Hermaphroditic freshwater mussel *Anodonta cygnea* does not have supranumerary open reading frames in the mitogenome

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**ABSTRACT**

The complete mitogenome of *Anodonta cygnea* is 15,613 bp long. This compact, circular molecule contains the set of 37 genes, typical for invertebrate mitogenomes, in the same order and orientation as in maternally inherited genomes of other bivalves from the same subfamily. There are only two unassigned regions longer than 200 bp (266 bp and 274 bp) and no indication of any supranumerary open reading frames.

*Anodonta cygnea* (Linnaeus, 1758) is a freshwater mussel from the family Unionidae, distributed in Eurasian waters. The family is several hundred species rich, but most of them are found in North America. They are usually gonochoristic, with the presence of two distinct mitochondrial lineages (M and F), inherited under DUI system (Skibinski et al. 1994; Zouros et al. 1994). This system has been faithfully operating in freshwater mussels for a long time, leading to extreme divergence of the two mitogenomes (Hoeh et al. 2002). Gender-specific anonymous open reading frames (FORF and MORF) have been described in both mitogenomes (Doucet-Beaupré et al. 2010). The few species with secondary hermaphroditism were described, and in case of North American mussels, these always lost the divergent, paternally inherited mitogenome. There were also substantial structural changes in the FORF (now denoted HORF) (Breton et al. 2011).

Here we announce, for the first time, the mitogenome of a European hermaphroditic species from the same family. We were unable to find a distinct paternally inherited mitogenome in sperm of this species so we assume the announced mitogenome to be the only one present.

The sample was taken in July 2009 from a pond in Hamryszko village, central Poland. Identification down to species level was based on diagnostic morphological characters (Piechocki and Dyduch-Falniowska 1993). The specimen is stored under voucher number 328 in the local collection at University of Szczecin. The taxonomic identity was confirmed by comparison of the barcoding *cox1* sequence with the references (Bogan and Roe 2008).

The sequencing strategy followed the previously published three-step protocol (Soroka and Burzyński 2010). Two parts of the mitogenome were amplified with universal primers and sequenced. Species-specific long-range primers were used to amplify the rest of the mitogenome. The LR-PCR products were sequenced by primer walking. The complete mitogenome was assembled in gap4 from Staden package (Staden et al. 2001). Annotations followed the established pipeline (Zbawicka et al. 2007) and were manually curated by comparison with the mitogenome of *A. anatina* (Soroka and Burzyński 2015).

The sequence has been deposited in GenBank under accession number MG385135. Comparative phylogenetic analysis was performed (Figure 1). The protein sequences encoded by the mitogenome differ from the closest relative (*A. anatina* F mitogenome) by approximately 10% (average p-distance, calculated in MEGA7 (Kumar et al. 2016)). No additional ORFs could be identified. In particular, the region containing FORF in *A. anatina* F mitogenome and HORF in *Utterbackia imbecillis* and *Lasmigona compressa* mitogenomes is much shorter and does not contain any ORF of appreciable length in *A. cygnea*.

Of the three cases of secondary hermaphroditism covered by the presented data set, the *A. cygnea* case seems to be the only one without the HORF and also the oldest one (Figure 1; Mitchell et al. 2016). It can be concluded that after the loss of DUI, the supranumerary ORFs can eventually degenerate. This reinforces the hypothesis of the involvement of gender specific mitochondrial ORFs in sex determination of these animals (Breton et al. 2011).
Acknowledgements

The authors thank Małgorzata Ostro (Kazimierz Wielki University in Bydgoszcz, Poland) for help with sample collection.

Disclosure statement

The authors declare no competing interests and are solely responsible for writing this manuscript.

Funding

This work was supported by the Polish Ministry of Science and Higher Education through a Grant no. N 303 364 33 to M. S.

