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Genetic Diversity in Mitochondrial Control Region Among Some Parrot Fish Species from Egyptian Red Sea

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ABSTRACT

The mitochondrial DNA (mtDNA) Control Region (CR) gene, also known as the D-loop, proved effective in distinguishing five parrotfish species: *Hipposcarus harid*, *Chlorurus sordidus*, *Scarus ferrugineus*, *Scarus psittacus*, and *Scarus fuscopurpureus*. The Red Sea parrotfish have been relatively understudied due to the difficulties associated with morphological identification, making genetic markers a reliable and efficient alternative for species identification. In this study, DNA sequence lengths ranged from 806 bp in *H. harid* to 1,050 bp in *S. fuscopurpureus*. The sequences were deposited in GenBank/NCBI under accession numbers (PV166662.1–PV166666.1). The average nucleotide composition across all species was A= 30.90%, T= 30.37%, C= 24.71%, and G= 14.03%, with an average A+T content of 61.27%, exceeding the C+G content. Pairwise genetic distances among the studied parrotfish species ranged from 0.0060 between *S. psittacus* and *S. fuscopurpureus* to 0.891 between *H. harid* and *S. ferrugineus*. These relationships were further supported by phylogenetic analysis using both Minimum Evolution (ME) and Neighbor-Joining (NJ) methods, which confirmed the distinct clustering of the species.

INTRODUCTION

The Red Sea is home to over 1,270 fish species **(Khalaf *et al.*, 1996)**, most of which inhabit coral reefs where they comprise a substantial portion of the fish community. Fish species richness, assemblage composition, and abundance vary considerably across the Red Sea's distinct zones **(Sheppard *et al.*, 1992)**. The family Scaridae is a characteristic feature of coral reefs and is dominant in terms of biomass and abundance in shallow reef habitats, despite comprising only ~80 species across 10 genera **(Russ, 1984; Choat & Bellwood, 1991)**. Parrotfishes are important food resources in

many tropical regions and make significant contributions to reef fisheries production **(Johannes, 1981; Reeson, 1983; Bellwood, 1988)**.

Scarids are considered among the most ecologically important fishes on modern coral reefs due to their feeding activities **(Bellwood, 1995; Hughes *et al.*, 2007; Hoey & Bellwood, 2008)**. **Choat and Randall (1986)** proposed that they form a natural group based on ecological and taxonomic similarities. However, accurate identification remains challenging, particularly within the genus *Scarus*.

Most parrotfish species are protogynous hermaphrodites, exhibiting distinct coloration patterns at different growth stages and sexes. The initial phase (IP) includes females and primary males, while the terminal phase (TP) consists of secondary males following sex change. Globally, there are 79 recognized species of parrotfish **(Bellwood, 1994)**. Although coloration is a key diagnostic trait, it must be used cautiously; colors fade rapidly post-mortem, and preservation can alter appearance. Furthermore, scarids undergo complex ontogenetic color changes associated with sexual development and growth **(Choat & Robertson, 1975)**.

Genetic diversity plays a vital role in species adaptation and survival. High genetic variation provides a wider range of alleles for natural selection, enhancing resilience to environmental changes, whereas low diversity increases vulnerability **(Frankham, 1996; Frankham *et al.*, 2002)**. Morphological and genetic studies have examined parrotfish impacts on coral reefs **(Streelman *et al.*, 2002; Andrew *et al.*, 2012)**, yet genetic identification and evolutionary relationships remain underexplored. Highly polymorphic molecular tools such as Inter-Simple Sequence Repeats (ISSR) offer a reliable approach for monitoring parrotfish genetic diversity as a commercial and ecological resource.

