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DNA barcoding post-larvae can improve the knowledge about fish biodiversity: an example from La Reunion, SW Indian Ocean

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ABSTRACT

The aim of this study was to demonstrate that fish larvae identified using their COI sequences offer a unique opportunity for improving the knowledge of local fish richness. Fish larvae were sampled at the end of their pelagic phase using light-traps set off the West Coast of La Reunion Island, southwestern Indian Ocean, once per month from October 2014 to March 2015. Among the 5174 larvae caught, 214 morphologically different specimens were selected, 196 successfully barcoded, giving a total of 101 different Barcode Index Numbers (BINs). Among these BINs, 55 had never been recorded in La Reunion exclusive economic zone (EEZ), and 13 were new for the BOLD database. Even if the sampling effort for collecting fish post-larvae during this study was relatively low, it allowed adding at least nine new species to an updated checklist of fishes of La Reunion EEZ.

Introduction

Most tropical marine reef fish species start their life as pelagic larvae that return to coastal habitats when they are about to settle. Catching larvae at the end of their pelagic phase thus offers a unique opportunity to assess, and/or survey, local fish biodiversity. Unfortunately, larvae strongly differ in morphology from the adults (Doherty 1991), and identifying them to the species level remains challenging (Ko et al. 2013; Leis 2015). The only guide of Indo-Pacific fish larvae (Leis and Carson-Ewart 2004) allows identification of early life stages mostly to the family level, more rarely to the genus level. Some more detailed guides exist, but they are often restricted to few species caught in specific geographic areas: Juncker (2007) for Wallis Islands, Maamaatuaiahutapu et al. (2006) for French Polynesia, IGREC Mer (2016) for French West Indies, Mwaluma et al. (2014) for Kenyan coastal waters, and Collet et al. (2013) for La Reunion. Moreover, these guides are, at the best, based on identification performed by rearing post-larvae until they reach a phenotype similar to adults; none of them are based on identification using molecular tools.

DNA barcoding, as proposed by Hebert et al. (2003), consists in identifying animals based on the sequence of their mitochondrial gene cytochrome c oxidase I (COI). DNA barcoding rapidly appeared to be a promising technique for identifying life stages of aquatic animals that are difficult to identify to the species level. This method proved its efficiency for identifying larvae of marine invertebrates for monitoring

invasive species (Harvey et al. 2009) and evaluating the biodiversity in MPA (Brandão et al. 2016). This method has also been used for identifying eggs (Burghart et al. 2014; Harada et al. 2015; Lewis et al. 2016), and larvae (Ko et al. 2013; Pappalardo et al. 2015; Ardura et al. 2016) of marine fish species, as well as tropical freshwater ones (Frantine-Silva et al. 2015; García-Dávila et al. 2015; Maggia et al. 2017). Studies of Hubert et al. (2010), Hubert et al. (2015), and Ayala et al. (2016) confirmed that DNA barcoding facilitates the identification of fish larvae at the species level, particularly for specimens that are difficult or impossible to identify morphologically. Ayala et al. (2016) even suggested that the higher species diversity they observed in the Sargasso Sea was partly due to the fact that larvae were identified using DNA barcoding. They estimated that without this technique, some individuals would have been identified at higher taxonomic levels, thus diminishing the estimates of species richness. For identifying fish larvae, a database with reliable reference COI sequences is required (Ardura et al. 2016). If barcode sequences of precisely identified adults are present in an accessible database, it becomes easy to give unknown larvae a Linnean taxonomic name (Hajibabaei et al. 2016). If the database is complete and accurate enough, DNA barcoding thus offers a fantastic opportunity to assess the local fish diversity by using the huge swarms the larvae form when they return to settle on coastal habitats.

In this context, the main aim of this study was to examine how fish larvae caught at the end of their pelagic stage, and

identified using DNA barcoding, could increase the knowledge of local fish diversity in La Reunion Island EEZ. This subtropical volcanic island in the Mascarene Archipelago, western Indian Ocean (WIO), is a place where fish biodiversity has been well studied (Letourneur et al. 2004; Fricke et al. 2009), but with only one study using DNA barcoding on adult fishes (Hubert et al. 2012). WIO is an interesting place for fish biodiversity studies as it is often considered as a secondary hotspot, especially in the area between Madagascar and the African east coastline (McClanahan 2015). Moreover, the number of cryptic species in the WIO may be much higher than previously thought (Zemlak et al. 2009), leading to incomplete local fish checklists thus to an underestimation of the local species diversity (Borsa et al. 2016). In order to reach our main aim, we had two preliminary objectives:

- verifying if the list of fish species present in La Reunion (Fricke et al. 2009) needed to be updated and assessing the barcoding effort made by the international scientific community on these fish species, and
- using the DNA barcoding approach for identifying specimens caught at the end of their pelagic stage (hereafter called 'post-larvae').

Materials and methods

Barcoding effort on fish species present in La Reunion exclusive economic zone (EEZ)

Before evaluating how many fish species present in La Reunion EEZ have already been barcoded, it appeared necessary to perform an update of the checklist established by Fricke et al. (2009). We thus used two more recent works: (a) the list of fish species caught and barcoded by Hubert et al. (2012) and (b) the list of fish species (Actinopterygii only) recorded as being present in La Reunion EEZ by the French Taxonomical Reference Tool, TAXREF v10.0 (Gargominy et al. 2016). All freshwater fish species present in the three lists were kept since all native freshwater fish species in La Reunion are diadromous (Teichert 2012) and individuals at the end of their larval stage are sometimes caught in coastal areas (Collet et al. unpublished data). The pertinence of each species' name present in Hubert et al. (2012) and/or TAXREF v10.0, and not present in the checklist established by Fricke et al. (2009), was assessed using the online version of the Catalog of Fishes (Eschmeyer et al. 2016) and the Global Biodiversity Information Facility (GBIF, <https://demo.gbif.org>), see supporting information Table S1 for more details. The final list of species present in La Reunion EEZ was finally matched to the fish species names present in the BOLD database (Ratnasingham and Hebert 2007, accessed 26 November 2016). BINs were recorded separately for COI sequences of specimens originating from La Reunion EEZ and of specimens caught elsewhere in the world.

Post-larvae sampling and processing in the lab

Sampling was conducted off the nearshore fringing reef of Saint Gilles, located on the west coast of La Reunion Island,

southWIO (Supporting information, Figure S1). This reef is part of a Marine Protected Area (MPA) created in 2007. Fish post-larvae were sampled monthly around the new moon from October 2014 to March 2015, the warm season being the period when post-larvae are the most abundant and diverse (Durville et al. 2002). Post-larvae were caught using light traps similar to the one described by Lecaillon (2004). At each of the six stations, one trap was attached to a buoy delimiting the perimeter of the MPA northern part (Supporting information, Figure S1). The water column depth varied between 30 and 60 m. All the traps were deployed before dusk and retrieved at sunrise. Post-larvae were kept alive in aerated seawater and returned immediately to the laboratory. All individuals were identified to the family level, sorted out by morphospecies, and counted.

For every morphospecies that had not been previously observed, an individual was euthanized in 0°C seawater, photographed, and a piece of fin was sampled, preserved in ethanol and stored at -20°C until processed for DNA extraction. When several individuals of the same morphospecies were present, the remaining individuals were placed in an aquarium in order to be grown. After some days, or weeks, juveniles grown in aquaria underwent the same treatment as post-larvae.

DNA extraction, amplification and sequencing

Samples were removed from alcohol and allowed for drying in individual well of a 2 ml deep-well block. Empty wells were kept as control for extraction contamination. Once dry, tissues were lysed and processed on a KingFisher Flex 96 (Thermo) using NucleoMag 96 Tissue kit (Macherey-Nagel). PCR was then performed including extraction controls as well as amplification controls using a cocktail of primers FishF1 and FishF2 in combination with FishR1 (Ward et al. 2005). The thermal program used consisted of an initial step of 5 min at 98°C followed by 35 cycles of 30 s at 94°C, 30 s at 57°C and 1 min at 72°C, followed by a final extension of 5 min at 72°C. After purification of the PCR products based on paramagnetic beads technology with the CleanPCR kit (Proteigene, Saint-Marcel, France), Sanger sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies, Carlsbad, CA). Electrophoregrams were obtained on an ABI3500 XL apparatus (Life Technologies, Carlsbad, CA). In order to lower the cost of analyses, bi-directional sequencing was not performed systematically. While all sequences were realized using the FishF1 primer, antisense sequences (using FishR1) were obtained for individuals presenting problematic sense sequences only.

Sequence analysis and specimens identification

Following Lewis et al. (2016), we used the BOLD system and its analytical tools for identifying species. COI sequences were uploaded to a dedicated BOLD database [BOLD public dataset DS-COLORRUN]. Each sequence >500 bp and without stop codon was assigned a Barcode Index Numbers (BINs, Ratnasingham and Hebert 2013) by a clustering algorithm

that generates operational taxonomic units (OTUs), and thus putative species. As the registry of BINs is integrated within the curated online BOLD database of specimen and taxonomic, each BIN should ideally correspond to one species' name only.

