

## Supplementary Material

### Mice

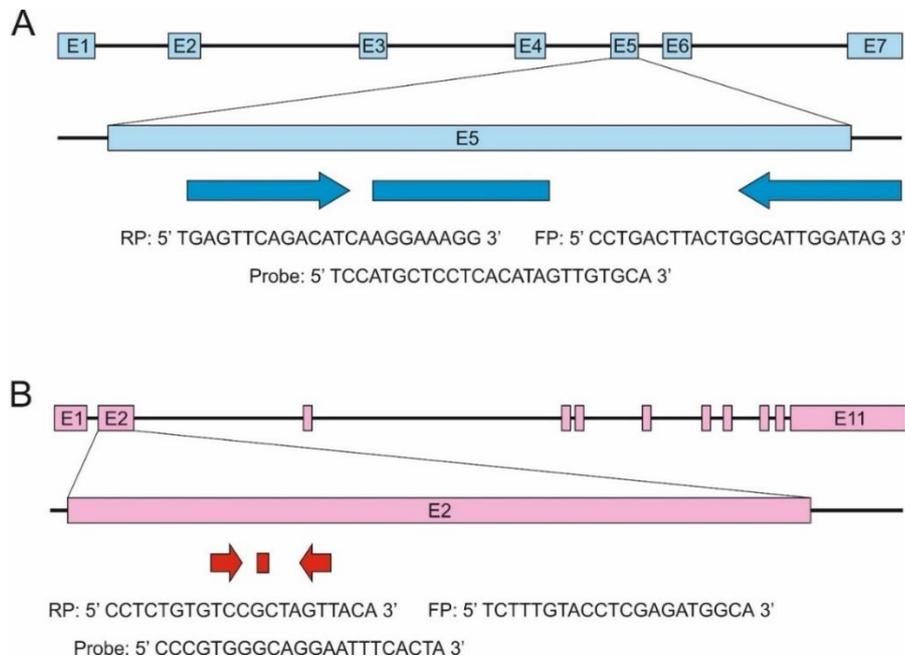
Details on the sex and age of the mice analysed in this study are given in Table S3. All individuals were weaned at 20 days and then housed singly until sacrifice, with food and water provided *ad libitum*. Cages were supplied with sawdust bedding, nesting material, and cardboard shelter. They were cleaned every 5-7 days and kept at room temperature 20°C and the 14h/10h light/dark cycle (light 6 am – 8 pm). At the age ~100 days (range 60–140 days, Table S3), the mice were sacrificed by cervical dislocation and immediately dissected. Parts of the liver were snap-frozen in liquid nitrogen and then stored at -80 °C until DNA extraction.

### Genomic DNA

About 3-mm<sup>3</sup> pieces of tissue were put in the 2-ml Reinforced Tubes with 2.8 mm Ceramic Beads (Omni International), 360 µl of the T1 Lysis Buffer (Macherey-Nagel) and 50 µl of Proteinase-K (Qiagen), homogenised using the MagNA Lyser Instrument (Roche), and incubated for 3–6 h at 56 °C in a shaker (200 rpm). Genomic DNA was then isolated using the NucleoSpin kit (Macherey-Nagel) following the manufacturer's instructions, with the final ddH<sub>2</sub>O elution. The concentration and purity of the extraction products were checked with a spectrophotometer (DeNovix DS 11). Subsequently, all samples were equalised to contain 50–100 copies of the reference gene per µl in each ddPCR well (see below).

