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Article

New Record of the Grey Cutthroat, *Synaphobranchus affinis* (Anguilliformes: Synaphobranchidae) from the East Mariana Basin, Western Pacific Ocean

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Abstract: Two deep-sea eels collected from the East Mariana Basin in the western Pacific Ocean are described in this study. Based on their morphological features, two eel specimens were assumed to belong to the Gray cutthroat eel family, Synaphobranchidae. Mitochondrial DNA (mtDNA) genes have been widely used as genetic markers to identify fish species. To accurately identify the species of the two eel specimens, we sequenced the mitochondrial cytochrome c oxidase subunit I (COI) and 16S ribosomal RNA (16S rRNA) genes from the two eel specimens. The sequences from the specimens were 100% identical. The molecular phylogenetic tree confirmed that the two eel specimens were closely related to *Synaphobranchus affinis* with a bootstrap value of 100%. This is the first study to report new records of *S. affinis* from the East Mariana Basin in the western Pacific Ocean.

Keywords: deep-sea eel; morphological characteristics; cytochrome c oxidase subunit 1; 16S ribosomal RNA; phylogenetic analysis

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1. Introduction

The deep sea (>200 m depth) is the Earth's largest ecosystem and a largely unexplored deep marine biosphere [1,2]. Deep-sea environments are extremely harsh, and deep-sea organisms survive with scarce food resources and under extreme pressure, cold, and constant darkness [3,4]. Deep-sea fishes are critical components of deep-water ecosystems, and their habitat range varies above a depth of 8200 m [5–7]. The identification and characterization of deep-sea fish species are important for understanding deep-sea biodiversity [8–10]. However, there is little information about deep-sea fish species because deep-ocean environments are difficult to access for collecting biological samples.

The eel family Synaphobranchidae is an important ecological component of the deep-sea fauna and is globally found in tropical and temperate seas [11]. In particular, Synaphobranchidae is widely distributed in the vertical ocean column from less than 100 m to several thousand meters [12]. The Synaphobranchidae family currently comprises 12 genera (about 40 species) [13,14]. Among them, the cutthroat eel genus *Synaphobranchus* belongs to the family Synaphobranchidae. Currently, *Synaphobranchus* eels include six valid species [13]: *S. kaupii* Johnson, 1862 (Kaup's arrowtooth eel) [15]; *S. affinis* Günther, 1877 (Gray cutthroat) [16]; *S. brevidorsalis* Günther, 1887 (Shortdorsal cutthroat eel) [17]; *S. dolichorhynchus* (Lea, 1913) [18]; *S. oregoni* Castle, 1961 [19]; and *S. calvus* Melo, 2007 [20].

To date, all species of this genus have been recorded in the North Atlantic Ocean, except for *S. calvus* [11,18]. In particular, *S. affinis* has been recorded in the Atlantic (western North Atlantic, eastern South Atlantic, eastern Atlantic, and eastern-central Atlantic), Pacific (western North Pacific, western South Pacific, eastern North Pacific, western South

Pacific) and Indian Oceans (southwestern Indian Ocean and eastern Indian Ocean) [1,21,22]. However, there was no evidence of the presence of *S. affinis* from the East Mariana Basin in the western Pacific Ocean so far. Nevertheless, we collected two cutthroat eels from off Mariana Basin in the western Pacific and identified them as *S. affinis*, based both on morphological and molecular characteristics.

Recently, DNA-based markers, especially mtDNA markers, have been extensively studied in fish taxonomy and phylogenetics. Mitochondrial COI and 16S rRNA genes are good genetic markers for identifying and differentiating deep-sea species, including fish [23–25]. DNA barcoding is used for the species identification of some deep-sea species, such as the squat lobsters, *Munida distiza* and *M. militaris* [26], and sea cucumber, *Benthodytes marianensis* [27]. Indeed, species identification of two deep-sea eels (*Bassozetus* sp. and *Synaphobranchus* sp.) has been described based on mitochondrial COI and 16S rRNA genes [28]. In addition, mtDNA genes were used to describe the molecular phylogenetics and evolution of 77 deep-sea fishes [29], indicating that DNA barcoding is very effective in identifying deep-sea species, including deep-sea eels. Therefore, mtDNA markers were considered a useful tool for the accurate identification of the two eel specimens from the East Mariana Basin in the western Pacific Ocean.

This study represents the first reliable record of *S. affinis* from the Mariana Basin. It provides species identification based on mitochondrial cytochrome c oxidase subunit I (COI) and 16S ribosomal RNA (16S rRNA) sequences and brief morphological information.

