ORIGINAL ARTICLE

First records of two gobies, *Eugnathogobius kabilia* and *Pseudogobius fulvicaudus*, in the Indian Ocean from Coringa Wildlife Sanctuary, India

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Abstract

Coringa Wildlife Sanctuary is one of the unique ecosystems in the State of Andhra Pradesh which is endowed with a complex of riverine, estuarine, mangrove, coastal and marine ecosystems with an area cover of 235.70 sq. km. During the faunal surveys in the mangrove forests of the sanctuary, two species of gobiid fishes viz., *Eugnathogobius kabilia* and *Pseudogobius fulvicaudus* were identified as new distributional record from the Indian Ocean. *Pseudogobius fulvicaudus* which had morphological similarity with *P. javanicus* was confirmed using molecular analysis.

Keywords: Andhra Pradesh, Eugnathogobius, Gobiiformes, Mangrove, Pseudogobius.

INTRODUCTION

Order Gobiiformes Linnaeus, 1758, is represented by a large taxonomic diversity, having nine families and a total of 2272 valid species. Fishes belonging to the family Gobiidae, under Gobiiformes are the true gobies, which is the largest diverse fish family contributing 258 valid genera and 1930 species of fishes from freshwater, brackish, and marine environments (Fricke et al. 2023). Whereas presently, Indian Gobiiformes diversity is only 134 species belonging to five families (including Eleotridae, Gobiidae. Kraemeriidae, Microdesmidae, Schindleriidae) (Chandra et al. 2020). Gobies found in diverse habitats ranging from mudflats to coral reefs (Herler et al. 2011). Mangrove estuaries being the most productive ecosystem also provides a good nursery ground for a diverse array of marine and estuarine fishes and in the Indo-Pacific region, gobies are one of the important contributors to fishes occurring in the mangrove or estuarine region because of their abundant distribution and diversity (Yokoo et al. 2012).

Species under the genus *Eugnathogobius* Smith, 1931, and *Pseudogobius* Popta, 1922 are recorded from mangroves, estuaries, as well as from intertidal areas, globally (Larson 2009; 2021) and come under the family Gobiidae. So far, nine valid species are

reported worldwide under Eugnathogobius (E. kabilia, E. illotus, E. indicus, E. siamensis, E. microps, E. mindora, E. stictos, E. variegatus, E. umbra). Fifteen species are recognised worldwide under the genus Pseudogobius (P. melanosticta, P. aquilonius, P. avicennia, P. cinctus, P. eos, P. fulvicaudus, P. olorum, P. poicilosoma, P. hoesei, P. jeffi, P. masago, P. minimus, P. rhizophora, P. taijiangensis, P. verticalis) (Fricke et al. 2023). Eugnathogobius has never been recorded and Pseudogobius is represented by two species i.e, P. gastrospilus (Bleeker, 1853) and P. javanicus (Bleeker, 1856) from India (Chandra et al. 2020; 2018). But both of these species are synonymous with P. poicilosoma (Bleeker 1849) (Fricke et al. 2023).

MATERIALS AND METHODS

The current study was carried out during the exploration of "Fauna of Coringa Wildlife Sanctuary, Andhra Pradesh" in December 2020. Coringa Wildlife Sanctuary is one of the 21 wildlife sanctuaries in Andhra Pradesh which is endowed with a complex of riverine, estuarine, mangrove, coastal, and marine ecosystems with an area cover of 235.70 sq. km. (Fig. 1).

Taxon sampling: The collections were made by manual search method and handpicking. The fishes

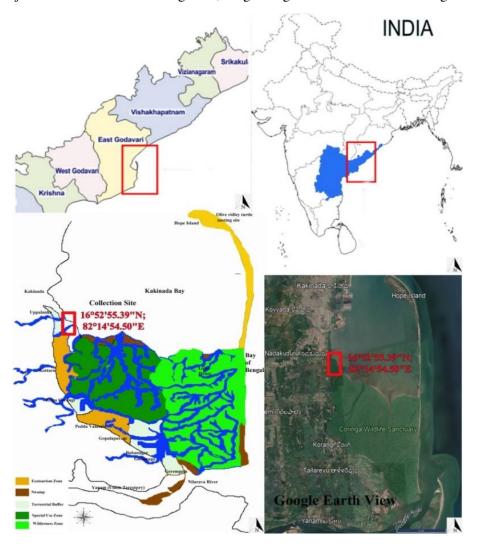


Fig.1. Map showing the collection site from Coringa Wildlife Sanctuary, Andhra Pradesh, India.

were asphyxiated with freshwater. The completely relaxed and freshly dead animals were fixed in 10% formalin (in seawater) solution and later preserved in 70% ethanol for long-term preservation. The animals/body parts for the DNA analysis were directly preserved in 100% ethanol. The preserved animals are deposited in the National Zoological Collections of the Zoological Survey of India. The specimens were identified based on meristic and morphometric characters provided by Larson (2009) and Larson & Hammer (2021). The maximum length of the specimens that has been mentioned in the study, is the length before preservation of the specimens.

