Brief Report

Complete mitochondrial genome assemblies for three species of searobins (genera *Bellator* and *Prionotus*) and their phylogenetic relationships within Triglidae: Prionotinae

Alan Marín[1], Ruben Alfaro[2], Eliana Zelada-Mázmela[1]

[1] *Laboratorio de Genética, Fisiología y Reproducción, Facultad de Ciencias, Universidad Nacional del Santa, Chimbote, Perú*

[2] *Ecobiotech Lab S.A.C. Trujillo, Perú*

Corresponding author: Alan Marín amarin@uns.edu.pe

Abstract

Species from the genera *Bellator* and *Prionotus*, commonly known as "searobins", are marine ray-finned fish that belong to the subfamily Prionotinae. These fish have evolved distinctive morphological features and specialized behaviors that enable them to walk along the seafloor while detecting buried prey. This unique adaptation makes them ideal candidates for studies in evolutionary genetics. However, their phylogenetic relationships remain poorly understood. In this study, we utilized publicly available genomic reads from the GenBank database to assemble the first complete mitochondrial genomes of three searobin species: *Bellator militaris*, *Prionotus alatus*, and *P. stephanophrys*. We conducted a comparative analysis of these mitochondrial genomes, including the first phylogenetic analysis of the Prionotinae based on complete mitogenomic data. The resulting circular contigs measured 16,765 base pairs for *B. militaris*, 16,602 base pairs for *P. alatus*, and 16,896 base pairs for *P. stephanophrys*. The three mitogenomes exhibited a typical vertebrate organization, which includes 13 protein-coding genes, 2 ribosomal RNAs, 22 transfer RNAs, and a putative control region. Notably, *P. stephanophrys* contained an additional tRNA-Leu and an extra non-coding region. A Bayesian phylogenetic analysis grouped all *Bellator* species into a monophyletic clade within Prionotinae, while the sister taxon *Prionotus* formed a separate monophyletic subclade. The findings of this research provide valuable insights into the phylogenetic relationships and evolutionary history of the genera *Bellator* and *Prionotus*. Clarifying the taxonomy of these species may also support future management and conservation efforts for these economically valuable species.

Keywords: Mitogenomics, Phylogeny, Scorpaenoidei, GenBank data mining

1. Introduction

Species from the genera *Bellator* Jordan & Evermann, 1896 and *Prionotus* Lacepède, 1801 (Triglidae), commonly known as "searobins", are the only accepted sister taxa within the subfamily Prionotinae (Portnoy et al. 2017). These species not only support important fisheries across their distribution ranges but also exhibit specialized morphological and biological characteristics. One notable feature is their wing-like pectoral fins, which have three modified lower rays that allow them to "walk" along the sea bottom (Portnoy et al. 2017). In some species, these modified rays are sensitive to chemical and mechanical stimuli, enabling them to detect buried prey (Portnoy et al. 2017; Allard et al. 2024). These unique features make searobins ideal biological models for studies in evolutionary genetics and adaptation (Herbert et al. 2024). Prionotinae species are exclusively found in the Americas (Portnoy et al. 2017). According to the World Register of Marine Species (WoRMS) database (available at https://www.marinespecies.org/index.php), there are currently eight extant *Bellator* species—four from the eastern Pacific and four from the western Atlantic. Additionally, there are 23 extant *Prionotus* species, with 16 located in the western Atlantic and seven in the eastern Pacific.

Taxonomic research on the classification of these species is still limited. Only a few studies have investigated their taxonomy using morphological traits (Imamura, 1996; Richards and Jones, 2002). Additionally, only one study has conducted a molecular phylogenetic analysis based on partial mitochondrial and nuclear gene fragments (Portnoy et al. 2017). While there has been some consistency between the findings of previous morphological studies and molecular phylogenetic analyses, the molecular research conducted by Portnoy et al. (2017) suggests that the subfamily Prionotinae has a monophyletic origin, with the genus *Bellator* nested within a paraphyletic *Prionotus*. The authors concluded that the currently recognized genus *Bellator* should be regarded as a junior synonym of *Prionotus*. These findings highlight

the need for further studies using robust phylogenetic methods and encompassing a broader species coverage to better clarify their taxonomic status.

Due to the limitations of phylogenetic relationships and limited taxonomic resolution derived from morphological characters or partial gene fragments, there is and increasing need for more comprehensive genome-wide data to address complex phylogenetic issues within fish lineages (Miya and Nishida, 2015). In this regard, nucleotide information extracted from complete mitochondrial genomes (mitogenomes) has been effective in clarifying the phylogeny of various fish taxa across different taxonomic levels (Miya and Nishida, 2015). Moreover, the unique characteristics of mitogenomes—such as their fast evolutionary rates, absence of recombination, and high copy numbers—make them an excellent source of powerful molecular markers. These markers are suitable for diverse applications, including accurate species identification, wildlife forensics, environmental DNA analysis, population genetics, and evolutionary studies (Maduna et al. 2022).

