2015

New Crayfish Species Records from the Sipsey Fork Drainage, Including Lewis Smith Reservoir (Alabama, USA): Native or Introduced Species?

Susan B. Adams
USDA Forest Service, Southern Research Station, Center for Bottomland Hardwoods Research, Oxford, Mississippi

Craig Roghair
USDA Forest Service, Southern Research Station, Stream Biota and Habitat Team, Blacksburg, Virginia

Colin Krause
USDA Forest Service, Southern Research Station, Center for Bottomland Hardwoods Research, Oxford, Mississippi

Melvin L. Warren Jr
1 USDA Forest Service, Southern Research Station, Center for Bottomland Hardwoods Research, Oxford, Mississippi

J. Allison Cochran
USDA Forest Service, National Forests in Alabama, Bankhead National Forest, Double Springs, AL

See next page for additional authors

Recommended Citation

Freshwater Crayfish 21(1):17–32, 2015 DOI: http://dx.doi.org/10.5869/fc.2015.v21-1.17

New crayfish species records from the Sipsey Fork drainage, including Lewis Smith Reservoir (Alabama, USA): native or introduced species?

ARTICLE · JANUARY 2015

DOI: 10.5869/fc.2015.v21-1.17

RESEARCH ARTICLE

Freshwater Crayfish 21(1):17–32, 2015
ISSN: 2076-4324 (Print), 2076-4332 (Online)
http://dx.doi.org/10.5869/fc.2015.v21-1.17

New Crayfish Species Records from the Sipsey Fork Drainage, Including Lewis Smith Reservoir (Alabama, USA): Native or Introduced Species?

Susan B. Adams,[1,*] Craig Roghair,[2] Colin Krause,[2] Melvin L. Warren Jr.,[1] J. Allison Cochran,[3] Andy Dolloff,[2] John Moran,[4] Stuart W. McGregor,[5] Guenter A. Schuster,[6] Michael Gangloff,[7] Dennis R. DeVries,[8] Michael R. Kendrick,[9] G. Lee Grove[8] and Russell A. Wright[8]

[1] USDA Forest Service, Southern Research Station, Center for Bottomland Hardwoods Research, Oxford, Mississippi 38655, USA.
*Corresponding Author.— *sadams01@fs.fed.us*

[2] USDA Forest Service, Southern Research Station, Stream Biota and Habitat Team, Blacksburg, Virginia 24060, USA.

[3] USDA Forest Service, National Forests in Alabama, Bankhead National Forest, Double Springs, AL 35553, USA.

[4] USDA Forest Service, National Forests in Alabama, Montgomery, AL, 36107, USA.

[5] Geological Survey of Alabama, Ecosystems Investigations Program, Tuscaloosa, Alabama 35486, USA.

[6] Department of Biological Sciences, Eastern Kentucky University, Richmond, KY 40475, USA.

[7] Appalachian State University, Biology Department, Boone, NC 28608, USA.

[8] School of Fisheries, Aquaculture & Aquatic Sciences, Auburn University, AL 36849, USA.

[9] Department of Biological Sciences, University of Alabama, Tuscaloosa, AL 35487, USA.

ABSTRACT

As part of a study of aquatic faunal community changes along riverine-lacustrine transition zones upstream of Lewis Smith Reservoir in northwest Alabama, USA, we collected crayfish from 60 sites in the Sipsey Fork, Brushy Creek, and selected tributaries (Black Warrior River system). After finding two unexpected and possibly-introduced crayfish species, we expanded our investigation of crayfish distributions to include crayfish obtained from stomachs of black bass (*Micropterus* spp.) caught at seven sites in the reservoir. To explore what crayfish species were in the drainage historically, we examined museum databases as well as stomach and intestinal contents of a variety of preserved fishes that were caught in the Sipsey Fork and Brushy Creek drainages upstream of the reservoir in the early 1990's. Of the seven crayfish species collected, one, *Orconectes (Procericambarus)* sp. nr *ronaldi*, was not previously reported from Alabama, and another, *O. lancifer*, was not reported from the Black Warrior River system prior to the study. Three are known or possibly introduced species. Upstream of the reservoir, the native species *Cambarus obstipus*, *C. striatus*, and *O. validus* were common. The same three species were found in fish collected in the 1990's. *Orconectes perfectus* was found only in the reservoir but may be native to the drainage. *Orconectes lancifer* was in the reservoir and in stream reaches influenced by the reservoir. Evidence points to *O. lancifer* being introduced in the drainage, but this is uncertain. *Orconectes* sp. nr *ronaldi* was found in a relatively small portion of Brushy Creek and its tributaries, in both flowing and impounded habitats, and may be introduced. *Orconectes virilis* is introduced in Alabama and was found only in stomachs of fish collected in the reservoir.

ARTICLE INFO

Article History:
Submitted: 10 JUN 2015
Accepted: 07 DEC 2015
Published Online: 31 DEC 2015
Published Print: 31 DEC 2015

Keywords:
crayfish;
distribution;
fish diets;
native;
non-native;
Orconectes;
reservoir;
stream;

INTRODUCTION

The southeastern United States leads the world in crayfish species diversity but also in the number of crayfish species that are listed as Data Deficient under International Union for the Conservation of Nature (IUCN) Red List criteria (Richman et al. 2015), meaning information on distribution, abundance, temporal trends, and threats are insufficient to allow a conservation ranking according to IUCN criteria. In Alabama, a long-term effort is underway to refine the taxonomy and better assess the distribution

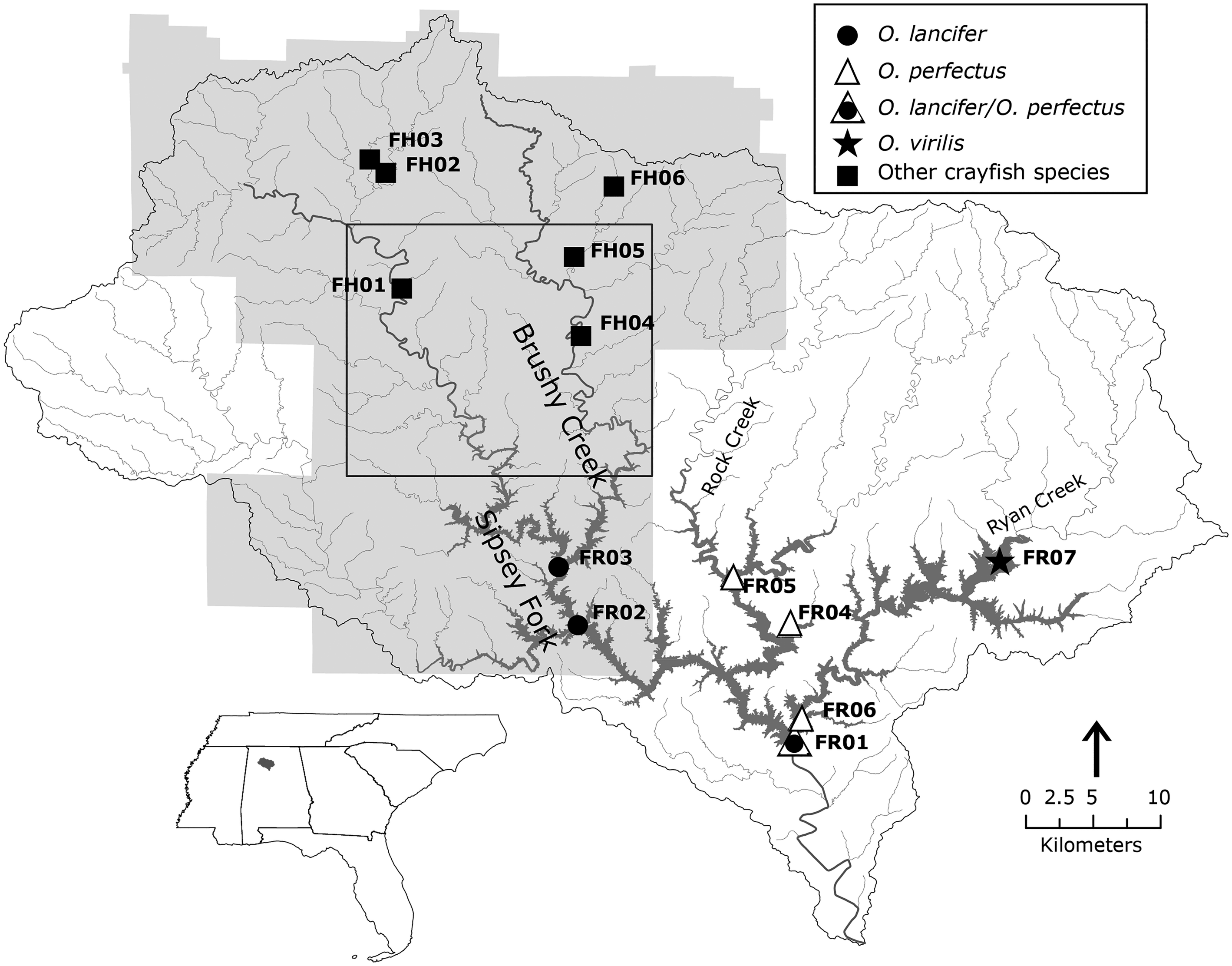

Figure 1. Sipsey Fork drainage, including Lewis Smith Reservoir, Alabama, USA, and indirect sampling sites (i.e., fish collection locations). Site codes as in Table 1. Symbols for individual species reflect distributions of species whose origin is uncertain. "Other crayfish species" indicates that the site yielded at least one of the known native species: *Cambarus obstipus*, *C. striatus*, or *Orconectes validus*. Shaded area is the Bankhead National Forest. Inset shows location of the study area in Alabama. Rectangle indicates area of direct sampling shown in Figure 2.

of the state's crayfish. The number of formally recognized crayfish species in the state currently hovers around 86. However, many gaps still exist in our knowledge of crayfish distributions, especially for certain crayfish groups. Primary burrowing species and species occupying large waterbodies are particularly underrepresented in the historic data, and new species and species records continue to be found in Alabama (Schuster et al. 2008, 2015).

