**Electronic supplement**

Polak et al: Molecular phylogeny of the cave beetle genus *Hadesia* (Coleoptera: Leiodidae: Cholevinae: Leptodirini), with a description of a new species from Montenegro.

**Table S1.** List of taxa and sequence data used in phylogenetic analysis.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Locality** | **wgs 84 X** | **wgs 84 Y** | **Voucher** | **28S rDNA I** | **28S rDNA II** | **Histone H3** | **COI I** | **COI I** | **16S rRNA** |
| *Anthroherpon cylindricollis* | Mračna pećina, Banja stijena, Rogatica, BIH | 18.8877 | 43.7728 | C-4518 | KX223625 | KX223637 | KX223673 | KX223661 | KX223648 | KX223613 |
| *Croatodirus bozicevici* | Kaverna u tunelu Učka, Učka, Opatija, CRO | 14.2499 | 45.3140 | C-3048 | KX223635 | KX223646 | KX223683 | KX223671 | KX223659 | KX223624 |
| *Hadesia asamo* | Vodena jama u Braveniku, Grab, Zubačko polje, BIH  | 18.4328 | 42.6191 | C-4504 | KX223628 | KX223639 | KX223676 | KX223664 | KX223651 | KX223616 |
| *Hadesia asamo* | Vodena jama u Braveniku, Grab, Zubačko polje, BIH  | 18.4328 | 42.6191 | C-4505 | KX223627 | NA | KX223675 | KX223663 | KX223650 | KX223615 |
| *Hadesia asamo* | Vodena jama u Braveniku, Grab, Zubačko polje, BIH  | 18.4328 | 42.6191 | C-4527 | KX223629 | KX223640 | KX223677 | KX223665 | KX223652 | KX223617 |
| *Hadesia lakotai* | Veliko Đatlo, Korita, Bileća, BIH | 18.4946 | 43.0618 | C-4531 | KX223630 | KX223641 | KX223678 | KX223666 | KX223654 | KX223619 |
| *Hadesia lakotai* | Veliko Đatlo, Korita, Bileća, BIH | 18.4946 | 43.0618 | C-4530 | KX223631 | KX223642 | KX223679 | KX223667 | KX223655 | KX223620 |
| *Hadesia vasiceki* | Vjetrenica, Zavala, Popovo polje, BIH  | 17.9839 | 42.8458 | C-2991 | KX223626 | KX223638 | KX223674 | KX223662 | KX223649 | KX223614 |
| *Hadesia vasiceki* | Vjetrenica, Zavala, Popovo polje, BIH  | 17.9839 | 42.8458 | C-4503 | NA | NA | NA | NA | KX223653 | KX223618 |
| *Hadesia weiratheri* | Vojvode Dakovića pećina, Grahovsko polje, MNE | 18.6758 | 42.6558 | C-3099 | KX223636 | KX223647 | KX223684 | KX223672 | KX223660 | NA |
| *Hadesia zetae* sp.n. | Lipska pećina, Lipa, Cetinje, MNE | 18.9525 | 42.3753 | C-4263 | KX223632 | KX223643 | KX223680 | KX223668 | KX223656 | KX223621 |
| *Hadesia zetae* sp.n. | Lipska pećina, Lipa, Cetinje, MNE | 18.9525 | 42.3753 | C-4502 | KX223633 | KX223644 | KX223681 | KX223669 | KX223657 | KX223622 |
| *Leptomeson drombowskii* | Vranjača, Kotlenice, Dugopolje, CRO | 16.6482 | 43.5622 | C-4254 | KX223634 | KX223645 | KX223682 | KX223670 | KX223658 | KX223623 |

**Table S2.** DNA partitions and their optimal substitution models as selected using the program PartitionFinder and used in subsequent phylogenetic analyses.

|  |  |
| --- | --- |
| **Molecular data partition** | **Selected model** |
| 16S rRNA | GTR + G |
| COI, I. codon site  | GTR+I+G |
| COI, II. codon site | GTR+I+G |
| COI, III. codon site | GTR+I+G |
| 28S rRNA  | GTR + G |
| Histone H3, I. + II. codon site | HKY+I |
| Histone H3, III. codon site | GTR + G |

