

# Assembly of the mitochondrial genome of the hydrothermal vent crab *Segonzacia mesatlantica* and detection of potential nuclear pseudogenes

Perrine Mandon, Laetitia Aznar-Cormano, Stephane Hourdez, Sarah Samadi

► **To cite this version:**

Perrine Mandon, Laetitia Aznar-Cormano, Stephane Hourdez, Sarah Samadi. Assembly of the mitochondrial genome of the hydrothermal vent crab *Segonzacia mesatlantica* and detection of potential nuclear pseudogenes. *mitochondrial DNA Part B*, Taylor & Francis Online, 2017, 2 (1), pp.291-293. <10.1080/23802359.2017.1318674>. <hal-01550571>

**HAL Id: hal-01550571**

**<http://hal.upmc.fr/hal-01550571>**

Submitted on 29 Jun 2017

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Assembly of the mitochondrial genome of the hydrothermal vent crab  
*Segonzacia mesatlantica* and detection of potential nuclear pseudogenes**

Perrine Mandon<sup>1,2,3</sup>, Laetitia Aznar-Cormano<sup>1</sup>, Stéphane Hourdez<sup>2,3</sup>, Sarah Samadi<sup>1</sup>

<sup>1</sup>*Muséum National d'Histoire Naturelle, Sorbonne Universités, Institut de Systématique, Evolution, Biodiversité, (ISYEB – UMR 7205 – CNRS, MNHN, UPMC, EPHE), 57 rue Cuvier, CP26, 75005 Paris, France*

<sup>2</sup>*Sorbonne Universités, UPMC Univ Paris 6, Station Biologique de Roscoff, 29680 Roscoff, France*

<sup>3</sup>*CNRS, UMR 7144, Adaptation et Diversité en Milieu Marin, Station Biologique de Roscoff, 29680 Roscoff, France*

Corresponding author: Perrine Mandon - Muséum National d'Histoire Naturelle, Sorbonne Universités - Institut de systématique, Evolution, Biodiversité (ISYEB – UMR 7205 – CNRS, MNHN, UPMC, EPHE) - Equipe Exploration, Espèces et Evolution (3E) - 57 rue Cuvier, CP26, 75005 Paris, France - pmandon@mnhn.fr - 01 40 79 36 59

Short biographies:

Perrine Mandon is a PhD student, who works on the phylogenetic relationships between hydrothermal vent taxa and their relatives from other environments.

Laetitia Aznar-Cormano is a PhD student, who works on comparative palaeogenomic of mammoths.

Stéphane Hourdez is working on the evolutionary adaptations of the hydrothermal vents organisms (e.g. Bythograeidae crabs, Polynoidae and Alvinellidae Polychaeta).

Sarah Samadi is a Professor working on speciation process and species delimitation of organisms from poorly known environments (e.g. deep-sea seamounts or organic enrichments).

Acknowledgments: This work was supported by the French-Taiwanese Project TFDeepEvo funded by ANR under Grant ANR 12-ISV7-0005-01 and Ministry of Science and Technology, Taiwan, R.O.C. We are grateful to Marie-Anne Cambon-Bonavita, cruise leader of Ifremer cruise BICOSE2014. We thank the Service of Molecular Systematic (SSM), UMS2700 CNRS-MNHN and Agnès Dettai for her helpful advises in data analysis.

Disclosure of interest: The authors do not report any conflict of interest. All material has been collected under appropriate collection permits and approved ethics guidelines. The authors alone are responsible for the content and writing of the paper.

## **Assembly of the mitochondrial genome of the hydrothermal vent crab *Segonzacia mesatlantica* and detection of potential nuclear pseudogenes**

We assembled the mitogenome of the Bythograeid crab *Segonzacia mesatlantica*, using long-range amplification of the mitochondrial genome. The mitogenome is 15,521 base pair long (33.8% A, 21.7% C, 10.5% G, 34%T) with 13 protein-coding genes, 2 ribosomal RNA genes, 22 transfer RNAs and a 624 bp AT-rich region. The gene arrangement is similar to other Brachyuran species. A whole genome shotgun sequencing approach revealed the presence of mitochondrial pseudogenes in the nuclear genome. This fifth mitogenome for a species of Bythograeidae should help resolve the puzzling question of the evolutionary origin of a family limited to deep-sea hydrothermal vents.

