Supplementary Material

Mice

All mice were housed in a conventional breeding facility at the Institute of Vertebrate Biology, Czech Academy of Sciences, in Studenec. They were maintained under standard conditions, with a light:dark regime of 14:10 hours (light 6 am – 8 pm), temperature of 22–23 °C, and humidity within the range of 40-70%. Mice had access to standard food pellets (Myška 1, VKS Podhledští Dvořáci, Hamry, Czechia) and tap water *ad libitum*. Mice were weaned at 20 days of age and then housed individually in transparent polycarbonate cages equipped with sifted sawdust, shredded paper for nest building and nest houses made of red transparent polycarbonate. The cages were cleaned every 5-7 days. The two subspecies were kept in different rooms. This facility is authorised for the use of experimental animals (Licence MZE-50144/2022-13143), as well as for the breeding and supply of experimental animals to third parties (MZE-50151/2022-13143). These licences are in compliance with the corresponding regulations and standards of the European Union, as specified in Council Directive 86/609/EEC. All animals were handled by authorised persons only (Licence No. CZ01271 for Z.H.).

At the age ~100 days (range 60–140 days, Table S3; basic statistics of the age structure are available upon request from the corresponding author), 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^3 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_2O 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.

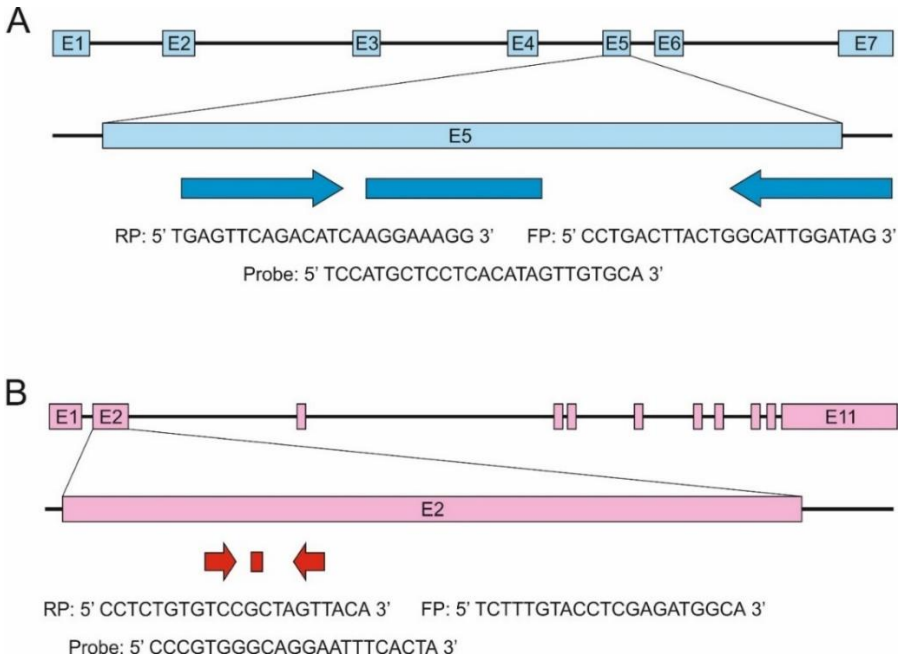

Fig. S1 Position of ddPCR primers and probes in *Mup* paralogs (A) and the *Tert* gene (B). RP = reverse primer, FP = forward primer, E = exon. Proportions of exons, introns, primers, and probes are not exactly to scale.

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 µl of ddPCR Supermix for Probes (Bio-Rad), 0.12 µl of the *Mup* assay, 0.12 µl of the *Tert* assay, 0.05 µl of *Mse*I 10 000 U/ml (New England Biolabs, Ipswich, MA, USA), 1.1 µl of genomic DNA (in some cases, the volume was adjusted to produce a desired load 50–100 copies/µl in the well), and ddH_2O to the final volume 24 µl.

