Complete mitochondrial DNA sequence analysis of Bluespotted seabream, *Pagrus caeruleostictus* (Perciformes: Sparidae)

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Abstract

Background Bluespotted seabream, *Pagrus caeruleostictus* is a benthopelagic marine species found in tropical regions and a member of the family Sparidae and genus *Pagrus*. In this article, a mitochondrial DNA sequence by high-throughput technique, Illumina Hiseq, was carried out on muscle of the above species and determined the complete mitogenome. Samples were obtained from species collected from the coast of Ghana, West Africa. Results The complete mitochondrial DNA sequence was 16,653 bases pairs in length (GenBank Accession number: MN319701) and comprises of 37 genes; 22 transfer RNA genes, 2 ribosomal RNA genes, 13 protein-coding genes as well as a control region as in a typical vertebrate mitochondrial DNA gene. The phylogenetic analysis showed *P. caeruleostictus* clustered with the clade of *P. auriga*. Conclusion This research will serve as the foundation for molecular genetic studies of Ghanaian fish species. Moreover, it will contribute to the phylogenetics of class Actinopterygii, order Spariformes, family Sparidae and genus *Pagrus*.

Background

Bluespotted seabream, *Pagrus caeruleostictus* is a bony fish species that belong to class Actinopterygii in the order Perciformes of the family Sparidae and genus *Pagrus*. Sparidae is family of bony fishes and according to FishBase (www.fishbase.se) and Integrated Taxonomic Information System (www.itis.gov/), 38 genera and 159 species exist within this family including genus *Pagrus*. However, Nelson and his colleagues reported 37 genera with 148 species within family Sparidae [1] whiles Parenti (2019) stated 426 present in family Sparidae consisting of 166 valid species in 39 genera, 5 subfamilies and 260 synonyms. Statistically, more than one third of this species are endemic along the coast of South Africa [2]. A total of 6 species are classified under genus *Pagrus*; *P. caeruleostictus* (Valenciennes, 1830), *P. major* (Temminck and Schlegel, 1843), *P. pagrus* (Linnaeus, 1758), *P. africanus* (Akazaki, 1962), *P. auratus* (Forster, 1801) and *P. auriga* (Valenciennes, 1843) [3,4 www.fishbase.org]. Apart from *P. major* and *P. auratus*, which are native to the Northwest Pacific and Indo-Pacific respectively, [3; www.fishbase.org], the remaining species are distributed in the Atlantic Ocean and the Mediterranean Sea. The Bluespotted seabream, *P. caeruleostictus* is a localized species of the eastern Atlantic Ocean, from Portugal to Angola, including the area around the Canaries, and also in warmer parts of the Mediterranean (Figure 1), aside from the Black Sea [4, 5 <https://www.fishbase.in/summary/4540>]. Conversely, in 2015, Yongxia and his colleagues,

discovered *P. caeruleostictus* in Chinese coastal waters precisely in the South China Sea. They associated the presence of these species to anthropogenic activities due to the low possibility to have expanded naturally from the eastern Atlantic to the South China Sea coupled with its absence in the Indian or Pacific Oceans [6].

As a high economic species, *P. caeruleostictus* is a benthopelagic marine species found in tropical regions. Commonly, they inhabit in rubble and sandy areas with a depth range of 15-50 m. The matured generation usually inhabit in the deeper part of this range while the young individuals can be located in the inshore areas. At full maturity, *P. caeruleostictus* attains an average standard length and a weight of 60 cm and 5 kg respectively. After two years of spawning, the young ones become sexually matured. Due to their oceanodromous nature, gravid fish migrate to shallow parts of the ocean with soft bottoms to spawn. Bivalves, crustaceans and other finfish are the principal feeds *P. caeruleostictus* survive on [2, 7]. This

Figure 1: Global geographic distribution of *P. caeruleostictus* (FAO Fisheries and Aquaculture Department website. Cited on July 16th, 2019 <https://www.aquamaps.org/>)

