The complete mitochondrial genome of the zebra seabream *Diplodus cervinus* (Perciformes, Sparidae) from the Mediterranean Sea

David Osca\textsuperscript{a}*, Luigi Caputi\textsuperscript{b,***}, Valentina Tanduo\textsuperscript{a}, Rosa Maria Sepe\textsuperscript{b}, Assunta Libert\textsuperscript{b}, Francesco Tiralongo\textsuperscript{c,d}, Iolanda Venuti\textsuperscript{e}, Marina Ceruso\textsuperscript{e}, Fabio Crocetta\textsuperscript{e}, Paolo Sordino\textsuperscript{f,} and Tiziana Pepe\textsuperscript{e}

\textsuperscript{a}Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Naples, Italy; \textsuperscript{b}Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Naples, Italy; \textsuperscript{c}Department of Biological, Geographical and Environmental Sciences, University of Catania, Italy; \textsuperscript{d}Scientific Organization for Research and Conservation of Marine Biodiversity, Ente Fauna Marina Mediterranea, Avola, Italy; \textsuperscript{e}Department of Veterinary Medicine and Animal Production, University of Naples "Federico II", Naples, Italy; \textsuperscript{f}Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Sicily Marine Centre, Messina, Italy

**ABSTRACT**

The complete nucleotide sequence of the mitochondrial (mt) genome of the demersal zebra seabream *Diplodus cervinus* (Lowe, 1838) was determined for the first time. The double stranded circular molecule is 16,559 base pairs (bp) in length and encodes for the typical 37 metazoan mitochondrial genes, and 2 non-coding regions (D-loop and L-origin). The gene arrangement of the *D. cervinus* mt genome follows the usual one for fishes. The nucleotide sequences of the mt protein coding and ribosomal genes of *D. cervinus* mt genome were aligned with orthologous sequences from representatives of the Sparidae family and phylogenetic relationships were inferred. Maximum likelihood analyses placed *D. cervinus* as a sister species of *Diplodus sargus* (LINNAEUS, 1758).

**CONTACT** Paolo Sordino\textsuperscript{g,} paolo.sordino@szn.it Stazione Zoologica Anton Dohrn, Sicily Marine Centre, Via Consolare Pompea 29, Messina 98167, Italy. 
\textsuperscript{g}Current address: Department of Biology, Faculty of Marine Sciences, Instituto Universitario de Estudios Ambientales y Recursos Naturales i-UNAT, University of Las Palmas de Gran Canaria, Canary Islands, Spain.

**ARTICLE HISTORY**

Received 9 June 2022 
Accepted 3 November 2022

**KEYWORDS** Mitogenome; gene order; base composition; demersal fishes; phylogeny

The zebra seabream *Diplodus cervinus* (Lowe, 1838) is a gregarious demersal marine fish of the family Sparidae Rafinesque, 1818, usually living in groups of 4–5 individuals over rocky bottoms up to 80 m depth, although it can be also found on muddy bottoms up to 300 m (Bauchot and Hureau 1986; Pajuelo, Lorenzo, and Domínguez-Seoane 2003). This thermophilic species is distributed in the eastern Atlantic Ocean from the Bay of Biscay to Cape Verde Islands, from Angola to South Africa, and around Madeira and Canary Islands, but it also occurs in the Mediterranean Sea, where it is recently widening its distribution (Bauchot and Hureau 1986; Pajuelo, Lorenzo, and Domínguez-Seoane 2003; Tiralongo et al. 2020). It reaches ~55 cm in length and 2.7 kg in weight, and it is a species of interest in small scale fisheries throughout its range of distribution, with scattered attempts to rear it using aquaculture techniques (Bauchot and Hureau 1986; IGFA 2001). This species, together with other ones of the genus *Diplodus*, represents a candidate with great potential for aquaculture, due to its market price and good adaptability to farming environment (Coutinho et al. 2016). In some coastal areas, like those of Canary Islands, this demersal species covers a relevant ecological role (Pajuelo, Lorenzo, Domínguez, et al. 2003).

