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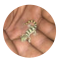

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A new obligate groundwater species of *Asellus* (Isopoda, Asellidae) from Iran

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Abstract

With only 43 described stygobionts and only two isopod species the obligate groundwater fauna of Iran, a vast country with over 10% of limestone surface, is inadequately known. Here, we report the discovery of *Asellus ismailsezarii* sp. nov. from Zagros mountains, the first eyeless and depigmented asellid isopod from Iran. The new species is morphologically similar to *Asellus monticola* Birstein, 1932, but it is eyeless and fully depigmented, has a slightly curved pereopod IV and does not bear any setae on proximal margins of exopodite of pleopods IV and V. Species phylogenetic relationships using original and datamined mitochondrial DNA and nuclear rDNA, and estimation of molecular divergences with other *Asellus* species, suggest that *A. ismailsezarii* sp. nov. is sister to a larger clade that also contains the European *A. aquaticus* species complex. Surface populations of *Asellus* have colonized groundwater at multiple occasions and localities, both in Europe and Asia, giving rise to species and subspecies that have evolved troglomorphisms, such as depigmentation and loss of eyes. Of the 37 formally described species and subspecies of *Asellus*, 15 are from groundwater, including *A. ismailsezarii* sp. nov. We predict that many more obligate groundwater *Asellus* taxa are yet to be discovered in Asia.

Keywords
Asia, Crustacea, groundwater, molecular phylogeny, taxonomy, troglomorphy

Introduction

Groundwater harbors a high diversity of metazoans that represents an important, yet underestimated, component of the Earth's freshwater biodiversity (Culver and Holsinger 1992; Dole-Olivier et al. 2009). Knowledge of groundwater biodiversity is uneven across the globe: species inventories are far more advanced in Europe, North America, and Australia, for example, than in South America, Africa and Asia (Zagmajster et al. 2018). We know of only 43 obligate groundwater species from Iran, despite the fact that 10.5% of the country (compared with approximately 1.7% of Europe) is underlain by carbonate rock formations. The obligate groundwater fauna includes four fish species, 23 amphipods of the genus *Niphargus* Schiødte, 1847, 12 copepods, one oligochaete, one gastropod and two isopods (Cichocka et al. 2015; Mousavi-Sabet et al. 2016; Malek-Hosseini and Zamani 2017; Vatandoust et al. 2019; Bargrizaneh et al. 2021; Malek-Hosseini et al. 2021). The only two obligate groundwater isopods known from Iran are *Microcharon raffaellae* Pesce, 1979 (Lepidocharontidae) collected from a well in Pliocenic sandstone and clay at Shahr-e-Kord city, Chaharmahal and Bakhtiari Province, and *Stenasellus tashanicus* Khalaji-Pirbalouty, Fatemi, Malek-Hosseini & Kuntner, 2018 (Stenasellidae) collected from Tashan karstic cave in Khuzestan province (Pesce 1979; Khalaji-Pirbalouty et al. 2018). In the present article, we describe the third obligate groundwater isopod from Iran belonging to the family Asellidae (Pancrustacea, Isopoda, Asellota).

The Asellidae is one of the few families of metazoans containing a large number of both surface and subterranean aquatic species. To date, the family contains 23 genera and 428 species and subspecies (Malard et al. in press). Of these 428 species and subspecies, 279 are eyeless and depigmented obligate groundwater species, 18 are mostly confined to groundwater, often showing reduced eyes and pigmentation, and 131 are occulated and pigmented surface water species. The family is widely distributed in the northern hemisphere with species in northern part of North America, Europe, northern Africa and Asia. The systematics of asellids is not yet fully established but morphological and molecular evidence support the existence of three major clades whose species occur in America, Europe and Northern Africa, and Asia, respectively (Morvan et al. 2013). One of these major clades to which Henry and Magniez (1995) referred to as the "*Asellus* pattern" contains 59 species and subspecies among which 26 inhabit surface waters and 33 groundwaters. They belong to the genera *Asellus* Geoffroy, 1762, *Calasellus* Bowman, 1981, *Columbasellus* Lewis, Martin & Wetzer, 2003, *Limnoasellus* (*Nomen nudum* in Hidding et al. 2003), *Mesoasellus* Birstein, 1939, *Nipponasellus* Matsumoto, 1962, *Phreatoasellus* Matsumoto, 1962, *Sibirasellus* Henry & Magniez, 1993 and *Uenasellus* Matsumoto, 1962.

With the exception of the genus *Asellus*, all genera within the "*Asellus* pattern" have relatively narrow distribution ranges either in Lake Baikal, Far East Russia, South Korea, Japan, or the Pacific Northwest coast of North America (Matsumoto 1963, 1966;

Bowman 1981; Henry and Magniez 1991, 1993, 1995; Hidding et al. 2003; Lewis et al. 2003; Sidorov and Prevorčnik 2016). The genus *Asellus* shows a wide geographic range, being present in Alaska (1 species, Bowman and Holmquist 1975), Asia (15 species, Henry and Magniez 1995; Sidorov and Prevorčnik 2016) and Europe (2 species). The occurrences of *Asellus* in Europe were all attributed to the *Asellus aquaticus sensu lato* species complex (Verovnik et al. 2005), with the exception of occurrences from two nearby surface water sites in eastern Ukraine, which were attributed to *Asellus* sp. (River Kharkiv and Pionersky pond; see Verovnik et al. 2005). The taxonomic status of *Asellus* populations within the *A. aquaticus* complex is not yet fully resolved. That complex actually contains two species, *A. aquaticus* Linnaeus, 1758 and *A. kosswigi* Verovnik et al., 2009, and eleven formally described subspecies (Sket 1965; Turk-Prevorčnik and Blejec 1998). COI-based species delimitation methods indicate that there may be many more species (Sworobowicz et al. 2015, 2020). Also, several *Asellus* populations belonging to that complex have colonized caves in Europe and have evolved traits such as loss of pigmentation and eye reduction (Balázs et al. 2021).

The eastern geographic boundaries of the *A. aquaticus* complex are not clearly established. The global biodiversity information facility does not report any records of *A. aquaticus* Linnaeus, 1758 in Iran (consultation date: 16/11/2021). However, the presence of that species was reported by Rémy (1941) at a pond located 6 km from Chahi, Mazanderan province, and more recently by Henry and Magniez (1995) at a surface freshwater site in the Gorgan province contiguous to Turkmenistan. Birstein (1945) described *Asellus aquaticus messerianus* from Turkmenistan Lakes Delili, Yashka and Karatogelek located near the northern border of Iran. Another asellid species, *Asellus monticola*, originally described by Birstein (1932) from freshwater surface sites in Armenia and Georgia was collected in Northern Iran at Bora Laan Spring, a locality located near the Turkish border (Henry and Magniez 1996).

Here, we report on the discovery of *Asellus ismailsezarii* sp. nov. (Asellidae, Asellota, Isopoda, Pancrustacea), the first eyeless and depigmented asellid isopod described from Iran. We also provide morphological comparisons with *A. monticola*, the species that morphologically resembles *Asellus ismailsezarii* sp. nov. as well as comparisons with European cave-species and subspecies belonging to the *A. aquaticus* complex. We then use molecular data to corroborate the species status of *A. ismailsezarii* sp. nov. and to document its phylogenetic relationship to the *A. aquaticus* complex.

Material and methods

Sampling

On several occasions from December 2018 to June 2019, we collected by hand four males, four females and 17 juveniles of *Asellus* at Ganow (Gandab) spring, Iran. This karstic spring is seasonal, flowing during winter and spring. The spring is located close to Tuveh village, Andimeshk, Khuzestan Province (geographic coordinates: 32°48'31"N, 48°43'32"E; altitude: 470 m above sea level) (Fig. 1).

Figure 1. Photo of Ganow spring, the type locality of *Asellus ismailsezarii* sp. nov., Tuveh village, Andimeshk, Khuzestan Province, Iran. Stream width is about one meter.

Morphological description

When necessary, pereopods I, IV and VII from one side of the body were dissected and heat-treated in a KOH solution, dyed with Trypan Blue for better visibility of spinulation and then temporary mounted on slides in glycerine. After dissection, also antennae I, II, pleopods, uropods and pleotelson, were mounted on slides in glycerine. Specimens and their body parts were photographed and measured using a Sony DXC390P digital camera mounted on a stereomicroscope or microscope (depending on the size of the structure), and measured with Leica Application suite – LAS EZ. The remains of the dissected specimens were then transferred to 70% ethanol for storage. In one of the specimens from the type locality, all appendages were dissected and prepared for drawing. All pereopods, as well as the trunk were heat-treated and dyed as described above, and then temporarily mounted in glycerine, alongside the rest of dissected specimen (mouth appendages, antennae, pleopods, uropods). Vector drawings were made from microphotographs using a graphics tablet (Wacom, Cintiq 13HD Creative Pen Display) and the free software KRITA 4.1.1 (https://krita.ord/). In males, we measured 90 morphometric characters (cf. Prevorčnik et al. 2004) for describing body proportions (trunk, appendages) and characterizing cuticular and sensory structures (number and length of spines, setae, aesthetascs). In antenna II, only the last two articles of the antennal basis (fourth and fifth) were included in the total antennal length together with the length of the flagellum. In pereopods IV and VII, length of the coxa was not included in the total pereopod length. We did not report full length of antenna II and pereopods because in most cases the basal articles of both appendages remained attached to the body during dissection. Throughout the ensuing description, we provide a single measurement value for the holotype, together with a range of values (in parentheses) referring to the males (♂♂) and females (♀♀) from the type material.

