The complete mitochondrial genome of the Formosan ferret-badger (*Melogale moschata subaurantiaca*)

台灣鼬獾整體粒線體基因組序列

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Abstract

A complete mitochondrial genome of the Formosan ferret-badger (*Melogale moschata subaurantiaca*) was obtained by PCR amplification and DNA sequencing. The genome spans 16,497 bp that includes 13 protein-coding genes, 22 tRNA genes, and two rRNA genes. Nucleotide sequence divergence (K2P distance) of mitochondrial genome was 3.57% with that of Chinese ferret-badger (*Melogale moschata*).

摘　要

台灣鼬獾整體粒線體基因組序列以聚合脢連鎖反應及核酸定序獲得，全長共 16,497 bp，包含 13 個蛋白基因、22 個 tRNA 基因與 2 個 rRNA 基因，與大陸鼬獾整體粒線體核酸序列歧異度為 3.57%。

Key words: mitochondrial genome, *Melogale moschata subaurantiaca*, Taiwan

關鍵詞：粒線體基因組、鼬獾、台灣

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Introduction

The Chinese ferret-badger (*Melogale moschata*, Mustelidae, Carnivora, Mammalia) comprises six subspecies and is distributed in China, India, Myanmar, Laos, Vietnam and Taiwan (Wozencraft 2005; Lariviere and Jennings 2009). It adapts well to tropical and subtropical forests, grasslands, and cultivated areas, and often lives near villages (Storz and Wozencraft 1999). Since 2008, *M. moschata* is confirmed as a rabies virus carrier and regarded as an important source of rabies transmission in southeastern China (Lei *et al.* 2008; Liu *et al.* 2010). Yu *et al.* (2011) had reported a complete mitochondrial genome of *M. moschata* from China, but without referring to its subspecies status.

The Formosan ferret-badger (*Melogale moschata subaurantiaca*) is an endemic and the only *Melogale* species in Taiwan Island (Wozencraft 2005; Lariviere and Jennings 2009). It is common and widely distributed across the whole island at low to high elevations (Lin 2008; Chiang *et al.* 2012). First case of rabies in ferret-badgers of Taiwan was detected in 2013 (Wu *et al.* 2014; Chiou *et al.* 2014). Because Taiwan Island had been free from canine rabies for 52 years (Wu *et al.* 2014), the cause of rabies re-emergence aroused the concern for relationship of ferret-badgers from Taiwan and China.

The genetic distance of mitochondrial genes between *M. moschata subaurantiaca* from Taiwan and *M. m. taxilla* from Vietnam reaches the interspecific level within the mustelid lineages, implying deep divergence among subspecies of *M. moschata* (Hosoda *et al.* 2011). We hereby report the complete mitochondrial genome of *M. moschata subaurantiaca* from Taiwan and compare it with that of *M. moschata* from China in divergence of sequences.

Materials and methods

Sampling and DNA extraction

Muscle tissue of Formosan ferret-badger (*Melogale moschata subaurantiaca*) was collected from a roadkill specimen near Yuan-Ton Temple in Fangshan Township, Pingtung County, southern Taiwan in 2012. Genomic DNA was extracted by using the Puregene DNA purification kit (Gentra) according to the manufacturer’s instructions.

Primer design, PCR amplification and DNA sequencing

To amplify the complete Formosan ferret-badger mitochondrial genome, 17 sets of primers were designed based on an alignment of ferret-badger mitochondrial genome sequences taken from GenBank with accession numbers HM106328 (*Melogale moschata*; Table 1). The PCR reactions were run on a GeneAmp 9700 PCR system (Applied Biosystems, Inc) and consisted of 1μl of template DNA (10–100ng), 2.5 μM of each primer, 0.4 mM of each dNTP, 1 unit of Taq polymerase and 1x buffer with 1.5mM $MgCl_2$, toally 25 μl. The PCR conditions were as follows: 5 minutes at 94℃, 35 cycles of 94℃ for 30 seconds, 45–50℃ for 30 seconds, and 72℃ for 1 minute, and a final 7 minutes extension at 72℃. PCR products were sequenced using an ABI Prism 3730 DNA automated sequencer (Applied Biosystems, Inc).

