



First record of roving coral grouper *Plectropomus pessuliferus* (Fowler, 1904) from Saint Martin's Island, Bangladesh based on Morphology and DNA Barcoding

MD JAYEDUL ISLAM, SHARMIN AKTER, PROVAKOR SARKAR, MOHAMMAD RASHED¹, IREEN PARVIN¹ AND KAZI AHSAN HABIB^{1*}

Aquatic Bioresource Research Lab (ABR Lab), Department of Fisheries Biology and Genetics
Sher-e-Bangla Agricultural University, Dhaka 1207, Bangladesh

¹Department of Fisheries Biology and Genetics,
Sher-e-Bangla Agricultural University, Dhaka 1207, Bangladesh

*Email: ahsan.sau@gmail.com; habibka@sau.edu.bd

Abstract. A new record of *Plectropomus pessuliferus* (Serranidae: Epinephelinae) was documented based on morphological characters and DNA barcoding. The species was collected during a regular survey for making an inventory of reef associated fishes in Saint Martin's Island, Bangladesh. This is the first report of roving coral grouper from the marine waters of Bangladesh validated by morpho-meristic analysis and DNA barcoding. This is also the first report from the northern Bay of Bengal.

Keywords: *Plectropomus pessuliferus*, Bay of Bengal, Epinephelinae, DNA barcoding

Introduction

Serranidae is one of the diverse groups of commercially important fish commonly known as groupers, rock cods, hinds and sea basses. They have a high morphological diversity and spectacular color variations, and are inhabitants on reefs throughout shallow tropical seas (Heemstra 1991). The fish belonging to any of a number of genera in the subfamily Epinephelinae of the family Serranidae are known as groupers. Epinephelinae is the second largest subfamily of the family Serranidae comprising 188 valid species present in the world (Craig *et al.* 2011, Fricke *et al.* 2020). Among these, 110 species of grouper were recorded in the Indo-Pacific region (Randall and Heemstra 1991). A total of 15 species of four genera belonging to the subfamily Epinephelinae have been recorded from Bangladesh waters. Initially, Hossain (1970) listed eight species of Epinephelidae in Bangladesh. After that, other scholars added nine more species in the last one decade (Rahman *et al.* 2009, Thompson and Islam 2010, Habib *et al.* 2017). *Plectropomus* (Oken 1817) is a genus of colorful serranid fishes that belongs to the subfamily Epinephelinae. Members of this genus are typically associated with shallow water reefs in 3–147 m, and rarely caught by trawlers (Heemstra and Randall 1993). Saint Martin's Island is the only island in Bangladesh which contains coral colony and has a rich biodiversity, especially coral-associated fish species.

The present paper reports a new record of one more grouper species, *Plectropomus pessuliferus* (Fowler 1904) for the first time from Bangladesh. This species is known to be distributed in the Indo-Pacific from East Africa to Tonga, including Zanzibar, Chagos,

Lacdives, St. Brandon's Shoals, Maldives, Sri Lanka, Andaman and Nicobar Island, Myanmar, Nazareth Bank, Sumatra, Fiji (Heemstra and Randall 1993, Rajan 2001, Randall *et al.* 2003, Rajan *et al.* 2013, Psomadakis *et al.* 2019, Frick *et al.* 2020). In the present study, the primary identification of *Plectropomus pessuliferus* was carried out observing morphological characters. DNA barcoding was then used as a tool for further confirmation of the morphological identification of the species since DNA barcoding is one of the most reliable methods for the confirmation of species identity (Hebert *et al.* 2003).

Material and Methods

Sample collection: In March 2018, one individual of *Plectropomus pessuliferus* was collected during a survey on the diversity of coral associated fish from Saint Martin's Island, the southernmost part of Bangladesh facing the Bay of Bengal and located in Cox's Bazar district (Fig 1).