Mitochondrial DNA (mtDNA) has been widely applied in phylogeography, which examines the spatial distribution of genetic diversity among closely related species **(Avise *et al.*, 1987; Avise, 2000; Avise, 2009)**, as well as in phylogenetic studies that assess evolutionary relationships across taxa **(Miya *et al.*, 2003; Inoue *et al.*, 2010; Cole *et al.*, 2019)**. **Mar'ie and Allam (2019)** demonstrated the usefulness of ISSR in differentiating six parrotfish species from the Egyptian Red Sea. In vertebrates, including fish, mtDNA is an extranuclear, closed circular double-stranded molecule composed of light and heavy strands **(Xiao & Zhang, 2000; Satoh *et al.*, 2016)**. The typical fish mitogenome is 15–18 kb, containing 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), two ribosomal RNAs (rRNAs), and one control region (CR or D-loop) **(Brown, 2008; Satoh *et al.*, 2016)**.

The objectives of this study were to (1) monitor genetic diversity in selected parrotfish species, (2) investigate their phylogenetic relationships, (3) estimate divergence times using mitochondrial genome data, and (4) expand scientific knowledge on parrotfish biodiversity by analyzing partial sequences of the mtDNA control region (D-loop).

MATERIALS AND METHODS

Sample preparation and *DNA* extraction

To assess the genetic diversity of the mitochondrial DNA (mtDNA) control region (CR) in the family Scaridae, five parrotfish species—*Hipposcarus harid*, *Chlorurus sordidus*, *Scarus ferrugineus*, *Scarus psittacus*, and *Scarus fuscopurpureus*—were collected from Hurghada, Red Sea, Egypt, and identified following the guidelines of prior studies **(Randall, 1982; Lin & Shao, 1999; Joshi *et al.*, 2011)**. Muscle tissue was dissected from the caudal peduncle, preserved at −20°C, and used for genomic DNA extraction. DNA was isolated from 15– 25mg of muscle tissue using the Biospin Genomic DNA Extraction Kit (Bioer, China), following the manufacturer's protocol.

PCR amplification of mtDNA control region

The mtDNA control region was amplified using primers designed by **Cheng *et al.* (2012)**. Each 50μL PCR reaction contained 1.0μL genomic DNA, 1.0μL of each forward and reverse primer, 25μL PCR Master Mix (Promega, USA), and nuclease-free water to volume. Thermal cycling conditions were as follows: initial denaturation at 94°C for 5 min; 30 cycles of denaturation at 94°C for 1min, annealing at 54°C for 1min, and extension at 72°C for 1min; and a final extension at 72°C for 5min. PCR products were visualized on 1.5% agarose gels stained with ethidium bromide and photographed under UV illumination.

DNA sequencing and phylogenetic analysis

PCR products were sequenced bidirectionally by Macrogen Inc. (Seoul, South Korea). The resulting sequences were deposited in GenBank/NCBI to obtain accession numbers. Multiple sequence alignment was performed in MUSCLE **(Edgar, 2004)** using default parameters. Phylogenetic relationships were reconstructed using two methods—Maximum Likelihood (ML) and Neighbor-Joining (NJ)—in MEGA v11 **(Tamura *et al.*, 2021)**. Bootstrap support was assessed with 1,000 replicates **(Felsenstein, 1985)**. Pairwise genetic distances were calculated using the Kimura 2-Parameter (K2P) model **(Kimura, 1980)**, and divergence patterns were visualized to represent interspecific variation.

RESULTS

PCR amplification and sequencing

PCR amplification and agarose gel electrophoresis of the mtDNA control region (CR) from five Scaridae species—*Hipposcarus harid*, *Chlorurus sordidus*, *Scarus ferrugineus*, *Scarus psittacus*, and *Scarus fuscopurpureus*—produced a single clear band for each species. The amplified fragment sizes ranged from 806 bp in *H. harid* to 1,050 bp in *S. fuscopurpureus*. The sequenced CR regions were deposited in GenBank/NCBI under the accession numbers (PV166662.1, PV166663.1, PV166664.1, PV166665.1,

PV166666.1), respectively. Sequence identity was confirmed using BLASTn at the National Center for Biotechnology Information (NCBI).

Base composition analysis revealed that adenine (A) content was the highest in all species, averaging 30.90%, except in *H. harid*, where it was 28.29%. Guanine (G) content was the lowest across all species, averaging 14.03%. The combined A+T content exceeded the combined G+C content in all species, with an overall mean of 61.27% (Table 1).