Rapidly changing species distributions add to the difficulty of documenting distributions over large areas. Factors contributing to changing distributions can include land use changes, hydrologic changes (e.g., impoundment of rivers, hydrologic connection of previously discrete river systems, alteration of water tables), and species introductions, both intentional and unintentional. Species introductions can contribute to sudden and dramatic distributional changes due not only to the addition of the non-native species, but also to the extirpation or range contraction of native species (Arcella et al. 2014; Richman et al. 2015).

We report on occurrences of crayfish species found in two mainstem streams that flow into Lewis Smith Reservoir, as well as in the reservoir itself, and compare those to previously known crayfish distributions from the streams and reservoir. The species were collected while working on a larger study examining changes in the aquatic faunal community composition along riverine-lacustrine transition zones (hereafter transition zones). The transition zones were stream segments that were inundated by the reservoir at high pool but flowing at low pool. The main objectives of this paper are to document recent occurrences of species previously unknown in the drainage and to discuss possible explanations for the new records.

MATERIALS AND METHODS

The study area in northwest Alabama, USA, lies within the Warrior Basin district of the Cumberland Plateau physiographic province, geologically dominated by Pottsville shale, sandstone, and coal (Boschung and Mayden 2004). Streams in the province typically flow through deeply entrenched valleys and have well-defined pool-riffle habitat sequences with substrate dominated by gravel, sand, slabrock, and bedrock (Haag and Warren 2008), along with some cobble and boulders. The Sipsey Fork, a major tributary to the Mulberry Fork of the Black Warrior River, was impounded by the 91 m high Lewis Smith dam in 1961, creating the 8,580 ha Lewis Smith Reservoir (Boschung and Mayden 2004). We studied two mainstem streams flowing into the reservoir, Sipsey Fork and Brushy Creek, as well as three tributaries to each stream and several sites throughout the reservoir (Figures 1 and 2). Both streams originated in the Bankhead National Forest, with much of the Sipsey Fork headwaters protected in the Sipsey Wilderness and the Sipsey Wild and Scenic River corridor. We defined river-reservoir transition zones as the stream segments impounded when the reservoir was at the summer full-pool elevation targeted by managers (155.5 m above sea level) but flowing at the typical winter low-pool elevation (153.0 m). We determined the transition zones based on both elevation contours and examination of habitat during reservoir full- and low-pool periods. Both streams had potential transition zones of about 8 km. The tributaries were relatively small (wetted widths 3 – 7 m), rocky streams that typically had little flow in late summer. Because they had steeper channel slopes than the larger streams, the transition zones in the tributaries were much shorter (< 0.1 – 0.6 km).

Direct Sampling for Crayfish

In the mainstem streams, we sampled every 1 – 3 km downstream of, in, and upstream of the transition zones. In three tributaries of each mainstem, we sampled from the impounded zone, if present, to well upstream of the transition zones. Sampling was most intensive and most effective for capturing crayfish during low pool in the autumn. We sampled the Sipsey Fork and its tributaries in September 2012, Brushy Creek and its tributaries and the Sipsey Fork upstream of the transition zone in September 2013, the lower transition zone mainstem and well upstream of the transition zones in tributaries in October 2014, and well upstream of the transition zones in mainstems and tributaries in April 2015.

We used a variety of methods to capture crayfish. Although some methods were quantitative, the overall effort was a qualitative sampling approach aimed at documenting species occurrences in a variety of lotic and lentic habitats. Sampling methods in deep habitats included boat electrofishing, trawling, and trapping (minnow traps with 3.2 cm openings and baited initially with canned dog food and later with pieces of fresh fish). In wadeable habitats, we used backpack electrofishing, seining, visual searches (using mask and snorkel, view buckets, rock flipping, and searches for molted carapaces along banks), and occasionally digging of burrows along banks.

Indirect Sampling for Crayfish

Our initial crayfish results led us to ask about the distributions of crayfish species in the continuously impounded portion of the reservoir. We obtained crayfish from an independent, concurrent study of black bass (*Micropterus henshalli* Baker et al. and *Micropterus salmoides* (Lacepède)) diets in the reservoir. Black

Table 1. Fish collection sites for indirect samples. Site codes beginning with "FH" indicate sites sampled from 1993 – 1995, and those beginning with "FR" indicate sites sampled from 2013 – 2014. All sites were in the Sipsey Fork drainage, Alabama. Coordinates are in the NAD83/WGS84 datum.

Site	Location	Latitude	Longitude	County
FH01	Sipsey Fork	34.25275	-87.36695	Winston
FH02	Flannagin Creek	34.33877	-87.38808	Lawrence
FH03	Borden Creek	34.32987	-87.37766	Lawrence
FH04	Brushy Creek	34.22108	-87.24703	Winston
FH05	Rush Creek	34.27388	-87.25187	Winston
FH06	Brown Creek	34.32082	-87.22515	Lawrence
FR01	Forebay	33.94851	-87.10441	Cullman
FR02	Sipsey A	34.02795	-87.24926	Winston
FR03	Sipsey B	34.06682	-87.26199	Winston
FR04	Rock A	34.02698	-87.10819	Winston
FR05	Rock B	34.06098	-87.14555	Winston
FR06	Ryan A	33.96499	-87.10091	Cullman
FR07	Ryan B	34.07066	-86.96745	Cullman

bass were captured via boat electrofishing at seven sites in the reservoir (Figure 1; Table 1) from July 2013 to September 2014 (Table 2). Bass were placed on ice and taken to the laboratory, where stomachs were dissected and contents stored in 95% ethanol. We attempted to identify all crayfish remains to species.

In an attempt to determine whether certain crayfish taxa were new to the study area, we also examined gut contents of potentially predaceous fishes that were collected in the Sipsey Fork, Brushy Creek, and their tributaries from October 1993 to July 1995, preserved in 5% formalin, and stored in 70% ethanol (in part, Haag and Warren 1998). These sites were farther upstream in the drainages than were most of our recently sampled sites (Figure 1). We dissected all fish deemed capable of eating adult crayfish (assessed subjectively based on fish size and gape size) and removed all crayfish and parts of undigested crayfish exoskeletons from stomachs and intestines. We attempted to identify whole crayfish specimens, as well as the parts, to species.

Genetic Analysis

We used a DNA bar-coding approach to assist in the identification of the *Orconectes* (*Procericambarus*) sp. collected. Initial morphological identifications suggested that these individuals were *Orconectes juvenilis* (Hagen). We extracted DNA from two specimens and amplified a region of the mitochondrial cytochrome oxidase subunit I gene (COI), a standard bar-coding region of the mitochondrial genome useful for diagnosing problematic or unknown specimens. Methods of DNA isolation, amplification, and sequencing are described in Taylor et al. (2014). Following methods described in Kessler et al. (this issue), we compared the COI sequences from our specimens to sequences from GenBank and from the Kessler et al. dataset, including each of the members of the *O. juvenilis* complex as well as other North American crayfish (see Table 2 in Kessler et al. in this issue, for details, including GenBank accession numbers). GenBank

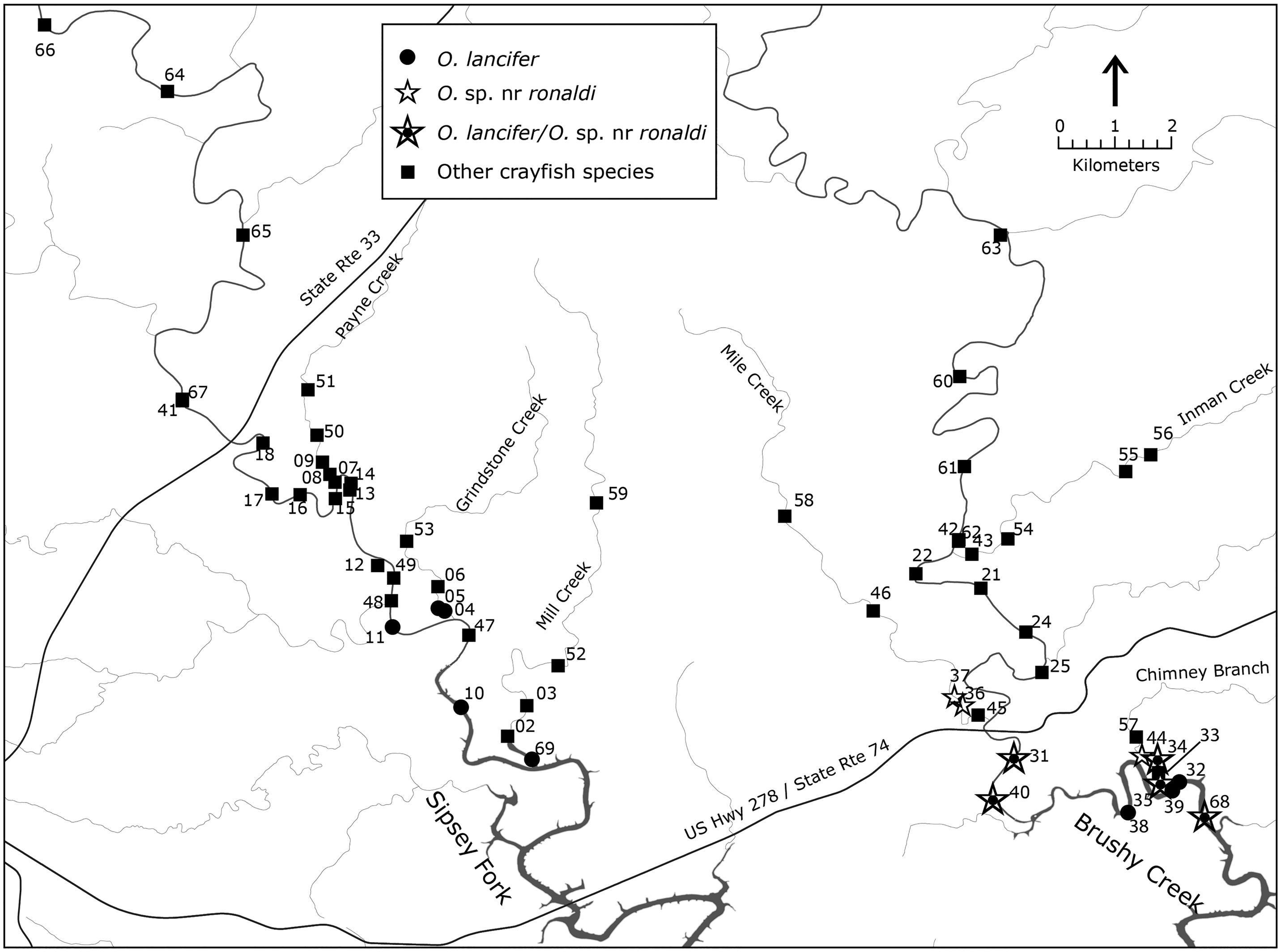

Figure 2. Direct sampling sites from which we collected crayfish in the Sipsey Fork, Brushy Creek, and their tributaries. "Other" represents sites where only known native species (as in Figure 1) were collected. Sites where no crayfish were collected are not shown. Site numbers coincide with those in Appendix 1, where collection details are provided.

accession numbers for the two specimens from Brushy Creek that we sequenced are KU168758 and KU168759.