For observation with scanning electron microscopy (SEM), Plp I and Plp II of the male holotype and paratype stored in 70% ethanol were air-dried and mounted on sample stubs using conductive double sided carbon tape. Mounted samples were sputter-coated with platinum and observed with a JEOL JSM-7500F field emission scanning electron microscope (Jeol. Japan) at the Department of Biology, Biotechnical Faculty, University of Ljubljana.

Molecular taxonomy

DNA extraction, amplification and gene sequencing

We used the last three pereopods of three specimens (specimens AS1, AS2 and AS3, see Material examined) of *A. ismailsezarii* sp. nov. to obtain sequences of the mitochondrial cytochrome oxidase subunit I (COI) gene and 28S nuclear rDNA gene. We performed molecular analyses at the Evolutionary Zoology Laboratory, Jovan Hadži Institute of Biology ZRC SAZU, Ljubljana (EZ LAB, Slovenia) and LEHNA laboratory, Villeurbanne (LEHNA, France). We crosschecked DNA extraction protocols for the COI gene using specimen AS1 and DNA extraction and PCR protocols for the 28S using specimens AS1 and AS2. Full detail of the molecular protocol for each gene and specimen is provided in Suppl. material 1: Table S1 and Suppl. material 2: Table S2.

We performed robotic DNA extraction at EZ LAB on the three specimens using Mag MAX™ Express magnetic particle processor Type 700 with DNA Multisample kit (Thermo Fisher Scientific kit) and modified protocols following Vidergar et al. (2014). We also extracted DNA from specimen AS1 at LEHNA using a modified Chelex protocol from Casquet et al. (2012). We incubated pereopods in a solution of 150 µl of 7% chelex and 10 µl of proteinase K at 15 mg / ml for 90 minutes at 56 °C, then 15 minutes at 90 °C.

We performed polymerase chain reactions (PCRs) for COI fragments at LEHNA using a previously optimized protocol (Calvignac et al. 2011). However, we used a Taq polymerase (Eurobiotaq) amount of 0.05 U instead of 0.15 U and a PCR volume of 25 µl instead of 35 µl. To detect amplification of nuclear paralogs of mtDNA, we used a mix of four half-nested PCR strategies applied on a dilution range of initial DNA template (pure DNA solution of 2 µl to 1/2500 dilution, primers in Suppl. material 1: Table S1 and Suppl. material 2: Table S2). We ran the first round PCRs with one step of 3 min at 95 °C; 37 cycles of 20 s at 95 °C, 30 s at 51 °C, 45 s at 72 °C; and one step of 5 min at 72 °C. The second round PCRs were run on 1 µl of the first round PCR product using the same parameters but 35 cycles. Service providers (BIOFIDAL, Vaulx-en-Velin, France) performed Sanger sequencing using PCR amplification primers.

We performed PCRs for 28S fragments at EZ LAB on the three specimens and at LEHNA on specimens AS1 and AS2. At EZ LAB we performed PCRs in 25 µl final volume using H2O: 18.3 µl, 10X Buffer: 2.5 µl, dNTPS (20 mM): 0.5 µl, Primer (10 µM): 0.6 µl forward and 0.6 µl reverse, polymerase (Eurobiotaq 5U/µl): 0.25 µl, and BSA (10 mg/ml): 0.25 µl (primers in Suppl. material 1: Table S1 and Suppl. material 2: Table S2). We applied the following PCR settings: one step of 3 min at 94 °C; 36 cycles of 45 s at 94 °C, 1 min at 47 °C, 1 min at 72 °C; and one step of 3 min at 72 °C. PCR products were sent to Macrogen Europe (Amsterdam, the Netherlands) for sequencing. At LEHNA, we amplified 28S fragments with 2 independent pairs of primers (Suppl. material 1: Table S1 and Suppl. material 2: Table S2) using the following settings: one step of 3 min at 95 °C; 37 cycles of 30 s at 95 °C, 30 s at 62 °C, 30 s at 72 °C; and one step of 5 min at 72 °C. BIOFIDAL performed Sanger sequencing. We visualized all chromatograms using FinchTV (Geospiza, Seattle, WA, USA). Sequences were submitted to GenBank (accession numbers OM640761–OM640764; OM501360–OM501366, Suppl. material 3: Table S3).

Molecular species delimitation

Species and higher taxa and their associated names represent hypotheses of independently evolving lineages that should ideally be supported by different lines of evidence (de Queiroz 2007; Padial et al. 2010; Fišer et al. 2018). In addition to morphological evidence, we used a COI-based molecular species delimitation approach to corroborate whether *A. ismailsezarii* sp. nov. was indeed a new species. In effect, we follow the diagnosable interpretation of the phylogenetic species concept (de Queiroz 2007).

COI sequences of *A. ismailsezarii* sp. nov. produced in this study were analysed with all COI sequences of taxa belonging to the "*Asellus* pattern" (*sensu* Henry and Magniez 1995) available in NCBI (Sequence extraction date: 25 August 2021). In addition to sequences of *A. ismailsezarii* sp. nov., we retrieved 581 sequences belonging to the *A. aquaticus sensu lato* species complex (including *A. kosswigi*, Verovnik et al. 2005, 2009; Sworobowicz et al. 2015, 2020; Lafuente et al. 2021), *Asellus* sp. from Ukraine, *Asellus hilgendorfii* from Japan, and two species from Lake Baikal, Russia – *Mesoasellus dybowskii* and *Limnoasellus poberezhnii* (*Nomen nudum* in Hidding et al. 2003) (Suppl. material 3: Table S3). We aligned the sequences with ClustalO in Seaview (Gouy et al. 2010) (Suppl. material 4: Table S4). We checked visually for the presence of anomalies, including stop codons and frameshifts, and removed ambiguous sites with the embedded Gblocks (with options for less stringed selection enforced) (Castresana 2000). COI maximum-likelihood phylogenies were reconstructed using PhyML (Guindon et al. 2010) under the GTR + G + I model of substitution with 4 substitution rate categories and a gamma shape parameter ($\alpha = 0.519$) as well as a proportion of invariant sites (0.392) estimated by maximum likelihood in PhyML. This model of evolution was previously used in several studies for delimiting species and reconstructing phylogenetic relationships among asellids (Lefébure et al. 2006; Morvan et al. 2013; Eme et al. 2018). We used MEGA (Kumar et al. 2018) as well, to find the best nucleotide substitution model and the optimal partitioning scheme for each marker. Molecular operational taxonomic units

(MOTUs) were delimited using the fixed threshold method implemented by Lefébure et al. (2006) for crustaceans. This method is based on the observation made from 1500 COI sequences belonging to 276 species of crustaceans that two clades diverging by more than 0.16 substitution per site, as measured by patristic distances, have a strong probability (ca. 0.99%) of belonging to different species. That method is conservative insofar as it identifies both fewer MOTUs and MOTUs that are more divergent than other delimitation methods, thereby limiting the risk of considering two populations of the same species as belonging to two distinct MOTUs. Several studies (Morvan et al. 2013; Sworobowicz et al. 2015; Eme et al. 2018) showed that evolutionary units of Asellids as delimited by distance-based methods, including that implemented by Lefébure et al. (2006), were further subdivided by tree-based methods such as the General Mixed Yule-Coalescent method (Pons et al. 2006) and the Poisson tree processes (Zhang et al. 2013). Our conservative estimate avoids potential oversplitting.

We used the R package “ape” (Paradis et al. 2004) to compute patristic distances and “cluster” to delimit MOTUs (Maechler et al. 2012).

To further assess the genetic differentiation of *A. ismailsezarii* sp. nov., we reconstructed the most likely phylogenetic relationships among taxa belonging to the “*Asellus* pattern” using all 28S sequences available in NCBI. Non-overlapping sequences (accession number KX467625) and short sequences (less than 300 bp, accession numbers AY739195 and HG322482) were excluded from the analyses. In addition to sequences of *A. ismailsezarii* sp. nov., we obtained 23 sequences belonging to the *A. aquaticus sensu lato* species complex (including *A. kosswigi*), *Asellus* sp. from Ukraine and *A. hilgendorfii* from Japan (Suppl. material 3: Table S3). As part of this study, we produced 28S sequences for *M. dybowskii* and *L. poberezhnii* using protocols described above for *A. ismailsezarii* sp. nov. We aligned sequences in Seaview with MAFFT (Katoh and Standley 2013) under the e-insi setting and removed ambiguous sites with the embedded Gblocks (with default options) (Suppl. material 5: Table S5). We reconstructed most likely relationships in the same way as for the partial COI gene.