Keywords: Mitochondrial genome, *Segonzacia mesatlantica*, Bythograeidae, Pseudogenes, Next-generation sequencing, Mid-Atlantic ridge vents.

With 6,793 species in 93 families, the Brachyura is the most diverse infra-order of Decapoda (Ng, Guinot & Davie 2008) but mitogenomes are available only for 51 species covering 22 families. We provide a sequence for the hydrothermal vent Bythograeidae crab *Segonzacia mesatlantica* Williams, 1988. *Segonzacia* is a monospecific genus and *S. mesatlantica* is the only crab found at Mid Atlantic Ridge vent sites (Desbruyères et al. 2006). Specimens were collected from Snake Pit (23°22'6.32"N; 44°57' 11.66"W) and TAG (26°08'14.07''N; 44°49'33.75''W) during the BICOSE2014 cruise. DNA was extracted from the cheliped of two specimens (MNHN-IU-2013-15617 & MNHN-IU-2013-15603).

We aligned the four available Bythograeidae mitogenomes (Kim et al. 2013; Kim et al. 2015; Yang et al. 2010; Yang et al. 2013) and used Geneious v.9.1.7 (Kearse et al., 2012) to define nine primers in addition to three universal primers LCOI, HCOI (Folmer et al. 1994), and 12S-R (Simon et al, 1994). These primers were used to amplify the whole mitogenome using a long-range amplification protocol. The products were sequenced using an Ion Torrent personal machine (PGM) (Life Technologies, Carlsbad, CA) with the Ion PGM™Hi-Q™ Sequencing Kit (Hinsinger et al. 2015). Reads were assembled by alignment with other Bythograeidae species.

The assembled mitogenome (KY541839) is 15,521-bp long. The base contents is similar to that of other Bythograeidae mitogenomes (33.8% A, 21.7% C, 10.5% G and 34%T). The annotation and boundaries of protein-coding genes (PCGs), determined with Geneious and checked with MITO webserver (Bernt et al. 2013b), were refined by manually checking for consistent reading frame (ORF). The genome contains 13 PCGs, 2 ribosomal RNA genes, 22 transfer RNAs and a putative AT-rich control region of 624 bp. The gene order is similar to most brachyurans, including the tRNA gene arrangement linking Crustaceans and insects (Boore et al. 1998). Two tRNA-Leu (TAA and TAG anticodons) and two tRNA-Ser (TCT and TGA) are found. The coding genes have ATC, ATG, ATT or GTG as start codons and TAA, TAG or T- (extended to TAA during posttranscriptional polyadenylation) as stop codons. Compared to other Bythograeidae, we found 20 instead of 5 to 9, non-coding intergenic nucleotides between tRNA-Leu (UAA) and COX2. Compared to other Bythograeidae, the COX2 gene is shorter, because of an early stop codon.

Libraries for a whole genome shotgun sequencing approach were generated using NEBNext® Fast DNA Fragmentation & Library Prep Set for Ion Torrent™ (E6285L, New England Biolabs), loaded onto Ion 316V2 chips and also sequenced on a

Ion PGM Sequencing platform (Hinsinger et al. 2015) and yielded 347,961 reads with an average of 160 pb. Only 397 reads were successfully mapped on the newly assembled mitogenome (25% minimum similarity). Each read was manually checked. Reads diverging by only a few nucleotides (sequencing errors or intra-specific polymorphism) were considered as mitochondrial reads. Reads for which the ORF gave misplaced stop codons or different amino acid pattern were considered as potential nuclear mitochondrial pseudogenes. Such potential pseudogenes were found for most of the genes, as already reported for other Bythograeidae crabs (Kim et al. 2013).

