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# The occurrence of branchiobdellidans on stone crayfish (*Austropotamobius torrentium*) in the Czech Republic

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**Abstract –** The stone crayfish *Austropotamobius torrentium* (Schrank, 1803) is one of only two native crayfish species in the Czech Republic. Epibiotic branchiobdellidans present on this crayfish species have not been studied before in the Czech Republic. Here we evaluate the occurrence, and basic population characteristics of branchiobdellidans inhabiting *A. torrentium* in 12 small streams in the Czech Republic. Three *Branchiobdella* species were found: *Branchiobdella parasita*, and *B. pentadonta* dominated. *B. hexadonta* was found only exceptionally, as we did not focus on species inhabiting the gill chamber. The overall abundance varied between 0.3 and 17 individuals per crayfish. The mean length of *B. parasita* was 3.58 (1.1–7.1) mm, of *B. pentadonta* 2.00 (0.9–4.9) mm, and of *B. hexadonta* 1.73 (1.4–2.2) mm.

**Keywords:** *Austropotamobius torrentium* / epibionts / branchiobdellidans / Czech Republic

**Résumé – Occurence de branchiobdellidés sur l'écrevisse des torrents (*Austropotamobius torrentium*) en République tchèque.** L'écrevisse des torrents *Austropotamobius torrentium* (Schrank, 1803) est l'une des deux espèces d'écrevisses natives de la République tchèque. Les branchiobdellidés épibiotiques présents sur cette espèce d'écrevisse n'ont pas été étudiés avant en République tchèque. Ici, nous évaluons l'apparition et les caractéristiques démographiques de base des branchiobdellidés épibiontes d'*A. torrentium* dans 12 petits cours d'eau en République tchèque. Trois espèces de Branchiobdelle ont été trouvées: *Branchiobdella parasita* et *B. pentadonta* ont dominé. *B. hexadonta* n'a été trouvée que de façon exceptionnelle, car nous ne nous sommes pas concentrés sur les espèces qui occupent la chambre branchiale. L'abondance globale variait entre 0,3 et 17 individus par écrevisse. La longueur moyenne de *B. parasita* était de 3,58 (1,1-7,1) mm, de *B. pentadonta* 2,00 (0,9-4,9) mm et de *B. hexadonta* 1,73 (1,4-2,2) mm.

**Mots-clés :** *Austropotamobius torrentium* / epibionte / branchiobdellidé / République Tchèque

## 1 Introduction

The stone crayfish *Austropotamobius torrentium* (Schrank, 1803) is one of only two native crayfish species in the Czech Republic (Souty-Grosset *et al.*, 2006; Kouba *et al.*, 2014). Presently, this species inhabits more than 40 streams in the western part of Bohemia, in Central Bohemia (streams in the Landscape Protected Area (LPA) Křivoklátsko and a few streams near the town of Prague), as well as in Bohemian Uplands (isolated localities in LPA České středohoří) and one introduced locality in the Giant Mountains (Kozák *et al.*, 2002; Vlach *et al.*, 2009; Petrusek *et al.*, 2017, see Fig. 1). The stone crayfish is a critically endangered species in the Czech Republic according to Czech law (114/1992), has an IUCN "data deficient" status (Füreder *et al.*, 2010) and is among priority species given by the Council Directive 92/43/EEC.

Branchiobdellidans are considered to be epizootic commensals with a transition to facultative parasitism or mutualistic cleaners, and they prefer to live on crayfish (Govendich *et al.*, 2010; Rosewarne *et al.*, 2012; Skelton *et al.*, 2013). These epibionts mainly invade the body surface, gills, and occasionally the gill chamber, using a sucker (Bádr, 2000; Ďuriš *et al.*, 2006; Lee *et al.*, 2009; Skelton *et al.*, 2013). The influence of branchiobdellidans can be negative if they feed on crayfish tissues, and in extreme cases they can even kill the crayfish (Bádr, 2000; Souty-Grosset *et al.*, 2006; Rosewarne *et al.*, 2012).