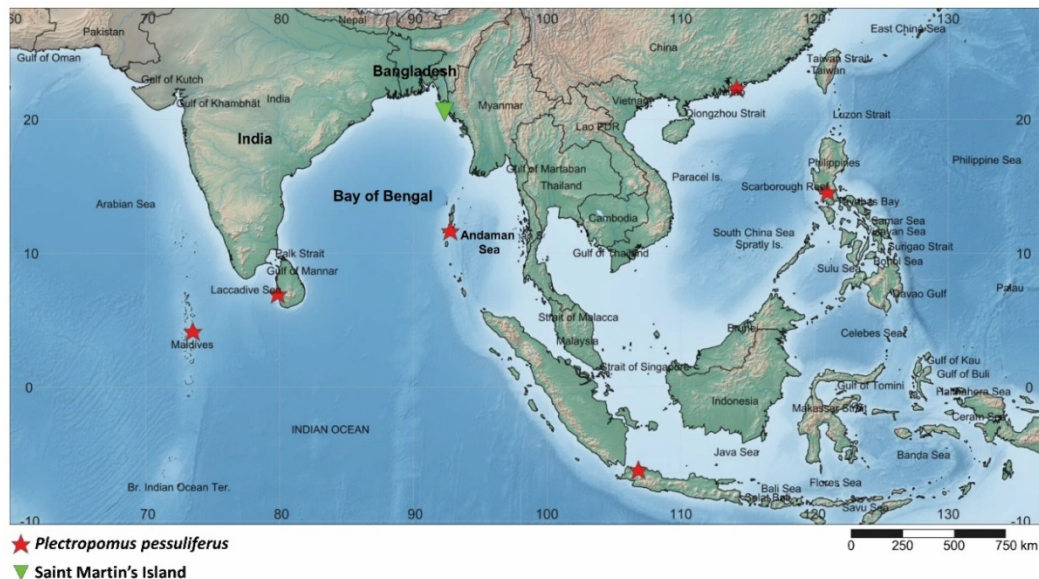


Fig. 1. Sampling location of *Plectropomus pessuliferus* in Saint Martin's Island, Bangladesh of the Northern Bay of Bengal (▼); and previously reported locations (★).

Morphological analysis: For morphological identification, taxonomic keys used by Randall and Heemstra (1991), Heemstra and Randall (1993), Baldwin and Johnson (1994) and Elamin *et al.* (2011) were followed. Abbreviations: D1- First dorsal fin, P1- Pectoral fin, P2- Pelvic fin, A- Anal fin, C- Caudal fin.

Molecular study: For molecular study, a piece of muscle tissue was collected and preserved in 95% ethanol. Genomic DNA was extracted from preserved tissue sample using TIANamp Marine Animals DNA Kit (TIANGEN). The DNA barcode region of mitochondrial COI gene was amplified using the primer pair F1: 5'-TCAACCAACCACAAAGACATTGGCAC-3' and R1: 5'-TAGACTTCTGG GTGGCCAAAGAATCA-3' (Ward *et al.* 2005). Polymerase chain

reaction (PCR) was performed in a 50 μ l reaction mixture in a thermal cycler (2720 Thermal Cycler, Applied Biosystems). Thermal cycling conditions included an initial denaturation temperature of 95°C for 2 minutes and subsequently, 35 cycles consisting 94°C for 40 seconds for denaturation, 54°C for 40 seconds for annealing and 72°C for 1 min for extension. Lastly, 72 °C for 10 min was maintained for final extension. PCR products were examined by 1% agarose gel electrophoresis with a 100 bp DNA ladder. Sequencing was conducted with the PCR primers by Sanger standard method in a normal automatic sequencer (Model 3730xI DNA analyzer). For molecular identification, we checked the DNA sequence using BLAST search engine provided by the National Center for Biotechnology Information (NCBI) and BOLD database. Finally, the obtained nucleotide sequence of COI barcode region was submitted to GenBank under the accession number of MK340678. For phylogenetic analysis, including the sequence of present study we used a total of five sequences of three species of the genus *Plectropomus* (i.e. *P. pessuliferus*, *P. leopardus*, *P. maculatus*) obtained from the BLAST search result in GenBank (Altschul *et al.* 1990). The Kimura-2 parameter (K2P) distance model (Kimura 1980) was used for calculating the genetic distance among the sequences using MEGA-7. Aconfamiliar species *Cephalopholis boenak* (Bloch 1790) was used as an outgroup. Sequences were aligned and phylogenetic tree was constructed by Neighbor Joining (NJ) method using MEGA7 (Kumar *et al.*, 2016). Robustness of the phylogenetic relationships was evaluated by bootstrap analysis with 10,000 replications (Felsenstein 1985). The examined specimen was deposited in the Aquatic Bioresource Research Lab., Department of Fisheries Biology and Genetics, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

