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Short Communication

Morphometric and molecular evidence confirm the occurrence of *Epinephelus erythrurus* (Valenciennes, 1828) along Odisha coast, India

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The present study reports the cloudy grouper, *Epinephelus erythrurus* for the first time from off Odisha coast. The species was confirmed by using morphometric analyses and COI gene sequencing. Hence the study updates the fish species diversity along the Odisha coast by adding another *Epinephelus* species to the family Epinephelidae faunal composition.

[**Keywords**: Bay of Bengal, Cloudy grouper, Epinephelidae, New record, Phylogeny]

Introduction

The groupers species plays a crucial role of regulation of structure and composition of coral ecosystem communities and forms an essential link to food chain[1]. The groupers are commercially important, carnivorous, and are mostly found in the coral reef areas, rocky reefs, muddy or silty-sand bottoms, and in estuarine regions[1]. The grouper family Epinephelidae comprises of 16 genera with 176 valid species worldwide[2]. The genus *Epinephelus* consists of ninety-seven species, of which seventy-seven species belong to the Indo-Pacific region[3]. The genus *Epinephelus* is represented by 47 species along the Indian coast[1,4]. Compilation of the state fauna of Odisha revealed 12 species under the genus *Epinephelus*[5-7], and two species in the genus *Cephalopholis*[8,9] in the family Epinephelidae. The present study confirms the occurrence of one more species namely *Epinephelus erythrurus* along the Odisha coast which increased the species diversity of groupers along the Odisha coast to 15 species. The study also provides first molecular sequence of the reported species from Indian waters.

Materials and Methods

In order to understand the coastal fish diversity of Odisha coast under the mandate of the Zoological Survey of India, some local surveys were conducted. During one such local survey, a single specimen of a grouper species, locally called Bantu (ବଣ୍ଟୁ), was collected from Arjipalli fish landing centre, Ganjam, Odisha (19°18'24.17" N; 84°57'47.91" E). According to the fishermen, the specimen was caught by hook and line in live condition. Fresh photographs of the specimen were taken immediately after collection from the landing centre and later deposited to the National repository of Estuarine Biological Regional Centre, ZSI, Odisha, for further study. Digital caliper and measuring tape were used for the morphometric measurements, and meristic counts were taken using Leica S9i microscope. Morphometric identification of the collected specimen was done by following standard literature[10]. Post-identification of the specimen, the fresh tissue sample was extracted and stored in –20 °C for the molecular study. The specimen was preserved in 70 % alcohol for further use. For the molecular analysis, the COI (cytochrome c oxidase subunit I) gene region was chosen for species identification as the COI gene is relatively well-conserved within a species but exhibits sufficient variation between species. The GenBank database comprises of several COI gene sequences for numerous species within the Genus *Epinephelus*, facilitating extensive comparative molecular analysis, which helped distinguishing between different species. DNA was isolated by the salting-out method[11]. Gel electrophoresis was done with 1.2 % agarose gel for the confirmation of the presence of DNA. Further, the quantity of DNA was measured using Quibt 4 Flourometer (Pub. No. MAN001217210). PCR amplification was carried out in Thermo fisher scientific PCR machine with a total volume of 25 µL [10 µL of Nuclease free water, 12.5 µL of 2X Hi G9 Taq PCR Master Mix, 0.5 µL of forward primer Fish F1 (5'TCAACCAACCACAAAGACATTGGCAC-3'), 0.5 µL of reverse primer Fish R1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3')[12] and 1.5 µL of DNA Template] with the condition initial denaturation at 94 °C for 3 m, 35 cycles at 94 °C for 30 sec, annealing at 54 °C for 30 sec and extension at 72 °C

for 1 m with a final extension at 72 °C for 10 m followed by the indefinite hold at 4 °C. Sanger sequencing method was followed for the sequencing of the amplified product through an out-sourcing method by HKP scientific. Sequencing, phylogenetic analysis, and GenBank data upload are done following Mohapatra *et al*.[13]. The phylogenetic analysis was carried out by taking 1000 bootstrap values with the best-fit model – HKY+G (Hasegawa–Kishono–Yano with Gamma distributed) nucleotide substitution model using MEGA 11 software. The COI gene sequence with accession number MW810350 and 699 bp is submitted to the NCBI database.

Results

Family: Epinephelidae
Genus: *Epinephelus* Bloch, 1793
Epinephelus erythrurus (Valenciennes, 1828)
Serranus erythrurus Valenciennes, in Cuvier & Valenciennes, 1828. *Hist Nat Poiss*, 2: 320. (Type locality: Malabar region, India).
Common name: Cloudy grouper

Material examined

EBRC/ZSI/F12410 (1 specimen, 240 mm TL), Arjipalli fish landing centre, Odisha, India, 10th November 2020.

