Journal of *Marine Science and Engineering*

MDPI

Article

DNA Barcoding Is a Useful Tool for the Identification of the Family *Scaridae* in Hainan

Bo Liu [1,2], Yali Yan [1], Nan Zhang [1,3], Huayang Guo [1,3], Baosuo Liu [1,3], Jingwen Yang [1], Kecheng Zhu [1,3] and Dianchang Zhang [1,3,4,5,*]

1 South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Key Laboratory of South China Sea Fishery Resources Exploitation and Utilization, Ministry of Agriculture and Rural Affairs, Guangzhou 510300, China
2 College of Fisheries and Life Science, Shanghai Ocean University, Shanghai 201306, China
3 Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), Guangzhou 511458, China
4 Tropical Aquaculture Research and Development Center, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Sanya 572018, China
5 Sanya Tropical Fisheries Research Institute, Sanya 572018, China
* Correspondence: zhangdch@scsfri.ac.cn

Abstract: Species markers can be quickly and accurately assessed using DNA barcoding. We investigated samples from the parrotfish family *Scaridae* using DNA barcoding in Hainan. A total of 401 DNA barcodes were analyzed, including 51 new barcodes generated from fresh material, based on a 533 bp fragment of the cytochrome c oxidase subunit I (*CO* I) gene. There were 350 *CO* I barcode clusters that matched 43 species from the Barcode of Life Data Systems (BOLD) and GenBank databases. The results showed the following average nucleotide compositions for the complete dataset: adenine (A, 22.7%), thymine (T, 29.5%), cytosine (C, 29.5%), and guanine (G, 18.2%). The mean genetic distance between confamilial species was nearly 53-fold greater than that between individuals within the species. In the neighbor-joining tree of *CO* I sequences, *Chlorurus sordidus* and *C. spilurus* clustered together, and all other individuals clustered by species. Our results indicated that DNA barcoding could be used as an effective molecular tool for monitoring, protecting, and managing fisheries, and for elucidating taxonomic problem areas that require further investigation.

Keywords: *Scaridae*; DNA barcoding; cytochrome c oxidase subunit I (*CO* I)

Citation: Liu, B.; Yan, Y.; Zhang, N.; Guo, H.; Liu, B.; Yang, J.; Zhu, K.; Zhang, D. DNA Barcoding Is a Useful Tool for the Identification of the Family *Scaridae* in Hainan. *J. Mar. Sci. Eng.* **2022**, *10*, 1915. https://doi.org/10.3390/jmse10121915

Academic Editor: Francesco Tiralongo

Received: 11 November 2022
Accepted: 18 November 2022
Published: 6 December 2022

1. Introduction

Parrotfish (family *Scaridae*), belonging to Actinopterygii: *Perciformes*, are herbivorous fish that live in tropical and subtropical coral reefs and are relatively abundant in biomass [1]. They play a crucial role in coral reef ecosystems [2,3], and as consumers of benthic algae, directly affect the structure and composition of benthic communities, and positively affect coral survival and growth [4]. Parrotfish are also involved in calcium carbonate cycling in coral reefs [5,6], and decompose coral reef skeletons into sand-sized sediments [7,8]. They maintain a coral-dominated community structure by feeding on fast-growing algae and can also influence reef development and complexity by decomposing reef carbonates [2,9]. Thus, sediments produced through parrotfish activities are an important source of island construction and maintenance in atoll coral reef environments, particularly in the setting of current rising sea levels and changes in island morphology [10]. The Healthy Reef Initiative (http://www.healthyreefs.org/cms/; accessed on 1 June 2022) uses parrotfish biomass as one of the key indicators in their coral reef health reports [11]. They have beautiful body shapes and bright colors, which greatly enhance the aesthetic quality of coral reefs and improve their economic value through ecotourism [3]. They are used as important food resources, and their biomass has recently decreased because of increased fishing [12,13].

Parrotfish, which are widely distributed in tropical, subtropical, and temperate areas [14], include approximately 100 species, divided among 10 genera [15]. There are 33 species of *Scaridae* in Taiwan and 31 species in mainland China [16]. The fish are known to change sex (from female to male) and color (inpectoral fin color from brown to blue) during growth [12,17]. Additionally, parrotfish have a behavioral mode called "grouping crypsis" (http://fishdb.sinica.edu.tw/; accessed on 1 June 2022). Juvenile parrotfish are discolored because of their social behavior. When they swim together, regardless of whether they are of the same species, their body color responds to the majority, becoming consistently grayish-brown, or exhibiting a longitudinal gradient in these colors. Once alone, juvenile fish instantly display beautiful and bright colors that blend into the surrounding environment, and these color changes occur very quickly. These extremely variable characteristics lead to difficulties in the morphological identification of *Scaridae*.

In the last decade, research on parrotfish has largely focused on their role and the mechanisms of their effects on coral reef ecosystems [9,18]. These fish can be excavators, scrapers, or grazers [19]. With increasing concern about parrotfish populations, the focus has been on coral reef health [20]. Furthermore, some researchers have explored the feeding ecology, habitat, diet, and habitat shifts of parrotfish by examining their fatty acid concentrations, composition, and levels [17]. Therefore, based on the economic, development and conservation of parrotfish, it is necessary to accumulate basic information about parrotfish and develop appropriate conservation and management measures [21]. A study using otoliths to identify parrotfish found that small otoliths were most similar to large *Scarus oviceps*, and least similar to large *Hipposcarus longiceps* [22]. However, there are several obvious shortcomings in the identification of otoliths: (i) there is no complete database of otolith morphology; (ii) because of the wide variety of parrotfish, it is difficult to find enough variability in otolith shape to identify species; and (iii) polishing otoliths is time-consuming, and the loss is large. Much experience is required to accurately identify otoliths.

