ABSTRACT
Cartilaginous fish are fascinating taxa, present in the folklore and art of many different cultures. Moreover, they display several unique anatomical, physiological, molecular, and behavioral characteristics making them extremely interesting from a biological perspective. Nevertheless, some crucial knowledge gaps remain, including phylogenetic relationships among extant species. Here, we produced the complete mitogenome sequence of the large-eyed rabbitfish, *Hydrolagus mirabilis* (Chimaeriformes). The complete mitogenome is 19,435 bp long and shows the same overall content, i.e., 13 protein-coding genes, 22 transfer RNA, and two ribosomal RNA genes, as all other examined Chondrichthyan mitogenomes. Phylogenetic reconstructions including 12 Holocephalan and three outgroup Elasmobranch mitogenomes place the *H. mirabilis* within the family Chimaeridae but revealed paraphyletic *Hydrolagus* and *Chimaera*, in line with a previous study, highlighting the importance for collecting additional molecular data to improve phylogenetic reconstruction in this group of vertebrates.

CONTACT L. Filipe C. Castro filipe.castro@ciimar.up.pt; CIIMAR/CIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros de Leixões, Av. General Norton De Matos s/n, 4450208 Matosinhos, Portugal; Elsa Froufe elsa.froufe@gmail.com; CIIMAR/CIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros de Leixões, Av. General Norton De Matos s/n, 4450208 Matosinhos, Portugal

ARTICLE HISTORY
Received 29 September 2020
Accepted 15 November 2020

KEYWORDS
Chondrichthyes; Holocephali; chimaera; mitogenome; phylogenetics

Shedding light on the Chimaeridae taxonomy: the complete mitochondrial genome of the cartilaginous fish *Hydrolagus mirabilis* (Collett, 1904) (Holocephali: Chimaeridae)

André Gomes-dos-Santos, Nair Vilas-Arrondo, André M. Machado, Ana Veríssimo, Montse Pérez, Francisco Baldo, L. Filipe C. Castro and Elsa Froufe

*CIIMAR/CIMAR – Interdisciplinary Centre of Marine and Environmental Research, Terminal de Cruzeiros de Leixões, Av. General Norton De Matos s/n, University of Porto, Matosinhos, Portugal; Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal; Instituto Español de Oceanografía, Centro Oceanográfico de Vigo, Vigo, Spain; Faculty of Biology, UVIGO, PhD Program “Marine Science, Technology and Management” (Do *MAR*), University of Vigo, Vigo, Spain; CIBIO – Research Centre in Biodiversity and Genetic Resources, Vairão, Portugal; Instituto Español de Oceanografía, Centro Oceanográfico de Cádiz, Puerto Pesquero, Muelle de Levante s/n, Cádiz, Spain

The complete mitogenome is 19,435 bp long and shows the same overall content, i.e., 13 protein-coding genes, 22 transfer RNA, and two ribosomal RNA genes, as all other examined Chondrichthyan mitogenomes. Phylogenetic reconstructions including 12 Holocephalan and three outgroup Elasmobranch mitogenomes place the *H. mirabilis* within the family Chimaeridae but revealed paraphyletic *Hydrolagus* and *Chimaera*, in line with a previous study, highlighting the importance for collecting additional molecular data to improve phylogenetic reconstruction in this group of vertebrates.
10% of the total PE reads. Afterwards, the complete mitogenome was retrieved by blast search (Altschul et al. 1990) against all Teleostei mitogenomes available on GenBank. Annotation was performed using MitoZ (v.2.3) (Meng et al. 2019).

All Holocephali mitogenomes publicly available (12 sequences), as well as three outgroup Elasmobranchii mitogenomes (i.e. Squalus brevirostris, Carcharhinus amblyrhynchos, and Raja radiata) were retrieved from GenBank (12-09-2020) and their 13 protein-coding genes (PCGs) aligned and concatenated using GUIDANCE (v.1.5) (Penn et al. 2010) and FASconCAT-G (https://github.com/PatrickKueck/FASconCAT-G), respectively (final length: 11,431 bp). The best partition-scheme for each gene and maximum-likelihood (ML) phylogeny were obtained using IQ-TREE (v.1.6.12) (Nguyen et al. 2015; Kalyaanamoorthy et al. 2017).

The complete mitogenome of *H. mirabilis* was deposited in GenBank (MW029477). The mitogenome length is 19,435 bp, which is within the expected length for holocephalans (16,758–24,889 bp), and the gene composition is in agreement with that of vertebrate mtDNA: 13 PCGs, 22 transfer RNA, and two ribosomal RNA genes. One PCG (NAD6) and eight tRNAs are encoded on the light strand. Furthermore, a Chimaeriformes-specific long noncoding insertion (2215 bp) between tRNAThr and tRNAPro genes is also present (Inoue et al. 2010).

