [1] and Jean-Dominique Durand [2]

1 Institut Halieutique et des Sciences Marines (IH SM), University of Toliara, BP 141–Route du Port, Av. De France, Tuléar 601, Madagascar; elise.andriamahazosoa@gmail.com (A.E.M.F.); raberinary@gmail.com (D.R.); faustinato@yahoo.fr (F.B.); sandra.ranaivomanana@gmail.com (S.R.); randriatsararoddymichel@gmail.com (R.M.R.); mbonyjovial001@gmail.com (J.M.); jamal.mahafina@ihsm.mg (J.M.); gildas.todinanahary@ihsm.mg (G.T.)
2 MARBEC, University Montpellier, CNRS, Ifremer, IRD, 34095 Montpellier, France; eliotruiz3@gmail.com (E.R.); fabien.rieuvilleneuve@umontpellier.fr (F.R.); jean-dominique.durand@ird.fr (J.-D.D.)
3 UMR ENTROPIE (IRD, University of La Reunion, CNRS, University of New Caledonia, Ifremer), c/o CRIOBE, Université de Perpignan, 66860 Perpignan, France; dominique.ponton@ird.fr
4 Organisme Consultant en Environnement Aquatique (OCEA) Consult, 97432 La Réunion, France; adeline.collet@ocea.re
5 Laboratoire d'Ecologie Evolutive, Freshwater and OCeanic Science Unit of research (FOCUS), Université de Liège, 4000 Liège, Belgium; henitsoa.jaonalison@gmail.com
6 UMR ENTROPIE c/o Institut Européen Universitaire de la Mer (IUEM), IRD, Technopôle, 29280 Brest-Iroise, France; marc.leopold@ird.fr
7 Harvard and Perry Institute for Marine Science, Waitsfield, VT 05673, USA; ahartmann@perryinstitute.org
* Correspondence: jean-jubrice-anissa.volanandiana@ird.fr or jvolanandiana@gmail.com; Tel.: +261-340464177

check for updates

Academic Editor: Michael Wink

Received: 23 June 2025
Revised: 10 July 2025
Accepted: 11 July 2025
Published: 18 July 2025

Citation: Volanandiana, J.J.A.; Ponton, D.; Ruiz, E.; Fiadanamiarinjato, A.E.M.; Rieuvilleneuve, F.; Raberinary, D.; Collet, A.; Behivoke, F.; Jaonalison, H.; Ranaivomanana, S.; et al. Building a DNA Reference for Madagascar's Marine Fishes: Expanding the COI Barcode Library and Establishing the First 12S Dataset for eDNA Monitoring. *Diversity* **2025**, *17*, 495. https://doi.org/10.3390/d17070495

Abstract

Madagascar harbors a rich marine biodiversity, yet detailed knowledge of its fish species remains limited. Of the 1689 species listed in 2018, only 22% had accessible cytochrome oxidase I (*COI)* sequences in public databases. In response to growing pressure on fishery resources, this study aims to strengthen biodiversity monitoring tools. Its objectives were to enrich the *COI* database for Malagasy marine fishes, create the first *12S* reference library, and evaluate the taxonomic resolution of different *12S* metabarcodes for eDNA analysis, namely MiFish, Teleo1, AcMDB, Ac12S, and 12SF1/R1. An integrated approach combining morphological, molecular, and phylogenetic analyses was applied for specimen identification of fish captured using various types of fishing gear in Toliara and Ranobe Bays from 2018 to 2023. The Malagasy *COI* database now includes 2146 sequences grouped into 502 Barcode Index Numbers (BINs) from 82 families, with 14 BINs newly added to BOLD (The Barcode of Life Data Systems), and 133 cryptic species. The *12S* library comprises 524 sequences representing 446 species from 78 families. Together, the genetic datasets cover 514 species from 84 families, with the most diverse being Labridae, Apogonidae, Gobiidae, Pomacentridae, and Carangidae. However, the two markers show variable taxonomic resolution: 67 species belonging to 35 families were represented solely in the *COI* dataset, while 10 species from nine families were identified exclusively in the 12S dataset. For 319 species with complete *12S* gene sequences associated with *COI* BINs (Barcode Index Numbers), *12S* primer sets were used to evaluate the taxonomic resolution of five *12S* metabarcodes. The MiFish marker proved to be the most effective, with an optimal similarity threshold of 98.5%. This study represents a major step forward in

documenting and monitoring Madagascar's marine biodiversity and provides a valuable genetic reference for future environmental DNA (eDNA) applications.

Keywords: fish diversity; DNA barcoding; reference library; eDNA marker taxonomic resolution

1. Introduction

Fishery resources are an indispensable source of income and subsistence in many regions, particularly in developing countries, where nearly 97% of the world's fishers are concentrated [1,2]. Marine resources provide essential protein and income for coastal communities, especially in the southwestern part of Madagascar [3]. In Madagascar, fishing constitutes the primary livelihood for many coastal communities. Due to recurrent droughts, some populations that traditionally depended on agriculture and livestock farming have shifted toward fishing, thereby increasing pressure on marine resources [4–6]. This shift has contributed to signs of resource overexploitation, prompting some fishers to adopt destructive practices such as mosquito net trawling and beach seining, which further accelerate ecosystem degradation. Over the past two decades, this growing pressure has led to a noticeable decline in both catch sizes [7] and total catch volumes [6]. This situation underscores the urgent need for sustainable management and conservation strategies. Interventions such as banning destructive gear and establishing marine protected areas (MPAs) could be effective, although their implementation may carry significant social implications.

Effective conservation efforts must be grounded in precise knowledge of the species being exploited, including their biology and ecology [8]. Accurate species identification and monitoring are essential for understanding the impacts of both anthropogenic and natural stressors on marine biodiversity. However, most current data are derived from fisheries-dependent monitoring or underwater visual censuses (UVC), both of which have significant limitations. These approaches often rely on morphological and meristic characters for species identification, which can be difficult to interpret, particularly for juvenile individuals (e.g., those collected with mosquito nets) or in underwater conditions where visual cues are limited.

In line with a global trend toward the integration of molecular tools into biodiversity monitoring, environmental DNA (eDNA) has increasingly been used as a complementary approach to traditional survey methods, including in the context of fisheries and marine protected area (MPA) monitoring (e.g., [9,10]). In Madagascar, eDNA offers promising opportunities to improve our understanding of fish diversity and community structure across coastal habitats by detecting species through genetic material shed into the environment via secretions, excretions, tissues, or carcasses. Over the past few decades, the technique has undergone rapid development [11] and is now widely applied for species detection and biodiversity monitoring [12–14]. Compared to traditional methods, eDNA is more sensitive and capable of detecting a broader spectrum of species, including cryptobenthic and rare taxa [15–17]. Nevertheless, several limitations persist, among which the incomplete taxonomic coverage of reference DNA barcode libraries remains a major constraint for assigning detected operational taxonomic units (OTUs) in water samples to species-level identities [18]. Despite global initiatives such as the International Barcode of Life (IBOL), which aim to establish comprehensive DNA barcode libraries for documenting biodiversity, the standard barcode marker for vertebrates (a fragment of the cytochrome c oxidase subunit I (*COI*) gene) has limited applicability for fish species detection via eDNA. Although *COI* is considered highly resolutive for species discrimination [19], its

relatively long fragment length renders it unsuitable for eDNA metabarcoding, where DNA is typically fragmented and degraded due to environmental conditions [20,21].

To overcome these limitations, alternative mitochondrial markers targeting shorter DNA fragments have been developed. Zhang et al. [22] reviewed 22 primer sets derived from four mitochondrial genes (*12S rRNA*, *COI*, *cytochrome b*, and *16S rRNA*) designed to amplify short variable regions for species discrimination while anchoring them in conserved flanking regions to enable broad taxonomic coverage [22,23]. Among these, markers within the *12S* rRNA gene have become particularly popular for fish eDNA surveys due to their balance of universality and taxonomic resolution [23–25].

In this context, building comprehensive *12S rRNA* reference libraries for marine fishes has become a priority, especially for supporting the implementation of eDNA-based biodiversity monitoring. In Madagascar, initiatives have recently emerged to develop DNA barcode libraries, with a focus on fish at early life stages [26] and on threatened species [27]. In 2022, of the 1689 marine and transitional water fish species recorded in Madagascar [28], 387 have *COI* barcodes available, and 307 have sequences generated from three mitochondrial genes (*COI*, *16S rRNA*, and *NADH2*). To date, however, no surveys have applied *12S rRNA* barcoding to assess fish diversity in Madagascar.

Relying solely on public databases such as GenBank for species-level assignment remains problematic due to issues such as taxonomic misidentifications and the absence of voucher specimens [29,30]. Accurate species identification from eDNA data requires a reference database that is both comprehensive and well-curated. In response, Blackman et al. [29] recommend the development of locally curated reference libraries and propose practical guidelines for enhancing the reliability of open-access databases through targeted curation.

The objectives of this study were fourfold. First, we aimed to enhance Madagascar's *COI* DNA barcode reference library for marine fish by expanding sampling efforts through the analysis of specimens obtained from artisanal fishers, with attention to fishing gear types and associated marine ecosystems. Second, we reviewed and curated previously assigned Barcode Index Numbers (BINs) to improve taxonomic accuracy within the existing DNA barcode database. Third, we generated the first *12S rRNA* reference library for Malagasy marine fishes by sequencing representative individuals from each BIN, thereby providing a foundational resource for future eDNA metabarcoding surveys. Finally, we assessed the taxonomic resolution of various *12S* metabarcodes to identify the most informative markers and to define optimal sequence similarity thresholds for accurate species-level assignment.

2. Materials and Methods

2.1. Tissue Sampling

To improve understanding of Malagasy marine fish diversity, this study utilized tissue samples collected during multiple research campaigns conducted in southwestern Madagascar (Table 1), consolidated under container MADFI (Reference DNA barcodes library of Malagasy marine fishes) in Barcode of Life Data Systems (BOLD). For the *COI* reference library, new samples were collected in Toliara and Ranobe Bays, with three field campaigns. This was sampling designed to increase the species representation in the database by expanding the geographic coverage, exploring diverse habitats, and employing a broader range of fishing gear than those used by Jaonalison et al. [26]. The first two campaigns followed artisanal pirogue fishers from eight villages in Toliara Bay (S23°25′0′, E 43°42′0) between May 2018 and April 2019, and 12 villages in Ranobe Bay (E43°30′, S23°00′, E43°38′, S23°18′) from October 2021 to November 2022. Fishing methods included beach seine, mosquito net trawl, gillnet, handline, and speargun. These efforts were complemented by samples obtained in the seagrass beds and along the mangrove fringes of Sarodrano (March 2022 and April 2023),

using mosquito net trawl and gillnet. Tissue samples and photographs were collected monthly in Toliara Bay, bimonthly in Ranobe Bay, and twice monthly in the Sarodrano area.

Table 1. List of DNA barcoding projects grouped under the Reference DNA barcodes library of Malagasy marine fishes (MADFI) container on the Barcode of Life Data Systems (BOLD) platform. Each project publicly available through the BOLD system v5 corresponds to a sampling and sequencing campaign targeting marine fish species from Madagascar, for molecular species identification and biodiversity monitoring. The sample is expressed as the number of pirogues surveyed during the barcoding campaigns.

Project Name in MADFI Container	Sampling Period	Sampling Gear	Sampling Areas	Number of Samples	Reference
CALMA	March 2019 to May 2019	light traps	Great barrier reef of Toliara and Anakao reef	60	Collet et al. [31]
COMAD	October 2014 to March 2015	mosquito seine nets and light traps	Great barrier reef of Toliara and Anakao reef	92	Jaonalison et al. [26]
RFSIO	November 2016 to April 2017, and November 2017 to April 2018	light traps	Great barrier reef of Toliara and Anakao reef	200	Jaonalison et al. [26]
IFBIO	November 2016 to April 2017, and November 2017 to April 2018	mosquito seine nets and light traps	Fishing landings in Toliara Bay	78	Jaonalison et al. [26]
GEOFI	May 2018 to April 2019	beach seine, mosquito trawl net, gillnet, handline, speargun	Fish landings in the eight villages along Ranobe Bay	252	present study
ARMSR	October 2021 to November 2022	beach seine, mosquito trawl net, gillnet, handline, speargun	Fish landings in the 12 villages along Ranobe Bay	597	present study
SAROD	March 2022 to April 2023	gillnet, mosquito trawl net	Seagrass beds and along the mangrove edges of Sarodrano	130	present study

To construct the *12S rRNA* reference library, one or more representatives of the different Molecular Operational Taxonomic Units (MOTUs) or BINs from the MADFI database in BOLD were selected for analysis. All *12S* sequences generated in this study are original and unpublished. They were obtained from two sources of DNA: (i) DNA extracts previously used in earlier studies to generate *COI* sequences [26,32], and (ii) newly extracted DNA from specimens collected specifically for the present study to expand the *COI* database.

2.2. Collection of Tissue Samples and Photographs

The same methodological protocol was applied across all sampling campaigns. All fish landed by each pirogue were systematically considered for tissue and photographic documentation. Following each fishing trip, the catch was sorted into morphologically similar groups within each family. For each group, a representative specimen was photographed, and a fin clip (typically from the pelvic or caudal fin) was taken for DNA extraction. Each tissue sample was preserved in a cryotube filled with 90% alcohol, labeled with a unique code, and stored at −20 °C. Photographs were imported into an image database using Adobe Lightroom, where image file names were renamed to match the corresponding tissue codes.

2.3. DNA Extraction, Amplification, and Sequencing

In the laboratory, genomic DNA was extracted from fin tissue samples using the Pure Link Genomic DNA Mini kit (Invitrogen, Waltham, MA, USA), following the manufacturer's protocol. Extracted DNA was stored at −20 °C until further analysis. Two

mitochondrial gene regions were targeted for amplification: the mitochondrial *COI* gene and the ribosomal *12S rRNA* gene.

A 650 base pair fragment from the 5′ region of the *COI* gene was amplified using the primer pairs FishF1 and FishF2 with FishR1, following the protocol established by Ward et al. [33]. PCR conditions for *COI* amplification included an initial denaturation at 98 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, extension at 72 °C for 1 min, and a final post-extension step at 72 °C for 5 min.

For the amplification of the complete fragment of the *12S rRNA* gene, a ~1000 base pair fragment was amplified from representative specimens of each BIN in the local database. Amplification was performed using the primer pair XRMUPheF1(5′-YAAAGCATAMCRCTGAAGATG-3′) [34] and TeleoR (5′-CTTCCGGTACACTTACCATG-3′) [35].

In cases of amplification failure, an alternative primer set, MiFish-U-F (5′-GTCGGTAAAACTCGTGCCAGC-3′) [24] and TeleoR, targeting a shorter ~600 base pair fragment, was employed. PCRs were carried out using the following thermal profile for 12S: initial denaturation at 92 °C for 5 min, followed by 35 cycles of 30 s denaturation, 30 s annealing (temperature ranging between 50 and 55 °C depending on primer set), and a final elongation step at 72 °C for 5 min. PCR products were visualized on 1% agarose gels stained with ethidium bromide, and successful amplifications showing clear and intense bands were selected for sequencing.

Selected PCR products were sent to GENOSCREEN (Lille, France) for Sanger sequencing, following procedures comparable to those described by Collet et al. [32] and Pham et al. [36]. The resulting sequences were retrieved, cleaned, aligned, and assigned taxonomic identities based on reference libraries.

2.4. Specimen Identification and Taxonomic Curation of the DNA Barcodes Library

All raw sequences obtained during this study were first examined and manually curated using Chromas software version 2.6.6 (http://technelysium.com.au/wp/chromas/ (accessed on 20 October 2022)), or 4peaks (https://nucleobytes.com/4peaks/index.html (accessed on 11 January 2020)). Cleaned sequences were then exported in FASTA format and aligned using MEGA 7.0 [37], where they were compared with their closest sequences available in GenBank. Any discrepancies between the newly obtained sequence and the reference were verified by checking chromatograms using the corresponding AB1 files.

The cleaned *COI* and 12S rRNA gene sequences were submitted to the BOLD database. Each *COI* sequence was automatically assigned to a BIN via the BOLD platform, based on the RESL (REfined Single Linkage) algorithm [38], which defines and delineates BINs in a standardized way. For each BIN, the associated names were reviewed (Figure 1).

When only one species name was associated with a BIN, and no other BINs were linked to that name, the species assignment was considered definitive. If multiple BINs were associated with the same species name, this raised the possibility of cryptic diversity or identification errors. These cases were further investigated using the "Taxon ID Tree" tool in BOLD, which allowed phylogenetic relationships to be reconstructed within relevant taxonomic groups (family or genus). In the resulting phylogenetic trees, each sequence was annotated with its geographic origin, BIN, GenBank accession number, and identifier, aiding in the critical assessment of the assigned names.

Each BIN was then taxonomically curated based on both molecular and phenotypic data. When identification was unambiguous, the sequence was assigned a Linnaean species name. In cases of uncertainty, the BIN was labeled at a higher taxonomic level (genus or family) followed by the BIN code. For species associated with multiple BINs, additional biogeographic analyses were conducted, including examination of their parapatric distribution and recent common ancestry. When possible, the type locality of each species was

verified via the Eschmeyer's Catalog of Fishes [39]. If the type locality corresponded to the BIN observed in Madagascar, the original Linnaean name was retained; otherwise, a "cf." designation was used (e.g., cf. species name [BIN]).

