



## North American Branchiobdellida (Annelida: Clitellata) or Crayfish Worms in France: the most diverse distribution of these exotic ectosymbionts in Europe

JEAN-FRANÇOIS PARPET<sup>1</sup> & STUART R. GELDER<sup>2,\*</sup>

<sup>1</sup>Royal Belgian Institute of Natural Sciences, Operational Directorate Taxonomy and Phylogeny, Vautier Street 29, B-1000 Brussels, Belgium.

<sup>2</sup>University of Maine at Presque Isle, 181 Main Street, Presque Isle, Maine 04769, USA. E-Mail: [stuart.gelder@maine.edu](mailto:stuart.gelder@maine.edu)

\*Corresponding author: [stuart.gelder@maine.edu](mailto:stuart.gelder@maine.edu)

### Abstract

Crayfishes and other invertebrates were collected during 2010 to 2016 from 519 sites located in the river basins of the Adour, Charente, Dordogne, Garonne, Loire, Seine, Rhône and Sélune in France. North American species included *Pacifastacus leniusculus* at 255 sites, *Faxonius limosus* at 206 sites, *Procambarus clarkii* at 56 sites, and the endemic *Austropotamobius pallipes* at two sites. However, branchiobdellidans were only recorded from 100 sites with 23 of these being sampled more than once, resulting in a total of 127 collections.

The widely distributed western North American, *P. leniusculus* carried four of its endemic branchiobdellidan species: *Cambarincola gracilis*, *C. okadai*, *Triannulata magna* and *Xironogiton victoriensis*. *X. victoriensis* was found at the majority of sites, with *C. okadai*, *C. gracilis*, and *T. magna* at fewer locations. Although *F. limosus* was the second most numerous crayfish species collected, it did not carry any of its endemic North American branchiobdellidan species. However, it was found cohabiting with *P. leniusculus* at four sites but carried *X. victoriensis* at only one of these. European *A. pallipes* only occurred at two sites with individuals harboring *X. victoriensis*, although no cohabiting exotic crayfish were found. Crayfish were also absent from three sites where free-living *X. victoriensis* were recovered from substrate samples. *Procambarus clarkii* appeared at sites scattered across the country, while specimens with *C. mesochoreus* were restricted to the Adour drainage.

This study has shown the widespread distribution of exotic branchiobdellidans in the wild and the virtual extinction of endemic species in France. In addition, we have provided the first European record of *T. magna*, and the first record of *C. mesochoreus* in France; the latter being only the second recording in Europe. With this comprehensive survey of their crayfish hosts in France, monitoring future exotic range expansions and endemic contraction or extinction can be traced. These data will be available to authorities for future planning in maintaining healthy freshwater bodies by reducing the damaging effects caused by exotic crayfishes.

**Keywords:** Ectosymbiosis, French distribution, *Triannulata magna*, *Cambarincola mesochoreus*, *Cambarincola gracilis*, *Cambarincola okadai*, *Xironogiton victoriensis*, North American crayfish, *Pacifastacus leniusculus*, *Procambarus clarkii*, *Faxonius limosus*, *Austropotamobius pallipes*

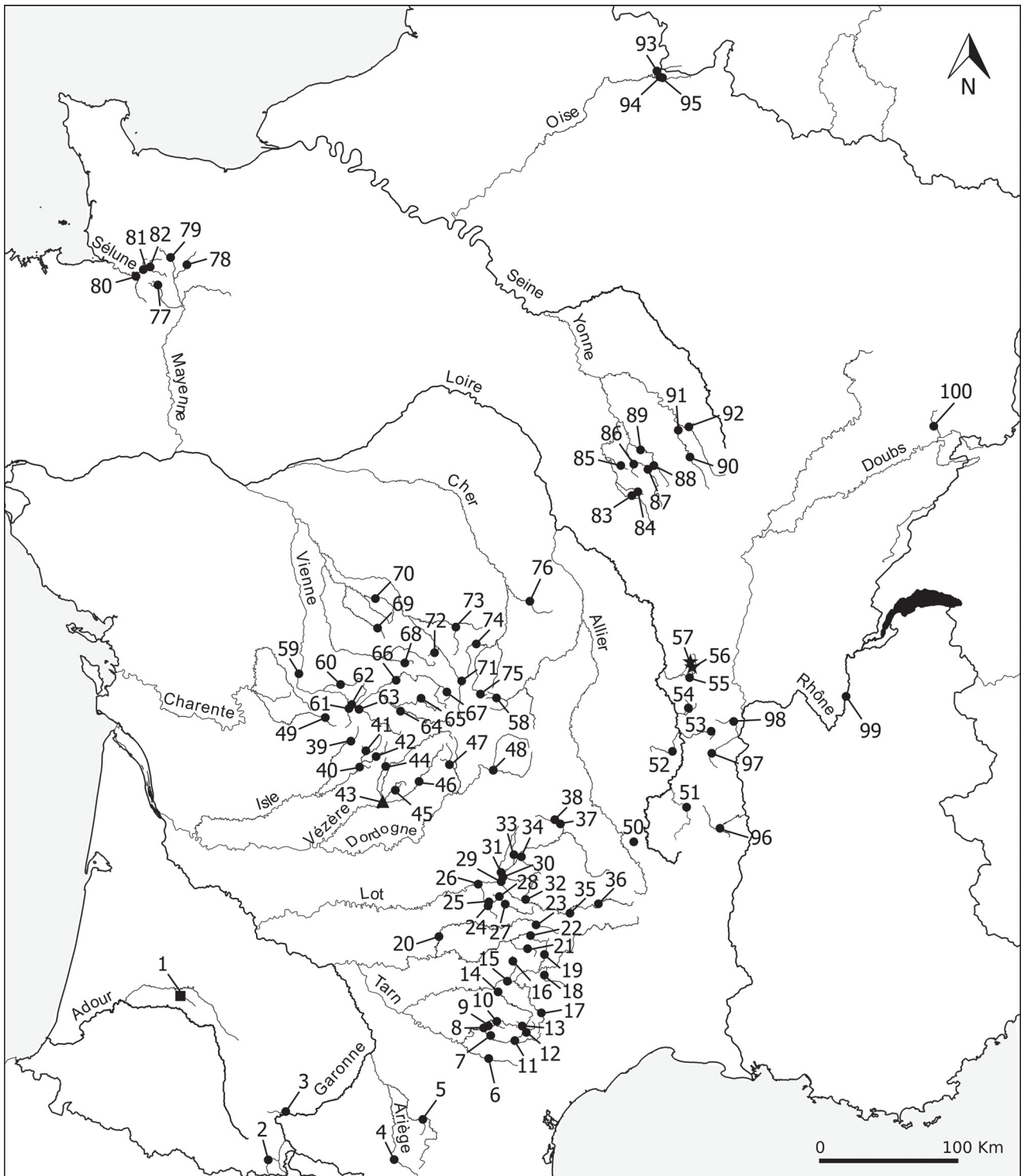
### Introduction

The French naturalist, Alphonse Odier, discovered specimens of *Branchiobdella astaci* Odier, on *Astacus astacus* (Linnaeus) in 1819 and this became the second species to be described in the Branchiobdellida (Annelida: Clitellata) or crayfish worms. Unfortunately reports of this and subsequent finds of *B. astaci*, *Branchiobdella hexadonta* Gruber, *Branchiobdella parasita* (Braun) and *Branchiobdella pentadonta* Whitman in the country were not accompanied by any detailed collection site information (Odier 1823;

Subchev 2014 & 2016). As a result, French distribution records on branchiobdellidans are very limited, consisting only of *Austropotamobius pallipes* (Lereboullet) carrying *B. astaci* at Grand Lac Laffrey, south of Grenoble, from the Druyes river, a tributary of the Yonne river, southeast of Paris (Subchev 2008), *A. pallipes* carrying *B. hexadonta* from an unidentified stream in an unlocated locality called “La Roche” (Subchev 2008), *A. pallipes* supporting *B. astaci* and *B. pentadonta* from an unidentified stream at Saint-Quentin-sur-Coole, Marne Department (Subchev 2016), an unknown crayfish host supporting *B. pentadonta* in an unknown stream at Strasbourg, Bas-Rhin Department (Subchev 2008) and finally with *A. astacus* supporting *B. astaci* in ponds at Thonnance-les-Joinvilles, Haute-Marne Department (J.-F. Parpet, unpub. data). Interest increased when exotic, North American branchiobdellidans were found on their translocated crayfish hosts. The first record in France of *Pacifastacus leniusculus* (Dana) carrying *Xironogiton victoriensis* Gelder and Hall, came from the River Lot, when a fisherman brought specimens to Laurent (2007) for identification; no details of location, date etc. were reported. Similarly, Subchev (2008) found the same association on a specimen from the River Dourbie, a tributary of the Tarn River, in the crayfish collection of the French National Museum of Natural History (Paris); again, collection details had not been recorded. However, collections of *P. leniusculus* from 11 recorded sites in the Rivers Lot, Tarn and Mayenne, recovered three branchiobdellidan species: *Cambarincola gracilis* Robinson, *Cambarincola okadai* Yamaguchi, and *X. victoriensis* (Gelder *et al.* 2012). Subsequently, Lecaplain and Noël (2015) reported *P. leniusculus* with the same three branchiobdellidans in Northwest France, probably from the Mayenne and Sélune River drainages. Following publication, they realized the report of *C. gracilis* in this region was an error (B. Lecaplain, pers. comm.). These imported crayfish were destined for human consumption, and the presence of branchiobdellidans mistakenly identified as parasitic leeches by consumers, reduced the public’s interest for their hosts (J.-F. Parpet, unpub. obs.). In reality, most branchiobdellidans feed on epibiota attached to the crayfish’s exoskeleton so they actually perform a beneficial host cleaning function thus forming a mutualistic association. However, the branchiobdellidan/ host relationship can vary, with periods of mutualism, commensalism or parasitism, depending on the species and habitat conditions (Skelton *et al.* 2013; Ames *et al.* 2015).

