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Short Communication: Species confirmation of juvenile cloudy grouper, *Epinephelus erythrurus* (Valenciennes, 1828), based on a morphologic analysis and partial CO1 gene sequencing

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Abstract. *Ariyanti Y, Farajallah A. 2019. Species confirmation of juvenile cloudy grouper,* Epinephelus erythrurus *(Valenciennes, 1828), based on a morphologic analysis and partial CO1 gene sequencing*. *Biodiversitas 20: 914-921*. The genus *Epinephelus* is the most species among the genera within the subfamily Epinephelinae. Species identification techniques for groupers, especially in the *Epinephelus*, are commonly based on color patterns and a suite of morphological characters, including body shape and the size and number of fins, scales and gill rakers. In some species, juveniles are morphologically distinct from adults of the same species, making morphological identification highly problematic. This present work will provide some morphological description or variations of juveniles that have been identified as *Epinephelus erythrurus* based on CO1 sequences. Further, the present study demonstrates that a molecular genetic technique, based on partial sequencing of the mitochondrial CO1 gene, may be used for the rapid species confirmation of every developmental stage and phase of an organism (juvenile *E. erythrurus*). Two DNA sequences of *E. erythrurus* from the Pangandaran District (7º43'8.31"S 108º30'11.59"E) have been submitted to GenBank under accession numbers KP998441 and KP998442.

Keywords: *Epinephelus erythrurus*, CO1, confirmation, grouper, juveniles

INTRODUCTION

The Subfamily Epinephelinae is a group of carnivorous marine fishes that belongs to the Family Serranidae. This group comprises about 159 species in 15 genera, commonly known as groupers, rockcods, hinds, and seabasses (Heemstra and Randall 1993). These commercially important fishes are bottom-associated which is found in tropical and subtropical waters. Most species occupy coral reefs, but some inhabit estuaries or on rocky reefs. Grouper has potential economic value in fisheries. However, the classification and their evolutionary relationship were often constrained by the incredible number of species, wide distribution, and lack of morphological key that it is used in classification. The genus *Epinephelus* is the most species among the genera within the subfamily Epinephelinae. The genus is represented in tropical and subtropical latitudes of all three major oceans. *Epinephelus* species are among the most important commercial fishes in global tropical fisheries (Carpenter and Niem 1999). However, many species within *Epinephelus* lack morphological specializations that are particularly used to identify individual species in the field.

Identification techniques for grouper species, especially *Epinephelus*, are commonly based on their color patterns and a suite of morphological characters, including body shape and the size and number of fins, scales and gill rakers (Craig et al. 2001; Heemstra and Randall 1993). The color pattern is usually distinctive enough to identify large adult groupers at the species level, but intra-specific variations in color pattern exist for each species (Heemstra and Randall 1993). Moreover, interspecific hybridizations are found among many grouper species (Ding et al. 2006). This has rendered to a great deal of taxonomic confusion within the genus.

In many cases, fishes, and especially their diverse developmental stages, are difficult to identify using morphological characters (Teletchea 2009). For example, the juveniles of some species of grouper are completely morphologically distinct from adults of the same species, making morphological identification extremely difficult (Govindaraju and Jayasankar 2004). Molecular genetic techniques, such as DNA sequencing of genes are known to complement morphological identification (Hebert et al. 2003). For example, a small sequence of the mitochondrial *cytochrome oxidase c subunit 1* (CO1) gene has been used in rapid analyses for commercial purposes, especially for the confirmation of fish species (Ward et al. 2005; Barber and Boyce 2006; Wong and Hanner 2008; Sachithanandam et al. 2012).

This present work will provide some morphological description or variations of juveniles that have been identified as *Epinephelus erythrurus* based on CO1

sequences. Partial of cytochrome oxidase c subunit 1 (CO1) gene were used as a molecular marker in order to confirm two *E. erythrurus* juveniles. We sought to show that molecular genetic techniques can be used to complement the morphological identification of juveniles of *E. erythrurus*. This species is very rarely caught so that the reports of the existence of this species is still lacking. Therefore, this species is listed as Least Concern (LC) status on the IUCN Red List. Another important result from this research is that the CO1 sequence for Indonesian *E. erythrurus*, which was previously absent from GenBank, is presented here for the first time.