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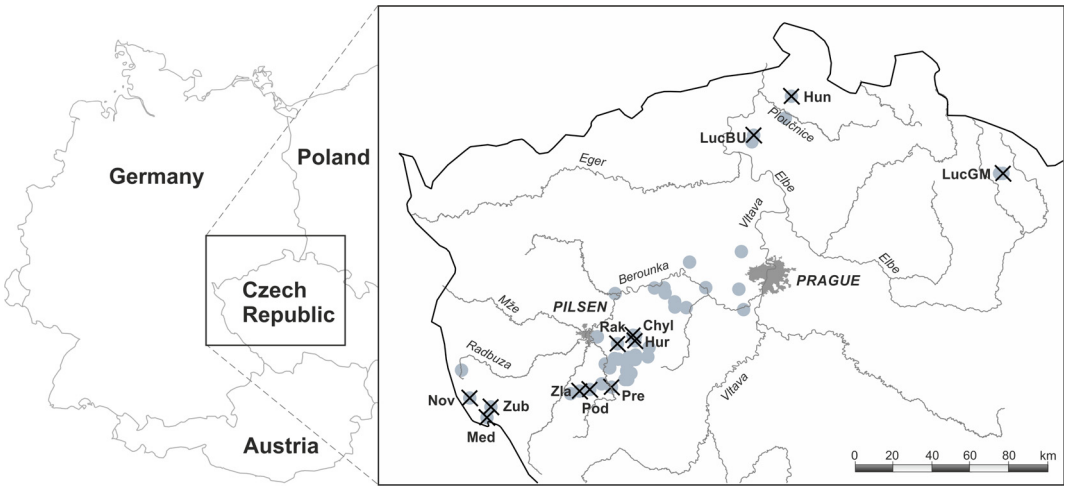


**Fig. 1.** Map of the Czech Republic with the locations of the examined streams. Grey circles: occurrence of *A. torrentium* in the Czech Republic according to Vlach *et al.* (2009), crosses: the examined streams (for abbreviations see Tab. 1).

However, other studies have found a positive influence associated with the removal of algae from crayfish gills (Lee *et al.*, 2009; Skelton *et al.*, 2013). In addition, there are studies describing a strong mutualistic relationship between branchiobdellidans and crayfish, expressing the fact that the high abundance of worms leads to good body condition of crayfish (Védia *et al.*, 2016).

Branchiobdellidans live in the holarctic region (Gelder, 1999; Füreder *et al.*, 2009). There are eight endemic species in Europe: *Branchiobdella parasita* (Braun, 1805), *B. astaci* Odier, 1823, *B. hexadonta* Gruber, 1883, *B. italica* Canegallo, 1928, *B. pentadonta* Whitman, 1882, *B. balcanica* Moszyński, 1938 (Gelder, 1999; Füreder *et al.*, 2009; Subchev and Gelder, 2010; Subchev, 2012, 2014), *B. kozarovi* Subchev, 1978 (Subchev, 1978, 2014; Fard and Gelder, 2011) and *B. papilosa* Nesemann and Hutter, 2002 (Nesemann and Hutter, 2002; Subchev, 2011).

Branchiobdellidans in the Czech Republic have not been studied intensively in the past, with only a few studies concerning these worms on *Astacus astacus* (Straškraba, 1956; Bádr, 2000) and *Orconected limosus* (Ďuriš *et al.*, 2006). An important contribution to Czech branchiobdellidans was made by Subchev (2012), who studied worms on crayfish from a collection in the London Natural History Museum. Moreover, there are some "grey" works focused on this topic, *e.g.*, Nováková and Fialová (2012), Šrámková (2014) or Ložek (2015). Most of these data were also reviewed by Subchev (2014). Based on these papers six species have been identified so far in the Czech Republic: *Branchiobdella astaci*, *B. balcanica*, *B. hexadonta*, *B. italica*, *B. parasite* and *B. pentadonta* (Straškraba, 1956; Bádr, 2000; Ďuriš *et al.*, 2006; Subchev and Gelder, 2010; Subchev, 2012, 2014).

The occurrence of branchiobdellidans on the stone crayfish *A. torrentium* was studied mainly in Southern and Eastern Europe, *e.g.*, in Greece (Subchev *et al.*, 2007), Macedonia (Subchev, 2012; Rimcheska *et al.*, 2014), Albania (Subchev, 2011), Croatia (Klobučar *et al.*, 2006) and Hungary (Kovács and Juhász, 2007). There is also some information from Central Europe, *e.g.*, Germany (Subchev, 2013) and Austria (Füreder *et al.*, 2009). Concluding the results of the above-mentioned papers, only three branchiobdellans were recorded on *A. torrentium*: *B. parasita*, *B. pentadonta* and *B. hexadonta*. However, two more *Branchiobdella* species, *B. italica* and *B. astaci* were found on a related species, *A. pallipes*, in Italy (Gelder, 1999; Mori *et al.*, 2001; Gherardi *et al.*, 2002; Oberkofler *et al.*, 2002; Skalici *et al.*, 2010), Croatia (Klobučar *et al.*, 2006) or in the UK (Rosewarne *et al.*, 2012).

