Complete Mitochondrial and Plastid DNA Sequences of the Freshwater Green Microalga *Medakamo hakoo*

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Running Head: The complete organellar genomes of the green alga *Medakamo hakoo*

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Abstract

Here we report the complete organellar genome sequences of *Medakamo hakoo*, a green alga identified in freshwater in Japan. It has 90.8-kb plastid and 36.5-kb mitochondrial genomes containing 80 and 33 putative protein coding genes, respectively, representing the smallest organellar genome among currently known core Trebouxiophyceae.

Body

The genomes of various organisms have been sequenced, and knowledge has been accumulated about not only biodiversity but also universal genes that are conserved among all organisms. These findings will be updated as new species are discovered; therefore, exploring new species will continue to be important. Recently, we discovered a green alga, *Medakamo hakoo*, in freshwater in Japan (1, 2). Based on the preliminary microphotometric observation, we predicted that it would have the smallest organellar and nuclear genomes among green plants, which prompted us to investigate its genomes. Whole-genome sequencing was performed with the PacBio RS II system (Pacific Biosciences, Menlo Park, CA, USA), and the read sequences were assembled *de novo* with the RS HGAP Assemble.3 program run by SMRT Analysis software 2.3.0 (Pacific Biosciences). Genes encoded by the chloroplast/mitochondrial genomes were annotated using GeSeq (3) with the following organellar genomes as references: *Chlorella vulgaris* (NC_001865/NC_045362), *Coccomyxa subellipsoidea* C-169 (NC_015084/NC_015316), and *Botryococcus braunii* (NC_025545/NC_027722) because *M. hakoo* belongs to Trebouxiophyceae, as described in Kato *et al.* (submitted). StructRNAfinder (4) and RNAweasel (5) were used to predict rRNA. We obtained two contigs representing the organellar genomes (Fig.1). The chloroplast genome (cpDNA) is 90,872 bp and has a GC content of 41.4%. We functionally annotated 80 protein-coding genes, including 31 photosynthesis-related genes (components of photosystem I and II, the cytochrome *b₆f* complex, and ATP synthase subunits) and 29 tRNA and 2 rRNA genes. The cpDNA of *M. hakoo* is slightly smaller than that of its most closely related alga, *Choricystis parasitica* (6); they share most genes but not their order. The mitochondrial genome (mtDNA) of *M. hakoo* is 36,544 bp and has a GC content of 35.2%. It is the smallest in size and the gene density appears high compared with other Trebouxiophyceae mtDNAs. It comprises 25 tRNAs, 3 rRNAs, and 33 predicted protein-coding genes, including 18 respiratory chain complex genes and ATP synthase, as well as *tatC* and a homing endonuclease gene.

Another notable characteristic of the mtDNA was that almost all the genes were localized to a single strand. This mtDNA characteristic is also observed among core Trebouxiiales, including Trebouxiophyceae sp. MX-AZ01 (7) and *Coccomyxa subellipsoidea* C-169 (8) and *Botryococcus braunii* (9,10); however, the group II introns of the *trnH* (gug), and *trnW* (cca) genes conserved in these species (10) were not predicted in *M. hakoo*. Normally in Trebouxiophyceae, *rpl10* gene was located next to *rps19* (10), but *M. hakoo* had another ORF that was not similar to other known genes. As expected, the size of the *M. hakoo* organellar genome was relatively small, and the gene density was high. It will be important for future algal research to focus on how mitochondrial genes encoded in a single strand are expressed (whether they form operons), and conversely how the separately encoded chloroplast rRNA genes, which should normally form an operon structure, exhibit coordinated transcription.

Public availability and accession numbers.

The cpDNA and mtDNA genome sequences were deposited in DNA Data Bank of Japan (<https://www.ddbj.nig.ac.jp/index-e.html>) with accession numbers LC604816 and LC604817, respectively.

Acknowledgments

This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (16H04813 and 19H03260) to T.K. and by JST-CREST (Grant Number JPMJCR20S6) to S.Matsunaga. The authors thank Mr. Brody Frink (Kyoto University) for editing a draft of this manuscript.

Figure legend

Fig. 1. Complete chloroplast and mitochondrial genomes of *Medakamo hakoo*. The both genomes were plotted using OrganellarGenomeDRAW (11). The inner gray circle illustrates the G+C content of the genome.

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