MATERIALS AND METHODS

Study area

Samples of fish belonging to the family Serranidae were collected from fisherman at Bojongsalawe Beach (7º43'8.31"S 108º30'11.59"E) in the Pangandaran District of West Java, Indonesia (Figure 1). Phenotypic characterization was conducted using the FAO species catalog of groupers of the world, and samples were stored in formalin. A portion of the dorsal muscle tissue was stored in 95% alcohol for the molecular genetics study.

DNA extraction and PCR

Total DNA was extracted from 0.30 g of ethanol-preserved muscle tissue using a DNA Extraction Kit for animal tissue (Geneaid Biotech Ltd., New Taipei City, Taiwan), following the manufacturer's protocol. Approximately 650-655 bp were amplified from the 5′ region of the CO1 gene using combinations of the primers AF282 and AF283 (modification of Ivanova 2007; FISH-BOL, http://www.fishbol.org).

The 25µL PCR mixes included 12.5 µL of 2X ReadyMix Green GoTaq® DNA Polymerase mix (0.05 U/µL, 3mM Mg^{2+},and0.4 mM each dNTP), 1 µL of each primer at a 10 µM/µl concentration, 1 µL of template DNA and 9.5 µL of nuclease-free water. The thermal regime consisted of an initial step of 2 min at 94 °C followed by 35 cycles of 0.5 min at 94 °C, 0.5 min at 56 °C, and 1 min at 72 °C, followed in turn by 7 min at 72 °C, with the samples then held at 4.0 °C in a Takara™ thermocycler (Otsu, Japan). Verification of the 2 µL amplicon was performed using a 6% polyacrylamide gel electrophoresis, ran at a voltage of 200 V for 40 min. Visualization was facilitated by silver staining (Byun et al. 2009).

All amplicons were sequenced commercially following the manufacturer's protocol. The DNA sequences were proofread, edited and assembled using MEGA6 (Tamura et al. 2013) and BioEdit (Hall 1999). A Kimura 2-parameter metric was employed for sequence comparisons (Kimura 1980), including genetic distance calculations and to generate neighbor-joining trees based on the CO1 region, with node frequencies calculated based on 1000 bootstrap replicates.

RESULTS AND DISCUSSION

Diagnostic features

Morphometric comparisons of *E. erythrurus* with the existing literature are shown in Table 1. Specimen voucher K7_PGR has a body depth of 2.74 in standard length (SL) and a head length of 2.37 in SL, with SL and the total length of 107 mm and 135 mm, respectively. Specimen voucher K8_PGR has a body depth of 2.58 in SL and a head length of 2.33 in SL, with SL and the total length of 93 mm and 125 mm, respectively. Both specimens have dorsal fins with XI spines and 16 rays, anal fins with III spines and 8 rays, pectoral fins with 19 rays, rounded caudal fins, and a dark gray body.

CO1-based analysis

The two *E. erythrurus* DNA sequences were submitted individually to GenBank under accession numbers KP998441 for the K7 PGR specimen voucher and KP998442 for the K8 PGR specimen voucher (Table 3). Out of the 640-651-bp basic taxonomic sequence length, we were able to obtain 548bp. BLAST searches using the two sequences indicated only nine sequences of *E. erythrurus* from Thailand and Malaysia in GenBank. The query coverage was 91%, and the maximum identity was 99-100% with existing *E. erythrurus* sequences in GenBank. Based on the partial CO1 sequences, a molecular phylogenetic tree was constructed using the neighbor-joining method (Kimura 2-parameter). The bootstrap confidence values of the nodes are indicated above each branch. Interestingly, Thai *E. erythrurus* had the smallest number of nucleotides (420 bp). We constructed two phylogenetic trees, with and without the Thai *E. erythrurus* sequences (Figures 2 and 3). Intra-and inter-specific genetic distances are shown in Table 2.

