sustainability

MDPI

Article

DNA Barcoding Revealing the Parrotfish (Perciformes: Scaridae) Diversity of the Coral Reef Ecosystem of the South China Sea

Yayuan Xiao [1,2,3,4], Chunhou Li [1,2,3,4], Teng Wang [1,2,3,4,*], Lin Lin [1,3,4], Jiatong Guo [1], Qiumei Quan [1] and Yong Liu [1,2,3,4,*]

[1] Key Laboratory of South China Sea Fishery Resources Exploitation & Utilization, Ministry of Agriculture and Rural Affairs, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Science, Guangzhou 510300, China
[2] Key Laboratory of Efficient Utilization and Processing of Marine Fishery Resources of Hainan Province/Sanya Tropical Fisheries Research Institute, Sanya 572018, China
[3] Scientific Observation and Research Station of Pearl River Estuary Ecosystem of Guangdong Province, Guangzhou 510300, China
[4] Guangdong Provincial Key Laboratory of Fishery Ecology Environment, Guangzhou 510300, China
* Correspondence: wangteng@scsfri.ac.cn (T.W.); liuyong@scsfri.ac.cn (Y.L.)

Abstract: Parrotfish are an important group in the coral reef ecosystem that maintain ecological stability and have a close relationship with coral reefs. Around the world, parrotfish and coral reefs are being destroyed due to human activities and global climate change. Here, we investigated the diversity of parrotfish in the South China Sea (SCS) using DNA barcoding, and initially established a DNA barcode database of the SCS parrotfish. A total of 1620 parrotfish specimens, belonging to 23 species of 6 genera in the family Scaridae, were collected in the SCS, accounting for 64% of the parrotfish species in the SCS. Genetic distance analysis at each taxonomic level showed that the average genetic distance was 0.23% within species, 8.52% within genus and 13.89% within family. The average inter-specific distance was 37.04-fold the intra-specific distance. Barcode gap analysis showed that 6 of the 21 parrotfish species with multiple samples had no barcode gap, resulting in an overall identification success rate of 74%. The ABGD analysis revealed that there could be 37 potential operational taxonomic units (OTUs) and the BIN analysis showed 32 identifiable taxonomic units, which by far exceed the number of morphologically previously known species. Overall, this study complements the lack of parrotfish DNA barcode sequences, and our findings provide an important stepping-stone to further study the diversity of parrotfish in South China.

Keywords: parrotfish; DNA barcoding; COI; the South China Sea; coral reef

check for updates

Citation: Xiao, Y.; Li, C.; Wang, T.; Lin, L.; Guo, J.; Quan, Q.; Liu, Y. DNA Barcoding Revealing the Parrotfish (Perciformes: Scaridae) Diversity of the Coral Reef Ecosystem of the South China Sea. *Sustainability* **2022**, *14*, 15386. https://doi.org/10.3390/su142215386

Academic Editors: Wen Xiong, Peter A. Bowler, Zhongxin Wu, Xiaoyu Li and Dongkui Gao

Received: 12 October 2022
Accepted: 11 November 2022
Published: 18 November 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The coral reef ecosystem, known as the "underwater rainforest", has a very high biodiversity and provides a habitat for numbers of aquatic organisms [1]. The coral reefs of the SCS account for 5% of the world's coral reef areas and consist of four main coral reef archipelagos, including at least 280 small reefs, with a total area of 38,462 km^2 [2,3]. The numerous coral reefs of the SCS provide a habitat for a wide variety of fish species. Among the four coral reef archipelagos of the SCS, 514 fish species were recorded from Dongsha islands, 632 species from Xisha and Zhongsha islands, and 548 species in Nansha islands, which may be much lower than the actual number of species [4]. Parrotfish (Scaridae), a family of brightly colored fish, are often closely associated with the health of coral reef ecosystems. There are 36 species of parrotfish living in the reefs of the SCS [5,6]. Parrotfish usually inhabit coral reefs in tropical seas, and only rarely live in subtropical zones [5]. They are the main herbivorous fish in coral reefs, feeding on algal substrates, macroalgae, shellfish, encrusting corallines and other organisms [5,7]. In the coral reef

ecosystem, macroalgae compete for space with reef-building corals, and macroalgae are more competitive [8]. Since most parrotfish feed on algae, parrotfish play an important role in regulating algae and coral reefs [8–10]. Studies have shown that when parrotfish and other herbivores are severely depleted, coral reefs can be severely degraded or destroyed by algal overgrowth [11]. In addition, parrotfish are a major factor in the bioerosion of coral reef carbonates and a major determinant of the benthic community structure of coral reefs [12–14]. Therefore, parrotfish are an important factor in maintaining the stability of coral reef ecosystems.

Unfortunately, coral reefs around the world are being degraded at an alarming rate [15]. In recent decades, the coral reefs of the SCS have been significantly degraded and fish biodiversity has been severely reduced due to human activities, such as overfishing, wastewater discharge and the greenhouse effect [16]. The overall coverage of coral reefs in the pelagic areas of the SCS has decreased from 60% in the early 2000s to about 20% in 2012, while the overall coverage in the offshore areas has decreased by about 80% from the 1980s to 2012 [3]. The decrease in the reef area has put reef fish under severe survival pressure. For parrotfish, the degradation of coral reefs is one of the most important factors leading to a significant decline in population size, change in age structure and decrease in reproductive quality [6]. This will inevitably reduce the ecological function of parrotfish and lead to the decline of coral reefs, thus creating a vicious cycle. Therefore, coral reefs in the SCS are in urgent need of attention and protection. Moreover, the study of parrotfish diversity not only provides important data for the conservation of parrotfish, but also provides insight into the health of coral reefs ecosystems.

