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NIPHARGUS SARII SP. N., A NEW SUBTERRANEAN NIPHARGID (CRUSTACEA: AMPHIPODA) FROM IRAN BASED ON MOLECULAR AND MORPHOLOGICAL CHARACTERS

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Niphargus sarii sp. n. was collected from Jo-Khanem Spring in Ilam Province. This species hypothesis is based on the analysis of morphological characters and 28S ribosomal DNA sequences. In this paper, we describe the morphological traits of this new species. Then, its taxonomic status within the genus is discussed in comparison to the 15 known Iranian species. Results revealed that *N. sarii* sp. n. is phylogenetically close to *N. sohrevardensis*. This species is easily distinguished from other Iranian species by some characters, in particular the equal length of rami in uropod I, lack of lateral robust setae on telson and the situation of dactylus to posterior margin of propodus in gnathopod II.

Keywords: *Niphargus*, Jo-Khanem Spring, Zagros Mountains, 28SrDNA, morphological characters, Iran.

INTRODUCTION

The Zagros Mountains range has a total length of 1600 km from north-east Iraq to the Strait of Hormuz and its elevation gradually declines toward the Persian Gulf (Raeisi 2004). This region is composed mainly of the karstic carbonate formations, and its karstic aquifers are among the most important karst reservoirs in the western part of Iran (Raeisi & Stevanovic 2010). Due to the wide geographical range and geological conditions coupled with the climatologically diverse environments, the Zagros Mountains range provides a great diversity of species in aquatic and terrestrial habitat types (Smith 1953, Sargeran *et al.* 2008, Akmali *et al.* 2011, Esmaeili-Rineh *et al.* 2016*a*, Esmaeili-Rineh *et al.* 2016*b*, Afroosheh *et al.* 2016, Fathipour *et al.* 2016, Shahabi *et al.* 2017, Mamaghani-Shishvan *et al.* 2017), and it is considered as a centre for the origin of numerous species (Coad & Vilenkin 2004).

The survey of species diversity in freshwater habitats indicates that large numbers of animal species are endemic in this part including the members of the genus *Niphargus* Schiödte, 1849 (Niphargidae, Amphipoda). These blind and depigmented animals normally live in subterranean freshwater ecosystems, and only a few niphargids of them occur in the surface waters (Sket 1981). They are distributed across most of Europe, and few species are known from the Middle East (Fišer 2012). Many of the 330 described species are known only from their type localities. According to CHRISTMAN *et al.* (2005), endemism or the restriction of taxa to a particular geographic area is very high in subterranean fauna due to the poor migratory capabilities.

So far, 16 species were described from Elburz (2 species) and Zagros Mountains ranges (14 species) in Iran. The described species have been found in the karstic areas including three from caves and the rest of the springs, but there are also species that are found in both cave and spring waters (e.g. *N. hosseiniei*). The most of them are endemic with narrow range. In contrast, some species such as *N. khwarizmi* have wide range, the distance between the two populations of this species is about 570 km (ESMAEILI-RINEH *et al.* 2015*a*). In this paper, we present results of a recent survey on niphargid fauna from Ilam Province and describe a new endemic species of this genus.

MATERIAL AND METHODS

Study area

The specimens were collected using a small hand net in Jo-Khanem Spring (33°19′N, 46°40′E) close to Chardavol City in Ilam Province (Fig. 1).The Ilam Province is located in the western and southwestern regions of the Iranian Plateau between 31°58′ to 34°15′ N and 45°24′ to 48°10′ E. Altitude ranges from 50 m a.s.l. in the south to 3062 m in the Kabir-

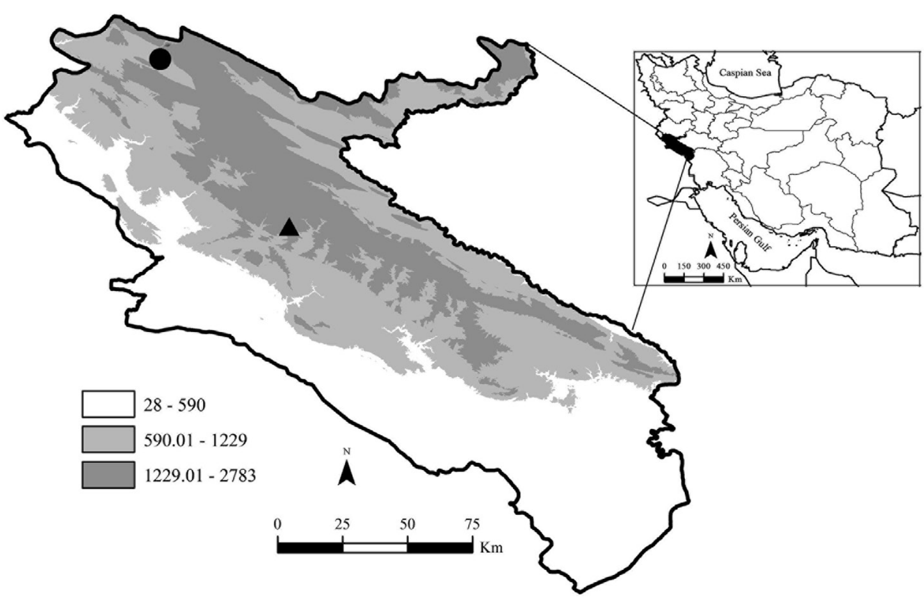

Fig. 1. Marks on the map are representing localities of (•) *N. ilamensis* and the newly described species (▲) *N. sarii* sp. n.

Kouh Mountain (to the east of the province). So far, *N. ilamensis* was found from this region in north part of Ilam Province.

Morphological and morphometric studies

Characters of individual specimens were measured according to Fišer *et al.* (2009) and then mounted on slides in a Euparal medium. Digital photos were taken with an Olympus LABOMED iVu 7000 camera fitted on an LABOMED Lx500 stereomicroscope. Measurements and counts were made using the computer program ProgRes CapturePro 2.7. The specimens used for the present study are deposited in the Zoological Collection of Razi University (ZCRU).

Phylogenetic analyses and molecular divergence

We extracted total genomic DNA from a part of the animal using Tissue Kits (GenNetBio™), following the manufacturer's instructions (Seoul, South Korea). A 810 bp of the first fragment of 28S ribosomal DNA were amplified and sequencing using the forward primer from Verovnik *et al.* (2005) and the reverse primer from Zakšek *et al.* (2007). Polymerase chain reactions (PCRs) in a final volume of 25 μl contained optimized amounts of PCR water, 12.5 μl of Master Mix kit (Sinaclon, Iran), 0.2 μl of each primer (10μM), and 50–100 ng of genomic DNA template. PCR cycling settings were as follows: initial denaturation of 94°C for 7 minutes, 35 subsequent cycles of 94°C for 45 seconds, 55°C for 30 seconds and 72°C for 1 minute, and a final extension of 72°C for 7 minutes. Purification of PCR products and sequencing were commercially performed by Macrogen Inc. (Korea).

In order to figure out the phylogenetic position of the new species, we analysed the acquired sequences within the data set of Esmaeili-Rineh *et al.* (2015*a*, 2017*a*) (Table 1, all samples included in analyses). All the sequences were edited and aligned using Clustal W (Thompson *et al.* 1994), as implemented in the Bioedit program sequence alignment editor (Hall 1999) using the default settings.