The *D. cervinus* specimen analyzed in this study was caught for research as a dead specimen from the fisherman’s market (36.7406 N, 15.1193 E), where it was supplied directly from local fishermen. Thus, it did not undergo any manipulation or experimentation in the laboratory. Its usage for scientific purposes is not included in the Article 2 of the Italian Legislative Decree n. 26 of 4 March 2014, national transposition of the European Directive 2010/63/UE. Complete mt genome sequence of *D. cervinus* and its annotation is presented here for the first time. The specimen was caught with trammel nets (1.5 m t depth) on a rocky bottom off Marzamemi (Syracuse, Sicily, Italy; Ionian Sea, Mediterranean Sea, ~36.7480 N, 15.1129 E) on 18 April 2020. It was morphologically identified based on species-specific diagnostic characters and subsequently deposited in the Darwin Dohrn Museum of the Stazione Zoologica Anton Dohrn of Naples (http://193.205.231.138/ZooColl/HTML/index.php, curator Andrea Travaglini, andrea.travaglini@szn.it) with the code number SZN-OST-0003. Total DNA was extracted from 25 mg of dorsal muscle tissue following Mascolo, Ceruso, Sordino, et al. (2019) methodology. The assembled and annotated mitogenome was obtained by high-throughput sequencing of enriched mitochondrial DNA with Illumina NextSeq 550 System (Illumina, San Diego, CA, USA). The obtained sequences were assembled using MegaHit (Li et al. 2015) through
The Galaxy server at https://usegalaxy.eu/ (Afgan et al. 2018). The final contig obtained was annotated by the MitoFish server (Iwasaki et al. 2013) and subsequently checked manually. The *D. cervinus* mitogenome was 16,559 bp long. The overall nucleotide composition was: 27.58% A, 29.30% C, 26.56% T, and 16.56% G, being similar to other Sparidae mitogenome data (Ceruso et al. 2018a, 2018b, 2020; Mascolo et al. 2018a, 2018b; Mascolo, Ceruso, Chirollo, et al. 2019; Caputi et al. 2021). As is the case for most metazoans (Boore 1999), the mtDNA encoded for 13 protein-coding genes, 22 tRNAs, and 2 rRNAs. Also, two non-coding regions (D-loop and L-origin) were present, in agreement with fish mitochondrial genomes (Satoh et al. 2016). The heavy strand of the mt genome encoded for 12 protein-coding genes, the majority of the tRNA genes, and the 2 ribosomal genes (12S and 16S). The NADH dehydrogenase subunit 6 (nad6) gene and the trnA, trnN, trnC, trnY, trnE, and trnC were encoded by the light strand. The mt genome organization followed those previously described (see Ceruso et al. 2020; Fietz et al. 2020; Caputi et al. 2021).

All protein-coding genes started with the codon ATG except for the subunit 1 of the cytochrome oxidase (cox1) that started with GTG. Some genes had complete stop codons (TAA in *nad4L* and *nad5*; TAG in subunit 8 of the ATP synthase (*atp8*), *nad1* and *nad6*; AGG in *cox1*), while other genes ended with a single T (cox2, cob, *nad3*, and *nad4*) or TA (*atp6*, *cox3*, and *nad2*), which presumably becomes

Figure 1. Phylogenetic relationships in the family Sparidae based on the complete mt genome sequences available in GenBank and that of *Diplodus cervinus* reported here (*Acanthopagrus latus* NC_010977; *Acanthopagrus schlegeli* JQ746035; *Dentex angolensis* MH593823; *Dentex gibbosus* MG653593; *Dentex tumifrons* NC_029479; *Diplodus puntazzo* MT319027; *Diplodus sargus* NC_057561; *Pagellus acarne* MG736083; *Pagellus bogaraveo* NC_009502; *Pagellus erythrinus* MG653592; *Pagrus auriga* AB124801; *Pagrus caeruleostictus* MN319701; *Pagrus major* NC_003196; *Parargyrops edita* EF107158; *Rhabdosargus sarba* KM272585; *Sparus aurata* LK022698). Five outgroup species (*Lutjanus peru* KR362299, *Lutjanus rivulatus* AP006000, *Lethrinus obsoletus* NC_009855, *Chaetodonoplus septentrionalis* NC009873, and *Chaetodon auripes* NC_009870) were selected. Maximum likelihood method was used with an automatic bootstrapping cutoff of 0.01.
functional by subsequent polyadenylation of the transcribed messenger RNA (Ojala et al. 1981).