Results

Taxonomy

Asellidae Rafinesque, 1815
***Asellus* E. L. Geoffroy, 1762**

***Asellus ismailsezarii* Malek-Hosseini, Jugovic, Fatemi & Douady, sp. nov.**
http://zoobank.org/5AD48986-0231-4775-9D8F-6F0D30169382
Figs 2–7

Material examined. *Holotype*: mature male, body length (BL) 8.2 mm (TU-SP.1), Ganow spring, Tuveh village, Andimeshk, Khuzestan Province, Iran, (32°48'31"N, 48°43'32"E, altitude 470 m above sea level). coll. Yaser Fatemi; 01. June.2019.

***Paratypes*:** 2 mature males, BL 8.2 mm (TU-SP.2), BL 6.5 mm (TU-SP.5), 1 juvenile male, BL 7.3 mm (TU-SP.7); 3 mature females, BL 8.1 mm (TU-SP.3), BL 8.1 mm (TU-SP.4), BL 6.7 mm (TU-SP.6); data same as holotype. ***Other material*:** 17 juvenile specimens (including specimens AS2 and AS3), data same as holotype. 1 mature female, BL 8.6 mm (specimen AS1), 01. December.2018, Same locality as holotype. coll. Mohammad Javad Malek-Hosseini and Yaser Fatemi. All material was preserved in 70% ethanol and deposited at UCBLZ (University Claude Bernard Lyon-Zoology, Lyon, http://cerese.univ-lyon1.fr/), under deposit numbers 2012.11.23.91–2012.11.23.99 and 2012.11.24.1–2012.11.24.47. We also deposited tubes containing stubs onto which pleopods I and II of holotype and paratype were mounted for producing SEM images.

Diagnosis of male. Species of *A. ismailsezarii* sp. nov. with depigmented body and complete loss of eyes (Fig. 2). Body elongated oval, more than three times (3.2–3.5) as long as wide. Head up to 1.4 times as wide as long; frontal margin bisinuate, medially concave, lateral margins rounded, each with posterolateral prominence with few weak setae. Pereonites I–III with slightly convex lateral margins, and pereonites IV–VII with straight lateral margins, with only few small simple short setae of about the same length. Pleomere I–II width about 60% of pereonite VII width. Pleotelson subrectangular, lateral margins densely fringed with simple short setae of about the same length, terminal edge rounded, with only weak median prominence. Antennae I and II, lengths about 15% and 70% of body length and with 8 and 55 flagellar articles, respectively. Propodus I slender ovoid, with weak apophysa on palmar margin. Carpus and propodus IV only slightly curved, superior margin and submarginal surface of propodus IV with few (6) short and weak simple setae. Length of longest spiniform

Figure 2. Habitus of *Asellus ismailsezarii* sp. nov. (Female AS1). The photo shows a specimen exposed to air after the stone it was under had been turned over. Body length is 8.6 mm.

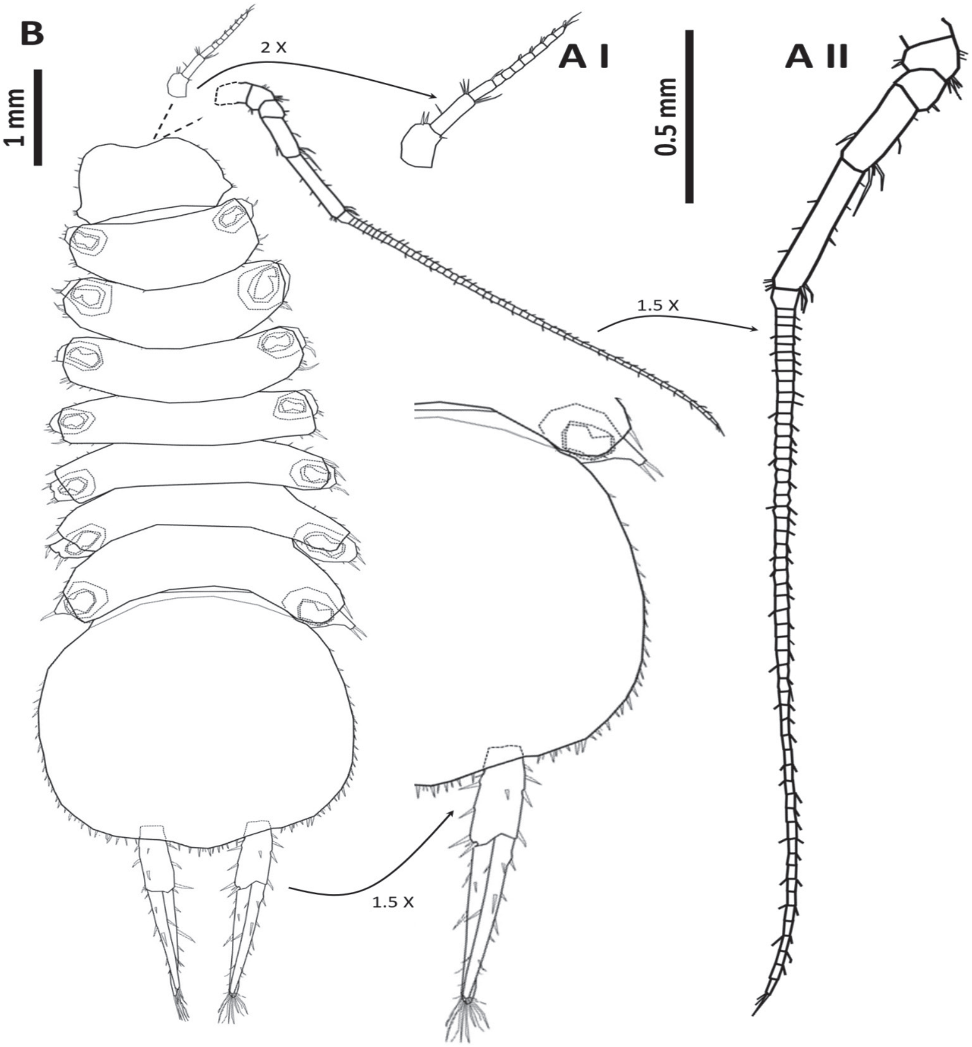

Figure 3. *Asellus ismailsezarii* sp. nov., Ganow spring, Western Iran. Holotype male, 8.2 mm: **A I, II** antennae I and II **B** body dorsal view.

robust seta on propodus VII inferior margin only about 3% pereopod VII length. Pleopod I exopodite without symmetric concavity on lateral margin, lateral margin being rather straight to slightly convex. Pleopod II architecture following typical "*Asellus* pattern" sensu Henry and Magniez (1995). Its endopodite with short (around 25% of protopodite length) horn-shaped basal spur (*processus calcariformis*). Pleopod IV and V with small respiratory areae, linea areae beginning and ending at the distal exopodite margin. Uropod short, up to about a quarter of body length.

Description of holotype and male type material (values in parenthesis). Body (Fig. 3B) 3.4 (♂♂: 3.3–3.5 ♀♀: 3.3–3.8) times as long as wide, elongated oval, without pigmentation. Head (Fig. 3B) width 1.3 (♂♂: 1.1–1.4; ♀♀: 1.3–1.4) times of its length. Frontal margin bisinuate, medially concave, without rostral process, lateral margins rounded, each with small posterolateral protuberance, with few small, short stiff setae. No eye structures visible.

Pereonites (Fig. 3B) I–III with straight to slightly convex lateral margins and weakly rounded antero- and posterolateral angles, slightly protruding forwards. Last three pereonites with antero- and posterolateral angles slightly protruding backwards. Pereonite VI widest. Anterolateral, lateral and posterolateral margins of pereonites almost bare, with only sparse short simple setae. Coxopods well developed, margins of all epimerae dorsally visible, last three the most prominent.

Pleomere I–II short but wide (Fig. 3B), their width about 62% of pereonite VII width, forming a stalk largely covered by posterior margin of pereonite VII. Pleotelson (Fig. 3B) subrectangular, its width 1.02 (♂♂: 1.02–1.13; ♀♀: 0.96–1.01) times of its length, terminal edge rounded, with only weak median prominence between uropods. Lateral margins in their anterior quarters with few short setae, other three quarters with 30 (♂♂: 26–30; ♀♀: 26–36) (left side of pleotelson) and 32 (♂♂: 25–32; ♀♀: 29–35) (right side of pleotelson) dense but minute spiniform setae of about the same length. Terminal edge with a series of 12 (♂♂: 11–15; ♀♀: 11–19) short setae between the uropods. Dorsal surface almost bare, without setation.

Antenna I length (Fig. 3AI) 15% (♂♂: 15–16%; ♀♀: 16–17%) of body length, with 3 peduncular segments. First segment robust, with curved superior (longer) and inferior (shorter) margin, other two segments cylindrical. Second segment 1.3 (♂♂: 1.26–1.33; ♀♀: 0.98–1.30) times as long as first and 1.9 (♂♂: 1.28–1.88; ♀♀: 1.43–2.08) times as long as third. Longest setae on segment 1 approximately as along as width of second article, and length of longest setae on segment 2 about as long as article 3. Flagellum of 8 segments (♂♂: 6–8; ♀♀: 7–8), with 5 (♂♂: 3–5; ♀♀: 1–4) aestetascs on distal segments. Aesthetascs shorter as their parallel segments.

