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**Derya Çetintürk**  
Department of Biology, Faculty  
of Science, University of Ankara,  
06100, Beşevler, Ankara, Turkey

## Mitochondrial dna variations in Turkish *Microtus hartingi* (Barret-hamilton 1903) and *Microtus lydius* (Blackler 1916)

**Derya Çetintürk**

### Abstract

Mitochondrial DNA variations of Turkish *Microtus hartingi* (Barret-Hamilton 1903) and *Microtus lydius* (Blackler 1916) species were investigated with regard to mutations and nucleotide diversities in synonymous and non-synonymous sites as well as synonymous and non-synonymous substitution rates (Ks and Ka values) in COXI, *cyt-b* and 12S rRNA regions. Analyses showed that synonymous substitution rates are greater than non-synonymous substitution rates and it can be concluded that synonymous substitutions are more observed which don't alter protein sequences, therefore selective pressure is negative on *M. hartingi* and *M. lydius* which have been considered same species by some authors.

**Keywords:** *Microtus hartingi*, *Microtus lydius*, Turkey, mtDNA, substitution, mutation

### 1. Introduction

Genus *Microtus* (Schrank 1798) is widely distributed in Turkey and 13 species have been described with recent studies (Kryštufek and Vohralík 2009, Yiğit *et al.* 2006, 2012) [5, 11, 12]. Guentheri group of subgenus *Sumeriomys* with 2n: 54 chromosomes (Golenischev 2002) [1] has three species *Microtus guentheri* (Dandford and Alston 1880), *Microtus hartingi* (Barret-Hamilton 1903) and *Microtus lydius* (Blackler 1916). Whereas *M. guentheri* distributed in Southeastern parts of Anatolia, *M. hartingi* and *M. lydius* are found in Thrace and Western Anatolia, respectively. More recently, phylogenetic relationships of Guentheri group voles was documented by Yiğit *et al.* (2017) [11, 12] and this study supported that these taxa are valid species. In this study, mitochondrial DNA variations of *M. hartingi* and *M. lydius* which are separated geographically by Bosphorus and Dardanelle Straits were investigated by the help of three mitochondrial genetic markers: COXI (Cytochrome oxidase-I), *Cyt-b* (Cytochrome-b) and 12S rRNA.

### 2. Materials And Methods

Total 43 samples from Ankara University Mammalian Research Collection (AUMAC, <http://www.mammalia.ankara.edu.tr>) and 5 *Cyt-b* sequences from GenBank were used for analyses. DNA isolation was carried out using GeneAll® Exgene™ Tissue SV mini kit (Atlas Biotechnology, Turkey) from frozen kidney and liver tissues. COXI gene (720 bp), *Cyt-b* gene (1100 bp) and 12S rRNA gene (1000 bp) were amplified using primers: BatL5310 (forward) and R6036R (reverse) primers (Robins *et al.* 2007) [7], L14727-SP (forward) and H15915-SP (reverse) primers (Jaarola and Searle 2002) [3] and L651 (forward) and 12GH (reverse) primers (Adkins *et al.* 2001), respectively. PCR mixture and reaction conditions were obtained from Yiğit *et al.* (2017) [11, 12].

Electrophoresis was performed in 0.8 % agarose gel for 1 hour at 70 V in 1×TAE, and using SYNGENE Bio Imaging System, viewing of PCR bands were performed.

Forward and reverse sequencing was carried out by MEDSANTEK and funded by Ankara University Scientific Research Projects Coordination Unit Project (15L0430002).

Forward and reverse sequences were visualised in Chromas Lite 2.1.1 ([www.technelysium.com.au](http://www.technelysium.com.au)). Clustalx 2.0 (Larkin *et al.* 2007) [6] and BIOEDIT 7.2.0 (Hall 1999) [2] programmes were used for alignment. Mutations and nucleotide diversities in synonymous and non-synonymous sites and Ks-Ka values (synonymous and non-synonymous substitution rates) were calculated using the program DNASP 5.10.01 (Rozas *et al.* 2010) [8].