Species accumulation curves were calculated using the vegan R package (Oksanen et al. 2016) with R version 3.3.2 (R Core Team 2016).

Results

Barcoding effort on fish species present in La Reunion EEZ

When compared to the checklist established by Fricke et al. (2009), the fish species listed by Hubert et al. (2012), or present in TAXREF v10.0, added 104 species, 23 categorized as 'present', 26 as 'probably present', and 55 as 'possibly present' (see supplementary material Table S1 for explanations). Only 13 species were categorized as 'doubtful' (Supplementary material Table S1) and were thus removed from the subsequent analyses.

As a result, a total of 1028 fish species were considered as being present, probably present, or possibly present in La Reunion EEZ (Supplementary material Table S2). Among these 1028 fish species, 238 (23.2%) did not have a COI sequence in BOLD (Table 1). The COI sequences of 187 fish species from La Reunion, and of 790 fish species captured elsewhere in the world were assigned at least one BIN in BOLD (Figure 1 and Supplementary material Table S2). Some of these fish species were assigned to more than one BIN, three from La Reunion, 278 captured elsewhere in the world (Figure 1). Among them *Anguilla bicolor*, *Plotosus lineatus*, *Trachinocephalus myops*, *Decapterus macarellus/D. russelli*, *Trichiurus lepturus*, *Gerres filamentosus* and *Sillago sihama* were assigned to five BINs or more (Supplementary material Table S2). The complex of species identified as *Mugil cephalus* was even assigned to 15 different BINs (Supplementary material Table S2). Interestingly, 72 BINs included representatives of two or more species (Supplementary material Table S3). These BIN merges occurred especially among Carangidae (eight different BINs including representatives of two species or more), Holocentridae, Lutjanidae, Serranidae (five BINs), and Tetraodontidae (four BINs, supplementary material Table S3).

Post-larvae caught and identified

Over the six sampling campaigns, a total of 5174 larvae were caught. A total of 214 specimens were selected for DNA barcoding, 114 (53%) selected at the post-larval stage because they presented different morphology, 100 (47%) selected among the reared individuals. Only 18 (8.4%) failed to amplify: eight Blenniidae, one Antennariidae, one Apogonidae, one Labridae, and one Monacanthidae, one Pomacentridae, and one Scorpaenidae. The 196 successfully barcoded individuals (102 post-larvae and 94 individuals reared between 1 and 111 days) gave COI sequences between 558 and 663 bp (mean = 640, Supporting information, Figure S2). Once

uploaded to BOLD, the sequences were assigned to a total of 101 different BINs (Table 2). It is interesting to note that the number of BINs obtained during this study increased steadily along the six campaigns without reaching a plateau (Figure 2). Over the 101 BINs obtained, 55 (54.5%) had never been recorded for specimens caught in La Reunion EEZ, increasing by almost one-third the information already present for this island in the BOLD database (Supplementary material Table S2). New BINs for La Reunion EEZ belonged to all the families except Labridae (only one species was caught, Table 2), Kuhliidae (only one species caught), Cirrhitidae (1), Balistidae (2), and Monacanthidae (3). A total of 13 BINs were completely new for the BOLD database (Table 2). The new BINs for BOLD were more abundant for Blenniidae with four new BINs, and Apogonidae with three new BINs (Table 1). All the BINs obtained for Caesionidae, Carapidae, Scorpaenidae, and Tripterygiidae were new for the BOLD database (Table 2). Logically, most of the new BINs were associated with specimens identified to the genus ($N=8$), or the family ($N=4$) level only (Table 2). The only new BIN associated to a species was BOLD:ACV6523 corresponding to individuals identified by their morphology and colours as *Pomachromis richardsoni* (one post-larvae and one juvenile reared for 28 days).

The COI sequences already present in the BOLD database did not allow identifying specimens with the same levels of precision. For 80 BINs (79.2% of the total), identification was possible at the species level without ambiguity (ID category I in Table 2). These species mostly belonged to Pomacentridae ($N=23$), Holocentridae ($N=9$), and Apogonidae ($N=7$, Table 2). Four BINs corresponded to several species (ID category II, Table 2); as a consequence identification at the species level remained uncertain. Five BINs corresponded to specimens for which identification at the species level was just impossible (ID category III, Table 2). Problems for identifying specimens at the species level occurred mostly among Apogonidae, Caesionidae, Carapidae, Holocentridae, Mullidae, Scorpaenidae, and Synodontidae.

New species for La Reunion EEZ

Sampling post-larvae allowed to add nine, possibly 10 (see below), new species to the checklist of fishes of La Reunion EEZ. Four of these species were identified without ambiguity at the species level (Table 2): *Sargocentron praslin* (BIN BOLD:AAC4647), *Plectroglyphidodon leucozonus* (BIN BOLD:AAC6436), *Crossorhombus valderostratus* (BIN BOLD:AAF8808), and *Psenes cyanophrys* (BIN BOLD:AAE0701). Three other species, *Petrosirtes* sp. BOLD:ACW9505, *Stanulus* sp. BOLD:ACW8877, and Atherinidae BOLD:ACW9771 are also probably new species for La Reunion EEZ. Indeed, only one species of the genera *Petrosirtes* and *Stanulus*, or of the family Atherinidae, is present in La Reunion but with another BIN. *Petrosirtes mitratus* is assigned to BIN BOLD:AAE6131, *Stanulus seychellensis* to BIN BOLD:AAH9965, and *Atherinomorus lacunosus* to BINs BOLD:ACJ4684 and BOLD:ACK7521 (Supplementary material Table S2). Among Apogonidae, three BINs (BOLD:ACW8182, BOLD:ACW9156, or BOLD:ACW9154) may correspond to new species for La

Table 1. Percentages of species per fish family recorded in La Reunion EEZ that are associated to at least one BIN in BOLD.

Families	Total number of species not associated to a BIN	Total number of species associated to a BIN	Percentage of species associated to at least one BIN
ALEPISAUROIDAE, ATHERINIDAE, AULOSTOMIDAE, BELONIDAE, BERYCIDAE, BYTHITIDAE, CAPROIDAE, CHANIDAE, CHLOPSIDAE, CREEDIIDAE, CYPRINIDAE, DACTYLOPTERIDAE, DREPANIDAE, ECHENEIDAE, ELOPIDAE, EPHIPPIDAE, GERREIDAE, GONORYNCHIDAE, GRAMMICOLEPIDIDAE, HAEMULIDAE, ISTIOPHORIDAE, KUHUIDAE, KYPHOSIDAE, LAMPRIDAE, LOBOTIDAE, MALACANTHIDAE, MEGALOPIDAE, MENIDAE, MOLIDAE, MONOCENTRIDAE, MONODACTYLIDAE, MURAESOCIIDAE, NEOSCOPELIDAE, NOMEIDAE, PARALICHTHYIDAE, PENTACEROTIDAE, PHOSICHTHYIDAE, PLESIOPIDAE, PLEURONECTIDAE, PLOTOSIDAE, POECLIIDAE, POLYMIXIDAE, PRIACANTHIDAE, RACHYCENTRIDAE, SALMONIDAE, SCIAENIDAE, SCOMBRIDAE, SIGANIDAE, SILLAGINIDAE, SPARIDAE, SPHYRAENIDAE, STERNOPTYCHIDAE, SYNODONTIDAE, TRICHIURIDAE, XIPHIIDAE, ZANCLIDAE	0	141	100
APOGONIDAE, BALISTIDAE, CARANGIDAE, CHAETODONTIDAE, MONACANTHIDAE	5	116	99-90
ACANTHURIDAE, ANGUILLIDAE, ANTENNARIIDAE, BOTHIDAE, CAESIONIDAE, CALLIONYMIDAE, CICHLIDAE, CIRRHITIDAE, CLUPEIDAE, DIODONTIDAE, GONOSTOMATIDAE, HOLOCENTRIDAE, LABRIDAE, LETHRINIDAE, LUTJANIDAE, MULLIDAE, MYCTOPHIDAE, OSTRACIDAE, PLATYCEPHALIDAE, POMACANTHIDAE, POMACENTRIDAE, SCORPAENIDAE, SERRANIDAE, TETRAODONTIDAE	71	361	89-75
ACROPOMATIDAE, ALBULIDAE, AMBASSIDAE, BLENNIIDAE, BRAMIDAE, CONGRIDAE, CORYPHAENIDAE, ELEOTRIDAE, EPIGONIDAE, GEMPYLIDAE, GOBIIDAE, HEMIRAMPHIDAE, LOPHOTIDAE, MORINGUIDAE, MUGILIDAE, MURAEINIDAE, NEMIPTERIDAE, OGCOCEPHALIDAE, OSPHRONEMIDAE, PEMPHERIDAE, PERISTEDIDAE, PINGUIPEDIDAE, SCARIDAE, SERRIVOMERIDAE, SOLENOSTOMIDAE, SYNAPHOBRANCHIDAE, URANOSCOPIIDAE	89	155	74-50
ENGRAULIDAE, EXOCOETIDAE, LEIOGNATHIDAE, OPHICHTHIDAE, OPHIDIIDAE, SOLEIDAE, SYNGNATHIDAE, TRIPTERYGIIDAE	45	20	49-13
ANOMALOPIDAE, CARAPIDAE, CHAUNACIDAE, CLINIDAE, CYNOGLOSSIDAE, DERICHTHYIDAE, GOBIESOCIDAE, LOPHIIDAE, MACROURIDAE, MORIDAE, NEMICHTHYIDAE, PEGASIDAE, PSEUDOCROMIDAE, SAMARIDAE, SCOMBERESOCIDAE, STOMIDAE, SYMPHYSANODONTIDAE, TRACHICHTHYIDAE, TRACHTERIDAE, TRICHONOTIDAE, TRIGLIDAE, TRIODONTIDAE	28	0	0

See Table S2 for the complete list of species and BINs.

