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DISTRIBUTION OF ENTOCYTHERIDAE (CRUSTACEA: OSTRACODA) IN THE NORTHERN PRAIRIES OF NORTH AMERICA AND REPORTS OF OPPORTUNISTIC CLITELLATE ANNELIDS ON CRAYFISH HOSTS

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ABSTRACT.—Northern crayfish, *Orconectes virilis* (Hagen, 1870), were collected from 89 sites across Alberta, Saskatchewan, Manitoba, Montana, North Dakota, and Minnesota. The entocytherid ostracod *Thermastrocythere riojai* (Hoff, 1943) was found on *O. virilis* at 45 of the 89 sites, distributed primarily in the eastern and southern portion of the study area. These observations of *T. riojai* greatly extend the known range of the species. The widespread distribution of *T. riojai* suggests that the dearth of entocytherid records from other parts of Canada is a result of nontargeted sampling rather than true absence. In addition, we report on observations of 3 noteworthy associations of oligochaetes with their crayfish hosts.

RESUMEN.—Se colectaron especímenes del acocil *Orconectes virilis* (Hagen, 1870) en 89 sitios a lo largo de Alberta, Saskatchewan, Manitoba, Montana, North Dakota y Minnesota. Encontramos el ostracódo entocitérido *Thermastrocythere riojai* (Hoff, 1943) en *O. virilis* en 45 de los 89 sitios, distribuidos principalmente en la parte sur y la parte este del área de estudio. Estas observaciones de *T. riojai* amplían considerablemente el área de distribución conocida de esta especie. La distribución extensa de *T. riojai* sugiere que la escasez de registros de entocitéridos en otras partes de Canadá es el resultado del muestreo inespecífico y no de una ausencia verdadera. Además, reportamos observaciones de 3 asociaciones sobresalientes entre los oligoquetos y los acociles huéspedes.

Entocytheridae is a family of ostracods that are obligate ectosymbionts of other crustaceans ranging across southern central Europe, southern India, Australasia, and North America (Hart and Hart 1974). Two entocytherid subfamilies have been described from North America: (1) Entocytherinae, commensal on freshwater crayfishes of the families Astacidae and Cambaridae and on one species of freshwater crab, *Pseudothelphusa veracruzana* Rodriquez and Smalley, 1970; and (2) Sphaeromicolinae, commensal on freshwater isopods of the family Cirolanidae (Hart and Hart 1974). The known distribution of entocytherids in North America extends from Cuba and Mexico north to a boundary that approximates to the United States–Canada border (reviewed in Hart and Hart 1974). Despite widespread occurrence of several potential host crayfish species (e.g., Crocker and Barr 1968, Taylor et al. 1996, Hamr 1998, Hamr 2002, Williams et al. 2011), few published records of entocytherids exist in Canada. The description of *Entocythere insignipes* (Sars, 1926) was the sole report from the country until 1970, when *Thermastrocythere riojai* (Hoff, 1943) was found on crayfish collected in the Swan River near the town of Swan River in western Manitoba (Delorme 1970d, Hart and Hart 1974). No further contributions to the knowledge of entocytherid ostracods in Canada have been made since.

Similarly, reports of entocytherids are lacking from large areas of the northern Great Plains of the United States, including Montana and North Dakota. Representatives of another group of crayfish ectosymbionts, branchiobdellidans or crayfish worms, were recently discovered in the Prairie Provinces of Canada (Williams et al. 2009) and in Montana and North Dakota (B.W. Williams unpublished observation). The wide distribution of these worms suggests that the prior dearth of reports was due to lack of targeted sampling. Therefore, it is likely that other crayfish-associated organisms, including entocytherid ostracods, have also gone unnoticed across the region.

The aim of the current study is to document the distribution of entocytherid ostracods associated with crayfish hosts in the Prairie Provinces of Canada, with additional reports from Montana, North Dakota, and Minnesota. We also provide information on incidental, yet notable, observations of 3 additional taxa associated with crayfish hosts.

