

Three new species of Niphargidae family added to the list of subterranean amphipods (Crustacea: Amphipoda) in Slovenia

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Abstract. Since the last comprehensive checklist of amphipods in 2021, three new species of subterranean amphipods have been confirmed for Slovenia: *Niphargus danielopolii*, *N. lurensis* and *N. julius*. Identification of all three species was based on molecular comparative analyses of the mitochondrial CO1 gene fragment, usually used in DNA barcoding identification. With these additions, we reached a total of 201 amphipod species confirmed for Slovenia, 60 of which are groundwater inhabitants, with 57 species belonging to the genus *Niphargus*.

Key words: groundwater, checklist, additional species, *Niphargus*, hotspot, barcoding, species richness

Izvešček. Tri nove vrste iz družine Niphargidae dodane na seznam podzemnih postranic (Crustacea: Amphipoda) Slovenije – Od zadnjega izčrpnega seznama postranic leta 2021 so bile za Slovenijo potrjene tri nove vrste podzemeljskih postranic: *Niphargus danielopolii*, *N. lurensis* in *N. julius*. Identifikacija treh vrst je temeljila na molekularnih primerjalnih analizah fragmenta mitohondrijskega gena CO1, ki se običajno uporablja pri identifikaciji s črtnim kodiranjem DNA. S temi dodatki je za Slovenijo potrjenih skupno 201 vrst postranic, od katerih je 60 podzemnih, s 57 vrstami iz rodu *Niphargus*.

Gljučne besede: podzemne vode, seznam, dodatne vrste, *Niphargus*, vroča točka, barkodiranje, vrstno bogastvo

Introduction

In the recently published checklist of amphipods of Slovenia, 198 different species inhabiting marine, brackish, freshwater, and terrestrial habitats have been listed (Fišer et al. 2021). More than one-fourth (57 species) of them inhabit groundwater and can be treated as obligatory subterranean species. All but three belong to the family Niphargidae. The genus *Niphargus* is the most diverse genus of freshwater amphipods in the world and is distributed throughout the western Palearctic region (Borko et al. 2021). Due to its wide distributional range and high species richness, *Niphargus* is the focal group for various taxonomical, ecological, evolutionary and biodiversity studies (e.g. Stoch et al. 2020; Borko et al. 2021, 2022).



Subsequently, Borko et al. (2022) published a study with an extensive list of *Niphargus* species with corresponding CO1 barcoding sequences. Although some sequences could have been ascribed to morphologically described species, many of them present potential new species based on molecular differences. Further sequencing of the *Niphargus* CO1 marker in Ljubljana (SubBioLab, at Biotechnical Faculty, University of Ljubljana) and elsewhere allowed us to link the identity of two other MOTUs (molecular operational taxonomic units) in Borko et al. (2022) with valid species names. To keep the amphipod checklist for Slovenia updated, we report herewith on all three additional species for the country.

Material and methods

Information on the new species for Slovenia was registered in the SubBioDB database, a spatial relational database managed by SubBioLab. Info on the new species and MOTUs were extracted from Borko et al. (2022), where the sequences were originally used in the analyses of biodiversity patterns of subterranean amphipods in the Western Balkans.

DNA extraction from amphipods stored in the SubBioLab collection was based on a single appendage; hence the specimens remained in the collection for potential subsequent morphological identification. The protocol of DNA extraction and amplification of the Folmer's CO1 barcoding fragment (Folmer et al. 1994) for two species followed the one described in Borko et al. (2021), with post-sequencing species identification using the BLAST tool (Altschul et al. 1990). Species identification was made based on widely used 97% match (Ratnasingham & Hebert 2007) with the barcoding fragments of CO1 stored in GenBank.

All sequences were deposited in GenBank, with access numbers given in the Results section.

Results and discussion

Three new groundwater amphipod species were recorded at four different localities in Slovenia (Tab. 1). One of them, *N. danielopolj*, was identified to the species level by Borko et al. (2022), while the other two were referred to as different MOTUs (Tab. 1). A comparison of acquired sequences with those stored in GenBank or originating in neighbouring countries (Stoch et al. 2020) allowed us to unambiguously identify two additional *Niphargus* species.

Niphargus danielopolj Karaman G., 1994 was described from Austria. It is a small species that has been reported from several localities geographically scattered in central Europe and northern Italy (Karaman 1994), and confirmed in Northern Slovenia (Borko et al. 2022).

Niphargus lurensis Schellenberg, 1935 was first described as a form of *N. tatrensis* Wrześniowski, 1888. In their molecular study, Stoch et al. (2020) provided evidence that this form should be treated as a valid species distributed in Styrian karst in Austria. Based on the barcoding fragment published by Stoch et al. (2020), the identification of material from Northeastern Slovenia revealed that this species is also present at two sites in Slovenia (Tab. 1).

