## Supplementary methods to: *Species limits and phylogeographic structure in two genera of solitary mole rats* (Uhrová et al. 2021)

### *PCR protocol and primers*

Each 10 µl PCR reaction contained 5 µl of Qiagen Multiplex PCR Master Mix or Qiagen HotStarTaq Master Mix Kit, 0.3 µl of each forward and reverse primer (10 µM), 0.5 µl of DNA, and 3.9 µl ddH2O. PCR conditions for cytochrome b marker followed instructions of Faulkes et al (1997). The thermocycling conditions for RAG1, DHCR, NAD SYN, SMO, and TRPV consisted of initial denaturation step at 95°C for 15min, 10 cycles of 94°C for 30s, 65°C for 30s (decreasing by 1°C with each cycle), 72°C for 1min, then 25 cycles of 94°C for 30s, 55°C for 30s, and 72°C for 1min, with the final step of 72°C for 10min. Amplification for FGB gene started with initial denaturation at 95°C for 15min, following by 35 cycles of 94°C for 40s, 59°C for 45s, 72°C 1min 30s, and ending with final extension at 72°C for 10min. The quality and lengths of fragments were verified by an electrophoresis in an 1% agarose gel. The purification of all amplified PCR products was performed by two enzymes (Exonuclease I, E.coli (Exo I, 20,000 units/ml) and Alkaline Phosphatase, Calf Intestinal (CIP, 10,000 units/ml) from New England BioLabs)) according to the following protocol: 0.05 µl Exo I, 0.1 µl CIP, 1 µl ddH2O and 5 µl of PCR product; 37°C for 30min, 85°C for 15min in thermocycler. All genes were commercially sequenced with forward primers and those with lower quality results, were sequenced from reverse side for the verification. The sequencing process was accomplished by GenSeq s.r.o. company.

**Table S1.** A list of nuclear markers with primers sequences and lengths of final sequences.

| Gene | Sequences of primers (5´-3´) | Alignment (bp) | | Reference |
|---|---|---|---|---|
| | | *Heliophobius* | *Georychus* | |
| CYTB | L: CGAAGCTTGATATGAAAAACCATCGTTG<br>H: AACTGCAGTCATCTCCGGTTTACAAGAC | 1116 | 920 | Irwin et al. 1991 |
| RAG1 | L: GCTTTGATGGACATGGAAGAAGACAT<br>H: GAGCCATCCCTCTCAATAATTTCAGG | 1083 | 1104 | Teeling et al. 2000 |
| DHCR | L: CAGGACATGCTGGTGCCCATGAA<br>H: CCTGGCTGGCTGGGCAGGATGAA | 351 | 350 | Rodríguez-Prieto et al. 2014 |
| FGB | L: GGGGAGAACAGAACCATGACCATCCAC<br>H: ACCCCAGTAFTATCTGCCATTCGGATT | 833 | 848 | Wickliffe et al. 2003 |
| NAD SYN | L: GTYCGYTACAAYTGCAGAGT<br>H: TCCTKSHCCAKGGGGTRAACCA | 568 | 565 | Rodríguez-Prieto et al. 2014 |
| SMO | L: GCCACCCTGCTCATCTGGAGGCG<br>H: TTGGCRATCATCTTGCTYTTCTTGA | 388 | 387 | Rodríguez-Prieto et al. 2014 |
| TRPV | L: TTACCRBACCACVGYGGACTACCT<br>H: CTGGAAGGAGCCRTCGAYGAAGA | 280 | 291 | Rodríguez-Prieto et al. 2014 |

**References:**

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