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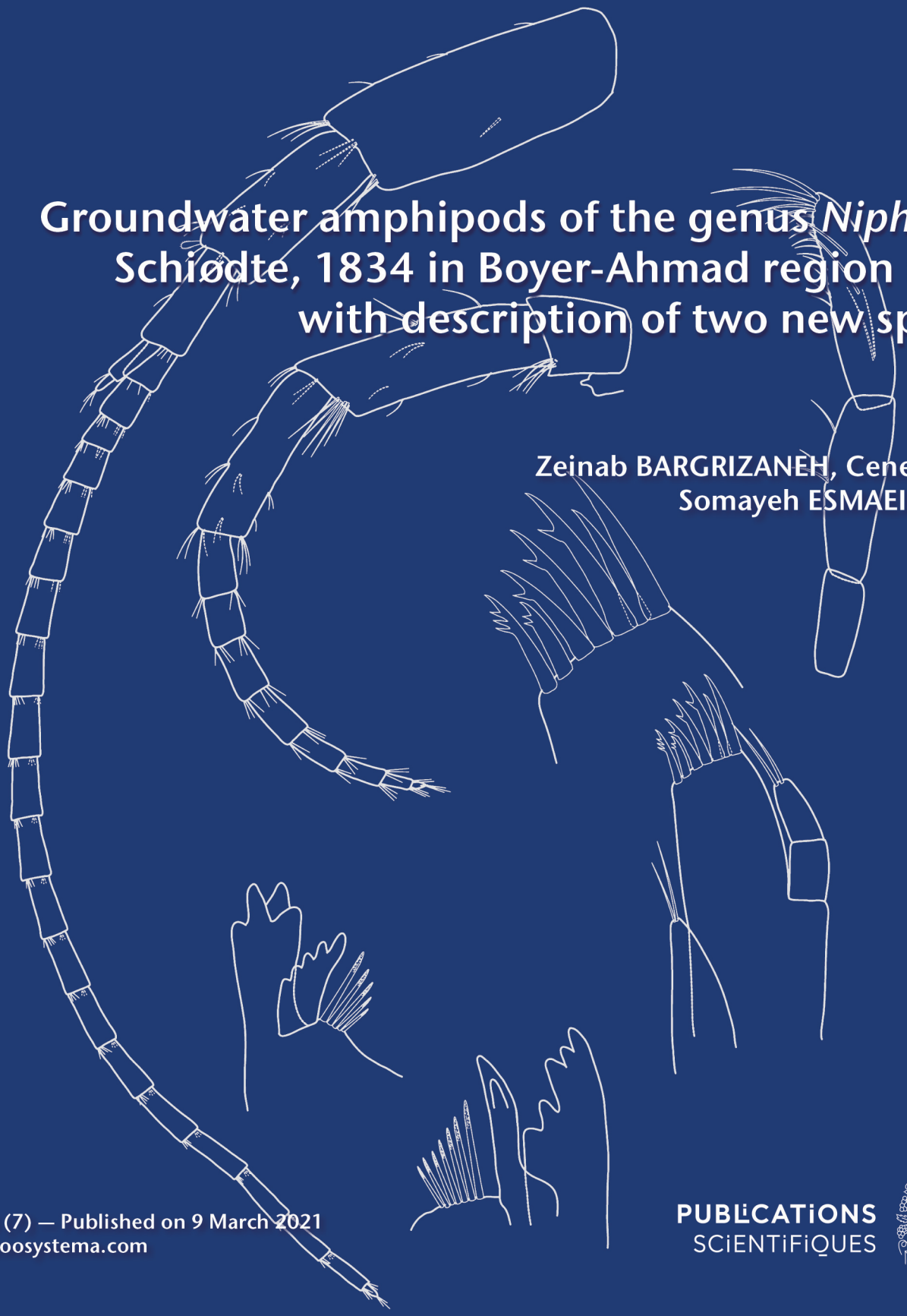

Groundwater amphipods of the genus *Niphargus* Schiødte, 1834 in Boyer-Ahmad region (Iran) with description of two new species

Zeinab BARGRIZANEH, Cene FIŠER & Somayeh ESMAEILI-RINEH

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Couverture / *Cover* :
Niphargus yasujensis n. sp., male 7 mm (holotype, ZCRU Amph. 1053) body parts.

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Groundwater amphipods of the genus *Niphargus* Schiødte, 1834 in Boyer-Ahmad region (Iran) with description of two new species

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ABSTRACT
The systematic research of amphipod genus *Niphargus* Schiødte, 1849 in Iran has begun only recently, and new field explorations result in discovery of new, yet undescribed species. Here we report on faunistic surveys in the Boyer-Ahmad Region that unveiled three species. Using molecular phylogeny (COI and 28S markers) and morphological analysis, we show that one species should be classified as the recently described *N. persicus* Esmaeili-Rineh, Sari, Fišer & Bargrizaneh, 2017, whereas the other two are new to Science. We show that both species, *N. yasujensis* n. sp. and *N. nasrullahi* n. sp., fall into the Middle East clade of species, as well as that both species are genetically and morphologically distinct from other species in the region. Both species are diagnosed, described and illustrated.

KEY WORDS
Niphargus, morphology, 28S, COI, phylogeny, Iran, new species.

RÉSUMÉ
Les amphipodes des eaux souterraines du genre Niphargus *Schiødte, 1834 dans la région de Boyer-Ahmad (Iran) avec la description de deux espèces nouvelles.*
Les recherches en systématique sur le genre d'amphipode *Niphargus* Schiødte, 1849 en Iran n'ont commencé que récemment, et de nouvelles études de terrain conduisent à la découverte d'espèces inédites non encore décrites. Dans le présent article, nous présentons les études faunistiques de la région de Boyer-Ahmad qui a révélé trois espèces. Par des analyses morphologiques et de phylogénie moléculaire (marqueurs COI et 28S), nous montrons que l'une des espèces peut être identifiée *N. persicus* Esmaeili-Rineh, Sari, Fišer & Bargrizaneh, 2017, nouvellement décrit, tandis que les deux autres sont nouvelles pour la Science. Ces deux espèces, *N. yasujensis* n. sp. et *N. nasrullahi* n. sp., appartiennent au clade des espèces du Moyen-Orient, et diffèrent tant génétiquement que morphologiquement des autres espèces de la région. Les deux espèces font l'objet d'une diagnose, d'une description et d'illustrations.

MOTS CLÉS
Niphargus, morphologie, 28S, COI, phylogeny, Iran, espèces nouvelles.

INTRODUCTION

Although freshwater ecosystems occupy only 0.8% of Earth's surface, they are estimated to harbour nearly 6% of all described species (Dudgeon *et al.* 2006). Data on the diversity of most invertebrate groups in freshwater including crustaceans, insects, and molluscs are inadequate (Dudgeon 1999, 2000; Benstead *et al.* 2003; Strayer *et al.* 2004). Groundwater represents about 30% of global freshwater resources (Shiklomanov 2000). This part of the aquatic ecosystems host numerous species that cannot be found in surface freshwaters (Culver *et al.* 2009; Trontelj *et al.* 2009; Esmaeili-Rineh *et al.* 2018). As in surface freshwater ecosystems, species lists are far from complete. Proper running of conservation and management programs for freshwater groups, however, depends on complete taxonomic lists of species, understanding boundaries of their ranges and how species distributions shaped diversity patterns (Cooke *et al.* 2012).

Niphargus Schiødte, 1849 is the largest freshwater amphipod genus in the world. Species and subspecies, living in subterranean waters of the Western Palearctic (Fišer *et al.* 2008), exhibit an extremely diversified morphology due to divergent selection (Sket 1999; Fišer *et al.* 2008; Esmaeili-Rineh *et al.* 2015a). *Niphargus* species cluster into more or less distinct morphotypes (Trontelj *et al.* 2018). Within each morphological type, however, cryptic species have been found (Trontelj *et al.* 2009; Delić *et al.* 2017a). Therefore, even basic faunistic and taxonomic surveys of *Niphargus* diversity critically depend on molecular analyses and integrative taxonomy.

Iran represents the eastern boundary of *Niphargus* range. Several studies during the past five years unveiled many new species (Esmaeili-Rineh *et al.* 2015a, b, 2017a, b, 2018; Mamaghani-Shishvan *et al.* 2017; Mamaghani-Shishvan & Esmaeili-Rineh 2019). In this paper, we report on further explorations of the niphargid fauna, specifically from Boyer-Ahmad region (Fig. 1). We describe two new species and report a new record of *N. persicus* Esmaeili-Rineh, Sari, Fišer & Bargrizaneh, 2017.

MATERIAL AND METHODS

Morphological and morphometric studies

We collected samples in Biareh, Mansurkhani, Chonarestan, Khong and Nahr springs, all situated in Boyer-Ahmad region (Fig. 1). The specimens were collected using hand nets in March-July 2015. The samples were fixed in 70% and 90% ethanol for morphological and molecular studies, respectively. We first examined samples morphologically, removed one appendage for DNA analyses and the rest of individuals were mounted on slides in Euparal® medium (Manchester, England).

The slides were studied using a Zeiss Primostar microscope and a LABOMED Lx500 stereomicroscope. Details were photographed using an Olympus LABOMED iVu7000 camera mounted on the stereomicroscope. Measurements and counts of morphological characters were performed using the digitized photos and the computer program ProgRes Capture Pro 2.7, referring to characters and landmarks defined by Fišer *et al.* (2009). Illustrations were prepared in Adobe Illustrator CS5, using photos as background pictures.

All specimens were deposited in the Zoological Collection of Razi University (ZCRU).

Molecular phylogenetic analyses

We extracted the total genomic DNA from a part of an animal using Tissue Kits (GenNet Bio™, Seoul, Korea) following the manufacturer's instructions. The modified primer pair LCO1490-JJ and HCO2198-JJ (Astrin & Stüben 2008) was used to amplify a fragment of the mitochondrial COI gene. We amplified and sequenced a fragment of 28S ribosomal DNA (rDNA) following Verovnik *et al.* (2005) and Zakšek *et al.* (2007), for the forward and reverse primer, respectively.

