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## MITOGENOME ANNOUNCEMENT

**PCR-free shotgun sequencing of the stone loach mitochondrial genome (*Barbatula barbatula*)**Jérôme Murienne<sup>1</sup>, Céline Jeziorski<sup>2,3</sup>, Hélène Holota<sup>1</sup>, Eric Coissac<sup>4</sup>, Simon Blanchet<sup>1,5</sup>, and Gaël Grenouillet<sup>1</sup><sup>1</sup>CNRS, Université de Toulouse III Paul Sabatier, ENFA, UMR5174 EDB (Laboratoire Evolution et Diversité Biologique), Toulouse, France, <sup>2</sup>INRA, UAR1209, Département de Génétique Animale, INRA Auzeville, Castanet-Tolosan, France, <sup>3</sup>GeT-PlaGe, Genotoul, INRA Auzeville, Castanet-Tolosan, France, <sup>4</sup>Laboratoire d'Ecologie Alpine, CNRS UMR, Grenoble, France, and <sup>5</sup>Station Expérimentale du CNRS à Moulis, U.S.R, Moulis, France**Abstract**

The complete mitochondrial genome of the stone loach *Barbatula barbatula* (Linnaeus, 1758) (Actinopterygii: Cypriniformes: Nemacheilidae) has been sequenced using a genome-skimming approach on an Illumina HiSeq 2500 platform. The mitochondrial genome of *B. barbatula* was determined to be 16,630 bp long and presents an organization typical of vertebrate mitogenomes. The mean coverage was 82× with a minimum coverage of 33× for the control region and 52× for the remaining part of the genome. A phylogenetic analysis of the Nemacheilidae family shows the monophyly of the *Barbatula* genus with strong support.

**Keywords**

Fish, genome skimming, illumina, next-generation sequencing

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The recent development of high-throughput sequencing has alleviated the need to rely on fastidious methods for amplifying and sequencing complete mitochondrial genomes. A relatively shallow sequencing allows us to easily retrieve the high copy fractions of the genomes, including the mitochondrial DNA. This “genome-skimming” approach has now been successfully applied to sequence complete mitochondrial genomes from a wide variety of animals including nematodes (Besnard et al., 2014), insects (Cally et al., 2014; Kocher et al., 2014, 2015) and pigeons (Besnard et al., 2015). We here performed a test case of the applicability of our approach to fishes using the stone loach *Barbatula barbatula*. Contrary to previous studies which are based at least partially on alternative sequencing technologies (e.g. Alam et al., 2014), our approach is solely based on the Illumina HiSeq platform (Illumina Inc., San Diego, CA).

Total genomic DNA was sent for library construction and sequencing to the Get-PlaGe core facilities of Genotoul (Toulouse, France). The library was hybridized and sequenced

on 1/24th of a lane of an Illumina HiSeq 2500 flow cell and the resulting data was stored on the NG6 platform (Mariette et al., 2012). From a total of over 14 million paired-end 100 pb reads, 13,570 bp could be assembled into a circular mitochondrial genome of 16,630 bp (GenBank accession KP715096) using a genome walking strategy (see Kocher et al., 2014, for more details). Our results demonstrate that for a relatively small cost (below 280 euros), we could efficiently assemble a complete fish mitogenome with sufficient coverage (the mean coverage was 82× with a minimum coverage of 33× for the control region and 52× for the remaining part of the genome) (Figure 1).

We conducted a maximum likelihood phylogenetic analysis of the family Nemacheilidae based on 12 protein coding genes (all PCGs except *atp8*) from all the complete mitochondrial genomes of the family available to date. The phylogenetic tree reconstructed with RAxML v8.1 (Stamatakis, 2014) shows the monophyly of the *Barbatula* genus (including our sample) with 100% bootstrap frequency.