Fish identification is usually an important and fundamental part of fish diversity surveys. Parrotfish have bright colors and complex patterns, and their colors change with the growth stage [17], making their morphological identification challenging. Traditional morphological identification methods rely on personal experience, but there are relatively few experienced professional taxonomists, which undoubtedly increases the difficulty of this work [18]. DNA barcoding, as a molecular identification method, does not require the user to have professional taxonomic knowledge, is easy to learn, and gradually has become an important tool for species identification. DNA barcoding does not depend on individual morphological characteristics, is independent of life cycle and sex and requires only a fragment of organism tissue for rapid identification [19,20]. Currently, DNA barcode marker genes have been developed for land plants, animals and fungi [21–24]. In fish identification, the COI (mitochondrial cytochrome oxidase subunit I) gene fragment is commonly used as a standard DNA barcode sequence and has been widely used in related fields [18,25,26]. A reliable DNA barcode reference library is the basic guarantee for the successful application of DNA barcoding. At present, many public DNA barcode databases have been established in the world, among which the BOLD (Barcode of Life Data System, http://www.barcodinglife.org/) database is a representative. For fish, the FISH-BOL (FISH-Barcode of Life Campaign) international cooperative program has been developed for DNA barcoding of fish groups. The establishment of these international databases have not only facilitated the sharing and communication among DNA barcoding researchers around the world, but also promoted the in-depth and wide application of DNA barcoding research. Parrot fish are a group of about 95 morphological fish species and the largest richness of species is in the Indo-Pacific. However, data on parrotfish in coral reefs are still scarce. Many parrotfish have virtually no available DNA barcodes. For example, there are less than 1700 DNA barcode sequences for only 38 parrotfish species in the BOLD database, and these sequences are unevenly distributed across species. This leaves DNA barcoding without significant data support for parrotfish diversity conservation applications.

Therefore, to supplement the public database with parrotfish DNA barcoding, we collected parrotfish samples from various coral reefs in the SCS and conducted DNA barcoding studies. Here, we reveal the diversity of parrotfish in the SCS, and initially build a DNA barcode reference database of parrotfish in the SCS, aiming to provide basic data for strengthening the conservation of coral reefs and fish diversity in the SCS.

2. Materials and Methods

2.1. Sample Collection and Morphological Identification

All samples were collected from five coral reef regions (Qilianyu, Meijijiao, Jinqingdao, Lingshui and Yongxingdao) (Figure 1) in Hainan Province, China from January 2018 to October 2020. A total of 1620 specimens were collected, mainly from commercial fishing harvests and scientific fishery surveys by multiple methods such as light trapping, bottom trawling and long-line fishing (Table S1 (Supplementary Materials)). The morphological identification of the specimens was mainly based on the monographs and the literature related to fish in the SCS [5,27,28]. Information about the fish species used in this study is shown in Table 1. A small piece of muscle tissue was excised from each specimen, preserved in 100% ethanol and subsequently used for genomic DNA extraction. All specimens were preserved in 95% ethanol and stored at the South China Sea Fisheries Research Institute, the Chinese Academy of Fishery Sciences (SCSFRI, CAFS).

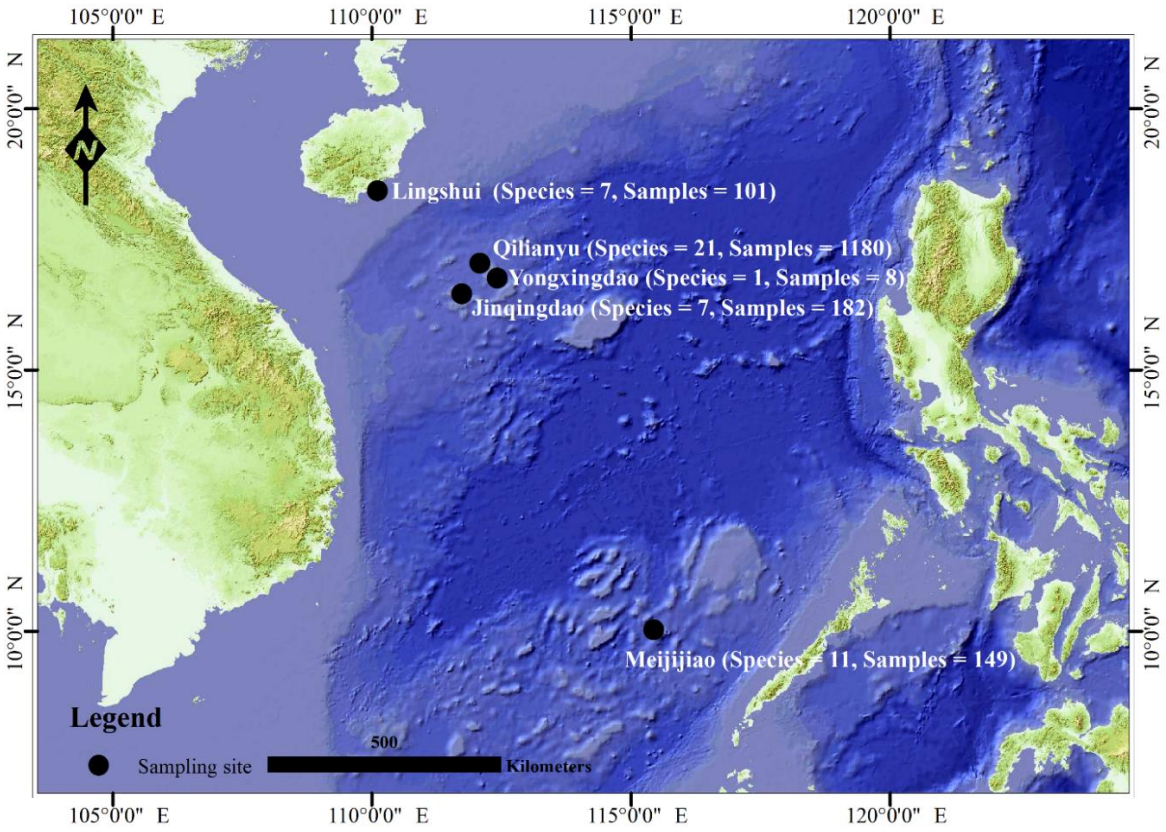

Figure 1. Sample collection sites in the SCS.

Table 1. Information of the fish species of Scaridae used in this study.