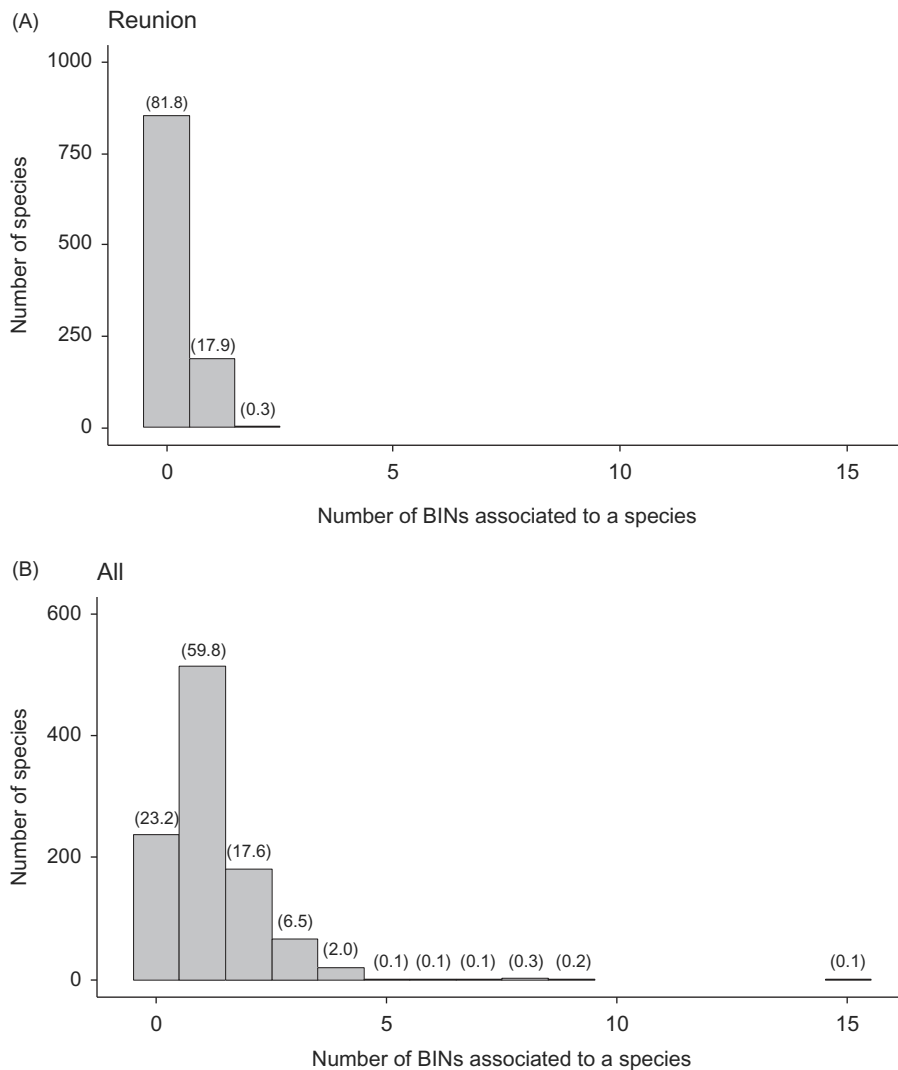


Figure 1. Numbers of BINs per species for (A) specimen caught in La Reunion, and (B) specimen caught anywhere. Heights of bars correspond to the number of species, numbers in brackets on their top indicate the corresponding percentage. See Table S2 for the complete list of species and BINs.

Reunion EEZ, but *Siphamia mossambica*, a species not yet barcoded, may be among them. Thus the number of new species of Apogonidae for La Reunion EEZ may be two or three.

Discussion

This present study first emphasizes the importance of (1) an up-to-date checklist of the species present in the studied area, (2) a database of COI sequences from unambiguously identified species. Several authors have stressed how a good database of COI sequences is important for identifying species using DNA barcodes (Goldstein and DeSalle 2011), especially when studying larval stages (García-Dávila et al. 2015). But to our knowledge, no study has focused on the fact that an up-to-date list of the species present in the studied area is also important. Indeed, comparing the identification performed using DNA barcoding to an up-to-date checklist avoid using names of species that are known to be restricted to totally different geographic areas. Such errors are sometime observed in scientific publications and, more problematically, these errors are transferred to international databases.

The updated checklist of fish species of La Reunion EEZ we built for this study is probably imperfect, as the status of several species still needs to be examined by specialists. Nevertheless, this list of species was helpful for verifying if the names associated with some COI sequences were pertinent or not. For example, in BOLD *Stegastes lividus* is assigned to BIN BOLD:AAC8561 but according to the Catalog of Fishes and GBIF, this species is present in Marquesas Islands only. The identification of the three specimens caught during this study and assigned to BIN BOLD:AAC8561 is thus more probably *S. punctatus*. This suggests that DNA barcoding will probably gain in efficiency if regularly updated lists of fish species for different areas become more accessible and are used for curating international databases such as BOLD and GBIF.

During this study, DNA barcoding did not allow to identify all the specimens with the same degree of precision. Among the 196 successfully barcoded individuals, 79.2% were assignable to a Linnean species-identifier name without ambiguity, 10.9% to the genus level only, and 9.9% to the family level only. As anticipated by Ratnasingham and Hebert (2013), 'discordant taxonomic assignments' were sometimes

Table 2. Results of post-larvae barcoding with final identification (retained name is underlined when several identifications correspond to a BIN) and process ID for each individual, corresponding best ID using full DB, BIN, distance to nearest BIN (in %), identification based on BIN and ID category.