The three most important commercial North American crayfishes translocated to Europe are: *P. leniusculus*, *Procambarus clarkii* (Girard) and *Faxonius limosus* (Rafinesque), and all of them have been reported in France (Holdich *et al.* 2009; Kouba *et al.* 2015). Details of *P. leniusculus* translocations from the USA’s northwestern states and subsequent multiple stockings in Europe, France in particular, were presented in Gelder *et al.* (2012). The European distribution of *P. clarkii* favors southwestern Europe which includes south and southwest France, while *F. limosus* extends from France in a wide band to Poland (Kouba *et al.* 2015). Recently these crayfishes were joined in northeastern France by *Faxonius juvenilis* (Hagen) in 2005, and *Faxonius immunis* (Hagen) in 2010 (Kouba *et al.* 2014), however, no branchiobdellidans have been reported on these new arrivals. Gelder (2004) listed the endemic branchiobdellidan species that were known on the three major commercial North American crayfishes and predicted their likely appearance in the importing countries. Currently four of these North American branchiobdellidan species have been recorded in Europe. The first one to be reported was *X. victoriensis* (= *X. instabilis* under various spellings) in Sweden by Franzén (1962), and subsequently in Finland (Kirjavainen & Westman 1999), Austria (Nesemann & Neubert 1999), Spain (Gelder 1999), Italy (Quaglio *et al.* 2002), Germany (Martens *et al.* 2006), Hungary (Kovács & Juhász 2007), France (Laurent 2007), Wales (James *et al.* 2015), Croatia (Dražina *et al.* 2018), Luxembourg (Dr. D. Templeman, pers. comm.) and Switzerland (R. Krieg, pers. comm.). This species has since been joined by *C. gracilis*, and *C. okadai*, in France (Gelder *et al.* 2012), with the latter species also appearing in Wales (James *et al.* 2015). Although *P. clarkii* is widespread in southwestern Europe, it is surprising there has been only one report of it carrying an endemic branchiobdellidan, and that was *Cambarincola mesochoreus* Hoffman, in northern Italy (Gelder *et al.* 1994). Continued translocations and natural range expansions are introducing these exotic crayfishes, and in some cases their branchiobdellidans, into new European freshwater habitats.

The present study in France was designed to extend the preliminary survey reported in Gelder *et al.* (2012) by sampling new sites and resampling previous sites at different times of year. These data will provide a detailed National baseline for local and state authorities to address the negative impact of exotic species on their freshwater habitats. Fortunately, these dangers to France’s endemic freshwater fauna have already been recognized resulting in many state and local authorities increasing their monitoring programs as a prelude to future conservation legislation.



**FIGURE 1.** Map of France showing numbered sites where branchiobdellidans were collected, while the other crayfish sites have been omitted for clarity: discs, *Pacifastacus leniusculus*; square, *Procambarus clarkii*; triangle, *Faxonius limosus* and stars, *Austropotamobius pallipes*.

### Material and methods

Five hundred and nineteen collection sites across France were located in the river basins of the Seine (Oise and Yonne drainages), Loire (Rhins, Allier, Cher, Vienne and Mayenne drainages), Dordogne (Isle and Vézère drainages), Garonne (Lot, Tarn and Ariège drainages), Rhône (Doubs) as well as the Sélune, Charente and

Adour basins (Table 1 and Fig. 1). Collections of crayfishes and their branchiobdellidan ectosymbionts were usually made along with other invertebrates as part of surface-water quality monitoring for French Water Agencies Adour-Garonne, Loire-Bretagne and Seine-Normandie during 2010-2016. Crayfishes were also captured during fish population monitoring in rivers for the Office National de l'Eau et des Milieux Aquatiques (ONEMA, today AFB, Agence Française pour la Biodiversité) in 2013-2016 (data access: <http://www.naiades.eaufrance.fr/acces-donnees#/hydrobiologie>). The majority of these collections were conducted during contract work by Asconit Consultants, Lyon, France, with additional collections being made by sport fishing enthusiasts. Specimens of the endemic *A. pallipes* were caught alive in the Drioule and Mardoret rivers (Rhône Department) during the springs of 2014 and 2016 as part of the fish and crayfish monitoring program of the Rhin's drainage (Loire basin) by the Fédération Départementale du Rhône et de la Métropole de Lyon pour la Pêche et la Protection du Milieu Aquatique, La Tour-de-Salvagny, France.

Exotic crayfishes, *P. leniusculus*, *P. clarkii* and *F. limosus*, were captured using one of four methods. Usually a Surber macro invertebrate stream net ("S" in Table 1) was used following the protocol described in Gelder *et al.* (2012), then captured crayfish were preserved in a 10% formalin solution for examination. Crayfishes immobilized by an electro-fishing protocol ("E" in Table 1) were transferred to holding tanks with dip nets. A collapsible Lift net or Balance method (Kozák *et al.* 2015) was used in streams with large crayfish populations ("B" in Table 1). A benthic nylon gill net ("G" in Table 1) was set between 1.7 and 2 m deep for about 12 hs overnight in Lake Bouchet, a crater lake at 1200 m altitude (site 55, Table 1 and Fig. 1), and on retrieving the net, crayfish were found eating any trapped fish. Crayfish caught by electro-fishing, balance and gill net, were preserved in 70% ethanol. Specimens of *A. pallipes* are protected and were hand collected ("H" in Table 1) alive at night with flashlights, marked and their body dimensions measured. Only their exposed surface was examined and any branchiobdellidans observed were removed before the crayfish were carefully returned to the river, thus preventing any bodily damage and minimizing handling stress.

In the laboratory all exotic crayfishes were identified, and their exposed surface and branchial chambers examined for branchiobdellidans; the worms found were transferred into 70% ethanol-containing tubes. Branchiobdellidans from each collection site were separated into groups based on body shape and size, and up to ten specimens were selected from each group. These were then dehydrated in a graded ethanol series from 70% to 99%, cleared in oil of wintergreen, infiltrated with Canada balsam, and mounted individually on a microscope slide under a cover-glass (Gelder & Williams 2015). Specimens were examined using an Olympus BX53F with Differential Interference Contrast (DIC) illumination and photographed with a Nikon 5100 camera mounted adapter lens #6144 providing 2.5X and 4X additional magnification. Unmounted branchiobdellidans were photographed using a Leica IC80 HD digital camera mounted on a Leica MZ95 zoom stereomicroscope with Leica CLS 150 X cold light source illumination. Morphological terminology used in the brief descriptions follows that in Gelder and Williams (2015). The results obtained were largely qualitative and therefore unsuitable for the statistical analyses usually applied to studies of branchiobdellidan-crayfish associations.

## Results

Crayfishes were collected from a total of 519 sites with the exotic *Pacifastacus leniusculus* being present at 255 sites, *Faxonius limosus* at 206 sites, *Procambarus clarkii* at 56 sites, and the endemic *Austropotamobius pallipes* at two sites. However, branchiobdellidans were only recorded from 100 sites (Table 1) and consisted of five North American species: *Cambarincola gracilis*, *C. mesochoreus*, *C. okadai*, *Triannulata magna* Goodnight, and *X. victoriensis*. Although collections were made at 100 numbered sites (Fig. 1), 23 of which were visited twice on different dates and two received three visits (Table 1), resulting in 127 collections. The multiple site visits provided an indication of the stability of species incidence.

Infected *P. leniusculus* were found in upland, cool waters mainly in the north facing Pyrénées, Massif Central, Plateau de Millevaches, Massifs Morvan and Jura, with an isolated group in southwestern Normandie and another near Saint-Michel, Aisne Department in northeast France (Fig. 1). These crayfish carried *X. victoriensis* at 95 sites, either as the only species at 58 sites or part of a cohabiting group at 37 others. Where *X. victoriensis* was the only species, 10 sites received more than one visit, usually separated by a year. These data establish *X. victoriensis* as the most widespread branchiobdellidan species in France with an abundance ranging from one, e.g., at site 15, to 784 individuals at site 97.



**TABLE 1.** Collection sites and numbers of alien Branchiobdellidians with their host crayfish species, total numbers, collectors, hosts and worms species identifiers, and catching method (CM) for each site by drainages. S = Surber net, G = Gill net, B = "Balance" net, E = Electrofishing, H = Hand. NC = No data communicated. (\* See Gelder *et al.*, 2012) Branchiobdellidians: Cg = *Cambarincola gracilis*, Co = *Cambarincola mesochoreus*, Cm = *Cambarincola okadai*, Cm = *Cambarincola mesochoreus*, Im = *Triannulata magna*, Xv = *Xironogiton victoricensis*. Crayfishes: Ap = *Austropotamobius pallipes*, Fl = *Faxonius limosus*, Pc = *Procambarus clarkii*, Pl = *Pacifastacus leniusculus*.