The third species, *N. julius* Stoch, 1997, has been reported from several caves and springs in the Nadiža/Natisone and neighbouring river valleys in northeastern Italy, close to the Slovenian border (Stoch 1997; Karaman 2020). Its presence in Slovenia was confirmed after comparing sequences of individuals collected in a cave close to Kobarid with sequences from Italy (Stoch et al. 2023; Tab.1).

Table 1. The list of new groundwater amphipod species confirmed as occurring in Slovenia, with reported details on findings (taken from the SubBioDB database). Coordinates (Lat: N latitude; Long: E longitude) are given in WGS84 decimal degrees. The Acc. No. refers to the sequence's GenBank access number and a code in brackets to the specimen's voucher number in the SubBioDB. The name, under which it was reported in Borko et al. (2022), is given in the last column.

Tabela 1. Seznam novih vrst podzemnih postranic, ki se potrjeno pojavljajo v Sloveniji, s podrobnejšimi podatki o najdbah (povzeto po podatkovni bazi SubBioDB). Koordinate (Lat: severna geografska širina; Long: vzhodna geografska dolžina) so podane v WGS84 decimalnih stopinjah. Okrajšava Acc. No. se označuje GenBank identifikacijsko številko, medtem ko se oznaka v oklepaju nanaša na številko osebkov (voucher) v SubBioDB. V zadnjem stolpcu je navedeno ime, pod katerim je bila vrsta navedena v Borko et al. (2022).

Species	Locality	Lat, Long	Date	Collectors	Acc. No. (voucher)	Name in Borko et al. (2022)
<i>Niphargus danielopoli</i> G. Karaman, 1994	Izvir Veliki Javornik, Javorniški rovt, Jesenice (spring)	46.458228, 14.108519	27.5.2017	Simona Prevorčnik	OK156541 (NC216), OK157026 (ND518), OK157027 (ND519)	<i>N. danielopoli</i>
<i>Niphargus lurensis</i> Schellenberg, 1935	Vodnjak pri hiši Cven 1c (well)	46.551063, 16.208525	14.8.2011	Jasmina Kotnik	OK156762 (ND186)	<i>N. spn</i> ND186
	Vodnjak ob hiši Ribiška 24 in Veržej (well)	46.586558, 16.165139	9.6.2017	Maja Zagmajster, Simona Prevorčnik, Nataša Mori	OK157130 (ND642)	<i>N. spn</i> ND186
<i>Niphargus julius</i> Stoch, 1997	Turjeva jama (Slovene cave Registry No. 821), Kred, Kobarid (cave)	46.241212, 13.500463	15.1.2018	Teo Delić, Špela Borko, Klemen Koselj, Maja Zagmajster	OK157116 (ND624)	<i>N. spn</i> ND624

Even though Slovenia has already been recognised as a hotspot of groundwater amphipod diversity in the Western Balkans (Bregović et al. 2019), the ongoing analyses, run by the SubBioLab members, revealed new species for the country. The new observations reported herein increase the number of amphipod species for Slovenia to 201, with 60 of them from groundwater, and 57 belonging to the genus *Niphargus*.

Povzetek

V zadnjem objavljenem pregledu postranic Slovenije je bilo naštetih 198 vrst iz različnih habitatov, od teh 57 iz podzemnih voda (Fišer et al. 2021). Od slednjih vse razen treh vrst pripadajo družini Niphargidae. Rod *Niphargus* je vrstno najpestrejši rod postranic, ki je razširjen po območju cele zahodne Palearktike (Borko et al. 2021). V študiji Borko et al. (2022) je bil uporabljen in objavljen seznam številnih vrst rodu *Niphargus* s pripadajočimi CO1 sekvencami. V tem seznamu je navedena nova vrsta za Slovenijo, še dve pa sta bili v tej študiji navedeni kot molekulski operativni taksonomski enoti, ki sta potencialno svoji vrsti. Za identifikacijo smo uporabili postopek BLAST (Altschul et al. 1990) in na podlagi 97-% ujemanja s sekvencami na GenBanku potrdili identiteto teh osebkov.

Skupno so bile identificirane tri nove vrste postranic za Slovenijo, najdene na štirih različnih lokalitetah (Tab. 1). *Niphargus danielopoli* Karaman G., 1994 je bil znan iz srednje Evrope. *Niphargus lurensis* Schellenberg, 1935 je razširjen po Štajerskem delu Avstrije, potrjena je bila tudi v severovzhodni Sloveniji. Tretja vrsta, *N. julius* Stoch, 1997, je bila znana iz doline reke Nadiže/Nattisone v Italiji, zdaj smo jo potrdili tudi v jami blizu Kobarida.

Nova opažanja so povečala skupno število potrjenih postranic v Sloveniji na 201, od katerih jih 60 živi v podzemnih vodah.

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