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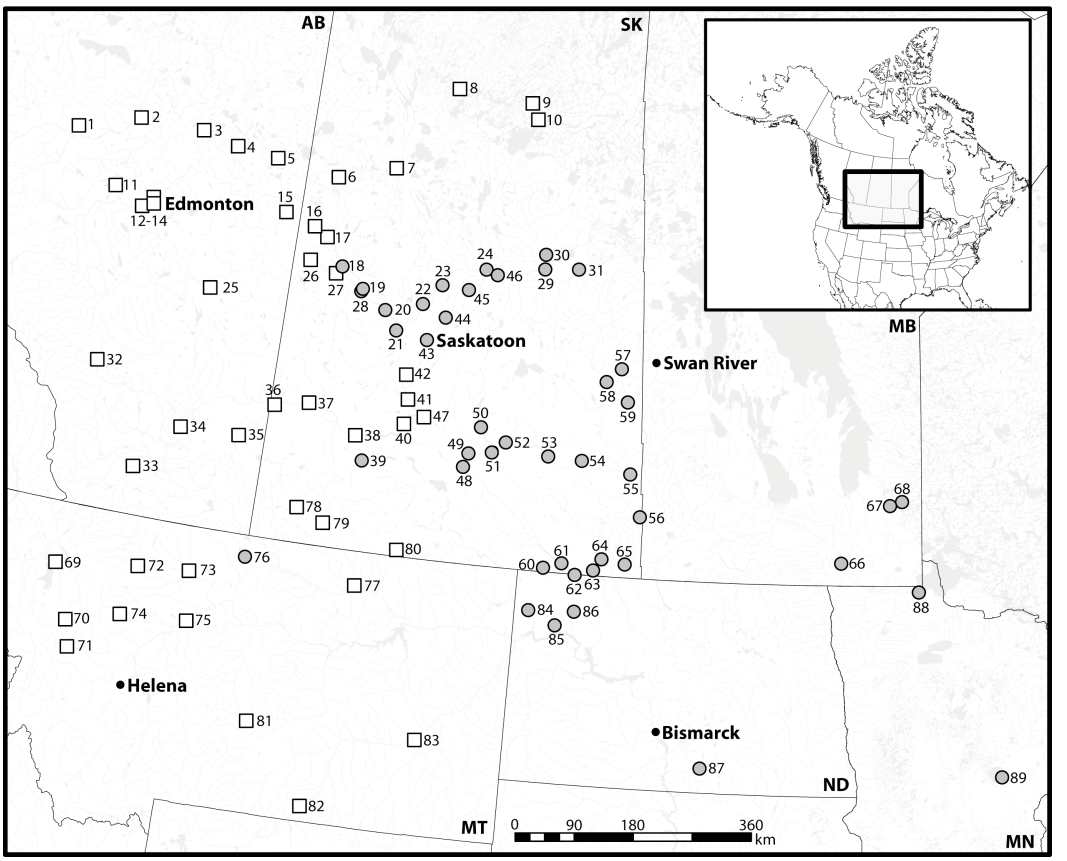

Fig. 1. *Orconectes virilis* (Hagen, 1870) sampling sites in the northern prairies of North America (Alberta, AB; Saskatchewan, SK; Manitoba, MB; Montana, MT; North Dakota, ND; Minnesota, MN) showing detection (shaded circles) and nondetection (open squares) of the entocytherid ostracod *Thermastrocythere riojai* (Hoff, 1943). Inset map delineates the study area in North America. Site numbers correspond to information provided in the appendix.

Northern crayfish, *Orconectes virilis* (Hagen, 1870), were collected as part of an ongoing population genetics study. A subset of specimens previously examined for branchiobdellidans (Williams et al. 2009; Appendix) were reexamined for entocytherid ostracods. Additional crayfish were collected by hand, by kick-netting, or in Gee minnow traps (Wildlife Supply Company, Buffalo, NY) deployed for up to 24 hours. The size of entrance holes in the minnow traps was increased to approximately 60 mm to allow access by adult crayfish. Crayfish were preserved individually in containers of 95% ethanol.

