European Journal of Taxonomy 546: 1–23
https://doi.org/10.5852/ejt.2019.546
ISSN 2118-9773
www.europeanjournaloftaxonomy.eu
2019 · Mamaghani-Shishvan M. & Esmaeili-Rineh S.

Research article

urn:lsid:zoobank.org:pub:5D316E52-39FF-4574-960B-5BD9A5F64E58

Two new species of groundwater amphipods of the genus *Niphargus* Schiödte, 1849 from northwestern Iran

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Abstract. This study was conducted to describe and illustrate two new species of groundwater amphipods from the northern parts of the Zagros Mountains in West Azerbaijan Province, Iran. Mitochondrial (COI) and nuclear (28S rDNA) fragments as well as several morphological traits were used to characterize *Niphargus urmiensis* sp. nov. and *Niphargus fiseri* sp. nov. The phylogenetic analyses showed that the nucleotide differences between the recently described species and their close allies are attributed to their distinctiveness. The molecular analysis also introduced that the new species are placed within the clade comprising Iranian species as a sister taxon. The genetic distances between *N. urmiensis* sp. nov. and *N. fiseri* sp. nov. are 7.6% and 1.6%, respectively based on the COI and 28S rDNA gene fragments.

Keywords. 28S rDNA and COI genes, morphological data, new niphargids, taxonomy, West Azerbaijan Province.

Mamaghani-Shishvan M. & Esmaeili-Rineh S. 2019. Two new species of groundwater amphipods of the genus *Niphargus* Schiödte, 1849 from northwestern Iran. *European Journal of Taxonomy* 546: 1–23. https://doi.org/10.5852/ejt.2019.546

Introduction

Groundwater accounts for about 40% of inland water in the world (Castany 1982), containing habitats which are narrowly distributed with a high endemism and a high diversity of fauna communities (Humphreys 2006; Asmyhr *et al*. 2014; Glanville *et al*. 2016). Groundwater fauna involve nearly all main taxonomic groups examined in surface waters, but, unfortunately, are poorly surveyed (Deharveng *et al*. 2009).

It is important to understand species delimitation especially in groups which are common in groundwater habitats (Witt *et al*. 2006). In these environments, convergent selective pressures result in cryptic speciation (Jörger & Schrödl 2013). Therefore, cryptic species are difficult to distinguish based on morphological characteristics. Recently, increasing use of molecular techniques in taxonomy has resulted in detecting cryptic species, especially in underground groups (Eme *et al*. 2017; Stokkan *et al*. 2018).

Most of the groundwater animals within crustaceans belong to *Niphargus* Schiödte, 1849, which is one of the most various and species-rich taxa in groundwater habitats in the Western Palearctic (Väinölä *et al*. 2008; Fišer *et al*. 2009a). The members of this taxon inhabit subterranean waters and represent a part of the groundwater biodiversity (Fišer *et al*. 2007; Brad *et al*. 2015).

In terms of biodiversity richness, Iran is considered an extremely complex area with extreme distributions in terms of altitude, climate and temperature (Frey & Probst 1986). The high biodiversity of the Iranian fauna is also the result of its location and influences of four ecozones (Palaearctic, Nearctic, Afrotropical and Oriental) (Madjnoonian *et al*. 2005). Iran is located in the eastern part of the geographical range of the genus *Niphargus*. The first species reported from Iran was *N. valachicus* (Karaman, 1998). Up to now, 18 species have been described from north (two species), west (13 species), northwest (two species) and south (one species) (Karaman 1998; Esmaeili-Rineh *et al*. 2015a, 2016, 2018; Mamaghani-Shishvan *et al*. 2017). Despite the large groundwater aquifers in Iran, the study of these taxonomic groups in those environments is a rather new scientific field. Obviously, most of these species have been identified from various parts of the Zagros Mountains in western Iran.

Some important factors influence the frequency of niphargid populations, including the underlying geology, annual precipitation and the quality of water resources, especially in the karstic areas (White 2007). Climatic and hydrological conditions in northwest Iran (Raeisi 2004) are well suited for members of the genus *Niphargus*. Until now, no comprehensive research has been conducted in northwestern Iran and only *N. khwarizmi* Hekmatara, Zakšek, Heidari & Fišer, 2013 and *N. hosseiniei* Esmaeili-Rineh, Sari, Fišer & Bargrizaneh, 2017 have been described from the Kahriz and Brolan Springs in this region, respectively. Both species are widely distributed and endemic to Iran.

In this paper, the results of a recent research on the niphargid fauna from West Azerbaijan in the northwest of Iran are presented, and two new species identified in this region are described according to morphology and two mitochondrial (COI) and nuclear genes (28S rDNA).

Material and methods

Morphological and morphometric studies

The samples were provided from Badin Abad and Randole Springs in West Azerbaijan Province, Iran (Fig. 1). The material was collected using a hand net. Badin Abad and Randole Springs are located respectively in the proximity of Piranshahr and near to Oshnavieh in West Azerbaijan. The distance between the localities is about 4.3 km in a straight line. The material was examined morphologically and mounted on slides in Euparal® medium.

An Olympus LABOMED iVu7000 camera fitted on a LABOMED Lx500 stereo microscope was used to take the digital photos. The computer program ProgRes Capture Pro ver. 2.7 was used to perform the measurements and the counts (Fišer *et al*. 2009b). The morphological details were analyzed, carefully following Fišer *et al*. (2009b). According to Karaman (2018), the morphological terminology of setae on the mandibular palpus article 3 and in the propodus of gnathopods I–II was formulated. All material was deposited in the Zoological Collection, Razi University (ZCRU).

Phylogenetic analyses and molecular divergence

The total genomic DNA was extracted from a part of the animal using Tissue Kits (GenNet Bio™) following the manufacturer's instructions to perform the molecular analyses (Seoul, Korea). The modified primer pair LCO1490-JJ and HCO2198-JJ was used to amplify the mitochondrial COI (Astrin & Stüben

2008). The first fragment of 28S ribosomal DNA (rDNA) was amplified and sequenced using the forward primer from Verovnik *et al.* (2005) and the reverse primer from Zakšek *et al.* (2007).

Each 25 µl reaction contained optimized amounts of PCR water, 12.5 µl of Master Mix kit (Sinaclon, Iran), 0.2 µl of each primer (10 µM), and 50–100 ng of genomic DNA template. An initial denaturation step at 94°C for three minutes was followed by 36 cycles of 40 seconds at 94°C, 40 seconds at 52.5°C and two minutes at 65°C, with a final extension step for eight minutes at 65°C to amplify the COI gene. Cycling parameters for the 28S rDNA gene were as follows: initial denaturation of 94°C for seven minutes, 35 subsequent cycles of 94°C for 45 seconds, 55°C for 30 seconds, 72°C for one minute, and a

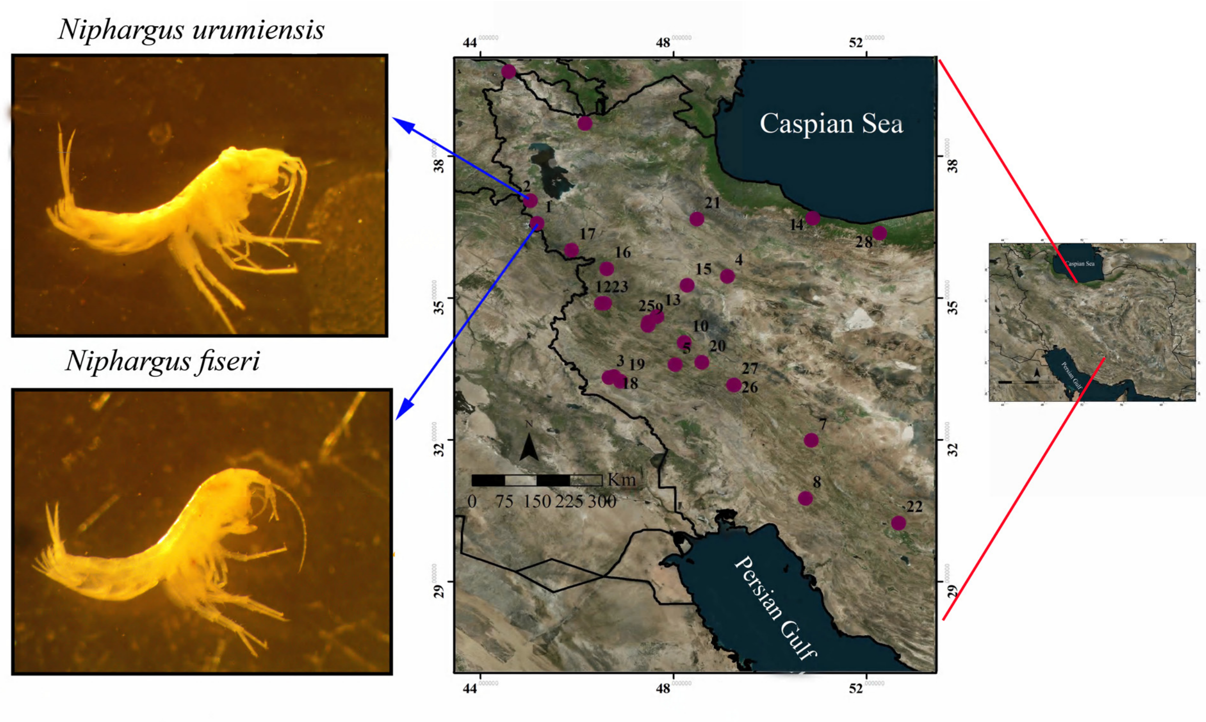

Fig. 1. Distribution map of the species of *Niphargus* Schiödte, 1849 in Iran. Localities are labelled as: 1. *N. fiseri* sp. nov. 2. *N. urmiensis* sp. nov. 3. *N. sarii* Esmaeili-Rineh, Mohammad-Niakan & Akmali, 2018. 4. *N. hakani* Esmaeili-Rineh, Mirghaffari & Sharifi, 2017. 5. *N. sharifii* Esmaeili-Rineh, Sari & Fišer, 2015. 6. *N. darvishi* Esmaeili-Rineh, Sari & Fišer, 2015. 7. *N. borisi* Esmaeili-Rineh, Sari & Fišer, 2015 (Siah population). 8. *N. borisi* (Belqais population). 9. *N. bisitunicus* Esmaeili-Rineh, Sari & Fišer, 2015. 10. *N. khwarizmi* Hekmatara, Zakšek, Heidari & Fišer, 2013 (Sarab-e-Niaz). 11. *N. khwarizmi* (Kahriz population). 12. *N. khayyami* Hekmatara, Zakšek, Heidari Baladehi & Fišer, 2013. 13. *N. kermanshahi* Esmaeili-Rineh, Heidari, Fišer & Akmali, 2016. 14. *N. daniali* Esmaeili-Rineh & Sari, 2013. 15. *N. alisadri* Esmaeili-Rineh & Sari, 2013. 16. *N. kurdistanensis* Hekmatara, Zakšek, Heidari Baladehi & Fišer, 2013 (Darvish-Olya population). 17. *N. kurdistanensis* (Shoei population). 18. *N. ilamensis* Esmaeili-Rineh, Sari, Fišer & Bargrizaneh, 2017 (Sarab-e-Kanipahn). 19. *N. ilamensis* (Sarab-e-Mord). 20. *N. sohrevardensis* Esmaeili-Rineh, Sari, Fišer & Bargrizaneh, 2017 (Razbashi population). 21. *N. sohrevardensis* (Sohrevard population). 22. *N. persicus* Esmaeili-Rineh, Sari, Fišer & Bargrizaneh, 2017. 23. *N. hosseiniei* Esmaeili-Rineh, Sari, Fišer & Bargrizaneh, 2017 (Ghori-Ghale population). 24. *N. hosseiniei* (Brolan population). 25. *N. hosseiniei* (Nojivaran population). 26. *N. lorestanensis* Esmaeili-Rineh, 2018 (Shol-Abad population). 27. *N. lorestanensis* (Ab-rahmeh population). 28. *N. valachicus* (Karaman, 1998).

final extension of 72°C for seven minutes. The purified PCR products were sequenced commercially by Macrogen Inc., Korea. Sequencing was performed using both primers mentioned above.