In the past two decades, molecular techniques have become a popular and critical method for identifying species and resolving taxonomic ambiguities [23]. Molecular methods are useful in elucidating phylogenetic relationships and evolutionary patterns for biological ecology where classical morphological methods are not applicable [24]. DNA barcoding methods have been used to complement or refine morphological species identifications [25,26]. Studies have shown that the identification and discrimination of DNA barcoding are accurate and rapid [27–29]. DNA barcoding has been used to identify species using cytochrome c oxidase subunit I (*CO* I) sequences [30]. Therefore, they have been widely used for species identification [31–34].

In this study, more parrotfish samples were investigated to further evaluate the effectiveness of DNA barcoding for distinguishing parrotfish. The objectives of this study were to examine the reliability of *CO* I as a DNA barcode in parrotfish gene composition, and to determine intra- and interspecific genetic distances, codon characteristics, and molecular phylogenetic trees. The DNA barcoding data generated can be used as an effective molecular tool to achieve better monitoring and conservation outcomes for the family *Scaridae*.

2. Material and Methods

2.1. Sample Collection

A total of 51 parrotfish were collected from Hainan and Sansha in China (collection information available in Table 1). All samples in this experiment were obtained with the assistance of local fishermen and buyers on 5 October 2018. Back muscles were collected from each sample and preserved in 95% ethanol prior to DNA extraction. All samples were preserved at the South China Sea Fisheries Research Institute, Chinese Academy of Fisheries Sciences, China.

Table 1. Voucher and sequence information for the 51 specimens. Process IDs are sequence numbers of voucher specimens in GenBank, and Voucher IDs are voucher numbers in the South China Sea Fisheries Research Institute. BOLD is a summary of identification based on the barcode sequence of each species obtained using the BOLD.

Genus	Species Studied	BOLD	Voucher ID	Process ID	Location
Calotomus	*Calotomus carolinus*	*Calotomus carolinus*	ss-18	MK765061	Sansha
Chlorurus	*Chlorurus japanensis*	*Chlorurus japanensis*	ss-67	MK765062	Sansha
	Chlorurus microrhinos	*Chlorurus microrhinos*	ss-53	MK765063	Sansha
	Chlorurus microrhinos	*Chlorurus microrhinos*	ss-54	MK765064	Sansha
	Chlorurus sordidus	*Chlorurus sordidus*	Ssfri-F0165-01	MK765065	Hainan
	Chlorurus sordidus	*Chlorurus sordidus*	Ssfri-F0165-02	MK765066	Hainan
	Chlorurus sordidus	*Chlorurus sordidus*	Ssfri-F0165-03	MK765067	Hainan
	Chlorurus sordidus	*Chlorurus sordidus*	Ssfri-F0165-04	MK765068	Hainan
	Chlorurus sordidus	*Chlorurus spilurus*	ss-16	MK765069	Sansha
	Chlorurus sordidus	*Chlorurus spilurus*	ss-44	MK765070	Sansha
	Chlorurus sordidus	*Chlorurus spilurus*	ss-45	MK765071	Sansha
Hipposcarus	*Hipposcarus longiceps*	*Hipposcarus longiceps*	ss-17	MK765072	Sansha
Scarus	*Scarus dimidiatus*	*Scarus dimidiatus*	ss-65	MK765073	Sansha
	Scarus chameleon	*Scarus chameleon*	Ssfri-F0158-02	MK765074	Hainan
	Scarus forsteni	*Scarus forsteni*	ss-48	MK765075	Sansha
	Scarus forsteni	*Scarus forsteni*	ss-57	MK765076	Sansha
	Scarus forsteni	*Scarus forsteni*	ss-66	MK765077	Sansha
	Scarus forsteni	*Scarus forsteni*	Ssfri-F0164-01	MK765078	Hainan
	Scarus forsteni	*Scarus forsteni*	Ssfri-F0164-02	MK765079	Hainan
	Scarus forsteni	*Scarus forsteni*	Ssfri-F0164-03	MK765080	Hainan
	Scarus forsteni	*Scarus forsteni*	Ssfri-F0164-04	MK765081	Hainan
	Scarus forsteni	*Scarus forsteni*	Ssfri-F0164-05	MK765082	Hainan
	Scarus forsteni	*Scarus forsteni*	Ssfri-F0164-06	MK765083	Hainan
	Scarus ghobban	*Scarus ghobban*	ss-14	MK765084	Sansha
	Scarus ghobban	*Scarus ghobban*	ss-15	MK765085	Sansha
	Scarus ghobban	*Scarus ghobban*	Ssfri-F0347-01	MK765086	Hainan
	Scarus ghobban	*Scarus ghobban*	Ssfri-F0347-02	MK765087	Hainan
	Scarus ghobban	*Scarus ghobban*	Ssfri-F0347-03	MK765088	Hainan
	Scarus ghobban	*Scarus ghobban*	Ssfri-F0347-04	MK765089	Hainan
	Scarus ghobban	*Scarus ghobban*	Ssfri-F0347-05	MK765090	Hainan
	Scarus niger	*Scarus niger*	ss-7	MK765091	Sansha
	Scarus niger	*Scarus niger*	ss-41	MK765092	Sansha
	Scarus niger	*Scarus niger*	ss-42	MK765093	Sansha
	Scarus oviceps	*Scarus oviceps*	ss-33	MK765094	Sansha
	Scarus oviceps	*Scarus oviceps*	ss-46	MK765095	Sansha
	Scarus psittacus	*Scarus psittacus*	Ssfri-F0171-01	MK765096	Hainan
	Scarus psittacus	*Scarus psittacus*	Ssfri-F0171-02	MK765097	Hainan
	Scarus globiceps	*Scarus rivulatus*	ss-93	MK765098	Sansha
	Scarus globiceps	*Scarus rivulatus*	Ssfri-F0161-03	MK765099	Hainan
	Scarus globiceps	*Scarus rivulatus*	Ssfri-F0161-04	MK7650100	Hainan
	Scarus globiceps	*Scarus rivulatus*	Ssfri-F0161-05	MK7650101	Hainan
	Scarus rivulatus	*Scarus rivulatus*	Ssfri-F0161-06	MK7650102	Hainan
	Scarus rivulatus	*Scarus rivulatus*	Ssfri-F0161-07	MK7650103	Hainan
	Scarus rivulatus	*Scarus rivulatus*	Ssfri-F0161-08	MK7650104	Hainan
	Scarus rivulatus	*Scarus rivulatus*	Ssfri-F0161-09	MK7650105	Hainan
	Scarus rivulatus	*Scarus rivulatus*	Ssfri-F0161-10	MK7650106	Hainan
	Scarus schlegeli	*Scarus schlegeli*	ss-20	MK7650107	Sansha
	Scarus schlegeli	*Scarus schlegeli*	ss-47	MK7650108	Sansha
	Scarus schlegeli	*Scarus schlegeli*	ss-51	MK7650109	Sansha
	Scarus schlegeli	*Scarus schlegeli*	ss-72	MK7650110	Sansha
	Scarus spinus	*Scarus spinus*	Ssfri-F0166-01	MK7650111	Hainan