Molecular analysis: DNeasy Blood and Tissue Kit (QIAGEN) is used to extract total genomic DNA. Amplification of the target 620-bp fragment of COI was done by polymerase chain reaction (PCR) using

universal primer pair FishF1-5'TCAACCAACCACAAAGACATTGGCAC3'and FishR15'TAGACTTCTGGGTGGCCAA

AGAATCA3' (Ward et al. 2005). Thermal cycle parameters for 25µl initial step of 3min at 95°C followed by 35 cycles of 60s at 94°C, 60s at 52°C and 60s at 72°C followed in turn by final extension of 10min at 72°C. PCR products were assessed using electrophoresis on an agar gel and, if a single band was observed, were purified using a QI Aquick PCR purification kit (QIAGEN Inc). Direct sequencing of PCR products was done in both directions by an automated sequencer (ABI Prism 3730 XL DNA Analyzer). The chromatograms were assessed for miscalls and ambiguities and assembled into contigs in Chromas 2.6.4. These sequences were individually checked and verified for protein-coding frame shifts

to avoid pseudogenes. Consensus files were aligned. Sequences were checked in the basic local alignment search tool (BLAST) to find the closest sequences available in the GenBank (www.ncbi.nlm.nih.gov). The sequence that is generated as a part of the study was deposited in GenBank (Accession Number: MW460017, MW460018).

RESULTS

The details of the newly reported species are given below:

Class: Actinopterygii Order: Gobiiformes Family: Gobiidae

Subfamily: Gobionellinae

Genus: Eugnathogobius Smith, 1931

Eugnathogobius kabilia (Herre, 1940)

1940. *Vaimosa kabilia* Herre [A.] New species of fishes from the Malay Peninsula and Borneo. Bulletin of the Raffles Museum No. 16: 5-26, Pls. 1-20 (Kabili River, Sabah, Borneo, East Malaysia).

1993. *Mugilogobius kabilia* (Herre 1940). Kottelat, M., Whitten, A.J., Kartikasari, S.N. & Wirjoatmodjo, S. Freshwater fishes of Western Indonesia and Sulawesi. Periplus Editions, Hong Kong. i-xxxviii + 1-259, Pls. 1-84.

2001. *Calamiana kabilia* (Herre 1940). Larson, H. K, A revision of the gobiid fish genus *Mugilogobius* (Teleostei: Gobioidei), and its systematic placement. Records of the Western Australian Museum Suppl. No. 62: i-iv + 1-233.

2009. *Eugnathogobius kabilia* (Herre, 1940). Larson, H. K, Review of the gobiid fish genera *Eugnathogobius* and *Pseudogobiopsis* (Gobioidei: Gobiidae: Gobionellinae), with descriptions of three new species. Raffles Bulletin of Zoology v. 57 (no. 1): 127-181.

Common Name: No common or colloquial name Conservation Status: Least Concern (IUCN Red List 3.1), date assessed 09 August, 2010 Economic Importance: Commercially not important

(India), Aquarium (Germany)

Material examined: ZSI/SbRC/KN3814, 1 ex., TL 26.2mm, SL 20.5mm, Coringa Wildlife Sanctuary, Andhra Pradesh (16°52'55.39"N; 82°14'54.50"E), 13.12.2020, Coll: Sreeraj C.R.

Diagnostic Characters: First dorsal spine 6; Second dorsal spine 1; Second dorsal soft rays 7; Anal spine 1; Anal soft rays 7; Pectoral Soft rays 11; Ventral Spine 1; Ventral soft rays 5; Caudal soft rays 16 (Segmented); Longitudinal scales 28; Transverse scales 11; Pre-dorsal scales 14. Slender body with cylindrical anterior end and a little compressed at the posterior part. Anteriorly depressed head and wider than deep. Head without pores. Caudal peduncle depth is three fourth of body depth. Pre-opercula with proper muscular lining and with a flat profile. Terminally placed oblique mouth is large in proportion with head. Jaws extend well below eye. Lips are fleshy, without fimbriae and almost free. Eyes placed dorso-laterally on the head with convex upper margin. Snout is wide and with flat margin. Small pointed teeth in both jaws. Gill opening restricted just behind the pectoral base and operculum with a straight margin. Interorbital area is large and flat. Caudal fin square (truncate).