Over the years, the mitochondrial genome sequence and analysis has evolved and keeps growing day-after-day due to increasing technological advancement in molecular biology [8–10]. Moreover, it has become less expensive and has gained appreciable recognition in the field of molecular biology. Cytochrome c oxidase I (*Cox1*) gene has ability of rapid mutation and its sequences remain unchanged even within closely related species [11]. In view of this, several researchers have used *Cox1* gene as a marker for species DNA barcoding [12–14]. Different studies have reported that the intraspecific variation of *Cox1* barcodes is small and highly distinguished from interspecific variation [18–20]. Additionally, complete mitogenome is utilized as a better marker in the study of phylogenetic analysis because it provides better phylogenetic resolution with high accuracy relative to traditional method of using single mitochondrial gene markers, for example, the control region (*CR*) and cytochrome b (*cyt b*) [9, 18–20] and can also distinguish intraspecific species with. The control region is also known as D-loop, relating to the structure formed when mitochondrial DNA replicates. This is a non-coding sequences region which functions as replication and transcription regulator within a mitogenome.

This region shows high mutation rate and therefore acts as a dominant region to determine intra-species variations [21].

Even though mitochondrial genome sequence has evolved rapidly and has become an ubiquitous technique in evolutionary studies of species, until now no molecular-based evolution studies has been conducted on *P. caeruleostictus* hence the need for this research. To understand the evolutionary and systematics of *P. caeruleostictus* within the family Sparidae and genus *Pagrus*, the complete mitogenome sequence was determined with high-throughput sequencing technology (Illumina Hiseq) and studied. Additionally, a phylogenetic analysis based on the *cox1* data was carried out on this species together with other species from Sparidae to investigate the phylogenetic position of *P. caeruleostictus* within the family Sparidae. The findings of this research outlined in this article will facilitate future investigations on population genetics studies and phylogenetic relationships of species within the Sparidae family and *Pagrus* genus.

Results

Mitogenome structure

The complete mitogenome of *P. caeruleostictus* was determined in this study. This mitogenome contains 37 genes (Table 1) which include 22 transfer RNA genes, 2 ribosomal RNA genes, 13 protein-coding genes in addition to a control region according to a characteristic vertebrate mitochondrial DNA gene [10, 22].

Table 1: 37 genes present in *P. caeruleostictus*

Name	Position	Strand	Length (bp)	Start codon	Stop codon	Anticodon	Intergenic nucleotide
trnF(ttc)	1-68	N	68			GAA	
rrnS	69-1022	N	954				-1
trnV(gta)	1022- 1093	N	72			TAC	39
rrnL	1133- 2790	N	1658				
trnL2(tta)	2791- 2863	N	73			TAA	
nad1	2864- 3838	N	975	ATG	TAA		5
trnI(atc)	3844- 3913	N	70			GAT	-1
trnQ(caa)	3913- 3983	J	71			TTG	-1
trnM(atg)	3983- 4052	N	70			CAT	
nad2	4053- 5099	N	1047	ATG	TAA		-1
trnW(tga)	5099- 5168	N	70			TCA	1
trnA(gca)	5170- 5238	J	69			TGC	1
trnN(aac)	5240- 5313	J	74			GTT	4
trnC(tgc)	5349- 5414	J	66			GCA	
trnY(tac)	5415- 5484	J	70			GTA	1

cox1	5486- 7048	N	1563	GTG	AGG	-9
trnS2(tca)	7040- 7110	J	71		TGA	2
trnD(gac)	7113- 7185	N	73		GTC	8
cox2	7194- 7884	N	691	ATG	T(AA)	
trnK(aaa)	7885- 7958	N	74		TTT	1
atp8	7960- 8127	N	168	ATG	TAA	-10
atp6	8118- 8801	N	684	ATG	TAA	-1
cox3	8801- 9586	N	786	ATG	TAA	-1
trnG(gga)	9586- 9657	N	72		TCC	
nad3	9658- 10008	N	351	ATG	TAG	-2
trnR(cga)	10007- 10077	N	71		TCG	
nad4l	10078- 10374	N	297	ATG	TAA	-7
nad4	10368- 11748	N	1381	ATG	T(AA)	
trnH(cac)	11749- 11817	N	69		GTG	
trnS1(agg)	11818- 11885	N	68		GCT	6

trnL1(cta)	11892- 11964	N	73			TAG	
nad5	11965- 13803	N	1839	ATG	TAA		-4
nad6	13800- 14321	J	522	ATG	TAA		
trnE(gaa)	14322- 14390	J	69			TTC	4
Cob	14395- 15535	N	1141	ATG	T(AA)		
trnT(aca)	15536- 15609	N	74			TGT	-1
trnP(cca)	15609- 15678	J	70			TGG	

The circle genome is 16,653 bp in length (Figure 2) which falls within the range of a typical vertebrate with the base composition of 27.92 % A, 28.67 % C, 16.47 % G, and 26.94% T [23]. The complete genome skewed to AT at 0.018 and to GC at -0.27.