### Mup Assay

The *Mup* assay was designed based on ~2.5 Mb long C57BL/6 sequence of Chromosome 4 (NC\_000070.7) bracketing the *Mup* cluster downloaded from NCBI GRCm38.p4 genome assembly. Geneious 9.1.5 (Biomatters) was used for the identification of *Mup* paralogs and pseudogenes as well as for designing primers and probes. The assay focused on the central *Mup* genes, on areas with the lowest SNP density and highest difference from pseudogenes. As the primer-design tool implemented in Geneious rendered several potential assays, we tested the proposed oligonucleotides with the online tool Oligo-Analyzer (Integrated DNA Technologies, IA, USA), retaining the two best candidates (pair of primers + probe). Finally, we used NCBI's Primer-BLAST tool (<http://www.ncbi.nlm.nih.gov/tools/primer-blast>) to check the specificity (Tables S1 and S2) using the GRCm39 built. The resulting assay 'Mup\_E5\_S1' (Exone 5, set 1; '*Mup* assay' in short) (Fig. S1A) was purchased as a custom-designed PrimeTime qPCR Assay (IDT, IA, USA) in primer-to-probe ratio 3.6 and resuspended upon delivery 20× with the recommended buffer.



**Fig. S1 Position of ddPCR primers and probes in *Mup* paralogs (A) and the *Tert* gene (B).** RP = reverse primer, FP = forward primer, E = exon. Proportions of exons, introns, primers, and probes are not exactly to scale.

## ddPCR

The telomerase reverse transcriptase gene (*Tert*), which is known to be present in two copies per diploid genome (Pezer et al. 2015), was used as standard (primers and probe shown in Fig. S1B). The ddPCR reactions were run in duplexes involving both the target (*Mup*, FAM-labelled) and standard (*Tert*, HEX-labelled). The *Mse*I restriction endonuclease was used to ensure an independent separation of *Mup* paralogs. The reaction mixture comprised 12  $\mu$ l of ddPCR Supermix for Probes (Bio-Rad), 0.12  $\mu$ l of the *Mup* assay, 0.12  $\mu$ l of the *Tert* assay, 0.05  $\mu$ l of *Mse*I 10 000 U/ml (New England Biolabs, Ipswich, MA, USA), 1.1  $\mu$ l of genomic DNA (in some cases, the volume was adjusted to produce a desired load 50–100 copies/ $\mu$ l in the well), and ddH<sub>2</sub>O to the final volume 24  $\mu$ l.

Before emulsion, the mixture was incubated at 37 °C for 10 min as recommended by the manufacturer (Bio-Rad 2015) ) and verified in an earlier study (Yanchukov et al., 2021). After the incubation, emulsions of droplets were prepared with the QX200 Droplet Generator (Bio-Rad). PCR reaction was then carried out in the Bio-Rad C1000 cycler using a deep-well block. Droplet fluorescence was screened in the QX200 Droplet Reader (Bio-Rad). Initially, several thermal gradients were tried to find the temperature at which both the target and standard showed sufficiently clear positive vs. negative droplet separation, needed for reliable CN estimation. The definitive conditions were: 10 min at 95 °C, followed by 40 two-step cycles with 30 sec at 94 °C and 1 min at 59 °C, with the final extension of 10 min at 98 °C. The ramp speed was set to 2 °C/sec for all steps and cycles.

**Table S1. List of Mup paralogs covered by the Mup assay, with sequences of the primers and probes.**  
The Mup paralogs are named according to the NCBI nomenclature. SNPs are marked in red.

