

Brief Report

Complete Mitochondrial DNA Genomes of Deep-Sea Eels *Synaphobranchus brevidorsalis* and *S. affinis* and New Record of *S. brevidorsalis* from the East Mariana Basin

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Abstract: In this study, we document the first recorded range extension of the genus *Synaphobranchus* from the East Mariana Basin in the Western Pacific Ocean. We sequenced the complete mitochondrial (mt) genome of two deep-sea eels (*Synaphobranchus brevidorsalis* and *S. affinis*) collected in the East Mariana Basin in the Western Pacific Ocean. The complete mt genomes of *S. brevidorsalis* and *S. affinis* were 16,686 bp and 16,677 bp in length, respectively, and consisted of 13 protein-coding genes, 22 tRNA genes, and two rRNA genes. Molecular phylogenetic analysis of the two deep-sea eel species was performed, based on the mt cytochrome c oxidase subunit I (COI) gene using the maximum likelihood method. The molecular phylogenetic tree demonstrated that *S. brevidorsalis* and *S. affinis* were congeneric species of *S. brevidorsalis* and *S. affinis* reported in previous studies with bootstrap values of 100% and 100%, respectively. This is the first report on the complete mt genomes of *S. brevidorsalis* and *S. affinis* collected in the East Mariana Basin in the Western Pacific Ocean. Overall, our study highlights the potential of molecular approaches in identifying species diversity and distribution in the Western Pacific Ocean.

Keywords: cytochrome c oxidase subunit I; phylogenetic analysis; East Mariana Basin; Western Pacific Ocean



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1. Introduction

The deep sea is generally defined as the depth at which light can no longer penetrate, which is typically 200 m. It is the largest ecosystem on Earth and a largely unexplored habitat [1–3]. The deep sea is recognized for its extremely harsh conditions, and deep-sea organisms survive with low food resources, high hydrostatic pressure, extreme cold, and permanent darkness [4–6]. Deep-sea fishes are essential components of biodiversity in deepwater ecosystems, and more than 30,000 species of fish exist worldwide [7,8]. The identification and characterization of new fish species are important for deep-sea biodiversity monitoring [9–11]. However, little information is available on deep-sea fish species because the exploration of the deep ocean and collection of biological samples are challenging tasks [12].

The eel family Synphobranchidae occupies a crucial trophic level and contributes to the biodiversity in the deep-sea ecosystem; in fact, this family is found in tropical and temperate areas around the world [13]. Synphobranchidae is particularly widely distributed in the vertical ocean column at depths ranging from less than 100 m to several thousand meters. The *Synphobranchidae* is currently represented by 12 genera and about 40 species [14,15]. Among them, *Synphobranchus* is a genus that belongs to the cutthroat eel family, Synphobranchidae, and among the 142 varieties are currently six valid species [14]: *S. kaupii* [16] (Kaup's arrowtooth eel), *S. affinis* [17], *S. brevidorsalis* [18], *S. dolichorhynchus* [19], *S. oregoni* [20], and *S. calvus* [21].

To date, analysis of morphological characteristics has been widely used as an important tool for species identification and distinguishing one species from another. Morphological characteristics alone are not sufficient to distinguish similar species and identify new species because of the morphological diversity and ontogenetic changes during fish growth [22–24].

Recently, DNA barcoding has been used for the accurate and reliable identification of unknown species, including rare and cryptic species, since mitochondrial DNA (mtDNA) is well conserved [25]. In addition, mtDNA-based barcoding can be extensively used in molecular biodiversity and conservation genetics studies [26]. In particular, the mitochondrial cytochrome c oxidase subunit I (COI) gene has been widely used for species identification and classification of marine species [27–30]. Furthermore, mtDNA genes have been used as DNA barcodes for the identification and classification of some deep-sea species (e.g., squat lobsters, sea cucumber, and deep-sea eels) [24,31–34]. Therefore, mtDNA genes can be applied to accurately identify deep-sea eel species from the East Mariana Basin in the Western Pacific Ocean.

In this study, we sequenced the complete mt genome of *Synaphobranchus brevidorsalis* and *S. affinis* collected in the East Mariana Basin in the Western Pacific Ocean. In addition, to accurately identify the species, we compared the mtCOI gene among deep-sea eel species using phylogenetic relationships.