Each 25 µl PCR mix comprised of 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 3 minutes (min) was followed by 36 cycles of 40 seconds (s) at 94 °C, 40 s at 52.5 °C and 2 min at 65 °C with a final extension step of 8 min at 65 °C to amplify the COI gene. Cycling parameters for the 28S rDNA gene were as follows: initial denaturation of 94 °C for 7 min, 35 subsequent cycles of 94°C for 45 s, 55°C for 30 s, 72°C for 1 min, and a final extension of 72°C for 7 min. PCR products were purified and sequenced by Macrogen Inc. (Korea), using the primer pairs mentioned above.

The chromatograms were edited in Bioedit, version 7.0.5.3. The acquired sequences (with GenBank accession numbers MT636103 to MT636107 for COI and MT652091 to MT652097 for 28S) were analysed within the data set of Esmaeili-Rineh *et al.* (2015b) and Mamaghani-Shishvan & Esmaeili-Rineh (2019), in order to identify the phylogenetic position of the newly collected specimens. The outgroup was selected on a basis of previous phylogenetic analyses. The NCBI available sequences for three species including *Niphargus krameri* Schellenberg, 1935, *Niphargus aquilex* Schiødte, 1855 and *Niphargus schellenbergi* S. Karaman, 1932 were used as outgroup (accession numbers: EF617274 and KF719253, EF617264 and JF420841, JF420854 and EU693321).

We sequenced and analysed seven individuals from five populations: Mansurkhani (two individuals), Chonarestan (two individuals), Khong (one individual), Biareh (one individual), and Nahr springs (one individual). All sequences were edited and aligned using ClustalW (Thompson *et al.* 1994), as implemented in the Bioedit, version 7.0.5.3, program sequence alignment editor (Hall 1999), using the default settings. Alignments were concatenated using Geneious Pro 5.6 (Biomatters, Auckland, New Zealand) and jModelTest, version 0.1.1 (Posada 2008) was used to select the optimal substitution model for each marker under the Akaike information criterion (AIC). The optimal substitution models were GTR + G and TIM3 + I + G for 28S and COI gene fragments, respectively.

Phylogenetic relationships were reconstructed using Bayesian inference in MrBayes, version 3.1.2 (Ronquist & Huelsenbeck 2003). Bayesian analyses were run for five million generations, with four chains, and the trees were sampled every 1 000 gen-

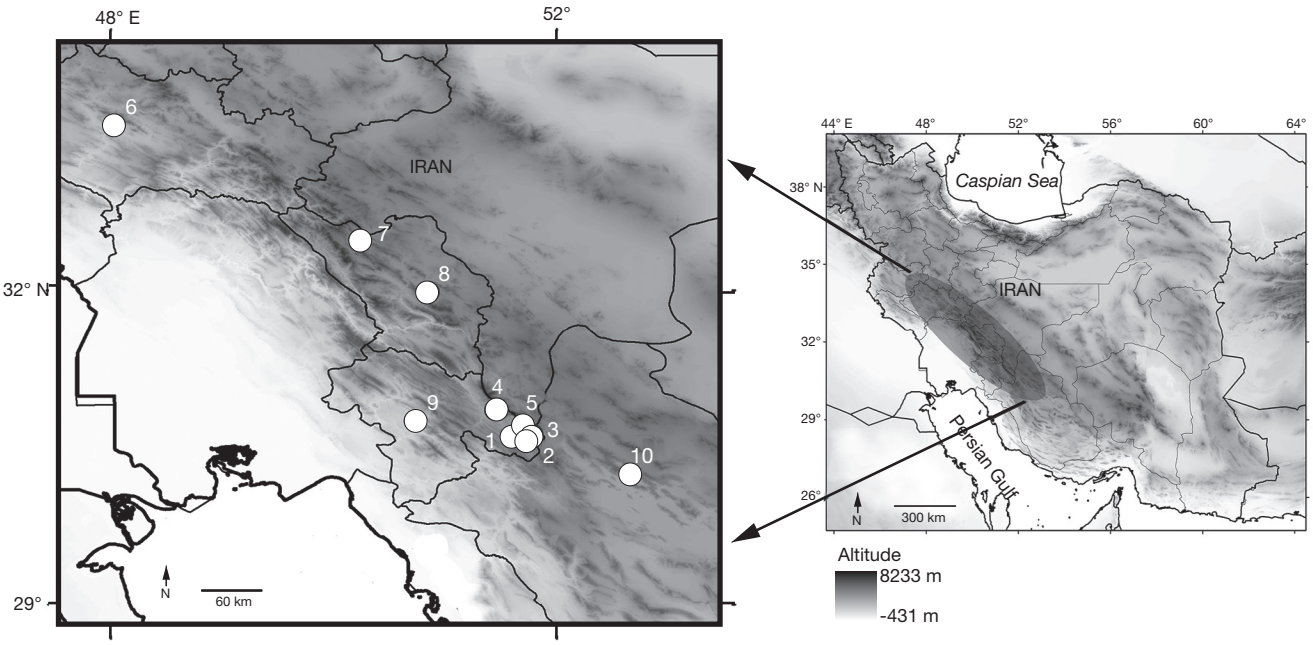

Fig. 1. — Distribution map of the *Niphargus* species. Localities are labelled as: **1**, *N. persicus* Esmaeili-Rineh, Sari, Fišer & Bargrizaneh, 2017 (Mansurkhani Spring); **2**, *N. yasujensis* n. sp. (Chonarestan Spring); **3**, *N. yasujensis* n. sp. (Khong Spring); **4**, *N. yasujensis* n. sp. (Biareh Spring); **5**, *N. nasrullahi* n. sp. (Nahr Spring); **6**, *N. sharifii* Esmaeili-Rineh, Sari & Fišer 2015; **7**, *N. darvishi* Esmaeili-Rineh, Sari & Fišer 2015; **8**, *N. borisi* Esmaeili-Rineh, Sari & Fišer 2015 (Belqais population); **9**, *N. borisi* (Siah population); **10**, *N. persicus*.

erations. The first 25% sampled trees were discarded as burn-in, and the subsequent tree likelihoods were examined for convergence in Tracer 1.5.0 (Rambaut & Drummond 2009). From the remaining trees, we calculated the 50% majority rule consensus tree and visualized it using FigTree v1.4.0.

In order to additionally characterize the two newly discovered species against other Iranian species, we also calculated genetic distances between individuals using uncorrected p-distance as implemented in MEGA ver. 5 (Tamura *et al.* 2011). Additionally, we tested species of the newly described species boundaries using the Automated Barcoding Gap Delimitation (ABGD) using COI sequences and the following settings: the parameter range Pmin = 0.001, Pmax = 0.1, gap width = 1, and Kimura two Parameter (K2P) correction of genetic distances. The genetic distance matrix was generated in MEGA ver. 5 (Tamura *et al.* 2011).

RESULTS

Phylogenetic analysis

The phylogenetic analysis showed that all the newly sequenced individuals are nested within the Middle East clade (Fig. 2) as defined by Esmaeili-Rineh *et al.* (2015a). The COI sequencing of individuals from Mansurkhani population failed. According to 28S sequences, these two individuals fell within the *N. persicus* subclade (Fig. 2). Although 28S is in general too conservative to accurately delimit species boundaries, we compared the degree of genetic differentiation between individuals from Mansurkhani population and its close relatives, i.e., *N. persicus* and *N. darvishi* Esmaeili-Rineh, Sari & Fišer, 2015. Members of the new population were approximately four times more similar to the nominal population of *N. persicus* (0.13% p-distance for 28S, Table 1) than to its sister species *N. darvishi* (0.38% p-distance for 28S, Table 1). Given that these individuals were morphologically similar to *N. persicus*, therefore we tentatively attributed the new finding to *N. persicus* as *N.* cf *persicus*, awaiting for further more detailed analyses.

Khong, Chonarestan and Biareh populations represented a sister lineage to *N. borisi* Esmaeili-Rineh, Sari & Fišer 2015 (Fig. 2). The genetic distances between these three populations was 1.56% for COI fragments. By contrast, genetic distances between the new species and *N. borisi* was 7.02-8.19% p-distance for COI gene. For comparison, the minimal p-distance between these *Niphargus* species was 3.70% (Table 1), implying that this new population could be considered as a new species. The ABGD analysis supported this result: individuals from these three populations comprised one putative species. Along with morphological differences (see below), we propose that these three populations constitute a new, yet undescribed species.

Finally, the specimens from Nahr population appeared to be a sister lineage of *N. borisi* and [Chonarestan + Biareh + Khong] populations (Fig. 2). Genetic distances between the population from Nahr and [Chonarestan + Biareh + Khong] populations were 8.38-8.87% p-distance for COI gene. The population from Nahr was even more divergent from *N. sharifii* Esmaeili-Rineh, Sari & Fišer 2015 with 13.45% p-distances for COI gene, again implying its species status. This result was confirmed by ABGD analysis that identified the individual from Nahr population as a distinct species. For this reason and due to distinct morphology, we considered Nahr population as another, undescribed species.