Table 1. Primers used in amplifying and sequencing the complete mitochondrial genome of *Melogale moschata subaurantiaca* from Taiwan

Primer name	Sequence (5’ → 3’)	Location	Product length (bp)
MMAF	GCCAGAGAACTACTAGCAAC	558-577	1200
MMAR	GGAGAAGTATTTCTTGTTACTCAT	1734-1757	
MMBF	CAACTCCTAATATTTTACTGGG	1677-1698	1059
MMBR	GAAGAGGATTTGAACCTCTGG	2715-2735	
MMCF	ATCTAGCCAGTCCCCTTCCT	2624-2643	1143
MMCR	GAAACAAGAGGGCTTGAACC	3745-3766	
MMDF	CCTATTATAACAGCAAGCATTC	3665-3686	1111
MMDR	TCAGTAGGGCCGTGATTGCC	4756-4775	
MMEF	TATCGCTATCACACACATGAAA	4593-4614	1181
MMER	AGGTCTACGGATGCTCCCGCATG	5751-5773	
MMFF	ATAATTGGGGGCTTTGGAAACTGA	5559-5582	1651
MMFR	ACTGCTTGGGCATCCATAGTACT	7187-7209	
MMGF	CAAAGTCAAATTATAGGTGGA	6991-7011	820
MMGR	ATCATGTTGAGGTATCTAGTTG	7789-7810	
MMHF	ACTTTGAGAAATGATCTGCCTC	7680-7701	891
MMHR	AATAAGGGCTACTGCAAATTCT	8549-8570	
MMIF	GATTGGAGGAGCTACCCTAGC	8462-8482	1037
MMIR	GTAAGGTTAGTGCTATGTTTAT	9477-9498	
MMJF	TGACACTTCGTAGACGTCGTCTG	9347-9369	1443
MMJR	ACGCTATTATGCATGCTAGTCA	10768-10789	
MMKF	CTATTCTACACCCTAGTAGGCTC	10630-10652	941
MMKR	CTATATTTACAGTAAATGGG	11551-11570	
MMLF	CACTACACAACGCGGAAAATAC	11418-11439	1009
MMLR	GTGGCAGCCAGTAGCAGGCCT	12406-12426	
MMMF	AACACATGGGACTTCCAACAAAT	12344-12366	1716
MMMR	TTAGCTTTGTAGGGTTTTCTTC	14038-14059	
MMNF	CCTAATCAAACTTTACTTCATA	13510-13531	976
MMNR	AGTATAATCCTCGTCCTACG	14466-14485	
MMOF	ACAGCCTTCTCGTCAGTCAC	14356-14375	1087
MMOR	ATAGTTAAATTAGAATGCCAGC	15421-15442	
MMPF	CCTATTAAAATGAAGAGTCTTTGT	15300-15323	984
MMPR	ACGTTGCATGTGCTTAGTTCTG	16262-16283	
MMQF	CCGTAACTTCAAAAGTATACAA	16208-16229	934
MMQR	TATCGATTATAGAACAGGCTC	624-644	

Sequence analysis

Forward and reverse sequences for the same section of mtDNA were edited using SeqMan (DNASTAR, LaserGene, Madison, WI, USA). The boundaries of each gene in the mitochondrial genome of *Melogale moschata subaurantiaca* were identified by sequence alignment of published Carnivora mitochondrial genome. Start and stop codons were used to define the protein-coding genes. Sequences were aligned and similarity analysis performed using the software BIOEDIT version 7.0.4 (Hall 1999). Numbers of tandem repeats were calculated using the program Tandem Repeats Finder (Benson 1999).

Results and discussion

General features of the *Melogale moschata subaurantiaca* mitochondrial genome

The complete mitochondrial genome of *Melogale moschata subaurantiaca* (Genbank accession no. KP726273) is 16,497 bp in length, shorter than *Melogale moschata* (Genbank accession no. HM106328) which is 16,587 bp (Table 2). The differences result from different lengths of the control region between two species. The genome contains 13 protein-coding genes, 22 tRNA genes, and two rRNA genes. Eight tRNA genes and one protein-coding gene are located on the light strand, with the remaining genes located on the heavy strand. The overall base composition of the heavy strand is 31.95% A; 26.62% C; 14.72% G; 26.71% T. Guanine (G) is the rarest nucleotide and the GC content is 41.43%.

