Ann. Limnol. - Int. J. Lim. 48 (2012) 315–322

DOI: 10.1051/limn/2012021

Available online at:
www.limnology-journal.org

First report of two North American branchiobdellidans (Annelida: Clitellata) or crayfish worms on signal crayfish in Europe with a discussion of similar introductions into Japan

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Received 7 March 2012; Accepted 6 June 2012

Abstract – Two species of North American branchiobdellidans, *Cambarincola gracilis* Robinson, 1954 and *Cambarincola okadai* Yamaguchi, 1933, have been reported in Europe for the first time. These branchiobdellidans together with *Xirogoniton victoriensis* Gelder and Hall, 1990 were found on signal crayfish, *Pacifastacus leniusculus* (Dana, 1852), collected from the Lot and Tarn River drainages in southern France. Specimens of *X. victoriensis* were also reported on the same host in the Mayenne River drainage in north-eastern France. Brief morphological descriptions of the three alien branchiobdellidan species are given. These introductions are briefly discussed and compared with similar alien ectosymbiotic associations found in Japan.

Key words: Annelida / *Cambarincola gracilis* / *C. okadai* / crayfish

Introduction

Although it is not known when alien or non-indigenous crayfish species (NICS) from North America first arrived in Europe, it must have predated the outbreak of crayfish plague, *Aphanomyces astaci* Schikora, 1903, that appeared in the Po Valley, northern Italy in 1859 (Evans and Edgerton, 2002). As the pathogen proceeded to kill endemic or indigenous crayfish species (ICS) around the continent, gastronomic demands were met by importing crayfishes from the USA starting in the late 19th century and continuing until quite recently (Henttonen and Huner, 1999). While further imports of these crayfish for food have now declined or stopped, interest in a variety of alien crayfishes as pets in freshwater aquaria has risen. As was to be expected some of these species have escaped or been released into the wild resulting in new alien species being reported and increases in the distribution of others (Holdich *et al.*, 2009). The concomitant introduction and spread of crayfish symbionts has recently taken on increased importance as veterinary health authorities attempt to control various introduced pathogens (Vogt, 1999; Evans and Edgerton, 2002); however, non-pathogenic organisms, in particular annelid branchiobdellidans or crayfish worms (Gelder, 2006; Govedich *et al.*, 2010), continue to receive only passing interest. These are small, leech-like ectosymbionts that live primarily on Holarctic freshwater crayfishes (Gelder, 1999).

To understand the complexities raised by the current study, it is necessary to review briefly the commercial history of signal crayfish in Europe and particularly in France. Originally they consisted of three species, *Pacifastacus leniusculus* (Dana, 1852) and *Pacifastacus trowbridgii* (Stimpson, 1857) ranging from southern British Columbia, Canada, through western Washington and Oregon, USA, and *Pacifastacus klamathensis* (Stimpson, 1857) in southwestern Oregon and northern California (Riegel, 1959). However, their morphological differences are so small that they were reduced to subspecies rank, *P. l. leniusculus, P. l. trowbridgii* and *P. l. klamathensis*. Through subsequent commercial and sport fishing introductions throughout the Pacific Northwest, USA, and successful inter-breeding, it is often impossible to recognize individual subspecies and so they are now referred to as *P. leniusculus* (Lewis, 2002). Lake Tahoe in California and Nevada, USA, was stocked in about 1895 with *P. leniusculus* from the Klamath River. More were added in 1909, probably from Oregon coastal streams, and a third infusion occurred in 1916 from unreported sources (Abrahamsson and Goldman, 1970). These were not the

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Table 1. Collection sites and numbers of alien *Pacifastacus leniusculus* with their branchiobdellidan species and total numbers from each site. *C.* = *Cambarincola*, *X.* = *Xironogiton*.

