The complete mitochondrial genome of the deep-water cartilaginous fish *Hydrolagus affinis* (de Brito Capello, 1868) (Holocephali: Chimaeridae)

André Gomes-dos-Santos,1,2,3 Nair Vilaes Arrondo,1,4 André M. Machado,1 Ana Veríssimo,1 Montse Pérez,2
Esther Román,2,3 L. Filipe C. Castro,1,2 and Elsa Froufe4

1CIIMAR/CIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal; 2Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal; 3AQUACOV, Instituto Español de Oceanografía, Centro Oceanográfico de Vigo, Vigo, Spain; 4UVIGO, PhD Program “Marine Science, Technology and Management” (Do “MAR), Faculty of Biology, University of Vigo, Vigo, Spain; 5CIBIO – Research Centre in Biodiversity and Genetic Resources, Vairão, Portugal

**ABSTRACT**

Cartilaginous fishes are a highly vulnerable vertebrate group but remain poorly studied, especially those occupying deep-water ecological niches. Here, we describe the complete mitogenome of the deep-water chimaeriform *Hydrolagus affinis* (de Brito Capello, 1868) (Holocephali: Chimaeridae). The mitogenome has 19,437 nucleotides and the same overall content, i.e. 13 protein-coding genes, 22 transfer RNA, 2 ribosomal RNA genes, as available for all cartilaginous fishes mitogenomes. Phylogenetic reconstructions including 615 cartilaginous fishes mitogenomes place the *H. affinis* within the family Chimaeridae but suggest that *Hydrolagus* and *Chimera* are not reciprocally monophyletic, highlighting the need for additional molecular data to improve phylogenetic reconstruction.

Research into Chondrichthyes Huxley, 1880 (cartilaginous fishes) biology is critical to understand the evolution of vertebrates. Their ecology, physiology, and evolutionary placement are decisive to understand the origin and evolution of gnathostomes (Inoue et al. 2010; Renz et al. 2013; Boisvert et al. 2019). The subclass Holocephali (chimaeras, ratfishes, and rabbitfishes) is composed of three families (Callorhinchidae Garman, 1901, Rhinichimeraeidae Garman, 1901, and Chimaeridae Bonaparte, 1831) widely distributed in the oceans, usually found below 200 m and up to 2000 m (Didier et al. 2012). Their deep-water habitat poses many challenges, resulting in a generalized lack of taxonomic, biological, ecological, and evolutionary knowledge (Venkatesh et al. 2014; Boisvert et al. 2019). Chimaeridae includes nearly 70% of known Holocephalan and comprises two genera, *Chimera* Linnaeus, 1758 and *Hydrolagus* Gill, 1862.

Application of molecular approaches, e.g. mitochondrial genomes (mtDNA), allowed a more comprehensive and detailed biodiversity assessment of cartilaginous fishes (Arnason et al. 2001; Inoue et al. 2010; Johri et al. 2019a, 2019b). Yet, and especially for holocephalans, most studies are still based on morphology or single mitochondrial markers (e.g. Didier et al. 2012; Walovich et al. 2017). Only 10 complete mtDNA have been sequenced for Holocephali; therefore, increasing taxonomic representation is essential to improve our understanding of their evolutionary relationships.

A male *Hydrolagus affinis* (de Brito Capello, 1868) specimen was captured in the Canadian North-Atlantic (47.3685 N; 46.6540 W) during the EU Groundfish Survey (Fletán Negro 3L-2018). Morphological identification was performed on board and later confirmed by COI mtDNA. Genomic DNA was extracted and used for whole-genome sequencing of 150 bp paired-end (PE) reads on Hiseq X Ten (BioProject PRJNA606208).

Mitogenome assembly was obtained using NOVOPlasty (v.3.7.1) (Dierckxsens et al. 2016) with 10% of the PE reads (SRR11071609) and annotated using MITOS2 (Bernt et al. 2013).

All available (614) mitogenomes of cartilaginous fishes were retrieved from GenBank (01 November 2019) and their 13 protein-coding genes (PCGs) aligned and concatenated using MAFFT (v.7.402) on XSEDE (Katoh and Standley 2013) and SequenceMatrix (v.1.7.8) (Vaiya et al. 2011), respectively (final length: 11,405 bp). The best partition-scheme for each gene was estimated using PartitionFinder2 (v.2.1.1) on XSEDE (Lanfear et al. 2016) and used for Bayesian phylogeny (GTR + I + G) in MrBayes (v.3.2.6), on XSEDE (Ronquist et al. 2012) with two independent runs (107 generations, sampling frequency one tree for 1000 generations) and maximum likelihood (ML) estimations on RAxML (v.8.2.12) (Stamatakis 2014) HPC Black Box, with a ‘halt bootstrapping automatically’ and
20 ML searches. All analyses were implemented in CIPRES (Miller et al. 2010). Genetic sequence divergence (uncorrected $p$-distance) was calculated using MEGAX (Kumar et al. 2018).

The new mitogenome was deposited in GenBank with accession number MT090368 (BioProject PRJNA606208), with a length of 19,437 bp, within the expected range for Holocephalan (16,758–24,889 bp). Gene content and orientation are expected for vertebrate mtDNA: 13 PCGs, 22 transfer RNA, and 2 ribosomal RNA genes. Only one PCG (NAD6) and eight tRNAs are encoded on the complementary strand.