The acquired sequences (with GenBank accession numbers MK911603 to MK911612) were analyzed within the data set by Esmaeili-Rineh *et al*. (2015b), to identify the phylogenetic position of the newly discovered materials. The NCBI available sequences for three species, *Synurella ambulans* (F. Müller, 1846), *Pontogammarus crassus* (Sovinsky, 1904) and *Gammarus fossarum* Koch, 1836, were used as outgroups (accession numbers: KF719240, KF719242 and KF71924). All sequences were edited and aligned using ClustalW (Thompson *et al*. 1994), as implemented in Bioedit ver. 7.0.5.3 program sequence alignment editor (Hall 1999) using the default settings. Alignments were concatenated and jModelTest ver. 0.1.1 (Posada 2008) was used under the Akaike information criterion (AIC) to select the optimal substitution model for each partition. The optimal substitution models were GTR+Γ and GTR+I+Γ for 28S and COI genes, respectively.

The phylogenetic form was reconstructed using the Bayesian inferences in Mr Bayes ver. 3.1.2 (Ronquist & Huelsenbeck 2003). Bayesian analyses were implemented for five million generations, with four chains, and the trees were sampled every 1000 generations. The first 1250 sampled trees were discarded as burn-in, and the subsequent tree likelihoods were examined for convergence in Tracer program ver. 1.5.0 (Rambaut & Drummond 2009). The remaining trees visualized by FigTree ver. 1.4.0 were applied to calculate a 50% majority rule consensus tree. The data on analyzed species are available in the Electronic Supplement of Esmaeili-Rineh *et al*. (2015b, 2017a, 2017b). The corrected genetic distances were calculated using Kimura two-parameter (K2P) model (Kimura 1980) as implemented in MEGA ver. 5 software to measure the divergence from other, already described Iranian species of *Niphargus* (Tamura *et al*. 2011).

Results

Phylogenetic position of the new species and their genetic distinctness

The dataset used in phylogenetic analyses comprised individuals from Iran and a single specimen from Lebanon constituting a monophyletic group. According to Esmaeili-Rineh *et al*. (2015b), the eastern half of the genus range was colonized several times independently and constitutes an unresolved relationship to other European species of *Niphargus*.

A total of five individuals, two from Randole and three from Badin Abad Springs were sequenced and analyzed. The two specimens from the Randole population indicated unique haplotypes in terms of both fragments of COI (513 bp) and 28S (954 bp) genes. However, the three specimens from the Badin Abad population showed three and two haplotypes based on the COI and 28S genes, respectively. The phylogenetic analyses on 43 specimens consistently led to the placement of the two new species into a clade with a sister relationship within the Middle East clade. The precise position of this clade is unknown within the Middle East clade. The phylogenetic relationships between this clade (the two new species) and other clades in the Middle East clade were mostly not resolved according to the combined data of 28S and COI gene fragments (1467 bp) (Fig. 2).

The two new species are obviously different from all other Iranian species. Table 1 shows the pairwise Kimura two parameter genetic distances between *N. urmiensis* sp. nov., *N. fiseri* sp. nov. and all other species for two genes. *Niphargus urmiensis* sp. nov. is the most genetically similar species to *N. fiseri* sp. nov. (7.6% and 1.6% in the studied COI and 28S rDNA genes fragment) and the most divergent species from *N. daniali* Esmaeili-Rineh & Sari, 2013 (18.6% and 10% based COI and 28S rDNA genes). In addition, the least genetic divergence to *N. fiseri* sp. nov. was observed in *N. urmiensis* sp. nov. (7.6%) and *N. alisadri* Esmaeili-Rineh & Sari, 2013 (1.3%) for COI and 28S rDNA, respectively. Also, *N. fiseri*

Fig. 2. Bayesian tree of 43 specimens as inferred from the 28S and COI gene sequences. Species are identified and named according to available taxonomic descriptions. Bayesian posterior probabilities more than 0.8 are indicated above branches.

Table 1. K2P genetic distances (%) between all Iranian species and the specimen from Lebanon of the genus *Niphargus* Schiödte, 1849 based on 28S ribosomal DNA gene (below diagonal) and mtDNA (COI) gene (above diagonal).

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1: *N. kurdistanensis*		12.1	13.0	15.1	14.7	11.6	12.1	13.2	15.7	13.2	11.6	12.5	19.9	9.8	–	–	–	12.1	12.1	11.6	12.5
2: *N. alisadri*	0.6		13.3	17.1	15.8	12.4	11.0	12.4	14.4	11.9	12.4	13.1	20.9	12.4	–	–	–	12.1	12.2	12.2	12.4
3: *N. darvishi*	1.1	0.8		14.1	15.6	9.6	10.5	12.3	13.5	12.3	4.7	10.7	17.6	10.2	–	–	–	9.3	10	10	10.5
4: *N. borisi*	2.0	2.2	2.7		13.0	16.3	14.5	18.1	18.6	17.6	13.9	15.6	23.5	14.8	–	–	–	13.7	14.8	14.4	15.3
5: *N. sharifii*	0.8	0.6	1.1	1.8		15.8	14.7	16.1	16.0	15.6	14.9	14.7	22.2	15.6	–	–	–	13.7	13.5	14	14.4
6: *N. khwarizmi*	1.4	1.0	1.3	2.6	1.1		11.2	12.1	15.8	12.1	9.0	9.0	19.9	10.5	–	–	–	9.1	11.4	11.4	11.8
7: *N. bisitunicus*	1.3	1.1	1.4	2.7	1.0	1.1		11.0	13.8	10.5	8.7	11.2	21.7	12.8	–	–	–	9.8	10.7	10.7	11.2
8: *N. khayyami*	1.7	1.3	1.5	2.6	1.1	1.0	1.1		10.3	4.0	11.6	14.0	22.2	13.7	–	–	–	14.2	13	13	13.5
8: *N. sohrevardensis*	1.4	1.0	1.0	2.6	1.1	0.8	0.9	0.8		9.9	14.6	13.6	22.0	16.4	–	–	–	15	14.5	14	14.5
10: *N. hosseinei*	1.7	1.4	1.4	2.8	1.5	1.1	1.3	1.1	0.4		12.1	14.0	22.2	14.2	–	–	–	13.7	12.5	12.5	13
11: *N. persicus*	1.3	0.9	0.3	2.8	1.3	1.4	1.5	1.7	1.1	1.5		10.1	17.8	9.6	–	–	–	8.9	10	10	10.5
12: *N. ilamensis*	1.7	1.3	1.3	2.8	1.4	1.0	1.1	1.3	0.5	0.9	1.4		22.0	11.9	–	–	–	10	13.5	13	13.2
13: *N. daniali*	9.7	9.7	10.0	10.0	9.6	10.4	10.4	10.4	10.4	10.8	10.2	10.4		17.1	–	–	–	18.6	19.4	19.6	19.9
14: *Niphargus* Lebanon	0.3	0.6	1.1	2.0	0.8	1.4	1.5	1.7	1.4	1.7	1.3	1.7	9.9		–	–	–	9.8	11.4	11.4	11.8
15: *N. sarii*	1.8	1.4	1.4	3.0	1.5	1.1	1.3	1.0	0.6	0.8	1.5	1.1	10.8	1.7		–	–	–	–	–	–
16: *N. kermanshahi*	1.1	1.0	1.3	2.6	1.1	0.8	0.6	1.3	0.8	1.1	1.4	1.0	10.5	1.4	1.1		–	–	–	–	–
17: *N. hakani*	1.9	1.5	2.1	3.1	1.7	1.4	1.7	1.8	1.3	1.7	2.2	1.5	10.1	1.9	1.9	1.5		–	–	–	–
18: *N. urumiensis* sp. nov.	2.2	2.1	2.3	3.4	1.9	2.2	1.9	2.3	2.1	2.3	2.5	2.3	10.0	2.2	2.5	2.3	1.9		7.6	7.6	8.5
19: *N. fiseri* 1 sp. nov.	1.7	1.3	1.8	3.0	1.7	1.8	1.7	1.8	1.4	1.5	1.9	1.5	9.8	1.7	1.9	1.8	1.8	1.6		0.4	0.8
20: *N. fiseri* 2 sp. nov.	1.8	1.4	1.9	3.1	1.8	1.9	1.8	1.9	1.5	1.7	2.0	1.7	9.9	1.8	2.0	1.9	1.9	1.6	0.1		0.8
21: *N. fiseri* 3 sp. nov.	1.7	1.3	1.8	3.0	1.7	1.8	1.7	1.8	1.4	1.5	1.9	1.5	9.8	1.7	1.9	1.8	1.8	1.6	0.0	0.1	

sp. nov. indicate the most genetically divergent species from *N. daniali* (19.9% and 9.9% in the studied COI and 28S rDNA genes fragment).

Species description

Order Amphipoda Latreille, 1816
Family Niphargidae Bousfield, 1977
Genus *Niphargus* Schiödte, 1849

Niphargus urmiensis sp. nov.
urn:lsid:zoobank.org:act:7C655030-3855-4B46-85D1-76DB596428AA
Figs 1, 3–6

Diagnosis (based on male only)

The palpus of maxilla I is short and does not reach the tip of the outer lobe. The size of coxae in gnathopods I and II is sub-similar. Gnathopods bear trapezoidal to rectangular shape of propodi. Gnathopds I to II dactyli have a single seta on the outer margin. Dorso-laterally, the urosomites I to II bear one and two setae, respectively. The inner ramus in uropod I is longer than the outer ramus. Epimeral plates I–II are partly angular but not produced. Lobes of telson have three distal spines each and no lateral spines. The telson cleft is more than half its length.