Site no.	Date	Site name	Department	Rivers	Latitude/longitude (degrees)	Worm species and No.		*Host No.*
Tarn drainage:								
1	13/09/11	Salvetat sur Agout	Hérault	Agout	N43.606684 E2.698347	*C. gracilis*	2	
						C. okadai	1	1
						X. victoriensis	3	
2	14/09/11	Lacaune	Tarn	Caunaise	N43.702322 E2.764630	*C. okadai*	12	4
						X. victoriensis	6	
3	01/08/11	Brusque	Aveyron	Dourdou	N43.789027 E2.938024	*X. victoriensis*	32	1
4	21/09/11	Curvalle	Tarn	Rance	N43.926207 E2.544494	*X. victoriensis*	30	3
5	01/08/11	Brousse le Château	Aveyron	Alrance	N43.996508 E2.628392	*C. okadai*	1	3
6	02/08/11	Alrance	Aveyron	Alrance	N44.127860 E2.677678	*X. victoriensis*	1	2
7	29/07/11	Saint Beauzély	Aveyron	Muze	N44.172432 E2.965483	*X. victoriensis*	12	2
Lot drainage:								
8	20/07/11	Balsièges	Lozère	Lot	N44.503892 E3.460192	*X. victoriensis*	9	1
9	17/08/10	St Félix de Lunel	Aveyron	Rau de Servan	N44.551966 E2.552527	*X. victoriensis*	4	4
10	18/08/10	Nauviale	Aveyron	Créneau	N44.490380 E2.448379	*X. victoriensis*	12	2
Mayenne drainage:								
11	24/06/11	Heusse	Orne	Rau de longueves	N48.506092 W0.856354	*X. victoriensis*	1	1

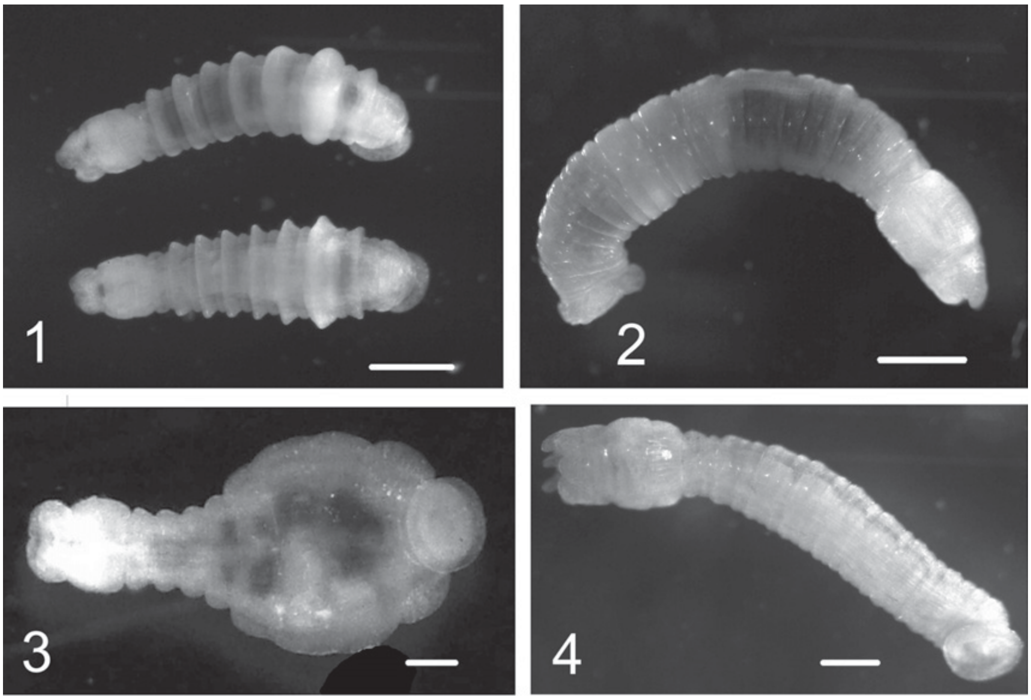
last introductions into Lake Tahoe, and other lakes and rivers in the region also received similar stockings. Fortunately Perry C. Holt examined crayfish collected from Lake Tahoe in 1964 and these were found to carry two branchiobdellidans, *Xironogiton victoriensis* Gelder and Hall, 1990 and *Sathodrilus attenuatus* Holt, 1981 (Gelder, 2005). Given the level of human transportation of crayfish in the Pacific Northwest, any chance of identifying the endemic distributions of the 15 branchiobdellidan species reported on *P. leniusculus* (Gelder *et al.*, 2002; Gelder, 2004) would be almost impossible. Therefore these exported crayfish can be expected to carry any of the branchiobdellidans listed in Gelder (2004).