Paralog	Primers, 5' → 3'	Probe, 5' → 3'
<i>Mup2</i>	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#1	TGAGTTCAGACATCAAGGAAAGG	
<i>Mup8</i>	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#2	TGAGTTCAGACATCAAGGAAAGG	
<i>Mup9</i>	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#3	TGAGTTCAGACATCAAGGAAAGG	
<i>Mup10</i>	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#4	TGAGTTCAGACATCAAGGAAAGG	
<i>Mup11</i>	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#5	TGAGTTCAGACATCAAGGAAAGG	
<i>Mup22</i>	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#6	TGAGTTCAGACATCAAGGAAAGG	
<i>Mup13</i>	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#7	TGAGTTCAGACATCAAGGAAAGG	
<i>Mup14</i>	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#8	TGAGTTCAGACATCAAGGAAAGG	
<i>Mup16</i>	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#9	TGAGTTCAGACATCAAGGAAAGG	
<i>Mup17</i>	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#10	TGAGTTCAGACATCAAGGAAAGG	
<i>Mup18</i>	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#11	TGAGTTCAGACATCAAGGAAAGG	
<i>Mup19</i>	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#12	TGAGTTCAGACATCAAGGAAAGG	
<i>Mup7</i>	CCTGACTTACTGGCATTGGATAG	TCCATGCTTCTCACATAGTTGTGCA
#13	TGAGTTCAGACATCAAGGAAAGG	
<i>Mup1</i>	CCTGACTTACTGGCATTGGATAG	TCCATGCTTCTCACATAGTTGTGCA
#14	TGAGTTCAGACATCAAGGAAAGG	
<i>Mup12</i>	CCTGACTTACTGGCATTGGATAG	TCCATGCTTCTCACATAGTTGTGCA
#15	TGAGTTCAGACATCAAGGAAAGG	
<i>Mup15</i>	CCTGACTTACTGGCATTGGATAG	TCCATGCTTCTCACATAGTTGTGCA
#16	TGAGTTCAGACATCAAGGAAAGG	
<i>Mup5</i>	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTTGTGCA
#17	TGAGTTCAGACATCAAGGAAAAG	
<i>MupPs19</i>	CCTGACTTACTGGCATTGGTTAG	TCCATGCTCCTCGCTTAGTTGTGCA
#18	TGAGTTCAGACATCAAGGAAAAG	
<i>MupPs21</i>	CCTGACTTACTGGCATTGGTTAG	TCCATGCTCCTCGCTTAGTTGTGCA
#19	TGAGTTCAGACATCAAGGAAAAG	
<i>Mup20</i>	CCTGACTTACTGGCATTGGTTAG	TCCATGCTCCTCGCTTAGTTGTGCA
#20	TGAGTTCAGACATCAAGGAAAAG	

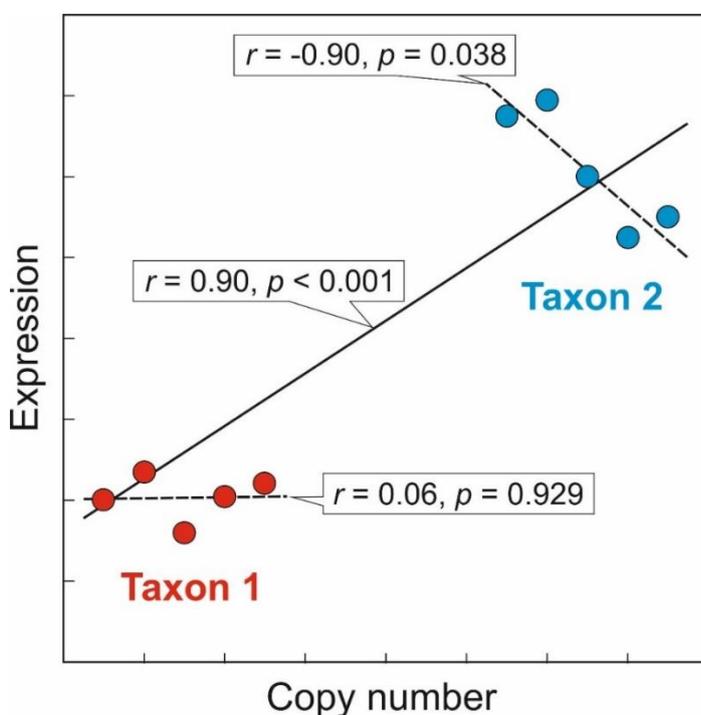
**Table S2. List of Mup pseudogenes of lower specificity to the GRCm39, with sequences of the primers and probes.** The loci are named according to the NCBI nomenclature; ‘Ps’ stands for pseudogene. SNPs are marked in red.