Etymology

The name 'urmiensis' refers to Urmia City, the center of West Azerbaijan province (Iran), where the species was found.

Material examined

Holotype

IRAN • ♂; West Azerbaijan Province, Oshnavieh City, Randole Spring; 37°03′25″ N, 45°02′48″ E; 10 Jul. 2016; M. Mamaghani-Shishvan leg.; ZCRU Amph.1071.

Paratypes

IRAN • 1 ♂, 1 juv. (body length 6.8 and 3.8 mm, respectively); same data as for holotype; ZCRU Amph.1071.

Description of holotype

Measurements. The total length of the holotype is 8 mm. Head represents 17% of the total body length (Fig. 3C).

Antennae. Antenna I (Fig. 3A) is 0.51 times body length. Peduncular articles 1–3 progressively shorter; length of peduncular article 3 exceeds half of peduncular article 2 (ratio 1.00 : 1.80). Main flagellum with 18 articles (most with short setae), articles with up to one aesthetasc. Accessory flagellum bi-articulated and reaching ¼ of article 4 of main flagellum; both articles with two and one setae, respectively. Antenna II with flagellum formed of seven articles, approximately half as long as antenna I. Flagellum length is 0.79 times length of peduncle articles 4 +5. Peduncular article 4 of antenna II is longer than article 5 (1.3 : 1.00), peduncle articles 4 and 5 with seven and five groups of setae, respectively (Fig. 3B).

Mouth parts. Labium (Fig. 4C) bi-lobate; with fine setae on tip of outer lobes. Inner plate of maxilla I with two long apical setae, outer plate with seven long spines with 0-3-1-3-0-0-2 lateral projections; palp bi-articulated, short and not reaching tip of outer lobe, with seven apical setae (Fig. 3D–E). Both plates of maxilla II with numerous long distal and lateral setae (Fig. 4E). Mandible: right mandible with

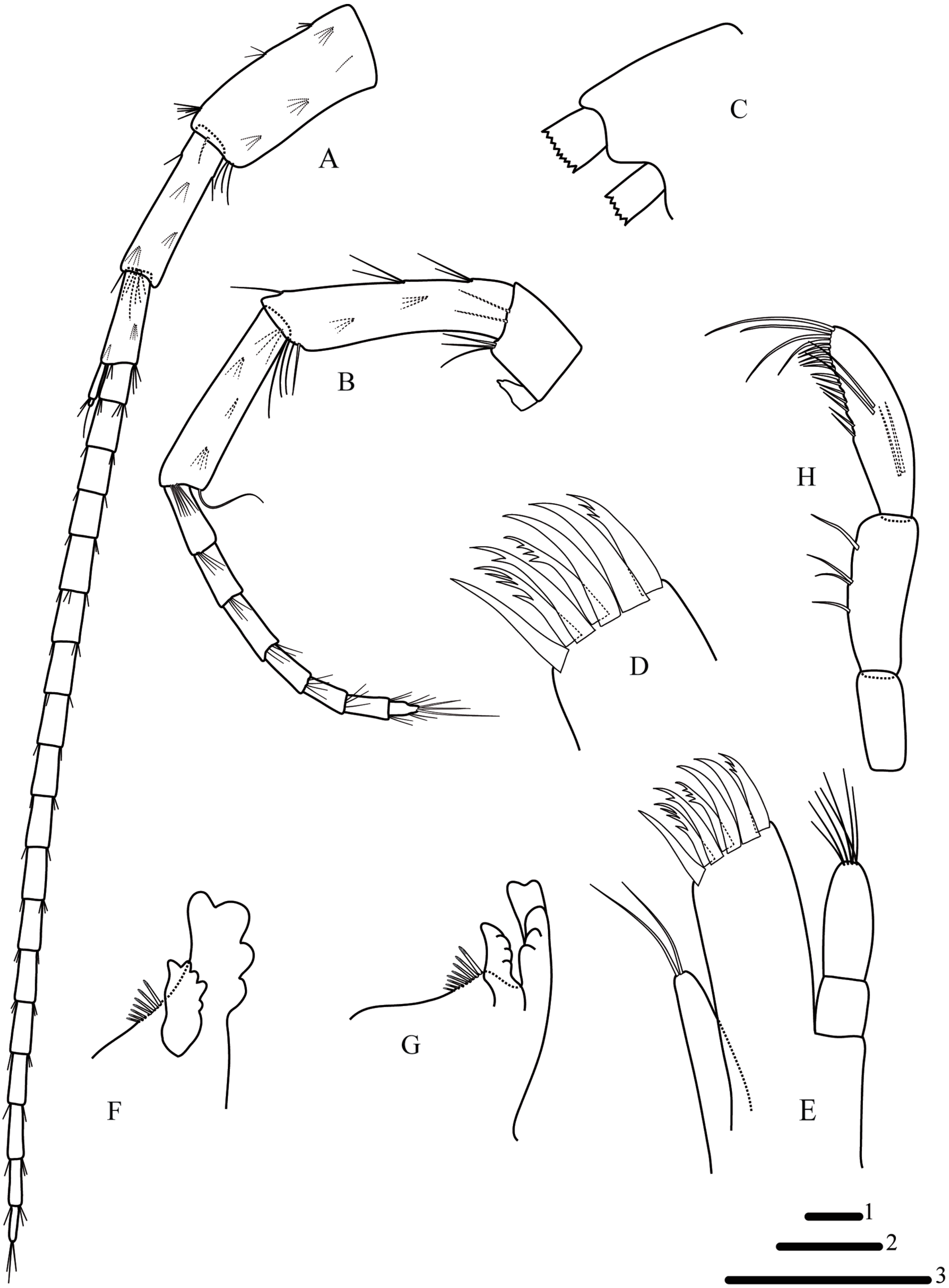

Fig. 3. *Niphargus urmiensis* sp. nov., holotype, ♂, 8 mm (ZCRU Amph.1071). **A**. Antenna I. **B**. Antenna II. **C**. Head. **D–E**. Maxilla I. **F**. Left mandible. **G**. Right mandible. **H**. Mandibular palp. Scale bars: 1 = 0.25 mm (F–G); 2 = 0.5 mm (C–E); 3 = 1 mm (A–B).

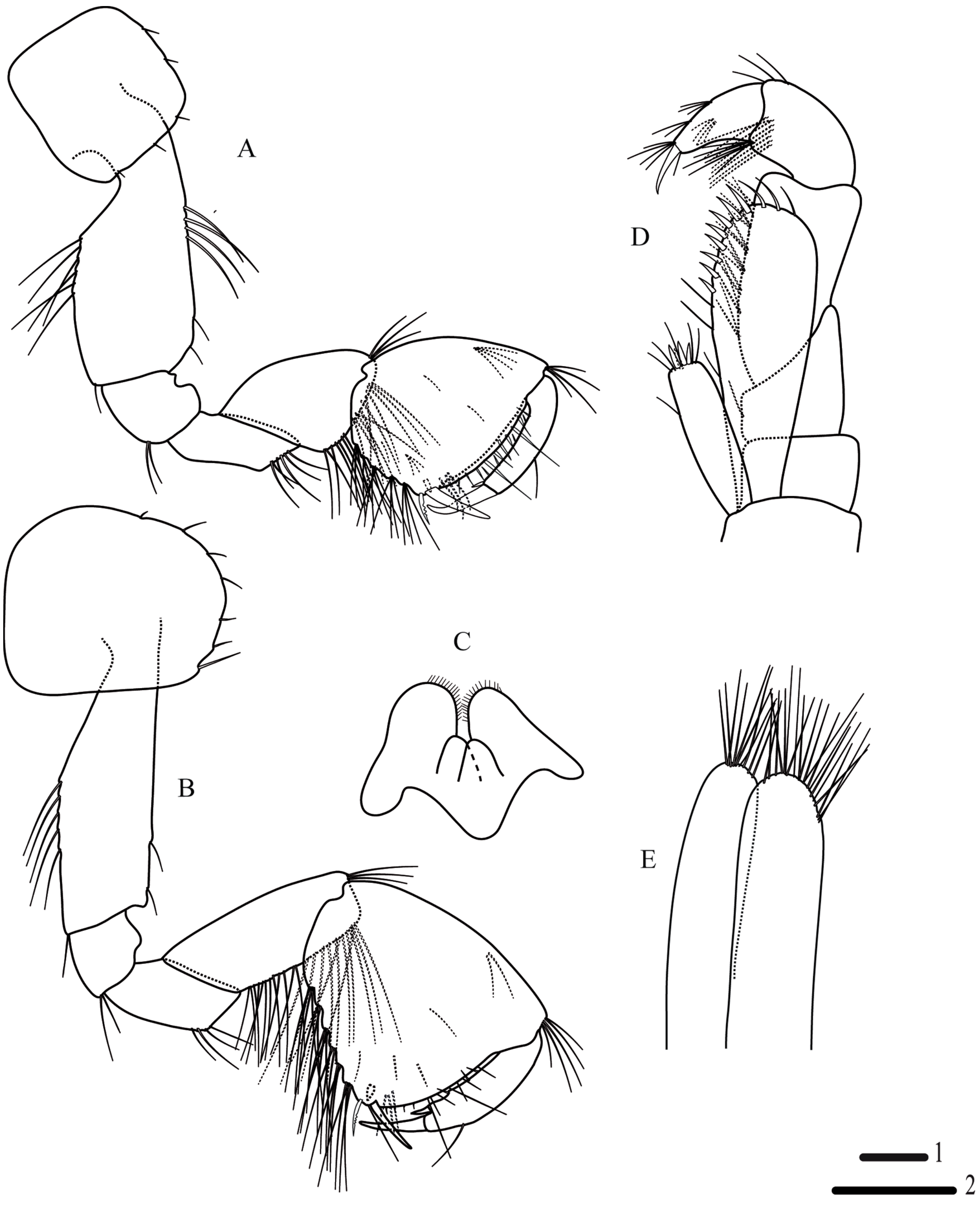

Fig. 4. *Niphargus urmiensis* sp. nov., holotype, ♂, 8 mm (ZCRU Amph.1071). **A**. Gnathopod I. **B**. Gnathopod II. **C**. Labium. **D**. Maxilliped. **E**. Maxilla II. Scale bars: 1 = 0.5 mm (C–E); 2 = 1 mm (A–B).

four teeth on incisor process, lacinia mobilis pluritoothed and row of seven setae with lateral projections (Fig. 3F). Left mandible with five teeth on incisor process, lacinia mobilis with four teeth and row of eight setae with lateral projections (Fig. 3G). Mandibular palp articles 1:2:3 represent 20%, 31% and 49% of total palp length, respectively. Proximal article without setae; second article with four setae along inner margin and third article with one group of two A-setae, one group of B-setae, no C-setae, 12 D-setae and four E-setae (Fig. 3H). Maxilliped with short inner plate bearing four distal spines intermixed with five distal setae and one long lateral seta sub-distally; outer plate exceeding half of palp article 2, with eight spines along inner margin and three setae distally; maxilliped palp article 3 at outer margin with one proximal and one distal group of long setae; palp terminal article with three groups of setae at outer margin and one seta at base of nail, nail shorter than pedestal (Fig. 4D).