A maximum likelihood (ML) analysis was implemented to elucidate the phylogenetic position of \textit{D. cervinus}. It was performed in RAxML-NG (Kozlov et al. 2019). The resultant phylogeny (Figure 1) placed \textit{D. cervinus} as sister species of \textit{D. sargus} (Linnaeus, 1758) with maximum support, and both species as sister group of \textit{Diplodus puntazzo} (Walbaum, 1792), again with maximum support. So, all three latter species formed a monophyletic group that corresponded to a clade including the species of the genus \textit{Pagellus} Rafinesque, 1810. This clade was placed in the topology as a sister group of a clade including the species of the genus \textit{Acanthopagrus} Peters, 1855, with a ML support of 94. This last clade was recovered as sister group of \textit{Pagellus acarne} (Risso, 1827) and \textit{Pagellus bogaraveo} (Nunner, 1768), similar to previous studies (Ceruso et al. 2020; Caputi et al. 2021).

Ethical approval

This study has been reviewed by the Ethical Animal Care and Use Committee of the University of Naples Federico II and received institutional approval (Notice No. PG/2022/0009423, July 27th 2022).

Author contributions

FC, TP and PS conceived and designed the project. VT, RMS, AL, FT, IV and MC performed labwork. DO, LC and FT performed data analyses and interpretation. DO drafted the manuscript. DO, TP, LC, VT, RMS, AL, FT, IV, FB, and PS revised the manuscript for intellectual content and were involved for final approval of the version to be published. All the authors agreed to be accountable for all aspects of the work.

Disclosure statement

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

Funding

The author(s) reported there is no funding associated with the work featured in this article.

Acknowledgements

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

Disclosure statement

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

References

Afgan E, Baker D, Batut B, van den Beek M, Bouvier D, Cech M, Chilton J, Clements D, Coraor N, Grünning BA, et al. 2018. The Galaxy platform for accessible, reproducible, and collaborative biomedical analyses: 2018 update. Nucleic Acids Res. 46(W1):W537–W544.

Bauchot ML, Hureau JC. 1986. Sparidae. In: Whitehead PJP, Bauchot ML, Hureau JC, Nielsen J, Tortorese E, editors. Fishes of the north-eastern Atlantic and the Mediterranean. Vol. 2. Paris: UNESCO, p. 883–907.

Boore J. 1999. Animal mitochondrial genomes. Nucleic Acids Res. 27(8):1767–1780.

Caputi L, Osca D, Cerso M, Venuti I, Sepe RM, Anastasio A, D’Aniello S, Crocetta F, Pepe T, Sordino P. 2021. The complete mitochondrial genome of the white seabream \textit{Diplodus sargus} (Perciformes: Sparidae) from the Tyrrenhian Sea. Mitochondrial DNA B Resour. 6(9):2581–2583.

Ceruso M, Mascoco C, Lowe EK, Palma G, Anastasio A, Pepe T, Sordino P. 2018b. The complete mitochondrial genome of the common Pandora \textit{Pagellus erythrinus} (Perciformes: Sparidae). Mitochondrial DNA B Resour. 3(2):624–625.

Ceruso M, Mascoco C, Palma G, Anastasio A, Pepe T, Sordino P. 2018a. The complete mitochondrial genome of the common dentex, \textit{Dentex} (Perciformes: Sparidae). Mitochondrial DNA B Resour. 3(1):391–392.

