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SHORT COMMUNICATION

Native European branchiobdellids on non-native crayfishes: Report from the Czech Republic

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ABSTRACT

Invasive alien species present a global threat to biodiversity, particularly where pathogens and symbionts are involved. Non-native crayfish species can increase their impact on the host ecosystem through introductions of symbiotic fauna. Conversely, non-native crayfishes could serve as hosts for native epibionts, thus substituting for disappearing native crayfishes as well. Here we report the presence of native branchiobdellids on naturalized populations of non-native crayfish *Orconectes limosus* and *Pacifastacus leniusculus* living in sympatry with native *Astacus astacus*. The native crayfish species probably served as a source of *Branchiobdella* spp. for non-native crayfishes. Two species of *Branchiobdella* (*B. parasita* and *B. pentadonta*) were found on *P. leniusculus* while only *B. parasita* was found on *O. limosus*. The level of colonization of inspected specimens and species was also evaluated. The mean number of branchiobdellids found on *P. leniusculus* and *O. limosus* was 26.0±15.9, and 64.8±27.7, respectively. *Orconectes limosus* was more heavily colonized by *Branchiobdella* despite being significantly smaller than *P. leniusculus*. Non-native species were corroborated as suitable to host the native epibionts.

Key words: *Branchiobdella parasita*; *Branchiobdella pentadonta*; ectosymbiont; signal crayfish; spiny-cheek crayfish; epibiosis.

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INTRODUCTION

Biological invasions generate ecological impacts challenging the conservation of biodiversity and natural resources (Dudgeon *et al.*, 2006; Gallardo *et al.*, 2016). Alien species which became invasive are impacting host ecosystems by their own activity. Additionally, they may add further concern through their associated symbionts which in turn infect native hosts (Prenter *et al.*, 2004). Conversely, non-native organisms could serve as hosts for native symbionts (Carrillo *et al.*, 2014). Branchiobdellida (Annelida: Clitellata) are ectosymbiotic clitellate worms living primarily upon freshwater crayfishes with a Holarctic distribution (Gelder and Williams, 2015). Eight *Branchiobdella* species are indigenous in Europe (Subchev, 2014). Together with their hosts - the signal crayfish *Pacifastacus leniusculus* (Dana, 1852), spiny-cheek crayfish *Orconectes limosus* (Rafinesque, 1817) and red swamp crayfish *Procambarus clarkii* (Girard, 1852), Nearctic (specifically North-American) branchiobdellidans were unintentionally introduced to the European continent. Thus, up to now only the non-native branchiobdellidans, represented especially by species belonging to the genera *Xironogiton* (*X. victoriensis* Gelder & Hall, 1990) and *Cambarincola* (*C. gracilis* Robinson, 1954, *C. okadai* Yamaguchi, 1933 and *C. mesochoreus* Hoffman, 1963), were recorded living on these non-native crayfishes from several European countries (Gelder *et al.*, 1994, 1999, 2012; James *et al.*, 2015). Studies reporting the occurrence of Palearctic branchiobdellids on non-native crayfish species are very rare and *Branchiobdella* spp. occurring on *O. limosus* were reported only by Ďuriš *et al.* (2006) from the Elbe River, Czech Republic and by Vogt (1999) from the Steinbach brook, Germany. Gelder *et al.* (1999) reported *Branchiobdella* spp. on *P. clarkii* in Canale di San Grato, Martinetto, Italy. To our knowledge, no records of native branchiobdellids have been reported on naturalized populations of signal crayfish. Generally, although several branchiobdellid species could be found together on one crayfish specimen (Gelder and Williams, 2015), still the branchiobdellidans actively discriminate among sympatric hosts (Brown and Creed Jr, 2004) and there are many factors driving the decision whether to switch to another host or to stay on the original host. Most of these factors are linked to behaviour and general activity of potential hosts (Brown and Creed Jr, 2004; Farrell *et al.*, 2014; Skelton *et al.*, 2015). Additionally, their occurrence is not limited to crayfish, but branchiobdellidans have been reported on other crustaceans such as freshwater crabs or isopods (for details see Young, 1966; Keller, 1992). Thus, we evaluated the hypothesis that native branchiobdellids can be transmitted by and live on naturalized non-native crayfish species populations of *O. limosus* and *P. leniusculus* living sympatrically with the native noble crayfish [*Astacus astacus* (Linnaeus, 1758)].

