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Short Communication — Open Access

Host shift to non-native species or ecological dead end? Endangered branchiobdellidans (Annelida: Clitellata) found on introduced Signal Crayfish in Japan

Tomoaki Konno[1],*, Kazunori Tanaka[2] and Itsuro Koizumi[1,3]

[1] Graduate School of Environmental Science, Hokkaido University, Sapporo, Japan
[2] Faculty of Humanities and Human Sciences, Hokkaido University, Sapporo, Japan
[3] Faculty of Environmental Earth Science, Hokkaido University, Sapporo, Japan

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Abstract – Symbionts, including parasites, are usually small and thus have been overlooked for their existence and scientific research. In the context of biological invasion, host shifts between native and non-native species occur. Since symbionts often affect host behaviour and survival, it is important to reveal the cryptic invasions and host shifts of such small symbionts. Here, we report the first evidence of the potential host shift of a native endangered branchiobdellidans, ectosymbiotic crayfish worms, to a non-native Signal Crayfish, *Pacifastacus leniusculus* (Dana 1852), in Hokkaido, Japan. *Pacifastacus leniusculus* is native to North America and was recently introduced to the Atsubetsu River system in Sapporo. In addition to the North American ectosymbiont, *Sathodrilus tetrodonta* (Pierantoni, 1906), we found an endangered Japanese species, *Cirrodrilus cirratus* complex on *P. leniusculus*. However, *C. cirratus* complex as well as *S. tetrodonta* were not found on the native Japanese Crayfish, *Cambaroides japonicus* (De Haan, 1841), in the same river system, indicating a host shift of the ectosymbiont to invasive crayfish. It remains unclear whether *C. cirratus* complex will persist for a long time with the non-native host or if this is only a transient leading to local extinction.

Keywords: Biological invasions / endangered species / symbiont / branchiobdellida / Japanese Crayfish

Recently, occurrences of native symbionts (including parasites) in introduced species (Kelly *et al*., 2009) or non-native symbionts in native species (Lymbery *et al*., 2014) have been reported. Because the presence or absence of symbionts often affects host behaviour and survival (Adamo, 2002; Prenter *et al*., 2004; Poulin, 2010; James *et al*., 2015), identifying secondary, often overlooked invasions and host shifts is important for the conservation and management of native ecosystems.

Some crayfish species are some of the most successful invaders due to anthropogenic translocations, and their range continues to expand globally (Lodge *et al*., 2012; Twardochleb *et al*., 2013). Crayfish in the Northern Hemisphere often carry branchiobdellidans (Annelida: Clitellata: Branchiobdellida), ectosymbiotic crayfish worm (Gelder and Williams, 2016), and new symbiotic relationships have been created as a result of translocations of the host crayfish and/or secondary invasions of the symbionts (Gelder, 2020). In Europe, several studies have documented the occurrence of native branchiobdellidans on invasive crayfish (Gelder *et al*., 1999; Vogt, 1999; Ďuriš *et al.,* 2006; Blaha *et al*., 2018). In North America, three species of native branchiobdelidans were found on non-native crayfish, where the native Signal Crayfish, *Pacifastacus leniusculus* (Dana 1852), and the non-native Snake River Pilose Crayfish, *Pacifastacus connections* (Faxon, 1914), are sympatric (Williams and Weaver, 2021). On the other hand, there is only one published report on the transfer of branchiobdellidans from non-native crayfish to native crayfish (Parpet and Gelder, 2020). Host transfer of branchiobdellidans typically occurs during aggressive or mating behaviour among host individuals (Hunt *et al*., 2018) or by indirect transmission via substrates (Gelder and Williams, 2016).

Pacifastacus leniusculus has been extensively introduced across Japan (Nakata *et al*., 2010; Usio *et al*., 2016), and secondary introductions of its branchiobdellidans have also been observed (Kawai *et al*., 2004; Ohtaka *et al*., 2005; Nakata *et al*., 2010). *Pacifastacus leniusculus* is a cold-water crayfish and new populations have been widely established in Hokkaido, one of the coldest regions of Japan (Usio *et al*., 2016). The native and endangered Japanese Crayfish, *Cambaroides japonicus* (De Haan, 1841), is present in Hokkaido, but *P. leniusculus* is competitively dominant and

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*Corresponding author: `tomoaki.konno@outlook.jp`.

