



*Matjaž Kuntner* | ORCID: 0000-0002-0057-2178

Jovan Hadži Institute of Biology, Research Centre of the Slovenian, Academy of Sciences and Arts, 1000 Ljubljana, Slovenia;

Department of Organisms and Ecosystems Research, National Institute of Biology, 1000 Ljubljana, Slovenia

*Cene Fišer* | ORCID: 0000-0003-1982-8724

SubBio Lab, Department of Biology, Biotechnical Faculty, University of Ljubljana, 1000 Ljubljana, Slovenia

### **Supplementary material**

ALIGNMENT S1      Fasta file of COI sequences used in our analyses.

ALIGNMENT S2      Fasta file of 28S rRNA sequences used in our analyses.

ALIGNMENT S3      Nexus matrix of concatenated COI and 28S rRNA sequences.











TABLE S1 Primer details with targeted gene, primer names (F: forward; R: reverse) and sequences, and reference information.

Gene	Primer F (Forward), R (Reverse)	Sequence	Reference
COI	F: LCO-1490  R: HCO-2198	5'GGTCAACAAATCATAAAGATATTGG-3'  (5'TAAACTTCAGGGTGACCAAAAAATCA-3')	Folmer et al., 1994;  Simon et al., 1994
28S	F: 28Sa  R: 28Sb	TTGGCGACCCGCAATTTAAGCAT  CCTGAGGGAACTTCGGAGGGAAC	Cristescu & Hebert 2005;  Hou et al., 2007
28S	F:28SF  R: 28SR	TTAGTAGGGGCGACCGAACAGGGAT  GTCTTTCGCCCCTATGCCCAACTGA	Hou et al., 2007

TABLE S2 PCR amplification protocols.

COI	28S
94°C: 1 min	94°C: 1 min
5 cycles	35 cycles
94°C: 30 s	94°C (30 s),
45°C: 90 s	45-50°C (45 s),
72°C: 1 min	72°C (1 min).
35 cycles:	
94°C: 30 s	
51°C: 90 s	
72°C: 1 min	
72°C (5 min).	72°C (5 min).
4°C: ∞	4°C: ∞