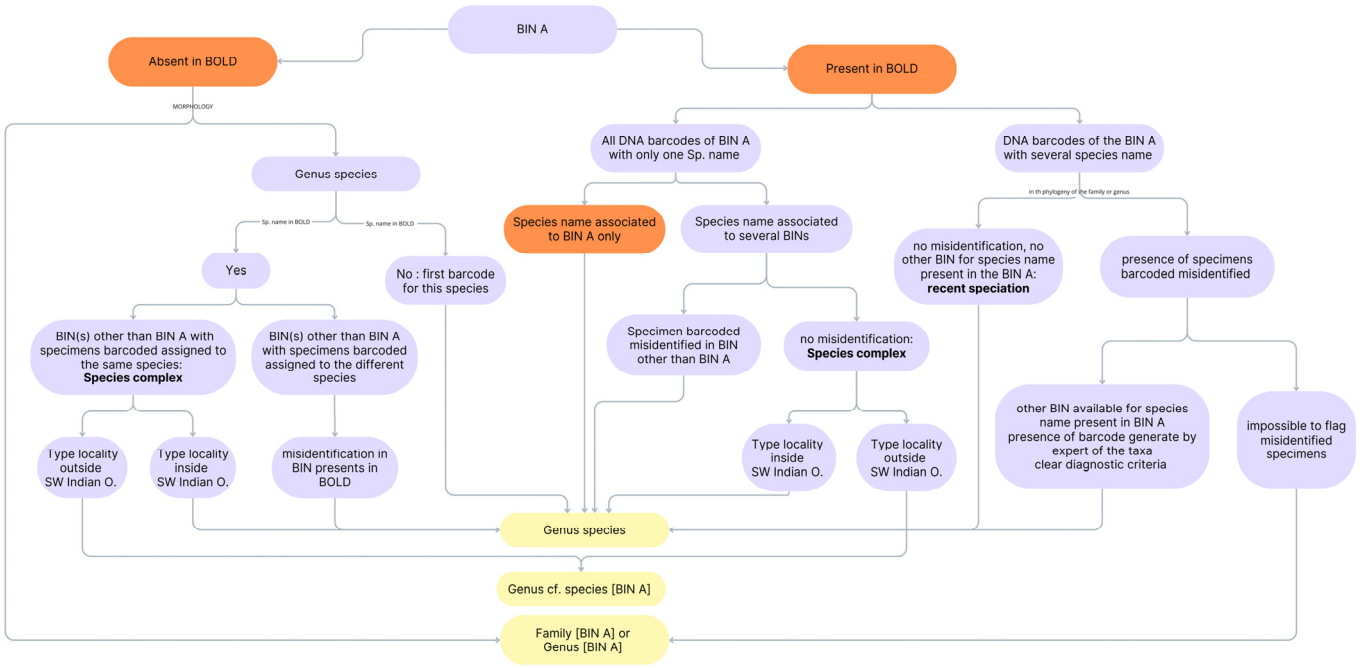

Figure 1. Decision tree illustrating the taxonomic identification method applied to each Barcode Index Number (BIN) for the curation of the reference database. Each BIN was initially generated automatically by the BOLD system. Taxonomic assignments (to species or genus) were then manually refined using an integrative approach combining: (i) morphometric traits and photographs of the barcoded specimens, (ii) the taxonomic composition within each BIN (consistency and frequency of species names), and (iii) phylogenetic relationships among BINs within the same genus or family (Table S1). This process allowed us to validate or correct initial identifications, resulting in a curated reference database suitable for reliable metabarcoding analyses.

For the 12S sequences generated in this study, the primary species identification was based on the BIN assignment of the corresponding *COI* sequence from the same specimen. This identification was then cross-validated using Basic Local Alignment Search Tool (BLAST) ((https://blast.ncbi.nlm.nih.gov/Blast.cgi), accessed on 27 March 2023) results against GenBank. When *COI* sequences were unavailable, identifications relied on morphospecies-level phenotypic features and the BLAST match of the *12S* sequence.

If identification remained uncertain, a conservative approach was taken:

- when sequence identity (seq ID) was greater than 99%, the species name was retained.
- when seq ID was between 95% and 99%, if only one genus appeared within the matches, the genus name was assigned. If multiple genera were listed, the identification was limited to the family level.
- when seq ID was between 90% and 95%, only the family name was used, provided it was consistently represented among the BLAST hits.

2.5. Evaluation of Taxonomic Resolution

To evaluate the taxonomic resolution of five *12S* primer sets commonly used in environmental DNA (eDNA) based species detection, namely MiFish (171 bp), Teleo1 (63 bp), AcMDB (281 bp), Ac12S (389 bp), and 12SF1/R1 (106 bp) (see Zhang et al. [22] for primer details and arrangement), we followed the methodological framework proposed by Ruiz et al. [40] (submitted). This approach enables standardized comparison of taxonomic resolution

across different metabarcodes using the Barcode Index Number (BIN) system established in BOLD as a reference, which is based on *COI* sequence clustering.

Briefly, in silico extractions of each metabarcode region were performed on complete 12S gene sequences from individuals already assigned to BINs, using the novel methodology of Ruiz et al. [40]. We then carried out efficient (i.e., k-mer presorting) pairwise sequence comparisons of each metabarcode region across a range of similarity thresholds (90% to 99%), using default parameters of the program VSEARCH [41].

This sequence delineation was compared to the BIN reference framework to evaluate congruence. Two types of discrepancies were quantified: false negatives (where individuals from different BINs were considered similar) and false positives (where individuals from the same BIN were considered different) (Figure 2). The total error rate for each metabarcode was defined as the sum of the false-positive and false-negative rates at each similarity threshold, providing an objective measure of taxonomic resolution across markers.

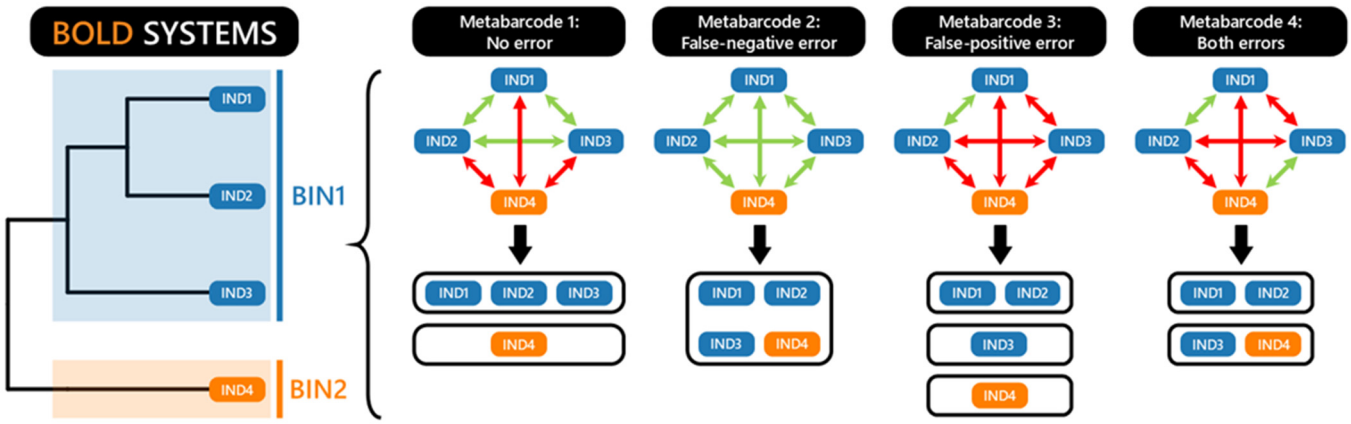

Figure 2. Illustration of different identification error scenarios in metabarcoding analyses, based on taxonomic clustering of Barcode Index Number(BINs). Green arrows indicate correct assignments, while red arrows represent errors. On the left, a phylogenetic tree shows the separation of four individuals (IND1 to IND4) into two BINs: BIN1 (IND1, IND2, IND3) and BIN2 (IND4). The four columns on the right represent different metabarcoding identification scenarios. Metabarcode 1 showed no error: all individuals were correctly identified as similar or different based on their BIN assignments. Metabarcode 2 showed a false-negative error, where IND4 (BIN2) was incorrectly considered similar to individuals from BIN1. Metabarcode 3 showed a false-positive error, where IND3 (BIN1) was incorrectly classified as different from other BIN1 individuals. Metabarcode 4 showed both error types: IND3 and IND4 were incorrectly identified as similar, and IND3 was also incorrectly considered different from other BIN1 individuals.

3. Results

3.1. Taxonomic Coverage of COI Sequences

The MADFI project currently comprises 2146 *COI* sequences, of which 953 were generated during the present study (Table 2).

Using the RESL algorithm, 502 distinct BINs were delineated from the complete *COI* dataset (Figure 3a). These BINs span two classes, 25 orders, 82 families, each family containing between one and 44 BINs, and 424 valid species names according to the World Register of Marine Species (WoRMS) (Figure 3a). The families with the most BINs in our database were Labridae (43), Apogonidae (41), Gobiidae (29), Pomacentridae (26), and Carangidae (22) (Figure 3d).

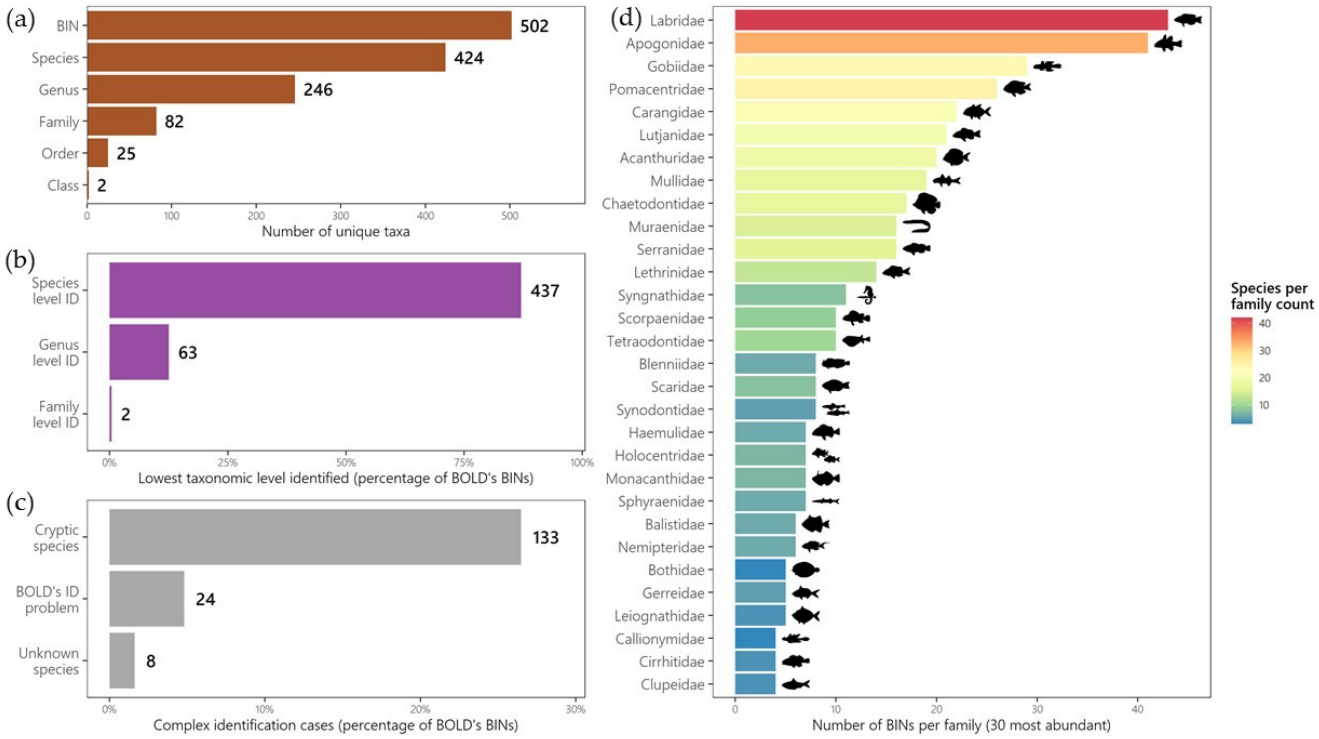

Figure 3. Overview of the taxonomic diversity and identification resolution of marine fish barcoded in the MADFI (Reference DNA barcodes library of Malagasy marine fishes) project in BOLD. (**a**): Number of taxa identified for each BIN. (**b**): Proportion of BINs for which identification was achieved at species, genus, or family level. (**c**): Proportion of complex identification cases, including potential cryptic species, inconsistencies related to BIN identification issues in BOLD (specimens misidentified), and BINs not identified at the species level. (**d**): Number of BINs and species richness per fish family for the 30 most represented families. The barplot shows the number of BINs per family, with color shading indicating species richness: blue bars represent families with lower species diversity, and red bars represent those with higher diversity. Silhouettes illustrate the general morphology of representative species within each family.

Of the 502 BINs identified in this study, 488 correspond to BINs already registered in the BOLD database as part of the MADFI project, and 14 are newly generated BINs not yet present in BOLD. Among the 502 BINs, 437 were identified at the species level, including 133 cryptic species, 63 at the genus level, and two at the family level (Figure 3b,c). Among the 488 BINs already registered in BOLD, 26 BINs are newly recorded for the West Indo-Pacific, and 290 BINs for Madagascar (Figure 4c).

The 14 new BINs, spanning 10 families, were generated from the new samples and include the following families: Gobiidae (4), Clupeidae (2), Apogonidae (1), Blenniidae (1), Bothidae (1), Cynoglossidae (1), Diodontidae (1), Labridae (1), Pseudochromidae (1), and Syngnathidae (1). Among these, five BINs were identified at the species level (including one cryptic species), eight at the genus level, and one at the family level (Syngnathidae).

Among the 488 BINs already registered in the BOLD database, 432 were assigned to the species level. These species include 132 BINs from 43 families corresponding to species belonging to cryptic species. The families most concerned with cryptic diversity were Apogonidae (23 BINs), Labridae (11 BINs), Gobiidae (10 BINs), Pomacentridae (6 BINs) and Serranidae (6 BINs), representing 26% of all putative species in the MADFI *COI* gene database. Additionally, 55 were identified only at the genus level, distributed across 31 families, mainly composed of Apogonidae (7), Gobiidae (3), Syngnathidae (3), and Synodontidae (3). Only one BIN was assigned at the family level (Exocoetidae).

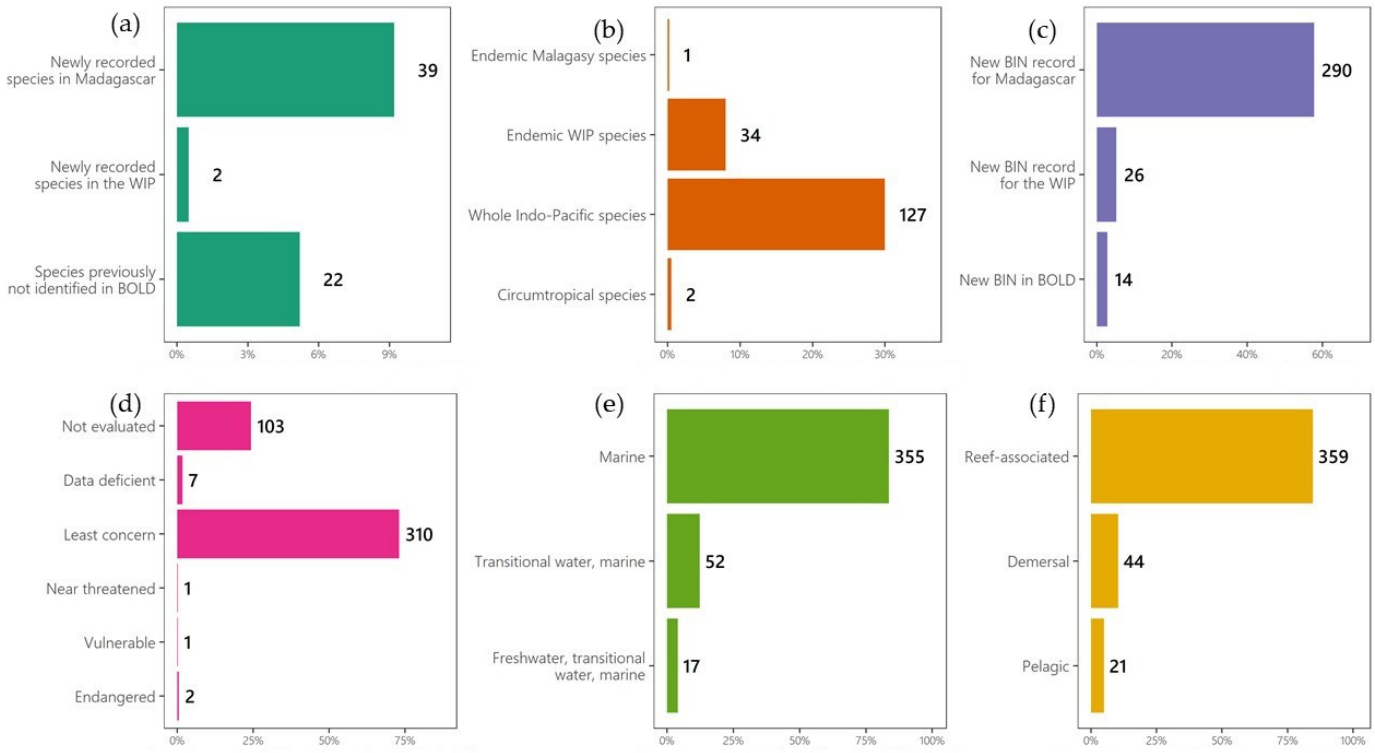

Figure 4. Summary of main characteristics of the 424 valid fish species (according to WoRMs) and 502 BINs recorded in the MADFI project. (**a**): Number of species newly recorded for Madagascar, in the Western Indian Pacific (WIP), or not previously identified in BOLD. (**b**): Biogeographic distribution of recorded species across four categories (endemic Malagasy, WIP endemic, whole Indo-Pacific, and circumtropical). (**c**): Novelty of BINs in BOLD (BINs newly recorded for Madagascar, the WIP, or globally). (**d**): IUCN Red List status of recorded species. (**e**): Habitat range based on FishBase. (**f**): Ecological lifestyle of the recorded species based on FishBase. (**a**,**b**,**d**–**f**): The X-axis in all panels represents the percentage of valid species (n = 424) as recognized by WoRMS (the X-axis in panel (**c**) represents the percentage of BOLD-registered BINs (n = 502)).