2.2. DNA Extraction

DNA was extracted from the muscle samples using a HiPure Mollusc DNA Kit (Axygen Biosciences, San Francisco, CA, USA) according to the manufacturer's instructions. All DNA samples were stored at −20 °C, followed by polymerase chain reaction (PCR) amplification.

2.3. PCR and DNA Sequencing

Fragments of the mitochondrial *CO*I gene were amplified using the following universal fish barcoding primers: forward fish-F 5′-TCRACYAAYCAYAAAGAYATYGGCAC-3′ and reverse fish-R 5′-ACTTCAGGGTGACCGAAGAATCAGAA-3′. The total volume and thermal cycle sequences of the PCR were performed as described previously [35]. The amplified PCR products were checked for optimal fragment sizes on 1.5% agarose gels. The PCR products with a single bright band were sent to Beijing RuiBiotec (Beijing, China) for sequencing in both directions. All sequences were loaded onto BOLD in the project.

2.4. Analysis of the Utility of the BOLD as an Identification Tool for Parrotfish

A total of 350 sequences belonging to 43 species of parrotfish with genus and species names assigned were found in a search on BOLD. In the case of these records, the number of barcoded index numbers (BIN) associated with each species was recorded. If a species was associated with a BIN, an assessment was conducted to determine whether the BIN was associated with any other species.

2.5. Data Analysis

SSR Hunter 1.3 software was used to edit the sequences, Clustal X 1.83 was used to align all the sequences, and redundant sequences at both ends were removed. Mega 7.0 was used to analyze the nucleotide composition, number of mutation sites and codon composition of all sequences. Based on the Kimura 2-parameter (K2P) model, inter and intraspecific genetic distances were calculated, molecular phylogenetic trees were constructed using the neighbor-joining (NJ) method, and their confidence was tested by 1000 repeated samplings.

2.6. Ethics Statement

All experiments in this study were conducted in accordance with the regulations and guidelines established by the Animal Care and Use Committee of the South China Sea Fisheries Research Institute of the Chinese Academy of Fishery Sciences (No. SCSFRI96-253).

3. Results

3.1. Sequence Characteristics of CO I Gene Fragment

A total of 401 *CO* I sequences were obtained, representing 44 species and 10 genera. All the sequences were trimmed to a consensus length of 533 bp. The mean nucleotide compositions for the complete data set were as follows: 22.7% adenine (A), 29.5% thymine (T), 29.5% cytosine (C), and 18.2% guanine (G). The highest percentage of G-C (55.69%) was detected in the first codon, whereas the lowest (42.96%) was detected in the second codon (Table 2). Within the 533-bp nucleotide sequences in the complete data set, there were conserved sites (327, 61.53%), variable sites (204, 38.27%), parsimony-informative sites (194, 36.40%), and singleton sites (10, 1.88%). Transitional pairs (si = 458) were present in greater numbers than transversional pairs (sv = 52). The ratio of si/sv (R) was 21.00 for the data set (Table 2).

Table 2. Sequence variation of the *CO* I gene and average nucleotide frequencies of *CO* I partial sequences of *Scaridae* (%).

Domain	ii	si	sv	R	T	C	A	G	Total
1		458.00	52.00	21.00	29.5	29.5	22.7	18.2	530.9
1st	174.00	4.00	0.00	9.40	19.0	27.5	25.3	28.2	178.0
2nd	175.00	2.00	0.00	5.89	42.1	28.8	15.0	14.1	178.0
3rd	109.00	46.00	20.00	2.23	27.5	32.2	27.9	12.3	175.0

Note: ii = Invariant pairs; si = Transitional pairs; sv = Transversional pairs; R = si/sv.

3.2. Genetic Distance between Species and within Species

Intraspecific K2P distances ranged from 0.000 to 0.015, and most intraspecific genetic distances were below 0.01. There were four species with intraspecific genetic distances between 0.01 and 0.02 (Figure 1). The mean intraspecific genetic distance was 0.003. Among the 44 species, *Scarus flavipectoralis* and *Nicholsina usta* had the greatest interspecific genetic distance of 0.248, while *Chlorurus sordidus* and *C. spilurus* had the lowest interspecific genetic distance (0.002). Most interspecific genetic distances were above 0.1. Overall, the mean interspecific genetic distance was 0.159, nearly 53 times higher than that among individuals within the same species (Supplementary Table S1).