RESULTS

Direct Crayfish Sampling

In Sipsey Fork/Brushy Creek sampling, we collected crayfish from 60 sites (Figure 2). Of the five crayfish species we found, three were expected [*Cambarus obstipus* Hall, *Cambarus striatus* Hay, and *Orconectes validus* (Faxon)] and two were not [*O. lancifer* (Hagen) and *O.* (*Procericambarus*) sp. nr *ronaldi*]. We were aware that *Orconectes virilis* (Hagen) was invasive elsewhere in the Black Warrior River system but did not find it in the Sipsey Fork or Brushy Creek upstream of the reservoir.

The three native species that we anticipated finding all occurred in perennially free-flowing stream segments upstream of any impoundment influences, as well as to varying extents in transition or impounded zones. *Cambarus obstipus* occurred frequently in the upper transition zones and upstream in both mainstem streams and their tributaries (Appendix 1). *Orconectes validus* had a similar distribution in Brushy Creek but also occurred in permanently impounded portions of the Sipsey Fork (Appendix 1). In the impounded and transition zones of the mainstems, *C. striatus* was found only in burrows near spring seeps; however, at one site in Brushy Creek nearly 10 km upstream of the transition zone, *C. striatus* was relatively abundant (Appendix 1). *Cambarus striatus* occurred in every tributary sampled, typically occurring upstream of the transition zones.

Orconectes lancifer occurred in the impounded portions of the Sipsey Fork and Brushy Creek, as well as in the transition zones of two tributaries - one in each watershed (Figure 2, Appendix 1). One individual also was caught a short distance upstream of the transition zone in Grindstone Creek.

We found *O.* sp. nr *ronaldi* only in the Brushy Creek watershed, where it occurred in the impounded and lower transition zones of the mainstem, as well as slightly upstream of the transition zones in both Brushy Creek tributaries that flowed into the impounded zone (Figure 2). We did not find it in collections farther upstream in those tributaries. We initially identified the species as *O. juvenilis* because, in addition to matching many other morphological features fairly closely, the incisor region of the mandible had a straight edge, an important diagnostic character distinguishing *O. juvenilis* from *Orconectes ronaldi* Taylor (Taylor 2000). Based on morphology, Dr. Chris Taylor (Illinois Natural History Survey) tentatively confirmed the identification. However, because the study area was far from the range of *O. juvenilis*, the possibility remained that the population represented a new species.

Comparisons of COI sequences from our Alabama specimens to those in Kessler et al. (this issue) and in GenBank indicated that the two sequenced individuals were most similar to *O. ronaldi*. On average, sequences from the two Alabama specimens differed (uncorrected p-distance) from *O. ronaldi* by 3.2% and from *O. juvenilis* by 6.6%. Re-examination of the specimens indicated that the form I male gonopod was consistent with *O. ronaldi*, although the mandible was not; *O. ronaldi* typically has a serrated edge on the incisor region of the mandible (Taylor 2000). As is often the case with crayfish, morphological and genetic information were at odds, so for the present, we refer to the specimens as *O.* sp. nr *ronaldi*.

Indirect Crayfish Sampling

We examined 18 crayfish from stomachs of 17 black bass collected in Lewis Smith Reservoir concurrently with the stream study. All 18 were *Orconectes*, representing four species; however, four individuals were not identified to species (Figure 1 and Table 2). Two of the species, *O. lancifer* and *O. validus*, also were found during our direct sampling, but the other two, *Orconectes perfectus* Walls and *O. virilis*, were not. *Orconectes perfectus* was found in fish stomachs from the Rock and Ryan creek arms of the reservoir as well as in the forebay of the dam and was collected over three months. We obtained a single *O. virilis* from a fish in the Ryan Creek arm of the reservoir. Given the generally high site fidelity and small home ranges documented for both largemouth bass and spotted bass in reservoirs (Warden and Lorio 1975; Winter 1977; Fish and Savitz 1983; Copeland and Noble 1994; Hunter and Maceina 2008), it is likely that most fish were collected within 2 km of where they fed on the crayfish.

At least 44 crayfish occurred in the stomachs and intestines of 32 individual fishes collected from streams in the 1990's. Nine of the crayfish could not be identified to genus, and 17 were identified to species with a high level of confidence. Because crayfish were removed from the intestines as well as the stomachs of these fishes, more small crayfish parts were encountered, relative to the samples from bass stomachs, making identifications more difficult. Both of the *C. obstipus* found in the fish guts came from fish captured in the Sipsey Fork mainstem, but six of the seven *C. striatus* came from fish collected in tributaries (Table 2). Four *O. validus* came from the Sipsey Fork mainstem, and the remaining 10 came from its tributaries. Because *O. lancifer* has the most distinctive rostrum, chelae, and gonopods of any crayfish in the study area, it is highly unlikely that it was overlooked in our evaluation of these samples.

Table 2. Crayfish obtained from fish guts. Site locations given in Table 1. Question marks indicate uncertainty about identifications. "Sp." indicates that crayfish or their parts were identified only to genus. "Cray" indicates the presence of crayfish parts that were not identified to genus. Crayfish were obtained from the following fish species (number of individuals): *Ambloplites ariommus* Viosca (4), *Ameiurus natalis* (Lesueur) (3), *Esox niger* Lesueur (1), *Lepomis cyanellus* Rafinesque (1), *Micropterus henshalli* (22), *M. salmoides* (1), and *M. warriorensis* Baker et al. (15).

Site	Location	Crayfish	Number	Date
FH01	Sipsey Fork	*C. obstipus*	1	6/13/1995
FH01	Sipsey Fork	*C. obstipus*	1	7/19/1995
FH01	Sipsey Fork	*C.* sp.	2	12/28/1993
FH01	Sipsey Fork	*C. striatus*?	1	4/14/1994
FH01	Sipsey Fork	cray	2	12/28/1993
FH01	Sipsey Fork	*O. validus*	2	7/19/1995
FH01	Sipsey Fork	*O. validus?*	2	12/28/1993
FH02	Flannagin	*C. striatus*	3	5/16/1995
FH02	Flannagin	*C. striatus?*	1	4/14/1994
FH02	Flannagin	cray	2	6/12/1995
FH02	Flannagin	*O.* sp.	2	4/2/1995
FH02	Flannagin	*O. validus?*	1	4/14/1994
FH03	Borden	*C.* sp.	2	10/26/1993
FH03	Borden	*C.* sp.	1	4/3/1995
FH03	Borden	*C. striatus*	1	4/3/1995
FH03	Borden	cray	1	4/3/1995
FH03	Borden	*O.* sp.	1	5/17/1995
FH03	Borden	*O. validus*	4	10/26/1993
FH03	Borden	*O. validus*	3	4/3/1995
FH03	Borden	*O. validus?*	1	10/26/1993
FH04	Brushy	*O.* sp.	1	10/27/1993
FH05	Rush	cray	1	5/15/1995
FH05	Rush	*O. validus*	1	4/5/1995
FH06	Brown	*C.* sp.	1	5/15/1995
FH06	Brown	*C.* sp.?	1	10/27/1993
FH06	Brown	*C. striatus*	1	10/27/1993
FH06	Brown	cray	1	10/27/1993
FH06	Brown	cray	1	5/15/1995
FH06	Brown	cray	1	6/12/1995
FH06	Brown	*O.* sp.	1	6/12/1995
FR01	Forebay	*O. lancifer*	2	7/21/2014
FR01	Forebay	*O. perfectus*	2	3/10/2014
FR02	Sipsey A	*O. lancifer*	1	9/25/2014
FR02	Sipsey A	*O.* sp.	2	9/25/2014
FR02	Sipsey A	*O. validus*	1	1/11/2014
FR02	Sipsey A	*O. validus*	1	9/25/2014
FR03	Sipsey B	*O. lancifer*	1	1/11/2014
FR04	Rock A	*O. perfectus*	2	9/25/2014
FR05	Rock B	*O. perfectus*	1	9/25/2014
FR05	Rock B	*O. perfectus*	1	11/6/2013
FR06	Ryan A	*O. perfectus*	1	9/24/2014
FR07	Ryan B	*O.* sp.	1	11/5/2013
FR07	Ryan B	*O.* sp.	1	7/8/2013
FR07	Ryan B	*O. validus*	1	7/21/2014
FR07	Ryan B	*O. virilis*	1	8/5/2013

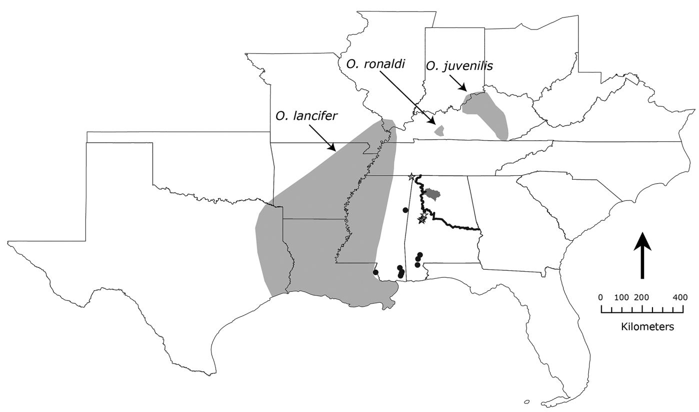

Figure 3. Approximations of the known native ranges of *Orconectes lancifer* (Pflieger 1996; Taylor and Schuster 2004; Morehouse and Tobler 2013; but see also Fitzpatrick 1987), *O. ronaldi*, and *O. juvenilis* (Taylor 2000). Black circles indicate additional records of *O. lancifer* from Mississippi and Alabama outside of the previously published native range and prior to our study. Stars indicate additional *O. lancifer* records outside of our study area (shaded area in northwest Alabama) collected after our study began. The bold line arcing through Alabama indicates the Fall Line, demarcating the Coastal Plain from upland provinces.