Table 1. Nucleotides bases content A+T% higher than G+C% in all samples

Specimens	Accession number	Base pair Length	Nucleotide Number %				A+T Content%
			A%	T%	C%	G%	
Hipposcarus harid	**PV166662.1**	**806**	**28.29**	**32.75**	**23.95**	**15.01**	**61.04**
Chlorurus sordidus	**PV166663.1**	**1014**	**31.07**	**29.09**	**26.63**	**13.21**	**60.16**
Scarus ferrugineus	**PV166664.1**	**1006**	**31.61**	**29.92**	**24.85**	**13.62**	**61.53**
Scarus psittacus	**PV166665.1**	**1014**	**31.46**	**30.38**	**23.96**	**14.2**	**61.84**
Scarus fuscopurpureus	**PV166666.1**	**1050**	**31.52**	**30.19**	**24**	**14.29**	**61.71**
Avg.		**978**	**30.9**	**30.37**	**24.7**	**14.03**	**61.27**

Pairwise nucleotide sequence alignments, showing match and mismatch scores, were performed in MEGA version 7.0.18 **(Kumar *et al.*, 2016)** using the Kimura 2-Parameter model with gamma correction. The overall genetic distance value among all examined species was 0.007%.

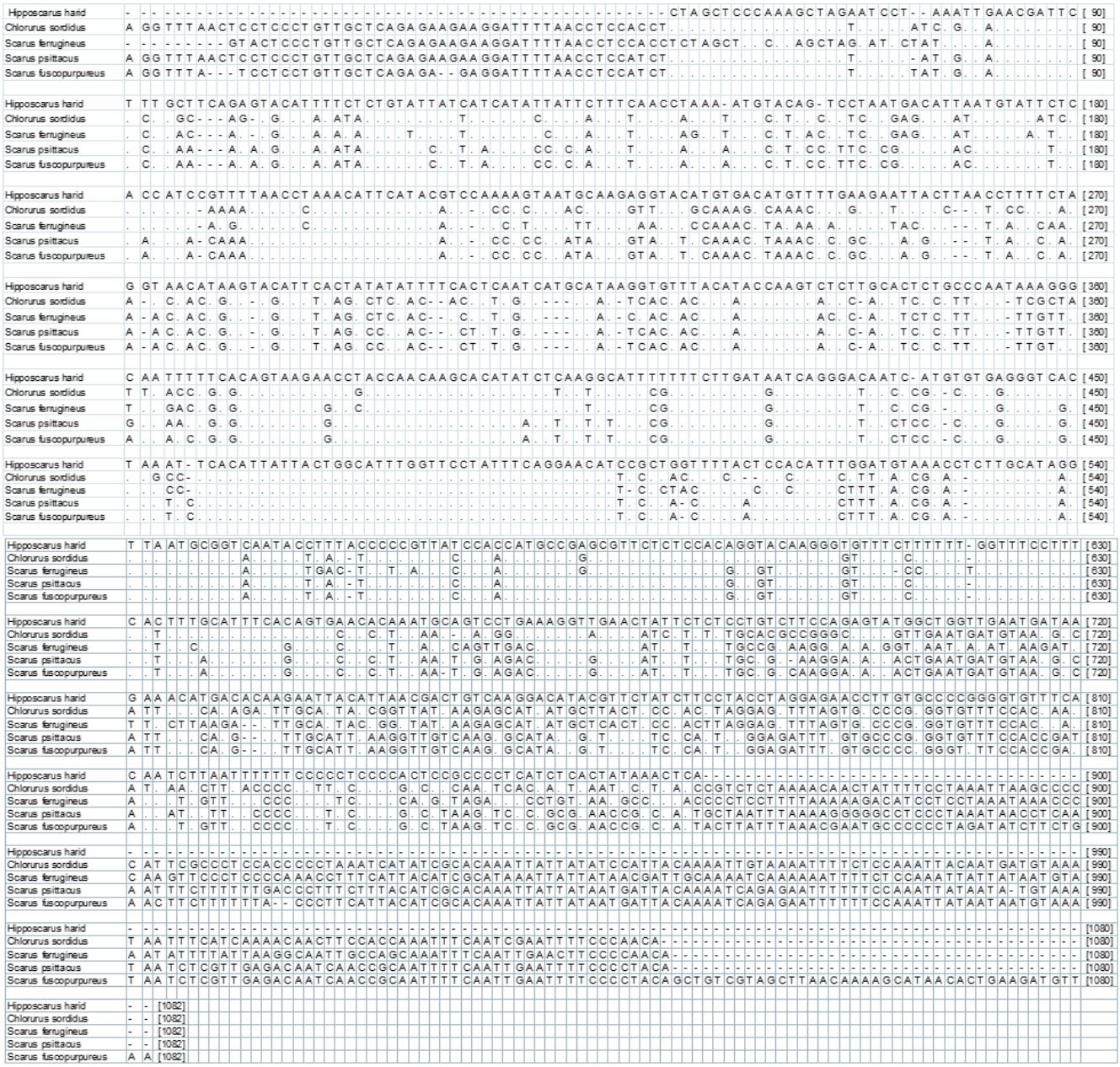

Fig. 1. Alignment of *mtDNA (CR)* control region sequences amongst five parrot fish species

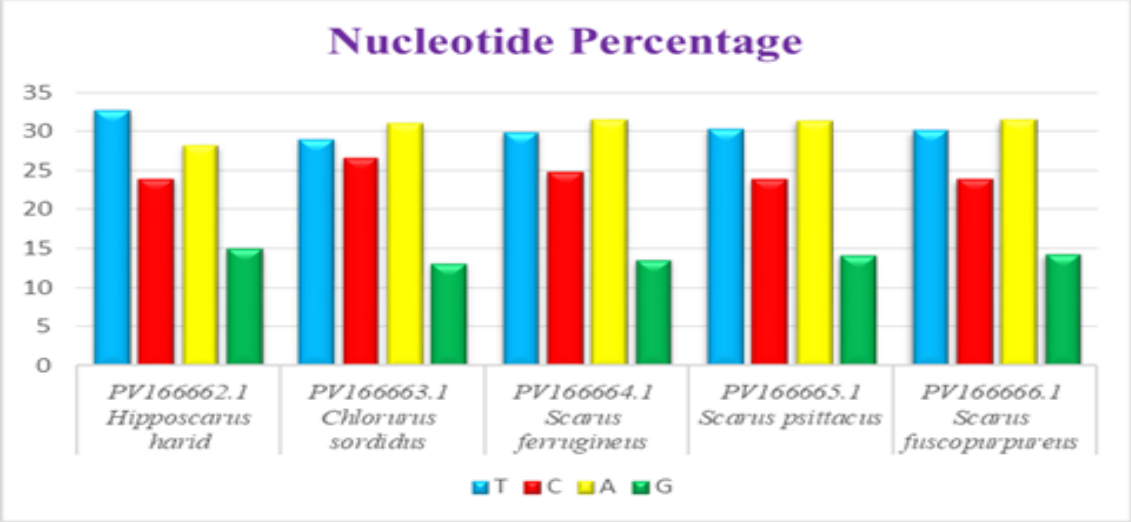

Fig. 2. The genetic similarity amongst five Parrot fish species by the nucleotide percentage

Pairwise genetic distances based on the mtDNA control region were calculated among the five parrotfish species examined in this study, along with sixteen additional *Scarus* species and two outgroup species from the order Acanthuriformes (family Lutjanidae). The smallest genetic distance (0.0060) was observed between *Scarus psittacus* and *Scarus fuscopurpureus*, indicating their close relationship, while the largest genetic distance (0.891) was recorded between *Hipposcarus harid* and *Scarus ferrugineus* (Table 2).