A detailed analysis of specimens assigned in BOLD systems to the 488 BINs revealed that:

- 243 BINs included specimens with a unique species name (one BIN, one species name). Among them, 236 were identified at the species level. Of these species names, 125 BINs were associated with a single BIN and could therefore be directly linked to the same species name, including only one cryptic species (*Ariosoma cf. meeki* [BOLD:ADD3087]). The remaining 118 BINs were associated with more than one BIN. Phylogenetic reconstructions at the family or genus level revealed that 87 species most probably corresponded to species complexes, accounting for 17% of all BINs. For 24 of the remaining BINs, after verification using the phylogenetic tree, they turned out to be misidentified specimens. However, seven BINs could only be assigned at the genus level.
- 212 BINs contained specimens with different species names. Phylogenetic reconstructions and further manual verifications confirmed that these specimens were misidentified. Among these, 188 BINs had another correct BIN available for the species name and a barcode generated by a taxonomic expert, with clear diagnostic criteria. All 188 BINs were identified at the species level, including 40 cryptic species. For the remaining 24 BINs, it was not possible to resolve the misidentifications, and their identification remained at the genus or family level.
- 33 BINs were either associated with a genus name or were not identified in the BOLD database. Among them, 25 were identified at the genus level, and eight at the species level, including four cryptic species.

3.2. Overall Profile of Fish Species in the MADFI Database According to the Fishbase

Among the 424 species validated through World Register of Marine Species (WoRMS) (Table S2), 39 are newly recorded for Madagascar, two are newly recorded for the entire Western Indo-Pacific (WIP) region, and 22, although already known, had not yet been identified in BOLD (Figure 4a). Regarding their biogeographic distribution, the majority of species (127) are widely distributed across the Indo-Pacific, 34 are endemic to the WIP, one species is strictly endemic to Madagascar, and two species have a circumtropical distribution (Figure 4b).

In terms of conservation status according to the International Union for Conservation of Nature (IUCN) via WoRMS, most species (310) are classified as "Least Concern," while 103 species have not yet been assessed. Very few are threatened: two are endangered (*Cheilinus undulates* and *Lethrinus mashena*), one is vulnerable (*Hippocampus histrix*), and one is near threatened (*Favonigobius melanobranchus*) (Figure 4d).

From an ecological perspective, 355 species are strictly marine, 52 occur in both transitional and marine zones, and 17 are euryhaline (present in freshwater, brackish, and marine environments) (Figure 4e). Finally, regarding ecological lifestyle, the majority of species (359) are reef-associated, 44 are demersal, and 21 are pelagic (Figure 4f).

3.3. Taxonomic Coverage of 12S Sequences

The *12S* barcode library comprises 524 sequences, of which 89% correspond to near full-length *12S* rRNA sequences (~1000 bp), obtained with long-range primers. The remaining 11% are shorter sequences (~600 bp), generated using internal primers when full-length amplification failed, likely due to mutations at primer binding sites or DNA degradation. These sequences represent 446 species from 78 families. The five most diverse families in terms of species were Labridae (40), Apogonidae (37), Gobiidae (26), Acanthuridae (19), and Pomacentridae (19) (Table 2). Among all BINs in the database, 86% (434 BINs from 76 families) were sequenced for the 12S gene. The complete set of fish DNA sequences for these two genes covered 513 species distributed across 84 families, including 436 species from 75 families with sequences available for both the *COI* and *12S* genes. Conversely, 67 species from 35 families were available only for the *COI* gene, while 10 species from nine families were available exclusively for the *12S* gene.

3.4. Taxonomic Resolution of Five Different 12S Primers

A total of 319 species have complete *12S* gene sequences associated with BINs defined by the *COI* marker. For these species, the taxonomic resolution of five sets of primers used for taxonomic assignments was evaluated by comparing their level of polymorphisms to species delimitation based on *COI* polymorphisms (BINs). For all metabarcodes, lowering the similarity threshold (ST) from 99.5% to 90% led to a reduction in false-negative errors, as expected (Figure 5). When the ST was set at 99% or less, both MiFish and ACMDB showed an increase in false-positive errors (overestimation of the number of species compared to the number of BINs). This false-positive error appeared at a lower ST (97%) for Teleo1, while no false-positive errors were observed for the AC12S and 12SF1R1 metabarcodes. As a result, all metabarcodes had different ST values at which false-negative and false-positive errors were well-balanced. Optimal ST values were 99.5% for Ac12S and 12SF1R1, 98.5% for AcMDB and Mifish, and 95.5% for Teleo1.

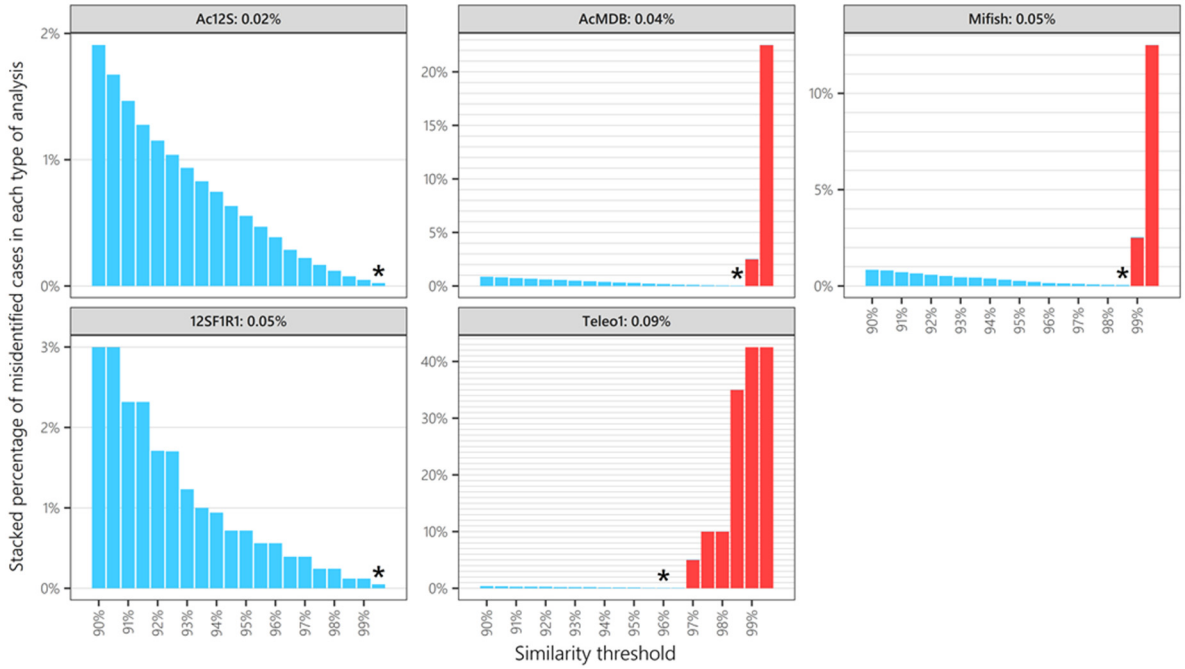

Figure 5. Proportions of false-positive errors (in red) and false-negative errors (in blue) for each metabarcode depending on similarity thresholds ranging from 99.5% to 90% for the five different metabarcodes (Ac12S, AcMBD, Mifish, 12SF1R1, and Teleo1). The black star represents the optimal threshold for each metabarcode, while the minimal error rate achieved at this threshold is indicated in the top panel.

Using similarity thresholds of 97% and 98%, AcMDB and MiFish metabarcodes were the most effective at discriminating BINs, while Ac12S and 12SF1R1 discriminated the most BINs at 99% similarity. For all three similarity thresholds (97%, 98%, and 99%), Teleo1 remained the least discriminating metabarcode (Figure 5).

The ability to distinguish different BINs within a family varied according to the metabarcodes used (Figure 6). An example of this variation is observed in the Carangidae family: no BIN discrimination errors were detected for the Ac12S and Mifish metabarcodes; one pair of BINs was not discriminated by both the AcMDB and 12SF1R1 metabarcodes; and four pairs of BINs were not discriminated for the Teleo1 metabarcode. The number of families (among 48) with BIN discrimination errors was 14, 15, 16, 16, and 19 for the Ac12S, Mifish, AcMDB, 12SF1R1, and Teleo metabarcodes, respectively. Pearson's correlation analysis revealed a moderate positive relationship between the number of non-discriminated BIN pairs and the number of BINs within each family, with a highly significant p-value ($r = 0.46$, $p < 0.001$). Regardless of the metabarcode, BIN discrimination errors were detected in the following 13 families (Figure 6). Outside of these 13 families, each metabarcode showed BIN discrimination errors in specific families. The Ac12S metabarcode showed errors in the Priacanthidae family. Errors were detected in several families across the different metabarcodes: AcMDB showed errors in Carangidae, Ephippidae, and Exocoetidae; MiFish in Ephippidae and Serranidae; and 12SF1R1 in Carangidae, Priacanthidae, and Serranidae. Finally, the Teleo1 metabarcode showed errors in the Carangidae, Ephippidae, Exocoetidae, Muraenidae, Pomacentridae, and Sphyraenidae families. The families with the highest number of non-discriminated BIN pairs were Lethrinidae for the Ac12S metabarcode, Labridae for the MiFish metabarcode, and Chaetodontidae for the metabarcodes AcMDB, 12SF1R1, and Teleo1.

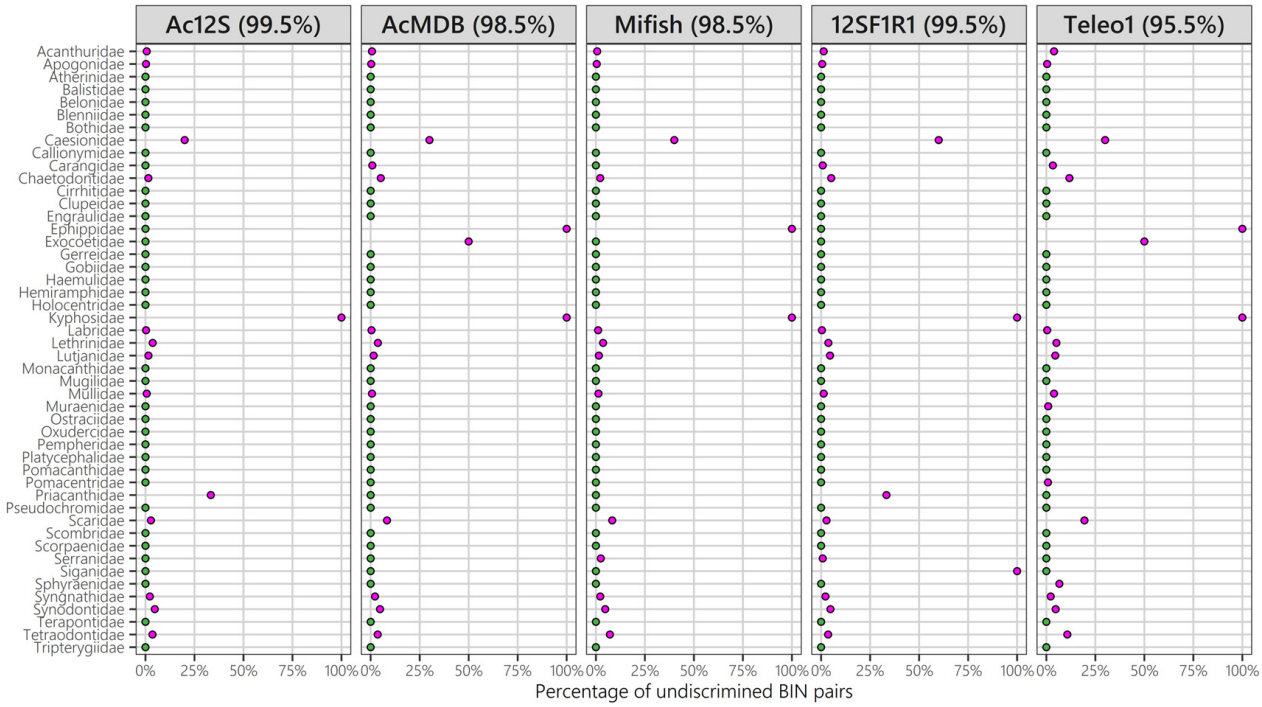

Figure 6. Percentage of BIN pair discrimination by family for each primer. The percentage indicated for each primer corresponds to its optimal similarity threshold used to assess taxonomic resolution across fish families. Ac12S (99.5%), AcMBD (98.5%), Mifish (98.5%), 12SF1R1 (99.5%), and Teleo1 (95.5%), Green indicates families for which all BINs are discriminated by the primer. Magenta indicates families containing BINs not discriminated by each primer.

4. Discussion

This study provides the most comprehensive molecular inventory to date of marine fish diversity in Madagascar. The newly assembled *COI* and *12S* reference libraries comprise 514 species spanning 84 families, 28 orders, and 2 classes. It constitutes the largest-scale DNA barcode reference dataset derived from specimens collected with a wide array of artisanal fishing gear over an extended sampling period exceeding 2 years. Despite earlier efforts in the same region of Toliara, this study stands out due to its unparalleled sampling intensity, its broad habitat coverage, and the integration of morphological and molecular identification methods. For example, Laroche and Ramananarivo [42], relying exclusively on morphological identification over a 7-month survey period, recorded only 35 fish families in Toliara Bay, less than half of the 74 reported here. Similarly, Jaonalison et al. [26] focused solely on larval and juvenile stages using light traps and mosquito net trawls. Although they successfully recorded 387 species from 66 families, their work captured only a portion of the local ichthyofauna. By contrast, this study revealed significantly greater taxonomic richness. The extended temporal scope allowed the detection of seasonal or rarely encountered species, consistent with findings by Laroche et al. [43], who documented temporal variation in fish assemblages in southwestern Madagascar. The use of diverse artisanal fishing methods further improved sampling coverage across ecological niches, including cryptic and reef-associated taxa. Broad-scale surveys may serve as an efficient way to rapidly increase the taxonomic coverage of DNA barcode libraries, as illustrated by Vences et al. [27]. They documented *COI* barcodes for 307 species across diverse locations in Madagascar. However, our findings demonstrate that high-frequency, site-specific sampling produces a more comprehensive and representative inventory of marine biodiversity.

Beyond contributing to the local biodiversity documentation, our findings also raise important concerns about the sustainability of current fishing practices. All specimens

analyzed in both Laroche & Ramananarivo [42] and the present study were collected from active fisheries. The marked increase in family-level richness observed may indicate a substantial rise in exploitation pressure over the intervening period. Notably, mosquito nets were not employed in 1995 [42], suggesting a shift toward more intensive or indiscriminate fishing methods. These results underscore the urgent need for systematic monitoring of fishery impacts and the integration of molecular tools into fisheries management strategies [44].

At a larger scale, such as that of the eastern Indian Ocean, our study represents a significant contribution to the genetic characterization of marine fish diversity. It notably led to the identification of 14 new BINs in the BOLD database. To date, substantial DNA barcoding efforts have been conducted in South Africa [45–47], Mozambique [48], the Red Sea [49], and the Persian Gulf [50,51]. Compared to the work carried out in Mozambique and South Africa, the sampling effort in Madagascar is more advanced than that of Mozambique. These two countries share 58 BINs, but each also harbors unique BINs: 444 BINs have been recorded exclusively in Madagascar, compared to 84 in Mozambique. When compared with the BINs recorded in South Africa, approximately half of those identified in Madagascar are also found there [47]. However, South Africa has a higher number of unique BINs, with 710 compared to 255 for Madagascar. Dissimilarities in biodiversity and taxonomic composition among these three countries can be explained by differences in sampling efforts and the biogeographic particularities of each region regarding ichthyological diversity. They also reflect that some species shared across multiple regions can vary significantly in local abundance. The relative abundance of a species in a given area directly influences its likelihood of being caught by fishers.