Genus	Species	Size	Sample Site and Size
Bolbometopon	*B. muricatum*	5	Qilianyu (5)
Calotomus	*C. carolinus*	41	Qilianyu (38); Meijijiao (3)
Cetoscarus	*C. bicolor*	4	Qilianyu (4)
	C. bleekeri	1	Meijijiao (1)
Chlorurus	*C. japanensis*	5	Qilianyu (1); Meijijiao (1); Lingshui (3)
	C. microrhinos	22	Qilianyu (21); Meijijiao (1)
	C. sordidus	362	Qilianyu (271); Meijijiao (43); Jinqingdao (48)
Hipposcarus	*H. longiceps*	135	Qilianyu (47); Meijijiao (12); Jinqingdao (75); Lingshui (1)

Table 1. *Cont.*

Genus	Species	Size	Sample Site and Size
Scarus	*S. altipinnis*	1	Lingshui (1)
	S. chameleon	31	Qilianyu (6); Meijijiao (17); Yongxingdao (8)
	S. dimidiatus	59	Qilianyu (48); Meijijiao (11)
	S. forsteni	276	Qilianyu (224); Meijijiao (35); Jinqingdao (14); Lingshui (3)
	S. frenatus	6	Qilianyu (6)
	S. ghobban	93	Qilianyu (15); Jinqingdao (2); Lingshui (76)
	S. globiceps	78	Qilianyu (78)
	S. niger	64	Qilianyu (59); Meijijiao (4); Lingshui (1)
	S. oviceps	196	Qilianyu (157); Jinqingdao (39)
	S. prasiognathos	9	Qilianyu (9)
	S. psittacus	12	Qilianyu (12)
	S. rivulatus	20	Qilianyu (20)
	S. rubroviolaceus	32	Qilianyu (13); Jinqingdao (3); Lingshui (16)
	S. schlegeli	158	Qilianyu (136); Jinqingdao (1); Meijijiao (21)
	S. spinus	10	Qilianyu (10)

2.2. DNA Extraction, PCR Amplification and Sequencing

Total genomic DNA was extracted from the muscle samples by proteinase K digestion followed by a standard phenol-chloroform method and further checked by 1.0% agarose gel electrophoresis. Approximately 660 bp of the 5′ end of the mitochondrial COI gene was amplified using various combinations of the following universal fish primers: FishF1-TCA ACC AAC CAC AAA GAC ATT GGA C; FishF2-TCG ACT AAT CAT AAA GAT ATC GGC AC; FishR1-TAG ACT TCT GGG TGG CCA AAG AAT CA; and FishR2-ACT TCA GGG TGA CCG AAG AAT CAG AA [29]. The 30 µL polymerase chain reaction (PCR) mixture contained 1.5 µL of each primer (10 mM), 1.5 µL dNTPs (2.5 mM each), 0.375 µL Taq DNA polymerase (2.5 U/µL, TaKaRa Bio, Shanghai, China), 1.0 µL DNA template (50–100 ng/µL), 3.0 µL 10× PCR buffer (including $MgCl_2$) and 21.125 µL sterilized ultrapure water. The PCR amplification procedures were as follows: 94 °C for 5 min, 32 cycles at 94 °C for 30 s, 53 °C for 30 s, 72 °C for 1 min and a final extension at 72 °C for 10 min. The PCR products were visualized on a 1.2% agarose gel. The successfully amplified products were separated from the gels, purified using the DNA Gel Extraction Kit (Tiangen, Wuhan, China) and sequenced on an ABI3730 XL DNA System with the above primers.

2.3. Molecular Data Analysis

The sequence chromatograms and alignments were visually inspected and verified using the DNASTAR Lasergene package (DNASTAR Inc., Madison, WI, USA). Sequences were aligned and trimmed to the same length using the software package MEGA 7.0 [30], and all alignments were translated to amino acids to confirm sequence validity and to detect the presence of nuclear DNA pseudogenes, insertions, deletions or stop codons. The aligned sequences were submitted to GenBank (accession numbers: OK346639-OK347213, OK347216-OK348260). Similarity of COI sequences was obtained by comparing the sequences of 23 species with homologous fragment sequences from the NCBI and BOLD databases to assess the accuracy of morphological identification. We adopted a general rule that defines top-matched sequences with at least 97% sequence similarity as potential species identification and used 3% as a relatively loose criterion [31,32].

All sequences' and specimens' collateral data were submitted to BOLD (project NHSHJ "DNA Barcodes of fish from coral reefs in the SCS"). The BOLD version 4 analytical tools were used for the following analyses. The distance summary with the default parameter setting, the BOLD aligner alignment option and pairwise deletion (ambiguous base/gap handling) were employed to estimate the Kimura 2-parameter (K2P) distances for taxonomic ranks whthin the species, genus and family levels. Barcode gap analysis was carried out with the setting of the parameter K2P; Kalign alignment option; pairwise deletion (am-

biguous base/gap handling) to construct the distribution of intraspecific and interspecific genetic distances (nearest neighbor (NN) analysis). The BIN analysis was also carried out with the default parameters.

To verify intra- and interspecific genetic distances, we also carried out the barcode gap analyses in ABGD [33] based on the K2P model with the transition/transversion ratio (TS/TV) set to 2.0, 10 recursive steps, X (relative gap width) = 1.0 and the remaining parameters were set to default values (Pmin = 0.001, Pmax = 0.1, Nb bins = 20).

The neighbor-joining (NJ) tree was constructed using MEGA 7 with 1,000 bootstrap replicates based on the K2P distance model to reveal the clustering relationships among all individuals, and *Labrus bergylta* (GenBank accession numbers: KU320024, JN231245) and *L. quadrilineatus* (GenBank accession numbers: MN560840, MN560884, MN560945, MF123934) from the Labridae family were used as outgroups. In addition, we downloaded four reliable parrotfish sequences (GenBank accession numbers: GU673732, GU673895, GU673816, MK657338) from NCBI to build the tree together. The NJ tree was edited and visualized using FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/ (accessed on 4 October 2016) [34].

When the molecular identification results were inconsistent with the morphological results, or the OTU delineation and phylogenetic relationship were abnormal, morphological examination was performed again for these ambiguous individuals.

3. Results

3.1. Morphology-Based Species Identification

A total of 23 species, belonging to 6 genera of the Scaridae family, were identified by morphological identification from 1620 parrotfish samples, with the *Scarus genus* accounting for 15, the *Chlorurus* genus for 4, and 1 each of the other genera (Table 1). Of the 1620 samples, the number of *C. sordidus* ranked the highest (362), *S. forstein* was the second (276) and the remaining species ranged from 1 to 196, of which *C. bleekeri* and *S. altipinnis* were single samples.

3.2. DNA Barcodes Identification

In this study, a total of 1620 COI barcode sequences with a length of 620 bp, representing 23 different morphological species (mean of approximately 68 samples per species), were successfully obtained. The GenBank and BOLD databases were used for sequence similarity comparison. For species-level identification, a total of 1620 COI barcode sequences representing 23 different species were employed (mean of approximately 68 samples per species). The identification results were consistent with morphology-based species identification.

