

Runcinidae and Facelinidae: Two complete mitogenomes of understudied and misleading heterobranch families (Gastropoda, Mollusca)

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Abstract

Here, we present the mitochondrial sequences of two ‘opisthobranch’ heterobranchs: *Runcina aurata* García-Gómez, López, Luque & Cervera, 1986 and *Facelina auriculata* (O. F. Müller, 1776), the latter type taxon of the genus. The mitochondrial genomes were 14,282 and 14,171bp in length respectively, the two of them with a complete set of 13 CDS, 2 rRNAs, and 22 tRNAs. None of the mitogenomes showed gene reorganization, keeping the standard heterobranch mitogenomic structure. The base composition was completely distant between them, with a 25.7% GC for *R. aurata*, becoming the mitogenome with more AT content to the date, and 35% for *F. auriculata*, supposing one case of extremely rich GC content in “opisthobranch” mitochondrial genomes.

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INTRODUCTION

Although traditional systematics used to rely on morphological features, molecular methods have demonstrated to provide a subjective tool to compare individuals, where only the stochasticity of mutation is taken in consideration, and the overweight of some morphological characters is somehow avoided. In this scenario, these disciplines have merged to give birth to an integrative taxonomy, which has become a key element in modern systematics (Dayrat, 2005). In this regard, integrative taxonomy allows the categorization of species according to both molecular and morphological differences and resemblances. Thus, it is of primal importance to know the taxonomical, but also the molecular nature of species, since the second provides a reliable standard template to compare with and to be compared to other organisms. Here, we aim to fill a knowledge gap in mitogenomes of poorly studied and misleading taxa. On one hand, we have *Runcina aurata* García-Gómez, López, Luque & Cervera, 1986 (Fig. 1), genus belonging to the order Runcinidae, which englobe close to 100 small sized species (1-4mm) described so far, although it is thought to include much more biodiversity (Araujo et al., 2022). The members of such order feed on macroalgae, contributing to the decomposition of dead matter, being a key element in the balance of the ecosystem (Araujo et al., 2019). However, due to the small size and difficulty to find them in their natural environment, few studies have focused on them so far. On the other hand, *Facelina auriculata* (O. F. Müller, 1776) (Fig. 1) is the type taxon of the family Facelinidae, which is considered one of the most diverse heterobranch families with more than 200 species (Karmeinski et al., 2021). Its distribution, ranging from the English coasts to the Spanish

Atlantic with some introgression in the Mediterranean, overlaps with the *R. aurata* 's one. Nevertheless, they do not compete with each other since *F. auriculata* feeds on cnidarians, from which they “steal” and store the cnidoblasts in specialized organs named ceratas (Goodheart et al., 2018). In view of the needs and scientific circumstances of both taxa, we present here the reference mitogenomes for the two species, which will allow direct comparisons of future genetic information, as well as provide a template for the synthesis of new specific primers.