ORDER	FAMILY	Final identification	BOLD Process ID	GenBank access number	Best ID (full DB)	BIN	Distance nearest BIN (%)	Identification based on BIN [N]	ID category
AULOPIFORMES									
SYNODONTIDAE									
		<i>Synodontidae</i> sp. (BOLD:ACY8238)	COLOR593-15	MF409619	No match	BOLD:ACY8238 ^{new}	3.64		IV
		<i>Trachinocephalus</i> sp. (BOLD:ACD1807)	COLOR589-15	MF409558	Aulopiiformes	BOLD:ACD1807 ^{new}	17.18	<i>Synodus</i> [1]	III
		<i>Trachinocephalus myops</i>	COLOR588-15	MF120944	<i>T. myops</i>	BOLD:AAA9578 ^{new}	1.93	<i>Trachinocephalus myops</i> [51]	I
MYCTOPHIFORMES									
MYCTOPHIDAE									
		<i>Bolinichthys supralateralis</i>	COLOR457-15	MF409617	<i>B. supralateralis</i>	BOLD:AAC5878 ^{new}	2.73	<i>Bolinichthys supralateralis</i> [21]	I
HOLOCENTRIFORMES									
		<i>Myripristis</i> sp. (BOLD:AAA9764)	COLOR591-15	MF409528	Holocentriiformes	BOLD:AAA9764	1.69	<i>Myripristis murdjan</i> [12]/ <i>M. hexagona</i> [11]/ <i>M. seychellensis</i> [6]	II
		<i>Myripristis berndti</i>	COLOR594-15	MF409625	<i>M. murdjan</i>	BOLD:AAA9763 ^{new}	3.05	<i>Myripristis berndti</i> [55]	I
		<i>Myripristis botche</i>	COLOR596-15	MF409553	<i>M. seychellensis</i>	BOLD:AAA9763 ^{new}	5.70	<i>Myripristis botche</i> [5]	I
		<i>Myripristis kuntee</i>	COLOR595-15	MF409590	<i>M. berndti</i>	BOLD:AAAX2837 ^{new}	4.98	<i>Myripristis kuntee</i> [32]	I
		<i>Neoniphon sammara</i>	COLOR533-15	MF409586	<i>M. botche</i>	BOLD:AAA9765 ^{new}	3.91	<i>Neoniphon sammara</i> [41]	I
		<i>Sargocentron</i> sp. (BOLD:AAB3426)	COLOR542-15	MF409630	<i>N. sammara</i>	BOLD:AAC8278	1.94	<i>Sargocentron ittodai</i> [2]/ <i>S. cf. ittodai</i> [2]/ <i>S. diadema</i> [11]/ <i>S. xanthythrum</i> [1]	II
		<i>Sargocentron caudimaculatum</i>	COLOR592-15	MF409484	<i>S. diadema</i>	BOLD:AAB3426 ^{new}	1.47	<i>S. caudimaculatum</i> [24]/ <i>S. spiniferum</i> [11]/ <i>S. violaceum</i> [7]	I
		<i>Sargocentron praslin</i> ^a	COLOR579-15	MF409503	<i>S. spiniferum</i>	BOLD:AAC2272	2.67	<i>Sargocentron praslin</i> [7]/ <i>S. melanospiilos</i> [2]	I
		<i>Sargocentron diadema</i>	COLOR545-15	MF409599	<i>S. praslin</i>	BOLD:AAC4647 ^{new}	2.78	<i>Sargocentron diadema</i> [28]	I
		<i>Sargocentron punctatissimum</i>	COLOR546-15	MF409545	<i>S. praslin</i>	BOLD:AAB3424	8.14	<i>Sargocentron punctatissimum</i> [31]	I
		<i>Sargocentron seychellense</i>	COLOR576-15	MF409539	<i>S. diadema</i>	BOLD:AAD3971	2.04	<i>Sargocentron seychellense</i> [4]	I
			COLOR590-15	MF409594	<i>S. diadema</i>	BOLD:AAF1416 ^{new}	15.81	<i>Carapidae</i> [1]	III
			COLOR534-15	MF409490	<i>S. punctatissimum</i>	BOLD:ACW9208 ^{new}	3.97		IV
			COLOR535-15	MF409640	<i>S. punctatissimum</i>	BOLD:ACW8182 ^{new}	11.02	<i>Apogonidae</i> [1]	III
			COLOR612-15	MF409486	<i>S. punctatissimum</i>	BOLD:ACW9154 ^{new}	4.91		IV
			COLOR577-15	MF409580	<i>S. seychellense</i>				
OPHIDIIFORMES									
		<i>Carapidae</i> sp. (BOLD:ACW9208)	COLOR484-15	MF409600	Ophidiiformes	BOLD:ACW9208 ^{new}	15.81	<i>Carapidae</i> [1]	III
KURTIFORMES									
APOGONIDAE									
		<i>Apogonidae</i> sp. (BOLD:ACW9156) ^a	COLOR403-15	MF409497	No match	BOLD:ACW9156 ^{new}	3.97		IV
		<i>Apogonidae</i> sp. (BOLD:ACW8182) ^a	COLOR387-15	MF409632	Actinopterygii	BOLD:ACW8182 ^{new}	11.02	<i>Apogonidae</i> [1]	III
		<i>Apogon</i> sp. (BOLD:ACW9154) ^a	COLOR399-15	MF409475	Kurtiformes	BOLD:ACW9154 ^{new}	4.91		IV
			COLOR400-15	MF409491	Kurtiformes				
			COLOR401-15	MF409480	Kurtiformes				
			COLOR426-15	MF409645	Kurtiformes				
			COLOR427-15	MF409487	Kurtiformes				
			COLOR454-15	MF409616	Kurtiformes				
			COLOR517-15	MF409557	Kurtiformes				
			COLOR518-15	MF409495	Kurtiformes				
			COLOR519-15	MF409563	Kurtiformes				
			COLOR520-15	MF409618	Kurtiformes				
			COLOR521-15	MF409521	Kurtiformes				
			COLOR522-15	MF409494	Kurtiformes				
			COLOR523-15	MF409540	Kurtiformes				
			COLOR524-15	MF409544	Kurtiformes				

(continued)

Table 2. Continued

ORDER FAMILY Final identification	BOLD Process ID	GenBank access number	Best ID (full DB)	BIN	Distance nearest BIN (%)	Identification based on BIN [N]	ID category
Apogon sp. (BOLD:ACW9155) <i>Apogon semiornatus</i> <i>Apogonichthys ocellatus</i> <i>Nectamia savayensis</i> <i>Ostorhinchus angustatus</i> <i>Ostorhinchus apogonoides</i>	COLOR525-15	MF409466	Kurtiformes				
	COLOR529-15	MF409568	Kurtiformes				IV
	COLOR526-15	MF409459	Kurtiformes	BOLD:ACW9155 ^{new}	7.64		
	COLOR465-15	MF409546	Kurtiformes				
	COLOR528-15	MF409531	<i>A. semiornatus</i>	BOLD:AAD2206 ^{new}	8.59	<i>Apogon semiornatus</i> [17]/ <i>A. lativittatus</i> [4]	I
	COLOR442-15	MF409467	<i>A. ocellatus</i>	BOLD:AAL6796	5.51	<i>Apogonichthys ocellatus</i> [3]	I
	COLOR428-15	MF409606	<i>N. savayensis</i>	BOLD:AAD9453	2.89	<i>Nectamia savayensis</i> [11]	I
	COLOR468-15	MF409505	<i>N. savayensis</i>				
	COLOR527-15	MF409577	<i>O. angustatus</i>	BOLD:AAD5116 ^{new}	2.50	<i>Ostorhinchus angustatus</i> [16]	I
	COLOR388-15	MF409458	<i>O. apogonoides</i>	BOLD:AAD5125 ^{new}	5.94	<i>Ostorhinchus apogonoides</i> [16]/ <i>O. cf. apogonoides</i> [3]	I
	COLOR389-15	MF409470	<i>O. apogonoides</i>				
	COLOR390-15	MF409453	<i>O. apogonoides</i>				
	COLOR417-15	MF409530	<i>O. novemfasciatus</i>	BOLD:AAD8453 ^{new}	1.22	<i>Ostorhinchus taeniophorus</i> [5]/ <i>O. novemfasciatus</i> [4] ^p	I
	COLOR418-15	MF409634	<i>O. novemfasciatus</i>				
	COLOR463-15	MF409488	<i>P. kallopterus</i>	BOLD:AAB4082	2.09	<i>Pristipogon kallopterus</i> [44]	I
COLOR464-15	MF409592	<i>P. kallopterus</i>					
Gobiiformes Gobiidae <i>Ptereleotris zebra</i>	COLOR392-15	MF409548	<i>P. zebra</i>	BOLD:AAC9880 ^{new}	10.59	<i>Ptereleotris zebra</i> [16]	I
	COLOR393-15	MF409482	<i>P. zebra</i>				
<i>Incertae sedis as to order within Ovalentaria</i> POMACENTRIDAE <i>Abudefduf</i> sp. (BOLD:ACK8109)	COLOR503-15	MF409633	<i>A. vaigiensis</i>	BOLD:ACK8109	2.76	<i>Abudefduf sexfasciatus</i> [64]/ <i>Abudefduf vaigiensis</i> [60]	II
	COLOR510-15	MF409571	<i>A. margariteus</i>	BOLD:AAJ2945	6.11	<i>Abudefduf margariteus</i> [2]	I
	COLOR582-15	MF409543	<i>A. sparoides</i>	BOLD:AAD7433	7.23	<i>Abudefduf sparoides</i> [9]	I
	COLOR476-15	MF409504	<i>C. opercularis</i>	BOLD:ACF0042 ^{new}	1.34	<i>Chromis opercularis</i> [9]/ <i>Chromis xanthochira</i> [6] ^p	I
	COLOR414-15	MF409517	<i>C. nigrura</i>	BOLD:AAD5022	1.93	<i>Chromis nigrura</i> [7]	I
	COLOR410-15	MF409610	<i>C. viridis</i>	BOLD:AAB4985	5.79	<i>Chromis viridis</i> [40]	I
	COLOR411-15	MF409462	<i>C. viridis</i>				
	COLOR431-15	MF409454	<i>C. viridis</i>				
	COLOR550-15	MF409502	<i>C. weberi</i>	BOLD:AAC3641 ^{new}	1.34	<i>Chromis weberi</i> [17]	I
	COLOR551-15	MF409639	<i>C. weberi</i>				
	COLOR552-15	MF409549	<i>C. weberi</i>				
	COLOR434-15	MF409516	<i>C. brownriggii</i>	BOLD:AAB6234	1.41	<i>Chrysiptera brownriggii</i> [13]	I
	COLOR505-15	MF409476	<i>C. brownriggii</i>				
	COLOR506-15	MF409465	<i>C. glauca</i>	BOLD:AAC4834	3.32	<i>Chrysiptera glauca</i> [7]	I
	COLOR507-15	MF409460	<i>C. glauca</i>				
COLOR412-15	MF409472	<i>D. aruanus</i>	BOLD:AAB4091	7.94	<i>Dasyllus aruanus</i> [47]	I	
COLOR443-15	MF409518	<i>D. aruanus</i>					
COLOR452-15	MF409533	<i>D. aruanus</i>					
COLOR481-15	MF409598	<i>D. aruanus</i>					
COLOR482-15	MF409596	<i>D. aruanus</i>					
COLOR555-15	MF409489	<i>D. aruanus</i>					
COLOR425-15	MF409512	<i>D. trimaculatus</i>	BOLD:AAB2685	4.05	<i>Dasyllus trimaculatus</i> [84]	I	
COLOR557-15	MF409506	<i>D. trimaculatus</i>					
COLOR558-15	MF409554	<i>D. trimaculatus</i>					

