The complete mitochondrial genome of *Barbatula quignardi* (Băcescu-Meşter, 1967) (Teleostei, Nemacheilidae)

by

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Abstract. – The complete mitochondrial DNA sequence of *Barbatula quignardi* (Teleostei, Nemacheilidae) was sequenced from a museum voucher caught in its type locality (Lez River). The sequence was 16,641 bp in length, consisted of 13 protein-coding genes, 22 transfer RNA genes including 2 tRNA-Leu and 2 tRNA-Ser, 2 ribosomic RNA genes and the control region. Intergenic space and overlapping gene sequences were found. The base composition of the whole mtDNA was 28.7% A, 26.2% T, 27.2% C and 17.9% G.

Résumé. – Le génome mitochondrial complet de *Barbatula quignardi* (Băcescu-Meşter, 1967) (Teleostei, Nemacheilidae).

Le génome mitochondrial complet de *Barbatula quignardi* (Băcescu-Meşter, 1967) (Teleostei, Nemacheilidae) provenant d'un spécimen de référence enregistré en collection et capturé dans sa localité type (Lez) a été séquencé. La séquence d'une longueur de 16 641 pb, contient 13 gènes codants, 22 ARN de transfert dont 2 ARNt-Leu et 2 ARNt-Ser, 2 gènes ARN ribosomiques et la région de contrôle. Un espace intergénique et des séquences de gènes se chevauchant ont été trouvés. La composition des bases du mitogénome est de 28,7% A, 26,2% T, 27,2% C et 17,9% G.

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INTRODUCTION

The Languedoc stone loach *Noemacheilus barbatulus quignardi* Băcescu-Meşter, 1967 was described from a population from the Lez River (Hérault department, South of France, near from Montpellier) (Băcescu-Meşter, 1967). According to morphological data, Kottelat and Freyhof (2007) erected this taxon as a species, *Barbatula quignardi* (Băcescu-Meşter, 1967) and extended its distribution from the Lez River to the South-West of France (Garonne, Adour and Mediterranean basins) and North-Eastern Spain. However, since then, molecular studies on several mitochondrial markers (cytochrome c oxidase subunit 1, cytochrome b and the ribosomic DNA 12S) highlighted independently the presence of several evolutionary lineages within this taxon, some of which at least are undescribed species like the recent *Barbatula leoparda* Gauliard, Dettaï, Persat, Keith & Denys, 2019 (Šedivá *et al*., 2008; Geiger *et al*., 2014; Gauliard *et al*., 2019).

In this paper, we describe the mitogenome of *B. quignardi* from a MNHN vouchered specimen from the type locality, obtained by a double-multiplexing approach. This mitogenome will be helpful to link the molecular studies on different markers (see Hinsinger *et al*., 2015) and, as molecular reference, for molecular identification from DNA barcoding *sensu* Hebert *et al*. (2003) to environmental DNA analyses (Schroeter *et al*., 2020).

MATERIAL AND METHODS

Voucher

The voucher specimen is stored in the National Ichthyological Collections of the Muséum national d'Histoire natu-

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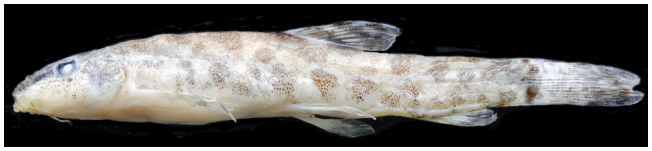

Figure 1. – Voucher of the sequenced mitogenome, MNHN-IC-2010-1064 (FFFtag4260), 41.7 mm SL, Lez River at Prades-le-Lez (Hérault Dept.), 24th Nov. 2010, Denys and ONEMA coll.

relle (MNHN) and catalogued MNHN-IC2010-1064 (field number FFFtag4260). The specimen (41.7 mm of standard length, SL) was caught in the type locality of *B. quignardi*: Lez River at Prades-le-Lez (Hérault Dept.) on the 24 November 2010, by Gaël Denys and the Office National de l'Eau et des Milieux Aquatiques (ONEMA) (Fig. 1). It was morphologically identified following Gauliard *et al.* (2019) with a pelvic-fin length of 15.4% SL, a caudal peduncle depth 1.7 times in its length and no blotch on the belly and the jugular area. These morphomeristic data as well as the origin (type locality) make certain its belonging to the right species.