Despite the availability of extensive genomic data from next-generation sequencing (NGS) in the GenBank database (Eldem and Balcı, 2024), there are only a few complete mitogenomes from Prionotinae species, and no studies have conducted a phylogenetic analysis based on mitogenomic information to investigate their evolutionary relationships. The objectives of the present work are: a) to assemble the mitochondrial genomes of commercially important searobin species using Sequence Read Archive (SRA) data mined from GenBank; b) to describe the characteristics of these mitogenomes and perform comparative analyses; and c) to investigate the phylogenetic relationships among Triglidae species by analyzing the assembled Prionotinae mitogenomes in conjunction with all available Triglidae mitogenomes in the GenBank database.

107 ## 2. Materials and methods

108 ### 2.1 *Genomic NGS reads mining, mitogenome assembly and annotation*

109 We mined short genomic read sequences (SRS) from publicly available SRA repository data
110 available in the GenBank database. Table 1 presents the metadata for the three searobin
111 specimens including the collection site and date, BioSample and experiment accessions, and
112 the sequencing technology platform. It also outlines the main characteristics of the short read-
113 sequences (pre and post processing) obtained from GenBank. The three searobin specimens
114 were collected by the Smithsonian National Museum of Natural History as part of their project
115 entitled "NOAA Genome Skimming of Marine animals inhabiting the US Exclusive Economic
116 Zone" (BioProject accession PRJNA720393). They consist of *B. militaris* (BioSample
117 SAMN36762063), *P. alatus* (BioSample SAMN36762230), and *P. stephanophrys* (BioSample
118 SAMN31811746).

119 The raw paired-end FASTQ genomic reads underwent quality control using FastQC and were
120 trimmed with BBDuk, as implemented in Geneious Prime v. 2025.0 (Biomatters Ltd.,
121 Auckland, New Zealand). To validate the mitogenomes assembled herein, we used two
122 different approaches ("map to reference" and a hybrid "map to reference/de novo" approach)
123 following Alfaro et al. (2025). The reference sequences information used in the "map to
124 reference" approach is shown in Table 1.

125 For the annotation of the mitochondrial genes, we used the "Annotate and Predict" feature of
126 Geneious Prime by comparing the novel *B. militaris*, *P. alatus*, and *P. stephanophrys*
127 mitogenomes to the complete mitogenomes of closely related species. The start and stop codon
128 positions of the protein-coding genes (PCGs) were confirmed using the Open Reading Frame
129 Finder tool (https://www.ncbi.nlm.nih.gov/orffinder/) using the vertebrate mitochondrial
130 genetic code. The positions of the transfer RNA genes (tRNAs) were corroborated using the
131 tRNAscan-SE tool (Chan and Lowe, 2019). The circular mitogenome maps were produced

132 with Organellar Genome Draw "OGDRAW" version 1.3.1 (Greiner et al. 2019) and then
133 subjected to manual edition with Inkscape macOS version 1.4 (Inkscape Project, 2020).
134
135
136 **Table 1.** Metadata information of the three searobin species, genomic read sequences retrieved
137 from the GenBank database, and mitochondrial genome assembly results.
138

	SPECIES		
	Bellator militaris	*Prionotus alatus*	*Prionotus stephanophrys*
METADATA			
BioSample	SAMN36762063	SAMN36762230	SAMN31811746
Submitted by	Smithsonian National Museum of Natural History	Smithsonian National Museum of Natural History	Smithsonian National Museum of Natural History
BioProject	PRJNA720393	PRJNA720393	PRJNA720393
Collection site	Atlantic Ocean, USA	Atlantic Ocean, USA	Pacific Ocean in El Salvador
Collection date	October 18, 2022	September 17, 2022	December 10, 2010
Coordinates	34.9233 N,75.4535 W	34.6094 N, 75.7052 W	12.7459 N, 89.0088 W
NGS Platform	Illumina NovaSeq 6000	Illumina NovaSeq 6000	Illumina NovaSeq 6000
Strategy	WGS	WGS	WGS
SRA	SRR25465203	SRR25465369	SRR22396965
Gbp	1.9	1.9	1.8
Reads (millions)	12.8	12.5	12.1
ASSEMBLY DATA			
Post trimming (millions)	12.7	12.49	12.07
Mapped to Ref Seq	*B. brachychir* (OP056895)	*P. nudigula* (NC_088019)	*P. evolans (*NC_083024)
Assembled reads	395,513	30,235	24,089
RESULTS			
GenBank accession	BK070174	BK069871	BK071384
Submission date	19 February 2025	25 January 2025	24 April 2025
Mito length bp	16,765	16,602	16,896
A %	26.9	26.3	26.6
C %	29.3	29.6	29.6
G %	17.6	17.3	17.6
T %	26.3	26.8	26.2