MATERIAL AND METHODS

The *Runcina aurata* specimen was collected in Swanage (S England, 50°36'28"N 1°56'45"W) at intertidal depth, whereas the *Facelina auriculata* individual was collected in Lagos (S Portugal, 37°05'00"N 8°39'57"W) intertidally. Both specimens were preserved in 96° ethanol. A piece of foot of *F. auriculata* and the whole individual of *R. aurata* were retrieved for DNA extraction, which was carried out with the kit QIAamp DNA mini Kit (QIAGEN Inc) following manufacturers' indications. Due to methodological procedures, *F. auriculata* tissue was deposited in the Museo Nacional de Ciencias Naturales de Madrid (MNCN) with the code MNCN15.05/94859 (Curator: Francisco Javier de Andrés Cobeta, mail: javiermol@mncn.csic.es), and the *R. aurata* DNA extraction was deposited in the same museum with the code MNCN:ADN118948 (Curator: Isabel Rey Fraile, mail: isabel.rey@csic.es). The genomic DNA was quantified using the NanoDrop One (ThermoScientific) and Qubit HS DNA kit (Invitrogen) systems. Total DNA was then sent out for library construction and sequencing to the Get-PlaGe core facilities of GenoToul (Toulouse, France). Library preparation was carried out for each species DNA sample using the Illumina TruSeq Nano DNA Library Prep Kit (Illumina) and sequenced in 2x150pb paired-ends on the high-speed sequencer Illumina HiSeq 3,000. Paired-end reads of both genomic libraries were then subjected to quality inspection using the FastQC software (Andrews, 2010), and the Velvet-1.2.10 de novo assembler (Zerbino & Birney, 2008) was used on each dataset independently in order to obtain contigs (long sequences). A local database including nudibranch mitochondrial genomes available in GenBank was used to screen the contigs collection after sequence assembly by running a BLAST search. The complete mitochondrial genome of *Runcina aurata* and *Facelina auriculata* was thus identified as single contigs of approximately 14 kb, with a mean depth coverage above 1500x in both cases (Supp Fig. 1). Contigs presumed to be the mitogenomes of the two species, were selected and annotated using MITOS2 (Donath et al., 2019). The annotation files were manually checked and curated in Geneious Prime. The mitogenomes sequence and their respective annotations were uploaded to Genbank (*F. auriculata* : OP661154, *R. aurata* : OP661155). We downloaded 44 heterobranch additional mitogenomes from Genbank, and generated a supermatrix of the nucleotide sequence of 13 CDS + 2 rRNA for the 46 gathered species. We used the maximum likelihood software IQtree-2 (Minh et al., 2020) to generate a phylogeny of the superorder, partitioning the genes according to their codon position, and rooting the topology with the superclass Acteonimorpha to highlight the clades Tectipleura and Ringipleura.

RESULTS AND DISCUSSION

The mitogenome of *Runcina aurata* spanned a total length of 14,282 bp. It was found to contain the typical 13 coding genes (CDS), the large and small ribosomal RNA genes, and 22 tRNA genes, all of them with the standard gene order of heterobranchs (Fig. 2). The overall GC% content was 25.7%, one of the lowest values in heterobranchs. When focusing on CDS, rRNA and tRNA this value shifted to 26.3%, 23.6% and 25.5% respectively. Parallely, *Facelina auriculata* mitochondrial genome length was 14,171, comprising the ordinary 13 coding genes, 2 ribosomal RNA and 22 tRNA, with the standard gene order of heterobranchs (Fig. 2). The overall GC% content of the mitogenome was 35%, in contraposition to the *R. aurata* 's one. The CDS of *F. auriculata* mitogenome were composed of 35.5% GC, the rRNA were 32% GC and the tRNA were 35.8% GC.

The phylogenetic analysis indicated, as previously reported (Varney et al., 2021), that *Runcina aurata* and therefore Runcinida is sibling taxon to a clade composed by Aplysiida and Cephalaspidea with a BS support value of 100 (Fig. 3). Following, *Facelina auriculata* is sibling to the other facelinid species *Sakuraeolis japonica* (Baba, 1937), with a bootstrap support value of 100, being facelinids sibling taxon to the other aeolidiaceans *Protaeolidiella atra* Baba, 1955 and *Hermisenda emurai* (Baba, 1937) (BS = 100) (Fig. 3)

(Carmona et al., 2013). With the *R. aurata* and *F. auriculata* mitochondrial reference genomes, the inner systematic relationships can be properly assessed and explored, while providing a valuable tool to design specific primers for mitochondrial targeted markers of these groups, which have been hampered when using the standard Folmer set for cytochrome oxidase I, and Palumbi set for 16S.