Figure 2: The mitogenome map of *P. caeruleostictus*

The mitochondrial gene arrangement of *P. caeruleostictus* indicated that all genes were encoded on heavy strand (OH) except for NADH dehydrogenase subunit 6 (ND6) and eight tRNA genes (tRNA-Gln, tRNA-Ala, tRNA-Asn, tRNA-Cys, tRNA-Tyr, tRNA-Ser, tRNA-Glu and tRNA-Pro) which occurred on the light strand (OL). This is in confirmation with other previous studies on vertebrate circular mitogenome sequence [24-27]. A total of thirteen (13) complete stop codons (TAA, TAA, AGG, TAA, TAA, TAA, TAG, TAA, TAA and TAA) and three (3) incomplete stop codons; T(AA), T(AA), and T(AA) were used in the protein-coding

genes. Moreover, it consisted of 22 anticodons; GAA, TAC, TAA, GAT, TTG, CAT, TCA, TGC, GTT, GCA, GTA, TGA, GTC, TTT, TCC, TCG, GTG, GCT, TAG, TTC, TGT, and TGG.

Features of 22 tRNA genes

During translation, making a protein from mRNA template, tRNA functions as a transporter of nucleotide from mRNA to amino acids. It functions as the bridge between mRNA and amino acid sequence to the ribosome where proteins are constructed [28]. The mitochondrial genome contained 22 tRNA genes (Table 2). The tRNA genes ranged in size from 65 to 75 nucleotides, which allowed the encoded tRNA to fold into the characteristic clover-leaf secondary structure [29].

Table 2: Present tRNA in the complete mitochondrion DNA of *P. caeruleostictus*

Product	Location	Length	Atypical pairings
tRNA-Phe	1-68	68	
tRNA-Val	1022-1093	71	
tRNA-Leu	2791-2863	72	UAA
tRNA-Ile	3844-3913	69	
tRNA-Gln (complement)	3913-3983	70	
tRNA-Met	3983-4052	69	
tRNA-Trp	5099-5168	70	
tRNA-Ala(complement)	5170-5238	68	
tRNA-Asn (complement)	5240-5313	73	
tRNA-Cys (complement)	5349-5414	65	
tRNA-Tyr (complement)	5415-5484	69	
tRNA-Ser	7040-7110	70	UGA
tRNA-Asp	7113-7185	72	
tRNA-Lys	7885-7958	73	
tRNA-Gly	9586-9657	71	
tRNA-Arg	10007-10077	70	
tRNA-His	11749-11817	68	
tRNA-Ser	11818-11885	67	GCU
tRNA-Leu	11892-11964	72	UAG
tRNA-Glu (complement)	14322-14390	68	
tRNA-Thr	15536-15609	73	
tRNA-Pro (complement)	15609-15678	69	

Features of rRNA

Ribosomal RNA (rRNA) combines with enzyme and proteins to form complex structure within the cytoplasm to form ribosomes which serve as protein synthesis site. These complex structures travel along the mRNA molecule during translation and facilitate the assembly of amino acids to form a polypeptide chain [30]. The ribosomal RNA (rRNA)

identified within the genome were 12S rRNA located at 69 bp to 1021 bp consisting 952 bp and 16S rRNA which also stationed at 1094 bp to 2790 bp and made up of 1696 bp.

Features of protein-coding genes

As is other vertebrate, a total of 13 protein-coding genes were present in the mitogenome of *P. caeruleostictus*. Within the obtained 13 protein-coding genes, 4 of them were paired, located adjacently to each other; *atp8-atp6*, *atp6-cox3*, *nad4l-nad4*, and *nad5-nad6*. Apart from *atp6-cox3*, all pairs had some overlap genes between them. The length of overlap between these 3 cases, *atp8-atp6*, *nad4l-nad4*, and *nad5-nad6* were 9 bp, 6 bp and 3 bp respectively (Table 3). All protein genes had an ATG initiation codon except for the cytochrome oxidase subunit I (*cox1*) gene which had GTG as its initiation codon.