We examined a total of 1376 crayfish collected from 89 sites across Alberta, Saskatchewan, Manitoba, Montana, North Dakota, and Minnesota between August 2006 and October 2010 (Fig. 1, Appendix). The study area included portions of the Arctic, Hudson Bay, and Gulf of Mexico ocean watersheds. We used a dissecting microscope to examine the external surface and branchial chambers of each crayfish and the debris at the bottom of sampling containers for presence of entocytherid ostracods. Entocytherids were transferred to separate containers of 95% ethanol for preservation and storage. Representative entocytherids were dehydrated in 100% ethanol, cleared in methyl salicylate, infiltrated with Canada balsam, and mounted on glass slides to museum standards. Species identification was made using the keys and information in Hart and Hart (1974). Slide mounts were deposited in the New Brunswick Museum, Saint John, New Brunswick, Canada, as catalogue numbers NBM-007156–007160.

Entocytherid ostracods were found at 50.1% (45 of 89) of surveyed sites (Fig. 1, Appendix) in Saskatchewan, Manitoba, Montana, North

Dakota, and Minnesota. Entocytherids were attached to setae along the abdomen; around legs, maxillipeds, bases of antennae, and chelae; and under the rostrum of their crayfish hosts. *Thermastrocythere riojai* was the only species of entocytherid ostracod found.

During our study, we observed additional organisms associated with *O. virilis*. The findings of 3 of these organisms are noteworthy, as all represent previously unreported associations. An enchytraeid oligochaete was found in the gill chamber of an *O. virilis* specimen collected from Nose Creek in Calgary, Alberta (site 32; Fig. 1). Although enchytraeids are usually free-living, *Lumbricillus catanensis* (Drago, 1887) has been reported in the branchial chambers of the freshwater crab, *Potamon fluviatilis* (Herbst, 1785) (= *Telphusa fluviatilis*) in Italy (Gelder 1980). We also noted specimens of the oligochaete *Chaetogaster limnaei* Baer, 1827, on the carapace of 3 *O. virilis* collected in Long Creek, southern Saskatchewan (site 60; Fig. 1). Although *C. limnaei* is known worldwide as an ectocommensal of freshwater molluscs, primarily in the gill chamber of gastropods (Gelder 1980), it has not previously been reported in association with crayfish. Lastly, we recovered additional small oligochaetes from the carapace of *O. virilis* from Long Creek. Although many of the small oligochaetes fragmented during preservation, we identified a subset as *Nais* sp., either *Nais alpina* Sperber, 1948 or *Nais simplex* Piguet, 1906. *Nais* sp. and other naidids have been observed on freshwater ectoprocts, which Sperber (1948) considered part of their normal foraging habits and which is consistent with our observations on crayfish. Both *Nais* species and *C. limnaei* have been previously reported from Canada (Brinkhurst 1986). As each of the above associations was observed only once, presence of these oligochaetes on *O. virilis* in our collection is unlikely to reflect a stable symbiosis. Conversely, our findings are likely a result of natural, random wanderings of the host and oligochaetes over the substratum.

Prior to this study, *T. riojai* was known in Canada only from a single site in western Manitoba (Delorme 1970d, Hart and Hart 1974). Our survey greatly extends the reported distribution of *T. riojai* westward into central and western Saskatchewan. We also provide new records of the species in areas of Montana, North Dakota, eastern Manitoba, and northern Minnesota. The species is now known to range from eastern Texas and Louisiana north through the northern Great Plains and western Great Lakes states, and into the eastern and central Prairie Provinces (Hart and Hart 1974). Detections of *T. riojai* at sites on the Winnipeg River in Manitoba (sites 67, 68) and Lake of the Woods in Minnesota (site 88) indicate that the species may also be present in adjoining waterways of southwestern Ontario.