Table 2. Pairwise distances among under studied parrot fish species and its related species and two outgroups

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Hipposcarus harid		0.0773	0.0924	0.0799	0.0799	0.0627	0.0710	0.0602	0.0594	0.0632	0.0701	0.0692	0.0731	0.0696	0.0794	0.0734	0.0920	0.0898	0.0879	0.0709	0.0969	2.2068	3.3782
Chlorurus sordidus	0.7927		0.0424	0.0271	0.0282	0.0245	0.0274	0.0258	0.0237	0.0228	0.0314	0.0331	0.0307	0.0299	0.0367	0.0289	0.0316	0.0402	0.0335	0.0294	0.0357	1.2675	1.0432
Scarus ferrugineus	0.8910	0.5044		0.0532	0.0513	0.0258	0.0294	0.0122	0.0184	0.0226	0.0263	0.0326	0.0217	0.0234	0.0378	0.0378	0.0295	0.0491	0.0448	0.0386	0.0345	0.9942	0.7610
Scarus psittacus	0.8029	0.3027	0.6273		0.0060	0.0256	0.0289	0.0246	0.0139	0.0219	0.0271	0.0313	0.0243	0.0289	0.0381	0.0300	0.0300	0.0362	0.0343	0.0316	0.0319	0.7620	0.5970
Scarus fuscopurpureus	0.7967	0.3180	0.6107	0.0322		0.0261	0.0280	0.0239	0.0145	0.0214	0.0271	0.0319	0.0235	0.0284	0.0385	0.0307	0.0302	0.0347	0.0326	0.0318	0.0306	0.7363	0.5844
Scarus quoyi	0.5446	0.1748	0.2057	0.2034	0.2084		0.0352	0.0243	0.0221	0.0285	0.0329	0.0275	0.0291	0.0280	0.0388	0.0280	0.0308	0.0392	0.0422	0.0379	0.0281	0.7176	0.6598
Scarus schlegeli	0.6126	0.2302	0.2491	0.2354	0.2264	0.3069		0.0347	0.0319	0.0312	0.0402	0.0430	0.0392	0.0341	0.0523	0.0414	0.0486	0.0506	0.0152	0.0503	0.0499	0.6023	0.5668
Scarus rubroviolaceus	0.5244	0.1940	0.0686	0.1836	0.1803	0.1895	0.2804		0.0201	0.0229	0.0246	0.0329	0.0214	0.0226	0.0341	0.0329	0.0329	0.0400	0.0419	0.0329	0.0311	0.8410	0.6167
Scarus psittacus	0.5240	0.1655	0.1321	0.0842	0.0906	0.1635	0.2530	0.1385		0.0189	0.0242	0.0280	0.0219	0.0269	0.0281	0.0277	0.0254	0.0388	0.0415	0.0265	0.0254	0.6823	0.6032
Scarus forsteni	0.5498	0.1623	0.1714	0.1636	0.1607	0.2164	0.2676	0.1653	0.1396		0.0278	0.0352	0.0233	0.0309	0.0369	0.0274	0.0276	0.0385	0.0467	0.0360	0.0273	0.8421	0.7478
Scarus ghobban	0.6100	0.2439	0.1980	0.2145	0.2172	0.2609	0.3286	0.1746	0.1744	0.2142		0.0380	0.0261	0.0347	0.0516	0.0369	0.0438	0.0373	0.0612	0.0477	0.0420	0.7664	0.6188