139

140 **2.2 *Bayesian phylogenetic analysis***

141 For the Bayesian phylogenetic analysis, we used the 13 PCGs from the three mitogenomes
142 assembled in this work along with those from all Triglidae species available in GenBank,
143 totaling 20 species (11 from Prionotinae and 9 from Triglinae). A Hoplichthyidae species,
144 *Hoplichthys gilberti*, was used as outgroup. The nucleotide sequences from each PCG obtained
145 from each mitogenome were multialigned using MEGA 7 (Kumar et al. 2016) and
146 concatenated with SeaView version 5.0.4 (Guoy et al. 2021). A final matrix of 11,415
147 nucleotides was obtained and used in subsequent phylogenetic analysis. The program
148 jModelTest 2 (Darriba et al. 2012) was used to find the most appropriate model of nucleotide
149 evolution for each one of the 13 PCGs under the corrected Bayesian Information Criterion
150 (BICc). To assess the levels of substitution saturation at single codon positions from each PCG
151 we used Xia´s method implemented in DAMBE7 (Xia, 2018). As a result, we observed that
152 the third codon positions of three PCGs (ATP8, ND3, and ND6) showed signs of saturation,
153 prompting us to conduct the Bayesian analysis with data partitioned by gene and codon position
154 excluding the third codon position from those genes. The Bayesian phylogenetic trees were
155 constructed with MrBayes 3.2.7 (Ronquist et al. 2012) using the CIPRES Science Gateway 3.3
156 server (Miller et al. 2010). Two independent runs of four Markov chains each were executed
157 for 10,000,000 generations, with a sampling frequency of 1,000. We discarded the first 25% of
158 the sampled trees as burn-in. The final consensus phylogenetic tree was visualized using
159 Figtree version 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

160

161 ## 3. Results and discussion

162 **3.1 *Mitochondrial genome assemblies***

163 In this work, we assembled and annotated the first mitochondrial genomes of three important
164 searobin species: the hornet searobin *B. militaris* (found from North Carolina through the Gulf

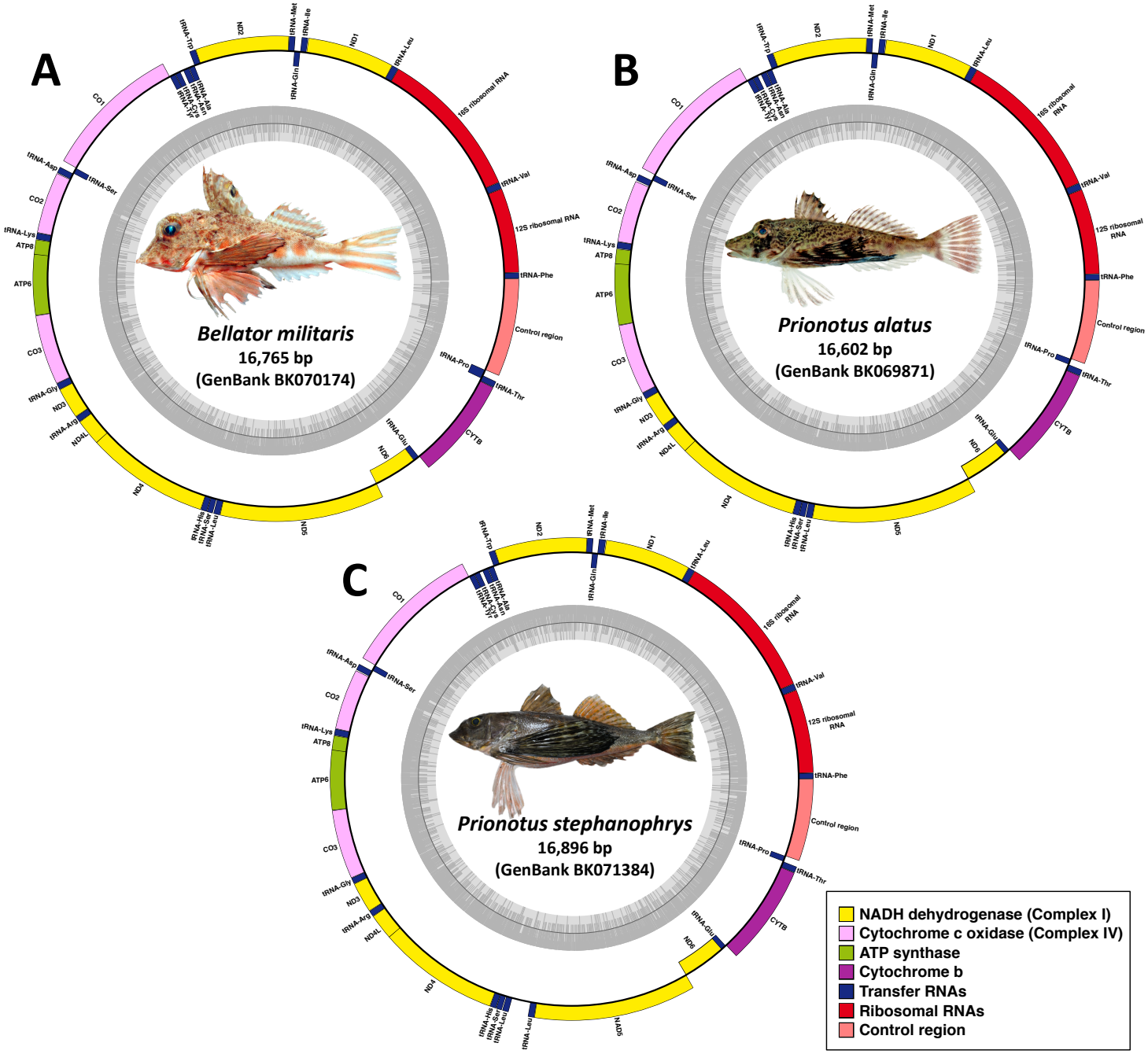
165 of Mexico), the spiny searobin *P. alatus* (occurring from Virginia in the USA to the Campeche
166 Shelf in Mexico), and the lumptail searobin *P. stephanophrys* (found from the Columbia River
167 in Washington in the USA to Ilo in Peru) (Russell et al. 1992; Chirichigno and Cornejo, 2001).
168 There were no differences between the consensus mitogenome contigs obtained from the two
169 different assembly approaches applied herein. The number of total assembled reads and the
170 base frequencies for each mitogenome are shown in Table 1. The mitogenome of *B. militaris*
171 consisted of a single circular contig of 16,765 (GenBank accession BK070174), for *P. alatus*
172 it was 16,602 bp (GenBank accession BK069871), and for *P. stephanophrys* it was 16,896 bp
173 (GenBank accession BK071384) (Table 1, Fig. 1). These lengths fall within the range reported
174 of for other *Bellator* and *Prionotus* mitogenomes, which vary from 16,599 bp in *P. nudigula*
175 (NC_088019) to 16,947 in *B. egretta* (NC_083033).
176
177 **3.2 *Mitochondrial genome characterization***
178 The mitogenomes showed a typical vertebrate organization, consisting of 13 PCGs, 2
179 ribosomal RNAs, 22 transfer RNAs, and a putative control region (Table 2). It is worth noting
180 that the mitogenome of *P. stephanophrys* displayed an additional tRNA-Leu and an extra non-
181 coding region. This extra tRNA-Leu was found on the positive strand, situated between a 220
182 bp non-coding region located downstream of the conserved block ND4/His/Ser/Leu and the
183 ND5 gene (Table 1 and Fig. 1). A similar non-coding region was also identified in the
184 mitogenomes of *P. rubio* (219 bp, Genbank OP056884), *P. tribulus* (219 bp, Genbank
185 OP056925), and *P. evolans* (235 bp, Genbank NC_080324), which are closely related to *P.*
186 *stephanophrys* (Fig. 2). Similarly, non-coding regions of 533 bp and 165 bp were reported
187 within the mitogenome cluster ND4/His/Ser/Leu/ND5 in the freshwater gobioid *Odontobutis*
188 *platycephala* (GenBank accession NC_010199; Ki et al. 2008) and in the threadfin
189 *Eleutheronema rhadinum* (Genbank accession MW845829; Zhong et al. 2021) respectively.