It is important to note that the crayfish from Swan River harboring *T. riojai* had been identified as the rusty crayfish, *Orconectes rusticus* (Girard, 1852) (Delorme 1970d). Originating from areas of north central North America (Tennessee, Kentucky, Indiana, Ohio, Michigan, and southern Ontario; Hobbs 1974), *O. rusticus* is thought to have been only recently introduced to southeastern Manitoba through its use as live bait; the first confirmed report of the species in the province was from Falcon Lake in 2007 (Lowdon 2009). As the lack of known voucher specimens precluded species verification of the original collection, we suggest that the crayfish collected from Swan River were likely *O. virilis*. *Orconectes virilis* is the only species found throughout much of the Prairie Provinces (Williams et al. 2011), including a sampling site in the current study (site 57 on the Swan River, Saskatchewan) approximately 50 km upstream from the town of Swan River, Manitoba. The calico crayfish, *Orconectes immunis* (Hagen, 1870), unsampled in this study, is also found in Manitoba but is restricted to the southeastern portion of the province in the Red River drainage and in 2 isolated areas in the Winnipeg River near the Ontario border.

Although we did not sample in western Manitoba, the distribution of *T. riojai* appears to be contiguous along the rivers of Saskatchewan and Manitoba (Fig. 1). On the basis of our collections and previous records summarized in Hart and Hart (1974), the general northern distribution likely includes the Red and Assiniboine River systems flowing into southern Hudson Bay, the Upper Great Lakes region draining to the Atlantic Ocean, and the Missouri and Upper Mississippi River systems flowing south toward the Gulf of Mexico. The western edge of the observed *T. riojai* distribution was inconsistent across sampled waterways relative to longitude. These differing observed range limits could result from incomplete sampling, such as the lack of collections

made along the Missouri River in eastern Montana and western North Dakota. However, particularly in rivers that were systematically sampled in this study (e.g., the North Saskatchewan and South Saskatchewan rivers), variation in range boundaries suggests differing barriers, such as physical (e.g., dams) or environmental barriers (e.g., tolerance limits).

The westernmost collection of *T. riojai* was from Fresno Reservoir on the Milk River in northern Montana (site 76, 48.685°N, 100.008°W; Fig. 1). However, this collection appears to be isolated and distant from the nearest collections of *T. riojai* to the north and east. No entocytherids were found elsewhere in Montana, including a second site on the Milk River (site 77; Fig. 1) and locations sampled in southern Saskatchewan in streams or rivers that drain into the Milk River (e.g., sites 78–80; Fig. 1). An identical distribution pattern was observed for the branchiobdellidan *Cambarincola vitreus* Ellis, 1919, which was found on *O. virilis* in Fresno Reservoir but on no other crayfish examined from Montana (B.W. Williams unpublished observation). Although nondetection does not necessarily correspond to true absence, the isolated observations of *T. riojai* and *C. vitreus* in Fresno Reservoir suggest an introduction of the *O. virilis* host from an area where both symbionts are sympatric (for distributions see Hart and Hart 1974, Gelder et al. 2002, Williams et al. 2009).

Orconectes virilis has been expanding its range westward in Montana and in the Prairie Provinces of Canada due to an apparent combination of natural dispersal and human-mediated introduction (Williams et al. 2011). In some cases, such as with *O. virilis* from the Fresno Reservoir, symbiont presence might be used to indicate likely crayfish introductions. A second host introduction might explain an apparent isolated collection of *T. riojai* in Swift Current Creek, Saskatchewan (site 39; Fig. 1). Two major dams, the Qu'Appelle River Dam and the Gardiner Dam, separate the Swift Current Creek collection from both nearest observed collections of *T. riojai* (site 43 on the South Saskatchewan River and site 49 on the Qu'Appelle River).

