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ORIGINAL ARTICLE

Mitochondrial genome of *Thecamoeba quadrilineata* – the first mt genome among the representatives of the order Thecamoebida (Amoebozoa, Discosea)

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Summary

We have sequenced and described the mitochondrial genome of *Thecamoeba quadrilineata* (Amoebozoa, Discosea, Thecamoebida). This is the first sequenced mitochondrial genome for amoebae of Thecamoebida lineage. The circular mitochondrial DNA of this species has 50942 bp in length and contains 23 protein-coding genes, 2 ribosomal RNAs, 18 transfer RNAs, and 26 open reading frames. In contrast with the shorter mitochondrial genomes of vannellid amoebae, it shows no evidence of RNA editing. This finding supports the hypothesis on the multiple origins of editing in different phylogenetic lineages of Amoebozoa.

Key words: Amoebozoa, Thecamoebida, mitochondrion, mitochondrial genome

Abbreviations: mt – mitochondria; *cox1-3* – cytochrome oxidase subunit I, II, and III genes; *tRNA* – transfer RNA genes; *rrnL, rrnS* – ribosomal RNA genes; ORFs – open reading frames; PCGs – protein-coding genes; *rps* – small ribosomal subunit protein genes; *rpl* – large ribosomal subunit protein genes

Introduction

Mitochondrial genomes (mt genomes) of Amoebozoa are sequenced for a limited number of phylogenetic lineages. The majority of known mt genomes belong to Centramoebida, Eumycetozoa and Vannellida lineages; there are only a few genomes belonging to the representatives of other Amoebozoa clades. Until now, far not all phylogenetic lineages have been sampled (reviewed by Bondarenko et al., 2019a). The mitochondrial genome of Amoebozoa shows significant differences in size and gene composition. While closely related amoebozoan species show almost identical mt genomes (Bondarenko et al., 2019b; Karlyshev, 2019), the level of mt genome synteny drops dramatically as the phylogenetic distance increases (Bondarenko et al., 2018a, 2018b, 2018c). Many of the sequenced mt genomes show extensive RNA editing, and among them the representative of Myxogastria – namely, *Physarum*

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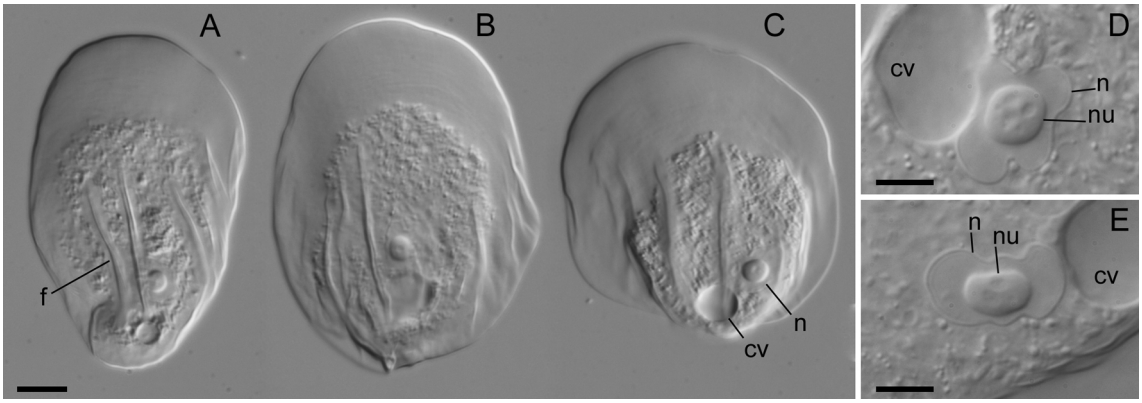

Fig. 1. Light microscopy of *Thecamoeba quadrilineata* strain CCAP 1583/10. A-C – Locomotive forms; D and E – higher magnification of the cell showing the granuloplasm and the nucleus. *Abbreviations*: cv – contractile vacuole, f – dorsal fold, n – nucleus, nu – nucleolus. Scale bars: A-C – 10 μm, D and E – 5 μm.

polycephalum – demonstrates one of the most complex and extensive patterns of RNA editing among eukaryotes (Takano et al., 2001; Houtz et al., 2018). However, lineages showing extensive editing are often located in the crown of the tree, while representatives of basal clades may show little or no editing (Bondarenko et al., 2019c). These data vote for the independent origin of editing in different lineages of Amoebozoa. It makes the study of the distribution, patterns and mechanisms of RNA editing among Amoebozoa especially fascinating and important and may lead to new findings in this field. However, these studies are seriously limited by the absence of a comprehensive picture of the distribution of RNA editing phenomena across the Amoebozoa tree.