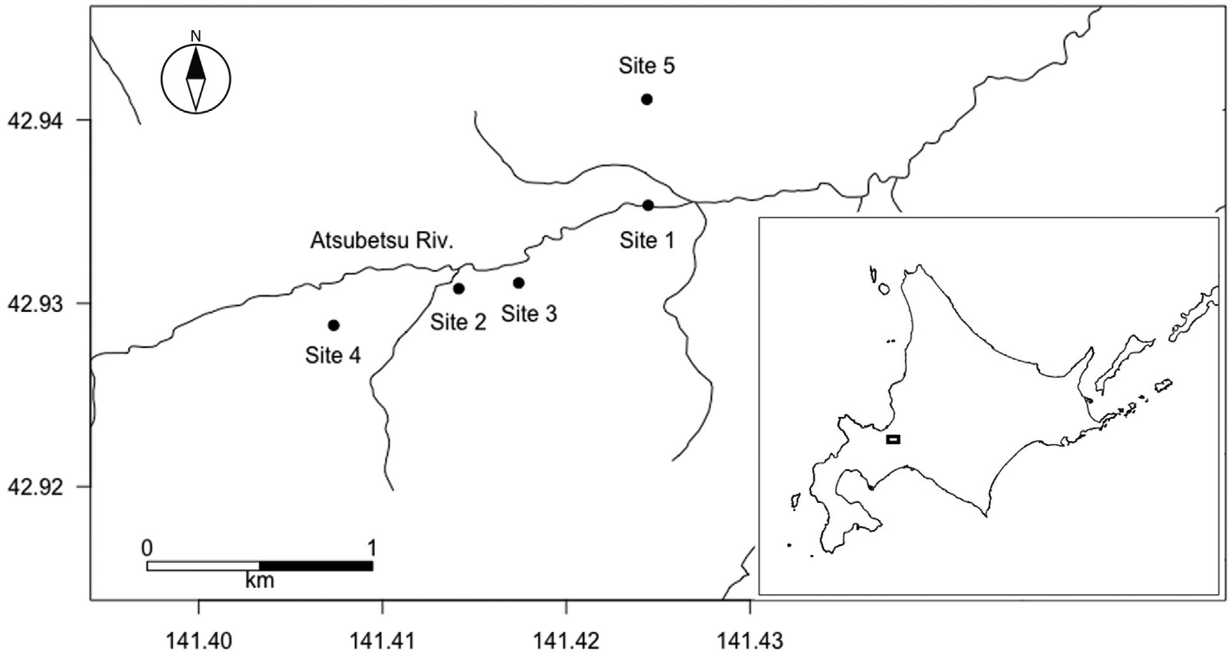

Fig. 1. Crayfish were captured in the Atsubetsu River system at the numbered sites. The collection area is indicated by a rectangle on the map of Hokkaido Island; insert bottom right.

has displaced *C. japonicus* in some sites (Kawai *et al.*, 2002; Nakata *et al.*, 2004). While direct interactions between the native and non-native crayfish have been suggested (Usio *et al.*, 2001; Nakata and Goshima 2003), no evidence has been reported regarding the transfer of native and non-native crayfish worms to each crayfish species.

In Japan, 11 valid species of branchiobdellidans have been found on *C. japonicus* (Ohtaka *et al.*, 2023; Ohtaka *et al.*, 2024). The Red List published by the Japanese Ministry of the Environment lists all these species as being at a higher risk of extinction than their host, *C. japonicus* (Ministry of the Environment, 2020). Biological invasions have a serious impact not only on *C. japonicus* but also on its branchiobdellidans. In the Atsubetsu River in Sapporo, Japan (mainstem, Site 1, Fig. 1), we found suspected Japanese and North American branchiobdellidans on two individuals of *P. leniusculus* collected on August 5, 2021. Therefore, we investigated the prevalence and species composition more detail. We also examined branchiobdellidans on native *C. japonicus*. We particularly focused if a transfer of the symbionts occurred between the two host species.