Gnathopods. Coxal plates of gnathopods I–II almost equal in size. Coxa of gnathopod I rectangular, longer than broad, anterior and ventral margins with five marginal setae. Basis with setae on anterior and posterior margins; ischium and merus with posterior group of setae. Carpus with one group of three setae antero-distally, bulge with long setae; carpus 0.55 times basis length and 0.87 times propodus length. Propodus of gnathopod I trapezoid shape and broader than long; anterior margin with four setae in one group in addition to antero-distal group of four setae. Palm convex, defined on outer surface by one strong long corner S-seta accompanied laterally by one L-seta with lateral projections and row of three facial M-setae, on inner surface by one short sub-corner R-seta. Dactylus reaching posterior margin of propodus, outer and inner margins of dactylus with one and four setae, respectively. Nail length 0.35 times total dactylus length (Fig. 4A). Coxal plate of gnathopod II with rectangular shape, longer than broad, anterior and ventral margins with seven setae. Basis with setae on anterior and posterior margins; ischium and merus with posterior group of setae. Carpus with one group of three setae antero-distally, bulge with long setae; carpus 0.69 times basis length and 0.85 times propodus length. Propodus longer than broad; anterior margin with two setae in one group in addition to antero-distal group of five setae. Palm slightly convex, defined on outer surface by one strong, long corner S-seta accompanied laterally by one L-seta with lateral projections and row of three facial M-setae, on inner surface by one short sub-corner R-seta. Dactylus reaching posterior margin of propodus, outer and inner dactylar margins with one and three setae, respectively; nail short, 0.28 times total dactylus length (Fig. 4B). Coxal plate III rectangular, length to width ratio is 1.08 : 1; antero-ventro-posterior margin with six setae. Coxal plate IV rectangular, antero-ventral margin with four setae, posterior concavity shallow and approximately 0.1 times coxa width (Fig. 5A–B). Coxal plate V with five and one setae on anterior and posterior lobes, respectively. Coxal plate VI with one simple seta on posterior lobe. Coxal plate VII with one simple seta (Fig. 5C–E).

Pereopods. Pereopod III : IV length ratio is 1.05 : 1 (Fig. 5A–B). Dactylus IV short, dactylus length 0.54 times propodus length, nail shorter than pedestal (Fig. 5B). Pereopods V : VI : VII length ratios 1 : 1.17 : 1.22, respectively. Pereopod VII is 0.53 times total body length. Pereopod bases V–VI each with six groups of spines along anterior margins and with eight and seven setae along posterior margins, respectively. Pereopod basis VII with six groups of spines along anterior margin and seven setae along posterior margin, respectively (Fig. 5C–E). Postero-ventral lobe of ischium in pereopods V–VII developed. Ischium, merus and carpus in pereopods V–VII with several groups of spines and setae along anterior and posterior margins; propodus of pereopod VI longer than these in V and VII, dactyli of pereopods V–VII with one spine and one short seta at base of nail on inner margin, nail length of pereopod VII 0.3 times total dactylus length (Fig. 5C–E).

Epimeral plates I–III (Fig. 6G). With angular postero-ventral corner, postero-ventral corners of plates I–III posteriorly with two, three and three spines and setae, respectively. Epimeral plates II–III with one and two spines along of ventral margins, respectively.

PLEOPODS. Peduncle of pleopods I–III with two-hooked retinacles at distal part of inner margins. Peduncle of pleopod I with one seta along of outer margin. Peduncle of pleopod III with two setae along of inner margin (Fig. 6A–C); rami of pleopods I–III each with five to eight articles (Fig. 6A–C).

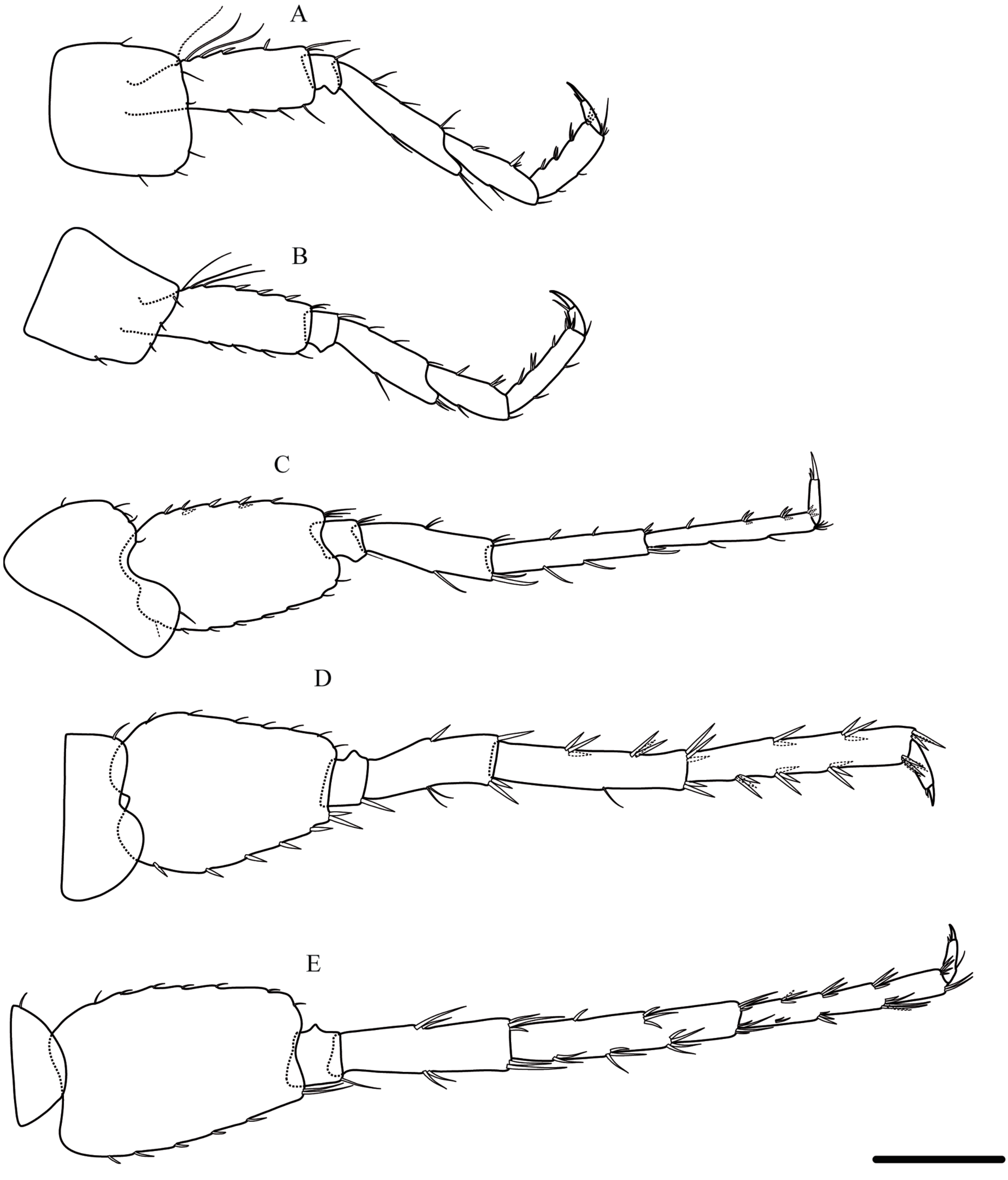

Fig. 5. *Niphargus urmiensis* sp. nov., holotype, ♂, 8 mm (ZCRU Amph.1071). **A**. Pereopod III. **B**. Pereopod IV. **C**. Pereopod V. **D**. Pereopod VI. **E**. Pereopod VII. Scale bar: 1 mm.