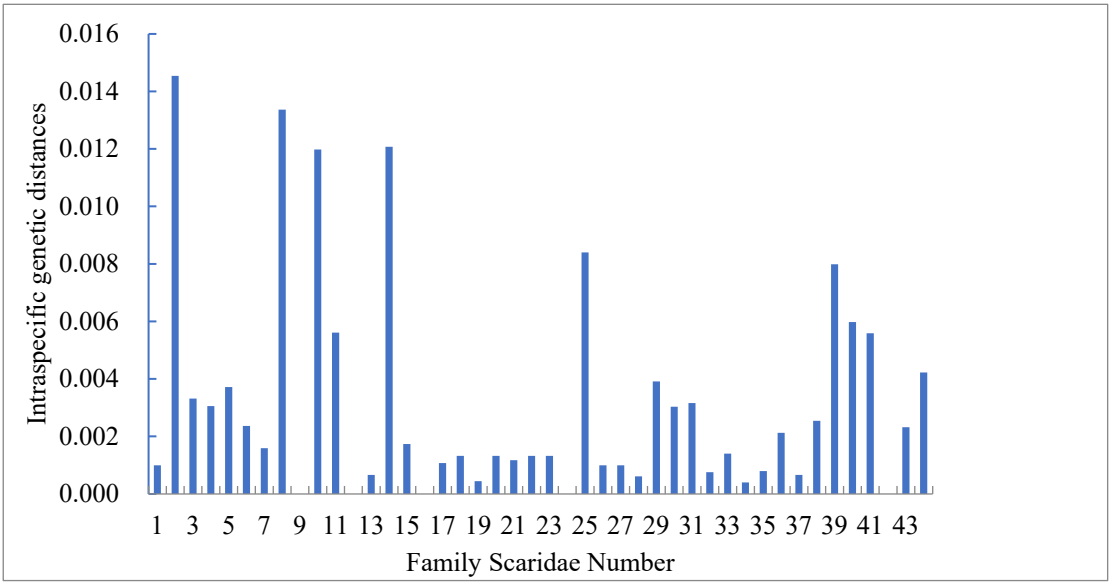

Figure 1. The intraspecific genetic distances of the family *Scaridae*. Note: The abscissa represents the species: 1. *Bolbometopon muricatum*, 2. *Cetoscarus ocellatus*, 3. *Hipposcarus longiceps*, 4. *Scarus iseri*, 5. *S. rubroviolaceus*, 6. *S. ghobban*, 7. *S. taeniopterus*, 8. *S. niger*, 9. *S. forsteni*, 10. *S. prasiognathos*, 11. *S. frenatus*, 12. *S. dimidiatus*, 13. *S. oviceps*, 14. *S. chameleon*, 15. *S. rivulatus*, 16. *S. globiceps*, 17. *S. quoyi*, 18. *S. flavipectoralis*, 19. *S. schlegeli*, 20. *S. fuscopurpureus*, 21. *S. psittacus*, 22. *S. pinus*, 23. *Chlorurus capistratoides*, 24. *C. japanensis*, 25. *C. bleekeri*, 26. *C. microrhinos*, 27. *C. frontalis*, 28. *C. spilurus*, 29. *C. sordidus*, 30. *Sparisoma radians*, 31. *S. aurofrenatum*, 32. *S. viride*, 33. *S. chrysopterum*, 34. *S. rubripinne*, 35. *S. rocha*, 36. *S. cretense*, 37. *S. atomarium*, 38. *Cryptotomus roseus*, 39. *Nicholsina denticulata*, 40. *N. usta*, 41. *Leptoscarus vaigiensis*, 42. *Calotlmus carolinus*, 43. *C. viridescens*, 44. *C. spinidens*.

3.3. Molecular Phylogenetic Tree

The NJ tree clustered *C. sordidus* and *C. spilurus* together, while the other individuals clustered by species (Figure 2). There were close relationships between *C. japanensis* and *C. capistratoides*, *C. carolinus*, and *C. viridescens*; *S. rivulatus* and *S. globiceps*; *S. rubroviolaceus* and *S. ghobban* and *S. schlegeli* and *S. ferrugineus*, which together formed a cohesive group with a moderately significant bootstrap value above 80%. Simultaneously, *Chlorurus*, *Cryp-*

totomus, *Nicholsina*, *Leptoscarus*, *Hipposcarus*, *Bolbometopon*, *Sparisoma*, *Calotomus*, *Cetoscarus*, and *Scarus* clustered into separate branches.

