





Mitochondrial genome of *Sabella spallanzanii* (Gmelin, 1791) (Sabellida: Sabellidae)

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ABSTRACT

We report the mitochondrial genome of *Sabella spallanzanii*, an invasive Mediterranean sabellid introduced to Australia and New Zealand. The mitogenome is 15,581 bp long and consists of 38 genes, including 13 protein coding genes, two rRNA genes, and 23 tRNA genes. It shows deviations from the putative annelid ground pattern, such as gene order re-arrangements and regions encoding on the negative strand. It is, however, very different from the mitogenome of the closely related serpulid, *Spirobranchus giganteus*. Phylogenetic analyses of the mitochondrial genes support a sister relationship of *Sabella spallanzanii* and *Spirobranchus giganteus*.

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Sabella spallanzanii (Gmelin 1791) (Sabellidae) is a feather duster worm, native to the Mediterranean, where it forms aggregations in natural habitats and on artificial structures. The species is a highly invasive pest in Australia and New Zealand (Read et al. 2011; Ah Yong et al. 2017). Since the first viable population was observed in Western Australia in 1965 (Clapin and Evans 1995), *Sa. spallanzanii* has established along the southern coast of Australia (Murray and Keable 2013). As one of three families within the sedentarian order, Sabellida (Sabellidae, Serpulidae and Fabriciidae), Sabellidae is sister to Serpulidae (calcareous tube worms), which together form a clade as sister to Fabriciidae (Tilic et al. 2020a). Herein we present the first mitochondrial genome for Sabellidae, which represents one of the suspension feeding lineages in Sedentaria. The only other mitochondrial genomes available for Sabellida are the Christmas tree worm *Spirobranchus giganteus* (Pallas 1766) (Serpulidae) and the freshwater *Manayunkia occidentalis* Atkinson, Bartholomew & Rouse, 2020 (Fabriciidae).

The studied specimen was collected from Western Australia (32°12'S, 115°40'E) and deposited in the Australian Museum, Sydney (W. 48385, Collection Manager Dr Stephen Keable, Stephen.Keable@Australian.Museum). Total genomic DNA was isolated from the posterior end of the worm with the DNeasy Blood & Tissue Kit (Qiagen) according to manufacturer's protocol. A library of total genomic DNA was prepared by the Australian Genome Research Facility (AGRF), Melbourne, and sequenced with 100 bp paired-end reads on an Illumina Hi-Seq 2000 platform (AGRF). Adapter sequences and low-quality bases were removed from the sequencing

reads using Trimmomatic (Bolger et al. 2014). *De novo* assemblies were conducted with CLC Genomics Workbench 7.0 (CLCbio, Aarhus, Denmark) using default settings. Mitochondrial protein-coding and rRNA gene sequences of all published annelids were used as tblastn queries to search for mitochondrial fragments in the *Sa. spallanzanii* assembly. The top-hitting contig identified by BLASTN recovered the entire mitochondrial genome of *Sa. spallanzanii*. The final contig was annotated using MITOS server under the mitochondrial code for invertebrate mitochondria (Bernt et al. 2013), including the protein coding genes and the secondary structure of tRNAs and rRNAs. Gene boundaries generated from automatic annotations were manually examined and adjusted, and the tRNAs identified by MITOS were rechecked via the tRNAscan-SE web server (Schattner et al. 2005).

Amino-acid sequences of protein-coding genes of 14 annelids belonging to Sedentaria (as selected by Tilic et al. (2020b) from available mitochondrial genomes), including *M. occidentalis*, and an outgroup *Marphysa sanguinea* (Montagu 1813) belonging to Errantia, were used for phylogenetic analysis. Sequences were aligned using Muscle (Edgar 2004), implemented in Geneious 2020.0.5. The concatenated matrix was partitioned by gene. The best fitting evolutionary model for each partition was selected and the maximum likelihood analysis was performed in IQ-TREE 1.3.4 (Nguyen et al. 2015). Bootstrap support was estimated using an ultrafast bootstrap algorithm (UFBoot) (Minh et al. 2013) for 1000 replicates.

The mitochondrial genome of *Sa. spallanzanii* (GenBank accession number MW002660), length 15,581 bp, is AT-rich (64.5%), with an overall base composition of 34.4% (A), 30.1%