For DNA barcoding studies, intra- and inter-specific genetic distance are a very important metric. When the minimum inter-specific genetic distance is larger than the maximum intra-specific genetic distance, a distinct gap area will be formed, namely barcode gap [35]. The genetic distance analysis at different taxonomic levels demonstrated the mean genetic distance within species was 0.23%, 8.52% within genus and 13.89% within family (Table 2). The mean intra-specific distance of all species was <2% (Table 3). The largest intra-specific distance was 11.92 for *C. microrhinos*, followed by 9.81 for *C. sordidus*, >2% for *C. japanensis*, *S. forsteni*, *S. globiceps*, *S. schlegeli* and 0 to 1.81 for the rest. In the barcode gap analysis, six species lacked a barcode gap, characterized by intra-specific K2P distance $\geq$ inter-specific distance (Figure 2 and Table 3), indicating that 81% of the investigated multi-individual species could be identified by the DNA barcode approach.

Table 2. Summary of K2P genetic distances (%) calculated for different taxonomic levels.

Level	N	Comparisons	Minimum	Mean	Maximum	SE
Within species	1618	157,247	0.00	0.23	12.08	0.00
Within genus	1435	473,979	0.32	8.52	21.60	0.00
Within family	1620	680,164	6.30	13.89	29.48	0.00

Table 3. Comparison of the mean and maximum intra-specific values with the nearest neighbor distance for each morphological species. Distances are highlighted if the nearest neighbor is less than 2% divergent, or when the distance to the nearest neighbor is less than the max intra-specific distance. N/A: the species is a singleton.

Species	Mean Intra-Species (%)	Max Intra-Species (%)	Nearest Species (%)	Distance to NN (%)
B. muricatum	0.1	0.16	*C. bicolor*	14.75
C. carolinus	0.06	0.32	*C. bicolor*	16.15
C. bicolor	0.27	0.49	*S. altipinnis*	13.29
C. bleekeri	N/A	0	*C. microrhinos*	3.16
C. japanensis	0.86	2.15	*C. microrhinos*	6.52
C. microrhinos	**1.3**	**11.92**	***C. bleekeri***	**3.16**
C. sordidus	**0.25**	**9.81**	***C. microrhinos***	**5.97**
H. longiceps	0.32	1.31	*C. sordidus*	14.78
S. altipinnis	N/A	0	*S. forsteni*	3.34
S. chameleon	0.08	0.49	*S. globiceps*	7.08
S. dimidiatus	0.11	0.65	*S. oviceps*	3.15
S. forsteni	**0.19**	**6.26**	***S. niger***	**2.48**
S. frenatus	0	0	*S. oviceps*	4.01
S. ghobban	0.18	0.98	*S. rubroviolaceus*	3.68
S. globiceps	**0.34**	**6.44**	***S. rivulatus***	**0.32**
S. niger	0.2	1.81	*S. forsteni*	2.48
S. oviceps	0.09	1.47	*S. forsteni*	2.65
S. prasiognathos	0.33	0.66	*S. forsteni*	3.68
S. psittacus	0.38	1.14	*S. schlegeli*	8.35
S. rivulatus	**0.13**	**0.65**	***S. globiceps***	**0.32**
S. rubroviolaceus	0.27	0.98	*S. ghobban*	3.68
S. schlegeli	**0.15**	**5.78**	***S. globiceps***	**5.58**
S. spinus	0.23	0.65	*S. globiceps*	9.55

Note: The bold type represents the species of no DNA barcode gaps.

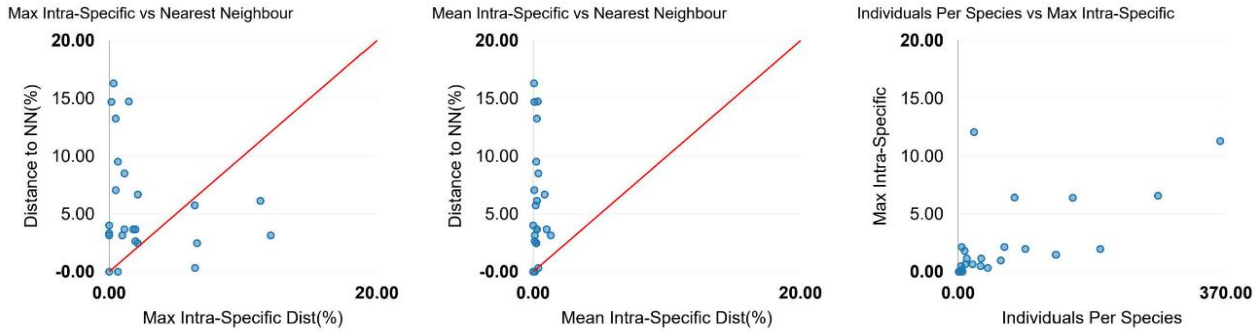

Figure 2. Barcoding gap: Maximum intraspecific Kimura 2-parameter (K2P) distances compared with the minimum interspecific K2P distances. The graphs show the overlap of the maximum and mean intra-specific distances with the inter-specific (NN = nearest neighbor) distances.

3.3. ABGD Analyses, BIN Analyses and Phylogenetic Tree Delimitation

The ABGD tool was used for the OTUs (Operational Taxonomic Units) partition. The prior maximal distance P ranged from 0.0010 to 0.0359, and the entire dataset was partitioned into 293 to 1. The partition with P = 0.0129 delimited the entire dataset into 37 putative OTUs (Figure 3 and Table S2). At P = 0.0129, the samples of *C. japanensis*, *C. microrhinos*, *C. sordidus*, *S. forsteni*, *S. globiceps* and *S. schlegeli* were partitioned into multiple OTUs (Table 4), and samples of *S. rivulatus* and part of *S. globiceps* were partitioned into one partition (OTU14) (Table 5), which was also reflected by high intraspecific (3.81%) and low interspecific (0.81%) genetic distances.Of the 23 OTUs identified based on morphology, 14 (58.3%) were clearly delineated by ABGD. The topology of the NJ phylogenetic tree was essentially the same. Consistent with ABGD, all individuals of *S. forsteni* and *S. globiceps* cannot be neatly divided into a clade, *S. forsteni* and *S. niger* are clustered on a clade,

S. globiceps and *S. rivulatus* are also together in a combined clade (Figure 4). The BIN analysis revealed 32 identifiable taxonomic units, including 8 unique BINs and 24 non-unique BINs (Table S3).

