Complete mitochondrial genome of the branching octocoral *Paramuricea grayi* (Johnson, 1861), phylogenetic relationships and divergence analysis

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**ABSTRACT**

The Gray’s sea fan, *Paramuricea grayi* (Johnson, 1861), typically inhabits deep littoral and circalittoral habitats of the eastern temperate and tropical Atlantic Ocean. Along the Iberian Peninsula, where *P. grayi* is a dominant constituent of circalittoral coral gardens, two segregating lineages (yellow and purple morphotypes) were recently identified using single-copy nuclear orthologues. The mitochondrial genomes of 9 *P. grayi* individuals covering both color morphotypes were assembled from RNA-seq data, using samples collected at three sites in southern (Sagres and Tavira) and western (Cape Espichel) Portugal. The complete circular mitogenome is 18,668 bp in length, has an A + T-rich base composition (62.5%) and contains the 17 genes typically found in Octocorallia: 14 protein-coding genes (*atp6*, *atp8*, *cob*, *cox1-3*, *mt-mutS*, *nad1-6*, and *nad4L*), the small and large subunit rRNAs (*rns* and *rnl*), and one transfer RNA (*trnV*). The mitogenomes were nearly identical for all specimens, though we identified a noteworthy polymorphism (two SNPs 9 bp apart) in the *mt-mutS* of one purple individual that is shared with the sister species *P. clavata*. The mitogenomes of the two species have a pairwise sequence identity of 99.0%, with *nad6* and *mt-mutS* having the highest rates of non-synonymous substitutions.

We recently determined the identity of multiple populations of *Paramuricea* in southern and western Portugal previously identified as *P. clavata* to be *P. grayi*, and identified two color morphotypes of *P. grayi* as segregating lineages based on multi-locus genotyping and phylogenomic analyses (Coelho et al. 2022). This highlights the need to further study genetic differentiation between *P. clavata* and *P. grayi*, as well as between the two segregating lineages of *P. grayi* at other mitochondrial genes. Here, we report the complete mitochondrial genome (mitogenome) of *P. grayi* assembled from RNA-seq read data generated in Coelho et al. (2022). In total, we assembled complete/partial mitogenomes of nine individuals of *P. grayi*, including specimens of both the yellow (*n = 7*) and purple (*n = 2*) morphotypes. The samples...
were collected at three sites in southern (Sagres and Tavira) and western (Cape Espichel) Portugal. A specimen of each color morphotype are deposited at the Biogeographical Ecology and Evolution team collection at the Centre of Marine Sciences (email: macoelho@ualg.pt) under voucher IDs 19-0054 and 19-0046. The mitogenomes of *P. clavata* collected in the Mediterranean (Spain, Italy and Croatia; Coelho et al. 2022) and of *P. biscaya* from the Gulf of Mexico (see DeLeo et al. 2018) were also assembled for phylogenetic analysis (see below). For additional information about the origin and collection of samples, as well as about RNA extraction and sequencing see Gómez-Gras et al. (in review), Coelho et al. (2022) and Supplemental Materials Section S1. Quality-filtered, ribosomal RNA-free read data for the samples from Coelho et al. (2022) used here are deposited on NCBI’s Sequence Read Archive (BioProject ID: PRJNA847883).

The mitogenomes were assembled with MITGARD (Mitochondrial Genome Assembly from RNA-seq Data) using default parameters (Nachtigall et al. 2021). MITGARD is an automated pipeline that allows mitochondrial sequence reads to be retrieved from RNA-seq data using a reference mitogenome as bait to subsequently generate de novo contigs and a consensus mitogenome assembly. We used the mitogenome sequence of *P. clavata* as a reference (Genbank Accession: NC_034749). The assembled mitogenomes were then visually curated by examining the alignment files of mapped reads, as well as of the assembled contigs and consensus mitogenome, onto the population consensus sequences (*P. clavata*: VAC, ALT and BALU; *P. grayi*: BAL; see Section S1 for details on population codes), as well as against a mitogenome assembled with long-read sequencing data for *P. grayi* (Costa et al. unpublished data). The assembled mitogenomes of both *P. grayi* and *P. clavata* were annotated using MITOS2 (Donath et al. 2019). Genes that were not identified (only rns), as well as gene boundaries in disagreement with available references (e.g. cox1 and rnl) were manually added in Geneious Prime. Finally, we performed a maximum likelihood (ML) phylogenetic analysis in IQ-TREE 2 (Minh et al. 2020) based on the mitogenomes of multiple octocorals (for details see Supplemental Materials, Section S3); and calculated the rate of synonymous (dS) and non-synonymous (dN) substitutions for protein-coding genes between *P. grayi* and *P. clavata* (NC_034749) using the online platform PAL2NAL (Suyama et al. 2006).