Antenna II length (Fig. 3AII) 62% (♂♂: 50–62%; ♀♀: 80–81%) of body length, with 6 peduncular and 55 (♂♂: 32–55; ♀♀: 47–55) flagellar segments. Sixth peduncular segment 1.6 (♂♂: 1.39–1.61; ♀♀: 1.39–1.43) times as long as fifth, both with only short setae, long setae present only on superior distal angles. Flagellum length 77% (♂♂: 66–77%; ♀♀: 76–77%) of antenna II length.

Mandibulae (Fig. 4MdbL) robust: *Pars molaris* (molar process) U-shaped, with toothed margin. *Pars incisiva* (incisor) formed by few blunt cusps arranged in semicircle. Left *lacinia mobilis* with few cups and spine row of about 15 biserrate setae, the distal ones being longest. Palp of three segments. First palpal segment widest, with few simple setae distally. Second palpal segment 1.6 times longer than first, without setation along external margin, with two simple setae distalo-mesially, and few simple setae along internal margin. Third palpal segment around half as long as second, with row of about 20 robust biserrate setae along external margin. Maxilla I (maxillule; Fig. 4MxI) lateral lobe with few smooth (the outer spines cone-shaped) and few weakly serrate robust spines. Distal part of outer margin with 1 long slender seta. Mesial lobe with 4 robust long plumose setae. Maxilla II (Fig. 4MxII) lateral and middle lobe with 19 slender and simple, and 13 curved pectinate robust setae, respectively, mesial lobe

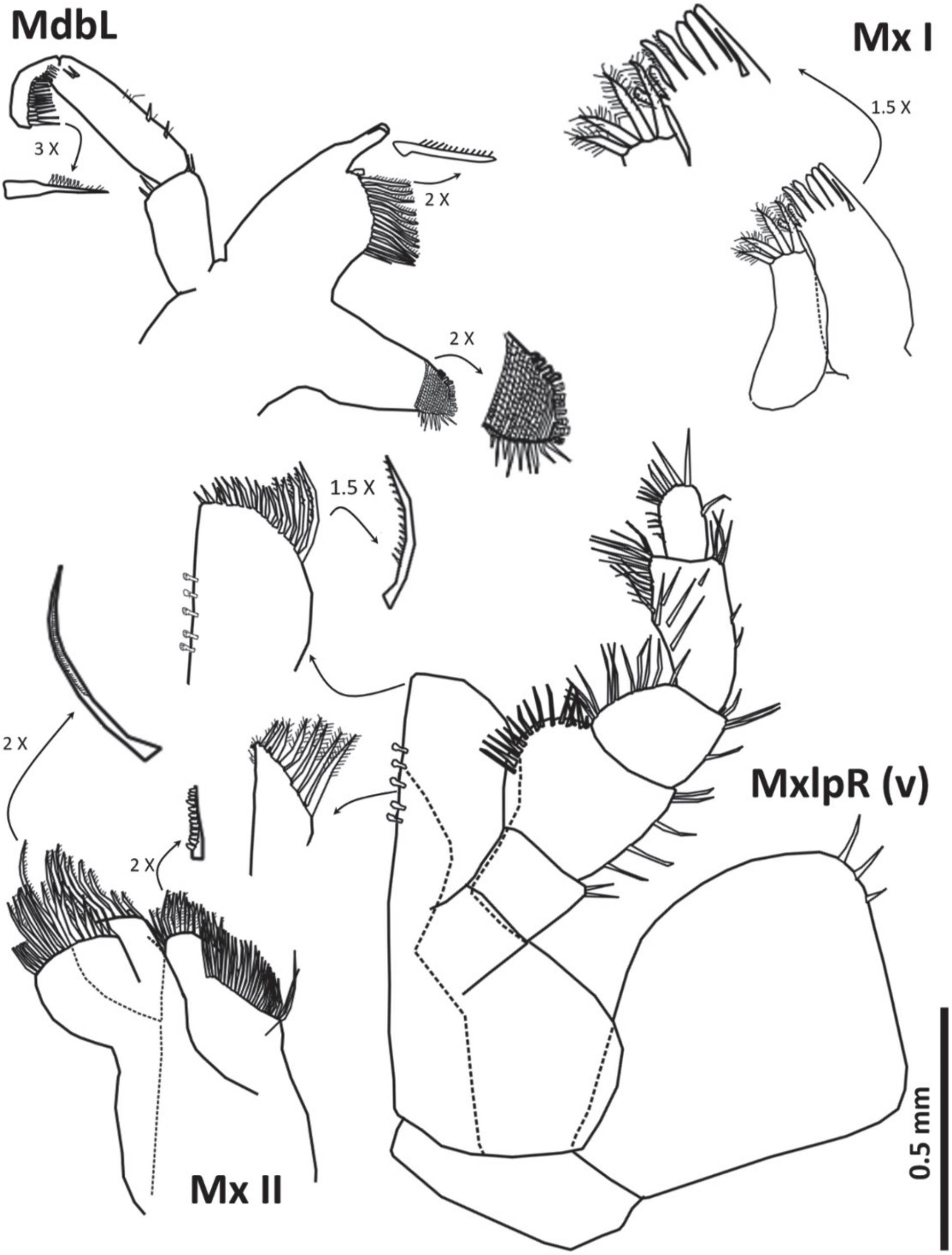

Figure 4. *Asellus ismailsezarii* sp. nov., Ganow spring, Western Iran. Paratype male, 8.1 mm (TU-SP. 2), mouthparts: MdL, left mandible; Mx I, maxilla I; Mx II, maxilla II, MxlpR (v), right maxilliped (ventrally).

with about 10 biserrate setae and parallel row of about 13 long simple setae along inner margin. Maxilliped (Fig. 4MxlpR) endite distal margin with about 11 biserrate robust setae, subapically with several rows of short simple setae. Mesial margin curved dorsally, with row of about 8 long biserrate setae, distomesial margin with setulose fringe and around 5 coupling hooks. Palp of five articles. First article with 2 short setae apically on outer margin; second about 2.5 times as long as first, subtrapezoidal, with 5 long stiff setae on outer margin and row of about 15 longer medially directed simple setae on inner margin. Third article a bit shorter as second, less broad, with 4 long stiff setae on outer margin and row of about 11 slender simple setae on inner margin. Fourth article approximately twice as long as third, slender, distally wider, with a row of 6 and about 20 long slender setae along outer and inner margin, respectively. Fifth article as long as first, ovoid, fringed with around 10 long slender setae and 2 longest stiff simple apical setae. Epipodite subrectangular, lateral margin almost bare, with only few (3) short simple setae.

With the exception of the first and the fourth pair, seven pairs of pereopods similar in construction and ambulatory, increasing in length towards posterior pairs. Pereopod I (Fig. 5PpI) grasping, subchelate. Propodus I (article 6) slender ovoid, 2.2 (♂♂: 2.2–2.5; ♀♀: 2.6–2.8) times as long as wide, with weakly expressed proximal apophysa with few stronger spiniform setae (♂♂: 3–4; ♀♀: 2–4) and other sparsely set row of shorter simple setae (about 10 in total). Dactylus I (article 7) length about 70% (♂♂: 70–82%; ♀♀: 70–78%) of propodus length, with 5 (♂♂: 4–6; ♀♀: 5) sparsely placed slender stiff robust setae along inferior margin (their length increasing towards unguis). Pereopod I length 31% (♂♂: 31–38%; ♀♀: 31–40%) of body length, length relations of articles from ischium (article 2) to dactylus (article 7): 1: 0.9 (♂♂: 0.7–0.9; ♀♀: 0.7–1.0): 0.5 (♂♂: 0.3–0.5; ♀♀: 0.4–0.5): 0.2 (♂♂: 0.15–0.20; ♀♀: 0.20–0.24): 0.9 (♂♂: 0.7–0.9; ♀♀: 0.8–1.0): 0.7 (♂♂: 0.7–0.8; ♀♀: 0.7–0.8), unguis length 18% (♂♂: 18–25%; ♀♀: 19–24%) of dactylus length.