Figure 2 shows a phylogenetic tree without the Thai *E. erythrurus* sequence. The two sequences in the present study were grouped with similar Malaysian *E. erythrurus* sequences. Some *E. coeruleopunctatus* sequences formed a sister group with *E. erythrurus*, while other sequences formed a separate clade. Furthermore, the intra-specific genetic distance of *E. erythrurus* ranged from 0.000 to 0.014 with the same species from other countries, while the inter-specific genetic distance between *E. erythrurus* and *E. coeruleopunctatus* ranged from 0.022 to 0.074. However, the phylogenetic tree with the first barcode for Thai *E. erythrurus* (JQ268576) (Figure 3) formed a species complex group with some *E. coeruleopunctatus*, distinct from the other separated clades of *E.erythrurus* and *E.coeruleopunctatus.* This suggests that *E. erythrurus* and *E. coeruleopunctatus* are not monophyletic. The genetic distance between pairs of Indonesian *E. erythrurus* and Thai *E. erythrurus* ranged from 0.066 to 0.068 (Table 2), suggesting two main genotypes of this species. Based on the two phylogenetic trees, both of our samples were grouped with the Indonesian and Malaysian *E. erythrurus* that possessed a genotype different from the Thai *E. erythrurus*.

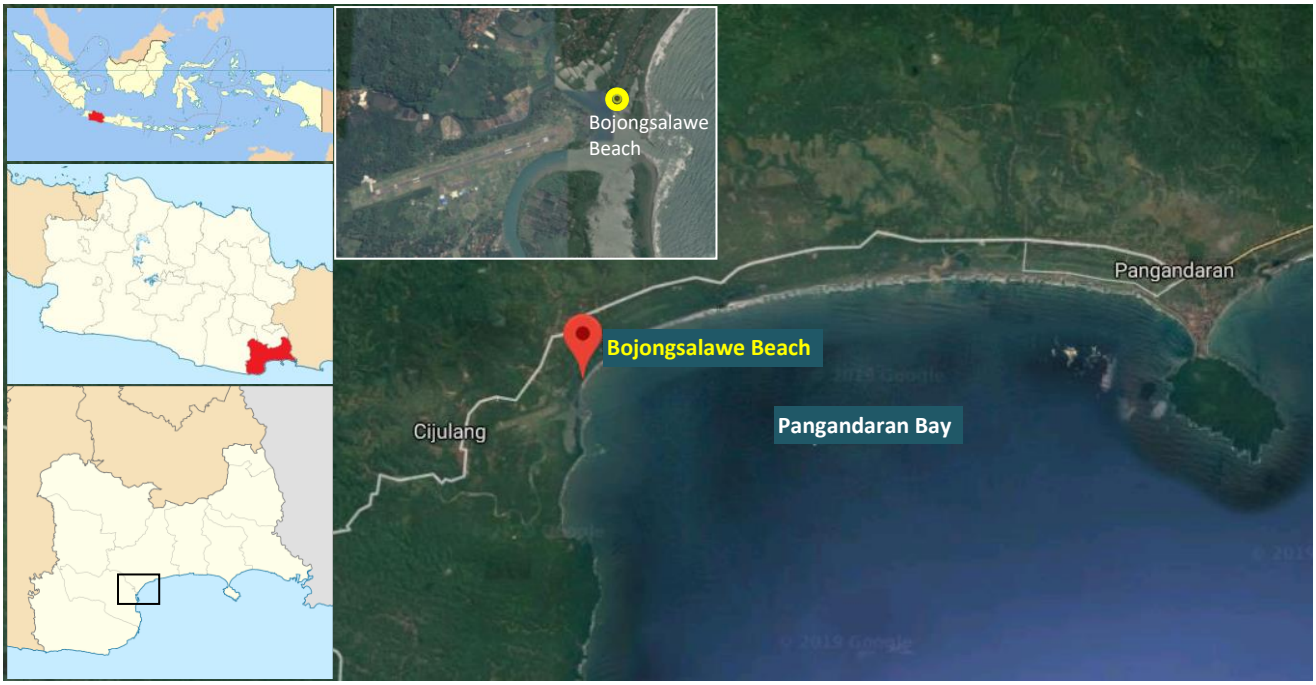

Figure 1. Location of Bojongsalawe Beach (7°43'8.31"S 108°30'11.59"E) in the Pangandaran District of West Java, Indonesia

Table 1. Morphometric comparison of the *E. erythrurus* specimens in the present study with those in the literature