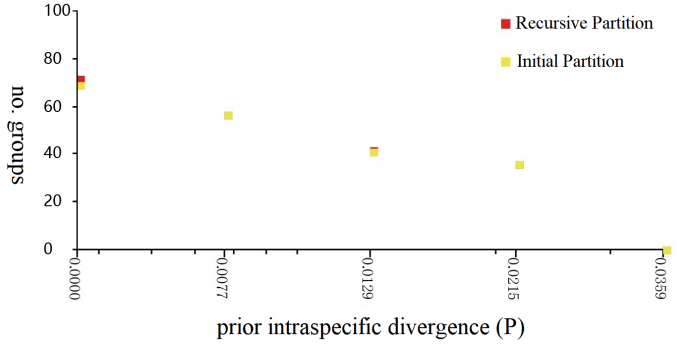

Figure 3. ABGD analysis assessed the number of OTUs as a function of P.

Table 4. List of the species with more than one OTU delimited including their Barcode Index Number in BOLD, maximum intraspecific and within OTU K2P genetic distances and K2P genetic distances to the nearest neighbor. For each OTU, the nearest neighbor distance corresponds to the distance to the most closely related, yet different, species in the data set.

OTU Information	Dist. (%). Max. Intra.	Dist. (%). Near. Neigh.
Chlorurus japanensis	2.15	6.52
OTU4 (BIN: ACK7947)	0.16	6.69
OTU5 (BIN: AAE8961)	-	6.52
Chlorurus microrhinos	11.92	3.16
OTU7 (BIN: AAJ5287)	9.81	3.16
OTU8 (BIN: AAD0850)	-	10.99
Chlorurus sordidus	9.81	5.97
OTU9 (BIN: AAB6670)		5.97
OTU10 (BIN: AEL9035)	-	14.56
OTU11 (BIN: AEM3067)	-	13.32
OTU12 (BIN: AAB6670)	-	8.37
Scarus forsteni	6.26	2.48
OTU18 (BIN: AAE4369)	1.16	2.48
OTU19 (BIN: AEL8860)	1.31	3.32
OTU20 (BIN: AAE4369)	-	3.60
OTU21 (BIN: AEL8858)	-	4.37
OTU22 (BIN: AEM1502)	-	6.44
OTU23 (BIN: AEL8859)	-	4.36
Scarus globiceps	6.44	0.32
OTU14 (BIN: ADB4663)	1.97	0.32
OTU26 (BIN: ADB4663)	-	3.31
OTU27 (BIN: AEM1397)	-	4.37
Scarus schlegeli	5.78	5.58
OTU33 (BIN: ACF2863)	0.98	7.73
OTU34 (BIN: AEL7735)	-	5.83
OUT35 (BIN: ACF2863)	-	11.74
OTU36 (BIN: ACF2863)	-	9.67

Note: "-" indicates that the OTU is only one individual, which is not applicable for genetic distance analysis.

Table 5. List of the BIN-sharing multiple species including maximum within OTU K2P genetic distances, K2P genetic distance to the nearest OTU and list of species detected for OTU. The nearest neighbor distance corresponds to the distance to the most closely related OTU without considering species boundaries.

OTU Information	Dist. (%). Max. Intra.	Dist. (%). Near. Neigh.	Species
OTU14	3.81	0.81	*Scarus globiceps* *Scarus rivulatus*

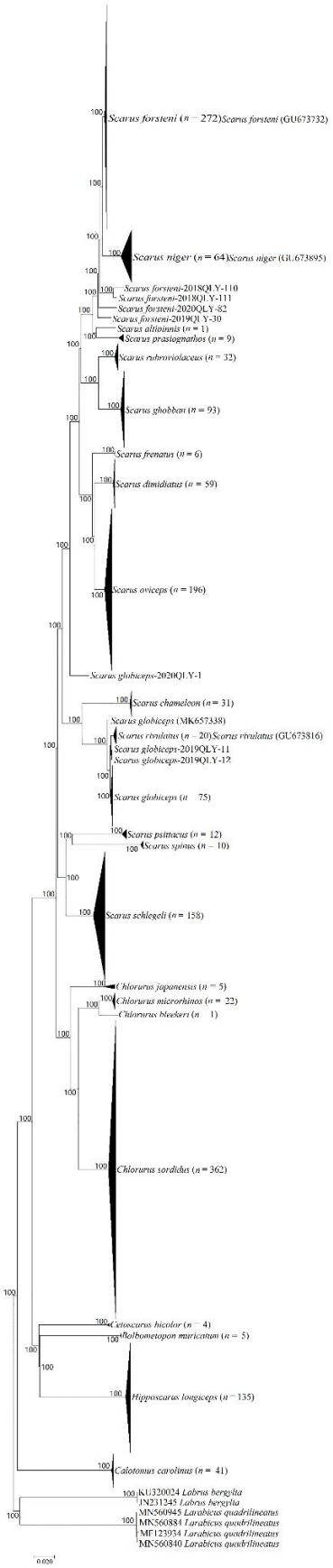

Figure 4. NJ tree based on DNA barcoding sequences of samples.

4. Discussion

In marine fish, DNA barcoding has been successfully applied to many geographical fauna, such as the Mediterranean Sea, the Caribbean Sea, and the Arctic Ocean, etc. [36–38]. It can also be successfully applied in the SCS [16]. When DNA barcoding is used to identify organisms, sequence divergence is an essential guarantee for successful species identification. Hebert et al. (2004) suggested that 10-fold sequence differences of the average interspecific and the average intraspecific divergence could be used as a standard COI threshold for animal species' identification [39]. The value of 37.04 was relatively high in this study compared to other marine areas, which meets the requirements of DNA barcoding identification [40,41]. In fish identification, the threshold is one of the important methods for DNA barcoding to identify individual samples. A genetic distance threshold of 2% usually identifies most species, i.e., individuals are the same species when the genetic distance between them is less than 2%, otherwise they are different species [20]. Among the 23 species investigated in this study, single-individual species, *C. bleekeri* and *S. altipinnis*, were not suitable for intraspecific genetic distance analysis, and 6 of the remaining 21 species had a maximum intraspecific genetic distance greater than 2%. Although the threshold method is widely used in DNA barcoding species identification, its limitations are still recognized. With further research on DNA barcoding, it has been found that the threshold is not always reliable in distinguishing closely related sister species [42]. In addition, it is difficult to have uniform criteria for thresholds across different groups and different DNA barcoding fragments [43]. In contrast, an alternative criterion based on genetic distance seems to be more convincing. In general, the intraspecific genetic distance of species is smaller than the interspecific genetic distance, and there is a gap region, i.e., a barcode gap, between them. In DNA barcoding studies, DNA barcode gaps are a strong guarantee for successful species identification [44]. In the present study, there were 6 out of the 21 multi-individual parrotfish without DNA barcode gaps, thus, the success rate of DNA barcoding was 74% for the 23 Scaridae species of the SCS. This success rate was lower than the 95.2% of Hou et al. (2018) for the identification of Perciformes of the SCS by DNA barcoding [16].