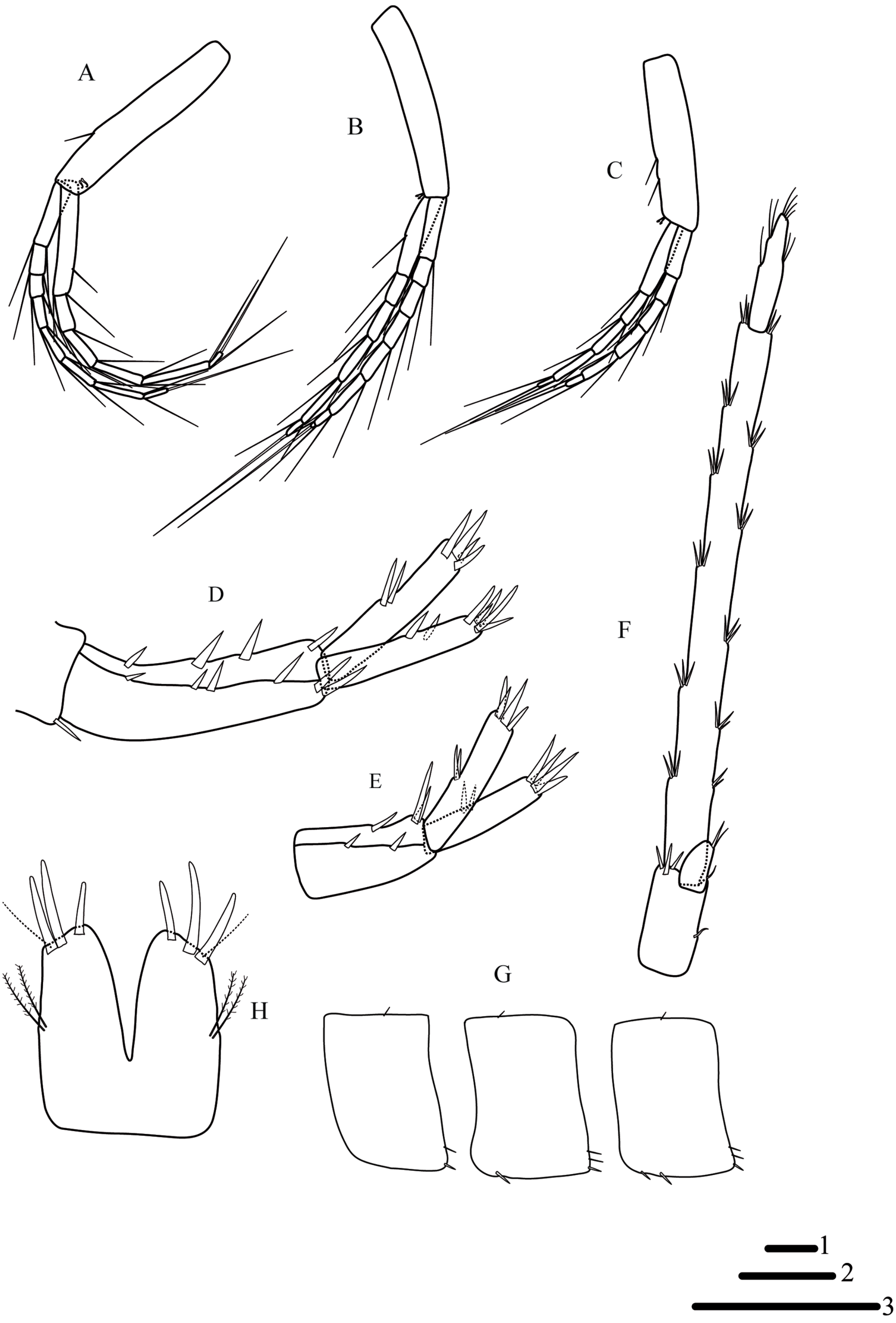

Fig. 6. *Niphargus urmiensis* sp. nov., holotype, ♂, 8 mm (ZCRU Amph.1071). **A**. Pleopod I. **B**. Pleopod II. **C**. Pleopod III. **D**. Uropod I. **E**. Uropod II. **F**. Uropod III. **G**. Epimeral plates. **H**. Telson. Scale bars: 1 = 0.5 mm (G–H); 2 = 1 mm (A–E); 3 = 2 mm (F).

PEREONITES. Pereonites I–VII without setae. Pleonites I–III each with one seta in middle of article on dorsal margin. Urosomites I–II with one and two setae on dorso-lateral margin, respectively. Urosomite III without setae. Urosomite I with one spine at base of uropod I.

UROPODS. Peduncle of uropod I with six and four large spines along dorso-lateral and dorso-medial margins, respectively. Outer ramus of uropod I slightly shorter than inner ramus (ratio 1 : 1.06); inner ramus with one group of two spines laterally and four spines distally; outer ramus with two groups of two spines laterally and five spines distally (Fig. 6D). Inner ramus in uropod II longer than outer, both rami with lateral and distal long spines (Fig. 6E). Uropod III long, almost 0.42 times body length. Peduncle of uropod III with three spines, outer ramus bi-articulated, distal article 0.26 times proximal article. Proximal article of outer ramus bearing each six groups of spines along inner and outer margins (Fig. 6F); distal article with setae laterally and four setae distally. Inner ramus short, with one distal spine and one lateral seta. Telson longer than broad, lobes slightly narrowing; each lobe with three spines and one seta distally, with two plumose setae laterally (Fig. 6H).

Female

Unknown.

Remarks

Niphargus urmiensis sp. nov. is characterized by two traits. The first one is the presence of one spine with lateral projections on the outer surface of the palmar corner in gnathopods I–II propodi. This character has been reported in *N. bisitunicus* Esmaeili-Rineh, Sari & Fišer, 2015 and *N. fiseri* sp. nov., however, both latter species bear the lateral spines in each telson lobe. Also, the maxillar palpus in *N. urmiensis* sp. nov. is short and not reaching the tip of the outer lobe of maxilla I, this character is rare among niphargids, but it has been reported in *N. kermanshahi* Esmaeili-Rineh, Heidari, Fišer & Akmali, 2016, *N. sohrevardensis* Esmaeili-Rineh, Sari, Fišer & Bargrizaneh, 2017, *N. borisi* Esmaeili-Rineh, Sari & Fišer, 2015 and *N. ilamensis* Esmaeili-Rineh, Sari, Fišer & Bargrizaneh, 2017 from Iran and *N. auerbachi* Schellenberg, 1934 from Switzerland. *Niphargus urmiensis* sp. nov., however, differs in the lower numbers of spines on the outer surface of the palmar corner of gnathopods I–II.

Niphargus fiseri sp. nov.

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Figs 1, 7–10

Diagnosis (based on male only)

Antenna I is shorter than half of the total body length. The palpus of maxilla I is as long as the outer lobe. The palmar corner of gnathopods I–II has two short supporting spines. Gnathopods I–II bear trapezoidal shape of propodi. Gnathopods I–II dactyli have a single seta on the outer margin. Pereopod VI is longer than pereopod VII. Urosomite I bears one simple seta and urosomite II bears two spines on dorso-lateral margin. The outer ramus in uropod I is shorter than the inner ramus. Epimeral plates I–II are partly pointed. The lobes of the telson bear three distal and one marginal spines. The telson cleft is more than half of the telson length.

Etymology

The species is named in honor of Dr. Cene Fišer, University of Ljubljana, Slovenia who dedicated his research to the examination and exploration of subterranean amphipod diversity.

Material examined

Holotype

IRAN • ♂; West Azerbaijan Province, Piranshahr City, Badin Abad Spring; 36°34′55″ N, 45°10′34″ E; 24 Aug. 2016; M. Mamaghani-Shishvan leg.; ZCRU Amph.1073.

Paratypes

IRAN • 2 ♀♀ (body length between 6.8 and 7.5 mm); same data as for holotype; ZCRU Amph.1073.

Description of holotype

Measurements. Total male body length is 8 mm. Head reperesents 18% of total body length (Fig. 7C). Antenna I is 0.39 times body length. Peduncular articles 1–3 progressively shorter; length of peduncular articles 3 exceeds half of peduncular article 2 (ratio 1.21 : 1); main flagellum with 16 articles (most with short setae), articles with up to one aesthetasc. Accessory flagellum bi-articulated and reaching ⅓ of article 4 of main flagellum, with one and two setae, respectively (Fig. 7A).

Antennae. Antenna II with flagellum formed of seven articles, approximately half as long as antenna I. Flagellum length is 0.90 of length of peduncle article 4 + 5. Peduncular article 4 slightly longer than article 5, with seven and five groups of setae, respectively (Fig. 7B).

Mouth parts. Labium (Fig. 8D) bi-lobate; with setae on the tip of lobes. Inner plate of maxilla I with two long apical setae; outer plate with seven spines with 3-2-2-1-0-1-0 lateral projections; palp bi-articulated, as long as outer lobe, with three long apical setae (Fig. 7D–E). Both plates of maxilla II with numerous distal setae and two lateral setae (Fig. 8E). Left mandible with five teeth on incisor process, lacinia mobilis with four teeth and a row of seven setae with lateral projections (Fig. 7F). Right mandible with four teeth on incisor process, lacinia mobilis pluritoothed and row of five setae with lateral projections (Fig. 7G). Mandibular palp articles 1 : 2 : 3 represent 22%, 35% and 43% of total palp length. Proximal article without setae, second article with five setae along inner margin and third article with one group of two A-setae, two groups of B-setae, no C-setae, 17 D-setae and five E-setae (Fig. 7H). Maxilliped with normal inner plate on which four distal spines intermixed with five distal and one simple long lateral setae subdistally; outer plate exceeding half of posterior margin of palp article 2, with nine spines along inner margin and three simple setae distally. Maxilliped palp article 3 with one proximal, inner and outer group of long simple setae at outer margin; palp terminal article with one simple seta at outer margin, nail shorter than pedestal (Fig. 8C).

Gnathopods. Gnathopod II larger than gnathopod I. Coxal plate of gnathopod I rounded, broader than long. Coxa I ventral margin with four setae. Basis with setae on anterior and posterior margins; ischium and merus with posterior group of setae. Carpus with one group of three setae antero-distally, bulge with long setae; carpus 0.53 times basis length and 0.88 times propodus length. Propodus longer than broad; anterior margin with three setae in one group in addition to antero-distal group of five setae. Palm slightly convex, defined on outer surface by one strong long corner S-seta accompanied laterally by one L-seta with lateral projections and row of three facial M-setae and by two short sub-corner R-setae on inner surface. Dactylus reaching posterior margin of propodus, outer and inner margins with row of one and two setae, respectively; nail short, 0.45 times total dactylus length (Fig. 8A). Coxal plate of gnathopod II slightly rounded, with five setae along antero-ventro-posterior margins. Basis with setae along anterior and posterior margins; posterior margins of ischium and merus with one posterior group of setae each. Carpus 0.64 times basis length and 0.90 times propodus length. Carpus with one group of two setae antero-distally. Propodus in gnathopod II larger than gnathopod I, rectangular and longer than broad; anterior margin with two setae in one group in addition to antero-distal group of four setae. Palm convex, defined on outer surface by one strong long corner S-seta accompanied laterally by one L-seta with lateral projections and row of three facial M-setae, on inner surface by two short sub-corner