Morphometric characters	***E. erythrurus* (KP998441 [K7_PGR]) (Present study)**	***E. erythrurus* (KP998442 [K8_PGR]) (Present study)**	***E. erythrurus* (Hemstra and Randall 1993)**	***E. erythrurus* (Carpenter and Niem 1998)**	***E. erythrurus* (Allen et al. 2003)**
Body depth	2.74 times in SL	2.58 times in SL	2.8-3.2 times in SL		
Standard length	107 mm	93 mm	110-280 mm		
Total length	135 mm	125 mm	-	430 mm	To 430 mm
Head length	2.37 times in SL	2.33 times in SL	2.4-2.7 times in SL		
Dorsal fin	XI	XI	XI		
Dorsal rays	16	16	15 or 17		
Anal spines	III	III	III		
Anal rays	8	8	8		
Caudal fin	Rounded	Rounded	Rounded		
Pectoral fins	19	19	17-19		
Lateral scales series	97	92	92-107		
Colour	Dark gray	Dark gray	Olive to reddish brown, usually with irregular pale spots and blotches that join randomly to form an irregular dark reticulum of the background color. Some specimens, especially the larger ones, nearly uniform brown or with the pale blotches on the body only faintly visible		Dark gray with irregular pale spots and randomly joined to form a maze-like pattern
Geographical distribution	Bojongsalawe Beach, Pangandaran District, Indonesia	Bojongsalawe Beach, Pangandaran District, Indonesia	Pakistan, India, Laccadive Island, Sri Lanka, Gulf of Thailand, Indonesia, Singapore, and Borneo	Pakistan, India, Laccadive Is. Sri Lanka, Gulf of Thailand, Indonesia, and Singapore	Pakistan, Laccadive Is. off India to Malaysian Peninsula and W. Indonesia
Habitat			Harbors and estuaries with muddy or silty-sand bottoms	In harbors and estuaries with muddy or silty-sand bottoms	Solitary, turbid harbors and estuaries with muddy or silty-sand bottoms
Depth					

Note: S:, Standard Length; mm: millimeter; m: meter

Table 3. DNA Sequences details of *E. erythrurus* in GenBank file version

Definition locus	Seq7 *Epinephelus erythrurus* CO1 gene CDS	Seq8 *Epinephelus erythrurus* CO1 gene CDS
Accession number	KP998441	KP998442
Submitted date	VRT 20-Mar-2015	VRT 20-Mar-2015
Classification	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Actinopterygii; Neopterygii; Teleostei; Euteleostei; Neoteleostei; Acanthomorpha; Eupercaria; Perciformes; Serranoidei; Serranidae; Epinephelinae; Epinephelini; Epinephelus	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Actinopterygii; Neopterygii; Teleostei; Euteleostei; Neoteleostei; Acanthomorpha; Eupercaria; Perciformes; Serranoidei; Serranidae; Epinephelinae; Epinephelini; Epinephelus
Alignment of partial Sequences of the CO1 Gene	>Seq7 AACCACAAAGACATCGGCACCCTTTATCTTGTATT TGGTGCCTGAGCCGGTATAGTAGGAACGGCTCTCA GCCTGCTTATTCGAGCCGAGCTTAGCCAACCAGGG GCTCTACTAGGTGACGATCAGATCTATAATGTAAT TGTTACAGCACACGCTTTTGTAATAATCTTTTTTA TAGTAATACCAATCATGATTGGTGGCTTTGGAAAC TGACTCATCCCGCTAATAATTGGTGCCCCAGATAT AGCATTCCCTCGAATAAATAATATGAGCTTCTGAC TTCTCCCCCCATCCTTCTTACTTCTTCTCGCTTCT TCTGGAGTAGAAGCCGGTGCTGGTACTGGCTGAAC GGTCTACCCACCCCTAGCCGGAAACCTAGCCCATG CAGGTGCATCTGTAGACTTAACTATCTTCTCATTA CATTTAGCAGGAATCTCATCAATTCTAGGTGCAAT CAATTTTATCACAACTATTATTAATATGAAACCCC CAGCTATCTCCCAATACCAAACACCTTTATTTGTA TGAGCGGTACTAATTACAGCAGTGCTCCTGCTCCT CTCCCTTCCTGTTCTCGCCGCCGGCATTACAATAC TACTTACAGATCGCAACCTTAACACCACTTTCTTT GACCCCGCT	>Seq8 AACCACAAAGACATCGGCACCCTTTATCTTGTATTT GGTGCCTGAGCCGGTATAGTAGGAACAGCTCTCAGC CTGCTTATTCGAGCCGAGCTTAGCCAACCAGGGGCT CTACTAGGTGACGATCAGATCTATAATGTAATTGTT ACAGCACACGCTTTTGTAATAATCTTTTTTATAGTA ATACCAATCATGATTGGTGGCTTTGGAAACTGACTC ATCCCGCTAATAATTGGTGCCCCAGATATAGCATTC CCTCGAATAAATAATATGAGCTTCTGACTTCTCCCC CCATCCTTCTTACTTCTTCTCGCTTCTTCTGGAGTA GAAGCCGGTGCTGGTACTGGCTGAACGGTCTACCCA CCCCTAGCCGGAAACCTAGCCCATGCAGGTGCATCT GTAGACTTAACTATCTTCTCATTACATTTAGCAGGA ATCTCATCAATTCTAGGTGCAATCAATTTTATCACA ACTATTATTAATATGAAACCCCCAGCTATCTCCCAA TACCAAACACCTTTATTTGTATGAGCGGTACTAATT ACAGCAGTGCTCCTGCTCCTCTCCCTTCCTGTTCTC GCCGCCGGCATTACAATACTACTTACAGATCGCAAC CTTAACACCACTTTCTTTGACCCCGCTGGAGGGGAG ACC
Translated protein sequence	NHKDIGTLYLVFGAWAGMVGTALSLLIRAELSQPG ALLGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGN WLIPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLAS SGVEAGAGTGWTVYPPLAGNLAHAGASVDLTIFSL HLAGISSILGAINFITTIINMKPPAISQYQTPLFV WAVLITAVLLLLSLPVLAAGITMLLTDRNLNTTFF DPA	NHKDIGTLYLVFGAWAGMVGTALSLLIRAELSQPGA LLGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWL IPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLASSGV EAGAGTGWTVYPPLAGNLAHAGASVDLTIFSLHLAG ISSILGAINFITTIINMKPPAISQYQTPLFVWAVLI TAVLLLLSLPVLAAGITMLLTDRNLNTTFFDPAGGE