Phylogenetic reconstruction was performed using the Bayesian inferences in MRBAYES, version 3.1.2 (Ronquist & Huelsenbeck 2003) and Maximum likelihood (ML) with PHYML, version 3.0 software (Guidon & Gascuel 2003). The appropriate model for BI and ML analysis was selected with jModelTest, version 0.1.1 (Posada 2008) using Akaike Information Criterion. The best fit model identified by AIC for phylogenetic reconstruction was GTR+I+G. Bayesian analyses were run for 5 million generations, with four chains, and trees sampled every 1000 generations. The first 1250 sampled trees were discarded as burn-in, and subsequent tree likelihoods were checked for convergence in Tracer 1.5.0 (Rambaut & Drummond *et al.* 2012). A fifty percent majority rule consensus tree was computed using the remaining trees and visualized by FigTree v1.4.0 software. In ML analysis a starting tree was obtained by BIONJ and nodal support was estimated from 1000 bootstrap replicates.

To assess interspecific divergence between the Iranian species of *Niphargus*, we calculated the genetic distances corrected with Kimura two-parameter (K2P) model (Kimura 1980) as implemented in MEGA ver. 5 (Tamura *et al.* 2011) and Patristic distances from a maximum likelihood (ML) tree as described in Fourment and Gibbs (2006) in the PATRISTIC v1.0 program. Patristic distances have been broadly used in *Niphargus* taxonomy so far; as such warrant compatibility of the results across different studies (Léfebure *et al.* 2006, Meleg *et al.* 2013).

Table 1. List of taxa used in the analyses and their geographic origin. Newly obtained sequences are written in bold.

Taxon	Acc. no. 28S	Origin of samples
N. alisadri Esmaeili-Rineh et Sari, 2013	KF581049	Alisadr Cave, Hamedan Prov., Iran
N. aquilex Schiödte, 1855	EF617264	North German Plain, Weser, Hessisch Oldendorf, Germany
N. arbiter G. Karaman, 1984	EF617287	Tounjčica Cave, Ogulin, Croatia
N. carniolicus Sket, 1960	EF617252	Lukenjska jama, Prečna, Slovenia
N. costozzae Schellenberg, 1935	EU693294	Grotta della Guerra Cave, Berici Mt., Italy
N. dalmatinus Shäfferna, 1922	EF617296	Vrana Spring, Zadar, Herzegovina.
N. daniali Esmaeili-Rineh et Sari, 2013	KF581033	Danial Cave, Mazandaran Prov., Iran
N. dimorphus Birstein, 1961	KF719273	Vodnjak v vrtnariji, Zarečnoe, ESE, Simferopol', Krym, Ukraine
N. dolichopus Fišer *et al.*, 2006	EU693297	Suvaja pećina, Lušci polje, Sanski most, Bosnia and Herzegovina
N. fontanus Bate, 1859	EF617265	Little stour, Kent, VB, Belgium
N. gabrovceci S. Karaman, 1952	EU693299	spring near village Gabrovčec, Krka, Slovenia
N. zagrebensis cf. gadina	EF617295	Žopenca-Gadina cave, Kočevje, Črnomelj, Slovenia
N. grandii Ruffo, 1937	EU693300	streamTorre, N of Ruda, Italy
N. hadzii Rejic, 1956	EU693301	»Pod orehom« spring, Verd, Vrhnika, Ljubljana, Slovenia
Niphargus hvarensis S. Karaman, 1952	EU693303	spring at the church, Slano, Coratia
N. illidzensis Schäferna, 1922	EU693304	spring of river Bosna, Ilidža, Sarajevo, Bosnia and Herzegovina
N. karamani Schellenberg, 1935	EU693305	well, Fram 119, Maribor, Slovenia
N. khayyami Hekmatara *et al.*, 2013	JX535353	Ghori- Ghale Cave, Ravansar-Paveh road, Kermanshah Prov., Iran
N. khwarizmi Hekmatara *et al.*, 2013	KF581056	Kahriz Spring, Khoranagh, E Azarbayejan, Iran
N. krameri Schellenberg, 1935	EF617274	subsidiary stream of river Fojba, Pazin, Croatia
N. lessiniensis Stoch, 1998	EF617300	Grotta dell Aqua, Ponte de Veja, Monti Lessini,Verona, Italy
N. longicaudatus cf. cres	EF617240	Retec spring, Lubenice, island of Cres, Bosnia and Herzegovina
N. longicaudatus Costa, 1951	EF617241	spring at the road, between Monte Faito and Vico Equense, Napoli, Italy
N.novomestanus S. Karaman, 1952	EU693314	Tominčev studenec spring, Dvor, Žužemberk, Slovenia
N. orcinus Joseph, 1869	EU693315	Križna jama, Lož, Slovenia
N. pasquinii Vigna-Taglianti, 1966	EF617244	Sorgenti di S spring. Vittorino, Rieti, Lazio, Italy
N. polymorphus Fišer *et al.*, 2006	EF617282	Obodska pećina cave, Rijeka Crnojevića, Montenegro

Table 1 (continued)

Taxon	Acc. no. 28S	Origin of samples
N. puteanus Koch, 1836	EF617302	spring at the guesthouse Zur Walba, Regensburg, Germany
N. rejici Sket, 1958	EF617283	spring at lake Podpeško jezero, Ljubljana, Slovenia
N. sanctinaumi S. Karaman, 1943	EU693320	Naum spring, Ohrid, Macedonia
N. schellenbergi S. Karaman, 1932	EU693321	Hessen, Kammerbacher Hohle, Germany
N. spinulifemur S. Karaman, 1954	EU693323	brook NE from village Hrastovlje, Slovenia
N. spoeckeri Schellenberg, 1933	EU693324	Pivka jama, Postojna, Slovenia
N. stygius Shiödte, 1847	EU693325	Pred jama, Postojna, Slovenia
N. subtypicus Sket, 1960	EU693326	Lukenjska jama, Prečna, Novo mesto, Slovenia
N. timavi S. Karaman, 1954	EU693327	Grotte di Trebiciano (Labodnica), Trst, Italy
N. trullipes Sket, 1958	EF617281	Vjetrenica cave, Zavala, Bosnia and Herzegovina
N. vadimi Birstein, 1961	KF719275	Skel'skaja peščera, Rodnikovo, Krym, Ukraine
N. valvasori S. Karaman, 1952	EU693328	Križna jama, Lož, Slovenia
N. vinodolensis Fišer *et al.*, 2006	EF61729	Stream below the bridge, Cerovići, Vinodol, Bosnia and Herzegovina
N. vjeternicensis S. Karaman, 1932	EU693329	Vjetrenica Cave, Zavala, Bosnia and Herzegovina
N. hosseiniei Esmaeili-Rineh *et al.*, 2017	KF581054	Brolan Spring, West Azarbayejan, Iran
N. hosseiniei Esmaeili-Rineh *et al.*, 2017	KF581055	Nojivaran Spring, Kermanshah Prov., Iran
N. hosseiniei Esmaeili-Rineh *et al.*, 2017	KF581036 KF581047	Tir-e-Bagh Spring, Fars Prov., Iran
N. ilamensis Esmaeili-Rineh *et al.*, 2017	KF581039	Sarab-e-Moord, Ilam Prov., Iran
N. ilamensis Esmaeili-Rineh *et al.*, 2017	KF581038	Sarab-e-Kanipahn, Ilam Prov., Iran
N. sohrevardensis Esmaeili-Rineh *et al.*, 2017	KF581035	Sohrevard Spring, Zanjan Prov., Iran
N. bisitunicus Esmaeili-Rineh *et al.*, 2015	KF581050	Sarab-e-Bisitun, Kermanshah Prov., Iran
N. khayyami Hekmatara *et al.*, 2013	KF581058	Ghori- Ghale Cave, Kermanshah Prov., Iran
N. darvishi Esmaeili-Rineh *et al.*, 2015	KF581043	Dimeh Spring, Chaharmahal- va- Bakhtiari Prov., Iran
N. sharifii Esmaeili-Rineh *et al.*, 2015	KF581048	Sarab-e-Robat, Lorestan Prov., Iran
N. khwarizmi Hekmatara *et al.*, 2013	KF581057 KF581051	Sarab-e-Niaz, Lorestan Prov., Iran
N. sohrevardensis Esmaeili-Rineh *et al.*, 2017	KF581034	Razbashi Spring, Lorestan Prov., Iran
N. sharifii Esmaeili-Rineh *et al.*, 2015	KF581037	Gahroo Spring, Chaharmahal- va- Bakhtiari Prov., Iran
N. borisi Esmaeili-Rineh *et al.*, 2015	KF581044	Belqais Spring, Kohgiloyeh- va- Boyerahmad Prov., Iran