A second major contribution of this study is the improvement of taxonomic resolution through the integration of 953 new *COI* sequences. This addition to the existing dataset enabled the development of the first high-resolution DNA barcode reference library for marine fishes in southwestern Madagascar. Among the 502 species catalogued, 132 were associated with multiple BINs in BOLD, revealing previously undetected cryptic diversity. These cryptic taxa account for approximately 26% of the observed species richness. This proportion is consistent with patterns reported in other Indo-Pacific regions, where morphologically indistinguishable sister species are increasingly being uncovered through molecular approaches [36,45]. In our dataset, cryptic diversity was especially pronounced within the families Apogonidae, Gobiidae, and Labridae. These families are not only ecologically significant (often composed of small, reef-associated species with high habitat specificity) but also taxonomically complex and geographically widespread. The prevalence of cryptic diversity in these groups is unsurprising and can be attributed to a combination of factors: (1) They represent some of the most species-rich lineages of reef fishes. (2) Their diversity is frequently the result of recent radiations, producing closely related species with limited morphological divergence but clear genetic distinctiveness (e.g., Duchene et al. [52], Winterbottom [53]). (3) Subtle morphological differences among species render traditional identification methods ineffective [54]. (4) These taxa often exhibit fine-scale ecological partitioning, with species occupying different microhabitats or depth zones. Such ecological specialization can reduce interspecific competition while promoting reproductive isolation and genetic divergence [55–57]. Together, these findings highlight the limitations of morphology-based taxonomy in resolving biodiversity patterns in such groups. They emphasize the importance of incorporating molecular tools into routine biodiversity assessments. The widespread occurrence of cryptic lineages also lends support to the hypothesis that allopatric speciation, driven by historical geographic barriers and ecological isolation, plays a central role in the diversification of reef fishes in the Western Indo-Pacific [58,59]. In our dataset, 82% of cryptic lineages appeared to be

allopatric, reinforcing the idea that spatial fragmentation and ecological segregation are key contributors to hidden biodiversity in this region [60].

In the context of environmental DNA (eDNA) metabarcoding, the accuracy of species detection and identification is critically dependent on both the quality and taxonomic completeness of DNA reference libraries. High-quality libraries must include sequences derived from rigorously validated voucher specimens, as misidentified individuals can introduce systematic errors into biodiversity assessments [61,62]. Equally important is taxonomic completeness (i.e., the comprehensive representation of species likely to occur in a given ecosystem), to reduce false negatives and improve detection accuracy, especially in species-rich and poorly documented regions such as the Western Indo-Pacific [58,60]. The widespread presence of cryptic species complexes in marine taxa further complicates the development of effective reference libraries. These taxa are frequently misidentified or overlooked in global repositories, which reduces the reliability of species-level assignments in eDNA studies. In addition, global databases often contain sequences from geographically disparate populations, thereby capturing high levels of intraspecific genetic variation. This geographic variation can obscure species boundaries and lower the accuracy of automated taxonomic assignments. Li et al. [63] highlighted this issue by demonstrating that pairwise genetic distances among local barcode sequences were three to five times lower than those from non-local sources. These limitations underscore the need for locally curated reference libraries based on regionally collected and accurately identified specimens. Such resources not only minimize misidentifications and account for cryptic diversity, but also allow for the calibration of locally relevant genetic divergence thresholds. In line with this rationale, we assembled a complementary 12S reference library to enhance eDNA-based species detection in Madagascar's marine ecosystems.

Lastly, because eDNA-based biodiversity monitoring commonly relies on short fragments of the *12S rRNA* gene, we conducted an in silico comparative assessment of five widely used primer sets (MiFish, Teleo1, AcMDB, Ac12S, and 12SF1/R1) to evaluate their taxonomic resolution. This analysis was performed using a curated dataset of full-length 12S sequences linked to COI-defined BINs. By comparing their performance across a range of similarity thresholds (90–99%), we were able to assess their discriminatory power and suitability for species-level identification in the context of Madagascar's marine biodiversity. No primer set performed optimally across all thresholds, but clear differences were observed. MiFish and Ac12S showed the lowest error rates at high thresholds, making them well-suited for high-resolution biodiversity assessments. In contrast, primers like 12SF1/R1, while offering broader taxonomic coverage, yielded higher error rates due to lower resolution among closely related species. These results illustrate the trade-off between universality and resolution in primer design [23]. In regions like Madagascar, where cryptic diversity is high, primers with high taxonomic discrimination (e.g., MiFish), coupled with localized reference databases, are essential for accurate eDNA-based biodiversity monitoring. The implementation of eDNA approaches in Madagascar's coastal waters will require careful primer selection, tailored to the ecological and taxonomic scope of each study. The reference frameworks and benchmarking results provided here offer essential guidance to ensure the accuracy and reliability of future biodiversity assessments.

5. Conclusions

This study represents a major step forward in documenting and understanding the marine fish biodiversity of southwestern Madagascar. By integrating extensive field sampling, morphological identification, and DNA barcoding (*COI* and *12S*), it provides the most comprehensive molecular reference databases available for the region to date. The resulting catalog of 514 species across 84 families not only enhances our knowledge of local species richness

but also reveals substantial cryptic diversity and biogeographic structuring. Although this represents a substantial contribution to understanding the island's marine biodiversity, it still only covers about 30% of the total marine and transitional water fish species listed by [26], highlighting the need for continued sampling and molecular characterization.

Beyond its taxonomic contributions, this work underscores several critical methodological insights. First, it highlights the limitations of global genetic databases such as GenBank and BOLD for accurate species identification in eDNA metabarcoding studies, especially in regions with high cryptic diversity. Second, it demonstrates the necessity of building locally curated reference libraries, both to reflect regional genetic diversity and to support more precise species delimitation. Third, it confirms the importance of long-term, spatially diverse sampling strategies, particularly in ecosystems experiencing intense and unregulated fishing pressure.

The *12S* reference library developed here, along with the in silico evaluation of commonly used primer sets, provides essential tools for the deployment of eDNA-based biodiversity monitoring in Madagascar. These molecular tools, when combined with traditional ecological knowledge and fisheries data, can greatly improve the accuracy of species detection, support sustainable management practices, and inform conservation strategies.

Table 2. List of species barcoded in the MADFI database on the BOLD platform. BIN: Barcode Index Number. *COI*: specimens barcoded with the COI gene. *12S*: specimens barcoded with *12S* gene. N *COI* in MADFI: Number of specimens barcoded with the *COI* gene in the MADFI database. N *12S* in MADFI: Number of specimens barcoded with the *12S* gene in the MADFI database. Nsp./BIN: number of species associated with each BIN in the BOLD. NBIN/Sp. In BOLD: number of BINs associated with each species (only if the species is linked to one BIN in the BOLD). New: new BINs in BOLD. NA: BINs that contain specimens not identified to the species level. Sp. complex: Y (Yes) indicates species complexes. Allo./Symp.: Cryptic species classified as allopatric (A) or sympatric (S).

Class		***COI***					***12S***
Order							
Family							
Species	**BIN**	**N *COI* in MADFI**	**Nsp./BIN in BOLD**	**NBIN/Sp. In BOLD**	**Sp. Complex**	**Allo./Symp.**	**N *12S* in MADFI**
Actinopterygii							
Acanthuriformes							
Acanthuridae							
Acanthurus blochii	BOLD:AAF0623	**1**	1	1			**1**
Acanthurus dussumieri	BOLD:AAE4046	**26**	1	>1			**1**
Acanthurus leucosternon	BOLD:AAB7142	**1**	>1				**1**
Acanthurus lineatus	BOLD:AAB3207	**1**	>1				**1**
Acanthurus mata	BOLD:AAE4025	**6**	1	>1			**1**
Acanthurus nigricauda	BOLD:AAB8729	**14**	1	1			**1**
Acanthurus nigrofuscus	BOLD:AAB0201	**3**	>1				**1**
Acanthurus tennentii	BOLD:AAD2621	**1**	1	1			**1**
Acanthurus triostegus	BOLD:AAA9362	**7**	1	1			**1**
Acanthurus xanthopterus	BOLD:AAC6467	**22**	>1				**1**
Ctenochaetus binotatus	BOLD:AAB9166	**2**	1	1			**1**
Ctenochaetus striatus	BOLD:AAB9167	**13**	>1				**2**
Naso annulatus	BOLD:AEN2475	**7**	1	1			**2**
Naso brevirostris	BOLD:AAC1635	**17**	1	1			**1**
Naso elegans	BOLD:AAA8635	**1**	>1				**1**
Naso unicornis	BOLD:AAC8042	**3**	1	1			**1**
Paracanthurus hepatus	BOLD:AAC3227	**1**	1	>1			**1**

Table 2. *Cont.*

Class		*COI*					*12S*
Order							
Family							
Species	**BIN**	**N *COI* in MADFI**	**Nsp./BIN in BOLD**	**NBIN/Sp. In BOLD**	**Sp. Complex**	**Allo./Symp.**	**N *12S* in MADFI**
Zebrasoma cf. *desjardinii* [BOLD:AAF6311]	BOLD:AAF6311	**2**	1	>1	Y	S	**1**
Zebrasoma cf. *desjardinii* [BOLD:ACV8450]	BOLD:ACV8450	**1**	>1		Y	S	**1**
Zebrasoma cf. *scopas* [BOLD:AAB3788]	BOLD:AAB3788	**5**	1	>1	Y	A	**0**
Zanclidae							
Zanclus cornutus	BOLD:ADI3027	**1**	1	1			**1**
Anguilliformes							
Congridae							
Ariosoma cf. *meeki* [BOLD:ADD3087]	BOLD:ADD3087	**1**	1	1	Y	A	**0**
Ariosoma scheelei	BOLD:AAJ1709	**14**	1	1			**1**
Conger cinereus	BOLD:AAL5915	**6**	1	>1	Y	A	**1**
Uroconger [BOLD:ACV7958]	BOLD:ACV7958	**1**	NA				**0**
Muraenidae							
Echidna nebulosa	BOLD:AAC5551	**3**	1	1			**2**
Echidna polyzona	BOLD:AAC9046	**4**	>1				**3**
Gymnomuraena zebra	BOLD:AAN1246	**1**	1	1			**1**
Gymnothorax [BOLD:ADB4048]	BOLD:ADB4048	**1**	NA				**1**
Gymnothorax cf. *chilospilus* [BOLD:AAC0198]	BOLD:AAC0198	**4**	1	>1	Y	A	**2**
Gymnothorax cf. *undulatus* [BOLD:AAC5500]	BOLD:AAC5500	**4**	>1		Y	S	**3**
Gymnothorax cf. *undulatus* [BOLD:AAC5502]	BOLD:AAC5502	**1**	>1		Y	S	**1**
Gymnothorax elaineheemstrae	BOLD:AAE6825	**2**	>1				**3**
Gymnothorax favagineus	BOLD:ADB3872	**3**	>1				**1**
Gymnothorax flavimarginatus	BOLD:AAE6819	**1**	>1				**1**
Gymnothorax javanicus	BOLD:AAE3588	**1**	1	1			**1**
Gymnothorax pictus	BOLD:AAE3580	**1**	1	>1			**1**
Gymnothorax richardsonii	BOLD:AAI6393	**3**	1	1			**1**
Gymnothorax robinsi	BOLD:AAY2654	**1**	1	1			**1**
Gymnothorax rueppelliae	BOLD:AAE3543	**2**	>1				**1**
Scuticaria tigrina	BOLD:AAH9730	**1**	1	1			**0**
Ophichthidae							
Pisodonophis cancrivorus		**0**					**1**
Atheriniformes							
Atherinidae							
Atherinomorus cf. *lacunosus* [BOLD:ACK7521]	BOLD:ACK7521	**3**	>1	Y	A		**1**
Hypoatherina [BOLD:AAL7563]	BOLD:AAL7563	**1**	>1				**1**
Hypoatherina [BOLD:ACV9758]	BOLD:ACV9758	**5**	>1				**1**
Aulopiformes							
Synodontidae							
Saurida cf. *gracilis* [BOLD:AAB1854]	BOLD:AAB1854	**4**	>1		Y	S	**1**
Saurida cf. *gracilis* [BOLD:AAE4190]	BOLD:AAE4190	**2**	>1		Y	S	**2**
Saurida nebulosa	BOLD:AAH0503	**11**	1	>1	Y	A	**1**
Synodus [BOLD:ACD1807]	BOLD:ACD1807	**2**	NA				**2**
Synodus dermatogenys		**0**					**2**
Synodus rubromarmoratus		**0**					**1**
Synodus variegatus	BOLD:AAB5069	**4**	>1				**1**
Trachinocephalus [BOLD:AAA9578]	BOLD:AAA9578	**2**	>1				**0**
Trachinocephalus [BOLD:ACY8623]	BOLD:ACY8623	**1**	1	>1			**1**
Trachinocephalus cf. *trachinus* [BOLD:ABX6347]	BOLD:ABX6347	**4**	>1		Y	A	**0**
Beloniformes							
Belonidae							
Ablennes cf. *hian [BOLD:AAB9824]*	BOLD:AAB9824	**1**	>1		Y	S	**1**
Tylosurus cf. *acus* [BOLD:AAH7713]	BOLD:AAH7713	**1**	1	>1	Y	S	**1**
Tylosurus crocodilus	BOLD:AAC4148	**7**	>1				**1**
Exocoetidae							
Cheilopogon [BOLD:ABZ7103]	BOLD:ABZ7103	**1**	>1				**1**
Cheilopogon cyanopterus	BOLD:ACK7919	**1**	>1				**1**
Exocoetidae [BOLD:AAK1099]	BOLD:AAK1099	**1**	>1				**1**
Parexocoetus brachypterus	BOLD:AAG2613	**1**	>1				**1**

Table 2. *Cont.*

Class		*COI*					*12S*
Order							
Family							
Species	**BIN**	**N *COI* in MADFI**	**Nsp./BIN in BOLD**	**NBIN/Sp. In BOLD**	**Sp. Complex**	**Allo./Symp.**	**N *12S* in MADFI**
Hemiramphidae							
Hemiramphus cf. *far* [BOLD:AAC0565]	BOLD:AAC0565	**6**	1	>1	Y	A	**1**
Hemiramphus lutkei	BOLD:ACK7284	**2**	>1	>1			**1**
Hyporhamphus affinis	BOLD:AAD0579	**5**	1				**1**
Blenniiformes							
Blenniidae							
Aspidontus dussumieri	BOLD:AAJ3001	**1**	1	1			**0**
Omobranchus [BOLD:ACY8418]	BOLD:ACY8418	**1**	NA				**1**
Omobranchus elongatus	BOLD:AFC2971	**2**	new				**1**
Petroscirtes cf. *mitratus* [BOLD:AAE6131]	BOLD:AAE6131	**19**	>1		Y	S	**1**
Petroscirtes cf. *mitratus* [BOLD:AAE6132]	BOLD:AAE6132	**7**	>1		Y	S	**1**
Plagiotremus tapeinosoma	BOLD:AAD0784	**2**	1	1			**0**
Salarias [BOLD:ADN2100]	BOLD:ADN2100	**1**	NA				**1**
Salarias cf. *fasciatus* [BOLD:ACV7965]	BOLD:ACV7965	**6**	1	>1	Y	A	**1**
Tripterygiidae							
Enneapterygius [BOLD:ACV9383]	BOLD:ACV9383	**2**	NA				**1**
Helcogramma [BOLD:AAT9889]	BOLD:AAT9889	**1**	NA				**1**
Carangiformes							
Carangidae							
Alectis ciliaris	BOLD:AAB7827	**2**	1	1			**1**
Atropus hedlandensis	BOLD:AAD6117	**2**	>1				**1**
Atropus mentalis	BOLD:AAD6118	**1**	>1				**1**
Atule mate	BOLD:AAB3382	**3**	>1				**1**
Carangichthys dinema	BOLD:AAO5886	**1**	>1				**1**
Caranx ignobilis	BOLD:AAB0587	**13**	>1				**3**
Caranx melampygus	BOLD:AAB0585	**6**	>1				**1**
Caranx papuensis	BOLD:ACF4541	**2**	>1				**1**
Caranx sexfasciatus	BOLD:AAB0584	**5**	>1				**1**
Caranx tille	BOLD:ACS0288	**1**	>1				**0**
Elagatis bipinnulata	BOLD:AAB3730	**1**	>1				**0**
Ferdauia ferdau	BOLD:AAE7640	**2**	1	1			**1**
Gnathanodon speciosus	BOLD:AAB7462	**2**	1	>1	Y	S	**1**
Parastromateus niger	BOLD:AAB3884	**1**	1	1			**1**
Platycaranx [BOLD:AAB4362]	BOLD:AAB4362	**2**	>1				**0**
Platycaranx chrysophrys	BOLD:AAB2977	**1**	>1				**1**
Scomberoides lysan	BOLD:AAB0512	**11**	1	>1			**0**
Selar cf. *crumenophthalmus* [BOLD:AAB0871]	BOLD:AAB0871	**2**			Y	S	**1**
Seriolina [BOLD:AAB8503]	BOLD:AAB8503	**1**	1	>1			**1**
Trachinotus blochii	BOLD:ACF4014	**1**	1	1	Y	S	**1**
Turrum cf. *coeruleopinnatum* [BOLD:AAD2297]	BOLD:AAD2297	**6**	>1		Y	S	**2**
Turrum fulvoguttatum	BOLD:AAC2745	**3**	1	1			**2**
Coryphaenidae							
Coryphaena hippurus	BOLD:AAA5277	**1**	>1				**1**
Echeneidae							
Echeneis naucrates	BOLD:AAB6121	**2**	>1				**1**
Sphyraenidae							
Sphyraena [BOLD:AAF8783]	BOLD:AAF8783	**1**	>1				**1**
Sphyraena [BOLD:ACV9716]	BOLD:ACV9716	**5**	NA				**1**
Sphyraena barracuda	BOLD:AAA6100	**6**	>1				**1**
Sphyraena chrysotaenia	BOLD:AAD0400	**2**	>1				**1**
Sphyraena flavicauda	BOLD:AAF8900	**6**	>1				**1**
Sphyraena putnamae	BOLD:AAB2694	**1**	>1				**1**
Sphyraena qenie	BOLD:AAD8414	**1**	1	1			**1**
Clupeiformes							
Chirocentridae							
Chirocentrus cf. *dorab* [BOLD:AAC2273]	BOLD:AAC2273	**2**	1	>1	Y	A	**1**
Clupeidae							