Discussion

We diagnosed both of our specimens as juvenile *E. erythrurus* based on the following criteria shown in Table 1. According to Carpenter and Niem (1999), females of this species mature at 15 cm of the SL. Also, the adult color pattern of this species is usually irregular pale spots and blotches that join randomly to form an irregular dark reticulum of the background color. Some specimens, especially larger ones, are nearly uniform brown or have pale blotches on the body that are only faintly visible (Randall and Heemstra 1993).

According to Noitokr et al. (2013), the top ten BLAST results in GenBank showed that the sequences of *E. erythrurus* were highly similar to those of *Epinephelus coeruleopunctatus.* In the current classification, an adult of *E. coeruleopunctatus* has XI dorsal spines with the longest on the third or fourth spine with its length of 2.7 to 3.6 times in head length, and 15 to 17 rays; anal fin with III spines and 8 rays; large and fleshy pectoral fins, with 17-19 rays; rounded caudal fin. The color is brownish grey; the body is covered with small pale spots overlain with large pale blotches; oblique black saddle on the rear half of peduncle; 4 to 5 indistinct black blotches at the base of dorsal fin, a prominent black streak on the maxillary groove. Meanwhile, juveniles (less than 20 cm standard length) are dark grey to black, covered with prominent pupil-size white spots and smaller white dots (Randall and Heemstra 1993). Based on the statement of Hebert et al. (2003), the CO1 gene sequence can delimit species when intraspecific genetic divergences are greater than 2% so that we suppose the specimens from Thailand are not *E. erythrurus*. It is probably due to a juvenile of *E. coeruleopunctatus* could not be easily differentiated from juvenile of *E. erythrurus*. Thus, it might be caused by misidentification between both of them. These COI sequences could provide genetic data to confirm and compare molecular and morphological traits. In case, the cloudy grouper has extremely different phenotypic traits between the juvenile and adult individual in same species, and it still becomes a serious problem on species determining particularly on its cloudy grouper specimen.

