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The complete mitochondrial genome of *Chlorostoma lischkei* (Gastropoda: Tegulidae) using Illumina HiSeq sequencing

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ABSTRACT

The complete mitochondrial genome of small sea snail, *Chlorostoma lischkei* (Tapparone Canefri, 1874) (Gastropoda: Tegulidae) was determined in this study, using Illumina Hiseq sequencing. The mitogenome length was 17,980 bp. Its gene arrangement was similar to that of mitogenome of Tegulids, having 13 protein-coding genes and 2 ribosomal RNAs. However, *C. lischkei* had 24 tRNA genes with two more tRNAs than others. It had an overall A+T content of 79.7% (27.3% A, 7.5% C, 12.8% G, and 52.4% T). Our results could be used for future taxonomic and evolutionary studies of subclass Vetigastropoda.

Keywords: *Chlorostoma lischkei*, Mitogenome, Vetigastropoda, Tegulidae

INTRODUCTION

Vetigastropods are the most diverse living gastropods with approximately 3,700 species (Knight *et al*., 1960; Ponder & Lindberg, 1997; Lee *et al*, 2016). Phylogenetic relationships of Vetigastropoda remain controversial due to the classification system initially based on morphological features (Salvini-Plawen & Haszprunar, 1987; Haszprunar, 1987a, b, c, 1988; Ponder & Lindberg, 1996, 1997; Hedegaard, 1997) and recent phylogenetic classification based on gene analysis (Harasewych *et al*., 1997; Colgan *et al*., 2003; McArthur & Harasewych, 2003; Geiger & Thacker, 2005; Yoon & Kim, 2005; Williams & Ozawa, 2006; Kano, 2008; Williams, Karube & Ozawa, 2008; Aktipis & Giribet, 2010, 2012; Aktipis, Boehm & Giribet, 2011; Williams, 2012; Uribe *et al*., 2016; Lee *et al* 2016). Especially, superfamily Trochoidea belonging to subclass Vetigastropoda has a very complex taxonomic history (Williams & Ozawa 2006; Kano 2008; Williams *et al*. 2008). Systematics of family Tegulidae in superfamily Trochoidea has been in a state of flux using morphological or molecular data. It has been the most difficult one to resolve. Complete mitogenome sequences can be used to reconstruct robust phylogenies if they are applied at proper taxonomic levels (Osca *et al*., 2015). They are now widely used for resolving branching lineages (Smith *et al*., 1993; Boore & Brown, 1994, 2000; Boore, Lavrov & Brown, 1998; Saccone *et al*., 1999; Stöger & Schrödl, 2013; Bernt *et al*., 2013; Lee *et al* 2016). In this study, we describe the complete mitogenome of *Chlorostoma lischkei* (Tapparone Canefri, 1874) in family Tegulidae through Illumina HiSeq sequencing.

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MATERIALS AND METHODS

1. Sample collection, mtDNA extraction and amplification

The specimen was collected from the intertidal zone of Goseong, Gangwon-do, Republic of Korea, on June 25th, 2016 . One live specimen of *Chlorostoma lischkei* was used for this study. Following three steps of mt DNA enrichment procedure (mitochondrial isolation, mt DNA extraction and amplification of mt DNA) of

