

## Mitochondrial DNA Part B Resources

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## The complete mitochondrial genome of the Atlantic spiny lumpsucker *Eumicrotremus spinosus* (Fabricius, 1776)

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### ABSTRACT

The complete mitogenome of the Atlantic spiny lumpsucker (*Eumicrotremus spinosus*) was generated using the PacBio Sequel II HiFi sequencing platform. The mitogenome assembly has a length of 19,281 bp and contains 13 protein-coding sequences, 22 tRNA genes, 2 rRNA genes, one control region containing the D-loop (2383 bp) and a duplicate control region (1133 bp). Phylogenetic analysis using maximum likelihood revealed that *E. spinosus* is closely related to the Siberian lumpsucker (*E. asperimus*). The mitogenome of the spiny lumpsucker will be useful in population genomics and systematic studies of Cyclopteridae, Liparidae, and Cottidae.

### ARTICLE HISTORY

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Cyclopteridae; *E. spinosus*;  
Mitogenome; PacBio

The Atlantic spiny lumpsucker, *Eumicrotremus spinosus* (Fabricius, 1776), is a deep-sea benthic fish native to the Arctic and coastal North Atlantic. Its morphological characteristics and particularly the presence of spiny tubercles and the ventral suction disk confirmed its taxonomic identification (Figure 1). To understand the phylogeny of Cyclopteridae family members, we sequenced the mitochondrial genome of *E. spinosus*. The specimen of *E. spinosus* was collected from the Barents Sea during an expedition as part of The Nansen Legacy project (<https://arvetternansen.com/>), which was conducted by trained scientists and according to the European Union animal experimentation guidelines on the protection of animals used for scientific purposes (directive 2010/63/UE). The *E. spinosus* specimen was deposited at the University of Oslo (contact person: Sissel Jentoft; email: [sissel.jentoft@ibv.uio.no](mailto:sissel.jentoft@ibv.uio.no)) under the voucher number 787912a2-9c81-11e8-9126-8c164557e466.

The whole fish was soaked in 96% ethanol and placed at 4°C for two weeks; then, the ethanol was decanted before storage at –80°C. DNA isolation and sequencing were performed by the Norwegian Sequencing Center. Genomic DNA was extracted from heart tissue using the nucleated tissue/blood protocol from the Circulomics Nanobind BIG DNA kit (Circulomics Inc.). The libraries were prepared using the Pacific Biosciences protocol for HiFi library prep using SMRTbell® Express Template Prep Kit 2.0. A total of 5.77 µg DNA was sheared into 15–20 kb fragments using Megaruptor 3. After clean-up, we had ~ 3 µg of fragmented DNA, which

was used for library preparation. The final library was size selected using BluePippin with an 11 kb cutoff, resulting in ~0.9 µg of genomic DNA. The library was sequenced on one 8M SMRT cell on a Sequel II instrument using Sequel II Binding kit 2.2 and Sequencing chemistry v2.0. Loading was performed by adaptive loading, with a movie time of 30 h.

Mitochondrial reads were extracted from the whole genome data using MitoHiFi v2.2 (Uliano-Silva et al. 2021) with the mitogenome of *Cyclopterus lumpus* (MN122882.1) as a mapping reference. Extracted reads were assembled with Flye v2.9.1 (Kolmogorov et al. 2019) to obtain a full-length mitogenome assembly. The genome was annotated using MITOS (Bernt et al. 2013) and MitoFish annotator (Iwasaki et al. 2013). The complete *E. spinosus* mitogenome consisted of 19.2 kb (GenBank accession no. OP728784) and had a typical mitochondrial genome structure consisting of 13 protein-coding genes PCGs, 22 transfer RNA (tRNA) genes, two ribosomal RNA (rRNA) genes, and three non-coding regions (ncDNA) (Figure 2). Additionally, the control region (CR) was duplicated.

The *E. spinosus* mitogenome was similar to that of *C. lumpus*, which also comprises three non-coding regions, two *trnL* and two *trnS* genes (Maduna et al. 2022). In the H-strand, two CR regions, one non-coding region (the homo polymer region), 12 PCGs, and 14 tRNA genes were present. The two control regions were adjacent to each other, with the CR1 region (2383 bp) located between the *trnT* and *trnP*, and the CR2 region (1133 bp) found between the *trnP* and *trnF* genes.