In the present study, *C. microrhinos*, *C. sordidus*, *S. forsteni*, *S. globiceps*, *S. schlegeli* and *S. rivulatus* had no DNA barcode gaps, which were due to the genetic overlap with related species within the genus. In addition, being divided into multiple OTUs in the ABGD and having a high intraspecific maximum genetic distance appear to be common characteristics of species lacking barcoding gaps. The emergence of cryptic species is always accompanied by changes in the genetic information, and the genetic distance between individuals must also change accordingly. In addition, intraspecific genetic distance is usually influenced by changes in intraspecific genetic structure. Although the ecological significance of parrotfish as an important group in coral reef ecosystems has received increasing attention, the genetic structure of parrotfish populations has been relatively little studied. Nevertheless, genetic divergence among parrotfish populations has been observed in previous studies. For example, there are significant genetic differences between the *C. sordidus* populations in the Indian and the Pacific Oceans [45]. *Scarus psittacus* populations had significant intraspecific genetic variation in the Indo-Pacific [46]. *Scarus trispinosus* from the Brazilian coast exhibited subtle genetic substructures at different latitudes [47]. Moreover, it was shown that some reef fish with a high dispersal capacity exhibited high genetic divergence among populations [48]. Parrotfish typically move less than 0.5–10 km and have a high dispersal capacity compared to other reef fish [49]. Therefore, the large genetic distance within some parrotfish species in this study may be affected by the population structure changes caused by their migration.

Moreover, the presence of cryptic species is also possible. The overestimation of the ABGD analyses may indicate that some potential cryptic species may be present in these parrotfish, especially *S. forsteni* and *S. globiceps*. On the NJ tree, some *S. forsteni* and *S. globiceps* individuals did not gather in a branch with most of the same species, but were scattered among other species of the genus *Scarus*. Some studies have shown that when closely related species of the same genus live in the same place or in a similar environment,

hybridization may occur due to the lack of effective isolation mechanisms and the increased probability of gene introgression between each other [50,51]. It should be noted that the dispersed *S. forsteni* and *S. globiceps* were collected from Qilianyu, which contains almost all of the species in this study and is the most abundant coral reef. Examples of interspecific hybridization among coral reef fish, including parrotfish, are numerous and not new in the Indo-Pacific [52–54]. Furthermore, some previous studies on the Eastern Pacific parrotfish have shown that regional interspecific hybridization has a profound effects on the species composition of local parrotfish [54]. Therefore, cryptic species or changes in genetic structure caused by interspecific hybridization could be a potential reason for the dispersal of *S. forsteni* and *S. globiceps* individuals and the overestimation of ABGD in this study.

Parrotfish are an important component of coral reef systems, and without their mutual interactions, coral reefs would decrease their merit for other organisms and eventually cease to exist. Worldwide, including in the SCS, the survival of parrotfish and coral reefs does not look promising due to human activities [6]. DNA barcoding, as a molecular biometric method, plays an important role in marine biodiversity conservation. However, parrotfish lack important DNA barcoding sequence data. In this study, a total of 23 parrotfish species, accounting for 64% of the SCS species, were collected, and 1620 DNA barcoding sequences were obtained. This provides a reasonable number of parrotfish DNA barcode sequences. In addition, a DNA barcoding database of parrotfish in the SCS was established, which provides data support for the conservation of parrotfish diversity and even the coral reefs' ecosystem.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su142215386/s1. Table S1: Samples Information; Table S2: OTUs numbers based on ABGD analysis; Table S3: BINs details for all samples.

Author Contributions: Conceptualization, Y.X., T.W. and Y.L.; Funding acquisition, C.L. and Y.L.; Investigation, Y.X., T.W., L.L., J.G. and Q.Q.; Methodology, Y.X., T.W. and L.L.; Writing—Original draft, Y.X.; Writing—Review and editing, Y.X., T.W. and Y.L. The manuscript and the order of authors listed in the manuscript has been read and approved by all named authors. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by National Key R&D Program of China (2018YFD0900803), National Key R&D Program of China (2019YFD0901201), National Natural Science Foundation of China (31702351), Fundamental and Applied Fundamental Research Major Program of Guangdong Province (2019B030302004-05), Hainan Provincial Natural Science Foundation (322CXTD530), Financial Fund of the Ministry of Agriculture and Rural Affairs, China (NFZX2021), Central Public-interest Scientific Institution Basal Research Fund, CAFS (2020TD16), Central Public-interest Scientific Institution Basal Research Fund, South China Sea Fisheries Research Institute, CAFS (2019TS28, 2021SD04).

Institutional Review Board Statement: The animal study protocol was approved by The Committee Laboratory Animal Welfare and Ethics of South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences (nhdf2022-14 and 13th October 2022).

Informed Consent Statement: Not applicable.

Data Availability Statement: All sequences and associated voucher data are available from BOLD (project NHSHJ "DNA Barcodes of fish from coral reefs in the SCS") and GenBank (accession numbers: OK346639-OK347213, OK347216-OK348260).

Acknowledgments: We are grateful for the assistance from Daning Li, Zhenhua Long and Da Huo of the Xisha Marine Science Comprehensive Experimental Station, South China Sea Institute of Oceanology, Chinese Academy of Sciences. We are also very grateful to many graduate students for their help in the collection of samples.