The complete circular mitogenome of *P. grayi* is 18,668 bp in length, has an A+T-rich base composition (62.5%) and contains the 17 genes typically found in Octocorallia: 14 protein-coding genes, including 13 energy pathway proteins (atp6, atp8, cob, cox1-3, nad1-6, and nad4L) and the mitochondrial homologue of the DNA mismatch repair MutS-like protein (mt-mutS); the small and large subunit rRNAs, rns and rnl, respectively; and one transfer RNA (trnM; methionine) (Figure 2). Like other members of family Paramuriceidae (formerly Plexauridae; see McFadden et al. 2022), *P. grayi* has the presumed ancestral octocoral gene order A (Brockman and McFadden 2012), with 12 genes encoded in the heavy strand (cox1-rns-nad1-cob-nad6-nad3-nad4L-mt-mutS-rnl-nad2-nad5-nad4) and 5 genes in the light strand (trnM-cox3-3atp6-atp8-cox2). In total, the intergenic regions (IGR) accounted for 618 bp of the mitogenome, ranging between 5 bp (rns-nad1 and rnl-nad2 IGRs) and 112 bp (cox2-cox1 IGR). The gene boundaries of nad2 and nad5 overlapped by 12 bp (Figure 2; Supplemental Materials, Section S4). The partial/complete mitogenome sequences of *P. grayi* were nearly identical for all nine individuals, including across the two segregating color morphotypes. Notable exceptions included low-confidence regions with low/no read coverage (Section S2); one single nucleotide polymorphism (SNP) in nad6 for one of the

Figure 1. Colonies of *Paramuricea grayi* belonging to the purple (A) and yellow (B) morphotypes corresponding to the segregating lineages identified in Coelho et al. (2022). Colonies were photographed at two distinct sites off Cape Espichel in western Portugal. The colonies of both color morphs tend to have relatively thin branches compared to the Mediterranean sister species *P. clavata* (though colonies with thick branches can also be observed in *P. grayi*, especially in the purple morphotype) and change color when dried or preserved (e.g. in etOH 96%), turning into a black/dark brown coloration. Photo credits: A.C. Ferreira.
seven individuals of the yellow lineage (BAL_8; 439 X coverage); and two SNPs 9 bp apart in mt-mutS for one of the two purple individuals (59X and 56X coverage), a polymorphism shared with P. clavata. Like previous studies, our ML analysis based on mitogenome sequence data show that recently diverged sister species (Poliseno et al. 2017; Coelho additional information about gene boundaries see Supplemental Materials, Section S5). In contrast, none of the mutations observed in cox1 resulted in amino acid replacements, whereas mt-mutS had 12 non-synonymous mutations (dN = 0.0058). Surprisingly, for the P. grayi-P. clavata comparison nad6 had the highest dN of all protein-coding genes (0.0071; Section S4), and together with cox2 the highest dN/dS ratios (Section S4; for a comprehensive overview of the evolution of mitochondrial protein-coding genes in octocorals see Muthye et al. 2022). With the rapid expansion of next- and third-generation sequencing projects, data from RNA-seq represent an underused, but valuable resource to assemble octocoral mitochondrial genomes and more broadly to study the evolution of the eukaryotic powerhouse.
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Author contributions

M.C., J.-B.L., J.G., E.S., and G.P. conceived the study. M.C., J.-B.L., J.B., D.P., D.G.-G., N.B., P.L.-S., C.C., S.K., and T. B.-P. contributed to data acquisition, including sample collection of the original study. M.C. and G.P analyzed the data. J.G. and E.S. contributed funding. M.C. drafted the manuscript. All authors critically revised the content of the manuscript, gave their approval for publication of the final version and agreed to be accountable for all aspects of the work.

Disclosure statement

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

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Data availability statement

Raw RNA-seq read data are available at NCBI’s SRA database (BioProject ID: PRJNA478883). The complete assembled mitogenomes of P. grayi (specimen BAL 3; Biosample [SRA] accessions: SAMN2889305 [SRR19977458] and SAMN2889308 [SRR19977454]) and P. clavata (specimen VAC_1; Biosample accessions: SAMN2889326 [SRR19977435] and SAMN28899232 [SRR19977438]) have been submitted to GenBank under accession numbers OP205061 and OP205062, respectively. Partial/com- plete mitogenome sequences of all the assemblies performed here (including P. biscaya) are available on Figshare (DOI: 10.6084/m9.figshare.20526978).

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