Zoogeography and Faunistics
Research Article

ACTA ZOOLOGICA BULGARICA
Acta zool. bulg., 70 (1), 2018: 45-50

Epigean Niphargids in Serbia: New Records of *Niphargus valachicus* Dobreanu & Manolache, 1933 (Amphipoda: Niphargidae), with Notes on its Ecological Preferences

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Abstract: *Niphargus valachicus* Dobreanu & Manolache, 1933 is an IUCN vulnerable epigean niphargid from the Danube River Basin and the Middle East. Although Serbia is one of countries within the geographical range of this species, since the middle of the 20th century its presence has not been confirmed. During an annual study of aquatic macroinvertebrates conducted by the Serbian Environmental Protection Agency (SEPA), a total of ten *N. valachicus* specimens were found at two localities in the Pannonian part of the country (Vojvodina). Nine specimens were found in two samples (August 2013 and July 2015) from a small tributary of the Tisza River (the Zlatica River) and one specimen was found in the sample from a small tributary of the Danube River (the Plazović River, September 2014). Regarding ecological characterisation and biological and physico-chemical assessment of these localities, it could be noted that *N. valachicus* tolerates slightly deteriorated habitats with moderate level of organic pollution and lower concentrations of dissolved oxygen. Based on recent distribution and ecological characteristics of this species, its wider distribution in Serbia could be expected.

Key words: vulnerable species, small watercourses, Pannonian Plain

Introduction

Niphargus Schiödte, 1847 is the most diverse genus of freshwater amphipods, with over 300 described species (Väinölä et al. 2008). The genus is widespread and common in Europe and the Middle East, primarily inhabiting various groundwaters. In Serbia, over 20 taxa of this genus (species and subspecies) were discovered from the springs, caves and other subterranean habitats (Karaman 2012). Most of the species are endemic and some are listed by IUCN as vulnerable taxa (Savić 2007). Among the numerous stygobiont species of this genus (Meijering 1995), there are a few epigean species (Fišer et al. 2006). At least three of these epigean species could be found in Central and South-eastern Europe: *N. hrabei* S. Karaman, 1932; *N. valachicus* Dobreanu & Manolache, 1933 (Copilas-Ciocianu et al. 2014) and *N. bulgaricus* Andreev, 2001 (Andreev 2001). Another epigean species has been recorded in Hungary, *N. mediodanubialis* Dudich, 1941, although its taxonomic status is doubtful, as it is regarded by some authors as a synonym of *N. valachicus* (Kontschan 2004, Gergely & Angyal 2013). Beside diverse ecology, *Niphargus* is characterised by diverse morphology as well as by numerous homoplasies, which makes its taxonomy difficult (Fišer et al. 2009).

Niphargus valachicus Dobreanu & Manolache, 1933 is considered to be closely related to *N. tatrensis*

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Wrzesniowsky, 1888 and initially it was described as a subspecies, i.e. *N. tatrensis valachicus* (Dobreanu & Manolache 1933, Gergely & Angyal 2013). Moreover, morphologically it resembles *N. puteanus* C. L. Koch, 1836 but it differs from these two species by having short second article of the exopodit (3rd uropod) (Cărăusu et al. 1955). The main morphological characters distinguishing *N. valachicus* from the other common epigean niphargids in the region, *N. hrabei*, are the dactylus of pereiopods VI and VII with row of spines (at least 5) and the pointed epimeres (Copilas-Ciocianu et al. 2014). Additional taxonomic characters of *N. valachicus* are the presence of an appendix at basipodit of male uropod I and tiny humps at the base of dorsolateral spines on uronite I (Cărăusu et al. 1955). Unlike the epigean niphargid from Bulgaria, *N. bulgaricus*, which is characterized by 9-10 groups of spines on posterior edge of gnathopods (Andreev 2001), *N. valachicus* has a lower number of these spines. An additional morphological feature separating *N. valachicus* from *N. bulgaricus* is the structure of uropods I and II in males, as in *N. bulgaricus* endopodits are twice longer than exopodits (Andreev 2001).