Table 2. Complete mitochondrial annotation and number of amino acids for each gene identified in the mitochondrial genomes of *Bellator militaris* (BM), *Prionotus alatus* (PA), and *P. stephanophrys* (PS). The plus "+" and minus "-" symbols represent the heavy and light strands, respectively. NCR: non-coding region.

Gene	Strand	Location						Size (bp)			Anticodon			Codon						Amino acids		
		BM		PA		PS		BM	PA	PS	BM	PA	PS	BM		PA		PS		PA	PS	BM
		Start	End	Start	End	Start	End							Start	Stop	Start	Stop	Start	Stop			
Phe (F)	+	1	68	1	68	1	68	68	68	68	GAA	GAA	GAA	—	—	—	—	—	—	—	—	—
12S rRNA	+	69	1014	69	1016	69	1017	946	948	949	—	—	—	—	—	—	—	—	—	—	—	—
Val (V)	+	1015	1088	1017	1090	1018	1091	74	74	74	UAC	UAC	UAC	—	—	—	—	—	—	—	—	—
16S rRNA	+	1089	2783	1091	2782	1092	2789	1695	1692	1698	—	—	—	—	—	—	—	—	—	—	—	—
Leu (L)	+	2784	2857	2783	2856	2790	2863	74	74	74	UAA	UAA	UAA	—	—	—	—	—	—	—	—	—
ND1	+	2858	3832	2857	3831	2864	3833	3833	975	975	—	—	—	ATG	TAA	ATG	TAA	ATG	TAG	324	324	324
Ile (I)	+	3837	3906	3836	3905	3843	3912	70	70	70	GAU	GAU	GAU	—	—	—	—	—	—	—	—	—
Gln (Q)	-	3906	3976	3905	3975	3912	3982	71	71	71	UUG	UUG	UUG	—	—	—	—	—	—	—	—	—
Met (M)	+	3976	4045	3975	4043	3982	4051	70	69	70	CAU	CAU	CAU	—	—	—	—	—	—	—	—	—
ND2	+	4046	5091	4044	5089	4052	5097	1046	1046	1046	—	—	—	ATG	TA-	ATG	TA-	ATG	TA-	348	348	348
Trp (W)	+	5092	5162	5090	5161	5098	5168	71	72	71	UCA	UCA	UCA	—	—	—	—	—	—	—	—	—
Ala (A)	-	5164	5232	5163	5231	5170	5238	69	69	69	UGC	UGC	UGC	—	—	—	—	—	—	—	—	—
Asn (N)	-	5234	5306	5233	5305	5440	5312	73	73	73	GUU	GUU	GUU	—	—	—	—	—	—	—	—	—
Cys (C)	-	5345	5409	5344	5408	5351	5415	65	65	65	GCA	GCA	GCA	—	—	—	—	—	—	—	—	—
Tyr (Y)	-	5410	5479	5409	5478	5416	5485	70	70	70	GUA	GUA	GUA	—	—	—	—	—	—	—	—	—
CO1	+	5481	7031	5480	7030	5487	7037	1551	1551	1551	—	—	—	GTG	TAA	GTG	TAA	GTG	TAA	516	516	516
Ser (S)	-	7032	7102	7031	7101	7038	7108	71	71	71	UGA	UGA	UGA	—	—	—	—	—	—	—	—	—
Asp (D)	+	7106	7178	7105	7177	7112	7184	73	73	73	GUC	GUC	GUC	—	—	—	—	—	—	—	—	—
CO2	+	7183	7873	7188	7878	7193	7883	691	691	691	—	—	—	ATG	T--	ATG	T--	ATG	T--	230	230	230