Site no.	Date Sampled	Site name	Department	River / Lake (L)	Latitude/Longitude degrees	Worm species (Number.)	Host species (Number.)	Host Collectors	Host/Worms Identifiers	CM
Adour Drainage :										
01	26/03/2014	Pujo-le-Plan	Landes	Aff. du Barrouquet	N43.848604 W0.338082	Cm (53)	Pc (14)	J. Rimour	J.-F. Parpet	B
Garonne Drainage :										
02	27/08/2012	Ôo	Haute-Garonne	Neste d'Ôo	N42.795583 E0.505650	Xv (1)	None	C. Rougé	R. Rudel	S
03	23/09/2014	Villeneuve-de-Rivière	Haute-Garonne	Lavet	N43.115995 E0.648545	Xv (96)	Pl (5)	S. Charansol	S. Charansol	S
Ariège Drainage:										
04	08/10/2013	Ormolac	Ariège	Ariège	N42.817577 E1.627303	Xv (168)	Pl (2)	L. Lopez	M. Bach	S
	29/09/2014	Ormolac	Ariège	Ariège	N42.816433 E1.628651	Xv (736)	Pl (1)	J. Revaud	R. Imbert	S
05	04/09/2013	Mirepoix	Ariège	Countirou	N43.084203 E1.877737	Xv (9)	Pl (1)	C. Blanco	Y. Duprat	S
Tarn Drainage:										
06	11/09/2012	Saint-Amans-Soult	Tarn	Thoré	N43.488106 E2.463361	Co (3) Xv (21)	Pl (1)	J. Rimour	O. Maingot	S
07	12/09/2012	Bez	Tarn	Agout	N43.639183 E2.480218	Cg (4) Co (10) Xv (10)	Pl (2)	J. Rimour	O. Maingot	S
08	12/09/2012	Vabre	Tarn	Gijou	N43.688927 E2.415895	Xv (14)	Pl (2)	J. Rimour	A. Morel	S
09	12/09/2012	Vabre	Tarn	Bertou	N43.702622 E2.458969	Xv (1)	Pl (2)	J. Rimour	A. Morel	S
	15/11/2013	Vabre	Tarn	Bertou	N43.702610 E2.458975	Xv (27)	Pl (2)	B. Vallée	J.-F. Parpet	E
10	13/09/2012	Lacaze	Tarn	Gijou	N43.732430 E2.534562	Xv (74)	Pl (2)	J. Rimour	O. Maingot	S
11*	13/09/2011	Salvetat-sur-Agout	Hérault	Agout	N43.606684 E2.698347	Cg (2) Co (1) Xv (3)	Pl (1)	M. Bach	J. Rimour	S
	14/09/2012	Salvetat-sur-Agout	Hérault	Agout	N43.606695 E2.698340	Co (14) Xv (15)	Pl (5)	J. Rimour	A. Meunier	S
12	13/09/2012	Murat-sur-Vèbre	Tarn	Vèbre	N43.660584 E2.802265	Xv (1)	None	J. Rimour	O. Maingot	S
13*	14/09/2011	Lacaune	Tarn	Caunaise	N43.702322 E2.764630	Co (6) Xv (12)	Pl (4)	M. Bach	C. Rougé	S
	13/09/2012	Lacaune	Tarn	Caunaise	N43.702333 E2.764624	Cg (27) Co (18) Xv (262)	Pl (2)	J. Rimour	A. Morel	S
14*	21/09/2011	Curvalle	Tarn	Rance	N43.926207 E2.544494	Xv (30)	Pl (3)	D. Bouché	J.-F. Parpet	S
	09/10/2013	Curvalle	Tarn	Rance	N43.926205 E2.544495	Co (2) Xv (433)	Pl (3)	S. Charansol	J.-F. Parpet	E
15*	01/08/2011	Brousse-le-Château	Aveyron	Alrance	N43.996508 E2.628392	Co (1)	Pl (3)	M. Daprey	F. David	S
	31/07/2012	Brousse-le-Château	Aveyron	Alrance	N43.996525 E2.628382	Xv (1)	Pl (3)	C. Rougé	C. Rougé	S

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TABLE 1. (Continued)

Site no.	Date Sampled	Site name	Department	River / Lake (L)	Latitude/Longitude degrees	Worm species (Number.)	Host species (Number.)	Host Collectors	Host/Worms Identifiers	CM
Tarn Drainage end:										
16*	02/08/2011	Alrance	Aveyron	Alrance	N44.127860 E2.677678	Xv (1)	P/ (2)	M. Daprey	F. David	S
17*	01/08/2011	Brusque	Aveyron	Dourdou de Camarès	N43.789027 E2.938024	Xv (32)	P/ (1)	M. Daprey	A. Burgnies	S
18	01/08/2012	Saint-Rome-de-Cernon	Aveyron	Cernon	N44.036835 E2.964661	Co (1)	P/ (2)	C. Rougé	J. Rimour	S
19*	29/07/2011	Saint-Beauzély	Aveyron	Muze	N44.172432 E2.965483	Xv (12)	P/ (2)	M. Daprey	A. Burgnies	S
20	02/08/2012	Saint-Beauzély	Aveyron	Muze	N44.172457 E2.965463	Xv (2)	P/ (1)	C. Rougé	J. Cayrou	S
20	03/09/2012	Monteils	Aveyron	Aveyron	N44.285585 E2.001088	Xv (14)	P/ (1)	J. Rimour	O. Maingot	S
21	10/10/2013	Curan	Aveyron	Vioulou	N44.210162 E2.811396	Co (57) Xv (768)	P/ (5)	A. Burgnies	J.-F. Parpet	E
22	24/07/2012	Séguir	Aveyron	Viaur	N44.296369 E2.838236	Co (1)	P/ (1)	C. Rougé	J. Cayrou	S
23	10/10/2013	Lugans	Aveyron	Aveyron	N44.366787 E2.888619	Xv (816)	P/ (2)	A. Burgnies	J.-F. Parpet	E
Lot Drainage :										
24*	18/08/2010	Nauviale	Aveyron	Créneau	N44.490380 E2.448379	Xv (12)	P/ (2)	M. Bach	M. Bach	S
25	20/05/2015	Nauviale	Aveyron	Créneau	N44.490266 E2.448386	Xv (193) on P/	P/ (2) & P/ (1)	E. Fievet	J.-F. Parpet	B
25	20/05/2015	Nauviale	Aveyron	Dourdou de Conques	N44.516353 E2.452528	Xv (2)	P/ (2)	E. Fievet	J.-F. Parpet	B
26	05/09/2012	Grand Vabre	Aveyron	Dourdou de Conques	N44.631991 E2.355842	Xv (8)	P/ (1)	J. Rimour	O. Maingot	S
27	19/05/2015	Rodelle	Aveyron	Dourdou de Conques	N44.502292 E2.605606	Xv (106)	P/ (1)	E. Fievet	J.-F. Parpet	B
28*	18/08/2015	Rodelle	Aveyron	Dourdou de Conques	N44.502292 E2.605606	Xv (76)	P/ (2)	Y. Pons	J.-F. Parpet	B
29	20/10/2015	Saint-Félix-de-Lunel	Aveyron	Rau de Servan	N44.551966 E2.552527	Xv (4)	P/ (4)	M. Bach	C. Rougé	S
29	20/10/2015	Entraygues-sur-Truyère	Aveyron	Truyère	N44.649800 E2.567268	Co (2) Xv (105)	P/ (1)	C. Roide	J.-F. Parpet	B
30	21/08/2014	Montpeyrroux	Aveyron	Selves	N44.679893 E2.583438	Xv (28)	P/ (1)	C. Roide	J.-F. Parpet	B
30	16/09/2014	Montpeyrroux	Aveyron	Selves	N44.679893 E2.583438	Co (6) Xv (61)	P/ (1)	C. Roide	J.-F. Parpet	B
30	20/05/2015	Montpeyrroux	Aveyron	Selves	N44.679893 E2.583438	Co (51) Xv (1307)	P/ (5)	C. Roide	J.-F. Parpet	B
31	13/05/2014	Saint-Hippolyte	Aveyron	Goul	N44.710404 E2.565347	Co (2) Xv (108)	P/ (1)	N. Boidin	J.-F. Parpet	B
31	19/08/2014	Saint-Hippolyte	Aveyron	Goul	N44.710411 E2.565349	Co (5) Xv (158)	P/ (1)	C. Roide	J.-F. Parpet	B
32	21/07/2015	Saint-Côme-d'Olt	Aveyron	Borale Flatiaguès	N44.533796 E2.792361	Co (1) Xv (147)	P/ (1)	C. Roide	J.-F. Parpet	B
33	20/05/2015	Brommat	Aveyron	Bromme	N44.827211 E2.683195	Xv (163)	P/ (1)	C. Roide	J.-F. Parpet	B
34	21/07/2015	Ste-Genève-sur-Argence	Aveyron	Argence Vive	N44.813568 E2.753040	Xv (489)	P/ (1)	C. Roide	J.-F. Parpet	B
35	19/07/2012	Banassac	Lozère	Lot	N44.443365 E3.198968	Xv (1)	P/ (5)	C. Blanco	J.-F. Parpet	S
36*	20/07/2011	Balsièges	Lozère	Lot	N44.503892 E3.460192	Xv (9)	P/ (1)	C. Blanco	M. Rossignol	S
	18/07/2012	Balsièges	Lozère	Lot	N44.503897 E3.460191	Xv (1)	P/ (1)	C. Blanco	C. Blanco	S

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TABLE 1. (Continued)