Table 3: Characteristics of the protein-coding genes

Region	A%	C%	G%	T%	A+T%	G+C%	AT skew	GC skew
<i>Rrns</i>	30.19	25.79	22.54	21.49	51.68	48.32	0.168	-0.067
<i>Rrnl</i>	33.78	24.43	20.99	20.81	54.58	45.42	0.238	-0.076
<i>nad1</i>	25.33	32.1	14.87	27.69	53.03	46.97	-0.044	-0.367
<i>nad2</i>	25.69	36.1	12.51	25.69	51.38	48.62	0	-0.485
<i>cox1</i>	24.12	26.36	18.43	31.09	55.21	44.79	-0.126	-0.177
<i>cox2</i>	27.79	27.5	16.79	27.93	55.72	44.28	-0.003	-0.242
<i>atp8</i>	28.57	35.12	12.5	23.81	52.38	47.62	0.091	-0.475
<i>atp6</i>	23.1	30.85	13.45	32.6	55.7	44.3	-0.171	-0.393
<i>cox3</i>	25.95	29.9	16.79	27.35	53.31	46.69	-0.026	-0.281
<i>nad3</i>	16.66	34.19	15.95	30.2	49.86	50.14	-0.211	-0.364
<i>nad4l</i>	21.21	34.01	15.49	29.29	50.51	49.49	-0.16	-0.374
<i>nad4</i>	26.29	31.21	13.9	28.6	54.89	45.11	-0.042	-0.384
<i>nad5</i>	28.98	30.29	13.32	27.41	56.39	43.61	0.028	-0.389
<i>nad6</i>	18.39	14.75	27.97	38.89	57.28	42.72	-0.358	-0.309
<i>Cob</i>	25.07	30.24	14.99	29.71	54.78	45.22	-0.085	-0.337

Features of D- loop

The D-loop of *P. caeruleostictus* was positioned at the end of tRNA-Pro (15679 to 16653) with a length of 974 bp. This correlates with other literature on teleost complete mitochondrial sequence [10, 31–33]. However, according to available literature, the D-loop in some vertebrates are located between tRNA-Pro and tRNA-Phe [34–36]. Conserved Sequence Blocks (CSBs) and High Variational Blocks (HVBs) were the findings of the multiple sequence alignment of the D-loops using *D. rerio* (GenBank Accession number: NC_002333.2) as the reference.

Table 4: Sequence of conserved regions of *P. caeruleostictus* and other teleost with *D. rerio* as reference.

Element	Species	Sequence									
CSB-1	<i>Danio rerio</i>	C	A	A	A	C	C	C	C	C	T
	<i>Pagrus caeruleotictus</i>	T	.	.	.	.	.	.	.	.	C
	<i>Oryzias curvinotus</i>	.	.	.	.	.	.	.	.	.	C
	<i>Gobiopterus lacustris</i>	.	.	.	.	.	.	.	.	.	C
	<i>Brycon henni</i>	T	.	.	.	.	.	.	.	.	C
	<i>Trichiurus lepturus</i>	T	.	.	.	.	.	.	.	.	C
	<i>Lutjanus russellii</i>	A	.	.	.	.	.	.	.	.	C
CSB-2	<i>Danio rerio</i>	G	G	G	A	C	A	T	C	A	A
	<i>Pagrus caeruleotictus</i>	.	.	.	.	.	.	A	G	.	.
	<i>Oryzias curvinotus</i>	.	.	.	.	.	.	A	A	.	.
	<i>Gobiopterus lacustris</i>	.	.	.	.	.	.	A	T	.	.
	<i>Brycon henni</i>	.	.	.	.	.	.	A	A	.	.
	<i>Trichiurus lepturus</i>	A	.	.	.	.	.	G	T	G	.
	<i>Lutjanus russellii</i>	.	.	.	.	.	.	A	T	.	.
CSB-3	<i>Danio rerio</i>	G	C	A	T	T	T	C	A	T	A
	<i>Pagrus caeruleotictus</i>	A	.	.	.	.	.	.	.	C	.
	<i>Oryzias curvinotus</i>	.	.	.	.	.	.	.	.	C	.
	<i>Gobiopterus lacustris</i>	.	.	.	.	.	.	.	.	C	.
	<i>Brycon henni</i>	A	.	.	.	.	.	G	G	.	C
	<i>Trichiurus lepturus</i>	A	.	.	.	.	.	.	.	G	.
	<i>Lutjanus russellii</i>	A	.	.	.	.	.	.	.	G	.

