New records of *Nevrorthus apatelios* H. Aspöck, U. Aspöck & Hölzel, 1977 (Neuroptera: Nevrorthidae) from Bosnia and Herzegovina

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Abstract. During the »Neretva Science Week« in summer 2022, two larvae of *Nevrorthus apatelios* were found in Eastern Bosnia and Herzegovina. These are the first confirmations of the species in the country since a single observation of one adult from Sarajevo almost 100 years ago. New records come from two sites of the upper course of the Neretva River, near Ulog village: one larva was found in a benthic fauna sample collected at the river bottom near Rastovac, while the second was pumped from the river interstitial at depths 30-60 cm, in the area near Cerova. Based on these findings we provide a description of the larva. *Nevrorthus apatelios* lives in highly oxygenated waters, and is a good indicator of clean waters. We discuss the potential reasons for rare observations of the species in the country, and conservation implications in light of ongoing environmental pressures in the currently pristine part of the upper course of Neretva River.

Key words: Nevrorthidae, new locality, Neretva River, conservation


Ključne besede: Nevrorthidae, nova lokaliteta, reka Neretva, varstvo
Introduction

The Nevrorthidae are a small neuropteran family that currently contains 19 described extant species assigned to four genera: *Nevrorthus* Costa, 1863, with five species distributed in the Mediterranean, *Nipponeurorthus* Nakahara, 1958, with 11 species on the Japanese islands, mainland China and Taiwan, *Austroneurorthus* Nakahara, 1958 with two species in eastern Australia, and *Sinoneurorthus* Liu et al., 2012, with one species in mainland China (Aspöck et al. 2017). The disjunct distribution of nevrorthid genera indicates an ancient, wide Pangaean distribution, which was subsequently fragmented by continental drift. Because of the great morphological similarity with fossil Nevrorthidae from Baltic amber, the extant species have even been considered »living fossils« (Aspöck & Aspöck 1994; Wichard et al. 2010).

The life cycle of Nevrorthidae includes aquatic and terrestrial life forms. From the first larval instar to the pupal stage they are bound to aquatic habitats, which is unique for Neuroptera. There are only two more families, Osmylidae and Sisyridae, having partially amphibious larval behavior or having aquatic larvae going ashore to pupate, respectively (Weißmair 1999; Wichard et al. 2009).

Larvae of Nevrorthidae are slender and elongated. They rapidly wriggle forward and backward through the gaps between gravel and coarse sand in fast-flowing streams (Malicky 1984). They are equipped with a complex joint between the head and the elongated anterior part of the pronotum, that allows them to move their head up quickly, but they have no sideways motions of the head. The function of this fast head movement in the life of the larva is not known (Zwick 1967). The conspicuous sucking stylets, a synapomorphy of neuropteran larvae, are curved at the tip and can move against each other. The association of the stylets with a venom gland suggests that they are carnivorous (Gaumont 1976), yet nothing is known about the type of prey (Malicky 1984). Gills are absent and the tracheal system is reduced. The abundance of oxygen in flowing water is apparently sufficient to be absorbed by the cuticle (Zwick 1967). The last instar larva spins a two-layered cocoon on the underside of stones in which air can be stored, covering the pupal body and thus enabling respiration (Wichard et al. 2010). The pupal stage probably only lasts a few days (Malicky 1984). The total length of development has not been appropriately investigated, Malicky (1984) and Popov (2005) estimate a duration of one year with the larva overwintering. The number of eggs and site for egg deposition are unknown (New 1978; Aspöck et al. 2017).
Adults are found on overhanging branches of deciduous trees, in bushes and low vegetation close to the water (Aspöck et al. 2017). Malicky (1984) often found adults on the leaves of alder species sticky with aphid exudates and thought it possible that they were feeding on honeydew. Additionally, the significant number of fungal spores that Monserrat (2005) reported in their digestive tract suggests a glycophagous diet, as some fungi develop in honeydew (Magyar et al. 2005). Furthermore, there is a special formation of the mouthparts, namely a secondary prolongation of the salivary system, which indicates that even dried honeydew can be liquefied and ingested (Randolf et al. 2014).


One of the countries where *Nevrorthus apatelios* had been reported, although only by a single observation, was Bosnia and Herzegovina. The specimen was an adult male, collected in 1929 from Sarajevo (Aspöck et al. 1977). Here we present new findings of the species in the upper Neretva River, during the studies conducted within the »Neretva Science Week« in summer 2022. They are confirming its presence in Bosnia and Herzegovina (BIH) after nearly 100 years since the first report, being the first observations of the larval stages for the country. We discuss the possible reasons for such rare observations of the species in its whole distribution area and conservation implications.

**Materials and methods**

From the 28.6. to 5.7. 2022, samples of benthic and interstitial macroinvertebrates were collected in the upper Neretva River, Eastern BIH, in the stretch of river between the confluence with Krupac River and the city of Konjic (see respective contributions of this special issue for details).