We performed this study using the following methods. We collected *P. leniusculus* in the Atsubetsu River on August 30, 2022 (Fig. 1, Tab. 1), using a cage trap, hand nets and backpack electrofisher (model 20B; Smith-Root Inc., Vancouver, WA, USA). Sardine heads and entrails were used as bait in the cage trap (50 cm [L] × 25 cm [W] × 25 cm [H], 2 mm mesh), and 100 V was used for electric fishing. The post-orbital carapace length, wet weight and sex of 14 captured *P. leniusculus* were recorded (Tab. 2), and each individual was fixed in 80% ethanol. Later, the fixative ethanol from each sample container was filtered through a 63 µm mesh, and the number of branchiobdellidans was counted. The species of all obtained branchiobdellidans were identified according to Yamaguchi (1934) and Holt (1981). Although *Cirrodrilus cirratus* Pierantoni, 1905 and *Cirrodrilus uchidai* (Yamaguchi, 1932) were described as separate species (Pierantoni, 1905; Yamaguchi, 1932; Gelder, 1987), recent studies have argued that the overlap in taxonomic traits between the two species makes it doubtful whether they are valid species or species complex (Ohtaka *et al.*, 2023). Therefore, in this study, we did not distinguish between the two species and considered *C. cirratus* complex.

From August 2021 to July 2022, we also surveyed the population of *C. japonicus* at four sites (tributaries, Site 2-5) near the site where *P. leniusculus* was found (Fig. 1; Tab. 1). Hand collecting was conducted for 30-100 minutes per site, with 2-3 people searching under stones and trees. Captured *C. japonicus* were temporarily submerged in 70% ethanol for a short time (30 s) to remove branchiobdellidans. This method can remove more than 90% of the attached branchiobdellidans without the death or growth reduction of the host crayfish (T. Konno, in preparation). The collected branchiobdellidans were preserved in 70% ethanol and brought back to the laboratory, and the species were identified according to Yamaguchi (1934). Host *C. japonicus* was rereleased at the site where they were captured.

From both surveys, we obtained the following results. A total of 75 individuals of *P. leniusculus* were captured in the mainstem of the Atsubetsu River (Site 1), while no *C. japonicus* was found in the mainstem (Fig. 1, Tab. 1). Individual information on 14 captured *P. leniusculus* and the number of branchiobdellidans from each host are summarised in Table 2. Two crayfish supported more *C. cirratus* complex than *S. tetrodonta*. The maximum number of *C. cirratus* complex on the host was 232, which was close to the number of *S. tetrodonta* on the same host individual.

Table 1. Number of crayfish captured at each site and the species of branchiobdellidans in the Atsubetsu River system..

				Host crayfish		Branchiobdellidans
Site	Date	Latitude	Longitude	Species	Number	Species
Site 1	30 Aug. 2022	42.93534 N	141.42446 E	*P. leniusculs*	75	*S. tetrodonta* *C. cirratus* complex
Site 2	24 Aug. 2021	42.93079 N	141.41415 E	*C. japonicus*	2	*C. digitatus*
Site 3	24 Aug. 2021	42.93111 N	141.41741 E	*C. japonicus*	4	*C. digitatus*
Site 4	7 Sep. 2021	42.92880 N	141.40734 E	*C. Japonicus*	4	*C. digitatus*
Site 5	15 Jul. 2022	42.94113 N	141.42438 E	*C. japonicus*	4	–

Table 2. Number of branchiobdellidans on individuals of *P. leniusculus* from Site 1 in the Atsubetsu River system.

					Number of branchiobdellidans		
Crayfish ID	MC	POCL (mm)	WW (g)	Sex	*S. tetrodonta*	*C. cirratus* complex	Total
1	BT	47.7	71.0	M	250	232	482
2	HN	43.7	48.5	F	151	82	233
3	EF	40.7	46.8	M	61	66	127
4	EF	43.0	43.3	F	39	21	60
5	HN	39.2	39.2	F	90	43	133
6	HN	39.9	38.4	F	16	19	35
7	HN	39.3	36.3	M	44	7	51
8	HN	38.4	33.9	F	25	1	26
9	BT	39.4	33.6	F	77	28	105
10	HN	36.8	28.3	F	38	8	46
11	BT	34.7	27.3	M	15	2	17
12	HN	33.9	22.7	F	16	6	22
13	HN	30.4	16.2	F	4	0	4
14	BT	26.5	10.3	F	9	7	16

MC: method of capture for crayfish (BT: box trap; HN: hand net; EF: electric fisher); POCL: postorbital carapace length; WW: wet weight.