Protein-coding genes

The mtDNA contains 13 protein-coding genes. One of these (ND6) is located on the light strand (Boore 2004), with the others all on the heavy strand. This arrangement is typical for vertebrates (Table 2). Among 13 protein-coding genes of *Melogale moschata subaurantiaca* and *M. moschata*, the common Met start codon (ATG) could be assigned as the start codon for all protein-coding genes except for ND2, ND3 and ND5, which begin with ATC, ATA and ATA respectively. Common stop codons (TAA or TAG) could be assigned to most of the putative protein-coding sequences. ND1, ND2, COIII and ND4 are terminated with incomplete stop codons (T--) (Table 2), which are accommodated post-transcriptionally in the mRNA maturation process by polyadenylation (Ojala *et al.* 1981; Broughton *et al.* 2001). Two protein-coding regions (ATP8/ATP6 and ND4L/ND4) overlap and in each case both genes are translated from the same cistronic mRNA.

Nucleotide and amino acid sequences divergence (K2P distance) of the 13 protein-coding genes between the two whole mitochondrial genome were 1.99–4.61% and 0.00–2.88% (Table 2). Among the 13 protein-coding genes, ATP8 is most conserved, followed by Cytb, COI and COIII, whereas ND2 has evolved most rapidly.

Table 2. Characteristics and comparison of gene length, GC content, the number of codons, start codon, stop codon, and nucleotide and/or amino acid sequence divergences (% Kimura two-parameter distance) between the two complete mitochondrial genomes of *Melogale moschata subaurantiaca* from Taiwan and *M. moschata* from China (*M. moschata subaurantiaca* / *M. moschata*)

