Rediscovery of *Arthroplea congener* Bengtsson, 1909 (Ephemeroptera, Arthropleidae) in the Pannonian lowland in SW Slovakia and the first record of *Ametropus fragilis* Albarda, 1878 (Ephemeroptera, Ametropodidae) from the Ipeľ (Ipoly) river

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Abstract

Although the mayfly fauna of Slovakia is relatively well-researched, there are still many endangered species which occurrence and distribution are relatively poorly known, and their latest records are more than three decades old. Therefore, in this study, we present the rediscovery of such mayfly species Arthroplea congener *in the National Nature Reserve Jurský Šúr in SW Slovakia and bring the first record of* Ametropus fragilis *from the Ipeľ (Ipoly) river, representing the only third known locality in Slovakia.*

Keywords

Ephemeroptera, endangered species, Arthropleidae, Ametropodidae, Central Europe

Introduction

Mayflies (Ephemeroptera) currently consist of more than 3700 species in approximately 450 genera and 42 families (Jacobus et al., 2021). They represent what is left of primitive ancestors (Ephemerida), dating back to the Carboniferous (Sartori & Brittain, 2015). The life cycle of all current representatives consists of aquatic eggs and larvae and terrestrial subadults and adults, with most of the life cycle taking place in an aquatic environment (Bauernfeind & Soldán, 2012).

Mayfly larvae - naiads inhabit almost all freshwater ecosystems except groundwater and heavily polluted water (Bauernfeind & Soldán, 2012). Most species prefer lotic habitats, where their naiads form an essential part of macrozoobenthos biomass (Baptista et al., 2006; Sartori & Brittain, 2015). Their naiads contribute to several processes, such as bioturbation and bioirrigation, decomposition, nutrient cycling, and simultaneously serve as a primary source of nutrients for numerous organisms (Wallace & Webster, 1996; Baptista et al., 2006; Jacobus et al., 2019). However, many of their ecosystem services are currently threatened by degradation and loss of natural habitats caused by hydromelioration, chemical pollution and the spread of invasive species (Carpenter et al., 2011). Due to their cosmopolitan distribution and high-quality water requirements, they also serve as important indicators of ecosystem health (Medina & Vallania, 2001; Nelson & Roline, 2003; Arimoro & Muller,

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2010; Snyder et al., 2014) and an integral part of biomonitoring protocols (Moog et al., 1997; Buffangi, 1997; Derka, 2003a; Menetrey et al., 2007; Mišíková Elexová et al., 2010, 2015).

The level of knowledge of mayfly diversity varies within different geographical regions. While North America and Europe are considered relatively well-known, there are still many undescribed species in other regions (Sartori & Brittain, 2015). The fauna of European mayflies, covering most of the Western Palaearctic, includes 369 species in 48 genera and 19 families (Bauernfeind & Soldán, 2012). The occurrence of 123 species in 33 genera and 16 families is currently reported in Slovakia. More than 60% belong to Baetidae and Heptagenidae, but up to 6 families are represented by only one species (Derka, 2003b; Krno & Derka, 2011). After reassessment of older records, eliminating dubious findings, and incorporating current knowledge of the mayflies taxonomy, we estimate that the number of valid species should be reduced to approximately 105. Concurrently, it is necessary to verify the occurrence of another species that has not been recorded in Slovakia for more than three decades, e.g. *Oligoneuriella polonica* Mol, 1984, *Cercobrachys minutus* (Tshernova, 1952) (Soldán, 1978), *Oligoneurisca borysthenica* (Tshernova, 1937) and *Behningia ulmeri* Lestage, 1929 (Soldán, 1981). The Red List of Slovakian mayflies includes 41 species (Deván, 2001), most of which live in lowland streams (Derka, 2005). Simultaneously, recent findings from Ukraine confirmed the importance of these habitats for rare species of mayflies (Martynov, 2018, 2020). Although the mayfly fauna of Slovakia is relatively well-researched, the actual distribution and population sizes of endangered species are poorly known.

This study, therefore, presents the rediscovery of endangered mayfly *Arthroplea congener* Bengtsson, 1909 in the two localities of Nature National Reserve Jurský Šúr in SW Slovakia after more than three decades (Krno, 1993) and the first record of critically endangered mayfly *Ametropus fragilis* Albarda, 1878 from the Ipeľ (Ipoly in Hungarian) river forming the border between Slovakia and Hungary, which represents the first record from the Ipeľ (Ipoly) river and only third known locality in Slovakia.