AUTHOR CONTRIBUTIONS

FP, MP, and JLC conceived the study. CG, FP, LC, AKA and MRM performed the data analysis. CG drafted the manuscript. All authors critically reviewed the article. All authors approved the final version of the manuscript.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

ETHICS STATEMENTS

None of the species use in the present study is included in any protected or threatened species on the IUCN Red List or the Spanish and Portuguese governments. Therefore, no specific permissions or licenses were needed for the sampling. We followed ethic procedures to ensure no substantial harm to the collecting individual.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov/genbank/>, under the accession numbers OP661154 for *Facelina auriculata* and OP661155 for *Runcina aurata*. The associated ****BioProject****, ****SRA****, and ****Bio-Sample**** numbers are PRJNXXXXXX, SXXXXXX, and SXXXXXX respectively.”

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Figure 1: Species reference images of the present study. A) *Runcina aurata* under the binocular lens. B) *Facelina auriculata* on its natural environment. C) *Facelina auriculata* under the binocular lens. Note that due to the small size of *Runcina auriculata*, it was not possible to take a picture on nature.

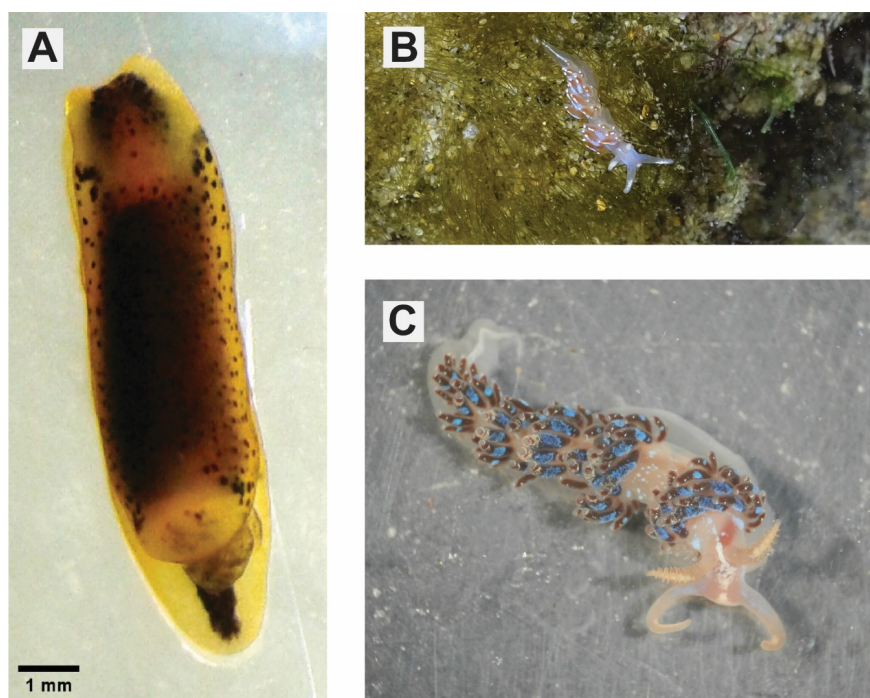


Figure 2. Gene map of the two newly generated mitogenomes. Above, the gene map of *Runcina aurata*. Below, the gene map of *Facelina auriculata*.