Table 1 (continued)

Taxon	Acc. no. 28S	Origin of samples
N. kermanshahi Esmaeili-Rineh *et al.*, 2016	KX232639 KX232640	Kangarshah Spring close to Sahneh City, Kermanshah Prov., Iran
N. sariei this study	–	Jo-Khanem Spring close to Chardavol City, Ilam Prov., Iran
N. hakani Esmaeili-Rineh *et al.*, 2017	KY629001 KY629002	Kheder-Goli spring, Razan city, Hamedan Prov., Iran
N. darvishi Esmaeili-Rineh *et al.*, 2015	KF581042 KF581071	Gholam-Abad Spring, Chaharmahal- va-Bakhtiari Prov., Iran
Niphargus sp.	KF581059 KF581081	Magharit cave, L-Mascat, Ras Chekka, Bat-roun, Lebanon
Gammarus fossarum Koch, 1935	EF617235 KF719241	stream near Dept. Biology, Ljubljana city, Slovenia
Pontogammarus crassus G. O. Sars, 1894	KF719277 KF719242	L. Razim, Jurilovka, Babadag, Italy
Synurella ambulans Müller, 1846	EF617236 KF719240	Biološko središče, Ljubljana, Slovenia

RESULTS

Niphargus sarii sp. n.
(Figs 2–5)

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Holotype: male specimen (11.5 mm) from Jo-Khanem Spring, Chardavol City, Ilam Province, Iran; (33° 19'N, 46° 40'E). Specimens were collected by A.M. Niakan in May 2014. Holotype with two paratypes is stored under catalogue number ZCRU Amph.1001 in the Zoological Collection of Razi University, Iran (ZCRU).

Diagnosis – The rami in uropod I have equal length. The urosomites I to II bear dorso-laterally one and two robust setae accompanied with one simple seta, respectively. The propods of gnathopods I to II are broader than long. The dactylus of gnathopod II is not reaching to the posterior margin of propodus.

Description of holotype – Total length of specimen 11.5 mm. Body strong and stout. Head length 13% of body length (Fig. 2). Antennae I (Fig. 2) 0.47 of body length. Peduncular articles 1–3 progressively shorter; peduncular articles 2: 3 ratio 2: 1; main flagellum with 37 articles (most of which with short setae); accessory flagellum biarticulated and reaching 1/2 of article 4 of main flagellum, flagellar articles 1 and 2 with two and three simple setae, respectively (Fig. 2). Length ratio of antenna I : II as 1 : 0.45. Peduncular article 4 as long as article 5, with nine and eight groups of simple setae, respectively; flagellum with 13 articles. Length of flagellum: length of peduncle article 4 + 5 as 0.56 : 1 (Fig. 2).

Labium (Fig. 3) with inner lobes and setae on tip of lobes. Inner plate of maxilla I with four long simple setae; outer plate with seven long robust setae with 1-0-2-0-2-5-0 apical lateral projections; palp biarticulated, longer than outer lobe, with three long distal simple setae (Fig. 2). Both plates of maxilla II with numerous distal simple setae, with four lateral simple setae (Fig. 3). Incisor in left mandible with five teeth, lacinia mobilis with four teeth; six setae with lateral projections between lacinia and triturative molar (Fig. 2). Incisor in right mandible with four teeth, lacinia mobilis pluritooth; seven setae with lateral projec-

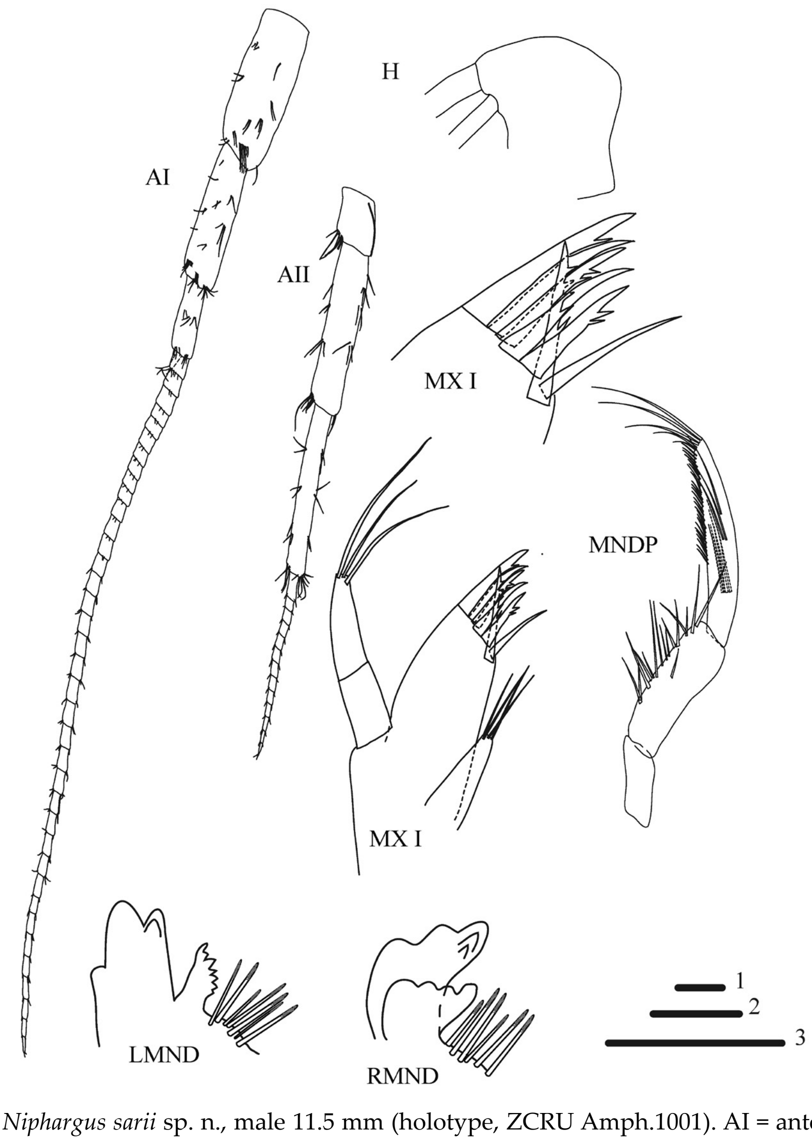

Fig. 2. *Niphargus sarii* sp. n., male 11.5 mm (holotype, ZCRU Amph.1001). AI = antenna I, AII = antenna II, H = head, MX I = maxilla I and outer plate of maxilla I, MNDP = mandibular palp, LMND = left mandible, RMND = right mandible. Scale bars: 1 = 0.25 mm (LMND, RMND), 2 = 0.5 mm (H, MX I, MNDP), 3 = 1mm (AI–AII)