Niphargus valachicus is a niphargid with wide geographical range associated with the Pannonian-Ponto-Caspian Region (Sket 1981). It is found primarily in the Danube River Basin countries: Slovenia, Croatia, Serbia, Bosnia and Herzegovina, Slovakia, Hungary, Romania and Bulgaria (Sket 1996, Copilas-Ciocianu et al. 2014, Karaman 2014) and with some distant findings further to the East as from Turkey (Akbulut et al. 2001, Fišer et al. 2009a) and Iran (Karaman 1998, Hekmatara et al. 2013). Due to a decreasing population trend, this niphargid taxon is marked as vulnerable according to IUCN (Sket 1996). Copilas-Ciocianu et al. (2014) provided an excellent overview of *N. valachicus* distribution in neighbouring Romania. According to these authors, the main habitats of this amphipod include ponds, canals and slow-flowing rivers with rich vegetation (e.g. *Salix* sp. roots and fallen leaves, submersed aquatic macrophytes, such as *Ceratophyllum* sp., *Myriophyllum* sp. and *Elodea* sp.) found at tributaries of the Danube and the Tisza Rivers and canals of the Danube River delta. In such habitats, this niphargid was often associated with another amphipod, *Synurella ambulans* (F. Müller, 1846) (Cărăusu et al. 1955; Akbulut et al. 2001, Gergely & Angyal 2013, Copilas-Ciocianu et al. 2014). Besides such permanent habitats, *N. valachicus* could be found in temporary waters (Copilas-Ciocianu et al. 2014) and euthropic pools (Gottstein et al. 2000). Niphargids are predators but being less competitive they generally avoid gammarids as cohabitants (Sket 1981, Fišer et al. 2006).

In Serbia, the most recent reported finding of this niphargid dates back to the middle of the 20th century, when S. Karaman (1950) reported *N. valachicus* from swamps of the Godomin Field (near Smederevo). Besides that report, the only other finding of this species in Serbia is from the Makiš, Belgrade Region (S. Karaman 1934).

The aim of this paper is to report the recent distribuition of *Niphargus valachicus* in Serbia and to evaluate its ecological preferences.

Materials and Methods

An extensive macroinvertebrate sampling within the regular annual monitoring of surface waters of Serbia, performed by the Serbian Environmental Protection Agency (SEPA) in the period 2011-2015. A total of 167 water bodies, situated on rivers, canals, lakes and accumulations in Serbia were sampled. Each year sampling was performed twice - in summer and in autumn. An additional sampling was performed in September 2014 in the northern Vojvodina within the Serbian-Hungarian Cooperation Programme field investigation.

The multi-habitat sampling procedure (Hering et al. 2004) and AQEM protocol (AQEM, 2002) were applied and a standard benthological hand net

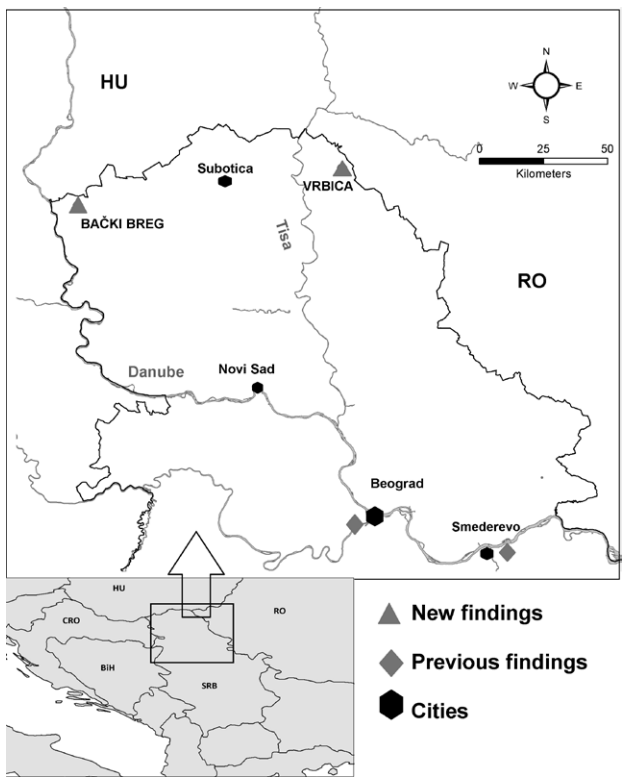

Fig. 1. Localities of *Niphargus valachicus* Dobreanu & Manolache, 1933 in Serbia.