DISCUSSION

Our results raise several questions about the origins of the crayfish in the drainage. Of the seven crayfish species collected in the study, one, *O.* sp. nr *ronaldi*, was not reported previously from Alabama, another, *O. lancifer*, was not reported from the Black Warrior River system prior to the study, and a third, *O. perfectus*, was known from only one tributary record (Ryan Creek) in the Sipsey Fork drainage (Smith et al. 2011). We placed crayfish species we collected into three categories: native, non-native, and unexpected species that may be native or non-native. Three possible scenarios could apply to the unexpected species: 1) they are native to the Sipsey Fork drainage but were previously undetected, 2) they are not native to the drainage but arrived through natural range expansions as aquatic habitats were drastically altered, or 3) they were introduced by humans, either intentionally or unintentionally. Below, we further explore these scenarios for the unexpected species.

The native species include *C. obstipus*, *C. striatus*, and *O. validus*. The Sipsey Fork is within their known native range, many previous records of these species exist, and all were found in fish guts from the 1990's. *Orconectes perfectus* has one previous record from the Sipsey Fork drainage, but is reported from numerous lotic habitats elsewhere in the Black Warrior River system above the Fall Line (Schuster et al. 2008; Smith et al. 2011). Hobbs (1989) noted that the species is widespread in the Tombigbee River system, although he did not indicate if it occurred upstream of the Fall Line. Although the origins of *O. perfectus* in the Sipsey Fork drainage may never be known with certainty, we have no basis for assuming that the species was introduced.

Orconectes virilis is not native to Alabama, but now occurs in lotic habitats in at least five river systems in the state (Schuster et al. 2008). Its native range is large, encompassing parts of the Mississippi, Missouri, and Ohio river basins and extending into New England and Canada (Pflieger 1996; Hamr 2002; Filipová et al. 2010), and it is also widely introduced, including introductions to at least three countries and seven states by 1989 (Hobbs et al. 1989). The previous collections of *O. virilis* in the Sipsey Fork drainage were made in 2010 in Borden Creek at Bunyan Hill Road in the Sipsey Wilderness, and in 2010 – 2011 in Ryan Creek (Smith et al. 2011), an eastern tributary of Lewis Smith Reservoir that flows near Cullman, AL, the largest city in the drainage. Given that *O. virilis* is a successful invader elsewhere in the southeastern US (e.g., Cooper and Russ 2013) and has two previous records in the drainage, it is somewhat surprising that it was not more prevalent in our samples. Evidently the populations in the drainage remain localized, and the disjunct records may be indicative of humans moving the species, possibly as bait, rather than of dispersal from one point in the drainage. We sampled Borden Creek at Bunyan Hill Road (latitude 34.3094, longitude -87.3950, map datum WGS84) in October 2015 (three person-hours of turning rocks and dipnetting) and found no *O. virilis*, suggesting that the population has not thrived, if it ever became established.

The *O.* sp. nr *ronaldi* population in Brushy Creek was disjunct, occurring far from the known native ranges of either *O. juvenilis* or *O. ronaldi* (lower Ohio and Cumberland river drainages, respectively; Figure 3). Because of the distance from either of those native ranges, it appears highly unlikely that the species arrived in the Sipsey Fork drainage by natural colonization. Further, the localized distribution of the species in the drainage points toward a recent introduction. Although unlikely because of its restricted distribution within the Sipsey Fork system, the possibility remains that the population represents a new species native to the study area; alternatively, it could be an introduced hybrid of *O. juvenilis* and *O. ronaldi*. The *juvenilis* complex of the subgenus *Procericambarus* also includes the highly invasive *O. rusticus,* a species that has spread rapidly, displaced native crayfish and other aquatic fauna, and caused a variety of ecosystem changes throughout much of its introduced range (Hamr 2002; Olden et al. 2006; Bobeldyk and Lamberti 2010); therefore, the presence of a closely related taxon from the same species complex in the Sipsey Fork drainage is cause for concern if it is non-native.

The origins of *O. lancifer* in the drainage are also unclear. The known native range of *O. lancifer* encompasses the Lower Mississippi Alluvial Valley (Taylor and Schuster 2004; Walls 2009) and the Gulf Coastal Plain from eastern Texas to Mississippi (Hobbs 1989) (Figure 3). Beyond the Mississippi River basin, *O. lancifer* records are scattered across the Coastal Plain in Mississippi and Alabama, especially near the Gulf Coast (Figure 3, Appendix 2). A 1972 record (USNM #146126; Appendix 2) from the Tombigbee River south of Columbus, Mississippi, predates construction of the Tennessee-Tombigbee Waterway. Other locality records prior to 1980 (the earliest from 1964) include one site in the Pascagoula River drainage in southeast Mississippi (USNM # 209191) and two sites in the lower Alabama and Mobile rivers in southwest Alabama (Figure 3) (Schuster et al. 2008; Smith et al. 2011). From 1980-1994, records of the species were found for neither Alabama nor Mississippi outside of the Mississippi River basin. From 1995-2011, *O. lancifer* was found in five additional sites east of the Mississippi River Basin: in the Pearl (one site) and Pascagoula (three sites) rivers in Mississippi (unpublished data; Fitzpatrick 2002) and the Tensaw River (one site), Alabama (Schuster et al. 2008; Smith et al. 2011)(Appendix 2). Since we found *O. lancifer* in the Sipsey Fork River system in 2012, the species has been found in six additional sites in Alabama outside of our study area (Figure 3 stars, Appendix 2). Five of the sites were along or downstream of the Fall Line in the Black Warrior River system, and one was in Pickwick Reservoir in the Tennessee River system below the Fall Line.

The *O. lancifer* records along the coast may indicate that the species is native along the coastal portions of Mississippi and Alabama. Indeed, Fitzpatrick (1987) showed the range extending in a narrow band slightly north of the coastline across southeast Mississippi and southwest Alabama. The 1972 record from the Tombigbee River also could be indicative of the native range extending farther north in that river or could reflect an early introduction to the system. In addition, the recent records from Tuscaloosa County, Alabama, could be taken as evidence that *O. lancifer* is native to the Black Warrior River system; however, likely introduction points are present near these sites. Sites in cities are likely candidates for introductions of crayfish used as bait, pets, or classroom animals. The Moon Lake site in the Black Warrior River system is <100 m from a former crayfish (*Procambarus clarkii* (Girard)) aquaculture pond, and the species could have spread from that location. The native ranges of *P. clarkii* and *O. lancifer* overlap substantially (Walls 2009; Green et al. 2011), and, thus, transport of *P. clarkii* for aquaculture could also inadvertently introduce *O. lancifer*.

Habitat in the native range of *O. lancifer* is generally characterized by low topographical relief, deep (> 0.5 m), sluggish or sometimes lentic waters, and mud or silt substrates (Pflieger 1996; Taylor and Schuster 2004; Walls 2009; Green et al. 2011). The Sipsey Fork's location on the Cumberland Plateau and its pre-impoundment habitat, including deep gorges (some incised more than 50 m below the surface of the plateau), rocky terrain, and well-developed riffle-run-pool habitats (Williams et al. 2008), are atypical for *O. lancifer*. Confirming the unsuitability of the natural, riverine habitat in the drainage, *O. lancifer* was not detected in our sampling upstream of the transition zones in either mainstem river, and only once in a tributary (<100 m upstream of the transition zone). Although *O. lancifer* occurred in fish stomachs from the reservoir in 2013-14, it did not occur in fish guts sampled far upstream of the reservoir influence in the 1990's. Taken together, the pre-impoundment habitat in the Sipsey Fork and the lack of historic records upstream of the Fall Line suggests that while *O. lancifer* may be native to the Tombigbee River system, it likely is not native to the Sipsey Fork.

If *O. lancifer* is not native to the Sipsey Fork, it could have arrived there via natural range expansion or introduction. As rivers were impounded in the Black Warrior River drainage, lentic habitats were created that were likely more favorable than the original rivers above the Fall Line for *O. lancifer*, and the species may have colonized these newly-altered habitats on its own; however, this scenario seems unlikely. If introduced, the introduction is probably not recent, because the population appears to be established throughout at least the western portion of the reservoir. The species occurred all the way from the dam up into the transition zones in both the Sipsey Fork and Brushy Creek arms of the reservoir. Curiously, we did not find the species in fish stomachs from the Rock or Ryan creek arms of the reservoir; however, sample sizes were small.

Although the source of any of the introduced or possibly-introduced crayfish species in the system may never be resolved, the results highlight two issues. The first is that large water bodies, both rivers and lakes, in the USA are poorly-sampled for crayfish, leading to situations where the native or introduced status of species is uncertain. The second is that a better understanding is needed of the pathways of crayfish introductions. Intentional introductions (e.g., release of pets or bait) of the species in our samples are possible, but unintentional introductions seem more plausible. These could happen by escape of live bait, but also by contamination of fish shipments during stocking operations. The latter has received little formal attention but is being increasingly suspected by biologists in the field.