2. Materials and Methods

2.1. Sampling Collection

During a recent expedition on board the RV *ISABU* (Supplementary Figure S1A) of the Korea Institute of Ocean Science and Technology (KIOST) to the East Mariana Basin in the Western Pacific Ocean in May 2021, *S. brevidorsalis* and *S. affinis* samples were collected (sampling sites are shown in Supplementary Figure S1 and Supplementary Table S1) using a trap baited with fish (Supplementary Figure S2) from different areas. Photographs of *S. brevidorsalis* and *S. affinis* are shown in Supplementary Figure S3. These specimens generated considerable scientific interest because they represented the first species of the *Synaphobranchus* genus from the East Mariana Basin to be recorded in the Western Pacific Ocean. Therefore, the specimens were kept for further examination to determine their taxonomic status. The specimens were preserved and stored at -20°C and transported to the laboratory where a tissue sample for DNA analysis was dissected from each specimen and stored in 95% ethanol.

2.2. DNA Extraction and Sequencing

The total genomic DNA was prepared from the muscle and anal fin using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA), according to 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). Whole-genome sequencing of the samples was performed on an Illumina NovaSeq 6000 platform (National Instrumentation Center for Environmental Management, Seoul, South Korea). The mitogenome of the synaphobranchid deep-sea eels was assembled and annotated using the MitoZ [35].

2.3. Phylogenetic Relationships

To determine the molecular phylogenetic tree of *S. brevidorsalis* and *S. affinis*, a total of 26 mtDNA COI sequences from the Synaphobranchidae eel family (genera: *Simenchelys*, *llyophis*, *Histiobranchus*, *Dysomma*, *Dysommima*, *Diastobranchus*, and *Synaphobranchus*) were obtained from the GenBank database and aligned with the sequences generated in this study. *Bassozetus compressus* and *Bassozetus glutionsus* (Ophidiidae eel family) were used as the outgroup. The nucleotide sequences of individual mt COI genes were aligned using the ClustalW algorithm of the 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 was estimated using a maximum likelihood (ML) analysis. According to the results of the model test, ML phylogenetic analyses were performed with the LG + G + I model using the MEGA software (ver. 10.0.1; Center for Evolutionary Medicine and Informatics). Rapid bootstrap analysis was conducted with 1000 replications with 48 threads running in parallel.

3. Results and Discussion

The complete mt genomes of *S. brevidorsalis* and *S. affinis* were sequenced and deposited in a database (accession numbers: OQ581844 and OQ581845, respectively). The lengths of the complete mt genomes of *S. brevidorsalis* and *S. affinis* were 16,686 bp and 16,677 bp, respectively. The complete mt genomes of *S. brevidorsalis* and *S. affinis* contained 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), and 2 ribosomal RNA genes (rRNAs) (Tables 1 and 2, Figure 1). The complete mt genome of *S. brevidorsalis* had the following base composition: A (24.9%), T (31.3%), C (16.4%), and G (27.4%). For *S. affinis*, the overall base composition was as follows: A (31.4%), C (27.3%), T (24.8%), and G (16.5%). Possible strain-specific differences may be related to the base composition of the complete mt genome. For example, the A + T and G + C contents of the 13 PCGs in the complete mt genome of *S. brevidorsalis* were 55.6% and 44.4%, respectively, while the contents in all sequences were 55.2% and 44.8%, respectively. In the case of *S. affinis*, the A + T and G + C contents of the 13 PCGs in the complete mt genome were 55.7% and 44.3%, respectively, while the contents in all sequences were 56.2% and 43.8%, respectively. In *S. brevidorsalis*, most PCGs (12 of 13 genes) initiated with the start codon ATG, while COX1 initiated with the start codon GTG. Eleven PCGs terminated with TAA/TAG, while ND4 and COX1 terminated with TAT and GAA. In the case of *S. affinis*, most PCGs (12 of 13 genes) started with ATG, whereas COX1 initiated with GTG. Ten PCGs terminated with TAA/TAG, while ND1, COX1, and ND4 terminated with AGT, AAG, and TAT, respectively. Therefore, we suggest that comparative analysis of the complete mt genomes of *S. brevidorsalis* and *S. affinis* revealed species-specific differences in the mt genomes of deep-sea eel species.

Table 1. Organization of the mitochondrial genome of *S. brevidorsalis*.