2. Materials and Methods

2.1. Sampling Collection

During an oceanographic cruise of the Korea Institute of Ocean Science and Technology (KIOST) to the western Pacific Ocean in May 2020, two eel specimens were collected in a baited trap (composed of meat and fish) from different areas. The sampling areas are shown in Figure 1 and Table 1.

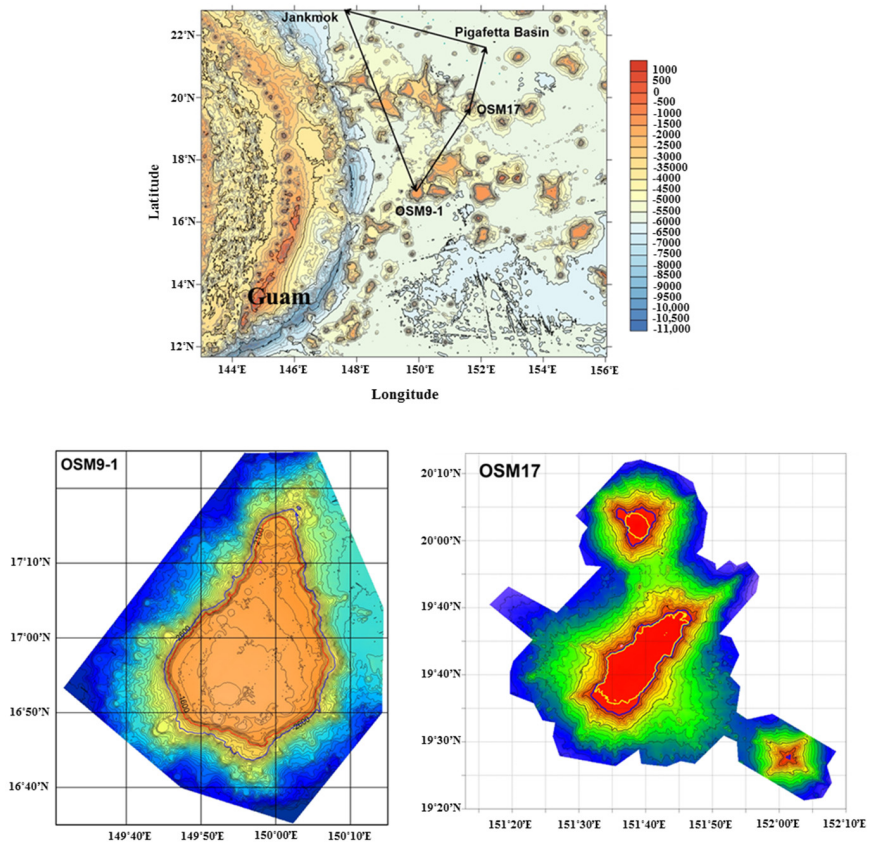

Figure 1. Map of the western Pacific Ocean and geographic distribution of sampling sites.

Table 1. List of samples and collection locations.

Years	ID Code	Geographical Area	Latitude	Longitude	Depth (m)
2020	BT02	OSM9-1	16°54′ N~17°27′ N	149°54′ E~150°12′ E	1572
2020	BT05	OSM17	19°27′ N~19°36′ N	151°27′ E~151°45′ E	1298

The pictures of the two eel specimens (sample IDs: BT02 and BT05) are shown in Figure 2. This study did not include live fish, and the samples had naturally died when they were collected. The dead fish were preserved and stored at −20 °C until morphological analysis and DNA isolation. For taxonomical examination, they were fixed in 10% formalin and transferred into 75% ethyl alcohol for long-term preservation. Counts, measurements, and terminology of the cephalic lateral canal were performed following the guideline reported in previous studies [11,30]. Vertebral count was based on radiographs (Softex, CMB-2, Japan). Morphometric data are presented in Tables 2 and 3.

Figure 2. Picture of the two deep-sea *Synaphobranchus* eels.

Table 2. Morphometric characters of the two eel specimens collected from the East Mariana Basin, western Pacific Ocean.

Morphometric Characters	BT02	BT05
Total length (mm, TL)	948.0	1023.0
In% TL		
Head length	14.1	14.5
Trunk	18.4	17.1
Preanal-fin distance	32.2	31.1
Predorsal-fin distance	51.6	52.1
Body depth	9.7	10.3
Pectoral fin length	5.1 *	5.2
Snout length	28.0	28.6
Eye diameter	10.3	10.5
Upper jaw length	70.2	68.8
Lower jaw length	69.9	71.4
Interorbtial width	20.3	21.1
Gill opening	21.1	25.5

*, Right side measured.