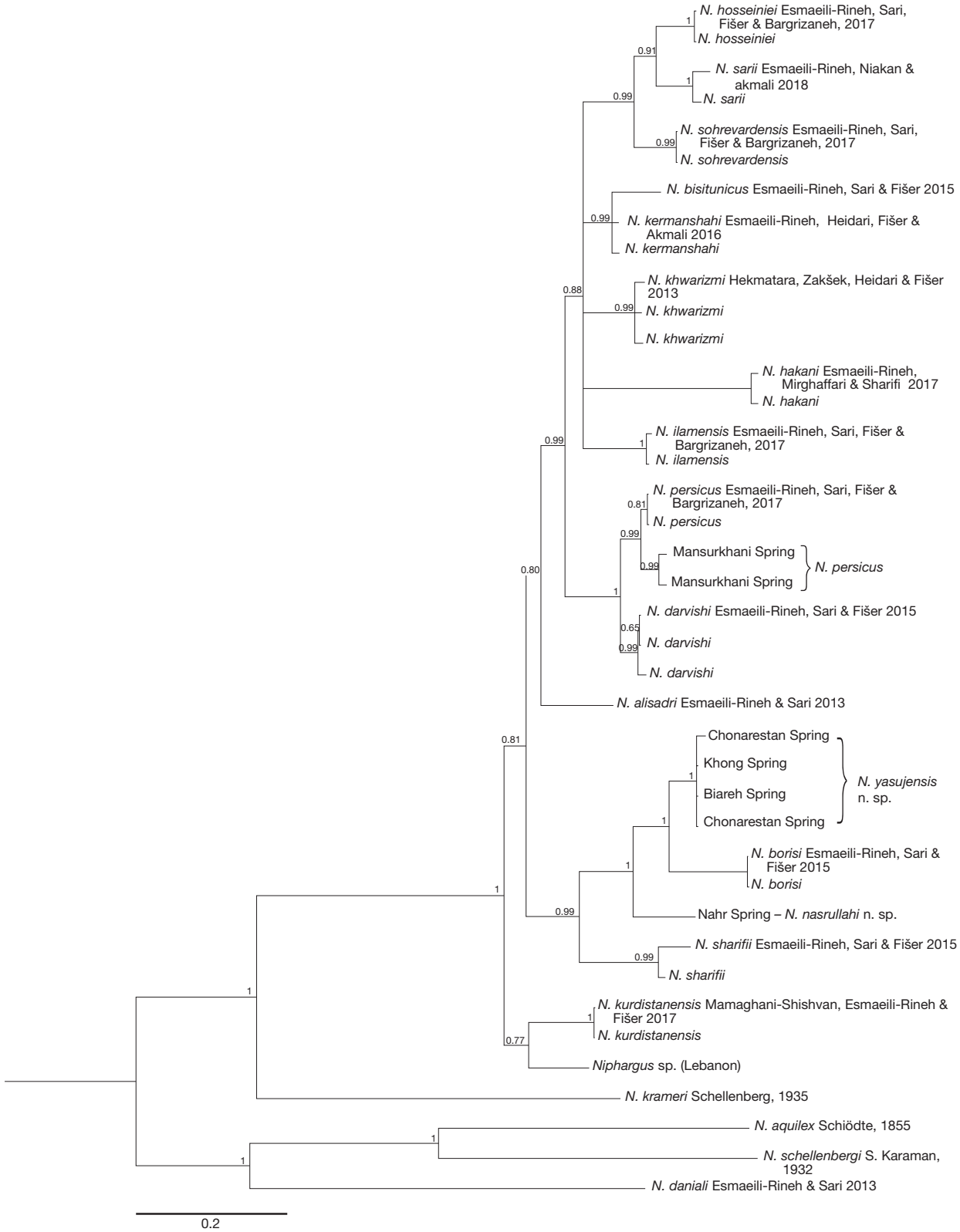

Fig. 2. — Phylogenetic tree of 40 specimens as inferred from the 28S and COI gene concatenated sequences, estimated within the Bayesian framework. Species are identified and named according to available taxonomic descriptions. Bayesian posterior probabilities are indicated above branches. Scale bar indicates number of substitutions per branch.

Table 1. — Uncorrected p-distances (%) between Iranian species (*N. sharifii* Esmaeili-Rineh, Sari & Fišer 2015, *N. borisi* Esmaeili-Rineh, Sari & Fišer 2015, *N. persicus* Esmaeili-Rineh, Sari, Fišer & Bargrizaneh, 2017, *N. darvishi* Esmaeili-Rineh, Sari & Fišer 2015) and new collected populations of the genus *Niphargus* Schiödte, 1849 (based on 28S ribosomal DNA gene (below diagonal) and mtDNA (COI) gene (above diagonal). Symbol: *, indicates that the sequencing has failed and comparison cannot be made.; **1**, *N. yasujensis* n. sp.; **2**, *N. nasrullahi* n. sp.; **3**, *N. persicus*.

	Chonarestan Spring (1)	Biareh Spring (1)	Chonarestan Spring (1)	Khong Spring (1)	Nahr Spring (2)	Mansurkhani Spring (3)	Mansurkhani Spring (3)	*N. sharifii*	*N. borisi*	*N. persicus*	*N. darvishi*
Chonarestan Spring (1)	–	1.56	1.56	1.56	8.77	*	*	14.04	8.19	12.87	11.70
Biareh Spring (1)	0.00	–	0.00	0.00	8.38	*	*	13.06	7.02	11.89	10.92
Chonarestan Spring (1)	0.00	0.00	–	0.00	8.38	*	*	13.06	7.02	11.89	10.92
Khong Spring (1)	0.00	0.00	0.00	–	8.38	*	*	13.06	7.02	11.89	10.92
Nahr Spring (2)	1.26	1.26	1.26	1.26	–	*	*	13.45	9.36	12.67	12.48
Mansurkhani (3)	2.14	2.14	2.14	2.14	1.64	–	*	*	*	*	*
Mansurkhani (3)	2.14	2.14	2.14	2.14	1.64	0.00	–	*	*	*	*
N. sharifii	1.26	1.26	1.26	1.26	0.75	1.38	1.38	–	11.70	13.45	13.45
N. borisi	1.13	1.13	1.13	1.13	1.76	2.64	2.64	1.76	–	12.67	12.09
N. persicus	2.27	2.27	2.27	2.27	1.51	0.13	0.13	1.26	2.77	–	3.70
N. darvishi	2.14	2.14	2.14	2.14	1.38	0.38	0.38	1.13	2.64	0.25	–

TAXONOMIC PART

Order AMPHIPODA Latreille, 1816
Family Niphargidae Bousfield, 1977
Genus *Niphargus* Schiødte, 1849

Niphargus yasujensis n. sp.
(Figs 3-6)

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Material examined. — **Holotype. Iran** • ♂ adult (7 mm); Biareh Spring, Sisakht City, Kohgiloyeh and Boyer Ahmad Province; 30°51'N, 51°27'E; Zeinab Bargrizaneh leg.; 5.IV.2015; ZCRU Amph.1053. **Paratypes. Iran** • 2 ♂; same data as for holotype; ZCRU Amph.1053.

Diagnosis. — A slender *Niphargus* species with elongated body. Pleonites with two setae along the dorso-posterior margin of each segment and with one strong spine at base of uropod I. Epimeral plates II-III slightly produced, posterior and ventral margins concave and convex, respectively. Antenna I shorter than half of the total body length. Outer lobe of maxilla I with seven spines with 0-3 lateral projection. Palpus of maxilla I short and not reaching the tip of the outer lobe (Fig. 3C, D). Gnathopods propodi of trapezoidal to rectangular shape (Fig. 4A, B). Propodus of gnathopod I with two equal palmar spines (Fig. 4A). Length of carpus and propodus of gnathopod I equal. Pereopods shorter than half of total body length, with one spine at the nail base. Outer ramus of uropod I slightly longer than inner ramus. Telson lobes with five distal spines each (Fig. 6H). Female unknown.

Diagnostic Coi sequences. — GeneBank Accession Numbers: MT636103-MT636106.

Etymology. — The name "yasujensis'" refers to Yasuj City, the center of Kohgiloyeh and Boyer-Ahmad province (Iran), where the species was found.

Description of holotype

Measurements

Total male body length: 7 mm. Head length representing 18% of total body length.

Antenna I (Fig. 3A)

40% of total body length. Peduncular articles 1-3 progressively shorter; length of peduncular article 2 more than half of peduncular article 3; most flagellum articles with one short aesthetasc. Main flagellum with 21 articles, most of which with short setae. Accessory flagellum bi-articulated, reaching beyond two-third of article 4 of main flagellum; distal article with three setae.

Antenna II (Fig. 3B)

Almost half of the length of antenna I. Peduncular articles 4 : 5 ratio equal to 1.36 : 1; peduncle articles 4 and 5 each with nine groups of simple setae. Flagellum of antenna II 0.89% of the length of peduncle articles 4 + 5, totalling eight articles.

Mouth parts (Figs 3, 4)

Labium (Fig. 4C). Bilobate; with developed inner lobes.

Left mandible (Fig. 3E). With five teeth on incisor process, with two teeth on lacinia mobilis and a row of seven serrated setae.

Right mandible (Fig. 3F). With four teeth on incisor process, with five teeth on lacinia mobilis and a row of five serrated setae.

Mandibular palp (Fig. 3G). Ratio of mandibular palp articles 1 : 2 : 3 equal to 1 : 1.72 : 2.06. Proximal article without setae; middle article with three simple setae medially; distal article with one A-group of two setae, two single separate B-setae, no C-setae, ten D-setae and five E-setae.