Base body colouration of live specimen is transparent. No distinct patterns on body. Each scale on the side of the body having a dusky posterior margin. Two distinct stripes appear at the posterior margin of the eye and extends backward. Another two stripe present from eye to snout. Edge of operculum and cheek with uniformly arranged brownish dots. Lower lip edge with a pale brownish outline and upper lip colour is light pinkish. Eye margins are brownish to pale green in colour. Dorsal fin transparent and with brown spots. Second dorsal with reddish margin. Ventral body is pale coloured. Anal fin is whitish. Caudal fin greyish with reddish rays and base with brownish blotches (Fig. 2). Detailed morphometric measurements of this specimen is given in Table 1.

Habitat: Temporary mangrove mud pools, created inland at the time of low tide having interconnected channels underground. Mud pools are associated to base of tree trunks and pneumatophores in shady areas of the mangrove. The specimen was collected using

small aquarium net and it was well camouflaged in muddy base with a very little movement in water; Brackish Water (Salinity: 9-15 ppt, Water temperature: 27.7°C-39.1°C, Mud Temperature – 22.9°C-26.2° C, pH: 8.1-8.3) (Fig. 3a)

Distribution: Sri Lanka, Thailand, Malaysian Borneo, Philippines, Indonesia (Huang et al. 2013).

Comments: Base colour of the specimen is transparent and size is very small. Only two to three animals can be found in the small mud pool and hence, finding them is quite tough. They prefer a composite and cryptic environment as all the mud pools does not harbour the same species. No juvenile specimens are found in the same mud pool. When pool water is stirred, they tend to hide in small interconnecting underground channel or they restrict their movement in muddy water not to be easily noticed. Can survive outside water for a couple of minutes. These specific mud pools are closely associated with mangrove trunks and pneumatophores. These pools are only created in the time of low tide. Species identification characters as described by Larson was "Headpores always absent; jaws greatly enlarged in males; head with at least two dark stripes, each body scale with fine brown bar or spot E. kabilia (Herre, 1940)" (Huang et al. 2013).

The species was described as *Vaimosa kabilia* by (Herre, 1940) from East Malaysia. Kottelat et al. (1993) placed the species in genus *Mugilogobius*. Again in 2001, the species was placed in the genus *Calamiana* by Larson H.K., but after some studies she replaced the species under *Eugnathogobius* in 2009. This species was poorly described from the wild and only aquarium specimen were described previously and there was no reference of fresh specimen description previously.

Hora (1923) described a new species from Chilika Lake region, *Glossogobius mas*. As the syntypes are destroyed comparing with *E. kabilia* is not possible but the descriptions are quite similar with *E. kabilia*. This specimen of *E. kabilia* is collected from almost the same region and as suspected by Larson (2009) there is a lot of chance that *Glossogobius mas*

described by Hora could be a *E. kabilia*. Very recently the works by Larson et al. (2022) proved that *Glossobius mas* should be considered as *Eugnathogobius mas* and *E. kabilia* can be differentiated from *E. mas* by having 17 segmented caudal fin rays and by the absence of dark bars on the cheek and dark blotch behind pectoral fin.

Genus: Pseudogobius Popta, 1922

Pseudogobius fulvicaudus Huang, Shao & Chen, 2014 2013. *Pseudogobius fulvicaudus* Huang [S.-P.], Shao [K.-T.] & Chen [I-S.] An annotated checklist of gobioid fishes from the mangrove estuary of Matang, Malay Peninsula, with comments on a new *Pseudogobius* (Teleostei: Gobiidae) species. Journal of Marine Science and Technology v. 21, Suppl. [2013]: 106-116 (Matang mangrove, Malaysia).

Common Name: Yellowfin snub-nose goby

Conservation Status: Not evaluated

Economic Importance: Commercially not important Material examined: ZSI/SbRC/KN3137, 1 ex., TL 23mm, SL 17.6mm, Coringa Wildlife Sanctuary, Andhra pradesh (16°52'55.39"N; 82°14'54.50"E), 11.12.2020; ZSI/SbRC/KN3121, 3 ex., TL 23.9-22.2mm, SL 18.1-17.6mm, Coringa Wildlife Sanctuary, Andhra Pradesh (16°51'55.39"N; 82°14'54.50"E), 09.12.2020, Coll: Sreeraj C.R.