The present paper reports data on the mitochondrial genome of *Thecamoeba quadrilineata* (Carter, 1856) Lepşi, 1960. This species shows no RNA editing. If we assume the current phylogeny of Discosea clade (Kang et al., 2017; Melton et al., 2018), our finding further evidences for the independent origin of editing in different Amoebozoa lineages.

Material and methods

The studied strain *Thecamoeba quadrilineata* CCAP 1583/10 was obtained from Dr. Rolf Michel. He maintained it as a host for *Nucleophaga amoebae* strain KTq2 (Michel, 2008; Michel et al., 2009). The culture was maintained on NN agar (Panreac agar-agar, American Type QB, Spain) as described by Page (1988) made on PJ medium (Prescott and James, 1955) on accompanying bacteria. Live cells were photographed on object slides (wet mounts in PJ medium) using a Leica DM2500 microscope (Fig. 1). The SSU sequence of this strain was obtained by Claudia Wylezich and deposited in GenBank under the number DQ122381 (see Walochnik et al., 2003; Michel et al., 2006; Kamyshatskaya et al., 2018).

To obtain a DNA sample, individual amoebae cells were collected from culture dishes using a tapered-tip Pasteur pipette, washed three times in Millipore-sterilized (0.2 μm pore) PJ medium, and placed with 1–2 μl of the medium in 200-μl PCR tubes. DNA was extracted using the Arcturus PicoPure DNA Extraction Kit (Thermo Fischer Scientific, USA). The extraction mixture was prepared according to the manufacturer's instructions; 10 μl of the mixture was added to the tube containing the single cell. Further, we performed whole genome amplification using the REPLI-g Single Cell DNA Amplification Kit (Qiagen, Hilden, Germany). Multiple Displacement Amplification (MDA) was performed according to the manufacturer's instructions. Approximately 127 million reads with a length of 150 bp were obtained using HiSeq 2500 sequencing system (Illumina). Quality control checking of raw sequence data was performed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), SPAdes assembler was used for *de novo* mitochondrial genome assembly (Bankevich et al., 2012). An annotation of the mitochondrial genome sequence was performed using the MITOS web server (Bernt et al., 2013a). Artemis was used to visualize annotation files, manual correction of gene boundaries, and open reading frames (ORFs) search (version 16.0; Rutherford et al., 2000). All

Table 1. Nucleotide composition characteristics of *Thecamoeba quadrilineata* mitochondrial genome.

Species	GC%	A%	T%	G%	C%	AT-skew	GC-skew
Thecamoeba quadrilineata	23.92	41.06	35.02	13.82	10.10	0.079	0.155

protein-coding genes (PCGs) boundaries were verified by manual comparison with the orthologs in other amoebozoans. Genes coding tRNAs were positioned with tRNAcan-SE Search Server v.1.21 (Lowe and Eddy, 1997). Strand asymmetry was calculated using the formulae: AT skew= [A-T]/ [A+T] and GC skew= [G-C]/ [G+C], for the H-strand (Perna and Kocher, 1995). The physical map of the mt genome was generated by our original script written in Python.

Results and discussion

The mitochondrial genome of *Thecamoeba quadrilineata* is a double-stranded circular DNA molecule with a length of 50942 bp (Fig. 2). It is the first sequenced mt genome among amoebae belonging to Thecamoebida lineage (order Thecamoebida). It shows GC content of 23.92% (Table 1), which is a rather low level. The prevalence of adenine over thymine and guanine over cytosine in the majority strand provides both positive AT-skew and GC-skew. This picture of AT-skew resembles the one observed in many other organisms (e.g., Bernt et al., 2013b; Bondarenko et al., 2019b). Therefore, the nucleotide composition of *Thecamoeba quadrilineata* mt genome is significantly biased toward A and T bases, which unavoidably leads to the predominance of certain codons and amino acids in proteins.