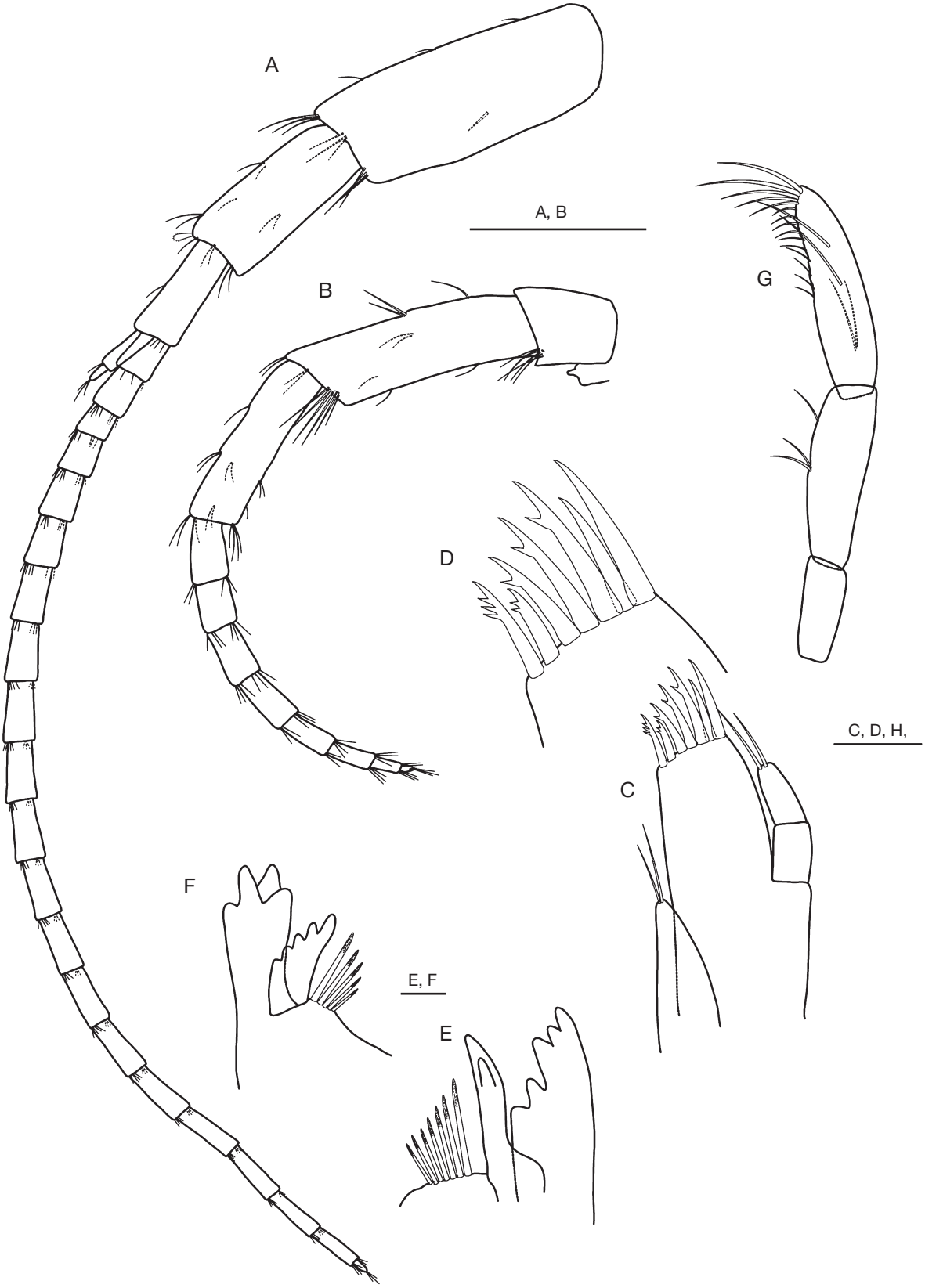

Fig. 3. — *Niphargus yasujensis* n. sp., male 7 mm (holotype, ZCRU Amph. 1053): **A**, antenna I; **B**, antenna II; C, D, maxilla I; **E**, left mandible; **F**, right mandible; **G**, mandibular palp. Scale bars: A, B, 1 mm; C, D, H, 0.5 mm; E, F, 0.25 mm; .

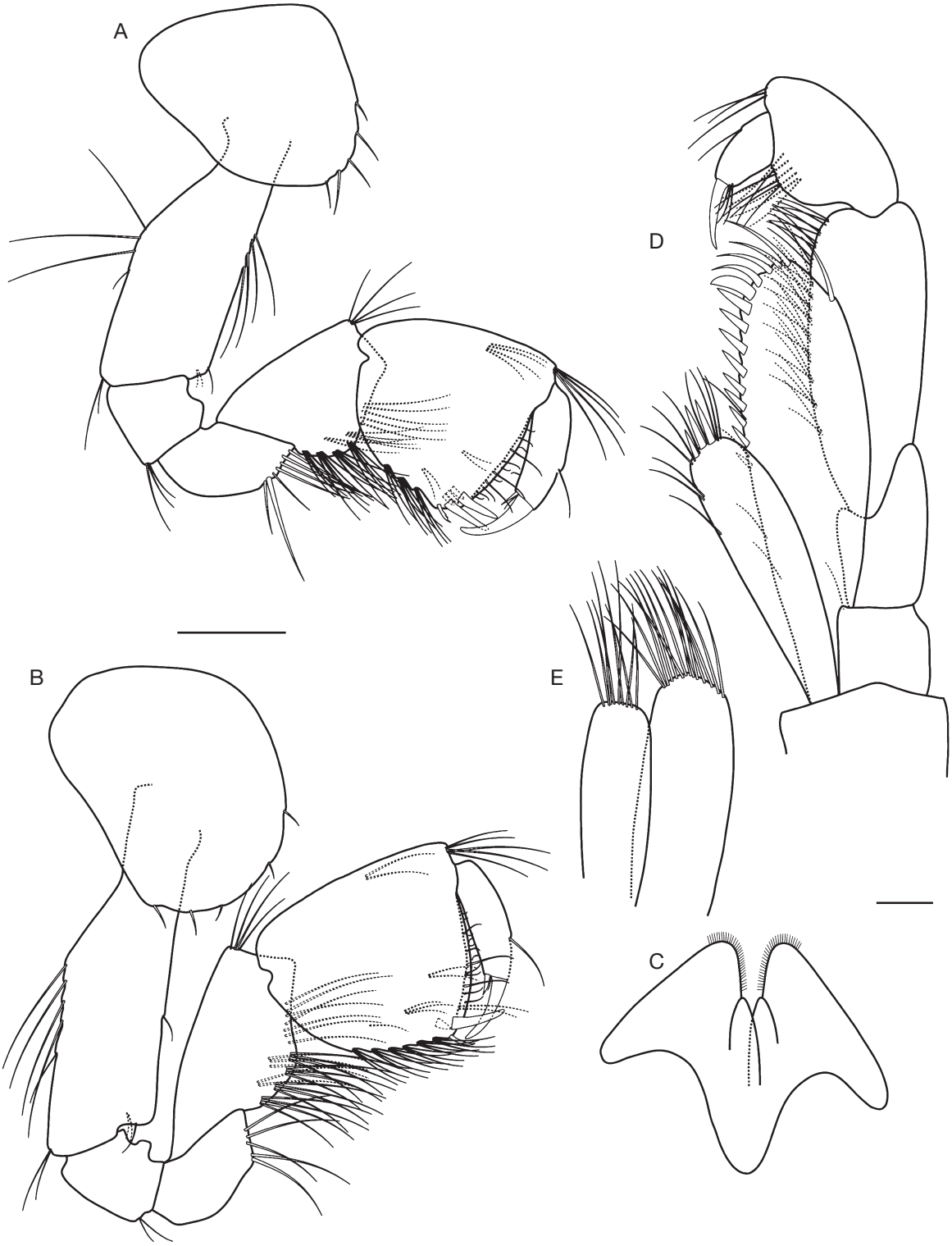

Fig. 4. — *Niphargus yasujensis* n. sp., male 7 mm (holotype, ZCRU Amph. 1053): **A**, gnathopod I; **B**, gnathopod II; **C**, labium; **D**, maxilliped; **E**, maxilla II. Scale bars: A, B, 1 mm; C-E, 0.5 mm.

Maxilla I (Fig. 3C, D). With two apical setae on distal palp article, inner plate with two apical setae, outer plate with seven long spines with 3-2-1-1-1-0-0 lateral projections; palp bi-articulated, not reaching mid-length of spines on lateral lobe.

Maxilla II (Fig. 4E). With inner lobe shorter than outer lobe; both lobes with numerous long apical setae.

Maxilliped (Fig. 4D). With three distal spines intermixed with seven distal setae on inner plate; outer plate exceeding proximal half of the palp article 2, with eleven marginal spines and three distal setae; palp article 3 at outer margin with one proximal and one distal group of long setae; palp terminal article with four setae in outer and inner margins, nail shorter than pedestal.

Gnathopod I (Fig. 4A). With rhomboid coxal plate, broader than long, with five marginal setae. Posterior and anterior margins of basis armed with single setae and groups of setae; posterior margin of ischium and merus with one posterior group of setae. Carpus length 60% of basis length and 93% of propodus length; anterior carpal margin with four antero-distal setae; carpus with posterior rows of setae on the proximo-posterior bulb and with a long row of setae along postero-medial margin. Propodus trapeozoid, slightly broader than long, anterior margin with one group of four setae in addition to four antero-distal setae. Posterior margin with three rows of setae. Palm convex, palmar corner with two strong palmar spines of equal length, three inner short accompanying spines and one outer denticulate spine. Dactylus reaching posterior margin of propodus, outer and inner margins of dactylus with one and three setae, respectively. Nail length 42% of total dactylus length.

Gnathopod II (Fig. 4B). With ovoid coxal plate wider than high, with five setae on antero-ventral margin. Basis armed with single setae along posterior and anterior margins. Ischium and merus with one posterior group of setae each. Carpus length 63% of basis length and as long as propodus length; anterior margin with single antero-distal group of setae; carpus with posterior rows of setae on the proximo-posterior bulb and with long row of setae along postero-medial margin. Propodus of gnathopod II longer than broad. Propodus of rectangular shape, anterior margin straight; palm and posterior margins slightly convex. Anterior margin with one group of two setae in addition to antero-distal group of five setae. Palmar corner with one strong long palmar spine and one short accompanying spine on inner surface, with one denticulate spine on outer surface. Dactylus reaching posterior margin of propodus, outer and inner margins with one and four setae, respectively; nail 45% of total dactylus length.

Coxae III-VII

Coxal plate III (Fig. 5A) with quadratic shape. Ventral margin with five setae. Coxal plate IV (Fig. 5B) rhomboid, depth : width ratio 1.22 : 1.00. Antero-ventro-posterior margins with six setae. Coxal plate IV posteriorly slightly concave. Coxal plates V-VI (Fig. 5C, D) with anterior lobe; anterior and posterior lobes of coxal plate V each with two marginal setae; coxal plate of VI with two and one setae on anterior and posterior lobes, respectively. Coxal plate VII (Fig. 5E) without lobe, with single seta posteriorly.