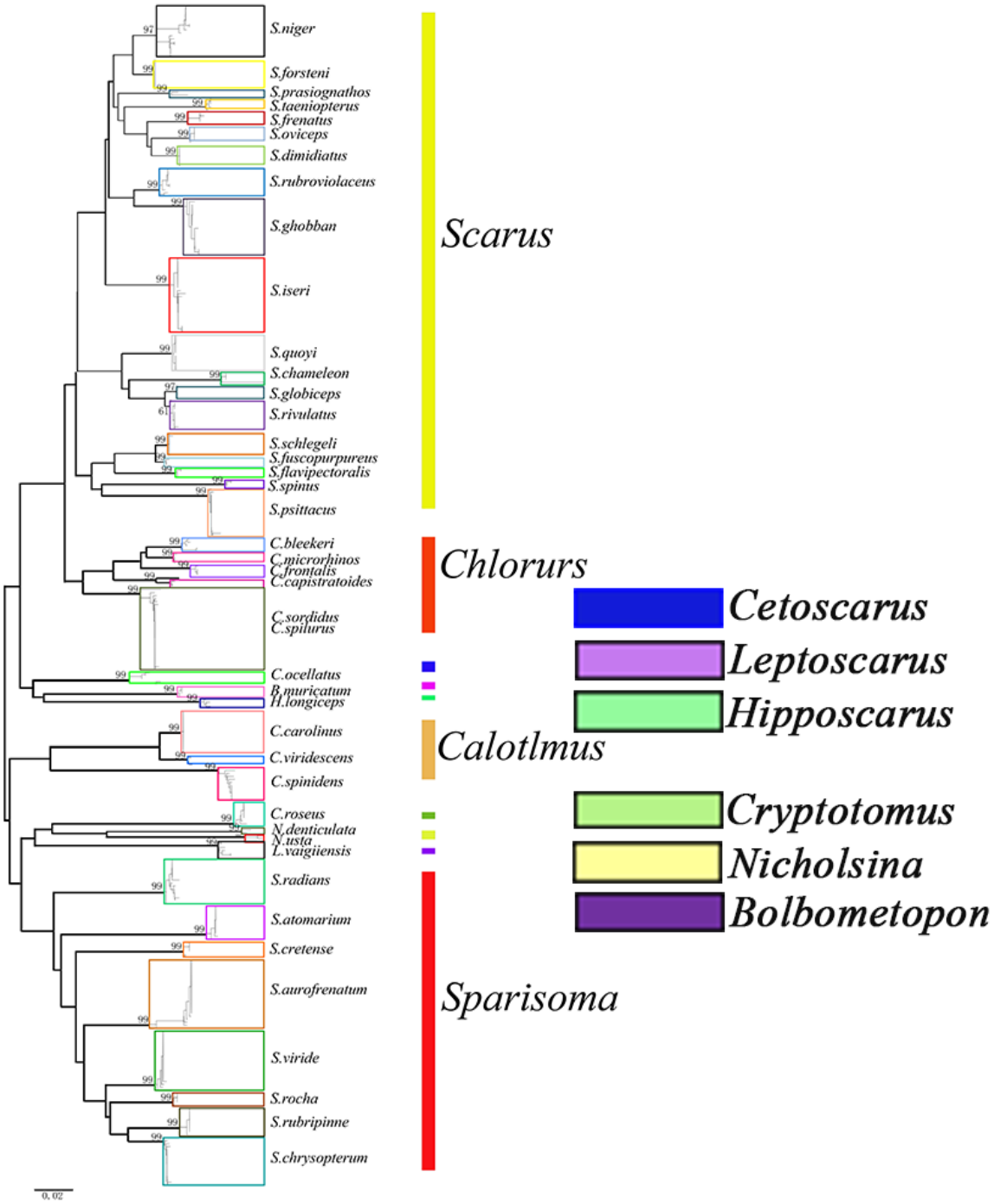

Figure 2. NJ tree resulting from analysis of the CO I gene for *Scaridae* species Bootstrap vaues Higher than 50% based on 1000 replicates are shown on the branches. The scale represents a genetic distance of 0.01 per million years.

3.4. New Country Records

Based on the samples collected, identified, and processed in this study, and a BLAST search in the BOLD and GenBank databases, three species, *C. carolinus*, *C. japanensis*, and *S. rivulatus*, are reported from mainland China for the first time. This increases the parrotfish diversity for mainland China species.

4. Discussion

CO I is commonly used as a barcode marker for animal species when the intraspecific K2P distance is below 1% and rarely exceeds 2% [36]. Hebert et al. suggested that the key point for the effective identification of species using *CO* I gene sequences is that the interspecific genetic distance must be greater than the intraspecific genetic distance, and the distances must differ by approximately 10 times [36,37]. The mean intraspecific genetic distance was 0.003 for the entire data set. The average interspecific genetic distance of the entire data set was 53 times that of average intraspecific genetic distance. The NJ tree clustered *C. sordidus* and *C. spilurus* together, and all other individuals clustered together by species, with high confidence. These results indicate that the *CO* I gene sequence can be used to effectively identify species in the family *Scaridae*.

On average, a G-C content of 47.75% was detected in the dataset. The base composition characteristics of the *CO* I gene were consistent with those reported for other teleosts, all of which had a GC content lower than AT content [38,39]. The first codon had the highest G + C content (48.3%), and the variation range was 53.39–56.74%. The second codon had the lowest G + C content (42.96%), and the variation range was 41.57–44.94%. The G + C content of the 3rd codon was 44.53%, ranging from 30.86–52.57%. In the CO I gene, si is often greater than sv, and the smaller the ratio R, the faster the evolutionary rate. In this study, the first and third codons had the largest and smallest R-values, respectively; the third codon had the fastest evolutionary rate, and the first codon the slowest. The possible explanation for this result might be that the variation range of G + C content is directly related to the evolution rate of codons. The larger the variation range of G + C content, the faster the evolution rate of codons.

The greatest genetic distance within the species was less than the smallest genetic distance between species, and a barcode gap was generated. Barcode gap is a key factor for the accurate identification of species from DNA barcodes. The intraspecific genetic distances of *C. sordidus* and *C. spirus* were 0.005 and 0.000, respectively. The interspecific genetic distance between the two species was 0.003. No barcode gaps were observed between the two species. In the NJ phylogenetic tree, *C. sordidus* and *C. spilurus* formed independent branches with a confidence level of 88. Therefore, the results of this study are identical to those of earlier studies, supporting the assertion that *C. spilurus* and *C. sordidus* are the same species [16].

