Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

New Records and Mitochondrial DNA Markers of Two Deep-sea eels Collected from the Western Pacific Ocean

Han-Jun Kim
Korea Institute of Ocean Science and Technology
jeonghoon han (jeonghoon@kiost.ac.kr)
Korea Institute of Ocean Science and Technology https://orcid.org/0000-0003-4022-0908
Byung-Jik Kim
National Institute of Biological Resources
Kyun-Woo Lee
Korea Institute of Ocean Science and Technology
Kiseong Hyeong
Korea Institute of Ocean Science and Technology
Young-Ung Choi
Korea Institute of Ocean Science and Technology

Short Report

Keywords: Deep-sea, eel, mitochondrial DNA, cytochrome c oxidase subunit 1, 16S ribosomal RNA, phylogenetic analysis

Posted Date: April 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-395813/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Version of Record: A version of this preprint was published at Molecular Biology Reports on July 22nd, 2021. See the published version at https://doi.org/10.1007/s11033-021-06559-8.

Abstract

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. To accurately identify the species of the deep-sea eel specimens, we sequenced the mitochondrial genes (cytochrome c oxidase subunit 1 (CO1) and 16S ribosomal RNA (16S rRNA)). Through molecular phylogenetic analysis based on mtDNA CO1 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. This is the first study to report new records of the genera *Bassozetus* and *Synaphobranchus* from the western Pacific Ocean based on mitochondrial DNA markers.

1. Introduction

The deep-sea (> 200 m depth) is the largest habitat on earth and is an unexplored environment [1, 2]. 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 [3, 4]. In general, fish are very important components of biodiversity in aquatic ecosystems, and more than 30,000 fish species exist worldwide [5]. However, there is little information on deep-sea fishes as deep ocean environments can be difficult to access and obtain biological samples [6]. Considering this, identification of new fish species becomes essential for ecological monitoring and understanding the deep-sea biodiversity [7, 8, 9].

To date, morphometric and meristic features have been used as traditional tools for fish species identification [10]. However, it is difficult to accurately identify closely related species [11] 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 [12, 13, 14]. Additionally, mitochondrial DNA (mtDNA) markers can be used in biodiversity research for monitoring and for studying the molecular phylogeny [15, 16, 17]. In particular, mtDNA cytochrome c oxidase subunit 1 (CO1) 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 [9, 13, 18, 19, 20, 21, 22, 23]. Therefore, genetic molecular marker-based species identification can be applied to accurately identify closely related fish species, in cases where traditional morphological classification methods lead to ambiguities.

In this study, we sequenced the mtDNA CO1 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.

2. Materials And Methods

2.1. Sampling collection and

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 October 2019 and May 2020, respectively. The sampling areas are shown in Fig. 1 and Table 1. Also, pictures of the two deep-sea eel specimens collected are shown in Fig. 2. These two deep-sea eel specimens were assumed to belong to the cusk-eel family Ophidiidae and cutthroat eel family Synaphobranchidae, respectively, based to the morphological data [24, 25, 26]. This study did not include live fish and the samples used were dead. The dead fish were preserved and stored at − 20°C until DNA isolation.

Table 1
Map with location of sample collection.

Years	ID Code	Geographical Area	Latitude	Longitude	Depth (m)
2019	EBS01	OSM19-1	16˚48N	150˚04E	1498
2020	BT04	OSM17	19˚40N	151˚38E	1298

2.2. 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).

2.3. PCR amplification and sequencing

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 cytochrome c oxidase subunit 1 (CO1) and 16S ribosomal DNA (16S rDNA) were used as the barcoding markers and were amplified with F and R primers given in Table 2 [27]. 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). All PCR products were sequenced by Macrogen (Seoul, South Korea).

Table 2
Primer set used in this study

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

2.4. Sequence alignment and phylogenetic analysis

To determine the phylogenetic relationships of the specimens of deep-sea cusk-eel *Bassozetus*, mitochondrial CO1 and 16S rRNA genes from the cusk eel family (genus: *Acanthonus*, *Aphyonus*, *Bassozetus*, *Dicrolene*, *Lamprogrammus*, *Neobythites*, and *Porogadus*) and cutthroat eel family Synaphobranchidae (genus: *Simenchelys*, *Ilyophis*, *Histiobranchus*, *Dysomma*, *Meadia*, *Dysommina*, *Diastobrachus*, and *Synaphobranchus*) were obtained from GenBank database. The nucleotide sequences of individual mitochondrial CO1 and 16S rRNA gene sequences from the eels were aligned using the ClustalW program 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).