Material and methods

Qualitative samplings of mayflies in the Jurský Šúr wetland was performed on 18. April and 29. May 2020, and 5. May 2021, and in the Ipeľ river on 15. May 2021. Sampling from the lotic habitats was performed by standard kicking technique according to Frost et al. (1971) with the hydrobiological net with a mesh size of 0.5 mm. Sampling from the Jurský Šúr lentic habitats was also performed by careful inspection of submerged debris. All specimens were selected on the site and preserved in 96% pure ethanol. Subsequently, all specimens were determined using a stereomicroscope and determination keys Bauernfeind & Humpesch (2001), Krno & Derka (2011).

Sampling area Jurský Šúr wetland is located in the NW part of Panonian lowland in close contact with the SW part of Carpathian Mts. It spreads on the area of 665 ha in altitude about 130 m a. s. l. Freshwater habitats are represented by peat bogs, swamps in alder forests, gravel pits, artificial channels, streams, ponds and several periodic water bodies of various types (Janský & David, 2010; Majzlan & Vidlička, 2010). In this study, we investigated three gravel pits (Štrkovisko 1 - Š1, 48°13'38.2"N, 17°12'06.89"E;

Štrkovisko 2 – Š2, 48°13'38.78"N, 17°12'17.57"E; Štrkovisko 3 – Š3, 48°13'37.89"N, 17°12'18.96"E), fish pond Šúrsky rybník – ŠR (48°13'50.05"N, 17°12'20.09"E) stream Račí potok – RP (48°13'41.62"N, 17°11'50.58"E), channel Blatina – BL (48°13'58.64"N, 17°12'11.27"E), and outflow channel Blahutov kanál – BK (48°14'07.84"N, 17°12'28.89"E), situated in the alder forest (Figures 1A, B).

The Ipeľ river is a left side Danube tributary situated in the southern part of central Slovakia. The essential part of the middle and lower reaches forms a natural border between Slovakia and Hungary (Figures 1A, C). The river's catchment area is 5151 km^2, with its dominant part (3648 km^2) in the territory of Slovakia (Fekete, 1972). In this study, we investigated three localities in the lowland reaches Ipeľ – Leľa (IL; 47°51'52.71"N, 18°47'32.03"E), Ipeľ – Kubáňovo (IK; 48°03'26.39"N, 18°48'45.83"E) and Ipeľ – Tešmák (IT; 48°03'19.57"N, 18°59'10.93"E).