METHODS

Species of Branchiobdellida were sampled from crayfish caught during routine monitoring for non-native crayfish presence at two Czech localities in May, 2008. Thirty adult *O. limosus* were collected by hand in Černovický brook (N 49.309344 E 14.860639; geographic reference system WGS-84) as well as sixty *P. leniusculus* caught in a pond close to Čáslavice village (N 49.255598 E 16.271148). Carapace length, wet mass (W) and sex of crayfish were recorded.

Crayfish were immersed individually to clove oil solution (4 ml L^{-1} for 10 min) to remove attached branchiobdellids. Branchiobdellids started to escape from their host immediately after exposure. After 10 min, the crayfish was removed from the solution and washed and the whole volume of the exposure tank was sieved (0.3 mm). The population of branchiobdellids from individual crayfish was then preserved in Eppendorf tubes containing 95% ethanol. The number, biomass and species of branchiobdellids were determined in the laboratory under a stereomicroscope (Olympus SZX9, Hamburg, Germany), using analytical scales KERN ABT 220-4M (Kern & Sohn GmbH, Balingen, Germany) and following the taxonomic keys by Neseman and Neubert (1999) and Gelder *et al.* (1994).

Non-parametric Mann-Whitney U tests were applied to reveal which crayfish species was more colonized by branchiobdellids using number and wet biomass of branchiobdellids. The data did not have a normal distribution, even after BOX-COX transformation (tested by the Shapiro-Wilks test). All statistical analyses were done using Statistica 12 (StatSoft Inc.).

RESULTS

In both sites, non-native crayfish species lived sympatrically with *A. astacus*, however sympatry had different patterns. *Astacus astacus* inhabited the upper stretch of the Černovický brook while *O. limosus* moved up from the lower stretch, pushing *A. astacus* upstream with only a thin contact zone. On the other hand, *P. leniusculus* co-occurred in the pond with *A. astacus* for more than 20 years, however the latter species also inhabited the source brook of the pond.

Two species of *Branchiobdella* (*B. parasita* (Braun, 1805) and *B. pentadonta* Whitman, 1882) were found on *P. leniusculus*, while only *B. parasita* was found on *O. limosus*. The mean number and biomass of branchiobdellids found on *P. leniusculus* and *O. limosus* were 26.0±15.9 (range 6-66), and 64.8±27.7 (34-115) individuals, respectively, and 0.005±0.003 and 0.016±0.011 g, respectively. *Branchiobdella parasita* slightly prevailed (58.7%) over *B. pentadonta* on *P. leniusculus*. Although individuals of *P. leniusculus* were significantly larger (CL=47.3±4.0; W=26.9±6.9 g) than individuals of *O. limosus* (CL=34.6±2.9; W=12.0±3.2 g), the number and biomass of branchiobdellids on *P. leniusculus* were significantly lower (Mann-Whitney; $Z = 7.68$, $P<10^{-6}$) showing that individuals of *O. limosus* were more colonized by branchiobdellids. To illustrate this difference, the mean number of branchiobdellids per gram of crayfish was 1.0±0.5 specimens g^{-1} in *P. leniusculus*, but 5.4±1.9 specimens g^{-1} in *O. limosus* ($Z=-6.69$, $P<10^{-6}$).