3. Results And Discussion

In this study, the mtDNA CO1 and 16S rRNA genes of deep-sea eel specimen (sample ID: EBS01) were sequenced and the lengths of the markers were 721 bp and 608 bp, respectively. For the deep-sea eel specimen (sample ID: BT04), the mtDNA CO1 and 16S rRNA genes were sequenced and the lengths of the markers were 655 bp and 600 bp, respectively. The amplified sequences of mitochondrial CO1 of two deep-sea eels were confirmed using BLAST searches on the NCBI website. One deep-sea eel (sample ID: EBS01) showed 87.22% identity with *Bassozetus zenkevitchi* (acc. no. AP004405.1) and the other deep-sea eel (sample ID: BT04) showed 94.66% identity with *Synaphobranchus kaupii* (acc. no. AP002977.2), suggesting that the two deep-sea eels belong to the genera *Bassozetus* and *Synaphobranchus*, respectively. Indeed, the molecular phylogenetic tree based on mtDNA CO1 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 CO1 gene sequence showed that this specimen clustered together with *S. kaupii*, *S. brevidorsalis*, and *S. affinnis* (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 *Bassozetus* (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 [28, 29, 30]. 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 [31]. 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 [19, 32, 33, 34, 35]. In this context, our two deep-sea eel specimens are morphologically similar to the genera *Bassozetus* and *Synaphobranchus*, respectively, which positively correlated with the phylogenetic analysis based on mitochondrial DNA markers. This suggests that mitochondrial DNA markers are useful for accurate species identification of the genera *Bassozetus* and *Synaphobranchus* collected from the western Pacific Ocean.

The deep-sea cusk-eel genus *Bassozetus* Gill 1883 (Ophidiiformes: Ophidiidae, Neobythitinae) currently comprises 13 species [36] and is commonly found at depths ranging between 1,000 and 5,500 m (except for *Bassozetus zenkevitchi* [37]) in tropical and temperate areas [24, 29]. To date, *Bassozetus* has been recorded in the Atlantic, Indian, and Pacific Oceans. For example, six species (*B. galatheae*, *B. glutinosus*, *B. compressus*, *B. elongatus*, *B. levistomatus*, and *B. robustus*) have been described in the western Indian Ocean [38]. *Bassozetus elongatus* was described from the Celebes (Sulawesi, Indonesia) (West Pacific) [39]. *Bassozetus compressus* and *B. elongatus* have also been described from the Indo-West Pacific Ocean [24, 29, 30, 40] and the Atlantic Ocean [24, 29], respectively. The cutthroat eel genus *Synaphobranchus* currently includes six valid species [41]: Kaup's arrowtooth eel, *S. kaupii* Johnson, 1862 [42]; Gray's cutthroat, *S. affinis* Günther, 1877 [43]; Shortdorsal cutthroat eel, *S. brevidorsalis* Günther, 1887 [44]; cutthroat eels, *S. dolichorhynchus* [45]; *S. oregoni* [46]; and *S. calvus* Melo, 2007 [25]. All species in *Synaphobranchus* have been recorded from the North Atlantic Ocean except for *S. calvus* [31, 45, 47]. However, to date, no species have been identified belonging to *Bassozetus* and *Synaphobranchus* from the western Pacific Ocean.

In conclusion, this study introduces two newly recorded specimens from the genera *Bassozetus* and *Synaphobranchus*, respectively, in the western Pacific Ocean. In addition, this study provides information on mitochondrial DNA markers (CO1 and 16S rRNA) for identifying deep-sea eels. However, more samples from the western Pacific Ocean are needed to accurately identify these species.

Declarations

Author contributions

J.H.: conception, analysis, interpretation of results and discussion. H.-J.K: carried out the experiments. B.-J.K.: carried out the morphological analysis. K.-W.L. and K.H.: Interpretation of results and discussion. Y.-U.C.: Conception, interpretation of results and discussion. All authors read and approved the manuscript.

Declaration of Competing 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.

Acknowledgements

This work was supported by the KIOST projects “Exploration of new marine biological/genetic resources and rare metal resources in the Area beyond national jurisdiction” (PE99924).