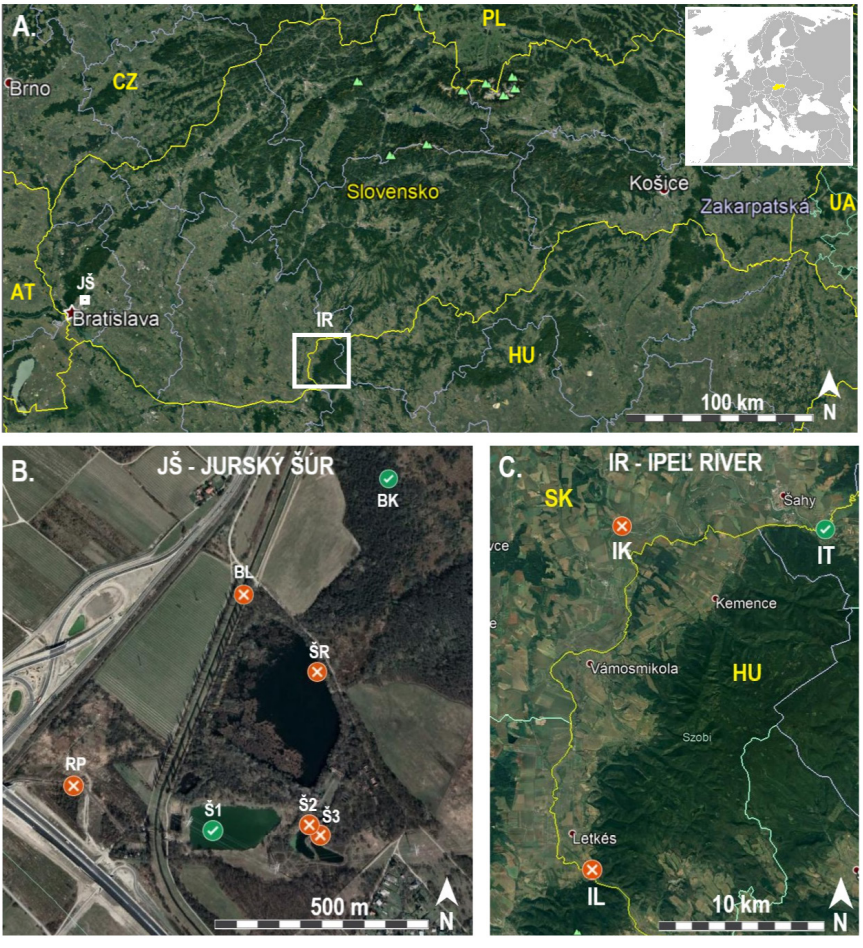

Figure 1. A. Map of Slovakia highlighting the study sites by squares (JŠ – Jurský Šúr, IR – Ipeľ river), B. Detail map of Jurský Šúr with exact sampled sites (RP – Račí

potok, Š1 – Štrkovisko 1, Š2 – Štrkovisko 2, Š3 – Štrkovisko 3, ŠR – Šúrsky rybník, BK – Blahutov kanál), C. Detail map of exact sampled sites in Ipeľ river (IL – Ipeľ Leľa, IK – Ipeľ Kubáňovo, IT – Ipeľ Tešmák). Legend: localitis with ✗ absent and ✓ present individuals of studied species.

Results and discussion

Rediscovery of *Arthroplea congener* Bengtsson, 1909 in Jurský Šúr

The occurrence of *Arthroplea congener* was confirmed in two out of seven investigated localities in Jurský Šúr wetland, which represents the only verified locality of this mayfly in Slovakia. The last findings date back to 1988 when six larvae from the Šúrsky rybník (fish pond) were recorded (Krno, 1993). One adult was allegedly recorded in the Eastern Slovakia on the lake Izra in the Slanské vrchy Mts. (Krno *in verb*), but our recent investigation did not confirm this finding. Naiads sampling is quite complicated because individuals often remain motionless and easily escape attention (Landa, 1969). It was confirmed during sampling in sampled site (Štrkovisko 1, Figures 1B, 2A), where, despite our great effort, we recorded only five naiads. However, we found an abundant population in shallow wetlands in the alder forest irrigated by the Blahutov channel (Figures 1B, 2B). This site represents a new locality of occurrence of *A. congener* in the area of Jurský Šúr. Here, the outflow channel from the neighbouring Šúrsky rybník spill between the roots of alder and cattail (*Typha* sp.) and creates a wetland with shallow, almost stagnant water (Figure 2B). The tiny brown-black naiads of *A. congener* were attached to the submerged twigs and leaves, where they became almost invisible. On the contrary, the fish-shaped niads of the other mayfly *Siphlonurus aestivalis* Eaton, 1903 were quite conspicuous and presented in relatively high abundance. Our subsequent investigations also confirmed the occurrence of the mayfly *Paraleptophlebia werneri* Ulmer, 1920.

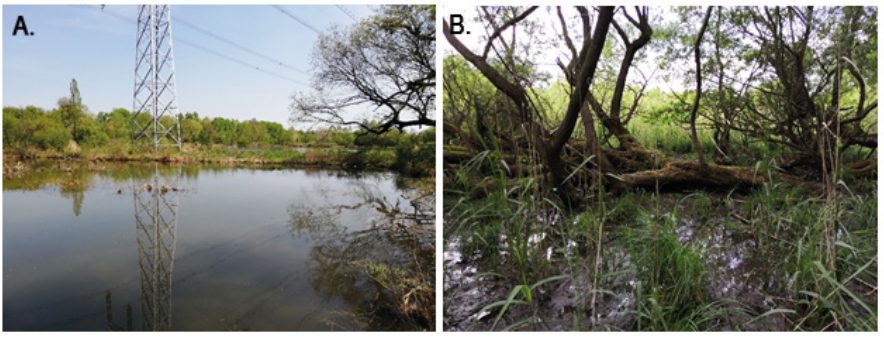

Figure 2. A. Gravel pit (Štrkovisko 1) with the occurrence of *A. congener*. B. Alder swamp (Blahutov kanál) with the occurrence of the abundant population of *A. congener.*

The morphology of *A. congener* larvae is unique and cannot be misinterpreted (Figures 3A, B). The highly specialized mouthparts form a filtering basket with long two-segmented maxillary palps, clearly visible in the dorsal view (Bauernfeind

& Soldán, 2012). The first segment extends behind the head and reaches the mesonotum. The second segment is exceptionally long, thin and sickle-shaped with numerous setae. Naiads move them intensively with a frequency of 50 up to 250 movements per minute, depending on the age. This way, *A. congener* actively filters food from the water column, including small crustaceans and rotifers. Concurrently, the enormous power of the maxillary palps is used to swim in defence, as if they were jumping and escaping predators (Soldán, 1979).