Pereopods III-IV (Fig. 5)

Pereopod III (Fig. 5A, B) longer than pereopod IV (pereopod III length: pereopod IV length ratio 1.00 : 0.95); dactyli of pereopods III-IV short, with one seta at outer margin in pereopods III-IV; nail length half of dactylus length in pereopod IV. Pereopods V-VII (Fig. 5C-E): Pereopods V:VI:VII length ratios as 1 : 1.11 : 1.12. Pereopod VII 48% of body length. Bases in pereopods V-VI with seven groups of spines along anterior margin each and with nine short setae along posterior margin each. Basis in pereopod VII with seven groups of spines along anterior margin and with eight short setae along posterior margin. Postero-ventral lobe of basis in pereopods V-VII slightly 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 propodus of V-VI, dactyli of pereopods V-VII similar to dactyli of pereopods III-IV with one seta at outer margin, robust seta at the base of nail; nail VII 28% of total dactylus length.

Pereonites

Pereonites I-VII with no setae.

Pleopods I-III (Fig. 6A-C)

Peduncle of pleopods I-III with two hooked retinacles; peduncle of pleopod III with two setae along the inner margin, rami of pleopods I-III with six to ten articles per ramus.
Pleonites I-III, each pleonite with two setae along the dorso-posterior margin.

Epimeral plates (Fig. 6G)

Epimeral plates I-III with pointed postero-ventral angles, posterior and ventral margins concave and convex, respectively. Posterior margins of plates I-III with two, three, and three setae, respectively; ventral margins of plates II-III each with three spines.

Uropods (Fig. 6D-F)

Uropod I (Fig. 6D) peduncle with five and two spines along dorso-lateral and dorso-medial margins, respectively. Outer ramus slightly longer than inner ramus (ratio as 1.11: 1.00). Inner ramus with three spines laterally and five spines and setae distally. Outer ramus with three groups of spines laterally and five spines distally. A single strong spine at the base of uropod I. Inner ramus in uropod II (Fig. 6E) slightly shorter than outer, both rami with lateral and distal long robust setae. uropod III (Fig. 6F) almost 20% of body length. Peduncle of uropod III with four distal spines. Proximal article of outer ramus with five and four groups of spines and setae along outer and inner margins, respectively. Distal article of outer ramus 30% proximal article. Distal article with marginal and distal setae. Inner ramus with one spine and seta distally and one seta laterally.

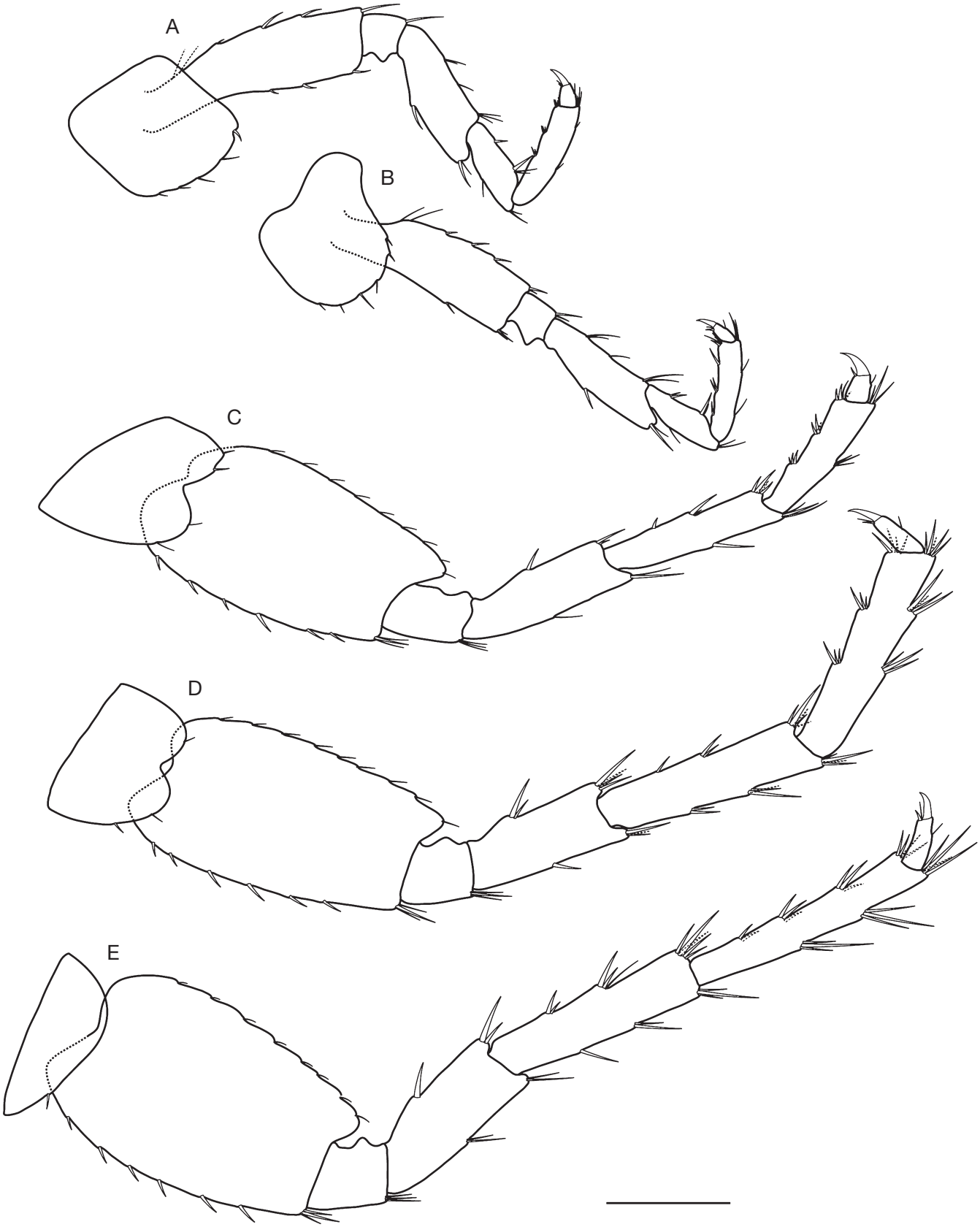

Fig. 5. — *Niphargus yasujensis* n. sp., male 7 mm (holotype, ZCRU Amph. 1053): **A**, pereopod III; **B**, pereopod IV; **C**, pereopod V; **D**, pereopod VI; **E**, pereopod VII. Scale bar: 1 mm.

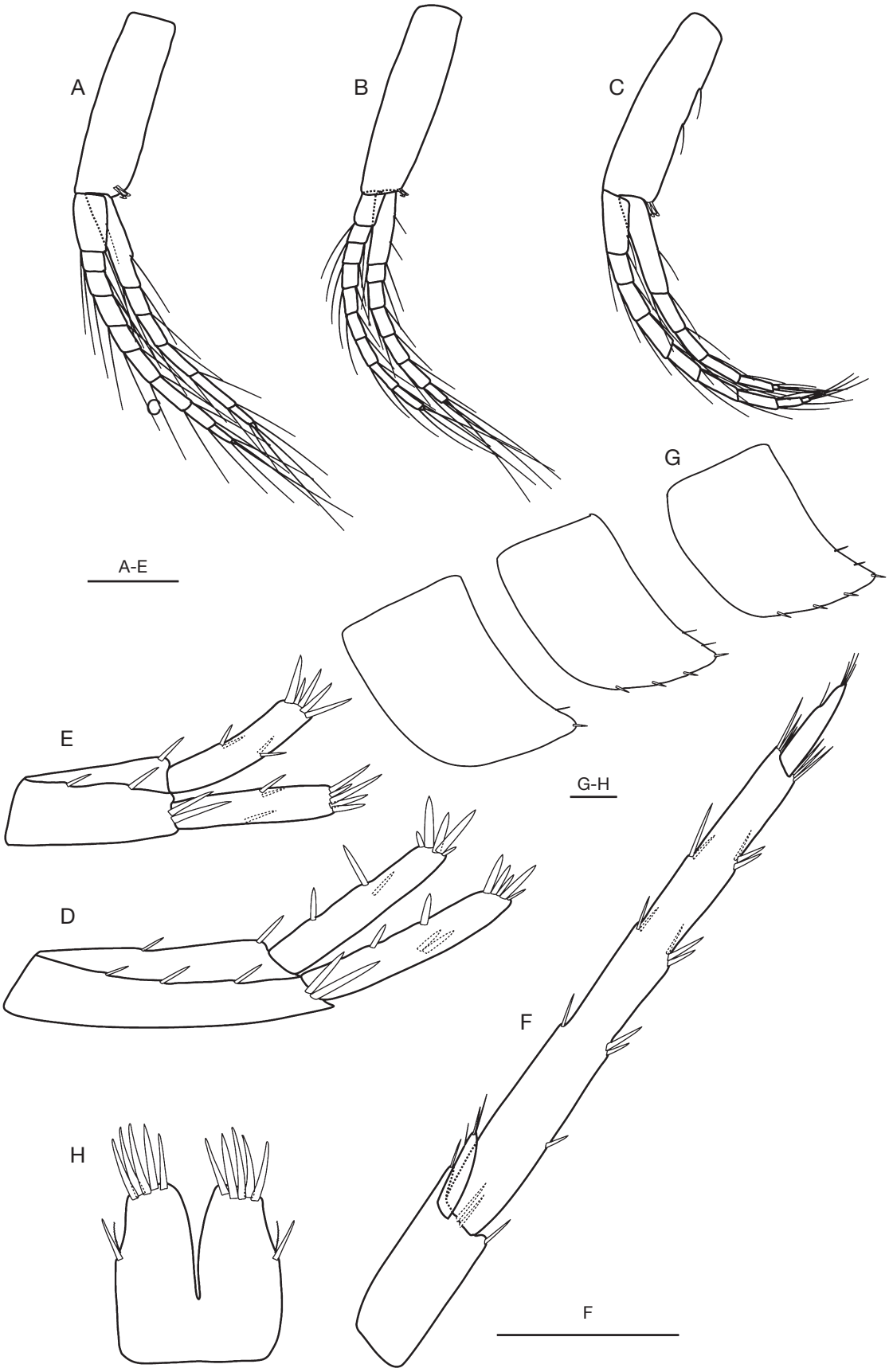

Fig. 6. — *Niphargus yasujensis* n. sp., male 7 mm (holotype, ZCRU Amph. 1053): **A**, pleopod I; **B**, pleopod II; **C**, pleopod III; **D**, uropod I; **E**, uropod II; **F**, uropod III; **G**, epimeral plates; **H**, telson. Scale bars: A-E, 1 mm; F, 2 mm; G-H, 0.5 mm.