Table 3. Meristic characters of the two eel specimens collected from the East Mariana Basin, western Pacific Ocean. Data based on specimens listed in Table 2.

Laterosensory Canal	BT02	BT05
Adnasal	1	1
Infraorbital canal	7	7
Preopercular-mandubula canal	12	12
Ethmoid	1	1
Supraorbtial canal	5	5
Frontal	2	2
Supratemporal canal	2	2
Lateral-line pores to anal-fin origin	34	32
Lateral-line pores to dorsal-fin origin	58	57
Total lateral-line pores	134	131
Total vertebrae	139	135

2.2. DNA Extraction, PCR Amplification and DNA Sequencing

Genomic DNA was extracted from the muscle with DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. The quantity and quality of the isolated DNA were analyzed and measured at 230, 260, and 280 nm using a spectrophotometer (NanoDrop One, Thermo Fisher Scientific Inc., Madison, WI, USA). The PCR amplification was carried out in a 50 µL reaction mixture containing 32.875 µL of sterilized distilled water, 6 µL of 10X Ex Taq Buffer (TaKaRa, Japan), 5 µL of dNTP mixture (2.5 mM each), 1 µL of each primer (5 µM), 0.125 µL of EX Taq DNA polymerase (5 units/µL), and 4 µL of DNA template. Two mitochondrial DNA genes (COI and 16S rDNA), as the barcoding marker genes for animals, were amplified with forward and reverse primers (Table 4) [31]. PCR cycling was performed using a thermal cycler PCR machine (C1000 Touch Thermal Cycler, Bio-Rad). The amplification conditions were as follows: initial denaturation for 3 min at 95 °C, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing for 30 s at 65 °C, and extension at 72 °C for 45 s. The final extension was performed at 72 °C for 5 min. PCR products were confirmed by 1.0% agarose gel electrophoresis and visualized using FluoroBox (Blue LED Gel doc, NeoScience, Gyeonngi-do, Seoul, South Korea). All PCR products were sequenced by Macrogen (Seoul, South Korea).

Table 4. Primer set used in this study.

Genes	Oligo Name	Sequence
16S	16S_F (16Sar-5′)	CGCCTGTTTATCAAAAACAT
	16S_R (16Sbr-3′)	CCGGTCTGAACTCAGATCACGT
CO1-3	VF2_t1	TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC
	FishF2_t1	TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC
	FishR2_t1	CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA
	FR1d_t1	CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA

2.3. Sequence Alignment and Phylogenetic Analysis

To determine the phylogenetic relationships of the two eel species, mitochondrial COI and 16S rRNA sequences from the eel family Synaphobranchidae (genus: *Simenchelys*, *llyophis*, *Histiobranchus*, *Dysomma*, *Dysommina*, *Diastobrachus*, and *Synaphobranchus*) were obtained from GenBank database. A total of 30 and 12 sequences for COI and 16S rRNA were selected and aligned using 'MAFFT' v7.450 with the default setting [32]. The most suitable models for each sequence were estimated using a Modeltest-NG [33] in raxmlGUI 2.0 software for Linux [34]. According to the Akaike information criterion (AIC), the best-fit model STMTREV + I + G4 + F of amino-acid and GTR + I of nucleotide substitution

were selected for COI and 16S rRNA sequences, respectively. Based on the best-fit model for each sequence, a maximum likelihood (ML) phylogenetic tree was constructed using RAxML-NG in raxmlGUI 2.0 software with 1000 bootstrap replicates and inferring the best scoring tree [35]. The *Simenchelys* species in the subfamily Simenchelyinae was used as an outgroup, as previously described [36,37].

3. Results and Discussion

The specimens collected from the Mariana Basin were assigned as a member of the genus *Synaphobranchus* based on the presence of two of the following features: gill slits confluent along the ventral midline; a dorsal fin originating from far behind the tip of the pectoral fin; 12 pores in the preoperculo-mandibular canal, 6 in the supraorbital branch of the supraorbital canal, and 2 in the supratemporal cross-commissure canal; the absence of temporal canal pore series; a pectoral fin longer than one-half of the gape length; interspace between the posterior margin of the orbit and a first trunk lateral-line pore shorter than the snout length; completed in paving stone array; a uniserial vomerine tooth patch, except irregularly biserial only in the anterior portion; a premaxillary-ethmoid tooth patch that is short and oval with teeth irregularly placed; and lateral-line pores to vertical of anus 29–32 [11].