Table 2. *Cont.*

Class		*COI*					*12S*
Order							
Family							
Species	**BIN**	**N *COI* in MADFI**	**Nsp./BIN in BOLD**	**NBIN/Sp. In BOLD**	**Sp. Complex**	**Allo./Symp.**	**N *12S* in MADFI**
Herklotsichthys [BOLD:AEQ8868]	BOLD:AEQ8868	**1**	new				**1**
Dussumieria cf. *elopsoides* [BOLD:AEP5161]	BOLD:AEP5161	**2**	new		Y	A	**2**
Herklotsichthys quadrimaculatus	BOLD:AAC2887	**8**	>1				**3**
Spratelloides cf. *delicatulus* [BOLD:ACV7999]	BOLD:ACV7999	**6**	1	>1	Y	A	**1**
Spratelloides cf. *gracilis*		**0**					**1**
Engraulidae							
Stolephorus belaerius	BOLD:AAG4825	**5**	1	>1			**1**
Thryssa baelama	BOLD:ABU9831	**1**	>1				**1**
Elopiformes							
Megalopidae							
Megalops cyprinoides	BOLD:AAC4501	**1**	1	>1			**1**
Gobiiformes							
Eleotridae							
Eleotris klunzingerii	BOLD:ACV7471	**1**	1	1			**1**
Gobiidae							
Amblygobius nocturnus		**0**					**1**
Amblygobius semicinctus	BOLD:AAB8727	**10**	>1				**1**
Amblygobius sphynx	BOLD:AAJ2321	**5**	1	>1			**1**
Asterropteryx [BOLD:AEU6891]	BOLD:AEU6891	**1**	new				**1**
Asterropteryx cf. *semipunctata* [BOLD:AAC0108]	BOLD:AAC0108	**12**	1	>1	Y	A	**1**
Bathygobius [BOLD:AAF8787]	BOLD:AAF8787	**1**	NA				**1**
Bathygobius cyclopterus	BOLD:AAB9729	**1**	>1				**1**
Callogobius flavobrunneus	BOLD:ACV9382	**2**	1	1			**1**
Cryptocentrus [BOLD:ADM8879]	BOLD:ADM8879	**1**	NA				**1**
Cryptocentrus [BOLD:AEP3749]	BOLD:AEP3749	**4**	new				**1**
Cryptocentrus [BOLD:AFC5965]	BOLD:AFC5965	**1**	new				**1**
Cryptocentrus cryptocentrus	BOLD:AAM4607	**1**	1	1			**1**
Drombus key	BOLD:AEF3691	**1**	new				**0**
Favonigobius cf. *melanobranchus* [BOLD:AAL8921]	BOLD:AAL8921	**1**	>1		Y	A	**1**
Favonigobius reichei	BOLD:AAY4455	**1**	1	1			**1**
Gunnellichthys copleyi	BOLD:ADM8209	**1**	1	1			**0**
Istigobius cf. *decoratus* [BOLD:ADM7236]	BOLD:ADM7236	**3**	NA		Y	A	**1**
Istigobius cf. *ornatus* [BOLD:AAD7940]	BOLD:AAD7940	**1**	1	>1	Y	A	**1**
Oplopomus [BOLD:AEX8266]	BOLD:AEX8266	**1**	NA				**1**
Oplopomus cf. *oplopomus* [BOLD:AAI3352]	BOLD:AAI3352	**13**	1	>1	Y	A	**2**
Palutrus reticularis	BOLD:AAL7906	**1**	>1				**1**
Psammogobius biocellatus	BOLD:AAC2888	**8**	>1				**1**
Ptereleotris evides	BOLD:AAD9105	**1**	1	1			**1**
Valenciennea cf. *puellaris* [BOLD:AAC4124]	BOLD:AAC4124	**1**	>1		Y	A	**1**
Valenciennea helsdingenii	BOLD:AAD4704	**1**	1	>1	Y	A	**1**
Valenciennea sexguttata	BOLD:AAC5611	**6**	1	1			**1**
Vanderhorstia ornatissima	BOLD:AAF0288	**4**	1	>1	Y	A	**1**
Yongeichthys audax	BOLD:AAJ2911	**1**	1	>1	Y	A	**0**
Yongeichthys nebulosus	BOLD:AAC3655	**2**	>1				**0**
Yongeichthys signatus	BOLD:ACX9631	**3**	NA				**1**
Oxudercidae							
Gnatholepis cf. *anjerensis* [BOLD:AAI5395]	BOLD:AAI5395	**10**	>1		Y	A	**1**
Oxyurichthys papuensis	BOLD:ACM4179	**3**	1	1			**2**
Holocentriformes							
Holocentridae							
Myripristis [BOLD:AAA9764]	BOLD:AAA9764	**1**	>1				**0**
Myripristis berndti	BOLD:AAA9763	**2**	>1				**1**
Myripristis kuntee	BOLD:AAA9765	**2**	>1				**1**
Neoniphon sammara	BOLD:AAC8278	**7**	1	>1	Y	A	**2**
Sargocentron caudimaculatum	BOLD:AEZ5902	**2**	>1				**1**
Sargocentron diadema	BOLD:AAB3424	**9**	1	>1	Y	A	**1**
Sargocentron praslin	BOLD:AAC4647	**3**	>1				**1**

Table 2. *Cont.*

Class		*COI*					*12S*
Order							
Family							
Species	**BIN**	**N *COI* in MADFI**	**Nsp./BIN in BOLD**	**NBIN/Sp. In BOLD**	**Sp. Complex**	**Allo./Symp.**	**N *12S* in MADFI**
Kurtiformes							
Apogonidae							
Apogon [BOLD:AAF8427]	BOLD:AAF8427	**5**	>1				**1**
Apogon [BOLD:AAJ8751]	BOLD:AAJ8751	**4**	1	>1			**2**
Apogon [BOLD:ACY1702]	BOLD:ACY1702	**3**	NA				**1**
Apogon cf. *crassiceps* [BOLD:ACW9154]	BOLD:ACW9154	**3**	1	>1	Y	A	**1**
Apogon erythrosoma	BOLD:ACC5690	**6**	>1				**1**
Apogon semiornatus	BOLD:AAD2206	**4**	1	>1			**1**
Apogonichthyoides timorensis	BOLD:ACV6948	**19**	1	>1			**1**
Apogonichthys [BOLD:ACW8182]	BOLD:ACW8182	**2**	NA				**1**
Apogonichthys cf. *ocellatus* [BOLD:AAL6796]	BOLD:AAL6796	**1**	1	>1	Y	A	**0**
Cheilodipterus cf. *quinquelineatus* [BOLD:ABU8169]	BOLD:ABU8169	**5**	1	>1	Y	A	**1**
Cheilodipterus macrodon	BOLD:AAB7874	**1**	1	>1	Y	A	**1**
Foa cf. *fo* [BOLD:ABU8857]	BOLD:ABU8857	**20**	1	>1	Y	A	**1**
Foa fo	BOLD:ABU8856	**6**	1	1			**1**
Fowleria cf. *marmorata* [BOLD:AAU0944]	BOLD:AAU0944	**1**	1	>1	Y	A	**0**
Fowleria cf. *vaiulae* [BOLD:AAD1017]	BOLD:AAD1017	**1**	1	>1	Y	A	**1**
Fowleria variegata	BOLD:AAD8726	**15**	1	>1	Y	A	**2**
Jaydia novaeguineae	BOLD:ACM4616	**1**	1	1			**1**
Neamia octospina	BOLD:ACY8539	**1**	>1				**1**
Nectamia cf. *fusca* [BOLD:AAL9262]	BOLD:AAL9262	**10**	1	>1	Y	A	**1**
Nectamia cf. *savayensis* [BOLD:AAD9453]	BOLD:AAD9453	**3**	>1		Y	A	**1**
Ostorhinchus [BOLD:AAJ1260]	BOLD:AAJ1260	**4**	1	>1			**1**
Ostorhinchus [BOLD:ADI1552]	BOLD:ADI1552	**3**	NA				**1**
Ostorhinchus aureus	BOLD:ACE9301	**1**	1	>1	Y	A	**1**
Ostorhinchus cf. *angustatus* [BOLD:AAD5116]	BOLD:AAD5116	**2**	1	>1	Y	A	**2**
Ostorhinchus cf. *apogonoides* [BOLD:AAD5125]	BOLD:AAD5125	**1**	>1		Y	A	**1**
Ostorhinchus cf. *cooki* [BOLD:AAC2084]	BOLD:AAC2084	**16**	1	>1	Y	A	**1**
Ostorhinchus cf. *cyanosoma* [BOLD:ACV9601]	BOLD:ACV9601	**14**	NA		Y	A	**3**
Ostorhinchus cf. *fasciatus* [BOLD:AAC1243]	BOLD:AAC1243	**1**	1	>1	Y	A	**1**
Ostorhinchus cf. *gularis* [BOLD:ACS5956]	BOLD:ACS5956	**2**	1	>1	Y	A	**1**
Ostorhinchus cf. *taeniophorus* [BOLD:AAD8453]	BOLD:AAD8453	**1**	>1		Y	S	**1**
Ostorhinchus cf. *taeniophorus* [BOLD:AAD8454]	BOLD:AAD8454	**7**	1	>1	Y	S	**1**
Ostorhinchus flagelliferus	BOLD:AAJ1254	**2**	1	1			**1**
Ostorhinchus fleurieu	BOLD:AAD5600	**5**	>1				**1**
Pristiapogon cf. *fraenatus* [BOLD:AAJ1264]	BOLD:AAJ1264	**3**	1	>1	Y	A	**1**
Pristiapogon kallopterus	BOLD:AAB4082	**5**	1	>1	Y	A	**1**
Pseudamia [BOLD:AFB6766]	BOLD:AFB6766	**3**	new				**2**
Siphamia [BOLD:ADO4802]	BOLD:ADO4802	**1**	NA				**0**
Siphamia mossambica	BOLD:AEF3435	**1**	NA				**0**
Taeniamia cf. *fucata* [BOLD:AAB8394]	BOLD:AAB8394	**10**	>1		Y	A	**1**
Taeniamia flavofasciata	BOLD:AAU1539	**5**	1	1			**1**
Zoramia cf. *leptacanthus* [BOLD:AAU1535]	BOLD:AAU1535	**4**	>1		Y	A	**1**
Lophiiformes							
Antennariidae							
Antennariidae		**0**					**1**
Antennarius cf. *striatus* [BOLD:AAO6018]	BOLD:AAO6018	**3**	1	>1	Y	A	**0**
Moroniformes							
Ephippidae							
Platax orbicularis	BOLD:AAC6496	**6**	1	1			**1**
Platax teira	BOLD:AAC5812	**1**	1	>1			**1**
Mugiliformes							
Mugilidae							
Crenimugil buchanani	BOLD:AAE3561	**1**	>1				**0**
Moolgarda crenilabis	BOLD:AAG6597	**3**	>1				**2**
Mugil cf. *cephalus [BOLD:AAA7833]*	BOLD:AAA7833	**8**	>1		Y	A	**1**
Ovalentaria							
Ambassidae							

Table 2. *Cont.*

Class		***COI***					***12S***
Order							
Family							
Species	**BIN**	**N *COI* in MADFI**	**Nsp./BIN in BOLD**	**NBIN/Sp. In BOLD**	**Sp. Complex**	**Allo./Symp.**	**N *12S* in MADFI**
Ambassis dussumieri	BOLD:AAJ2348	**2**	>1				**1**
Plesiopidae							
Plesiops coeruleolineatus	BOLD:AAE4785	**5**	1	>1			**1**
Pomacentridae							
Abudefduf sparoides	BOLD:AAD7433	**2**	1	1			**1**
Abudefduf vaigiensis	BOLD:ACK8109	**6**	>1				**1**
Amblyglyphidodon [BOLD:ACF1984]	BOLD:ACF1984	**2**	1	>1			**1**
Amblypomacentrus annulatus	BOLD:AAF2723	**12**	1	1			**1**
Amphiprion allardi	BOLD:AAC4980	**2**	1	1			**1**
Chromis [BOLD:ACF0042]	BOLD:ACF0042	**4**	>1				**1**
Chromis atripectoralis	BOLD:AAB9018	**1**	1	1			**0**
Chromis cf. *ternatensis* [BOLD:AAC6972]	BOLD:AAC6972	**1**	1	>1	Y	A	**0**
Chromis fieldi	BOLD:AAC0458	**1**	>1				**1**
Chromis ternatensis	BOLD:AAF3462	**1**	1	>1	Y	A	**1**
Chromis viridis	BOLD:AAB4985	**7**	1	1			**1**
Chrysiptera cf. *brownriggii* [BOLD:AAB6234]	BOLD:AAB6234	**2**	>1		Y	A	**0**
Dascyllus abudafur	BOLD:AAB4091	**6**	>1				**1**
Dascyllus carneus	BOLD:AAD7114	**5**	1	>1	Y	A	**0**
Dascyllus trimaculatus	BOLD:AAB2685	**13**	>1				**1**
Neoglyphidodon melas	BOLD:AAC5328	**1**	1	1			**1**
Neopomacentrus cyanomos	BOLD:ABX6100	**1**	1	>1	Y	A	**1**
Neopomacentrus fuliginosus	BOLD:AAI3160	**9**	>1				**0**
Neopomacentrus sororius	BOLD:AAC8318	**6**	>1				**1**
Plectroglyphidodon cf. *lacrymatus* [BOLD:AAB6988]	BOLD:AAB6988	**8**	1	>1	Y	A	**1**
Plectroglyphidodon dickii	BOLD:ACC1132	**1**	1	1			**1**
Pomacentrus aquilus	BOLD:AAY3550	**33**	>1				**1**
Pomacentrus baenschi	BOLD:AAC9672	**1**	1	1			**0**
Pomacentrus caeruleus	BOLD:AAB9539	**5**	>1				**1**
Pomacentrus sulfureus	BOLD:AAD2952	**2**	1	1			**0**
Pycnochromis nigrurus	BOLD:AAD5022	**1**	1	1			**1**
Pseudochromidae							
Halidesmus [BOLD:AEU2441]	BOLD:AEU2441	**1**	new				**1**
Halidesmus [BOLD:AAU1500]	BOLD:AAU1500	**1**	NA				**0**
Pseudochromis cf. *kristinae* [BOLD:ADI5215]	BOLD:ADI5215	**11**	NA		Y	A	**2**
Pseudochromis madagascariensis	BOLD:AAU3299	**3**	1				**1**
Perciformes							
Caesionidae							
Caesio cf. *caerulaurea* [BOLD:AAB4823]	BOLD:AAB4823	**12**	>1		Y	S	**2**
Caesio cf. *caerulaurea* [BOLD:AAB4822]	BOLD:AAB4822	**2**	>1		Y	S	**1**
Caesio lunaris	BOLD:AAJ8622	**1**	1	1			**0**
Caesio xanthonota	BOLD:AAE8330	**1**	1	1			**0**
Dipterygonotus balteatus	BOLD:AAD3666	**1**	1	1			**1**
Pterocaesio chrysozona	BOLD:AAE8474	**1**	>1				**0**
Pterocaesio marri	BOLD:AAC2204	**3**	>1				**1**
Pterocaesio trilineata	BOLD:AAE8473	**1**	>1				**1**
Chaetodontidae							
Chaetodon auriga	BOLD:AAB1540	**7**	1	1			**2**
Chaetodon blackburnii	BOLD:AAE1178	**3**	1	1			**1**
Chaetodon cf. *bennetti* [BOLD:ACE8647]	BOLD:ACE8647	**1**	1	>1	Y	A	**1**
Chaetodon guttatissimus	BOLD:AAD3872	**1**	>1				**1**
Chaetodon kleinii	BOLD:AAC2841	**3**	>1				**1**
Chaetodon lineolatus	BOLD:ABZ0931	**1**	1	1			**1**
Chaetodon lunula	BOLD:AAB6339	**2**	>1				**1**
Chaetodon madagaskariensis	BOLD:AAC2634	**2**	>1				**1**
Chaetodon melannotus	BOLD:AAC2848	**5**	>1				**1**
Chaetodon trifasciatus	BOLD:AAB7102	**5**	>1				**2**