Description

D XI, 17; A III, 8; P I, 5; C 18. Body deep, moderately elongated and laterally compressed. Body depth 2.9 times in Standard Length (SL) (225 mm). Dorsal fin originates at the level of the pectoral fin origin, and pre-dorsal length (78 mm) 2.9 times in SL. Pre-anal length (131 mm) 1.7 times in SL. Head prominent and large (85 mm), 2.6 times in SL. Eyes large (14 mm), 6.1 times in Head Length (HL). Inter-orbital length (10 mm), 8.5 times in HL; snout length (21 mm), 4.0 times in HL. Pectoral fin bear 17 rays, its length 5.0 times in SL and 1.9 times in HL. Operculum contains three spines; the middle one is situated halfway between the level of the upper and lower jaw. Second anal fin spine slightly longer than third. Scales on lateral line 104. Tip of the pelvic fin not touching the anus. Caudal fin rounded.

Colour

Body reddish brown in colour with irregular pale spots. Both dorsal and anal fins are mottled. Three dark streaks athwart the gill cover, the widest and darkest runs from the eye to the lower edge of the gill cover, the second runs from the lower edge of the eye to subopercle, and the third runs from the dark streak on the upper jaw to the lower edge of preopercle (Fig. 1).

Fig. 1 — *Epinephelus erythrurus* (240 mm TL), Arjipalli fish landing centre, Odisha, India

Distribution

In India, the species *Epinephelus erythrurus* was earlier reported from Gujarat[14], Maharashtra[15], Karnataka[16], Andhra Pradesh[17], Andaman and Nicobar Islands[18], and West Bengal[19] along the Indian coast. The present study confirms the distribution of *E. erythrurus* along the Odisha coast.

Molecular identification

The DNA sequencing of the collected specimen *Epinephelus erythrurus* was done, and the result of COI gene sequence analysis supports the morphometric identification. The obtained cytochrome C oxidase subunit I gene (COI) sequence having 699 bp shows 99–100 % similarity with previously uploaded sequences of *E. erythrurus* from China, Myanmar, Viet Nam, and Malaysia. The Kimura-2-Parameter (K2P) distance varies 0–1.5 %. Thus, the molecular identification confirms it as *E. erythrurus*, and this is the first-ever sequence (MW810350) uploaded from the coastal waters of India.

Discussion

Groupers are uppermost predators, steady growing, sluggish in movement, and long living fishes[10]. They are commercially important due to their use in food, medicinal purposes, and ornamental display, which led to their over-exploration. The loss of grouper population can have a serious impact on the coral reef ecosystem[20] as groupers are the at the apex level of the food chain in the coral reef habitat. The genus *Epinephelus* is represented by 47 species along the Indian coast[1,21], of which 18 species (45 %) were reported from the east coast[17,19,22], while Odisha represents only 12 species (30 %). The present study added one more species, *i.e*., *E. erythrurus*, which increased the number to 13 species under the genus *Epinephelus* along the Odisha coast.

Groupers are typically identified by the shape of their bodies and heads, their colour patterns, and the size of their fin elements[10]; hence, there is a chance of misidentification due to close colour patterns in multiple species.

The molecular analysis revealed that the uploaded sequence (MW810350) in NCBI forms the lonely sequence of *E. erythrurus* from India. In the present study, the obtained sequence of *E. erythrurus* closely matched with the reported sequence of *E. erythrurus* from China, Myanmar, Viet Nam, and Malaysia with 0.0 to 1.5 % K2P distance (Fig. 2). However, three sequences from Mumbai, India submitted with the name *Epinephelus coeruleopunctatus* also appear in the same clade, indicative of a case of misidentification. It may be noted that misidentification of species within this family can result in misleading stock assessments and conservation programs[23]. The *E. erythrurus* differs from the *E. coeruleopunctatus* in having 1 or 2 dark streaks from eye to lower edge of the gill cover which is absent in *E. coeruleopunctatus*; mid-lateral part of lower jaw with 2–3 rows of teeth (vs 3–5 rows in *E. coeruleopunctatus*); small white spots absent on the head, body, and dorsal fin (present in the