Table 2. Genetic distance of CO1 sequences from Indonesian *E. erythrurus* and reference sequences from GenBank (below the diagonal), with standard error estimates (above the diagonal)

Accession number	**KP998441**	**KP998442**	**JN208613**	**JN208612**	**JN208609**	**JN208611**	**JN208614**	**JN208608**	**JN208607**	**JN208610**	**JQ268576**	**JQ349961**	**JQ349962**	**JX674992**	**JX674993**	**JX674990**	**JX674991**	**KF929848**	**JF493438**	**JX093908**
KP998441 *E. erythrurus* (Indonesia)		0.002	0.002	0.000	0.005	0.006	0.006	0.002	0.002	0.002	0.013	0.014	0.013	0.008	0.008	0.008	0.008	0.013	0.013	0.012
KP998442 *E. erythrurus* (Indonesia)	0.002		0.003	0.002	0.005	0.005	0.005	0.000	0.003	0.003	0.013	0.014	0.012	0.007	0.007	0.007	0.007	0.012	0.012	0.012
JN208613 *E. erythrurus* (Malaysia)	0.002	0.005		0.002	0.006	0.006	0.006	0.003	0.000	0.000	0.013	0.014	0.013	0.008	0.008	0.008	0.008	0.013	0.013	0.012
JN208612 *E. erythrurus* (Malaysia)	0.000	0.002	0.002		0.005	0.006	0.006	0.002	0.002	0.002	0.013	0.014	0.013	0.008	0.008	0.008	0.008	0.013	0.013	0.012
JN208609 *E. erythrurus* (Malaysia)	0.012	0.010	0.014	0.012		0.002	0.002	0.005	0.006	0.006	0.013	0.014	0.012	0.005	0.005	0.005	0.005	0.012	0.012	0.011
JN208611 *E. erythrurus* (Malaysia)	0.014	0.012	0.017	0.014	0.002		0.000	0.005	0.006	0.006	0.013	0.014	0.012	0.006	0.006	0.006	0.006	0.012	0.012	0.012
JN208614 *E. erythrurus* (Malaysia)	0.014	0.012	0.017	0.014	0.002	0.000		0.005	0.006	0.006	0.013	0.014	0.012	0.006	0.006	0.006	0.006	0.012	0.012	0.012
JN208608 *E. erythrurus* (Malaysia)	0.002	0.000	0.005	0.002	0.010	0.012	0.012		0.003	0.003	0.013	0.014	0.012	0.007	0.007	0.007	0.007	0.012	0.012	0.012
JN208607 *E. erythrurus* (Malaysia)	0.002	0.005	0.000	0.002	0.014	0.017	0.017	0.005		0.000	0.013	0.014	0.013	0.008	0.008	0.008	0.008	0.013	0.013	0.012
JN208610 *E. erythrurus* (Malaysia)	0.002	0.005	0.000	0.002	0.014	0.017	0.017	0.005	0.000		0.013	0.014	0.013	0.008	0.008	0.008	0.008	0.013	0.013	0.012
JQ268576 *E. erythrurus* (Thailand)	0.068	0.066	0.071	0.068	0.066	0.068	0.068	0.066	0.071	0.071		0.007	0.005	0.011	0.011	0.011	0.011	0.005	0.004	0.011
JQ349961 *E. coeruleopunctatus*	0.074	0.071	0.076	0.074	0.071	0.074	0.074	0.071	0.076	0.076	0.019		0.006	0.012	0.012	0.012	0.012	0.007	0.005	0.011
JQ349962 *E. coeruleopunctatus*	0.063	0.061	0.066	0.063	0.058	0.061	0.061	0.061	0.066	0.066	0.010	0.014		0.011	0.011	0.011	0.011	0.005	0.002	0.010
JX674992 *E. coeruleopunctatus*	0.024	0.022	0.027	0.024	0.012	0.014	0.014	0.022	0.027	0.027	0.052	0.057	0.045		0.000	0.000	0.000	0.011	0.011	0.012
JX674993 *E. coeruleopunctatus*	0.024	0.022	0.027	0.024	0.012	0.014	0.014	0.022	0.027	0.027	0.052	0.057	0.045	0.000		0.000	0.000	0.011	0.011	0.012
JX674990 *E. coeruleopunctatus*	0.024	0.022	0.027	0.024	0.012	0.014	0.014	0.022	0.027	0.027	0.052	0.057	0.045	0.000	0.000		0.000	0.011	0.011	0.012
JX674991 *E. coeruleopunctatus*	0.024	0.022	0.027	0.024	0.012	0.014	0.014	0.022	0.027	0.027	0.052	0.057	0.045	0.000	0.000	0.000		0.011	0.011	0.012
KF929848 *E. coeruleopunctatus*	0.063	0.061	0.066	0.063	0.061	0.063	0.063	0.061	0.066	0.066	0.012	0.019	0.010	0.053	0.053	0.053	0.053		0.004	0.011
JF493438 *E. coeruleopunctatus*	0.061	0.058	0.063	0.061	0.058	0.061	0.061	0.058	0.063	0.063	0.007	0.012	0.002	0.045	0.045	0.045	0.045	0.007		0.010
JX093908 *E. corallicola*	0.060	0.060	0.063	0.060	0.055	0.058	0.058	0.060	0.063	0.063	0.055	0.060	0.047	0.058	0.058	0.058	0.058	0.055	0.047	