References

1. Rex MA (1981) Community structure in the deep-sea Benthos. Annual Review of Ecology and Systematics 12 (1): 331–353
2. Ramirez-Llodra E, Brandt A, Danovaro R, Mol BD, Escobar E, German CR, Levin LA, Arbizu PM, Menot L, Buhl-Mortensen P, Narayanaswamy BE, Smith CR, Tittensor DP, Tyler PA, Vanreusel A, Vecchione M (2010) Deep, diverse and definitely different: unique attributes of the world’s largest ecosystem. Biogeosciences 7:2851–2899
3. Sanders HL, Hessler RR (1969) Ecology of the deep-sea benthos. Science 163(3874): 1419–1424
4. Robison BH (2004) Deep pelagic biology. J Exp Mar Biol Ecol 300: 253–272
5. Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN (2005) DNA barcoding Australia’s fish species. Philos Trans R Soc Lond B Biol Sci 360:1847–1857
6. Danovaro R, Corinaldesi, C, Dell'Anno A, Snelgrove, PVR (2017) The deep-sea under global change. Curr Biol 27(11):R461-R465
7. Elphick CS (2008) How you count counts: the importance of methods research in applied ecology. J Appl Ecol 45:1313–1320
8. Farnsworth EJ, Chu M, Kress WJ, Neill AK, Best JH, Pickering J, Stevenson RD, Courtney GW, VanDyk JK, Ellison AM (2013) Next-Generation Field Guides. Bioscience 63:891–899
9. Austen GE, Bindemann M, Griffiths RA, Roberts DL (2016) Species identification by experts and non-experts: comparing images from field guides. Sci Rep 20(6):33634

10. Strauss RE, Bond CE (1990) Taxonomic methods: morphology. In: Schreck CB, Moyle PB (eds) Methods for fish biology. American Fisheries Society, Maryland, pp 109–140
11. Callejas C, Ochando MD (2001) Molecular identification (RAPD) of the eight species of the genus Barbus (Cyprinidae) in the Iberian Peninsula. J Fish Biol 59:1589–1599
12. Matarese AC, Spies IB, Busby MS, Orr JW (2011) Early larvae of *Zesticelus profundorum* (family Cottidae) identified using DNA barcoding. Ichthyol Res 58: 170–174
13. Triantafyllidis A, Bobori D, Koliamitra C, Gbandi E, Mpanti M, Petriki O, Karaiskou N (2011) DNA barcoding analysis of fish species diversity in four north Greek lakes. Mitochondrial DNA 22:37–42
14. Zhang JB, Hanner R (2011) DNA barcoding is a useful tool for the identification of marine fishes from Japan. Biochemical Systematics and Ecology 39(1):31–42
15. Meusnier, I., Singer, G.A., Landry, J., Hickey, D.A., Hebert, P.D.N., Hajibabaei, M., 2008. A 683 universal DNA mini-barcode for biodiversity analysis. BMC Genom. 9, 214
16. Rivera J, Currie DC (2009) Identification of Nearctic black flies using DNA barcode (Diptera: Simuliidae). Mol Ecol Resour 9:224–236
17. Trivedi S, Aloufi AA, Ansari AA, Ghosh SK (2016) Role of DNA barcoding in marine biodiversity assessment and conservation: An update. Saudi J Biol Sci 23:161–171
18. Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proc R Soc Lond B. 270:313–322
19. Lakra WS, Goswami M, Gopalakrishnan A (2009) Molecular identification and phylogenetic relationships of seven Indian Sciaenids (Pisces: Perciformes, Sciaenidae) based on 16S rRNA and cytochrome c oxidase subunit I mitochondrial genes. Mol Biol Rep 36:831–839
20. Bingpeng X, Heshan L, Zhilan Z, Chunguang W, Yanguo W, Jianjun W (2018) DNA barcoding for identification of fish species in the Taiwan Strait. PLoS One 13(6): e0198109
21. Lee MY, Munroe TA, Shao KT (2014) Description of a new cryptic, shallow-water tonguefi sh (Pleuronectiformes: Cynoglossidae: *Symphurus*) from the western North Pacific Ocean. J Fish Biol 85:563–585
22. Wu R, Zhang H, Liu J, Niu S, Xiao Y, Chen Y (2018) DNA barcoding of the family Sparidae along the coast of China and revelation of potential cryptic diversity in the Indo- West Pacific oceans based on COI and 16S rRNA genes. J Oceanol Limnol 36:1753–1770
23. Shen Y, Hubert N, Huang Y, Wang X, Gan X, Peng Z, He S (2019) DNA barcoding the ichthyofauna of the Yangtze River: insights from the molecular inventory of a mega-diverse temperate fauna. Mol Ecol Resour 19:1278–1291
24. Nielsen JG, Cohen DM, Markle DF, Robins CR (1999) Ophidiiform fishes of the world (order Ophidiiformes). An annotated and illustrated catalogue of pearlfishes, cusk-eels, brotulas and other ophidiiform fishes known to date. FAO species catalogue. FAO Fisheries synopsis no. 125, volume 18. Rome: FAO. 178 pp.