Pereopod IV (Fig. 5PpIV) grasping, with parallel, but only slightly curved superior and inferior margins of propodus. Pereopod IV length 36% (♂♂: 36–47%; ♀♀: 42–47%) of body length, length relations of articles from ischium (article 2) to dactylus (article 7): 1: 0.6 (♂♂: 0.6–0.8; ♀♀: 0.6–0.7): 0.4 (♂♂: 0.4–0.5; ♀♀: 0.4–0.5): 0.7 (♂♂: 0.7–0.9; ♀♀: 0.7–0.8): 0.8 (♂♂: 0.8–0.9; ♀♀: 0.8–1.0): 0.4 (♂♂: 0.3–0.4; ♀♀: 0.3–0.4), unguis length 32% (♂♂: 32–39%; ♀♀: 29–41%) of dactylus length. Carpus IV superiodistal angle with 5 (♂♂: 5–6; ♀♀: 5–6) spiniform setae, longest one 26% (♂♂: 18–26%; ♀♀: 17–26%) of carpus length. Propodus IV inferior margin and mesial surface with 6 (♂♂: 3–6; ♀♀: 3–4) acute stiff robust setae, longest robust seta 7% (♂♂: 7–10%; ♀♀: 8–11%) of propodus length, inferodistal surface with 3 (♂♂: 2–4; ♀♀: 0–5) short simple and penicilate setae, superior margin and submarginal surface with 6 (♂♂: 6–13; ♀♀: 12–13) short simple and penicilate setae, superior distal angle with 4 (♂♂: 4–6; ♀♀: 5–6) long simple setae and 1 penicilate seta. Dactylus IV inferior margin with 2 (♂♂: 2; ♀♀: 1–2) robust stiff setae, superior margin distally with 2–5 simple setae.

Pereopod VII (Fig. 5PpVII) with long slender articles, its length 70% (♂♂: 67–81%; ♀♀: 65–72%) of body length, length relations of articles along pereopod VII (given as in pereopod IV): 1: 0.9 (♂♂: 0.9–1.0; ♀♀: 0.8–0.9): 0.7 (♂♂: 0.6–0.7; ♀♀: 0.6–0.7): 1 (♂♂: 1.0–1.1; ♀♀: 0.9–1.1): 1.4 (♂♂: 1.2–1.4; ♀♀: 1.1–1.4): 0.3 (♂♂: 0.3–0.4; ♀♀: 0.3), unguis length 30% (♂♂: 30–39%; ♀♀: 33–43%) of dactylus length. Ischium VII with 8 (♂♂: 6–8; ♀♀: 6–6) spiniform setae along its margins,

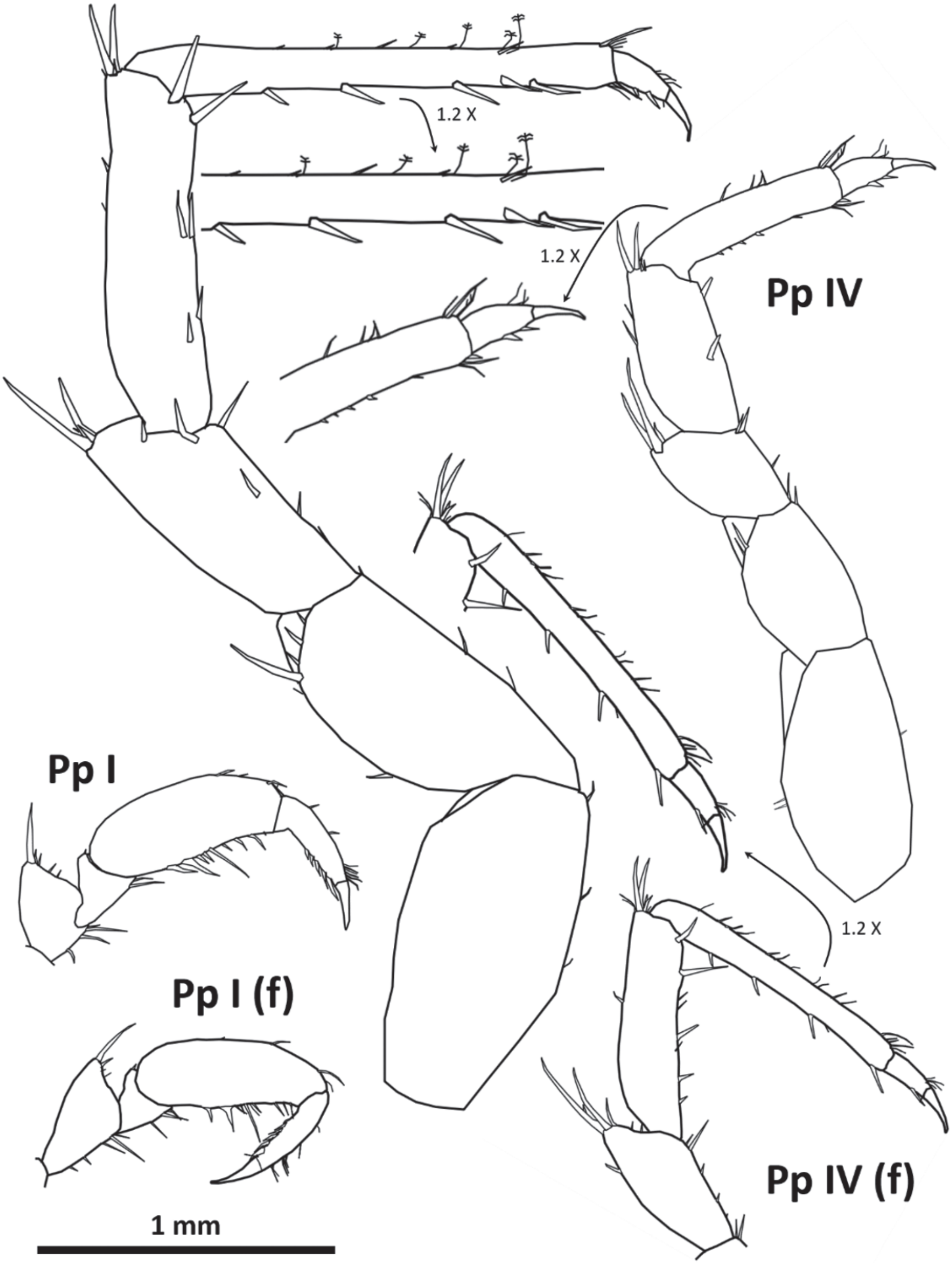

Figure 5. *Asellus ismailsezarii* sp. nov., Ganow spring, Western Iran. Holotype male, 8.2 mm: Pp I, IV, VII – pereopods I (distal articles), IV, VII. Female, 8.1 mm: Pp I, IV (f) – distal articles of pereopods I, IV.

with around 7 spiniform setae along inferodistal margins of merus, and a group of around 4 spiniform setae on superiodistal angle of merus VII, longest one 42% (♂♂: 42–58%; ♀♀: 44–53%) of merus length. Carpus VII with around 6 strong spiniform setae along inferior and inferodistal margins, few (around 2) weaker spiniform setae along superior margin, and a group of (around two strong and two weak) spiniform setae at superior-distal angle. Propodus VII inferior margin with row of 6 (♂♂: 6; ♀♀: 6) acute stiff robust setae, longest robust seta 10% (♂♂: 9–11%; ♀♀: 11–15%) of propodus length, mesial surface and inferodistal angle with 6 (♂♂: 2–6; ♀♀: 3–5) submarginal simple setae, with few weak simple setae at inferodstal angle. Superior margin and submarginal surface with 10 (♂♂: 7–10; ♀♀: 3–12) short simple and penicilate setae, superior distal angle with 1 (♂♂: 1–2; ♀♀: 1–2) simple setae and around 5 penicilate seta. Dactylus VII inferior margin with 2 (♂♂: 2; ♀♀: 1–2) robust stiff setae, longest 30% (♂♂: 30–39%; ♀♀: 33–43%) of dactylus length, superior margin distally with around 5 (♂♂: 4–6; ♀♀: 4–5) simple setae.

Male pleopod I (Fig. 6PlpI, Fig. 7) protopodite 0.92 (0.88–0.96) times as wide as long, retinacle on medial margin of 5 (4–5, exceptionally less) hooks. Exopodite elongated ovoid, its width 77% (57–77%) of its length, with 19 (6–23) simple setae along lateral and distal margins, and 7 (7) plumose terminal marginal setae. Without concavity on lateral margin, lateral margin being rather straight to slightly convex. In juvenile males (Fig. 6PlpI (juv)), pleopod I exopodit external margin proximally elongated in a finger-like projection, with one plumose seta on it, and around 8 plumose setae along lateral and distal margins.

Male pleopod II (gonopod; Fig. 6PlpII, Fig. 7) protopodite subtrapezoidal, with rounded angles, its width 77% (76–83%) of its length. Lateral and medial margins without setae. Exopodite oval, almost twice (1.76–1.96) as long as wide, its proximal segment with around 2 simple setae on it its lateral margin; lateral and medial margins of distal segment fringed with 11 (6–11) simple and 6 (0–6) long plumose setae, respectively. A well-developed and rounded catch lobe on the dorsal side and medial margin of the distal segment of exopodite. This catch lobe partly overlaps the proximal segment of exopodite. Endopodite elongated ovoid, its length 60% (60–71%) of protopodite length, with a short horn-shaped basal spur (*processus calcariformis*): 43% (37–55%) of endopodite length. Dorsal side of endopodite entirely smooth. Ventral side with a finger-like labial spur (*processus cylindriformis*), an anterior lobe densely covered with serrated scales, and a spoon-shaped *Capitulum.* Surface of *Capitulum* is smooth and without spines. A short but large-diameter *Cannula* is clearly visible with scanning electron microscopy between the anterior lobe and *Capitulum*. In juvenile males, pleopod II (Fig. 6PlpII (juv)) without endopodite, exopodite almost without setation.