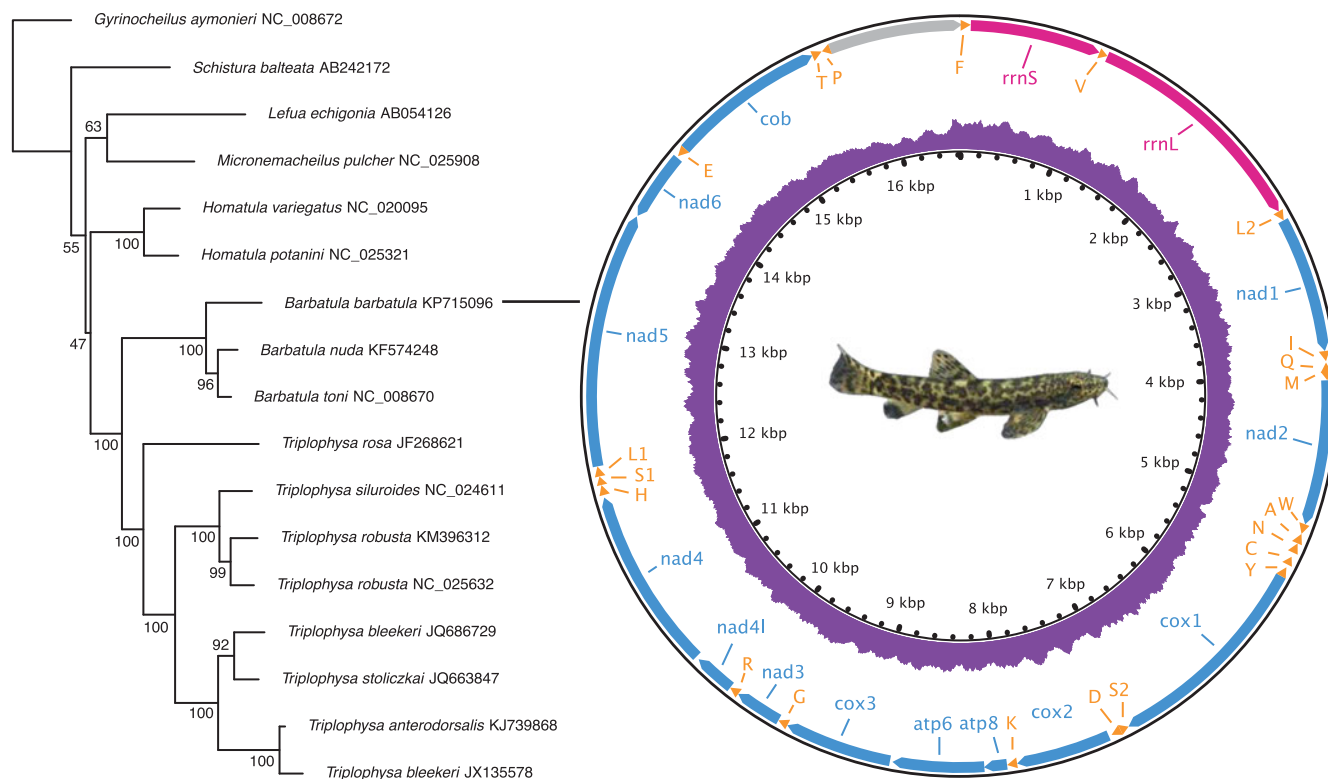


Figure 1. Left panel: maximum likelihood phylogeny of the family Nemacheilidae inferred from all available complete mitochondrial genomes. Posterior probabilities are displayed for each internal node. Right panel: map of the mitochondrial genome of *Barbatula barbatula*. The tRNAs are labeled according to the IUPC-IUB single-letter amino acid codes. The inner graph represents the coverage (min = 33×, max = 115×, mean = 82×).

## Declaration of interest

This work was supported by “Investissement d’Avenir” grants managed by Agence Nationale de la Recherche (CEBA, ref. ANR-10-LABX-25-01; TULIP, ANR-10-LABX-41, and ANR-11-IDEX-0002-02) as well as project METABAR (ANR-11-BSV7-0020). The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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