The divergence between *Chlorurus* and *Scarus* was quite close, 6.0–7.4 mya, and the genera *Chloruus* and *Scarus* showed most of the variation after 3–5 million years [40]. However, Bellwood regarded the genera *Chlorurus* and *Scarus* as two distinct monophyletic lineages [41]. The topological structure reinforced the morphological diagnosis that these two genera belong to a monophyletic lineage, and together form a good clade [39]. Bayesian analysis was consistent with previous studies that provided strong support for confirming the identity of *Chlorurus* and *Scarus* [42]. In our study, *Chlorurus* and *Scarus* clustered into two independent branches, verifying the morphological diagnosis. In the phylogenetic tree, fish species of each genus formed an independent branch. Therefore, *CO* I is also suitable for identification at the genus level in the *Scaridae*.

The International Barcode of Life (iBOL, http://ibol.org/; accessed on 2 June 2022) is the global leader in DNA barcode work, determining species based on DNA barcodes, and sharing results freely [43]. Notably, the development of DNA barcode libraries is based on community efforts, and the use of the BOLD has led to DNA barcode technology being regarded as the standard for species recognition [38]. In BOLD, barcode sequences are stored and associated with other taxonomic data (voucher images, location data, etc.) to improve the accuracy of species recognition [44]. The BOLD has accelerated exchanges between countries worldwide, enabling global resources to be interoperable and species identification to be more standardized. BOLD is an accessible database for the analysis and search of DNA barcode data [45]. International life barcodes have several shortcomings: (i) Data sharing is not timely. Data provided by countries to the BLOD can be made public after only two years, because researchers hope to disclose the data only after analysis

or article publication. (ii) According to the BLOD standards for data management, each DNA barcode must have complete voucher specimen information, acquisition information, and the original files of the sequenced peak map. However, many research groups and researchers from China cannot strictly follow these requirements and standards, and the quality of the data is greatly reduced. (iii) The data were not updated in time to match the genetic sequences of the same species, which were still identified by a previous name. Therefore, the entries were not unified. Although *C. sordidus* and *C. spilurus* belong to the same genus, their genetic distance is very small, and *C. spilurensis* syn. nov. is therefore synonymized with *C. sordidus* [16], and they cluster together in a molecular evolutionary tree. However, when the sequences are aligned in BOLD, they do not have the same species names.

5. Conclusions

By analyzing the sequences of the *CO* I gene of 401 parrotfish, we found that the average intraspecific genetic distance was 0.003, and the average interspecific genetic distance was 0.159, approximately 53 times the average intraspecific genetic distance. The NJ tree shows that *C. sordidus* and *C. spilurus* of blue-headed green parrotfish gather together, and individuals of other species grouped together, with high support, and different species can be effectively distinguished. The results showed that DNA barcode technology based on the *CO* I gene could be used to identify species of *Scaridae*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jmse10121915/s1, Table S1: Mean percent genetic distances between Scaridae species under the corrected K2P.

Author Contributions: Data curation, B.L. (Bo Liu).; formal analysis, N.Z.; investigation, H.G.; methodology, J.Y.; resources, Y.Y., N.Z., H.G., B.L. (Baosuo Liu) and K.Z.; validation, B.L. (Baosuo Liu); visualization, J.Y.; writing—original draft, B.L. (Bo Liu); writing—review & editing, D.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Financial Fund of Ministry of Agriculture and Rural affairs of China (NHYYSWZZZYKZX2020), National Marine Genetic Resource Center, and China-ASEAN Maritime Cooperation Fund.

Institutional Review Board Statement: All experiments in this study were approved by the Animal Care and Use Committee of South China Sea fisheries Research Institute, Chinese Academy of fishery Sciences (no. SCSFRI96-253) and performed according to the regulations and guidelines established by this committee. Written informed consent was obtained from the owners for the participation of their animals in this study.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed con-sent has been obtained from the patients to publish this paper.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

Acknowledgments: This work was supported by the Financial Fund of Ministry of Agriculture and Rural affairs of China (NHYYSWZZZYKZX2020), National Marine Genetic Resource Center, and China-ASEAN Maritime Cooperation Fund.

Conflicts of Interest: No potential conflict of interest was reported by the authors.

References

1. Francini-Filho, R.B.; Moura, R.L. Evidence for spillover of reef fishes from a no-take marine reserve: An evaluation using the before-after control-impact (BACI) approach. *Fish. Res.* **2008**, *93*, 346–356. [CrossRef]
2. Bellwood, D.R.; Hoey, A.S.; Choat, J.H. Limited functional redundancy in high diversity systems: Resilience and ecosystem function on coral reefs. *Ecol. Lett.* **2003**, *6*, 281–285. [CrossRef]
3. Carlon, D.B.; LippÉ, C. Isolation and characterization of 17 new microsatellite markers for the ember parrotfish *Scarus rubroviolaceus*, and cross-amplification in four other parrotfish species. *Mol. Ecol. Notes* **2007**, *7*, 613–616. [CrossRef]