**Correspondence**  
**Derya Çetintürk**  
Department of Biology, Faculty  
of Science, University of Ankara,  
Beşevler, Ankara, Turkey

### 3. Results And Discussions

Synonymous and non-synonymous substitution rates have been supposed as parameters to clarify sequence differentiation among species. While synonymous substitutions defined as “silent” don't affect altering protein sequences, non-synonymous substitutions change protein sequences (Wang *et al.* 2009) [9]. In this study, we

investigated synonymous and non-synonymous substitution rates (Ks and Ka values) as well as number of mutations and nucleotide diversities in synonymous and non-synonymous sites based on Jukes and Cantor Parameter (Jukes and Cantor 1969) [4] in Turkish *M. hartingi* and *M. lydius* species. These species were analysed both together and separately and obtained results were given for three gene region in Table 1-3.

**Table 1:** Number of mutations and nucleotide diversities (Pi) in synonymous and non-synonymous sites of COXI gene in Turkish *M. hartingi* and *M. lydius* samples.

	Number of codons and sites*	Number of synonymous sites	Pi* (synonymous sites)	Number of mutations	Number of non-synonymous sites	Pi* (non-synonymous sites)	Number of mutations
<i>M. hartingi</i>	190 (570)	130,17	0,001	1	439,83	0,000	0
<i>M. lydius</i>	190 (570)	132,88	0,018	14	437,12	0,014	39
Total	189 (567)	131,56	0,021	15	434,44	0,012	39

\*: Numbers in brackets show number of protein-coding sites analysed.

**Table 2:** Number of mutations and nucleotide diversities (Pi) in synonymous and non-synonymous sites of Cyt-*b* gene in Turkish *M. hartingi* and *M. lydius* samples.

	Number of codons and sites*	Number of synonymous sites	Pi* (synonymous sites)	Number of mutations	Number of non-synonymous sites	Pi* (non-synonymous sites)	Number of mutations
<i>M. hartingi</i>	153 (459)	106,74	0,002	1	352,26	0,002	3
<i>M. lydius</i>	136 (408)	94,58	0,023	13	313,42	0,017	40
Total	142 (426)	99,02	0,021	19	326,98	0,016	48

\*: Numbers in brackets show number of protein-coding sites analysed.

**Table 3:** Number of mutations and nucleotide diversities (Pi) in synonymous and non-synonymous sites of 12S rRNA gene in Turkish *M. hartingi* and *M. lydius* samples.

	Number of codons and sites*	Number of synonymous sites	Pi (synonymous sites)	Number of mutations	Number of non-synonymous sites	Pi (non-synonymous sites)	Number of mutations
<i>M. hartingi</i>	191 (573)	123,83	0,000	0	449,17	0,000	0
<i>M. lydius</i>	187 (561)	121,06	0,003	6	439,94	0,001	10
Total	184 (552)	118,43	0,009	6	433,57	0,003	10

\*: Numbers in brackets show number of protein-coding sites analysed.

In addition, synonymous and non-synonymous substitution rates (Ks and Ka values) of three gene regions were calculated. According to the results, almost all Ks values are greater than Ka values (Table 4). It can be inferred that synonymous substitutions occur much more than non-synonymous substitution in this situation, therefore selective pressure is negative. According to Yiğit *et al.* (2017) [11, 12],

differentiation level between *M. hartingi* and *M. lydius* are notable low based on phylogeny and genetic distance, so Thrace and Western Anatolia populations could be identified as the same species *Microtus hartingi* (Barret-Hamilton 1903) according to the priority rule. Assuming the negative selection is effective, probability of speciation between these populations is significantly low.

**Table 4:** Ks (synonymous substitution rate) and Ka (non-synonymous substitution rate) values of COXI, Cyt-*b* and 12S rRNA regions.

Species	COXI		Cyt- <i>b</i>		12S rRNA	
	Ks	Ka	Ks	Ka	Ks	Ka
<i>M. hartingi</i>	0-0,0077	0	0-0,0192	0-0,0087	0	0
<i>M. lydius</i>	0-0,0970	0-0,0596	0-0,0706	0-0,0649	0-0,0300	0-0,014
Total	0-0,978	0-0,598	0-0,1413	0-0,1299	0-0,0388	0-0,040

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