Brief material and method

DNA extraction was carried on a fin-clip stored in 95% ethanol on an EpMotion Robot using a MN Biomedical extraction kit and protocol. Three long overlapping PCRs of

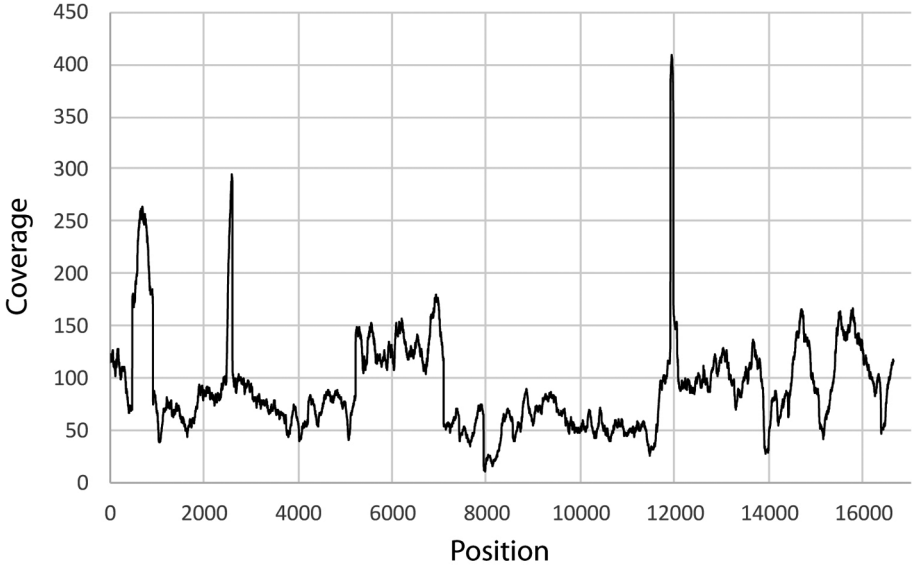

Figure 2. – Coverage depending on position in the mitogenome assembly.

Table I. – Long PCR Primers used to amplify the mitogenome of *Barbatula quignardi*.

Long PCR	Primer name	5'-3' sequence	Source
Mt1	12S-L1091R	AAACTGGGATTAGATACCCCACTAT	Kocher *et al.* (1989)
	MtH7061 LOF	TGGTTATGTGACTGGCTTGAAAC	This study
Mt2	MtL5231 LOF	TAGATGAGAAGGCCTCGATCCTACA	
	MtH11944 LOF	CATAGCTTCCACTTGGATTTGCACCA	
Mt3	MtL11910 LOF	CAGCTTATCCATTGGTCTTAGGAAC	
	12S-H1478 LOF	GTGACAGGGGAGAGTGACGGGCGGTGTGT	

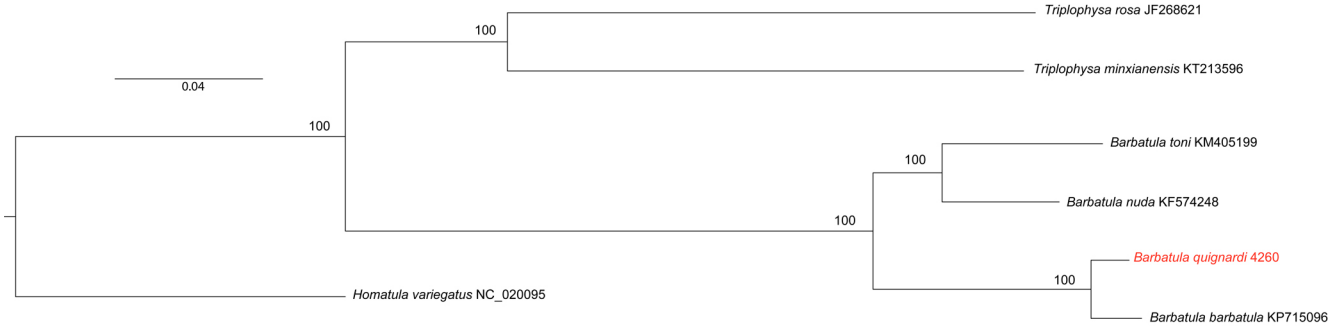

Figure 3. – Maximum Likelihood phylogenetic tree of *Barbatula* mitogenomes; bootstrap values beside the nodes.

6,7 kbp were done using primers in table I. Sequencing was performed using a double-multiplexing approach in which sequences of several distant teleosts (including *Barbatula* spp.), insects, birds and mammal organisms were included in the library in order to reduce the costs on an Ion Torrent PGM (Hinsinger *et al.,* 2015). The reads were mapped on a reference sequence (GenBank accession number KP715096) with the Geneious 11.2.2 software (Kearse *et al*., 2012) and quality controlled. The consensus sequence was annotated using MitoAnnotator (Iwasaki *et al*., 2013).

Table II. – Gene composition of the complete mitochondrial genome of *Barbatula quignardi* (GenBank Accession Number MW288293) detailing position, length, direction, and the number of differences with *B. barbatula* KP715096.

