The mitochondrial genome of the ciliate *Pseudourostyla cristata* (Ciliophora, Urostylida)

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**ABSTRACT**

The mitochondrial genomes (mitogenomes) of ciliates are linear and relatively large (~80 kb). Until now, only 11 ciliate mitogenomes, either partial or complete, have been reported. The aim of the present study was to characterize the mitogenome of *Pseudourostyla cristata* (Ciliophora, Urostylida). The resulting mitogenome represents the first complete mitogenome for order Urostylida and is the largest sequenced ciliate mitogenome (~76 kb), containing 31 protein-coding genes, 9 transfer RNAs (tRNAs), 2 ribosomal RNAs (rRNAs), and 21 unclassified open reading frames. Among the 11 ciliates whose mitogenomes have been identified, the mitogenome of *P. cristata* is most similar to that of *Oxytricha trifallax*, which is in the same subclass. However, the mitogenome of *P. cristata* is missing two genes (nad3 and nad6) and three split genes (nad4, nad5, and rpl6) that are found in the mitogenome of *O. trifallax*. Furthermore, the locations of the protein-coding and tRNA genes in *P. cristata* are different than those of the same genes in either *Euplotes minuta* or *E. crassus*, even though the species belong to the same class. This suggests that mitogenome structure is unlikely conserved in class level phylogenetic comparison.

*Pseudourostyla cristata* (Jerka-Dziadosz 1964) Borror, 1972 is a freshwater ciliate that was isolated from Mungyeong Saejae Provincial Park (36°56’45.77”N, 128°4’29.65”E), South Korea, in June 2014. Mitochondria were isolated from ~200 live *P. cristata* specimens. Mitochondrial DNA (mtDNA) extraction, whole genome amplification, and mtDNA sequencing were performed according to Song et al. (2016). DNA of specimen has been stored in the Korea Polar Research Institute (KOPRI), South Korea.

SOAP denovo, SSPACE 2.0 scaffold, and Gap Closer tool were used (Boetzer and Pirovano 2014). The mitogenome was reconstructed using Geneious 6.1.3 (Biomatters, Ltd., Auckland, New Zealand) and the partial cytochrome b gene of *P. cristata* as an initial bait.

The complete mitogenome was annotated using MFANNOT (Beck and Lang 2010), and transfer RNA (tRNA) genes were identified using tRNAscan-SE1.2.1 (Lowe and Eddy 1997) with the default search mode. Gene locations were determined using the genoPlotR package in R (Guy et al. 2010).

The phylogenetic analysis for mitogenome sequences was performed for the five ciliate species including our species (Figure 1). Sequences were aligned using MAFFT (Katoh and Standley 2013). All gaps were removed using Gblocks 0.91b (Talavera and Castresana 2007). The phylogenetic relationships were reconstructed using the maximum likelihood method in the RAxML (Stamatakis 2006).

The resulting mitogenome of *P. cristata* represents the first complete mitogenome for order Urostylida and is the largest ciliate mitogenome (~76 kb) sequenced to date (GenBank accession number: MH888186).

The mtDNA of *P. cristata* included 19 protein-coding genes with functions in the respiratory chain (complex I – nad1, nad2, nad3, and nad6) and three split genes (nad4, nad5, and rpl6) that are found in the mitogenome of *O. trifallax*. Furthermore, the locations of the protein-coding and tRNA genes in *P. cristata* are different than those of the same genes in either *Euplotes minuta* or *E. crassus*, even though the species belong to the same class. This suggests that mitogenome structure is unlikely conserved in class level phylogenetic comparison.
gene sequences and locations, and *E. minuta* and *E. crassus*, which belong to the same genus, have identical protein-coding genes. Although there are not enough published ciliate mitogenomes to compare or analyze phylogenetically, more information of related species at the level of family as well as genus could be useful for more detailed study of mitogenome evolution and phylogenetic relationships in ciliates.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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