Pleopod III (Fig. 6PlpIII) exopodite rounded triangular, about 1.6 times as long as wide, with almost straight medial margin. Medio-distal, terminal and latero-distal margins with around 8 long plumose setae. No setation along medial and lateral margins. Endopodite length about 0.7 of exopodite length.

Pleopod IV (Fig. 6PlpIV) exopodite broadly ovoid, about 1.6 times as long as wide, its area equally shaped as in pleopod V. Without setation along margins. Endopodite subrectangular, its length about 0.8 of exopodite length.

Pleopod V (Fig. 6PlpV) exopodite ovoid, 1.6 (♂♂: 1.6–1.7; ♀♀: 1.5–1.6) times as long as wide, its margins without setation. Respiratory area small, its surface 21% (♂♂: 21–28%; ♀♀: 22–28%) of exopodite surface, linea areae beginning and ending

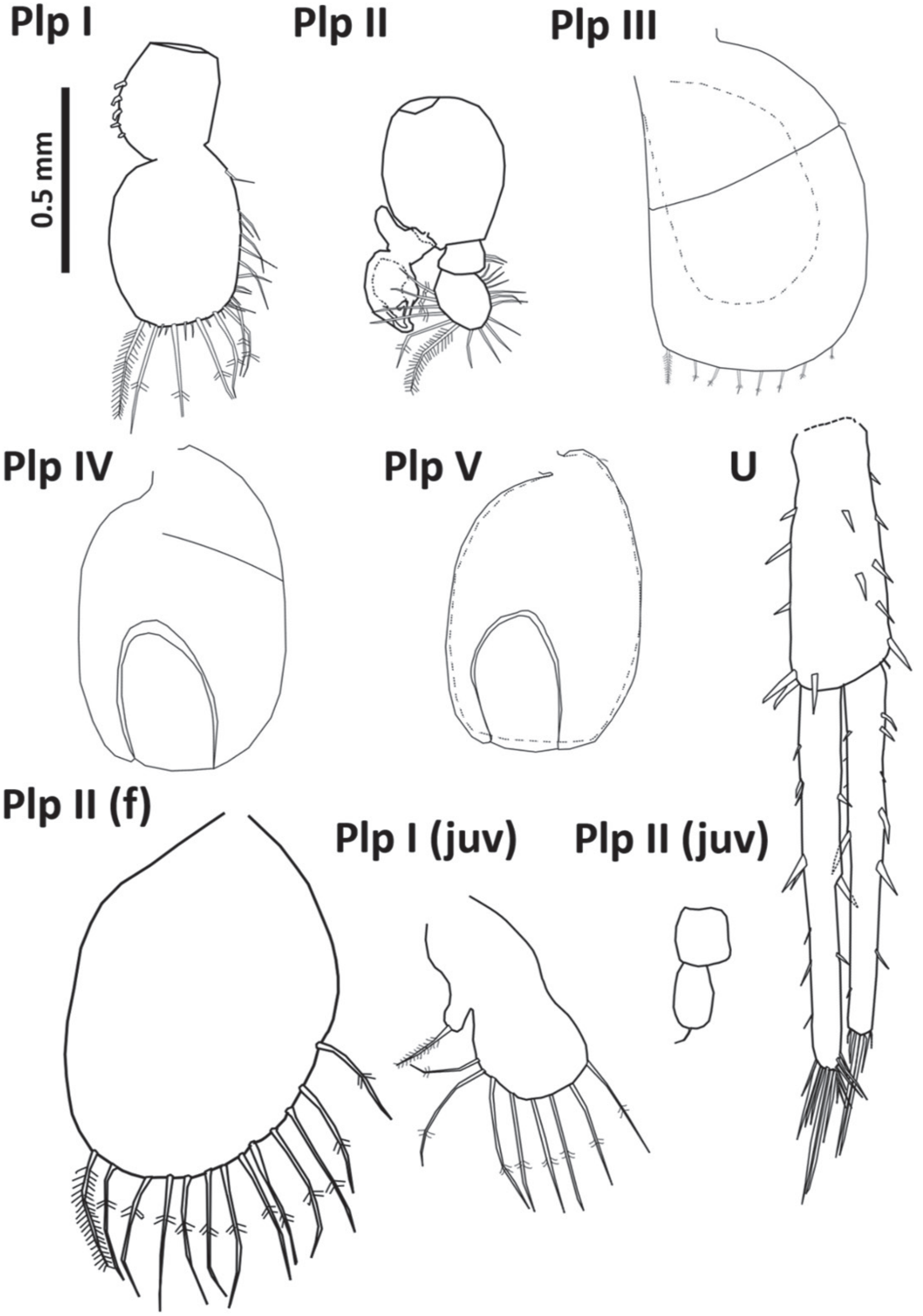

Figure 6. *Asellus ismailsezarii* sp. nov., Ganow spring, Western Iran. Holotype male, 8.2 mm: Plp I, II; II, IV, V – pleopods I, II, III, IV, IV; U – uropod (pleopod VI). Female, 8.1 mm: Plp II (f) – pleopod II. Juvenile male, 7.3 mm: Plp I, II (juv) – pleopods I, II.

on the distal exopodite margin. Endopodite suboval, its length almost as long (holotype: 97%) as exopodite.

Uropod (Fig. 6U) length 24% (♂♂: 24–25%; ♀♀: 17–24%) of body length. Proto-, endo- and exopodite length relations: 1:1.52 (♂♂: 1.40–1.52; ♀♀: 1.53–1.77):1.49 (♂♂: 1.25–1.49; ♀♀: 1.30–1.56). Endopodite with 9 (♂♂: 9; ♀♀: 8–9) spiniform simple setae and 7 (♂♂: 7–9; ♀♀: 8–9) penicilate setae, longest simple seta length 14% (♂♂: 14–15%; ♀♀: 10–13%) of endopodite length.

Female type material. Body length 6.7–8.1 mm (3.3–3.8 times of body width), almost identical to male except, antenna II seems longer than in males, around 4/5 of body length, but with similar number of flagellar segments (47–55). Pereopod I (Fig. 5PpI(F)) propodus with less expressed proximal apophysa, slender and longer propodus, 2.6–2.8 times as long as wide (♂♂: 2.2–2.5). Pereopod IV (Fig. 5PpIV(f)) not for grasping, ambulatory, little longer than the preceding pairs. Without pleopod I. Pleopod II (Fig. 6PlpII(f)) suboval, about 1.3 (1.3–1.4) times as long as wide, with 12–13 long marginal plumose setae. Uropods seem a bit shorter than in males (♂♂: 24–25%; ♀♀: 17–24%), with relatively longer endopodite compared to protopodite (♂♂: 1.40–1.52 *versus* ♀♀: 1.53–1.77 times as long as protopodite).

Morphological comparison with other *Asellus* species. Among the 18 species of *Asellus* presently described, *A. ismailsezarii* sp. nov. resembles morphologically *A. monticola* Birstein, 1932 (Suppl. material 6: Table S6). Pleopods I and II appear to be almost identical between the two species, although Henry and Magniez (1996) did not provide SEM photos of these pleopods for *A. monticola*. In both species, the endopodite of pleopod II has a spoon-shaped *Capitulum* with no spines. However, *A. ismailsezarii* sp. nov. differs from *A. monticola* in that it is eyeless and fully depigmented, has a slightly curved pereopod IV and bears no setae on proximal margins of exopodite of pleopods IV and V. These morphological differences are sufficient to consider the studied specimens as belonging to a distinct species. We also provided in Suppl. material 6: Table S6, a morphological comparison with *A. kosswigi*, *A. aquaticus aquaticus*, *A. aquaticus infernus* and *A. aquaticus cavernicolus* showing that *A. ismailsezarii* sp. nov. shows many morphological differences with both surface and subterranean aquatic species and subspecies of the *A. aquaticus* complex. Contrary to *A. ismailsezarii* sp. nov., all members of the *A. aquaticus* complex show a helical *Capitulum*, suggesting the new species does not belong to that complex.

Molecular species delimitation. We found eleven MOTUs within the "*Asellus* pattern", including one MOTU corresponding to *A. ismailsezarii* sp. nov. (Fig. 8A). Maximal patristic distances among COI sequences within the same MOTU (i.e., within-MOTU distances) ranged from 0 to 0.19 (0.01 for *A. ismailsezarii* sp. nov.) (Suppl. material 7: Table S7). Minimal patristic distances among sequences belonging to *A. ismailsezarii* sp. nov. and sequences belonging to the other MOTUs (i.e., between-MOTU distances) ranged from 0.76 to 1.00 (Suppl. material 7: Table S7). The considerable gap between within- and between-MOTU patristic distances supports our taxonomic assessment that *A. ismailsezarii* sp. nov. is a distinct species.

