Mitochondrial DNA

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MITOGENOME ANNOUNCEMENT

# PCR-free shotgun sequencing of the stone loach mitochondrial genome (*Barbatula barbatula*)

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**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

**History**

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The recent development of high-throughput sequencing has alleviated the need to rely and 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 an 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.

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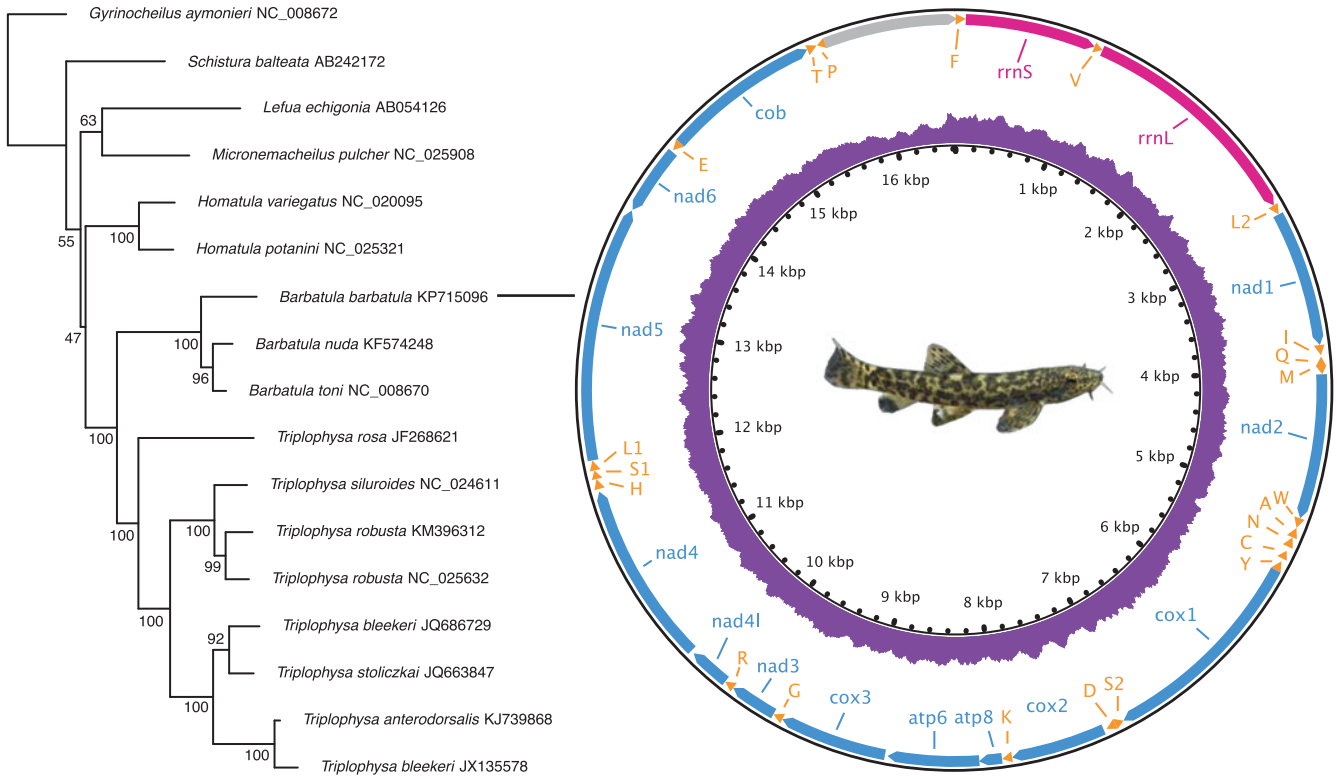


Figure 1. Left panel: maximum likelihood phylogeny of the family Nemacheilidae inferred from all available complete mitochondrial genomes. Posterior probabilities are displayed for each internal node. Right panel: map of the mitochondrial genome of *Barbatula barbatula*. The tRNAs are labeled according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



## Declaration of interest

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## References

Alam MT, Petit III RA, Read TD, Dove AD. (2014). The complete mitochondrial genome sequence of the world's largest fish, the whale shark (*Rhincodon typus*), and its comparison with those of related shark species. Gene 539:44–9.

Besnard G, Bertrand J, Delahaie B. (2015). Valuing museum specimens: High-throughput DNA sequencing using historical collections of New Guinea crowned pigeons (*Goura*). Biol J Linn Soc 115. (in press). doi: 10.111/bij.12494.

Besnard G, Jühling F, Chapuis E, Zedane L, Lhuillier E, Mateille T, Bellafiore S. (2014). Fast assembly of the mitochondrial genome of a plant parasitic nematode (*Meloidogyne graminicola*) using next generation sequencing. C R Biol 337:295–301.

Cally S, Lhuillier E, Iribar A, Garzón-Orduña I, Coissac E, Murienne J. (2014). Shotgun assembly of the complete mitochondrial genome of the neotropical cracker butterfly hamadryas epinome. Mitochondrial DNA. [Epub ahead of print]. doi: 10.3109/19401736.2014.971262.

Kocher A, Guilbert E, Lhuillier E, Murienne J. (2015). Sequencing of the emitochondrial genome of the avocado lace bug *Pseudacysta perseae* (Heteroptera, Tingidae) using a genome skimming approach. C R Biol 338:149–60.

Kocher A, Kamilari M, Lhuillier E, Coissac E, Péneau J, Chave J, Murienne J. (2014). Shotgun assembly of the assassin bug *Brontostoma colossus* mitochondrial genome (Heteroptera, Reduviidae). Gene 552: 184–94.

Mariette J, Escudié F, Allias N, Salin G, Noirot C, Thomas S, Klopp C. (2012). NG6: Integrated next generation sequencing storage and processing environment. BMC Genomics 13:462.

Stamatakis A. (2014). RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–13.