The invertebrates of the river bottom were sampled using the Kick and Sweep (K&S) method, from all microhabitat types using the standardised approach of handnet sampling of aquatic macroinvertebrates (EN 27828:1994). The biological material was transferred to sample containers (size 250, 500 ml) and preserved with 96 or 70% ethanol. Invertebrates were sampled with this method at nine different locations.
The river interstitial was sampled using Bou-Rouch pumping, conducted at multiple points of each gravel bar, and at two different depths to which the metal pole with holes was dug in: 30-60 cm and 60-90 cm. At each point and depth, at least 30 l of water with sediments and organisms was pumped out and filtered through a net of 0.5 and 0.1 mm mesh size. The remaining material was stored in 96% ethanol, keeping the information on each sampling point and depth. All together, five gravel bars of Neretva were sampled with Bou-Rouch pumping for a total of 30 taken samples (see respective contribution of this special issue for details).

All the samples were checked in the laboratories, using the stereomicroscope, and organisms sorted according to taxonomic levels. Identification of the aquatic macroinvertebrates was done based on morphological characteristics to the lowest possible systematic level using appropriate identification keys., ZEISS Stemi 2000C (x50) and Nikon SMZ 800N (x75) binocular stereoscopic magnifiers, ZEISS Axio Lab. A1 (x630) microscope.

Photographs of the specimen were taken with a Leica DFC490 camera attached to a Leica Z16APO optics carrier, using Leica Application Suite V3.8, and were subsequently stacked with ZereneStacker 64-bit, and processed with Adobe Photoshop 7.0.

Results and discussion

Localities of *N. apatelios* in BIH

The only previous locality of the specimen in BIH was reported from »Sarajevo«, with no more details on the labels (Tab. 1; Fig.1). Considering the habitat requirements and behaviour of *N. apatelios*, this adult male was probably collected along a mountain stream in the vicinity of the city. Two new specimens of *N. apatelios* in BIH were found at two localities of the upper Neretva River (Tab. 1; Fig. 1), about 55 km south from Sarajevo. While the first one was found in the water pumped from the 60-90 cm depth of the river interstitial, the second one was found in the sample taken from the bottom of the Neretva River.
Description of the *N. apatelios* larva

Here we describe the morphology of the larva from the Neretva gravel bar at Cerova, since the two larvae do not differ in their morphological characteristics. The larva is slender and about 9 mm long, with thin bristles all over the body, which are up to 2 mm long on the last abdominal segment (Fig. 2). The head is dorsoventrally flattened, the sucking stylets are longer than the head capsule. Head, neck and pronotum are heavily sclerotised, while on the meso- and metanotum only one oval spot is sclerotised on each side. The thoracic sterna are completely unsclerotised. On both sides of the thoracic segments two small pleural sclerites form the articulation sites for the coxae. The abdominal segments, except for the last one, are unsclerotised. They are white, dorsally with a pattern of brownish pigmentation with one light transverse and two light lateral longitudinal stripes.
Table 1. All *N. apatelios* localities in BIH, with two recent ones from summer 2022 sampling at upper Neretva River. Details on sampling points, dates, legators (Leg.) and determinators (Det.) are given. Acronyms refer to: BR – Behare Rexhepi, BT – Bojana Tubić, EP – Ester Premate, HA – Horst Aspöck, HH – Herbert Hözel, MZ – Maja Zagmajster, SA – Stefan Andjus, SR – Susanne Randolf, ŠB – Špela Borko, UA – Ulrike Aspöck, VM – Vojo Milanović, Z – Zerny, NHMW – Natural History Museum Vienna, IBISS – Institute for Biological Research »Siniša Stanković«, Belgrade.


<table>
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<tr>
<th>No.</th>
<th>Locality</th>
<th>Coordinates</th>
<th>Description</th>
<th>Date</th>
<th>Leg.</th>
<th>Det.</th>
<th>Depository</th>
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<tr>
<td>1</td>
<td>Sarajevo, BIH</td>
<td>18.416667, 43.866667</td>
<td>1 adult male</td>
<td>16.7.1929</td>
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<td>2</td>
<td>Neretva gravel bar at Cerova, Ulog, Kalinovik, BIH</td>
<td>18.356472, 43.378611</td>
<td>Bou-Rouch pump at the river bank, depth 60-90 cm (Point 5); 1 larva</td>
<td>1.7.2022</td>
<td>BR,</td>
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<td>3</td>
<td>Neretva River above the old wooden bridge, Rastovac Ulog, Kalinovik, BIH</td>
<td>18.369990, 43.365230</td>
<td>hand-net benthic sample in about 40 cm depth; 1 larva</td>
<td>30.6.2022</td>
<td>SA</td>
<td>BT</td>
<td>IBISS</td>
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Figure 2. *Nevrorthus apatelios*, larva from the Neretva gravel bar at Cerova, dorsal view (photo: H. Bruckner).