Paralog	Primers, 5' → 3'	Probe, 5' → 3'
<i>MupPs18</i>	CCTGAGTTACTGGCATTGGATAG TGAGTTCAGACATCAAGGAAAAG	TGCATGCTCCTCACATAATTTGCA
<i>MupPs12</i>	CCTGACTTACTGGCATTAGATAG TGAGTTCAGACATCAAGGAAGAG	TCCATGCTCCTCACATAGTTTTC
<i>MupPs1</i>	CCTGACTTACTGGCATTAGATAG TGAGTTCAGACATCAAGGAAGAG	TCCATGCTCCTCAATAGTTTTC
<i>MupPs2</i>	CCTGACTTACTGGCATTAGATAG TGAGTTCAGACATCAAGGAAGAG	TCCATGCTCCTCAATAGTTTTC
<i>MupPs10</i>	CCTGACTTACTGGCATTAGATAG	TCCATGCTCCTCAATAGTTTTC
<i>LOC108168721</i>	TGAGTTCAGACATCAAGGAAGAG	

### CNV estimation

All samples were run in triplicates (technical replicates), and data obtained were processed in the Quantasoft Software (Bio-Rad). For optimisation, we employed the same laboratory strain as we used for designing the *Mup* assay, i.e. C57BL/6.

On each plate, we used C57BL/6 gDNA as a positive control and for setting the threshold. The Quantasoft Software was employed to merge the triplicates, estimate CN, and calculate Poisson measurement errors (see Table S3). Only estimates with errors of less than 10% were accepted as valid.



**Fig. S2 A hypothetical example of the discrepancy between correlations at different levels.** While there is no correlation between the number of genes and their expression in Taxon 1, and this correlation is even significantly *negative* in Taxon 2, we detect a highly *positive* correlation in the total sample.

Table S3. CN estimates for each individual studied.

ID	Taxon	WDS	Total CN	+Poisson error	-Poisson error
SZ♂0922	<i>M. m. musculus</i>	BUSNA	34	1,3	1,3
SZ♂8455	<i>M. m. musculus</i>	BUSNA	32	1,5	1,5
SZ♂8456	<i>M. m. musculus</i>	BUSNA	35	1,8	1,7
SZ♂7552	<i>M. m. musculus</i>	MBK	23	0,9	0,9
SZ♂8569	<i>M. m. musculus</i>	MBK	22	0,9	0,9
SZ♀1605	<i>M. m. musculus</i>	MBK	22	0,9	0,8
SZ♂1561	<i>M. m. musculus</i>	MDH	26	1,1	1,1
SZ♂7092	<i>M. m. musculus</i>	MDH	23	1,1	1,1
SZ♂7832	<i>M. m. musculus</i>	MDH	24	0,8	0,8
SZ♂0788	<i>M. m. musculus</i>	MPB	24	1,0	0,9
SZ♂7398	<i>M. m. musculus</i>	MPB	27	1,2	1,1
SZ♂8772	<i>M. m. musculus</i>	MPB	28	1,3	1,2
SZ♂2157	<i>M. m. musculus</i>	STUF	22	0,9	0,9
SZ♂8789	<i>M. m. musculus</i>	STUF	24	1,2	1,2
SZ♂8790	<i>M. m. musculus</i>	STUF	23	1,0	0,9
SZ♂1155	<i>M. m. domesticus</i>	DDO	27	1,1	1,1
SZ♂7605	<i>M. m. domesticus</i>	DDO	28	1,1	1,1
SZ♂7612	<i>M. m. domesticus</i>	DDO	28	1,1	1,1
SZ♂7305	<i>M. m. domesticus</i>	DROS	35	1,6	1,6
SZ♂8265	<i>M. m. domesticus</i>	DROS	40	1,6	1,6
SZ♀8268	<i>M. m. domesticus</i>	DROS	33	1,3	1,3
SZ♂1005	<i>M. m. domesticus</i>	SCHUNT	22	0,8	0,7
SZ♂7274	<i>M. m. domesticus</i>	SCHUNT	21	0,9	0,9
SZ♂7405	<i>M. m. domesticus</i>	SCHUNT	22	0,9	0,8
SZ♂1363	<i>M. m. domesticus</i>	STRA	29	1,1	1,1
SZ♂7484	<i>M. m. domesticus</i>	STRA	29	1,4	1,3
SZ♂7700	<i>M. m. domesticus</i>	STRA	27	1,1	1,0
SZ♂1907	<i>M. m. domesticus</i>	WLA	18	0,8	0,7
SZ♂7156	<i>M. m. domesticus</i>	WLA	17	0,6	0,6
TA♂0051	<i>M. m. domesticus</i>	WLA	18	0,8	0,6
SZ♂2484	<i>M. m. castaneus</i>	CIM	7	0,3	0,2
SZ♂2486	<i>M. m. castaneus</i>	CIM	7	0,4	0,3
SZ♂9032	<i>M. m. castaneus</i>	CIM	8	0,3	0,2
SZ♂1551	<i>M. m. castaneus</i>	CKN	14	0,7	0,6
SZ♂8515	<i>M. m. castaneus</i>	CKN	13	0,5	0,6
SZ♀8517	<i>M. m. castaneus</i>	CKN	14	0,7	0,5
SZ♂1527	<i>M. spretus</i>	SEB	5	0,2	0,2
SZ♂7696	<i>M. spretus</i>	SEB	5	0,2	0,2
SZ♂8111	<i>M. spretus</i>	SEB	5	0,2	0,2
SZ♂0722	<i>M. spretus</i>	SMON	5	0,2	0,2
SZ♂1620	<i>M. spretus</i>	SMON	5	0,2	0,2
SZ♂7048	<i>M. spretus</i>	SMON	5	0,2	0,2
SZ♂2268	<i>M. spicilegus</i>	ZPB	8	0,4	0,4
SZ♀2271	<i>M. spicilegus</i>	ZPB	11	0,4	0,5
SZ♂6098	<i>M. spicilegus</i>	ZRU	6	0,3	0,2
SZ♂6187	<i>M. spicilegus</i>	ZRU	6	0,3	0,3
SZ♀8325	<i>M. spicilegus</i>	ZRU	6	0,2	0,2