Results

Material examined: Specimen collected from Bangladesh: Cox`'s Bazar, Saint Martin`s Island. Coordinate 20°34'50.1" N, 92°20'28.0" E (DMS) (Fig 1); Collected by Provakor Sarkar. 10 March 2018; one specimen; specimens voucher nos. F1803SM-43. GenBank Acession No. M340678. We identified our specimens as *Plectropomus pessuliferus* (Fowler 1904) (Roving coral grouper) based on morphological study.

Habitat: This fish was found in shallow waters of Saint Martin`s Island.

Diagnostic Characters: D1 VIII, 11; P1 16; P2 I, 5; A III, 8; C 16. Body large, elongated and laterally compressed; its depth 10.3 in standard length (Fig 2). Ventral profile is more convex than ventral dorsal profile. Dorsal profile of head gently sloped. Mouth large, oblique, superior, and protractile. Preopercular edge serrated. Opercular flap present, opercle with three flat spines. Mouth gap large. Head large in comparison with body and numerous spot present (Fig. 2a&b). Scale ctenoid and cover the whole body. Teeth canine and present in front of both the lower and upper jaw. Jaws large and lower jaw is larger than the upper jaw. Barble absent. Lateral line single, complete and curved. Eye large and completely dorsal in position. Dorsal fin single, large and continuous and starts at the middle of the pectoral fin. Anal-fin spines weak, first and second spines covered by skin. Caudal fin emarginated.