25. Melo MRS (2007) A new synaphobranchid eel (Anguilliformes: Synaphobranchidae) from Brazil, with comments on the species from the Western South Atlantic. Copeia 2007:315–323
26. Ho, H. -C., Smith, D. G., McCosker, J. E., Hibino, Y., Loh, K. -H., Tighe, K. A., & Shao, K. -T. (2015). Annotated checklist of eels (orders Anguilliformes and Saccopharyngiformes) from Taiwan. *Zootaxa*, 4060(1), 140–189
27. Ivanova NV, Zemlak TS, Hanner R, Hebert PDN (2007) Universal primer cocktails for fish DNA barcoding. Mol Ecol Notes 7:544–548
28. Cohen DM, Nielsen JG (1978). Guide to the identification of genera of the fish order Ophidiiformes with a tentative classification of the order. NOAA Tech Rep NMFS Circ 417:1–72
29. Nielsen JG, Merrett NR (2000) Revision of the cosmopolitan deep-sea genus *Bassozetus* (Pisces: Ophidiidae) with two new species. Galathea Rep 18:7–56
30. Nielsen JG, Møller PR (2008) New and rare deep-sea ophidiiform fishes from the Solomon Sea caught by the Danish Galathea 3 Expedition. Steenstrupia 30:21–46
31. Sulak KJ, Shcherbachev YN (1997) Zoogeography and systematics of six deep-living genera of synaphobranchid eels, with a key to taxa and description of two new species of Ilyophis. Bulletin of Marine Science 60:1158–1194
32. Siraj SS, Esa YB, Keong BP, Daud SK (2007) Genetic characterization of the two colour-type of Kelah. Malaysian Applied Biology 36:23–29
33. Svendsen F, Byrkjedal (2013) Morphological and molecular variation in *Synaphobranchus* eels (Anguilliformes: Synaphobranchidae) of the Mid-Atlantic Ridge in relation to species diagnostics. Mar Biodiv 43:407–420
34. Robins CH (1971) The Comparative Morphology of the Synaphobranchid Eels of the Straits of Florida. Proceedings of the Academy of Natural Sciences of Philadelphia 123:153–204
35. Smith MA, Wood DM, Janzen DH, Hallwachs W, Hebert PDN (2007) DNA barcodes affirm that 16 species of apparently generalist tropical parasitoid flies (Diptera, Tachinidae) are not all generalists. Proc Natl Acad Sci USA 104:4967–4972
36. Tomiyama S, Takami M, Fukui A (2016) Redescription of *Bassozetus compressus* (Gu¨nther 1878), a senior synonym of *Bassozetus elongatus* Smith and Radcliffe 1913 (Ophidiiformes: Ophidiidae). Ichthyol Res 63:218–226
37. Rass TS (1955) Deep-sea fishes of the Kurile-Kamchatka trench. Trud Inst Okeanol 12:328–339
38. Gill TN (1883) Diagnosis of new genera and species of deep-sea fishlike vertebrates. Proc US Natn Mus 6:253–260
39. Radcliffe L (1913) Descriptions of seven new genera and thirty-one new species of fishes of the families Brotulidae and Carapidae from the Philippine Islands and the Dutch East Indies. Proc US Natn Mus 44:135–176
40. Nielsen JG (1997) Deepwater ophidiiform fishes from off New Caledonia with six new species. Mem Mus Natn Hist Natl 174:51–82