Lys (K)	+	7874	7947	7879	7952	7884	7957	74	74	74	UUU	UUU	UUU	—	—	—	—	—	—	—	—	—
ATP8	+	7949	8116	7956	8123	7959	8126	168	168	168	—	—	—	ATG	TAA	ATG	TAA	ATG	TAA	55	55	55
ATP6	+	8107	8789	8114	8796	8117	8799	683	683	683	—	—	—	ATG	TA-	ATG	TA-	ATG	TA-	227	227	227
CO3	+	8790	9574	8797	9581	8800	9584	785	785	785	—	—	—	ATG	TA-	ATG	TA-	ATG	TA-	261	261	261
Gly (G)	+	9575	9646	9582	9653	9585	9655	72	72	71	UCC	UCC	UCC	—	—	—	—	—	—	—	—	—
ND3	+	9647	9995	9654	10002	9656	10004	349	349	349	—	—	—	ATG	T--	ATG	T--	ATG	T--	116	116	116
Arg (R)	+	9996	10064	10003	10071	10005	10073	69	69	69	UCG	UCG	UCG	—	—	—	—	—	—	—	—	—
ND4L	+	10065	10361	10072	10368	10074	10370	297	297	297	—	—	—	ATG	TAA	ATG	TAA	ATG	TAA	98	98	98
ND4	+	10355	11735	10362	11742	10364	11744	1381	1381	1381	—	—	—	ATG	T--	ATG	T--	ATG	T--	460	460	460
His (H)	+	11736	11804	11743	11812	11745	11813	69	70	69	GUG	GUG	GUG	—	—	—	—	—	—	—	—	—
Ser (S)	+	11805	11871	11813	11879	11814	11880	67	67	67	GCU	GCU	GCU	—	—	—	—	—	—	—	—	—
Leu (L)	+	11880	11952	11887	11959	11887	11959	73	73	73	UAG	UAG	UAG	—	—	—	—	—	—	—	—	—
NCR	+/-	—	—	—	—	11960	12179	—	—	220	—	—	—	—	—	—	—	—	—	—	—	—
Leu (L)	+	—	—	—	—	12180	12252	—	—	73	—	—	UAG	—	—	—	—	—	—	—	—	—
ND5	+	11953	13791	11960	13798	12253	14091	1839	1839	1839	—	—	—	ATG	TAG	ATG	AGA	ATG	TAA	612	612	612
ND6	-	13788	14309	13794	14315	14088	14609	522	522	522	—	—	—	ATG	TAA	ATG	TAG	ATG	TAA	173	173	173
Glu (E)	-	14310	14378	14316	14384	14610	14677	69	69	68	UUC	UUC	UUC	—	—	—	—	—	—	—	—	—
CYTB	+	14386	15526	14388	15528	14683	15823	1141	1141	1141	—	—	—	ATG	T--	ATG	T--	ATG	T--	380	380	380
Thr (T)	+	15527	15598	15529	15600	15824	15895	72	72	72	UGU	UGU	UGU	—	—	—	—	—	—	—	—	—
Pro (P)	-	15599	15668	15601	15670	15896	15965	70	70	70	UGG	UGG	UGG	—	—	—	—	—	—	—	—	—
Control region	+	15669	16765	15671	16602	15696	16896	1097	932	1200	—	—	—	—	—	—	—	—	—	—	—	—

195



196

197 **Fig. 1**. Circular mitochondrial genome organization of A) *Bellator militaris*, B) *Prionotus*
198 *alatus* (specimen photographs kindly provided by R. Robertson; Robertson and van Tassell,
199 2015), and C) *P. stephanophrys* (photo by R. Alfaro). Genes located in inner circle (negative
200 strand) are transcribed in a clockwise direction, while those on the outer circle (positive strand)
201 are transcribed counterclockwise. The innermost circle of the GC content graph indicates a
202 50% threshold, with darker and lighter grey shades representing GC and AT content,

respectively. The different gene types are illustrated with colored bars: ND dehydrogenase (yellow), cytochrome c oxidase (pink), ATP synthase (green), cytochrome b (purple), transfer RNA (blue), and ribosomal RNA (red). The control region is represented by a coral red bar.