Gene	Strand	Position	Length (bp)	Start	Stop	Intergenic Region *
trnP	H	624–693	70	-	-	5
trnT	L	699–771	73	-	-	2
CYTB	L	774–1913	1140	ATG	TAA	4
trnE	H	1918–1986	69	-	-	1
ND6	H	1988–2509	522	ATG	TAG	−4
ND5	L	2506–4344	1839	ATG	TAA	0
trnL	L	4345–4417	73	-	-	0
trnS	L	4418–4486	69	-	-	0
trnH	L	4487–4555	69	-	-	0
ND4	L	4556–5936	1381	ATG	TAT	−7
ND4L	L	5930–6226	297	ATG	TAA	0
trnR	L	6227–6296	70	-	-	−2
ND3	L	6295–6645	351	ATG	TAG	0
trnG	L	6646–6717	72	-	-	−1
COX3	L	6717–7502	786	ATG	TAA	−1
ATP6	L	7502–8185	684	ATG	TAA	−10
ATP8	L	8176–8343	168	ATG	TAA	1
trnK	L	8345–8419	75	-	-	−14
COX2	L	8406–9110	705	ATG	TAA	6
trnD	L	9117–9186	70	-	-	5
trnS	H	9192–9262	72	-	-	−11
COX1	L	9252–10,845	1594	GTG	GAA	1
trnY	H	10,847–10,917	71	-	-	1
trnC	H	10,918–10,982	65	-	-	−73
trnN	H	11,020–11,092	73	-	-	2

Table 1. Cont.

Gene	Strand	Position	Length (bp)	Start	Stop	Intergenic Region *
trnA	H	11,095–11,163	69	-	-	3
trnW	L	11,167–11,238	72	-	-	-2
ND2	L	11,237–12,283	1047	ATG	TAG	0
trnM	L	12,284–12,353	70	-	-	-1
trnQ	H	12,353–12,423	71	-	-	0
trnI	L	12,424–12,492	69	-	-	11
ND1	L	12,504–13,472	969	ATG	TAG	0
trnL	L	13,473–13,547	75	-	-	0
l-rRNA	L	13,548–15,259	1712	-	-	0
trnV	L	15,260–15,330	71	-	-	1
s-rRNA	L	15,332–16,289	958	-	-	0
trnF	L	16,290–16,360	71	-	-	-

* Negative numbers indicate overlapping nucleotides between adjacent genes.

Table 2. Organization of the mitochondrial genome of *S. affinis*.

Gene	Strand	Position	Length (bp)	Start	Stop	Intergenic Region *
trnF	H	342–413	71	-	-	0
s-rRNA	H	414–1371	959	-	-	1
trnV	H	1373–1442	70	-	-	0
l-rRNA	H	1443–3152	1710	-	-	0
trnL	H	3153–3227	75	-	-	0
ND1	H	3228–4197	970	ATG	AGT	9
trnI	H	4207–4275	69	-	-	0
trnQ	L	4276–4346	71	-	-	-1
trnM	H	4346–4415	70	-	-	0
ND2	H	4416–5462	1047	ATG	TAG	-2
trnW	H	5461–5532	72	-	-	3
trnA	L	5536–5604	69	-	-	2
trnN	L	5607–5679	73	-	-	35
trnC	L	5715–5779	65	-	-	0
trnY	L	5780–5850	71	-	-	1
COX1	H	5852–7443	1595	GTG	AAG	-9
trnS	L	7435–7505	71	-	-	5
trnD	H	7511–7580	70	-	-	6
COX2	H	7587–8283	697	ATG	TAA	-6
trnK	H	8278–8352	75	-	-	1
ATP8	H	8354–8521	168	ATG	TAA	-10
ATP6	H	8512–9195	684	ATG	TAA	-1
COX3	H	9195–9980	786	ATG	TAA	-1
trnG	H	9980–10,051	72	-	-	0
ND3	H	10,052–10,402	351	ATG	TAG	-2
trnR	H	10,401–10,470	70	-	-	0
ND4L	H	10,471–10,767	297	ATG	TAA	-7
ND4	H	10,761–12,141	1381	ATG	TAT	0
trnH	H	12,142–12,210	69	-	-	0
trnS	H	12,211–12,279	69	-	-	0
trnL	H	12,280–12,352	73	-	-	0
ND5	H	12,353–14,191	1839	ATG	TAA	-4
ND6	L	14,188–14,710	522	ATG	TAG	0
trnE	L	14,711–14,779	69	-	-	4
CYTB	H	14,784–15,923	1140	ATG	TAG	2
trnT	H	15,926–15,998	73	-	-	5
trnP	L	16,004–16,073	70	-	-	-

* Negative numbers indicate overlapping nucleotides between adjacent genes.