41. Eschmeyer W, Fricke, R (2011) Catalog of fishes electronic version. http://research.calacademy.org/research/ichthyology/catalog/ fishcatmain.asp. Accessed 30 Nov 2011
42. Johnson JY (1862) Descriptions of some new genera and species of fishes obtained at Madeira. Proceedings of the Zoological Society of London 1862:167–180
43. Günther A (1877) Preliminary notes on new fishes collected in Japan during the Expedition of H.M.S. “Challenger”. The Annals and magazine of natural history 20(4):433–446
44. Günther A (1887) Report on the deep-sea fishes collected by H.M.S. Challenger during the years 1873–1876. Zoology 5(22): 1–335
45. Lea E (1913) Muraenoid Larvae: From the "Michael Sars" North Atlantic Deep-Sea Expedition, 1910. Rep Sci Res "Michael Sars" N Atl Deep Sea Exped
46. Castle PHJ (1961) Deep-water eels from cook strait, New Zealand. Zoology Publications from Victoria University Wellington 27:1–30
47. Robins CH, Robins CR (1989) Family Synaphobranchidae. *In*: Fishes of the western North Atlantic, Part 9, Orders Anguilliformes and Saccopharyngiformes (Böhlke E.B., ed.), pp. 207-253. New Haven: Sears Foundation for Marine Research, Yale Univ