(continued)

Table 2. Continued

ORDER	FAMILY	Final identification	BOLD Process ID	GenBank access number	Best ID (full DB)	BIN	Distance nearest BIN (%)	Identification based on BIN [N]	ID category
		<i>Plectrogliphidodon dickii</i>	COLOR511-15 COLOR512-15 COLOR603-15 COLOR604-15 COLOR422-15 COLOR423-15 COLOR424-15 COLOR605-15 COLOR560-15 COLOR466-15 COLOR606-15 COLOR395-15 COLOR396-15 COLOR615-15 COLOR599-15 COLOR601-15	MF409567 MF409552 MF409627 MF409608 MF409585 MF409534 MF409456 MF409451 MF409643 MF409576 MF409474 MF409559 MF409620 MF409471 MF409547 MF409473	<i>P. dickii</i> <i>P. dickii</i> <i>P. dickii</i> <i>P. dickii</i> <i>P. imparipennis</i> <i>P. imparipennis</i> <i>P. imparipennis</i> <i>P. imparipennis</i> <i>P. lacymatus</i> <i>P. leucozonius</i> <i>P. leucozonius</i> <i>P. phoenixensis</i> <i>P. phoenixensis</i> <i>P. phoenixensis</i> <i>P. randalli</i> <i>S. fasciolatus</i> ^e	BOLD:ACY9039 BOLD:ACY9012 BOLD:AAB6988 ^{new} BOLD:AAC6436 ^{new} BOLD:ABX5644 BOLD:AAE4626 BOLD:ABZ0285 BOLD:AAB9539	11.46 10.17 3.99 8.35 1.95 8.70 1.83 3.52	<i>Plectrogliphidodon dickii</i> [18] <i>P. imparipennis</i> [15]/ <i>P. sagmarius</i> [6] <i>P. lacymatus</i> [9] <i>P. leucozonius</i> [26] <i>P. phoenixensis</i> [1] <i>Plectrogliphidodon randalli</i> [6] <i>Stegastes fasciolatus</i> ^e [9]/ <i>Pomacentrus agassizii</i> [8] <i>Pomacentrus caeruleus</i> [12]/ <i>P. alleni</i> [8]	I I I I I I I I I I I I I I
		<i>Plectrogliphidodon imparipennis</i>	COLOR435-15 COLOR533-15 COLOR554-15 COLOR415-15 COLOR409-15 COLOR441-15 COLOR573-15 COLOR574-15 COLOR575-15 COLOR416-15 COLOR397-15 COLOR398-15 COLOR578-15 COLOR419-15 COLOR420-15	MF409526 MF409595 MF409455 MF409626 MF409624 MF409623 MF409514 MF409602 MF409485 MF409613 MF409584 MF409561 MF409535 MF409452 MF409501	<i>P. caeruleopunctatus</i> ^f <i>P. caeruleopunctatus</i> ^f <i>P. caeruleopunctatus</i> ^f <i>S. limbatus</i> <i>Pomachromis</i> <i>S. punctatus</i> <i>S. punctatus</i> <i>S. punctatus</i> <i>S. limbatus</i> <i>S. nigricans</i> <i>S. nigricans</i> <i>S. nigricans</i> <i>S. pelcieri</i> <i>S. pelcieri</i>	BOLD:AAC8561 BOLD:AAD4445 BOLD:ACV6563 ^{new} BOLD:AAC8562 BOLD:AAF2692	3.53 3.88 9.63 3.47 4.01	<i>Stegastes limbatus</i> [7] <i>Stegastes lividus</i> ^g [12]/ <i>S. punctatus</i> [10] <i>Stegastes nigricans</i> [10] <i>Stegastes pelcieri</i> [6]	I IV I I I
		<i>Stegastes caeruleus</i>	COLOR460-15 COLOR459-15	MF409566 MF409641	No match No match	BOLD:ACW8441 ^{new} BOLD:ACX0083 ^{new}	9.31 5.32		IV IV
		<i>Stegastes limbatus</i>	COLOR471-15 COLOR458-15 COLOR472-15 COLOR513-15 COLOR408-15 COLOR413-15 COLOR469-15 COLOR436-15 COLOR429-15 COLOR430-15 COLOR407-15 COLOR432-15 COLOR433-15	MF409636 MF409605 MF409496 MF409604 MF409642 MF409520 MF409601 MF409493 MF409529 MF409454 MF409523 MF409542 MF409511	No match No match <i>B. gibbifrons</i> <i>B. periorphthalimus</i> <i>C. castaneus</i> <i>C. castaneus</i> <i>C. castaneus</i> <i>E. lineatus</i> <i>E. brevis</i> <i>E. brevis</i> No match Blenniiformes Blenniiformes	BOLD:ACW9250 ^{new} BOLD:ACW8602 ^{new} BOLD:AAU2943 BOLD:AAU4015 BOLD:AAU0601 BOLD:AAE2835 BOLD:ACV8875 ^{new} BOLD:AAF6818 BOLD:ACW9505 ^{new} BOLD:ACW8877 ^{new}	15.7 4.82 2.49 10.00 7.91 4.52 7.11 13.71 3.15 11.76	<i>Blenniella gibbifrons</i> [1] <i>Blenniella periorphthalimus</i> [4] <i>Cirripectes castaneus</i> [7] <i>Cirripectes stigmaticus</i> [10]/ <i>C. castaneus</i> [4] <i>Ecsenius lineatus</i> [1] <i>Exallias brevis</i> [18]	IV IV I I I I I I I IV IV
		<i>Stegastes nigricans</i>	COLOR471-15 COLOR458-15 COLOR472-15 COLOR513-15 COLOR408-15 COLOR413-15 COLOR469-15 COLOR436-15 COLOR429-15 COLOR430-15 COLOR407-15 COLOR432-15 COLOR433-15	MF409636 MF409605 MF409496 MF409604 MF409642 MF409520 MF409601 MF409493 MF409529 MF409454 MF409523 MF409542 MF409511	No match No match <i>B. gibbifrons</i> <i>B. periorphthalimus</i> <i>C. castaneus</i> <i>C. castaneus</i> <i>C. castaneus</i> <i>E. lineatus</i> <i>E. brevis</i> <i>E. brevis</i> No match Blenniiformes Blenniiformes	BOLD:ACW9250 ^{new} BOLD:ACW8602 ^{new} BOLD:AAU2943 BOLD:AAU4015 BOLD:AAU0601 BOLD:AAE2835 BOLD:ACV8875 ^{new} BOLD:AAF6818 BOLD:ACW9505 ^{new} BOLD:ACW8877 ^{new}	15.7 4.82 2.49 10.00 7.91 4.52 7.11 13.71 3.15 11.76	<i>Blenniella gibbifrons</i> [1] <i>Blenniella periorphthalimus</i> [4] <i>Cirripectes castaneus</i> [7] <i>Cirripectes stigmaticus</i> [10]/ <i>C. castaneus</i> [4] <i>Ecsenius lineatus</i> [1] <i>Exallias brevis</i> [18]	IV IV I I I I I I I IV IV
		<i>Stegastes pelcieri</i>	COLOR471-15 COLOR458-15 COLOR472-15 COLOR513-15 COLOR408-15 COLOR413-15 COLOR469-15 COLOR436-15 COLOR429-15 COLOR430-15 COLOR407-15 COLOR432-15 COLOR433-15	MF409636 MF409605 MF409496 MF409604 MF409642 MF409520 MF409601 MF409493 MF409529 MF409454 MF409523 MF409542 MF409511	No match No match <i>B. gibbifrons</i> <i>B. periorphthalimus</i> <i>C. castaneus</i> <i>C. castaneus</i> <i>C. castaneus</i> <i>E. lineatus</i> <i>E. brevis</i> <i>E. brevis</i> No match Blenniiformes Blenniiformes	BOLD:ACW9250 ^{new} BOLD:ACW8602 ^{new} BOLD:AAU2943 BOLD:AAU4015 BOLD:AAU0601 BOLD:AAE2835 BOLD:ACV8875 ^{new} BOLD:AAF6818 BOLD:ACW9505 ^{new} BOLD:ACW8877 ^{new}	15.7 4.82 2.49 10.00 7.91 4.52 7.11 13.71 3.15 11.76	<i>Blenniella gibbifrons</i> [1] <i>Blenniella periorphthalimus</i> [4] <i>Cirripectes castaneus</i> [7] <i>Cirripectes stigmaticus</i> [10]/ <i>C. castaneus</i> [4] <i>Ecsenius lineatus</i> [1] <i>Exallias brevis</i> [18]	IV IV I I I I I I I IV IV
		<i>Petroscirtes</i> ^h sp. (BOLD:ACW9505) ^a	COLOR471-15 COLOR458-15 COLOR472-15 COLOR513-15 COLOR408-15 COLOR413-15 COLOR469-15 COLOR436-15 COLOR429-15 COLOR430-15 COLOR407-15 COLOR432-15 COLOR433-15	MF409636 MF409605 MF409496 MF409604 MF409642 MF409520 MF409601 MF409493 MF409529 MF409454 MF409523 MF409542 MF409511	No match No match <i>B. gibbifrons</i> <i>B. periorphthalimus</i> <i>C. castaneus</i> <i>C. castaneus</i> <i>C. castaneus</i> <i>E. lineatus</i> <i>E. brevis</i> <i>E. brevis</i> No match Blenniiformes Blenniiformes	BOLD:ACW9250 ^{new} BOLD:ACW8602 ^{new} BOLD:AAU2943 BOLD:AAU4015 BOLD:AAU0601 BOLD:AAE2835 BOLD:ACV8875 ^{new} BOLD:AAF6818 BOLD:ACW9505 ^{new} BOLD:ACW8877 ^{new}	15.7 4.82 2.49 10.00 7.91 4.52 7.11 13.71 3.15 11.76	<i>Blenniella gibbifrons</i> [1] <i>Blenniella periorphthalimus</i> [4] <i>Cirripectes castaneus</i> [7] <i>Cirripectes stigmaticus</i> [10]/ <i>C. castaneus</i> [4] <i>Ecsenius lineatus</i> [1] <i>Exallias brevis</i> [18]	IV IV I I I I I I I IV IV
		<i>Stanulus</i> ^h sp. (BOLD:ACW8877) ^a	COLOR471-15 COLOR458-15 COLOR472-15 COLOR513-15 COLOR408-15 COLOR413-15 COLOR469-15 COLOR436-15 COLOR429-15 COLOR430-15 COLOR407-15 COLOR432-15 COLOR433-15	MF409636 MF409605 MF409496 MF409604 MF409642 MF409520 MF409601 MF409493 MF409529 MF409454 MF409523 MF409542 MF409511	No match No match <i>B. gibbifrons</i> <i>B. periorphthalimus</i> <i>C. castaneus</i> <i>C. castaneus</i> <i>C. castaneus</i> <i>E. lineatus</i> <i>E. brevis</i> <i>E. brevis</i> No match Blenniiformes Blenniiformes	BOLD:ACW9250 ^{new} BOLD:ACW8602 ^{new} BOLD:AAU2943 BOLD:AAU4015 BOLD:AAU0601 BOLD:AAE2835 BOLD:ACV8875 ^{new} BOLD:AAF6818 BOLD:ACW9505 ^{new} BOLD:ACW8877 ^{new}	15.7 4.82 2.49 10.00 7.91 4.52 7.11 13.71 3.15 11.76	<i>Blenniella gibbifrons</i> [1] <i>Blenniella periorphthalimus</i> [4] <i>Cirripectes castaneus</i> [7] <i>Cirripectes stigmaticus</i> [10]/ <i>C. castaneus</i> [4] <i>Ecsenius lineatus</i> [1] <i>Exallias brevis</i> [18]	IV IV I I I I I I I IV IV