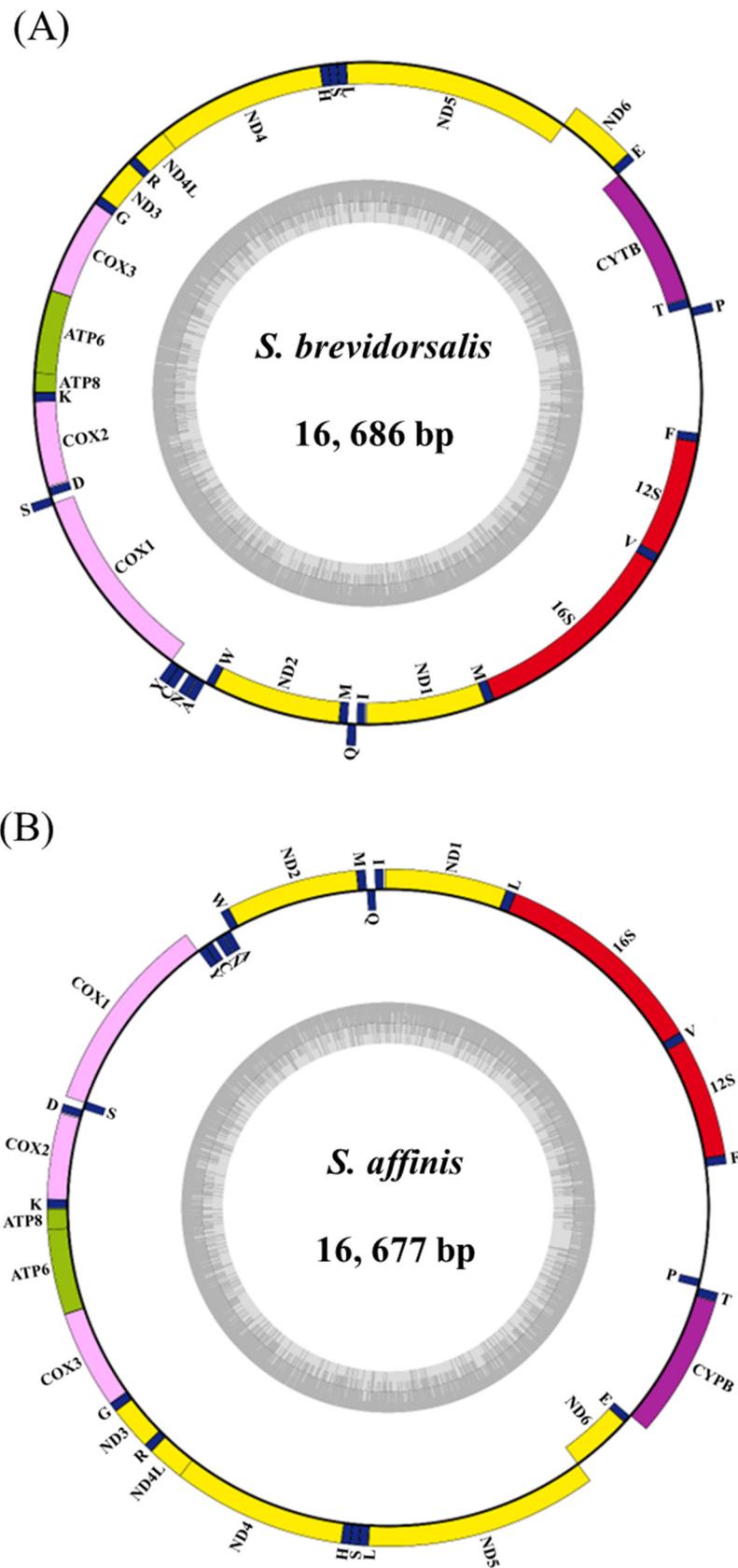


Figure 1. Organization of the mitochondrial genome of two deep-sea eel specimens. (A) *S. brevidorsalis* and (B) *S. affinis*. Genes encoded by the heavy (H) strand are shown outside the circle, and those encoded by the light (L) strand are shown inside the circle.