Table 2. *Cont.*

Class		***COI***					***12S***
Order							
Family							
Species	**BIN**	**N *COI* in MADFI**	**Nsp./BIN in BOLD**	**NBIN/Sp. In BOLD**	**Sp. Complex**	**Allo./Symp.**	**N *12S* in MADFI**
Chaetodon ulietensis	BOLD:AAC8636	**1**	>1				**1**
Chaetodon vagabundus	BOLD:AAB3198	**8**	1	1			**2**
Chaetodon xanthocephalus	BOLD:AAE1213	**5**	1	1			**1**
Chaetodon zanzibarensis	BOLD:AAE9260	**1**	1	1			**1**
Heniochus cf. *acuminatus* [BOLD:AAB5716]	BOLD:AAB5716	**4**	>1		Y	A	**1**
Heniochus diphreutes	BOLD:AAB5717	**1**	>1				**1**
Heniochus monoceros	BOLD:AAC7417	**1**	1	1			**1**
Cirrhitidae							
Cirrhitus cf. *pinnulatus* [BOLD:AAC5875]	BOLD:AAC5875	**2**	>1		Y	A	**2**
Cyprinocirrhites cf. *polyactis* [BOLD:AAF8813]	BOLD:AAF8813	**1**	1	>1	Y	A	**1**
Paracirrhites arcatus	BOLD:AAC6007	**2**	1	1			**1**
Paracirrhites forsteri	BOLD:AAC5873	**1**	>1				**1**
Gerreidae							
Gerres cf. *filamentosus* [BOLD:AAC0377]	BOLD:AAC0377	**3**	>1		Y	A	**0**
Gerres filamentosus	BOLD:AAC0380	**2**	>1				**1**
Gerres longirostris	BOLD:AAE6359	**8**	1	>1	Y	A	**3**
Gerres oblongus	BOLD:AAE6346	**2**	1	1			**1**
Gerres oyena	BOLD:AAC1291	**2**	1	>1	Y	A	**0**
Haemulidae							
Diagramma [BOLD:AAD4477]	BOLD:AAD4477	**12**	>1				**2**
Plectorhinchus cf. *vittatus* [BOLD:AAE4709]	BOLD:AAE4709	**1**	1	>1	Y	A	**1**
Plectorhinchus [BOLD:AAF8797]	BOLD:AAF8797	**1**	>1				**1**
Plectorhinchus flavomaculatus	BOLD:AAC4020	**10**	1	1			**1**
Plectorhinchus gaterinus	BOLD:AAH9156	**12**	1	1			**1**
Plectorhinchus plagiodesmus	BOLD:ACC0439	**1**	1	1			**0**
Pomadasys cf. *furcatus* [BOLD:AAD1382]	BOLD:AAD1382	**1**	1	>1	Y	A	**1**
Kyphosidae							
Kyphosus cinerascens	BOLD:ABX5727	**1**	>1				**1**
Kyphosus vaigiensis	BOLD:AAC3456	**2**	>1				**1**
Labridae							
Anampses caeruleopunctatus	BOLD:AAB9969	**1**	>1				**0**
Bodianus cf. *perditio* [BOLD:AAC7631]	BOLD:AAC7631	**1**	1	>1	Y	A	**1**
Cheilinus cf. *chlorourus* [BOLD:AAB4186]	BOLD:AAB4186	**8**	>1		Y	A	**1**
Cheilinus oxycephalus	BOLD:AAB4187	**3**	>1				**1**
Cheilinus trilobatus	BOLD:AAB4188	**5**	>1				**1**
Cheilinus undulatus	BOLD:AAF3078	**1**	1	1			**1**
Cheilio inermis	BOLD:AAA6101	**5**	1	1			**3**
Coris caudimacula	BOLD:AAC2101	**4**	1	1			**1**
Cymolutes cf. *torquatus* [BOLD:ADM8951]	BOLD:ADM8951	**1**	1	>1	Y	A	**1**
Cymolutes praetextatus	BOLD:AAF7751	**7**	1	>1			**1**
Cymolutes torquatus	BOLD:AAI9445	**3**	>1				**1**
Epibulus cf. *insidiator* [BOLD:AAB8858]	BOLD:AAB8858	**8**	1	>1	Y	S	**1**
Epibulus cf. *insidiator* [BOLD:ADU6797]	BOLD:ADU6797	**1**	1	>1	Y	S	**1**
Gomphosus caeruleus	BOLD:AAD5965	**2**	1	1			**2**
Halichoeres [BOLD:AAT9960]	BOLD:AAT9960	**1**	>1				**1**
Halichoeres cf. *hortulanus* [BOLD:AAB3085]	BOLD:AAB3085	**2**	1	>1	Y	A	**1**
Halichoeres cf. *nebulosus* [BOLD:AAC7896]	BOLD:AAC7896	**3**	1	>1	Y	A	**1**
Halichoeres cf. *zeylonicus* [BOLD:AAF7654]	BOLD:AAF7654	**5**	1	>1	Y	A	**1**
Halichoeres lapillus	BOLD:AAE3021	**1**	1	1			**1**
Halichoeres scapularis	BOLD:AAC0139	**6**	1	>1	Y	A	**1**
Halichoeres timorensis	BOLD:AAU1265	**1**	>1				**1**
Hemigymnus cf. *fasciatus* [BOLD:ACE5917]	BOLD:ACE5917	**1**	1	>1	Y	A	**0**
Hemigymnus melapterus	BOLD:AAB5264	**1**	1	1			**1**
Hologymnosus doliatus	BOLD:AAD1381	**1**	>1				**1**
Iniistius bimaculatus	BOLD:ACT0048	**2**	1	1			**1**
Iniistius naevus	BOLD:ACY5613	**1**	new				**0**
Iniistius pavo	BOLD:AAD8586	**1**	1	>1			**0**

Table 2. *Cont.*

Class		*COI*					*12S*
Order							
Family							
Species	**BIN**	**N *COI* in MADFI**	**Nsp./BIN in BOLD**	**NBIN/Sp. In BOLD**	**Sp. Complex**	**Allo./Symp.**	**N *12S* in MADFI**
Novaculichthys taeniourus	BOLD:AAB3259	**3**	1	1			**1**
Novaculoides macrolepidotus	BOLD:AAD8577	**15**	1	1			**1**
Oxycheilinus cf. *bimaculatus* [BOLD:AAC3195]	BOLD:AAC3195	**26**	>1		Y	A	**1**
Oxycheilinus digramma	BOLD:AAC0639	**1**	1	1			**1**
Pseudojuloides argyreogaster	BOLD:ADI3805	**6**	1	1			**1**
Pteragogus flagellifer	BOLD:AAZ4093	**11**	>1				**1**
Pteragogus taeniops	BOLD:AAV2736	**1**	1	1			**1**
Pteragogus trispilus	BOLD:ADI3844	**2**	1	1			**1**
Stethojulis albovittata	BOLD:AAD4824	**8**	1	1			**1**
Stethojulis interrupta	BOLD:AAC2336	**7**	>1				**1**
Stethojulis strigiventer	BOLD:AAE2186	**12**	1	1			**3**
Stethojulis		**0**					**1**
Thalassoma amblycephalum	BOLD:ADI4249	**1**	>1				**1**
Thalassoma hardwicke	BOLD:AAB0061	**2**	1	1			**1**
Thalassoma hebraicum	BOLD:AAC2788	**2**	1	1			**1**
Thalassoma lunare	BOLD:AAB0673	**4**	1	1			**2**
Thalassoma trilobatum	BOLD:AAC2785	**1**	>1				**1**
Leiognathidae							
Aurigequula fasciata	BOLD:AAB2489	**1**	>1				**0**
Deveximentum cf. *insidiator* [BOLD:ACF0053]	BOLD:ACF0053	**1**	1	>1	Y	A	**1**
Equulites klunzingeri	BOLD:AAC0238	**1**	>1				**0**
Equulites [BOLD:AAX8534]	BOLD:AAX8534	**1**	NA				**0**
Gazza [BOLD:AAB7096]	BOLD:AAB7096	**2**	>1				**0**
Lutjanidae							
Aprion virescens	BOLD:AAB8692	**1**	1	1			**1**
Lutjanus argentimaculatus	BOLD:AAB2440	**3**	1	1			**1**
Lutjanus bengalensis	BOLD:AAB7901	**1**	>1				**1**
Lutjanus bohar	BOLD:AAB4501	**2**	>1				**0**
Lutjanus cf. *fulvus* [BOLD:AAB7015]	BOLD:AAB7015	**2**	1	>1	Y	A	**1**
Lutjanus cf. *kasmira* [BOLD:ACC0930]	BOLD:ACC0930	**4**	>1		Y	A	**1**
Lutjanus cf. *lutjanus* [BOLD:AAA8168]	BOLD:AAA8168	**4**	>1		Y	A	**2**
Lutjanus fulviflamma	BOLD:ADF5681	**18**	>1				**2**
Lutjanus gibbus	BOLD:AAB3276	**10**	>1				**1**
Lutjanus monostigma	BOLD:AAB2907	**1**	>1				**1**
Lutjanus notatus	BOLD:AAF7731	**6**	>1				**1**
Lutjanus rivulatus	BOLD:AAB7684	**1**	1	>1			**1**
Lutjanus sebae	BOLD:AAB5388	**1**	1	1			**1**
Monodactylidae							
Monodactylus argenteus	BOLD:AAA9698	**3**	1	>1	Y	A	**1**
Mullidae							
Mulloidichthys flavolineatus	BOLD:AAB2592	**3**	>1				**2**
Parupeneus [BOLD:AAB2590]	BOLD:AAB2590	**15**	>1				**1**
Parupeneus [BOLD:ADM7122]	BOLD:ADM7122	**2**	NA				**1**
Parupeneus cf. *barberinus* [BOLD:AAB5978]	BOLD:AAB5978	**7**	>1		Y	S	**1**
Parupeneus cf. *barberinus* [BOLD:AAB5980]	BOLD:AAB5980	**6**	>1		Y	S	**2**
Parupeneus cyclostomus	BOLD:AAD1433	**2**	>1				**0**
Parupeneus fraserorum	BOLD:AAF8776	**2**	1	1			**1**
Parupeneus indicus	BOLD:AAB0334	**5**	1	1			**1**
Parupeneus macronemus	BOLD:ACF0223	**7**	>1				**1**
Parupeneus pleurostigma	BOLD:AAD6266	**6**	1	1			**1**
Parupeneus rubescens	BOLD:AAC1405	**6**	1	1			**1**
Parupeneus trifasciatus	BOLD:AAI4266	**2**	1	1			**1**
Upeneus cf. *margarethae* [BOLD:AAB9714]	BOLD:AAB9714	**3**	>1		Y	A	**1**
Upeneus guttatus	BOLD:AAH7551	**1**	>1				**1**
Upeneus moluccensis	BOLD:AAB6469	**4**	>1				**1**
Upeneus pori	BOLD:AAC1406	**4**	>1				**1**
Upeneus supravittatus	BOLD:ABZ7416	**3**	>1				**1**

Table 2. *Cont.*

Class		*COI*					*12S*
Order							
Family							
Species	**BIN**	**N *COI* in MADFI**	**Nsp./BIN in BOLD**	**NBIN/Sp. In BOLD**	**Sp. Complex**	**Allo./Symp.**	**N *12S* in MADFI**
Upeneus tragula	BOLD:AAB9327	**1**	>1				**1**
Upeneus vittatus	BOLD:ACV4665	**3**	>1				**1**
Pempheridae							
Parapriacanthus [BOLD:AAC7599]	BOLD:AAC7599	**3**	>1				**1**
Pempheris connelli	BOLD:AAC6084	**1**	1	1			**1**
Pempheris ibo	BOLD:AAF8820	**3**	>1				**0**
Pempheris mangula	BOLD:AAD1777	**3**	>1				**1**
Polynemidae							
Polydactylus plebeius	BOLD:AAC6244	**4**	1	>1			**0**
Polydactylus sextarius	BOLD:AAB7311	**1**	>1				**0**
Pomacanthidae							
Centropyge multispinis	BOLD:AAD3135	**2**	1	1			**1**
Pomacanthus chrysurus	BOLD:AAL9941	**1**	1	1			**1**
Pomacanthus semicirculatus	BOLD:ACK8181	**5**	1	1			**1**
Priacanthidae							
Heteropriacanthus carolinus	BOLD:AAB1874	**2**	>1				**1**
Priacanthus cf. *arenatus* [BOLD:AAB1642]	BOLD:AAB1642	**2**	>1		Y	A	**1**
Priacanthus hamrur	BOLD:AAB1643	**10**	>1				**1**
Scaridae							
Calotomus carolinus	BOLD:ADI4703	**9**	>1				**1**
Calotomus spinidens	BOLD:AAD4765	**23**	1	1			**1**
Chlorurus atrilunula	BOLD:AAE8961	**2**	>1				**1**
Chlorurus cyanescens	BOLD:AEW3945	**2**	1	1			**1**
Leptoscarus vaigiensis		**0**					**3**
Scarus [BOLD:AAD0849]	BOLD:AAD0849	**18**	>1				**2**
Scarus cf. *ghobban* [BOLD:ABY4451]	BOLD:ABY4451	**12**	1	>1	Y	A	**2**
Scarus psittacus	BOLD:AAB8901	**6**	1	1			**1**
Scarus scaber	BOLD:AAE9524	**1**	>1				**1**
Serranidae							
Aethaloperca rogaa	BOLD:AAD9665	**3**	1	>1	Y	A	**1**
Cephalopholis argus	BOLD:AAC4474	**1**	1	>1			**1**
Cephalopholis cf. *sonnerati* [BOLD:AAB5431]	BOLD:AAB5431	**1**	1	>1	Y	A	**1**
Cephalopholis miniata	BOLD:AAC0216	**2**	1	1			**1**
Cephalopholis nigripinnis	BOLD:AAC4202	**1**	>1				**1**
Epinephelus areolatus	BOLD:AAA9822	**2**	>1				**1**
Epinephelus coeruleopunctatus	BOLD:ADL0994	**2**	>1				**1**
Epinephelus fasciatus	BOLD:AAB1334	**1**	1	>1	Y	A	**1**
Epinephelus flavocaeruleus	BOLD:AAD1767	**2**	>1				**1**
Epinephelus longispinis	BOLD:AAD8800	**1**	>1				**1**
Epinephelus macrospilos	BOLD:AAE1882	**2**	1	>1	Y	A	**1**
Epinephelus merra	BOLD:AAB8387	**1**	>1				**1**
Epinephelus rivulatus	BOLD:ACZ9919	**5**	1	>1	Y	A	**1**
Epinephelus spilotoceps	BOLD:AAC3590	**1**	1	>1			**1**
Plectropomus punctatus	BOLD:AAN4283	**3**	1	1			**1**
Variola louti	BOLD:AAC5719	**1**	1	>1	Y	A	**1**
Siganidae							
Siganus argenteus	BOLD:AAC6461	**2**	1	>1			**2**
Siganus luridus	BOLD:AAL9467	**2**	>1				**0**
Siganus stellatus	BOLD:AAB2341	**4**	>1				**1**
Siganus sutor	BOLD:AAB6556	**20**	>1				**1**
Terapontidae							
Pelates quadrilineatus	BOLD:AAA9700	**2**	>1				**1**
Terapon [BOLD:ACV8977]	BOLD:ACV8977	**1**	>1				**1**
Terapon jarbua	BOLD:AAA9351	**1**	1	>1	Y	A	**1**
Pleuronectiformes							
Bothidae							
Bothus cf. *pantherinus* [BOLD:AAC9155]	BOLD:AAC9155	**13**	>1		Y	A	**1**
Crossorhombus valderostratus	BOLD:AAF8808	**1**	1	1			**1**