(continued)

Table 2. Continued

ORDER	FAMILY	Final identification	BOLD Process ID	GenBank access number	Best ID (full DB)	BIN	Distance nearest BIN (%)	Identification based on BIN [N]	ID category
	ATHERINIFORMES								
	ATHERINIDAE	<i>Atherinidae</i> ^b sp. (BOLD:ACW9771) ^a			No match	BOLD:ACW9771 ^{new}	5.17		IV
		<i>Atherinomorus lacunosus</i>	COLOR461-15 COLOR462-15	MF409492 MF409622	<i>A. lacunosus</i>	BOLD:ACK7521 ^{new}	7.11	<i>Atherinomorus lacunosus</i> [12]	I
	CARANGIFORMES								
	CARANGIDAE	<i>Caranx papuensis</i>	COLOR662-15	MF409562	<i>C. papuensis</i>	BOLD:ACF4541 ^{new}	1.16	<i>Caranx papuensis</i> [108]	I
	PLEURONECTIFORMES								
	BOTHIDAE	<i>Bothus pantherinus</i>	COLOR530-15 COLOR609-15	MF409609 MF409532	<i>B. pantherinus</i> <i>Bothus</i>	BOLD:AAC9155 ^{new}	15.41	<i>Bothus pantherinus</i> [37]	I
		<i>Crossorhombus valderostratus</i> ^a	COLOR474-15 COLOR475-15	MF409536 MF409578	<i>C. valderostratus</i> <i>C. valderostratus</i>	BOLD:AAF8808 ^{new}	15.89	<i>Crossorhombus valderostratus</i> [18]	I
	SCOMBRIFORMES								
	NOMEIDAE	<i>Psenes cyanophrys</i> ^a	COLOR531-15 COLOR536-15	MF409457 MF409570	<i>P. cyanophrys</i> <i>P. cyanophrys</i>	BOLD:AAE0701 ^{new}	6.42	<i>Psenes cyanophrys</i> [41]	I
	LABRIFORMES								
	LABRIDAE	<i>Halichoeres cosmetus</i>	COLOR394-15	MF409522	<i>H. cosmetus</i>	BOLD:AAC1194	4.17	<i>Halichoeres cosmetus</i> [13]	I
	PERCIFORMES								
	MULLIDAE	<i>Parupeneus</i> ^b sp. (ACW9573)	COLOR532-15	MF409515	No match	BOLD:ACW9573 ^{new}	7.44		IV
		<i>Parupeneus trifasciatus</i>	COLOR538-15	MF409607	<i>P. trifasciatus</i>	BOLD:AAI4266	2.98	<i>Parupeneus trifasciatus</i> [8]	I
		<i>Upeneus</i> sp. (BOLD:AAH7551)	COLOR453-15	MF409481	<i>U. cf. margarethae</i>	BOLD:AAH7551 ^{new}	6.61	<i>Upeneus cf. margarethae</i> [5]/ <i>Upeneus margarethae</i> [2]	II
	KUHLIIDAE								
	KUHLIIDAE	<i>Kuhlia mugil</i>	COLOR456-15	MF409525	<i>U. cf. margarethae</i>				
	SERRANIDAE								
		<i>Epinephelus rivulatus</i>	COLOR598-15	MF409597	<i>K. mugil</i>	BOLD:AAC5673	5.88	<i>Kuhlia mugil</i> [63]	I
		<i>Grammistes sexlineatus</i>	COLOR404-15 COLOR405-15	MF409527 MF409612	<i>E. rivulatus</i> <i>E. rivulatus</i>	BOLD:ACZ9919 ^{new}	1.30	<i>Epinephelus rivulatus</i> [20]	I
			COLOR568-15 COLOR569-15 COLOR570-15	MF409509 MF409461 MF409631	<i>G. sexlineatus</i> <i>G. sexlineatus</i> <i>G. sexlineatus</i>	BOLD:AAC7810	4.33	<i>Grammistes sexlineatus</i> [32]	I
	CHAETODONTIDAE								
		<i>Chaetodon xanthocephalus</i>	COLOR479-15	MF409603	<i>C. xanthocephalus</i>	BOLD:AAE1213 ^{new}	4.96	<i>Chaetodon xanthocephalus</i> [4]	I
	LUTJANIDAE								
		<i>Lutjanus gibbus</i>	COLOR539-15 COLOR540-15	MF409565 MF409615	<i>L. gibbus</i> <i>L. gibbus</i>	BOLD:AAB3276 ^{new}	9.50	<i>Lutjanus gibbus</i> [51]	I
	CAESIONIDAE								
		<i>Caesio</i> sp. (BOLD:ACW9576)	COLOR483-15	MF409469	<i>Caesio suevica</i> ^d	BOLD:ACW9576 ^{new}	1.93	<i>Caesio suevica</i> [3]	III
	CIRRHITIDAE								
		<i>Paracirrhites arcatus</i>	COLOR564-15 COLOR607-15 COLOR608-15	MF409500 MF409524 MF409499	<i>P. arcatus</i> <i>P. arcatus</i> <i>P. arcatus</i>	BOLD:AAC6007	10.51	<i>Paracirrhites arcatus</i> [24]	I
	SIGANIDAE								
		<i>Siganus luridus</i>	COLOR514-15	MF409629	<i>S. luridus</i>	BOLD:AAL9467 ^{new}	7.18	<i>Siganus luridus</i> [12]/ <i>S. sutor</i> [2]	I
		<i>Siganus sutor</i>	COLOR508-15 COLOR515-15 COLOR563-15	MF409513 MF409582 MF409508	<i>S. sutor</i> <i>S. sutor</i> <i>S. sutor</i>	BOLD:AAB6556 ^{new}	3.19	<i>Siganus sutor</i> [25]	I

(continued)