An experimental aquaculture project in 1959 introduced *P. leniusculus* from the Sacramento area, California, USA, into Sweden (Lewis, 2002; Holdich *et al.*, 2009). Following this successful trial, additional stocks were imported from Lake Tahoe and released into numerous Swedish lakes. Similarly, stocks from Lakes Tahoe and nearby Hennessy, California, were also introduced into Finnish Lakes between 1967 and 1969 (Westman, 1973). Commercial success in rearing these crayfish and decreasing populations of European crayfishes led to Swedish-reared signal crayfish being transported to many other European countries. However, some countries obtained their stocks directly, whether legally or illegally, from sources other than Lake Tahoe in the Pacific Northwest (Holdich *et al.*, 2009). The first report of an alien North American branchiobdellidan on *P. leniusculus* in Europe came shortly after their Swedish introduction when Franzén (1962) identified *Xironogiton instabilis* (Moore, 1894), subsequently renamed *X. victoriensis*. Populations of *X. victoriensis* flourished along with their hosts, resulting in both being introduced into various European countries (Gelder, 1999). Subsequent reports show that *X. victoriensis* is more widely distributed than thought with recent sightings in the Italian Tyrol (Quaglio *et al.*, 2001; Oberkofler *et al.*, 2002), Manzanas, Zamora and Ebro rivers and tributaries, eastern Spain (Rosa Cubo E., unpublished data), and Rivers Lot (Laurent, 2007) and Dourbie in southern France (Subchev, 2008). Although *X. victoriensis* has remained the only alien species recognized from *P. leniusculus* on the continent, Kirjavainen and Westman (1999) raised the possibility that a cambarincolid had been seen in Finland when they reported, "either *Xironogiton* [*victoriensis*] *instabilius* Moore or *Cambarincola* sp., both of which were found in [on] signal crayfish imported from California in 1967–1969".

As our paper is focused on France, the first importations of *P. leniusculus* came from Sweden and they were released into northern and eastern regions of the country in 1972. Additional stocks came directly into central France from Lakes Tahoe and Donner, California, USA, in 1974 (Arrignon *et al.*, 1999) and these were followed by illegal introductions directly from Oregon, USA (Holdich *et al.*, 2009). From these populations we report an expanded distribution of *X. victoriensis* in France, and the first records of two *Cambarincola* species on *P. leniusculus* in Europe. Brief morphological descriptions of the three species are presented for ease of identification by European researchers, and these alien branchiobdellidan introductions are compared with a similar situation in Japan.

Material and methods

Pacifastacus leniusculus were collected as part of surface water quality monitoring studies for the Adour Garonne and Loire Bretagne Water Agencies in 2010 and 2011 by Asconit Consultants, Ramonville Saint-Agne, France. Sites were selected along rivers in the Tarn, Lot and Mayenne drainages, France (Table 1 and Fig. 9), with 12 samples taken from available habitats at each site.



Figs. 1–4. Fig. 1. *Cambarincola gracilis* adults in lateral and dorsal aspects, bar = 0.5 mm. **Fig. 2.** *Cambarincola okadai* adult in lateral aspect, bar = 1.0 mm. **Fig. 3.** *Xironogiton victoriensis* adult in ventral aspect, bar = 0.4 mm. **Fig. 4.** *C. okadai* juvenile in oblique ventral aspect, bar = 0.5 mm.

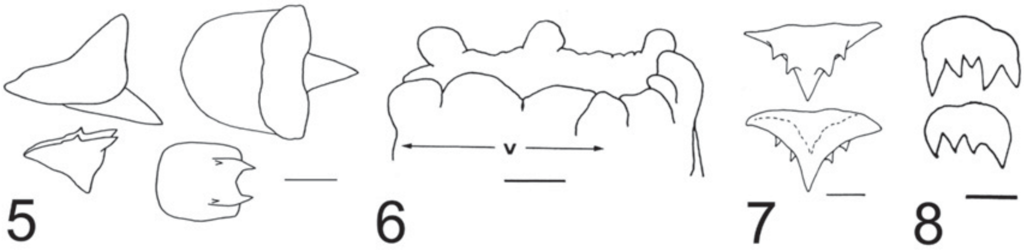
Benthic fauna was sampled using a Surber sampler covering a 20 cm square substrate that was agitated by hand for about 1 min with displaced macrofauna and debris being carried by the current into a 0.5 mm mesh net. Captured organisms and sediments were transferred directly into bottles containing a 10% formalin solution, subsequently washed in running water in the laboratory and then any macrofauna observed under a stereomicroscope were removed and transferred into 70% ethanol for storage. This protocol is mandated by the Water Agencies. Crayfish were subjected to additional detailed examination to remove any branchiobdellidans remaining on the host and these were added to individuals recovered from the debris. Insufficient numbers of *P. leniusculus* were recovered by Surber sampling to provide branchiobdellidan population data, therefore this is a preliminary study to establish only the distribution of alien branchiobdellidan species in the areas surveyed.