We recommend redoubled efforts to understand the pathways of crayfish introductions into the Sipsey Fork drainage and at a broader scale. Once non-native crayfish species are established in a large watershed where desirable crayfish are also present, eradication is not feasible and control is expensive (Sandodden and Johnsen 2010; Lodge et al. 2012), making prevention a high priority. However, effective prevention efforts require first understanding how introductions are occurring. We also recommend establishing a crayfish monitoring program in and upstream of Lewis Smith Reservoir to document any spread of the non-native populations and effects on native crayfish species. Finally, we suggest that increased effort to sample crayfish in large water bodies is essential to understanding the distributions of native crayfish and the extent to which crayfish translocations are an issue in North America.

ACKNOWLEDGMENTS

Funding was provided via the Alabama Power FERC relicensing agreement with the National Forests of Alabama. Thanks to Jeff Baker, Alabama Power, for providing crayfish specimens from traps in Lewis Smith Reservoir. Wendell Haag (US Forest Service [FS]) assisted with study design and sampling. Chris Taylor (Illinois Natural History Survey) assisted with identification of *O.* (*Procericambarus*) sp. nr *ronaldi* and extracted and sequenced DNA for genetic analyses. Mickey Bland (FS) dissected preserved fishes. Gayle Henderson (FS) created maps. Thanks to the following individuals for assistance with sampling: Mickey Bland, Gordon McWhirter, Zanethia Barnett, Amy Carson, Preston Chrisman, Brandon Fair, Mike Knoerr, Jacoby Lipscomb, Travis McDonald, Dan Nuckols, David Pfuhler, Carl Smith (FS), Cal Johnson (initially Geological Survey of AL [GSA] and later Alabama Department of Environmental Management), Rebecca Bearden, Anne Wynn, and Brett Smith (GSA).

LITERATURE CITED

Arcella TE, Perry WL, Lodge DM and Feder JL (2014). The role of hybridization in a species invasion and extirpation of resident fauna: hybrid vigor and breakdowns in the rusty crayfish, *Orconectes rusticus*. *Journal of Crustacean Biology* 34(2):157–164.

Bobeldyk AM and Lamberti GA (2010). Stream food web responses to a large omnivorous invader, *Orconectes rusticus* (Decapoda, Cambaridae). *Crustaceana* 83(6):641–657.

Boschung HT and Mayden RL (2004). Fishes of Alabama. Smithsonian Books, Washington D.C., USA.

Cooper JE and Russ WT (2013). Four crayfishes (Decapoda: Cambaridae) new to the North Carolina fauna, with notes on other species in the state. *Journal of the North Carolina Academy of Science* 129(2):53–57.

Copeland JR and Noble RL (1994). Movements by young-of-year and yearling largemouth bass and their implications for supplemental stocking. *North American Journal of Fisheries Management* 14(1):119–124.

Filipová L, Holdich DM, Lesobre J, Grandjean F and Petrusek A (2010). Cryptic diversity within the invasive virile crayfish *Orconectes virilis* (Hagen, 1870) species complex: New lineages recorded in both native and introduced ranges. *Biological Invasions* 12(5):983–989.

Fish PA and Savitz J (1983). Variations in home ranges of largemouth bass, yellow perch, bluegill, and pumpkinseeds in an Illinois lake. *Transactions of the American Fisheries Society* 112(2A):147–153.

Fitzpatrick Jr. JF (1987). The subgenera of the crawfish genus *Orconectes* (Decapoda: Cambaridae). *Proceedings of the Biological Society of Washington* 100(1):47–74.

Fitzpatrick Jr. JF (2002). The conservation status of Mississippi crawfishes (Crustacea: Decapoda: Cambaridae). *Louisiana Academy of Sciences* 63:25–36.

Green CC, Gautreaux KM, Pérez RAP and Lutz CG (2011). Comparative physiological responses to increasing ambient salinity levels in *Procambarus clarkii* (Girard) and *Orconectes lancifer* (Hagen). *Freshwater Crayfish* 18(1):87–92.

Haag WR and Warren Jr. ML (2008). Effects of severe drought on freshwater mussel assemblages. *Transactions of the American Fisheries Society* 137(4):1165–1178.

Haag WR and Warren Jr. ML (1998). Role of ecological factors and reproductive strategies in structuring freshwater mussel communities. *Canadian Journal of Fisheries and Aquatic Sciences* 55(2):297–306.

Hamr P (2002). *Orconectes*. Pp. 585–608, *In:* Holdich DM, (ed.) Biology of Freshwater Crayfish. Blackwell Science, Ltd., Ames, Iowa.

Hobbs Jr. HH, Jass JP and Huner JV (1989). A review of global crayfish introductions with particular emphasis on two North American species (Decapoda, Cambaridae). *Crustaceana* 56(3):299–316.

Hobbs Jr. HH (1989). An illustrated checklist of the American crayfishes (Decapoda: Astacidae, Cambaridae and Parastacidae). *Smithsonian Contributions to Zoology* 480:1–236.

Hunter RW and Maceina MJ (2008). Dispersal of tournament-displaced largemouth bass and spotted bass in Lake Martin, Alabama. *North American Journal of Fisheries Management* 28(3):678–687.

Kessler RA, IV, Black TR, Fraley SJ and Gangloff MM (this issue). Molecular and morphological analyses reveal cryptic crayfish invasions in the Appalachian Mountains. *Freshwater Crayfish* 21(1):51–62.

Lodge DM, Deines A, Gherardi F, Yeo DCJ, Arcella TE, Baldridge AK, Barnes MA, Chadderton WL, Feder JL, Gantz CA, Howard GW, Jerde CL, Peters B, W,, Peters JA, Sargent LW, Turner CR, Wittmann ME and Zeng Y (2012). Global introductions of crayfish: Evaluating the impact of species invasions on ecosystem services. *Annual Review of Ecology, Evolution, and Systematics* 43:449–472.

Morehouse RL and Tobler M (2013). Crayfishes (Decapoda: Cambaridae) of Oklahoma: Identification, distributions, and natural history. *Zootaxa* 3717(2):101–157.

Olden JD, McCarthy JM, Maxted JT, Fetzer WW and Vander Zanden MJ (2006). The rapid spread of rusty crayfish (*Orconectes rusticus*) with observations on native crayfish declines in Wisconsin (USA) over the past 130 years. *Biological Invasions* 8(8):1621–1628.

Pflieger WL (1996). The Crayfishes of Missouri. Missouri Department of Conservation, Jefferson City.

Richman NI, Böhm M, Adams SB, Alvarez F, Bergey EA, Bunn JJS, Burnham Q, Cordeiro J, Coughran J, Crandall KA, Dawkins KL, DiStefano RJ, Doran NE, Edsman L, Eversole AG, Füreder L, Furse JM, Gherardi F, Hamr P, Holdich DM, Horwitz P, Johnston K, Jones CM, Jones JPG, Jones RL, Jones TG, Kawai T, Lawler S, López-Mejía M, Miller RM, Lara CP, Reynolds JD, Richardson AMM, Schultz MB, Schuster GA, Sibley PJ, Souty-Grosset C, Taylor CA, Thoma RF, Walls J, Walsh TS and Collen B (2015). Multiple drivers of decline in the global status of freshwater crayfish (Decapoda: Astacidea). *Philosophical Transactions of The Royal Society B Biological Sciences* 370:20140060.

Sandodden R and Johnsen SI (2010). Eradication of introduced signal crayfish *Pasifastacus leniusculus* using the pharmaceutical BETAMAX VET. *Aquatic Invasions* 5(1):75–81.

Schuster GA, Taylor CA and Adams SB (2015). *Procambarus* (*Girardiella*) *holifieldi*, a new species of crayfish (Decapoda: Cambaridae) from Alabama, with a revision of the Hagenianus Group in the subgenus *Girardiella*. *Zootaxa* 4021(1):001–032.

Schuster GA, Taylor CA and Johansen J (2008). An annotated checklist and preliminary designation of drainage distributions of the crayfishes of Alabama. *Southeastern Naturalist* 7(3):493–504.

Smith JB, Schuster GA, Taylor CA, Wynn EA and McGregor SW (2011). A preliminary report on the distribution and conservation status of the Alabama crayfish fauna. Geological Survey of Alabama, Tuscaloosa, AL. Open-file report 1102.

TAYLOR CA (2000). Systematic studies of the *Orconectes juvenilis* complex (Decapoda: Cambaridae), with descriptions of two new species. *Journal of Crustacean Biology* 20(1):132–152.

TAYLOR CA, ADAMS SB AND SCHUSTER GA (2014). Systematics and biogeography of *Orconectes*, subgenus *Trisellescens*, in the southeastern United States, a test of morphology-based classification. *Journal of Crustacean Biology* 34(1):1–14.

TAYLOR CA AND SCHUSTER GA (2004). The crayfishes of Kentucky. Illinois Natural History Survey Special Publication No. 28, Champaign, IL.

WALLS JG (2009). Crawfishes of Louisiana. Louisiana State University Press, Baton Rouge.

WARDEN RL, JR. AND LORIO WJ (1975). Movements of largemouth bass (*Micropterus salmoides*) in impounded waters as determined by underwater telemetry. *Transactions of the American Fisheries Society* 104(4):696–702.

WILLIAMS JD, BOGAN AE AND GARNER JT (2008). Freshwater mussels of Alabama and the Mobile basin in Georgia, Mississippi, and Tennessee. The University of Alabama Press, Tuscaloosa, AL.

WINTER JD (1977). Summer home range movements and habitat use by four largemouth bass in Mary Lake, Minnesota. *Transactions of the American Fisheries Society* 106(4):323–330.

SUPPPLEMENTARY INFORMATION

The data in the appendices below are also available in electronic format from the publisher's website.

Appendix 1. Crayfish direct sampling sites, locations, dates, methods, and crayfish collected. All sites were in Winston County, Alabama, USA. Latitude and longitude are in decimal degrees in the NAD83 or WGS84 datum. "Total crayfish" gives the number of individuals of the species in the collection. US Forest Service, Center for Bottomland Hardwoods Research (CBHR) catalog numbers are given. "Electrofish" indicates backpack electrofishing unless "boat electrofish" is specified. Question mark indicates uncertainty about identification.