At the majority of sites where *T. riojai* was observed, the species was common and found on the majority of crayfish examined. In contrast, entocytherids were rare at the western edge of the observed range, suggestive of environmental tolerances or few founders at a leading edge of a range expansion. For example, a total of 5 *T. riojai* were found among 21 crayfish examined from site 18 (Fig. 1). In contrast, *T. riojai* were numerous (>5 per crayfish) in the 2 isolated populations observed in Fresno Reservoir (site 76) and Swift Current Creek (site 39), despite the examination of only 2 *O. virilis* hosts from the former site.

Delorme's (1970a, 1970b, 1970c, 1970d) 4-part review of freshwater ostracods of Canada included 84 species from 8 families but only a single species of entocytherid, *T. riojai*, from Swan River, Manitoba. The earliest recorded entocytherid from Canada, *Cytherites insignipes* Sars, 1926, was a new species description based on examination of 3 female specimens; however, collector, locality (other than "Canada"), date of collection, host, and male morphology were all unknown. Hoff (1944) reassigned *C. insignipes* to the genus *Entocythere*, but due to the lack of taxonomically distinguishing male morphological characters, the species is currently considered *incertae sedis* (Hart 1962).

Several entocytherid species are reported from areas immediately south of the United States–Canada border, including *Uncinocythere occidentalis* (Kozloff and Whitman, 1954) and *Uncinocythere columbia* (Dobbin, 1941) in northern Washington; *Uncinocythere stubbsi* Hobbs and Walton, 1966, and *T. riojai* in the Upper Great Lakes states; and *Donnaldsoncythere scalis* Hobbs and Walton, 1963, in upper New England (ranges summarized in Hart and Hart 1974). Several potential crayfish hosts are found throughout southern Canada as extensions of known distributions in the United States. Therefore, it is inevitable that additional records and range extensions of entocytherid ostracods will appear as researchers begin targeted examination of crayfishes in Canada.

AUTHOR CONTRIBUTIONS

BWW made the majority of the field collections across Alberta and Saskatchewan, Canada, identified the crayfish and entocytherid species, and prepared the manuscript. KLW assisted with field collection and laboratory sampling of the entocytherids. SRG identified the additional oligochaetes and assisted with manuscript preparation. HCP provided laboratory space and resources.

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See Appendix on pages 281–282.

APPENDIX. Detection (+) or nondetection (–) of the entocytherid ostracod *Thermastrocythere riojai* (Hoff, 1943) on *Orconectes virilis* (Hagen, 1870) collected across the northern prairies region, with information on sample location (latitude and longitude, NAD83) and number of hosts examined (*N* hosts). Site numbers correspond to locations in Figure 1.