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References

An C, Ouyang S, Zhou C-H, Wu X-P. 2016. The complete F-type mitochondrial genome of Chinese Anodonta arcaeformis (Bivalvia: Unioniidae: Anodontinae). Mitochondrial DNA Part A. 27:1552–1553.

Bogan AE, Roe KJ. 2008. Freshwater bivalve (Unioniformes) diversity, systems, and evolution: status and future directions. J N Am Benthol Soc. 27:349–369.

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D, Suchard MA, Rambaut A, Drummond AJ, Prlic A. 2014. ASUS: a software platform for Bayesian evolutionary analysis. PLoS Comput Biol. 10:e1003537.

Breton S, Beaupré HD, Stewart DT, Piontkivska H, Karmakar M, Bogan AE, Blier PU, Hoeh WR. 2009. Comparative mitochondrial genomics of freshwater mussels (Bivalvia: Unionoidia) with doubly uniparental inheritance of mtDNA: gender-specific open reading frames and putative origins of replication. Genetics. 183:1575–1589.

Breton S, Stewart DT, Shepardson S, Trdan RJ, Bogan AE, Chapman EG, Ruminas AJ, Piontkivska H, Karmakar M, Bogan AE, Blier PU, Hoeh WR. 2011. Comparative mitochondrial genomics of freshwater mussels (Bivalvia: Unionoidia) with doubly uniparental inheritance of mtDNA: gender-specific open reading frames and putative origins of replication. Genetics. 183:1575–1589.

Breton S, Stewart DT, Shepardson S, Trdan RJ, Bogan AE, Chapman EG, Ruminas AJ, Piontkivska H, Hoeh WR. 2009. Comparative mitochondrial genomics of freshwater mussels (Bivalvia: Unionoidia) with doubly uniparental inheritance of mtDNA: gender-specific open reading frames and putative origins of replication. Genetics. 183:1575–1589.

Burzyński A, Soroka M, Mioduchowska M, Kaczmarszczak A, Soll J. 2017. The complete maternal and paternal mitochondrial genomes of Unio crassus: mitochondrial molecular clock and the overconfidence of molecular dating. Mol Phylogenet Evol. 107:605–608.

Doucet-Beaupré H, Breton S, Chapman EG, Blier PU, Bogan AE, Stewart DT, Hoeh WR. 2010. Mitochondrial phylogenomics of the Bivalvia (Mollusca): searching for the origin and mitogenomic correlates of doubly uniparental inheritance of mtDNA. BMC Evol Biol. 10:50.

Fonseca MM, Lopes-Lima M, Eackles MS, King TL, Frouve E. 2016. The female and male mitochondrial genomes of Unio delphinus and the
phylogeny of freshwater mussels (Bivalvia: Unionida). Mitochondrial DNA Part B. 1:954–957.

He F, Wang G, I, Li J. 2016. Complete F-type mitochondrial genome of Chinese freshwater mussels Lamprotula gottschei. Mitochondrial DNA Part A. 27:246–247.

Hoeh WR, Stewart DT, Guttman SI. 2002. High fidelity of mitochondrial genome transmission under the doubly uniparental mode of inheritance in freshwater mussels (Bivalvia: Unionoidea). Evol Int J Org Evol. 56:2252–2261.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 33:1870–1874.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, et al. 2007. Clustal W and Clustal X version 2.0. Bioinformatics. 23:2947–2948.

Lee JH, Choi EH, Kim SK, Ryu SH, Hwang UW. 2012. Mitochondrial genome of the cockscomb pearl mussel Cristaria plicata (Bivalvia, Unionoida, Unionidae). Mitochondrial DNA. 23:39–41.

Lopes-Lima M, Fonseca MM, Aldridge DC, Bogan AE, Gan HM, Ghamizi M, Sousa R, Teixeira A, Varandas S, Zanatta D, et al. 2017. The first Margaritiferidae male (M-type) mitogenome: mitochondrial gene order as a potential character for determining higher-order phylogeny within Unionida (Bivalvia). J Molluscan Stud. 83:249–252.