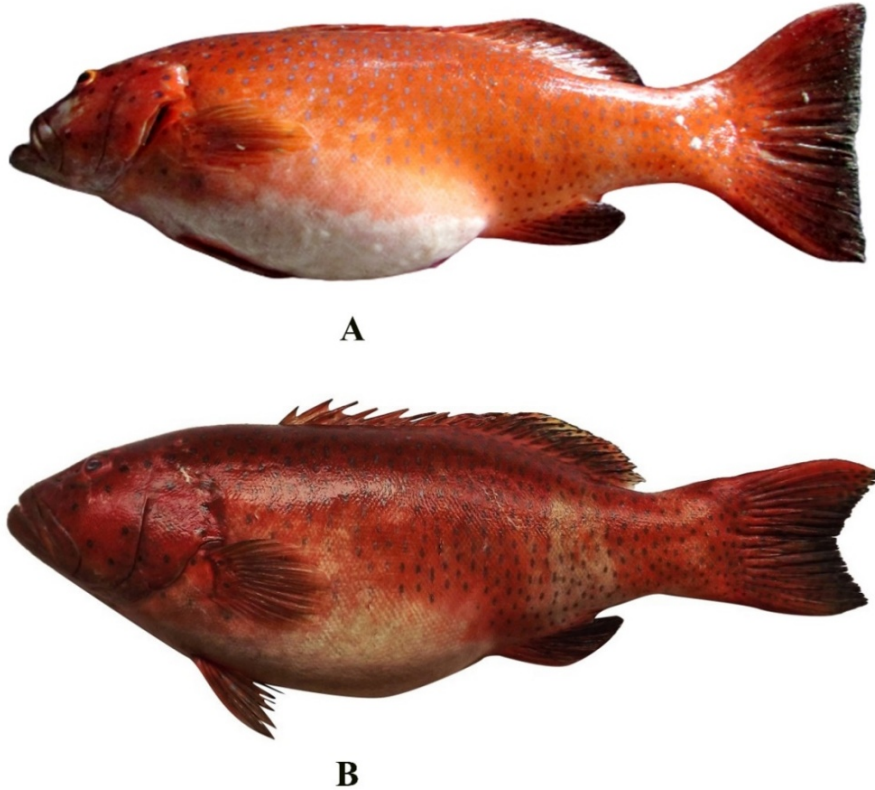


Fig. 2. **A.** Lateral view of fresh specimen of *Plectropomus pessuliferus*, and **B.** Lateral view of preserved *P. pessuliferus* collected from Saint Martin's Island. SL 454.0 mm.

Color: Coloration of body orange and ventral side is whitish. Numerous dark edged blue spot present along the body. Abundant blue spots found on all the fins. Head orange color; dorsal fin orange color and contains blue spot. Pectoral and pelvic fin somewhat grey in color. Anal and caudal fin dark orange color.

The morpho-meristic counts and measurements of the collected specimens of *P. pessuliferus* and its comparison with the records of Elamin *et al.* (2011) are given in Table I.

Table I. Comparison of morphological and meristic characters of collected specimen *P. pessuliferus* of the present study and the records of Elamin *et al.* (2011)

Parameter	<i>P. pessuliferus</i> Present study	<i>P. pessuliferus</i> (Elamin <i>et al.</i> 2011) n= 165
Total length (TL)	552.0	569.0 (326-900)
Standard length (SL)	454.0	462.9 (267-750)
Percentage of SL		
Body depth (BD)	44.7	35.90 (33.7-38)
Body width (BW)	20.0	
Head length (HL)	35.9	35.10 (33.4-37.33)
Inter-orbital length (IOL)	6.83	
Pre-orbital length (PrOL)	12.78	
Postorbital length (PoOL)	20.48	
Eye diameter (ED)	4.41	4.41 (3.96-5.06)
Snout length (SL)	6.83	10.33 (8.61-12.0)
Caudal-peduncle length (CPL)	16.74	
Dorsal-fin base length (DBL)	43.61	
Pectoral-fin base length (P1BL)	6.17	
Pelvic-fin base length (P2BL)	5.73	
Anal-fin base length (ABL)	15.64	
Caudal-fin base length (CPL)	17.40	
Longest dorsal-fin length (DFL)	11.23	
Longest pectoral-fin length (P1FL)	17.62	15.45 (14.99-16.10)
Longest pelvic-fin length (P2FL)	15.64	14.02 (14.02-14.68)
Longest anal-fin length (AFL)	14.54	
Caudal-peduncle depth (CPD)	13.66	11.15 (11.16-12.0)
Meristic Study		
	<i>P. pessuliferus</i> (Present study)	Heemstra and Randall (1993)
Dorsal-fin spines	VIII	VII-VIII
Dorsal-fin rays	11	10-12
Pectoral-fin rays	16	15-16
Pelvic-fin spine	I	
Pelvic-fin rays	5	
Anal-fin spines	III	III
Anal-fin rays	8	8

Molecular identification: A clear nucleotide sequences of 611bp of mtDNA COI gene was obtained after removing ambiguous sequences near the primer ends and submitted to GenBank. No insertions, deletions or stop codons were observed in the sequence. The lack of stop codons is consistent with amplified sequence being functional mitochondrial COI sequence. To verify the result of morphological identification, our identified COI barcode region of *P. pessuliferus* specimen with other conspecific and congeneric species retrieved from the GenBank were compared. In the phylogeny, *P. pessuliferus* sequence of the present study (i.e. the Bay of Bengal) formed a single clade with two sequences of Australian individuals (Fig 3). No nucleotide difference was observed in these three sequences (i.e. single haplotype). Each of the three congeneric species of the genus *Plectropomus* made three separate clades in the neighbor joining tree. Genetic divergence was found 1.3% (SE±0.005) between the *P. pessuliferus* of present study and phylogenetically the most closely related species *Plectropomus leopardus* reported from Malaysia. On the other hand, the genetic distance was calculated as 5.1% (SE±0.009) between *P. pessuliferus* and *Plectropomus maculatus* of Malaysia in our study. Overall mean distance among the sequences of COI gene used in the study was 2.4%.

