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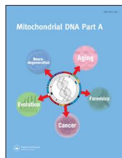

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RESEARCH ARTICLE

Mitochondrial signatures for identification of grouper species from Indian waters

V. S. Basheer[a], N. Vineesh[a], K. K. Bineesh[a], Rahul G. Kumar[a], C. Mohitha[a], S. Venu[b], A. Kathirvelpandian[a], A. Gopalakrishnan[c] and J. K. Jena[d]

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ABSTRACT
Groupers are important commercial fish in many parts of the world. Accurate identification is critical for effective conservation assessment and fisheries management. Genetic barcodes provide a simple and reproducible method for the identification of species even in the absence of taxonomic expertise. The generation of reference barcodes from properly identified specimens is an important first step in this direction. Here, 36 species belonging to the subfamily Epinephelinae (Family: Serranidae) were collected from landings on the west coast of India and Port Blair, Andaman, and partial nucleotide sequence data of the mitochondrial cytochrome C oxidase subunit I (*COI*) gene was generated. Barcodes for 13 species were developed from Indian waters for the first time. Analysis using the *COI* gene produced phylogenetic trees in concurrence with other multi-gene studies. *Epinephelus fasciatus* and *E. areolatus* were found to be a species complex, as hypothesized in other studies. The DNA barcodes developed in the study can be used for identifying species within Epinehelinae, where taxonomic ambiguity still exists.

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COI; DNA barcoding; groupers; India

Introduction

Groupers are economically important fish the world over and are highly priced. Some species are also used as ornamental fish. Many species, such as *Epinephelus bleekeri, E. areolatus, E. malabaricus, E. coioides, E. fuscoguttatus, E. lanceolatus, E. tauvina, Plectropomus leopardus,* and *P. maculatus* have been prioritized as potential species for aquaculture (Noikotr et al., 2013; Pierre et al., 2008). Although India is rich in grouper diversity, species-specific catch data is not available due to difficulties in field level identification (Heemstra & Randall, 1993). Groupers are identified based on morphological characteristics and color pattern (Heemstra & Randall, 1993), but, overlapping meristic counts and changes in color pattern during various life stages contributes to misidentification (Craig et al., 2001; Heemstra & Randall, 1993). The presence of species complexes and synonymies, as well as doubts over some generic placements also makes assessment for conservation and fisheries management difficult (Craig & Hastings, 2007; Schoelinck et al., 2014). Due to over fishing, some grouper species face the threat of extinction in the wild. Out of 163 grouper species reported across the globe, red list assessment estimates that 20 species are at the risk of extinction and another 22 species are considered to be nearly threatened (Sadovy de Mitcheson et al., 2013). As groupers are a commercially important group of fishes, several low value fishes are mislabeled as grouper for enhanced market value (Ropicki et al., 2010). Accurate species identification is, therefore, essential for biodiversity assessment, fishery management, and population dynamic analysis. In such cases, DNA barcoding– a global bio-identification system for animals using mitochondrial gene cytochrome c oxidase I (COI), can play a key role in identifying up to the species level accurately (Hebert et al., 2003). DNA barcoding is helpful in identifying individuals at different life stages, incomplete specimens, and cryptic species (Basheer et al., 2014; Chakraborty & Ghosh, 2014; Hebert et al., 2004; Lakra et al., 2009; Noikotr et al., 2013; Persis et al., 2009; Ward et al., 2005) and can prove very useful in identifying mislabeled fish products and for identifying the illegal catch of protected species (Civera, 2003; Filonzi et al., 2010). DNA barcoding is a sequencing-based technique which can be utilized for the identification for a wide range of species and it represents the largest attempt to catalog biodiversity using molecular approaches. Although many markers have been employed for DNA barcoding, the use of COI gene fragment has proven to be most effective in the identification of 98% marine fish species and 93% fresh water fish species (Ward et al., 2009).

Only a few molecular studies have been carried out on groupers. Craig et al. (2001) reported the first molecular analysis of Epinephelinae and the evidence for paraphyly in *Cephalopholis* and *Epinephelus*. Maggio et al. (2005) presented a hypothesis of relationships for few Eastern Atlantic species from the genus *Epinephelus* and *Mycteroperca*. Craig & Hastings (2007) carried out a molecular analysis and revised the

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classification of Epinephelini and resurrected the genus *Hyporthodus*. Schoelinck et al. (2014) carried out phylogenetic analysis using five genes. Alcantara & Yambot (2014) barcoded important grouper species from Philippines. There are many reports about identification using traditional taxonomic tools, fishery, and biology of groupers from Indian waters (James et al., 1996; Kirubasankar et al., 2013; Rajan, 2001; Roy & Gopalakrishnan, 2011; Sujatha et al., 2004), but molecular studies are limited. Govindaraju & Jayasankar, (2004) studied the taxonomic relationship of seven species of *Epinephelus* using RAPD finger printing and Lakra et al., (2009) barcoded seven species belonging to the genus *Epinephelus*. Reference sequences are critical to the success of any DNA barcoding program. This study was undertaken to generate reference sequences of properly identified specimens of groupers within Epinehelinae from Indian waters to aid in the identification of these fishes in the future.