A preliminary phylogenetic tree is provided based on the alignment of the 62 available and non-redundant brachyuran mitogenomes (Figure 1). Based on this very incomplete taxonomic sampling the monophyly of Bythograeidae is well supported but relationships with other families are not resolved.

## References:

- Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsich G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: Improved de novo metazoan mitochondrial genome annotation. *Mol Phylogenet Evol.* 69:313–319.
- Boore JL, Lavrov DV, Brown WM. 1998. Gene translocation links insects and crustaceans. *Nature.* 392:667.
- Desbruyères D, Segonzac M, Bright. 2006. Handbook of deep-sea hydrothermal vent fauna. Linz: Desbruyères D, Segonzac M, Bright.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol.* 3:294–299.
- Hinsinger DD, Debruyne R, Thomas M, Denys GPJ, Mennesson M, Utge J, Dettai A. 2015. Fishing for barcodes in the Torrent: from COI to complete mitogenomes on NGS platforms. *DNA Barcodes* 3:170–186
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, et al. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics.* 28:1647–1649.
- Kim S-J, Kim HS, Ju S-J. 2013. Mitochondrial genome of the hydrothermal vent crab *Austinograea alayseae* (Crustacea: Bythograeidae): genetic differences between individuals from Tofua Arc and Manus Basin. *Mitochondrial DNA.* 25:251–252.
- Kim S-J, Lee KY, Ju S-J. 2013. Nuclear mitochondrial pseudogenes in *Austinograea alayseae* hydrothermal vent crabs (Crustacea: Bythograeidae): effects on DNA barcoding. *Mol Ecol Resour.* 13:781–787.

- Kim S-J, Moon J-W, Ju S-J. 2015. Complete mitochondrial genome of the blind vent crab *Gandalfus puia* (Crustacea: Bythograeidae) from the Tonga Arc. *Mitochondrial DNA Part A*. 27:2719–2720.
- Ng PKL, Guinot D, Davie, P.J.F. 2008. Systema Brachyurorum: Part I. An annotated checklist of extant brachyuran crabs of the world. *Raffles Bull Zool*. 17:1–286
- Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann Entomol Soc Am* *Ann Entomol Soc Am*. 87: 651-701.
- Yang J-S, Lu B, Chen D-F, Yu Y-Q, Yang F, Nagasawa H, Tsuchida S, Fujiwara Y, Yang W-J. 2013. When Did Decapods Invade Hydrothermal Vents? Clues from the Western Pacific and Indian Oceans. *Mol Biol Evol*. 30:305–309.
- Yang J-S, Nagasawa H, Fujiwara Y, Tsuchida S, Yang W-J. 2010. The complete mitogenome of the hydrothermal vent crab *Gandalfus yunohana* (Crustacea: Decapoda: Brachyura): a link between the Bythograeoidea and Xanthoidea. *Zool Scr*. 39:621–630



Figure 1. RaxML topology of Brachyura phylogenetic relationships based on the alignment of the concatenated 13 mitochondrial coding genes for 63 Brachyuran and 4 non Brachyuran decapods. The model GTR+G+I was used in both maximum likelihood and Bayesian analysis. 500 rapid bootstrap resampling were set for RaxML analysis. Convergence of the 2 run Bayesian analysis was verified by the ESS values > 200 with Tracer V.1.6. Node values correspond to Bayesian posterior probabilities/bootstrap. Asterisks indicate nodes with complete support in the considered analysis. A hyphen denotes a node not recovered in the considered analysis. All non-redundant available complete mitogenomes were selected and a primary RaxML analysis with a concatenated dataset was performed. The Leucosiidae *Pyrhila pisum* (NC\_030047) generates a long branch and was thus excluded. Each gene was then separately analysed to detect potential long-branch artefacts. This analysis conducted to exclude the Cytb and ND6 genes of *Metopaulias depressus* (NC\_030535). The dataset was completed with *Sinopotamon yangtsekiense* (JF909980) for which the sequences of ND1 and ND2 are lacking.