Gene	From	To	Length (bp)	Codon			Intergenic nucleotides*	Strand†	Nucleotide divergence (%)	Amino acid divergence (%)
				No. of codons	Start	Stop				
tRNA Phe	1/1	69/69	69/69	–	–	–	–	H/H	0	–
12SrRNA	70/70	1030/1029	961/960	–	–	–	0/0	H/H	1.25	–
tRNAVal	1031/1030	1097/1096	67/67	–	–	–	0/0	H/H	0	–
16SrRNA	1097/1096	2666/2664	1570/1569	–	–	–	-1/-1	H/H	1.78	–
tRNA Leu(UUR)	2667/2665	2741/2739	75/75	–	–	–	0/0	H/H	0	–
ND1	2744/2742	3698/3696	955/955	318/318	ATG/ATG	T– –/T– –	2/2	H/H	3.14	0.00
tRNA Ile	3699/3697	3768/3766	70/70	–	–	–	0/0	H/H	1.43	–
tRNA Gln	3766/3764	3839/3837	74/74	–	–	–	-2/-2	L/L	4.05	–
tRNA Met	3841/3839	3910/3908	70/70	–	–	–	1/1	H/H	0	–
ND2	3911/3909	4952/4950	1042/1042	347/347	ATC/ATC	T– –/ T– –	0/0	H/H	4.61	2.88
tRNA Trp	4953/4951	5019/5017	67/67	–	–	–	0/0	H/H	0	–
tRNA Ala	5029/5027	5097/5095	69/69	–	–	–	9/9	L/L	0	–
tRNA Asn	5099/5097	5171/5169	73/73	–	–	–	1/1	L/L	1.36	–
OL	5172/5170	5207/5205	36/36	–	–	–	0/0	–/–	0	–
tRNA Cys	5205/5203	5271/5269	67/67	–	–	–	-2/-2	L/L	0	–
tRNA Tyr	5271/5269	5338/5336	68/68	–	–	–	-1/-1	L/L	0	–
COI	5340/5338	6884/6882	1545/1545	515/515	ATG/ATG	TAA/TAA	1/1	H/H	2.91	0.49
tRNA Ser(UCN)	6881/6879	6956/6954	76/76	–	–	–	-3/-3	L/L	1.32	–
tRNA Asp	6961/6959	7027/7025	67/67	–	–	–	4/4	H/H	1.49	–
COII	7028/7026	7711/7709	684/684	228/228	ATG/ATG	TAA/TAA	0/0	H/H	3.51	0
tRNA Lys	7715/7713	7781/7779	67/67	–	–	–	3/3	H/H	0	–
ATP8	7783/7781	7986/7984	204/204	68/68	ATG/ATG	TAA/TAA	1/1	H/H	1.99	1.49
ATP6	7944/7942	8624/8622	681/681	227/227	ATG/ATG	TAA/TAA	-43/-43	H/H	3.08	0.88
COIII	8624/8622	9407/9405	784/784	261/261	ATG/ATG	T– –/T– –	-1/-1	H/H	2.93	0
tRNA Gly	9408/9406	9476/9474	69/69	–	–	–	0/0	H/H	1.45	–
ND3	9477/9475	9824/9822	348/348	116/116	ATA/ATA	TAG/TAA	0/0	H/H	4.31	0
tRNA Arg	9825/9823	9892/9890	68/68	–	–	–	0/0	H/H	2.94	–
ND4L	9893/9891	10189/10187	297/297	99/99	ATG/ATG	TAA/TAA	0/0	H/H	4.04	0
ND4	10183/10181	11560/11558	1378/1378	459/459	ATG/ATG	T– –/T– –	-7/-7	H/H	4.06	1.53
tRNA His	11561/11559	11629/11627	69/69	–	–	–	0/0	H/H	0	–
tRNA Ser(AGY)	11630/11628	11691/11689	62/62	–	–	–	0/0	H/H	1.61	–
tRNA Leu(CUN)	11692/11690	11761/11759	70/70	–	–	–	0/0	H/H	0	–
ND5	11753/11751	13585/13580	1833/1830	611/610	ATA/ATA	TAA/TAA	-9/-9	H/H	3.11	1.80
ND6	13569/13564	14102/14097	526/526	175/175	ATG/ATG	TAA/TAA	-17/-17	L/L	3.99	1.14
tRNA Glu	14103/14098	14171/14166	61/61	–	–	–	0/0	L/L	0	–
Cytb	14176/14171	15315/15310	1140/1140	378/378	ATG/ATG	TAA/TAA	4/4	H/H	2.82	1.06
tRNA Thr	15316/15311	15383/15378	68/68	–	–	–	0/0	H/H	2.94	–
tRNA Pro	15384/15379	15448/15443	65/65	–	–	–	0/0	L/L	3.08	–
Control region	15449/15444	16497/16587	1048/1144	–	–	–	0/0	–	12.63	–
PCG‡	–	–	11413/11410	3794/3793	–	–	–	–	3.40	1.01
Entire genome	–	–	16497/16587	–	–	–	–	–	3.57	1.01

Notes: Bold letters indicate different lengths of genes between the two taxa. * Numbers correspond to the nucleotides separating different genes. Negative numbers indicate overlapping nucleotides between adjacent genes; † H and L denote heavy and light; ‡ Combined sequences of 13 protein-coding genes (PCGs).

RNA genes

There are 22 tRNA genes identified in the *Melogale moschata subaurantiaca* mitochondrial genome, with lengths ranging from 61bp (tRNA-Glu) to 76 bp (tRNA-Ser). The 12S rRNA and 16S rRNA genes are 961 bp and 1,570 bp respectively. tRNA-Ile and tRNA-Gln are overlapping with separate transcripts in opposite directions. This arrangement is typical for mammalian mitochondrial genomes (Anderson *et al.* 1981; Parma *et al.* 2003, Tsai *et al.* 2009). (Table 2).

Control region

The origin of L-strand replication (O_L) of *Melogale moschata subaurantiaca* was located between tRNA-Asn and tRNA-Cys, which is 36bp in size and similar to those of other vertebrates (Hou *et al.* 2007; Delisle and Strobeck 2002; Yue *et al.* 2006). The sequence of the control region (CR) of *M. moschata subaurantiaca* located between tRNA-Pro and tRNA-Phe is 1,048 bp in size and contains three sets of repeats in position 569-616, 568-726 and 568-726 by online software TANDEM REPEATS FINDER (Benson 1999).

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