Materials and methods

Sample collection

Fresh specimens of grouper species including juveniles and adults were collected from Kochi (9°56′21.5″N 76°15′45.6″E), Mangalore (12°53′14.4″N 74°48′49.5″E), and Andaman & Nicobar islands (11°58′75.2″N, 92°61′46.7″E), India, from August 2013 to June 2014. All specimens were identified using morphomeristic characters and color pattern (Craig et al., 2011; Heemstra & Randall, 1993; Randall & Heemstra, 1991), following the classification scheme proposed by Craig et al. (2011). All specimens were photographed, numbered, and voucher specimens were preserved in 10% formalin and maintained in the collections of the Peninsular and Marine Fish Genetic Resource Center of National Bureau of Fish Genetic Resources, Kochi. Kerala, India. For DNA extraction, a piece of tissue (fin clips and muscle, approx. 5 × 5 mm size) was excised prior to formalin fixation and preserved in absolute alcohol (MERCK).

DNA isolation, PCR amplification, and sequencing

Total DNA was extracted from the muscle samples following the procedure of Miller et al. (1988) with minor modifications. The extracted DNA was checked through 0.8% agarose gel electrophoresis with ethidium bromide incorporated in 1 × TBE buffer. The quality and the quantity of the extracted DNA were measured with an UV spectrophotometer (Beckman, Brea, CA) by taking the optical density (OD) at 260 nm and 280 nm. Subsequently, the DNA was diluted to 100 ng/µl for further use.

Approximately 655 bp of 5′ region of mitochondrial Cytochrome C Oxidase subunit I was PCR amplified using primers Fish F1 (5′-TCA ACC AAC CAC AAA GAC ATTGGC AC-3′) and Fish R1 (5′-TAG ACT TCT GGG TGG CCA AAG AATCA-3′) (Ward et al., 2005). The amplifications were performed in 25 µl reactions containing 1 × assay buffer (100 mM Tris, 500 mM KCl, 0.1% gelatin, pH 9.0) with 25 mM MgCl2 (ThermoFischer Scientific, Mumbai, India), 10 pmoles of each primer, 200 µM of each dNTP (Thermo Fisher Scientific, Mumbai, India), 1.5U Taq DNA polymerase and 40 ng of template DNA. The thermocycler conditions included initial preheating at 95 °C for 5 min, denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s, and extension at 72 °C for 45s, repeated for 32 cycles, followed by a final extension for 5 min at 72 °C.

About 5 µl PCR product along with marker (100 bp DNA ladder; ThermoFischer Scientific, Mumbai, India) were electrophoresed in 1.5% agarose gel (with ethidium bromide) using 1 × TBE buffer for 30 min at constant voltage (90 V). The gel was visualized and documented using BIORAD Gel Doc ™ XR + with Image Lab Software (Bio-Rad Laboratories, Inc., Berkeley, CA). The remaining PCR product was purified using GeneJET PCR Purification Kit (Thermo Fisher Scientific, Mumbai, India) following the instructions given by the manufacturer. Products were labeled using the BigDye Terminator V.3.1 Cycle sequencing Kit (Applied Biosystems Inc., Foster City, CA) and sequenced bidirectionally using ABI 3730 capillary sequencer, following the instructions of the manufacturer.

Data analysis

The raw DNA sequences were edited using BioEdit sequence alignment editor version 7.0.5.2 (Hall, 1999). Multiple alignments of sequences were performed using CLUSTAL X version 2.0 alignment editor (Applied Biosystems Inc., Foster City, CA) as implemented in BioEdit. Phylogenetic and molecular evolutionary analysis was carried out using MEGA V.6.0 software (Tamura et al., 2013). The standard error of pairwise sequence divergence among populations was calculated according to Kimura two-parameter model in MEGA (Kimura, 1980). The rate of transitions/transversions was also calculated using MEGA. The number of polymorphic sites and nucleotide diversity (Pi) and nucleotide composition between species were determined by DnaSp ver 3.0 (Rozas et al., 2006). Neighbor-joining (NJ) trees of K2P distance were generated to provide graphical representation of divergence with 1000 replications. The reference sequences of mitochondrial COI of genus *Mycteroperca* were taken from NCBI for comparison and analysis.

Results

In this study, we barcoded 36 grouper species, belonging to six genera, including seven species listed in the IUCN red list under the threatened category. A total of 107 sequences were generated and submitted to GenBank (Table 1). Sequencing of the *COI* gene produced an average length of 655 nucleotide base pairs. As expected, all variable changes within species were third codon position transitional substitutions. The intrageneric and intergeneric divergences were calculated (Tables 2 and 3). The nucleotide frequencies were found to be 24.46% (A), 29.47% (T), 27.84% (C), and 18.22% (G). The transition/transversion ratios were estimated as $k_1 = 0.142$ (purines) and $k_2 = 6.921$ (pyrimidines). The overall transition/transversion bias was $R = 2.348$. Altogether a total of 54 haplotypes were recorded. The haplotype diversity and nucleotide diversity was found to be 0.9830 and 0.14465, respectively.