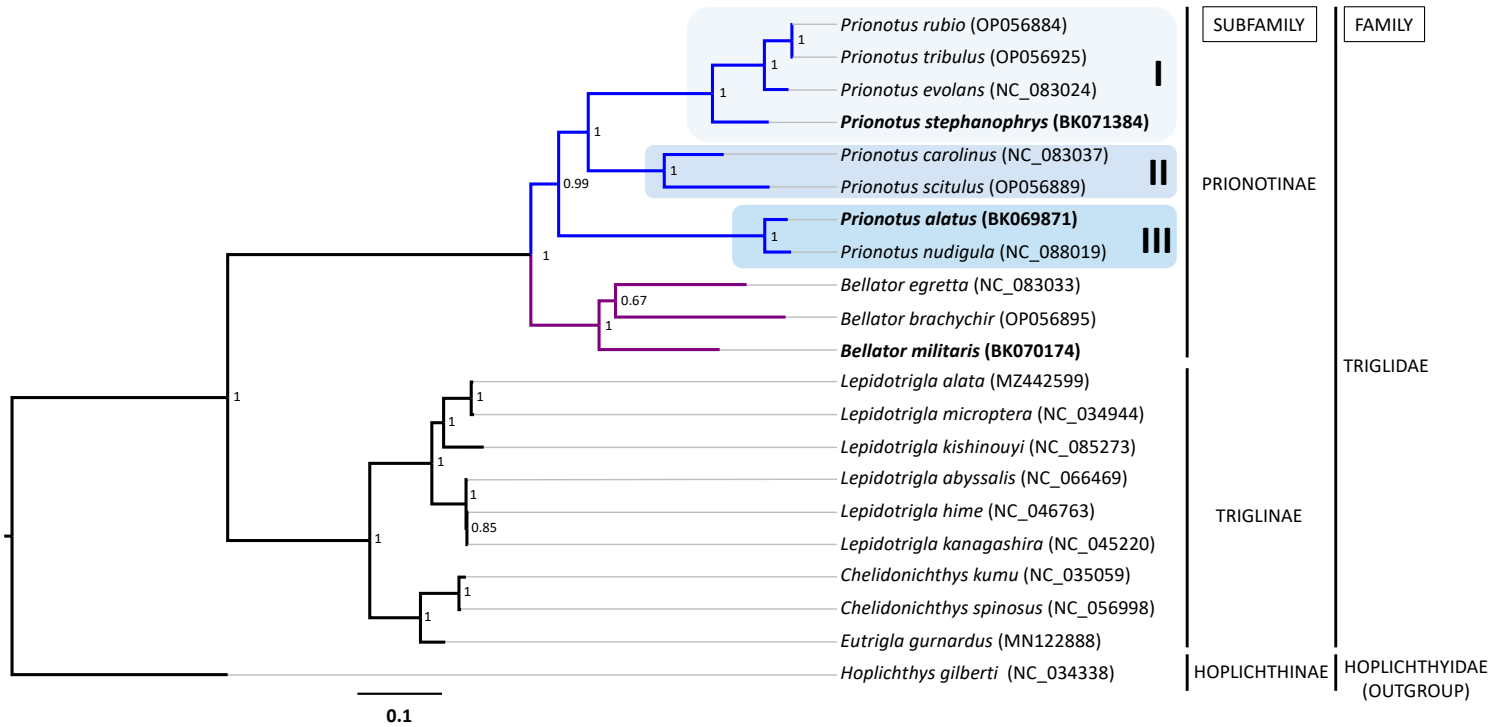
Although the unusual non-coding region observed in the four previously mentioned *Prionotus* species may have resulted from an unknown evolutionary process due to their close phylogenetic relationship (Fig. 2), we cannot entirely dismiss the possibility that it could have an artefactual origin. This is particularly relevant given the presence of a C homopolymer at the 5' end of the non-coding region, which is known to introduce biases in short-read sequencing (Laehnemann et al. 2016).

The sizes of the PCGs ranged from 168 bp (55 amino acids) in the ATP8 gene to 1,839 bp (612 amino acids) in the ND5 gene from the three mitogenomes. All PCGs were encoded in the heavy strand and transcribed counterclockwise, except for the ND6 gene, which was located in the light strand and transcribed in a clockwise direction. Most PCGs initiated with the start codon ATG; however, the CO1 gene started with the alternative codon GTG in all mitogenomes. The use of alternative stop codons was observed only in the ND5 gene of the *B. militaris* mitogenome, which ended with the TAG codon. In the mitogenome of *P. alatus*, two genes, the ND5 and the ND6, ended with the AGA and TAG codons, respectively. In the mitogenome of *P. stephanophrys*, the ND1 gene also used the TAG stop codon (Table 2). In the three mitogenomes, the heavy strand contained the two rRNA and 14 tRNA genes, which included an additional tRNA-Leu found only in *P. stephanophrys*. The light strand contained the remaining 8 tRNA genes in all the reported mitogenomes.

229 **3.3 *Phylogenetic analysis***

230 The Bayesian phylogenetic analysis provided strong to maximum statistical support for the
231 clades Prionotinae and Triglinae (Fig. 2). In our phylogenetic tree, the subfamily Triglinae,
232 which includes 9 species across 3 genera (*Chelidonichthys*, *Eutrigla*, and *Lepidotrigla*), was
233 positioned basally within the family Triglinidae. This finding aligns with part of the molecular
234 phylogeny proposed by Portnoy et al. (2017), which classified the Triglinae group as more
235 basal than Prionotinae. However, this result contrasts with the morphological phylogenetic
236 analysis conducted by Richards and Jones (2002), which identified the Prionotinae as the most
237 ancestral subfamily within Triglidae.