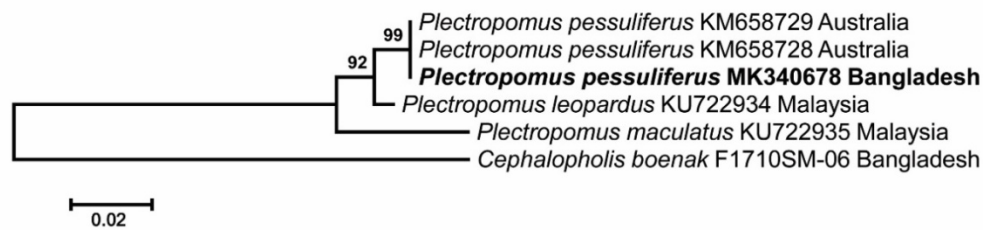


Fig. 3. Neighbor-joining tree for DNA barcode sequence (601 bp) of COI gene of *Plectropomus pessuliferus*. Accession number and the country of each sequence are given beside species name. Bootstrap support above 90% are shown above branches. Scale bar represents the substitution rate per site.

Discussion

A new record of *Plectropomus pessuliferus* (Serranidae: Epinephelinae) in Bangladesh is reported on the basis of morphological characters and DNA barcoding. During the survey, only one specimen was found which was then prepared for further analysis. Depending on the record of a single specimen for including a species in a region's or country's fish inventory was also carried out in other studies such as Vella *et al.* (2016), Sen *et al.* (2019) and De La Cruz *et al.* (2020). The members under subfamily Epinephelinae are usually identified by their colour pattern, morphological characters and size of the fins, the shape and relative size of the head and various parts of the head and body. Sometimes they exhibit different colors and morphological counts in the juvenile stage. The morphometric characters were counted, measured and photographed, then compared with the records of Elamin *et al.* (2011) and Heemstra and Randall (1993). All meristic characters are in agreement with previous record of this species (Table I). Although some morphological characters do not exactly similar to the range recorded by Elamin *et al.* (2011), the majorities of characters either overlap or span the range, which is reasonable given the differences in growth rate.

COI sequencing is considered as an effective and reliable procedure in identifying species (Hebert *et al.* 2003). The query sequence showed a high identity (> 99%) with 2 sequences of *Plectropomus pessuliferus* deposited in the GenBank database (accession numbers: KM658728 and KM658729; Fig. 3). COI sequences of *P. pessuliferus* clustered into a monophyletic group that was clearly separated from other species of same genus. The results provide strong evidence for the species validity of *P. pessuliferus* by genetic study. The phylogentic analysis also showed that the sequences of *P. pessuliferus* was differentiated from other species of the same genus with the genetic distance ranged from 1.3% to 5.1%. A list of the Epinephelid species currently known and their first report from Bangladesh are given in Table II. The list shows, 16 species under four genera (including the present study), namely *Cephalopholis* (3 species), *Cromileptes* (1 species), *Epinephelus* (10 species), *Plectropomus* (2 species) were recorded from Bangladesh which are relatively low in number compared to Indian waters. Rajan *et al.* (2017) recorded a total of 54 numbers of Epinephelids from Indian waters. However, India is a large country having several times larger maritime area than Bangladesh.