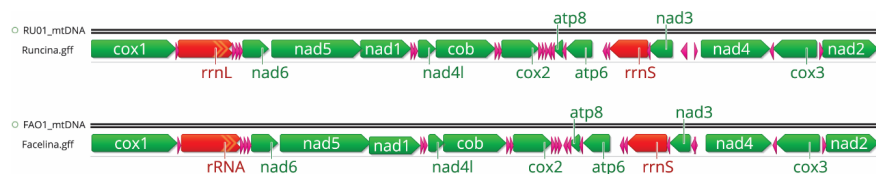
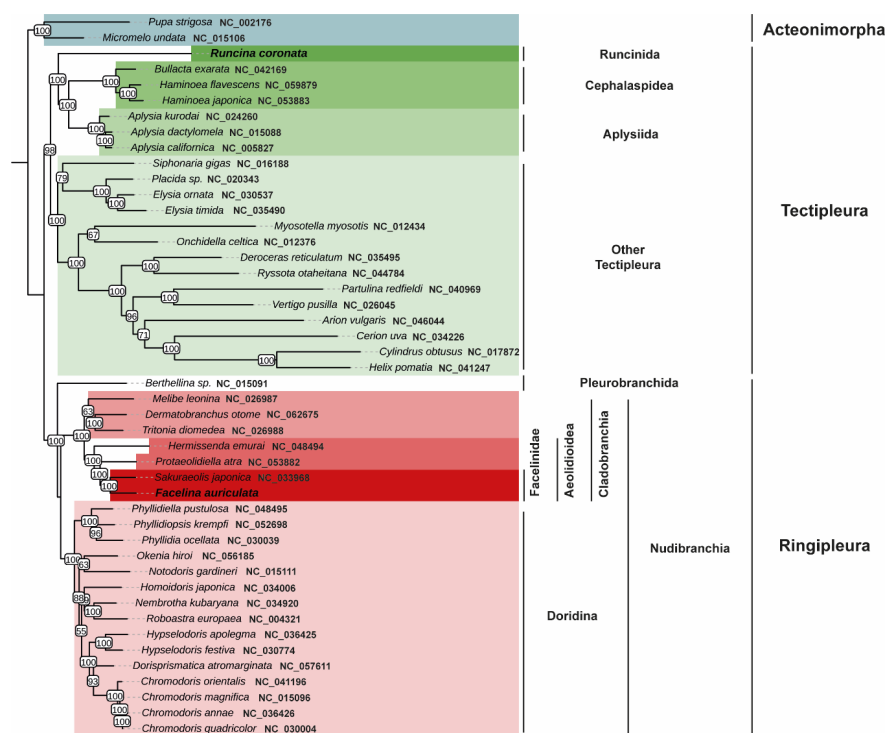


Figure 3. Best Maximum Likelihood phylogeny recovered from 46 heterobranch CDS+rRNA sequences. In bold font are indicated the species newly included in this study. Accession numbers: *A. californica*:NC_-005827.1, *A. dactylomela* : NC_015088.1, *A. kurodai*:NC.024260.1, *A. vulgaris*: NC_046044.1, *Berthellina* sp.: NC_015091.1, *B. exarata* : NC_042169.1, *C. uva*:NC_034226.1, *C. annae* : NC_036426.1, *C. magnifica* : NC_015096.1, *C. orientalis* : NC_041196.1, *C. quadricolor*:NC_030004.1, *C. obtusus*: NC_017872.1, *D. otome* : NC_062675.1, *D. reticulatum*: NC_035495.1, *D. atromarginata* : NC_057611.1, *E. timida* : NC_035490.1, *E. ornata*: NC_030537.1, *H. flavescens* : NC_059879.1, *Haminoea japonica* : NC_053883.1, *H. pomatia*: NC_041247.1, *H. emurai*: NC_048494.1, *Homoidoris japonica*:NC_034006.1, *H. apolegma* : NC_036425.1, *H. festiva* : NC_030774.1, *M. leonina*: NC_026987.1, *M. undata*:NC_015106.1, *M. myosotis*: NC_012434.1, *N. kubaryana* : NC_034920.1, *N. gardineri*: NC_015111.1, *O. hiroi*:NC_056185.1, *O. celtica*: NC_012376.1, *P. redfieldi*:NC_040969.1, *P. ocellata*: NC_030039.1, *P. pustulosa* : NC_048495.1, *P. krempfi*: NC_052698.1, *Placida* sp.: NC_020343.1, *P. atra* : NC_053882.1, *P. strigosa*:NC_002176.1, *R. europaea*: NC_004321.1, *R. otaheitana*:NC_044784.1, *S. japonica*: NC_033968.1, *S. gigas* : NC_016188.1, *T. diomedea*: NC_026988.1, *V. pusilla*:NC_026045.1.



Supplementary Figure 1: Base by base coverage along the mitogenome. On the left panel, it is displayed the *Runcina aurata* mitogenome coverage. On the right panel, it is displayed the *Facelina auriculata* mitogenome coverage.