In this study, a molecular phylogenetic tree was constructed according to ML, based on the mtCOI gene (Figure 2). *S. brevidorsalis* and *S. affinis* from the Western Pacific Ocean were genetically distinct and belonged to the genus *Synaphobranchus*, according to the mtDNA COI sequences. In particular, the phylogenetic tree of mtDNA COI showed that *S. brevidorsalis* and *S. affinis* were congeneric species of *S. brevidorsalis* and *S. affinis* reported in previous studies, with bootstrap values of 100% and 100%, respectively. Previously, identification of *S. affinis* from the East Mariana Basin in the Western Pacific Ocean was reported based on DNA barcoding and morphological characteristics [25]. In this study, we further determined that mtDNA barcoding markers may be useful for rapid and accurate identification of *S. brevidorsalis* and *S. affinis* collected in the Western Pacific Ocean. Therefore, supported by the molecular phylogenetic relationships of mtCOI, we suggest that the two deep-sea eels are *S. brevidorsalis* and *S. affinis*. However, morphological feature analysis of *S. brevidorsalis* is needed for accurate species identification.

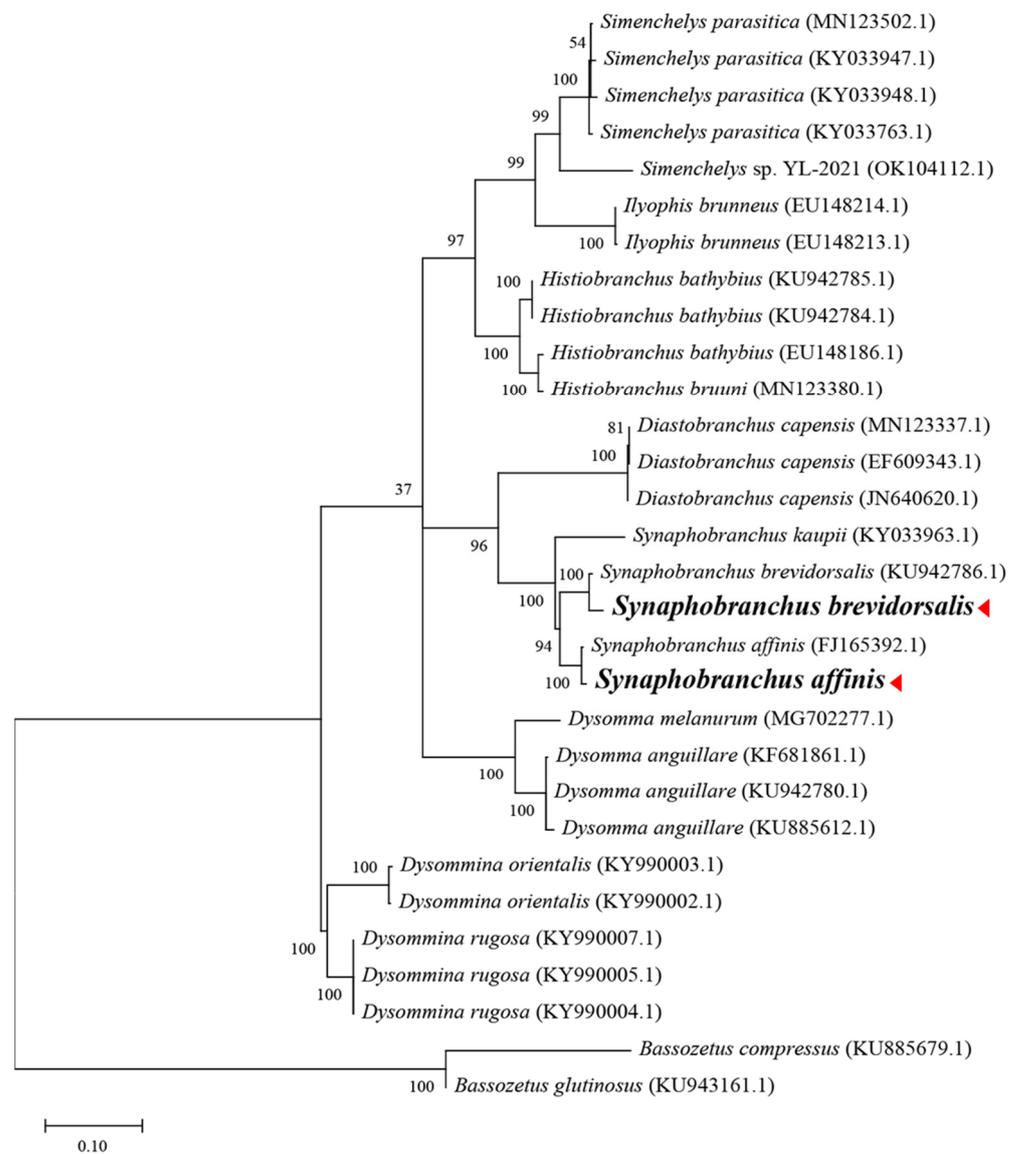


Figure 2. Phylogenetic tree based on mitochondrial COI using the MEGA software (ver. 10.0.1). Phylogeny was generated using maximum likelihood estimation. Published mtDNA COI sequences from the Synaphobranchidae eel family were used as reference. The red triangles indicate the *Synaphobranchus* species analyzed in this study.

To date, the diversity and distribution of fishes belonging to genus *Synaphobranchus* are likely to be incompletely documented because the deep sea, which is Earth's largest ecosystem, remains poorly explored [1,3]. Despite the extreme difficulties associated with sampling in the deep sea, there are growing efforts globally to characterize these habitats and document their diversity. This is evidenced by several expeditions that have been undertaken in the diverse ocean regions over the past decade. Several species have been discovered and described, and the geographical ranges of many species have also been extended because of these increased sampling efforts [33,34]. Indeed, *S. brevidorsalis* has been reported in the Indo-Pacific, North Atlantic, and Western Central Atlantic Ocean [13,36]. However, there has been no record of *S. brevidorsalis* in the East Mariana Basin in the Western Pacific Ocean.