Table 2. Continued

ORDER	FAMILY	Final identification	BOLD Process ID	GenBank access number	Best ID (full DB)	BIN	Distance nearest BIN (%)	Identification based on BIN [N]	ID category
SCORPAENIFORMES									
SCORPAENIDAE									
		Scorpaenidae ^h , sp. (BOLD:ACV9178)							
ACANTHURIFORMES									
ACANTHURIDAE									
		<i>Acanthurus nigrofuscus</i>	COLOR421-15	MF409479	Actinopterygii ⁱ	BOLD:ACV9178 ^{new}	12.95	Actinopterygii [1]	III
		<i>Acanthurus nigrofuscus</i>	COLOR543-15	MF409510	<i>A. nigrofuscus</i>	BOLD:AAB0201	8.35	<i>Acanthurus nigrofuscus</i> [121]	I
		<i>Acanthurus nigrofuscus</i>	COLOR544-15	MF409638	<i>A. nigrofuscus</i>	BOLD:AAB0201	8.35	<i>Acanthurus nigrofuscus</i> [121]	I
		<i>Acanthurus triostegus</i>	COLOR501-15	MF409541	<i>A. triostegus</i>	BOLD:AAA9362	3.09	<i>Acanthurus triostegus</i> [166]	I
		<i>Acanthurus triostegus</i>	COLOR502-15	MF409588	<i>A. triostegus</i>	BOLD:AAA9362	3.09	<i>Acanthurus triostegus</i> [166]	I
		<i>Acanthurus xanthopterus</i>	COLOR584-15	MF409573	<i>A. xanthopterus</i>	BOLD:AAC6467 ^{new}	3.80	<i>Acanthurus xanthopterus</i> [45]	I
		<i>Acanthurus xanthopterus</i>	COLOR547-15	MF409644	<i>Z. desjardinii</i>	BOLD:AAC6467 ^{new}	3.80	<i>Acanthurus xanthopterus</i> [45]	I
		<i>Zebrasoma desjardinii</i>	COLOR548-15	MF409621	<i>Z. desjardinii</i>	BOLD:AAF6311	1.28	<i>Zebrasoma desjardinii</i> [10]	I
		<i>Zebrasoma desjardinii</i>	COLOR549-15	MF409478	<i>Z. desjardinii</i>	BOLD:AAF6311	1.28	<i>Zebrasoma desjardinii</i> [10]	I
SPARIFORMES									
LETHRINIDAE									
		<i>Lethrinus mahsena</i>	COLOR473-15	MF409468	<i>L. mahsena</i>	BOLD:AAB6438 ^{new}	3.80	<i>Lethrinus mahsena</i> [12]	I
		<i>Lethrinus mahsena</i>	COLOR559-15	MF409569	<i>L. mahsena</i>	BOLD:AAB6438 ^{new}	3.80	<i>Lethrinus mahsena</i> [12]	I
		<i>Lethrinus rubrioperculatus</i>	COLOR509-15	MF409579	<i>L. rubrioperculatus</i>	BOLD:AAB6439 ^{new}	3.69	<i>Lethrinus rubrioperculatus</i> [35]	I
		<i>Lethrinus rubrioperculatus</i>	COLOR585-15	MF409611	<i>L. rubrioperculatus</i>	BOLD:AAB6439 ^{new}	3.69	<i>Lethrinus rubrioperculatus</i> [35]	I
LOPHIIFORMES									
ANTENNARIIDAE									
		<i>Histrio histrio</i>	COLOR444-15	MF409574	<i>H. histrio</i>	BOLD:AAB7990 ^{new}	4.61	<i>Histrio histrio</i> [42]	I
		<i>Histrio histrio</i>	COLOR445-15	MF409556	<i>H. histrio</i>	BOLD:AAB7990 ^{new}	4.61	<i>Histrio histrio</i> [42]	I
		<i>Histrio histrio</i>	COLOR446-15	MF409593	<i>H. histrio</i>	BOLD:AAB7990 ^{new}	4.61	<i>Histrio histrio</i> [42]	I
		<i>Histrio histrio</i>	COLOR447-15	MF409591	<i>H. histrio</i>	BOLD:AAB7990 ^{new}	4.61	<i>Histrio histrio</i> [42]	I
		<i>Histrio histrio</i>	COLOR448-15	MF409614	<i>H. histrio</i>	BOLD:AAB7990 ^{new}	4.61	<i>Histrio histrio</i> [42]	I
		<i>Histrio histrio</i>	COLOR449-15	MF409555	<i>H. histrio</i>	BOLD:AAB7990 ^{new}	4.61	<i>Histrio histrio</i> [42]	I
		<i>Histrio histrio</i>	COLOR451-15	MF409560	<i>H. histrio</i>	BOLD:AAB7990 ^{new}	4.61	<i>Histrio histrio</i> [42]	I
TETRAODONTIFORMES									
OSTRACIIDAE									
		<i>Lactoria cornuta</i>	COLOR480-15	MF409635	<i>L. cornuta</i>	BOLD:AAB2988 ^{new}	10.34	<i>Lactoria cornuta</i> [30]	I
BALISTIDAE									
		<i>Ballistoides viridescens</i>	COLOR556-15	MF409575	<i>P. flavimarginatus</i>	BOLD:AAD0474 ^{new}	11.61	<i>Ballistoides viridescens</i> ^k [21]/ <i>Pseudoballistes flavimarginatus</i> [8]	I
		<i>Odonus niger</i>	COLOR586-15	MF409637	<i>B. viridescens</i>	BOLD:AAB5804 ^{new}	3.94	<i>Odonus niger</i> [40]/ <i>Melichthys niger</i> [5]	I
		<i>Odonus niger</i>	COLOR587-15	MF409581	<i>P. flavimarginatus</i>	BOLD:AAB5804 ^{new}	3.94	<i>Odonus niger</i> [40]/ <i>Melichthys niger</i> [5]	I
		<i>Odonus niger</i>	COLOR580-15	MF409483	<i>O. niger</i>	BOLD:AAB5804 ^{new}	3.94	<i>Odonus niger</i> [40]/ <i>Melichthys niger</i> [5]	I
		<i>Odonus niger</i>	COLOR581-15	MF409564	<i>O. niger</i>	BOLD:AAB5804 ^{new}	3.94	<i>Odonus niger</i> [40]/ <i>Melichthys niger</i> [5]	I
		<i>Odonus niger</i>	COLOR613-15	MF409507	<i>O. niger</i>	BOLD:AAB5804 ^{new}	3.94	<i>Odonus niger</i> [40]/ <i>Melichthys niger</i> [5]	I
		<i>Rhinecanthus aculeatus</i>	COLOR583-15	MF409538	<i>R. aculeatus</i>	BOLD:AAB6992	2.41	<i>Rhinecanthus aculeatus</i> [34]	I
		<i>Rhinecanthus aculeatus</i>	COLOR611-15	MF409498	<i>R. aculeatus</i>	BOLD:AAB6992	2.41	<i>Rhinecanthus aculeatus</i> [34]	I
		<i>Sufflamen chrysopteron</i>	COLOR610-15	MF409463	<i>S. chrysopteron</i>	BOLD:AAB1339	6.42	<i>Sufflamen chrysopteron</i> [54]	I
MONACANTHIDAE									
		<i>Cantherhines pardalis</i>	COLOR439-15	MF409589	<i>C. pardalis</i>	BOLD:AAB9564	2.23	<i>Cantherhines pullus</i> ^m [39]/ <i>C. pardalis</i> [28]	I
		<i>Cantherhines pardalis</i>	COLOR602-15	MF409587	<i>C. pardalis</i>	BOLD:AAB9564	2.23	<i>Cantherhines pullus</i> ^m [39]/ <i>C. pardalis</i> [28]	I
		<i>Oxymonacanthus longirostris</i>	COLOR485-15	MF409477	<i>O. longirostris</i>	BOLD:AAF3040	ⁿ	<i>Oxymonacanthus longirostris</i> [?] ⁿ	I
		<i>Pervagor aspricaudus</i>	COLOR614-15	MF409537	<i>P. aspricaudus</i>	BOLD:AAU0195	2.31	<i>Pervagor aspricaudus</i> [5]	I
TETRAODONTIDAE									
		<i>Arothron hispidus</i>	COLOR561-15	MF409550	<i>A. hispidus</i>	BOLD:AAB9202 ^{new}	3.85	<i>Arothron hispidus</i> [58]	I

(continued)

Table 2. Continued

ORDER	FAMILY	Final identification	BOLD Process ID	GenBank access number	Best ID (full DB)	BIN	Distance nearest BIN (%)	Identification based on BIN [N]	ID category
	<i>Canthigaster valentini</i>		COLOR562-15 COLOR571-15 COLOR572-15	MF409628 MF409464 MF409519	<i>A. hispidus</i> <i>C. valentini</i> <i>C. valentini</i>	BOLD:AAC9721	3.42	<i>Canthigaster valentini</i> [20]	I

Orders and families are organized following Nelson et al. (2016). Numbers of COI sequences present in BOLD for each species (this project and an identical project run in parallel in Madagascar excluded, database accessed on 01 and 02 March 2017) are indicated within brackets. ID categories are I: identification to species level possible, II: identification to species level is uncertain when BIN corresponds to several species, III: identification to species level is impossible when BIN corresponds to specimens identified at the genus or family level, and IV: new BIN for BOLD. ^{new} indicates a BIN which had never been recorded for La Reunion Island before.

^aA species which is new, or may be new (see text for details), for La Reunion EEZ.

^bNot present in WIO.