4. Mumby, P.J.; Dahlgren, C.P.; Harbome, A.R.; Kappel, C.V.; Micheli, F.; Brumbaugh, D.R.; Holmes, K.E.; Mendes, J.M.; Broad, K.; Sanchirico, J.N.; et al. Fishing, Trophic Cascades, and the Process of Grazing on Coral Reefs. *Science* **2006**, *311*, 98. [CrossRef]
5. Goatley, C.; Bellwood, D. Biologically mediated sediment fluxes on coral reefs: Sediment removal and off-reef transportation by the surgeonfish *Ctenochaetus striatus*. *Mar. Ecol. Prog. Ser.* **2010**, *415*, 237–245. [CrossRef]
6. Glynn, P.; Manzello, D. Bioerosion and coral reef growth: A dynamic balance. In *Coral Reefs in the Anthropocene*; Springer: Dordrecht, The Netherlands, 2015; pp. 67–97. [CrossRef]
7. Bellwood, D.R. Production and reworking of sediment by Parrotfish (family Scaridae) on the Great Barrier Reef, Australia. *Mar. Biol.* **1996**, *125*, 795–800. [CrossRef]
8. Perry, C.T.; Kench, P.S.; O'Leary, M.J.; Morgan, K.; Januchowski-Hartley, F.A. Linking reef ecology to island building: Parrotfish identified as major producers of island-building sediment in the Maldives. *Geology* **2015**, *43*, 503–506. [CrossRef]
9. Bellwood, D.R.; Hughes, T.P.; Folke, C.; Nystrom, M. Confronting the coral reef crisis. *Nature* **2004**, *429*, 827–833. [CrossRef] [PubMed]
10. Morgan, K.M.; Kench, P.S. Parrotfish erosion underpins reef growth, sand talus development and island building in the Maldives. *Sediment. Geol.* **2016**, *341*, 50–57. [CrossRef]
11. Vallès, H.; Oxenford, H.A. Simple family-level parrotfish indicators are robust to survey method. *Ecol. Indic.* **2018**, *85*, 244–252. [CrossRef]
12. Ebisawa, A.; Ohta, I.; Uehara, M.; Nakamura, H.; Kanashiro, K.; Yasui, R. Life history variables, annual change in sex ratios with age, and total mortality observed on commercial catch on Pacific steephead parrotfish, *Chlorurus microrhinos* in waters off the Okinawa Island, southwestern Japan. *Reg. Stud. Mar. Sci.* **2016**, *8*, 65–76. [CrossRef]
13. Edwards, C.B.; Friedlander, A.M.; Green, A.G.; Hardt, M.J.; Sala, E.; Sweatman, H.P.; Williams, I.D.; Zgliczynski, B.; Sandin, S.A.; Smith, J.E. Global assessment of the status of coral reef herbivorous fishes: Evidence for fishing effects. *Proc. Biol. Sci.* **2014**, *281*, 20131835. [CrossRef] [PubMed]
14. Carpenter, K.E.; Niem, V.H. (Eds.) FAO Species Identification Guide for Fishery Purposes. Bony Fishes Part 4 (Labridae to Latimeriidae), Estuarine Crocodiles, Sea Turtles, Sea Snakes and Marine Mammals. In *The Living Marine Resources of the Western Central Pacific*; FAO: Rome, Italy, 2001; Volume 6, pp. 468–3492. Available online: https://ci.nii.ac.jp/ncid/BA67023968 (accessed on 1 June 2022).
15. Nelson, J.S.; Grande, T.; Wilson, M.V.H. *Fishes of the World*; John Wiley & Sons: Hoboken, NJ, USA, 2016; Volume 15, pp. 429–430. [CrossRef]
16. Wu, H.L.; Shao, G.Z.; Lai, C.F.; Chong, D.H.; Lin, P.L. *Latin-Chinese Dictionary of Fish Names by Classification System*; China Ocean University Press: Qingdao, China, 2017; pp. 319–320. Available online: http://cpfd.cnki.com.cn/Article/CPFDTOTAL-ZGHI201209001002.htm (accessed on 2 June 2022).
17. Arai, T.; Amalina, R.; Bachok, Z. Similarity in the feeding ecology of parrotfish (Scaridae) in coral reef habitats of the Malaysian South China Sea, as revealed by fatty acid signatures. *Biochem. Syst. Ecol.* **2015**, *59*, 85–90. [CrossRef]
18. Rotjan, R.D.; Dimond, J.L. Discriminating causes from consequences of persistent parrotfish corallivory. *J. Exp. Mar. Biol. Ecol.* **2010**, *390*, 188–195. [CrossRef]
19. Francini-Filho, R.; Moura, R.; Ferreira, C.M.; Coni, E.O.C. Live coral predation by Parrotfish (Perciformes: Scaridae) in the Abrolhos Bank, eastern Brazil, with comments on the classification of species into functional groups. *Neotrop. Ichthyol.* **2008**, *6*, 191–200. [CrossRef]
20. Adam, T.C.; Deron, E.B.; Ruttenberg, B.I.; Paddack, M.J. Herbivory and the Resilience of Caribbean Coral Reefs: Knowledge Gaps and Implications for Management. *Mar. Ecol. Prog. Ser.* **2021**, *520*, 1–20. [CrossRef]
21. Roos, N.C.; Carvalho, A.R.; Lopes, P.F.M.; Pennino, M.G. Modeling sensitive parrotfish (Labridae: Scarini) habitats along the Brazilian coast. *Mar. Environ. Res.* **2015**, *110*, 92–100. [CrossRef]
22. Jawad, L.A. A comparative morphological investigation of otoliths of six parrotfish species (Scaridae) from the Solomon Islands. *J. Fish Biol.* **2018**, *93*, 1046–1058. [CrossRef]
23. Barman, A.S.; Singh, M.; Pandey, P.K. DNA barcoding and genetic diversity analyses of fishes of Kaladan River of Indo-Myanmar biodiversity hotspot. *Mitochondrial DNA A* **2018**, *29*, 367–378. [CrossRef]
24. Ritchie, P.; Bargelloni, L.; Meyer, A.; Taylor, J.A.; Macdonald, J.A.; Lambert, D.M. Mitochondrial Phylogeny of Trematomid Fishes (Nototheniidae, Perciformes) and the Evolution of Antarctic Fish. *Mol. Phylogenet. Evol.* **1996**, *5*, 383–390. [CrossRef]
25. Kochzius, M.; Seidel, C.; Antoniou, A.; Botla, S.K.; Campo, D.; Cariani, A.; Vazquez, E.G.; Hauschild, J.; Hervet, C.; Hjorleifsdottir, S.; et al. Identifying Fishes through DNA Barcodes and Microarrays. *PLoS ONE* **2010**, *5*, e12620. [CrossRef]
26. Chen, W.T.; Ma, X.H.; Shen, Y.J.; Mao, Y.T.; He, S.P. The fish diversity in the upper reaches of the Salween River, Nujiang River, revealed by DNA barcoding. *Sci. Rep.* **2015**, *5*, 17437. [CrossRef] [PubMed]
27. Ma, H.Y.; Ma, C.Y.; Ma, L.B. Molecular identification of genus Scylla (Decapoda: Portunidae) based on DNA barcoding and polymerase chain reaction. *Biochem. Syst. Ecol.* **2012**, *41*, 41–47. [CrossRef]
28. Khedkar, G.D.; Jamdade, R.; Naik, S.; David, L.; Haymer, D. DNA barcodes for the fishes of the Narmada, one of India's longest rivers. *PLoS ONE* **2014**, *9*, e105490. [CrossRef]
29. Dhar, B.; Ghosh, S.K. Genetic assessment of ornamental fish species from North East India. *Gene* **2015**, *555*, 382–392. [CrossRef]
30. Hebert, P.D.N.; Cywinska, A.; Ball, S.L.; de Waard, J.R. Biological identification through DNA barcodes. *Proc. R. Soc. Lond.* **2003**, *270*, 313–321. [CrossRef]