SZ♂1952	<i>M. macedonicus</i>	MACSO	5	0,3	0,3
SZ♀3157	<i>M. macedonicus</i>	MACSO	4	0,2	0,1
SZ♂0793	<i>M. macedonicus</i>	XBS	6	0,3	0,2
TA♂0671	<i>M. macedonicus</i>	XBS	6	0,3	0,2
TA♂1148	<i>M. macedonicus</i>	XBS	6	0,3	0,3

**Table S4. The results of Tukey HSD post-hoc pairwise tests of estimated Mup CN between the mouse (sub)species:** The p-values significant at the alpha = 0.05 level are marked in red.

	<i>mus</i>	<i>dom</i>	<i>cas</i>	<i>spr</i>	<i>spi</i>	<i>mac</i>
<i>M. m. musculus</i>	--					
<i>M. m. domesticus</i>	1,0000	--				
<i>M. m. castaneus</i>	0,0001	0,0001	--			
<i>M. spretus</i>	0,0001	0,0001	0,2960	--		
<i>M. spicilegus</i>	0,0001	0,0001	0,8542	0,9535	--	
<i>M. macedonicus</i>	0,0001	0,0001	0,3675	1,0000	0,9679	--

**Table S5. The results of Kruskal-Wallis tests of the total MUP levels between the mouse (sub)species:** p-values of multiple comparisons for males (top) and females (bottom). The values significant at the alpha = 0.05 level are marked in red.