Slika 2. *Nevrorthus apatelios*, larva iz neretvanskog šljunka na Cerovi, pogled s leđne strane (foto: H. Bruckner).
Comments of the new findings

The habitat where Nevrorthidae larvae are found is limited to perennial, oxygen rich and clean water systems with gravel in various forms, larger blocks and coarse sand in non-urbanised areas with intact natural vegetation such as maquis, montane forests, or deciduous forests, usually in coastal mountain ranges (Zwick 1967; New 1978; Aspöck & Aspöck 1983; Malicky 1984; Letardi et al. 2005; Jones & Devetak 2009; Gavira et al. 2012; Monserrat & Gavira 2014). The two locations on the Neretva River fit this description – both are situated in the natural river course, surrounded by the well-preserved natural riparian and deciduous forests, with almost no human settlements (Fig. 3). The Nevorthidae are tolerant towards wide ranges of water temperatures, but sensitive to pollution, so they can be regarded as indicators of clean water (Malicky 1984). Their presence in the upper Neretva part supports the pristine and non-polluted nature of this part of the river.

Figure 3. One new locality of *N. apatelios* on the Neretva River at Cerova (Loc. 2, Fig. 1, Tab. 1), with gravel bars and surrounding forests (photo: M. Zagmajster).

Slika 3. Pogled na novo nahajališče vrste *N. apatelios* na reki Neretvi pri Cerovi (Loc. 2, Sl. 1, Tab. 1), s prodišči in okoliščini gozdovi (foto: M. Zagmajster).
Although a high abundance has been reported for some species of Nevorthidae at certain localities (e.g. *N. fallax* in Corsica and Sardinia; Mosely 1932; Zwick 1967; Malicky 1984), this is not likely to be the general case. Nevorthidae are rarely collected even in targeted searches and appear to be very locally distributed (Popov 2002; Jones & Devetak 2009; Gavira et al. 2012; Monserrat & Gavira 2014). This is especially true for *N. apatelios*, having only a few confirmed records for most countries within its range. Even though in some countries of its range, ecological river monitoring is conducted also by standardised monitoring of benthic communities, this is not reflected in more common findings of the species. But, it is most likely that a lack of studies of river invertebrates within Bosnia and Herzegovina is the main reason for the paucity of records and the rediscovery of the species after almost 100 years.

The finding of the larva in a deep interstitial zone indicates that in this developmental stage the species occurs in deeper interstitial habitats, too, which are rarely sampled by regular river monitoring practices. Even if the specimen was pumped in from the surrounding area of the Bou-Rouch pole at 60-90 cm depths, this would still present a subterranean interstitial layer. The slender body of the larvae and known ecology of living between the pieces of sand and gravel support the possibility of the species actively entering this kind of habitat. On the other hand, it is not only the larvae that are rarely found, but also the adults (Letardi et al. 2005, Jones & Devetak 2009). So, if larvae in deep interstitial zones had been overlooked on a large scale, one would expect to find numerous adults – which evidently does not seem to be the case.

General paucity of Nevorthidae records and their sensitivity and dependence on clean waters (Zwick 1967; New 1978; Aspöck & Aspöck 1983; Malicky 1984; Letardi et al. 2005; Jones & Devetak 2009; Gavira et al. 2012; Monserrat & Gavira 2014) may also indicate the possibility of their disappearance due to human activities, as assumed by Aspöck & Aspöck (2010). This makes the two findings within the upper course of the Neretva River even more important, and supports protection of the pristine and unaltered nature of the river and its wider area.

To our knowledge, *N. apatelios* has no conservation status within Bosnia and Herzegovina, nor within the countries in other parts of its range. A first attempt to protect this species was made in Bulgaria, where it is listed as »vulnerable« in the Red Data Book of the Republic of Bulgaria with B2ab(ii) conservation status according to IUCN criteria (Golemanski et al. 2011; Marković et al. 2016). Due to its scattered distribution, rarity of individuals found at the site, and assumed dependence on clean non-polluted habitats, *N. apatelios* would have to be put on the IUCN Red List of Threatened Species. However, more targeted studies, directed toward sampling larvae as well as adult forms, would be needed to achieve a better understanding of species distribution and ecology.
Povzetek

Edini zapis o vrsti Nevrorthus apatelios v Bosni in Hercegovini, odraslem samcu, sega skoraj sto let v preteklost. V Tednu znanosti na Neretvi 2022 sta bili med vodnimi makroskopskimi nevretenčarji najdeni tudi dve ličinki vrste N. apatelios. To je izjemno odkritje, saj gre za redko vrsto, ki se pojavlja le zelo lokalno. Njena redkost in odvisnost ličink od čiste, neonesnažene vode poudarjata pomen varovanja neokrnjene narave te reke in njene okolice.

Sažetak


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