Site no.	Date Sampled	Site name	Department	River / Lake (L)	Latitude/Longitude degrees	Worm species (Number.)	Host species (Number.)	Host Collectors	Host/Worms Identifiers	CM
Lot Drainage end:										
37	21/05/2015	Saint-George	Cantal	Lander	N45.029645 E3.112954	Xv (1474)	Pl (3)	C. Roide	J.-F. Parpet	B
	19/08/2015	Saint-George	Cantal	Lander	N45.029645 E3.112954	Xv (38)	Pl (1)	Y. Pons	J.-F. Parpet	B
38	17/07/2014	Andelat	Cantal	Lander	N45.057361 E3.061141	Xv (144)	Pl (6)	O. Maingot	J.-F. Parpet	E
	19/08/2015	Andelat	Cantal	Lander	N45.057361 E3.061141	Co (59) Xv (487)	Pl (1)	Y. Pons	J.-F. Parpet	B
Isle Drainage:										
39	19/08/2013	Le Chalaud	Haute-Vienne	Isle	N45.558120 E1.154582	Co (3) Xv (2)	Pl (1)	C. Rougé	R. Imbert	S
	17/06/2014	Le Chalaud	Haute-Vienne	Isle	N45.558170 E1.154675	Co (2) Xv (61)	Pl (1)	O. Maingot	J.-F. Parpet	E
40	20/08/2013	Payzac	Dordogne	Rau des Belles-Dames	N45.389256 E1.241430	Xv (1)	Pl (12)	C. Rougé	S. Marty	S
41	09/07/2013	Coussac-Bonneval	Haute-Vienne	Rau de Marcognac	N45.497117 E1.296632	Xv (4)	Pl (4)	C. Blanco	C. Blanco	S
42	21/08/2013	Lubersac	Corrèze	Auvézère	N45.460608 E1.393369	Co (3)	Pl (3)	C. Rougé	S. Charansol	S
Vézère Drainage:										
43	26/09/2013	Saint-Pantaléon-de-Larche	Corrèze	Corrèze	N45.167843 E1.463142	Xv (3) on Fl	Pl (1) & Fl (1)	J. Rimour	J. Rimour	S
44	22/08/2013	Troche	Corrèze	Loyre	N45.397322 E1.484695	Co (1) Xv (3)	Pl (6)	C. Rougé	C. Rougé	S
	09/07/2014	Troche	Corrèze	Loyre	N45.397468 E1.485017	Co (1)	Pl (1)	C. Blanco	Y. Duprat	S
45	17/09/2014	Sainte-Féréole	Corrèze	Maumont Noir	N45.242352 E1.578193	Co (4) Xv (3)	Pl (4)	C. Blanco	S. Charansol	S
46	09/09/2013	Les Angles-sur-Corrèze	Corrèze	Corrèze	N45.301877 E1.797793	Cg (9) Co (8) Xv (118)	Pl (6)	J. Rimour	J. Rimour	S
	25/06/2015	Les Angles-sur-Corrèze	Corrèze	Corrèze	N45.301566 E1.797724	Cg (9) Co (4) Xv (133)	Pl (1)	O. Maingot	J.-F. Parpet	E
Dordogne Drainage:										
47	08/10/2013	Egletons	Corrèze	Rau d'Egletons/Millet	N45.415133 E2.078524	Co (28) Xv (452)	Pl (1)	O. Maingot	J.-F. Parpet	E
48	13/08/2014	Bort-les-Ogues	Corrèze	Rhue	N45.380969 E2.487629	Cg (25) Co (92) Xv (236)	Pl (6)	O. Maingot	J.-F. Parpet	E
Charente Drainage:										
49	06/08/2015	Champagnac-la-Rivière	Haute-Vienne	Tardoire	N45.708720 E0.909530	Cg (21) Co (3) Xv (133)	Pl (1)	C. Roide	J.-F. Parpet	B
Loire Drainage:										
50	22/08/2013	Cayres	Haute-Loire	Lac du Bouchet (L)	N44.909501 E3.790820	Co (23) Xv (657)	Pl (2)	A. Bijon	J.-F. Parpet	G
51	25/06/2014	Tence	Haute-Loire	Lignon du Velay	N45.132275 E4.284021	Co (42) Xv (43)	Pl (1)	O. Maingot	J.-F. Parpet	E
	27/07/2015	Tence	Haute-Loire	Lignon du Velay	N45.132275 E4.284021	Xv (137)	Pl (1)	O. Maingot	J.-F. Parpet	E

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TABLE I. (Continued)

Site no.	Date Sampled	Site name	Department	River / Lake (L)	Latitude/Longitude degrees	Worm species (Number.)	Host species (Number.)	Host Collectors	Host/Worms Identifiers	CM
Loire Drainage End:										
52	27/08/2014	Saint-Marcelin-en-Forez	Loire	Mare	N45.500300 E4.160510	Xv (275)	Pl (3)	N. Boidin	J.-F. Parpet	E
53	25/08/2014	Larajasse	Rhône	Coise	N45.627998 E4.525517	Xv (792)	Pl (1)	N. Boidin	J.-F. Parpet	E
54	01/07/2014	Panisières	Loire	Charpasonne	N45.783705 E4.315772	Xv (584)	Pl (14)	N. Boidin	J.-F. Parpet	E
	30/10/2015	Panisières	Loire	Charpasonne	N45.783705 E4.315772	Xv (31)	Pl (1)	Y. Pons	J.-F. Parpet	E
	16/06/2016	Panisières	Loire	Charpasonne	N45.783705 E4.315772	Xv (31)	Pl (1)	C. Roide	J.-F. Parpet	E
Rhins Drainage :										
55	07/08/2015	Amplepuis	Rhône	Rhins	N45.982623 E4.332064	Xv (157) on Pl	Pl (1) & Fl (1)	Y. Pons	J.-F. Parpet	E
56	07/08/2014	NC	Loire	Drioule	NC	Xv (51)	Ap (1)	J.-P. Faure	J.-F. Parpet	H
57	26/07/2016	NC	Loire	Mardoret	NC	Xv (12)	Ap (1)	J.-P. Faure	J.-F. Parpet	H
Allier Drainage:										
58	31/07/2015	Saint-Avit	Puy de Dôme	Ru de Letrade	N45.856011 E2.515899	Xv (58)	Pl (1)	O. Maingot	J.-F. Parpet	E
Vienne Drainage:										
59	28/08/2014	Ansac-sur-Vienne	Charente	Vienne	N45.991501 E0.649968	Xv (1)	None	N. Gouneau	J.-F. Parpet	S
60	23/06/2015	Oradour-sur-Glane	Haute-Vienne	Glane	N45.928387 E1.044815	Co (1) Xv (74)	Pl (1)	O. Maingot	J.-F. Parpet	E
61	19/06/2014	Aixe-sur-Vienne	Haute-Vienne	Aixette	N45.772382 E1.129334	Xv (278)	Pl (2)	O. Maingot	J.-F. Parpet	E
	24/06/2015	Aixe-sur-Vienne	Haute-Vienne	Aixette	N45.772375 E1.129324	Xv (11)	Pl (1)	O. Maingot	J.-F. Parpet	E
62	12/06/2014	Aixe-sur-Vienne	Haute-Vienne	Aurence	N45.798651 E1.154958	Xv (1)	Pl (1)	C. Gerret	S. Estevenon	S
	18/06/2014	Aixe-sur-Vienne	Haute-Vienne	Aurence	N45.798655 E1.154954	Co (3) Xv (2)	Pl (17)	O. Maingot	J.-F. Parpet	E
63	24/06/2015	Condat-sur-Vienne	Haute-Vienne	Briance	N45.768548 E1.224145	Cg (9) Co (18) Xv (105)	Pl (1)	O. Maingot	J.-F. Parpet	E
64	08/09/2016	Neuvic-Entier	Haute-Vienne	Ru de Vergnas	N45.761578 E1.613662	Cg (3) Co (23) Xv (34)	Pl (3)	J. Cayrou	M. Rossignol	S
65	17/06/2014	Saint-Martin-Château	Haute-Vienne	Maulde	N45.848667 E1.804524	Xv (8)	Pl (1)	O. Maingot	J.-F. Parpet	E
	21/08/2014	Saint-Martin-Château	Haute-Vienne	Maulde	N45.848654 E1.804522	Xv (1)	Pl (4)	O. Maingot	O. Maingot	S
66	23/06/2015	Saint-Martin-Ste-Catherine	Haute-Vienne	Taurion	N45.963863 E1.566920	Xv (87)	Pl (1)	O. Maingot	J.-F. Parpet	E
67	21/08/2014	Vallières	Creuse	Banize	N45.892132 E2.046535	Xv (29)	Pl (4)	O. Maingot	O. Maingot	S
68	28/10/2014	Mouroux-Vieilleville	Creuse	Ardour	N46.078932 E1.645914	Co (5)	Pl (2)	C. Gerret	M. Daprey	S
69	27/10/2014	Saint-Sulpice-les-Feuilles	Haute-Vienne	Benatze	N46.304217 E1.382553	Xv (19)	Pl (2)	C. Gerret	S. Charansol	S
70	19/05/2014	Sacterges-Saint-Martin	Indre	Abloux	N46.497754 E1.355734	Xv (60)	Pl (1)	A. Burgnies	A. Burgnies	S
71	22/08/2014	Aubusson	Creuse	Rau d'Aubusson	N45.965823 E2.184793	Xv (2)	Pl (4)	O. Maingot	O. Maingot	S

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TABLE I. (Continued)