Scarus ovifrons	0.5849	0.2598	0.2555	0.2479	0.2514	0.2052	0.3427	0.2570	0.2154	0.2723	0.2945		0.0362	0.0390	0.0457	0.0382	0.0433	0.0544	0.0528	0.0424	0.0406	0.7446	0.6235
Scarus oviceps	0.6133	0.2404	0.1609	0.1884	0.1821	0.2237	0.3244	0.1500	0.1634	0.1738	0.1859	0.2834		0.0245	0.0392	0.0323	0.0346	0.0369	0.0500	0.0387	0.0302	0.9323	0.7166
Scarus festivus	0.5895	0.2445	0.1905	0.2326	0.2288	0.2280	0.2936	0.1723	0.2057	0.2381	0.2723	0.3079	0.1815		0.0480	0.0368	0.0447	0.0439	0.0528	0.0463	0.0496	1.2481	1.4706
Scarus festivus	0.5456	0.2278	0.2399	0.2488	0.2488	0.2386	0.3554	0.2085	0.1692	0.2418	0.3415	0.2919	0.2536	0.3079		0.0333	0.0260	0.0366	0.0527	0.0059	0.0324	0.4438	0.4898
Scarus coelestinus	0.5201	0.1786	0.2475	0.1926	0.1969	0.1551	0.2786	0.1881	0.1616	0.1505	0.2311	0.2245	0.1793	0.2213	0.1974		0.0282	0.0227	0.0355	0.0271	0.0196	0.7191	0.8836
Scarus rivulatus	0.6357	0.1940	0.1823	0.1883	0.1927	0.1762	0.3298	0.2002	0.1457	0.1681	0.2785	0.2595	0.2228	0.2930	0.1547	0.1592		0.0347	0.0446	0.0243	0.0306	0.5756	0.6592
Scarus trispinosus	0.6158	0.2592	0.3302	0.2369	0.2273	0.2316	0.3211	0.2373	0.2315	0.2256	0.2267	0.3149	0.2124	0.2668	0.2266	0.1218	0.2079		0.0465	0.0299	0.0335	0.6902	0.9749
Scarus schlegeli	0.6288	0.2303	0.3252	0.2329	0.2206	0.2895	0.0606	0.2761	0.2703	0.3179	0.4027	0.3434	0.3312	0.3527	0.3452	0.2441	0.2970	0.3129		0.0404	0.0481	0.6570	0.7557
Scarus chameleon	0.5056	0.1866	0.2626	0.2066	0.2066	0.2263	0.3331	0.1988	0.1554	0.2296	0.3098	0.2623	0.2419	0.2912	0.0117	0.1606	0.1366	0.1851	0.2716		0.0302	0.4864	0.5898
Scarus perrico	0.6096	0.1949	0.1877	0.1885	0.1783	0.1500	0.3197	0.1657	0.1338	0.1400	0.2503	0.2270	0.1640	0.2901	0.1805	0.0884	0.1651	0.1780	0.2980	0.1552		0.8119	0.9859
Lutjanus fulvus	4.6093	4.1573	3.7527	3.5026	3.4178	3.1436	2.8200	3.2976	3.0731	3.2035	3.2029	3.1187	3.4476	3.5857	2.1472	2.5451	2.4056	2.5284	2.5450	2.2843	2.4808		0.0113
Lutjanus lemniscatus	4.7672	3.2755	2.8804	2.6647	2.6383	2.6851	2.5380	2.5427	2.6054	2.7324	2.6493	2.5886	2.7878	3.1427	2.2465	2.6780	2.5310	2.7742	2.6477	2.4398	2.6480	0.0532	