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, https://doi.org/10.1101/2021.12.22.473661). 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´
Mup2	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#1	TGAGTTCAGACATCAAGGAAAGG	
Mup8	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#2	TGAGTTCAGACATCAAGGAAAGG	
Mup9	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#3	TGAGTTCAGACATCAAGGAAAGG	
Mup10	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#4	TGAGTTCAGACATCAAGGAAAGG	
Mup11	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#5	TGAGTTCAGACATCAAGGAAAGG	
Mup22	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#6	TGAGTTCAGACATCAAGGAAAGG	
Mup13	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#7	TGAGTTCAGACATCAAGGAAAGG	
Mup14	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#8	TGAGTTCAGACATCAAGGAAAGG	
Mup16	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#9	TGAGTTCAGACATCAAGGAAAGG	
Mup17	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#10	TGAGTTCAGACATCAAGGAAAGG	
Mup18	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#11	TGAGTTCAGACATCAAGGAAAGG	
Mup19	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTTGTGCA
#12	TGAGTTCAGACATCAAGGAAAGG	
Mup7	CCTGACTTACTGGCATTGGATAG	TCCATGCT**T**CTCACATAGTTGTGCA
#13	TGAGTTCAGACATCAAGGAAAGG	
Mup1	CCTGACTTACTGGCATTGGATAG	TCCATGCT**T**CTCACATAGTTGTGCA
#14	TGAGTTCAGACATCAAGGAAAGG	
Mup12	CCTGACTTACTGGCATTGGATAG	TCCATGCT**T**CTCACATAGTTGTGCA
#15	TGAGTTCAGACATCAAGGAAAGG	
Mup15	CCTGACTTACTGGCATTGGATAG	TCCATGCT**T**CTCACATAGTTGTGCA
#16	TGAGTTCAGACATCAAGGAAAGG	
Mup5	CCTGACTTACTGGCATTGGATAG	TCCATGCTCCTCACATAGTT**T**TGCA
#17	TGAGTTCAGACATCAAGGAAA**A**G	
MupPs19	CCTGACTTACTGGCATTGG**T**TAG	TCCATGCTCCTC**G**C**T**TAGTTGTGCA
#18	TGAGTTCAGACATCAAGGAAA**A**G	
MupPs21	CCTGACTTACTGGCATTGG**T**TAG	TCCATGCTCCTC**G**C**T**TAGTTGTGCA
#19	TGAGTTCAGACATCAAGGAAA**A**G	
Mup20	CCTGACTTACTGGCATTGG**T**TAG	TCCATGCTCCTC**G**C**T**TAGTTGTGCA
#20	TGAGTTCAGACATCAAGGAAA**A**G	

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´
MupPs18	CCTGAGTTACTGGCATTGGATAG	TGCATGCTCCTCACATAATTTTGCA
	TGAGTTCAGACATCAAGGAAAAG	
MupPs12	CCTGACTTACTGGCATTAGATAG	TCCATGCTCCTCACATAGTTTTTCA
	TGAGTTCAGACATCAAGGAAGAG	
MupPs1	CCTGACTTACTGGCATTAGATAG	TCCATGCTCCTCAAATAGTTTTGCA
	TGAGTTCAGACATCAAGGAAGAG	
MupPs2	CCTGACTTACTGGCATTAGATAG	TCCATGCTCCTCAAATAGTTTTGCA
	TGAGTTCAGACATCAAGGAAGAG	
MupPs10	CCTGACTTACTGGCATTAGATAG	TCCATGCTCCTCAAATAGTTTTGCA
LOC108168721	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.