Appendix 2. Collection records for *Orconectes lancifer* in Mississippi and Alabama outside of the most commonly published native range. Latitude and longitude are in decimal degrees. Where known, the map datum is indicated. Month, day, and year of collection are given. The name of person identifying (ID) the specimens and the year identified are indicated, if known. Georeferencing information refers to assigning latitude and longitude to locality descriptions from museum databases. Source abbreviations are as follows: GSA - Geological Survey of Alabama, Tuscaloosa, AL; INHS - Illinois Natural History Survey, Champaign, IL; MMNS - Mississippi Museum of Natural Science, Jackson, MS; USNM - National Museum of Natural History, Washington, DC; and TUMNH - Tulane Museum of Natural History (crayfish collection now housed at MMNS). Export date is date records were extracted from original database and sent to authors.

Appendix 1. Crayfish direct sampling sites, locations, dates, methods, and crayfish collected. All sites were in Winston County, Alabama, USA. Latitude and longitude are in decimal degrees in the NAD83 or WGS84 datum. "Total crayfish" gives the number of individuals of the species in the collection. US Forest Service, Center for Bottomland Hardwoods Research (CBHR) catalog numbers are given. "Electrofish" indicates backpack electrofishing unless "boat electrofish" is specified. Question mark indicates uncertainty about identification.

Site	Species	Stream	Location	Total crayfish	Collection date	CBHR catalog #	Latitude	Longitude	Method
21	*C. obstipus*	Brushy Creek	at approx. river km 21.9 (upstream of where southern branch of trail meets Brushy Creek).	3	16-Sep-13	5478	34.19469	-87.24933	visual search
22	*C. obstipus*	Brushy Creek	at approx. river km 23. West end of large, sharp curve in river.	2	16-Sep-13	5480	34.19702	-87.25967	visual search
24	*C. obstipus*	Brushy Creek	at approx. river km 20.8 near mouth of tributary on river left.	1	17-Sep-13	5481	34.18768	-87.24213	visual search
42	*C. obstipus*	Brushy Creek	at river km 24. Started at coordinates and sampled about 250 m upstream.	12	19-Sep-13	5498	34.20242	-87.25282	visual search
61	*C. obstipus*	Brushy Creek	at river km 25.4.	1	28-Apr-15	5783	34.21409	-87.25194	electrofish/ seine
62	*C. obstipus*	Brushy Creek	at river km 24; approximately 200 m upstream of Inman Creek.	1	30-Apr-15	5784	34.20219	-87.25283	electrofish
63	*C. obstipus*	Brushy Creek	at river km 33.8; near Capsey Creek confluence.	2	29-Apr-15	5792	34.25100	-87.24619	electrofish/seine
33	*C. obstipus*	Chimney Branch	Above transition zone.	1	18-Sep-13	5490	34.16752	-87.22110	visual search
06	*C. obstipus*	Grindstone Creek	Above transition zone.	1	25-Sep-12	5446	34.19498	-87.33590	electrofish/seine
06	*C. obstipus*	Grindstone Creek	Above transition zone.	4	25-Sep-12	5447	34.19498	-87.33590	visual search
53	*C. obstipus*	Grindstone Creek	at river km 2.0.	12	20-Oct-14	5695	34.20219	-87.34089	electrofish
55	*C. obstipus*	Inman Creek	at river km 4.2.	3	21-Oct-14	5699	34.21329	-87.22623	electrofish
56	*C. obstipus*	Inman Creek	at river km 4.8.	2	21-Oct-14	5702	34.21600	-87.22218	electrofish
36	*C. obstipus*	Mile Creek	Upper transition site. From about 150 m downstream of County Road 13 (FS Rd. 121) xing to about 50 m downstream of xing (end at coordinates).	3	18-Sep-13	5497	34.17622	-87.25222	visual search
37	*C. obstipus*	Mile Creek	Just upstream of FS Road 121 crossing to about 100 m upstream (end near coordinates). "Above transition" site.	3	18-Sep-13	5501	34.17752	-87.25349	visual search
46	*C. obstipus*	Mile Creek	Upstream of "Above transition" site.	2	27-Apr-13	5549	34.19109	-87.26645	electrofish
58	*C. obstipus*	Mile Creek	at river km 6.1.	22	21-Oct-14	5708	34.20618	-87.28057	electrofish
03	*C. obstipus*	Mill Creek	Above transition zone.	2	25-Sep-12	5439	34.17597	-87.32172	electrofish/seine
52	*C. obstipus*	Mill Creek	at river km 3.7.	18	21-Oct-14	5692	34.18235	-87.31671	electrofish
59	*C. obstipus*	Mill Creek	at river km 7.3.	7	22-Oct-14	5709	34.20829	-87.31060	electrofish
07	*C. obstipus*	Payne Creek	Lower transition zone. Near mouth - inundated by reservoir level of ~502 feet elevation.	1	24-Sep-12	5448	34.21164	-87.35231	visual search
50	*C. obstipus*	Payne Creek	at river km 1.0.	4	20-Oct-14	5686	34.21909	-87.35521	electrofish
51	*C. obstipus*	Payne Creek	at river km 2.0.	5	20-Oct-14	5690	34.22638	-87.35664	electrofish
14	*C. obstipus*	Sipsey Fork	at approximately river km 18.8.	1	19-Sep-12	5462	34.21140	-87.34976	visual search
16	*C. obstipus*	Sipsey Fork	Upper transition zone- high gradient riffle appears to represent a boundary between frequently vs rarely impounded segments. From mouth of Payne Cr. upstream to approximately river km 21.	4	24-Sep-12	5466	34.20959	-87.35790	visual search
18	*C. obstipus*	Sipsey Fork	Upper transition zone; from the reservoir-river transition zone to Hwy. 33 bridge (about river km 21-23.2)	10	26-Sep-12	5470	34.21789	-87.36381	visual search

41	*C. obstipus*	Sipsey Fork	at river km 24. Started at coordinates given.	5	19-Sep-13	5505	34.22461	-87.37668	visual search
48	*C. obstipus*	Sipsey Fork	at river km 16.3-16.4.	1	23-Oct-14	5683	34.19271	-87.34334	trap
49	*C. obstipus*	Sipsey Fork	at river km 16.6-16.8.	1	23-Oct-14	5685	34.19630	-87.34294	trap
65	*C. obstipus*	Sipsey Fork	at river km 29; about 200 m downstream of confluence with Hurricane Creek. Accessed from FS Rd. 228.	1	28-Apr-15	5788	34.25102	-87.36697	electrofish/seine
63	*C. striatus*	Brushy Creek	at river km 33.8; near Capsey Creek confluence.	8	29-Apr-15	5793	34.25100	-87.24619	electrofish/seine
33	*C. striatus*	Chimney Branch	Above transition zone.	4	18-Sep-13	5489	34.16752	-87.22110	visual search
34	*C. striatus*	Chimney Branch	Upper transition zone. From upper end of mucky reservoir substrate (coordinate location) upstream to 155.4 m elevation.	1	18-Sep-13	5492	34.16529	-87.22094	visual search
53	*C. striatus*	Grindstone Creek	at river km 2.0.	1	20-Oct-14	5696	34.20219	-87.34089	electrofish
55	*C. striatus*	Inman Creek	at river km 4.2.	3	21-Oct-14	5700	34.21329	-87.22623	electrofish
56	*C. striatus*	Inman Creek	at river km 4.8.	1	21-Oct-14	5701	34.21600	-87.22218	electrofish
58	*C. striatus*	Mile Creek	at river km 6.1.	2	21-Oct-14	5707	34.20618	-87.28057	electrofish
52	*C. striatus*	Mill Creek	at river km 3.7.	1	21-Oct-14	5693	34.18235	-87.31671	electrofish
09	*C. striatus*	Payne Creek	reach upstream of reservoir full pool extent (155.4 m elevation).	1	24-Sep-12	5452	34.21479	-87.35432	electrofish/seine
51	*C. striatus*	Payne Creek	at river km 2.0.	1	20-Oct-14	5689	34.22638	-87.35664	electrofish
12	*C. striatus*	Sipsey Fork	at approximately river km 17; about 2 km upstream of Grindstone Cr.	1	25-Sep-12	5460	34.19831	-87.34554	visual search
18	*C. striatus*	Sipsey Fork	upstream of frequently-impounded zone; from the reservoir-river transition zone to Hwy. 33 bridge (about river km 21-23.2).	1	26-Sep-12	5471	34.21789	-87.36381	visual search
66	*C. striatus*	Sipsey Fork	at river km 38.5; downstream of Cranal Rd. (AL 60) crossing.	1	28-Apr-15	5790	34.28453	-87.39864	electrofish/seine
57	*C. striatus?*	Chimney Branch	at river km 1.3.	5	22-Oct-14	5704	34.17099	-87.22453	electrofish
25	*O.* sp. nr *ronaldi*	Brushy Creek	at river km 20.	1	17-Sep-13	5483	34.18129	-87.23953	visual search
31	*O.* sp. nr *ronaldi*	Brushy Creek	near river km 15.8.	4	17-Sep-13	5484	34.16775	-87.24405	trap
40	*O.* sp. nr *ronaldi*	Brushy Creek	at river km 15 in Lewis Smith Reservoir.	1	18-Sep-13	5507	34.16116	-87.24739	trap
33	*O.* sp. nr *ronaldi*	Chimney Branch	Above transition zone.	4	18-Sep-13	5488	34.16752	-87.22110	visual search
34	*O.* sp. nr *ronaldi*	Chimney Branch	Upper transition zone. From upper end of mucky reservoir substrate (coordinate location) upstream to 155.4 m elevation.	11	18-Sep-13	5493	34.16529	-87.22094	visual search
34	*O.* sp. nr *ronaldi*	Chimney Branch	Upper transition zone. From upper end of mucky reservoir substrate (coordinate location) upstream to 155.4 m elevation.	1	27-Apr-15	5795	34.16529	-87.22094	visual search
34	*O.* sp. nr *ronaldi*	Chimney Branch	Upper transition zone. From upper end of mucky reservoir substrate (coordinate location) upstream to 155.4 m elevation.	1	27-Apr-15	5795	34.16529	-87.22094	visual search
35	*O.* sp. nr *ronaldi*	Chimney Branch	Lower transition site. From halfway through low-pool reservoir inlet (coordinates) to downstream end of site 34.	2	16-Sep-13	5778	34.16368	-87.22068	electrofish
35	*O.* sp. nr *ronaldi*	Chimney Branch	Transition zone.	2	16-Sep-13	5778	34.16368	-87.22068	electrofish

Appendix 1. Continued.