The specimens used in this study are most similar to *S. affinis* among the six valid species of the genus *Synaphobranchus*, based on the sharing of the diagnostic characters of the discriminating species, such as the elongated oval scales on the body, 135–139 vertebral numbers, and uniserial vomerine teeth (Figure 3 and Tables 2 and 3). In addition, they are easily differentiated from both *S. calvus* and *S. oligolepis* (without scales) due to the presence of a scaly head, and from both *S. brevidorsalis* (oval) and *S. oregoni* (rounded) because of the elongated oval scales on the body. The present specimens, in accordance with that of *S. kaupii*, show a dorsal fin origin located well behind from a vertical at the vent. In addition, the vertebral number of *S. kaupii* (143–153) is higher than that of *S. affinis* (128–140). However, *Synaphobranchus* eels are difficult to distinguish at the species levels because of the broad range of vertebral values and similar external morphologies [38,39]. To date, morphological characteristics have been used as basic tools for identifying species and distinguishing among them. However, distinguishing between similar species and identifying new species based on morphological characteristics is challenging because of the diversity in morphological characteristics and the ontogenetic changes in morphology during fish development [23,40,41]. Therefore, although our two eels are closely related to *S. affinis*, it is difficult to accurately identify the species solely based on morphological characteristics.

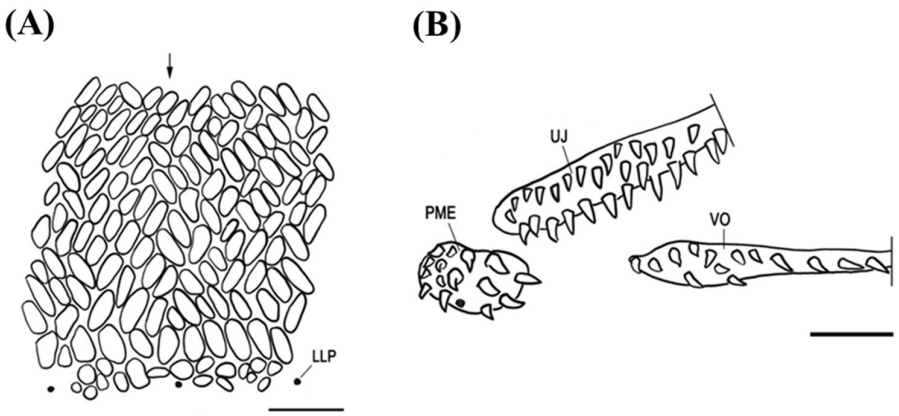

Figure 3. Scale pattern (**A**) below origin of dorsal fin (arrow) and teeth (**B**) on premaxillary-ethmoid maxillary (PME), upper jaw (UJ), and vomer (VO) of *Synaphobranchus affinis*, BT05, 1023 mm in total length. LLP, lateral line pore. Posterior parts of UJ and VO omitted. Scales bars indicate 5 mm.

In this study, the mitochondrial COI and 16S rRNA genes of the two *Synaphobranchus* eels were 655 base pairs (bp) and 611 bp in length, respectively. The partial mtDNA COI and 16S rRNA gene sequences were sequenced and deposited at GenBank (BT02 [COI; MZ221136 and16S rRNA; MZ227814] and BT05 [COI; MZ221137 and16S rRNA; MZ227815]). The mitochondrial COI and 16S rRNA sequences from the two eel specimens showed 100% identity, indicating that the specimens were from the same species. Based on sequence analysis of the amplified mitochondrial COI and 16S, molecular phylogenetic trees were constructed according using the maximum likelihood (ML) (Figure 4).