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

References

1. Wilkinson, C.R. *Status of Coral Reefs of the World: 2008*; Australian Institute of Marine Science: Townsville, Australia, 2008.
2. Hong, X.; Guoning, S.; Baolin, L.; Gang, C.; Shuyi, D.; Longwei, Q.; Qi, L.; Jinxiong, L.; Jian, S.; Xiaoqi, Z. Coral-coral reefs in China seas: The biodiversity characteristics of coral-coral reefs in the central South China Sea. *J. Palaeogeogr. Chin. Ed.* **2021**, *23*, 771–788.
3. Hughes, T.P.; Huang, H.U.I.; Young, M.A.L. The wicked problem of China's disappearing coral reefs. *Conserv. Biol.* **2013**, *27*, 261–269. [CrossRef] [PubMed]
4. Li, Y.Z.; Shi, Y.R.; Ai, H.; Dong, L.N.; Li, N.N.; Li, X.; Gao, T.X. Large scale distribution patterns of taxonomic diversity of fish in coral reef waters, South China Sea. *J. Fish. Sci. China* **2011**, *18*, 619–628. [CrossRef]
5. Wang, Z.M.; Kong, X.Y.; Huang, L.M. Taxonomic revision of parrotfishes (Perciformes: Scaridae) in China seas. *J. Trop. Oceanogr.* **2013**, *32*, 22–32.
6. Quan, Q.M.; Wang, T.; Liu, Y.; Guo, J.T.; Xie, Y.F.; Li, C.R.; Li, C.H. The community structure of parrotfish in typical islands and reefs of the South China Sea. *Chin. J. Ecol.* **2021**, *40*, 2133–2145.
7. Mumby, P.J. Herbivory versus corallivory: Are parrotfish good or bad for Caribbean coral reefs? *Coral Reefs* **2009**, *28*, 683–690. [CrossRef]
8. Rotjan, R.D.; Lewis, S.M. Parrotfish abundance and selective corallivory on a Belizean coral reef. *J. Exp. Mar. Biol. Ecol.* **2006**, *335*, 292–301. [CrossRef]
9. Sánchez, J.A.; Gil, M.F.; Chasqui, L.H.; Alvarado, E.M. Grazing dynamics on a Caribbean reef-building coral. *Coral Reefs* **2004**, *23*, 578–583.
10. Bruckner, A.W.; Bruckner, R.J.; Sollins, P. Parrotfish predation on live coral: "spot biting" and "focused biting". *Coral Reefs* **2000**, *19*, 50. [CrossRef]
11. Mumby, P.J. The impact of exploiting grazers (Scaridae) on the dynamics of Caribbean coral reefs. *Ecol. Appl.* **2006**, *16*, 747–769. [CrossRef]
12. Gromova, E.S.; Makhotin, V.V. Morphofunctional Features of the Visceral Apparatus in Ember Parrotfish Scarus rubroviolaceus (Scaridae). *J. Ichthyol.* **2020**, *60*, 204–229. [CrossRef]
13. Roos, N.C.; Longo, G.O.; Pennino, M.G.; Francini-Filho, R.B.; Carvalho, A.R. Protecting nursery areas without fisheries management is not enough to conserve the most endangered parrotfish of the Atlantic Ocean. *Sci. Rep.* **2020**, *10*, 19143. [CrossRef] [PubMed]
14. Brock, R.E. An experimental study on the effects of grazing by parrotfishes and role of refuges in benthic community structure. *Mar. Biol.* **1979**, *51*, 381–388. [CrossRef]
15. Szmant, A.M. Nutrient enrichment on coral reefs: Is it a major cause of coral reef decline? *Estuaries* **2002**, *25*, 743–766. [CrossRef]
16. Hou, G.; Chen, W.T.; Lu, H.S.; Cheng, F.; Xie, S.G. Developing a DNA barcode library for perciform fishes in the South China Sea: Species identification, accuracy and cryptic diversity. *Mol. Ecol. Resour.* **2018**, *18*, 137–146. [CrossRef] [PubMed]
17. Choat, J.H.; Robertson, D.R. *Protogynous Hermaphroditism in Fishes of the Family Scaridae*; Springer: Berlin/Heidelberg, Germany, 1975; pp. 263–283.
18. Shen, Y.; Hubert, N.; Yan, H.; Wang, X.; He, S. DNA barcoding the ichthyofauna of the Yangtze River: Insights from the molecular inventory of a mega-diverse temperate fauna. *Mol. Ecol. Resour.* **2018**, *19*, 1278–1291. [CrossRef]
19. Ferri, G.; Alu, M.; Corradini, B.; Licata, M.; Beduschi, G. Species Identification Through DNA 'Barcodes'. *Genet. Test.* **2009**, *13*, 421–426. [CrossRef] [PubMed]
20. Hebert, P.D.N.; Cywinska, A.; Ball, S.L.; deWaard, J.R. Biological identifications through DNA barcodes. *Proc. B* **2003**, *270*, 313–321. [CrossRef]
21. Hebert, P.D.; Ratnasingham, S.; De Waard, J.R. Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **2003**, *270*, S96–S99. [CrossRef]
22. Chase, M.W.; Salamin, N.; Wilkinson, M.; Dunwell, J.M.; Kesanakurthi, R.P.; Haidar, N.; Savolainen, V. Land plants and DNA barcodes: Short-term and long-term goals. *Philos. Trans. R. Soc. B Biol. Sci.* **2005**, *360*, 1889–1895. [CrossRef]
23. Mir, R.A.; Bhat, K.A.; Rashid, G.; Ebinezer, L.B.; Masi, A.; Rakwal, R.; Shah, A.; Zargar, S.M. DNA barcoding: A way forward to obtain deep insights about the realistic diversity of living organisms. *Nucleus* **2021**, *64*, 157–165. [CrossRef]
24. Schoch, C.L.; Seifert, K.A.; Huhndorf, S.; Robert, V.; Spouge, J.L.; Levesque, C.A.; Chen, W.; Consortium, F.B. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 6241–6246. [CrossRef]
25. Shadrin, D. DNA Barcoding: Applications. *Russ. J. Genet.* **2021**, *57*, 489–497. [CrossRef]
26. Yancy, H.F.; Zemlak, T.S.; Mason, J.A.; Washington, J.D.; Tenge, B.J.; Nguyen, N.L.T.; Barnett, J.D.; Savary, W.E.; Hill, W.E.; Moore, M.M. Potential use of DNA barcodes in regulatory science: Applications of the Regulatory Fish Encyclopedia. *J. Food Prot.* **2008**, *71*, 210–217. [CrossRef] [PubMed]
27. Zheng, B.S. *Fishes of South China Sea*; Science Press: Beijing, China, 1962.
28. Yang, J.J. *The Fishes of the Islands in the South China Sea*; Science Press: Beijing, China, 1979.
29. Ward, R.; Zemlak, T.; Innes, B.; Last, P.; Hebert, P. DNA barcoding Australia's fish species. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* **2005**, *360*, 1847–1857. [CrossRef]