Here we report the occurrence and basic morphometry of branchiobdellidans on the stone crayfish *A. torrentium* in the Czech Republic, based on newly determined data from worm collections that were analysed in the unpublished works of Nováková and Fialová (2012) and Šrámková (2014).

## 2 Material and methods

Branchiobdellidans were collected from 2011 to 2013. In total, worms from 12 stream inhabited by *A. torrentium* (from more than 40 of these streams in the Czech Republic, see Vlach *et al.*, 2009) were analysed. The names and abbreviations of the streams, as well as the number of crayfish used for analyses are given in Table 1, while the basic morphological character of the examined streams are described in Vlach *et al.* (2009).

At each locality, individuals of *A. torrentium* were caught using a standard method consisting of searching through all the possible shelters (*e.g.*, turning over stones) using hands and a hand net. Crayfish were collected as a part of standard *A. torrentium* monitoring programme led by Nature Conservation Agency of the Czech Republic (collectors were also the owners of the exception to the Czech law no. 114 enabling them to manipulate with this critically endangered species). From the crayfish caught, a set of 9–19 specimens was chosen depending on the availability of crayfish in each stream. Following the method of Gelder *et al.* (1994), individuals of this set were anaesthetized in a solution of brook water and carbonated water (bottled mineral water) mixed in a ratio of 1:1 for 2 min. The total length of each crayfish (TL) was measured using a calliper. Individuals of branchiobdellidans dwelling on the crayfish surface (not in the gill chamber) were then removed with tweezers and fixed in 70% ethanol.

**Table 1.** The examined localities, abbreviations, numbers of *A. torrentium* analysed, the mean total length in mm (TL) and standard deviation (SD) of *A. torrentium* from which branchiobdellidans were collected.

| Locality | Abbreviation | *N* | TL (mm) | SD |
|---|---|---|---|---|
| Luční brook (Giant Mnts.) | LucGM | 15 | 66.8 | 16.4 |
| Huníkovský brook | Hun | 14 | 60.2 | 9.9 |
| Luční brook (Bohem. Uplands) | LucBU | 15 | 63.7 | 8.0 |
| Chýlava | Chyl | 11 | 55.1 | 11.2 |
| Hurecký brook | Hur | 15 | 56.4 | 10.7 |
| Rakovský brook | Rak | 9 | 42.3 | 9.6 |
| Přešínský brook | Pre | 15 | 59.7 | 8.1 |
| Podhrázský brook | Pod | 15 | 64.9 | 8.3 |
| Zlatý brook | Zla | 15 | 54.8 | 8.5 |
| Zubřina | Zub | 18 | 63.7 | 16.1 |
| Novosedlský brook LT | Nov | 19 | 52.4 | 11.1 |
| Medvědí brook | Med | 19 | 48.6 | 10.0 |

**Table 2.** Numbers of individual species, total numbers and the mean abundance of branchiobdellidans per one crayfish in the studied streams (Bpar: *B. parasita*, Bpen: *B. pentodonta*, Bhex: *B. hexadonta*, and BpC: the mean number of branchiobdellidans per one crayfish specimen).

| Locality | Bpar | Bpen | Bhex | Total | BpC |
|---|---|---|---|---|---|
| Luční brook (Giant Mnts.) | 86 | 35 | 0 | 121 | 8.1 |
| Huníkovský brook | 57 | 20 | 3 | 80 | 5.7 |
| Luční brook (Bohem. Uplands) | 48 | 1 | 0 | 49 | 3.3 |
| Chýlava | 46 | 45 | 0 | 91 | 8.3 |
| Hurecký brook | 25 | 4 | 0 | 29 | 1.9 |
| Rakovský brook | 25 | 6 | 0 | 31 | 3.4 |
| Přešínský brook | 71 | 6 | 0 | 77 | 5.1 |
| Podhrázský brook | 22 | 0 | 0 | 22 | 1.5 |
| Zlatý brook | 15 | 1 | 1 | 17 | 1.1 |
| Zubřina | 14 | 292 | 0 | 306 | 17.0 |
| Novosedlský brook LT | 35 | 0 | 0 | 35 | 1.9 |
| Medvědí brook | 3 | 2 | 0 | 5 | 0.3 |
| Total | 447 | 412 | 4 | 863 | 4.8 |

The identification of all removed branchiobdellidans was performed using an optical microscope (magnification 100–400×, Intraco Micro LM 600) on the basis of body shape and morphology, the configuration of cuticle teeth, as well as the shape of the spermathecal, as used by, *e.g.*, Subchev (1984) and Gelder *et al.* (1994), while taking into account new European species and their morphology (Nesemann and Hutter, 2002; Subchev and Gelder, 2010; Subchev, 2014).