Thecamoeba quadrilineata mt genome contains the set of 23 PCGs (*atp1, 6, 9, cob, cox1-3, nad1-5,7,9,11, rpl* and *rps* genes), 18 tRNA, two rRNA genes (*rrnL* and *rrnS*) and 28 open reading frames (ORFs) (Table 2). All genes and ORFs are located on H-strand.

The set of PCG genes differs from other known Amoebozoa mt genomes by the absence of *nad4l, nad5, nad6* and *cob* genes. BLAST search for *nad4l* provides a set of low-significant matches in the region cor-responding to *orf 9* (Table 2, Fig. 2). The set of *rpl* and *rps* genes in the mt genome of this strain also differs from that in other known Amoebozoa mt genomes (Burger et al., 1995; Ogawa et al., 2000; Greninger et al., 2015; Tanifuji et al., 2017; Bondarenko et al., 2018a, 2018b, 2018c). The total length of all PCGs in *Thecamoeba quadrilineata* mt genome, excluding termination codons, is 22022 bp, which constitutes 43.2% of the total genome length. It is due to the fact that over 40% of the mt genome is occupied by ORFs (total length is 20460 bp) and the remaining 6.5% are tRNA, rRNA and intergenic regions. This mt genome of *Thecamoeba quadrilineata* differs from other known Amoebozoa mt genomes by high density in genes and ORFs. The mt genome of this species has a significant number of gene overlaps and only two non-coding regions longer than 100 bp (Table 2). The largest overlap is 44 bp; it is located between *orf7* and *orf8*. The non-coding regions constitute 1398 bp in total and 2.74% of the total mt genome size (Table 2). The largest non-coding region is 454 bp long; it is located between $tRNA^{His}$ and *orf5*. Also, the mt genome contains two rather long ORFs with lengths 2163 and 3216 bp (*orf3* and *orf 7*, respectively). All ORFs are unique to this mt genome and have no homologs among other Amoebozoa.

There are two alternative start codons in *Thecamoeba quadrilineata* mt genome. Most PCGs and ORFs use ATG as a start codon, while *orf8* uses ATA, and orf15 uses ATT as a start codon. There are only two stop codons in *Thecamoeba quadrilineata* mt genome (TAA and TAG); TGA stop codon was not found in this mt genome. In contrast to most of sequenced mt genomes of Amoebozoa, mt genome in *Thecamoeba quadrilineata* uses the genetic code 1 (the standard code).

The large and the small ribosomal RNA genes (*rrnL* and *rrnS*) in *Thecamoeba quadrilineata* mt genome are located close to each other between $tRNA^{Met}$ and $tRNA^{Ala}$ (Table 2, Fig. 2). The length of *rrnL* and *rrnS* is 2874 bp and 1911 bp, respectively. tRNA genes have a total length of 1191 bp, and most of them are located between *rrnS* and *orf5* genes. All tRNAs have the typical cloverleaf secondary structure. This mt genome contains additional arginine, serine, and three methionine tRNA genes. These features are not unique. Three methionine tRNA genes are also known in other Amoebozoa mt genomes (Pombert et al., 2013; Bondarenko et al., 2018c, 2021). We observed significant differences in the nucleotide composition between $tRNA^{Met}$ and the