**Table S3.** List of primers and protocols used in the molecular analysis.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Molecular partition** | **Primer name** | **Direction** | **Sequence** | **Reference** | **Protocol** |
| 16S rRNA | 16S ar | Forward | CGCCTGTTTA5CAAAAACAT (5\* INOSINE) | Simon et al. 1991  | 30 s at 94°C, 30 s at 47°C, 45 s at 72°C, for 35 cycles followed by final extension at 72°C for 5 min |
|  | 16S br | Reverse | CCGGTCTGAACTCAGATCA | Simon et al. 1991 |
| COI | LCO 1490 | Forward | GGTCAACAAATCATAAAGATATTG | Folmer et al. 1994 | 45 s at 94°C, 1 min s at 45°C, 1 min at 72°C, for 35 cycles followed by final extension at 72°C for 2 min |
|  | HCO 2198 | Reverse | TAAACTTCAGGGTGACCAAAAAAT | Folmer et al. 1994 |
|  | Jerry | Forward | CAACATTTATTTTGATTTTTTGG | Simon et al. 1991 | 1 min at 94°C, 1 min at 46°C, 2 min at 72°C, for 35 cycles followed by final extension at 72°C for 7 min |
|  | Pat | Reverse | TCCAATGCACTAATCTGCCATATTA | Simon et al. 1991 |
| 28S rRNA | 28S lev2 | Forward | CAAGTACCGGTGAGGGAAAGTT | Verovnik et al. 2005 | 45 s at 94°C, 30 s at 50°C, 1 min at 72°C, for 30 cycles followed by final extension at 72°C for 3 min |
|  | 28S des2 | Reverse | GTTCACCATCTTTCGGGTC  | Zakšek et al. 2007 |
|  | 28S lev3 | Forward | GCCCTTAAAATGGATGGCGCT | Fišer et al. 2013 | 45 s at 94°C, 1 min at 50°C, 2 min at 72°C, for 30 cycles followed by final extension at 72°C for 3 min |
|  | 28S des5 | Reverse | CCGCCGTTTACCCGCGCTT | Fišer et al. 2013 |
| Histone H3 | H3aF2 | Forward | ATGGCTCGGTACCAAGCAGAC | Colgan et al. 1998 | 45 s at 94°C, 1 min 20 s at 46°C, 1 min at 72°C, for 35 cycles followed by final extension at 72°C for 3 min |
|  | H3aR2 | Reverse | ATRTCCTTGGGCATGATTGTTAC | Colgan et al. 1998 |

**References:**

COLGAN, D.J., McLAUCHLAN, A., WILSON, G.D.F., LIVINGSTON, S.P., EDGECOMBE, G.D., MACARANAS, J., G. CASSIS & M.R. GRAY 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Australian Journal of Zoology **46**: 419–437.

FIŠER, C., M. ZAGMAJSTER, M. & V. ZAKŠEK 2013. Coevolution of life history and morphology in female subterranean amphipods. – Oikos **122**: 770–778.

FOLMER, O.M., BLACK, M., HOEH, R., R. LUTZ & R. VRIJEHOEK 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. – Molecular Marine and Biotechnology **5:** 304–313.

SIMON, C., A. FRANKE & A. MARTIN 1991. The polymerase chain reaction: DNA extraction and amplification. Pp. 329–355 in: G.M. Hewitt, A.W.B. Johnson & J.P.W. Young (eds.), Molecular techniques in taxonomy. – Springer-Verlag, Berlin.

VEROVNIK, R., B. SKET & P. TRONTELJ 2005. The colonization of Europe by the freshwater crustacean *Asellus aquaticus* (Crustacea: Isopoda) proceeded from ancient refugia and was directed by habitat connectivity. – Molecular Ecology **14:** 4355–4369.

ZAKŠEK, V., B. SKET & P. TRONTELJ 2007. Phylogeny of the cave shrimp *Troglocaris*: evidence of a young connection between Balkans and Caucasus. – Molecular Phylogenetics and Evolution **42**, 223–235.

**Fig. S1.** Phylogenetic relationships among studied Anthroherponina taxa inferred by Bayesian analysis based on COI, 16S rRNA, 28S rRNA and H3 molecular markers. Values at nodes indicate posterior probabilities. Species names are followed by voucher codes in parentheses.