	<i>mus</i>	<i>dom</i>	<i>cas</i>	<i>spr</i>	<i>spi</i>	<i>mac</i>
<i>M. m. musculus</i>	--					
<i>M. m. domesticus</i>	1,0000	--				
<i>M. m. castaneus</i>	1,0000	1,0000	--			
<i>M. spretus</i>	0,2556	0,0213	0,9828	--		
<i>M. spicilegus</i>	0,0458	0,0039	0,2177	1,0000	--	
<i>M. macedonicus</i>	1,0000	0,6235	1,0000	1,0000	1,0000	--

	<i>mus</i>	<i>dom</i>	<i>cas</i>	<i>spr</i>	<i>spi</i>	<i>mac</i>
<i>M. m. musculus</i>	--					
<i>M. m. domesticus</i>	0,4538	--				
<i>M. m. castaneus</i>	1,0000	0,1403	--			
<i>M. spretus</i>	0,7177	0,0045	1,0000	--		
<i>M. spicilegus</i>	0,1269	0,0008	1,0000	1,0000	--	
<i>M. macedonicus</i>	1,0000	0,7926	1,0000	1,0000	1,0000	--

**Table S6. Total MUP levels in the urine (ng/ml).**

<b>Taxon</b>	<b>WDS</b>	<b>Sex</b>	<b>Total MUP</b>
<i>M. m. musculus</i>	BUSNA	male	2,46517E+11
<i>M. m. musculus</i>	BUSNA	male	1,98267E+11
<i>M. m. musculus</i>	BUSNA	male	1,94918E+11
<i>M. m. musculus</i>	BUSNA	female	90922726300
<i>M. m. musculus</i>	BUSNA	female	1,11731E+11
<i>M. m. musculus</i>	BUSNA	female	3,14495E+11
<i>M. m. musculus</i>	MBK	male	3,55171E+11
<i>M. m. musculus</i>	MBK	male	2,71233E+11
<i>M. m. musculus</i>	MBK	male	1,54604E+11
<i>M. m. musculus</i>	MBK	female	1,07276E+11
<i>M. m. musculus</i>	MBK	female	41659054400
<i>M. m. musculus</i>	MBK	female	54580663570
<i>M. m. musculus</i>	MDH	male	4,18523E+11
<i>M. m. musculus</i>	MDH	male	11293057560
<i>M. m. musculus</i>	MDH	male	96256335100
<i>M. m. musculus</i>	MDH	female	3,09352E+11
<i>M. m. musculus</i>	MDH	female	21681455230
<i>M. m. musculus</i>	MDH	female	20432230660
<i>M. m. musculus</i>	MPB	male	2,39306E+11
<i>M. m. musculus</i>	MPB	male	3,7173E+11
<i>M. m. musculus</i>	MPB	male	4,02615E+11
<i>M. m. musculus</i>	MPB	female	88221641470
<i>M. m. musculus</i>	MPB	female	2,55394E+11
<i>M. m. musculus</i>	MPB	female	96296607800
<i>M. m. musculus</i>	PWD	male	2,58023E+11
<i>M. m. musculus</i>	PWD	male	3,01607E+11
<i>M. m. musculus</i>	PWD	female	29577503510
<i>M. m. musculus</i>	PWD	female	72959030670
<i>M. m. musculus</i>	STUF	male	1,86953E+11
<i>M. m. musculus</i>	STUF	male	1,33419E+11
<i>M. m. musculus</i>	STUF	male	85705173340
<i>M. m. musculus</i>	STUF	female	1,00333E+11
<i>M. m. musculus</i>	STUF	female	28554232670
<i>M. m. musculus</i>	STUF	female	21152495230
<i>M. m. domesticus</i>	DDO	male	1,58505E+11
<i>M. m. domesticus</i>	DDO	male	1,96881E+11
<i>M. m. domesticus</i>	DDO	male	2,28704E+11
<i>M. m. domesticus</i>	DDO	female	31608436030
<i>M. m. domesticus</i>	DDO	female	67062170000
<i>M. m. domesticus</i>	DDO	female	1,92464E+11
<i>M. m. domesticus</i>	DROS	male	2,03232E+11
<i>M. m. domesticus</i>	DROS	male	4,03498E+11
<i>M. m. domesticus</i>	DROS	male	2,18228E+11
<i>M. m. domesticus</i>	DROS	female	1,51353E+11
<i>M. m. domesticus</i>	DROS	female	1,77458E+11
<i>M. m. domesticus</i>	DROS	female	1,97522E+11
<i>M. m. domesticus</i>	SCHUNT	male	4,31599E+11