S. affinis is circumglobally distributed, except for the Northeastern Pacific [34]. Recently, *S. affinis* was reported in the East Mariana Basin in the Western Pacific Ocean [25]. However, information on the complete mt genome of *S. affinis* from the East Mariana Basin was lacking. Our results suggest that *S. brevidorsalis* is widely distributed in the Pacific Ocean. In addition, the complete mt genomes of *S. brevidorsalis* and *S. affinis* will provide molecular information for further research on deep-sea marine species diversity, evolutionary abundance, and distribution in the Western Pacific Ocean. However, these preliminary genetic results suggest that the two deep-sea eel specimens from the East Mariana basin in the Western Pacific Ocean may potentially represent a species to science; however, determining their taxonomic distinctiveness requires application of an integrative taxonomic approach that includes sequences for topotypes of all currently known species in the *Synaphobranchus* genus, as well as detailed osteological and morphological examinations of the types of specimens.

In summary, we determined the first complete mt genome of *S. brevidorsalis* and *S. affinis* collected in the East Mariana Basin in the Western Pacific Ocean. Importantly, mtCOI sequencing was sufficient to distinguish between *S. brevidorsalis* and *S. affinis*, supporting the notion that mtDNA sequencing is a useful method for accurate species identification. Overall, this study presents a new record of *S. brevidorsalis* and the complete mt genomes of *S. brevidorsalis* and *S. affinis* from off the East Mariana Basin, Western Pacific Ocean.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/jmse11040860/s1>, Figure S1: (A) The research vessel *ISABU*. (B) Sampling sites of *Synaphobranchus brevidorsalis* and *Synaphobranchus affinis* on the East Mariana Basin in the western Pacific Ocean.; Figure S2: Fish sampling using bait traps (composed of fish); Figure S3: Photographs of two deep-sea eel specimens. (A) *Synaphobranchus brevidorsalis* and (B) *S. affinis*. Table S1: List of the species and sample sites.

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Institutional Review Board Statement: The animal study protocol was approved by the Institutional Animal Care and Experimental Committee of Korea Institute of Ocean Science and Technology (KIOST).

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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Conflicts of Interest: The authors declare no conflict of interest.

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