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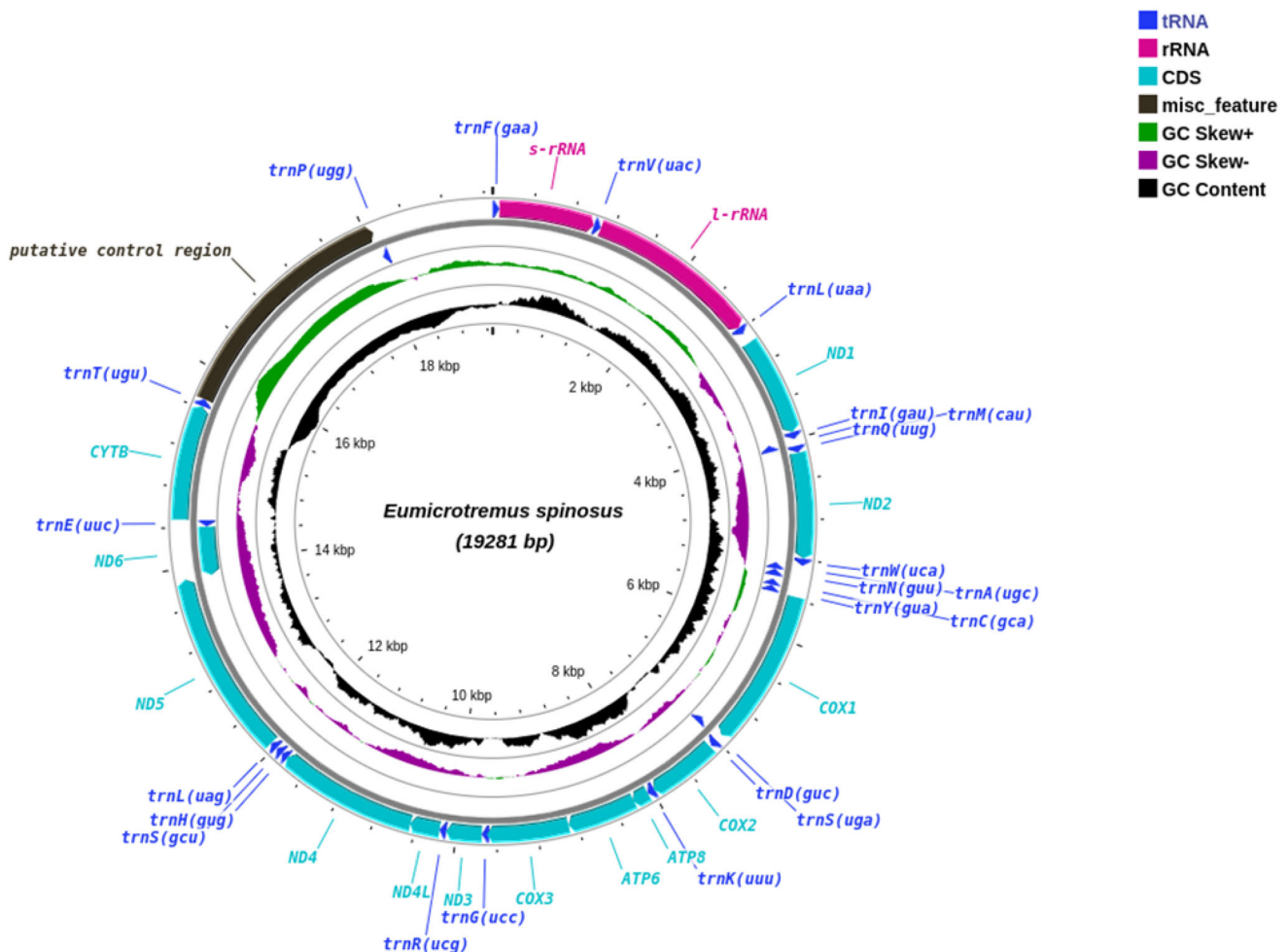
The presence of two CR regions is linked to concerted evolutionary events in non-coding regions (Li et al. 2015). Also, a third non-coding region (intergenic spacer) of 133 bp with a C-tract homopolymer was present between *trnL* and the *ND1* gene on the H-strand. Three non-coding regions have also been observed in other fish species (Maduna et al. 2022).