238



239 **Fig. 2.** Bayesian phylogenetic tree constructed based on the concatenation of 13 mitochondrial
240 protein-coding genes from 20 Triglidae species (GenBank accessions are provided in
241 parenthesis). The three species with newly determined mitogenomes, *Bellator militaris*,
242 *Prionotus alatus*, and *P. stephanophrys* (subfamily Prionotinae), are highlighted in bold. The
243 nodes and branches from the *Bellator* and *Prionotus* groups are shown in violet and blue colors

respectively. The three subclades formed within the *Prionotus* group are shaded in sky blue panels and denoted with Roman numbers (I, II, and III). Posterior probabilities are shown at the corresponding nodes. The Gilbert's spiny flathead, *Hoplichthys gilberti*, was used as outgroup.

All species within the genera *Bellator* and *Prionotus* were classified under a major monophyletic Prionotinae clade, supported by a maximum Bayesian posterior probability (BPP) value of 1. Notably, our results revealed that the *Bellator* group, which includes *B. egretta*, *B. brachychir*, and *B. militaris*, has a monophyletic origin and shows a well-supported sister group relationship with *Prionotus* (Fig. 2). This supports the current classification of *Bellator* as a distinct genus, in line with the morphological taxonomic studies conducted by Miller and Richards (1991), Imamura (1996), and Richards and Jones (2002). However, these results contrast with the partial gene-based phylogeny reported by Portnoy et al. (2017), which found *Prionotus* to be paraphyletic within Prionotinae and placed *Bellator* within the genus *Prionotus*.

The analysis of the eight *Prionotus* species revealed three distinct subclades, as illustrated in Fig. 2. These subclades are as follows: subclade I includes *P. rubio*, *P.* tribulus, *P. evolans*, and *P. stephanophrys*; subclade II comprises *P. carolinus* and *P. scitulus*; and subclade III consists of *P. alatus* and *P. nudigula*. Our findings indicate that *P. alatus* and *P. stephanophrys* are positioned in different subclades within the Prionotinae. Specifically, *P. alatus* has a closer evolutionary relationship with *P. nudigula* (subclade III), while *P. stephanophrys* is more closely related to *P. evolans* (subclade I). The estimated divergence levels (using the Kimura two-parameter model) between *P. alatus* and *P. nudigula* is 0.05, whereas the divergence between *P. stephanophrys* and *P. evolans* is 0.12. It is important to note that *P. stephanophrys* is the only Pacific species of *Prionotus* included in our study, and there are still several Atlantic species whose mitogenomes have yet to be sequenced. Therefore, we cannot identify which

Atlantic species serves as the transisthmian sister taxon of *P. stephanophrys*. However, based on findings reported by Portnoy et al. (2017), it appears that *P. longispinosus* is the closest Atlantic relative of *P. stephanophrys* and likely represents its transisthmian sister species.

The shortest interspecific genetic distance (K2P=0.0014; 23 variable sites) was observed between the mitogenomes of *P. rubio* (Genbank accession OP056884, Biosample SAMN29555344) and *P. tribulus* (Genbank accession OP056925, BioSample SAMN29555068), both of which share the same length of 16,891 bp. A further analysis of the CO1 gene sequence from both mitogenomes in the BOLD database identified both sequences as *P. tribulus*. These findings suggest that Biosample SAMN29555344, which is identified as *P. rubio*, may be actually a hybrid of a male *P. rubio* and a female *P. tribulus*. Hybridization between different species of *Prionotus* has been reported both in the wild and under laboratory conditions (McClure and McEachran, 1992; Herbert et al. 2024), indicating that potential hybridization between the sympatric species *P. rubio* and *P. tribulus* is plausible.

4. Conclusion

We conducted the first complete mitogenome-based phylogenetic analysis of species within the Prionotinae subfamily. Our findings indicate a monophyletic origin for both sister taxa, *Bellator* and *Prionotus*, which aligns with some previous studies. However, this monophyletic relationship contradicts the conclusions of Portnoy et al. (2017), who suggested a paraphyletic origin for *Prionotus* and proposed that *Bellator* should be considered a junior synonym of *Prionotus*. It is worth noting that out of the three *Bellator* species included in both our analysis and that of Portnoy et al. (2017), *B. militaris* is the only *Bellator* species analyzed in both studies. Therefore, more extensive molecular phylogenetic analyses, incorporating a wider range of taxa and complete mitogenome sequences of additional *Bellator* and *Prionotus* species,

are necessary to clarify the current genus status of *Bellator* and to validate the proposed monophyletic origin of *Prionotus*.

Funding

Not applicable.

Ethical approval

Not applicable.

Informed consent

Not applicable.

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.

Data availability

Nucleotide sequence data reported are available in the Third Party Annotation Section of the DDBJ/ENA/GenBank databases under the accession number TPA: BK069871, BK070174, and BK071384.

References

Alfaro R, Mantilla G, Marín A (2025) A complete mitochondrial genome-based phylogeny of gooseneck barnacles confirms the sister group relationship between the eastern Pacific *Pollicipes elegans* and the eastern Atlantic *P. pollicipes*. Gene Rep 40, 102238.