Branchiobdellidans were divided into three groups with each group being characterized by having one of the following features: body racquet-shaped, peristomial tentacles, or transverse segmental ridges. Then up to 10 individuals from each group were selected and prepared for microscopical examination and identification. Specimens were dehydrated in a graded water: ethanol series from 70 to 100%, then cleared in methyl salicylate (oil of wintergreen), infiltrated with Canada balsam, mounted on a microscope slide under a cover-glass (Govedich *et al.*, 2010), and observed under a Nikon compound microscope using differential interference illumination (DIC). Photographs of unmounted branchiobdellidans were taken using a digital camera (Leica IC80 HD) mounted on a zoom stereomicroscope (Leica $MZ9_5$) under a cold light source illumination (Leica CLS 150X). Morphological terminology used in the brief descriptions follows that given in Govedich *et al.* (2010).

Results

Three species of North American branchiobdellidans were identified on *P. leniusculus* collected in France: *Cambarincola gracilis* Robinson, 1954 (Fig. 1), *Cambarincola okadai* Yamaguchi, 1933 (Figs. 2 and 4) and *X. victoriensis* Gelder and Hall, 1990 (Fig. 3), and this is the first record of the two *Cambarincola* species in Europe.

X. victoriensis is the most common branchiobdellidan found on *P. leniusculus* with 30 or more individuals on a host. Although the worms have a characteristic racquet shape (Fig. 3), preservation can sometimes cause them to appear more tubular. As a result, it is easy to overlook less frequently occurring cambarincolids even when they are present. However, *C. gracilis* often contracts upon preservation causing prominent transverse segmental ridges to appear (Fig. 1), and *C. okadai* is significantly longer (Fig. 2) than the other two species. Juvenile specimens are usually shorter than adults but the head size in some species (*i.e., C. okadai*) is almost the same as that in a fully grown individual (Figs. 2 and 4). Maturity is established when reproductive organs are observed in segments 5 and 6, otherwise individuals are referred to as immature or juveniles.



Figs. 5–8. Fig. 5. *Cambarincola gracilis* jaws in lateral aspect (left) and dorsal aspect (right) with teeth pointing posteriorly, bar = 25 μm; redrawn from Robinson (1954). **Fig. 6.** *Cambarincola okadai* with peristomium ("v", ventral lip) in ventral aspect, bar = 50 μm. **Fig. 7.** *C. okadai* ventral aspect of jaws (teeth pointing posteriorly), bar = 40 μm; Figures 6 and 7 redrawn and modified from Gelder and Ohtaka (2000). **Fig. 8.** *Xironogiton victoriensis* dorsal aspect of jaws (teeth pointing posteriorly), bar = 20 μm; redrawn from Gelder and Hall (1990).

Brief descriptions of the three exotic North American branchiobdellidans

All three North American species have spermatozoa in body segments 5 and 6, and can be easily distinguished from endemic European *Branchiobdella* which carry spermatozoa only in body segment 5. Each alien species has a unique combination of morphological characters: body length and shape, peristomial lip appendages and jaw structure.

C. gracilis are rod-shaped, 1.3–4.5 mm long, usually with dorsal transverse ridges on body segments; these are frequently prominent following preservation. The dorsal peristomial lip has four small lobes which may be partially withdrawn during preservation. The triangular dorsal jaw is large (about 2.5 × the ventral) with a large median tooth, and the rectangular ventral jaw has two pairs of lateral teeth, a small anterior pair and a larger posterior pair (Fig. 5).

C. okadai are rod-shaped, 3.4–7.0 mm long, possessing no body segment ridges. The peristomial dorsal lip has four distinct lobes, two pairs of lateral lobes and a ventral lip partitioned into four ill-defined lobes (Fig. 6). Both jaws are similar sized, triangular, each with a large median tooth and two pairs of smaller lateral teeth (Fig. 7).

X. victoriensis are racquet-shaped and 1.8–2.8 mm long. Peristomial dorsal lip has four slight lobes in life, but usually appears smooth when preserved while lateral lobes are absent and the ventral lip has a slight median cleft. Jaws are similar sized, rounded rectangular, with irregular and randomly arranged teeth, usually five dorsal and four ventral (Fig. 8).