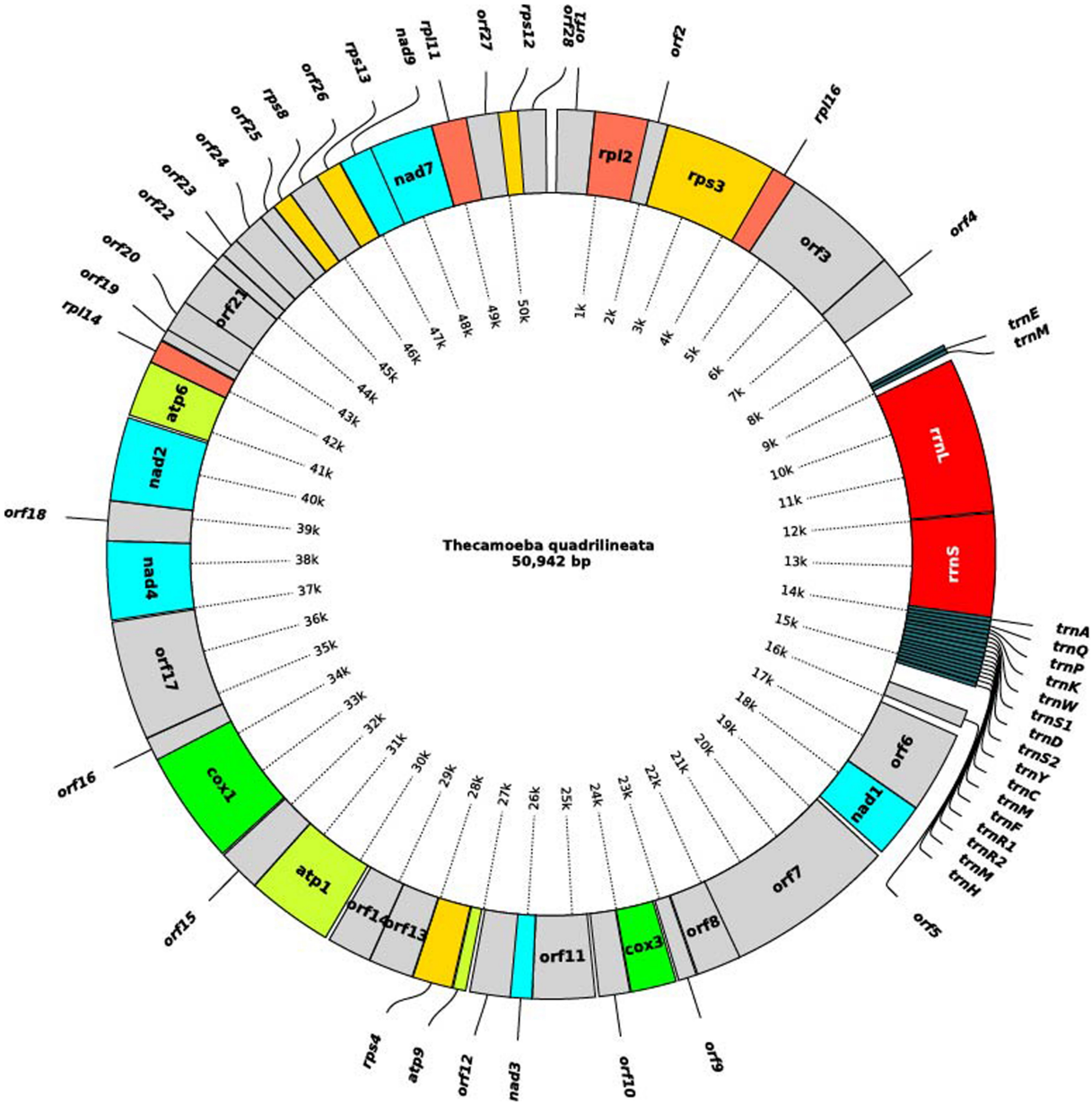

Fig. 2. Mitochondrial genome map of *Thecamoeba quadrilineata*. The tRNA genes are labeled based on the IUPACIUB single-letter amino acid codes.

other two duplicates of this gene. These differences and location of obtained duplications suggest the ancient nature of $tRNA^{Met}$ duplication compared with $tRNA^{Arg}$ and $tRNA^{Ser}$, which show much smaller differences in the nucleotide composition between the duplicates of these genes.

If we accept the current phylogeny of Discosea clade as revealed by Kang with coauthors (2017) and Melton with coauthors (2018), our finding further evidences for the independent origin of editing in different Amoebozoa lineages. Among the lineages constituting the Flabellinia clade, up to now editing is known in Vannellida. Available data on Dactylopodida clade (sister clade to Vannellida) show no evidence of RNA editing in sequenced mitochondrial genomes (Bondarenko et al., 2019c). Yet, several sequences of the Cox I gene of *Korotnevella* spp. deposited with the GenBank suggest the presence of frameshift in poly-T areas. Also, it might be a sequencing problem (Zlatogurski et al. 2016). Another case of RNA editing is known in Acanthopodida, which belongs to Centramoebia clade (Burger et al., 1995; Fučíková and Lahr, 2016). The present finding is the first evidence

Table 2. *Thecamoeba quadrilineata* mitochondrial genome organization.