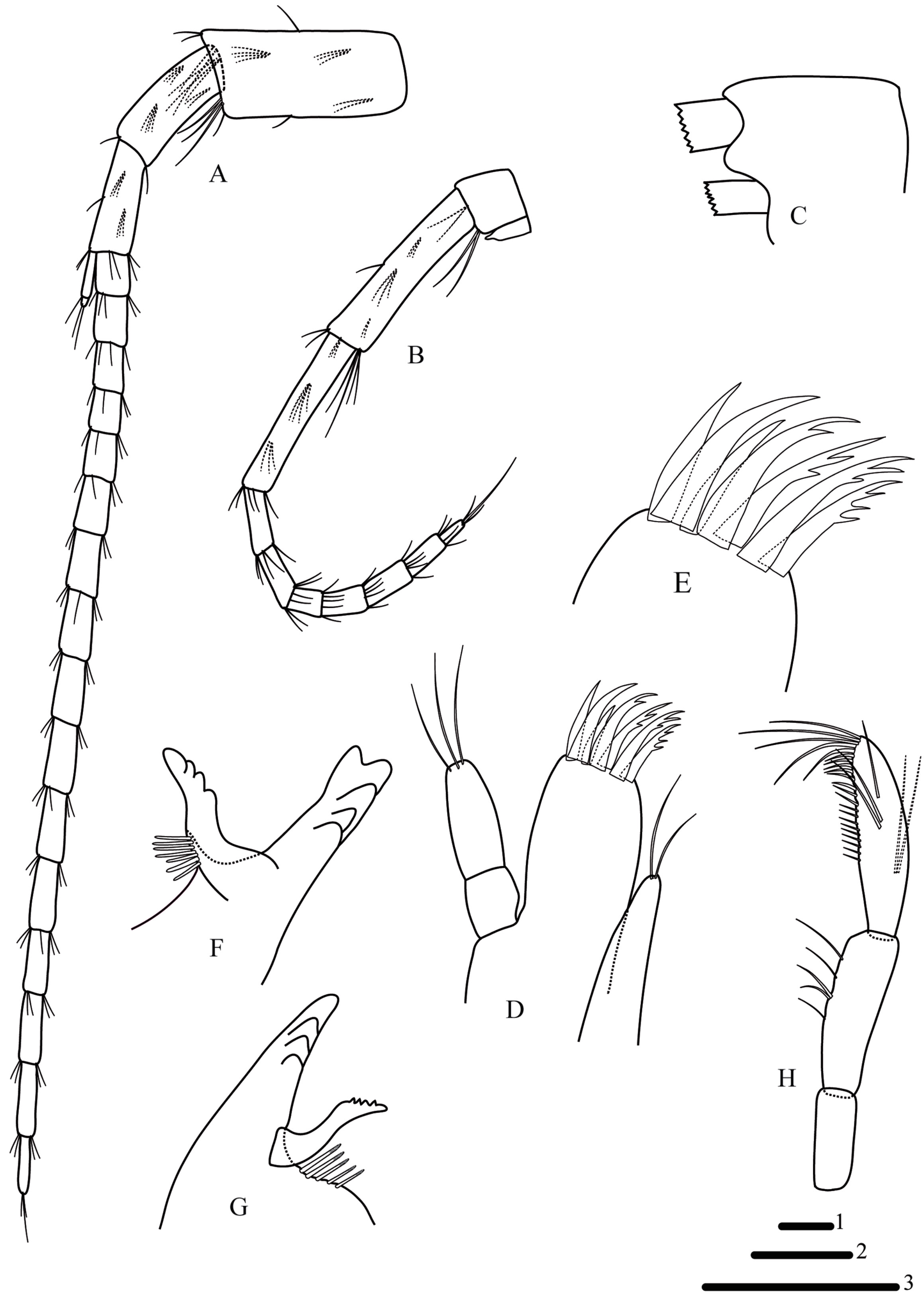

Fig. 7. *Niphargus fiseri* sp. nov., holotype, ♂, 8 mm (ZCRU Amph.1073). **A**. Antenna I. **B**. Antenna II. **C**. Head. **D–E**. Maxilla I. **F**. Left mandible. **G**. Right mandible. **H**. Mandibular palp. Scale bars: 1 = 0.25 mm (F–G); 2 = 0.5 mm (C–E, H); 3 = 1 mm (A–B).

R-setae. Dactylus reaching posterior margin of propodus, outer and inner margins of dactylus with one and three setae, respectively. Nail length 0.45 of total dactylus length (Fig. 8B). Coxal plate III with rectangular shape, length to width ratio is 1.42 : 1; antero-ventral margin with five setae. Coxal plate IV with rectangular shape, ventral margin with four setae, posterior concavity shallow and approximately 0.1 of coxa width (Fig. 9A–B). Coxal plates V–VI with two and one setae on anterior and posterior lobes, respectively. Coxal plate VII with one seta (Fig. 9C–E).

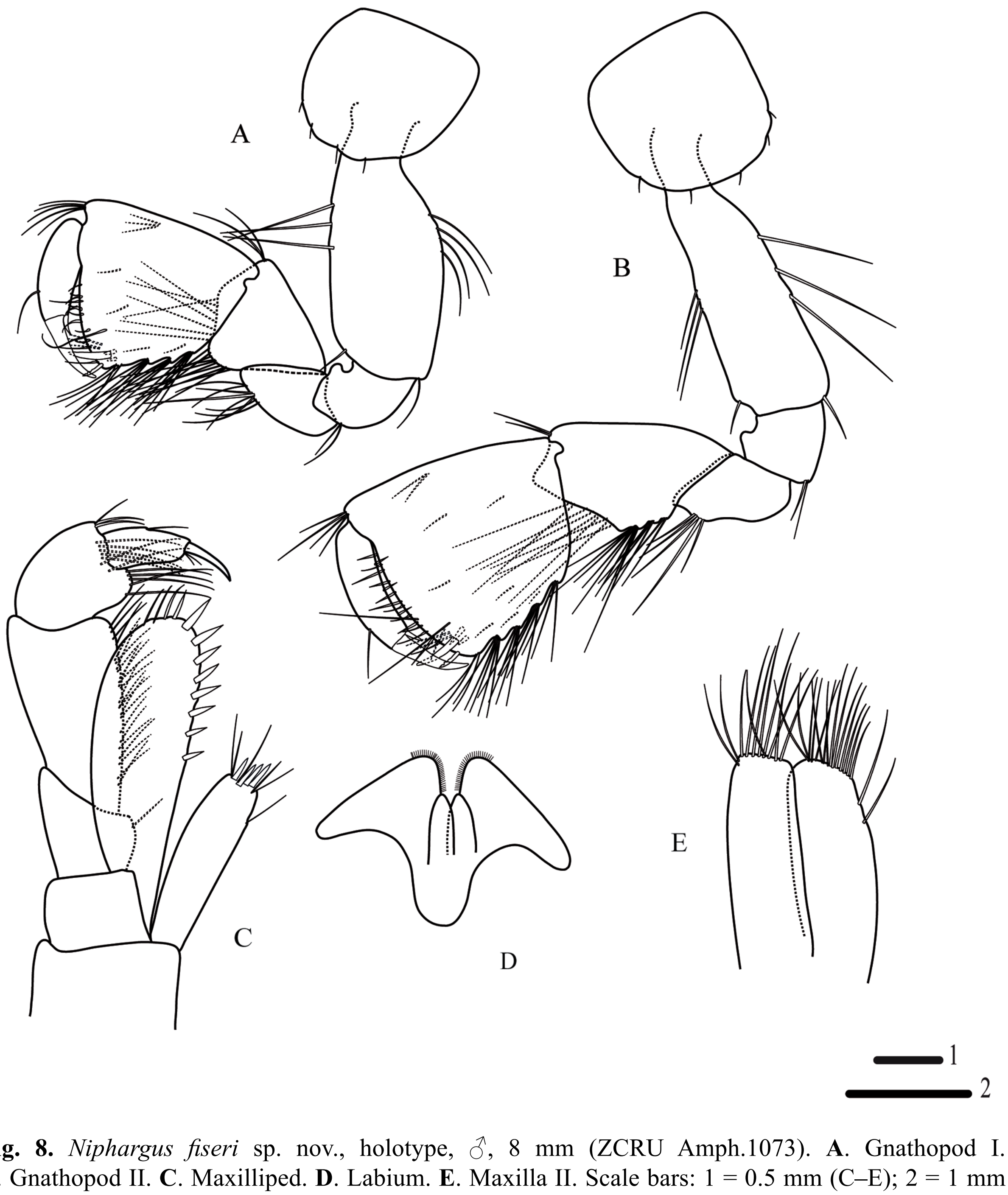

Fig. 8. *Niphargus fiseri* sp. nov., holotype, ♂, 8 mm (ZCRU Amph.1073). **A**. Gnathopod I. **B**. Gnathopod II. **C**. Maxilliped. **D**. Labium. **E**. Maxilla II. Scale bars: 1 = 0.5 mm (C–E); 2 = 1 mm (A–B).

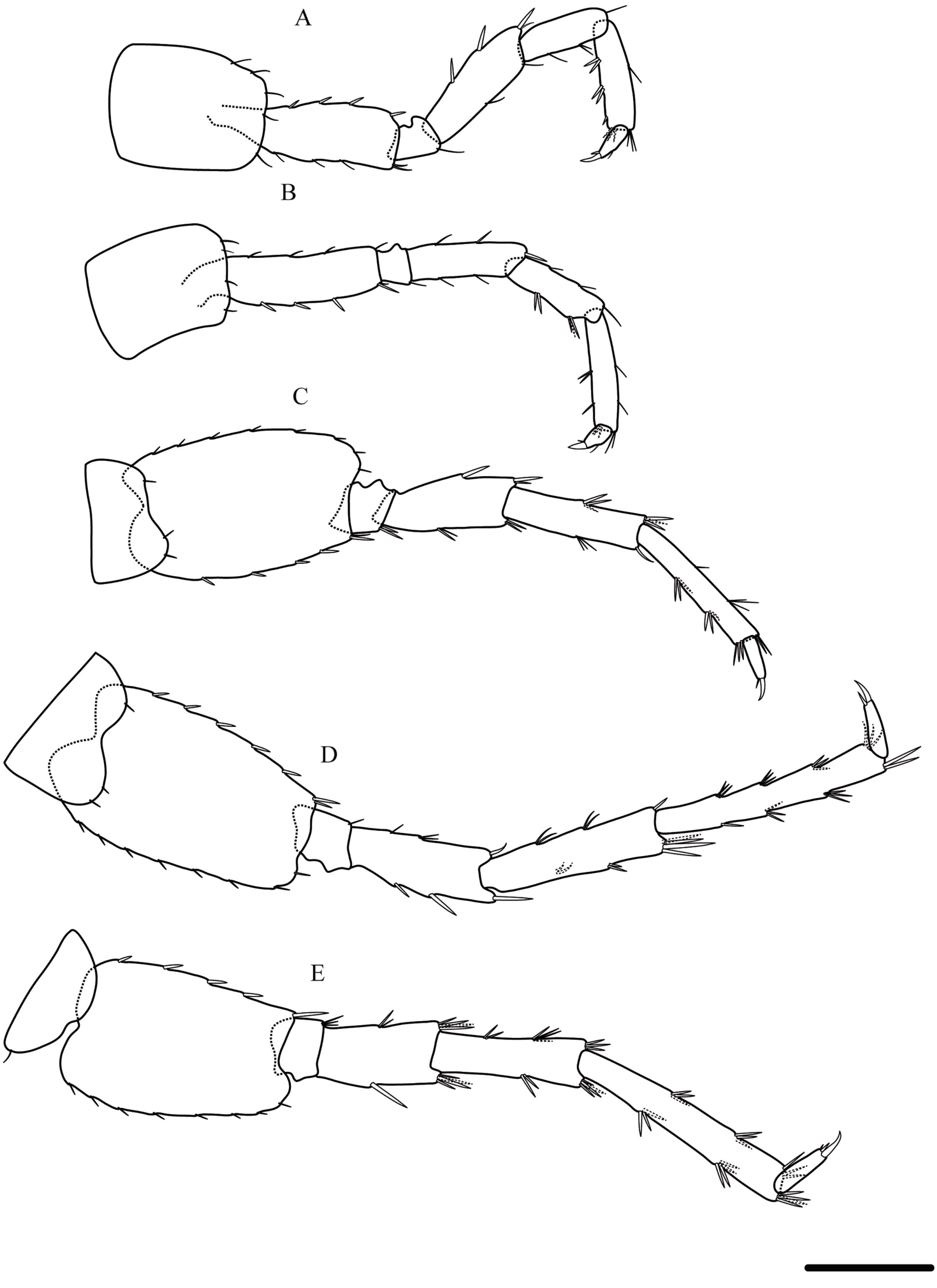

Fig. 9. *Niphargus fiseri* sp. nov., holotype, ♂, 8 mm (ZCRU Amph.1073). **A**. Pereopod III. **B**. Pereopod IV. **C**. Pereopod V. **D**. Pereopod VI. **E**. Pereopod VII. Scale bar = 1 mm.