tions between lacinia and triturative molar (Fig. 2). Mandibular palp articles ratios 1 : 2 : 3 as 1 : 1.58 : 2.41. The proximal article has no setae, the second article with 16 setae along inner margin and the third article with one group of four A-setae, two groups of B-setae, no C-setae, 25 D-setae and four E-setae (Fig. 2).

Maxilliped with short inner plate on which are three distal robust setae intermixed with six distal simple setae; outer plate exceeding the proximal half of the posterior margin of palp article 2, with 12 robust setae along inner margin and four simple setae distally. Palp article 3 of maxilliped with one proximal, inner and outer group of long simple setae at outer margin; terminal article of palp with one simple seta at outer margin, nail shorter than pedestal (Fig. 3).

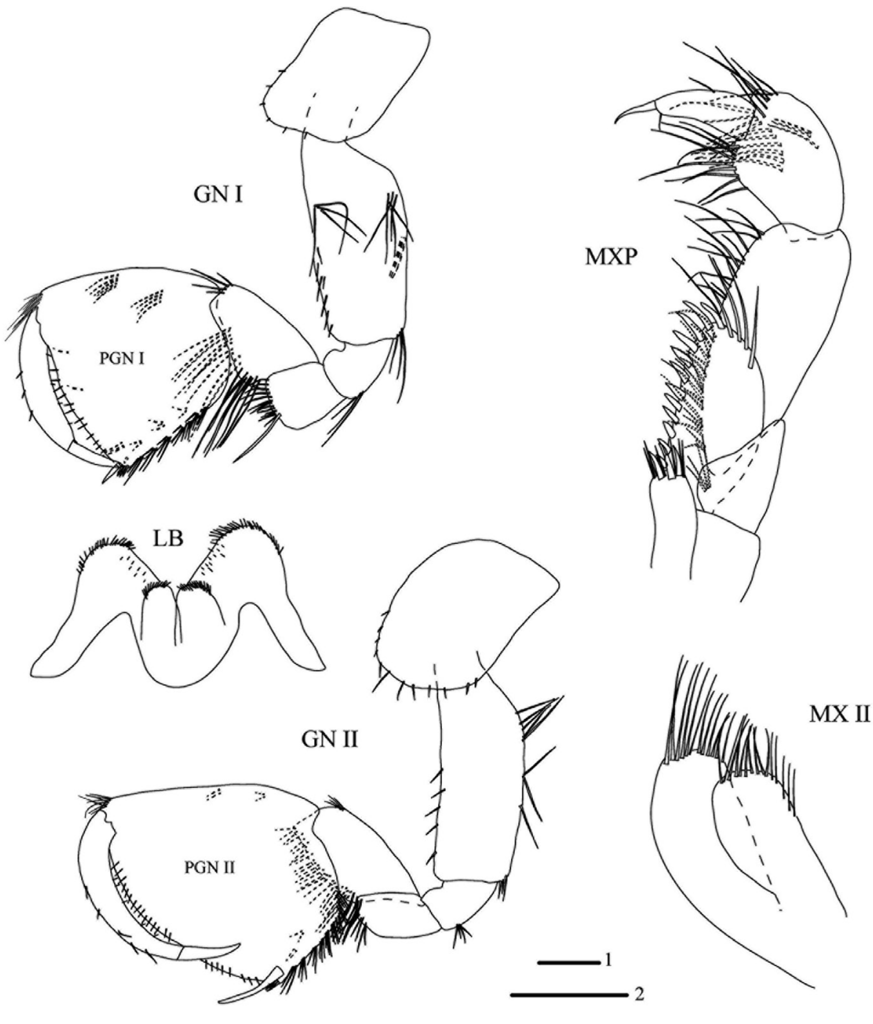

Fig. 3. *Niphargus sarii* sp. n., male, holotype, 11.5 mm. Legend: GN I = gnathopod I, GN II = gnathopod II, LB = labium, MXP = maxilliped, MX II = maxilla II. Scale bars: 1 = 0.5 mm (LB, MXP, MX II), 2 = 1 mm (GN I–II)

Coxa of gnathopod I shorter than coxa of gnathopod II. Coxa I rectangular, longer than broad, ventral to anterior margin each with three simple setae. Basis with setae along anterior and posterior margins; ischium and merus with posterior group of setae. Carpus with one group of four setae anterodistally, a bulge with long simple setae; carpus 0.54 of basis length and 0.71 of propodus length. Propodus slightly broader than long; anterior margin with nine setae in two groups in addition to anterio-distal group of nine simple setae. Palm slightly convex, with one strong long palmar robust seta, one short supporting robust seta on inner surface, with three robust setae with lateral projections on outer surface; two simple setae under supporting robust seta in palmar corner. Dactylus reaching posterior margin of propodus, outer and inner margins with a row of three and five simple setae, respectively; nail short, 0.23 of total dactylus length (Fig. 3).

Coxa of gnathopod II rectangular, with 12 setae along anterio-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 of basis length and 0.69 propodus length. Carpus with one group of four setae antero-distally. Propodus in gnathopod II larger than gnathopod I, trapezoid shape and broader than long; anterior margin