The development of *A. congener* is fast and short, lasting approximately two months. In the Jurský Šúr, the first naiads hatch in April and disappear at the beginning of June. The rest of the year they spend in the egg diapause. Their development in northern Europe is shifted, and it takes time from the beginning of May to July or August (Bauernfeind & Soldán, 2012).

The family Arthropleidae is defined as monophyletic, with *A. congener* and *A. bipunctata* (McDunnough, 1924) in Europe and North America, respectively (Bauernfeind & Soldán, 2012). The distribution of *A. congener* is Palearctic with a continuous area ranging from Scandinavia through Northern Russia to Western Siberia. In this area, *A. congener* represents a typical lowland species but exceptionally occurs outside the lowlands. Simultaneously, it occurs in the United Kingdom and Central Europe, including Germany, Austria, Czech Republic, and Slovakia. Here, it inhabits localities from the lowlands to approximately 800 m a.s.l. Generally, the distribution of *A. congener* roughly follows the last glaciation's southern limits (Bauernfeind & Soldán, 2012).

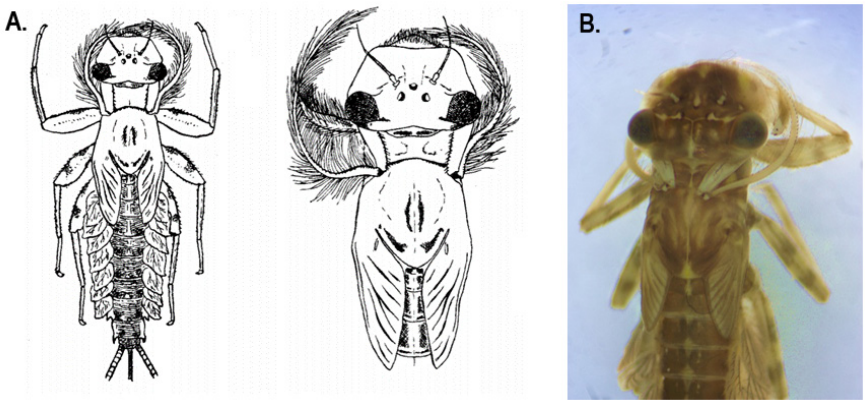

Figure 3. A. The original larvae illustration of *A. frankenbergeri* (synonym of *A. congener*) from the Jurský Šúr by Balthasar (1937), B. Larvae of *A. congener* recently sampled in the Jurský Šúr.

The population of *A. congener* in Jurský Šúr was first discovered by Balthasar (1937), who originally described it as a new species *A. frankenbergeri* (Figure 3A). However, Landa (1954) compared material from Central Europe with the description of *A. frankenbergeri* and the original description of *A. congener* and concluded that the *A. frankenbergeri* probably represent a junior synonym of *A. congener*. Currently,

A. frankenbergeri is considered a junior subjective synonym of *A. congener* (Landa, 1969; Bauernfeind & Soldán, 2012). Therefore, the Central European populations probably represent glacial relicts, not endemic species.
Finally, our newly discovered locality in the alder forest probably represents the site where most of the population of *A. congener* survives. Previously published rare findings from the other sites in the Jurský Šúr (Balthasar, 1937; Landa, 1969; Krno, 1993) probably represented the peripheral areas of the *A. congener* population or an accidental occurrence.

The first record of *Ametropus fragilis* Albarda, 1878 from the Ipeľ river
Four fully developed larvae of *A. fragilis* were found in the Ipeľ river near the Tešmák (Figures 1C, 4). The occurrence of this species was not confirmed in the two other sampled localities near the Leľa and Kubáňovo (Figure 1C). Although the Ipeľ river has been subjected to relatively intensive hydrobiological research (Landa, 1969; Landa & Soldán, 1989; Kovács et al., 2002; Kovács & Kovács, 2006; Kovács, 2011; Mišíková Elexová et al., 2010, 2015), this species was not recorded. The first record of *A. fragilis* from Slovakia was published by Soldán (1978), who recorded one male adult and one female subadult at the Latorica river near the Leles, South-East Slovakia. Recent data confirm its occurrence from the Tisa river near the Malé Trakany (Mišíková Elexová et al., 2010, 2015), which represents the border river between Hungary and Slovakia. Therefore, our findings represent the third known locality of *A. fragilis* in Slovakia. We can speculate whether the population represents a relict of a former larger population decimated by river regulations and contamination or a current dispersion from the Danube river. The first option seems more likely because the species is considered extinct in the Danube (Kovács, 2011).