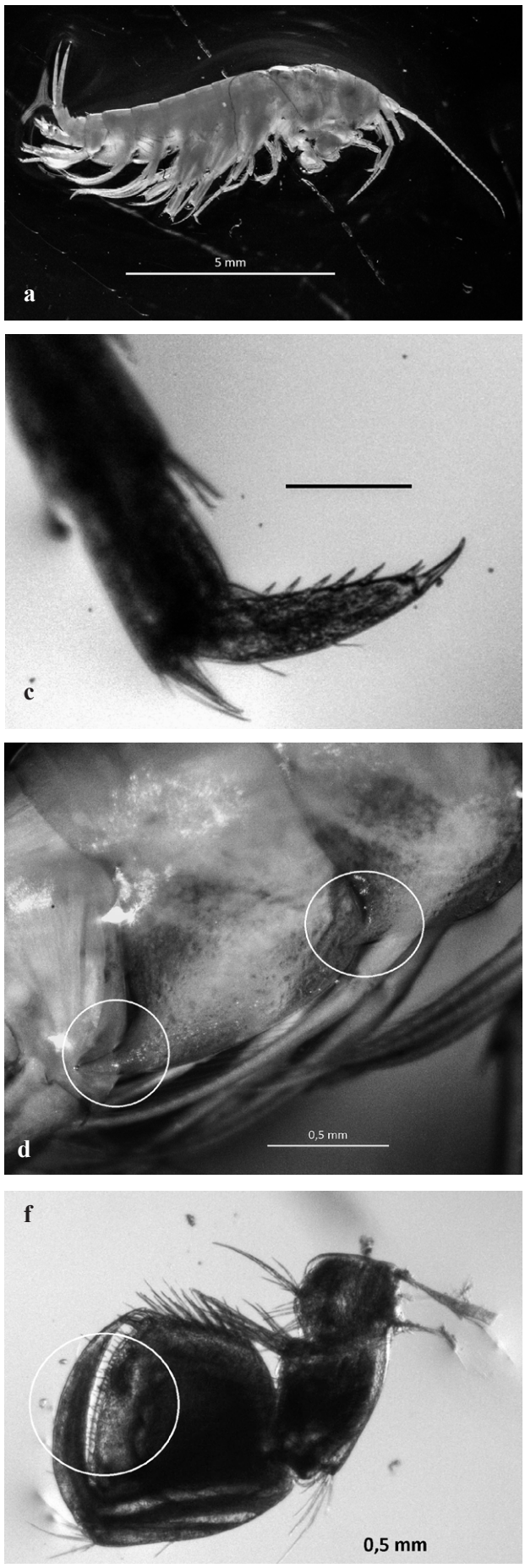

Fig. 2. A. Male specimen of *Niphargus valachicus* from the Zlatica River (Vrbica locality); scale bar 5 mm. B. Uropod III; scale bar 0.5 mm. C. Dactylus of pereiopodes VI; scale bar 2mm. D. Pointed epemeral plates (II and III); scale bar 0.5 mm. E. Uropod I; scale bar 0.5 mm. F. Gnathopod I; scale bar 0.5 mm.

Fig. 3. A typical habitat of *Niphargus valachicus* – a small Pannonian river with plenty of vegetation (the Zlatica River at the Vrbica locality).

Table 1. Selected physical and chemical parameters of water at the Vrbica locality; in bold are parameters used for ecological status assessment according to the national legislation (74/2011).

Physical and chemical parameters (the Vrbica locality)	**05.08.2013**	**Average for 2013**
Temperature (°C)	24.2	13.1
Dissolved oxygen (mg/dm^3)	**1.4**	**7.1**
Oxygen saturation (%)	17	61
Total hardness (mg/dm^3)	318	380
Dissolved CO2 (mg/dm^3)	6.7	5.1
pH	**7.8**	**7.9**
Conductivity (mS/cm)	1241	1430
Ammonia (NH_4-N) (mg/dm^3)	**0.02**	**0.07**
Nitrites (NO_2-N) (mg/dm^3)	0.007	0.01
Nitrates (NO_3-N) (mg/dm^3)	**0.06**	**1.37**
Orthophosphates (mg/dm^3)	**0.29**	**0.23**
Total phosphorus (TP) (mg/dm^3)	**0.39**	**0.34**
Total organic carbon (TOC) (mg/dm^3)	**10.3**	**10.36**
Chlorides (mg/dm^3)	**143**	**164**
BOD-5 (mg/dm^3)	**3**	**2.82**

(25x25 cm, 500 μm mesh size) was used. All the samples were preserved in 70% ethanol for further processing in the laboratory. The identification of the crustacean taxa was done in the laboratory of the Department of Hydroecology and Water Protection of the Institute for Biological Research "Siniša Stanković" (IBISS). A binocular magnifier Carl Zeiss Stemi 2000-C (75x magnification) with digital camera AxioCamERc 5s and ZEN 2011 software were used to photograph the crustaceans. Identified specimens are deposited at IBISS.

Results

Nine specimens of *N. valachicus* were collected from two localities (Fig. 1) in the northern part of Serbia (Vojvodina). Three specimens were collected on 05.08.2013 and six specimens were found in a sample from 26.07.2015 from the Zlatica River (at the Vrbica locality; N 45°58'46.92", E 20°19'44.78",76 m a.s.l., 34 km from the river mouth). Only one specimen was found in the Plazović River (Bački Breg locality; N 45°54'28.00", E 18°58'25.08", 86 m a.s.l., 20 km from the river mouth; 10.09.2014).

As demonstrated by a specimens from the Zlatica River (Fig. 2a), the collected individuals exhibited the main taxonomic characteristics of *N. valachicus*, i.e. uropod III (Fig. 2b), spinulate dactylopodites (Fig. 2c), pointed epimeral plates (Fig. 2d), uropod I (Fig. 2e) and gnathopod I (Fig. 2f).