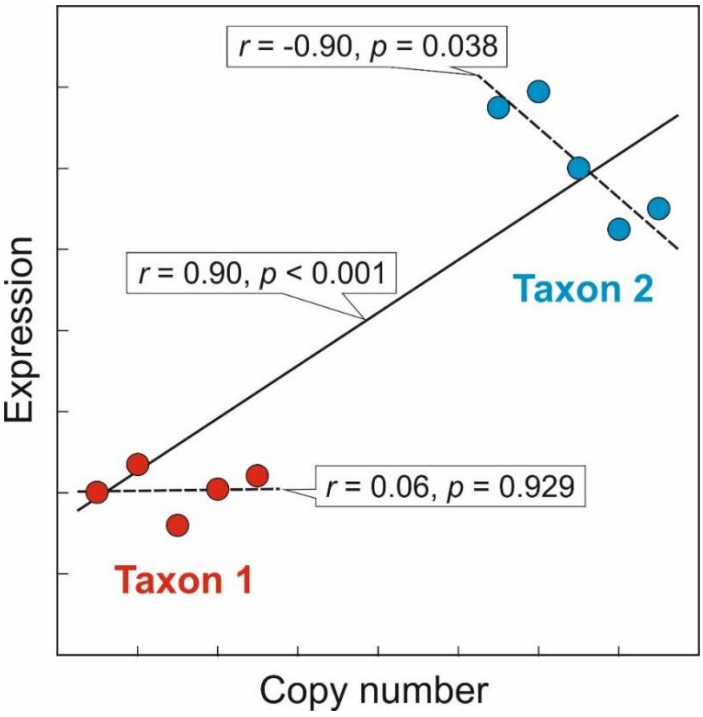

Fig. S2 A hypothetical example of the discrepancy between correlations at different levels. While there is no correlation between the number of genes and their expression in Taxon 1, and this correlation is even significantly *negative* in Taxon 2, we detect a highly *positive* correlation in the total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

Table S3. CN estimates for each individual studied.

ID	Taxon	WDS	Age (days)	Total CN	+Poisson error	-Poisson error
SZ♂0922	*M. m. musculus*	BUSNA	140	34	1.3	1.3
SZ♂8455	*M. m. musculus*	BUSNA	98	32	1.5	1.5
SZ♂8456	*M. m. musculus*	BUSNA	98	35	1.8	1.7
SZ♂7552	*M. m. musculus*	MBK	80	23	0.9	0.9
SZ♂8569	*M. m. musculus*	MBK	119	22	0.9	0.9
SZ♀1605	*M. m. musculus*	MBK	136	22	0.9	0.8
SZ♂1561	*M. m. musculus*	MDH	139	26	1.1	1.1
SZ♂7092	*M. m. musculus*	MDH	99	23	1.1	1.1
SZ♂7832	*M. m. musculus*	MDH	134	24	0.8	0.8
SZ♂0788	*M. m. musculus*	MPB	62	24	1.0	0.9
SZ♂7398	*M. m. musculus*	MPB	88	27	1.2	1.1
SZ♂8772	*M. m. musculus*	MPB	111	28	1.3	1.2
SZ♂2157	*M. m. musculus*	STUF	113	22	0.9	0.9
SZ♂8789	*M. m. musculus*	STUF	84	24	1.2	1.2
SZ♂8790	*M. m. musculus*	STUF	110	23	1.0	0.9
SZ♂1155	*M. m. domesticus*	DDO	131	27	1.1	1.1
SZ♂7605	*M. m. domesticus*	DDO	108	28	1.1	1.1
SZ♂7612	*M. m. domesticus*	DDO	108	28	1.1	1.1
SZ♂7305	*M. m. domesticus*	DROS	87	35	1.6	1.6
SZ♂8265	*M. m. domesticus*	DROS	106	40	1.6	1.6
SZ♀8268	*M. m. domesticus*	DROS	106	33	1.3	1.3
SZ♂1005	*M. m. domesticus*	SCHUNT	136	22	0.8	0.7
SZ♂7274	*M. m. domesticus*	SCHUNT	69	21	0.9	0.9
SZ♂7405	*M. m. domesticus*	SCHUNT	60	22	0.9	0.8
SZ♂1363	*M. m. domesticus*	STRA	124	29	1.1	1.1
SZ♂7484	*M. m. domesticus*	STRA	84	29	1.4	1.3
SZ♂7700	*M. m. domesticus*	STRA	102	27	1.1	1.0
SZ♂1907	*M. m. domesticus*	WLA	122	18	0.8	0.7
SZ♂7156	*M. m. domesticus*	WLA	96	17	0.6	0.6
TA♂0051	*M. m. domesticus*	WLA	85	18	0.8	0.6
SZ♂2484	*M. m. castaneus*	CIM	120	7	0.3	0.2
SZ♂2486	*M. m. castaneus*	CIM	100	7	0.4	0.3
SZ♂9032	*M. m. castaneus*	CIM	100	8	0.3	0.2
SZ♂1551	*M. m. castaneus*	CKN	118	14	0.7	0.6
SZ♂8515	*M. m. castaneus*	CKN	95	13	0.5	0.6
SZ♀8517	*M. m. castaneus*	CKN	95	14	0.7	0.5
SZ♂1527	*M. spretus*	SEB	118	5	0.2	0.2
SZ♂7696	*M. spretus*	SEB	102	5	0.2	0.2
SZ♂8111	*M. spretus*	SEB	115	5	0.2	0.2
SZ♂0722	*M. spretus*	SMON	65	5	0.2	0.2
SZ♂1620	*M. spretus*	SMON	136	5	0.2	0.2
SZ♂7048	*M. spretus*	SMON	81	5	0.2	0.2
SZ♂2268	*M. spicilegus*	ZPB	110	8	0.4	0.4
SZ♀2271	*M. spicilegus*	ZPB	110	11	0.4	0.5
SZ♂6098	*M. spicilegus*	ZRU	123	6	0.3	0.2
SZ♂6187	*M. spicilegus*	ZRU	62	6	0.3	0.3
SZ♀8325	*M. spicilegus*	ZRU	129	6	0.2	0.2

