The complete mitogenomes of the spinyhead blenny, *Acanthemblemaria spinosa* (chaenopsidae) and the lofty triplefin, *Enneanectes altivelis* (Tripterygiidae)

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**ABSTRACT**

The blennies, *Acanthemblemaria spinosa* (Chaenopsidae) and *Enneanectes altivelis* (Tripterygiidae) are representative members of two families spanning the deepest node of the Blennioidae tree. The mitogenomes of 16,507 bp for *A. spinosa* and 16,529 bp for *E. altivelis* each consisted of 37 genes and one control loop region. Phylogenetic analysis confirmed the placement of Chaenopsidae and Tripterygiidae within the Blenniformes, however, there was instability in the placement of the triplefins between reconstruction methods, likely due to low taxon sampling. These mitogenomes represent an important milestone in uncovering relationships within Blenniformes and Ovalentaria.

The complete mitogenomes presented here are from an *A. spinosa* individual collected from Curacao (12.12208 N, −68.96851 W) and an *E. altivelis* individual collected from New Providence, Bahamas (25.00719 N, −77.54846 W), both were stored in 95% ethanol at the Yale Peabody Museum of Natural History (YPM ICH 23707 and YPM ICH 23717, respectively; Gregory Watkins-Colwell, gregory.watkins-colwell@yale.edu). Animal collection and handling were done in accordance with IACUC permit 2014-0133. The DNA was extracted from lateral muscle using a Qiagen DNeasy Extraction Kit and used for whole-genome sequencing of 150 bp paired-end reads on an Illumina Hi-Seq 4000 at Texas A&M University’s AgriLife genomics core facility. MITOBim (Hahn et al. 2013) was used for mitogenome assembly.

Genome annotation was performed using MitoAnnotator (Iwasaki et al. 2013) and checked using Geneious 9.0.5 (https://www.geneious.com). The 12 PCGs on the heavy strand of the mitochondrial genome of *A. spinosa*, *E. altivelis*, and 18 other species from within the Ovalentaria available on GenBank, were aligned individually using MAFFT (Katoh and Standley 2013). The 12 nucleotide alignments were concatenated into a single alignment for phylogenetic reconstruction using Bayesian (BEAST 2.6.3; Bouckaert et al. 2019) and Maximum Likelihood (IQ-TREE; Nguyen et al. 2015) methods.

The circular mitogenomes of *A. spinosa* and *E. altivelis* were 16,507 bp (GenBank Accession: MZ315025) and 16,529 bp (GenBank Accession: MZ365315), respectively. The mitogenomes are composed of 23% A, 30% C, 19.2% G, and 27.8% T bases for *A. spinosa* and 26% A, 28.5% C, 17.2% G, and 28.3% T bases for *E. altivelis*, both exhibit AT bias (49.2% GC, *A. spinosa*; 45.7% GC, *E. altivelis*). AT bias has been found in numerous other fish mitochondrial genomes (Satoh et al. 2016). The mitogenomes of both species consisted of 13 protein-coding genes, two rRNA genes, 22 tRNA genes, and one D-loop control region. Only ND6 and eight tRNAs are found on the complementary strand. The gene order is the same as the typical vertebrate mitochondrial genome. In *A. spinosa* and *E. altivelis*, the 12S rRNA genes are 944 and 950 bp, the 16S rRNA genes are 1670 and 1690 bp, and the D-Loops are 892 and 862 bp, respectively.

In *A. spinosa*, 10 protein-coding genes use the start codon ATG, while the remaining three genes use the start codon **CONTACT** Megan A. Sporre, sporrema@tamu.edu

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GTG. In *E. altivelis*, eleven PCGs use the start codon ATG and two use the start codon GTG. Three genes in *A. spinosa* and two genes in *E. altivelis* share the stop codon TAG, two genes in *A. spinosa* and four genes in *E. altivelis* share the stop codon TAA, and the ND4 gene in *A. spinosa* has the stop codon AGG. The remaining seven genes in both species have incomplete stop codons but are followed by an encoded tRNA gene or another PCG, on the same strand that may allow transcription to terminate without a complete stop codon (Pereira 2000; Satoh et al. 2016).

Both reconstruction methods recovered the Blenniiformes as a monophyletic group with Gobiesocidae as sister to the Blennioidei (Wainwright et al. 2012; Eytan et al. 2015). However, there was inconsistency in the placement of the Tripterygiidae; in the ML reconstruction Tripterygiidae and Chaenopsidae are sisters to each other and in the Bayesian reconstruction Tripterygiidae are sisters to the other Blenniiformes (Figure 1). These mitochondrial genomes represent the first complete mitochondrial genome reported from both Chaenopsidae and Tripterygiidae.

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**Author contributions**

Megan A. Sporre: investigation, formal analysis, writing—first draft and revisions, and visualization. Ron I. Eytan: conceptualization, methodology, investigation, and writing—review.

**Data availability statement**

Raw reads and metadata for *Acanthemblemaria spinosa* can be found under the BioProject: PRJNA785178 (BioSample: SAMN23551158; SRA: SRR17173289) and mitogenomic sequence data can be found in GenBank under the accession no. MZ315025. Raw reads and metadata for *Enneanectes altivelis* can be found under the BioProject: PRJNA785173 (BioSample: SAMN23551083; SRA: SRR17171571) and mitogenomic sequence data can be found in GenBank under the accession no. MZ365315.

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