Name	Type	First nucleotide	Last nucleotide	Length (bp)	Direction	Number of differences with *B. barbatula*
tRNA-Phe	tRNA	1	69	65	forward	0
12S rRNA	rRNA	70	1019	950	forward	9
tRNA-Val	tRNA	1020	1091	72	forward	0
16S rRNA	rRNA	1092	2771	1680	forward	22
tRNA-Leu	tRNA	2772	2846	75	forward	1
NADH1 gene	gene	2847	3821	975	forward	35
tRNA-Ile	tRNA	3829	3900	72	forward	0
tRNA-Gln	tRNA	3899	3969	71	reverse	0
tRNA-Met	tRNA	3971	4039	69	forward	0
NADH2 gene	gene	4040	5084	1045	forward	55
tRNA-Trp	tRNA	5085	5154	70	forward	1
tRNA-Ala	tRNA	5157	5225	69	reverse	1
tRNA-Asn	tRNA	5227	5299	73	reverse	0
tRNA-Cys	tRNA	5330	5395	66	reverse	1
tRNA-Tyr	tRNA	5419	5486	68	reverse	2
COI gene	gene	5488	7038	1551	forward	30
tRNA-Ser	tRNA	7039	7109	71	reverse	0
tRNA-Asp	tRNA	7111	7183	73	forward	1
COII gene	gene	7196	7886	691	forward	14
tRNA-Lys	tRNA	7887	7962	76	forward	0
ATP8 gene	gene	7964	8131	168	forward	1
ATP6 gene	gene	8122	8804	683	forward	16
COIII gene	gene	8805	9588	784	forward	12
tRNA-Gly	tRNA	9589	9661	73	forward	0
NADH3 gene	gene	9662	10012	351	forward	6
tRNA-Arg	tRNA	10015	10084	70	forward	0
NADH4L gene	gene	10085	10381	297	forward	3
NADH4 gene	gene	10375	11756	1382	forward	36
tRNA-His	tRNA	11757	11826	70	forward	1
tRNA-Ser	tRNA	11827	11893	67	forward	2
tRNA-Leu	tRNA	11895	11967	73	forward	1
NADH5 gene	gene	11968	13806	1839	forward	52
NADH6 gene	gene	13803	14324	516	reverse	13
tRNA-Glu	tRNA	14326	14394	69	reverse	0
CYTB gene	gene	14399	15539	1141	forward	31
tRNA-Thr	tRNA	155310	15610	71	forward	3
tRNA-Pro	tRNA	15609	15678	70	reverse	0
control region D-loop	D-loop	15679	16641	967	forward	29

Sequence quality

7,928 reads (average length: 190) were obtained from the 3 long range PCRs and assembled. The read coverage throughout the entire sequence ranges from 12 to 407, with a mean read coverage of 190.0 fold (Fig. 2).

Phylogenetic reconstruction

Mitogenomes from three other *Barbatula* species: *B. barbatula* (Linnaeus, 1758), *B. nuda* (Bleeker, 1864) and *B. toni* (Dybowski, 1869) plus a sequence of *Triplophysa minxianensis* (Wang & Zhu, 1979), *T. rosa* Chen & Yang, 2005 and *Homatula variegatus* (Dabry de Thiersant, 1874) were retrieved from GenBank.

MUSCLE alignment (Edgar, 2004), nucleotide diversity and pairwise distances were performed in Geneious 11.2.2. The best evolutionary model was inferred in jModelTest (Darriba *et al*., 2012) and was GTR+G for both Akaike and Bayesian information criterion. Phylogenetic analysis was inferred by Maximum Likelihood (ML) using RAxML (version 8.2.10) (Stamatakis, 2014) with the GTR+G model and 1000 bootstrap iterations on the CIPRES Science Gateway (Miller *et al.,* 2010) online platform.

RESULTS AND DISCUSSION

Sequence description and phylogenetic analysis

The newly obtained mitogenome has a total length of 16,641 bp and follows the standard vertebrate order. It includes 13 protein-coding genes, 22 transfer RNA genes including 2 tRNA-Leu and 2 tRNA-Ser, two ribosomal RNA genes and a control region (Fig. 2; Tab. II).

Intergenic spaces and overlapping sequences were found. Six oding genes have an incomplete codon stop: NADH2, COII, ATP6, NADH3, NADH4 and Cyt b. The base composition of the entire genome was 28.7% for A, 26.2% for T, 17.9% for G and 27.2% for C.

The ML phylogeny shows high support values for all nodes (Fig. 3) and is consistent with Murienne *et al*. (2016). The *Barbatula* sequences form two monophyletic groups. The first groups the newly sequenced mitogenome with *B. barbatula,* both European. The second includes the two Asiatic nominal species *B. toni* and *B. nuda*. The new sequence clusters with the *B. barbatula* sequence (GenBank Accession number KP715096) with a divergence of 2.3% (pairwise *p*-distance) and 381 differences (Tab. II). The differences between the two sequences are more compatible with affiliation to two distinct lineages or species.

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