Site no.	Date Sampled	Site name	Department	River / Lake (L)	Latitude/Longitude degrees	Worm species (Number.)	Host species (Number.)	Host Collectors	Host/Worms Identifiers	CM
Vienne Drainage End:										
72	22/06/2015	Sainte-Feyre	Creuse	Ru de la Pisciculture	N46.149065 E1.925434	Xv (202)	Pf (1)	O. Maingot	J.-F. Parpet	E
73	22/06/2015	Domcyrot	Creuse	Verraux	N46.319225 E2.124189	Xv (108)	Pf (1)	O. Maingot	J.-F. Parpet	E
Cher Drainage:										
74	09/09/2014	Lussat	Creuse	Vouzeize	N46.209530 E2.319701	Co (1)	Pf (1)	O. Maingot	O. Maingot	S
75	13/06/2014	Saint-Oradoux-près-Crocq	Creuse	Tardes	N45.880548 E2.362425	Xv (10)	Pf (2)	C. Gerret	S. Estevenon	S
76	30/08/2013	Cosne-d'Allier	Allier	Aumance	N46.491960 E2.825308	Co (13), Xv (15)	Pf (3)	O. Maingot	J.-F. Parpet	S
Mayenne Drainage:										
77*	24/06/2011	Heussé	Orne	Rau de Longueues	N48.506092 W0.856354	Xv (1)	Pf (1)	J. Martin	M. Rossignol	S
78	27/08/2013	Saint-Bômer-les-Forges	Orne	Varenne	N48.647277 W0.579295	Co (8), Xv (6)	Pf (6)	X. Jaladon	J. Rimour	S
79	15/07/2015	Beauchêne	Orne	Égrenne	N48.690112 W0.744126	Co (14), Xv (204)	Pf (12)	F. Ogier	J. Rimour	S
Sélune Drainage :										
80	26/07/2012	Les Loges-Marchis	Manche	Airon	N48.555000 W1.077222	Co (1), Xv (4)	Pf (1)	F. Martignac	J. Cayrou	S
81	24/07/2013	Milly	Manche	Gueuche	N48.602002 W1.007757	Co (1), Xv (1)	Pf (1)	S. Bouron	C. Blanco	S
82	25/07/2012	Romagny	Manche	Cance	N48.621666 W0.941388	Xv (14)	Pf (1)	F. Martignac	C. Blanco	S
Yonne Drainage :										
83	16/06/2014	Montreuilon	Nièvre	Yonne	N47.183043 E3.805829	Xv (112)	Pf (1)	L. Baraillé	J.-F. Parpet	E
84	11/07/2012	Mhère	Nièvre	Aff. de l'Anguison	N47.207784 E3.864033	Xv (176)	Pf (1)	A. Morel	R. Rudel	S
85	10/09/2015	Nuars	Nièvre	Armanche	N47.381277 E3.701388	Xv (33)	Pf (1)	C. Roide	J.-F. Parpet	B
86	16/07/2012	Saint-André-en-Morvan	Nièvre	Rau de la Brinjame	N47.389479 E3.827269	Xv (43)	Pf (4)	A. Meunier	A. Burgnies	S
87	17/07/2013	Saint-André-en-Morvan	Nièvre	Rau de la Brinjame	N47.389548 E3.828473	Xv (20)	Pf (1)	O. Maingot	A. Burgnies	S
88	29/08/2016	Marigny-L'Église	Nièvre	Cure	N47.354071 E3.963813	Cg (8), Xv (66)	Pf (1)	C. Henry	J.-F. Parpet	B
89	11/09/2015	Saint-Léger-Vauban	Yonne	Trinquelin/Cousin	N47.378440 E4.020953	Cg (27), Co (8), Xv (137)	Pf (1)	C. Roide	J.-F. Parpet	B
90	16/07/2012	Avallon	Yonne	Cousin	N47.482590 E3.894428	Xv (1)	Pf (1)	O. Maingot	A. Burgnies	S
91	02/07/2015	Montigny-sur-Armançon	Côte-d'Or	Armançon	N47.431230 E4.371562	Xv (2)	Pf (3)	A. Bijon	J.-F. Parpet	B
92	02/07/2015	Quincy-le-Vicomte	Côte-d'Or	Armançon	N47.608609 E4.262646	Xv (23)	Pf (2)	A. Bijon	J.-F. Parpet	B
92	17/06/2014	Marmagne	Côte-d'Or	Rau de Fontenay	N47.629237 E4.366302	Xv (93)	Pf (4)	A. Bijon	J.-F. Parpet	S

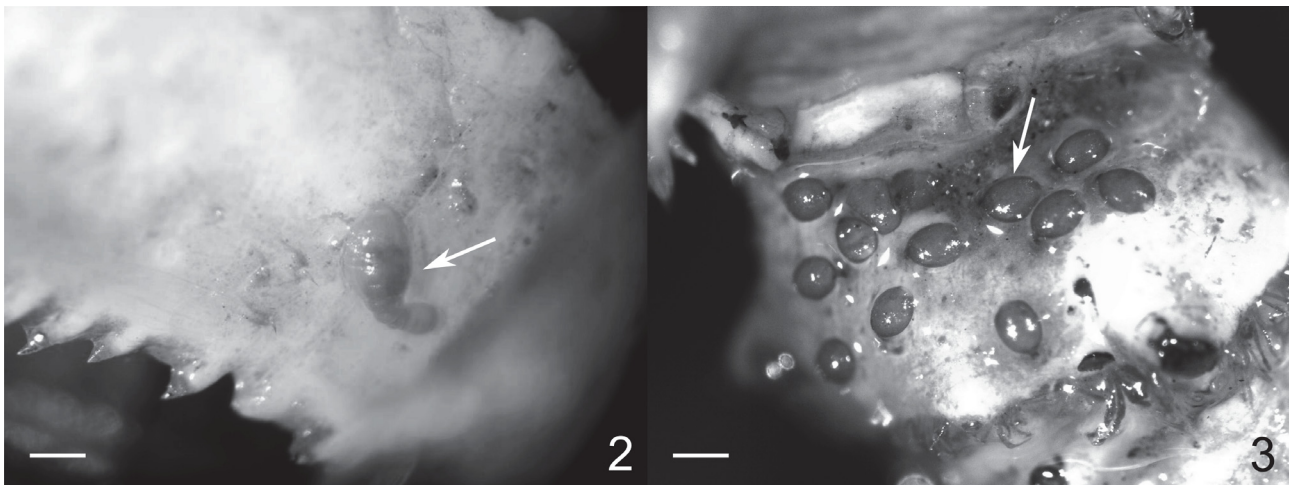
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TABLE 1. (Continued)

Site no.	Date Sampled	Site name	Department	River / Lake (L)	Latitude/Longitude degrees	Worm species (Number.)	Host species (Number.)	Host Collectors	Host/Worms Identifiers	CM
Oise Drainage:										
93	31/07/2013	Hirson	Aisne	Oise	N49.965933 E4.104384	<i>Co</i> (9) <i>Xv</i> (33)	<i>Pl</i> (10)	E. Golembecki	J. Rimour	S
	28/07/2014	Hirson	Aisne	Oise	N49.965712 E4.104116	<i>Co</i> (4) <i>Xv</i> (9)	<i>Pl</i> (6)	E. Golembecki	J.-F. Parpet	S
94	16/07/2014	Saint-Michel	Aisne	Gland	N49.927497 E4.133415	<i>Cg</i> (67) <i>Co</i> (66) <i>Xv</i> (261)	<i>Pl</i> (4)	T. Mattioni	J.-F. Parpet	B
95	28/07/2014	Saint-Michel	Aisne	Gland	N49.922060 E4.159413	<i>Cg</i> (3) <i>Co</i> (3) <i>Im</i> (2) <i>Xv</i> (48)	<i>Pl</i> (1)	E. Golembecki	J.-F. Parpet	S
	02/07/2015	Saint-Michel	Aisne	Gland	N49.921970 E4.159182	<i>Cg</i> (1) <i>Co</i> (8) <i>Im</i> (7) <i>Xv</i> (38)	<i>Pl</i> (1)	E. Golembecki	J.-F. Parpet	S
Rhône Drainage:										
96	31/03/2015	Lamastre	Ardèche	Grozon	N44.989567 E4.589638	<i>Xv</i> (142)	<i>Pl</i> (1)	B. Vallée	J.-F. Parpet	B
97	19/08/2015	Saint-Chamond	Loire	Gier	N45.483054 E4.527758	<i>Xv</i> (784)	<i>Pl</i> (1)	B. Vallée	J.-F. Parpet	E
98	19/06/2015	Auberge du Garon	Rhône	Garon	N45.689119 E4.738934	<i>Xv</i> (1267)	<i>Pl</i> (2)	J.-P. Faure	J.-F. Parpet	E
99	27/10/2015	Culoz	Savoie	Rhône	N45.832643 E5.799751	<i>Xv</i> (305)	<i>Pl</i> (1)	N. Hendrick	J.-F. Parpet	E
Doubs Drainage:										
100	10/08/2016	Héricourt	Haute-Saône	Lizaine	N47.581416 E6.743084	<i>Xv</i> (857) on <i>Pl</i>	<i>Pl</i> (1) & <i>Fl</i> (1)	C. Roide	J.-F. Parpet	B

Although *C. okadai* was the sole species on *P. leniusculus* at sites 18, 22, 68 and 74, it was more usual to find it cohabiting along with *X. victoriensis* as reported at sites 45, 47, 50, 60, 76, 78, 79, 80, 81 and 87. In addition, two collections about a year apart were made at sites 31, 32, 39 and 93, indicating the stability of the cohabiting populations. In contrast, only *C. okadai* was recorded at site 15 in 2011, followed by only *X. victoriensis* in 2012. While at other site examinations separated by about a year, *X. victoriensis* was found initially and then both *C. okadai* and *X. victoriensis* at sites 30, 38 and 62, with the reverse occurring at sites 44 and 51 with *C. okadai* initially then *C. okadai* and *X. victoriensis*. A triple species cohabitation of *C. gracilis*, *C. okadai*, and *X. victoriensis* was identified at eight sites (7, 46, 48, 49, 63, 64, 88 and 94), while one of the double collections at sites 11 and 13, lacked *C. gracilis*. Site 95 near Saint-Michel in northeast France was unique in that two collections, one in 2014 and the other in 2015, both captured a single crayfish each time carrying four species, *C. gracilis*, *C. okadai*, *T. magna* and *X. victoriensis*.