Table 1. Groupers used in phylogenetic analysis with GenBank accession numbers.

S. no.	Species	Accession number	No. of specimens
1	*Aethaloperca rogaa*	KM226213–KM226216	4
2	*Cephalopholis aurantia*	KM226217–KM226218	2
3	*Cephalopholis argus*	KM226219	1
4	*Cephalopholis formosa*	KM226220	1
5	*Cephalopholis miniata*	KM226221–KM226225	5
6	*Cephalopholis nigripinnis*	KM226226–KM226230	5
7	*Cephalopholis sonnerati*	KM226231–KM226234	4
8	*Epinephelus areolatus*	KM226235–KM226238	4
9	*Epinephelus bleekeri*	KM226239–KM226243	5
10	*Epinephelus chlorostigma*	KM226244–KM226245	2
11	*Epinephelus coioides*	KM226246–KM226248	3
12	*Epinephelus diacanthus*	KM226249–KM226253	5
13	*Epinephelus epistictus*	KM226254–KM226260	7
14	*Epinephelus fasciatus*	KM226261–KM226265	5
15	*Epinephelus flavocaeruleus*	KM226266–KM226268	3
16	*Epinephelus latifasciatus*	KM226269–KM226270	2
17	*Epinephelus longispinis*	KM226271–KM226276	6
18	*Epinephelus macrospilos*	KM226277–KM226279	3
19	*Epinephelus malabaricus*	KM226280	1
20	*Epinephelus melanostigma*	KM226281	1
21	*Epinephelus miliaris*	KM226282–KM226283	2
22	*Epinephelus morrhua*	KM226284–KM226285	2
23	*Epinephelus poecilonotus*	KM226286–KM226287	2
24	*Epinephelus polylepis*	KM226288–KM226292	5
25	*Epinephelus polyphekadion*	KM226293	1
26	*Epinephelus quoyanus*	KM226294	1
27	*Epinephelus radiatus*	KM226295–KM226297	3
28	*Epinephelus spilotoceps*	KM226298–KM226300	3
29	*Epinephelus tauvina*	KM226301	1
30	*Epinephelus undulosus*	KM226302–KM226303	2
31	*Hyporthodus octofasciatus*	KM226304–KM226307	4
32	*Plectropomus areolatus*	KM226308	1
33	*Plectropomus leopardus*	KM226309–KM226310	2
34	*Plectropomus laevis*	KM226311	1
35	*Variola albimarginata*	KM226312–KM226315	4
36	*Variola louti*	KM226316–KM226319	4

Aethaloperca

The genus *Aethaloperca* is monotypic. Barcodes for *A. rogaa* were generated. The average nucleotide frequency was observed to be T = 30.7, C = 27.1, A = 25.1, and G = 17.2. Out of 654 sites, 653 were invariable and remaining one was parsimony informative site. The intra-specific variation was as 0.1%.

Cephalopholis

Six species belong to the genus *Cephalopholis* were barcoded. Inter-specific variation was found to be in the range of

Table 2. Intra-generic divergence of different genus of groupers in this study.

	Genus name	Mean distance
1	*Aethaloperca*	0.000
2	*Cephalopholis*	0.066
3	*Epinephelus*	0.101
4	*Hyporthodus*	0.000
5	*Plectropomus*	0.057
6	*Variola*	0.038

Table 3. Inter generic divergence of different genus of groupers in this study.

	1	2	3	4	5	6
1. *Aethaloperca*						
2. *Cephalopholis*	0.118					
3. *Epinephelus*	0.129	0.137				
4. *Hyporthodus*	0.109	0.13	0.109			
5. *Plectropomus*	0.168	0.171	0.158	0.165		
6. *Variola*	0.15	0.156	0.161	0.148	0.171	