Note: Dot represent the same nucleotide as the reference species, *D. rerio*

Phylogenetic analysis

In order to ascertain the evolutionary features of *P. caeruleostictus* mitogenomes, complete mitochondrial and *Cox1* sequences of other species from Order Perciformes were downloaded in FASTA format from the database of National Center for Biotechnology Information (NCBI-Blast) (<https://blast.ncbi.nlm.nih.gov>) and Barcode of Life Database

(BOLD) (<http://www.barcodinglife.org>) systems respectively, these database contains comprehensive data on species gene sequence for molecular phylogenetic analysis [37]. Using MEGA version 6.0v, nucleic acid sequences were aligned using ClustalW with the Alignment Explorer tool and constructed a neighbor-joining trees at the order-level [38]. As shown in Figure 3 and 4 below, *P. caeruleostictus* clustered closest with *P. auriga* as supported by a high bootstrap value (99%) in both cases.

Figure 3: Neighbor-joining phylogenetic tree based on *Cox1* sequences.

Figure 4: Neighbor-joining phylogenetic tree based on complete mitochondrial sequences with *L. fulviflamma* as the out group.

Discussion

Illumina HiSeq technology utilized during the sequencing is one of high-throughput sequencing (HTS) methods, also known as next-generation sequencing. Successfully, this technique was able to sequence the complete mitochondrial DNA from the specimen, obtaining a total read of 16,653 bp length. This is similar to the mitogenomic length of other vertebrates including teleost [22, 39–41]. A major disadvantage of this method is the price involved due to the use of sophisticated equipment and highly skilled personnel. Furthermore, the cost for sequencing is determined by the types of libraries desired, either single-end read, paired-end read or mate-pair libraries as well as the number of cycles requested [42].

The MitoAnnotation detected the presence of 2 tRNA-Leu and 2 tRNA-Ser (Table 2). The same result was obtained in a study on *Lutjanus russelli* [34]. Both species belong to the Order Perciformes.

The result of the D-loop multiple sequence alignment depicts the presence of both Conserved Sequence Blocks (CSBs) (Table 4) and High Variation Blocks (HVBs). Furthermore, an extended termination associated sequence domain (ETAS), a central conserved sequence block domain (CSB-D, CSB-E and CSB-F) and a conserved sequence block domain (CSB-1, CSB-2 and CSB-3) were identified. This result is same as the result of

the other teleost compared with [10, 31, 34, 39, 43, 44]. Additionally, the non-coding region with a light strand (L-strand), was located amongst a cluster of tRNA genes (the WANCY region), that is between the tRNA- Asn and tRNA-Cys genes as found in a most vertebrate [45]. This consisted of 31 bp in length (TCTTTCCCCCGCCCTGGGGGCGGAAAGGCG).

The resultant phylogenies (Figure 3 and 4) indicated *P. caeruleostictus* shares a close relationship with *P. auriga*. Additionally, in Figure 4, *Pagrus* species together with *Dentex* species formed a clade based on complete mitogenome representing these genera evolved from a common ancestor. This result confirms the findings on phylogenetic relationship between *Pagrus* species and *Dentex* species[46–48]. With the formation of a monophyletic group between both genera, authors hypothesize a degree of resemblance not only morphologically but on the molecular level. In view on this, further molecular based studies within these genera are recommended since both genera belong to the same family, Sparidae.

Conclusions

In the current study, muscle of bluespotted seabream, *P. caeruleostictus*, was sampled from the landings of commercial boats from the coast of Ghana. Muscle sample was subjected to high-throughput sequencing (Illumina Hiseq) and successfully acquired the complete mitogenome of this species. This article throws more light on the taxonomy, systematics, global distribution and evolution based on complete mitogenome phylogenetics analysis of *P. caeruleostictus*. This research will contribute to the phylogenetics of class Actinopterygii, order Perciformes, family Sparidae and genus *Pagrus*. As a novel published paper on the complete mitogenome of *P. caeruleostictus*, it lays a foundation for further research and a better understanding on the molecular genetics of *P. caeruleostictus*. Furthermore, it illustrates the beneficial effects of an increased taxon sampling coupled with the use of more realistic nucleotide and amino acid substitution models for the reconstruction of animal phylogeny.