Urosomites I-III
Urosomites I and II postero-dorso-laterally with one and three spines, respectively. Urosomites II with additional postero-dorso-lateral seta. Urosomite III without setae.

Telson (Fig. 6H)
Longer than broad, lobes slightly narrowing apically, each lobe with five distal long spines; lateral margins with one spine and one plumose seta each.

Female
Unknown.

Remarks
Niphargus yasujensis n. sp. is diagnosed exclusively by a combination of some characters including the presence of two subequal palmar spines on propodi of gnathopod I (Fig. 4A) and five apical spines in each telson lobe (Fig. 6H). Double palmar spines have been observed in *N. hosseiniei* Esmaeili-Rineh, Sari, Fišer & Bargrizaneh, 2017 from Iran which has triangular propodi of gnathopods (Esmaeili-Rineh *et al.* 2015b), in contrast to *N. yasujensis* n. sp., which has trapezoid to rectangular propodi. Also, the lengths of size carpus and propodus in gnathopod I are similar, a trait not found among Iranian species so far. An additional and potentially unique trait among Iranian species are the five apical spines on the telson lobe in *Niphargus yasujensis* n. sp. (Fig. 6H). Elevated number of spines per telson lobe has been noted in Europe (e.g. *N. podogoricensis* S. Karaman, 1934; *N. kusceri* S. Karaman, 1950) but not in combination with double palmar spines. By contrast, most of Iranian species have three distal spines per telson lobe. To our knowledge only *N. daniali* Esmaeili-Rineh & Sari 2013 and *N. kurdistanensis* Mamaghani-Shishvan, Esmaeili-Rineh & Fišer 2017 have four distal spines per lobe.

Niphargus nasrullahi n. sp.
(Figs 7-10)

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Material examined. — **Holotype. Iran** • ♂ (10 mm); Nahr Spring, Kakan Village, Boyer-Ahmad City, Kohgiloyeh and Boyer-Ahmad Province; 30°40'N, 51°43'E; Zeinab Bargrizaneh leg.; 28.VII.2015; ZCRU Amph.1055.
Paratypes. Iran • 2 ♂; same data as for holotype; ZCRU Amph.1055.

Diagnosis. — A mid-sized *Niphargus* with slender body. Epimeral plates II-III slightly inclined, posterior and ventral margins concave and convex, respectively; ventro-posterodistal corner distinct but not produced (Fig. 10G). Antenna I shorter than half of total body length. Article 5 of antenna II longer than article 4. Maxilla I outer lobe with seven strong spines, each with more than 3 subapical denticles. Palpus of maxilla I (Fig. 7C) short and not reaching the tip of outer lobe. Gnathopods with large propodi of trapezoid to triangular shape (Fig. 8A, B). Pereopods half of the total body length. Length of pereopods V and VI (Fig. 9C, D) nearly equal. Pleonites with one seta along the dorso-posterior margin of each segment and with one strong spine at base of uropod I. Urosomite I postero-dorso-laterally with two spines; urosomite II postero-dorso-laterally with two spines and one seta. Inner ramus of uropod I shorter than outer ramus (Fig. 10D).
Female unknown.

Diagnostic Coi Sequence. — GeneBank Accession Number: MT636107.

Etymology. — The name "nasrullahi" was chosen in honour of the Iranian zoologist Prof. Nasrullah Rastegar-Pouyani, Razi University, who dedicated his research to herpetology in Iran.

Geographic Distribution. — Apart from the type locality, the species was found also in two other localities along Zagros Mountains: Chonarestan Spring and Khong Spring (Fig. 1). The geographic distance between Chonarestan and Biareh Springs, Chonarestan and Khong Springs and Khong and Biareh Springs is 32.2 km, 15.5 km and 40.6 km, respectively.

Variability. — The individuals from Chonarestan and Khong springs have inner ramus of uropod I longer than outer ramus (1.07 : 1 : 00).

Description of holotype
Measurements
Total male body length 10 mm. Head length equal to 10% of total body length.

Antennae I (Fig. 7A)
43% of total body length. Peduncular articles 1-3 progressively shorter; length of peduncular article 2 more than half of peduncular article 3; most flagellum articles with one short aesthetasc. Main flagellum with 19 articles, most of which with short setae. Accessory flagellum bi-articulated, reaching beyond one-third of article 4 of main flagellum; second article with two setae.

Antennae II (Fig. 7B)
Roughly half of the length of antenna I (length ratio of antenna I : II equal to 1 : 0.53). Ratio of peduncular articles 4 : 5 length equal to 1.00 : 1.20; peduncle articles 4 and 5 with ten and thirteen groups of setae, respectively. Flagellum 70% of the length of peduncle articles 4 and 5, counting nine articles.

Mouth parts (Figs 7-8)
Labium (Fig. 8D). Bilobate; with inner developed lobes.

Left mandible (Fig. 7E). With five teeth on incisor process, with four large teeth on lacinia mobilis and a row of six serrated setae.

Right mandible (Fig. 7F). With four teeth on incisor process, lacinia mobilis pluri-toothed, with a row of seven serrated setae.

Mandibular palp (Fig. 7G). Ratio of mandibular palp articles 1 : 2 : 3 as 1 : 1.85 : 2.09. Proximal article without setae; middle article with thirteen setae medially; distal article with one A-group of three setae, three groups of B-setae, no C-setae, 23 D-setae and five E-setae.

Maxilla I (Fig. 7C, D). With two apical setae on distal palp articles. Inner plate with two apical setae, outer plate with seven long spines with 4-4-3-4-3-4 and more than five lateral

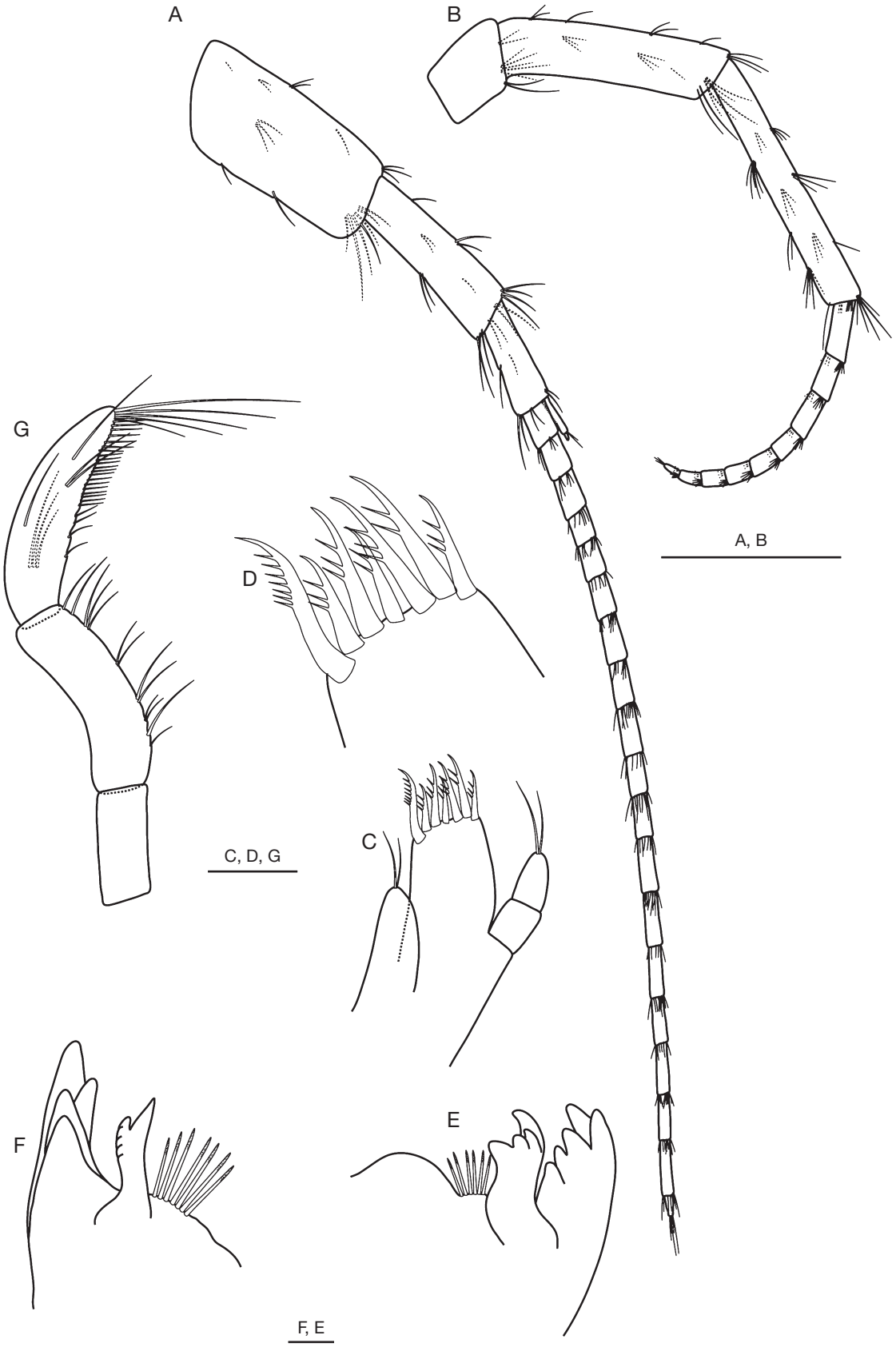

Fig. 7. — *Niphargus nasrullahi* n. sp., male 10 mm (holotype, ZCRU Amph. 1055): **A**, antenna I; **B**, antenna II; **C**, **D**, maxilla I; **E**, left mandible; **F**, right mandible; **G**, mandibular palp. Scale bars: A, B, 1 mm; C, D, G, 0.5 mm; E, F, 0.25 mm.