^c*D. aruanus* is replaced by *D. abudafur* in western Indian Ocean, see Borsa et al. (2014).

^dPresence confirmed in La Reunion.

^e*S. fasciolatus* is present in western Pacific only.

^f*P. caeruleopunctatus* is present in Madagascar only.

^g*S. lividus* is present in Marquesas Islands only.

^hIdentification based on the morphology of the post-larvae.

ⁱEndemic from the Red Sea.

^jUnidentified larvae caught in KwaZulu-Natal, South Africa (BOLD process ID DSLAG968-10).

^kAt least one unambiguous image of adult *B. viridescens* in BOLD public data.

^lNo unambiguous image of adult *P. flavimarginatus* in BOLD public data.

^m*Cantherhines pullus* is only present in the Atlantic Ocean, from Massachusetts (U.S.A.) to Brazil, and west Africa.

ⁿInformation concerning this BIN unavailable on 22 June 2017.

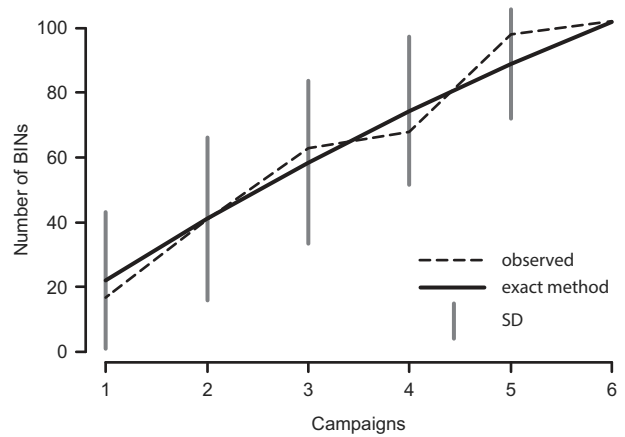


Figure 2. Accumulated number of Barcoding Index Numbers obtained along the six sampling campaigns during the 2014–2015 warm season. The dotted line connects the actual values when the solid line corresponds to BIN accumulation curve with the exact method, also called Mao Tau estimate by Colwell et al. (2012). Vertical grey lines correspond to standard deviation (SD).

encountered during this study and unfortunately, the ‘majority rule’ they suggested in order to evaluate the validity of specimen identification was not always functional. Indeed, for some BINs an almost equal number of specimens were found to be assigned to different species. When these identifications were performed by few taxonomists, and when no obvious error was detected about the geographic distribution of each species (see above), it became difficult to decide which name was more likely to be correct. As a result, the identification was restricted to the genus, or even family level. The problem of ‘ambiguous match’, i.e. COI sequences with 100% of similarity to sequences attributed to two (or more) species has already been detected as a problem for identifying fish larvae by Hubert et al. (2015). This problem has been fully discussed by these authors and other in the context of the limits of using mitochondrial DNA for species identification (due to hybridization for example). But it is probable that some of these ‘discordant taxonomic assignments’ correspond to identification performed by laboratories that were working on specimens belonging to the same species, but identifying them under different names (Collins and Cruickshank 2012). Indeed, Leis (2015) stressed that misidentifications of Indo-Pacific fishes in GenBank and BOLD is a problem, and Hubert et al. (2015) concluded their publication by stressing that information needs to be curated in BOLD, especially for some fish families. Superimposed on this curation effort, it appears clearly that regional sampling effort of fish DNA barcoding is essential so that the full range of intra-specific genetic variation is represented (Barber and Boyce 2006). This effort in DNA barcoding must be associated to a better traceability of the way specimens are identified by adding to each record the reference of the guides, or keys, that were used.

Specimens assigned to a BIN without a corresponding scientific name may also correspond to either a new species for science, or a known species that has not been DNA barcoded yet. A lot of tropical fish species remain to be barcoded, either because of the low effort performed in some areas, this is the case for la Reunion EEZ, or because some species

are rare (Bringloe et al. 2016). Specimens assigned to a BIN without a corresponding scientific name may also correspond to ‘insufficiently studied species’ which actually represent two – or more – true species (Ayala et al. 2016). For example, Zemplak et al. (2009) estimated that 300 fish species that are believed to occur from South Africa to Australia actually represent two taxa. Asgharian et al. (2011) detected high degrees of divergence between five regions of the Indo-West Pacific Ocean for 16 fish species over the 76 they collected, suggesting they were cryptic species. It is thus probable that the recent resurrection of the Indian Ocean humbug damselfish, *Dascyllus abudafur*, from synonymy with its Pacific Ocean sibling, *D. aruanus*, by Borsa et al. (2014) is a good example of what may happen to several species that are presently thought to have an Indo-Pacific distribution. In the near future, the addition of new sequences will increase the clarity of the BIN boundaries in the sequence space (Ratnasingham and Hebert 2013).

Although not all specimens were identified with the same degree of precision during this study, they were all assigned to a BIN. Hubert et al. (2015) regretted that taxonomic incompleteness of reference libraries in BOLD may limit the automated identification of Indo-Pacific fishes. Their remark is pertinent only if specimens need to be identified to Linnaean taxa. Taxa defined by a BIN (i.e. a Molecular Operational Taxonomic Unit or ‘MOTU’; Blaxter et al. 2005) can be used for biodiversity and ecological surveys as any other precisely identified specimen (Blaxter 2016). Indeed, values of occurrence and abundance can be assigned to each BIN as to any other Linnaean names. When using BIN, the resulting data tables are more homogeneous than those based on specimens identified to different taxonomic levels, as it is often the case in fish larvae studies. BIN can also be used for analysing diversity according to multiple spatial scales as stressed by Bringloe et al. (2016). Moreover, as BINs are traceable, and curated in an international database, it will be easy to replace them by Linnaean names once new species are precisely identified and barcoded. This will allow to ‘move from anonymous sequence to ecosystem biology’ as forecasted by Blaxter et al. (2005).

Even if the sampling effort for collecting fish post-larvae during this study was relatively low, nine of the obtained BINs, and possibly 10, corresponded to species that are new for La Reunion EEZ. DNA barcoding of larval specimens has already demonstrated its efficiency for increasing the knowledge of species richness in different groups. For example, DNA barcoding larvae of gonodactylid stomatopods (mantis shrimp), Barber and Boyce (2006) demonstrated that the species richness in this well-studied group is underestimated. For fishes, DNA barcoding larvae caught near the surface allowed to get access to deep living species of Pempheridae, Melanocetidae, Myctophidae, and Nomeidae (Hubert et al. 2015). Using the same approach, a new species of Serranidae living in deep habitats was even discovered by Baldwin and Johnson (2014). DNA barcoding thus appears mature enough for making a question such as ‘How many species are there?’ tractable (Adamowicz and Scoles 2015), and this, using different stages of the life cycle. In this present study, four of these potentially new

species for La Reunion EEZ were identified without ambiguity. The first species is *Plectroglyphidodon leucozonus*. This species had been initially identified as present in La Reunion by Letourneur et al. (2004) but Fricke et al. (2009) considered the correct identification was *P. randalli*. This present study proves that both species are present in La Reunion coastal areas: *P. leucozonus* assigned to BIN BOLD:AAC6436 and *P. randalli* to BIN BOLD:AAE4626. The second new species for La Reunion is *Sargocentron praslin*. According to the online version of the Catalog of Fishes (Eschmeyer et al. 2016), this species is present in Mauritius only but due to the high level of connectivity within the Mascarene Archipelago (Crochelet et al. 2016), it is logical to collect post-larvae of this species even if adults have not yet been recorded in La Reunion coastal habitats. The distribution of the third species, *Psenes cyanophrys*, is considered as circumglobal in tropical and warm temperate seas (Eschmeyer et al. 2016); their presence in light-trap samples is also logical. Oppositely, the presence of post larvae of the last species, *Crossorhombus valderostratus*, may appear as more problematic. Indeed, according to the online version of the Catalog of Fishes (Eschmeyer et al. 2016), the distribution of this species is restricted to Sri Lanka and southern Japan. But the GBIF database indicates that the species is present in the WIO and the 12 specimens of this species present in BOLD all originate from South Africa. As BOLD:AAF8808 is the only BIN associated to this species at the moment in BOLD, it is difficult to assess whether the identification of the South African specimens is problematic, or the geographical distribution of this species is more extended than previously thought. The other potential new species for La Reunion EEZ discovered during this study have not been identified to the species level. They correspond to small, often cryptic, species (Apogonidae BOLD:ACW8182, BOLD:ACW9156, and BOLD:ACW9154, *Petrosirtes* sp. BOLD:ACW9505, and *Stanulus* sp. BOLD:ACW8877) or species that are sometimes difficult to identify such as Atherinidae BOLD:ACW9771.

In conclusion, our study not only confirmed that COI barcoding is useful for identifying fish larvae and juveniles (Ko et al. 2013) but it also demonstrated the usefulness of this technique for improving the knowledge of local fish richness. As the number of BIN did not reach a plateau during this short study, it is probable that pursuing the collection of fish larvae about to settle in coastal habitats will increase this knowledge.

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