<i>M. m. domesticus</i>	SCHUNT	male	39843145360
<i>M. m. domesticus</i>	SCHUNT	male	3,81514E+11
<i>M. m. domesticus</i>	SCHUNT	female	1,40508E+11
<i>M. m. domesticus</i>	SCHUNT	female	4,47635E+11
<i>M. m. domesticus</i>	SCHUNT	female	3,01116E+11
<i>M. m. domesticus</i>	STRA	male	5,94708E+11
<i>M. m. domesticus</i>	STRA	male	3,64625E+11
<i>M. m. domesticus</i>	STRA	male	3,57001E+11
<i>M. m. domesticus</i>	STRA	female	3,52241E+11
<i>M. m. domesticus</i>	STRA	female	4,67474E+11
<i>M. m. domesticus</i>	STRA	female	3,90032E+11
<i>M. m. domesticus</i>	WLA	male	3,8744E+11
<i>M. m. domesticus</i>	WLA	male	4,43308E+11
<i>M. m. domesticus</i>	WLA	male	25712726630
<i>M. m. domesticus</i>	WLA	female	84959764600
<i>M. m. domesticus</i>	WLA	female	1,63757E+11
<i>M. m. domesticus</i>	WLA	female	2,63347E+11
<i>M. m. castaneus</i>	CIM	male	1,72722E+11
<i>M. m. castaneus</i>	CIM	male	1,92545E+11
<i>M. m. castaneus</i>	CIM	male	3,13676E+11
<i>M. m. castaneus</i>	CIM	female	90957106500
<i>M. m. castaneus</i>	CIM	female	38601120090
<i>M. m. castaneus</i>	CIM	female	25533672800
<i>M. m. castaneus</i>	CKN	male	2,27304E+11
<i>M. m. castaneus</i>	CKN	male	11024921640
<i>M. m. castaneus</i>	CKN	male	3,91792E+11
<i>M. m. castaneus</i>	CKN	female	5617526230
<i>M. m. castaneus</i>	CKN	female	53824091000
<i>M. m. castaneus</i>	CKN	female	1,51756E+11
<i>M. spretus</i>	SEB	male	82471517000
<i>M. spretus</i>	SEB	male	58464708700
<i>M. spretus</i>	SEB	male	1,22234E+11
<i>M. spretus</i>	SEB	female	47492290000
<i>M. spretus</i>	SEB	female	94988258550
<i>M. spretus</i>	SEB	female	7644034850
<i>M. spretus</i>	SMON	male	41354940440
<i>M. spretus</i>	SMON	male	57883152100
<i>M. spretus</i>	SMON	male	26390689530
<i>M. spretus</i>	SMON	female	22936536000
<i>M. spretus</i>	SMON	female	10536046730
<i>M. spretus</i>	SMON	female	7388313180
<i>M. spicilegus</i>	ZBP	male	35700809700
<i>M. spicilegus</i>	ZBP	female	5217857200
<i>M. spicilegus</i>	ZRU	male	6674888600
<i>M. spicilegus</i>	ZRU	male	14893201300
<i>M. spicilegus</i>	ZRU	male	14137819000
<i>M. spicilegus</i>	ZRU	female	9291124830
<i>M. spicilegus</i>	ZRU	female	15389113500
<i>M. spicilegus</i>	ZRU	female	8772840360
<i>M. macedonicus</i>	MACSO	male	98631559600

<i>M. macedonicus</i>	MACSO	female	43432965500
<i>M. macedonicus</i>	XBS	male	1,48994E+11
<i>M. macedonicus</i>	XBS	male	68465748000
<i>M. macedonicus</i>	XBS	male	1,37363E+11
<i>M. macedonicus</i>	XBS	female	1,66277E+11
<i>M. macedonicus</i>	XBS	female	32202607900
<i>M. macedonicus</i>	XBS	female	26921836039

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