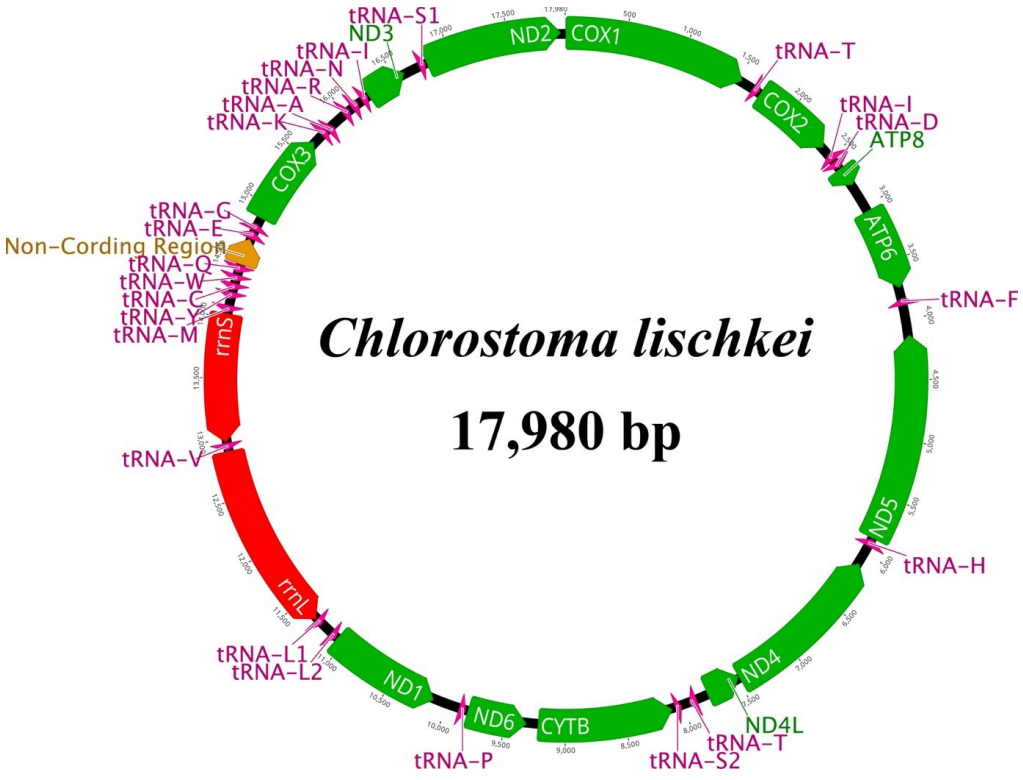

Fig. 1. Circular representation of the complete mitochondrial genome for *Chlorostoma lischkei*.

mt DNA enrichment procedure, mtDNA was extracted and amplified using a Qproteome Mitochondrial Isolation Kit (Qiagen Co., Germany), an E.Z.N.A Mollusc DNA Kit (Omega Co., USA), and a REPLI-g Mitochondrial DNA Kit (Qiagen Co., Germany) according to the manufacturer's protocol, respectively.

2. Next-generation sequencing (NGS), data analysis and annotation

Amplified mt DNA of *C. lischkei* was sequenced using Illumina HiSeq2000 platform at Biomedic (Bucheon, Korea). Short-read DNA sequences were assembled using SOAPdenovo2 (Luo *et al*., 2012) and analyzed using Geneious 9.1.8 (Biomatters Ltd,

Table 1. Nucleotide composition of the mitochondrial genome of *C. lischkei*

Nucleotide	Length (bp)	T (%)	C (%)	A (%)	G (%)	A+T (%)	G+C (%)
Entire sequence	17,980	52.4	7.5	27.3	12.8	79.7	20.3
Protein coding sequence	11,430	39.7	15.7	24.8	19.8	64.5	35.5
Codon position							
1st	3,810	32.0	15.3	25.1	27.1	57.1	42.9
2nd	3,810	44.0	20.6	17.5	17.9	61.6	38.4
3rd	3,810	43.0	11.1	31.6	14.3	74.6	25.4
Ribosomal RNA gene sequence	2,674	34.9	12.0	33.1	20.0	68.0	32.0
Transfer RNA gene sequence	1,655	33.2	14.6	30.3	21.9	63.5	36.5
Non-coding region	234	30.8	17.1	39.7	12.4	70.5	29.5