Table II. List of species from subfamily Epinepheninae in Bangladesh waters

Species Name	Reference
<i>Cephalopholis boenak</i> (Bloch 1790)	Hussain 1969, Habib <i>et al.</i> 2017
<i>Cephalopholis ormosa</i> (Shaw 1812)	Rahman <i>et al.</i> 2009
<i>Cephalopholis sonnerati</i> (Valenciennes 1828)	Hussain 1970
<i>Cromileptes livilis</i> (Valenciennes 1828)	Hussain 1970
<i>Epinephelus coioides</i> (Hamilton 1822)	Habib <i>et al.</i> 2017
<i>Epinephelus erythrurus</i> (Valenciennes 1828)	Habib <i>et al.</i> 2017
<i>Epinephelus hexagonatus</i> (Forster 1801)	Tomascik 1997
<i>Epinephelus lanceolatus</i> (Bloch 1790)	Hussain 1970
<i>Epinephelus atifasciatus</i> (Temminckand Schlegel 1842)	Hussain 1970
<i>Epinephelus malabaricus</i> (Bloch and Schneider 1801)	Hussain 1970
<i>Epinephelus merra</i> (Bloch 1793)	Hussain 1970
<i>Epinephelus morrhua</i> (Valenciennes 1833)	Hussain 1970
<i>Epinephelus polyphkadion</i> (Bleeker 1849)	Tomascik 1997
<i>Epinephelus quoyanus</i> (Valenciennes 1830)	Molony <i>et al.</i> 2006
<i>Plectropomus leopardus</i> (Lacepède 1802)	Molony <i>et al.</i> 2006
<i>Plectropomus pessuliferus</i> (Fowler 1904)	Present study

The nearby areas of the Bay of Bengal from where the species *P. pessuliferus* was known to occur are Andaman and Nicobar Island, Srilanka and Myanmar but these species has no valid record from the northern part of the Bay of Bengal. This study confirms the presence of *P. pessuliferus* in the Saint Martin's Island of the north Bay of Bengal and indicates the possibility

of existence of more species of the genus *Plectropomus* in Bangladeshi marine water that have been overlooked in past surveys.

Acknowledgements: This study was supported by UNESCO/Korean Funds-in-Trust funded DRMREEF project coordinated by IOC Sub-Commission for the Western Pacific (WESTPAC).

Literature Cited

- Altschul, S.F., W. Gish, W. Miller, E.W. Myers and D.J. Lipman, 1990. Basic local alignment search tool. *J. Mol. Biol.*, 215 (3): 403–410.
- Baldwin, C.C. and G.D. Johnson, 1994. FAO Species Catalogue. Vol. 16. Groupers of the World (Family Serranidae, Subfamily Epinephelinae). An Annotated and Illustrated Catalogue of the Grouper, Rockcod, Hind, Coral Grouper, and Lyretail Species Known to Date. *Copeia*, 4: 1058– 1061.
- Craig, M.T. and P.A. Hastings, 2007. A molecular phylogeny of the groupers of the subfamily Epinephelinae (Serranidae) with a revised classification of the Epinephelinae. *Ichthyol. Res.*, 54: 1–17.
- De La Cruz, J., R. Gonzalez, F. Garcia and V. Cota, 2020. First Record of aspecimen of the shortbill spear fish *Tetrapturus angustirostris* Tanaka, 1915 in the Pacific Coast of Mexico. *Lat. Am. J. Aquat. Res.*, 47(4):
- Elamin, S.M., M.A. Ambak, M.A. Samoilys, and M.E. Hamza, 2011. Some Morphometric Relationships of Coral Trouts *Plectropomus pessuliferus* and *Plectropomus areolatus* Inhabiting Sudanese Red Sea. *Adv. Environ. Biol.*, 5(9): 2860–2865.
- Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39(4): 783–791.
- Fricke, R., W.N. Eschmeyer and R. Van der Laan (eds.), 2020. Eschmeyer's Catalog of Fishes: Genera, Species, References. (<http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp>). Electronic version accessed 01 September 2020.
- Habib, K.A., C.G. Kim, J. Oh, A.K. Neogi and Y.H. Lee, 2017. Aquatic Biodiversity of Sundarbans, Bangladesh. Korea Institute of Ocean Science and Technology (KIOST). 394 p.
- Hebert, P.D.N., A. Cywinska, S.L. Ball and J.R. deWaard, 2003. Biological identification through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences, 270: 313-321.
- Heemstra, P.C. and J.E. Randall, 1993. FAO species catalogue. Groupers of the world (Family Serranidae, Subfamily Epinephelinae) FAO Fisheries Synopsis. No. 125, Vol. 16. Rome, FAO. 382 p.
- Heemstra, P.C., 1991. A taxonomic revision of the eastern Atlantic groupers (Pisces: Serranidae). *Bol. Mus. Mun. Funchal.*, 43: 5–71.
- Hussain, M.M., 1970. The Marine and Estuarine Fishes of North East part of the Bay of Bengal. Scientific Researches. East Regional Laboratories, Dhaka. 52 p.
- Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.*, 16(2): 111–120.
- Kumar, S., G. Stecher and K. Tamura, 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.*, 33(7): 1870–1874.