Site	Species	Stream	Location	Total crayfish	Collection date	CBHR catalog #	Latitude	Longitude	Method
44	*O.* sp. nr *ronaldi*	Chimney Branch	Above transition zone; approximately 850 m upstream of confluence with Brushy Creek.	1	16-Sep-13	5510	34.16815	-87.22363	electrofish
68	*O.* sp. nr *ronaldi*	Brushy Creek	in impounded zone, about 1.1 km downstream of Chimney Branch.	1	07-May-14	5779	34.15842	-87.21362	trap
36	*O.* sp. nr *ronaldi*	Mile Creek	Upper transition zone. From about 150 m downstream of County Road 13 (FS Rd. 121) xing to about 50 m downstream of xing (end at coordinates).	3	18-Sep-13	5496	34.17622	-87.25222	visual search
37	*O.* sp. nr *ronaldi*	Mile Creek	Just upstream of FS Road 121 crossing to about 100 m upstream (end near coordinates). “Above transition” site.	2	18-Sep-13	5500	34.17752	-87.25349	visual search
31	*O. lancifer*	Brushy Creek	near river km 15.8.	1	17-Sep-13	5485	34.16775	-87.24405	trap
32	*O. lancifer*	Brushy Creek	near river km 10; up- and downstream of private boat ramp on river left (Burns Road).	1	18-Sep-13	5486	34.16381	-87.21762	visual search
38	*O. lancifer*	Brushy Creek	at river km 12.0.	1	18-Sep-13	5502	34.15893	-87.22583	boat electrofish
39	*O. lancifer*	Brushy Creek	at confluence with Chimney Branch.	1	18-Sep-13	5503	34.16243	-87.21877	boat electrofish
40	*O. lancifer*	Brushy Creek	at river km 15 in Lewis Smith Reservoir.	1	18-Sep-13	5504	34.16116	-87.24739	trap
33	*O. lancifer*	Chimney Branch	Above transition zone.	1	18-Sep-13	5487	34.16752	-87.22110	visual search
35	*O. lancifer*	Chimney Branch	Transition zone.	2	16-Sep-13	5777	34.16368	-87.22068	electrofish
35	*O. lancifer*	Chimney Branch	Lower transition site. From halfway through low-pool reservoir inlet (coordinates) to downstream end of site 34.	2	16-Sep-13	5777	34.16368	-87.22068	electrofish
35	*O. lancifer*	Chimney Branch	Lower transition site. From halfway through low-pool reservoir inlet (coordinates) to downstream end of site 34.	1	18-Sep-13	5512	34.16368	-87.22068	visual search
04	*O. lancifer*	Grindstone Creek	near mouth - inundated by reservoir level of ~153.0 m elevation.	1	25-Sep-12	5440	34.19105	-87.33481	visual search
05	*O. lancifer*	Grindstone Creek	Transition zone between reach flooded at reservoir elevation of 153.0 m and reach never impounded.	1	25-Sep-12	5442	34.19150	-87.33579	electrofish/ seine
69	*O. lancifer*	Mill Creek	near mouth, in impounded zone.	1	20-May-14	5780	34.16740	-87.32092	trap
10	*O. lancifer*	Sipsey Fork	at approximately river km 13; roughly 1/2 way between Mill and Grindstone creeks.	1	25-Sep-12	5453	34.17571	-87.33222	visual search
11	*O. lancifer*	Sipsey Fork	at approximately river km 16; about 1.1 km upstream of Grindstone Cr.	1	26-Sep-12	5455	34.18854	-87.34310	trap
21	*O. validus*	Brushy Creek	at approx. river km 21.9 (upstream of where southern branch of trail meets Brushy Creek).	1	16-Sep-13	5477	34.19469	-87.24933	visual search
22	*O. validus*	Brushy Creek	at approx. river km 23. West end of large, sharp curve in river.	1	16-Sep-13	5479	34.19702	-87.25967	visual search
24	*O. validus*	Brushy Creek	at approx. river km 20.8 near mouth of tributary on river left.	2	17-Sep-13	5482	34.18768	-87.24213	visual search
42	*O. validus*	Brushy Creek	at river km 24. approximately 200 m upstream of Inman Creek.	4	19-Sep-13	5499	34.20242	-87.25282	visual search
42	*O. validus*	Brushy Creek	at river km 24; approximately 200 m upstream of Inman Creek.	4	30-Apr-15	5785	34.20219	-87.25283	electrofish
42	*O. validus*	Brushy Creek	at river km 24. approximately 200 m upstream of Inman Creek.	4	19-Sep-13	5499	34.20242	-87.25282	visual search

42	*O. validus*	Brushy Creek	at river km 24; approximately 200 m upstream of Inman Creek.	4	30-Apr-15	5785	34.20219	-87.25283	electrofish
60	*O. validus*	Brushy Creek	at river km 29.7; roughly 4 km downstream of Capsey Creek.	1	30-Apr-15	5781	34.22849	-87.25266	electrofish
61	*O. validus*	Brushy Creek	at river km 25.4.	2	28-Apr-15	5782	34.21409	-87.25194	electrofish/seine
63	*O. validus*	Brushy Creek	at river km 33.8; near Capsey Creek confluence.	5	29-Apr-15	5791	34.25100	-87.24619	electrofish/seine
33	*O. validus*	Chimney Branch	above reservoir transition zone.	3	18-Sep-13	5491	34.16752	-87.22110	visual search
34	*O. validus*	Chimney Branch	Upper transition site. From upper end of mucky reservoir substrate (coordinate location) upstream to 155.4 m. elevation.	3	18-Sep-13	5494	34.16529	-87.22094	visual search
44	*O. validus*	Chimney Branch	upstream of transition zone; approximately 850 m upstream of confluence with Brushy Creek.	2	16-Sep-13	5509	34.16815	-87.22363	electrofish
57	*O. validus*	Chimney Branch	at river km 1.3.	8	22-Oct-14	5705	34.17099	-87.22453	electrofish
05	*O. validus*	Grindstone Creek	transition zone between reach flooded at reservoir elevation of 153.0 m and reach never impounded.	16	25-Sep-12	5443	34.19150	-87.33579	electrofish/seine
05	*O. validus*	Grindstone Creek	transition zone between reach flooded at reservoir elevation of 153.0 m and reach never impounded.	4	25-Sep-12	5441	34.19150	-87.33579	visual search
06	*O. validus*	Grindstone Creek	reach upstream of reservoir full pool (155.4) extent.	4	25-Sep-12	5445	34.19498	-87.33590	electrofish/seine
06	*O. validus*	Grindstone Creek	reach upstream of reservoir full pool (155.4) extent.	1	25-Sep-12	5444	34.19498	-87.33590	visual search
53	*O. validus*	Grindstone Creek	at river km 2.0.	16	20-Oct-14	5694	34.20219	-87.34089	electrofish
43	*O. validus*	Inman Creek	upstream of transition zone; approximately 275 m upstream of confluence with Brushy Creek.	1	17-Sep-13	5508	34.20013	-87.25073	electrofish
54	*O. validus*	Inman Creek	at river km 1.0.	2	22-Oct-14	5697	34.20255	-87.24500	electrofish
55	*O. validus*	Inman Creek	at river km 4.2.	19	21-Oct-14	5698	34.21329	-87.22623	electrofish
56	*O. validus*	Inman Creek	at river km 4.8.	2	21-Oct-14	5703	34.21600	-87.22218	electrofish
36	*O. validus*	Mile Creek	Upper transition site. From about 150 m downstream of County Road 13 (FS Rd. 121) xing to about 50 m downstream of xing (end at coordinates).	5	18-Sep-13	5495	34.17622	-87.25222	visual search
45	*O. validus*	Mile Creek	in reservoir transition zone.	1	17-Sep-13	5511	34.17445	-87.24974	electrofish
46	*O. validus*	Mile Creek	upstream of "above transition" site.	1	27-Apr-13	5550	34.19109	-87.26645	electrofish
58	*O. validus*	Mile Creek	at river km 6.1.	4	21-Oct-14	5706	34.20618	-87.28057	electrofish
02	*O. validus*	Mill Creek	transition zone between reach flooded at reservoir elevation of 153.0 m and reach never impounded.	18	25-Sep-12	5437	34.17111	-87.32478	electrofish/seine
03	*O. validus*	Mill Creek	reach upstream of reservoir full pool (155.4) extent.	7	25-Sep-12	5438	34.17597	-87.32172	electrofish/seine
52	*O. validus*	Mill Creek	at river km 3.7.	18	21-Oct-14	5691	34.18235	-87.31671	electrofish
59	*O. validus*	Mill Creek	at river km 7.3.	14	22-Oct-14	5710	34.20829	-87.31060	electrofish
07	*O. validus*	Payne Creek	near mouth - inundated by reservoir level of ~502 feet elevation.	3	24-Sep-12	5449	34.21164	-87.35231	visual search
08	*O. validus*	Payne Creek	transition zone between reach flooded at reservoir elevation of 502 feet and reach never impounded.	1	24-Sep-12	5450	34.21286	-87.35311	electrofish/seine
08	*O. validus*	Payne Creek	transition zone between reach flooded at reservoir elevation of 502 feet and reach never impounded.	1	24-Sep-12	5450	34.21286	-87.35311	electrofish/seine
09	*O. validus*	Payne Creek	reach upstream of reservoir full pool (510 feet) extent.	9	24-Sep-12	5451	34.21479	-87.35432	electrofish/seine

Appendix 1. Continued.