The normality of sample distribution was examined using the Kolmogorov–Smirnov test. The length structures of stone crayfish from individual streams were compared using nonparametric one-way analysis of variance (Kruskal–Wallis ANOVA). The length structure of particular *Branchiobdella* species was also compared using one-way analysis of variance (ANOVA). A multiple comparison was also made using Tukey–Kramer test. All tests were carried out at a significance level of 5% ($P < 0.05$) using the NCSS 9.0 software pack.

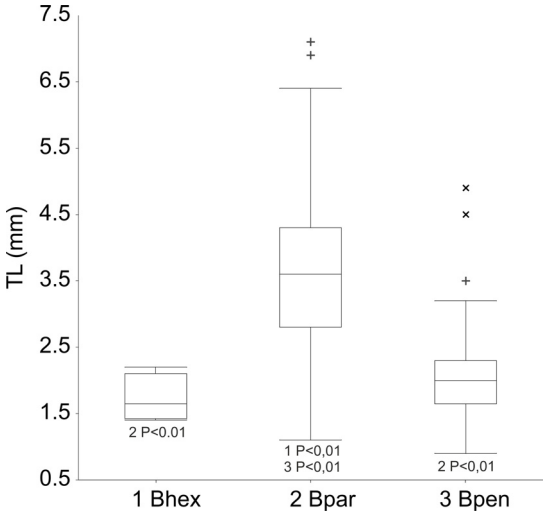


**Fig. 2.** The length structure of particular *Branchiobdella* species. Bhex: *B. hexadonta*, Bpar: *B. parasita*, and Bpen: *B. pentadonta* (median, inter-quartile range (IQR), the largest value/the smallest value smaller/bigger than 1.5 times IQR, extremes + lower (×higher) than 3 times IQR).

## 3 Results

Branchiobdellidans were found at all localities where crayfish were caught and examined. A total of three *Branchiobdella* species were recorded: *B. parasita* and *B. pentadonta* dominated, while *B. hexadonta* occurred exceptionally.

*B. parasita* occurred at all observed localities and dominated at most (LucGM, Hun, LucBU, Rak, Pre, Pod, Zla, Nov). In two streams, there were equal occurrences of *B. parasita* and *P. pentadonta* (Hur, Med), and *B. pentadonta* dominated in one stream (Zub). *B. hexadonta* was observed in only two streams (Hun, Zla). The mean abundance of branchiobdellidans per one crayfish individual varied between 0.3 (Med) and 17.0 (Zub). For more details see Table 2.

In addition, the body lengths of particular branchiobdellidans differed significantly ($H = 216.32$, $P < 0.01$, Fig. 2). The mean length of *B. parasita* was 3.58 mm (minimum 1.1–maximum 7.1), which was significantly larger than other species (Tukey–Kramer multiple test, $P < 0.01$). The mean length of *B. pentadonta* was 2.00 (0.9–4.9), and the mean length of *B. hexadonta* was 1.73 (1.4–2.2). The body lengths of these species did not significantly differ ($P = 0.85$).

## 4 Discussion

Six species of *Branchiobdella* species have been found so far in the Czech Republic. Historically, Straškraba (1956) described *B. hexadonta* and *B. pentadonta*. Bádr (2000) found *B. parasita* and *B. italica* on *A. astacus*. However, the identification of *B. italica* was only carried out on the basis of

cuticle teeth, and was later corrected by Čermáková and Bádr (2002). The occurrence of *B. italica* was also reported by Nováková and Fialová (2012), with the same misidentification; all *B. italica* reported from the Přešínský brook were in fact *B. pentadonta*. In fact, *B. italica* have not yet been found on *A. torrentium*, but on *A. pallipes* in Italy and Croatia (Gelder, 1999; Mori *et al.*, 2001; Gherardi *et al.*, 2002; Oberkofler *et al.*, 2002; Klobučar *et al.*, 2006; Skalici *et al.*, 2010).