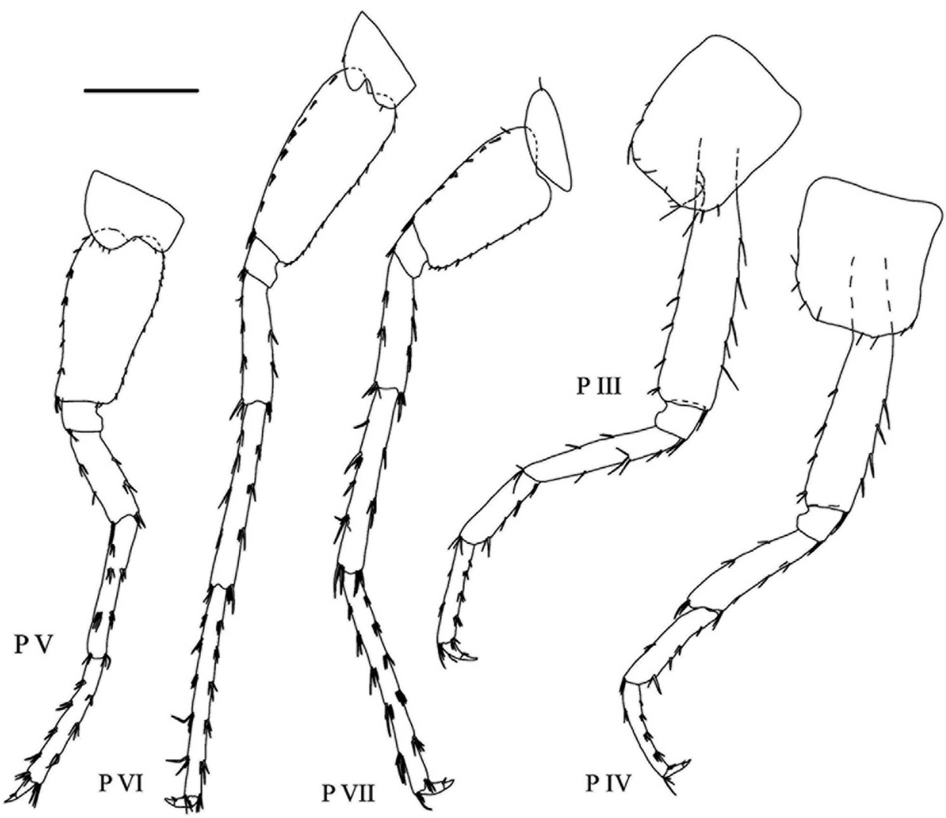

Fig. 4. *Niphargus sarii* sp. n., male, holotype, 11.5 mm. Legend: P III = pereopod III, P IV = pereopod IV, P V = pereopod V, P VI = pereopod VI, P VII = pereopod VII. Scale bar: 1 mm (P III–P VII)

with totally four setae in two groups in addition to antero-distal group of eight simple setae. Palm nearly convex, with one strong palmar robust seta, one supporting robust seta without lateral projections on inner surface, with two robust setae with lateral projections

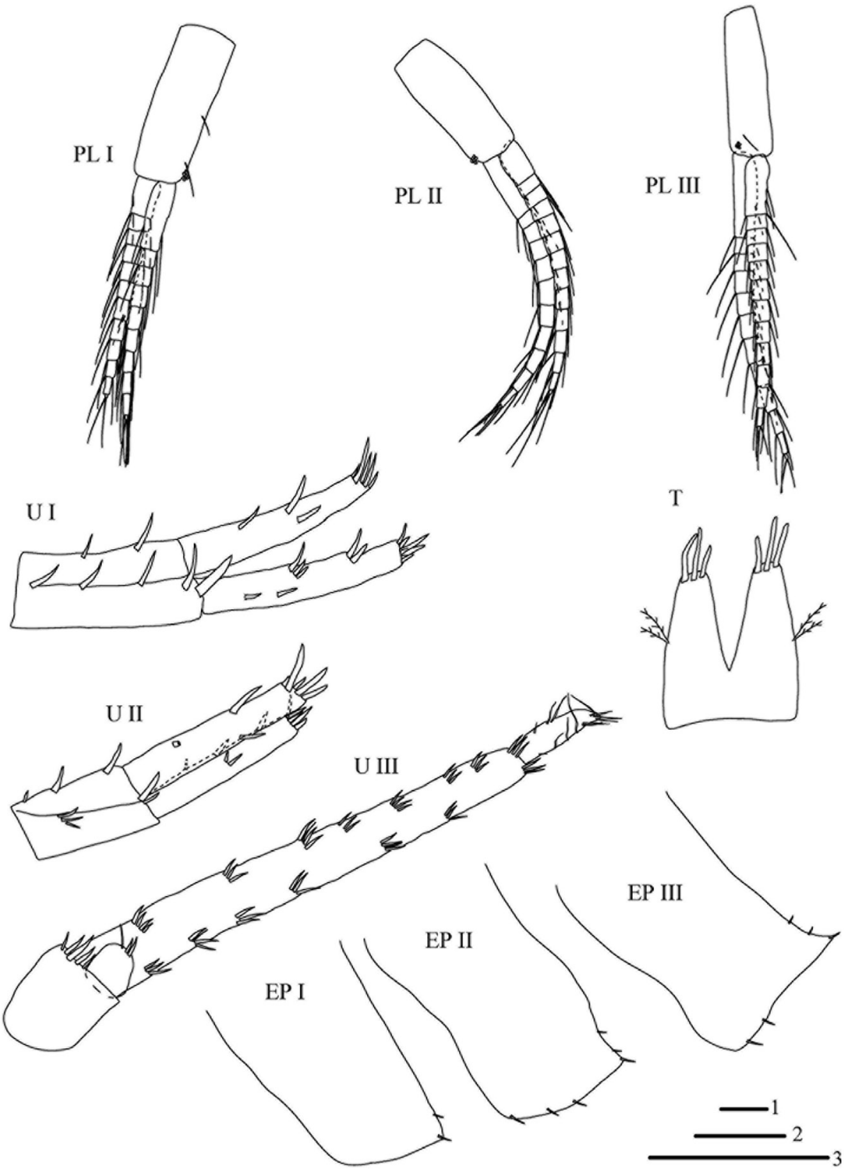

Fig. 5. *Niphargus sarii* sp. n., male, holotype, 11.5 mm. Legend: PL I = pleopod I, PL II = poleopod II, PL III = pleopod III, U I = uropod I, U II = uropod II, U III = uropod III, EP I–III = epimeral plates I–III, T = telson. Scale bars: 1 = 0.5 mm (EP I–III, T), 2 = 1 mm (PL I–III, U I–II), 3 = 2 mm (U III)

on outer surface; two setae under supporting robust seta in palmar corner. Dactylus is not reaching posterior margin of propodus, outer and inner margins of dactylus each with five simple setae. Nail length 0.25 of total dactylus length (Fig. 3).

Coxa III rectangular, length to width ratio as 1.24 : 1; antero-ventro-posterior margin with 10 simple setae. Coxa IV rectangular, length to width ratio as 1.36 : 1, antero-ventro-posterior margin with eight simple setae, posterior concavity shallow and approximately 0.1 of coxa width (Fig. 4). Coxa V with anterior lobe, with four and one simple setae on anterior and posterior lobes, respectively. Coxa VI with anterior lobe, with one simple seta on anterior and posterior lobes each. Coxa VII with one simple seta (Fig. 4).

Pereonites I–VI without setae. Pereonite VII with one simple seta along dorsal surface. Pereopod III : IV lengths ratio as 1.08 : 1 (Fig. 4). Dactylus IV short, length of dactylus 0.36 of propodus, nail shorter than pedestal (Fig. 4). Pereopods V: VI: VII length ratios as 1 : 1.26 : 1.17, respectively. Pereopod VII 0.52 of body length. Pereopod bases V-VI each with eight groups of robust setae and pereopod basis VII with 12 simple setae along anterior margin, respectively. Pereopod bases V-VI with 13 and 14 simple setae and pereopod basis VII with eight groups of robust setae along posterior margin, respectively (Fig. 4). Postero-ventral lobe of ischium in pereopods V–VII weakly developed. Ischium, merus and carpus in pereopods V–VII with several groups of robust and simple setae along anterior and posterior margins; propodus of pereopod VII longer than these in V-VI, dactyli of pereopods V–VII with one robust and one short simple seta at the base of nail on inner margin, nail length of pereopod VII 0.29 of total dactylus length (Fig. 4).