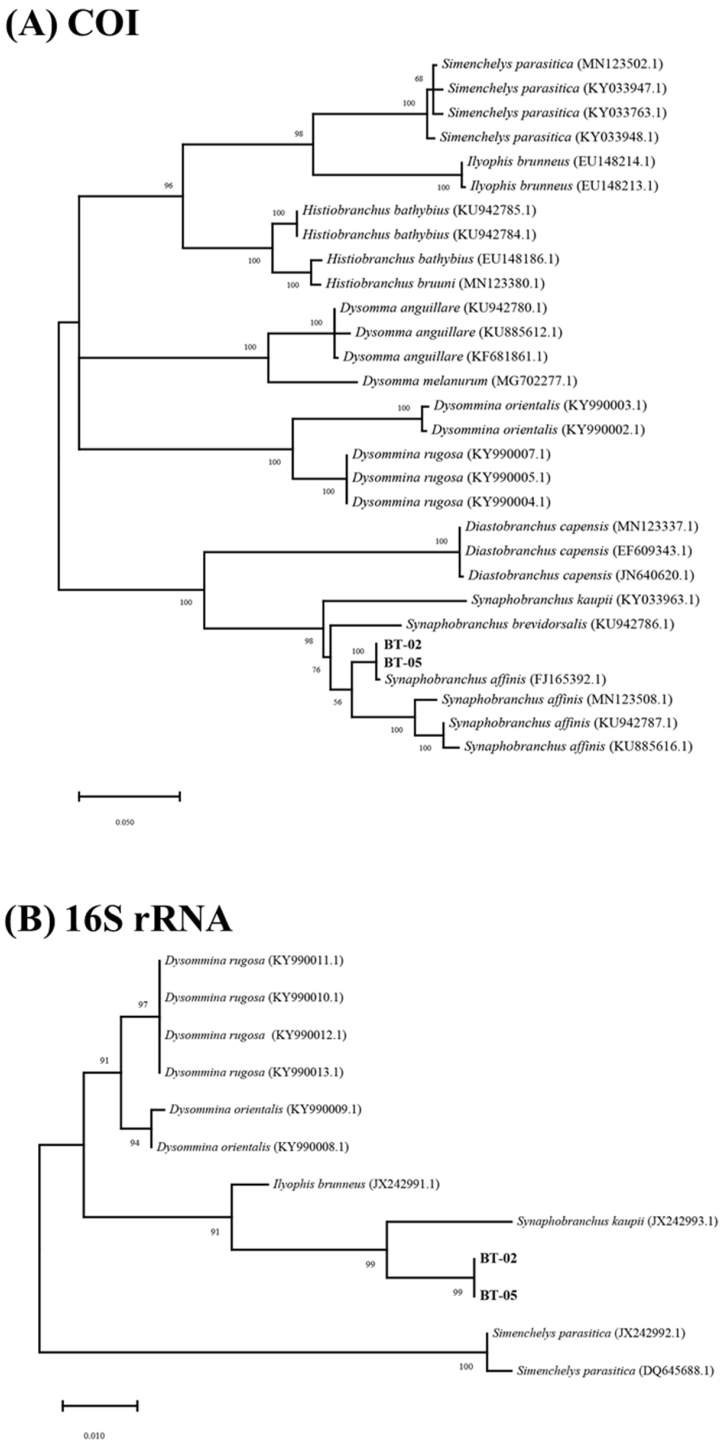

Figure 4. Phylogenic tree based on (**A**) mitochondrial CO1 and (**B**) 16S rRNA gene sequences using RAxML-NG in raxmlGUI 2.0 software.

The two eel specimens genetically belonged to the genus *Synaphobranchus*, based on the mitochondrial COI and 16S rRNA sequences. The phylogenetic tree of mitochondrial COI and 16S rRNA showed that the two eel species were deeply nested within *Synaphobranchus* eels. The phylogenetic tree of the mitochondrial COI showed that the two eel specimens were closely related to *S. affinis* with a 100% bootstrap value. The sequences from the two eel specimens converged with the one branch containing one individual *S. affinis*. Despite the non-monophyletic relationship among the four *S. affinis*, the two specimens (e.g., BT02 and BT05) clearly formed a separate clade from *S. brevidorsalis*, suggesting that the two newly sampled species are congeneric species to *S. affinis*. Taken together, supported by the phylogenetic tree of mitochondrial COI and 16S rRNA, the two eel specimens are *S. affinis*.

In summary, *S. affinis* was reported for the first time from the East Mariana Basin in the western Pacific Ocean based on molecular and morphological characteristics. The mitochondrial COI and 16S rRNA sequences were sufficient to identify the Synaphobranchus species collected from the East Mariana Basin in the western Pacific Ocean, supporting the usefulness of the mtDNA-based method in fish species identification. This study provides insights into the deep-sea fish species diversity in the western Pacific Ocean. However, replicate samples from the western Pacific Ocean are needed for accurate species identification and a better understanding of species distribution.

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Institutional Review Board Statement: All experiments were conducted in compliance with the guidelines of the Institutional Animal Care and Experimental Committee of Korea Institute of Ocean Science and Technology (KIOST)" that approved the experimental protocol.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are available via the data repository of the KIOST. Requests for material should be made to the corresponding author.

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