Figures

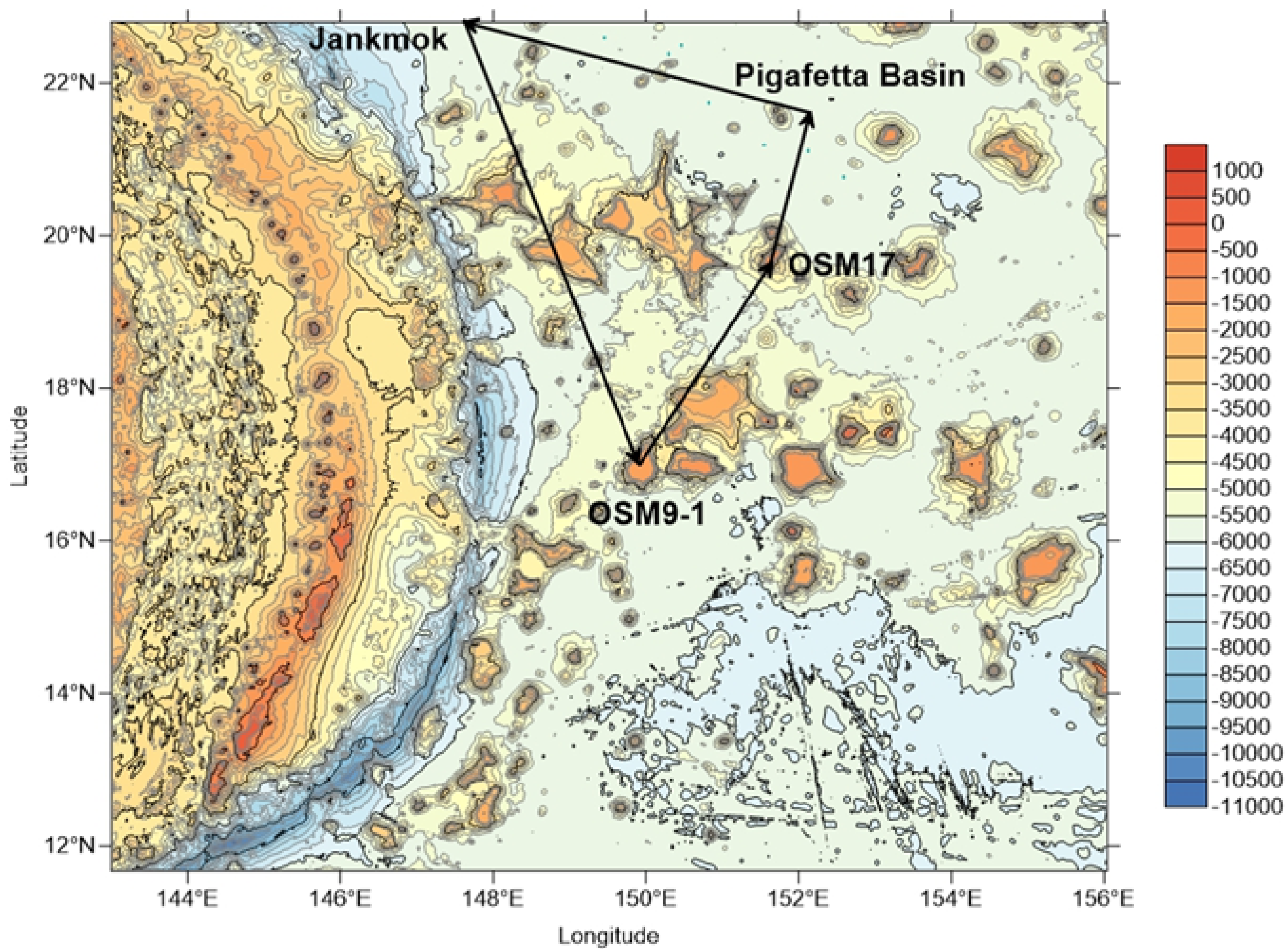


Figure 1

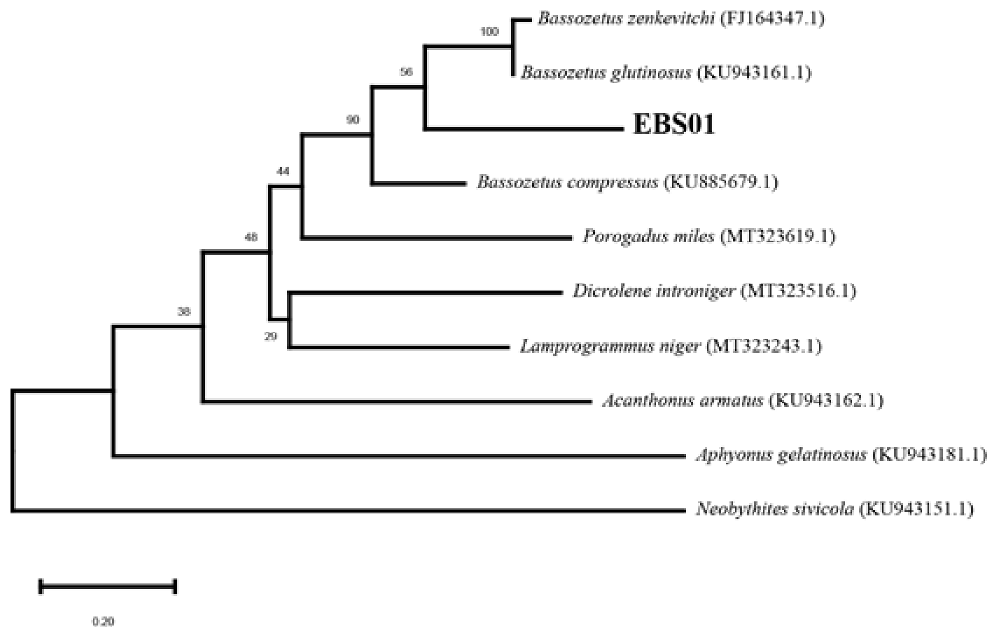
Map of the western Pacific Ocean and geographic distribution of sampling locations. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

A)

B)

Figure 2

Pictures of the two deep-sea eel specimens collected. (a) Sample EBS01. (b) Sample BT04.