Epimeral plates I–III (Fig. 5) with angular postero-ventral corner, anterior and ventral margins convex; postero-ventral corners of plates I-III with one robust seta each, and with one, two and two simple setae posteriorly, respectively. Epimeral plates II-III with three and two robust setae along of ventral margins, respectively.

Pleonites I–III each with one to two simple setae along dorsal surface. Peduncle of pleopod I with two simple setae and two-hooked retinacles along of inner margin (Fig. 5); peduncle of pleopod II with two-hooked retinacles at distal part of inner margin; peduncle of pleopod III with two-hooked retinacles and with one simple seta at distal part of inner margin; rami of pleopods I–III each with 10 to 14 articles (Fig. 5).

Laterally, urosomites I-III with one robust seta, two robust setae and one simple seta and without setae, respectively. Peduncle of uropod I with five and two large robust setae along dorso-lateral and dorso-medial margins, respectively. Outer ramus as long as inner ramus; inner ramus with three groups of robust setae laterally and five robust setae distally; outer ramus with four groups of seven robust setae laterally and five robust setae distally (Fig. 5). Inner ramus in uropod II longer than outer, both rami with lateral and distal long robust setae (Fig. 5). Uropod III long, almost 0.5 of body length. Peduncle of uropod III with five robust setae. Outer ramus biarticulated, distal article 0.19 proximal article. Proximal article of outer ramus bearing seven and eight groups of robust setae along inner and outer margins (Fig. 5); distal article with simple setae laterally and four simple setae distally. Inner ramus short, with two robust and one simple distal setae. Telson longer than broad, lobes slightly narrowing; each lobe with three robust setae distally and with two plumose setae laterally (Fig. 5).

Etymology – The species is named in honour of Professor Alireza Sari, University of Tehran, Iran, to acknowledge his many years of contribution to the zoology of Iran, especially his work on crustaceans.

Phylogenetic relationships and molecular divergence

We analysed 53 *Niphargus* species (63 individuals) based on 810 base pairs of the first fragment of the 28S ribosomal DNA gene. Two phylogenetic analyses (Bayesian inference and maximum likelihood) yielded similar topologies. The topology resulting from Bayesian inference analysis is presented in Figure 6. The newly described *N. sarii* sp. n. is nested within the main Iranian clade plus the specimen from Lebanon (which is juvenile and its identity could not be determined). This species shares an ancestor with *N. hosseiniei*, *N. khayyami* and *N. sohrevardensis* (Fig. 6). Pairwise uncorrected Kimura 2-parameter genetic distances and patristic distances on a 28s maximum likelihood tree between all Iranian species and the Lebanon sample are presented in Table 2. The K2P genetic distances between the *N. sarii* and other species ranged between 0.60% and 10.80%, and the patristic distance is between 0.90% and 28.60%. The new species is genetically most similar to *N. sohrevardensis* (0.60% K2P and 0.90% patristic divergence in the studied 28 rDNA gene fragment) and the most divergent species from *N. daniali*, (10.08% K2P and 28.60% patristic divergence). Minimum interspecific distance was observed between two previously known species *N. persicus* and *N. darvishi* with the least divergence of 0.30% in both K2P and patristic divergence.

DISCUSSION

The study of collected populations from Ilam Province proposes a new species of *Niphargus* genus, on the basis of morphological characters and the DNA sequences of 28S ribosomal DNA (rDNA) gene. The DNA sequences confirmed the relative taxonomic position of *N. sarii* sp. n. with *N. sohrevardensis*, *N. hosseiniei* and *N. khayyami*. The Bayesian and ML analyses indicate that this species is phylogenetically distinct of other relative species. All Iranian species (except *N. daniali* plus a sample from Lebanon) constitute a large, strongly supported monophyletic clade that is separated from European taxa. Two individuals of the newly described species constitute a strongly supported monophylum that differs from *N. sohrevardensis* in 0.60% and 0.90% of base pairs based on Kimura two parameter and patristic distances, respectively. According to Hou and Li (2010) and Esmaeili-Rineh *et al.* (2015*a*, 2016), this is within the same range of divergence for 28S gene as in well-defined *Gammarus* and *Niphargus* species.

→

Fig. 6. Bayesian consensus tree of 53 *Niphargus* species (52 taxa from Esmaeili-Rineh *et al.* 2015*a*, 2017*a*), based on the 28S ribosomal DNA sequences. Species are identified and named according to the valid taxonomic descriptions. Numbers above line indicate the posterior probabilities of the nodes in the Bayesian inference analysis. Numbers below line are the bootstrap values of the nodes in the Maximum likelihood analysis