Phylogenetic tree analysis based on CR (D-loop) sequences was conducted for the five Scaridae species examined in this study, together with 16 related *Scarus* species and two outgroup species obtained from GenBank/NCBI (as listed in Table 2). To provide a more comprehensive view of phylogenetic relationships, two methods—Minimum Evolution (ME) and Neighbor-Joining (NJ)—were applied. Both methods produced largely consistent topologies, differing only in some support values, and revealed four main features:

1. The outgroup species formed a distinct cluster, separate from the Scaridae taxa.
2. The understudied *Scarus psittacus* and *Scarus fuscopurpureus* grouped together in a single main clade.
3. *Hipposcarus harid* formed a sister clade with *Scarus ovifrons* (GenBank accession AY324599.1).
4. *Scarus ferrugineus* occupied a basal clade with *Scarus rubroviolaceus* (GenBank accession AY324604).

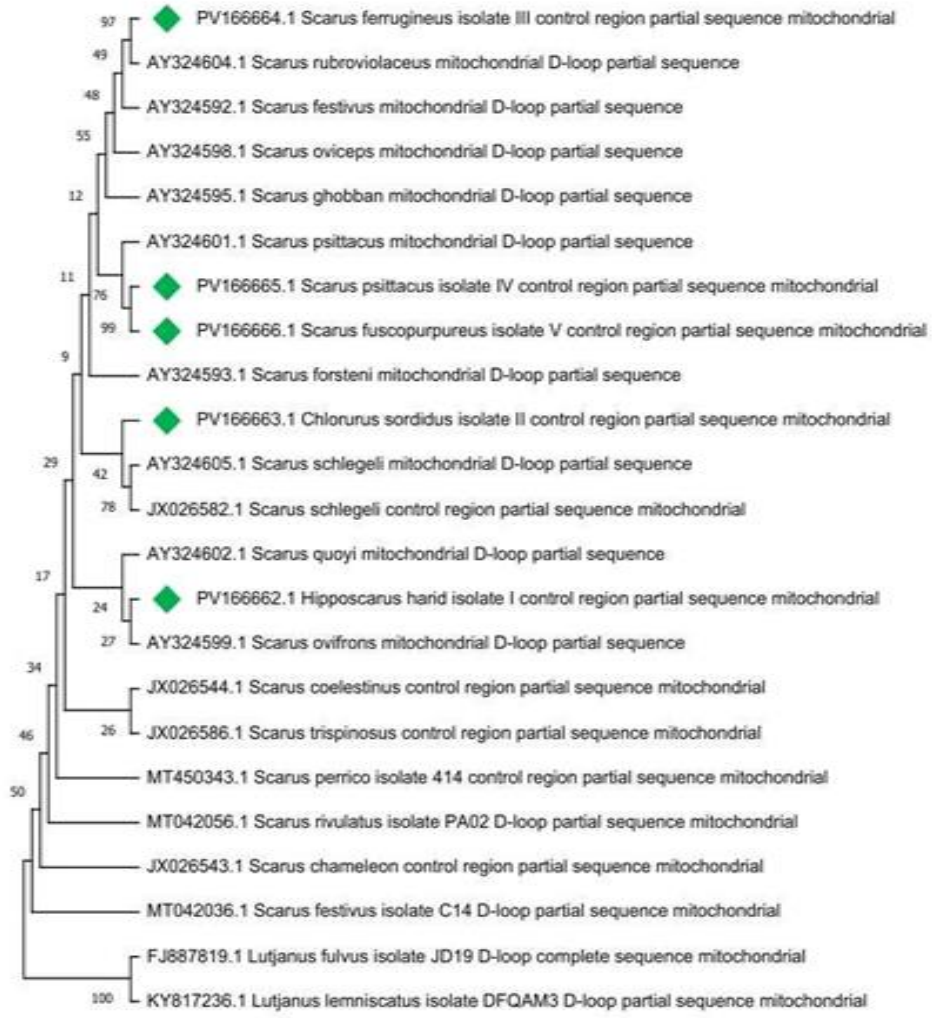

Fig. 3. Neighbor-joining tree amongst five parrot fish and their linked species with two outgroups