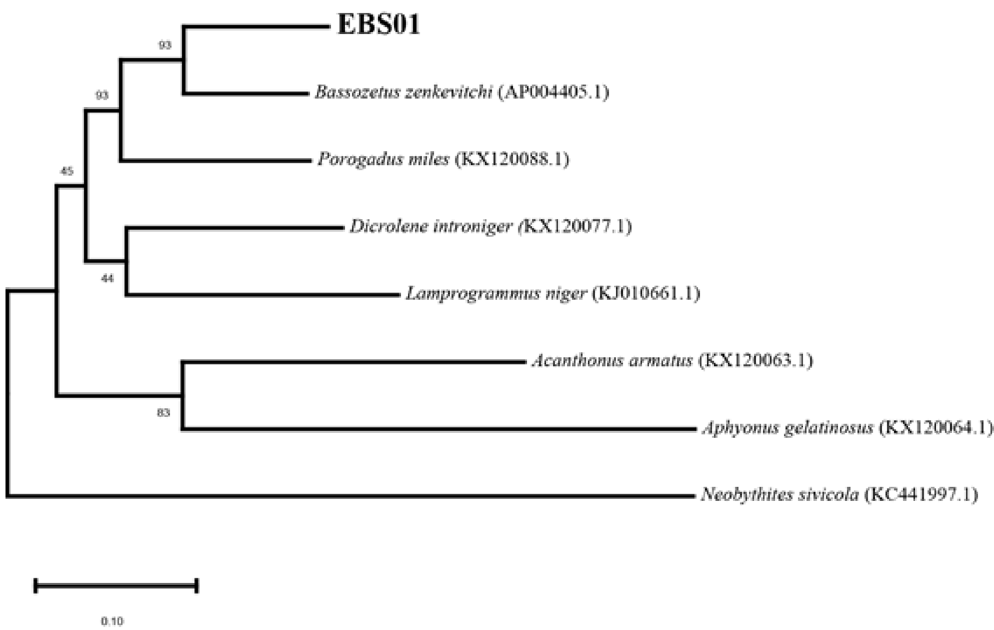
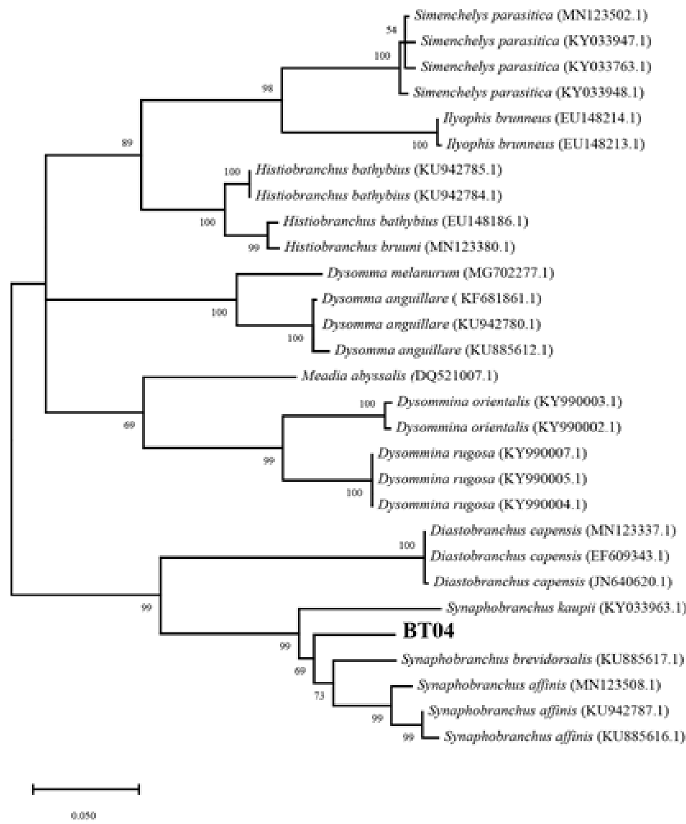


Figure 3

Phylogenic analyses of sample ID: EBS01. (a) mtDNA CO1 gene sequence. (b) mitochondrial 16S rRNA.

A) COI



B) 16S rRNA

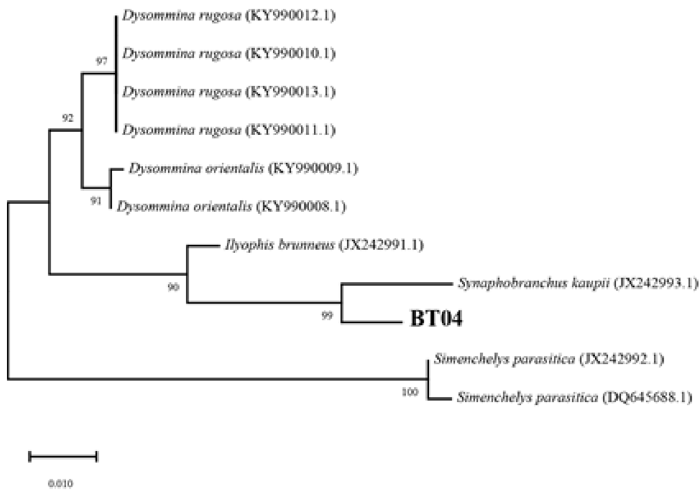


Figure 4

Phylogenic analyses of sample ID: BT04. (a) mtDNA CO1 gene sequence. (b) mitochondrial 16S rRNA.