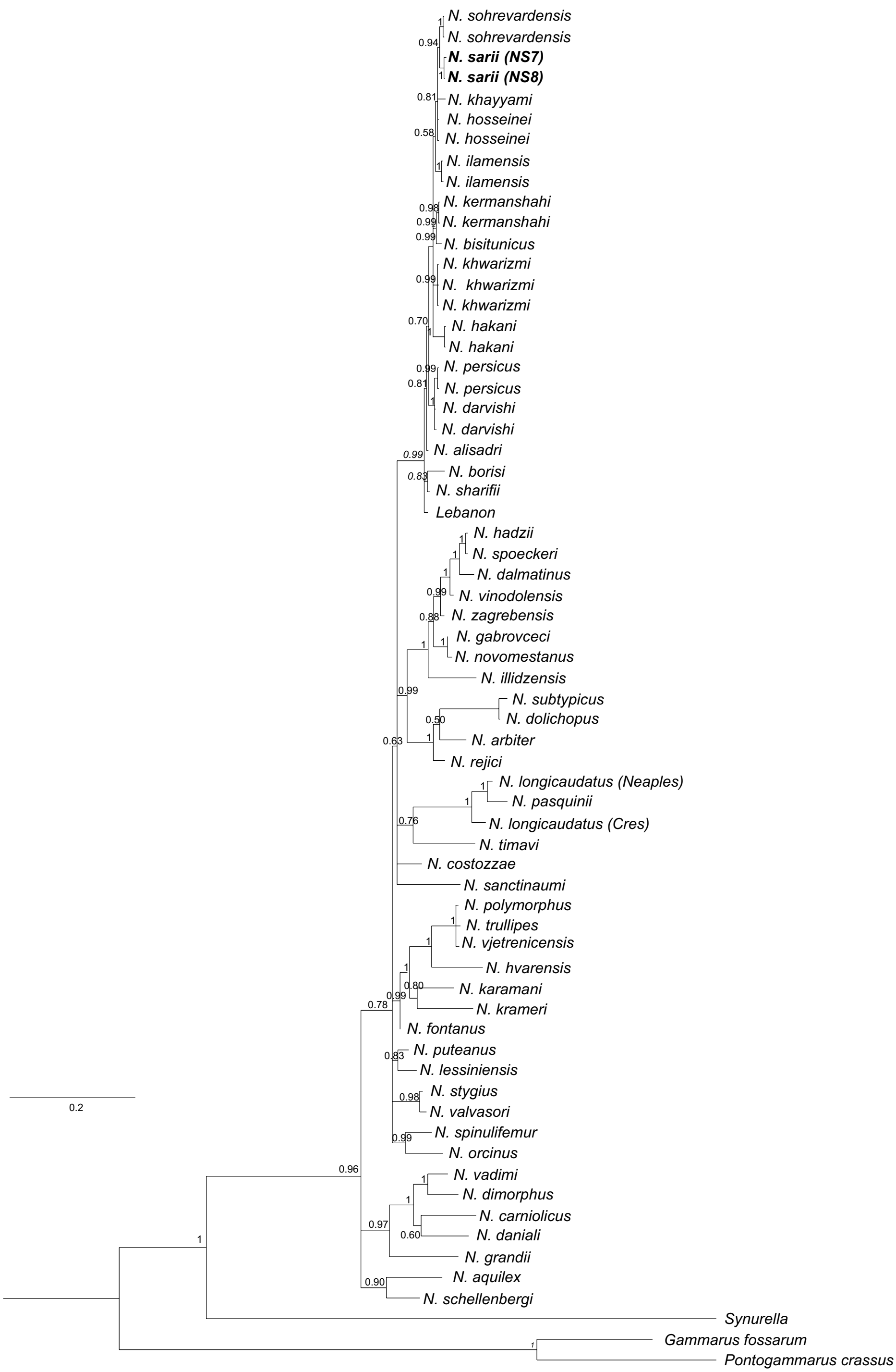
N. sohrevardensis
N. sohrevardensis
N. sarii (NS7)
N. sarii (NS8)
N. khayyami
N. hosseinei
N. hosseinei
N. ilamensis
N. ilamensis
N. kermanshahi
N. kermanshahi
N. bisitunicus
N. khwarizmi
N. khwarizmi
N. khwarizmi
N. hakani
N. hakani
N. persicus
N. persicus
N. darvishi
N. darvishi
N. alisadri
N. borisi
N. sharifii
Lebanon
N. hadzii
N. spoeckeri
N. dalmatinus
N. vinodolensis
N. zagrebensis
N. gabrovceci
N. novomestanus
N. illidzensis
N. subtypicus
N. dolichopus
N. arbiter
N. rejici
N. longicaudatus (Neaples)
N. pasquinii
N. longicaudatus (Cres)
N. timavi
N. costozzae
N. sanctinaumi
N. polymorphus
N. trullipes
N. vjetrenicensis
N. hvarensis
N. karamani
N. krameri
N. fontanus
N. puteanus
N. lessiniensis
N. stygius
N. valvasori
N. spinulifemur
N. orcinus
N. vadimi
N. dimorphus
N. carniolicus
N. daniali
N. grandii
N. aquilex
N. schellenbergi
Synurella
Gammarus fossarum
Pontogammarus crassus
0.2

Table 2. Pairwise molecular divergences were calculated using K2P genetic distances (below diagonal) and Patristic distances (upper diagonal) on a 28s maximum likelihood tree between all Iranian species and the Lebanon sample of the genus *Niphargus*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1: *N. daniali*	–	0.266	0.268	0.290	0.267	0.277	0.288	0.282	**0.286**	0.284	0.288	0.277	0.282	0.277	0.274	0.277
2: *N. lebanon*	0.099	–	0.010	0.032	0.009	0.019	0.030	0.025	**0.028**	0.026	0.030	0.019	0.025	0.019	0.016	0.019
3: *N. sharifii*	0.096	0.008	–	0.026	0.009	0.019	0.030	0.025	**0.028**	0.027	0.031	0.019	0.025	0.019	0.016	0.019
4: *N. borisi*	0.100	0.020	0.018	–	0.031	0.041	0.052	0.047	**0.050**	0.049	0.053	0.041	0.047	0.041	0.038	0.041
5: *N. alisadri*	0.097	0.006	0.006	0.022	–	0.014	0.025	0.019	**0.023**	0.021	0.025	0.014	0.019	0.014	0.011	0.014
6: *N. sohrevardensis*	0.104	0.014	0.011	0.026	0.010	–	0.011	0.005	**0.009**	0.011	0.018	0.011	0.016	0.011	0.018	0.021
7: *N. khayyami*	0.104	0.017	0.011	0.026	0.013	0.008	–	0.016	**0.019**	0.021	0.029	0.021	0.026	0.021	0.028	0.032
8: *N. hosseinei*	0.108	0.017	0.015	0.028	0.014	0.004	0.011	–	**0.011**	0.016	0.023	0.016	0.021	0.016	0.023	0.026
9: *N. sarii* sp.n.	**0.108**	**0.017**	**0.015**	**0.030**	**0.014**	**0.006**	**0.010**	**0.008**	–	**0.019**	**0.027**	**0.019**	**0.025**	**0.019**	**0.026**	**0.030**
10: *N. ilamensis*	0.105	0.015	0.013	0.027	0.011	0.004	0.011	0.008	**0.010**	–	0.025	0.018	0.023	0.018	0.025	0.028
11: *N. hakani*	0.101	0.019	0.017	0.031	0.015	0.013	0.018	0.017	**0.019**	0.014	–	0.022	0.027	0.022	0.029	0.032
12: *N. khwarizmi*	0.104	0.014	0.011	0.026	0.010	0.008	0.010	0.011	**0.011**	0.009	0.014	–	0.016	0.011	0.018	0.021
13: *N. bisitunicus*	0.104	0.015	0.010	0.027	0.011	0.009	0.011	0.013	**0.013**	0.010	0.017	0.011	–	0.009	0.023	0.026
14: *N. kermanshahi*	0.105	0.014	0.011	0.026	0.010	0.008	0.013	0.011	**0.011**	0.009	0.015	0.008	0.006	–	0.018	0.021
15: *N. darvishi*	0.100	0.011	0.011	0.027	0.008	0.010	0.015	0.014	**0.014**	0.011	0.021	0.013	0.014	0.013	–	0.003
16: *N. persicus*	0.102	0.013	0.013	0.028	0.009	0.011	0.017	0.015	**0.015**	0.013	0.022	0.014	0.015	0.014	0.003	–

Table 3. Diagnostic characters in identification of Iranian species of the genus *Niphargus*.