GenBank Accession Number: MW460017; 614 bp; cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial; voucher ZSI-SbRC-KN3121; Sreeraj, C.R.; Raghunathan, C.; Deepa, J. & Karuthapandi.

Diagnostic Characters: First dorsal spine 6; Second dorsal spine 1; Second dorsal soft rays 8; Anal spine 1; Anal soft rays 7; Pectoral Soft rays 15; Ventral Spine 1; Ventral soft rays 5; Caudal soft rays 9 (Segmented)/ 8 (Branched); Lateral scales 23 - 25; Transverse scales 7; Pre-dorsal scales 7. Opercula with 3 rows of cycloid scales. Naked cheek. Teeth in both jaws pointed; Upper jaw teeth in two rows. Slender body with compressed profile. Head large and wider than deep. Interorbital area is moderately wide. Caudal peduncle depth is five fourth of body depth. Small sub terminal mouth placed slightly oblique and



Fig.2. Live specimen; a - Eugnathogobius kabilia (Herre, 1940); b - Pseudogobius fulvicaudus Huang, Shao & Chen, 2014.

jaws reach up to middle of eye. Lips are thin, fused with chin anteriorly. Eyes placed dorso-laterally almost at the top of the head with convex upper margin. Rounded snout with a flat outer margin. First dorsal low and triangular in shape with largest 2nd ray. Pectoral fins oval shaped. Caudal fin with a rounded edge and upper half is larger than lower. Gill opening up to middle of opercula is straight and pointed. Scales on body are ctenoid and predorsal scales are cycloid.

Base body colouration is translucent to transparent and ventrally white. Six light dark blotches appear at the dorsal part. Upper margin of the eye is greenish brown. Presence of black pigments all over the head. Margin of the lower lip is with two rows of black pigments and upper lip with only one row. Base colour of the head is somewhat brownish. Yellowish green patch over the mid of opercula. Posterior part of the scales are marked with light grey. Black band over the membrane between 5th and 6th spine of first dorsal is diffused to black pigments, but the pattern shape is prominent and the pigmented bar extends over the

body up to mid of pectoral base. Pectoral base with black pigments. Fin ray colours are reddish brown. All the fins are transparent coloured. Ventral part of the body after anus is with 2 light black blotches. Caudal peduncle end is quite distinguished with the presence of yellow in a half moon shape. Five pigmented blotches over the side of the body starting from 2nd dorsal base to caudal peduncle. Lowed part of the caudal peduncle ending is demarcated with the presence dark pigments in a round shape (Fig 2). Colouration of the preserved materials is almost similar but without the yellow colouration. Detailed morphometric measurements of this specimen is given in Table 1.

Habitat: Temporary mangrove mud pools or at the side of small channels inside the mangrove creeks which are visible during full low tide, created inland at the time of low tide having interconnected channels underground. Mud pools are associated to nearby small channels or small holes connecting the pool to the channel. The specimen was collected using small

Table 1. Morphometric measurements of the collected specimens ($n = 1$ each).

Characters	E. kabilia	P. fulvicaudus Sample 1	P. fulvicaudus Sample 2	P. fulvicaudus Sample 3	P. fulvicaudus Sample 4
TL - Total Length (mm)	26.2	23	23.9	23	22.2
SL - Standard Length (mm)	20.5	17.6	18.1	18	17.6
HL - Head Length (mm)	6.1	4.8	4.9	4.5	4.1
Eye Diameter (% of HL)	22.95	24.25	24.69	24.88	24.39
Inter Orbital Length (% of HL)	26.22	16.16	18.36	17.77	16.63
Snout Length (% of HL)	19.67	22.91	25.53	25.66	23.63
Upper Jaw Length (% of HL)	44.26	32.33	32.65	29.88	29.82
Pectoral Fin Length (% of SL)	20.97	24.43	26.51	24.44	24.15
Pelvic Fin length (% of SL)	14.63	20.45	21.75	21.11	19.88
Anal Fin Length (% of SL)	1.95	4.54	4.62	4.44	4.47
Pre-dorsal Length (% of SL)	35.6	39.27	40.09	40.66	39.65
Pre-anal Length (% of SL)	46.34	50.22	52.59	51.66	50
Pre-pectoral Length (% of SL)	25.36	25.13	25.51	25.55	25.56
Pre-pelvic Length (% of SL)	21.95	28.97	29.96	28.44	28.86
Body Depth (% of SL)	19.51	22.15	22.03	21.44	21.47
Caudal Peduncle Length (% of SL)	7.31	15.06	16.57	16.55	15.2
Caudal Peduncle Depth (% of SL)	14.63	12.77	13.25	12.49	12.22
Caudal Fin Length (% of SL)	29.75	25.56	25.7	24.93	24.86