31. Stein, E.D.; Martinez, M.C.; Stiles, S.; Miller, P.E.; Zakharov, E.V. Is DNA Barcoding Actually Cheaper and Faster than Traditional Morphological Methods: Results from a Survey of Freshwater Bioassessment Efforts in the United States. *PLoS ONE* **2014**, *9*, e95525. [CrossRef] [PubMed]
32. Sethusa, M.T.; Yessoufou, K.; der Bank, M.V.; der Bank, H.V.; Sethusa, M.T.; Millar, I.M.; Jacobs, A. DNA barcode efficacy for the identification of economically important scale insects (Hemiptera: Coccoidea) in South Africa. *Afr. Entomol.* **2014**, *22*, 257–266. [CrossRef]
33. Decru, E.; Moelants, T.; De Gelas, K.; Vreven, E.; Verheyen, E.; Snoeks, J. Taxonomic challenges in freshwater fishes: A mismatch between morphology and DNA barcoding in fish of the north-eastern part of the Congo basin. *Mol. Ecol. Resour.* **2016**, *16*, 342–352. [CrossRef] [PubMed]
34. Nigro, L.M.; Angel, M.V.; Blachowiak-Samolyk, K.; Hopcroft, R.R.; Bucklin, A. Identification, Discrimination, and Discovery of Species of Marine Planktonic Ostracods Using DNA Barcodes. *PLoS ONE* **2016**, *11*, e0146327. [CrossRef] [PubMed]
35. Liu, B.; Yang, J.W.; Liu, B.S.; Zhang, N.; Guo, L.; Guo, H.Y.; Zhang, D.C. Detection and identification of marine fish mislabeling in Guangzhou's supermarkets and sushi restaurants using DNA barcoding. *J. Food Sci.* **2022**, *87*, 2440–2449. [CrossRef]
36. Hebert, P.D.N.; Ratnasingham, S.; de Waard, J.R. Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. Biol. Sci.* **2003**, *270*, 96–99. [CrossRef]
37. Zhang, Y.H.; Qin, G.; Zhang, H.X.; Wang, X.; Lin, Q. DNA barcoding reflects the diversity and variety of brooding traits of fish species in the family Syngnathidae along China's coast. *Fish. Res.* **2017**, *185*, 137–144. [CrossRef]
38. Chambers, E.A.; Hebert, P.D. Assessing DNA Barcodes for Species Identification in North American Reptiles and Amphibians in Natural History Collections. *PLoS ONE* **2016**, *11*, e0154363. [CrossRef] [PubMed]
39. Sun, L.Y.; Yang, T.Y.; Meng, W.; Yang, B.Q.; Zhang, T. Analysis of the mitochondrial genome characteristics and phylogenetic relationships of eight sciaenid fishes. *Mar. Sci.* **2017**, *41*, 48–54. Available online: https://oversea.cnki.net/HYKX201703007 (accessed on 2 June 2022).
40. Smith, L.L.; Fessler, J.L.; Alfaro, M.E.; Streelman, J.T.; Westneat, M.W. Phylogenetic relationships and the evolution of regulatory gene sequences in the Parrotfish. *Mol. Phylogenet. Evol.* **2008**, *49*, 136–152. [CrossRef] [PubMed]
41. Bellwood, D.R. A phylogenetic study of the parrotfish family Scaridae (Pisces: Labroidea), with a revision of genera. *Rec. Aust. Mus.* **1994**, *20*, 1–86. [CrossRef]
42. Choat, J.H.; Klanten, O.S.; Herwerden, L.V.; Robertson, D.R.; Clements, K.D. Patterns and processes in the evolutionary history of Parrotfish (Family Labridae). *Biol. J. Linn. Soc.* **2012**, *107*, 529–557. [CrossRef]
43. Ma, K.P. DNA barcode: From species to biome. *Biodivers. Sci.* **2015**, *23*, 279–280. [CrossRef]
44. Barco, A.; Raupach, M.J.; Laakmann, S.; Neumnn, H.; Knebelsberger, T. Identification of North Sea molluscs with DNA barcoding. *Mol. Ecol. Resour.* **2016**, *16*, 288–297. [CrossRef]
45. Zhang, J.B.; Hanner, R. DNA barcoding is a useful tool for the identification of marine fishes from Japan. *Biochem. Syst. Ecol.* **2011**, *39*, 31–42. [CrossRef]