Mitchell A, Guerra D, Stewart D, Breton S. 2016. In silico analyses of mitochondrial ORFans in freshwater mussels (Bivalvia: Unionoida) provide a framework for future studies of their origin and function. BMC Genomics. 17:579.

Piechocki A, Dyduch-Falniowska A. 1993. Mieczaki (Mollusca). Malże (Bivalvia). [Mollusca (Mollusca). Bivalves (Bivalvia) Fauna Słodkowodna Polski. Warszawa (Poland): PWN. Polish.

Rambaut A. 2009. FigTree, a graphical viewer of phylogenetic trees [Internet]. Institute of Evolutionary Biology, University of Edinburgh. Available from: http://tree.bio.ed.ac.uk/software/figtree

Skibinski DOF, Gallagher C, Beynon CM. 1994. Mitochondrial DNA inheritance. Nature. 368:817–818.

Song X-L, Ouyang S, Zhou C-H, Wu X-P. 2016. Complete maternal mitochondrial genome of freshwater mussel Anodonta lucida (Bivalvia: Unionoidae: Anodontinae). Mitochondrial DNA Part A. 27:549–550.

Soroka M. 2010. Characteristics of mitochondrial DNA of unionid bivalves (Mollusca: Bivalvia: Unionidae). I. Detection and characteristics of doubly uniparental inheritance (DUI) of unionid mitochondrial DNA. Folia Malacol. 18:147–188.

Soroka M, Burzyński A. 2010. Complete sequences of maternally inherited mitochondrial genomes in mussels Unio pictorum (Bivalvia, Unionidae). J Appl Genet. 51:469–476.

Soroka M, Burzyński A. 2015. Complete female mitochondrial genome of Anodonta anatina (Mollusca: Unionidae): confirmation of a novel protein-coding gene (F ORF). Mitochondrial DNA. 26:267–269.

Soroka M, Burzyński A. 2017. Doubly uniparental inheritance and highly divergent mitochondrial genomes of the freshwater mussel Unio tumidus (Bivalvia: Unionidae). Hydrobiologia, In press. Available from: https://link.springer.com/article/10.1007/s10750-017-3113-7

Staden R, Judge DP, Bonfield JK. 2001. Sequence assembly and finishing methods. In: Baxevanis AD, Ouellette BFF, editors. Bioinformatics: a practical guide to the analysis of genes and proteins, Vol. 43, 2nd ed. New York (UK): Wiley.

Wang G, Cao X, Li J. 2013. Complete F-type mitochondrial genome of Chinese freshwater mussel Lamprotula tortuosa. Mitochondrial DNA. 24:513–515.

Wang G, Guo L, Li J. 2016a. The F-type complete mitochondrial genome of Arconaia lanceolata. Mitochondrial DNA Part A. 27:322–323.

Wang G, Xue T, Chen M, Guo L, Li J. 2016b. Complete F-type mitochondrial genome of freshwater mussels Unio douglasiae. Mitochondrial DNA Part A. 27:4021–4022.

Wu R-W, An C-T, Wu X-P, Zhou C-H, Ouyang S. 2016. Complete maternal mitochondrial genome of freshwater mussel Aculamprotula tientsinensis (Bivalvia: Unionidae: Unioninae). Mitochondrial DNA Part A. 27:4520–4521.

Zbawicka M, Burzyński A, Wenne R. 2007. Complete sequences of mitochondrial genomes from the Baltic mussel Mytilus trossulus. Gen. 406:191–198.

Zhou C-H, Ouyang S, Wu X-P, Ding M-H. 2016. The complete maternal mitochondrial genome of rare Chinese freshwater mussel Lepidodesma languii (Bivalvia: Unionidae: Unioninae). Mitochondrial DNA Part A. 27:4615–4616.

Zouros E, Ball AO, Saavedra C, Freeman KR. 1994. Mitochondrial DNA inheritance. Nature. 368:818.