Ďuriš *et al.* (2006) reported *B. parasita*, *B. pentadonta*, *B. hexadonta* and *B. balcanica* on *O. limosus* in the Elbe River in Obříství (Central Bohemia). Subchev (2012) recently studied crayfish from the Czech Republic in the London Natural History Museum collections; in this collection he found three species (*B. pentadonta*, *B. hexadonta* and *B. parasita*) and also found *B. astaci* on the noble crayfish *Astacus astacus*. This was the first description of this *Branchiobdella* species from the Czech Republic.

In this study we identified *B. parasita*, *B. pentadonta* and *B. hexadonta*. No other branchiobdellans have been found on *A. torrentium* as mentioned by many authors (Klobučar *et al.*, 2006; Kovács and Juhász, 2007; Subchev *et al.*, 2007; Füreder, 2009; Subchev, 2011, 2012, 2013; Rimcheska *et al.*, 2014; also reviewed by Subchev, 2014).

The most common species at each locality were *B. parasita* and *B. pentodonta*. These species are among the most common species of the *Branchiobdella* genus in Europe, and their occurrence has been reported by many authors in different European countries (*e.g.*, Gelder *et al.*, 1994; Ďuriš *et al.*, 2006; Klobučar *et al.*, 2006; Subchev *et al.*, 2007; Subchev and Gelder, 2010; Subchev, 2012 and reviewed by Subchev, 2014).

*Branchiobdella hexadonta* is also very common European branchiobdellidans (Subchev, 2014). We found only a few individuals of this species, as we only collected worms dwelling on the crayfish body and not in the gill chamber where this species usually occur if present (Skelton *et al.*, 2013; Subchev, 2014). The species was recorded for the first time on *A. torrentium* in the Czech Republic. *B. astaci* is another species inhabiting the gill chamber (Subchev, 2014) and Šrámková (2014) mentioned the occurrence of this species in the left tributary of the Novosedlský brook. Nevertheless, this individual was identified incorrectly and the studied worm was most likely *B. parasita* (M. Subchev, in lit.). In fact, the presence of *B. astaci* in the Czech Republic has only been recorder once (Subchev, 2012). On the other hand, *B. astaci* was frequently found in the UK on the related crayfish, *A. pallipes*, as this worm is the only European one living in the UK, except for two invasive species *Xironogiton victoriensis* and *Cambarincola* aff. *okadai* inhabiting introduced *Pacifastacus leniusculus* (James *et al.*, 2015) there.

Despite the findings of Ďuriš *et al.* (2006) and other findings of *B. balcanica* reported in many Central European countries by Subchev (2014), we were unable to confirm the occurrence of this worm. Taking into account the Balcan origin of the *A. torrentium* in Luční brook in the Giant Mountains (Petrusek *et al.*, 2017), we assumed a possibility of detecting *B. balcanica* there. Nevertheless, *B. balcanica* has never been reported as being present on *A. torrentium*. *B. kozarovi* has also never been found on *A. torrentium*, and its presence has never been observed in the Czech Republic. This is not surprising considering its East European distribution (Kolesnikova and Utevsky, 2003; Fard and Gelder, 2011; Subchev, 2012, 2014).

In conclusion, only three *Branchiobdella* species were found on *A. torrentium* until now. However, as other *Branchiobdella* species are present on ecologically similar or related crayfish species, inhabiting the same watershed or living sympatrically, there is no reason not to expect the presence of other species.

The mean abundance of branchiobdellidans in the particular streams studied here varied significantly, but the values are comparable to abundances mentioned in other papers. Skalici *et al.* (2010) reported 6.9 individuals per crayfish in northern Italy, while Gelder *et al.* (1994) even found abundances in the range of 9.8–20 branchiobdellidans per one crayfish. The largest population was observed in Greece, with an abundance of 145.5 individuals per one crayfish (Subchev *et al.*, 2007). This variability is not surprising as the branchiobdellidans abundance depends on many factors; Védia *et al.* (2016) pointed out the positive relationship between biochemical oxygen demand, phosphates, coliforms, potassium and branchiobdellans abundance. Moreover, a higher abundance of these worms on large crayfish individuals is known generally, providing them with more surface for their life and reproduction and also avoiding frequent molting (Mori *et al.*, 2001; DeWitt *et al.*, 2013; Védia *et al.*, 2016).

The body lengths of branchiobdellidans found here mostly correspond to those from other studies (Subchev, 1984, 2014; Füreder *et al.*, 2009).

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