New records of two deep-sea eels collected from the Western Pacific Ocean based on COI and 16S rRNA genes

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Abstract

Background Two deep-sea eels collected from the Western Pacific Ocean are described in this study. Based on their morphological characteristics, the two deep-sea eel specimens were assumed to belong to the cusk-eel family Ophidiidae and the cutthroat eel family Synaphobranchidae.

Methods and results To accurately identify the species of the deep-sea eel specimens, we sequenced the mitochondrial genes (cytochrome c oxidase subunit I [COI] and 16S ribosomal RNA [16S rRNA]). Through molecular phylogenetic analysis based on mtDNA COI and 16S rRNA gene sequences, these species clustered with the genera Bassozetus and Synaphobranchus, suggesting that the deep-sea eel specimens collected are two species from the genera Bassozetus and Synaphobranchus in the Western Pacific Ocean, respectively.

Conclusions This is the first study to report new records of the genera Bassozetus and Synaphobranchus from the Western Pacific Ocean based on COI and 16S rRNA genes

Keywords Deep-sea · Eel · Mitochondrial DNA · cytochrome c oxidase subunit I · 16S ribosomal RNA · Phylogenetic analysis

Introduction

The deep-sea (> 200 m depth) is the largest habitat on earth and is an unexplored environment [1]. Deep-sea is subject to extremely harsh conditions and the organisms here adapt to survive despite food shortage, high pressure, extreme cold, and constant darkness [2]. In general, fish are very important components of biodiversity in aquatic ecosystems, and more than 30,000 fish species exist worldwide [3]. However, there is little information on deep-sea fishes as deep ocean environments can be difficult to access and obtain biological samples are problematic [4]. Considering this, identification of new fish species becomes essential for ecological monitoring and understanding the deep-sea biodiversity [5]. To date, morphometric and meristic features have been used as traditional tools for fish species identification [6]. However, it is difficult to accurately identify closely related species [7] because their morphological characteristics are generally very similar. Recently, DNA barcoding has been used as a powerful tool for the simple and accurate identification of fish species and for phylogenetic construction. Additionally, mitochondrial DNA (mtDNA) markers can be used in biodiversity research for monitoring and for studying the molecular phylogeny. In particular, mtDNA cytochrome c oxidase subunit I (CO I) and 16S ribosomal RNA (16S rRNA) genes are widely used in fish taxonomy and phylogenetics since these genes are extremely conserved and as they help identify and differentiate closely related species [8]. Therefore, genetic molecular marker-based species identification can be applied to accurately identify closely related species.
related fish species, in cases where traditional morphological
classification methods lead to ambiguities.

In this study, we sequenced the mtDNA COI and 16S
rRNA genes of the two deep-sea eel specimens collected
from the Western Pacific Ocean. The species identification
and phylogenetic relationship were analyzed using mtDNA
genes and compared with those of other deep-sea eel species.

Materials and methods

Sampling collection

The sampling areas are shown in Fig. 1 and Supplementary
material 1. The deep-sea eel specimens were collected with
an epibenthic sledge (EBS) and baited trap (composed of
meat and fish) from the Western Pacific Ocean, in Octo-
ber 2019 and May 2020, respectively. The pictures of the
two deep-sea eel specimens (sample IDs: EBS01 [length:
15.5 cm and weight: 3.53 g] and BT04 [length: 53.6 cm and
weight: 180.69 g]) collected are shown in Fig. 2. These two
deep-sea eel specimens (sample IDs: EBS01 and BT04)
were assumed to belong to the cusk-eel family Ophidiidae
and cutthroat eel family Synaphobranchidae, respectively,
based to the morphological data [9, 10]. This study did not
include live fish and the sample had died naturally when
it was collected. The dead fish were preserved and stored
at −20 °C until DNA isolation.

DNA extraction

Genomic DNA was extracted from the muscle and anal fin
using DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA,
USA) following the manufacturer’s protocol. The quantity
and quality of isolated DNA were analyzed and measured at
230, 260, and 280 nm using a spectrophotometer (NanoDrop
One, Thermo Fisher Scientific Inc., Madison, USA).

PCR amplification and DNA sequencing

PCR amplification of each sample was carried out in a 50
μL reaction mixture containing 32.875 μL of sterilized dis-
tilled 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),

Fig. 1  Map of the western
Pacific Ocean and geographic
distribution of sampling loca-
tions

Fig. 2  Pictures of the two deep-sea eel specimens collected. a Sample
EBS01. b Sample BT04
and 4 µL of DNA template. Two mitochondrial DNA genes cytochrome c oxidase subunit I (COI) and 16S ribosomal RNA (16S rRNA) were used as the barcoding markers and were amplified with F and R primers given in Supplementary material 2 [11]. 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 94 °C for 30 s, annealing for 30 s at 65 °C, and an 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, South Korea). Sequencing was performed in both forward and reverse directions for each sample using the PCR primers. DNA sequencing was performed at the DNA synthesis and Sequencing Facility, Macrogen (Seoul, Korea), using an ABI3700 automated DNA sequencer (PE Applied Biosystems, Foster City, CA, USA). The DNA sequence fragments were edited and assembled into contigs using Geneious Pro 4.7.1 software (Biomatters Ltd.).