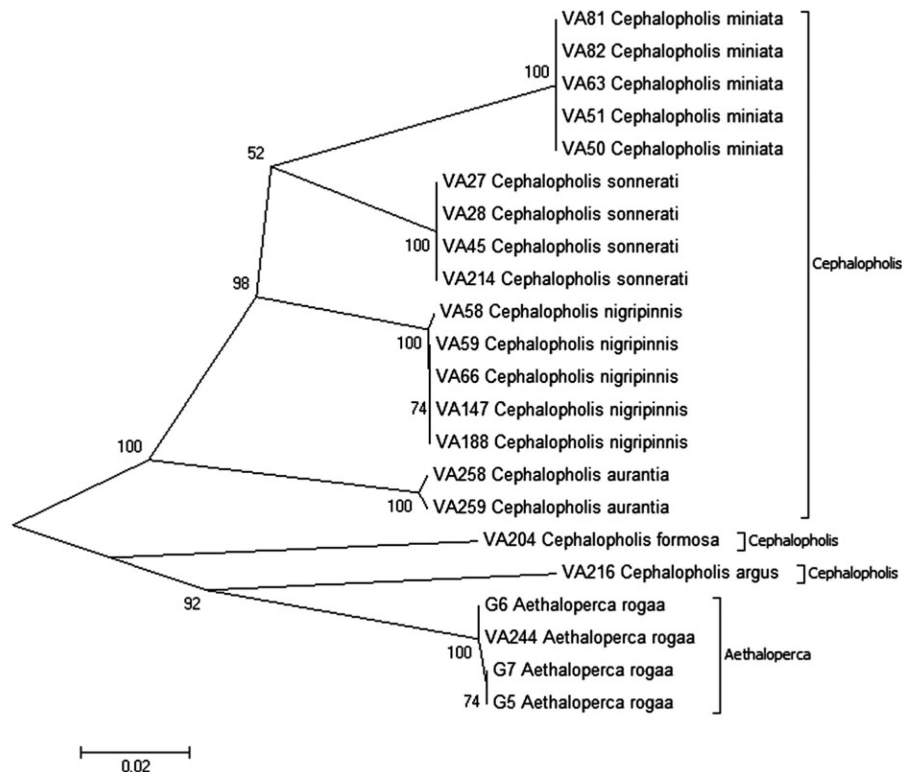

Figure 1. NJ tree of *COI* gene sequences of the genus *Aethaloperca and Cephalopholis* using K2P distances.

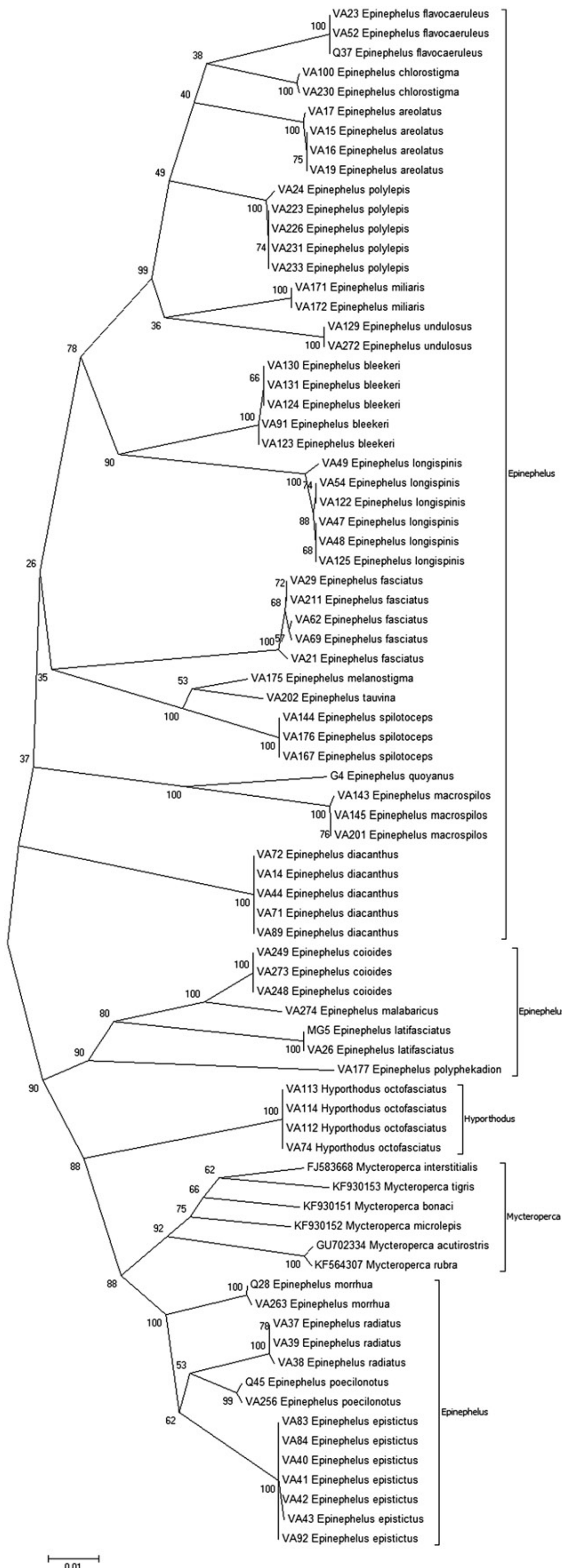

Figure 2. NJ tree of *COI* gene sequences computed using K2P distances of delete genus *Epinephelus, Hyporthodus,* and compared with NCBI sequences of *Mycteroperca*.

6.5–20.3% and intra-specific variation up to 0.3%. The average nucleotide diversity in the genus was T = 31.1, C = 27.6, A = 23.6, and G = 17.7. The sequences consisted of 655 base pair with 483 constant sites, 167 variable sites, 47 singleton, and 98 parsimony informative sites. Out of 18 sequences generated, there were eight haplotypes with haplotype diversity, Hd: 0.856, and nucleotide diversity, Pi: 0.082. The phylogenetic tree of *Aethaloperca sp* and *Cephalopholis spp* forming sister clades which indicate that these two genera are closely related. (Figures 1 and 4).