Characters	Urosomites I-III(number of dorso-postero-lateral setae)	Telson setal pattern (A – apical robust setae, L – lateral robust setae)	Size of coxa of GNI to GNII	Maxilla I - number of denticles on R. S of outer plate (from outer towards inner)	Uropod I (ratio of uropod rami)
Species					
N. sarii sp. n.	I: 1 R.S; II: 2 R.S, 1 S.S; III: No setae	3A	smaller	1,0,2,0,2,5,0	subequal
N. khayyami	I: 1-2 S.S; II: 2-3 S.S	3:00 de.	smaller	1,others more than 5	Inner>outer
N. sharifii	I:1 S.S; II: 3 R.S; III: No seta	3:00 de.	smaller	5,5,5, others more than5	Inner>outer
N. darvishi	I–II:2R.S, 2S.S	3:00 de.	smaller	0,2,2,3,3,4,5	Inner>outer
N. hosseiniei	I:1 S.S; II: 3 R.S; III: No setae	3:00 de.	smaller	4,0,1,2,0,1,2	Inner>outer
N. persicus	I: 1 R.S; II: 2 R.S; III: No setae	3:00 de.	similar	More than5,4,4,4,3,3,0	Inner>outer
N. ilamensis	I:1 R.S; II: 2 R.S; III: 1R.S	3:00 de.	similar	4,3,1,1,2,2,0	Inner>outer
N. hakani	I: 2 R.S; II: 5 R.S;III: 2 R.S	3 A, 1 L	smaller	0,0,0,2,3,4,more than 5	Inner>outer
N. bisitunicus	I:1R.S,2S.S; II:3R.S,2 S.S III: No setae	3 A, 1 L	smaller	0,0,1,1,1,1,2	Inner<outer
N. borisi	I–II: 4 R.S; III: 2 R.S	3 A, 1 L	smaller	0,0,0,1,1,2,2	Inner>outer
N. sohrevardensis	I:1 R.S; II: 2 R.S, 1S.S; III: No setae	3 A, 1 L	smaller	4,1,1,2,2,0,0	Inner>outer
N. valachicus	I-II: 1 R.S; III: No setae	3 A, 1 L	smaller	1,1,1,1,1,1,1	Inner<outer
N. kermanshahi	I:3 S.S; II: 3 S.S, 2R.S III: No setae	3 A, 1 L	of similar size	0,1,1,1,2,2,5	Inner<outer
N. alisadri	I-III: 6 R. S	3 A, 2 L	smaller	0,1,1,1,2,2,5	Inner>outer
N. khwarizmi	I:1 S.S; II: 2 R.S; III: No setae	3 A, 2 L	smaller	0,1,1,1,1,1,1	Inner<outer
N. daniali	I-II: 1 S.S;III: No setae	4 A, 2 L	smaller	0,0,0,1,1,1,3	Inner>outer

Legend: A = apical, GNI–II = gnathopods I–II, L = lateral, R. S. = robust seta, S = simple seta, SRS = supporting robust setae

Table 3 (continued)

Characters	No. of SRS in GNII	Length of maxilla I palpus	Shape of propods of GNI	Shape of propods of GNII	Uropod III (length of distal article of outer ramus)
Species					
N. sarii sp. n.	1	longer than outer lobe of maxilla I (without R. S)	trapezoid	trapezoid	approx. 0.20 of the first article
N. hakani	1	longer than outer lobe of maxilla I (without R. S)	trapezoid	trapezoid	approx. 0.52 of the first article
N. valachicus	1	longer than outer lobe of maxilla I (without R. S)	trapezoid	trapezoid	approx. 0.33 of the first article
N. khwarizmi	1	longer than outer lobe	trapezoid	trapezoid	0.65 of the first article
N. darvishi	1	longer than outer lobe of maxilla I (without R. S)	trapezoid	trapezoid	0.08 of the first article
N. persicus	1	longer than outer lobe of maxilla I (without R. S)	trapezoid	triangular	0.14 of the first article
N. daniali	1	longer than outer lobe of maxilla I (without R. S)	rectangular	subquadrangular	approx. 0.33 of the first article
N. hosseinei	1	longer than outer lobe of maxilla I (without R. S)	rectangular	triangular	0.14 of the first article
N. khayyami	1	as long as outer lobe of maxilla I (without R. S)	trapezoid	rectangular	0.1 of the first article
N. sohrevardensis	1	shorter than outer lobe of maxilla I (without R. S)	rectangular	rectangular	0.18 of the first article
N. bisitunicus	2	longer than outer lobe	rectangular	rectangular	approx. 0.33 of the first article
N. sharifii	2	as long as outer lobe	rectangular	rectangular	0.16 of the first article
N. kermanshahi	2	shorter than outer lobe of maxilla I (without R. S)	trapezoid	trapezoid	approx. 0.5 of the first article
N. ilamensis	2	shorter than outer lobe of maxilla I (without R. S)	rectangular	triangular	0.15 of the first article
N. alisadri	3	as long as outer lobe of maxilla I (without R. S)	rectangular	triangular	as long as the first article.
N. borisi	3	shorter than outer lobe of maxilla I (without R. S)	rectangular	rectangular	0.14 of the first article

Legend: A = apical, GNI–II = gnathopods I–II, L = lateral, R. S. = robust seta, S = simple seta, SRS = supporting robust setae

N. sarii sp. n. is geographically far from its sister species *N. sohrevardensis*. This is a surprise, as *Niphargus* species are in general poorly vagile, and closely related species often share the same geographic region (Fišer *et al.* 2008, Trontelj *et al.* 2009, Meleg *et al.* 2013). The distance between the two species is 323.5 km. Moreover, the distances between *N. sarii* with *N. hosseiniei* and *N. khayyami* are 127.3 and 121.4 km, respectively.

The species is also morphologically distinct from other Iranian species. A shortened comparison of diagnostic traits for the Iranian species is presented in Table 3. Both species share several morphological traits in the ratio of second to first article of the outer ramus of uropod III, the number of supporting robust setae in the palmar corner of gnathopods I–II and the number of robust and simple setae in dorso-lateral urosomites I–III surface. However, this species differs from *N. sohrevardensis* in the shape of propodus of gnathopods I–II, by the absence of lateral robust setae in telson, by the ratio of rami length in uropod I and by the outer plate longer than the palpus in maxilla I (Esmaeili-Rineh *et al.* 2017*b*). Thus, the evidence from both morphological and molecular studies supports *N. sarii* sp. n. as new species (Table 3).

The new species resembles *N. hosseiniei* in distal robust setae in telson, the ratio of maxillar palpus to outer plate length in maxilla I and in the shape of the postero-ventral angle of epimeral plates. The two species differ from each other in the ornamentation of the lateral projections of the robust setae in outer plate of maxilla I, the number of robust and simple setae in dorso-lateral urosomites I–III surface and the ratio of the inner to outer ramus of uropod I and the shape of propodus in gnathopods I–II. *N. hosseiniei* is recognizable by rectangular and triangular propodi in gnathopods I–II, respectively, whereas *N. sarii* sp. n. bear trapezoidal shape propodi in both gnathopods. Also, *N. hosseiniei* individuals bear double palmar robust setae that was not observe in other Iranian species (Esmaeili-Rineh *et al.* 2017*b*). *Niphargus khayyami* differs from *N. sarii* sp. n. by the ratio of inner to the outer ramus of uropod I, by the ratio of palpus to outer plate length in maxilla I and ornamentation of lateral projections of robust setae in outer plate of maxilla I (Hekmatara *et al.* 2013).

An important diagnostic trait of *N. sarii* sp. n. is the equal ratio of the length of rami in uropod I. This character was not observed in other Iranian species (Karaman 1998, Hekmatara *et al.* 2013, Esmaeili-Rineh *et al.* 2015*b*, 2016*a*). This character seen only in *N. nadarini favitor* in the Middle East (Karaman 2012), but, two taxa differ in the ratio of palpus to outer plate length in maxilla I, the number of robust setae at the base of nail in pereopods IV–VII and the number of robust setae in outer surface of palmar corners in gnathopods I–II.

Finally, we describe the new taxon and increase the Iranian niphargid fauna to 17 species. Most of the species are endemic to Iran. Endemic species are an important part of the natural heritage of the country. Therefore, it is necessary to identify the biodiversity of this area before habitat destruction.

*

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