Although site 43 was cohabited by *P. leniusculus* and *F. limosus*, *X. victoriensis* was only present on the latter host with adult worms occupying their usual position on the chelae and cocoons on the protected inner surface (Figs 2 and 3). A more unexpected adoption was found at sites 56 and 57 where the North American *X. victoriensis* had populated the European indigenous white-clawed crayfish, *A. pallipes*. In addition to monitoring invertebrates, which included crayfishes, samples of substrate were also collected from many sites for mesofaunal examination. Although no crayfish were captured from sites 2, 12 and 59, specimens of *X. victoriensis* were recovered from the substrate collections. *Procambarus clarkii* was only found at site 1 in the central Adour basin, southwest France (Fig. 1, Table 1) where it carried *Cambarincola mesochoreus*, making this the first record of *C. mesochoreus* in France.



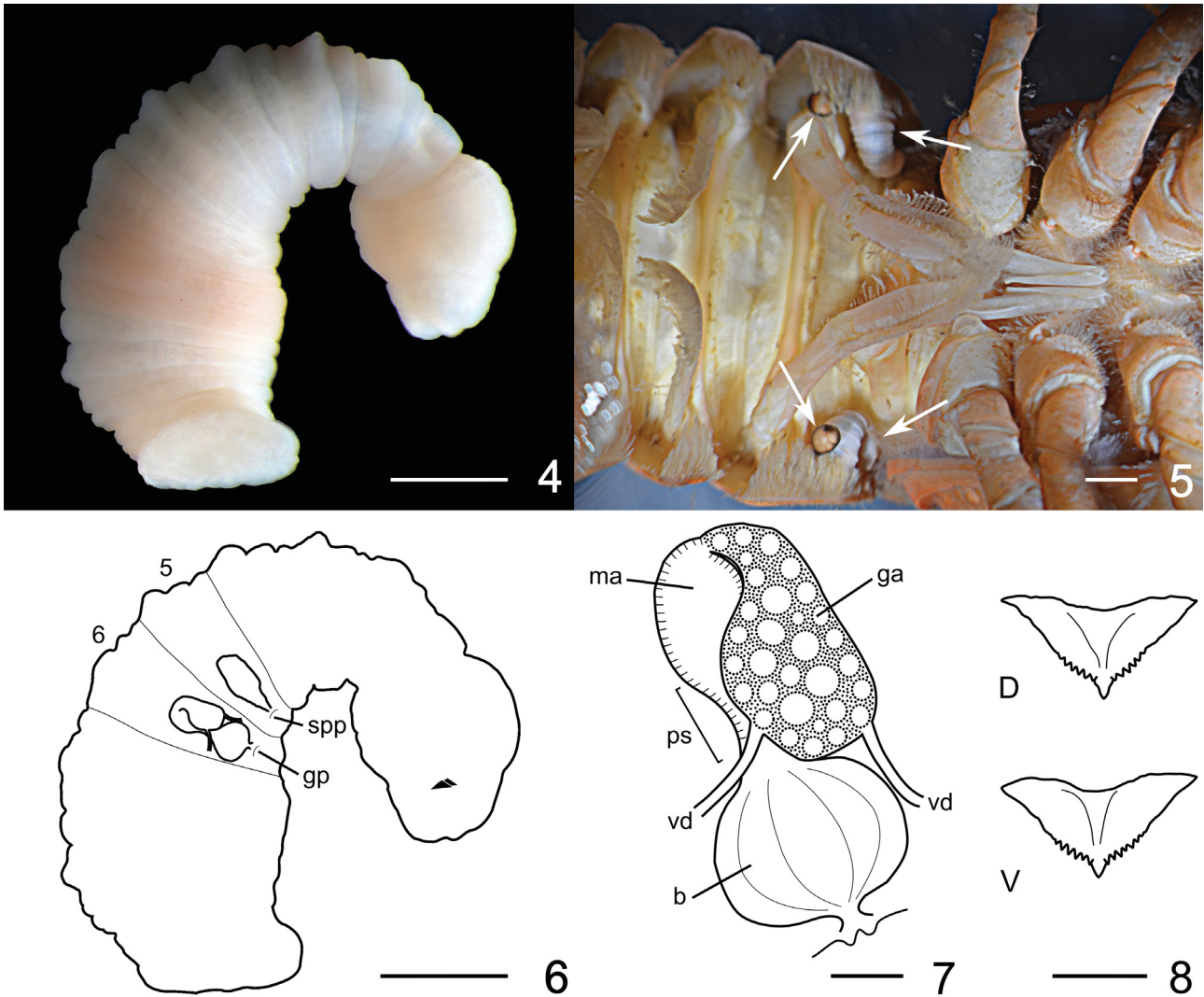
**FIGURES 2-3.** *Xironogiton victoriensis*. **Fig. 2.** Adult in dorsal aspect on the external surface of *Faxonius limosus* cheliped's merus, bar = 1mm. **Fig. 3.** Cocoons on *F. limosus* internal surface of cheliped's carpus, bar = 40µm.

#### *Brief descriptions and observations of the two newly reported exotic North American branchiobdellidan species*

Previously Gelder *et al.* (2012) provided diagnostic descriptions for *C. gracilis* and *C. okadai* reported in France, and so similar information are presented here for *T. magna* and *C. mesochoreus*. As juvenile worms lack male organs, they can only be tentatively identified by their jaws when collected along with adult specimens; cocoons are similarly recognized by association and inference. Reference specimens of the five exotic North American branchiobdellidan species collected in France were submitted to Professor Jean-Loup Justine, Curator of the Annelida Collection at the Museum National d'Histoires Naturelles (MNHN), Paris, France, on April 25<sup>th</sup>, 2017. The museum's registration number of a specimen is followed in parentheses by its site number taken from Table 1: *Cambarincola gracilis*, HEL647 (site 11), HEL651 (site 7) and HEL652 (site 95); *C. mesochoreus*, HEL655 (site 1); *C. okadai*, HEL648 (site 11), HEL653 (site 7) and HEL654 (site 95); *Triannulata magna*, HEL649 (site 95) and HEL656 (site 95) and *Xironogiton victoriensis*, HEL650 (site 36) and HEL657 (site 95).

*Triannulata magna* Goodnight, 1940: Adults have a large triangular head and elongated ovoid body,

measuring 3.5 to 5.5mm long, with three annuli per segment and no transverse segmental ridges (Figs 4 and 6). The dorsal peristomial lip is without lobes and the jaws have a dental formula of 8-1-8/8-1-8 (Figs 6 and 8). Both dorsal and ventral jaws are triangular with a large median tooth and eight pairs of very small lateral teeth (Fig. 8), however, the lateral teeth may not be visible resulting in an apparent dental formula of 1/1. Spermatozoa occur in segments 5 and 6, while the male organs consist of a sac-shaped glandular atrium (ga), a muscular atrium of similar length and spherical bursa containing an eversible penis in segment 6 (Figs 6 and 7) (emended from Holt 1974).



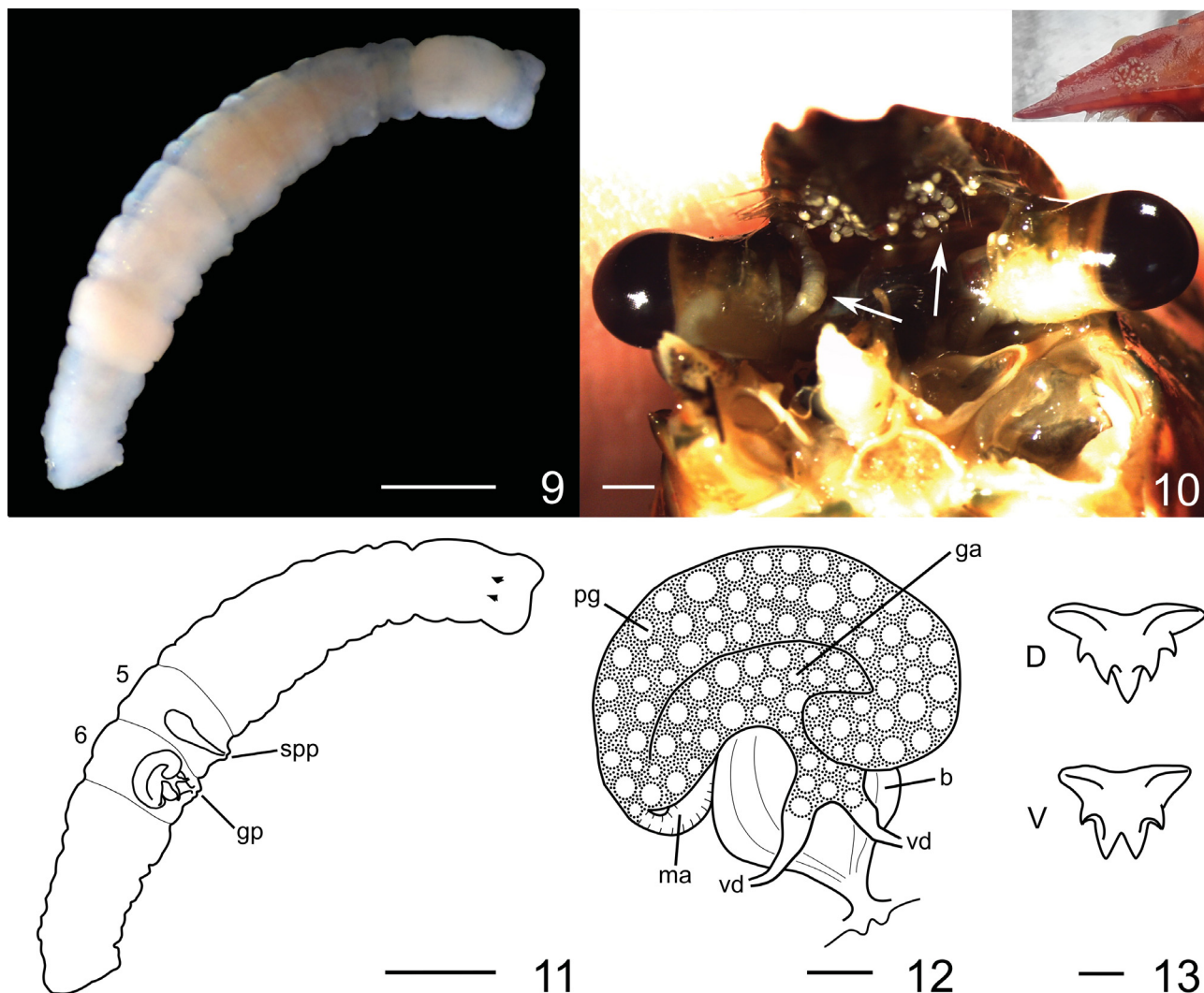
**FIGURES 4-8.** *Triannulata magna*. **Fig. 4.** Adult in latero-ventral view (MNHN-HEL656), bar = 1.0mm. **Fig. 5.** Adults (right arrows) near cocoons (left arrows) on ventral abdomen of *Pacifastacus leniusculus*, bar = 2.0mm. **Fig. 6.** Diagram of an adult in latero-ventral view showing position of jaws, spermatheca in segment 5 and major male organs in segment 6: gp, genital pore, spp, spermatheca pore, bar = 1.0mm. **Fig. 7.** Diagram of adult male organs in lateral view: b, bursa; ga, glandular atrium; ma, muscular atrium; ps, penial sheath; vd, vas deferens, bar = 100µm. **Fig. 8.** Juvenile jaws in ventral view with teeth pointing posteriorly: D, dorsal jaw; V, ventral jaw, bar = 50µm.