Distribution of branchiobdellidans

Branchiobdellidans were found at all 11 sites sampled in the upper Garonne and eastern Loire basins in southern and northwest France, respectively (Table 1 and Fig. 9, rectangles in inset); however, the ectosymbionts were not found on every signal crayfish collected. Each site supported populations of *X. victoriensis* except for site 5 (Fig. 9), where the three crayfish examined yielded only one *C. okadai*. This contrasted with site 1 where all three species were found on the single crayfish collected, and this was the only record of *C. gracilis* in the survey. As expected, *X. victoriensis* is the most wide spread and numerous (maximum of 32 individuals on a single host) of the three species detected in the Rivers Tarn, Lot and Mayenne (Table 1). Numbers of branchiobdellidans on each crayfish were not recorded and so ectosymbiont abundance and other population data are not available; however, being a preliminary study future monitoring will address these omissions.

Discussion

Prior to 1962, the only branchiobdellidans recorded in Europe were six endemic species of *Branchiobdella*. Subsequently, *X. victoriensis* on *P. leniusculus* (Franzén, 1962) and *Cambarincola mesochoreus* Hoffman, 1963 on *Procambarus clarkii* (Girard, 1852) (Gelder *et al.*, 1994) were found in Europe. Both crayfishes had also been commercially introduced into Japan, *P. clarkii* having arrived in 1927 (Kawai and Kobayashi, 2006) and *P. leniusculus*, in the late 1920s (Kawai, 2002). The first and believed to be the only introduction of *P. clarkii* from New Orleans, Louisiana, USA, into Japan involved about 20 individuals being release into ponds in Kanagawa Prefecture (Kawai, 2002; Kawai and Kobayashi, 2011) where they reproduced rapidly and have since become widely distributed throughout the country. Kawai and Kobayashi (2006, 2011) examined *P. clarkii* from across Japan and did not find any branchiobdellidans. This supports the hypothesis that any branchiobdellidans leaving Louisiana in 1927 died in transit due to poor transportation conditions. In contrast, there is evidence that *P. leniusculus* were introduced into Japan on at least five separate occasions before imports were banned in 1930 (Kawai *et al.*, 2004). Signal crayfish introduced into Hokkaido Island came from the Columbia River area in Oregon; however, the sources of other introductions from the Pacific Northwest, USA, are unknown (Kumakawa *et al.*, 2011). The description of *C. okadai* on an "American crayfish" from Lake Chuzenji, central Honshu Island, Japan, by Yamaguchi (1933) raised some problems. The morphological description was incomplete;