Fig.3. Collection habitat of a - *Eugnathogobius kabilia* (Herre, 1940); b - *Pseudogobius fulvicaudus* Huang, Shao & Chen, 2014.

aquarium net and it was well camouflaged in muddy base because of its almost transparent body; Brackish Water (Salinity: 9-15 ppt, Water temperature: 27.7°C-39.1°C, Mud temperature – 22.9°C-26.2°C, pH: 8.1-8.3) (Fig. 3b).

Distribution: Vietnam, Thailand, Malaysia, Brunei, Singapore and northern Australia (Larson et al. 2020). **Comments:** Base colour of the specimen is transparent and size is very small. Only two to three animals can be found in the small mud pool and hence, finding them is quite tough. They prefer a composite and cryptic environment as all the mud pools does not harbour the same species. Three different specimens of the same species has been collected from three different pools nearby. No juvenile specimens are found in the same mud pool. When pool water is stirred, they tend to hide in small interconnecting underground channel or they restrict their movement in muddy water not to be easily noticed. Sometimes they used to escape to the main channel through underground channels. Can survive outside the water for almost one minute. Species identification

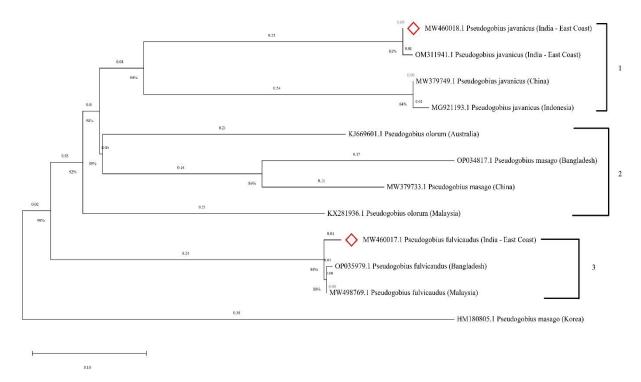


Fig.4. Phylogenetic relationships of *Pseudogobius* sp. examined in the present study with other *Psedogobius* sp. (available in NCBI); derived by NJ analysis of the COI sequences.

The species was firstly recorded in 2013 from Matang, Malay Peninsula and described by Huang et al. (2013). The specimen was identified through only morphological characters but it had many overlapping characters with *Pseudogobius javanicus* (Bleeker, 1856), so identification is further confirmed with COI genetic sequence and has been studied and updated to Gen Bank. Larson 2021 confirmed that *Pseudogobius javanicus* (Bleeker, 1856) is a synonym of *Pseudogobius poicilosoma* (Bleeker 1849) and *P. fulvicaudus* has almost exact same coloration as well as morphological pattern with this species.

Molecular analysis was exclusively conducted for *Pseudogobius* species gathered from the Coringa

wildlife sanctuary because relying solely on morphological characteristics proved inadequate for distinguishing between *P. javanicus* and *P. fulvicaudus* due to their remarkable similarity. Conversely, the identification of *E. kabilia* was accomplished using traditional taxonomic methods, as the morphological and morphometric traits provided sufficient distinction, making molecular sequencing unnecessary for this species.

COI phylogenetic analysis was carried out using 10 COI sequences of *Psedogobius* sp. available from the NCBI database (Accession Numbers: MW379749, MG921193, KJ669601, OP034817, MW379733, KX281936, MW460017, OP035979, MW498769, HM180805) with two current study sequence (Accession Numbers: MW460017, MW460018). The analysis is based on 710-character alignments. Within the group 182 sites are persimony informative, 237 are variable and 437 are conserved sites. The NJ analyses of the sequence data yielded a phylogenetic tree with three major clades in the group with an outgroup (Fig. 4).