Epinephelus

A total of 23 species belonging to the genus *Epinephelus* were barcoded and 69 sequences were generated. Intra and inter-specific levels of variation were recorded as 0–0.5% and 2.5–13.4, respectively. The average nucleotide diversity was estimated as T = 29.8, C = 28.0, A =24.0, and G =18.2. Sequences revealed 409 invariable, 216 variable, nine singleton variable sites, and 207 parsimony informative sites. Haplotype analysis of sequences revealed 37 haplotypes with haplotype diversity, Hd: 0.975 and nucleotide diversity, Pi: 0.12. The phylogenetic NJ tree depicting status of species under *Epinephelus* is given in Figures 2 and 4.

Hyporthodus

One species belonging to this genus, *Hyporthodus octofasciatus,* was barcoded. The average nucleotide diversity in the genus *Hyporthodus* was T = 31.8, C = 26.6, A = 23.5, and G= 18.2. Phylogenetic tree of the species under genera *Hypothrodus* is given in Figures 2 and 4.

Plectropomus

Three species belonging to the genus *Plectropomus* were barcoded. The sequences showed no variation within the species and between the species level of variation was ranged from 8.4% to 11.6%. The average nucleotide diversity in the genus *Plectropomus* was T =30.3, C = 27.3, A =24.8, and G =17.7. The sequence with 655 bp showed 567 invariable sites, 87 variable sites, 18 parsimony informative sites, and 69 singleton variable sites. A total of four sequences were analyzed and showed three haplotypes with haplotype diversity, Hd: 0.833 and nucleotide diversity, Pi: 0.071.

Variola

Two species belonging to the genus *Variola* were barcoded and they showed within species variation up to 0.3% and between species up to 8.6%. The average nucleotide diversity in *Variola* was T = 31.9, C = 26, A = 23.9, and 18.2. The sequence analysis showed 599, 53 52, and 1 sites with alignment gaps or missing data, invariable sites, variable sites, parsimony informative, and singleton variable sites. A total of eight sequences were generated with three haplotypes. Phylogenetic tree of the genus *Plectropomus* and *Variola* is given in Figure 3.

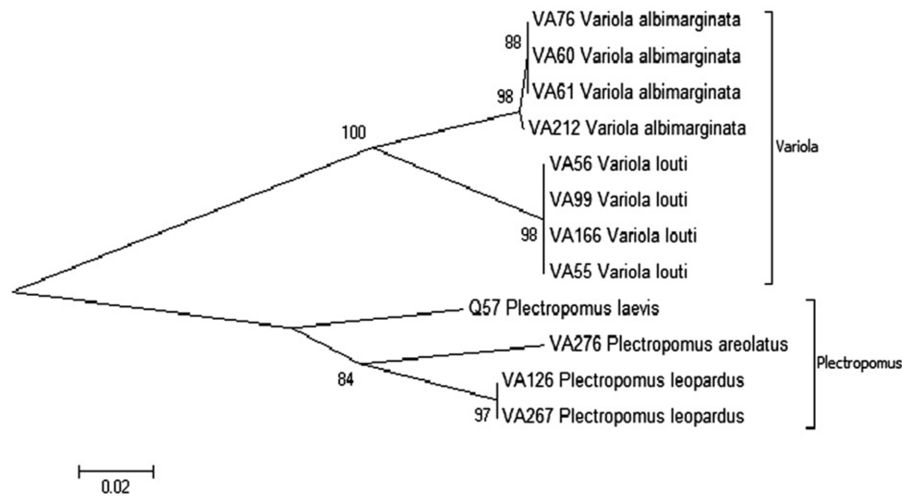

Figure 3. NJ tree of *COI* gene sequences computed using K2P distances genus *Plectropomus* and *Variola*.

Discussion

A total of 36 species of grouper were barcoded in this study. Phylogenetic analysis showed six major clades of grouper species (Figure 4). Out of 23 species of *Epinephelus*, barcodes of *E. polylepis* and *E. miliaris* were generated for the first time. *Epinephelus polylepis*, described from the northwest Indian Ocean (Randall, 1991), has not been reported from Indian waters since its original description. It is very closely allied with *E. chlorostigma*. The specimens which we identified as *E. polylepis* (lateral-line scales 65–72; lateral-scale series 126–137) and *E. chlorostigma* (lateral-line scales 48–53; lateral-scale series 96–122) genetically differ by 4% in COI analysis. From this, we conclude that both species are present in commercial grouper landings in India, and that DNA barcodes can be used to reliably differentiate the two species in the commercial trade.