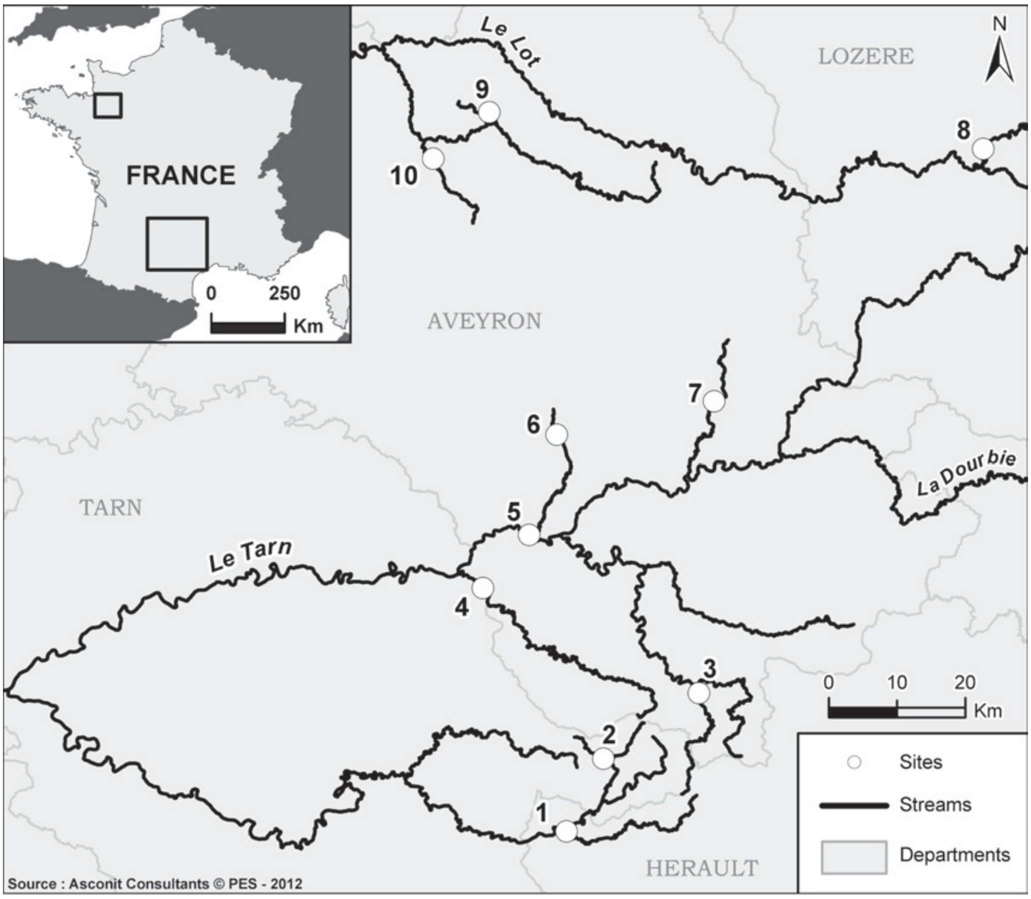


Fig. 9. The two study areas are rectangles in the map of France (inset), with collection locations indicated on Lot and Tarn rivers in the eastern Garonne Basin (sites 1–10) and Mayenne River in the western Loire Basin (site 11, inset).

he did not designate any type specimen, and no comparable branchiobdellidan was known in the Pacific Northwest. Following the rediscovery of Professor H. Yamaguchi's slide collection, Gelder and Ohtaka (2000) were able to recognize his specimens, designate them as syntypes, make a detailed redescription of *C. okadai* and thus reduce the Northwestern USA species, *Triannulata montana* (Goodnight, 1940) to a junior synonym.

Given these reports of alien branchiobdellidans in Europe and East Asia, Gelder (2004) recognized the likelihood that other North American branchiobdellidans almost certainly had survived transportation and remained to be found. He listed all the branchiobdellidans reported on three North American crayfishes most frequently involved in commercial exports. This consisted of six branchiobdellidan species on *P. clarkii*, 15 on *P. leniusculus* and four on *Orconectes limosus* (Rafinesque, 1817). Shortly after, Ohtaka *et al.* (2005) found two additional branchiobdellidan species, *Sathodrilus attenuatus* Holt, 1981 in Hokkaido Island, and *X. victoriensis* in Nagano Prefecture, Honshu Island, Japan, both on *P. leniusculus*. Of the 15 branchiobdellidan species reported on *P. leniusculus* in the Pacific Northwest, USA, only seven are widely distributed. As *C. okadai* and *X. victoriensis* have been reported in both Europe and Japan, only *C. gracilis* in Europe and *S. attenuatus* in Japan, this leaves *Sathodrilus inversus* (Ellis, 1919), *Triannulata magna* Goodnight, 1940 and *Uglukodrilus hemophagus* (Holt, 1977) as potential species to be discovered.

Branchiobdellidans occur as single or multi-species populations on a host and within a population of crayfish at a site, therefore not every individual will have branchiobdellidans or necessarily the same species composition (Govedich *et al.*, 2010). This variability means not every crayfish batch that is exported will necessarily carry all the branchiobdellidan species present at the rearing source. Crayfish are more tolerant to temperature extremes, dehydration, and low oxygen tensions than branchiobdellidans resulting in their having lower survival rates following transportation, *e.g.*, the absence of branchiobdellidans on *P. clarkii* across the Japanese Archipelago, and from *P. leniusculus* in Shiga Prefecture, central Honshu Island, Japan. Any one or a combination of these factors may account for the different species reported at our sites, and those of Laurent (2007) and Subchev (2008) in nearby locations.