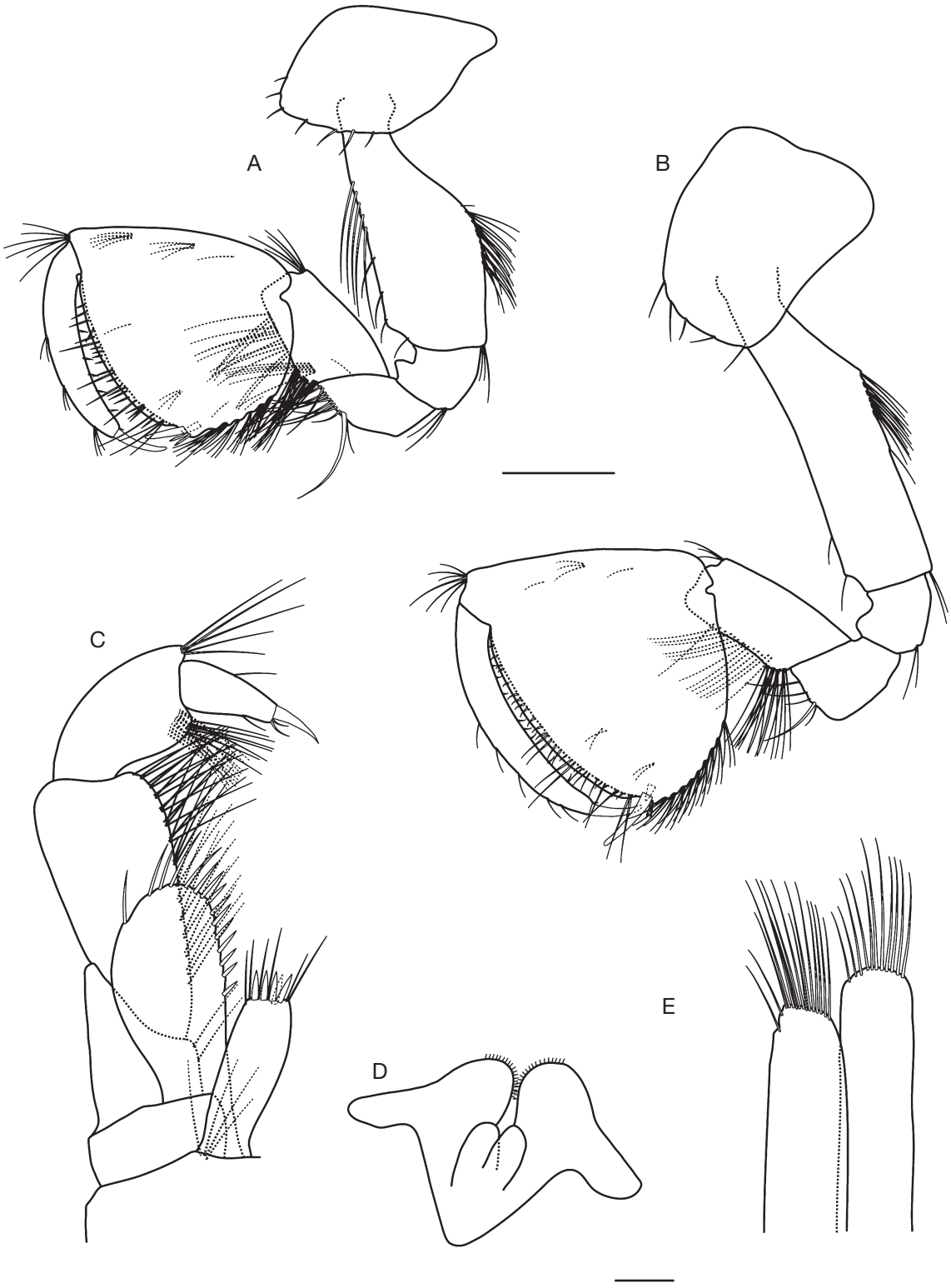

Fig. 8. — *Niphargus nasrullahi* n. sp., male 10 mm (holotype, ZCRU Amph. 1055): **A**, gnathopod I; **B**, gnathopod II; **C**, maxilliped; **D**, labium; **E**, maxilla II. Scale bars: A, B, 1 mm; C-E, 0.5 mm.

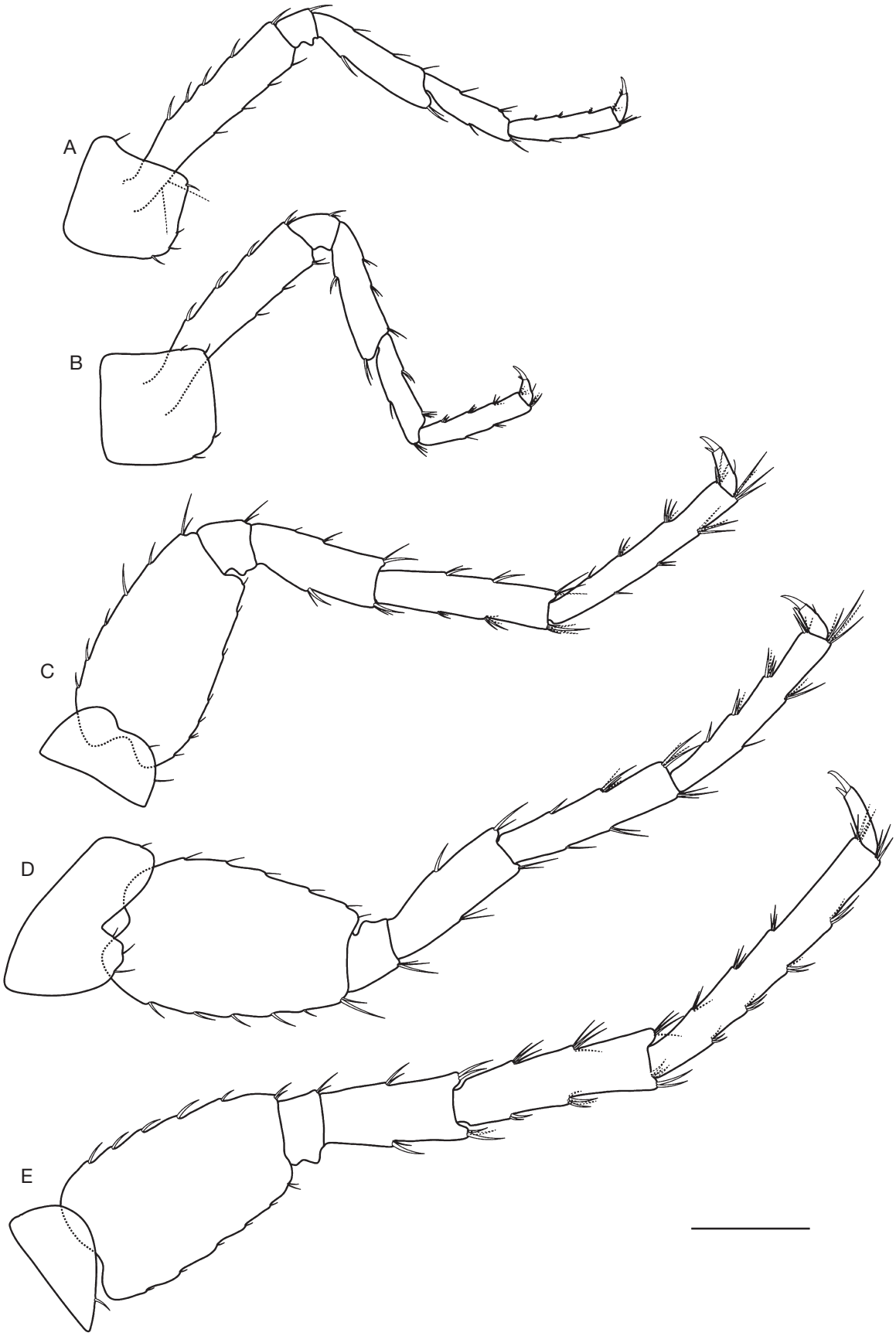

Fig. 9. — *Niphargus nasrullahi* n. sp., male 10 mm (holotype, ZCRU Amph. 1055): **A**, pereopod III; **B**, pereopod IV; **C**, pereopod V; **D**, pereopod VI; **E**, pereopod VII. Scale bar: 1 mm.