Phylogenetic relationships as inferred from the COI gene indicated that *A. ismailsezarii* sp. nov. does not belong to the *A. aquaticus* complex, which itself contained

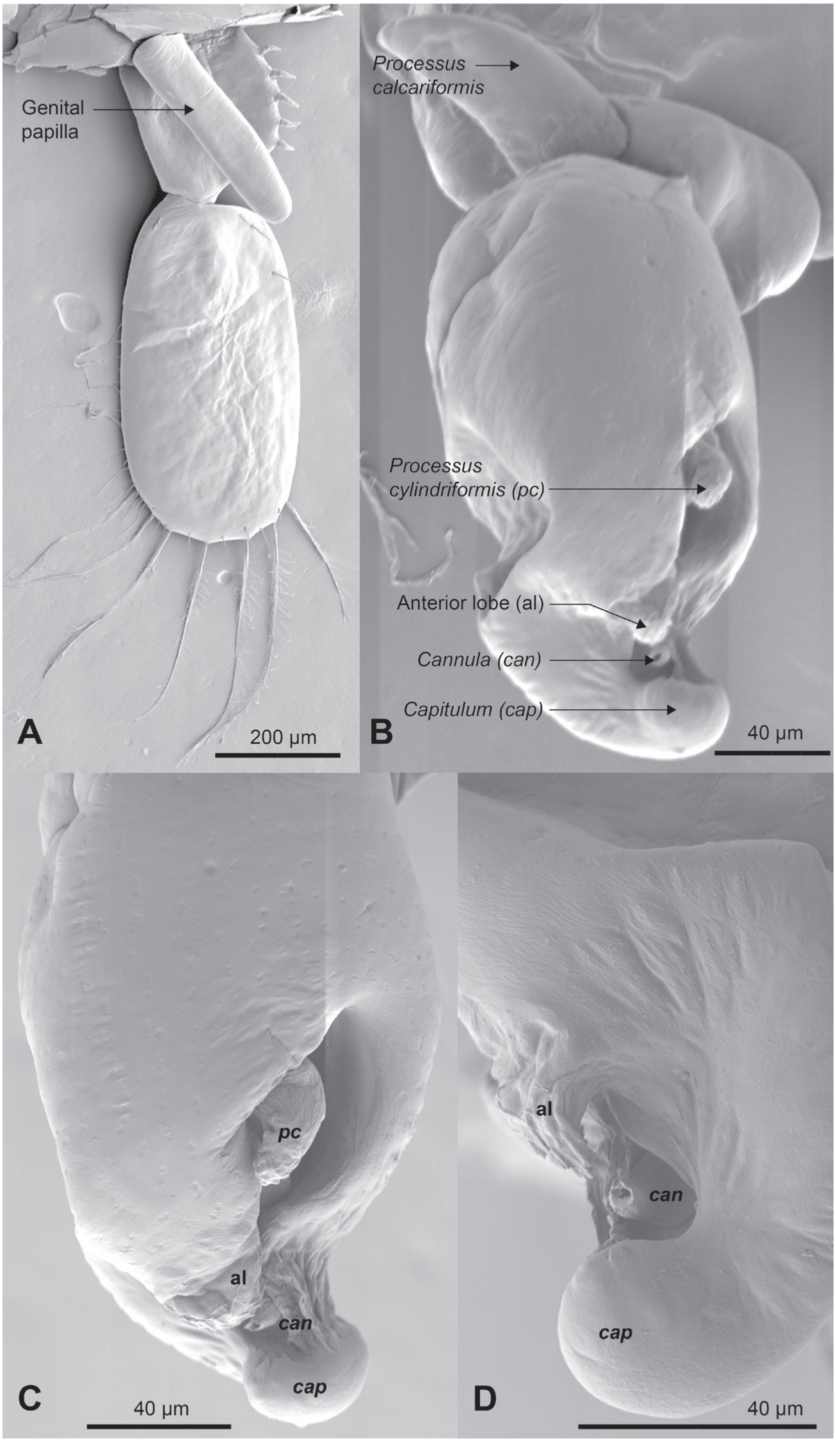

Figure 7. *Asellus ismailsezarii* sp. nov., male; SEM micrographs of (**A**) pleopod I (paratype male BL 8.2 mm: TU-SP.2). Photo also shows one genital papilla inserted on ventral side of pereionite VIII **B, C, D** pleopod II endopod (holotype male BL 8.2 mm: TU-SP.1) **B, C** ventral view and **D** lateral view.

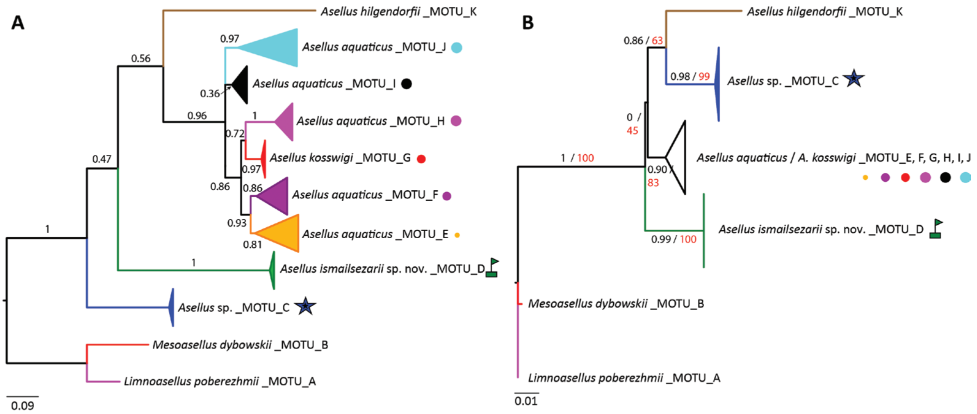

Figure 8. Phylogenetic relationships among taxa belonging to the *Asellus* pattern derived from Maximum likelihood analysis of (**A**) 585 COI mtDNA sequences, (**B**) thirty 28S rDNA sequences. The clade comprised of *M. dybowskii* and *L. poberezhnii* was used as outgroup. In A, branches to molecular operational taxonomic units (MOTU) as delimited with the fixed threshold method implemented by Lefébure et al. (2006) were collapsed. In B, branches to the main clades were collapsed. Colors and symbols as in Fig. 9. Branch supports are approximate likelihood ratio test (aLRT) values (black) and bootstrap values (red).

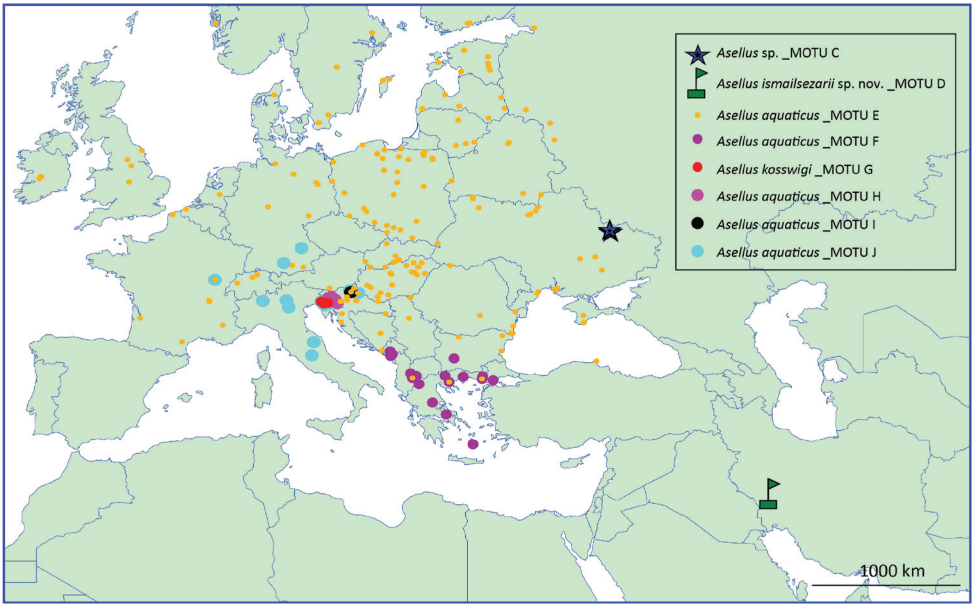

Figure 9. Map showing the distribution of molecular operational taxonomic units (MOTU) within the "*Asellus* pattern". Site locations for *Asellus hilgendorfii* (Japan) and *Mesoasellus dybowskii* and *Limnoasellus poberezhnii* (Lake Baikal, Russia) are not shown. Colors and symbols as in Fig. 8.

six MOTUs, including *A. kosswigi* (Fig. 8A). Within that complex, only MOTU E corresponding to *A. aquaticus* Linnaeus, 1758 *sensu stricto* was widely distributed across Europe (Fig. 9). The resulting tree of the nuclear 28S rDNA sequences agreed well with the COI phylogeny, again indicating that *A. ismailsezarii* sp. nov. was a sister species of the *A. aquaticus* complex (Fig. 8B). Patristic distances derived from the 28S loci also corroborated COI results by revealing a strong divergence between *A. ismailsezarii* sp. nov. and the other MOTUs.

Etymology. The name of the new species is a patronym for ''Mohammad Ismail Sezari", the legendary guard of the train tracks from the area of the type locality. In year 1986, he sacrificed his life to save 750 others that were on a collision course train.