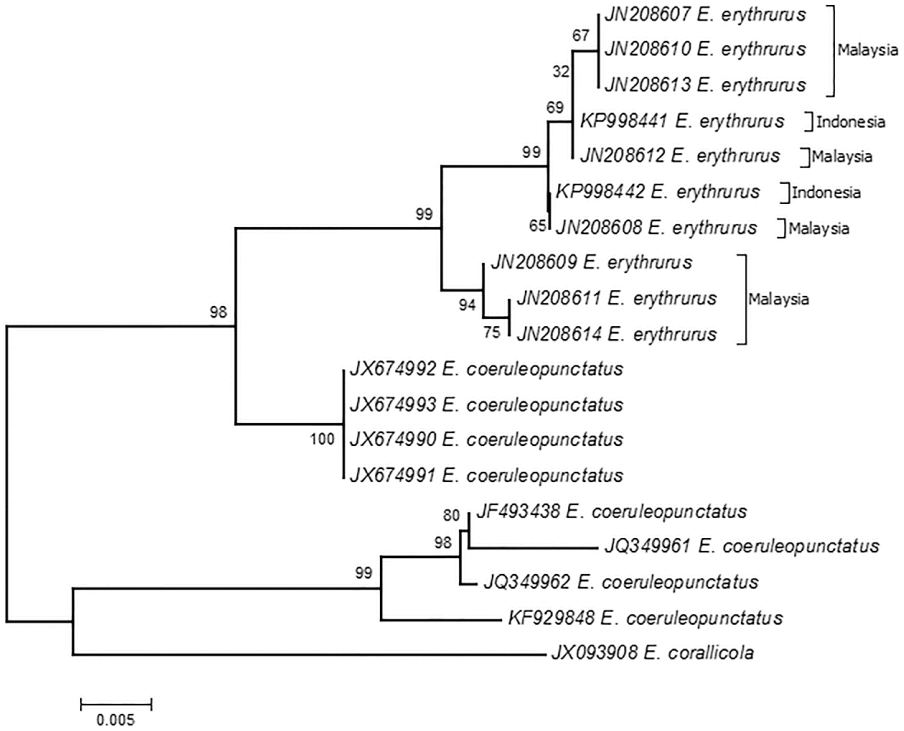

Figure 2. Neighbor-joining tree based on the CO1 nucleotide sequences of the *E. erythrurus* specimens analyzed in the present work and of species from GenBank (without Thai *E. erythrurus*). The numbers at the nodes indicate bootstrap values for 1000 replicates