Table 2. *Cont.*

Class		*COI*					*12S*
Order							
Family							
Species	**BIN**	**N *COI* in MADFI**	**Nsp./BIN in BOLD**	**NBIN/Sp. In BOLD**	**Sp. Complex**	**Allo./Symp.**	**N *12S* in MADFI**
Engyprosopon [BOLD:AD1616]	BOLD:ADI1616	**5**	NA				**1**
Engyprosopon [BOLD:ADM7915]	BOLD:ADM7915	**3**	NA				**1**
Engyprosopon [BOLD:AFI7142]	BOLD:AFI7142	**1**	new				**0**
Cynoglossidae							
Cynoglossus [BOLD:AFB7676]	BOLD:AFB7676	**1**	new				**1**
Soleidae							
Pardachirus marmoratus	BOLD:AAI5984	**1**	1	1			**0**
Scombriformes							
Nomeidae							
Psenes cyanophrys	BOLD:AAE0701	**1**	>1				**1**
Scombridae							
Auxis thazard	BOLD:AAB0928	**1**	>1				**1**
Rastrelliger kanagurta	BOLD:AAA9666	**1**	>1				**1**
Trichiuridae							
Trichiurus cf. *lepturus* [BOLD:AAB0165]	BOLD:AAB0165	**2**	>1		Y	A	**2**
Scorpaeniformes							
Platycephalidae							
Papilloculiceps longiceps	BOLD:AAI6160	**20**	1	>1			**2**
Platycephalus [BOLD:AAB2371]	BOLD:AAB2371	**5**	1	>1			**1**
Rogadius cf. *pristiger* [BOLD:ACY6212]	BOLD:ACY6212	**10**	1	>1	Y	A	**1**
Sunagocia otaitensis	BOLD:AAD2134	**1**	1	1			**1**
Scorpaenidae							
Ablabys binotatus	BOLD:AAF8834	**2**	1	1			**0**
Dendrochirus cf. *brachypterus* [BOLD:AAC9564]	BOLD:AAC9564	**6**	1	>1	Y	A	**1**
Parascorpaena [BOLD:AAU1214]	BOLD:AAU1214	**7**	NA				**1**
Pterois miles	BOLD:AAB8146	**5**	>1				**2**
Scorpaenopsis cf. *possi* [BOLD:AAD4142]	BOLD:AAD4142	**1**	1	>1	Y	A	**1**
Scorpaenopsis diabolus	BOLD:AAE1598	**3**	1	1			**1**
Scorpaenopsis longispina	BOLD:AAD7048	**2**	>1				**1**
Scorpaenopsis venosa	BOLD:AAD9168	**3**	1	1			**1**
Sebastapistes cf. *strongia* [BOLD:AAC4542]	BOLD:AAC4542	**14**	1	>1	Y	S	**1**
Sebastapistes cf. *strongia* [BOLD:AAC4543]	BOLD:AAC4543	**41**	1	>1	Y	S	**1**
Synanceia verrucosa	BOLD:AAE2821	**3**	>1				**1**
Siluriformes							
Plotosidae							
Plotosus cf. *lineatus* [BOLD:ACF3921]	BOLD:ACF3921	**8**	1	>1	Y	A	**2**
Spariformes							
Lethrinidae							
Lethrinus		**0**					**1**
Gnathodentex aureolineatus	BOLD:AAC8927	**1**	1	1			**1**
Gymnocranius cf. *microdon* [BOLD:AAB5193]	BOLD:AAB5193	**1**	1	>1	Y	A	**1**
Gymnocranius elongatus	BOLD:AAB5194	**3**	>1				**1**
Lethrinus [BOLD:AAC1547]	BOLD:AAC1547	**5**	NA				**2**
Lethrinus [BOLD:AAC8078]	BOLD:AAC8078	**19**	>1				**1**
Lethrinus borbonicus	BOLD:AAB0511	**50**	>1				**3**
Lethrinus cf. *lentjan* [BOLD:ABZ0131]	BOLD:ABZ0131	**11**	>1		Y	A	**1**
Lethrinus cf. *nebulosus* [BOLD:ABY6363]	BOLD:ABY6363	**12**	1	>1	Y	A	**0**
Lethrinus harak	BOLD:AAC1521	**34**	1	>1	Y	A	**2**
Lethrinus mahsena	BOLD:AAB6438	**24**	>1				**1**
Lethrinus obsoletus	BOLD:AAC1522	**1**	>1				**1**
Lethrinus rubrioperculatus	BOLD:AAB6439	**2**	>1				**1**
Lethrinus xanthochilus	BOLD:AAC1546	**4**	1	>1			**1**
Monotaxis grandoculis	BOLD:ABZ0166	**1**	1	>1	Y	A	**1**
Lobotidae							
Lobotes surinamensis	BOLD:AAC1878	**1**	1	1			**0**
Nemipteridae							
Nemipterus bipunctatus	BOLD:AAF2507	**1**	>1				**1**

Table 2. *Cont.*

Class		*COI*					*12S*
Order							
Family							
Species	**BIN**	**N *COI* in MADFI**	**Nsp./BIN in BOLD**	**NBIN/Sp. In BOLD**	**Sp. Complex**	**Allo./Symp.**	**N *12S* in MADFI**
Nemipterus elaine	BOLD:AEH3406	**2**	NA				**1**
Nemipterus zysron	BOLD:AAD1868	**1**	>1				**0**
Scolopsis [BOLD:AAC3574]	BOLD:AAC3574	**1**	>1				**0**
Scolopsis bimaculata	BOLD:AAD6249	**4**	1	1			**0**
Scolopsis ghanam	BOLD:AAC4767	**5**	1	1			**0**
Sillaginidae							
Sillago cf. *sihama* [BOLD:AAA7598]	BOLD:AAA7598	**1**	1	>1	Y	A	**1**
Sparidae							
Argyrops spinifer	BOLD:AAB3720	**1**	1	>1			**1**
Crenidens cf. *crenidens* [BOLD:AAE4408]	BOLD:AAE4408	**4**	1	>1	Y	A	**1**
Syngnathiformes							
Aulostomidae							
Aulostomus chinensis	BOLD:AAB9194	**1**	1	1			**0**
Callionymidae							
Callionymus [BOLD:AAI6959]	BOLD:AAI6959	**2**	>1				**1**
Callionymus [BOLD:ADN1799]	BOLD:ADN1799	**2**	NA				**1**
Callionymus cf. *filamentosus* [BOLD:ACZ1215]	BOLD:ACZ1215	**4**	1	>1	Y	A	**1**
Callionymus		**0**					**2**
Diplogrammus infulatus	BOLD:AAN1422	**4**	1	1			**2**
Centriscidae							
Aeoliscus punctulatus	BOLD:AAD3585	**4**	1	1			**1**
Dactylopteridae							
Dactyloptena orientalis	BOLD:AAB5966	**9**	>1				**1**
Fistulariidae							
Fistularia commersonii	BOLD:AAB5992	**7**	1	1			**1**
Pegasidae							
Eurypegasus draconis		**0**					**1**
Solenostomidae							
Solenostomus cf. *cyanopterus* [BOLD:ACG8874]	BOLD:ACG8874	**4**	1	>1	Y	A	**1**
Syngnathidae							
Acentronura [BOLD:ADI1740]	BOLD:ADI1740	**7**	NA				**1**
Corythoichthys cf. *conspicillatus* [BOLD:AAI8860]	BOLD:AAI8860	**7**	>1		Y	A	**1**
Corythoichthys haematopterus	BOLD:AAF0628	**8**	>1				**2**
Hippichthys [BOLD:AAE5342]	BOLD:AAE5342	**5**	>1				**2**
Hippichthys cf. *cyanospilos* [BOLD:AAK6079]	BOLD:AAK6079	**11**	1	>1	Y	A	**3**
Hippocampus [BOLD:ACE6993]	BOLD:ACE6993	**8**	>1				**1**
Hippocampus camelopardalis	BOLD:AAX0875	**20**	1	1			**3**
Hippocampus cf. *histrix* [BOLD:AAE5356]	BOLD:AAE5356	**1**	1	>1	Y	A	**1**
Syngnathidae [BOLD:AEF4074]	BOLD:AEF4074	**1**	new				**0**
Syngnathoides biaculeatus	BOLD:AAD7359	**2**	>1				**3**
Trachyrhamphus cf. *bicoarctatus* [BOLD:ADG5410]	BOLD:ADG5410	**2**	NA		Y	A	**1**
Tetraodontiformes							
Balistidae							
Balistapus undulatus	BOLD:AAC2755	**2**	1	1			**2**
Balistoides viridescens	BOLD:AAD0474	**8**	>1				**1**
Pseudobalistes fuscus	BOLD:ACZ6203	**5**	1	1			**1**
Rhinecanthus aculeatus	BOLD:AAB6992	**2**	1	1			**1**
Rhinecanthus rectangulus	BOLD:AAD6918	**1**	1	1			**1**
Sufflamen chrysopterum	BOLD:AAB1339	**3**	>1				**1**
Diodontidae							
Lophodiodon calori	BOLD:AEW6267	**1**	new	NA			**0**
Monacanthidae							
Amanses cf. *scopas* [BOLD:ADX8121]	BOLD:ADX8121	**1**	1	>1	Y	A	**1**
Cantherhines pardalis	BOLD:AAB9564	**4**	>1				**1**
Paraluteres prionurus	BOLD:AAC3185	**1**	1				**1**
Paramonacanthus frenatus	BOLD:AAI6357	**14**	1	1			**1**

Table 2. *Cont.*

Class		*COI*					*12S*
Order							
Family							
Species	**BIN**	**N *COI* in MADFI**	**Nsp./BIN in BOLD**	**NBIN/Sp. In BOLD**	**Sp. Complex**	**Allo./Symp.**	**N *12S* in MADFI**
Paramonacanthus pusillus	BOLD:AAV6227	**2**	1	>1	Y	A	**1**
Pervagor melanocephalus	BOLD:AAD6159	**2**	>1				**1**
Pseudalutarius cf. *nasicornis* [BOLD:AAI4531]	BOLD:AAI4531	**9**	>1		Y	A	**0**
Ostraciidae							
Lactoria cornuta	BOLD:AAB2988	**8**	1	1			**1**
Lactoria fornasini	BOLD:AAF2668	**2**	>1				**1**
Ostracion cubicus	BOLD:AAC2246	**5**	>1				**1**
Tetrosomus concatenatus	BOLD:AAD3999	**1**	>1				**1**
Tetraodontidae							
Arothron hispidus	BOLD:AAB9202	**18**	>1				**3**
Arothron immaculatus	BOLD:AAD2857	**2**	>1				**1**
Arothron stellatus	BOLD:AAC8066	**2**	1	1			**1**
Canthigaster bennetti	BOLD:AAC6218	**3**	1	1			**2**
Canthigaster petersii	BOLD:AAD2222	**7**	>1				**1**
Canthigaster punctatissima	BOLD:ACC1190	**2**	>1				**1**
Canthigaster valentini	BOLD:AAC9721	**1**	1	1			**0**
Lagocephalus [BOLD:AAC5565]	BOLD:AAC5565	**1**	>1				**1**
Torquigener flavimaculosus	BOLD:AAG3690	**5**	>1				**1**
Torquigener hypselogeneion	BOLD:ADC5749	**1**	1	1			**0**
Trachiniformes							
Pinguipedidae							
Parapercis hexophtalma	BOLD:AAD6753	**3**	1	1			**1**
Parapercis maculata	BOLD:AAE3727	**3**	1	>1			**2**
Trichonotidae							
Trichonotus [BOLD:ACG8296]	BOLD:ACG8296	**1**	NA				**0**
Uranoscopidae							
Uranoscopus guttatus	BOLD:ACX9882	**1**	NA				**1**
Elasmobranchii							
Myliobatiformes							
Dasyatidae							
Neotrygon indica	BOLD:AAA5611	**2**	>1				**1**
Pristiformes							
Rhinobatidae							
Acroteriobatus andysabini	BOLD:AAG4398	**4**	>1				**2**
Torpediniformes							
Torpedinidae							
Torpedo [BOLD:AAU1234]	BOLD:AAU1234	**3**	1	>1			**1**
Total		**2146**					**524**

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d17070495/s1, Table S1: Detailed information on all fish BINs from Madagascar in the MADFI database on the BOLD system. Information presented: the names associated with each BIN in BOLD, the number of BINs linked to a single species name (in cases where BINs are assigned to only one species name). The type localities of the BINs according to the Catalog of Fishes, the hypothetical species name assigned after morphological identification, the final species name attributed to each BIN, as well as comments regarding each BIN and its level of identification. The numbers in brackets in column E indicate the number of specimens available in BOLD for each species associated with each BIN listed in the MADFI database. The numbers in brackets in column F indicate the number of BINs corresponding to the species name when only one species name is in column E. Table S2: Details of the main characteristics of the 424 fish species validated by WoRMS and the 502 BINs recorded in the MADFI container on BOLD. The information presented includes: taxonomic novelty, biogeographic distribution of the recorded species,

species status according to the IUCN Red List, and the distribution of species based on their habitats and ecological lifestyles, according to WoRMS (World Register of Marine Species) data.

Author Contributions: Project leader: J.-D.D.; Conceptualization, J.J.A.V., J.-D.D., D.R. and D.P.; Development of the data collection protocol: J.J.A.V., J.-D.D., D.P., M.L., D.R., A.C., H.J., F.B., S.R. and A.E.M.F.; Data collection: J.J.A.V., A.C., H.J., F.B., R.M.R., S.R., J.M. (Jovial Mbony) and A.E.M.F.; Preparation of sample export permits: J.J.A.V., J.-D.D., D.P., A.C., H.J., S.R., A.E.M.F., J.M. (Jamal Mahafina) and G.T.; Molecular laboratory analyses: J.J.A.V., J.-D.D., F.R., H.J. and A.C.; Data analyses: J.J.A.V., J.-D.D. and E.R. Writing—original draft preparation: J.J.A.V. and J.-D.D.; Writing—review and editing: J.J.A.V., J.-D.D., E.R., D.P., A.H., M.L., H.J., A.C. and S.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded through the JEAI-ACOM/IH.SM/IRD grant and the International Laboratory (LMI) MIKAROKA, funded by Institut de Recherche pour le Développement (IRD), France. Additional support came from the Belmont Forum by the ARMSRestore project in Madagascar via the National Science Foundation (RISE-2022717 to AH), the South African National Research Foundation (BF-CRA 12854 to GT), Svenska FORMAS (2019-02394), the Rose Service Learning Fellowship at Harvard University, Good Planet Foundation. The University of Montpellier through the MARBEC laboratory and the Regional Cooperation Fund (CALMA project) also contributed financial support. The COMAD, RFSIO, and IFBIO projects were funded by the Critical Ecosystem Partnership Fund (grant MG 66341), the Rufford Foundation (grant 2ac29a-1), the Western Indian Ocean Marine Science Association (WIOMSA, MARG II contract 1/2016), and the European Union (grant POE 2.10 POCT FED–FEDER "Biodiversity of the Indian Ocean").

Institutional Review Board Statement: The specimens analyzed in this study were fin clips from dead fish recovered from artisanal fisheries' catches (in the areas of Toliara Bay, Ranobe, and the Anakao reef), in accordance with the international Nagoya Protocol, under the following approval codes: ABSCH-CPC-FR-264975-1 and ABSCH-CPC-FR-265446-1.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the BOLD (Barcode of Life Data System) database and will be submitted to GenBank.