- Molony, L., 2006. A National Project Professional Personnel. St Martin's Island ECA Conservation Management Plan. Coastal and Wetland Biodiversity Management Project, Department of Environment, Dhaka, Bangladesh.
- Psomadakis, P.N., H. Thein, B.C. Russell and M.T. Tun, 2019. Field identification guide to the living marine resources of Myanmar. FAO Species Identification Guide for Fishery Purposes. FAO and MOALI, Rome. 694 p.
- Rahman, A.K.A., S.M.H. Kabir, M. Ahmad, A.T.A. Ahmed, Z.U. Ahmed, Z.N.T. Begum, M.A. Hasan and M. Khondker, (eds.), 2009. Encyclopedia of Flora and Fauna of Bangladesh. Vol. 24. Marine Fishes. Asiatic Society of Bangladesh, Dhaka. 485 p.
- Rajan, P.T., 2001. Field guide to grouper and snapper fishes of Andaman and Nicobar Islands. Zoological Survey of India. 103 p.
- Rajan, P.T., C.R. Sreeraj and T.I.T.U.S. Immanuel, 2013. Fishes of Andaman Andaman and Nicobar Islands: a checklist. *J Andaman Sci Assoc.*, 18(1): 47–87.
- Rajan, P.T., S.S. Mishra and K.K. Bineesh, 2017. First records of two species of groupers, *Cephalopholis nigripinnis* and *Epinephelus retouti* (Perciformes: Epinephelidae) from India, with a note on Epinephelids from Andaman and Nicobar Islands. *Rec. Zool. Surv. India*, 117(3): 289–294.
- Randall, J.E. and P.C. Heemstra, 1991. Revision of Indo-Pacific groupers (Perciformes: Serranidae: Epinephelinae), with descriptions of five new species. *Indo-Pac. Fish.*, 20: 332.
- Randall, J.E., J. Williams, D.G. Smith, M. Kulbicki, G.M. Tham, P. Labrosse, M. Kronen, E. Clua and B.S. Mann, 2003. Checklist of the shore and epipelagic fishes of Tonga. *Atoll Res. Bull. Nos.* 502.
- Sen, S., G. Dash and S.A. Bharadiya, 2019. First Record of Longspine Grouper, *Epinephelus longispinis* (Kner, 1864) from Gujarat, North-West Coast of India. *Thalassas: An Intl. J. Mar. Sc.*, 35: 287–289.
- Thompson, P.M. and M.A. Islam, (eds.), 2010. Environmental Profile of St. Martin's Island, United Nations Development Programme, Dhaka. 150 p.
- Tomascik, T., 1997. Management Plan for Resources of NarikelJinjira (St. Martin's Island) Final Report, National Conservation Strategy Implementation Project -1 Ministry of Environment and Forest, Government of Bangladesh. 125 p.
- Vella, A., N. Vella and S. Agius Darmanin, 2016. The first record of the African sergeant, *Abudefduf hoefleri* (Perciformes: Pomacentridae), in the Mediterranean Sea. *Mar. Biodivers. Rec.*, 9(15):