SZ♂1952	*M. macedonicus*	MACSO	121	5	0.3	0.3
SZ♀3157	*M. macedonicus*	MACSO	91	4	0.2	0.1
SZ♂0793	*M. macedonicus*	XBS	61	6	0.3	0.2
TA♂0671	*M. macedonicus*	XBS	99	6	0.3	0.2
TA♂1148	*M. macedonicus*	XBS	77	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.

	mus	*dom*	*cas*	*spr*	*spi*	*mac*
M. m. musculus	--					
M. m. domesticus	1.0000	--				
M. m. castaneus	0.0001	0.0001	--			
M. spretus	0.0001	0.0001	0.2960	--		
M. spicilegus	0.0001	0.0001	0.8542	0.9535	--	
M. macedonicus	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.

	mus	*dom*	*cas*	*spr*	*spi*	*mac*
M. m. musculus	--					
M. m. domesticus	1.0000	--				
M. m. castaneus	1.0000	1.0000	--			
M. spretus	0.2556	0.0213	0.9828	--		
M. spicilegus	0.0458	0.0039	0.2177	1.0000	--	
M. macedonicus	1.0000	0.6235	1.0000	1.0000	1.0000	--

	mus	*dom*	*cas*	*spr*	*spi*	*mac*
M. m. musculus	--					
M. m. domesticus	0.4538	--				
M. m. castaneus	1.0000	0.1403	--			
M. spretus	0.7177	0.0045	1.0000	--		
M. spicilegus	0.1269	0.0008	1.0000	1.0000	--	
M. macedonicus	1.0000	0.7926	1.0000	1.0000	1.0000	--

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

Taxon	WDS	Sex	Total MUP
M. m. musculus	BUSNA	male	2.46517E+11
M. m. musculus	BUSNA	male	1.98267E+11
M. m. musculus	BUSNA	male	1.94918E+11
M. m. musculus	BUSNA	female	90922726300
M. m. musculus	BUSNA	female	1.11731E+11
M. m. musculus	BUSNA	female	3.14495E+11
M. m. musculus	MBK	male	3.55171E+11
M. m. musculus	MBK	male	2.71233E+11
M. m. musculus	MBK	male	1.54604E+11
M. m. musculus	MBK	female	1.07276E+11
M. m. musculus	MBK	female	41659054400
M. m. musculus	MBK	female	54580663570
M. m. musculus	MDH	male	4.18523E+11
M. m. musculus	MDH	male	11293057560
M. m. musculus	MDH	male	96256335100
M. m. musculus	MDH	female	3.09352E+11
M. m. musculus	MDH	female	21681455230
M. m. musculus	MDH	female	20432230660
M. m. musculus	MPB	male	2.39306E+11
M. m. musculus	MPB	male	3.7173E+11
M. m. musculus	MPB	male	4.02615E+11
M. m. musculus	MPB	female	88221641470
M. m. musculus	MPB	female	2.55394E+11
M. m. musculus	MPB	female	96296607800
M. m. musculus	PWD	male	2.58023E+11
M. m. musculus	PWD	male	3.01607E+11
M. m. musculus	PWD	female	29577503510
M. m. musculus	PWD	female	72959030670
M. m. musculus	STUF	male	1.86953E+11
M. m. musculus	STUF	male	1.33419E+11
M. m. musculus	STUF	male	85705173340
M. m. musculus	STUF	female	1.00333E+11
M. m. musculus	STUF	female	28554232670
M. m. musculus	STUF	female	21152495230
M. m. domesticus	DDO	male	1.58505E+11
M. m. domesticus	DDO	male	1.96881E+11
M. m. domesticus	DDO	male	2.28704E+11
M. m. domesticus	DDO	female	31608436030
M. m. domesticus	DDO	female	67062170000
M. m. domesticus	DDO	female	1.92464E+11
M. m. domesticus	DROS	male	2.03232E+11
M. m. domesticus	DROS	male	4.03498E+11
M. m. domesticus	DROS	male	2.18228E+11
M. m. domesticus	DROS	female	1.51353E+11
M. m. domesticus	DROS	female	1.77458E+11
M. m. domesticus	DROS	female	1.97522E+11
M. m. domesticus	SCHUNT	male	4.31599E+11