Allard CA, Herbert AL, Krueger SP, Liang Q, Walsh BL, Rhyne AL, ... Bellono NW (2024) Evolution of novel sensory organs in fish with legs. Curr Biol 34(19): 4349-4356.

Chan PP, Lowe TM (2019) tRNAscan-SE: Searching for tRNA Genes in Genomic Sequences. Methods Mol Biol 1962: 1-14.

Chirichigno N, Cornejo RM (2001) Catálogo comentado de los peces marinos del Perú. Lima: Instituto del Mar del Perú. 2001: 314 pp.

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and high-performance computing. Nat Methods 9(8): 772.

Eldem V, Balcı MA (2024) Mining NCBI Sequence Read Archive Database: An Untapped Source of Organelle Genomes for Taxonomic and Comparative Genomics Research Divers 16(2): 104.

Gouy M, Tannier E, Comte N, Parsons DP (2021) Seaview version 5: a multiplatform software for multiple sequence alignment, molecular phylogenetic analyses, and tree reconciliation. Multiple sequence alignment: Methods Protoc 241-260.

Greiner S, Lehwark P, Bock R (2019) OrganellarGenomeDRAW (OGDRAW) version 1.3. 1: expanded toolkit for the graphical visualization of organellar genomes. Nucleic Acids Res 47(W1): W59-W64.

342 Herbert AL, Allard CA, McCoy MJ, Wucherpfennig JI, Krueger SP, Chen HI, ...Kingsley DM
343 (2024) Ancient developmental genes underlie evolutionary novelties in walking fish. Curr
344 Biol 34(19): 4339-4348.

345 Imamura H (1996) Phylogeny of the family Platycephalidae and related taxa (Pisces:
346 Scorpaeniformes). Species Divers 1(2): 123-233.

347 Inkscape Project (2020) Inkscape. Retrieved from https://inkscape.org. Accessed 30 January
348 2025.

349 Ki JS, Jung SO, Hwang DS, Lee YM, Lee JS (2008) Unusual mitochondrial genome structure
350 of the freshwater goby *Odontobutis platycephala*: rearrangement of tRNAs and an
351 additional non-coding region. J Fish Biol 73(2): 414-428.

352 Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis
353 version 7.0 for bigger datasets. Mol Biol Evol 33(7): 1870-1874.

354 Laehnemann D, Borkhardt A, McHardy AC (2016) Denoising DNA deep sequencing data—
355 high-throughput sequencing errors and their correction. Brief Bioinform 17(1): 154-179.

356 Maduna SN, Vivian-Smith A, Jónsdóttir ÓDB, Imsland AK, Klütsch CF, Nyman T, ...Hagen
357 SB (2022) Mitogenomics of the suborder Cottoidei (Teleostei: Perciformes): improved
358 assemblies, mitogenome features, phylogeny, and ecological
359 implications. Genomics 114(2): 110297.

360 McClure MR, McEachran JD (1992) Hybridization between *Prionotus alatus* and *P. paralatus*
361 in the Northern Gulf of Mexico (Pisces: Triglidae). Copeia 1992(4): 1039-1046.

362 Miller GC, Richards WJ (1991) Revision of the western Atlantic and eastern Pacific genus
363 *Bellator* (Pisces: Triglidae). Bull Mar Sci 48(3): 635-656.

364 Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for
365 inference of large phylogenetic trees. In: 2010 Gateway Computing Environments
366 Workshop (GCE) New Orleans, LA, USA, 2010 (pp. 1-8). doi:
367 10.1109/GCE.2010.5676129.

368 Miya M, Nishida M (2015) The mitogenomic contributions to molecular phylogenetics and
369 evolution of fishes: a 15-year retrospect. Ichthyol Res 62: 29-71.

370 Portnoy DS, Willis SC, Hunt E, Swift DG, Gold JR, Conway K.W (2017) Molecular
371 phylogenetics of New World searobins (Triglidae; Prionotinae). Mol Phylogenet Evol 107:
372 382-387.

373 Richards WJ, Jones DL (2002) Preliminary classification of the gurnards (Triglidae:
374 Scorpaeniformes). Mar Freshw Res 53(2): 274-282.

375 Robertson DR, Van Tassell J (2015) Shorefishes of the Greater Caribbean: online information
376 system. Version 3.0 Smithsonian Tropical Research Institute, Balboa, Panamá.

377 Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, ...Huelsenbeck JP
378 (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a
379 large model space. Syst Biol 61(3): 539-542.

380 Russell M, Grace M, Gutherz EJ (1992) Field Guide to the searobins (*Prionotus* and *Bellator*)
381 in the western North Atlantic. NOAA Technical Report NMFS 107. U.S. Department of
382 Commerce. March 1992.

383 Xia X (2018) DAMBE7: New and improved tools for data analysis in molecular biology and
384 evolution. Mol Biol Evol 35(6): 1550-1552.

385 Zhong L, Wang M, Li D, Tang S, Chen X (2021) Mitochondrial genome of *Eleutheronema*
386 *rhadinum* with an additional non-coding region and novel insights into the
387 phylogenetics. Front Mar Sci 8: 746598.