Table 2. Mitochondrial genome organization of *C. lischkei*

Gene / region	Position		Size		Codons		Intergenic sequence
	Start	Finish	No. of nt	No. of aa*	Initiation	Termination	
cox1	1	1,536	1,536	511	ATG	TAA	104
trnT	1,641	1,706	66				6
cox2	1,713	.2,408	696	231	ATG	TAA	63
trnI	2,472	2,542	71				8
trnD	2,551	2,626	76				0
atp8	2,627	2,803	177	58	ATG	TAG	241
atp6	3,045	3,743	699	232	ATG	TAA	75
trnF	3,819	3,886	68				248
nad5	4,135	5,877	1,743	580	ATG	TAA	0
trnH	5,878	5,945	68				159
nad4	6,105	7,496	1,392	463	ATG	TAA	-7
nad4L	7,490	7,789	300	99	ATG	TAA	98
trnT	7,888	7,961	74				57
trnS$_2$	8,019	8,084	66				15
cytb	8,100	9,239	1,140	379	ATG	TAA	83
nad6	9,323	9,289	507	168	ATG	TAG	5
trnP	9,835	9,901	67				221
nad1	10,123	11,076	954	317	ATG	TAA	1
trnL$_2$	11,078	11,145	68				96
trnL$_1$	11,242	11,309	68				0
rrnL	11,310	12,904	1,595				0
trnV	12,905	12,972	68				0
rrnS	12,973	14,051	1,079				0
trnM	14,052	14,120	69				46
trnY	14,167	14,233	67				3
trnC	14,237	14,308	72				6
trnW	14,315	14,381	67				9
trnQ	14,391	14,459	69				0
NCR	14,460	14,693	234				0
trnE	14,694	14,763	70				5
trnG	14,769	14,836	68				41
cox3	14,878	15,657	780	259	ATG	TAG	78
trnK	15,736	15,803	68				-5
trnA	15,799	15,868	70				117
trnR	15,986	16,054	69				23
trnN	16,078	16,146	69				28
trnI	16,175	16,243	69				4
nad3	16,248	16,601	354	117	ATG	TAA	128
trnS$_1$	16,730	16,797	68				3
nad2	16,801	17,952	1,152	383	ATG	TAA	28

*:Stop codons were not included. nt; nucleotide, aa; amino acid

Auckland, New Zealand). Thirteen mt protein coding genes (PCGs) were annotated by identifying their open-reading frames (ORF) and then comparing them with other reported mollusk mt genomes using DOGMA (Wyman *et al*., 2004). All transfer RNA (tRNA) genes were identified with tRNAscan-SE 1.21 (Schattner *et al*., 2005) and ARWEN 1.2 (Laslett & Canbäck, 2008) to infer cloverleaf secondary