*Triannulata magna* is endemic on *P. leniusculus* in the Pacific Northwest, USA, and this is not only the first record of *T. magna* in Europe, but also in the Palearctic region. Specimens were found in the lateral depressions along the ventral abdominal segments (Fig. 5) and are consistent with its reported ventral body microhabitat. Close to adult *T. magna* were three cocoons (Fig. 5) with a length, including stalk, of 2.8 to 3.0mm and containing 8 to 15 embryos. In one cocoon an embryo was visible and measured about 0.8mm long, with a well-formed triangular head, triple body annulations, and triangular jaws with crenelated sides



and large medium tooth (Fig. 8). These observed morphological characters provide a virtual assurance the cocoons were *T. magna*. Without seeing such details in an embryo, it could have been possible that other cohabitants, *C. gracilis* and *C. okadai*, had deposited their cocoons in the area.

*Cambarincola mesochoreus* Hoffman, 1963: Adults have a distinct head and a rod-shaped body (Figs 9 and 11), measuring 2.5 to 4.2mm long, with two annuli per segment and no transverse segmental ridges. There are four small lobes on the dorsal peristomial lip, which may not always be visible due to preservation effects, and jaws with a dental formula of 5/4 (Fig. 13). The dorsal jaw is triangular with a large median tooth and two pairs of slightly smaller lateral teeth, while the ventral jaw is trapezoidal and has two pairs of teeth that interdigitate with those on the dorsal. Spermatozoa occur in segments 5 and 6, with the spermatheca located in segment 5 and male organs in 6 (Fig. 11). The male organs consist of a curved glandular atrium (ga) and a longer reflexed prostate gland (Fig. 12, pg). These organs are shown overlaying a muscular atrium and bursa containing a protrusible penis.



**FIGURES 9-13.** *Cambarincola mesochoreus*, for abbreviations see Figs. 4-8. **Fig. 9.** An adult in lateral view, bar = 0.5mm. **Fig. 10.** In foreground, an adult (left arrow) with cocoons (right arrow) under the rostrum of *Procambarus clarkii* (the antennae and antennules were removed), bar = 1mm. Thumbnail in top right, cocoons on the rostrum of *P. clarkii*, picture with Julien Rimour used permission. **Fig. 11.** Diagram of an adult in lateral view showing jaws, spermatheca in segment 5 and major male organs in segment 6, bar = 0.5mm. **Fig. 12.** Diagram of male organs in lateral view: pg, prostate gland, bar = 50µm. **Fig. 13.** Jaws in ventral aspect with teeth pointing posteriorly, bar = 20µm.

*Cambarincola mesochoreus* is endemic on *P. clarkii* in the southeast USA and adjacent Mexico, and this is the first record of the association in France, although the crayfish are much more widely distributed in the country. Branchiobdellidans were observed over most of the exposed body surface, but the rostrum area



appeared to be a favored microhabitat for both individuals and cocoons (Fig. 10: arrows and top right thumbnail).

## Discussion

The spread of exotic species and establishment of breeding populations through commercial and accidental translocations is resulting in the reduction or extinction of endemic species and consequent modification or destruction of traditional habitats (Keith & Allardi 1997; Lévêque 1997). This is particularly noticeable in Europe where large scale commercial introductions of North American crayfishes started about 1960 and have continued to date (Holdich *et al.* 2009; Kouba *et al.* 2015). Such translocations of crayfish are always accompanied by their endemic pathogenic and non-pathogenic symbionts (Longshaw 2011). While ectosymbiotic branchiobdellidans are generally non-pathogenic mutualists, a few species have a mainly parasitic association with their host (Gelder & Williams 2016).

Branchiobdellidans are found in most areas of North America (Gelder 2016) and the majority on crayfish species. However, only a few of these hosts are of commercial interest; Gelder (2004) listed a total of 24 endemic branchiobdellidan species reported on the three most popular commercial crayfishes: *P. leniusculus* (15), *P. clarkii* (6) and *F. limosus* (4). Since then, additional exotic crayfishes have been reported in Europe (Kouba *et al.* 2015), principally *F. immunis*, *F. juvenilis*, and *Faxonius virilis* (Hagen) with at least 10 more ornate species imported by the pet trade (Holdich *et al.* 2009), some of which also harbor branchiobdellidans. A revision of the endemic branchiobdellidans recorded on the newer crayfish imports has increased the total potential number of North American worms from 24 to about 50 (S.R. Gelder, unpub. data). Some of these are less than 1.5mm in length and will only be seen during a careful host examination using a magnification of x20 or greater. Up to the 1950s, branchiobdellidans found wild in Europe were assuredly members of the *Branchiobdella*, but as large-scale translocations of exotic North American crayfishes started, their endemic branchiobdellidans began to be observed on the continent (Franzén 1962). Identification of these exotic branchiobdellidans presented researchers with a challenge as some species had not been described at the time of their collection. For example, Franzén (1962) used the name of an eastern North American species, but he actually studied *X. victoriensis* which was only described 28 years later by Gelder and Hall (1990). Although being aware of this background, some reviewers still cite the original name, and this perpetuates the confusion in the literature. The taxonomic situation of North America branchiobdellidans has greatly improved with the availability of a recently published key to Nearctic species (Gelder 2016). Brief descriptions of the commonly occurring exotic European branchiobdellidans were included here for *C. mesochoreus* and *T. magna*, and in Gelder *et al.* (2012), to assist in the accurate identification and recognition of these species as their ranges expand.

During this freshwater faunal survey, four species of crayfish – *P. leniusculus*, *F. limosus*, *P. clarkii* and *A. pallipes* – were found carrying North American branchiobdellidans at 105 sites in France. *Pacifastacus leniusculus* tends to be found in colder, upper and headwaters of the Massif Central, Plateau de Millevaches, Massif Morvan, Basse Normandie and near Saint-Michel in northeast France. The three sites near Saint-Michel in northeast France are quite close together and each contains a multiple species cohabitation but with differing compositions: site 93 – *C. okadai* and *X. victoriensis*, site 94 – *C. gracilis*, *C. okadai* and *X. victoriensis*, and site 95 – *C. gracilis*, *C. okadai*, *T. magna* and *X. victoriensis*. In addition, sites 93 and 95 were sampled twice and the same species composition was found. Site 95 is unique as it is the first exotic, quadruple cohabitation of branchiobdellidans on *P. leniusculus* to be found in Europe, and also contains the first record of *T. magna* in the Palaearctic realm. It is probable that *P. leniusculus* and its four branchiobdellidan species were introduced to these sites at the same time, but why one and two species were lost at sites 93 and 94 respectively, is unknown. Similarly, the disparate distribution of triple species cohabitations at sites 7, 11, 13, 46, 48, 49, 63, 64, and 88, with two or single species at nearby sites is most likely the result of the same combination of unknown factors. One approach that might explain these variations in worm species on *P. leniusculus* is to trace the host's original locations back in the Pacific Northwest, USA. Unfortunately, the endemic distribution of *P. leniusculus* is complex and not fully understood (Larson and Williams 2016) and this is compounded as wild crayfish were collected from a number of waterbodies and held for subsequent translocation, thus resulting in both a taxonomic and geographic mixing. Finally, after the initial translocation into Europe, many secondary introductions, both legal and illegal occurred, not only from the USA but also from breeding stocks in Europe. An attempt at

tracking these movements in Europe was attempted using fragments of the mitochondrial gene for cytochrome *c* oxidase subunit I (COI) by Petrusek *et al.* (2017), but no clear distribution of haplotypes was found that corresponded to the various introductions. Therefore, any possibility of tracing the observed branchiobdellidan species combinations found in this study to their origins in the Pacific Northwest USA was discounted.