According to the national watercourse typology (Official Gazette of the Republic of Serbia, 74/2011), both watercourses belonged to the Type 5 – Pannonian Plain watercourses except large rivers with domination of fine sediment. The habitat at the Vrbica locality was characterised by the presence of dense riparian and aquatic vegetation (Fig. 3), including the following macrophyte associations: *Scirpo-Phragmitetum* W. Koch, 1926, *Glycerietum maximae* Hueck, 1931 and *Lemno-Spirodeletum* W. Koch, 1954. Representatives of submerged macrophytes, such as *Ceratophyllum demersum* L., 1753 and *Elodea* Michaux are present as well. Besides *N. valachicus*, other seven taxa, mainly of the orders Coleoptera and Diptera were found (Annual Water Quality Report 2013). The Amphipoda community consisted of two species, *N. valachicus* and *Synurella ambulans* (F. Müller, 1846). An overall ecological status of the locality, based on aquatic macroinvertebrates and physical and chemical characteristics of the water (Table 1), could be assessed as moderate (class III), according to the national legislation (Official Gazette of the Republic of Serbia, 74/2011). Regarding physical and chemical characteristics of the habitat (Table 1), very low dissolved oxygen (probably due to higher temperature and worsened saprobic situation of the water in the summer) indicating very low ecological status could be noted. Considering a broader time scale (i.e. yearly mean values), water temperature and dissolved oxygen content were within the boundaries of the good ecological status. A good ecological status, based on low concentrations of organic nutrients, particularly nitrogen, in the water (on both time scales) was registered.

The Bački Breg locality was characterised by a significantly more diverse macroinvertebrate com-

munity (a total of 42 taxa recorded), including an abundant population of *Asellus aquaticus* (L., 1758). The high taxon diversity suggested better ecological status of this locality, while the mass presence of the isopod *A. aquaticus*, an alfa-mesosaprobic taxon (Maltby 1991, AQEM 2002), could point to a moderate organic pollution.

Discussion

Based on an ecological characterisation and assessment of *N. valachicus* habitats in Serbia, we could conclude that this species is tolerant of moderate level of organic pollution and slightly deteriorated habitats, which is in accordance with literature data. Particularly important is the tolerance of *N. valachicus* to pronounced fluctuations in dissolved oxygen contents (common in smaller Pannonian rivers and canals with dense vegetation). Thus, in Hungary and Romania where *N. valachicus* is rather common, it has been found in various habitats but mostly in smaller canals and swamps (Musko 1994, Borza et al. 2010, Copilas-Ciocianu et al. 2014). Gottstein et al. (2000) reported exclusively findings of this species in eutrophic pools during their study of the Drava and Mura River Basins in Croatia. In regard to ecological preferences of the other crustaceans found coexisting with *N. valachicus* during our study, it should be noted that *S. ambulans* is considered beta-mesosaprobic with preference for lower river stretches (metapotamal), while *A. aquaticus* is as an alpha-mesosaprobic and has no clear preference for any particular habitat type/stream stretch (AQEM 2002). Although *N. valachicus* was not assessed in the AQEM database, regarding its ecological and saprobiological preferences, our findings and the available literature data (Cărăusu et al. 1955, Akbulut et al. 2001, Gergely & Angyal 2013, Copilas-Ciocianu et al. 2014) suggest that it could be classified as metapotamal and beta to alpha-mesosaprobic taxon.

The rather frequent recent records of *N. valachicus* in neighbouring countries, particularly in the Pannonian part of Romania and in Hungary (e.g. Musko 1994, Borza et al. 2010, Copilas-Ciocianu et al. 2014), suggest supposedly wider and more common presence of the species in Serbia as well (primarily in Vojvodina). Although this species tolerates moderate level of anthropogenic impacts and could be found in even temporary waters and ponds, its IUCN status as vulnerable could be justified as its main distribution area (Pannonian Plain) is influenced by many negative and intense anthropogenic pressures (Liška et al. 2008, 2015).

Due to lack of records of epigean niphargids from the territory of Serbia (the latest report dates back from 1950s), our findings represent a contribution to the knowledge regarding these crustaceans in Serbia and the region. Moreover, our results are of importance for the assessment of *N. valachicus* as vulnerable on a global scale (the IUCN Red List). Future studies, including molecular/genetic analysis and more extensive and detailed field surveys, should provide more reliable data regarding presence and status of this rare species in Serbia.

Acknowledgements: We thank Matija Petković (Faculty of Biology, Belgrade University) for his valuable help during the preparation of this manuscript. We are grateful to two anonymous reviewers for their suggestions which helped us to improve the manuscript.

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Received: 11.01.2017
Accepted: 20.03.2017