Ceruso M, Venuti I, Osca D, Caputi L, Anastasio A, Crocetta F, Sordino P, Pepe T. 2020. The complete mitochondrial genome of the sharpsnout seabream \textit{Diplodus puntazzo} (Perciformes: Sparidae). Mitochondrial DNA B Resour. 5(2):2379–2381.

Coutinho F, Peres H, Castro C, Pêrez-Jiménez A, Pousão-Ferreira P, Oliveira-Teles A, Enes P. 2016. Metabolic responses to dietary protein/carbohydrate ratios in zebrafish (\textit{Diplodus cervinus}, Lowe, 1838) juveniles. Fish Physiol Biochem. 42(1):343–352.

Fietz K, Trofimenko E, Gueirin PE, Armal V, Torres-Oliva M, Lobréaux S, Pérez-Ruzafe A, Manel S, Puebla O. 2020. New genomic resources for three exploited Mediterranean fishes. Genomics. 112(6):4297–4303.

IGFA. 2001. Database of IGFA angling records until 2001. Fort Lauderdale (FL): IGFA.

Iwasaki W, Fukunaga T, Isagoozawa R, Yamada K, Maeda Y, Satoh T, Sado T, Mabuchi K, Takeshima H, Miyai M, et al. 2013. MitoFish and MitoAnnotator: a mitochondrial genome database of fish with an accurate and automatic annotation pipeline. Mol Biol Evol. 30(11):2531–2540.

Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A. 2019. RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. Bioinformatics. 35(21):4453–4455. DOI: 10.1093/bioinformatics/btz305.

Li D, Liu CM, Luo R, Sadakane K, Lam TW. 2015. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics. 31(10):1674–1676.

Mascolo C, Ceruso M, Chirollo C, Palma G, Anastasio A, Sordino P, Pepe T. 2019. The complete mitochondrial genome of the Angolan dentex \textit{angolensis} (Perciformes: Sparidae). Mitochondrial DNA B Resour. 4(1):1245–1246.

Mascolo C, Ceruso M, Palma G, Anastasio A, Sordino P, Pepe T. 2018a. The complete mitochondrial genome of the axillary seabream, \textit{Pagellus acarne} (Perciformes: Sparidae). Mitochondrial DNA B Resour. 3(1):434–435.

Mascolo C, Ceruso M, Palma G, Anastasio A, Sordino P, Pepe T. 2018b. The complete mitochondrial genome of the Pink dentex \textit{gibbosus} (Perciformes: Sparidae). Mitochondrial DNA B Resour. 3(2):525–526.

Mascolo C, Ceruso M, Sordino P, Palma G, Anastasio A, Pepe T. 2019. Comparison of mitochondrial DNA enrichment and sequencing methods from sparid fishes. Food Chem. 294:333–338.

Ojala D, Montoya J, Attardi G. 1981. tRNA punctuation model of RNA processing in human mitochondria. Nature. 290(5806):470–474.

Pajuelo JG, Lorenzo JM, Domínguez-Seoane R. 2003. Age estimation and growth of the zebra seabream \textit{Diplodus cervinus} (Lowe, 1838) on the Canary Islands shelf (Central-east Atlantic). Fish Res. 62(1):97–103.

Pajuelo JG, Lorenzo JM, Domínguez R, Ramos A, Gregoire M. 2003. On the population ecology of the zebra seabream \textit{Diplodus cervinus} (Lowe 1838) from the coasts of the Canadian archipelago. North West Africa. Environ. Biol. Fish. 67(4):407–416.

Satoh TP, Miyai M, Mabuchi K, Nishida M. 2016. Structure and variation of the mitochondrial genome of fishes. BMC Genom. 17(1):20.

Satoh TP, Miyai M, Mabuchi K, Nishida M. 2016. Structure and variation of the mitochondrial genome of fishes. BMC Genom. 17(1):1–20.

Tiralongo F, Crocetta F, Riginella E, Lillo AO, Tondo E, Macali A, Mancini E, Russo F, Coco S, Paolillo G, et al. 2020. Snapshot of rare, exotic and overlooked fish species in the Italian seas: a citizen science survey. J Sea Res. 164:101930.