Gene	Strain	Position (start-stop)	Length (bp)	Intergenic space (bp)	Start codon	Stop codon
orf1	+	110-799	690	209	ATG	TAG
rpl2	+	803-1807	1005	-3	ATG	TAA
orf2	+	1788-2153	366	-20	ATG	TAA
rps3	+	2159-4249	2091	-5	ATG	TAA
rpl16	+	4242-4709	468	-8	ATG	TAA
orf3	+	4710-6872	2163	0	ATG	TAA
orf4	+	6875-7678	804	2	ATG	TAA
trnE	+	8771-8841	71	-8		
trnM1	+	8860-8931	72	18		
rrnL	+	9077-11950	2874	45		
rrnS	+	11978-13890	1911	27		
trnA	+	13890-13961	72	-1		
trnQ	+	13970-14041	72	8		
trnP	+	14048-14119	72	6		
trnK	+	14125-14197	73	5		
trnW	+	14214-14284	71	16		
trnS1	+	14291-14375	85	6		
trnD	+	14381-14453	72	5		
trnS2	+	14463-14547	85	9		
trnY	+	14555-14637	83	7		
trnC	+	14644-14714	71	6		
trnM2	+	14719-14790	72	4		
trnF	+	14795-14867	73	4		
trnR1	+	14870-14944	75	2		
trnR2	+	14944-15015	72	-1		
trnM3	+	15027-15098	72	11		
trnH	+	15119-15189	71	20		
orf5	+	15653-16015	363	454		
orf6	+	16140-17657	1518	124	ATG	TAA
nad1	+	17662-18675	1014	4	ATG	TAA
orf7	+	18770-21961	3192	-6	ATG	TAA
orf8	+	21918-22748	831	-44	ATA	TAA
orf9	+	22773-23111	339	23	ATG	TAA
cox3	+	23160-24005	846	48	ATG	TAA
orf10	+	24025-24597	573	19	ATG	TAA
orf11	+	24664-25818	1155	66	ATG	TAA
nad3	+	25824-26237	414	5	ATG	TAA
orf12	+	26221-26967	747	-17	ATG	TAA
atp9	+	27042-27278	237	74	ATG	TAG
rps4	+	27302-28039	738	23	ATG	TAA
orf13	+	28052-28885	834	12	ATG	TAA
orf14	+	28892-29713	822	6	ATG	TAA
atp1	+	29790-31397	1608	76	ATG	TAG
orf15	+	31402-32187	786	4	ATG	TAA
cox1	+	32224-34326	2123	36	ATG	TAA
orf16	+	34289-34744	456	-38	ATT	TAA

Table 2. (Continuation).

orf17	+	34752-36962	2211	7	ATG	TAG
nad4	+	36998-38449	1452	35	ATG	TAA
orf18	+	38453-39187	735	3	ATG	TAG
nad2	+	39194-40735	1542	6	ATG	TAA
atp6	+	40790-41830	1041	54	ATG	TAA
rpl14	+	41834-42262	429	3	ATG	TAA
orf19	+	42282-42509	228	19	ATG	TAA
orf20	+	42499-43095	597	-11	ATG	TAA
orf21	+	43088-43936	849	-8	ATG	TAA
orf22	+	43942-44199	258	5	ATG	TAA
orf23	+	44206-44550	345	6	ATG	TAA
orf24	+	44555-45169	615	4	ATG	TAA
orf25	+	45172-45471	300	2	ATG	TAA
rps8	+	45480-45854	375	8	ATG	TAA
orf26	+	45859-46434	576	4	ATG	TAA
rps13	+	46436-46918	483	1	ATG	TAA
nad9	+	46930-47559	630	11	ATG	TAA
nad7	+	47546-48727	1182	-14	ATG	TAA
rpl11	+	48733-49386	654	5	ATG	TAA
orf27	+	49379-50011	633	-8	ATG	TAG
rps12	+	49972-50358	387	60	ATG	TAG
orf28	+	50330-50842	513	-29	ATG	TAA

for the absence of editing in Thecamoebida. The Thecamoebida clade belongs to Flabellinia, but branches earlier than Dactylopodida/Vannellida clade (Kang et al., 2017; Melton et al., 2018). So, if the absence of editing in Thecamoebida is further confirmed with sequences or a larger number of mt genomes, it would prove that the point of origin of RNA editing in Vannellida, belonging to Flabellinia clade, is isolated in the phylogenetic tree from that in Acanthopodida, which belongs to Centramoebia clade.

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