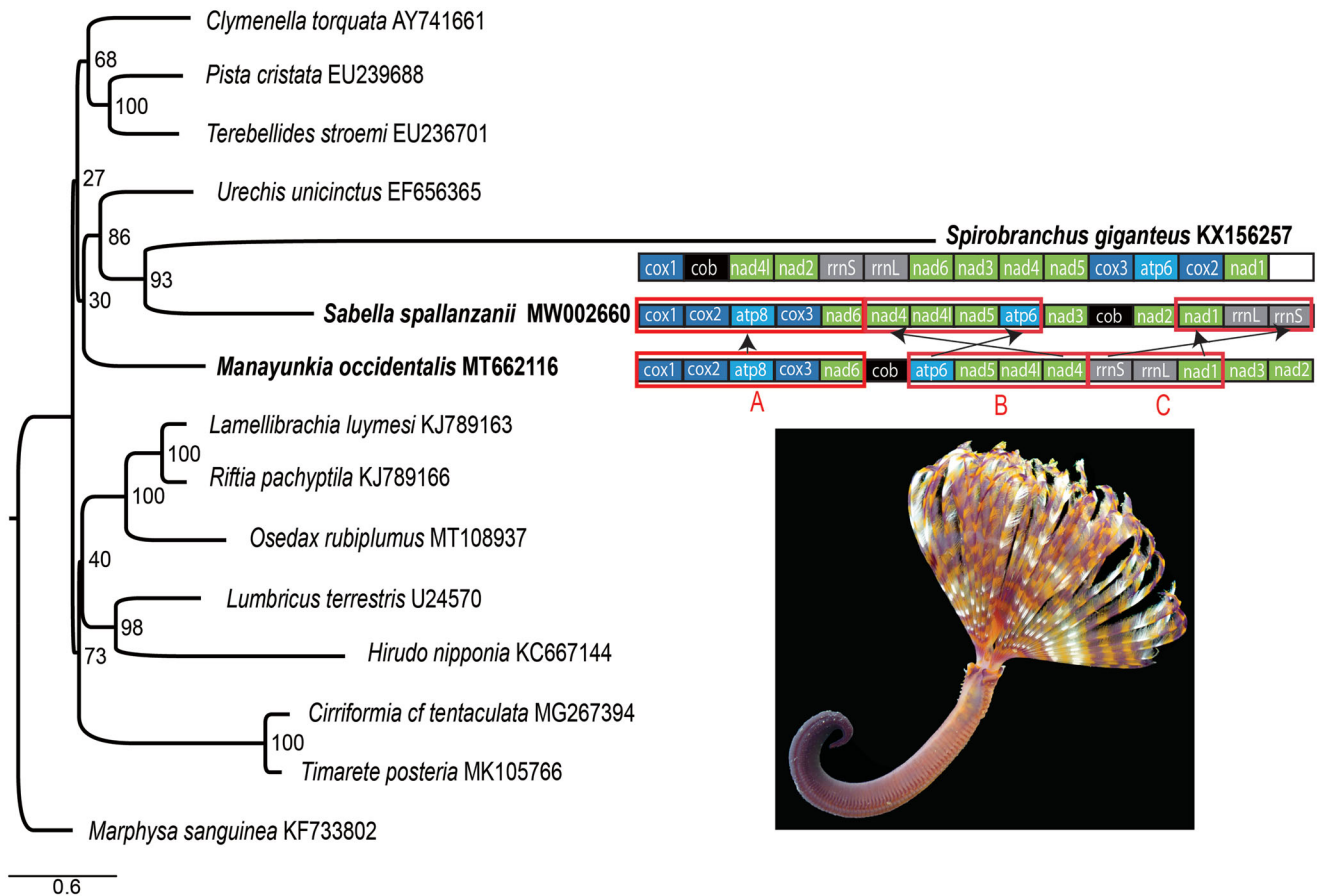


Figure 1. Maximum likelihood (ML) tree based on the concatenated amino acid sequences of mitochondrial protein-coding genes. Bootstrap support values are indicated at each node. *Marphysa sanguinea* (KF733802) was chosen as an outgroup. Gene order rearrangements in the Sabellida (Serpulidae + Sabellidae) relative to the annelid putative ground pattern (PGP) *sensu* Weigert et al. (2016) exemplified by *Manayunkia occidentalis*. Photo by S. Ahnyong.

(T), 12.3% (G) and 23.1% (C). The genome has negative GC-Skew (−0.31) and a positive AT-Skew (0.07). We identified 13 protein coding genes, 2 rRNAs and 22 tRNAs. Two copies of *trnM* were found. Two gene blocks '*cox1-cox2-atp8-trnK-cox3-trnD-trnE-trnW-trnV*' and '*trnQ-trnA-trnY-trnM-trnL1-trnN-trnT*' and gene *nad3* is found on the positive strand, while the other genes are transcribed from the negative strand. Encoding regions in the negative strand are unusual features of *Sa. spallanzanii*, because in other annelid mitochondrial genomes, all regions encode in the positive strand, except for some tRNAs in *Owenia fusiformis* Delle Chiaje, 1844, *Magelona mirabilis* (Johnston, 1865) (Weigert et al. 2016), and *Laonereis culveri* (Webster, 1879); (Seixas et al. 2016).

The order of mitochondrial protein-coding genes and two rRNA genes in *Sa. spallanzanii* differs from the putative ground pattern (PGP) of Errantia and Sedentaria (Weigert et al. 2016) that is also observed in *M. occidentalis* (Tilic et al. 2020b). The genome of *Sa. spallanzanii* has three blocks: A–C. Block A (*cox1-cox2-atp8-cox3-nad6*) corresponds to the PGP. The gene orders in block B (*atp6-nad5-nad4l-nad4*) and C (*rrnS-rrnL-nad1*) in *Sa. spallanzanii* are reversed relative to the PGP (Figure 1). The *Sa. spallanzanii* mitogenome differs from that of *Sp. giganteus* where gene order is remarkably divergent from those of other annelid lineages (Seixas et al. 2017).

Maximum likelihood analyses recovered *Sa. spallanzanii* as sister group to *Sp. giganteus* (Figure 1). This placement

supports the results of Tilic et al. (2020a), finding Sabellidae to be closer to Serpulidae than Fabriciidae. The Sabellidae + Serpulidae clade, however, did not group with *M. occidentalis*, but was recovered as sister to Echiura. Given that the high genetic distances between the mitogenome of *Sp. giganteus* and other annelids suggests rapid sequence evolution in serpulids (Seixas et al. 2017), the present result may be an artifact of rate heterogeneity on the long serpulid branch.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov/> under the accession no. MW002660. The associated BioProject number is PRJNA670556.

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