Acknowledgments: This study was carried out under a doctoral fellowship from the ARTS program (IRD), within the framework of a research partnership between IH.SM, the MIKAROKA international laboratory, and the MARBEC laboratory at the University of Montpellier (France). We gratefully acknowledge the Belmont Forum for its financial support, which facilitated tissue sample collection and the preparation of export permits as part of the ARMSRestore project. Special thanks go to the administrative staff of IH.SM (Institut Halieutique et des Sciences Marines) for their assistance with export documentation, as well as to the staff of Madagascar's DREDD (Direction Régionale de l'Environnement et du Développement Durable) and DAPRNE (Direction des Aires Protégées, des Ressources Naturelles Renouvelables et des Écosystèmes) for their collaboration. We are especially grateful to Léa Jeanine RAVAOARISOA, former principal secretary of IH.SM, for her invaluable support in preparing these documents. We warmly thank all the technicians from IH.SM and the NGO Reef Doctor for their dedicated assistance in the field, in the laboratory, and during data collection. Our sincere thanks also go to the local authorities of each village involved in the study, and to the local assistants who facilitated communication and cooperation with the fishing communities. Finally, we express our deepest gratitude to the fishers for their generous welcome, availability, and active participation, without whom this research would not have been possible.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Béné, C.; Macfadayen, G.; Allison, E.H. *Increasing the Contribution of Small-Scale Fisheries to Poverty Alleviation and Food Security*; FAO: Rome, Italy, 2007; p. 141.
2. Pomeroy, R.; Andrew, N. *Small-Scale Fisheries Management: Frameworks and Approaches for the Developing World*; CABI: Oxford, UK, 2011. [CrossRef]
3. Barnes-Mauthe, M.; Oleson, K.L.L.; Zafindrasilivonona, B. The Total Economic Value of Small-Scale Fisheries with a Characterization of Post-Landing Trends: An Application in Madagascar with Global Relevance. *Fish. Res.* **2013**, *147*, 175–185. [CrossRef]
4. Mahafina, J. Perception et comportement des pêcheurs pour une gestion durable de la biodiversité et de la pêcherie récifale: Application au niveau des réserves marines temporaires du Sud Ouest de Madagascar. Ph.D. Thesis, Thèse en co-tutelle. Université de La Réunion, France et Université de Toliara, Madagascar, Toliara, Madagascar, 2011.
5. Vasseur, P. Écosystèmes Côtiers En Danger Dans La Région de Tuléar. Analyse Des Agressions Humaines et Problèmes de Gestion. In *Milieux et Sociétés Dans Le Sud-Ouest de Madagascar*; Îles et Archipels, 23; Centre de Recherches sur les Espaces Tropicaux (Université Michel de Montaigne, Bordeaux 3): Talence, France, 1997; pp. 97–120.
6. Behivoke, F. Caractérisation spatio-temporelle de la pêche aux poissons récifo-lagonaires par trajectométrie dans la baie de Toliara (sud-ouest de Madagascar). Ph.D. Thesis, Université de Toliara, Toliara, Madagascar, 2022.
7. Ranaivomanana, H.S.; Jaquemet, S.; Ponton, D.; Behivoke, F.; Randriatsara, R.M.; Mahafina, J.; Léopold, M. Intense Pressure on Small and Juvenile Coral Reef Fishes Threatens Fishery Production in Madagascar. *Fish. Manag. Ecol.* **2023**, *30*, 494–506. [CrossRef]
8. Tsoupas, A.; Papavasileiou, S.; Minoudi, S.; Gkagkavouzis, K.; Petriki, O.; Bobori, D.; Sapounidis, A.; Koutrakis, E.; Leonardos, I.; Karaiskou, N.; et al. DNA Barcoding Identification of Greek Freshwater Fishes. *PLoS ONE* **2022**, *17*, e0263118. [CrossRef] [PubMed]
9. Jacquemot, L.; Hunt, B.P.V.; Li, S.; Schulze, A.D.; Deeg, C.M.; Sutherland, B.J.G.; Tabata, A.; Lovejoy, C.; Miller, K.M. Mapping Biodiversity Coast-to-Coast-to-Coast Across Canada's Three Oceans Using eDNA Metabarcoding. *Environ. DNA* **2024**, *6*, e70028. [CrossRef]
10. Sahu, A.; Singh, M.; Amin, A.; Malik, M.M.; Qadri, S.N.; Abubakr, A.; Teja, S.S.; Dar, S.A.; Ahmad, I. A Systematic Review on Environmental DNA (eDNA) Science: An Eco-Friendly Survey Method for Conservation and Restoration of Fragile Ecosystems. *Ecol. Indic.* **2025**, *173*, 113441. [CrossRef]
11. Wang, S.; Yan, Z.; Hänfling, B.; Zheng, X.; Wang, P.; Fan, J.; Li, J. Methodology of Fish eDNA and Its Applications in Ecology and Environment. *Sci. Total Environ.* **2021**, *755*, 142622. [CrossRef] [PubMed]
12. Barnes, M.A.; Turner, C.R. The Ecology of Environmental DNA and Implications for Conservation Genetics. *Conserv. Genet.* **2016**, *17*, 1–17. [CrossRef]
13. DiBattista, J.D.; Coker, D.J.; Sinclair-Taylor, T.H.; Stat, M.; Berumen, M.L.; Bunce, M. Assessing the Utility of eDNA as a Tool to Survey Reef-Fish Communities in the Red Sea. *Coral Reefs* **2017**, *36*, 1245–1252. [CrossRef]
14. West, K.; Travers, M.J.; Stat, M.; Harvey, E.S.; Richards, Z.T.; DiBattista, J.D.; Newman, S.J.; Harry, A.; Skepper, C.L.; Heydenrych, M.; et al. Large-scale eDNA Metabarcoding Survey Reveals Marine Biogeographic Break and Transitions over Tropical Northwestern Australia. *Divers. Distrib.* **2021**, *27*, 1942–1957. [CrossRef]
15. Cantera, I.; Cilleros, K.; Valentini, A.; Cerdan, A.; Dejean, T.; Iribar, A.; Taberlet, P.; Vigouroux, R.; Brosse, S. Optimizing Environmental DNA Sampling Effort for Fish Inventories in Tropical Streams and Rivers. *Sci. Rep.* **2019**, *9*, 3085. [CrossRef]
16. Fediajevaite, J.; Priestley, V.; Arnold, R.; Savolainen, V. Meta-analysis Shows That Environmental DNA Outperforms Traditional Surveys, but Warrants Better Reporting Standards. *Ecol. Evol.* **2021**, *11*, 4803–4815. [CrossRef]
17. Watson, D.L.; Harvey, E.S.; Anderson, M.J.; Kendrick, G.A. A Comparison of Temperate Reef Fish Assemblages Recorded by Three Underwater Stereo-Video Techniques. *Mar. Biol.* **2005**, *148*, 415–425. [CrossRef]
18. Marques, V.; Milhau, T.; Albouy, C.; Dejean, T.; Manel, S.; Mouillot, D.; Juhel, J. GAPeDNA: Assessing and Mapping Global Species Gaps in Genetic Databases for eDNA Metabarcoding. *Divers. Distrib.* **2021**, *27*, 1880–1892. [CrossRef]
19. Andújar, C.; Arribas, P.; Yu, D.W.; Vogler, A.P.; Emerson, B.C. Why the COI Barcode Should Be the Community DNA Metabarcode for the Metazoa. *Mol. Ecol.* **2018**, *27*, 3968–3975. [CrossRef] [PubMed]
20. Deagle, B.E.; Jarman, S.N.; Coissac, E.; Pompanon, F.; Taberlet, P. DNA Metabarcoding and the Cytochrome c Oxidase Subunit I Marker: Not a Perfect Match. *Biol. Lett.* **2014**, *10*, 20140562. [CrossRef]
21. Thomsen, P.F.; Willerslev, E. Environmental DNA—An Emerging Tool in Conservation for Monitoring Past and Present Biodiversity. *Biol. Conserv.* **2015**, *183*, 4–18. [CrossRef]
22. Zhang, S.; Zhao, J.; Yao, M. A Comprehensive and Comparative Evaluation of Primers for Metabarcoding eDNA from Fish. *Methods Ecol. Evol.* **2020**, *11*, 1609–1625. [CrossRef]
23. Collins, R.A.; Bakker, J.; Wangensteen, O.S.; Soto, A.Z.; Corrigan, L.; Sims, D.W.; Genner, M.J.; Mariani, S. Non-specific Amplification Compromises Environmental DNA Metabarcoding with COI. *Methods Ecol. Evol.* **2019**, *10*, 1985–2001. [CrossRef]

24. Miya, M.; Sato, Y.; Fukunaga, T.; Sado, T.; Poulsen, J.Y.; Sato, K.; Minamoto, T.; Yamamoto, S.; Yamanaka, H.; Araki, H.; et al. MiFish, a Set of Universal PCR Primers for Metabarcoding Environmental DNA from Fishes: Detection of More than 230 Subtropical Marine Species. *R. Soc. Open Sci.* **2015**, *2*, 150088. [CrossRef]
25. Chavez, F.; Min, M.; Pitz, K.; Truelove, N.; Baker, J.; LaScala-Grunewald, D.; Blum, M.; Walz, K.; Nye, C.; Djurhuus, A.; et al. Observing Life in the Sea Using Environmental DNA. *Oceanography* **2021**, *34*, 102–119. [CrossRef]
26. Jaonalison, H.; Durand, J.-D.; Mahafina, J.; Valade, P.; Collet, A.; Cerqueira, F.; Ponton, D. Application of DNA Barcoding for Monitoring Madagascar Fish Biodiversity in Coastal Areas. *Diversity* **2022**, *14*, 377. [CrossRef]
27. Vences, M.; Stützer, D.; Rasoamampionona Raminosoa, N.; Ziegler, T. Towards a DNA Barcode Library for Madagascar's Threatened Ichthyofauna. *PLoS ONE* **2022**, *17*, e0271400. [CrossRef] [PubMed]
28. Fricke, R.; Mahafina, J.; Behivoke, F.; Jaonalison, H.; Léopold, M. Annotated Checklist of the Fishes of Madagascar, Southwestern Indian Ocean, with 158 New Records. *Fish Taxa* **2018**, *3*, 1–432.
29. Blackman, R.C.; Walser, J.; Rüber, L.; Brantschen, J.; Villalba, S.; Brodersen, J.; Seehausen, O.; Altermatt, F. General Principles for Assignments of Communities from EDNA: Open versus Closed Taxonomic Databases. *Environ. DNA* **2023**, *5*, 326–342. [CrossRef]
30. Pentinsaari, M.; Ratnasingham, S.; Miller, S.E.; Hebert, P.D.N. BOLD and GenBank Revisited—Do Identification Errors Arise in the Lab or in the Sequence Libraries? *PLoS ONE* **2020**, *15*, e0231814. [CrossRef]
31. Collet, A.; Ponton, D.; Salager, B.; Yven, C.; Pierre, V. *SLEEP COLOR Développement et Test d'un Système Lumineux Electronique Evolutif (Multi Spectral) Pour l'échantillonnage Des Post-Larves et Juvéniles de Poissons de Récifs. Rapport de Phase 3: Calibration Spatio-Temporelle de La Stratégie d'échantillonnage*; Organisme Consultant en Environnement Aquatique: Saint Pierre, La Réunion, 2022. [CrossRef]
32. Collet, A.; Durand, J.-D.; Desmarais, E.; Cerqueira, F.; Cantinelli, T.; Valade, P.; Ponton, D. DNA Barcoding Post-Larvae Can Improve the Knowledge about Fish Biodiversity: An Example from La Reunion, SW Indian Ocean. *Mitochondrial DNA Part A* **2018**, *29*, 905–918. [CrossRef]
33. Ward, R.D.; Zemlak, T.S.; Innes, B.H.; Last, P.R.; Hebert, P.D.N. DNA Barcoding Australia's Fish Species. *Philos. Trans. R. Soc. B Biol. Sci.* **2005**, *360*, 1847–1857. [CrossRef]
34. Xia, R.; Durand, J.-D.; Fu, C. Multilocus Resolution of Mugilidae Phylogeny (Teleostei: Mugiliformes): Implications for the Family's Taxonomy. *Mol. Phylogenetics Evol.* **2016**, *96*, 161–177. [CrossRef]
35. Valentini, A.; Taberlet, P.; Miaud, C.; Civade, R.; Herder, J.; Thomsen, P.F.; Bellemain, E.; Besnard, A.; Coissac, E.; Boyer, F.; et al. Next-generation Monitoring of Aquatic Biodiversity Using Environmental DNA Metabarcoding. *Mol. Ecol.* **2016**, *25*, 929–942. [CrossRef]
36. Pham, M.H.; Hoang, D.H.; Panfili, J.; Ponton, D.; Durand, J.-D. Diversity of Fishes Collected with Light Traps in the Oldest Marine Protected Area in Vietnam Revealed by DNA Barcoding. *Mar. Biodivers.* **2022**, *52*, 30. [CrossRef]
37. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870–1874. [CrossRef] [PubMed]
38. Ratnasingham, S.; Hebert, P.D.N. A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System. *PLoS ONE* **2013**, *8*, e66213. [CrossRef]
39. Fricke, R.; Eschmeyer, W.N.; Van-Der-Laan, R. Eschmeyer's Catalog of Fishes: Genera, Species, References. 2025. Available online: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp (accessed on 15 April 2025).
40. Ruiz, E.; Lamy, T.; Mouillot, D.; Durand, J.-D. Not One Threshold to Rule Them All! Benchmarking the Taxonomic Resolution of Fish eDNA Metabarcodes Against COI Barcodes. *Mol. Ecol. Resour.* 2025, *submitted*.
41. Rognes, T.; Flouri, T.; Nichols, B.; Quince, C.; Mahé, F. VSEARCH: A Versatile Open Source Tool for Metagenomics. *PeerJ* **2016**, *4*, e2584. [CrossRef] [PubMed]
42. Laroche, J.; Ramananarivo, N. A Preliminary Survey of the Artisanal Fishery on Coral Reefs of the Tulear Region (Southwest Madagascar). *Coral Reefs* **1995**, *14*, 193–200. [CrossRef]
43. Laroche, J.; Razanoelisoa, J.; Fauroux, E.; Rabenevanana, M.W. The Reef Fisheries Surrounding the South-west Coastal Cities of Madagascar. *Fish. Manag. Ecol.* **1997**, *4*, 285–299. [CrossRef]
44. Attingli, A.H.; Ahouansou Montcho, S.; Vissin, E.W.; Zinsou, L.H.; Laleye, P.A. Influence des engins et techniques de pêche sur l'abondance relative des espèces dans la Basse Vallée de l'Ouémé au Bénin. *Afr. Crop Sci. J.* **2017**, *25*, 47. [CrossRef]
45. Zemlak, T.S.; Ward, R.D.; Connell, A.D.; Holmes, B.H.; Hebert, P.D.N. DNA Barcoding Reveals Overlooked Marine Fishes. *Mol. Ecol. Resour.* **2009**, *9*, 237–242. [CrossRef]
46. Cawthorn, D.-M.; Steinman, H.A.; Witthuhn, R.C. Evaluation of the 16S and 12S rRNA Genes as Universal Markers for the Identification of Commercial Fish Species in South Africa. *Gene* **2012**, *491*, 40–48. [CrossRef]
47. Steinke, D.; Connell, A.D.; Hebert, P.D.N. Linking Adults and Immatures of South African Marine Fishes. *Genome* **2016**, *59*, 959–967. [CrossRef]

48. Muhala, V.; Guimarães-Costa, A.; Macate, I.E.; Rabelo, L.P.; Bessa-Silva, A.R.; Watanabe, L.; Dos Santos, G.D.; Sambora, L.; Vallinoto, M.; Sampaio, I. DNA Barcoding for the Assessment of Marine and Coastal Fish Diversity from the Coast of Mozambique. *PLoS ONE* **2024**, *19*, e0293345. [CrossRef]
49. Coker, D.J.; DiBattista, J.D.; Sinclair-Taylor, T.H.; Berumen, M.L. Spatial Patterns of Cryptobenthic Coral-Reef Fishes in the Red Sea. *Coral Reefs* **2018**, *37*, 193–199. [CrossRef]
50. Asgharian, H.; Sahafi, H.H.; Ardalan, A.A.; Shekarriz, S.; Elahi, E. Cytochrome c Oxidase Subunit 1 Barcode Data of Fish of the Nayband National Park in the Persian Gulf and Analysis Using Meta-data Flag Several Cryptic Species. *Mol. Ecol. Resour.* **2011**, *11*, 461–472. [CrossRef] [PubMed]
51. Rabaoui, L.; Yacoubi, L.; Sanna, D.; Casu, M.; Scarpa, F.; Lin, Y.; Shen, K.; Clardy, T.R.; Arculeo, M.; Qurban, M.A. DNA Barcoding of Marine Fishes from Saudi Arabian Waters of the Gulf. *J. Fish Biol.* **2019**, *95*, 1286–1297. [CrossRef] [PubMed]
52. Duchene, D.; Klanten, S.O.; Munday, P.L.; Herler, J.; Van Herwerden, L. Phylogenetic Evidence for Recent Diversification of Obligate Coral-Dwelling Gobies Compared with Their Host Corals. *Mol. Phylogenet. Evol.* **2013**, *69*, 123–132. [CrossRef] [PubMed]
53. Winterbottom, R. Trimma Tevegae and T. Caudomaculatum Revisited and Redescribed (Acanthopterygii, Gobiidae), with Descriptions of Three New Similar Species from the Western Pacific. *Zootaxa* **2016**, *4144*, 1–53. [CrossRef]
54. Troyer, E.M.; Coker, D.J.; Berumen, M.L. Comparison of Cryptobenthic Reef Fish Communities among Microhabitats in the Red Sea. *PeerJ* **2018**, *6*, e5014. [CrossRef]
55. Tornabene, L.; Ahmadia, G.N.; Berumen, M.L.; Smith, D.J.; Jompa, J.; Pezold, F. Evolution of Microhabitat Association and Morphology in a Diverse Group of Cryptobenthic Coral Reef Fishes (Teleostei: Gobiidae: Eviota). *Mol. Phylogenet. Evol.* **2013**, *66*, 391–400. [CrossRef]
56. Luehrmann, M.; Cortesi, F.; Cheney, K.L.; De Busserolles, F.; Marshall, N.J. Microhabitat Partitioning Correlates with Opsin Gene Expression in Coral Reef Cardinalfishes (Apogonidae). *Funct. Ecol.* **2020**, *34*, 1041–1052. [CrossRef]
57. Doll, P.; Munday, P.; Bonin, M.; Jones, G. Habitat Specialisation and Overlap in Coral Reef Gobies of the Genus Eviota (Teleostei: Gobiidae). *Mar. Ecol. Prog. Ser.* **2021**, *677*, 81–94. [CrossRef]
58. Hubert, N.; Meyer, C.P.; Bruggemann, H.J.; Guérin, F.; Komeno, R.J.L.; Espiau, B.; Causse, R.; Williams, J.T.; Planes, S. Cryptic Diversity in Indo-Pacific Coral-Reef Fishes Revealed by DNA-Barcoding Provides New Support to the Centre-of-Overlap Hypothesis. *PLoS ONE* **2012**, *7*, e28987. [CrossRef]
59. Hubert, N.; Dettai, A.; Pruvost, P.; Cruaud, C.; Kulbicki, M.; Myers, R.; Borsa, P. Geography and Life History Traits Account for the Accumulation of Cryptic Diversity among Indo-West Pacific Coral Reef Fishes. *Mar. Ecol. Prog. Ser.* **2017**, *583*, 179–193. [CrossRef]
60. Durand, J.-D.; Borsa, P. Mitochondrial Phylogeny of Grey Mullets (Acanthopterygii: Mugilidae) Suggests High Proportion of Cryptic Species. *C. R. Biol.* **2015**, *338*, 266–277. [CrossRef] [PubMed]
61. Collins, R.A.; Cruickshank, R.H. The Seven Deadly Sins of DNA Barcoding. *Mol. Ecol. Resour.* **2013**, *13*, 969–975. [CrossRef]
62. Hanner, R.; Becker, S.; Ivanova, N.V.; Steinke, D. FISH-BOL and Seafood Identification: Geographically Dispersed Case Studies Reveal Systemic Market Substitution across Canada. *Mitochondrial DNA* **2011**, *22*, 106–122. [CrossRef]
63. Li, F.; Zhang, Y.; Altermatt, F.; Zhang, X.; Cai, Y.; Yang, Z. Gap Analysis for DNA-Based Biomonitoring of Aquatic Ecosystems in China. *Ecol. Indic.* **2022**, *137*, 108732. [CrossRef]