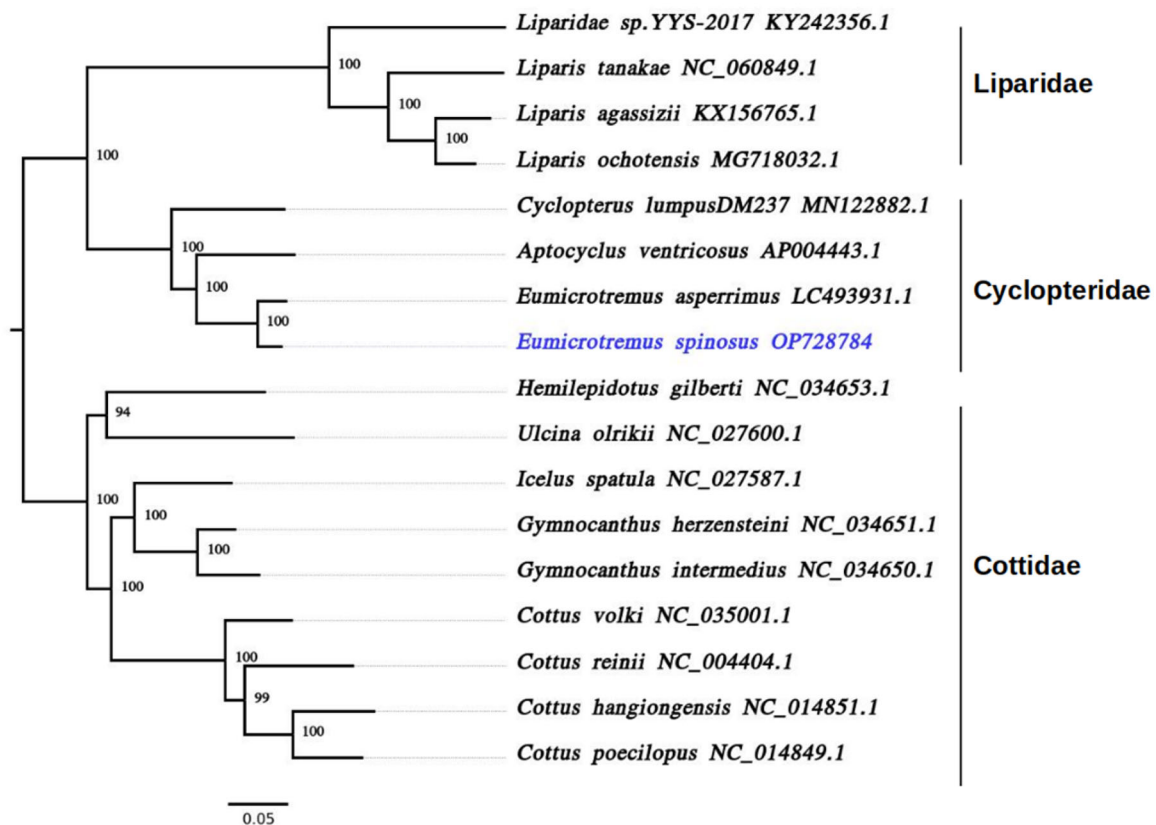
**Figure 1.** Photograph of the *E. spinosus* specimen used in the present study. The morphological traits typical of this species are spiny tubercles and a suction disk on the ventral side.

The L-strand encoded the origin of replication (OL) of 43 bp, 8 tRNA genes and the *ND6* protein-coding gene. The mitogenome arrangement and structure were very similar to a typical vertebrate mitogenome, but the presence of three non-coding regions contribute to our knowledge about the diversity and organization of mitochondrial genomes.

To understand the phylogenetic relationships among the members of Cyclopteridae, Liparidae, and their closest Cottidae relatives, all their mitochondrial PCGs were aligned using MAFFT v7.471 (Kato and Standley 2013), trimmed with trimAL (Capella-Gutierrez et al. 2009) to remove poorly aligned, divergent, and ambiguous regions, and then a maximum likelihood phylogenetic tree was generated using IQ-TREE2 v2.1.2 (Minh et al. 2020). The best-fitting evolution model was automatically selected (JC+SYM+K80) and the phylogenetic reconstruction was performed with the parameters -B 1000, -wbt and 1000 bootstrap replicates (Nguyen et al. 2015; Hoang et al. 2018). Our phylogeny followed the currently accepted taxonomic relationships between all the closely related group members of the Cyclopteridae, Liparidae, and Cottidae families (Maduna et al. 2022) with more than



**Figure 2.** Circular plot of the Atlantic spiny lump sucker (*E. spinosus*) mitogenome displaying both heavy (outer circle) and light (inner circle) strands. It has a typical vertebrate mitochondrial structure with 13 PCGs, two rRNA genes and 22 tRNA genes.



**Figure 3.** Maximum likelihood phylogeny of 17 infraorder Cottales (Teleostei: Perciformes) species based on their mitogenomes. The tree has more than 94% bootstrap support for each node. Accession numbers for each species are shown after the name of the species. The genome sequence generated in this study (OP728784) is labeled in violet and the branches are indicated in black.

94% bootstrap support for each node, as shown in Figure 3. Moreover, it confirmed that Cyclopteridae and Liparidae are sister clades and *E. spinosus* is most closely related to the Siberian lumpsucker (*E. asperrimus*). The data generated from this study will be a valuable resource for future comparative genomics and phylogenetics studies of molecular evolution and systematics in deep-sea fish.

### Ethics statement

The specimen used in this study was collected in a responsible manner, i.e. in connection to the first joint research cruise of the Nansen Legacy project (<https://arvenetternansen.com/nb/arven-etter-nansen/>), as part of larger hauls for stock assessments. The fish were humanely sacrificed before sampling in accordance with the guidelines set by national and international animal welfare laws (e.g. [www.norecopa.no](http://www.norecopa.no)), and thus no specific legislation were needed.

### Authors' contributions

The study was planned by S.J., J.M.O.F., and L.R.N. L.R.P. and M.F.M. performed the analysis, and L.R.P. wrote the manuscript. We especially thank S.J. and S.N.K.H. who sampled the specimen used and initiated the sequencing. Finally, I thank J.M.O.F. for his overall support. S.J., S.N.K.H., M.F.M., J.M.O.F., and L.R.N. have reviewed the manuscript.

### Disclosure statement

No conflict of interest was reported by the author(s).

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### Data availability statement

The mitogenome sequence data can be found in GenBank under the accession number (OP728784).

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