Branchiobdellidans have been reported as being crayfish host species specific (McManus, 1960). While a few species may be, others exhibit a preference for certain hosts (Bishop, 1968; Gelder and Smith, 1987; Brown and Creed, 2004), but most branchiobdellidans are opportunistic regarding a crayfish host. Moreover, a small number of North American and East Asian branchiobdellidans have adopted non-crayfish hosts such as freshwater isopods, shrimps and crabs (Gelder, 1999; Gelder and Messick, 2006). Although a non-crayfish host–branchiobdellidan ectosymbiosis has not been reported in Europe, adoption of alien crayfish partners in Europe is known. *C. mesochoreus* on *P. clarkii* was discovered in northern Italy (Gelder *et al*., 1994) in the same area where *Austropotamobius pallipes* (Lereboullet, 1858) live carrying *Branchiobdella parasita* (Braun, 1805) and *Branchiobdella pentodonta* Whitman, 1882. Within 4 years *B. parasita* and *B. pentodonta* had replaced *C. mesochoreus* on *P. clarkii* at Torino (site 15), Italy (Gelder *et al*., 1999). A probable explanation is *P. clarkii* ate the endemic *A. pallipes* and the two endemic *Branchiobdella* species got transferred to a new host during contact. As *B. parasita* and *B. pentodonta* would recognize each other having been sympartic on *A. pallipes*, *C. mesochoreus* would appear alien and be vulnerable to predation by the larger *B. parasita*. Vogt (1999) found the same *Branchiobdella* species on *Austropotamobius torrentium* (Schrank, 1803) and on the alien *O. limosus* in the same stretch of Steinbach Creek, Hesse, Germany. Similarly, and with two additional species, *Branchiobdella hexodonta* Grüber, 1883 and *Branchiobdella balcanica* Moszyński, 1938, Ďuriš *et al*. (2006) recorded them on *O. limosus* in the northern Czech Republic. Although this North American crayfish species was introduced into Europe in the late 1890s and is quite widespread (Holdich *et al*., 2009), none of its endemic North American branchiobdellidans have ever being reported on the continent. Surprisingly no comparable adoption of alien or endemic branchiobdellidans and crayfishes has been reported in East Asia.

One ectosymbiosis not found in Europe or Japan is a shrimp–branchiobdellidan association; however, two examples are present in China where *Caridinophilus unidens* Liang, 1963 and *Holtodrilus truncatus* (Liang, 1963) both occur on freshwater shrimps (Liang, 1963). Recently *H. truncatus* was discovered on imported Chinese *Neocaridina* spp. (Niwa *et al*., 2005; Niwa and Ohtaka, 2006; Ohtaka *et al*., 2012) in central Honshu Island, Japan. As these shrimps are used for bait in freshwater sport fishing across Japan, escapes into local waters along with their branchiobdellidans has already started. At some point endemic *Cambaroides japonicus* (De Haan, 1841) and its branchiobdellidans will come into contact with alien *P. leniusculus* and *Neocaridina* spp. and their branchiobdellidans; what impact this will have on the ecology of these symbioses and diversity of Japanese freshwater species, remains to be seen. So far an endemic shrimp–branchiobdellidan association has not been reported in Europe, although the potential for one exists in the wild. However, when *B. astaci* were removed from *A. pallipes* and placed repeatedly on a troglodytic shrimp's (*Troglocaris* sp.) carapace, they reacted violently and refused to attach (Gelder, 1999). How common this response is for endemic branchiobdellidans on European shrimps remains to be determined.

Freshwater benthic monitoring programmes are likely to provide the first reports of further North American branchiobdellidans in Europe, but it is also important for monitors to be aware that particular branchiobdellidans can adopt available crayfish species and recognize the potential for other crustaceans to become hosts. Branchiobdellidan adoption of alien hosts shows a degree of behavioural and physiological tolerance not generally recognized by researchers. Investigations into these areas would not only advance our understanding of various ectosymbiotic associations but also help to predict the spread of introduced branchiobdellidans into the wider endemic fauna.

Acknowledgements. Our thanks to collection teams (Mathilde Bach, Marion Rossignol, Christelle Rougé, Aurélie Burgnies, Charline Blanco, Jennifer Martin, Julien Rimour, Frank David, Jérome Cayrou, David Bouché) and Philippe Espy for producing Figure 9 at Asconit Consultants, France, and Drs Andrea M. Gorman, University of Maine at Presque Isle, USA and T. Kawai, Wakkanai Fisheries Institute, Japan for manuscript suggestions. We also thank Jacky Durocher, Water Agency Loire Bretagne and Jean-Pierre Rebillard, Water Agency Adour Garonne, France, for permission to publish survey results and Dr. E. Rosa y Cubo, Estudios y Proyectos, S.L., Valladolid, Spain.

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