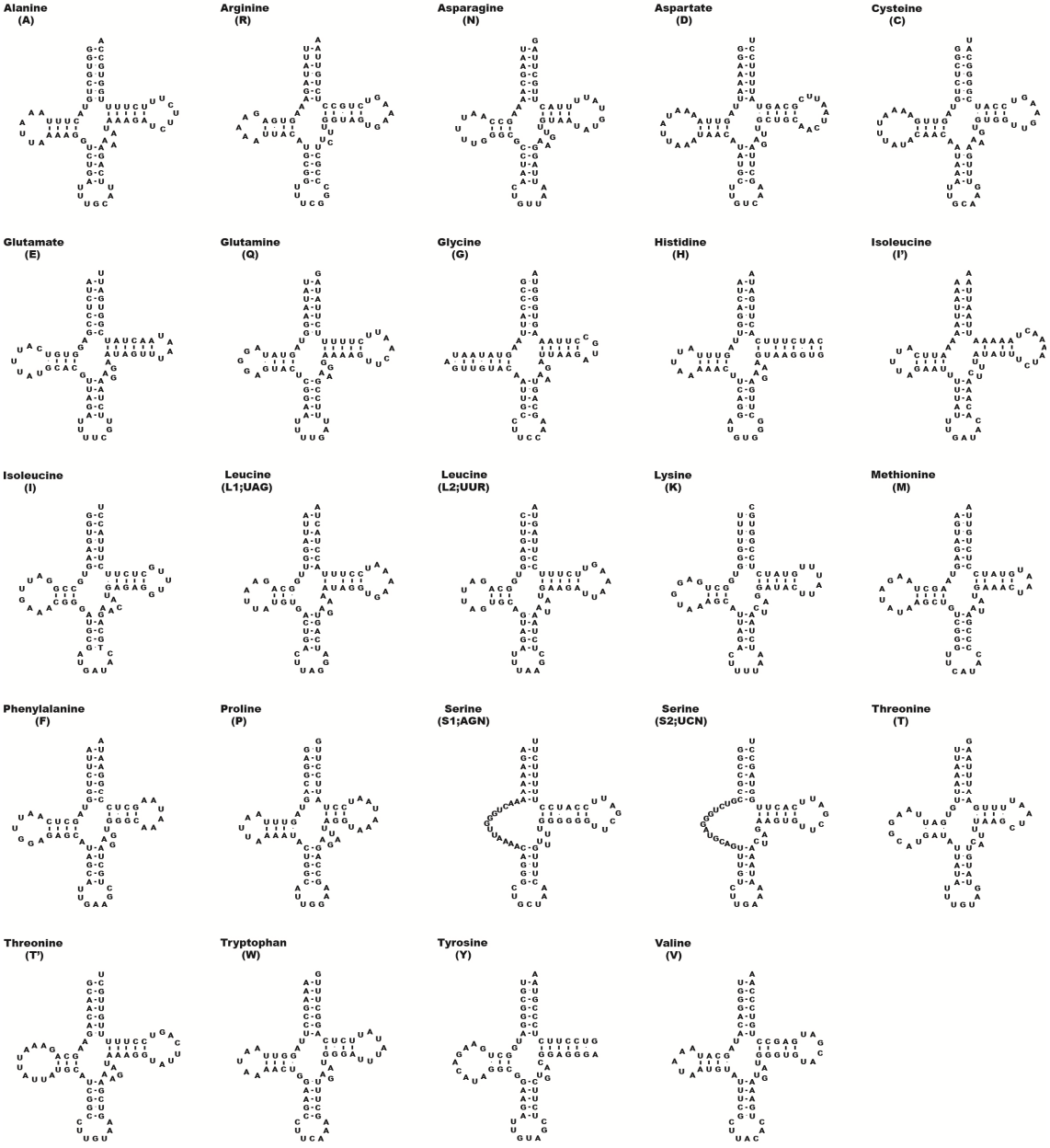

Fig. 2. Putative secondary structures of 24 mitochondrial transfer RNAs of *Chlorostoma lischkei*.

structures. The ribosomal RNA (rRNA) genes were identified by sequence comparison with other reported mollusk mt genomes and assumed to extend to the boundaries of neighboring genes.

3. Phylogenetic analysis

The mitogenome of *C. lischkei* was included in phylogenetic analyses with 19 vetigastropod mitogenomes available from GenBank. Nucleotide sequences of all 13 protein-coding genes were used to reconstruct a phylogenetic tree. Nucleotide sequences were aligned using the automatic multiple-alignment program MUSCLE (Edgar, 2004). PartitionFinder v. 2.1.0 (Lanfear *et al*., 2012) was used to find the best partition