Figure 4. Ipeľ river near the Tešmák with the occurrence of *A. fragilis*.

The morphology of *A. fragilis* larvae, as the only known European representative of the family Ametropodidae, is unique thanks to the relatively short forelegs with curved claws and characteristic ventral process on coxa (coxal pad, Figures 5A, B). Fish-shaped larvae reach a body length of 13 to 16 mm. Body colouration is light brownish with a reddish-brown triangular spot or mediolongitudinal line, more detailed description in Bauernfeind & Soldán (2012).

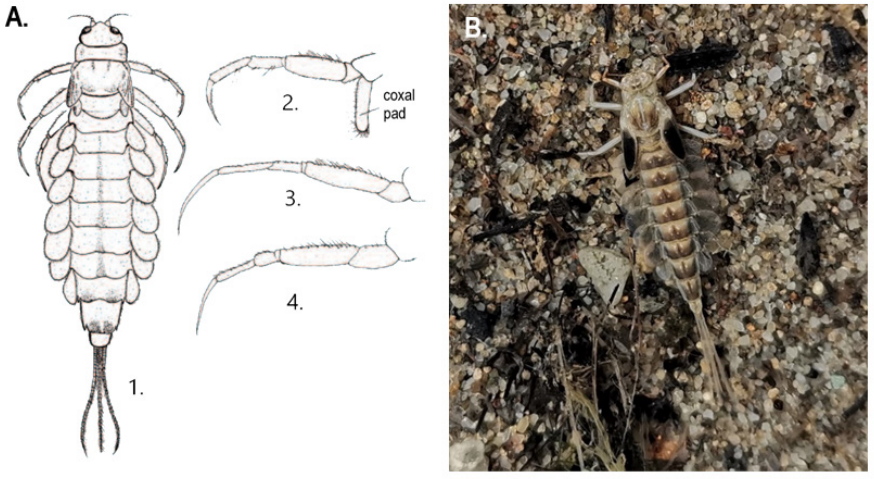

Figure 5. A. The illustration of *A. fragilis* larvae by Wang et al. (2013). Legend: 1. habitus (dorsal view), 2. foreleg, 3. midleg, 4. hindleg, B. Larvae of *A. fragilis* from the Ipeľ river.

The growth of the larvae takes place from August to May, and adults occur in late April and May. The larvae are psamophilous and sand-dwelling and usually occur near the river banks with the preferred microhabitats such as sand with an admixture of detritus, clay, gravel, submerged logs, alder twigs, cones or accumulations of leaves. Larvae swim exceptionally quickly but usually remain hidden in the substratum (semi-burrowers), only antennae and eyes extruding from the bottom (Jadzewska, 1973; Bauernfeind & Soldán, 2012). Jadzewska (1973) also reported that the most accompanying species are larvae of mayfly *Ephemera lineata* Eaton, 1870, dragonfly of *Gomphus* Leach, 1815 and the waterbug *Aphelocheirus aestivalis* (Fabricius, 1794), and we confirmed her observations. Simultaneously at the sampling site, we recorded other ten mayfly species: *Baetis buceratus* Eaton, 1870, *B. fuscatus* (Linnaeus, 1761), *Procloeon bifidum* (Bengtsson, 1912), *Potamanthus luteus* (Linnaeus, 1767), *Heptagenia sulphurea* (Müller, 1776), *H. longicauda* (Stephens, 1836), *Ephemerella ignita* (Poda, 1761), *Paraleptophlebia submarginata* (Stephens, 1835), *Caenis luctuosa* (Burmeister, 1839) and *Brachycercus harrisellus* Curtis, 1835.

The worldwide monophyletic family Ametropodidae includes only one genus with five nominal species, and *A. fragilis* represents the only known Holarctic species (Bauernfeind & Soldán, 2012). The distribution of *A. fragilis* includes North-Western Canada and the USA, Central and Eastern Europe, Ural Mts. and Siberia (subspecies *A. eatoni*). The distribution in Europe covers Estonia, Latvia, Lithuania, Poland, Germany, Austria, Slovakia, Hungary, Italy, Bulgaria and Romania (Thomas & Belfiore, 2013) and Croatia (Ćuk et al., 2015).

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