Sequence alignment and phylogenetic analysis

To determine the phylogenetic relationships of the unknown specimens of presumably deep-sea cusk-eel and cuttroat eel, additional available sequences of mitochondrial COI and 16S rRNA genes from the cusk-eel family (genus: Acanthonus, Aphyonus, Bassozetus, Dicrolene, Lamprohamus, Neobythites, and Porogadus) and cutthroat eel family (genus: Simenchelys, Ilyophis, Histobranchus, Dysomnia, Meadia, Dysommina, Diastobranchus, and Synaphobranchus) were obtained from GeneBank database. The nucleotide sequences of individual mitochondrial COI and 16S rRNA genes from the eels were aligned using the ClustalW algorithm in MEGA software (ver. 10.0.1; Center for Evolutionary Medicine and Informatics, Tempe, AZ, USA). To establish the best-fit substitution model for phylogenetic analysis, the model with the lowest Bayesian information criterion (BIC) and Akaike information criterion (AIC) scores were estimated using a maximum-likelihood (ML) analysis. According to the results of model test, maximum-likelihood phylogenetic analyses were performed with the LG + G + I model using MEGA software (ver. 10.0.1; Center for Evolutionary Medicine and Informatics, Tempe, AZ, USA).

Results and discussion

In this study, the partial mtDNA COI and 16S rRNA in two deep-sea eel specimens (sample IDs: EBS01 and BT04) was sequenced and deposited at GenBank (Supplementary material 3). All nucleotide sequences were trimmed and aligned COI and 16S rRNA fragments. The mtDNA COI and 16S rRNA genes of deep-sea eel specimen (sample ID: EBS01) were sequenced and the lengths of the markers were 721 and 608 bp, respectively. For the deep-sea eel specimen (sample ID: BT04), the mtDNA COI and 16S rRNA genes were sequenced and the lengths of the markers were 655 and 600 bp, respectively. The sequences of mitochondrial COI and 16S rRNA of two deep-sea eels were confirmed using BLAST searches on the NCBI website. One deep-sea eel (sample ID: EBS01) showed high identity matches with genus Bassozetus and the other deep-sea eel (sample ID: BT04) showed high identity matches with genus Synaphobranchus, suggesting that the two deep-sea eels belong to the genera Bassozetus and Synaphobranchus, respectively. Indeed, the molecular phylogentic tree based on mtDNA COI gene sequence showed that the deep-sea eel specimen (sample ID: EBS01) clustered together with B. zenkevitchi, B. glutinosus, and B. compressus (Fig. 3A). In addition, the phylogenetic tree based on 16S rRNA showed that the deep-sea eel specimen (sample ID: EBS01) and Bassozetus zenkevitchi were placed together as sister groups, suggesting that EBS01 belongs to the genus Bassozetus (Fig. 3B). Regarding the sample BT04, the molecular phylogenetic tree based on the mtDNA COI gene sequence showed that this specimen clustered together with S. kaupii, S. brevidorsalis, and S. affinis (Fig. 4A). The phylogenetic tree based on 16S rRNA showed that the deep-sea eel specimen (sample ID: BT04) and Synaphobranchus kaupii were placed together as sister groups, suggesting that BT04 belongs to the genus Synaphobranchus (Fig. 4B).

Species identification of the genus Bassozetus has been described based on significant morphological characteristics, such as large head, eyes much smaller than the snout, 9–22 long gill rakers on the anterior arch, dorsal margin of the maxilla sheathed by skin of the cheek region, elongated body tapering caudally, opercula spine absent or weak, and 21–29 pectoral-fin rays not reaching the anus [12, 13]. Additionally, the genus Synaphobranchus has been described based on morphological characteristics, such as a conical head, slender and large mouth, dark brown color, oval scales, gill slits confluent along the ventral midline, and irregularly placed teeth [14]. Although taxonomical information of the genera Bassozetus and Synaphobranchus, based on morphological characteristics, are available, it is difficult to accurately identify the similar species only by morphological characteristics due to morphological diversity and ontogenetic change during development [15]. In this context, our two deep-sea eel specimens are morphologically similar to the genera Bassozetus and Synaphobranchus, respectively, which supported the phylogenetic analysis based on mitochondrial DNA markers. This suggests that mitochondrial DNA markers may be useful for accurate identification of...
Fig. 3  Phylogenic analyses of sample ID: EBS01. A mtDNA COI gene sequence. B mitochondrial 16S rRNA
Fig. 4  Phylogenic analyses of sample ID: BT04. A mtDNA COI gene sequence. B mitochondrial 16S rRNA
the genera *Bassozetus* and *Synaphobranchus* collected from the Western Pacific Ocean.

The deep-sea cusk-eel genus *Bassozetus* Gill 1883 (Ophidioformes: Ophidiidae, Neobythitinae) currently comprises 13 species [16] and is commonly found at depths ranging between 1000 and 5500 m (except for *Bassozetus zenkevitchi* [17]) in tropical and temperate areas [9, 13]. To date, *Bassozetus* has been recorded in the Atlantic, Indian, and Pacific Oceans. For example, six species (*sozetus* has been recorded in the Atlantic, Indian, and Pacific

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Author contributions H-JK: conception, carried out the experiments. JH: conception, analysis, interpretation of results and discussion. B-JK: carried out the morphological analysis. K-WL and KH: interpretation of results and discussion. Y-UC: conception, interpretation of results and discussion. All authors read and approved the manuscript.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval This study did not require ethical approval as we do not use live animals.
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