Cephalopholis nigripinnis (Valenciennes 1828) has for long been considered a synonym of *C. urodeta* (Forster 1801) (Heemstra & Randall, 1993; Randall & Heemstra, 1991). They have been recently considered as distinct species mainly based on the color of the caudal fin (Allen & Adrim, 2003, Allen & Erdmann, 2012). Sequences generated for species we identified as *C. nigripinnis* showed a less than 1% difference when compared with sequences of *C. urodeta* from GenBank (FJ583012–FJ583015, JQ349869–JQ349871). A similar situation exists with *Hyporthodus octofasciatus* (Griffin 1926) and *H. ergastularius* (Whitley 1930), which are very similar based on the comparison of our sequences of *H. octofasciatus* and that of *H. ergastularius* from GenBank (DQ107881 and DQ107882). More detailed analysis using specimens from across the distribution ranges of these species are called for to resolve the identities of these species.

Epinephelus coioides often gets misidentified with *E. tauvina* and *E. malabaricus.* All the three species possess aquaculture importance, and reports indicate that they are being cultured in different countries without proper identification at the species level (Heemstra & Randall, 1993; Randall & Heemstra, 1991). In our study, we developed barcodes for all three species. COI analysis of *E. coioides* (Pyloric caeca 50–60) and *E. tauvina* (Pyloric caeca 16–18,) showed 15% genetic variability. The barcode of *E. fasciatus* from our study showed only 92% similarity with the barcodes of specimens reported from South Africa (GU805082, JX093907, and KF489582) and 96% similarity with specimens from Australia and French Polynesia (DQ107875, DQ107876, and JQ431717). Heemstra & Randall (1993) reported six populations of *E. fasciatus* based on the color pattern and scale counts. Our results support their conclusions and suggest that further research is required to determine taxonomic status of the *Epinephelus* species which are currently synonymized with *E. fasciatus*. Similarly, the sequences of *E. areolatus* match 100% (KJ607969), 99% (HQ945841 and HQ149838), 97% (KC970469, KC593374, and DQ107870), and 95% (KJ594969) with the sequences of *E. areolatus* available in GenBank, hence we suggest further review of specimens currently assigned to *E. areolatus* from the Red sea, Indian Ocean, and Indo-West Pacific for validating this species currently synonymized under this name. Sujatha et al. (2008) reported *Epinephelus magniscuttis* as a new record from Vishakapatnam, India. On comparing meristic counts of *E. mangniscuttis,* originally described from Reunion Island, Indian Ocean, with the description and photograph in the publication, we suspect that it is more likely to be *E. epistictus.*

Maggio et al. (2005) also reported a close relationship between the genus *Epinephelus* and *Mycteroperca* using mitochondrial molecular markers and proposed to change the generic status of *M. rubra* to the genus *Epinephelus* based on the evolutionary relationships with *E. costae*. Craig & Hastings (2007) considered *E. albomarginatus, E. caninus, E. costae, E. goreensis, E. marginatus, E. morrhua, and E. radiatus* to be members of the genus *Mycteroperca*. Schoelinck et al. (2014) proposed *E. poecilonotus* be merged with the genus *Mycteroperca*, using mitochondrial (COI, 16S) and nuclear genes (TMO-4C4, Rhodopsin, and pkd1). From our analysis, we also found that the species *Epinephelus epistictus, E. morrhua, E. poecilonotus*, and *E. radiatus* from Indian waters form a clade with the genus *Mycteroperca* (Figures 2 and 4). *Epinephelus epistictus* was not included in analysis of Craig & Hastings (2007) and Schoelinck et al. (2014). We agree with the findings of Craig & Hastings (2007) based on the analysis of *COI*