Site	Species	Stream	Location	Total crayfish	Collection date	CBHR catalog #	Latitude	Longitude	Method
50	*O. validus*	Payne Creek	at river km 1.0.	3	20-Oct-14	5687	34.21909	-87.35521	electrofish
51	*O. validus*	Payne Creek	at river km 2.0.	8	20-Oct-14	5688	34.22638	-87.35664	electrofish
10	*O. validus*	Sipsey Fork	at approximately river km 13; roughly 1/2 way between Mill and Grindstone creeks.	1	25-Sep-12	5454	34.17571	-87.33222	visual search
11	*O. validus*	Sipsey Fork	at approximately river km 16; about 1.1 km upstream of Grindstone Cr.	1	25-Sep-12	5457	34.18854	-87.34310	boat electrofish
11	*O. validus*	Sipsey Fork	at approximately river km 16; about 1.1 km upstream of Grindstone Cr.	1	25-Sep-12	5458	34.18854	-87.34310	visual search
11	*O. validus*	Sipsey Fork	at approximately river km 16; about 1.1 km upstream of Grindstone Cr.	2	26-Sep-12	5456	34.18854	-87.34310	trap
12	*O. validus*	Sipsey Fork	at approximately river km 17; about 2 km upstream of Grindstone Cr.	1	26-Sep-12	5459	34.19831	-87.34554	trap
13	*O. validus*	Sipsey Fork	at approximately river km 18.4-18.9; downstream side of Moody's Bend; end about 0.5 km downstream of Payne Creek confluence.	2	25-Sep-12	5461	34.21037	-87.34995	boat electrofish
14	*O. validus*	Sipsey Fork	at approximately river km 18.8.	2	19-Sep-12	5463	34.21140	-87.34976	visual search
15	*O. validus*	Sipsey Fork	at approximately river km 19.5; about 0.3 km upstream of Payne Cr.	3	19-Sep-12	5464	34.20900	-87.35224	visual search
16	*O. validus*	Sipsey Fork	Upper transition zone- high gradient riffle appears to represent a boundary between frequently vs rarely impounded segments. From mouth of Payne Cr. upstream to approximately river km 21.	9	24-Sep-12	5465	34.20959	-87.35790	visual search
17	*O. validus*	Sipsey Fork	at approximately river km 21; transition zone.	7	26-Sep-12	5467	34.20974	-87.36238	electrofish/seine
18	*O. validus*	Sipsey Fork	upstream of frequently-impounded zone; from the reservoir-river transition zone to Hwy. 33 bridge (about river km 21-23.2).	3	26-Sep-12	5468	34.21789	-87.36381	electrofish/seine
18	*O. validus*	Sipsey Fork	upstream of frequently-impounded zone; from the reservoir-river transition zone to Hwy. 33 bridge (about river km 21-23.2).	7	26-Sep-12	5469	34.21789	-87.36381	visual search
41	*O. validus*	Sipsey Fork	at river km 24. near end of access from AL Hwy 33.	8	19-Sep-13	5506	34.22461	-87.37668	visual search
41	*O. validus*	Sipsey Fork	at river km 24; near end of access from AL Hwy 33.	3	27-Apr-15	5794	34.22461	-87.37668	electrofish/seine
47	*O. validus*	Sipsey Fork	at river km 14.5-14.0.	3	23-Oct-14	5682	34.18723	-87.33098	trap
49	*O. validus*	Sipsey Fork	at river km 16.6-16.8.	1	23-Oct-14	5684	34.19630	-87.34294	trap
64	*O. validus*	Sipsey Fork	at river km 34.8.	3	27-Apr-15	5786	34.27396	-87.37902	electrofish/seine
65	*O. validus*	Sipsey Fork	at river km 29; about 200 m downstream of confluence with Hurricane Creek. Accessed from FS Rd. 228.	2	28-Apr-15	5787	34.25102	-87.36697	electrofish/seine
66	*O. validus*	Sipsey Fork	at river km 38.5; downstream of Cranal Rd. (AL Hwy 60) crossing.	3	28-Apr-15	5789	34.28453	-87.39864	electrofish/seine

Appendix 2. Collection records for *Orconectes lancifer* in Mississippi and Alabama outside of the most commonly published native range. Latitude and longitude are in decimal degrees. Where known, the map datum is indicated. Month, day, and year of collection are given. The name of person identifying (ID) the specimens and the year identified are indicated, if known. Georeferencing information refers to assigning latitude and longitude to locality descriptions from museum databases. Source abbreviations are as follows: GSA - Geological Survey of Alabama, Tuscaloosa, AL; INHS - Illinois Natural History Survey, Champaign, IL; MMNS - Mississippi Museum of Natural Science, Jackson, MS; USNM - National Museum of Natural History, Washington, DC; and TUMNH - Tulane Museum of Natural History (crayfish collection now housed at MMNS). Export date is date records were extracted from original database and sent to authors.

Entry	Crayfish form													Map			
#	M1	M2	JM	F	JF	U	SiteName	Location	County	State	Drainage	Lat	Long	datum	Month	Day	Year
1							Oxbow Lake	at Choctaw Bluff Camp (Stimpson Game Preserve).	Clarke	AL	Mobile	31.3681	-87.7611	—	7	3	1964
2	0	0	0	0	3	0	Buzzards Island	Tombigbee River, about 4 air miles S of Columbus.	Lowndes	MS	Tombigbee	33.4206	-88.4251	—	7	12	1972
3	0	5	0	6	0	0	Holley Cr.	just above confluence with Alabama River.	Baldwin	AL	Mobile	31.1825	-87.8542	—	8	9	1973
4	1	0	0	0	0	0	Sioux Bayou	—	Jackson	MS	Pascagoula	30.4195	-88.6497	—	1	10	1979
5	0	0	0	1	0	0	Caswell Lake	on Pascagoula River. T5S 6W Section 27.	Jackson	MS	Pascagoula	30.6010	-88.5909	—	8	9	1995
6	0	2	0	0	0	0	Old Dead River	in Pascagoula River. Three Rivers: T6S R6W Section 17.	Jackson	MS	Pascagoula	30.5195	-88.6116	—	8	11	1995
7	0	0	0	4	0	0	pond	of channel leading to Perch Lake in Pascagoula River. T3S R7W Section 25.	George	MS	Pascagoula	30.7817	-88.6842	—	8	14	1995
8	0	1	0	0	0	0	East Branch Pearl River	7 miles WNW Picayune, Walkiah Bluff Recreational Area.	Pearl River	MS	Pearl	30.5710	-89.7895	—	6	19	1996
9	0	0	0	1	0	0	Dennis Cr.	2.2 km NW of junction of Kilcrease Road & AL State Route 225.	Baldwin	AL	Mobile Bay	30.9112	-87.8761	WGS84	9	29	2006
10	1	0	0	2	0	0	Sipsey Fork River	in the headwaters of Lewis Smith Lake, at US Hwy 278 crossing.	Winston	AL	Tombigbee	34.1408	-87.3140	WGS84	10	24	2012
11	0	0	0	0	0	2	Pickwick Reservoir	in Panther Creek embayment at boat ramp, among milfoil, adjacent to Lauderdale County Road 105 (aka Panther Creek Road).	Lauderdale	AL	Tennessee	34.9600	-88.1528	WGS84	10	9	2013
12	0	0	0	1	0	0	Black Warrior River	at US 82 near Bama Belle at inlet.	Tuscaloosa	AL	Tombigbee	33.2143	-87.5699	—	10	14	2013
13	3	0	0	4	0	0	Moon Lake	at Fosters, Alabama.	Tuscaloosa	AL	Tombigbee	33.0472	-87.6352	WGS84	6	5	2014
14	0	0	0	1	0	0	Elliott's Creek tributary	at Allen Acres.	Hale	AL	Tombigbee	32.9768	-87.6878	WGS84	6	30	2015
15	0	2	0	1	0	0	Little Keaton Lake	at Allen Acres (Oxbow; Elliot's Creek).	Hale	AL	Tombigbee	32.9841	-87.6900	WGS84	6	30	2015
16	0	0	0	1	0	0	Black Warrior River	in Black Warrior Lock 9 wetland/ backwater at Allen Acres.	Hale	AL	Tombigbee	32.9899	-87.7049	WGS84	6	30	2015

Appendix 2. Extended.

Entry #	Collected by	ID by	ID year	Georeferenced by	Georeferencing notes	Data source	Original ID	Export date
1	RD Suttkus, Environmental Biology class	–	–	–	–	TUMNH	TUMNH #374	5/27/2015
2	H Boschung	HH Hobbs, Jr.	1974	JG McWhirter	Coordinates indicate general location - collection was pre-TennTom waterway. Island may no longer exist.	USNM	USNM# 146126	08/16/2005
3	–	JF Fitzpatrick, Jr.	–	–	–	USMN	USNM# 177553	5/27/2015
4	J Underwood	JF Fitzpatrick, Jr.	1980	JG McWhirter	Coordinates locate Sioux Bayou at only road crossing.	USNM	USNM# 209191	08/16/2005
5	MS Peterson et al.	–	–	JG McWhirter	Changed township from T15S.	MMNS	CatNo 1155	02/25/2008
6	MS Peterson et al.	–	–	AM Carson	–	MMNS	CatNo 1148	02/25/2008
7	MS Peterson et al.	–	–	JG McWhirter	Changed county from Jackson to George.	MMNS	CatNo 1149	02/25/2008
8	MH Sabaj, JW Armbruster, TJ Near, JM Serb	CA Taylor	1996	JG McWhirter	–	INHS	INHS# 5615	09/01/2007
9	GSA	GA Schuster	–	–	–	GSA	–	5/27/2015
10	G Schuster, SW McGregor	GA Schuster	2012	–	–	INHS	–	–
11	SW McGregor, CC Johnson	–	–	–	–	McGregor	–	10/20/2015
12	A Espy-Brown et al.	GA Schuster	2013	–	–	GSA	–	5/27/2015
13	MR Kendrick			–	–	Kendrick	–	
14	MR Kendrick, R Bearden, N Brook	GA Schuster	2015	–	–	Kendrick	–	10/20/2015
15	MR Kendrick, R Bearden, N Brook	GA Schuster	2015	–	–	Kendrick	–	10/20/2015
16	MR Kendrick, R Bearden, N Brook	GA Schuster	2015	–	–	Kendrick	–	10/20/2015