DISCUSSION

Molecular sequencing has emerged as a valuable tool for identifying different species, especially in the case of gobioid species, which often have hidden or overlapping physical characteristics. In our current investigation, we utilized COI gene sequencing to confirm the identity of two species, namely P. javanicus and P. fulvicaudus, collected from Coringa, India. Our phylogenetic analysis shed light on the relationships among Pseudogobius species, revealing the presence of three distinct clades and one outgroup in the phylogenetic tree. In Clade 1, we found *P. javanicus* exclusively from the eastern Indian Ocean and western Pacific Ocean. The high degree of phylogenetic similarity between *P. javanicus* in Clade 1 and *P. fulvicaudu*s in Clade 3 (92%) provides strong evidence of their morphological resemblance. This similarity may be attributed to shared habitats and environmental conditions, particularly in mangrove ecosystems (Woodroffe 1988; Sarker et al. 2019). Clade 2 comprised two species, P. masago (Tomiyama, 1936) and P. olorum (Sauvage, 1880), collected from the South Eastern Indian Ocean and the West Pacific Ocean. The similarities observed between the South Asian and Australian Pseudogobius fauna align with previous research by Larson and Hammer (2021), highlighting the connection between these regions. Clade 3 exclusively included P. fulvicaudus, reaffirming the significant role of habitats in speciation. Once again, the similarities in the mangrove habitat of the eastern Indian Ocean appear to be a key driver of this genetic grouping (Tovar Verba et al. 2023; Martinez et al. 2018). While the reason for the presence of the outgroup, P. masago from Korea, remains unclear, our discussion underscores the strong similarity among **Pseudogobius** species. South Asian investigations into the habitats of these cryptic species are needed to solidify this hypothesis (Dad et al. 2023; Islam et al. 2022; Anderson et al. 2017).

India being one of the biodiversity reach countries, harbours a various kinds of habitats and mangrove is one of them (Beatley 1991). Considering mangrove as

one of the most productive ecosystems, studies focused on mangrove-associated species diversity is quite less. India covering 4921 sq. km. area of mangrove belt harbours the largest patch of mangrove, i.e. Sunderban (2114 sq. km.) and the second most large mangrove patch in India is Coringa Wild Life Sanctuary (235.7 sq. km.) situated at Godavary estuarine region (404 sq. km.) (Chandra et al. 2019). A total of 135 species of gobies had been reported from coasts of India, whereas 95 species belonging to two families have been reported from Indian mangroves and 53 gobies are reported from present study site, i.e. Godavary estuarine region - Coringa Wildlife Sanctuary (Padmavathi 2017; Chandra et al. 2019, 2020;). However, worldwide data, which is 2272 valid species of gobiies, Indian diversity is very less (Fricke et al. 2023). Fish diversity of India is well studied still diversity of order Gobiiformes is very little known to us for Indian region.

From the available data, it is evident that the estuarine gobies are not well documented from the Northern Indian Ocean Region, because of their cryptic habitat choice, camouflaging appearances and small size.

Most part of the Indian mangroves are not properly accessible to researchers and this is a major reason for the untapped diversity of mangrove gobies. As well as mangroves are near to the estuarine regions and these locations are commonly used as waste disposal sites, which are affecting the species diversity a lot. Other major mangrove forests of India viz., Andaman Nicobar Islands, Bhitarkanika, Coringa, Pichavaram, Gulf of Kachchh, Sunderban etc. also need more attention for finding out the mangrove associated gobiid diversity for their proper biological study and to develop conservation tactics.

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مقاله كامل

اولین گزارش دو گونه گاو ماهی، Eugnathogobius kabilia و گونه گاو ماهی، fulvicaudus و شده در پناهگاه حیات وحش کورینگا، هند

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چکیده: پناهگاه حیات وحش کورینگا یکی از اکوسیستمهای منحصر بهفرد در ایالت آندرا پرادش است که دارای مجموعهای از اکوسیستمهای رودخانهای، مصبی، حرا، ساحلی و دریایی با مساحت ۲۳۵٬۷۰ کیلومتر مربع است. طی بررسیهای فونستیکی در پناهگاه جنگلهای حرا، دو گونه گاو ماهی یعنی ۲۳۵٬۷۰ کیلومتر مربع است. طی بررسیهای فونستیکی در پناهگاه جنگلهای حرا، دو گونه گاو ماهی یعنی P. javanicus با و اقیانوس هند شناسایی شدند. Pseudogobius fulvicaudus که شباهت ریختی با Preudogobius fulvicaudus که شباهت ریختی با داشت با استفاده از تجزیه و تحلیل مولکولی تأیید شد.

كلمات كليدى: آندرا پرادش، Eugnathogobius، گاوماهى شكلان، حرا، Pseudogobius