Discussion

The present study provides morphological and molecular evidence supporting the species status of *Asellus ismailsezarii* sp. nov. This newly discovered species shows diagnostic morphological features of the *Asellus* genus and *Asellus* subgenus (Magniez and Henry 1970; Henry and Magniez 1993, 1996). In addition to being eyeless and fully depigmented, *A. ismailsezarii* sp. nov. differs from the surface aquatic species *A. monticola* to which it is supposedly most closely related, by the shape of its pereopod IV and the lack of setation on pleopods IV and V. Most but not all groundwater species lack a grasping pereopod IV, suggesting adult males no longer engage in precopulatory mate guarding (Henry 1976). The occurrence of a still curved pereopod IV among some groundwater asellids could indicate that groundwater colonization has occurred recently. However, this hypothesis deserves to be tested using a large number of population and/or species (see for example Balázs et al. 2021). We also present morphological evidence that *Asellus ismailsezarii* sp. nov. is distantly related to species belonging to the *A. aquaticus* complex. All species of that complex are characterized by a helical *Capitulum*, whereas both *Asellus ismailsezarii* sp. nov. and *A. monticola* have a spoon-shaped *Capitulum.*

In addition to morphological evidence, we show that patristic distances for the COI gene between *A. ismailsezarii* sp. nov. and any other MOTUs delimited within the "*Asellus* pattern" largely exceed the 0.16 threshold value above which two clades can be considered to belong to distinct species (Lefébure et al. 2006). Phylogenetic relationships as inferred from the COI and 28S gene confirm that *A. ismailsezarii* sp. nov. does not belong to the *A. aquaticus* complex.

Our findings together with previous asellid reports from Iran and neighboring countries (Turkmenistan, Georgia, Armenia) suggest that the Caspian Sea region is a contact zone between species of the European *A. aquaticus* complex, including *A. aquaticus* Linnaeus, 1758 and *A. aquaticus messerianus* and Asian species, including *A. monticola* and *A. ismailsezarii* sp. nov. Although the number of MOTUs within the *A. aquaticus* complex varies according to the species delimitation method – Sworobowicz et al. (2015) recognized 13 MOTUs using the automatic barcode gap discovery – one MOTU corresponding to the neotype population of *A. aquaticus* Linnaeus, 1758 (see Verovnik et al. 2009) systematically shows a large distribution throughout Europe. According to records by Rémy (1941) and Henry and Magniez (1995), *A. aquaticus* Linnaeus, 1758 is also present in Iran. Further sampling and sequencing of asellids in the Caspian See region is necessary to ascertain the existence of that contact zone between species of the European *A. aquaticus* complex and Asian species.

The molecular systematics of the *Asellus* and more largely of the “*Asellus* pattern” is yet to be established since most of its species have not yet been sequenced. Hence, except from the fact that *A. ismailsezarii* sp. nov. does not belong to the *A. aquaticus* complex, its phylogenetic position within the *Asellus* remains to be more precisely determined. We expect that *A. ismailsezarii* sp. nov. is phylogenetically closely related to *A. monticola* because the two species share many morphological characters. This hypothesis can be tested when fresh material of *A. monticola* becomes available for DNA sequencing. Ancestral populations of *A. monticola* might have colonized groundwater on multiple occasions and localities. If so, many more obligate groundwater *Asellus* taxa could be discovered in Iran.

We have several reasons to expect many more groundwater species discoveries in Iran as sampling effort increases. First, the number of 43 obligate groundwater species presently known from Iran is exceedingly low considering the extent and diversity of groundwater habitats (Vardanjani et al. 2017). Of the 2000 registered caves and many more wells and springs in Iran, a tiny proportion has actually been sampled. Second, about 70% of the species were described in the past eight years when sampling and taxonomic efforts intensified. Third, the number of species is very unevenly distributed among taxa, reflecting a strong taxonomic bias in description effort. Of the 43 obligate groundwater species, 23 belong to the genus *Niphargus* Schiødte, 1847, due to a recent increase in species description activity among Iranian experts (Malek-Hosseini and Zamani 2017; Zamanpoore et al. 2020; Bargrizaneh et al. 2021). Yet, recent species descriptions of Stenasellidae, aquatic Isopoda (Khalaji-Pirbalouty et al. 2018), Moitessieriidae, aquatic snails (Fatemi et al. 2019), Agnaridae, terrestrial Isopoda (Kashani et al. 2013) and Carabidae, terrestrial Coleoptera (Malek-Hosseini et al. 2021) indicate that many animal groups are likely to have presently unknown subterranean representatives in Iran. Local but repeated sampling is also revealing the presence of potentially species-rich aquifers with high conservation value. The karst aquifer feeding the Ganow spring, the type locality of *A. ismailsezarii* sp. nov., also harbors three described species of cave fish (Mousavi-Sabet et al. 2016; Vatandoust et al. 2019) as well as yet-undescribed species of gastropods (in first author collection). This paper contributes a piece in the puzzle of the Iranian cave biodiversity.

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Supplementary material 1

Table S1. DNA extraction and PCR protocols

Authors: Mohammad Javad Malek-Hosseini, Jure Jugovic, Yaser Fatemi, Matjaž Kuntner, Rok Kostanjšek, Christophe J. Douady, Florian Malard

Data type: pdf. file

Explanation note: Molecular protocols indicating for each gene (COI and 28S) and specimen of *Asellus ismailsezarii* sp. nov. (AS1, AS2 and AS3) the DNA extraction protocol (see text), PCR protocol (see text), primer names, sequencing institutions and sequence codes.

Link: https://doi.org/10.3897/subtbiol.42.79447.suppl1

Supplementary material 2

Table S2. List of primers

Authors: Mohammad Javad Malek-Hosseini, Jure Jugovic, Yaser Fatemi, Matjaž Kuntner, Rok Kostanjšek, Christophe J. Douady, Florian Malard

Data type: pdf. file

Explanation note: List of primers used in this study.

Link: https://doi.org/10.3897/subtbiol.42.79447.suppl2

Supplementary material 3

Table S3. COI and 28S sequence data set with sampling localities

Authors: Mohammad Javad Malek-Hosseini, Jure Jugovic, Yaser Fatemi, Matjaž Kuntner, Rok Kostanjšek, Christophe J. Douady, Florian Malard

Data type: GenBank codes (excel file)

Explanation note: COI and 28S sequence data set used in the present study. TH: Molecular operational taxonomic units (MOTU) as delimited with the 16% COI divergence threshold; Long.: longitude of the locality, in decimal degrees; Lat.: latitude of the locality, in decimal degrees, There may be more than one locality per sequence in case hapoltype sequences were desposited to GenBank.

Link: https://doi.org/10.3897/subtbiol.42.79447.suppl3

Supplementary material 4

Table S4. COI alignment

Authors: Mohammad Javad Malek-Hosseini, Jure Jugovic, Yaser Fatemi, Matjaž Kuntner, Rok Kostanjšek, Christophe J. Douady, Florian Malard

Data type: mase. file

Explanation note: COI alignment data.

Link: https://doi.org/10.3897/subtbiol.42.79447.suppl4

Supplementary material 5

Table S5. 28S alignment

Authors: Mohammad Javad Malek-Hosseini, Jure Jugovic, Yaser Fatemi, Matjaž Kuntner, Rok Kostanjšek, Christophe J. Douady, Florian Malard

Data type: mase. file

Explanation note: 28S alignment data.

Link: https://doi.org/10.3897/subtbiol.42.79447.suppl5

Supplementary material 6

Table S6. Morphological comparison with other *Asellus* species

Authors: Mohammad Javad Malek-Hosseini, Jure Jugovic, Yaser Fatemi, Matjaž Kuntner, Rok Kostanjšek, Christophe J. Douady, Florian Malard

Data type: pdf. file

Explanation note: Comparison of *Asellus ismailsezarii* sp. nov. with *A. monticola*, *A. kosswigi*, and *Asellus aquaticus* and its known hypogean subspecies from Europe (*A. a. infernus*, *A. a. cavernicolous*). Abbreviations: A II – antenna II, Prp IV, VII – pereopods IV and VII, Plp IV, V – pleopods IV and V. Most discriminative features between *A. ismailsezarii* and any other species analysed here are presented in bold (note value overlapping).

Link: https://doi.org/10.3897/subtbiol.42.79447.suppl6

Supplementary material 7

Table S7. Genetic distances among and within MOTUs

Authors: Mohammad Javad Malek-Hosseini, Jure Jugovic, Yaser Fatemi, Matjaž Kuntner, Rok Kostanjšek, Christophe J. Douady, Florian Malard

Data type: excel file

Explanation note: Genetic distances among MOTUs. Letters correspond to MOTUs as shown in Figs 8 and 9. Below diagonal: Minimum patristic distances among MOTUs. Above diagonal: minimum uncorrected distances (p-distance) among MOTUs. Diagonal: maximum patristic distances within MOTUs / maximum uncorrected distances (p-distance) within MOTUs.

Link: https://doi.org/10.3897/subtbiol.42.79447.suppl7