Pereopods. Pereopod III : IV length ratio 1.03 : 1 (Fig. 9A–B). Dactylus IV short, dactylus length 0.43 times propodus length, nail shorter than pedestal (Fig. 9B). Pereopods V : VI: VII length ratios 1 : 1.27 : 1.15, respectively. Pereopod VII 0.45 times body length. Pereopod bases V and VII each with five groups of spines along anterior margins and with eight setae along posterior margins, respectively (Fig. 9C, E). Pereopod base VI with six groups of spines along posterior margin and with eight setae along anterior margin (Fig. 9D). Postero-ventral lobe of ischium in pereopods V–VII developed. Ischium, merus and carpus in pereopods V–VII with several groups of spines and setae along anterior and posterior margins; propodus of pereopod VII longer than these in V–VI, dactyli of pereopods V–VII with one spine and one short seta at base of nail on inner margin, nail length of pereopod VII 0.50 times of total dactylus length (Fig. 9C–E).

Epimeral plates I–III (Fig. 10G). With angular postero-ventral corner, anterior and ventral margins convex; postero-ventral corners of plates I–III posteriorly with two, two and three spines and setae, respectively. Epimeral plates II–III each with two spines along of ventral margins. Peduncle of pleopods I–III with two-hooked retinacles at distal part of inner margins. Peduncle of pleopods II–III with one and two setae along of inner margin (Fig. 10A–C); rami of pleopods I–III each with five to nine articles (Fig. 10A–C).

Pereonites. Pereonites I–VII without setae. Pleonites I–III each with one seta in middle of article on dorsal margin. Urosomites I–II with one seta and two spines dorso-laterally, respectively. Urosomite III without setae. Urosomite I with one spine at base of uropod I.

Uropods. Peduncle of uropod I with four and three large spines along dorso-lateral and dorso-medial margins, respectively. Inner ramus of uropod I longer than outer ramus (ratio 1 : 1.04); inner ramus with two groups of three spines laterally and five spines distally; outer ramus with two groups of three spines laterally and five spines distally (Fig. 10D). Inner ramus in uropod II longer than outer, both rami with lateral and distal long spines (Fig. 10E). Uropod III normal, almost 0.40 times body length. Peduncle of uropod III with five spines, outer ramus bi-articulated, distal 0.17 proximal articles. The proximal article of outer ramus bearing five and four groups of spines along inner and outer margins, respectively (Fig. 10F); distal article with four setae distally. Inner ramus short, with one distal spine and one lateral seta. Telson two times as long as broad, lobes slightly narrowing; each lobe with three spines distally, with one long spine and two plumose setae laterally (Fig. 10H).

Female

Unknown.

Remarks

Niphargus fiseri sp. nov. is diagnozed mainly by two characters. The first one is the presence of two supporting spines (R-setae sensu Karaman 2018) in both gnathopods, so far observed only in one species from Europe (*N. kenki* Karaman, 1952), but it seems to be more common in Iran. This character has been reported in *N. bisitunicus* Esmaeili-Rineh, Sari & Fišer, 2015 and *N. lorestanensis* Esmaeili-Rineh, 2018. However, in the latter two species, the propodi in gnathopod I are more rectangular and more elongated. The second character is the length of the nail to pedestal ratio in the dactyli of gnathopods I to II that includes more than ⅓ of the dactyli in *N. fiseri* sp. nov. So far, this character has not been described in Iranian species.

Discussion

The West Azerbaijan Province is located in northwest of Iran between 35°58′ to 39°46′ N and 44°03′ to 47°23′ E. The populations collected in this study showed the presence of two new species of the genus

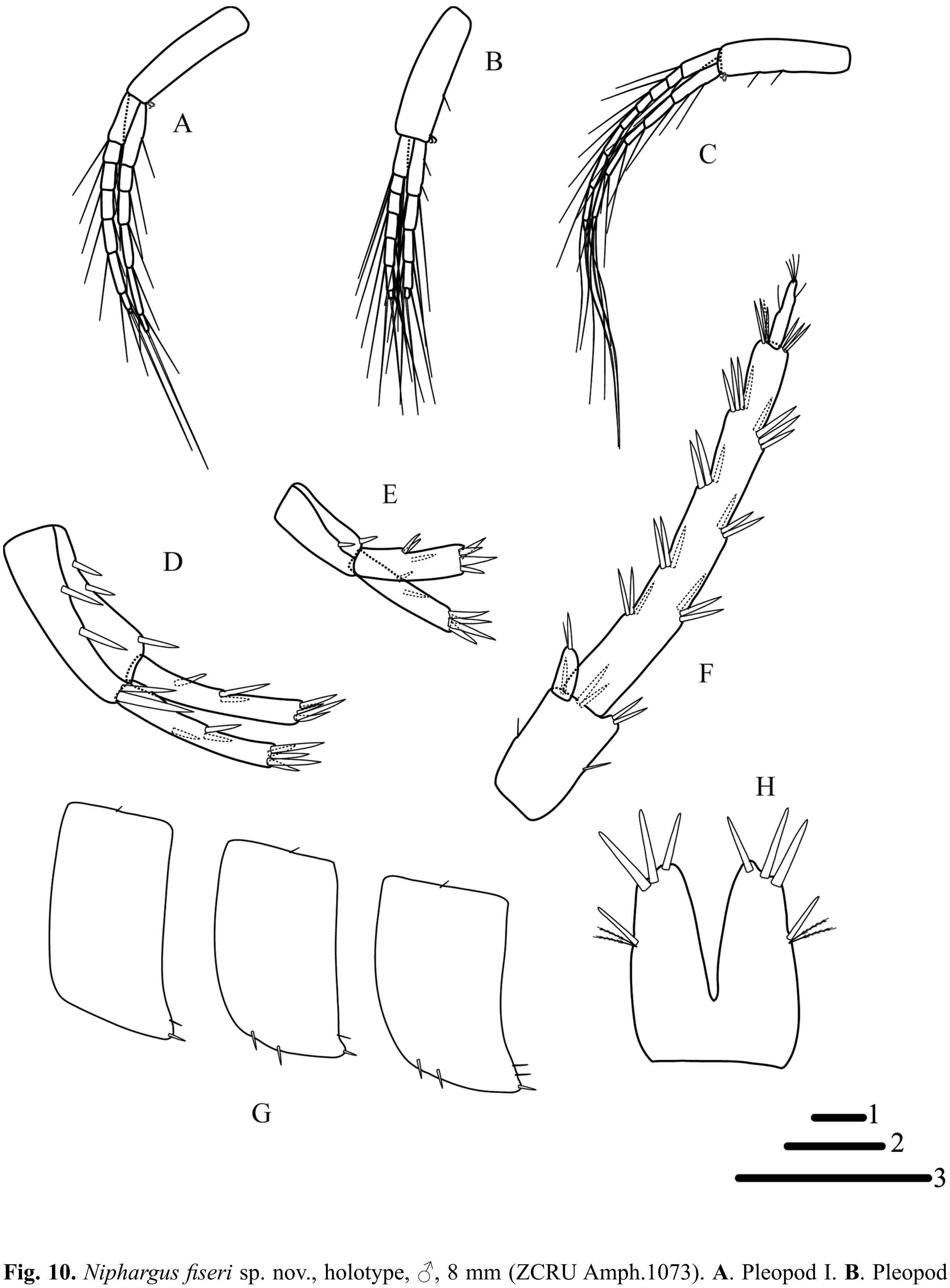

Fig. 10. *Niphargus fiseri* sp. nov., holotype, ♂, 8 mm (ZCRU Amph.1073). **A**. Pleopod I. **B**. Pleopod II. **C**. Pleopod III. **D**. Uropod I. **E**. Uropod II. **F**. Uropod III. **G**. Epimeral plates. **H**. Telson. Scale bars: 1 = 0.5 mm (G–H); 2 = 1 mm (A–E); 3 = 2 mm (F).

Niphargus. The DNA sequences confirmed the related taxonomic position of *N. urmiensis* sp. nov. and *N. fiseri* sp. nov. The Bayesian analysis showed that the two new species are phylogenetically different from other related species.

Genetically, based on COI and 28S rDNA genes (Table 1), *N. urmiensis* sp. nov. and *N. alisadri* are the most similar species to *N. fiseri* sp. nov. *Niphargus fiseri* sp. nov. resembles *N. alisadri* in terms of equal length of the maxillar palpus to the outer plate in maxilla I and the number of distal spines on the telson lobes (each with three distal spines). However, epimeral plates I–III are inclined in *N. fiseri* sp. nov. and there is a lower number of spines on the ventral margin of epimeral plates II–III. In comparison, *N. alisadri* bears pointed epimeral plates I–III with more spines on the ventral margin of epimeral plates II–III. The gnathopods in *N. alisadri* bear rectangular- to triangular-shaped propodi, but these structures in *N. fiseri* sp. nov. are trapezoidal-shaped. Moreover, *N. fiseri* sp. nov. is separated from *N. alisadri* in terms of the number of spines and simple setae on the dorso-lateral surface of urosomites I–III. Also, *N. fiseri* sp. nov. bears one lateral spine in each telson lobe, but there are two lateral spines in *N. alisadri* (Esmaeili-Rineh & Sari 2013).

Nevertheless, *N. urmiensis* sp. nov. and *N. fiseri* sp. nov. share several morphological features regarding the number of spines on the outer surface of the palmar corner in gnathopods I–II propodi (each with one spine), the number of distal spines in the telson lobes (each with three spines), the longer size of the inner to outer ramus in uropod I, and reaching the dactyli at the posterior margin of gnathopods I–II propodi. However, *N. urmiensis* sp. nov. differs from *N. fiseri* sp. nov. in a number of features, such as the absence of lateral spines on the telson: there is one lateral spine in each telson lobe in *N. fiseri* sp. nov. Outer plate to palpus ratio in maxilla I is shorter in *N. urmiensis* sp. nov. compared with *N. fiseri* sp. nov.