The phylogenetic tree (Figure 1), recovered the two major Chondrichthyan subclasses as reciprocally monophyletic, i.e. Holocephali and Elasmobranchii. As previously described, the Callorhinichidae, Rhinochimaeridae, and Chimaeridae families were retrieved as three well-supported clades within Holocephali (Inoue et al. 2010). The paraphyletic status of *Hydrolagus* and *Chimaera* was also recovered, as recently reported by Gomes-dos-Santos et al. (2020). The newly sequenced *H. mirabilis* mitogenome ranges in the percentage of sequence divergence from 12.8% (unc p-distance) from *H. affinis* to 15.4% from *H. leumures*. The prevalence of this paraphyly in the Chimaeridae, reinforce the scenario of a possible misidentification of one of the specimens, most likely *Chimaera phantasma*. Yet, the authors are engaged at raising the few existing molecular data from this group of gnathostomes to clarify the evolutionary relationships between extant lineages.

**Acknowledgements**

The authors would like to thank Jaime Moreno Aguilar and the staff involved in the research survey PORCUPINE 2019 of the Spanish Institute of Oceanography (IEO) on board the R/V Vizconde de Eza (Ministry of Agriculture, Fisheries and Food, Spain).

**Disclosure statement**

The authors declare no conflict of interest.

**Funding**

This work was funded 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) and A.V. (DL57/2016). The Spanish Bottom Trawl Survey on the Porcupine Bank (SP-PORC-Q3) was funded in part by the EU through the European Maritime and Fisheries Fund (EMFF) within the Spanish National Program of Collection, Management and Use of Data in the Fisheries Sector and Support for Scientific Advice Regarding the Common Fisheries Policy.

**ORCID**

André Gomes-dos-Santos http://orcid.org/0000-0001-9973-4861
Ana Veríssimo http://orcid.org/0000-0003-3396-9822
Elsa Froufe http://orcid.org/0000-0003-0262-0791
Data availability statement

The data produced in this study are available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov, reference number MW029477 or from the corresponding authors, L. Filipe C. Castro and Elsa Froufe.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol. 215(3):403–410.
Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prijibelski AD, et al. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 19(5):455–477.
Boisvert CA, Johnston P, Trinajstic K, Johanson Z. 2019. Chondrichthyan evolution, diversity, and senses. Cham: Springer; p. 65–91.
Catarino D, Jakobsen K, Jakobsen J, Giacomello E, Menezes GM, Diogo H, Canha A, Porteiro FM, Melo O, Stefanní S. 2020. First record of the opal chimaera, Chimaera opalescens (Holocephali: Chimaeridae) and revision of the occurrence of the rabbitfish Chimaera monstrosa in the Azores waters. J Fish Biol. 97:763–775.
Davidson LNK, Krawchuk MA, Dulvy NK. 2016. Why have global shark and ray landings declined: improved management or overfishing? Fish Fish. 17(2):438–458.
Dulvy NK, Fowler SL, Musick JA, Cavanagh RD, Kyne PM, Harrison LR, Carlson JK, Davidson LN, Fordham SV, Francis MP, et al. 2014. Extinction risk and conservation of the world’s sharks and rays. Elife. 3:e00590.
Finucci B. 2020. Hydrologus mirabilis. IUCN red list of threatened species. 2020:e.T63104A124458962, [accessed 2020 Sep]. https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T63104A124458962.en
Freitas M, Almeida AJ, Delgado J, González JA, Santana JI, Biscoito M. 2011. First record of Hydrologus affinis (Holocephali: Chimaeriformes: Chimaeridae) from Madeira and the seine seamount (North Atlantic Ocean). Acta Ichth Piscat. 41(3):255–257.
Gomes-dos-Santos A, Arrondo NV, Machado AM, Veríssimo A, Pérez M, Román E, Castro LFC, Froufe E. 2020. The complete mitochondrial genome of the deep-water cartilaginous fish Hydrologus affinis (de Brito Capello, 1868) (Holocephali: Chimaeridae). Mitochondrial DNA Part B. 5(2):1810–1812.
Inoue JG, Miya M, Lam K, Tay BH, Danks JA, Bell J, Walker TI, Venkatesh B. 2010. Evolutionary origin and phylogeny of the modern holocephalans (Chondrichthyes: Chimaeriformes): a mitogenomic perspective. Mol Biol Evol. 27(11):2576–2586.
James K, Ebert DA, Long DJ, Didier DA. 2009. A new species of chimaera, Hydrologus melanophasma sp. nov. (Chondrichthyes: Chimaeriformes: Chimaeridae), from the eastern North Pacific. Zootaxa. 2218(1):59–68.
Kalyaanamoorthy S, Minh BQ, Wong TKF, Von Haeseler A, Jermiiin LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods. 14(6):587–589.
Lopes-Marques M, Delgado ILS, Ruivo R, Torres Y, Sainath SB, Rocha E, Cunha I, Santos MM, Castro LFC. 2015. The origin and diversity of Cpt1 genes in vertebrate species. PLOS One. 10(9):e0138447.
Meng G, Li Y, Yang C, Liu S. 2019. MitoZ: a toolkit for animal mitochondrial genome assembly, annotation and visualization. Nucleic Acids Res. 47(11):e63.
Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 32(1):268–274.
Penn O, Privman E, Ashkenazy H, Landan G, Graur D, Pupko T. 2010. GUIDANCE: a web server for assessing alignment confidence scores. Nucleic Acids Res. 38(Web Server Issue):W23–W28.