30. Sudhir, K.; Glen, S.; Koichiro, T. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870–1874.
31. Shen, Y.J.; Guan, L.H.; Wang, D.Q.; Gan, X.N. DNA barcoding and evaluation of genetic diversity in Cyprinidae fish in the midstream of the Yangtze River. *Ecol. Evol.* **2016**, *6*, 2702–2713. [CrossRef]
32. Ratnasingham, S.; Hebert, P.D.N. BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). *Mol. Ecol. Notes* **2007**, *7*, 355–364. [CrossRef]
33. Puillandre, N.; Lambert, A.; Brouillet, S.; Achaz, G. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Mol. Ecol.* **2012**, *21*, 1864–1877. [CrossRef]
34. Rambaut, A. *FigTree v1. 4.0. A Graphical Viewer of Phylogenetic Trees*; University of Edinburgh: Edinburgh, UK, 2012.
35. Meyer, C.P.; Paulay, G. DNA Barcoding: Error Rates Based on Comprehensive Sampling. *PLoS Biol.* **2005**, *3*, e422. [CrossRef]
36. Landi, M.; Dimech, M.; Arculeo, M.; Biondo, G.; Martins, R.; Carneiro, M.; Carvalho, G.R.; Brutto, S.L.; Costa, F.O. DNA barcoding for species assignment: The case of Mediterranean marine fishes. *PLoS ONE* **2014**, *9*, e106135. [CrossRef] [PubMed]
37. Weigt, L.A.; Baldwin, C.C.; Driskell, A.; Smith, D.G.; Ormos, A.; Reyier, E.A. Using DNA barcoding to assess Caribbean reef fish biodiversity: Expanding taxonomic and geographic coverage. *PLoS ONE* **2012**, *7*, e41059. [CrossRef] [PubMed]
38. Walczyńska, K.S.; Mańko, M.K.; Weydmann, A. Arctic Ocean biodiversity and DNA barcoding—A climate change perspective. In *YOUMARES 8–Oceans across Boundaries: Learning from Each Other*; Springer: Cham, Switzerland, 2018; pp. 145–153.
39. Hebert, P.D.N.; Stoeckle, M.Y.; Zemlak, T.S.; Francis, C.M.; Godfray, C. Identification of birds through DNA barcodes. *PLoS Biol.* **2004**, *2*, e312. [CrossRef] [PubMed]
40. Wang, L.; Wu, Z.; Liu, M.; Liu, W.; Zhao, W.; Liu, H.; You, F. DNA barcoding of marine fish species from Rongcheng Bay, China. *PeerJ* **2018**, *6*, e5013. [CrossRef]
41. Rock, J.; Costa, F.O.; Walker, D.I.; North, A.W.; Hutchinson, W.F.; Carvalho, G.R. DNA barcodes of fish of the Scotia Sea, Antarctica indicate priority groups for taxonomic and systematics focus. *Antarct. Sci.* **2008**, *20*, 253–262. [CrossRef]
42. Neigel, J.; Domingo, A.; Stake, J. DNA barcoding as a tool for coral reef conservation. *Coral Reefs* **2007**, *26*, 487. [CrossRef]
43. Collins, R.A.; Cruickshank, R.H. The seven deadly sins of DNA barcoding. *Mol. Ecol. Resour.* **2013**, *13*, 969–975. [CrossRef]
44. Čandek, K.; Kuntner, M. DNA barcoding gap: Reliable species identification over morphological and geographical scales. *Mol. Ecol. Resour.* **2015**, *15*, 268–277. [CrossRef]
45. Bay, L.K.; Choat, J.H.; Herwerden, L.; Robertson, D.R. High genetic diversities and complex genetic structure in an Indo-Pacific tropical reef fish (*Chlorurus sordidus*): Evidence of an unstable evolutionary past? *Mar. Biol.* **2004**, *144*, 757–767. [CrossRef]
46. Winters, K.L.; Herwerden, L.V.; Choat, J.H.; Robertson, D.R. Phylogeography of the Indo-Pacific parrotfish Scarus psittacus: Isolation generates distinctive peripheral populations in two oceans. *Mar. Biol.* **2010**, *157*, 1679–1691. [CrossRef]
47. Bezerra, I.M.; Gramacho, K.P.; Barreto, M.A.; Hackradt, C.W.; Leão Feitosa, J.L.; Torres, R.A.; Ferreira, B.P.; González-Wanguemert, M.; Félix-Hackradt, F.C. Genetic diversity and gene flow of the threatened Brazilian endemic parrotfish Scarus trispinosus (Valenciennes, 1840). *Mar. Environ. Res.* **2018**, *142*, 155–162. [CrossRef] [PubMed]
48. Visram, S.; Yang, M.C.; Pillay, R.M.; Said, S.; Henriksson, O.; Grahn, M.; Chen, C.A. Genetic connectivity and historical demography of the blue barred parrotfish (*Scarus ghobban*) in the western Indian Ocean. *Mar. Biol.* **2010**, *157*, 1475–1487. [CrossRef]
49. Green, A.L.; Maypa, A.P.; Almany, G.R.; Rhodes, K.L.; Weeks, W.; Abesamis, R.A. Larval dispersal and movement patterns of coral reef fishes, and implications for marine reserve network design. *Biol. Rev.* **2015**, *90*, 1215–1247. [CrossRef]
50. Gong, Y.Y.; Zhang, Q.; Cao, Y.; Lv, J.L.; Yang, X.S. Application of Mitochondrial Cytochrome Oxidase Subunit I sequence in DNA barcoding *Upeneus* in the South China Sea. *Mar. Fish.* **2016**, *38*, 113–119.
51. Bostrom, M.A.; Collette, B.B.; Luckhurst, B.E.; Reece, K.S.; Graves, J.E. Hybridization between two serranids, the coney (Cephalopholis fulva) and the creole-fish (*Paranthias furcifer*), at Bermuda. *Fish. Bull.* **2002**, *100*, 651–661.
52. Hobbs, J.-P.A.; Frisch, A.J.; Allen, G.R.; Van Herwerden, L. Marine hybrid hotspot at Indo-Pacific biogeographic border. *Biol. Lett.* **2009**, *5*, 258–261. [CrossRef]
53. DiBattista, J.D.; Whitney, J.; Craig, M.T.; Hobbs, J.-P.A.; Rocha, L.A.; Feldheim, K.A.; Berumen, M.L.; Bowen, B.W. Surgeons and suture zones: Hybridization among four surgeonfish species in the Indo-Pacific with variable evolutionary outcomes. *Mol. Phylogenetics Evol.* **2016**, *101*, 203–215. [CrossRef]
54. Carlon, D.B.; Robertson, D.R.; Barron, R.L.; Choat, J.H.; Anderson, D.J.; Schwartz, S.A.; Sánchez-Ortiz, C.A. The origin of the parrotfish species Scarus compressus in the Tropical Eastern Pacific: Region-wide hybridization between ancient species pairs. *BMC Ecol. Evol.* **2021**, *21*, 7. [CrossRef]