In *N. fiseri* sp. nov., epimeral plates I–III are inclined whereas *N. urmiensis* sp. nov. has partly angular epimeral plate I–III. Also, multiple spines were observed in *N. fiseri* sp. nov. on the medial surface of the palmar corner (two supporting spines), a feature which was not observed in *N. urmiensis* sp. nov. The mentioned feature has been reported so far from one species in Europe (*N. kenki*) only, but it seems to be more common in Iran (Esmaeili-Rineh *et al*. 2015b, 2016, 2017b). The shape of gnathopod II is highly different between the two species. The propodi of *N. fiseri* sp. nov. are broader, whereas those in *N. urmiensis* sp. nov. are more rectangular and longer. The two new species differ in terms of the number of spines and simple setae on the dorso-lateral urosomites I–III margin.

These differences developed when the amphipod species become reproductively isolated (Fišer *et al*. 2017). In addition to the morphological distinctiveness, the molecular divergence further supported the hypothesis that *N. urmiensis* sp. nov. and *N. fiseri* sp. nov. deserve an independent species status.

Acknowledgements

This project was financially supported by Razi University as a part of the MSc thesis of first author. Special thanks go to Dr. Vahid Akmali for his help regarding molecular analyses. We are especially grateful to two anonymous referees for their valuable comments.

References

Asmyhr M., Linke S., Hose G. & Nipperess D. 2014. Systematic conservation planning for groundwater ecosystems using phylogenetic diversity. *PLoS ONE* 9 (12): e115132.
https://doi.org/10.1371/journal.pone.0115132

Astrin J.J. & Stüben P.E. 2008. Phylogeny in cryptic weevils: molecules, morphology and new genera of Western Palaearctic Cryptorhynchinae (Coleoptera: Curculionidae). *Invertebrate Systematics* 22 (5): 503–522. https://doi.org/10.1071/IS07057

Brad T., Fišer C., Flot J-F. & Sarbu S.M. 2015. *Niphargus dancaui* sp. nov. (Amphipoda, Niphargidae) – a new species thriving in sulfidic groundwaters in southeastern Romania. *European Journal of Taxonomy* 164: 1–28. https://doi.org/10.5852/ejt.2015.164

Castany G. 1982. *Principes et méthodes de l'hydrogéologie*. Dunod Université, Paris.

Deharveng L., Stoch F., Gibert J., Bedos A., Galassi D., Zagmajster M., Brancelj A., Camacho A., Fiers F., Martin P., Giani N., Magniez G. & Marmonier P. 2009. Groundwater biodiversity in Europe. *Freshwater Biology* 54: 709–726. https://doi.org/10.1111/j.1365-2427.2008.01972.x

Eme D., Zagmajster M., Delić T. & Malard F. 2017. Do cryptic species matter in macroecology? Sequencing European groundwater crustaceans yields smaller ranges but does not challenge biodiversity determinants. *Ecography* 41 (2): 424–436. https://doi.org/10.1111/ecog.02683

Esmaeili-Rineh S. 2018. A new data of freshwater amphipods of genus *Niphargus* Schiödte, 1849 from Lorestan Province in Iran. *Zootaxa* 4531 (2): 242–250. https://doi.org/10.11646/zootaxa.4531.2.5

Esmaeili-Rineh S. & Sari A. 2013. Two new species of *Niphargus* Schiödte, 1849 (Crustacea: Amphipoda: Niphargidae) from two caves in Iran. *Journal of Natural History* 47: 2649–2669. https://doi.org/10.1080/00222933.2013.802041

Esmaeili-Rineh S., Sari A. & Fišer C. 2015a. Making future taxonomy of *Niphargus* (Crustacea: Amphipoda: Niphargidae) in the Middle East easier: DELTA database of Middle East species with description of four new species from Iran. *Zootaxa* 4020: 401–430. https://doi.org/10.11646/zootaxa.4020.3.1

Esmaeili-Rineh S., Sari A., Delić T., Moškrič A. & Fišer C. 2015b. Molecular phylogeny of the subterranean genus *Niphargus* (Crustacea: Amphipoda) in the Middle East: a comparison with European niphargids. *Zoological Journal of the Linnaean Society* 175: 812–826. https://doi.org/10.1111/zoj.12296

Esmaeili-Rineh S., Heidari F., Fišer C. & Akmali V. 2016. Description of new endemic species of the genus *Niphargus* Schiödte, 1849 (Amphipoda: Niphargidae) from a karst spring in Zagros Mountains in Iran. *Zootaxa* 4126: 338–350. https://doi.org/10.11646/zootaxa.4126.3.2

Esmaeili-Rineh S., Mirghaffari S.A. & Sharifi M. 2017a. The description of a new species of *Niphargus* from Iran based on morphological and molecular data. *Subterranean Biology* 22: 43–58. https://doi.org/10.3897/subtbiol.22.11286

Esmaeili-Rineh S., Sari A. Fišer C. & Bargrizaneh Z. 2017b. Completion of molecular taxonomy: description of four amphipod species (Crustacea: Amphipoda: Niphargidae) from Iran and release of database for morphological taxonomy. *Zoologischer Anzeiger* 271: 57–79. https://doi.org/10.1016/j.jcz.2017.04.009

Fišer C., Zakšek V., Zagmajster M. & Sket B. 2007. Taxonomy and biogeography of *Niphargus steueri* (Crustacea: Amphipoda). *Limnology* 8: 297–309. https://doi.org/10.1007/s10201-007-0221-5

Fišer C., Çamur-Elipek B. & Özbek M. 2009a. The subterranean genus *Niphargus* (Crustacea, Amphipoda) in the Middle East: a faunistic overview with descriptions of two new species. *Zoologischer Anzeiger* 248: 137–150. https://doi.org/10.1016/j.jcz.2009.03.003

Fišer C., Trontelj P., Luštrik R. & Sket B. 2009b. Toward a unified taxonomy of *Niphargus* (Crustacea: Amphipoda): a review of morphological variability. *Zootaxa* 2061: 1–22. https://doi.org/10.11646/zootaxa.2061.1.1

Fišer C., Konec M., Alther R., Švara V. & Altermatt F. 2017. Taxonomic, phylogenetic and ecological diversity of *Niphargus* (Amphipoda: Crustacea) in the Hölloch cave system (Switzerland). *Systematics and Biodiversity* 15: 218–237. https://doi.org/10.1080/14772000.2016.1249112

Frey W. & Probst P. 1986. A synopsis of the vegetation of Iran *In*: Kiirschner H. (ed) *Contributions to the Vegetation of Southwest Asia*: 9–43. Dr. Ludwig Reichert Verlag. Wiesbaden.

Glanville K., Schulz C., Tomlinson M. & Butler D. 2016. Biodiversity and biogeography of groundwater invertebrates in Queensland, Australia. *Subterranean Biology* 17: 55–76. https://doi.org/10.3897/subtbiol.17.7542

Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.

Humphreys W.F. 2006. Groundwater fauna. Australian State of the Environment Committee, Department of the Environment and Heritage, Canberra.

Jörger K.M. & Schrödl M. 2013. How to describe a cryptic species? Practical challenges of molecular taxonomy. *Frontiers in Zoology* 10 (1): 59. https://doi.org/10.1186/1742-9994-10-59

Karaman S. 1952. Podrod *Stygoniphargus* u Sloveniji i Hrvatskoj, Poseban otisak iz 25. knjige Prirodoslovnih istraživanja. *Academia Scientiarum et Artium Slavorum Meridionalium* 1: 5–38.

Karaman G.S. 1998. First discovery of the family Niphargidae (Gammaridea) in Iran (Contribution to the knowledge of the Amphipoda 234). *Glas Od Prir Nauka–Crnog Akad Nauka Umjet* 12: 9–22.

Karaman G.S. 2018. Further discovery of new or partially known taxa of the genus *Niphargus* Schiödte, 1849 (fam. Niphargidae) in Greece (Contribution to the knowledge of the Amphipoda 302). *Agriculture and Forestry* 64 (2): 5–31.

Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111–120. https://doi.org/10.1007/BF01731581

Madjnoonian H., Kiabi B.H. & Danesh M. 2005. *Readings in Zoogeography of IraN. Part I*. Department of Environment, Iran.

Mamaghani-Shishvan M., Esmaeili-Rineh S. & Fišer C. 2017. An integrated morphological and molecular approach to a new species description of amphipods in the Niphargidae from two caves in west of Iran. *Zoological Studies* 56: 33. https://doi.org/10.6620/ZS.2017.56-33

Posada D. 2008. jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253–1256. https://doi.org/10.1093/molbev/msn083

Raeisi E. 2004. Iran cave and karst. *In*: Gunn J. (ed.) *Encyclopedia of Cave and Karst*: 460–461. Fitzroy Dearborn, New York.

Rambaut A. & Drummond A.J. 2009. Bayesian Evolutionary Analysis Sampling Trees (BEAST). Version 1.7.4.

Ronquist F. & Huelsenbeck J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Stokkan M., Jurado-Rivera J.A., Oromí P., Juan C., Jaume D. & Ponsa J. 2018. Species delimitation and mitogenome phylogenetics in the subterranean genus *Pseudoniphargus* (Crustacea: Amphipoda). *Molecular Phylogenetic and Evolution* 127: 988–999. https://doi.org/10.1016/j.ympev.2018.07.002

Tamura K., Peterson D., Peterson N., Stecher G., Nei M. & Kumar S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28: 2731–2739. https://doi.org/10.1093/molbev/msr121

Thompson J.D., Higgins D.G. & Gibson T. 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680. https://doi.org/10.1093/nar/22.22.4673

Väinölä R., Witt J.D.S., Grabowski M., Bradbury J.H., Jazdzewski K. & Sket B. 2008. Global diversity of amphipods (Amphipoda; Crustacea) in freshwater. *Hydrobiologia* 595: 241–255. https://doi.org/10.1007/s10750-007-9020-6

Verovnik R., Sket B. & Trontelj P. 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. https://doi.org/10.1111/j.1365-294X.2005.02745.x

White W.B. 2007. Groundwater flow in karstic aquifers. *In*: Delleur J.W. (ed.) *The Handbook of Groundwater Engineering*. 2nd edition 21: 1–47. CRC Press & Taylor and Francis Group.

Witt J.D.S., Threloff D.L. & Hebert P.D.N. 2006. DNA barcoding reveals extraordinary cryptic diversity in an amphipod genus: implications for desert spring conservation. *Molecular Ecology* 15: 3073–3082. https://doi.org/10.1111/j.1365-294X.2006.02999.x

Zakšek V., Sket B. & Trontelj P. 2007. Phylogeny of the cave shrimp *Troglocaris*: evidence of a young connection between Balkans and Caucasus. *Molecular Phylogenetics and Evolution* 42: 223–235. https://doi.org/10.1016/j.ympev.2006.07.009

Manuscript received: 24 January 2019
Manuscript accepted: 1 July 2019
Published on: 27 August 2019
Topic editor: Rudy Jocqué
Desk editor: Pepe Fernández