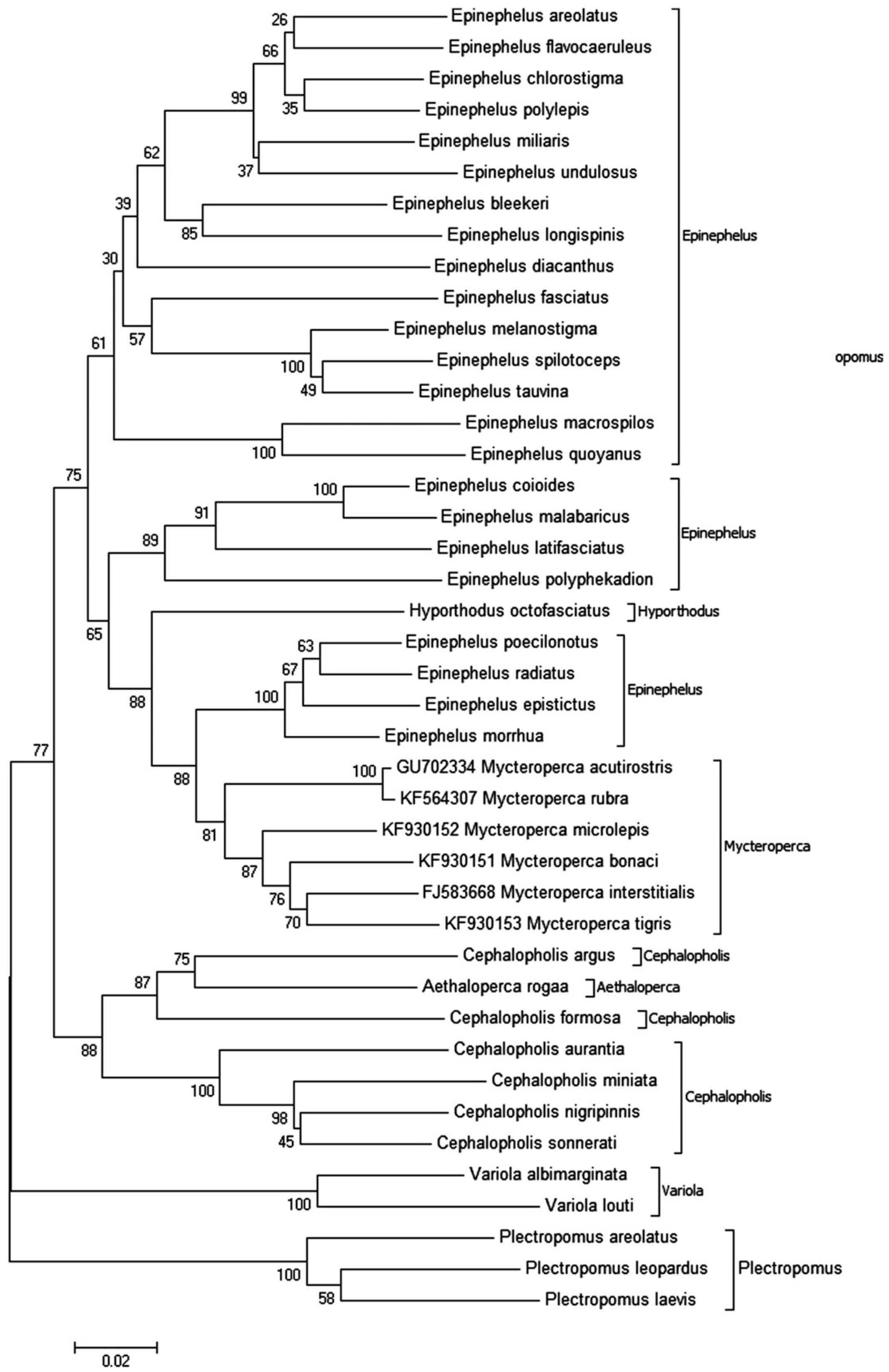

Figure 4. NJ tree of *COI* gene sequences computed using K2P distances of the groupers barcoded.

gene and also support their observation on the number of anal fin rays alone is not a good character for distinguishing *Epinephelus* and *Mycteroperca*.

Similarly, NJ tree of *Aethaloperca* and *Cephalopholis* showed two sister clades consisting of *C. miniata, C. sonnerati, C. nigripinnis,* and *C. auratia* in the first one and *C. formosa, C. argus,* and *A. rogaa* in the other, supporting the grouping of *Aethaloperca* with the genus *Cephalopholis* as reported by Craig & Hastings (2007) and Schoelinck et al. (2014). The species belonging to the genera *Variola* and *Plectropomus* showed significant genetic variability than other genera hence there is no ambiguity among the species belonging to these genera.

The molecular data analysis of Epinephelinae from the present study deviates from the morphological hypothesis and based on the molecular data, we suggest a reevaluation is needed in the morphological characteristics used for taxonomic identification of grouper species from Indian waters.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

Alcantara SG, Yambot AV. (2014). DNA barcoding of commercially important Grouper species (Perciformes, Serranidae) in the Philippines. Mitochondrial DNA 1–9.

Allen GR, Erdmann MV. (2012). Reef fishes of the East Indies, vol. I–III. Perth Australia: Tropical Reef Research. v. I: x + 1-424 + end note; v. II: 425–855. http://dx.doi.org/10.3109/19401736.2014.958672.

Allen GR, Adrim M. (2003). Coral reef fishes of Indonesia. Zool Stud – Taipei 42:1–72.

Basheer VS, Mohitha C, Vineesh N, Divya PR, Gopalakrishnan A, Jena JK. (2014). Molecular phylogenetics of three species of the genus *Rastrelliger* using mitochondrial DNA markers. Mol Biol Rep 42:873–9.

Chakraborty M, Ghosh SK. (2014). An assessment of the DNA barcodes of Indian freshwater fishes. Gene 537: 20–8.

Civera T. (2003). Species identification and safety of fish products. Vet Res Commun 27:481–9.

Craig MT, Hastings PA. (2007). A molecular phylogeny of the groupers of the subfamily Epinephelinae (Serranidae) with a revised classification of the Epinephelini. Ichthyol Res 54:1–17.

Craig MT, Pondella IIDJ, Franck JP, Hafner JC. (2001). On the status of the Serranid fish genus *Epinephelus:* Evidence for paraphyly based upon 16S rDNA sequence. Mol Phylogenet Evol 19:121–30.

Craig MT, Sadovy de Mitcheson YJ, Heemstra PC. (2011). Groupers of the world: A field and market guide. NISC. i-xx + 1-356 + A:1-A: 47

Filonzi L, Chiesa S, Vaghi M, Nonnis Marzano F. (2010). Molecular barcoding reveals mislabelling of commercial fish products in Italy. Food Res Int 43:1383–8.