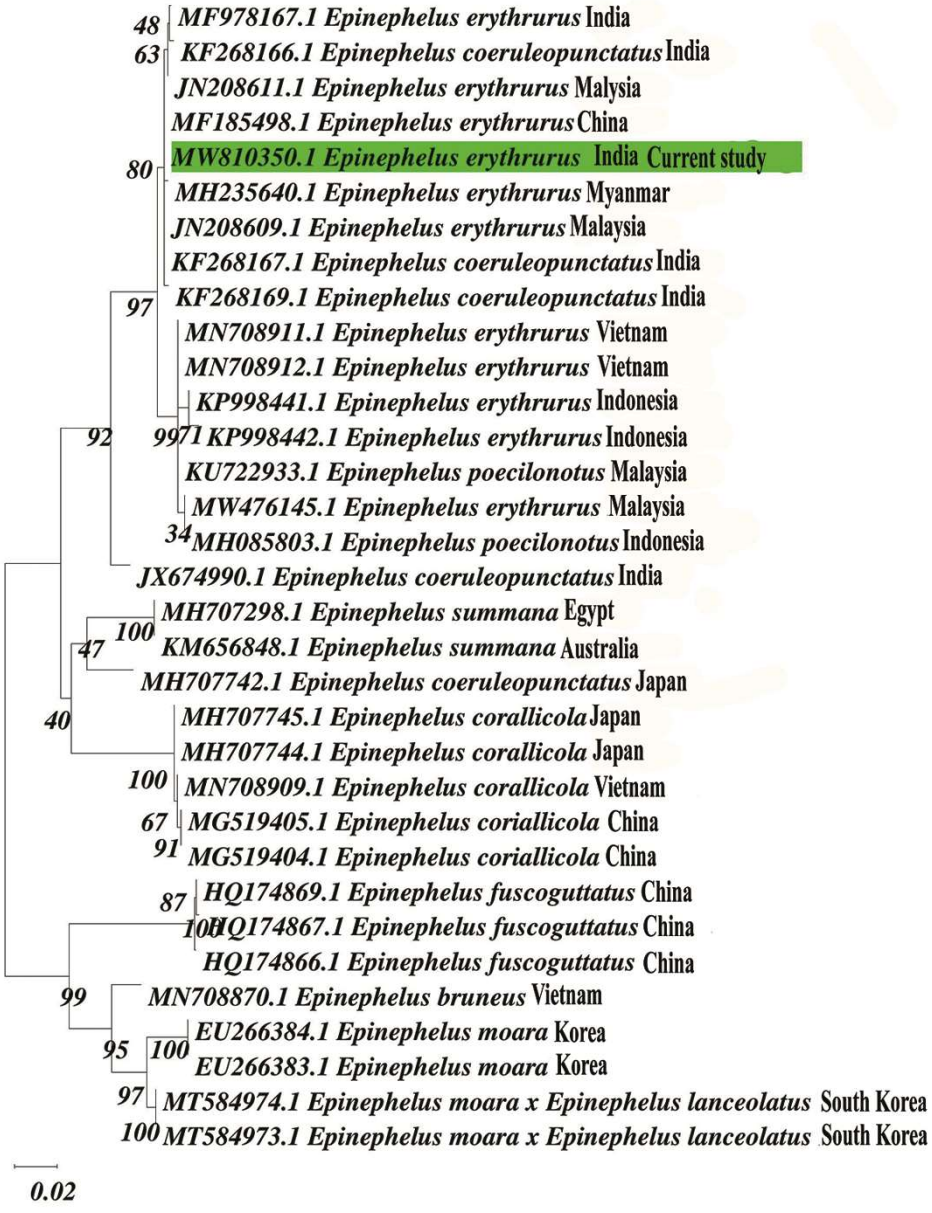

Fig. 2 — ML tree showing the relationship of *Epinephelus erythrurus* with congeneric species

E. coeruleopunctatus)[3]. The sequence uploaded as *Epinephelus poecilonotus* from Indonesia shows 99.1 % nucleotide similarities with a recent study and also comes under the same clade in the maximum likelihood tree having 99 bootstrap values with *E. erythrurus* most possibly be another case of misapplied nomenclature that needs to be evaluated with specimens (Fig. 2). All the sequences except the recent study compared from different localities in Figure 2 have been taken from NCBI. *Epinephelus poecilonotus* differs from the *E. erythrurus* in having large oval dark brown blotch in small specimens and a group of small spots in larger specimens at the base of the mid-dorsal spine; dorsal fin rays 14 – 15 (vs 17 in current study); length of the head 2.3 – 2.5 times in SL (vs 2.6 times in SL in present study); lateral scale series with 110 – 121 scales (vs 104 scales in current study)[10].

Conclusion

The present study added an additional species to the known diversity of genus *Epinephelus* (family Epinephelidae) along the Odisha coast with report of *Epinephelus erythrurus* on the basis of both morphometric and molecular level identification raising the species number to thirteen.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Statement

The species being studied is not included in any protected categories or under schedule list and is collected dead from the fish landing centre; hence the ethical clearance certification is not required.

Author Contributions

RKB, SRM & SA: Collection, preservation, identification, DNA analysis and manuscript preparation; and SP, SSM & AM: Identification, manuscript preparation and critical analysis.

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