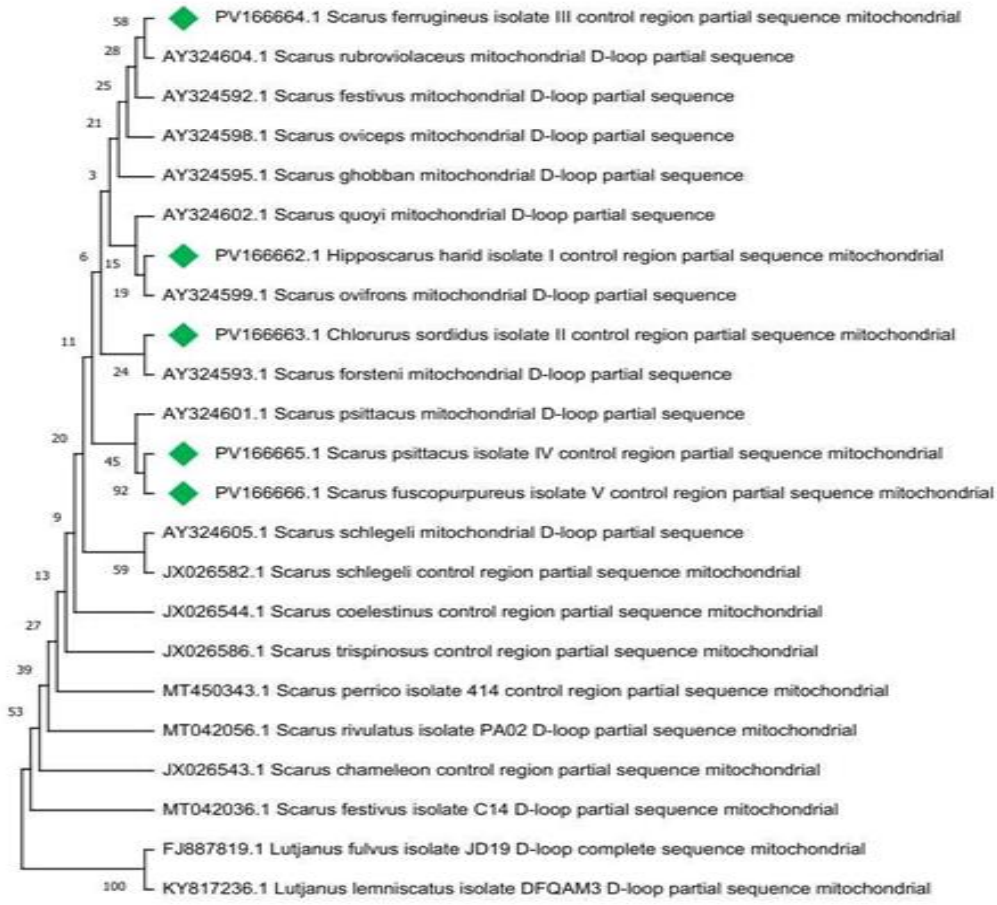

Fig. 4. Minimum-evolution tree among five parrot fish and their linked species with two out-groups

DISCUSSION

Genetic diversity studies on the parrotfish species are limited compared with research on their taxonomy and evolution **(Almeida *et al.*, 2017)**. In the present study, we examined the genetic diversity of five parrotfish species—*Hipposcarus harid*, *Chlorurus sordidus*, *Scarus ferrugineus*, *Scarus psittacus*, and *Scarus fuscopurpureus*. The mitochondrial control region (CR), also known as the D-loop, is a non-coding region within the mitochondrial genome that has been widely used to investigate genetic diversity and relationships among different parrotfish species and genera. Its high variability among populations and closely related species has made the CR a useful genetic marker in phylogenetic and population genetics studies.

Natural and sexual selection may also have contributed to the diversification of parrotfishes **(Gao *et al.*, 2023)**. However, substitution saturation can reduce the phylogenetic signal **(Salemi, 2003)**. **Gao *et al.* (2023)** reported that the CR in the parrotfish exhibits significant substitution saturation and recommended avoiding its use

for species identification in future studies. In contrast, **Allam and Mahrous (2021)** analyzed phylogenetic relationships among six parrotfish species from the Egyptian Red Sea using the divergent domain D11 of the 28S rRNA gene and concluded that D11 has strong potential as a genetic marker for DNA barcoding of parrotfish.

Our results showed that the A+T content was higher than the C+G content, consistent with **Alyamani (2024)**, who reported the same trend when sequencing the tRNA-Val gene in five labrid species. Similarly, **Aziz *et al.* (2025)** found that the A+T ratio exceeded the C+G ratio in the CR of freshwater catfish, highlighting the usefulness of the CR in catfish phylogenetic studies.

CONCLUSION

The results of this study demonstrate that the mtDNA control region (CR) is both unique and highly variable among the parrotfish species. Variation in intergenic spacers between species and the wide divergence observed among clades within the same *Scarus* species, highlight the substantial genetic diversity contained within this region of the mitochondrial genome.

These findings confirm the utility of the mtDNA CR as a valuable molecular marker for studying the genetic diversity of parrotfishes. This marker can support accurate species identification and inform conservation strategies, contributing to the sustainable management of parrotfish populations.

STATEMENT OF ETHICS

Procedure inclusive of animal care was approved in accordance with the guidelines of the Bioethics Committee of Faculty of Science belonging to South Valley University, Qena, Egypt **(Code No. 004/02/2024)**.

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