Following *P. clarkii*'s Spanish introduction in 1973 from Louisiana, USA, it has spread across the Iberian Peninsula, while subsequent introductions of *P. leniusculus* onto the Peninsula tended to extend across the northern half of the region (Kouba *et al.* 2015). *Xironogiton victoriensis* on *P. leniusculus* was first reported in the Basque Country (Gelder 1999) and later in the Ebro Basin, northeastern Spain, by Oscoz *et al.* (2010). Although the two crayfish species typically live in different habitats, both have shown an adaptability which has allowed them to cohabit in certain areas. Vedia *et al.* (2014) reported such an area in the River Piedra, Zaragoza, Spain, where *X. victoriensis* were found on both crayfishes. Iván Vedia (pers. comm.) also observed that this branchiobdellidan occupied the same microhabitat on the chelae on the adopted *P. clarkii*. This find is consistent with our observation on the exotic *F. limosus* and the endemic *A. pallipes* and that of the first published record of such an association on this last crayfish recently described in Teruel, eastern Spain, by Martín-Torrijos *et al.* (2018).

It is most likely that some illegal introductions *P. clarkii* into France came from Spain (Holdich *et al.* 2009; Kouba *et al.* 2015) and were probably supplemented by translocations from Louisiana, USA. Our study indicates *P. clarkii* are predominantly found in southern and southwestern France, with scattered records across the rest of the country (Kouba *et al.* 2015), but only at site 1 in the Adour drainages was it found to carry *C. mesochoreus* (Table 1). Subsequent collections in the same southwest area have shown this is not an isolated occurrence (J.-F. Parpet, unpub. obs.). Although this crayfish has an almost worldwide distribution (Kouba *et al.* 2015), endemic branchiobdellidans on translocated stocks are surprisingly rare with the only other European record being in northern Italy (Gelder *et al.* 1994), and a recent one in Japan (Ohtaka *et al.* 2017). A subsequent study of the northern Italian population of *P. clarkii* (Gelder *et al.* 1999) found the association unchanged, but in a second population a short distance away, the *C. mesochoreus* population had been replaced by European *Branchiobdella italica* Canegallo and *B. parasita*. Unfortunately, events leading to this replacement were not observed, but it became the first report of *Branchiobdella* species adopting *P. clarkii*. Although *C. mesochoreus* is the most common species found on *P. clarkii*, it is not the only one, and so specimens need to be carefully examined for sympatric *Cambarincola barbarae* Holt and *Cambarincola pamela* Holt. All three species have similar body shapes and a dental formula of 5/4, but each has differing shaped jaws which can be only recognized if the specimens are correctly orientated on a microscope slide. Hence an examination should also include the male reproductive system to confirm the species identification and determine if a multi-species cohabitation has been found (Holt & Opell 1993).

The first European introduction of *F. limosus* from the USA was made into Poland in 1890, followed by an unsuccessful attempt into northern France in 1896. A subsequent translocation of stock from Germany to France resulted in a breeding population being established shortly after (Kouba *et al.* 2015). To date, none of its endemic branchiobdellidans have been found on *F. limosus* in Europe, even though it was the second most numerous species to be collected in our survey. Twenty-one sites were found with *F. limosus* and *P. leniusculus* cohabiting, while at four of these sites (24, 55 and 100), *X. victoriensis* only populated *P. leniusculus*, in contrast with site 43, where only *F. limosus* carried *X. victoriensis*. This observation of *X. victoriensis* transferring to another host species could be explained if the *P. leniusculus* was unhealthy or molting. Unfortunately, no record of its condition was made, but whatever the reason, this is the first record of the western North American *X. victoriensis* being found on an exotic eastern *F. limosus*. This crayfish's acceptability also extends to endemic *Branchiobdella* species when it cohabits waters with European crayfishes (Bláha *et al.* 2017; Ďuriš *et al.* 2006; Dr. W. Struzynski, unpub. obs.; Vogt 1999). The reverse is also true as *X. victoriensis* has adopted the European *A. pallipes* at two closely located sites (56 and 57) making it the first report of such an ectosymbiotic combination in France and follows that in Spain (Martín-Torrijos *et al.* 2018). This association appears to be stable, as it was observed in samples taken at the sites two years apart. Although no exotic crayfishes were captured at sites 56 and 57, at site 55 in an adjacent tributary, *F. limosus* and *P. leniusculus* were found, with *X. victoriensis* on the latter species. Whether this reflected a host preference is difficult to say. However, it should be remembered that crayfish can travel overland from one freshwater sources to another, e.g., ponds, streams, seeps, and as demonstrated experimentally (Hunt *et al.* 2018) are capable of carrying viable branchiobdellidans with them.

These observations demonstrate conclusively that most branchiobdellidans are not species specific to a particular crayfish host, or even to one from its endemic region. Therefore, endemic associations once restricted to a region can no longer be assumed, as uncontrolled and new crayfish translocations continue to occur. The usual method of branchiobdellidan transfer from one host to another is during direct contact (Hunt *et al.* 2018). Alternatively, branchiobdellidans will leave the host following molting, bodily damage and death, to live on the substratum until another crayfish comes in contact with them, whether endemic or acceptable exotic. Such a temporary free-living situation was reported at sites 2, 12 and 59, where *X. victoriensis* was found in substrate samples, but no crayfish were captured. It is most probable crayfish were present, but they eluded capture during the collection period. Although free-living branchiobdellidans have been recorded in similar situations (Holt 1973; Timm 1991) and experimentally by Hunt *et al.* (2018), other extensive examinations of substrate samples have not found these worms (James *et al.* 2017, Gelder unpub. data). As the majority of branchiobdellidans have a diet of micro-flora and -fauna, food would not be a limiting factor while waiting for a host to make contact with them. Indeed *in vitro* observations of some branchiobdellidan species have shown they can be maintained alive for months (Gelder and Williams, 2015). However, it is most likely survival time is both species and conditions dependent. James *et al.* (2017) observed that *X. victoriensis* survived for over 21 weeks and *C. okadai* for less than two weeks while cohabiting on *P. leniusculus*, but when kept separately *in vitro* without a host, both ectosymbionts lasted for about the same amount of time (14 weeks). This free-living period would be adequate under most conditions for them to survive while awaiting contact with a new host. Such habitat adaptability and capacity to adopt either local endemic or exotic astacoidean crayfish favors the survival and range expansion of branchiobdellidans in Europe.

A consideration not so far discussed is the potential for branchiobdellidans in Europe to adopt commercially imported Australian crayfish. Yabbys, or *Cherax destructor* Clark were imported into Spain in 1983 from a farm in California, USA, and Redclaws, *Cherax quadricarinatus* (von Martens), primarily into Italy (Kouba *et al.* 2015). Although Australian crayfishes do not have endemic branchiobdellidans, they do carry flatworm temnocephalidans which fill the same niche (Gelder 1999). This ectosymbiotic association has already been reported three times in Europe, with *Temnosewellia minor* Haswell (synonym *Temnocephala minor*) on *C. destructor* in Italy (Quaglio *et al.* 1999; Scalici *et al.* 2009; Chiesa *et al.* 2015), but it is unlikely that these are the only examples of the association in Europe. Whether endemic or exotic branchiobdellidans are capable of adopting the southern parastacoidean crayfish is not known at this time. However, Vayssière (1898) did find branchiobdellidans and temnocephalidans cohabiting a specimen of *Procambarus digueti* Bouvier in Mexico. This demonstrates that *Temnocephala* sp. have found a northern cambarid crayfish an acceptable host, therefore it is predicted that branchiobdellidans may well adopt *Cherax* species if the opportunity arises.

The demand for crayfish is being driven by increased human consumption and the pet trade, and as a result the business of illegally translocating crayfishes is thriving. *Pacifastacus leniusculus* and *P. clarkii* have been known in Britain since 1970 (Holdich & Lowery 1988) and extensive studies have followed (Holdich *et al.* 2014); therefore, if exotic branchiobdellidans were present they would have been reported. As the first report of *P. leniusculus* carrying both *C. okadai* and *X. victoriensis* only occurred recently in south Wales (James *et al.* 2015), the population must have been a very recent translocation from outside the UK. This assessment is further supported as extensive sampling of Welsh rivers over the previous 35 years have yielded no branchiobdellidans (James *et al.* 2017). One explanation for these south Wales populations of infected *P. leniusculus* is that specimens were acquired for gastronomic reasons, probably from France, and then some individuals were either released or escaped into local waterbodies to form a starter population. This hypothesis is certainly supported by our reported presence of *C. okadai* and *X. victoriensis* on *P. leniusculus* in numerous areas of France. In response to the public demand in France, particularly at holiday times, fish markets offer both endemic and exotic live crayfish under the label “European crayfish” (J.-F. Parpet, unpub. obs.). Technically this would be true if the exotic species were raised in Europe, and so the chances they were imported into the country are high. However, a recent decree bans the import, transport, sale and purchase of live specimens of *F. limosus*, *P. leniusculus* and *P. clarkii* in metropolitan France (decree of February 14, 2018).

Although most European countries have stringent legislation banning uncontrolled importation and transportation of exotic crayfish species for human consumption and the pet trade, public demand appears to be greater than current enforcement efforts. In areas not yet invaded by the exotic crayfishes, active

enforcement of regulations has a chance to stop further range expansions. However, such measures need to be accompanied by a coordinated public education program on the environmental damage and financial costs these invasives cause to recreational areas and natural resources. Exotic crayfishes continue to be introduced for commercial reasons, but the hidden costs resulting from such biological invasions, e.g., the ancillary introduction of “crayfish plague”, have resulted in much higher indirect damages than is generally recognized.

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