M. m. domesticus	SCHUNT	male	39843145360
M. m. domesticus	SCHUNT	male	3.81514E+11
M. m. domesticus	SCHUNT	female	1.40508E+11
M. m. domesticus	SCHUNT	female	4.47635E+11
M. m. domesticus	SCHUNT	female	3.01116E+11
M. m. domesticus	STRA	male	5.94708E+11
M. m. domesticus	STRA	male	3.64625E+11
M. m. domesticus	STRA	male	3.57001E+11
M. m. domesticus	STRA	female	3.52241E+11
M. m. domesticus	STRA	female	4.67474E+11
M. m. domesticus	STRA	female	3.90032E+11
M. m. domesticus	WLA	male	3.8744E+11
M. m. domesticus	WLA	male	4.43308E+11
M. m. domesticus	WLA	male	25712726630
M. m. domesticus	WLA	female	84959764600
M. m. domesticus	WLA	female	1.63757E+11
M. m. domesticus	WLA	female	2.63347E+11
M. m. castaneus	CIM	male	1.72722E+11
M. m. castaneus	CIM	male	1.92545E+11
M. m. castaneus	CIM	male	3.13676E+11
M. m. castaneus	CIM	female	90957106500
M. m. castaneus	CIM	female	38601120090
M. m. castaneus	CIM	female	25533672800
M. m. castaneus	CKN	male	2.27304E+11
M. m. castaneus	CKN	male	11024921640
M. m. castaneus	CKN	male	3.91792E+11
M. m. castaneus	CKN	female	5617526230
M. m. castaneus	CKN	female	53824091000
M. m. castaneus	CKN	female	1.51756E+11
M. spretus	SEB	male	82471517000
M. spretus	SEB	male	58464708700
M. spretus	SEB	male	1.22234E+11
M. spretus	SEB	female	47492290000
M. spretus	SEB	female	94988258550
M. spretus	SEB	female	7644034850
M. spretus	SMON	male	41354940440
M. spretus	SMON	male	57883152100
M. spretus	SMON	male	26390689530
M. spretus	SMON	female	22936536000
M. spretus	SMON	female	10536046730
M. spretus	SMON	female	7388313180
M. spicilegus	ZBP	male	35700809700
M. spicilegus	ZBP	female	5217857200
M. spicilegus	ZRU	male	6674888600
M. spicilegus	ZRU	male	14893201300
M. spicilegus	ZRU	male	14137819000
M. spicilegus	ZRU	female	9291124830
M. spicilegus	ZRU	female	15389113500
M. spicilegus	ZRU	female	8772840360
M. macedonicus	MACSO	male	98631559600

M. macedonicus	MACSO	female	43432965500
M. macedonicus	XBS	male	1.48994E+11
M. macedonicus	XBS	male	68465748000
M. macedonicus	XBS	male	1.37363E+11
M. macedonicus	XBS	female	1.66277E+11
M. macedonicus	XBS	female	32202607900
M. macedonicus	XBS	female	26921836039