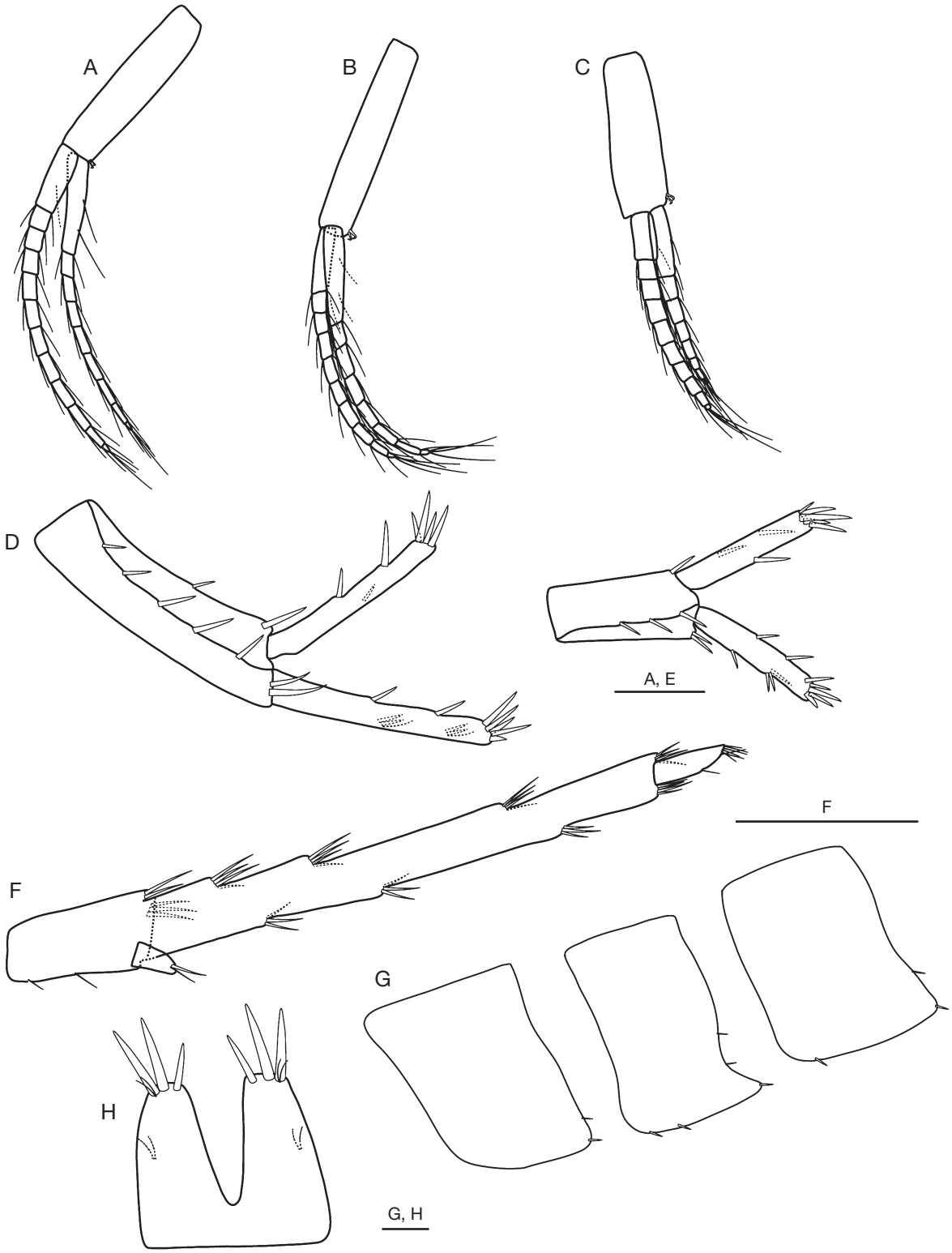

Fig. 10. — *Niphargus nasrullahi* n. sp., male 10 mm (holotype, ZCRU Amph. 1055): **A**, pleopod I; **B**, pleopod II; **C**, pleopod III; **D**, uropod I; **E**, uropod II; **F**, uropod III; **G**, epimeral plates; **H**, telson. Scale bars: A-E, 1 mm; G-H, 0.5 mm; F, 2 mm.

projections; palp bi-articulated, not reaching mid-length of spines on lateral lobe.

Maxilla II (**Fig. 8E**). With inner lobe shorter than outer lobe; both lobes with numerous long apical setae.

Maxilliped (**Fig. 8C**). Inner plate with five distal spines intermixed with six distal setae; outer plate not exceeding proximal half of the palp article 2, with ten marginal spines and five distal setae; palp article 3 at outer margin with one proximal and one distal group of long setae; palp terminal article with one seta in outer and inner margins, nail shorter than pedestal.

Gnathopod I. Coxal plate of gnathopod I (Fig. 8A) quadratic, with seven marginal setae. Posterior margin of basis with setae in groups; anterior margin with single setae; posterior margin of ischium and merus with one posterior group of setae. Carpus length 59% of basis length and 69% of propodus length; anterior carpal margin with antero-distal group of setae; carpus with posterior rows of setae on the proximo-posterior bulb and with a long row of setae along postero-medial margin. Propodus trapezoid, broader than long, anterior margin with three groups of ten setae in addition to six antero-distal setae. Posterior margin with six rows of setae. Palm convex, palmar corner with one strong palmar spine, one inner short accompanying spine and two outer denticulate spines. Dactylus reaching posterior margin of propodus, outer and inner margins of dactylus with three groups of setae and five individual setae, respectively. Nail length 22% of total dactylus length.

Gnathopod II (**Fig. 8B**). With rectangular coxal plate wider than high. Ventral and anterior margins with five setae. Basis with single setae along anterior margin and with setae in groups along posterior margin. Ischium and merus with one posterior group of setae each. Carpus length equal to 50% of basis length and 70% of propodus length; anterior margin with single antero-distal group of setae; carpus with posterior rows of setae on the proximo-posterior bulb and with a row of setae along postero-medial margin. Propodus of gnathopod II broader than long. Propodus of trapezoid, almost almond shape: anterior margin straight; palm and posterior margins slightly convex and palmar angle inclined. Anterior margin with three groups of totally five setae in addition to antero-distal group of six setae. Palmar corner with one strong long palmar spine, one short accompanying spine on inner surface and with two denticulated spines on outer surface. Dactylus reaching posterior margin of propodus, both, outer and inner margins each with a row of five setae; nail short, 20% of total dactylus length.

Coxae III-VII (Fig. 9A-E).
Coxal plates III and IV quadratic. Antero-ventro-posterior margins with five setae on coxa III and with four setae on coxa IV. The latter shallowly concave posteriorly, approximately 10% of coxa width. Coxal plates V-VI with anterior lobe; anterior lobes V-VI with zero and three marginal setae, respectively; posterior lobes V-VI with two and one marginal setae, respectively. Coxa VII without lobe, with single seta posteriorly.

Pereopods III - IV (Fig. 9)
Pereopods III : IV length ratios as 1.08 : 1; dactyli of pereopods III-IV short, with small spine at the base of the nail and one seta of outer margin at pereopod IV; length of nail IV 38% of total dactylus IV length. Pereopods V : VI : VII length ratios as 1 : 1.05 : 1.11. Pereopod VII 44% of body length. Bases V-VII with six groups of spines along anterior margins each and with seven, six and six short setae along posterior margins, respectively. Postero-ventral lobe of basis of pereopods V-VII weakly 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 propodi of V and VI, dactyli of pereopods V-VI similar to dactyli of pereopods III with one seta at outer margin and with spine at the base of nail; nail VII 25% of total dactylus length.

Pereonites
Pereonites I-VI without setae. Pereonite VII with one postero-ventral seta.

Pleopods (Fig. 10A-C)
Peduncle of pleopods I-III with two hooked retinacles; rami of pleopods I-III with eight to twelve articles per ramus. Pleonites I-III, each pleonite with one seta along the dorso-posterior margin.

Epimeral plates (Fig. 10G)
Epimeral plates I-III with angular postero-ventral corner, posterior and ventral margins concave and convex, respectively. Posterior margins of plates I-III with two, three, and two setae, respectively; ventral margins of plates II-III with two and one spine, respectively.

Uropods (Fig. 10D-F)
Uropod I (Fig. 10D) peduncle with seven and two spines along dorso-lateral and dorso-medial margins, respectively. Outer ramus shorter than inner ramus (ratio as 1.00 : 1.15). Inner ramus with three spines laterally and four spines distally. Outer ramus with four groups of spines and setae laterally and five spines distally. A single strong spine at the base of uropod I. Inner ramus in uropod II (Fig. 10E) slightly longer than outer, both rami with lateral and distal long spines. Uropod III (Fig. 10F) almost 31% of body length. Peduncle of uropod III with five spines distally and with two setae laterally. Proximal article of outer ramus with four groups of spines and plumose setae along of outer and inner margins each. Distal article of outer ramus short, proximal : distal ratio as 6.0 : 1. Distal article with marginal and distal setae. Inner ramus with one spine and one seta distally.

Urosomites I-III
Urosomite I postero-dorso-laterally with two spines, urosomite II postero-dorso-laterally with two spines and one seta, urosomite III without setae.

Telson (Fig. 10H)
As long as broad, lobes slightly narrowing apically, each lobe with three distal long spines; lateral margins with pairs of plumose setae each.

Female
Unknown.

Remarks
Niphargus nasrullahi n. sp. is difficult to diagnose and we suggest it is best characterized by its diagnostic COI and 28S sequences. The new species is lacking unique morphological traits or at least a unique combination of distinct morphological traits. The shape of gnathopods, coxal plates, appendages, telson and setal patterns on the body are not distinctive and can be observed in other species of the genus. The newly described species most closely resembles the Iranian species *N. darvishi* Esmaeili-Rineh, Sari & Fišer, 2015. The two species share some characters including the short maxillary palpus, not reaching the tip of the outer lobe of maxilla I, multi-denticulated spines on maxilla I, shape of propodi in gnathopods and the number of apical spines on each telson lobe. However, *N. nasrullahi* n. sp. differs from *N. darvishi* in the lack of pectinate dactyli of pereopods III-VII (only one spine in *N. nasrullahi* n. sp.; Fig. 9A-E) and setae in groups on gnathopod I dactylus (single setae in *N. darvishi*; Fig. 8A, B). Moreover, *N. nasrullahi* n. sp. resembles both sister species (*N. borisi* and *N. yasujensis* n. sp.) by short palpus on maxilla I (Fig. 7C), however it clearly differs from both species by larger and differently shaped propodi of gnathopods I-II (Fig. A, B).

DISCUSSION

Both molecular and morphological data suggest that we discovered two species new to Science. Clearly, the species inventory of *Niphargus* from Iran is not yet complete: nearly all field expeditions to new sites unveil new species. Given the high diversity of *Niphargus* in karstic massifs of Western Balkans (Bregović *et al.* 2019), the observed high diversity in karstic Zagros Mountains, which parallels to Western Balkans in size, is not a surprise. Given that most of subterranean aquatic species have small ranges, at times nearly point distributions (Mamaghani-Shishvan & Esmaeili-Rineh 2019), we hypothesize that the exploration of Iranian *Niphargus* has just begun and that many more species still await formal description.

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