Site	Waterbody	Latitude (°N)	Longitude (°W)	*T. riojai*	*N* hosts
1	McLeod Lake	54.293	115.651	–	29
2	Beyette Lake	54.592	114.199	–	15
3	Amisk Lake	54.604	112.647	–	22
4	Amisk River	54.461	111.772	–	23
5	Beaver River	54.389	110.755	–	21
6	Beaver River	54.260	109.221	–	26
7	Beaver River	54.510	107.868	–	12
8	Churchill River	55.733	106.565	–	1
9	Churchill River	55.643	104.734	–	30
10	Churchill River	55.418	104.561	–	28
11	East Pit Lake	53.584	114.464	–	5
12	North Saskatchewan River	53.370	113.751	–	16
13	Bearspaw Lake	53.443	113.505	–	26
14	North Saskatchewan River	53.502	113.561	–	28
15	North Saskatchewan River	53.659	110.337	–	6[a]
16	North Saskatchewan River	53.523	109.618	–	19
17	North Saskatchewan River	53.396	109.293	–	29
18	North Saskatchewan River	53.245	105.433	+	20
19	North Saskatchewan River	52.743	108.284	+	13
20	North Saskatchewan River	52.491	107.699	+	19
21	Eagle Creek	52.232	107.380	+	26
22	North Saskatchewan River	52.646	106.842	+	18
23	North Saskatchewan River	52.946	106.435	+	24
24	North Saskatchewan River	53.182	105.162	+	22
25	Battle River	52.409	111.810	–	20[a]
26	Battle River	53.046	109.601	–	25
27	Battle River	52.907	108.949	–	20
28	Battle River	52.717	108.310	+	21
29	Saskatchewan River	53.237	104.464	+	17
30	Torch River	53.539	104.069	+	19
31	Carrot River	53.366	103.264	+	26
32	Nose Creek	51.086	114.047	–	16
33	Henderson Lake	49.688	112.790	–	24
34	Lake Newell Reservoir	50.379	111.911	–	20
35	South Saskatchewan River	50.399	110.589	–	13
36	South Saskatchewan River	50.913	109.890	–	4
37	South Saskatchewan River	51.024	109.134	–	36[a]
38	South Saskatchewan River	50.656	107.975	–	27[a]
39	Swift Current Creek	50.308	107.769	+	31
40	South Saskatchewan River	50.905	106.917	–	22
41	South Saskatchewan River	51.259	106.896	–	10
42	South Saskatchewan River	51.613	107.008	–	14
43	South Saskatchewan River	52.137	106.646	+	12[a]
44	South Saskatchewan River	52.491	106.283	+	25
45	South Saskatchewan River	52.923	105.805	+	22
46	South Saskatchewan River	53.182	105.162	+	25
47	South Saskatchewan River	51.034	106.494	–	21
48	Moose Jaw Creek	50.394	105.497	+	24
49	Qu'Appelle River	50.595	105.411	+	22
50	Last Mountain Lake	50.992	105.180	+	21
51	Qu'Appelle River	50.630	105.007	+	12
52	Qu'Appelle River	50.804	104.581	+	20
53	Qu'Appelle River	50.662	103.603	+	20
54	Qu'Appelle River	50.642	102.847	+	21
55	Qu'Appelle River	50.499	101.728	+	10
56	Pipestone Creek	49.886	101.449	+	28
57	Swan River	51.998	102.075	+	21
58	Assiniboine River	51.792	102.408	+	15
59	Assiniboine River	51.533	101.877	+	20
60	Long Creek	49.062	103.498	+	4
61	Rafferty Dam Reservoir	49.145	103.098	+	25

APPENDIX. Continued.

Site	Waterbody	Latitude (°N)	Longitude (°W)	*T. riojai*	*N* hosts
62	Short Creek Dam	48.992	102.784	+	12
63	Souris River	49.079	102.399	+	17
64	Moose Mountain Creek	49.261	102.239	+	21
65	Antler River	49.193	101.711	+	6
66	Rat River	49.317	96.945	+	2
67	Winnipeg River	50.160	95.867	+	1
68	Winnipeg River	50.210	95.588	+	13
69	Echo Lake	48.121	114.036	–	3
70	Lake Alva	47.316	113.582	–	3
71	Clearwater River	46.945	113.431	–	3
72	Lake Frances	48.285	112.264	–	3
73	Tiber Reservoir	48.342	111.163	–	3
74	Willow Creek Reservoir	47.557	112.443	–	3
75	Missouri River	47.627	111.035	–	4
76	Fresno Reservoir	48.685	110.008	+	2
77	Nelson Reservoir	48.495	107.546	–	2
78	Conglomerate Creek	49.507	109.047	–	4
79	Frenchman River	49.335	108.417	–	27
80	Weatherall Creek	49.093	106.738	–	1
81	Deadmans Basin	46.339	109.426	–	2
82	Yellowtail Dam	45.228	108.072	–	5
83	Settling pond, Miles City	46.387	105.867	–	2
84	Blacktail Dam	48.433	103.735	+	3
85	Kota-Ray Dam	48.239	103.143	+	3
86	White Earth Dam	48.456	102.744	+	3
87	Beaver Creek	46.297	99.866	+	7
88	Lake of the Woods	48.899	95.240	+	3
89	Mille Lacs	46.211	93.528	+	7

[a]Crayfish collected for Williams et al. (2009) re-examined for presence of entocytherids.