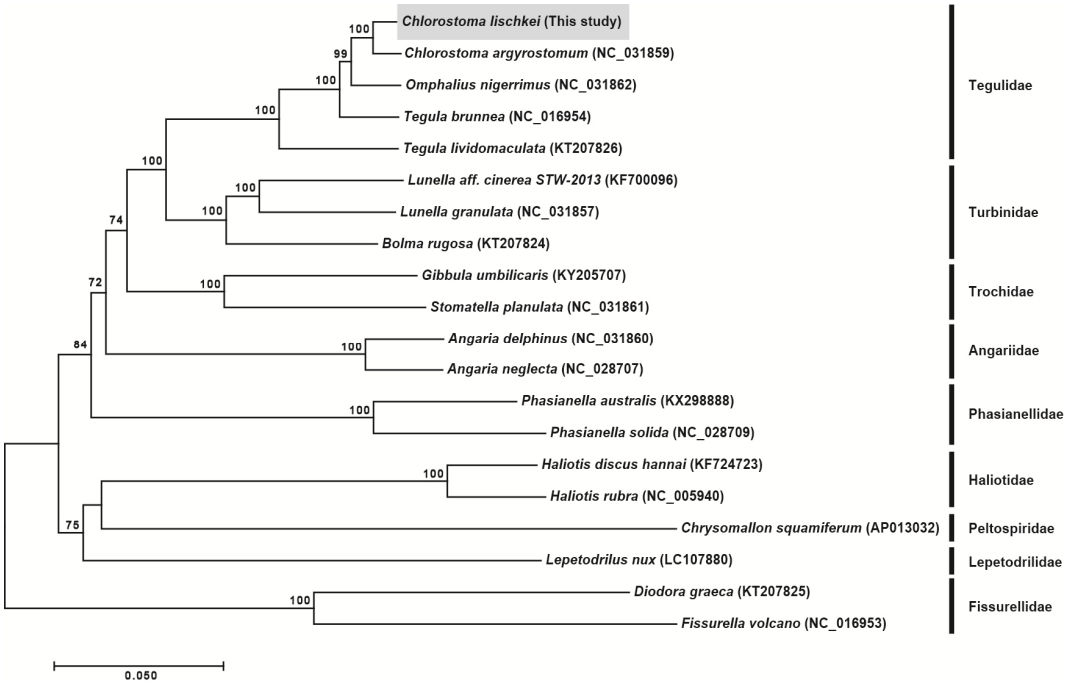

Fig. 3. Molecular phylogeny of *Chlorostoma lischkei* and the others related species in Vetigastropoda using concatenated 13 PCGs nucleotide dataset. The phylogenetic tree is constructed by Maximum likelihood (ML) method based on 20 mitogenome sequences including *C. lischkei* (This study). Bootstrap replicates were performed 1,000 times. Bootstrap values above 60% were indicated on the cladogram.

scheme and the best-fit model for nucleotide sequence alignments. A phylogenetic tree of Vetigastropods including *C. lischkei* was reconstructed using the maximum likelihood (ML) method in MEGA7 (Kumar *et al.*, 2016).

Results and Discussion

The complete mitogenome of *C. lischkei* was found to be 17,980 bp in length (Fig. 1). The overall base composition was 27.3% of A, 52.4% of T, 7.5% of C, and 12.8% of G, showing a slight A-T bias (79.7%) (Table 1). The genome contained full complements of 13 protein coding genes (PCGs), two ribosomal RNA genes, and one non-coding region (NCR). However, *C. lischkei* had two more tRNAs than others. It had a total of 24 tRNAs including tRNA-Thr (*trnT*) and tRNA-Ile (*trnI*). Mitochondrial genome organization of *C. lischkei* is shown in Table 2. The heavy strand (H-strand) encodes *cox1-3, atp6, atp8, nad2, nad3, trnD, trnT*1, *trnT*2, *trnE, trnG*, the cluster KARNI (*trnK, trnA, trnR, trnN*, and *trnI*1), and *trnS*1. The light strand (L-strand) encodes remaining protein coding genes (*nad1, nad4, nad4L, nad5, nad6* and *cytb*), the two rRNA genes *(rrnS* and *rrnL*), *trnF, trnH, trnS*$_2$, *trnP, trnL*$_1$, *tnrL*$_2$, *trnV, trnI*2, and the cluster MYCWQ (*trnM, trnY, trnC, trnW*, and *trnQ*). Almost all PCGs start with ATG (*cox1-3, nad1, nad3, nad4, nad4L, nad5, atp6,* and *atp8*). However, *nad2* and *nad6* start with ATT and *cob* starts with ATA. Except for *nad3* and *atp8* (TAG), the most common stop codon is TAA.

The total length of these 13 protein-coding genes was found to be 11,430 bp in the mitogenome sequence of *C. lischkei*, which accounted for 63.6% of its total mitogenome length (Table 1). All 24 tRNAs found in the mitochondrial genome of this species were 66 to 76 bp in size (Fig. 2). All tRNA genes had a clover-leaf structure except for *trnS*$_1$ and *trnS*$_2$, in which its DHU arm simply formed a loop. Both *rrnL* and *rrnS* were located between *trnL*$_1$ and *trnM* and separated by *trnV*. The non-coding region was found between *trnQ*

and *trnE* with a length of 234 bp. The A-T content of the NCR was 70.5 %.

Phylogenetic analyses of *C. lischkei* were performed using concatenated 13 PCGs nucleotide sequences with 19 vetigastropods registered in NCBI. The ML tree with a GTR+G+I model showed that *C. lischkei* was grouped into family Tegulidae and genus *Chlorostoma* with strong bootstrap support values (Fig. 3). Our results could be used for future taxonomic and evolutionary studies for both the order of Vetigastropoda and the family of Tegulidae.

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