Govindaraju GS, Jayasankar P. (2004). Taxonomic relationship among seven species of groupers (Genus Epinephelus; Family Serranidae) as revealed by RAPD fingerprinting. Mar Biotechnol 6:229–37.

Hall TA. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–8.

Hebert PDN, Cywinska A, Ball SL, deWaard JR. (2003). Biological identifications through DNA barcodes. Proc Biol Sci 270:313–21.

Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. Proc Natl Acad Sci USA 101:14812–7.

Heemstra PC, Randall JE. (1993). 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. Rome: Food and Agriculture Organization of the United Nations.

James PSBR, Murty VS, Nammalwar P. (1996). Groupers and snappers of India: biology and exploitation. ICLARM Conference Proceedings, vol. 48. p. 106–36.

Kimura M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–20.

Kirubasankar R, Dam Roy S, George G, Sarma K, Krishnan P, Ramkumar S, Kaliyamoorthy M, Goutham Bharathi MP. (2013). Fishery and exploitation of Malabar Grouper, *Epinephelus malabaricus* (Bloch & Schneider 1801) from Andaman islands. Asian Fish Sci 26:167–75.

Lakra WS, Goswami M, Gopalakrishnan A. (2009). Molecular identification and phylogenetic relationships of seven Indian Sciaenids (Pisces: Perciformes, Sciaenidae) based on 16S rRNA and cytochrome c oxidase subunit I mitochondrial genes. Mol Biol Rep 36:831–9.

Lakra WS, Verma MS, Goswami M, Lal KK, Mohindra V, Punia P, Gopalakrishnan A, et al. (2011). DNA barcoding Indian marine fishes. Mol Ecol Resour 11:60–71.

Maggio T, Andaloro F, Hemida F, Arculeo M. (2005). A molecular analysis of some Eastern Atlantic grouper from the *Epinephelus* and *Mycteroperca* genus. J Exp Mar Biol Ecol 321:83–92.

Miller SA, Dykes DD, Polesky HF. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215

Noikotr K, Chaveerach A, Pinthong K, Tanomtong A, Sudmoon R, Tanee T. (2013). RAPD and barcode analyses of groupers of the genus *Epinephelus.* Genet Mol Res 12:5721

Persis M, Chandra Sekhar Reddy A, Rao LM, Khedkar GD, Ravinder K, Nasruddin K. (2009). COI (cytochrome oxidase-I) sequence based studies of Carangid fishes from Kakinada coast, India. Mol Biol Rep 36:1733–40.

Pierre S, Gaillard S, Prévot-D'Alvise N, Aubert J, Rostaing-Capaillon O, Leung-Tack D, Grillasca JP. (2008). Grouper aquaculture: Asian success and Mediterranean trials. Aquat Conserv 18:297–308.

Rajan PT. (2001). A field guide to grouper and Snapper fishes of Andaman and Nicobar Islands. New Delhi: Zoological Survey of India.

Randall JE, Heemstra PC. (1991). Revision of Indo-Pacific groupers (Perciformes: Serranidae: Epinephelinae) with descriptions of five new species. In: Indo-Pacific fishes 20. p. 1–332.

Ropicki AJ, Larkin SL, Adams CM. (2010). Seafood substitution and mislabeling: WTP for a locally caught grouper labeling program in Florida. Marine Resour Econ 25:77–92.

Roy TSC, Gopalakrishnan A. (2011). Identification of groupers based on pyloric caeca differentiation. J Fish Biol 79:1334–9.

Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R. (2006). DNA sequence polymorphism version 4.10.9. Bioinformatics 19:2496–7.

Sadovy de Mitcheson Y, Craig MT, Bertoncini AA, Carpenter KE, Cheung WW, Choat JH, John HC, et al. (2013). Fishing groupers towards extinction: a global assessment of threats and extinction risks in a billion dollar fishery. Fish Fish 14:119–36.

Schoelinck C, Hinsinger DD, Dettaï A, Cruaud C, Justine JL. (2014). A phylogenetic re-analysis of groupers with applications for Ciguatera fish poisoning. PloS One 9:e98198

Sujatha K. (2004). Grouper off Visakhapatnam, North-East coast of India. J Mar Biol Ass India 46:87–92.

Sujatha K, Padmavathi P, IswaryaDeepti VA, Shrikanya KVL. (2008). *Epinephelus magniscuttis* Postel, Fourmanoir and Gueze, 1963 – New record. Indian J. Fish 55:341–3.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. (2013). MEGA6: Molecular evolutionary genetics analysis Version 6.0. Mol Biol Evol 30:2725–9.

Ward RD, Hanner R, Hebert PDN. (2009). The campaign to DNA barcode all fishes, FISH-BOL. J Fish Biol 74:329–56.

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN. (2005). DNA barcoding Australia's fish species. Philos Trans R Soc Lond, B, Biol Sci 360:1847–57.