

Additional file 2.

Sequence alignment of mtDNA *control region* amplicons generated with the primer set HRM_Rod [11]. IRH control samples sequence; IRQ questing nymphs sequence. Sequences 1-13: *Apodemus* spp.; 9 -13 *Apodemus* spp. amplicons (as resulting from BLASTn search) with deletions and mutations resulting in a deviating melting pattern leading to misidentification by HRMA as *M. glareolus*; 14-15: *M. glareolus* amplicons. Reported are also melting temperature, in bold refer to QIAgen extracted samples, while normal to ThermoScientific extracted samples.

	10	20	30	40	50	60	70
1-IRH_A.flavicolis	CTGGTTCTTACTTCAGGGCCATCAAATGCGTTATC-GCCCATACGTTCCCCCTTAAATAAGACATCTCG-A						
2-IRQ
3-IRQ
4-IRQ
5-IRQ
6-IRQ
7-IRQ
8-IRQ
9-IRQ
10-IRQ
11-IRQ
12-IRQ
13-IRQ
14-IRH_M.glareolus
15-IRQ
Clustal Consensus	****	****	*****	*	*****	*****	*

	80	90	100	110	120	130	140
1-IRH_A.flavicolis	TGGTACGGGTCTAATCAGCCAAGACTTC-CATAACTGTGGTCTCGGGCAGTTGGTATTTTTTATTTT						
2-IRQ
3-IRQ
4-IRQ
5-IRQ
6-IRQ
7-IRQ
8-IRQ
9-IRQ
10-IRQ
11-IRQ
12-IRQ
13-IRQ
14-IRH_M.glareolus
15-IRQ
Clustal Consensus	*****	*****	*	*	*	*	*****

	150	160	170	T_m (°C)
1-IRH_A.flavicolis	GGATGCTGTGACTCAGCATAGCCGTC AAGCATGAA			81.9
2-IRQ	81.90
3-IRQ	81.50
4-IRQ	82.06 ^a
5-IRQ	82.20
6-IRQ	82.44
7-IRQ	82.40
8-IRQ	82.46
9-IRQ	82.00
10-IRQ	81.30
11-IRQ	81.10
12-IRQ	81.34
13-IRQ	82.00
14-IRH_M.glareolus	81.12
15-IRQ	81.00
Clustal Consensus	*****	*	*	*****

^a despite no mutation this sample showed a lower melting temperature than reference samples, possibly because of abnormal condition in the HRMA run.