DISCUSSION

Here we reported European branchiobdellids (*Branchiobdella* spp.) found on naturalized populations of non-indigenous crayfish species, namely *O. limosus* and *P. leniusculus* (for the first time on the latter species). In both localities surveyed in this study, non-native crayfishes lived in sympatry with native *A. astacus*. Thus, *A. astacus* served most likely as a source of studied ectosymbionts, however we did not analyse the branchiobdellid community from *A. astacus*. Similarly, Jurek (2014) found branchiobdellids only on individuals of *P. leniusculus* living in sympatry with *A. astacus*. In Černovický brook, however, the high colonization level on *O. limosus* can most likely be explained by the decreasing number of original host *A. astacus* at the locality, where branchiobdellids were forced to find a new suitable host for that reason in order to survive. To illustrate this situation, the numbers of *O. limosus* and *A. astacus* captured at Černovický brook site were ca. 1300 and 90 specimens, respectively. On the other hand, at the Čáslavice pond locality, four thousand individuals of *P. leniusculus* and one thousand individuals of *A. astacus* were caught. This could mean that there were still enough native crayfish enabling survival of branchiobdellids, which had adopted the non-indigenous crayfish as a host. Moreover, the sympatry lasted here for more than 20 years and the community of branchiobdellids had not been faced with a rapid and critical decrease of its host, as in the Černovický brook site. The transmission of branchiobdellidans between sympatric species is known to be highly variable, most probably driven by cost/benefits relationships and explained in terms of fitness outcomes for individual ectosymbionts (Brown and Creed Jr, 2004; Skelton *et al.*, 2015). In contrast, sympatry of native and non-native species does not automatically mean that non-native species were colonized by branchiobdellids. Adámek and Řehulka (2000) found sympatry of *A. astacus* and *O. limosus* in the small brook Pšovka and although individuals of *A. astacus* were highly colonized by *Branchiobdella* spp. (up to 362 individuals), the non-native *O. limosus* carried no branchiobdellids. It is a question whether these two species came into physical contact enabling host switching, or there

could still be host preferences or other reasons, *e.g.* different activity, grooming behaviour, encounter of predators (Skelton *et al.*, 2013; Farrell *et al.*, 2014; Skelton *et al.*, 2015). Nonetheless, the high infestation of *A. astacus* was most likely caused by decreasing numbers of this species, which had been dying of crayfish plague. *Branchiobdella* from dying individuals had probably transferred to live specimens. Reports of *O. limosus* having no branchiobdellidans are more frequent from different parts of Europe (Burba, 1997; Oberkofler *et al.*, 2002; Klobučar *et al.*, 2006). Only Ďuriš *et al*. (2006), Vogt (1999) and this study reported *Branchiobdella* spp. on *O. limosus*. Generally, more active crayfishes are colonized less than are less active species (Brown and Creed Jr, 2004), and non-native species (*O. limosus* and *P. leniusculus*) are considered as more active than native *A. astacus* (Lozán, 2000; Styrishave *et al.*, 2007; Musil *et al.*, 2010). Thus, probably only critical events increase the transmission rate for ectosymbiotic fauna to other possible hosts, which, otherwise, would not be infested at all. Further, specimens of *O. limosus* were taken and used for another study (Buřič *et al.*, 2011) and after the end of this experiment (ca. six months) crayfish were found to be heavily colonized by adults and cocoons of *Branchiobdella parasita* (Fig. 1). It is important to note that, with respect to the relatively short life span of *O. limosus* (Smith, 1981),

Fig. 1. The level of *Branchiobdella parasitica* colonization on *Orconectes limosus*: a) side view; b) dorsal view.

the number of crayfish declined during the experiment and afterwards and branchiobdellids may have moved to live crayfish, resulting in quite a high number of *Branchiobdella* on the single remaining specimen depicted in Fig. 1. The population of *B. parasita* seemed to be viable and stable in the Černovický brook, using non-indigenous crayfish species as possible hosts.

CONCLUSIONS

We report here the occurrence of *Branchiobdella* spp. on the non-indigenous crayfish *O. limosus* and *P. leniusculus*. In both sites, non-native species lived sympatrically with native *A. astacus*, which most likely served as a source for the branchiobdellids. Although *O. limosus* is reported to be colonized less frequently by *Branchiobdella* spp., we found *B. parasita* establishing a viable population using this species. Furthermore, this is the first report of *P. leniusculus* with an established viable population of two *Branchiobdella* species (*B. parasita*, *B. pentadonta*). This also raises the question whether the native branchiobdellids colonize non-native invaders i) by chance; ii) naturally substitute for the disappearing native crayfishes in order to survive, since the colonization of a non-native host could be initiated by the gradual disappearance of native hosts; or iii) exhibit a natural dispersal strategy exploiting more possible niches for living. The same patterns can be expected also for future introduced epibionts colonizing native biota in order to survive.

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