Cambaroides japonicus was found in four small tributaries (Site 2-5) near the site of the mainstem (Site 1) where *P. leniusculus* was captured (Fig. 1, Tab. 1). The density of *C. japonicus* was very low in every site we surveyed. No sympatric sites were observed between the native and non-native crayfish. Japanese branchiobdellidans were found in three populations of *C. japonicus* (Site 2-4), and all were identified as *Cirrodrilus digitatus* (Pierantoni, 1906) based on the morphology of the jaws.

This is the first record of a native branchiobdellid species being found on a non-native host in Japan. The symbiotic relationships of branchiobdellidans and *P. leniusculus* have been studied at 21 sites in Japan, but only historically associated ectosymbionts, *S. tetrodonta* and *Xironogiton victoriensis* Gelder and Hall, 1990, were detected (Kawai *et al*., 2004; Ohtaka *et al*., 2005; Nakata *et al*., 2010). One of the reasons why Japanese branchiobdellidans have not been found on *P. leniusculus* in past studies could be that it has been a long time since crayfish was introduced (*e.g.*, 70–80 yr after initial introduction (Usio *et al*., 2007)). For example, Ďuriš *et al.* (2006) reported that four species of native branchiobdellidans – *Branchiobdella italica* Canegallo, 1923, *Branchiobdella pentadonta* Whitman, 1882, *Branchiobdella parasita* (Braun, 1805) and *Branchiobdella hexadonta* Grube, 1883 – were found on the non-native crayfish *Faxonius limosus* (Rafnesque, 1817) in 2001. However, only *B. italica* was observed in 2003, and no branchiobdellidans were detected in subsequent surveys. This suggests that novel symbiotic relationships between crayfish and branchiobdellidans may not last long. The population of *P. leniusculus* was established in the Atsubetsu River system relatively recently (less than 10 yr ago, K. Tanaka, unpublished data). Not much time has been passed since the first interactions between the non-native and native crayfish, and the branchiobdellidans population might have been transiently supported by *P. leniusculus*.

No individuals of *C. cirratus* complex were found on *C. japonicus* during this study. Because we investigated only four populations of *C. japonicus*, exploring more sites in this river system may identify the source population of the branchiobdellidans transmitting to *P. leniusculus* from *C. japonicus*. However, the very low densities of *C. japonicus* in the studied area suggest that the host population from which the source of *C. cirratus* complex might have been already extinct, or that *C. cirratus* complex became extinct before *C. digitatus* due to the decline of host crayfish. Hinosawa *et al.* (2023) estimated that *C. japonicus* in northern Honshu Island in Japan will be largely extinct by 2050, suggesting that *C. japonicus* is at a greater risk of extinction than previously thought. In Hokkaido, population extinction has already been confirmed at several sites (Kawai *et al.*, 2002; Nakata *et al.*, 2004), and the estimation in Hinosawa *et al.* (2023) could apply to Hokkaido populations as well. Since symbionts are known to go extinct earlier than their hosts in some cases (Rózsa and Vas, 2005; Lafferty, 2012), it is highly possible that the extinction of *C. cirratus* complex occurred earlier than that of *C. japonicus* in the Atsubetsu River system.

Japanese branchiobdellidans are specifically ectosymbiotic with *C. japonicus* and could only live in lakes or small streams near water sources inhabited by this host. On the other hand, *P. leniusculus* can inhabit mainstems, and *C. cirratus* complex could permanently use *P. leniusculus* as a host; they have expanded their habitat range to mainstem reaches, where it was originally difficult for them to inhabit. This finding is a unique example of a native symbiont that would normally be lost along with its native host as a result of biological invasions avoiding extinction by shifting hosts to the invasive species as well as expanding the living area of the river due to the high dispersal ability of the new host.

When native endangered symbiotic species transfer to non-native hosts, the removal of non-native species means driving the endangered species to extinction at the hands of humans. This finding suggests that there is a dilemma between the extermination of non-native species and the protection of symbionts caused by an overlooked host shift. Nevertheless, the main goal of the management of introduced species is to eradicate or reduce their population sizes, which leads to the restoration of native biodiversity (Hulme, 2006; Prior *et al.*, 2017). A proper understanding of complex ecosystems includes symbiotic organisms and clarifying on what to conserve and what to eliminate, sometimes at the expense of native species, is important for ecosystem management.

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