Materials And Method

Specimen sampling and sampling site

In the present study, *P. caeruleostictus* specimen was sampled from the coast of Sekondi-Takoradi, Ghana, West Africa in December 2018. Geographically, samples were taken from the coordinates 4° 54' 59" N, 1° 42' 06" W from the landings of a commercial fishing boat. A total of 18 samples were collected and next generation sequencing was carried out on one to obtain the complete mitogenome.

The muscle was used to extract DNA [49]. The specimen was stored in absolute ethanol whiles been transported to the laboratory where it was stored in -20 °C refrigerator upon arrival until used.

DNA Extraction, Amplification, and Sequencing

Total DNA was extracted using the method of proteinase K digestion from fish muscle. The DNA quality test was carried out to determine the concentration and purity using Thermo Scientific NanoDrop 2000. The integrity was detected by agarose gel electrophoresis and Agilent Bioanalyzer (Agilent Technologies, CA, USA). Covaris machine was used to interrupt DNA and for DNA fragmentation. Using a combination of 3'-5' exonuclease and polymerase, DNA fragments were cleaved with a protruding end for double end repair. A single base "A" was introduced at the 3' end, and the 3' terminus of the linker containing a single base "T", enabling the DNA fragments and linkers to connect through "A" complementary pairing. DNA fragments and linker were attached to ligase. Enriched DNA fragments with ligated ends and simultaneously amplified DNA libraries were selected after library PCR amplification and the quality was inspected using Agilent Bioanalyzer to detects library size and quantifies total library concentration. Illumina Hiseq was selected for sequencing. The single-strand library was used as a template for bridge PCR amplification and sequencing while sequencing.

The complete mitochondrion DNA sequence was submitted to the GenBank database and can be accessed via the accession number MN319701.

Sequence assembling and Annotation

Multiple sequence analysis was carried out on the D-loop region of *P. caeruleostictus* together with D-loop regions from other species; *Trichiurus nanhaiensis*, *Oryzias curvinotus*, *Lutjanus russellii*, *Gobiopterus lacustris* and *Brycon henna* using MEGA version 6.0v [38].

Declarations

Ethics approval and consent to participate

The fish specimen under study in this article was obtained from commercial boats and is not involve endangered or protected species (www.iucnredlist.org). Samples collection and maintenance were performed in strict accordance with the recommendations of the government of Ghana on Conservation, Management and Utilization of Ghana's wildlife resources. No ethics approval was required for the public sequence data used.

Consent for publication

Not applicable

Availability of data and materials

The article contains all of the datasets supporting the results whiles the sequence obtained from the species under study was submitted and assigned the accession number MN319701 on GenBank. Supplementary files are uploaded together with the manuscript.

1. Additional file 1: Sequence file
2. Additional file 2: Figure S1. Map of mitogenome map (<http://mitofish.aori.u-tokyo.ac.jp>)
3. Additional file 3: Figure S2. Multiple sequence analysis of *caeruleostictus* with both *Pagrus* species and other teleost based on Cox1 sequences (MEGA file)
4. Additional file 4: Figure S3. Multiple sequence analysis of *caeruleostictus* with other teleost based on complete mitochondrial DNA sequences (MEGA file)
5. Additional file 5: Table S1. Multiple sequence analysis of *caeruleostictus* and other teleost with *D. rerio* as reference for conserved sequence block (MEGA file)
6. Additional file 6: Table S2. List of species examined in this study with GenBank accession numbers

Competing interests

The authors report no competing interest.

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Authors' contributions

ZW, GA, and YG conceived and designed the study. CAL collected the sample. GA, ZW and FKAK carried out the data analysis, molecular work and wrote the article. ZW supervised this study.

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Figures

Figure 1

Global geographic distribution of *P. caeruleostictus* (FAO Fisheries and Aquaculture Department website. Cited on July 16th, 2019 <https://www.aquamaps.org/>) 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.

Figure 2

The mitogenome map of *P. caeruleostictus*

Figure 3

Neighbor-joining phylogenetic tree based on Cox1 sequences.

Figure 4

Neighbor-joining phylogenetic tree based on complete mitochondrial sequences with *L. fulviflamma* as the out group.

Supplementary Files

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- [4.Multiplesequenceanalysisbasedcompletemitogenome.mas](#)
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- [3.MultiplesequenceanalysisbasedonCox1.mas](#)
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