Congruent BI and ML phylogenetic trees, rooted at the split between Holocephali and the remaining cartilaginous fishes (Figure 1), recovered the two subclasses as reciprocally monophyletic, i.e. Holocephali and Elasmobranchii (Boisvert et al. 2019).

In Holocephali, three well-supported clades were identified, Callorhinchidae, Rhinocormorphaidae, and Chimaeridae, supporting the most comprehensive Holocephalan phylogenetic reconstructions (Arnason et al. 2001; Inoue et al. 2010). Although Hydrologus and Chimaera cluster within the Chimaeridae group (Figure 1), neither genus was recovered as monophyletic. The two Hydrologus species show a sequence divergence of 14%. Hydrologus affinis clusters together with C. montrosa and C. fluva, showing between 10% and 9% $p$-distances. Conversely, H. lemuers clusters with C. phantasma, showing only 5% $p$-distance. Together these results reinforce the need for multidisciplinary approaches to further clarify Holocephali systematics and taxonomy.

**Acknowledgements**

We thank all the participants and crew of the cruise Demeralse19 performed on board the R/V Miguel Oliver.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).
Funding
This oceanographic campaign was funded by ERDEM [2015 00000003, Instituto Español de Oceanografía]. This work was financed by the Project The Sea and the Shore, Architecture and Marine Biology: The Impact of Sea Life on the Built Environment [PTDC/ART-DAQ/29537/2017] from FCT/MCTES through National Funds (PIDDAC) and co-financing from the European Regional Development Fund (FEDER) [POCI-01-0145-FEDER-029537], in the aim of the new partnership agreement PT2020 through COMPETE 2020 – Competitiveness and Internationalization Operational Program (POCI), and by FCT – Fundação para a Ciência e a Tecnologia; UIDB/04423/2020, UIDP/04423/2020 which also supported A.G.S. [SFRH/BD/137935/2018]. EU-Spain NAFO Groundfish survey has been co-funded by the European Union through the European Maritime and Fisheries Fund (EMFF) within the National Program of collection, management and use of data in the fisheries sector and support for scientific advice regarding the Common Fisheries Policy and IEO-BIOPELE Project.

ORCID
André Gomes-dos-Santos https://orcid.org/0000-0001-9973-4861
L. Filipe C. Castro https://orcid.org/0000-0001-7697-386X
Elsa Froufe https://orcid.org/0000-0003-0262-0791

References
Arnason U, Gullberg A, Janke A. 2001. Molecular phylogenetics of gnathostomous (jawed) fishes: old bones, new cartilage. Zool Scripta. 30(4):249–255.
Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol. 69(2):313–319.
Boisvert CA, Johnston P, Trinajstic K, Johanson Z. 2019. Chondrichthyan evolution, diversity, and senses. Cham: Springer.
Didier AD, Kemper MJ, Ebert AD. 2012. Phylogeny, biology, and classification of extant Holocephalans. In: Carrier J, Musick J, Heithaus M, editors. Biology of sharks and their relatives. Boca Raton: CRC Press; p. 97–122. http://www.crcnetbase.com/doi/abs/10.1201/b11867-11.
Dierckxsens N, Mardulyn P, Smits G. 2016. NOVOPlasty: de novo assembly of organelle genomes from whole genome data. Nucleic Acids Res. 45:e18.
Inoue JG, Miya M, Lam K, Tay BH, Danks JA, Bell J, Walker TJ, Venkatesh B. 2010. Evolutionary origin and phylogeny of the modern Holocephalans (Chondrichthyes: Chimaeriformes): a mitogenomic perspective. Mol Biol Evol. 27(11):2576–2586.
Johri S, Doane M, Allen L, Dinsdale E. 2019a. Taking advantage of the genomics revolution for monitoring and conservation of Chondrichthyan populations. Diversity. 11(4):49.
Johri S, Solanki J, Cantu VA, Fellows SR, Edwards RA, Moreno I, Vyas A, Dinsdale EA. 2019b. Genome skimming’ with the MinION hand-held sequencer identifies CITES-listed shark species in India’s exports market. Sci Rep. 9(1):4476.
Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 30(4):772–780.
Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 35(6):1547–1549.
Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2016. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol Biol Evol. 34:722–773.
Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. 2010 Gateway Computing Environments Workshop, GCE 2010. IEEE. p. 1–8. http://ieeexplore.ieee.org/document/5676129/.
Renz AJ, Meyer A, Kuraku S. 2013. Revealing less derived nature of cartilaginous fish genomes with their evolutionary time scale inferred with nuclear genes. PLOS One. 8(6):e66400.
Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 61(3):539–542.
Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 30(9):1312–1313.
Vaidya G, Lohman DJ, Meier R. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-genre datasets with character set and codon information. Cladistics. 27(2):171–180.
Venkatesh B, Lee AP, Ravi V, Maurya AK, Lian MM, Swann JB, Ohta Y, Flajnik MF, Sutoh Y, Kasahara M, et al. 2014. Elephant shark genome provides unique insights into gnathostome evolution. Nature. 505(7482):174–179.
Walovich KA, Ebert DA, Kemper JM. 2017. Hydrologus erithacus sp. Nov. (Chimaeriformes: Chimaeridae), a new species of chimaerid from the south-eastern Atlantic and Southwestern Indian oceans. Zootaxa. 4226(4):509–520.