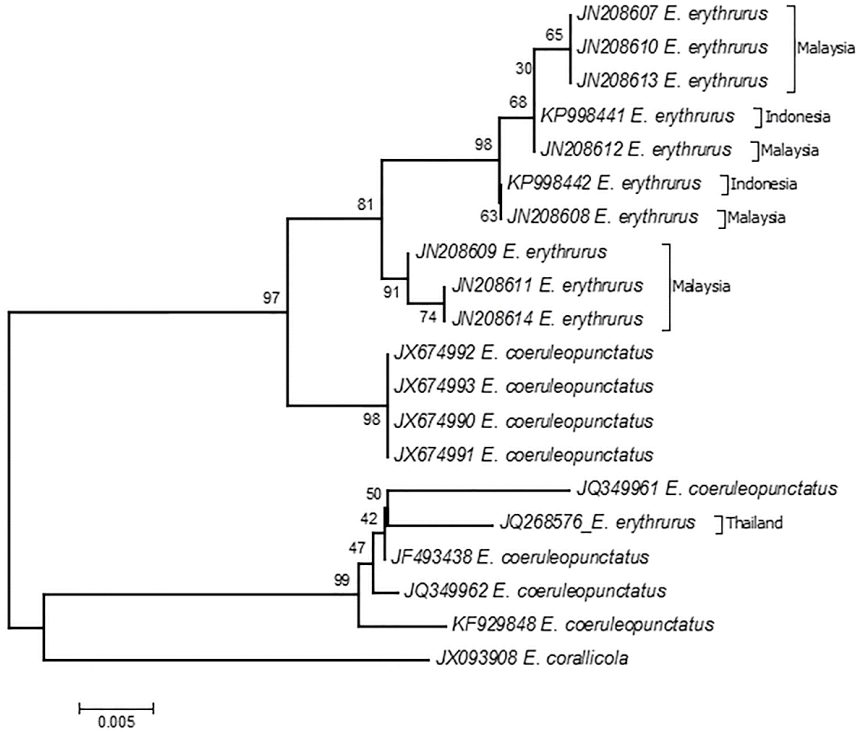

Figure 3. Neighbor-joining tree based on the CO1 nucleotide sequences of the *E. erythrurus* specimens analyzed in the present work and of species from GenBank (with Thai *E. erythrurus*). The numbers at the nodes indicate bootstrap values for 1000 replicates

The taxonomic classification of the cloudy grouper, *E. erythrurus* (Valenciennes 1828) is class Pisces, subclass Osteichthyes (bony fishes), order Perciformes (perch-like fishes), family Serranidae (sea basses and groupers) and subfamily Epinephelinae (grouper). This species is a minor commercial importance fish (Heemstra and Randall 1993), most often caught with other grouper species. These species inhabit harbors and estuaries with muddy or salty-sand bottoms, but nothing has been published on its biology. Geographical distribution of this species is known from Pakistan, India, Laccadive Island, Sri Lanka, the Gulf of Thailand, Indonesia, Singapore, Borneo and the Malaysian Peninsula (Heemstra and Randall 1993; Carpenter and Niem 1999; Allen et al. 2003). Based on the FishBase database, *E.erythrurus* was recorded in Indonesia from Sulawesi to Java. In museums, we identified RMNH 13525 (Java, Batavia), RMNH 13524 (Surabaya market), SU 61470 (Sangi Island), FMNH 22515-17 (Borneo, Kalimantan, Balikpapan Harbour), AMS I.19355-039 (Sabah, Sandakan Island), USNM 183241 (North Borneo) and FMNH 51717. In 2003, Allen and Adrim stated that the distribution of the species in Indonesia stretched from Sulawesi to Sumatra, and specimens were stored in the Western Australia Museum. In Thailand, these species were recorded in a preliminary checklist of coral reef fishes of the Gulf of Thailand (Satapoomin 2000) and also for the first karyological analysis and chromosomal characteristics of NORs research (Pinthong et al. 2015). Hegde et al. (2013) reported that these species included in the list of the new record along with their habitats from Goa, West coast of India. Sluka (2013) also stated that *E. erythrurus* was recorded in the three locations in near-shore rocky or corral habitats of western India. The report on this species can be used to supplement the Data Deficient (DD) status in the International Union for Conservation of Nature and Natural Resources (IUCN) Red List.

The samples in the present study originated from Bojongsalawe Beach (7º43'8.31"S 108º30'11.59"E) in the Pangandaran District of West Java, Indonesia. The shoreline of Bojongsalawe Beach directly faces the Indian Ocean. Due to the lack of report, based on IUCN Red List (2015), the species was categorized as "Data Deficient" (DD) status. To the light sight, based on the 2018 assessment data, the current status of this species categorized as Least Concern (LC) for the IUCN Red List (Russell 2018). A least concern (LC) species is a species which has been categorized by the IUCN as evaluated but not qualified for any other category. They do not qualify as threatened, near threatened, or (before 2001) conservation dependent. The significant result from this research is that the barcode sequence for Indonesian *E. erythrurus*, which was previously absent from GenBank, is presented here for the first time. The DNA analysis based on partial mitochondrial CO1 gene sequencing successfully identified and confirmed *E. erythrurus* juveniles; these DNA sequences have been submitted individually to GenBank. Partial sequencing of the mitochondrial CO1 gene may be used in rapid analyses for commercial species purposes, especially species identification at various developmental stages.

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